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CONSULTANCY REPORT ON FISH NUTRITION PROGRAM

at
Aquaculture Unit, Primary Production Department
Ministry of National Development
Changi Fisheries Complex, Singapore

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CONSULTANCY REPORT ON FISH NUTRITION PROGRAM

Aquaculture Unit, Primary Production Department, Singapore

1. Introduction

The author visited the Philippines, Singapore, Malaysia and Sri Lanka for consultation on the fish nutrition programs at the respective Institutions in the period of August 12 - September 12, 1982 following a request from the International Development Research Centre (IDRC) of Canada. The visits to Singapore and Malaysia were to follow up a previous consultation visit made in 1980.

This report is a written summary of discussions and recommendations already made to the researchers at the Institutions while the author was visiting. Because of the similarity of the discussions and recommendations made to the 4 Institutions, the common subjects were listed in the GENERAL Section. The specific comments on each Institution are given in the SUMMARY Section. Since the steps for the initial approach in fish nutrition and diet development are the same, several parts of the previous report following the 1980 visit are reproduced. The Manufacturing Specifications for Fish Feed which are employed by the Ontario Ministry of Natural Resources, Fisheries Branch are included in Appendix 1 as a guide-line for

feed manufacture.

As mentioned in the previous report there is no doubt about the potential of aquaculture in South-East Asia as many of the environmental conditions for various fish species are very advantageous in this region. There is also a large demand for fisheries products in this area.

The breeding program, including the induced spawning technique, of suitable fishes for the Philippines, Singapore and Malaysia seemed to be well established. Furthermore, the weather in the region is very suitable for producing natural food for larvae. Therefore, to promote more intensive fish culture rather than traditional extensive rearing, several problems associated with husbandry and management have to be solved. Fish nutrition, particularly diet formulation and feed manufacturing, need to be developed in the immediate future if there is to be a further advance of fish culture and production in this region. This report includes recommendations for future research and experiments in the areas of nutrition. In addition several other areas specified by IDRC for the consultancy are clarified.

A reference for the principle and methodology of diet formulation and evaluation with respect to energy is reproduced in Appendix 2 of this report for future information.

The opinions and recommendations made in this report are strictly those of the author and do not necessarily represent those of the Ontario Ministry of Natural Resources or the University of Guelph.

2. COMMENTS

In most cases, personnel, facilities, and raw materials are available to supply USABLE production diets for local fish culture. In other words, if husbandry techniques suitable for local fish culture are refined and all available resources are mobilized, there would be no great difficulty, in the author's view, in supplying usable feed for local fish production. At all locations there exist nearby well-established animal and poultry feed mills. Whatever the reason, however, there was virtually no manufactured fish feed supply except in kilogram quantities of 'hand-made' experimental feed. In some cases even existing laboratory pellet mills have hardly been utilized.

In most institutions there were well-educated professional and technical staff available. However, many of them seemed to be occupied with either administration or academic research and no one seemed to be actually exerting a CONTINUOUS effort in applied nutrition research and diet development.

Of course, the author realizes that there is little basic nutritional information for warm water fishes of the region, but

there are several ways to start the formulation of a usable practical diet for immediate use. The simplest way is to modify catfish and salmonid diets for local conditions. By use of these modified diets, trial and error types of experiments can produce a reasonable production diet in a relatively short period of time. At the same time, nutritional studies can be carried out for future application. If one expects to study basic nutrient requirements first, at least a decade will pass before practical diets based on available feedstuffs can be formulated.

A fundamental question is identifying today's problems for the advancement of aquaculture in terms of socio-economic necessity. Then a continuous and concerted effort must be made to solve these problems and to achieve these goals for socio-economic benefits.

3. GENERAL RECOMMENDATIONS

3.1 Diet Formulation

3.1.1 Feedstuff Evaluation

An evaluation of the nutritional value of feedstuffs is the most important and should be the first step in diet formulation. (Appendix 2) The chemical composition, digestibility coefficient and physical and organoleptic properties of each

ingredient must be determined. The source of raw materials preferably local ingredients which will be employed in the fish diet formula, and the method of processing must also be considered. Proximate analyses for dry matter, crude protein, crude fat, crude fibre, ash, Ca and P should be performed on each batch to confirm the declared composition. Then the digestibility coefficient must be measured (see 3.1.2) to establish the ingredients' usefulness as a source of nutrients for the fish (Appendix 2). The physical properties of feedstuffs are also important since pelletability, durability and water stability are very critical to the preparation of dry fish feeds. Any negative organoleptic characteristics of raw materials should be considered before the ingredients are incorporated into the diet. Some of the key ingredients such as fish meal and oil must meet strict quality standards as shown in the Appendix 1 (Table 3), and these may have to be imported if necessary, to mantain fish feed quality.

3.1.2 Digestibility Study

Different species of fish have different digestive capabilities, particularly for carbohydrates. Therefore, digestibility coefficients must be determined for most available feedstuffs for each species of fish. There are several different methods for measurement of digestibility and one of them is described in detail in section 3 of Appendix 2. As soon

as possible, the digestibility coefficients for local feedstuffs which can be used in fish diets should be repeatedly determined so that preliminary results would be available for the initial diet formulation studies. Eventually, the accumulated information on ingredient digestibility coefficients will allow the prediction of nutrient availability in diets made from any combination of tested feedstuffs.

3.1.3 Diet Formulae

Lack of information about locally available ingredients, nutrient requirements of individual fish species at various stages of growth and feedstuff prices, make it difficult to formulate practical types of dry pelleted diets in a precise way. However, with the minimal information that is available, a diet can be formulated from locally available feedstuffs for immediate use, for testing and further modifications. Some of the practical diets for salmonids which have been successfully used in Canada are included in Appendix 1 for reference.. These formulae could be modified by substituting local ingredients and considering factors peculiar to the species of fish under consideration.

3.1.4 Feeding and Feeders

There are no suitable feeding guides for warmwater and marine fishes in tropical regions. Feeding guides can only be produced over a period of several years by using hand feeding with

careful observation. As with coldwater species, water temperature is an important consideration in developing such a feeding guide. Under normal growth, the feed:gain ratio should be between 1.2 and 1.5. Otherwise overfeeding, or underfeeding are indicated. The actual condition for optimal feed:gain ratio for various species at different water temperatures can only be established by several controlled experiments and field tests. To determine the desirable feed efficiency, the dry matter, protein and fat content of fish carcass samples and the conversion ratio and growth rate of the fish must also be considered.

Use of mechanical (automatic) feeders is desirable for certain circumstances, such as cage culture operations. However, feeders must be regularly calibrated for the amount of feed dispensed and the frequency of activation adjusted using feed intake data from the hand-fed experiments. There are several types of mechanical feeder; electrical, pneumatic, battery operated with sunlight activation, and pendulum (demand) feeders. Battery operated or pendulum feeder types are most suitable for cage culture. For intensive pond culture, the pneumatic feeder system may be more desirable.

3.1.5 Growth Experiments

To obtain the growth pattern of different fish species and strains under the local environmental conditions, it is necessary to conduct several growth experiments under well-

defined conditions, using standardized experimental procedures. A diet should be formulated using all information available in the scientific literature and the results of any experiments carried out as described in the previous sections. The results of these applied studies will indicate the expected growth at different stages and also help to develop norms for performance parameters and experimental techniques and procedures. Furthermore, the growth curves obtained will be valuable to evaluate experimental and commercial performance. These simple growth trials prior to more experimental nutrition investigations (such as a more precise definition of nutrient requirements etc.) are needed to meet the immediate necessity of developing practical diets and methodology. After a specific diet, formulated as described as above, has been evaluated and has been shown to produce a predictable growth response, important local ingredients may be tested by substituting them in the diet for a known ingredient. In this way, the effect of the test ingredient can be measured in growth trials. Over a period of several years, local feedstuffs of potential use in practical fish diets can be identified.

3.2 Feed Manufacturing

3.2.1 Quality Control of Feed

For the manufacture of fish feed, several important guidelines must be followed by the feed mills in regards to

quality control of fish diets. Specifications for feed ingredients, particularly fish meal and fish oil (Appendix 1, Table 3) must be strictly observed to ensure high quality of the feeds and to prevent serious problems in the well-being of the fish. Many of the problems associated with the diets and also sometimes with health originate with the use of poor quality raw materials. All feed ingredients must be free from contaminants such as chlorinated hydrocarbons, herbicides, insecticides, pesticides and heavy metals etc. Raw materials also should be harvested, processed and stored properly to preserve the freshness and quality of the available nutrients in the feedstuffs. (See also Section 3.1.1)

3.2.2 Grinding and Pelleting

The most important process prior to mixing a diet is grinding all ingredients to finer than 0.25 mm particle size (Appendix 1). A fine particle size improves pelletability and durability of the pellet and the digestibility of the feed. A coarse particle size reduces the water stability of pellets. Therefore a hammer mill is essential for fish feed processing. To improve pellet durability and water stability, steam pelleting is required for both experimental and production diets. However, laboratory pellet mills are not equipped with a hopper designed for steam pelleting nor are they equipped with a steam generator. Therefore to provide steam-pelleting capability, the pellet chamber housing may be modified by drilling 1/2 inch

threaded holes to allow injection of steam and using an old autoclave as a steam generator. These modifications were made at several institutions and this arrangement functioned satisfactorily and the pelleted feed was of good quality. Personnel were therefore trained how to steam-pellet. However, finely-ground ingredients and steam pelleting do not always guarantee durable and water-stable pellets. A binding agent is sometimes required to serve this specific purpose. There are many binders: lignosol, gelatinized starch, wheat gluten, guar gum, agar, alginate and other synthetics etc. A selection must be made in association with a specific formula, and repeated pelleting tests.

3.2.3 Drying and Storage

Steam pelleted feed contains 10-15% water, and moist pelleted feed contains 30-40% water, and most of this moisture must be removed in a well-ventilated area without direct sunlight to avoid destruction of some nutrients by the microbial fermentation which may occur. Therefore it is essential to have drying chamber for pellets. Carefully manufactured experimental feed, and other moisture and heat sensitive ingredients, particularly vitamin premixes must be stored in a refrigerator or freezer. Otherwise, all efforts to ensure quality production may be wasted, especialy in hot and humid weather conditions.

3.2.4 Extruded (Floating) Pellets

Extruded pellets have the desirable characteristic of floating on the water surface for some time before sinking to the bottom where they stay as a pellet which is moist and spongy without disintegrating. These characteristics make it easy to observe overfeeding of surface feeders and to give pellets more water stability. Floating pellets are manufactured by gelatinizing the starch fraction of the ingredients with steam, extrusion and expansion of the pellet and drying at high temperature. Therefore, some of the essential nutrients are destroyed and more raw starch is made available to the fish creating glycogen accumulation in the liver, particularly in carnivorous fish. Also higher levels of protein and fat in the marine fish diet make it difficult to extrude feed, and the manufacturing cost is higher than steam pelleting. Extrusion machinery is not common at farm animal feed mills and is expensive to set up. For these reasons, floating pellets are NOT recommended except in special circumstances. Steam pelleting with a suitable binder can achieve the necessary durability and water stability in dry feeds.

3.3 Experimental Nutrition Research

Determination of protein, fat and energy requirements (Appendix 2) for growth of various species of fish is necessary before proceeding to further research into the requirements for other nutrients such as amino acids, fatty acids and vitamins. Therefore, experiments to establish these requirements may be

planned while proceeding with the applied experiments mentioned in Section 3.1.5.

3.4 Facilities and Equipment Required

Proper and adequate facilites and equipment for wet and biochemical laboratories are crucial to the success of any future nutrition program. However, this does not mean elaborate modern biochemical equipment is essential in the initial stages of fish nutrition research to develop diets. Basic facilities with fish tanks, ponds and cages, small scale feed mill, proximate analytical services and some basic laboratory equipment for biochemical analysis etc. will satisfy most of the requirements at this stage of the research program. Much of the available resouces should be devoted to obtaining essential basic information for diet development and a data base for future nutrition research rather than modernizing facilities. However, gradual acquisiton of sophisticated instruments for future research when the need arises may be justified. (See also SUMMARY).

3.5 Personnel Training

Further training for some personnel in the applied fish nutrition field is required to improve their professional experience. Very desirably, they should be trained at an institution where specific objectives can be fulfilled.

Otherwise valuable time and financial resources may not be utilized for the best interest. Each case must be considered objectively and a search for an appropriate research institution must be made.

3.6 Progress Reports and Publicatons

Most progress reports are in abstract form which describes only the type of work and researcher's interpretation. Hence, it is difficult to evaluate the validity of the design, methods and results and adequacy of the conclusions. Therefore, it is strongly suggested that all progress reports of nutrition research for IDRC be written in a pre-publication format which includes design of experiment, diet compositions, method and materials, including details of diet processing, summarized results and discussion or conclusion. Also, many of the reports should be available to other scientists in the region to share the informaton for common benefit. Scientific progress reports should not be regarded as an administrative document.

3.7 Co-operative Program with Canadian Institutons

There are several institutons in Canada which can establish co-operative programs for fish nutrition projects even though they are mostly working with salmonids. However, this is not a disadvantage as the principles of fish nutrition are the same no matter what species are studied. Therefore, it will be

beneficial to establish a cooperative program for fish nutrition research and training staff at one or more Canadian institutions. The Canadian Working Group for Finfish Nutrition, an asso-ciation of finfish nutritionists, may give advice on individual programs or make arrangements with Canadian institutions interested in these cooperative programs. (Contact the author).

4. SUMMARY

Aquaculture Unit, Primary Production Department, Singapore

4.1 Visited Aquaculture Unit, Changi Point during period of August 23rd to 28th and September 6th to 7th. Used this time to review the progress since last visit and to meet and have discussions with scientists and research staff who are actively involved in fish nutrition and aquaculture research. These included:

Mr. Leslie Cheong, Head, Aquaculture Unit

Mrs. Renee Chow, Senior Officer
- Fish Nutrition and Diet Development

Mr. Lim Lian-Chuan, Senior Officer - Fish Breeding

Mrs. Law-Yu Wen Wei, Laboratory Services
Pig and Poultry Research and Training Institution

Dr. Ching Ai Lee, Veterinary Research Officer
Pig and Poultry Research and Training Institution

4.2 Also made a site visit to 2 cage fish farms, a fish meal factory, 3 feed mills and 2 pellet and hammer mill engineering companies to discuss the production possibilities and the supply of equipment.

4.3 All scientific and support staff were well-educated and showed confidence and enthusiasm in the project. However, proper opportunity for experience in applied nutrition and diet processing for the nutritionist will be very beneficial for the nutrition program in the future.

4.4 The progress made in the planned schedule of the phase II project was very good and the recommendations made in the previous Consltancy Report were mostly implemented for the nutrition program. The author praises all personnel who are involved directly and indirectly for this achievement.

4.5 Had daily meetings with Mrs. Chou and her staff to discuss fish nutrition experiments and various aspects of diet development and processing. The topics discussed are as follow:

1) Experimental Methods

Carcass composition

Total lipid determination

Usual proximate analyses

Digestibility of feeds and feedstuffs

Feces collection

Chromic oxide determination

Oxidation of feedstuffs and oils

Peroxide value and anisidine value

Storage of feeds and feedstuffs

Storage of oils and stabilization

Calculation of feed efficiency with adjustment of mortality

Vitamin premixing

Glycogen in liver - measurement using kit

Fatty acid determination for oils

Total pesticides and aflatoxins in feedstuffs

Importance of thiaminase activity in trash fish

Growth curves and growth index

2) Experimental Design

Protein/lipid experiment (CP:45/50%, Lipid:5/10/15%)

Tallow substitution

Improvement of present practical diet

Reformulation of C505 formula

Effect of moisture in diets

Infiltration of lipid in fish liver-wild and cultured fish

3) Demonstrations

Commissioning of CPM Laboratory Pellet Mill with steam

Commissioning of steam generator

4) Facilities

Modification of present tank system for feces collection

Choice of mixers (bowl/ribbon/vertical)

Choice of hammermill

5) Review of locally available feedstuffs

Corn gluten meal

Brewer's dried yeast

Wheat pollard

Fish meal

6) Brief discussions

Nutrition of tropical fish - requirement of lipid

Importance of hand-feeding experimental fish

Automatic and demand feeders

Larval feeds - send larval diet samples

Salt content of fish meals

Ash in diets and feedstuffs

Review of protein requirement of grouper

Growth promoters

7) Discussion with IDRC

Hammermill

Cold room

Laboratory equipment for lipid and chromate dterminations

Co-operative program with the University of Guelph, Canada Fish Nutrition Workshop in 1983

4.6 Discussed production of practical feed on a larger scale at commercial feed mills and also made initial contact with 2 local feed mills which could produce fish feed with reasonable effort. Diet quality, demand by fish farmers and raw material supply will be important factors which determine the interest of fish feed manufacturing by feed producers.

4.7 Fish farming (mainly grouper and seabass/cage culture) was increased in Singapore and most of the farmers seemed to be successful financially. However, supply of fry and feed were the main problems to expand aquaculture. All fish farmers were relying on chopped or ground raw fish. Developing acceptable dry pelleted diet and moist pelleted diet partly based on fish silage were urgent requisites for the future of aquaculture.

4.8 Facilities

Most wet laboratories were almost complete and they were well-constructed and arranged in a practical manner.

However, a biochemical laboratory was not initiated yet. The Analytical Facility at the Pig and Poultry Research and Training Institute may be utilized only temporarily because they are loaded to full capacity. Therefore, the Aquaculture Unit should acquire its own biochemical laboratory in the near future and the availability of this facility will be crucial for the fish nutrition program within a short time. All agencies supporting this program must fund for a moderately equipped laboratory to improve the efficiency of their total support.

4.9 Co-operative Program and Personnel Training

This cooperative program will potentially be beneficial for the fish nutrition group and an avenue for further personnel training. However, the program must be established after identifying the needs of the overall program in the near future. Without clearly identifying needs and objectives, it is difficult to arrange a program at a Canadian institution which has appropriate resources.

It is recommended that the Aquaculture Unit submit a proposal for a cooperative program with the Canadian institutions which have a fish nutrition program. It is also suggested that arrangements for a cooperative program be made with the Fish Nutrition Laboratory, Fisheries

Branch, Ontario Ministry of Natural Resources/University of Guelph for the development of larval diets, digestibility of feeds and amino acids and fatty acids requirements.

The program can be also used for technical services such as analyses of amino acids, fatty acids and vitamins etc. in Canada to overcome the problems of purchasing and maintaining costly equipment.

5. ACKNOWLEDGEMENTS

The author is grateful to Mrs. Renee Chou and Mr. Leslie Cheong and their associates at Aquaculture Department for their cooperation. The author also asknowledges the Ontario Ministry of Natural Resources and the University of Guelph for allowing him to undertake this consultancy.

APPENDICES

Appendix 1

Manufacturing Specifications for Fish Feed

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The contractor's mill must comply with the Standards of the Plant Products Division, Agriculture Canada, for 'Feed Mill Inspection Report'.

EQUIPMENT AND METHODS

1. Premix Equipment: The mixer must be the batch type with complete clean-out features. Premix equipment must include adequate scales to weigh micro-ingredients in gram quantity.

2. Premix Preparation: Premixes are used to facilitate dispersion of vitamins, minerals, and other trace materials normally required in smaller quantity. A premix must be finely ground (less than 0.25 mm) and a uniform mixture of micro-ingredients with a carrier of such physical properties that separation does not occur. A premix must consist of at least 0.5 percent of the entire mix; for example, one tonne of feed requires a premix of 5 kg containing accurately weighed additives plus, as a diluent, a cereal ingredient contained in the diet. The premix is then blended with a quantity of feedstuffs equal to 3-5% of the total mix. Introduction of this diluted premix into a batch system must be made midway in the loading of ingredients which comprise the formula.

If the premixes are supplied by a second company this clause will apply to that company's manufacturing practices.

3. Manufacturing Equipment: The feed mixer may be batch or continuous system which must meet ASAE Standard: S303.1 (Agricultural Engineers Yearbook, 1973) and all scales must meet the Canadian scale code.

4. Mixing, Pelleting and Crumbling of Feed: The following production records should be provided after completion of mixing:

a. Formulation code.
b. Ingredient identifications and weight in each mix.
c. Total weight of each mix.
d. Date of mixing and batch number.
e. Measure of actual yield in bags or bulk of finished product.

Starter formula shall be usually made as 0.5, 1 or 1.5 mm granules. Grower formula shall be usually made as 2 mm granules or larger. When the particle size is greater than 3 mm feed should be made in pellet form. Brood stock formula shall be made as 6.4 x 7 mm pellets (see Table 1). All ingredients shall be carefully and thoroughly mixed and pulverized (less than 0.25 mm) before mixing into the diets.

The mix shall be processed into pellets, using live dry steam from a high pressure boiler to make a pellet of the right texture that will be hard enough to hold together in packaging, transporting and storing. When the pellets are extruded through a die they must be moist enough to be spongy when pressed between fingers. Shiny surfaced, flinty pellets are not of this type. Steam temperature must be approximately 90C and pellets must be cooled immediately after pelleting.

Granules shall be manufactured by crumbling 4 or 5 mm pellets. 'Fines' which result from the manufacture of pellets or granules of an intended size must not be used as part of starter feed and must be recirculated continuously so as to cause a minimum alteration in formulation from that intended.

If necessary, a binder specified by the Ministry may be used substituting for wheat middlings.

To make a pellet or granule of satisfactory durability it is necessary that major part of the oil be sprayed on the feed following pelleting, crumbling and screening.

Bags shall contain not more than 10% oversize and/or undersize granules. 'Fines' content (defined as particle size less than 0.4 mm) shall not exceed 2% of feed.

5. Bagging and Loading: Fish feed will be shipped in bags or by bulk truck at buyer's discretion. If not speficied, shipment will be in PLAIN (unmarked) 25 kg bags. The pellets and granules are not to be bagged or loaded for bulk delivery until cooled to 3C above ambient air temperature and moisture content is never to exceed 10%. If bagged, the pellets and granules shall be sacked in bags with a polyethylene lining and a non-slip outer covering. The net weight of feed in each bag shall be 25 kg.

6. Labelling: Each bag shall be clearly labelled with Formula name (e.g. MNR-82S), pellet or granule size (e.g. 3 GR or 4 PT) and date processed (e.g. 82-07-01) using glue-on sticker or stamp. Label must be located less than 10 cm from the bottom so that the labels can be read on the stack. The size of letters for formula and feed size should be larger than 2 square centimeters.

DELIVERY

Delivery will be required on the date as designated in each order of the contract, WITHIN 14 DAYS OF MANUFACTURING DATE. All deliveries of fish feed, under award made as a result of this invitation for bid, shall be made only by licensed common carriers or contractor-owned trucks.

All feed (bulk and/or bagged) shall be loaded on suitable trucks

at the feed mill and delivered directly to the hatchery on the same trucks unless otherwise specified in the bidding schedule.

The contractor must arrange his shipment schedule to avoid deliveries on Saturdays, Sundays and Government holidays. Shipments shall be scheduled to arrive not later than 16:00 hr Monday through Friday. Feed trucks arriving after 16:00 hr will not be unloaded until the following work day. Twenty-four hour advance notice of delivery date is required.

CONDITIONS FOR AWARD

1. The Ministry reserves the right to inspect the bidders' plants and production facilities prior to releasing tender documents and award of the contract and to reject bids by contractor whose plant facilities do not comply with the Standards (Table 2).

2. It will be assumed that the equipment described in the sections above is functional and operational at the time of application for the contract, otherwise the application will not be considered. The contractor must be able to demonstrate the capability to manufacture all or part of the contract to the satisfaction of the Ministry if requested to do so.

3. Award will NOT necessarily be made on the basis of the LOWEST TENDER and will be made only to RECOGNIZED FEED MANUFACTURERS in Canada.

4. The quantity specified herein is estimated; the Government, therefore, reserves the right to increase or decrease the quantity by as much as 20% without changes in the tendered price.

5. The Ministry intends to award this contract to a single supplier. However, the Ministry reserves the right to award contract to several suppliers if it is in the best interest of the Ministry to do so.

6. The contractor shall submit CONFIRMATION OF PURCHASE of a sufficient quantity of the quality fish meal and fish oil to assure supply for whole contract period by June 1, 1982.

7. The formulae supplied for this contract are the property of the Ministry and confidential. However, permission may be granted upon written request to manufacture feeds for sale using these formulae.

INSPECTION AND QUALITY CONTROL

1. The Ministry reserves the right to inspect the contractor's plant facilities, equipment, inventories and invoices from his

suppliers during the contract period. The contractor will supply or make available labels and representative samples of all ingredients used upon request by personnel authorized by the Ministry.

2. Thirty days prior to manufacturing the contractor must submit a certificate of analysis of all ingredients, primarily fish meal and fish oil (see Table 3), provided by an independent testing laboratory specifying the LOT NUMBER, detailed sample DESCRIPTION (e.g. herring or capelin meal), ORIGIN of the ingredient to be used in the formulae and the DATE samples received by the laboratory. Each shipment of ingredients must be analyzed separately.

3. The contractor shall notify the Fisheries Branch, Ministry of Natural Resources at least 14 days in advance of exact mixing dates to allow arranging for inspection of ingredients, manufacture and finished products.

4. The contractor shall keep representative samples both of all ingredients used in each production run and of final products until 6 months after the end of the contract year and supply representative samples if requested by the Ministry.

5. Quality, including the level of contaminants, of the ingredients and fish feed shall be solely the responsibility of the contractor who selects the ingredients and manufactures the fish feed (see Table 3).

6. The contractor shall keep a record of the detailed formula of any other feed manufactured, using the same equipment prior to the processing of the Ministry's fish feed.

7. The contractor must follow the supplied formulae stricly and any modification or substitution must be authorized by the Ministry prior to manufacturing.

8. It is the responsibility of the contractor to ensure that competent personnel are employed and they are fully informed of these 'Manufacturing Specifications'.

VIOLATION OF CONTRACT CONDITIONS

The Ministry reserves the right to cancel any contract with a contractor for the supply of fish feed if any of the terms and/or conditions of the contract are violated. The contractor will be notified on 7 days written notice.

Prices are to remain firm for the period of the contract. In the event of any unavoidable price increase, the Ministry requests 30 days written notice to enable to ask for a second tender for the product lines affected.

The Ministry reserves the right to change formulae specifications as necessary during the life of the contract, on 30 day notice.

If the Ministry requires changes, the contractor may:
a. Continue to supply at the original price for the new product line(s).
b. Decline to supply the new formulae at the original price.

In case (b), the Ministry may make alternate supply arrangements for the product line(s) affected as it sees fit; and the successful contractor will continue to supply the other product line(s) that are not changed at the original tendered prices.

Contractor should therefore tender prices on an individual 'product line' basis.

In the event that the contractor supplied fish feed which does not meet formulae specifications the Ministry also reserves the right to either:

1) return the feed shipment without payment to the contractor at the contractor's expense if the Ministry decides that the feed is unacceptable or
2) accept the feed shipment, but impose a penalty equivalent to 3 times the retail value of the nutrients not meeting contact specifications.

Table 1

Feed Sizes and Sieve Openings

Feed I.D.	Feed Sizes	Std.Sieve No.	Sweco No.	Screen Opening
1. BROOD STOCK PELLETS:				
7 PT	6.4x7mm long	5/16"	-	8 mm
		#3.5	# 4 MG	5.7
2. GROWER PELLETS:				
6 PT	6.4x6mm long	5/16"	-	8 mm
		#3.5	# 4 MG	5.7
5 PT	4.8x5	# 4	# 4 MG	4.8
4 PT	3.4x4	# 6	# 6 MG	3.4
3 PT	2.4x3	# 8	# 8 MG	2.4
3. GROWER GRANULES:				
3 GR	3 mm	# 6	# 6 MG	3.4 mm
		# 8	# 8 MG	2.4
2 GR	2	# 12	# 12 MG	1.7
4. STARTER GRANULES:				
1.5 GR	1.5 mm	# 12	# 12 MG	1.7 mm
		# 16	# 18 TBC	1.2
1 GR	1	# 25	# 28 TBC	0.7
0.5 GR	0.5	# 40	# 46 TBC	0.4
Fines				

1. Feed I.D. and sizes are used at Fish Culture Stations, Ontario Ministry of Natural Resourses
2. 7 & 6 PT=1/4", 5 PT=3/16", 4 PT=1/8", 3 PT=3/32", 3 GR = #5 2 GR = #4, 1.5 GR = #3, 1 GR = #2, 0.5 GR = # 1 & Starter

C.Y.Cho/82.02

Table 2

CHECK LIST
for
Fish Feed Manufacturing Facilities

Manufacturer No.	1	2	3	NOTE
House-keeping	___	___	___	
Grinder	___	___	___	
Premixer	___	___	___	
Mixer	___	___	___	
Scale	___	___	___	
Steam supply				
Boiler HP	___	___	___	
Pressure	___	___	___	
Trap & piping	___	___	___	
Pelleting				
Dies	___	___	___	
Prod. capacity	___	___	___	
Cooling	___	___	___	
Crumbling				
Crumbler	___	___	___	
Screens	___	___	___	
Fat application				
Sprayer	___	___	___	
Meter	___	___	___	
Storage bins	___	___	___	
Ingredients supply	___	___	___	
Production records	___	___	___	
Personnel	___	___	___	
Laboratory	___	___	___	
Others	___	___	___	

G=good A=acceptable U=unacceptable CYC/79

Signed:_____ Date:

Table 3

Required Quality of Fish Meal and Oil
for Salmonid Diets

Compounds	Levels
Fish Meal:	
Crude Protein(%N x 6.25)	Higher than 68 %
Crude Fat	Less than 10 %
Ash, Total	" 13 %
Salt(NaCl)	" 3 %
Ammonia-N	" 0.2 %
Moisture	" 10 %
Antioxidant(sprayed liquid form)	200 ppm
Check Heavy Metals	
Steam Processed, ground finer than 0.25 mm	
Fish Oil:	
Peroxide Value	Less than 5 meq/kg
Anisidine Value	" 10
Total Pesticides	" 0.4 ppm
P C Bs	" 0.6 ppm
Nitrogen	" 1 %
Moisture	" 1 %
Antioxidant(liquid form)	500 ppm
Deaerate and mix antioxidant by bubbling nitrogen gas before storage in air-tight container	
No fortification of vitamins	

CYC/80

ONTARIO MINISTRY OF NATURAL RESOURCES

Starter Diet for Salmonids

FORMULA: MNR-82S

Ingredient	kg	
Fish meal, herring or capelin * (>68% C.P., 13% ash)	46	
Feather meal, hydrolyzed (80% C.P.)	8	
Soybean meal, dehulled (48% C.P.)	9	
Corn gluten meal (60% C.P.)	8	
Brewer's dried yeast (45% C.P.)	5	
Whey, spray dried (12% C.P.)	10	
DL-methionine	0.2	
Vitamin premix (VIT-8204) *	1.5	
Mineral premix (MIN-8204) *	1	
Fish oil, herring, caplin or salmon *	3	
Fish oil, herring, caplin or salmon * sprayed on pellets/granules	8.3	
TOTAL	100 kg	kg

* See attached specifications
1. All ingredients and premixes must be ground finer than 0.25mm
2 DL-methionine may be included in vitamin premix
3. 54% crude protein and 17% lipid (dry matter basis)

C.Y. Cho/82

ONTARIO MINISTRY OF NATURAL RESOURCES

Grower Diet for Salmonids

FORMULA: MNR-82G

Ingredient	kg
Fish meal, herring or capelin * (>68% C.P., 13% ash)	27
Feather meal, hydrolyzed (80% C.P.)	8
Soybean meal, dehulled (48% C.P.)	10
Corn gluten meal (60% C.P.)	10
Brewer's dried yeast (45% C.P.)	5
Whey, spray dried (12% C.P.)	7
Wheat middlings (17% C.P., 8% fiber)	21.8
DL-methionine	0.2
Vitamin premix (VIT-8204) *	1
Mineral premix (MIN-8204) *	1
Fish oil, herring, caplin or salmon *	3
Fish oil, herring, caplin or salmon * sprayed on pellets/granules	6
TOTAL	100 kg

* See attached specifications

1. All ingredients and premixes must be ground finer than 0.25mm
2. DL-methionine may be included in vitamin premix
3. 45% crude protein and 14% lipid (dry matter basis)

C.Y. Cho/82

ONTARIO MINISTRY OF NATURAL RESOURCES

Brood Diet for Salmonids

FORMULA: MNR-82B

Ingredient	kg
Fish meal, herring or capelin * (>68% C.P., 13% ash)	35
Feather meal, hydrolyzed (80% C.P.)	8
Soybean meal, dehulled (48% C.P.)	9
Corn gluten meal (60% C.P.)	7
Alfalfa meal (17% C.P., 24% fiber)	6
Brewer's dried yeast (45% C.P.)	5
Whey, spray dried (12% C.P.)	8
Wheat middlings (17% C.P., 8% fiber)	13
DL-methionine	0.2
Vitamin premix (VIT-8204) *	2
Mineral premix (MIN-8204) *	0.8
Fish oil, herring, caplin or salmon * sprayed on pellets/granules	6
TOTAL	100 kg

* See attached specifications

1. All ingredients and premixes must be ground finer than 0.25mm
2 DL-methionine may be included in vitamin premix
3. 49% crude protein and 11% lipid (dry matter basis)

C.Y. Cho/82

ONTARIO MINISTRY OF NATURAL RESOURCES

Vitamin Premix for MNR-82s' Salmonid Diets

Formula: VIT-8204

Ingredient	g/kg premix
Vitamin A (as acetate)	500,000 IU
Vitamin D3	300,000 IU
Vitamin E (dl-alpha-tocopheryl acetate)	10,000 IU
Vitamin K (menadione sodium bisulfate)	3
Vitamin B12	0.003
Ascorbic acid	40
Biotin	0.05
Folic acid	1
Niacin	20
Pantothenic acid (as D-Calcium salt)	15
Pyridoxine (as HCl salt)	3
Riboflavin	5
Thiamin (as HCl salt)	3
Choline Chloride (50%)*	300
Wheat middlings	+
TOTAL PREMIX	1000 g / kg

All ingredients and premixes must be ground finer than 0.25 mm
* Choline Chloride (50%) may be added to the main mix directly

C.Y. CHO/82

ONTARIO MINISTRY OF NATURAL RESOURCES

Mineral Premix for MNR-82s' Salmonid Diets

Formula: MIN-8204

Ingredient	g/kg premix
Copper (as CuSO4.5H2O)	2.5
Iron (as FeSO4.7H2O)	6.3
Manganese (as MnSO4.H2O)	8.6
Iodide (as KI)	0.8
Zinc (as ZnSO4.H2O)	14.4
Salt (99% NaCl)*	300
Wheat middlings	+
TOTAL PREMIX	1000 g kg

All ingredients and premixes must be ground finer than 0.25mm
* Salt may be added to the main mix directly C.Y. CHO/82

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BIOENERGETICS OF SALMONID FISHES: ENERGY INTAKE, EXPENDITURE AND PRODUCTIVITY

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1. INTRODUCTION

Life exists in a thermodynamically-unstable state and its continuation is associated with an increase in the entropy of the environment, with the release of energy as heat. In animals this energy is derived from the catabolism of the food. In addition to the heat which is generated as a consequence of the life maintaining processes, animals need energy to grow, reproduce, and for physical activity. At any one moment in time the animal's energy expenditure may be either greater or less than the energy content of the food it consumes, resulting in either an overall loss, or an overall gain in energy. Bioenergetics is the study of the balance between energy supply in the food and energy expenditure, and requires an examination of the physiological processes by which energy is transformed in living organisms.

Energy expended as heat by animals is measured by calorimetry which is a general technique for measuring heat flux between two bodies. Lavoisier (1783) based his classical conclusion that life is a process of combustion: "*La respiration c'est donc une combustion*", upon simultaneous measurements of heat flux, and of oxygen consumption. His observation laid the foundation for modern nutrition. Brody (1945) reviewed the evidence that bioenergetics are governed by the Laws of Thermodynamics and showed that, ultimately, life was supported by physical processes.

The catabolism of food is organized within the animal to conserve free energy for use in anabolic and other life sustaining processes. The physiological mechanisms which achieve this are sublimely complex, but allow the catabolism of an infinite variety of food molecules using the finite number of enzyme systems which are found in animals (Krebs & Kornberg, 1957).

Nutritionists working at the Weende Agricultural Experimental Station in Germany, in the nineteenth century recognized that the components of foods which make a significant contribution to the energy supply of the animal could be characterized as three proximate principles: proteins, fats and carbohydrates. The stoichiometry of the oxidation of these classes of compounds allows calculation of the energy released as heat from measurements of respiratory exchange: oxygen consumption and carbon dioxide production, along with measurement of urinary nitrogen excretion. This method of measuring heat production is referred to as indirect (or respiration) calorimetry. Rubner (1894) validated this approach to calorimetry by showing that the heat produced by a dog equalled the heat of combustion of the fat and protein catabolized, minus the heat of combustion of the urine: the urine being the vehicle for the excretion of the incompletely oxidized nitrogen products of protein catabolism.

Animals use energy not only to sustain the life process but also to support physical activity. The incremental effect of varying levels of physical work upon heat production and substrate oxidation was shown by Atwater & Benedict (1903) using human subjects working with bicycle ergometers. Growth—the formation of new tissues—requires energy inputs, both as the new components of the tissue itself (their heats of combustion) and to sustain the anabolic processes leading to the elaboration of the new tissue components. Measurements of the energy cost of growth, particularly in domestic livestock and fish in captivity, is of great practical importance and the methodology and results have been summarized recently by Kleiber (1975).

Ege & Krogh (1914) applied the principles of bioenergetics to fishes, and Ivlev (1939) extended these studies to carp. More recently there have been reports of studies of energy utilization and expenditure for several species of fish by Spoor (1946), Brett (1962), Beamish (1964), Warren & Davis (1967), Niimi & Beamish (1974), Cho *et al.* (1976) and Smith *et al.* (1978a,b).

Many of these investigations have been primarily concerned with the energy cost of swimming by the fish and have largely ignored the level of feeding and the type of diet. Characteristically these studies measured oxygen consumption by a fish swimming involuntarily either without food, or after receiving a single meal. Calculation of heat production from oxygen consumption measured under these circumstances in which an undefined fraction of the heat would be due to catabolism of food components, with the balance being provided by breakdown of body tissue, precludes definitive statements of the energy cost of swimming for fish in their natural surroundings, or under the conditions employed for intensive fish culture.

An understanding of the bioenergetics of any animal, including fish, is necessary as a basis for providing an adequate dietary regime for any particular

physical environment. Full definition of the nutrient requirements depends upon a knowledge of the partition of the dietary components between catabolism as fuels and storage in tissues. Successful fish culture depends upon the provision of diets containing an appropriate balance of nutrients and adequate energy to permit the most efficient growth of the fish.

2. DIETARY FUELS

Nutritionists deal with defined chemical entities which play a role in the animal either as tissue components or their precursors, or as fuels which upon catabolism lead to the formation of useful intermediates to permit the completion of energy requiring processes. The completion of these energy requiring processes results in either an increase in the energy content of the animal (weight gain) or to the release of heat. Thus the utilization of dietary fuels does not cause any change in the energy content of the system (the animal, its food, or its surroundings). What has been changed is the form of the energy: the chemical energy of the food has been wholly or partly converted to heat. The heat energy is less useful than the chemical energy of the food, and this change is described by the increase in the entropy of the system. Schroedinger (1944) summarizes this by stating that "animals consume negative entropy". Thus animals do have a requirement for fuels which can be expressed as their energy equivalents, or even as an energy requirement.

All the organic compounds in the food release heat upon combustion, but for salmonid fish the fats and proteins are the dietary components which are the main fuels. All of these components play some part in the structure of the animal, but the need for energy can preclude their incorporation into tissues and lead to their catabolism. Thus the utilization of the components of each diet depends both upon the level of intake and upon the make-up of the diet. In effect, both the quantity and quality of the diet influence the metabolic partition of the components between storage, and catabolism as fuels. It is this flexibility on the part of the animal to use all three components of the diet as fuels which leads to the complexities of the interactions among proteins, fats and carbohydrates. As a consequence, the energy value of the diet to the animal must be defined before drawing any conclusions as to the effect of the diet upon the growth of the animal. In fact, if the diet provides less energy to the animal than is needed to sustain the life processes, and to support its physical activities, tissue components will be catabolized in addition to the food. This over-riding importance of the food as fuel means that the major factor regulating the amount of food consumed is its energy value in relation to the animal's energy needs. As a consequence, the concentration of the nutrients which must be provided in the diet to adequately meet the animal's requirements depends upon the fuel value of the diet to the animal.

The animal's energy needs are influenced by the stage of the life cycle, by the season and by the environment. Young growing animals require more energy per unit weight than mature animals, although reproduction increases the energy needs of mature animals. Animals whose habits are greatly influenced by the season of the year require different amounts of energy from their diets at different times; for example, hibernating animals increase their food intake and store energy as fat in response to declining day length and temperature. Environmental temperature plays an obvious role in influencing the fuel required to maintain body temperature in homeotherms but has an even greater influence in poikilotherms, such as fish in which the rate of metabolism can change many-fold in response to change in water temperature.

The energy value of the food to the animal depends upon its chemical composition, the heats of combustion of starch, egg protein and animal fat being: 17.2, 23.4 and 39.2 kJ/g respectively (Davidson *et al.*, 1979). However, the inherent chemical makeup of the food influences only its heat of combustion or gross energy. Digestion, absorption and utilization of the amino acids, fatty acids and sugars derived from the food are associated with various energy expenditures and losses of materials, which if retained and catabolized, would yield energy. Thus the measurement of the energy value of foods needs to be assessed by both chemical and biological assays.

3. METHODS FOR MEASURING ENERGY METABOLISM

The free energy changes which occur in animals as the chemical energy of the diet is used to support the life processes cannot be measured directly, however, ultimately the dietary energy is either voided as feces and metabolic wastes, dissipated as heat, or stored by the deposition of new tissues. Energy metabolism is studied by comparing dietary intake with fecal and other waste outputs; measurement of the energy values of the diet and wastes by combustion of samples in calorimeters allows calculation of the digestible and metabolizable energy intakes. The ultimate usefulness of the dietary energy in promoting an increase in the energy value of the fish can be determined by either measuring the fraction of the energy intake liberated as heat, or by measuring the fraction retained by the fish over a part of its growth period.

3.1. *Measurement of digestibility*

The first task in evaluating the potential of any foodstuff for inclusion in a diet is the measurement of its digestibility. In fish it is difficult to separate the feces from the water, and to avoid contamination of the feces with the uneaten feed. This problem has required very different approaches to those used in the measurement of digestibility for mammals and birds. Nose (1960) collected samples of rectal contents by manually stripping the fish, and squeezing out the fecal material from the rectum. Windell *et al.* (1978) obtained samples of rectal contents by applying suction to the annus or by disecting fish. The feces obtained by both these techniques involved handling the fish and exposing them to considerable stress. Forced evacuation of their rectum would result in the addition of an excess of enzymes, body fluid and intestinal epithelium to the rectal contents.

Smith (1971) confined the fish in metabolic chambers and collected the feces which were voided normally into the water, Ogino *et al.* (1973) collected

the feces by passing the effluent water from the fish tanks through a filtration column. Cho *et al.* (1975) used a settling column to separate the feces from the effluent water and Choubert *et al.* (1979) used a mechanically rotating screen to filter out the fecal material.

The system developed by Cho *et al.* (1975) to allow the measurement of digestibilities by collecting fecal material in a settling column is shown in Fig. 1. There are three tanks in each unit which all drain through a common drain pipe and a single stand pipe placed over an acrylic settling column (10 cm dia. × 40 cm high). The base of the settling column can be surrounded by a cooling jacket to minimize degradation of the fecal material. The tanks each measure 55 × 10 × 35 cm and have a sloping bottom, and each tank is loaded with 5–10 kg of fish. The velocity of the water flow is adjusted to minimize settling of feces in the drain pipe and maximize the recovery of feces in the settling column whilst maintaining appropriate levels of dissolved oxygen and ammonia in the water. In normal operation it is observed that the larger particles of feces are trapped in the settling column within 2 min of being voided by the fish.

The fish are accustomed to the tanks and the dietary regime for several days before a collection is begun. The fish are fed three daily meals between 09.00 and 16.00 hr, diets being offered only as long as the fish are actively feeding, to avoid wastage. Once the fish are consuming a uniform daily amount of feed the collection period is begun. The fish are fed normally through the day, and one hour after the last meal the drain pipe, and the settling column are brushed out to remove feed residues and feces from the system. One third of the water in the tanks is drained out to assure this cleaning procedure. At 08.30 the following day the settled feces and surrounding water are gently withdrawn from the base of the settling column into a centrifuge tube. These feces are free of uneaten feed particles, and are considered to be a representative sample of the feces produced throughout the 24 hr period. Immediately after collection of the feces the fish are fed again as normal, allowing repeated sampling over 6–9 days.

The feces are centrifuged at 10,000 *g* for 20 min at 5°C and the supernatant discarded, the feces are freeze-dried and ground for determination of chromic oxide concentration, nutrient analysis (dry matter, nitrogen and fat), and for gross energy determination.

A series of eight such units allows the determination of digestibility coefficients for up to 7 ingredients at any one time; one unit being devoted to the reference diet. This facility allows the examination of the influence of fish size, meal size, and water temperature upon the digestibility of feeds. The fish are under the normal culture regime which closely resembles that in the facilities used for other nutritional experiments, allowing the application of laboratory standards for levels of feed consumption and weight gain to the fish being used in the digestibility determinations.

Table 1 shows the results for the determinations of digestibility coefficients using different methods in a number of different laboratories. The studies all included herring fish meal, although the different studies used different samples. The crude protein contents of all the samples except that used by Smith *et al.* (1980) were very similar. The results for five of the studies allowed calculation of the digestibility of the dry matter of the diets to give an indication of their overall digestibilities and there were variations due to the procedures used: trough netting gave the highest digestibility coefficient suggesting overestimation by "break-up or handling" loss of the feces; however stripping the fish to remove the rectal contents gave the lowest digestibility coefficient suggesting underestimation by increased loss of endogenous material with the rectal contents: principally nitrogenous and

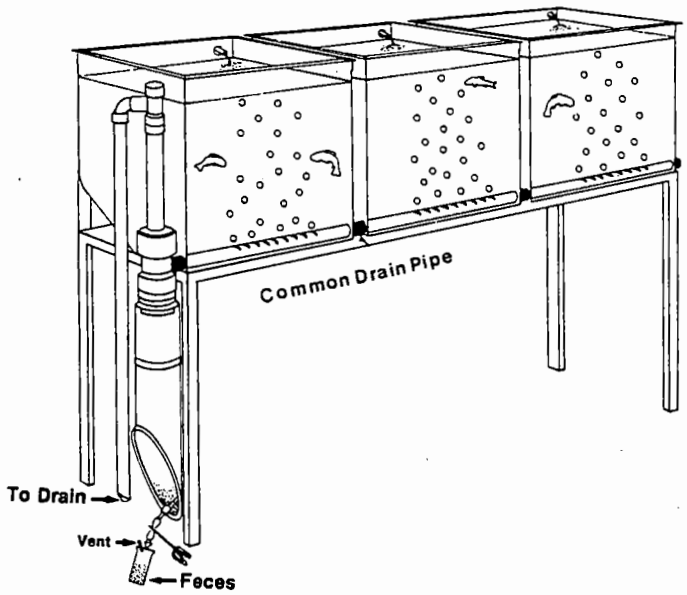

Fig. 1. CYAQ-2 digestibility system consisting of three fish tanks with common drains to feces settling column for the determination of digestibility coefficients of feedstuffs (Cho *et al.*, 1975).

Table 1. Apparent digestibility coefficients of herring meal determined with rainbow trout (Cho & Slinger, 1979)

Feces collection method	Crude protein in herring meal	Digestibility coefficient		
		Dry matter	Crude protein	Lipid
	(%)	(% Mean ± SE)		
1. Metabolism chamber	75.8	—	86.7 ± 0.9	—
2. Intestinal disection	67.9	80.3 ± 1.0	90.2 ± 0.4	96.7 ± 0.5
3. Anal suction	67.9	79.1 ± 0.4	90.4 ± 0.1	97.4 ± 0.3
4. Trough netting	67.9	84.4 ± 0.1	94.6 ± 0.3	96.8 ± 0.2
5. Stripping	67.9	73.3 ± 1.6	77.5 ± 1.0	62.2 ± 5.1
6. Stripping (Guelph)	66.7	—	88.2 ± 1.7	—
7. CYAQ-2 Guelph system	66.7	80.2 ± 2.4	91.0 ± 0.8	97.3 ± 1.0

1. Smith *et al.* (1980).
2–5. Windell *et al.* (1978).

lipid compounds. There were no differences between the digestibility coefficients for the dry matter determined by intestinal dissection, suction, or use of a settling column to collect the fecal material. The differences in the digestibility coefficients for the crude protein were even more marked than for the dry matter; the lowest value being that obtained by stripping the rectal contents from the fish, confirming that there was an increased loss of endogenous nitrogen as a result of the manipulation. The results of the other techniques were similar, except that trough netting to collect the feces, again gave the highest estimate of digestibility. The digestibility coefficients for the fat were all high except for the value determined by stripping.

Advantages of the settling column are that it allows the fish to feed normally, there is no need to handle the fish, it allows repeated determinations, and the evaluation of different mixed diets can be carried out at the same time as observations of growth rate. However, a criticism of this method is that soluble material would be lost from the feces by leaching. The close agreement between the results obtained by intestinal dissection, and by collecting rectal contents by suction for the digestibilities of dry matter, crude protein and lipids with the results obtained using the settling column indicates that leaching is not an important source of error. The major cause of leaching is "break-up" of the feces particles by physical handling which must be minimized.

3.2. *Assay diets and measurement of feed ingredient digestibility*

Very few potentially useful feed ingredients can be fed as the sole component of a diet; it is almost always necessary to make up a mixture of feeds into a diet. Thus determination of the digestibility values of a feed requires the comparison of the digestibilities of a reference and a test diet. The test diet being a mixture of the reference diet and the test ingredient. Table 2 shows the formulation of the reference diet used by Cho *et al.* (1974). The diet has been mixed for 20 separate experiments over a 5-yr period, and the complete diet has been analyzed for each experiment, the coefficient of variation for these analyses confirms the difficulty of obtaining uniform diets from the same formulation and emphasizes the need to include control diets in all nutritional experiments. The digestibility coefficients for the proximate components and for the gross energy show that the protein and fat are better digested than the dry matter or the energy. This is because of the poor digestibility of the wheat middlings which are included in the diet to allow the preparation of a water-stable pellet. To provide some internal verification of the system for determining digestibility, the digestion coefficients for the proximate components of each of the feedstuffs used in the reference diet were determined, by making the ingredients the test ingredients in a series of diets (see Table 6). These coefficients were then used to calculate the total level of digestible dry matter, protein, lipid and energy in the reference diet, comparison of the determined levels of digestible nutrients in the diet, with those calculated as the sum of the individual components ranges from 95 to 104% showing that the procedure employed produces internally consistent results and confirms that there were no important interactions between the reference diet and the ingredients when they were included as the test materials in the assay diets. Substitution of the test ingredient for a part of the reference diet allows calculation of the contribution of the test ingredient to the levels of digestible nutrients in the test diet. Table 3 illustrates the use of chromic oxide as a digestion indicator; inclusion of 1% of this inert material allows the digestibility coefficients of the nutrients in the diets to be calculated from measurements of the nutrient to indicator ratios in diet and feces. Edin (1918) introduced the use of chromic oxide as a digestion indicator to obviate the need to quantitate dietary intake and feces output. Austreng (1978) confirmed the suitability of this procedure for measuring digestibilities in fish. Once these coefficients have been calculated for the reference and test diets, the corresponding digestibility coefficients can be calculated for the nutrients in the feed being tested: in the example shown, the test ingredient was substituted for 30% of the reference diet.

The use of a reference diet assumes that there are no interactions between the components of the diet during digestion, but the adoption of this procedure allows the preparation of an adequately balanced diet in which to test the susceptibility of the feedstuff to

Table 2. Apparent digestibility coefficients of reference diet determined and calculated with rainbow trout

		Dry matter (%)	Protein (%)	Lipid (%)	Energy (MJ/kg)
C201-Guelph Reference Diet			(Mean ± %CV)		
Proximate analysis		91.5 ± 1.8	39.1 ± 4.5	15.2 ± 5.6	20.6 ± 2.5
Digestibility coefficient		70.9 ± 8.4	90.7 ± 3.1	92.2 ± 2.1	78.7 ± 6.0
Determined Digestible nutrient		65.7	35.5	14.0	16.2
(Formulation)	(%)		(Digestible Nutrient)*		
Fish meal, herring (70% CP)	35	27.7	22.9	3.8	6.6
Soybean meal, solvent extracted (49% CP)	20	13.4	9.4	0.3	2.7
Wheat middlings (16% CP)	32	10.0	5.2	1.2	2.7
Herring oil	10	9.4	0	9.0	3.6
Vitamin/methionine premix	2	1.8	0	0	0
Mineral premix	1	0.6	0	0	0
Calculated Total	100	62.9	37.5	14.3	15.6
$\frac{\text{Determined}}{\text{Calculated}}$ (%)		104	95	98	104

Data are based on 20 experiments over a period of 5 yr.
* See Table 6 for the digestibility coefficients of ingredient.

digestion. In determinations using such reference and substituted diets measurement of feed intake and growth rate allowed confirmation of the nutritional adequacy of the experimental diets.

Table 4 lists the digestible energy values determined for a number of different feedstuffs using rainbow trout. The digestible energy values approached the gross energy values for the high protein materials such as blood meal, herring meal, cooked full-fat soybeans, and a soybean protein concentrate indicating a high degree of digestion and absorption; however, for the feedstuffs which contained a substantial level of carbohydrate such as yellow corn, corn gluten feed and rapeseed meal, the digestible energy values were less than half of the gross energy values, confirming that starch and fibre are poorly digested by rainbow trout. The determined values were higher than the calculated values for the poorly digested feeds, by a large margin in the case of yellow corn. These differences could be explained if leaching losses were more subjected to the larger quantity of feces provided from these poorly digested feed ingredients.

Table 3. Reference and test diets for digestibility studies

	Reference diet	Test diet
	(% composition)	
Reference diet (Table 2)	99.0	69.3
Test ingredients	0	29.7
Chromic oxide	1.0	1.0

Feeding: 9–16 hr; feces collection: 16–9 hr. Experiment period: 3 days adjusting and 9 days collection. No. of fish: 3 × 50–150 fish/test ingredient. Water temp.: 15°C in flow-through system or other temperature. Feces analysis: mean values based on 3 consecutive, 3-day collections, each being pooled. Diets: cold-pelleted with 20–30% water and dried.

The digestibility coefficient for a nutrient in either the reference or test diet is calculated from the ratios of nutrient to indicator (chromic oxide) using the following expression:

$$\frac{\dfrac{\%\text{ nutrient in diet}}{\%\text{ indicator in diet}} - \dfrac{\%\text{ nutrient in feces}}{\%\text{ indicator in feces}}}{\dfrac{\%\text{ nutrient in diet}}{\%\text{ indicator in diet}}} \times 100.$$

The digestibility coefficient for a nutrient in the ingredient can be calculated from the digestibility coefficients for the reference and test diets on the basis of the 30% substitution of the test ingredient for the reference diet using the following expression:

$$\frac{100}{30}\left(\text{dig. coeff. of test diet} - \frac{70}{100}\,\text{dig. coeff. of ref. diet}\right).$$

Table 4. Digestible energy of ingredients determined and calculated with rainbow trout

Ingredient name	International feed number	Gross energy (MJ/kg)	Digest. coeff. (%)	Digest. energy (MJ/kg)	Ratio Determined/Calculated* (%)
Alfalfa meal	1-00-023	17.9	43	7.7	99
Blood meal, spray-dried	5-00-381	21.8	89	19.4	98
flame-dried	5-00-381	21.8	50	10.9	109
Brewers dried yeast	7-05-527	18.1	77	13.9	95
Corn, yellow	4-02-935	17.0	39	6.6	138
Corn gluten feed	5-02-903	18.6	29	5.4	106
Corn gluten meal	5-09-318	21.2	83	17.6	99
Corn dist. dried sol.	5-02-844	20.9	51	10.7	97
Feather meal, poultry	5-03-795	22.4	70	15.7	96
Fish meal, herring	5-02-000	20.7	91	18.8	94
Meat & bone meal	5-09-321	17.6	85	15.0	93
Poultry byproduct meal	5-03-798	19.6	71	13.9	103
Rapeseed meal	5-03-871	18.1	45	8.1	106
Soybean, fullfat, cook.	5-04-597	22.3	85	19.0	100
Soybean meal, dehull.	5-04-612	18.0	75	13.5	95
Wheat middlings	4-05-205	16.5	46	7.6	109
Whey, dehydrated	4-01-182	14.3	94	13.4	82
Soybean protein concentrate		18.3	84	15.4	96
C201-Guelph Reference diet		20.6	79	16.3	97

* Digestible Energy calculated = (23.4 MJ × % dig. prot.) + (39.8 MJ × % dig. fat) + (17.2 MJ × % dig. NFE).

3.3. *Fish calorimetry*

The nutrients absorbed from the digestive tract are either catabolized or stored as new tissue components. The energy released by catabolism of the nutrients, ultimately being released as heat so that energy balance can be determined either by measuring heat production or by estimating the change in body energy content from weight and carcass composition changes. The latter system is referred to as body balance and requires observations over an appreciable portion of the animals' growth phase. The results from these studies are difficult to interpret in terms of the animals' response to changes in dietary composition, or the changes in feeding regime. Animal calorimetry is the preferred method for nutritionists to measure energy balance of animals over the short time period for which an individual meal exerts its effect. Classically, calorimetry is the measure of heat flow between two objects—from the animal to the environment.

A direct calorimeter has been designed to measure the heat production of fish (Smith *et al.*, 1978a,b), but from data presented, the high heat capacity of this calorimeter made it less sensitive to changes in metabolic rate than methods based on measurements of oxygen consumption (Brett & Groves, 1979). Direct measurements of heat production require measurement of small temperature changes (0.02–0.03°C) of the water as a result of changes in metabolic rates from feed ingestion. An indirect method of estimating heat production depends upon the stoichiometry of nutrient catabolism and oxygen consumption: the consumption of 1 g of oxygen being associated with release of 13.6 kJ energy. The application of this principal to terrestrial animals, and the measurement of both oxygen consumption and carbon dioxide production allows the calculation of respiratory quotients (carbon dioxide produced/oxygen consumed) and from these the relative proportions of carbohydrate and fat being oxidized can be calculated because the respiratory quotients of carbohydrate and fat are characteristically 1.0 and 0.7.

Several indirect calorimeters for fish have been described (Brett *et al.*, 1971; O'Hara, 1971; Solomon & Brafield, 1972; Pierce & Wissing, 1974; Cho *et al.*, 1975). These calorimeters allow the oxygen consumption to be measured for fish under varying degrees of physical constraint. A few calorimeters have been described which allow measurements of oxygen consumption by fish swimming under different conditions (Blazka *et al.*, 1960; Brett, 1964); Braaten (1979) reviewed comprehensively the methodology of fish bioenergetics. A complete description of the energy balance of the fish needs measurement of food intake and fecal and other excreta losses to allow energy to be partitioned on the basis of digestible or metabolizable energy intakes and few of these calorimeters incorporate this feature.

Figure 2 shows the completely automated fish respirometer developed by Cho *et al.* (1975) to allow measurement of oxygen consumption by fish swimming in standard experimental tanks similar to those described for the collection of feces. The tank is covered to prevent any exchange of oxygen between the water surface and the atmosphere. The influent water is aerated or deaerated using air, oxygen or nitrogen to maintain a constant dissolved oxygen level regardless of water temperature in a mixing and reservoir tank below the fish tank and is then pumped into the fish tank through a filter and flowmeter. The difference in the dissolved oxygen concentration between the in-flow and out-flow, multiplied by the water flow rate indicates the rate of oxygen consumption by the fish. The output signals from the dissolved oxygen meter, flowmeter and thermometer being fed

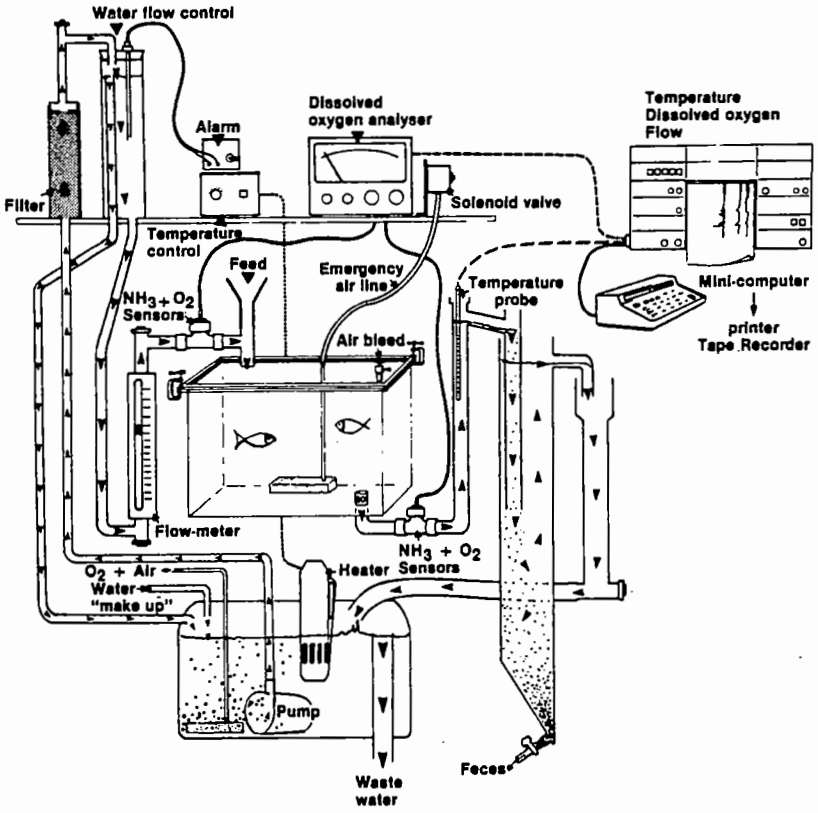

Fig. 2. CYAQ-1 automated fish respirometer equipped with a fish tank, feces settling column and instruments to measure oxygen consumption and water temperature (Cho *et al.*, 1975).

into a mini-computer which monitors and processes data every minute. In case of equipment failure an emergency air line controlled by a solenoid valve can supply air to maintain the fish.

The operating parameters for the facility are shown in Table 5; the 14-day schedule allows one week of observations on the fed fish, followed by three days of fasting to establish baseline values for each of the determinations; at the end of each determination the fish are removed from the system which is then run for a further day to measure the biochemical oxygen demand of the system. Figure 3 shows the results of an experiment in which three diets were compared. There was an increase in oxygen consumption for the first 4 days after the fasting period, and thereafter the oxygen consumption rate was stable for the remainder of the feeding period.

Measurements of energy balance using a respirometer can make an important contribution to nutritional evaluations of different dietary formulations. Often diets with similar levels of digestible energy and nutrients support different levels of performance, and a frequent explanation of such differences is in the rates of heat production from the diets.

4. UTILIZATION OF DIETARY ENERGY IN SALMONID FISH

The nutrient content of a feed can be determined by chemical analysis; such analyses measure the total

Table 5. Experimental conditions for respirometry

Fish	Rainbow trout—hatchery reared
Activity level	Resting
Initial body weight	15–150 g/fish
Total biomass	3 kg ± 10%
Water volume	30 l (50 × 30 × 20 cm)
Water flow rate	3–6 l/min
Water temp. control	±0.1°C
D.O. in influent water	9 ± 0.2 mg/l regardless water temperature
Lighting	300 lux–24 hr/day
Feeding	Near satiety/3–4 meals per day
Data collection	1320 readings by computer in 22 hr/day
Experimental period	3 days adjusting 7 days feeding 3 days fasting 1 day B.O.D. reading

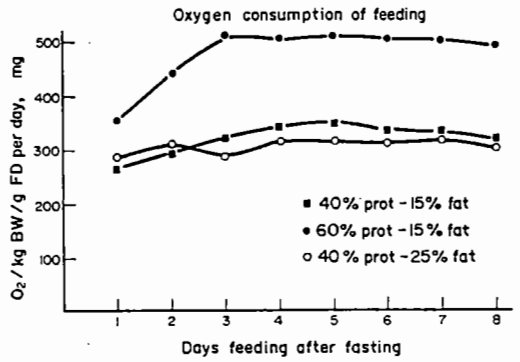

Fig. 3. Typical results of continuous measurements of oxygen consumption by groups of rainbow trout over an 8-day period after 3-days fasting (Cho *et al.*, 1976).

amounts of the nutrients in the feed. In most cases the value of the feed as a source of nutrients is less than that indicated by chemical analysis because a part of the nutrient may be "unavailable". This usually implies that the nutrient is present in the feed in combination with some other component which resists the digestive processes of the fish. In other cases the complexed form of the nutrient may be absorbed, but remain unused by the fish because in its complexed form it cannot enter into the metabolic processes of fish; such compounds are usually excreted through the gill or in the urine. However, because of the multiplicity of the physiological processes involved in the utilization of the dietary fuels an elaborate scheme has been developed, and generally accepted by convention, to describe the losses which occur in the utilization of dietary energy for tissue growth in animals. Measurements of these energy losses involve the use of animals since this aspect of assessing nutritive value of a feed is a biological evaluation rather than physical or chemical (Blaxter, 1967). This convention is described along with a comprehensive glossary by the NRC-NAS (1981a).

Using these terms a modified scheme is proposed (Fig. 4) to describe the physiological consequences to the fish of the diet which it ingests upon bioenergetics. The energy balance may be summerized as follows:

$$ME = RE + HE$$

where

ME is metabolizable energy intake,
RE is energy retained as new tissue,
HE is energy dissipated as heat.

4.1. *Gross intake energy (IE)*

The gross energy content of a food is measured by combustion, usually in an atmosphere of compressed oxygen. Under these conditions the carbon and hydrogen are fully oxidized to carbon dioxide and water, as they are *in vivo*. However, the nitrogen is converted to oxides which is not the case *in vivo*. The oxides of nitrogen interact with water to produce strong acids which can be estimated by titration, allowing a correction to be applied for the difference between combustion in an atmosphere of oxygen and catabolism *in vivo*. The gross energy intake of an animal is simply the product of food consumption and its heat of combustion.

The gross energy values of carbohydrates and proteins are quite similar (17.2 and 23.4 kJ/g respectively), but that of fat is much higher (39.2 kJ/g), thus variation in fat content of the food has a great influence upon its gross energy value, whereas equivalent exchanges between protein and carbohydrate result in only small changes in gross energy value. Obviously the concentration of minerals (ash) in the food influences its gross energy value because the inorganic components of the food are not combustible. This can explain some of the differences between certain types of food; for example, the fish meals used in the preparation of commercial diets can vary widely in ash content, and hence in gross energy values.

4.2. *Digestible energy (DE)*

Before the feed components can serve as fuels for fish they must be digested and absorbed from the digestive tract. Some feed components resist digestion and a large portion of these pass through the digestive tract to be voided as feces. The energy which would have been liberated by the combustion of the fecal material is lost to the animal and is referred to as the fecal loss (FE). The difference between the gross energy of the food, and the gross energy of the feces derived from a unit quantity of food is termed the digestible energy value: for a well digested food its digestible energy value would approach its gross energy value.

Feces are a mixture of the undigested food components and the unreabsorbed residues of body origin. These residues are the remains of mucosal cells, the remains of digestive enzymes and other secretions released into the digestive tract by the animal, together with the residues of the microflora which inhabit the digestive tract. The residues not arising directly from ingested food but from the metabolic activities of the animal are referred to as metabolic residues. The heat of combustion of these materials represents a loss of energy due to the process of digestion, but which is not derived from the food. This energy loss is designated fecal energy of metabolic origin (FmE) and is influenced by the characteristics of the food and the level of feed intake. In practice, fasting animals void such small amounts of feces that the fecal energy of metabolic origin is of little significance for animals receiving normal amounts of feed (Guillaume & Summers, 1970). However, it does allow the description of "corrected" or "true" (a misleading term) digestible energy values which are greater than "apparent" digestible energy values.

"Apparent" digestible energy = IE − FE

"Corrected" digestible energy = IE − (FE − FmE).

Variation in the digestibility of foods is generally the major factor affecting the variation in their usefulness as energy sources to the animal, since the fecal energy loss is the major loss of the ingested gross energy. Therefore digestible energy values and the digestibilities of individual nutrients such as protein, fat and carbohydrate should be used to estimate levels of available nutrients in the feed ingredients for the formulation of diets.

The commercially formulated diets normally used in fish culture result in the loss to the animal of

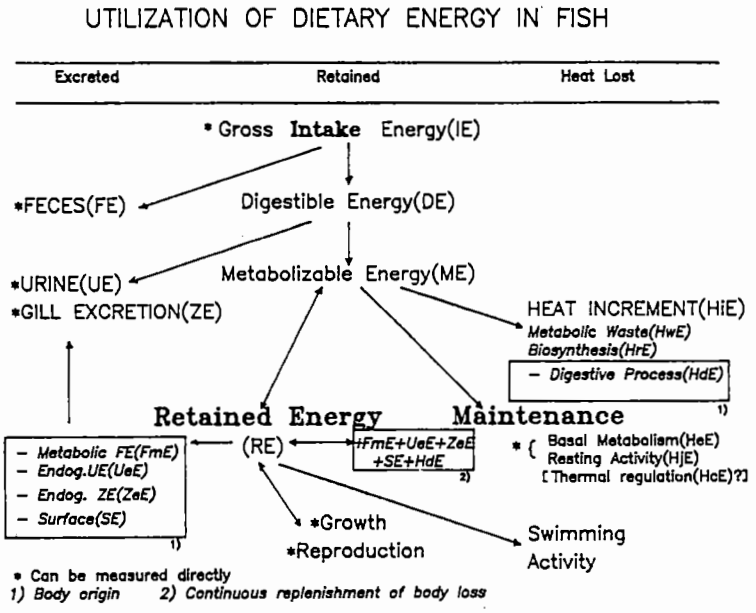

Fig. 4. Schematic presentation of the fate of dietary energy for fish categorizing the losses of energy which occur as food is digested, and metabolized leaving a fraction of the dietary energy to be retained as new tissue (Cho, 1981).

between 10 and 40% of the gross energy in the feces. There are some feedstuffs in which as much as 60–80% of the dietary energy is lost as feces. Fish, particularly salmonids, digest protein and fat very well (Table 6) and simple sugars and hydrolyzed or cooked starch are also digested by rainbow trout (Table 7). However, raw starch is completely undigested by rainbow trout (Cho & Slinger, 1979) and thus the level of starch should not be included in calculations to predict the energy values of formulated diets. Some grain by-products such as wheat middlings are included as "binders" to enhance the stability of the pelleted diets used in fish culture. Clearly the appropriate level at which to include these poorly-digested materials represents a compromise between the nutritional value of the diet, and practical considerations, such as the mechanical stability of the feed aggregate. Table 6 shows the apparent digestibi-

Table 6. Apparent digestibility coefficients and digestible energy value of ingredients measured with rainbow trout

Ingredient name	International feed number	Dry matter (%)	Crude protein (%)	Lipid (%)	Gross energy (%)	Digestible energy (MJ/kg)
Alfalfa meal	1-00-023	39	87	71	43	7.7
Blood meal, animal						
spray-dried	5-00-381	91	99	—	89	19.4
flame-dried	5-00-381	55	16	—	50	10.0
Brewers dried yeast	7-05-527	76	91	—	77	13.9
Corn, yellow	4-02-935	23	95	—	39	5.0
Corn gluten feed	5-02-903	23	92	—	29	5.4
Corn gluten meal	5-09-318	80	96	—	83	17.6
Corn dist. dried sol.	5-02-844	46	85	71	51	10.7
Feather meal, poultry	5-03-795	75	58	—	70	15.7
Fish meal, herring	5-02-000	85	92	97	91	18.8
Meat & bone meal	5-09-321	78	85	73	85	16.0
Poultry byproduct meal	5-03-798	52	68	79	71	13.9
Rapeseed meal	5-03-871	35	77	—	45	8.1
Soybean, fullfat, cooked	5-04-597	78	96	94	85	19.0
Soybean meal, dehulled	5-04-612	74	96	—	75	13.5
Wheat middlings	4-05-205	35	92	—	46	7.0
Whey, dehydrated	4-01-182	97	96	—	94	16.0
Fish protein concentrate		90	95	—	94	17.2
Soybean protein concentrate		77	97	—	84	15.4
*C201-Guelph Reference diet		71	91	92	79	16.0

1 MJ = 239 kcal.

Table 7. Digestibility of carbohydrates by rainbow trout (Singh & Nose, 1967)

Carbohydrate levels in diet (%)	20	30	40	50	60
			(%)		
Glucose	99	99	99	100	100
Sucrose	100	99	99	99	99
Lactose	94	95	97	97	96
Dextrin	77	75	60	50	46
α-Starch, potato	69	65	53	38	26

lity coefficients for commonly used feed ingredients in the diets for salmonid fish. The overall digestibility of the feedstuffs, shown as dry matter digestibilities, varied from 23% for yellow corn to 97% for dehydrated whey. There was less variation in the digestibility coefficients for the crude protein than for the dry matter, only flame-dried blood meal showing a low protein digestibility. The digestible energy values of the feedstuffs were closely correlated with the dry matter digestibilities. The yellow corn had the lowest digestible energy value (5.0 MJ/kg), but the highest values were for the feedstuffs containing highly digestible protein and fat such as fish meal, spray-dried blood meal and fullfat soybeans.

4.3. *Metabolizable energy (ME)*

Digestion of a diet leads to the absorption of amino acids, fatty acids and sugars which are the principal metabolic fuels for the body. Catabolism of fat and carbohydrate results in the formation of carbon dioxide and water, the fully oxidized products of carbon and hydrogen respectively. However, the catabolism of amino acids yields ammonia, in addition to carbon dioxide and water, and the excretion of the ammonia, or of its detoxification product, urea, leads to the loss of combustible material by the fish. The loss of these compounds either through the gill (ZE) or through the kidney (UE) means that the digestible energy value of a diet overestimates its fuel value to the fish. The physiologically available fuel value of the diet to the fish is the metabolizable energy value defined as follows:

$$ME = IE - (FE + UE + ZE) \quad \text{(see Fig. 4).}$$

The loss of combustible matter in the feces depends upon the susceptibility of the feed components to digestion and absorption by the fish, and there are few interactions, of any significance, between feeds mixed together to form a diet, which influence their digestibility. Thus the digestible energy value of a feedstuff is relatively independent of the composition of the diet in which it is fed. In contrast, the loss of combustible matter through the gill, or in the urine depends upon the biological value of the total protein in the diet, and this is influenced by the proportions of different feedstuffs, particularly the level and type of fat, which are included in the diet. As a consequence the metabolizable energy value of a given feedstuff in a diet is not independent of the composition of the diet since it is the overall balance of the amino acids and energy in the diet which influences the retention of protein by the body and hence governs the loss of nitrogen products through the gill or in the urine.

The variation due to differences in nitrogen excretion following the consumption of mixed diets can be taken into account by measuring the nitrogen excreted and then calculating nitrogen retention as the difference between nitrogen intake and excretion. Combustible material lost through the gill or in the urine by fish receiving diets in which only a fraction of the absorbed nitrogen is retained can then be compared by calculating how much energy would have been lost if all the dietary nitrogen had been excreted. Metabolizable energy values corrected to zero nitrogen retention (MnE) can be calculated from nitrogen retention and the heats of combustion of the nitrogenous excretion products which are 21, 23 and 34 kJ/g N for ammonia, urea and uric acid, respectively. Values corrected in this way represent the fuel values of the diet to the animal if all the protein is catabolized, a concept which has been criticized by Kleiber (1975).

There is some controversy amongst animal nutritionists as to the relative utility of digestible and metabolizable energy values as a means of comparing the fuel value to the animal of different feed ingredients. Metabolizable energy values have been chosen by poultry nutritionists for the practical reason that birds void the feces and urine together. These values have been used for the last two decades as a basis for the prediction of the effects upon "productivity" of changing dietary formulations. Measurement of metabolizable energy values of diets and feedstuffs is relatively simple in poultry. In mammals, for example pigs, the measurement of metabolizable energy requires the separate collection and analysis of urine, and for most diets formulated to contain normal protein levels for the pig there is a constant relationship between digestible and metabolizable energy values (Diggs *et al.*, 1965), metabolizable energy equalling 96% of the digestible energy. Thus, the extra experimental work involved in determining metabolizable energy for pigs does not appear to be warranted.

Determination of metabolizable energy values of diets for fish is technically difficult because of the need to quantitate both gill and urinary losses. However, Smith (1971) attempted to overcome these difficulties and developed a procedure which allowed the estimation of the metabolizable energy values of a number of feedstuffs using rainbow trout of 165–530 g body weight. Before the assays, the fish were anaesthetized to allow the insertion of a cannula for urine collection. The fish were then confined in a chamber with a diaphragm placed around the outside middle

of the fish in such a way as to separate excretions from the front and the rear of the body; they were force-fed the feed being tested as a single daily meal. The metabolizable energy values determined by this procedure as a fraction of the digestible energy values ranged from 0.72 to 0.93 (mean = 0.87) (Table 8). This shows that for most feedstuffs a much higher proportion of the combustible energy of the absorbed food components was excreted through the gill and kidney than was excreted in the urine of the pig.

It is usual to feed pigs with diets containing from 13 to 18% protein, which is much lower than the protein levels of from 35 to 50% which are common in the diets fed to salmonid fish species. Thus a large part of the energy intake of fish is provided as nitrogenous compounds which lead to the need to excrete ammonia; more than 85% of the nitrogen derived from protein catabolism is excreted as ammonia through the gills (Forster & Goldstein, 1969). An additional factor contributing to the larger difference between metabolizable and digestible energy values for rainbow trout (Smith *et al.*, 1980) than for pigs could be the procedures employed to separate and collect nitrogen excreted via the gill and kidney. These procedures (including force-feeding) involve considerable handling, are stressful to the fish and lead to an increase in nitrogen loss (Hunn, 1981) thus enhancing the loss of combustible matter. The resulting increase in nitrogen output through gill and kidney together with the low food intake attained by the force-feeding of a single daily meal, might be expected to result in a negative nitrogen balance and a low ratio of metabolizable to digestible energy values for many of the feed ingredients studied (Table 8). This strongly suggests that energy losses via the gill and kidney were relatively much greater than would be the case for unrestrained fish feeding normally.

Thus fish nutritionists who must ascribe fuel values to different "feedstuffs" to formulate balanced diets should employ digestible energy values. They should avoid such values (or metabolizable energy values) as have been obtained by methods susceptible to gross error as indicated by excessive nitrogen losses or other signs of disturbed protein metabolism.

It is recognized that, theoretically, metabolizable energy is the appropriate measure of fuel availability in evaluating the potential energy utilization of "formulated" diets. However, further development of techniques to obtain reliable measures of metabolizable energy are necessary before values for this parameter can be safely applied to formulated diets. Until such reliable metabolizable energy values become available, digestible energy values should be used as a first approximation of the relative fuel values of different feed ingredients and diets.

5. FATE OF METABOLIZED ENERGY

Heat is liberated by animals as a consequence of the metabolic transformation of dietary substrates into tissue components, as a result of tissue turnover, and as a result of physical activity. The rate at which heat is liberated is termed the metabolic rate and this varies with the activity of the animal. An important concept is that of basal metabolic rate which, as the term implies, is the minimum rate of metabolic activity needed to sustain the structure and function of the body tissues. Any activity results in an increase in metabolic rate. The ingestion of food increases metabolic rate as a consequence of the extra work due to the ingestion, digestion and utilization of the food. This increase is termed the heat increment of feeding. Physical activity also increases metabolic rate due to work done against internal and external frictional

Table 8. Digestible and metabolizable energy and ratio measured with rainbow trout (Smith *et al.*, 1980 and NRC-NAS, 1981b)

Ingredient name	International feed number	Digestible energy*	Metabolizable energy (MJ/kg)	ME/DE*
Alfalfa meal	1-00-023	8.1	5.8	0.72
Blood meal, spray-dried	5-00-381	19.4	16.8	0.87
Corn gluten meal	5-09-318	16.9	14.9	0.88
Corn dist. solubles	5-02-844	10.3	9.6	0.93
Cotton seed meal	5-07-874	12.4	10.3	0.83
Fish meal, anchovy	5-01-985	19.1	16.8	0.88
herring	5-02-000	19.8	17.3	0.87
salmon	5-02-012	16.8	14.9	0.89
whitefish	5-02-025	14.6	12.4	0.85
Fish solubles, dehy.		15.5	14.0	0.90
Rapeseed meal, sol. extracted	5-03-871	12.5	11.3	0.90
Soybean meal, dehulled	5-04-612	12.5	10.7	0.86
Soybean, fullfat,	5-04-597			
roasted, 232°C, 8 min.		18.1	16.4	0.91
Jetsploder, 204°C		18.6	17.1	0.92
Wheat, hard, clears		7.9	6.6	0.84
Wheat middlings	4-05-205	10.3	9.4	0.91
Wheat germ meal	5-05-218	12.6	11.5	0.91
Whey, dehydrated	4-01-182	11.3	10.0	0.88
low lactose	4-01-186	11.1	9.5	0.86
Yeast, brewers	7-05-527	15.9	12.2	0.77
torula	7-05-534	15.4	14.1	0.92

* Calculated from the data given. 1 MJ = 239 kcal.

forces. These three components of animal metabolism lead to the release of energy as heat (HE) from the metabolizable energy derived from the food, and clearly energy released as heat is not available for weight gain (increase in body energy). This can only occur if the dietary metabolizable energy intake exceeds the rate of heat production. As might be anticipated, if the metabolizable energy intake is less than the rate of heat production the difference will be provided by catabolism of body tissues and weight loss will ensue. An intake of metabolizable energy in excess of heat production will be stored in the body as the energy retained in new tissues.

5.1. *Energy requirement for maintenance* ($HeE + HcE + HjE$)

Fish require a continuous supply of energy for those functions of the body immediately necessary for maintaining life regardless of whether or not it is consuming food. A fish deprived of food obtains this energy by catabolizing body reserves of fat and protein; however in the fed fish this requirement for maintenance energy is supplied by the food thus obviating the catabolism of body tissue. Among requirements for maintenance a major portion of the energy is spent for basal metabolism (HeE), thermoregulation of body temperature (HcE) in the case of homeotherms, and a smaller portion of energy is spent for involuntary or resting activity (HjE) such as minor bodily movements and muscular activity.

By definition, the basal metabolism is the minimum rate of energy expenditure needed to keep the animal alive and this has highest priority in the maintenance requirement. The basal metabolic rate is measured when the animal is in the post-absorptive state, and is in a state of muscular repose at an environmental temperature which is thermoneutral. Definition of basal metabolic rate for fish precludes the latter condition but makes it necessary to specify the temperature at which the metabolic rate is measured.

Brett (1972) showed that the maintenance requirements of poikilothermic animals were 10–30 times lower than those of mammals which maintain body temperatures of 35°C. It is more difficult to ensure that the fish is in a state of muscular repose because they need to maintain their orientation in the water and this entails some muscular activity. Thus basal metabolism can be measured by extrapolation to zero activity from fish swimming at different rates. However, fish such as rainbow trout will spend considerable periods resting on the bottom of their tanks, maintaining their position in quiet water with minimal activity. The energy expenditure of fasting fish under these conditions can be regarded as a close approximation to basal metabolism.

There have been several reports of measurements of basal metabolic rates in fish. Cho *et al.* (1976) found that fasting, resting rainbow trout at 15°C released 59–63 kJ/kg per day as heat. These measurements were made with groups of fish whose weights were in the range 96–145 g. Smith *et al.* (1978a,b) studied rainbow trout with weights in the range 0.85–57 g, and found that fasting heat production at 15°C increased as the size of the fish increased; their estimates ranged from 54 to 136 kJ/kg per day. They developed equations to relate Heat Production to body weight (W):

For fish 1 to 57 g body weight

$$\text{Heat production (kJ/d)} = 204\ W^{0.75}\ (r = 0.92).$$

A relationship between heat production and a fractional coefficient of body weight indicates that surface area rather than body weight may be the important factor contributing to the basal metabolic rate of fishes, as is the case in terrestrial animals (Kleiber, 1975).

Variation in water temperature had a great effect on the fasting heat production of fish. Smith *et al.* (1978a,b) found that increasing the temperature from 3 to 18°C almost doubled the heat production of Atlantic salmon and rainbow trout. In their studies with lake trout and brook trout the lowest water temperature was 6°C, and the fasting heat production of these fish was doubled by an increase in water temperature to 18°C. The plots of heat production against water temperature show that the metabolic rates of Atlantic salmon and rainbow trout increased more slowly than those for either lake trout or brook trout as water temperature increased. Cho & Slinger (1980) measured the fasting heat production of rainbow trout (live weights from 47 to 136 g) at temperatures of 7.5, 10, 15 and 20°C (Table 9). Temperature rise had its largest effect on fasting heat production between 7.5 and 10°C, such an increase leading to a doubling of heat production, with a further increase in water temperature to 15°C resulting in a 50% increase in heat production; no further increase in heat production occurred in response to increasing water temperature from 15 to 20°C.

5.2. *Heat increment of feeding* ($HiE = HdE + HrE + HwE$)

Ingestion of food by an animal which has been fasting results in an increase in the animal's heat production. This expenditure of energy due to feeding is referred to by several terms: heat increment of feeding (HiE), specific dynamic action (SDA), calorigenic effect and dietary thermogenesis. The factors contributing to heat increment of feeding are designated (1) the digestion and absorption processes (HdE), (2) transformation and interconversion of the substrates and their retention in tissues (HrE) and (3) formation and excretion of metabolic wastes (HwE). The main biochemical basis for heat increment is the energy required for the ingested amino nitrogen to be deaminated and excreted (HwE) (Kleiber, 1975); the energy expenditures associated with food ingestion and digestion (HdE) are very small compared to that associated with the metabolic work (HwE + HrE) (Brody, 1945). The physiological basis of this increased heat production are the post-absorptive processes related to ingested food, particularly protein-rich food—mainly metabolic work required for the synthesis of proteins and fats in the tissues from the newly absorbed, food-derived substrates such as amino acids and fatty acids and the formation of excretory nitrogen products. Figure 5 emphasizes the importance of protein's contribution to heat production. Diets containing 6% fat and either 30 or 47% of digestible protein resulted in similar rates of oxygen consumption. However, increasing the fat level in the lower protein diet resulted in a substantial reduction in oxygen consumption: the classical effect of fat upon

Table 9. Influence of water temperature upon the utilization of digestible energy by rainbow trout for gain, heat increment and maintenance (Cho & Slinger, 1980)

Water Temperature (°C)	7.5	10	15	20
Energy retained (% of DE intake)	44	49	53	58
Heat increment				
(% of DE intake)	20	14	11	15
(kJ/g N intake)	50	34	27	38
Maintenance (kJ/kg BW/day)	18	37	61	56

Diet contains 38% digestible protein and 9% digestible fat; 1 g oxygen = 13.6 kJ.

heat increment of feeding, whereas increasing the fat level in the higher protein diet had practically no effect upon oxygen consumption, presumably because of the metabolic work associated with the higher influx of amino acids provided by the high protein diet.

In fish there have been several studies of heat increment of feeding designed to separate the biochemical aspects of the post-absorptive processes from the physical or mechanical aspects of feeding and digestion. These studies involved either "sham feeding" or feeding non-digestible materials such as kaolin or cellulose. A standard diet for largemouth bass was diluted 1:5 with cellulose and the effects of increasing meal volumes of the standard and diluted diets upon heat increment of feeding were compared. The difference between the heat productions resulting from the two types of food being referred to as "mechanical SDA" (Tandler & Beamish, 1979). This approached 10–30% of the total heat increment and this study assumed that the effects of the standard diet and cellulose in the diluted diet on the HiE were additive. Smith *et al.* (1978a,b) with rainbow trout, and Jobling & Speer (1980) with plaice, found that neither sham feeding, nor kaolin feeding increased the resting metabolic rate of their fish. In studies with rainbow trout, Cho *et al.* (1976) and Cho & Slinger (1980) found that sham feeding did increase heat production, but only by about 1–2% of the increase found when the fish were fed normally. Clearly one effect of providing food to fasting fish is an increase in physical activity, but the magnitude of this effect in relation to the physiological effects of "normal" food ingestion depends upon the experimental procedures employed. Sham feeding and non-digestible materials elicit minimal response compared to the use of the "diluted" low protein diet used by Tandler & Beamish.

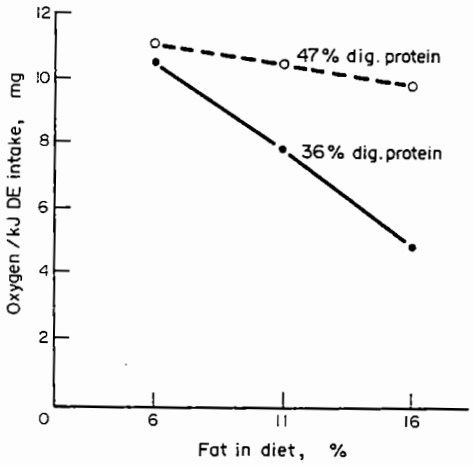

Fig. 5. Influence of dietary protein and fat levels upon oxygen consumption (heat production) of rainbow trout (Cho, 1981).

Since its introduction by Rubner (1902) the term "Specific Dynamic Action" of food has caused confusion, but his idea was useful because it showed that the chemical work of the glands was important as a source of heat in contrast with the original belief that the increased heat production was due to the mechanical work of the digestive tract. His concept was based upon the observation that feeding bone meal to dogs did not increase heat production. This conclusion was reinforced by the observation that intravenous infusion of amino acids increased heat production to the same extent as oral administration of the amino acids (Benedict & Emmes, 1912; Borsook 1936).

The length of time for which consumption of food exerts an influence upon heat production depends upon many factors; chief amongst these are the quantity and quality of the food, and the water temperature. Saunders (1963) found that oxygen consumption of Atlantic cod (*Gadus morhua*) remained above the fasting level for 7 days at 10°C following consumption of a large meal of herring whereas in water at 15°C the effect lasted for only 4–5 days. The heat increment of feeding depends to a large extent upon the balance of dietary nutrients and the plane of nutrition (Brody, 1945). Therefore attempts to measure the heat increment of individual feed ingredients which give a nutritionally unbalanced diet or measurements made under forced activity conditions have doubtful meaning.

In mammals and birds the heat increment of feeding is greater for high protein diets than for diets with low protein concentrations because of the deamination of the amino acids to be used as a source of energy and the need to detoxify the ammonia by synthesis of urea or uric acid; the energy cost of synthesis for these products being 13 and 10 kJ/g N respectively (Martin & Blaxter, 1965). Urea and uric acid are concentrated for excretion by the kidneys in terrestrial animals, requiring the expenditure of further energy. In contrast ammonia is the primary waste product of protein catabolism in fish and thus they do not require energy to detoxify or concentrate this waste (Cowey, 1975). As a result, values for heat increment of feeding for fish are lower, ranging from 8 to 12% of IE (Table 10) than those encountered for terrestrial animals which can dissipate as much as 30% of the dietary energy as heat (Farrell, 1974).

The carnivorous fish consume high protein diets and excrete much of the digested nitrogen. Table 11 shows that although the heat increment of feeding, as a fraction of the digestible energy, varies with the level of protein and fat in the diet, it is remarkably independent of dietary composition when expressed on the basis of nitrogen intake. This confirms the suggestion that protein metabolism is the major factor contributing to the heat increment of feeding. The data in this table shows that the maintenance requirements of

Table 10. Influence of dietary protein and fat levels upon the partition of gross energy intake by rainbow trout (Cho *et al.*, 1976)

Digestible protein (%)	34	34	55
Digestible fat (%)	13	22	13
Energy:	(% of intake energy)		
Digestible*	70	78	85
Metabolizable	62	70	77
Retained	37	49	46
Heat lost:			
Maintenance	16	13	18
Heat increment	9	8	12

* Apparent digestibility; water temperature: 15°C.

the fish were not influenced by the level of fat in the diet, but appeared to decrease as the protein level was increased from 34 to 55% of the diet.

To date many of the estimates of the heat increment of feeding are widely variable. The approaches used preclude generalizations concerning the loss of energy associated with this aspect of the food utilization. The situation was admirably summarized by Brett & Groves (1979) "..... that unless serious attention to the effect of diet composition on heat increment is the aim, it is doubtful if the necessary effort to correctly partition feeding metabolism into its respective components is warranted". This is an area of fish bioenergetics which needs careful study to show the total magnitude of the heat increment of feeding under the various nutritive and temperature regimes under which fish are cultured. The data in Table 9 shows that increasing the water temperature from 7.5 to 15°C halved the proportion of digestible energy dissipated as heat. This suggests that this is an area deserving further study.

5.3. *Growth and energy retention (RE)*

Metabolizable energy taken in as food which is not dissipated as heat is retained within the body as new tissue elements. In growing animals part of the retained energy is stored as protein and part as fat, but as the animal approaches its mature size an increasing proportion of the retained energy is stored as fat; the relative importance of protein and fat deposition depends upon a great number of factors in addition to the maturity of the animal. The balance of the available amino acids of the dietary protein, and the amount by which the dietary energy intake exceeds the energy expended as heat are the two major factors. Proteins of higher biological value promote greater protein deposition than those of low value. Marginal excesses of energy intake over energy expended as heat, result in the deposition of a larger proportion of the retained energy as protein. As the excess energy intake increases, the total amount of protein deposited increases, but the proportion of the energy retained as fat increases at an even faster rate so that increasing energy intake leads to an increase in the amount of energy retained as fat.

The water temperature also influenced the proportions of the dietary energy retained and dissipated. Table 9 shows that increasing the water temperature from 7.5 to 20°C increased energy retention from 44 to 58% of digestible energy intake. The composition of the diet also influences energy retention: Table 11 shows that increasing digestible fat levels from 13 to 22% of the diet resulted in an increase in energy retention. Watanabe *et al.* (1979) fed diets with a range of protein and fat levels to growing rainbow trout; they found maximum protein retention and an optimum protein to fat ratio with diets containing 35% protein and 15–20% fat.

Energy expended in swimming reduces that which can be retained as new body tissues. The energy expended in swimming can exceed the dietary energy intake, the balance being supplied by the mobilization of body tissue reserves. Over a long term the energy reserves accumulated may be depleted when the fish migrate, often without feeding, to the spawning ground. The implication of this aspect of the energy expenditure in swimming of the fish has been comprehensively reviewed by Brett (1972), Beamish (1979, 1980) and Brett & Groves (1979).

In almost all cases retention of energy and the deposition of new tissue results in an increase in the weight of the animal; and the weight gain of young fish is usually a reliable indicator of the adequacy of the nutritional and management regimes. Unfortunately the rate of weight gain is not a quantitative measure of energy retention for two reasons, firstly because deposition of fat reduces the water content of the body thus changing the energy value per unit weight of the living animal, and secondly because the energy contents of fat and protein are so different. Fat is usually deposited in adipose tissue in association with relatively little water resulting in a heat of combustion for fish adipose tissue of 31 kJ/g, whereas protein is usually deposited in such tissues as viscera and muscle in association with a great deal of water resulting in a heat of combustion for fish muscle tissue of 6 kJ/g.

This problem of interpreting productivity data is further complicated by the different metabolic processes by which dietary energy is stored as either fat or protein: the relatively simple synthesis of fat being 74% efficient whereas the much more complex synthesis of protein is only 44% efficient as shown by Pullar & Webster (1977) for rats. This means that it is impossible to equate weight gain with energy retention without a simultaneous estimate of body composition. Hence comparisons of weight gain per unit of food consumed (feed efficiency) are only useful if the

Table 11. Influence of dietary protein and fat levels upon the partition of digestible energy intake by rainbow trout, showing the importance of nitrogen intake as a factor determining the heat increment of feeding (Cho *et al.*, 1976)

Digestible protein (%)	34	34	55
Digestible fat (%)	13	22	13
Energy retained (% of DE intake)	53	63	54
Heat increment			
(% of DE intake)	11	8	13
(kJ/g N intake)	28	26	29
Maintenance (kJ/kg BW/day)	63	63	59

Water temperature: 15°C; 1 g oxygen = 13.6 kJ.

energy value of the food and of weight gain are known.

Reproduction involves the synthesis and temporary storage of new material which is formed almost regardless of the level of dietary energy intake, the necessary energy being withdrawn from other body tissues if the dietary supply is insufficient. Consequently the redistribution of tissue energy which takes place in the breeding season can influence measurements of energy balance.

In fish, particularly in cultured fish, there are few studies of exact energy retention from the diet. It is much more difficult to compare the results of growth studies carried out with fish than with those carried out with other animals, not only because of the profound influence of temperature, but also because of the difficulties of accurately assessing food intake. To allow the establishment of a basis for comparison of data with the same species of fish it is necessary to define reference growth curves which are "normal" for particular conditions. Figure 6 shows the growth curves for rainbow trout under laboratory conditions at three different temperatures: at 10°C the fish weighed only 4 kg/100 fish after 20 weeks whereas at 15°C they reached this weight after 12 weeks. Similar results have been obtained under large scale fish farming conditions. Since the maintenance requirement is a fixed cost for the fish at any given temperature, the rate of energy retention, and hence of growth depends on the level of dietary energy intake. Culture regimes which promote high levels of food consumption by provision of well balanced diets, in a stable, prehensible form, to fish maintained in facilities which minimize the stresses upon them, will clearly promote more rapid growth and hence have a higher net efficiency of energy retention than regimes which fail to provide these conditions.

6. EVALUATION OF FEEDS FOR FISH

The value of a diet depends upon the levels and availabilities of the more than 40 nutrients which have been shown to be needed by fishes. However, the fuel value of the food for the fish is especially difficult to define because of the interactions occurring between the food-derived substrates after they have been absorbed from the digestive tract. It is important to define the energy value of the diet because this is of overriding importance in determining the amount of feed consumed by the fish because they adjust their food intake to satisfy their needs for energy. Thus the actual intake of all the nutrients is regulated by the available energy level of the diet.

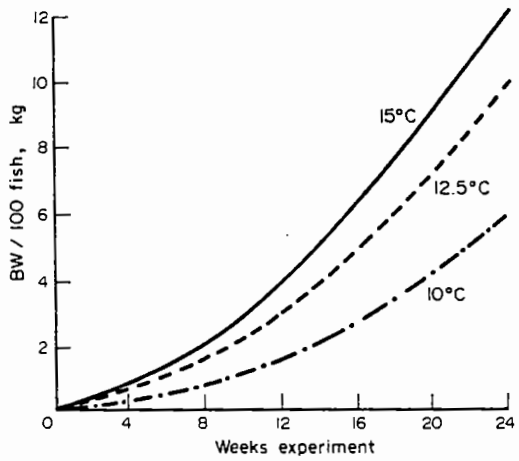

Fig. 6. Influence of water temperatures upon growth of rainbow trout under similar environmental conditions and receiving the same diet.

The simplest measure of the available energy level of a diet is its digestible energy value since this appears not to be influenced by level of feeding above the maintenance level, or by other environmental factors, but this simple measure does not provide any indication of the interactions which can influence the proportion of the dietary energy intake which is retained by the fish and used for growth. The advantage of using digestible energy values to compare different feed ingredients is that these values are additive: if the formula of the diet and digestible energy values of the ingredients are known, the digestible energy value of the mixed diet can be calculated.

Figure 7 shows that the loss of feces from the diet is the primary reason for variation in the nutritional values of foods. Hence measurement of digestibility gives a good indication of the nutritional quality of feedstuffs, allowing a rational basis for the formulation of diets to meet specified standards of available nutrient levels. The losses which occur after digestion of the food, such as losses through the gills, or in the urine depend upon the appropriateness of the nutrient balance, the level of feeding, and upon the physiological status of the fish. None of these factors are directly attributed to the inherent characteristics of the feedstuffs included in the diet, but depend upon the skill of the nutritionist in formulating the feedstuffs into a diet balanced with respect to the animal's nutrient needs.

The effectiveness of diets formulated upon the basis of the digestibilities of the nutrients and energy in the component feeds can be evaluated by observation of weight gain, feed efficiency and body composition of fish receiving the diets under particular culture regimes. Only if the diets formulated in this way fail to support standard levels of productivity should the whole area of post-absorptive losses be examined. This will require measurement of the non-fecal losses and the energy dissipated as the heat increment of feeding. The preparation of a diet with an inadequate amino acid balance may be the cause of excessive nitrogen losses through the gills and in the urine, or an inadequate balance of energy and protein may be the cause of excessive energy lost as heat increment of feeding. Measurements of these post-absorptive losses require much more sophisticated facilities and employ more invasive techniques leading to stress for the fish as compared with the simpler measurement of digestibility.

Although metabolizable energy values are used to compare feedstuffs before their inclusion in diets for some domestic livestock, the practical difficulties of measuring the non-fecal energy losses by fish suggest that both mixed diets and feedstuffs be compared for fish on the basis of their digestible energy values until the many problems of determining metabolizable

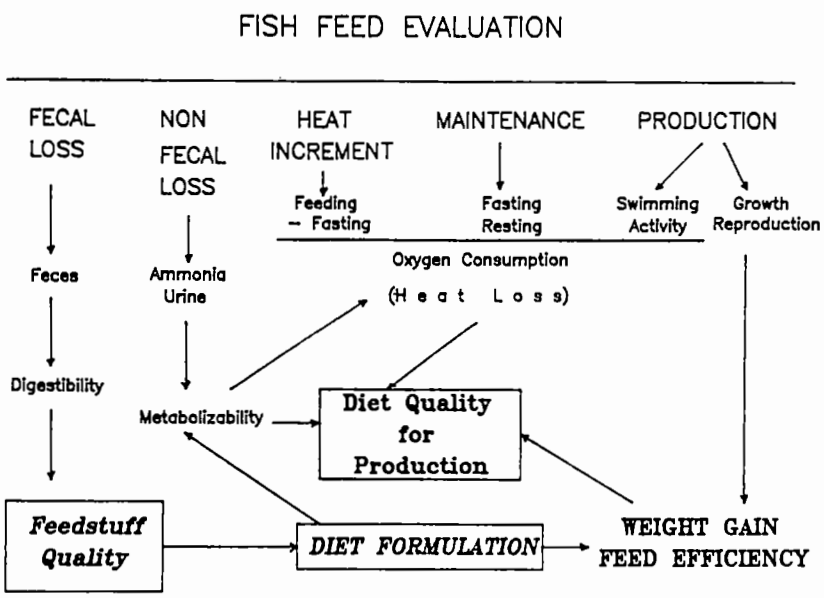

Fig. 7. Summary of a scheme to evaluate feedstuffs for their potential as components of fish diets (Cho, 1981).

energy values of feeds for fish have been overcome. The advantages of having metabolizable energy values rather than digestible energy values for fish feeds are not sufficient to offset the large artifacts inherently associated with present methods of their determination. Therefore feedstuffs which are believed to have potential for use in fish diets may be satisfactorily evaluated using the following scheme:

(1) Measure digestibilities of feed ingredients.
(2) Formulate balanced diets in combination with other feedstuffs.
(3) Observe the levels of productivity supported by such diets.
(4) Measure weight gains, feed efficiency and calculate nutrient and energy retention by analyzing the fish carcasses.

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