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SUITABILITY OF SELECTED RAW MATERIALS AND BY-PRODUCTS IN FORMULATED FEEDS FOR NILE TILAPIA *Oreochromis niloticus* AND AFRICAN CATFISH *Clarias gariepinus.*

By

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This thesis is dedicated to the everlasting memory of my dearest father Abdel-Moeze and to my wonderful mother who has supported my studies throughout my school and college years. Also my loving family, my wife Awatef and my dearest children (Islam, Omar and Motaz)

ABSTRACT

Suitability of selected raw materials and by-products in formulated feeds for Nile tilapia *Oreochromis niloticus* and African catfish *Clarias gariepinus*.

Abdel-Wahab A. Abdel-Warith

The current status of global aquaculture production was reviewed with a special emphasis on Africa and in particular Egypt. The main species of interest in this study were tilapia *Oreochromis niloticus* and African catfish *Clarias gariepinus* which are gaining popularity and are of considerable importance in the market of farmed fish in this continent and of economic relevance to Egypt and other Middle Eastern countries. Research was principally directed to establishing the suitability of specific feed ingredients and materials that could be included in balanced diets for both species. Various animal and plant by-products were selected to evaluate their nutritional value for either species.

The experimental protocols, materials and methods and techniques employed are described for nutritional investigations with tropical freshwater fish. These included the various parameters assessed in the growth and digestibility studies relevant to the species in question. These include Specific Growth Rates (SGR), Feed Conversion Ratios (FCR), Protein Efficiency Ratio (PER) and Apparent Net Protein Utilisation (ANPU).

An initial investigation to determine the coefficients of digestibility of protein, amino acids and energy was first undertaken using tilapia as the model warmwater fish species. This investigation was able to provide useful data and information as a prelude for successive growth trials with both tilapia and catfish. Fishmeal, soyabean meal, corn gluten meal, poultry by-products including feathermeal and blood meal were all tested at a variety of inclusion levels in successive trials.

Apparent digestibility coefficients (ADC %) for tilapia fed diets containing 60% LT 94 fishmeal and 40% of each ingredient are reported. ADC of dry matter (DM) and protein (CP) and energy (E) for the reference fishmeal diet were 83.99 DM; 92.60 CP; and 93.31E respectively. For each test ingredient, these values were as follows; 1- PBM (56.99 DM; 69.30 CP & 73.47 E), 2- Feathermeal (54.09 DM, 45.53 CP & 49.11E), 3- Blood meal (76.13 DM; 85.79 CP and 75.96 E), 4- Solvent extracted soyabean meal (85.83 DM; 93.46 CP & 82.16 E), 4- Full fat soyabean meal (75.86 DM; 86.99 CP & 74.84 E).

The amino acid availability coefficients reflected the same trends as protein digestibility, and these varied from >87% on average for the essential amino acids in fishmeal, 83% for maize gluten and 85% for solvent extracted soyabean meal with an average of 63% for feathermeal and only 61% for poultry meat meal.

The importance of plant protein sources and especially soyabean meal was the focus of a complete nutritional study with juvenile tilapia, The influence of full fat soyabean meal (FFSB) inclusion on growth performance, feed utilisation and the gastrointestinal digestive enzymes was also measured in this experiment. It was found that soyabean meal levels above 50% could reduce growth performance and adversely affect gut enzyme activities. Tilapia fed a series of diets with FFSB (58, 63 and 63% + DL-methionine did not perform as well as the control group. SGR values ranged between 2.42 to 2.12, and ANPU between 39.41-34.46. Supplementation of the diet with

methionine did not restore performance. Hepatic trypsin and amylase enzyme activity was affected with FFSB (from 12.64-1.43 Units and 4.99-2.76 Units respectively). No affects were detected on general proteolytic activity for stomach, intestine and liver.

For studies with African catfish, it was first necessary to assess the different grades of fishmeal that could be employed in suitable reference diets for this species. A Poultry by-product meal (PBM) was further evaluated as a fishmeal replacement source (0-100%) for this species.
Catfish fed dry and wet diets of two types of fishmeal showed significant differences in growth performance. Catfish fed dry diets performed better than those receiving wet diets for both LT94 and white fishmeal sources. SGR were (2.80 and 2.75 dry) and (2.46 and 2.57 wet). FCR (0.97 and 0.80 dry) and (1.30, 1.30 wet), ANPU (41.85, 52.94 dry) and (31.43, 30.9 wet) for LT94 and White fishmeal respectively.
The PBM fed catfish showed significant differences in weight gain and feed utilisation. SGR was between 3.57 to 2.83, FCR between 1.61 to 2.25 and ANPU fell from 28.90 to 18.82 for groups' fed the control fishmeal diet towards the maximum level of PBM substitution. Histological examination of liver tissue showed alterations in hepatic morphology with respect to sinusoids and fat accumulation for catfish fed higher amounts of PBM.
A restricted inclusion of up to 40% poultry by-product meal could therefore be suggested for practical diet formulations.

Further investigations were undertaken to assess the potential for either maize gluten meal (MG) or soyabean meal as substitute protein sources for the African catfish. Catfish fed higher inclusions of MG displayed SGR's ranging between 5.28 to 2.79, FCR between 0.81 to 1.53 and ANPU values from 52.33 to 24.99%. All lower performance data were obtained for 75% MG substitution of LT94 fishmeal protein. Further histological examination of liver tissue revealed alterations in hepatic structure associated with higher levels of MG. It was suggested that no more than 25% substitution of fishmeal with maize gluten meal is feasible under the present conditions.
In a separate study, catfish fed diets containing different levels of FFSB (58, 63 and 63% + DL-methionine) at the expense of fishmeal (LT94), showed significant differences in weight gain. SGR ranged between 3.11 to 2.78, FCR 0.82-0.83 and ANPU between 54.48 to 48.60. Also trypsin activities for intestine ranged between 2.75 to 1.71 Units, liver 1.37 to 1.05 Units and stomach 4.09 to 2.29 Units of activity for increasing levels of FFSB. Hepatic amylase was also reduced from 4.49 to 2.46 Units. General proteolytic activities however, did not show any significant differences between catfish fed different levels of FFSB for the stomach, intestine and liver.

The conclusions from each of the nutritional trials were considered and comparisons between the response of tilapia and catfish were made. The advantages of plant based protein concentrates was stressed due to the problems currently existing for animal sources and the expense of fishmeal. There were many similarities for the tilapia and catfish and it would seem that both fish species could greatly benefit from improved diet formulations that may meet with their nutritional requirements whilst minimising cost of production.
A future strategy of research is presented that includes further work to identify more feed ingredients for potential use in these species.

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AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.

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PUBLICATIONS

A. A. Abdel-Warith, P. M. Russell & S. J. Davies. (2001). Inclusion of a commercial poultry by-product meal as a protein replacement of fishmeal in practical diets for African catfish, *Clarias gariepinus*. *Aquaculture Research*, 32 (Supplement 1), 296-305 (published).

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A. A. Abdel-Warith & S. J. Davies. Nutrient digestibility coefficients and amino acid avialability of some plant and animal proteins for Nile tilapia *Oreochromis niloticus*. (In preparation for submission).

A. A. Abdel-Warith & S. J. Davies. Influence of dietary inclusion of full-fat soybean meal on growth and digestive enzyme activity for Nile tilapia *O. niloticus* (In preparation for submission).

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The Ninth International Conference on Nutrition and Feeding in Fish, Miyazaki, Japan, May 2000. Poster presented: Optimum inclusion levels of poultry by product meal as a protein concentrate for African catfish *Clarias gariepinus* in practical diets.

Signed Date

Abdel-Wahab 3/5/2002

Abdel-Wahab A. Abdel-Warith

CHAPTER 1

GENERAL REVIEW AND INTRODUCTION

CHAPTER 1

1. GENERAL REVIEW AND INTRODUCTION

1.1. Status of world aquaculture

Aquaculture contributes an increasingly important role in world fishery production. In 1990 this contributed over 10% of total landed fish, 15.6% of total landed crustaceans, 37% of total landed molluscs, and 73.5% of total landed aquatic plants, with an estimated value of US$ 26.5 billion (FAO, 1993). More recently, Tacon (2002) reported that according to the latest statistical information available from FAO more than half of total global aquaculture production in 1999 revealed that finfish (21.46 million metric tones (mmt) or 50.2% total production), followed by molluscs (10.13mmt or 23.7%), aquatic plants (9.46mmt or 22.1%), crustaceans (1.58mmt or 3.7%), and miscellaneous aquatic animals (0.13mmt or 0.3%) accounted for this production. Global data showed that finfish and crustacean aquaculture production increased by 96% from 4,66 to 9,13 mmt between 1984 and 1990, representing an average annual growth rate of 16% per year. However, it should also be stated that the growth of the aquaculture sector has reduced somewhat since the mid eighties, with a modest increase of 8.0% and 6.1% reported between 1984 and 1990 for total fish and crustacean aquaculture production.

Nevertheless, aquaculture production has shown impressive growth over the last few decades. Fish farming probably has a 4,000-year history, but it is only in the last 50 years that it has developed into an important worldwide industry. Figure 1.1 displays aquaculture production development from 1988 to 1997 (FAO 1999).

In 1999, FAO (Food and Agriculture Organization of the United Nations) statistics are available that show 28.808 mmt of fish and shellfish were producted in 1997 (Figure 1.1).

Recently, Tacon (2002) reported that the latest statistical data from FAO, total aquaculture production in 1999 was reported as 42.77 million metric tonnes (mmt) by

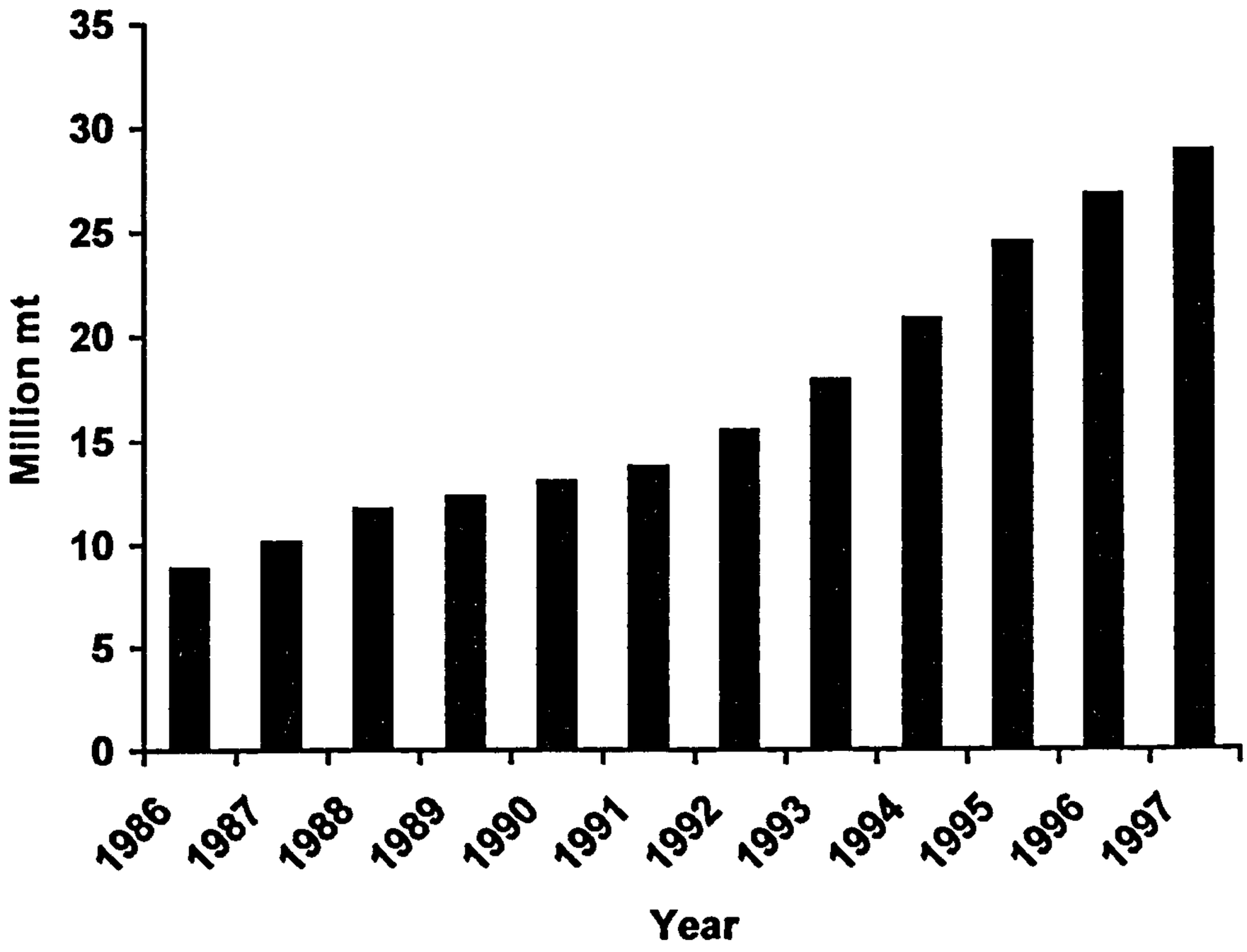

Figure 1.1 World Aquaculture production development from 1988 to 1997 (FAO, 1999).

weight and estimated at US $ 53.56 billion, with production increasing by 8.9% by weight since 1998 (compared with 6.9% for wild capture fisheries). Currently aquaculture products represent 31.3% of total global fisheries landings by weight. According to the region over 90.9% of total production of aquaculture was realized within the Asian region (38.89mmt) followed by Europe (2.10mmt or 4.9%), North America (0.73mmt or 1.7%), South America (0.63mmt or 1.5%), Africa (0.28mmt or 0.7%) and Oceania (0.14mmt or 0.3%).

In the early years of the twentieth century, several forms of fish culture were fairly well established, such as milkfish farming in southeast Asia, carp polyculture in China, carp monoculture in Europe, tilapia culture in tropical Africa, the rearing of indigenous finfish and crustaceans in estuarine impoundments in Asia and southeast Asian coastal areas, and hatchery rearing of salmonid species mainly in North America and Western Europe. Top ten farmed finfish and crustaceans in 1999 were, silver carp (3.38mmt, production increase by 2.2% since 1998), grass carp (3.16mmt, production increase by 9.2%), common carp (2.56mmt production increase by 6.8%), bighead carp (1.61mmt, production increase by 1.7%), crucian carp (1.24mmt, production increase by 19.6%), Nile tilapia (0.89mmt, production increase by 13.8%), Atlantic salmon (0.797mmt, production increase by 15.9%), rohu (0.767mmt, production increase by 1.9%), catla (0.636mmt, production increase by 1.1%), and the giant tiger prawn (0.576mmt, production increase by 3.9% since 1998) (Tacon, 2002).

With the exception of salmonid culture, these forms of aquaculture were generally extensive where the nutrient inputs into the system were restricted or limited to fertilizers and crude sources of foods, and yields were generally low (Lovell, 1998). Aquaculture made its greatest advancements during the latter part of the twentieth

century. New species were being continually introduced, novel technologies for more intensive production have been developed, and a large research base has been established. Commercial investment is being attracted from both the government and private commercial factors, such that aquaculture is now remarked as the fastest growing sector of agribusiness (Lovell, 1998).

Aquaculture is now recognized as a viable and profitable enterprise globally. For example, channel catfish farming in the United States has grown from almost obscurity in 1970 to an annual yield of over 223,000 tons in 1996 (USDA, 1997). Farming of penaeid (marine) shrimp, primarily in South and Central America and Asia, is the fastest growing aquaculture enterprise worldwide, supplying approximately 43% of the world's consumption. Ocean pen culture of salmon is a thriving industry in Norway and other areas of Western Europe, where it provides 90% of the salmon consumed, and in regions of North and South America as well as Australia. High value marine species such as sea bream, sea bass, turbot, and yellow tail tuna, are also being cultured on a large commercial scale in Europe and Japan. A broad range of tilapia species is produced mainly for export from tropical areas of America and Asia.

1.2.Aquaculture in Africa

At the first FAO/IDRC workshop on Research priorities for African aquaculture held in Dakar, Senegal, October 13-16, 1986, Dadzie & Oduol (1987) pointed out that the prognosis for this indicator is encouraging, but Africa had no reason to look forward to the turn of the century for a solution to its protein deficiency through a fish culture 'boom', because its contribution to global aquaculture is distressingly low. However, there has been a resurgence of interest in furthering the development of this industry,

and efforts are now directed in a number of African countries to upgrade their aquaculture programmes.

Egypt, Kenya, and Malawi, have the earliest recorded history of fish farming in Eastern Africa, dating back to the beginning of the century. Between 1940 and 1960 aquaculture also started in Rwanda, Uganda, Zambia, Zimbabwe and Tanzania.

Recently, FAO (1999) reported that aquaculture production in some Africa nations have increased i.e. production of fish and shellfish increased from 52,200mt in 1988 to 73, 454mt in 1997 for Egypt compared to South Africa, Nigeria, Zambia and Madagascar (Figure 1.2) (FAO 1999).

The main aquaculture systems in practice are; monoculture, polyculture, using tilapia as the main species, mono or polyculture of tilapia with different terrestrial systems and rice-cum-fish culture. Aquaculture research and training schemes are now conducted in universities, research, institutions and Government fisheries training colleges in most countries. The major common constraints to aquaculture development are biological, infrastructural and economic.

Chimatiro (1998) stated that aquaculture in Africa accounts for less than 2% of total domestic fish production. Although its contribution has expanded significantly from 59,000mt in 1985 to 85,000mt in 1990, the estimated potential is 3.5mmt per year. Of the 20 major species cultured, only Nile tilapia, African catfish and common carp are farmed throughout Africa.

The leading fish culture producers, Cote d'Ivoire, Egypt, Kenya, Nigeria, South Africa, Tunisia and Zambia, accounted for more than 95% of production in 1990. Africa contributes only 0.2% towards the world aquaculture production, and this is from only some of the countries, notably Kenya, Madagascar, Nigeria, Zambia and South Africa, such that in 1994, sub-Saharan Africa produced only 33,000 tonnes of fish (FAO 1997).

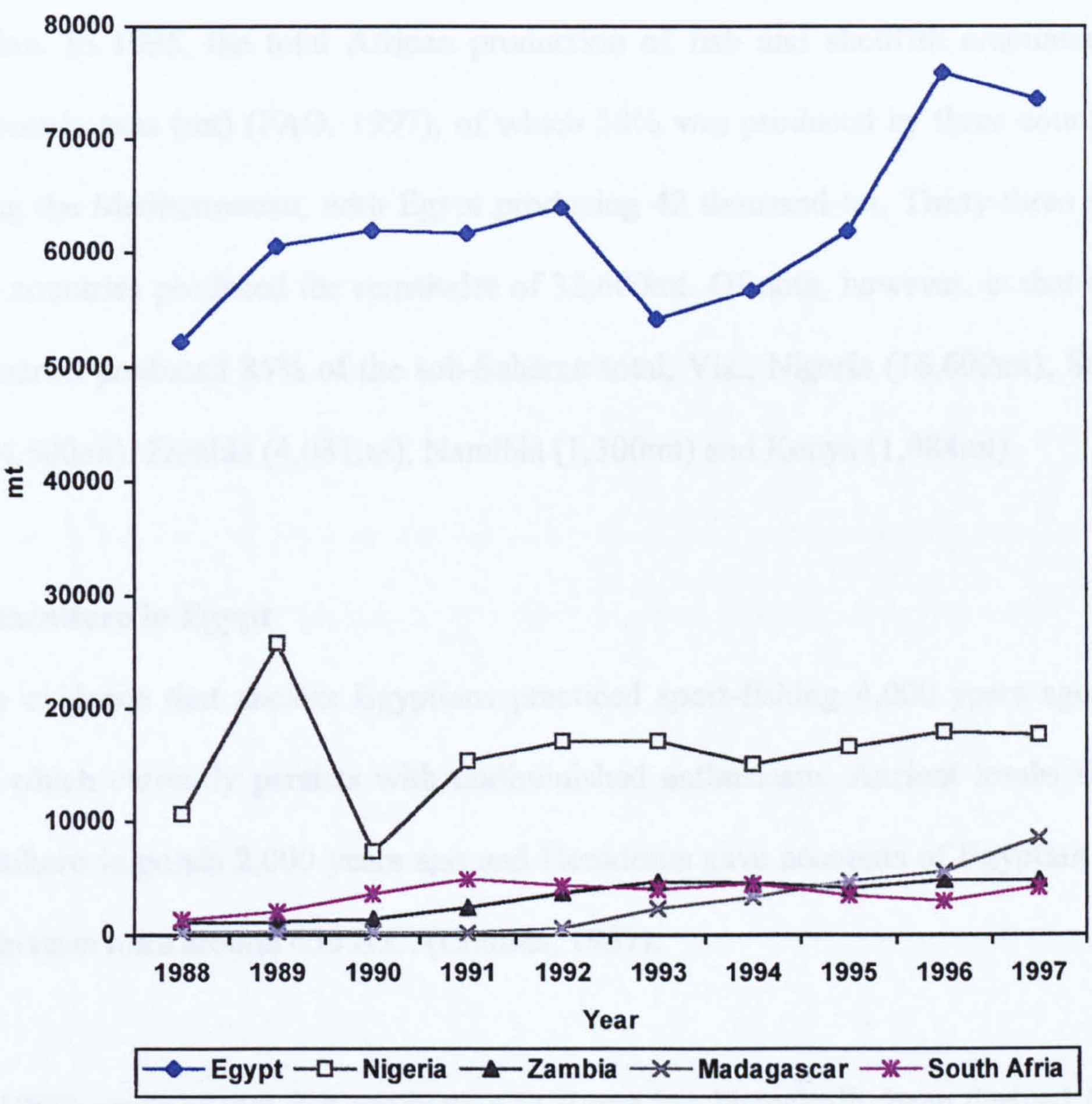

Figure 1.2 The trend in aquaculture production in some selected African states (FAO, 1999).

Although, the total aquaculture production for the total continent may have been as high as 96,000 tonnes increasing to 105,000; 121,000 and 122,000 in 1995, 1996 and 1997 respectively (FAO 1999), the last figure being only 0.34% of world production.

In comparison to the rest of the world, aquaculture in Africa is relatively insignificant. The continent as whole contributes a mere 0.4% to the total world aquaculture production. In 1995, the total African production of fish and shellfish amounted to 77,761 metric tons (mt) (FAO, 1997), of which 58% was produced by three countries bordering the Mediterranean, with Egypt producing 42 thousand mt. Thirty-three sub-Saharan countries produced the remainder of 32,600mt. Of note, however, is that only five countries produced 85% of the sub-Saharan total, Viz., Nigeria (16,600mt), South Africa (4,500mt), Zambia (4,081mt), Namibia (1,300mt) and Kenya (1,084mt).

1.3 Aquaculture in Egypt

There is evidence that ancient Egyptians practiced sport-fishing 4,000 years ago, an activity which currently persists with undiminished enthusiasm. Ancient tombs show tilapia culture in ponds 2,000 years ago and Herodotus gave accounts of Egyptian fish culture in reservoirs around 450 B.C. (Chimits, 1957).

Sadek (1989) reported that fish production in Egypt has historically been derived from natural marine and freshwater systems. Currently the area under fish production is approximately about 2.4 million-hectares and is more than the land area under agriculture. Altogether, 134,000mt of fish were landed in 1982 from inland fresh and brackish water fisheries (77.7%), marine fisheries (18.3%) and aquaculture (4%). While capture fisheries production in Egypt is near its maximum sustained level, aquaculture can provide a substantial increase in fish production within a relatively short period.

Egypt has great potential for aquaculture, especially in the coastal wastelands, which are not suitable for traditional agricultural practices.

The main water resources are along the extensive shoreline of the Mediterranean Sea. Many lagoons in this region are ideal for mariculture activates. Coastal lakes and other brackish water resources are potentially available for fish culture (Hamza & Zaki, 1987). Over the next 10 to 15 years, fish farming may expand to a significant proportion of the total animal production of the country. As in many other countries of the world, aquaculture production in Egypt has rapidly expanded in last few years (Figure 1.3).

Fresh and brackish water fish cultures are much more developed in Egypt than specialist mariculture systems. At present, brackish water fish farming is gaining more prominence due to the increasing demand for land and for fresh water needs of the population (Wassef, 2000).

The future of fish farming depends on the interplay of many technical, commercial, and political factors, which influence supply and demand (Sadek, 1984). In 1987, fish farming following the semi-intensive, intensive and integrated fish culture techniques, covered about 251 thousand hectare and 10 thousand m^2 of cages in Egypt. The private cage culture, in the district of Ismalia (Egypt) regularly generates a cage production of 35-40 kg m^3 of fish, mixied common carp and tilapias, after 4 months duration (Moreau & Costa-Pierce, 1997).

Species cultured in Egypt; with the wide range of environmental conditions that exist in the country, several types of fish are popular for culture. In the fresh water areas, a polyculture system of tilapia, the common carp *Cyprinus carpio,* mullets (*Mugil cephalus* and *M.capito)* and the catfish (*Clarias lazera)* has been established (Eisawy *et al.,* 1974). Recently, El-Sayed (2001) reported tilapia as the most important fish species cultured in Egypt. The production of this fish increased from 21,505 in 1992 to 52,755mt in 1998.

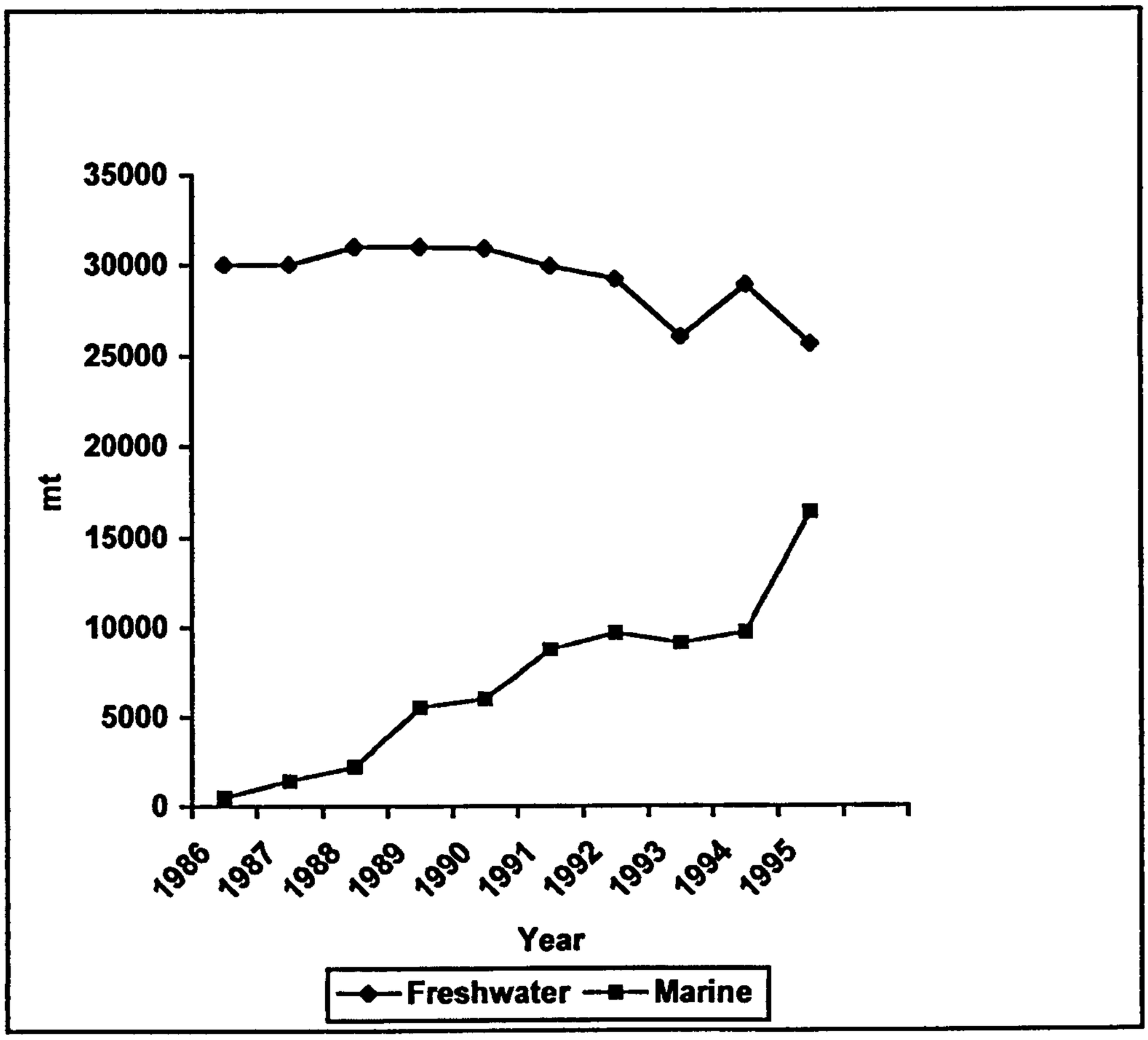

Figure 1.3 Total aquaculture production of freshwater and marine species in Egypt between 1986 and 1995 (FAO, 1997).

These figures represented 37.9% of total aquaculture production in Egypt and 5.42% of the world aquaculture production. Egypt is the fourth largest producer of cultured tilapia in the world, after China, the Philippines and Indonesia (El-Sayed, 2001). The Chinese carp, the silver carp, *Hypophthalmichtys molitrix,* the grass carp *Ctenopharyngodon idellus* and the bighead carp *Aristichthys nobilis* have been recently introduced into the country.

These species were also included in the freshwater polyculture system. The occurrence of *C. lazera* and *Lates niloticus* occasionally takes place in freshwater fishponds. The four species of tilapia, which are found in aquaculture operations in Egypt, are *Oreochromis niloticus, Sarotherodon galilaeus, Tilapia zillii* and *O. aureus.*

Attempts are being made to culture some endemic Nile fishes namely *Barbus bynni, Labeo niloticus* and *Lates niloticus.* In the saline areas, the grey mullets (*M. cephalus* and *M. capito)* are used predominantly with some marine species namely the seabream (*Sparus auratus),* the sea bass *(Dicentrarchus labrax),* and the saline tolerant *T. zillii,* Sole (*Solea solea)* has also been introduced and evaluated for rearing and artificial breeding by Hamza & Zaki (1987). Considerable attention is being given to the culture of the seabream and sea bass in cages (Hamza *et al.*; 1988) in Alexandria, Egypt as practiced in several European countries notably Greece, Cyprus, Portugal, Spain and Italy. Tilapia is the most favoured species for domestic consumers (Figure 1.4) Tilapia production in 1997 was 30,500mt, representing 41.4% of total production by weight, and valued at more than US$ 63,000. Among tilapia, Nile tilapia (*Oreochromis niloticus)* is the most important species due to its higher growth rate and better performance. Blue and white tilapias (*Oreochromis aureus* and *Sarotherodon gallilaeus*) are also good candidates for farming and follow Nile tilapia in the order of importance (Wassef, 2000).

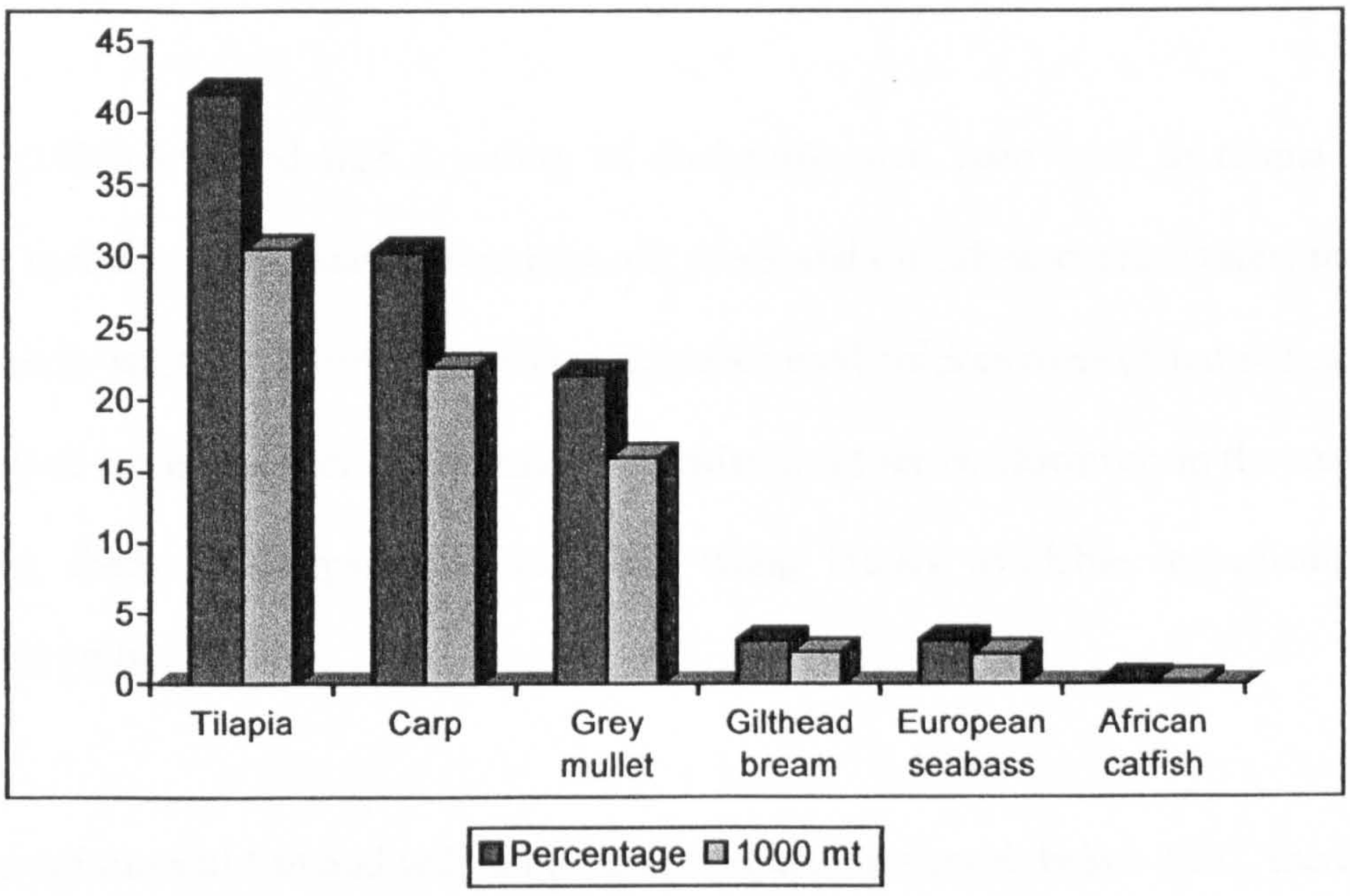

Figure 1.4 Production of the primary fish cultured species in Egypt in 1997 (Wassef, 2000).

1.4 The Nile Tilapia *Oreochromis niloticus* (Trewavas, 1982)

Tilapia (family *Cichlidea*) are native to Africa and Palestine. They have been introduced into a large number of tropical and sub-tropical countries around the world in the last four or five decades, either accidentally or deliberately. Aquaculturally, this group of species has had a rather checkered history. Tropical aquaculturists who experienced considerable problems in controlled spawning of fishes were initially excited by the availability of a species that could breed in almost any type of water body. Being herbivorous or omnivorous, it was comparatively easy to feed the species of this group. They were found hardy and could be reared in fresh, brackish and even seawater (Pillay, 1990).

Lovell (1998) reported that a variety of feedstuffs have been used in tilapia pond rearing, including plant leaves, rice bran, oil seeds and oil cakes, copra wastes, manioc and brewery wastes. Culturists have in some cases used chicken diets (often mixed with protein rich ingredients) or rarely, more expensive trout feeds. However, in the majority of cases, feeds are prepared on the farm using locally available ingredients and feedstuffs (Pillay, 1990).

Tilapias are tropical fish and will not tolerate temperatures much below 12^0C, therefore, their culture is limited to tropical and subtropical regions or thermally controlled environments in temperate regions (Lovell, 1998). More recent studies provided evidence that water temperature also governed the phenotypic sex on *Oreochromis* spp. A large majority of investigations demonstrated that high temperatures favoured the production of almost monosex male progenies of *O. niloticus* (Baroiller *et al.*, 1996) and *O. aureus* (Desprez & Melard, 1998). Also, Baras *et al.* (2001) suggested that the advantage of producing faster growing males of Nile tilapia at high temperature would

hardly compensate the loss of production incurred in the masculinising treatment, unless after the selection of the most thermosensitive strains or breeders.

Tilapias are endemic to Africa, but are presently found in most warm regions of the world. They are a popular fish for culture in the tropics and areas where supplemental feeds are cost restrictive because of their efficient use of natural aquatic foods, fast growth, propensity to consume a variety of supplemental feeds, herbivorous nature, resistance to diseases and handling, ease of reproduction in capacity, and tolerance to wide ranges of environmental conditions. Some of the cultured species have been shown to survive dissolved oxygen concentrations of $0.1mgl^{-1}$ and tolerate unionized ammonia concentrations of $2.4mgl^{-1}$ (Lovell, 1998). Although tilapias are able to grow well in saline water if properly acclimated, they are all unable to reproduce effectively below 20^0C and their feeding response usually ceases below 16^0C Lovell (1998). Their activity and feeding is considerably compromised at such low temperatures.

Most cultured tilapias are grouped into two genera (Trewavas, 1982):

Tilapia, which are macrophagous and substrate-spawners; and *Oreochromis*, which are microphagous and mouth-brooders. About 70 different species have been identified under these two genera; however, only two tilapia species, *rendalli* and *zillii,* and three *Oreochromis* species, *mossambicus*, *niloticus*, and *aureus,* have been used widely in practical culture. Pillay (1990) reported two alternative classifications were proposed; one includes five genera, *Tilapia, Sarotherodon, Oreochromis, Tristromella* and *Danakilia* and the other only one genus, *Tilapia* with sub-genera: *Heterotilapia, Pelmatilapia, Sarotherodon, Oreochromis, Nyasalapia, Alcolapia* and *Neotilapia* (Fishelson & Yaron, 1983). The most popular culture species is *Oreochromis niloticus*. In monosex culture, males are preferred because they have a faster growth rate than

females. This can be managed by manual sexing, hybridization, or sex-reversal of genotypic females with the use of hormones (Lovell, 1998).

1.5 The African catfish: *Clarias gariepinus* (Burchell 1822)

Clarias gariepinus, known as the African catfish, sharp tooth catfish or the Nile catfish is a recent addition to aquaculture in Africa, which has been largely dominated by tilapia. Though its potential for farming has been demonstrated, its culture presently seems to be restricted to the central African countries, the Ivory Coast and, on an experimental scale, to Egypt. Other catfish, such as *Chrysicthys* spp., are also being experimented with for pond and cage culture (Pillay, 1990). *Clarias gariepinus* can best be described as an omnivore, often feeding on vegetable matter, aquatic invertebrates, small fish, detritus, etc. Though it has a normal capacity to utilize dissolved oxygen, it often comes to the surface and breathes atmospheric air when the oxygen concentration of the water becomes low. These fish have to survive environments that may be turbid, turbulent, subject to fluctuations in temperature, water chemistry and oxygen saturation. Britz & Hecht (1987) reported that African sharptooth catfish *Clarias gariepinus* preferred temperature of 30^0C which corresponds exactly to the temperature at which fastest growth was observed. This temperature might be optimal for most physiological functions.

A characteristic feature of clariid catfish is their ability to breathe air and tolerate low dissolved oxygen levels (Bruton, 1979). This is an important factor within aquaculture as air contains approximately 30 times more oxygen per unit volume than water. Catfish have been observed to reach over 130cm in length and 12.8kg in weight. A high degree of hardiness, the ability to feed on a variety of feedstuffs and good growth and survival in poorly oxygenated waters have made it an attractive fish for rural aquaculture.

The species can grow in brackish water in salinities of 10ppt and survive in salinities up to 29ppt. The most common system of culture for this catfish is in pond farms, either in monoculture or in combination with tilapia, which has been shown to be a compatible species under pond conditions. Experiments have shown that the species is highly suitable for high-density tank culture Hogendoorn *et al.* (1983). Under natural conditions, the annual breeding season is limited to several months during spring and summer (De Leeuw *et al.*, 1985). *Clarias gariepinus* and *C. anguilaris* are the most important Clarias species used in African aquaculture today, although locally other species may also be utilized (e.g. *Clarias ebriensis* in the Oueme River in Benin Republic).

Production of *Clarias gariepinus* can be economical only when its qualitative and quantitative feed requirements are known. Aquaculture of *C. gariepinus* should ideally be based on a low-grade feed compound of locally available agricultural by-products. The growth and production of *C. gariepinus*, the effect of stocking density and pond size and polyculture with tilapia *(Oreochromis niloticus)* were studied extensively under field conditions by Hogendoorn & Koops (1983). Aquaculture production of *Clarias gariepinus* in Egypt increased from 462mt in 1991 to 2000mt in 1995 (FAO, 1997).

One of the most important attributes of the African catfish for culture is its highly efficient feed conversion and growth rate. In low-input systems, the species is an opportunistic omnivore and feeds on almost any available food with a preference for animal material. As well as filtering feed, using small, bony growths on its gill arches as tiny rakes, a catfish will forage over a wide area, shoveling detritus with its flat, bony head, and catching any organisms it disturbs (Haylor, 1990)

In more developed systems with supplementary feeding, a wide range of under-utilized local products can serve as cheap feed ingredients. These include brewery wastes, rice bran, cotton seed cake, blood meal, ground nut cake, etc. Adding high-energy supplementary feeds allows more of the protein resulting from enhanced natural productivity to be used for growth. The African catfish efficiently converts such feeds into fish flesh. Growth and development is very fast; catfish fry can reach 10g within 56 days of first feeding.

Catfish can be subsequently on-grown, in ponds to market size. In Southern Africa, catfish are marketed at about 1kg (at some 32 weeks` old, on the best farms). In West Africa, the target size is about 330g, which may be possible with good management, in four months. At this early stage in developing *C. gariepinus* culture, procedures and production vary considerably. Although the most common stocking density to date, is between 10,000 and 20,000 fry ha^{-1} high yields have been achieved in Southern Africa by stocking 100,000 fish ha^{-1}, with good management, fertilization and supplementary feeding, there is no evidence to suggest that production declines relative to stocking density up to this level. So, there appears to be many reasons for promoting the African catfish (C. *gariepinus)* for African aquaculture projects.

For this to be achieved, however, entrepreneurs and aid agencies must invest in research as well as interactive extension and training programmes (Haylor, 1990). Certainly, if African aquaculture is to fulfill its potential, a broader range of indigenous candidate species, amenable to low input culture, must be evaluated, and their culture developed under different circumstances.

1.6 Fish nutrition in aquaculture

Clearly, finfish culture involves a number of diverse species suitable for domestication. Each of these species has its own specific nutritional requirements, particular feeding

behaviour and food preferences. The species cultured range from strict carnivores to those feeding very low in the food chain. When the global finfish aquaculture industry is considered, the dominance of non-carnivorous species is obvious: they currently constitute about 85% of the global and nearly 90% of Asian finfish production (FAO, 1990, 1991).

The matter is further complicated because supplemental feeds can range from a single ingredient to a mix of ingredients, presented in various forms, to a properly formulated practical diet (New *et al.*, 1993). The role of supplementary feeding in pond fish culture of non-carnivores was aptly dealt with by Hepher (1988), when the dearth of knowledge on this subject was highlighted.

As with all other forms of animal production, the growth and production of farmed fish or shrimp is dependent upon the dietary intake of feed, which is based on at least forty or more essential dietary ingredients. Either in the form of endogenously produced live food organisms or exogenously supplied artificially compounded diets. It follows therefore that if aquaculture production, and in particular finfish and crustacean production is to maintain its current growth rate into the next decade then corresponding inputs of fertilizer and aquafeed will also have to be provided (FAO, 1993).

1.6.1 Dietary energy

All terrestrial animals and fish require energy for all physiological functions including digestion, maintenance of cellular functions and tissue synthesis for growth and replacement (Cho & Kaushik, 1985). Steffens (1989) reported that all metabolic reactions, whether concerned with requiring energy for the maintenance of body functions, the replacement of spent materials such as hormones, enzymes, etc., or the

building of new body tissues, are closely interconnected and cannot be divided one from another.

Fish are known to utilize protein preferentially to lipid or carbohydrate as an energy source. Nevertheless, it is important from a nutritional, environmental and economical point of view to enhance protein utilization for tissue synthesis rather than for energy purposes. The optimization of dietary digestible protein/ digestible energy ratio (DP/DE) has proven to have an important role on protein and energy utilization (Kaushik & Medale, 1994). The increase of DE content in fish diets, by lipid supplementation has been shown to have a protein sparing effect, therefore decreasing nitrogen losses to the environment (Cho & Kaushik, 1990).

However, some authors have observed no protein sparing effect of lipid supplementation (Kissil & Gropp, 1984; Andersen & Alsted, 1993; Lanari *et al.*, 1998), others found an increased protein utilization efficiency with high digestible energy (DE) diets (Vergara *et al.*, 1996; Dias *et al.*, 1998).

A decrease of the dietary protein requirements for maximum growth, by increasing the level of dietary non-protein energy, is termed " protein-sparing". Jauncey (1998) reported that at moderate dietary protein levels, increased amounts of dietary energy may lead to retention of carcass lipid and undesirable changes in carcass composition. The design of practical feeds is thus a compromise between a protein level that promotes high growth, with little conversion to energy, and an energy level that permits high rates of protein synthesis but does not lead to undesirably high levels of carcass lipids. Inclusion of non-protein energy has been shown to spare dietary protein from catabolism to provide energy and improve its utilization for growth, (Millikin, 1983; Dias *et al.*, 1998; Grisdale-Helland & Helland, 1998; Helland & Grisdale-Helland, 1998).

The relationship between the protein and energy levels in the diet is usually expressed as the protein:energy ratio (P:E) (units of mg of protein per kJ of metabolisable or digestible energy) (Jauncey, 1998). Meyer-Burgdorff *et al.* (1989) working with Nile tilapia *O. niloticus,* concluded that increasing the feeding rate, and thus energy ingested, resulted in a decrease in the availability of gross energy. They concluded that tilapia should not be fed more than 400kJ(ME) $kg^{-1}d^{-1}$.

Anderson *et al.* (1991) studied the measurement and prediction of digestible energy values of a range of feedstuffs for *O. niloticus* and reported that the level of a component in test diets for digestibility measurement had little or no effect on digestible energy (DE) values. However, they also reported that soybean DE was significantly higher after a 15 week adaptation period and that "typical" 28 day digestibility studies may not be sufficient to determine the DE in this species for different raw materials. The DE values for 16 feedstuffs were determined and are presented in (Table 1.1). In common the plant material DE's were higher than reported for either trout or channel catfish whilst the animal material DE's were generally lower.

1.6.2 Dietary protein and amino acid requirements

Fish are fed higher percentages of protein in their diets compared to terrestrial animals. The main reasons for this are not so much that fish have higher protein requirements (%) than terrestrial animals *per se*, but that fish have lower energy requirements. For example, broiler chickens will consume about 3.4 times as much energy but only 1.6 times as much protein as growing channel catfish on a kg^{-1} of body weight basis. Production diets for cultured fish will contain 30-35% amino acid-balanced protein whereas comparable feeds for poultry or pigs will typically contain 18-23% or 14-16%, respectively (NRC, 1983).

Table 1.1 Digestible energy contents of some feedstuffs for *O. niloticus.*

Feedstuff	DE (MJ kg^{-1} dry matter)
Cassava[1]	13.4
Copra cake[1]	6.5
Copra[2]	15.6
Fish meal[1]	16.2
Fish meal[2]	13.9
Groundnut cake[1]	17.9
Maize[1]	13.1
Maize[2] (corn)	14.5
Meat & bone meal[1]	9.1
Palm kernel meal[1]	1.7
Poultry by-product meal[1]	10.0
Rapeseed meal[1]	10.6
Rice bran[1]	5.0
Rice bran[2]	17.9
Shrimp meal[1]	10.4
Soybean meal[1]	14.4
Soybean meal[2]	14.6
Sorghum[1]	12.4
Sunflower seed meal[1]	3.6
Wheat[1]	13.3
Wheat bran[1]	6.1
Wheat middlings[1]	10.4

1 Anderson *et al.* (1991)
2 Kamarudin *et al.* (1989)

Mangalik (1986) found that the amount of digestible, amino acid-balanced protein $kcal^{-1}$ of digestible energy (DE) consumed for maximum growth of channel catfish was 0.10g for 3g fish and decreased only slightly to 0.09g for 200g fish. While protein-energy ratio changes only slightly with size, daily protein consumption decreased significantly as size increased, from 16.4gkg^{-1} for 3g fish to 5.2gkg^{-1} for 200g fish. Fish appear to require the same 10 essential amino acids as warm-blooded animals. The full

quantitative amino acid requirements have been established for five fish species (NRC, 1981, 1983, 1993). These are presented in (Table1.2) along with comparable values for swine and chickens. Except for arginine, the amino acid requirements of fish are relatively similar to other animals. With the exception of arginine and methionine plus cysteine, the amino acid requirements of channel catfish *Ictalurus punctatus* are similar to those of chinook salmon *Oncorhynchus tschawytscha*. Recently, Izquierdo *et al* (2001) suggested that although fish is the basic diet in many countries, the nutritive requirements of some valuable species are not well established. With respect to the amino acid profiles of studied species a high concentration of lysine, histidine, and methionine was shown, tilapia appears to need high levels of valine and isoleucine, even higher than regular requirements. However, a low content of methionine was observed for all species used in this study for tilapia (Izquierdo *et al.*, 2001).

However, the requirements for common carp *Cyprinus carpio* and Japanese eel *Anguilla japonica,* expressed as a percentage of the protein, are somewhat higher than those of channel catfish and chinook salmon.

Various studies indicate that the dietary protein requirement for channel catfish *Ictalurus punctatus* ranges from about 24 to 50%. Protein requirements vary due to numerous reasons including differences in water temperature, feed allowance, fish size, amount of non-protein energy in the diet, protein quality, natural food available, and management practices (Lovell, 1998). However, 28 or 32% protein feeds are typically used for fish during grow-out to marketable weights (Robinson & Li, 1996). Levels as low as 24% have been shown adequate for table -size catfish provided the fish are fed to satiation (Li, & Lovell, 1992). Some studies indicated that weight gain of catfish is only slightly reduced using feeds containing as low as 16% protein. In order to maximize profits, the optimum dietary protein level should not necessarily be based on maximum weight gain but rather on the most economic gain.

Table 1.2 Essential amino acid requirements of several fishes, chicken, and swine (expressed as % of protein).

Amino acid	Japanese eel[1]	Common carp[1]	Channel catfish[1]	Rainbow trout[2]	Chinook salmon[1]	Nile tilapia[2]	Chicken[1]	Swine[1]
Arginine	4.5	4.2	4.3	4.4	6.0	4.2	5.6	1.2
Histidine	2.1	2.1	1.5	2.1	1.8	1.7	1.4	1.2
Isoleucine	4.0	2.3	2.6	2.6	2.2	3.1	3.3	3.4
Leucine	5.3	3.4	3.5	4.1	3.9	3.4	5.6	3.7
Lysine	5.3	5.7	5.1	5.3	5.0	5.1	4.7	4.4
Methionine + cysteine	5.0	3.1	2.3	2.9	4.0	3.2	3.3	2.3
Phenylalanine + tyrosine	5.8	6.5	5.0	5.3	5.1	5.5	5.6	4.4
Threonine	4.0	3.9	2.0	2.4	2.2	3.8	3.1	2.8
Tryptophan	1.1	0.8	0.5	0.6	0.5	1.0	0.9	0.8
Valine	4.0	3.6	3.0	3.5	3.2	2.8	3.4	3.2

[1]Source: NRC (1981 and 1983).
[2]Source: (NRC, 1993).

Lysine is generally the first limiting amino acid and if feeds are formulated to meet a minimum lysine requirement, all other amino acid requirements are met or exceeded if traditional feed ingredients are employed (Lovell, 1998). Cysteine can replace about 60% of the methionine and tyrosine can replace about 50% of the phenylalanine in the protein. In a practical feed, amino acid requirements are best met by feeding a mixture of feedstuffs or by using a mixture of feedstuffs supplemented with the appropriate crystalline amino acid decreased to be limiting. Robinson & Li (1993) reported that synthetic amino acids, such as lysine HCl, are effectively used by catfish when supplemented in a practical feed.

1.6.3 Dietary lipid

All vertebrates have requirements for n-3 and n-6 fatty acids the main variation among species being the amount of fatty acids necessary to include in the diet formulation and

the bioconversion capability of different fatty acids in a given species (Sargent *et al.*, 1999). According to species, fish require fatty acids mainly of the n-3 and n-6 type. Lipid is well digestible energy source for fish. By appropriate protein:energy ratio (high energy feed) favourable diet utilization, fast growth and efficient protein and energy utilization can be achieved (Steffens, 1996). Fish are known to utilize protein preferentially to lipid or carbohydrate as an energy source. Moreover, it is important from a nutritional, environmental and economic point of view to improve protein utilization for tissue synthesis rather than for energy purposes.

The increase of DE content of fish feeds, by lipid supplementation, has been shown to have a protein sparing effect, therefore reducing protein losses to the environment (Cho & Kaushik, 1990).

Peres & Oliva-Teles (1999) observed no growth differences in sea bass fed diets ranging from 12 to 24% lipids, while with 30% lipid a growth reduction occurred. Other authors also studying in sea bass found no beneficial effects of increasing dietary lipid levels above 12% (Metailler *et al.*, 1981; Pérez *et al.*, 1997). However, in Atlantic salmon, levels of lipid in the diets up to 30% were shown to significantly improve feed and protein utilization efficiencies and to decrease pollution load from nitrogen, phosphorous and organic matter (Johnsen & Wandsvik, 1990). According to the phospholipid requirement, it has been observed that the supplementation of polar lipid to artificial diets improved growth and survival of larval red seabream (Kanazawa *et al.*, 1983) and common carp (Geurden *et al.*, 1995). It has also been observed that dietary *n*-3 HUFA in the polar lipid fraction improved the growth rate of gilthead sea bream larvae *Sparus aurata* (Salhi *et al.*, 1995).

Koven *et al.* (1993) reported that by supplementation of diets with phospholipids one observes an improved incorporation of oleic acid into tissue lipids and a higher content

of *n*-3 HUFA, mainly due to the DHA in the polar lipids, as well as in gilthead sea bream (Salhi *et al.*, 1995).

1.6.4 Dietary carbohydrates

Fish do not use carbohydrates as efficiently as terrestrial animals and birds, although fish have a limited capability to digest and utilize carbohydrates (Wilson, 1994). Carbohydrates constitute one of the three nutrient components of the fish diet that are used as an energy source to support growth. Fish in general utilize carbohydrate poorly. Although, different types of carbohydrate may not be equally assimilated by fish, in warm water fish particularly tilapia, *Oreochromis niloticus* × *O. aureus*, a number of factors appear to be associated with carbohydrate utilization (Shiau, 1997). It is now established for the majority of fish species used in aquaculture, that the efficiency of protein utilization can be improved by increasing the proportion of conventional energy sources (lipid and carbohydrate) in the diets (Cho & Kaushik, 1990; Kaushik & Médale, 1994).

Nowadays, much attention has been focused on studying the use of various carbohydrate sources in fish diets. For instance, chromium supplementation in diet containing glucose as the carbohydrate source for tilapia, *Oreochromis niloticus* × *O. aureus*, has been improved weight gain, energy deposition and liver glycogen (Shiau & lin, 1993). In addition, the effects of different types of technological processing in order to improve their utilization by fish has been investigated (Kim & Kaushik 1989; Takeuchi *et al.*, 1990; Bergot, 1991; Schwarz & Kirchgessner, 1991). The actual reasons for the poor ability of fish to utilize carbohydrate are still unknown despite much research.

Moreover, different types of carbohydrate might not be equally available to fish. For example, the availability of starch has been shown to be higher than that of glucose and

dextrin for carp, red sea bream (Furuichi & Yone, 1982) and tilapia (Anderson *et al.*, 1984). Channel catfish were observed not to utilize simple sugars efficiently (Wilson & Poe, 1987). The European eel *Anguilla anguilla* has been observed to be able to use relatively high dietary carbohydrate levels (Hidaglo *et al.*, 1993; Sanz *et al.*, 1993).

García Gallego *et al.* (1991) reported that in a comparative study on the ability of trout and eel to utilize diets with different carbohydrate levels, eels were comparatively better adapted to use higher dietary carbohydrate levels although results of dietary utilization may have been affected by the greater hardness of the dietary pellets as carbohydrate content increased.

Carbohydrate digestibility is generally better in warmwater and freshwater fish than in coldwater and marine species (Hofer & Sturmbauer, 1985), and it is related to the nature of the carbohydrate source (Furuichi & Yone, 1982; Wilson & Poe, 1987).

Carbohydrate digestibility of maltose is much higher than that of crude starch in sea bass (Alliot *et al.*, 1984) and decreases with an increase of dietary carbohydrate level. Starch gelatinization enhances carbohydrate digestibility in sea bass (Peres, 2000), and extrusion improves wheat carbohydrate digestibility in sea bass (Santinha, 1997). In rainbow trout *Oncorhynchus mykiss*, gelatinized starch has been shown to be as effective as lipid as an energy source (Pieper & Pfeffer, 1980).

1.6.5 Dietary vitamins

Vitamins are a select group of organic components, which are essential for normal metabolism in animals. They are also important in the same manner as EAA or EFA in that they cannot be synthesised at all by a particular species or they are not synthesised fast enough to meet the animals requirements (Jauncey, 1998). The vitamins are generally classified into two groups according to their solubility i.e. water or lipid

soluble. Water-soluble vitamins are not stored to any significant extent in animals tissues and their turnover is rapid during the course of metabolism (Jauncey, 1998).

In contrast, fat soluble vitamins, are absorbed along with dietary lipid and stored in the fatty tissues of animals (NRC, 1993). Recently, Oliva-Teles (2000) reported that the available data of vitamin requirements of marine fish is much less than other fish. Only for yellowtail *Seriola quinquerradiata* have the quantitative requirements for the majority of vitamins been determined (Shimeno, 1991). A requirement for vitamin C was also observed in both sea bass and sea bream but not quantified (Alexis *et al.*, 1997; Henrique *et al.*, 1998).

Recently, Fournier *et al.* (2000) reported that in sea bass a minimum of 5mgkg^{-1} diet of ascorbic acid was required for high growth rate and to maintain normal skin collagen concentration although higher levels were required based on whole body hydroxyproline and concentration of liver ascorbic acid. Baker & Davies (1996) showed that at the dietary lipid level employed, supplementation of vitamin E in the form of α-tocopheryl acetate results in a concomitant increase in tissue α-tocopheryl content and progressive protection of tissues of African catfish *Clarias gariepinus* against the peroxidative damage of oxy-radicals both *in vivo* and under-forced oxidation *in vitro*.

Dickson (1987) reported that a supplementation of vitamins might be unnecessary in feeds for intensively farmed tilapia in Zambia. There are also many other reports of the non-essential of added dietary vitamins for tilapia (Wee & Ng, 1986; Campbell, 1985; Wannigama *et al.*, 1985).

Jauncey (1998) suggested that there are still some unanswered questions concerning the vitamin requirments of tilapia species and it is exceedingly difficult to make general recommendations as to the requirement level in specific diet formulation. Vitamins are generally supplemented to fish diets in the form of supplementary premix that contains

all of the necessary vitamins in the correct relative proportion. Cowey (1992) showed that determinations of vitamin requirement made in trout (and other salmonids) were much higher than those of omnivorous birds and mammals.

1.6.6 Dietary minerals

Minerals are required for the normal life process, maintenance of normal metabolic and physiological function for all animals and therefore fish also need these inorganic elements (Watanabe *et al.*, 1997). Determination of the mineral requirements of fish is complicated by the ability to take up certain ions from the water. Ca, Mg, Na, K, Fe, Zn, Cu and Se can absorbed by fish from the water via the gills if they are present; phosphorus with chlorine and sulphur probably together are best supplied in the food (Cowey, 1992). The minerals are important for skeletal formation, maintenance of colloidal system, regulation of acid-base equilibrium and for biologically important compounds such as enzymes and hormones (Watanabe *et al.*, 1997). Cristina Rowena *et al.* (1996) showed that the reduction of dietary calcium and phosphorous was found to have a significant affect on growth and feed utilization. However, these latter workers did not clarify if the effect was due to either calcium or phosphorous, or their combined effect.

The dietary requirement for phosphorous in tilapia varies from 0.9% (Watanabe *et al.*, 1980), 0.45-0.6% (Viola & Arieli, 1983), 0.3-0.5% (Robinson *et al.*, 1987) to 0.46% (Haylor *et al.*, 1988) depending on species, fish size, diet composition or expression of reported requirement whether as available or total dietary phosphorous.

Although plant protein sources such as soybean meal contain sufficient amounts of phosphorous, about two-thirds is phytate phosphorous which is in bound form (Lovell, 1977 cited in Kim & Oh, 1985) and is poorly available to fish (Akiyama, 1988). The beneficial effect of supplementation of phosphorous to soybean-based diets has been

observed by (Kim & Oh, 1985) for carp and Hung (1989) for tilapia. Storebakken *et al.* (2000b) reported that lower dietary P concentration and P intake in the fish fed the soy-protein concentrate SPC diet, both faecal and metabolic excretion of P were lower in the fish fed SPC diet than in fish fed fish meal diet for Atlantic salmon *Salmo salar.* Although fish can partially absorb P from their environment (Lall, 1989 and 1991).

1.7 Fish meal quality

Much of the feed cost is due to the extensive use of fish meal in the diet (Tacon, 1994; Higgs *et al.*, 1995) and attempts have been made to develop viable alternative protein sources and energy for inclusion in fish diets in order to reduce this dependency. Considerable efforts are also being made to improve fishmeal quality so that maximum nutritive value can be obtained from this expensive dietary component. These protein concentrates have high palatability and nutritional value but are expensive and not always readily available (Lim & Dominy, 1990). From 1984 to 1990, world aquaculture production increased by an average rate of about 14% yearly, and the total production will be dramatically increased at least by the year 2005 (Hardy, 1999). However, the fishmeal production in the world is not expected to increase further (Pauly *et al.*, 2000)

Fishmeal quality varies, both among sources and among batches from the same sources, and the reasons for this variation include: (1) Dissimilar composition and degree of freshness of the raw material before conversion into fishmeal; (2) Variable proportions of whole fish, offal, and filleting residues in the raw material; (3) Differences in processing conditions (cooking and drying temperatures employed during meal manufacturing). (4) Variation in the amounts of solubles added to the presscake to make whole fish meals. (5) Possible alterations in the general quality of the fishmeal due to the addition of poor quality fish solubles. (6) Inappropriate levels of antioxidants and

moisture in the meals; and (7) Sub-optimal meal storage and transportation conditions (Higgs *et al.* 1995).

Fish meal of the highest quality results from the processing of extremely fresh whole fish under low-temperature cooking and drying conditions (McCallum & Higgs 1989; Anderson *et al.* 1993). Meal products manufactured under these conditions are designated low- temperature (LT) type meals, but even within this category there can be variation in quality standards.

There are, however, still uncertainties regarding the extent to which fluctuations in the composition and freshness of the raw material, the cooking and drying condition during meal preparation, and the subsequent meal storage conditions, influence the quality of fish meals.

1.8 Economic diet formulation and alternative protein sources

Fishmeal has traditionally been a major ingredient in compounded aquafeeds because of its high protein quality and palatability to fish. High quality fishmeal is well recognized as the best source of protein for salmonids (Pike *et. al.*, 1990), but the best quality meals are expensive (Higgs *et al.*, 1988). Partial or total replacement of fishmeal protein with alternate sources of protein could be of considerable economic advantage, even if this approach was associated with a moderate reduction in efficiency.

Computerized linear programming techniques are now often used to guide diet formulation for terrestrial livestock species with well-defined nutritional requirements (Waldroup, 1984). Linear programming cannot be used to determine optimum aquaculture diets of most aquatic species, however, because their specific dietary requirements are not yet known (for an exception, Barbieri & Cuzon, 1980). Instead of using mathematical methods, aquaculture nutritionists often rely on trial and error screening of various combinations of "off the shelf" feeds and basic nutrient

specifications of raw materials. Feeding costs and resulting growth rates, growth efficiencies and mortality vary appreciably for fish species fed different diets. These parameters are routinely determined in nutrition studies designed to evaluate the performance of various feeds.

Studies of non-algal feeds for bivalves have largely ignored such economic considerations. When economics have been considered, it has been by selecting diets that minimize food costs or to maximize revenues (De Pauw *et al.,* 1983; Urban and Langdon, 1984). Instead, the primary economic criterion for diet selection should be to maximize profit, because reducing food cost may decrease conversion efficiencies or growth rates, or increase mortality, resulting in decreased profit.

Much research has been focused to evaluate new protein sources as substitutes for fishmeal in complete diets for warmwater fish species of commercial importance. Vukina & Anderson (1993) reported that cross-commodity hedging between fishmeal and soybean meal, the approach uses successively updated out-of-sample forecasts to approximate subjective price expectations, and forecast error variance-covariance matrices to measure risk.

Animal by-products have been either of terrestrial, avian or marine animal origin. They constitute the most important (and often the most expensive) ingredients of aquaculture feeds. These ingredients are necessary to balance the amino acid profile and vitamin deficiencies in cereals and other plant products in complete diets (Lovell, 1998).

Animal by-products, particularly fishmeal appear to contain unidentified growth factors. Some examples are meat meal, blood meal, feather meal, poultry by-product meals, fishmeal, , raw fish, fish oils, fish silage, shrimp meal and meal by-product. As with some of the plant by-products available, certain animal by-products also demonstrate imbalances of essential amino acids (De Silva & Anderson, 1998). For example blood

meal, meat and bone meal are all deficient in methionine, while hydrolysed feather meal is deficient in lysine (Lovell, 1998).

In addition, the sole use of certain animal by-products can result in serious dietary imbalance. For example, blood meal is very rich in leucine but contains only low levels of isoleucine. Leucine acts antagonistically to isoleucine. Accordingly, when high levels of blood meal are incorporated into a diet, the antagonism between leucine and isoleucine will result in the fish suffering from isoleucine deficiency (De Silva & Anderson, 1998). Poultry by-product meals are rendered products of poultry processing waste, made from inedible portions of poultry, excluding feathers. PBM has been studied as a partial fish meal replacement in the diets of channel catfish (Brown *et al.*, 1985), rainbow trout (Alexis *et al.*, 1985), and European eels (Gallagher & Degani, 1988). Generally, when PBM was added as a high percentage replacement for fishmeal protein, growth was compromised. Fowler (1982) reported that supplementing the diets (15% fishmeal, 35% PBM) with methionine and lysine, two amino acids suspected to be deficient in the diet, did not have a positive effect on growth of chinook salmon fry. The maximum replacement level of PBM for fishmeal in a practical diet for juvenile fall chinook salmon (*O. tshawytscha*) was 20% (Fowler, 1991).

It has also been tested in diets for chinook salmon (Fowler 1981a,b, 1990, 1991), coho salmon (*O. kisutch)* (Higgs *et al.;* 1979), and Atlantic salmon (*Salmo salar)* (Bergström, 1973). Additional results have been achieved with many other freshwater fish species. A standard poultry by- product meal available in the market, was also used as one of the main protein sources in all diets tested and it has also has a high protein level 58%. (NRC, 1993). Poultry feathers are an other commonly available by-product that is rich in protein (>80%). Unfortunately, this protein source is not being utilized for any productive purposes due to poor digestibility characteristics.

Poultry-feather meal has been included up to 10% level in the concentrates for dairy cattle (Gohl, 1981). It has also been used as a protein source in the diets for rainbow trout (*Oncorhynchus mykiss*) (Koops *et al.*, 1982), coho salmon (Higgs *et al.*, 1979) and chinook salmon (Fowler, 1990).

Blood meal has also been used for numerous species in aquafeed formulations. Spray-dried blood meals have desirable characteristics as a feed ingredient because they are high in digestible protein 99%, (Cho, 1990) and have a low phosphorus content 0.26% (Luzier *et al.*, 1995). Thus, commercial supplies of spray-dried animal blood cells (SBC) are available and not heretofore tested as an ingredient in fish diets.

This product is a high protein, (92%), high lysine (9.0% of protein content) feedstuff, and it contains only 0.33% phosphorous. However, SBC ($800/ton) is a more expensive product than flash-dried blood meals ($600/ton), (Feedstuffs, 1997).

Although blood by-products have many good qualities, they have a high iron content. The iron content of SBC (2.700mg kg^{-1}) is 23.7 times greater than herring meal (114mg kg^{-1}) and 19.3 times greater than soybean meal (140mg kg^{-1}) (NRC 1993). Increasing iron concentration of rainbow trout diets may increase lipid oxidation (Desjardins *et al.*, 1987). Oxidation of fish oils, which contain 20-25% polyunsaturated fatty acids, produces pro-oxidants that induce free radicals and peroxides (Lovell, 1988). These compounds react with other nutrients, reducing their biological value Goddard (1996), and resulting in poor growth and health (Lovell, 1988). Baker *et al.* (1997) have shown the negative effects of high iron diets on the African catfish *Clarias gariepinus*.

Among the protein sources studied, those of plant origin appear to be of most interest in spite of their lower total protein content and a possible deficiency of certain essential amino acids as compared to most animal protein sources. The efficiency of various alternative protein sources as partial or complete dietary replacements for fish meal has

been evaluated in fish diets, e.g. rapeseed meal for tilapia (Davies *et al.*, 1990) and salmon (Higgs *et al.*, 1979), pea seed meal for sea bass (Gouveia & Davies, 1998, 2000), soybean meal, spirulina meal, cotton seed meal and cake and other oilseed by products, including groundnut, sunflower, rapeseeds, sesame seeds, copra, macadamia and palm kernel (El-Sayed, 1990, 1994, 1999).

Hossain & Jauncey (1989) evaluated mustard, linseed and sesame oil cakes of Bangladeshi origin as dietary protein sources for the common carp, *Cyprinus carpio* fingerlings. Nevertheless, many of these ingredients have been used as dietary protein sources for other fish species, i.e. linseed Hasan *et al.*, (1989, 1991), mustard and sesame (Hasan *et al.*, 1991), and groundnut meal (Jackson *et al.*, 1982).

Among the plant protein sources, soybean meal has been shown to possess an acceptable amino acid profile for growth of several fish species (Wilson & Poe, 1985; Murai *et al.*, 1986) and may be used as the major protein source in many fish diets (Lovell, 1988). Nevertheless, soybean meals are not a local protein source for many countries and soybean meal dependence, as well as fish meal, have indicated the need for evaluating new alternative protein sources. In that sense, lupin and cottonseed meals as well as corn gluten meal appear to be interesting protein sources in certain circumstances. Lupin seed protein has been fed to rainbow trout at substitution levels for fish meal of up to 40% of a fish meal protein diet without any loss of feed conversion or protein retention efficiency (Moyano *et al.*, 1991). Digestibility trials are used extensively to assess the potential nutritive value of feedstuffs. However, the use of plant materials of high protein content in fish diets is desirable due to their relatively low price and continuous market availability.

The most widely tested plant materials are leguminous seed, and especially soybean, either in extracted or full fat form, due to their high protein content and good amino acid

profile. Soybean meal is a concentrated source of protein and energy and is lower in crude fibre than other oilseed meals. The higher protein, energy and lower fibre content of soybean meal enable formulation of diets that are proven to be more efficient in the conversion of feed to meat in most livestock.

Soybean meal is available in two different grades. Meals are produced following the same basic process, the only difference being that the 44% grade has some hulls blended back into the meal. Dehulled 47% protein soy meal is preferred by most nutritionists who which to achieve a dense ration, as there is no space for soy hulls in a dense ration designed for high production. Full fat soy is the whole bean toasted and provides a high protein source and high fat source, but should be carefully limited so the fat content does not go beyond normal levels in the total ration (Cooper & Benson, 2000).

Protein is a major nutrient of soybeans but is ranked second after oil in terms of economic value. Whole soybeans contain approximately 40% crude protein on a dry matter basis. Some strains of soybeans have as high as 48% crude protein (Pryde, 1983). These varieties, however, produce lower yields of crop. The most economically valuable component of soybeans is the oil, which is approximately 20% of their dry weight (Table1.3). Although oil and protein are components of major interest, full fat soybean meal (FFSB) contains substantial amounts of carbohydrates, approximately 30% on a dry weight basis.

The carbohydrate fraction of soybeans is usually classified in two categories, the soluble carbohydrates or oligosaccharides and the insoluble carbohydrates or polysaccharides. Sucrose, raffinose and stachyose are the major soluble carbohydrates in soybeans. Mature soybean seeds contain about 10% soluble carbohydrates, which comprise

approximately 55% sucrose, 1% raffinose and 45% stachyose (Snyder & Kwon, 1987). Raffinose and stachyose are not digested and absorbed by monogastric animals due to the lack of endogenous alpha-galactosidase. These sugars subsequently pass into the large intestine where microbial fermentation converts them into CO_2 and H_2 causing flatulence (Wolff, 1983; Snyder & Kwon, 1987).

Table 1.3 Nutrient composition of soybean products (NRC, 1982).

	Seeds	Seeds, heat process (FFSB)	Meal, mech. Extracted (Soybean cake)	Meal, solvent Extracted With hulls	Meal, solvent Extracted Without hulls
International Feed number	5-04-610	5-04-597	5-04-600	5-04-637	5-04-612
Dry matter	92.0	90.0	90.0	89.0	90.0
Crude protein	39.2	38.0	42.9	44.6	49.7
Ether extract	17.2	18.0	4.8	1.4	0.9
Crude fibre	5.3	5.0	5.9	6.2	3.4
Ash	5.1	4.6	6.0	6.5	5.8

In addition, soy protein concentrate (SPC) has been used as a protein source in diets for many fish species. Faster growth was reported in Atlantic salmon (Storebakken *et al.*, 1998) fed diets with 75% of total protein substitution from SPC.

Kaushik *et al.*, (1995) reported that rainbow trout *Oncorhynchus mykiss* can be fed diets containing up to 100% of dietary protein from SPC without negative effects on growth rate. Inclusion of 40% SPC for juvenile white sturgeon *Acipenser transmontanus* resulted in poorer growth compared to a purified test diet Stuart & Hung (1989). For yellowtail and for juvenile common carp *Cyprinus carpio,* it was shown that lower inclusion levels of SPC in the diets (20 and 40% respectively) were necessary to obtain sufficient growth (Takii *et al.*, 1990). However, high level of plant protein feedstuffs have been shown to reduce growth, especially due to lower feed intake (Reigh & Ellis, 1992; Gomes *et al.*, 1995).

Corn gluten meal has also been shown to be a suitable dietary protein source in fish diets (Ketola, 1982; Alexis *et al.*; 1985; Moyano *et al.*; 1991). Corn gluten meal is a high protein by-product of corn processing, during which most of the starch, bran, and germ are removed. The protein level ranges from 41-43% with the fat level remaining below 3%. There is also a 60% protein corn gluten meal available after further processing and refinement (Halver, 1989).

Refstie *et al.* (1997, 1998) reported that both rainbow trout and Atlantic salmon, *Salmo salar* adapt to soybean meal diets, and achieve equal feed intake after an adaptation period compared with a fishmeal diet. However, voluntary feed intake has been improved in European sea bass fed plant protein rich diets by supplementation with an attractant mix (Dias *et al.*, 1997).

The main limitations in the use of plant materials are certain amino acid deficiencies (Harris, 1980), and the presence of chemical compounds collectively known as anti-nutritional factors (ANF's) (Liener, 1980), which lower the nutritional quality and sometimes cause mortalities due to their toxic characteristics. Specification of the reduction in fish performance and determination of the maximum dietary levels of plant materials compatible with good fish growth are the first steps for further possible improvement of their quality and maximizing their inclusion levels in fish diet formulations.

1.9 Central objectives and aims of study

The review of the aquaculture potential and status of warmwater fish production in the African region and in Egypt especially has highlighted a number of important aspects that require further explanation and in-depth research of a nutritional nature.

Central to the development of sustainable and economic production of tilapia and catfish in Egypt, detailed knowledge of fish feed formulations based on locally available

materials and by-products is required. It is understood that there is a definite need to rationalize the use of fishmeal and other expensive imports. Therefore alternative protein sources must be considered and their potential value assessed for these important species of commercial value.

As stated previously, the major ingredients at our disposal are those derived from animal and protein sources. Chiefly among these, various by-products associated with the rendering industries e.g. feather meals, poultry meat meals, poultry by- product meal, blood meals are important commodities. Plant protein concentrates are largely obtained from soybean and corn based products and are of special concern too.

The central theme of the research programme was the assessment of specific ingredients for these groups in a series of comprehensive experiments designed to evaluate their use in tilapia and catfish feeds.

It was the prime objective to determine the optimum inclusion rates of selected protein sources within balanced diets under controlled experimental conditions.

Secondarily, the effects of inclusion level on production efficiency in terms of growth rates, feed utilization and general health of the fish species in question are an important consideration.

The main aims central to the programme of investigation may be summarized as:

1) Determine the digestibility coefficients of the nutrient components in raw materials and feed ingredients appropriate to tilapia.

2) Select those materials offering the best potential to replace fishmeal in successive nutrition trials applicable to both tilapia and African catfish for comparative purposes.

3) Explore the effects of fishmeal replacement on the general health of fish in terms of histological assessments and fish quality as determined by gross body composition.

4) To develop a better understanding of gastro-intestinal biochemistry associated with the feeding of novel ingredients to fish such as tilapia and catfish.

These collective aims and objectives serve to provide more detailed knowledge to advance our ability to develop better nutritional information for warmwater fish. The methodology and experimental techniques were in accordance with those advocated for fish nutrition studies and also include new approaches and developments where applicable.

CHAPTER 2

GENERAL MATERIALS, METHODS AND SPECIFIC TECHNIQUES

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2.1 Experimental animals

African catfish (*Clarias gariepinus*), were bred in the fish nutrition aquarium, Davy Building, Faculty of Sciences at the University of Plymouth.

The husbandry of the African catfish and spawning techniques are outlined as follows.

(1) Healthy male and female catfish >1kg in weight were selected and confined to separate tanks. The temperature was raised to 30^{0}C and water levels reduced in each tank to initiate pre-spawning. (2) The technique includes hormone injection. The hormone was prepared as recommended by Manickam and Joy (1989). 1mg of LH-RHa (Lutenizing hormone-releasing hormone analogue) was dissolved in 1ml of de-ionized water. This was then divided into 10 portions of 100 microlitres and stored at –20^{0}C.

The carrier solution was made by adding 5mg pimozide to a solution of 0.8g sodium chloride, 0.1g sodium metabisulphite and 0.25g bovine serum albumin in 100ml de-ionized water. Using a 3ml syringe, 100 microlitres of hormone solution dissolved in 2ml carrier solution was injected at the base of the fish's pectoral fin and massaged into the surrounding tissue. This dosage is suitable for a 1kg fish. After 16h, female fish were hand stripped and male fish sacrificed for milt. Fertilized eggs were transferred to rearing tanks until first feeding stages.

After hatching, fry were fed on brine shrimp (Artemia) for 2 to 3 weeks (in the period of yolk sac) and then fed on granular pelleted trout diets, trout and salmon starter (00). Fish were acclimated to the rearing tanks and grown to fingerling size prior the commencement of each experimental trial as shown in Figure 2.1.

Figure 2.1 African catfish *Clarias gariepinus* typically used in the experimental studies.

Tilapia fry and juvenile stock were obtained from the School of Biological Sciences, Department of Genetics, University of Swansea (Wales). The strain of tilapia was lake Manzala, Egypt, the *Oreochromis niloticus* were collected from lake Manzala by staff from the Institute of Aquaculture in Stirling in the mid 1970`s and have been maintained as a genetically pure strain ever since. Swansea University received these fish in 1983 with a few later introductions of the same stock from Stirling over the past 10 years (Figure 2.2).

2.2 Feed ingredients and standard diet preparations

All experimental diets were formulated according to the nutritional constraints typical for most warm water fish species (NRC, 1993). Diets justified to contain approximately level of protein and oil (36% CP; 15% lipid) and a low temperature fishmeal LT94 was the basic ingredient for all reference diets.

Isonitrogenous, energetic substitution of the fish meal component of the diet was made using each of the test protein concentrations evaluated. All diets were processed by blending the dry ingredients into a homogenous mixture with a Hobart A120 food processor. The required supplement oil for each diet was added gradually and after few minutes of mixing, 300ml of water was added to prime the binder per 1kg diet.

Once a homogenous mixture was obtained, the diets were extruded through a mincer into standard die holes of different diameters and broken into smaller pellets as required. The pellets were dried by air convection in an oven at 70^0C. After cooling, the diets were packed in a sealed airtight container and stored at -20^0C until used. Prior to feeding, the diets were further broken into small pellets (1-2mm diameter) to suit the mouth size of small fish. Table 2.1 shows all ingredients used throughout the experimental studies.

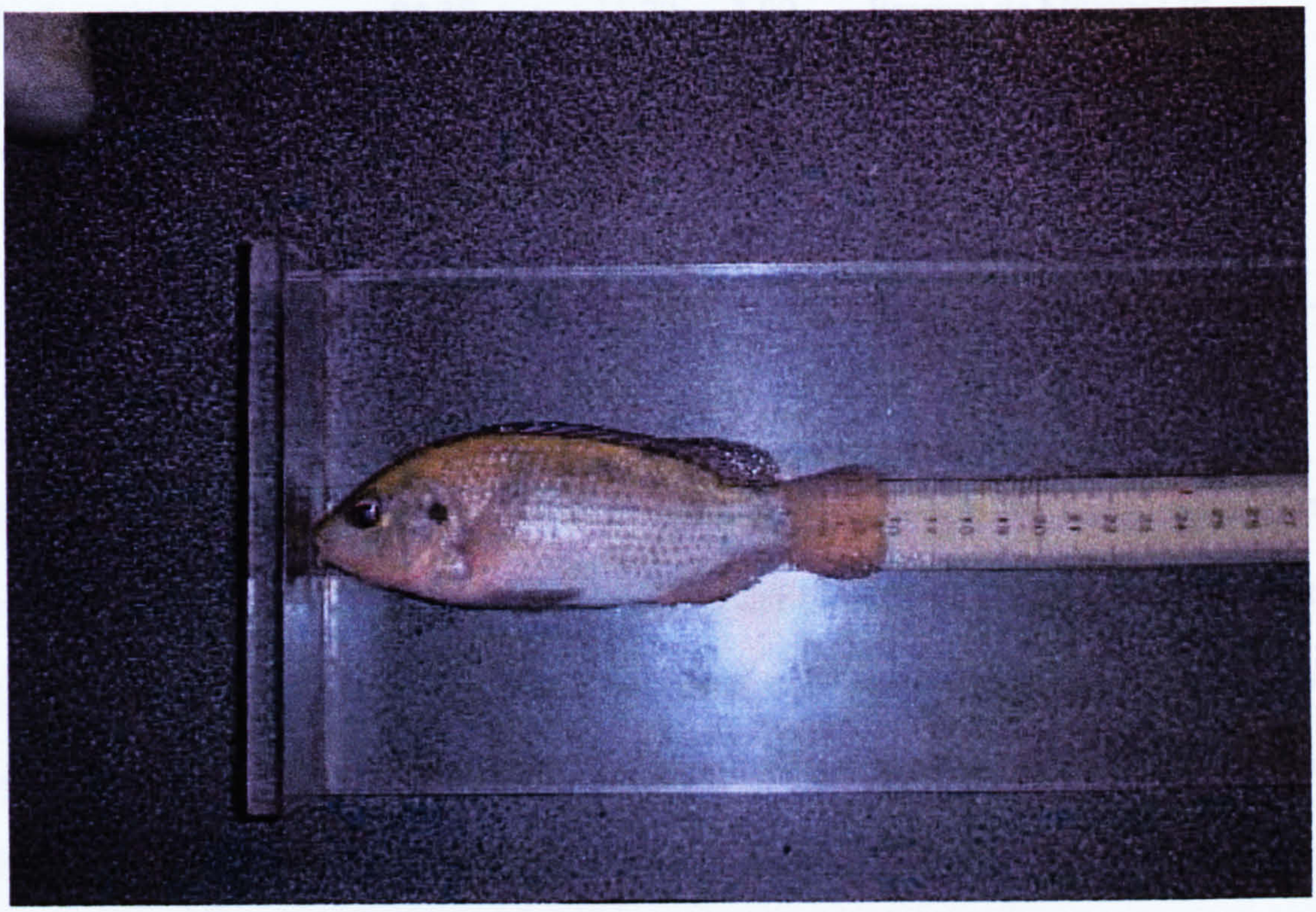


Figure 2.2 The Nile tilapia *Oreochromis niloticus* used in the experimental studies.

Table 2.1 proximate composition for practical ingredients (g100g^{-1} dry weight) used for digestibility determination and other experiments.

Ingredients	% Protein	Lipid	Ash	Source
Fishmeal (LT94)	71.30	11.3	13.00	Norwegian
White fishmeal	63.75	5.82	22.10	White fishmeal Ltd Hull, UK
Sopropeche fishmeal	70.26	16.20	5.60	CTPP Boulogne, France
Haem blood meal (Spray dried)	94.60	-	3.00	American Protein Corporation (Des Moines Iowa, USA)
Poultry by-product meal	60.00	19.50	15.60	Prosper de Mülder Ltd, UK
Feather meal	84.30	11.70	3.00	Prosper de Mülder Ltd, UK
Maize gluten meal	62.30	1.50	1.60	Cargill Ltd.
Solvent extracted soybean	48.00	3.00	8.20	Central Soya Michigan, USA
Full fat soybean	38.20	19.30	5.7	Central Soya Michigan, USA

2.3 Experimental system

During the course of the investigation, fry were acclimatized to the tank environment one-week prior to each trial and were fed on standard trout fry diets (Trouw 00, starter) until the feeding response was uniform. The trials were conducted in eight or ten round tank recirculation systems within the aquarium of the Davy building, University of Plymouth. The tanks were constructed from fibreglass being approximately 75 litres, and were suspended over a 1000 litre bio-filter (Figure 2.3). Water entered each tank via a spray bar after filtration and an aerator was placed in the centre of the tank. Partial water changes amounted approximately 20% of the systems volume per week. Filters of the systems were cleaned daily to avoid the build up of nitrate levels in the water.

A 12h light : dark cycle was provided by fluorescent lighting. The water temperature was maintained at 27± 1^{0}C by a thermostatically controlled immersion heater and pH, ammonia (NH_3), nitrite (NO^{-}_2) and nitrate (NO^{-}_3), were monitored and remained twice a week at defined levels throughout the experimental period.

Figure 2.3 photograph of main recirculation system used for the fish feeding and digestibility trials.

2.4 Proximate composition analysis

2.4.1 Determination of moisture content

The moisture content of feed and fish carcasses were determined as outlined in the A.O.A.C. handbook (1990). Thus, in summary, samples of feed materials, tissues or whole fish carcasses were weighed and dried to a constant final weight at 105^0C inside a fan assisted Pickerstone E70F oven (R E Pickerstone Ltd, Thetford, Norfolk). The percentage moisture in the sample was calculated thus:

Moisture (%) = weight of the moist sample (g) - weight of the dry sample (g) / weight of moist sample (g) × 100

Where;

Weight of moist sample = combined weight of the tray and contents - weight of tray.

Weight of dry sample = constant final weight - weight of tray.

2.4.2 Determination of protein content

The protein content of feed and fish carcasses was determined by the automatic Kjeldahl method. 500 mg of dried feed or carcass were weighed into borosilicate digestion tubes containing 20 ml of concentrated H_2SO_4 and 2 Kjeldahl catalyst tablets (2 × 3g K_2SO_4, 105mg $CuSO_4.5H_2O$ and 105mg TiO_2. Thompson and Capper Ltd, Runcorn, Cheshire). Digestion was carried out in a Gerhardt kjeldatherm digestion block which contained 20 places (Gerhardt Laboratory Instruments, Bonn, Germany) for 30 minutes at 250^0C followed by a further 2 hours at 380^0C with the acid fumes collected and neutralized by 15 % NaOH in a Gerhardt Turbosog unit.

After cooling, using a Gerhardt Vapodest 3S distillation unit, the sample was diluted with distilled water and reduced with 40 % NaOH. The ammonia in the sample was then collected into 50ml of saturated orthoboric acid (H_3BO_3) by steam distillation. Using BDH '4.5' indicator, the distillate was titrated against 0.20M HCl and the percentage protein in the dry sample determined thus:

% Crude protein = [titre sample (ml) - titre blank (ml)] × [0.2] × 14.007 × 6.25 /

weight of sample (mg) × 100

Where:

[0.2] = (HCl) in moles.

6.25 = Constant describing relationship between nitrogen and protein content of sample.

14.007 = Relative molecular mass of nitrogen.

Determination of protein content by micro Kjeldahl

The protein content of feed and fish carcasses was determined by micro Kjeldahl method. 100-150mg of dried feed or carcass was weighed into borosilicate digestion tube containing 10ml of concentrated H_2SO_4 and 1 Kjeldahl catalyst tablet (1 × 3g K_2SO_4, 105mg $CuSO_4$. $5H_2O$ and 105mg TiO_2. Thompson and Capper Ltd, Runcorn, Cheshire).

Digestion was carried out in a Gerhardt kjeldatherm digestion block which containing 40 places (Gerhardt Laboratory Instruments, Bonn, Germany) for 30 minutes at 250^{0}C followed by a further 1 hours at 380^{0}C with the acid fumes collected and neutralised by 15 % NaOH in a Gerhardt Turbosog unit.

After cooling, using a Gerhardt Vapodest 40 distillation unit, the sample was diluted with distilled water and neutralized with 37 % NaOH. The ammonia in the sample was then collected into 50ml of 4% orthoboric acid (H_3BO_3) by steam distillation, which have been added automatically by the machine. Using BDH `4.5` indicator, the distillate was titrated against 0.10M HCl.

% Crude protein = [Titre sample (ml) - Titre blank (ml)] × [0.1] × 14.007 × 6.25 /

weight of sample (mg) ×100

Where:

[0.1] = (HCl) in moles.

6.25 = Constant describing relationship between nitrogen and protein content of sample.

14.007 = Relative Molecular mass of nitrogen.

2.4.3 Determination of total lipid

Total lipid in samples of feed and carcass was determined by Folch *et al.* (1957). 500mg of dry material was weighed into a 50ml Erlenmeyer flask to which 10ml of chloroform: methanol (2:1) was added. The flasks were sealed and left overnight at room temperature to allow the lipids to be dissolved out of the samples. At the end of this period, the extract was filtered through a Whatman ashless filter paper into a test tube and the residue in the Erlenmeyer quantitatively removed using a further 10ml of 2:1 chloroform: methanol. Duplicate 5ml aliquots were transferred to a pre-weighted test tube. All the test tubes were placed onto a rack in a water bath with the temperature at 55^0C, to allow total evaporation of the chloroform and methanol.

The test tubes were then air-dried and reweighed. The lipid content of the test samples could then be calculated using the following equation:

% Lipid = weight of lipid residue collected (g) / weight of sample (g) × 100

2.4.4. Determination of ash content

The ash content of the dry material was determined as outlined in the A.O.A.C. handbook (1990). Thus, 500mg of dry sample were weighed into a crucible and ignited for 12 hours at 600^0C in a Carbolite GLM 11/7 furnace (Carbolite Furnaces Ltd, Bamford, Sheffield). The residual weight of the crucible was proportional to the ash content of the sample and hence the percentage of ash in the sample was calculated thus:

Ash (%) = Weight gained by crucible (g) / Weight of sample (g) × 100

2.4.5 Energy content

Analysis was performed in an adiabatic Bomb Calorimeter (Gallenkamp and Co. Ltd, Loughborough, England). Here a known quantity of the test material is placed in an insulated metal container and oxidized by combustion to carbon dioxide, water, ash, nitrogen, and other gases, and the liberated heat energy is measured.

Approximately 0.5g of benzoic acid as standard was pressed into a pellet. Then the pellet was suspended by gun cotton from a platinum wire connecting the anode and cathode inside the bomb also approximately the same amount of diet or faeces were pelleted. Absorption of the combustion gases was achieved by inclusion of 1ml of water in the bomb. The bomb was then filled with pure oxygen to 30 bar and immersed in a water jacket of known temperature. The bomb was fired and the maximum temperature reached by the water jacket was recorded. The energetic value of benzoic acid standard, diet and faecal samples was calculated using the following formula:

Energy value in J/g (dry weight) = Δt Sample × 26456.39 / (Δt (Benzoic acid) / g Benzoic acid) × weight of sample (g)

T= Temperature rise (^{0}C)

To convert Joules to calories divided by 4.1868

Convert Mkg^{-1}.

2.4.6 Determination of amino acids

The amino acid contents of the diets were determined in acid hydrolysates. Approximately 20-25mg of ground sample were weighed into 5ml vials with 4ml (6.6M) of HCl + 1ml (0.1 M) phenol to protect tyrosine, each vial was sealed and placed in an oven for 22 hours at 110^{0}C. Amino acids were assayed at Loughborough University, Department of Chemical Engineering, using a Kontron Chromakon 500

automatic amino acid analyser (column 250 × 4.6mm, cation ion-exchange resin material (AS70).

The mobile phase was a gradient of sodium citrate-based buffers according to the following composition: 1) 0.22M Sodium citrate, adjusted to pH 3.2 with concentrated HCl, + 8% v/v methanol; 2) 0.067M Sodium citrate + 0.5M Sodium chloride, adjusted to pH3.79 with concentrated HCl; 3) 0.067M Sodium citrate + 1.4M Sodium chloride adjusted to pH4.3 with concentrated HCl.

Detection was by a post column reaction with ninhydrin (in 4M lithium acetate buffer pH5.2 flow rate 12mlh^{-1}) at 115^{0}C in a reaction oven followed by visible absorption measurement at 570nm and 440nm to produce a mean signal for quantitative integration. Dilution was made by loading buffer (2.2 pH sodium citrate buffer) and suitable 100µl aliquots injected onto the column. The amino acid composition (expressed as g 16 gN^{-1}).

Amino acid of all samples was calculated using the following formula.

Amino acid mg100g^{-1} sample =

$$\frac{\text{Area sample peak}}{\text{Area standard peak}} \times \frac{\text{concentrate of standard} \times 5 \times \text{dilution}}{\text{weight of sample (mg)}} \times 100$$

2.5 Determination of liver glycogen

Glycogen was determined using a method derived from that outlined in Plummer (1987).

Glycogen was liberated from the tissue by heating with KOH and then precipitated with ethanol using sodium sulphate as a co-precipitant to give a quantitative yield.

The glycogen was then acid hydrolysed to liberate the glucose for determination by the glucose oxidase method. 0.5g of tissue was weighed into a calibrated centrifuge tube containing 2ml of KOH (300gl^{-1}) and was heated for 20 minutes with occasional shaking in a boiling water bath.

The tubes were cooled on ice and 200µl of saturated Na_2SO_4 were added with shaking. 5.0ml of ethanol (95 % v/v) was then added and, following vortex mixing, the tubes were allowed to stand on ice for five minutes prior to centrifugation at 1500 ×g.

The supernatant was discarded and the pellet was then resuspended with gentle warming in 5ml of distilled water before dilution to a final volume of 10ml.

Using a centrifuge tube calibrated to 10ml, 1ml of the glycogen solution was added to an equal volume of HCl (1.2mol l^{-1}) and heated in a boiling water bath for 2 hours. At the end of this period, the hydrolysate was neutralised with NaOH (0.5mol l^{-1}) using phenol red as an indicator. The solution was then made up to a final volume of 5.0ml and 25µl was then withdrawn to concentration of glucose in the hydrolysate by glucose oxides method as outlined in 2.5.1.

2.5.1 Determination of glucose

The concentration of glucose in the test solution was determined by the glucose oxidase method as described by the Sigma procedure No. 541. For the test, standard and blank 25µl of sample, glucose standard (100mgdl^{-1}) and water respectively were added to 0.5ml of distilled water. 5.0 ml of combined enzyme colour reagent was added to all the tubes which were then incubated at 37 ^{0}C for 30 minutes.

The absorbance of sample was then read against blank at 450nm using a Cecil Series 5000 U.V. Vis. spectrophotometer. Having demonstrated that the response of the assay was linear within a glucose concentration of 300mgdl^{-1}, the concentration of glucose in the sample was determined thus:

$$\text{glucose (mgdl}^{-1}) = \frac{\text{Absorbance of test solution}}{\text{Absorbance of standard}} \times 100^{a}$$

[a] Where (standard) = 100 mgdl^{-1}

The glycogen content of the tissue (g/g wet weight) was then determined thus:

Weight glycogen =

(Glucose) in hydrolysate ($mgdl^{-1}$) × 50 × 0.9[a](g/g wet weight) / Weight of sample (g)

[a] Where due to the difference in molecular weight, 0.9 is the factor allowing the estimation of glycogen from the measured glucose content of the tissue.

2.6 Determination of gastro-intestinal enzymes for fish fed experimental diets

2.6.1 Enzyme sample preparation

Five fish from each group were dissected at the end of each nutrition trial were enzymatic profile of the gastro-intestinal tract were assayed. Stomach, liver, and gut were removed and the organs were cleaned as much as possible of remaining fat, faeces or food and kept in small plastic bags individually before freezing (-80^{0}C) until use for the enzyme assays.

2.6.2 Extraction of enzymes

Frozen samples of stomach, liver and gut were weighed into propylene tubes with 5-10 volume (200$mgml^{-1}$- 100$mgml^{-1}$) of cooled distilled water depending on organ size. The samples were homogenized separately in an electrical homogeniser (Ultra-Turrax-T8 IKA).

The tubes were surrounded by ice during homogenization. Homogenate samples were centrifuged at 30000 ×g (Jouan KR 22 with Rotor AK 16-20) for 30 minutes at 4^{0}C. Supernatant was removed and frozen at -20^{0}C for further analysis.

2.6.3 Trypsin activity

Trypsin activity was assayed in test tubes using benzoyl-Arg-*p*-nitroanilide (BAPNA) as substrate according to (Erlanger *et al.*, 1961). BAPNA substrate: prepared by dissolving 0.044g of BAPNA (Sigma 4875) was dissolved in 1ml dimethyl sulphoxide (DMSO) to

make a 1mM solution of the substrate and then made to 100ml with 50mM Tris-HCl buffer, 20mM $CaCl_2$, (BDH 262244W) pH7.5, at 37^{0}C, to allow substrate solubilization.

2.6.3.1 Procedures

Duplicate of 0.5ml of enzyme extract were mixed in 15ml test tubes with 2ml of BAPNA substrate and incubated in a water bath at 37^{0}C for 10 minutes. After incubation 1ml of trichloroacetic acid (8%) was adding to each tube to stop the reaction and tubes were then centrifuged for 5 minutes at 13000rpm 1ml of supernatant was read using (UNICAN HELIOS UV-VISIBLE) a spectrophotometer at 410nm against a blank. Trypsin activity was expressed as the amount of tyrosine (μg) liberated by 0.5ml of enzyme solution at pH7.5 per minute at 37^{0}C.

2.6.4 Proteolytic activity

Total proteolytic activity was measured using the casein hydrolysis method of Kunitz, (1947) as modified by Walter (1984). The determination was conducted using a range of pH values. The buffers used were (pH 1.5) 0.1 M KCl-HCl, (pH 3.0) 0.2 M glycine-HCl, (pHs4.0 & 7.0) 0.1M citrate-0.2M phosphate, (pHs8.5 & 9.0) 0.1M Tris-HCl and (pH10.0) 0.1M glycine-NaOH.

2.6.4.1 Casein substrate

Substrate was prepared by dissolving 1 gram of casein (Sigma 8654) in 100ml of distilled water. The suspension was heated in a water bath at 60^{0}C for complete solution of casein.

2.6.4.2 Procedure

The enzyme reaction mixture consisted of 1% (w/v) casein in distilled water (0.25ml), buffer (0.25ml), and enzyme sample (0.1ml) were incubated for 1 hour at 37^{0}C. The reaction was stopped by adding 0.6ml of 8% (w/v) trichloroacetic acid. After holding for 20 minutes on ice, samples were centrifuged at 1800 ×g for 10 minutes (Mistral 3000 centrifuge). Absorbance of the supernatant was recorded at 280nm (UNICAN HELIOS UV-VISIBLE). All samples were assayed in triplicate and two blanks with no incubation were used by adding the supernatant just before stopping the reaction with trichloroacetic acid.

2.6.4.3 Tyrosine standard

Prepared by dissolved 0.02g of Tyrosine (Sigma 3379) in 20ml of 0.2M HCl. Several dilutions with 0.2M HCl were prepared to obtain a 10, 25, 50, 75, 100, 250 and 500µg concentration of tyrosine in 1ml to construct a standard curve. One ml of each dilution was treated as sample.

2.6.4.4 Determination of protein

Protein determination of gut, liver and stomach supernatant was determined according to the Lowry method (Lowry *et al.*, 1951), 0.5 ml of supernatant of sample was mixed with 0.5ml of Lowry reagent (Sigma 1013) in 15ml test tubes. The tubes were left to stand for 20 minutes at room temperature and then 0.250ml of Folin & Ciocalteu's phenol reagent (Sigma 9252) were added to each tube with rapid and immediate mixing. The tubes were left at room temperature for another 30 minutes to develop the colour. The colour formed was read on a spectrophotometer (Model V-530) at 500nm against blank. The blank was prepared in the same manner except 0.5ml of water was used instead of 1ml of supernatant.

2.6.4.5 Protein standard

Protein standard was prepared by dissolving 2mg of bovine serum albumin (Sigma 7656) in 5ml of distilled water. Several dilutions with distilled water were made to obtain a 50, 100, 200, 300 and 400µg concentration of protein in 1ml to construct a standard curve. One ml of each dilution has been treated as a sample and blank.

2.6.5 Lipase activity

Lipase activity was assayed with the aid of a Sigma diagnostic test-kit (procedure No. 800). The procedure depends upon the hydrolysis of triglycerides in olive oil into fatty acids and diglycerides. The amount of fatty acids formed, under the specific conditions of the test, is a measure of lipase activity in the sample. The fatty acids formed are determined by titration with 0.05N sodium hydroxide.

2.6.5.1 Procedures

Triplicates of 1ml of enzyme extract were mixed in 15ml test tubes with 3ml of Sigma lipase substrate (Sigma 800-1) and 1ml of Trizma buffer (Sigma 800-2). Tubes were incubated in a water bath at 30^0C for 3 hours. At the end of the incubation time 3ml of 95% ethanol were added to each tube to terminate the reaction. Samples then were subjected to titration with 0.05N sodium hydroxide. Prior to titration 6 drops of thymolphthalein indicator solution (Sigma 800-3) were added to the sample.

The blank sample was incubated alongside the tested sample and treated in the same manner except that 1ml of enzyme extract solution was added at the end of incubation time. The difference in titration volume between sample and blank was to determine the quantity of fatty acids liberated during incubation time by 1ml of extracted enzyme solution.

2.6.6 Amylase activity

Amylase activity was determined by the starch hydrolysis method according to Tietz (1970). The procedure depends on the liberation of maltose from the substrate by extracted enzyme solution under standard conditions.

2.6.6.1 Starch substrate

Substrate was prepared by dissolving 1gram of starch (Sigma 9765) in 100ml 0.1M phosphate citric buffer (pH7.5). Concentrated HCl and 0.5N NaOH were used to adjust the final pH.

2.6.6.2 Dinitrosalicylic acid reagent

The reagent was prepared by dissolving 5grams of 3,5-dinitrosalicylic acid (Sigma 0550) and 150grams of sodium potassium tartrate (Sigma 6170) in 150ml of distilled water and 200ml of 1M sodium hydroxide. The mixture was refluxed in a water bath at 60^0C until all components were totally dissolved and then made up to 500ml with distilled water.

2.6.6.3 Maltose standard

Maltose standard was prepared by dissolving 20mg of maltose (Sigma 9171) in 20ml of distilled water. Several dilutions with distilled water were made to obtain a 1.0, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.05mg concentration of maltose in 1ml to construct a standard curve. One ml of each dilution was treated as sample.

2.6.6.4 Procedure

The enzyme reaction mixture consisted of 1% (w/v) starch solution (0.125ml) in 0.1 phosphate citric buffer, pH7.5 (0.125ml) and enzyme sample (0.05ml). The reaction mixture were incubated for 1 h at 37^0C. After this incubation time 2ml of dinitrosalicylic

acid reagent were added to terminate the reaction. Samples were then incubated in boiling water bath for 5 minutes. The absorbance of sample was measured at 600nm. All samples were assayed in triplicate. Maltose was used as the standard and amylase activity was expressed as µg maltose released from starch ml^{-1} $minute^{-1}$.

2.7 Growth performance indicators

Several nutritional parameters relevant to growth and feed utilization efficiency were employed throughout the current programme of work and these are defined accordingly.

2.7.1 Specific growth rate

Specific growth rate (SGR%) is used to compare growth of fish on a relative daily basis expressed as percent increase in initial live weight over a defined period of time and hence reflecting the instantaneous rate of growth.

$$SGR = In\ W2 - In\ W1 / T \times 100$$

Where,

W2 = Final weight (g)

W1 = Initial weight (g)

T = Defined time period (days)

2.7.2 Feed efficiency

Feed efficiency relates the ability of the feed to support weight gain with respect to the amount of feed consumed or put simply, the extent to which feed is utilized for growth. Feed efficiency may be expressed as the feed conversion efficiency (FCE) or as the feed conversion ratio (FCR). The latter term is widely accepted in practical fish and animal nutrition field trials.

FCE (%) = Live weight gain (g)/ feed intake (g) × 100

FCR = Feed intake (g) /Weight gain (g)

2.7.3 Protein utilization

The utilization of protein for growth may be expressed as either the protein efficiency ratio (PER) or the apparent net protein utilization (ANPU). Apparent net protein utilization (ANPU) is a more precise index of the amount of the dietary protein utilized or retained by the fish. Also, ANPU known as protein retention (%PPV), is a better measure of the feed quality than protein efficiency ratios (Lie *et al.*, 1988). It is given by the formula:

ANPU (%) = (% final body protein × final body weight) - (% initial body protein × initial body weight) / total protein intake (g) × 100

PER = Increase in body weight (g) / Protein consumption (g)

2.8 Histological studies

2.8.1 Histological preparation and staining techniques

At the termination of the feeding trial, five fish from each group were sacrificed and their livers and gut removed for histological examination for comparative purposes of catfish and tilapia, and also processed according to the following procedures. All livers were dissected to give an equal size piece of tissue with 5mm long square faces. This piece of tissue was immediately fixed in buffered formol saline. Subsequently all the pieces were processed together, separately labelled and encased, in a Shandon Elliot Hypercentre II tissue processor (Shandon Southern Products Ltd, Runcorn. UK). This allowed dehydration in a graded series of alcohols, clearing in xylene and infiltration in Fibrowax. Then the pieces were embedded in Fibrowax Mpt 56^0C. Sectioning was at 10 and 7μm using disposable blades and a Leitz rotary microtome (R.JUNG). Short ribbons of sections were placed into a heated water bath to flatten the sections. Then

they were mounted onto glass slides and dried before staining. According to Peacock (1973), Mallory's trichrome was used to elucidate the general histology of the livers.

2.8.2 Image analysis

Stained slides were examined with a Zeiss photomicroscope II and the images captured using an Hitachi 3CCD colour camera. The analogue signal was then imported into a Quantimet Q570 image analyser (Cambridge Instuments, Cambridge. UK). Once the binary image was created it was measured for lipid area using a macro to ensure reproducability of result. The results of the feature measurement menu were saved to disc and imported into Microsoft Excel for statistical analysis.

2.8.3 Photomicrographs

Photomicrographs were taken using the Zeiss photomicroscope II and a Nikon 950 coolpix digital camera at an objective magnification of ×40 and photo-eyepiece ×2.5. A green filter was used for all photomicrographs and the condenser iris position kept constant.

2.9 Statistical treatment of data

Statistical evaluation of the data was conducted using the computer software application Statgraphics plus (Statistical Graphics Corporation, USA). ANOVA was used to identify any statistical differences ($p< 0.05$) in weight and body composition etc., resulting from feeding each test diet formulation. Duncan's New Multiple Range Test was subsequently used to identify the significance differences between treatment mean values for selected parameters. Where appropriate, arcsine transformation of data was applied. For example, percent nutrient composition of fish carcass were data is presented as mean percentage values for moisture, protein lipid and ash respectively.

CHAPTER 3

NUTRIENT DIGESTIBILITY COEFFICIENTS AND AMINO ACID AVAILABILITY OF SOME PLANT AND ANIMAL PROTEINS FOR NILE TILAPIA *Oreochromis niloticus.*

3.1 Introduction

The digestibility of various feed ingredients by several species of fish has been previously reported (NRC, 1993), and there appear to be considerable differences among species in their ability to digest and absorb nutrient components from the array of materials available. It is important to evaluate the nutrient value of alternative ingredients in order to more accurately formulate complete diets and reduce our dependence on fishmeal as a protein concentrate in tropical fish diets. Cho & Kaushik (1990) stated that our knowledge was still largely insufficient and much more research is required especially for warmwater fish species.

Rodrigues (1994) reviewed the status of digestibility studies in fish from a physiological and applied context and reported data for many feed ingredients. There is a myriad of feed ingredients and materials available for fish diets and many new products are being considered as technology improves and even traditional feedstuffs are upgraded in quality and consistency. Determination of the digestibility of nutrients in raw materials is not only important to enable accurate diet formulation but may allow maximization of nutrient availability and contribute towards reducing waste.

This is especially important with respect to the reduction of organic matter output and also nitrogen and phosphorus limitation as the two primary excretion products of concern (Ketola, 1985).

In terms of practical use for feed formulation, Nengas *et al.* (1995) were able to obtain digestibility coefficients (DC) for a selection of ingredients for Gilthead seabream, *Sparus aurata*. Likewise Lupatsch *et al.* (1997) reported DC values for various animal and plant protein concentrates for use in the same species.

Nutrient digestibility of common feedstuffs in extracted diets for sunshine bass *Morone chrysops* × *M. saxatilis* were also comprehensively determined by Rawles & Gatlin (2000). These workers obtained data for the ADC's for protein, lipid, carbohydrate,

gross energy, and organic matter in a variety of feedstuffs in extrusion-processed diets. Included in the study was a low temperature fishmeal, meat and bone meal, fishmeal analogue, de-hulled soybean meal, cottonseed meal, corn grain and wheat flour.

The major factor influencing the digestibility values of nutrient components in ingredients are the types of processing and technology applied in their preparation. The majority of raw materials are greatly affected by such processes and this is true of the main proteins such as fishmeal and various animal by-products and plant/cereal by-products conventionally used in aquafeeds. Most investigations have focused on the latter concentrates since plant protein sources demonstrate greatest changes as a consequence of processing. The source and level of protein and carbohydrates are principal determinates of the apparent digestibility coefficients (ADC) values for both these nutrient components and consequently, for energy and dry matter (Hajen *et al.*, 1993; Arnesen *et al.*, 1995). Indeed it was stated by McGoogan & Reigh (1996) that protein digestibility was highest in feedstuffs with relatively high protein content (>60%) and low fibre content (<2%). In their studies with red drum (*Sciaenops ocellatus*), these latter researchers found that apparent energy digestibility values were somewhat lower for animal and certain plant by- products compared to premium grade fishmeals.

Practical diets for fish depend on a broad range of materials, ingredients and commodities from the marine environment, agriculture and biotechnological industries. Fishmeal is by far the standard ingredient that is employed in the majority of aquafeed formulations and its contribution to the protein component of diets destined for carnivorous species and especially high value marine fish is significant. Fishmeal is also important for other species such as carp, tilapia and African catfish but may be appreciably reduced in practical diets for warm-water omnivorous fish. The variation in fishmeal quality is reflected by its digestibility, but this remains very high in general.

Watanabe *et al.* (1996) reported that digestibility of protein in white fishmeal, local fish meal (Japanese) and meat meal was more than 90% for all species investigated by these workers (i.e. rainbow trout, carp, tilapia and Ayu) with the exception of meat meal in rainbow trout. However, digestibility of fishmeal in rainbow trout was lower (Smith *et al.*, 1980) than in feeds for channel catfish *Ictalurus punctatus* (Brown *et al.*, 1985).

It should be noted that Gaylord & Gatlin (1996) found that the apparent digestibility coefficients of the protein in different types of fishmeal were variable for the red drum (*Scianops ocellatus*) and this is also the case for most fish. Fishmeal digestibility (LT) serves as an important criterion and reference in our assessment of most protein rich alternatives and Pike *et al.* (1990) has cautioned the rationale of comparing alternative protein and energy concentrates when lower quality fishmeals were used as the reference material.

The most promising class of feed ingredients for which digestibility data has been reported for fish are those derived from the rendering industries, i.e. animal by-products and various meat, blood and feather meals. These offer a consistent source of digestible protein and fat, but may be affected by the nature of the industrial processing applied during the extraction and separation stages together with the heat treatments associated with drying and grinding.

Aksnes *et al.* (1997) noted lower protein digestibility in pelleted feeds than in extruded feeds in gilthead sea bream *Sparus aurata* for a number of animal protein sources. Hajen *et al.* (1993) reported greater digestibility of organic matter, crude protein and gross energy in Kansas poultry by-product meal than in local poultry by-product meal for chinook salmon *Oncorhynchus tshawytscha*. However the feather meal protein evaluated was reasonably well digested by this carnivorous marine fish species. The

apparent digestibility of crude protein and gross energy in blood meal however was quite low for the chinook salmon.

Studies on the apparent digestibility of crude protein and gross energy of blood meal by rainbow trout *Oncorhynchus mykiss* and Atlantic salmon, *Salmo salar* report conflicting results (Cho & Slinger, 1979; Smith *et al.*, 1980; Cho *et al.*, 1982; Lall *et al.*, 1984; Asgard & Austreng, 1986). However, protein and energy digestibility of poultry by-product meal (PBM) appeared low for red drum compared to values reported for rainbow trout and chinook salmon (NRC, 1993).

Allan *et al.* (2000) reported that compared to fishmeal, dry matter, energy, nitrogen and amino acid availability for several animal meals were somewhat lower for the Australian silver perch, *Bidyanus bidyanus.* For ingredients with relatively high total protein content (e.g., poultry offal meal, feather meal, blood meal and gluten meal), total digestibility of dry matter and energy was similar to fishmeal, and protein digestibility was in the range 85-99%, compared with 89-94% for fish meal. However, essential amino acid content, profile and availability of these ingredients were lower than in fishmeal.

The quality of animal by-product meals may vary considerably depending on the consistency and relative ratio of different waste products and offal that comprise the meals. Dong *et al.* (1993) noted a range of protein digestibility values from 64 to 78% for poultry by-product meal from different processing sources fed to rainbow trout *Oncorhynchus mykiss.*

Renewed interest continues to examine the value of animal and poultry based by-products for fish by means of establishing their digestibility. For example, Serwata, Davies & Jauncey (unpublished data) recently confirmed excellent digestibility for protein and amino acids for poultry by-product meals (PBM); feathermeal/PBM blended products and a refined blood meal for rainbow trout.

Plant proteins have always been viewed as good secondary ingredients that may substitute for fishmeal in aquafeeds. As stated in the introductory Chapter, they have a reasonably balanced amino acid profile but contain a number of anti-nutritional factors (ANF's).

An extensive amount of research effort has been directed towards evaluating the digestibility of protein, essential amino acids and energy content of various oil-seed and legume derived protein by-products for fish.

Digestibility profiles of plant feedstuffs and by-products are known to be particularly affected by the source of the ingredient, processing techniques and the target fish species under consideration. There have been numerous investigations focussing on plant protein concentrates with a majority of the trials characterising the digestibility of soyabean meals and associated products. There are many other plant protein ingredients important to aquaculture that require evaluation with respect to the digestibility of their nutrient composition for fish.

Most experiments have shown that soybean can partially substitute fishmeal in salmonids without adverse effects (Cho *et al.*, 1974; Pongmaneerat & Watanabe, 1992, 1993a; Viyakarn *et al.*, 1992; Oliva-Teles *et al.*, 1994) and for tilapia (Shiau *et al.*, 1987, 1990). Soybean meal was also highly digested in fish as already found by many researchers. Gaylord & Gatlin (1996) demonstrated that protein digestibility values for red drum *Sciaenops ocellatus* for dehulled soybean meal and cottonseed meal were approximately similar.

However, values for other species have been reported for soybean meal ranged 77% for chinook salmon to 93% for channel catfish, and values for cottonseed meal have ranged from 76% for rainbow trout to 83% for channel catfish (NRC, 1993). Wheat protein digestibility has been high for all fish species studied (NRC, 1993).

Few studies report apparent digestibility coefficients of feed ingredients for tilapia (Kamarudin *et al.*, 1989; Hossain *et al.*, 1992; Sintayehu *et al.*, 1996; Degani *et al.*, 1997 and Fagbenro, 1998). Sintayehu *et al.* (1996) recorded better protein digestibility for soybean meal than of Hanley (1987) in Nile tilapia *Oreochromis niloticus.*

Apparent soybean meal protein digestibility in eel *Anguilla anguilla* (Schmitz *et al.*, 1984) and crayfish (Reigh *et al.*, 1990) as well as the results of (Sintayehu *et al.* 1996) with tilapia were almost similar. Although the ADC values of the legume seeds were not different between different types of legume seeds, the essential amino acid availability measurements were quite variable. The ADC for gross energy in full fat soybean, winged bean and African locust been seed meals were fairly high for tilapia (Fagbenro, 1998).

Similarly, protein digestibility and amino acid availability was determined by Sadiku & Jauncey (1998 & 1995) for African catfish fingerlings *Clarias gariepinus* and tilapia, *Oreochromis niloticus* fed soybean flour-poultry meat meal blend. They reported agreement between the pattern of overall protein digestibility and average amino acid availability for this species. It was also cautioned that the level of dietary marker can affect the results. The optimum level in experiments for both tilapia and catfish need to be established. They also reported that the apparent amino acid availability for tilapia, *Oreochromis niloticus* was highest for methionine while the lowest was for the amino acid cysteine.

Wilson *et al.* (1981) studied the availability of amino acids in soybean meal for channel catfish *Ictalurus punctatus* and reported that arginine was the most available while the lowest available was glycine. The digestibility characteristics of grain legumes, various pulses such as peas and beans have been determined for some fish groups and individual species.

De la Higuera *et al.* (1988) also reported that up to 30% lupin seed meal as a replacement for fishmeal can be used successfully in diets for rainbow trout.

Burel *et al.* (2000) determined apparent digestibility coefficient for whole-extracted peas and de-hulled extracted lupins for rainbow trout. Whilst Gouveia & Davies (1998) found that juvenile European sea bass can be fed diets containing up to 40% ground whole peas *Pisum sativum* without any apparent effects on digestibility of dietary protein and little if any effect on digestibility of dietary energy.

Recently, it was demonstrated that no appreciable differences between the digestibility of dietary protein, energy or carbohydrate for juvenile sea bass occurred when fed up to 30% of an extracted, de-hulled and de-fibred pea seed flour (Gouveia & Davies, 2000).

Fontaínhas-Fernandes *et al.* (1999) showed that extracted pea seed meal and de-fatted soybean meal exhibited the highest ADC values of the plant proteins tested, while micronized wheat and full-fat toasted soybean showed slightly lower digestibility values for Nile tilapia.

Carbohydrate digestibility is also worthy of investigation due to the fact that many feed ingredients such as cereals, pulses and grains are sources of starch and sugars that provide an appreciable form of available energy. Fish vary considerably with respect to their ability to effectively digest and assimilate carbohydrates from the diet. Nonetheless, such ingredients are often included in the compound diets of farmed fish as either a source of energy or as a 'filler' component that aids in the expansion process associated with extrusion technology. This is important for the production of slow sinking pellets, or as a means of including higher oil levels in feeds.

Over 30 years ago, Kesamaru & Fukuda (1971) demonstrated a high digestibility for wheat germ in diets for carp and related fish. Indeed, carbohydrate was widely used in an array of fish feeds across the species range and even in diets for salmonids at up to

20%. Digestibility of starches in carnivorous fish has been shown to be increased by gelatinization (Inaba *et al.*, 1963; Bergot & Breque, 1983; Kim & Kaushik, 1992; Hajen *et al.*, 1993). Also, Arnesen *et al.* (1995) reported that Atlantic salmon *Salmo salar* utilized significantly more starch from a diet with oat and maize mix than from diets with only oats or maize.

Degani & Revach (1991) reported that there are differences in the abilities of carp, *Cyprinus carpio* L., tilapia, *Oreochromis aureus* x *O. niloticus,* and catfish *Clarias gariepinus,* to digest carbohydrates. This also showed that tilapia which represents an omnivorous fish, digest carbohydrates fairly efficiently. (Anderson *et al.*, 1984) and (Viola & Arieli, 1983) have shown that tilapia can digest animal protein (fish and poultry meal) better than carp. Carp *Cyprinus carpio* is also omnivorous in nature, but finds most of its food from benthic sources, the natural food of tilapia contains a higher percentage of plant material. However, African catfish digested a high animal protein diet efficiently, but not a plant protein diet, while utilizing fish meal better than poultry meal (Degani *et al.*, 1989).

Investigations that address the need to determine the digestibility profile of potential ingredients for use in fish diets are a pre-requisite step prior to more intensive studies involving long-term fish feeding trials of the classical approach. A majority of studies report the apparent coefficients for key nutrients in separate experiments where the test ingredient is included at a set level within a reference diet usually based on a fishmeal protein source (Cho & Slinger, 1979). These experiments are often of a short duration and involve a faecal collection stage. Digestibility trials with fish are fraught with difficulties associated with the recovery of faeces and accurate assessment of nutrients voided. The scientific literature reports many approaches ranging from simple recovery of faeces from special tank arrangements, traps and siphons, to more advanced

mechanical removal of voided waste matter that involves sophisticated apparatus connected to the fish holding system (Austreng, 1978). For salmonids, it is possible to expel faeces by gentle pressure on the abdomen from individual fish. Additionally, there is the need to consider the correct use of inert marker on which all measurements of digestibility are ultimately based (Bowen, 1978). There is much controversy regarding the choice of marker in animal nutritional studies and although a number of claims about the advantages and disadvantages of certain compounds, chromic oxide remains the favoured choice for most investigators working with fish and was therefore adopted in the current study.

Before serious appraisal of the nutritional values of ingredients can be made, there is a preliminary requirement for accurate data to characterise the digestibility coefficients for the major nutrients in ingredients commonly used in tropical fish diet specifications species.

For technical reasons (due to the compact nature of the faeces), tilapia was selected as the main experimental animal to examine a series of diets containing proportionate levels of selected test ingredients considered to have merit in practical feeds for this species and the African catfish. It was envisaged that the information obtained would be helpful in the selection of ingredients for the future nutritional assessments based on conventional feeding trials. The digestibility of protein, energy, amino acids and dry matter was thus determined according to standard protocols.

3.2 Materials and methods

3.2.1 Experimental fish

Nile tilapia (*Oreochromis niloticus*) as described in Chapter 2 section 2.1 were used.

Fifteen fish were placed in each tank with an average body weight 77.72 ± 1.02g.

Graded fish were acclimated to a control diet for one week and fed the experimental diets for 3 days prior to faecal collection.

3.2.2 Experimental system

The experimental facilities described in section 2.3 were used to evaluate eight experimental diets recording the apparent digestibility coefficients (ADC) values for plant and animal protein sources for the Nile tilapia.

3.2.3 Diet formulation

Eight diets were formulated with different protein levels depending on the protein levels in each ingredient replaced by fishmeal. The ratio was 40% of each ingredient (full-fat soybean, extracted soybean, maize gluten meal, poultry meat meal, feather meal, haem blood meal and Sopropêche fishmeal-France) included within a fishmeal (LT94) based reference diet. The effective ratio of 40:60 [test ingredient protein: fishmeal protein] was held for each of the experimental diets evaluated.

These diets also contained 0.5% chromium oxide (Cr_2O_3) as the inert indicator together with a vitamin and mineral premix as supplements. These were the 'fixed' component of the diet. All experimental diets are shown in Table 3.1 and diet formulation and preparation was as described in section 2.2.

Table 3.2 shows the essential amino acid composition (expressed as % of protein) of the experimental diets.

3.2.4 Experimental procedure

Fish were weighed periodically for the assessment of biomass to enable accurate feed intake. Faeces collection, nutrient analysis, determination of chromium oxide and calculation of apparent digestibility coefficients (ADC's) are described below.

3.2.4.1 Faeces collection

Fish were starved one day prior to faecal collection in order to promote complete evacuation of digestive tract of *Oreochromis niloticus*. All fish were then fed with respective diets in the afternoon (1.00pm) employing a fixed feeding regime of 3% of body weight. The fish were subsequently allowed to feed for 1 hour, then uneaten feed (if any) was cleaned from the bottom of the tanks. Faecal matter was collected at (9.00am) in the next morning by siphoning from the bottom of each tank (52 days of faeces collection throughout 8 weeks), weighed and immediately oven dried overnight at 105^0C prior to nutrient determination and Cr_2O_3 analyses.

3.2.4.2 Nutrient analysis

Nutrient analysis of ingredients, diets & combinations of diets, and faeces were as described in Chapter 2 according to AOAC protocols. These determined the protein, amino acid, and energy content of the respective materials.

3.2.4.3 Chromic oxide determination

The chromic oxide (Cr_2O_3) content of both the test diets and faecal material was determined by the analysis for chromium in samples using atomic absorption spectroscopy. Due to the inert nature of the chromic oxide this could be carried out after the samples had undergone a form of the wet acid digestion first described by Furukawa & Tsukahara (1966). Triplicate 50-100mg of the test diets and faecal materials were weighed into weighing boat, and then transferred to the borosilicate digestion tube. To each tube, 6ml of nitric acid (HNO_3) (Aristar grade) was added prior to their being heated to 120^0C for 75 minutes in a digestion block (Gerhardt Vapodest 40).

After digestion all of the organic matter was seen to have disappeared, the tubes containing clear solution and varying amounts of green precipitate. The samples were

then allowed to cool at room temperature. To each tube, a further 3ml of concentrated sulphuric acid (H_2SO_4) and 3ml of 70% Perchloric acid (Analar grade) was added. All tubes were subsequently reheated to 220^{0}C for another 75 minutes, at which point a yellow, orange solution was obtained, this indicates all the chromic oxide has been raised to hexivalent chromium. After cooling, the samples are carefully de-canted into 50ml volumetric flasks. The digestion tubes were each washed with distilled water and any residue poured into the volumetric flask, made up to 50ml and transferred into 50ml polypropylene tubes. The tubes were centrifuged at 3000rpm using a bench top centrifuge (Mistral 3000). The resulting supernatant solutions were stored in plastic bottles in the darkness and refrigerated at 2^{0}C until required for chromium analysis.

The samples were analyzed for chromium using a Varian (model No. AA-600) Atomic Absorbtion Spectrophotometer that was fitted with a chromium lamp set at a wavelength of 425nm.

3.2.4.4 Calculation of apparent digestibility coefficients (ADC)

Percentage apparent dry matter, nutrient digestibility and amino acid availability were calculated using the following formulas:

Apparent dry matter digestibility (%) = 100-(100× (%Cr_2O_3 in feed / %Cr_2O_3 in faeces)

ADC (%) of nutrients =100-(100 × (%Cr_2O_3 in feed / % Cr_2O_3 in faeces) × (% nutrient in faeces / (% nutrient in feed)

Apparent amino acid availability (AAAA %)=

100-(100 × (%Cr_2O_3 in feed / % Cr_2O_3 in faeces) × (amino acid in faeces / amino acid in diet)

The $ADC_{protein}$ and ADC_{energy} in test ingredient was calculated based upon the 60:40 ratio of reference diet mixture and test feedstuff in each of the test diets as follows:

$ADC_{nutrient}$ =100/40($ADC_{test\text{-}diet}$ - 60/100 $ADC_{reference\ diet}$).

Table 3.1 Composition and proximate analysis of the control and test diets ($g100^{-1}g$ dry weight).

Ingredients	D1 100% LT94	D2 60: 40%* FFSB	D3 60:40% SESB	D4 60:40% MG	D5 60:40% PMM	D6 60:40% FTHM	D7 60:40% BM	D8 60:40% SFM
Fishmeal (LT94)[1]	80.0	48.00	48.00	48.00	48.00	48.00	48.00	48.00
Full fat soybean[2]		32.00						
Solvent extracted soybean[3]			32.00					
Maize gluten meal[4]				32.00				
Poultry meat meal[5]					32.00			
Feather meal[6]						32.00		
Haem blood meal[7]							32.00	
Sopropêche fishmeal[8]								32.00
Fish oil[9]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Starch	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Dextrin	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin premix[10]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix[11]	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Carboxymethyl cellulose	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Proximate composition (as fed)								
Moisture	5.84	5.74	5.83	6.22	6.41	6.24	6.14	5.43
Protein	56.6	47.3	49.87	53.3	53.43	60.7	64.02	63.83
Lipid	11.45	13.65	8.95	8.42	13.62	11.72	8.36	12.88
Ash	11.46	8.81	9.86	7.94	11.02	8.21	8.56	9.39
Gross energy (KJg^{-1})	19.76	20.42	18.54	20.21	20.01	20.05	20.18	20.70

FM (fishmeal LT94), FFSB (full-fat soybean), SESB (solvent extracted soybean), MG (maize gluten), PMM (poultry meat meal), FTHM (feather meal), BM (blood meal) and SFM (Sopropêche fishmeal).

*60 LT94: 40% of each ingredient.

[1]Fish meal LT94, Trouw Aquaculture (Nutreco company).

[2]Full fat soybean, Central Soya Michigan, USA.

[3]Solvent extracted soybean, Central Soya Michigan, USA.

[4]Maize gluten meal, Cargill, Ltd.

[5]Poultry meat meal, Prosper de Mulder Ltd, UK

[6]Feather meal, Prosper de Mulder Ltd, UK

[7]Haem blood meal, American Protein

[8]Sopropeche fishmeal, CTPP Boulogne, French

[9]Fish oil- seven pure cod liver oil

[10]Vitamin premix, Trouw Aquaculture (Nutreco company).

[11]Mineral premix, Trouw Aquaculture (Nutreco company).

3.2.5 Statistical analysis

Statistical analyses were performed as described in Chapter 2 section 2.9.

Table 3.2 Essential amino acid composition (expressed as % of protein) of the experimental diets.

	D1	D2	D3	D4	D5	D6	D7	D8
	100%	60:40%	60:40%	60:40%	60:40%	60:40%	60:40%	60:40%
	LT94	FFSB	SESB	MG	PMM	FTHM	BM	SFM
Arginine	6.49	7.00	7.08	5.63	7.38	6.65	5.56	6.15
Histidine	2.88	2.70	2.68	2.72	2.56	1.83	5.22	2.29
Isoleucine	4.57	4.92	4.54	4.79	4.49	4.76	2.54	4.34
Leucine	7.63	8.53	7.79	1.59	7.92	8.19	10.81	7.43
Lysine	7.58	7.66	6.97	5.53	7.54	5.23	7.85	7.09
Methionine + Cysteine	3.01	2.78	2.84	3.40	3.38	3.07	2.37	3.30
Phenylalanine + Tyrosine	6.76	8.72	7.39	9.00	7.18	7.64	7.99	7.25
Threonine	4.91	5.29	4.52	4.27	4.62	4.66	3.91	4.31
Valine	5.42	5.21	5.24	5.38	5.66	5.96	6.98	4.79
Tryptophan	ND	ND	ND	ND	ND	ND	ND	ND

ND not detected
FM (fishmeal LT94), FFSB (full-fat soybean), SESB (solvent extracted soybean), MG (maize gluten), PMM (poultry meat meal), FTHM (feather meal), BM (blood meal) and SFM (Sopropêche fishmeal).

3.3 Results

3.3.1 Apparent digestibility coefficients (ADC%)

The apparent digestibility coefficients for dry matter, crude protein and gross energy of all control and test diets are displayed in Table 3.3. Apparent protein digestibility for fishmeal as the principal control diet showed no significant differences between this group (92.60%), tilapia fed on diet containing 40% solvent extracted soybean (92.94%) and fish fed on the diet containing 40% Sopropêche fishmeal hydrolysate (93.35%). However, tilapia fed on diets containing two other plant protein sources, (full fat soybean, and maize gluten meal) produced slightly lower protein digestibility. This was

90.36% and 88.77% respectively. Whereas tilapia fed on various animal protein sources showed significant differences compared to fish fed on the basal control diet. Fish fed on poultry meat meal (83.28%), and feather meal, which had the lowest protein digestibility (73.77%).

Table 3.3 displays the apparent digestibility coefficients for the diet/test ingredient mixture and these demonstrate the variation in digestibility values for dry matter, protein and energy. These were all quite high except for the feather meal diet/blend and this was appreciable lower for all nutrient components compared to the other treatments.

Table 3.3 Apparent digestibility coefficient (ADC%) (mean ±SD, *n*=2) for protein, dry matter and energy in control and test diets.

	Dry matter	Protein	Energy
Control (CON LT94)	83.99 ±1.15^{bc}	92.60 ±0.53^{de}	93.31 ±0.30^{c}
60% of FM (LT94) + 40% Full-fat soybean	80.73 ±0.52^{b}	90.36 ±0.25^{cde}	85.91 ±0.07^{b}
+ 40% Solvent extracted soybean	84.72 ±0.20^{bc}	92.94 ±0.09^{de}	88.84 ±0.55^{b}
+ 40% Maize gluten	84.59 ±1.10^{bc}	88.77 ±0.80^{c}	87.96 ±0.54^{b}
+ 40% Poultry meat meal	73.19 ±4.07^{a}	83.28 ±2.54^{b}	85.36 ±4.04^{b}
+ 40% Feather meal	72.03 ±5.05^{a}	73.77 ±4.74^{a}	74.54 ±1.28^{a}
+ 40% Haem blood meal	80.84 ±1.13^{b}	89.88 ±0.22^{cd}	85.42 ±0.85^{b}
+ 40% Sopropêche fish meal	86.62 ±0.41^{c}	93.35 ±0.56^{e}	92.50 ±1.73^{c}

Values with the same superscript in the same column are not significantly different (p> 0.05).

ADC for dry matter, crude protein and gross energy of all feedstuffs tested are shown in Table 3.4. Apparent digestibility dry matter coefficients were reliable indicators for energy digestibility for all ingredients. Except for PMM and feather meal, which had low dry matter digestibility (due to high ash content) 56.99 and 54.09% and for energy 73.47 which is quite high depending on high lipid content in this ingredient and 49.11 respectively. Whereas, crude protein digestibility of these animal by-products showed as well significant differences this were 69.30 for PMM and 45.53% for feather meal. However, blood meal was slightly higher than other animal protein sources for dry matter, protein and energy.

Table 3.4 Apparent digestibility coefficients (ADC%) (mean ±SD, n=2) of dry matter, protein and energy of tested ingredients.

	Dry matter	Protein	Energy
Fish meal (LT94)	83.99 ±1.15^{bc}	92.60 ±0.53^{de}	93.31 ±0.30^{c}
Full-fat soybean meal (FFSB)	75.86 ±1.30^{b}	86.99 ±0.62^{cde}	74.84 ±0.19^{b}
Solvent extracted soybean meal (SESB)	85.83 ±0.50^{bc}	93.46 ±0.23^{de}	82.16 ±1.37^{b}
Maize gluten meal (MG)	85.50 ±2.74^{bc}	83.03 ±2.00^{c}	82.36 ±1.34^{b}
Poultry meat meal (PMM)	56.99 ±10.18^{a}	69.30 ±6.35^{b}	73.47 ±10.1^{b}
Feather meal (FTHM)	54.09 ±12.64^{a}	45.53 ±11.85^{a}	49.11 ±3.20^{a}
Blood meal (BM)	76.13 ±1.03^{b}	85.79 ±0.55^{cd}	75.96 ±2.12^{b}
Sopropêche fish meal (SFM)	90.56 ±2.83^{c}	94.48 ±1.41^{e}	92.70 ±4.32^{c}

Values in the same column with the same superscript are not significantly different (p> 0.05).

The highest digestibility obtained for tilapia was with the Sopropêche fishmeal and fishmeal (LT94) which together showed a significant difference between their digestibility compared with animal and plant feedstuffs. Solvent extracted soybean meal was well accepted by tilapia with comparatively high digestibility by these fish for ADC dry matter, nutrient and energy and there were no significant differences between this ingredient and fishmeals.

Table 3.5 showed apparent availability coefficients for amino acids in control and test diets fed to *Oreochromis niloticus*. Amino acid availability reflected crude protein digestibility. However, for some feedstuffs, there were some major differences in the apparent availability of specific amino acids. For fishmeal, there seemed to be a reduced availability for glycine (68.83%) compared with other amino acids. For diets containing plant protein sources such as soybean meals, there were lower availability coefficients for cysteine particularly in FFSB (28.41%) while maize gluten MG (64.55%) showed slightly higher than control diet (55.75%) for cysteine. For animal by-products, amino acid availability was quite different for blood meal and two other ingredients (PMM and feather meal) but, blood meal showed slightly higher for most amino acid availability than other two animal by-products except fishmeal.

All animal by-products showed lowest values of isoleucine (PBM, feather meal and BM) 73.97, 76.27 and 77.07% respectively compared with fishmeal diets 89.10 of control and 88.95 of partial substitution of Sopropêche fishmeal. While plant protein sources which included FFSB, SESB and MG resulted in slightly lower 85.40, 86.55 and 84.92% than the control diet. Diets containing poultry by-products (PMM and feather meal) showed the lowest digestibility value for valine (73.56 and 68.85%) respectively compared with other diets. In general PMM and feather meal produced lower availability values for all the amino acids determined in feeds tested for tilapia.

Table 3.5 Apparent availability coefficients of amino acids (%) in control and test diets fed to *Oreochromis niloticus*.

	D1 (FMLT94)	D2 (60 FM: 40% FFSB)	D3 (60 FM: 40% SESB)	D4 (60 FM: 40% MG)	D5 (60 FM: 40% PMM)	D6 (60 FM: 40% FTHM)	D7 (60 FM: 40% BM)	D8 (60 FM: 40% SFM)
Essential amino acid								
Arginine	87.07	89.93	88.45	89.46	79.07	76.63	83.46	90.13
Histidine	90.00	86.65	90.30	88.91	81.37	85.46	90.30	88.39
Isoleucine	89.10	85.40	86.55	84.92	73.97	76.27	77.07	88.95
Leucine	89.98	86.39	85.87	84.38	77.22	74.07	88.52	90.09
Lysine	93.32	92.72	93.48	92.81	87.16	89.28	90.85	94.25
Methionine	84.62	88.83	85.80	86.59	78.73	86.10	75.97	87.11
Phenylalanine	83.80	85.42	83.53	82.75	70.09	72.73	84.84	86.43
Threonine	87.11	85.69	86.62	84.14	74.36	71.81	79.13	86.36
Valine	85.39	82.97	82.22	82.39	73.56	68.85	83.04	84.15
Tryptophan	ND	ND	ND	ND	ND	ND	ND	ND
Non-essential amino acids								
Alanine	83.35	87.44	85.11	83.65	72.98	73.11	79.80	81.46
Aspartic acid	87.38	86.65	85.47	87.68	76.61	75.98	84.95	87.72
Cysteine	55.75	28.41	53.92	64.55	55.61	44.39	64.72	74.80
Glutamic acid	83.97	85.70	78.25	84.30	76.46	76.85	80.82	89.13
Glycine	68.83	87.32	84.97	81.88	67.63	68.70	69.34	82.41
Proline	72.07	23.73	70.78	80.12	67.80	62.14	72.33	80.69
Serine	84.69	85.08	81.61	85.98	74.19	66.38	81.76	87.85
Tyrosine	79.69	80.04	73.58	76.77	60.69	71.85	79.12	83.74

ND (not detected).
FM (fishmeal LT94), FFSB (full-fat soybean), SESB (solvent extracted soybean), MG (maize gluten), PMM (poultry meat meal), FTHM (feather meal), BM (blood meal) and SFM (Sopropêche fishmeal).

Table 3.6 shows apparent availability coefficients of amino acids (%) in test ingredients fed to Nile tilapia. All essential and non essential amino acid availability values were slightly lower in all ingredients particularly animal ingredients with exception of glycine which had a higher availability in plant ingredients compare to animals ingredients.

3.4 Discussion

Evaluation of apparent digestibility coefficient of ingredients utilized in fish diets is one of the most important preliminary steps in the formulation of properly balanced diets to meet the nutrient requirements of fish (Cho *et al.*, 1982). In order obtain optimum digestibility, the nutritional components of fish diets should be thoroughly investigated and formulated separately for each species. The determination of digestibility and chemical analysis together normally more accurately estimates the value of ingested protein, lipid or carbohydrate sources (Plakas & Katamaya, 1981).

Whereas, these latter workers observed the digestibility of diet formulations only in carp, *Cyprinus carpio* that has lower digestibility for all components compared to tilapia (Degani *et al.*, 1997). This agrees with the current investigation that indicates Nile tilapia as having a higher digestive capability than many other fish, especially for some plant and animal by-products when fishmeal is replaced up to 40%.

The suitability of fishmeal as the major protein source in feed formulation for aquaculture is easily understood by the high digestibility of energy, protein and amino acids.

Digestibility trials and nutrient balance investigations have been routinely used to assess the quality and nutritional value of feedstuffs for domestic farmed livestock (Henken *et al.*, 1986). Moreover, determining nutrient digestibility has also been used successfully with fish for many years (Windell *et al.*, 1978; Smith *et al.*, 1980).

Table 3.6 Apparent availability coefficients of amino acids (%) in test ingredients fed to *Oreochromis niloticus*.

	FM	FFSB	SESB	MG	PMM	FTHM	BM	SFM
Essential amino acid								
Arginine	87.07	94.22	90.51	93.03	67.06	60.96	78.05	94.72
Histidine	90.00	81.64	90.76	87.29	68.44	78.65	90.77	85.97
Isoleucine	89.10	79.85	82.72	78.66	51.27	57.01	59.02	88.71
Leucine	89.98	81.01	79.70	75.98	58.06	50.20	86.31	90.24
Lysine	93.32	91.81	89.62	87.04	77.91	83.21	87.15	95.65
Methionine	84.62	85.14	87.57	89.55	69.91	88.32	62.99	90.85
Phenylalanine	83.80	87.85	83.13	81.17	49.54	56.13	86.41	90.38
Threonine	87.11	83.55	85.88	79.68	55.23	48.86	67.15	85.23
Valine	85.39	79.36	77.48	77.89	55.81	44.04	79.51	82.28
Tryptophan	ND	ND	ND	ND	ND	ND	ND	ND
Non-essential amino acids								
Alanine	83.35	83.58	87.75	84.09	57.42	57.76	74.49	78.63
Aspartic acid	87.38	85.56	82.61	88.14	60.46	58.90	81.30	88.25
Cysteine	55.75	-12.60	51.17	77.74	55.40	27.35	78.18	103.38
Glutamic acid	83.97	88.30	69.68	84.80	65.21	66.16	76.10	96.87
Glycine	68.83	115.05	109.18	101.46	65.83	68.51	70.10	102.77
Proline	72.07	-48.79	68.85	92.20	61.40	47.24	72.72	93.62
Serine	84.69	85.66	76.98	87.91	58.43	38.90	77.36	92.59
Tyrosine	79.69	80.55	64.41	72.39	32.19	60.09	78.25	89.82

ND (not detected).
FM (fishmeal LT94), FFSB (full-fat soybean), SESB (solvent extracted soybean), MG (maize gluten), PMM (poultry meat meal), FTHM (feather meal), BM (blood meal) and SFM (Sopropêche fishmeal).

The ADC of the nutrient components in a diet is calculated from the ratio of an inert indicator to nutrient levels in the food and associated faeces (Furakawa & Tsukahara, 1966; Hanley, 1987).

The chromium oxide method was used in the present study and has been widely applied in many studies of fish feeds to determine digestibility (Austreng, 1978), although other types of inert marker substances have been compared by some researchers (Tacon & Rodrigues, 1984).

The results for Nile tilapia fed fishmeal as a basal reference diet agree well with high digestibility coefficients obtained for this diet, as reported for other species including salmonid fish (Cho *et al.*, 1982; Hajen *et al.*, 1993; Gomes *et al.*, 1995; Smith, 1995; Sugiura *et al.*, 1998), red drum (McGoogan & Reigh, 1996), hybrid striped bass (Sullivan & Reigh, 1995), channel cat fish (Robinson 1989; Wilson, 1991), European eel (Schmitz *et al.*, 1984), tilapia (Eid & Matty, 1989; Hanley, 1987; El-Sayed & Teshima, 1991; Luquet, 1991) and carp (Jauncey, 1982).

Digestibility of many ingredients, including some animal meals and plant protein, was similar to fishmeal for tilapia. For these feedstuffs with relatively high total protein content, for example blood meal, poultry meat meal, feather meal, full-fat soybean, and maize gluten meal, ADC values for dry matter, nutrient and energy were different, particularly for protein which ranged between 45-86% compared with 92- 94 % for fishmeal. The disadvantage of these good digestibility coefficients for high protein ingredients compared with fishmeal was that the essential amino acid contents profile and availability for specific EAA's in these feedstuffs were often inferior to fishmeal.

In the present investigation, it was found that the average essential amino acid (EAA) availability coefficients reflected the same trends as protein digestibility for the test ingredients. This varied from >87% on average for the essential amino acids in

fishmeal, 77% for blood meal, 83% for maize gluten, 85% for solvent extracted soyabean meal, 84% for full fat soyabean meal with an average of 63% for feathermeal and only 61% for poultry meat meal. Clearly, there are discrepancies in the overall digestion or availability of each amino acid and therefore their contribution to a balanced protein within the diet.

This latter point may have profound consequences in maintaining the correct balance of EAA's for fish when individual ingredients are substituted on the basis of crude protein only.

For plant ingredients used in this study solvent extracted soybean had the highest ADC values while full-fat soybean and maize gluten meal showed slightly lower digestibility values than in the control diet.

There are explanations for this; one possibility is that the concentrations of enzymatic and other digestive factors would be lower in the diets containing plant ingredients. A second reason is that plant ingredients contain anti-nutritional factors even after processing these inhibit digestive enzymes, resulting in reduced.

The high ADC values of extracted soybean may be due to the effect of the extrusion treatment on elimination of anti-nutritive factors (Melcion *et al.*, 1988; Pongmaneerat *et al.*, 1993; Pongmaneerat & Watanabe, 1993a). Low protein digestibility in some plant ingredients can be due to excessive heat during processing that can damage proteins and amino acids particularly lysine, and also contribute to low nitrogen digestibility of some animal meals (Carpenter & Booth, 1973). This agrees with the current results which lower protein digestibility by tilapia of PMM and feather meal Table 3.4. In addition, protein from bone, feathers and connective tissue may not be as well digested as protein from muscle (NRC, 1993). Opstvedt *et al.* (1984) noted that cooking pollock or mackerel decreased protein digestibility and amino acid availability for rainbow trout and postulated that this was due to heat-induced denaturation and cross-linkage.

Robaina *et al.* (1999) found that ADC of protein from wheat gluten was very high (close to 100%), for sea bass (*Dicentrarchus labrax*), as has been reported with other finfish (Pfeffer *et al.*, 1995; Davies *et al.*, 1997) or crustaceans (Akiyama *et al.*, 1989). ADC measured in the present study was affected by the diet incorporation for each ingredient with fishmeal. For example, protein digestibility of plant ingredients was higher approximately (5%) than the protein digestibility for this ingredient when it has been calculated individually. This high digested of incorporation diets may be indicating that amino acid balanced. While, animal by-products have showed a very high digestibility in diet formulation especially for PMM and feather meal about 15 and 30% respectively than individually ingredients by tilapia.

The results in this study were agreement with Fontaínhas-Fernandes *et al.* (1999) who found extracted pea seed and defatted soybean meal had the highest ADC values of the vegetable proteins tested, however, micronized wheat and FFSB gave slightly lower digestibility values for Nile tilapia, *Oreochromis niloticus*.

On the other hand, this present study showed agreements with the results noted by (Fontaínhas-Fernandes *et al.*, 1999) which indicated that the full-fat toasted soybean showed lower digestibility coefficients than defatted soybean meal for Nile tilapia. These data confirm earlier observations that soya is a plant protein with high potential for utilization in fish diets. Many investigations have shown that this ingredient can partially substitute for fishmeal in salmonids with no adverse effects on growth and feed utilization (Cho *et al.*, 1974; Pongmaneerat & Watanabe, 1992, 1993b; Viyakarn *et al.*, 1992; Oliva-Teles *et al.*, 1994). The same conclusion has also been reported in tilapia, *O. niloticus* × *O. aureus* (Shiau *et al.*, 1987, 1990) and for Nile tilapia (Fontaínhas-Fernandes *et al.*, 1999).

The highest digestibility for animal by-products was found for blood meal as a diet component for tilapia. However, diets containing PMM and feather meal showed slightly lower digestibility compared with fishmeal in the control diet and blood meal groups. This might be due to the fact that PMM and feather meal have a high ash content and these is also a reduction in some amino acids particularly histidine and lysine in diets containing feather meal. In contrast, Hajen *et al.* (1993) reported that blood meal is very poorly digested by chinook salmon *Oncorhynchus tshawytscha* in complete diet formulations. In general, apparent crude protein and energy digestibility of the high protein plant products showed better values than those obtained for animal by-products (excluding fish meals) by tilapia.

Hanley (1987) reported a study on juvenile Nile tilapia (34g), which found lower digestibility than in the present study. He observed the protein digestibility of various protein sources such as, soybean meal 91%; fishmeal 86%; poultry meal 74% and wheat flour 75%. While in the current study on bigger Nile tilapia (78g) digestibility coefficients were: - solvent extracted soybean 93%; fishmeal 92%; PMM 69% and maize gluten meal 83%. There are some possibilities to account for these lower results. This may indicate that the digestive enzymes for more mature fish are more developed than for young tilapia which may affect the digestibility and absorption efficiency.

Amino acid availability coefficients tended to reflect digestibility coefficients for protein for highly digestible ingredients, fishmeal (LT94), Sopropêche fish hydrolyzate, solvent extracted soybean and blood meal.

In this study on amino acid availability for tilapia, the lowest values were reported for glycine, proline and cysteine respectively. These results agree with Allan *et al.* (2000) that reported similar trends for silver perch, *Bidyanus bidyanus*. It sould be noted that the PMM and feather meal exhibited lower availability coefficients for lysine than any other ingredients. This might indicate heat damage to lysine through the rendering

process (Carpenter & Booth, 1973; Opstvedt *et al.* 1984) or possibly reduced protein digestibility of bone fragments. For blood meal, the imbalance in isoleucine and leucine (very high isoleucine and very low leucine) was compounded by the poor availability of isoleucine compared with leucine.

Trypsin and chymotrypsin inhibitors as well as condensed tannins can stimulate excretion of nitrogen leading to underestimates of apparent protein digestibility in monogastric livestock (van Berneveld *et al.*, 2000). This investigation has not been reported with fish. Some discrepancy was observed between the respective digestibility of protein and the amino acid availability of plant and animal protein sources. In these cases the coefficients of protein digestibility were higher than determined based on amino acid availability (values for seventeen amino acids). The higher variance associated with the measurement and calculation of protein digestibility for these feedstuff products.

This study has been agreement with (Mu *et al.*, 2000) who found large variations in apparent amino acid availability of individual amino acids within a protein source and among protein sources.

These results suggest that amino acid availability values are more useful than protein digestibility values for comparing the quality of protein. To more effectively formulate a cost-effective diet for tilapia or any fish with similar requirements, amino acid availability must be considered even though protein digestibility values were sometimes similar between protein sources.

A critical aspect of the current study was the use of fixed proportions of each test ingredient relative to the basal or reference diet. The original technique adopted by (Cho & Slinger, 1979) employed a 70:30 ratio between the reference diet and test ingredient. This has been the basis of most of the literature cited, but in the present trial with tilapia

it was thought more realistic to make a comparison at a dietary level of 40% inclusion in order to obtain a more sensitive effect of the ingredient within the basal mixture. The nutritional experiments that follow test levels in excess of 40% and therefore it was imperative to examine a dietary inclusion level that was at a mid-point in the range. It would however be most pertinent to obtain digestibility measurements for low, medium and high inclusions if any nutrient interactions are likely. It has been suggested that specific amino acid- amino acid, protein -starch, and lipid- protein, lipid-carbohydrate interactions exist between different ingredients. These may significantly alter the profiles of ingredients with respect to coefficients of digestibility in complex mixtures.

All digestibility values in fish trials typically rely on the ratio of nutrient to marker in the diet and faeces. The choice of marker may be important for some species particularly those that have a relatively long GI tract and where there is a possibility of differential flow between marker and digesta. This is of concern in studies with tilapia and carp compared to species such as salmonids with short intestines and defined stomach compartment. It is essential that the marker is representative of the digestive state of the feed and test ingredient and uniformly proportional to the assimilation of respective nutrients. Indeed even the equation used by previous experimenters has also been questioned. Forster (1999) recently expressed the view that the relationship commonly applied by most researchers and also used in the current study is open to error in some circumstances. Although the points made are pertinent, the standard formula was chosen for consistency and comparison of the data reported in this Chapter with those in the literature.

The other area of contention is the duration of the acclimation period in which tilapia are conditioned to the various diet combinations. In this study fish were fed the

experimental feeds for at least 2-weeks prior to faecal collection and this was taken as a reasonable period for adjustment to the diet compositions and feeding regimes.

The gastrointestinal tract of tilapia is adaptable to diets of varying quality and composition although it is possible that certain enzyme systems may need to be activated and induced by the presence of different nutrients such as indigestible carbohydrates and fibre in some materials.

The aim of replacing fishmeal with alternative protein sources must therefore consider the response of fish to different feeds in respect of their direct effects on the digestive system. The subsequent studies include determination of specific enzymes associated with the digestion sequence and the long- term effects of feeding novel proteins in association with standard nutritional investigations based on feeding trials. Such knowledge can be used to develop an *in-vitro* approach to screen ingredients based on techniques that involve simulation of the gastro-intestinal system of either tilapia or catfish using validated enzyme activity profiles in buffered solutions exposed to the test ingredient. Similar ideas and approaches have been made by other workers notably (Grabner & Hofer, 1985) to offer a reliable and cost effective method for routine digestibility measurements for tropical fish. De Silva & Perera (1984) have also suggested these for tilapia.

Finally, the size of the tilapia used in the study and environmental temperature employed were consistent and these factors are clearly of prime importance with respect to their effects on food intake, gut passage time and nutrient assimilation. Fish of different sizes have different relative metabolic rates and may also differ in their capacity to process and absorb nutrients. Davies (unpublished data) has demonstrated that the digestibility of protein and energy is influenced by elevated temperature in a closed rearing system.

Clearly, further research is needed to examine all of these potential factors on digestibility and nutrient absorption. Digestibility governs the effective use of the nutrient components of the ingredients used in complete formulations and will be one of the main considerations in limiting their potential in aquafeeds for tropical fish such as the tilapia and African catfish.

CHAPTER 4

INFLUENCE OF DIETARY INCLUSION OF FULL-FAT SOYBEAN MEAL ON GROWTH AND DIGESTIVE ENZYME ACTIVITY FOR NILE TILAPIA, *Oreochromis niloticus*.

4.1 Introduction

Soybean meals are extensively used in practical feeds for many species of fish in aquaculture. The reason for this is mainly due to their relatively high protein content, well-balanced amino acid profile and fairly consistent composition. The cost of this ingredient is usually lower than that fishmeal, and is also quite favourable compared to other animal or plant ingredients available. In general, high substitutions of plant protein sources as a complete replacement for fishmeal protein have resulted in poor growth and feed efficiency in fish (Jackson *et al.*, 1982; Viola *et al.*, 1983; Dabrowski *et al.*, 1989). In the case of soybean meals, this is attributed to the indigestible carbohydrate fraction consisting of oligosaccharides such as stachyose, raffinose etc and also several anti-nutritional factors (ANF's) that may reduce digestibility and disturb digestive enzyme activity in fish (Storebakken *et al.*, 2000a).

Soybean products have been successfully used as a low level component in diets for the production of large salmonids (Hendriks *et al.*, 1990). However, recently improved processing technology has partly overcome the limitations to soybean utilization in diets for fish species, making soybean protein more attractive as a secondary protein source for diet formulation in aquaculture.

Specific anti-nutritional factors (ANF's) present in most leguminous seeds, like protease inhibitors, lectins and oligosacharides, limit their use in diet formulation as the main protein source for most animals (Saini, 1989).

These protease inhibitors are inactivated by proper heat treatment of soybean meal. High trypsin inhibitor activity in inadequately heated soybean meal decreases both protein and energy digestibility in rainbow trout, *Oncorhynchus mykiss* (Smith *et al.*, 1980) and growth performance in carp, *Cyprinus carpio* (Viola *et al.*, 1983).

It was demonstrated that solvent extruded SBM, with or without methionine supplementation could successfully substitute up to 75% for Nile tilapia fry fed test

diets (Tacon *et al.*, 1983), *Oreochromis mossambicus* (Jackson *et al.*, 1982) and 67% of the protein in diets for tilapia hybrids (Shiau *et al.*, 1989).

Viola & Arieli (1983) and Teshima & Kanazawa (1988) observed that supplementing tilapia diets with crystalline essential amino acids did not improve fish performance. Soy protein concentrate (SPC) has also been evaluated as a protein source for some fish species especially salmonids. Rapid growth was observed in Atlantic salmon *Salmo salar* by Storebakken *et al.* (1998).

However, there is a paucity of information on the efficiency of SPC as an alternative protein source in feeds for tilapia except for the investigation by Davies *et al.* (1989). Digestibility of crude protein in diets with SPC and other soy products in Atlantic salmon ranges from a level commonly found in low-temperature-dried fishmeal (85%) (Storebakken *et al.*, 1998) to value within the range of (60-70%) (Olli *et al.*, 1994).

While dietary protein is in excess, the amino acid profile of soy protein is not limiting for growth for fish. Storebakken *et al.* (1998) noted that growth in Atlantic salmon fed diets with the majority of the protein from SPC was comparable to that obtained with salmon fed a fishmeal-based diet.

With respect to the essential amino acid requirements of fish, methionine is the first limiting amino acid in soy protein (Perkins, 1995) when compared to the amino acid requirement in rainbow trout (Pack *et al.*, 1995). Moreover, alcohol-washed soy concentrate contains about 1.4g methionine per 100g crude protein (Perkins, 1995), while low temperature fishmeal LT contains double this amount of methionine (2.8g $100g^{-1}$ protein) (Pike *et al.*, 1990; Mundheim & Opstvedt, 1993).

The inclusion level of SBM in tilapia feeds is affected by dietary protein level. Davis & Stickney (1978) noted that inclusion of soybean meal at 15% dietary protein decreased growth of blue tilapia *Tilapia aurea,* however, at 36% protein, SBM could totally replace fishmeal in the diets without reduction in fish performance.

The authors suggested that the nutritional value of the feed varied at low protein levels and became similar at the highest protein level and the essential amino acid level in the 36% crude protein SBM based diet was above the normal fish requirement. It was interesting that Viola *et al.* (1994) reported that the supplementation of lysine to SBM based diets fed to tilapia hybrids was ineffective at 25 and 30% dietary protein. While, at 35% crude protein, reducing the lysine/to protein ratio resulted in impaired fish growth.

Improvement of the nutritional value of both plant and animal feed ingredients can be attributed to amino acid supplementation of the diet with crystalline L-amino acids (Guillaume, 1997; Millamena *et al.*, 1998). Also combining different protein sources containing complementary EAA profiles such as legumes and grains is also a feasible approach (Audesirk & Audesirk, 1996). Chen *et al.* (1992) reported that binding supplemental amino acids in various polymers or plasteins is a possible way to improve protein utilization. Teshima *et al.* (1992) employed transgenic seed plants with better protein digestibility characteristics or expressing growth factors in aquaculture species. The addition of specific attractants to improve the palatability of diets containing soybean meal may also be important (Lee & Meyers, 1997).

The omnivorous species channel catfish *Ictalurus punctatus* was found to have progressively greater weight gain as increasing levels of fishmeal replaced a soybean-corn mixture (Mohsen & Lovell, 1990). In addition, several animal proteins tested in this previous study increased palatability over a soybean-corn diet alone.

Moreover, Webster *et al.* (1995) were able to replace fishmeal totally with soybean meal in diets for blue catfish *Ictalurus furcatus* without reduction in growth rates compared to fish fed diets containing 15% fishmeal. For carnivorous species such as salmon, lower levels of soybean have been included in diets to avoid growth reduction. Fowler (1980) reported that full-fat and dehulled soybean meals could not effectively

substitute fishmeal in diets for chinook salmon *Oncorhynchus tshawytscha* and coho salmon *Oncorhynchus kisutch.*

However, significant growth reduction has been observed in rainbow trout *Oncorhynchus mykiss* when 50% of the fishmeal was replaced by soybean (Dabrowski *et al.*, 1989). This probably was a function of the processing methodology used which has been shown to affect utilization (Viola *et al.*, 1983; Olli & Krogdahl 1994). In contrast, other authors have reported that diets containing a considerable amount of soybean meal have actually improved growth performance of salmonids (Cho *et al.*, 1974; Reinitz, 1980; Watanabe & Pongmaneerat, 1993b).

Recently, El-sayed (1999) also reported that the contradiction between researchers regarding the use of soybean meal as a plant protein source for fish may be due to the quality and processing methods of SBM, fish species, size of fish and culture systems. For example, it has been noted that the method of processing of SBM has a significant effect on its nutritive value. Wassef *et al.* (1988) observed that the germination and defatting of SBM reduced the activity of protease inhibitors and other deleterious factors that impair digestion.

Heating SBM also helps to rupture the cellulose membrane structure surrounding the cell and releases the cell contents making them more available for digestion and absorption (Tacon & Jackson, 1985). Also Liener (1980) reported that adequate heating inactivates and destroys the anti-nutritional factors found in raw soybean meal. The quality of full-fat SBM heated at 100°C for 1h was greatly improved and reduced trypsin inhibitor activity for Nile tilapia (Wee & Shu, 1989).

Digestive fluids and enzymes are essential components of the gastric, pancreatic, bile, and intestinal secretions in fish as in all animals. Acid gastric fluid production occurs in

most fish, except in those without a defined stomach when neither HCl nor pepsin is formed in the gut (De Silva & Anderson, 1998).

The digestive enzymes are generally hydrolases, i.e.: capable of catalyzing hydrolytic reactions to degrade macro-molecules. Based on their respective physiological function they are divided into proteases, lipases, esterases, and carbohydrases respectively.

Apart from the principal locations, enzymes may be produced in other tissues, e.g. amylase is also produced in the liver of some fish. It is also known that enzymes present in animals, that form part of the diet of a fish may enhance endogenous enzyme activity. This is particularly the case in very young fish, where the gastrointestinal tract is not fully developed (De Silva and Anderson, 1998).

The principal objective of the experiment described in this Chapter was to evaluate the replacement of fishmeal with full fat soybean meal in balanced diet formulations for tilapia. The criteria for assessment included the obvious key nutritional parameters such as growth, feed utilization and carcass composition as well as a comprehensive study to examine the effects of soybean on the digestive enzyme profile in different regions of the gastro-intestinal tract of tilapia.

4.2 Materials and methods

4.2.1 Experimental fish

Nile tilapia (*Oreochromis niloticus*) as described in Chapter 2 section 2.1 were used. Twenty fish were placed in each tank as duplicate groups with an average body weight 10.92 ±SD 2.01g. The fish were acclimated to the control diet (LT94) for one week and subsequently fed the experimental diets (Table 4.1).

4.2.2 Experimental system

The experimental facilities described in section 2.3 were used to test the four experimental diets containing different levels of full-fat soybean (% of dietary protein).

4.2.3 Diet formulation

Four approximately isoenergetic and isonitrogenous diets were formulated for different levels of FFSB replacing fishmeal. Table 4.1 displays the formulation and proximate composition of control and test diets, and Table 4.2 shows the essential amino acids as a % of protein for each diet.

These included the control diet 1 (LT94 fishmeal), diet 2 (58%) protein of FFSB, diet 3 (63%) and diet 4 (63%) with amino acid supplementation (1% DL-methionine) to evaluate the growth performance, enzyme activity in stomach, liver and gut, and to estimate the alterations in proximate and distal gut.

4.2.4 Experimental procedure

Fish were weighed bi-weekly and fed a ration of 2.25% of body weight per day. The trial was conducted over a 12-week period and the feed intake adjusted according to the biomass. At the termination of the feeding trial, five fish from each group were sacrificed and the gut removed for histological examination following the methods described in section 2.8. The other five fish were killed and gut, liver and stomach were removed and frozen at -80^{0}C for enzymatic analysis as described in section 2.6. Also a similar group was killed for carcass composition.

4.2.5 Proximate composition

Proximate compositions of diets and fish tissue for moisture, protein, lipid, ash and gross energy were determined as described in Chapter 2 section 2.4.

4.2.6 Determination of enzymes

Analysis of gastro-intestine, stomach and liver for proteolytic, trypsin, amylase and lipase activities were described in section 2.6 Chapter 2.

4.2.7 Statistical analysis

Statistical analyses were carried out as described in section 2.9.

Table 4.1 Composition and proximate analysis of the control and test diets ($g100g^{-1}$ dry weight).

Ingredients	D1 0% FFSB	D2 58% FFSB	D3 63% FFSB	D4 63% FFSB +DL-Met.
Fish meal[1]	43.00	23.00	16.00	16.00
Full-fat soybean meal[2]		41.00	57.00	57.00
Wheat meal[3]	32.00	20.00	17.50	16.50
Corn oil[4]	8.77	2.30		
Cod liver oil[5]	0.70	2.90	2.50	2.50
Vitamin premix[6]	2.00	2.00	2.00	2.00
Mineral premix[7]	1.00	1.00	1.00	1.00
DL-Methionine				1.00
Binder[8]	2.00	2.00	2.00	2.00
∝-Cellulose[9]	10.03	4.80		
Proximate composition (% as fed)				
Moisture	4.23	3.25	3.85	4.05
Protein	36.55	35.73	35.51	36.42
Lipid	14.33	15.18	14.79	14.56
Ash	7.41	7.29	8.28	8.22
Gross energy $MJkg^{-1}$	20.78	20.97	20.77	20.37

[1] Fish meal LT94, Trouw Aquaculture (Nutreco company).
[2] Full fat soybean, Central Soya Michigan, USA.
[3] Wheat meal, Kalpro S™. Orsan, Paris, France
[4] Mazola- pure corn oil
[5] Fish oil- seven pure cod liver oil
[6] Vitamin premix, Trouw Aquaculture (Nutreco company)
[7] Mineral premix, Trouw Aquaculture (Nutreco company)
[8] Carboxymethyl Cellulose (CMC).
[9] Sigma Chemical Co., Poole, Dorset.

Table 4.2 Essential amino acid composition (expressed as % of protein) of the control and test diets fed to tilapia *Oreochromis niloticus* and their requirements.

	D1 0% FFSB	D2 58% FFSB	D3 63% FFSB	D4 63%FFSB +DL-Met.	Tilapia Requirements*
Arginine	5.99	5.39	5.33	5.76	4.20
Histidine	2.52	2.23	2.25	2.65	1.72
Isoleucine	4.01	3.79	3.55	3.65	3.11
Leucine	6.99	6.70	6.19	6.56	3.39
Lysine	6.03	4.83	4.62	5.30	5.12
Methionine	2.22	1.80	1.33	3.29	2.69
Methionine + Cysteine	2.51	2.15	1.78	3.75	
Phenylalanine	3.90	4.29	4.00	4.19	3.75
Phenylalanine + Tyrosine	6.33	7.30	6.74	7.03	
Threonine	4.36	3.79	3.38	3.84	3.75
Tryptophan	ND	ND	ND	ND	

* source: Santiago & Lovell (1988).
ND (not detected)

4.3 Results

4.3.1 Growth performance

Growth performance and feed utilization data for Nile tilapia fed the four respective diets are shown in Figure 4.1 & Table 4.3. There was a significant difference between the final average body weights between fish fed the control diet and the other groups. Fish fed the fishmeal (LT94) based control diet demonstrated the highest mean final body weight (72.21g) resulting in a 7- fold increase in weight from the start of the study.

The specific growth rate (SGR%) values further supported this trend, with SGR reduced from 2.45 for the control diet fed fish to 2.14, 2.17 and 2.15 for the fish fed the other three diets which have been presented in Table 4.3 and Figure 4.2. No mortality was

observed during the experimental period and the overall health of the fish appeared normal.

4.3.2 Feed consumption and feed utilization

The control diet was well accepted by the tilapia, while diets containing the partial replacement of FFSB were less palatable so that fish were fed only 2% of body weight for first six weeks increased to 2.5% for the last six weeks. Mean daily feed intake ranged between 0.93 and 0.78gfish^{-1} day^{-1}. There was a noticeable effect of the dietary inclusion of alternative protein sources on feed intake (Table 4.3). Feed intake for tilapia fed on control diet containing the highest amount of fishmeal (LT94) was significantly better than those observed for fish fed diets including FFSB even with amino acid supplementation. FCR values also differed significantly between the control group and fish fed on diets containing FFSB.

Protein efficiency ratio (PER) was noticeably different between treatments and supported the same trend. The fish fed the control diet displayed superior PER (2.32) while fish receiving the different levels of FFSB exhibited PERs of 2.06, 2.14 and 2.06. Apparent net protein utilization (ANPU%) values also showed a reduction when fishmeal was replaced by the full fat soybean source. These values ranged from 39.41 to 34.46 (Table 4.3).

It should be noted that in general, the essential amino acid profile of the experimental diets shows a declining level for most the amino acids with each FFSB increment in the diets. However, the diet supplemented with 1% DL-methionine showed the closest levels to the control diet (Table 4.2). This was especially apparent for the total sulphur amino acids (Met. + Cys.) which showed 2.51 for the control diet while tilapia have a requirement for methionine of 2.69% of protein and other diets containing different

levels of FFSB showed methionine deficiency, while, diet containing 1% DL-methionine showed the highest methionine value which was 3.75, sufficient for tilapia requirement of methionine.

Table 4.3 Weight increase, feed consumption, nutritive utilization of feed of Nile tilapia *Oreochromis niloticus* fed experimental diets (mean ±SD *n=2*) for 12 weeks.

.	D1 0% FFSB	D2 58% FFSB	D3 63% FFSB	D4 63% FFSB +DL-Met.
Mean initial weight (g)	10.93 ±2.07	10.9 ±2.03	10.91 ±2.09	10.93 ±1.86
Mean final weight (g)	72.21 ±4.16[b]	58.25 ±1.55[a]	57.48 ±1.74[a]	56.95 ±1.29[a]
Mean weight gain (g)	61.29 ±4.18[b]	47.34 ±1.60[a]	46.55 ±1.71[a]	46.06 ±1.30[a]
Mean daily feed Intake ($gfish^{-1}\ d^{-1}$)	0.93 ±0.04	0.80 ±0.02	0.80 ±0.01	0.78 ±0.01
SGR (%)	2.42 ±0.08[b]	2.15 ±0.04[a]	2.13 ±0.04[a]	2.12 ±0.03[a]
FCR	1.18 ±0.02[a]	1.32 ±0.02[b]	1.33 ±0.03[b]	1.36 ±0.01[b]
PER	2.32 ±0.05[b]	2.06 ±0.02[a]	2.14 ±0.03[a]	2.06 ±0.04[a]
ANPU (%)	39.41 ±0.75[b]	35.43 ±0.39[a]	35.07 ±0.69[a]	34.46 ±0.29[a]

Values in the same row with the same superscript are not significantly different ($p > 0.05$).

Lysine also support this trend which has improved the lysine deficiency found in diets including 58% of FFSB and 63% without methionine supplementation which resulted in 4.83 and 4.62% of protein respectively. Whereas, the requirement was 5.12, and these results have been listed in Table 4.2.

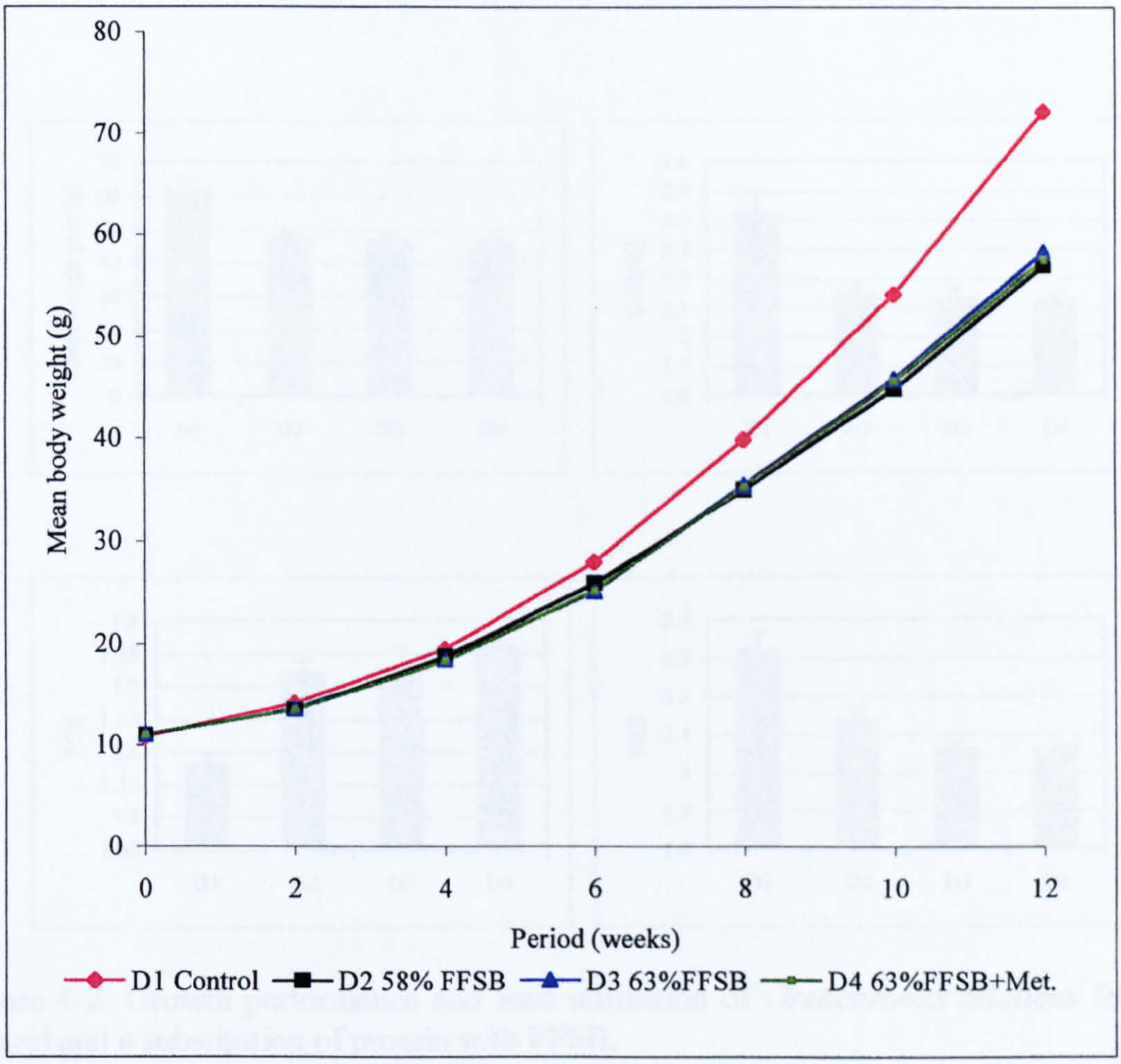


Figure 4.1 Growth performance of *O. niloticus* fed a fishmeal based control diet and a substitution of protein with full fat soybean (FFSB).

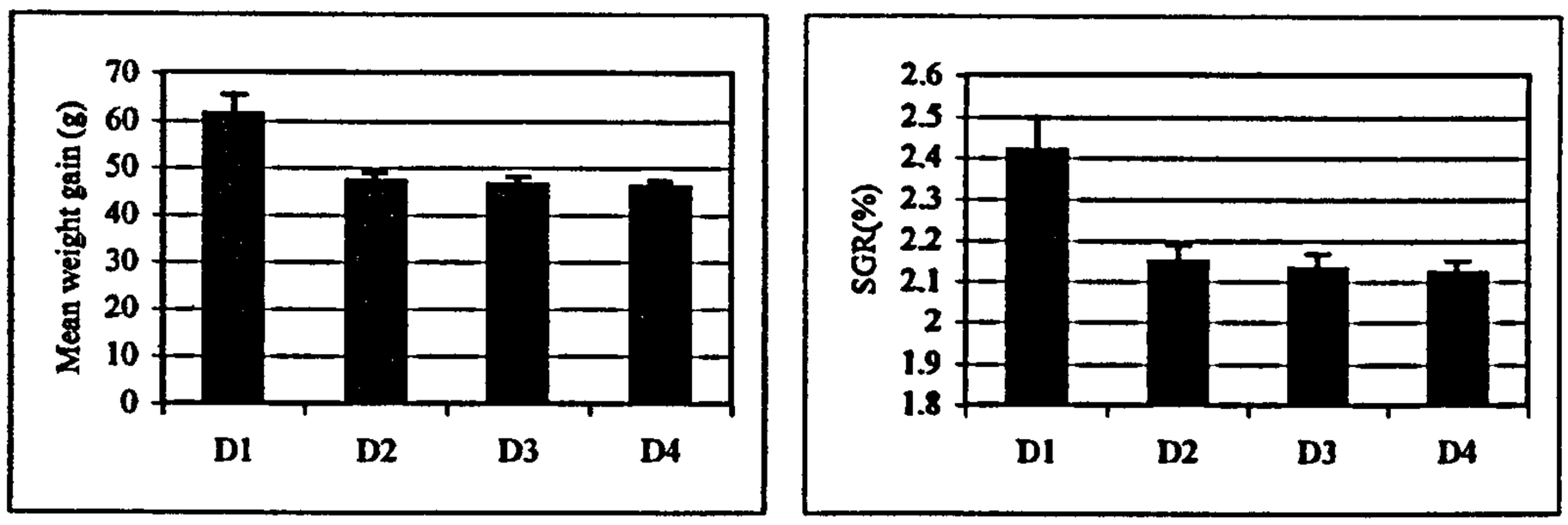


Figure 4-2: Growth performance and feed utilization of *Oreochromis niloticus* fed on control and a substitution of protein with FFSB.
Bars denote mean values ±SD (*n*=2).

D1 (control diet LT94 fishmeal), D2 (58% protein of FFSB), D3 (63% protein of FFSB) and D4 (63% protein of FFSB+ 1%-DL-methionine).

4.3.3 Fish body composition

Initial and final carcass composition of the fish fed the experimental diets is presented in Table 4.4. The final carcass composition showed little significant variation of their proximate composition as a result of the diet formulations.

Fish fed the fishmeal based control diet and different levels of FFSB diets did not yield any variations in the moisture, protein content ($P>0.05$) but was a significant reduction in percentage lipid from 9.70 to 8.88% on the higher FFSB diets, whilst ash content showed slight differences among groups (Table 4.4).

Table 4.4 Body composition of Nile tilapia *O. niloticus* fed graded levels of FFSB (mean ± SD *n=2* five fish per duplicate).

	Initial fish	D1	D2	D3	D4
Moisture	72.35	71.23 ±2.89^{a}	72.16 ±1.20^{a}	72.74 ±0.91^{a}	72.03 ±2.35^{a}
Protein	12.11	16.72 ±1.68^{a}	15.79 ±0.40^{a}	15.45 ±0.36^{a}	15.39 ±1.10^{a}
Lipid	7.96	9.70 ±0.61^{b}	9.64 ±0.89^{ab}	8.76 ±0.96^{a}	8.88 ±0.69^{a}
Ash	2.85	3.55 ±0.40^{a}	3.47 ±0.32^{a}	3.71 ±0.15^{a}	3.52 ±0.28^{a}

Values in the same row with the same superscript are not significantly different ($p>0.05$).
(After arcsine transformation of original data)

4.3.4 Gastro-intestinal enzyme activity

Table 4.5 shows total proteolytic, trypsin, amylase and lipase activities in the intestine, liver and stomach. Total proteolytic (sum of pIIs 1.5, 3, 4, 7, 8.5, 9, and 10) activity of the intestine was higher than the activity in the liver and stomach and ranged between 7.92 to 10.45 µg tyrosine minute^{-1} mg^{-1} protein. However, average proteolytic activity among fish fed the four experimental diets did not show any significant differences ($p>$ 0.05) for the intestine and stomach (Table 4.5 and Figure 4.3).

Liver proteolytic activity was lower than stomach activity, and also the mean of proteolytic activity showed a significant difference among fish fed the control diet and test diets. Fish fed on control diet and diet 2 (58%) FFSB showed similar results 0.57 and 0.50 μg tyrosine^{-1} minute^{-1} mg^{-1} protein respectively however fish fed diet 3 and 4 showed a significant difference among these groups and control diets 0.26 and 0.18 respectively. Figure 4.4 shows the enzymatic activity determined at different pHs for tilapia fed control and test diets. For the intestine, the higher proteolytic activity was at neutral and alkaline pHs, whereas only very low activity was shown at acid pHs. However, liver proteolytic activity was appreciably higher at alkaline pHs only for control and diet 2, whereas diet 3 and 4 showed lower values for all pHs. In contrast, higher proteolytic activity was observed at acid pHs whereas the lower values were observed at alkaline pH (Figure 4.4).

Table 4.5 Total proteolytic, trypsin, amylase and lipase activities in intestine, liver and stomach of tilapia *O. niloticus* fed control and test diets determined at 37^{0}C (mean ±SD *n=2* five fish per duplicate).

	Proteolytic activity (mean)	Proteolytic Sum of pHs* (µg tyrosine min^{-1} mg^{-1} protein)	Trypsin activity (µg tyrosine min^{-1} mg^{-1} protein)	Amylase activity (µg maltose ml^{-1} min^{-1})	Lipase activity (Sigma/Tietz/unit/L) min^{-1} ml^{-1}
Intestine					
D1	1.13±0.71^a	7.92	17.94±3.37^a	0.80±0.21^{ab}	1.74±0.62^b
D2	1.41±0.78^a	9.90	17.90±1.96^a	0.79±0.28^{ab}	1.78±0.72^b
D3	1.39±0.88^a	9.74	17.82±4.45^a	0.65±0.04^a	1.67±0.36^b
D4	1.49±1.04^a	10.45	17.80±2.58^a	0.83±0.12^b	0.95±0.30^a
Liver					
D1	0.57±0.30^c	3.98	10.44±2.25^b	4.99±0.79^b	1.18±0.30^c
D2	0.50±0.29^{bc}	3.47	9.05±1.41^b	3.98±1.62^{ab}	0.66±0.33^b
D3	0.26±0.04^{ab}	1.83	2.95±1.72^a	2.76±1.35^a	0.44±0.25^{ab}
D4	0.18±0.02^a	1.25	1.43±0.37^a	3.18±1.32^a	0.26±0.19^a
Stomach					
D1	0.68±0.36^a	4.76	ND	0.59±0.06^{ab}	ND
D2	0.70±0.42^a	4.92	ND	0.64±0.08^b	ND
D3	0.77±0.39^a	5.40	ND	0.52±0.12^a	ND
D4	0.63±0.58^a	4.38	ND	0.67±0.15^b	ND

ND (not detected).

Values in the same column with the same superscript are not significant ($p>0.05$).

D1 (control diet LT94 fishmeal), D2 (58% protein of FFSB), D3 (63% protein of FFSB) and D4 (63% protein of FFSB+ 1%-DL-methionine).

*Total proteolytic activity was obtained as the sum of those determined at pH 1.5, 3, 4, 7, 8.5, 9, and 10.

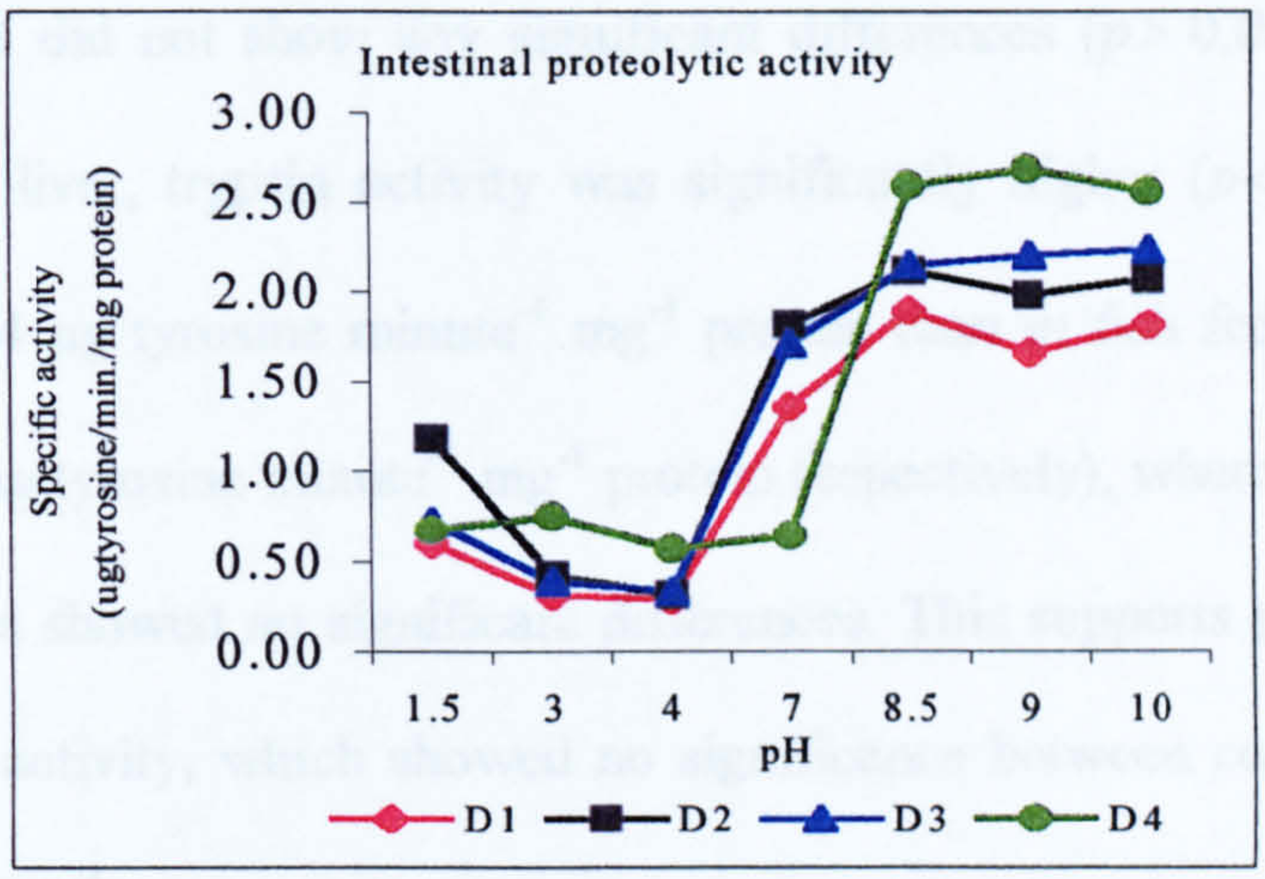
Total proteolytic activity was obtained as the sum of those determined at pH 1.5, 3, 4, 7, 8.5, 9, and 10.

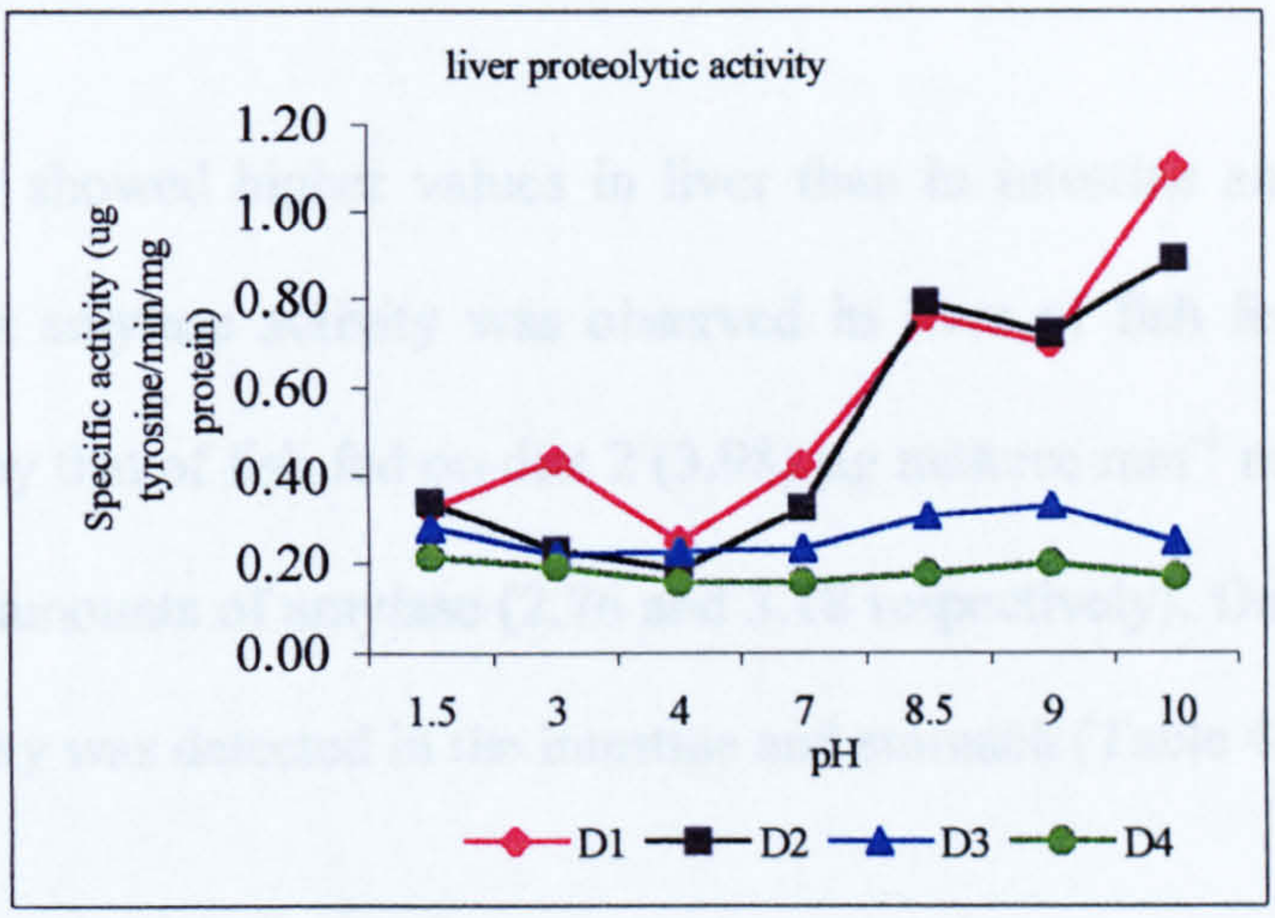
Proteolytic activity was expressed as the amount of tyrosine (µg) digested by 100µl of enzyme solution minute^{-1} mg^{-1} protein at acid, natural and alkaline pHs at 37^0 C.

Trypsin activity was expressed as the amount of tyrosine (µg) liberated by 0.5ml of enzyme extract per minute mg^{-1} protein at 37^0 C.

Amylase activity was expressed as the amount of maltose liberated by 50µl of enzyme extract minute^{-1} ml^{-1} at 37^{0}C.

Lipase activity was expressed as the amount of fatty acids neutralized by 0.05 NaOH liberated by 1ml enzyme solution minute^{-1} at 37^0 C.





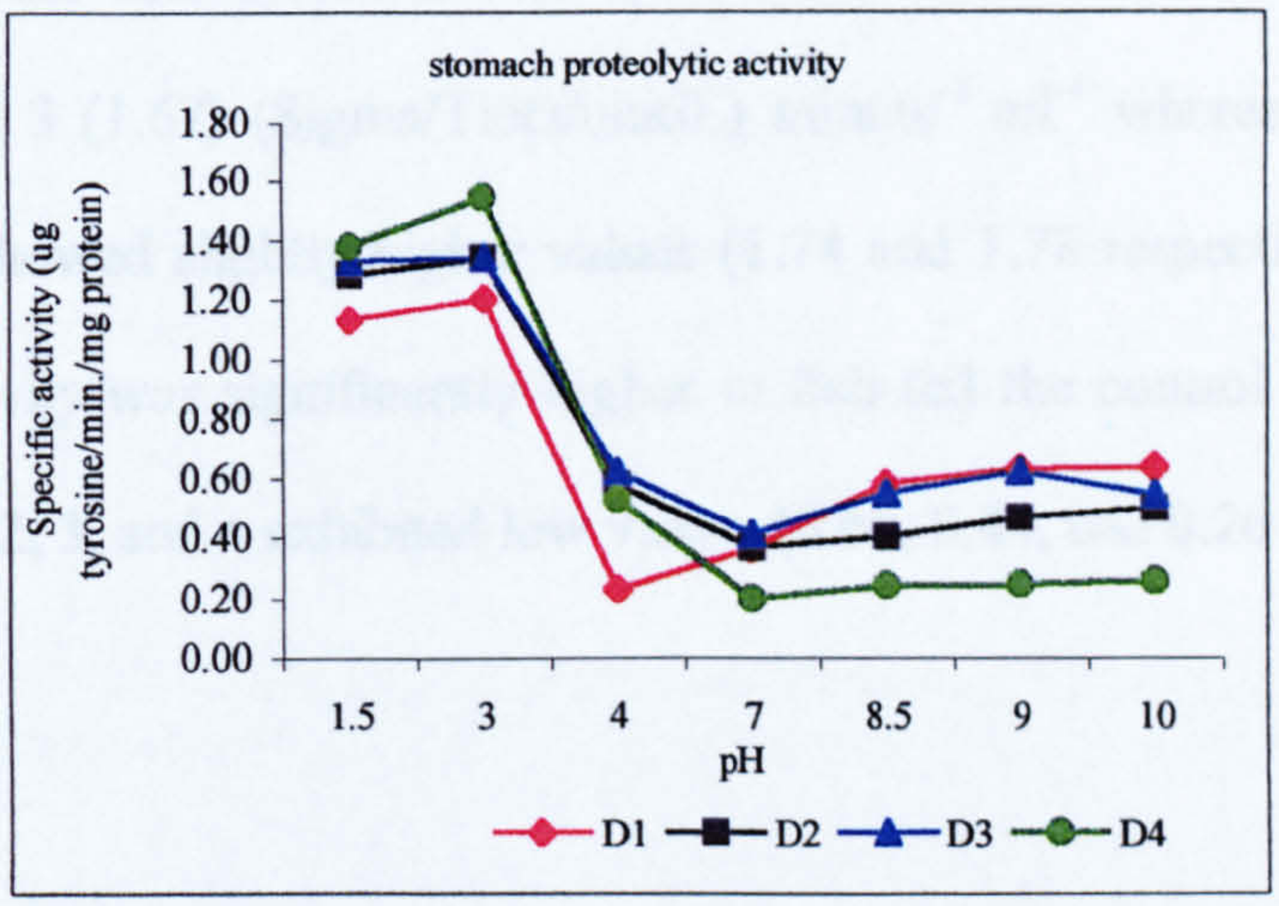


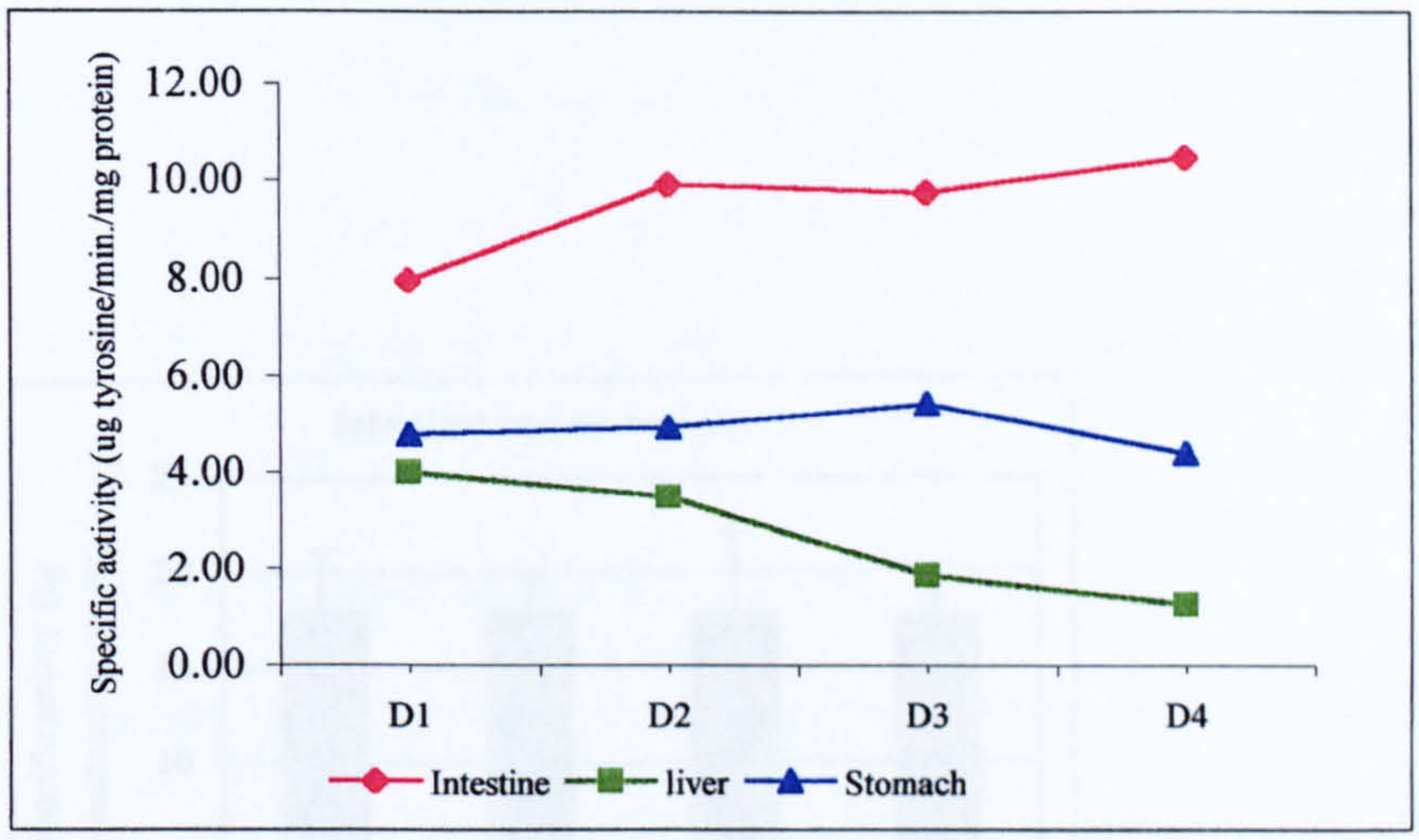
Figure 4.3 Proteolytic activity in intestine, liver and stomach in tilapia fed different levels of FFSB for control and test diets affected by different pHs (mean values ±SD *n=2* five fish per duplicate).

D1 (control diet LT94 fishmeal), D2 (58% protein of FFSB), D3 (63% protein of FFSB) and D4 (63% protein of FFSB+ 1%-DL-methionine).

Moreover, trypsin activity was also observed to be higher in the intestine than the liver and stomach, but did not show any significant differences ($p > 0.05$) among groups. However, in the liver, trypsin activity was significantly higher ($p < 0.05$) in fish fed control diet 10.44 µg tyrosine minute^{-1} mg^{-1} protein than in fish fed on diets 3 and 4 (2.95) and 1.43 µg tyrosine minute^{-1} mg^{-1} protein respectively), whereas fish fed on diet 2 and control diet showed no significant differences. This supports previous trends for liver proteolytic activity, which showed no significance between control diet and fish fed 58% FFSB protein.

Amylase activity showed higher values in liver than in intestine and stomach (Table 4.5). The highest amylase activity was observed in liver of fish fed the control diet (4.99) followed by that of fish fed on diet 2 (3.98) µg maltose min^{-1} ml^{-1}. Fish fed diet 3 and 4 had lower amounts of amylase (2.76 and 3.18 respectively). Only a small amount of amylase activity was detected in the intestine and stomach (Table 4.5).

For intestinal lipase activity the lowest level was observed in fish fed diet 4 (0.95) followed by diet 3 (1.67) (Sigma/Tietz/unit/L) minute^{-1} ml^{-1} whereas fish fed control diet and diet 2 showed slightly higher values (1.74 and 1.78 respectively) (Table 4.5). Liver lipase activity was significantly higher in fish fed the control diet 1.18 whereas fish fed on diets 2, 3, and 4 exhibited low values (0.66, 0.44, and 0.26 respectively).



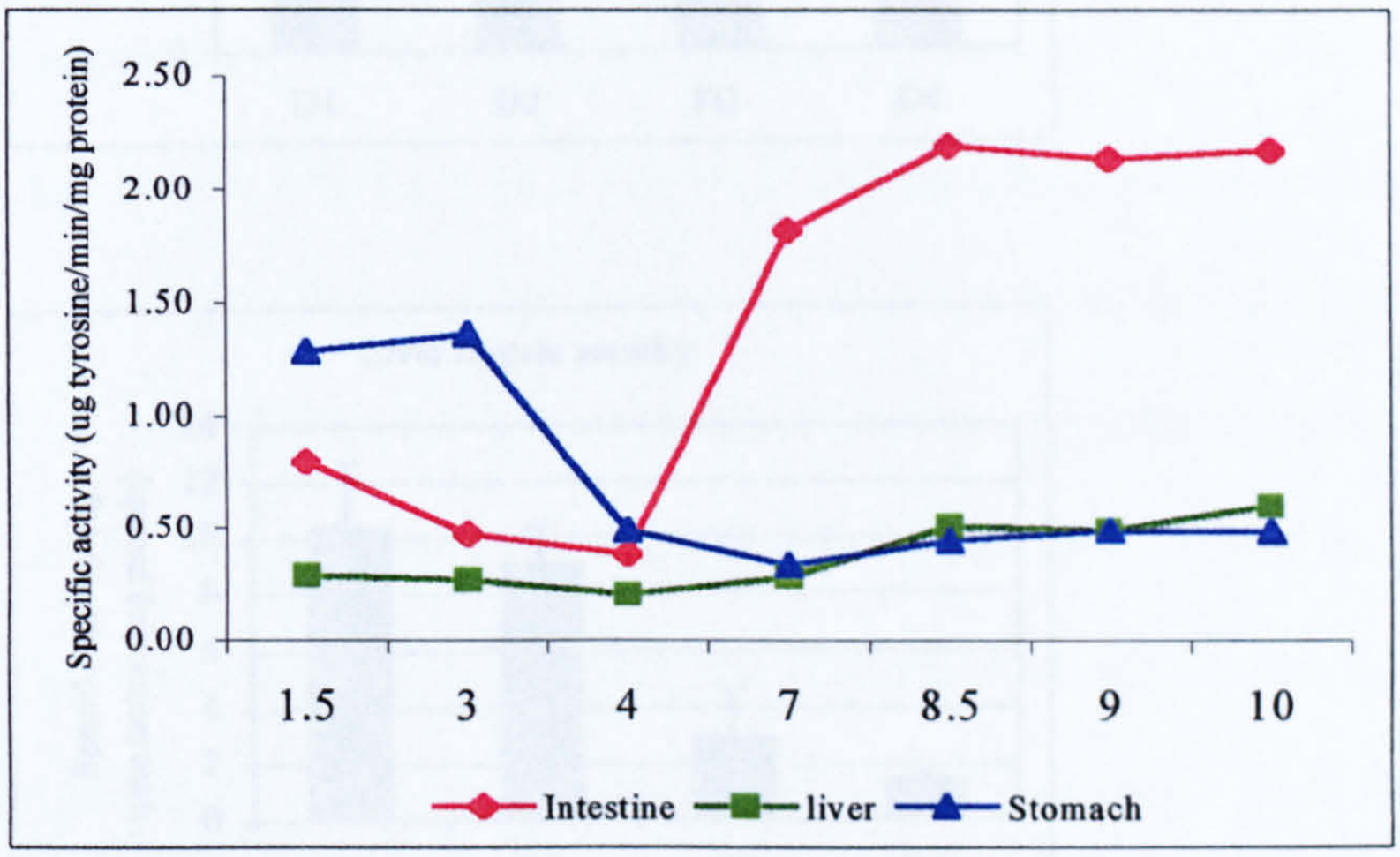
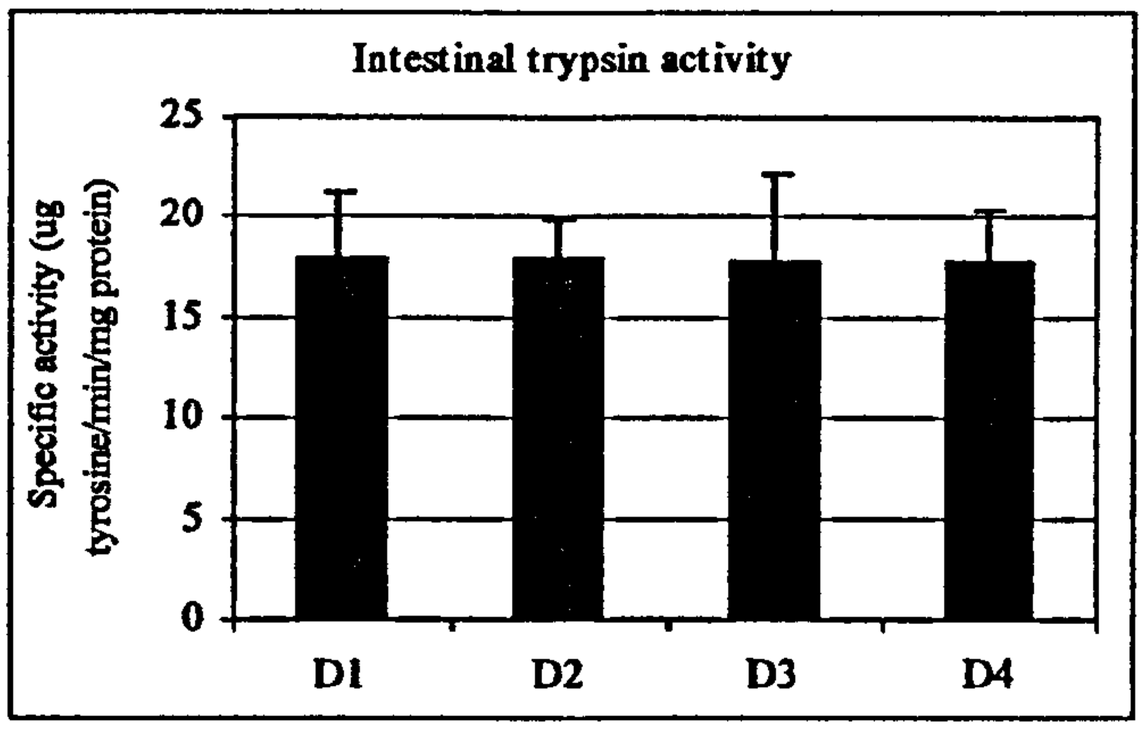


Figure 4.4 Total proteolytic activity in intestine, liver and stomach in tilapia fed different levels of FFSB (Top) is total proteolytic activity (PA) for control and test diets, (Bottom) is average PA affected by different pHs (mean values ±SD *n=2* five fish per duplicate).

D1 (control diet LT94 fishmeal), D2 (58% protein of FFSB), D3 (63% protein of FFSB) and D4 (63% protein of FFSB+ 1%-DL-methionine).



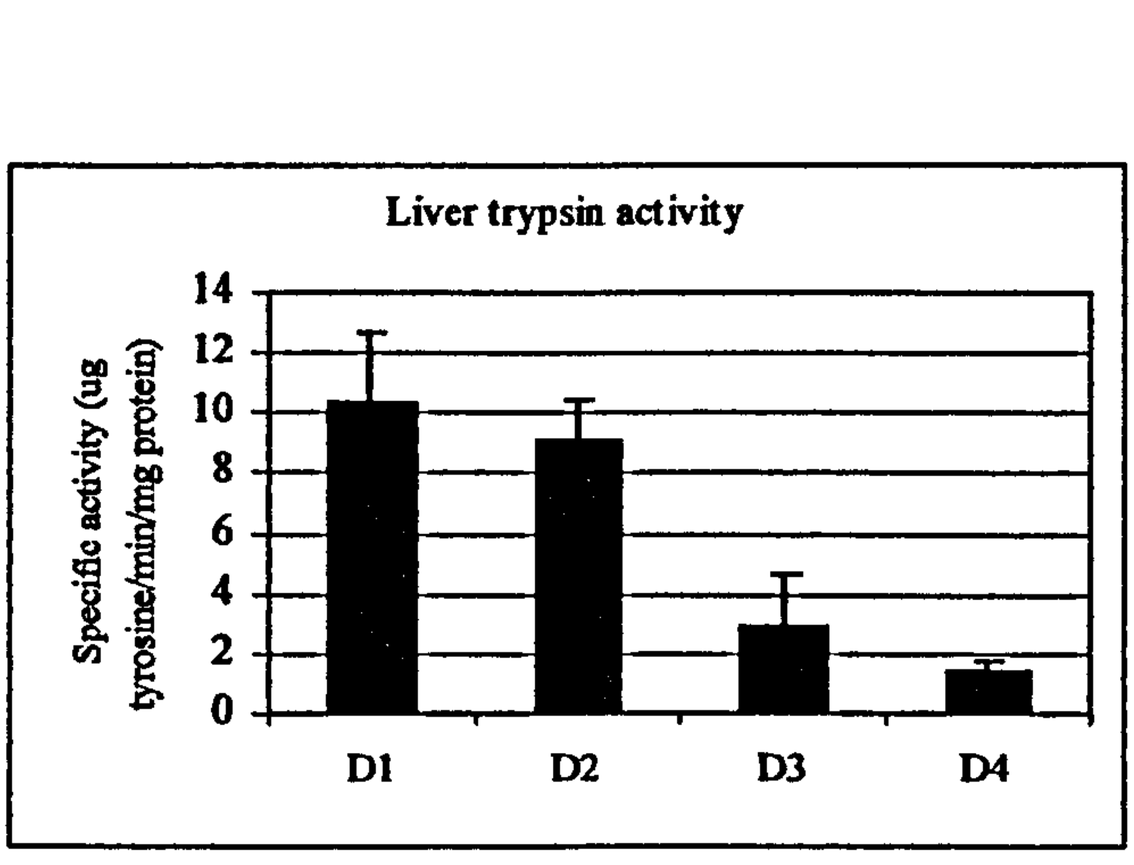


Figure 4.5 Effect of FFSB in diets on trypsin activity in Nile tilapia intestine, liver and stomach (mean values ±SD *n=2* five fish per duplicate).
Bars denote mean values ±SD.

D1 (control diet LT94 fishmeal), D2 (58% protein of FFSB), D3 (63% protein of FFSB) and D4 (63% protein of FFSB+ 1%-DL-methionine).

4.4 Discussion

Previous workers have extensively investigated the use of alternative protein sources in the substitution of fishmeal for many fish species. The main concerns are that increasing plant protein levels to replace fishmeal has the net effect of reducing growth rate and feed utilization. In addition, most of the alternative ingredients have inherent anti-nutritional (ANF's) factors that affect feed efficiency feed utilization and ultimately compromise growth rates of fish.

In this study, the results obtained demonstrate that plant protein sources such as full fat soybean are unable to replace 58% of a high quality fishmeal protein (LT94) in diets for tilapia. Inferior growth and feed utilization occurred when over 60% of the protein from fishmeal was substituted with the full fat soybean meal.

Additionally, tilapia fed 63% FFSB with DL-methionine supplementation did not show any improvement in the growth performance compared to the unsupplemented diet.

Other investigations have suggested that weight gain, feed conversion ratio, protein efficiency ratio and protein digestibility in Tilapia *O. niloticus* × *O. aureus* can be supported by diets containing full-fat soybean and defatted soybean levels up to 30% replacement of fishmeal (Shiau *et al.*, 1990). Lower levels of soybean meal inclusion was not tested in this experiment but may have been feasible. It is possible that FFSB protein replacement of fishmeal up to 30%.would have been acceptable.

A possible reason for the poor performance associated with the high substitution of plant proteins is an imbalance of nutrients, especially protein composition. This could be related to a less adequate dietary amino acid profile when FFSB is added to the formula as this latter ingredient is considered deficient in both lysine and methionine.

The results from the current trial with Nile tilapia demonstrate a trend towards a poorer feed conversion ratio and protein efficiency ratio for the diets containing FFSB at the expense of fishmeal. Also apparent net protein utilization values were all consistent and

similar to those reported by other workers. The data obtained with tilapia in this study would indicate that methionine supplementation was insufficient to improve the protein quality.

There are a number of reasons that could explain the decreased growth rates and poor feed consumption observed at higher levels of plant protein source inclusion such as FFSB. In addition, palatability of FFSB in tilapia was certainly lower compared to fishmeal based diets. Our results are in agreement with those obtained with yellowtail by Shimeno *et al.* (1993) who also reported reduced palatability in fish fed plant proteins.

These workers observed that diets containing the animal protein source meat and bone meal (MBM) supported higher feed utilization than those containing a similar level of corn gluten meal. A second reason for a decrease in feed utilization of FFSB is lower digestibility of plant proteins and energy, which was described in Chapter 3.

In addition, certain anti-nutritional factors (ANF's) are also known to specifically interfere with the digestive enzymes in the gastro-intestinal tract and associated organs such as the liver and pancreas thereby suppressing digestion and absorption.

Furthermore, in relation to the present study a decrease in growth rate was observed in all fish fed FFSB diets when compared with the LT94 fishmeal reference diet. Although the ANF's content of FFSB remains unquantified even after heat processing and advanced technology, it would not be unreasonable to assume that the ANF's content of FFSB is at least partially responsible for the poor growth performance of tropical fish fed the FFSB test diets. However, it should not necessarily be interpreted as a primary reason for the suppression of growth.

According to Tacon (1993) there are three factors which produce a reduction in growth performance and poor feed utilization in fishes fed high dietary inclusion levels of soybean protein. These are: - (1) amino acid imbalance, especially lysine and

methionine; (2) presence of endogenous anti-nutritional factors; and (3) lower digestibility of protein and carbohydrates.

In addition, (Shiau *et al.*, 1990) reported that there was a high fibre content (2.63 and 4.61%) in FFSB and defatted soybean respectively. Poorly processed FFSB probably inhibited the normal digestion process as fibre is negatively correlated with digestibility. Furthermore, FFSB was poorly digested with average protein and dry matter digestibilities as reported previously in Chapter 3.

However, one reason for fat supplementation is that when plant proteins such as maize gluten or FFSB are employed to replace fish meal on a nitrogenous basis, fat must be added to the diet to elevate the energy level because plant meals are generally lower in digestible energy than fish meal (Chapter 3). Investigations of digestive enzyme activities constitute an essential aspect of understanding the physiology of the digestive tract and the nutritional requirements of specific stages of development (Le Moullac *et al.*, 1996).

Uys & Hecht (1987) found that of the various digestive enzymes, protein digestion occurs mainly in the stomach and foregut in African catfish. This latter study agrees with the current study, which observed a high amount of proteolytic actually present in the intestine and stomach compared to the liver.

As stated previously, protease inhibitors are common anti-nutrient substances in many plant derived nutritional ingredients of potential value, especially the legumes (Norton, 1991). Also protease inhibitors (both trypsin and chymotrypsin inhibitors) particularly in legumes are known to decrease the growth performance in fish (Liener, 1994).

Hidalgo *et al.* (1999) also found no observable differences in proteolytic activity to justify fish classification as either omnivorous or carnivorous. However, Kuz`mina (1990) observed a high proteolytic potential in non-carnivorous fish. This should be

understood, on the basis that vegetable proteins are more difficult to digest compared to animal protein.

Nevertheless, omnivorous fish need less protein than carnivorous fish as a concentration of diet and protein has to be well utilized for fish that feed continuously.

Moyano *et al.* (1999) reported that Nile tilapia *O. niloticus* displayed a greater sensitivity to protease inhibitors present in defatted soybean meal, corn gluten meal and wheat bran on alkaline protease activity than sea bream *Sparus aurata* and African sole *Solea senegalensis*.

Recently, El-Sayed *et al.* (2000) reported that tilapia fed different sources of soybean, unheated full fat soybean, heated full fat soybean, soaked full fat soybean and commercial defatted soybean meals showed considerable differences in total activity of digestive protease between diets.

It should be noted that proteolytic activity was influenced at different pHs in different organs and tissues examined in tilapia for each dietary treatment at the end of the study. Only minor activity at acid pHs was detected in the intestine and liver whereas a high amount was actually observed in the stomach. It was interesting that the liver showed the lowest amount of protease at acid pHs due possibly to the fact that there are some intra-cellular enzymes with an optimal acid pH (Kuz`mina, 1990).

It is believed that acidity in the stomach also causes lysis of plant cell walls in macrophyte feeding fish (e.g. tilapias). Hydrolysis of the cell walls by HCl, facilitated by partial crushing of the ingested material by pharyngeal teeth, allows the plant cell contents to be subjected to the actions of the proteolytic enzymes (De Silva & Anderson 1998). In contrast, at alkaline pHs intestine and liver both showed a higher proteolytic activity.

The present findings agree with results observed by (Moyano *et al.*, 1999) who reported that the high sensitivity for protease activity in the digestive tract of tilapia were optimum at alkaline pH. On the other hand, Hidalgo *et al.* (1999) reported that the proteolytic activity in the digestive tract of eel was detected at an acid pH (pH 1.5) however trout showed opposite results which were more active at alkaline pHs.

Activity of some enzymes in carp has been shown to be related to the dietary composition and frequency of feeding. Das & Tripathi (1991) reported that optimum protease activity was recorded between pH 7.6 and 8.4 in both fingerling and adult grass carp *Ctenpharyngodon idella.* However, in the present experiment, the optimum protease activity was recorded in different organs which showed a different activity i.e. for intestine and liver, optimum pH ranged between 7.0-8.5, stomach 1.5-3.0 in tilapia. These findings are in agreement with Das & Tripathi (1991) who also found the optimum protease activity was recorded between pH 7.6 and 8.4 in both fingerling and adult grass carp *Ctenopharyngodon idella.* However, in the thick walled muscular stomach such as in the African catfish the pH is fairly high, around 4 (Uys & Hecht 1987).

According to some authors (Clark *et al.*, 1985; Uys & Hecht 1987 and Martínez & Sierra, 1989), the optimum pH for the trypsin-like activity is higher than that of chymotrypsin-like enzyme. The current results have demonstrated that the higher enzymatic activity at pH 8.5 than at 7.0 for tilapia agree with other species such as carp, trout, and sea bream (Hidalgo *et al.*, 1999). The detected results at high alkaline pHs (9.0 and 10.0) might be attributed to alkaline protease having carboxypeptidase, elastase or collagenase-like activities, as have previously been noted (Clark *et al.*, 1985).

In all fish species, the stomach takes time to develop and respond to varying diet intake and nutrient composition. Until it does, there will be inadequate digestion of protein in

the early stages of larval development. Similarly, in stomachless fish, there is initially low activity of alkaline proteases. In addition, stomachless species such as carp do not secrete either hydrochloric acid or pepsinogen for protein digestion, while fish possessing a stomach such as tilapia do produce copious amounts of acid secretion.

Commercial soybean products mostly show trypsin inhibitors (TI's) (Snyder & Kwon, 1987), TI's are present in the plant kingdom and in most legumes and cereals. The commonly cultured fish species show different results in their ability to tolerate dietary trypsin inhibitors. In this study with tilapia it can be seen that this species is quite sensitive to the amount of trypsin inhibitor in intestine and liver. Generally a considerable reduction of trypsin activities was found in the liver for tilapia fed high inclusion levels of FFSB.

In agreement with these findings Robaina *et al.* (1995) observed that gilthead sea bream fed diets containing soybean meal showed a reduction in trypsin activity and protein digestibility when substitution levels increased.

Takii *et al.* (1998) also conformed the general findings in this study, they demonstrated that soybean trypsin inhibitors (SBTI's) inhibited trypsin and basic proteinase in the hepatopancreas, pyloric ceaca and intestine of cultured marine and freshwater fish. Also Wilson & Poe (1985) observed that the growth rate and PER values improved when the trypsin inhibitor activity of the soybean meal decreased to tolerable levels for channel catfish. Also they found channel catfish can utilize soybean meal with higher trypsin inhibitor activity than carp with respect to nutrient digestibility and growth response.

The amylase activities in various organs (intestine, liver and stomach) also varied for tilapia in this study. The higher levels were shown in the liver rather than intestine and stomach and amylase activity in liver was affected in fish fed high FFSB inclusion levels in diet formulation for tilapia. The low amount of amylase in the stomach

indicates that very little starch is digested before the food reaches the foregut. This supports the findings of other authors (Uys & Hecht, 1987). Also Fraisse *et al.* (1981) reported that starch digestion and glucose absorption occurs mainly in the anterior part of intestine of fish which possess a stomach. Moreover, that there is a decreasing gradient in amylase activity from the anterior towards the posterior part of the intestine.

Al-Owafeir (1999) reported that α-amylase activity was present in Nile tilapia, and that may indicate that Nile tilapia is more adapted to utilization and digestion of carbohydrate more than in the African catfish. In agreement with this study, Steffens (1989) reported that the amylase activity in the hepatopancreas is so regulated that carbohydrate of dietary origin is well assimilated and hence levels of amino-acid decomposition and gluconeogenesis in the hepato-pancreas diminished in carp.

Nile tilapia exhibits a negative reaction to carbohydrate levels in response to high dietary levels of starch, but a positive reaction in respect of both α-glucosidase and β-galactosidase activity in response to elevated lactose content (Steffens, 1989).

Early studies on lipase activity in the intestinal tract of tilapia have reported varied results. Moriarty (1973) found no lipase activity in the gut of *T. nilotica.* However, in agreement with the current study Al-Hussaini & Kholy (1953) and Nagase (1964) have previously demonstrated this enzyme in tilapia, with the activity most prominent in the proximal and middle parts of the intestine but these studies are not conclusive. Recently, Tengjaroenkul *et al.* (2000) suggested that lipolytic activity in *O. niloticus* is definitely present, and occurs mainly in the anterior half of the intestinal tract. The relatively restricted distribution of lipase enzyme in the Nile tilapia may be due to the fact that lipase activity is lowest in herbivorous fish (Opuszynski & Shireman, 1995), related to the low fat content in plant materials naturally consumed by tilapia (Vonk & Western, 1984; Opuszynski & Shireman, 1995).

Barnard (1973) maintained that pancreatic lipase is the major enzyme involved in the digestion of animal and plant triglycerides in all vertebrates and that it is present in the zymogen granules. Das & Tripathi (1991) reported that lipase activity was generally highest in the hepatopancreas of both the adult and fingerling grass carp *Ctenopharyngodon idella.* This latter study supported the present findings, which found lipase activity was present in gut and liver for tilapia, however the amount of lipase was decreased by the substitution of FFSB. In contrary, Yamamoto & Akiyama, (1995) noted that lipase activity in stomach and intestine for fingerling Japanese flounder *Paralichthys olivaceus* fed three different diets containing carboxymethylcellulose, gelatinized potato starch (α-starch) and wheat gluten did not show a difference among groups.

Lipase activity has been found in extracts of the pancreas, pyloric caecae and upper intestine, but not necessarily in all three sites in all species. Lipase activity is almost non-existent in the stomach, and in the intestine the principal site for lipase is the mucosal layer. In general, fish utilize dietary lipid effectively as an energy source (Sargent *et al.*, 1989).

In summary: - the investigation has demonstrated that tilapia are unable to grow fairly effectively with high replacement (58-63%) of the fishmeal component of the diet with full fat soybean meal. Growth was appreciably compromized and feed utilization was much lower than the control diet group of fish. The physiological processes of digestion were noticeably impaired and specific gastro-intestinal enzymes were affected by the change in diet composition.

CHAPTER 5

EVALUATION OF FISHMEAL AS A REFERENCE PROTEIN SOURCE AND SUBSTITUTION WITH POULTRY BY-PRODUCT MEAL IN DIETS FOR AFRICAN CATFISH *Clarias gariepinus.*

5.0 Introduction

Fish require high levels of protein in their diets (25-55%) (Wilson & Halver, 1986) and fishmeal constitutes the main protein source and its highest cost component (Crampton, 1985). Efforts to completely substitute fish meal with ingredients of plant or animal by-products have not been completely successful in a number of fish species (Higgs *et al.*, 1988).

Fish meals are known to vary considerably in their protein and nutrient composition such as amino acid content depending on the freshness of the raw material, amount of remaining lipid, drying process and temperature, and whether the meal was made from whole fish or the waste from some other treatment operation. Condition and length of storage may also affect quality (Pedersen & Eggum, 1983). However, production of fish meal already involves approximately 35% of the total global fish catch (Tacon & Dominy, 1999).

As a consequence of the increasing cost of high quality fishmeal, however, there is a real problem for economic and effective feed formulation. Feed represents the major cost in modern intensive aquaculture. It is generally recognized that over-heating during the drying of fish meals probably causes complexing of limiting amino acids and adversely reduces protein quality. Since lysine is a major amino acid in many proteins, and is particularly affected by heat damage, its availability has been extensively studied (Carpenter & Booth, 1973). Heating also influences the status of cysteine/ cystine residues in diets and further reduces protein utilization by fish (Opstvedt *et al.*, 1984).

The traditional practice in animal husbandry is to partially or totally replace animal protein with less expensive plant protein sources to obtain least cost diet formulation without decreasing the quality of the feed. These include supplementation of one or more limiting essential amino acids in diet formulation for fish species, combining various ingredients to restore an optimum dietary amino acid profile and processing

plant proteins to remove anti-nutritional factors. In the last few years the quality of fish meals produced in the world has been improved and developed significantly, but relatively few investigations have been undertaken to evaluate their protein quality compared to high-quality Norwegian fishmeal products. Norse-LT94 is a high quality fishmeal produced under the strict guidelines of the Norwegian Herring Oil and Meat Institute for use in aquaculture (Pike *et al.*, 1990). Diets made with Norse-LT94 have been observed to produce approximately a 15% increase in growth rate in Atlantic salmon compared to feeds based on a regular steam-dried fish meal (Pike *et al.*, 1990).

Anderson *et al.* (1997) have also reported that the apparent digestibility coefficients for protein in Atlantic salmon fed LT meals were higher than fish fed herring fishmeal.

The protein quality of fish meals (AOAC, 1984) may be determined *in vitro* using individual proteolytic enzymes or a combination of them (Pedersen & Eggum, 1983) and depends on an estimation of the extent of proteolysis. Generally, the assay is made in a closed system, where the protein digestibility is estimated by nitrogen or amino acid analysis of the various digestion fractions. Thus, the amount of proteolysis determined varies according to the procedure used. Optimum activity of the proteolytic enzymes used is pH-dependant; keeping pH constant ensures uniform enzyme activities (Pedersen & Eggum, 1983).

Much of the cost of aquafeed production is due to the extensive use of fishmeal in the feed (Tacon, 1994; Higgs *et al.*, 1995). Therefore, numerous attempts have been made to develop economical alternative protein and energy sources for inclusion in fish diets. Considerable efforts are also being made to improve fishmeal quality so that maximum nutritive value can be obtained from this expensive dietary component. Historically, fish meal has been the most desirable protein concentrate in formulated feeds; however, this

is an expensive resource for use in warm water fish diets. The demand for fish meal is expected to raise the price of compounded feeds even higher than present levels due to the rapid expansion of aquaculture (Tacon, 1996).

This will become an increasing problem in countries that rely on the impact of this resource for fish feed manufacture. Alternative feed ingredients will become even more cost effective for incorporation in diets especially those destined for warm water fish species such as carp, tilapia and catfish (Rumsey, 1993).

Gouveia (1991) critically reviewed the use of animal by-products in diets for salmonids. This author described the use of various protein concentrates that originated from the rendering industries.

One of the more promising sources is poultry by-product meal (PBM), the rendered product of poultry processing waste, made from inedible portions of poultry, excluding feathers. PBM, has been previously evaluated in diets for chinook salmon, *Oncorhynchus tshawytscha* (Fowler 1981a,b, 1990, 1991), coho salmon, *Oncorhynchus kisutch* (Higgs *et al.*, 1979), and Atlantic salmon, *Salmo salar* (Bergström, 1973). PBM has been tested as a partial fishmeal replacement in diets of channel catfish, *Ictalurus punctatus* (Brown *et al.*, 1985) and rainbow trout *Oncorhynchus mykiss* (Alexis *et al.*, 1985).

However, compared to fishmeal, animal by-products may be deficient in one or more essential amino acids (Davies *et al.*, 1991). More recently, Nengas *et al.* (1999) reported that diets containing poultry meat meal (PMM), poultry feather meal (PFM) mixture and PBM at 40% replacement of fishmeal in gilthead seabream, *Sparus aurata* indicated that the first limiting essential amino acid (EAA) was methionine.

Investigations concerning the use of animal by-product meals have been conducted with other freshwater fish species mainly carp and tilapia with good results (Rodriguez-Serna *et al.*, 1996). There is little information available for the use of such products in practical diet formulations for the African catfish, *Clarias gariepinus*. A poultry by-product meal available in the UK was evaluated as an alternative protein source for African catfish.

5.1 Experiment 1: - Assessment of fishmeal quality for African catfish- materials and protocols

5.1.1 Experimental fish

African catfish, *Clarias gariepimus* as described in Chapter 2 section 2.1 were used in the investigation. Fifteen fish were graded and transferred to each tank with an average weight of 33g. The dietary treatments were tested on duplicate groups of fish. A similar group of twenty fish was killed using a lethal concentration of benzocaine and kept frozen at -20^{0}C to determine initial carcass composition.

5.1.2 Experimental diet

Four experimental diets were formulated to contain a variable type of fishmeal (LT94 and white fishmeal), as either wet or dry diets. These are described as D1 (LT94 dry), D2 (LT wet), D3 (white fishmeal dry) and D4 (white fishmeal wet). The wet diets were frozen at -20^{0}C, and remained in the freezer with the daily aliquot removed as necessary. Samples of all diet types were withdrawn immediately after preparation and stored at –20^{0}C prior to analysis of proximate composition.

All diets were designated to be isoenergetic and isonitrogenous and were adjusted at appropriate levels and to contain 40 % crude protein and 15 % lipid.

Table 5.1.1 shows the proximate feed formulation and chemical composition of the experimental diets and Table 5.1.2 shows the proximate chemical composition of the fish meals used.

5.1.3 Feeding regime

Culture facilities were described in section 2.3. Fish were fed for one week for acclimation using a commercial trout diet (Trouw aquaculture 2mm pellet). At the end of the acclimation period, the fish were weighed and then started on the respective experimental diets. Each of eight tanks was randomly assigned to one of the dietary treatments.

Fish were hand fed twice daily, to satiation, and were weighed individually every fourteen days. Feed was withdrawn on the day of weighing and feed intake was recorded over the 12-week period. At the end of the experiment, the final weights of fish were measured following a 24 hour starvation period. Five fish were collected from each treatment, (after 8 weeks and 12 weeks), randomly selected from each treatment to carcass composition analyses.

5.1.4 Proximate composition analysis

Proximate compositions of diets and fish tissues for moisture, protein, lipid, ash and gross energy were determined as described in Chapter 2 section 2.4.

5.1.5 Determination of amino acids

The amino acid contents of the diets were determined as described in Chapter 2 section 2.4.6. Essential amino acid compositions have been listed in Table 5.1.3.

Table 5.1.1 Feed formulation (as fed) and results of proximate analysis ($g100g^{-1}$ dry weight).

Ingredients	D1 LT94 (dry)	D2 LT94 (wet)	D3 WFM (dry)	D4 WFM (wet)
Fish meal[1]	58.71	–	67.95	–
Wheat meal[2]	18.01	–	18.01	–
Corn oil[3]	4.94	–	5.52	–
Cod liver oil[4]	4.94	–	5.52	–
Vitamin premix[5]	2.00	–	2.00	–
Mineral premix[6]	1.00	–	1.00	–
∝ cellulose[7]	10.40	–	–	–
Proximate composition (as fed)				
Moisture	4.93	36.67*	4.82	29.76*
Protein	41.51	26.29	40.95	28.76
Lipid	17.20	10.89	16.31	11.45
Ash	11.6	7.34	18.4	12.92
Gross Energy ($MJkg^{-1}$)	21.20	13.42	20.02	14.05
Phosphorus ($g100g^{-1}$)	1.38	0.87	3.25	2.28
Calcium ($g100g^{-1}$)	1.30	0.82	3.71	2.61

[1] Fish meal LT94, Trouw Aquaculture (Nutreco Company).
[2] Wheat meal, Kalpro S™. Orsan, Paris, France
[3] Mazola- pure corn oil
[4] Fish oil- seven pure cod liver oil
[5] Vitamin premix, Trouw Aquaculture (Nutreco Company)
[6] Mineral premix, Trouw Aquaculture (Nutreco Company)
[7] Sigma Chemical Co., Poole, Dorset.
* Added water.

Table 5.1.2 Proximate composition analysis for fish meals ($g100g^{-1}$ dry weight)

	Moisture	protein	lipid	Ash
LT94 fish meal	8.67	72.3	11.3	13.00
White fishmeal	8.66	63.75	5.82	23.30

Table 5.1.3 Essential amino acid composition (expressed as % of protein) of the two types of fishmeal in diet composition (LT94 and white fishmeal dry & wet) fed to African catfish *Clarias gariepinus*.

	D1 & D2 Lt94 (dry & wet)	D3 & D4 WFM (dry & wet)	African catfish Requirements*
Arginine	7.40	8.14	-
Histidine	3.13	3.59	-
Isoleucine	5.85	6.13	-
Leucine	10.61	10.24	-
Lysine	7.17	7.53	5.70[1]
Methionine	3.53	3.64	3.20[1]
Methionine + Cysteine	4.35	4.13	-
Phenylalanine	6.26	6.67	-
Phenylalanine + Tyrosine	10.85	11.39	-
Threonine	5.68	5.88	-
Valine	5.83	5.91	-
Tryptophan	ND	ND	-

* Requirements for all amino acids have not been determined for African catfish.
[1]Source: Fagbenro *et al.* (1998a,b).
ND (not detected).

5.1.6 Determination of minerals

Phosphorus and calcium contents of diets and carcasses were determined using a Varian Atomic Absorption Spectrophotometer (AA-600). Triplicate 0.07-0.10g samples were weighed into weighing boats and then transferred to Teflon PFA digestion tubes. To each of these tubes, 2ml of nitric acid (69% w/w) were added, the tubes had tight lids and were placed upright in the microwave rack and put in a Microwave (CEM) Innovators in Microwave Technology (MDS-2000) for half an hour. After the samples were digested, volumes were adjusted to 10ml with distilled water and samples were stored at 5^0C until subsequent analysis.

Calcium and phosphorous ($g100g^{-1}$) were determined thus:

= absorbance × dilution / weight of sample (g)

5.2 Experiment 2: - Evaluation of poultry by-product meals for catfish-materials and protocols

5.2.1 Experimental fish

African catfish *Clarias gariepinus* as described in Chapter 2 section 2.1 were used. Twenty fish with an average weight of 16.5g were graded and transferred to each tank. All dietary treatments were tested on duplicate groups of graded fish of uniform size.

5.2.2 Experimental diets

The experimental diets were formulated as described in Chapter 2 section 2.2. Six experimental diets were formulated to contain a variable proportion of PBM to partially and totally replace the fishmeal component of the diet.

Diet I is a control containing (LT94 fishmeal), D2 (20% PBM), D3 (40% PBM), D4 (60% PBM), D5 (80% PBM) and D6 (100% PBM). PBM was obtained from a commercial source (Sun Valley Poultry Ltd.). This is defined as the milled dry rendered material originating from the processing of ground, rendered clean parts of the carcass of slaughtered chicken, turkey and duck. This is inclusive of heads, feet, undeveloped ova and intestines (exclusive of feathers) as offal. The material is cooked at a temperature of 125^{0}C at 1Atm for approximately 3hours.

The product typically consists of 16% ash and 50-60% crude protein with a fat content of about 18%. All diets were designed to be isoenergetic and isonitrogenous in gross nutrient terms and were adjusted to appropriate levels to contain 37% crude protein and 15% lipid.

Table 5.2.1 shows the proximate feed formulation and composition of the experimental diets. A control diet based on fishmeal (LT94) served as the reference source of dietary protein used to substitute with PBM.

Table 5.2.1 Composition and proximate analysis of the control and test diets ($g100g^{-1}$ dry weight).

Ingredients	D1 0% PBM	D2 20% PBM	D3 40% PBM	D4 60% PBM	D5 80% PBM	D6 100% PBM
Fish meal[1]	40.00	32.00	24.00	18.00	10.00	–
PBM[2]	–	9.00	17.00	24.00	33.00	45.50
Wheat meal[3]	38.00	38.00	40.00	41.00	41.00	38.00
Blood meal	2.00	2.00	2.00	2.00	2.00	2.00
Corn oil[4]	4.41	4.12	3.91	3.66	3.37	2.94
Cod liver oil[5]	4.41	4.12	3.91	3.66	3.37	2.94
Vitamin premix[6]	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[7]	1.00	1.00	1.00	1.00	1.00	1.00
Binder[8]	2.00	2.00	2.00	2.00	2.00	2.00
∝-cellulose[9]	6.18	5.76	4.18	2.68	2.26	3.62
Proximate composition (% as fed)						
Moisture	4.81	4.44	4.73	3.25	4.38	4.54
Protein	34.98	36.63	35.60	36.25	35.92	35.94
Lipid	17.49	17.6	17.44	16.50	15.11	15.28
Ash	7.35	8.03	7.96	8.69	8.93	9.72
Gross energy ($MJkg^{-1}$)	21.02	24.61	21.08	20.60	19.58	19.80

[1] Fish meal LT94, Trouw Aquaculture (Nutreco company).
[2] Poultry by-product meal (PBM). Prosper de Mulder Ltd, UK
[3] Wheat meal, Kalpro S™. Orsan, Paris, France
[4] Mazola- pure corn oil
[5] Fish oil- seven pure cod liver oil
[6] Vitamin premix, Trouw Aquaculture (Nutreco company).
[7] Mineral premix, Trouw Aquaculture (Nutreco company).
[8] Carboxymethylcellulose.
[9] Sigma Chemical Co., Poole, Dorset.

Table 5.2.2 shows the EAA composition of experimental diets as determined. A ground wheat meal was also included as the main carbohydrate source and bulk filler component. Five diets were formulated with an incremental substitution of fishmeal with poultry by-product meal up to 100% replacement.

Table 5.2.2 Essential amino acid composition of protein component (expressed as % of protein) of the experimental diets.

	D1 0% PBM	D2 20% PBM	D3 40% PBM	D4 60% PBM	D5 80% PBM	D6 100% PBM
Arginine	6.51	5.13	4.83	4.57	4.64	4.59
Histidine	3.38	2.81	2.58	2.36	2.23	2.13
Isoleucine	4.20	3.40	2.66	2.80	2.41	2.59
Leucine	8.43	6.70	5.80	5.69	5.24	5.58
Lysine	6.53	4.94	4.38	3.94	3.74	2.85
Methionine + Cysteine	2.83	2.71	2.78	1.96	1.98	1.88
Phenylalanine + Tyrosine	7.97	6.49	6.40	5.72	5.65	5.48
Threonine	5.06	3.73	3.38	3.02	2.93	3.14
Valine	5.33	4.03	3.50	2.90	2.90	3.16
Tryptophan	ND	ND	ND	ND	ND	ND

ND (not detected).

5.2.3 Feeding regime & experimental protocol.

Fish were fed for one week for acclimation to each test diet within the experimental system and to free their gastrointestinal tract from the pre-experimental diet regime (Trouw aquaculture 2mm pellet, salmon starter-54% CP & 15% lipid) until the feeding response was uniform. Culture facilities were as described in section 2.3.

Twenty fish with an average weight of 16.5g were graded and transferred to each tank. All dietary treatments were tested in duplicate groups of graded fish of uniform size.

Initially thirty fish were killed using a lethal concentration of benzocaine and stored frozen (at -20^0C) to determine initial carcass composition. Six fish were randomly collected from each treatment at the end of feeding trial to analyze the carcass and muscle composition. Fish were fed twice daily by hand at a rate of 4% body weight per day for the first 4 weeks and this was subsequently reduced to 3% for the remaining 4 week period and finally to 2.5% in the last 2 weeks of the trial.

The fish were weighed bi-weekly and were not fed on the day prior to weighing. The experiment was undertaken for a total period of 10 weeks. On termination of the study, the final weight of fish was measured following a 24-hour starvation period.

5.2.4 Proximate composition

Proximate compositions of diets and fish tissue for moisture, protein, lipid, ash and gross energy were determined as a described in Chapter 2 section 2.4.

5.2.5 Determination of amino acids

The amino acid contents of the diets were determined as described in Chapter 2 section 2.4.6.

5.2.6 Histological studies histological preparation and staining techniques

At the termination of the feeding trial, five fish from each group were sacrificed and their livers removed for histological examination. Hepatic tissues were sampled and also processed as described in Chapter 2 section 2.8.

Image analysis was applied for assessment of hepatic tissues as described in Chapter 2 section 2.8.2.

5.2.7 Photomicrographs

Photomicrographs were taken using a Zeiss photomicroscope II and a Nikon 950 coolpix digital camera at an objective magnification of ×40 and photo-eyepiece ×2.5. A green filter was used for all photomicrographs and the condenser iris position kept constant.

5.2.8 Statistical treatment of data

Statistical analyses were carried out as described in Chapter 2 section 2.9.

5.3 Experiment 1: - Results

5.3.1 Growth performance and feed utilization

The growth performance and feed utilization data for African catfish fed the four diets are presented in Figure 5.1.1 & Table 5.3.1. This experiment examined the differences between two types of fishmeal, LT94, and white fishmeal (WFM). There was an observed difference between the final average body weights amongst catfish fed on the experimental diets. Fish fed on dry fishmeal diets of (LT94) and white fish meal (WFM) resulted in an increased in final average body weight compared with the LT94 wet, and white fish meal (WFM) on a wet basis. All diets showed no significant differences between treatments ($p > 0.05$). With respect to feed intake, the catfish exhibited a preference for the LT fishmeal source for both dry and wet diets tested. Voluntary feed intake (VFI) (% bw/d^{-1}) was 4.51% and 4.35% for LT94 diets respectively compared to 3.53 and 3.83% respectively for the white fishmeal sources.

Specific growth rate (SGR%), feed conversion ratio (FCR) and protein efficiency ratio (PER) were all inferior for catfish fed the wet fishmeal based diets. SGR valued from 2.80-2.46% bwd^{-1} whilst PER ranged from 3.17-2.21 across the treatments Figure 5.1.2. Apparent net protein utilization (ANPU%) showed a significant difference between

treatments, which support the similar trend with other feed utilization parameters which was higher for catfish fed dry diets compared to catfish fed wet diets (Table 5.3.1).

Table 5.3.1 Weight increase, feed consumption, nutritive utilization of feed and protein, and hepatosomatic index (HSI) (mean ±SD *n=2*).

	D1 LT94 (dry)	D2 LT94 (dry)	D3 WFM (dry)	D4 WFM (wet)
Mean initial weight (g)	32.61 ±3.41	33.23 ±4.52	32.99 ±3.89	32.89 ±3.72
Final weight (g)	290.51^{b}	225.77^{a}	282.37^{b}	244.59^{ab}
Weight gain (g)	257.90 ±14.93^{b}	192.54 ±20.90^{a}	249.38 ±19.79^{b}	211.70 ±18.6^{ab}
Voluntary feed intake (% of body weight)	4.51	4.35	3.53	3.83
SGR (%)	2.80 ±0.05^{b}	2.46 ±0.06^{a}	2.75 ±0.06^{b}	2.57 ±0.05^{a}
FCR	0.97 ±0.09^{a}	1.30 ±0.13^{b}	0.80 ±0.08^{a}	1.30 ±0.15^{b}
PER	2.54 ±0.22^{a}	1.99 ±0.19^{a}	3.13 ±0.28^{b}	2.18 ±0.01^{a}
ANPU (%)	41.85 ±3.91^{b}	31.43 ±3.08^{ab}	52.94 ±4.80^{c}	30.9 ±3.66^{a}
Liver glycogen mg g^{-1} wet weight.	60.67 ±2.32^{c}	52.07 ±2.67^{b}	31.33 ±3.30^{a}	33.68 ±2.36^{a}
HSI (%)	0.93 ±0.19^{a}	0.89 ±0.03^{a}	0.82 ±0.06^{a}	0.70 ±0.02^{a}

Values in the same row with the same superscript are not significantly different ($p > 0.05$).

Liver glycogen in fish fed on the LT94 diet was higher compared to fish fed WFM diet. These trends showed also in the weight gain figures, which decreased when the fish fed diet inclusion WFM. No mortality was observed during the experimental period. Feeding rates obtained in fish fed the diet containing fishmeal (LT94) dry were superior compared to those fed other diets.

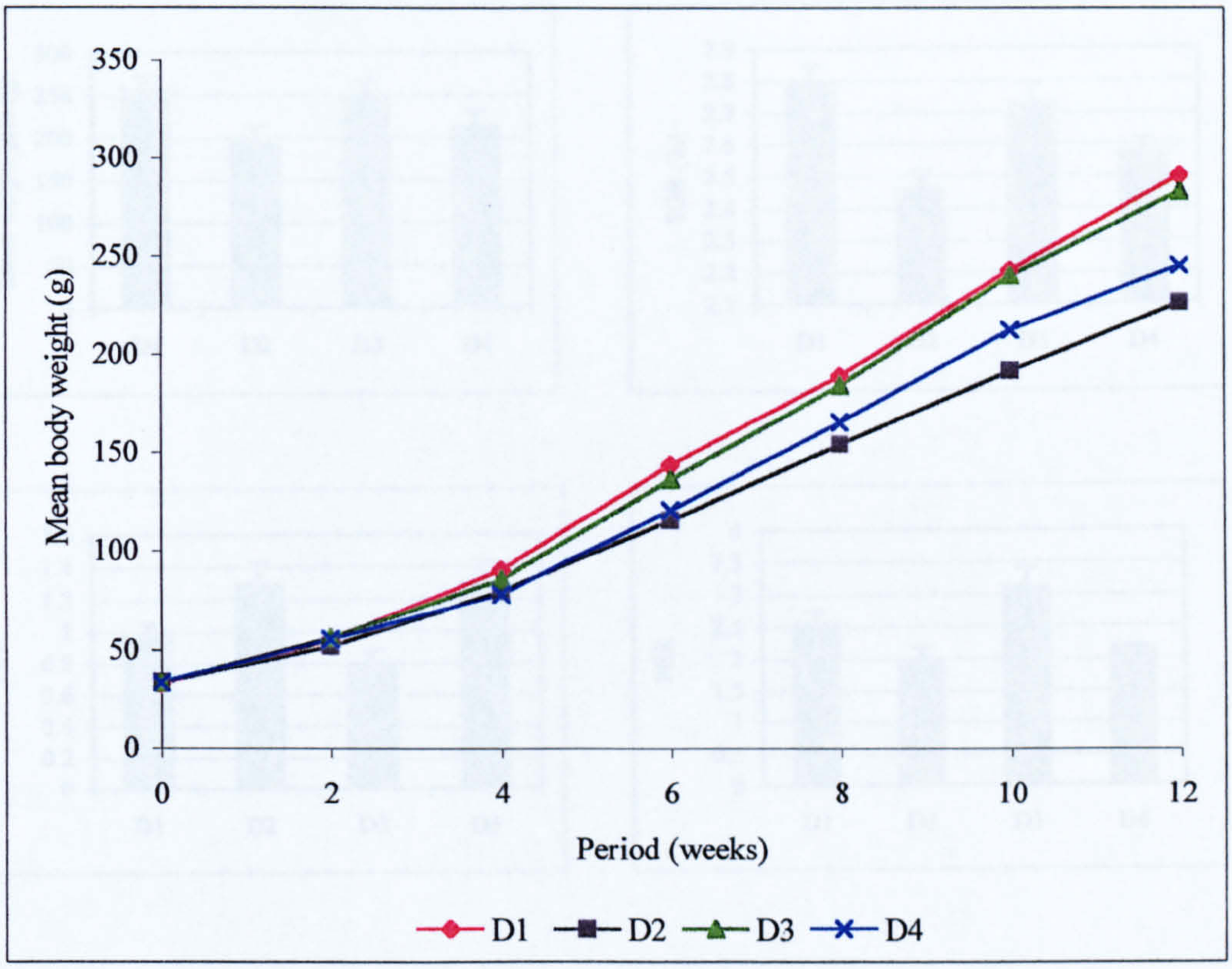


Figure 5.1 Growth performance of African catfish fed diets containing different types of fishmeal (wet & dry) over 12 weeks.

D1 (LT94 fishmeal dry), D2 (LT94 fishmeal wet), D3 (white fishmeal dry) and D4 (white fishmeal wet).

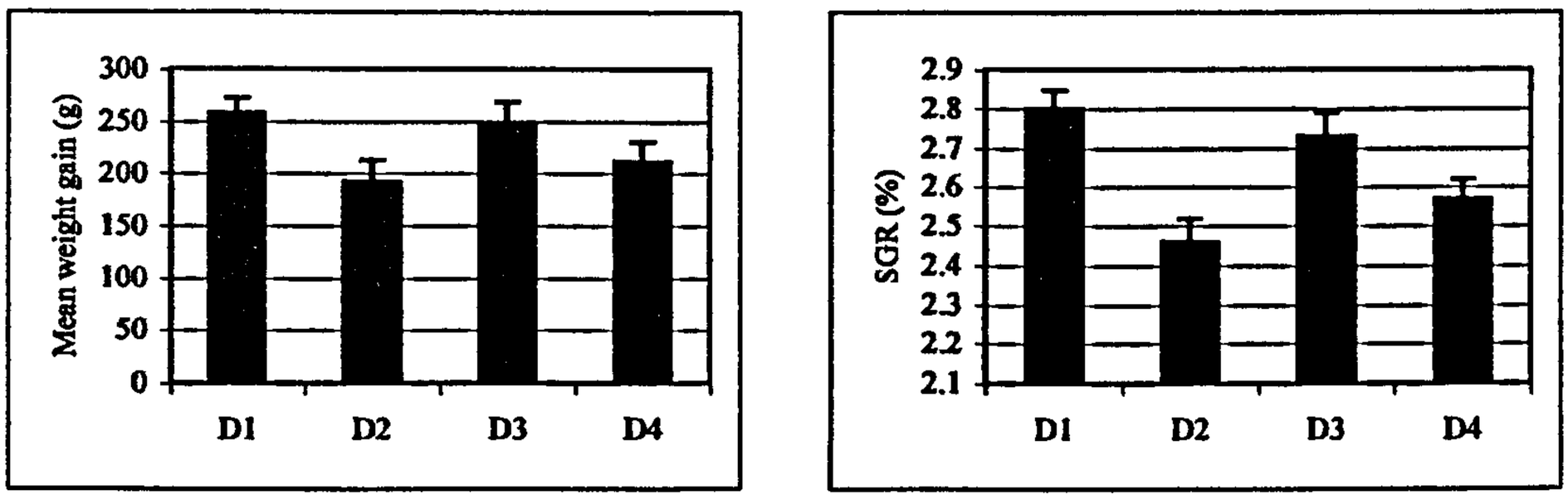


Figure 5.2 Growth and feed performance for *Clarias gariepinus* fed different source of fishmeal.
Bars denote mean values ±SD (*n=2*).

D1 (LT94 fishmeal dry), D2 (LT94 fishmeal wet), D3 (white fishmeal dry) and D4 (white fishmeal wet).

5.3.2 Fish body composition

The initial and final carcass composition of the fish fed the experimental diets is presented in Table 5.3.2. The carcass composition was determined after 8 weeks and 12 weeks. The final carcass composition showed slight differences in the nutrient components determined.

Table 5.3.2 Carcass Composition ($g100g^{-1}$ wet weight) of Catfish (means ±SD *n=2* five fish per duplicate) (8 & 12 Weeks).

Proximate composition (% wet weight)	Initial fish	D1 LT94(dry)	D2 LT94(wet)	D3 WFM (dry)	D4 WFM (wet)
8 weeks					
Moisture	76.30	73.21 ±0.80^{a}	74.33 ±0.96^{b}	73.71 ±0.97^{ab}	74.11 ±1.51^{ab}
Protein	14.68	16.35 ±0.44^{a}	16.36 ±0.58^{a}	16.05 ±0.45^{a}	16.40 ±0.94^{a}
Lipid	5.71	6.56 ±0.95^{b}	5.58 ±0.94^{a}	6.28 ±0.84^{ab}	5.97 ±1.22^{ab}
Ash	3.02	3.46 ±0.16^{a}	3.58 ±0.23^{a}	3.53 ±0.17^{a}	3.54 ±0.21^{a}
12 weeks					
Moisture		73.05 ±1.0^{a}	74.18 ±1.20^{a}	73.32 ±0.98^{a}	74.07 ±1.85^{a}
Protein		16.43 ±0.10^{ab}	16.50 ±0.79^{ab}	16.96 ±0.39^{b}	16.02 ±0.50^{a}
Lipid		6.85 ±1.10^{a}	5.76 ±1.30^{a}	5.39 ±1.22^{a}	6.05 ±1.55^{a}
Ash		3.78 ±0.19^{a}	3.75 ±0.38^{a}	4.10 ±0.25^{a}	4.08 ±0.28^{a}
Phosphorus ($g100g^{-1}$)	2.61	2.45 ±0.28^{a}	2.16 ±0.67^{a}	2.94 ±0.76^{a}	2.45 ±0.31^{a}
Calcium ($g100g^{-1}$)	2.46	2.12 ±0.51^{a}	2.07 ±0.42^{a}	2.23 ±0.48^{a}	2.67 ±0.23^{a}

Values in the same row with the same superscript are not significantly different ($p>0.05$).
(After arcsine transformation of original data)

The fish fed on the LT94 showed slightly lower ash content in carcass after 12 weeks which, was 3.78 and 3.75% for the dry diet and wet diets respectively, which was related to the fishmeal ash content, while the fish fed WFM showed high ash content which was 4.10 and 4.05% for dry and wet diets respectively. Moisture, protein and lipid were all very similar after 8 weeks and 12 weeks feeding of the different diets.

5.4 Experiment 2: - Results

5.4.1 Growth performance

The growth performance and feed utilization data for African catfish fed the six respective diets are shown in Figure 5.3 & Table 5.3. There was a significant differences between the final average body weights amongst the fish fed each of the experimental diets. Fish fed the fishmeal (LT94) based control diet demonstrated the highest mean final body weight (175.5 g) resulting in a 10- fold increase in weight from the start of the study.

However, the lowest value (103.8g) was observed for catfish fed the 100% inclusion level of PBM in the diet replacing the entire fishmeal component.

The control diet supported the highest weight gain of 158.9 g, while fish fed the PBM 100% diet exhibited the lowest weight gain of 87.3g. It was apparent that above 40% inclusion, PBM resulted in a significant reduction in growth performance for a final weight gain.

The specific growth rate (SGR%) values further supported this trend, with SGR reduced from 3.47 for the control diet fed fish to 2.83 for the fish fed the 100% PBM protein diet. Fish fed the 40 and 60% PBM protein performed better than those on the 80 and 100% level of (PBM), while the 20% inclusion level was very similar to the control group. No mortality was observed during the experimental period and the overall health of the fish appeared normal.

5.4.2 Feed consumption and feed utilization

All diets were well accepted by the catfish, except diet (6), containing the maximum amount of poultry by-product meal. Mean daily feed intake ranged between 3.93 and 3.02 g100g^{-1} fish. There was a noticeable effect of the higher inclusion of alternative protein sources on feed intake (Table 5.4.1). Feed intake for catfish fed the diet containing the highest amount of fishmeal (LT94) was significantly better than observed for fish fed diets including PBM.

Table 5.4.1 Weight increase, feed consumption, nutritive utilization of feed and protein, and hepatosomatic index (HSI) of diets fed to African catfish (mean ±SD n=2).

	D1 0% PBM	D2 20% PBM	D3 40% PBM	D4 60% PBM	D5 80% PBM	D6 100% PBM
Mean initial weight (g)	16.50 ±0.12^{a}	16.50 ±0.10^{a}	16.50 ±0.14^{a}	16.40 ±0.10^{a}	16.50 ±0.11^{a}	16.50 ±0.10^{a}
Mean final weight (g)	175.5 ±2.7^{de}	180.76 ±2.5^{e}	167.46 ±2.6^{cd}	157.37 ±2.0^{c}	143.1 ±1.5^{b}	103.82 ±1.9^{a}
Weight gain (g)	158.90 ±2.84^{e}	164.30 ±2.47^{e}	151.00 ±2.69^{d}	140.90 ±2.31^{c}	126.60 ±1.56^{b}	87.30 ±1.91^{a}
Mean daily feed intake (g 100g^{-1} fish^{-1} d^{-1})	3.93	3.99	3.78	3.78	3.52	3.02
SGR	3.57 ±0.06^{de}	3.68 0.03^{e}	3.56 ±0.06^{cd}	3.48 ±0.03^{c}	3.32 ±0.02^{b}	2.83 ±0.06^{a}
FCR	1.61 ±0.04^{ab}	1.58 ±0.04^{a}	1.63 ±0.07^{ab}	1.74 ±0.06^{bc}	1.86 ±0.07^{c}	2.25 ±0.08^{d}
PER	1.78 ±0.11^{c}	1.73 ±0.08^{c}	1.72 ±0.06^{c}	1.58 ±0.08^{bc}	1.50 ±0.08^{b}	1.24 ±0.06^{a}
ANPU (%)	28.90 ±0.85^{d}	28.18 ±0.48^{d}	27.20 ±1.21^{cd}	25.94 ±0.83^{c}	23.37 ±0.88^{b}	18.82 ±0.74^{a}
HSI (%)	1.58 ±0.06^{bcd}	1.50 ±0.08^{abc}	1.63 ±0.06^{cd}	1.31 ±0.10^{a}	1.40 ±0.14^{ab}	1.74 ±0.08^{d}

Values in the same row with the same superscript are not significantly different (p>0.05).

FCR values also differed significantly amongst the groups of fish. The poorest FCR was obtained for catfish fed the test diet containing 100% PBM protein with a value of 2.25. Superior FCR (< 2) were obtained for the remaining diets (control diet and 20% PBM showed the best FCR, at 1.61 and 1.58 respectively) (Table 5.4.1 and Figure 5.4).

Protein efficiency ratio (PER) was noticeably different between treatments. The fish fed the control diet displayed superior PER (1.78) while fish receiving the highest level of PBM resulted in a PER of 1.24 (Figure 5-4). Apparent net protein utilization (ANPU%) also showed significant differences between treatments for (control diet and 20, 40, 60, 80, and 100% PBM resulted ANPU 28.90, 28.18, 27.20, 25.94, 23.37 and 18.82 respectively, which decreased with diet whilst inclusion levels of PBM increased.

It should be noted that the essential amino acid profile of the experimental diets clearly shows a declining level of each amino acid at each PBM increment. This was especially apparent for the total sulphur amino acids (Met. + Cys.) and also lysine above a 60% inclusion of PBM protein.

The hepato-somatic index (HSI%) did not reflect any trend in catfish sampled at the end of the study. Fish fed the highest level of PBM showed the highest value for HSI (1.74). Fish fed the other diets revealed no significant relationship after 10 weeks.

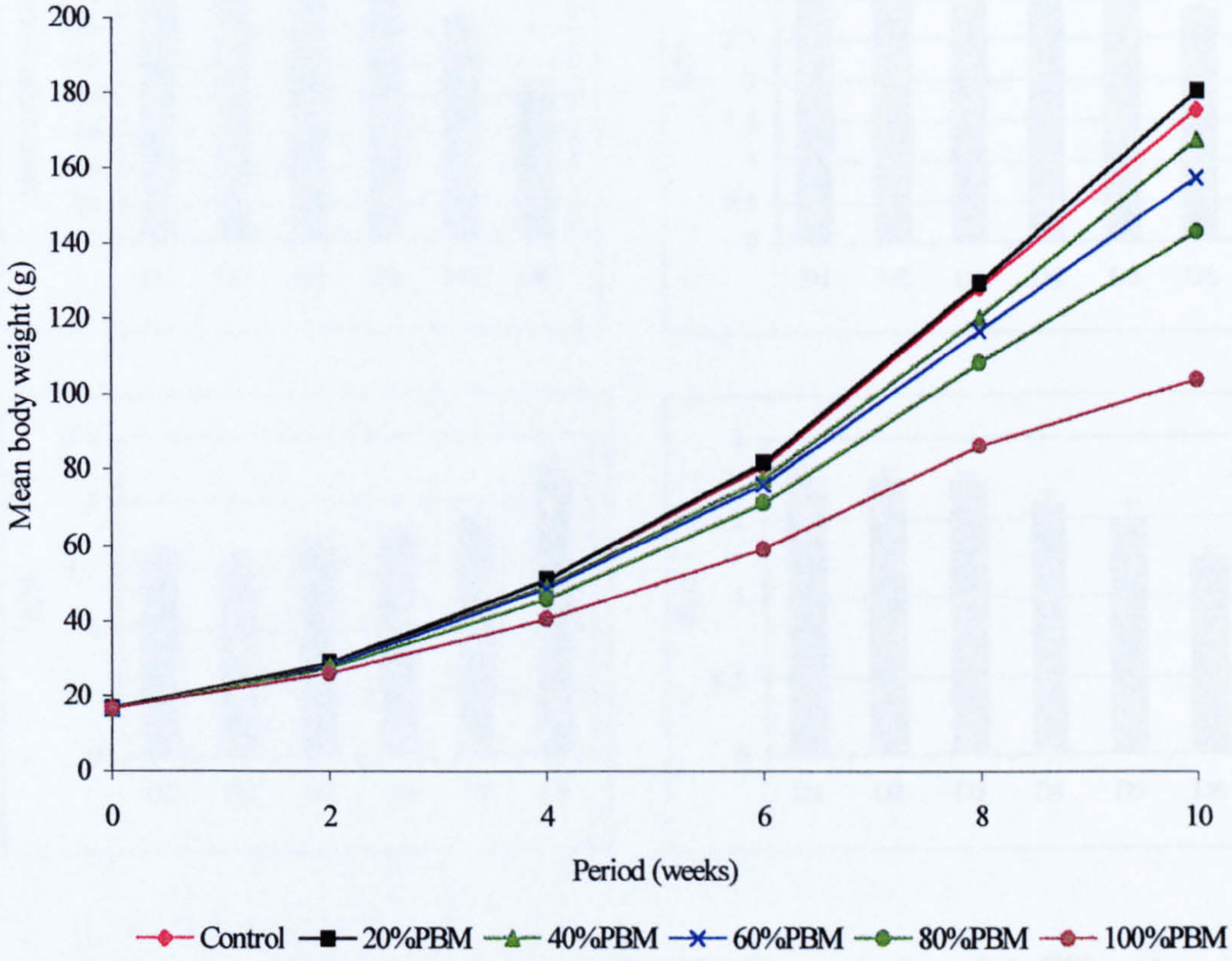


Figure 5.3 Growth performance of African catfish fed diets containing graded levels of poultry by-product meal (PBM).

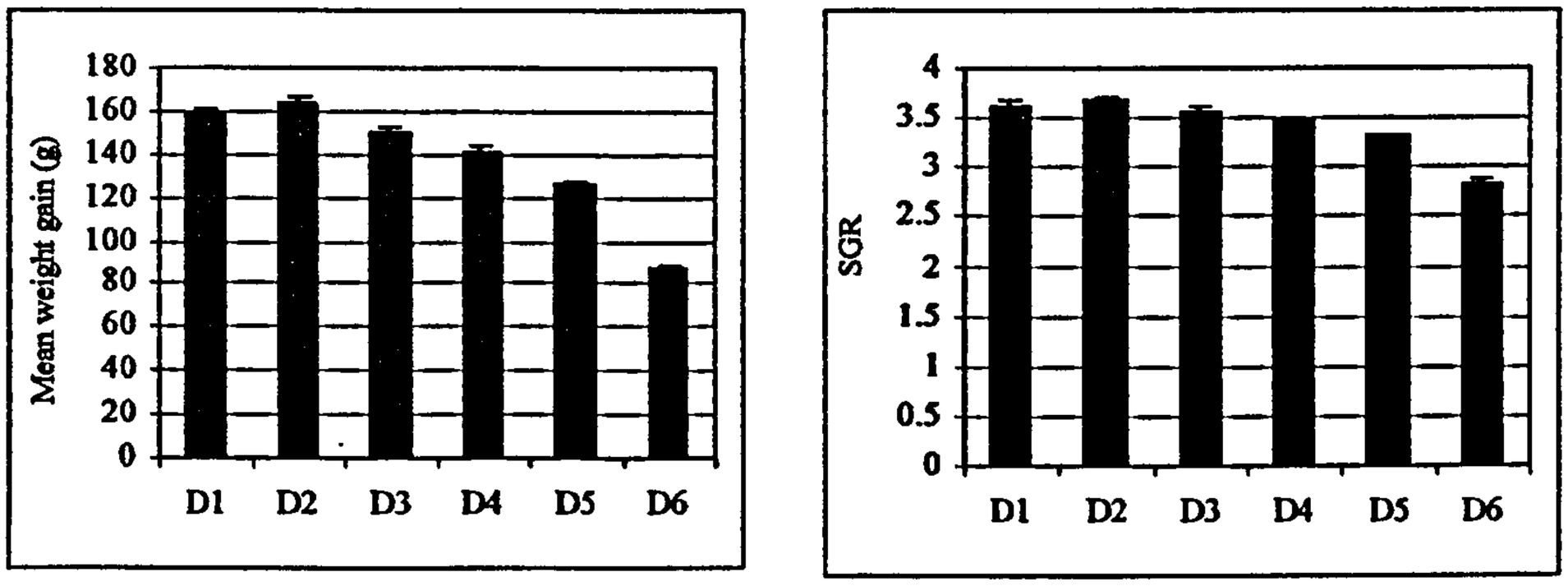


Figure 5.4 Growth and feed performance for *Clarias gariepinus* fed different source of poultry by-product meal (PBM) (mean ±SD *n=2*).
Bars denote mean values ±SD (*n=2*).

D1 (control LT94 fishmeal), D2 (20% PBM), D3 (40% PBM), D4 (60% PBM), D5 (80% PBM) and D6 (100% PBM).

5.4.3 Fish body composition

Initial and final carcass composition of the fish fed the experimental diets is presented in Table 5.4.2. The final carcass composition showed a treatment effect on proximate composition with respect to protein and ash content only. Fish fed the fishmeal based control diet and the 20, 40 and 60% PBM protein diets did not yield any variations in the protein content whilst fish fed the 80, and 100% PBM diets had slightly lower protein in their final carcass composition.

Table 5.4.2 Fish carcass composition ($g100g^{-1}$ wet weight) of whole fish fed experimental diets (mean ±SD *n=2* five fish per duplicate).

Proximate composition (% wet weight)	Initial fish	D1 0% PBM	D2 20% PBM	D3 40% PBM	D4 60% PBM	D5 80% PBM	D6 100% PBM
Moisture	77.62	69.83 ±0.62^{ab}	70.67 ±0.88^{bc}	70.89 ±0.94^{c}	69.56 ±0.74^{a}	69.37 ±0.77^{a}	69.55 ±0.61^{a}
Protein	11.95	15.86 ±0.33^{c}	15.81 ±0.33^{c}	15.39 ±0.44^{b}	15.89 ±0.23^{c}	15.18 ±0.29^{b}	14.70 ±0.55^{a}
Lipid	6.77	10.33 ±0.96^{ab}	10.30 ±0.53^{ab}	10.10 ±0.61^{a}	10.63 ±0.52^{bc}	11.01 ±0.49^{c}	10.53 ±0.53^{abc}
Ash	2.07	2.63 ±0.10^{a}	2.94 ±0.25^{b}	3.10 ±0.27^{b}	3.42 ±0.30^{c}	3.51 ±0.13^{c}	3.65 ±0.25^{c}

Values in the same row with the same superscript are not significantly different ($p>0.05$).
(After arcsine transformation of original data)

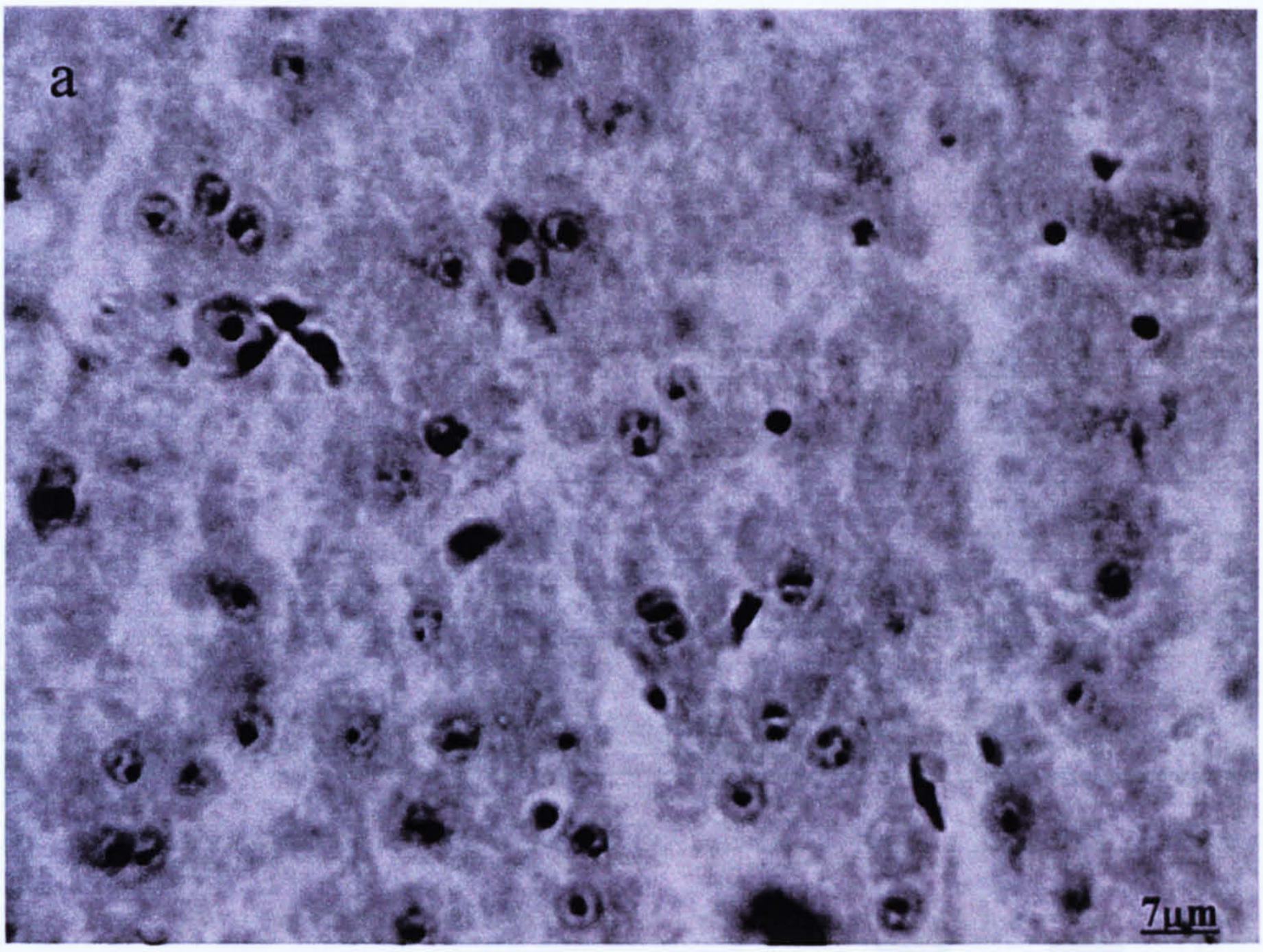
Elevated levels of PBM resulted in no discernible trend on lipid content compared to lower PBM diets or the fishmeal fed control group respectively (Table 4). Similarly, there was no obvious trend in carcass moisture content (*P>0.05*) in relation to substitution level. However, the ash content in the carcasses of fish fed the control diet was appreciably lower (2.63%) compared to catfish fed higher levels of PBM. This was found to be significant (*P<0.05*) above the 40 % inclusion level with values ranging from 3.10- 3.65.

5.4.4 Histological studies

Sections of liver tissue showed alterations in the liver structure of those fish fed diets with higher levels of PBM, as shown in Figure 5.5a & b for typical gross architecture of hepatic tissue from representative fish. Hepatic cells of fish fed the control diet and 20% PBM protein were well defined in shape, well organized and there was no sign of shrinkage or necrosis.

Catfish fed diets with high inclusion levels of PBM protein (80, 100%) showed appreciable alterations in liver tissue.

The relative size of hepatocytes increased as the proportion of the PBM in the diets increased and this was associated with a much greater hepatic lipid deposition. Polarization and isolated necrosis in hepatocytes were also observed when the diets included higher levels (80 and 100% PBM). These changes were all quantified by the use of the image analyzer and the mean relative area (μm^2) of lipid deposition is shown in Figure 5.6.



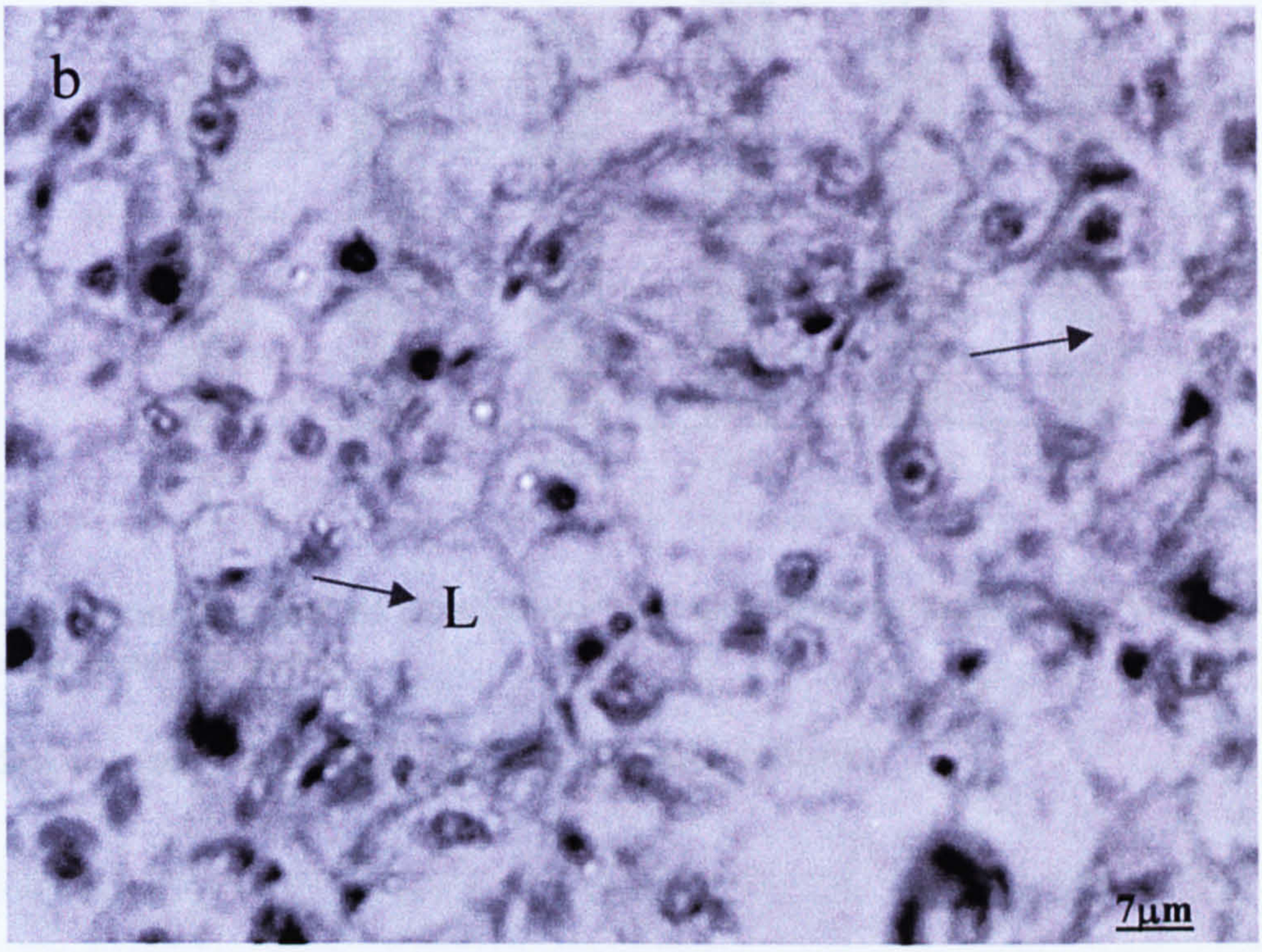


Figure 5.5 (a) Section of liver from *Clarias gariepinus* fed the fish meal based control diet showed no visible intracellular lipid deposition, (b) for fish fed diet 6 (100% PBM) showed severe intracellular lipid deposition (L). (Nikon camera CoolPix 950 with Adapter MD lens- MEIJI techno. Company) magnification ×40.

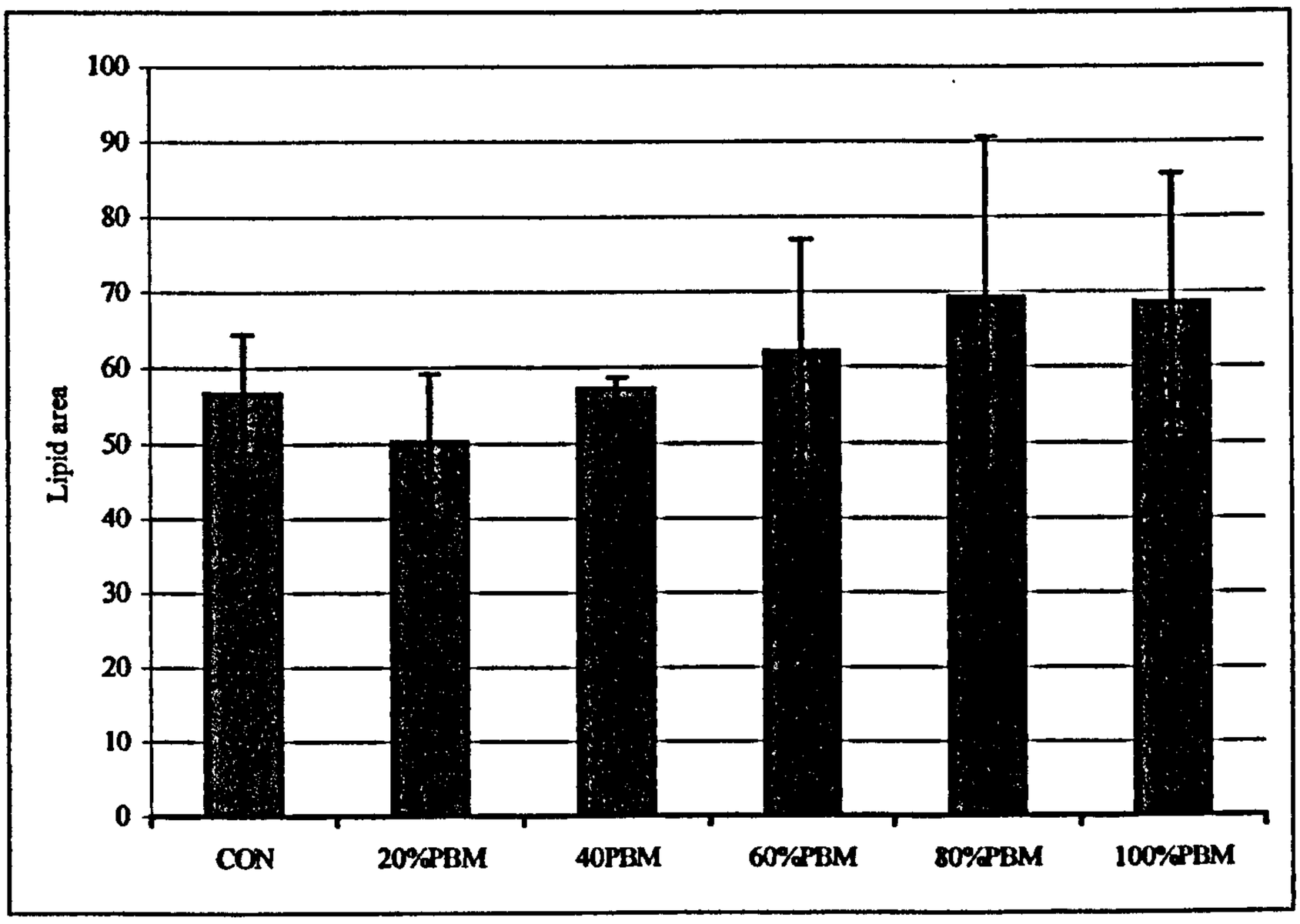


Figure 5.6 Mean area μm^2 of intracellular lipid deposition in the liver of African catfish fed on experimental diets.

Bars denote mean values (±SD *n=2* five fish per duplicate).

5.5 Discussion

5.5.1 Experiment 1

Fish meals are well-established high quality protein sources for all terrestrial farm animals and fish for the following reasons. (1) Fishmeal is highly palatable for fish. (2) fish meal is an excellent source of high quality protein and has an essential amino acid (EAA) profile that approximates almost exactly to the known dietary EAA requirements of farmed fish. In the current study, the EAA levels shown in Table 5.1.3 support this rationale as EAA levels in the diets, which contain two types of fishmeal, were high for all amino acids. In contrast to other commercially available feed ingredients, fishmeal can therefore be used as the main source of dietary protein (therefore simplifying the dietary formulation) with no additional requirement for supplementation with synthetic amino acids (FAO, 1993). (3) Fish meal is an excellent source of digestible energy for fish, and in the case of fish meals produced from oily-fish has an essential fatty acid (EFA) profile which approximates almost exactly to the known dietary EFA requirements of carnivorous fish species. (4) FM is a good source of essential minerals and trace elements, including calcium, phosphorus, magnesium, iodine, zinc, manganese, selenium, and trivalent chromium. (5) FM is a good source of essential vitamins, including choline, vitamin B_{12}, inositol, vitamin A, vitamin D_3 and to a lesser extent niacin, thiamine, riboflavin, pyridoxine, pantothenic acid, biotin and vitamin E (FAO, 1993). All diets were well accepted by fish, and the investigations with African catfish confirm their high feeding value in this respect.

In this study Norwegian fishmeal (Norse-LT94) was the primary high quality protein source for catfish and was marginally better than white fish meal resulting in higher growth performance and feed utilization.

This experiment was a foundation for the assessment of fishmeal used in the subsequent experiments evaluating both animal and plant by-products. Fish fed a wet diet can

compensate to obtain similar dry matter by eating more, voluntary feed intake was therefore similar on a dry matter basis, but feed efficiency, SGR, PER and apparent net protein utilization (ANPU) were inferior in fish fed wet diets. This might indicate that fish fed wet diets simply empty their stomachs faster than fish fed dry diets and for fish fed wet diets there is some nutrient leaching so that the fish could not receive all the nutrient in the diets.

It should be noted that the white fish meal (WFM) diets contain a much higher ash content than LT94 diets. WFM is consistently higher in mineral content (over twice the level of calcium and phosphorous). Elevated ash in digestion may impair nutrient uptake and also promote decreased gastro-intestinal transit time. This might explain the much reduced hepatic glycogen levels found in African catfish fed the WFM diets. It is concluded that no advantage could be obtained by feeding a wet version of either fishmeal based diet and that superior results occur with a dry pellet for this species based on a low temperature (LT94) high quality fishmeal product.

One of the main aims of this study was an attempt to improve the biological value of plant and animal sources for catfish by concurrently supplementing this protein source in practical experimental diets for this species.

5.5.2 Experiment 2

The inclusion of alternative protein sources for the partial or direct replacement of fishmeal has been studied in previous investigations for numerous fish species. These have concluded that increasing animal derived protein to replace fishmeal has a detrimental effect on growth rate and feed utilization above certain constraints although partial substitution is quite feasible. The feasibility of poultry by-products in fish diets was found to depend on fish species and size as well as composition and processing

techniques. Lu & Kevern (1975) found that a diet containing 30% PBM and 70% salmon feed lowered the growth rate of channel catfish, *Ictalurus punctatus*.

On the other hand, up to 75% of fishmeal could be replaced by defatted PBM in coho salmon, *Oncorhynchus kisutch* diets, without adverse effects on growth (Higgs *et al.* 1979). At a 100% substitution level, growth was significantly compromised. However, a mixture of PBM and feather meal supplemented with essential amino acids effectively replaced fishmeal in rainbow trout diets (Gropp *et al.*, 1976). However, Higgs *et al.* (1979) reported at least 28% of PBM may be included in the diet of coho salmon, without amino acid supplementation.

In the present study, the results for African catfish *Clarias gariepinus* demonstrate that PBM can replace up to 40% of a high quality fishmeal protein without amino acid supplementation whilst not compromising growth performance and feed utilization. Sadiku & Jauncey (1995) investigated the nutritional value of a feather meal and poultry by-product meal blend for *C.gariepinus*. These authors reported similar conclusions to our investigations. The highest mean final weight, SGR, PER, and FCR values were recorded for fish fed the control diet and 20% PBM protein diets. The improved performance of these diets was probably due to a more favourable essential amino acid (EAA) balance than fishmeal alone.

In the present investigation, it was found that PBM substitution levels exceeding 40% had a marked effect on apparent net protein utilization (ANPU) which was in accordance with a reduced protein retention due probably to an inferior biological value for the dietary protein. This was attributed to the changes in the amino acid composition of the diet and also due to a possible effect on protein digestibility.

The methionine and lysine requirements for the African catfish have recently been determined by Fagbenro *et al.* (1998a,b) and stated these to be 3.2 and 5.7% of the total

protein respectively, based on semi-purified diets. In the present study, these specific amino acids were appreciably lower than this for all PBM inclusions above 40% of the protein component of the diet.

It should also be noted that several other amino acids became progressively reduced at high PBM inclusions and may have contributed to the inferior protein utilization (PER) and growth performance of catfish.

Fowler (1991) was successful in rearing chinook salmon, *Oncorhynchus tschawytscha* with a diet containing 20% PBM meal without additional amino acid supplementation. Alexis *et al.* (1985) working with rainbow trout, *Oncorhynchus mykiss,* obtained very good results with a feed containing 20% PBM meal, with methionine supplementation alone.

The inferior performance of the fish receiving the higher PBM diets compared with groups receiving fishmeal in most studies is possibly a result of the lower availability of nutrients and amino acid imbalance. Alexis, *et al.* (1985) listed the digestibility of some of the main animal by-products tested in their study. These workers gave values of about 85% digestible protein for fishmeal but only 60% for PBM in rainbow trout. Gaylord & Gatlin (1996) also reported the protein digestibility coefficients of selected animal protein sources, including meat and bone meal, and found them to be close to values previously reported for various fish species.

The protein digestibility of PBM in their study appeared low at 49% for red drum, *Sciaenops ocellatus,* compared to values for rainbow trout and chinook salmon of 68% and 74%, respectively (NRC, 1993). There have been few studies to determine the digestibility coefficients for protein and energy in ingredients for tropical fish such as tilapia and catfish.

Hanley (1987) reported such values for tilapia *O. niloticus* and found that the digestibility of protein and energy was appreciably lower (74% & 59% respectively)

compared to fishmeal (86% & 80% respectively). In the current study, this is probably one explanation for the reduced growth performance associated with increased level of PBM for African catfish. The palatability of low fishmeal diets for fish is also a problem and should be addressed for even omnivorous fish such as the African catfish. At high inclusion levels of PBM in the current study there was a reduction in feed intake.

The carcass composition of catfish was not affected by substitution of fishmeal with PBM with respect to either moisture content or lipid. Also the protein component remained consistent for each of the groups receiving the experimental diets. This was consistent with the similar protein and energy levels employed in the feed formulations. It should be noted that Belal *et al.* (1995) also found that the replacement of fishmeal with chicken offal silage for tilapia *O. niloticus* did not compromise growth performance or carcass composition. There was however, a progressive significant increase in the ash content of catfish fed PBM. This elevated mineral retention reflected the inorganic component of the diets. Future studies should also address the mineral composition of whole carcass and selected tissues.

The accumulation of lipid observed in the liver histology of African catfish fed the higher levels PBM diets could be related to a dietary imbalance between saturated and unsaturated fatty acids, as a consequence of the high ratio of saturated fatty acids present in this ingredient.

These results agree with those reported by Shimeno *et al.* (1993) who found a higher liver lipid content in yellowtail seabream *Seriola quinqueradiata* fed meat and bone meal compared to fish fed corn gluten meal diets at the same dietary inclusion level. Interestingly, in the present experiment, isolated necrosis in hepatocytes was found in livers of fish fed higher levels of PBM in the diet, indicating possible irreversible effects on fish health due to nutritional imbalances (Mosconi-Bac, 1987; 1990). Also Robaina

et al. (1998) have reported that the increase in the *n-3/n-6* fatty acid ratio with about 30% soybean meal improved the utilization of liver lipids thus reducing liver histological alterations in gilthead sea bream.

It should be noted that the high fat content of PBM (18-20%) contributes significantly to the total lipid content in diet formulations in which the PBM inclusion rate is above 25%. Since poultry by-product meal is predominately a source of *n-9* (oleic acid), this could have an important consequence for the longer term feeding of such ingredients.

However, the supplementary fish /vegetable oils remained relatively high in our diets and would have provided the *n-3, n-6* fatty acid requirements for this species.
The objective was to determine the optimum level consistent with good growth performance without compromising the feed utilization efficiency and health of these fish. It was not thought that the lipid composition of the experimental diets containing PBM was detrimental to catfish under the defined conditions of this investigation.
In conclusion, the results from this study would indicate that fishmeal LT94 should be used in African catfish diets. Also PBM is an acceptable ingredient for the partial replacement of fishmeal protein in practical diets for juvenile African catfish. PBM can be used in balanced diet formulations for this species with up to 40% replacement of fishmeal protein before limitations in growth performance and health criteria are observed.
Further work is required to obtain reliable digestibility data for protein, amino acids, lipid and energy components for this ingredient to realize its full potential in practical diets particularly for African catfish. Also, more work could consider the amino acid requirements for this species. This would necessitate investigations with various size classes of fish representing the complete production cycle.

CHAPTER 6

MAIZE GLUTEN AND FULL FAT SOYBEAN MEALS AS PROTEIN SOURCES IN DIETS FOR AFRICAN CATFISH *Clarias gariepinus* INCLUDING EFFECTS ON GASTROINTESTINAL ENZYMES AND HISTOPATHOLOGY.

6.0 Introduction

Investigations of the total or partial substitution of fishmeal by alternative protein sources in diets of fish are numerous (Tacon, 1994). Although there is evidence of some differences between and within fish species in the utilization of plant products, most studies confirm scope for successful replacement of fishmeal by plant protein ingredients with a high protein component. Special attention should be paid to the use of appropriate technological processes for the deactivation and removal of endogenous anti-nutritional factors. In order to increase the nutritional value, there is a need to consider dietary amino acid and mineral supplementation to overcome possible nutritional imbalance (Kaushik, 1990; Rumsey *et al.*, 1993; Tacon, 1994). Commercial fish feeds usually contain fishmeal, which is expensive and not always available. Replacement of fishmeal with plant proteins in fish feed has been an important objective of several investigations (Webster *et al.*, 1992; Wu *et al.*, 1995). Moreover, many studies have reported that high levels of replacement of fishmeal with plant protein sources often lead to reduced growth, attributed to a lower voluntary feed intake, that may be related to feed palatability (Reigh & Ellis, 1992; Davis *et al* 1995; Gomes *et al.*, 1995).

In addition, plant ingredients possess various anti-nutritional factors (ANFs) which have a negative affect on growth performance, digestibility and enzyme activities. Normally corn is deficient in certain amino acids especially lysine and tryptophan for humans, fish, and non-ruminant animals, but high lysine varieties contain higher levels of lysine and tryptophan than normal corn (Mertz *et al.*, 1964). Substitution of high lysine corn for normal corn can reduce protein supplementation for swine feed. It is therefore an attractive proposition to use high lysine corn instead of normal corn to reduce the need for other protein supplement (Wu *et al.*, 1999). Also Dias *et al.* (1997) studied the beneficial effects of incorporation of an attractant amino acid mixture (AA Mix) by

replacement of an equivalent amount of basal dietary mixture on feed intake, growth performance and protein utilization in a marine teleost, the European sea bass *Dicentrarchus labrax*, fed with plant protein rich diets. High replacement levels of fishmeal by plant proteins in diets for European sea bass juveniles decreases the growth performance and protein utilization (Dias *et al.*, 1997).

Previous investigations have reported a range of different fish species fed relatively high inclusions of plant proteins. This includes work on tilapia fed corn gluten (Wu *et al.*, 1999) gilthead seabream (Robaina *et al.*, 1997), rainbow trout (Alexis *et al.*, 1985; Moyano *et al.*, 1992), Nile tilapia (Wu *et al.*, 1995), channel catfish (Robinson *et al.*, 2001) striped bass *Morone saxatilis* (Papatryphon & Soares, 2001) and African catfish (Falaye & Oloruntuyi, 1998).

Among plant protein sources tested, some studies noted that partial substitution of dietary fish meal with corn gluten meal has led to satisfactory results with respect to the growth rate and feed utilization in diets for rainbow trout (Alexis *et al.*, 1985; Moyano *et al.*, 1992). Corn gluten meal, which contains more than 60% protein, is the major protein fraction obtained from the wet milling process that is used to separate corn into starch, germ, protein, and fibre fractions.

Corn gluten meal in fish diets, including tilapia and catfish diets, has been studied by a number of workers. Lorico-Querijero & Chiu (1989) reported that the true digestibility of corn gluten meal was high for Nile tilapia. Furthermore, (Papatryphon & Soares 2001) determined the effect of phytase on apparent dry matter, crude protein and phosphorus digestibility of four plant feedstuffs (isolated soyprotein, soybean meal, corn gluten meal and wheat middlings) fed to striped bass *Morone saxatilis*. These results showed lower dry matter digestibility for SBM and wheat middlings when compared

with corn gluten meal and isolated soy protein, and was not influenced by phytase supplementation. Also the crude protein digestibility was not affected by phytase supplementation and was lower for SBM compared with fish fed diets containing isolated soy protein.

Wu *et al.* (1995) reported that corn gluten was incorporated in diets containing 32 and 36% protein fed to Nile tilapia and they observed that 32 and 36% protein diets containing corn gluten feed produced weight gains and feed conversion ratio similar to a control diet of a commercial (36% protein) catfish feed. In addition, channel catfish can efficiently utilize corn gluten feed at a level of 50% in the diet without adverse effect on feed palatability, weight gain or FCR (Robinson *et al.*, 2001).

African catfish fed a diet containing maize and plantain peel meal showed a decrease in weight gain with increased inclusion levels of these ingredients compared with a control diet (without plantain peel meal) also PER and PPV have been affected (Falaye & Oloruntuyi, 1998).

Fagbenro (1999) reported the use of mature winged bean seeds subjected to different moist heat treatments (autoclaving, boiling, cooking, quick cooking). These meals were then fed to African catfish with different levels of substitution by menhaden fishmeal; the results showed carcass composition of catfish was not diet related, and additionally liver composition did not show any lipid or glycogen accumulation at the end of the feeding trial. Fagbenro (1999) was also able to show that the African catfish was capable of digesting the energy and protein in winged bean meal as effectively as soybean meal in experimental diets.

More recently Fagbenro & Davies (2001) tested soybean flour (dehulled, solvent-extracted meal) as a fish meal substitute in practical diets for African catfish. These latter authors evaluated growth, feed utilization and digestibility. Supplemental methionine was added to the diet formulation in which soybean flour replaced up to 75% of the diet protein. At 75% fishmeal replacement with soybean flour (without methionine supplementation), growth performance and feed efficiency were compromised. However, it was evident that the addition of methionine confirmed that this was the rate limiting amino acid, thus allowing a higher inclusion level of soybean in the diet. These results raise interesting possibilities for fish diet formulation.

As stated in Chapter 4, numerous workers have explored the potential of using different soybean meal products for a variety of fish species. Tilapia comprise the main warm water fish of interest and Nyirenda *et al.* (2000) successfully grew tilapia, *Oreochromis karongae* on a practical diet that contained a maximum of 10% soybean meal replacing both fishmeal and meat and bone meal without the need for amino acid supplementation. There are considerable differences in soybean quality and selected grades of concentrate meals exist for aquafeeds. Protein and energy content vary in soybean meal depending on protein level of the beans, residual fat after processing and whether or not hulls have been removed (Swick & Tan, 1995).

The objective of the current investigation was to study the partial substitution of fishmeal (LT94) by two alternative plant protein sources (maize gluten and full fat soybean meals) in separate feeding trials for African catfish to evaluate maximum inclusion levels of both ingredients for this species. The first experiment was conducted to study fish growth, feed utilization and liver histology. The second experiment described in this Chapter was to evaluate the replacement of fishmeal with full fat soybean meal in balanced diet formulations for catfish.

The criteria for assessment included the key nutritional parameters such as growth, feed utilization and carcass composition as well as a comprehensive study to examine the effects of soybean on the digestive enzyme profile in different regions of the gastrointestinal tract of catfish.

6.1 Experiment 1: - Assessment of maize gluten meal for African catfish-materials and protocols

6.1.1 Experimental fish

African catfish *Clarias gariepinus* as described in Chapter 2 section 2.1 were used in the investigation. Each of eight tanks was randomly assigned to each dietary treatment. Thirty fish were graded and transferred to each tank with an average weight of 5.97g, the dietary treatments were tested on duplicate groups of fish. A similar group of twenty fish was killed using a lethal concentration of benzocaine and kept frozen at -20^{0}C to determine initial carcass composition.

6.1.2 Experimental diet

Four experimental diets were formulated to contain a variable proportion of maize gluten meal (MG) to achieve partial replacement of fishmeal. The four experimental diets were isoenergetic and isonitrogenous and were adjusted at appropriate levels to contain 37% crude protein and 15% lipid. Table 6.1 shows the feed formulation and proximate chemical composition of the experimental diets. The control diet was based on fishmeal (LT94) as the main source of dietary protein, D2 (25% MG), D3 (50% MG) and D4 (75%MG). Although wheat meal was also included, this did not have a major effect due to its low content of protein and lipid.

6.1.3 Feeding regime

Fish were fed for one week to acclimate them to the test diets, the system and also to free their gastrointestinal tract from the pre-experimental diet (Trouw aquaculture pellet). At the end of the acclimation period, the fish were weighed and then started on the experimental diets. Fish were fed twice daily by hand, and were fed 4% of body weight.

The fish were weighed individually every fourteen days but were not fed on the day of weighing, the experiment was undertaken for an 8 week period and daily feed intake was recorded throughout. At the end of the experiment, the final weight of fish was measured and following a 24 hour starvation period, five fish from each treatment were randomly selected from each experimental tank for carcass analysis. A further ten fish of each treatment were killed and the liver was removed for glycogen determination in liver and general hepatic morphology as described in Chapter 2 section 2.8.

6.1.4 Proximate composition

Proximate compositions of diets and fish tissue for moisture, protein (Automatic Kjeldahl method, Gerhardt Vapodest 3S), lipid, ash and gross energy were determined as a described in Chapter 2 section 2.4.

6.1.5 Determination of amino acids

The amino acid contents of the diets were determined as described in Chapter 2 section 2.4.6.

Table 6.1 Composition and proximate content of the control and test diets (g100g^{-1} dry weight).

	D1 0% MG	D2 25% MG	D3 50% MG	D4 75% MG
Fish meal[1]	42.00	31.50	21.00	10.00
Maize gluten[2]		10.50	21.00	35.00
Wheat meal[3]	35.00	35.00	35.00	32.00
Blood meal	2.00	2.00	2.00	2.00
Corn oil[4]	4.40	4.45	4.57	4.65
Cod liver oil[5]	4.40	4.45	4.57	5.65
Vitamin premix[6]	2.00	2.00	2.00	2.00
Mineral premix[7]	1.00	1.00	1.00	1.00
Binder[8]	2.00	2.00	2.00	2.00
αCellulose[9]	7.20	7.10	6.86	6.70
Proximate composition				
(% as fed)				
Moisture	3.73	4.98	6.72	4.11
Protein	37.48	36.30	35.38	38.35
Lipid	16.24	16.66	18.71	17.86
Ash	7.30	6.16	4.95	3.90

[1] Fish meal LT94,. Trouw Aquaculture (Nutreco Company).
[2] Maize gluten, Cargill Ltd.
[3] Wheat meal, Kalpro S™. Orsan, Paris, France
[4] Mazola- pure corn oil
[5] Fish oil- seven pure cod liver oil
[6] Vitamin premix, Trouw Aquaculture (Nutreco Company).
[7] Mineral premix, Trouw Aquaculture (Nutreco Company).
[8] Carboxymethyl Cellulose (CMC).
[9] Sigma Chemical Co., Poole, Dorset.

Table 6.2 Essential amino acid composition (expressed as % of protein) of control and test diets containing different levels of maize gluten meal fed to African catfish.

	D1 0% MG	D2 25% MG	D3 50% MG	D4 75% MG	African catfish Requirements*
Arginine	5.65	4.92	5.19	4.37	-
Histidine	2.81	2.58	3.31	2.81	-
Isoleucine	3.64	3.58	4.59	3.86	-
Leucine	7.37	8.90	16.54	13.87	-
Lysine	6.39	4.24	4.21	3.58	5.70[1]
Methionine	2.38	2.23	2.82	2.35	3.20[1]
Methionine + Cysteine	2.71	2.65	3.63	3.01	-
Phenylalanine	3.91	4.74	7.29	6.19	-
Phenylalanine + Tyrosine	6.48	8.02	12.94	10.76	-
Threonine	4.00	4.17	4.63	4.33	-
Valine	4.42	4.14	5.29	4.39	-
Tryptophan	ND	ND	ND	ND	-

* Requirements for all amino acids have not been determined for African catfish.
[1]Fagbenro *et al.* (1998a,b).
ND (not detected).

6.1.6 Histological studies

Histological preparation and staining techniques

At the termination of the feeding trial, five fish from each group were sacrificed and their livers removed for histological examination. Hepatic tissues were sampled and also processed as described in Chapter 2 section 2.8.

Photomicrographs were taken using the VANOX model AHBT olympus microscopes objective magnification of ×200 and photo-eyepiece × 2.5.

6.1.7 Statistical treatment of data

Statistical analyses were carried out as described in Chapter 2 section 2.9.

6.2 Experiment 2: - Evaluation of full fat soybean meals for catfish-materials and protocols

6.2.1 Experimental fish

African catfish *Clarias gariepimus* as described in Chapter 2 section 2.1 were used in the investigation. Each of eight tanks was randomly assigned to each dietary treatment. Twenty fish were graded and transferred to each tank with an average weight of (7.82g± SD 2.02). The dietary treatments were tested on duplicate groups of fish. A similar group of twenty fish was killed using a lethal concentration of benzocaine and kept frozen at -20^{0}C to determine initial carcass composition. Fish were similarly removed at the end of the experiment for final carcass compositional analysis.

6.2.2 Experimental system

The experimental facilities described in Chapter 2 section 2.3 were used to test four experimental diets containing different levels of full-fat soybean (% of dietary protein).

6.2.3 Diet formulation

Four isoenergetic and isonitrogenous diets were formulated for different levels of FFSB replaced by fishmeal. Chapter 4 Table 4.1 shows the formulation and proximate composition of control and test diets and Table 4.2 shows the essential amino acids as a % of protein for experimental diets.

6.2.4 Experimental procedure

Fish were weighed bi-weekly and fed 3.5% of body weight for the first six weeks decreased to 3% for the last six weeks with an average (3.25%). At the termination of the feeding trial, five fish from each group were sacrificed and the gut removed for histological examination. Tissues were processed for histological examination

according to the method described in Chapter 2 section 2.8. A further five fish were killed and their intestine, liver and stomach were removed and frozen at -80^{0}C for enzymatic analysis as described in section 2.6.

6.2.5 Proximate composition

Proximate compositions of diets and fish tissue for moisture, protein (Micro Kjeldahl method, Gerhardt Vapodest 40), lipid, ash and gross energy were determined as described in Chapter 2 section 2.4.

6.2.6 Determination of amino acids

The amino acid contents of the diets were determined as described in Chapter 2 section 2.4.6.

6.2.7 Determination of enzymes

Determination of gastro-intestine and liver for proteolytic, trypsin, amylase, and lipase were described in section 2.6 Chapter 2.

6.2.8 Statistical analyses

Statistical analyses were carried out as described in section 2.9.

6.3 Experiment 1: - Results

6.3.1 Growth performance and feed utilization

The growth performance and feed utilization data for African catfish fed the four diets are displayed in Figure 6.1 & Table 6.3. There was a significant difference between the final average body weight amongst the fish fed on the experimental diets. Fish fed on a fishmeal (LT94) based control diet resulted in the highest increase in final average body

weight (92.70g). However, the lowest value was observed for the fish fed the 75% inclusion level of plant protein source (Maize gluten) in the diet replacing the fishmeal component (25.47g). These fish had only approximately 4 fold increase in weight after 8 weeks of feeding. These trends were also evident with respect to the absolute weight gain figures, which decreased as the level of plant protein inclusion was elevated. The control diet supported the highest weight gain of 86.73g, while the MG 75% diet produced the lowest weight gain of 19.50g.

The specific growth rate (SGR%) values further supported this trend, where SGR decreased from 5.28 for the control diet fed fish, to 2.79 for the fish fed the 75% MG diet. Fish fed the 50% level inclusion of plant protein source (MG) performed better than those on the 75% level of (MG), while for 25% level inclusion, there was very little difference between this treatment and the control diet (Table 6.3 & Figure 6.2). No mortality was observed during the course of the experimental period.

6.3.2 Feed consumption and feed utilization

All diets were well accepted by fish, except diet four, which included the highest level of maize gluten (75% MG). Mean daily feed intake ranged between 1.32 and 0.55 g $fish^{-1}$ day^{-1}. There was a higher effect due to the inclusion of alternative protein sources on feed intake, as shown in Table 6.3.

Feeding rates obtained in fish fed the diet containing the highest amount of fishmeal (LT94) were superior compared to those observed for fish fed diets containing the plant protein source (MG). The feed conversion ratio (FCR) differed significantly between treatment and values followed the same apparent trend, in common with final weight gain and specific growth rate. The poorest food conversion ratio was obtained for catfish fed the test diet containing 75%MG with a value of 1.53, significantly better

values were obtained for the other three remaining diets. Fish fed the control diet showed the best FCR, 0.81 compared to the maize gluten diets.

Table 6.3 Weight increase, feed consumption, nutrient utilization of feed and protein, and hepatosomatic index (HSI) (mean ±SD n=2).

	D1 0% MG	D2 25% MG	D3 50% MG	D4 75% MG
Mean initial weight (g)	5.97	5.98	5.95	5.97
Mean final weight (g)	92.70 ±0.59^{d}	78.34 ±1.52^{c}	59.59 ±2.01^{b}	25.47 ±1.10^{a}
Mean weight gain (g)	86.73 ±1.60^{d}	72.36 ±1.53^{c}	53.64 ±2.01^{b}	19.50 ±1.03^{a}
Daily feed intake (gfish^{-1} day^{-1})	1.32 ±0.02^{d}	1.17 ±0.04^{c}	0.98 ±0.02^{b}	0.55 ±0.02^{a}
SGR (%)	5.28 ±0.01^{d}	4.97 ±0.04^{c}	4.43 ±0.06^{b}	2.79 ±0.07^{a}
FCR	0.81 ±0.01^{a}	0.83 ±0.01^{a}	0.94 ±0.01^{b}	1.53 ±0.02^{b}
PER	3.33 ±0.02^{c}	3.33 ±0.03^{c}	3.00 ±0.04^{b}	1.71 ±0.02^{a}
ANPU (%)	52.33 ±0.98^{c}	50.84 ±0.55^{c}	44.30 ±0.61^{b}	24.99 ±0.19^{a}
Liver glycogen (mg g^{-1} wet weight)	34.02 ±1.66^{a}	37.00 ±1.10^{a}	50.83 ±1.70^{b}	53.07 ±1.80^{b}
HSI (%)	1.11 ±0.07^{a}	1.15 ±0.05^{a}	1.74 ±0.21^{b}	2.71 ±0.79^{c}

Values in the same row with the same superscript are not significantly different ($p > 0.05$).

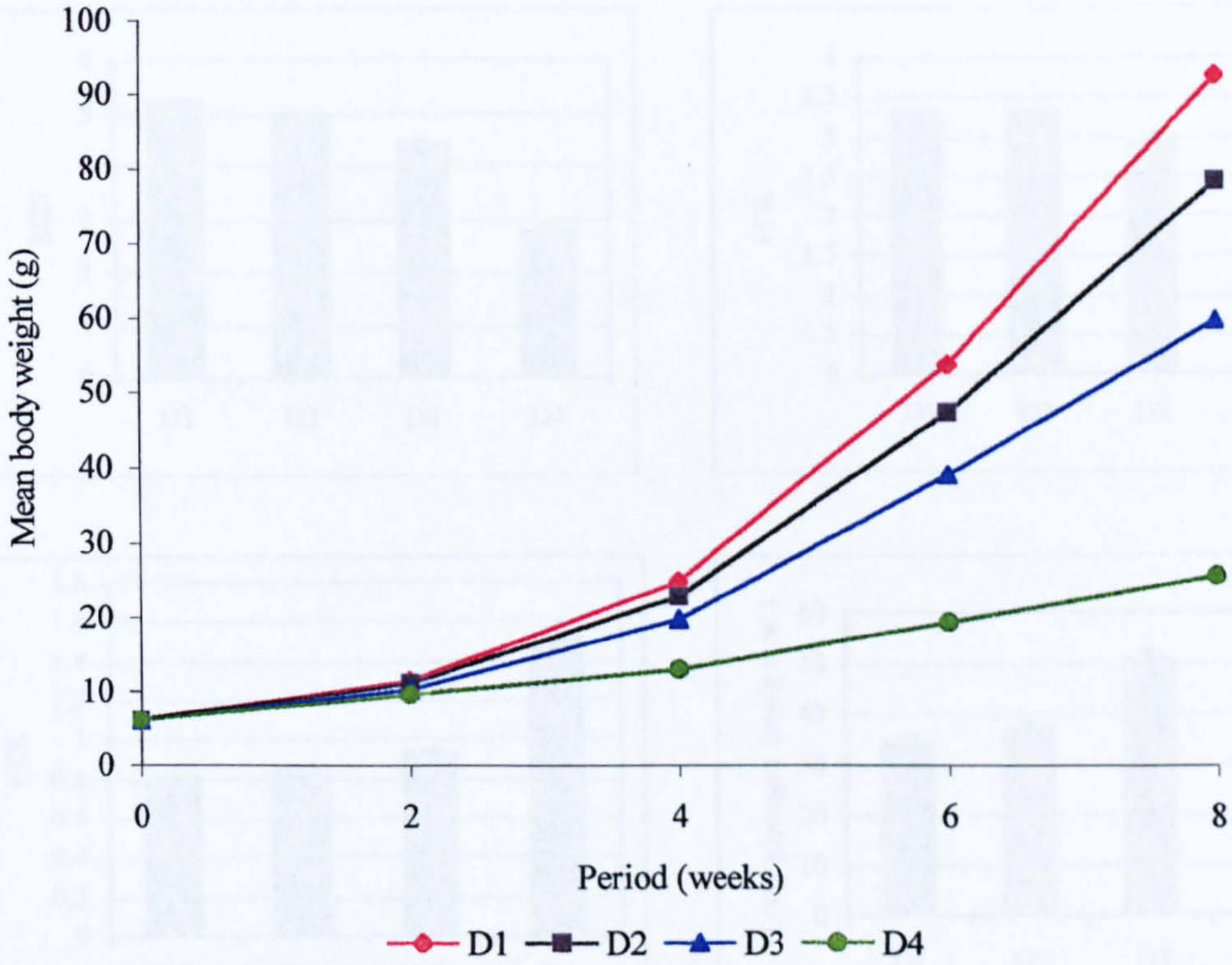


Figure 6.1 Growth performance of African catfish *C.gariepinus* fed on the control and test diets containing different levels of maize gluten (MG) for the 8 weeks period.

D1 (control- 0% MG), D2 (25% MG), D3 (50% MG) and D4 (75% MG).

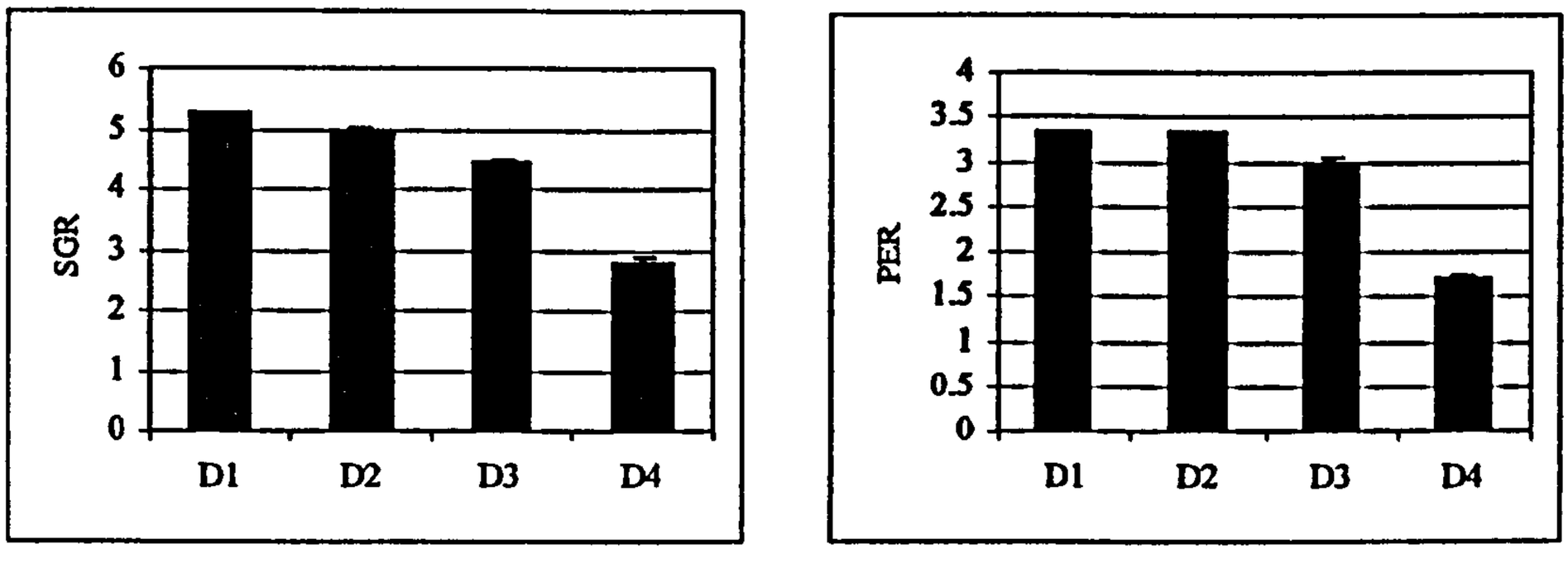


Figure 6.2 Growth, feed performance and liver glycogen for *C. gariepinus* fed different levels of maize gluten meal.

Bars denote mean values ±SD (*n=2*).

D1 (control diet 0% MG), D2 (25% MG), D3 (50% MG) and D4 (75% MG).

Protein efficiency ratio (PER) also showed differences between treatments. Fish fed the control and diet two displayed superior PER (3.33), while fish fed high levels of plant protein (MG) showed lowest values (1.71) (Figure 6.2). Apparent net protein utilization (ANPU%) was also shown to be significantly different between treatments in the same manner with PER. The highest ANPU yielded of catfish fed control diet (LT94 fishmeal) (52.33) compared to other treatments fed different levels of maize gluten specially for catfish fed high inclusion level (75%MG) which yielded the lowest ANPU (24.99) (Table 6.3).

The hepatosomatic index (HSI%) has also supported this trend between the dietary groups. The fish fed the highest level of plant protein source (MG) showed the highest value for HSI, which was 2.71, while the fish fed on the control diet observed the lowest values of HSI, which was 1.11.

Also liver glycogen content supports these trends in fish fed on the control diet and 25% of maize gluten showed lower 34.02 and 37mg g^{-1} wet weight values respectively. However, fish fed 50% and 75% maize gluten diet showed higher results 50.83 and 53.07mg g^{-1} wet weight respectively (Table 6.3 and Figure 6.2).

6.3.3 Fish body composition

Initial and final carcass composition of the fish fed on the experimental diets are presented in Table 6.4. Moisture and lipid levels were inversely correlated with lipid increasing at higher levels of MG inclusion. Protein and ash levels decreased with increasing MG inclusion. Most of these trends were significant ($P<0.05$). Fish fed diets including MG showed a peculiar yellow orange colouration on skin, operculum and base of the fins, with increasing intensity as the total amount of this feedstuff increased in the diet. This colouration was absent in fish fed the other treatments, including the control diet.

Table 6.4 Fish composition ($g100g^{-1}$ wet weight) of whole fish fed experimental diets (mean ±SD *n=2* five fish per duplicate).

	Initial fish	D1 0% MG	D2 25% MG	D3 50% MG	D4 75% MG
Moisture	77.2	73.18 $\pm0.84^{b}$	72.76 $\pm0.92^{b}$	70.93 $\pm1.05^{a}$	70.81 $\pm0.82^{a}$
Protein	11.5	15.21 $\pm0.57^{c}$	14.99 $\pm0.41^{c}$	14.43 $\pm0.38^{b}$	13.40 $\pm0.40^{a}$
Lipid	6.77	8.76 $\pm0.81^{a}$	9.20 $\pm0.64^{a}$	12.02 $\pm0.80^{b}$	13.02 $\pm0.63^{c}$
Ash	2.07	2.51 $\pm0.08^{c}$	2.31 $\pm0.14^{b}$	2.08 $\pm0.15^{a}$	2.00 $\pm0.14^{a}$

Values in the same row with the same superscript are not significantly different ($p> 0.05$).
(After arcsine transformation of original data)

6.3.4 Histological studies

Sections of liver tissue showed alterations in the liver structure of those fish fed diets with higher MG as shown in Figures 6.3a & b for typical gross architecture of hepatic tissue from representative fish. Hepatic cells of fish fed the control diet and 25% MG were well defined in shape, well organized and there was no sign of shrinkage or necrosis. Catfish fed diets with high inclusion levels of MG (75%) showed appreciable alterations in the liver tissue. The relative size of hepatocytes increased as the proportion of the MG in the diets increased and this was associated with a much greater hepatic lipid deposition. Polarization and isolated necrosis in hepatocytes were also observed when the diets included higher level (75% MG) (Figure 6.3a&b).

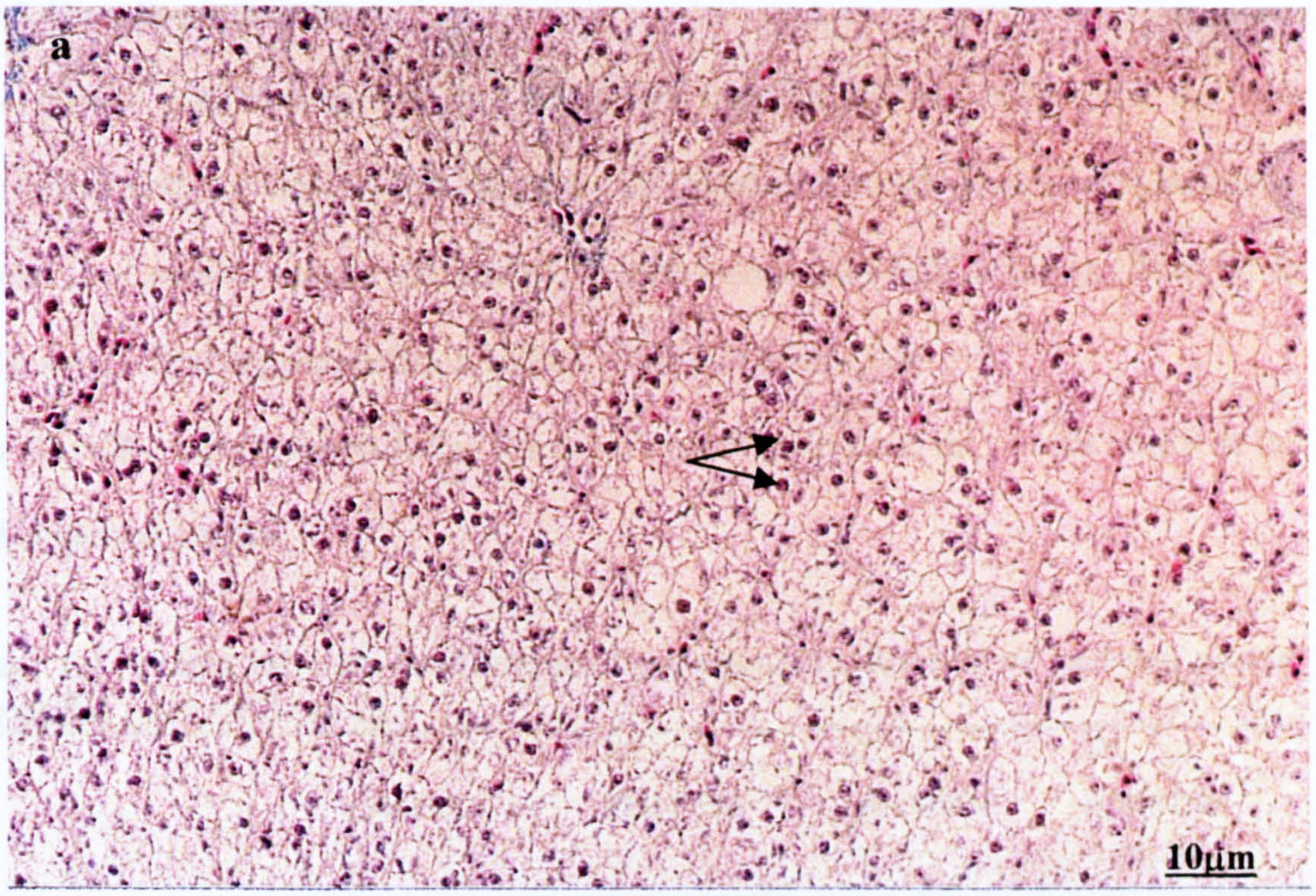


Figure 6.3 (a): Section of liver from *Clarias gariepinus* fed the fish meal based control diet showed no visible intracellular lipid deposition and normal nucleus (arrow), (b) for fish fed diet 4(75% MG) showed severe intracellular lipid deposition (L) and abnormal nucleus. (VANOX model AHBT Olympus microscopes objective magnification ×200).

6.4 Experiment 2: - Results

6.4.1 Growth performance

The growth performance and feed utilization data for African catfish fed the four respective diets are displayed in Figure 6.4 & Table 6.5. Growth decreased significantly (mean final weight, weight gain and SGR) with increasing FFSB inclusion (from D1 to D3). Amino acid supplementation had no effect (D4 versus D3).

No mortality was observed during the experimental period and the overall health of the fish appeared normal.

Table 6.5 Weight increase, feed consumption, nutritive utilization of feed and protein for catfish (mean ±SD *n=2*).

	D1 0% FFSB	D2 58% FFSB	D3 63% FFSB	D4 63% FFSB + DL- Met.
Mean initial weight (g)	7.85 ±2.31	7.83 ±1.76	7.88 ±1.99	7.8 ±2.02
Mean final weight (g)	88.69 ±4.27^{c}	79.7 ±1.09^{b}	70.82 ±1.42^{a}	68.29 ±3.54^{a}
Mean weight gain (g)	80.86 ±4.25^{c}	71.87 ±1.03^{b}	62.94 ±1.34^{a}	60.49 ±3.54^{a}
Mean daily feed Intake ($gfish^{-1}\ d^{-1}$)	0.85 ±0.01	0.80 ±0.01	0.73 ±0.03	0.72 ±0.01
SGR (%)	3.11 ±0.06^{c}	2.98 ±0.01^{b}	2.82 ±0.01^{a}	2.78 ±0.07^{a}
FCR	0.82 ±0.05^{a}	0.87 ±0.02^{ab}	0.91 ±0.02^{ab}	0.93 ±0.06^{b}
PER	3.36 ±0.13^{b}	3.24 ±0.09^{ab}	3.12 ±0.07^{ab}	2.97 ±0.18^{a}
ANPU (%)	54.48 ±1.01^{b}	51.88 ±1.53^{ab}	52.63 ±1.23^{ab}	48.60 ±2.89^{a}

Values in the same row with the same superscript are not significantly different ($p > 0.05$).

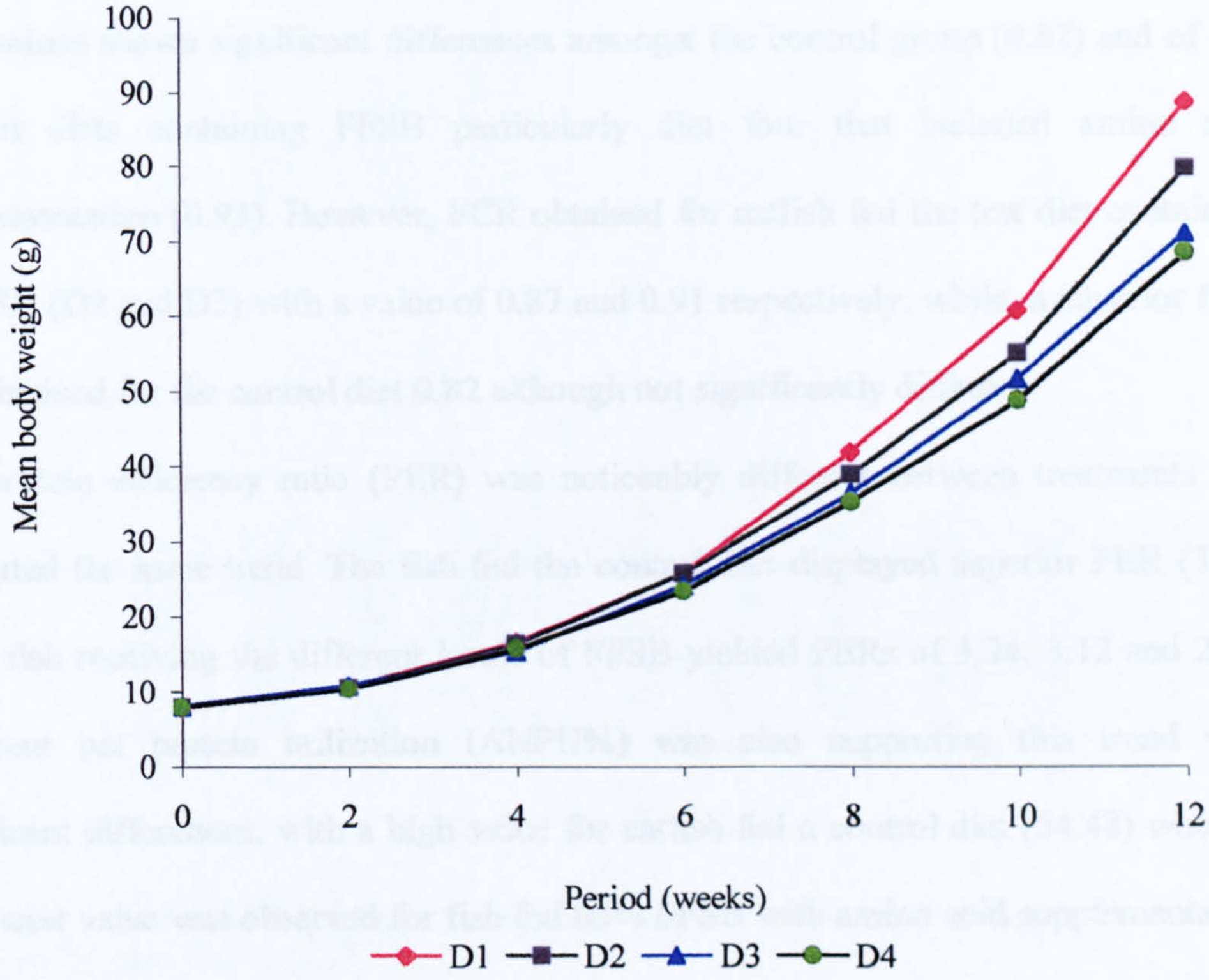


Figure 6.4 Growth performance of African catfish *C.gariepinus* fed different levels of FFSB in diets over 12 weeks.

D1 (control diet 0% FFSB), D2 (56% protein of FFSB), D3 (63% protein of FFSB) and D4 (63% protein of FFSB+ 1%-DL-methionine).

6.4.2 Feed consumption and feed utilization

The control diet was well accepted by the catfish while diets containing partial replacement with FFSB were less palatable. Fish were fed 3.5% of body weight for the first six weeks increased to 3% for the last six weeks. Mean daily feed intake ranged between 0.85 and 0.72g $fish^{-1}$ day^{-1}. There was a noticeable effect of inclusion of FFSB on feed intake (Table 6.5). The control diet (LT94) was significantly better than diets including FFSB even with amino acid supplementation.

FCR values shown significant differences amongst the control group (0.82) and of fish fed on diets containing FFSB particularly diet four that included amino acid supplementation (0.93). However, FCR obtained for catfish fed the test diet containing of FFSB (D2 and D3) with a value of 0.87 and 0.91 respectively, while, a superior FCR was obtained for the control diet 0.82 although not significantly different.

The protein efficiency ratio (PER) was noticeably different between treatments and supported the same trend. The fish fed the control diet displayed superior PER (3.36) while fish receiving the different levels of FFSB yielded PERs of 3.24, 3.12 and 2.97. Apparent net protein utilization (ANPU%) was also supporting this trend with significant differences, with a high value for catfish fed a control diet (54.48) whereas the lowest value was observed for fish fed 63% FFSB with amino acid supplementation (48.6) (Table 6.5).

It should be noted that in general the essential amino acid profile of the experimental diets shows a declining level for most of the amino acids at each FFSB increment in the diets. However, the diet supplemented with 1% DL-methionine showed the closest level to the control diet (Table 4.2 Chapter 4) whilst not achieving a positive affect on feed utilization for African catfish fed this dietary treatment.

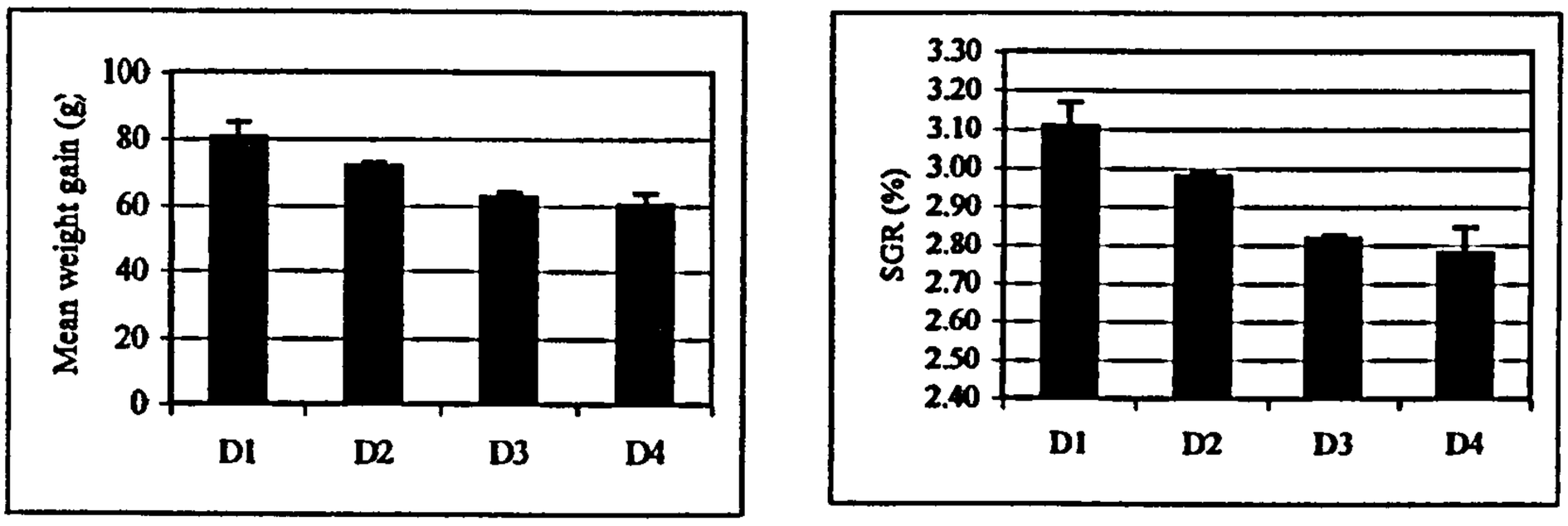


Figure 6.5 Growth performance and feed utilization of African catfish fed on a control diet and different levels of FFSB.
Bars denote mean values ±SD (*n=2*).

D1 (control diet 0% FFSB), D2 (58% protein of FFSB), D3 (63% protein of FFSB) and D4 (63% protein of FFSB+ 1%-DL-methionine).

6.4.3 Fish body composition

Initial and final carcass composition of the fish fed the experimental diets is presented in Table 6.6. Final carcass composition showed little significant variation as a result of the diet formulation they were respectively fed. Fish fed the fishmeal based control diet and different levels of FFSB diets did not yield any variations in the moisture, protein and lipid content ($P>0.05$) whilst ash content showed slight differences among groups (Table 6.6).

Table 6.6 Body composition of catfish fed graded levels of FFSB ($g100g^{-1}$ wet weight) of whole fish experiment diets (mean ± SD $n=2$ five fish per duplicate).

	Initial fish	D1 0% FFSB	D2 58% FFSB	D3 63% FFSB	D4 63% FFSB + DL-Met.
Moisture	76.45	72.24 ±1.26^{a}	72.71 ±1.81^{a}	72.97 ±1.19^{a}	73.81 ±1.39^{a}
Protein	12.01	16.01 ±0.27^{ab}	15.82 ±0.48^{a}	16.55 ±0.55^{b}	16.11 ±0.49^{ab}
Lipid	7.40	9.75 ±1.05^{bc}	10.24 ±0.96^{c}	9.24 ±0.55^{b}	7.99 ±0.33^{a}
Ash	2.46	3.27 ±0.10^{b}	2.96 ±0.15^{a}	3.04 ±0.19^{a}	2.96 ±0.10^{a}

Values in the same row with the same superscript are not significantly different ($p>0.05$).
(After arcsine transformation of original data)

6.4.4 Gastro-intestinal enzyme activity

Table 6.7 shows total proteolytic, trypsin, amylase and lipase activities in intestine, liver and stomach for catfish fed the experimental diets. The total proteolytic (sum of pHs 1.5, 3, 4, 7, 8.5, 9, and 10) activity of the intestine was higher than the activity in the liver and stomach and ranged between 5.68 to 2.98 μg tyrosine^{-1} minute^{-1} mg^{-1} protein. However, the average proteolytic activity among fish fed the four experimental diets did not show any significant difference ($p>0.05$) for the intestine and stomach, although the

fish fed on the control diets showed slightly higher activities than the other experimental diets (Table 6.7 and Figure 4.5). It should be noted that liver proteolytic activity was lower than stomach activity, also the mean of proteolytic activity showed no significant difference among fish fed the control and test diets. In general, total proteolytic activity obtained in this study with African catfish seem to be lower than corresponding proteolytic activities in tilapia (Chapter 4).

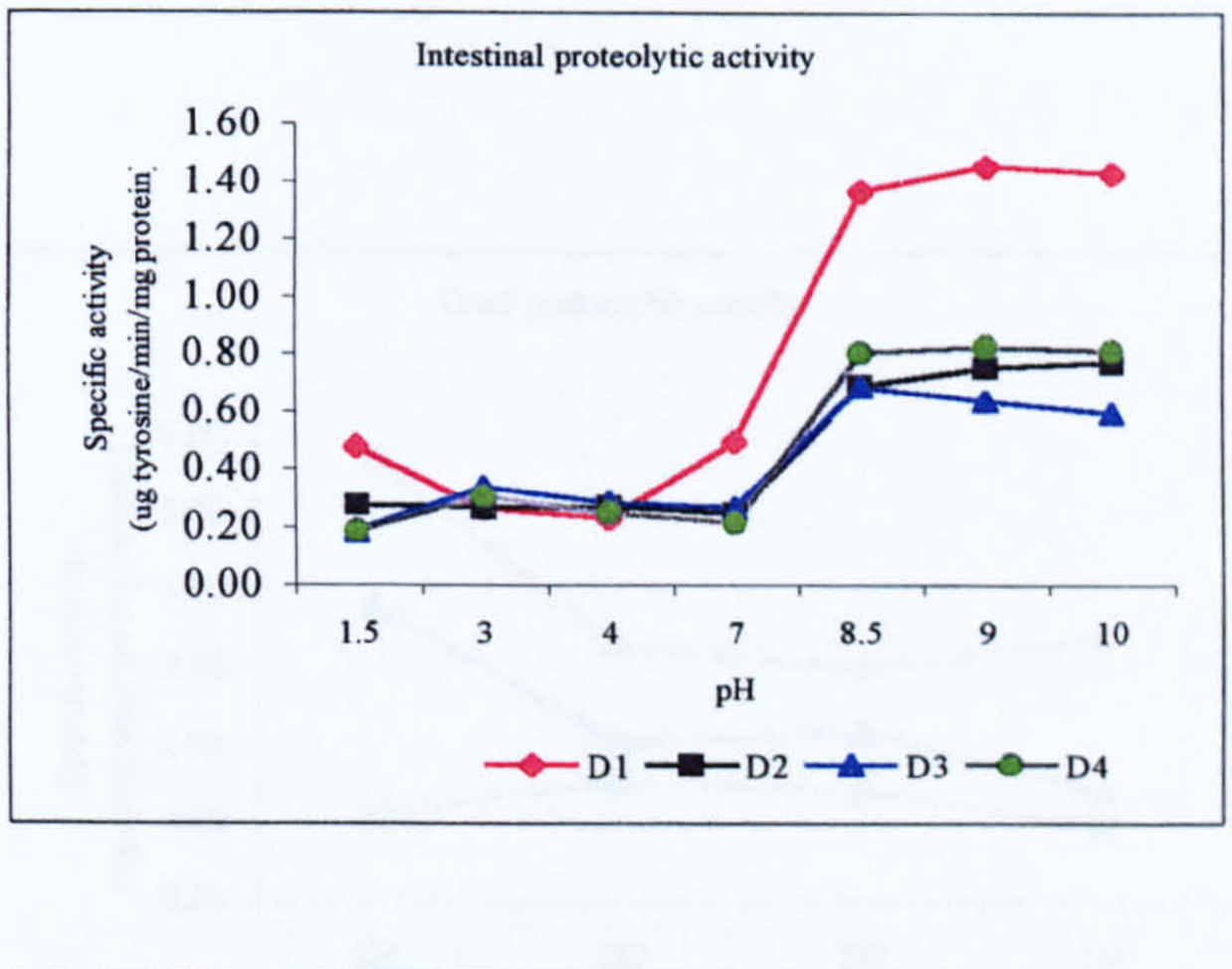
Figure 6.6 shows the enzymatic activity determined at different pHs for catfish fed control and test diets. For the intestine, the higher proteolytic activity was only at alkaline pHs, whereas only very low activity was shown with acid pHs. In contrast, the results with tilapia in Chapter 4 showed that the highest proteolytic activity was at neutral and alkaline pHs.

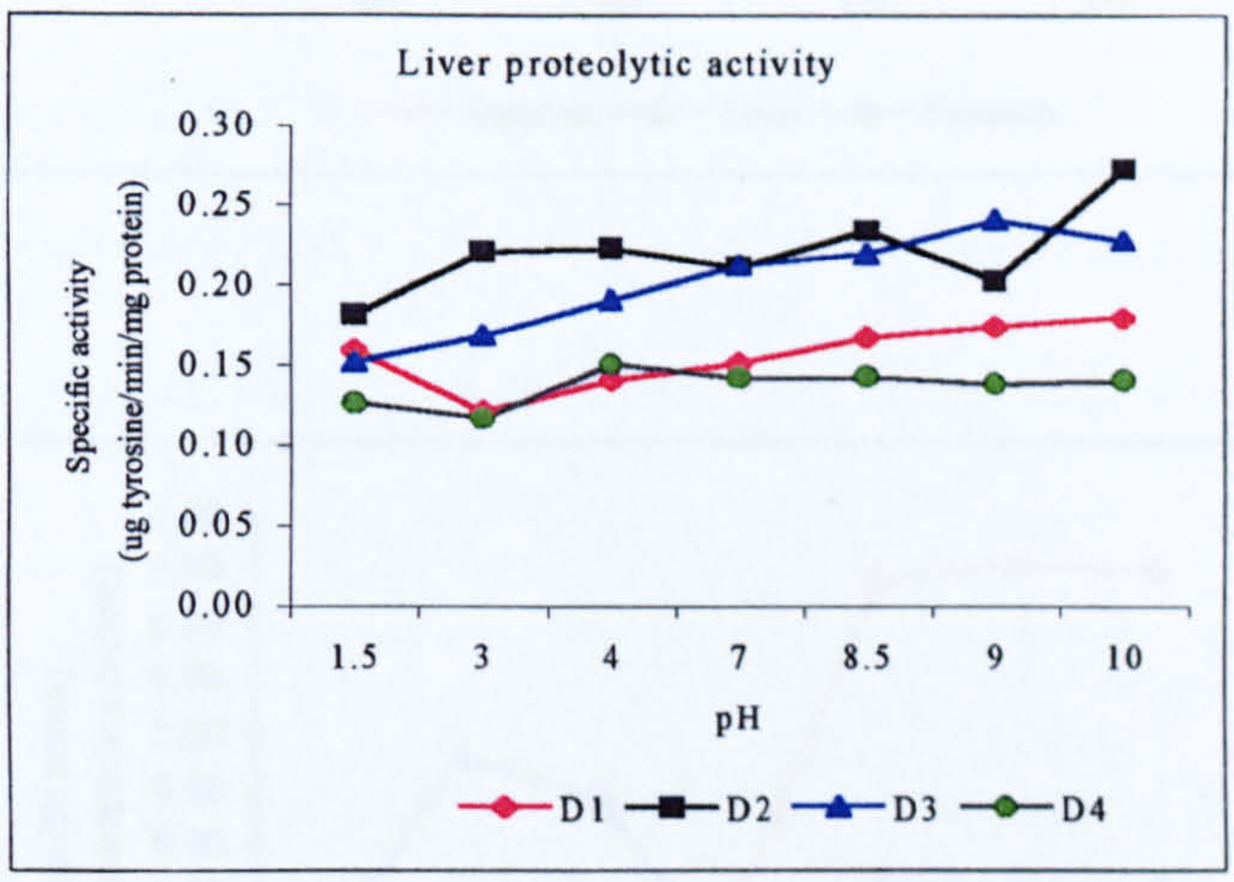
However, liver proteolytic activity showed approximately similar results at acid and alkaline pHs. In contrast, the higher proteolytic activity was observed at acid pHs (3.0 and 4.0 μg tyrosine^{-1} minute^{-1} mg^{-1} protein) whereas lower values were observed at neutral pH 7.0 (Figure 6.6) for catfish fed on the experimental diets.

Table 6.7 Total proteolytic, trypsin, amylase and lipase activities in intestine, liver and stomach (mean ±SD *n=2*) five fish per duplicate) of catfish fed control and test diets determined at 37^{0}C.

	Proteolytic activity (mean)	Proteolytic Sum of pHs* (µg tyrosine min^{-1} mg^{-1} protein)	Trypsin activity (µg tyrosine min^{-1} mg^{-1} protein)	Amylase activity (ug maltose ml^{-1} min^{-1})	Lipase activity (Sigma/Tietz/unit L^{-1}) min^{-1} ml^{-1}
Intestine					
D1	0.81±0.57^a	5.68	2.75±0.18^b	0.96±0.37^b	1.87±0.49^b
D2	0.47±0.25^a	3.26	2.39±0.66^{ab}	1.01±0.30^b	1.37±0.38^{ab}
D3	0.43±0.20^a	2.98	2.07±0.97^{ab}	0.67±0.19^a	1.14±0.25^a
D4	0.48±0.31^a	3.38	1.71±0.73^a	0.94±0.29^b	1.07±0.18^a
Liver					
D1	0.15±0.02^a	1.08	1.37±0.31^a	4.49±1.17^c	1.01±0.40^a
D2	0.22±0.03^b	1.54	1.42±0.39^a	2.94±0.98^{ab}	0.92±0.16^a
D3	0.20±0.03^b	1.41	1.33±0.29^a	2.46±0.86^a	1.12±0.31^a
D4	0.14±0.01^a	0.95	1.05±0.65^a	3.66±0.63^{bc}	0.97±0.43^a
Stomach					
D1	0.54±0.17^b	3.79	4.09±0.79^a	0.76±0.18^{ab}	1.10±0.55^a
D2	0.29±0.24^a	2.03	2.77±0.66^a	0.82±0.18^b	1.18±0.57^a
D3	0.31±0.13^a	2.17	2.27±0.43^a	0.65±0.14^a	1.21±0.45^a
D4	0.20±0.09^a	1.40	2.29±0.46^a	0.69±0.10^{ab}	1.11±1.13^a

D1 (control diet 0% FFSB), D2 (58% protein of FFSB), D3 (63% protein of FFSB) and D4 (63% protein of FFSB+ 1%-DL-methionine).
Values in the same column with the same superscript are not significant ($p>0.05$).
*Total proteolytic activity was obtained as the sum of those determined at pH 1.5, 3, 4, 7, 8.5, 9, and 10.
Total proteolytic activity was obtained as the sum of those determined at pH
1.5, 3, 4, 7, 8.5, 9, and 10.
Proteolytic activity was expressed as the amount of tyrosine (µg) digested by 100µl of
enzyme solution minute^{-1} mg^{-1} protein at acid, natural and alkaline pHs at 37^0 C.
Trypsin activity was expressed as the amount of tyrosine (µg) liberated by 0.5ml of enzyme extract per minute mg^{-1} protein at 37^0 C.
Amylase activity was expressed as the amount of maltose liberated by 50µl of enzyme extract minute^{-1} ml^{-1} at 37^0 C.
Lipase activity was expressed as the amount of fatty acids neutralized by 0.05 NaOH liberated by 1ml enzyme solution minute^{-1} at 37^0 C.





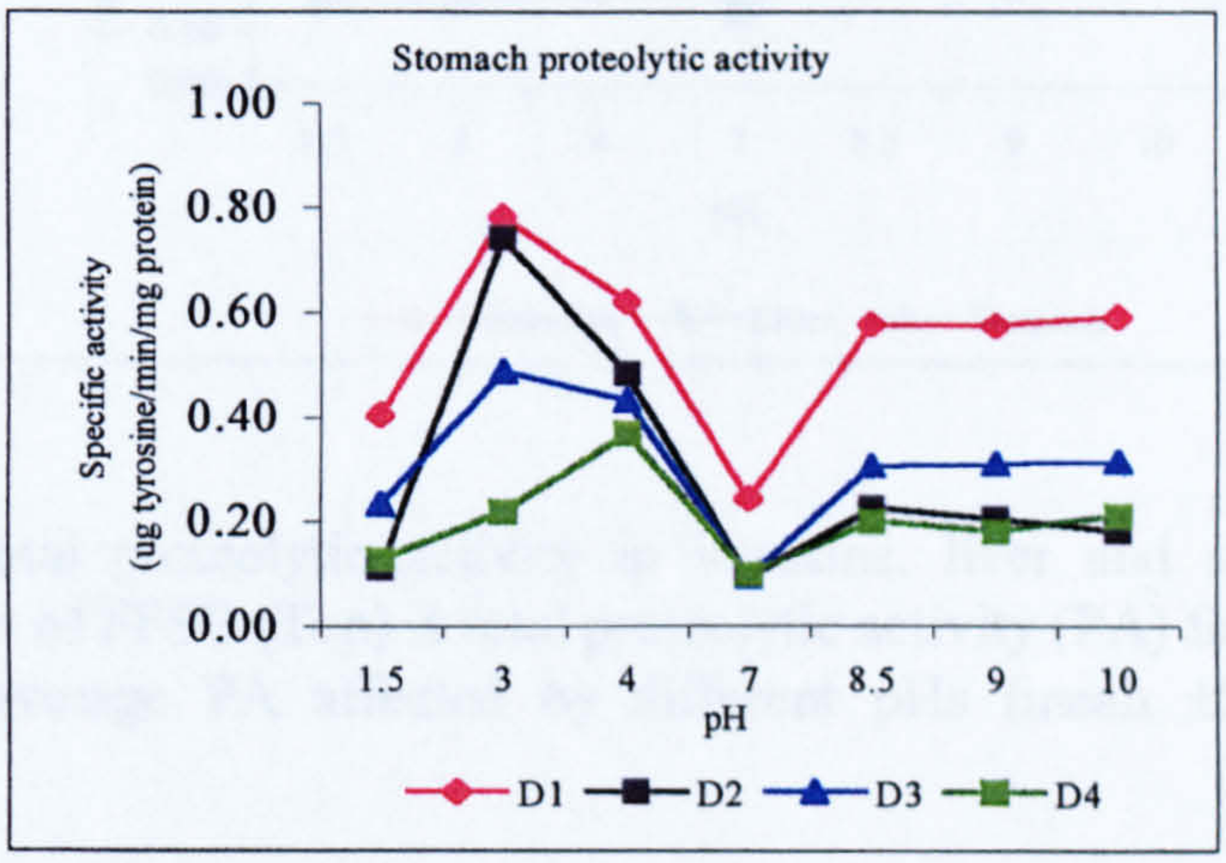
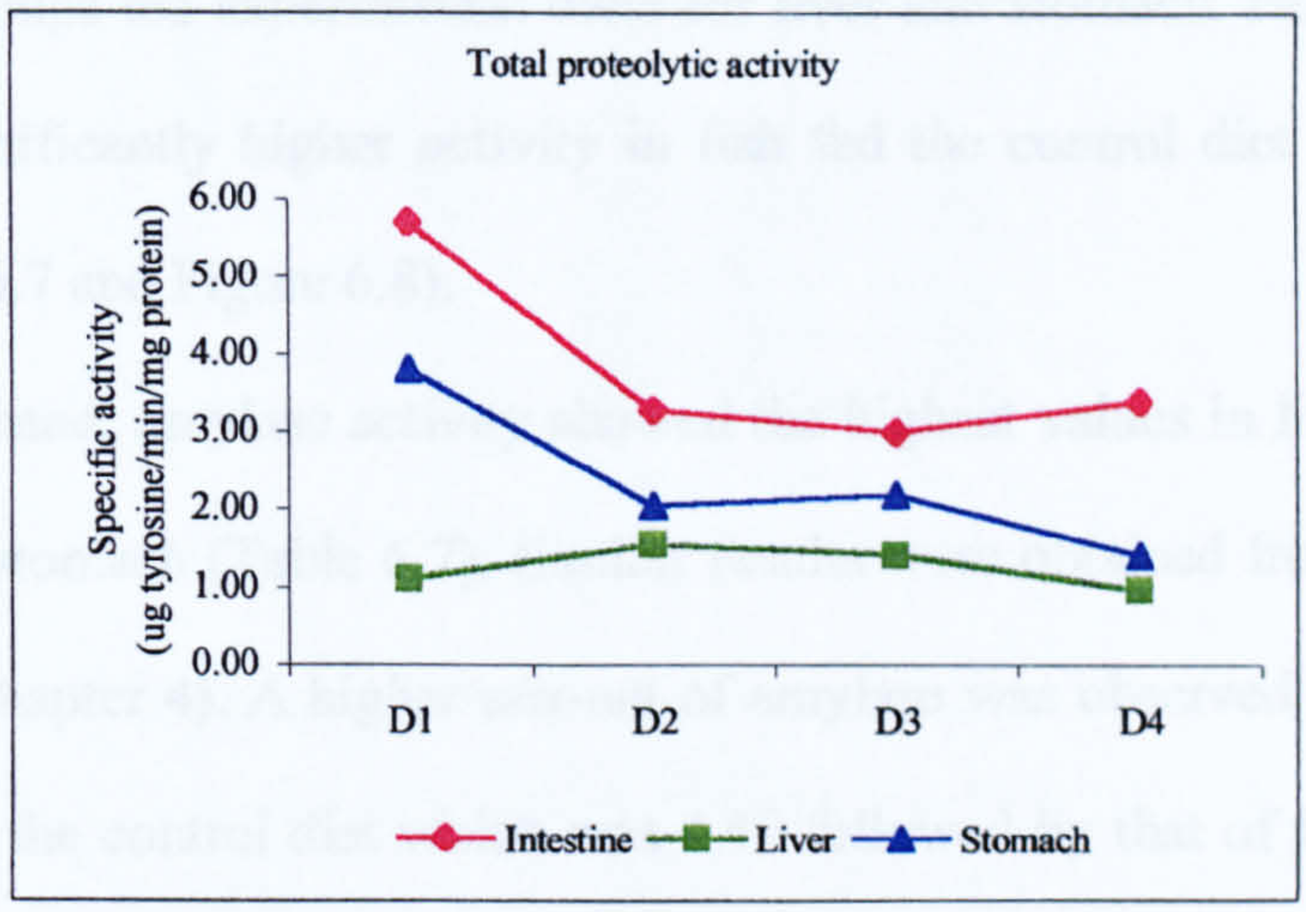


Figure 6.6 Proteolytic activity in intestine, liver and stomach in African catfish fed different levels of FFSB for control and test diets affected by different pHs (bars are means ±SD *n=2* five fish per duplicate).

D1 (control diet 0% FFSB), D2 (58% protein of FFSB), D3 (63% protein of FFSB) and D4 (63% protein of FFSB+ 1%-DL-methionine).



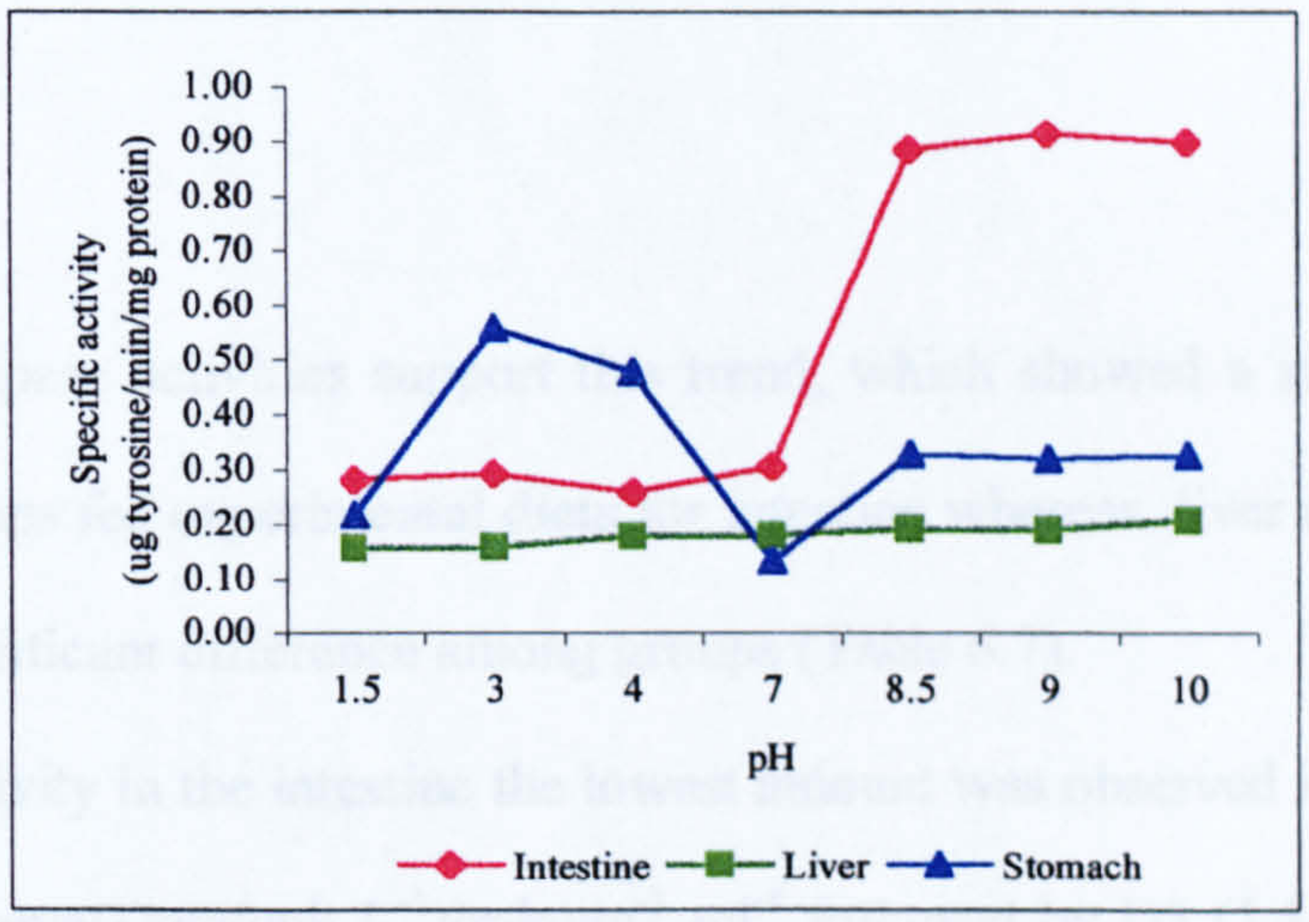


Figure 6.7 Total proteolytic activity in intestine, liver and stomach in catfish fed different levels of FFSB (Top) is total proteolytic activity (PA) for control and test diets, (Bottom) is average PA affected by different pHs (mean ±SD *n=2* five fish per duplicate).

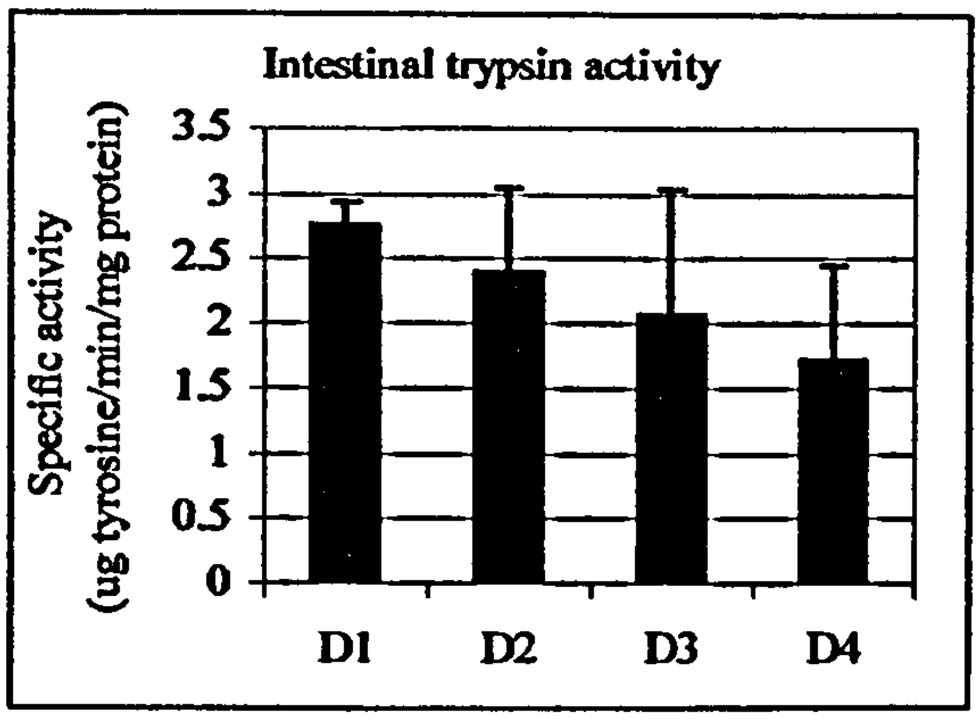
D1 (control diet 0% FFSB), D2 (58% protein of FFSB), D3 (63% protein of FFSB) and D4 (63% protein of FFSB+ 1%-DL-methionine).

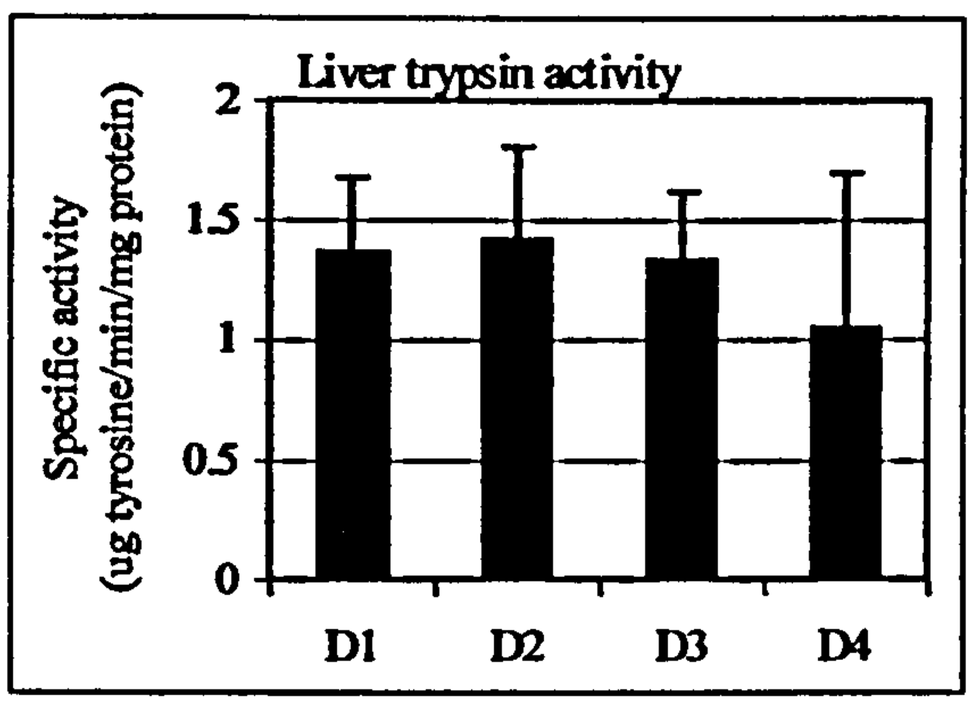
Moreover, trypsin activity was also observed to be in the higher range for the stomach followed by intestine and liver, but did not show any significant differences ($p > 0.05$) among the groups fed experimental diets for liver and stomach. However, the intestine showed a significantly higher activity in fish fed the control diet containing fishmeal alone (Table 6.7 and Figure 6.8).

On the other hand, amylase activity showed the highest values in liver compared to the intestine and stomach (Table 6.7). Similar results were obtained from tilapia fed on the same diets (Chapter 4). A higher amount of amylase was observed in the hepatic tissue of catfish fed the control diet which was 4.49 followed by that of fish fed on D2 (2.94 μg maltose^{-1} minute^{-1}ml^{-1}). Fish fed diets 3 and 4 showed lower amounts of amylase. However, a small amount of amylase activity was detected in the intestine and stomach (Table 6.7).

In addition, lipase activities support this trend, which showed a significant difference between, groups fed experimental diets for intestine whereas, liver and stomach did not show any significant difference among groups (Table 6.7).

For lipase activity in the intestine the lowest amount was observed in fish fed D4 (1.07) fatty acid (Sigma/Tietz/unit L^{-1})/minute^{-1} ml^{-1} followed by D3 (1.14), whereas fish fed the control diet and D2 showed slightly higher activities, ie:- 1.87 and 1.37 respectively (Table 6.7).





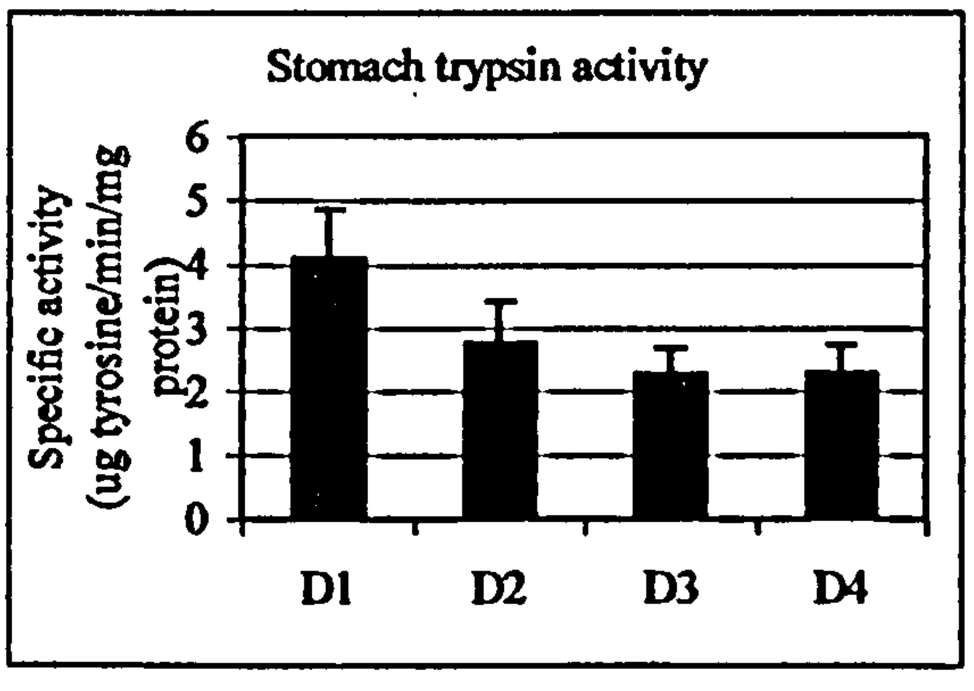


Figure 6-8 Effect of FFSB in diets on trypsin activity in African catfish intestine, liver and stomach.
Bars denote mean values (±SD *n=2* five fish per duplicate).

D1 (control diet 0% FFSB), D2 (58% protein of FFSB), D3 (63% protein of FFSB) and D4 (63% protein of FFSB+ 1%-DL-methionine).

6.5 Discussion

6.5.1 Experiment 1

Previous workers have extensively investigated the use of alternative protein sources to substitute for fishmeal. The concensus view is that increasing plant protein level to replace fishmeal has a negative effect on growth rate and feed utilization. However, a number of researchers have demonstrated the feasibility of using many plant proteins for fish at moderate inclusion levels. In this work with African catfish, the results obtained demonstrate that plant protein sources such as maize gluten can be effective in replacing up to 25% of high quality fish meal protein (LT94) in diets for catfish, without serious depression of growth rate. Other workers have also recommended gluten levels of 20-25% (Gropp *et al.*, 1979; Alexis *et al.*, 1985) and even 40% (Morales, 1993) in diets for rainbow trout. This study was in agreement with the results previously obtained by Davies *et al.* (1989) using soy protein concentration for tilapia, and by Gallagher (1994) using soybean meal for hybrid striped bass (*Morone saxatilis* × *M. chrysops*) in which there were restrictions for the use of a plant protein concentrate.

Robinson *et al.* (2001) suggested that corn gluten can be used as an effective plant protein source for channel catfish *Ictalurus punctatus* at levels up to 50% without affecting feed palatability, weight gain and feed utilization. High replacement levels with plant protein sources radically reduced growth rate in channel catfish. The possible reason for the poor performance associated with the higher substitution of plant proteins in the fishmeal based control diet is the imbalance of nutrients especially protein composition. This may be related to a less adequate dietary amino acid profile when maize gluten (MG) is added to the formula as this ingredient is considered deficient in lysine.

There are a number of other causes for decreased growth rates and feed consumption observed at higher levels MG substitution. The palatability of MG by catfish was certainly decreased compared to fishmeal based diets. Our results are an agreement to those obtained with yellowtail *Seriloa quinqueradiata* by Shimeno *et al.* (1993). These workers observed that diets containing animal protein sources such as meat and bone meal (MBM) supported higher feed intake than those containing a similar level of corn gluten meal. A second reason for a decrease in feed utilization of MG is the lower digestibility of plant proteins and carbohydrate and the interaction of specific nutrient components during the course of digestion.

Maize gluten meal is a high protein by-product of the milling industry (Davies *et al.*, 1997). In this manner, MG may be considered a partially purified ingredient. Due to the application of heat treatment and subsequent extraction of carbohydrate, maize gluten has a relatively lower level of the anti-nutritional factors (ANF's) compared to other plant protein sources such as soybean meal. These specific endogenous anti-nutritional factors are known to decrease the digestibility and the efficiency of protein utilization in fish (Saini, 1995). Furthermore, in relation to the present study, a decrease in growth rate was observed in all fish fed maize gluten test diets with high levels, when compared with the LT94 fishmeal reference diet. Although the ANF content of maize gluten is at least partially responsible for the poor growth performance of tropical fish fed the MG test diets. However, it should not necessarily be interpreted as a primary reason for the suppression of growth.

Fagbenro (1999) reported that the presence of anti-nutritional factors in winged beans did not pose a nutritional problem if the beans were adequately heat treated before inclusion in fish diets.

In the present investigation, the protein efficiency ratio (PER) of the control group was 4.21 as compared to the fish fed on MG 75%, which was only 2.09. The PER showed a linear trend with the feed conversion ratio (FCR), which increased at each level of inclusion of plant proteins. The reduction in apparent net protein utilization (ANPU) supports the reduced protein efficiency with respect to utilization and is in accordance with data from the literature. This indicated a progressive reduction in food quality, as can be confirmed from the lipid content in the carcass composition. The results of the body composition of fish in this experiment are in accordance with the growth performance results and show an overall reduction in protein and energy retention.

The fish fed the 75%MG diet produced lower body moisture, but higher fat content and this is a typical relationship in most fish composition measurements. Instead of utilizing the proteins for growth, they may be diverted for energy storage purposes. Storing the unused energy of the diets in the fish body may also increase the fat content.

A high level of body fat is undesirable from the standpoint of storage because it increases rancid off-flavour and thus reduces consumer acceptance. In this present study, growth and final lipid content of fish were affected by diet formulation.

Shearer (1994) also observed that fish weight was the sole determine of the amount of body protein. In the present study, final protein deposition in the carcass was higher than the initial fish. Furthermore, the protein content in the final carcass did show a trend between fish groups fed the different diets of treatment. Fish fed on the MG 75% diet showed the lowest protein content in the whole body.

The protein content value in the carcass only indicated the partition of how protein in the diets was utilized it does not, however, provide an indication of how protein is utilized for maintenance. A further reason for poor growth performance and nutrient utilization was the possible imbalance of fatty acids between animal and plant oil within the diet formulation. However, one reason for oil supplementation is that when plant

proteins such as maize gluten, are employed to replace fish meal on a isonitrogenous basis, oil must be added to the diet to elevate the energy level because plant meals are lower in digestible energy than fish meal.

The yellow- orange colouration observed in operculum, skin and fins of fish fed high on level of MG diets could be explained by the presence of carotenoids in this meal, mainly zeaxanthin and lutein (Latscha, 1990). Robaina *et al.* (1997) found similar colour changes in gilthead seabream fed diets containing high levels of corn gluten meal. Different studies suggest that dietary carotenoids produce yellowish colouration in fish muscle and skin, with a relationship between colour intensity and carotenoid content of the diet (Lee, 1987).

The accumulation of lipid observed in the liver histology of African catfish fed the higher level MG diets could be related to a higher available carbohydrate component in this ingredient, These results agree with those reported by Shimeno *et al.* (1993) who found an elevated liver lipid content in yellowtail seabream *Seriola quinqueradiata* fed meat and bone meal compared to fish fed corn gluten meal diets at the same dietary inclusion level.

Interestingly, in the present experiment, isolated necrosis in hepatocytes were located in livers of fish fed higher levels of MG in the diet, indicating possible irreversible effects on fish health due to nutritional imbalances (Mosconi-Bac, 1987; 1990). Also Robaina *et al.* (1998) have reported that the increase in the *n-3/n-6* fatty acid ratio with about 30% soybean meal improved the utilization of liver lipids thus reducing liver histological alterations in gilthead sea bream. However, the supplementary fish /vegetable oils remained relatively high in our diets and would have provided the *n-3, n-6* fatty acid requirements for this species.

6.5.2 Experiment 2

Most plant ingredients have inherent anti-nutritional factors (ANF's) that affect feed efficiency, feed utilization and ultimately compromise growth rates of fish.

In this study, the results obtained demonstrate that plant protein sources such as full fat soybean can be effective in the replacement of between 40 to 50% of the high quality fishmeal protein (LT94) in diets for catfish. Catfish growth was inferior with a diet containing about 58 and 63% protein from FFSB compared with fishmeal as the only protein source.

However in this study, catfish fed 63% FFSB with DL-methionine supplementation did not improve their growth performance compared to the unsupplemented diet. These results may be compared with those obtained by Fagbenro & Davies (2001) when a standard type of solvent extracted dehulled soybean meal was used to replace fishmeal in a series of diets for juvenile African catfish. Only 50% of the dietary protein could be supplied by soy protein without methionine supplementation. In contrast with our data however, methionine supplementation was feasible and extended the use of soybean to 75% inclusion. In the current study with catfish, ANPU values were not greatly affected except with methionine supplementation. This result could indicate an imbalance if methionine is added in excess of requirement levels promoting inferior protein utilization efficiency.

Aquatic species, like monogastric terrestrial animals do not tolerate raw soybeans in their diets. In general, most fish species including African catfish exhibit poor growth when fed diets containing raw or under heated soybean meal. Balogun & Ologhobo (1989) observed considerable differences between the utilization of raw and cooked soybean meal by *C. gariepinus*. Although these workers used solvent extracted soybean meal in their studies, full fat soybean meal (FFSB) also requires to be effectively cooked prior to incorporation into aquafeeds.

Viola *et al.* (1983) reported that the nutritive value of properly heated FFSB for mirror carp was essentially the same as those of commercial soybean meal or soya protein concentrate reconstituted with soybean oil simulating full fat soybean (FFSB). Generally, fishmeal based reference diets in these investigations outperformed the soya diets due to a better balanced amino acid profile and superior digestibility of protein and energy.

Although studies using solvent extracted soybean meals are quite common, there have been relatively few investigations to assess a full fat soybean meal for tropical fish. Reinitz & Orme (1978). Compared de-fatted and full-fat soybean meals for rainbow trout with favourable results. The inherent oil content in the flaked-ground meal had higher digestibility characteristics and the meal was more effectively heat processed to denature the trypsin inhibitors and other related ANF's often associated with soybeans. The benefits of using full fat soybean meals in aquaculture was reviewed by Lim & Akiyama (1989) who stated that the nutritional value of the protein and oil was good for fish. FFSB due to its high fat content, nature of its fatty acids and presence of phospholipids could play a major role in the nutrition of aquatic animals.

High replacement levels with plant protein sources radically reduce the growth rate in catfish such as maize gluten meal, as described in experiment 1. Other reasons for the poor performance associated with the high substitution of plant proteins in warm water fish diets is reviewed in Chapter 4.

In addition, the palatability of FFSB by catfish is less than was found for tilapia, which was observed from daily feed intake records. However, catfish grew faster and better compared to tilapia on similar dietary regimes. Also palatability was certainly decreased compared to fishmeal based diets for both species. Our results are in general agreement with those obtained with yellowtail by Shimeno *et al.* (1993) who also reported a reduced palatability in fish fed plant protein substitutes.

These workers observed that diets containing animal protein sources such as meat and bone meal (MBM) supported higher feed utilization than those containing a similar level of corn gluten meal. A second reason for a decrease in feed utilization of FFSB is the lower digestibility of plant proteins and energy, which was noted in Chapter 3.

In addition, FFSB may be considered as a partially processed and complete ingredient due to the application of heat treatment and subsequent grinding of the whole bean. Full fat soybean meal has a relatively higher amount of the anti-nutritional factors (ANF's) compared to other plant protein and animal protein sources. These specific endogenous anti-nutritional factors are known to decrease the digestibility and the efficiency of protein utilization in fish (Saini, 1995).

In relation to the present study, a decrease in growth rate was observed in all fish fed FFSB diets when compared with the LT94 fishmeal reference diet. Although the ANF content of FFSB remains unquantified even after heat processing and advanced technology. This was discussed in Chapter 4 and related to similar trials conducted with tilapia.

Proteolytic activity showed no differences among fish fed different levels of FFSB with and without amino acid supplementation for intestine and stomach. However, liver proteolytic activity was different between fish fed on the control diet and test diets respectively. This seems to agree with our previous results with tilapia reported in Chapter 4.

Hidalgo *et al.* (1999) also found no differences in proteolytic activity to justify fish classification as either omnivorous or carnivorous. This was in agreement with previous and current studies with tilapia and catfish which demonstrated a marked difference in the amount of proteolytic activity between herbivorous and carnivorous fish. In these experiments, tilapia revealed a high amount of proteolytic activity for all organs

compared to catfish. These findings were confirmed by Kuz'mina (1990) who also observed a high proteolytic potential in non-carnivorous fish. This should be understood on the basis that vegetable proteins are more difficult to digest compared to animal proteins within the gastrointestinal tract for the maintenance of metabolism and achievement of rapid growth.

Indeed, Al-Owafeir (1999) reported that in general, proteolytic and amylase activity in Nile tilapia was higher than in the African catfish species. These probably indicate that tilapia (herbivorous fish) is more adapted to use carbohydrates than African catfish (carnivorous fish) when presented with higher dietary levels of soybean meal. Although, the same author also noted that proteolytic activity in African catfish is sometimes higher than Nile tilapia and this might be due to the diet nutrient or protein efficiency. This may also be attributed to the natural feed of African catfish which consists mainly of fish, crustaceans and molluscs. Many species of tilapia however, depend on plant matter or detritus of plant origin as the typical diet (Jauncey & Ross, 1982).

Moreover, this may give an indicator that carnivorous fish are enzymatically adapted to the high protein levels present in their natural foods despite the source.

With respect to the digestive enzyme profile of the gastrointestinal tract of catfish, it should be noted that most investigations have focussed on other warmwater fish species such as the tilapia, catfish, eel and some marine fish species. Relatively few studies have been undertaken for the African catfish (*Clarias gariepinus*). In Chapter 4, it was stated that El-sayed *et al.* (2000) had reported the effects of various treated soybean products on the digestive enzymes of the tilapia.

Given that catfish are carnivorous compared to tilapia, it is necessary to make comparison between the results obtained in both studies. As mentioned previously, Hidalgo *et al.* (1999) demonstrated that the eel (*Anguilla anguilla*) had high proteolytic

activity associated with a low gastrointestinal tract pH together with significant activities for proteolytic enzymes at higher gastrointestinal pH's.

Das & Tripathi (1991) reported that optimum protease activity was recorded between pH 7.6 and 8.4 in both fingerling and adult grass carp *Ctenpharyngodon idella*. However, in this experiment, the optimum protease activity was recorded in different organs which showed a different activity in catfish i.e. for intestine optimum pH ranged between 8.5 to 10 in contrast with tilapia fed on the same diet that showed 7.0-8.5. Also the catfish stomach showed optimum proteolytic activity at pH 3.0 and 4.0 however tilapia showed 1.5-3.0., this gives an indicator that in the thick walled muscular stomach such as African catfish the pH is fairly high, around 4 (Uys *et al.*, 1987).

The optimum pH for the trypsin-like activity is higher than that of chymotrypsin-like enzyme (Uys & Hecht 1987). The current results have demonstrated that the higher enzymatic activity at pH 8.5, 9.0 and 10 rather than at 7.0 for catfish which seems to be in agreement with data for other species such as carp, trout, and sea bream (Hidalgo *et al.*, 1999).

Generally, an appreciable reduction of trypsin was found in intestine and stomach tissue however, tilapia showed the greater reduction exhibited in liver when fed high inclusion levels of FFSB compared to African catfish.

In agreement with this result Robaina *et al.* (1995) observed that gilthead sea bream fed diets containing soybean meal showed a reduction in trypsin activity and protein digestibility when substitution levels were increased.

The amylase activities in various organs (intestine, liver and stomach) also varied for catfish in this study. The highest levels were measured in the liver compared to the intestine and stomach. This agrees with the previous study with tilapia which support

this trend. Amylase activity in this experiment showed that the liver was affected in fish fed high FFSB inclusion levels in diet formulations for catfish. However, catfish fed diet 4 containing a high level of FFSB with amino acid supplementation showed higher values than other fish fed diets containing FFSB without AA supplementation. The low amount of amylase in the stomach indicates that very little starch is digested before the food reaches the foregut. This supports the findings reported by other authors (Uys & Hecht 1987).

Al-Owafeir (1999) recently reported that α-amylase activity was especially present in Nile tilapia; this may indicate that Nile tilapia is more adapted to use and digest carbohydrates better than African catfish as discussed in Chapter 4.

Relatively few investigations have been undertaken for lipase activity in African catfish. In this study, lipase activity was shown to be present in the intestine, liver and stomach and slightly higher in the intestine than liver and stomach. Recently, Tengjaroenkul *et al.*, (2000) suggested that lipolytic activity in *O. niloticus* is definitely present, and occurs mainly in the cranial half of the intestinal tract. The relatively restricted distribution of lipase enzyme in the Nile tilapia may be due to the fact that lipase activity is lowest in herbivorous fish (Opuszynski & Shireman, 1995), related to the low fat content in plant materials naturally consumed by tilapia (Vonk & Western, 1984; Opuszynski & Shireman, 1995). This seems to agree with the current two experiments for tilapia and catfish that showed a higher amount of lipolytic activity in catfish (carnivorous fish) than for Nile tilapia (herbivorous fish).

However, in the present study the results for lipase activity are in disagreement with the results obtained by Al-Owafeir (1999) who found that lipase activity in Nile tilapia was higher than African catfish. There appears to be no information in the literature that either agrees or disagrees with data for these fish. However, the natural food for

carnivorous fish contains high amounts of protein and lipid and small amounts of carbohydrates (Smith, 1989). This might give an indicator that carnivorous fish have a high lipase activity compared to herbivorous fish since enzyme activities are usually correlated to the natural food habits of the species. More investigations are needed in this area to clarify and validate such findings.

This was an interesting trend and may have been due to the fact that the contribution of lipid from full fat soybean meal was not as available compared to that in fishmeal and supplementary oil (fish oil and corn oil). Although it has been suggested by Reinitz & Orme (1978) that the lipid in full fat soybean is potentially a better source of energy for trout, research has also shown that the digestible energy coefficients for full fat soybean meal was lower in gilthead sea bream, *Sparus aurata* (Nengas *et al.*, 1995) compared to solvent extracted soybean meal.

In summary: - the first feeding trial was able to show that African catfish responded to diets of varying maize gluten incorporation and grew favourably up to an inclusion level of about 25-30% replacing the fishmeal component. However, higher inclusion rate affected growth adversely and also resulted in substantial changes in the hepatic ultrastructure of these fish.

The similar investigation using full fat soybean meal also demonstrated a limitation due to the nutritional value of soybean protein when above a threshold of >50% dietary protein replacement. These appeared to be no advantages in supplementation of high soybean diets with crystalline synthetic amino acids such as methionine under the present conditions. The anti-nutritional factors (ANF's) associated with FFSB possibly caused a further depression in growth rate, feed utilization efficiency and also for several key enzyme activity levels associated with the gastrointestinal tract.

CHAPTER 7

GENERAL DISCUSSION & SUMMATION

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The programme of research to evaluate the suitability of selected ingredients for inclusion in diets for tilapia and catfish was based on the needs outlined in the introductory chapter. The expansion of global aquaculture and in particular the role of fish farming in the Middle East and such countries as Egypt will become increasingly dependent on minimising imports of commodities such as fishmeal and soyabean produced in the USA and South America. Although a significant trade balance exists for the purchase of fishmeal, soyabean meal and animal proteins by Egypt, there is a growing pressure to utilise and develop the domestic sources of protein concentrates that could safely be incorporated into feeds and diet formulations for finfish and shrimp. These are usually reared under semi-intensive and of course under intensive operations in this region of the African continent.

There has been a historical interest in the research and development of alternative protein sources in the most developed nations of Africa with an aquaculture interest. In countries such as Nigeria, Kenya, Zambia, Zimbabwe, Uganda, Botswana and South Africa, where an emphasis on locally available materials and resources has resulted in complete aquafeed formulations using plant and animal based proteins. The replacement of fishmeal by various pulses, legumes, and grains has been quite successful for many species and allowed significant savings and reductions in cost.

Most efforts have been focussed on the use of soyabean meal by-products and other investigations on sunflower seed meals, safflower, mustard seed, various types of beans, peas and gluten rich protein by-products from wheat and corn extractions. These have all yielded promising results and are now established ingredients for inclusion in practical fish diets.

Research on animal protein sources for potential use in fish feeds has a long record and was the original interest of the early pioneers of fish nutrition work. This was obvious due to the fact that salmonid diets were originally dependent on fish meal and animal proteins from abattoirs. Agricultural wastes were seen as acceptable alternatives closely matching the nutritional value of marine proteins. Meat & bone meals, meat meals, blood meals and poultry by-products such as poultry by- product and feathermeals proved to be reliable sources, but with some definite limitations in the diet depending on composition and processing. Most of these proteins can be included from 7-50% of the protein component without causing adverse effects on growth and nutrient utilisation in fish.

Animal proteins have a very good amino acid profile and are usually well digested by fish with little effects on palatability and feed intake. However, variations in processing technology do influence their properties and in the past, blood meal and feathermeals were poorly digested in some species and therefore their inclusion in fish diets may be limited to below 10%. Other animal derived proteins that have also been considered include spray- dried milk protein powders, egg powders and whey. Unfortunately these are very expensive commodities and are not economical to be of practical consideration. More recently, there has been major public concern regarding all animal proteins for use in the rearing of domestic farm animals, and this was mainly due to the BSE outbreak in Europe that originated in the United Kingdom. Animal proteins for feeds are now largely prohibited in most of the EU states and only fishmeal is allowed for mono-gastric animals such as swine and poultry. The legislation has also been extended to cover fish and trout and salmon diets are formulated to contain high quality fishmeals and soyabean as the chief sources of protein. Meat & bone meals, meat meals and poultry by-products are still used routinely in the USA, Canada, Chile and Australia for

salmonid diets and they are widely respected in both temperate and tropical fish species in SE Asia, Africa, China and Japan. However there are growing concerns by countries where the importation of animal proteins originated in Europe and especially the UK that might be a risk to the industry and public safety. Recently, the Egyptian agriculture ministry has proposed a ban on such imports but will seek to develop similar by-products from its domestic animal farming operations. Therefore animal proteins will be of importance in Egypt and are worth exploring as ingredients for fish and crustacea.

Egypt has a considerable amount of arable farming and corn and wheat are produced for both human consumption and animal feed. Soyabean, cottonseed and rice are also produced and all these crops have recognised importance in aquaculture. The research project was therefore primarily directed towards the assessment of both animal proteins and plant based ingredients for tropical fish of relevance to Egypt. It was the aim to provide detailed formation that could be used for optimising their inclusion rates in compound feeds for the tilapia and African catfish species.

Tilapia are now stated to be the fastest growing aquacultured fish species in the world with the fish found in almost every continent as well as its native Africa. Tilapia is intensively grown in re-circulation systems in the USA and Europe or more commonly in either open tank systems, raceway or earthen pond culture typically in Africa and SE Asia. Their versatility, fecundity and fast growth rates are appropriate for the expansion of aquaculture where water and space is limited. Similarly, the African catfish species is also regarded as a high value fish in many countries and serious investment is being made in the future of this important fish. It is cultured intensively in Holland and parts of Europe, but is mainly found in Africa and several SE Asian countries. Egyptian farming of *Claridae* is expected to grow in the future and special locally produced diets for both tilapia and catfish are urgently required to meet their nutritional requirements for efficient production to marketable weights.

In the research programme, it was essential to proceed with a study to evaluate a selection of ingredients that might have the potential to meet the needs of tropical fish diets. Most fish feeding experiments can be criticised because they assume equal availability of nutrients from ingredients and are designed to test the replacement of protein and energy from various sources by substitution of values on a gross nutrient basis. Preliminary investigations should therefore be applied to determine the digestibility of protein; amino acids and energy in separate ingredients and feed materials prior to feed formulation for practical diets. Experiments should provide information for linear least cost diet formulations by computer that will allow exchanges of ingredients without changing the digestible ratios of protein, amino acids and energy.

Such information is only partially available for even the most established fish species and so this is quite important for such fish as tilapia and catfish where relatively little information exists in the scientific literature.

The digestibility trial described in the third chapter of this work was undertaken to characterise the apparent digestibility coefficients for the tilapia as a model fish with a similar mode of feeding and nutritional expectations. Tilapia were fed a range of experimental diets that were based on a reference diet that incorporated separate test ingredients in a defined ratio. It was assumed that no interactions occurred between the nutrient components and that the level of inclusion provided a realistic compromise to the inclusion levels that could be expected in balanced practical diets for this species. Of course, these assumptions may not be true, and many complex interactions between starch, protein, amino acids and fats/oils are likely in the digesta of fish under various conditions.

The method of digestibility assessment is also of crucial importance since it can be the principal cause of differences found between the experimental results of investigators. The collection of faeces by natural output into the tank and subsequent siphoning is common but may result in over- estimation of digestibility due to leaching losses and other errors. Alternative methods suitable for salmon and trout are the recovery of faeces by manual stripping and various suction and metabolic chamber approaches. These also have their respective problems, and the former may even under-estimate the digestibility of diets and feed ingredients because of incomplete digestion or presence of contaminating body fluids.

Apparent digestibility also fails to consider the matter of endogenous losses of energy and protein associated with enzyme secretions and cellular degradation. This could be a significant source of error for those fish where the diet is relatively low in protein. True digestibility would be preferable measurement but would necessitate the feeding of a very low or essentially protein-free diet to fish. In the current study, it was decided to adopt the standard apparent digestibility approach for consistency and comparison with data from other workers.

The data obtained for tilapia showed that the technique and protocol used generated useful data on the digestibility profiles of protein and energy for soyabean, maize gluten, poultry meat meals, blood meals and sopropeche fishmeal and feathermeal proteins in combined test diets. The weighting factor of 40:60 for the ingredient: reference diet mixtures were satisfactory for the calculation of individual feed ingredient digestibility coefficients, and these proved similar for values reported in the literature for tilapia and other warmwater fish. These initial experiments served as the basis for selecting the ingredients that would be tested in successive nutrition trials in which a more full appraisal of the replacement value for the ingredients could be made.

In this respect, different ratios of test ingredient should be assessed compatible with the expected levels of ingredient that is likely to be found in the final production diet.

Nutrition investigations were based on the standard concept of designing a balanced diet that meets the known requirements of the fish in question. For tilapia and catfish, a control or reference diet was based on fishmeal.

A low temperature LT fishmeal was used throughout all of the experiments for consistency of comparison. Fishmeal is recognised as a reliable protein that provided an ideal amino acid profile and quality for ensuring maximum growth and feed utilisation and feed intake. Experimental diets conformed to the NRC (1993) recommendations for tilapia and African catfish and also on the latest research papers on these or related species. Diets were principally iso-nitrogenous and iso-energetic in terms of their gross nutrient specifications and it was decided not to formulate diets for tilapia using the digestibility data obtained, so that uniform presentation of nutrition trial data was maintained for all investigations and ease of comparison between fish species.

The vitamin and mineral premixes were of a commercial source for all experiments and these were always included at levels to guarantee a supply of each micro- nutrient in excess of recommended levels.

The diets were all made under laboratory conditions and cold pressed with added water before drying by air convection. It should be appreciated that this does not replicate the industrial manufacture of aquafeeds and the extrusion technology employed in modern diet production uses higher temperatures and pressures that can alter the physical and chemical properties of the feed ingredients and consequently the nutritional value of the product to fish. Laboratory scale diets are still valid and enable effective comparisons of different ingredients for small feeding trials etc. but should be cautioned if correlations

are to be made with extruded or expanded feeds and those in which certain oils and fats are added to the pellets separately.

The main concern is the changes that extrusion can make to the digestibility of protein and starch in feeds and therefore in future, more studies are required to screen ingredients subjected to various technologies.

The diets developed for the fish in the present study were all accepted very well and there were never any problems of palatability of control diets composed of fishmeal or for diets containing low inclusion levels of test materials. The pellets were all stable and of the correct dimension for feeding trials and appropriate to all size classes of fish stock used.

Experimental fish were all obtained from designated stock and of a defined source and strain. This was a very important concern and enabled proper consistency across all of the studies. Tilapia was produced to be all male stock from Swansea University and developed from a super-male YY parent as F1 progeny. *Oreochromis niloticus* is an acceptable species and is widely used in tilapia farming operations worldwide. Male tilapia is preferred due to their fast and uniform growth rates. There was little problem of hierarchy or differential growth effects in the studies reported. African catfish were all generated at Plymouth by artificial fertilisation and were a pure stock source.

In all cases, juvenile fish were chosen for the growth trial studies to ensure a rapid increase in biomass in a reasonable time frame. A significant growth increment could be achieved in a typical 2-3 month period and any effects of feed ingredient and diet composition could be assessed with confidence. Fish were usually weighed at weekly or bi-weekly intervals and husbandry related stress minimised as far a possible.

The nutritional parameters associated with experimental studies with fish included the calculations of growth rate, feed conversion and net protein utilization efficiencies.

Each experiment also included a full analysis of the carcass composition and health evaluation by histological examination of major organs and tissues where appropriate. The incorporation of detailed histological assessment of the liver was seen to be an important step in order to check on the more subtle changes that may occur due to dict modulation and variations in nutrient partitioning.

The standard AOAC methods and protocols for nutrient analysis of raw materials, ingredients, completed diets, fish tissues, and faeces were applied according to the recognised practice for fish nutrition experiments. However in this project, it was sometimes necessary to utilise specialised biochemical assays for digestive enzyme activities that may have been affected by some of the feed ingredients tested.
Where appropriate micro analytical methods such as that for lipids in diets and fish carcass was used instead of the AOAC method for large samples and accuracy.

The fish trials were all performed indoors under controlled conditions of lighting, temperature and water quality. Re-circulation systems were provided and these seldom varied in terms of the environmental conditions presented to both tilapia and catfish over the 4-years of the research programme.
It can be therefore stated with confidence that the rearing conditions were within the normal expectations of these species and indeed provided a realistic environment capable of supporting optimum growth and health. The results from the successive experiments could at least be compared between the trials and interpretations made between the differences in growth and feed utilisation of fish fed diets with varying composition.
Water temperature was constant and was maintained at 26-28 ^{0}C at all times and a regular 14h light 10h dark photoperiod held for all experiments. It should be argued

however, that future research work must show that the laboratory experiments are supportive of outside field conditions and fish farm situations where fish are raised in tanks, ponds, raceways and cages at ambient conditions. Also, the whole production cycle including fry, grower stages and broodstock should ideally be tested on diets containing the various sources of animal and plant proteins.

Feeding trials with tilapia were concerned with bettering our understanding of replacing fishmeal with a full fat soy protein. The effects of soybean meal inclusion on growth performance and feed utilisation was assessed in a comprehensive study that included measuring the influence of soybean protein on gastro-intestinal enzyme activities and nutrient retention or balance. The results clearly showed that interactions occurred due to diet, and that appreciably negative effects on digestion could account for a reduction in fish performance. Soyabean contains a host of anti-nutritional factors that were not determined in any of these studies but are the likely cause of the problems and constraints associated with legumes of this type.

The conclusions made were that soyabean could not support optimum growth rates of tilapia despite supplementation with methionine and that replacement of over 50% of the fishmeal component of the diet may not justifiable with this ingredient. Other work has supported this finding and it is widely accepted that amino acid supplementation is less feasible in warmwater fish species due to problems of absorption and post prandial assimilation at a metabolic level. However there is a considerable contradiction regarding the merit of amino acid additions to feeds where they are limiting and better results have been obtained with temperate carnivorous species such as trout and salmon where a more acidic stomach digestion phase is dominant. The value of amino acid supplementation remains to be explored for warmwater fish such as tilapia and catfish.

More research effort is needed to test coated amino acids that may allow a delay in absorption and a more uniform uptake of crystalline amino acids with the digested complete protein of the diet.

The next area of investigation centred on catfish and the feasibility of both animal and plant protein alternatives to fishmeal were explored in a sequence of nutrition trials based on the principles described earlier. First, a quality source of poultry by-product meal was tested by an incremental replacement of fishmeal in balanced diets and fed to juvenile catfish. Poultry by-product meal was very well accepted by this species and an optimum inclusion of up to 40% found as a result. There was no evidence of pathology or negative health problems although some changed in liver ultra-structure are reported at the end of the two-month study.

Plant proteins of interest included a study on maize (corn) gluten meal and a full fat soyabean product.

Corn gluten meal was tested at varying levels and 75% inclusion was found to promote inferior performance compared to lower levels in the feed. In this trial, changes in hepatic tissue structure and general morphology were noticed. Maize gluten represented an important protein concentrate for potentialy use in Egypt and it was accepted that it is fairly low in anti-nutritional factors except for its lower content of lysine. In the associated experiment with soyabean meal, it was again found that high inclusions in test diets were detrimental to catfish and that amino acid supplementation was not beneficial to diets where soy protein substitution was greater than 60% of the protein.

Most of the investigations conformed to numerous findings in the literature and supported the arguments and constraints associated with the ingredients selected in the course of the programme of research. It was evident that there were many reasons for the problems encountered and that there was much scope for improvement and

development for the concept of replacing fishmeal in diets for warmwater fish of commercial importance. The catfish and tilapia were both sensitive to high inclusion levels of either animal or plant protein rich ingredients. This demonstrated that the same principles of nutritional limitations were occurring for these respective species such as protein and energy digestibility reductions, differences in amino acid balances and also effects on palatability and feed intake. In general, carcass compositional changes were only slightly affected by diet and this may have been due to the fact that most experiments ended with relatively small fish below production sizes close to market weights where fat content would have been significant.

It should be mentioned that one criticism of the experimental design is that every ingredient was evaluated as a separate component of the diet formulation at the expense of fishmeal in increments or at a fixed level. Practical diets are formulated to have many different ingredients in a complex manner and they may in many cases be additive in quality. This is especially true of complementary proteins where amino acid imbalances can be offset and the effect is synergistic and allows greater use of protein blends to replace fishmeal. More research is required to examine this property for various protein sources for the species under consideration. Linear Least Cost formulation is a technique that can calculate the optimum use of these materials and even suggest the correct ratios of protein ingredients that can be recommended to reduce the fishmeal. The upper dietary inclusion levels for the animal and plant proteins tested here in the study on tilapia and catfish would however enable the maximum amounts to be stated. Optimisation would require further work to obtain essential amino acid digestibility data for each raw material, so as to formulate directly on this basis.

The anti-nutritional factors associated with plant proteins are large in number and vary considerably in levels between different sources. These were not measured for soyabean

despite the significant effects of soyabean on the growth of the fish species and the negative effects on proteolytic enzymes of the gastro-intestinal tract. Experiments are required to study the role of ANF's on both tilapia and catfish in more detail and this would necessitate the isolation of these compounds from plant based by-products and their testing in semi-purified diets at various levels.

Such studies would be very important and provide evidence for the changes described for the investigations reported in the present trials. The histo-pathological examination of such tissues as the gut lining (mucosa), villi, liver, pancreas etc would be interesting and would aid in confirming the limitations for the inclusion of these ingredients at high levels. .

There have been considerable improvements in the processing technology of raw materials in western countries over the last decade and this has resulted in a range of feed ingredients with better protein levels, higher digestibility coefficients for protein, amino acids, and energy. Feed manufacturers and milling companies have developed new types of concentrates, micronised, extruded and flaked products from various legumes and grains such as those originating from soyabeans, peas, beans and different grains for use in aquafeeds. Some blended, co-extruded products based on rapeseed meal and beans have also proved to be interesting and worthy of consideration.

Lately, renewed interest in animal proteins has led to refinements in their processing to yield a range of better and improved products for use in fish diets. This is applicable to blood meals based on spray-dried haem protein concentrate, enzyme hydrolysed feathermeals, specially rendered poultry meat meals and other animal protein derived materials.

Controversial areas of concern are the use of genetically engineered organisms (GMO) that may be introduced into the food chain. Many large companies are researching the development of soyabean and corn crops that are higher in protein, oil and also starch. It is also possible to genetically engineer the removal of certain anti nutritional factors such as the protease inhibitors, haemoglutinins and saponins and reduce the levels of indigestible oligosaccharides. These products could be 'tailored' for the fish feed market.

Although acceptable possibilities for the future, GM cereals and crops are not allowed to be used at present due to public resistance and consumer pressure.

However, the production of new by-products is a continuing source of interest and leads to a constant requirement for assessment in feed trials for fish. There is no doubt that for countries like Egypt, considerable scope exists for the improvement of animal and plant proteins, and only premium grade materials should be considered for high value fish and shrimp species.

Conclusion

In summary, the research programme described in this thesis has served to address the rationale of utilising a select choice of animal and plant derived ingredients for inclusion in diets for two important fish species commonly farmed in Egypt, namely tilapia and African catfish. The materials reflect highly processed products of good quality obtained in the UK, Europe and USA and may not be representative of the fish meals, poultry meat meals, feathermeals soyabean meals, corn gluten meals etc available in Egypt or indeed Africa. However they are nonetheless typical of what is imported in most countries and demonstrate the potential for replacement of fishmeal in practical diets. The economics of interchanging ingredients within least cost diet

formulations needs more attention and would allow costs to be assessed on a per unit of protein basis and live weight production on a per unit of protein intake. This is very important with respect to locally manufactured feeds in Egypt where costs must be reduced and feed conversion efficiency, growth rate (time to reach market weight) balanced against production levels and fish quality.

It is likely that eventually as in the West, consumer demand for quality fish with respect to taste, texture and appearance will determine the production methods and nature of the diet composition. Welfare of fish and hygiene will also be important factors in the future if fish are to be exported from Egypt to countries within the EU for instance where there is stricter legislation controlling the product and type of feed sources. Harvesting techniques including the slaughter methods of fish and legislation controlling feed additives and supplements may also be issues of importance.

The investigations described here have demonstrated the range of techniques and methods fundamental for the proper nutritional assessment of novel protein ingredients in complete diets for the fish species examined. There were compatible trends for both the tilapia and catfish in general, and the data presented should provide useful information for the basis of future work. Obviously, there are numerous ingredients that require comprehensive evaluation and this should be applied to fish within the full range of weight classes of the production cycle. The expansion and success of the aquafeed industry in Egypt will clearly be dependent on such research and development in the university, government and private sector at technical and advanced scientific levels.

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