NUTRITIONAL STUDIES IN THE AFRICAN CATFISH *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT

A purified diet, the raw material zein and two diets composed of complex foodstuffs (fishmeal, soy, wheat), were tested over a seven and a half (7.5) month period, to obtain the *in vivo* Apparent Digestibility Coefficients (ADC) for dry matter, crude protein, lipid, carbohydrates, ash, energy and the ADCs of their amino acids, for the African catfish *Clarias gariepinus* (Burchell 1822). Faeces were collected by sedimentation and digestibility coefficients calculated by use of chromium oxide as external indicator. Apparent digestibility coefficients ranged between 54% and 96%. Crude protein ADCs (81-90%) recorded for diets in this experiment were similar to those previously recorded. Ash digestibility (54-89%) varied significantly between diets probably as the result of the reduced bioavailability of minerals and trace elements in their constituent raw materials. ADCs for individual amino acids ranged between 82% and 99%. Zein, as an experimental feed ingredient for African catfish, showed acceptable ADCs for both gross nutrients and amino acids. When the nutrient level in the test ingredient was very different from that in the reference diet, calculation of ADCs based on relative nutrient contributions appeared necessary. Dextrin (an intermediate product of starch hydrolysis) was the prevalent carbohydrate source in the purified diet. The high carbohydrate digestibility for this diet (72%) suggests high starch digestibility in African catfish and confirms previous hypothesis of good starch utilization in the species due to elevated amylase levels in the anterior part of the intestine.

A 26-day experiment was conducted on triplicate groups of sub-adult catfish (102.2g) using two feeding frequencies, twice per day and three times per day with fish fed to appetite on a purified diet. Fish fed twice ate 1.42 % of b. w. d^{-1}, those fed three times consumed 1.27% of b. w. d^{-1}. Fish fed twice also exhibited better growth and food conversion. Despite the use of purified diets, performance indices for the groups fed twice per day were regarded as good compared to previously reported data.

Administration of seven diets differing in protein: energy levels over a seventy-six day period, indicated that *C. gariepinus* (120-233g) performed best when fed diets containing gross energy between 22-24 $kJ.g^{-1}$, P:E ratio of 21.5-23 mg $protein.kJ^{-1}$, crude protein at 46%, crude lipid at 10-17% and carbohydrate at 26-32%. These data served to confirm the carnivorous nature of *C. gariepinus* compared to the less carnivorous North American catfish *Ictalurus punctatus*. However, performance indices for the fastest growing groups where lower than those achieved in experiments by previous researchers, possibly due to the weight range of the experimental animals used, the available tank surface and the employed stocking densities. Carbohydrate levels (26-32%) of the best performing diets during these experiments were much higher than used by previous researchers (16-18%) for the same species and still higher than those employed for other carnivores (15-25%) (salmonids, sea bass, sea bream). Taking into account the high carbohydrate digestibility of all the diets used in these experiments more comprehensive use of carbohydrates in *Clarias* catfish diet formulations is suggested. The increase of carcass lipid as a result of increased dietary non-protein energy demonstrated for other species has also been demonstrated for African catfish.

Blood parameters (haematocrit and total hemoglobin) proved not to be valuable performance indices in relation to general nutritional parameters associated with growth and food utilization.

Low carcass incorporation values for arginine and methionine resulted in low requirements (1.97 and 0.26 $g.100g^{-1}$ dietary Crude Protein, respectively) as determined by the carcass deposition technique. The same technique revealed the following values, as g required $Amino\ Acid.100g^{-1}$ dietary Crude Protein: Histidine 1.39, Isoleucine 1.56, Leucine 4.87, Lysine 4.49, Phenylalanine 4.56, Threonine 2.04, Tryptophan 2.59, Valine 2.08. Diets used in these experiments were characterized by lower levels of arginine, lysine, methionine, cystine, threonine and tryptophan to those proposed by most recent research.

It is suggested that the sequence of pre-experimental adaptation and starvation might have exerted an irreparable loss for some of those amino acids and consequently lower values as determined requirements. Furthermore, mediocre oxygen levels prevailing in the employed experimental system in conjunction with sampling manipulations (prolonged emmersion) might have created a shift to ureotelism with a result in low arginine tissue levels.

Results of this study could be used to optimize formulations based on the "economically optimal protein level" and the peculiarities of individual culture systems. Such formulations could be based on both conventional and non-conventional foodstuffs of a standard supply and price for the selected country-area, which would standardize production practices, production levels and costs.

Use of labelled substrates coupled with further experimentation on the required dietary carbohydrate: lipid ratios and the optimum dietary fatty acid profile will clarify the underlying metabolic pathways and probably lead to better elucidation of the deposition of essential amino acids.

More information on the digestibility of various raw materials in conjunction with the determined dietary requirements, will lead to the manufacture of more balanced and cost-effective diets for the African catfish *C. gariepinus*.

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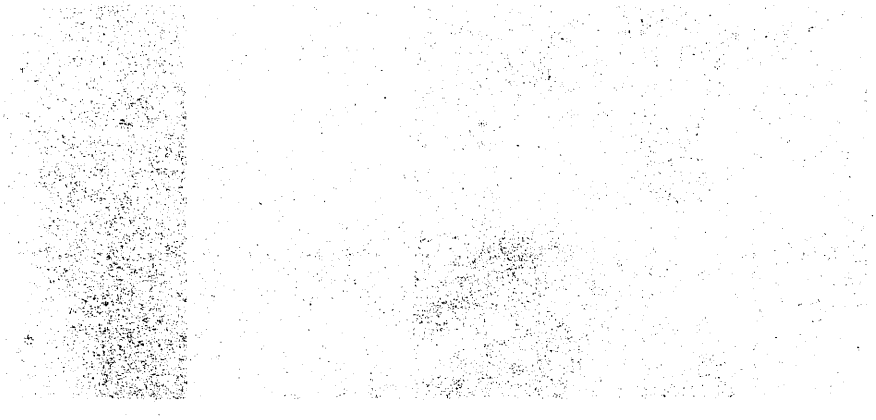
"The ultimate aim of nutritional research is the provision of a balanced diet, which will meet the requirements of the animal with respect to any one of a number of physiological functions ranging from growth to reproduction. This aim cannot be attained without an understanding of the chemical role of each food component and a broad comprehension of the interplay between different food components. The nature of the interaction between the cells, tissues and organs of the body and their food supply are equally germane to this end. Only when these relationships are understood, will it be possible to provide for optimal performance of those functions with respect to which any particular diet might be designed or balanced".

Cowey, C. B. & Sargent, J. R. 1972



CHAPTER 1

1.1 INTRODUCTION AND LITERATURE REVIEW

1.1.1 Introduction

In recent years total world fish production (from capture fisheries and aquaculture) has continued to increase (Figure 1) with estimated total landings of 122 million metric tonnes in 1997 (FAO 1999).

However, the Annual Percent Rate (APR) of increase between 1996 and 1997 was much lower (0.99%) than the APRs recorded for the previous years (Figure 1). This is attributable to the total fish production from capture fisheries which levelled off in 1996 at 94.63 million tonnes (Figure 1) whereas total fish production from aquaculture continued to rise at an APR of 7.1% between the years 1996 and 1997 (FAO 1999).

Asia's dominant role in aquaculture has been perpetuated through the centuries. Asia's contribution towards total world aquaculture production increased from 83.8% to 90.1% from 1984 to 1995 (Tacon 1997). Production of finfish, shellfish and aquatic plants from culture in Asia increased from 8.4 million Mt. in 1984 to over 25 million Mt. in 1995, denoting an Annual Percent Rate (APR) of 10.4 (Rana 1997a). A similar increase (APR 12.7) was recorded in Africa, from 25,000 Mt. in 1984 to 107,000 Mt. in 1995 (Rana 1997a). Compared with the 2.6% APR for capture fisheries in Africa, aquaculture in this continent has grown at a much faster pace. However, it is still a small sector when compared with the 5.4 million Mt. landed in 1995 from capture fisheries in African countries (Pedini 1997).

The contribution of freshwater species to total aquatic production is high in many Asian countries (FAO, 1997). In China freshwater finfish production has shown rapid growth since 1991 and in 1995 it accounted for 53% of total aquaculture production (Rana 1997b). In Southeast Asia (Brunei Darussalam, Cambodia, Indonesia, Laos, Malaysia, Philippines, Singapore, Thailand, Viet Nam) freshwater fish dominate aquaculture production as they account for 29% (by weight) of the total production (Subasinghe *et al.*1997). In South Asia (Bangladesh, Bhutan, India,

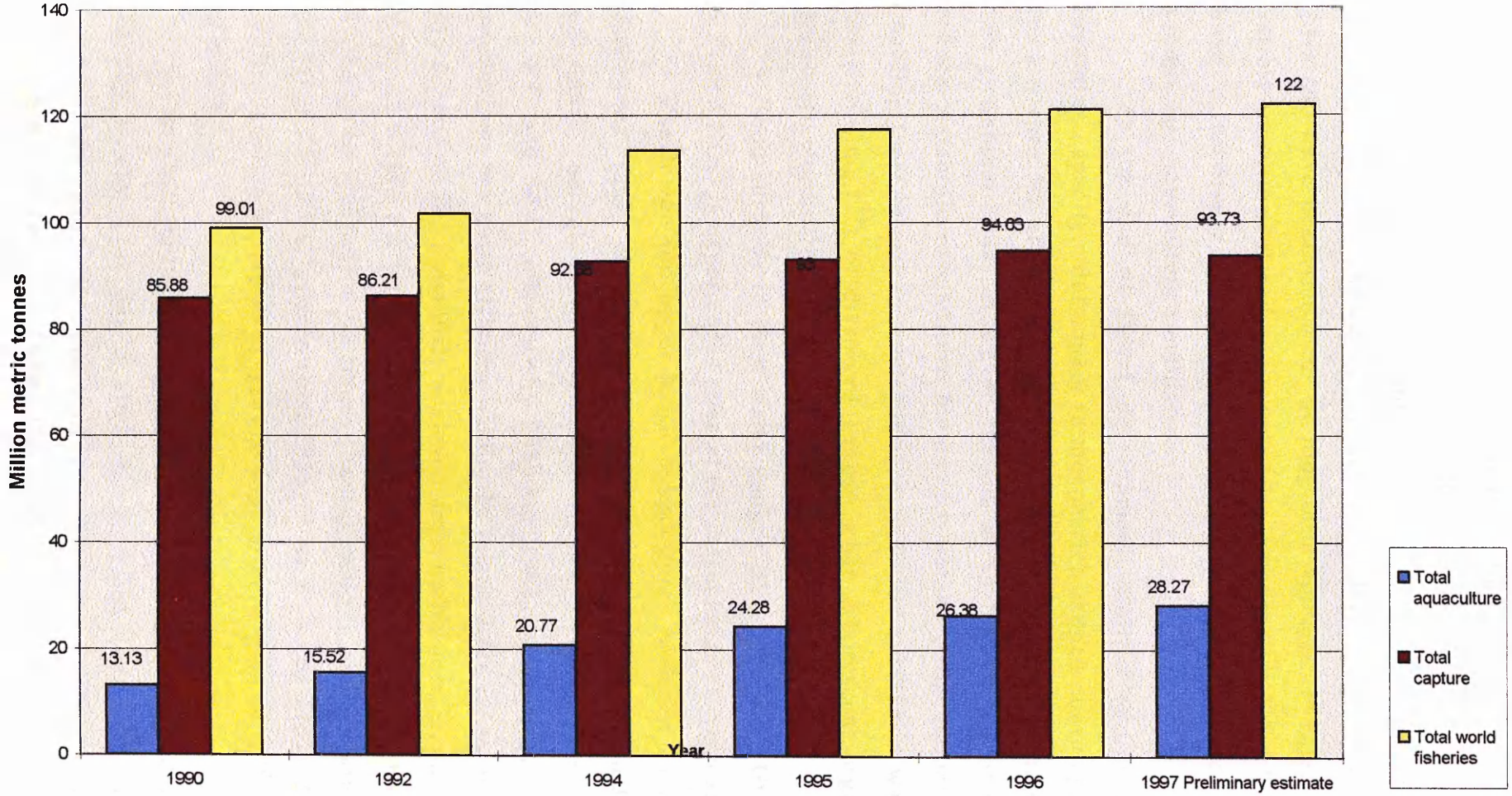


Figure 1. World fisheries production (FAO 1999)

Maldives, Nepal, Pakistan, Sri Lanka) freshwater fish accounted for 94.2% of the total aquaculture production in 1995 (Subasinghe 1997).

Clariids are among these freshwater species with a world production of more than 80,000 Mt. per annum. (Beveridge & Haylor 1998). *Clarias* species, commonly known as catfish, are widely distributed in Africa and Asia and have been the focus of long-term aquaculture interest. Besides a variety of historical and socio-economic reasons (1.1.1.1 &1.1.1.2) catfish have a relatively unique piscine physiology that renders them very promising aquaculture candidates:

- At the end of larval development, *Clarias sp.* develops an air breathing capacity. Pulmonary (atmospheric) oxygen consumption accounts for less than 5% of the total oxygen consumption in individuals of less than 100 g (Babiker 1984). However, the suprabranchial organs of adult *Clarias* reduce metabolic expenditure for oxygen uptake (Hogendoorn *et al.*1983), enabling them to utilise atmospheric oxygen and survive in water with low levels (0-3mg.L^{-1}) of dissolved oxygen when arborescent organs are fully grown (Haylor & Oyegunwa, 1993; Viveen *et al.*1986).

- Good survival in poor water quality (0.1 ppm NH_3, 10-15ppm NO_2, 300 ppm NO_3) is experienced even at the larval stage (Viveen *et al.*1986).

- The lower and upper lethal temperatures recorded under laboratory conditions (and evidenced as changes in branchial and pulmonary ventilation, neural, morphological, biochemical and functional symptoms) are 6^0C and 50^0C, respectively (Babiker 1984). *Clarias* thermoregulatory mechanisms are quite efficient within the temperature range of 20^0 - 30^0 C and depend on the previously achieved acclimation temperature, being more efficient within the temperature range of 20-25^0 C (Klyszejko *et al.*1993). At the larval and postlarval stages *Clarias* exhibits high growth rates within the range 25^0- 33°C (Greenwood 1955; Britz & Hecht 1987). Juveniles and adults experience improved growth and protein gain at 27^0C (Degani *et al.*1989).

- The short larval development period of 14-15 days and weaning time starting at day 4 or day 5 after the onset of exogenous feeding, both considerably reduce the costs of live food production in the hatchery (Verreth & Van Tongeren 1989 ; Haylor 1993a, 1993b).

• *Clarias sp.* is able to exploit both animal and plant dietary protein sources. Its adaptation to detritivory is also a valuable culture characteristic as it creates increased levels of gut microflora resulting in enhanced intestinal cellulytic activity (Clay 1981; Uys 1989)

• *Clarias sp.* are characterized by high gastric lysozyme levels (Uys 1989; Uys & Hecht 1987). This can be beneficial for both increased microbial resistance and good utilization of single cell proteins (yeast and bacteria) as protein sources in commercial fish feeds (Lindsay 1984; Lindsay 1986; Uys 1989).

• *Clarias sp.* generally exhibit high rates of growth (160 to 200% weight gain within a 20 to 78 day time period and for stages between 20 and 200g wet weight) and consequently short production cycles (Degani *et al.*1989; Greenwood 1955; Uys 1989).

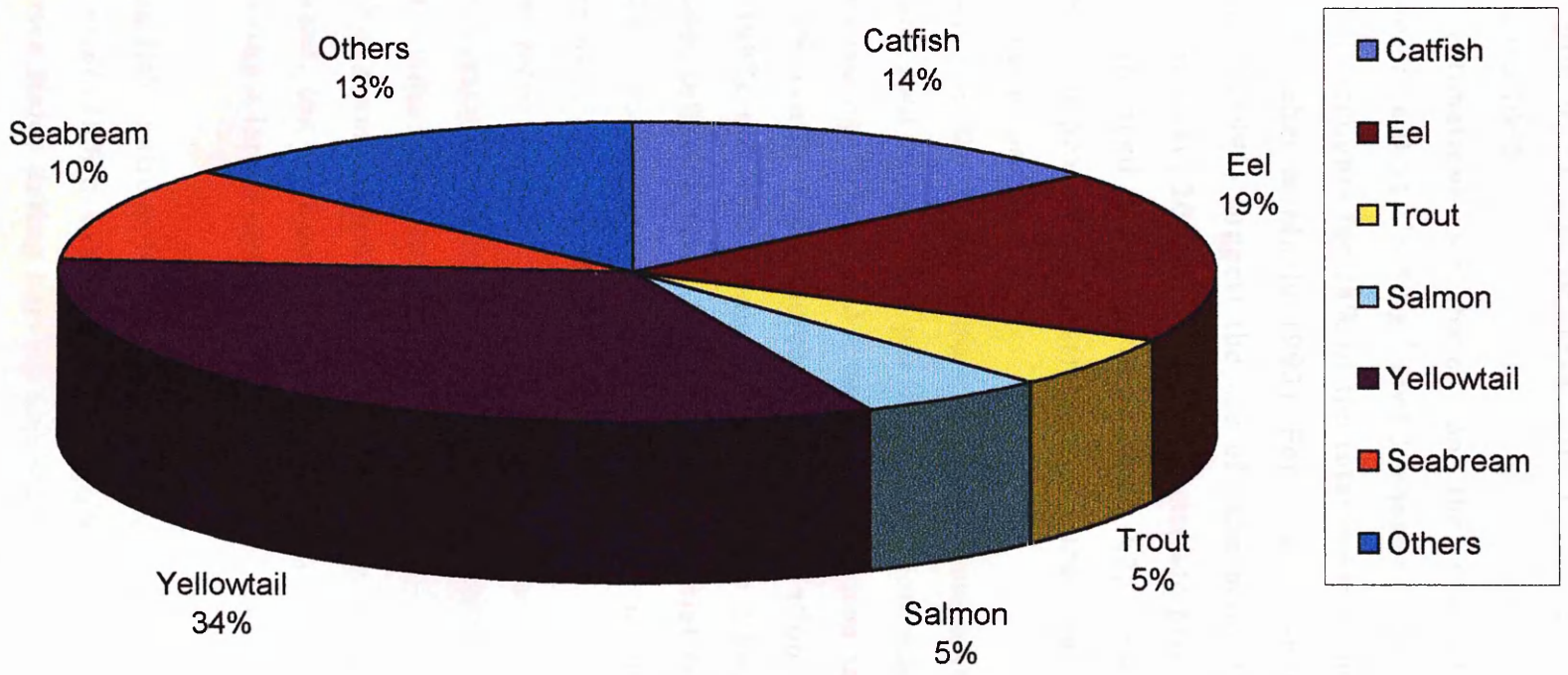
1.1.1.1 Catfish culture in Asia and the Pacific

In 1990, the culture of catfish species in the Pacific and Asian region (Figure 2) accounted for 13.8 % (75,000 t) of the total 510,000 tonnes of cultured carnivorous species in the area (FAO 1992). New & Csavas (1993a) predict that catfish culture in the same area will increase by 80 % within the next decade reaching 135,000 tonnes by 2000. This represents 18.3 % of the predicted 736,000 tonnes of all carnivorous species that will be cultured by the year 2000 and indicates their increasing financial importance in the region.

The main *Clarias sp.* producing Asian countries are Bangladesh, Thailand, Philippines and Malaysia. Culture practices in Cambodia, India and Vietnam have not been successful so far and are limited to small scale agricultural polyculture systems (New *et al.* 1993).

In 1990, the culture of catfishes in Bangladesh accounted for 10.1% (19,000 t) of the total aquaculture production of the country and was based mainly on *C. batrachus*, *Heteropneustes fossilis*, *Mystus aor*, *M. tengra*, *Ompok pabda* and the African *C .gariepinus* (Zaher & Mazid 1993). Pond semi-intensive systems were mainly employed with an average output

Figure 2. Cultured carnivorous fish in Asia and the Pacific by major species groups in 1990 (FAO 1992)



capacity of 800 kg.ha^{-1}.yr^{-1} of final fresh product. Though, with a total production cost of US $ 1,833.ha^{-1} and a market price of no more than 2.0 US $.kg^{-1} of fresh product, the profitability of such systems is in question as the total production cost of fresh catfish fluctuates around 2.3 US$.kg^{-1} (Zaher & Mazid 1993).

These estimations are based on the use of commercial feeds (approximate price 0.51 US $.kg^{-1} feed) where the feed cost (0.63US$.kg $^{-1}$ fresh product) accounts for 28% of the total production cost (2.25US$.kg $^{-1}$ fresh product; Zaher & Mazid 1993). For these systems to be sustainable, Zaher & Mazid (1993) suggest the use of farm-made feeds (40% fishmeal, 40% mustard oil cake, 20% rice bran; approximate price 0.2 US$. kg^{-1} feed) which allows the feed cost to drop down to 0.25 US$ .kg $^{-1}$ fresh product (11% of the total production cost), reducing the total production cost and allowing a broader profit margin.

According to the same authors, fish production in Bangladesh has to increase many fold to meet the per capita fish consumption demands. This can be achieved by at least a 5% annual production increase. Taking into account a 5% yearly price increase due to inflation (US Department of Commerce 1997), the total production cost will be 2,843 US$. ha^{-1} by 2000. With a similar inflation rate, the cost of commercial feeds will increase to 0.75 US$.kg $^{-1}$ feed with a respective increase in the feed cost to 0.94 US$.kg $^{-1}$ of fresh product. Based on a farm gate value of approximately 1.8 US$.kg $^{-1}$ of fresh product (pers. com. with Bangladeshi researchers at the Institute of Aquaculture, University of Stirling, 1997), catfish production in Bangladesh, under semi-intensive systems, is still profitable despite the high cost of commercial feeds (per kg of final product). If farm-made feeds are to be used, the feed cost can be reduced to 0.37 US $.kg $^{-1}$ of fresh product, leaving a larger profit margin for the catfish producer.

The catfish culture situation in Thailand is well described by Panayotou *et al.*(1982): catfish were traditionally obtained from sources including rice fields during harvest and from canals but supply from these sources has been adversely affected by use of agricultural pesticides and the filling up of canals for road construction. An increase in demand led to the

culture of the species in ponds, with a brief boom in the late 60s and early 70s. High pond stocking densities, the use of trash fish as the main feeding input and an inability to improve water quality (due to higher fuel costs for pumping water), resulted in a high incidence of disease and an unavoidable decline in total production between 1973 and 1976 (Panayotou *et al.*1982).

In 1987, increased consumer preference for *C. macrocephalus* coupled with bottlenecks in fry availability and slow growth, led to the introduction of *C. gariepinus* from Laos for the purpose of hybrid creation (Mohidin 1995). These hybrids (female *C. macrocephalus* X male *C. gariepinus*) were characterized by faster growth, high resistance to environmental conditions and intermediate morphological characteristics and meat quality compared to the parent species (Mohidin 1995). This hybrid catfish is of considerable commercial importance in Thailand with over 80.000 tonnes produced each year since 1990 (Mohidin 1995).

Jantrarotai & Jantrarotai (1993) classify feeds of choice for intensive or semi-intensive *Clarias* farming in Thailand, into three categories: commercial, fresh feeds and farm-made feeds.

Fresh feed materials include poultry viscera, fish gills and viscera, kitchen refuse and bread. Most of these are highly palatable to catfish (Jantrarotai & Jantrarotai 1993). Poultry viscera have been proved "quite nutritious" creating fish with a fatty carcass with shiny yellow skin, which is in high demand (Jantrarotai & Jantrarotai 1993). Despite the constraints that fresh feeds impose (seasonality in supply and quality, deterioration in water quality with an unavoidable higher risk of disease), they are still in use by many farmers because of their low purchase cost and labour free process practices (Jantrarotai & Jantrarotai 1993; Somsueb 1993).

According to the same authors, the manufacture of commercial feeds in Thailand in 1993 was more organized than in Bangladesh. However, insufficient domestic production of some important ingredients such as soybean, fishmeal and sesame meal kept the cost of these feeds quite high (Somsueb 1993). Therefore, the main constraint faced by farmers as a result of the use of commercial feeds was the high cost of feeding (US$0.57-0.85.kg^{-1} of fish produced) which could exceed the farm gate value of the

product ranging from US $ 0.36 to $1.0.kg^{-1} of fish produced (Jantrarotai & Jantrarotai 1993).

According to Jantrarotai & Jantrarotai (1993) farm-made aquafeeds were preferred by many catfish farmers in Thailand, despite their varying quality and the labour involved in their preparation. The most frequently used raw materials were trash fish, rice bran, broken rice, soybean cake, chicken bone, noodle waste, moist tofu waste, corn, cassava chips, fish meal and freshwater fish oil. Exceptionally high yields of 62.5t.ha^{-1} in 1.6 ha ponds have been achieved by some farmers using such diets (Jantrarotai & Jantrarotai 1993). A farm-gate value of 0.88-1.00 US$.kg^{-1} of final product, a total production cost of 0.79 US$.kg^{-1} of final product and a feed cost of 0.05 US$.kg^{-1} of final product, enabled these farmers to stay profitable and supported the use of farm-made feeds in semi-intensive or intensive culture pond systems (Jantrarotai & Jantrarotai 1993).

Using a 5% inflation rate the total production cost at year 2000 can rise to 1.16-1.17 US$. kg^{-1} of final product. As today's farm-gate value fluctuates between 1.0-1.5 US$. kg^{-1} of fresh product (Beveridge & Little 1998, pers. comm.), catfish production in Thailand can still be profitable under the systems employed.

Catfish production in the Philippines is characterized by similarities to Thailand (pers. comm. with M. M. Tayamen of the Philippines' Bureau of Fisheries and Agricultural Research, 1997), although there are some notable differences:

"Filipinos" are rice and fish consumers (ideal combination for cheap cereal and protein sources) with a preference for catfishes, particularly the native species *C. macrocephalus* (more palatable and softer meat than the exotic *C. batrachus* and *C. gariepinus*). However, problems related to seed production of the native species resulted in importation of *C. batrachus* from Thailand in the 1970s and hybridisation with the local species. Furthermore, *C. gariepinus* was brought into the country by the private sector without any governmental approval. As a result exotic species have gradually eliminated the native one from natural reservoirs and kept the price of the final product high (between 4.5 and 6.0 US$.kg $^{-1}$ in 1996). In

addition, the need for a high protein diet (compared to other freshwater cultured species, i.e. tilapia, milkfish) keeps production costs high (trash fish or artificial diets are quite expensive) and catfish is considered a luxury fish, a highly prized delicacy (Tayamen 1997; pers. comm.).

1.1.1.2 Catfish culture in Africa

According to Pedini (1997) aquaculture in Africa should be viewed as an activity of two sub-regions: North Africa (Algeria, Egypt, Libyan Arab Jamahiriya, Morocco, Tunisia) and sub-Saharan Africa (all the remaining 37 African countries). These differ markedly with regard to general characteristics of production, constraints and development potential. Resource limitations (freshwater and suitable inland sites) in the North African region impelled development towards the farming of marine and estuarine species, mainly for export to the European markets. On the contrary, aquaculture in sub-Saharan Africa has been oriented to domestic markets and practised mainly by small-scale farmers (Pedini 1997).

The status of *C. gariepinus* culture in Africa has been affected by these constraints and is quite different from that in Asia.

C. gariepinus, an endemic African species, has the widest natural latitudinal range of all freshwater fishes and is cultured commercially in 15 African countries, especially Nigeria, Ghana, Cote d'Ivoire and South Africa (Beveridge & Haylor 1998). *C. gariepinus* used to be one of the most important freshwater fish in traditional subsistence and commercial fisheries in Africa (Uys 1984). In East and Central Africa it used to make up to 28% of fish consumed by local populations (Uys 1984).

In 1995, the African catfish accounted for 4.6% of the main cultured species in North Africa (Shehadeh 1997). In the sub-Saharan countries catfishes were the second most important group with 7,000 Mt., of which 4,000 Mt. represent the cultured African catfish *C. gariepinus,* 12.5% of the total finfish production in the area (Pedini 1997). Between 1990-1995, production of *C. gariepinus* in the sub-Saharan region grew with an APR of 35.5, showing the level of interest in this species especially in Nigeria (Pedini 1997).

Culture of *C. gariepinus* has been practised in both semi-intensive and intensive systems, consisting either of the direct stocking of eggs and larvae

in fertilised ponds or the stocking of fry after rearing in controlled hatchery conditions (Haylor 1992).

Although finfish culture in North Africa is oriented towards the farming of marine species, there is a growing interest in the production of catfish, especially in Egypt (Shehadeh 1997). Should aquaculture be recognized as a legitimate resource user, and consequently integrated into the management of natural resources as with any other farming activity, catfish culture in Egypt might be stimulated provided that shortage of seed and related know-how problems are also solved (Shehadeh 1997).

Shortage of good quality freshwater, integration of aquaculture in agriculture planning and in the management of natural resources are also problems encountered in the countries of the sub-Saharan region. However, the underlying reasons for erratic aquaculture in the region are far more complex and many are not specific to aquaculture alone (Haylor 1992; Pedini 1997):

- adverse climatic conditions (insufficient and fluctuating levels of rainfall, diverse ecological conditions within individual countries) frequently ending in natural disasters
- unstable social and financial conditions leading to weak institutional frameworks and economic problems.
- the economies of most countries rely on small-scale family or tribe based agricultural systems, where supplies are produced rather for short distance, local markets, than large-scale markets.
- lack of aquaculture development plans and firm commitment to its promotion
- rural aquaculture development strategies inconsistent with the needs and circumstances of rural communities and family economies
- lack of transportation and communication infrastructure
- heavy dependence on external support for both research and development, often leading to erratic development efforts due to differences in expectations and priorities between donors and host countries and to collapse of development efforts on conclusion of external support.

Therefore catfish culture in the countries of the sub-Saharan region has always been retained within the rural, small-scale type of exploitation (Haylor1992).Recent Geographical Information Systems (GIS) -based evaluations (Pedini 1997) estimate that the physical potential for expansion of production based on this form of aquaculture is much larger than the present levels of production. Successful increase in production levels of *C. gariepinus* in Mali by integration of aquaculture in irrigation schemes suggests that catfish culture in the sub-Saharan region may be expanded if emphasis is given to (Pedini 1997):

- integration of fish culture with agriculture using family level technologies applied in schemes that involve water storage practices, including micro-irrigation and small ponds
- encouraging investment-oriented aquaculture by progressive farmers and entrepreneurs with the aim of supplying urban centres with fresh fish
- aquaculture-based stock enhancement in small and medium-size water bodies, using local species and including the development of cage culture

1.1.2. Recent catfish research

The culture of *C. gariepinus* has received considerable attention since the early 1970s and 1980s (Micha 1972, 1973,1975; De Kimpe & Micha 1974; Kouassi & Ville 1975; Richter 1976; Clay 1977,1979, 1981; Pham & Raugel 1977) with all these studies confirming the considerable culture potential of the species. Since then, catfish research has focused on the following areas.

1.1.2.1 Broodstock management, fertilization and genetic manipulation

Significant attention has been given to establishment of successful propagation techniques (Hogendoorn 1977, 1979, 1980, 1981; Hogendoorn & Vismans 1980; Schoonbee *et al.*1980). Behavioural peculiarities of the species (aggressiveness and territoriality; Hecht & Appelbaum 1988) resulted in higher growth rate and better feed utilization in mono-sex all-male than all-female cultures with the performance of mixed-sex groups (1:1) being as good as those of all-male groups (Henken *et al.*1987a).

Maturation requires the deposition of nutrients to gonadal instead to other tissues, with possible effects on the observed growth rates and feed efficiency. Therefore, the creation of a final product of uniform size and quality was investigated through ploidy and sex reversal manipulation (Henken *et al.*1987b; Mohidin 1995). Based on a successful triploid induction protocol (Richter *et al.*1986) Henken *et al.*(1987b) concluded that although triploids gave a higher carcass yield after gutting, they experienced similar growth rates and feed conversion ratios to their diploid siblings. On the contrary, triploids deposited less protein, more fat and more energy per gram fresh body weight gain, implying that their nutritional requirements are different from those of their sibling diploids. Mohidin (1995) applied similar triploid induction protocols in the hybrid catfish which is highly valued in Thailand (female *C. gariepinus* x male *C. macrocephalus*): low survival rates observed in the group of the successful protocol and total mortality in the rest of the applied treatments-protocols suggest that more experimentation is needed towards successful polyploidy induction in hybrid catfish. Furthermore, the induction of phenotypic males by oral administration of androstan and androsten in the gynogenetic hybrid catfish (female *C. gariepinus* x male *C. macrocephalus*) and the higher growth rates observed in the phenotypic male group when compared to the control gynogenetic group, suggest that sex reversal is an effective way to enhance growth in this hybrid catfish (Mohidin 1995).

1.1.2.2 General husbandry and culture techniques

Polyculture techniques have been successfully identified (Hogendoorn & Koops, 1983). Increased interest in the culture of the species has led to investigation of culture techniques, feeding regimes and practices of the early life stages, in order to establish standard production procedures (Appelbaum & Van Damme 1988; Haylor 1991; Haylor 1992a; Haylor 1992b; Haylor 1993a; Haylor & Oyegunwa, 1993; Uys & Hecht, 1985; Verreth & Bieman 1987; Verreth *et al,* 1987; Verreth & Van Tongeren 1989).

1.1.2.3 Nutritional research

Determination of suitable feeding regimes is a prerequisite for any successful commercial operation or research facility. Based on commercial feeds, Hogendoorn (1981) concluded that the African catfish is a nocturnal feeder. He also established (Hogendoorn *et al.*1983) a ration size between 0.9 and 2.6 % body weight.day $^{-1}$ for an average fish body weight of 100g (see also 2.4)

Verreth *et al.*(1992) investigated the morphological and functional development of *C. gariepinus* larvae. They concluded that in the short two day period until the end of the yolk sac stage, the entire gastrointestinal tract (with exception of the stomach) differentiated into its final segments. Within the same period, the pancreatic and intestinal hormones regulating metabolism and digestive secretion (insulin, glucagon, C-t-gastrin, cholecystokinin) were active. At the start of exogenous feeding significant levels of intestinal enzymes (trypsin, chymotrypsin, aminopeptidase, non-specific esterase and ATP-ase) suggest that *Clarias* larvae have active protein and lipid digestion at this life stage. However, the development of gastric acid secretion and the formation of a functional stomach (adequate levels of pepsin secretion) is completed within the four first days of exogenous feeding and coincides with the weaning time found in previous studies (Van Damme *et al.*1989; Verreth & Van Tongeren 1989).

Similar work was undertaken by Uys (1989) focusing on the digestive enzyme activities of the adult *C. gariepinus* (350-600g). Uys concluded that:

- *Clarias* proteolytic enzyme activities correspond with those of carnivorous fishes with pepsin activity prevailing in the stomach and trypsin and chymotrypsin activity prevailing in the intestine. The thick- walled muscular stomach with relatively little acid secretion (pH of ≅4) implies that *Clarias* relies on enzymatic digestion and mechanical crushing rather than acid hydrolysis of the food in its stomach.
- Amylase is secreted by the pancreatic tissue (low levels in liver tissue and bile) in its active form and not as zymogen with a prevailing activity in the anterior part of the intestine. Since the

recorded amylase activity was considered high, Uys concluded, "starch can be well utilized as energy source in practical diets for this species".

- Very high activities of alkaline phosphatase were found in the anterior part of the intestine with a typical decreasing proximo-distal gradient being evident. This suggests efficient dietary lipid utilization. In addition, increased gastric lysozyme activity suggests an adaptation towards detritivory, making this fish a true opportunistic omnivore.

During the same experimental work, Uys (1989) tried to establish the protein and lipid requirements as well as the optimum Protein: Energy ratio, for animals ranging from 1g to 160g and by employing 21day experimental periods. By creating diets with the entire nutrients stable but one, he concluded that juvenile and sub-adult *C. gariepinus* have a dietary protein requirement between 44-48% of the diet and a lipid requirement between 12% and 14% of the diet. After a multi-factorial Protein: Energy experiment he concluded that the best performing diet was one with 43% Crude Protein, 13.25% Crude Lipid, 18.36% Nitrogen Free Extracts (NFE) and a Protein: Energy ratio of 26.4 mg Protein. kJ^{-1}. His work was based on diets made from complex foodstuffs-raw materials (maize, wheat bran, fish meal, carcass meal, blood meal, molasses) under the least cost objective 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).

Similar constraints apply to the work of Chotiyarnwong & Chuapoehuk (1981), Chuapoehuk (1987), Boonyaratpalin (1988) and Mollah & Hossain (1990), as they all used similar raw materials to establish the protein requirements of *C. batrachus*. In his published article, Chuapoehuk (1987) admits that diets based on complex foodstuffs are susceptible to changes in protein content (due to the variability of their raw materials) and therefore not recommended for the determination of the nutritional requirements of a species.

Machiels & Henken (1985) investigated the effect of Protein: Energy ratios on growth rate, feed utilization and energy metabolism of sub-adult *C. gariepinus* (40-120g) using purified diets and for a total experimental period of 56 days. They tested five protein levels (from 19 to 38.5%) combined with five lipid levels resulting in three ranges of total dietary lipid: from 4.2% to 11.7%, from 11.5% to 22.1% and from 21.4% to 29.3% of the diet. Gross Energy levels were 20, 23 and 24 $kJ.g^{-1}$ for each range respectively.

They concluded that growth rate and protein utilization were better at the 23 $kJ.g^{-1}$ Gross Energy level, at a Crude Protein level of over 40% of the diet, a Crude Lipid level between 11.5% and 14% of the diet, a Carbohydrate level between 12% and 14% and at a Protein: Energy ratio of at least 16 mg Protein.kJ^{-1}. They also concluded that at the highest range of dietary lipid level, growth rate, digestibility, Protein efficiency and Energy gain were considerably reduced, resulting in excess fat deposition.

Ancillary to the effort of establishing the nutritional requirements of the species by using purified materials, came the research by Fagbenro *et al.* (1998a;1998b;1999a;1999b). Diets based on casein and gelatin and supplemented with crystalline amino acids were formulated to a crude protein content of 400 $g.kg^{-1}$ and a gross energy content of 12 $kJ.g^{-1}$, on a dry matter basis. In those diets, graded levels of methionine (20-40 g. kg^{-1}), lysine (40-65 g. kg^{-1}), arginine (40-65 g. kg^{-1}) and tryptophan (3-13 g. kg^{-1}) were incorporated and fed to juvenile African catfish (18.7±1.8g, 15.4±1.6g, 16.6±1.8g and 11.5±1.1 respectively). Methionine, lysine, arginine and tryptophan requirements were estimated, by the broken point analysis, as 32,57,45 and 11g. kg^{-1} of dietary protein, respectively.

The ability of *C. gariepinus* to digest and utilize carbohydrate (Uys 1989) was also shown for *C. batrachus* fed a farm-made feed comprised of broken rice, trash fish and rice bran with a total carbohydrate level of 49% (Jantrarotai *et al.*1992). Only when dietary carbohydrate was increased to 54%, was reduction in growth observed (Jantrarotai *et al.*1992).

The indispensable vitamins required by *C. batrachus* and *C. macrocephalus* were determined by Butthep *et al.*(1983) and Sitasit *et al*

(1984). Although the required dose (RD) for every vitamin was not determined (but only for a few of them), it was shown that riboflavin (B_2), pyridoxine (B_6), pantothenic acid (B_3), folic acid, niacin (B_5) and ascorbic acid (vitamin C) are all required to be incorporated in the diet. On the contrary thiamine (B_1) deficient diets did not create severe symptoms (as they do in salmon and trout) except for a dark skin colour. It was speculated that thiamine might be produced by intestinal micro-organisms (Butthep *et al.*1983).

Research on the vitamin and mineral requirement of *C. gariepinus* was also performed by Uys (1984; 1989) for both larval and adult stages.

For the larval stages, Uys (1984) used a semi-purified basic diet composed of Torula yeast, cod liver oil, soya bean oil, antioxidant (Vitamin E), stabilizers, emulsifiers, bactericides, and crystalline methionine. He then incorporated a vitamin premix at various levels. The vitamin levels in the best performing diet (Table 5) are uniformly high in comparison with the requirements of warm water fish in general (Piper *et al.*1982). Even though no severe heat treatment was involved during the manufacturing process of these diets some loss of water-soluble vitamins due to leaching, oxidation and heat denaturation can still be expected (Uys 1984). Therefore Uys (1984) further suggests that "research on minimum, specific vitamin requirements of *C. gariepinus* larvae and the effect of processing, storage and leaching on dietary vitamins is also recommended as the vitamin supplement to the established feed makes up to 24% of the cost of the ingredients".

Later, the same author (Uys 1989) followed similar methodology regarding the vitamin requirements of adult *C. gariepinus* but using diets based on complex foodstuffs. The established vitamin levels are shown in Table 5 (page 62). However, he admits that this particular experiment is not conclusive for two basic reasons:

- the duration of the experiment (21 days) was not long enough for dietary vitamin and mineral deficiencies to manifest themselves in the fish
- the use of complex foodstuffs for diet formulation enabled unknown quantities of vitamins to be incorporated in the diets.

Furthermore, the exact quantities of incorporated dietary vitamins were not analytically determined, leaving an ambiguity regarding the precision of the established vitamin requirements.

In his effort to establish the ascorbic acid requirement of *C. gariepinus,* Mgbenka (1991) supplemented fish waste diets with graded levels of L-ascorbic acid and concluded that the optimal level is 60 $mg.kg^{-1}$ of diet. However, the use of fish waste as the basal diet imposes the same restrictions and ambiguities as does in Uys (1989) work.

A more precise determination of *C. gariepinus* ascorbic acid requirement, proved to be the one by Baker & Davies (1997). Applying graded levels of all-rac-alpha-tocopheryl acetate in practical-type diets and by employing broken-line analysis for muscle and liver lipid peroxidation, the authors found a requirement of 30-40 $mg.kg^{-1}$ of dry diet, within the range of 10-120 g live weight.

As previously mentioned (1.1.1.1 and 1.1.1.2) the development, and in some cases the intensification, of *Clarias* culture as a means to solve the overwhelming problem of fish resource depletion, have imposed continuous pressure on the production of low-cost fish feeds. Therefore, the substitution of expensive raw materials (fishmeal, fish oil) with cheaper plant proteins and their by-products has been the objective of several researchers.

Bureau *et al.* (1995) conclude that juvenile *C. gariepinus* (average weight 4.6g) are able to tolerate up to 10% cassava leaves in diets mainly comprised of soybean meal, cassava chips, fishmeal, rice bran and rice oil. In addition, incorporation of peanut vines up to 20% in diets mainly comprised of soybean, corn, fishmeal, rice bran and rice oil, did not compromise the performance of the animals and created equally high weight gain and low Food Conversion Ratios compared to the Control (0 % peanut vines).

Similar research was performed on the incorporation of household wastes in low cost diets for the mud catfish *C. isheriensis* in south-western Nigeria (Fagbenro & Arowosoge 1991). It was concluded that maize chaff had the greatest potential and desirability as a replacement for yellow maize

as the energy source in low-cost diets for this species. Further research revealed that cocoa-pod husk meal can be incorporated at levels up to 30% in diets mainly comprised of yellow maize, blood meal and shrimp by-products without any significant adverse effect on the performance of *C. isheriensis* (Fagbenro 1992)

The excess of waste fish (tilapia *Oreochromis niloticus*) in the same geographical area, led to the investigation of the effect of fish silage on the performance of *C. gariepinus*.

Juvenile catfish *C. gariepinus* fed Moist Diets (34% moisture level) consisting of fermented tilapia silage blended with fishmeal, 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 and blended with fishmeal at various incorporation levels (Fagbenro 1994; Fagbenro & Jauncey 1994; Fagbenro *et al.* 1997). 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).

These results are in accordance with the good performance of *C.batrachus* fed moist fermented tilapia silage diets (Wee *et al.*1986).

In conclusion, nutritional research on any of the African or 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 (family type) or large scale (big continental market) exploitation.

1.2. THEORETICAL BACKGROUND REGARDING THIS STUDY

1.2.1 Physiological basis of food intake, gastric evacuation rate and feeding frequency. Their importance in nutritional research.

The amount of food taken within a meal and the frequency of meals within a day, influence the utilization of a diet in relation to the digestive system-type of the animal in question.

Therefore, a comprehensive understanding of gastric evacuation and return of appetite mechanisms enable maximization of feed utilization and the expression of any beneficial effect on the growth of the animal.

In mammals it was established long ago (Peter 1979; McHugh & Moran 1979; Hunt 1980; Jobling 1986) that food intake is regulated by the caloric content and the nutrient content of the ration, so that body weight is maintained about some set-point level. Mammalian gastric evacuation and return of appetite, is mainly controlled by two mechanisms (Jobling 1986):

- Duodenal receptors tuned to respond to variations in pH, osmotic pressure, fatty acid anions and certain amino acids especially tryptophan and phenylalanine and able to generate contractions of the smooth musculature of the stomach wall, the pyloric and duodenal musculature. Therefore, nutrients have an inhibitory effect (direct slow down) on gastric emptying as a result of their action on these receptors. This coincides with the mechanical stomach fullness, or else, satiation.
- Many of the above substances known to slow gastric emptying and initiate intestinal processes, lead to release of an array of peptides in the blood [gastrins, cholecystokinin (CCK), secretin, motilin, glucagon and vasoactive intestinal peptide (VIP)]. The release of CCK results in two contradictory cascading effects:

-the direct slow down of gastric emptying (satiety effect, continuation of intestinal processes) and

-the stimulation of gall bladder contraction and the secretion of pancreatic enzymes. Bile salts from the gall bladder will neutralise the acid chyme and remove the contractile stimulus (for receptors

responsive to low pH) on the smooth musculature of the stomach wall, the pyloric and duodenal musculature, which in turn will remove the slowing down of gastric emptying, enhancing the gastric emptying and the return of appetite.

These events allow the entry of a new pulse of gastric chyme into the upper intestine. This, in turn, begins a new and sequential cycle of secretion and inhibition.

In fish it is not known whether cells capable of responding to these stimuli (low pH, osmotic pressure, fatty acids, aromatic amino acids) are found in the intestine, but if receptors are present it is difficult to envisage how all types could be functionally operative in all species, i.e. pH receptors would be of little value to the fish species which are not capable of acid secretion. Similarly, it is difficult to envisage how receptors responding to osmotic properties could provide an effective regulation of gastric emptying in marine species where the osmotic properties of the gastric chyme would be expected to be influenced by the drinking of sea water (Jobling 1986).

But gastric evacuation and return of appetite in fish are also regulated by other factors not always conclusive or even contradictory in their effects:

- gastric evacuation rate increases with temperature (Hofer *et al.*1982; Ross & Jauncey, 1981; Santulli *et al.*1993) and is empirically described by an exponential function (Dos Santos & Jobling 1991a; Paakkonen & Marjomaki 1997)
- according to some researchers gastric evacuation time increases with fish size (Ross & Jauncey, 1981; De Silva & Owoyemi 1983), whereas others have reported that there is no significant effect of fish size on evacuation rate (Dos Santos & Jobling 1991b; Buckel & Canover 1996)
- increasing meal size decreases the gastric evacuation rate (Dos Santos & Jobling 1991b, 1992; Paakkonen & Marjomaki 1997) but is not always the case as reported by Brett & Higgs (1970), Talbot *et al.* (1984) and Bromley (1988).
- gut evacuation rate decreased as the time of pre-prandial starvation increased (Elliot 1972; Talbot *et al.* 1984). Furthermore, post-prandial

starvation led to a reduction in gut evacuation time (Corazza & Nickum 1983; Talbot *et al.*1984)

Therefore, using feeding tables and feeding on a percentage body weight per day basis, without taking into account all the aforementioned factors entails a twofold risk: overfeeding which eventually leads to overestimation of nutrient requirements, or restricted feeding (growth responses might be biased towards the diets with the higher protein and energy levels).

Related to the gastric evacuation time and consequently the food intake is the rhythmicity of digestive and other metabolic processes in relation to feeding frequency:

-in fishes with a very long delay-response between feed ingestion and their peak digestive enzyme activity, like *Cyprinus carpio* and *Anguilla japonica*, it is important to keep a continuous, low quantity, feeding regime as infrequent large meals will result in poor food utilization due to the slow secretory response of their digestive enzymes (Onishi *et al.*1973a,b, 1976; Takii *et al.*1985)

-on the contrary, in *C. gariepinus*, the secretory enzyme response is relatively fast: Uys (1989) showed that gastric, pancreatic and foregut protease activity started rising after 1 hour and had a peak 4 hours after meal ingestion time. 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. Therefore, *C. gariepinus* is physiologically equipped to cope with infrequent and irregular meals. However, Hossain *et al.*(1999) showed that fingerlings (113.48±1.87 mm total length) kept at 30^0 C, 12:12 L:D and fed alternatively on a continuous 24 h - daytime basis, followed a diel cycle in voluntary feed intake with the majority of food taken during the dark phase.

It should be remembered (1.1.2.3) that in contrast to herbivores[1] (whose stomach is acidic), the pH of the catfish stomach is relatively high (≅4) meaning that *C. gariepinus* depends more on the enzymatic activity of

[1] With the massive exclusion of the effectively agastric cyprinids

pepsin and lysozyme than on acid hydrolysis of the substrates (Uys 1989).Its thick walled muscular stomach is typical of fishes that employ this strategy.

Finally, feed utilization and all the above mentioned factors affecting gastric evacuation and return of appetite, are influenced by several other biotic and abiotic factors as intra- and inter- specific social interactions, migration, spawning, salinity changes, changes in dissolved oxygen levels and light (in salmonids it has been demonstrated that the overall energy balance and growth is greater at high light intensities, 1600 lux), water flow rate and the characteristics and design of the holding facility (Peter 1979).

1.2.2 Crude fibre, carbohydrates and Nitrogen Free Extracts (NFE). Controversial aspects and rationalization.

The crude fibre method is of uncertain origin (Tyler 1975 cited at Van Soest & Robertson, 1980) and has been used for at least 150 years. According to Harris (1970) (cited at Anderson, 1985), crude Fibre is the insoluble material (mainly cellulose and the rest lignin and hemicellulose) that remains after treating feed samples with hot dilute sulphuric acid and hot sodium hydroxide under specified conditions. But during this extraction sequence, 20-50% of cellulose, 50-90% of lignin and 85% of hemicellulose are dissolved by the procedure and non detected (NRC 1982). Consequently, they are calculated in the Nitrogen Free Extract (NFE) fraction to which they give a false increased value, as NFE is supposed to represent the easily digestible carbohydrates of the feed (NRC 1982). Therefore the principal problem of the "crude fibre" system is the distribution of the organic nonlipid, nonprotein fraction between crude fibre and NFE, which fails to provide a meaningful separation of the carbohydrates according to their nutritive value (NRC 1982).

For all the above reasons, very early in the '40s and later on in the '80s both the Association Of Analytical Chemists (A.O.A.C) and the subcommittee on Feed Composition of the USA (NRC 1982) recommended that reporting of NFE be discontinued.

Similarly, the International Agency for Research on Cancer (IARC) of the European Community (Van Soest & Robertson, 1980) has endorsed the Van Soest (1978) definition of crude fibre as "the plant polymeric

substances resistant to animal digestive enzymes". This definition conforms to the "plant cell wall" definition and contains more than lignin, cellulose and hemicelluloses. It includes pectins, gums and galactans, which although not affected by the digestive enzymes, are affected by bacterial action of the lower digestive tract and therefore do not contribute to the true indigestible faecal fraction of crude fibre but accounted as NFE as well.

Therefore, when the objective is to obtain the most accurate estimate of digestibility, the IARC recommended the use of the Detergent system: the truly indigestible components of feed are recovered in the neutral-detergent residue (Neutral Detergent Fibre / NDF= ADF+ hemicellulose), which in turn under an acid detergent procedure permits the calculation of hemicellulose and cell wall proteins. The resulting residue of Acid Detergent Fibre (ADF) under consecutive extraction with 72% $H_2 SO_4$ and $KMnO_4$ reveals the cellulose and lignin fractions (Anderson 1985).

However, according to Van Soest and Robertson (1980), NFE calculations based on ADF have no scientific validity, the hemicelluloses, metabolic faecal matter and available carbohydrates having been confounded. According to the same authors, the use of ADF as a predictor of digestibility is not founded on any solid theoretical basis other than statistical association.

A more accurate description of the NFE fraction can be found in the original Van Soest's analysis (Van Soest 1963; Van Soest & Wine 1967) by the equation:

Organic Matter (OM)= Cell Contents (CC) + Cell Wall Constituents (CWC) (1) which can be transformed to : (CC) = (OM) - (CWC) (2) because

(CC) = Protein + Lipid + Carbohydrates and

(CWC) = Cellulose + Hemi-cellulose + Lignin = $NDF_{ash\ free}$ equation (2) can be transformed to :

Carbohydrates = (OM) - (Cellulose + Hemi-cellulose + Lignin + Protein + Lipid) (3)

As all the components in equation (3) can be measurable, a more precise Carbohydrate / NFE value can be achieved. However this approach is labour and time intensive as it requires the estimation of all the components: lignin (Van Soest 1963), cellulose (= $ADF_{ash\ free}$- lignin),

hemi-cellulose (=$NDF_{ash\ free}$ - $ADF_{ash\ free}$), Organic Matter, Protein and Lipid (AOAC 1995).

In addition, the results of the above mentioned extractions depend on the nitrogen content of the various extracts that varies considerably from material to material. These problems illustrate the difficulty of designing a single system of analysis for all conditions, and a necessary result is that a single analytical protocol cannot satisfy all conditions.

In this study, most of the diets used were purified having dextrin as the sole source of available CHO. Therefore, the determination of available carbohydrates in diets or faeces was determined by an analytical method, modified from McCready *et al.* (1950) and described in Appendix III.

The credibility and repeatability of this method was assessed by applying it to various diets and comparing the results to the NFE fraction estimated by subtraction (Appendix III).

1.2.3 Differential energy concepts between fish and mammals. Their implications for nutritional research and methodology

1.2.3.1 The importance of seafood products in human health. Factors affecting their quality and the underlying metabolic pathways

During the last three decades biomedical research has elucidated many of the facts and practices that prolong human life. The role of polyunsaturated fatty acids (PUFAs) in human health and as essential molecules for neural development in infants has been detailed (Innis 1991; Innis *et al.*1994; Uauy-Dagach & Valenzuela 1992; 1996; Nettleton 1992; Steffens 1997) and encouraged consumers towards seafood products and eventually aquaculture products. Therefore, people of European and other "western" type economies have been increasingly aware of the quality of food products and associated criteria (Haard 1992; Nettleton 1992). Surveys by the Food Marketing Institute (FMI) in the USA (Food Marketing Institute 1989,1990) revealed that consumers have been changing their diets towards more fruits and vegetables (57% of respondents), less meat (34%), less fats,

oils (27%) and sugar (19%), more chicken (19%), fish (18%) and fibre (16%) and less salt and cholesterol (15%). The main seafood issues that consumers were facing in the 90s, were fat and cholesterol, omega-3 fatty acids, the source of the fish, i.e. farmed versus wild, fresh versus frozen and raw versus cooked fish (Mackie *et al.*1986; Nettleton 1992).

Fat represents a paradox. The public is being urged to trim its fat intake to no more than 30% calories from fat (Nettleton 1992). Therefore, one has to convince the consumer about the qualitative differences between terrestrial animal and fish fat and between plant and seafood omega-3 fatty acids (Nettleton 1991, 1992).

Consequently, research efforts were focused in identifying the factors affecting fish carcass quality. As a result of this effort the following points can be highlighted:

a) feeding levels affect carcass composition, i.e. increasing ration size in rainbow trout (0.5-1.0 kg body weight) increases the percentage of carcass fat and carcass dry matter without significantly affecting the carcass protein levels (Storebakken & Austreng 1987)

b) Several species (rainbow trout, plaice, channel catfish, common carp and red drum) experience some sparing of protein under increasing levels of dietary non-protein energy but at the expense of an increasingly fatty carcass (Cowey 1993). On the contrary, salmon (*Salmo salar*) carcass fat levels seem to be unaffected by a high lipid intake (Johnsen & Wandsvik 1991)

c) There is an heterogeneity of lipid distribution in fatty fillets originating from different body regions (Henderson & Tocher 1987). Furthermore, carcass quality is affected by specific categories of fatty acids, as administration of dietary medium chain triglycerides (MCT / 8-12 carbon atoms) in some species *(Plecoglossus altivelis, Oreochromis niloticus)* suppressed carcass lipid accumulation without reduction in growth and feed efficiency (Nematipour *et al,* 1989; Nakagawa & Kusunoki 1980; Nakagawa & Kimura 1993). In addition fillets of farmed fish were characterized by increasing proportion of the omega-3 fatty acids and decreasing proportion of

the corresponding monoenes, when compared to fillets of wild fish of the same species (Lie *et al.*1986; Shahidi *et al.*1992)

d) The proportions of muscle lipid and glycogen are affected by the dietary energy levels and influence the quality of the fillets. A near linear correlation exists between texture and ultimate post-mortem pH. Excess glycogen in the muscle decreases post-mortem pH and eventually deteriorates the texture quality of the fillet (Shahidi *et al.*1992). In addition, muscle lipid levels affect hydrolysis and peroxidation of lipids in frozen fish fillets (Haard 1992).

In order to minimize carcass lipid deposition in fish in favour of a high quality final product, a better understanding of the energy partitioning, the underlying lipogenetic mechanisms and their implications for diet formulation is required. This imposes an extra challenge for the fish nutritionist who has to be able to differentiate between energy metabolism in fish and mammals.

1.2.3.2 Energy metabolism in mammals

1.2.3.2.1 Introduction

Energy metabolism in mammals is characterized by the utilization of dietary carbohydrates and lipids either as an immediate energy fuel or as a storage fuel tank (Leech 1977).

Generally, the predominant energy storage polysaccharide in animals is glycogen, which under physiological conditions is highly hydrated. Despite its low energetic value (17.24 kJ $.g^{-1}$ dried glycogen) it is the only storage substance that can be used by anaerobic cells (Leech 1977). Glucose in turn, is an important metabolic fuel in vertebrate animals and various mechanisms have evolved to ensure that it is constantly supplied to serve the needs of highly glucose-dependent tissues (Suarez & Mommsen 1987). Glucose may originate from the gut during digestion and absorption of ingested carbohydrates. It may be produced by liver and kidney as a result of glycogenolysis (the breakdown of glycogen), or through gluconeogenesis from lactate, amino acids and glycerol (Suarez & Mommsen 1987).

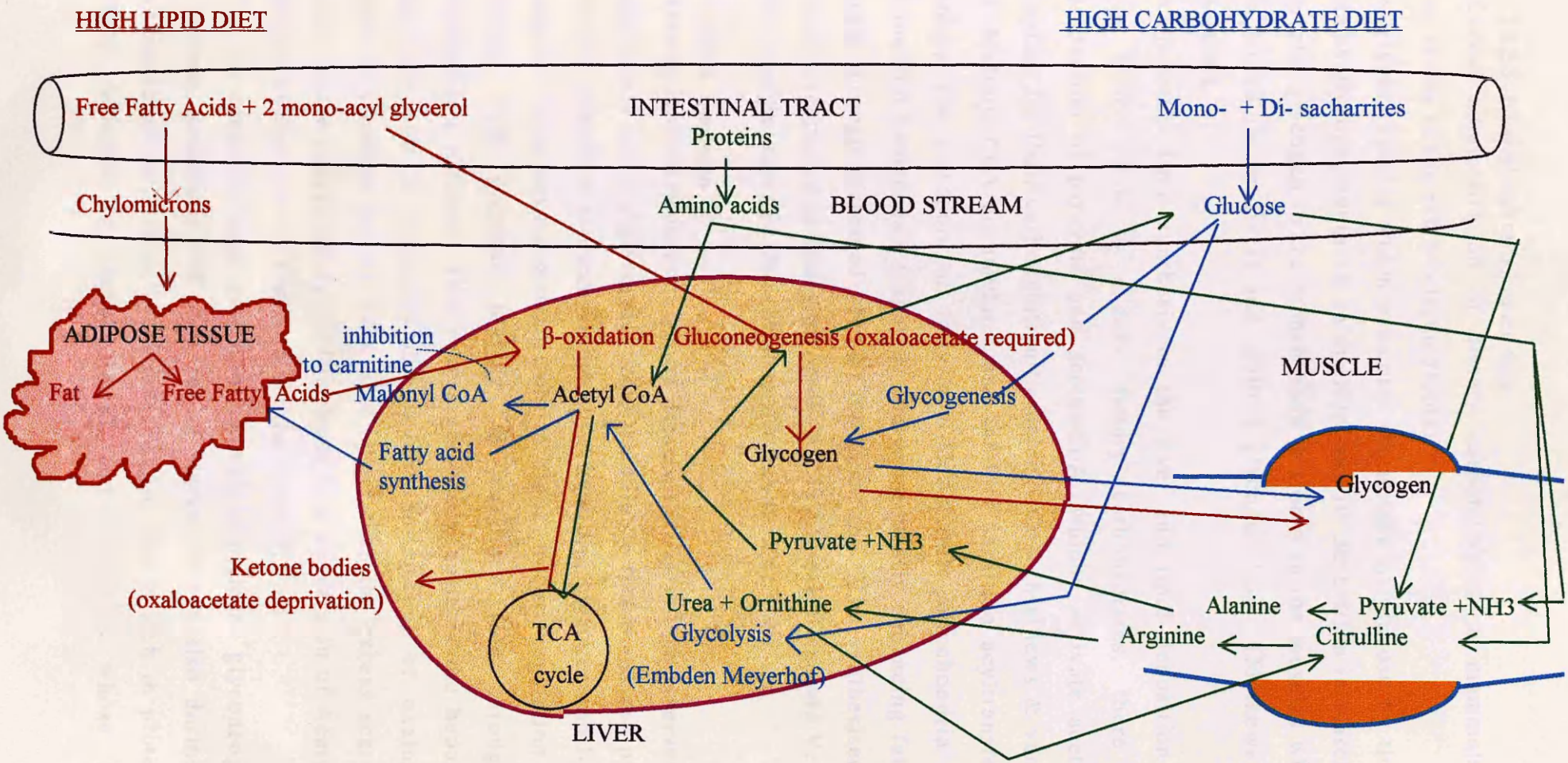
Triglyceride presents a much more economical proposition as a fuel. Not only does its energetic value (38.92 $kJ.g^{-1}$) greatly exceed that of glycogen, but it is stored free from water. Despite its suitability as an energy storage substance, triglyceride has the disadvantage that it cannot be used to generate ATP in the absence of oxygen. In mammals triglyceride is stored predominantly in adipose tissue, the cells of which may contain 90% lipid (Leech 1977).

1.2.3.2.2 Effect of high lipid diets

Excess administration of dietary lipids in mammals enhances the action of intestinal pancreatic lipases that convert triacylglycerol to two free fatty acids and 2-monoacylglycerol (Mathews & van Holde 1990). 50-60% of the dietary lipids are absorbed through the intestine as 2-monoacylglycerol whereas the rest are completely hydrolyzed to free fatty acids by a 2-monoacylglycerol lipase. In the intestinal epithelium triacylglycerols are resynthesized and transported in the circulatory system in the form of chylomicrons (Leech 1977; Karlson 1980; Figure 3). Chylomicrons are broken down by a special lipoproteinic lipase bound to the cellular membranes of the adipose tissue. As transport of fat to storage depots is not regulated (Mathews & van Holde 1990) the liberated fatty acids are deposited in the adipose tissue while the rest are transferred to other organs (Karlson 1980). Monoacylglycerol is converted through the gluconeogenesis pathway to glycogen produced in the liver and eventually deposited as such in the muscle (Mathews & van Holde 1990; Figure 3).

In the case of a high lipid diet, the excess free fatty acids not bound to the adipose tissue or used by other organs, are transported to the liver and converted to acetyl-CoA through the β-oxidation pathway (Leech 1977). Because of the depletion of oxaloacetate (due to gluconeogenesis), the excess acetyl-CoA is not able to enter the TriCarboxylic Acid (TCA) cycle with a result in the production of ketone bodies (acetoacetate, β-hydroxybutyrate; Figure 3). These compounds are the only form of lipid that can be utilized by the mammalian brain. If not used they are eventually excreted in the urine (Leech 1977).

FIGURE 3. Metabolic pathways occurring as a result of a high lipid or a high carbohydrate diet in mammals (text or arrows in red and blue represent pathways as a result of a high lipid or a high carbohydrate diet, respectively. (Dashed arrows represent inhibitory actions)



1.2.3.2.3 Effect of high carbohydrate diets

Excess administration of dietary carbohydrates in mammals (high glucose levels in the extra-cellular fluids):

- triggers insulin which promotes the uptake of glucose by the liver, stimulates glycogen synthesis in the liver and its deposition in muscles (3/4 of the total glycogen in the human body is stored in the muscles while the rest is stored in the liver) and inhibits glycogenolysis (Mathews & van Holde 1990).

- increases lipid synthesis in the liver and lipid deposition in the adipose tissue: due to excess dietary carbohydrates, there is an overproduction of pyruvate and acetyl-CoA, which activate acetyl-CoA carboxylase for their carboxylation to malonyl-CoA (Mathews & van Holde 1990). Malonyl-CoA accumulates and inhibits carnitine acyltransferase I, preventing the transport of fatty acyl-CoAs into mitochondria for β-oxidation and ketogenesis (Figure3) and consequently enhancing fatty acid synthesis through increased acetyl-CoA quantities. The synthesised Fatty Acids are transported as triacylglycerols to the adipose tissue via Very Low Density Lipoproteins (VLDL) (Mathews & van Holde 1990).

1.2.3.2.4 Starvation

During starvation mammals first deplete their glycogen reserves. Next the body starts using triglycerides from the adipose tissue and end products of protein catabolism and transamination. Therefore, glycogen and lipids of the adipose tissue have a primary role in blood glucose regulation during starvation, with proteins playing an auxiliary role through the glyconeogenesis pathway. This excess gluconeogenesis in the hepatocytes during starvation in mammals diminishes the amount of oxaloacetate available to combine (in the citric acid cycle) with the excess acetyl-CoA produced by the excess lipolysis. This leads to a conversion of Acetyl-CoA into ketone bodies that are removed via the urine (1.2.3.2.2.).

On the contrary, some groups of animals experience glyconeogenesis from protein precursors not only during starvation but also during their normal metabolic activities: carnivores (whose diet is rich in protein but relatively deficient in carbohydrates) and ruminants whose dietary

carbohydrate will be converted to short-chain fatty acids (acetate, propionate and butyrate) by the bacterial flora of the rumen, rendering the animal chronically short of carbohydrate (Leech 1977; Mathews & van Holde 1990).

Diabetes in mammals is usually attributed to low production of insulin or to increased production of glucagon, somatotropin or cortizone which act antagonistically to insulin. Diabetic mammals experience similar metabolic pathways as normal ones do under fasting conditions. Though the cause is different: in starvation the main cause is the lack of adequate dietary energy, whereas in diabetes there is glucose in the blood which cannot be mobilised into the tissues, due to lack or inhibition of insulin (Mathews & van Holde 1990).

1.2.3.3 Energy metabolism in fish

Similar biochemical metabolic pathways to mammals generally characterize fish. However, the utilization of nutrients as energy sources is different and quite varied, almost species specific.

Fish have little adipose tissue and they store triglycerides in their liver and muscles (Leech 1977). In addition, their muscle glycogen levels are generally lower than the respective liver ones (Shahidi *et al.*1992). Consequently, fish do not seem to have the ability for long-term high storage levels of energy fuels, as seen in mammals.

Experiments in carp (Nagai & Ikeda 1971a; Nagai & Ikeda 1971b; Nagai & Ikeda 1972) showed that this fish is characterized by an inherent inability to convert glucose to glycogen: during these experiments animals fed a high carbohydrate diet and after eight hours of starvation, had higher levels of liver glycogen than ones fed a medium or a low carbohydrate diet, though, a very low proportion of this glycogen was of dietary glucose origin. On the contrary, most of the liver glycogen was of protein (gluconeogenesis from aminoacids) dietary origin, with the dietary carbohydrates being used as immediate energy yielding sources (TCA cycle, CO_2 production).

As a result the blood glucose image in carp is quite different from that of mammals: a high carbohydrate diet creates low blood glucose levels, which tend to increase considerably under a high protein diet, as blood glucose is the result of gluconeogenesis from dietary amino acids. Simultaneously,

liver lipid is higher under the high protein diet as it is a product of fatty acid synthesis from amino acid precursors.

It is obvious then that during starvation in carp, dietary carbohydrates (CHOs) do not play the same regulatory role regarding blood glucose levels, as they do in mammals, because most of the dietary CHOs are immediately catabolized as energy yielding sources. As a result, carp is considered a fish, which is utilising CHOs quite efficiently, though quite differently from mammals.

As carbohydrates (CHOs) have a key role in energy metabolism, Cowey & Walton (1989) and Wilson (1994) reviewed carbohydrate metabolism and utilization in fish. The following points should be highlighted:

a) Carbohydrate utilization depends on carbohydrate digestibility, which in turn is affected by the source-type, dietary level and heat treatment of carbohydrate. Some mono- and disaccharides are not always used better than complex polysaccharides: chinook salmon experienced better growth rates when fed glucose and the disaccharides maltose and sucrose at a level of 20% of the dry diet followed in descending order by dextrin, fructose, galactose and potato starch (Buhler & Halver 1961 cited at Wilson 1994). On the contrary channel catfish have been shown to utilize dextrin or corn starch as an energy source but not glucose, fructose, maltose or sucrose (Wilson & Poe 1987).

In general it has been observed that fresh and warm water fish have much higher intestinal amylase activity and consequently higher CHO digestibility than marine and cold water fish (Shimeno *et al.*1977; Hofer & Sturmbauer 1985; Wilson & Poe 1985). Therefore a dietary level of $\leq$ 20% digestible CHO appears to be optimal for marine and cold-water species, whereas higher levels are used by fresh and warm water ones. However, this conclusion cannot be generalized, as it applies only to the two above mentioned cases without taking into account the rest of possible combinations found in nature (marine warm water and fresh cold water species).

b) In order to investigate CHO utilization in fish many researchers employed oral glucose tolerance tests. Experiments in rainbow trout (Palmer

& Ryman 1972), common carp, red sea bream, yellowtail (Shimeno *et al.* 1977; Furuichi & Yone 1981) and channel catfish (Wilson & Poe 1987) concluded that in each case the oral administration of glucose resulted in persistent hyperglycaemia. Therefore it was suggested that fish resemble diabetic animals by having insufficient insulin for maximum carbohydrate utilization. However, the development of radio-immunoassay methods has shown that plasma insulin levels in fish are similar to, or often higher than, those observed in mammals (Mommsen & Plisetskaya 1991) and a non-insulin-dependent diabetes mellitus (NIDDM) situation was suggested (Hilton *et al.*1987). In addition, Gutierrez *et al.*(1991) have been able to show that rainbow trout muscle tissue has only 3-10% of the insulin receptors found in rat (per microgram of membrane protein) in either white or red skeletal muscle and that the overall insulin-receptor binding in trout is lower than that reported for mammals. However, these workers could not demonstrate a difference in insulin-receptor binding in skeletal muscle of trout fed a high-carbohydrate diet as compared to those fed a low-carbohydrate diet and concluded that glucose intolerance in fish does not seem to be a consequence of either insulin deficiency or impaired hormone-receptor binding and it may result from some defects in the postreceptor glucose-transport system (Gutierrez *et al.*1991).

Furthermore, researchers suggested that certain amino acids appear to be the most potent secretagogues[2] of insulin in fish (Cowey & Walton 1989; Mommsen & Plisetskaya 1991) while others have demonstrated that in certain fish (catfish, eel and salmon) somatostatin producing pancreatic D-cells are more sensitive to glucose than insulin-producing cells (Ronner & Scarpa 1984, 1987; Sheridan *et al.*1991). As somatostatin inhibits insulin release (Sheridan *et al.*1987) it is possible that this accounts for the lack of an increase, and sometimes a decrease, in plasma insulin levels following glucose administration in fish (Sheridan *et al.*1991).

An additional reason for the high blood glucose levels (low blood glucose tolerance) in fish is the different existence and utilization of the appropriate enzymes. In mammals, two hepatic enzymes serve as key

[2] (Med) Substance, e.g. hormone, which stimulates secretion. (Larousse Dictionary of Science and Technology, M. B. Walker ed., 1995, Edinburgh, EH7 4AZ)

enzymes in the regulation of blood glucose: hexokinase and glucokinase (Wilson 1994). Hexokinase has low specificity and a low K_m[3] for the sugar substrate whereas glucokinase is specific for glucose and has a much higher K_m. Although hexokinase activity has been measured in a variety of fish species, glucokinase has not been detected in fish tissue (Cowey *et al.*1977; Furuichi & Yone 1982). As simple CHOs are absorbed rapidly through the intestine, they result in high levels of blood glucose as the hexokinase enzyme is easily saturated by small substrate quantities and feedback inhibited by glucose-6-phosphate (Tung & Shiau 1991; Wilson 1994).

On the contrary complex CHOs are digested and absorbed slowly resulting in a better CHO utilization but at a much slower rate than the one that homeothermic animals experience (Wilson 1994).

c) Glucose tolerance and utilization can also be dependent on water temperature. Research on Atlantic salmon *Salmo salar* L., (Hemre *et al.*1995) revealed an increased starch adaptation and an improved protein-sparing effect from starch during warm-water periods compared with cold-water ones. In addition, the increased liver somatic indices and liver glycogen levels in the treatments with the better CHO utilization, suggest that salmon store excess glucose in the liver. The poor CHO utilization in certain combinations of dietary CHOs and temperatures was reconfirmed by the elevated plasma glucose levels even 72 hours post intra-peritoneal injection of glucose (Hemre *et al.*1995)

In conclusion, the long-term strategies of fish with regard to carbohydrates can be divided into three categories (Sheridan & Mommsen 1991):

- rapid starvation-induced glycogen depletion (similar to that observed in mammals)
- partial protection of glycogen reserves with up to 50-60% loss (*Anguilla japonica, Esox lucius, Gadus morhua, Oreochromis mossambicus*)

[3] K_m = Michaelis constant. The substrate concentration at which the speed of the reaction creating the enzyme-substrate complex, has reached half of its maximum value (Karlson 1980)

- complete protection of glycogen reserves for prolonged time periods of up to 6 months (*Anguilla anguilla, Anguilla rostrata, Chrysophrys major*)

When glycogen is conserved, energy demands are met by mobilization of lipid and gluconeogenesis from protein precursors (Sheridan & Mommsen 1991).

1.2.4 Recent understanding of protein and amino acid requirements

1.2.4.1 Body protein store and amino acid pool. Estimation of nutritional requirements

According to Cowey and Sargent (1972), "Dietary protein is necessary for three main purposes (i) maintenance, the making good of tissue wear and tear, (ii) the repletion of depleted tissues and (iii) growth or the formation of new additional protein".

The existence of a protein store in the body of mammals has been established long ago (Martin & Robison 1922 cited by Cowey & Sargent 1972): when a subject in nitrogen equilibrium on a high protein diet is transferred to a low protein diet, there is a lag period before nitrogen excretion attains a new low equilibrium level. When this *new, low* nitrogen equilibrium is attained and the animal is reverted to a high protein diet there is again an excretory lag phase and less nitrogen is excreted than ingested, for a time. This lag phase and the fact that nitrogen excretion follows an exponential pattern advocates the existence of a protein store in the body.

This protein store (which is in fact the cytoplasmic protein itself) does not seem to be very effective in all the different species of fish, as the interchange of low and high protein diets in plaice did not generate a similar pattern of liver enzymes, related to the intermediary metabolism of protein (Cowey & Sargent 1972). One of the possible reasons is that the allocation of dietary substrates for energy utilisation in fish seems to be quite different from that of farmed terrestrial animals with the result of considerable loss of dietary protein for energy purposes through the gluconeogenesis pathway (see also 1.2.4.3 & 4.4). Another possible explanation is that the control of aminoacid metabolism by deaminating

tissue enzymes is not uniform among the various fish species (see for the concept of "Coarse control" and its implications under the Paragraph 1.2.4.4).

Therefore, the concept of the body protein store can actually be extended to the concept of body amino acid pools (DeSilva & Anderson 1995). These pools rely mainly on three sources: dietary amino acids, amino acids from the catabolism of body proteins and synthesis of non-essential amino acids. The achieved steady state equilibrium in these pools is effected through a continuous exchange of the amino acids incorporated into the tissue proteins with the tissue free amino acids and eventually with the blood free amino acids. Some of the blood free amino acids are transported preferentially to the red blood cells (DeSilva & Anderson 1995).

A major concern to nutritionists engaged in nutritional research is the minimum amount of nutrient needed to produce maximal growth.

The evaluation of this nutrient requirement is usually based on the growth response plot of fish fed diets containing graded levels of the nutrient in question. This growth response plot usually takes the form of a dose- response curve that has been constructed by plotting growth against the concentration of nutrient supplied in the diet. Consequently, various regression techniques are employed for the analyses of dose-response data.

The broken-line response as a regression technique for estimating amino acid requirements has been widely used (Wilson *et al.*1980; Robinson *et al.*1981; Walton *et al.*1984; Coloso *et al.* 1991; Lall *et al.*1994; Tibaldi *et al.*1994; Ruchimat *et al.*1997). By this technique the requirement for a particular nutrient (essential amino acid) is determined by calculating the break point obtained by plotting the growth of the fish against the concentration of the nutrient. This break point is the one at which increased inclusion of the nutrient (essential amino acid) does not promote any further increase in the growth response and a plateau is reached. Requirements of several essential amino acids in various species were determined on such a linear relation between nutrient intake and weight gain. On this basis, amino acid requirements of fish have been expressed as a percentage of dietary protein, as well as on a dry-matter basis (Cowey 1994).

Alternatively a second order polynomial (quadratic) curve could be used, which is parabolic and takes into account the fact that some nutrients maybe toxic in high concentrations (Jobling 1998). Another non-linear model is the four-parameter logistic model employing the concept of saturation kinetics: the growth response is dependent upon the result of the outcome of a series of enzymatic steps, one of which is rate limiting (Finke *et al.*1987a; Finke *et al.*1987b; Finke *et al.*1989; Ghal *et al.*1991; Jobling 1998). This exponential model supports a more accurate assessment of the diminishing returns area of the response curve and of the maximum response when compared to the broken line response (Finke *et al.*1989; Cowey 1994).

Estimates of requirements obtained by using broken-line plots are usually lower than those obtained by the non-linear models (Asgard & Shearer 1997).

As indicated at the beginning of this paragraph, proteins are required firstly for growth (protein deposition) and secondly for a number of processes that are described as maintenance. As fish expend minimal energy for thermo-regulation, the maintenance component of their total protein requirement is much less than the respective one of monogastric terrestrial animals and birds, creating a higher piscine protein requirement for growth. This protein requirement for growth in fish varies directly with the water temperature and the salinity (Cowey & Walton 1989). The increase in the requirement of the dietary protein levels for growth, due to the increase in temperature, can partially be attributed to the concomitant increase in the K_m (Michaelis constant see page 29) values of deaminating enzymes. This entails an unavoidable decrease in their affinity for their respective dietary amino acid substrates. In order to compensate for this, more dietary substrate is needed, i.e. higher dietary protein levels.

1.2.4.2 The dietary protein: energy ratio and protein quality as a decisive factor in animal nutrition.

According to Boorman (1980) "Energy and Protein interact because dietary protein is a source of dietary energy, because dietary energy is needed for protein turnover and deposition and because deposited protein represents part of the body's energy store".

Metabolizable energy (ME) (Figure 4) is that portion of Digestible Energy (DE) which is capable of undergoing transformation within the body and represents the amount of energy that is effectively utilized for heat production (HiE and Hem) and growth (RE) by the organism (Cho & Kaushik 1985). Therefore it entails the quantitative assessment of the non-faecal energy losses: branchial (ZE) and urinary nitrogen excretion (UE) (Figure 4.).

Metabolizable energy (ME) values of diets have (and are) been widely used with terrestrial farmed animals (especially avian species) quite successfully. This is largely attributed to the terrestrial "environment" which facilitates the collection of non-faecal nitrogen excreta and estimation of their respective energy values. On the contrary, the collection of branchial and urinary excreta is extremely difficult in the aquatic environment. Therefore, researchers (Jobling 1983) have suggested the use of formulae such as:

Metabolizable Energy (kcal.g^{-1} feed) = $[D_E E - X(D_P P - Y)] / W$

where

D_E is the digestibility coefficient for energy (%)

D_P is the digestibility coefficient for protein (%)

E is the total energy consumed (kcal)

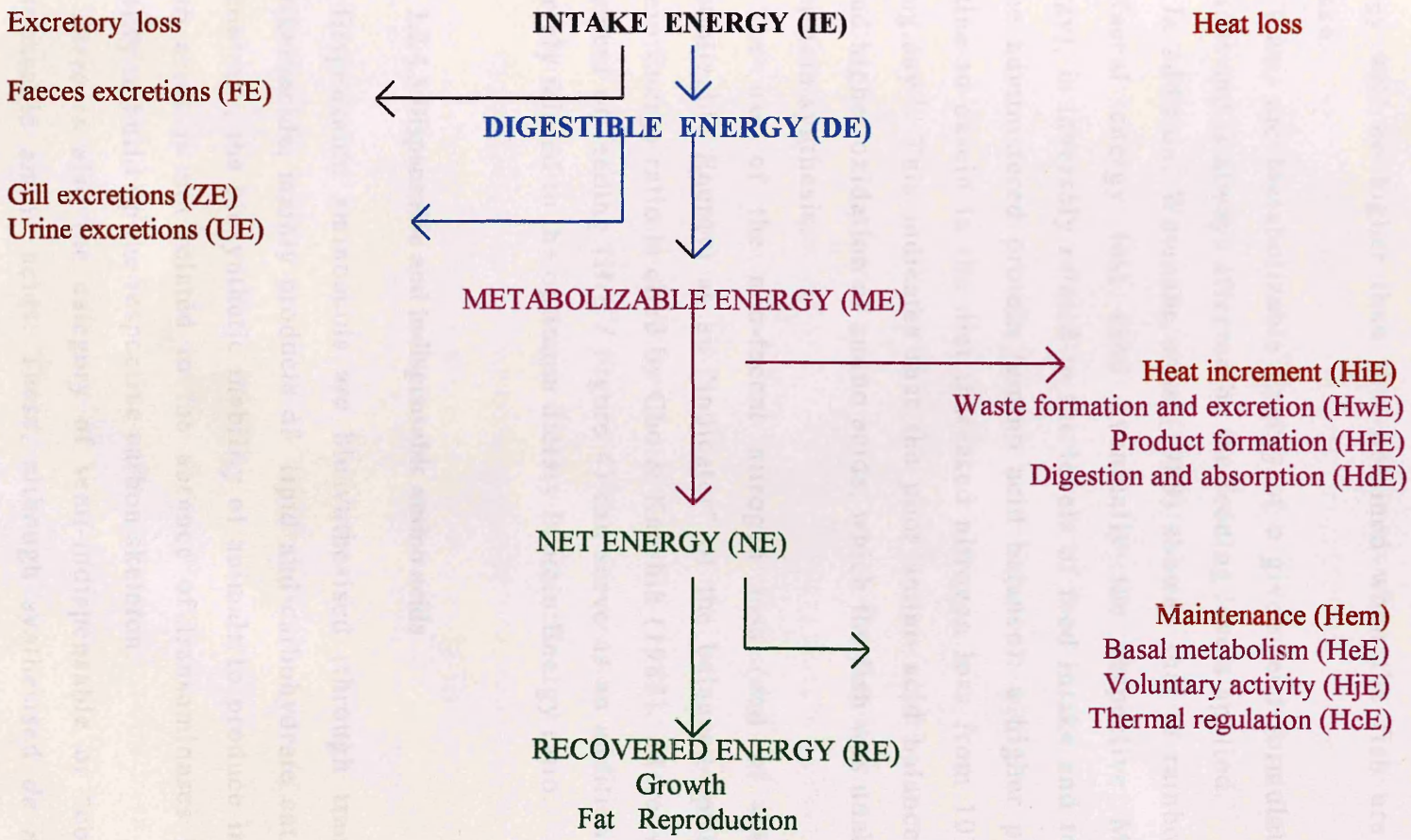
P is the weight of protein consumed (g)

X is the energy loss of non-faecal nitrogenous products per gram of protein deaminated (usually 0.95 kcal g^{-1} according to Elliot & Davison 1975)

Y is the weight of protein retained as growth (g)

W is the weight of food consumed (g)

FIGURE 4. Schematic presentation of the fate of dietary energy for fish, categorising the losses that occur as feed is digested and metabolised leaving a fraction of the energy to be retained as new tissue (after "Nutrient Requirements of Fish" NRC, 1993)



From the above formula, it is obvious that at one specific level of feeding fish will be in nitrogen balance, i.e. Y=0. That means that all the digested protein has been deaminated and the remaining energy used metabolically. At higher feeding levels, a certain proportion of the protein will be deposited as growth (Y > 0), hence the estimate of Metabolizable Energy will be higher than that obtained when the fish are in nitrogen balance.

Thus, the Metabolizable Energy of a given feed formulation is not a constant and is always affected by the feeding levels applied.

In addition, Watanabe *et al.*(1983) showed that in rainbow trout, the non-faecal energy loss (and eventually the respective Metabolizable Energy), is inversely related to the levels of feed intake and to the quality of the administered protein (amino acid balance): a higher proportion of gelatine to casein in the diet increased nitrogen loss from 107mg.day^{-1} to 153mg.day^{-1}. This indicates that the poor amino acid balance of gelatine caused higher oxidation of amino acids, which the fish was unable to utilize for protein synthesis.

The use of the non-faecal nitrogen loss (and of the respective Metabolizable Energy) as an "indicator" of the balanced-optimum dietary Protein:Energy ratio is cited by Cho & Kaushik (1985). Moreover, the Heat Increment of feeding (HiE / Figure 4) can serve as an additional indicator, inversely related to the optimum dietary Protein:Energy ratio.

1.2.4.3 Dispensable and indispensable amino acids

Dispensable aminoacids are biosynthesised (through transamination) by α-ketoacids, mainly products of lipid and carbohydrate catabolism. On the contrary, the biosynthetic inability of animals to produce indispensable amino acids is not related to the absence of transaminases but to their inability to build up the respective carbon skeleton.

There is also the category of semi-indispensable or "conditionally" indispensable amino acids. These, although synthesised *de novo* by the organism, are needed in such high levels, because of metabolic impairment or during specific developmental stages, that the animal's biosynthetic

ability cannot fulfil the requirements (Visek 1984). Such amino acids include arginine for rats and swine and cysteine and tyrosine for birds (Borman *et al.*1946; Mertz *et al.*1952; NRC 1984, 1988).

Furthermore, meeting the amino acid requirements of a certain animal at a certain life stage should be thoroughly considered as some essential amino acids (methionine and phenylalanine) are readily converted to non-essential ones (cysteine and tyrosine respectively) if the latter are lacking (DeSilva & Anderson 1995). Because cysteine and tyrosine are exclusively synthesized from their precursors methionine and phenylalanine, they have the characteristics of indispensable amino acids if their precursors are not present in a diet in adequate amounts (Harper 1983). Therefore the term "semi-indispensable" is considered by many researchers as self-contradictory, unsatisfactory and should be replaced by the term "conditionally" indispensable (Harper 1983).

In addition, the distinction between dispensable and indispensable amino acids is species- and amino acid-dependent as most of the indispensable ones (except lysine and threonine) were successfully replaced by their alpha keto analogues in chicks and other animals (Bell *et al.*1981; Boebel *et al.*1982; Close 1974).

Protein synthesis is also regulated by the "limiting" amino acid. This is defined as that which is in the lowest proportion (among the rest of amino acids) when compared to its respective level in an "ideal" protein, usually egg albumin or the experimentally determined requirement. In this case the levels of this limiting amino acid dictate the quantities of amino acids used for protein synthesis and their surplus is degraded for energy purposes or even lipid synthesis (Hepher 1988). The concept of first or second limiting amino acid also varies (within the same species) with the age and developmental stage of the animal. In rats fed Mormon Cricket Meal (*Anabrus simplex*, Haldeman) as the protein source in purified diets, methionine is clearly the first limiting amino acid when used for growth, but not the one when used for maintenance (Finke *et al.*1987b).

Fish have the same qualitative essential amino acid requirements as young mammals.

Fish and birds lack the urea cycle, which in growing mammals can serve as a source of about 75% of the arginine needed. Arginine requirements in marine teleosts (Alliot & Pasteureaud 1984) and euryhaline teleosts kept in sea water (Kaushik 1979; Kaushik *et al.*1988) were found to be considerably lower than those of freshwater species. This suggests increased activity of urea-cycle enzymes in marine teleosts (Huggins *et al.* 1969). However, the apparently large spread in the values of the arginine requirements of marine or euryhaline species studied to date implies that there is no general evidence for concluding that salinity *per se* influences the arginine requirement of fish (Tibaldi *et al.*1994)

As can be seen from Table1, arginine requirements show the widest variation among different species. The arginine requirement of fishes and birds (as % of the dietary protein) is similar and high. The arginine requirement of growing mammals is low.

Fish appear to have the lowest tryptophan requirement when compared to terrestrial species (0.45%-1% of the dietary protein).

Differences in leucine and isoleucine requirements are also noted in Table 1. Fingerling salmon, juvenile carp and channel catfish have a lower isoleucine requirement than the terrestrial species whereas chicks have a high leucine and methionine requirement possibly due to feathering (NRC 1984). Chicks also differ from the other three species in having an absolute requirement for either glycine or serine.

Despite these inter-specific differences in amino acid requirements expressed as % of the dietary protein (Table 1), there is a similarity in the metabolism of nitrogenous compounds between animals that appear quite distant on the phylogenetic scale (Buttery 1979). Furthermore the mechanisms of protein synthesis appear to differ only in minor aspects throughout the animal kingdom (Visek 1984; Munro 1976). There appears to be great similarity in content of essential amino acids in tissues whether one examines food producing ruminants or single stomached mammals or even poultry (Smith 1980). Therefore Smith (1980) suggests "the apparent differences in composition of organs or histologically similar tissues are mainly due to analytical methods" and "it is likely that a standard animal

can be conceived to predict amino acid needs and to guide research in areas where data are not available from direct observations".

Table 1. Amino acid requirements of different species [a]

Amino Acids	*Swine*[1]	*Chicken*[2]	*Rat*[3]	*Salmon*[4]	*Common Carp*[5]	*Channel Catfish*[6]	*Nile Tilapia*[7]
Arginine	2.22	5.58	1	5.4	4.3	4.3	4.2
Histidine	1.38	1.42	2.1	1.57	2.1	1.5	1.72
Isoleucine	2.94	3.33	3.9	1.97	2.5	2.6	3.1
Leucine	3.89	5.58	4.5	3.52	3.3	3.5	3.39
Lysine	5.27	3.75	5.4	4.5	5.7	5.1	5.12
Methionine + Cystine	2.67	3.33	3	5.85	3.1[b]	2.3[b]	2.68+0.54
Phenylalanine + Tyrosine	4.28	5.58	5.3	5.5	6.5+2.6	5+1.25	3.75+1.79
Threonine	3.11	3.083	3.1	2.03	3.9	2	3.75
Tryptophan	0.78	0.92	1	0.45	0.8	0.5	1
Valine	3.11	3.42	3.1	2.93	3.6	3	2.8

a. Expressed as percentage of the dietary protein. Original authors can be traced on the given referred sources as they vary from one amino acid to another within the same species

b. In the absence of dietary cystine

1. Requirements for a live weight range of 10-20 kg and based on a diet of 18% Crude Protein / After NRC (1988) and (Church) 1991
2. Requirements for Leghorn-Type chickens of 14-20 weeks and based on a diet of 12% Crude Protein / Chicken also have a Glycine+Serine requirement of 0.47% of the diet (as fed). / After NRC (1984)
3. After Gahl *et al,* 1991 and based on various levels of dietary Crude Protein ranging from 13 to 20%
4. Requirements of juvenile Chinook Salmon *Oncorhynchus tshawytsha* based on a 40% and 41% Crude Protein diet (NRC 1993)
5. Requirements of juvenile common carp based on a 38.5 % Crude Protein diet (NRC 1993)
6. Requirements of juvenile channel catfish and based on a 24% Crude Protein diet (NRC 1993)
7. Requirements of juvenile Nile tilapia and based on a 28% Crude Protein diet (NRC 1993)

1.2.4.4 Control of amino acid metabolism- Intra-specific amino acid relationships

1.2.4.4.1 Control of amino acid metabolism and intra-specific amino acid relationships in terrestrial animals

In omnivorous mammals (Cowey & Walton 1989; Hoar *et al.* 1987; Krebs 1972) control of amino acid catabolism is considered to depend on two factors:

a. a coarse control, that is the tissue level of amino acid deaminating and degrading enzymes

b. a fine control, which consists of the control that the K_m values of these deaminating enzymes exert on the breakdown of their respective aminoacid substrates (and all the factors that control the change of the K_m values).

Coarse control implies that at high protein intake, levels of amino acid deaminating enzymes in the tissues of omnivorous mammals increase by several fold compared with their levels in animals given a low protein intake (there is actually a threshold in protein intake above which proteolytic enzymes are activated and is based on the theory of enzyme activation by an increased substrate level). This is especially true of enzymes that degrade essential amino acids; under conditions of dietary protein restriction, when conservation of essential amino acids is necessary, these enzymes are found in very low concentrations (Krebs 1972; Aebi & Berger 1980) .

Omnivorous mammals are able to control amino acid catabolism according to their protein requirements, the level of protein intake and the biological value of ingested protein. If requirements for at least one of the essential amino acids (EAA) are not met, the utilisation of all the rest of the EAA is impaired as well, which leads to inefficient protein utilisation and an increase in protein intake. Though, in the case of limiting aminoacids, mammals are able to use selectively these aminoacids for protein synthesis and not for energy purposes (gluconeogenesis) (Kalaisakis 1982).

Because of all the above regulating mechanisms in mammals, plasma aminoacid levels have been used in the past in order to estimate the amino

acid fluxes among various tissues and the integration of whole-body amino acid metabolism in general. It was found that plasma surely reflects amino acid transport but it does seem to underestimate total blood amino acid transport. There was at least some blood cell transport of glutamine, glutamate, taurine, glycine and leucine and in the case of the first three the whole-blood transport exceeded that of the respective plasma (Bergman & Heitmann 1980).

On the contrary, in humans alanine transport is effected by way of plasma at a rate of 75 to 93%, whereas in sheep, alanine transport accounts for 86% of the whole blood transport (Bergman & Heitmann, 1980).

The whole-blood (or plasma) alanine concentration, as a reflecting image of the quantity hydrolysed in the intestinal lumen and the respective quantity taken by the tissues, can easily be distorted due to the alanine cycle (Figure 5) and due to an energy deficient diet. A non-balanced dietary Protein: Energy ratio will result in excessive gluconeogenesis from amino acids. Blood glucose and alanine will be *de novo* synthesized and disproportionately increased, without reflecting the dietary levels and the respective tissue requirements.

Similar problems can be encountered in the arginine whole blood-plasma image as a result of urea cycle amino acid transport among tissues of sheep (Figure 6). Kidneys, and to a lesser extent muscle, remove citrulline and ornithine from the blood and add arginine. Conversely, liver removes arginine and adds citrulline, ornithine and urea.

Another critical factor affecting the whole-blood or plasma amino acid levels as a reflecting image of the quantity hydrolysed in the intestinal lumen and the respective quantity taken by the tissues, is the intestinal transport mechanism involved in the absorption of amino acids (Mathews 1991; Munck & Munck 1994; Jobling 1998). These intestinal transport mechanisms are classified into systems transporting several amino acids within the same category (neutral, basic, acidic, imino acids). Therefore competition exists among amino acids for binding to the same transport sites. In addition, similar transport mechanisms exist for the absorption of di- and tripeptides, larger peptides or even whole proteins (Jobling 1998).

FIGURE 5. Diagram illustrating alanine and glutamine as major forms of amino acid and nitrogen transport between tissues of sheep (adapted from Bergman and Heitmann, 1980)

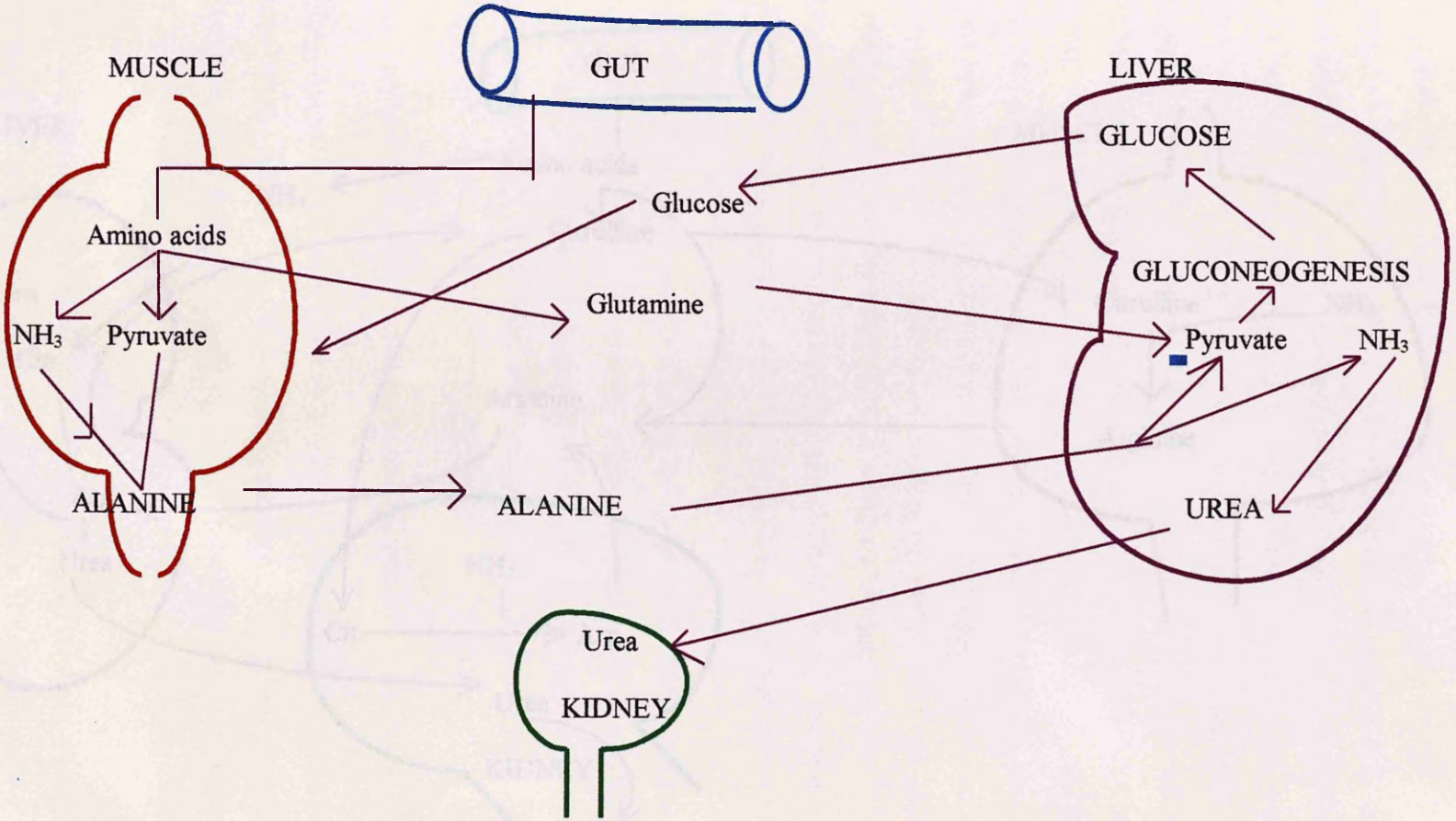
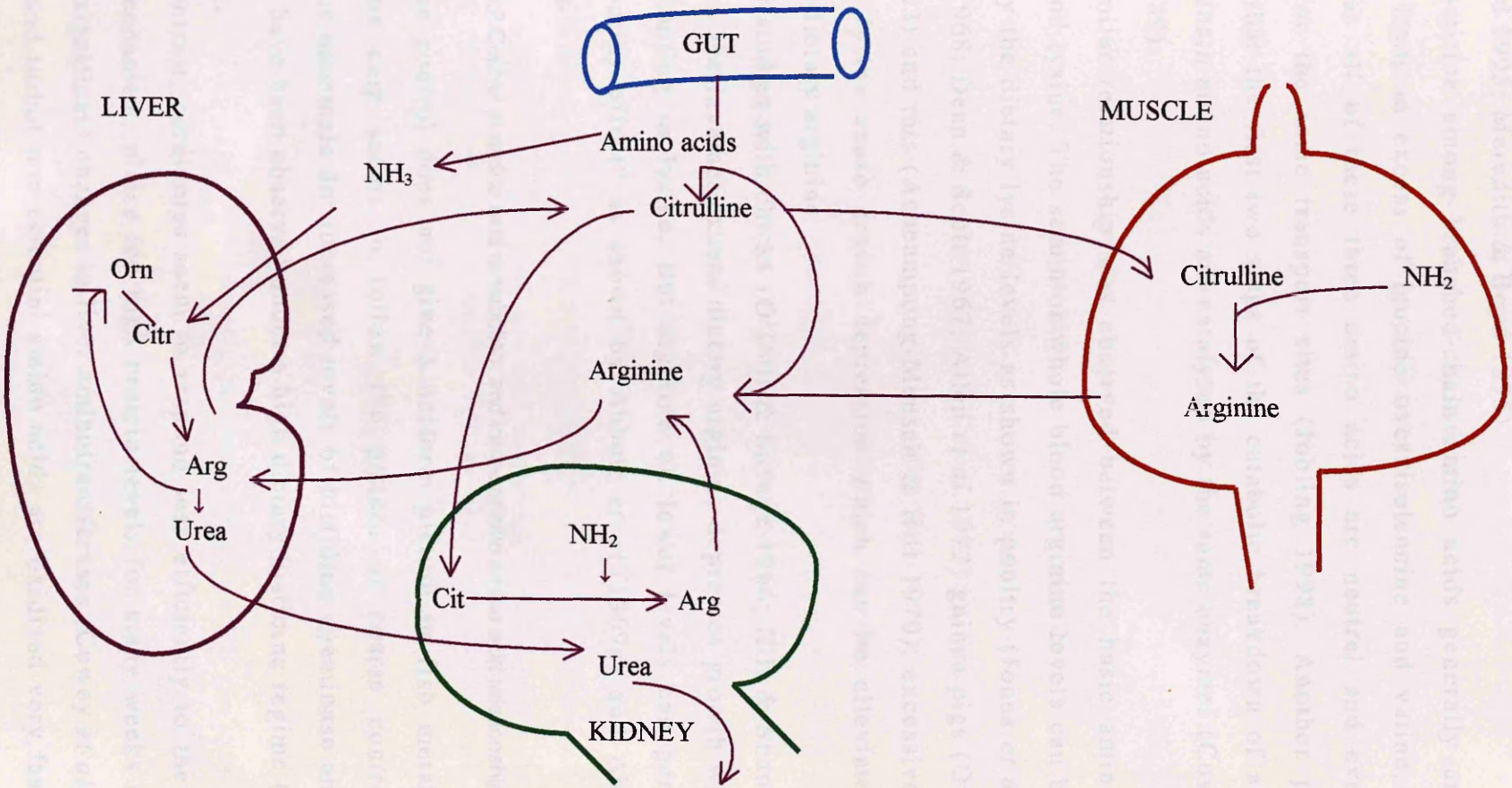


FIGURE 6. The urea cycle amino acids as a form of nitrogen transport between tissues of sheep. Arginine transports ammonia in a non-toxic form from kidneys and muscle so that the liver forms urea and releases ornithine and citrulline. (adapted from Bergman and Heitmann, 1980)



Many researchers have concluded that di- and tripeptide transport is more effective through the gastrointestinal tract and therefore of higher significance than the transport of free amino acids (Matthews 1991; Hirst 1993; Boyd 1995; Meredith & Boyd 1995)

Competition among branched-chain amino acids generally arises in mammals from an excess of leucine over isoleucine and valine; this is expected as all of these three amino acids are neutral and eventually compete for the same transport sites (Jobling 1998). Another possible reason is that the first two steps of the catabolic breakdown of all three branched-chain amino acids are catalyzed by the same enzymes (Cowey and Walton 1989).

A similar relationship was observed between the basic amino acids arginine and lysine. The serum or whole blood arginine levels can be quite affected by the dietary lysine levels as shown in poultry (Jones *et al.*1967; Nesheim 1968; Dean & Scott 1967; Allen *et al.*1972) guinea pigs (O'Dell & Regan 1963) and rats (Acheampong-Mensah & Hill 1970): excessive levels of dietary lysine cause growth depression which can be alleviated with additional dietary arginine.

Other studies with chicks (O'Dell & Savage 1966; Hill & Shao, 1968) have shown the inverse: excess dietary arginine depresses growth when fed in diets limiting in lysine. But arginine on lower levels can perform a "lysine sparing effect" as shown by Abbott *et al.*(1969a) and Abbott *et al.*(1969b).

1.2.4.4.2 Control of amino acid metabolism and intra-specific amino acid relationships in fish

Coarse control does not give a uniform picture in fish metabolism. Omnivorous carp seems to follow the pattern of coarse control that omnivorous mammals do: increased levels of histidine apaminase and liver urocanase have been observed under a high dietary histidine regime (Cowey 1980).

In contrast, carnivores seem to respond less efficiently to the coarse control mechanism: plaice fed high protein levels for many weeks did not show any significant changes in liver aminotransferases (Cowey *et al.*1974). In plaice and turbot non essential amino acids are oxidized very fast (50%

of their total quantity in the first 24 hours) and EssentialAminoAcids were oxidized irrespective of their levels in the diet (Cowey 1980).

A series of investigations regarding the effects of dietary protein content on the activities of a number of enzymes revealed that fish fed a high protein - low carbohydrate diet had decreased levels of glycolytic enzymes, increased levels of gluconeogenic enzymes whereas there was little effect on the enzymes of the TCA cycle or those enzymes which initiate amino acid catabolism (Cowey & Walton 1989).

The existing transport mechanisms in mammals (1.2.4.4.1) for the absorption of di- and tripeptides, larger peptides or even whole proteins, have been verified for fish as well (Ash 1985). As a result the emerged "peptide uptake theory" suggested that the absorption of small peptides (resulting from hydrolysis of protein in the gastrointestinal tract) by the intestinal mucosa is more efficient and less energy consuming than the absorption of free amino acids (Walton & Wilson 1986; Murai *et al.*1981). Some species are even able to absorb macromolecules of protein (Boge & Peres 1983). Noaillac-Depeyre & Gas (1973) have found that molecules of horseradish peroxidase are transported through the epithelium of an adult common carp mid-gut without modification, either directly into the blood circulation or to be accumulated in supra-nuclear vacuoles of the epithelium where they are decomposed by lytic enzymes. Therefore, the feeding of diets high in crystalline amino acids may preclude their optimal intestinal absorption (Plakas & Katayama 1981; Murai *et al.*1982a). This in turn will negatively affect the rates of protein synthesis, tissue deposition and growth, as simultaneous presentation of the EAA at the site of protein synthesis is required. On the contrary when the rapid absorption of free amino acids is retarded or the amino acids are part of natural proteins, tissue imbalances do not arise and the dietary protein is well utilized for growth (Wilson 1989; Cowey 1994).

Cowey *et al.* (1977) showed that trout is able to perform better utilisation of EAA when fed a low protein diet: the decrease in the concentration of EAA in the plasma, was greater than that of non-essential amino acids so that some selective tissue uptake-conservation of essential amino acids occurred.

This picture was confirmed for certain amino acids in the channel catfish *Ictalurus punctatus*: the concentration of the serum free amino acids lysine, L-methionine , L-cystine and threonine remained relatively low following the administration of deficient (in these amino acids) diets; however the serum concentration of these amino acids increased as their dietary requirement was exceeded (Wilson *et al.*1977; Wilson *et al.*1978; Harding *et al.*1977).

This relationship was not confirmed for leucine in the channel catfish where the serum free leucine levels remained constant regardless of dietary leucine intake (Wilson *et al.*1980). There was, however, an effect of dietary leucine on the serum free isoleucine and valine levels: the utilization of isoleucine and valine was impaired (elevated serum concentrations) below the 0.7% dietary leucine level and was restored only after a dietary leucine level of 1.2% or above was fed. Therefore it appears that leucine facilitates both the tissue uptake of branched-chain amino acids as well as their intra-cellular metabolism.

Data on leucine- isoleucine competition in other species are contradictory: the isoleucine requirement of Chinook salmon *Oncorhynchus tshawytscha* increased slightly with increasing concentrations of dietary leucine (Chance *et al.*1964). In lake trout valine deficient fish poorly utilized leucine and isoleucine unless valine was increased (Hughes *et al.*1983). In contrast rainbow trout showed a high tolerance for dietary leucine; no growth depression occurred when concentrations were as high as 9.2%. Even with excessive leucine concentrations of up to 13.4%, the concentrations of free valine and isoleucine in plasma, liver and muscle were not depressed (Choo 1990).

The effects of feeding disproportionate levels of lysine and arginine (1.2.4.4.1) have been evaluated for a few fish species as well: Robinson *et al.* (1981) showed that adult channel catfish (200g) are not as sensitive to lysine and arginine unbalanced ratios as was observed in other animals. Kaushik & Fauconneau (1984) presented evidence for a lysine-arginine antagonism in rainbow trout on the level of ureagenesis, while Kim *et al.* (1992) found the arginine requirement of fingerling rainbow trout to be

higher when dietary lysine exceeded the requirement value by up to 85%. Kaushik *et al.* (1988) reported a slight growth depression when 50% excess lysine was fed in a diet adequate in arginine for 100g rainbow trout. Excessive arginine intake has been shown to depress growth rate and feed conversion in Nile tilapia (Santiago & Lovell 1988) and milkfish (Borlongan 1991). On the contrary excessive arginine intake had no adverse effect on the performance of sea bass (*Dicentrarchus labrax*) fingerlings suggesting a lack of sensitivity of the species to moderate disproportion of dietary arginine and lysine (Tibaldi *et al.*1994).

All these data suggest varied protein utilisation among various species and consequently varied levels of application regarding the concepts of "protein and amino acid body pool". Alternatively, many of these inter-specific differences in protein utilization, maybe artefacts of experimental design and not true species differences (Jauncey 1999, pers. communication). Cowey (1994) suggested the following factors as the most likely cause of these variations: a. the very low growth rates achieved during some of these experiments b. environmental and water physico-chemical factors classified as "laboratory variances" c. variable availability of amino acids as a result of different "reference proteins" d. variable energy densities among diets of different experiments.

As "coarse control" of amino acid metabolism is very often variable among different fish species (beginning of this paragraph 1.2.4.4.2), the required metabolizable energy varies considerably as well. Therefore, differences in amino acid requirements as % of the dietary protein (Table 1, page 38) have little comparative value and should be better expressed as % of dietary energy intake (Cowey 1994). This further supports the statement of Smith (1980) regarding the uniform levels of tissue amino acids among various species in the animal kingdom (1.2.4.1, pp.31).

The concept of amino acid pool was proved for histidine by Nose (1979) when trying to establish the quantitative amino acid requirements for *carp*: individuals raised on an histidine deficient diet did not loose any

weight during the first two weeks of the trial, most probably because of the large amount of free histidine stored in the tissues.

Similar research by Kaushik (1979) for rainbow trout showed that the incorporation of arginine in the diet above the required levels creates an abrupt rise in the free arginine levels of muscle and blood plasma and therefore increases the arginine tissue pool. On the contrary, Robinson *et al.* (1981), failed to demonstrate a similar pattern for the serum arginine levels in the channel catfish *Ictalurus punctatus*, as a result of graded dietary arginine levels.

It is quite understandable that the rate of amino acid appearance in fish blood is not only affected by the nature of the diets used (purified materials, crystalline amino acids, commercial raw materials) but also by the digestive system-type of the specific fish. Zebian (1977) found that the level of circulating plasma amino acids (except arginine and lysine) in carp fed either casein or an amino acid mixture, peaked 4 hr after feeding. In contrast, when carp were fed a commercial diet, the maximum blood concentration of most essential amino acids (except histidine) was achieved 24 hr after feeding.

Furthermore it was shown that carp fed a crystalline amino acid mixture experienced poorer growth rates than when fed a casein diet (Aoe *et al.*1970; Plakas *et al.*1980). The observed amino acid competition due to the incorporation of crystalline amino acids in the diet, was confirmed by the plasma EAA and ammonia levels: all plasma amino acids (except alanine) were in higher concentrations in the fish fed the casein diet than in those fed the amino acid diet. In addition plasma amino acids of the fish fed the casein diet reached their maximum levels 4 h after feeding whereas the plasma amino acids of the animals fed the amino acid diet reached their peak levels between 2 and 4 hours after feeding, implying a non uniform utilization of the dietary amino acids (AA) as a result of variations in the timing of gastrointestinal absorption of individual AA (Plakas *et al.*1980). This was also reconfirmed by the higher plasma ammonia levels of fish fed the amino acid diet at 2 hours after feeding, indicating a higher rate of amino acid degradation after the administration of such a diet. Further research by Plakas & Katayama (1981) reconfirmed the previous hypothesis

of differential timing of gastrointestinal absorption giving more evidence in favour of the "peptide uptake theory".

Rainbow trout shows an efficient utilization of crystalline synthetic amino acid mixtures, casein, or even fishmeal based diets. It was suggested that a very slow release of amino acids from protein takes place in rainbow trout stomach and amino acids given in a free form in the diet, have been retained in the stomach 14 hr after a meal (Dabrowski & Dabrowska 1981). This is an extra advantage for a better-synchronized presentation of amino acids in the sites of protein synthesis according to the "peptide uptake theory"

In conclusion, the mechanisms of protein and amino acid metabolism in fish, although quite similar to these of terrestrial animals, vary widely and tend to be more species specific.

1.2.4.5 Methodologies employed for the determination of amino acid requirements and "troubleshooting" arising from these

1.2.4.5.1 Determination of the quantitative EAA requirements based on diets composed of purified materials, crystalline amino acids and graded levels of the amino acid in question

One of the most common methodologies employed (Chance *et al.*1964; Jauncey *et al.*1983; Wilson *et al.* 1980; Tibaldi *et al.*1994) is the creation of a basic diet comprised of casein, gelatine, and crystalline amino acids and with an amino acid profile similar to that of whole egg protein and adjusted for the optimum total protein level required for the species in question. Additional isonitrogenous and isocaloric diets are created by altering the ratios of the non-essential amino acids and non protein materials (i. e. dextrin) in order to create graded levels of the amino acids in question. The performance of fish fed these diets is compared with those fed the basic diet and conclusions are drawn based on various indices.

Complementary to the above methodology is the picture created by the plasma free amino acid levels and in some cases, urea levels as well:

Amino acids have the tendency to be stored in the aminoacid pool of the body, especially when the dietary intake is in excess of the respective requirements (1.2.4.1). In addition, in some teleosts (sea bass *Dicentrarchus labrax*) characterized by increased activity of urea-cycle enzymes (1.2.4.3), plasma urea levels disproportionately increase when amino acid requirements are met and the excess amino acids are degraded for energy purposes (Tibaldi *et al.*1994).

Therefore many researchers (Kaushik 1979; Walton & Wilson, 1986) have successfully employed plasma and tissue free amino acid levels as a means of confirming the results obtained by the comparison of performance indices.

Another useful index regarding the dietary required amino acid levels and their efficient utilization is the Heat Increment (HI / 1.2.4.2), as it is related to the biochemical oxidation processes that follow the ingestion of a meal and it is influenced by the quality, quantity and balance of dietary

components. Therefore, HI is in part a measure of the energy cost for the incorporation of amino acids into proteins. Kaczanowski & Beamish (1996) showed that in rainbow trout (*Oncorhynchus mykiss*), excessive levels of dietary lysine create poor utilization of arginine and the rest of the dietary amino acids, as increased levels of plasma arginine and Heat Increment were observed.

Problems arising from the above methodology:

Diets containing large quantities of free amino acids have frequently been found to result in growth rates inferior to those obtained by diets composed of commercially available proteins and of similar overall amino acid composition (1.2.4.4.2). The most plausible explanation is the competition among amino acids of similar chemical affinity for transport sites in the intestinal mucosa with a result in differential rates of presentation of these amino acids at sites of protein synthesis (1.2.4.4.1 & 1.2.4.4.2). In addition, a high level of free amino acids may act as an appetite depressant (Lall 1991a).

Another problem arising from the above methodology is the limited number of essential amino acids whose requirements can be determined at a time: if the requirements of all the ten EEA are to be determined within the same experiment, the total number of experimental diets to be prepared will be at least sixty (six graded levels of the same amino acid multiplied by ten EAA) requiring a minimum of hundred eighty replicates (sixty diets multiplied by three for a triplicate) and a minimum of eighteen hundred fish (180 x 10) in order to fulfil a proper experimental design-methodology.

"Troubleshooting" related to the plasma free amino acid levels

As mentioned earlier (1.2.4.4.1. & 1.2.4.4.2) the picture created by the plasma free amino acid levels is not very clear when dealing with amino acids of similar chemical affinity (i.e. valine, leucine and isoleucine, methionine, valine and threonine, lysine and arginine) due to the antagonism and species-specific inter-relations of these.

1.2.4.5.2 Determination of the quantitative EAA requirements by correlation of the tissue pattern and the requirement pattern

This methodology assumes that the EAA pattern of whole fish and muscle is equivalent to the requirement pattern (Boorman 1980; Cowey & Tacon 1983; Mambrini & Kaushik 1995). Therefore, after having established the requirement for one amino acid and the EAA profile of tissue and muscle, one can extrapolate and find the requirements for the rest of amino acids, based on their relative proportions within their total sum (Jauncey *et al.*1983).

1.2.4.5.3 Determination of the quantitative EAA requirements by the "daily deposition" method

This methodology was first introduced by Ogino (1980) and later employed by other researchers as well (Jauncey *et al.* 1983). It assumes that the EAA requirements of a certain species are similar to the daily deposition rate of these EAA on the entire carcass and that all absorbed EAA are selectively conserved and retained in the carcass as protein and not catabolized for energy purposes.

1. 3 RESEARCH AIMS OF THIS STUDY

Feeding cost accounts for up to 40% of total cost of aquaculture production (Shang 1981). Low feed cost does not necessarily mean low cost of feeding as growth performance and efficient nutrient utilization determine the profitability of the diet. In Asian countries where *Clarias gariepinus* is prized as a highly valued product, feeding cost for the species varies between 5% to 100% of the farm gate value of the final product and depends on the production system, the source and type of raw materials used in feed formulation and the feed manufacture practices (1.1.1.1). A cost-effective catfish production system necessitates the standardization of feed raw materials and feed manufacture practices. This will be realized only after the exact determination of the nutrient requirements of the species has been achieved. At the conclusion of his experiments, Uys (1989) enunciates that "it can be said with certainty that the dietary protein requirement of this animal is in excess of 40%". Furthermore, the results (1.1.2.3) of Machiels & Henken (1985) based on purified diets suggest a vague nutrient profile of above 40% Crude Protein and a Crude Lipid level between 11.5% and 14% of the diet. Therefore, it was felt advantageous to establish an optimum dietary protein and energy ratio, based on purified diets, with a much greater degree of certainty than complex foodstuffs (fishmeal, soya, wheat) have, so far, permitted (1.1.2.3). Results of a well substantiated Protein: Energy experiment based on purified diets would inevitably improve the cost-effectiveness of feeds for this species and optimise the culture conditions under any form of aquaculture exploitation (semi-intensive or intensive).

Therefore the following experimental procedure was followed:

- Establishment of the digestibility of specific raw materials and of the diets to be used in the Protein: Energy experiments
- Experiments in order to define the optimum dietary Protein: Energy ratio for maximum growth
- Determination of the quantitative amino acid requirements by the "daily deposition method" and based on the results of the previous experiment

CHAPTER 2. GENERAL MATERIALS AND METHODS

2.1 WATER QUALITY PARAMETERS

Samples were filtered through a Glass microfibre GF/C 2 micron filter paper. A Technicon II Autoanalyzer was used for the determination of total Ammonia, Nitrites and Nitrates employing spectrophotometric methods as described by Golterman (1978) (for total Ammonia) and Strickland and Parsons (1972) (for nitrites and nitrates).

2.2 FISH STOCK, EXPERIMENTAL FACILITIES AND HUSBANDRY

In all but the digestibility experiments, fish were kept in 50 litre cylindrical tanks, as shown in Plate One. These tanks were part of a recirculated water system in the Tropical Aquarium of the Institute of Aquaculture, Stirling University (IoASU).

Fish were obtained from broodstock kept at the IoASU Tropical Aquarium. Production of experimental fish involved the following stages (Haylor 1992).

2.2.1 Selection of Broodstock

A "plump" female was selected and anaesthetized (2.2.7). A canula (outside diameter 2.0-2.5mm, bore 1.2-1.5mm) was inserted 2-4cm into the ovary via the oviduct. By applying suction to the canula and whilst withdrawing it eggs, if present, were retained within the canula. Good quality eggs should be green-brown and not soft. If the female was considered suitable it was isolated in a separate lidded tank.

2.2.2 Hormone preparation

2.2.2.1 Carrier solution

0.8 g NaCl, 0.1g Sodium metabisulphite and 0.25g bovine serum albumen (Sigma A-2153) were dissolved in 100 ml de-ionized water. This solution was stored in stoppered bottle in the fridge.

PLATE 1. The recirculating system located at the Tropical Aquarium of the Institute of Aquaculture University of Stirling and comprised of 50 L cylindrical tanks.

2.2.2.2 LHRHa

1mg LHRHa (Sigma L-4513) was dissolved in 1ml cold de-ionized water and divided into 100 µl aliquots, each containing 100 µg LHRHa . These were stored in a freezer at - 20^0 C.

2.2.2.3 Preparation of solution for injection (treatment for 1kg fish)

Using a dust mask and gloves, 5mg pimozide were weighed (Sigma P-1793) into a small screw-topped container. 2ml carrier solution was added and the container agitated vigorously to suspend pimozide. The suspension was stored on ice until required. A 100 µl aliquot of LHRHa was thawed and mixed thoroughly with carrier / pimozide suspension and the final suspension was kept on ice until used.

2.2.3 Injection of Broodstock

This usually took place around 5.00 pm.

An isolated female was anaesthetized (2.2.7), weighed and injected with the final hormone suspension at a rate of 2.0 ml per 1kg of fish. The dose was administered behind the base of the pectoral fin; fish were allowed to recover from anaesthesia and returned to their tank.

2.2.4 Collection of milt

This usually took place at 9.00 am the day following injection.

A male was sacrificed and the testes removed intact, kept dry until used and all blood and connective tissue removed.

2.2.5 Stripping and fertilization

According to previous experience of the personnel of the Tropical Aquarium of IoASU, stripping of the injected female usually takes place not earlier than sixteen hours from the injection time. A practical way to assess the right timing is the condition of the uro-genital papilla, which is distended, with eggs coming out free from the genital pore and with minimum pressure on the abdominal area.

The female was stripped into a dry bowl using only enough force to expel the eggs. Testes were laid on a clean piece of fine netting and sliced open with a scalpel. The net was formed into a bag around the testes and the milt was squeezed out over the eggs.

About 100ml of clean water was added to the eggs and milt and mixed thoroughly. Eggs were left for 1-2 minutes and then poured into an incubation tray.

2.2.6 Incubation, hatching and larval rearing

Incubation took place in plastic trays (50 x 76 x 6 cm) suspended on a metallic frame (1.2m x 0.84m). These formed part of a small recirculating system (Plate Two) comprising a 60L sump tank, a second tank similar to the former and connected to it, full of structured bio-rings[4] to act as a biofilter (Robertson 1992) and two heating elements (300Watt / 50-60Hz) to keep the temperature constant at 25-26^0 C. A small pump with an integrated mechanical filter ensured recirculation of water in the system at a rate of 1L.min^{-1} per tray. An Ultra Violet system (Ultra Violet Sterilizer Model 30/ 240 V /30 W, Tropical Marine Centre Ltd., Solesbridge Lane, Chorleywood, Herts) was interpolated between the pump and the pipe used as the main inflow of the system.

Eggs were laid on a rectangular framed mesh (0.5mm) sitting in the above-mentioned trays, with an approximate density of 300,000 eggs.m^{-2}. Hatching started at 27 h (25-26^0 C) and was generally completed 30h after fertilization. On completion of hatching the framed mesh was removed. Larvae were kept in the dark until the onset of exogenous feeding (Haylor 1992).

First feeding by *Artemia salina* hatched cysts was effected 48h after hatching. Larvae were kept in the hatching trays until Day 3 of exogenous feeding (Haylor 1992) and thereafter transferred to the rearing tanks as shown in Plate One, at an initial stocking density of 25 larvae. L^{-1} (Haylor 1992a). The volume of these tanks (original volume of 50L) was diminished to 5L by adjusting the height of the outflow pipe in order to achieve a diameter to depth ratio of 10 (Haylor 1992c).

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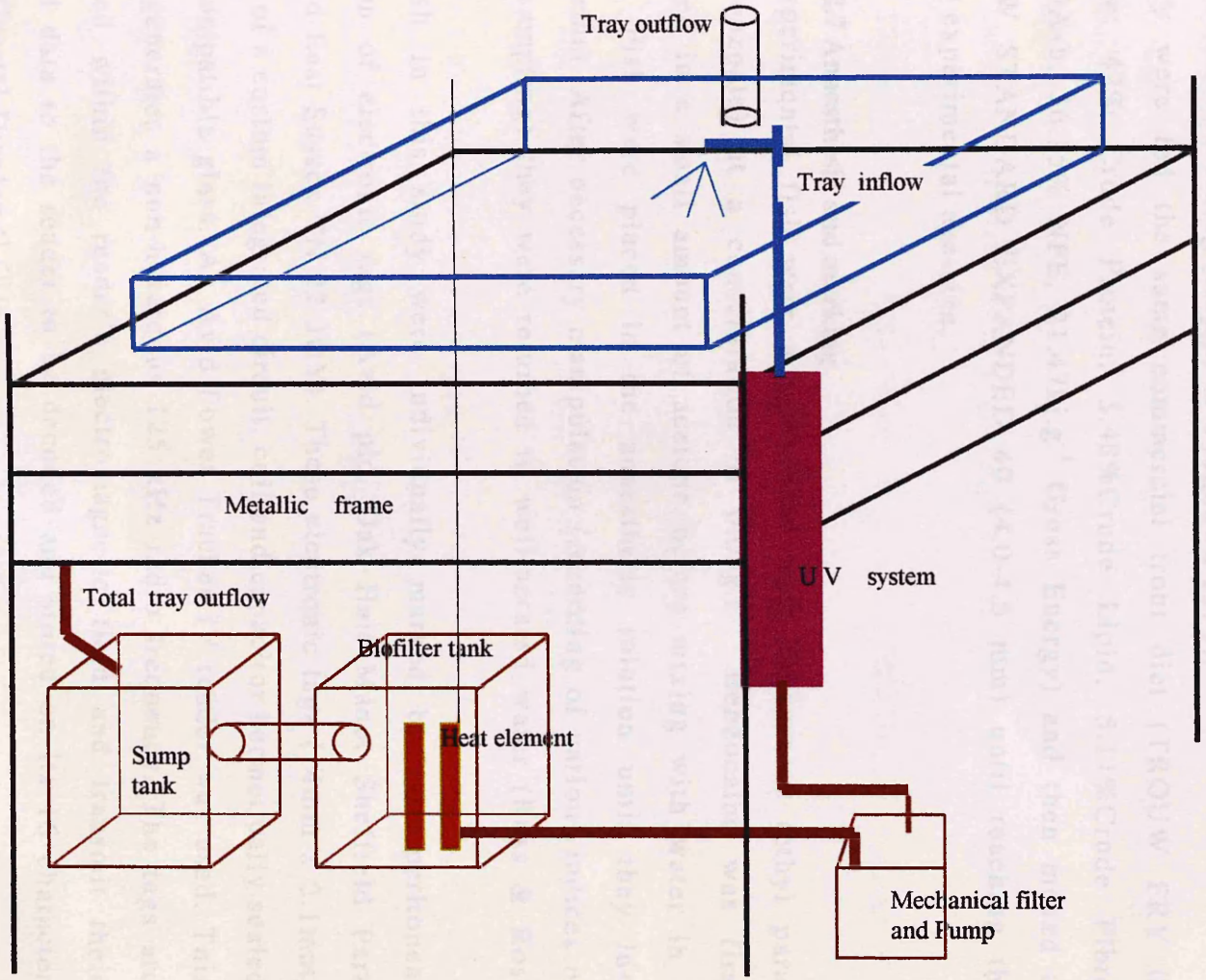
[4] Cascade Filterpak YTH 1150 bio-rings are a random dumped polypropylene media characterized by a surface area of 200 m^2. m^{-3} and ideally suited for nitrification applications as well as remedial carbonaceous BOD_5 removal. They are distributed by MASS TRANSFER INTERNATIONAL, Heversham, Cumbria, LA7 7EB, England.

PLATE 2. Egg incubation and hatching system used in the Tropical Aquarium of the IoASU (not to scale)



Larvae were fed Artemia until Day 6. On Day 7 first weaning with a commercial dry feed (TROUW FRY 02 crumble 1.1-1.5 mm) ground to 250 µm was attempted and between Day 11 and Day 14 animals were moved progressively to a 500 µm dry feed based on the previously used commercial diet.

The end of larval period (Day 15-Day 17) was characterized by the onset of aerial breathing (Haylor & Oyegunwa 1993).

Fry were fed the same commercial trout diet (TROUW FRY 02 crumble: 42% Crude Protein, 5.48%Crude Lipid, 5.11%Crude Fibre, 11.25%Ash, 36.15% NFE, 21.47kj.g^{-1} Gross Energy) and then moved to TROUW STANDARD EXPANDED 40 (4.0-4.5 mm) until reaching the desired experimental age-size.

2.2.7 Anaesthesia and marking

Experimental fish were anaesthetized with benzocaine (ethyl para-aminobenzoate) at a concentration of 0.05g.L^{-1}. Benzocaine was first dissolved in a small amount of acetone before mixing with water in a bucket. Fish were placed in the anaesthetic solution until they lost equilibrium. After necessary manipulation (recording of various indices or blood sampling) they were returned to well-aerated water (Ross & Ross 1983).

Fish in this study were individually marked by intra-peritoneal injection of electronic tags (Avid plc, Oak Hall Manor Sheffield Park Uckfield East Sussex TN 22 3QY). These electronic tags (14mm x 2.1mm) consist of a custom integrated circuit, coil and capacitor hermetically sealed in biocompatible glass. An Avid Power Tracker IV reader was used. This reader generates a non-hazardous 125 kHz radio frequency. The tags are energized within the reader's electromagnetic field and transmit their encoded data to the reader to be decoded and stored on its 16 character Liquid Crystal Display (LCD).

2.3 PERFORMANCE INDICES AND STATISTICAL ANALYSES

Evaluation of performance of fish in all experiments was based on the following indices:

Specific Growth Rate 100 x [ln P_t - ln P_o]/ number of feeding days where P_t the weight at time t and P_o the weight at the start of the feeding period (Steffens 1989)

Thermal Unit Growth Coefficient [$P_t^{0.333}$ - $P_0^{0.333}$]/ Sum [temp C^0 x days] Cowey 1992)

Percentage Weight Gain 100 x [P_t - P_o] / P_o (Steffens 1989)

Food Conversion Ratio [Total dry weight of ration consumed / unit of live fish weight gain] (Steffens 1989)

Protein Efficiency Ratio [Live body weight gained /crude protein fed] (Steffens 1989)

Apparent Net Protein Utilization [protein retained / unit of protein intake] x 100 (Bender & Miller 1953)

Hepatosomatic Index 100 x [liver wet weight / total body wet weight] (Pfeffer *et al,* 1991)

Feed intake 100 x [daily feed intake per fish /((final fish weight + initial fish weight)/2)]

Body Composition The percentages of Carcass Crude Protein and Carcass Crude Lipid are very useful indices as they better describe the allocation of dietary nutrients and the quality of the final product.

Chemical Score The biological value of a protein can be estimated by the Chemical Score of each individual amino acid expressed by the following fraction (Cowey & Sargent 1972):

[Percentage of amino acid in the protein / Percentage of the respective amino acid in egg albumin]

As fish were tagged individually (2.2.7), performance of each group was estimated on the average index as a product (arithmetic mean) of the individual indices of each fish within the group. In the Protein: Energy experiments performance indices were also calculated on a cumulative tank basis (see 4.3.1 & 4.3.6).

For statistical evaluation of results SPSS for Windows Statistical Software Package (Release 6.1.3) was used. Multiple comparisons were effected by use of Duncan's multiple range test (Zar 1996). For statistical evaluation of Weight Gain, all percentages were arcsine transformed as described by Zar (1996).

2.4 DETERMINATION OF THE ADOPTED FEEDING LEVELS, RATION SIZE AND FEEDING FREQUENCY

Hogendoorn (1981) established that the best feeding regime for fingerling catfish (0.5 to 10.0 g) is at satiation on a continuous 24 hour basis with the second best being a nocturnal regime on a continuous twelve hour basis (at a stocking density of 0.8-1.5 g. L^{-1}). Later on (and by adopting the nocturnal twelve hour continuous feeding strategy), he established appropriate feeding levels (percentage body weight per day) for size groups of 1 to 200g, temperatures between 20^0 and 35^0 C and stocking densities of 0.83 g. L^{-1} up to 12 g. L^{-1} (Hogendoorn *et al,* 1983). These results suggested that vision is not essential for successful feeding in this species. Similarly, Britz & Pienaar (1992) and Bruton (1979a) observed that African catfish is a nocturnal tactile feeder characterized by a distinct crepuscular activity pattern. However, if food or prey are available only during the light phase, *C. gariepinus* adopts a daily searching and feeding behaviour pattern (Bruton 1979b; Britz and Pienaar 1992).

Uys (1989) adopted a feeding frequency of three times daily (sunrise, noon and sunset) in outdoor tanks with feeding levels based on "visually determined satiation" (no stocking densities are stated). Furthermore, he concludes, "physiological data (quick and strong digestive response subsequent to feeding 1.2.1) provide no evidence that nocturnal feeding schedules would have to be implemented in commercial culture, whereas behavioural data might indicate otherwise".

The adoption of a continuous (on a twelve or twenty four hour basis) feeding regime for this study was not felt expedient for the following reasons:

- The continuous feeding regime, demonstrated by Hogendoorn (1981) to be the optimum, was based on fingerlings and low stocking densities. He then, theoretically, extrapolated his results to larger fish (125g) and at much higher stocking densities (12 g. L^{-1}) without further experimental substantiation. As fish used in this study were within the size range of 90-200 g, and the available individual tank volume was 50 L, the adoption of such a continuous feeding regime as the optimum one, would be arbitrary rather than substantiated.
- The diets used by both previous researchers were manufactured based on commercial raw materials and eventually characterized by high palatability and acceptability on behalf of the animals. Such diets are expected to create high food intake and usually favour high feeding frequencies. Diets used in this study were mostly based on purified or semi-purified materials, which are less palatable and are generally expected to result in lower food intake and potentially lower feeding frequencies.
- A continuous feeding regime would require the provision of automatic feeders and the problem of estimating the proper ration size to be released to meet variations in feed demand of the fish at different times of the day, would still exist (Jobling 1998). The use of "demand" feeders would overcome this difficulty but would still leave unclear the question to whether the food released had been consumed. An "interactive" automatic feeding system might be more efficient in this direction: the system consists of a computerized control system, a feed trap with a sensor capable of detecting uneaten food and a feed hopper. Feed released from the hopper is regulated in accord to the amount of waste detected by the sensor and this ensures a readjustment of the quantities delivered according to fish appetite and consumption (Jobling 1998). The use of such a system was not felt expedient in this particular study, mainly on financial grounds. In addition it is accepted that "the easiest way to study food consumption is to feed the fish by hand at regular time intervals and make direct observations on whether or not the food is

eaten" (Tuene & Nortvedt 1995; Jobling 1998)

- X- radiography data based on incorporation of "ballotini" glass beads in prepared diets (Hossain *et al.*1999) showed that voluntary food intake in African catfish follows a diel cycle. It was demonstrated that although the majority of food ingested, occurred during the phase of darkness, two distinct peaks in feeding activity were observed: at the onset of the dark phase (between 20.00-23.00 h) and before the onset of the light phase (06.00-08.00am).

For all the above reasons, it was decided to investigate the ration size and feeding frequency on the basis of hand feeding at distinct daily time intervals.

2.4.1 Determination of ration size

The ration size in this experiment was determined experimentally. Fish groups were fed a pre-weighed ration of a certain diet. Each individual tank was monitored for 10 minutes after first administration and if the entire initial ration was consumed, an additional ration was administered and monitored 5-10 minutes later. After every meal any uneaten food was removed, dried, and subtracted (on a dry matter basis) from the initial quantity administered.

This practice was followed in order to ensure that all fish within a tank population would adequately be fed, as catfish is an extremely aggressive and territorial species creating strong behavioural and eventually feeding hierarchies within any group or population (Hecht & Appelbaum 1988).

The same methodology was employed in order to determine the daily ration during the Protein: Energy experiments (see 4.2.3).

2.4.2 Determination of the feeding frequency

In order to determine the optimum number of rations per day (feeding frequency) the following experiment was performed.

2.4.2.1 Materials and methods

Thirty four (34) fish with an average weight of 102.18g (± 30.48g) and an average stocking density of 2.04g.L^{-1} (±0.61) were allocated to six tanks following a Completely Randomized Experimental Design (Woolf 1968; Zar 1984). The animals were individually tagged as described in 2.2

The average weights and average stocking densities of the six groups were not significantly different (P>0.05) (Table 2)

Table 2. Average Weights and Stocking Densities of the six experimental groups [(1)] *

Groups	*Weights (g)*	*Stocking Density (g . L^{-1})*
Group 1	96.81 [a] (28.75)	1.93 [a] (0.57)
Group 2	99.70 [a] (36.25)	1.99 [a] (0.73)
Group 3	95.33 [a] (32.68)	1.91 [a] (0.65)
Group 4	102.86 [a] (15.08)	2.05 [a] (0.30)
Group 5	96.37 [a] (38.65)	1.93 [a] (0.77)
Group 6	122.25 [a] (29.09)	2.44 [a] (0.58)

1.values in the same column and with the same superscript are not significantly different (P>0.05)

* numbers in parentheses represent Standard Deviation (n=4-6)

Fish were fed the diet 40:10, prepared for the Protein: Energy experiments (4.2.3), at two different feeding frequencies:

-Groups 1, 3 and 5 were fed approximately every twelve (12) hours (twice per day); practically the time interval employed was 11.30 hours as fish were fed shortly after lights went on (08.00 am) and half an hour before lights went off (19.30pm)

-Groups 2, 4 and 6 were fed approximately every six (6) hours (three times per day); the second interval between the afternoon feeding time and the late evening time was 5.30 hours as fish were fed half an hour before lights went off (19.30pm)

The ration size was established by the methodology previously described (2.4.1). A 26-day experiment took place in a recirculating system of the Tropical Aquarium of the IASU, as described in 2.2. Evaluation of the optimum feeding frequency was based on the indices as described in 2.3. A detailed account of the achieved indices of each individual within each group is given in Appendix I.

Statistical Analysis of the results was performed as described in 2.3 and based on the individual performance indices of each fish within each group.

2.4.2.2 Results and Discussion

The feed intake 1.42% b.w.day^{-1} (SD 0.49 / n=19), established for the 12h interval Group was significantly higher (P>0.05) than the 1.27% (SD 0.27 / n=15) established for the 6h interval Group.

Table 3 shows the established indices for the various experimental groups.

It is obvious that feeding every twelve hours, twice daily, resulted in significantly higher Specific Growth Rates, Weight Gains and significantly lower Food Conversion Ratios implying better feed utilization (Figure 7). Therefore this feeding frequency was adopted for the Protein: Energy experiments.

These results partially reconfirm the (previously mentioned) findings of Hossain *et al.*(1999), who showed that two distinct peaks exist in the feeding activity of the African catfish: at the onset of the dark phase (between 20.00-23.00 h) and before the onset of the light phase (06.00-08.00am). Both of these peaks are quite close, to the feeding times employed in the experiments of this study.

Table 3. Comparison of the performance indices among all the groups of the feeding frequency experiment[1]

Treatment	*Specific Growth Rate*	*Thermal Growth Coefficient*	*%Weight Gain* [2]	*Food Conversion Ratio*
12 h interval	1.24^{a} (0.08)	0.019^{a} (0.013)	38.51 ^{a} (2.96)	0.81 ^{a} (0.17)
6 h interval	0.81^{b} (0.002)	0.013^{a} (0 .0003)	23.50 ^{b} (0.24)	1.54 ^{b} (0.082)

1. values in the same column and with the same superscript are not significantly different (P>0.05). Values in parentheses represent Standard Deviation (n=15-19)
2. Percentages of weight Gain have been arcsine transformed (Zar 1996)

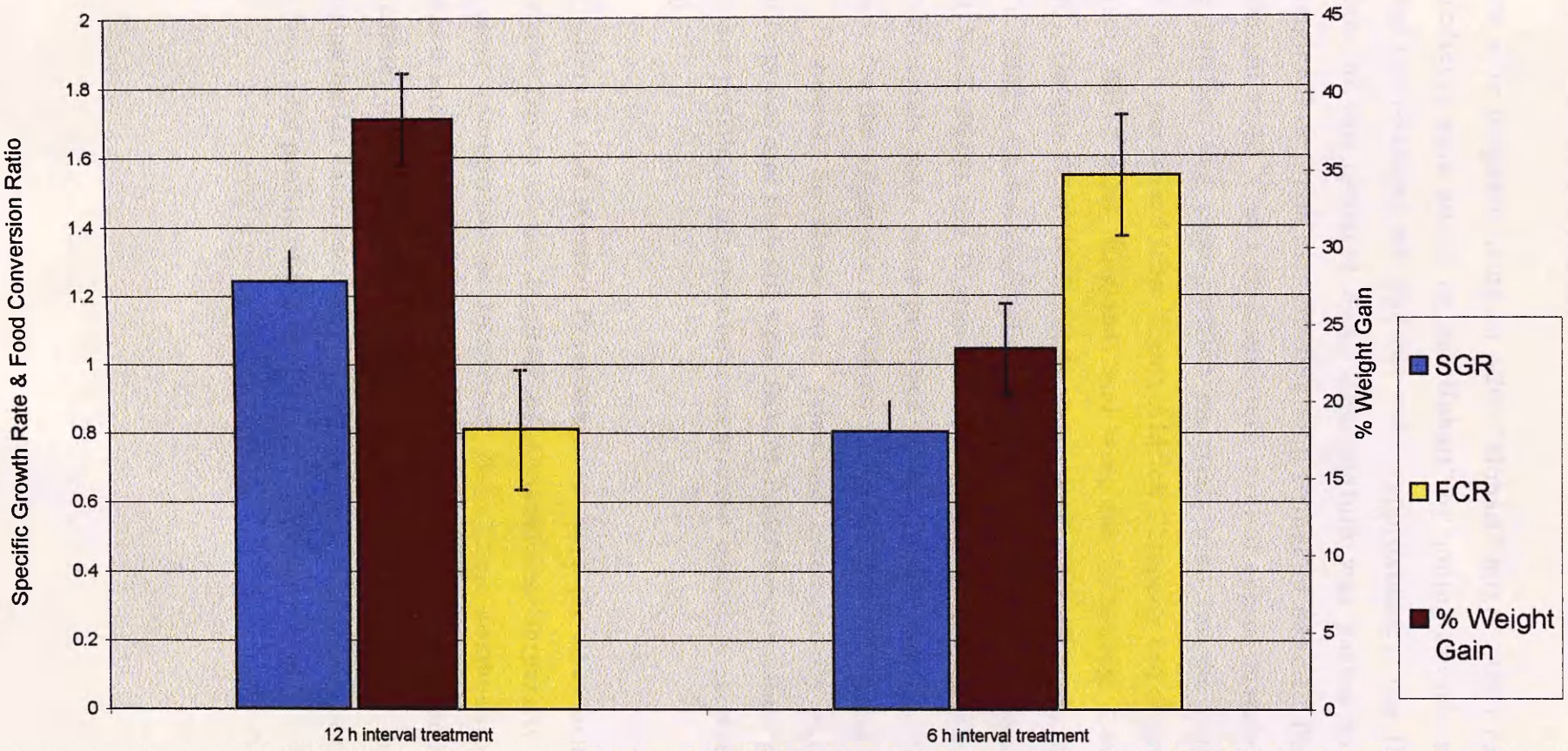


Figure7. Comparison of performance indices between treatments (error bars represent Standard Deviation)

2.5 DIET PREPARATION

Diets were prepared using an A200 "Hobart" mixer. After careful weighing, ingredients were mixed in the "Hobart" by adding the necessary (according to the formulation) oils and hot water (approximately one third of the total weight of the prepared diet). The mixture was passed in the "Hobart" through a 3 or 5 mm die, creating long strings of pellets. Pellets were dried overnight at 40 ^{0}C to a final moisture level of approximately 8-10 %.
The purified and semi-purified materials used for the preparation of the diets were purchased from Sigma-Aldrich Company Ltd (Fancy Road, Poole, Dorset, BH12 4QH, England) and were the following: Casein (Cat No C 3400), Gelatin (Cat No G 2625), Dextrin (Cat No D 2131), α-cellulose (Cat No C 8002), Carboxymethylcellulose -CMC Binder (Cat No C 5013), Zein (Cat No Z 3625) and Gluten (Cat No G 5004). The complex, non-purified raw materials used, were provided by BOCM-PAULS (Renfrew, Glasgow), except for the vegetable oil which was a TESCO Rapeseed Pure Vegetable Oil. Fishmeal, Soyabean meal (standard mechanically extracted), Wheat (whole grain) and Fish Oil were BOCM-PAULS aquaculture grade.
Nutrient profile of all the above-mentioned materials is given in Appendix Two.

The Vitamin and Mineral Premixes used were the ones established at the Nutrition Group of the Institute of Aquaculture University of Stirling, as the most appropriate for freshwater fish. Their composition is shown in Tables 4 and 5. Eighty five 85% percent of the Vitamin Premix, consisted of the carrier α-cellulose having an increased adsorbance for the vitamins and ensuring better incorporation and intermixing with the other raw materials.
The fatty acid profile of the Oils used is shown in Table 6.

Table 4. Profile of Mineral Premix [1]

Mineral formula		*g.100g^{-1} of premix*
$CaHPO_4.2H_2O$	calcium orthophosphate	72.77
$MgSO_4.7H_2O$	magnesium sulphate	12.75
NaCl	sodium chloride	6.0
KCl	potassium chloride	5.0
$FeSO_4 .7H_2O$	iron sulphate	2.5
$ZnSO_4 .7H_2O$	zinc sulphate	0.55
$MnSO_4.4H_2O$	manganese sulphate	0.255
$CuSO_4.5H_2O$	copper sulphate	0.0785
$CoSO_4.7H_2O$	cobalt sulphate	0.0477
$CaIO_3 .6H_2O$	calcium iodate	0.0295
$CrCl_3 .6H_2O$	chromic chloride	0.0127
	TOTAL	100.00

1.incorporated at x % of diet

Table 5. Profile of the Vitamin Premix[1]

	g.100g^{-1} of premix (this study)	*g.100g^{-1} of diet (this study)*	*g.100g^{-1} of diet (Uys 1984)*	*g.100g^{-1} of diet (Uys 1989)*
CyanocobalaminB_{12}	0.000125	0.000002	0.00019	0.0011
Ascorbic	3.75	0.075	0.15	0.1
Cholecalciferol (D)	0.0004	0.000008	0.00003	n.s.[2]
Tocopherolacetate (E)	0.7	0.014	n.s. [2]	0.04
Vitamin K	0.15	0.003	n.s.	0.004
Thiamine Hydrochloride (B_1)	0.425	0.0085	0.00357	0.005
Rivoflavin (B_2)	0.3	0.006	0.00707	0.02
Pyridoxine Hydrochloride (B_6)	0.125	0.0025	0.00188	0.005
Calcium Pantothenate	0.525	0.0105	0.0445	0.05
Niacinamide	1.25	0.025	0.059	0.075
Biotin	0.009	0.00018	0.00107	0.0005
Folic acid	0.1	0.002	0.00132	0.0015
Choline Chloride	7.405	0.1481	0.85	0.5
MyoInositol	0.25	0.005	0.286	0.2
Ethoxyquin	0.001999	0.000039	n. s.	n. s.
VitaminA	0.008	0.00016	0.002236	n. s.
Total	14.99952	0.299989	1.406836	1.0021

1. incorporated at x % of diet 2. None stated

Table 6. Profile of the oils used for the preparation of diets [1]

Fatty acid species		*Vegetable oil* [2]	*Fish oil* [3]
Saturates			
Myristic	14:0	n. d	9.87 (0.17)
Pentadecanoic	15:0	n. d	0.38 (0.08)
Palmitic	16:0	11.85 (0.25)	17.13 (0.78)
Heptadecanoic	17:0	n. d	n. d *
Stearic	18:0	3.16 (0.22)	1.84 (0.17)
Nonadecanoic	19:0	n. d	n. d
Arachidic	20:0	n. d	n. d
Heneicosanoic	21:0	n. d	n. d
Behenic	22:0	0.13 (0.07)	n. d
Lignoceric	24:0	n. d	n. d
Monounsaturates			
Myristoleic	14:1	n. d	n. d
cis-10 Pentadecanoic	15:1	n. d	n. d
Palmitoleic	16:1n-7	n. d	n. d
	16:1**	n. d	6.79 (0.17)
cis-12-octadecenoic	18:1n-6	n. d	n. d
cis-11-octadecenoic (vaccenic)	18:1n-7	n. d	1.69 (0.16)
Oleic	18:1n-9	27.19 (0.82)	7.06 (0.04)
cis-10 nonadecanoic	19:1	n. d	n. d
cis-13-Eicosenoic	20:1n-7	n. d	0.06 (0.06)
Eicosenoic	20:1**	0.16 (0.06)	12.78 (0.38)
Erucic	22:1n-9	n. d	21.89 (1.56)
Polyunsaturates			
Linoleic	18:2n-6	52.26 (1.42)	1.61 (0.12)
gamma-linolenic	18:3n-6	n. d	n. d
Linolenic	18:3n-3	3.84 (0.23)	0.87 (0.21)
cis- 6,9,12,15-Octadecatetraenoic	18:4n-3	n. d	2.11 (0.45)
cis-11,14,17-Eicosatrienoic	20:3n-3	n. d	n. d
cis-5,8,11,14,17-Eicosapentaenoic	20:5n-3	n. d	4.41 (1.14)
cis-11,14-Eicosadienoic	20:2n-6	n. d	0.19 (0.04)
cis-8,11,14 Eicosatrienoic	20:3n-6	n. d	n. d
Arachidonic	20:4n-6	n. d	0.25 (0.08)
cis-4,7,10,13,16,19 DHA	22:6n-3	n. d	5.56 (0.96)
cis-7,10,13,16-docosatetraenoic acid	22:4n-6	n. d	n. d
Nervonic	24:1n-9	n. d	0.33 (0.11)
Unknown peaks		1.30 (0.50)	5.13 (0.2)
Total saturates		15.21 (0.10)	29.23 (1.05)
Total monounsaturates		27.35 (0.88)	50.62 (2.18)
Total (n-6) PUFA		52.27 (1.42)	2.06 (0.25)
Total (n-3) PUFA		3.84 (0.02)	12.96 (2.78)
Ratio (n-3) / (n-6)		0.073 (0.001)	6.26 (0.59)

1. as % methylesters of total fatty acid methylesters. Numbers in parentheses represent Standard Deviation * none detected ** position of double bond not determined 2. TESCO Rapeseed Pure Vegetable Oil 3. BOCM-PAULS aquaculture grade

2.6 DIET ANALYSES

Prior to analyses, samples of each diet were ground with a mortar and pestle to pass a 1mm sieve. All analyses were performed on a dry matter basis in triplicate.

Moisture content in raw materials and prepared diets was determined by freeze-drying to constant weight. As faeces appeared to be burnt when the standard procedure was applied (oven drying at 135^0 C for 2 hours / AOAC, 1990, Method 930.15) it was decided to employ freeze drying for all the materials used (Cho *et al.*1982), except for carcass analysis (see 2.7). Freeze drying was achieved by the following layout: a Speedivac 2 Rotary Vacuum Pump (Edwards High Vacuum International) was attached to a Modulyo 4K Freeze Dryer (Edwards High Vacuum International, Manor Royal West Sussex RH10 2LW) able to create a working vacuum of 0.1 mbar at a -40^0 C temperature.

Total Nitrogen content in raw materials was estimated by the Kjeldahl method (Tecator Application Note / 09-09-1987 / AN 30/87) using a Tecator-Kjeltec Auto Analyzer 1030 unit (PERSTORP Analytical Ltd, Highfield House, Foundation Park, Roxborough Way, Berkshire SL6 3UD). Digestion was effected by using mercury tablets as catalyst (Fisher, Cat No K/0130/80 / FISHER Scientific, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG)[5] in a Tecator Digestion System 40 / 1006 Heating Unit.

In order to convert the determined Nitrogen into Crude Protein the following factors were used for the respective raw materials (Takeuchi 1988; Osborne & Voogt 1978): Casein 6.38, Gelatin 5.55, Fishmeal 6.25, Soya 5.71, Wheat 5.70, Zein 5.70, Gluten 5.70.

For the determination of Crude Protein in the prepared diets, a weighted average of the aforementioned factors was used, taking into account the percentage of incorporation of each material in the final formulation.

Crude Lipid in raw materials and the respective diets was determined as initially described in the E.E.C Methods (18.1. 84 / Official Journal of

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[5] Each tablet contains 1g sodium sulphate and the equivalent of 0.1g mercury as mercuric oxide

the European Communities No L15/29) and applied by the Tecator Application Note 92/87: samples are subjected to *Acid Hydrolysis* (in a Tecator Soxtec 1047 Hydrolysing Unit) by 3.3 N hydrochloric acid and filtered using Celite or Hyflo-supercel to avoid loosing any oil and fat. In the case of extremely oily samples (>10%) pre-extraction by petroleum spirit is suggested. After the acid hydrolysis phase, the main extraction is carried out in a Soxhlet Unit (Tecator Soxtec System HT 1043 Extraction Unit), using petroleum spirit as the extraction solvent (Tecator Application Note 67 /83).

Crude Fibre was determined in a Tecator Fibertec System M / 1020 Hot Extractor Unit as described by the Tecator Application Note 01/ 78: samples are treated with hot dilute (1.25%) sulphuric acid for 30 minutes and hot sodium hydroxide (1.25%) for a further 30 minute period. Samples are washed with boiling deionized water, dried overnight at 110^0 C, and ashed in the muffle furnace at 550^0 C for two hours. The percentage of Crude Fibre in the sample is determined as the difference between the dried insoluble material (remaining after the acid and alkaline hydrolysis) and its respective ashed inorganic fraction.

Carbohydrates were determined by the Anthrone-Sulphuric acid reagent method as initially described by McCready *et al.* (1950) (Appendix III) whereas Ash was determined by incineration in a muffle furnace at 600^0 C for two hours (AOAC, 1990, Method 942.05)

The amino acid profile of raw materials and diets was determined by hydrolyzing the dried samples with 6N hydrochloric acid for 24 hr at 110^0 Celsius. After drying for another 24 hr over sodium hydroxide pellets, samples were dissolved in sodium citrate buffer, filtered through 0.2 μm centrifuge filters and loaded into the Amino Acid Analyzer (Pharmacia LKB Biochrom Ltd., 4151 Alpha Plus model / PHARMACIA Biotech, 23 Grosvenor Road, St.Albans, Herts ALI 3AW). Samples were eluted through a stainless steel (202 mm in length by 0.6mm diameter) Ultra pack 8 cation exchange resin column (sodium form) and determined spectrophotometrically by the intensity of the colour produced following a ninhydrin reaction. As external standard a protein hydrolysate (Sigma-

Aldrich Cat No A9781) was used containing 0.5 μmol. ml $^{-1}$ of each amino acid except for L-Cystine, which was contained at 0.25 μmol .ml $^{-1}$.

Tryptophan was determined colourimetrically as described by Fischl (1960).

Fatty Acid Methyl Esters (FAMEs) were prepared by the acid esterification method (Christie 1982): after extraction by the method of Folch *et al.*(1957) total lipids were separated into neutral and polar classes by Thin Layer Chromatography (TLC) on 20x20 cm glass silica gel G coated plates (BDH-Merck,Cat No 15555 2J / MERCK Ltd, Hunter Boulevard, Magna Park, Lutterworth, Leicester LE17 4XN) using hexane: diethyl ether: glacial acetic acid (80:20:2 by vol.) as developing solvent. Bands of adsorbent containing the lipid classes were scraped off the glass plates, lipids were isolated by chloroform:methanol 2:1 (v/v), purified by potassium chloride and sodium hydrogen carbonate and subjected to acid catalyzed transesterification (1% v/v sulfuric acid in methanol) for 16 h at 50^0 C. The resulting methyl esters were purified using 20x20 cm TLC silica gel G plates (BDH-Merck Cat No 15555 2J) with hexane: diethyl ether: acetic acid (90:10:1 v/v) as developing solvent and quantified by comparison with known standards (Sigma-Supelco Cat Nos. 4-7885-U, 4-7033, 4-7085-U, T 6543, E 3512, D 3659, N 1256, O 9881, N 5377, N 6519, E 6885, 189-1, 189-4, 189-13, 189-16) on a Carlo Erba gas-liquid chromatographer (Carlo Erba Strumentazione, Erba Science, Swindon SN2 6JQ, UK) equipped with a Chrompack CP-WAX 58 CB (Cat No 7716) capillary column (ID 0.25 mm - Length 25 m - df 0.2 μm).A thermal gradient of 160^0 C-240^0 C was used. FAME identity was confirmed where possible by GC-MS (Thermo-Quest / Finnigan, Trace 2000 Series).

2.7 CARCASS COMPOSITION ANALYSES

Carcass Moisture was determined by oven drying at 135 ^{0} C (AOAC, 1990, Method 930.15) until constant weight (approximately 24 hr) and the Dry Matter determined accordingly.

Total Carcass Nitrogen content was estimated by the Kjeldahl method as previously described (2.6). Crude Protein was calculated by multiplying the determined Nitrogen by the factor 6.25 (AOAC, 1995, Method 954.01)

Carcass Crude Lipid was estimated as Ether Extracts by the use of a Soxhlet Unit (Tecator Soxtec System HT 1043 Extaction Unit) and petroleum spirit as the extraction solvent (Tecator Application Note 67 /83) whereas Carcass Ash was estimated by incineration in a muffle furnace at 600^0C for two hours (AOAC, 1990, Method 942.05)

Liver Lipid was determined by the method of Folch *et al.* (1957) and Liver Glycogen by the method of the anthrone- sulphuric acid reagent as initially described by Good *et al.* (1933) and later modified by Seifter *et al.* (1950), Hassid & Abraham (1957) (Appendix IV).

Carcass amino acid profile was determined by the same methodology, employed for the raw materials and diets (2.6)

All fish within each Group were analyzed individually. Final values of each experimental Group represent the arithmetic mean of individuals' values within the specific Group.

2.8 DETERMINATION OF ENERGY BY THE METHOD BASED ON THE ENERGY CONTENT OF ORGANIC CARBON IN THE SAMPLE.

2.8.1 Introduction

There are three different approaches to determine the energy content of fish carcass, faeces and feed:

a) calculation based on the proximate biochemical composition using the conversion factors 5.65, 9.45 and 4.2 kcal g^{-1} for protein, fat and total carbohydrates respectively (Henken *et al.*1986; Craig *et al.*1978),

b) bomb calorimetry

c) chemical oxidation

Bomb calorimetry is the most often used method whose results are referred to as Gross Energy. Although widely accepted as a method, it is characterized by some constraints: the success in ignition of the prepared pellets is heavily influenced by their stability, which in turn varies according to the level of moisture of the materials in question, and their "mechanical properties" (i.e. it is quite difficult to create stable pellets out of purified materials like casein, gelatin, zein). Furthermore bomb

calorimetry requires relatively large samples (approximately 3 g for triplicate determination). This becomes unfeasible in digestibility studies where the quantity of faeces available for analyses is often restricted.

Chemical oxidation, a method using the oxidizing agent potassium dichromate, is also characterized by some constraints: according to O'Shea & Maguire (1962), sample moisture can cause variation in the titration values. Furthermore the credibility of the determined values relies on the stability of the chemicals used (especially potassium dichromate and sodium thiosulphate) whose normality has to be regularly assessed, especially if they are not to be used on the same day of preparation.

It is widely accepted that the end product of amino acid nitrogen in chemical oxidation is ammonia as compared to free nitrogen in bomb calorimetry (Maciolek 1962; Craig *et al.*1978). In oxygen bombs, ammonia is oxidized further according to the equation

$$2\ NH_3 + 1.5\ O_2 \dashrightarrow N_2 + 3\ H_2O$$

and the specific enthalpy of combustion of ammonia to water is -5.94 cal. mg $^{-1}$ (Kersting, 1972). Therefore the energy values obtained by chemical oxidation are usually lower than those determined by bomb calorimetry, requiring a correction of 7.7-30% for incomplete oxidation of protein (Craig *et al.*1978).

The extra energy obtained in this way in a bomb calorimeter is not of any metabolic use to the animal in question and is usually accounted as part of the total heat loss due to urine excretions (Figure 4.)

Rationale of the method used in this study

The method used in this study is a modification of the methodology described by Salonen *et al.* (1976). Organic carbon is supposed to have a uniform energy value among all biological materials. As the ratio of inorganic to total carbon varies considerably among different materials, it is necessary to estimate both the percentages of total and inorganic carbon in this material in order to evaluate the respective fraction of organic carbon. At the same time the gross energy of the material can be established by the use of an adiabatic bomb calorimeter and transformed to units of energy per unit of organic carbon. If this procedure is repeated for various raw materials, an average uniform value of energy per unit weight of organic

carbon can be established. This can be used in the future as a conversion factor in order to convert the percentage of sample organic carbon to sample energy.

This method has the advantage of avoiding using the bomb calorimeter once the conversion factor is established. A CHN/O combustion analyzer is used instead, in order to establish the carbon fractions of ashed and non-ashed samples. By using the combustion autoanalyzer, one can minimize the sample size needed (30-45 mg for a triplicate) with minimal compromise in the accuracy and reliability of the results. The Elemental Analyzer accurately determines the carbon, nitrogen and hydrogen content of organic compounds by detecting and measuring their combustion products, i.e.CO2, N_2, H_2O. Combustion occurs in pure oxygen and the products are analyzed automatically in a self-integrating thermal conductivity analyzer.

2.8.2 Materials and methods

Thirteen different materials (Table 7) were prepared (2.6), freeze-dried, and ashed (see 2.6).

For the determination of total carbon in dried ashed and non-ashed samples a Perkin-Elmer 2400 (Perkin-Elmer Ltd, Post Office Lane, Buckinghamshire, HP9 1QA) CHN/O combustion analyzer was used.

Bomb energy values were established by using a Gallenkamp Autobomb System M-T adiabatic bomb calorimeter (GALLENGAMP Ltd, Belton Road, West Loughborough, Leicestershire, LE11 OTR).

All analyses were performed in duplicate except for ash determination where triplicates were employed.

A detailed account of all the calculated parameters for each raw material or diet is given in Appendix II.

2.8.3 Results and discussion

The established conversion factors for the thirteen raw materials and diets are shown in Table 7. The average value 47.08 $kJ.g^{-1}$ organic carbon of these thirteen values was considered as the future reference conversion factor. Such a conversion factor was established for aquatic invertebrates by Salonen *et al.* (1976) as 46 $kJ.gr^{-1}$ organic carbon. Ross (1982) established a

conversion factor of 43 kJ.gr^{-1} of organic carbon, based on samples of *Littorina rudis* (Maton).

Table 7. Conversion factors used for the determination of energy content in raw materials and diets

Raw material [1]	***Energy in kJ. g^{-1} Organic Carbon***	***Standard Deviation***
Gluten	47.8	0.43
Zein	50.3	0.72
Casein	50.9	2.96
Gelatin	48.6	0.22
Fishmeal	50.8	2.98
Soya	42.7	0.28
Wheat	40.7	1.54
Feathermeal	51.6	2.56
Purified Diet 1	43.4	1.17
Purified Diet 2	45.5	1.11
Purified Diet 3	45.0	1.32
Commercial Diet 1	46.5	0.48
Commercial Diet 2	48.0	0.18
Average Value	47.1	3.45

1.The proximate analysis of the raw materials and diets used is given in Appendix II.

Based on the above, and by knowing the percentage of organic carbon in each sample, one can estimate the value of Gross Energy in kJ.g^{-1} Dry Sample (without knowing the bomb values), because

Gross Energy (kJ.g^{-1}Dry Sample) = 47.08 x (%Total Organic Carbon :100)

An example of this procedure is given below

1. Percentage of Total Carbon by the use of CHN/O combustion analyzer: 46.38 (45.97 46.8)

2. Percentage of Inorganic Carbon in Ashed Samples by the use of CHN/O combustion analyzer: 9.61 (7.96 11.26)

3. Percentage of Ash in the Diet: 10.53

4. Percentage of Inorganic Carbon in the Diet: 9.61 x (10.53: 100) = 1.011

5. Percentage of Organic Carbon in the Diet: 46.38 - 1.011 = 45.368

6. Gross Energy (kJ. g^{-1} Dried Diet): 47.08 x (45.36: 100) = 21.3

CHAPTER 3. DIGESTIBILITY TRIALS

3.1. INTRODUCTION

A basic prerequisite for nutritional experiments is an understanding of the assimilation efficiency of the experimental animals towards various raw materials and (or) diets.

The aims of the digestibility trials in this study were twofold.

First to assess digestibility of raw materials and of respective diets prepared for the Protein: Energy experiments

Second to assess variations arising from differences in the digestibility values between a diet based on complex foodstuffs and one based on purified ingredients (1.2.4.4.2). Therefore the digestibility of two types of diets was assessed: a diet consisting of purified materials and another one consisting of complex foodstuffs.

Diet formulation by purified materials, frequently experiences problems due to the imbalanced amino acid profile of the materials used. Gelatine is characterized by low quantities of Leucine, Isoleucine, Lysine, Methionine and Tyrosine which can be amended by the higher levels of those amino acids in casein (Appendix II). Even so, the combination of casein and Gelatine can not match the requirements of certain species in Methionine and Cystine (Table 47). In this case, corn gluten and corn zein, characterized by higher levels of Methionine and Cystine (Appendix II) can be used as complementary materials in diet formulation. Zein is a semi-purified corn by-product, low in Arginine, totally deficient in Tryptophan and Lysine but quite high in Methionine and Cystine and having the rest of Essential Amino acids in sufficient quantities (Appendix II). It is also characterized by low carbohydrate and lipid levels and therefore useful in nutritional experimental work where exact levels of required nutrients are desired (Borlongan & Benitez 1990; Lopez-Alvarado *et al.*1994; Ruchimat *et al.*1997).Therefore, the digestibility of zein was also investigated in order to evaluate its use in related nutritional experimental work.

3.2 THE DIGESTIBILITY METHODOLOGY

Digestibility of dietary nutrients has always been a major concern in animal nutrition. Its importance is recognized in fish nutrition as well (Cho & Kaushik, 1985): "...it is a necessity to determine ingredient digestibility prior to any attempt to formulate diets for fish as the digestibility coefficients of nutrients in the individual dietary ingredients solely determine the potentially available nutrients and energy values of the diets ...". However, the same authors admit that the digestibility values for the nutrients and energy of each individual ingredient, indicate, but do not necessarily measure to the same extent, the digestibility for the same nutrients of the complete diet and its potential productivity.

The use of "true" digestibility values by taking into account the "metabolic" faecal material (residues of mucosal cells, digestive enzymes and other secretions released into the digestive tract) is also in question (Cho & Kaushik 1985). The energy loss contained in residues represents a loss of energy due to the process of digestion and is minimally related to the food when animals are fed with a balanced diet. This faecal metabolic energy (FmE) represents only 2-3% of the total faecal energy (FE) loss (Figure 4., page 34) when the nutritional profile of the diet falls within the range of the nutrient requirements of the species (Cho & Kaushik 1985). Therefore what is most decisive in the magnitude of faecal energy is the energy intake. When normal dietary energy levels are applied, the proportion of FmE in faeces is negligible.

Faeces collection methodology can be categorized as :

- the direct method, consisting on the quantitative collection of faeces that correspond to one or several meals (Ogino *et al.*1973).
- the indirect method which obviates the quantitative collection problems by using an inert, internal or external, tracer and by estimating nutrient digestibility based on the relative proportions of the tracer in the feed and faeces (Maynard & Loosli 1972).

Both methods suffer by the collection of representative fish faeces (De La Noue & Choubert 1986). The direct method also suffers by the problem of dissolution of faeces that starts rapidly in the water leading to an underestimation of 10-15% of the measured nitrogen and therefore

overestimation of digestibility coefficients (Windell *et al.*1978; Smith *et al.* 1980). The indirect method suffers from fragmentation and loss of material (external marker chromic oxide and dry matter) during the collection of faeces. A loss of 10% of faecal chromic oxide would lead to an Apparent Digestibility Coefficient about 2% lower than the expected (De La Noue & Choubert 1986).

Therefore faeces' collection methodology has been highly variable and closely related to the digestive physiology of the species in question:

Channel catfish researchers (Wilson *et al.*1981; Wilson & Poe 1985) employed the dissection method and removal of the faecal sample from the rectal area, as it was previously established that absorption of nutrients takes place throughout the intestine and up to that area. In rainbow trout it was shown that stripping in the area between the ventral fins and up to the anus could be quite a reliable method as no significant contamination with urine occurs (Austreng 1978).

All these methods avoid overestimation of the digestibility values due to leaching of nutrients into the water. Though, according to other researchers, stripping of faeces or even catheterization, involves the risk of potential underestimation of apparent nutrient digestibility due to body fluid contamination, addition of an excess of enzymes and intestinal epithelium to the rectal contents (Cho & Slinger 1979; Cho *et al.*1982; Spyridakis *et al.*1989; Hajen *et al.*1993a).

The development of the Guelph system (Cho *et al.*1975; Cho *et al.*1982) appeared as a method that minimized handling and exposure of fish to considerable stress, as well as precluding the alteration of faeces' biochemical composition: faeces are collected through a drain pipe and recovered in a settling column within 2 min of being voided by the fish. Comparative data of digestibility values for rainbow trout obtained by intestinal dissection, catheterization, or the Guelph settling column, showed that leaching is not an important source of error in the Guelph system (Cho *et al.*1982). The major cause of leaching is "break-up" of the faeces particles by physical handling which is minimized by the Guelph system. The same system has been proved sufficiently reliable for estimating foodstuff digestibility in chinook salmon (*Oncorhynchus tshawytscha*),

provided that faeces are rapidly and completely carried to the settling column and care is exercised to minimize faecal pellet break-up at the time of collection (Hajen *et al.*1993a).

In *C. gariepinus,* the stripping method (in the area between the ventral fins and up to the anus) would involve the risk of underestimation of apparent nutrient digestibilities as it was proven (Uys & Hecht 1987) that amylase, trypsin , chymotrypsin and lysozyme are showing a decreasing gradient activity from the anterior towards the posterior part of the intestine (proximo-distal gradient) . In addition, stripping trials with the initial population of available catfish at the Tropical Aquarium facilities of the IASU revealed that the rhythmicity of gastric evacuation was affected by these manipulations and the weight of the fish (1.2.1). Gastric evacuation time ranged from 8.20 h after feeding, for big size fish (300-500g) to 9.45 h after feeding for small size fish (80-140g) unavoidably affecting the credibility of digestibility values following this method.

Dissection at the rectum area of *C. gariepinus* might generate credible digestibility values (Henken *et al.*1985). However, the drawback of expending a large number of animals lead to the adoption of the sedimentation technique (Guelph system) for the additional reason that fish are not stressed at all by any kind of handling or manipulation. This has the extra advantage of not interfering with the gut transit and gastric evacuation time and eventually not influencing the digestibility of the diet.

Most digestibility estimates in fish have been achieved by use of "inert" foreign markers, primarily (Cr_2O_3) chromic oxide. The validity of those estimates depends on the assumptions that, the inert material introduced does not interfere with the digestive metabolism of the experimental subject, it is not absorbed or metabolized and the rate of its passage through the gut is the same as the passage rate of the feed (Maynard & Loosli 1972 cited at De Silva & Perera 1983).

Early studies have raised doubts with regard to the suitability of chromic oxide as a dietary marker due to its differential passage along the digestive tract with respect to the digesta (Knapka *et al.*1967; Bowen 1978; Leavitt 1985). However, what seems to be decisive is the level of inclusion in the

diet: Tacon & Rodrigues (1984) showed that chromic oxide at an inclusion level between 0.5% and 1% of the diet, exhibited excellent replication within individual treatments. Furthermore, apparent digestibility coefficients for all the nutrients were not significantly statistically different at the 0.5% and 1% chromic oxide inclusion level, whereas they were statistically higher ($P<0.05$) at the 2% inclusion level, implying a faster chromic oxide transit rate relative to the digesta (Tacon & Rodrigues 1984). Sadiku & Jauncey (1995) showed that in tilapia (*Oreochromis niloticus* L.) the increase of Cr_2O_3 inclusion level from 0.5 to 1.0% in diets made by a blend of soybean flour, poultry meat meal and wheat flour, affected negatively protein digestibility but positively ash digestibility.

It has also been shown that chromic oxide affects faecal lipid in Arctic charr, *Salvelinus alpinus* (L.): total feacal lipid, triacylglycerols and diacylglycerols where higher in the groups whose diet was supplemented with 1% Cr_2O_3 and kept either in fresh or saltwater, compared to the respective feacal nutrients of the group whose diet was devoid of Cr_2O_3. Furthermore, Cr_2O_3 inclusion affected the digestibility of only 22:1(n-9) and 22:1(n-11) in Arctic charr kept in seawater but had an effect on the digestibility of a wide range of fatty acids in fish kept in freshwater, indicating that salinity affects the passage of chromic oxide in respect to the fatty acid unsaturation level (Ringo 1993a; 1993b).

Contradictory are the experimental data on another external digestibility marker, the acid insoluble ash (or acid washed sand): Tacon & Rodrigues (1984) showed significantly erratic variation in nutrient digestibility coefficients over the range of dietary inclusion levels tested (0.5%, 1% and 2%) in rainbow trout. On the contrary Halver *et al.*(1993) endorsed acid insoluble ash as the more reproducible and more convenient method to use, when compared to chromic oxide, iron oxide, magnesium ferrite, or dietary fibre.

Because of the above criticism, the use of various indigenous dietary components as "inert" markers, has been examined: silica (Hickling 1966), cellulose (Buddington 1979), hydrolysis resistant organic matter (Buddington 1980; De Silva & Perera 1983), hydrolysis resistant ash

(Bowen 1981; De Silva & Perera 1983) and crude fibre (De Silva & Perera 1983; Tacon *et al.*1983; Tacon & Rodrigues 1984). Due to the biochemical structure of these "indigenous" markers, their validity tends to be more species-specific: fish in general, lack cellulases, chitinases and lignin hydrolyzing substances in their digestive tract (Stickney & Shumway 1974; NRC 1993; Lindsay & Harris 1980; Lindsay 1984). Therefore, hydrolysis resistant organic matter (HROM), consisting of cellulose and chitin, proved to have a 100% faecal recovery in the cichlid *Etropus suratensis* (De Silva & Perera 1983), while crude fibre (CF), consisting of cellulose and lignin, has being proved to be a credible marker for rainbow trout (Tacon & Rodrigues 1984; Tacon *et al.*1983).

However their use as "indigenous" digestibility markers, would be of limited value to species which have shown some levels of similar enzymatic activity (of endogenous or exogenous-bacterial origin) : cellulolytic activity in *Ictalurus punctatus* (Smith & Lovell 1972 cited at Buddington 1980 ; Stickney & Shumway 1974), cellulolytic and chitinase activity in *Clarias gariepinus* (Uys 1989), limited cellulolytic activity by *Tilapia mossambica* (Bowen 1981), some cellulolytic activity by *Scardinius erythrophthalmus* and *Rutilus rutilus* (Niederholzer & Hofer 1979).

Digestibility values are also influenced by the nutrient levels in the diet and their interaction in respect to the digestive system-type of the species. In red drum (*Sciaenops ocellatus*), protein digestibility was highest in foodstuffs of high protein content (>60%) and low fiber content (<2%), whereas apparent digestibility of dry matter and energy was positively influenced by protein and lipid content and negatively influenced by crude fiber content (McGoogan & Reigh 1996). In the Brazilian pacu *Piaractus mesopotamicus* protein digestibility was increased from 58.64% to 85.01% due to an increase in the energy level of the diet (Carneiro *et al.*1994). On the contrary, high energy diets with a protein: energy ratio below 19.8 $mg.Kj^{-1}$ yielded lower protein digestibility values in tilapia *Oreochromis niloticus* (Lorico-Querijero & Chiu 1989).

Similarly, the Asian catfish *C. batrachus* showed increased protein digestibility as a result of increasing dietary protein levels (within the range

of 19.15% to 39.95% Crude Protein) provided by semi-purified diets (Singh & Singh 1992).

Digestive system of fish shows a gradual adaptation to the presented diets resulting in better nutrient assimilation over time. This was shown for the Asian cichlid *Etroplus suratensis* (Bloch) fed on an aquatic macrophyte (De Silva & Perera 1983) and the milkfish *Chanos chanos* fed on semi-purified diets (Ferraris *et al.* 1986). Although desired in the long-term, digestive adaptation can lead to erroneous digestibility results during the transitional phase that fish are transferred from one diet to another. Therefore, sufficient experimental time, replication and allocation of different diets to different fish groups is required, to counteract variability generated from digestive adaptation.

Limited number of available individuals and facilities (tanks) in the present experiments imposed replication in time. Allocating two experimental periods to each diet intermittently separated by at least one experimental period of a different diet and a one-week adaptation period would eliminate any digestive adaptation artefacts. In addition, collection of faeces from the same individuals fed the same diet for at least a two week time period would also eliminate any variability in the faeces' chromic oxide content, as a result of differential passage of this marker along the gastro-intestinal tract (Knapka *et al.*1967; Bowen 1978; Leavitt 1985).

3.3. MATERIALS AND METHODS

3.3.1 Experimental diets

Three diets were prepared, one based on purified materials, one based on complex foodstuffs and the third one (zein combined diet) based on a combination of complex foodstuffs and zein. Diets were prepared as described in 2.5.

The composition and proximate analyses of the prepared diets are shown in Table 8.

Table 8. Composition & nutritional profile (g.100g $^{-1}$ DryMatter) of the prepared diets

Nutritional profile	*Purified Diet*	*Experimental basal reference Diet*	*Zein Combined Diet*
Casein [1]	31.0	-	-
Gelatine	6.5	-	-
Gluten	8.0	5.5	3.85
Zein	3.5	0.5	30.4
Fishmeal	-	26.0	18.2
Soya	-	15.0	10.5
Wheat	-	15.0	10.5
Dextrin	31.0	21.0	14.7
α- cellulose	3.0	3.0	2.1
Carboxymethylcellulose	0.5	1.0	0.7
Vegetable Oil [2]	5.0	3.0	2.1
Fish Oil [2]	5.0	3.0	2.1
Vitamin premix [3]	2.0	2.0	1.4
Mineral premix [3]	4.0	4.0	2.8
Chromic Oxide	0.5	1.0	0.7
Dry matter	94.67 (0.14)	95.58 (0.04)	95.42 (0.23)
Crude Protein	41.66 (0.35)	34.25 (0.26)	49.55 (0.06)
Crude Lipid	13.69 (0.16)	6.14 (0.22)	7.31 (0.001)
Carbohydrates	28.82 (0.22)	43.35 (4.18)	31.65 (2.06)
Ash	11.34 (2.17)	9.32 (0.24)	6.44 (0.005)
Crude Fibre	4.47 (2.16)	3.49 (0.30)	4.32 (0.32)
Chromic Oxide	0.45 (0.03)	1.07 (0.07)	0.74 (0.02)
Gross Energy	21.12 kJ. g^{-1} (0.03)	20.35 kJ.g^{-1} (0.04)	22.43 kJ.g^{-1} (0.05)
Protein: Energy ratio	19.73 mg Prot. kJ^{-1}	16.84 mg Prot. kJ^{-1}	22.09 mg Prot. kJ^{-1}
Energy: Protein ratio	50.67 kJ .g^{-1} Protein	59.4 kJ.g^{-1} Protein	45.27 kJ.g^{-1} Protein

1.Source and types of raw materials used are described in 2.5. Their proximate analyses is given in Appendix II. Numbers in parentheses represent Standard Deviation (n=3).
2.The source and fatty acid profile of the oils used is given in Table 6.
3.The composition of Vitamin and Mineral premixes used are described in Tables 4 and 5

3.3.2 Analytical Methods

Water quality analyses are detailed in 3.2.4.

Analyses of diets were performed as described in 2.6.

The marker chromic oxide in diets and faeces was determined as described by Furukawa & Tsukhara (1966).

Gross Energy was determined based on the content of Organic Carbon in the sample as described in 2.8

3.3.3 Experimental Design and Methodology

In order to assess the digestibility of the prepared diets (Table 8) the marker chromic oxide was incorporated at a percentage between 0.5 and 1.0% (Tacon & Rodrigues 1984).

The apparent digestibility coefficients (ADC) were calculated based on the formula (NRC 1993):

ADC= 100-[100 x (%marker in feed x % nutrient in faeces) / (%marker in faeces x %nutrient in feed)]

For the determination of ADC of the Dry Matter the above formula is used after eliminating the factors "nutrient in feed", "nutrient in faeces" (Windell *et al.* 1978).

For the evaluation of zein digestibility, the zein combined diet was formulated (Table 8). The methodology was that described by Wilson & Poe (1985): after having established the digestibility coefficients for the nutrients of a reference diet (in this case the experimental basal reference diet /Table 8), a combined diet for a specific ingredient (zein in this case) is prepared by substituting 30% of the reference diet with the ingredient in question.

The calculation of the nutrient ADCs for the specific ingredient is based on the formula (Wilson *et al.*1981):

Apparent Digestibility Coefficient =(100:30) x [digestion coefficient of combined diet - (70:100) x digestion coefficient of reference diet] (1)

This method has advantages over using single source diets, in that any synergistic effect of feeding the ingredient in combination with other diet components may be realized and prevent the outcome of underestimated digestibility values due to decreased utilization of the single ingredient source (Wilson *et al.* 1981). Equation (1) assumes that the nutrient digestibility of the combined diet is the average of the nutrient digestibility of the reference diet and the test ingredient, weighted by the proportion of each in the combined diet (70:30).

However, according to Forster (1999) the above mentioned equation (1) does not account for the relative contribution of the nutrient from the

reference diet and the test ingredient to the combined diet. Therefore, Forster (1999) suggested the use of the following formula:

$$ADCN_{ingr} = [(a+b) \times ADCN_{com} - a \times ADCN_{ref}] \times b^{-1} \quad (2)$$

where i= level of test ingredient in combined diet (%)

a= nutrient contribution of reference diet to nutrient content of combined diet [=level of nutrient in reference diet x (100-i)]

b= nutrient contribution of test ingredient to nutrient content of combined diet [=level of nutrient in test ingredient x i]

(a + b) = level of nutrient in combined diet (%)

$ADCN_{ingr}$ = Apparent digestibility coefficient of a nutrient in the ingredient under investigation

$ADCN_{com}$ = Apparent digestibility coefficient of a nutrient in the combined diet

$ADCN_{ref}$ = Apparent digestibility coefficient of a nutrient in the reference diet

The purified diet used in the first and fifth experiments (Table 8) was initially destined to be used as a reference diet. When a combined zein diet was made by substituting 30% of the purified diet (Table 8) with zein or gluten (Wilson & Poe 1985), the resulting diet was not accepted by the animals. Due to the palatability problems created by excess zein and gluten, a diet based on complex foodstuffs (Table 8) was prepared and considered the reference diet, instead.

During a total experimental period of 7.5 months, the digestibility of three diets was assessed within six distinct experimental sub-periods:

- First 40-day experimental period: Assessment of the Purified diet.
- Second 60-day experimental period: Assessment of the basal reference diet based on complex foodstuffs.
- Third 25-day experimental period: Assessment of the zein combined semi-purified diet.
- Fourth 45-day experimental period: Reassessment of the basal reference diet based on complex foodstuffs.
- Fifth 40-day experimental period: Reassessment of the Purified diet.
- Sixth 20-day experimental period: Reassessment of the zein combined semi-purified diet.

This experimental design was followed in order to extinguish any variation generated from adaptation of the digestive system to various raw materials and the levels of these materials within the respective diets (3.2). In addition, the collection of faeces from the same individuals fed the same diet for at least a two week time period would also eliminate any variability in the faeces' chromic oxide content, as a result of differential passage of this marker along the gastro-intestinal tract (3.2).

3.3.4 The system

The system used (Plate Three) was a purpose-built recirculating system consisting of twelve 10 L tanks, and two 100 L tanks full of structured plastic bio-rings (2.2.6) and limestone gravel (in order to keep the pH at a neutral range). Standing on a frame and 2m above, there was a 100 L header tank. The overflow of the header tank and the overflows of the twelve individual chamber-tanks were discharged to the first of the biofilter tanks, which additionally acted as a sedimentation tank.

Fish (3.3.5) were kept in the twelve 10 L tanks. The bottom of each tank-chamber was replaced by a 3mm mesh screen and a plastic transparent funnel. At the apex of the funnel short length tubing with a valve was attached for the collection of faeces (Plate Four).

Water was recirculated through a 75Watt Beresford submersible pump (BERESFORD Pumps, Carlton Road, Foleshill, Coventry, CV6 7FL), of a head of 4m with a max flow rate of 3,400 L. h^{-1} (780gph or 57 $L.min^{-1}$).

Temperature was kept constant at 26-27^0 C by using heating elements in both the biofilter tanks and the header tank.

Water from the header tank was sampled once fortnightly in order to assess the water quality parameters (2.1). During the 7.5 months of total experimental period fifteen (15) samplings were performed and the following values recorded:

Nitrites (NO_2): Average value 0.14 ppm (SD 0.057)

Minimum 0.044 ppm, Maximum 0.24 ppm

Nitrates (NO_3): Average value 48.5 ppm (SD 9.85)

Minimum 26 ppm, Maximum 65 ppm

Unionized ammonia: Average value 0.66 ppm (SD 0.11)

PLATE 3. OUTLINE OF THE DIGESTIBILITY SYSTEM (not to scale)

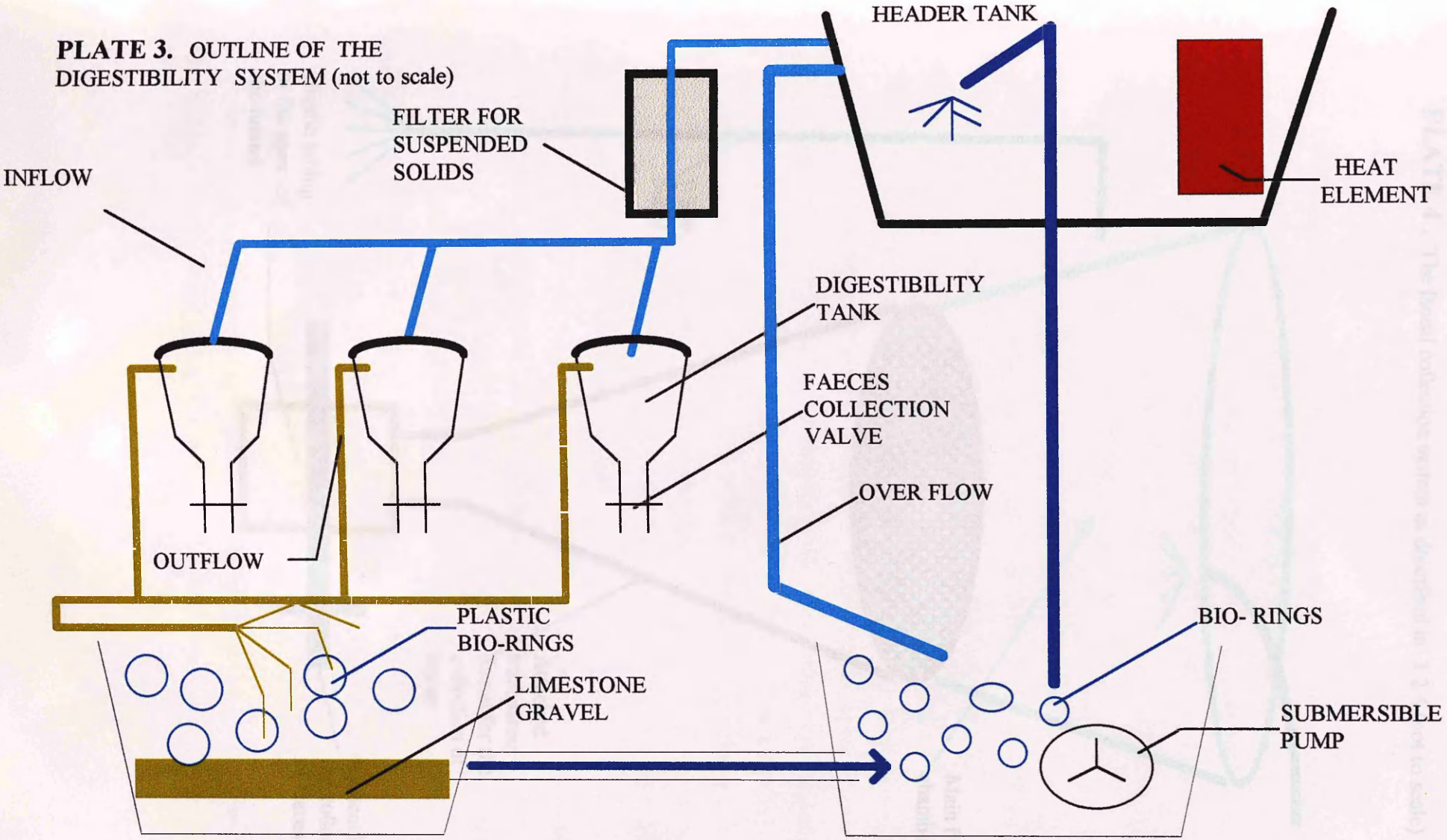
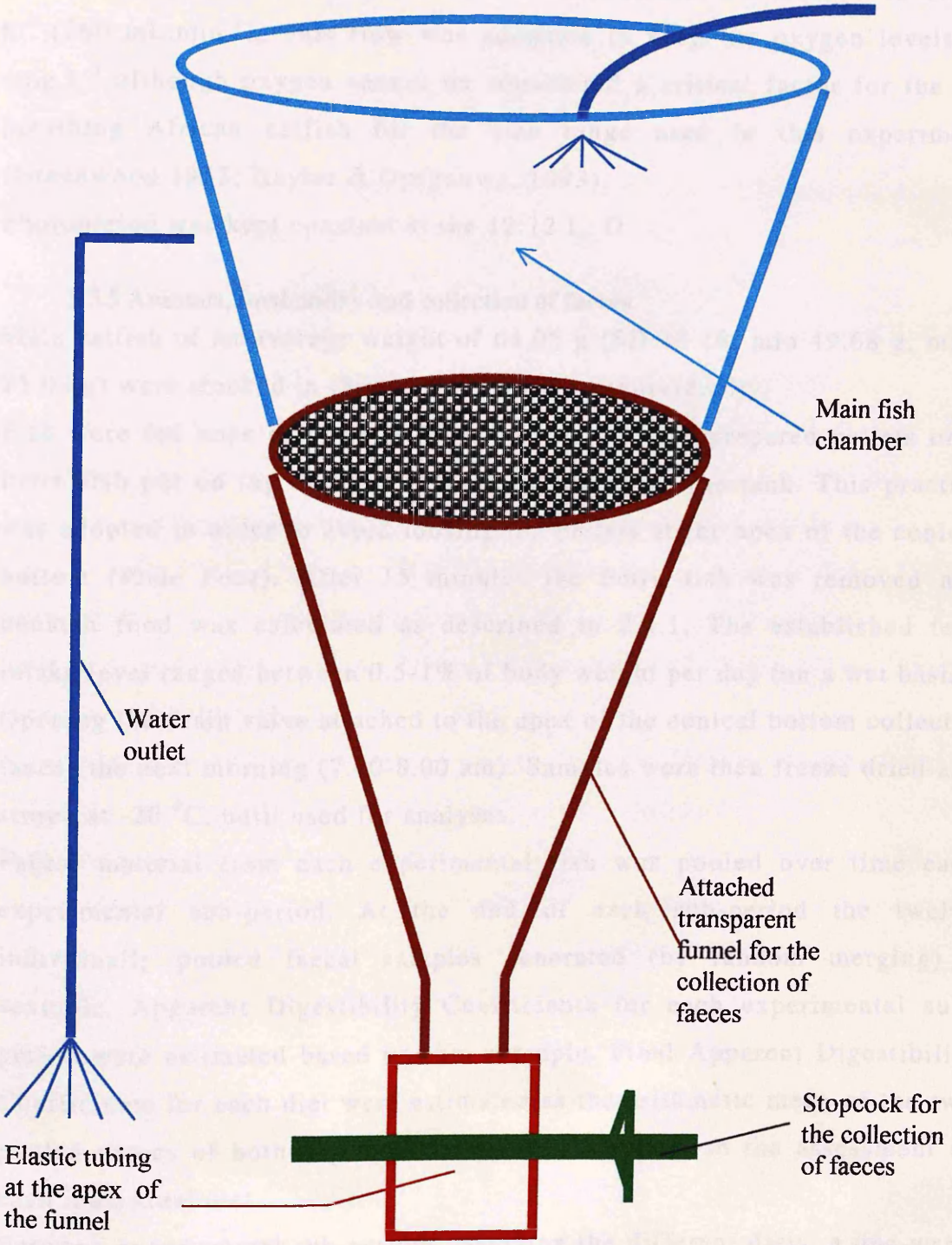


PLATE 4 . The faecal collection system as described in 3.2.4 (not to scale)



Minimum 0.25 ppm, Maximum 0.77 ppm

pH : Average value 6.6 (SD 0.047)

Minimum 6.5 Maximum 6.7

The flow rate in each of the individual fish chambers did not exceed 15 L. h^{-1} (260 $mL.min^{-1}$). This flow was adequate to keep the oxygen levels at 4$mg.L^{-1}$ although oxygen cannot be considered a critical factor for the air breathing African catfish for the size range used in this experiment (Greenwood 1955; Haylor & Oyegunwa, 1993).

Photoperiod was kept constant at the 12:12 L: D

3.3.5 Animals, husbandry and collection of faeces

Male catfish of an average weight of 64.05 g (SD 10.18, min 49.68 g, max. 83.03 g) were stocked in the twelve chambers, individually.

Fish were fed once daily at 19.00 h by inserting the prepared pellets on a Petri dish put on top of the mesh screen bottom of the tank. This practice was adopted in order to avoid loosing the pellets at the apex of the conical bottom (Plate Four). After 15 minutes the Petri dish was removed and uneaten food was calculated as described in 2.4.1. The established feed intake level ranged between 0.5-1% of body weight per day (on a wet basis). Opening the drain valve attached to the apex of the conical bottom collected faeces the next morning (7.00-8.00 am). Samples were then freeze dried and stored at -20 ^{0}C, until used for analyses.

Faecal material from each experimental fish was pooled over time each experimental sub-period. At the end of each sub-period the twelve individually pooled faecal samples generated (by random merging) a sextuple. Apparent Digestibility Coefficients for each experimental sub-period were estimated based on this sextuple. Final Apparent Digestibility Coefficients for each diet were estimated as the arithmetic mean of the two pooled values of both experimental periods involved in the assessment of each individual diet.

Between experimental sub-periods assessing the different diets, a one-week acclimatization period (from one diet to the other) was adopted.

3.4. RESULTS

The established Apparent Digestibility Coefficients (ADCs) for the three experimental diets and the calculated ADCs for zein are shown in Table 9.

The comparison of the ADCs among the various diets and zein revealed the following (Table 10):

The purified diet used in the first and fifth experiment showed the highest Crude Protein digestibility. The rest of the diets and zein showed no significant difference in Crude Protein digestibility.

All diets and zein are characterized by statistically insignificant ($P>0.05$) differences in Crude Lipid and Dry Matter digestibility coefficients.

Higher carbohydrate digestibility was observed in the basal reference diet comprised of complex foodstuffs, whereas the purified and the semi-purified zein combined diet showed lower carbohydrate digestibility.

Significantly ($P< 0.05$) higher Ash digestibility values were recorded for the purified diet and the raw material zein.

Only the raw material zein showed significantly ($P<0.05$) higher Energy digestibility values.

Estimation of zein digestibility values by the Forster (1999) method [equation (2), page 80], revealed significantly lower and higher ADC values for carbohydrates and ash, respectively (Table 10) when compared to the ADCs of the manufactured diets. However, the Forster (1999) method did not reveal any statistically significant differences ($P<0.5$) for the rest of nutrients, between zein and the manufactured diets.

Table 9. Apparent Digestibility Coefficients (%) for the diets of the digestibility experiments[1]

Purified Diet	*Dry Matter*	*Crude Protein*	*Crude Lipid*	*CHO*	*Ash*	*Energy*
ADC 1st experiment	78.51 (2.58)	92.88 (1.5)	96.02 (0.27)	67.14 (3.2)	89.36 (1.23)	82.65 (0.32)
ADC 2nd experiment	71.54 (2.4)	86.65 (0.23)	95.36 (0.32)	76.39 (2.87)	88.17 (1.55)	82.92 (0.57)
Average ADC	75.02 (4.92)	89.76 (3.12)	95.80 (0.33)	71.77 (4.63)	88.96 (0.59)	82.78 (0.13)
Basal reference Diet						
ADC 1st experiment	64.67 (4.53)	76.97 (1.29)	87.51 (0.77)	79.8 (3.67)	52.31 (0.95)	71.85 (2.15)
ADC 2nd experiment	71.61 (3.12)	88.88 (1.14)	95.08 (0.47)	85.06 (2.95)	55.62 (0.78)	78.71 (0.27)
Average ADC	68.14 (8.57)	82.93 (5.96)	91.29 (5.35)	82.43 (2.63)	53.96 (1.65)	75.28 (4.85)
Zein combined Diet						
ADC 1st experiment	68.96 (3.12)	81.22 (1.24)	90.85 (2.89)	74.39 (0.76)	53.26 (4.47)	79.61 (0.65)
ADC 2nd experiment	72.36 (3.84)	83.49 (0.55)	93.71 (0.90)	79.25 (0.93)	68.12 (11.84)	79.85 (0.56)
Average ADC	70.66 (2.40)	82.36 (1.60)	92.28 (2.02)	76.83 (2.43)	60.68 (10.51)	79.73 (0.12)
Calculated Zein ADCs *						
ADC 1st experiment	78.96 (12.73)	91.14 (7.78)	98.65 (6.88)	61.75 (8.57)	55.46 (16.38)	97.69 (2.48)
ADC 2nd experiment	74.09 (11.27)	70.92 (11.95)	90.52 (2.07)	65.72 (6.90)	97.30 (37.66)	82.51 (0.63)
Average ADC	76.53 (3.44)	81.03 (14.30)	94.58 (5.75)	63.73 (1.98)	76.38 (29.58)	90.10 (10.73)
Calculated Zein ADCs **						
ADC 1st experiment	78.79 (12.63)	85.32 (4.05)	104.16 (9.93)	32.49 (28.41)	62.93 (56.95)	94.83 (2.20)
ADC 2nd experiment	74.06 (11.16)	78.30 (7.06)	88.26 (2.91)	34.41 (22.87)	196.42 (125.33)	82.08 (0.52)
Average ADC	76.43 (3.34)	81.81 (4.96)	96.21 (11.24)	33.45 (1.35)	129.67 (94.39)	88.46 (9.01)

1.Numbers in parentheses represent Standard Deviation (for each experimental sub-period n=6 except for the Calculated Zein ADCs where n=3 / Standard Deviation of average ADCs are based on n=2)

* Calculation based on Wilson *et al.* (1981) ** Calculation based on Forster (1999)

Table 10. Comparison of the Apparent Digestibility Coefficients (ADC) among the diets of the digestibility trials[1]

Nutrients	*Purified diet*	*Basal reference diet*	*Zein combined diet*	*Zein**	*Zein***	*SD****
Dry Matter	75.02[a]	68.14[a]	70.66[a]	76.53[a]	76.43[a]	8.13
Crude Protein	89.76[a]	82.93[b]	82.36[b]	81.03[b]	81.81[b]	8.08
Crude Lipid	95.80[a]	91.29[a]	92.28[a]	94.58[a]	96. 21[a]	6.24
Carbohydrate	71.76[b]	82.43[a]	76.82[b]	63.73[b]	33.45[c]	22.34
Ash	88.96[b]	53.96[c]	60.68[c]	76.38[b]	129.67[a]	32.31
Energy	82.78[b]	75.28[b]	79.73[b]	90.10[a]	88.46[b]	6.71

Values in the same row and with the same superscript are not significantly different (P>0.05). * Calculation based on Wilson *et al.* (1981) ** Calculation based on Forster (1999) *** Standard Deviation of the multiple comparisons

The established ADCs for the amino acids of three experimental diets and zein are shown in Tables 11, 12, 13 and 14.

Table 11. Apparent digestibility coefficients for the Amino Acids of the purified diet[1]

	ADC of 1st experiment	*ADC of 2nd experiment*	*Average ADCs*
Essential AminoAcids			
L-Arginine	99.12 (0.72)	97.28 (1.53)	98.21 (1.30)
L-Cystine (NEAA)	90.45 (0.34)	88.63 (0.44)	89.54 (1.29)
L-Histidine	98.42 (2.30)	96.34 (1.22)	97.38 (1.47)
L-Isoleucine	97.63 (2.01)	94.58 (1.53)	96.10 (2.16)
L-Leucine	96.38 (0.66)	95.66 (0.78)	96.20 (0.51)
L-Lysine	98.02 (0.97)	96.52 (0.68)	97.27 (1.06)
L-Methionine	97.47 (0.78)	95.89 (0.83)	96.68 (1.12)
L-Phenylalanine	97.93 (2.40)	93.63 (2.75)	95.78 (3.04)
L-Threonine	98.50 (1.60)	95.60 (1.90)	97.05 (2.05)
L-Tyrosine(NEAA)	98.46 (0.52)	96.20 (1.01)	97.33 (1.60)
L-Valine	97.86 (0.38)	97.25 (0.29)	97.55 (0.43)
Non Essential AminoAcids			
L-Alanine	97.65 (0.54)	95.30 (0.89)	96.47 (1.66)
L-Aspartic acid	97.45 (0.82)	95.52 (1.02)	96.48 (1.36)
L-Glutamic acid	98.97 (0.32)	98.31 (0.43)	98.64 (0.46)
Glycine	98.61 (1.23)	97.13 (0.86)	97.87 (1.05)
L-Proline	97.76 (0.22)	96.64 (0.53)	97.20 (0.79)
L-Serine	98.10 (0.72)	95.69 (0.65)	96.89 (1.70)

1. Numbers in parentheses represent the Standard Deviation (n=2)

Table 12. Apparent Digestibility Coefficients for the amino acids of the Basal reference diet[1]

	ADC 1st experiment	*ADC 2nd experiment*	*Average ADCs*
Essential AminoAcids			
L-Arginine	92.30 (0.52)	91.36 (0.83)	91.83 (0.46)
L-Cystine (NEAA)	88.17 (2.47)	97.13 (1.36)	92.65 (4.47)
L-Histidine	88.25 (0.65)	88.43 (1.12)	88.34 (0.09)
L-Isoleucine	88.73 (0.68)	87.24 (1.49)	87.98 (0.75)
L-Leucine	91.47 (1.7)	87.72 (2.16)	89.59 (1.87)
L-Lysine	90.42 (0.97)	87.50 (2.05)	88.96 (1.46)
L-Methionine	96.67 (0.34)	97.28 (0.92)	96.97 (0.31)
L-Phenylalanine	84.93 (1.12)	80.99 (2.18)	82.96 (1.96)
L-Threonine	88.15 (0.51)	90.28 (0.79)	89.22 (1.07)
L-Tyrosine (NEAA)	87.68 (0.66)	88.13 (1.02)	87.91 (0.23)
L-Valine	87.85 (1.43)	86.70 (0.67)	87.27 (0.57)
Non Essential AminoAcids			
L-Alanine	88.17 (0.45)	85.39 (1.02)	86.78 (1.39)
L-Aspartic acid	87.82 (0.98)	87.85 (1.29)	87.84 (0.01)
L-Glutamic acid	92.80 (0.98)	91.17 (0.73)	91.98 (0.82)
Glycine	86.89 (0.26)	87.09 (0.53)	86.99 (0.10)
L-Proline	92.53 (1.32)	91.67 (1.95)	92.10 (0.43)
L-Serine	88.77 (0.83)	85.68 (1.09)	87.23 (1.54)

1.Numbers in parentheses represent the Standard Deviation (n=2)

Table 13. Apparent Amino Acid Digestibility Coefficients for the zein combined diet[1]

	ADCs 1st experiment	*ADCs 2nd experiment*	*Average ADCs*
Essential AminoAcids			
L-Arginine	92.94 (1.56)	93.73 (1.72)	93.35 (0.55)
L-Cystine (NEAA)	90.78 (1.43)	91.36 (0.63)	91.07 (0.41)
L-Histidine	91.35 (0.48)	90.32 (0.37)	90.83 (0.73)
L-Isoleucine	90.83 (1.67)	89.66 (0.78)	90.24 (0.83)
L-Leucine	91.29 (0.56)	90.10 (0.73)	90.69 (0.84)
L-Lysine	90.34 (0.68)	90.43 (0.93)	90.38 (0.06)
L-Methionine	97.15 (0.73)	97.89 (0.89)	97.52 (0.53)
L-Phenylalanine	85.48 (1.25)	86.34 (0.84)	85.91 (0.61)
L-Threonine	90.48 (1.03)	91.85 (1.36)	91.17 (0.96)
L-Tyrosine(NEAA)	90.98 (0.28)	90.28 (0.32)	90.63 (0.50)
L-Valine	89.43 (0.92)	90.13 (0.68)	89.78 (0.49)
Non Essential AminoAcids			
L-Alanine	89.25 (0.87)	88.86 (1.02)	89.26 (0.55)
L-Aspartic acid	91.24 (0.42)	90.88 (0.35)	91.06 (0.25)
L-Glutamic acid	91.46 (0.23)	92.74 (0.15)	92.10 (0.90)
Glycine	89.90 (0.43)	90.42 (0.38)	90.16 (0.37)
L-Proline	89.74 (1.84)	88.53 (2.01)	89.13 (0.85)
L-Serine	89.65 (0.83)	88.74 (0.72)	89.19 (0.64)

1.Numbers in parentheses represent the Standard Deviation (n=2)

Table 14. Apparent Amino Acid Digestibility Coefficients of zein (as a raw material) for the African catfish *C. gariepinus*

	1st experiment	*2nd experiment*	*Average values*[1]	
Essential AminoAcids				
L-Arginine	94.43	99.26	96.85	(3.41)
L-Cystine (NEAA)	96.87	77.89	87.38	(13.42)
L-Histidine	98.58	94.73	96.65	(2.72)
L-Isoleucine	95.73	95.31	95.52	(0.30)
L-Leucine	90.87	95.65	93.26	(3.38)
L-Lysine	90.15	97.26	93.71	(5.03)
L-Methionine	98.27	99.31	98.79	(0.74)
L-Phenylalanine	86.76	98.82	92.79	(8.50)
L-Threonine	95.91	95.51	95.71	(0.29)
L-Tyrosine(NEAA)	98.68	95.29	96.98	(2.40)
L-Valine	93.11	98.13	95.62	(3.54)
Non Essential AminoAcids				
L-Alanine	91.77	96.95	94.36	(3.67)
L-Aspartic acid	99.22	97.95	98.58	(0.89)
L-Glutamic acid	88.33	96.40	92.36	(5.71)
Glycine	96.93	98.19	97.55	(0.89)
L-Proline	83.23	81.20	82.21	(1.43)
L-Serine	91.86	95.88	93.87	(2.84)

1.Numbers in parentheses represent the Standard Deviation (n=2)
Calculation based on Wilson *et al.* 1981

The comparison of amino acid digestibility coefficients among the three diets and zein showed the following (Table 15):

The highest Apparent Digestibility Coefficients (ADC) for Histidine, Tyrosine, Isoleucine, Valine, Glycine, Threonine and Aspartic acid were recorded for the Purified diet (1st and 5th experiment) and the raw material zein. On the contrary, Glutamic acid, Methionine, Cystine, Leucine, Phenylalanine were characterized by non statistically different ADC values among all the diets and the raw material in question (zein).

Except for Arginine, Cystine, Methionine and Glutamic acid the ADCs for the rest of the amino acids were higher for the Purified diet than for the Basal reference diet made from complex foodstuffs.

Fifteen out of the seventeen (88.2%) determined amino acids of zein had similar and non-statistically different ADCs (Table 15) with the Purified diet. In addition, calculation of zein's ADCs by the Forster (1999) equation (2), revealed statistical differences for only three amino acids when compared to their respective values estimated by equation (1) (Wilson *et al.* 1981)

Table 15. Comparison of the Amino acid ADCs among the diets and zein[1]

Amino Acids	*Purified diet*	*Basal reference diet*	*Zein combined diet*	*Zein* [2]	*Zein* [3]	*SD*[*]
Essential AminoAcids						
L-Arginine	98.21[a]	91.83[a]	93.35[a]	96.85[a]	97.82[a]	3.28
L-Cystine (NEAA)	89.54[a]	92.65[a]	91.07[a]	87.38[a]	89.49[a]	5.61
L-Histidine	97.38[a]	88.34[b]	90.83[b]	96.65[a]	95.62[a]	3.96
L-Isoleucine	96.10[a]	87.98[c]	90.24[c]	95.52[a]	92.41[b]	3.36
L-Leucine	96.02[a]	89.59[b]	90.69[b]	93.26[ab]	91.15[ab]	2.81
L-Lysine	97.27[a]	88.96[b]	90.38[b]	93.71[b**]	n.d.	4.02
L-Methionine	96.68[b]	96.97[ab]	97.52[ab]	98.79[a]	98.52[ab]	1.03
L-Phenylalanine	95.78[a]	82.96[b]	85.91[ab]	92.79[ab]	87.72[ab]	5.90
L-Threonine	97.05[a]	89.22[c]	91.17[bc]	95.71[a]	93.87[ab]	3.16
L-Tyrosine (NEAA)	97.33[a]	87.91[c]	90.63[bc]	96.98[a]	92.37[b]	3.98
L-Valine	97.55[a]	87.27[c]	89.78[bc]	95.62[a]	93.55[ab]	4.23
Non Essential AminoAcids						
L-Alanine	96.47[a]	86.78[c]	89.26[bc]	94.36[ba]	90.13[bc]	4.03
L-Aspartic acid	96.48[a]	87.84[c]	91.06[b]	98.58[a]	96.17[a]	4.22
L-Glutamic acid	98.64[a]	91.98[a]	92.10[a]	92.36[a]	92.18[a]	3.46
Glycine	97.87[a]	86.99[c]	90.16[b]	97.55[a]	99.46[a]	5.20
L-Proline	97.20[a]	92.10[b]	89.13[c]	82.21[d]	87.39[c]	5.29
L-Serine	96.89[a]	87.23[c]	89.19[bc]	93.87[ab]	91.11[bc]	3.85

1. Values in the same row and with the same superscript are not significantly different ($P>0.05$ / n=2). 2. Calculation based on Wilson *et al.* 1981 3. Calculation based on Forster 1999

* Standard Deviation of the multiple comparisons ** Lysine of endogenous origin as zein is totally deficient in this amino acid (Appendix II)

n.d. not determined as coefficient "b" in Forster's formula (2) equals zero

3.5. DISCUSSION

Information on the Apparent Digestibility Coefficients of either experimental or common ingredients and their respective macronutrients for clariid catfishes has advanced in recent years:

Henken *et al.* 1985 recorded similar apparent digestibility values for Dry Matter, Crude Protein and Energy (73-83%) to the ones of the present experiments, by experimenting on adult African catfish (136.92±3.89g) fed a diet comprised of complex foodstuffs (50% Crude Protein, 22.52 $kJ.g^{-1}$ Energy) and by using a similar faecal collection system. However, these results cannot be directly comparable to the results of this study due to the different procedures employed: the experiment lasted for only 21 days, fish were fed on a nocturnal (19.00-09.00h) continuous basis by applying

varying feeding levels and digestibility values were estimated by the direct method of quantitative collection of faeces as initially described by Ogino *et al.* 1973.

Fagbenro (1996) showed varying Crude Protein (58-92%) and Energy (50-93%) ADC values for animal and plant-based foodstuffs in *Clarias isheriensis* (47.5-51.2g) using chromic oxide as an indicator and the rectal dissection method (Henken *et al.* 1985) for faeces collection. In his effort to reduce catfish/feed production costs by fishmeal replacement, he also tested various oilseed cakes/meals in *C.gariepinus* fingerlings (47.5-51.2g) and revealed Crude Protein (80-87%) and Energy (65-79%) ADCs similar to the ones of the present study (Fagbenro 1998).

The Crude Protein ADCs recorded in this experiment are also similar to the ones (70-86.6%) recorded for fingerlings (≅ 21g) fed diets based on a mixture of algal and blood meal at various combinations and with protein levels ranging from 9.7 to 63% (Ufodike & Ekokotu 1986). The protein digestibility of those algal and blood meal diets was directly proportional to the dietary protein level until an optimum of protein ADC was achieved at 50.2% dietary protein (Ufodike & Ekokotu 1986). However this has not been the case with the diets of this experiment, as the basal reference diet and the zein combined diet showed equal ADCs for Crude Protein, despite their difference in Crude Protein level (34% and 49.5% respectively).

Lower Crude Protein (69.5% - 75%) and Crude Lipid (63.9% -60.1%) ADCs were recorded for agricultural by-products (plantain peelings- *Musa sp.*, yam peelings-*Dioscorea sp.*) and fish waste fed to juvenile African catfish, whereas the Carbohydrate ADCs for these agricultural by-products were similar (65.5%-73.2%) to those recorded in this experiment (Mgbenka 1991).

The Apparent Digestibility Coefficients (ADCs) for Crude Protein for all the diets and zein in these experiments, were higher than those reported for moist diets based on fermented tilapia silage and tested in *C. gariepinus* of an average weight of 18.5 ±1.3 g (Fagbenro & Jauncey 1994). On the contrary, dry diets based on co-dried fermented tilapia silage:soyabean flour, blended with fishmeal and corn starch and tested in *C. gariepinus* of

an average weight of 10.8±0.3g (Fagbenro *et al.*1994; Fagbenro 1994) had almost equal Crude Protein and Energy ADCs with those reported in this study. This could be attributed to the near neutral pH (6.3-6.5) of the dried blend tilapia silage: soyabean flour and the better control of protein autolysis in these diets (Non Protein Nitrogen / NPN; Backhoff 1976, Gildberg & Raa 1979), when compared to the semi-moist diets, based on fermented tilapia silage and which are characterized by lower pH and higher levels of free amino acids (NPN). These high levels of free amino acids and peptides, resulting from proteolysis, can interfere with protein absorption and result in lower ADC values, as has been reported for salmonids fed dried acid silages (Hardy *et al.*1983;1984). Furthermore, the availability and biological value of amino acids from silages may be negatively affected due to the loss of some amino acids as a result of the Maillard reaction (reactions between a-amino groups and sugar aldehyde groups) (Kalaisakis 1982).

Protein availability in soy containing diets may be largely controlled by the presence of soy crystalline globular proteins that act as trypsin inhibitors (Liener & Kakade 1980). Both the Basal Reference Diet and the zein Combined Diet used in these experiments contained soy in such quantities that could probably have exerted an inhibitory action towards protein and amino acid digestibility and eventually explain the higher ADCs for Crude Protein and most of the amino acids of the Purified Diet. However, levels of trypsin inhibitors in the "commercial aquaculture grade" soy used in these experiments, should have been rather low and therefore not much in the way of protein absorption (Jauncey 1999 per. com.)

Although the Cr_2O_3 inclusion level, in any of the diets, was not >2%, it is possible that a faster (relative to the digesta) Cr_2O_3 transit rate took place, resulting in the overestimation of Crude Lipid ADCs (Ringo *et al.*1993a,b). However, the applied experimental design in these trials should have eliminated this component of variation.

The low Ash ADC values observed for the basal reference diet and the zein combined diet could have been the effect of the low bio-availability of minerals and trace elements in their constituent raw materials: the apparent availability of phosphorus in whole-fish meals for salmonids may range from 20 to 70% according to the form of the phosphate salt (monobasic-dibasic-tribasic) and the ash (bone) content of the fishmeal (Lall 1991; Nordrum *et al.* 1997; Riche & Brown 1996; Yamamoto *et al.* 1997; Yone & Toshima 1979). In plant proteins a large proportion of phosphorus is present as organically bound phytate. Not only is phytic acid phosphorus largely biologically unavailable, but phytic acid also has the capacity to chelate other trace elements (i.e. iron, copper, zinc, cobalt, molybdenum) and by so doing may render them biologically unavailable to the fish during digestion (Spinelli 1980; Lovell 1989; Hossain & Jauncey 1991). Both, basal reference diet and the zein combined diet contained fishmeal and grain-meals (soy, wheat) whose minerals might have been biologically unavailable and therefore the cause of lower Ash ADC values compared to the ones of the Purified Diet.

The significant variation that was observed in zein's ADC values for Carbohydrates and Ash by using Forster's (1999) equation (2) compared to the values estimated by equation (1) (Wilson *et al.*1981) has mainly been caused by the disparity in the values of those two nutrients between the Basal reference diet and the test ingredient (zein) (Table1 & Appendix II). This disparity resulted in a very unequal nutrient contribution (a, b, in equation 2) of each component (reference diet-zein) to the nutrient level (a+b, in equation 2) of the zein combined diet (Forster 1999). Another source of variation between the ADC values obtained by the two equations for those two zein nutrients, is the difference between the nutrient ADC values of the Basal reference diet and the zein combined diet (Table 10).
Calorific content of Protein, Lipid and Carbohydrate constitute the main components of Energy in any material. Therefore, the significantly higher Energy ADC value for zein estimated by Wilson *et al.*1981 when compared to the lower respective one estimated by Forster's equation, cannot be

reasoned based on its component ADC values for Protein, Lipid and Carbohydrate.

On the contrary, the lack of significant difference ($P<0.05$) between zein's ADC values for the Dry Matter, Crude Protein, and Crude Lipid when estimated by the two equations (1) and (2), can be attributed to the minimized difference between the component values "a" and "b" for those nutrients.

Furthermore, the lack of statistical difference in the amino acid ADC values of zein (but for three amino acids;Table 15) when estimated by both methods (Wilson *et al.* 1981; Forster 1999) can be attributed to the minor differences of those amino acid values between the material zein and the basal reference diet.

It can be concluded that calculation of nutrient ADCs for a single ingredient by Forster's method can be a useful tool in digestibility studies, particularly when the nutrient profile of the examined ingredient deviates from the nutrient profile of the reference diet.

Dextrin, an intermediate product of starch hydrolysis to maltose and *d*-glucose, is characterized by similar glycoside linkage to starch and was the prevalent carbohydrate source in the purified digestibility diet. Therefore the high carbohydrate digestibility of this diet (71.76% ±4.63) suggests a similar high starch digestibility for the African catfish *C. gariepinus* and confirms the Uys (1989) hypothesis of good starch utilization in the species due to elevated amylase levels in the anterior part of the intestine.

Higher ADCs for crude protein and most amino acids in the purified diet used in these experiments, coupled with its equally high ADCs for other nutrients and good palatability, indicate that such a purified experimental diet can be an efficient tool in assessing assimilation efficiency in African catfish. However an unsuccessful attempt of incorporating gluten and zein in excess of 30% in such a purified diet, unveils an unpalatability element, taking effect beyond a certain incorporation threshold of those two semi-purified materials.

As digestibility *per se* does not reveal anything related to the time-course of absorption, further investigation of the serum free amino acid concentrations following the administration of such diets will better elucidate their intra-specific amino acid relationships and their effect on protein utilization.

Maize, which is of high zein content, is usually characterized by low protein digestibility values as zein is not easily broken down by enzymes due to the absence of lysine and tryptophan (Morrison 1950;Clay 1981).

However, the zein used in these experiments was a semi-purified feed ingredient with no apparent negative effect on digestive processes: both the zein combined diet and zein (as a material) showed acceptable ADCs for their nutrients and amino acids. As 88.2% of zein amino acids had similar and non-statistically different ADCs to those of the purified diet, use of zein in related nutritional experimental work can be recommended. Furthermore, the recorded ADCs for zein could be used as a broadly applied index of its digestibility in African catfish.

CHAPTER 4 INVESTIGATION FOR THE DETERMINATION OF THE OPTIMUM PROTEIN:ENERGY RATIO

4.1 INTRODUCTION

Digestibility experiments (Chapter 3) showed that adult catfish *C. gariepinus* accepts purified diets comprised mainly (48-50%) of purified proteins with high digestibility values for most nutrients (3.4). However, digestibility *per se* does not guarantee good performance of animals fed such diets. A dietary nutritional profile fulfilling the nutritional requirements of the animal will further support good performance of the species in question.

Increasing the protein level of the diet does not result in better protein utilization. The magnitude of metabolizable energy (ME) and its fraction, the recovered energy (RE) will dictate protein deposition (Figure 4;1.2.4.2). Protein quality will affect energy utilization and therefore replacing good quality dietary protein with lipid or carbohydrate might create a protein-sparing effect (Watanabe *et al.*1979; Degani & Viola 1987; Nematipour *et al.*1992; Cowey 1993).

Ratios of digestible protein to digestible energy for maximum weight gain of several fish species range between 19.35 mg protein.kJ^{-1} to 27.96 mg protein.kJ^{-1} (NRC 1993). These ratios are substantially higher than those for swine and poultry ranging from 9.55 to 14.34 mg protein.kJ^{-1} (NRC 1984; 1988), as fish require less energy for maintenance (1.2.4.2).

Excessive dietary energy can lead to increased lipid deposition (Watanabe 1982), deterioration of carcass quality (1.2.3.1) and reduced growth (Daniels & Robinson 1986), although some workers have indicated that differences in fat deposition may be a reflection of differences in growth rates and fish sizes, rather than being a direct effect of dietary treatment (Shearer 1994; Einen & Roem 1997; Johansen & Jobling 1998). Fat deposition in rainbow trout is affected both by feeding rates-fish size (Kiessling *et al.*1989; Storebakken *et al.*1991) and dietary fat content (Jobling *et al.*1998). Excess carcass lipid due to increased dietary energy has also been shown for channel catfish *Ictalurus*

punctatus (Garling & Wilson 1977; Mohsen & Lovell 1990), common carp *Cyprinus carpio* (Takeuchi *et al.*1979), red drum *Sciaenops ocellatus* (Serrano *et al.*1992) and rabbitfish *Siganus canaliculatus* (Osman *et al.*1996). In Atlantic salmon (*Salmo salar*) carcass fat levels are affected by the life stage: use of a diet with a digestible energy content of more than 20 kJ g^{-1}during smoltification increased carcass fat (Helland & Grisdale-Helland 1998), whereas during the sea water phase carcasses were significantly affected by feeding rate but not by dietary fat (Johnsen & Wandsvik 1991; Hillestad *et al.* 1998).

Precise definition of the appropriate dietary Protein: Energy ratio (based on purified diets) would inevitably improve the cost-effectiveness of feeding (1.3). Therefore, the purpose of this experiment was to assess the performance of African catfish fed varying Protein to Energy ratios, at graded dietary protein levels. Three protein levels (30, 40 and 46%) resulting in six protein to energy ratios were tested ranging from 12.85 mg Protein.kJ^{-1} (53.77mg Protein.$kcal^{-1}$) to 22.74 mg Protein.kJ^{-1} (95.16 mg Protein.$kcal^{-1}$). These levels were selected based on the results of Machiels & Henken (1985) and Uys (1989) (1.1.2.3) who concluded that growth and protein utilization were better at a Crude Protein level of over 40% but not higher than 48% of the diet and a Protein:Energy ratio between 16mg and 26.4mg Protein.kJ^{-1}.

Utilization of nutrients as energy sources in fish is different from that in mammals and almost species specific (1.2.3.3). Therefore, it was felt that a more in depth investigation of the biochemical composition of vital body organs (liver) coupled with blood glucose levels and achieved performance parameters would better elucidate the utilization of carbohydrates and other dietary nutrients and facilitate decisions regarding the balanced dietary profile of a cost-effective diet.

Another ancillary tool in nutritional research is haematology. It is widely accepted (Schaperclaus 1991; Roberts 1989) that haematological investigation

does not have as great a prominence in fish studies as it does in human and veterinary medicine. This is mainly attributed to the fact that haematopoiesis in teleosts is different from that in higher vertebrates: it takes place in the stroma of the spleen and the interstitium of the kidney and to a lesser extent in the periportal areas of the liver, the intestinal submucosa and the specialized lymphoid organ, the thymus (Schaperclaus 1991). Moreover, there are no special regulatory processes, which keep immature blood cells from entering the blood vessels of the peripheral blood (Schaperclaus 1991). On a practical level, the total blood volume available for sampling, in fish is quite small, involving the risk of excess stress and irreversible damage for individuals in the experimental population.

Evidently, marked variations in haematological data of fish can occur due to the different haematopoiesis mechanisms involved (compared to those of higher vertebrates). However, investigation of haematocrit as an haematological parameter revealed that intra-specific (within individuals of the same species and experimental group) variations can occur due to a multitude of other factors: the diet and nutritional stage (Eiras *et al.*1983), breed and genetic factors (Larsson *et al.*1976), salinity (Dendrinos & Thorpe 1985; Zanuy & Carrillo 1985), temperature (Houston & Koss 1984), oxygen level in the medium (Larsson *et al.*1976), stocking density (Kjartansson *et al.* 1988), sex (Larsson *et al.*1976), seasonal changes and daily cycles (Murray 1984; Zanuy & Carrillo 1985; Sandnes *et al.*1988) and weight or age of the fish (Romestand *et al.* 1983; Zanuy & Carrillo 1985).

The values of the haematological parameters can also be affected by the techniques used for sample collection (cardiac or venous puncture, section of the caudal peduncle, with or without anaesthesia, use/non-use of an anticoagulant) (Hoffman *et al.*1982; Hoffman & Lommel 1984; Duthie & Tort 1985) or the analytical methods involved (Nomura & Kawatsu 1977).

Therefore, "normal" haematological values are meaningful only under specific environmental conditions and age groups for each individual species.

Despite the variations involved, haematocrit has been repeatedly used as a performance index and proved to be successful in revealing amino acid deficiencies and imbalances (Chance *et al.*1964; Ruchimat *et al.*1997), other feed related anaemias (Plumb *et al.*1986) or responses to specific dietary regimes and diets (Eiras *et al.*1983; Alexis *et al.*1985; Hemre *et al.* 1995). Although the diets used in this study were not expected to create pathological problems it was considered worthwhile to examine two basic parameters, haematocrit and total haemoglobin. These results might be indicative of the usefulness of these indices as potential "performance indicators" in nutritional research related to the African catfish.

4.2 MATERIALS AND METHODS

4.2.1 Diets, diet preparation and feeding regime

Seven diets made from purified materials (2.5) and one, the Control, made from complex foodstuffs (2.5) were prepared and used in this experiment. Diet analyses were performed as described in 2.6.

The composition and proximate analyses of the diets was as shown in Table 16. Formulation of the diets was based on previously established successful formulations (Uys 1984;Uys 1989;Machiels & Henken 1985) but utilizing purified materials that give a much greater degree of certainty than complex foodstuffs (fishmeal, soy, wheat, maize, carcass meal, blood meal) have so far permitted (1.1.2.3). Levels of nutrients were also based in previous experimentation with the aim of narrowing down the already achieved proportions-incorporation ratio (4.1). Selection of complex foodstuffs for the preparation of the Control diet was considered worthwhile in order to establish a degree of comparison between the performance of animals fed the purified materials and those fed an artificial diet under real conditions in a commercial operation.

The ration size adopted was determined by the methodology described in 2.4.1. Fish were fed twice a day as described in 2.4.2.

Before the start of the experiment, fish were acclimatized to the purified diets for 20 days (by using the maintenance diet / Table 16 & Plate 5) and the administration of the prepared experimental diets started after a starvation period of four (4) days (Kaushik 1979). Administration of the maintenance diet during the 20-day preconditioning period established a rough guideline for the experimental ration size. With the onset of the experiment this ration size was readjusted by the procedure described in 2.4.1.

Table 16. Composition and proximate analyses of Diets (as % of Dry Matter) used in the Protein: Energy experiment[1]

Nutritional profile	32:10	32:16	40:10	40:16	46:10	46:16	Control	Maintenance
Casein	19.5	19.5	27.5	27.5	31	31.0	-	18.0
Gelatin	5.0	5.0	4.5	4.5	6.5	6.5	-	5.0
Gluten	8.0	8.0	8.5	8.5	8.0	8.0	-	7.0
Zein	3.0	3.0	3.5	3.5	3.5	3.5	-	3.0
Fishmeal	-	-	-	-	-	-	23.0	-
Soya	-	-	-	-	-	-	40.5	-
Wheat	-	-	-	-	-	-	11.0	-
Dextrin	44.5	38.5	36.0	30.5	31.0	25.5	7.0	47.0
α- cellulose	3.0	3.0	3.0	3.0	3.0	3.0	1.0	3.0
Carboxymethylcellulose	1.0	1.0	1.0	1.0	1.0	1.0	2.0	1.0
Vegetable Oil [2]	5.0	8.0	5.0	8.0	5.0	8.0	5.0	5.0
Fish Oil [2]	5.0	8.0	5.0	7.5	5.0	7.5	4.5	5.0
Vitamin premix [2]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix [2]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Dry matter	86.67 (0.16)	88.65 (0.04)	85.82 (0.002)	94.81 (0.06)	92.05 (0.33)	91.62 (0.22)	94.36 (0.06)	91.66 (0.03)
Crude Protein	31.93 (0.28)	30.78 (0.04)	41.32 (0.35)	40.32 (0.11)	45.98 (0.05)	46.26 (1.31)	36.22 (0.36)	28.16 (0.18)
Crude Lipid	9.64 (1.24)	17.10 (0.70)	10.75 (1.04)	17.50 (1.12)	11.65 (0.82)	16.58 (0.07)	13.37 (0.41)	8.36 (1.13)
Carbohydrates	48.92 (0.34)	41.33 (0.48)	42.71 (0.78)	36.88 (0.13)	31.39 (1.08)	26.42 (0.74)	33.60 (0.82)	52.75 (1.63)
Ash	4.15 (0.06)	5.35 (2.04)	4.17 (0.005)	4.07 (0.06)	5.55 (2.29)	5.19 (1.22)	10.53 (0.38)	5.44 (1.88)
Crude Fibre	5.34 (0.26)	5.35 (0.26)	5.22 (0.32)	5.3 (0.48)	5.43 (0.18)	5.32 (0.29)	6.29 (0.31)	5.32 (0.53)
Gross Energy kJ. g^{-1}	22.77	23.95	23.06	24.47	22.42	23.75	21.26	22.59
Digestible Energy [3] kJ. g^{-1}	18.85	19.83	19.09	20.25	18.56	19.66	17.6	18.7
Protein: Energy ratio mg Prot. kJ^{-1}	14.03	12.85	19.89	18.37	22.74	21.53	18.82	13.83

1.numbers in parenthesis represent Standard Deviation (n=3) . The source of raw materials used is described in 2.5

2. The composition of Vitamin and Mineral premixes is given in Tables 4 and 5. The fatty acid profile of the oils used is given in Table 6.

3. Digestible Energy values were estimated according to the digestibility experiments (Chapter 3)

4.2.2 Fish population, the tank system and experimental design

Fifty-five (55) fish were individually tagged (2.2.7) and allocated to eighteen (18) - 50 L cylindrical tanks (2.2, Plate One). The nine selected treatments (Table 17) were duplicated according to the Randomized Block experimental design (Woolf 1968; Zar 1996) where plots are allocated within each block to the number of treatments (2 blocks x 9 treatments = 18 tanks).

The initial weight of the basic "core" experiment (Groups 1-6 and Control) was between 130 and 181 g with average initial weights not statistically different from each other (Table 17).

Two extra fish groups were selected: Group 7 / 46:10 and Group 8 / 46:16 (Table 17). The starting weight of those two groups was between 122 and 134 g. The main purpose of selecting these extra groups was the estimation of the amino acid requirements of fish fed a diet with a high Protein: Energy ratio (Plate 5). Table 17 also shows the variation of weights within each of these treatments. Initial weights of the individuals in all the experimental Groups are shown in Appendix V.

Before comparing the mean weights of each group by multiple range tests, Bartlett's test for homogeneity of variances (Zar 1996) was employed among the initial weights of all groups in order to assess the validity and strength of the expected results (Appendix V).

Statistical comparisons (Table 17 and Appendix V) revealed no differences among the average initial weights of all the groups and advocated for homogeneity of variances within the experimental population.

PLATE 5. Flow Diagram of Protein: Energy Trials

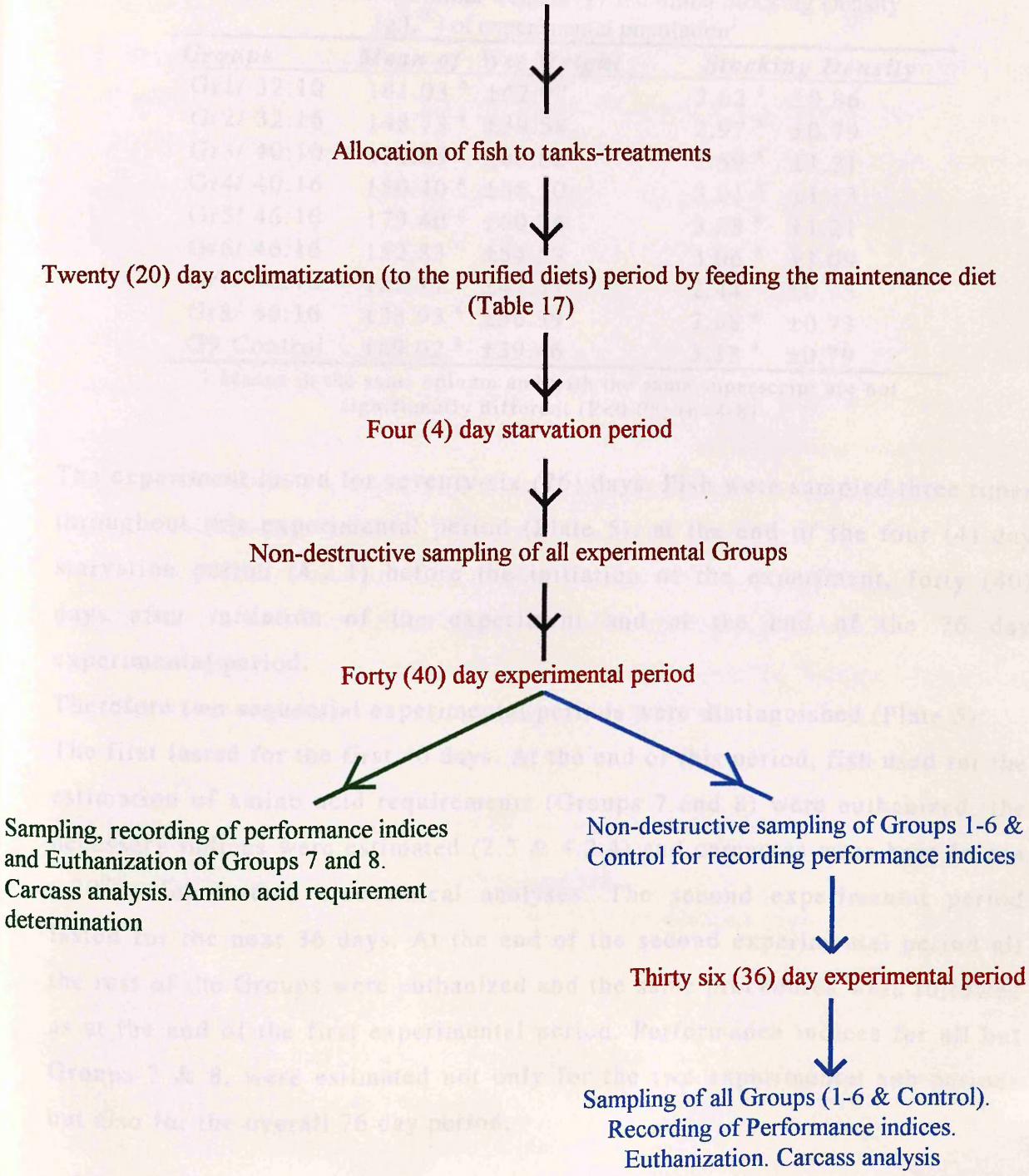


Table 17. Average initial weights (g) and initial Stocking Density ($g.L^{-1}$) of experimental population[1]

Groups	*Mean of Wet Weight*	*Stocking Density*
Gr1/ 32:10	181.03 [a] ±42.97	3.62 [a] ±0.86
Gr2/ 32:16	148.73 [a] ±39.58	2.97 [a] ±0.79
Gr3/ 40:10	130.08 [a] ±60.82	2.59 [a] ±1.21
Gr4/ 40:16	150.40 [a] ±56.50	3.01 [a] ±1.13
Gr5/ 46:10	179.40 [a] ±60.76	3.58 [a] ±1.21
Gr6/ 46:16	152.83 [a] ±54.59	3.06 [a] ±1.09
Gr7/ 46:10	122.17 [a] ±47.31	2.44 [a] ±0.95
Gr8/ 46:16	133.93 [a] ±36.39	2.68 [a] ±0.73
G9 Control	169.02 [a] ±39.46	3.38 [a] ±0.79

1.Means in the same column and with the same superscript are not significantly different ($P<0.05$) (n=4-8)

The experiment lasted for seventy-six (76) days. Fish were sampled three times throughout this experimental period (Plate 5): at the end of the four (4) day starvation period (4.2.1) before the initiation of the experiment, forty (40) days after initiation of the experiment and at the end of the 76 day experimental period.

Therefore two sequential experimental periods were distinguished (Plate 5):

The first lasted for the first 40 days. At the end of this period, fish used for the estimation of amino acid requirements (Groups 7 and 8) were euthanized, the necessary indices were estimated (2.3 & 4.2.4) and carcasses were kept frozen (-20^0C) for further biochemical analyses. The second experimental period lasted for the next 36 days. At the end of the second experimental period all the rest of the Groups were euthanized and the same procedures were followed as at the end of the first experimental period. Performance indices for all but Groups 7 & 8, were estimated not only for the two experimental sub-periods but also for the overall 76 day period.

4.2.3 Physicochemical parameters

Temperature was kept constant at 26-27^0 C, photoperiod was set at 12:12 L:D and oxygen levels ranged between 4-4.5 mg.L^{-1}. As the experimental tanks were part of a recirculated system of the Tropical Aquarium of IoASU (2.2 , 4.2.2, Plate One) water from the header tank of the system was sampled fortnightly in order to assess water quality parameters (2.1). During the 76 days of the experiment five (5) samplings were performed. Values of the recorded parameters are found in Appendix V.

4.2.4 Performance indices and Statistical Analyses

Performance indices used to evaluate the results of this experiment are described in 2.3. In addition, animals of all experimental Groups were analyzed for liver glycogen and liver lipid, as these two parameters can be indicative of the utilization of dietary nutrients by fish (1.2.4.2).

Carcass analyses were performed in triplicate for each individual within a certain treatment-Group. Final values for each Group represent the arithmetic mean of values of all individuals within the specific Group. Statistical analyses were performed as described in 2.3.

4.2.5 Biochemical analyses

Biochemical analyses were performed as described in 2.7.

4.2.6 Haematological and serological analyses

Sampling for haematological parameters was performed eight hours after the last feeding.

Fish were anaesthetized with benzocaine (2.2.7). 0.5 to 1.00 ml of blood was collected from the caudal artery, by a non-heparinized syringe and divided in almost equal portions into two heparinized 1.5mL Eppendorfs, with or without sodium fluoride, both kept in crushed ice.

Haematocrit was determined by transferring blood from the heparinized (without sodium fluoride) Eppendorf to non-heparinized haematocrit capillary

tubes, sealing one end of the capillary tubes with Critoseal and centrifuging in a micro-Centaur centrifuge for 5 min at 10,000 rpm.

The percent packed cell volume, following centrifugation, was determined using a Graphic Reader (Klontz 1994).

Plasma glucose was determined from aliquots of the heparinized Eppendorfs containing sodium fluoride (to minimize glycolysis due to the blood cells). The glucose oxidase method (Sigma Kit Cat No 315-100) was used as described by Trinder (1969).

Haemoglobin was determined using Drabkin's Reagent (Sigma Cat No 541-2) from blood aliquots of the heparinized (without sodium fluoride) Eppendorfs. Drabkin's Reagent contains potassium ferricyanide, potassium cyanide and sodium bicarbonate. Most forms of haemoglobin are converted into methaemoglobin by the action of ferricyanide, which in turns reacts with cyanide to form cyanmethaemoglobin. The absorbance is read at 540 nm and is proportional to the haemoglobin content in blood.

For both glucose and Haemoglobin determinations, a UVIKON 810 / KONTRON spectrophotometer was used.

Each haematological parameter of each Group presented in the Results (4.3), is the arithmetic mean of the values of all the fish within this specific Group. Except for the Groups 7 & 8 (which were euthanized at the end of the first experimental period), the haematological parameters for the rest of the Groups were evaluated at the termination of the second experimental period (4.2.2).

4.3 RESULTS

4.3.1 First Experimental Period

Comparison of the performance parameters during the first experimental period (Table 18) revealed the following:

At the starting weight range of 130-181g (core experiment) and up to a final weight of 170-234g, Group 6 (fed the 46:16 Diet) outperformed all the other treatments, except the Control, in terms of Specific Growth Rate (SGR), Thermal Growth Coefficient (TGC) and %Weight Gain.

The observed Food Conversion Ratios (FCR) of the core experimental population (calculated either on an individual basis or on cumulative tank data♠ / Table 18) were not significantly different among the treatments except for Group 1 (32:10) which was characterized by a higher FCR.

Despite the fact that the initial weight of Groups 7 & 8 (122-133g initial weight up to 196-256g final weight) was not significantly different from the initial weight of the rest of the groups (Table 17), their SGR, TGC, %Weight Gain and PER (calculated on a individual basis) were significantly higher ($P<0.05$) than those of the core experimental group (130-180g). The FCR of Groups 7 & 8 calculated either on an individual basis or on cumulative tank data, was not significantly different from the FCR of the rest of the Groups and differed only from Group 1 (32:10). PER estimated on cumulative tank data was significantly higher ($P<0.05$) only for Group 8.

When Groups 7 & 8 are not taken into consideration, the Control Group outperformed all the rest in terms of the parameters observed, except for FCR and PER (Table 18).

♠ FCR & PER were estimated as described in 2.3 and in a two-fold approach: either
by dividing the total ration or crude protein consumed by the number of residing individuals in each tank while fish weight gain was estimated on individual records due to the tagging technique (2.2.7) or
total ration, crude protein consumed and fish weight gain were estimated collectively for the biomass of each tank

Table 18. Comparison of the performance parameters observed during the first 40 days of the experiment*

Groups	SGR[1] ***	TGC[1] ***	%Weight Gain**	FCR[1] ***	PER[1] ***	FCR[1] ****	PER[1] ****
Gr1/32:10	0.29 [d] (0.13)	0.008 [d] (0.004)	20.18 [e] (4.93)	3.75 [a] (2.51)	1.23 [c] (0.76)	2.71 [a] (1.26)	1.29 [b,c] (0.60)
Gr2/32:16	0.38 [d] (0.14)	0.011 [d] (0.004)	23.94 [e] (5.09)	2.65 [b] (1.26)	1.55 [c] (0.82)	2.42 [a,b] (1.48)	1.65 [b,c] (1.00)
Gr3/40:10	0.50 [d] (0.23)	0.012 [d] (0.005)	27.77 [e] (7.82)	2.18 [b] (0.77)	1.22 [c] (0.41)	2.08 [a,b] (0.3)	1.17 [c] (0.17)
Gr4/40:16	0.53 [d] (0.19)	0.014 [d] (0.005)	28.93 [e] (6.48)	2.16 [b] (1.05)	1.42 [c] (0.73)	1.74 [a,b] (0.38)	1.46 [b,c] (0.32)
Gr5/46:10	0.51 [d] (0.17)	0.014 [d] (0.004)	28.11 [e] (5.74)	1.58 [b] (0.43)	1.46 [c] (0.42)	1.54 [a,b] (0.25)	1.43 [b,c] (0.24)
Gr6/46:16	0.81 [c] (0.41)	0.022 [c] (0.01)	37.47 [d] (15.82)	2.49 [b] (3.96)	2.07 [c] (0.99)	1.05 [a,b] (0.31)	2.05 [a,b,c] (0.61)
Gr7/46:10	1.09 [b] (0.38)	0.029 [b] (0.13)	49.19 [b] (14.70)	1.39 [b] (1.02)	2.58 [b] (1.74)	0.85 [b] (0.09)	2.58 [a,b] (0.30)
Gr8/46:16	1.67 [a] (0.34)	0.047 [a] (0.008)	81.33 [a] (31.81)	0.83 [b] (0.22)	3.02 [a] (0.7)	0.70 [b] (0.07)	3.11 [a] (0.33)
G9Control	0.84 [c] (0.32)	0.024 [b] (0.008)	39.81 [c] (12.65)	1.51 [b] (0.55)	2.06 [c] (0.97)	1.33 [a,b] (0.45)	2.20 [a,b,c] (0.75)
SD*****	0.45	0.012	19.66	1.85	0.95	0.85	0.75

[1] SGR=Specific Growth Rate / FCR= Food Conversion Ratio / TGC= Thermal Growth Coefficient / PER= Protein Efficiency Ratio

*values in the same column and with the same superscript are not significantly different (P<0.05) / numbers in parentheses represent Standard Deviation

** expressed as the arcsine transformed numbers of the real weight gain percentages

*** the ration consumed and fish weight gain were estimated on an individual basis (n=4-8) as fish were electronically tagged (2.2.7)

**** the ration consumed and fish weight gain were estimated on cumulative tank data (n=2) (see 4.3.1)

***** Standard Deviation of the multiple comparison

4.3.2 Second experimental period

Comparison of performance parameters during the second experimental period (Table 19) revealed the following:

Starting at the range of 155-233 g and up to a final weight of 177-298g, fish fed the Control Diet outperformed all other Groups in terms of SGR, TGC and % Weight Gain. Fish of Group 5 (46:10 Diet) were characterized by the second highest values for SGR and % Weight Gain, whereas the rest of treatments were characterized by statistically insignificant ($P>0.05$) differences as far as the same indices are concerned. Food Conversion Ratios and Protein Efficiency Ratios were not significantly different ($P<0.05$) among all the experimental Groups, whether estimated on an individual or on a cumulative tank data basis (Table 19).

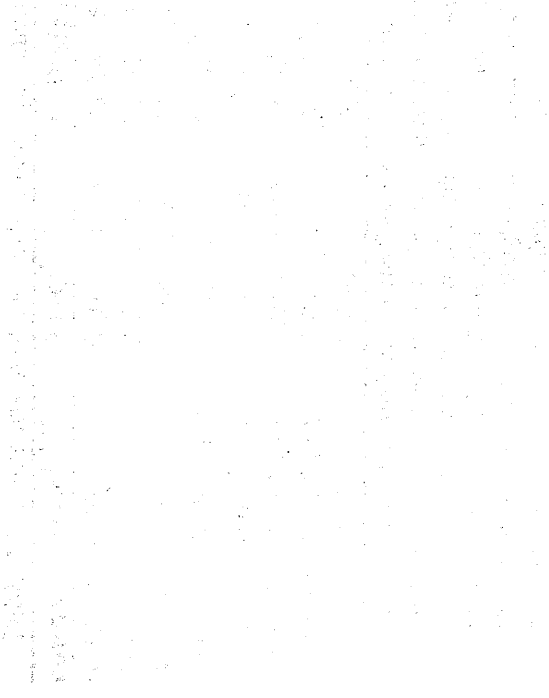


Table 19. Comparison of the performance parameters observed during the last 36 days of the experiment*

Groups	SGR[1] ***	TGC[1] ***	% Weight Gain**	FCR[1] ***	PER[1] ***	FCR[1] ****	PER[1] ****
Gr1/32:10	0.36 [c] (0.15)	0.01 [b] (0.005)	21.54 [b] (5.26)	2.72 [a] (1.93)	1.78 [a] (1.16)	1.90 [a] (0.95)	1.88 [a] (0.94)
Gr2/32:16	0.39 [c] (0.16)	0.01 [b] (0.004)	22.51 [b] (5.24)	2.38 [a] (1.22)	1.84 [a] (1.12)	2.20 [a] (1.58)	1.98 [a] (1.43)
Gr3/40:10	0.39 [c] (0.10)	0.009 [b] (0.003)	22.81 [b] (3.52)	2.33 [a] (1.15)	1.30 [a] (0.66)	2.01 [a] (1.02)	1.37 [a] (0.69)
Gr4/40:16	0.48 [c] (0.07)	0.013 [b] (0.003)	25.61 [b] (2.27)	1.88 [a] (0.84)	1.59 [a] (0.80)	1.54 [a] (0.21)	1.63 [a] (0.23)
Gr5/46:10	0.63 [b] (0.19)	0.018 [a] (0.007)	30.97 [a] (6.01)	1.34 [a] (0.53)	2.29 [a] (1.04)	0.93 [a] (0.48)	2.71 [a] (1.42)
Gr6/46:16	0.51 [c] (0.31)	0.014 [b] (0.008)	26.43 [b] (9.63)	1.61 [a] (1.05)	1.74 [a] (0.92)	1.32 [a] (0.49)	1.67 [a] (0.62)
G9Control	0.67 [a] (0.21)	0.019 [a] (0.006)	31.31 [a] (6.39)	1.59 [a] (0.97)	2.49 [a] (1.54)	1.27 [a] (0.86)	2.83 [a] (1.92)
SD*****	0.21	0.0065	6.60	1.24	1.12	0.79	1.00

[1] SGR= Specific Growth Rate / FCR= Food Conversion Ratio / TGC= Thermal Growth Coefficient / PER= Protein Efficiency Ratio

*values in the same column and with the same superscript are not significantly different ($P<0.05$) / numbers in parentheses represent Standard Deviation

** expressed as the arcsine transformed numbers of the real weight gain percentages

*** the ration consumed and fish weight gain were estimated on an individual basis (n=4-8) as fish were electronically tagged (2.2.7)

**** the ration consumed and fish weight gain were estimated on cumulative tank data (n=2) (see 4.3.1)

***** Standard Deviation of the multiple comparison

4.3.3 Total 76 day period

Performance indices based on the overall 76 day period (Table 20) suggest that the Control group outperformed all the rest in terms of SGR, TGC, %Weight Gain and PER (estimated on a individual basis). Furthermore Group 6 fed the high protein-high energy diet (46:16) had significantly higher ($P>0.05$) SGR and % Weight Gain than the rest (except for the Control).

Fish fed the 46:10 Diet (Groups 5 & 7) were characterized by better protein utilization (ANPU; Table 21) and higher carcass protein levels than the rest of groups (Table 22; Figure 8) for the overall 76 day period. In addition fish fed the 46:10 Diet (Groups 5 & 7) were characterized by lower carcass lipid levels (Table 22; Figure 8) compared to those fed the 46:16 Diet (Groups 6 & 8).

Comparison of the hepatosomatic indices (Table 23) did not reveal any statistical differences among the various experimental groups (except for Group 4). Performance of each experimental group over time revealed significant differences ($P>0.05$) in the % Weight Gain of the 76day period when compared to the other two experimental periods (Table 24), whereas SGR, FCR and PER (estimated either on individual or cumulative tank data) revealed no significant differences among the observed experimental phases and within each experimental group, except for the FCR of the Control (Tables 25,26,27,28,29).

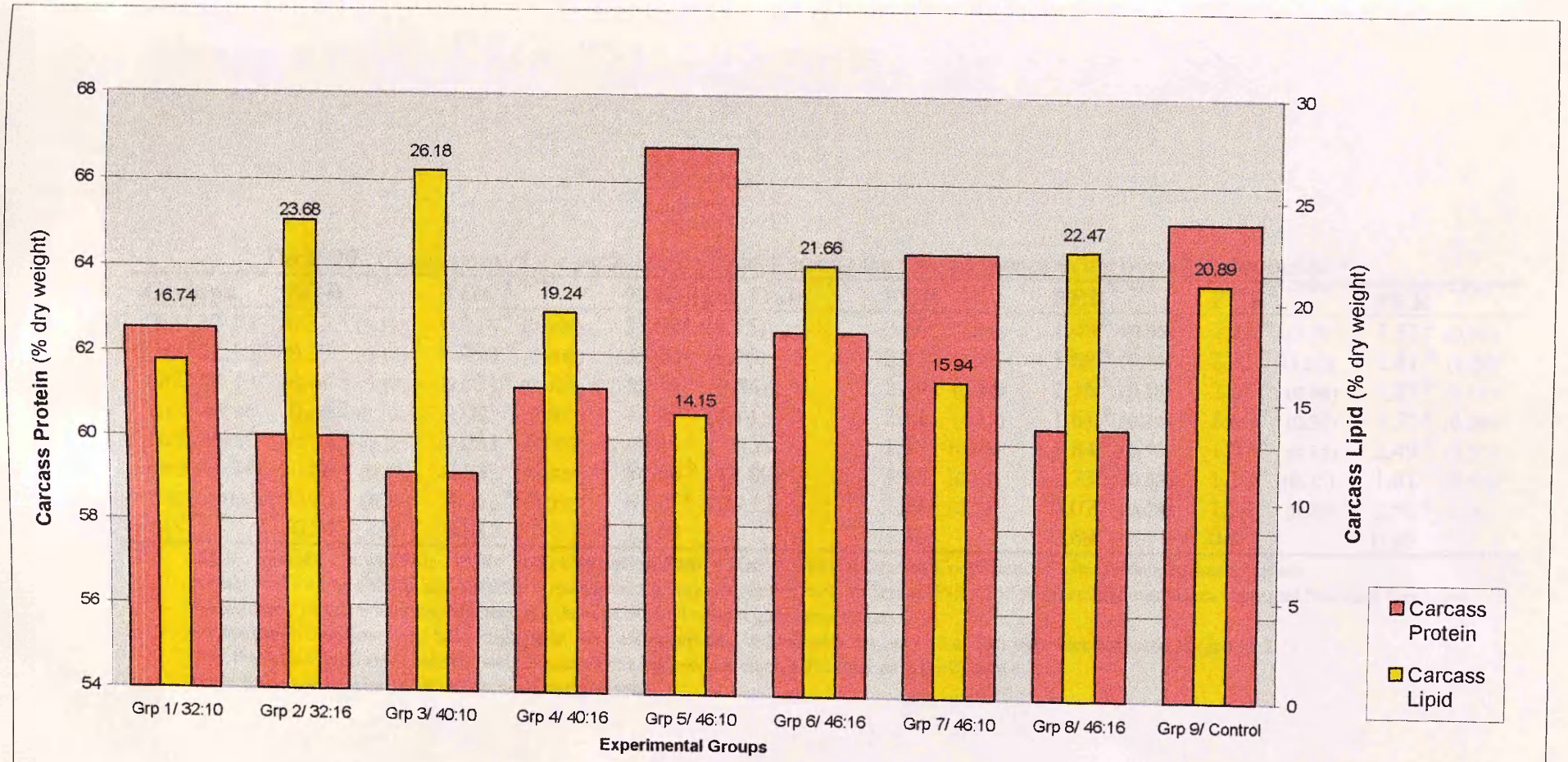


Figure 8. Carcass Protein and Lipid levels of the experimental groups during the Protein:Energy experiments (76d data)

Table 20 . Comparison of the performance indices among the various groups in the overall 76day period *

Groups	SGR[1] ***	TGC[1] ***	%Weight Gain**	FCR[1] ***	PER[1] ***	FCR[1] ****	PER[1] ****
Gr1/ 32:10	0.32 [c] (0.13)	0.018 [c] (0.009)	31.69 [c] (8.75)	3.66 [a] (2.54)	1.49 [b] (0.95)	2.25 [a] (1.09)	1.57 [a] (0.76)
Gr2/ 32:16	0.37 [c] (0.15)	0.020 [c] (0.008)	35.90 [c] (9.36)	2.83 [b] (1.39)	1.69 [b] (0.96)	2.31 [a] (1.53)	1.81 [a] (1.20)
Gr3/ 40:10	0.44 [c] (0.15)	0.021 [c] (0.005)	39.36 [c] (9.54)	2.49 [c] (0.44)	1.16 [b] (0.18)	2.01 [a] (0.66)	1.27 [a] (0.42)
Gr4/ 40:16	0.49 [c] (0.12)	0.025 [c] (0.007)	42.95 [c] (8.14)	2.11 [c] (1.43)	1.61 [b] (0.74)	1.66 [a] (0.32)	1.52 [a] (0.29)
Gr5/ 46:10	0.55 [c] (0.11)	0.031 [b] (0.007)	46.35 [c] (7.43)	1.37 [c] (0.36)	1.84 [b] (0.54)	1.07 [a] (0.65)	2.49 [a] (1.53)
Gr6/ 46:16	0.60 [b] (0.19)	0.032 [b] (0.009)	50.60 [b] (13.89)	1.45 [c] (0.55)	1.73 [b] (0.58)	1.19 [a] (0.35)	1.81 [a] (0.53)
G9/Control	0.84 [a] (0.27)	0.043 [a] (0.012)	67.71 [a] (29.12)	1.44 [c] (0.28)	2.07 [a] (0.78)	1.28 [a] (0.67)	2.50 [a] (1.3)
SD*****	0.24	0.012	25.49	1.46	0.69	0.8	0.85

[1] SGR= Specific Growth Rate / FCR= Food Conversion Ratio / TGC = Thermal Growth Coefficient / PER= Protein Efficiency Ratio

*values in the same column and with the same superscript are not significantly different (P<0.05) / numbers in parentheses represent Standard Deviation

** expressed as the arcsine transformed numbers of the real weight gain percentages

*** the ration consumed and fish weight gain were estimated on a individual basis (n=4-8) as fish were electronically tagged (2.2.7)

**** the ration consumed and fish weight gain were estimated on cumulative tank data (n=2) (see 4.3.1)

***** Standard Deviation of the multiple comparison

Table 21. Estimation of the Apparent Net Protein Utilization (ANPU) in the overall 76day experimental period[1]

Groups	*ANPU*	
Group 1 / 32 : 10	22.75 [a]	(8.32)
Group 2 / 32 : 16	17.61 [b]	(2.65)
Group 3 / 40 : 10	18.77 [b]	(2.57)
Group 4 / 40 : 16	18.67 [ab]	(9.27)
Group 5 / 46 : 10	42.26 [c]	(7.77)
Group 6 / 46 : 16	27.10 [a]	(9.70)
Group 7 / 46 : 10	32.84 [c]	(6.67)
Group 8 / 46 : 16	16.37 [ab]	(8.82)
Group 9 / Control	22.78 [a]	(1.75)

1. Values have been arcsine transformed. Values in the same column and with the same superscript are not significantly different (P<0.05) / numbers in parenthesis represent Standard Deviation (n=4-8)

Table 22. Comparison of the carcass protein levels and carcass lipid levels (% DM basis) among the various experimental groups*

Groups	*Carcass Protein*		*Carcass Lipid*	
Group 1 / Diet 32:10	62.54 [c]	(1.63)	16.69 [f]	(0.58)
Group 2 / Diet 32:16	60.00 [d]	(1.16)	23.68 [b]	(0.41)
Group 3 / Diet 40:10	59.15 [d]	(1.69)	26.18 [a]	(0.65)
Group 4 / Diet 40:16	61.19 [d]	(2.62)	19.24 [e]	(0.96)
Group 5 / Diet 46:10	66.79 [a]	(2.64)	14.15 [g]	(0.48)
Group 6 / Diet 46:16	62.56 [c]	(3.04)	21.66 [d]	(1.53)
Group 7/ Diet 46:10	64.40 [b]	(2.22)	15. 94 [f]	(0.33)
Group 8/ Diet 46:16	60.36 [d]	(2.21)	22.47 [c]	(0.48)
Group 9 / Control	65.16 [b]	(0.96)	20.89 [d]	(1.16)

* values in the same column and with the same superscript are not significantly different (P<0.05) / numbers in parenthesis represent Standard Deviation (n=4-8)

Table 23. Comparison of the hepatosomatic indices among the various experimental groups *.

Groups	*Hepatosomatic Index*	
Group1 / Diet 32:10	1.71 [a]	(0.13)
Group2 / Diet 32:16	1.57 [a]	(0.16)
Group3 / Diet 40:10	1.60 [a]	(0.11)
Group4 / Diet 40:16	1.88 [b]	(0.12)
Group5 / Diet 46:10	1.64 [a]	(0.14)
Group6 / Diet 46:16	1.56 [a]	(0.23)
Group7 / Diet 46:10	1.62 [a]	(0.19)
Group8 / Diet 46:16	1.68 [a]	(0.16)
Group9 / Control	1.43 [a]	(0.16)

*values in the same column and with the same superscript are not significantly different (P<0.05) / numbers in parenthesis represent Standard Deviation (n=4-8)

Table 24. Comparison of the % Weight Gain (arscine transformed data) among the three experimental periods*

Groups	*1st 40 day experimental period*	*2nd 36 day experimental period*	*Overall 76 day experimental period*	*SD***
Gr1/ 32:10	20.18 [a] (4.93)	21.54 [a] (4.96)	31.69 [b] (8.75)	8.08
Gr2/ 32:16	23.94 [a] (5.09)	22.51 [a] (5.24)	35.90 [b] (9.36)	8.95
Gr3/ 40:10	27.77 [a] (7.83)	22.81 [a] (3.52)	39.36 [b] (9.54)	9.96
Gr4/ 40:16	28.93 [a] (6.48)	25.61 [a] (2.27)	42.95 [b] (8.14)	9.64
Gr5/ 46:10	28.11 [a] (5.74)	30.97 [a] (5.83)	46.35 [b] (7.43)	10.18
Gr6/ 46:16	37.47 [a] (15.82)	26.43 [a] (9.63)	50.60 [b] (13.89)	16.15
Gr9/Control	39.81 [a] (12.65)	31.31 [a] (6.39)	67.71 [b] (29.12)	23.9

*values in the same row and with the same superscript are not significantly different (P<0.05)
/ numbers in parenthesis represent Standard Deviation (n=4-8)
** Standard Deviation of the multiple comparisons

Table 25. Comparison of the Specific Growth Rate among the three experimental periods*

Groups	*1st 40 day experimental period*	*2nd 36 day experimental period*	*Overall 76 day experimental period*	*SD***
Gr1/ 32:10	0.29 [a] (0.13)	0.36[a] (0.15)	0.33 [a] (0.14)	0.13
Gr2/ 32:16	0.39 [a] (0.14)	0.38 [a] (0.16)	0.38 [a] (0.15)	0.14
Gr3/ 40:10	0.50 [a] (0.23)	0.39 [a] (0.10)	0.44 [a] (0.15)	0.16
Gr4/ 40:16	0.53 [a] (0.19)	0.47 [a] (0.07)	0.49 [a] (0.12)	0.13
Gr5/ 46:10	0.50 [a] (0.17)	0.55 [a] (0.17)	0.55 [a] (0.11)	0.14
Gr6/ 46:16	0.64 [a] (0.48)	0.52 [a] (0.31)	0.55 [a] (0.11)	0.33
Gr9/Control	0.84 [a] (0.33)	0.67 [a] (0.22)	0.84 [a] (0.27)	0.27

*values in the same row and with the same superscript are not significantly different (P<0.05)
/ numbers in parenthesis represent Standard Deviation (n=4-8)
** Standard Deviation of the multiple comparisons

Table 26. Comparison of the Food Conversion Ratio (estimated on an individual basis) among the three experimental periods*

Groups	*1st 40 day experimental period*	*2nd 36 day experimental period*	*Overall 76 day experimental period*	*SD***
Gr1/ 32:10	3.75 [a] (2.52)	2.72 [a] (1.94)	3.66 [a] (2.54)	2.28
Gr2/ 32:16	2.65 [a] (1.26)	2.38 [a] (1.22)	2.83 [a] (1.39)	1.24
Gr3/ 40:10	2.18 [a] (0.77)	2.33 [a] (1.15)	2.48 [a] (0.41)	0.99
Gr4/ 40:16	2.28 [a] (1.17)	1.88 [a] (0.85)	2.11 [a] (1.43)	1.09
Gr5/ 46:10	1.58 [a] (0.43)	1.13 [a] (0.53)	1.37 [a] (0.32)	0.44
Gr6/ 46:16	2.48 [a] (3.96)	1.61 [a] (1.05)	1.45 [a] (0.56)	2.29
Gr9/Control	1.52 [a] (0.55)	1.59 [a] (0.97)	1.44 [b] (0.28)	0.64

*values in the same row and with the same superscript are not significantly different (P<0.05) / numbers in parenthesis represent Standard Deviation (n=4-8) (see 4.3.1)
** Standard Deviation of the multiple comparisons

Table 27. Comparison of the Food Conversion Ratio (estimated on cumulative tank data) among the three experimental periods*

Groups	*1st 40 day experimental period*		*2nd 36 day experimental period*		*Overall 76 day experimental period*		*SD***
Gr1/ 32:10	2.71 [a]	(1.26)	1.90 [a]	(0.95)	2.25 [a]	(1.09)	0.93
Gr2/ 32:16	2.42 [a]	(1.48)	2.20 [a]	(1.58)	2.31 [a]	(1.53)	1.19
Gr3/ 40:10	2.08 [a]	(0.30)	2.01 [a]	(1.02)	2.01 [a]	(0.66)	0.56
Gr4/ 40:16	1.74 [a]	(0.38)	1.54 [a]	(0.21)	1.66 [a]	(0.32)	0.26
Gr5/ 46:10	1.54 [a]	(0.25)	0.93 [a]	(0.48)	1.07 [a]	(0.65)	0.47
Gr6/ 46:16	1.05 [a]	(0.31)	1.32 [a]	(0.49)	1.19 [a]	(0.35)	0.33
Gr9/Control	1.33 [a]	(0.45)	1.27 [a]	(0.86)	1.28 [a]	(0.67)	0.53

*values in the same row and with the same superscript are not significantly different (P<0.05) / numbers in parenthesis represent Standard Deviation (n=2) (see 4.3.1)
** Standard Deviation of the multiple comparisons

Table 28. Comparison of the Protein Efficiency Ratio (estimated on individual data) among the three experimental periods *

Groups	*1st 40 day experimental period*		*2nd 36 day experimental period*		*Overall 76 day experimental period*		*SD***
Gr1/ 32:10	1.23 [a]	(0.76)	1.78 [a]	(1.16)	1.49 [a]	(0.95)	0.95
Gr2/ 32:16	1.55 [a]	(0.82)	1.84 [a]	(1.12)	1.69 [a]	(0.96)	0.93
Gr3/ 40:10	1.22 [a]	(0.42)	1.30 [a]	(0.66)	1.16 [a]	(0.18)	0.44
Gr4/ 40:16	1.42 [a]	(0.73)	1.59 [a]	(0.80)	1.61 [a]	(0.74)	0.71
Gr5/ 46:10	1.46 [a]	(0.42)	2.29 [a]	(1.04)	1.84 [a]	(0.54)	0.76
Gr6/ 46:16	2.07 [a]	(0.99)	1.74 [a]	(0.92)	1.73 [a]	(0.58)	0.81
G9/Control	2.06 [a]	(0.99)	2.49 [a]	(1.57)	2.07 [a]	(0.78)	1.15

*values in the same row and with the same superscript are not significantly different (P<0.05) / numbers in parenthesis represent Standard Deviation (n=4-8) (see 4.3.1)
** Standard Deviation of the multiple comparisons

Table 29. Comparison of the Protein Efficiency Ratio (estimated on cumulative tank data) among the three experimental periods *

Groups	*1st 40 day experimental period*		*2nd 36 day experimental period*		*Overall 76 day experimental period*		*SD***
Gr1/ 32:10	1.29 [a]	(0.60)	1.88 [a]	(0.94)	1.57 [a]	(0.76)	0.66
Gr2/ 32:16	1.65 [a]	(1.00)	1.98 [a]	(1.43)	1.81 [a]	(1.20)	0.96
Gr3/ 40:10	1.17 [a]	(0.17)	1.37 [a]	(0.69)	1.27 [a]	(0.42)	0.38
Gr4/ 40:16	1.46 [a]	(0.32)	1.63 [a]	(0.23)	1.52 [a]	(0.29)	0.23
Gr5/ 46:10	1.43 [a]	(0.24)	2.71 [a]	(1.42)	2.49 [a]	(1.53)	1.12
Gr6/ 46:16	2.05 [a]	(0.61)	1.67 [a]	(0.62)	1.81 [a]	(0.53)	0.49
Gr9/Control	2.20 [a]	(0.75)	2.83 [a]	(1.92)	2.50 [a]	(1.30)	1.13

*values in the same row and with the same superscript are not significantly different (P<0.05) / numbers in parenthesis represent Standard Deviation (n=2) (see 4.3.1)
** Standard Deviation of the multiple comparisons

4.3.4 Results of the haematological and serological analyses

Haematocrit values showed an erratic variation not only among groups but also among individuals of the same group (except for Groups 3 and 8, Table 30).

Table 30. Comparison of the haematocrit values (as percent cell packed volume / Hc %) among the experimental groups*

Gr1 / 32:10	*Gr2 / 32:16*	*Gr3 / 40:10*	*Gr4/ 40:16*	*Gr5 / 46:10*
25.00 ^{c} (2.68)	26.93 ^{c} (1.9)	29.86 ^{a} (1.78)	23.71 ^{c} (1.82)	27.37 ^{b} (1.93)
28.29 ^{cb} (1.71)	35.43 ^{b} (1.17)	32.00 ^{a} (0.42)	29.50 ^{b} (1.88)	32.68 ^{ba} (3.38)
29.29 ^{cb} (1.17)	35.68 ^{b} (3.5)	33.19 ^{a} (0.97)	30.05 ^{b} (1.27)	33.59 ^{a} (0.77)
31.91 ^{ba} (2.41)	40.74 ^{a} (3.66)	31.15 ^{a} (2.62)	34.79 ^{ab} (2.39)	37.00 ^{a} (1.13)
35.76 ^{a} (0.75)	41.94 ^{a} (3.98)	30.93 ^{a} (2.73)	37.86 ^{a} (2.18)	
			35.00 ^{ab} (2.83)	

Gr6 / 46:16	*Gr7 / 46: 10*	*Gr8 / 46:16*	*Gr9 / Control*
44.36 ^{a} (1.64)	19.75 ^{b} (1.76)	21.00 ^{a} (0.35)	25.00 ^{b} (2.54)
42.94 ^{a} (1.75)	26.33 ^{a} (1.17)	22.75 ^{a} (1.76)	33.00 ^{ab} (3.45)
29.44 ^{b} (2.32)	27.75 ^{a} (0.35)	24.00 ^{a} (5.65)	35.00 ^{ab} (6.76)
30.50 ^{b} (1.98)	28.75 ^{a} (1.76)	28.50 ^{a} (2.12)	38.00 ^{a} (1.70)
			40.00 ^{a} (2.12)

*values in the same column and with the same superscript are not significantly different ($P<0.05$) / numbers in parenthesis represent Standard Deviation (n=2)

Similar results were shown for total haemoglobin (Table 31)

Table 31. Total haemoglobin ($g.dL^{-1}$) in the various experimental groups*

Gr1/ 32:10	*Gr2/ 32:16*	*Gr3 / 40:10*	*Gr4 / 40:16*	*Gr5 / 46:10*
10.85 ^{d} (0.36)	11.43 ^{b} (1.08)	13.31 ^{a} (1.79)	9.38 ^{a} (2.18)	13.74 ^{b} (1.59)
11.53 ^{d} (0.53)	13.22 ^{b} (0.45)	13.27 ^{a} (1.68)	8.26 ^{a} (0.72)	13.68 ^{b} (0.26)
12.39 ^{c} (0.13)	13.76 ^{b} (2.05)	12.25 ^{a} (1.76)	11.04 ^{a} (0.05)	14.59 ^{ba} (0.98)
13.65 ^{b} (0.47)	14.39 ^{b} (0.68)	11.09 ^{a} (0.66)	9.12 ^{a} (1.34)	15.00 ^{a} (0.46)
14.35 ^{b} (0.1)	14.78 ^{a} (0.18)	11.69 ^{a} (2.13)	10.43 ^{a} (0.34)	
15.04 ^{a} (0.50)	11.14 ^{b} (2.05)			

Gr6 / 46:16	*Gr7/ 46:10*	*Gr8 / 46:16*	*Gr9 / Control*
12.36 ^{b} (0.47)	9.75 ^{b} (0.65)	11.33 ^{b} (1.79)	15.34 ^{a} (1.48)
12.94 ^{b} (0.76)	9.34 ^{b} (0.76)	12.35 ^{b} (1.15)	8.95 ^{c} (3.89)
14.54 ^{a} (0.37)	13.15 ^{a} (0.97)	10.63 ^{b} (0.79)	14.07 ^{ba} (2.18)
14.50 ^{a} (0.28)	12.75 ^{a} (1.14)	13.78 ^{a} (0.68)	10.43 ^{cb} (0.39)
			7.82 ^{c} (0.39)
			8.58 ^{c} (2.22)
			8.62 ^{c} (1.16)
			11.64 ^{abc} (0.59)

* Values in the same column and with the same superscript are not significantly different ($P<0.05$). Numbers in parenthesis represent Standard Deviation (n=2)

On the contrary blood glucose levels were uniform among animals of the same experimental Group, whereas statistical differences were observed between the high and low energy diets on the 46% Crude Protein level (Tables 32 and 33).

Table 32. Blood glucose values among the experimental animals expressed as mg. dL^{-1} *

Gr1/ 32:10	*Gr2/ 32:16*	*Gr3 / 40:10*	*Gr4 / 40:16*
44.86 [a] (2.49) ±1.02	58.13 [a] (3.23) ±9.06	59.61 [a] (3.31) ±4.18	53.69[a] (2.98) ±1.39
47.39 [a] (2.63) ±6.15	57.64 [a] (3.19) ±15.32	64.35 [a] (3.57) ±11.68	65.65[a] (3.64) ±7.38
49.26 [a] (2.74) ±2.09	60.59 [a] (3.36) ±20.89	55.22 [a] (3.06) ±7.38	52.96[a] (2.94) ±6.62
52.59 [a] (2.92) ±0.65	41.84 [a] (2.32) ±18.76	55.17 [a] (3.06) ±4.87	59.56[a] (3.31) ±3.69
	38.26 [a] (2.13) ±11.68		52.61[a] (2.92) ±2.46
			65.52[a] (3.64) ±8.36

Gr5/ 46:10	*Gr6 / 46:16*	*Gr7 / 46:10*	*Gr8 / 46:16*
129.87[a] (7.21) ±1.25	106.72[a] (5.93) ±1.21	97.89[a] (5.43) ±10.55	88.42[a] (4.91) ±12.65
130.62[a] (7.25) ±1..34	111.61[a] (6.19) ±1.87	96.32[a] (5.35) ±12.02	89.47[a] (4.7) ±2.23
130.46[a] (7.24) ±1.57	107.41[a] (5.96) ±2.74	66.84[a] (3.71) ±8.94	84.74[a] (4.71) ±20.84
126.72[a] (7.03) ±1.31	111.28[a] (6.17) ±0.99		88.95[a] (4.94) ±11.91

* Values in the same column and with the same superscript are not significantly different / numbers in parenthesis represent blood glucose values in mmol. L^{-1} (n=2)

Table 33. Comparison of blood glucose (mg. dL^{-1}) among the various groups*

Groups	*Blood glucose values*
Group1 / Diet 32:10	48.53 [d] (3.25)*
Group2 / Diet 32:16	51.29 [d] (10.4)
Group3 / Diet 40:10	58.58 [d] (4.37)
Group4 / Diet 40:16	58.33 [d] (6.16)
Group5 / Diet 46:10	129.42 [a] (1.83)
Group6 / Diet 46:16	109.26 [b] (2.55)
Group7 / Diet 46:10	87.02 [c] (7.5)
Group8 / Diet 46:16	87.89 [c] (2.15)
Standard Deviation **	28.75

*Values in the same column and with the same superscript are not significantly different (P<0.05)
Numbers in parenthesis represent Standard Deviation (n=4-6)
** Standard Deviation of the multiple comparison

4.3.5 Biochemical composition of the liver

The glycogen and lipid composition of liver is shown in Table 34:

Group 1 (fed a diet of 32%Protein: 46%CHOs: 22.7kJ.g^{-1} Energy), Group 3 (fed a diet of 40%Protein: 37.8%CHOs: 23kJ.g^{-1} Energy) and Group 5 (fed a diet of 46%Protein: 32.5CHOs: 22.4kJ.g^{-1} Energy) were characterized by increasing levels of liver glycogen. On the contrary, liver glycogen levels

within groups fed the same protein level showed an erratic distribution of these values. In addition the liver lipid values did not show any cohesive pattern related to the origin of the dietary energy.

Table 34. Comparison of liver glycogen and liver lipid values (% on a Dry Matter basis) among the various groups*

Groups	*Glycogen*	*Lipid*
Group1 / Diet 32:10	24.26 ^{d} (12.20)	12.86 ^{e} (1.27)
Group2 / Diet 32:16	9.71 ^{e} (4.10)	30.59 ^{b} (4.96)
Group3 / Diet 40:10	44.50 ^{c} (10.25)	27.25 ^{c} (6.76)
Group4 / Diet 40:16	64.89 ^{a} (8.92)	24.98 ^{c} (3.60)
Group5 / Diet 46:10	60.16 ^{b} (9.42)	15.88 ^{e} (2.45)
Group6 / Diet 46:16	44.66 ^{c} (4.31)	19.34 ^{d} (2.57)
Group7 / Diet 46:10	48.81 ^{c} (5.15)	30.53 ^{c} (8.88)
Group8 / Diet 46:16	43.37 ^{c} (7.54)	34.68 ^{a} (2.75)
Standard Deviation **	19.05	22.81

* Values in the same column and with the same superscript are not significantly different (P<0.05)
Numbers in parenthesis represent Standard Deviation (n=4-8)
** Standard Deviation of the multiple comparison

4.3.6 Established feed intake levels during the Protein: Energy experiments

Feed intake levels were estimated as described in 2.3, in a two-fold approach:

- On an individual basis. Total food consumption for each tank was divided by the number of residing individuals in that tank and further divided by individual weights accurately recorded due to electronic tagging (2.2.7)
- On a cumulative tank basis. Food consumption was calculated as total food consumption of the residing tank biomass.

Established feed intake levels during the two experimental phases, indicate the following:

No significant differences (P<0.05) were found in the feed intake levels among the various treatments, within the same experimental phases and calculated either on individual or on cumulative tank data (Tables 35, 36 & 37). Similarly, no significant differences (P<0.05) were found in the feed intake levels within each experimental group over time (except for the Control) and estimated either on individual or on cumulative tank data (Table 38 & 39)

When feed intake levels are expressed as g of dry ration consumed per g of live fish per day, the best-fit regression (Appendix Six) is expressed as a power one:

$$Y = 0.8031 * X^{(-0.871)}$$

Where X= g of live fish weight and Y = g of dry ration consumed. g^{-1} live fish weight . day^{-1}

Table 35. Established feed intake levels (as % of live weight per day) during the first 40 days of the experiment *

	Based on individual tank data (n=4-8)***	***Based on cumulative tank data (n=2)******
Group1 / 32:10	0.97 [a] (0.27)	0.91 [a] (0.11)
Group2 / 32:16	1.004 [a] (0.25)	0.95 [a] (0.20)
Group3 / 40:10	1.28 [a] (0.56)	1.08 [a] (0.68)
Group4 / 40:16	1.08 [a] (0.34)	0.97 [a] (0.08)
Group5 / 46:10	0.97 [a] (0.43)	0.75 [a] (0.35)
Group6 / 46:16	0.84 [a] (0.25)	0.79 [a] (0.06)
Group7 / 46:10	1.25 [a] (0.55)	1.08 [a] (0.21)
Group8 / 46:16	1.31 [a] (1.29)	1.33 [a] (0.17)
Group9 / Control	1.23 [a] (0.37)	1.13 [a] (0.40)
Standard Deviation **	0.38	0.28

*Values in the same column and with the same superscript are not significantly different (P<0.05)
** Standard Deviation of the multiple comparison
*** See 4.3.6

Table 36. Established feed intake levels (as % of live weight per day) during the last 36 days of the experiment*

	Based on individual tank data (n=4-8)***	***Based on cumulative tank data (n=2)******
Group1 / 32:10	0.86 [a] (0.28)	0.79 [a] (0.13)
Group2 / 32:16	0.87 [a] (0.24)	0.82 [a] (0.23)
Group3 / 40:10	1.07 [a] (0.45)	0.92 [a] (0.54)
Group4 / 40:16	0.89 [a] (0.29)	0.80 [a] (0.08)
Group5 / 46:10	0.78 [a] (0.34)	0.60 [a] (0.27)
Group6 / 46:16	0.68 [a] (0.14)	0.66 [a] (0.04)
Group9 / Control	0.93 [a] (0.30)	0.85 [a] (0.34)
Standard Deviation **	0.29	0.23

*Values in the same column and with the same superscript are not significantly different (P<0.05)
** Standard Deviation of the multiple comparisons
*** See 4.3.6

Table 37. Established feed intake levels (as % of live weight per day) in the overall of the 76-day experimental period*

	Based on individual tank data (n=4-8)***		***Based on cumulative tank data (n=2) ******	
Group1 / 32:10	0.91 [a]	(0.27)	0.84 [a]	(0.12)
Group2 / 32:16	0.93 [a]	(0.24)	0.87 [a]	(0.22)
Group3 / 40:10	1.08 [a]	(0.37)	1.09 [a]	(0.65)
Group4 / 40:16	0.97 [a]	(0.37)	0.88 [a]	(0.08)
Group5 / 46:10	0.76 [a]	(0.28)	0.65 [a]	(0.31)
Group6 / 46:16	0.82 [a]	(0.25)	0.77 [a]	(0.10)
Group9 /Control	1.26 [b]	(0.21)	0.97 [a]	(0.37)
Standard Deviation **	0.44		0.27	

*Values in the same column and with the same superscript are not significantly different (P<0.05)
** Standard Deviation of the multiple comparisons
*** See 4.3.6

Table 38. Comparison of the feed intake levels (as % of live weight per day) among the three experimental periods within the same groups and based on individual tank data *

Groups	***1st 40 day experimental period***		***2nd 36 day experimental period***		***Overall 76 day experimental period***		***SD****
Gr1/ 32:10	0.97 [a]	(0.27)	0.86 [a]	(0.27)	0.91 [a]	(0.27)	0.34
Gr2/ 32:16	1.004 [a]	(0.25)	0.87 [a]	(0.24)	0.93 [a]	(0.24)	0.24
Gr3/ 40:10	1.28 [a]	(0.57)	1.07 [a]	(0.45)	1.08 [a]	(0.37)	0.45
Gr4/ 40:16	1.08 [a]	(0.34)	0.89 [a]	(0.29)	0.97 [a]	(0.37)	0.32
Gr5/ 46:10	0.97 [a]	(0.43)	0.78 [a]	(0.34)	0.76 [a]	(0.28)	0.34
Gr6/ 46:16	0.84 [a]	(0.25)	0.68 [a]	(0.14)	0.82 [a]	(0.25)	0.22
G9/Control	1.23 [b]	(0.37)	0.94 [b]	(0.30)	1.26 [a]	(0.21)	0.32

*Values in the same row and with the same superscript are not significantly different (P<0.05)
Numbers in parenthesis represent Standard Deviation (n=4-8) (see 4.3.6)
** Standard Deviation of the multiple comparisons

Table 39. Comparison of the feed intake levels (as % of live weight per day) among the three experimental periods within the same groups and based on cumulative tank data *

Groups	***1st 40 day experimental period***		***2nd 36 day experimental period***		***Overall 76 day experimental period***		***SD****
Gr1/ 32:10	0.91 [a]	(0.11)	0.79 [a]	(0.13)	0.84 [a]	(0.12)	0.11
Gr2/ 32:16	0.95 [a]	(0.20)	0.82 [a]	(0.23)	0.87 [a]	(0.22)	0.18
Gr3/ 40:10	1.08 [a]	(0.68)	0.92 [a]	(0.54)	1.09 [a]	(0.65)	0.49
Gr4/ 40:16	0.97 [a]	(0.08)	0.80 [a]	(0.08)	0.88 [a]	(0.08)	0.10
Gr5/ 46:10	0.75 [a]	(0.35)	0.60 [a]	(0.27)	0.65 [a]	(0.31)	0.25
Gr6/ 46:16	0.79 [a]	(0.06)	0.66 [a]	(0.04)	0.77 [a]	(0.10)	0.10
Gr9/Control	1.13 [a]	(0.40)	0.85 [a]	(0.34)	0.97 [a]	(0.37)	0.31

*Values in the same row and with the same superscript are not significantly different (P<0.05)
Numbers in parenthesis represent Standard Deviation (n=2) (see 4.3.6)
** Standard Deviation of the multiple comparisons

4.4 DISCUSSION

Based on purified materials and for the range of 120 to 298g (total 76-day experimental period), a minimum of 46% dietary Crude Protein level within a dietary Energy range of 22-24 kJ. g^{-1} is required for maximal growth of African catfish. This is a more refined range than that previously proposed (Uys 1989) 44% - 48% Crude Protein (based on commercial raw materials and for similar weight ranges / 1.1.2.3 / Table 40 pp.120), supersedes the 40% Crude Protein at a 23 kJ.g^{-1} Gross Energy dietary level based on purified materials and proposed by Machiels & Henken (1985; 1.1.2.3) and seems to be closer to the 50% Crude Protein level with a lower dietary Gross Energy level of 18.2 kJ.g^{-1} based on semi-purified materials but for the smaller weight range of 20 - 60 g (Ufodike & Ekokotu 1986 ; Table 40 pp.120).

High Energy diets (46:16) enhance the growth of African catfish at a starting weight of 130-181g (4.3.1/ Table 18). African catfish at the starting weight of 120-130g (4.3.1/Groups 7&8,Table 18) and fed both the High (46:16) and Low Energy (46:10) diets, experience higher growth rate and weight gain than those starting at 130-180g. As both weight groups were characterized by insignificant differences ($P>0.05$) in initial average weight and initial stocking density (Table 16) such results could possibly be explained by the difference in tank surface available for each individual within these groups (Appendix V), taking into account the territorial nature of the species and its sedentary feeding habits (Hecht & Appelbaum 1988; Viveen *et al.* 1986). Groups 7&8 consisted only of four individuals per group, inevitably allocating more tank surface per individual (Appendix V). Results of the second experimental period suggest that *C. gariepinus* at higher starting weights (155-233g) is benefiting more from a diet with a higher P:E ratio (Diet 46:10) in terms of growth rate and weight gain (4.3.2 / Table 19).

The overall superiority of the Control group (except for the Groups 7 & 8) is not surprising as diets based on commercial raw materials are more palatable and consequently consumed in higher quantities than those prepared with purified materials. This higher feed intake for the Control has been confirmed over the 76-day experimenal period and when consumption was estimated on individual tank data.

Within each experimental period, comparison of FCR (estimated either on individual or cumulative tank data / Tables 18,19 & 20) and Feed Intake (estimated either on individual or cumulative tank data / Tables 35,36 & 37) did not reveal any significant differences ($P<0.05$) among the best performing groups, except for the Feed Intake of the Control when estimated on individual data and for the overall 76 day experimental period (Table 37). Similarly FCR, SGR and Feed intake did not reveal any significant differences within the same groups, over time (Tables 25,26,27, 38 &39), except for the FCR and Feed Intake of the Control. As % Weight Gain can approximately be expressed by the fraction [Feed Intake: FCR], the significant increase of % Weight Gain in the best performing groups should anticipate either an increase in the Feed Intake or a decrease in the FCR of the respective groups. This was not the case in these experiments.
All fish in these experiments were individually tagged (2.2.7) therefore % Weight Gain values of any Group during any experimental period can be considered robust. On the contrary, evaluation of Feed Intake (and consequently FCR) was estimated on the daily consumption of each replicate tank equally divided by the number of residing individuals (2.3; 4.3.1; 4.3.6) and not on the real consumption of each individual for which there were no means of estimation. The intra-specific territoriality and aggressiveness of *C. gariepinus* (Hecht & Appelbaum 1988) unavoidably leads to unequal food consumption among individuals in any small or large population. This methodological inconsistency in the evaluation of these parameters could be the main cause for the inconsistent relation among %Weight Gain, Feed Intake and FCR when estimated on an individual basis. However, when Feed Intake and FCR were estimated on cumulative numbers deriving from the performance of each replicate tank as a population (total

tank biomass, total ration consumed per tank, weight gain of the total tank biomass; Tables 18,19,20,35,36,37; Appendix VI), again, no significant differences for these indices were recorded. Therefore, the inconsistent relation among %Weight Gain, Feed Intake and FCR might be the result of another parameter not properly controlled or monitored during this experiment. It is possible that this parameter has been the previously mentioned factor "available tank surface" (see 2nd paragraph of this Discussion). This parameter has probably prevented fish from optimum diet utilization and consequently optimal Feed Intake levels.

The higher performance indices (SGR & %Weight Gain) observed by previous researchers (Table 40) and for experimental periods of almost similar time length, can be a reflection of the lower initial weights of the animals used in those experiments combined with the administration of diets made from complex foodstuffs (increased consumption due to increased palatability-acceptability). Furthermore, some culture conditions in this experiment (stocking density in relation to available per fish tank surface) may have become limiting for the best performing groups towards the end of the experimental period. However, the achieved SGR, FCR and PER for Group 8 compare favourably with respective values previously observed by other researchers (Table 40) and place the results of this experiment within the range of acceptable experimental values for the species.

Blood glucose levels in this experiment fall within the "normal" range of blood glucose values for rainbow trout (Alexis *et al.*1985; Kaushik *et al.* 1989). Although Groups 1, 2, 3 and 4 were fed decreasing levels of dietary carbohydrates (49%, 42%, 42%, and 37%) with slightly different levels of energy, they had equally low blood glucose levels. This is indicative of carbohydrate utilization similar to that of carp whose blood glucose is the result of gluconeogenesis from dietary amino acids (1.2.3.2). Furthermore, Groups 5,6,7 and 8 fed lower dietary carbohydrate levels (32%, 26.5%) than Groups 1,2,3 and 4, almost the same dietary energy levels (22.4-23.7kJ.g^{-1}) and similar dietary lipid levels, were characterized by the highest blood

glucose levels most probably a result of the highest protein levels (46%) in their diets. The liver glycogen levels among all the experimental Groups (Table 34) partially reconfirm the blood glucose picture, as Groups 5,6,7 and 8 (high dietary protein levels 46%) had higher levels of liver glycogen than Groups 1 and 2, probably a result of higher liver glyconeogenesis from amino acid precursors (1.2.4.4.1 / Figure 5). But this was not the case with Group 3 which had equal levels of liver glycogen compared to Groups 6 and 8 fed the High Protein - High Lipid diet and Group 4 which was characterized by the highest liver glycogen values among the rest of the groups.

Rainbow Trout and Atlantic salmon have the ability to store excess dietary carbohydrates (within a certain range) in the liver (Bergot 1979; Kaushik *et al.* 1989; Hemre *et al.*1995). On the contrary, although African catfish has the ability to store liver glycogen, this is rather of gluconeogenic origin from amino acid precursors. Dietary carbohydrates are utilized rather as an immediate energy-yielding source, than as a fuel storage source. This is reconfirmed by the results of these Protein: Energy experiments, where groups administered the high carbohydrate diets showed better Apparent Net Protein Utilization to the ones fed the low carbohydrate - high lipid diets (within the same protein levels).

Protein utilization (ANPU) and Protein carcass deposition are higher under a high P: E ratio Diet (Diet 46:10 / 22.74mg Protein.kJ^{-1}) with a concomitant decrease in the carcass lipid levels. This reconfirms the findings of Machiels & Henken (1985) who observed excess carcass lipid incorporation after the administration of a high energy (24 $kJ.g^{-1}$)- low P: E ratio Diet (7.9-16 mg Protein.kJ^{-1}) with dietary crude lipid levels ranging between 20% and 24%, carbohydrate levels between 16% and 16.5% and within a dietary protein range of 19% to 38.5% of the diet (on a dry matter basis). Similarly, Degani *et al.* (1989) concluded that an increase of protein in the diet, increases the carcass protein retention and protein efficiency and decreases the lipid in the muscle, suggesting that more research is needed towards the protein-sparing action of fat.

With a total energy content between 23.75 and 22.42 $kJ.g^{-1}$ (567 and 535 $kcal .100\ g^{-1}$) of dry diet and a dietary Protein: Energy ratio between 21.53 and 22.74 mg $protein.kJ^{-1}$ (90 and 95 mg $protein.kcal^{-1}$) (Diets 46:16 and 46:10 respectively), *C. gariepinus* confirms its carnivorous dietary requirements compared to the less carnivorous North American catfish *Ictalurus punctatus* (11.5 $kJ.g^{-1}$ of dry diet and a P: E ratio of 20.3 mg $protein.kJ^{-1}$; Garling & Wilson, 1976).

Despite its carnivorous tendencies compared to the North American *I. punctatus*, the African catfish seems to be using dietary carbohydrate energy quite efficiently. This protein-sparing effect of dietary carbohydrates coupled with significant high carbohydrate digestibility (high pre-feeding intestinal amylase activity; 3.4) advocates more comprehensive use of carbohydrates in catfish diet formulation. More experimentation is needed (by the use of radiolabelled substances) in order to clarify the utilization of carbohydrates, the glyconeogenesis pathway from amino acid precusors and the lipogenesis pathway from both protein and carbohydrate precursors.

Haematocrit values observed in all the groups of this experiment, fall within the range of haematocrit values recorded for clinically healthy individuals of other species (Eiras *et al.*1983; Alexis *et al.*1985; Klontz 1994). They are also similar to the haematocrit values observed in *C. gariepinus* adults fed diets containing co-dried tilapia silage:soyabean flour blend, partialy or entirely substituting fishmeal (Fagbenro *et al.*1997). Though, apart from Groups 3 and 8, where haematocrit values were not significantly different among individuals of these groups, fish of the rest of the groups showed significant difference in their haematocrit values within each group (4.3.4 / Table 30). This response is similar to that of Atlantic salmon adapted to various dietary carbohydrate levels at different temperatures as no variations in the haematocrit were found as a function of increased dietary carbohydrate or season but only as a function of the individuality of each fish within each experimental Group (Hemre *et al.*1995). On the contrary such variations, as a function of diet, nutritional state, temperature and weight have been suggested for rainbow trout (Eiras *et al.*1983; Garcia *et al.*1992; Houston & Koss 1984; Papoutsoglou *et al.*

1987). Similar variation was observed for the haemoglobin values within each group of this experiment, except for Groups 3 and 4 where haemoglobin values have also not been significantly ($P<0.05$) different (Table 31). The haemoglobin values observed in this experiment were higher than the respective ones observed for rainbow trout (Eiras *et al.*1983), Atlantic salmon (Wagboo *et al.*1994) and *C. gariepinus* adults (Fagbenro *et al.* 1997).

Evidently, African catfish showed significant variation for both these parameters (haematocrit and haemoglobin) within each group. Therefore in cases of nutritional studies with this species, where severe pathologies (due to nutrient deficiencies or imbalances) are not expected, haematocrit and haemoglobin values are not useful due to individual variation.

In conclusion, *C. gariepinus* of 120-233g performed best when fed diets containing Gross Energy between 22-24 $kJ.g^{-1}$, P: E ratio of 21.5-23 mg $protein.kJ^{-1}$, Crude Protein 46%, Crude Lipid 10-17% and Carbohydrate between 26-32%. These diets indicate a protein-sparing effect as a result not only of lipid but also of a better dietary carbohydrate utilization compared to the carbohydrate utilization of the diets used by Uys (1989), Machiels & Henken (1985). Furthermore, the increase of carcass lipid as a result of increased dietary non-protein energy demonstrated for other species (rainbow trout, plaice, channel catfish, common carp and red drum / see 1.2.3.1) has also been demonstrated for African catfish (Table 22; Figure 8).
Overall, dietary nutrient ratios should be an important consideration when carcass quality of the final product is also of decisive nature.

Table 40. Comparison of various performance indices between this experiment and experiments by previous researchers

Experiments	*SGR*	*%Weight Gain**	*FCR*	*PER*
Group 9 / Control this experiment [a]	0.84	79.73	1.44	2.08
Group 5 / 46:10 this experiment [a]	0.55	52.23	1.37	1.84
Group 6 / 46:16 this experiment [a]	0.60	59.27	1.45	1.73
Group 8 / 46:16 this experiment [b]	1.67	96.84	0.82	3.01
Ufodike & Ekokotu (1986)[1]	1.2	160.0	2.43	0.8
Degani et al. (1989)[2]	1.31	127.14	2.31	1.43
Degani et al. (1989)[3]	1.44	203.6	1.35	1.87
Uys 1989[4]	5.42	212.1	0.95	2.46

a. Overall 76 day period

b. First 40 day period

* Not arcsine transformed

1. 84 day experimental period for the range of 20 - 60 g. The proximate analysis of this diet was: Protein 50.2%, Fat 10.6%, Crude Fibre 5.0%, Ash 9.1%, Carbohydrates 10.3%, Gross Energy 18.22 k J. g^{-1}. The composition was: Algae 32%, Cow Blood meal 52%, Groundnut Oil 6.0%, Cod Liver Oil 4.0%, Minerals 2.0%, Vitamins 2.0%, Starch 1.5%, Cr_2O_3 0.5%

2. 78 day experimental period for the range of 10 - 37 g. Proximate analsysis of the diet used: Protein 30.0%, Fat 2.4%, Crude Fibre 11.0%, Ash 10.0%, Carbohydrates 42% , Gross Energy 10.46 kJ.g^{-1}. The composition of the diet was: Fishmeal 8.96%, Soyabean meal 43.42%, Corn Meal 37.52%, Cellulose 8.19%, Vitamins 1.0%, Guar gum 1.0%

3. 78 day experimental period for the range of 10 - 37 g. Proximate analysis of the diet used: Protein 34.9%, Fat 3.5%, Crude Fibre 10.8%, Ash 9.8%, Carbohydrates 40.12%, Gross Energy 10.46% kJ.g^{-1}. The composition of the diet was: Fishmeal 17.03%, Soyabean meal 43.94%, Corn meal 29.03%, Cellulose 8.0%, Vitamins 1.0%, Guar gum 1.0%

4. 21day experimental period for groups of 1.0 to 160g. Proximate analysis of the diet used: Protein 43%, Fat 13.25%, Crude Fibre 1.74%, NFE 18.36%, Ash 14.8%, Gross Energy 20.0 kJ.g^{-1}. The composition of the diet was: Corn 5.4%, Wheat Bran 5.4%, Fish meal 52.9%, Carcass meal 5.0%, Blood meal 5.0%, Fish Oil 6.9%, Vitamins 0.2%, Minerals 0.2%, Molasses powder 19.0%

CHAPTER 5. DETERMINATION OF THE QUANTITATIVE ESSENTIAL AMINO ACID REQUIREMENTS of the African Catfish *Clarias gariepinus*

5.1 INTRODUCTION

The lack of data on the nutritional requirements of the various life stages of *C. gariepinus* constitutes the major constraint for further cost-effective diet formulation (1.1.2.3; Wilson & Moreau 1996). Only recently (Fagbenro *et al.*1998a; 1998b; 1999a; 1999b) have efforts been made for the determination of arginine, methionine, lysine and tryptophan requirements of juvenile African catfish. These results may be questionable as the diets used were formulated on the basis of nutrient requirements determined by the use of complex foodstuffs and the constraints that this method implies (1.1.2.3). Therefore, information generated from previous experiments in this study (Chapter 4) on optimum Protein: Energy ratios using purified diets, can now be used for determination of the Essential Amino Acid (EAA) requirements of the species in the adult phase.

This experiment formed part of the previously described Protein: Energy experiment (Chapter 4), employing Groups 7 and 8 as experimental fish stocks over the first 40-day period (4.2.2; Plate 5). EEA requirements have been evaluated by the "daily deposition" method (1.2.4.5.3) using Group 7 (46:10) as the best performing group of high performance parameters (SGR, TGC, % Weight Gain and PER; Table 18), good Protein Utilization (ANPU; Table 21), high carcass protein levels and low carcass lipid levels (Table 22).

5.2 MATERIALS AND METHODS

Diets, facilities, husbandry protocol, physicochemical parameters, performance indices and statistical analyses, were as described in Protein: Energy experiments (4.2.1, 4.2.2 & 4.2.3)

The analytical methods followed were as described in 4.2.5

5.3 ESTIMATION OF THE EAA CARCASS DEPOSITION.

Before the start of the Protein: Energy experiment, six fish of the preconditioned stock (fed the maintenance purified diet; Table 16), of an average weight of 110.66±39.9 g, were fasted for 4 days (Kaushik 1979), euthanized and analyzed. Their average body composition and amino acid composition was as shown in Tables 41 and 42.

Table 41. Body composition of preconditioned fasted animals before the start of the experiment

	% on a dry matter basis	*% on a live weight basis*	*Standard Deviation (n=6)*
Dry Matter	-	34.68	5.12
Crude Protein	58.81	20.39	2.33
Crude Lipid	22.86	7.93	3.12
Ash	17.65	6.12	3.13
Gross Energy $kJ.g^{-1}$	21.36	7.40	0.65

Table 42. Carcass amino acid profile of the preconditioned fasted animals before the start of the experiment [1]

Essential Amino Acids	*g EAA.100g^{-1} dried carcass*	*g EAA.100g^{-1} carcass protein*	*g EAA.100g^{-1} total EAA*
L-Arginine	2.58 (0.12)	4.39 (0.32)	12.56 (0.43)
L-Cystine (NEAA)	0.045 (0.01)	0.07 (0.05)	0.22 (0.07)
L-Histidine	0.86 (0.03)	1.46 (0.04)	4.16 (0.053)
L-Isoleucine	1.85 (0.15)	3.14 (0.18)	8.98 (0.22)
L-Leucine	3.25 (0.25)	5.52 (0.89)	15.76 (1.34)
L-Lysine	3.57 (0.37)	6.07 (0.73)	17.35 (1.65)
L-Methionine	0.70 (0.01)	1.20 (0.075)	3.44 (0.74)
L-Phenylalanine	1.90 (0.05)	3.24 (0.61)	9.24 (1.08)
L-Threonine	1.73 (0.41)	2.94 (0.95)	8.41 (1.22)
L-Tyrosine (NEAA)	1.49 (0.08)	2.53 (0.12)	7.22 (1.33)
Tryptophan	0.55 (0.03)	0.93 (0.045)	2.66 (0.44)
L-Valine	2.057 (0.09)	3.49 (1.05)	9.99 (1.65)
Total A.A. %	20.59	35.01	100.00

1. Numbers in parenthesis represent Standard Deviation (n=6)

Similarly, fish of the best performing group (Group 7), were euthanized after an overnight fasting at the end of the experiment, and analyzed for both proximate carcass composition and amino acid carcass composition (Tables 43 & 44).

Table 43. Body composition of best performing animals (Group 7)

	% on a dry matter basis	*% on a live weight basis*	*Standard Deviation (n=4)*
Dry Matter	-	27.05	5.57
Crude Protein	64.41	17.42	2.21
Crude Lipid	15.94	4.31	0.33
Ash	14.27	3.86	3.57
Gross Energy $kJ.g^{-1}$	22.59	6.11	1.48

Table 44. Carcass amino acid profile of the best performing group (Group 7) at the end of the experiment[1]

Essential Amino Acids	*g EAA.100g^{-1} dried carcass*	*g EAA.100g^{-1} carcass protein*	*g EAA.100g^{-1} total EAA*
L-Arginine	3.63 (0.22)	5.16 (0.34)	10.13 (1.77)
L-Cystine (NEEA)	0.24 (0.13)	0.34 (0.20)	0.64 (0.21)
L-Histidine	1.58 (0.001)	2.26 (0.001)	4.46 (1.05)
L-Isoleucine	2.66 (0.36)	3.77 (0.55)	7.38 (0.75)
L-Leucine	5.77 (1.27)	8.13 (0.94)	15.81 (0.21)
L-Lysine	5.93 (0.61)	8.41 (1.98)	16.48 (2.21)
L-Methionine	0.85 (0.17)	1.22 (0.27)	2.43 (1.04)
L-Phenylalanine	4.25 (0.23)	5.86 (0.35)	11.16 (1.11)
L-Threonine	2.80 (0.41)	3.96 (0.63)	7.74 (0.71)
L-Tyrosine(NEEA)	3.74 (2.04)	5.13 (3.82)	9.73 (7.45)
Tryptophan	1.89 (0.01)	2.71 (0.016)	5.33 (1.28)
L-Valine	3.15 (0.56)	4.45 (0.87)	8.68 (0.51)
Total A.A. %	36.53	51.41	100.00

1. Numbers in parenthesis represent Standard Deviation (n=4)

Based on the carcass amino acid profile of both these groups at the beginning and the end of the experiment, the following daily amino acid deposition rate was estimated (Table 45)

An analytical explanation of the involved calculations is given in Appendix VII

Table 45. Daily rate of deposition of Essential Amino Acids in the carcass of the African Catfish *Clarias gariepinus* (n=4)

Essential Amino Acids	*mg EAA g^{-1} DryWt.day^{-1}*	*mg EAA g^{-1} LiveWt.day^{-1}*
L-Arginine	0.266	0.022
L-Cystine (NEEA)	n.d.*	n.d.
L-Histidine	0.187	0.034
L-Isoleucine	0.211	0.020
L-Leucine	0.657	0.113
L-Lysine	0.606	0.094
L-Methionine	0.036	0.004
L-Phenylalanine	0.615	0.128
L-Threonine	0.275	0.040
L-Tyrosine (NEEA)	n.d.	n.d.
Tryptophan	0.350	0.083
L-Valine	0.281	0.035
Totals	3.487	0.568

* Not determined as NEEA can not be estimated by the carcass deposition method

5.4 CONVERSION OF THE DAILY DEPOSITION RATES TO EAA REQUIREMENTS AS % OF THE DIETARY PROTEIN OR AS % OF THE ADMINISTERED DIET.

Having estimated the daily deposition rates, the daily feed intake (4.3.6), the dietary protein level and the amino acid digestibility of the best performing diet (Table 11), the EAA requirements were established as shown in Table 46 (Appendix VII)

Table 46. Essential Amino Acid requirements of the African Catfish *C. gariepinus* for the weight range of 100-130g, based on a diet of 46% Crude Protein level (on a DM basis) and composed of purified materials (n=4)

Essential Amino Acids	*g.$100g^{-1}$ dietary Crude Protein*	*g.$100g^{-1}$ total required EAA*	*g.$100g^{-1}$ body weight gain. day^{-1}*	*g.$100g^{-1}$ body weight.day^{-1}*
L-Arginine	1.97	7.63	2.76	0.026
L-Cystine(NEEA)	n.d.*	n.d.	n.d.	n.d.
L-Histidine	1.39	5.37	1.94	0.018
L-Isoleucine	1.56	6.06	2.19	0.021
L-Leucine	4.87	18.86	6.82	0.065
L-Lysine	4.49	17.39	6.29	0.060
L-Methionine	0.26	1.03	0.37	0.003
L-Phenylalanine	4.56	17.65	6.38	0.061
L-Threonine	2.04	7.88	2.85	0.027
L-Tyrosine(NEEA)	n.d.	n.d.	n.d.	n.d.
Tryptophan	2.59	10.04	3.63	0.035
L-Valine	2.08	8.06	2.92	0.028
Totals	25.85	100.00	36.19	0.35

* Not determined as NEEA can not be estimated by the carcass deposition method

5.5 DISCUSSION

Results of the Protein: Energy experiments (4.3, 4.4) revealed that adult (100-130 g) *C. gariepinus* experience better Protein utilization and higher Protein carcass deposition fed a high P: E ratio Diet (46:10;Table 16). The established amino acid requirements estimated on animals fed this diet seem to be lower than expected (Table 47) especially for the amino acids arginine, methionine, whereas phenylalanine and the rest of EAA were determined in levels comparable to those of other fish species (Tables 47 & 48).

Table 47. Amino Acid requirements (g.100g^{-1} dietary Crude Protein) of various species (NRC 1993) compared to the established ones for *C. gariepinus*[a]

	Chinook Salmon[1]	*Common Carp*[2]	*Channel Catfish*[3]	*Nile Tilapia*[4]	*African Catfish*[5]
Arginine	6.0	4.3	4.3	4.2	1.97
Cystine(NEEA)	2.5	n .s.[b]	n.s.	0.54	n.d.*
Histidine	1.8	2.1	1.5	1.72	1.39
Isoleucine	2.2	2.5	2.6	3.11	1.56
Leucine	3.9	3.3	3.5	3.39	4.87
Lysine	5.0	5.7	5.1	5.12	4.49
Methionine	4.0	3.1[c]	2.3[c]	2.68	0.26
Phenylalanine	5.1	6.5	5.0	3.75	4.56
Threonine	2.2	3.9	2.0	3.75	2.04
Tryptophan	0.5	0.8	0.5	1.0	2.59
Tyrosine(NEEA)	0.97	2.59	1.25	1.79	n.d.*
Valine	3.2	3.6	3.0	2.8	2.08
Totals	37.37	38.39	31.05	33.85	25.85

a. Original authors can be traced on the referred source as they vary from one amino acid to another within the same species b. none stated c. in the absence of cystine

1. With 40% and 41% Protein 2.with 38.5% Protein 3.with 24% Protein 4.with 28% Protein 5. Results of this experiment with 45.98% Protein

* Not determined as NEEA can not be estimated by the carcass deposition method

Table 48. Chemical score (Cowey & Sargent 1972) of the EAA requirements of various species when compared to those of *C. gariepinus* [1]

	Chinook Salmon	*Common Carp*	*Channel Catfish*	*Nile Tilapia*	*Clarias gariepinus*
Arginine	0.90	0.65	0.65	0.64	0.29
Histidine	0.69	0.81	0.57	0.66	0.54
Isoleucine	0.37	0.42	0.44	0.53	0.26
Leucine	0.44	0.37	0.39	0.38	0.55
Lysine	0.64	0.73	0.65	0.66	0.57
Methionine	1.25	0.97	0.72	0.84	0.08
Cystine(NEEA)	1.19	0	0	0.26	n.d.*
Phenylalanine	0.93	1.18	0.90	0.68	0.83
Tyrosine(NEEA)	0.26	0.68	0.33	0.47	n.d.*
Threonine	0.45	0.79	0.41	0.76	0.42
Tryptophan	0.36	0.57	0.36	0.72	1.85
Valine	0.45	0.51	0.42	0.39	0.29
Average	0.66	0.69	0.49	0.58	0.47

1. Chemical score with reference to egg albumin (2.3). Sources and other explanations are the same as in Table 47

* Not determined as NEEA can not be estimated by the carcass deposition method

The main nitrogen (N) containing body components are non-collagenous cells, collagen and keratin. Collagen content increases with age, therefore the relative proportions of these components vary and create concomitant variations in whole-body EAA composition (Smith 1980). Such differences have been reported but were small (Smith 1980). Because inter-specific whole-body EAA differences are small as well (1.2.4.3), Smith (1980) created the "standard animal" assuming that nitrogen is provided by cellular protein at 66%, collagen at 30% and keratin at 4%. These proportions were based on the keratin values for rat and chicken whereas cellular protein was given by a mean value for the flesh of sheep, cattle, pig and chicken. The calculated whole-body EAA composition of the "standard animal" (Table 49) is very similar to whole-body compositions of the rat, pig and calf and not very different from that of the chicken. Furthermore, these whole-body EAA values of the "standard animal" are very close to the ones established for *C.gariepinus*, except for methionine which is dramatically low in the carcass of the best performing Group 7 at the end of this experiment (Tables 44 & 49).

Low carcass deposition of EAA (as percentage of total deposited EAA) would only be observed if the best performing diets were deficient in total energy or had P: E ratios that would lead in catabolism of amino acids for energy purposes (gluconeogenesis). However, even under these conditions the body amino acid pool (1.2.4.1; 1.2.4.4.2) would be able to compensate for such losses, though for a restricted time period. This period having had expired, a decline in the retained carcass protein, weight gain and growth rate should be observed.

Group 7 during the first 40-day experimental period had the optimum combination of high performance parameters (SGR, TGC, % Weight Gain and PER; Table 18), good Protein Utilization (ANPU; Table 21), high carcass protein and low carcass lipid levels (Table 22). Although no body protein loss has been observed, % Weight Gain has been acceptable but lower than the respective ones achieved in previous research (Table 40). This reduced growth could be the effect of a prolongued exposure to deficient, in certain EAA, diets, which depleted the body amino acid pool and affected growth. During these experiments, animals have been subjected to a 20-day adaptation pre-experimental period fed on a maintenance diet (Table 16), followed by a 4-day starvation period and the 40-day first experimental one (4.2.1; Plate 5). Both, maintenance and 46:10 diets were characterized by lower levels of arginine, lysine, methionine, cystine, threonine and tryptophan (3.9-4.0%, 4.0-4.5%, 0.83-0.88%, 0.96-1.0%, 3.46-3.60%, 0.8-0.87% of dietary protein) to the ones (4.5%, 5.7%, 3.2%, 1.33%, 4.26%, 1.1% of dietary protein) proposed by Fagbenro *et al.* (1998a; 1998b; 1999a; 1999b). It is possible that the sequence of adaptation, starvation and main first experimental period (20+40=60 days) exerted an combinatory effect on the body amino acid pool, with irreparable loss for some of those amino acids (Table 49) and consequently lower values as determined requirements (Table 47).

Low body arginine incorporation could also be related to another metabolic dysfunction-peculiarity: aquatic teleosts excrete most of their nitrogenous

waste products in the form of ammonia, which is mediated predominantly through the gills (Smith 1929). However, during periods of emersion the

continued production of ammonia, as the primary end product of excretion, should be eliminated for two basic reasons: to prevent desiccation (as ammonia excretion demands considerable loss of water) and to stop ammonia accumulation, as it will eventually become toxic (Davenport & Sayer 1986).

An alternative strategy for excretion in species that regularly encounter conditions of drought, is the production of urea, via the ornithine-urea cycle, which of course is metabolically expensive (Mommsen & Walsh 1992). By converting ammonia to urea, the nitrogenous waste products can be stored until re-immersion. Urea can be stored (Saha & Ratha 1989) and is found in the tissues of such species at much higher concentrations than ammonia (Mommsen & Walsh 1992). Functional ornithine-urea cycles have been found in the freshwater, air-breathing teleosts *Heteropneustes fossilis* (Saha *et al.*1988), *Clarias batrachus*, *Amphipnous cuchia, Anabas testudineus* (Saha & Ratha 1989) as well as in the seawater species *Periophthalmus sobrinus, Sicyases sanguineus* and *Periopthalmus cantonensis* (Gordon et al. 1969; 1970; 1978).

As previously stated (1.1.1), *Clarias gariepinus* develops an air breathing capacity at the end of larval development. Yolk-sac larvae of *C. gariepinus* are predominantly ammoniotelic with urea excretion accounting for 19±7% of the total nitrogen excretion. During exogenous feeding and until 180-205h post fertilization, *C. gariepinus* larvae increase the relative urea excretion to 44 ±13% of their total nitrogen excretion (Terjesen *et al.*1997). This high degree of ureotelism in larvae has been considered an adaptive mechanism to a habitat of temporal water shortage.

However, this level of ureotelism remains in adult (119.9±30.6g) *C. gariepinus*. Buttle *et al.* (1996) showed that the non-ammonia fraction represented 37% of the total nitrogenous waste products during continuous immersion, was higher than the one of other air-breathing species and even higher than that of *C. batrachus*, implying a more intense ornithine-urea cycle. During 180 min of emersion, *C.gariepinus* lowers the rate of its

nitrogen metabolism in order to decrease the accumulation of metabolic end products and consequently to limit the metabolic cost of converting them to non-toxic forms (Buttle *et al.*1996).

It is speculated that mediocre oxygen levels prevailing in the employed experimental system (4.2.2&5.2) in conjunction with sampling manipulations (prolonged emmersion), created a shift to ureotelism with a result in low arginine tissue levels throughout the experiment and consequently low determined values as arginine requirements for the species.

Table 49. Whole body EAA composition (g.100g^{-1} total EAA) of various animals (Smith 1980)[1] and *C.gariepinus*

	Rat	***Pig***	***Calf***	***Chicken***	***Standard animal****	***Clarias gariepinus***[2]
Arginine	n.s[3]	n.s	n.s	n.s	n.s	10.13
Cystine	4	2	3	6	4	0.64
Histidine	7	7	7	4	6	4.46
Isoleucine	9	10	8	10	11	7.38
Leucine	17	18	19	17	17	15.81
Lysine	17	18	18	16	18	16.48
Methionine	5	5	5	4	5	2.43
Phenylalanine	9	9	10	10	9	11.16
Threonine	10	9	10	10	10	7.74
Tyrosine	8	6	7	6	7	9.73
Tryptophan	2	3	2	2	2	5.33
Valine	12	13	11	15	11	8.69
Met+Cys	9	7	8	10	9	3.07
Phe+Tyr	17	15	17	16	16	20.89

1. Original authors can be traced on the given referred source as they vary from one amino acid to another within the same species.
2. As determined during this study for the best performing group (Table 44).
3. None stated
* As described by Smith (1980) (5.5 Discussion)

CHAPTER 6. CONCLUSIONS

6.1 ACHIEVEMENTS IN THIS STUDY

A most important aspect of feed management in aquaculture operations is the cost of feeds which accounts for 55-60% of the running costs in intensive systems and 40% in semi-intensive systems (Shang 1981).

The cost of feeding in catfish culture practiced in Asian and African countries is highly variable due to a variety of production systems and the multitude of foodstuffs used originating from different sources and processed in different ways (1.1.1.1; 1.1.1.2).

Wilson & Moreau (1996) acknowledge that the lack of data on the nutritional requirements of the various life stages of African catfish constitutes a major constraint for further development of low-cost practical feeds. Protein requirements and protein: energy ratios using either purified materials (Machiels & Henken 1985) or complex foodstuffs (Uys 1989) have been defined within a wide range. Consequently, establishment of a dietary protein to energy ratio based on purified diets would inevitably improve the cost-effectiveness of feeds for the species and optimize the culture conditions under any form of aquaculture exploitation. However, diet formulation using purified materials experience problems due to the imbalanced amino acid profile of the materials used (3.1;NRC 1993). Zein and gluten are semi-purified corn by products, deficient in tryptophan and lysine but high in methionine and cystine and complete for the rest of amino acids. Therefore, the digestibility of purified diets based on casein, gelatin, zein and gluten has been investigated.

Least-cost feeds used by large scale catfish farmers are formulated from a mixture of animal and plant-based protein foodstuffs (blood meal, meat and bone meal, hydrolyzed feather meal, yeast, soybean meal, groundnut cake, cottonseed cake, rice bran, cassava chips) low in methionine, cystine, lysine and tryptophan which may act as first or second limiting amino acids (1.2.4.3; DeSilva & Anderson 1995; Fagbenro *et al.*1998a, b). Therefore an

insight into the EAA requirements of the species has also been attempted, using data generated from the two previous experiments.

The purified diets used in the experiments of this study proved to be palatable and therefore acceptable by *C. gariepinus*. The recorded Apparent Digestibility Coefficients (ADCs) for amino acids and most nutrients, coupled with the acceptable performance indices observed in the protein: energy experiments, advocate the use of purified diets of similar composition in experimental work on African catfish. Due to the absence of lysine and tryptophan (Morrison 1950; Clay 1981) enzymes do not easily break down zein, major maize component. However, the zein used in these experiments had no apparent negative effect on digestive processes: both the zein combined diet and zein (as a material) showed acceptable ADCs for their nutrients and amino acids which can be used as a broadly applied index of its digestibility for the species. Furthermore, calculation of nutrient ADCs for a single ingredient by Forster's method can be a useful tool in digestibility studies when the nutrient profile of the examined ingredient deviates from the nutrient profile of the reference diet.
Finally, the high carbohydrate digestibility of the purified diet in these trials confirmed the good starch utilization suggested by Uys (1989).

The protein: energy experiments confirmed the carnivorous nature of *C.gariepinus* compared to the less carnivorous North American catfish *I.punctatus* and better elucidated dietary nutrient levels required for optimal growth. Both the high (46:16) and low (46:10) energy diets in this experiment suggested that the best performing diets for *C. gariepinus* at a starting range of 130-180g, are those of a total gross energy between 22-24 $kJ.g^{-1}$, a P: E ratio of 21.5-23 mg protein.kJ^{-1}, a crude protein level of 46%, a crude lipid level of 10-17% and a carbohydrate level of 26-32%. The weight range of the individuals used in conjunction with the available tank surface and the employed stocking densities, may have become limiting for the fast growing groups towards the end of the observed experimental periods, imposing lower performance indices when compared to the indices

achieved in experiments by previous researchers. Available tank surface could also have prevented fish from optimum diet utilization, optimal feed intake levels and resulted in an inconsistent relation among % Weight Gain, feed intake and FCR.

The higher blood glucose levels and liver glycogen levels observed in the animals fed the low carbohydrate-high protein diets suggest carbohydrate utilization similar to that of carp *Cyprinus carpio* (1.2.3.3). In such species dietary carbohydrates are employed as an immediate energy yielding nutrient and liver glycogen is likely to be of gluconeogenetic origin from amino acid precursors. Such an interpretation is also supported by carcass composition data and improved protein utilization of fish fed the high carbohydrate-low lipid diets rather than the low carbohydrate-high lipid ones (within the same protein levels). Although excessive gluconeogenesis may create a drawback in protein utilization, carbohydrate levels (26-32%) of the best performing diets during these experiments were much higher than used by previous researchers (16-18%) for the same species and still higher than the ones (15-25%) employed for other carnivores (salmonids, sea bass, sea bream; NRC 1993). Therefore and by taking into account the high carbohydrate digestibility of all the diets used in these experiments, a more comprehensive use of carbohydrates in catfish diet formulation is suggested.

In many species, haematocrit and total haemoglobin vary according to the levels of incorporated dietary nutrients, with water temperature and the weight range of the individuals involved. During the present experiments both these blood parameters revealed that they are not particularly valuable performance indices for African catfish in relation to general nutritional parameters associated with growth and food utilization. Only severe nutrient imbalance and/or deficiency might be expected to cause related changes in either haematocrit or total haemoglobin.

An attempt to determine the quantitative essential amino acid requirements of the species has been partially successful.

The diets proved to be energetically balanced promoting good growth rates and protein deposition and therefore not expected to negatively affect carcass essential amino acid deposition ratios. However, more recent information on the EAA of the species (Fagbenro *et al.*1998a; 1998b; 1999a; 1999b) suggests that the best performing diets have been low particularly in lysine, methionine and cystine. The prolonged sequence of adaptation, starvation and main first experimental period (60 days) might have exerted an combinatory effect on the body amino acid pool, with irreparable loss for some of those amino acids (Table 49) and consequently lower values as determined requirements (Table 47).

Low body arginine incorporation could also be related to another metabolic dysfunction-peculiarity: aquatic teleosts excrete (through the gills) most of their nitrogenous waste products in the form of ammonia (Smith 1929). Yolk sac and exogenously feeding larvae of *C.gariepinus* increase the level of ureotelism up to 44±13% of their total nitrogen excretion (Terjesen *et al.*1997), which remains functional in their sub-adult and adult stages as well (Buttle *et al.*1996). It is possible that physicochemical parameters in the aquarium systems (4.2.2 & 5.2) and handling of the experimental population created a shift to urea production via the ornithine-urea cycle at the expense of tissue arginine.

6.2 FUTURE TRENDS IN CATFISH PRODUCTION AND RESEARCH

The aquaculture industry in Asia and the Pacific has been embarking on an increasing phase of intensification driven by a multitude of economic forces amongst which is an increasing need for even the small-scale farmer to transform from subsistence aquaculture to an income generating enterprise. Therefore, semi-intensive systems, the most widespread in Asian countries for catfish production, will have to be at least optimized if not fully intensified. In such systems, supplementary feeding is the form of external-artificial dietary input aiming at preventing deficiencies. However, due to increasing competition for primary resources (land and water) compounded feeds (containing significant fishmeal proportions and nutrient profiles meeting requirements) become increasingly popular. Consequently,

development of optimized formulations becomes increasingly important as well (De Silva 1993).

Albrecht & Breitsprecher (1969) showed that mean protein, CHO and lipid content of fish food organisms in ponds were 52.1, 27.3 and 7.7% respectively with a calorific value ranging between 6.69 and 23.85 $kJ.g^{-1}$ (DM basis) and therefore able to meet the nutritional requirements of most of the semi-intensively cultured organisms in Asia. However, the utilization efficiency of a supplementary feed in such semi-intensive systems is also dependent on the standing crop and body weight of cultured organisms (Hepher 1978). Furthermore, use of supplementary ingredients *per se* is becoming less important as most of those materials are of plant origin characterized by low digestibility values and consequently by low FCR. Such ingredients are now commonly referred to as non-conventional foodstuffs for compounded feeds (Table 50) aiming at either replacing a significant proportion of fishmeal or/and complete the profile of compounded feeds in terms of nutrient requirements. As these compounded feeds are expected to supplement natural food production in a pond environment, use of a nutritionally balanced feed is questionable. On the contrary, there is an urge for a "bottom-up" approach where research will be directed towards the "economically optimal protein level" within the food chain of the semi-intensive system in question and with other parameters (standing crop, feeding level and feeding frequency) be equally important to an acceptable nutritional profile (De Silva 1993).

Farm-made feeds are the common choice in Asian catfish systems (1.1.1.1). Mostly composed of non-conventional foodstuffs these are either overformulated or have a profile far from nutritionally balanced for the species (Table 51). Despite their low price, Diets 1 and 2 have low total energy compared to their protein levels and consequently equally high P: E ratio. In addition levels of Methionine, Cystine, Phenylalanine, Tyrosine and Threonine are lower than recently recommended (Fagbenro *et al.*1998a; 1998b; 1999a; 1999b). Diets 3, 4 and 6 have improved amino acid profile and P: E ratio, however fishmeal incorporation and low energy levels do not seem to reason the price increase for such semi-intensive systems. Diet 5

Table 50. Proximate analysis (on a DM basis) and price of foodstuffs used in the Asian and Pacific region*

Foodstuff	Cr. Protein	Cr. Lipid	Cr. Fibre	Ash	NFE	Energy in kj.g^{-1}	Price in US $. Kg^{-1}
Blood meal	63.15	0.56	0	20.7	15.59	17.78	0.03
Cassava leaves	31.85	7.98	12.43	8.0	39.73	14.98	0.16
Chicken viscera	52.85	11.2	2.7	4.56	-	12.59	0.13
Corn Gluten	60	0.82	1.92	1.33	35.94	16.37	0.89
Fishmeal (A1 grade)	59.61	11.22	2.17	22.76	4.24	19.88	1.18
Fish Oil	0	100	0	0	0	37.66	1.84
Flour	19.73	0.38	0	0.4	79.49	18.28	0.37
GroundNut cake	36.15	8.85	15.33	6.43	33.24	14.95	0.21
Kitchen refuse	20.26	5	-	5.88	41.17	16.53	0.005
Leuceana leaf meal	34.96	5.05	13.57	10.99	35.43	13.68	0.02
Linseed oil cake	26.45	7.78	14.33	15.51	35.93	18.33	0.22
Maize Meal	10.98	4.64	4.64	1.74	78.04	16.64	0.26
Moist tofu waste	27.45	0.82	19.33	4.12	48.28	12.98	0.04
Mollasses	4.45	0	0	11.93	83.62	15.16	0.16
Mustard oil cake	30.33	13.44	12.12	9.73	34.38	20.84	0.16
Rice bran	10.56	10.82	15.79	16.4	46.43	17.73	0.18
Sesame cake	27.2	13.18	11.18	13.47	34.97	19.89	0.49
Soya bean meal	46.70	10.57	5.46	7.30	29.96	16.81	0.59
Soya Oil	0	100	0	0	0	37.66	0.42
Tapioca flour	3.1	2.3	2	2.3	78.8	14.57	0.2
Starch flour	0.47	0.54	0	0.057	98.94	16.84	0.37
TrashFish	65.3	11.2	0.9	19.2	3.4	15.72	0.32
Yeast Brewery	53.26	0	0	0	41.46	15.86	0.98
Wheat bran coarse	14.96	3.7	13.95	6.29	61.92	14.13	0.16

* Data are based on the following authors: Jantrarotai & Jantrarotai (1993); Djunaidah (1993); Pascual (1993); Nandeesha (1993); Chou (1993); prices are estimated on referred prices adding a 5% inflation rate per annum (US Dpt of Commerce 1997)

seems to be more balanced in terms of most parameters (total energy, protein, and lipid, P: E ratio and amino acids). Again the price increase due to fishmeal incorporation questions its profitability and cost-effectiveness as standing crop, fish weight and density will affect its final performance. In addition cost-effectiveness of such diets relies on their fluctuating price and availability among countries (New & Csavas 1993b) and water quality and disease problems due to their rapid deterioration (1.1.1.1).

The results of this study could be used to optimize formulations based on the "economically optimal protein level" and the peculiarities of individual culture systems. Such formulations could be based on both conventional and non-conventional foodstuffs (Table 50) of a standard supply and price for the selected country-area, which would standardize production practices, production levels and costs. Such a standardized diet is shown in Table 51. This diet ("proposed diet") has lower total energy than Diet 5, but almost equal levels of total protein, amino acids and a much lower price due to fishmeal elimination. Although its cost-effectiveness within a semi-intensive system will have to be tested *in situ*, it seems promising.

Despite the significant input of this study, numerous questions will have to be answered before African catfish nutrition is deemed complete:

- The proposed comprehensive use of carbohydrates in catfish diet formulation (4.4) calls for more extensive experimentation on nutrient digestibility of raw materials, particularly of plant origin.
- Use of radiolabelled substances will clarify the metabolic pathways and nutrient utilization existing under various dietary regimes.
- Further experimentation on the required dietary carbohydrate: lipid ratios and the optimum dietary fatty acid profile will confirm the previous findings and probably better elucidate the deposition of essential amino acids.

Production of such data coupled with data regarding the digestibility of various raw materials will lead to the manufacture of more balanced diets for the African catfish. Cost-effectiveness of such diets will have to be *in situ* tested, in order to finalize the optimum ones for each production system.

Table 51. *Composition and expected proximate analyses of catfish farm made feeds used in the Asian and Pacific region*[1]

Nutritional profile	Diet 1 [2]	Diet 2 [2]	Diet 3 [2]	Diet 4 [3]	Diet 5 [4]	Diet 6 [4]	Proposed diet
Cassava chips	-	-	10.00	-	-	-	-
Chicken viscera	-	-	-	-	-	-	15.00
Corn Gluten	-	-	-	-	-	-	5.00
Fishmeal	-	-	15.00	40.00	56.00	15.00	-
Fish Oil	-	-	2.00	-	4.00	-	1.50
Leuceana leaf meal	-	-	5.00	-	-	3.00	-
Maize meal	-	-	22.00	-	-	24.00	1.00
Mustard oil cake	-	-	-	40.00	-	-	14.00
Rice bran	20.00	40.00	15.00	20.00	12.00	10.00	-
Soyabean meal	-	-	30.00	-	12.00	45.00	35.00
Soya oil	-	-	-	-	-	-	1.50
Starch flour	-	-	-	-	14.00	-	5.50
TrashFish	80.00	60.00	-	-	-	-	20.50
Vitamin premix	-	-	-	-	1.00	1.00	-
Mineral premix	-	-	1.00	-	1.00	2.00	1.00
Crude Protein	54.35	43.40	29.47	25.96	40.32	32.06	40.68
Crude Lipid	11.12	11.05	10.54	6.65	12.92	8.78	10.78
NFE	12.00	20.61	43.39	50.98	27.38	44.70	39.04
Ash	18.64	18.08	9.79	12.38	15.60	9.08	7.26
Crude Fibre	3.87	6.85	6.80	4.02	3.77	5.37	2.22
Gross Energy kJ. g^{-1}	16.12	16.52	17.30	11.50	19.19	16.72	14.07
Arginine [1]	5.07	5.22	6.34	10.11	6.60	7.19	7.10
Lysine	5.65	5.62	5.85	9.38	7.28	6.68	6.17
Methionine+Cystine	3.02	3.05	3.18	5.76	3.82	3.54	3.74
Phenylalanine+Tyrosine	4.74	5.17	7.32	10.22	7.21	8.37	7.46
Threonine	2.72	2.82	3.67	5.84	4.06	4.18	3.91
Tryptophan	1.04	1.04	1.17	1.06	1.13	1.39	1.17
Protein: Energy ratio mg Prot. kJ^{-1}	33.72	26.27	19.89	18.37	21.00	19.18	28.91
Price in $ US. Kg^{-1}	0.29	0.26	0.53	0.51	0.95	0.64	0.43

1. Prices are estimated on referred prices adding a 5% inflation rate per annum (US Dpt of Commerce 1997).
Composition of raw materials as shown in Table 50. Amino Acids expressed as % of Crude Protein
2. Jantrarotai & Jantrarotai (1993) 3. Zaher & Mazid (1993) 4. Somsueb (1993)

APPENDICES

APPENDIX ONE

Indices for fish in the feeding frequency experiment.

Group 1	Initial weight (g)	Initial stocking density g. L^{-1}	Final weight (g)	SGR[1]	TGC[2]	%Weight Gain	FCR[3]
Male	115.8	2.3	175	1.58	0.026	51.12	0.49
Male	98.4	1.97	130	1.07	0.016	32.11	0.93
Female	107.2	2.14	137.68	0.96	0.015	28.44	0.96
Male	106	2.12	138.64	1.03	0.016	30.79	0.81
Female	114	2.28	181.5	1.78	0.030	59.21	0.39
Male	39.5	0.79	49.89	0.89	0.010	26.30	0.86

Group 2	Initial weight (g)	Initial stocking density g. L^{-1}	Final weight (g)	SGR	TGC	%Weight Gain	FCR
Male	153.9	3.078	188.8	0.78	0.014	22.67	0.95
Female	83.6	1.67	104.03	0.84	0.012	24.45	1.63
Male	77.5	1.55	96.35	0.84	0.012	24.32	1.66
Female	83.8	1.67	102.3	0.76	0.011	22.07	1.70

Group 3	Initial weight (g)	Initial stocking density g. L^{-1}	Final weight (g)	SGR	TGC	%Weigh t Gain	FCR
female	70.3	1.41	94.00	1.12	0.016	33.72	1.04
female	70.7	1.41	103.80	1.47	0.021	46.82	0.74
male	71.0	1.42	105.20	1.52	0.022	48.17	0.72
male	89.2	1.78	128.38	1.40	0.021	43.92	0.63
female	147.0	2.94	209.30	1.36	0.025	42.38	0.34
female	123.8	2.47	167.38	1.16	0.019	35.20	0.56

Group 4	Initial weight (g)	Initial stocking density g. L^{-1}	Final weight (g)	SGR	TGC	%Weight Gain	FCR
female	105.5	2.11	135.0	0.95	0.0151	27.96	1.09
male	93.9	1.87	115.0	0.78	0.0118	22.47	1.52
male	96.7	1.94	108.6	0.45	0.0068	12.31	2.56
male	127.9	2.56	166.84	1.02	0.0174	30.48	0.83
female	90.3	1.81	111.56	0.82	0.0122	23.54	1.52

Group 5	Initial weight (g)	Initial stocking density g. L^{-1}	Final weight (g)	SGR	TGC	%Weight Gain	FCR
Male	74.3	1.48	102.30	1.30	0.017	37.69	1.03
Male	84	1.68	128.60	1.64	0.024	53.09	0.64
Male	56	1.12	70.40	0.88	0.011	25.72	1.99
Female	74	1.48	114.40	1.67	0.024	54.59	0.71
Male	102.7	2.05	129.55	0.89	0.014	26.15	1.07
male	110	2.20	138.50	0.88	0.014	25.90	1.01
female	173.6	3.47	221.73	0.94	0.017	27.73	0.59

Group 6	Initial weight (g)	Initial stocking density g. L^{-1}	Final weight (g)	SGR	TGC	%Weight Gain	FCR
female	131.7	2.64	158.0	0.70	0.012	19.97	1.36
female	149.8	2.99	207.0	1.24	0.022	38.18	0.62
male	153	3.06	173.3	0.48	0.008	13.27	1.76
male	122.8	2.46	149.0	0.75	0.013	21.33	2.58
female	80.8	1.61	89.9	0.41	0.006	11.26	2.54
female	95.4	1.91	132.27	1.25	0.019	38.65	0.97

[1] SGR= Specific Growth Rate
[2]TGC = Thermal Growth Coefficient
[3] FCR = Food Conversion Ratio

APPENDIX TWO

Determination of the Energy Content of Organic Carbon in Samples.

All the parameters needed for the calculation of Organic Carbon in raw materials and samples are given in the following Table

Raw material	*Bomb values as kJ.g^{-1} sample*	*Carbon as g.100g^{-1} sample*	*Carbon as g.100g^{-1} Ashed sample*	*Organic Carbon as g.100g^{-1} sample*	*Energy in kJ.g^{-1} Organic Carbon*	*Standard Deviation*
Gluten [1]	23.2	48.77	31.80	48.46	47.82	0.42
	22.8	48.31	32.35	47.75		
Zein	27.5	55.06	42.77	54.20	50.33	0.71
	25.8	52.91	44.46	51.89		
Casein	25.0	50.65	42.86	47.23	50.9	2.96
	23.8	50.89	40.12	48.77		
Gelatin	20.8	43.11	11.07	43.06	48.64	0.22
	20.7	42.57	11.43	42.51		
Fishmeal	22.9	47.69	6.39	47.01	50.81	2.97
	24.5	47.01	6.29	46.29		
Soya	19.8	46.33	2.78	46.13	42.75	0.28
	19.4	45.80	2.30	45.65		
Wheat	17.3	43.59	2.67	43.55	40.76	1.53
	18.2	43.60	1.75	43.57		
Feathermeal	25.9	52.12	1.52	52.08	51.65	2.56
	27.6	51.61	1.85	51.57		
Purified Diet 1	17.7	40.18	0.03	40.18	43.42	1.17
	18.0	42.35	0.04	42.35		
Purified Diet 2	21.7	48.76	1.83	48.69	45.55	1.11
	21.7	46.95	1.74	46.88		
Purified Diet 3	20.6	46.79	1.24	46.74	45.00	1.32
	21.5	46.86	1.19	46.83		
Commercial Diet 1	23.0	50.03	1.26	49.89	46.47	0.48
	23.9	51.24	1.15	51.12		
Commercial Diet 2	21.6	44.91	1.11	44.78	48.04	0.17
	21.4	44.74	1.09	44.62		
Average Value					47.08	3.45

1. Source of raw materials as given in 2.5

Proximate analyses of the raw materials and diets used for the determination of the Energy content

Values are expressed as $g.100g^{-1}$ on a dry matter basis [1]

	Crude Protein	*Crude Lipid*	*Crude Fibre*	*Ash*	*Carbohydrates*
Gluten [2]	78.79 (0.09)	5.56 (0.28)	1.69 (0.03)	1.48 (0.83)	12.47 (1.02)
Zein	81.38 (0.14)	3.53 (0.25)	0.16 (0.03)	2.08 (0.19)	12.84 (0.32)
Casein	92.83 (0.58)	1.42 (0.17)	None Detected	5.13 (0.53)	0.62 (0.08)
Gelatin	98.77 (0.15)	0.239 (0.07)	None Detected	0.875 (0.04)	0.11 (0.05)
Fishmeal	72.41 (1.32)	10.61 (0.55)	0.44 (0.05)	11.11 (0.77)	5.41 (0.95)
Soya	44.57 (1.73)	4.54 (0.52)	3.88 (0.19)	7.04 (0.19)	39.94 (2.13)
Wheat	11.86 (0.55)	1.93 (0.08)	1.74 (0.14)	1.30 (0.04)	83.16 (0.65)
Feathermeal	89.86 (0.76)	6.18 (0.02)	1.30 (0.34)	1.93 (0.21)	0.72 (0.42)
Purified Diet 1	0.26 (0.15)	10 (0.14)	78.93 (0.41)	5.85 (0.16)	4.96 (0.41)
Purified Diet 2	53.79 (2.02)	10.63 (0.16)	5.13 (0.15)	3.84 (0.04)	26.59 (2.05)
Purified Diet 3	40.76 (0.27)	9.34 (0.25)	1.37 (0.02)	3.93 (0.05)	44.59 (0.56)
Commercial Diet 1	53.23 (0.16)	10.28 (0.08)	5.01 (0.17)	10.55 (0.16)	20.92 (0.27)
Commercial Diet 2	41.14 (0.12)	10.77 (0.14)	5.01 (0.25)	15.03 (2.76)	28.06 (2.41)

1.Numbers in parentheses represent Standard Deviation 2. Source of raw materials as referred in 2.5

Amino acid profile of some raw materials used in these experiments

Values expressed as $g.100g^{-1}$ Crude Protein on a dry matter basis [1]

Essential Amino Acids	*Fishmeal*	*Casein*	*Gelatin*	*Gluten*	*Zein*
L-Arginine	4.88 (0.26)	3.28 (0.18)	7.86 (1.38)	3.69 (0.70)	1.98 (0.78)
L-Cystine (NEAA)	0.48 (0.06)	0.58 (0.03)	0.58 (0.04)	2.97 (0.87)	1.28 (0.05)
L-Histidine	1.84 (0.10)	2.50 (0.80)	1.26 (0.60)	2.31 (0.48)	1.21 (0.78)
L-Isoleucine	3.00 (0.04)	4.23 (0.75)	1.98 (0.35)	3.72 (0.56)	4.09 (0.88)
L-Leucine	5.76 (0.08)	7.18 (1.35)	3.47 (0.43)	5.56 (0.66)	16.59 (2.32)
L-Lysine	6.90 (0.18)	6.44 (0.12)	3.59 (0.15)	1.56 (0.05)	0.00
L-Methionine	1.86 (0.05)	0.67 (0.03)	0.39 (0.02)	1.86 (0.04)	1.22 (0.33)
L-Phenylalanine	3.37 (0.38)	4.15 (0.15)	2.17 (0.22)	4.69 (0.43)	6.67 (0.55)
L-Tyrosine (NEAA)	2.67 (0.04)	5.16 (0.20)	1.14 (0.03)	4.17 (0.19)	5.09 (0.23)
L-Threonine	3.48 (0.40)	4.19 (0.35)	2.24 (0.75)	2.63 (0.48)	2.71 (0.23)
L-Valine	3.93 (0.06)	6.09 (0.45)	3.09 (0.06)	4.23 (0.09)	3.37 (0.56)
Non Essential Amino Acids					
L-Alanine	5.21 (0.30)	2.92 (0.28)	8.55 (1.27)	3.05 (0.77)	9.46 (1.38)
L-Aspartic	8.03 (0.50)	7.49 (0.43)	6.60 (0.46)	3.79 (0.83)	4.81 (0.90)
L-Glutamic	10.82 (0.2)	20.75 (1.25)	10.06 (1.34)	23.94 (2.23)	19.64 (0.98)
Glycine	5.37 (0.62)	1.84 (0.03)	16.93 (4.13)	3.15 (0.53)	1.52 (0.78)
L-Proline	4.79 (1.68)	11.56 (0.98)	8.03 (2.40)	13.25 (0.99)	9.9 (1.35)
L-Serine	3.70 (2.70)	7.47 (2.01)	2.01 (1.32)	7.92 (2.02)	4.57 (1.88)
Total A.A. %	76.09(2.30)	96.50 (1.89)	79.95 (1.69)	92.49 (2.05)	94.11 (2.23)

1. Source of raw materials as given in 2.5. The analytical methodology employed for the amino acid determination as described in 2.6. Number in parentheses represent Standard Deviation (n=2)

APPENDIX THREE

Determination of Available Carbohydrates

This method is a modification of the method of McCready *et al.* (1950) and based also on the methodology employed by Morris (1948); Seifter *et al.* (1950); Hassid & Abraham (1957); Good *et al.* (1933); Clegg (1956).

1.Reagents

Perchloric acid 52%

Ethyl alcohol 80%

Anthrone-Sulphuric acid 0.2% reagent (0.2 g anthrone-BDH/MERCK Prod No 27246- in 100 ml concentrated sulphuric acid)

Alternatively (especially if one is dealing with soft materials, i. e. purified diets) a more diluted Anthrone-Sulphuric acid 0.1% reagent can be used (Clegg 1956)

Glucose Standard (SIGMA Cat. No 16-300)

2.Preparation of the glucose standards for the soluble sugars (all these quantities are based on the assumption that 1ml of glucose standard equals 1mg of glucose):

Blanks: auto zero the spectrophotometer with a deionized water blank
B/ 0.00mg Glucose standard: 0.75ml water + 4.25ml of 80% ethanol
1/ 0.1mg Glucose standard: 0.1ml of the Glucose standard + 0.735ml of water + 4.165ml of 80% ethanol
2/ 0.2mg Glucose standard: 0.2ml of the glucose standard +0.72ml of water + 4.080ml of 80% ethanol
3/ 0.3mg Glucose standard: 0.3ml of the glucose standard + 0.705ml of water + 3.995ml of 80% ethanol
4/ 0.4mg Glucose standard: 0.4ml of the glucose standard + 0.69ml of water + 3.910ml of 80% ethanol
5/ 0.5mg Glucose standard: 0.5ml of the glucose standard + 0.675ml of water + 3.825ml of 80% ethanol
6/ 0.6mg Glucose standard: 0.6ml of the glucose standard + 0.660ml of water + 3.740ml of 80% ethanol
7/ 0.7 mg Glucose standard: 0.7 ml of the glucose standard + 0.645ml of water + 3.655ml of 80% ethanol
8/ 0.8mg Glucose standard: 0.8ml of the glucose standard + 0.630ml of water + 3.670ml of 80% ethanol
9/ 0.9mg Glucose standard: 0.9ml of the glucose standard + 0.615ml of water + 3.485ml of 80% ethanol

10/ 1.0mg Glucose Standard: 1.0ml of the glucose standard + 0.6ml of water + 3.400ml of 80% ethanol

3.Remove and estimate the soluble sugars: a 30mg sample (containing approximately 12mg of total CHO) is put in a 50ml centrifuge tube. 0.5ml of 80% heated (60^0 C) ethanol is added to wet the ground material so as to prevent clumping. 4.5ml of water is added and the mixture is thoroughly stirred. 25ml of hot 80% ethanol is added again, the mixture is stirred again , let to stand for 5minutes and centrifuged (3000rpm for 5 min).
The alcoholic solution is decanted to a 200ml flask and the ethanol extraction is repeated twice more, always decanting the alcoholic solution to the same flask. The volume of this solution (b) is accurately recorded.
The above solution (in the 200ml flask containing the soluble sugars) is filtered by suction and the first 5ml of the filtrate is discarded. A 5ml aliquot of the filtrate is taken into a 20ml glass borosilicate test tube and 10ml of the anthrone reagent is added (while keeping the tube in a cold water bath).
10ml anthrone reagent is added to the standards and blanks as well (also in the cold bath).
All the tubes are heated to 100°C for 7.5min. Tubes are cooled rapidly to 25°C in a water bath and the optical density is read on a spectrophotometer, at 620 nm.
If "a" is the amount of total soluble sugars (in mg) of the initial sample, "b" is the volume of the alcoholic solution in the 200ml flask, "c" is the final quantity (in mg) of soluble sugars determined by the spectrophotometer, then a%= (c x b) / (d x 0.05) where d = sample size in mg .

4. Preparation of blanks and standards for the starch determination:
Blanks: auto zero the spectrophotometer towards net deionized water blank
B/ 0.00mg Glucose standard: 4.675ml H_2O: 0.325ml perchloric acid
1/ 0.05mg Glucose Standard: 0.05ml glucose standard + 4.625 H_2O +0.325ml perchloric acid 52%
2/ 0.075mg Glucose Standard: 0.075ml glucose standard +4.60ml H_2O +0.325 ml perchloric acid 52%
3/ 0.1mg Glucose Standard: 0.1ml glucose standard +4.575ml H_2O +0.325ml perchloric acid 52%
4/ 0.125mg Glucose Standard: 0.125ml glucose standard +4.550ml H_2O + 0.325 ml perchloric acid 52%
5/ 0.15mg Glucose Standard: 0.15ml glucose standard + 4.525ml H_2O + 0.325 ml perchloric acid 52%

6/ 0.175mg Glucose Standard: 0.175mg glucose standard + 4.5ml H_2O + 0.325 ml perchloric acid 52%
7/ 0.2mg Glucose Standard: 0.2ml glucose standard + 4.475ml H_2O +0.325ml perchloric acid 52%
8/ 0.225mg Glucose Standard: 0.225ml glucose standard +4.450ml H_2O +0.325ml perchloric acid 52%
9/ 0.25mg Glucose Standard: 0.25ml glucose standard + 4.425ml H_2O + 0.325 ml perchloric acid 52%
10/ 0.275mg Glucose Standard: 0.275ml glucose standard + 4.400ml H_2O + 0.325 ml perchloric acid 52%
11/ 0.3mg Glucose Standard: 0.3ml glucose standard + 4.375ml H_2O + 0.325 ml perchloric acid 52%

5. Extraction and hydrolysis of starch: The sugar free residue is then treated with 5ml of water and stirred with a glass rod. At the same time 6.5ml of 52% perchloric acid are added, while keeping the centrifuge tube in an ice bath. Stirring is continued for 5 min and then occasionally for the next 15min. 20ml of water are added and centrifuged again.

The aqueous solution (which contains the starch) is filtered on a dry filter paper or sintered glass (by suction) to a 100ml flask, and the extraction of the residue is repeated with the same quantities (5ml of water and 6.5ml of the dilute perchloric acid reagent) for about 25min (in the ice bath). The contents of the tube are washed-filtered again into the 100ml flask containing the first extract and made up to 100ml with distilled water.

6. Determination of starch: A 10ml aliquot of the filtered starch solution is taken and 10ml of distilled water are added.

5ml of this last dilution are transferred into a 20ml glass borosilicate test tube and 10ml of the anthrone reagent is added (keeping the borosilicate tubes in a ice bath) (Seifter *et al.* 1950). The anthrone reagent is added to the blanks and standards as well. All the tubes are heated at 100°C for 7.5min. Tubes are cooled rapidly to 25°C in a water bath and the optical density is read in a spectrophotometer, at 620 nm. Convert the glucose readings (mg) into starch multiplying by 0.9

If the % of starch in the initial sample is "a", the final amount determined by the spectrophotometer, is "c", and "d" is the sample size then

$a\% = (100 \times c) / (0.025 \times d)$

7.Total carbohydrates of the sample: Add the two previous results (total soluble sugars + total starch).

"Available carbohydrate" determinations[1]

Nutritional profile	*Purified Diet 1*	*High CHO Purified Diet*	*Zein Purified Diet*
Casein	20.0	23.0	21.70
Gelatin	19.5	21.0	4.55
Gluten	-	4.0	5.60
Zein	-	4.0	32.45
Fishmeal	-	-	-
Soya	-	-	-
Wheat	-	-	-
Dextrin	43.0	38.0	21.7
α- Cellulose	-	3.0	1.75
Carboxymethylcellulose	2.5	1.0	0.7
Vegetable Oil [2]	5.0	-	3.5
Fish Oil [2]	4.0	-	3.5
Vitamin premix [3]	2.0	2.0	1.4
Mineral premix [3]	4.0	4.0	2.8
Dry matter	96.13 (0.2)	91.90 (0.06)	97.27 (0.33)
Crude Protein	34.56 (0.5)	49.29 (0.25)	56.72 (0.16)
Crude Lipid	10.39 (0.14)	1.02 (0.32)	7.56 (0.07)
Ash	4.24 (1.10)	4.74 (0.25)	4.86 (0.05)
Crude Fibre	6.13 (1.16)	7.70 (0.30)	2.03 (0.32)
Determined CHOs	42.22 (3.80)	37.35 (0.26)	22.25 (1.003)
Expected NFE	44.68 (0.23)	37.25 (1.18)	28.83 (2.06)
Gross Energy	18.77 $kJ.gr^{-1}$ (0.03)	21.7 $kJ.gr^{-1}$ (0.08)	21.54 $kJ.gr^{-1}$ (0.06)

1. Percentage on a Dry Matter basis. Types of raw materials used are described in 2.5. Numbers in parentheses represent Standard Deviation (n=2).
2. The fatty acid profile of the oils used is given in Table 6.
3. The composition of Vitamin and Mineral premixes used are described in Tables 4 and 5.

Nutritional profile[1]	*Commercial ingredients Diet 1*	*Commercial ingredients Diet 2*
Casein	-	-
Gelatin	-	-
Gluten	-	5.5
Zein	-	0.5
Fishmeal	24.0	26.0
Soya	40.0	15.0
Wheat	22.0	15.0
Dextrin	1.0	21.0
α- cellulose	-	3.0
Carboxymethylcellulose	2.0	2.0
Vegetable Oil [2]	3.0	3.0
Fish Oil [2]	2.0	3.0
Vitamin premix [3]	2.0	2.0
Mineral premix [3]	4.0	4.0
Dry matter	94.74 (0.4)	95.57 (0.09)
Crude Protein	49.29 (0.7)	34.25 (0.25)
Crude Lipid	8.37 (0.14)	6.13 (0.32)
Ash	10.82 (1.08)	9.32 (0.25)
Crude Fibre	3.75 (1.25)	4.49 (0.30)
Determined CHOs	25.78 (7.78)	43.35 (4.18)
Expected NFE	27.77 (3.25)	45.81 (3.18)
Gross Energy	18.56 $kJ.gr^{-1}$ (0.03)	18.16 $kJ.gr^{-1}$ (0.08)

1. Percentage on a Dry Matter basis. Types of raw materials used are described in 2.5. Numbers in parentheses represent Standard Deviation (n=2).
2. The fatty acid profile of the oils used is given in Table 6.
3. The composition of Vitamin and Mineral premixes used are described in Tables 4 and 5.

Estimation of the values by the t-test:

The x^2 chi square goodness of fit test gave the following results:

42.22	22.25	37.35	25.78	43.35	Observed
44.68	28.83	37.25	27.77	45.81	Expected

Degrees of freedom: 5-1=4

$X^2 = 0.144183+1.505287+0.000316+2.312854+0.130781=4.093421$

$0.25 < P(X^2 >= 4.093421) < 0.5$

Therefore the null hypothesis that the observed values do not deviate significantly from the expected is accepted.

Conclusion: the available CHO method seems to have repeatability and success, especially when dealing with purified diets

APPENDIX FOUR

Determination of Liver Glycogen.

This is a method described by Seifter *et al.* (1950) and Hassid & Abraham (1957). It is actually a modification of the method originally described by Good *et al.* (1933) for tissues of relatively high glycogen content. The method has the advantages of brevity and simplicity as compared with other procedures since it eliminates the necessity of both glycogen precipitation and hydrolysis, and requires few and simply prepared reagents.

1.Reagents

30% KOH solution

97.7%ethanol

Anthrone-Sulfuric acid 0.2% reagent (anthrone BDH/MERCK Prod No 27246)

Glucose Standard (SIGMA Cat. No 16-300)

2.Preparation of the glucose standards for the soluble sugars (all these quantities are based on the assumption that 1ml of glucose standard equals 1mg of glucose)

Blank 0.00mg glucose standard: 0.03ml KOH+0.04ml ethanol+ 4.93mlH2O

S1 0.0250mg Glucose standard: 0.0250ml Glucose Standard+ 0.03mlKOH+ 0.045ml ethanol+ 4.9ml H2O

S2 0.05mg Glucose Standard: 0.05ml Glucose Standard +0.03 mlKOH +0.045ml ethanol+4.875ml H2O

S3 0.075mg Glucose Standard: 0.075ml Glucose Standard + 0.03mlKOH+ 0.045ml ethanol+4.85ml H2O

S4 0.1mg Glucose Standard: 0.1ml Glucose Standard+ 0.03mlKOH+ 0.045ml ethanol+ 4.825ml H2O

S5 0.125mg Glucose Standard: 0.125ml Glucose Standard+ 0.03mlKOH + 0.045ml ethanol+ 4.8ml H2O

S6 0.150mg glucose Standard: 0.150ml Glucose Standard+0.03ml KOH+ 0.045ml ethanol+ 4.775ml H2O

S7 0.175mg glucose Standard: 0.175ml glucose Standard+0.03ml KOH+ 0.045ml ethanol+ 4.75ml H2O

S8 0.2mg glucose Standard: 0.2ml glucose Standard +0.03ml KOH + 0.045 ml ethanol +4.725ml H2O

S9 0.225mg glucose Standard: 0.225ml glucose Standard + 0.03 ml KOH + 0.045ml ethanol + 4.7ml H2O

3.Digestion of the tissue sample : 300mg of fresh liver sample is placed into a centrifuge tube containing 3ml of 30% KOH.

Digest by heating the tube for 20min in a boiling water-bath, cool the digest, and add 3.75ml of 97.7% ethanol. Bring to boil in a hot water bath, cool again and centrifuge for 15min at 3000rpm. Decant the supernatant and filter to a graduated cylinder.

Dilute to 250ml.

Take an aliquot of 5ml.

4.Anthrone sulphuric acid reaction: While keeping in a cold bath add 10ml of the anthrone reagent in samples, blanks and standards as well. The tubes are then covered and heated for 7.5min in a boiling water bath. Cool again and read at 620 nm. Multiply by 50 (dilution factor) in order to extrapolate the readings to the total sample size. Divide the final quantity of glucose by the factor 1.11 to convert to glycogen (or multiply by 0.92 / Hassid & Abraham 1957). Divide by the sample size "d" in order to have the final % of glycogen in the sample.

APPENDIX FIVE

Initial weights of all the individuals of the experimental population (4.2)

Group1/ 32:10	*Group2/ 32:16*	*Group3/ 40:10*	*Group4/ 40:16*	*Group9/Control*
Male 236.5	Male 172.4	Male 90.7	Male 116	Male 148.87
Male 173	Male 111.4	Female 243.3	Male 236	Male 163.07
Male 117.5	Female 119	Male 71.9	Male 144	Male 125.18
Female 154.72	Female 126.3	Male 113	Male 168.1	Male 256
Female 225	Male 119	Male 115.8	Female 87.9	Male 142
Male 205.5	Male 214	Female 145.8		Female 176.2
Male 155	Female 179			Female 180
				Female 160.88
Group5/ 46:10	***Group6/ 46:16***	***Group7/ 46:10***	***Group8 / 46:16***	
Male 176	Male 245	Female 144.5	Male 99.2	
Female 95	Female 131	Male 81	Male 150.7	
Female 154	Female 125	Female 85	Female 177	
Male 253	Male 160	Male 178.2	Male 108.8	
Female 219	Male 172			
	Male 84			

Bartlett's test for homogeneity of variances among the treatments of the Protein: Energy experiment

	Group 1/ 32:16	*Group2/ 32:10*	*Group3/ Control*	*Group4 / 40:10*	*Group 5 / 40:16*	*Group 6 / 46:10*	*Group 7 / 46:16*	*Group 8 / 46:16*	*Group 9 / 46:10*
Average weight	148.728	181.031	169.02	129.966	150.4	122.175	133.925	152.833	179.4
Ss_i	9401.09	11079.381	10897.34	18552.773	12771.2	6714.567	3973.94	14902.8	14765.2
v_i	6	6	7	5	4	3	4	5	4
ΣSs_i	103058.36								
Σv_i	44								
s_i^2	1566.84	1846.56	1556.7	3710.55	3192.8	2238.18	993.486	2980.56	3691.3
$Logs_i^2$	3.19502	3.26636	3.1922	3.56943	3.5041	3.34989	2.99716	3.47429	3.5671
$v_i Logs_i^2$	19.1701	19.5981	22.345	17.8471	14.016	10.0496	11.9886	17.3714	14.268
$1/ v_i$	0.16666	0.16666	0.1428	0.2	0.25	0.33333	0.25	0.2	0.25
$\Sigma v_i Logs_i^2$	146.656								
$\Sigma 1/ v_i$	1.95952								
s_p^2	2342.23								
$\log s_p^2$	3.36963								
B=	3.70119								
C=	1.09222								
Bc=	3.38866								
0.9<P<0.95									

Because $x^2_{0.05,\ 8} = 15.507$ we do not reject H_0 and conclude that all the sample variances estimate the same population variance.

Comparison of initial surface stocking density as total tank biomass per square centimetre

Groups	*Stocking Density ($g.cm^{-2}$)*
Gr1 / 32:10	0.57 $^{a\ b}$ ±0.06
Gr2 / 32:16	0.47 $^{a\ b}$ ±0.01
Gr3 / 40:10	0.35 $^{a\ b}$ ±0.001
Gr4 / 40:16	0.34 $^{a\ b}$ ±0.15
Gr5 / 46:10	0.40 $^{a\ b}$ ±0.25
Gr6 / 46:16	0.41 $^{a\ b}$ ±0.05
Gr7 / 46:10	0.22 ^{b} ±0.02
Gr8 / 46:16	0.24 ^{b} ±0.20
Gr9 Control	0.61 ^{a} ±0.23

Values of water quality parameters of the experimental system (4.2.2)

Nitrites (NO2) : Average value 0.2 ppm (SD 0.05)

Minimum 0.12 ppm Maximum 0.29 ppm

Nitrates (NO3) : Average value 19.28 ppm (SD 4.84)

Minimum 12.77ppm Maximum 28.16 ppm

Unionized ammonia : Average value 0.75 ppm (SD 0.06)

Minimum 0.65 ppm Maximum 0.86 ppm

pH : Average value 7.07 (SD 0.05)

Minimum 6.97 Maximum 7.12

APPENDIX SIX

Performance indices estimated by two different methodological approaches (4.3)

Performance indices for the 40 day period							
	Control	Gr6 / 46:16	Gr5 / 46:10	Gr4 / 40:16	Gr3 / 40:10	Gr2 / 32:16	Gr1 / 32:10
%WeightGain	39.81	37.47	28.11	28.93	27.77	20.18	23.94
FeedIntake:FCR / individual data	81.46	33.74	61.39	50	58.71	37.88	25.86
FeedIntake:FCR / cumulative data	84.96	75.24	48.70	55.75	51.92	39.26	33.58
Performance indices for the 36 day period							
	Control	Gr6 / 46:16	Gr5 / 46:10	Gr4 / 40:16	Gr3 / 40:10	Gr2 / 32:16	Gr1 / 32:10
%WeightGain	31.31	26.43	30.97	25.61	22.81	22.51	21.54
FeedIntake:FCR / individual data	58.49	42.23	58.21	47.34	45.92	36.55	31.62
FeedIntake:FCR / cumulative data	66.93	50	64.53	51.95	45.77	37.27	41.58
Performance indices for the 76 day period							
	Control	Gr6 / 46:16	Gr5 / 46:10	Gr4 / 40:16	Gr3 / 40:10	Gr2 / 32:16	Gr1 / 32:10
%WeightGain	67.71	50.6	46.35	42.95	39.36	35.9	31.69
FeedIntake:FCR / individual data	87.5	56.55	55.47	45.97	43.37	32.86	24.86
FeedIntake:FCR / cumulative data	75.78	64.71	60.75	53.02	54.23	37.66	37.33

Comparison of various best fit regressions for the relation between live fish weight and feed consumption (4.3.6)

1. Method. LOGARITHMIC Dependent variable. CONSUMPTION

List wise Deletion of Missing Data
Multiple R 0.75868
R Square 0.57560
Adjusted R Square 0.57108
Standard Error 0.00216

Analysis of Variance:

	DF	Sum of Squares	Mean Square
Regression	1	0.00059288	0.00059288
Residuals	94	0.00043714	0.00000465

F = 127.48860 Signif F = 0.0000

---------- Variables in the Equation ----------

Variable	B	SE B	Beta	T	Sig T
WEIGHT	-0.007663	0.000679	-0.758682	11.291	0.0000
(Constant)	0.049099	0.003556		13.807	0.0000

Y=0.049099-0.0077*ln (X)

2. Method. POWER Dependent variable. CONSUMPTION
List wise Deletion of Missing Data
Multiple R 0.77083
R Square 0.59418
Adjusted R Square 0.58986
Standard Error 0.23589

Analysis of Variance:

	DF	Sum of Squares	Mean Square
Regression	1	7.6584551	7.6584551
Residuals	94	5.2306978	0.0556457

F = 137.62882 Signif F = 0.0000

---------- Variables in the Equation ----------

Variable	B	SE B	Beta	T	Sig T
WEIGHT	-0.870993	0.074244	-0.770830	-11.732	0.0000
(Constant)	0.803131	0.312412		2.571	0.0117

$Y = 0.8031 * X^{(-0.871)}$

3. Method.. EXPONENTIAL Dependent variable.. CONSUMPTION
List wise Deletion of Missing Data
Multiple R 0.76225
R Square 0 .58103
Adjusted R Square 0.57657
Standard Error 0.23968

Analysis of Variance:

	DF	Sum of Squares	Mean Square
Regression	1	7.4890054	7.4890054
Residuals	94	5.4001475	0.0574484

F = 130.36061 Signif F = 0.0000

---------- Variables in the Equation ----------

Variable	B	SE B	Beta	T	Sig T
WEIGHT	-0.004574	0.000401	-0.762254	-11.418	0.0000
(Constant)	0.020741	0.001709		12.137	0.0000

Hi-Res Chart # 1:Curvefit for consump

$Y = 0.020741 * e^{(-0.0046)}$

APPENDIX SEVEN

Calculation of various parameters related to the EAA requirements

1.Calculation of the daily deposition rate of Arginine (5.3)

a. Concentration of Arginine in the carcass of the initially fasted animals (Table 42) A= 25.86 $mg.g^{-1}$ Dry Weight

b. Concentration of Arginine in the carcass of the best performing Group 7 (Table 44) B= 36.31 $mg.g^{-1}$ Dry Weight

c. Daily rate of carcass deposition of Arginine for the 40 day period C= (B-A) / 40 = 0.2613 mg Arginine.g^{-1} Dry Weight.day^{-1}

d. Correction based on the Apparent Digestibility Coefficient (Table 11) of Arginine D= (C * 100) / 98.21 = 0.266 mg Arginine.g^{-1} Dry Weight. day^{-1} or 0.0266 g Arginine.100 g^{-1} Dry weight.day^{-1} (Table 45)

2. Conversion of the daily deposition rate of Arginine to EAA requirements

a. Feed intake E = 1.348 g Dry Crude Protein consumed.100 g^{-1} Dry Weight.day^{-1}

b. Arginine requirement as % of the Dietary Crude Protein (DM basis; Table 46) F= [D/10)*100]/E= (D *10) / E = 1.973 g Arginine.100 g^{-1} Crude Protein

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