Chapter 1

General Introduction

1. INTRODUCTION

1.1 Importance and Status of Aquaculture

Aquaculture plays a vital role in many countries by offering better nutrition, higher income, earning foreign exchange and better employment opportunities. Fish has long been valued as a source of high quality animal protein, relatively cheaply, for human nutrition. Consumption of fish generally cuts across ecological, socio-economic, cultural and religious boundaries, leading to its prominent role as a source of animal protein. Fish proteins are essential and critical components of the human diet in some densely populated countries where the total protein intake level may be low. Presently fish accounts for more than, or close to, 50% of the total animal protein consumed in most countries of the world. *Per capita* fish supply, based on reported production, has increased dramatically over the last 20 years indicating the growing importance of fish as food (FAO, 2000).

Aquaculture has become recognised as a growth area of economic importance in many countries and has attracted the attention of both the private and public sectors. The development plans of most producing countries are aimed at increasing fish supplies from aquaculture for local and export markets, and at increasing the sector's contribution to food security in rural areas. The potential of aquaculture to meet the challenges of food security and to generate employment and foreign exchange has been clearly demonstrated by the rapid expansion of this sector (Rana, 1997). On the other hand, the world's population has been increasing more quickly than total fish production. Present world *per capita* food fish supply stands at 15.8kg / year, which still leaves a negative balance between consumption (demand) and supply (FAO, 2000).

Aquaculture is one of the fastest growing food production activities in the world. It has grown rapidly during the last decade (Figure 1.1). Between 1990 and 1999 aquaculture production expanded by about 150%. In recent years world fish production from capture fisheries and aquaculture has continued to increase (Figure 1.1) with estimated total landings of 125 million metric tonnes in 1999 (FAO, 2000). Reported global aquaculture production in 1999 was estimated at 32.9 million metric tonnes, 26% of the total fisheries production. Aquaculture is supplementing or replacing capture fishery production of over-exploited fish and shellfish stocks. Reported global capture fisheries and aquaculture production contracted from a figure of 122 million tonnes in 1997 to 117 million tonnes in 1998 (Figure 1.1). This was mainly due to decline of some major marine capture fisheries. However, production recovered in 1999 for which the preliminary estimate is about 125 million tonnes. The production increase of 20 million tonnes over the last decade was mainly due to aquaculture, as capture fisheries production remained relatively stable (FAO, 2000).

According to FAO (1998) global aquaculture production continues to be dominated by Asia (Figure 1.2) whose role in aquaculture has been perpetuated through the centuries. Asia's contribution towards total world production increased from 82% to 89% from 1989 to 1998, while Europe's share is 6.33% of global aquaculture production; Africa, South America and North America contributed only 4.38% in 1998. The low contribution from such continents is attributed to the fact that Europe, Africa, South America and North America do not have long histories of aquaculture like Asia. For example, in China the aquaculture industry goes back thousands of years and has not only become an important industry to that country but has also become part of the nation's culture. In addition, China has reported increases in production of 0.7 million tonnes per year until 1992 and 2.6 millions tonnes per year thereafter.

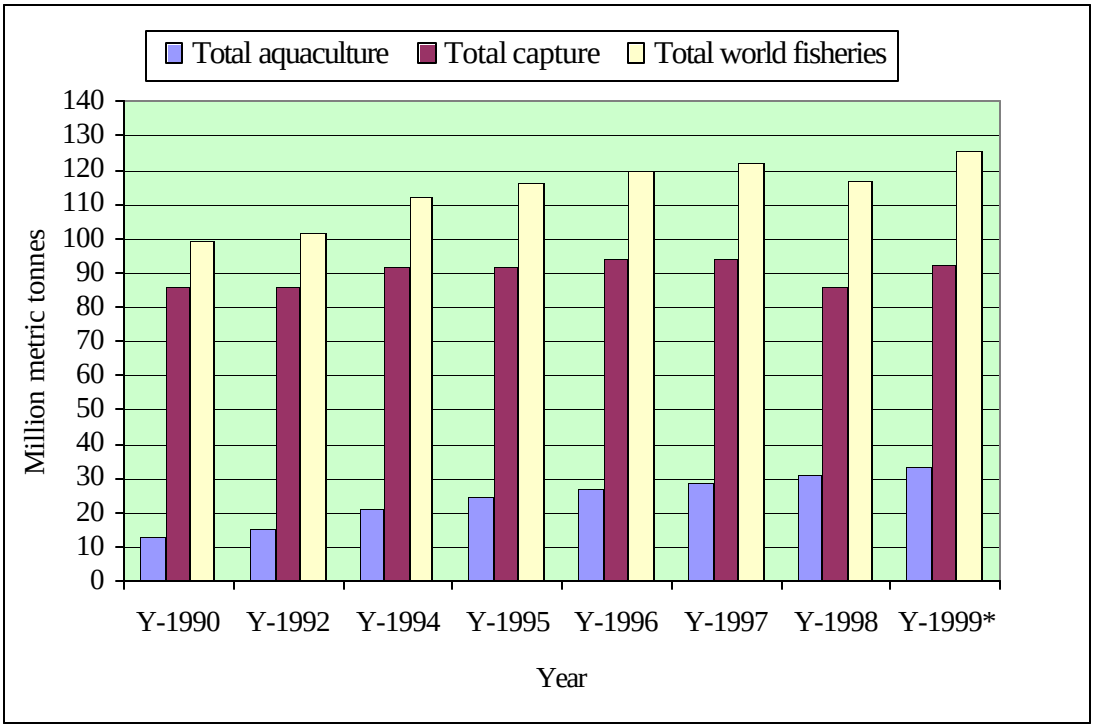

Figure 1.1 World Fisheries Production (FAO, 2000)

* Preliminary estimate

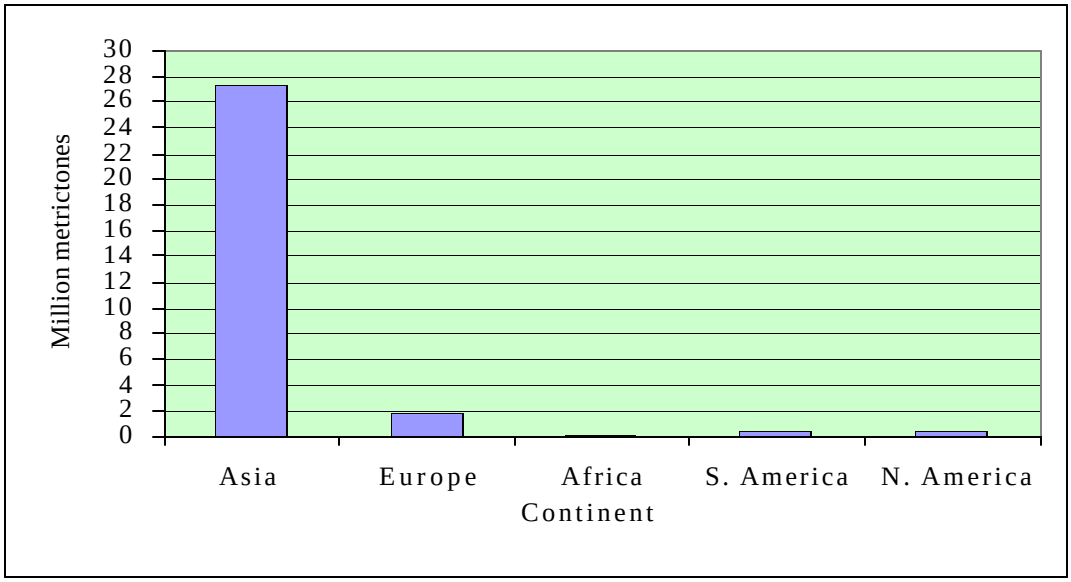

Figure 1.2 World Aquaculture Production in 1998 by continent (FAO, 1998)

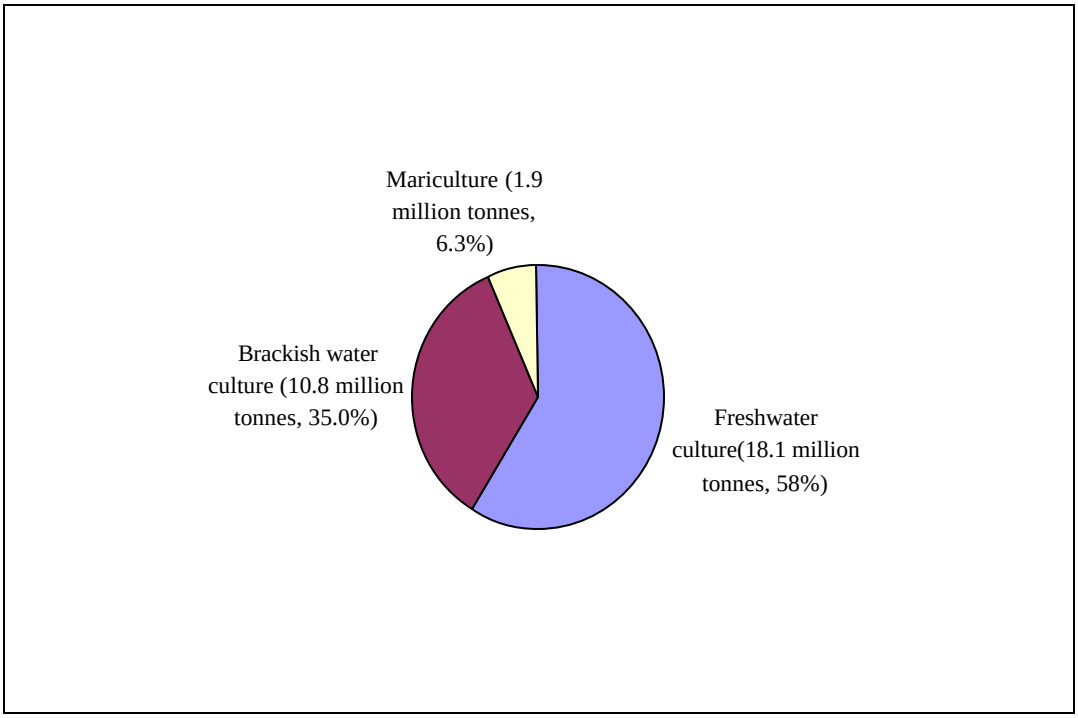

Figure 1.3 World Aquaculture Production in 1998: Breakdown by Environment (FAO, 2000).
Note: Data do not include aquatic plants

For the rest of the world, combined growth in production has averaged 0.4 million tonnes per year (FAO, 2000). However, statistical data reported by FAO (1998) show that aquaculture production expanded rapidly from 1989 to 1998 in Asia (169%), Africa (90%), Europe (32%), South America (400%) and North America (27%).

Most aquaculture has developed in freshwater environments (Figure 1.3), and mainly in Asia. The development of inland aquaculture is seen as an important source of food security in Asia particularly in land-locked countries. Fresh water aquaculture production, dominated by finfish, has contributed to high total aquatic production in many Asian countries (FAO, 2000). In China freshwater finfish production has shown rapid growth since 1991 and in 1995 it accounted for 53% of total aquaculture production (Rana, 1997). In Southeast Asia (Brunei Darussalam, Cambodia, Indonesia, Laos, Malaysia, Philippines, Singapore, Thailand, Viet Nam) freshwater fish dominate aquaculture production and account for 29% (by weight) of the total production (Subasinghe *et al.*, 1997). In South Asia (Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, Sri Lanka) freshwater fish accounted for 94.2% of the total aquaculture production in 1995 (Subasinghe, 1997). In 1995, world production of Clariidae catfish (the subject of this thesis) was more than 0.2 million MT which was the second most important group of farmed catfish in the world (FAO, 1997).

World aquaculture production will surely increase to meet the increasing demand for animal protein. The growth of the human population has lead to an intensified search for methods of producing animal protein, other than conventional animal livestock and capture fisheries, as both face limits in production. The potential of aquaculture as a supplementary producer of protein is attracting more interest than ever. Therefore, aquaculture will presumably expand geographically, in terms of species cultured and technologies used, as time passes.

1.2 Aquaculture Nutrition

Aquaculture has made its greatest advance during the latter part of the 20th century. New species are being cultured, new technologies for more intensive culture have been introduced, large-scale research has been established, and commercial investment is being attracted into aquaculture. Aquaculture is now recognised as a viable and profitable enterprise worldwide. It will presumably continue to grow and supply an increasingly larger percentage of fishery products as time passes (Lovell, 1998).

Aquaculture nutrition research has also developed significantly over the past 35 years, but the nutrition of terrestrial animals has been studied for a far longer time. Robinson (1989) indicated that scientifically, channel catfish nutrition was at a level where domestic animal nutrition was more than 30 years previously. Most of the early fish nutrition research was conducted with salmonid fishes. More recently attention has also been paid to other important species of fish cultured in different parts of the world, as well as new fish species with aquaculture potential (NRC, 1983,1993; Steffens, 1989).

The purpose of fish culture is to increase the weight of fish in the shortest possible time under economically acceptable conditions. A necessary condition is the optimal satisfaction of all physiological metabolic requirements of the organisms, as is attempted for example by the provision of favourable ambient conditions, and feeding with specially formulated diets (Steffens, 1989). As aquaculture technology has evolved, traditional extensive culture, which has been practiced by farmers for generations, is being replaced by semi-intensive and intensive production systems in which there has been a trend toward higher yields and faster growth. This has led to enhancing the natural food available by fertilisation (extensive systems), supplementation of natural food with moist or dry feed materials (semi-intensive

systems), or supplying all the nutrients to the fish in a prepared diet (intensive systems). As the fish become more dependent on prepared feeds, the need for nutritionally complete feeds becomes more critical. Thus, availability of least-cost, well balanced feeds designed for practical production systems and good husbandary practices are fundamental in achieving the expected production goals (Lim, 1994; Lovell, 1998).

The nutritional quality of diets and their adequate presentation is the foundation of fish farming and can largely determine the success or failure of fish husbandry. Nutrition influences behaviour, structural integrity, general health, reproduction, environmental impact and growth in fish (Weatherley and Gill, 1987). Therefore it is necessary to establish more precisely the nutritional requirements of fish, establish knowledge on nutrient bioavailability of various feedstuffs, their availability and cost, and feed technology under cultured conditions. This is in order that nutritionally adequate cost-effective diets can be formulated to maximise growth and also maintain fish in good health.

Generally, fish are more efficient converters of feed to body weight than terrestrial animals, achieving whole body weight food conversion ratios of between 1:1 and 2:1, compared to 2.2:1 for chickens, 3:1 for pigs and 7:1 for beef cattle and sheep (New, 1986). Conversion ratios on an edible flesh / food intake basis are more favourable for fish, being about 2:1 compared to 5:1 for chicken and 20:1 for beef (New, 1986; Lovell, 1998).

A major determinent of successful intensification of aquaculture is feed. It accounts for a major part of the total operation cost of an average fish farm. The performance of a feed is not only dependent on its quality but also on feeding management. Good quality, nutritionally adequate feed can give poor performance unless proper feeding practice (feed allowance,

feeding frequency and method, and daily feeding schedules) are employed (Lim and Poernomo, 1985). Thus, particular attention must be directed towards the development of feeding strategies necessary to obtain economical production and maintain a clean environment.

The continued expansion and improvement in efficiency of aquacultural production requires continued improvements in nutritional formulation and feed technology. Up to the present time more than 300 different species of finfish have been cultivated, all with different feed and nutritional requirements and it is clear that much research has to be carried out in order to achieve a basic knowledge of their nutrition (Watanabe, 1982). However, nutritional studies have demonstrated that any diet must, in order to promote growth, include an energy source, essential amino acids, essential fatty acids, certain vitamins and minerals. The science of aquaculture nutrition is concerned with the supply of these dietary nutrients to cultured animals (Lovell, 1998).

1.2.1 Introduction to Dietary Requirements

Fish require dietary sources of energy and other nutrients for growth, reproduction and health. Growth is characterised primarily by an increase in protein, minerals and water. Energy-yielding nutrients such as lipid and carbohydrate are important to support the growth process, and an adequate supply of vitamins is also required. These nutrients may come from natural aquatic organisms or prepared feed, however, in contemporary aquaculture, prepared feeds from commercial foodstuffs are the primary sources. Thus a familiarisation with the nutrients and their sources, requirements and roles in metabolism are necessary for successful aquaculture (Lovell, 1998).

Growth of fish and feed conversion together with carcass composition are generally affected by species, genetic strain, sex, stage of reproductive cycle, etc., leading to different nutritional requirements. Growth is also greatly affected by quality of diets in terms of nutrient balance, energy content, bioavailability of each nutrient, etc. and environmental conditions. The total requirement for a given nutrient during growth must include the amount needed for maintenance as well as the amount required for the new tissue formed (Jauncey, 1998).

Dietary requirements for energy, protein and amino acids, vitamins, essential lipids and minerals have been established for several fish species of commercial importance. With few exceptions, the nutrient requirements of fish (per unit weight gain or per unit protein gain) are similar to those of monogastric terrestrial animals although energy requirements for fish are lower. Fish are poikilothermic animals and consequently do not have to expend a large proportion of ingested energy in maintaining body temperature in contrast to warm-blooded vertebrates (De Silva and Anderson, 1995). In addition, the primary end-product of nitrogen metabolism, ammonia, is rapidly excreted by passive diffusion through the gills, and consequently fish employ less energy in protein catabolism than do terrestrial animals, which must convert ammonia to non-toxic substances such as urea or uric acid (Brett and Groves, 1979). These two important metabolic differences between these vertebrate groups, according to Tacon and Cowey (1985), contribute to the high energetic efficiency of fish and thus, the absolute difference in requirements of fish and homeothermic vertebrates would reside in their requirements for energy, not protein (Cho and Kaushik, 1985).

The nutritional quality of diets depends upon the levels of available nutrients that have been shown to be needed by fish. This is also greatly affected by the energy value of the diets because fish generally appear to adjust their food intake to satisfy their need for energy. The

available energy level of the diets and the energy requirements of the fish regulate the actual nutrient intake (Grove *et al.*, 1978; Smith, 1989). Not all fish exhibit such effects experimentally, for example Koskela *et al.*, (1998) did not find reduced absolute feed consumption in whitefish, *Coregonus lavaretus* fed higher energy level diets.

Nutrients should be balanced so that the fish will have enough essential nutrients for optimum growth when energy needs are satisfied. Thus, all types of formulated fish diets must satisfy the nutritional requirements of the cultured species in terms of protein (essential amino acids), lipid (essential fatty acids), energy, vitamins and minerals.

1.2.2 Protein

Proteins are the major organic materials in most fish tissue, and most necessarily form an important component of the diet. One of the major requirements of fish culture is the efficient transformation of dietary protein into tissue protein (Weatherley and Gill, 1987). However, protein is essential for normal tissue function, for the maintenance and renewal of fish body protein and for growth. Because of the cost of the protein the feed will be more cost effective if all the protein is used for tissue repair and growth and little catabolised for energy (Jauncey, 1998).

The dietary protein requirements of fish, for maximum growth, appear, as a percentage of the diet, to be much higher than those of terrestrial farmed animals. This is principally an artifact attributable to the relatively lower energy requirements (Cowey and Sargent, 1979; Cowey and Luquet, 1983; Tacon and Cowey, 1985; Jauncey, 1998). Since the protein component is the most expensive major ingredient in an animal feedstuff, some investigators claim that this requirement tends to lessen the advantages of fish as an efficient feed converter (Halver, 1976;

Steffens, 1981). However, when dietary protein requirements are expressed in terms of protein intake rate (grams of protein per kilogram body weight per day) or the weight gain achieved per weight of protein ingested (grams of protein per kilogram live weight gain), the dietary protein requirements of fishes appear similar to those of monogastric terrestrial farm animals (Jauncey, 1998).

1.2.2.1 Quantitative Dietary Protein Requirements

'Optimal growth' is generally used as the criterion for estimating dietary protein requirement for fish (Tacon and Cowey, 1985). The optimal protein requirement has been defined as the minimum amount of dietary protein, expressed as a percentage of diet, needed to supply adequate amino acids and produce maximum growth (NRC, 1993); also as the protein level in the diet which tends to maximise simultaneously growth and protein deposition (Ogino, 1980; Weatherley and Gill, 1987). However, a considerable proportion of dietary amino acids are not used by fish for anabolic processes, but are catabolised as an energy source (Cowey, 1980; 1995). Thus Cowey and Sargent (1979) suggested that a better way of expressing the optimum dietary protein level is in terms of the proportion of energy it contributes (i.e., protein energy: total dietary energy), seeing that protein acts both as a growth nutrient and as an energy source. Similarly, Weatherley and Gill (1987) indicated that protein requirements should be expressed as suggested by Cowey and Sargent (1979) or on the basis of weight gain (grams of ingested protein : Kg live weight gain).

The estimated protein requirements of several fish species are summarised in Table 1.1. In almost all of these studies investigators have utilised various semi-purified and purified diets to estimate the protein requirements of fish. Some of the requirement values listed in Table 1.1

may have been overestimated and also are difficult to compare due to one or more of the of the following reasons:

(1) Generally the level of a nutrient, e.g., protein, that produces maximum growth is defined as the optimum. However, optimum growth does not coincide with maximum protein utilisation.

(2) In many studies, different protein levels were obtained by substitution of protein sources with carbohydrate such as dextrin or starches, the assumption being that the two are isoenergetic for fish. In fact dietary protein, at least for salmonids and probably for many other species, has higher metabolizable energy values than do carbohydrates (Rumsey, 1978). Thus, fish fed higher protein levels may have used protein for energy purposes in a higher proportion than those fish fed the lower protein diets (Cowey, 1995).

(3) Energy requirements are not uniformly expressed (GE, ME or DE), hampering objective comparison. Various investigators have used estimated gross energy (GE) values in formulating their diets (Davis and Stickney, 1978; Jauncey, 1982a; De Silva and Perera, 1985; Daniels and Robinson, 1986; Degani *et al.*, 1989; Khan and Jafri, 1990); other authors used estimated metabolisable energy (ME) values (Garling and Wilson, 1976; Machiels and Henken, 1985; Archdekin *et al.*, 1988; Fagbenro, 1992; Hassan *et al*., 1995); and also estimated digestible energy (DE) values (Winfree and Stickney, 1981; Jantrarotai *et al.*, 1996; Samantaray and Mohanty, 1997)

(4) Most experiments have been carried out on young fish, which should be growing rapidly, and may not extrapolate to larger fish.

Table 1.1 Estimated dietary protein requirement of selected fish species (for maximum growth and expressed as a percentage of the diet)

Species	Initial body wt.(g)	Protein source	Requirement (% dry wt.)	Reference
Tilapia:				
Oreochromis mossambicus	0.5 - 1.0	Fish meal	40	Jauncey (1982a)
Oreochromis niloticus	0.024	Fish meal	28 - 30	De Silva and Perera (1985)
Oreochromis niloticus	0.56	Casein- gelatin	35	Teshima *et al.,* (1985b)
Oreochromis aureus	0.3 - 0.5	Soy/fish meal	36	Davis and Stickney (1978)
Oreochromis aureus	2.5 - 7.5	Casein/albumin	34	Winfree and Stickney (1981)
Tilapia zillii	1.3 - 3.5	Casein	35	Mazid *et al.,* (1979)
Major carp:				
Cirrhinus mrigala	3.0 - 4.0	Casein/gelatin	40	Hassan *et al.,* (1995)
Catla catla	1.0 - 1.5	Casein/gelatin	30-35	Seenappa and Devaraj (1995)
Labeo rohita	0.06- 1.3	Casein	45	Sen *et al.,* (1978)
Common carp:				
Cyprinus carpio	0.01- 7.0	Casein	45	Sen *et al.,* (1978)
Cyprinus carpio	4.0- 10.0	Casein	32	Takeuchi *et al.,* (1979)
Grass carp:				
Ctenopharyngodon idella	0.2	Casein	41 - 43	Dabrowski (1977)
Catfish:				
Clarias gariepinus	40-120	Casein	40	Machiels and Henken (1985)
Clarias gariepinus	10 - 12	Fish meal	40	Degani *et al.,* (1989)
Clarias macrocephalus × *Clarias gariepinus*	2.5	Fishmeal / Soybean meal	40	Jantrarotai *et al.,* (1996)
Clarias batrachus	13-15	Casein / gelatin	40	Khan and Jafri (1990)
Clarias batrachus	26	Fish meal	40	Singh and Singh (1992)
Clarias isheriensis	32	Fish meal	40	Fagbenro (1992)
Heteropneustes fossilis	3.0 – 4.0	Fish meal	39	Sakthivel (1994)
Channel catfish:				
Ictalurus punctatus	200.0	Whole egg protein	32 – 36	Garling and Wilson (1976)
Snakehead:				
Channa striata	10 – 12	Fish meal	40	Samantaray and Mohanty (1997)
Rainbow trout:				
Oncorhynchus mykiss	6.9	Fish meal / Casein/gelatin	40	Satia (1974)
Chinook salmon:		-		
Oncorhychus tschawytscha	1.5 – 5.0		40	Archdekin *et al.,* (1988)
Gilthead sea bream:				
Pagrus aurata	3.0	Casein, fish - protein conc.	40	Sabaut and Luquet (1973)
Red sea bream:				
Chrysophrys major	16 - 45	Casein	55	Yone (1976)
European sea bass:				
Dicentrarchus labrax	5.5	Casein / gelatin	40	Alliot *et al.,* (1979)
Yellow tail:				
Seriola quinqueradiata	65	Sand eel and fish meal	55	Takeda *et al.,* (1975)
Red drum:	52	Menhaden	35	Daniels and Robinson (1986)
Sciaenops ocellatus	46	Meal / Casein	44	
Striped bass:				
Morone saxatilis	2.5	Fish meal and Soy proteinate	55	Millikin (1982)

(5) The protein sources may not contain an adequate balance of essential amino acids.

(6) A restricted or fixed feeding regime may have favoured the growth of those fish fed the higher protein diets, as fish eat to satisfy their energy requirements (Cowey and Sargent, 1979)

(7) Protein digestibility coefficients may vary as different protein sources are employed (Hunt, 1980; Tacon and Cowey, 1985; Wilson, 1985; De Silva and Anderson, 1995).

The optimum dietary protein requirement in fish diets is influenced by fish species, protein quality (digestibility, available essential amino acid profile), dietary protein to energy ratio, the amount of non-protein energy in the diets, the physiological state of the animal (size / age, reproductive state), environmental status (water temperature, salinity, etc.), and the level of food intake (Tacon and Cowey, 1985; Steffens, 1989; Cowey, 1995; Jauncey, 1998).

A fixed feeding regime directly influences the outcome of the observed dietary protein requirement. With tilapia maximum growth was achieved at a level of 25% dietary protein when the feeding rate was 3.5% body weight per day, while for a feeding rate of 2.9% the optimum dietary protein level was 30% (Wang *et al.*, 1985).

The influence of dietary protein to energy ratio and protein digestibility on protein requirements will be discussed in Sections 1.2.4 and 1.2.5 respectively.

1.2.2.2 Qualitative Dietary Protein Requirements

Much effort has been devoted to determining the qualitative amino acid requirements of fish, and for all the fish species so far investigated the same ten amino acids are essential. In alphabetical order: Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan and Valine. These amino acids cannot be synthesised *de novo* by fish and must be therefore supplied pre-formed in the diet (NRC, 1993; Jauncey, 1998; Lovell, 1998).

Quantitative dietary requirements for all ten essential amino acids have been established for only a few fish species, namely chinook salmon, common carp, Japanese eel, tilapia, rainbow trout, chum salmon and channel catfish (NRC, 1993; Jauncey, 1998; Lovell, 1998). The requirements for several amino acids show differences among species.

Amino acid requirements are influenced by interactions among the essential amino acids themselves, between essential and non-essential amino acids, and between amino acids and other nutrients. For example, if cystine is deficient in the diet, it can be synthesised by fish from methionine; the requirement for methionine is therefore partially dependent on the cystine content of the diet. A similar relationship also exists between phenylalanine and the non-essential amino acid tyrosine (Tacon and Cowey, 1985).

1.2.2.3 Dietary Protein Sources

The most appropriate level and source of protein to include in formulated diets is of particular concern. The protein source will inevitably influence the cost and nutritional quality of artificial diets. The protein fraction is typically the most expensive component of diets. The utilization of feeds that contain proteins appropriate to the growth environment, the species

cultured and its stage of development, and which consist of low-cost protein sources, is an essential requirement for developing a cost effective production system (De Silva and Anderson, 1995; Jauncey, 1998).

Historically fishmeal has provided the major portion of the protein in fish feeds. Commercial feed manufacture currently tend to utilize high quality fishmeal as the major portion of the protein source in fish diets. Unfortunately, attempts by nutritionists to replace the fishmeal component of practical fish feeds with alternative sources have met with only variable success. Protein sources which have been considered include meat and bone meal, blood meal, soy bean meal, silk worm pupae, various oil cakes, cotton seed meal, poultry by-product meal, dried brewers yeast, hydrolyzed feather meal, corn gluten meal and fish silage. These proteins have generally been termed secondary protein sources and as such are commonly incorporated at low levels in practical fish diets (Tacon and Jackson, 1985).

Apart from amino acid profiles that are often imbalanced, endogenous anti-nutritional factors and palatability or feed acceptability are among those factors limiting the use of these alternative protein sources, especially plant feed stuffs (Tacon and Jackson, 1985).

However, the increased availability of new processing technologies such as micronisation, extrusion and expansion or the supplementation with limiting essential amino acids of alternative conventional feed ingredients, as well as the use of new, unconventional ingredients as protein sources (single cell proteins, plant protein concentrates, invertebrates and animal and food processing wastes), together with the use of dietary feeding stimulants or attractants, will not reduce the cost of the finished diet (Tacon and Jackson, 1985).

Fishmeal is generally rich in protein (essential amino acids), lipid (polyunsaturated fatty acids), minerals, and vitamins and low in fibre and carbohydrate. Apart from fishmeal there are no animal or plant feed proteins available to the fish diet compounder with an essential amino acid (EAA) profile approximating the dietary EAA requirements of farmed fish. However, fishmeal as a protein source in fish diets is often necessary to avoid nutrient deficiencies and / or to enhance palatability of diets, so ensuring good growth and nutritional performance of the fish.

1.2.3 Protein and Energy

The process of protein synthesis results in an energy demand in addition to the basal metabolic rate. Unless a form of non-protein energy is provided in the diet, an animal has to direct amino acids into energy-liberating pathways in order to provide the energy needed for basal metabolism and growth; this will limit the scope for growth. The alternative energy sources that can be included in the diet to meet these needs are carbohydrate and lipid (De Silva and Anderson, 1995).

The ability or capacity to utilise carbohydrate and lipid for energy varies between species. Inclusion of non-protein energy sources in a formulation may allow reduction in the protein content of the diet. This capacity is called the protein-sparing effect and it is of great interest to those involved in formulating fish diets. The relative prices of ingredients containing high levels of protein, high levels of fat and high levels of carbohydrates are different enough to make the protein-sparing effect of lipid and carbohydrates cost-effective (De Silva and Anderson, 1995).

1.2.3.1 Protein Metabolism for Energy

Metabolism is the term given to the sequence, or succession, of chemical process that take place in the living organism. Some of the process involve the degradation of complex substances to simpler materials and are designated by the general term catabolism. Anabolism describes those metabolic processes in which complex compounds are synthesized from simpler substances. Waste products arise as a result of metabolism and these have to be chemically transformed and ultimately excreted; the reactions necessary for such transformations form part of general metabolism. As a result of the various metabolic processes energy is made available for mechanical work, and for chemical work such as the synthesis of carbohydrates, proteins and lipids (McDonald *et al.*, 1988).

The starting points for metabolism are the substances produced by the digestion of food. Digestion of dietary protein by fish enzymes such as pepsin, trypsin, chymotrypsin and carboxypeptidases, results in the production of free amino acids and peptides in the digestive system, which are then absorbed by passive and active diffusion via the intestinal villi into the blood stream (Weatherley and Gill, 1987; Hepher, 1988). These are then carried to the liver where they may then be used for protein synthesis or may pass into the systemic blood and join the amino acids produced as a result of tissue catabolism in providing the raw material for synthesis of protein and other biologically important nitrogen compounds. Amino acids in excess of this requirement are carried to the liver and broken down to ammonia and keto acids (carbon skeletons). The keto acid may in some circumstances be used for lipid synthesis (lipogenesis), carbohydrate synthesis (gluconeogenesis), amino acid synthesis or to produce energy.

However, the major fate of the keto acids is oxidation to CO_2 and H_2O via the tricarboxylic acid (TCA) cycle, which produces energy (Fig. 1.4) (Cowey and Walton, 1989). The end products of amino acid catabolism, principally ammonia, are eliminated by fish mainly through the gills (60 - 90%), the rest being excreted with urine, faeces and through skin (Cowey and Walton, 1989).

Protein metabolism takes place in many organs of the body, most importantly the liver and muscles. So many catabolic pathways for amino acids are localised in the liver that this organ must be considered the major catabolic site for the body and also most plasma proteins are synthesised in the liver. Although the rate of protein metabolism in muscle may be slower than in the liver, the mass of muscles so much exceeds that of other tissues that it makes this tissue also quantitatively the most important site of protein synthesis. Also, much of the degradation and catabolism of amino acids take place in the muscle (Hepher, 1988).

In contrast, Fauconneau (1985) suggests that analysis of protein synthesis in whole fish shows the overall quantity of protein synthesised by non-muscular tissues is more than twice as high as that synthesised by muscle which represents 40-60% of the body. Consequently, the greater part of dietary protein is used by fish to ensure protein turnover in non-muscular tissues.

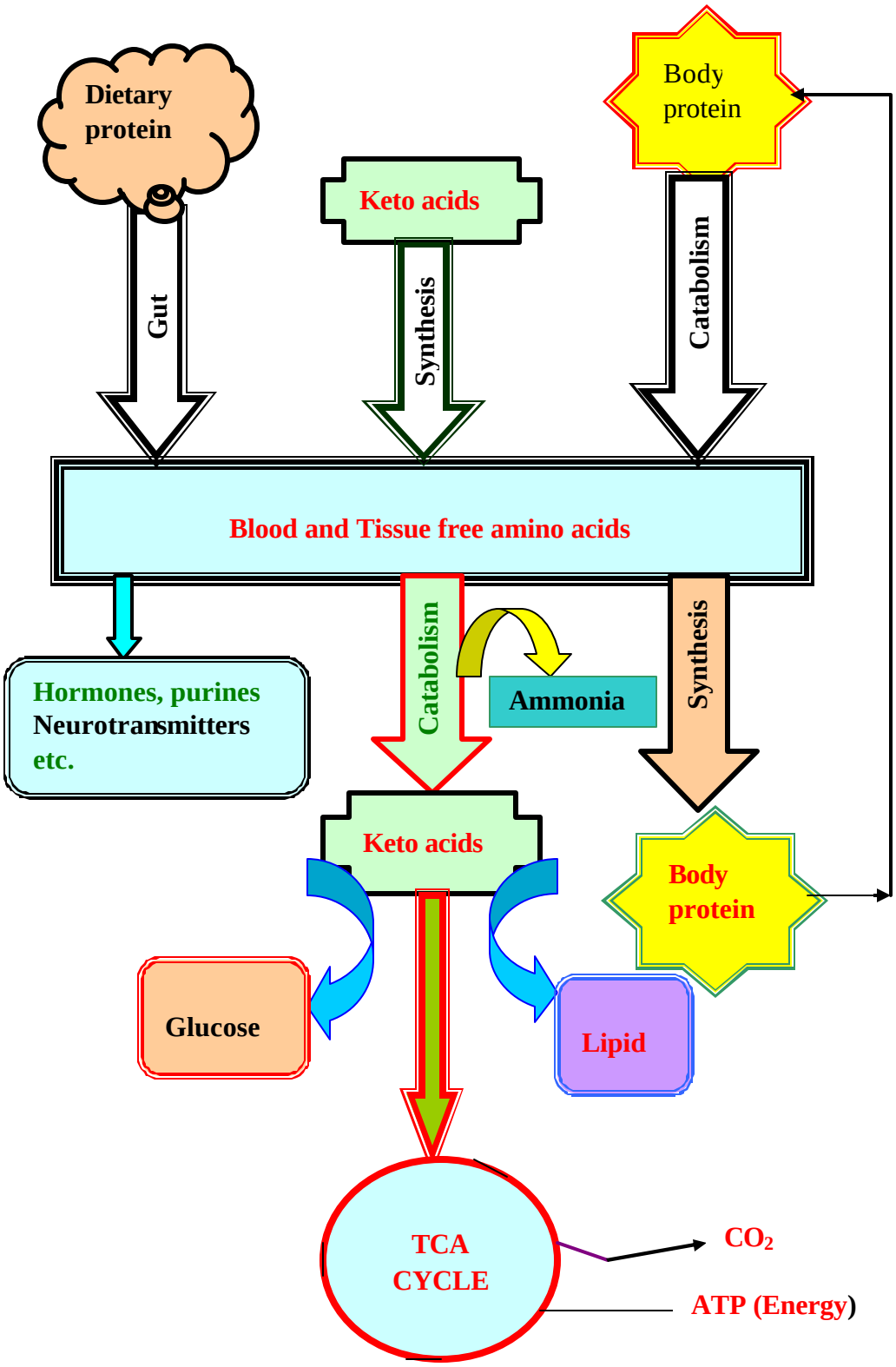

Figure 1.4 Main pathways of protein (amino acid) metabolism (adapted from Cowey and Walton, 1989)

The body pool of free amino acids is relatively small compared with the pool of amino acids held in protein and is derived from three sources; from the diet, from catabolism of body proteins and from synthesis of non-essential amino acids (De Silva and Anderson, 1995). Catabolism of body proteins provides less than 50% of free amino acids in fish (Cowey and Luquet, 1983) and they are therefore highly dependent on dietary sources for free amino acids.

Thus the dynamic state of body protein is achieved through two processes: protein synthesis and protein breakdown. The differences between these two processes are used as a measure of "protein turnover". Mechanisms involved in protein synthesis are similar in fish and in mammals, protein synthesis rates in fish being higher in liver, gill, digestive tract, kidney and spleen than in heart, red muscle and white muscle (Fauconneau, 1985).

The actual proportions of protein anabolised and catabolised depend on the protein requirement of the fish, the content of protein in the diet and the proportions of various amino acids within it, the energy requirement, and the amount of energy available from other sources such as fat and carbohydrate. Amino acid catabolism is favoured by a lack of sufficient energy from dietary carbohydrates and fats. Such diets, even though adequate in proteins, will be utilised for energy (Hepher, 1988).

In animal production regimes, dietary amino acids are utilised for maintenance and growth. From a practical point of view, the ideal situation should tend to maximise the use of dietary protein for growth, minimising the use of proteins for functional protein synthesis, gluconeogenesis, lipogenesis and energy (Lovell, 1998).

If adequate protein is not provided in the diet, there is a rapid reduction in or cessation of growth and a loss of weight due to withdrawal of protein from less vital tissues to maintain the functions of more vital tissues. On the other hand, if too much protein is supplied in the diet, only part of it will be used to make new proteins and the remainder will be catabolised to produce energy (Wilson, 1989; NRC, 1993).

1.2.3.2 Dietary Lipids as Energy Sources

Lipids are an extremely diverse group of compounds many of which function as important sources of metabolic energy. Of all the various types of lipid it is the simple, glycerol based, fats and oils that are of most interest in terms of general nutrition (Jauncey, 1998). Lipids normally occur in foodstuffs and in the fat deposits of most animals in the form of triglycerides, which are esters of fatty acids and glycerol (McDonald *et al.*, 1988).

Thus, dietary lipids provide a source of indispensable nutrients, the essential fatty acids. In addition, they also act as carriers of certain non-fat nutrients, notably the fat-soluble vitamins A, D, E and K and they are also an important source of energy (New, 1986). Lipids contain more energy per unit weight than any other biological compound e.g., one gram of lipid contains almost twice as much total energy as either one gram of carbohydrate or one gram of protein (Jauncey, 1998).

Energy that is not utilised immediately is stored for future use as glycogen and carcass fat. Since glycogen reserves are fish are usually low, the main energy stored is fat. Experiments show that during starvation or food restriction in fish most of the metabolic energy is derived from lipid and, to a more limited degree, protein and carbohydrate (De Silva and Anderson, 1995).

Lipid is digested and metabolised with greater relative ease and so serves as a much better source of energy for protein sparing than carbohydrate. This fact has been widely studied, and many experiments carried out with fish species have shown that by increasing the dietary lipid content, the optimum level of protein can be reduced due to the high effectivity of lipids as "energy-yielding" nutrients (Section 1.2.4). The main protein sparing effect of dietary lipids is to replace protein which would otherwise have been catabolised and used both for energy and to synthesise lipid.

Per unit of energy provided, fat is cheaper than protein thus it makes both nutritional and economic sense to use fat to satisfy the energy requirement of fish and to reserve the dietary protein contents, as much as possible, for growth. There is now increasing evidence to indicate that significant benefits can arise from feeding the correct types and levels of dietary fat. Recently 30-35% lipid in Atlantic salmon diets has been recommended and has given optimum protein utilisation and growth rates with no undesirable gross alterations in carcass composition (BioMar Ltd., 2000). Increased dietary lipid levels have also been reported to increase metabolisable energy, recovered energy, energy retention efficiency and support better protein conversion, indicating improved energy utilisation (Cho, 1987).

The main products of lipid digestion in fish intestines are free fatty acids, most of which contain a single carboxylic acid group (COOH) and a straight unbranched carbon (C) chain. This may in turn contain no double bonds (saturated fatty acids), one double bond (mono-unsaturated fatty acids), or more than one double bond (polyunsaturated fatty acids, PUFA). The latter may be divided into three major families: the oleic (18:1n-9) series, the linoleic (18:2n-6) series, and the linolenic (18:3n-3) series (Tacon, 1990; Jauncey, 1998).

In common with other vertebrates, fish are incapable of "*de novo*" synthesis of fatty acids of the linoleic (18:2n-6) and linolenic (18:3n-3) series. Hence one or both of these fatty acids must be supplied pre-formed within the diet (NRC, 1993; Jauncey, 1998).

With the possible exception of some carnivorous fish species, fish are able to chain elongate and further desaturate 18:2n-6 or 18:3n-3 to the corresponding highly unsaturated fatty acids (HUFA) 20:4n-6 and 20:5n-3 or 22:6n-3, respectively (Watanabe, 1982). In general, freshwater fish require either dietary linoleic acids (18:2n-6), or linolenic acid (18:3n-3) or both, whereas stenohaline marine fish require dietary eicosapentaenoic acid (20:5n-3), and / or docosahexaenoic acid (22:6n-3) (NRC, 1993).

1.2.3.2.1 Lipid Metabolism for Energy

Dietary lipids, mainly in the form of triglycerides, are hydrolysed to three fatty acids and glycerol by pancreatic lipase, aided by the saponifying and emulsifying action of bile acids in the digestive tract. Absorption generally occurs primarily in the anterior ileum including the caecum (Cowey and Walton, 1989). Lipids are transported in the bloodstream either as lipoprotein complexes called very low-density lipoproteins (VLDLs) or as very small droplets called chylomicrons. The triglycerol components of VLDL and chylomicrons are hydrolysed to free fatty acids and glycerol in the target tissues (generally adipose tissue and skeletal muscle) outside of the cell by an enzyme called lipoprotein lipase (LPL). The other source of long chain fatty acid is synthesis (lipogenesis) from acetyl-CoA derived from carbohydrate (glucose), mainly in adipose tissue and the liver (De Silva and Anderson, 1995) (Figure 1.5).

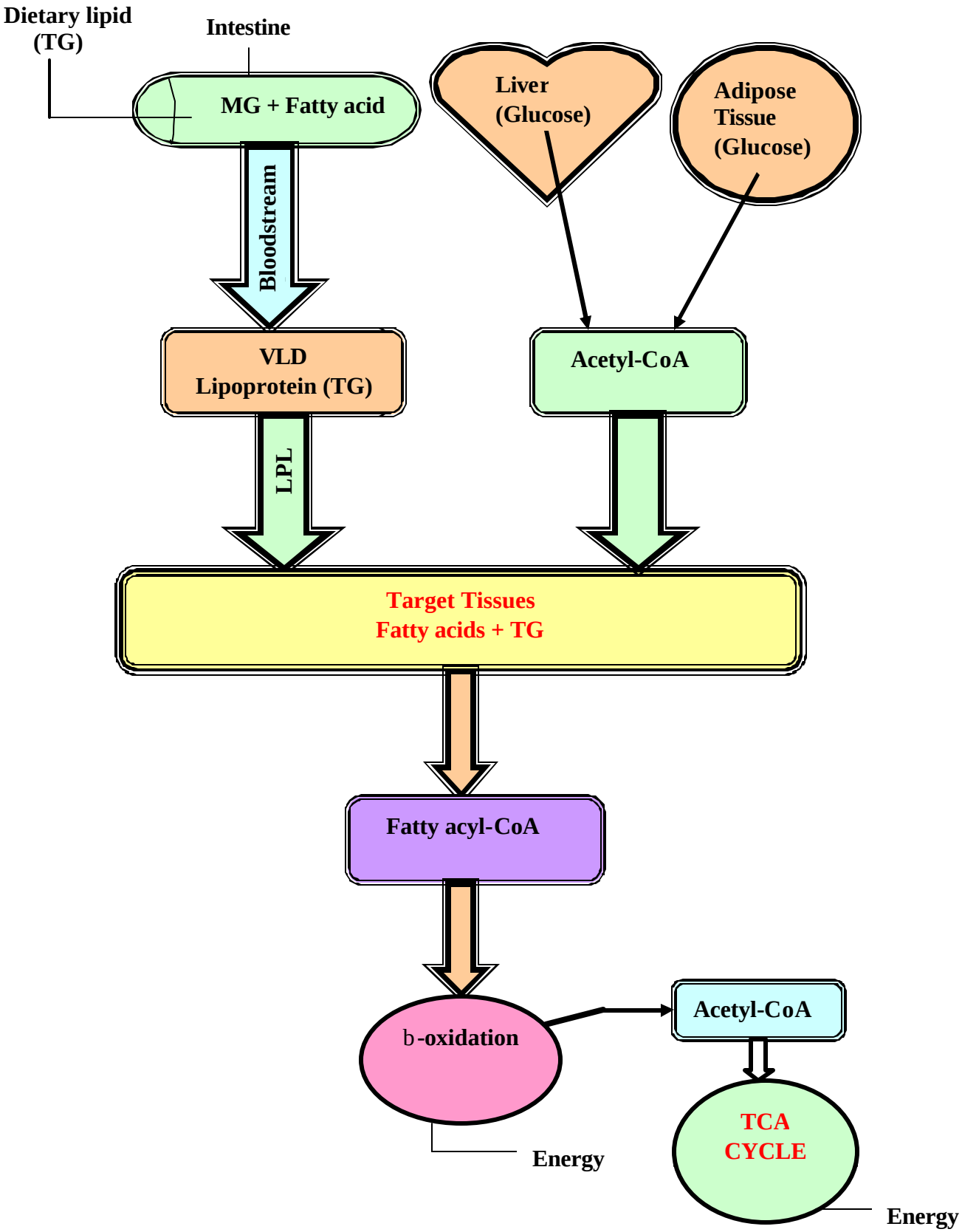

Figure 1.5 Main pathways of lipid (fatty acids) metabolism (MG, monoacylglycerol; TG, triacylglycerol; VLD, very low–density; LPL, Lipoprotein lipase (adapted from Sargent *et al.*, 1989; De Silva and Anderson, 1995)

Fatty acid oxidation in mitochondria leads to the generation of large quantities of ATP by a process called β-oxidation that cleaves acetyl-CoA units sequentially from fatty acid chains. The acetyl-CoA is then oxidised further in the citric acid cyle (TCA cycle) to release energy (Figure 1.5) (De Silva and Anderson, 1995). In the tissues fatty acids may be oxidised to acetylCoA (β- oxidation) or esterified to acylglycerols where as triacylglycerol (fat) they constitute the body's main caloric reserve. Acetyl-CoA formed by β-oxidation has several important fates:

(1) In the case of acetyl-CoA derived from CHO, it is oxidised completely to $CO_2 + H_2O$ via the citric acid cycle. Fatty acids yield considerable energy both in β-oxidation and in the citric acid cycle and therefore are very effective tissue fuel (Lehniger *et al.*, 1993).

(2) It is a source of the carbon atoms in cholesterol and other steroids (Lehniger *et al.*, 1993).

(3) In the liver, it forms ketone bodies, alternative water-soluble tissue fuels that become important sources of energy (glucose) under certain conditions (eg., starvation). Notably, ketone body formations do not generally occur in fishes (De Silva and Anderson, 1995).

In higher vertebrates, and probably also in fish, there are two pathways for body fat deposition: (a) from dietary fats, without modification (the exogenic pathway); (b) *de novo* synthesis of fat from non-fat nutrients derived from various substrates (glucose, pyruvate, acetate). Acetate is an important intermediate metabolite, which serves as a precursor for *de novo* synthesis of higher fatty acids. The hepatic tissue seems to be the major site for fatty acid synthesis from carbohydrates (Hepher, 1988). On a whole-body basis, the liver is a more

important site of fatty acid synthesis than adipose tissue in fish. Overall the fine details concerning the generation of acetyl-CoA for fatty acid synthesis in fish are still not clear (Sargent *et al.*, 1989).

The oxidation of fatty acids plays a prominent role in the provision of energy in fish tissues. In mammals, fatty acid oxidation is carried out mainly in the liver. In fish, however, the red lateral line muscle and heart have similar high capacities to oxidise fatty acids, being much higher than those of white muscle, kidney, or liver (Sargent *et al.*, 1989).

1.2.3.3 Dietary Carbohydrates as Energy Sources

Carbohydrates are an important and inexpensive source of energy-supplying nutrients. Since most fish have the ability to use carbohydrates as an energy source, the introduction of varying levels / sources of carbohydrate into fish feed may lead to a reduction in the cost of formulated diets (Wilson, 1994). Although carbohydrates are 'non-essential' dietary nutrients for fish they should still be included in fish feed for a number of reasons (Jauncey, 1998):

(a) They are the least expensive source of dietary energy (in terms of cost per kJ),
(b) They can, to some extent, 'spare' dietary protein for growth,
(c) They improve the pelletability and water stability of feeds,
(d) They are essential as 'bulking' agents to make formulation add up to 100%.

Since the early work of Phillips and Brockway (1959), carbohydrates, the major source of metabolisable energy in nutrition of mammals and birds, have been considered to be of relatively little value in fish nutrition. Possible reasons why carbohydrates might be utilised with low efficiency may be:

(a) Insufficient enzymatic break-down in the digestive tract

(b) Insufficient absorption of the end products of digestion

(c) Insufficient metabolisability of absorbed monosaccharides

The nutritional value of carbohydrates varies among fish. Warmwater fish are able to utilise much higher levels of dietary carbohydrate than coldwater or marine fish. No specific dietary requirement for carbohydrate has been demonstrated in fish; however, certain fish species exhibited reduced growth rates when fed carbohydrate-free diets. If cabohydrates are not provided in the diets other nutrients such as protein and lipids are catabolised for energy and to provide metabolic intermediates for the synthesis of other biologically important compounds usually derived from carbohydrates (NRC, 1993; Wilson, 1994). However, carbohydrates may serve as precursors for the dispensable amino acids and nucleic acids, which are metabolic intermediates necessary for growth (NRC, 1993). Thus, it is important to provide the appropriate concentration of carbohydrate in the diet of the fish species being cultured.

Dietary carbohydrate levels beyond a certain point have been reported to depress protein digestibility, feed efficiency and growth, increase liver glycogen deposition, liver size, and even cause eventual mortality (Shimeno *et al.,* 1979; Hilton and Atkinson, 1982; Kaushik *et al.,* 1989;). On the other hand, some species seem to have a certain ability to use dietary carbohydrate, with levels in the range 10-40% promoting higher growth and better protein efficiency ratios than diets with no carbohydrates at all (Garling and Wilson, 1977; Alliot *et al.,* 1979; Shimeno *et al.,* 1979; Berger and Halver, 1987).

Fish exhibit varying ability to adapt to and utilise carbohydrate for energy purpose (Buhler and Halver, 1961; Phillips *et al.,* 1966), storing energy reserves as glycogen in the liver and muscle for use in time of metabolic need, and converting excess energy to fat in the body. The utilisation of carbohydrate in fish varies depending on its complexity, source, level in the diet, pre-treatment and degree of gelatinisation. The ability of fish to utilise carbohydrate also differs greatly between species and life stage as a consequence of the marked variations in the anatomy of the digestive tract and in the food habits (Steffens, 1989; Wilson, 1994). It is also thought that herbivorous and omnivorous fish species utilise carbohydrate better than carnivorous fishes (Shimeno *et al.,* 1979; El-Sayed and Garling, 1988). Unlike protein and fat, carbohydrate as a nutrient was not considered essential to fish because of their ability to synthesize carbohydrate metabolites (glucose/glycogen etc.) from excess dietary energy obtained from protein/fat. Compared to the farmed terrestrial animals, the utilisation of dietary carbohydrates in fish is limited, but the inclusion of carbohydrate in fish feeds has certain beneficial effects (Wilson, 1994).

An adequate supply of carbohydrate can minimise the use of expensive protein for energy purposes. The protein-sparing action of dietary carbohydrates for growth in fish feed has been reported for some fish species (Cowey *et al.,* 1975; Shimeno *et al.,* 1993; Shiau and Peng, 1993; Wilson, 1994; Shimeno *et al.,* 1995a; Erfanullah and Jafri, 1995; Seenapa and Devaraj, 1995). However, reports in the literature on the maximum levels of dietary carbohydrates that can be tolerated by different species of fish often appear to be contradictory. This is probably due to the variable digestibility of the different carbohydrate sources used and the fact that not all studies used isoenergetics diets (Martinez-Palacios, 1987).

Thus for elevated levels of dietary protein and energy contents in carp feeds, the large-scale replacement of carbohydrates by fat had negative effects on growth. This might be explained by the fact that carbohydrate is to some extent essential for the formation of oxaloacetate and reduced nicotinamide adeninedinucleotide phosphate ($NADPH_2$) and in this respect can only be replaced by protein not by fat (Steffens, 1989).

The inability of fish to utilise dietary carbohydrate has been illustrated by glucose tolerance tests. Oral administration of glucose to different fish species led to linear increase of blood glucose concentration, with a poor response of plasma insulin levels. This imples that glucose levels in blood are poorly regulated by fish, their response being frequently similar to diabetic mammals (Palmer and Ryman, 1972; Bergot, 1979; Cowey and Walton, 1989).

Other carbohydrates such as fibres, hemicellulose, lignin and pentosans generally form indigestible fractions in the feed, often as pellet binders. Some fish species can tolerate up to 8% of dietary fibre and depressed growth may occur when the fibre content reaches 20% (NRC, 1993; Wang *et al.*, 1985; Davis, 1985). However, small amounts of cellulose (fibre) have a beneficial effect on growth due possibly to the presence of trace elements, partial digestion, and improvement of digestion and absorption of other nutrients by increasing retention time in the gut (Steffens, 1989; D'Abramo and Lovell, 1991).

Thus, the main sources of energy in fish seem to be proteins and lipids, the value of carbohydrates being considered uncertain and probably limited (Jauncey, 1998; Walton, 1987). Typical values of heats of combustion (gross energy) for carbohydrate, protein and lipid are 17.2, 23.6 and 39.5 Kilojoules per gram, respectively (Jobling, 1983; Steffens, 1989; Jauncey, 1998).

Data on the available energy values of various types of carbohydrates from different sources at various levels, although available for some species, are by no means sufficient. Research to determine the optimum levels of digestible carbohydrate in practical fish diets is required. This would allow maximum use of carbohydrates in the diets, thereby reducing feed costs in fish culture (Lim, 1994).

1.2.3.3.1 Carbohydrate Metabolism for Energy

Carbohydrates are absorbed through the wall of the digestive tract. They are absorbed and enter the bloodstream as monosaccharides (i.e. glucose, fructose, galactose, etc.), then they are transported to the liver where the initial processes of metabolism occur. Of the monosaccharides, glucose is the most important, acting as a major metabolic energy source circulating in all vertebrates. Some tissues (e.g. brain) use only glucose as an energy source and so maintenance of blood glucose levels is a very important process. Four pathways exist to assist: glycolysis - the breakdown of glucose liberating energy; gluconeogenesis - the synthesis of glucose from other molecules; glycogen synthesis - the storage of excess glucose in glycogen molecules; and glycogenolysis - the breakdown of glycogen to provide free glucose (De Silva and Anderson, 1995).

Glycolysis is the major route and pentose phosphate pathways the minor route of glucose catabolism in fish tissues (Cowey and Walton, 1989). The utilisation of glucose for energy is carried out in two stages. The glucose is first converted, through a chain of reactions of the Embden-Meyerhoff-Parnas glycolytic pathway (glycolysis), into pyruvic acid. These processes are carried out in the cell cytoplasm and do not require oxygen. The second stage is carried out through another series of reactions of the Krebs or tricarboxylic acid cycle (TCA cycle), in the cell mitochondria. In these processes pyruvate is converted to acetyl coenzyme

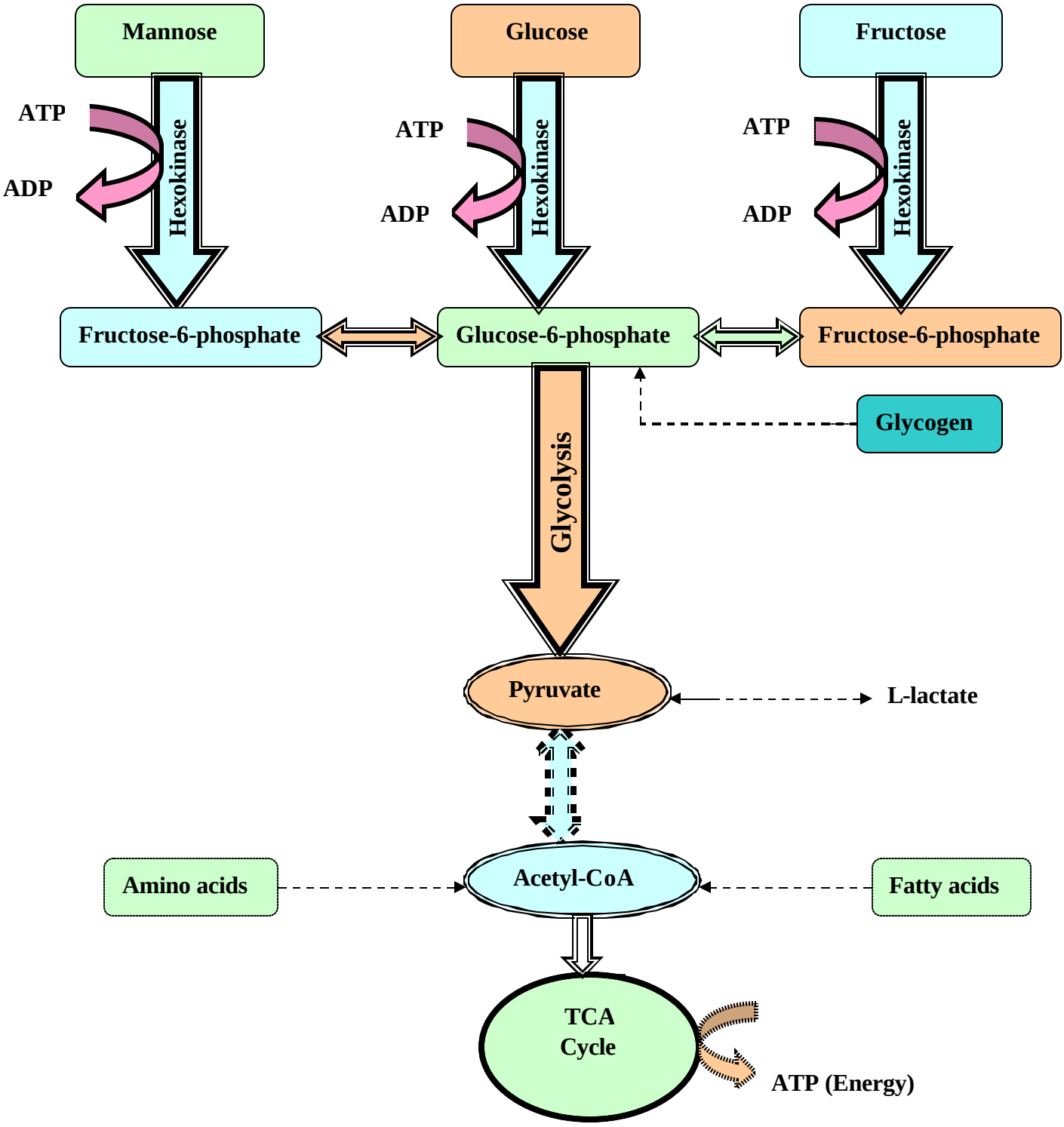

Figure 1.6 Main pathways of carbohydrate metabolism
Underline compounds – dietary precursor; heavy lines – main process direction; broken lines – process includes intermediate metabolites (adapted after Hepher, 1988)

A, which is further oxidised to carbon dioxide and water, producing reduced nicotinamide adenine dinucleotide ($NADH\text{-}H^+$) and flavin adenine dinucleotide ($FADH_2$), which through oxidative phosphorylation in the cytochrome system converts adenosine diphosphate (ADP) into the energy-rich adenosine triphosphate (ATP) (Figure 1.6). The processes of oxidative phosphorylation are aerobic and require oxygen (Hepher, 1988).

The first portion of the Embden-Myerhoff reaction (glycolysis) yields eight moles of ATP per mole of glucose catabolised. Together with the final part of this pathway (Krebs or TCA cycle) one mole of glucose results in 38 moles of ATP (the high-energy molecule of cells). (Krebs and Kornberg, 1957; Axelrod, 1967). Figures 1.6 gives a schematic representation of the main energy pathways.

Although glycogen (the storage form of carbohydrate) is the major energy source (through glycolysis) in fish white muscle during peak activity, fish liver and muscle tissues store little glycogen (less than 1% of wet tissue) (Cowey and Sargent, 1979). Fish do not mobilise liver glycogen stores during starvation. In fact it has been frequently stated that carbohydrate metabolism in fish resembles that of diabetic terrestrial animals (Jauncey, 1998).

Apart from dietary glucose and products of enzymatic hydrolysis of dietary starch and other carbohydrates, for example sucrose, metabolic glucose may be derived from stored liver glycogen or other substrates, for example protein, in most vertebrates, including fish (Cowey and Sargent, 1972; Cowey and Walton, 1989). The process of mobilisation of glucose monophosphate from glycogen is termed glycogenolysis, whilst the reaction sequences which result in the production of glucose or glucose monophosphate from amino acid substrates are collectively termed gluconeogenesis (Cowey and Sargent, 1972; Newsholme and Start, 1979).

Liver glycogen reserves are particularly depleted after night starvation in mammals, while they are scarely altered in fish even after several months of starvation (Chang and Idler, 1960; Dave *et al.*, 1975). It is probable that the energy demand of tissues such as brain and nervous tissue, which catabolise glucose, are met in fish by gluconeogenesis (from amino acids and lipids) rather than by glycogenolysis (Walton and Cowey, 1982). It also seems that the energy source of starved fish must be tissue lipid and protein transformed into energy through gluconeogenesis, rather than glucose or glycogen (Hepher, 1988).

Shimeno *et al.*, (1993, 1995a) observed an effect of carbohydrate to protein ratios and carbohydrate to lipid ratios on intermediary metabolism in carp and tilapia respectively. They suggested that as dietary carbohydrate levels increase, this accelerates glycolysis and lipogenesis and decelerates gluconeogenesis and amino acid degradation in the hepatopancreas and the liver.

1.2.4 Protein and Energy Ratio

Although the utilisation of proteins for basal energy metabolism is a well-established phenomenon, conventional "energy-yielding" nutrients like fats and carbohydrates can reduce the oxidation of protein to satisfy the energy needs of fish and thus improve the utilisation of dietary protein (Cho and Kaushik, 1985). The percentage of the total dietary energy required from either protein or non-protein ingredients may be used to express the relationship between protein and energy in the diet.

Evidence of the use of dietary protein as an important energy source is widespread (Cowey, 1979, 1980; Wilson, 1989), and it has been suggested that the high protein requirement is an artefact of the low energy requirement in fish (Section 1.2.2). In addition, if the dietary protein to energy ratio is unbalanced so that non-protein energy is inadequate, dietary protein may be catabolised and used as an energy source to satisfy maintenance before growth (Cowey and Sargent, 1979; Shepherd and Bromage, 1988; NRC, 1993). In contrast, excessive dietary energy can reduce feed consumption and thus lower the intake of the necessary amount of protein and other essential nutrients for maximum growth. Excessively high ratios of energy to nutrients can also lead to deposition of large amounts of body fat, which can be undesirable in food fish (NRC, 1993; Jauncey, 1998).

Equally important is that the inclusion of appropriate levels of non-protein energy sources in the diet determines the efficiency of protein utilisation (Steffens, 1981; Wilson and Halver, 1986). Any imbalance with respect to non-protein energy sources and / or their levels of inclusion may have a direct effect on growth, conversion efficiencies, nutrient retention, and body composition. It is thus imperative to determine the optimum dietary lipid to carbohydrate ratio that produces maximum growth.

As the use of protein as an energy source is wasteful from the both nutritional and economical points of view, it seems worthwhile to supply as much as possible of the required non-protein energy as carbohydrates and lipids rather than protein and thus reduce the proportion of protein in the diet to the level needed for growth (NRC, 1993).

The beneficial effects of the incorporation of protein-sparing nutrients have been widely studied and optimal ratio between protein and energy (P/E ratio) have been proposed for some species of fish. The most favourable or optimal P/E ratios (in mg of protein / kJ of energy) in the diets for selected fish species are shown in Table 1.2. These results are based on experiments where dietary lipid was the main source of non-protein energy and fish were fed diets containing a range of dietary protein levels (in all of them but in those from Takeuchi *et al.*, 1978b; Watanabe *et al.*, 1979; where all diets contained a fixed dietary protein level), combined with a range of dietary lipid levels. In all the experiments shown in Table 1.2 the energy content of the diets was calculated using typical nutrient energy contents, except in those by Daniels and Robinson (1986), where calorimetry was used. Digestible energy values were determined by separate digestibility trials in the work by Wang *et al.*, (1985).

It appears from these data that there are considerable differences between species and, in general, the requirement of the fish for protein per unit energy intake (digestible or metabolizable) lies well above 20, and is greater than that of terrestrial animals (14-18 for chicken, 10-16 for swine and 6-10 mg of protein per Kilojoule for cattle) (Cho and Kaushik, 1985).

Table 1.2 Optimum protein : energy ratios (P/E ratios) in the diets for various fish species. (CP = Crude protein; GE = Gross energy; DE = Digestible energy; ME = Metabolizable energy, as the basis of calculation)

Species	Initial body wt.(g)	P/E ratio (mg P. kJ^{-1})	Crude protein (CP, %); Energy ($kJ.g^{-1}$) as the basis of calculation	Reference
Tilapia:				
Oreochromis aureus	2.5	29	56% CP ; 19.2 $kJ.g^{-1}$ DE	Winfree and Stickney (1981)
Oreochromis aureus	7.5	26	34% CP; 13.4 $kJ.g^{-1}$ DE	Winfree and Stickney (1981)
Oreochromis niloticus	6.0	16-17	30% CP; 19.6 $kJ.g^{-1}$ DE	Wang *et al.*, (1985)
Oreochromis niloticus	12.0 mg	26	45% CP; 16.7 $kJ.g^{-1}$ GE	El-Sayed and Teshima (1992)
O.niloticus × *O. aureus*	1.6	25	24% CP; 10.8 $kJ.g^{-1}$ ME	Shiau and Huang (1990)
Major carp:				
Cirrhinus mrigala	3.0 - 4.0	26.6	40% CP; 15.0 $kJ.g^{-1}$ ME	Hassan *et al.*, (1995)
Common carp:				
Cyprinus carpio	6.0-20.0	20-25	32-37% CP; 14 $kJ.g^{-1}$ DE	Watanabe *et al.*, (1987)
Cyprinus carpio	3.0	24	34% CP; 14 $kJ.g^{-1}$ DE	Murai *et al.*, (1985)
Cyprinus carpio	4.0-10.0	21-23	32% CP; 12.2 $kJ.g^{-1}$ DE	Takeuchi *et al.*, (1979)
Catfish:				
Clarias gariepinus	1.0-14.0	26-29	40-42%CP;14-16$kJ.g^{-1}$DE	Uys (1989)
Clarias gariepinus	40-120	31	40% CP; 13 $kJ.g^{-1}$ ME	Machiels and Henken (1985)
Clarias batrachus	13-15	25	40% CP; 16 $kJ.g^{-1}$ GE	Khan and Jafri (1990)
Clarias batrachus	-	23-31	40% CP; 13-17 $kJ.g^{-1}$ GE	Patra and Ray (1988)
Clarias isheriensis	32	28-31	37-40%; 13 $kJ.g^{-1}$ ME	Fagbenro (1992)
Hybrid catfish:				
Clarias macrocephalus × *Clarias gariepinus*	2.5	34.0	40% CP; 11.7 $kJ.g^{-1}$ DE	Jantrarotai *et al.*, (1996)
Clarias macrocephalus	2.0	25.7	35% CP; 13.6 $kJ.g^{-1}$ DE	Jantrarotai *et al.*, (1998)
× *Clarias gariepinus*	4.0	34.7	40% CP; 11.52 $kJ.g^{-1}$ DE	Jantrarotai *et al.*, (1998)
Channel catfish:				
Ictalurus punctatus	60	20	26% CP; 12.9 $kJ.g^{-1}$ DE	Li and Lovell (1992)
Ictalurus punctatus	200	21-28	32-36% CP; - $kJ.g^{-1}$ ME	Garling and Wilson (1976)
Snakehead:				
Channa striata	10 - 12	21.7	40% CP; 18.4 $kJ.g^{-1}$ DE	Samantaray and Mohanty (1997)
Rainbow trout:				
Salmo gairdneri	15	19	35% CP; 18 $kJ.g^{-1}$ DE	Takeuchi *et al.*, (1978b)
Salmo gairdneri	15	18	35% CP; 18 $kJ.g^{-1}$ DE	Watanabe *et al.*, (1979)
Oncorhynchus mykiss	10-15	22	33% CP; 15 $kJ.g^{-1}$ DE	Cho and Kaushik (1985)
Red drum:				
Sciaenops ocellatus	52	21	35% CP; 17 $kJ.g^{-1}$ GE	Daniels and Robinson (1986)
Gilthead sea bream:	46	26	44% CP; 17 $kJ.g^{-1}$ GE	Daniels and Robinson (1986)
Sparus aurata	3.0	23	GE	Kissil and Groop (1984)
Striped bass:				
Morone saxatilis	11-16	28	52% CP; 19 $kJ.g^{-1}$ ME	Berger and Halver (1987)
Yellow tail:				
Seriola quinqueradiata	65	34	55% CP; 16 $kJ.g^{-1}$ ME	Takeda *et al.*, (1975)
Seriola quinqueradiata	89	38	57% CP; 15 $kJ.g^{-1}$ ME	Shimeno *et al.*, (1985)

Hybrid tilapia *Oreochromis niloticus* × *O. aureus* showed satisfactory growth performance when the dietary protein content was decreased from 24 to 21% and dietary lipid raised from 9 to 15% (Shiau and Huang, 1990). Hybrid *Clarias* catfish *Clarias macrocephalus* × *C. gariepinus* also showed a protein-sparing effect when a decrease from 35 to 40% in dietary protein gave similar performance when lipids were increased from 11 to 15% (Jantrarotai *et al.,* 1998).

A similar protein-sparing effect was reported for channel catfish (Garling and Wilson, 1976), when a decrease from 36 to 24% in dietary protein gave similar performance when lipids were increased from 5 to 10%. Dietary protein level could be reduced from 50 to 45% when lipid level was increased from 14 to 17% in striped bass (Berger and Halver, 1987).

In some experiments carried out with rainbow trout reported in Table 1.2, dietary protein level could be reduced to 35% with little effect on growth performance by adding lipid to the experimental diets at 18% (Takeuchi *et al.,* 1978; Watanabe *et al.,* 1979). All works listed showed some protein-sparing effect of dietary lipid. A similar protein-sparing effect was reported for yellowtail (Takeda, 1975; Shimeno *et al.,* 1985), although the minimum optimum dietary protein level achieved ranged between 55 and 57% given dietary lipid contents of 15-17%. The protein-sparing action of dietary lipid seems well documented, proving that in many cases the optimum level of dietary protein can be reduced at higher lipid inclusion rates. The optimum levels (i.e., those which support the best growth rate and food conversion) of protein and lipid in a fish diet, depend on many factors such as the environmental conditions (temperature probably being the most important), fish age and size, genetic factors and the aquaculture system / techniques being used, among others (New, 1986).

The specific requirements for protein and energy in a particular fish species and size must be considered. The ratio of protein to energy in the diet for individual species is predictable only on the basis of diets with optimal protein and energy content. The rate of feed supply also exerts a quite decisive influence (Steffens, 1989).

1.2.5 Nutrient Digestion and Digestibility

Digestibility is the quantification of the digestive processes. Digestibility gives relative measures of the extent to which ingested food and its nutrient components have been digested and absorbed by the animal. Part of the food consumed by fish will pass through the gastrointestinal tract without being digested and absorbed, i.e., part of the ingested food will be lost as faeces. If digestibility is high then faecal losses will be low and *vice versa* (De Silva and Anderson, 1995).

The nutrient content of a diet yields no information on whether or not the nutrients are made "available" to fish through the digestive process (Cho and Kaushik, 1985). Digestive processes basically involve the mechanical and chemical breakdown of food particles and their nutrients to provide the animal with the simple forms of such essential nutrients as amino acids, fatty acids, and glucose and also fulfil a major dietary function in supplying digestible energy which is fuel for metabolic activity (McDonald *et al.*, 1988).

In most carnivorous fish the digestion of food commences in the stomach. The gastric mucosa of the stomach secretes protective mucus, plus pepsin, a protease. The glands of the stomach produce hydrochloric acid, which maintains the pH of the stomach contents within the optimal range for pepsin activity (pH 2 to 4). Protein digestion proceeds in the intestine in an alkaline

medium by the action of pancreatic trypsin and chymotrypsin. Trypsin is one of the most important proteases for protein digestion, and polypeptides are further broken down to peptides and free amino acids by intestinal peptidase and pancreatic carboxypeptidase (Weatherley and Gill, 1987).

Pancreas and liver are concerned with the production of those digestive enzymes, which include carbohydrases, which convert oligo and polysacharides into simpler sugars and lipases to hydrolyse triglycerides (lipid) into diglycerides, monoglycerides, glycerol and free fatty acids (Shepherd and Bromage, 1988). Enzymes for carbohydrate digestion are apparently present in fish. The enzymes for the major carbohydrate metabolic pathways, such as glycolysis, tricarboxylic cycle, pentose phosphate shunt, gluconeogenesis, and glycogen synthesis, have been demonstrated (Shimeno, 1974; Cowey and Walton, 1989). Amylase is the enzyme responsible for the hydrolysis of starch into glucose. Spannhof and Plantikow (1983) have observed a positive correlation between intestinal α-amylase levels, and dietary carbohydrate concentration (about 0-30%) in rainbow trout, *Salmo gairdneri*. They also concluded that high dietary starch concentration tended to inhibit intestinal α-amylase activity.

The dietary nutrients, broken down into simple compounds in the digestive tract, are absorbed through the intestinal epithelium by diffusion or by active transport. The waste material, or faeces, voided from the intestine via the anus consists mainly of water, undigested food residues, digestive secretions, epithelial cells from the gastrointestinal tract, inorganic salts and bacteria (McDonald *et al.*, 1988).

1.2.5.1 Protein Digestibility

Proteins in most feedstuffs that have been properly processed are highly digestible to fish. The digestion coefficients for protein in protein-rich feedstuffs are usually in the range of 75 to 95 percent. Protein digestibility tends to be depressed as the concentration of dietary carbohydrate increases (NRC, 1993). On the other hand, food composition seems to more significantly affect the level of protein digestibility. Thus, increased amounts of dietary lipids have produced increased protein digestibility (Takeuchi *et al.*, 1978a; Watanabe *et al.*, 1979). In rainbow trout high levels of starch reduced the protein digestibility (Kitamikado *et al.*, 1964), and the higher the protein content and the lower the carbohydrate content are, the higher is apparent protein digestibility of compound feedstuffs. This seems to be true for most canivorous fish (Shimeno *et al.*, 1979; Kaushik *et al.*, 1989) and some herbivorous fish (Page and Andrews, 1973). The negative effect of indigestible crude fibre has been repeatedly reported for many fish species (Jobling, 1981; Hilton *et al.*, 1983; Wang *et al.*, 1985; Ferraris *et al.*, 1986).

1.2.5.2 Lipid Digestibility

Digestibility of lipids ranges from 85 to 95% in most fish species (NRC, 1993). Long-chain fatty acids exhibit a higher digestibility than short-chain ones. Polyunsaturated fatty acids such as 20:5 or 22:6 acids are up to 100% digested by rainbow trout (Austreng *et al.*, 1980) and, in general, the essential PUFA show a very high digestibility in this fish species (Ellis and Smith, 1984). Most of the time, this high digestibility of lipids is little affected by their inclusion level in the diet or diet composition. Thus, for rainbow trout lipid digestibility coefficients range between 93 and 98% (Higuera *et al.*, 1977; Takeuchi *et al.*, 1978a). For carp these values oscillate between 83 and 90% (Takeuchi *et al.*, 1979; Kirchgessner *et al.*, 1986), between 64

and 94% for channel catfish (Andrews *et al.*, 1978), and around 93% for tilapia (*O. niloticus*) (Hanley, 1987).

1.2.5.3 Carbohydrate Digestibility

Source or type, dietary level, and heat treatment affect the digestibility of carbohydrates in fish (Wilson, 1994). Considerable differences in carbohydrate digestibility between the various fish species can be expected as a consequence of the marked variations in the anatomy of the digestive tract and in the native diet (Steffens, 1989). A factor, which has a major effect on carbohydrate digestibility in fish, is the degree of polymerisation. The monosaccharides are well absorbed by fish, while dextrin is only moderately digestible and crude starches have comparatively low digestibilities (NRC, 1983; Singh and Nose, 1967). In addition, there may also be antinutrients found in natural feedstuffs which can inhibit the action of some hydrolases, as for example the amylase inhibitor found in raw wheat (Tacon, 1985).

For salmonids the carbohydrate digestibility diminishes with increasing molecular weight (99% for glucose, 22% for polysaccharides) (Singh and Nose, 1967). The digestibility of polysaccharides is dependent on the magnitude of their contribution to the diet. Dextrin and starch are digested less well as their proportion in the diet increases, and cooked starch is significantly better digested than raw starch in rainbow trout (Buhler and Halver, 1961; Singh and Nose, 1967; Smith, 1971). Other carbohydrates, such as fibre, are considered indigestible fractions in the feed (NRC, 1993). However, a complete absence of crude fibre must be regarded as unfavourable (Furuichi *et al.*, 1983), as crude fibre delays absorption of carbohydrate and protein, and nutrients can be more effectively employed for energy production and biosynthesis. Fibre may also improve the pelletability of feed (Jauncey and Ross, 1982).

1.2.5.4 Factors Affecting Digestion of Food in Fish

As for any metabolic process, digestibility is expected to be influenced by both biological and environmental factors. However, unlike the influence of biological and environmental factors on metabolic activities such as respiration, that on digestibility is less well defined and little understood. To complicate matters, digestibility varies from day to day, even when all environmental factors are kept unchanged and when fed on the same diet. This variation was not found to be correlated to consumption (De Silva and Anderson, 1995).

Digestion of food in fish depends on the following three main factors: (1) the ingested food and the extent to which it is susceptible to the effect of the digestive enzymes; (2) the activity of the digestive enzymes; (3) the length of time the food is exposed to the action of digestive enzymes. Each of these main factors is affected by a number of secondary factors, such as fish species (carnivorous versus omnivorous / herbivorous species), age, size and physiological condition. Other factors are related to environmental conditions such as water temperature; and some are related to the food itself, i.e., its composition (the relative proportion of nutrients in the diet), particale size, feeding rate and amount eaten (Hepher, 1988; Page and Andrews, 1973).

1.2.5.4.1 Fish species

Nutrient digestibility may vary among fish species due to differences in the digestive system and its digestive enzymes, and to the different foods consumed. Despite these differences and the lack of pepsin in the fish without stomachs, variations in the digestibilities of proteins and lipid among species are small. Much more pronounced variations are found in digestibility of carbohydrates, especially those with high molecular weight (Hepher, 1988).

1.2.5.4.2 Fish age and size

Enzymatic activity may vary with fish age and size, proteolytic and amylolytic activities are usually lower during first development stage of fish than in later stage. This clearly affects digestibility of different nutrients (Hepher, 1988).

1.2.5.4.3 Physiological conditions

Stressed fish, due either to excessive handling or to disease, may have disturbed digestibility. Job (1977) stated that tilapia (species not given) which were fished in natural water bodies and transferred into tanks showed increased defaecation until acclimated. A long period of starvation may also affect enzyme secretion and digestibility. Seasonal variations in digestive enzyme activity may also occur (Hepher, 1988).

1.2.5.4.4 Water temperature

Increasing the temperature may increase both enzyme secretions and enzyme activity, and decrease enzyme-substrate affinity (Nordlie, 1966; Smith, 1967; Trofimova, 1973). Temperature may also affect the rate of absorption of digested nutrients through the intestinal wall (Smith, 1970; Smith and Kemp, 1971). However, the higher the temperature, the more rapid is the transport of food and the shorter its exposure time to the digestive enzymes (Elliot, 1972; Ross and Jauncey, 1981). Cho (1987) found no effect of water temperature variation on diet digestibility for rainbow trout.

1.2.5.4.5 Water salinity

Not much is known of the effect of salinity or other factors related to water composition on digestibility. MacLeod (1978) found that the digestibility of dry matter, energy and protein by

rainbow trout fell linearly with increasing water salinity, but this could be due to an indirect stress effect (Hepher, 1988).

1.2.5.4.6 Food composition

The relative proportions of nutrients in the diet may affect their digestibilities, and increased amounts of dietary lipids are known to support increased protein, carbohydrate, lipid and energy digestibility (Takeuchi *et al.,* 1978a; Watanabe *et al.,* 1979). Relatively high carbohydrate contents in the diet have been reported to reduce protein digestibility (Shimeno *et al*., 1979; Kaushik *et al.,* 1989). The explanation seems to be that the undigested portion of the carbohydrates passes more rapidly through the alimentary canal, carrying with it some of the proteins (Hepher, 1988). Other carbohydrates, such as fibres, generally form the indigestible fraction in the feed (NRC, 1993). Various feeds may also contain digestive enzyme inhibitors which reduce digestibility (NRC, 1993). Food particle size is generally positively correlated with lower digestibility values (Hepher, 1988).

1.2.5.4.7 Feeding rate and frequency

According to Jobling (1994), the efficiency with which food is digested and absorbed can be affected by feeding rate. An increase in the rate of ingestion may result in reduced digestibility. Henken *et al.,* (1985) found that apparent digestibility of protein (and dry matter) by African catfish (*Clarias gariepinus*) was negatively correlated with feeding level. On the contrary, other works suggest that there is no direct effect of feeding rate on digestibility (Cho and Kaushik, 1990; Windell *et al.,* 1978). Feeding frequency also does not seem to affect digestibility greatly (Hundon and De La Noue, 1984).

1.3 Catfish, *Clarias* species

Catfish of the genus *Clarias* are widely distributed in Africa and Asia and have been the focus of long-term aquaculture interest. A variety of species of the genus *Clarias* and their hybrids is cultured, for reasons of their high growth rate, disease resistance and amenability to high density culture, related to their air-breathing habits (Haylor, 1993; Huisman and Richter, 1987). The principal cultured species in this group are *Clarias gariepinus, Clarias batrachus, Clarias macrocephalus, and Clarias aguillaris.* Alongside a variety of historical and socioeconomic reasons *Clarias* catfish have a relatively unique piscine physiology that renders them very promising aquaculture candidates.

1.3.1 The African Catfish, *Clarias gariepinus*

The African catfish, *Clarias gariepinus* (Order: *Siluriformes*, Family: *Clariidae*), is a freshwater eurytopic species. The superior performance of *Clarias gariepinus* compared to other *Clarias* species in terms of growth rate has probably contributed to fact that *C. gariepinus* has been widely introduced to areas outside its natural range (Verreth *et al.,* 1993). It has a sedentary life style and lives in a wide variety of habitats from temperate to tropical streams, rivers, pans, swamps, underground sinkholes, shallow or deep lakes (Uys, 1989), ponds, submerged rice-fields and impoundments. Ecological studies and studies in ponds (Bruton, 1979; Mbewaza-Ndawula, 1984; Uys, 1989) have shown than juvenile *C. gariepins* feed in decreasing order of preference on insects and crustacea, molluscs, detritus and plankton. Subadults and adults feed mainly on fish. *C gariepinus* can vary its food according to availability (Clay, 1979) and the species is thus considered an opportunistic omnivore (Uys, 1989).

Culture of African catfish, *Clarias gariepinus* has received considerable attention since the early 1970s and 1980s (Micha, 1972; De Kimpe and Micha, 1974; Richter, 1976; Clay, 1977, 1979, 1981) with all these studies confirming the considerable culture potential of the species. African catfish is a suitable alternative to tilapia in subsistence fish farming in Africa and using low grade feed composed of some local agricultural by-products, the yields of catfish from ponds could be as much as 2.5 times higher than those of tilapia (Hogendoorn, 1983a). Hogendoorn (1983a) summarised the attributes of the species for culture:

- It matures and is easy to reproduce in captivity,
- It grows fast and efficiently,
- It tolerates high densities,
- It is hardy, and
- It survives in adverse water quality conditions.

From the biological and socioeconomic points of view, the African catfish is highly suitable for aquaculture, with good prospects for both developing and developed countries.

At present this species is cultured on a commercial and subsistence basis in at least twelve African countries, the most important of which, in terms of tonnage produced, are Mali, Nigeria, Ethiopia and Ghana (FAO, 1997). Among Asian countries it is farmed mainly in Thailand, the Philippines, China, Malaysia and Indonesia. Recently countries such as Bangladesh, India and the Czech Republic have been begun to farm the species on both an extensive and intensive basis. In Europe, it has been cultured in the Netherlands, Germany, Belgium (Verreth *et al.*, 1993) and in Latin America in Brazil (Hecht *et al.*, 1996). Research activities, experimental and commercial culture have been widely undertaken throughout Africa as well as in Asia - China, Thailand, India and Bangladesh and Europe - the Netherlands and Scotland (Haylor, 1992).

Despite considerable research effort and the availability of well developed technical knowledge in different fields of African catfish culture systems, total production in 1995 (39,218 MT) was very low in terms of world freshwater fish production (18,145,100 MT). It accounts for less than one fifth of total Clariid catfish production (200,294 MT) (FAO, 1997).

1.3.2 Nutrition of African Catfish, *Clarias gariepinus*

Initial research on African catfish, *Clarias gariepinus* (Burchell, 1822), nutrition was mainly carried out in the Netherlands, S. Africa and Israel (Hogendoorn *et al.*, 1983; Hogendoorn, 1983b; Henken *et al.*, 1986; Machiels and Henken, 1985, 1986; Uys, 1989; Degani *et al.*, 1988, 1989). Since then, an increasing amount of work has been done covering several aspects involved in the nutritional requirements of this species.

Verreth *et al.*, (1992) investigated gut morphology and development of functional digestive enzymes in *C. gariepinus* larvae. The anatomy of the digestive tract, as well as the activity of some enzymes involved in digestive system of adult *C. gariepinus*, has also been studied (Uys *et al.*, 1987; Uys and Hecht, 1987; Uys, 1989). Uys and Hecht (1987) reported that protein digestion in *C. gariepinus* occurs mainly in the stomach and foregut; that starch digestion occurs mainly in the foregut, and that the pancreas plays an important role in digestive enzyme secretion. The secretory digestive enzyme response in *C. gariepinus* is relatively faster than in carp. Uys (1989) found that gastric, pancreatic and foregut protease activity started rising after 1 hour and had a peak 4 hours after meal ingestion. In addition, no evidence could be found for an inherent rhythmic cycle in digestive enzyme activities. According to the same author, changes in digestive enzyme activities appeared to be induced solely by food intake. In another study on the digestive enzymes, Uys *et al.*, (1987) concluded that *C. gariepinus* is physiologically equipped to utilise infrequent and irregular meals effectively, since *C.*

gariepinus has no intrinsic digestive enzyme cycle, and since it has a relatively rapid digestive enzyme secretory response.

Reports in the technical literature indicate that the optimum level of protein in feeds for growth of *Clarias gariepinus* ranges from 40 to 42% (Section, 1.2.2.1; Tables 1.1 & 1.2). Energy levels range from 11 to 16 kJ GE/g, resulting in protein-energy (P/E) ratios of between 26 and 36 mg/kJ GE for *Clarias gariepinus* (Section, 1.2.4; Table 1.2). Evaluation criteria in Tables 1.1 & 1.2 of all studies for *Clarias gariepinus* are growth rate, feed conversion efficiency and protein utilisation.

Fats or oils and carbohydrates are sources of non-protein energy. Uys (1989) varied fat percentage at constant dietary protein level (42% CP) and arrived at an optimum fat percentage for *Clarias gariepinus* of 10-12%. In fact, in other most studies in this species diets reported seem to have similar or slightly lower fat contents (Degani *et al.*, 1988, 1989; Heinsbroek *et al.*, 1990). The African catfish, *Clarias gariepinus* has limited possibilities to include high inclusion levels of dietary fat (above 20%) due to the ensuing reduced feed intake (Uys, 1989).

In general, teleosts have a limited capacity to assimilate and metabolize dietary carbohydrates (Cowey and Cho, 1993) and there is some controversy about the ability of *C. gariepinus* to utilise dietary carbohydrates efficiently. Uys (1989) reported that the natural diet of especially juvenile *Clarias* may contain considerable amounts of carbohydrates and his studies on digestive enzyme activities point to a carbohydrate digesting capacity. Degani and Revach, (1991) compared digestive capabilities of tilapia (*Oreochromis aureus* × *O. niloticus*), common carp (*Cyprinus carpio*) and African catfish (*Clarias gariepinus*), and found the latter

to have carbohydrate digestive capabilities lower than tilapia but higher than carp, whereas fat was digested better than by tilapia but less well than by carp. On the other hand, Machiels and Van Dam (1987) mention that *C. gariepinus* has a low ability to metabolize glucose rapidly. Despite the existing controversy, carbohydrate levels in *Clarias gariepinus* diets are often substantial , and reportedly range from 15 to 35% (Balogun and Ologhobo, 1989; Heinsbroek *et al.,* 1990; Fagbenro *et al.,* 1993).

Bioenergetic studies in *Clarias gariepinus* revealed that the high ratio between metabolizable energy for production (MEp) and that for maintenance (MEm), and the high efficiency of conversion of MEp into retained energy, largely explain the highly efficient feed conversion of *Clarias gariepinus* (Hogendoorn, 1983b). The ratio MEp/MEm varies with body weight and temperature, due to an interactive effect of feeding level and temperature on the weight exponents in the allometric relations of feed intake and metabolism with body weight. Hogendoorn (1983b) calculated an efficiency of conversion of MEp into retained energy of 0.8 in *C. gariepinus*, as compared to 0.7 in carp and rainbow trout. This efficiency in *C. gariepinus* is independent of body weight, feeding level and temperature, and these factors therefore affect growth mainly through maintenance requirements and maximum feed intake or metabolism (Heinsbroek, 1987).

With respect to protein to energy ratio, Machiels and Henken (1985) reported that at the intermediate energy 21 kJ. g^{-1} of GE level, at a crude protein level of 40%, a crude lipid level 11.5% of the diet (P/E ratio = 19mg protein kJ^{-1} of GE) supported better growth rate and protein utilisation of sub-adult 40-120g *C. gariepinus* than higher energy diets. They also concluded that at the highest dietary lipid level, growth rate, digestibility, protein efficiency and energy gain were considerably reduced, resulting in excess fat deposition. Fish were fed

for 8 weeks, using purified diets and different P/E ratios were primarily achieved by varying levels of protein (20 to 40%) and lipid (4.2 to 29.3%).

Uys (1989) investigated the dietary protein and lipid requirements as well as optimum protein to energy ratio for *Clarias gariepinus* ranging from 1g to 160g by employing 21-day experimental periods. By creating diets with the entire nutrients stable but one, he concluded that juvenile and sub-adult *C. gariepinus* have dietary protein requirements between 44-48% and a lipid requirement between 12% and 14% of the diet. After a multi-factorial protein to energy ratio experiment he concluded that the better performing diets contained 40-42% crude protein, 14-16 $kJ.g^{-1}$ of DE, and resulting protein to energy ratios of 26-29 mg protein kJ^{-1} of DE. His work was based on diets made from complex foodstuffs (maize, wheat bran, fish meal, carcass meal, blood meal, molasses) under "least cost" objectives and the constraints that this method implies (lack of precision in added nutrients to the formulated diets, existence of uncontrolled components, i.e. growth factors and anti-nutritional factors).

Recently Pantazis (1999) tried to establish the optimum protein to energy ratio in 120-233g adult *C. gariepinus* using purified diets and for a total experimental period of 76 days. In the experiment, he tested six purified diets with three protein levels (32, 40 and 46%), each protein level with two lipid levels (10 and 17%) resulting gross energy levels 22-24 $kJ.g^{-1}$ of GE, and a control diet (36% CP, 13% lipid, 21 kJ. g^{1} of GE) using natural foodstuffs (fish meal, soybean, wheat dextrin etc.). He concluded that *Clarias gariepinus* performed best when fed diets containing 46% crude protein, 10-17% crude lipid, 26-32% carbohydrate and resulting gross energy 22-24 kJ. g^{1} of GE and P/E ratio 21.5 - 23 mg protein kJ^{-1} of GE. He also concluded that these diets indicate a protein-sparing effect as a result not only of lipid but also of a better dietary carbohydrate utilisation.

In addition, Henken *et al.,* (1986) reported that optimum P/E ratios are also affected by temperature for *Clarias gariepinus* (e.g., 25 mg protein kJ^{-1} of ME at 24°C and 34.7 mg protein kJ^{-1} of ME at 29°C). Other studies have confirmed the relationship between temperature and growth of the species (Degani *et al.,* 1989).

Pantazis (1999) also determined the quantative requirements for essential amino acids in feeds for *Clarias gariepinus.* Reported values, as a percentage of dietary protein, were: Histidine 1.39, Isoleucine 1.56, Leucine 4.87, Lysine 4.49, Phenylalanine 4.56, Threonine 2.04, Tryptophan 2.59 and Valine 2.08. He determined these requirements by using the daily deposition technique (Jauncey *et al.,* 1983).

Machiels and Henken (1986) developed a dynamic simulation model to predict the relationship between feeding level and growth, and metabolism of *C. gariepinus* of different weight classes at different temperatures. *C. gariepinus* were fed a commercial diet, based upon nutrient intake, digestion, absorption, biochemical reactions in intermediate metabolism and the ultimate deposition of body constituents. Body weight, body fat percentage, feed composition, feeding level and temperature were input values yielding growth, protein gain, fat gain and oxygen consumption as output values. The model adequately predicted the relationship mentioned above.

In a subsequent study (Machiels and Henken, 1987) the effect of feed composition (protein, fat and carbohydrates) was incorporated. At high dietary fat levels (22% or more) fresh weight gain decreased, because of reduced food intake caused by a rapidly increasing body fat percentage. This had been observed earlier for *C. gariepinus* (Machiels and Henken, 1985) and led the authors to propose a feed intake regulation model based on body fat percentage.

Machiels and Van Dam (1987) therefore proceeded to adapt the model to account for the effect of body composition, assuming maximum feed intake would be regulated by lipostatic and glucostatic mechanisms, the former at low dietary carbohydrate levels, the latter at high dietary carbohydrate levels. Using this model, fresh weight gain of *C. gariepinus* fed diets with different compositions, can be predicted.

Juvenile African catfish, *Clarias gariepinus* fed moist diets (34% moisture) consisting of fermented tilapia silage blended with fish meal, soybean meal, corn starch and fish oil, had poorer performance indices (weight gain, specific growth rate, food conversion ratio, protein efficiency ratio), than juveniles fed a dry diet consisting of co-dried tilapia silage and soybean flour blended with fish meal at various incorporation levels (Fagbenro *et al.*, 1997; Fagbenro and Jauncey, 1994; Fagbenro, 1994). Furthermore, total replacement of fishmeal by the co-dried fish silage and soybean flour resulted in poor growth, whereas the best growth was observed at the 50% replacement level (Fagbenro *et al.*, 1997).

To further improve performance of *C. gariepinus* maintenance requirements could be lowered, through selection or through improvement of husbandry systems (e.g., to develop appropriate feeding management strategies, reduction of stress or improvement of water quality). Maximum feed intake could be addressed, e.g., by fine tuning feed composition to regulate feed intake (Machiels and Van Dam, 1987; Machiels and Henken, 1987) and body composition changes with age/size (Hogendoorn, 1983b). In conclusion, nutritional research on any of the African and Asian catfish species would seem to be a prerequisite for more cost effective diets that will reduce production costs and create a good quality product suitable for any small scale or large scale exploitation.

1.4 Aims and Objectives of the Present Study

From the foregoing discussion it is clear that one of the major problems faced by rapidly growing aquaculture in the world is the availability of feeds, since feeds are the major cost in any culture system (Shang, 1981) as with other cultured species. In Asian countries where *Clarias gariepinus* is prized as a highly valued product, feeding costs for the species vary between 5 and 100% of the farm gate value of the final product. This depends on the production system, the source and type of raw materials used in feed formulation and feed manufacture practices (Pantazis, 1999). Thus, a cost-effective catfish production system necessitates the standardisation of feed raw materials and their nutrients, and feed manufacture practices. This will be realized only after the exact determination of the nutrient requirements and appropriate feeding management strategies of the species have been achieved.

Thus, the initial interest in the African catfish, *Clarias gariepinus* as a potential candidate for intensive aqaculture has lead to a significant research effort devoted to different aspects concerning its nutritional requirements and feeding strategies. However, there is still a lack of detailed research work into major aspects of catfish nutrition. Quantative essential amino acid and essential fatty acid requirements, vitamin and mineral requirements, specific dietary protein to energy ratio, specific dietary lipid to carbohydrate ratio as well as the interactions of the different nutrients, metabolic effects of different nutrients, digestibility of different feedstuffs and bioenergetics and effects of feeding regimes, are among these.

The ovarall objective of the present study was to carry out orderly nutritional research with African catfish, *Clarias gariepinus* to improve the cost-effectiveness of feeds for this species and optimise the culture conditions under semi-intensive or intensive aquaculture exploitation. Specifically the aims of the present research were to study:

(1) the effect of dietary protein and energy levels on growth and protein utilisation in *C. gariepinus*

(2) the effect of non-protein, lipid and carbohydrate levels on growth and protein utilisation in *C. gariepinus*

(3) to evaluate the effect of feeding regimes on dietary protein and energy for this species

(4) to evaluate the effect of these nutrients and feeding regimes on digestibility, digestive enzymes, and blood plasma components and

(5) the possible histological changes in fish intestine and liver resulting from ingestion of the various nutrients and by various feeding regimes under study

In order to achieve these goals four key experiments were identified. The first experiment was to carry out approaches to optimising dietary protein to energy ratio in *Clarias gariepinus* (Chapter 3). In Chapter 4, approaches to optimising non-protein energy, lipid to carbohydrate ratio in *C. gariepinus* were investigated. Approaches to optimising feeding regimes in *C. gariepinus* with various concentrations of dietary protein and energy are presented in Chapter 5. Finally, in a follow-up experiment (Chapter 6), an evaluation of mixed feeding schedules with respect to compensatory growth, feed conversion, nutrient utilisation and body composition in *Clarias gariepinus* applying the results from previous experiments was carried out.