Fish Nutrition Research in Asia

Proceedings of the Third Asian Fish Nutrition Network Meeting

Edited by S.S. De Silva

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Contents

Foreword

This publication provides a summary of the discussions held at the Asian Institute of Technology near Bangkok, Thailand, 6-10 June 1988. This workshop was the third in a series funded by the International Development Research Centre (IDRC) to develop a more coordinated regional fish nutrition research program in Asia.

The workshop consisted of two parts: review papers on topics selected at the Second Fish Nutrition Workshop (Manila, 1986) and invited research papers from other scientists in the region. The review papers were part of an attempt to develop more standardized terminology and research protocols for the region. The invited papers were opportunities for some scientists to present their latest research results and seek critical inputs from their colleagues under the workshop format.

In addition, an economist (D. Atapattu) and a fish disease worker (M. Shariff) from the region joined the workshop to present papers related to nutrition and to make the nutrition researchers aware of issues from these related disciplines. It had been agreed at the earlier workshops that broader issues need to be considered and this was a first attempt on this approach. Other disciplines will likely be encouraged to participate in future meetings.

A variety of follow-up actions were discussed at this meeting and these are summarized in the discussion sessions. A fourth meeting in 12-18 months was one of the suggested future activities.

The meeting agreed to formalize an Asian Fish Nutrition Network (AFNN) to provide a more formal structure for this activity. It is hoped that the AFNN can be affiliated with the Asian Fisheries Society initially as a study group and later as a section. IDRC has agreed to provide partial funding for some of the proposed AFNN follow-up activities. Asian fish nutrition scientists interested in collaborating with AFNN should contact Dr. S.S. De Silva at the Faculty of Science, University of Ruhuna, Matara, Sri Lanka.

IDRC is very pleased with the developments taking place under the AFNN. IDRC would like to thank Dr. T. Watanabe and Dr. C. Young Cho for acting as resource persons at this workshop, Dr. Kok Leong Wee for his assistance in making the local arrangements at AIT and Dr. S.S. De Silva for most of the work in arranging the program, scientific papers and producing the proceedings.

The Asian Fisheries Society was a natural partner for the Network in publishing these proceedings. The Second Finfish Nutrition Workshop from which many of the papers here were derived was held during the Society's First Asian Fisheries Forum and this close linkage is expected to increase if the Network forms a section of the Society in the future.

This publication is the fourth in the Society's Special Publication series, which are provided free to members. In this way the Asian Fisheries Society helps its members overcome the difficulties associated with keeping up with advances in their field.

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Introduction

More than 50% of the world aquaculture production is attributed to Asia, the cradle of aquaculture. However, in most Asian countries aquaculture is practiced at a subsistence level. Conflicting demands for space and water in particular have called upon intensification of the traditional practices; supplementary feeding is basic to intensification. The highest recurring cost in intensified practices is attributed to feed cost, and therefore nutritional research in the region is becoming increasingly important.

Development of suitable, low cost feeds for fin and shellfish species cultured in most of Asia is still in the early stages. Basic research into investigations on nutritional requirements are not known for many of the cultured species in the region. Unlike in temperate waters the contribution from natural production in the ponds is likely to play a significant role in the nutrition of most cultured species; quantification of this component will enable a saving in the feed cost.

This volume contains papers presented at the Third Workshop on Fish Nutrition held at the Asian Institute of Technology, Bangkok, Thailand in June 1988. The volume is divided into two parts for convenience and clarity. The first part includes presentations made on basic nutritional requirements and methodologies related to experimental design in the laboratory as well as in outdoor facilities, and the second part contains the presentations made in ongoing original nutrition research in the region. Attempts have been made to obtain a fairly representative cross-section of the ongoing research on fish nutrition in the region, species-wise, depicting the immediate nutrition needs for intensification of the practices in the region.

This volume, like its predecessors, does not claim to be a treatise for fish nutrition researchers. This is an addition to the much needed relevant and appropriate documentation for the growing number of researchers in the region. It is however, hoped that in the course of the next few years these volumes will provide a comprehensive reference work on fish nutrition research in tropical Asia.

I am thankful to Drs. C. Young Cho, T. Watanabe, Kok Leong Wee and F. Brian Davy for their help in the editing.

The proceedings were edited when I was on attachment to the Department of Zoology, National University of Singapore (NUS), and I am grateful to the NUS for giving me this opportunity.

SENA S. DE SILVA

Part I

Nomenclature, Terminology and Definitions Appropriate to Animal Nutrition

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Devendra, C. 1989. Nomenclature, terminology and definitions appropriate to animal nutrition, p. 1-10. *In* S.S. De Silva (ed.) Fish Nutrition Research in Asia. Proceedings of the Third Asian Fish Nutrition Network Meeting. Asian Fish. Soc. Spec. Publ. 4, 166 p. Asian Fisheries Society, Manila, Philippines.

The paper attempts to present a comprehensive coverage of nomenclature, terminology and definitions appropriate to animal nutrition which are relevant to fish production. Particular focus is made to the expression of energy and protein that are currently used, inter-conversion of energy values and methods of measuring protein quality. Of the energy systems, the metabolizable energy (ME) system is the most widely applied. Concerning proteins, digestible crude protein (DCP) and true protein values are also widely used. Reference is also made to efficiency as it applies to the overall production process and especially to feed efficiency, including various measures of it. A glossary of common terms used in animal nutrition is also included.

In a previous publication (Devendra 1986), the general approaches to animal nutrition research and their relevance to fish production were discussed with reference to four objectives as follows:

(i) Identification and definition of the feed resources.
(ii) Assessment of nutritive value.
(iii) Utilisation in efficient and economic feeding systems, and
(iv) Determination of nutrient requirements.

The paper also referred to the significance of nutrient variables that influence production responses, the importance of feeding standards and a strategy for fish feed formulation. Reference is also made in this context to the publication by Cho et al. (1985) on the methodological details to research and development in fish nutrition.

In a continuation of the focus on fish nutrition, this paper is concerned with nomenclature, terminology and definitions appropriate to animal nutrition. The reference to these aspects has a bearing on an understanding of research in fish nutrition.

It is not proposed to deal exhaustively with all aspects of the subject since much of the information can be found in various books on animal nutrition, although this is likely to be scattered. Thus, for example, little or no reference will be made to identification and definition of the various types of agro-industrial by-products, including non-conventional feeds, which are potentially useful as energy or protein feeds for fish. Attention will be focused on the energy and protein systems in use, and the importance of efficiency in animal nutrition which may be of relevance to fish nutrition.

Expressions of Energy

Quantitatively, energy is the most important nutrient in the diet of animals. Several expressions of energy systems exist. It is now known that over a wide range of forages and concentrate feeds, the assessment of energy values using different systems is variable and inaccurate. In general the efficiency of energy utilization increases with dietary, energy density. This does not occur at a constant rate and is dependent on the physiological conditions and functions of the animal. The following expressions of energy are used:

(i) Starch Equivalent (SE) - United Kingdom
(ii) The Scandinavian Fodder Unit (SFU) - Denmark
(iii) Fattening Fodder Units (FFU) - Denmark
(iv) The French systems of Fodder Equivalent - France
(v) Rostock NEF system - Germany
(vi) Net Energy of lactation system - Netherlands
(vii) California system - USA
(viii) Total Digestible Nutrients (TDN) - USA
(ix) Metabolisable Energy (ME) - United Kingdom
(x) Digestible Energy (DE) - United Kingdom.

Alderman (1983) has reviewed the status of the newer European feeding systems and concluded that the ME value was basic to all systems. More recently, Alderman (1985) has also reviewed the different methods for the prediction of the energy value of compound feeds for ruminants, poultry and pigs. Of the systems in use, it is clear that the ME system is the most widely applied presently. It has become the preferred expression of feed value (MAAF 1975; Van Eys et al. 1978; ARC 1980; Sibbald 1982; Dewhurst et al. 1986) since the deduction of energy losses in urine and fermentation gases from DE provide a more accurate estimate of productive energy. ME can be calculated from DE from the equation ME = 0.81 DE based on methane and urine energy as a constant fraction (Armstrong 1964). Evidence that the ME system is superior to the SE system is seen in the results of studies by Brabender et al. (1978) and Hill (1977). The former used 55 different diets of dairy cows to compare the total energy intake with each animal's requirements for maintenance and production within each system. It was found that the ME system fitted the actual energy requirements better than the SE system. Similar results were also reported by Hill (1977) for dairy cows. Both reports indicated that the SE system underestimated the energy requirements of the cows.

ME or DE for each type of animal is usually calculated from the average ME or DE in dry matter or from various published equations. For poultry, it can also be calculated from the average true ME (TME) using the method of Sibbald (1982). DE is calculated as follows:

DE = Gross energy of feed in DM x Gross energy digestion coefficient.

Energy Conversions

It is appropriate to keep in perspective that it is feasible to convert different feed units in terms of energy, with the full knowledge that these are approximations and subject to error. The interconversion of energy values enables the expression of nutritive values of the same feed in different laboratories and locations. The following conversions are important:

1 kg starch equivalent (SE)	=	5.082 Mcal DE
	=	4.167 Mcal ME
	=	1.15 kg TDN

	=	1.10 kg DOM
	=	2.356 kcal NE
1 kg Scandinavian feed unit (SFU)	=	2.820 Mcal ME
1 feed fattening unit (FFU)	=	1.650 Mcal ME
1 kg TDN	=	4.409 Mcal DE
	=	3.620 Mcal ME
1 Kilo joule (KJ)	=	0.239 Kcal
1 Kcal	=	4.184 joule
1 Mcal	=	4.184 MJ
1 Mcal ME	=	0.81 Mcal DE

Expressions of Protein

A number of expressions of protein value exit. They are:

(i) Digestible crude protein (DCP)
(ii) True protein (TP)
(iii) Protein efficiency ratio (PER)
(iv) Net protein retention (NPR)
(v) Gross protein value (GPV)
(vi) Protein replacement value (PRV)
(vii) Biological value (BV)
(viii) Net protein utilisation (NPU)
(ix) Net protein value (NPV)
(x) Biological assays
(xi) Protein equivalent (PE)
(xii) Degradability

It is perhaps appropriate to focus on those methods that are more relevant. DCP for example is not an entirely satisfactory method of expressing protein value since the efficiency with which the absorbed protein is used differs from one species to another and even breeds within a species. It also does not indicate the usefulness to the animal. In comparison to DCP, true protein values are also used. The latter separates out the non-protein nitrogen (NPN) portion within the total protein. DCP is calculated as follows:

$$\text{(a) DCP} = \frac{\text{\% protein in DM x Digestibility coefficient of protein}}{100}$$

In addition to DCP, and in view of the limitations in it, other methods are used as follows:

$$\text{(b) PER} = \frac{\text{Gain in body weight (g)}}{\text{Protein consumed (g)}}$$

$$\text{(c) NPR} = \frac{\text{Weight gain of TPG - weight loss of NPG}}{\text{Weight of protein consumed}}$$

Where TPG = group fed on test protein
NPG = non-protein group

$$\text{(d) GPV} = \frac{A}{A_O}$$

Where A = g increased weight gain/g test protein
A_O = g increased weight gain/g casein

$$\text{(e) PRV} = \frac{A - B}{\text{N intake}}$$

Where A = N balance for standard protein in mg/basal KJ
B = N balance for protein under investigation in mg/basal KJ

$$\text{(f) BV} = \frac{\text{N intake - (faecal N - MFN) - (urinary N - EUN)}}{\text{N intake - (faecal N - MFN)}}$$

Where MNF = Metabolic faecal nitrogen
EUN = Endogenous urinary nitrogen

(g) NPU = Digestibility x BV

(h) NPV = NPU x crude protein

$$\text{(i) PE} = \frac{\text{\% DCP + \% Digestible protein}}{2}$$

Of these expressions, GPV is the most commonly used biological method for evaluating proteins. GPV measures the ability of proteins to supplement diets containing mainly cereals. The use of protein degradability is a relatively recent one, and one which is more appropriate to ruminant nutrition. The extent of the degradability of a protein source within the rumen reflects the usefulness in terms of amino acid supply posterior to the rumen. This is influenced by such factors as solubility and also the tanin content.

Efficiency

With reference to dietary nutrient supply to meet the requirements for maintenance and production, and the manner in which these are utilized, the final expression of these aspects is reflected in the general term efficiency. It is pertinent therefore to consider this issue briefly.

Efficiency in animal and fish production is the sum total of a number of interacting factors, genetic and phenotypic. It is developed through research aimed at maximizing genetic, nutritional and physiological potential of individual animal species. It is also dependent on improved management practices, reduced effects of the environment and disease factors.

The components which influence the measurement of efficiency are:

(i) Efficiency of feed conversion, which is influenced by the nutritional quality of the diet, level of feeding, processing of ingredients, use of feed additives, prices and genetic potential.

(ii) Ability to reproduce efficiently, which is the sum total of age at first breeding, conception rate, number of offspring born and breeding interval. Survival of the young and wastage in adult life are influenced by several factors of which environmental adaptation, management and disease resistance are particularly important.

(iii) Age, duration and size of maturity for biomass production.

(iv) Reduced disease incidence.

Feed Efficiency

The efficiency with which farm animals convert feedstuffs into food for man is a subject of much interest, and has particular significance in animal and fish nutrition. In recent years, it is a subject that has received much attention (see for example Reid 1970; Holmes 1971).

The efficiency of feed conversion is influenced by several factors and include *inter alia* inherent genetic capacity, diet quality, level of feeding, processing and level of ingredient used, potential response and price of the product. Related to these is the time scale involved.

There are several measures of efficiency. The type of index is influenced by the type of units used. The indices below reflect the more common ones used in animal nutrition. Reference is made to each method as well as how each one is derived:

$$\text{(a) Apparent efficiency (meat)} = \frac{\text{Amount of meat produced}}{\text{Quantity of feed consumed}}$$

$$\text{(b) Gross efficiency (meat)} = \frac{\text{Apparent efficiency}}{\text{Dressing percentage}}$$

$$\text{(c) Gross energy (GE) \%} = \frac{\text{Edible energy}}{\text{Total GE consumed}} \times 100$$

$$\text{(d) Metabolisable energy (ME) \%} = \frac{\text{Edible energy}}{\text{Total ME consumed}} \times 100$$

$$\text{(e) Edible protein (\%)} = \frac{\text{Edible protein}}{\text{Total feed protein consumed}} \times 100$$

$$\text{(f) Edible protein (g/MJ ME)} = \frac{\text{Edible protein}}{\text{Total ME consumed (MJ)}}$$

$$\text{(g) Edible protein (g/MJ GE)} = \frac{\text{Edible protein}}{\text{Total GE consumed (MJ)}}$$

Glossary of Terms

A wide variety of terms are used in animal nutrition associated with nomenclature, terminology, various expressions of dietary energy and protein, and measures of efficiency. It is impossible and also impractical to draw reference to all the terms used, however, an attempt is made to bring together the ones that are more commonly used. Appendix I presents this compilation to reflect the range of terms used, an understanding of the use of the terms, especially with regard to description of research methodology, results and discussion appropriate to animal nutrition. It is hoped that this compilation will be helpful to fish nutritionists.

References

ARC. 1980. The nutrient requirements of ruminant livestock. Agriculture Research Council. Commonwealth Agricultural Bureau, Farnham Royal, Bucks, England, xvi + 351 p.

Alderman, G. 1983. Status of the newer European feeding systems. Proceedings 2nd INFIC Symposium, Sydney, Australia, p. 305-321.

Alderman, G. 1985. Prediction of the energy value of compounds feeds. *In* W. Haresign and D.J.A. Cole (eds.) Recent advances in animal nutrition, p. 1-52. Butterworth, London, England.

Armstrong, D.G. 1964. Evaluation of artificially-dried grass as a source of energy for sheep. Journal of Agricultural Science, Cambridge 62: 399-416.

Brabander, D.L., R.J. Moerman, J.-V. Aerts and F.X. Buyne. 1978. Is the new energy system (VEM) for dairy cows better than the starch value system? Revue de l'Agriculture 31: 619-629.

Cho, C.Y., C.B. Cowey and T. Watanabe. 1985. Fish nutrition in Asia. International Development Research Centre, Ottawa, Canada, 154 p.

Devendra, C. 1986. General approaches to animal nutrition research and their relevance to production in the Asian region. *In* S.S. De Silva (ed.) Fish nutrition research in Asia. Proceedings Fish Nutrition Workshop, Manila, Philippines. 129 p.

Dewhurst, R.J., A.J.F. Webster, F.W. Woinman and P.J.C. Dewey. 1986. Prediction of true metabolisable energy concentration in forages for ruminants. Animal Production 43: 183-194.

Hill, R. 1971. Comparison of the starch equivalent (SE) and metabolisable energy (ME) systems in feeding cattle. Veterinary Records 101: 381-386.

Holmes, W. 1971. Animals for food. Proceedings Nutrition Society 29: 237-244.

MAAF. 1975. Ministry of Agriculture, Fisheries and Food. Energy allowances and feeding systems for ruminants. Technical Bulletin, 33, HMSO, London, United Kingdom.

Reid, J.T. 1970. The future role of ruminants in animal production. *In* A.T. Phillipson (ed.) Physiology of digestion and metabolism in the ruminant, p. 1-22. Oriel Press, Newcastle upon Tyne, England.

Sibbald, I.R. 1982. Measurement of bioavailable energy in poultry feeding stuffs: a review. Canadian Journal of Animal Sciences 62: 983-1048.

Van Eys, A.J.H., M. Vermorel and H. Bickel. 1978. Feed evaluation for ruminants: new energy systems in the Netherlands, France and Switzerland. Livestock Production Science 5: 527-330.

APPENDIX I

Glossary

Additives	-	Feed materials such as antibiotics, colouring matter, flavours, hormones and medicants.
Biological value (BV)	-	of a protein is a measure of protein retention to protein absorption.
Cake	-	The residue that results from pressing seeds or meat to extract oil, fat and other liquids.
Calorie	-	The unit for measuring the energy in feeds. 1000 cal = 1 Kcal 1000 Kcal = 1 Mcal 1 cal = 4.184 joules (J)
Concentrate	-	Class of feeds that are low in crude fibre and high in total digestible nutrients such as cereal grains and high quality by-products like fish meal and groundnut cake. It is usually used to improve the total nutritive value of a diet for production.
Crude fibre	-	The ash-free residue of a food which remains after boiling for 30 minutes successively in 1.25% sulphuric acid and 1.25% sodium hydroxide. With most foods, crude fibre represents some 90 to 95% cellulose, the remainder being hemicellulose, lignin and other plant cell wall constituents.
Crude protein	-	Refers to the true protein component and all the nitrogen (N) in the feed. It is determined by multiplying the total N by 6.25; the latter represents the average N content in most foods.
Degradability	-	Of dry matter or of a protein is the measure of the extent to which it is degraded in the rumen of ruminants.
Digestibility	-	Is that proportion of a feed which is not excreted in the faeces and is assumed to be absorbed by the animal.
Digestible crude protein	-	Refers to the protein of the (DCP) dietary proteins which are digested and absorbed.
Digestible energy (DE)	-	The portion of the gross food energy (GE) minus the faecal energy that has been appa-rently absorbed. DE = (GE of feed per unit dry wt. x dry wt. of feed) - (GE of faeces per unit dry wt. x dry wt. of faeces).

Digestible nutrients	-	The portion of the dietary nutrients which is digested and absorbed by the animal body. These usually refer to carbohydrates, fats and proteins.
Dry matter (DM)	-	Refers to the moisture-free residue of animal sample. It is determined by keeping a sample in an oven at 105 C until it reaches constant weight.
Essential amino acids (EAA)	-	Are amino acids which are essential to the animal and which the animal body cannot synthesize fast enough to meet the requirements. These include arginine, histidine, leucine, isoleucine, threonine, phenylalanine, threonine, tryptophan and valine.
Energy feeds	-	Ingredients with less than 20% protein and less than 18% crude fibre.
Ether extract	-	Also called crude fat. The material extractable with any anhydrous solvent, for example, petroleum spirit or di-ethyl ether. It contains neutral fats and all fat-soluble materials.
Feed efficiency	-	Refers to the ability with which animals can convert the feed consumed into edible and other products. Its accurate determination is dependent on the inputs used and outputs derived.
Feeding standards	-	Statements of the amounts of nutrients required by animals and fishes. The term is synonymous with nutrient requirements and allowances.
Gross energy (GE)	-	Is the amount of heat released from a feed when it is completely oxidized in a bomb calorimeter containing 25 to 30 atmospheres of oxygen.
Heat increment (HI)	-	Is the increase in heat production following consumption of a feed or ration when the ani-mal is in a thermally neutral environment. It consists of increased heats of fermentation produced in the digestive tract as a result of microbial action and during intermediary meta-bolism and absorption. HI is wasted except when the temperature of the environment is below the animal's critical temperature.
Intake	-	Refers to the amount of feed consumed and available for digestion, usually expressed in DM. Intake is the result of either restricted or *ad libitum* feeding.
Maintenance	-	State of energy equilibrium of an animal when there is no net gain or loss of energy in body tissues.
Meal	-	Describes the physical form of a feed that has been reduced to a particle size larger than that of flour.

Metabolisable energy (ME)	-	The portion of the gross food energy (GE) minus faecal energy, minus energy in the gaseous products of digestion minus urinary energy. ME = (GE of feed) - (GE of faeces + GE of gaseous products + GE of urine).
Minerals	-	Are elements that have a metabolic role in the body. They include macro-minerals and microminerals; the latter include trace minerals.
Net energy (NE)	-	Is the amount of energy used either for maintenance or for production or both. NE = ME - HI. NE is available for maintenance (NE_m) or production (NE_p). The latter refers to growth, fattening draught or in the production of milk, eggs and fibre.
Nitrogen-free extract (NFE)	-	The food fraction that is calculated as the difference between the dry matter of the sample and the sum of the determined ash, crude protein, ether extract and crude fibre. Its chief components are starches, variable amounts and lignin and hemicellulose. NFE (%) = 100 - % ash - % crude protein - % ether extract - % crude fibre.
Non-essential amino acids	-	Are amino acids which are not needed in the diet but which are essential to the animal, e.g., aspartic acid, alanine, cystine, glycine and proline.
Non-protein nitrogen (NPN)	-	Compounds which are not true protein in nature but contain N and can be converted to protein by bacterial action, e.g., urea and biuret.
Nutrient balance	-	Condition which describes a diet that makes available various nutrients in the right amounts to fulfill the physiological needs of animal to meet both maintenance and production requirements (growth, pregnancy, meat, milk, fibre, eggs or work).
Pellets	-	Refers to physical form during which a feed or combination of feeds are compacted by mechanical means. Different size pellets can be made depending on die openings.
Pressed	-	Process of compaction by pressure or extraction under pressure.
Protein quality	-	Refers to the amount and ratio of amino acids in the protein.
Protein supplement	-	Products which contain 20% or more protein from plant or animal origins.

Roughages	-	Class of feeds, usually plant materials, that are very fibrous, bulky and contain usually more than 18% crude fibre. They are low in total digestible nutrients, such as straws and stovers.
Supplement	-	A feed, either alone or in combination which is used to increase the availability of nutrients and also performance of animals. Can be an energy, protein, mineral and/or vitamin supplement.
Total digestible nutrients (TDN)	-	Is the sum of all digestible organic materials (proteins, fibre, fat and NFE). TDN = Digestible crude protein x 1% + digestible crude fibre x 1% + digestible NFE x 1% and digestible ether extract x 2.25%.
True protein	-	The protein of the protein source which is composed only of amino acids.
Vitamins	-	Are important organic compounds in animal nutrition that are associated in enzyme systems and metabolism for various body functions.

Protein Requirements of Fish and Prawns Cultured in Asia*

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Pandian, T.J. 1989. Protein requirements of fish and prawns cultured in Asia, p. 11-22. *In* S.S. De Silva (ed.) Fish Nutrition Research in Asia. Proceedings of the Third Asian Fish Nutrition Network Meeting. Asian Fish. Soc. Spec. Publ. 4, 166 p. Asian Fisheries Society, Manila, Philippines.

In general, fish are better convertors than prawns. Tropical fish grow faster at relatively lower optimum dietary protein density but less efficiently than temperate fish. Penaeids grow faster but less efficiently than Palaemonids. Optimum dietary protein density requirement of brooder prawns is higher than the juveniles. Prawns lose over 20% of the converted body substance at moulting. Nitrogen requirement per unit ingested food (kJ) is in the range of 15 and 21 mg for fish and prawns, respectively.

Feed constitutes the major fraction of the operational cost in aquaculture. Protein is generally the most expensive component of artificial diets. Unlike in mammals, protein acts both as a structural component and as an energy source in fish (Brett and Groves 1979) and decapods (Castell and Budson 1974). Consequently, the dietary protein requirements of these organisms are higher. However, an excess dietary protein may not only cost more but also increase the energy cost of assimilation by hiking the Specific Dynamic Action (LeGrow and Beamish 1986). Therefore, this paper presents a critical summary of available information on protein requirements of fish and decapods cultured in Asia.

Available information on the qualitative (Phillips 1969; Cowey and Sargent 1972; Halver 1972; Cowey 1979) and quantitative (e.g., Brett and Groves 1979; Pandian 1987) aspects of fish nutrition has been critically summarized from time to time by different authors. Unfortunately, relatively less information is available on decapod nutrition and even that is scattered in the literature, and a comprehensive review is yet to be published. Hence this review gives greater importance to decapod nutrition. A hierarchy of terms are now used; Jobling (1983) reviewed the terms used to indicate the rate or efficiency of a process of energy transformation and recommended specific terms as well as methodology to be used in fish growth and nutrition studies. A corresponding review for decapod nutrition is not available. Therefore, the reviewer has to subjectively depend on values reported from selected publications.

Protein Requirements of Fish

The protein requirements of fish are about 2 to 3 times higher than that of mammals (Pandian 1987). Table 1 summarizes available information on protein requirement of finfish

*Dedicated to Prof. S. Krishnaswamy on his 61st birthday.

Table 1. Protein requirements of some fish (from Pandian 1987; modified).

Species	Tested diet	Mid body wt (g)	Feeding rate (% body wt d^{-1})	Protein requirement (% dry wt of food)
Carnivores				
Anguilla japonica	casein	4.4	ad lib	45
A. anguilla	-	-	-	62
Channa micropeltes	fish meal	21	1.8	52
Chrysophrys aurata	casein/amino acids	8.9	1.8	52
C. major	-	-	-	55
Fugu rubripes	casein	3.2	3.2	47
Morone saxatilis	fish/soymeal	6.0	2.8	55
Oncorhynchus tshawytscha	-	-	-	48
Pleuronectes platessa	cod muscle	22.3	1.8	50
Salmo gairdneri	casein/gelatin	12.6	ad lib 1.7	43
S. salar	-	-	-	40
Salvelinus alpinus	fish meal	-	-	40
Seriola quinqueradiata	-	-	-	50
S. gairdneri[1]	Tubifex/Pellet	3.5	2.5, 4, 5.5	-
S. gairdneri[2]	Pellet	6.5	5.0	40.7
Oncorhynchus kisutch[3]	Pellet	4.3	-	51
Omnivores				
Cyprinus carpio	fish meal	13.6	2.9	54
C. carpio[4]	Pellet	150	-	-
Ictalurus punctatus	-	-	-	40
I. punctatus[5]	pellet	24	2-6	40
I. punctatus[6]	*Hermetia illucens*/ Pellet	-	-	30
Micropterus salmoides	fish/gelatin/a. acids	3.5	2.9	40
M. dolomieu	fish/gelatin amino acids	3.6	2.4	45
M. salmoides[7]	Pellet	14.4	-	35.8
M. salmoides[7]	Pellet	14.4	-	35.9
Tilapia aureus[8]	fish/soymeal	4.4	3.5	36
T. aurea	casein/albumin	2.5	2.0	56
T. aurea	casein/albumin	2.5	10.0	34
T. aurea	*Hermetia illucens*	30	3.0	-
Herbivores				
Chanos chanos	casein	0.11	8.2	39
Ctenopharyngodon idella	casein	0.34	-	42
Tilapia mossambicus[9]	fish meal	5.1	4.7	42
T. mossambica	fish meal	6-30	3	35
T. niloticus	fish meal	0.31	6.4	35
Oreochromis niloticus[10]	pellet	0.185	2.5-5.5	30
Sarotherodon mossambicus	pellet	5.1	6	40

Arabic numbers (1,2,3,4,5,6,7,8,9 and 10) are additions and the corresponding references are: 1 Philips & Butler (1979), 2 Zeitoun et al. (1976), 3 Fagerlund et al. (1983), 4 Schwarz et al. (1985), 5 Andrews& Stickney (1972), 6 Bondari & Sheppard (1981), 7 Tandler & Beamish (1978), 8 Davis & Stickney (1978), 9 Jauncey (1982), 10 De Silva (1985).

species. Pandian (1987) pointed out that most workers have chosen juvenile fish, fixed low ration and used diets with limited protein sparing action. He concluded that the protein requirement of culturable Asian fish like the carps and tilapias, the mullets and milkfish, which are herbivores/omnivores, is in the range of 25-30%, as against 30-40% protein required by the carnivorous fish like the salmon and trout, that are cultured in developed countries. It may also be noted that the protein content of natural food of tropical fish like *Oreochromis mossambicus* (De Silva et al. 1984) and temperate fish like *Salmo gairdneri* (Yurkowski and Tabachek 1979) is recorded as 25 and 42%, respectively.

Herbivorous/omnivorous fish are reported to economize their protein requirements by (i) reabsorbing digestive enzymes and (ii) reducing protein metabolites. Herbivorous fish, especially

those with a long intestine, reabsorb over 90% of the digestive enzymes, as against 40% by the carnivores (Hofer and Schiemer 1981). The fraction of food nitrogen lost through ammonia is in the range of 3.5% for herbivores (e.g., Caulton 1978; Hofer et al. 1985), which is half of that (7%) excreted by carnivorous fish (Brett and Groves 1979).

Production Rate and Efficiency

Although several authors have reported the protein requirements of fish, adequate information on specific growth rate and conversion efficiency of the tested species was not always provided. Figs. 1 and 2 lead one to conclude (i) with respect to rate and efficiency of growth, finfish are 2 to 3 times better convertors than decapods, and (ii) the range of optimal dietary protein requirement of decapods is nearly 2 times wider than that of fish (Fig. 1). Hence protein (= feed) and time costs of fish production are cheaper than decapods. Not surprisingly, many developing countries like India domestically consume the low cost fish, and export the high cost decapods.

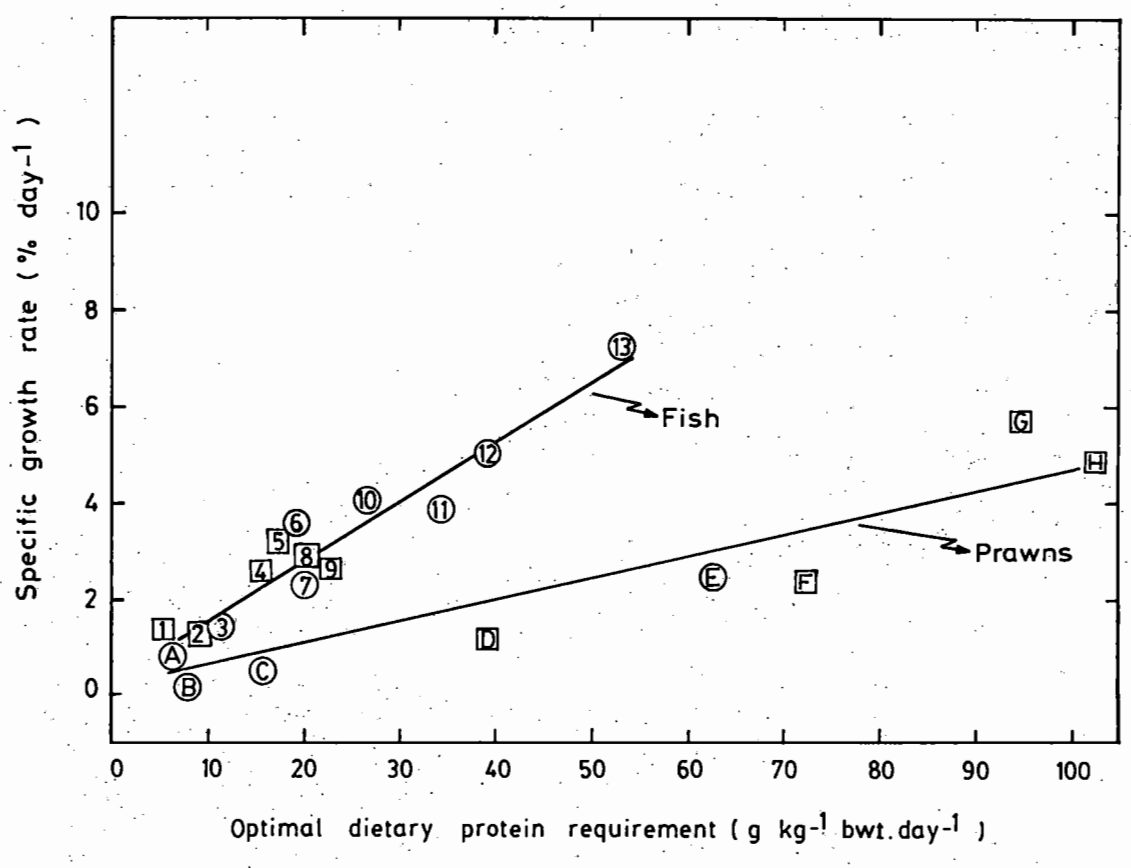

Fig. 1. Relationship between optimal dietary protein requirement and specific growth rate of fish and prawns: Fish - 1 - *Pleuronectes platessa* (Cowey et al. 1972); 2 - *Chrysophrys aurata* (Sabaut & Luquet 1973); 3 - *Channa microspeltes* (Wee and Tacon 1982); 4 - *Micropterus salmoides* (Anderson et al. 1981); 5 - *Morone saxatilis* (Millikin 1982); 6 - *Cyprinus carpio* (Jauncey 1981); 7 - *Tilapia zilli* (Mazid et al. 1979); 8 - *Salmo gairdneri* (Satia 1974); 9 - *Micropterus dolomieu* (Anderson et al. 1981); 10 - *Oreochromis mossambicus* (Jauncey 1982); 11 - *Tilapia aurea* (Davis and Stickney 1978); 12 - *Chanos chanos* (Lim et al. 1979); 13 - *Oreochromis niloticus* (Santiago et al. 1982): Prawns - A - *Macrobrachium nobilii* (Pandian and Murugadass, in prep.); B - *M. rosenbergii* (Boonyaratpalin and New 1982); C - *M. rosenbergii* (Manik 1976);D - *Penaeus monodon* (Lim 1983); E - *M. rosenbergii* (Millikin et al. 1980); F - *P. aztecus* (Venkataramiah et al. 1975);; G - *P. setiferus* (Fennucci et al. 1980); H - *P. stylostoris* (Fennucci 1981).

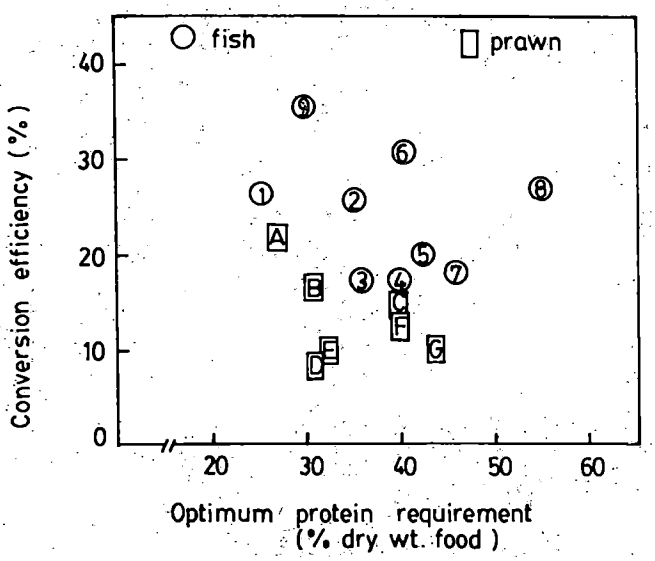

Fig. 2. The relationship between optimal dietary protein requirement and conversion efficiency values reported for fish and prawns: Fish - 1 - *Ictalurus punctatus* (Page and Andrews 1973); 2 - *Ictalurus punctatus* (Page and Andrews 1973); 3 - *Tilapia aureus* (Davis and Stickney 1978); 4 - *Sarotherodon mossambicus* (Jauncey 1982); 5 - *Tilapia mossambicus* (Jauncey 1981); 6 - *Salvelinus alpinus* (Jobling and Wandsvik 1983); 7 - *Salmo gairdneri* (Bromley and Smart 1981); 8 - *Morone saxatilis* (Millikin 1982); 9 - *Salmo gairdneri* (March et al. 1985): Prawn - A - *Macrobrachium rosenbergii* (Clifford and Bricks 1979); B - *M. nobilii* (Pandian and Murugadass, in prep.); C - *Penaeus aztecus* (Venkataramiah et al. 1975); D - *Penaeus setiferus* (Fennucci et al. 1980); E - *Penaeus stylirostris* (Fennucci et al. 1980); F - *Penaeus monodon* (Alava and Lim 1983); G - *Penaeus indicus* (Colvin 1976).

A linear relationship between optimal dietary protein requirements and specific growth rate for fish was first reported by Tacon and Cowey (1985). Our observation summarized in Fig. 1 not only confirms this but also points out that a similar linear relationship also holds good for decapods. Most tropical fish like tilapias, carps and milkfish display a faster specific growth rate than temperate fish such as trout. The relatively faster growth displayed by tropical fish demand nearly two times higher dietary protein requirements than their temperate counterparts. This is further confirmed by Pandian and Vivekanandan (1985), who observed that the feeding rate of tropical fish is 180% higher than that of temperate species, and that the elevation in feeding rate is nearly 2.5 times higher than that (70%) observed for maintenance metabolism. They concluded that a considerable fraction of this difference may contribute to faster, if not more efficient growth, a characteristic of tropical fish.

As may be seen from Fig. 1, dietary protein requirements of decapods are reliably estimated only for about 8 *Macrobrachium* and *Penaeus* species. In general, the requirements of *Macrobrachium* spp. are nearly a third of that for penaeids. Not surprisingly, *Penaeus* spp. grow faster than *Macrobrachium* spp. Another important point to be noted is that the dietary protein requirement of *Penaeus* spp. is nearly two times higher than the maximum required among fish.

The highest conversion efficiency values exhibited by prawns and fish, when fed at their respective optimum dietary protein requirements are shown in Fig. 2. Temperate species like salmonids are more efficient convertors than tropical fish. Likewise, the relatively slow-growing *Macrobrachium* spp. exhibit higher efficiency values than the penaeids. Apparently, tropical fish are tuned to exhibit faster growth rate but at low efficiency, and the temperate ones higher efficiency but low rate of growth. Likewise, the penaeids are tuned to grow faster but less efficiently, in comparison to *Macrobrachium* spp. Therefore, our major efforts with reference to fish and penaeids must be to provide nutritional and environmental conditions that would further accelerate the rate process of growth and the efficiency process of *Macrobrachium* spp.

Effect of Protein Density

The available information on protein requirements of some decapods is summarized in Table 2. Three species of Palaemonidae, 7 species of *Penaeus* and *Homarus americanus* have been tested on diets containing protein densities ranging from 0 to 76%. Using different culture

Table 2. Protein requirements of some tested decapod crustaceans. All *Macrobrachium* were tested in freshwater containing less than 2‰ at 26 ± 3°C. Others were tested at 29 ± 3‰ at 27 ± 3°C (O.P.L. - Optimum protein level).

Species	Range of protein level (% dry wt)	O.P.L. (% dry wt)	K_1 (%)	Authority
Macrobrachium rosenbergii	15-40	25	21.5	Clifford & Brick (1979)
M. rosenbergii	15-35	35	-	Balazs & Ross (1976)
M. rosenbergii	23-49	40	-	Millikin et al. (1980)
M. rosenbergii	25-40	35	-	Balazs et al. (1973)
M. rosenbergii	15-35	35	-	Boonyaratpalin & New (1982)
M. rosenbergii	23-30	25	-	Manik (1976)
M. rosenbergii	10-30	25	-	Perry et al. (1984)
M. nobilii	10-50	30	16.1	Pandian & Murugadass (in prep)
Palaemon serratus	12-69	35	-	Forster & Beard (1973)
Penaeus japonicus	60-76	60	-	Deshimaru & Shigano (1972)
P. japonicus	25-50	50	-	Deshimaru & Kuroki (1974)
P. japonicus	52-70	55	-	Deshimaru & Yone (1978)
P. japonicus	25-40	35	-	Balazs et al. (1973)
P. aztecus	25-40	40	-	Balazs et al. (1973)
P. aztecus	40-80	40	16	Venkataramiah et al. (1975)
P. aztecus	18-35	27	-	Shewbart et al. (1973)
P. setiferus	14-52	30	-	Andrews et al. (1972)
P. setiferus	29-37	30	7.3	Fennuci et al. (1980)
P. monodon	25-60	40	13.4	Alava & Lim (1983)
P. indicus	21-53	43	8.2	Colvin (1976)
P. merguiensis	34-42	42	-	Sedgwick (1979)
P. merguiensis	29-55	50	-	Aquacop (1978)
P. stylirostris	29-37	30	7.5	Fennuci et al. (1980)
Homarus americanus	0-60	60	-	Castell & Budson (1974)
H. americanus	11-60	53	-	Gallagher et al. (1976)
H. americanus	30-53	30.5	-	D'Abramo et al. (1981)
H. americanus	24-57	40	-	Conklin et al. (1975)

techniques, terminologies and methods, different workers have evaluated nutritional values of these decapods and reported dietary optimum protein densities that range from 25 to 60%.

The following generalizations could be made from Fig. 3; *Macrobrachium* spp. are better convertors than *Penaeus* spp; they exhibit higher efficiency values of about 16%, when fed on diet containing 20-30% protein. *Penaeus* spp. display lower efficiency values ranging from 7-14%, when fed 40-50% protein diets. Hence, *Macrobrachium* spp. display higher efficiency values at lower protein density than *Penaeus* spp. (Fig. 3). Briefly, the production cost of *Macrobrachium* spp. is likely to be cheaper than *Penaeus* spp.

Dietary Protein and Egg Production

Freshly spawned decapod eggs are known to contain 50% protein and 25% lipid (Pandian 1967, 1970 a,b, 1972; Katre 1977; Mathavan et al. 1986). Hence the reproductive growth, namely vitellogenesis must be a lipoprotein demanding process (Dehn et al. 1983). Evidently, the dietary protein requirement of brooders is likely to be higher than that of juvenile decapods. Although commercial firms are already selling specific diets for larvae, juveniles and brooders, to the best of the reviewer's knowledge, no worker has so far reported dietary protein requirement of brooders with specific reference to egg production in decapods. Murugadass (1989) is perhaps the first to experimentally determine the protein requirements of juveniles and brooders of *Macrobrachium nobilii*. Irrespective of changes in feeding regime and other culture conditions, *M. nobilii,* a diecdysic riverine prawn, attains sexual maturity when it grows to 600 mg weight. Therefore, it is possible to separately determine the dietary protein requirement of juveniles (< 600 mg) and brooders (> 600 mg).

The trends obtained for growth rate and conversion efficiency of juvenile and brooder *M. nobilii* fed on diets containing 10, 15, 25, 35 and 50% protein are shown in Fig. 4. Both from the points of rate and efficiency of growth, brooders proved to be better convertors than juveniles. The brooders are most efficient, when fed 45-50% protein diet, but in juveniles conversion is best when fed 35% protein diet (upper panel). Consequently, the optimum protein requirement level shifts from 35% for the juveniles to 46.5% for the brooders. Expectedly, fecundity per intermoult period is higher when the brooders are fed 45-50% protein diet (Fig. 5). Quality of the eggs, as noted by the dry weight is maximal when the brooders are fed the optimum protein diet.

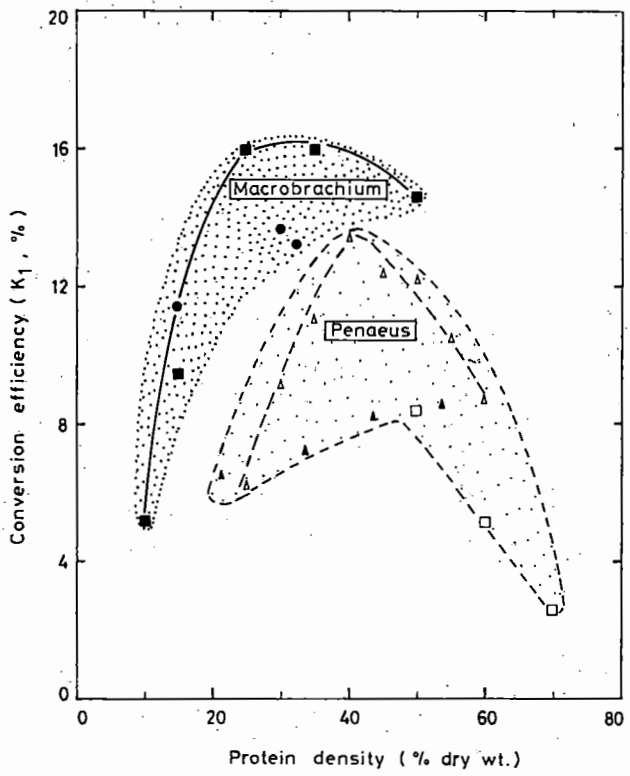

Fig. 3. Relationship between dietary protein density and conversion efficiency of *Macrobrachium* and *Penaeus* spp. *M. rosenbergii* (•); *M. nobili* (■); *Penaeus aztecus* (▲); *P. indicus* (Δ); *P. setiferus* (□).

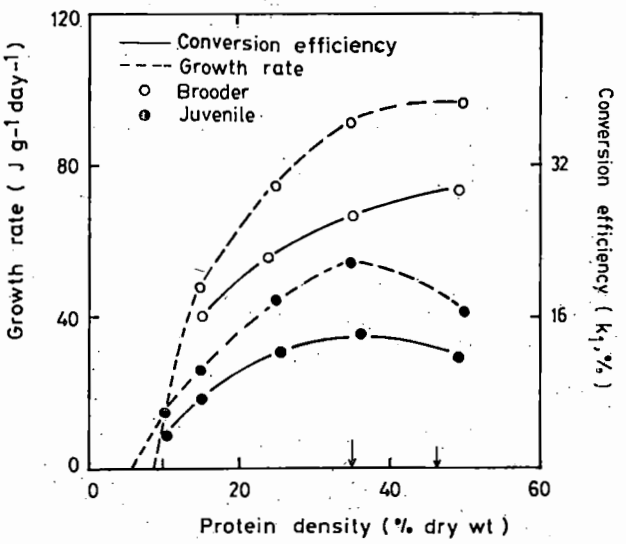

Fig. 4. Relationship between dietary protein density and rate and efficiency of growth in juveniles and brooder *M. nobilii* (from Murugadass 1987; modified).

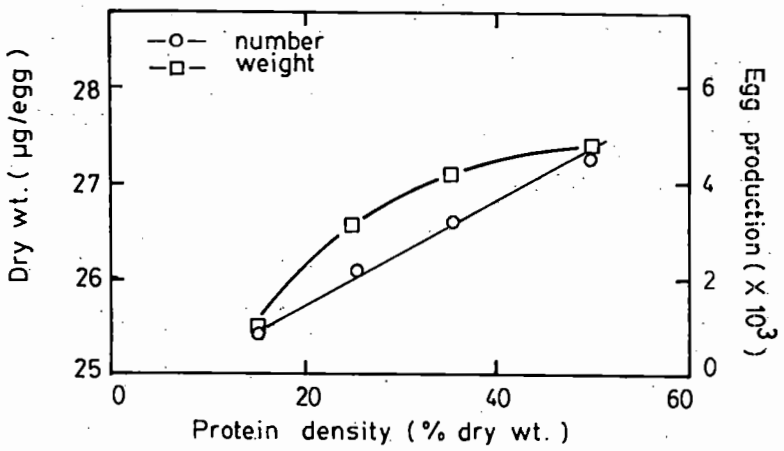

Fig. 5. Relationship between protein density, egg production (from 5 intermoult periods) and dry weight of egg of *M. nobilii* (Murugadass 1987; modified).

Energy Partitioning

As stated elsewhere, finfish are better convertors than decapods. A major reason for this poor performance of decapods is that they lose a significant fraction of the converted energy at each moult. Estimations on the energy cost of moulting should include the structural and functional costs of moulting. The estimation reported on the functional cost of moulting in *M. nobilii* by Sindhu and Pandian (1988) stands as a single report in the literature; from respirometric studies, they estimated the functional cost of moulting as equivalent to 4667 J for juvenile, i.e. 40% of total energy cost of moulting. For want of corresponding information, the reviewer has concentrated on structural cost of moulting in tested decapods. Several workers have not cared to indicate the fraction of food energy lost on exuvia (e.g., Andrews et al. 1972; Balazs and Ross 1976; Colvin 1976; Clifford and Brick 1979; Fennuci et al. 1980; Alava and Lim 1983); however, a few (Ponnuchamy et al. 1981; Sindhu and Pandian 1987; Pandian and Murugadass, in prep.) have reported the structural energy cost of exuvia, but it is not clear, whether this was included in the calculation of growth rate or growth efficiency. Table 3 shows the structural energy cost of moulting in some crustaceans. The cost appears to depend on dietary protein and life stage. For instance, it fluctuates between 56 and 83% in a juvenile *Macrobrachium nobilii,* and 33 and 45% in a brooder (Fig. 6). It should be pointed out that in future studies considerable effort should be made to estimate the structural energy cost of moulting.

Table 3. Structural cost (exuvia production) of moulting in some decapods (Ex - exuvia as % of conversion; *, ** represent animals fed on 10 or 50% dietary protein).

Species	Life Stage	Ex	Authority
Macrobrachium lanchesteri	Juvenile	37	Ponnuchamy et al. (1981)
M. nobilii	Juvenile*	83	Murugadass (1989)
	**	56	
	Adult *	-	
	**	30	
Caridina cajulhari	Juvenile	25	Ponnuchamy et al. (1984)
Panulirus homarus	Adult	64	Vijayakumaran & Radhakrishnan (1984)
Menippe mercenaria	Zoea & megalopa	23	Mootz & Epifanio (1974)
Rhithropanopeus harrisii	Zoea & megalopa	23	Levine & Sulkin (1979)
Carcinus maenas	Adult	39	Eriksson & Edlund (1977)
Carcinus maenas	Zoea	8	Dawirs (1983)
Cancer irroratus	Zoea I to III	3 to 12	Johns (1982)

Converted energy is partitioned between somatic growth and exuvial output in a decapod juvenile but between somatic growth, exuvial output and egg production in a brooder. The energy partitioning patterns in juvenile and brooder *M. nobilii,* when fed on diets containing different protein levels is shown in Fig. 6. In the brooders fed on diets containing 45-50% protein a good fraction of the converted energy is channelled for egg production. It may be noted that it is wasteful to feed juveniles 25% protein diet, as more than 80% of the converted energy is lost on structural cost of moulting. On the whole, there is an urgent need for more information on brooders of a few more decapods before generalizations could be made.

Energy-Protein Interaction

In the recent years, attempts have also been made to study the energy-protein interaction and to estimate the optimum protein requirement per unit of ingested food equivalent to 1 kJ. Two

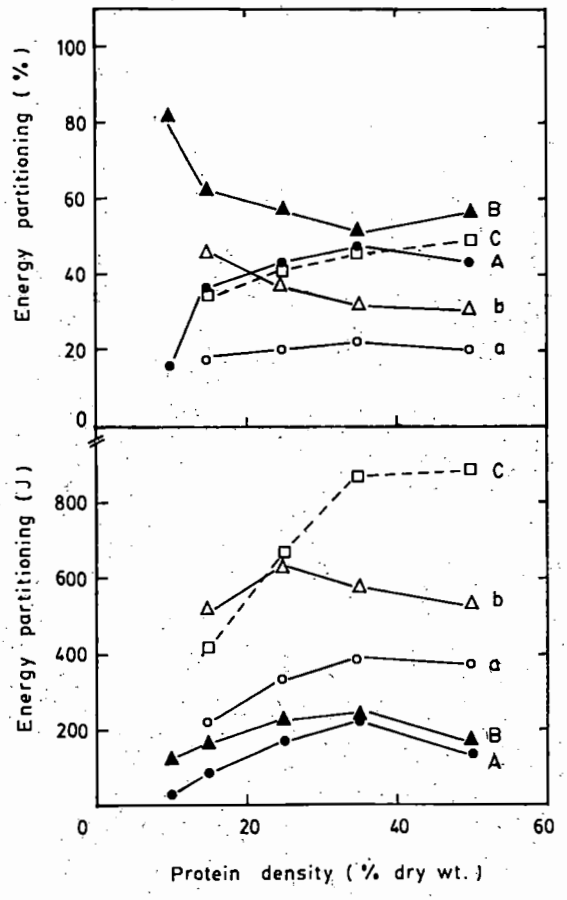

Fig. 6. Relationship between dietary protein density and pattern of energy partitioning in juvenile and brooder *M. nobilii* (from Murugadass 1987; modified). Circles (o), triangles (Δ) and squares (□) represent somatic growth, exuvial production and egg production, respectively. Closed circles (•) and triangles (Δ) represent juveniles. Open circles (o), triangles (Δ) and squares (□) represent the contribution of brooders.

kinds of experiments have been undertaken; in the first, a fish or decapod is fed on a wide range of isocaloric but anisonitrogenous diets, as well as isonitrogenous but anisocaloric diets. In the second experiment, the energy requirement of the tested fish or decapod is previously determined; subsequently, the composition of the determined unit of diet is altered to estimate the optimum nitrogen required per kJ by it (LeGrow and Beamish 1986). The optimum level is believed to let least energy loss on the account of Specific Dynamic Action.

A substantial fraction of energy is lost in the form of heat after food ingested by the animal. The energy loss in this process is termed as Specific Dynamic Action (SDA). The magnitude of SDA is dependent on quantity and quality of food, especially the protein content, as deamination is energetically a costly process. When the rate of ingestion of amino acids exceeds the rate of their utilization in protein synthesis, the excess amino acids are deaminated (Cowey and Sargent 1972). Therefore, when the dietary protein level exceeds the optimum, it may not result in a concomitant increase in the efficiency but may even decrease it. In this context, it is relevant to consider the hypothesis of De Silva (1985) that daily presentation of high protein diet could be wasteful and that presentation of high and low protein diets alternatively might provide a means of reducing the feed cost, perhaps by depressing the SDA. Selected examples for the nitrogen requirements of fish and prawns are presented in Fig. 7. Critical examination of very limited data available on protein-energy interaction indicate that per unit (kJ) ingested food, nitrogen requirement is in the range of 15-20 mg for fish (Adron et al. 1976; Jobling 1983; Jobling and Wandsvik 1983; LeGrow and Beamish 1986) and about 21 mg for prawns like *Macrobrachium nobilii* (Murugadass 1989).

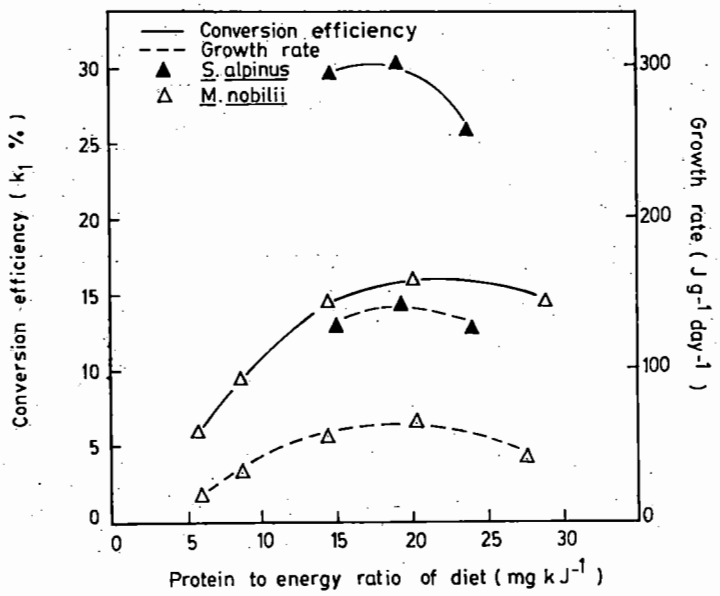

Fig. 7. Rate and efficiency of growth of selected fish and prawns as functions of protein-energy ratio of diet. Original data collected for *M. nobilii* from Murugadass (1987) and for *S. alpinus* from Jobling and Wandavik (1983).

Conclusions

1. From the points of specific growth rate and conversion efficiency, fish are proved to be better convertors than decapods.

2. Tropical fish grow faster at relatively lower optimum dietary protein density, while temperate fish grow more efficiently but requires relatively higher optimum protein levels. Penaeids grow faster than palaemonids.

3. Efficiency values of decapods fed on 25-70% protein diet indicate that in general, palaemonids are more efficient and require lower protein diet than penaeids.

4. Optimum dietary protein density required for brooder is higher (≈ 50%) than the juvenile (35%) *Macrobrachium nobilii*. Brooders receiving optimum protein diet display the highest efficiency and are most fecund and produce better quality eggs.

5. A substantial fraction (≈ 20%) of converted energy is lost on moulting. Decapods receiving low protein diet (< 25%) almost exhaust the converted energy on exuvia production.

6. Limited studies on energy-protein interaction indicate that nitrogen required per unit ingested food (kJ) is in the range of 15-20 mg for fish and 21 mg for decapods to achieve optimum growth.

Acknowledgements

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References

Adron, J.W., A. Blair, C.B. Cowey and A.M. Shanks. 1976. Effects of dietary energy level and dietary energy source on growth, feed conversion and body composition of turbot *Scophthalmus maximus*. Aquaculture 7: 125-132.

Alava, V.R. and C. Lim. 1983. The quantitative dietary protein requirement of *Penaeus monodon* juveniles in a controlled environment. Aquaculture 30: 53-61.

Andrews, J.W., L.V. Sick and G.J. Baptist 1972. The influence of dietary protein and energy levels on growth and survival of penaeid shrimp. Aquaculture 1: 341-347.

Andrews, J.W. and R.R. Stickney. 1972. Interactions of feeding rates and environmental temperature on growth, food conversion and body composition of channel catfish. Transactions American Fisheries Society 101: 94-99.

Anderson, R.J, E.W. Kleinholz and S.A. Flickinger. 1981. Protein requirements of smallmouth bass and largemouth bass. Journal of Nutrition 111: 1085-1097.

Aquacop. 1978. Study of nutritional requirements and growth of *Penaeus merguiensis* in tanks by means of purified and artificial diets. Proceedings World Mariculture Society 8: 225-234.

Balazs, G., F. Ross and C. Books. 1973. Preliminary studies on the preparation and feeding of crustacean diets. Aquaculture 2: 369-377.

Balazs, G. and F. Ross. 1976. Effect of protein source and level on growth and performance of captive freshwater prawn, *Macrobrachium rosenbergii*. Aquaculture 7: 229-313.

Bondari, K. and D.C. Sheppard. 1981. Soldierfly larvae as feed in commercial fish production. Aquaculture 24: 103-109.

Boonyaratpalin, M. and M.B. New. 1982. Evaluation of diets for *Macrobrachium rosenbergii* reared in concrete ponds. *In* M.B. New (ed.) Giant Prawn Farming, p. 249-256. Elsevier Scientific Publishers, New York.

Brett, J.R. and T.D.D. Groves. 1979. Physiological energetics. *In* W.S. Hoar and R.J. Randall (eds.) Fish physiology, Vol. 8, p. 279-352. Academic Press, New York.

Castell, J.D. and S.D. Budson. 1974. Lobster nutrition: the effect on *Homarus americanus* of dietary protein levels. Journal of the Fisheries Research Board, Canada 31: 1363-1370.

Caulton, M.S. 1978. The importance of habitat temperatures for growth in the tropical cichlid *Tilapia rendalli* Boulenger. Journal of Fish Biology 13: 99-112.

Clifford, H.C. and R.W. Brick. 1979. A physiological approach to the study of growth and bioenergetics in the freshwater shrimp. Proceedings World Mariculture Society 10: 701-719.

Conklin, D.E., K. Devers and R.A. Shleser. 1975. Initial development of artificial diets for the lobster *Homarus americanus*. Proceedings 6th Annual Meeting World Mariculture Society, p. 237-248.

Colvin, P.M. 1976. Nutritional studies on penaeid prawns: protein requirements in compounded diets for juvenile *Penaeus indicus* (Milne-Edwards). Aquaculture 7: 315-326.

Cowey, C.B. 1979. Proteins and amino acid requirements of finfish. *In* J.E. Halver and K. Tiews (eds.) Finfish Nutrition and Fishfeed Technology, Vol. I., p. 3-16.

Cowey, C.B. and J.R. Sargent. 1972. Fish nutrition, p. 383-492. *In* F.S. Russel and M. Yonge (eds.) Advances in Marine Biology, 10. Academic Press, London.

Cowey, C.B., J.A. Pope, J.W. Adron and A. Blair. 1972. Studies on the nutrition of marine flatfish. The protein requirements of plaice *(Pleuronectes platessa)*. British Journal of Nutrition 28: 447-456.

D'Abramo, L.R., D.E. Conklin, C.E. Bordner, N.A. Baum, and A. Norman-Boudreau. 1981. Successful artificial diets for the culture of juvenile lobsters. Proceedings World Mariculture Society 12: 325-332.

Davis, A.T. and R.R. Stickney. 1978. Growth response of *Tilapia aurea* to dietary protein quality and quantity. Transactions American Fisheries Society 107: 478-483.

Dawirs, R.R. 1983. Respiration, energy balance and development during growth and starvation of *Carcinus maenus* L. larvae (Decapoda: Portunidae). Journal of Experimental Marine Biology and Ecology 69: 105-128.

Dehn, P.F., D.E. Aiken and S.L. Waddy. 1983. Aspects of vitellogenesis in the lobster *Homarus americanus*. Canadian Technical Report, Fisheries and Aquatic Sciences 1161: 1-24.

Deshimaru, O. and K. Shigeno. 1972. Introduction to the artificial diet for prawn *Penaeus japonicus*. Aquaculture 1: 115-133.

Deshimaru, O. and K. Kuroki. 1974. Studies on a purified diet for prawn. I. Basal composition of diet. Bulletin Japanese Society of Scientific Fisheries 40: 413-419.

De Silva, S.S. 1985. Performance of *Oreochromis niloticus* L. fry maintained on mixed feedings schedules of differing protein content. Aquaculture and Fisheries Management 16: 331-340.

De Silva, S.S., M.K. Perera and P. Maitipe. 1984. The composition, nutritional status and digestibility of the diets of *Sarotherodon mossambicus* from nine man-made lakes in Sri Lanka. Environmental Biology of Fishes 11: 205-219.

Eriksson, S. and A. Edlund. 1977. On the ecological energetics of O group *Carcinus maenus* L. Gullman Fjord, Sweden. Journal of Experimental Marine Biology and Ecology 30: 233-248.

Fagerlund, U.H.M., D.A. Higgs, J.R. McBride, M.D. Plotnikoff, B.S. Dosanjh and J.R. Market. 1983. Implications of varying dietary protein, lipid and 17-methyltestosterone content on growth and utilization of protein and energy in juvenile coho salmon *Oncorhynchus kisutch*. Aquaculture 30: 109-124.

Fennuci, J.L., Z.P. Zein-Eldin and A.L. Lawrence. 1980. The nutritional response of two penaeid species to various levels of squid meal in a prepared feed. Proceedings World Mariculture Society 11: 403-409.

Forster, J.R.M. and T.W. Beard. 1973. Growth experiments with the prawn *Palaemon serratus* fed with fresh compounded food. Fisheries Investigation Series II, 27: 1-16.

Gallagher, M.L., D.E. Conklin and W.D. Brown. 1976. The effects of pelleted protein diets on growth, moulting and survival of juvenile lobsters. Proceedings World Mariculture Society 6: 363-378.

Halver, J.E. 1972. Fish nutrition. Academic Press, New York, N.Y., p. 713.

Hofer, R. and F. Schiemer. 1981. Proteolytic activity in the digestive tract of several species of fish with different feeding habits. Oecologia 48: 342-345.

Hofer, R., G. Krewedl and F. Koch. 1985. An energy budget for an omnivorous cyprinoid, *Rutilus rutilus* L. Hydrobiologia 122: 53-59.

Jauncey, K. 1981. The effects of dietary composition in the river carp *(Cyprinus carpio)* maintained in thermal effluents and laboratory recycling system. Proceedings World Symposium and Aquaculture on Heated Effluents Recirculating Systems, 1980. Vol. 2. Heenemann, Berlin, p. 247-261.

Jauncey, K. 1982. The effect of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapia *Sarotherodon mossambicus*. Aquaculture 27: 43-54.

Jobling, M. 1983. A short review and critique of methodology used in fish growth and nutrition studies. Journal Fish Biology 23: 685-703.

Jobling, M. and A. Wandsvik. 1983. An investigation of factors controlling food intake in Arctic charr, *Salvelinus alpinus* L. Journal Fish Biology 16: 629-638.

Johns, D.M. 1982. Physiological studies on *Cancer irroratus* larvae III. Effects of temperature and salinity on the partitioning of energy resources during development. Marine Ecology, Progress Series 8: 75-85.

Katre, S. 1977. Yolk utilization in the freshwater prawn *Macrobrachium lamarrei*. Journal Animal Morphology and Physiology 24: 13-20.

LeGrow, S.M. and F.W.H. Beamish. 1986. Influence of dietary protein and lipid on apparent heat increment of rainbow trout *Salmo gairdneri*. Canadian Journal of Fisheries and Aquatic Sciences 43: 19-25.

Levine, D.M. and S.D. Sulkin. 1979. Partitioning and utilization of energy during the larval development of the Zanthid crab, *Rhithropanopeus harrisii*(Gould). Journal of Experimental Marine Biology and Ecology 40: 247-257.

Lim, C., S. Sukhawongs and E.P. Pascual. 1979. A preliminary study on the protein requirements of *Chanos chanos* Forsskal fry in a controlled environment. Aquaculture 17: 195-201.

Manik, R. 1976. Preliminary studies on the effect of different pelletized formulated feeds on the growth of *Macrobrachium rosenbergii*. Bulletin Shrimp-Culture Research Centre 187-193.

March, B.E., C. Macmillan and F.W. Ming. 1985. Techniques for evaluation of dietary protein quality for the rainbow trout *(Salmo gairdneri)*. Aquaculture 47: 275-292.

Mathavan, S., S. Murugadass and M.P. Marian. 1986. Ontogenic changes in composition and energy density of the commercially important riverine prawn, *Macrobrachium malcolmsonii*. *In* J. Maclean, L.B. Dizon and L.V. Hosillos (eds.) First Asian Fisheries Forum, p. 647-650. Asian Fisheries Society, Manila.

Mazid, M.A., Y. Taneka, M. Asadur, K. Rahman, L. Simpson and C.O. Chichester. 1979. Growth response of *Tilapia zillii* fingerlings fed isocaloric diets with variable protein levels. Aquaculture 18: 115-122.

Millikin, M.R. 1982. Qualitative and quantitative nutrient requirements of fishes, a review. Fish. Bull. 80: 653-684.

Millikin, M.R., R. Fortner, P.H. Fair and V. Sick. 1980. Influence of dietary protein concentration on growth, feed conversion and general metabolism of juvenile prawn. Proceedings World Mariculture Society 11: 385-391.

Mootz, C.A. and C.E. Epifanio. 1974. An energy budget for *Menippe mercenaria* larvae fed *Artemia nauplii*. Biological Bulletin (Woods Hole, Mass.) 146: 44-55.

Murugadass, S. 1989. Growth, moulting and egg production of *Macrobrachium nobilii* fed on different dietary protein densities (under preparation).

Pandian, T.J. 1967. Changes in chemical composition and calorific content of developing eggs of the shrimp *Crangon crangon*. Helgolander Wiss Meeresuntersuchung 16: 216-224.

Pandian, T.J. 1970a. Ecophysiological studies on the developing eggs and embryos of the European lobster *Homarus gammarus*. Marine Biology 5: 157-167.

Pandian, T.J. 1970b. Yolk utilization and hatching time in the Canadian lobster *Homarus americanus*. Marine Biology 7: 249-254.

Pandian, T.J. 1972. Egg incubation and yolk utilization in the isopod *Ligia oceanica*. Proceedings Indian National Science Academy 38: 430-441.

Pandian, T.J. 1987. Fish energetics, p. 357-465. *In* T.J. Pandian and F.J. Vernberg (eds.) Animal energetics, Vol. 2. Academic Press, New York.

Pandian, T.J. and E. Vivekanandan. 1985. Energetics of feeding and digestion. *In* P. Tytler and P. Calow (eds.) Fish energetics: New perspectives, pp. 99-124. Croom Helm Publishers, London.

Pandian, T.J. and S. Murugadass 1989. Influence of dietary protein densities on moult, growth and egg production of *Macrobrachium nobilii* (under preparation).

Perry, W.G., J.V. Hunter and J.W. Avault. 1984. Production trials of prawns comparing a marine reaction, catfish diet and agricultural range pellet. Journal World Mariculture Society 15: 120-128.

Phillips, A.M. 1969. Nutrition, digestion and energy utilization. *In* W.S. Hoar and D.J. Randall (eds.) Fish Physiology, Vol., 1. pp. 391-432. Academic Press, New York.

Phillips, G.R. and D.R. Butler. 1979. Influence of dieldrin on the growth and body composition of fingerling rainbow trout *Salmo gairdneri* fed Oregon moist pellets or tubificid worms (*Tubifex* spp.). Journal Fisheries Research Board, Canada 36: 77-80.

Ponnuchamy, R., S.R. Reddy and S. Katre. 1981. Effects of eyestalk ablation on growth and food conversion efficiency of the freshwater prawns *Macrobrachium lanchesteri* (de Man). Hydrobiologia 77: 77-80.

Ponnuchamy, R., S.R. Reddy, S. Katre and K.V. Anantharaman. 1984. Laboratory studies on the food utilization of *Caridina rajadhari* (Decapoda: Atyidae). Comparative Physiology and Ecology 9: 417-420.

Sabaut, J.J. and P. Luquet. 1973. Nutritional requirements of the gilthead bream *Chrysophrys aurata*. Quantitative protein requirements. Marine Biology 18: 50-54.

Santiago, C.B., M. Beues-Aldeba, M.A. Laron. 1982. Dietary crude protein requirement of *Tilapia nilotica* fry. Philippine Journal of Biology 11: 255-259.

Satia, B.P. 1974. Protein requirements of rainbow trout. Progressive Fish Culturist 36: 80-86.

Schwarz, F.J., M.H. Zeritler and M. Kirchgessner. 1983. Wachstum und Nahrstoffaufwand bei karpfen (*Cyprinus carpio* L.) mit underschieldlicher protein und Energieversorgung. 2. Mitteilung Gewichtsentwicklumg, Futterreerwertung, Protein and Energieaufwant. Zeitschrift fur Tierphysiologic Tierernahrung. Futtermmittelkunde 49: 88-98.

Sedgwick, R.W. 1979. Influence of dietary protein and energy on growth, food consumption and food conversion efficiency in *P. merguiensis* de Man. Aquaculture 16: 7-30.

Shewbart, K.L., W.L. Mies and P.D. Ludwig. 1973. Nutritional requirements of the brown shrimp, *Penaeus aztecus*. U.S. Dept. Comm. Rep. No. Com-73-11794. NOAA, Office of Sea Grants, Rockville, MD. 52 p.

Sindhu, K. and T.J. Pandian. 1987. Effect of unilateral eyestalk ablation on moulting, growth, reproduction and energy budget of *Macrobrachium nobilii*. Asian Fisheries Science 1: 1-17.

Sindhu, K. and T.J. Pandian. 1988. Moulting and eyestalk ablation in *Decapod crustaceans*, a review. Advances in Aquatic Biology and Fisheries, p. 293-312.

Tacon, A.C.J. and C.B. Cowey. 1985. Protein and amino acid requirements. *In* P. Tytler and P. Calow (eds.) Fish Energetics: new perspectives, pp. 155-184. Croom Helm press. London.

Tandler, A. and F.W.H. Beamish. 1979. Mechanical and biochemical components of apparent specific dynamic action in largemouth bass, *Micropterus salmoides* Lacepede. Journal Fish Biology 14: 343-350.

Vijayakumaran, M. and E.V. Radhakrishnan. 1984. Effect of eyestalk ablation in the spiny lobster *Panulirus homarus* (Linnaeus): 2. On food intake and conversion. Indian Journal of Fisheries 31: 148-155.

Venkataramiah, A., G.J. Lakshmi and G. Gunter. 1975. Effect of protein level and vegetable matter on growth and food conversion efficiency of brown shrimp. Aquaculture 6: 115-125.

Wee, K.L. and A.C.J. Tacon. 1982. A preliminary study of dietary protein requirement of juvenile snakehead. Nippon Suisan Gakkishi 48: 1463-1482.

Yurkowski, M. and J.L. Tabachek. 1979. Proximate and amino acid composition of some natural fish foods. Proc. World Symp. on finfish nutrition and fish. Vol. I., p. 435-448. Heenemann, Berlin.

Zeitoun, I.H., D.E. Ullrey, W.T. Magee, J.L. Gill and W.G. Berge. 1976. Quantifying the nutrient requirement of fish. Journal of Fisheries Research Board, Canada 33: 167-172.

Amino Acid and Fatty Acid Profiles in Aquaculture Nutrition Studies

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The amino acid profile is an important parameter in the evaluation of protein quality and in requirement studies. Amino acid profiles are usually determined in two steps: hydrolysis of the protein to constituent amino acids followed by quantitative analysis of the amino acids in the hydrolysate. The ten amino acids known to be essential in most animals have been found to be essential in all fish so far studied. The reference amino acid profiles used in the amino acid requirement studies of various fish species include that of whole chicken, egg, fish egg and fish muscle. The amino acid profile of fish muscle provides a useful first approximation of the amino acid requirement of the young, growing fish in which the greatest proportion of weight gain is in the form of muscle.

Fatty acids are important components of lipids. Determination of fatty acid profiles involve the extraction of total lipids with organic solvents and the transesterification of the lipid to form the fatty acid methyl esters which are then analyzed by gas chromatography. The fatty acid composition of fish is dependant on such factors as temperature, salinity and diet. Coldwater fish contain higher levels of polyunsaturated fatty acids than warmwater fish. Freshwater fish tend to have higher levels of saturated fatty acids than marine fish. The essential fatty acid requirement (EFA) of fish seem to vary from species to species. Coldwater and marine fishes require medium chain ω3 or highly unsaturated long chain ω3 fatty acids. The EFA requirement of warmwater fish is more diverse. Some fish require ω6 and others require a combination of ω3 an ω6 fatty acids.

Amino Acid Profiles

Proteins are a major component of fish tissues constituting about 65-75% of the total on a dry weight basis. Dietary proteins which constitute up to 50% of the diets are converted to free amino acids through the action of various digestive enzymes. The free amino acids released are absorbed in the intestinal tract and used by various tissues for synthesis of tissue proteins. Excess amino acids are used as an energy source. Proteins often constitute the most expensive item of fish diets. Thus, research in aquaculture nutrition invariably starts with the determination of gross protein requirement of the fish. This information however, is of limited value without data on essential amino acid requirements since protein quality depends largely on its amino acid composition and digestibility. The determination of amino acid profiles is helpful in the design of amino acid test diets for research studies on amino acid requirements. It is also an important parameter in the evaluation of protein quality of feedstuff.

*Editorial note: Immediately after submission of this paper, Dr. L.V. Benitez died in an air-crash. As a tribute to her the article is published in the original form.

Determination of Amino Acid Profiles

The determination of amino acid profiles usually consists of two steps: hydrolysis of the protein to constituent amino acids followed by the quantitative estimation of the amino acids in the hydrolysate.

Protein Hydrolysis

The complete hydrolysis of proteins may be accomplished by use of strong acid, strong base or proteolytic enzymes; strong acid is normally preferred. The most frequently employed acid is constant boiling 6N hydrochloric acid.

The reaction is carried out in evacuated sealed tubes at about 110°C for periods ranging from 20 to 70 hours. The tubes have to be thoroughly cleaned with chromic acid and rinsed with distilled water and with 1N HC1. The residual HC1 is removed in an air oven at 100°C. Thick-walled Pyrex test tubes have been used in the hydrolytic reaction. About 5 mg of proteins is suspended in 1 ml of 6N HCI and a section of the tube 3 cm from the top is constricted to about 1 mm bore using an oxygen flame. The lower half of the tube is then frozen in a dry ice-ethanol bath. When the sample is frozen, the tube is connected into a vacuum line and the entire system is evacuated. The tube is sealed under vacuum after frozen sample is allowed to thaw slowly and then refrozen to allow complete degassing (Glazer et al. 1976).

The tedious method of evacuating and sealing of test tubes for hydrolysis has been largely simplified by the use of specially designed hydrolysis tubes with teflon screw seals and with side-arm for rapid evacuation.

The three sulfur containing amino acids cysteine, cystine and methionine are susceptible to oxidation. Even when great care has been taken to remove oxygen from hydrolysis tubes, considerable losses of cysteine and cystine are found after acid hydrolysis and this prevents direct quantification of these amino acids. Total cysteine and half-cystine content may be determined as cysteic acid after performic acid oxidation. Methionine will be present as methionine sulfone cysteine and cystine as cysteic acid.

Performic acid is prepared by allowing a mixture of 1.0 ml of 30% (w/v) H_2O_2 and 9.0 ml of 88% (w/v) formic acid to stand at room temperature for 1 hour after which it is cooled to 0°C. A known amount of protein, usually the amount used for routine hydrolysis is dissolved in about 2 ml of the reagent in a cooled hydrolysis tube. The mixture is kept at 0°C for 4 hours after which excess performic acid is destroyed by addition of 0.15 ml of cold 48% (w/v) HBr per ml of performic acid used. The bromine which forms, as well as the formic acid can be removed by rotary evaporation under high vacuum (More 1963). Acid hydrolysis of the oxidized sample and analysis of the resulting hydrolysates are performed in the usual manner.

Tryptophan is completely destroyed by acid hydrolysis, thus, for accurate determination of this amino acid alkaline hydrolysis would have to be resorted to. Alkaline hydrolysis is however, of limited application because cysteine, cystine, serine and threonine are decomposed in the process.

For this procedure, the proteins (1-5 mg) are hydrolyzed at 110°C in about 0.6 ml of 4.2N NaOH containing 25 mg of hydrolyzed starch. The addition of 5 µl of 1% 1-octanol was observed to greatly suppress foaming during evacuation. The reaction vessel is a thin-walled Pyrex test tube fitted with a polypropylene inner lining. The Pyrex test tube, as in acid hydrolysis was constricted with an oxygen flame to about 2 mm. A thin-walled tube is preferred since this constriction can be accomplished rapidly without overheating the plastic insert.

The lower portion of the tube was cooled in an acetone-dry ice bath chill, but not to freeze the solution. The tube was evacuated with a high vacuum line and then sealed. The sealed hydrolysis tubes were heated in an oven at 110°C. For most proteins, 16 hours of hydrolysis is adequate. Peptides containing tryptophan bound to isoleucine and valine are very resistant to hydrolysis and require a longer reaction time, sometimes as much as 98 hours. The long-term hydrolysis required at 110°C may be avoided if the temperature is increased to 135°C for about 48 hours (Hugli and Moore 1972).

There is an alternative procedure which affords high recovery of tryptophan and precise analysis of amino acid composition from a single hydrolysate. This method utilizes 4N methanesulfonic acid containing 0.2% 3-(2-aminoethyl) indole rather than 6N HC1 as a catalyst for hydrolysis. The hydrolysis is carried out in vacuum at 115°C for 22 to 72 hours. Half-cystine is determined as S-sulfocysteine by treating the hydrolysate with dithiothreitol followed by excess tetrathionate. The values of all amino acids including tryptophan and half-cystine were close to expected theoretical values (Simpson et al. 1976).

Amino Acid Analysis

After complete hydrolysis of a protein the analysis is completed by obtaining quantitative analysis of each of the amino acids. Both acid and alkaline hydrolysate would have to be neutralized prior to amino acid analysis. The separation of a mixture of amino acids is readily done by ion-exchange chromatography usually on a cationic exchanger. The amino acid mixture is often taken up in an acid buffer and loaded on the ion exchange column.

The more basic amino acids are most tightly bound and those with acidic groups the least. The column is then eluted gradually with buffers of increasing pH and ionic strength. The acidic amino acids such as glutamic acid and aspartic acid are removed readily from the resin followed by neutral amino acids and finally by basic amino acids. Each amino acid can be cleanly separated from the other. As they are eluted from the column, they are collected individually in an automatic fraction collector. Once separated, the individual amino acids are quantified by reaction with ninhydrin and resultant color intensity is measured by spectrophotometry. Commercial amino acid analyzers automatically separate amino acids on an ion exchange column, control and programme column temperature during elution, programme shift in buffers, collect eluted amino acids, add ninhydrin, heat to develop the color, record color intensity in the form of a plot and integrate area under each curve.

The identity of each amino acid is established on the basis of its position on the chromatogram and is estimated quantitatively on the basis of area under each curve. The more recent computerized amino acid analyzers have most of the analytical operations preprogrammed and finely controlled. Amino acid analysis will not be described in detail in this paper. Several publications can be consulted for the detailed description of amino acid analysis (Moore and Stein 1983; Bailey 1967; Glazer et al. 1976).

The amino acid composition of some feedstuffs were studied by seven collaborating laboratories. The feedstuff included casein, egg white, beef, soy isolate, rapseed concentrate, pea flour and wheat flour. Samples were hydrolyzed with 6N HCl, performic acid + 6N HCl and 4.2N NaOH. Amino acids were then determined by ion-exchange chromatography using automatic analyzers. Interlaboratory variation of tryptophan (coefficient of variation, CV, up to 24%), cystine and methionine (CV up to 17%) was greater than that of most other amino acids (CV up to 10%). Within each laboratory, the variation for all amino acid was less than 5% (Sarwar et al. 1983).

Amino Acid Profile in Fish Nutrition Studies

The nutritive value of a dietary protein is dependent on the extent to which the composition of its essential amino acids fulfill the requirement of the organism. The closer the profile from the requirement, the greater is the nutritional value. The amino acid profile is therefore, a valuable index in the assessment of nutritive quality of dietary proteins and is particularly useful when information on the amino acid requirement of the animal is known.

The first successful amino acid test diet for fish was reported by Halver in 1957. Halver compared test diets containing 70% cyrstalline L-amino acid formulated on the amino acid patterns of whole chicken egg protein, chinook salmon egg protein and chinook yolk-sac fry protein. The test diet based on whole chicken egg gave the best growth and feed efficiency for chinook salmon. Subsequently, Halver et al. (1957) used this test diet to determine the qualitative amino acid requirement of chinook salmon. Several workers subsequently used a similar test diet for determining the essentiality of some amino acids in other fishes.

An altogether different approach was used by Cowey et al. (1970) to determine the qualitative amino acid requirement of plaice and sole. Small fish were injected intraperitoneally with radioactively labelled ^{14}C glucose and fed on a natural diet for 7 days. The fish were then sacrificed, homogenized and the protein isolated. A sample of the protein was hydrolyzed and constituent amino acids were separated by chromatography and counted for radioactivity. Significant radioactivity was incorporated into non-essential amino acids. The essential amino acids remained unlabelled. Similar techniques for determining essential amino acids were applied to sea bass, *Dicentrarchus labrax* (Metailler et al. 1973)

All finfish studied so far have a requirement for the same ten amino acids which are known to be essential for most other animals (Table 1). These are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Wilson and Halver 1986).

Table 1. Finfish known to require the same ten essential amino acids.

Species			Kind of Study
Channel catfish	-	*I. punctatus*	Growth
Chinook salmo	-	*O. tshawytscha*	Growth
Common carp	-	*C. carpio*	Growth
European eel	-	*A. anguilla*	Growth
Japanese eel	-	*A. japonica*	Growth
Plaice	-	*P. platessa*	^{14}C-labelling
Rainbow trout	-	*S. gairdneri*	Growth
Red sea bream	-	*C. major*	Growth
Sea bass	-	*D. labrax*	^{14}C-labelling
Sockeye salmo	-	*O. nerka*	Growth
Sole	-	*S. solea*	^{14}C-labelling
Tilapia	-	*T. zillii*	Growth

Adapted from Wilson and Halver (1986).

Studies on quantitative amino acid requirement used the basic method developed by Halver (1957). The procedure involved the use of purified diets containing either all crystalline amino acids or a mixture of casein and gelatine and crystalline amino acids formulated so that the amino acid profile is identical to whole chicken egg protein. A series of feeds is then formulated to contain graded levels of one amino acid whose requirement is to be determined.

Other investigators have used semi-purified and practical test diets instead of purified diets. The semi-purified diets include a major protein source which is deficient in certain amino acids such as zein (Dabrowski 1981) or corn gluten (Ketola 1983). The practical diet involves normal feedstuff to furnish most of the amino acids using a fixed amount at a desired protein level and the remainder is made up of cystalline amino acids (Jackson and Capper 1982; Walton et al. 1984).

Several reference amino acid patterns have been used by various investigators. As mentioned earlier, the amino acid profile of whole chicken egg proved useful in the design of amino acid test diets of chinook salmon. The amino acid profile of fish egg has been used for formulating feeds for amino acid requirement studies in some fish (Ketola 1982). The composition of fish egg also provided an effective guide for successful amino acid supplementation of protein in fish feeds for Atlantic salmon and rainbow trout.

Cowey and Tacon (1983) have identified several problems in the accurate determination of amino acid requirements of fish based on growth studies. Some of these problems are:

i. Growth rates commonly observed in the amino acid test diets are frequently inferior or lower than those observed with proteins (Murai et al. 1981; Walton et al. 1982).

ii. Some of the amino acids in test diets may leach out during feeding.

iii. The interpretation of the break-point on the growth response curve is frequently subjective.

In young growing animals, the greatest proportion of body weight is in the form of muscle. It is reasonable to infer that the dietary essential amino acid requirement will be closely related to and or governed by the amino acid profile of muscle protein (Cowey and Tacon 1983). This hypothesis was examined by Boorman (1980) for pigs and chicken. Amino acid requirement is closely correlated to the amino acid profile of muscle protein (Fig. 1). Based on this relationship Boorman concluded that, "in the absence of more detailed information, the pattern of essential amino acid in the product can be used for the pattern in the diet".

A similar correlation is shown in Fig. 2 for common carp *(Cyprinus carpio)*. The requirement values for 10 essential amino acids were obtained from dose-response curves using amino acid test diets (Nose 1979). The relationship of the essential amino acid requirement with

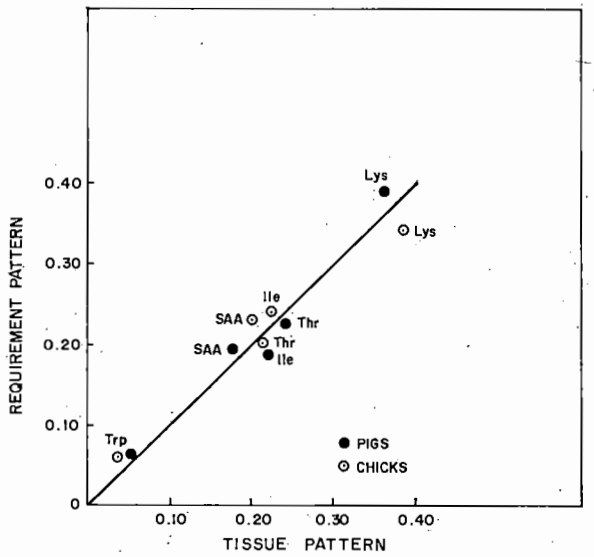

Fig. 1. Relationships between pattern of amino acid requirements determined/deduced from feeding experiments for lysine (Lys), methionine plus cysteine (SAA), isoleucine (Ile), threonine (THr) and trypthophan (Trp) and the pattern of the same amino acids in muscle tissues of growing pigs and chicks. (Adapted from Boorman 1980).

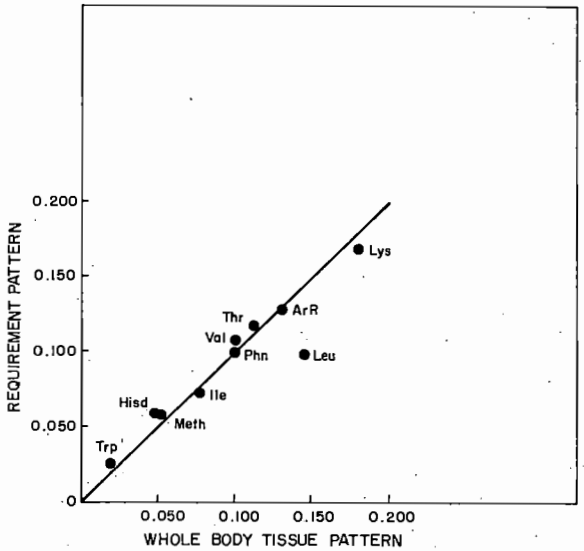

Fig. 2. Relationships between pattern of requirements determined/deduced from feeding experiments for ten essential amino acids as determined by using amino acid test diets and the pattern of the same amino acids in the whole body of growing carp *(Cyprinus carpio)* (adapted from Nose 1979).

the amino acid profile of muscle or whole body tissue is very close except in the case of leucine and lysine where the requirement appears to be lower than the level body tissue (Nose 1979). The amino acid profile for muscle protein provides a very useful first approximation for the amino acid requirement of fish.

Fatty Acid Profiles

Lipids are major sources of metabolic energy in fish. They are also the source of essential fatty acids for cell structure and membrane function and are carriers of oil-soluble vitamins. Fatty acids are among the most well-studied components of lipids. The fatty acid profile like the amino acid profile can be used as an index of quality of dietary lipids. Many useful inferences on dietary lipid requirements can be derived from data on fatty acid profiles of fish tissues.

Lipid Extraction

To obtain a fatty acid profile of animal tissues, it is necessary to first extract the total lipid from the particular tissues. Lipids exist in tissue in a variety of forms. In adipose tissues of animals for example, lipids are found as discrete droplets in separate compartments from most other cellular constituents. In other tissues, lipids occur in association with other cell components especially proteins and may be strongly bound to them (Christie 1984).

Lipids can be isolated quantitatively from tissues by extraction with suitable organic solvents. The extracts must be freed of non-lipid contaminants by appropriate washing procedures. The solvents selected must be sufficiently polar to disrupt the association between lipids and other tissue components but should not react with lipids in any way. Single pure solvents are rarely used for lipid extractions. The most commonly used solvent mixture for lipid extraction is chloroform-methanol although the water present in a given tissue is considered a third component of the solvent mixture.

Under optimum conditions, tissues should be extracted as soon as possible to minimize oxidative and hydrolytic changes that may alter the lipid composition of the samples. If immediate extraction is not possible, the sample should be stored in glass containers, frozen rapidly at -20°C in an atmosphere of nitrogen.

One of the more commonly used procedures for total lipid extraction of fish tissues is the procedure of Bligh and Dyer (1959). This procedure is preferred when rapid extraction of a large amount of sample and less than complete recovery of lipid is acceptable. Recoveries of most lipids are likely to be about 95%. The fresh tissue is assumed to contain 80% water. The schematic diagram of the procedure is presented in Fig. 3.

The extraction procedure of Folch et al. (1957) is most often used when more exhaustive recoveries of lipids are required. This procedure which is schematically described in Fig. 4 gives recoveries of the order of 95 to 99% with most animal tissues.

Preparation of Methyl Esters

Fatty acids in lipids are mostly in the form of glycerol esters. However, for many analytical purposes, it is preferable that fatty acids be converted to less polar and more volatile methyl ester derivatives. It is not necessary to hydrolyze lipids prior to esterification as they can be easily transesterified directly in the presence of an excess of methanol by means of a suitable catalyst (Christie 1984). A method for preparing methyl esters (Metcalfe et al. 1966) from lipids by transesterification is outlined in Fig. 5, and utilizes boron trifluoride as a catalyst.

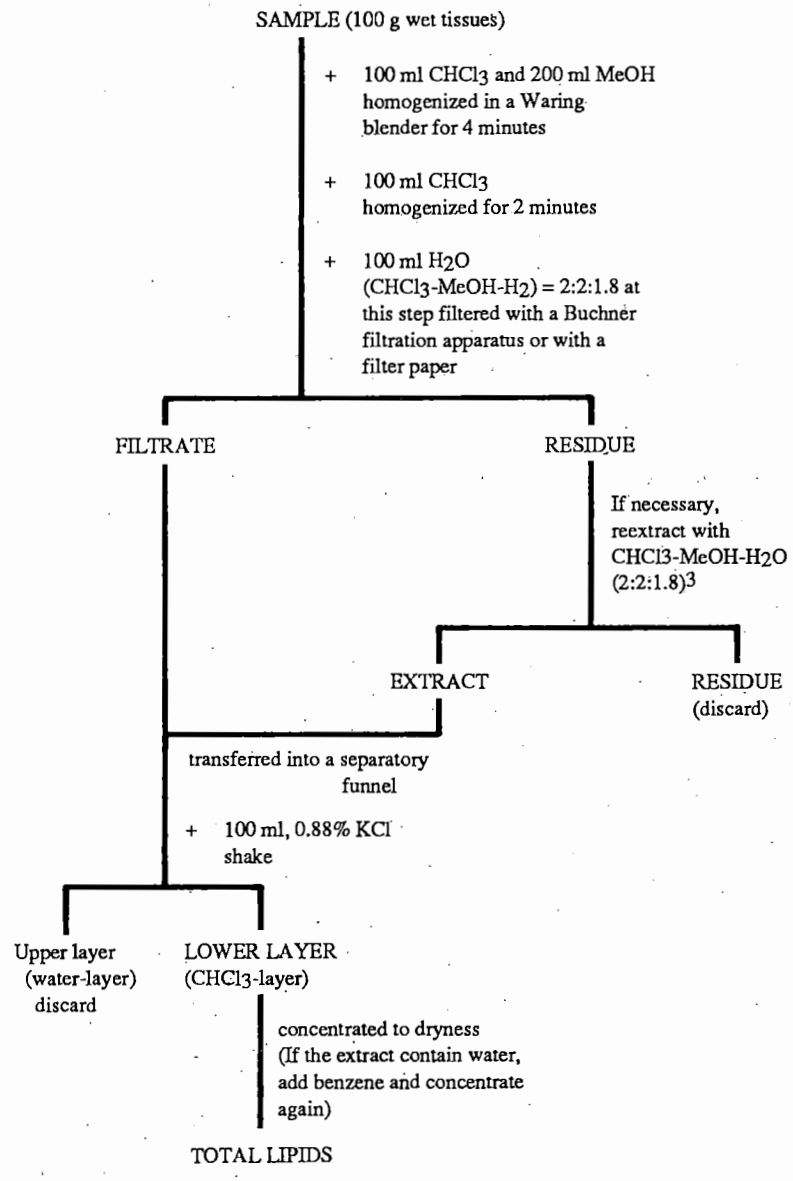

Fig. 3. Schematic diagram indicating the method of extraction of lipids (Bligh and Dyer 1959).

Fatty Acid Analysis

Complex mixtures of fatty acid methyl esters derived from lipids can be quantitatively determined by gas chromatography. The procedure of gas chromatography (GC) will not be described in detail in this paper as there is much written on this subject (see for example: James 1960; Mangold et al. 1984). Gas chromatography is essentially a process of partition on which components to be separated are distributed between two immiscible phases, one phase is held stationary in the column, the other is a moving phase-the carrier gas, in gas chromatography.

The essential features of the technique include:

i. a source of carrier gas at constant pressure,
ii. a column containing the stationary phase which is liquid but non-volatile at column temperature and provides inert support for the stationary phase,
iii. a system for heating the column and detector,

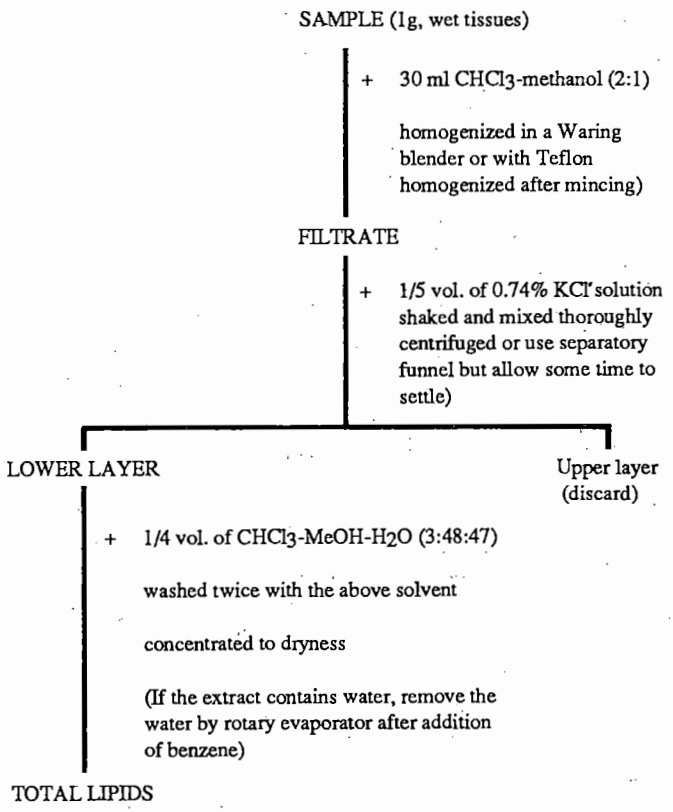

Fig. 4. Schematic diagram indicating the method of extraction of lipids (Folch et al. 1957).

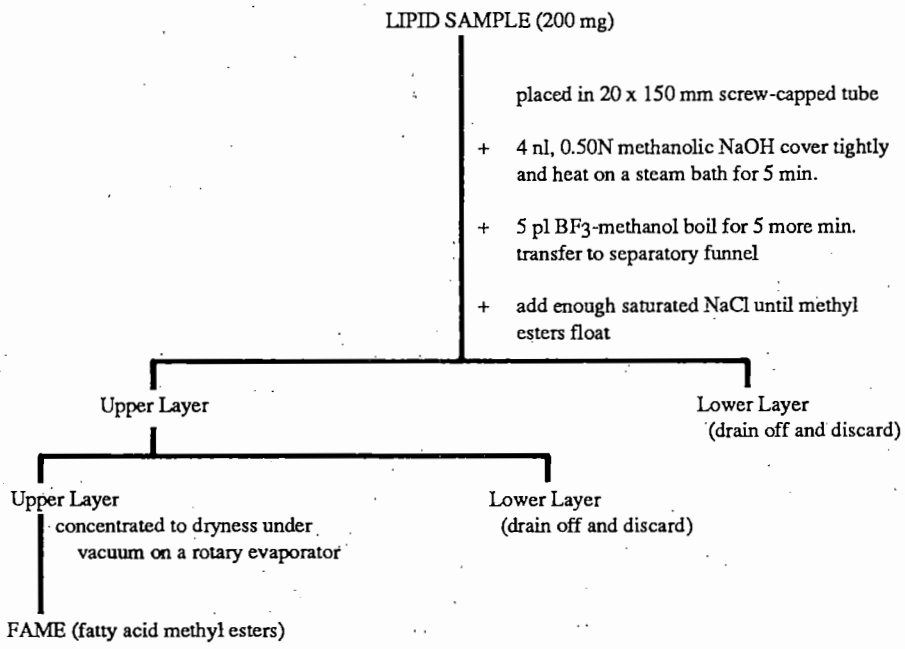

Fig. 5. Schematic diagram indicating the steps involved in the preparation of fatty acid methyl esters.

iv. the detector whose function is to measure the concentration of vapour in each zone leaving the column,
v. a recorder that responds to the emergence of compounds in the form of amplified electrical signals and records this in the form of a chromatogram.

The chromatogram is the basis for qualitative and quantitative information on the fatty acid composition of the sample. As in the amino acid analyzer gas chromatographs are now computerized and controlled with better precision.

Fatty Acid Profiles in Fish Nutrition Studies

Fish oils contain a greater variety of fatty acids in comparison with other oils or fats. Fish oils also contain highly polyunsaturated fatty acids of considerably longer carbon chain (20 or 22 carbon chain length), most of which belong to the ω3 family of fatty acids (Stansby 1982). The long chain fatty acids generally make up one-fourth to one-third of all fatty acids in fish oils. This contrasts sharply with the proportion of long chain fatty acids in most vegetable oils which seldom exceeds 5% and is frequently less than 1%. Likewise, polyunsaturated fatty acids in fish oils generally occur to an extent of 15-30%, yet they almost never total even 1% in vegetable oils. Many useful inferences on the dietary lipid requirement of fish can be derived from their fatty acid profiles. The composition of fatty acids in fish is affected by a number of environmental factors foremost of which are salinity, temperature and diet. Fish live in environments of varying salinity, the extreme being represented by freshwater and marine conditions. A comparison of the fatty acid composition of some freshwater and marine fishes is shown in Table 2. The general trend suggests that the total saturated fatty acids are slightly higher in freshwater species than marine species. Freshwater fish have higher levels of medium

Table 2. Distribution of major fatty acids in various fish lipids.

Fatty Acids	Percent Fatty Acids					
	Freshwater Species			Marine Species		
	A	B	C	X	Y	Z
14:0	2.8	6.7	2.1	5.1	3.7	2.2
14:1	1.0	0.7	0.8	0.4	0.1	0.2
16:0	16.6	14.6	11.9	10.9	12.6	17.0
16:1	17.7	14.7	8.2	12.0	9.3	4.1
18:0	3.3	1.5	4.1	1.2	2.3	3.2
18:1	26.1	18.2	19.8	12.6	22.7	21.4
18:2ω6	4.3	3.7	4.6	0.7	1.5	2.0
18:3ω3	3.6	3.6	5.2	0.3	0.6	1.0
20:1	2.4	1.6	3.0	16.1	7.5	5.4
20:4ω6	2.6	2.4	2.2	0.4	1.4	0.9
20:5ω3	2.7	8.2	5.0	7.4	12.9	6.7
22:1	0.3	0.4	1.3	19.4	6.2	9.4
22:6ω3	2.0	6.0	19.0	3.9	12.7	16.1
Total saturated	22.7	22.8	18.1	17.2	18.6	22.4
Total monoenoic						
Medium	44.8	33.6	28.8	25.0	32.2	25.7
Long	2.7	2.0	4.3	35.5	13.7	14.8
Total ω3	10.3	19.3	31.8	12.7	27.9	26.1
Total ω6	7.3	6.5	7.4	1.1	4.1	3.5
Ratio ω3/ω6	1.4	3.0	4.3	11.5	6.8	7.5
Total unsaturated	65.1	61.4	72.3	74.3	77.5	70.1

A - sheepsherd; B - alewife; C - rainbow trout; X - Atlantic herring; Y - Atlantic cod; Z - Chinook salmon (adapted from Ackman 1967)

chain monolenoic acids than marine species. However, marine species contain more longer chain polyunsaturated fatty acids than freshwater species. The ratio of ω3/ω6 fatty acids is greater for marine species than freshwater species (Ackman 1967; Castell 1979). The same trend in fatty acid composition is expressed in fish that migrate from freshwater to marine environments and vice versa. Table 3 shows changes in fatty acid composition in sweet smelt *(Plecoglossus altivelis)* as it migrates from marine to freshwater (Ota and Takagi 1977) and for masu salmon *(Oncorhynchus masu)* as they migrate from freshwater to seawater (Ota 1976). The most significant change is observed in the ratio of ώ3/ω6. For smelts migration from marine to freshwater was accompanied by a decrease in the ω3/ω6 ratio from 7.5 to 3.5. In contrast, an increase in the ω3/ω6 ratio from 3.8 to 10.0 was observed in salmon during its migration from freshwater to seawater. The general observation that ω3/ω6 ratio is higher for marine species holds true even for migratory fish where a change in salinity seems to cause a change in fatty acid profile.

Table 3. Changes in fatty acid composition in triglycerides of migratory fishes.

Fatty Acids	Percent Fatty Acids			
	Smelt		Masu Salmon	
	Marine	Freshwater	Freshwater	Marine
	April	May	May	June
14:0	8.0	10.0	5.2	5.7
16:0	21.6	18.7	19.9	20.0
16:1	10.0	17.0	11.6	8.7
18:0	2.8	2.9	4.6	3.9
18:1	12.8	11.5	23.3	21.7
18:2ω6	2.8	4.3	3.9	1.7
18:3ω3	3.0	5.1	3.0	1.3
20:1	1.1	-	3.0	6.7
20:4ω6	1.4	1.5	1.0	0.6
20:4ω3	1.9	1.8	1.5	1.2
20:5ω3	8.2	6.3	4.2	7.0
22:1	-	-	1.9	4.2
22:5ω6	-	1.1	-	-
22:5ω3	1.4	1.2	1.8	2.4
22:6ω3	12.1	5.2	6.7	9.0
Total saturated	32.4	31.6	29.7	29.6
Total monoenoic				
Medium	22.8	28.5	34.9	30.4
Long	1.1	-	4.9	10.9
Total ω3	31.7	23.9	18.6	23.2
Total ω6	4.2	6.9	4.9	2.3
Ratio ω3/ω6	7.5	3.5	3.8	10.0
Total unsaturated	59.8	59.3	63.3	66.8

Adapted from Castell 1979.

Temperature is a major factor widely known to cause differences in fatty acid composition. Fish that live in warmer waters contain more saturated fatty acids. A decrease in temperature induces an increase in the degree of unsaturation in fish lipids, the accumulation of long chain (C_{20} and C_{22}) polyunsaturated fatty acids and an increase in the ω3/ω6 ration (Knipprath and Mead 1968; Patton 1975; Farkas and Csengeri 1976).

The results suggest that fish and crustaceans alike manipulate their levels of polyunsaturated fatty acids to maintain membrane integrity and function in the cold (Farkas 1979). The effect of temperature on fatty acid composition has been studied using the same fish fed the same diet but acclimated at different temperatures. Table 4 shows that fish reared at higher temperature had more saturated fatty acids accumulated than the cold-acclimated fish (Castell 1979).

Table 4. Effect of temperature on fatty acid composition of guppies.

Fatty Acids	Guppies 17°C	24°C
14:0	1.5	0.9
16:0	22.9	0.9
16:1	15.9	36.0
18:0	8.2	9.8
18:1	18.3	15.0
18:3ω3	1.4	0.8
20:4ω6	2.0	2.0
20:5ω3	4.8	4.6
22:5ω3	6.1	7.3
22:6ω3	16.5	11.5
Total saturated	32.6	46.7
Total monoenoic	34.2	23.9
ω3	28.8	24.2
ω6	3.3	3.0
ω3/ω6	8.7	8.0
Total unsaturated	66.3	51.1

Adapted from Castell 1979.

If the trends in fatty acid composition can be taken as a clue to the essential fatty acid requirements of fish, the ω3 requirement would be greater for coldwater fish. Warmwater fish may do better with a mixture of ω6 and ω3 (Castell 1979). Coldwater fish are likely to be more demanding in their requirement for essential fatty acids than warmwater fish because constraints imposed in maintaining membrane fluidity are greater at low temperature (Hazel 1979).

The essential fatty acid (EFA) requirement in fish has been reviewed by several workers (Castell 1979; Watanabe 1982). Studies have demonstrated that the EFA requirements of fish differ considerably from species to species. The EFA requirements of rainbow trout is the best known. It was shown that the EFA requirement was about 1% 18:3ω3 in the diet and no combination of 18:3ω3 and 18:2ω6 resulted in as fast a growth rate or efficient feed conversion as 1% 18:3ω3 (Castell et al. 1972a,b; Watanabe et al. 1974).

On the other hand, carp *(Cyprinus carpio),* one of the most important cultured fish in Japan had an EFA requirement of both 18:2ω6 and 18:3ω3 (Watanabe et al. 1975). The best weight gain and feed conversion were obtained in fish receiving a diet with both 1% 18:2ω6 and 1% 18:3ω3. The eel *(Anguilla japonica),* another important cultured warmwater fish also has a requirement for both 18:2ω6 and 18:3ω3 at a level of 0.5% each (Takeuchi et al. 1980). A tropical herbivore, *Tilapia zillii* was found to require ω6 rather than ω3 fatty acids. The dietary requirement for 18:2ω6 or 20:4ω6 was about 1% in the diet (Kanazawa et al. 1980).

Highly unsaturated ω3 fatty acids with more than 20 carbon atoms (ω3 HUFA) are essential in the nutrition of some marine fish such as red sea bream and yellow tail. The requirement of red sea bream for ω3 HUFA is about 0.5% in the diet (Yone 1978). The requirement for long chain polyunsaturated fatty acids has been traced to the limited ability of some marine fishes to chain elongate and desaturate medium chain fatty acids (Cowey et al. 1976; Yamada et al. 1980).

Most coldwater and marine fish require either ω3 or ω3 HUFA fatty acids. The situation with warmwater fish is presently more confused. Some species require both ω3 and ω6 fatty acids, others ω6. Moreover, growth inhibition at certain levels of ω3 and ω6 has been observed (Stickney and Hardy 1987). Clearly, different species require different EFAs and the diversity is much more so in warmwater than in coldwater fishes.

References

Ackman, R.G. 1967. Characteristics of the fatty acid composition and biochemistry of some freshwater fish oils and lipids in comparison with marine oils and lipids. Comparative Biochemistry and Physiology 22: 907-922.

Bailey, J.L. 1967. Techniques in protein chemistry. Elsevier Publishing Co., Amsterdam 62-109.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911-917.

Boorman, K.N. 1980. Dietary constraints on nitrogen retention. *In* P.S. Buttery and D.B. Lindsay (eds.) Protein Deposition in Animals, pp. 147-166. Butterworth and Co. Ltd., London.

Castell, J.D. 1979. Review of lipid requirements of finfish. *In* J.E. Halver and K. Tiews (eds.) Finfish Nutrition and Fishfeed Technology. Vol. I, pp. 59-84. Proceedings of World Symposium, Hamburg, Germany.

Castell, J.D., D.J. Lee and R.O. Sinnhuber. 1972a. Essential fatty acids in the diet of the rainbow trout *(Salmo gairdneri):* lipid metabolism and fatty acid composition. Journal of Nutrition 102: 93-100.

Castell, J.D., R.O. Sinnhuber, J.H. Wales and D.J. Lee. 1972b. Essential fatty acids in the diet of rainbow trout *(Salmo gairdneri):* growth, feed conversion and some gross deficiency symptoms. Journal of Nutrition 102: 77-86.

Christie, W.W. 1984. Extraction and hydrolysis of lipids and some reactions of their fatty acid components in lipids. *In* H.K. Mangold, G. Zweig and I. Sherma (eds.) CRC Handbook of Chromatography. CRC Press, Inc., Florida, USA.

Cowey, C.B. and A.G.J. Tacon. 1983. Fish nutrition - relevance to marine invertebrates. *In* G.D. Pruder, C.J. Langdon and D.E. Conklin (eds.) Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition, pp. 13-30. Baton Rouge, Louisiana State University.

Cowey, C.B., J.W. Adron and A. Blair. 1970. Studies on the nutrition of marine flatfish. The essential amino acid requirements of plaice and sole. Journal of the Marine Biology Association of the United Kingdom 50: 87-95.

Cowey, C.B., J.W. Adron, J.M. Owen and R.J. Roberts. 1976. The effect of different dietary oils on tissue fatty acids and tissue pathology in turbot, *Scophthalmus maximus*. Comparative Biochemistry and Physiology 53B: 399-403.

Dabrowski, K. 1981. Tryptophan requirement of common carp (*Cyprinus carpio* L.) fry. Zeitschrift fur Tierphysiologie Tierrenahrung and Futtermittelkunde, 46: 64-71.

Farkas, T. 1979. Adaptation of fatty acid compositions to temperature - a study on planktonic crustaceans. Comparative Biochemistry and Physiology 64B: 71-76.

Farkas, T. and I. Csengeri. 1976. Biosynthesis of fatty acids by carp, *Cyprinus carpio* L. in relation to environmental temperature. Lipids 11: 401-407.

Folch, J., M. Lees and G.H. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226: 497-509.

Glazer, A.M., R.G. Delange and D.S. Sigman. 1976. Chemical modification of protein. *In* T.S. Work and J.E. Work (eds.) Laboratory Techniques in Biochemistry and Molecular Biology, pp. 1-206. North-Holland Publishing Co., Amsterdam.

Halver, J.E. 1957. Nutrition of salmonid fishes. IV. An amino acid test diet for chinook salmon. Journal of Nutrition 62: 245-254.

Halver, J.E., D.C. Delong and E.T. Mertz. 1957. Nutrition of salmonid fishes. V. Classification of essential amino acids for chinook salmon. Journal of Nutrition, 63: 95-105.

Hazel, J.R. 1979. Influence of thermal acclimation on membrane lipid composition of rainbow trout liver. American Journal of Physiology 236: 91-101.

Hugli, E.T. and S. Moore. 1972. Determination of the tryptophan content of proteins by ion exchange chromatography of alkaline hydrolysate. Journal of Biological Chemistry 247: 2828-2834.

Jackson, A.J. and B.S. Capper. 1982. Investigation into the requirements of tilapia, *Sarotherodon mossambicus* for dietary methionine, lysine and arginine in semisynthetic diets. Aquaculture 29: 289-297.

James, A.T. 1960. Qualitative and quantitative determination of fatty acids by gas-liquid chromatography. *In* Glick, D. (ed.) Methods of Biochemical Analysis, pp. 1-59. Interscience Publishers, Inc., New York, USA.

Kanazawa, A., S. Teshima, M. Sakamoto and Md. A. Awal. 1980. Requirement of *Tilapia zillii* for essential fatty acids. Bulletin of the Japanese Society of Scientific Fisheries 46: 1353-1356.

Ketola, H.G. 1982. Amino acid nutrition of fishes: requirement and supplementation of diets. Comparative Biochemistry and Physiology 73B: 17-24.

Ketola, H. 1983. Requirement for dietary lysine and arginine by fry of rainbow trout. Journal of Animal Science 56: 101-107.

Knipprath, W.G. and J.F. Mead. 1968. The effect of environmental temperature on the fatty acid composition and on the incorporation of (1-^{14}C) acetate in goldfish. Lipids 3: 121-128.

Mangold, H.K., Zweig and J. Sherma. 1984. Lipids: CRC Handbook of Chromatography. CRC Press Inc., Florida, USA.

Metailler, R., A. Febvre and E. Alliot. 1973. Preliminary note on the essential amino acids of the sea bass, *Dicentrarchus labrax* (Linn.). Standard Review GFCM 52: 91-96.

Metcalfe, L.D., A.A. Schmitz and J.R. Pelka. 1966. The rapid preparation of fatty acid methyl esters from lipids for gas chromatographic analysis. Analytical Chemistry 38: 514.

Moore, S. 1963. On the determination of cystine as cysteic acid. Journal of Biological Chemistry 238: 235-237.

Moore, S. and W.H. Stein. 1963. Chromatographic determination of amino acids by use of automatic recording equipment. *In* S.P. Colonick and N.O. Kaplan (eds.) Methods of Enzymology, Vol. VI, pp. 819-831. Academic Press, New York.

Murai, T., T. Akiyama and T. Nose. 1981. Use of crystalline amino acids coated with casein in diets of carp. Bulletin of the Japanese Society of Scientific Fisheries 47: 523-527.

Nose, T. 1979. Summary report on the requirements of essential amino acids for carp. *In* J.E. Halver and K. Tiews (eds.) Finfish Nutrition and Fishfeed Technology, Vol. 1, pp. 145-156. I.H. Heeneman GmbH and Co., Berlin.

Ota, T. 1976. Lipids of masu salmon. IV. Changes of lipid composition and fatty acid composition in flesh lipids of juvenile masu salmon in the early stages of seawater life. Bulletin of the Faculty of Fisheries, Hokkaido University 27: 30-36.

Ota, T. and T. Takagi. 1977. A comparative study on the lipid class composition and fatty acid composition of sweet smelt, *Pleocoglossu altivelis* from marine and freshwater habitat. Bulletin of the Faculty of Fisheries, Hokkaido University 28: 47-56.

Patton, J.S. 1975. The effect of pressure and temperature on phospholipid and triglyceride fatty acids of fish white muscle: a comparison of deepwater and surface marine species. Comparative Biochemistry and Physiology 52B: 105-110.

Sarwar, G., D.A. Christensen, A.J. Finlayson, M. Friedman, L.R. Hackler, S.L. Mackenzie, P.L. Pellett and R. Tkachuk. 1983. Inter and intra-laboratory variation in amino acid analysis of food proteins. Journal of Food Science 48: 526-531.

Simpson, R.J., M.R. Neuberger and T.Y. Liu. 1976. Complete amino acid analysis of proteins from a single hydrolysate. Journal of Biological Chemistry 251: 1936-1940.

Stansby, M.E. 1982. Properties of fish oils and their application to handling of fish and to nutritional and industrial use. *In* R.E. Martin, G.J. Flick, C.E. Hebard and D.R. Ward (ed.), pp. 75-92. Api Publishing Co., Westpoint, Conn., USA.

Stickney, R.R. and R.W. Hardy. 1987. Lipid requirement of fishes: the enigma of warmwater species (abstract). International Symposium on Feeding and Nutrition in Fish, Bergen, Norway.

Takeuchi, T., S. Arai, T. Watanabe and Y. Shimma. 1980. Requirement of eel, *Anguilla japonica* for essential fatty acids. Bulletin of the Japanese Society of Scientific Fisheries 46: 345-353.

Walton, M.J., C.B. Cowey and J.W. Adron. 1982. Methionine metabolism in rainbow trout fed diets of differing methionine and cystine contents. Journal of Nutrition 112: 1525-1535.

Walton, M.J., C.B. Cowey and J.W. Adron. 1984. The effect of dietary lysine levels on growth and metabolism of rainbow trout *(Salmo gairdneri)*. British Journal of Nutrition 52: 115-122.

Watanabe, T. 1982. Lipid nutrition in fish. Comparative Biochemistry and Physiology 73B: 3-15.

Watanabe, T., F. Takashima and C. Ogino. 1974. Effect of dietary methyl linolenate on growth of rainbow trout. Bulletin of the Japanese Society of Scientific Fisheries 40: 181-188.

Watanabe, T., T. Takeuchi and C. Ogino. 1975. Effects of dietary methyl linoleate and linolenate on growth of carp. Bulletin of the Japanese Society of Scientific Fisheries 41: 263-269.

Wilson, R.P. and J.E. Halver. 1986. Protein and amino acid requirements of fishes. Annual Review of Nutrition 6: 225-244.

Yamada, K., K. Kobayashi and Y. Yone. 1980. Conversion of linolenic acid to w3 highly unsaturated fatty acids in marine fishes and rainbow trout. Bulletin of the Japanese Society of Scientific Fisheries 46: 1231-1233.

Yone, Y. 1978. Essential fatty acids and lipid requirements of marine fish. *In* Koseisha-Koseihaku (ed.) Japan Society of Scientific Fisheries, Tokyo 43-59.

Digestibility Evaluations of Natural and Artificial Diets

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Digestibility is the quantitative evaluation of the usefulness of a diet and/or its nutrient components. Digestibility could be estimated directly where the total food and or nutrient(s) consumed and that voided as faeces are determined and or indirectly by utilizing a marker. The markers or indicators used are either external when a foreign non-digestible substance is added to the diet or internal when a non-digestible component of the diet itself is used. The merits and demerits of the direct and the indirect methods and the use of internal and external markers are discussed. Factors that are known to affect digestion are dealt with briefly and the need for standardization of the techniques involved in digestibility estimates are discussed.

Digestion includes the process by which the ingested food material is broken down into suitably absorbable nutrients across the gut wall into the blood system. The digestive processes are essentially enzymatic, and the biochemistry of enzyme digestion and nutrient absorption in fish have been adequately reviewed by Fange and Grove (1979). Digestion has to be considered separately from the passage of food along the gut and their digestibility. The latter is a more quantitatve aspect of the digestive process and refers essentially to the degree to which a food or its nutrient components are made absorbable by an individual; in other words it is a measure of the nutritional usefulness of food. The nutritional usefulness of a given food material is variable between and within a species and is dependent on a host of other factors. Also the differences between digestibility and rate of digestion and rate of passage of food through the gut, also known as the gut transient time, should be clearly understood, in spite of a degree of dependence of digestibility on these parameters (Kapoor et al. 1975).

Studies on digestibility in fish are relatively recent and commenced as a sequel to intensification in aquaculture which went hand in hand with development and dependance on artificial diets. A knowledge of digestibility of basic dietary nutrients is essential for the study of fish energetics and for evaluation of the dietary value of foodstuffs and feed ingredients; digestibility studies are pivotal to feed development. Apart from its use in aquaculture, digestibility studies of naturally ingested food material of natural or quasi-natural populations are proving to be useful to evaluate and understand the success/failure of a species (Bowen 1981; De Silva 1985a).

Terminology

Generally, digestibility is not determined for mineral elements and vitamins, and it is not a term that is equally useful and descriptive for all nutrients. For example dietary protein could be broken down in the alimentary canal by digestive fluids as well as micro organisms. As a result, the faecal material could contain protein of bacterial origin. In addition, previously digested proteins could be metabolized and re-excreted into the digestive tract as enzymes and get voided with faeces. Faeces could also contain nitrogen from the abraded gut mucosa. Therefore, it is evident that when digestibility is estimated, the extent to which the dietary protein alone was digested is not accurately reflected.

Sugar and or some other nutrients for example may behave in different ways; sugar is normally absorbed fully and metabolic by-products are eliminated completely either as CO_2 or as water, unlike fats or minerals such as calcium. Apparent digestibility of total dry matter or a nutrient component is the fraction of the ingested amount that is not recovered in the faeces; when this fraction is expressed as a percentage of the ingested amount it is known as the coefficient of digestibility.

Basic Methodology

Aspects of basic methodology for digestibility studies were recently reviewed by Cho et al. (1985) and Talbot (1985) amongst others.

The digestibility coefficient (D%) could be determined directly or indirectly. Directly by determining the amount of feed (or nutrient) ingested and voided as faeces.

$$D\% = \frac{\text{amount of food (nutrient) ingested - amount of food (nutrient) egested}}{\text{amount of food (nutrient ingested)}} \times 100$$

This method however, demands that food consumed and faecal material voided are accurately determined, collected and quantified. This is time consuming and subjected to a high degree of experimental error. The quantitative collection of faecal material and maintenance of quantitative records of feed intake is avoided with the use of indicator substances or markers. Markers were originally utilized for domestic animals by Edin (1918). Since then a wide variety of markers have been used in digestibility estimations in fish. An evaluation was made by Leavitt (1985) on the gravimetric and inert marker techniques in digestibility studies. Noue and Choubert (1986) made a similar comparison of the two methods and concluded that reliable digestibility estimates could be obtained by the direct method (total collection of faeces) with an automatic fish faeces collector. In essence both methods pose the common difficulty of obtaining a collection of a representative faecal sample.

Markers could also be of two forms; artificial foreign substances introduced into the feed in small quantities or substances that are a component of the feed itself (Table 1). In either case, the markers have to conform to four basic criteria in that they should not

(i) interfere with the digestive metabolism of the animal,

(ii) be absorbed through the lumen,

(iii) move along the gut at a differential velocity from the rest of the food material, and

(iv) be toxic.

Table 1. Different types of internal (indigenous) and external markers used in digestibility studies and authorities for chemical determination (cd) of some of the more widely used components (* De Silva 1985a).

Marker (Abbreviation)	Used by	cd
Internal		
Hydrolysis Resistant Ash (HRA) or Acid Insoluble Ash (AIA)	1,3,8,9	14,18*
Hydrolysis Resistant Organic Matter (HROM)	4,5,8,9	5*
Crude Fibre (CF)	4,8,9,15	14*
Ash	9	9
Cellulose	4,17	4,7*
Silica	12	11
External		
Chromic Oxide	(numerous) e.g., 2,6	10
32P (insoluble Ammonium molybdate)	12	
Titanium IV oxide	13	
Celite (as a supplementation to AIA)	1	
Polythene	16	

1 - Atkinson et al. (1984); 2 - Austreng (1978); 3 - Bowen (1981); 4 & 5 - Buddington (1979, 1980); 6 - Cho et al. (1974); 7 - Crampton & Maynard (1938); 8 - De Silva & Perera (1983); 9 - De Silva et al. (1984); 10 - Fukurawa & Tsukahara (1966) ; 11 - Hickling (1966); 12 - Hirao et al. (1960); 13 - Lied et al. (1979); 14 - Tacon & Ferns (1979); 15 - Tacon et al. (1983); 16 - Tacon & Rodrigues (1984); 17 - Van Keulen & Young (1977); 18 - Van Dyke & Sutton (1979).

Even though Cr_2O_3 is widely used and continues to be used as an external marker, Bowen (1978) found that in *Tilapia mossambica* Cr_2O_3 moved at a different rate than the rest of the food. Hilton et al. (1981) found that extruded-pellet feed moved slower than steam-pelleted feed due to the lower density of the latter. De Silva and Owoyemi (1983) reported that the specific gravity of the diet affects its rate of passage through the gut. The above observations raise some doubt regarding the use of external markers. This points out to the need of more extensive controlled experiments to determine and evaluate the degree of accuracy in digestibility estimates obtained using external markers as against the use of internal markers and the direct method.

Of the internal markers (Table 2) that are generally used in digestibility estimations, CF has shown to be assimilated to a very small extent at least by certain species (Niederholzer and Hofer 1979; Van Dyke and Sutton 1977) and similarly even a small fraction of HROM from the detrital aggregate by *Tilapia mossambica* (Bowen 1981). Thonney (1981) demonstrated that deliberate addition to supplement an endogenous marker, in this case a supplement to HRA (or Acid Insoluble Ash-AIA) increased the precision of the measurements. These observations were endorsed by Atkinson et al. (1984) who used celite to supplement HRA in their practical diets for rainbow trout. De Silva and Perera (1983) compared the validity of HROM, HRA and CF as markers based on experiments with the Asian cichlid, *Etroplus suratensis* (Bloch) fed the aquatic weed, *Hydrilla* and concluded that HROM was the most appropriate on the basis of the degree of precision that could be achieved in quantification.

Table 2. The chief individual components of internal markers used in digestibility studies.

Marker	Components
CF	Cellulose and lignin fractions
HROM	Cellulose, chitin
HRA (= AIA)	Minerals, ash resistant to acid digestion; primarily silica

The use of indigenous markers in digestiblity estimates are gradually gaining favour (Atkinson et al. 1984; Hilton and Slinger 1986). This is partly because of the increasing awareness of the hazards involved in the determination of Cr which is the most widely used external marker, and the increasing degree of precision that could be achieved with internal markers with or without supplementation. Moreover, it has been shown that in poultry, external indicators tend to separate from the feed or are excreted with a diurnal pattern variation thereby making complete recovery of the indicator in the faeces difficult (Thonney et al. 1984).

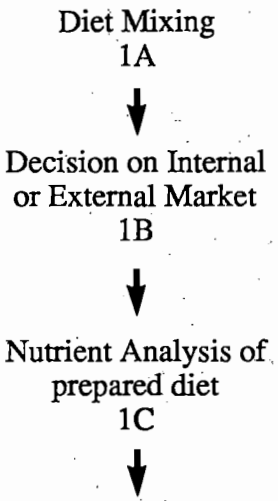
In essence there is still a controversy as to whether internal or external indicators are more suitable and or reliable and which specific ones are to be used in digestibility estimations in fish. The balance of evidence tends to indicate that indigenous markers, which are found in appreciable quantity in the diet, are more suitable than external markers. For example, when using AIA as a marker Thonney et al. (1984) recommend that it is desirable that the diet contains AIA in excess of 0.75%. Utilization of an internal and or an indigenous marker also eliminates an additional step of mixing the marker into the diet.

Chemical Analysis

Whichever indicator is finally chosen upon, apart from dry-matter digestibility estimates made using total faecal collections all the other estimates of dry-matter and nutrient digestibilities involve chemical analyses of feeds and the faecal matter for the indicators as well as for the nutrient component(s) that are being evaluated. It is not intended here to go into methods of detailed chemical analyses. The original authorities for the indigenous markers are cited in Table 1 and summarized by De Silva (1985b). In addition most of the methods are given in the AOAC (1980).

Digestibility Experiments

Digestibility experiments are basically of two kinds; those experiments which are designed to determine the digestibility of a diet and its nutrient components and those designed to evaluate the digestibility of a test ingredient and its nutrients. The steps involved for the former estimations could be schematically indicated as follows:



Commence
feeding
1D
(minimum five days
acclimatization to the diet)

↓

Collection of faeces-
non-quantitative
1E
(time of collection, pooling
of faecal samples, etc., to be
decided on the basis of the
experiment, the amount of faecal
material collected, etc.)

↓

estimation of dry matter,
nutrients and indicator
estimations in the faeces
1F

↓

Calculations
(Cho et al. 1985)
1G

Evaluation of Digestibility of a Single Dietary Ingredient and its Nutrients

A single ingredient, particularly a plant component when fed to fish, is not readily accepted and may also influence the digestive physiology so that a true indication of the extent of its digestibility may not be obtained. Therefore, to evaluate the digestibility of a single ingredient a test diet and a reference diet are used, their dry-matter and nutrient digestibilities determined independently as described earlier. The steps involved could be summarized as follows:

Reference diet (RD) -- as used in the earlier case
Test diet (TD) -- 70% RD + 30% test ingredient.

Group I -- determine digestibility of RD as indicated earlier (steps 1A to 1G)

Group II -- feed TD -- collect faeces and analyze as in step 1F

↓

Calculations
(Cho et al. 1985)

Briefly, the Apparent Digestibility Coefficient (ADC) of the Test Ingredient is calculated by the ratio:

$$\text{ADC of Test Ingredient} = \frac{\text{ADC of TD} - 0.7\ \text{ADC of RD}}{0.3\ \text{ADC of TD}}$$

General Aspects

Within the framework of the above basic concepts the experiments could differ in detail, depending on the objective. Details such as, for example, the time of collection of faeces, pooling of faecal samples, the feeding regime to be adopted, etc. could vary. Nevertheless, in all digestibility experiments certain basic precautions need to be adopted. These could be summarized as follows:

(i) fish should be acclimatized to the diet and the selected feeding regime for a minimum of 3 to 5 days, preferably more,

(ii) a host of factors are known to influence apparent digestibility of diets and nutrients (Table 3) and therefore, care should be taken to minimize/reduce these influences: such as for, e.g., the fish should be of uniform size, and

(iii) the design of the experimental tanks should be such that leaching of nutrients from faeces, mixing of food particles and faeces, and mechanical breaking up of faecal particles when collections are made minimized. That is the system should be geared for collection of a representative faecal sample. The "Guelph System (CYAQ-2)"

Table 3. Some factors known to affect or investigated for their influence on the apparent dry-matter or nutrient digestibility.

Factor	Species	Authority
Feeding level, meal size	*Clarias gariepinus*	Henken et al. (1985)
	Cyprinus carpio	von Gongnet et al. (1987)
	Salmo gairdneri	Windell et al. (1978)
Size, age, density	*S. gairdneri*	Hastings (1969); Windell et al. (1978)
Dietary components - protein, lipid, fibre, etc.	*S. gairdneri*	Rychly & Spannhof (1979)
		Cho et al. (1976)
	Oreochromis niloticus	De Silva & Perera (1984)
	S. gairdneri	Beamish & Thomas (1984)
	S. gairdneri	Hanley (1987)
	O. niloticus	De Silva & Perera (1984)
Type of nutrient (e.g., protein)	*S. gairdneri*	Nose & Toyama (1966)
Physical state of diet	*S. gairdneri*	Bergot & Breque (1983)
Protein: Energy ratio	*C. carpio*	von Gongnet et al. (1987)
Temperature, salinity	*S. gairdneri*	Windell et al. (1978)
	Colisa fasciatus	Pandey & Singh (1980)
	O. niloticus	De Silva & Perera (1984)

developed by Cho et al. (1976) meets most of the above criteria and also permits three replicates to be carried out simultaneouly. The methods used for obtaining a representative faecal sample has been summarized by Noue and Choubert (1986) and the merits and demerits enumerated. It is claimed by these authors that the automatic faeces collector developed by Choubert et al. (1982) meets most of these inadequacies and also permits collection of faeces over a long-term, and therefore enables the use of the direct method of digestibility estimation.

(iv) the time gap between collection of faeces and the last feeding should be kept constant, and

(v) as far as possible the experimental tanks should be distributed randomly and favourable environmental conditions such as adequate oxygen, proper pH, constant light and dark regimes, etc. provided.

Estimation of Digestibility of Food in Natural Populations

As mentioned earlier estimations of digestibility in natural populations could be relevant to understanding its failure or success. It is also becoming increasingly apparent that under tropical culture conditions the naturally produced food in the system is utilized (Little and Muir 1987). Therefore, estimations of this contribution will provide a further tool for manipulation of this naturally produced resource to the benefit of the farmer.

Digestibility studies on natural fish populations are very few (Bowen 1981; De Silva et al. 1984; De Silva 1985a). In digestibility estimations of natural populations, it is assumed that contents in the stomach are representative of the previously ingested material and material about to be voided in the rectal region. Based on this assumption, the concentration of an indigenous marker such as CF, HROM, HRA and or ash (De Silva et al. 1984) in the rectal contents as compared to that in the stomach contents is estimated. Similarly, the nutrient components in the stomach and the rectal contents are evaluated and the digestibility (total) of the individual nutrients calculated using an indigenous component as the marker. The very narrow coefficients of variation in the estimations between individual fish taken from the same locality is perhaps indicative of the validity of the assumptions made as well as the validity of the method. This approach has the advantage that the individual fish are not disturbed before their capture (death) and as such the results obtained will be a reasonable reflection of the natural state. De Silva et al. (1984) also pointed out that ash could be used reliably, to estimate digestibility, and argued that this was possible because the ingested material in *O. mossambicus* has a high proportion of ash; even if ash was assimilated to a small extent, the final estimations would not be affected to a significant extent. Use of ash as a marker relieves the investigators and saves the problem of performing intricate chemical analyses. The validity of this observation however, has to be tested for other species with comparable food habits.

Future Trends in Digestibility Research

There is an increasing amount of research in the region, as elsewhere on the evaluation of the suitability of locally available plant products incorporated into practical diets.

The first step in this screening would be to determine the digestibility (total and nutrient) of the individual ingredients by different species, and within a species at different stages of growth. However, screening of all plant ingredients with a potential would be an uphill task for any one group of researchers. It is suggested therefore, that basic criteria be developed to determine the potential suitability of plant ingredients based on the proximate composition, amino acid profile, presence of growth inhibitors, fibre content, etc. and ingredients representative of each group be chosen for further experimentation. In determining the suitability for further experimentation the availability, cost and experimental work carried out hitherto on these should be taken into consideration.

Unlike in terrestrial animals, husbandry practices have received little attention in fish culture practices. It has been demonstrated that the daily food consumption in fish is variable (Smagula and Adelman 1982; De Silva et al. 1986). This daily variability is likely to have an influence on digestibility (De Silva and Perera 1984) about which little is known. Investigations on diurnal variation in digestibility may also provide another means of adjusting the quantity of food presented or provide mixed dietary regimes of low and high protein content and thereby provide a means of reducing feed costs.

Standardization of the techniques in faecal collection, pooling of faecal collection for chemical analysis and time of collection of faeces are desirable. In the scientific literature in most instances these details are not presented. It is possible that somewhat contradictory observations that have been made on the influence of various environmental and other factors such as size on digestibility could be at least partially a result of such differences.

An aspect of digestibility in fish which has received scanty attention is the role of microbes. Although microbes may be of lesser or insignificant importance in digesting pelleted, low fibre diets they may be of importance for herbivorous and omnivorous species (Moriarty 1976; Trust et al. 1979). In tropical Asia, a good number of cultured fish are herbivore/omnivore types and are known to obtain significant quantities of food from what is produced naturally in the system. In such a context, it will be important to assess the role of microbes in digestion with a view to providing more optimal conditions for effective utilization of such food materials.

References

Association of Official Analytical Chemists. 1980. Official methods of analysis, Washington, DC, USA.

Atkinson, J.L., J.W. Hilton and S.J. Slinger. 1984. Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout *(Salmo gairdneri)*. Canadian Journal of Fisheries and Aquatic Sciences 41: 1384-1386.

Austreng, E. 1978. Digestibility determinations in fish using chromic oxide marker and analysis of contents from different segments of the gastrointestinal tract. Aquaculture 13: 265-272.

Beamish, F.W. and E. Thomas. 1984. Effect of dietary protein and lipid nitrogen losses in rainbow trout, *Salmo gairdneri*. Aquaculture 41: 359-371.

Bergot, F. and J. Breque. 1983. Digestibility of starch by rainbow trout: effects of physical state of starch and of the intake level. Aquaculture 34: 203-212.

Bowen, S.H. 1978. Chromic acid in assimilation studies - a caution. Transactions American Fisheries Society 107: 755-756.

Bowen, S.H. 1981. Digestion and assimilation of periphytic detrital aggregate by *Tilapia mossambica*. Transactions American Fisheries Society 110: 239-245.

Buddington, R.K. 1979. Digestion of an aquatic macrophyte by *Tilapia zilli*. Journal of Fish Biology 15: 449-456.

Buddington, R.K. 1980. Hydrolysis resistant organic matter as a reference for measurement for fish digestive efficiency. Transactions American Fisheries Society 109: 653-656.

Cho, C.Y., H.S. Bayley and S.J. Slinger. 1974. Partial replacement of herring meal with soybean meal and other changes in a diet for rainbow trout *(Salmo gairdneri)*. Journal of Fisheries Research Board, Canada 31: 1523-1528.

Cho, C.Y., H.S. Bayley and S.J. Slinger. 1976. Influence of level and type of dietary protein, and level of feeding on feed utilization by rainbow trout. Journal of Nutrition 106: 1547-1556.

Cho, C.Y., C.B. Cowey and T. Watanabe. 1985. Methodological approaches to research and development. *In* C.Y. Cho, C.B. Cowey and T. Watanabe (eds.) Finfish Nutrition in Asia, pp. 10-80. International Development Research Centre (Canada), Ottawa.

Choubert, G., J. de la Noue and P. Luquet. 1982. Digestibility in fish: improved device for the automatic collection of feces. Aquaculture 29: 185-189.

Crampton, E.W. and L.A. Maynard. 1938. The relation of cellulose and lignin content to the nutritive value of animal feeds. Journal of Nutrition 15: 383-395.

De Silva, S.S. 1985a. Body condition and nutritional ecology of *Oreochromis mossambicus* (Pisces, Cichlidae) populations of man-made lakes in Sri Lanka. Journal of Fish Biology 27: 621-633.

De Silva, S.S. 1985b. Evaluation of the use of internal and external markers in digestibility studies. *In* C.Y. Cho, C.B. Cowey and T. Watanabe (eds.) Finfish Nutrition in Asia, pp. 96-102. International Development Research Centre (Canada), Ottawa.

De Silva, S.S. and A.A. Owoyemi. 1983. Effect of dietary quality on the gastric evacuation and intestinal passage in *Sarotherodon mossambicus* (Peters) fry. Journal of Fish Biology 23: 347-355.

De Silva, S.S. and M.K. Perera. 1983. Digestibility of an aquatic macrophyte by the cichlid *Etroplus suratensis* with observations on the relative merits of three indigenous components as markers and daily changes in protein digestibility. Journal of Fish Biology 23: 675-684.

De Silva, S.S. and M.K. Perera. 1984. Digestibility in *Sarotherodon niloticus* fry: effect of dietary protein level and salinity with further observations on daily variability in digestibility. Aquaculture 38: 293-306.

De Silva, S.S., M.K. Perera and P. Maitipe. 1984. Food, nutritional status and digestibility of *Sarotherodon mossambicus* populations of twelve man-made lakes in Sri Lanka. Environmental Biology of Fishes 11: 205-219.

De Silva, S.S., C. Keembiyahetty and R.M. Gunasekera. 1986. Optimum ration and feeding frequency in *Oreochromis niloticus* young. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum, pp. 559-564. Asian Fisheries Society, Philippines.

Edin, H. 1918. Orienterande forsok over anvandbarheten av en pa "ledkroppsprincipen" grundad metod att bestamma en foderblandings smaltbarhet. Forsoksvasendet Jordbruk Stockholm Medd: stands 165: 1-28.

Fange, R. and D. Grove. 1979. Digestion. *In* M.B. Brown (ed.) The Physiology of Fishes, Vol. VIII, pp. 162-260. Academic Press, London.

Furukawa, A. and H. Tsukahara. 1966. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility in fish feed. Bulletin of the Japanese Society of Scientific Fisheries 32: 502-506.

Hanley, F. 1987. The digestibility of foodstuffs and the effects of feeding selectivity on digestibility determinations in tilapia, *Oreochromis niloticus* Aquaculture 66: 163-179.

Hastings, W.H. 1969. Nutritional scores. *In* O.W. Neuhaus and J.C. Halver (eds.) Fish in Research, p. 263-293. Academic Press, New York.

Henken, A.M., D.W. Kleingeld and P.A.T. Tijssen. 1985. The effects of feeding level on apparent digestibility of dietary dry matter, crude protein and gross energy in the African catfish *Clarias gariepinus* (Burchell 1822). Aquaculture 51: 1-11.

Hickling, C.F. 1966. On the feeding process in the white amur *Ctenopharyngodon idella*. Journal of Zoology, London 148: 408-419.

Hirao, S., J. Yamada and R. Kikuchi. 1960. On improving efficiency of feed for fish culture. I. Transit and digestibility of diet in eel and rainbow trout observed by use of P32. Bulletin of the Tokai Regional Fisheries Laboratory 7: 67-74.

Hilton, J.W. and S.J. Slinger. 1986. Nutrition and feeding of rainbow trout. Canadian Fisheries and Aquatic Sciences, Special Publication 55: 15 p.

Hilton, J.W., C.Y. Cho and S.J. Slinger. 1981. Effect of extrusion processing and steam pelleting diets on pellet durability, pellet water absorption and physiological response of rainbow trout (*Salmo gairdneri* R.). Aquaculture 25: 185-194.

Kapoor, B.G., H. Smit and I.A. Verighina. 1975. The alimentary canal and digestion in teleosts. *In* F.S. Russel and C.M. Young (eds.) Advances in Marine Biology, pp. 105-239. Academic Press, London.

Leavitt, D.F. 1985. An evaluation of gravimetric and inert marker techniques to measure digestibility in the American lobster. Aquaculture 47: 131-142.

Lied, E., K. Julshamn and O.R. Braekkan. 1979. Determination of protein digestibility in Atlantic cod *(Gadus morhua)* with internal and external indicators. Canadian Journal of Fisheries and Aquatic Sciences 39: 854-858.

Little, D. and J. Muir. 1987. A guide to integrated warm water aquaculture. University of Stirling, Scotland, 238 p.

Moriarty, D.J.W. 1976. Quantitative studies on bacteria and algae in the food of the mulet *Mugil cephalus* L. and prawn *Metapenaeus bennettae* (Racek & Dall). Journal of Experimental Marine Biology and Ecology 22: 131-143.

Niederholzer, R. and R. Hofer. 1979. The adoptation of digestive enzymes to temperature, season and diet in roach *Rutilus rutilus* L. and rudd *Sardines erythrophthalamus* L. Cellulase. Journal Fish Biology 15: 411-416.

Noue, J. de la and G. Choubert. 1986. Digestibility in rainbow trout: Comparisons of the direct and indirect methods of measurement. Progressive Fish-Culturist 48:190-195.

Nose, T. and K. Toyama. 1966. Protein digestibility of brown fishmeal in rainbow trout. Bulletin Freshwater Fisheries Research Laboratory 15: 213-224.

Pandey, H.S. and R.P. Singh. 1980. Protein digestibility by Khosti fish *Colisa fasciatus* (Pisces, Anabantidae) under the influence of certain factors. Acta Hydrochimica Hydrobiologia 8: 583-585.

Rychly, J. and L. Spannhof. 1979. Nitrogen balance in trout. 1. Digestibility of diets containing varying levels of protein and carbohydrates. Aquaculture 16: 39-46.

Smagula, O.M. and I.R. Adelman. 1982. Day to day variation in food consumption by largemouth bass. Transactions American Fisheries Society 111: 543-548.

Tacon, A.G.J. and P.N. Ferns. 1979. Activated sewage sludge - a potential animal foodstuff. 1. Proximate and mineral content: seasonal variation. Agriculture and Environment 4: 257-269.

Tacon, A.G.J. and A.M.P. Rodrigues. 1984. Comparison of chromic oxide, crude fibre, polythylene and acid-insoluable ash as dietary markers for the estimation of apparent digestibility coefficient in rainbow trout. Aquaculture 43: 391-399.

Tacon, A.G.J., J.V. Haaster, P.B. Featherstone, K. Kerr and J.A. Jackson. 1983. Studies on the utilization of full-fat soybean and solvent extracted soybean meal in a complete diet for rainbow trout. Bulletin of the Japanese Society for Scientific Fisheries 49: 1437-1443.

Talbot, C. 1985. Laboratory methods in fish feeding and nutritional studies. *In* P. Tytler and P. Calow (eds.) Fish Energetics: New Perspectives, p. 125-154. Croom Helm Publishers, London.

Thonney, M.L. 1981. Acid insoluble ash as a digestion marker. Proceedings Cornell Nutrition Conference for Feed Manufacturs, p. 118-120. Cornell University, Ithaca, New York.

Thonney, M.L., B.A. Palhof, M.R. Decarlo, D.A. Ross, N.L. Firth, R.L. Quaas, D.J. Perosio, D.J. Duhaime, S.R. Rolline and A.Y.M. Nour. 1984. Sources of variation of dry matter digestibility measured by the acid insoluble ash marker. Journal of Dairy Science 69: 661-668.

Trust, T.J., M. Bull, B.R. Currie and J.T. Buckley. 1979. Obligate anaerobic bacteria in the gastrointestinal microflora of the grass carp *(Ctenopharyngodon idella)*,goldfish *(Carassius auratus)*, and rainbow trout *(Salmo gairdneri)*. Journal Fisheries Research Board, Canada 36: 1174-1179.

Van Dyke, J.M. and D.L. Sutton. 1977. Digestion of duckweek (*Lemna* spp.) by grass carp *(Ctenopharyngodon idella)*. Journal of Fish Biology 11: 273-278.

Van Keulen, J. and B.A. Young. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. Journal of Animal Science 44: 282-287.

Von Gongnet et al. 1987. The influence of different protein/energy rations and increasing feeding level on nitrogen excretion in growing mirror carp (*Cyprinus carpio* L.) Journal of Animal Physiology and Animal Nutrition 58: 173-188.

Windell, J.T., J.W. Foltz and J.A. Sarokon. 1978. Effect of fish size, temperature and amount fed on nutrient digestibility of a pelleted diet by rainbow trout, *Salmo gairdneri*. Transactions American Fisheries Society 107: 613-616.

Considerations for Feeding Experiments to Quantify Dietary Requirements of Essential Nutrients in Fish

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Standard methods in feeding experiments conducted to quantify nutrient requirements in fish are needed so that results are widely applicable and comparisons can be made between studies. Environmental factors that affect growth should be optimal. These parameters, including aspects of the experimental setup that influence water quality should be reported. Characteristics of the fish that influence its growth response including strain, size, state of maturation, sex, among others need to be specified. The composition of diets and feeding managements should provide for maximum growth. Both feeding rate and feeding frequency contribute to differences in growth response and feed efficiency. Consideration of the objectives in an investigation should be made in the choice of method and diet. Other parameters such as tissue levels of nutrients can be used as adjuncts to the analysis of the growth response but cannot be used alone. The experiment should attempt to answer one question and the design should allow for proper statistical analysis. Nutrient interaction contributes to differences in requirements and the response to deficiency, so levels of interacting nutrients should be reported.

The worldwide expansion of fish culture has resulted in a considerable increase in its contribution to fish production. Information on the nutrition of fish is important in promoting further development of fish culture, but has lagged behind that of other farmed animals. Among fish, the nutrient requirements of coldwater chinook salmon and rainbow trout (NRC 1981), and warmwater channel catfish and common carp (NRC 1983) have been most studied. Scanty information is available on other species such as the tilapias, milkfish and the different species of Indian and Chinese carp that are widely cultured in Asia. Most of the reported requirement levels have been determined using feeding studies with growth as the primary response parameter. Feeding trials remain the most useful method in obtaining results that have direct application to feeding practices. Thus, the validity of other parameters of measurement, such as carcass deposition or tissue and plasma levels of nutrients are often verified by comparison with the conventional growth response curve. Although widely accepted, this method takes time to conduct and permits the evaluation of one nutrient at a time.

There are more species of fish than there are of mammals and their dietary nutrient requirements and feeding habits are as varied as those of mammals. Many of the features of methods for assessing nutrient requirements in fish however, are common because the aquatic environment provides common limitations and problems. Standardizing methods for requirement studies would result in the increased applicability of results and allow the comparison of results,

intra and inter specifically. Much effort has been directed towards this goal and guidelines for the standardization of experimental design and evaluation have been formulated (Castell and Tiews 1980).

The objective of this paper is to identify the major issues in nutrient requirement studies involving feeding trials. The factors that influence the reported requirements are emphasized, so that these parameters can be defined and considered in the planning of experimental designs.

Environmental Conditions

In feeding experiments, the experimental tanks should be set up such that the environment is favorable for maximum growth of the fish. Environmental factors influence growth through four different ways: 1) temperature and pH, as controlling factors, govern rates of reaction and consequently the rate of food consumption, 2) oxygen, carbon dioxide, ammonia and nitrite, as limiting factors for growth are involved in restricting the supply or removal of metabolites as links in the metabolic chain, 3) salinity and turbidity are masking factors in that they modify the effect of other environmental factors through regulation, and 4) light quality and photoperiod, as directive factors, cue the fish to select or respond to particular characteristics of the environment (Fry 1971; Brett 1979).

The quality of water is influenced by the fish biomass (weight x density), the velocity of water flow and the source of water. Water may be recirculated or flowing through, exposed to treatment (filtration, exposure to O_3, UV, etc.) or not at all. A flow through system, designed to remove extraneous sources of nutrients is preferred. The various environmental parameters that affect rate of growth should be uniform in all tanks and should be defined.

Experimental Fish

The species used in feeding studies should be identified by its scientific and common name. The size, age, state of maturation, sex and strain affect growth and therefore should be reported. Body stores of nutrients can affect nutrient requirement as high levels may reduce the minimum level for maximum response or lengthen the assay time to produce a deficiency. This can be defined by determining preassay levels of nutrients, or providing information on the source of fish and previous rearing conditions including the feed used and the conditions during acclimation. Gradual conditioning to the experimental diet and set-up is necessary in attaining maximum growth response.

Experimental Diet

Identification and analysis of diets and ingredients

In order to standardize the identification of diets and dietary ingredients, the full recognized name and International Feed Number of all ingredients should be given for all prepared feeds. If this is not possible, source, species and part of material from which ingredients were prepared should be given together with details of stage of maturity, grade and processing method. The chemical formula and quality of mineral components and forms and quality of vitamins should be reported. If a commercially prepared diet or ingredient is used, the full name of the diet and manufacturer, with the manufacturer's code and lot number should be given (Castell and Tiews 1980).

The chemical composition of diets is usually defined by the standard proximate composition (AOAC 1984) following the analysis of diets and feedstuff by the Weende System. This broadly divides the material into six fractions, namely: moisture, crude protein, crude fibre, crude fat or ether extract, mineral matter or ash and nitrogen-free extract. The moisture content is defined as the loss of weight of a sample dried to constant weight but not longer than 24 hr at 104°C. Crude protein is defined as the product of 6.25 (assumes protein contains 16% N) times the nitrogen content of the sample, usually analyzed using the Kjeldahl method. It provides a crude estimate of protein content because not all protein contains 16% N and not all N-containing compounds are proteins (e.g., ammoniacal nitrogen containing materials such as urea, amino acids, amides, glycosides, etc.). The crude fibre fraction includes all materials that are insoluble in boiling weak acids and bases, less the ash content of this residue. Although it mainly consists of the fibrous materials like cellulose, lignin and chitin, the separation is not complete and is at best an approximation of materials that are of low digestibility. Crude fat is determined by extracting ground samples continuously for a few hours with ether. The ash fraction is that portion remaining after burning off all organic material in a muffle furnace beyond 500°C. The ash fraction does not indicate the specific elements present and may include carbon from organic matter as carbonate when base forming minerals are in excess. The nitrogen-free extract (NFE) generally includes the more digestible carbohydrates, mostly sugars and starches. It also includes some of the more soluble hemicellulose and lignin. This fraction is not determined directly but is determined by the difference between 100 and the other five fractions. The errors of the other analyses would therefore be added in the determination of NFE.

The composition of feeds may be expressed on the basis of its dry matter content and this should be specified either as: 1) fed on wet basis, 2) air-dry basis or 3) oven-dry or moisture-free basis. Moisture content should be provided on the first two expressions so that data can be converted to dry basis.

The standard proximate analysis does not define the concentration of the individual required nutrients. Additional analyses to determine the concentration of nutrients under study of the nutrients that may affect the requirement levels should be conducted. The pH of the diet should be monitored and adjusted to neutrality. Studies have shown that diets which are acidic result in poor growth and feed efficiency (Nose et al. 1974; Wilson et al. 1977; Deshimaru et al. 1982).

Fish, like many terrestrial animals, regulate food consumption to attain a certain level of energy intake, so that expressing some nutrient concentration relative to the dietary energy level is very useful. This is exemplified by expressions of dietary protein requirements (Cowey 1979). At present, researchers express dietary energy as total, digestible or metabolizable energy and use many different methods for estimating these values (Jobling 1983). The experimental determination of metabolizable energy has been performed extensively only for trout (NRC 1981) and reported values for other species have been calculated from unreliable assumptions. Jobling (1983) noted that the use of caloric conversion coefficients and assumed digestibility can lead to serious errors since these values change with many factors including the species of fish, quality of nutrient and rate of inclusion. He suggested that total and digestible nutrients should be measured directly. Consideration should also be made on the protein intake and the balance between digestible protein and energy because these affect the metabolizable and net energy of fish diets (Cho and Kaushik 1985). In many cases, it is not possible to perform digestibility measurements prior to a nutrient requirement study because of technical, financial or manpower problems. Because ingredients used for nutrient requirement studies are relatively pure and digestible and are added within certain levels which are known to be optimal, it would be better to assume digestibility values to attain diets of an approximate energy content than not to consider dietary energy at all. The values used by Jauncey (1982) for tilapia, namely: 4.5 kcal/g protein (Smith 1971), 8.5 kcal/g dextrin (Chiou and Ogino 1975) would assume about 90%

digestibility for protein and fat and 85% digestibility for carbohydrate and would probably be well within the actual digestibility range for many species.

Diet preparation and physical form

When preparing a diet, one should bear in mind the need to attain a homogeneous distribution and to produce a diet in a physical form that is easily accepted by the fish. Preparing a premix would greatly help in mixing micronutrients. The equipment and procedure used in the preparation of the diet, the form of the diet (wet, moist or dry feed), particle size and the storage conditions need to be stated.

Diet composition

The basal diet should supply all essential nutrients in quantities required for maximum growth and control over the nutrients should be possible so that only the nutrient factor to be tested is varied. If possible, a common reference diet should be used to facilitate comparisons between experiments, species, locations, researchers and other factors and conditions. It is important that the quality and quantity of all nutrients should be the same except for the test nutrient and the nutrient that replaces it. Experimental diets should be isocaloric unless the dietary energy is the subject of the study. These are possible only with the use of purified and semi-purified diets and only after all essential nutrients have been identified. Purified nutrient sources include casein, gelatin, purified soybean protein and isolated fish protein as typical protein sources; glucose, sucrose, dextrin and starch as typical carbohydrate sources; lard, marine fish oils and other oils as fat sources, crystalline amino-acids as sources of amino acids and chemically pure compounds as sources of vitamins and minerals. Studies on the qualitative and quantitative requirements for amino acids in fish were first conducted after the development of an amino acid test diet (Halver 1957; Halver et al. 1957). Quantitative vitamin requirements can be defined only with the use of vitamin-free ingredients. Likewise mineral requirements can be defined when the diet is free of the mineral being investigated. Such studies are complicated by environmental sources of minerals which are absorbed by fish. Presently, only seven of the 20 or so inorganic elements needed to maintain the structural and metabolic functions of vertebrates have been found to be required in fish diets. In many instances the use of semi-purified or purified diets results in poorer growth response and feed efficiency than a practical diet. A purified diet can be completely successful for a given species only when all the requirements, rate of utilization and interactions of nutrients are known and when the diet is palatable and of suitable physical nature. In the case of amino acid test diets, poorer growth may be attributed to the inefficient utilization of free crystalline amino acids than of protein-bound amino acids by some species of fish (Aoe et al. 1970; Andrews et al. 1977).

Non-nutrient components may be required to improve the stability of nutrients and acceptability of the diet. Antioxidants such as ethoxyquin, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are added at levels not exceeding 0.02 percent of the feed to protect the diets from lipid peroxidation and destruction of fat-soluble nutrients (Rumsey 1980). Binders, such as carboxymethylcellulose or algin products are added to improve stability in water and minimize the leaching of nutrients. The feeding behavior in different species of fish is stimulated by different chemical substances (Carr 1982). Chemical attractants in feed, particularly those containing pure or semi-purified ingredients will be needed to improve palatability so that maximum consumption can be achieved.

Table 1. Factors that have been demonstrated to alter nutritional requirements of fish.

Species	Required Nutrient	Change in Requirement	Parameter	Change in Parameter
Ictalurus punctatus[1]	protein	35% - >25%	fish size	14 g - >114 g
Cyprinus carpio[2]				
Salmo gairdneri[2]	protein	50% - >35%	rate of feeding	2.5% - >3.5% of body weight
Salmo gairdneri[3]	protein	50-60 - >40%	rate of feeding	restricted - >satiated
Onchorynchus tshawytscha[4]	protein	40% - >55%	temperature	8.3°C - >14.4°C
Salmo gairdneri[5]	protein	40% - >45%	salinity	10 ppt - >20 ppt
Salmo gairdneri[6]	arginine	3.0% - >2.5%	salinity	freshwater - >seawater
Salmo gairdneri[7]	arginine	4.2% - >3.5%	rate of feeding	restricted - >satiated
Salmo gairdneri[7]	arginine	increase	electrolyte balance (Na + K - Cl)	0 meg/kg - >200 meg/kg
Salmo gairdneri[8]	tocopherol	increase	temperature	decrease

1-Page and Andrews 1973; 2-Ogino 1980; 3-Cho et al. 1976; 4-De Long et al. 1973; 5-Zeitoun et al. 1973; 6-Kaushik 1979; 7-Chiu et al. 1988; 8-Bell and Cowey 1985.

Management of Feeding

Proper management of feeding is important for the attainment of maximum growth and feed efficiency. It involves the management of feeding rate and frequency. Both parameters vary with many factors including the species and size of fish, water temperature and dietary energy level. Even with the same species of fish, management of feeding practices vary among different groups of researchers.

Feeding rate

The rate of feeding affects nutrient intake and feed efficiency. The accuracy of measurement of these two parameters on the other hand, depends on the feeding rate as excess feeding may lead to leaching. Limiting the amount of feed available may not permit the fish to reach its full growth potential. The method of determination of feeding rate would therefore depend on the objective of specific studies.

The methods of determining feeding rate can generally be classified into: 1) feeding at a given percentage of body weight, and 2) feeding to satiation. Feeding at a certain percentage of body weight is a simple and common practice. The method does not take into consideration the exponential ($Y = a\text{-}b \log X$) relation between the capacity of the stomach (in % of body weight) and fish weight (Brett 1971), so that the level of dietary restriction is not the same (greater at the earlier stage). The feed given can be restrictive, thereby limiting the potential for growth resulting from an experimental diet; or it can be in excess of consumption. The latter makes it difficult to quantify any depression of feed intake or to measure feed efficiency and many even cause the degradation of water quality. Feeding to satiation corrects these problems but requires more effort to conduct. However, the determination of the point of satiation is subjective.

Nutrient requirements of fishes have been demonstrated to differ with feeding rate. Lower requirement for protein (Cho et al. 1976; Ogino 1980) and for arginine (Chiu et al. 1988) has been demonstrated when trout is fed at a higher rate (Table 1). At the required levels, the total arginine fed per day using the two feeding rates were found to be comparable (Chiu et al. 1988). The requirements for nutrients should therefore not only be expressed in concentrations such as percentage but also in terms of intake (g nutrient kg body weight^{-1} day^{-1}).

Feeding frequency

Growth and feeding frequency are positively related. Fish at the higher feeding regime naturally grow faster. However, there is a maximum limit to extensive feeding at which the increase is negligible when considering the amount of food given (Chua and Teng 1978), and this is defined as the optimal feeding frequency. Optimal feeding frequency differs among different species of fish which vary considerably in gut morphology and is influenced by other factors such as fish size and meal size (Table 2). The use of optimal feeding frequency to attain the best growth response in nutrient requirement studies is preferred; however, the main objective of the experiment should take priority. For example, increased number of feedings per day is suggested when a study involves the use of amino acids because the utilization of crystalline amino acids improve with increased feeding (Yamada et al. 1981). Increased number of feedings is also suggested for fishes at the earlier stages of development to minimize leaching of diets prepared in fine particulates.

Table 2. Feeding frequency required to promote optimum growth and feed efficiency in different species of fish.

Species of fish	Optimum feeding frequency	Initial fish size	Reference
Cyprinus carpio	3 x / day	0.17 g	Charles et al. 1984
Chanos chanos	8 x / day	0.60 g	Chiu et al. 1987b
Salmo gairdneri	2 x / day	7.6-16.0 g	Grayton and Beamish 1977
Epinephelus tauvina	1 x 2 / days	70 g	Chua & Teng 1978
Heteropneustes fossilis	1-2 x / day	4.5-9 g	Marian et al. 1982 Singh & Srivastava 1984
Ictalurus punctatus	2 x / day		Andrews and Page 1975
Channa striatus	1 x / day	0.66 g	Sampath 1984
Clarias lazera	continuous	0.5 g	Hogendoorn 1981
Oreochromis niloticus	1 x / day	6.8 g	De Silva et al. 1986

Response Parameters

Growth is the most widely accepted response used to evaluate nutrient status. Measurements of body weight and/or length to determine growth should be done in regular subperiods, preferably no less than twice a month. Measurements at the start and end of the experiment are inadequate and would not give any information of the growth pattern as influenced by the diet under the experimental conditions. The procedure for measurement, the frequency of measurement and the duration of the experiment should be described. Growth can be expressed in absolute terms as the difference (gain or loss) in the initial and final weight or length or as the partial difference in each sub-period. Growth is sometimes expressed in relative terms, so that comparisons can be made with fish having different initial weights. Relative growth is growth expressed as a percentage of the initial body weight. When the growth of fish is characterized by an exponential curve, a constant instantaneous or specific rate can be derived as the difference in the natural logarithm of the initial and final weight over a period. Equations for growth measurements are summarized as follows:

Absolute growth : $W_2 - W_1$

Absolute growth rate : $\dfrac{W_2 - W_1}{t_2 - t_1}$

$$\text{Relative growth} : \frac{W_2 - W_1}{W_1}$$

$$\text{Relative growth rate} : \frac{W_2 - W_1}{W_1 (t_2 - t_1)}$$

$$\text{Instantaneous or specific growth rate} : \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

where W_1 and W_2 are weights at periods t_1 and t_2, respectively.

The efficiency of food utilization can strictly be quantified only when feeding is managed so that excess food is negligible. Two most widely used parameters are feed conversion ratio (FCR) and feed efficiency. FCR is the dry weight of feed per unit wet weight gain (feed/gain) while feed efficiency is its reciprocal (gain/feed). The latter is preferred in scientific papers because it conforms to the usual understanding about efficiency in that it is an expression of output over input, value increases with a better diet and the expression becomes zero when the fish is not growing, while FCR becomes infinity (Baker 1986). FCR is preferred in practice because it allows one to easily relate the quantity of feed and increase in weight to the cost of production.

When a measure of the biological value of protein is involved, protein efficiency ratio (PER = weight gain/protein intake) and productive protein value (PPV = N retained/N consumed) would be useful parameters. Like feed efficiency, higher values for both parameters indicate better efficiency of utilization. Both parameters provide for a relative rating of protein utilization but do not take into consideration the requirements for maintenance.

The number of fish lost during the experiment should be indicated. Any difference in percentage mortality attributable to dietary treatment should be noted. When mortality is substantial, gross and histological necropsy of mortalities should be conducted to provide an explanation. Deficiency symptoms of a nutrient should be monitored.

Growth is a poor indicator of the status of some nutrients such as for some minerals (Baker 1986). Response parameters specific to certain nutrients can be as adjuncts to growth measurements in evaluating nutrient requirements. For many vitamins, which have a coenzyme function, the activity of the enzyme would be an indicator of vitamin status. However, requirements based on such measurements do not always coincide with that derived from the growth response (Cowey 1976). The use of other biochemical parameters for other nutrients likewise do not always provide a response comparable to that of growth and would not be advisable to use on its own in requirement determinations (Walton et al. 1986).

Experimental Design

Identification of objectives and experimental design

An experiment should attempt to answer only one clearly defined question at a time. Where facilities permit two questions can be tested through a factorial design. The experimental design is determined by the question(s) asked and replicates should be provided to allow one to take advantage of proper statistical analysis. In feeding experiments where tanks are the experimental

units, consideration of replication should be made on the number of fish per tank and the number of tanks per treatment. The goals are to set a level of significance (usually 0.05) that will be used to ascertain whether a treatment has an effect and to provide the number of replicates needed in an experiment to detect important differences. The number of replicates would therefore depend on the variation associated with the experimental unit and on the size of the difference which is of importance to the study. Calculations can be made following the method described by Cochran and Cox (1957). A complete description of the statistical method used including the reference for the method should be provided.

Determination of nutrient requirement from growth data

Growth, derived from a feeding trial involving dose-response of the species in question, still remains the most useful and practical method for determining nutrient requirements. The comparison of response can be predictive if the nutrient does and observed response are quantified and their relationships expressed mathematically through models. Regression analysis is favored over the analysis of variance and multiple comparisons of the mean from dose-response data because it is continuous and more accurate. The broken line method, with a horizontal and a positively sloping vertical line fitted by the method of least squares is more commonly used. It is based on the assumption that a growing animal will respond linearly to additions of a limiting, indispensable nutrient until the exact requirement is met, after which no further growth response will be observed (Robbins et al. 1979). In many cases, the response curve cannot fit into the broken line model, and a curvilinear model is needed. For example, in some cases a certain high concentration of nutrient can result in growth depression so that non-linear models such as a parabolic (second order polynomial) curve would be more suitable. Requirements may be defined as the concentration resulting in maximum growth or as the minimum nutrient level that does not reflect any significant difference from maximum growth. The latter which would result in a lower requirement level has been successfully used to determine economic protein requirement (Zeitoun et al. 1976), tryptophan (Poston and Rumsey 1983) and arginine (Chiu et al. 1988) requirements of rainbow trout. Other curvilinear models such as the sigmoidal (Robbins et al. 1979) and the four-parameter kinetic model (Mercer et al. 1978) have been tested and found useful. The final choice of the model should be based on the simplest model that can describe the responses of fish to a wide range of dosages.

Identification of relevant nutrient parameters

Information regarding the qualitative requirement for nutrients is needed for the least cost formulation of diets. The nutrients that have been found to be essential for fish are listed in Table 3. The essential nutrients are common among different species of fish except for essential fatty acids which differ according to the type of fish (Kanazawa 1985). The essentiality of some micronutrients such as minerals is difficult to demonstrate because of possible absorption from environmental sources.

Among the species of fish for which nutrient requirements have been determined, many of the reported requirement levels from different laboratories differed significantly. Differences may be attributed to dietary components and the manner of expression of concentration (Tables 3 and 4) or to other parameters including the rate of feeding, size of fish, temperature and salinity

Table 3. Major considerations in the study of essential nutrients.

Required Nutrients		Major Research Considerations
Protein		*Requirements depend on the quality of protein, with a "balanced" amino acid profile resulting in lower requirement. Thus, a common high quality protein (standard reference protein) is preferred. *Requirement can be reduced with adequate dietary energy. The relative protein sparing action of carbohydrate and lipids vary considerably among different species of fish.
Amino acids: Arginine Histidine Isoleucine Leucine Lysine	 Methionine Phenylelanine Threonine Tryptophan Valine	*A constant relationship exists between amino acid requirement and protein intake, so requirement should also be expressed as a percentage of dietary protein *Considerable variation in the ability to utilize free amino acids exists among different species of fish. This may cause problems in quantifying requirements because crystalline amino acids are used to vary dietary level (Aoe et al. 1970).
Fatty acids: 18:2ω6/ 18:3ω3/ 20:5ω3/		*Essential fatty acid (EFA) requirement is dependent on total lipid level, so expressing it as a percentage of total lipid would be useful (Takeuchi and Watanabe 1977). *Different species of fish have different requirement for fatty acids. Generally, EFA requirement can be grouped as 18:2ω6 tilapia, 18:3ω3 rainbow trout - and 20:3ω5 red sea bream types. Lecithin is an essential dietary component of some larval fish (Kanazawa 1985).
Vitamins: Fat soluble - A,D,E,K Water soluble - ascorbic biotin choline cobolamine folic acid	 niacin pantothenic acid pyridoxine riboflavin thiamin	*Vitamin requirement vary with species of fish size, age, environment and state of maturation. Requirements for maximum growth under optimum conditions increase up to tenfold when fish are exposed to conditions of stress or disease (Halver 1985). *The allowance needed to compensate for leaching and processing losses may result in significantly higher requirements. The losses vary in different vitamins and is particularly high in ascorbic acid (Slinger et al. 1979). Leaching losses are particularly greater in larval feeds.
Minerals: macrominerals - Ca, P, Mg, Na, K, Cl, S microminerals - Fe, Zn, Cu, Mg Se, I, Ni, Co, Mb, Cr, F, Si, Va, As		*Studies on requirement are masked by the contribution of minerals from the environment. Thus, only Ca, P, Mg, Fe, Zu, Cu, Mn, Se and I have been demonstrated to be required by fish. *Deficiency is associated with deficiency symptoms and in most cases, growth is a poor indicator to mineral status.

(Table 1). These factors have to be clearly defined in experiments, for purposes of standardization. If an optimum condition is known to exist, the experiment should be set-up to provide for it. In investigations involving nutrients known to have a marked interaction with another, a factorial design involving both parameters is preferred. Nutrient interactions result in the alternation of the requirement of a nutrient by a second, often closely related nutrient or in the exacerbation of the deficiency of one nutrient by another (Table 4). If a factorial design is not possible, the level of the interacting nutrient should be specified.

When scanty information is available regarding the nutrient requirement of a cultured fish, it is best to start out with nutrients that are limiting and are a major cost in diets. Studies on the nutrients, of which a great difference in requirements has been observed in other species, would be very useful. Protein is one of the most expensive dietary components, especially for fish which have high dietary protein requirements compared to terrestrial animals (Rumsey 1981). Thus, the number of studies conducted to determine protein requirement is enormous, but insufficient attention has been given to major factors that affect it; protein quality, dietary energy from the carbohydrate and food intake. On the other hand, nutrients needed in trace quantities

Table 4. Nutrient interactions demonstrated in fish.

Essential Nutrient	Interacting Nutrient	Nutrient Relationship	Observed response in fish	Reference
Protein	Dietary energy	Fats and carbohydrates can spare protein as a source of energy	Increase in protein requirement at low dietary energy	NRC 1981; NRC 1983
Essential fatty acid (EFA)	Lipid	EFA requirement is dependent on total lipid level	Increase in EFA requirement at high lipid level	Takeuchi and Watanabe 1977
Methionine (Met)	Cystin (cys)	Met is a precursor of Cys so Cys can spare some Met	Met requirement is lower in the presence of Cys	Harding et al. 1977 Rumsey et al. 1983
Phenylalanine (Phe)	Tyrosine (Tyr)	Phe is a precursor of Tyr so Tyr can spare some Phe	Phe requirement is lower in the presence of Tyr	Robinson et al. 1980
Lysine (Lys)/ Arginine (Arg)	Arginine (Arg)/ Lysine (Lys)	Lys and Arg are basic and thus, cationic	Arg exacerbated the growth depression due to excess Lys	Chiu et al. 1987a
Leucine (Leu)	Isoleucine (Ile) and Valine (Val)	All here are branch-chain amino acids	Excess leucine depressed growth of fish fed diets deficient in Ile and Val	Robinson et al. 1984
Calcium (Ca)	Phosphorus (P)	High levels of P affect Ca absorption; need to consider Ca:P ratio	Ca requirement, with higher dietary P	Sakamoto and Yone 1973
Magnesium (Mg)	Ca	Reduction of Mg availability	Increase in Mg requirement, poor growth, renal calcinosis	Cowey et al. 1977
Zinc (Zn)	Tricalphos phytic acid	Reduction of Zn availability	Increase in Zn requirement, poor growth, cataracts	Ketola 1979 Satoh et al. 1987a Richardson et al. 1985
Ca and P	Counteron on compound to which they are bound	Difference in binding and solubility	Difference in availability, response varies with fish species	Nakamura and Yamada 1980 NRC 1983
Ca	Vitamin D	Stimulation of binding	Vitamin D deficiency may result in Ca deficiency	Lovell and Li 1978
α-Tocopherol	Lipid PUFA	α-tocopherol acts as lipid-soluble intra-cellular anti-oxidant	α-tocopherol requirement increases with increasing dietary lipid, particularly unsaturated fat	Watanabe et al. 1977 Satoh et al. 1987b

may not be a major cost factor and may be extremely difficult to evaluate because of the difficulty of producing diets that are adequately low in the nutrient but contain adequate levels of all other nutrients to attain maximum growth. Apparently there is a need to define common reference diets for the determination of the requirement for specific nutrients.

References

Andrews, J.W. and J.W. Page. 1975. The effects of frequency of feeding on culture of catfish. Transactions of the American Fisheries Society 104: 317-321.

Andrews, J.W., J.W. Page and M.W. Murray. 1977. Supplementation of a semi-purified casein diet for catfish with free amino acids and gelatin. Journal of Nutrition 107: 1151-1156.

AOAC (Association of Official Analytical Chemists). 1984. Official methods of analysis. 14th Edition S. Williams (ed.), Arlington, Virginia, 1141 p.

Aoe, H., I. Matsudo, I. Abe, T. Saito, T. Toyoda and K. Kitamura. 1970. Nutrition of protein in young carp. 1. Nutritive value of free amino acids. Nippon Suisan Gakkaishi 26: 407-413.

Austreng, E. 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents of different segments of the gastrointestinal tract. Aquaculture 13: 265-272.

Baker, D.H. 1986. Problems and pitfalls in animal experiments designed to establish dietary requirements for essential nutrients. Journal of Nutrition 116: 2339-2349.

Bell, J.G. and C.B. Cowey. 1985. Roles of vitamin E and selenium in the prevention of pathologies related to fatty acid oxidation in salmonids. *In* C.B. Cowey, A.M. Mackie and J.G. Bell (eds.) Nutrition and feeding in fish, pp. 333-347. New York, Academic Press.

Brett, J.R. 1971. Satiation time, appetite and maximum food intake of sockeye salmon *(Oncorhynchus nerka)*. Journal of the Fisheries Research Board of Canada 32: 409-415.

Brett, J.R. 1979. Environmental factors and growth. *In* W.S. Hoar, D.J. Randall and J.R. Brett (eds.) Fish physiology, Vol. VII, p. 599-675. Academic Press, New York.

Carr, W.E.S. 1982. Chemical stimulation of feeding behavior. *In* T.S. Hara (ed.) Chemoreception in fishes, p. 259-273. Elsevier Scientific Publication, New York.

Castell, J.D. and K. Tiews (eds.). 1980. Report of the EIFAC, IUNS and ICES Working Group on the standardization of methodology of fish nutrition research. Hamburg, Federal Republic of Germany, 21-23 March, 1979. EIFAC Tech. Paper (36). 24 p.

Charles, P.M., S.M. Sebastian, M.C.V. Ray and M.P. Marian. 1984. Effects of feeding frequency on growth and food conversion of *Cyprinus carpio* fry. Aquaculture 40: 293-300.

Chiou, J.D. and C. Ogino. 1975. Digestibility of starch in carp. Nippon Suisan Gakkaishi 41: 465-466.

Chiu, Y.N., R.E. Austin and G.L. Rumsey. 1987a. Interactions among dietary minerals, arginine and lysine in rainbow trout *(Salmo gairdneri)*. Fish Physiology and Biochemistry 4: 45-55.

Chiu, Y.N., N.S. Sumagaysay and M.A.S. Sastrillo. 1987b. Effect of feeding frequency and feeding rate on the growth and feed efficiency of milkfish *Chanos chanos* Forsskal juveniles. Asian Fisheries Science 1: 27-31.

Chiu, Y.N., R.E. Austin and G.L. Rumsey. 1988. Effect of feeding level and dietary electrolytes on arginine requirement in rainbow trout *(Salmo gairdneri)*. Aquaculture 69: 79-91.

Cho, C.Y. and S.J. Kaushik. 1985. Effects of protein intake on metabolizable and net energy values of fish diets. *In* C.B. Cowey, A.M. Mackie and J.G. Bell (eds.) Nutrition and feeding in fish, p. 95-117. Academic Press, New York.

Cho, C.Y. and S.J. Slinger and H.S. Bayley. 1976. Influence of level and type of dietary protein, and of level of feeding on feed utilization of rainbow trout. Journal of Nutrition 106: 1547-1556.

Chua, T.E. and S.K. Teng. 1978. Effects of feeding frequency on the growth of young estuarine grouper, *Epinephalus tauvina* (Forsskal), cultured in floating cages. Aquaculture 14: 31-47.

Cochran, W.G. and G.M. Cox. 1957. Experimental designs, Second Ed. New York, John Wiley and Sons, 611 p.

Cowey, C.B. 1976. Use of synthetic diets and biochemical criteria in the assessment of nutrient requirements of fish. Journal of the Fisheries Research Board of Canada 33: 1040-1045.

Cowey, C.F. 1979. Nutrition. *In* W.S. Hoar, D.J. Randall and J.R. Brett (eds.) Fish physiology Vol. VIII, pp. 1-69. Academic Press, New York.

Cowey, C.B., D. Knox, J.W. Adron, S. George and B. Pirie. 1977. The production of renal calcinosis by magnesium deficiency in rainbow trout *(Salmo gairdneri)*. British Journal of Nutrition 38: 127-135.

De Long, O.C., J.E. Halver and E.T. Mertz. 1958. Nutrition of salmonid fishes. VI. Protein requirements of chinook salmon at two water temperatures. Journal of Nutrition 65: 589-599.

Deshimaru, O., K. Katsunobu and Y. Yone. 1982. Purified basal diet for yellow tail. Nippon Suisan Gakkaishi 48: 1151-1154.

De Silva, S.S., R.M. Gunasekara and C. Keembiyahetty. 1986. Optimum ration and feeding frequency in *Oreochromis niloticus* young. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum, p. 559-564. Asian Fisheries Society, Manila, Philippines.

Fry, F.E.J. 1971. The effect of environmental factors on the physiology of fish. *In* W.S. Hoar and D.J. Randall (eds.) Fish physiology. Vol. 6, p. 1-98. Academic Press, New York.

Grayton, B.O. and F.W.H. Beamish. 1977. Effect of feeding frequency on food intake, growth and body composition of rainbow trout *(Salmo gairdneri)*. Aquaculture 11: 159-172.

Halver, J.E. 1957. Nutrition of salmonid fishes. IV. An amino acid test diet for chinook salmon. Journal of Nutrition 62: 245-254.

Halver, J.E., D.C. Delong and E.T. Mertz. 1957. Nutrition of salmonid fishes. V. Classification of essential amino acids for chinook salmon. Journal of Nutrition 63: 95-105.

Halver, J.E. 1985. Recent advances in vitamin nutrition and metabolism in fish. *In* C.B. Cowey, A.M. Mackie and J.G. Bell (eds.) Nutrition and feeding in fish, pp. 414-429. Academic Press, New York.

Harding, D.E., O.W. Allen and R.P. Wilson. 1977. Sulfur amino acid requirement of channel catfish. L-methionine and L-cystine. Journal of Nutrition 107: 2031-2035.

Hogendoorn, H. 1981. Controlled propagation of the African catfish, *Clarias lazera* (C. and V.). IV. Effect of feeding regime in fingerling culture. Aquaculture 24: 123-131.

Jauncey, K. 1982. The effect of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapias *(Sarotherodon mossambicus)*. Aquaculture 27: 43-54.

Jobling, M. 1983. A short review and critique of methodology used in fish growth and nutrition studies. Journal of Fish Biology 23: 685-703.

Kanazawa, A. 1985. Essential fatty acid and lipid requirement of fish. *In* C.B. Cowey, A.M. Mackie and J.G. Bells (eds.) Nutrition and feeding in fish, p. 281-298. Academic Press, New York.

Kaushik, S. 1979. Application of biochemical method for the estimation of amino acid needs in fish: quantitative arginine requirements of rainbow trout in different salinities. *In* J.E. Halver and K. Tiews (eds.) Finfish Nutrition and Fishfeed Technology, Vol. II, pp. 198-208. I.H. Heenemann GmbH and Co., Berlin.

Ketola, H.G. 1979. Influence of dietary zinc on cataract in rainbow trout *(Salmo gairdneri)*. Journal of Nutrition 109: 965-969.

Lovell, R.T. and Y.P. Li. 1978. Essentiality of vitamin D in the diets of channel catfish *(Ictalurus punctatus)*. Transactions of the American Fisheries Society 107: 809-811.

Marian, M.P., A.G. Ponniah, R. Pitchairaj and M. Narayanan. 1982. Effect of feeding frequency on surfacing activity and growth in the air breathing fish *Heteropneustes fossilis*. Aquaculture 26: 237-244.

Mercer, L.P., N.W. Flodin and P.H. Morgan. 1978. New methods for comparing the biological efficiency of alternative nutrient sources. Journal of Nutrition 108: 1244-1249.

Nakamura, Y. and J. Yamada. 1980. Effects of dietary calcium level Ca/P ratios and calcium components on the calcium absorption rate in carp. Bulletin of the Faculty of Fisheries, Hokkaido University 31: 277-282.

National Research Council. 1981. Nutrient requirements of coldwater fishes. National Academy of Sciences 16, 63 p.

National Research Council. 1983. Nutrient requirement of warmwater fishes. National Academy of Sciences 16, 102 p.

Nose, T., S. Arai, D. Lee and Y. Hashimoto. 1974. A note on amino acid essential for growth of young carp. Nippon Suisan Gakkaishi 40: 903-908.

Ogino, C. 1980. Protein requirement of carp and rainbow trout. Nippon Suisan Gakkaishi 40: 383-388.

Page, J.W. and J.W. Andrews. 1973. Interactions of dietary levels of protein and energy on channel catfish *(Ictalurus punctatus)*. Journal of Nutrition 103: 1339-1346.

Poston, H.A. and J.L. Rumsey. 1983. Factors affecting dietary requirement and deficiency signs of L-tryptophan in rainbow trout. Journal of Nutrition 113: 2568-2577.

Richardson, N., D.A. Higgs, R.M. Beames and J.R. McBridge. 1985. Influence of dietary calcium, phosphorus, zinc and sodium phytate level on cataract incidence, growth and histopathology of juvenile chinook salmon *(Onchorynchus tshawytscha)*. Journal of Nutrition 115: 553-567.

Robbins, K.R., H.W. Norton and D.H. Baker. 1979. Estimation of nutrient requirements from growth data. Journal of Nutrition 109: 1710-1714.

Robinson, E.H., R.P. Wilson and W.E. Poe. 1980. Total aromatic amino acid requirements, phenylalanine requirement and tyrosine replacement value for fingerling channel catfish. Journal of Nutrition 110: 1805-1812.

Robinson, E.H., W.E. Poe and R.P. Wilson. 1984. Effects of feeding diets containing on imbalance of branched-chain amino acids on fingerling channel catfish. Aquaculture 37: 51-62.

Rumsey, G.L. 1980. Antioxidants in compounded feeds. *In* T.V.R. Pillay (ed.) Fish Feed Technology. Aquaculture development and Coordination Programme ADCP/REP/80/11, pp. 177-182. FAO, Rome.

Rumsey, G.L. 1981. Why does the salmonid require a high protein diet? Salmonid 5: 20-24.

Rumsey, G.L., J.W. Page and M.L. Scott. 1983. Methionine and cystine requirements of rainbow trout. Progressive Fish-Culturist 45: 139-143.

Sakamoto, S. and Y. Yone. 1973. Effect of dietary calcium/phosphorus ratio upon growth, feed efficiency and blood serum Ca and P level in red sea bream. Nippon Suisan Gakkaishi 39: 343-348.

Sampath, K. 1984. Preliminary report on the effects of feeding frequency in *Channa striatus*. Aquaculture 40: 301-306.

Satoh, S., K. Tabata, K. Izume, T. Takeuchi and T. Watanabe. 1987a. Effect of dietary tricalcium phosphate on availability of zinc to rainbow trout. Nippon Suisan Gakkaishi 53: 119-1205.

Satoh, S., T. Takeuchi and T. Watanabe. 1987b. Requirement of *Tilapia* for α-Tocopherol. Nippon Suisan Gakkaishi 53: 119-124.

Singh, R.P. and A.K. Srivastava. 1984. Effect of feeding frequency on the growth, consumption and gross conversion efficiency in siluroid catfish *Heteropneustes fossilis* (Bloch). Bamidgeh 36: 80-90.

Slinger, S.J., A. Razzaque and C.Y. Cho. 1979. Effect of feed processing and leaching on losses of certain vitamins in fish diets. *In* J.E. Halver and K. Tiews (eds.) Finfish Nutrition and Fishfeed Technology. Vol. II, pp. 425-434. I.H. Heenemann GmbH and Co., Berlin.

Smith, R.R. 1971. A method for measuring digestibility and metabolizable energy of fish feeds. Progressive Fish-Culturist 33: 132-134.

Takeuchi, T. and T. Watanabe. 1977. Dietary levels of methyl laurate and essential fatty acid requirement of rainbow trout. Nippon Suisan Gakkaishi 43: 893-898.

Walton, M.J., C.V. Cowey, R.M. Coloso and J.W. Adron. 1986. Dietary requirement of rainbow trout for tryptophan, lysine and arginine determined by growth and biochemical measurements. Fish Physiology and Biochemistry 2: 161-169.

Watanabe, T., T. Takeuchi, M. Matsui, C. Ogino and T. Kawabata. 1977. Effect of α-tocopherol deficiency on carp. VII. The relationship between dietary levels of linoleate and α-tocopherol requirement. Nippon Suisan Gakkaishi 43: 935-946.

Wilson, R.P., D.E. Harding and D.L. Garling, Jr. 1977. Effect of dietary pH on amino acid utilization and lysine requirement of fingerling channel catfish. Journal of Nutrition 107: 166-170.

Yamada, S., Y. Tanaka and T. Katayama. 1981. Feeding experiments with carp fry fed on amino acid by increasing the number of feeding per day. Nippon Suisan Gakkaishi 47: 1247.

Zeitoun, I.H., J.E. Halver, D.E. Ullrey and P.I. Tack. 1973. Influence of salinity on protein requirement of rainbow trout *(Salmo gairdneri)* fingerlings. Journal of the Fisheries Research Board of Canada 30: 1867-1873.

Zeitoun, I.H., D.E. Ullrey, W.T. Magee, J.L. Gill and W.G. Bergen. 1976. Quantifying nutrient requirement of fish. Journal of the Fisheries Research Board of Canada 33: 167-172.

Methodologies for Vitamin Requirement Studies

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An overview of the methodologies used for the study of the vitamin requirements of fish is provided, with an emphasis on the composition of test diets and on the evaluation of results.

Vitamins are organic compounds required in small quantities in animal diets for normal growth or function. The group was named Vitamins by Funk who thought they were the chemical compound amines vital for life. The name vitamin comes from "vital amines". It is known that vitamins are essential to all fish species. All vitamins are not essential to all fish species, and a vitamin becomes essential when it cannot be synthesized in the body.

Qualitative vitamin requirement experiments generally aim at determining the essential vitamins needed by the fish are limited by the experimental capacity. The experimental design normally uses one-way classification where the vitamins studied are classified as treatments and a complete diet serves as a control diet. In quantitative vitamin requirement experiments a two-factorial design may be recommended; for example testing of two vitamins, each with 4 levels, resulting in 2 x 4 = 8 treatments, the levels should be graded to cover the expected-requirement level.

Number of Fish and Replications for Treatment

A general recommendation on the number of fish/replicates cannot be given since it depends on experimental conditions such as size of tank, water exchange rate, size of fish and duration of feeding trial amongst others. Nevertheless, utilization of a small number of fish/replicates may invalidate the conclusions for practical conditions and this should be taken into account in the design of the experiment.

The number of replicates/treatments depends on the degree of differences expected between the treatments. For large-scale experiments with 50-180 fish, 2 replicates may suffice if a big difference between the treatments is expected, but 3-4 replicates are desirable. Replication is of outstanding importance because it enables the experimenter to perform reliable statistical analysis.

Statistical Evaluation

Experiments must be designed to allow statistical analysis. No experiment should be considered as completed before statistical analysis is performed. For the majority of fish nutrition experiments, due to the limited experimental capacity, range tests such as Duncan's multiple range test or orthogonal comparison seem more suitable than distribution tests. However, it is desirable to give the standard errors of the means whenever possible.

Experimental Diet Formulation

The control diet should cover all known nutrient requirements with a certain margin of safety. In the experimental diets only the factor to be tested should be varied. For the species for which nutrient requirements are unknown the experimental diet can be formulated based on data such as the following:

i. Modifications of dietary formulae derived for fish, such as salmon and channel catfish, can be employed to develop suitable test diets for seabass and *Clarias* sp.

ii. The composition of natural food consumed and the carcass composition of healthy fish provides a certain amount of information about the nutrition requirements of fish.

Using the information obtained from (i) and/or (ii) experimental diets for initial experimentation can be formulated. As a starting point, the test diet (H-440) which the author refers to is that used by Halver (1957) (Tables 1 and 2).

Table 1. Composition of vitamin test diet for salmon and red sea bream (MDF-molecular distilled fish oil).

Ingredients	H-440[a] (g)	YR-1[c] (g)	H-440[b] (g)
Vitamin-free casein	38	52	38
Gelatin	12	11	12
Corn Oil	6	-	-
Tristearin	-	-	6
Cod liver oil	3	-	-
MDF	-	-	3
Pollack residual oil	-	9	-
White dextrin	28	8	28
α-cellulose	8	2.3866	8
Vitamin Mix[d]	1	0.6134	1
Mineral Mix[e]	4	8	4
L-Phe	-	0.6	-
L-Arg. HCl	-	1.3	-
L-Cys	-	0.7	-
L-Try	-	0.2	-
L-His. $HCl.H_2O$	-	0.2	-
DL-Ala	-	1.3	-
L-Asp. Na	-	1.0	-
L-Lys.HCl	-	0.6	-
L-Val	-	0.7	-
Gly	-	0.4	-
Water	200	200	200

a-Composition of Halver (1957) water-soluble vitamin test diet; b-Composition of Halver (p.c.) fat-soluble vitamin test diet; c-Composition of Yone (1975) vitamin test diet; d-Composition shown in Table 2; e-Composition shown in Table 3.

Table 2. Composition of vitamin mix for test diets from Halver 1957.

	H-440 (mg/100 g feed)
Thiamin-HCl	5
Riboflavin	20
Pyridoxin-HCl	5
Choline Chloride	500
Nicotinic acid	75
Calcium pantothenate	50
Inositol	200
Biotin	0.5
Folic acid	1.5
L-ascorbic acid	100
Vitamin B_{12}	0.01
Menadione (K)	4
α-Tocopherol acetate (E)	40

Yone et al. (1971) found that Halver's (1957) test diet for salmonids was apparently not tasteful to red sea bream, *Chrysophrys major* and they attempted to modify it (Yone et al. 1974). The modifications were: (a) addition of phenylalanine and aspartic acid capable of attracting the fish, (b) replacement of corn oil with a pollack residual oil, (c) modification of the mineral mix (Table 3) and, (d) a further additional supplement of certain essential amino acids such as arginine, tryptophan and valine as shown in diet YR-1 (Table 1). Red sea bream accepted the modified diet very well, and the growth and feed efficiency were considerably improved. In their studies, YR-1 was primarily employed as the basal diet. Vitamin test diets prepared from purified ingredients (except pollack residual oil), to which all crystalline vitamins have been added are used as a basal diet to determine essentiality of water-soluble vitamins for fishes compared with vitamin deleted diets (Table 4). Cod liver oil has various levels of vitamin A and D. Therefore in fat-soluble vitamin requirement experiments vitamin A and D free oil should be used. For quantitative vitamin requirement experiments, graded levels (amounts) of deleted vitamins are added to the test vitamin-free diet and fed to the experimental fish. An example is shown in Table 5.

Table 3. Composition of the mineral mix for vitamin test diet.

Ingredient	USP No. 12 (g/100 g of mixture)	YR-1
NaCl	4.33	-
$MgSO_4.7H_2O$	13.63	-
$NaH_2PO_4.2H_2O$	8.67	30.81
KH_2PO_4	23.86	-
$Ca(H_2PO_4)_2.H_2O$	13.51	-
KCl	-	5.18
Fe-Citrate	2.95	1.49
Ca-Lactate	32.53	19.74
$AlCl_3$	0.015	0.009
KI	0.015	0.0085
CuCl	0.010	0.0055
$MnSO_4$	0.080	0.0400
$CoCl_2$	0.100	0.0525
$ZnSO_4$	0.300	0.1785
α-cellulose	-	42.486

Table 4. Composition of qualitative water soluble vitamins (Thiamine, Riboflavin and Pyridoxine) requirement test diets.

Ingredient	Diet			
	Basal (%)	-B_1 (%)	-B_2 (%)	-B_6 (%)
Vitamin free casein	38	38	38	38
Gelatin	12	12	12	12
Corn Oil	6	6	6	6
Cod liver oil	3	3	3	3
White dextrin	28	28	28	28
CMC	3	3	3	3
Cellulose	5	5	5	5
Complete Vitamin Mix	1	-	-	-
B_1-Free Vitamin Mix	-	1	-	-
B_2-Free Vitamin Mix	-	-	1	-
B_6-Free Vitamin Mix	-	-	-	1
Mineral Mix	4	4	4	4

Table 5. Composition of test diets for quantitative vitamin requirement studies (MDF-molecular distilled fish oil).

Ingredient	Diet No. (g) 1	2	3	4	5	6
Vitamin Free Casein	38	38	38	38	38	38
Gelatin	12	12	12	12	12	12
Tristearin	6	6	6	6	6	6
MDF	3	3	3	3	3	3
White dextrin	28	28	28	28	28	28
CMC	3	3	3	3	3	3
A acetate free vitamin mix	1	1	1	1	1	1
Mineral mix	4	4	4	4	4	4
Vitamin A acetate (IU)	0	50	100	200	300	500
α-cellulose			(---------- balance ----------)			

Since some fish species will not accept the semi-purified diet at the commencement of an experiment there should be an acclimatization period during which the practical-type diet is gradually replaced with the semi-purified test diet. If the test diets are still not accepted readily the practical-type diets with and without tested vitamins or graded levels of test vitamins should be used (Tables 6, 7). The requirement was found to be a function of food intake in salmon and catfish. Vitamin requirements are expressed in mg of vitamin per kg of feed (mg vit./kg feed). Theoretically, the vitamin requirements are best expressed in concentration per unit dietary energy. However, many factors (taste, texture) override the tendency of fish to eat to satisfy energy needs (Cowey 1987).

Table 6. Composition of qualitative vitamin test diet for seabass.

Ingredient	Diet No. 1 (%)	2 (%)	3 (%)	4 (%)	5 (%)
Fish meal	76.35	76.35	76.35	76.35	76.35
Mineral mix	4.00	4.00	4.00	4.00	4.00
CMC	6.00	6.00	6.00	6.00	6.00
α-starch	7.00	7.00	7.00	7.00	7.00
Fish oil	4.65	4.65	4.65	4.65	4.65
Vitamin mix	2.00	-	-	-	-
B_1-free vitamin mix	-	2.00	-	-	-
B_2-free vitamin mix	-	-	2.00	-	-
B_6-free vitamin mix	-	-	-	2.00	-
C free vitamin mix	-	-	-	-	2.00

Table 7. Composition of quantitative vitamin test diet for seabass.

Ingredient	Diet No. 1 (%)	2 (%)	3 (%)	4 (%)	5 (%)
Fish meal	76.35	76.35	76.35	76.35	76.35
Mineral mix	4.00	4.00	4.00	4.00	4.00
CMC	6.00	6.00	6.00	6.00	6.00
α-starch	7.00	7.00	7.00	7.00	7.00
Fish oil	4.65	4.65	4.65	4.65	4.65
Ascorbic acid free vitamin mix	1.40	1.40	1.40	1.40	1.40
Ascorbic acid (mg)	0.00	0.05	0.10	0.15	0.20
α-cellulose	0.60	0.55	0.50	0.45	0.40

Feeding Procedure and Frequency

Factors affecting the amount of feed consumed are temperature, fish size, water quality, pellet density, energy content of feed, nutrients in the feed, amount of feed consumed on the previous day, number of times fed per day, flavor of feed amongst others (Lovell 1977, 1979). For fish farming, feeding procedures are as important as feed formulation. Fish farmers face problems which other animal raisers do not in that uneaten or unassimilated feed uses oxygen, contaminates the environment and is hazardous to fish.

The feeding methods employed in nutritional experiments are *ad libitum* feeding or feeding a fixed amount ("set") twice daily six or seven days per week. Since separation of feed intake response and metabolic efficiency response is not possible and the feeding rates for the fish tested are not established *ad libitum* feeding may be more desirable. By feeding slowly and carefully watching as the fish eat, the experimenter can feed the fish closer to their maximum rate of consumption without overfeeding and is able to observe possible nutritional disease problems. "Set" feeding is more practical for fish species whose feeding rates are set, but might result in over feeding on the vitamin-deficient group and under feeding to the vitamin-complete group once appetites are affected by the treatment. Studies on salmon and catfish vitamin requirements are based on feeding small fish a "set" amount.

Growth, feed efficiency and size variation are affected by feeding procedure or feed intake (Table 8). Therefore for highly cannibalistic fish such as seabass *ad libitum* feeding is more suitable because high variation in size will primarily cause changes in feeding and schooling behaviour and, secondarily cause cannibalism.

Table 8. Growth and food conversion in rainbow trout given diets differing in riboflavin content and feeding (Woodward 1983).

	Feeding regime	Initial weight (g)	14 week weight (g)	Gain/ feed
Riboflavin deficient (a)	satiation	11.0	33.7	0.56
Riboflavin sufficient (b)	satiation	10.9	57.1	0.77
Riboflavin sufficient (c)	pair fed with (a)	10.9	46.3	1.11

Experimental Fish

Good genetic strains and healthy young fish with an average initial weight range from 1-5 g are preferable because they grow fast and their small size permits the use of a larger number of fish per replicate. The number of fish per experimental unit or per volume of water depends on the experimental period and the capacity of experimental system, which is related to the final biomass and water exchange rate, respectively. In seabass nutrition experiments where a water exchange rate of 1 l/min is used fish grow at a satisfactory rate from 1 g to 20 g in 8 weeks at a stocking density of approximately 1 fish per 2 l of water.

Fish should be acclimatized to the experimental feed and conditions prior to grading and random stocking in the experimental units. Treatments are randomly assigned to the experimental units by the completely random design when there is no difference in environmental effects among experimental units or by the randomized complete-block design when possible.

Experimental Units

In controlled environment experiments glass aquaria or circular fibreglass tanks located in an indoor wet laboratory could be used. Each aquarium or tank (experimental unit) is ideally equipped with an individual air and water supply at the opposite end from the water drain or with central drainage. A continuous flow through system is desirable.

Small circular fibreglass tanks are preferable to glass aquaria for many reasons: they are easier to keep clean, lasts longer and most importantly according to our observations fish grow faster.

Management

Each test diet should be fed in 3-4 replicate tanks containing 20-50 fish each. The fish should be fed twice daily, 6-7 days per week at "set" or *ad libitum*, and the amount consumed recorded daily; fish weighed fortnightly and observed for gross signs of test vitamin deficiencies (Cho et al. 1985; NRC 1983; Robinson 1984; Tacon 1985). The insides of all tanks should be scrubbed twice weekly to minimize algal, fungal and bacterial growth. Fortnightly when the fish are removed for weighing, the tanks are emptied of water and cleaned thoroughly. Fish are fed the experimental diet until deficiency signs develop, when half of the replicates or a portion of fish are separated and transferred to the complete diet. The experiment should be terminated after the growth and deficiency signs have disappeared in the latter group.

In quantitative vitamin requirement experiments data pertaining to blood enzyme activities, blood parameters, level of vitamins stored in the liver or kidney should be monitored with a view to interpreting the results. These essential parameters will be considered as judgment criteria for the level of vitamin required in each situation.

Evaluation of Vitamin Requirement Experiments

The principles of experimental evaluation depend on the design establishing experimental criteria. Growth rate, feed consumption and feed efficiency are the most important but nevertheless rather approximate parameters of feeding success are also useful. In addition, biological criteria such as liver or kidney vitamin storage are highly desirable for water-soluble vitamin experiments. Furthermore, clinical (blood parameters, enzymes) and histological assessment of the experimental fish is deemed necessary. All tests giving information about qualitative or quantitative metabolic alterations of the organism are of value for a better understanding of the influence of the feeding regimes.

Growth Rate

Measurement of body weight at the start and the end of the experiment appears insufficient. Weighing in subperiods (once every 2-3 weeks) is preferred. For small scale experiments taking the total weight of all the fish in each replicate is recommended. When the minimum dietary vitamins required for maximum growth can be estimated. Worthwhile additional information may be obtained by measuring length distribution at the end of the experiment. Feeding fish with vitamin-deficient diet may result in large variations in size at the end of an experiment. The experiment should be continued until there has been a ten or twenty fold increase in animal size. Growth rate (weight and length) is preferably recorded in absolute terms (Figs. 1 and 2).

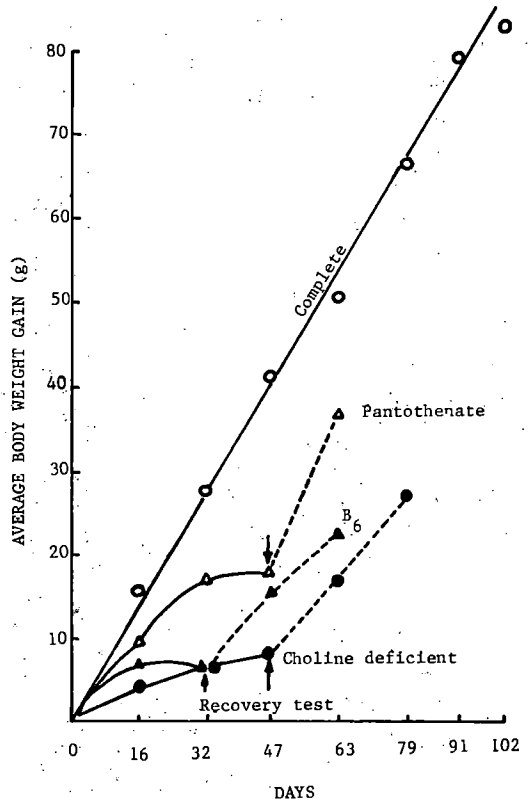

Fig. 1. Growth of fish fed diets deficient in pantothenate, choline or vitamin B_6 (Yone 1975).

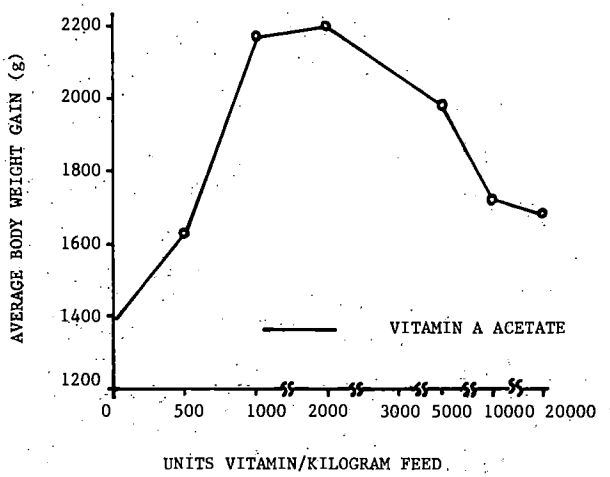

Fig. 2. Effect of vitamin A acetate on the growth of channel catfish. Growth was linear with vitamin A acetate content of the purified diets from 0 to 1,000 units/kg feed. Higher levels of vitamin suppressed growth (Dupree 1975).

Feed Intake

The feed intake must be recorded daily or for each subperiod as cumulative figures. An understanding of which vitamin effects feed intake and an optimal feeding table can be obtained from feed intake and feed efficiency information.

Gross Deficiency Signs, Mortality and Blood Parameters

Gross deficiency signs and number of dead fish are examined and recorded during daily feeding. Possible factors affecting mortality must be recorded. If the rate of mortality exceeds 5%, additional comments or examination of gross necropsy using animal tissue techniques and histochemical techniques and disease diagnosis are required. An explanation for increased mortality should be attempted.

Blood parameters such as hematocrit, hemoglobin, erythrocyte fragility, erythrocyte fragmentation, are made on individual fish or groups of fish on vitamin (E, C, Folic acid, B_{12}, Biotin) requirement experiments.

Feed Efficiency

Feed efficiency has traditionally been given special attention but is unfortunately often presented in an undefined form. Two terms seem to dominate the literature.

$$\text{Feed conversion ratio} = \frac{\text{Feed intake}^a}{\text{Weight gain}^b}$$

$$\text{or Feed conversion efficiency} = \frac{\text{Weight gain}^b}{\text{Feed intake}^a}$$

[a] Feed intake = feed eaten by the fish on a dry matter basis;
[b] Weight = a weight increase on wet matter basis.

As they contain the same factors in inverse relation, they are frequently mixed up. Feed conversion efficiency has been recently preferred by more researchers since it is positively correlated with growth and PER. In addition, if weight gain is zero, weight gain/feed intake is zero but feed intake/weight gain is infinite, which is difficult to interpret biologically.

Body Storage of Vitamins

Liver or kidney storage of vitamins is affected by the dietary vitamin level (Fig. 3). Water-soluble vitamins are stored in the liver up to the point of saturation; then the excess is excreted through urine, except for vitamin C where liver storage does not level off but accumulates at a very low rate. High levels of vitamin C in the diet and in the liver are required to provide resistance to diseases. Therefore the water-soluble vitamin requirement level can be estimated from the lowest dietary vitamin level which gives the highest or acceptable tissue storage.

This value is relatively higher than the level of dietary vitamins required for maximum vitamin-related enzyme activity and for maximum growth.

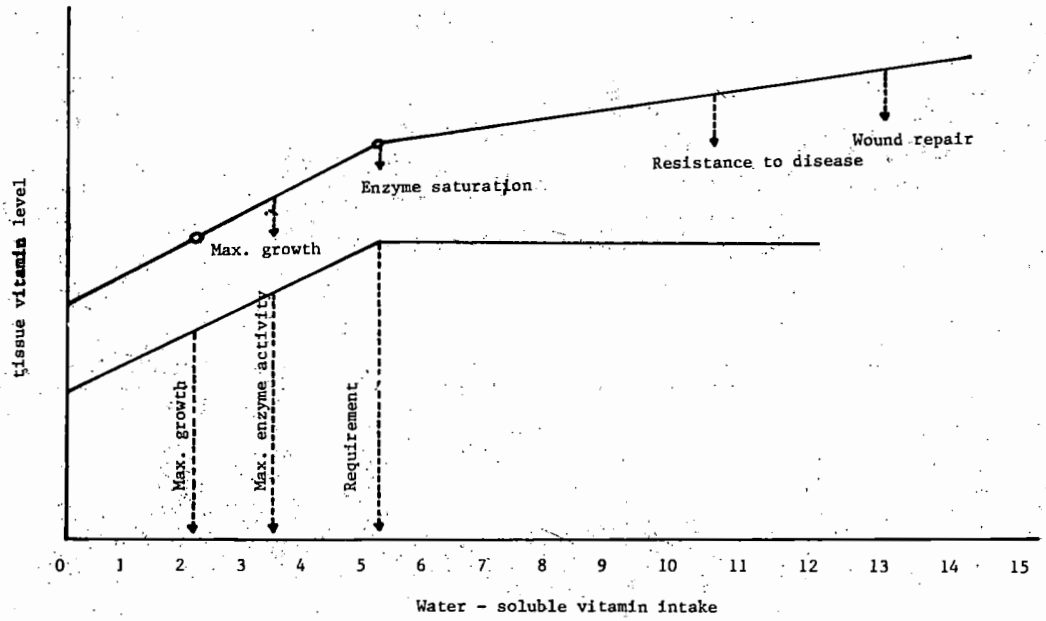

Fig. 3. Tissue vitamins storage level in fish fed grade level of dietary water soluble vitamin (Halver, pers. comm.)

0 - 0 vitamin C
- vitamin B

There is debate over what is the most appropriate definition of the dietary vitamin requirement of fish. Some nutritionists believe that since vitamins are expensive only the minimum amount required for maximum growth should be incorporated into feeds. Halver (1980) believes that sufficient quantities of vitamins should be incorporated into feeds to allow maximum liver storage.

Liver vitamin levels are determined by microbiological or chemical assay or high-pressure liquid chromotography at adequate intervals, i.e., at start middle, break point and at the end of the experiment.

Vitamin Related Enzymes

Maximal activities of vitamin-dependent enzymes in serum, liver or muscles of fish which were fed graded level of test vitamin diets should be determined by pooling the fish collected from each experimental unit. This clinical chemistry assay should be carried out at adequate experimental intervals, with enzyme tests for specific vitamins (Table 9). For example, Erythrocyte transketolase; glutathione reductase and/or liver D-amino acid oxidase; muscle aspartate amino transperase; Acetyl coenzyme A; blood serum alkaline phosphatase; and liver pyruvate carboxylase for B_1; B_2; B_6; Pantothenic; ascorbic acid; and biotin; respectively.

Table 9. Effect of graded levels of dietary riboflavin on rainbow trout (Cowey 1987).

Dietary riboflavin (mg/kg)	Initial weight (g)	Final weight (g)	liver flavin (mg/kg)	DAOO activity unit/q liver x 10^2	
				-FAD	+FAD
0.57	60.5	189.8*	4.40*	0.16*	0.64*
1.62	56.8	220.6	9.92	4.15	6.68**
3.22	60.4	221.4	11.35	9.63	11.00
5.81	57.0	221.1	11.81	7.68	9.86

Requirements

The dietary vitamin level which gives the maximal activity of vitamin dependent enzymes is the amount required not only for maximum growth but also for good health condition. The dietary vitamin level required for maximum liver storage is relatively higher (the highest) since vitamins are stored in the liver when more than the amount for immediate metabolic needs is present. This level of vitamins is recommended when the dietary intake is low or when the animal is threatened by stress and disease or when a rapid increase in metabolic activity occurs.

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References

Cho, C.Y., C.B. Cowey and T. Watanabe, Editors. 1985. Finfish nutrition in Asia. Methodological Approaches to Research and Development. International Development Research Centre, Ottawa, Canada, 154 p.

Cowey, C. 1987. Recommended dietary vitamin requirements, relevance and biochemical significance. Presented at International Symposium on Feeding and Nutrition in Fish, Bergen, Norway. (in press)

Dupree, H.K. 1975. Studies on nutrition and feeds of warmwater fish. *In* K.S. Price, W.N. Shaw and K.S. Danberg (eds.) Proceedings of the First International Conference on Aquaculture Nutrition, pp. 60-74. Lewes/Rehoboth, University of Delaware.

Halver J.E. 1957. Nutrition of salmonid fishes. III. Water soluble vitamin requirements of chinook salmon. Journal of Nutrition 62: 225-243.

Halver, J.E. 1980. The vitamins. *In* Fish Feed Technology. Rome, UNDP/FAO ADCP/REP/80/11: 65-103.

Lovell, R.T. 1977. Feeding practices. *In* R.R. Stickney and R.T. Lovell (eds.) Nutrition and feeding of channel catfish. Southern Cooperative Series Bulletin No. 218, pp. 50-55. Auburn University.

Lovell, R.T. 1979. Factors affecting voluntary food consumption by channel catfish. Proceedings of the Annual Conference of Southeast Association of Fish and Wildlife Agency 33, 563-571.

National Research Council (NRC). 1983. Nutrient requirement of warmwater fishes and shellfishes. Washington, D.C., National Academy Press, 102 p.

Robinson, E.H. 1984. Vitamin requirement. *In* E.M. Robinson and R.T. Lovell (eds.) Nutrition and feeding of channel catfish. Southern Cooperative Services Bulletin No. 296, pp. 21-25.

Tacon, A.G.J. 1985. Nutritional fish pathology. Rome UNDP/FAO ADCP/REP/85/22: 33 p.

Woodward, B. 1983. Sensitivity of hepatic D-amino acid oxidases and glutathione reductase to the riboflavin status of the rainbow trout *(Salmo gairdneri)*. Aquaculture 34: 193-201.

Yone, Y., M. Furuichi and K. Shitanda. 1971. Studies on the nutrition of red seabream. III. Nutrient value and optimum content of lipids in diet. Report of the Fisheries Research Laboratory, Kyushu University (Japan) 1: 49-60.

Yone Y., S. Sakamoto and M. Furuichi. 1974. Studies on nutrition of red sea bream-IX. The basal diet for nutrition studies. Report of the Fisheries Research Laboratory, Kyushu University 2: 13-24.

Yone, Y. 1975. Nutrition studies on red sea bream. *In* K.S. Price, W.N. Shaw and K.S. Danbert (eds.) Proceedings of the First International Conference on Aquaculture Nutrition, pp. 34-59. Lewes/Rehoboth, University of Delaware.

Pond Experiment Methodododology

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Aquaculture as an art began centuries ago in China but as a science is less than a hundred years old. Pond based aquaculture research is even more recent, starting approximately 50 years ago. Aquacultural research in ponds is thus in its infancy compared to agricultural research in which the design, execution and evaluation of experiments are relatively well understood. Production techniques employed in agronomy and animal husbandry have largely been standardized. In contrast, pond experimentation remains to be standardized with different workers seldom conducting experiments in a comparable manner. Different confounded components in the treatments are therefore, almost inevitable even if basic experimental designs are the same.

A substantial amount of information has been collected over the past 100 years in agronomy and animal husbandry but the data base for aquaculture remains weak, particularly in the tropics. Comparatively little is known about the two major components fish and the environment, and their interaction. To reach the level of competency of agricultural research, aquacultural scientists need to conduct more basic research about the interactions and relationships of fish-fish, fish-environment and environmental changes to generate a sufficiently large information base from which the rapidly developing aquaculture industry can extract details for its needs. The deficiency in standardized methodology has long been recognized and the need for the formulation of a set of standards in designing, operating and evaluating aquacultural (mainly pond) research was identified more than twenty years ago at the FAO World Symposium on Warmwater Pond Fish Culture held in Rome in 1966 (Prowse 1968; Alikunhi 1968; Swingle 1968).

The purpose of this paper is to discuss fish pond experiment methodology with emphasis on specific problems normally encountered in the operation of such experiments.

Basic Experimental Design

Aquacultural research seeks answers to key questions in production which could lead to significant changes and improvements in existing aquacultural practices. The key questions to be answered are generally expressed as a statement or hypothesis that has to be verified or

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disproved through experimentation. Hypothesis are usually suggested by past experience and observations, and at times by theoretical considerations (Gomez and Gomez 1984). Experimental methodology has the following steps:

(a) Formulation of a hypothesis
(b) Planning an experiment to objectively test the hypothesis
(c) Careful observation and collection of data
(d) Interpretation of experimental results to confirm, reject or alter the hypothesis
(e) Recycling the procedure until a suitable explanation or solution is achieved.

A well planned experiment should be simple. Measurements of parameters should be done with an appropriate degree of precision without systematic error, and should provide an adequate range of validity of conclusions and a provision for calculation of the degree of uncertainty (Cox 1966).

The following steps should be considered in planning an experiment (Little and Hills 1978):

1. Definition of problem
2. Statement of objective
3. Selection of treatments
4. Selection of experimental materials
5. Selection of experimental design
6. Selection of the unit for observation and the number of replications
7. Control of the effects of adjacent units on each other
8. Consideration of data to be collected
9. Outline of statistical analyses
10. Conduct of the experiment
11. Analysis the data and interpretation of the result
12. Preparation of the report

The selection of an appropriate experimental design depends on the objective of the experiment. Some basic equations such as the species of fish and the types of feeds or fertilizers to use depend on the culture system to be studied. However, the experimenter should be familiar with the subject matter and be observant during the experiment for unexpected results. The experimenter should be aware of possible solutions to the specific question as well as others that may be suggested during the course of the experiment. Above all the scientist should have an open mind, devoid of personal bias towards a certain expected or desirable result. Personal bias in scientific experimentation can be reduced by the establishment of not one but a series of initial hypotheses.

Experimental Variation

Widely variable production among replicates of a single treatment is a common occurrence in aquaculture. Some variability may be due to human experimental error in conducting the experiment, but biological material is variable in itself and in its response to the environment. The variation due to chance discussed above needs to be separated from that due to treatment effects in the experiments.

To overcome the problems which arise from variability, three basic principles need to be incorporated into the experiment: (a) control (b) replication and (c) randomization (Roberts 1983).

Control

The word control can be used in two contexts in experimentation. Firstly, it can mean the standard or normal treatment of the experiment, the norm (an "untreated control") against which, the other treatment (a deliberate variation of the experimental conditions which is to be investigated) is to be compared. An untreated control is essential to provide an internal standard of performance against which the novel treatments can be compared, otherwise the latter can only be compared with vague concepts such as "typical" or an "average" performance from other separate experiments in which experimental conditions may have varied.

The second meaning of control is in the sense of control over experimental variability such as carrying out the experiment in uniform conditions, by a systematic approach to experimental procedures and by using uniform stock. Experimental fish should be of the same breed, the same age and weight, same sex and from the same origin, as far as possible. However, the search for uniformity of the stock fish should not be too rigidly followed, e.g., highly inbred stock may be uniform but may be so different in respect from commercial stock that their reaction to accuracy would have limited applicability (Roberts 1983).

A dilemma frequently faced by the scientist in pond experimentation is that the environmental conditions change to such an extent in some treatments during the experiment, usually in response to increasing eutrophication, that fish mortality occurs. The desire to interfere with the initial treatment to "save the fish" should be resisted. Some treatments need to lead to suboptimal growth, or even mortality, to enable the fish production response to a wide range of treatments to be properly ascertained.

Replication

Replication, or the repetition of treatments on a number of subjects has two distinct benefits. Firstly, it has the effect of reducing the inherent variability, thereby improving the expected accuracy of the experiment. For example, a sample of 5 fish is taken from a population and their average weight taken. This can be repeated for a number of samples and it will be observed that the averages show less variation than do the separate members. Secondly, it enables the internal variability of an experiment to be measured. It is necessary to assess how extensive the effects of chance may be to determine if an experimental effect did not occur by chance.

This leads to the question most asked by aquacultural scientists, how many replicates are required? It is possible to calculate the number of replicates required if the following are known: (a) the size of difference (between means of treatments) which is to be detected, (b) the size of the standard deviation for experimental error in the unit concerned or the coefficient of variation where the standard deviation is expressed as a percentage of the mean (S.D./mean x 100%), and (c) the level of probability of detection of the difference, if any. Roberts (1983) derived a table from Davies (1954) which gives the replication appropriate to the variation of the named quantities (Table 1). Both the required difference and the standard deviation are expressed as percentages of the mean. Typical coefficients of variation derived from experiments with different species of livestock, conducted over many years, are available for livestock nutrition studies to calculate the required replication. Therefore, prior information on experimental error is important in determining the degree of replication required. However, it is difficult in a new or relatively new area such as aquacultural pond research to obtain this estimate of variation in advance.

However, a considerable amount of information on experimental error in aquaculture has accumulated through the years at Auburn University (Tables 2 and 3; Shell 1983). Although

Table 1. Number of replicates required to detect a difference between treatments (significance level 0.05) (from Roberts 1983).

Difference to be detected (expressed as % of mean)	Probability of detection 0.9	0.8	0.5
Coefficient of variation 2%			
2%	23	17	9
3%	11	9	5
4%	7	6	4
5%	5	4	
6%	4	4	
7%	4	3	
8%	3		
Coefficient of variation 4%			
2%	86	64	32
3%	39	29	15
4%	23	17	9
5%	16	12	7
6%	11	9	5
7%	8	6	4
9%	6	5	
10%	5	4	
11%	4	4	
12%	4	4	
15%	3		
Coefficient of variation 6%			
2%			87
3%	86	64	32
4%	44	34	17
5%	34	26	14
6%	23	17	9
7%	16	12	7
8%	14	11	6
9%	11	9	5
10%	9	7	4
11%	8	6	4
12%	7	6	4
15%	5	4	
20%	4	3	
25%	3		

Difference to be detected (expressed as % of mean)	Probability of detection 0.9	0.8	0.5
Coefficient of variation 8%			
2%			124
3%		100	50
4%	86	64	32
5%	60	45	23
6%	39	29	15
7%	27	21	11
8%	23	17	9
9%	19	14	8
10%	16	12	7
11%	12	10	6
12%	11	9	5
15%	7	6	4
20%	5	4	
25%	4	4	
30%	3		
Coefficient of variation 10%			
3%			87
4%		100	50
5%	86	64	32
6%	60	45	23
7%	44	34	17
8%	34	26	14
9%	27	21	11
10%	23	17	9
11%	19	14	8
12%	16	12	7
15%	11	9	5
20%	7	6	4
25%	5	4	
30%	4	4	
35%	4	3	
40%	3		

direct use of the information in other parts of the world may not be valid, it may serve as a guide for the magnitude of variation in response that might be expected. Coefficients of variation in production experiments in various types of experimental units were variable, although a majority were in a relatively narrow range with 31 of 51 of the coefficients 15% or less. Apparently, there is a degree of constancy in coefficients of variation in production experiments with fish as well as corn (Snedecor and Cochran 1967). However, coefficients obtained from earthen pond experiments varied widely within a range of 4.2 to 34.6% and were similar for all species of fish studied, which reinforces that wide variations can be observed in pond studies which makes it difficult to interpret results from such studies. Feeding seemed to result in lower coefficients in earthen ponds as compared to fertilization. The lowest coefficients were obtained in rather artificial environments such as cages or troughs where the fish were stocked at high rates and fed a nutritionally complete ration.

The actual number of replicates per treatment used in experiments can also be influenced by other non-statistical factors such as (a) the availability of sufficient number of ponds for

Table 2. Range of coefficients of variation obtained in a number of production experiments with several species of fish and in several types of experimental units (from Shell 1983).

Species of fish	Experimental unit					
	Earthen pond		Plastic pools		Cages	Troughs
	Feed	Fertilizer	Feed	Fertilizer	Feed	Feed
Channel catfish	15.1-15.7	11.5-23.3	-	56.6-81.5	3.2-5.5	1.6-6.8
White catfish *(Ictalurus catus)*	9.4		-	-	-	-
Common carp *(Cyprinus carpio)*	-	8.6-18.3 12.3-22.7	-	-	-	-
Largemouth bass *(Micropterus salmoides)* and bluegill *(Lepomis macrochirus)*	-	4.2-10.1 10.4-24.8	-	-	-	-
Java tilapia *(Tilapia mossambica)*	-	-	-	11.6-32.3	-	-
Fathead minnow *(Pimephales promelas)*	3.1-11.5	-	-	11.9-51.9	-	-

Table 3. Frequency of occurrence of coefficients of variation from 13 fish production experiments recorded in Table 2 (from Shell 1983).

Class values of coefficients	Frequency
1- 5	8
6-10	13
11-15	10
16-20	7
21-25	6
26-30	4
31-35	3

replication, (b) the logistics of operating a large number of ponds, each needing to be sampled uniformly, e.g., sampling dissolved oxygen concentrations at dawn in large numbers of ponds, and (c) the experimental design, i.e., the number of treatments designated. The CRSP (Collaborative Research Support Program) - Pond Dynamics/Aquaculture program had seven stations in various regions of the world, each having 10 or 12 ponds for experimentation (Egna et al. 1987). The first of the series of experiments, to determine baseline data on pond dynamics using one fertilizer (P_2O_5) at a fixed rate of 8 kg/ha/mo, had 10 or 12 replicates depending on the sites because there was only 1 treatment. Subsequent experiments were designed to look at varying levels of fertilizer inputs which thus increased the number of treatments and decreased the number of replicates per treatment to 2 treatments with 6 replicates (C. Kwei Lin, pers. comm).

A perusal of selected papers in the literature indicated that treatments in most experiments had only 2 or 3 replicates, with no justification given for the degree of replication used.

In view of the wide range of coefficient of variation within treatments in earthen fish ponds, which may be high on occasion, the maximum number of replicates that is logistically feasible should be chosen. Although a minimum of two replicates is necessary to calculate experimental error the usual replication of treatments in duplicate and triplicate should be considered as an

absolute minimum. The problem of inadequate availability of ponds and the need for replication is compounded with the need for factorial experiments which are essential in fish nutrition experiments with the need for different fish stocking densities with diets of varying nutritional value, and with different rates of feeding.

Randomization

Replication reduces systematic error and enables it to be estimated. Randomization of treatments to experimental units can further reduce systematic error and by special procedures such as randomized blocking and Latin squares systematic error can be reduced even further.

Experimental Units

Size

Ideally, the appropriate size of the experimental units (ponds) should approximate the size of the ponds in which the results will be utilized; i.e., commercial size ponds. However, large ponds are problematic for experimentation in terms of (a) filling with water which may take several days in series of ponds, e.g., in Malaysia (Anon. 1965) 0.4 ha ponds, the quality of the source of water may change with time, (b) difficulty in sampling of fish for growth determination and feeding rate calculations, (c) supply of water in areas where water is a premium and (d) cost of experimentation increases with pond size.

Therefore, the size of experimental ponds should be commensurate with being manageable in terms of filling with water, sampling for fish and water quality measurements, cost of the experiment including feed cost if supplementary feed is given, and draining during harvesting. Ponds should be of a size to permit the construction of adequate numbers to enable proper replication of treatments. In other words, the most appropriate size is the smallest one which permits a range of replicated treatments from which the data are representative of usually larger commercial size ponds. From a survey of the literature, it can be noted that ponds vary in size at institutes where aquaculture research is carried out:

Israel (DOR)	mainly 0.04 ha
Thailand (AIT)	mainly 0.02 ha
USA (Auburn University)	0.02 to 0.4 ha
CRSP (six regions)	0.02 to 0.1 ha

Problems may arise when data obtained from experiments conducted in ponds of varying size are converted to a common denominator, normally an extrapolation to one ha. Little research has been done to show whether such extrapolations are valid. Since replicate ponds of the same size often show results with considerable variation in experiments, ponds of different sizes undoubtedly would result in even more variation (Shell 1983).

While information concerning pond dimensions is usually given, often the volume of water can not be calculated because water depth is not provided. The volume of water may be more important than surface area in experiments in which fish are provided supplementary feed because the natural food production depends ultimately on solar radiation which is more a function of pond area. For experiments concerning benthos feeding species, the degree of slope on the bottom of ponds may be important because shallow water may be more productive than deeper water as a function of surface area, although this information is seldom provided.

Age and past use of ponds

Age of pond and the history of use are important in pond experimentation. Natural productivity in ponds is important in experiments concerned with herbivorous/omnivorous species, which comprise most species of fish farmed. Water quality and the level of natural productivity may be affected by residual fertilizer effects from past treatments if the ponds are not properly treated prior to experimentation.

Sediment built up from the previous experiment should be removed before a new experiment is commenced although this is seldom the practice. However, it is the normal procedure at AIT to remove sediment between experiments to avoid residual fertilizer effects, especially in large ponds. While sediment removal is considered to be of vital importance in pond experimentation at AIT sediment build-up might be desirable to a certain extent in commercial farms as it increases the fertility of the ponds.

Water sources

Ponds should be filled with water from the same source, and at the same time, as the nutrient content of the water may differ with source and may also change with time. Another problem related to water source is sometimes encountered when there is considerable water loss through seepage, usually in new ponds. This necessitates continual topping up with water which may change the environmental conditions with a high degree of replacement.

Weed species and predators

Competitor and predator organisms should be controlled as their entry into experimental ponds could ruin properly and carefully designed experiments. Fences of fine mesh nylon netting surround experimental ponds at AIT to keep out predatory fish such as snakehead *(Channa striata),* walking catfish (*Clarias* spp.) and climbing perch *(Anabas testudineus).* Entry of carnivorous fish particularly during the initial stages of the experiment when stocked fish are normally small, can seriously disrupt an experiment. Water should be properly screened when filling ponds to prevent entry of wild fish. Ponds should be properly drained and all extraneous organisms eliminated through poisons before the start of a new experiment.

Dense growth of aquatic macrophytes in ponds can drastically reduce the production of most fish (Huner and Dupree 1984). Macrophytes take up nutrients from the water that could be used in the production of phytoplankton, and limit the access of the fish to food organisms produced on and in the pond bottom soils. Dense vegetation interferes with the feeding of prepared rations and may contribute to lowered water quality if the fish cannot reach feed that settles on the vegetation. Aquatic macrophytes also compete with fish for space and for oxygen at night. Seining is severely hampered by vegetation and may interfere with pond draining by clogging screens and drains and by trapping fish. Another consequence of aquatic weed infestation is the increase in experimental error when the infestation is unequal and varies from pond to pond. The prime consideration in the control of vegetation is prevention. The ponds should be constructed with the smallest possible area of shallow water (less than 1 m deep) commensurate with stable dikes.

Experimental Fish

Experimental fish should be stocked at a uniform rate unless the determination of the optimum stocking rate is the object of the experiment when a range of stocking rates would be the treatments. Stocking rates should normally be similar to those used on commercial farms to enable meaningful utilization of data. Stocking rates should also be at a magnitude at which they can be managed easily in terms of sampling and harvesting, without financial constraints.

Experimental fish should have the same dietary history. This is particularly important in feeding trials using essential nutrient formulation as fish with different nutritional backgrounds may have varying amounts of nutrients such as fat soluble vitamins, fatty acids, amino acids or minerals stored in their body. The deficiency syndrome would not manifest itself until these stores were exhausted.

The initial stocking weight is of some importance in designing experiments. Too low an initial stocking weight may exert little if any effect, particularly during the early part of the experimental period. Hepher (1967) suggested that low population density in experimental ponds may explain the lack of effect of fertilization on food production of fish food organisms. Increasing food supply through fertilization will have little effect on production when fish have such a low density that they already have adequate food. Too high an initial stocking weight has the opposite effect in that the carrying capacity of the pond may be reached before the end of the experimental period and other factors such as deteriorating water quality may play a significant role.

Duration of Experiment

The duration of experiments can be varied depending on the type of research. If the research concerns some sequence of the production cycle, then the duration should approximate those normally experienced on commercial farms. Other factors may be considered, such as budget constraints, if the experiment is more basic in nature. The desired treatment effects may not appear if the experimental period is too short whereas the effects may be blurred if it is too long. For example, if the duration of the experiment is too long, those experimental fish in a treatment more conducive to growth may go into the growth plateau phase of the growth curve, whilst fish in treatments less conducive to growth may still be growing. Dupree's (1966) work on the vitamin B_{12} requirement of channel catfish illustrated the significance of the duration of the experiment in arriving at a valid conclusion (Fig. 1). Channel catfish required vitamin B_{12} but it was not apparent for 21 weeks until those fish which had not been provided with it had depleted the stored supply in their body. The need for vitamin B_{12} would not have been demonstrated had the experiments been terminated before 21 weeks.

Frequency of Sampling for Growth Calculations

Sampling is stressful to fish and changes the pond environment by stirring up sediment which affect water quality, fertility, and the production of natural food. Hence the frequency of sampling should be a compromise between minimizing fish stress and disturbance of the water column, and determination of accurate growth rates. In experiments in which supplementary feeding is provided the interval between sampling is of importance. Feeding rate normally calculated as percent of feed given per body weight of fish and the amount to be given to each

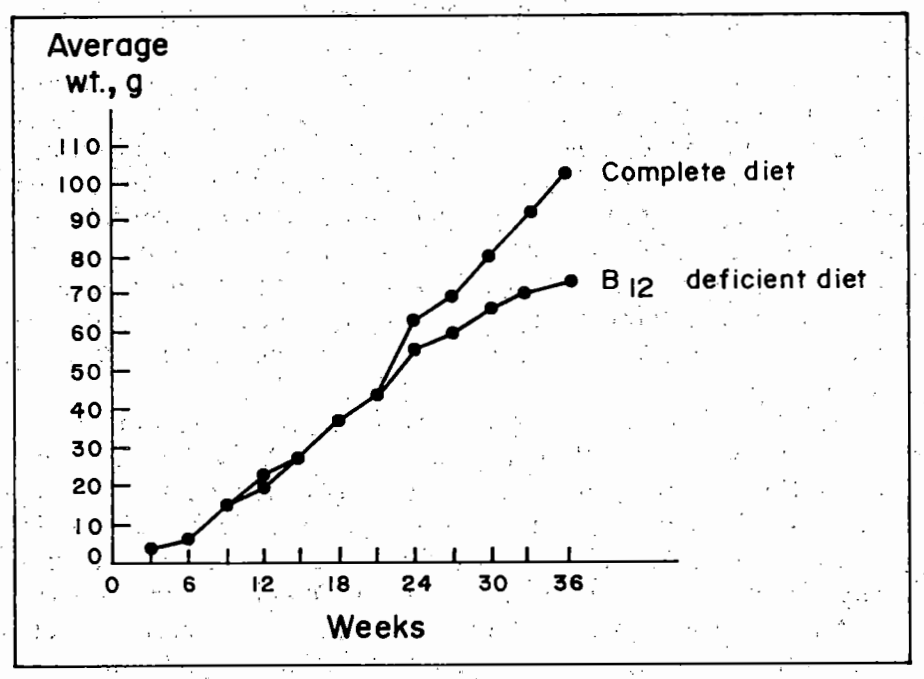

Fig. 1. Average weights of channel catfish fed a vitamin-complete diet and a vitamin B-12 deficient diet. Data from Dupree (1966) (Figure obtained from Shell 1983).

pond is thus obtained from the average weight of sampled fish extended to the biomass of fish in the pond. A lengthy interval between sampling actively growing fish results in the fish getting less than the required or intended amount towards the later part of the period as the fish have grown since the last sampling; in fact, the daily feeding rate steadily becomes underestimated. On the other hand, the feeding rate immediately after adjustment is probably wasteful, although accurate, as the fish acclimatize themselves to the sudden increase in the amount of feed.

A minimum frequency of sampling should be two weeks if supplementary feed is provided. A two-week sampling frequency could also be used in relatively short term experiments in which the experimental stocked fish are in the exponential phase of growth. Less frequent monthly sampling could be used in longer duration experiments without supplementary feed.

Sample Size

The appropriate sample size depends on the number of fish in the pond, decreasing with increasing number. A sample of 20-30 fish per pond is usually considered sufficient at AIT. Prowse (1966) suggested a sample size of 30% of the stocked fish as the minimum. However, in sampling, especially using a seine net it is not always possible to capture the desired number in one seining. Repeated seining has disadvantages of stressing the fish as well as disturbing the water column. In this context, large ponds are more difficult to sample for fish than small ponds. The capture method also needs to be standardized as fish learn to escape the nets and the sample may not be truly representative of the fish present in the pond. In addition, some species are more difficult to catch than others, e.g., tilapia is more elusive than Chinese carps. In studies on polyculture, it is difficult to get the desired number of fish of the desired species without numerous attempts at capture.

Evaluating Experiments

Methods of expressing data

1. *Standing crops*

Standing crop is defined as the fish biomass at a given instant of time, e.g., after one month. The standing crop measured when the fish biomass in a pond ceases to increase is the carrying capacity. Maximum standing crop can be defined as the maximum weight of fish which can be sustained by a pond, this weight of fish being at balance, without gain or loss in weight, with the food produced by the pond or made available to the fish (Hickling 1971). It is a most useful parameter because it represents fish biomass under a certain set of pond conditions.

2. *Net production*

Net production is defined as 'the increase in weight of fish in the experimental unit during the experiment'. It is the difference between the stocking weight and the standing crop and hence indicates by how much the biomass changed which is the basis for aquaculture. Its disadvantage is that it does not give any idea of the weight of fish supported in a pond at the time of final harvest, the standing crop at harvest.

3. *Relative weight gain*

Relative weight gain can also be called relative growth rate or percentage growth rate. It is defined as the net production divided by the initial stocking weight expressed as a percentage. It measures the degree to which initial weight is compounded during the experimental period. Relative weight gain is useful as it adjusts, to some degree, mathematically the problem of unequal stocking weights. However, it does not correct for biological effects of the differences in weights of fish stocked, i.e., conditions in a pond which contain a higher weight of stocked fish may be different from those in a pond with a lower stocking weight. The advantage of the method is that it is a measure of the dynamics of biomass production. Unfortunately, the quantity expressed is without units.

4. *Weight gain per day*

Weight gain per day is a method of expressing growth or yield, i.e., the net production over a given period of time divided by the number of days in the period. A dilemma in using this method is whether to calculate the average gain per day over the whole experimental period as defined, or to monitor changes at regular intervals as the gain per day changes with time, i.e., the overall average gain per day calculated at the end of the experiment may be quite different from the gain per day at specific time periods during the experimental period.

5. *Growth curves*

The growth curve shows the change in biomass with time and is an estimate of the weight of fish in the experimental unit plotted against time at intervals throughout the experimental period.

This method enables one to see the response to treatments at various stages of the experiment. An advantage of this method is that it is possible to determine the approximate time in which the treatments begin to exert a significant effect on growth or production, and where the effect of the treatments appear early, the experiment can be terminated early, thus reducing cost. Growth curves are essentially a series of standing crop data obtained over time. When fish are confined in relatively small experimental units such as cages and tanks where weighing of all fish is feasible, an accurate measure of the standing crop is possible. However, in ponds it is difficult to estimate standing crops periodically because the fish are only sampled which does not give a measure of mortality of stocked fish.

Miscellaneous Problems That May Be Encountered During Experiments

Reproduction of fish in the pond

Reproduction of stocked fish can lead to difficulty in evaluation of the results. As the reproduction is uncontrolled, variable number of young may be produced resulting in variable yields. As such reproduction of stocked fish should be avoided where possible. However, it would appear that only tilapia species breed in ponds within a short experimental period. In other experiments, reproduction of stocked fish is expected and may be beneficial as the overall net production increases although relative growth rate of individual fish declines. For example, experiments where the objectives are to produce fish biomass for animal feeds or for domestic consumption where the size of harvested fish is unimportant.

Changes in environmental conditions

It may be possible to observe changes in environmental conditions within experimental units during the experimental period. In static water systems supplied with fertilization and supplementary feeding, the water quality changes with time. This may culminate in fish mortality in some treatments as a result of lack of dissolved oxygen in treatments with high fertilizer and/or feed inputs.

Mortality of stocked fish

Mortality of experimental fish which is not treatment related is often observed in pond experiments. In some cases, when the mortality is monitored, the dead fish can be replaced without loss of information with fish from the same stock or with the same history of those that died. In this case, a replication of stocked fish may be stocked for replacement purposes. A major problem is how much mortality can be tolerated with the replication remaining as part of the experiment.

Another problem is if the mortality went unnoticed, as not all dead fish float and dead fish could be removed by animals before being recorded. This problem is compounded in feeding experiments as feeding rates are calculated using average fish weights obtained during regular sampling of the surviving population/community. If the mortality rate is not known, the feeding rate may be overestimated and feed given wasted which could lead to misleading poor feed utilization efficiency when in fact the feed was not even consumed (Edwards et al. 1988).

The best solution is to prevent mortality by making certain that healthy stock fish are purchased and handled with minimum care during stocking and sampling.

References

Alikunhi, K.H. 1968. Standardization of biological studies in fish culture research. Proc. of the World Symposium on Water-Water Pond Fish Culture. FAO Fish Bulletin 44(4): 430-474.

Anonymous. 1965. Tropical Fish Culture Research Institute, Malacca. Report for 1964-1965.

Cox, D.R. 1966. Planning of Experiment. Wiley International Edition, p. 308.

Dupree, H.K. 1966. Vitamins Essential for the Growth of Channel Catfish *(Ictalurus punctatus)*. U.S. Bureau of Sport Fish and Wildlife. Technical Paper 7.

Edwards, P., C. Polprasert and K.L. Wee. 1987. Resource Recovery and Health Aspects of Sanitation. AIT Research Report No. 205.

Egna, H.S., N. Brown and M. Leslie. 1987. Pond dynamics/aquaculture Collaborative Research Data Report. Volume I. Office of International Research and Development, Snell Hall, Oregon State University, U.S.A.

Gomez, K.A. and A.A. Gomez. 1984. Statistical Procedures for Agricultural Research. 2nd ed. John Wiley & Sons, Inc. New York, p. 680.

Hepher, B. 1967. Some Biological Aspects of Warm-Water Pond Management. *In* S. Gerking (ed.) The Biological Basis of Freshwater Fish Production, pp. 417-427. John Wiley and Sons, New York, N.Y.

Hickling, C.F. 1971. Fish Culture. 2nd ed. Faber and Faber, London. 317 p.

Huner, J.V. and H.K. Dupree. 1984. Pond Management. *In* H.K. Dupree and J.V. Huner (ed.) Third Report to the Fish Farmers, p. 270. U.S. Fish and Wildlife Service, Washington, D.C.

Little, T.M. and F.J. Hills. 1978. Agricultural Experimentation - Design and Analysis. John Wiley & Sons, New York, N.Y.

Prowse, G.A. 1968. Standardization of Statistical Methods in Fish Culture Research. Proc. of the World Symposium on Warm-Water Pond Fish Culture. FAO Fisheries Bulletin 44(4): 386-396.

Roberts, P. 1983. The Number of Replicates and Other Considerations in the Design of Field Trials. *In* W. Haresign (ed.) Studies in the Agricultural and Food Science Series - Recent Advances in Animal Nutrition, 242 p. Butterworths, England.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical Methods. The Iowa State Univ. Press. Ames, Iowa.

Swingle, H.S. 1968. Standardization of Biological Methods in Fish Culture Research. Proc. of the World Symposium on Warm-Water Pond Fish Culture. FAO Fish Bulletin 44(4): 422-429.

Status of Shrimp Nutrition and Feed Development in Southeast Asia

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World demand for shrimp has increased significantly in the last decade. Of the 32 species investigated, the most commonly cultured in Southeast Asia is the *Penaeus monodon*. Formulated feed constitutes around 50% of the operational costs in shrimp culture and hence there is a need to develop low, cost effective feeds. However, due to limited information on feeding habits and nutritional requirements, development of feeds for *P. monodon* has been mainly dependent on data derived from other penaeid species.

Studies on *P. monodon* nutrition and feed development and constraints to shrimp culture common to the Southeast Asian region are reviewed. Standardization of methodologies for nutrition research and husbandry to hasten collection of data is recommended.

The total world crustacean production in 1985 approximates 265.7 thousand mt, 74.7% of which is attributed to Asia (Table 1). The production of shrimp since 1975 has increased to around 201.1 thousand mt in 1985 and is projected to be 800 thousand mt by year 2000. (Table 1). Most of the crustaceans produced in this region are of high value and exported to major developed countries, like Japan, U.S.A. and Western Europe.

Table 1. Cultured crustacean production in Asia and estimated production by 2000 from Csavas (1988) (figures within parantheses are estimations).

Country	YEAR 1975	1980	1985	2000
Bangladesh	(1.6)	2.7	7.6	60
Burma	(0.2)	0.3	(0.7)	5
China, PR of	0.5	1.2	42.7	200
India	4.0	(7.0)	(17.0)	50
Indonesia	10.0	24.0	38.0	120
Japan	0.9	1.6	2.1	3
Korea, Rep of	0.1	0.1	0.1	5
Malaysia	0.1	0.1	0.2	8
Philippines	1.1	1.4	29.9	100
Singapore	0.0	0.0	0.3	2
Taiwan PC	0.3	3.8	31.0	85
Thailand	3.3	8.1	18.5	110
Vietnam	(3.0)	5.4	13.0	30
TOTAL (mt)	25.1	55.5	201.1	800

World demand for shrimp products increased significantly and importation was 80% higher in 1986 than in 1976 (Cruz 1987). It is estimated that by 1990 the demand will exceed 2 million mt. Presently around 5-6% of shrimps in the world market is from aquaculture and is expected to rise to 15.20% by 1990.

There are some 32 species that have been investigated and cultured throughout the world (Liao 1987). Seven species are now commercially cultured: *Penaeus monodon, P. indicus, P. merguiensis, P. japonicus* and *P. orientalis* in Asia (Table 1). *Macrobrachium rosenbergii,* a freshwater prawn is also extensively cultivated in Thailand and *P. vannamei, P. stylirostris* and *P. schmitti* in Latin America.

Shrimp farming was previously dependent on the collection of wild post-larvae and gravid females. But when broodstock and larvae were produced in captivity shrimp farming was intensified.

The information gained from the culture of *P. japonicus* has been used in the culture of other penaeids. The knowledge gathered about *P. japonicus,* as well as its nutritional requirements and feed technology, has made it the most widely studied of all cultured penaeids.

Technology Constraints

In 1972 three research priorities for intensification of prawn culture were identified; completing the life cycle, nutritional requirements and development of economical commercial feeds.

Still limitations exist in the technology and Liao (1987) identified these constraints as lack of:

i. hatchery bred larvae and post-larvae,

ii. efficient, complete, and inexpensive feeds suitable for each stage in the life cycle,

iii. studies on optimal culture conditions,

iv. preventive measures against pollution, contamination and fish vaccination,

v. a gene pool and knowledge of artificial insemination for continuous supply of fry,

vi. research in genetic engineering for production of disease as well as cold resistant, fast growing shrimps with high survival rates for better market value,

vii. aquaculture technology itself as aquaculture is still an art rather than a technology, and

viii. aqua engineering information for better pond design, harvesting equipment and post-harvest handling.

Formulated feeds constitute the most expensive component of the shrimp industry, approximating 50-60% of operational costs. This makes it highly desirable to study nutrient requirements as well as to develop feeds from locally available material and on industrial waste products with a view to producing cheaper and more effective feeds.

The problem of larval rearing is, "not to be able to produce postlarvae but to optimize the techniques" (Aquacop 1985). To simplify hatchery operations, research must be focused on full replacement of natural food by inert feeds such as microbound and microencapsulated diets.

For nursery systems better feeds are needed, efficient counting systems, pond design for easy control and harvesting, and benthic algal control to avoid trapping of postlarvae. In earthen ponds, quantification of the role of natural productivity in relation to soil preparation, fertilization and water management and harvesting should be studied.

The most important phase in the economics of the prawn industry is the grow-out phase which involves major costs of labor, feeding, the development of the low cost commercial feeds using local products and by-products of the industry, feeding frequencies, distribution techniques and the adjustment of the feeding rate to pond conditions.

It is therefore, evident that more studies in nutrition, feeds and husbandry are urgently needed. *P. monodon* is the penaeid most commonly cultured in Southeast Asia. Known as the tiger prawn in the Philippines and the grass shrimp in Taiwan, it is sturdy and grows to a larger size than other prawns in the area. I will attempt to review what is known in terms of nutritional requirements and feed development for *P. monodon*. Although feeds for grow out have been developed in Taiwan, Chuang et al. (1986) have indicated the need for more studies on nutrition requirements for *P. monodon* to be able to formulate more efficient feeds. To develop a suitable feed for shrimps, knowledge of feeding habits, and nutrient requirements are necessary.

Food and Feeding Habits

Prawn larvae are omnivorous; they prefer phytoplankton from zoea to mysis, and zooplankton such as rotifers from mysis to post-larvae (Villaluz et al. 1969). Feeding habits of different developmental stages of *P. monodon* in the wild are summarized in Table 2. Gut transient time for 95% of food ingested from the foregut is around five hours (Marte 1980).

Table 2. Food habits of *P. monodon* in the wild at different developmental stages.

Life stages	Food of study	Location	Authors
Zoea to Mysis	Phytoplankton	Philippines	Villaluz et al. 1969
Mysis to Postlarvae	Zooplankton and small crustaceans	Philippines	Villaluz et al. 1969
Postlarvae	Small crabs Shrimp Molluscs Polychaetes Ophiuroids Fish debris Sand Silt	Philippines	Marte 1980
Adults	Crustaceans Annelids Algae Mud Unidentified matter	Sudanese Red Sea Coast and Kerapuglia Estuary, India	El Hag 1984 Thomas 1972
	Molluscs Crustaceans Fish remains	Philippines	Marte 1982

Healthy prawns feed on the weak ones. Exuviae are reinjested but this remains to be determined (Cuzon pers. comm.). Prawns move around the perimeter of the pond in the late afternoon and evening and although they feed at any time during the day, they prefer to bottom feed when there is light (Apud et al. 1980).

Nutritional Requirements

Due to lack of information on the nutrient requirements of *P. monodon* feeds have been formulated using the limited information available and quantities derived from other species, mainly those gathered from *P. japonicus*.

Protein and amino acids

High protein diets such as Artemia which have been found to be suitable for *P. monodon* larvae seem to suggest that higher amounts of protein are required by the larvae. The reported nutrient requirements for *P. monodon* are summarized in Table 3. Ten essential amino acids for *P. monodon* are known (Coloso and Cruz 1980). These are similar to those reported by Deshimaru (1981) for *P. japonicus* and *P. aztecus* (Shewbart et al. 1972). Several investigators have analyzed the amino acid pattern of *P. monodon* (Catedral and Penaflorida 1977; Kanazawa and Teshima 1981; Coloso and Cruz 1980). Preliminary results on the required amount of arginine and histidine in the diet showed that a level nearest the amount in the amino acid pattern of the prawn post-larvae gave the best results (Pascual, unpublished). According to Penaflorida (pers. comm.) except for arginine the essential amino acid pattern is similar throughout the life cycle of *P. monodon*. A semi-purified type of complete diet was formulated by following the amino acid pattern of *P. monodon* post-larvae compared to diets that were deficient in single amino acid (Pascual and Kanazawa 1986). Although mean percentage weight gains and survival were not significantly different from each other, some trends indicate that some amino acids seemed to be more critical in the diet than others.

Lipid and Fatty Acids

Generally, the fatty acid composition of post-larvae is related to the fatty acid composition of the diets (Yashiro 1982). Millamena and Quinitio (1985) reported a decrease in lipid content with developmental stage (egg to post-larvae) indicating that lipids are utilized for energy during larval development and metamorphosis.

There is a predominance of higher long chain polyunsaturated fatty acids (PUFA) arachidonic, eicosapentaenoic and docosahexaenoic acids in prawn broodstock ovaries, hepatopancreas and tail muscle from the wild (Millamena et al. 1985).

Other lipids needed by prawns are cholesterol and lecithin (Table 3).

Carbohydrates

Sucrose, dextrin, maltose, molasses, cassava starch, corn starch, sago palm starch, trehalose and glucose are carbohydrates that have been studied by Pascual et al. (1983) and Alava and Pascual (1987). Diets with 10% molasses caused mortality within ten days of culture, whereas sucrose and sago palm starch at the same level gave better survival rates. Sucrose and trehalose

Table 3. Protein and lipid requirements of *P. monodon*.

Nutrient % of dry diet	Stage	Source of Nutrient	Authors
Protein	juvenile		
40		Shrimp meal Squid meal Fish meal Casein Soybean meal	Alava & Lim (1983)
40-50	juvenile	Casein & Gelatin	Bautista (1986)
55	juvenile	Prawn muscle Casein Gelatin Whole egg Protein	Nezaki (1986a)
50-55	broodstock	Fish meal Squid meal Shrimp head meal	Millamena et al. (1986)
Lipid	juvenile		
5-10		Cod liver oil and soy- bean oil	Bautista (1986)
11.7	juvenile	Cod liver oil and soy- bean oil	Mendoza (1982)
12	broodstock		Millamena et al. (1986)
Lecithin	juvenile	Soy lecithin	
3			Pascual (1986)
4	juvenile	Soy lecithin	Nezaki (1986b)
Cholesterol 0.5-1			Nalzaro (1982)
Polyunsaturated fatty acids, n3 series 0.5-1			Chuen (1986)

proved to be the better sugars compared to glucose at 20% of the diet (Alava and Pascual 1987). Abdel-Rahman et al. (1979) concluded that glucose remains in the bloodstream for as long as 24 hr and therefore, is detrimental to *P. japonicus*.

Energy

Energy content between 2.85 and 3.70 Kcal/kg resulted in good growth, survival and feed conversion ratio depending on the protein, carbohydrate and fat content. The diet that contained 40 to 50% protein, 5-10% lipid and 20% carbohydrate gave the best growth, survival and feed conversion ratio (Bautista 1986).

Vitamins and Minerals

Only exploratory, preliminary work on vitamins and minerals have been done on *P. monodon*. Preliminary studies with juveniles fed under laboratory conditions showed that some vitamins may not be needed in practical formulated diets. Growth efficiency of the diets was comparable to the growth obtained for prawns fed a complete diet when one of each of the vitamins was omitted from the diet (Pascual, unpublished). Catacutan and Kanazawa (1985) obtained similar results with purified diets.

Diet Development

Apart from the basic nutritional requirements other factors have to be considered in the development of formulated or artificial diets. Amongst them are, the physical characteristics such as water stability, attractability, size, shape, density and texture of the diets. Such factors would differ from one stage of the life cycle of the prawn-from larval to grow-out to broodstock.

Larval, Grow-out and Broodstock Diets

Larvae that are pelagic and swim continuously within the water column need diets that are suspended in the water column for a certain period.

A microparticulate feed containing 58% crude protein, 16% crude fat, 1% crude fiber, 8% crude ash, nitrogen free extract of 0.2% and 8% moisture was compared to Artemia as feed for shrimp fry. Preliminary results showed survival rates of 40% for fry fed a combination of microparticulate diets, and was 20% for those fed only Artemia (Kuo 1986).

Acetis sp. frozen, fresh and dried when fed to larvae was found to give good survival (Kungvankij et al. 1986) while Quinitio et al. (1983) found that soybean meal is a good substitute for algal food in the larval stages.

Various larval feeds have been developed - microparticulate, microencapsulated, microbound diets. There are commercially available larval diets but refined techniques for total algal replacement are still necessary.

Use of non-live foods are associated with problems of water pollution; however, with proper management microparticulate diets offer a potential substitute for traditional algal food (Kanazawa 1985). Bautista (unpublished) has developed a microbound diet that is presently being tested.

Physical Characteristics of Pellets
Water Stability

Sweet potato meal, cassava starch, extract of shark fins, gracilaria, gum arabic, alginate, glutinous rice, alpha-potato starch, carboxymethyl cellulose, carrageenan, corn starch, polymethylolcarbamide (Pascual et al. 1978; Pascual and Tabbu 1979; Murai et al. 1981; Pascual and Sumalangcay 1982; Pascual, unpublished) and sago palm starch (Lim and Destajo 1979) have been studied for their possible use as binders in practical and/or semi-purified diets. Sweet potato starch at 5% of the diet has poor binding capacity. Other binders are either too expensive or not commercially available. Steaming the diet has been found to further increase water stability of the pellet.

Attractants

Shrimp, mussel, squid, fish extract, and mussel extract in purified diets have been found to be good attractants (Pascual 1980). Krill meal, earthworm meal, glycine, sucrose and mussel water were used as attractants in a practical diet by Murai et al. (1983). Glycine and mussel significantly improved attractability while krill meal, earthworm meal and sucrose improved attractability only to a certain extent.

Feed and Feedstuff Resources

Protein sources

Squid meal, shrimp meal, mussel meat, fish meal, shrimp head meal and earthworm meal have been found to be good animal protein sources (Lim et al. 1979; Pascual and Destajo 1979; Pascual 1985). They also provided the attractants and contain essential amino and fatty acids. Shrimp meal and other crustacean meals contain astaxanthin, the carotenoid that gives the bright red-orange color to the prawn when cooked (Benjamin 1982). Pascual et al. (1986) showed that soybean meal can substitute for fish meal up to 45% in the diet.

Lipid sources

Mangalik (1979) reported that fish oil is best for juvenile prawns, followed by beef tallow, soybean oil, copra oil and pork lard in descending order. A 1:1 ratio of cod liver oil and soybean oil, preferably the crude degummed soya oil, has been found effective in diets for juveniles (Pascual 1986). Better survival and growth were obtained with diets fortified with *Artemia* oil compared to those fed soybean oil (Chen and Tsai 1986).

Apparent Digestibility

Apparent digestibility of some protein sources were analyzed for male and female prawn of approximately 30 g. Peruvian fish meal was poorly digested, while full fat and defatted soybean meal were equally digestible (Catacutan, pers. comm.).

Although in the last few years researchers and food producers have been successful in the development of various formulated feeds sufficient to sustain present needs, many improvements are still needed. Aquacop (1985) identified the following research priorities:

i. Determination of nutritional requirements for each species in each stage of the life cycle and standardization of nutritional tests,

ii. reduction in the leaching of water-soluble products,

iii. finding specific growth factors,

iv. development of adequate and low cost feeds for larvae, broodstock and grow out by replacement of costly animal protein with plant protein, and

v. improving digestibility of carbohydrates.

With the use of various feed ingredients most of which are industrial by-products, and the keen competition in the marketing of shrimp, color and flavor of the shrimp may become very important factors in determining its marketability. Thus, post-harvest handling studies and consumer acceptance will have to be pursued.

Shrimp culture activity is just emerging from its infancy. The success of the broiler industry was achieved only after more than 20 years of intensive scientific and commercial effort and the shrimp industry is likely to follow the same. The rate of development will depend on how closely researchers will work with producers to identify the constraints and to integrate available techniques according to the socio-economic conditions of each country. This has been clearly shown to be so in the development of the shrimp culture industry in Taiwan (Chien and Liao 1987).

In the Philippines for example, although formulated diets are developed under laboratory conditions, there is a fear in the take off into pond conditions as no fish farmer or few of them are willing to spend for research. They would rather rely on funding agencies like the government. Research support is hard to come by unless applied research is pursued. Verification of these feeds takes a long time and is fraught with tie-ups. It is more lucrative for the business to rely on technology that can be imported from other countries than to develop one's capabilities as this is going to take time and money.

The lack of trained, dedicated labor force, peace and order situation, socio-economic conditions, are deterrents to the progress of the shrimp industry.

Constraints to shrimp culture development will vary from country to country. Common to the region are:

i. inadequate supply of wild spawners,

ii. lack of efficient formulated feeds for broodstock, grow out and larval feeds,

iii. presence of diseases in hatcheries and rearing ponds under intensive method of culture,

iv. lack of culture techniques for *P. monodon*.

Although there are techniques, these have to be refined, standardized, packaged and tested in different environments and disseminated to shrimp farmers. Techniques developed or established in one country may not be directly applicable to other countries. Hence, shrimp farming techniques are often a secret and will remain undisclosed until such time that aquaculturists and researchers are confident that research results can be packaged into a standard technology. Furthermore, many of the feed formulations are proprietary to the company and are trade secrets.

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References

Abdel-Rahman, S.H., A. Kanazawa and S. Teshima. 1979. Effects of dietary carbohydrates on the growth and the levels of the hepatopancreatic glycogen and serum glucose of prawns. Bulletin Japanese Society of Scientific Fisheries 45(12): 1491-1494.

Alava, V.R. and C. Lim. 1983. The quantitative dietary protein requirement of *Penaeus monodon* juveniles in a controlled environment. Aquaculture 30(1): 53-61.

Alava, V.R. and F.P. Pascual. 1987. Carbohydrate requirements of *P. monodon* Fabricius juveniles. Aquaculture 61: 211-217.

Apud, F.D., N. Deatras and R.G. Gonzales. 1980. Feeding behavior and food preferences of *Penaeus monodon* Fabricius with scrap *Tilapia mossambica*. Quarterly Research Report Southeast Asian Fisheries Development Center, Aquaculture Department, 4(3): 19-21.

Aquacop. 1985. Overview of penaeid culture research: impact on commercial culture activity. *In* Y. Taki, J.H. Primavera, J.A. Llobrera (eds.) Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps, p. 3-10. Iloilo City, Philippines.

Bautista, M.N. 1986. The response of *Penaeus monodon* juveniles to varying protein/energy ratios in test diets. Aquaculture 53: 229-242.

Benjamin, G. 1982. Ensilage treatment of shrimp head wastes. Abstract, M.S. Thesis, Louisiana State University, 95 p.

Catacutan, M. and A. Kanazawa. 1985. Effect of some water soluble vitamins on the growth of *Penaeus monodon* juvenile. *In* Y. Taki, J.H. Primavera and J.A. Llobrera (eds.) Proceedings of the First International Conference on the Culture of Penaeid Prawn/Shrimps. October 1985, Iloilo City, Philippines. p. 182.

Catedral, F. and V. Penaflorida. 1977. Amino acid analysis of *P. monodon* muscle. Quarterly Research Report Southeast Asian Fisheries Development Center, Aquaculture Department 1(4): 1-2.

Chen, H.Y. and R.H. Tsai. 1986. The dietary effectiveness of *Artemia nauplii* and microencapsulated foods for postlarval *P. monodon*. *In* J.L. Chuang and S.Y. Shian (eds.) Research and Development of aquatic Animal Feeds in Taiwan Vol. 1, p. 73.

Chien, Yew-Hu and I.C. Liao. 1987. Bioeconomic consideration of prawn farming. Paper presented in "Prawn Farming for Profit - Potential for Success" Seminar in 1987 (Taipei, Taiwan). Manuscript.

Chuang, J.L., P.W. Yuan and S.L. Shin. 1986. Aquaculture in Taiwan - Review and Outlook. *In* J.L. Chuang and S.Y. Shian (eds.) Research and Development of Aquatic Animal Feed in Taiwan Vol. 1, p. 15-28.

Chuen-Herng Wu. 1986. Requirement of lipids and cholesterol in diet grass shrimp. *In* J.L. Chuang and S.Y. Chian (eds.) Research and Development of Aquatic Animal Feed in Taiwan Vol. 1, p. 69.

Coloso, R.M. and L.J. Cruz. 1980. Preliminary studies in some aspects of amino acid biosynthesis in juveniles of *Penaeus monodon* Fabricius. Bulletin: Philippine Biochemical Society 3(1&2): 12-22.

Cruz, S.P.F. 1987. Status and prospects of the shrimp industry. Technical Considerations for the Management and operations of Intensive Prawn Farms. U.P. Aquaculture Society, College of Fisheries, University of the Philippines in the Visayas, Iloilo City, Philippines, p. 7-10.

Csavas, I. 1988. Shrimp farming developments in Asia. Infofish International, 2 (March/April) 1988; p. 11-16.

Deshimaru, O. 1981. Studies on nutrition and diet for prawns, *Penaeus japonicus*. Memoirs Kagoshima Prefecture Fisheries Experiment Station. No. 12, Dec. 118 p.

El Hag, E.A. 1984. Food and food selection of the penaeid prawns *Penaeus monodon* (Fabricius). Hydrobiologia 110: 213-217.

Kanazawa, Akio. 1985. Nutrition of penaeid prawns and shrimps. *In* Y. Taki, J.H. Primavera, J.A. Llobrera (eds.) Proceedings of the First International Conferences on the culture of Penaeid Prawns/Shrimps, pp. 123-130. Iloilo City, Philippines, October 1985.

Kanazawa, A. and S. Teshima. 1981. Essential amino acid of the prawns. Bulletin of the Japanese Society of Scientific Fisheries 47: 1375-1377.

Kungvankij, P.A., G. Tacon, K. Corre, B.P. Pudadera, G. Taleon, E. Borlongan and I.O. Potestas. 1986. Ascetes as prime food for *Penaeus monodon* larvae. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum, pp. 581-584. Asian Fisheries Society, Manila, Philippines.

Kuo, Chin-Chao. 1986. Microparticulated diet for grass shrimp larvae. *In* J.L. Chuang and S.J. Shian (eds.) Research and Development of Animal Aquatic Feed in Taiwan, Vol. 1, p. 81.

Liao, I. Chiu. 1987. Future technology in prawn production. Paper presented at the Special Session during the 18th Annual Meeting of the World Aquaculture Society Guayaquil, Ecuador 18-23 Jan.

Lim, C. and W.H. Destajo. 1979. Effects of crude, semi-purified, and purified starch of sago (*Metroxylon sagu* Rottb) on the water stability of pelleted shrimp diets. Fisheries Research Journal of the Philippines 4(1): 19-21.

Lim, C., P. Suraniranat and R.R. Platon. 1979. Evaluation of various protein sources of *P. monodon* postlarvae. Kalikasan Philippine Journal of Biology 8: 29-36

Mangalik, A. 1979. Effects of various lipid sources on the growth and survival rates of *Penaeus monodon* Fabricius from post larvae to juveniles in a controlled environment. M.S. Thesis. College of Fisheries, University of the Philippines, 65 p.

Marte, C.L. 1980. The food and feeding habit of *Penaeus monodon* Fabricius collected from Makato River, Aklan, Philippines (Decapoda: Natantia). Crustaceana 38: 225-236.

Marte, C.L. 1982. Seasonal variation in food and feeding *Penaeus monodon* Fabricius (Decapoda: Natantia). Crustaceana 42: 250-255.

Mendoza, E.C. 1982. Quantitative dietary lipid requirement of *P. monodon* juveniles in a controlled environment. M.S. Thesis, College of Fisheries, University of the Philippines in the Visayas, 33 p.

Millamena, O. and E.T. Quinitio. 1985. Lipids and essential fatty acids in the nutrition of *Penaeus monodon* larvae, p. 180. *In* Taki, Y., J.H. Primavera and J.A. Llobrera (eds.) Proceedings of the First International Conference on the culture of Penaeid Prawn/Shrimps. Oct. 1985, Iloilo City, Philippines.

Millamena, O.M., T. Pudadera, M. Catacutan, F. Pascual, P. and K. Simpson. 1985. The fatty acid composition and tissue of lipid content of unablated and ablated *P. monodon* broodstock from the wild. In press. World Mariculture Society.

Millamena, O.M., J.H. Primavera, R.A. Pudadera and R.V. Caballero. 1985. The effect of diet on the reproductive performance of pond-reared *Penaeus monodon* Fabricius broodstock, p. 593-596. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Murai, T., A. Sumalangcay and F.P. Pascual. 1981. The water stability of shrimp diets with various polysaccharides as binding agents. Quarterly Research Report SEAFDEC AQD 5(2): 18-22.

Murai, T., A. Sumalangcay and F.P. Pascual. 1983. Supplement of various attractants to a practical diet for juvenile *Penaeus monodon* Fabricius. Fisheries Research Journal of the Philippines 8: 2-6.

Nalzaro, G.G. 1982. Quantitative dietary cholesterol requirement of *Penaeus monodon* juveniles. M.S. Theses, College of Fisheries, University of the Philippines in the Visayas, 47 p.

Nezaki, G. 1986a. Nutritional requirements of prawn (*Penaeus monodon* Fabricius)-II. Effect of dietary protein and carbohydrate. Unpublished Terminal Report. Aqua Dept. SEAFDEC, Tigbauan, Iloilo, Philippines.

Nezaki, G. 1986b. Nutritional requirement of prawn (*Penaeus monodon* Fabricius)-IV. Effect of dietary lecithin. Unpublished Terminal Report. Aqua. Dept. SEAFDEC, Tigbauan, Iloilo, Philippines.

Pascual, F.P. 1980. Attractants in purified diets. Quarterly Research Report SEAFDEC AQD 6(1): 7.

Pascual, F.P. 1985. An evaluation of three annelids as feed ingredients in formulated diet for juvenile *Penaeus monodon*. Fisheries Research Journal of the Philippines 10(1-2): 9.

Pascual, F.P. 1986. Effect of supplemental lecithin and lipid sources on growth and survival of *P. monodon* juveniles. *In* J.L. Maclean, L.B. Dizon, & L.V. Hosillos (eds.) The First Asian Fisheries Forum, pp. 615-618. Asian Fisheries Society, Manila, Philippines.

Pascual, F.P., R. Coloso and C. Tamse. 1983. Survival and some histological changes in *P. monodon* Fabricius juveniles fed various carbohydrates. Aquaculture 31: 169-180.

Pascual, F.P. and W. Destajo. 1979. Growth and survival of *Penaeus monodon* postlarvae fed shrimp head meal and fish meal as primary animal sources of protein. Fisheries Research Journal of the Philippines 4(1): 29-36.

Pascual, F.P. and A. Kanazawa. 1986. Specific amino acid free semi-purified diets for *P. monodon* juveniles. Memoirs Kagoshima University, Research Center for the South Pacific 7: 65-72.

Pascual, F.P. and A. Sumalangcay, Jr. 1982. Gum arabic, carrageenan of various types and sago palm starch as binders in prawn diets. Fisheries Research Journal of the Philippines 7: 54.

Pascual, F.P. and M. Tabbu. 1979. Fishwater and agar as binders in a prawn diet. Quarterly Research Report SEAFDEC AQD 3(1): 1.

Pascual, F., P. Bandonil and W.H. Destajo. 1978. The effect of different binders on the water stability of feeds for prawns. Quarterly Research Report SEAFDEC AQD 2(1): 31-35.

Pascual, F.P., E.M. Cruz and A. Sumalangcay, Jr. 1986. Practical diets for *P. monodon* juvenile containing various levels of defatted soybean meal (Unpublished manuscript).

Quinitio, E.T., D. de la Peña and F. Pascual. 1983. The use of substitute feeds in larval rearing of *Penaeus monodon*, p. 337-342. *In* G.L. Rogers, R. Day, A. Lim (eds.) Proceedings at the First International Biennial Conference on Warmwater Aquaculture, Crustacea. Loie, Hawaii.

Shewbart, K.L., W.L., Mies and P.D. Ludwig 1972. Identification and quantitative analysis of the amino acids present in protein of the brown shrimp, *Penaeus aztecus*. Marine Biology 16: 64-67.

Thomas, M.M. 1972. Food and feeding habits of *Penaeus monodon* Fabricius from Korapuzlia estuary. Indian Journal Fisheries 19: 202-204.

Villaluz, D.K., A. Villaluz, B. Ladrera, M. Sheik and A. Gonzaga. 1969. Reproductive larval development and cultivation of sugpo (*Penaeus monodon* Fabricius). Philippine Journal Science 98(3-4): 205-233.

Yashiro, R.H. 1982. The effect of Artemia fed with different diets on the growth and survival of *Penaeus monodon* Fabricius postlarvae. M.S. Thesis University of the Philippines in the Visayas 48 p.

Economic Parameters in Nutritional Studies

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Economic analysis of aspects on nutrition can play an important role in determining the commercial viability of aquaculture production systems. Changes in feed formulation and methods of feeding must be subjected to an economic analysis before they are recommended for use by aquaculture producers. Economic analysis techniques are readily available for this purpose. This paper focuses mainly on the application of economic analysis to the following issues: (a) estimation of input-output relationships and determination of economically optimal level of feed use (b) determination of least cost combination of different feeds (c) determination of output mix and, (d) derivation of minimum cost feed formulation.

Finfish culture is an animal husbandry process. It has many similarities to other types of land based animal husbandry, one of them being that prepared feeds is an important, if not the most important input in the production process. The objectives of providing prepared feeds in aquaculture are similar to land based animal production, i.e., maximize growth rates, increase production per unit area per unit time, increase reproductive efficiency, increase resistance to disease, and minimize mortality (Cole and Ronning 1986). The cost of feeds in aquaculture can often exceed 50 percent of total production costs, rising to as high as 75 percent (Shang 1981). This is comparable to hog, beef cow, feeder cattle and caged layer livestock enterprises (Herbst 1968).

The preparation of feeds requires scarce resources, i.e., feed ingredients, labour, capital. All of these resources have alternative uses and 'values' associated with those uses. If they are not allocated to preparing fish feeds, they could be used for other livestock feeds, other farming and/or industrial activities. Economics is the science of the allocation of scarce resources among competing uses. With reference to animal feeds and feeding methods economic analysis is an accepted and integral part of commercial livestock operations and experimental research in both developed and developing countries (Fine and Lattimore 1982).

While the authors cannot claim to have conducted an extensive review of the finfish nutrition literature in Asia, it seems fairly safe that economic analysis of commercial and

experimental finfish feeding practices in Asia is very limited. There are, of course, exceptions (see Pullin and Shehadeh 1980; Hopkins and Cruz 1982). There are probably three main reasons for this. First, a historical lack of trained and experienced aquaculture economists in Asia; second and perhaps more important is that the state of knowledge of tropical Asian finfish nutrition itself, for both laboratory and producer level environmental conditions, is significantly less as compared to temperate species and producer culture environments. This means that the specific (species, culture system) objectives of feeding are not as well defined, nor are the technical relationships and their efficiencies between feed and species performance. While recognizing that the known nutritional requirements for all commercial temperate species is far from complete, it appears to be even less so for the tropical species (Cho et al. 1985). This means that economic analysis of feeds and feedings, as an aquaculture enterprise management practice provides more accurate estimates of probability of economic feasibility in proportion to the certainty of the feed input/species performance relationship. Third, tropical Asian finfish aquaculture is not relatively 'industrialized' as for example, the non-ruminant livestock sectors. This relates to feeds in the sense that the quality of feed used by producers in aquaculture is highly variable due to local formulation, non-standard processing (ingredient content, balance of ingredients, storage, etc.), varying local availability on ingredients for use as feeds and a wide range of mostly manual feeding methods (Cho et al. 1985). A high degree of variability in feed quality both within and between aquaculture enterprises growing similar species makes it extremely difficult for the economist or aquaculture producer to formulate least-cost feed formulations or diets, with broad application. Unless there is a minimum level of certainty as to the feed (or ingredient) quality, it is not possible to calculate a least cost ration. Such a calculation (as will be shown later) depends on an estimate of the marginal rate of technical substitution of alternative (feed) inputs to maintain a given level of species performance. The authors are not arguing for highly industrialized finfish production in Asia, but stressing the need for the development of feeds of relatively known quality when used by producers, otherwise the economic feasibility of the use of those feeds is very difficult to calculate.

In addition to the economic analysis of specific technical objectives for providing feeds (as stated above), there is an additional economic analysis of finfish that is important. That is the comparison of the economics of supplementary feeding to achieve species/system performance as compared to alternative, i.e., capital intensive technologies. In Asia, where access to large water areas and/or borrowed capital to develop extensive and/or capital intensive (controlled environment) systems is often not feasible, supplementary feeding is probably the principal means of intensifying production per unit area per unit time. This is especially true for small scale rural producers. Also, supplementary feeding provides producer with a high degree of flexibility and control in varying his or her production and total production costs. This allows the producer to react more economically to changes in local demand (quantities and price) for fish.

It appears from this discussion that economic analysis of nutrition can play a very important role in determining the commercial viability of aquaculture production systems. The major objective of this paper is to examine the application of economic theory and techniques to the decision making problems faced by fish farmers with respect to the use of supplementary feeds as a major input in their production systems. Basically these decisions are concerned with the following issues related to finfish nutrition:

a. Estimation of input-output relationships and determination of economically optimal level of feed use

b. Determination of least cost combination of different feeds

c. Combination of fish species output using a single type of feed, and

d. Derivation of minimum cost feed formulations.

The areas of microeconomics dealing with the theory of production and costs are obviously useful in making decisions on these issues. The applications of economic theory to analyse each of the above issues are presented in this paper.

Estimation of Input-Output Relationships and Determination of Economically Optimal Level of Feed

One of the most important practices in aquaculture is the use of supplementary feed to improve the productivity and nutritional level of fish. Decisions on inputs and outputs cannot of course, be taken independently as there are technological relationships between inputs and outputs which restrict the options available to management. This technological relationship between input and output is commonly referred to as the production function. Production function specifies the maximum possible output that can be produced for a given amount of inputs or, alternatively, the minimum quantity of inputs necessary to produce a given level of output. Production functions are determined by the technology available to the firm. Any improvement in technology results in a new production function.

The basic properties of production functions can be illustrated by examining a simple unconstrained single-output, single-input production process. Consider a simple aquaculture production system where the output of fish (Q) is dependent upon the quantity of supplementary feed (X) used. All other inputs and technical knowledge are assumed to be fixed during the period of production. The production function for this system can be expressed as the following unspecified relationship:

$$Q = f(X)$$

Where Q is quantity of fish produced and X is quantity of supplementary feed consumed. Table 1 illustrates a hypothetical production function for this single-input, single-output production system. This Table shows the maximum quantity of fish (Q) that can be produced with a specific quantity of supplementary feed (X) while other factors remain constant.

Table 1. Hypothetical production function for a single input, output system.

Units of Feed (Bags)	Units of Fish (kg)	Marginal Product (MP) (kg)	Average Product (AP) (kg)	Price of Fish ($/kg)	Marginal Revenue Product (MRP) $	Marginal Factor Cost (MFC) $/Bag	Total Revenue $	Total Cost $	Profit $
1	9	9	9	2.00	18.00	38.00	18.00	38.00	-20
2	32	23	16	2.00	46.00	38.00	64.00	76.00	-12
3	63	31	21	2.00	62.00	38.00	126.00	114.00	12
4	96	33	24	2.00	66.00	38.00	192.00	152.00	40
5	125	31	25	2.00	62.00	38.00	250.00	190.00	60
6	144	19	24	2.00	38.00	38.00	288.00	228.00	60
7	147	3	21	2.00	6.00	38.00	294.00	266.00	28

This production function can be expressed mathematically as: $Q = 10X^2 - X^3$.

The input-output relationship given in Table 1 can also be displayed graphically as shown in Fig. 1 assuming that the underlying production function is continuous in nature. Fig. 1 shows that as more of supplementary feed is employed while all other inputs being held constant output of fish will first tend to rise but eventually, at least, a point will be reached where additional quantity of feed will yield diminishing marginal contribution to total output.

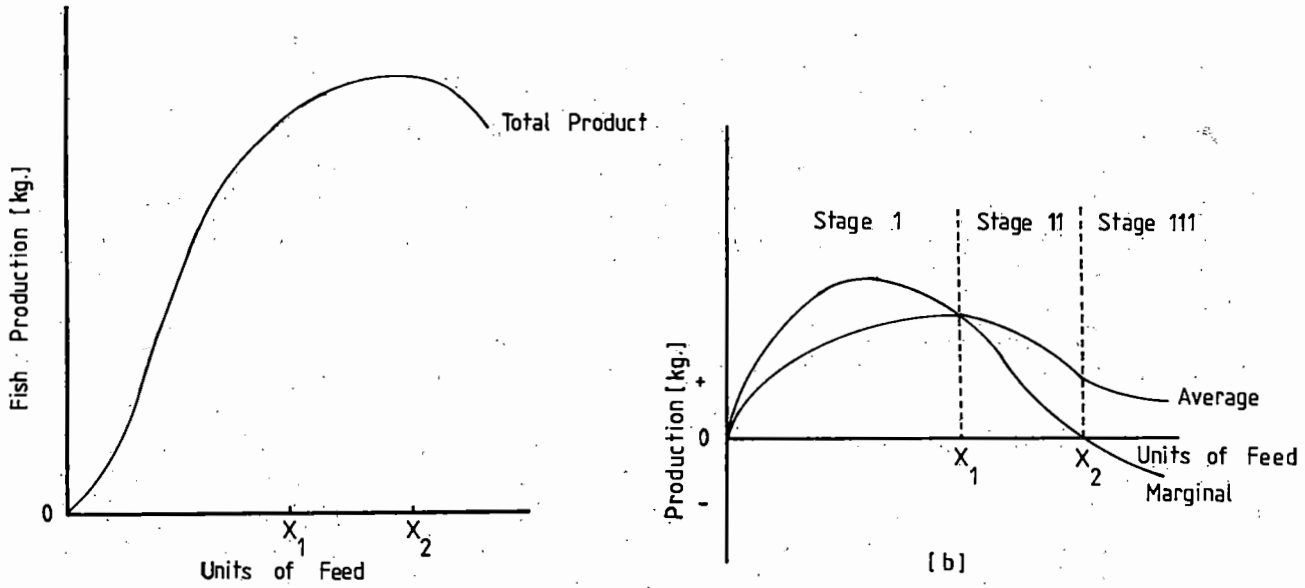

Fig. 1. The production function and its relationship to marginal and average product.

This relation is known in production theory as the "Law of Diminishing Returns". The marginal product of an input X (MPx) is defined as the change in output (Q) resulting from a very small change of this input keeping all other things constant. For a discrete total product function, the marginal product is estimated by the relationship:

$$MPx = \frac{\Delta Q}{\Delta X}$$

For a continuous total product function the marginal product can be derived by taking the partial derivative of the production function with respect to the input. Thus the marginal product of input X is given by:

$$MPx = \frac{\Delta Q}{\Delta X}$$

Graphically the marginal product is equal to the slope of the total product curve. In our hypothetical production function presented in Table 1, marginal product of feed continues to increase until four bags of supplementary feed have been added but starts to decline when the fifth bag is added. Therefore, with the fifth bag we encounter diminishing returns, eventhough the total fish output continues to increase until the seventh bag of feed is used.

Another important concept which is frequently used in input-output relationship is the average product (AP) and defined as the total output (Q) divided by the number of units of variable input (X) used:

$$APx = \frac{Q}{X}$$

The average and marginal product curves that correspond to the total product curve in Fig. 1 (a) are shown in Fig. 1 (b). We can see from Fig. 1 (b) that there are three ranges of input utilization which can be used to identify the rational and irrational stages of production. Stage I is characterized by an excessive amount of fixed input relative to the quantity of variable input employed. Average product of the variable input in this stage is increasing, compelling the producer to increase the use of variable input within this stage which extends from the origin to X_1. The input utilization in Stage I does not lead to cost minimization for any level of production.

The operation in Stage III is also irrational as the marginal product of the variable input is negative in that range. Stage II lies between X_1 and X_2 is characterized by diminishing returns to the variable input over its entire range which is known as the area of rational economic production. Therefore, if production is to take place it will occur somewhere in Stage II.

In order to realize maximum profits a firm must find out the rates at which to apply the inputs. The production function relationships discussed so far are insufficient to determine the optimal (profit maximizing) input use in a production system. It is necessary to combine this technological information with economic data such as the prices of inputs and outputs prevailing in the factor and product markets in order to determine the economically efficient input level the producer should use. To achieve this, the firm must balance the returns from employing supplementary feed against the cost of feed.

The addition to a firm's total revenue when one more unit of variable input (feed) is employed is called the marginal revenue product (MRP) of that input. It is equal to the marginal product of the variable input multiplied by the firm's marginal revenue (if the firm is a price taker in the product market, firm's marginal revenue will be identical to the market price of the product). The corresponding addition to the firm's total cost resulting from applying additional unit of variable input (feed), all other inputs unchanged, is called the marginal factor cost (MFC). A firm is in profit maximizing position with respect to input utilization, if the marginal revenue product of the variable input is equal to its marginal factor cost (Asimakopulos 1978). This equilibrium condition can be written as:

$$MRP_x = MFC_x$$

So long as marginal revenue product exceeds marginal factor cost, profits must increase and the firm will increase the employment of inputs if it aims at profit maximization. Similarly, when the marginal revenue product is less than the cost of the factor, marginal profit is negative, so the firm would decline to employ additional units of that factor. As shown in Table 1, profits are maximized with the use of six units of feed when MRP equals MFC.

Least-Cost Combination of Different Feed Inputs

The presence of more than one complementary/substitutable feed inputs in aquaculture production systems makes the analysis of optimal combination of feed inputs more intricate.

When there are only two feed inputs in the production function, the choice of optimal combination of two inputs can be analysed graphically using isoquants and isocost curves. An isoquant is a line joining all points representing combinations of two variable inputs which when combined efficiently, produce a specified level of output. In Fig. 2 a production function with two variable inputs is depicted in the form of a set of isoquants. The isoquant labeled Q_1 shows the various combinations of rates of inputs X and Y that produce 300 kg of fish.

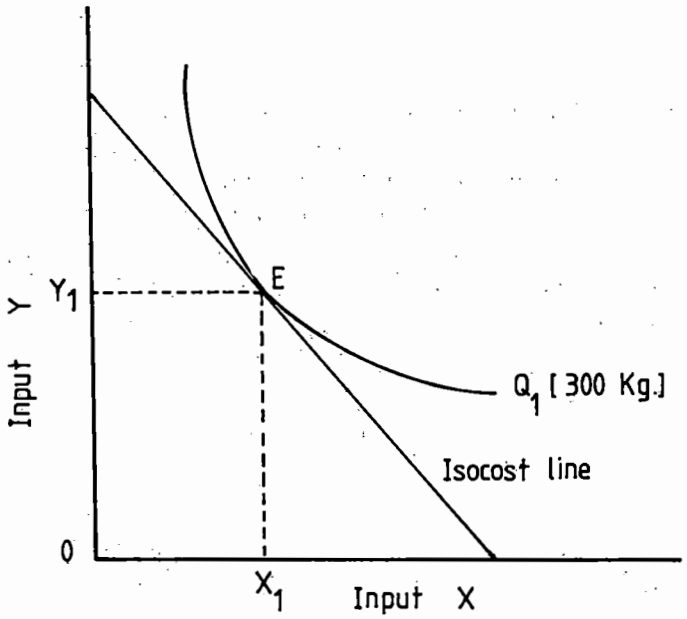

Fig. 2. Optimal input combination.

The numerical value of the slope of an isoquant provides the key to the substitutability of the two inputs. The slope of the isoquant shown in Fig. 2 is simply the change in input Y (ΔY) divided by the change in input X (ΔX). This relationship known as the marginal rate of technical substitution ($MRTS_{xy}$) of the two variable inputs provides a measure of the amount of one input factor that must be substituted for one unit of the other factor if output is to remain unchanged. Thus:

$$MRTS_{xy} = \frac{\Delta Y}{\Delta X} = \frac{dY}{dX} = \text{Slope of the isoquant.}$$

It can be deducted that the MRTS is equal to the ratio of the marginal products of the two inputs as follows:

$$MRTSxy = \frac{\Delta Y}{\Delta X} = \frac{MP_x}{MP_y}$$

The production function shown in the form of a set of isoquants alone cannot determine the optimal combination of the two inputs producing a specified level of output. Data on input prices are required to determine the optimal input combination. Graphically, input prices can be

introduced into the production function by adding an isocost line to the diagram of production isoquants. Isocost line is the focus of all combinations of factors the firm can purchase for the same expenditure. The slope of the isocost line is equal to the relative prices of the inputs, X and Y. Thus:

$$\text{Slope of isocost line} = \frac{P_x}{P_y}$$

Combining the production isoquants with the isocost line, it is possible to determine the optimal input combination required to produce a specified level of output. In Fig. 2, the optimal input combination occurs at point E where the isocost line and the isoquant are tangent. The optimal combination of factors required to produce the level of output Q_1 is X_1 and Y_1. At the point of tangency (E), the slope of the isocost line (relative input price: P_x/P_y) is equal to the slope of the isoquant (ratio of marginal products: MP_x/MP_y). Therefore, for optimal input combination, the ratio of prices of inputs must be equal to the ratio of their marginal products (Pappes and Brigham 1979). That is:

$$\frac{P_x}{P_y} = \frac{MP_x}{MP_y}$$

Or, alternatively, the ratio of marginal product to price must be equal for each input:

$$\frac{MP_x}{P_x} = \frac{MP_y}{P_y}$$

The economic principle for least-cost combination of inputs as shown above states that the firm employs various inputs in such a way that as the last dollar spent on each input contributes the same amount to output as a dollar spent on any other input. For example, with P_x = \$2 and P_y = \$4, a solution could be 6/\$2 = 12/\$4 or 2/\$2 = 4/\$4, which means the last dollar spent on each input will bring forth the same amount of output.

When the number of variable inputs in the production system is greater than two, it is not possible to use the graphical method to determine the optimality though the requirements for production at least cost would still be the same. This optimality condition can be expressed when the number of variable inputs is *n*, as:

$$\frac{MP_x}{P_x} = \frac{MP_y}{P_y} = \ldots\ldots = \frac{MP_n}{P_n}$$

When there are a number of inputs in the production system, the marginal productivity of different inputs are to be derived from an empirically estimated production function through differential calculus. Estimation of production functions involves the application of statistical techniques to cross-section or time series data (Heathfield 1971).

Combination of Fish Species Output Using a Single Type of Feed

A fish farmer may have been engaged in raising two types of fish species (A and B) using a single type of feed input. The farmer can produce more of either A or B product by reallocating its feed inputs between the two outputs. Graphically, this can be represented by a production possibility frontier (PP') as shown in Fig. 3. A production possibility curve shows all possible combinations of two products (A and B) that can be produced with all inputs available to the firm. When there is a resource constraint on the producer engaged in producing two products, he must find the optimal product mix which brings maximum profits.

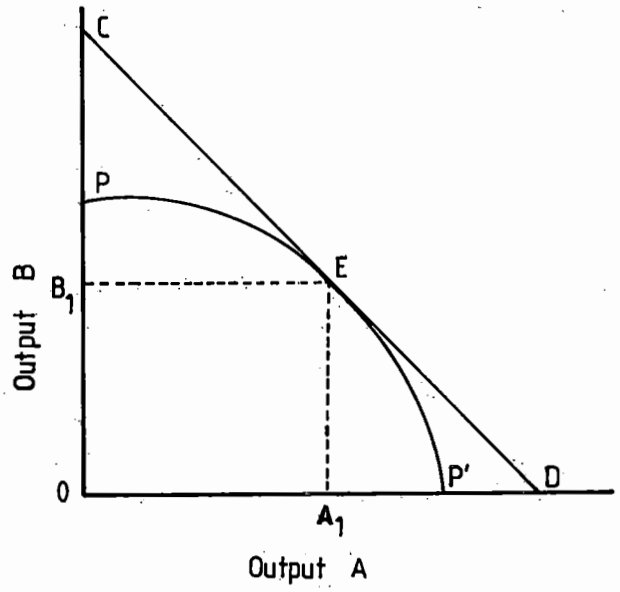

Fig. 3. Determination of optimal product mix.

The slope of the production possibility curve reflects the marginal rate of product substitution (MRPS) which indicates the amount one product (B) changes in quantity when the other product (A) is increased by successive equal units, when total resources used remain constant. MRPS is equal to the ratio of marginal products of the resource used. Thus:

$$MRPS_{A \text{ or } B} = \frac{\Delta B}{\Delta A} = \frac{MP_B}{MP_A}$$

To determine the optimal product mix, we need an additional tool, the isorevenue line which represents all possible combinations of two products (A and B) that, if sold, will give a fixed amount of revenue to the firm. This is drawn as C-D in Fig. 3. The slope of the isorevenue line is determined by the price ratios of the two products P_A and P_B. The isorevenue line may be derived from the equation:

$$R = P_A (A) + P_Y (B)$$

where R is total revenue; P_A and P_B are prices of A and B respectively and A and B are quantities of two products.

Graphically the optimal condition is defined by the point of tangency of the production possibility curve (P-P') and the isorevenue line (C-D). This point of tangency (E) fulfills the profit maximization criterion and leads to the most profitable combination of two products, A_1 and B_1. At the optimal point, the slopes of production possibility curve and the isorevenue line are equal:

$$\frac{MP_B}{MP_A} = \frac{P_A}{P_B}$$

This equilibrium condition known as the principle of equimarginal returns states that the last unit of expenditure (or cost of feed) on producing one product generates the same revenue as it would if applied to any other product, that is:

$$MP_A \cdot P_A = MP_B \cdot P_B.$$

(Allen et al. 1984).

Minimum Cost Feed Formulations: Linear Programming Approach

Linear programming is a class of mathematical programming models concerned with the efficient allocation of limited resources to known activities with the objective of meeting desired goals such as maximizing profit or minimizing cost. The distinct characteristic of linear programming models is that the functions representing the objective and the constraints are linear. Linear programming technique can be applied to the problems dealing with the determination of optimal feed mix for meeting the desired nutritional objectives at the least cost for the aquatic organisms. The application of this technique for optimization problems can best be explained by means of a simple illustration.

Assume for the sake of simplicity, that there are two types of feed stuffs or ingredients from which the supplementary feed is formulated. The nutritional elements to be considered for this formulation are protein and fat. The nutritive content and price of each ingredient are known. The constraint of the model is the minimum daily nutrient requirements for fish. Suppose the daily requirement of supplementary feed mix is 100 kg, now the problem is to determine the combination of two feed ingredients which will satisfy the daily nutrient requirements and entail the least-cost. The above information is summarized in Table 2.

The problem can be stated mathematically as follows:

$$\text{Minimize } C = 0.6X_1 + 0.8X_2$$

Table 2. Hypothetical example of cost and nutritive content of two feed ingredients.

Ingredient	Units per kg of ingredient Protein	Fat	Cost ($) per kg
X_1	0.10	0.40	0.60
X_2	0.50	0.08	0.80
Minimum daily requirement	22%	8%	

Subject to

$$0.40X_1 + 0.08X_2 >/ 0.08 \times 100$$
$$0.10X_1 + 0.50X_2 >/ 0.22 \times 100$$
$$X_1 + X_2 = 100$$

and

$$X_1, X_2 >/ 0$$

The first equation represents the cost function based on price information of feed ingredients and constitutes the objective function of the linear programme. The inequalities that follow are the constraints necessitated by daily requirements of nutrients. Let X_1 and X_2 be the amounts of feed ingredients used in producing 100 kg of feed mix. The last inequality refers to the non-negativity restriction. The above mathematical representation of least cost feed formulation problem can be easily solved with the help of a computer programme which is readily available at present.

Use of Micro Computers

Micro Computers and related software are valuable tools for conducting economic analysis of feed formulation and supplementary feeding trials. They are used for similar research in the livestock and food processing industries (Castle and Becker 1972). They have also been used in aquaculture (Allen et al. 1984). Hand held programmable calculators are often sufficient for analysing results of aquacultural feeding trials where quantity and type of feed are varied (and perhaps one or two other variables i.e. stocking density and species). The micro computer is an efficient way of analysing large quantities of survey data which attempts to identify the contribution of feed and feeding technology to output, using production function analysis (IDRC 1982; Hopkins and Cruz 1982). The authors recommend that fish nutritionists in the economics of supplementary feeding first attempt to involve a national fisheries economist in their work. If that is not possible, then contact a scientific research group known to be involved in livestock nutrition. It is quite likely that they will have an associate agricultural economist involved in related economic analysis or will be using software prepared and/or recommended by the economist for the analysis. Such software could be modified to fish nutrition/feeding research. The exception could be multi-species polyculture aquaculture systems with complex feeding relationship, due to the inclusion of species that feed at different 'levels' of the food chain. Special software would have to be written for these.

Conclusions and Recommendations

Economic analysis of new feeds, changes in feed formulations and methods of feeding are essential before they are recommended for use by aquaculturists. Given the significant cash costs of feeds relative to a producer's total cost, and that often these feeds have potential value in food production (i.e., fertilizer) or as human food their use must be as economic as possible in order to maximize both private and social benefits (i.e., improved nutrition) from increase in finfish production. It is recognized that adequate feeding is essential in order to have sufficient output and revenue to provide returns to other non-feed input use (i.e., fixed costs for pen, cage or pond construction). Supplementary feeding may also be economically justified if it can be

demonstrated to be the most economic means of increasing production, compared to alternative extensive and/or capital intensive means. These latter methods often have associated 'social costs', i.e., environmental, abuse of traditional land/water use rights, income distribution; which can be partially avoided by intensifying economic production from "small scale" rural producers.

Economic analysis techniques are readily available for application to finfish nutrition research. Agricultural economists involved in livestock nutrition or preferably fisheries economists, should be associated with the definition and results analysis (and interpretation of the results) of experiments that evaluate new feed technology. Micro Computer technology is now within the means of most research institutions, and can be used for the economic analysis.

Given the above, there is no reason why the next few years should not see a significant increase in publication of finfish nutrition research results that includes the economic assessment of the new feed technology. Published nutrition research that excludes an economic assessment will be of less direct interest to individual producers, the aquaculture industry, or government, all of whom are potential investors in the new nutrition technology.

References

Allen, P., W. Botsford, A.M. Louis, A. Schuur and W.E. Johnston. 1984. Bioeconomics of aquaculture. Elsevier Science Publishers, Amsterdam, 351 p.

Asimakopulos, A. 1978. Microeconomics. Oxford University Press, Toronto, 450 p.

Brown, E.E. 1983. World fish farming: cultivation and economics. AVI Publishing Company Inc., Westport, 516 p.

Castle, E.N. and H.M. Becker. 1972. Farm business management. Macmillan, New York, 320 p.

Cho, C.Y., C.B. Cowey and T. Watanabe. (eds.). 1985. Finfish nutrition in Asia: methodological approaches to research and development. IDRC (Canada), 154 p.

Cole, H.H. and M. Ronning. (eds.). 1986. Animal agriculture. W.H. Freeman and Company, San Francisco.

Fine, J.C. and R.G. Lattimore. (eds.). 1982. Livestock in Asia: issues and policies. IDRC (Canada).

Heathfield, D.F. 1971. Production functions. Macmillan, London, 91 p.

Herbst, J.H. 1968. Farm management. Stipes Publishing Company, Illinois.

Hopkins, K.D. and E.M. Cruz. 1982. Integrated animal-fish farming project: final report. ICLARM Technical Reports 5. International Center for Living Aquatic Resources Management, Philippines. 96 p.

IDRC. 1982. Aquaculture economic research in Asia: Proceedings of a Workshop Held in Singapore 2-5 June, 1981, 128 p.

Kifle, W.B., G.R. Potts and R.M. Drysdale. (eds.). 1983. By-product utilization for animal production. IDRC (Canada), 158 p.

Pappes, J.L. and E.F. Brigham. 1979. Managerial Economics. Dryden Press, Illinois, 656 p.

Pullin, R.S.V. and Z.H. Shehadeh (eds.) 1980. Integrated agriculture-aquaculture farming systems: ICLARM Conference Proceedings No. 4. International Center for Living Aquatic Resources Management, Philippines. 258 p.

Shang, Y.C. 1981. Agriculture economics. Westview Press, Colorado.

Some Basic Concepts on Fish Disease for Nutritionists

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Disease is one of the most serious limiting factors in the development of aquaculture. Disease is not only restricted to commercial farms which practice high stocking density but also causes mortalities in laboratory and other experimental systems. Interplay between three main factors, the fish (the host), the environment, and the pathogen determines the outbreak of disease. When in a stable situation these factors may interact without resultant disease. However, when there is a change in one or more of the three factors, the delicate balance among them is disrupted and disease may result. Basic understanding of these three factors and their interrelationships is important in planning for prevention and control of disease and in making a correct diagnosis.

Studies in fish nutrition require testing and analysing the performance of diets and this is usually done with the use of live fish. Fish, like any other animal is susceptible to diseases. The outbreak of disease during an experiment can result in the loss of all or some of the test animals, while fish that survive are usually not suitable for future experiments.

It is unfortunate that frequently, nutritionists, when planning experimental designs and methodology do not ensure that experimental animals are disease free and that they will not encounter any disease throughout the study. Lack of awareness of the need for disease free fish and their proper maintenance can lead to an outbreak of disease. Experiments may have to be repeated, doubling or tripling the research budget and costing the nutritionist valuable time.

In some instances, disease occurs due to the use of a new feed which may have either excessive elements or which is lacking in some essential components. In both instances disease may result. Sometimes disease may not be evident due to lack of obvious clinical signs and thus may go unrecorded. Thus it is important to recognise an abnormal situation and to seek assistance to interpret the results correctly. Some knowledge about fish diseases thus forms an integral part of the basic information required by a nutritionist.

This paper will provide guidelines on the possible causes of disease and will discuss how the balance between the environment, host and pathogen can be altered to cause disease. Basic understanding of disease diagnosis, prevention, and some aspects of control will also be discussed.

Causes of Disease

Disease is a complex interaction between fish, disease agent and the environment (Warren 1983). A pathogen can be present on the host, but will not cause disease if the balance among these factors is well maintained. A change in any factor may disrupt the balance of the relationship, resulting in the outbreak of disease. Therefore, it is best to understand the relationship between the three factors involved and the causes that could create an imbalance.

Environment

Water forms the immediate environment that surrounds the fish. Adequate supply of clean water is essential for the maintenance of good fish health. Thus water used in experimental tanks or fish ponds must be of acceptable quality.

pH, light, salinity, alkalinity, temperature, depth of water and other physiological requirements must be maintained within the optimal limits for each host species. In addition, the water must also be free of pollutants, pathogens and their larval stages. Some of the important water quality parameters for fishes are listed in Table 1.

Table 1. Selected environmental parameters for fish health and their recommended safe levels. (Adapted from Munro 1978, Wedemeyer et al. 1976).

Water chemistry	Upper limits for continuous exposure	
	Freshwater	Seawater
pH	6.5-8.5 optimal 6.0-9.0 permissible	+ 0.5 units of normal range
Cadmium (mg/l)	3.0 in hard water	0.2
Chlorine (mg/l)	0.003	-
Chromium (mg/l)	0.05	0.05
Copper	0.1 X LC_{50} of water	0.01 X LC_{50} of water
Cyanide (mg/l)	0.005	0.005
Lead (mg/l)	0.03	0.01
Mercury (mg/l)	0.05	unknown
Nitrite (mg/l)	0.1	0.1
Polychlorinated biphenyls (PCBs)	0.002 ppm	-
Suspended solids		
High level of protection	< 25	The nature of solid material may greatly influence safe level
Moderate level of protection	25-80	
Low level of protection	80-400	
Undissociated ammonia (mg/l)	0.02	0.01
Undissociated hydrogen sulphite (mg/l)	0.002	0.005

Any rapid change in the environment will cause stress to fish. Stress as defined by Brett (1958) ".... is a stage produced by an environmental or other factors which extends the adaptive responses of an animal beyond the normal range, or which disturbs the functioning to such an extent that the chances of survival are significantly reduced." Any form of stress thus increases the susceptibility of fish to disease. Wedemeyer et al. (1976) identified several factors which in their opinion can cause stress and hence predispose fish to disease. These factors are: handling, crowding, low oxygen levels, low temperatures, elevated ammonia, low total water hardness, excessive size variation of fish in ponds, chronic sublethal levels of heavy metals, pesticides and suspended solids. Besides these factors, Ingram (1988) reported that uneven distribution of feed among fish can also cause stress.

Sudden drastic variations in temperature over short intervals or a wide range of changes over 24 hours will cause stress. Low temperatures over a long duration (e.g., during the rainy season) or a hot dry period can also cause stress. High rates of ulceration were observed in situations where there was rapid change in salinity and temperature (Rodgers and Burke 1981). Extremes of salinity and temperature lead to high malformation rates in fish larvae (Koo and Johnston 1978). The author has frequently observed the occurrence of white spot disease in his experimental tanks during the rainy season, when the water temperature was 2-3°C below the normal range. A survey on fish mortalities at the breeding stations in Malaysia revealed that higher mortalities were encountered during periods of high temperature fluctuation (Shariff and Vijarungam 1986). Thus in tropical countries, where temperature fluctuation is diurnal and seasonal (wet or dry), temperature should be considered as an important factor in inducing disease outbreaks.

Several examples will illustrate a wide range of stress factors and their effects on fish. Low oxygen levels in conjunction with high ammonia levels were reported to cause severe ulcerations and mortalities in a riverine fish population (Shariff and Law 1981). Milkfish *Chanos chanos* fingerlings, when subjected to stress during transport due to high stocking density, a drop in dissolved oxygen levels, increased water turbidity and abrupt changes in salinity suffered increased susceptibility to secondary infection by bacteria and subsequent mortalities (Llobrera 1987). Environmental gill disease is considered to be caused by high population density, low dissolved oxygen levels, water temperature fluctuation, and environmental pH (Snieszko 1974). High temperatures (34-35°C), and on another occasion, oxygen depletion as a result of pollution and silting, resulted in mass mortalities in a reservoir in Sri Lanka (Balasuriya 1987).

Poor water quality, a result of the breakdown of a circulation water pump, resulted in low digestibility of feed in *Leptobarbus hoeveni* (Bleeker) (Dr. A.T. Law, pers. comm).

Pathogens

Many of the pathogens that cause disease in fishes are normally present in the water, host, substrate or the pond bottom. They are mainly facultative and will cause disease only when the host is under stress. Some pathogens however, are virulent and will cause disease on contact with the host. Pathogens can be categorised into bacteria, fungi, parasites and viruses. Many of these pathogens cause infectious diseases which can spread with ease via water, or by direct physical contact.

The most commonly reported diseases of fish in the South and Southeast Asian region are caused by ectoparasites and bacteria (Arthur 1987). Recently a virus has also been implicated as a cause of the wide spread epizootic ulcerative syndrome (Roberts et al. 1986). Ectoparasites, mainly protozoans and monogeneans, are more frequently the cause of mortalities than are bacteria. The common ectoparasites in South and Southeast Asia are the protozoans, *Ichthyophthirius, Cryptocaryon, Trichodina* and related genera, *Chilodonella, Piscinoodinium, Ichthyobodo, Epistylis* and *Zoothamnium,* the monogeans and the crustaceans, *Dactylogyrus* and *Gyrodactylus, Argulus, Lernaea, Ergasilus* and *Nerocila* (Arthur 1987).

Ubiquitous protozoans,when present in small numbers, do not cause measurable harm to the host. However, when the host is subjected to stress, these parasites may multiply and cause disease. *Tetrahymena* have been reported to cause 100% mortalities in fish larvae in which feeding was delayed by 3-5 days in an experimental trial (Dr. P. Arumugam pers. comm.). Death in these instances, usually occurs due to hypoxia, i.e., when large numbers of parasites cover the gill filaments completely. *Chilodonella* infection causing mortalities was reported to be associated with incorrect feeding (Shariff 1984).

Some parasites can cause severe damage to host tissue during feeding or while penetrating the host. These parasites, when present even in small numbers can cause severe mortalities of fish fry. Invasion by other parasites that do not cause mortality can result in slow growth rates; for example fish infected with *Lernaea,* have been reported to have 35% lower growth rates as compared to non-infected fish (Shariff and Sommerville 1986).

Bacteria, such as *Aeromonas hydrophila, Pseudomonas fluorescens,* and *Flexibacter columnaris* are commonly isolated from freshwater fishes, while *Vibrio parahaemolyticus* and *V. alginolyticus* are typically recovered from marine fishes (Leong 1987). However, none of these bacteria have been implicated as primary pathogens. The occurrence of vibriosis is generally secondary in nature, occurring as a sequel to trauma or primary infection by Protozoa (Anon 1987).

Aeromonas hydrophila, which is always isolated from fish with epizootic ulcerative syndrome (EUS), is believed to be a secondary pathogen. In Malaysia, EUS occurs during the drought season when water temperatures are higher than normal. In contrast the same disease is prevalent during cool temperatures in Thailand and the Philippines. Thus occurrence of EUS is believed to be the result of depression of the immune system of the host due to viral infection which leads to the outbreak of disease during adverse environmental conditions. Bacteria and ectoparasites are secondary infectious agents.

The author has also observed the frequent occurrence of bacterial infections in the presence of abundant organic matter together with high stocking densities in pond and tanks. Organic matter in these situations is either accumulation from droppings or excess feed.

Host

When the host is provided clean water and high quality nutrition and in the absence of stress factors or virulent strains of pathogens, it will usually not succumb to disease. Fish have several non-specific defence mechanisms that provide protection against infectious agents. The mucus covering the body contains lysozymes which provide protection against microbial pathogens (Ellis 1978). Removal of mucus due to rough handling can provide an opportunity for microbes to invade the host. Several other chemicals and immune mechanisms that provide protection to the host are described by Ellis (1978). In addition, it is also worth mentioning that inflammation, a very common phenomena, is among the first line of defence that provides protection to the host. Inflammation is the reaction of the host's tissue to any form of irritation. The series of changes that takes place during inflammation helps dilute the effect of the irritant (infectious agent), restrict its spread to surrounding tissue and restore the injured tissue to its normal form.

The fish in its healthy state is thus able to protect itself against microbial invasions. It is when the host is subjected to stress that its defence mechanisms respond poorly and, as a consequence, the host succumbs to disease. Stress causes immunodepression due to release of corticosteriod hormones (Ellis 1981). Temperature also has a marked effect on the immune response in fish. Optimum temperatures are reported to produce the best response in vaccination of fish (Ellis 1982) while low temperatures delay or completely inhibit antibody production (Ellis 1978). Other stress factors that suppress antibody production are high stocking density, handling and abnormal light conditions (Ellis 1978).

Nutritional Disorders

Nutritional requirements differ with species and age of fish. If an unbalanced diet is provided, the fish may not feed and die of starvation. The various components of the diet are also

important factors for the normal growth of fish. Essential amino acids, vitamins, essential fatty acids and minerals have to be given in the right combination for a particular species and age of fish. Malnourished fish cannot maintain good health regardless of the quality of the environment.

Fish subjected to nutritional deficiencies or imbalances of diet may not show obvious symptoms and the condition may go undetected. Under these circumstances, if the host is infected by secondary infection with biological agents, the situation can be misinterpreted. Thus the nutritional condition could be camouflaged and diagnosed as a disease caused by a secondary pathogen. Lipoid liver degeneration, avitaminosis, toxicity of minerals, mycotoxins and bacterial toxicosis are some of the conditions frequently described. Cowey and Roberts (1978) have described the causes of nutritional diseases.

Transmission of Diseases Via Feeding Trash Fish

Fish feeds could also be a source of biological agents causing diseases. Diseases that can be transmitted by feeds are viral haemorrhagic septicaemis, mycobacteriosis (tuberculosis), furunculosis, vibriosis, ichyophoniasis, amebiasis, capillariosis and acanthocephalosis (Ghittino 1979).

Recognizing Disease Conditions

In the event of an outbreak of disease, one should be able to recognise the disease condition, its probable cause or causes and take necessary measures so as to ensure that it does not occur again.

The most common symptoms in many disease conditions are:

1. Anorexia - i.e., the fish does not feed.
2. Lethargy.
3. Loss of colour or darker body colour.
4. Change in swimming behaviour.
5. Loss of weight.

Since most of the symptoms mentioned are common to many disease conditions, it is important that a thorough investigation be conducted before any conclusions are made as to the cause or causes of a disease.

A basic understanding of the time frame of mortalities and its causes will be useful.

1. Preacute: Sudden mortalities without any prior clinical signs.

   High mortalities over short periods are usually due to sudden changes in environmental factors which can lead to severe stress, e.g., salinity or temperature change. Other common causes of preacute mortalities are:

   a) hypoxia, due to low oxygen levels,
   b) toxic conditions such as high ammonia levels,

c) other toxic chemicals such as pesticides, and
d) viral infection.

2. Acute: Mortalities occur within two or three days on the appearance of clinical signs.

The following are the common causes of acute mortalities:

a) lower degree or levels of physicochemical factors such as those causing preacute mortalities,

b) biological agents; viruses, bacteria and ectoparasites,

As the time frame indicates, acute and preacute mortalities can cause severe losses in short periods without advance warning.

Initially virulent strains of pathogens cause severe mortalities when first introduced into a population, but after some time the host adapts or acquires resistance against them. These strains will then cause disease only when the host is exposed to other stress factors.

Many of the protozoan ectoparasites multiply by binary fission and several thousand parasites can develop within a short period. Some protozoans can cause suffocation when present in large numbers on the gills. On the other hand, some ectoparasites, e.g., *Lernaea*, can also cause tissue damage and in small fish only a few are needed to cause death. The other groups of ectoparasites are the monogeneans and the crustaceans.

3. Chronic: Incidences of mortalities are spread over a long period, a few weeks or months, after the appearance of clinical signs. This pattern of mortality may be caused by:

a) endoparasites,

b) nutritional diseases,

c) mycobacteriosis (piscine tuberculosis),

d) less deleterious levels of physicochemical factors as compared to those found to cause acute mortalities, and

e) *Ichthyophonus* (fungal infection)

Diagnosis

There are many causes for acute and chronic mortalities and the symptoms seen may differ or be similar for different diseases. Anorexia and retarded growth may be the only symptoms seen for nutritional diseases and these symptoms may seem similar to those for other chronic diseases. Thus it would be appropriate to obtain the assistance of a specialist to investigate and diagnose the condition.

1. Diagnosis of a disease condition is not a simple procedure. It involves a systematic approach whereby all possible causes are kept in mind and all aspects are examined to

pinpoint the cause or causes of the disease. The systematic approach is the golden rule to correct diagnosis.

2. The systematic approach includes the following studies;

   a) investigation of environmental factors, water quality and stress related factors;

   b) investigation for possible biological agents (parasites, bacteria, viruses and fungi); and

   c) histopathology.

   In many instances histopathological studies are used to confirm the diagnosis. Most nutritional deficiency conditions can be diagnosed by histopathological studies.

3. The cause of preacute mortalities is difficult to diagnose. In most instances, by the time attempts are made to examine the case, the evidence is lost. However, efforts should be made to examine the environment thoroughly for changes in oxygen levels, pH, ammonia levels, salinity and other water quality parameters. Minor changes in water quality may escape detection when examined by crude methods such as the HACH kit, though such changes may result in mortalities in fingerlings, which are sensitive to minor changes. It it important to monitor the environmental parameters regularly so as to be familiar with normal standards. This will also allow detection of abnormal fluctuation with ease.

4. Although ectoparasites can be easily identified by preparing a wet mount of the mucus from the skin and gills of the infected fish, their presence may not necessarily mean that they are the primary cause of disease. The opinion of a specialist should always be sought.

5. Selection of a laboratory where samples can be submitted for diagnosis is also important. For a systematic approach, it is important that samples be submitted to a laboratory which is equipped to study all possible causes of disease. An ideal laboratory should have facilities for water quality determination, parasitology, pathology, bacteriology, mycology and virology. In instances where some of these facilities may not be available, samples should be also sent to other laboratories to have a complete result.

6. Information on the history of the disease condition contributes significantly towards making a correct diagnosis. The following are some important particulars that should be provided to the laboratory:

   a) source of fish fingerlings,

   b) age group affected,

   c) clinical signs,

   d) percentage affected,

   e) percentage mortalities (preacute, acute, or chronic),

f) feeding habits,

g) type of feed provided,

h) water quality,

i) pond condition, and

j) other relevant information such as stocking density, etc.

7. All fish samples must be sent live to the laboratory. These should be packed in the same water as the fish were collected from. The laboratory should be informed of the shipment of samples before hand so that arrangements can be made to examine them immediately upon arrival. Never send dead fish to a laboratory for diagnosis.

8. In circumstances where live fish cannot be sent to the laboratory, live fish should be sacrificed and fixed immediately in 10% buffered formalin (40% formaldehyde 100 ml, distilled water 900 ml, $NaH_2PO_4H_2O$ 4g, Na_2HPO_4 6 g). The tissue should be no larger than 5 mm thick and should be from all organs, including the gills, skin, eyes and the alimentary tract. These should be placed in a bottle containing 10% buffered formalin which is at least 10 times the volume of the tissue, labelled correctly and sent to the laboratory. Do not attempt to substitute normal 10% formalin for buffered formalin as this may give rise to artifacts in the sections and may result in an inconclusive diagnosis.

Prophylaxis

With knowledge on the factors involved in the outbreak of diseases, one should be able to take precautionary measures to minimize the occurrence of disease. The following are some guidelines which should be made a common practice.

1. *Use clean fish*

It is important to start an experiment with fish that are free of pathogens. Viral infections are particularly difficult to diagnose and the host may be a carrier showing no signs of the disease condition. Under these circumstances if would be advisable to obtain fish from a stock that is known to be free of virus.

2. *Treat for ectoparasites*

Since ectoparasites are a common cause of mortalities in the region, all fish for experimental purposes must be treated to remove them. Simple dip or bath in chemical solution will rid the host of most ectoparasites. Formalin at 100 ppm/hr (10 ml/100 l) or 50 ppm/24 hr (5 ml/100 l) has been used in our laboratory routinely to remove ectoparasites.

3. *Hygienic measures*

During the experiment, care must be taken to ensure that no new pathogens are introduced via new introductions of infected fish or via net or by other means. Strict hygienic measures,

such as the use of individual nets for each tank and discouraging visitors and animals to the laboratory must be adopted to ensure that no new pathogens are introduced into the system.

4. *Ideal system*

a) Since the environment, the pathogen and the host are interrelated in the outbreak of disease, it is important to consider the three factors and other related factors that could cause their imbalance in designing a system.

b) The system should, as far as possible, meet all physiological requirements for fish. Good water quality that is free of pathogens must be a priority. It is critical in the hatchery that more emphasis should be placed on the water quality because fingerlings are sensitive and easily susceptible to diseases. It is important that water is filtered to remove organic matter together with larval and adult stages of pathogens. The water may also be treated with ultraviolet light to remove smaller pathogens which cannot be removed by gravel filter.

c) It is important to have an area for quarantine where new fish can be examined and treated before they are introduced into ponds or mixed with other fish.

5. *Good management*

a) Tanks should be disinfected and dried after every experiment. Similarly, ponds should be dried and treated with lime. Maintain ideal condition to avoid all possible stress factors affecting fish.

b) As far as possible avoid using antibiotics for prophylaxis. Constant use of antibiotics usually results in development of resistant strains.

c) Wild fish, and other animals that are intermediate or final host (snails, birds and mammals) should be kept out of the ponds,

d) Broodstock and fingerlings or fry should be maintained separately. Broodstock which may carry disease with no apparent symptoms are a health hazard when kept together with young fish.

e) Never try to look for a disease causing agent without giving consideration to other factors, such as the environment, which may be causing stress to the host.

f) Treatment, without proper diagnosis may further aggravate the situation, as addition of chemicals into water causes further stress on fish. In a pond condition, chemotherapy may not be practical due to the large surface area. Prevention is always better than cure.

g) During an outbreak of disease, as far as possible avoid handling the fish, as this will subject them to additional stress.

h) Unnecessary stress and injury to fish can be avoided by using an anaesthestic agent such as MS-222 (tricaine methane sulphonate), quinaldine (2 methylquinone) or benzocaine before handling or during transport.

In Case of Emergency

Disease that results in mortalities can occur at any moment without prior warning. Losses can be minimized by following the guidelines set below:

1. If there is an outbreak of disease, get immediate help from a disease specialist. If a specialist is unable to come immediately provide details over the phone to get instructions on how to reduce the mortalities.

2. In the case of ectoparasitic infection, flushing of the pond or tank will remove the larval stages and thus prevent further infection.

3. Increased aeration will provide more dissolved oxygen and help fish with infected gills in uptake of oxygen, thus reducing a prime stress factor.

4. Reduce stocking densities. This will help prevent the spread of infectious disease through physical contact and will also reduce the possibility of fish being subjected to stress.

5. Check for all possible stress factors such as high organic level and drastic fluctuation in temperature and take appropriate measures to overcome the problem. It is important that stress factors be removed before any chemicals are introduced into the pond/tank for treatment. Introduction of chemicals without prior removal of stress factors will further increase mortalities.

6. Maintain records of all activities (observations, treatment, dosage applied, etc.) as they will serve as a valuable reference for the future.

Summary

Successful disease control involves an understanding of the basic concepts of fish diseases and a careful program of fish health management that will prevent the introduction of infectious agents and reduce stress factors by maintaining optimum conditions. The outbreak of disease may indicate poor fish health management.

The systematic approach is essential for a correct diagnosis and in many instances, the disease may not be primarily caused by an infectious agent but by a complex interaction between host, environment and the pathogen.

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References

Anonymous. 1987. Current status of problems related to mortalities in farmed marine food fish in Singapore. *In* J.R. Arthur (ed.), Fish quarantine and fish diseases in south and southeast Asia: 1986 update. Asian Fisheries Society Special Publication No. 1, pp. 29-35. Asian Fisheries Society, Manila, Philippines.

Arthur, J.R. 1987. Fish quarantine and fish diseases in South and Southeast Asia: 1986 update. Asian Fisheries Society Special Publication No. 1, 86 p. Asian Fisheries Society, Manila, Philippines.

Balasuriya, L.K.S.W. 1987. Current fish disease problems in Sri Lanka. *In* J.R. Arthur (ed.) Fish quarantine and fish diseases in Southeast Asia: 1986 update. Asian Fisheries Society Special Publication No. 1, pp. 36-40. Asian Fisheries Society, Manila, Philippines.

Brett, J.R. 1958. Implications and assessments of environmental stress in the investigation of fish power problems. H.R. Mac Millan lectures on fisheries, University of British Colombia.

Cowey, C.B. and R.J. Roberts. 1978. Nutritional pathology of teleosts. *In* R.J. Roberts (ed.) Fish Pathology, pp. 216-226. Bailliere Tindall, London.

Ellis, A.E. 1978. The immunology of teleosts. *In* R.J.Roberts (ed.) Fish pathology, pp. 92-104. Bailliere Tindall, London.

Ellis, A.E. 1981. Stress and the modulation of the immune response in fish. *In* A.D. Pickering (ed.) Stress and Fish, pp. 147-169. Academic Press, London.

Ellis, A.E. 1982. Differences between the immune mechanisms of fish and higher vertebrates. *In* R. J. Roberts (ed.) Microbial diseases of Fish, pp. 1-29. Academic Press, London.

Ghittino, P. 1979. Fish disease vectors in feed. *In* J.E. Halver and K. Tiews (eds.) Finfish nutrition and fishfeed technology. Vol. 11, pp. 330-333. Satz and Druck, H. Heenemann GmbH & Co, Berlin.

Ingram, I. 1988. The stress factor: fish or farmer? Fish Farming International 15: 36-37

Koo, T.S. and M.L. Johnston. 1978. Larval deformity in striped bass, *Morone saxatilis* (Walbaum), and blueback herring, *Alosa estivalis* (Mitchill), due to heat shock treatment of developing eggs. Environmental Pollution 16: 137-149.

Leong, T.S. 1987. Recent fish disease problems in south and southeast Asia and their economic impact. *In* J.R. Arthur (ed.) Fish quarantine and fish diseases in South and Southeast Asia: 1986 update, pp. 1-3. Asian Fisheries Society Special Publication No. 1. Asian Fisheries Society, Manila, Philippines.

Llobrera, A.T. 1987. Current fish disease problems in the Philippines and their eonomic impact. *In* J.R. Arthur (ed.) Fish quarantine and fish diseases in South and Southeast Asia: 1986 update. Asian Fisheries Society Special Publication no. 1, pp. 22-28.

Munro, A.L.S. 1978. The aquatic environment, p. 1-12. *In* R.J Roberts (ed.) Fish Pathology. Bailliere Tindall, London.

Rodgers, L.J. and J.B. Burke. 1981. Seasonal variation in the prevalence of "red spot" disease in estuarine fish with particular reference to the sea mullet, *Mugil cephalus* L. Journal Fish Diseases 4: 297-307.

Roberts, R.J., J. Macintosh, K. Tonguthai, S. Boonyaratpalin, N. Tayaputch, M.J. Phillips and S.D. Millar. 1986. Field and laboratory investigations into ulcerative fish disease in the Asian-Pacific Region. Tech. Rep. FAO Project TCP/RAS/4508, Bangkok. 214 p.

Shariff, M. and A.F. Vijiarungam. 1986. Occurrence of parasites at the fish-breeding stations in Peninsular Malaysia and their control. *In* Chan Hooi Har, Ang Kok Jee, Law Ah Theem, Mohd Ibrahim Hj. Mohamed, Ishak Hj. Omar (eds.) International Conference on Development and Management of Tropical Living Aquatic Resources, pp. 68-73, University Pertanian Malaysia, August 2-5, 1983.

Shariff, M. 1984. Occurrence of *Chilodonella hexasticha* (Kiernik 1909) (Protozoa, Ciliata) from big head carp *Aristichthys nobilis* (Richardson) in Malaysia. Tropical Biomedicine 1: 69-75.

Shariff, M. and A.T. Law. 1981. An incidence of fish mortality in Bekok River, Johore, Malaysia. *In* M. Nordin, A. Latiff, M.C. Mahani and S.C. Tan (eds.) "Proceedings of International Symposium on Conservation Inputs from Life Sciences", pp. 153-162. University of Kebangsaan Malaysia, Bangi, Malaysia.

Shariff, M and C. Sommerville. 1986. Effects of *Lernaea polymorpha* on the growth of bighead carp, *Aristichthys nobilis*. *In* M.J. Howell (ed.) Handbook of Sixth International Congress of Parasitology, p. 227. Australian Academy of Science. (abstract)

Snieszko, S.F. 1974. The effect of environmental stress on outbreak of infectious diseases of fishes. Journal of Fish Biology 6: 197-208.

Warren, J.W. 1983. The nature of fish diseases. *In* F.P. Meyer, J.W. Warren and T.G. Carey. (eds.) A guide to intergrated fish health management in the Great Lake Basin. Great Lakes Fisheries Commision, Michigan. Special Publication No. 82-3, pp. 7-13.

Wedemeyer, G.A., Meyer, F.P. and L. Smith. 1976. Environmental stress and fish diseases. *In* S.F. Sneiszko and H.R. Axelrod (eds.) Diseases of Fishes. T.F.H. Publications Inc., Ltd. Neptune City.

Part II

Nutritional Evaluation of Sri Lankan *Artemia parthenogenetica* for Use in Larval Rearing

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In Sri Lanka *Artemia parthenogenetica*, a parthenogenetic strain, is found in solar salt ponds in Hambantota (6°8'N;81°7'E). Cyst production from 83ha of salt pans was 256 kg and 309 kg wet weight in 1986 and 1987, respectively. Cyst diameter was 267.9 µm and length of Instar 1 nauplius was 475.4 µm. Hatching percentage of cysts was 77.9 and hatching efficiency was 175,360 nauplii/g product.

The protein content of cysts was 45%, essential amino acids were present at 51.8 g/100 g protein and total amino acids amounted to 43.9 g/100 g cysts. Freshly-hatched nauplii contained 33.5% total lipids. The total amount of ω3-Highly Unsaturated Fatty Acids (HUFA > 20:3ω3) in nauplii was 22.8 mg/g dry weight. HUFA 20:5ω3 considered essential in the nutrition of marine fish and crustacean larvae was present at 15.8 mg/g dry weight. HUFA 22:6ω3 generally absent in most strains, was present at 0.9 mg/g dry weight. On the basis of the above results, the Sri Lankan Artemia can be classified as of the 20:5ω3 type (or the marine type) and is considered suitable for marine fish and crustacean larvae.

The last few years have witnessed the expansion of fin-fish and shellfish culture to an industrial scale in developed as well as in developing countries (Versichele et al. 1985). The development of the aquaculture industry was hampered for a considerable period due to the inconsistent supply of naturally available fry. This has been overcome to a large extent with development of hatching techniques so that a reliable supply of hatchery reared fry is increasingly becoming available to the commercial aquaculturist. The brine shrimp *Artemia* (Anostraca, Artemiidae), has been widely used as a suitable and convenient source of nutrition for the successful rearing of fish and shellfish fry in hatcheries (Kinne 1977; Leger et al. 1986; Sorgeloos 1980; Versichele et al. 1985). *Artemia* is available in many parts of the world, yet important biological and nutritional quality variations have been found to exist among strains from different geographical origins (Schaeur et al. 1980; Vanhaecke and Sorgeloos 1980; Leger et al. 1986). *Artemia* cysts are imported into Sri Lanka at a high cost - in 1986 imports to a value of Sri Lankan Rupees 6.56×10^6 (US$ 2.2×10^5) have been recorded[a].

[a]External Trade Statistics, 1986, Sri Lanka Customs.

The present study evaluates the nutritional quality, hatching characteristics and availability of *Artemia parthenogenetica* cysts in a saltern in Sri Lanka with a view to provide culturists with a suitable low-cost feed.

Materials and Methods

The Mahalewaya salterns at Hambantota, located in the southern coast of Sri Lanka (6°8'N;81°7'E) is a major solar saltern of 265 ha. Investigations were carried out in three shallow condenser pans of 42.5, 20.8 and 19.6 ha, respectively. The mean depth of water in these ponds during the dry production months was 15 cm. Cysts were collected in 1986 (MLI) and in 1987. The results relating to nutritional quality, biometrics and hatching are based on cysts collected in 1986 (MLI).

Artemia cysts were harvested when available in 1986 and 1987 during the morning hours using hand nets of 220 µm mesh. The wet weight of harvested cysts was recorded and the cysts were stored at the salterns in a cement tank containing super-saturated brine, and later transported to the laboratory for processing and analysis.

Brine shrimp cysts were hydrated with diluted sea water (10 ppt) for two hours and kept overnight after addition of 1m1 1% Lugol solution (Vanhaecke et al. 1980). The diameter of 100 cysts was determined using a micrometer eyepiece. Cysts were decapsulated following the procedure given by Sorgeloos et al.(1986). Decapsulation solution was made of 71 g bleaching powder, 67 g Na_2CO_3 and 1400 ml sea water per 100 g cysts. For estimating naupliar length, Instar 1 larvae were obtained by incubating hydrated cysts in normal sea water (35 ppt) at 25°C under constant illumination, gentle aeration and a pH of 8.2. The body length of 100 larvae was measured microscopically using a micrometer eyepiece.

The hatching percentage and hatching efficiency (number of nauplii/g cysts) were estimated using the methods given by Sorgeloos et al. (1986).

The protein content of decapsulated *Artemia* cysts, and the amino acid content were analysed at the Laboratorium voor Bromatologie, Ghent, Belgium. Lipids were extracted and the fatty acid composition determined according to the method of Leger and Candreva (1987) at the *Artemia* Reference Centre, Ghent, Belgium.

Results

The salterns yielded a total of 256 kg wet weight of cysts from June to October 1986. In 1987, a total 309 kg wet weight of cysts was collected from April to October. Cyst production and salinity ranges during the months of cyst production in 1986 and 1987 are shown in Fig. 1 and some relevant data on the cysts and nauplii are summarized in Table 1. The amino acid profile is presented in Table 2. The total amino acid content was 43.96 g/100 g cysts (decapsulated), and the essential amino acids (EAA) 51.8 g/100 g protein. Table 3 gives the Fatty Acid Methyl Ester (FAME) composition of freshly-hatched nauplii. The total amount of ω3-Highly Unsaturated Fatty Acids (HUFA > 20:3ω3) amounted to 22.8 mg/g dry weight. HUFAs *20:5ω3* and *22:6ω3* were present at 15.8 and 0.9 mg/g dry weight, respectively. *18:3ω3* was low at 0.7 mg/g dry weight in nauplii.

Discussion

Artemia cyst production season in the Mahalewaya coincides with the season of salt production. In the Mahalewaya, the average cyst production was 3.1 and 3.73 kg/ha in 1986 and

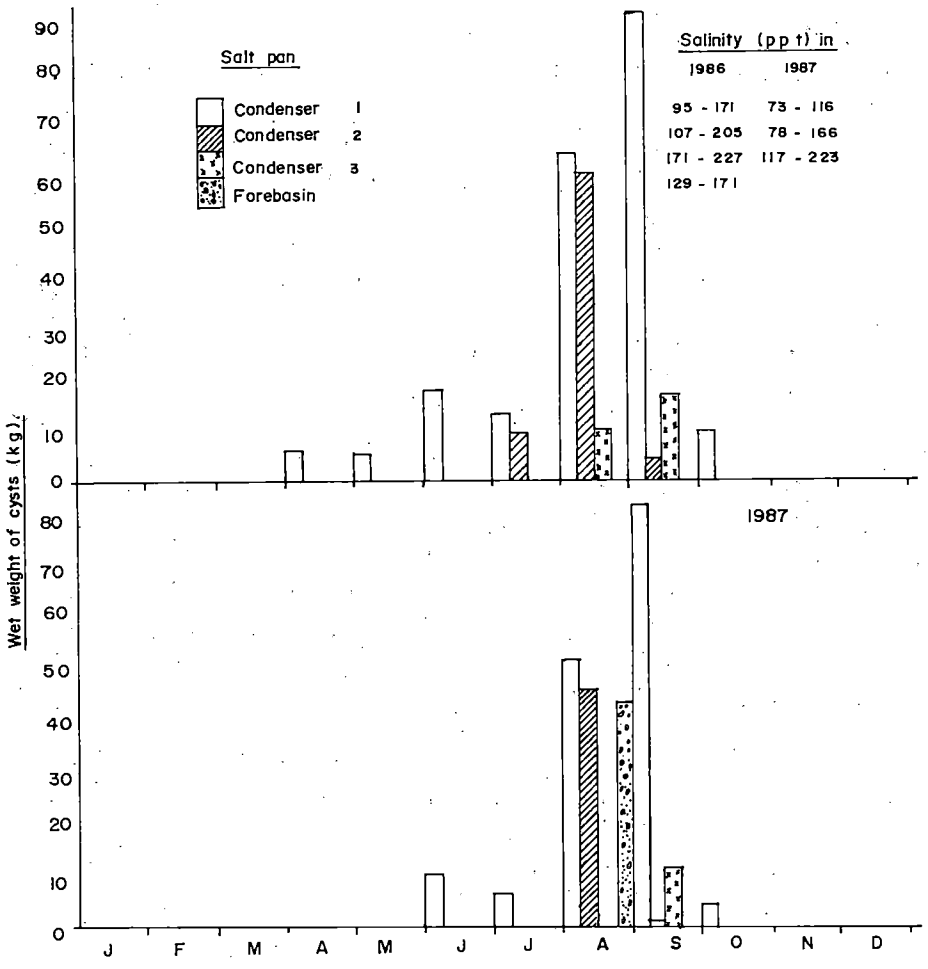

Fig. 1. Production of *Artemia* cysts and salinity (ppt) values in four salt pans in the Mahalewaya salterns of Hambantota, Sri Lanka.

Table 1. Biometrics, hatching characteristics and food quality data for *Artemia parthenogenetica* from Sri Lanka.

Feature	1	2	3
Diameter (µm)	267.9	256.2	-
Length (µm)	-	-	475.4
Hatching (%)	77.9	-	-
Hatching Efficiency (nauplii/g cysts)	175.360	-	-
Protein (%)	-	45.0	-
Total lipid (%)	-	-	33.5

1, 2 and 3 refer to hydrated cysts, decapsulated cysts and instar 1 nauplii, respectively.

1987 respectively. Persoone and Sorgeloos (1980) suggested a production of 10-20 kg cysts/ha/season from a good *Artemia* biotope. The low production reported here may be attributed to the fact that the condenser pans in the Mahalewaya are shallow resulting in high production of phytobenthos which on breaking float towards the surface hindering phytoplankton productivity.

Table 2. Amino acid composition of decapsulated cysts of *Artemia parthenogenetica* from Mahalewaya salterns in Hambantota, Sri Lanka.

Amino Acid	g/100 g cysts	g/100 g protein
Asp	3.76	8.57
Thr	2.09	4.75
Ser	2.72	6.18
Glu	6.12	13.95
Pro	2.06	4.70
Gly	2.18	4.96
Ala	2.14	4.87
Val	3.51	7.98
Cys	0.30	0.68
Met	1.23	2.79
Ile	2.13	4.86
Leu	3.29	7.49
Tyr	1.75	3.98
Phe	2.0	4.55
Orn	0.11	0.26
Lys	3.68	8.39
His	1.11	2.52
Arg	3.73	8.50
Ess. AA	22.77	51.83
Tot. AA	43.91	99.98

Table 3. Fatty Acid Methyl Ester (FAME) composition of *Artemia parthenogenetica* nauplii. (Nauplii were hatched from cysts produced in Mahalewaya Salterns in Hambantota, Sri Lanka ; HUFA > 20:3ω3 = 22.8 mg/g dry weight).

FAME	mg/g Dry weight	FAME	mg/g Dry weight
14:0	1.8	19:4	0.6
14:1	1.0	20:0	1.4
15:0	0.9	18:3ω6	0.6
15:1	1.2	20:1	0.6
14:2	0.4	18:3ω3	0.7
16:0	17.2	18:4	3.9
16:1ω7	12.8	20:3ω6	0.2
17.0	1.9	20:3ω3	0.4
16:2	5.8	20:4ω6	0.2
16:3	4.4	20:4ω3	5.6
17:1	0.3	22:1	0.2
18:0	5.7	21:5	0.2
18:1ω9	24.6	20:5ω3	15.8
18:1ω7	12.9	22:5ω3	0.1
18:2	7.9	22:6ω3	0.9

Sorgeloos et al. (1986) recommended a minimum water depth of 40-50 cm for ponds of 100-180 ppt range for *Artemia* production. Also constant flooding of condenser pans with low salinity water during the salt production season creates sudden decreases in salinity and may lead to lowered cyst production (Sorgeloos et al. 1986). The cyst dimensions may vary considerably between strains and even among batches of the same strain (Vanhaecke and Sorgeloos 1980), the cyst diameter varying from 223.9-284.9 µm. The diameter of MLI cysts (267.9 µm) is smaller than those reported for the parthenogenetic strains from India-Tuticorin (283.8 µm) and Italy-Margherita de Savoia (MS) (284.9 µm). It has been noted (Vanhaecke and Sorgeloos 1980) that the characteristic of a large cyst size in parthenogenetic strains may be correlated with the degree of ploidy. The diameter values of decapsulated cysts are also higher in the parthenogenetic strains. Vanhaecke and Sorgeloos (1980) found a variation of 428-517 µm in naupliar length of various geographical strains. The naupliar length of 475.4 µm is larger than those reported for most bisexual strains. Experiments on *Menidia* larvae by Beck et al. (1980) showed that the larvae grew faster when fed with large nauplii from MS, great Salt Lake (GSL) and Shark Bay (Australia), as compared to those fed with smaller nauplii from San Francisco Bay (SFB) and Macau (Brazil). According to Beck and Bengtson (1982) 20% or more mortality could be expected when nauplii larger than 480 µm were fed as a first food to fish larvae.

The hatching percentage of 77.9 compares well with values recorded for other strains, most of which are above 70% except for Chaplin Lake cysts having a considerably low value 19.5% at 35 ppt salinity (Sorgeloos et al. 1986). Sorgeloos et al. (1978) considered the hatching efficiency as the weight of cysts required to produce a million nauplii. Accordingly, Sri Lankan *Artemia* has a hatching efficiency of 175,360 nauplii/g product and 5.7 g cysts are needed to obtain one million nauplii and this hatching efficiency is lower than that reported for most bisexual strains. Low hatching efficiency is attributed to the larger cyst size associated with parthenogenetic strains (Sorgeloos et al. 1986).

Nutritional quality of *Artemia* for marine and freshwater fish and shellfish is well documented (Beck et al. 1980; Johns et al. 1980; Seidel et al. 1980; Fenucci et al. 1981; Juario et al. 1985; Leger et al. 1985; Van Ballaer et al. 1985). The protein content ranges from 37.4-71.4% in various *Artemia* strains (Leger et al. 1986).

Most of the values estimated for amino acids in Sri Lankan *Artemia* compared favourably with those reported by Seidel et al. (1980) for *Artemia* from different geographical locations, while lower values were found for the EAAs leucine, tyrosine, phenyl alanine, histidine, lysine and arginine. Valine was exceptionally high at 7.98 g/100 g protein. Watanabe et al. (1978a) found 3.2 g valine/100 g protein in *Artemia salina*. In the Sri Lankan *Artemia,* all the amino acid values specially the EAA values were higher than those estimated by Watanabe et al. (1978a) for *A. salina* and other living organisms used as food in fish culture in Japan.

Freshly hatched nauplii of MLl cysts contained 33.5% total lipids. This is higher than 23.3% (Royan 1980) recorded for the India-Tuticorin parthenogenetic strain. The lipid content of nauplii from various strains range from 11.6 to 30.0% (Leger et al. 1986).

The nutritional quality of *Artemia* has been assessed based on the HUFA content of the various strains. Accordingly *Artemia* has been classified into two types: one with a large amount of the HUFA *18:3ω3* which is required for freshwater fish and the other with a large amount of *20:5ω3* essential for marine fish (Watanabe et al. 1978b).

ω3-Fatty Acid Methyl Esters (FAME > *20:3ω3*) are considered to be Essential Fatty Acids (EFA) for marine fish and prawns. The present results indicate a high total value of 22.8 mg/g dry weight of ω3-FAME content in the Sri Lankan *Artemia* nauplii. Leger et al. (1985) observed 2.8 mg/g dry wt. of total ω3-FAME in SFBB 1628 nauplii. These nauplii when fed with an enrichment diet yielded 15.1 mg/g dry wt. of total ω3-FAME. Watanabe et al. (1978) demonstrated the importance of the FAME *20:5ω3* for survival of the red seabream. High mortality in these larvae was observed when the amount of *20:5ω3* was lower than that of *18:3ω3* in the diet. *Penaeus stylirostris* has shown a high requirement for *20:5ω3* during post-larval metamorphosis and higher amounts of *22:6ω3* have been effective in promoting better growth (Leger et al. 1985). In the Sri Lankan *Artemia,* 15.8 and 0.9 mg/g dry wt. of *20:5ω3* and *22:6ω3* respectively have been observed. Reports indicate that in most *Artemia* strains the level of *20:5ω3* is inversely related to the level of *18:3ω3* (Watanabe et al. 1978b; Siedel et al. 1980). This relationship has been observed in the Sri Lankan *Artemia* too, the latter amounting to 0.7 mg/g dry wt. Schaeur and Simpson (1985) mentioned that *18:3ω3* is converted to *20:5ω3* in freshwater fish and that this conversion is very low in marine fish.

Work on *P. stylirostris* using formulated diets has shown that the FAME *18:2ω6* is also important in the diet of prawns (Fenucci et al. 1981). According to Fujita et al. (1980) the food value of *Artemia* is significantly affected by its EFA content.

The single most important factor so far identified in defining the nutritional quality of *Artemia* nauplii for marine fish and crustaceans is the content of essential fatty acids such as *20:5ω3* which should be in quantities above 4% (Leger et al. 1986). As stated earlier the Sri Lankan *Artemia* strain contains high amounts of *20:5ω3*. Based on these results, the Sri Lankan *Artemia* can be said to possess a high nutritional quality for aquaculture purposes. It can be classified as the *20:5ω3* type or the marine-type and can be highly recommended for marine fish and crustacean larvae.

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References

Beck, A.D. and D.A. Bengtson. 1982. International study on *Artemia* XXII. Nutrition in aquatic toxicology - diet quality of geographical strains of *Artemia*. *In* J.G. Pearson, R.B. Foster and W.E. Bishop (eds.) Aquatic toxicology and Hazard Assessment, pp. 161-169. American Society for Testing and Materials, Philadelphia, USA.

Beck, A.D., D.A. Bengtson and W.H. Howell. 1980. International Study on *Artemia*. V. Nutritional value of five geographical strains of *Artemia*: effects on survival and growth of larval Atlantic silverside, *Menidia menidia*. *In* G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds.) The Brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in aquaculture, pp. 249-259. Universa Press, Wetteren, Belgium.

Fenucci, J.L., A.L. Lawrence, and Z.P. Zein-Eldin. 1981. The effects of fatty acid and shrimp meal composition of prepared diets on growth of juvenile shrimp, *Penaeus stylirostris*. World Mariculture Society 1: 315-324.

Fujita, S., T. Watanabe and C. Kitajima. 1980. Nutritional quality of of Artemia from different localities as a living feed for marine fish from the viewpoint of essential fatty acids. *In* G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds.) The Brine Shrimp *Artemia*. Vol. 3. Ecology, Culturing, use in Aquaculture, pp. 277-290. Universa Press, Wetteren, Belgium.

Johns, D.M., M.E.J. Peters and A.D. Beck. 1980. International Study on *Artemia*. VI. Nutritional value of geographical and temporal strains of *Artemia:* Effects on survival and growth of two species of Brchyuran larvae. *In* G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds.) The Brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture, pp. 291-304. Universa Press, Wetteren, Belgium.

Juario, J.V., M.N. Duray, V.M. Duray, J. Nacario and J.M.E. Almendras. 1985. Breeding and larval rearing of the rabbitfish, *Siganus guttatus* (Bloch). Aquaculture 44: 91-101.

Kinne, O. (ed.). Marine Ecology, Vol. 3, Part 2, p. 743-761. John Wiley & Sons, 1977.

Leger, P., D.A. Bengtson, K.L. Simpson and P. Sorgeloos. 1986. The use and nutritional value of *Artemia* as a food source. *In* Margaret Barnes (ed.) Oceanography and Marine Biology, Annual Review 24, pp. 521-623. Aberdeen University Press, Aberdeen, UK.

Leger, P., D.A. Bengtson, P. Sorgeloos, K. Simpson and A.D. Beck. 1985. The Nutritional value of *Artemia:* A review. Paper presented at the 2nd International Symposium on the Brine Shrimp *Artemia*, Antwerp, (Belgium); Sept. 1-5, 1985.

Leger, P., G.F. Bieber and P. Sorgeloos. 1985. International Study on *Artemia*. XXXIII. Promising results in larval rearing of *Penaeus stylirostris* using a prepared diet as algal substitute and for *Artemia* enrichment. Journal World Mariculture Society 16: 354-367.

Leger, P. and P. Candreva. 1987. Sample preparation for total lipid extraction and Fatty Acid Methyl Ester Analysis (unpublished). Artemia Reference Centre, Rozier 44, B-9000 Ghent, Belgium.

Persoone, G. and P. Sorgeloos. 1980. General aspects the ecology and biogeography of *Artemia*. *In* G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds.) The Brine Shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture, pp. 3-24. Universa Press. Weteren, Belgium.

Royan, J.P. 1980. Laboratory and field studies on an Indian strain of the brine shrimp *Artemia*. *In* G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds.) The Brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture, pp. 223-230. Universa Press, Wetteren, Belgium.

Schaeur, P.S. and K.L. Simpson. 1985. Bioaccumulation and bioconversion of dietary labelled fatty acids in *Artemia* and winter flounder *(Pseudopleuronectes americanus)*. Canadian Journal of Fisheries and Aquatic Sciences 42: 1430-1438.

Schaeur, P.S., D.M. Johns, C.E. Olney, and K.L. Simpson. 1980. International Study on *Artemia*, p. 365-373. IX. Lipid level, energy content and fatty acid composition of the cysts and newly hatched nauplii from five geographical strains of *Artemia*. *In* G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds.) The Brine shrimp Artemia. Vol. 3. Ecology, Culturing, Use in Aquaculture. Universa Press, Wetteren, Belgium.

Seide, C.R., J. Kryznowek and K.L. Simpson. 1980. International study on *Artemia*. XI. Amino acid composition and electrophoretic protein pattems of *Artemia* from five geographical locations. *In* G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds.) The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture, pp. 375-382. Universa Press, Wetteren, Belgium.

Sorgeloos, P. 1980. The use of the brine shrimp *Artemia* in aquaculture. *In* G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds.) The Brine Shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture, pp. 25-46. Universa Press, Wetteren, Belgium.

Sorgeloos, P., P. Lavens, P. Leger, W. Tackaert and D. Versichele. 1986. Manual for the Culture and Use of Brine Shrimp Artemia in Aquaculture. *Artemia* reference Centre, Wettem, Belgium, 319 p.

Sorgeloos, P., G. Persoone, M. Baeza, E. Bossuyt and E. Bruggeman. 1978. The Use of Artemia cysts in Aquaculture; The Concept of 'Hatching efficiency' and Description of a new method for cyst processing. *In* J.W. Avault, Jr. (ed.) Proceedings. 9th Annual Meeting World Mariculture Society, pp. 715-721. Louisiana State University, Baton Rouge, LA.

Van Ballaer, E., F. Amat, F. Hontoria, P. Leger, and P. Sorgeloos. 1985. Preliminary results on the nutritional evaluation of w3-HUFA-Enriched *Artemia nauplii* for larvae of the sea bass, *Dicentrarchus labrax*. Aquaculture 49: 223-229.

Vanhaecke, P. and P. Sorgeloos. 1980. International study on *Artemia*. IV. The biometrics of *Artemia* strains from different geographical origin, p. 393-405. *In* G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds.) The Brine shrimp *Artemia*. Vol 3. Ecology, Culturing, Use in Aquaculture. Universa Press, Wetteren, Beligium.

Vanhaecke, P., H. Steyaert and P. Sorgeloos. 1980. International Study on Artemia. III. The use of Coulter Counter equipment for the biometrical analysis of *Artemia* cysts, Methodology and mathematics. *In* G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds.) the brine shrimp *Artemia*. Vol. 1 Morphology, Genetics Radiobiology, Toxicology, pp. 107-115. Universa Press, Wetteren, Belgium.

Versichele, D., P. Sorgeloos, P. Baert, P. Lavens, P. Leger and W. Tackaert. 1985. Improved aquaculture production of marine fish and crustaceans through progress in Artemia research and applications. Proceedings 'Progress in Belgium Oceanographic Research', Brussels, March 1985.

Watanabe, T., T. Arakawa, C. Kitajima and S. Fujita. 1978a. Nutritional evaluation of proteins of living feeds used in seed production of fish. Bull. Japan Soc. Sci. Fish. 44: 985-988.

Watanabe, T., F. Oowa, C. Kitajima and S. Fujita. 1978b. Nutritional quality of brine shrimp, *Artemia salina*, as a living feed from the view - point of essential fatty acids for fish. Bulletin Japanese Society of Scientific Fisheries 44: 1115-1121.

The Digestibility of Several Feedstuffs in Red Tilapia

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A digestibility study of red tilapia on six different feedstuffs were conducted. The feedstuffs tested were fishmeal, shrimp meal, soybean meal, copra meal, corn meal and ricebran. The test diet composed of 30% of the test feedstuff and 70% of the reference diet of 40% protein. One percent chromic oxide was added to each diet as an internal indicator.

The results showed that the apparent digestion coefficients of dry matter in the feedstuffs used were above 80% except for soybean meal (63.90%). The digestion coefficients of protein in ricebran was 99.85%, followed by fish meal (99.45%), shrimp meal (99.42%), copra meal (99.09%), soybean meal (91.56%) and corn meal (89.40%). The mean fat digestion coefficients for ricebran, copra meal, fishmeal and shrimp meal were significantly higher ($P < 0.01$) than those values for corn meal and soybean meal. Carbohydrates in soybean meal and fishmeal were poorly digested by red tilapia while those in corn meal and ricebran were well digested. The mean ash digestion coefficients of ricebran and copra meal were significantly higher ($P < 0.01$) than those of other feedstuffs. Fishmeal and shrimp meal gave significantly higher energy digestion coefficients ($P < 0.01$) than other feedstuffs for red tilapia.

Feed cost is one of the major constraints in aquaculture. To reduce the price of a well-balanced feed, locally available ingredients should be included in the feed, especially agricultural by-products. Being an agricultural-based country, Malaysia has a number of agricultural by-products that can be used as animal feed and may also be suitable for fish (Devendra 1975). Other than looking at growth responses, the digestibility of nutrients contained in the feedstuffs could be used to assess the suitability of the feedstuffs as ingredients in fish feeds. Few studies had been carried out to determine the nutrient digestibility of local feedstuffs in several fish species commonly cultured in Malaysia (Law 1984, 1986: Law et al. 1985, 1987). The results showed that the digestibility of a feedstuff differs with species.

Red tilapia is one of the most important and popular cultured food fish in Malaysia (Siraj et al. 1987). It is easy to culture, has a fast growth rate and readily accepts artificial feed. Furthermore, it commands a high market price. However, there is little known on its response to local feedstuffs. In this study, the nutrient digestibility of several feedstuffs in red tilapia was determined.

Materials and Methods

Six funnel-shaped experimental tanks as described by Law (1984) were used in the study. Twenty fish with an average weight of 36.6 g were placed in each tank. The average water flow into each tank was 201/hr.

Six test diets, each composed of 30% of the test feedstuff and 70% of the reference diet (Table 1) were prepared. The feedstuffs tested were fishmeal, shrimp meal, soybean meal, copra meal, corn meal and ricebran. A 40% protein diet was used as the reference diet (Yahaya 1986). 1% chromium oxide (Cr_2O_3) was added to all diets as an internal marker. Each diet was assigned to a tank at random and 3 runs were made for each diet.

Table 1. Composition of the reference (control) diet.

Ingredients[1]	%
Fishmeal	28.89
Shrimp meal	28.89
Soybean meal	28.89
Corn meal	3.03
Rice bran	3.03
Copra meal	3.03
Vitamin premix[2]	0.60
Mineral premix[2]	3.65

[1]The proximate compositions of the feedstuffs were as follows: Fishmeal - 62.5% protein, 5.12% lipid, 19.8% ash, 12.4% moisture, 3483.0 cal/g energy; shrimp meal - 27% protein, 2.27% fat; 44.3% ash; 10.8% moisture, 2639.7 cal/g; soybean meal - 44.1% protein, 1.6% fat, 6.0% ash, 12.3% moisture, 4523.4 cal/g energy; corn meal - 9.07% protein, 4.5% fat, 2.2% ash, 11.5% moisture, 4253.6 cal/g: ricebran - 10.9% protein, 10.79% fat; 12.3% ash; 11.8% moisture, 4859.1 cal/g energy; copra meal - 16.8% protein, 8.4% fat; 6.39% ash; 11.8% moisture, 4565.4 cal/g energy.

[2] As recommended by NRC (1977).

The fish were fed twice daily (Siraj et al. 1987) at 2% body weight/day at 0700 and 1300 hr while the faeces were collected at 1230 and 1830 hr. The procedures for feeding and fecal collection, the analyses of crude protein, fat, ash, carbohydrate, dry matter, gross energy and chromic oxide were similar to those described by Law (1984).

The apparent digestibility of dry matter and nutrients in the diets were determined according to Schneider and Flatt (1975) while the apparent digestion coefficients of the nutrients in the feedstuffs were estimated according to Cho and Slinger (1979). All data were analysed using 1-way ANOVA (Schefler 1980) and the differences between the means were tested using Duncan's New Multiple Range Test (Duncan 1955).

Results and Discussion

The proximate analyses of the feed and faeces of the reference and test diets are given in Table 2 while the apparent digestion coefficients of the nutrients and dry matter of the tested feedstuffs in red tilapia are given in Table 3.

Table 2. Proximate analyses of feed and faeces of the reference diet and test diets in red tilapia (dry weight basis).

	Control (Reference diet)		Control + Fishmeal		Control + Shrimp meal	
	Feed	Faeces	Feed	Faeces	Feed	Faeces
Protein (%)	39.80* ± 0.29	12.99 ± 0.91	50.31 ± 0.28	13.90 ± 0.41	37.81 ± 0.28	9.75 ± 0.30
Fat (%)	2.40 ± 0.06	1.47 ± 0.08	3.38 ± 0.04	1.73 ± 0.10	2.71 ± 0.04	1.40 ± 0.06
Carbohydrate (%)	29.81 ± 0.74	45.52 ± 0.43	21.15 ± 1.19	48.50 ± 0.43	23.75 ± 0.57	38.19 ± 0.06
Ash (%) (%)	27.98 ± 0.40	40.02 ± 0.58	25.15 ± 0.90	35.86 ± 0.58	35.73 ± 0.80	50.66 ± 0.39
Gross energy (cal/g)	3564.27 ± 70.55	1906.25 ± 89.59	3849.70 ± 84.95	1956.70 ± 67.03	3142.35 ± 58.19	1558.60 ± 81.90
Cr_2O_3 (%)	0.77 ± 0.03	3.29 ± 0.04	0.78 ± 0.03	3.91 ± 0.02	0.73 ± 0.01	3.41 ± 0.05

*Mean ± Standard Deviation

Continued

Table 2. (continued)

Control + Soybean meal		Control + Corn meal		Control + Copra meal		Control + Ricebran	
Feed	Faeces	Feed	Faeces	Feed	Faeces	Feed	Faeces
45.04 ± 0.14	13.04* ± 0.54	32.67 ± 0.20	13.26 ± 0.59	35.30 ± 0.41	8.94 ± 0.12	34.38 ± 0.37	9.42 ± 0.41
2.37 ± 0.06	1.57 ± 0.05	2.79 ± 0.13	1.88 ± 0.13	5.24 ± 0.04	1.78 ± 0.16	5.79 ± 0.62	2.35 ± 0.12
29.01 ± 0.18	56.80 ± 0.69	44.00 ± 0.45	57.15 ± 1.09	37.15 ± 0.86	63.99 ± 0.39	35.56 ± 0.42	55.96 ± 0.55
23.58 ± 0.18	28.59 ± 0.31	20.55 ± 0.15	27.71 ± 0.60	22.30 ± 0.47	25.29 ± 0.44	24.27 ± 0.33	32.28 ± 0.49
3756.60 ± 58.15	2146.55 ± 54.80	3708.67 ± 69.84	2518.10 ± 57.54	3789.77 ± 70.76	2433.27 ± 8.36	3805.13 ± 75.48	2437.53 ± 72.85
0.80 ± 0.02	2.94 ± 0.04	0.79 ± 0.03	3.76 ± 0.03	0.77 ± 0.01	3.47 ± 0.05	0.78 ± 0.01	4.07 ± 0.05

Table 3. Apparent digestion coefficients of dry matter and nutrients in the tested feedstuffs.

Nutrient	Feedstuff					
	Fish meal	Shrimp meal	Soyabean meal	Com meal	Copra meal	Rice bran
Dry matter	88.11^{d*} ±0.34	83.24^{bc} ±1.05	63.90^{a} ±1.23	84.57^{c} ±0.56	80.63^{b} ±1.07	90.72^{d} ±0.79
Protein	99.45^{b} ±0.54	99.42^{b} ±0.57	91.56^{a} ±1.09	89.40^{a} ±1.27	99.09^{d} ±0.25	99.85^{b} ±0.25
Fat	98.95^{c} ±1.33	96.57^{c} ±1.58	73.36^{a} ±1.92	86.26^{b} ±3.27	100^{c} ±0.00	100^{c} ±0.00
Carbohydrate	30.90^{b} ±1.36	68.64^{d} ±0.19	5.69^{a} ±2.00	92.42^{f} ±1.74	55.98^{c} ±0.78	82.86^{e} ±0.99
Ash	83.30^{c} ±1.54	76.93^{b} ±0.78	68.13^{a} ±1.20	83.67^{c} ±2.05	94.22^{d} ±1.46	93.14^{d} ±1.29
Gross energy	95.41^{c} ±1.16	93.89^{c} ±1.97	77.38^{a} ±1.32	81.65^{a} ±1.09	81.71^{a} ±0.75	88.28^{b} ±1.23

*Mean ± Standard Deviation Means within the same row and followed by a same letter were not significantly different ($p > 0.01$).

The results show that the mean apparent digestion coefficients of dry matter in the tested feedstuffs were between 63.90% and 90.72%. The apparent dry matter digestion coefficient was highest in ricebran, followed by fishmeal, corn meal, shrimp meal, copra meal and soybean meal. Contrary to these results, Law (1986) and Law et al. (1987) noted that ricebran was poorly digested by grass carp and giant gourami but soybean meal was well digested by both species. Law (1986) also found that copra meal was poorly digested by grass carp.

The proteins in all the tested feedstuffs were very well digested by red tilapia. More than 99% of the proteins in ricebran, fishmeal, shrimp meal and copra meal were digested while 91.56% and 89.40% of the proteins in soybean meal and corn meal were digested, respectively. Similar observations were made by Law et al. (1987) in giant gourami. In jelawat, only fishmeal protein was well digested (Law 1984) while only fishmeal and soybean meal proteins were well digested in grass carp (Law 1986).

The fat contents in copra meal and ricebran were all digested by red tilapia. Except for soybean meal, the fats contained in other feedstuffs were also well digested. The mean apparent fat digestion coefficients of the feedstuffs were between 73.36 and 100%. In contrast, fats in ricebran were poorly digested by jelawat (Law 1984) while fats in corn meal were slightly digested by grass carp (Law 1986). The apparent fat digestibility of soybean meal were also lower than fishmeal in giant gourami and jelawat (Law 1984; Law et al. 1987).

The mean apparent carbohydrate digestion coefficient was highest in corn meal, followed by ricebran, shrimp meal, copra meal, fishmeal and soybean meal. The results showed that red tilapia did not efficiently digest the carbohydrates contained in the high protein feedstuffs. Similar observations were also seen in giant gourami and grass carp (Law 1986; Law et al. 1987). However, they also noted that ricebran carbohydrates were poorly digested in both species.

The results also showed that copra meal and ricebran had very high ash digestion coefficients in red tilapia. In contrast, the ash contents of copra meal and ricebran were not digestible in grass carp (Law 1986) and were poorly digested by giant gourami (Law et al. 1987). The minerals of copra meal were also poorly digested by jelawat (Law 1984).

The apparent energy digestion coefficients of all feedstuffs were high. Except for fishmeal and shrimp meal, these values were much higher than those of jelawat (Law 1984). Fishmeal had the highest apparent digestion coefficient for energy (95.41%) while soybean meal had the lowest (77.38%). In other studies, fishmeal had also the highest energy digestion coefficients (Law 1984, 1986; Law et al. 1987).

The study showed that ricebran was the most digestible feedstuff for red tilapia in terms of dry matter and nutrients. Ricebran is one of the local agricultural by-products. It is cheap and abundant. Thus ricebran should be one of the major ingredients for red tilapia feed.

Despite low digestibility of carbohydrates, fishmeal was the second most digestible feedstuff. This suggests that fishmeal is the most important protein source in red tilapia feed. Soybean meal, though not well digested by the fish, had a high protein digestion coefficient. Therefore, the results indicated that soybean meal could be used to replace some portion of fishmeal in the feed. Such substitutions of fishmeal in fish feeds has been increased lately (Viola et al. 1982) and it would, of course, reduce the cost of feed. In general, all the tested feedstuffs are suitable ingredients for formulating a well-balanced red tilapia feed.

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References

Cho, C.Y. and S.J. Slinger 1979. Apparent digestibility measurement in feedstuffs for rainbow trout. *In* J.E. Halver and K. Tiews (eds.) Proc. World Symposium on Finfish Nutrition and Fishfeed Technology, Vol. II, pp. 239-247. Heenemann, Berlin.

Devendra, C. 1975. Agricultural by-products for animal feeding in Malaysia. MARDI Report No. 31. 44 p.

Duncan, D.B. 1955. Multiple range and multiple F tests.Biometrics 11: 1-42.

Law, A.T. 1984. Nutritional study of jelawat (*Leptobarbus hoevenii* Bleeker), fed pelleted feed. Aquaculture 41: 227-233.

Law, A.T. 1986. Digestibility of low-cost ingredients in pelleted feed by grass carp *(Ctenopharyngodon idella)*. Aquaculture 51: 97-103.

Law, A.T. S.H. Cheah and K.J. Ang. 1985. An evaluation of the apparent digestibility of some locally available plants and a pelleted feed in three finfish in Malaysia. *In* C.Y. Cho, C.B. Cowey and T. Watanabe (eds.) Finfish Nutrition In Asia, pp. 90-95. IDRC (Canada), Ottawa.

Law, A.T, K.K. Chong and K.J. Ang. 1987. Digestibility of ingredients in a pelleted feed by giant gourami, *Osphronemus goramy* (Lacepede). *In* R.I. Hutagalung, C.P. Chen, W.M. Wan Embong, A.T. Law and S. Sivarajasingam (eds.) Proceedings 10th Annual Conference Malaysian Society of Animal Production, pp. 206-209.

Schefler, W.C. 1980. Statistics for the biological sciences. Addison-Wesley Publ. Co., 260 p.

Schneider, B.H. and W.P. Flatt. 1975. The evaluation of feed through digestibility experiments. University of Georgia Press, Athens, 423 p.

Siraj, S.S., Z. Kamaruddin, M.K.A. Satar and M.S. Kamarudin. 1987. Effects of feeding frequency on growth, food conversion and survival of red tilapia fry. Presented in the 2nd International Symposium on Tilapia in Aquaculture. 16-20 March, 1987. Bangkok. Thailand.

Yahaya, M.Z. 1986. Optimal dietary protein level for red tilapia cultured in glass aquaria. B.S. Thesis. Univ. Pertanian Malaysia. 44 p.

Growth Response, Feed Conversion and Metabolism of the Air-Breathing Fish, *Anabas testudineus* (Bloch) to Different Dietary Protein Sources

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A 60 day laboratory feeding trial was conducted to examine the effects of different dietary protein sources on the weight gain, feed conversion, protein efficiency ratio (PER), protein synthesis and protease activity of the air-breathing fish, *Anabas testudineus*. Specific growth rate, feed conversion and protein efficiency ratios were highest in fish fed with diet D4 (containing carcass waste, ricebran and groundnut oil cake) followed by fish fed with diet D3 (dried goat blood, ricebran, wheat bran and ground nut oil cake) and D1 (soybean meal, wheat bran and mustard oil cake) in decreasing order. Rates of protein synthesis in terms of amino acid (^{14}C L-leucine) incorporation (cpm/mg tissue protein) in the liver and muscle increased significantly ($P < 0.001$) with all the diets except with D1 where the increase was not significant in the muscle. Protein synthesis rates followed the same trend as growth rates. The protease activity in the hepatopancreas, stomach and intestine also increased significantly with all the diets following a similar trend.

The dietary formulation of a nutritionally balanced diet to meet the requirements is one of the major aspects in the development of aquaculture. Protein is considered as the most important and expensive component of fish diets. The characteristics of the protein sources used in fish diets should also be considered, since the response of the fish could vary depending on protein quality and gross protein level of the diet. The present experiment was therefore designed to study the influence of diets with different protein sources on the growth, feed conversion, protein synthesis and protease activity of the air-breathing fish, *Anabas testudineus* (Bloch).

Materials and Methods

Four isonitrogenous (crude protein level ~ 35%) experimental diets using either soybean meal or fish meal or dried goat blood or carcass waste as the main protein source were formulated (Table 1) and pelleted.

Table 1. Proportion of different ingredients (% dry matter) and proximate composition of the experimental diets.

INGREDIENTS	DIETS			
	D1	D2	D3	D4
Soybean meal	50.94	-	-	-
Mustard oil cake	30.58	-	25.49	-
Groundnut oil cake	-	27.27	-	24.79
Wheat bran	17.68	10.18	16.84	-
Rice bran	-	10.00	20.00	37.09
Fish meal	-	51.75	-	-
Dried goat blood	-	-	36.87	-
Carcass waste	-	-	-	37.32
Vitamin and mineral mixture+	0.80	0.80	0.80	0.80
Proximate composition++(%)				
Moisture	5.27	3.90	6.05	3.72
Dry matter (DM)	94.73	96.10	93.95	96.28
Ash	5.95	16.28	8.97	16.09
Protein	34.68	33.67	34.84	34.83
Crude lipid	13.60	6.99	5.45	12.80
Crude fibre	6.59	6.67	8.13	13.13
Nitrogen free extract (NFE)	33.91	32.50	36.56	19.43
Total carbohydrate	40.50	39.17	44.69	32.56
Calcium	1.62	6.09	2.05	3.27
Phosphorus	0.96	0.96	0.56	1.17
Gross energy (K Cal/g)	04.90	4.17	4.37	4.51
P/E ratio (mg protein/K Cal)	70.70	80.80	79.69	77.22
Cost (Rs/Kg)	3.99	3.09	2.60	2.66

+ Vitaminate forte, Roche India Ltd.
++ On dry matter basis.

A. testudineus (average weight 10.0 g and total length 10.0 cm) were obtained locally and after acclimatization for 10 days, were segregated and divided into 4 groups of 20 fish each and kept in specially designed fibre glass aquaria (76 x 40 x 35 cm) in approximately 70 l of water. Each treatment was conducted in duplicate. Initial body weight and total length of the fish were recorded prior to commencement of the experiment.

Each diet was fed to the experimental fish at 5% of the total body weight throughout the experimental period of 60 days adjusting the ration every fortnight on the basis of average weight of the fish. Left over food was collected after four hours of feeding and weighed after drying in an air oven. Individual fish were weighed every fortnight.

Aquaria were drained out every 24 hrs through the outlet situated at the bottom through a filter of 22 Bolt silk cloth to collect faecal matter. The faecal matter was dried in an air oven at 100 ± 5°C and weighed. For each feeding trial, a 15 day composite faecal matter was pooled for digestibility estimates. Digestibility study was terminated after 15 days but feeding was continued upto 60 days. The faecal samples were analysed for proximate composition following the methods of AOAC (1975). Digestibility was calculated as :

$$\text{Digestibility (\%)} = \frac{\text{Nutrient intake - nutrient in faecal matter x 100}}{\text{Nutrient intake}}$$

At the end of 60 days protease activity was measured in the hepatopancreas, stomach and intestine using the method of Moore and Stein (1948). Initial activity of the enzyme was measured prior to the commencement of the feeding experiment.

Protein synthesis in the liver and muscle was measured as incorporation of ^{14}C L-leucine (specific activity 282 mCi/ m mol: obtained from Bhabha Atomic Research Centre, Trombay, Bombay). The fish from each group were given intramuscular injection of the isotope at the rate of 0.05 μCi/100 g both before commencement and at the termination of feeding experiment. The injected fish were sacrificed after 2 and 4 hr for counting the radioactivity in the liver and muscle, respectively. Protein was finally precipitated with 5% concentration of trichloroacetic acid. The precipitate was processed and the radioactivity was counted in an automatic EC (Electronic Corporation of India) Liquid Scintillation Counter (Model No. LSS-20). Protein was estimated by the method of Lowry et al. (1951).

The water temperature during the experimental period ranged from 29.5 to 35.5°C. The pH, dissolved oxygen and alkalinity of the water ranged between 7.0 and 7.1, 3.25 and 5.85 mg/l and 104.04 and 116.13 mg/l, respectively.

Results

The general performance of the fish on different diets is presented in Table 2. The average daily dry matter intake per 100 g of fish was highest for diet D4 followed by D1, D3 and D2. The growth performance of the fish in terms of average weight gain (%) is presented in Fig. 1. The average weight gain was highest in fish fed diet D4 (161.43%) and lowest (79.99%) with diet D1. The overall specific growth rate was also higher with diet D4 (1.60), followed by diets D3 (1.24), D2 (1.18) and D1 (0.98).

Table 2. Evaluation of different experimental diets for *A. testudineus*.

PARAMETERS	DIETS D1	D2	D3	D4
Feed intake and weight gain				
Initial body wt.(g)	10.56	10.46	10.64	10.67
Live wt. gain (g)	8.45	10.73	11.73	17.22
Weight gain (%)	79.99	102.56	110.11	161.43
Specific growth rate (%/day)	0.98	1.18	1.24	1.60
Average daily DM intake (g/100 g body wt/day)	2.6	2.37	2.58	3.33
Average daily faeces release (mg/100 g body wt/day)	300	631	511	579
Digestibility co-efficients (%)				
Crude protein	86.88	77.34	84.64	86.81
Crude lipid	89.11	79.51	87.14	87.55
Gross energy	86.93	74.74	84.07	84.66
Nitrogen balance				
N-intake (mg/100 g fish/day)	146	128	144	186
Faecal-N (mg/100 g fish/day)	15	29	22	25
N-absorbed (mg/100 g fish/day)	131	99	122	161
Energy balance				
Energy intake (K Cal/100 g fish/day)	12.753	9.892	11.292	15.023
Faecal energy (K Cal/100 g fish/day)	1.319	2.475	1.816	2.292
Energy absorbed (K Cal/100 g fish/day)	11.434	7.417	9.476	12.731
Nutritive value				
Feed conversion ratio (FCR)	1.93	1.39	1.41	1.24
Protein efficiency ratio (PER)	1.50	2.14	2.04	2.32

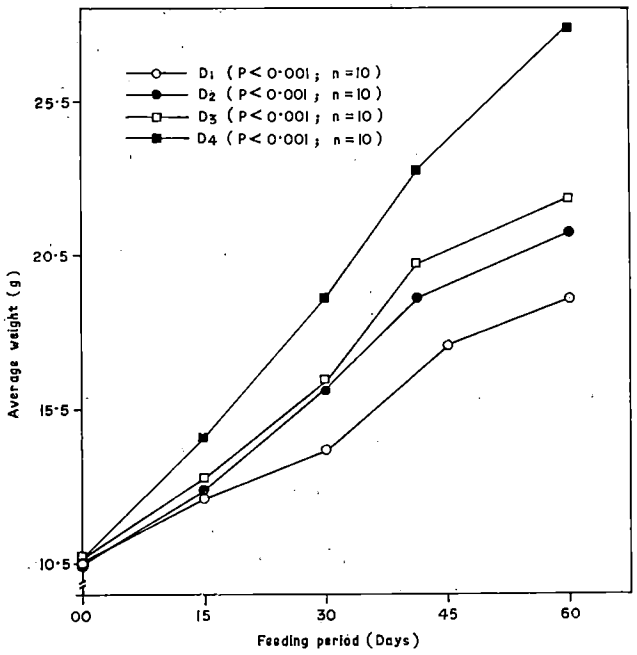

Fig. 1. Average weight gain of *A. testudineus* fed with experimental diets.

The best conversion ratio was obtained with diet D4 followed by D2, D3 and D1. Protein efficiency ratio, on the other hand, showed a different trend. PER was highest with diet D4, followed by D3, D2 and D1 (Table 2).

Digestibility values of protein and lipid did not show a marked difference for the diets D1, D3 and D4 although their values were little higher for diet D1 (Table 2). A lower value of the protein and lipid digestibility was, however, recorded with diet D2.

The specific protease activity in the hepatopancreas, stomach and intestine increased significantly ($P < 0.001$) in all the feeding trials. The activity was, however, higher in fish fed with diet D4 (Table 3).

The rates of protein synthesis in the liver were significantly higher ($P < 0.001$) in fish fed with the experimental diets. Protein synthesis rates in the muscle of fish fed with the diet D1 did not increase significantly. The rates increased significantly in the muscle of fish fed with other three diets being higher with diet D4, followed by D2 and D3 (Fig. 2).

Table 3. Specific protease activity (mean ± S.E) in the hepatopancreas, stomach and intestine of *A. testudineus* after 60 days of feeding experiment (µg of glycine liberated/hour/mg of protein ; $^{+}p < 0.001$, n = 5).

Organ	Initial	Final			
		D1	D2	D3	D4
Hepatopancreas	31.716 ± 0.030	33.567+ ± 0.032	41.129+ ± 0.018	41.015+ ± 0.543	47.128+ ± 0.037
Stomach	45.652 ± 0.308	47.114+ ± 0.024	53.437+ ± 0.037	52.812+ ± 0.058	62.216+ ± 0.028
Intestine	28.708 ± 0.031	31.937+ ± 0.029	32.311+ ± 0.028	33.109+ ± 0.044	41.630+ ± 0.006

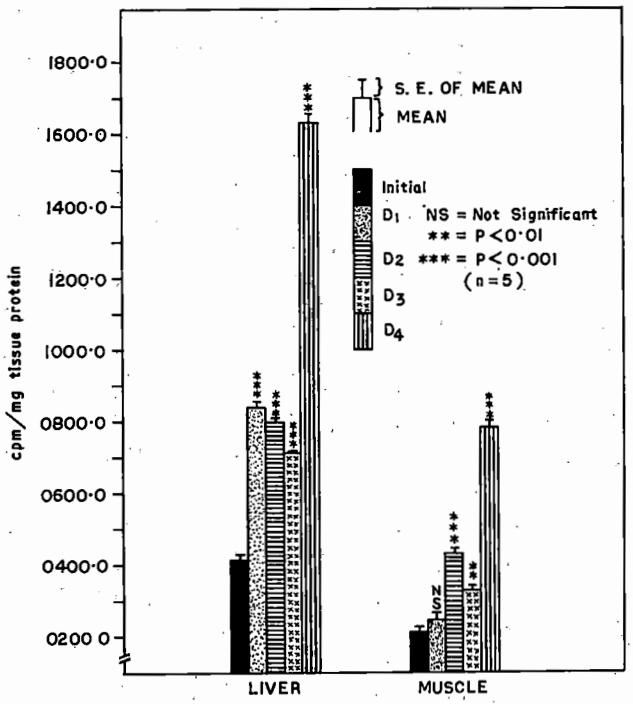

Fig. 2. Incorporation of ^{14}C L-Leucine in the liver and muscle of *A. testudineus* fed the experimental diets.

Discussion

All four diets used in the present study were isonitrogenous (35% crude protein). This level of crude protein has been reported to result in good growth in *A. testudineus* of similar size (Patra and Ray 1988). Since all the diets were isonitrogenous, the differences in growth performance in the various groups of fish may be expected to be related mainly to the differences in protein quality.

The better growth of fish recorded with carcass waste based diet (D4) indicates the relatively poor nutritional status of the other three diets. The lowest growth rate was recorded in fish fed with diet containing plant protein sources (D1). The results indicate that among the animal protein sources, the protein in carcass waste (D4) was efficiently utilized by the fish followed by goat blood (D3) and fish meal (D2) diets. Partial replacement of fish meal with plant and animal sources have resulted in almost equal or higher growth in a number of fish species in comparision with fishmeal based diets (Gropp et al. 1976; Akiyama et al. 1984; Viola and Zohar 1984; Nandeesha et al. 1986). The superior growth of *A. testudineus* recorded with diets D4 and D3 as compared to the fish meal diet (D2) indicates that the fish meal can be totally replaced by either carcass waste or dried goat blood in *A. testudineus* diets. Venkatesh et al. (1986) however, considered fish meal and silk worm pupae as better sources of protein in the diets of *Clarias batrachus*. The plant protein based soybean meal diet resulted in low growth rate and poor feed conversion and protein efficiency ratios. Venkatesh et al. (1986) also recorded reduced growth rate, feed conversion and protein efficiency ratios in *C. batrachus* with a plant protein based oil cake diet. Protein from soybean meal diet, therefore, appears to be nutritionally poor for growth of *A. testudineus*.

The digestibility values of protein and lipid did not differ significantly in the test diets. Protein digestibility however, increased for diet D1 (containing plant protein source) where rice bran was not used. Wannigama et al. (1985) also reported a higher value of protein digestibility in *Sarotherodon niloticus* for diets devoid of rice bran. But the cause of increase in protein digestibility for diets D4 and D3 is inexplicable because both diets contained a high proportion of rice bran (37.09 and 20.0%, respectively). Andrews et al. (1978) showed that ability of channel catfish to digest fat may be influenced by temperature and the level of fat in the diet. An increased value of lipid digestibility for diet D1 (soybean meal diet) may also be a result of an increased level of crude lipid (13.60%) in the diet.

The rates of protein synthesis in the liver and muscle in fish fed with different diets increased significantly except on diet D1. The increase in rates of protein synthesis, nevertheless, followed a similar trend exhibited by the growth rate. This indicates that *A. testudineus* can efficiently utilize the animal protein source which in turn increases the rates of protein synthesis in the liver and muscle. Venkatesh et al. (1986) also reported higher rates of protein synthesis in the liver of *C. batrachus* when maintained on diets prepared with animal protein components.

The present investigation indicates that the level of protease activity was higher in fish fed with predominantly animal protein diets. Although the dietary protein level is known to influence the protease activity in fish (Kawai and Ikeda 1972; Mukhopadhyay et al. 1978; Steffens 1981), there is no evidence to show any relationship between the protein source and protease activity in fish. However, variations in the enzyme activity may be related to the structure of protein and duration of retention of feed in the digestive tract which in turn depends on the fibre content and physical consistency of the diet (Venkatesh et al. 1986). A higher protease activity in the intestine of the fish fed on diet D4 may be due to a high crude fibre content in the diet (13.13%).

The present study indicates that a predominantly plant protein diet is not nutritionally adequate for *A. testudineus*. A comparison of the growth rate, feed conversion efficiency and physiological parameters of the fish fed with diets D2 (fish meal diet), D3 (goat blood diet) and D4 (carcass waste diet) indicates that carcass waste diet compares well with those of the goat blood and fish meal diets. Fowler and Banks (1976) and Asgard and Austreng (1986) recorded better growth in rainbow trout and salmon fed with diets containing protein from the blood of cattle in comparison to diets without blood. It can be concluded that carcass waste and goat blood are better sources of protein in the diet of *A. testudineus* than a mixture of soybean meal, mustard oil cake and wheat bran.

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References

AOAC. 1975. Official Methods of Analysis. Association of Official Agricultural Chemists, Washington, D.C.

Akiyama, T., T. Murai, Y. Hirasawa and T. Nose. 1984. Supplementation of various meals to fish meal diet for chum salmon fry. Aquaculture 37(3): 217-222.

Andrews, J.W., M.W. Murray and J.M. Davis. 1978. The influence of dietary fat levels and environmental temperature on digestible energy and absorbability of animal fat in channel catfish. Journal of Nutrition, 108: 749-752.

Asgard. T. and E. Austreng. 1986. Blood, ensiled or frozen, as feed for salmonids. Aquaculture 55: 263-284.

Fowler, L. G. and J.L. Banks. 1976. Animal and vegetable substitutes for fishmeal in the Abernaty diet, 1973. Progressive Fish-Culturist 38: 123-126.

Gropp, J., H. Koops, K. Tiews and H. Beck. 1976. Replacement of fish meal in trout feeds by other feed stuffs. *In* T.V.R. Pillay and W.A. Dill (eds.) Advances in Aquaculture, pp. 596-600. Fishing News Book Limited, Farnham, Surrey, England.

Kawai, S. and S. Ikeda. 1972. Studies on digestive enzymes of fishes. II. Effect of dietary change on the activities of digestive enzymes in carp intestine. Bulletin of Japanese Society of Scientific Fisheries 38: 265-270.

Lowry, D.H., N.J. Rosenborough, A.L. Farr and R.J. Randall. 1951. Protein measurement with folin phenol reagent. Journal of Biological Chemistry 193: 265-275.

Moore, S. and W.W. Stein. 1948. Photometric ninhydrin method for use in the chromatography of amino acids. Journal of Biological Chemistry 176: 367-388.

Mukhopadhyay, P.K., P.V. Dehadrai and S.K. Banerjee. 1978. Studies on intestinal protease: isolation, purification and effect of different dietary proteins on alkaline protease activity of the air-breathing fish, *Clarias batrachus*. Hydrobiologia 57: 11-15.

Nandeesha, M.C., K.V. Devaraj and N.S. Sudhakara. 1986. Growth response of four species of carps to different protein sources in pelleted feeds. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) First Asian Fisheries Forum, pp. 603-608. Asian Fisheries Society, Manila, Philippines.

Patra, B.C. and A.K. Ray. 1988. Influence of varying levels of dietary protein on the muscle protein and lipid contents in the air-breathing fish, *Anabas testudineus* (Bloch). Indian Biologist 20(1): 38-43.

Steffens, W. 1981. Protein utilisation by rainbow trout *(Salmo gairdneri)* and carp *(Cyprinus carpio):* a brief review. Aquaculture 23: 337-345.

Venkatesh, B., A.P. Mukherji, P.K. Mukhopadhyay and P.V. Dehadrai. 1986. Growth and metabolism of the catfish *Clarias batrachus* (Linn.) fed with different experimental diets. Proceedings of Indian Academy of Science (Animal Science) 95: 457-462.

Viola, S. and G. Zohar. 1985. Nutrition studies with market size hybrids of tilapia *(Oreochromis)* in intensive culture.3. Protein levels and sources. Bamidgeh 36: 3-15.

Wannigama, N.D., D.E.M. Weerakoon and G. Muthukumarana. 1985. Cage culture of S.niloticus in Sri Lanka: effect of stocking density and dietary crude protein levels on growth. *In* C.Y. Cho, C.B. Cowey and T. Watanabe (eds.) Finfish Nutrition in Asia: methodological approaches to research and development, Part II, pp. 113-118. IDRC, Ottawa, Ontario, Canada.

Effect of Feeding Regimes on Growth and Survival of Bighead Carp (*Aristichthys nobilis* Richardson) Fry

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Two five-week feeding trials were undertaken to evaluate growth and survival of bighead carp fry of 1.9-2.4 mg mean weight reared on various feeding regimes. In Treatment 1, the carp fry were fed with *Brachionus* alone. In Treatment 2, 3, 4 and 5, the fry were fed with *Brachionus* for 2, 4, 6 and 10 days, respectively, and then with an artificial diet for the remaining period. The carp fry were fed with the combination of *Brachionus* and artificial diet in Treatment 6 and with artificial diet alone in Treatment 7. Results showed that the combination of *Brachionus* and artificial diet was the best feeding regime in enhancing the growth of the bighead carp fry. Mean weights of the fry fed with *Brachionus* for 2,4,6 and 10 days prior to the shifting to artificial diet were similar to that of the fry fed with *Brachionus* alone or artificial diet alone. There was no distinct trend in survival as a function of feeding regime. However, *Brachionus* alone gave the highest survival rate in both trials.

The demand for bighead carp *(Aristichthys nobilis)* fingerlings in the Philippines has increased in recent years. In areas surrounding Laguna Lake alone, about 16 privately-owned carp hatcheries with a capacity to produce from 0.1 to 1.5 million fingerlings every six months have been set up to serve the requirements for stocking in an aggregate area of 1,862 ha devoted to bighead carp grow-out as of 1985-1986 (Almazora 1987). Assuming a minimum stocking density of 10,000 fish/ha, some 19 million bighead carp fingerlings would be needed every 6-8 months (Fermin 1987).

Bighead carp larvae are produced by induced spawning of carp broodstock. Larvae are usually reared in indoor nursery tanks for 30-45 days before they are harvested for stocking in cages (Fermin 1987). One serious problem in nursery operations in the Philippines, and probably elsewhere, is the shortage or unavailability of live food organisms when needed. Various artificial diets have been tested to partially or completely replace live foods for larvae of different species of carp (Appelbaum and Dor 1978; Appelbaum and Uland 1979; Dabrowski et al. 1983; Szlaminska and Przylbyl 1986; Csengeri and Petitjean 1987).

The present study was conducted to determine the response of bighead carp fry to different feeding regimes.

Materials and Methods

Bighead carp fry were obtained in two batches from the hatchery of the SEAFDEC Aquaculture Department, Binangonan Freshwater Station, for two feeding trials. The fry were stocked in glass aquaria containing 20 l of tap water at 200 fry/aquarium. For each trial, there were seven feeding treatments (Table 1) with three replicates each.

Table 1. Mean body weight (BW), total length (TL) and survival rate (SR) of bighead carp fry on different feeding regimes for 5 weeks (Trial I)*. Values with the same superscript are not significantly different at 5% level.

Treatment	Feeding regime	BW (mg)	TL (mm)	SR (%)
1.	*Brachionus* alone	40.9^{B}	18.5^{B}	86.2^{A}
2.	*Brachionus* for 2 days, then artificial diet	26.5^{B}	14.8^{C}	38.0^{C}
3.	*Brachionus* for 4 days, then artificial diet	29.1^{B}	14.9^{C}	20.3^{D}
4.	*Brachionus* for 6 days, then artificial diet	38.0^{B}	16.0^{C}	40.0^{C}
5.	*Brachionus* for 10 days, then artificial diet	36.1^{B}	16.1^{C}	62.2^{B}
6.	Combination of *Brachionus* and artificial diet	139.9^{A}	23.7^{A}	64.2^{B}
7.	Artificial diet alone	25.5^{B}	14.6^{C}	32.0^{C}

* Initial mean body weight was 1.9 mg and total length was 8.2 mm.

Brachionus plicatilis, a marine rotifer, was cultured in tanks containing brackish water using the combination of baker's yeast and *Chlorella. Brachionus* was harvested by siphoning the culture medium which was filtered with a plankton net. The concentrated rotifers were washed and presented to the carp fry twice a day such that the density in the rearing medium ranged from 5-8 rotifers/ml for the first five treatments and 2-4 rotifers/ml for Treatment 6. The artificial diet (Table 2) was a slight modification of the M-2 diet formulated previously for milkfish fry (Santiago et al. 1983). Daily feeding rate ranged from 100-30% of the fish biomass. The ration was suspended in about 50 ml of water before it was given to the fry. Each feeding trial lasted for five weeks.

Table 2. Percentage composition of the artificial diet fed to bighead carp fry.

Ingredient	g/100 g diet
Fish meal	56.6
Soybean meal	11.4
Shrimp meal	9.0
Rice bran	12.7
Cod liver oil	2.5
Corn oil	2.5
Starch	1.0
Vitamin mix[1]	0.7
Mineral mix[1]	3.6
Nutrients (as-fed basis)	
Crude protein	41.5
Crude fat	11.9
Ash	12.5
Nitrogen-free extract	24.5

[1] Composition as reported for common carp and other warmwater fishes (NRC 1983).

Fish were sampled at weekly intervals to determine growth and for adjustment of the ration. Daily mortalities were recorded. Aquaria were provided with adequate aeration and cleaned once or twice daily with two-thirds of the water replenished.

In Trial I, water temperature ranged from 26-31°C; dissolved oxygen (D.O.), 5.5-7.5 mg/l; pH, 7.8-8.6; total NH_3-N, 70-1242 µg/l. In Trial II, water temperature ranged from 26-30°C; D.O., 5.4-7.5 mg/l; pH, 7.5-8.0; total NH_3-N, 30-941 µg/l.

Results

Trial I

Bighead carp fry fed with the combination of *Brachionus* and artificial diet (Treatment 6) consistently had the highest mean body weight among all treatments from week 1 to week 5 (Fig. 1). Feeding the carp fry with *Brachionus* for 2, 4, 6 or 10 days and artificial diet on the

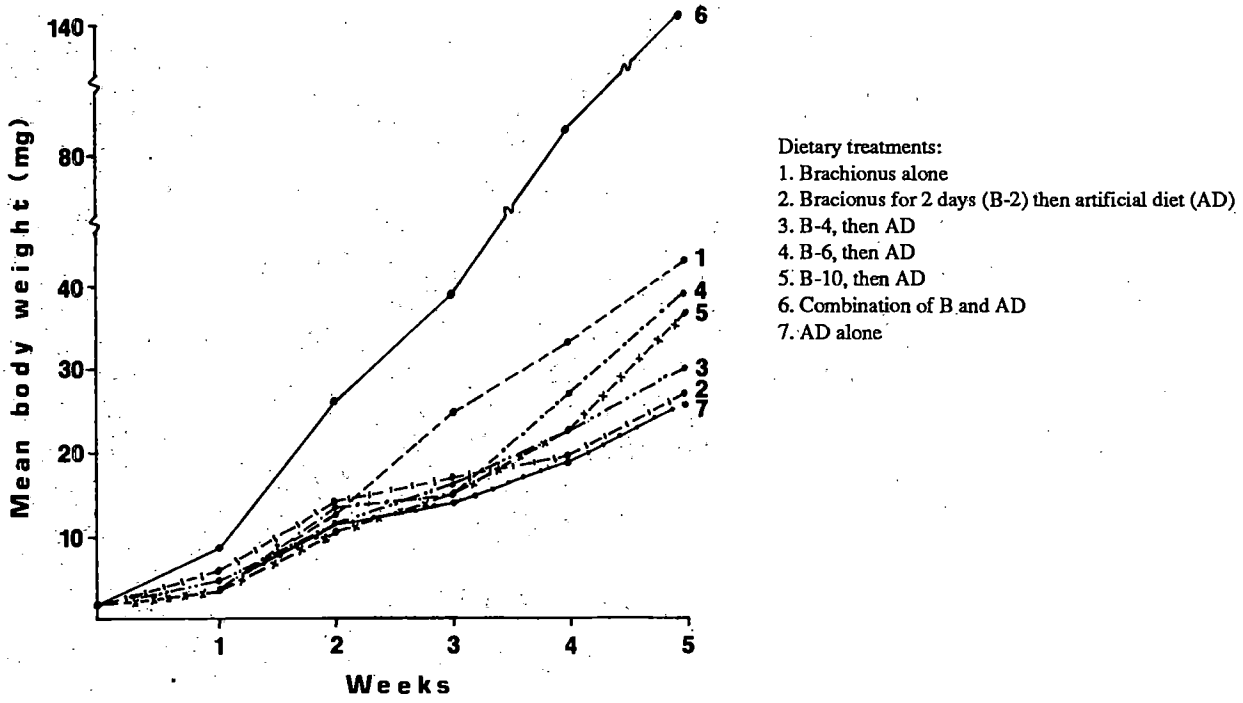

Fig. 1. Growth of bighead carp fry on various feeding regimes in Trial I.

succeeding days (Treatments 2, 3, 4 and 5) did not increase the body weight beyond that attained by the fry fed solely with *Brachionus* (Treatment 1). Fry fed the artificial diet alone had the lowest final mean body weight which was not significantly different ($p > 0.05$) from that of fry in Treatments 1 to 5 (Table 1). The final mean total length was likewise highest for fry fed with the combination of *Brachionus* and artificial diet followed by those fed with *Brachionus* alone. For all other treatments, the total lengths were not significantly different from each other (Table 1).

Survival rate was highest for fry fed with *Brachionus* alone followed by fry fed with the combination of *Brachionus* and artificial diet, and fry fed with *Brachionus* for 10 days prior to artificial diet (Table 1). All other treatments had significantly low survival rates.

Trial II

The general trend of growth in Trial II was similar to that in Trial I (Fig. 2). Again, highest body weight and total length were attained by fry on the combination of *Brachionus* and artificial diet. Growth of fry in all other treatments were not significantly different ($p > 0.05$), although it was slightly increased by offering *Brachionus* for several days prior to the artificial diet (Table 3).

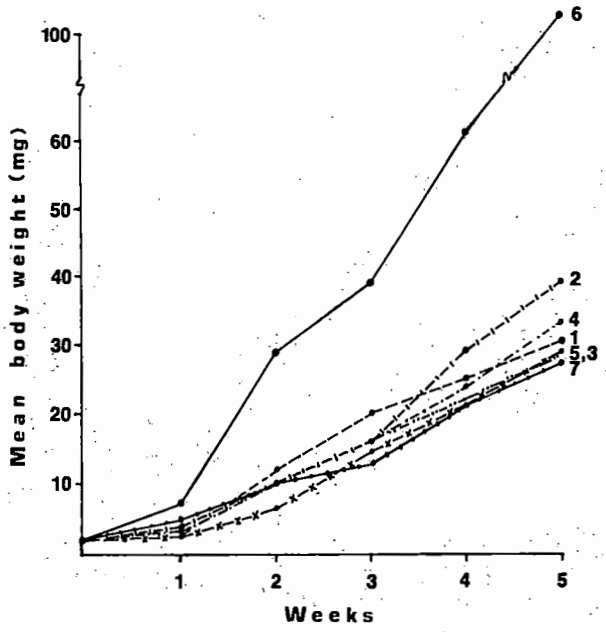

Fig. 2. Growth of bighead carp on various feeding regimes in Trial II.

Table 3. Mean body weight (BW), total length (TL) and survival rate (SR) of bighead carp fry on different feeding regimes for 5 weeks (Trial II)*. Values with the same superscript are not significantly different at 5% level.

Treatment	Feeding regime	BW (mg)	TL (mm)	SR (%)
1.	*Brachionus* alone	30.5^{B}	16.3^{B}	94.0^{A}
2.	*Brachionus* for 2 days, then artificial diet	39.3^{B}	16.7^{B}	66.5^{B}
3.	*Brachionus* for 4 days, then artificial diet	28.9^{B}	15.5^{B}	57.8^{CD}
4.	*Brachionus* for 6 days, then artificial died	33.4^{B}	15.8^{B}	54.2^{D}
5.	*Brachionus* for 10 days, then artificial diet	29.0^{B}	15.3^{B}	62.9^{BC}
6.	Combination of *Brachionus* and artificial diet	103.3^{A}	21.8^{A}	63.2^{BC}
7.	Artificial diet alone	27.9^{B}	15.1^{B}	54.0^{D}

* Initial mean body weight was 2.4 mg and total length was 8.5 mm.

Survival rates were higher in Trial II than in Trial I (Tables 1 and 3). As in Trial I, fry fed with *Brachionus* had the highest survival (Table 3). Fry fed with artificial diet alone had the lowest survival rate but it was not significantly different ($p > 0.05$) from that of fry in Treatments 3 and 4.

Discussion

The combination of *Brachionus* and artificial diet was the best feeding regime in accelerating the growth of bighead carp fry. It took only 2-3 weeks for the carp fry given the feed combination to attain the same growth achieved in 5 weeks by those fed with *Brachionus* alone.

Survival rates were generally increased by feeding the fry with *Brachionus* for 2, 4, 6 or 10 days prior to feeding with artificial diet. In both trials, survival rate of the fry fed with *Brachionus* for 10 days and then artificial diet for the remaining days was near the survival rate of the fry given the feed combination. It was observed in the course of the feeding trials that high mortality occurred from one to two hours after feeding the fry with the artificial diet; typically the fry would feed vigorously, eventually swim erratically, settle at the bottom, and die. It was presumed that the physical quality of the diet such as particle size when wet rather than its nutritional quality was directly responsible for such mortalities.

B. plicatilis is a favored live food for larval fish due to its small size ranging from 92-276 µm depending on the strain and food of the rotifer (Yufera 1982). The cladoceran, *Moina macrocopa,* is much larger but was also found suitable for bighead carp larvae (Baldia et al. 1985). The artificial diet offered to the bighead carp fry in the present study ranged in size from 21-370 µm when dry. About 85% of the particles measured less than 150 µm.

Bryant and Matty (1981) reported that common carp *(Cyprinus carpio)* larvae with an initial body weight ≥ 9.5 mg have better ability to utilize and survive on artificial diet alone than smaller larvae. An initial weight of 15 mg was considered the common carp larvae's adaptation weight to an artificial diet. For bighead carp fry in our study the weight of about 15 mg was attained after three weeks of feeding with *Brachionus* alone or *Brachionus* for 2, 4, 6 or 10 days and then the artificial diet.

Dabrowski (1984) has demonstrated that the larvae of four cyprinids could be transferred directly to a dry diet at a much lower individual wet weight (5-6 mg), which meant 15 days of feeding with zooplankton in the case of bighead carp larvae. Although feeding of bighead carp larvae with artificial diets for 12 days from an initial weight of 5.6 mg gave high final body weights (about 79-110 mg), survival rates were low (23-38%) after a total of 27 days inclusive of 15 days of feeding with zooplankton. In comparison, bighead carp fry primed with *Brachionus* prior to giving artificial diet in our study had relatively higher survival rates after five weeks but growth was lower.

Lieder and Helms (1981 in Dabrowski 1984) observed good growth of silver carp *(Hypophthalmichthys molitrix)* and bighead carp reared on a dry diet alone or in combination with live phytoplankton and zooplankton, but survival was rarely above 50%. There are other studies that have shown that the combination of live food and artificial diet is a much better food for the larvae of Chinese carps than artificial diet alone.

Growth rate of grass carp *(Ctenopharyngodon idella)* fry was higher when fed for 20 days with an excess of a mixed diet consisting of coconut meal and filtered zooplankton (1:3 by wet weight) than when fed with coconut meal alone (De Silva and Weerakoon 1981). The study of Lubzens et al. (1984) likewise showed that the common carp larvae raised on a combination of a commercial dry food and rotifers *(B. plicatilis)* grew three times faster than those on the artificial food alone for 16 days. In a seven-day experiment, Szlaminska and Przybyl (1986) found better growth of common carp fry fed with a mixed food (encapsulated dry diet and zooplankton of mixed species) compared to those fed with the encapsulated diet alone or zooplankton alone. It was observed further that the encapsulated diet alone, consisting mainly of Torula yeast and lyophilized pork liver and pancreas, and zooplankton alone as feeds had an additive effect on body weight of common carp larvae. The combination of *Brachionus* and artificial diet in our

present work on bighead carp fry had a synergistic effect on growth since the final mean body weight of those given the feed combination was approximately twice as much as the sum of the mean weights of fry given *Brachionus* alone and artificial diet alone for 35 days. Bighead carp fry was also shown earlier to grow best on a combination of *Moina macrocopa* and an artificial feed (40% crude protein), and mean survival rate after 12 weeks was 24% (Fermin 1985). When "green water" was added to *Moina* + artificial feed, survival rate increased to 53% but growth did not improve. It was postulated that "green water" enhanced survival rate by maintaining good water quality.

Panov et al. (1969 in Szlaminska and Przybyl 1986) reported that bighead and silver carp larvae started to take food when the density of living prey in the water was 500 individuals/dm^3 and the optimal density of zooplankton was 1000 individuals/dm^3. In terms of density per cc or ml, this amounts to 0.5-1 individual/ml which is much lower than what was used in our present study. Lubzens et al. (1984) found that *B. plicatilis* supplied at 5/ml with artificial food seemed optimum for common carp larvae based on growth and the development of dorsal and caudal fins; increasing the density of the rotifers to 20/ml in the presence or absence of artificial food decreased growth rates.

In practical nursery operations, the ultimate objective is to promote both fast growth and high survival rate because young fish of a particular size are usually sold or stocked based on number rather than on biomass. It was shown in our present study that the combination of *Brachionus* and artificial diet given throughout the rearing period was the best feeding regime in enhancing the growth of bighead carp fry. The survival rate, however, needs improvement. Although *Brachionus* alone as feed for the fry gave the highest survival rate, it also gave a significantly lower growth. The artificial diet alone as feed gave a relatively low growth which was not significantly different from that of fry given *Brachionus* alone or *Brachionus* for a few days (2-10 days) followed by the artificial diet. Thus, future studies should be geared towards further improvement of the nutritional quality as well as the physical characteristics of artificial diets for the carp fry.

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References

Almazora, R.R. 1987. A project feasibility study for a carp hatchery and grow-out financing scheme. MBA Thesis. Asian Institute of Management. Makati, Metro Manila, Philippines. 136 p.

Appelbaum S. and U. Dor. 1978. Ten-day experimental nursing of carp (*Cyprinus carpio* L.) larvae with dry feed. Bamidgeh 30(3): 85-88.

Appelbaum, S. and B. Uland. 1979. Intensive rearing of grass carp larvae *Ctenopharyngodon idella* (Valenciennes 1844) under controlled conditions. Aquaculture 17: 175-179.

Baldia, S.F., J.B. Pantastico and J.P. Baldia. 1985. Acceptability of selected zooplankton and phytoplankton for growing larvae/fry of bighead carp *(Aristichthys nobilis)*. Paper presented at the Asian Symposium on Freshwater Fish culture. Oct. 10-15, 1985. Beijing, China. 11 p.

Bryant, P.L. and A.J. Matty. 1981. Adaptation of carp *(Cyprinus carpio)* larvae to artificial diets. I. Optimum feeding rate and adaptation age for a commercial diet. Aquaculture 23: 275-286.

Csengeri, I. and M. Petitjean. 1987. Fresh liver powder: a new starter diet for the larvae of a cyprinid fish. Aquaculture 65: 189-192.

Dabrowski, K. 1984. Influence of initial weight during the change from live to compound feed on the survival and growth of four cyprinids. Aquaculture 40: 27-40.

Dabrowski, K., R. Bardega and R. Przedwojski. 1983. Dry diet formulation study with common carp (*Cyprinus carpio* L.) larvae. Zeitschrift fur Tierphysiologie, Tierernahrung und Futtermittelkunde 50(1): 40-52.

De Silva, S.S, and D.E.M. Weerakoon. 1981. Growth, food intake and evacuation rates of grass carp, *Ctenopharyngodon idella* fry. Aquaculture 25(1): 67-76.

Fermin, A.C. 1985. Growth and survival of bighead carp, *(Aristichthys nobilis)* fry fed different types of feed and their combination. M.S Thesis. Central Luzon State University, Nueva Ecija, Philippines. 49 p.

Fermin, A.C. 1987. Broodstock development and management and seed production of carp and tilapia. Report on ADSEA '87 Seminar on Aquaculture Development in Southeast Asia, p. 272-292.

Lubzens, E., G. Sagie, G. Minkoff, E. Meragelman and A. Schneller. 1984. Rotifers *(Brachionus plicatilis)* improve growth rate of carp *(Cyprinus carpio)* larvae. Bamidgeh 36(2): 41-46.

National Research Council (NRC). 1983. Nutrient Requirements of Warmwater Fishes and Shellfishes. National Academy Press, Washington, D.C. 102 p.

Santiago, C.B., M.B. Aldaba and E.T. Songalia. 1983. Effect of artificial diets on growth and survival of mikfish fry in fresh water. Aquaculture 34: 247-252.

Szlaminska, M. and A. Przybyl 1986. Feeding of carp (*Cyprinus carpio* L.) larvae with an artificial dry food, living zooplankton and mixed food. Aquaculture 54: 77-82.

Yufera, M. 1982. Morphometric characterization of a small sized strain of *Brachionus plicatilis* in culture, Aquaculture 27: 55-61.

Growth Performance of an Indian Major Carp, *Catla catla* (Ham.) on Fishmeal-Free Diets

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Three experimental diets incorporated with either defatted soybean meal (pellet SY), non-defatted silkworm pupa (pellet SP) or defatted silkworm pupa (pellet DP) as the principal ingredient were evaluated in comparison to a fishmeal based reference diet (pellet FM) on the Indian major carp, *Catla catla* (Ham.). The rearing experiment was conducted for 156 days in cement cisterns (25 m^2), with catla fry (0.8 g) at a density of 10,000 ha^{-1} and fed with one of the test diets at 5% body weight day^{-1}. The growth of fish was consistently higher with pellet SP, while the other diets induced almost equal growth. The final average weights attained were 97.96, 87.63, 84.63, and 84.56 g with pellets SP, SY, DP, and FM, respectively. There was no significant difference ($P < 0.05$) between treatments. The average specific growth rates were 3.08, 3.01, 2.99, and 2.99 in treatments SP, SY, DP, and FM respectively. The feed conversion ratio was better in treatment DP (3.50), followed by SY (3.64), SP (3.68) and FM (3.75).

The mean panel scores for organoleptic evaluations based on color, texture, odour and flavour indicated that fish grown on pellet FM could be graded as the best, followed by pellets SY, DP, and SP. Flesh protein values in fish grown on pellets SY and DP were higher when compared to the other two treatments. The fish grown on non-defatted silkworm pupa based diet had a higher fat content.

In India, the area under carp culture is increasing rapidly; yet there is no standard fish feed available in the market. The industry is now in urgent need of nutritionally balanced, cost-effective feeds as a further step in increasing production. Though fishmeal incorporated diets are known to induce good growth in carps, in recent years the availability of fishmeal has become uncertain and costly. Therefore, investigations on alternative cheaper sources of protein are needed to develop cost-effective diets. With this objective, defatted soybean meal, non-defatted silkworm pupa and defatted silkworm pupa were evaluated by conducting a feeding experiment on the Indian major carp, *Catla catla* (Ham.).

Materials and Methods

Fishmeal based diet was taken as the standard reference diet (FM). In the test diets, fishmeal was replaced with the ingredients selected for screening (Table 1) and levels adjusted as to make

all test diets isonitrogenous (Table 2). The experimental diets were designated as SY (defatted soybean meal), SP (non - defatted silkworm pupa) and DP (defatted silkworm pupa). The diets were prepared in pellet form following the procedure described by Jayaram and Shetty (1981).

Table 1. Chemical composition of the principal test ingredients (%)*.

Ingredient/ component	Defatted soybean meal	Non-defatted silkworm pupa	Defatted silkworm pupa	Fish meal
Moisture	7.43	6.90	6.49	8.50
Crude protein	53.77	41.23	63.25	55.04
Crude fat	1.87	17.94	1.26	7.60
Ash	10.03	11.84	18.65	16.41

*Average of three values.

Table 2. Ingredient proportion and proximate composition of experimental diets.

Ingredients (%)	Diet SY	SP	DP	FM
Defatted soybean flour	27	-	-	-
Silkworm pupa	-	30	-	-
Defatted silkworm pupa	-	-	24	-
Fish meal	-	-	-	24
Groundnut cake	25	25	25	25
Rice bran	37	34	40	40
Tapioca flour	10	10	10	10
Vitamin and mineral mix*	1	1	1	1
% Composition**				
Moisture	7.54	6.24	6.02	6.95
Crude protein	29.31	28.17	30.91	29.92
Crude fat	4.44	8.79	4.31	6.11
Crude fibre	9.22	8.47	10.88	10.21
Ash	12.77	13.82	15.80	15.22
Nitrogen free extract	36.72	34.51	32.08	31.59
Caloric content (Kcal/g)	3.33	3.58	3.22	3.31

* Nuvimin Forte - Supplied by Sarabhai Chemicals Ltd., India.
** Average of three values.

The growth of fish was assessed on test diets over a period of 156 days, employing uniform sized duplicate cement cisterns of 25 m^2 (5 x 5 x 1 m). The cisterns were devoid of soil bed and stocked with uniform sized catla fry at 10,000 fish/ha (25 fish/cistern) and fed with the test diets at 5% body weight day^{-1} throughout the experimental period. The feed quantity was regulated based on fortnightly sampling.

Moisture, protein, fat, ash and fibre were analysed employing AOAC (1975) methods and nitrogen free extract was calculated according to Hastings (1979). The water stability of the feeds was determined following the wet durability test of Hastings (1964). Statistical analysis was carried out using Duncan's multiple range test (Duncan 1955).

On termination of the experiment, fish under different treatments were judged by 14 trained panelists for qualities such as colour, texture, odour and flavour of raw and cooked (in 1.5% salt solution) flesh. In addition, fish grown on non-defatted silkworm pupa diet and fishmeal based diet were fried in Indian style and served to the same group of panelists. The differences in individual attributes were tested employing the method recommended by Kramer and Twigg (1970) and the overall quality was assessed according to Udupa and Jayaram (1979).

Results and Discussion

The water temperature in the experimental cisterns varied from 24.0 to 29.5°C during the period of rearing. After fortnightly sampling, water in the cisterns was replenished partially.

Water stability of all the diets was found to be satisfactory even at the end of six hours (Table 3).The higher fat content of diets SP and FM and better gelatinization of carbohydrate content of diet SY appear to have improved the stability of these pellets as compared to that of diet DP. The fat content is known to give compactness to the pellets by preventing the entry of water (Jayaram and Shetty 1981). Diets which are stable for at least 2-4 hours are preferred for major carps since they are slow feeders.

Table 3. Stability of formulated diets.

Diet	Time			
	1 hr	2 hr	4 hr	6 hr
SY	92.73^{b}	90.26^{b}	88.11^{b}	82.66^{b}
SP	90.11^{a}	88.66^{a}	86.20^{b}	80.42^{b}
DP	90.09^{a}	88.42^{a}	84.85^{c}	79.29^{a}
FM	90.61^{a}	89.01^{ab}	85.52^{a}	81.23^{c}
SEM*	0.48	0.41	0.09	0.13

Figures in the same column having the same superscript are not significantly different ($P < 0.05$).
*Standard error of means.

Growth studies

Since there was no significant difference in growth of fish between replicates, the data were averaged (Fig. 1). Non-defatted silkworm pupa based diet induced the highest growth in weight of catla. The second best growth was recorded with diet SY, except for a slight fall back between the 42 and 84 days. In DP treatment,though the growth was relatively poor until the 84th day, it improved satisfactorily, ultimately attaining slightly higher weight than in the FM treatment at the termination of the experiment. The overall average specific growth rate for weight was found to be better with diets SP and SY, while it was almost equal with diets DP and FM.Though the food conversion rate (dry weight of food ÷ wet body weight gain) was found to be slightly better with diet DP (3.50), there was no marked difference between other diets. The overall survival of fish was better in SP and DP treatments (Table 4).

Table 4. Growth, food conversion and survival of catla fed on different diets.

Parameter	Diet			
	SY	SP	DP	FM
Final average weight (g)	87.63	97.96	84.63	84.56
Net weight gain (g)	86.83	97.16	83.83	83.76
Average specific growth rate (%)	3.01	3.08	2.99	2.99
Daily weight gain (g/fish/day)	0.56	0.62	0.54	0.54
Daily food intake (g/fish/day)	2.04	2.28	1.89	2.03
Food conversion ratio	3.64	3.68	3.50	3.75
Overall survival (%)	86.00	98.00	96.00	84.00

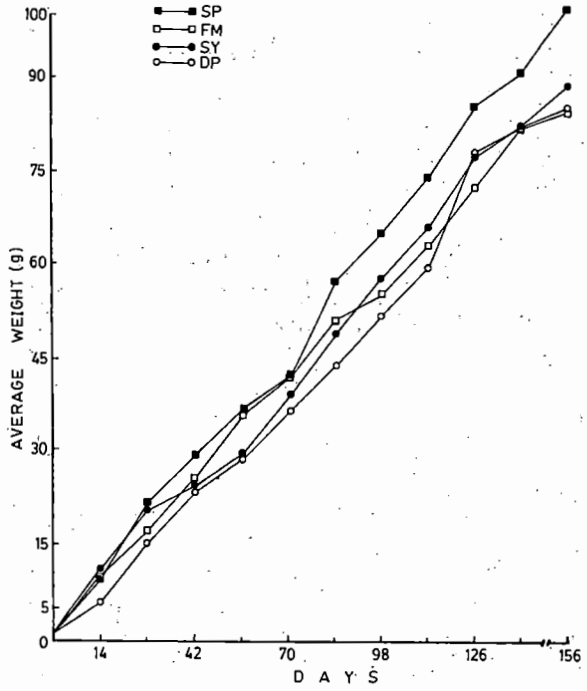

Fig. 1. The mean weight of catla fry reared under different food regimes.

Silkworm pupa is extensively used in carp diets in China and Japan (Hora and Pillay 1962) and is known to induce good growth in carps. The superior growth of catla on diet SP appears to be due to its high fat content, sparing protein for growth. The silkworm pupa is known to contain some attractants and appetite stimulants (Ina 1976; Tsushima and Ina 1978) which are known to result in better acceptability of the pellets and hence growth. In a rearing experiment using catla, rohu *(Labeo rohita)* and common carp *(Cyprinus carpio),* Jayaram and Shetty (1980b) recorded better growth in catla and common carp with a diet containing non-defatted silkworm pupa. In contrast to non-defatted silkworm pupa, defatted silkworm pupa based diet induced poorer growth of catla in the present experiment, though it was comparable to fishmeal based diet. It is possible that in defatted pupa the attractants and appetite stimulants are lost in the process of oil extraction and probably this accounts for relatively low growth. Interestingly soybean meal based diet induced good growth though it is known to be poor in lysine and methionine contents. Viola et al. (1982) suggested lysine and methionine supplementation and oil enrichment of soybean meal based diets to obtain the growth of common carp equivalent to that of fishmeal based diet. In a feeding trial using soybean meal based diet, Bhat et al. (1986) obtained growth of silver carp *(Hypophthalmichthys molitrix),* rohu and common carp comparable to that with fish meal based diets. Though soybean is known to contain some antinutritional factors other than trypsin inhibitors (Wilson and Poe 1985) proper cooking removes these factors (Spinelli et al. 1979).

It may be of interest to state here that some progressive fish farmers in Andhra Pradesh, India, are using soybean meal in carp diets with encouraging results. Nandeesha et al. (1987) obtained highly significant growth of common carp when soybean meal was combined with squilla meal *(Oratosquilla nepa)* which is rich in lysine and adequate in methionine. Though it may be too early to conclude that soybean meal could completely replace fishmeal in major carp diets, the encouraging results obtained in the present experiment suggest the need for further investigations on this aspect.

Proximate composition of fish flesh

The moisture and fat contents were higher and protein content lower in fish raised on diets SP and FM as compared to the other two treatments (Table 5). An inverse relationship between moisture and protein is observed in catla in contrast to that between moisture and fat in tilapia (Appler and Jauncey 1983; Edwards et al. 1985). The higher fat content of diets SP and FM appears to have resulted in higher fat deposition. The ash content was lower only in the SY treatment. The chemical composition of flesh mainly depends on the composition of diets and digestibility of nutrients (Dabrowski and Kozak 1979; Jayaram and Shetty 1980a).

Table 5. Proximate composition of flesh of catla.

Diet	Body composition (%) Moisture	Protein	Fat	Ash
SY	78.66^{ab}	16.82^{b}	2.58^{c}	1.12^{a}
SP	79.81^{c}	15.01^{a}	3.27^{b}	1.69^{b}
DP	78.55^{a}	16.91^{b}	2.25^{a}	1.93^{c}
FM	79.47^{bc}	15.17^{a}	2.80^{d}	1.99^{d}
SEM*	0.24	0.17	0.08	0.02

Figures in the same column having the same superscript are insignificantly different ($P < 0.05$).
*Standard error of means.

Organoleptic evaluation

The overall quality of flesh of catla grown on fish meal based diet was superior (Table 6). Based on the mean panel scores it could be observed that diet SP resulted in comparatively poor flesh quality. The overall quality of raw flesh of the SP treatment was significantly inferior to SY and FM treatments. However, no statistical difference could be observed in cooked flesh. Among the individual attributes, the texture of cooked flesh and colour of raw flesh were significantly better in fish grown on diets FM and SY. However, no difference in odour, flavour and texture could be noticed by the panelists when the fish grown on diets SP and FM were fried. Non-defatted silkworm pupa is known to induce some off-flavours in the flesh of carps when its incorporation in the diet is high (Hora and Pillay 1962). It appears that its incorporation upto 30% level has no adverse effect on odour and flavour. It is reported that in Japan fish are depurated before marketing when silkworm pupa is used in the diet to avoid the odour problem (Spinelli 1979).

Table 6. Mean panel scores for overall quality and individual attributes of fish flesh (a-colour and gloss of skin).

Diet	a Raw	Colour Raw	Cooked	Odour Raw	Cooked	Texture Raw	Cooked	Flavour Cooked	Overall quality Raw	Cooked
SY	3.50	3.50^{c}	3.00	3.21	3.14	3.57	3.36^{ab}	3.07	3.39^{ab}	3.07
SP	3.07	2.86^{a}	2.79	2.86	3.00	3.43	3.07^{a}	2.93	3.05^{c}	2.88
DP	3.14	3.00^{ab}	3.00	2.86	3.07	3.29	2.43^{c}	3.07	3.09^{bc}	3.00
FM	3.50	3.36^{bc}	3.14	3.36	3.14	3.71	3.79^{b}	3.14	3.48^{a}	3.13

Figures in the same column having the same superscript/without super script are not significantly different ($P < 0.05$).

The present investigation indicates the possibility of utilizing silkworm pupa and soybean meal in the diet of catla. These ingredients are 40-50% cheaper than fishmeal, and their use in carp diets would reduce the cost of production significantly.

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References

Appler, H.N. and K. Jauncey. 1983. The utilization of a filamentous green algae (*Cladophora glomerata* (L) Kutzin) as a protein source in pelleted feeds for *Sarotherodon* (Tilapia) *niloticus* fingerlings. Aquaculture 30: 21-30.

Association of Official Analytical Chemists (AOAC). 1975. Official methods of analysis. Washington, DC, USA, 1094 p.

Bhat, K.G., H.P.C. Shetty and M.C. Nandeesha 1986. Formulation and evaluation of pelleted fish feed based on soybean meal and squilla meal. *In* J.L. Maclean, L.B. Dizon, L.V. Hosillos (eds.) First Asian Fisheries Forum, pp. 539-542. Asian Fisheries Society, Manila, Philippines.

Dabrowski, K and B. Kozak. 1979. The use of fish meal and soybean meal as a protein source in the diet of grass carp fry. Aquaculture 18: 107-144.

Duncan, D.B. 1955. Multiple range and multiple F-tests, Biometrics 11: 1-42.

Edwards, P., M. Kamal and K.L. Wee. 1985. Incorporation of composted and dried water hyacinth in pelleted feed for tilapia *Oreochromis niloticus* (Peters). Aquaculture and Fisheries Management 16: 233-248.

Hastings, W.B. 1964. Fish feed processing research. Feed Stuffs 36: 13.

Hastings, W.H. 1979. Fish nutrition and fish feed manufacture. *In* T.V.R. Pillay and W.A. Dill (eds.), pp. 568-574. Fishing News books Ltd., Farnham, Surrey, England.

Hora, S.L. and T.V.R. Pillay. 1962. Handbook on fish culture in the Indo-pacific fisheries region. FAO Fisheries Biology Technical paper, No. 14, p. 203.

Ina, K. 1976. Fish behaviour. Kagaku To Seibutsu 14: 648-653.

Jayaram, M.G. and H.P.C. Shetty. 1980a. Studies on the growth rates of catla, rohu and common carp fed on different formulated feeds. The Mysore Journal of Agricultural Sciences 14: 589-606.

Jayaram, M.G. and H.P.C. Shetty 1980b. Influence of different diets on the proximate body composition of *Catla catla, Labeo rohita* and *Cyprinus carpio*. The Mysore Journal of Agricultural Sciences 14: 381-384.

Jayaram, M.G. and H.P.C. Shetty. 1981. Formulation, processing and water stability of two new pelleted fish feeds. Aquaculture 23: 355-359.

Kramer, A. and B.A. Twigg. 1970. Quality control for the food industry. Vol. I. AVI publishing Co., Westport, 566 p.

Nandeesha, M.C., N. Basavaraja, P. Keshavanath, T.J. Varghese, H.P.C. Shetty and G.K. Srikanth. 1987. Influence of soybean meal and squilla meal based diets enriched with sardine oil on the growth and organoleptic quality of common carp, *Cyprinus carpio* (Linn.) Paper presented at the international Symposium on Feeding and Nutrition in Fish. 23-27 August, Bergen, Norway.

Spinelli, J. 1979. Influence of feed on finfish quality. *In* J.E. Halver and K. Tiews (eds.) Proceedings of the World Symposium on Finfish Nutrition and Fishfeed Technology, Vol. II, pp. 346-352. Hamburg, Berlin.

Spinelli, J., C. Mahnken and M. Steinberg. 1979. Alternate sources of proteins for fishmeal in salmonial diets. *In* J.E. Halver and K. Tiews (eds.). Proceedings of the world Symposium on Finfish Nutrition and Fishfeed Technology, Vol. II, pp. 132-139. Hamburg, Berlin.

Tsushima, J. and K. Ina. 1978. Survey of feeding stimulants for carp, *Cyprinus carpio*. Nippon Nogei Kagaku Kaishi, 52: 225-229.

Udupa, K.S. and M.G. Jayaram. 1979. A note on the statistical analysis of panel test data of food products by sensory evaluation. Current Research 18: 106-107.

Viola, S., V. Mokady, U. Rappaport and Y. Arieli. 1982. Partial and complete replacement of fishmeal by soybean meal in feeds for intensive culture of carp. Aquaculture 26: 223-236.

Wilson, R.P. and W.E. Poe. 1985. Effects of feeding soybean meal with varying trypsin inhibitor activities on growth of fingerlings channel catfish. Aquaculture 46: 19-25.

Utilization of Aquatic Weed Mixture Pellet as Feed for Nile Tilapia *(Oreochromis niloticus)* and Pig

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Chiayvareesajja, S., C. Wongwit, A. Cronin, K. Supamataya, C. Tantikitti and R. Tansakul. 1989. Utilization of aquatic weed mixture pellet as feed for Nile tilapia *(Oreochromis niloticus)* and pig, p. 143-147. *In* S.S. De Silva (ed.) Fish Nutrition Research in Asia. Proceedings of the Third Asian Fish Nutrition Network Meeting. Asian Fish. Soc. Spec. Publ. 4, 166 p. Asian Fisheries Society, Manila, Philippines.

Dry aquatic weed *(Ceratophyllum demersum)*, rice bran and fish meal were mixed in a ratio of 4:3:1 in order to produce aquatic weed mixture pellet for the rearing of Nile tilapia *(Oreochromis niloticus)* and pig. Two culture systems; tilapia culture in ponds with formulated feed at a feeding rate of 10% body weight per day; and an integration of pig-tilapia using the same formulated feed as pig feed at a feeding rate of 10% body weight per day were carried out in triplicate. During the six-month culture period, pig and tilapia growth, water quality parameters (pH, temperature, DO, conductivity and turbidity) were monitored at monthly intervals. Tilapia production in the integrated system was slightly higher than in the monoculture system; production from the monoculture system and the integrated system being 4467 and 5499 kg/ha respectively. The pig grew well on the formulated feed and a good quality carcass was obtained. The results suggest that the integration of pig-tilapia using aquatic weed mixture pellet as pig feed is practical and useful.

In certain areas in Thailand, such as Thale Noi, pigs are fed with fresh *Ceratophyllum demersum,* one of the most abundant aquatic macrophytes in the uppermost part of Songkhla Lake in Thale Noi. The nutritive value of *C. demersum* is relatively low (Boyd 1968). The moisture content, crude protein, crude lipid and ash contents of sun dried *C. demersum* in order were 15.72%, 16.23%, 1.48%, 19.66 and 8.25% (Chiayvareesajja et al. 1987).

This study was undertaken in order to evaluate the utilization of this aquatic weed incorporated into pellet form as feed for the rearing of Nile tilapia, *Oreochromis niloticus* (L.) and pig. Furthermore, in order to maximize the limited resources, tilapia production using pig manure was also studied.

Materials and Methods

Two earthen ponds, 480 m² each, were partitioned into three compartments with plastic sheets. One pond was used for monoculture of tilapia, while the other was used for the integration of pig and tilapia. A pigsty of 4 m² was constructed over each compartment and adjacent to the bank in the latter pond. Both ponds were dried and limed with quick lime (10-15 kg/compartment) before stocking. Tilapia (approximate length 5 cm) were stocked at a density of 640 fish/compartment (160 m²). Three pigs, approximately 6 kg each, were stocked in each pigsty.

Sun-dried *C. demersum,* rice bran and fish meal were mixed in a ratio of 4:3:1 and 1 g of livestock vitamin supplement (OVIMIN) was added for every 4 kg feed. The semi-moist pellet was made daily with a simple extruder. Tilapia in the monoculture system and pigs in the integrated system were fed this formulated feed at a rate of 10% body weight/day, twice a day. Piggery waste was directly dispersed into the integrated pond. The amount of feed was adjusted after each sampling period.

The experiment lasted 6 months during which pig and tilapia growth, loadings of pig manure into the pond and water quality parameters were monitored at monthly intervals. The water quality parameters pH, temperature, dissolved oxygen (DO), conductivity and turbidity were monitored by a Horiba water checker (Horiba U-7, Horiba Ltd., Japan).

Proximate composition of formulated feed and pig faeces were determined according to the methods recommended by AOAC (1975), and gross energy with a ballistic bomb calorimeter (Gallenkamp). The statistical significance of tilapia growth and production were analysed using the Micro Quasp package.

Results

Water quality parameters in the two systems are presented in Table 1. The ranges observed of the different parameters were comparable in the two systems. In both systems the ranges did appear to exceed beyond the tolerance limit of *O. niloticus*.

Table 1. The range in water quality parameters in the two culture systems from 30 May to 22 November 1987.

Culture System	pH	Temperature (°C)	DO (mg/l)	Conductivity mS/cm	Turbidity (NTU)
Monoculture	6.30-6.55	30.2-30.8	3.48-6.12	0.07-0.48	17.50->100
Integrated	6.11-6.60	30.0-30.8	2.82-8.67	0.13-0.57	0.76-50.0

The nutritive value of formulated feed was much higher than that of pig faeces. The crude protein and lipid contents of the formulated feed and pig faeces were (on dry weight basis) 24.25% and 12.69% and 14.15% and 5.66%, respectively (Table 2.).

All the pigs grew well and the percentage weight gain was high (1074.68%). The amount of piggery waste loading increased as the pig grew (Fig. 1). The average loadings of pig faeces and urine were 6.42 kg/3 pigs/day and 6.24 kg/3 pigs/day, respectively.

Growth performance of tilapia in the two systems was not apparently different (Fig. 2). However, although there was no significant difference between tilapia production in the two systems the production in the integrated system was slightly higher than in the integrated system (87.99 and 71.47 kg/compartment, respectively: Table 3). The percentage weight gain of tilapia

Table 2. Proximate composition of formulated feed and pig faeces (mean ± SE), air dry basis.

Item	Formulated Feed	Pig Faeces
Moisture (%)	42.97 ± 2.13	79.40 ± 1.27
Crude protein (%)	24.25 ± 4.06	12.69 ± 0.12
Lipid (%)	14.15 ± 5.44	5.66 ± 1.86
Crude fibre (%)	9.75 ± 0.29	14.86 ± 0.76
Ash (%)	19.24 ± 0.76	26.09 ± 1.01
Ca (%)	2.29 ± 0.53	3.62 ± 1.29
P (%)	3.30 ± 0.79	4.62 ± 0.54
Gross energy (Kcal/kg)	4660.72 ± 130	4457.18 ± 275

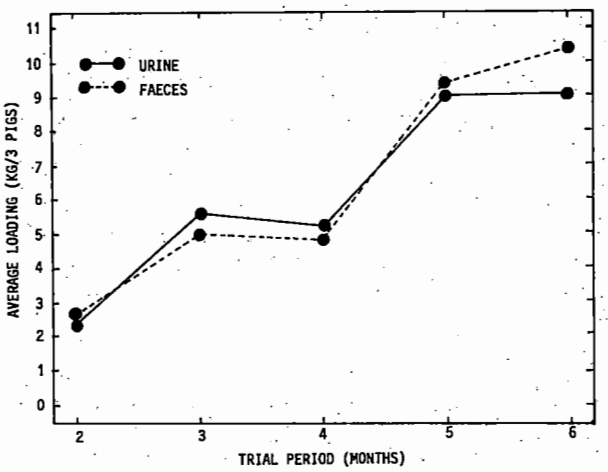

Fig. 1. Daily loading of piggery waste into the integrated system.

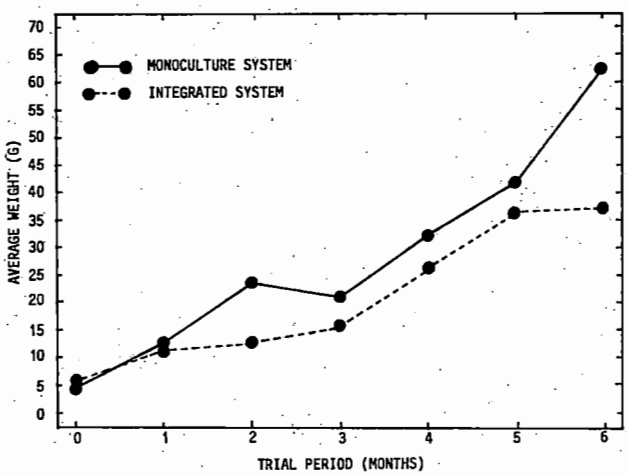

Fig. 2. Growth performance of tilapia in the two systems.

Table 3. Growth performance of tilapia and pig (mean ± SE) during 30 May to 22 November 1987.

Item	Tilapia	Integration	
		Tilapia	Pig
Initial number (No/compartment)	640	640	3
Final catch (No/compartment)	1155 ± 185	2360 ± 450	3 ± 0
% by number of final catch			
5 - 10 cm class	11.10 ± 1.21	11.85 ± 1.79	
10 - 15 cm class	42.93 ± 1.27	72.08 ± 1.60	
> 15 cm class	45.97 ± 1.24	16.08 ± 2.47	
Average Initial weight**	4.39 ± 0.21[a]	5.60 ± 0.13[b]	5.92 ± 0.20
Average Final weight**	62.39 ± 1.73[c]	37.11 ± 0.71[d]	69.44 ± 0.44
Average % weight gained**	1329.80 ± 102.74[e]	563.82 ± 26.88[f]	1074.68 ± 34.25
Total Production (kg/unit)**	71.47 ± 9.77[n]	87.99 ± 17.75[n]	208.33 ± 0.28
Food Conversion Rate	4.05 ± 0.69	25.77 ± 4.25	7.39 ± 0.28

** unit of initial or final weight g/individual for tilapia and kg/individual for pig; for values along each horizontal line those with a common superscript was not significantly different at $P < 0.05$.

in the monoculture system was much higher than in the other. The food conversion ratio (FCR) of tilapia, on the formulated feed (4.05) was much better than in tilapia fed on piggery waste (25.77). The FCR of 7.39 of pigs fed on aquatic weed mixture pellet was relatively high.

At the final harvesting, the number of tilapia in both systems were higher than the number stocked, and that in the integrated system was twice that of tilapia in the monoculture system, (2360 and 1155 fish/compartment, respectively). Furthermore, most tilapia in the integrated system (72.08%) were of 10-15 cm, and in the other (45.97%) were of >15 cm.

Discussion

The growth of pigs fed on aquatic weed mixture pellet is considered to be poorer than growth obtained in general commercial pig farming in Thale Noi. Generally, pigs are fed with a commercial feed and it takes only 4 months to reach the marketable size (over 100 kg). The FCR of pigs fed on the commercial feed is about 4.1 (Anon. n.d.). In this study the poor growth of pigs could be due to a poor management and a nutritionally inadequate feed as compared to that in commercial pig farms. While the formulated feed contains 24.25% crude protein, a commercial pig feed (Hogdonal 252) contains 37.76% crude protein (Chiayvareesajja et al. 1987). After a 6-month period on a formulated feed, pigs were fed a commercial pig feed at *ad libitum* for one month when they reached the marketable size. These results show that there is a potential for utilization of aquatic weed mixture pellet as pig feed.

It is very difficult to analyse the significance of tilapia growth in the two systems since there were a large number of young produced. The average final weight of tilapia in the monoculture system was significantly higher than in the integrated system but the total final number of fish was much lower in the first system.

The production of tilapia in the integrated system was slightly higher than in the monoculture system. This may be due to a big difference of nutrient loading into each system. The average value of daily loading of pig faeces was 6.42 kg/compartment, which was equal to 0.81 kg crude protein/compartment/day while in the monoculture system it was 1.46 kg of feed/compartment/day which was equal to 0.35 kg crude protein/compartment/day.

These results suggest that the integration of pig-tilapia using aquatic weed mixture-pellet as pig feed is practical and useful on the basis of maximizing the limited resources and reducing the fish production costs. The cost of supplemental fish feed is the largest operating cost in fish culture, usually 50% of the total operating costs. Polyculture of common carps and Chinese carps (Woynarovich 1980) and/or Indian carps and Chinese carps (Jhingran and Sharma 1980) are known to utilize all available food types resulting in a higher production. The introduction of a new fish species instead of tilapia in the integration of pig-fish farming is also feasible. Fang et al. (1986) reported that pig manure is suitable for the growth of *Carassius cuvieri*. In order to avoid oxygen depletion and to maintain an adequate supply of nutrient loading into fish ponds the amount of piggery waste loading should be adjusted. In manure loaded ponds which produced high fish yields, manure (dry organic matter) was added daily at a rate of about 2 to 4% of fish biomass (Schroeder 1980). Woynarovich (1980) reported that the total manure loading per ha of pond surface is between 40 and 80 pigs/ha which is similar to that reported by Cruz and Shehadeh (1980) with 60 pigs/ha, and Jhingran and Sharma (1980), with 35-40 pigs/ha. Chen and Li (1980) reported that waste loading can be as high as 250 pigs/ha. Moreover, some carnivorus species such as *Ophicephalus striatus* (Cruz and Shehadeh 1980) and *Lateolabrax japonicus* (Chen and Li 1980) are stocked in the ponds in order to control overcrowding of tilapia. Cruz and Shehadeh (1980) reported that the highest net yields were obtained with the 60 pigs/20,000 fish (85% *Sarotherodon niloticus*, 14% *Cyprinus carpio* and 1% *Ophicephalus striatus*)/ha.

Acknowledgement

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References

Anonymous. n.d. Manual for rearing of fattening pig. Division of Advertising and Marketing Promotion, Charoen Pokphand Industry Co. Ltd. Bangkok. 24 pp. (in Thai).

Association of Official Analytical Chemists (AOAC). 1975. Official methods of analysis. 12 edition. Washington, DC. USA. 1094 pp.

Boyd, C.E. 1968. Fresh water plants: a potential source of protein. Economic Botany 22: 239-168.

Chen, T.P and Y. Li. 1980. Integrated Agriculture-Aquaculture Studies in Taiwan. *In* R.S.V. Pullin and Z.H. Shehadeh (eds.) Integrated Agriculture-Aquaculture Farming Systems, pp. 239-241. ICLARM Conference Proceedings 4, Manila, Philippines.

Chiayvareesajja, S., B. Sirikul, P. Sirimontrapom, S. Rakkeaw, R. Tansakul, and A. Somprasit. 1987. Tilapia Cage Culture with Aquatic weed Mixture Pellet in Thale Noi, Thailand. Paper presented at the Second International Symposium on Tilapia in Aquaculture, during 16-20 March, 1987. Bangkok. (in press).

Cruz, E.M. and Z.H. Shehadeh. 1980. Preliminary Results of Integrated Pig-Fish and Duck-Fish Production Tests. *In* R.S.V. Pullin and Z.H. Shehadeh (eds.) Integrated Agriculture-Aquaculture Farming systems, pp. 225-238. ICLARM Conference Proceedings 4, Manila, Philippines.

Fang, Y.X., X.Z. Guo,, J.K. Wang, X.Z. Fang, and Z.Y. Liu. 1986. Effects of Different Animal Manures on Fish Farming. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum, pp. 117-120. Asian Fisheries Society, Manila, Philippines.

Jhingran V.G. and B.K. Sharma. 1980. Integrated Livestock-Fish Farming in India. *In* R.S.V. Pullin and Z.H. Shehadeh (eds.) Integrated Agriculture-Aquaculture Farming Systems, pp. 135-142. ICLARM Conference Proceedings 4, Manila, Philippines.

Schroeder, G.L. 1980. Fish Farming in Manure-Loaded Ponds. *In* R.S.V. Pullin and Z.H. Shehadeh (eds.) Integrated Agriculture-Aquaculture Farming Systems, pp. 73-86. ICLARM Conference Proceedings 4, Manila, Philippines.

Woynarovich, E. 1980. Utilization of Piggery Waste in Fish Ponds. *In* R.S.V. Pullin and Z.H. Shehadeh (eds.) Integrated Agriculture-Aquaculture Farming Systems. pp. 125-128. ICLARM Conference Proceedings 4, Manila, Philippines.

Improvement of Meat Quality of Grass Carp, *Ctenopharyngodon idellus* (Cuv. et Val.)

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Deterioration of the meat quality of grass carp in recent years is mainly attributed to improper feed formulation which results in the production of big-belly grass carp.

Big-belly grass carp is stout, with protuberant belly. Ratio of viscera and mesentery fat to body weight is 11% and 4.2% respectively. Water content is 80.6% and protein 16.6%. The musculature is flabby and lacks elasticity. Histological examination of muscle tissues reveals that the arrangement of myofibrils is loose, with 7-9 fibrils within 0.01 mm, the spacings between the fibrils are wide and the zonation of dark and bright bands is indistinct.

Grass carp fed experimental formulation as compared to those fed green manure show little difference in body shape. However, in the former, the ratio of viscera and of mesentery fat to body weight is 7.9% and 1.8%; and in the latter the ratio is 6.2% and 0.8%. The moisture, protein and total amino acid content of muscle differ in the two group.

In grass carp fed experimental formulations the arrangement of myofibrils in muscle is compact, with 12-14 fibrils within 0.01 mm; the spacings narrower; the zonation of dark and bright bands distinct and the muscle elastic. The fish has a better taste than green manure fed grass carp.

With the development of grass carp culture it has been a common practice for fish farmers to use mono-feedstuff as the main feed source. Consequently, the resulting nutritional imbalance leads to a deterioration of meat quality and the production of the kind of grass carp referred to as big-belly grass carp.

Morphologically big-belly grass carp is stout, with protuberant belly and a high accumulation of mesenteric fat. The edible part of the fish is thus reduced and the taste is 'flat'.

This phenomenon is attributable to different factors. In the present study the effects of three different feedstuffs, greens, mono-feedstuff and well- balanced feed on the meat quality of grass carp were investigated.

Materials and Methods

Eighty to one hundred grass carp of 200-300 g in weight were stocked in three ponds each of 533 m^2 and 1.5 m deep. The experiment period lasted from May to November when the fish had grown to a marketable size.

Three different feeds were applied through the experimental period: green manure, mainly grass of monocotyledon, mono-diet, soaked whole corn or wheat, or chopped beancake and pellet feed of experimental formulation.

At the end of the experiment all fish were measured and weighed. The viscera were then dissected and weighed and the hepatopancreas and adipose tissues were separated from the mesenteries. Samples of liver and muscle taken from apiaxial were dried at 105°C. Crude protein, fat and ash contents were determined and tissues and liver were fixed in Bouin's solution and paraffin-sectioned for histological studies.

Results

The belly of fish fed on mono-feedstuff is swollen and drooping (Plate 1), unlike in the other groups, the condition factor being 2.00. The condition factor of those fed greens was 1.81 and of those fed mixed pellets 1.79.

The proportion of the viscera to the body weight in the mono-feedstuff group was 11.8%, whereas that in the balanced diet group 8.86% and in the green manure feeding group 6.5%.

The proportions of the liver and the mesenteric adipose tissue to the body weight in grass carp fed on mono-feedstuff are the highest, being 3.2% and 4.2%, as compared with those in the balanced diet group which were 2.3% and 1.6% and those in the grass feeding group 2.3% and 0.8%.

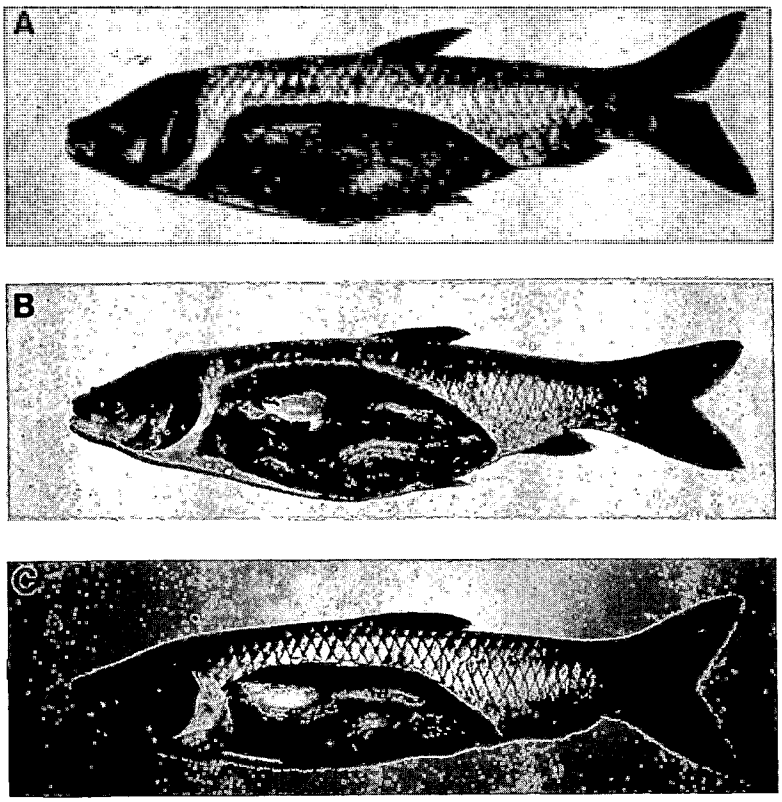

Plate 1. External features and gross anatomy of grass carp fed on three different feedstuffs. A. Grass carp fed on mono-feedstuff. B. Grass carp fed on green manure. C. Grass carp fed on balanced diet.

The moisture content of fish fed on balanced diet is the lowest, 77.8%, as compared with that in the mono-feedstuff group which was 80.6% and 78.9% of that in the grass fed group. The crude protein content was the highest in the balanced diet group (Table 1).

The glutamic acid content of 15.0%, in the muscles of the fish that gives the fish its delicious taste was highest in the balanced diet group. The proline content of 5.45%, and the histidine content of 2.89% were also the highest in this group (Table 2).

The phenylalanine content was lowest in the balanced diet group.

The histological structure of the muscles of fish maintained on three different feeds shows a marked difference (Table 3).

Grass carp fed on mono-feedstuff has a higher lipid content in the liver, amounting to 8.65% against 6.90% in fish fed on the balanced diet (Table 4).

Table 1. Moisture and proximate composition of fish maintained on three different feeds expressed as % of the fresh weight.

Feed	Moisture	Protein	Fat	Ash
Mono-feedstuff	80.58 ± 0.80	16.64	1.56 ± 0.04	1.12
Greens	78.98 ± 0.48	18.82 ± 0/09	0.45 ± 0.08	1.19 ± 0.05
Balanced diet	76.84 ± 0.24	19.41 ± 0.47	1.36 ± 1.41	1.22 ± 0.05

Table 2. Amino acid in the muscles of fish maintained on three different feeds (dry wt. %).

Feed	Total amino acids	Glutamic acid	Proline	Phenyla-lanine	Histidine
Mono-feedstuff	81.16	12.60	0.16	4.51	2.08
Greens	82.52	12.70	2.48	5.11	2.19
Balanced diet	88.56	15.0	5.45	4.27	2.89

Table 3. Histological structure of the muscles of fish maintained on three different feeds

Feed	Number of fibrils within 0.01 mm	Spacings	Zonation	Elas-ticity
Mono-feedstuff	7-9	wide	indistinct	poor
Greens	11-13	narrow	distinct	good
Balanced diet	12-14	narrow	distinct	good

Table 4. Liver composition of fish maintained on three different feeds expressed as % of the fresh weight

Feed	Water	Crude protein	Crude fat	Ash
Mono-feedstuff	71.21 ± 2.68	10.42	8.56 ± 2.39	1.58
Greens	72.80 ± 3.18	11.35 ± 1.63	2.36 ± 2.14	1.12 ± 0.04
Balanced	66.26 ± 3.43	12.10 ± 0.31	6.90 ± 0.81	1.02 ± 0.07

Histological studies reveal that among the three groups the liver cells of grass carp maintained on mono-feedstuff are the most seriously deteriorated because of the infiltration of lipoid into the hepatopancreas. The deterioration was manifested in the dislocation of the nucleus, the distortion of hepatic muralia and the loss of cytoplasm staining affinity. As to the balanced diet fed grass carp, the situation was not so serious though there was an accumulation of lipoid in the liver cells and some cells increased in size. The grass fed fish seem to be the least affected as the liver cells contain little lipoid (Plate 2).

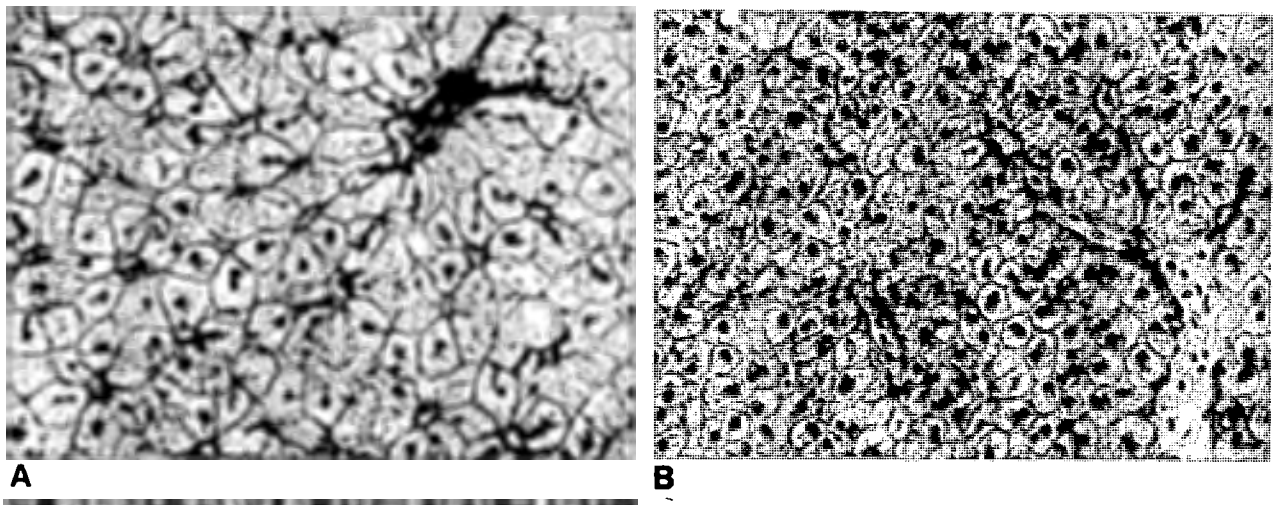

Plate 2. Photomicrograph of liver cells of grass carp fed on three different feedstuffs. A. Liver cells of grass carp fed on mono-feedstuff x 250. B. Liver cells of grass carp fed on green manure x 250. C. Liver cells of grass carp fed on balanced diet x 250.

Discussion

The improvement of meat quality of aqua-products compels attention. Factors affecting the quality of aqua-products are manifold, among which imbalanced diet seems to be the chief one. Results of the present investigation show that it is feasible to improve the meat quality of grass carp through the application of a balanced diet.

Food intake and energy expenditure are more or less in a state of constant turnover in wild animals (Heisuke 1985). Voluntary activities expend much energy and thus the energy reserve in the body is reduced. But grass carp under cultivation and maintained on mono-feedstuff or imbalanced diet tend to have excessive energy, probably more than the optimal level. The nutrients may be improperly utilized and transferred into accumulated fat.

Ingredients of mono-feedstuffs used in China are mainly vegetative; such as corn, wheat middlings and a small proportion of soybean cake and peanut cake. The P/E ratio is not balanced, as the diet is composed of low level dietary protein and high level carbohydrate. Excessive carbohydrate may stimulate lipogenic enzyme activities both in the liver and in the mesenteric adipose tissues. The indispensable amino acids in the vegetative protein source are not balanced as well, and the low level methionine can not satisfy the needs of the fish for CH3.

Hidetoshi (1985) in his studies on the improvement of meat quality of yellowtail *Seriola quinqueradite* (T. et S.) pointed out that differently formulated feedstuffs have different effects on the fat accumulation. Fat accumulates when fish are fed with low protein and high fat diets. It could be inferred that a balanced diet should take into consideration the balance of protein and energy as well as the overall balance of amino acids. Quick transformation of the diet can reduce excessive energy and in the end improve upon the fatness of the fish.

It is generally acknowledged that nutrients in the diet have a distinct effect on the fat accumulation in the fish muscle. Our experiments show that the water and fat contents in the muscles of fish fed on mono-feedstuff or imbalanced diet are high while the protein content is low.

The differences in histological structure between the muscle of grass carp fed on mono-feedstuff and those fed with experimental formulations are distinct. Balanced diet has a positive effect on the arrangement of the myofibrils, the zonation of dark and bright bands and the degree of elasticity. Toshiyuki (1985) in his paper on the meat quality of red bream snapper *Pagrus major* (T. et S.) studied the relationship of feedstuff and muscle elasticity. He compared the degree of muscle deterioration of cultured fish with that of sea caught fish. The myofibrils of the former started to deteriorate on the first day after the death of the fish, and became fractionized on the third day, while in the latter the myofibrils remained in good condition thirteen days after the death of the fish. In our experiment the firmness of the meat of grass carp maintained on different diets was tested in boiling water. The meat of different diet groups of fish was sliced into pieces of about 1 mm in thickness and boiled separately for 2-3 min. Results show that the meat of fish fed or an imbalanced diet soon fractured but not so in fish fed on balanced diet.

The protein content and some free amino acids, such as glutamic acid, proline and histidine, give fish its delicious taste. Balanced diet improves the meat quality of grass carp because the muscles of the fish thus fed are firm and because some free amino acids are found in higher quantities. Hidetoshi (1985) in his studies on the improvement of meat of yellowtail *S. quinqueradite* obtained similar results.

The improvement of meat quality poses a challenging and intriguing problem for researchers of nutritional physiology of fish. The effect of feeds on fish meat quality is but one factor affecting the meat quality of aqua-products, and shows it is necessary to encourage further interdisciplinary coordination among researchers.

References

Heisuke, N. 1985. The importance of meat quality of cultured fishes. Fish Culture 11: 48-51.

Hidetoshi, K. 1985. Studies on the importance of meat quality of yellowtail *Seriola quinqueradite* (T. et S.). Fish Culture 11: 52-55

Liao Xianghua, Lin Ding and Mao Yongqing. 1985. On the nutrition of grass carp *Ctenopharyngodon idellus* (C. et V.) and mud carp *Cirrhinus molitorella* (C. et V.). Asian Symposium on Freshwater fish Culture, October 10-15, 1988. Beijing (in press).

Lin Ding, Mao Yongqing and Cai Fasheng. 1985. On the lipid liver disease of grass carp *Ctenopharyngodon idellus* (C. et V.). Asian Symposium on Freshwater Fish Culture, October 10-15, 1985. Beijing (in press).

Toshiyuki, K.. 1985. Studies on the importance of meat quality of red bream snapper *Pagrus major* (T. et S.). Fish Culture 11: 56-59.

Culture of Banana Prawn *(Penaeus merguiensis)* and Tilapia *(Oreochromis niloticus)* by Using Aquatic Weed Mixture Pellet

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Chiayvareesajja, S. and R. Tansakul. 1989. Culture of banana prawn *(Penaeus merguiensis)* and tilapia *(Oreochromis niloticus)* by using aquatic weed mixture pellet, p. 153-156. *In* S.S. De Silva (ed.) Fish Nutrition Research in Asia. Proceedings of the Third Asian Fish Nutrition Network Meeting. Asian Fish. Soc. Spec. Publ. 4, 166 p. Asian Fisheries Society, Manila, Philippines.

Culture of banana prawn *(Penaeus merguiensis)* and Nile tilapia *(Oreochromis niloticus)* in 0.43 m^3 plywood tanks at 20 ppt salinity on an aquatic weed mixture pellet was studied. The aquatic weed mixture pellet composed of dry weed *(Ceratophyllum demersum)*, rice bran and fish meal in a ratio of 4:3:1. Experiments were carried out on monoculture of prawns, fish and polyculture of prawn and fish in ratios of 1:1, 1:2 and 1:4, for 9 weeks. It was found that the tilapia in all treatments grew well. A poor growth rate and survival rate of prawns were observed in the polyculture systems.

Aquatic macrophytes may be used directly as human food, as livestock fodder, as fertilizer and as food for herbivorous fishes (Edwards 1980). Aquatic weeds can also in addition be used for water purification (Little 1979). In our aquatic plants utilization project in Songkhla Lake, it was found that *Ceratophyllum demersum,* a submerged aquatic plant, can be used as feed for herbivorous fish *(Puntius gonionotus, Oreochromis niloticus, Labeo rohita),* chicken and pigs (Tansakul 1987).

This preliminary study attempts to evaluate the utilization of dry *C. demersum* as fish pellet for the banana prawn *(P. merguiensis),* and the feasibility of culturing Nile tilapia *(O. niloticus)* in the same system with penaeus shrimp. If the system is proven to be feasible, it will benefit the small farmers and induce them to take up more profitable penaeus shrimp culture.

Materials and Methods

The experimental design consisted of 5 treatments with 3 replicates of each. In treatment 1, 14 *P. merguiensis* were reared in a marine plywood tank (10 shrimp/m^2). In treatment 2, only *O. niloticus* were reared in a tank (14 tilapia/tank). In treatments 3, 4 and 5 shrimp and fish were

reared in an integrated system with ratios of shrimp:fish, 1:1, 1:2 and 1:4 (14 shrimp with 14, 28, 56 fish, respectively).

Banana prawn *(P. merguiensis)* collected from Songkhla Lake at 7 cm total length and tilapia *(O. niloticus)* of 7 cm from the Department of Aquatic Science, Prince of Songkla University were acclimatized in 20 ppt sea water for 7 days before commencing the experiments. The experiment was done in a small closed system of 1.2 x 1.2 x 0.4 m marine plywood tanks with 30 cm water depth. Aeration was carried out as shown in Fig. 1. The system was covered by fine polyethylene nets to prevent the fish jumping, and the salinity maintained at 20 ppt during the experiment.

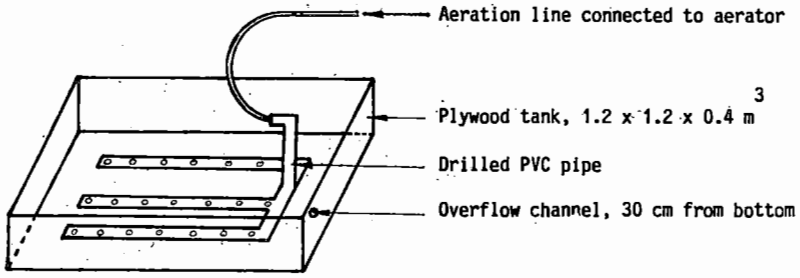

Fig. 1. Plywood tank for rearing of prawn and tilapia.

Experimental aquatic weed pellets composed of dry *C. demersum,* rice bran and fish meal in a ratio of 4:3:1 by weight plus vitamins and minerals (OVIMIN) supplementation up to 2% (Table 1) was used as the feed. Fish and shrimp were fed at 5% of body weight, 2 times daily at 0900 hr and 1700 hr.

The water quality of the system was checked for DO, pH, temperature with a HORIBA water checker (Horiba Co., Japan) every 3 weeks. Fish and shrimp were weighed for growth every third week and the ration adjusted accordingly.

Table 1. Proximate composition of *C. demersum* and the formulated feed, air dry basis.

	C. demersum			Feed*
	Fresh[1]	Fresh[2]	Dry[3]	
Moisture (%)	94.80	96.39	15.72	9.95
Crude protein (%)	21.70	29.08	16.23	23.18
Lipid (%)	5.97	11.91	1.48	5.20
Crude fibre (%)	-	-	8.25	9.69
Ash (%)	20.60	-	19.66	23.09
Gross energy (Kcal/Kg)	3710.00	-	-	3127.02**

[1] Boyd, 1968; [2] Sitasit et al. 1982; [3] Chiayvareesajja et al. 1987; * analysed by A.O.A.C. methods (A.O.A.C., 1975); ** analysed with a Ballistic bomb calorimeter (Gallenkamp).

Results

The experiment was carried out from 5 September to 26 November 1987 for 9 weeks. It was found that in the integrated system shrimp lost weight and survival was low, while the system with only shrimp or fish showed better growth (Table 2 and Fig. 2). In treatment 1, which had only shrimp *P. merguiensis* the body weight increased by 17.85% and 69.05% survival was recorded (Table 2).

Tilapia in every treatment showed an increase in the body weight (Table 2 and Fig. 3). treatment 3 (fish: shrimp, 1:1) showed the best growth (426.33%) and survival rate (97.62%).

Table 2. Growth and survival rate (mean ± SE) of banana prawn and Nile tilapia from 25 September to 26 November 1987.

Treatment	Weight (g)		Gain (%)	Survival (%)
	Initial	Final		
Banana prawn *(P. merguiensis)*				
T1	3.53 ± 0.24	4.16 ± 0.35	17.85	69.05 ± 2.38
T2	0	0	0	0
T3	3.14 ± 0.19	2.34 ± 0.02	-25.48	26.19 ± 2.38
T4	3.23 ± 0.10	2.46 ± 0.06	-23.84	21.43 ± 7.14
T5	3.15 ± 0.10	3.09 ± 0.05	- 1.90	9.52 ± 2.38
Nile tilapia *(O. niloticus)*				
T1	0	0	0	0
T2	3.07 ± 0.05	12.82 ± 1.57	317.59	85.71 ± 7.14
T3	3.19 ± 0.16	16.79 ± 0.46	426.33	97.62 ± 2.38
T4	2.85 ± 0.10	12.45 ± 0.60	336.84	91.67 ± 5.19
T5	2.86 ± 0.07	8.33 ± 0.23	191.26	93.45 ± 2.59

T1 = Monoculture of prawn; T2 = Monoculture of tilapia; T3 = Polyculture of prawn and tilapia in a ratio of 1:1; T4 = Polyculture of prawn and tilapia in a ratio of 1:2; T5 = Polyculture of prawn and tilapia in a ratio of 1:4.

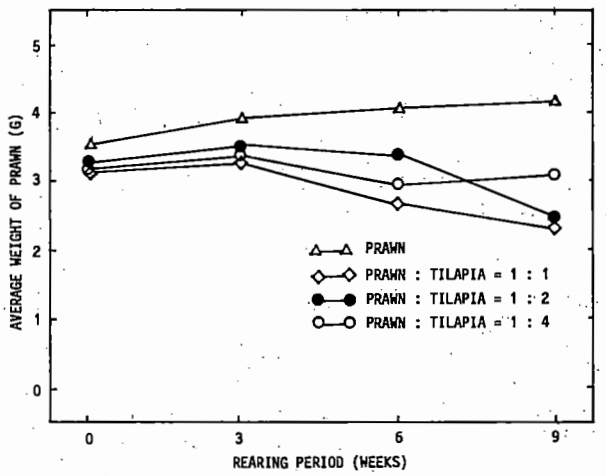

Fig. 2. Growth of prawn in each treatment.

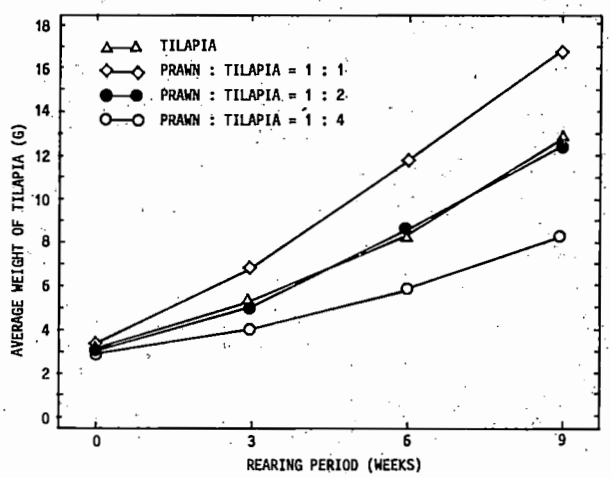

Fig. 3. Growth of tilapia in each treatment.

Water quality parameters in the 5 treatments during the experiment were not different; DO between 8.00 and 9.00 am was in the range of 4.47-5.83 ppm, temperature ranged from 26.23 to 30.10°C and pH ranged from 6.10 to 7.33.

Discussion

It was found that dry *C. demersum* can be utilized in pellet form for feeding *P. merguiensis* and *O. niloticus*. However, the growth of shrimp and fish were not as good as expected, probably due to lower protein content of the pellet. Boonyaratapalin (1987) recommended that pellets for *P. merguiensis* should have a crude protein level of 30-42%. Also the crude protein in fish pellets for tilapia should be 30-35% (Jauncey and Ross 1982). De Silva and Perera (1985) have shown that young *O. niloticus* grow best at 10-15 ppt salinity and that a protein level of 30% is optimal. Modification of weed pellet formula could improve the growth of the *Penaeus* shrimp and tilapia in the system.

There appears to be a potential to use this small closed system for *Penaeus* prawn culture. The low capital investment and low running costs, and practical method of operation were among some of the reasons to demonstrate *Penaeus* prawn culture for small farmers in fishing villages in Thailand. If this system can be improved to make it profitable, it will be of great benefit to small farmers.

In this study, it was also found that banana prawn and Nile tilapia may be reared together as in other polyculture systems. The best system for shrimp and fish rearing was treatment 3 (shrimp:fish = 1:1), in which tilapia gained 426.33% of the body weight but shrimp weight was reduced by 25.48% and the survival rate was only 26.19%. This low growth rate may be due to the shallow water depth used in the system (30 cm) which was probably insufficient for shrimp and tilapia to separate their feeding territories. When tilapia stocking density/ratio was increased to 2-4 times of the shrimp, it resulted in lower shrimp growth and survival. Integrated culture of *Penaeus* shrimp and tilapia has also been studied in earthen ponds in the Philippines (Pudadera et al. 1986) and it was found that the best ratio for shrimp and tilapia was 5,000 *P. indicus* to 1,500 *O. niloticus* and 200 *Chanos chanos* in 1,000 m^2 earthen pond. It is hoped that integrated systems of *Penaeus* shrimp and tilapia or even with pig and chicken rearing will be developed in the near future.

Acknowledgement

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References

Association of Official Analytica Chemists. 1975. Official methods of analysis. 12th Edition. Washington, DC, USA. 1094 p.

Boonyaratapalin, M. 1987. Nutrition and feeding techniques for tiger prawns. Extension sheet, National Inland Fisheries Institute, Department of Fisheries, Ministry of Agriculture and Cooperative, Bangkok. 41 p. (in Thai).

Boyd, C.E. 1968. Fresh water plants: a potential source of protein. Economic Botany 22: 359-368.

Chiayvareesajja, S., B. Sirikul, P. Sirimontraporn, S. Rakkeaw, R. Tansakul and A. Somprasit. 1988. Comparison between natural feeding done and supplemental feeding with pellets containing locally available ingredients for cage culture of *Oreochromis niloticus* in Thale Noi, Thailand, p. 323-327. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, 623 p.

De Silva, S.S. and W.M.K. Perera. 1985. Effects of dietary protein level on growth, feed conversion and protein use in young *Tilapia nilotica* at four salinities. Transactions of the American Fisheries Society 114: 584-589.

Edwards, P. 1980. Food potential of aquatic macrophytes. International Center for Living Aquatic Resources Management, Manila, Philippines. 51 p.

Jauncey, K. and B. Ross. 1982. A guide to tilapia feeds and feeding. Institute of Aquaculture, University of Stirling, Scotland. 111 p.

Little, E.C.S. 1979. Handbook of utilization of aquatic plants. FAO Fisheries Technical Paper No. 187. 176 p.

Pudadera, B.J.J., K.C. Corre, E. Coniza and G.A. Taleon. 1986. Integrated farming of broiler chickens with fish and shrimp in brackishwater ponds. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum, pp. 141-144. Asian Fisheries Society, Manila, Philippines.

Sitasit, P., M. Boonyaratapalin and N. Ounprasert. 1982. Fish nutrition. National Inland Fisheries Institute, Department of Fisheries, Ministry of Agriculture and Cooperative. Bangkok. p. 77, 88. (in Thai).

Tansakul, R. 1987. Aquatic weeds (Thailand) Phase II, first year report. Department of Biology, Faculty of Science, Prince of Songkla University, Haad Yai. 37 p.

Summary of the Discussions

The workshop was divided into six sessions, in which the review papers were presented in the first two and the original papers in the other four. This summary entails the main points that were discussed.

1. STANDARDIZATION

Fish nutrition researchers in Asia, as elsewhere tend to use a wide array of terminology and as such there is confusion in the manner in which data are presented; such as for example the amount of feed presented and/or consumed, efficiency of food utilization etc. are expressed in different forms by different authors. The workshop was of the view that it would be desirable to agree upon common terminology so that exchange and comparisons of data from different studies would be facilitated.

2. LABORATORY TRIALS

It was pointed out that when conducting feeding experiments, in particular in relation to evaluation of diets under laboratory conditions it would be desirable to:

a) consider the economic aspects in the formulation of the experimental and or test diets;

b) conduct growth experiments over an appreciable period of time, such as for example until a 5 to 10 fold increase in the mean weight is obtained;

c) take extreme care to minimize food wastage and to feed at least twice a day to satiation (unless the experiments are designed to evaluate the ration size and/or feeding frequency);

d) avoid undue stress on the fish by weighing individual fish at regular intervals, for example, fortnightly; it was felt that depending on the size of the fish and the duration of the experiment, intermediate weighings are best restricted to 'group weighings', if possible without anaesthetization, and

e) provide optional evironmental conditions such as pH, dissolved oxygen and lighting.

3. POND TRIALS

In experiments associated with supplemental feeding, mostly in ponds, attempts should be made to monitor the key environmental 'variables' such as temperature, dissolved oxygen and natural food production through estimation of chlorophyll, algal and zooplankton biomass regularly. It was also pointed out that in such experiments regular feeding regimes need to be maintained and as far as possible and 10-20% of the fish should be sampled at regular intervals to monitor growth. The difficulties in assessing the fish biomass at times of sampling was discussed and it was felt that no exact guidelines could be provided for such experiments and the researcher's alertness would play a key role in the success of the experiment.

4. LARVAL EXPERIMENTS

It was apparent that at present most marine fish larvae can not be economically raised on artificial diets and that live foods such as rotifers are still preferred; nutrient requirement studies on larval fish often require bioenrichment of live foods such as rotifers as exemplified by the fatty acid requirement evaluations of the yellow tail (*Seriola* sp.) of T.Watanabe. It was also pointed out that generally, to date *Brachionus* or comparable live feeds are taken as the reference feeds and that if results contrary to this are obtained in larval growth experiments the researchers should repeat the experiments and carefully consider all possible errors that could have occurred before interpretation of data. In particular one would have to consider the size of the live food in relation to mouth-size and whether sufficient quantities of food were presented and the like.

In growth and feed response experiments with larvae and fry in particular, it is necesary to account for and make allowance for food ingested by those animals which die in the course of the experiment; if not the food conversion and protein efficiency ratios are likely to be influenced significantly, resulting in erroneous interpretation of the data.

5. DIGESTIBILITY

It was agreed that there is no rationale for using the terms coefficient with respect to the dry matter and/or nutrient digestibility which are expressed as percentages. The difficulties encountered in defining the differences between 'true' and 'apparent' digestibilities were dealt with and it was agreed that evaluation of the apparent digestibility of the total feed, ingredients or their nutrient components were sufficient. It was noted that:

a) in evaluation of the digestibility of an ingredient that an experimental diet composed of 70% of a well-defined reference diet and 30% of the ingredient to be tested should be used;

b) when results on ingredient digestibilities are presented the proximate/nutrient composition of them should be made available; and

c) when diet formulation is made with ingredients whose digestibilities are not known it was best to make estimates based on their proximate composition and equivalent energy values used in recent comparable studies but not on the 'physiological fuel equivalents'.

6. GENERAL

The role of zeolite in shrimp feeds was discussed. It was agreed that its main role was in retention and uniform distribution of the lipids. In addition, zeolite was also thought to reduce the ammonia, hydrogen sulphide and other toxic compounds in the water.

On site (on farm) preparation of supplemental feeds and aspects related to storage of commercial feeds were discussed. It was agreed that commercial feeds should be stored in a manner that ground contact is minimized and in places with a good air-circulation. The feeds are best utilized within three months of the date of manufacture. It was noted that in most feeds manufactured in Asia the date of manufacture is not recorded.

In general, it was felt that there is a potential in the use of a variety of waste-products as base ingredients for supplemental feeds and that more detailed investigation - one on these as well as on the variety of materials used indigenously by small- scale farmers and the practice employed would be desirable.

All participants were in agreement that attempts should be made to disseminate the results of studies on fish nutrition in internationally accepted journals and that it would be desirable to make it imperative for results of studies funded by donor agencies to attempt to publish the findings in international journals. Such attempts by themselves would in the longer term, cater to a standardization of the methodologies used and upliftment in fish nutritional research.

Participants recognized that recommendations made at the 2nd Fish Nutrition Workshop held in Manila, in May 1986 have been taken into consideration as apparent from the increasing research effort on aspects on use of non-conventional resources in feeds, supplemental feeding and a narrowing of the number of species worked on. It was however, felt that the problems of standardization of the terminology and research methodology have been insufficiently addressed, as yet.

Apart from the above discussions on the individual presentations considered specific elements of each, which undoubtedly would be taken into account by the individual researchers.

Workshop Recommendations

The Workshop recognized that:

1. There is a lack of standard texts, and that something similar to a manual in fish nutrition in relation to the Asian context is needed. As a result of this deficiency a high degree of variability is apparent in the research methodology amongst researchers from different countries in the region thereby hindering the use of research findings elsewhere.

   To partially overcome this situation the workshop recommended that Part A of IDRC publication 233e (Finfish Nutrition in Asia. Methodological Approaches to Research and Development by C.Y. Cho, C.B. Cowey and T. Watanabe (editors), 1985, 184 pp.) be revised and updated with examples from Asia incorporated into the text, where appropriate.

   The workshop also recommended that a quick reference manual be prepared by a group of researchers drawn from the region on the more important themes in fish nutrition, and that the IDRC liaises with ADCP as ADCP is considering a related effort.

2. The group was of the view that in most institutions in the region sophisticated analytical instruments are available but are underutilized; the underutilization often resulted from malfunctioning of the instruments and the lack of trained technicians.

   The workshop recommended that provision of analytical services (for most nutrition analyses) between institutions in the region should be encouraged, that future grantees should be encouraged to make estimates on the cost of obtaining analytical services within the region and the IDRC to explore the possibilities of providing selected technician training in institutions where appropriate in return for providing a particular service(s) to other researchers in the region.

3. The participants were of the view that the degree of staff exchanges between institutions involved in fish nutrition research in the region is very limited, and recommended that such exchanges should be encouraged where appropriate, perhaps through the 'Networking' system.

   The workshop also recommended that where possible researchers should be encouraged to select post-graduates from other countries in the region for scholarships which would limit 'inbreeding' and also that it would be an effective means of transferring and exchanging research methodologies and approaches.

4. The participants were in agreement that there was a dearth of training courses available in the region in fish nutrition. The workshop recommended that:

   a) Attachments to institutions for middle and senior researchers be encouraged and such attachments should be, where relevant, to institutions in the region;

   b) 'Satellite' courses in Experimental Design, Research Methods and Data Analysis be conducted; and

   c) Attempts should be made to develop a comprehensive training course in fish nutrition in a regional institution, commencing with a 3-4 week course, initially, once the needed type and numbers for training are better defined.

5. The participants endorsed the need to form an Asian Fish Nutrition Network and approved that they be the initial 'core' group. It was generally agreed that the network would function in a fashion similar to the other disciplinary research networks in the region, with the primary objective being to promote and adopt mechanisms that would upgrade and provide direction to fish nutrition research that is of relevance and application in the region. The document prepared by Prof. Sena S. De Silva on the Fish Nutrition Network (Annex) was adopted.

6. The workshop considered that there was a need to facilitate ways and means of making information transfer among scientists in the region more effective and the need to utilize the information services already available in the region. It was agreed that as a first step, copies of reprints authored by the participants would be exchanged.

7. The Workshop also recommended that regular meetings and workshops should be held to bring fish nutrition researchers together to enable them to interact effectively. The Workshop recommended that future workshops:

   a) should be conducted in a similar fashion to this meeting including sufficient time being allotted for interaction and discussion;

   b) that the practice of presenting full papers be continued;

   c) have a main workshop theme, but time be also allotted for presentation of other work on fish nutrition;

   d) to have a person(s) deliver a keynote address(es) on a subject related to the main theme of the workshop; and

   e) that all attempts be made to 'draw-in' and encourage younger scientists in fish nutrition research.

8. The Workshop recommended that the main theme for the next meeting be on aspects related to development and use of home made or on-farm prepared feeds, and be held toward the end of 1989 or in early 1990.

ANNEX

ASIAN FISH NUTRITION NETWORK

(1) BACKGROUND:

Two fish nutrition workshops have been held todate, the first in Singapore in September 1983 and the second in Manila in May 1986. It was agreed during these Workshops that it would be desirable to have more coordination amongst fish nutrition researchers in the region with a view to optimizing research inputs within the region and to bring about a more effective exchange of information amongst the researchers. This is of particular relevance to the Asian region which is embarking on intensification of the aquacultural practices even at the rural fish farmer level. Intensification goes hand in hand with increasing feed inputs into the 'systems'. It is accepted that feed costs is one of the highest, if not the highest recurring costs in fish culture. There is therefore, a need for an increasing emphasis on finfish nutrition research in the region; to develop low cost diets, to evaluate optimal husbandry practices for diffent systems and cultivated species, to evaluate the role of natural food in the system - to name a few priorities.

(2) RATIONALE:

In accordance with the recommendations made by researchers in the region, and in recognition of the need to increase the emphasis on fish nutrition research and coordination of activity amongst fish nutrition researchers in the region, the International Development Research Centre (Canada) has come forward to partially support the establishment of an Asian Fish Nutrition Network.

(3) OBJECTIVES:

The primary objectives of the Asian Fish Nutrition Network would be

(a) to bring together periodically researchers in fish nutrition in the region to develop a more coordinated research framework among Asian fish nutrition scientists;

(b) to provide information of on-going fish nutrition research in the region;

(c) to prepare and guide the preparation and publication of material/manuals of reference for fish nutrition research in the region; and

(d) to effect exchange of information and collaboration between researchers in other specialities which are of direct relevance to fish nutrition.

(4) The above is only a brief outline on the Asian Fish Nutrition Network. You will appreciate that a lot more effort is needed to make it a functional and an effective unit which would hopefully have a direct bearing on increasing the protein availability, at a reasonable price, to the rural poor in the region, as well as increasing the standard of living of the small fish farmer.

Obviously, the success of the network in meeting its objectives depends on each of us and our concerted efforts and commitment to the cause.

I shall be grateful if you would come back to me with your own ideas and suggestions on the network.

24 Nov. 1987

PROF. SENA S. DE SILVA

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- **Fish Nutrition Research in Asia.** 1989. Edited by S.S. De Silva. Asian Fish. Spec. Publ. 4, 166 p. Price: Members, free. Nonmembers, US$8.

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CANADA

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