USE OF SOYBEAN FLOUR-POULTRY MEAT MEAL BLENDS IN PRACTICAL DIETS OF *OREOCHROMIS NILOTICUS* AND *CLARIAS GARIEPINUS*

BY

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DEDICATION

To Humanity

DECLARATION

I do hereby declare that this thesis is a compilation of my original research work and that it has not been presented for any other qualification, anywhere. Information from the works of others (published and unpublished) and their contributions have been duly acknowledged.

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ABSTRACT

The present study evaluated the use of improved soybean flour (SF) through autoclaving and blending with a readily available low cost animal protein - poultry meat meal (PMM). Enrichment of such blends with dl-methionine, mineral supplementation and substitution of fishmeal with these blends in practical diets for *Oreochromis niloticus* and *Clarias gariepinus* were also investigated. Nutrient apparent digestibility coefficients (ADC) and apparent amino acid availability (AAAA) of diets based on 25:75, 50:50 and 75:25 SF-PMM were assessed. Lipid had the highest ADC while ash had the lowest. Best protein ADC and AAAA were obtained in the 0.5% Cr_2O_3 treatment which corresponded to the lowest level of faecal crude protein. Average AAAA agreed with the pattern of overall protein digestibility in the fingerlings of both species. Methionine was the most available while cystein was least.

Study on utilisation of SF-PMM Blends as dietary protein source in practical diets of *O.niloticus* and *C. gariepinus* showed that 50:50 SF:PMM was best utilised with lowest food conversion ratio (FCR), highest specific growth rate (SGR), and best protein efficiency ratio (PER). Autoclaving of raw SF before blending with PMM did improve nutrient utilisation of the blends as diets containing autoclaved SF did better than those containing raw SF. Substitution of fishmeal at 25%, 50% and 75% with these blends showed best utilisation of the 25:75 SF:PMM blend replacing 25% fishmeal in *O.niloticus* and 50:50 SF:PMM blend replacing 25% fishmeal in *C.gariepinus*. There was consistently better utilisation of dl-methionine supplemented diets in most cases. The essential

amino acid profile in the diet correlated positively with that of the carcass. ($P<0.05$). Reconstitution of mineral premix in 50:50 blend based diet did not affect nutrient utilisation ($p>0.05$). However, there was significant correlation of dietary and carcass phosphorus in both species and that of calcium only in *C. gariepinus* ($P<0.05$). In conclusion, there was enhanced utilisation of SF by both fish species with these treatments.

INTRODUCTION

CHAPTER ONE

INTRODUCTION

1.0 Introduction

Fish has long been valued as a source of animal protein for human nutrition. Consumption of fish generally cuts across ecological, socio-economic, cultural and religious boundaries, leading to its predominant role as an animal protein. Presently fish accounts for over 50% of total animal protein consumed in most countries of the world (FAO, 1991a). Fish is a first class animal protein and relatively the cheapest source (Osajuyigbe, 1981).

Present world per capita seafood consumption of fish stands at 19kg/year. The world fisheries harvest kept pace with growing population demand for seafood, but now we are approaching the limits of the wild fisheries. Landings from the world's capture fisheries are expected to plateau at 100 million mt by the year 2000, and after that the gap between supply and demand will have to be filled by aquaculture (Chamberlain, 1993).

Aquaculture has been conducted since pre-historic times and stood at 15.3 million mt in 1990 (De Silva and Anderson, 1995). Presently, it accounts for 20% of the worldwide fish production and is expected to reach 25% by the year 2000 (Ratafia, 1995). With its growth rate of over 5% annually, production from aquaculture by the year 2000 is projected at 20 million mt. This still leaves a negative balance

between consumption (demand) and supply of seafood (Chamberlain, 1993). Intensification of aquaculture to increase the production capacity of our aquacultural resources to match increasing seafood demand seems a solution. It entails increasing primary, intermediate and terminal productivity capacities of our natural aquatic ecosystem and creation of productive artificial aquatic ecosystems through proper planning, development and management.

A major determinant of successful intensification of aquaculture is fish feed. It accounts for a major part of the total operation cost of an average fish farm (Rumsey,1993) - of 30-70%. Fish species, unlike domesticated terrestial farm animals are ectotherms with no energy requirement for endothermy. Warm water fishes require 30-56% protein in their diets (Lim and Dominy, 1993). Traditionally, animal protein sources have been the major ingredients of fish feed, particularly fishmeal (Lim and Dominy,1991). Ironically, fishmeal is one of the most expensive ingredients in prepared fish diets. In addition, fishmeal supply is likely to be declining by 5% annually between 1900 and 2000, and this can no longer meet the demand from the expanding fish feed industry (Lim and Dominy, 1993; Rumsey, 1993). Therefore, there is the need to check overdependence on fishmeal through its reduction in fish feeds and finding suitable replacements. Replacement with available and cheaper vegetable proteins has been the goal of

aquaculture (Wee and Wang, 1987; Lim and Dominy, 1989; Ng and Wee, 1989).

Plant proteins such as soybean meal, peanut meal, cottonseed meal, sunflower seed meal, rapeseed meal and *Leucaena* leaf meal have been identified as possible alternatives to fishmeal. However, high plant protein levels in fish diets have resulted in retarded fish growth and poorer feed efficiency compared to fishmeal containing diets (Lim and Dominy, 1991). This has made it seemingly impossible to replace animal protein totally without compromising the performance of the fish. Therefore, an economically and nutritionally viable fish diet will require a good combination of both plant and animal protein.

Of the plant proteins, soybean meal is the most promising. Presently, it is the major protein source in catfish diet in the United States. It has been postulated that an economic improvement in soybean meal based diets for catfish could be achieved by the incorporation of lower cost animal protein than fishmeal (Mohsen and Lovell, 1990). Whether there would be a nutritional improvement in such an approach needs to be studied. This study is therefore intended to investigate plant-animal protein blend nutritional improvement and its utilisation in the diets of *Oreochromis niloticus* and *Clarias gariepinus,* detail of which is provided in section 1.8.

1.1 Aquaculture Production

On the basis of common usage with practical distinctions between hunting and gathering on one hand and agriculture on the other, aquaculture has been defined thus: "Aquaculture is the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated. For statistical purposes, aquatic organisms which are harvested by an individual or corporate body which has owned them throughout their rearing period contribute to aquaculture, while aquatic organisms which are exploitable by the public as a common property resource, with or without appropriate licences, are the harvest of fisheries" (FAO,1994).

Aquacultural practice antedates recorded history as it was in place as far back as 2798 B.C. in China with the culture of milkfish. It was not until 475 B.C. that the first treatise on aquaculture written by Fan Lai appeared (Chackroff,1980) and since then much attention has been given to aquaculture. It has fastly become a high-return enterprise over the last two decades, spreading across countries with suitable land and water resources. It has an annual growth rate of 8.7% and this compares favourably with other animal and plant food

production increments of 1.7-3.3% and 1.7-3.8% respectively (Akiyama, 1991). By continent, current world aquaculture production is Asia, 85%; Europe, 7-10%; Africa, <1%; South America, <1% and Oceania, <1%. Over 100 countries from these regions are engaged in active aquaculture and China is presently the world leading producer of aquaculture commodities producing 7.2 million mt annually. By species, carps topped the list with 5 million mt production. Tilapias and catfishes accounted for 391,000 mt and 165,000 mt respectively (FAO, 1992). The future challenges facing the aquaculture industry are the development of environmentally sustainable management practices, free of disease, pollution and global warming hazards (Chamberlain,1993).

1.2 Aquaculture in Africa

Aquaculture in Africa is a relatively new industry, presently of minor importance but with the prospect of becoming a major one in the future (FAO, 1991b). Aquaculture in Africa has an ancient history dated as far back as 2500 BC in Egypt (Maar et al., 1966). Pond fish culture in sub-Saharan Africa first started in Kenya in 1924 and later spread to other parts of the continent (Huisman, 1986; Jackson, 1988). Fish contributes about half of the animal protein intake in Africa (FAO,1991b). Capture fisheries has been the major source of domestic fish production. However, slow growth of African capture fisheries has

been projected. This, coupled with the heightened interest of both African government and donor agencies, has led to anticipation that priority will be accorded to aquaculture in order to meet the ever increasing fish protein demand in the continent (FAO, 1991b).

Aquaculture accounts for less than 10% of the total domestic fish production in Africa (Satia, 1989) and only 0.1% of the total world aquaculture production (FAO, 1991b). Annual aquaculture production in sub-Saharan Africa in 1990 was estimated at 12,000 mt with Nigeria, Zambia and Zimbabwe as the major producers (FAO, 1991b). Until recently, African aquaculture practice has been at a subsistence level to meet animal protein demand at individual and family levels and has been dominated by small scale subsistence farming of tilapia species (FAO, 1991b). The active participation of African government and donor agencies, such as FAO, has brought the benefits of modern aquaculture and aquacultural technology to expand the scope of African aquaculture. Additional cultivatable species now include clariid catfishes and exotic carps (Balarin and Hatton, 1979; Vanden Boscche & Bernacsek, 1990) enhanced with acquisition of artificial breeding know-how. Large scale aquaculture is now in practice in Nigeria, Kenya, Zambia, Zimbabwe and Cote d'Ivoire (Huisman, 1986; Kutty, 1986)

1.3 Aquaculture in Nigeria

Fish farming has been practised at a subsistence level in Nigeria for generations using flood plains and tidal ponds. Presently, Nigerian aquaculture production stands at 16,588 mt. However, the history of modern aquaculture is a recent one. In 1951, the government of Northern Nigeria started the construction of pilot fish farm at Panyam. At about the same time, the governments of Western and Eastern Nigeria encouraged the construction of homestead fish ponds. FAO responded to federal government requests to initiate the development of brackish water fish culture in the Niger Delta area in 1965 and another project in Lagos in 1968. Cage culture was also initiated by FAO in Kainji Lake due to a sharp decline in the commercial catches from the lake from 28,638 mt in 1971 to 10,905 mt in 1973 (Ita, 1975).

Initially, seeds and fingerlings were obtained from the wild. There was an abundance of seeds of tilapias , *Mugil* spp. *Liza* spp, and *Hemichromis* spp. from the wild. About 2.5 million mullet fry per annum were obtained from Lagos area and an adequate number to stock 10,000 ha pond yr^{-1} in the Niger Delta. Artificial breeding of carp was introduced in 1954 at Panyam fish farm (Ezenwa, 1975) and its scope is widening with time. Since then, there has been a steady growth in

the number of fish ponds and fish farms all over the federation (Dada, 1975; Sagua, 1976). From a humble beginning with a 20 ha fish farm at Panyam near Jos in 1954, producing less than 800kg.ha^{-1} yr^{-1}, there were over 3000 homestead sandcrete ponds, 2,000 small earthen ponds and 60 commercial farms (>3ha) in 1990 (Satia, 1990) producing 7,735 mt (FAO, 1992).

Freshwater species cultured were the tilapias; *Oreochromis niloticus, Tilapia melanopleura* and *Sarotherodon galilaeus*; carps; *Cyprinus carpio* (European carp), *Labeo* sp. (African carp); *Heterotis niloticus* (bony tongue); *Gymnarchus niloticus* (trunk fish); *Lates niloticus* (Nile perch) and Clariid fishes; *Clarias* sp. and *Heterobranchus* species. Brackish water species cultured were: finfishes; *Chrysichthys nigrodigitatus* (silver catfish); *Gymnarchus* sp.; *Hemichromis fasciatus* (jewel fish); *Lutjanus* sp. (snappers); *Elops* sp.; *Ethmalosa fimbriata* (bonga fish); *Heterotis* sp; *Mugil* sp (mullets); and shellfishes; *Penaeus duararum* (shrimp); and *Macrobrachium* sp (prawn) (Dada, 1975) and recently *Ophiocephalus* sp (channa, snakeheads) (FAO, 1991c). *O. niloticus* and *C. gariepinus* dominate aquaculture production in Nigeria, contributing about 49.06% and 19.82% respectively of fish production in 1990 (FAO, 1992).

Nigeria has a population of 88.5 million people and a per capita fish consumption of 5kg yr^{-1} well below the official "optimum" of 11.5 kg

individual^{-1} yr^{-1} in 1992 (Satia, 1990). Additional 90,000 and 340,000 mt of fish per year respectively was required in excess of the official capture fishery MSY (estimated at 971,000 mt) to maintain these levels with the balance expected from aquaculture (Satia, 1990). Aquaculture development,just as in any other agricultural subsector, reflects global and national economies. Still in its infancy, it has gone through periods of boom and bust.

Aquaculture in Nigeria has recorded two growth patterns of steady increase from its inception in 1951 to 1980 and fluctuation between boom and bust from then until the present. A "bust" between 1980-83 was due to massive importation of fish that almost crippled domestic fish production in the government's bid to supply enough protein to its entire citizenry and correct the per capita fish consumption deficit. After 1983, the trend maintained a plateau phase. A total of 900,000 mt of fish was imported in 1980 (Ita,1986). The global oil glut of 1983 discouraged importation of fish and encouraged domestic fish production. This attracted both the government and donor agencies to aquaculture. Programmes such as the "Inshore Fishing Project" (I.F.P.) to provide pond construction equipment at subsidised rate to fish farmers, the Fish Seed Multiplication Scheme (F.S.M.S.) - aimed at providing fish seeds and fingerlings to farmers through hatchery development and the Homestead Fish Farming Programme (H.F.F.P.) to promote "backyard" fish farming at the household level (Adesimi

and Aderinola, 1983) were floated and these culminated into revitalised aquaculture in 1984. There has been steady production of 5-10 thousand mt since then except for 1989 when an arguably sharp production figure of 25.5 thousand mt was recorded (FAO,1992).

The aquaculture potential of the country has been grossly underutilised, with only 1,000 ha used out of an available 443,406 ha of water area suitable for aquaculture (Ita et al., 1985). With the current effort to revitalise the economy at global and national levels, improved active participation of the government and donor agencies, increasing interest of fish farmers, there is every likelihood of a significantly improved production before the end of the century.

1.4 Aquaculture Feeds and Feedstuffs

The increase in aquaculture production has been made possible by the intensification of aquaculture that requires feed application either as total or supplementary. There exists controversey over present aquafeed production levels. Aquaculture feed production increased from 1.9 to 4.0 million mt from 1980 to 1988. This has been projected to hit 6.6 million mt by the year 2000. Asia produced 2.6 million mt with the Peoples Republic of China, Republic of China and Japan topping the list in descending order (Akiyama,1992). New et al. (1993) found the 1988 production level an overestimation and assumed less than 3

million mt figure for the same year. The consensus however is that of expanding aquaculture feed production to match aquaculture production.

The major feedstuff in aquafeed production is fishmeal, particularly in marine shrimp, salmon, marine fish, trout and eel industries. Fishmeal usage is likely to increase by 50% from about 0.8 million mt in 1990 to 1.2 million mt by the year 2000, and fish oil production to 363,000 mt (an increase of 77% from 1990) (Chamberlain, 1993). Fishmeal is most preferred for its excellent nutritional quality and palatability (Lim and Dominy,1993).

1.5 Fishmeal in Aquaculture

The major fishmeal producing countries are Peru, Chile, Norway, U.S.A, Japan, Iceland, Denmark and South Africa. Fismeal can be produced from whole fish or fish processing wastes. The major forms of fishmeal available are the flame dried and steam dried menhaden fishmeal from U.S.A., mostly steam dried herring fishmeal from Canada, flame dried and steam dried anchovy fishmeal from Peru and Chile, pilchard fishmeal from South Africa, low temperature dried herring and capelin fishmeal from Norway and Iceland, and sardine fishmeal from Japan (Hardy and Masumoto, 1991).

Fishmeal is presently the major feedstuff in the aquaculture feed and provides the major portion of protein in commercial diets for aquatic animals (Lim and Dominy, 1991). Presently, aquaculture consumes only 10% of the total fishmeal production which amounts to 6 million mt/yr (Barlow, 1989; Rumsey, 1993). 60% of fishmeal production goes to the poultry industry mostly in China (Hardy, 1991). Fishmeal production has increased moderately over 20 years (by 27%) and is now expected to decline by 5% annually (FAO, 1991; IAFMM, 1991; New, 1991). Ironically, the aquaculture industry has grown rapidly in the last decade (Lovell, 1991). This has led to high demands for fishmeal but the concommitant decline in fishmeal production poses an imminent danger to the industry. Fishmeal consumption for aquaculture feeds will double by the year 2000 while production is expected to remain constant (Barlow, 1989; Rumsey, 1993).

Fishmeal demand is highly elastic. This has been estimated at 0.7% for fish meal in poultry feed production (Crowder, 1990). In otherwords, a 10% price increase would lead to 7% decline in demand. Fishmeal has a more variable demand than any other animal or plant protein, i.e its demand is more price sensitive than others (Starkey, 1990). Concerns over future fishmeal cost and availability for aquaculture feeds have exerted tremendous pressure to use fishmeal as efficiently as possible and to try and identify suitable alternatives.

Making efficient use of fishmeal involves its characterisation in terms of freshness, lipid oxidation, protein digestibility, effects of cooking and hydrolysis, and meeting fish meal quality requirements for various aquaculture species (Chamberlain, 1993). An enormous range and number of alternatives to fishmeal have been investigated in a wide variety of aquaculture feeds.

1.6 Soybean Products in Aquaculture

Soybean is sometimes known as "the Miracle Crop" internationally, the "Cow Crop" of China, the "Cinderella Crop" of the West or the "Pearls of the Orient" and originated from China 2000 years before Christ. Soybean is the world's most valuable and widely grown oil seed legume (Osho, 1991). Total soybean production was 11.8 million mt in 1985 with major producing nations being the United States, 56% ; Brazil, 18% ; Peoples' Republic of China, 10% ; and Argentina, 6% (Vohra and Kratzer, 1991). In contrast to dwindling fish meal production, soybean production has been on the increase for the past 20 years with a 176% gain and is expected to increase by another 40% by the year 2000 (Alexandratos, 1988). Some of the wide range of commercially available soybean meals are full-fat and toasted soybean meal, hexane extracted and extruded soybean meal, alcohol extracted soybean meal, full-fat and puffed soybean meal and hexane extracted soybean meal (Tacon, et al.,1983).

Soybean is the most widely grown oilseed with about 50% of world oilseed production (FAS, 1991).

Despite the possible economic advantages of plant protein utilisation in aquaculture feeds, high inclusion levels have generally resulted in growth retardation and poorer feed efficiency than with fishmeal controls. These results have been attributed to improper balance of essential amino acids and minerals, presence of toxic substances or antinutritional factors, reduced water stability and palatability (Lim and Dominy, 1991). These inherent defects may be remedied to some extent by processing of soybean to remove toxic substances and antinutritional factors, and addition of deficient nutrients through supplementation of single or combined nutrients or incorporation of low cost animal proteins that are richer in such nutrients. Such products include poultry by-product meal, meat and bone meal (Hardy and Masumoto, 1991).

Soybean production and utilisation research have been going on in Nigeria since the early seventies at the International Institute of Tropical Agriculture, Ibadan - Nigeria. In the mid seventies, breeders were able to overcome the barrier that frustrated earlier attempts to adapt the American bred variety to the tropics. Roots of the US variety were later found not to be congenial to the nitrogen fixing bacteria of Africa. A high yielding, better nodulating variety was later

developed. This new variety is capable of yielding 2.5 tons per hectare (IITA, 1990a).

The International Development Research Centre funded a research project on soybean utilisation in 1985, carried out by IITA and Institute of Agricultural Research and Training (I.A.R. & T) - Ibadan. Several products and end uses of soybean in human nutrition were developed, viz: raw soy flour, cooked soy flour, soy milk, soy vegetable soup, soy "ogi"(cooked plain paste or milk residue of dehulled soybean flour), soy "eba"(cooked mixture of soy "gari" and soy paste or milk residue), soy "moinmoin"(spiced and steam-cooked mixture of maize, wheat or cassava flour and soy paste or milk residue from dehulled soybean - cooking oil added), soy "akara" (cake), soy "iru" (spice from fermented soy) and soy "gari" (blend of soybean and cassava fibrous flour) (IITA, 1990a, 1990b, 1990c; Osho, 1991).

In Nigeria, there is great enthusiasm for soybean production and utilisation of soybean in human nutrition. Using soybean products as fish feedstuffs in the growing Nigerian aquaculture industry may help to convert soybean protein into much needed fish protein. With adequate planning soybean in aquaculture nutrition in Nigeria could complement its popular utilisation in human nutrition.

1.7 Poultry Products and By-products in Aquaculture

In Nigeria, there is dwindling supply of poultry products and by-products as a result of a declining poultry industry. Feed supply to the poultry industry is dependent on fishmeal as this industry consumes 60% of the total world fishmeal production (Hardy, 1991). Shortages of fishmeal, and its rising cost, has adversely affected the poultry industry. Invariably, the poultry industry is facing the same problem, but much worse as the aquaculture industry.

Finding suitable alternatives to fishmeal may also boost poultry production. This may eventually make available poultry products and by-products which could serve as cheap sources of animal protein in aquaculture feeds, as their demand is less elastic than that of fishmeal (Starkey, 1990). Such products could then be incorporated into fish feed and where combined with soybean, it would provide deficient nutrients such as essential amino acids - methionine, and minerals - phosphorus.

The poultry industry in Nigeria shares the same features as that in the rest of the world. Poultry production is now in its bust cycle and is expected to boom once the problem of feed supply is resolved. This will make available cheaper animal protein sources to be utilised in the aquaculture industry.

1.8 Objectives

From the foregoing the following points are evident: Fish meal production and supply are declining and it is becoming unaffordable; sourcing alternatives is a current research need; promising alternative protein sources are low cost animal and plant protein; soybean is the most promising vegetable protein and its production is ever increasing. Soybean, despite its high nutritional value lacks essential nutrients and has anti-nutritional factors.

Generally, the objectives of this study are to investigate nutritional improvement of soybean flour through blending with poultry meat meal, utilisation of such products and their suitability to replace fish meal in practical diets of *O. niloticus* and *C. gariepinus*. These are achievable through;

1. Enriching soybean flour (SF) by blending with poultry meat meal (PMM) in different ratios, to naturally raise deficient nutrient levels that are low in SF but high in PMM and evaluate the digestibility, and utilisation of these blends in practical diets of *Oreochromis niloticus* and *Clarias gariepinus*, to establish the best blending ratio.

2. Establish suitable replacement levels of fish meal with these SF-PMM blends in practical diets of *O. niloticus* and *C. gariepinus.*

3. Enrich SF-PMM blend based diets with dl-methionine as a way of ameliorating its methionine deficiency, and evaluating its utilisation by *O.niloticus* and *C. gariepinus.*

4. Reconstitute supplemented mineral premix, to evaluate the effect of mineral supplementation on the utilisation of these blends in practical diets of *O.niloticus* and *C. gariepinus*

5. Detoxifying SF through heat processing to remove heat labile anti-nutritional factors and assess the effect of this on its utilisation and as blends with PMM in diets of *O. niloticus* and *C. gariepinus.*

6. Predict the environmental impact assessment of such diets using nutritional models.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

Fishmeal provides much of the protein feedstuff demand of the animal feed industry. Much of the nutritionists attention was therefore originally focused on fishmeal development. The dwindling fisheries resources to provide enough feedstuff for the expanding feed industry prompted a search for alternatives to fishmeal. Current fishmeal research needs are therefore development of quality fishmeal, replacement of fishmeal with vegetable proteins and the effects of antinutritional factors on plant proteins utilisation.

Presently, quite enormous research has been conducted on plant proteins in aquaculture feeds. This ranges from the search for suitable plant proteins in the feed for aquaculture species, to their development and utilisation. Several plant proteins have been characterised and documented (See NRC, 1993). Current plant protein research focuses on nutritional improvement of these plant proteins and their utilisation. The American Soybean Association has been engaged in soybean improvement and utilisation research, the findings of which have been well documented in their biennal conference proceedings.

Development research into soybean either through "self" improvement by further processing and "cross" improvement by simple nutrient (pure

nutrient) or compound nutrient (nutrients in other feedstuffs - complementary to soybean) supplementation is now widespread. The terms self, cross, simple and compound used here are for convenience and clarity. Information on the nutritive value of soybean, improvement of soybean by supplementation and blending, autoclaving to remove anti-nutritional factors and their utilisation in the diets of tilapia and catfish either as sole protein source or as partial replacer of fishmeal follows thus:

2.1 Soybean Products and Other Vegetable Proteins in Aquaculture Feeds

Commercially formulated fish feeds contain 20-45% crude protein and protein sources make up to 60% or more of the ingredients (Lovell, 1990) and even up to 50% crude protein - particularly diets for salmonids. However, the maintenance energy requirements of ectothermic fish are lower than those of endothermic land animals and fish use fat more efficiently as an energy source than carbohydrate (Lovell, 1990).

Historically fishmeal has provided the major portion of the protein in fish feeds. Fishmeal production is presently predicted to decline by 5% between 1990 and the year 2000. Fishmeal supply stagnating while the aquaculture feed industry grows (Rumsey, 1993). There is imminent

danger of shortage of supply of feedstuffs to the aquaculture feed industry. Reduction of overdependence on fishmeal, as is being gradually done in the poultry industry, is necessary to avert this danger. Alternatives being investigated include animal by-product meals (Lovell, 1990) and cheaper plant proteins (Lim and Dominy, 1991).

2.1.1 Nutritive Value of Soybean Meal

Of all vegetable proteins, soybean is the most widely grown. It constitutes 50% of total oil seed production (FAS, 1991). It is equally the most widely used in aquaculture feeds where it forms 30 - 40% of feed for warmwater fishes. In addition, Soybean has better nutritive value than other plant proteins in terms of essential amino acid balance (EAA) (Lim and Dominy, 1991) and is richer in polyunsaturated fatty acids as well (Snyder and Kwon, 1987; Lovell, 1989). Tables 2.1 and 2.2 present the EAA and trigyceride profiles of common plant proteins.

Table 2.1 Essential amino acid composition of some plant seed meals.[1]

	Roasted Full-fat Soybean	Dehulled solv. extd. soybean	Peanut meal	Cottonseed meal	Sunflower seed meal	Rapeseed meal
International Feed Number	5-04-597	5-04-612	5-03-650	5-01-621	5-03-871	5-04-739
Essential amino acids (% Protein)						
Arginine	7.4	7.4	9.5	10.2	9.6	5.6
Histidine	2.7	2.5	2.0	2.7	2.7	2.7
Isoleucine	5.7	5.0	3.7	3.7	4.9	3.7
Leucine	6.8	7.5	5.6	5.7	8.3	6.8
Lysine	6.3	6.4	3.7	4.1	4.2	5.4
Methionine	1.4	1.4	0.9	1.4	2.5	1.9
Cystein	2.8	2.9	2.4	3.3	4.1	2.7
Phenylalanine	5.5	4.9	4.2	5.9	5.1	3.8
Tyrosine	8.7	8.3	7.4	7.9	8.1	6.0
Threonine	4.4	3.9	2.4	3.4	4.2	4.2
Tryptophan	1.4	1.4	1.0	1.4	1.3	1.2
Valine	5.3	5.1	3.9	4.6	5.6	4.8

[1]NRC (1982, 1983, 1993).

Table 2.2 Percentage fatty acid composition of the triglyceride fraction of some plant oils.[1]

	Soybean	Peanut meal	Cottonseed meal	Sunflower seed meal	Rapeseed meal
Saturates	14.0	14.5	30.0	17.0	4.5
Monosaturates	23.2	53.0	18.5	29.0	55.5
Polyunsaturates	62.8	27.5	51.5	52.0	39.5
Linoleic	54.5	27.5	51.5	52.0	29.5
Linolenic	8.3	-	-	-	10.0

[1] Pryde (1983); Snyder and Kwon (1987); Lovell (1989)

i Protein and Amino Acids

Full fat soybean meal is 38 - 42.2% protein (NRC, 1993), and dehulling and defatting could enrich this to 49% (Lovell, 1990; NRC, 1982). Soybean protein is 85% digestible in channel catfish (Lovell, 1977), rainbow trout (Smith, 1976) and tilapia (Popma, 1982) and is high in all EAA except methionine + cystine- a better EAA profile than other vegetable proteins such as peanut and cottonseed that are lower in all EAA except arginine. Soybean meal has also better essential amino acid balance in terms of meeting the requirements of some fishes than some fishmeal - like menhaden fishmeal that has lower phenylalanine + tyrosine, tryptophan and histidine than soybean meal (Lovell, 1990).

ii Lipid, Carbohydrate and Energy

Soybean (full-fat) contains 18-20% lipid (NRC,1993) comprising a mixture of n-3 and n-6 fatty acids whilst fish meal has predominantly n-3 fatty acids. Soybean oil contains 8% n-3 (18:3) and 55% (18:2) fatty acids (Castell, 1978; Snyder and Kwon, 1987). *Tilapia zilii* has been found to prefer 1% 18:2n-6 or 20:4n-6 fatty acids or their combination (Kanazawa et al, 1980; Cho et al, 1985) and has no requirements for n-3 fatty acids. Soybean oil is thus a good lipid source in tilapia diets. Channel catfish prefers menhaden fish oil, beef tallow oil and olive oil

to linseed oil (more n-3 than n-6) and sunflower oil (more n-6 than n-3) (Cho, 1985).

However, there is limited use of triglycerides in tilapia diets. Tilapia requires more digestible carbohydrate energy than lipid energy and thus less of triglycerides than digestible carbohydrate in its diet (Lovell, 1991; Jauncey et al, 1993). Whole soybean has less digestible carbohydrate than indigestible carbohydrate and therefore not a good carbohydrate energy source for warmwater fish such as tilapia. Catfish digests carbohydrate well (Lovell, 1990). Soybean is a source of good quality protein. It is therefore a protein energy source for both fish.

Table 2.3 Nutrient composition of soybean products.[1]

	Seeds	Seeds, heat process (FFSMB)	Meal, mech. extd.(soybean cake)	Meal,solv.extd with hulls	Meal,solv.extd without hulls
Int. feed No.	5-04-610	4-04-597	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

[1]NRC(1983; 1993)

iii Micro - nutrients (Minerals and Vitamins)

Phosphorus is the first limiting mineral in soybean meal diets for fishes due to poor availability. Of 0.6% present, only 0.2% is available to fish while most fish require 0.5% (Lovell, 1978). Certain trace minerals are equally unavailable especially zinc. They are chelated by phytic acid as detailed in 2.2.2. Consequently, trace mineral supplementation is recommended in diets with less than 15% fishmeal or terrestial animal by-products (Lovell, 1990). Other minerals are not so critical as they are available and some of their requirements by fish are met from water, viz; iron, magnesium, cobalt, potassium and sodium (NRC, 1981, 1983).

Soybean meal is a poor source of most of the 15 vitamins so far identified. It is a good source of choline, tocopherol (vitamin E) and menadione (vitamin K) (Tacon, 1991). Fishes require most, if not all, of the 15 vitamins (Lovell, 1990) and therefore soybean meal based diets need to be supplemented. However, evidence exists that tilapia may not require vitamin premixes in their diets.

Table 2.4 Minerals and vitamins contents of soybean meal (full fat)

Minerals. [1]		Vitamins (Water soluble vitamin content of mature soybean mg100g^{-1}. [2]	
Calcium (Macrominerals - %)	0.25	Ascorbic acid	20
Magnesium "	0.21	Biotin	0.06
Phosphorus "	0.59	Choline	340
Potassium "	1.70	Folic acid	0.23
Sodium "	0.03	Inositol	190-260
Sulphur "	0.22	Niacin	2.0-2.6
Copper (Microminerals-mgkg^{-1})	16.0	Panthothenic acid	1.2
Iron "	80.0	pyroxidine	0.64
Manganese "	30.0	Riboflavin	0.23
Selenium "	0.11	Thiamin	1.1-1.7
Zinc "	54.0		

[1] NRC (1982, 1983, 1993) ; [2] Snyder and Kwon (1987)

2.1.2 Utilisation of Soybean Meal in Aquaculture Feeds

Considerable research has been conducted on the use of soybean in fish diets. Such studies range from its use in its raw form, through processed forms (dehulling, toasting and defatting) to supplemented forms (synthetic methionine, cystine and lysine supplementation). Soybean has been used as a sole protein supplement or to partially replace animal protein, particularly fishmeal, with more success than failure (Lovell, 1990; Lim and Dominy, 1991).

Viola and Arieli (1983) observed that up to 50% of fishmeal could successfully be replaced in a 25% crude protein tilapia feed. Similarly, fishmeal in tilapia (*O. niloticus* x *O. aureus*) diets could be partially replaced with soybean meal at suboptimal protein level (24% crude protein) without compromising performance but substitution at the optimal protein level (32% crude protein) depressed growth and feed efficiency (Shiau et al, 1987). Reduced feed efficiency and growth response were recorded with 50% replacement of fish meal with soybean meal in diets of *Oreochromis mossambicus* (Jackson et al, 1982). This was attributed to low level of sulphur amino acids and the presence of anti-nutritional factors.

Lovell (1974) reported growth depression and lower feed efficiency in channel catfish fed soybean meal compared to those fed diets containing fish meal. Similarly Andrew and Page (1974) recorded reduced feed efficiency and growth when fish meal based diet was isonitrogenously replaced with soybean. The reverse order of substitution in another study gave increased growth and feed efficiency as fish meal in the diet increased to 20% (Mohsen, 1988). This was also attributed to low sulphur containing amino acids in soybean (NRC,1977).

From the foregoing, low levels of sulphur amino acids and oil, and the presence of anti-nutritional factors were identified as factors militating against optimum utilisation of soybean meal. These problems have been confirmed in further investigations. In most cases, supplementing sulphur containing amino acids to diets containing soybean meal improved performance (Lim and Dominy, 1989).

Supplementing soybean meal with either coated or uncoated methionine significantly improved growth and feed efficiency of common carp and channel catfish with coated methionine doing better only in carp (Murai et al, 1982). However, earlier investigations showed no difference in the performance of channel catfish fed diets containing soybean meal supplemented with crystalline methionine, cystine and lysine (Andrew and Page, 1974). This discrepancy may be attributed to the higher methionine and cystine levels in the basal diets in the later investigation. Growth depression, caused by methionine deficiency, in tilapia was alleviated when diets for *O. niloticus,* in which 75% of the fishmeal was replaced with soybean meal, were supplemented with 0.8% dl-methionine (Tacon et al, 1983).

Heat treatment has been identified as an effective method of removing most antinutritional factors (ANF) in soybean. Haemagglutinins, trypsin inhibitors, phytate, goitrogens and anti-vitamins D, E and B_{12} have been identified as heat labile ANF (Tacon

et al, 1982; Lovell, 1990). However, heat treatment reduces available lysine levels due to the Maillard reaction (Maynard et al 1979; Bondi,1987). Commercial soybean meals currently in use in aquaculture feeds are heat treated (177°C at 15 lb per inch^2 for ten minutes or 105°C at 15 lb per inch^2 for 20 minutes) (Lovell, 1990). Such heat treatment has been observed to improve soybean meal utilisation in aquaculture feeds.

Utilisation of heat treated soybean has been studied and found successful. Poorly heated hexane extracted soybean flour depressed growth in channel catfish fed 25 and 35% crude protein diets with graded levels of trypsin inhibitor (TI) activities, brought about by heating for different durations (Wilson and Poe , 1985). No utilisation differentia were recorded between the control and the test diets of channel catfish when 64% commercial soybean meal and 10% fish meal in the control were replaced with 50% and 100% heat treated soybean meal (Saad, 1979).

2.2 Antinutritional Factors in Soybean Products and Vegetable Products.

Anti-nutritrional factors identified in soybean and other plant products of agricultural importance are: i, Protein ANFs; protease inhibitors (trypsin inhibitor) and haemagglutonins; ii, Glycosides; goitrogens,

cyanogens, saponins and estrogens iii, Phenols; gossypols and tannins iv, Miscellaneous; anti-minerals, anti-vitamins, anti-enzymes, food allergens, microbial/plant carcinogens and toxic amino acids (Liener et al, 1981; Tacon, 1985). These have been further classified into heat and non-heat labile ANFs. Heat labile ANFs are Haemagglutonins, phytate, trypsin inhibitor, goitrogens and anti-vitamins while the non heat labile ones are saponins, estrogens, lathyrus and flatulence factors (Tacon, 1982).

2.2.1 Trypsin Inhibitor

The unparalleled nutritive value of soybean amongst the plant proteins has been bedeviled by the presence of heat labile anti-nutritional factors in raw or inadequately heated soybean. Poor growth and pancreatic hypertrophy are physiological responses of animals fed unheated soybean (Kakade et al, 1973). Trypsin inhibitor constitutes 6% of the total protein. There exists controversy over the extent of the contribution made by trypsin inhibitor to the deleterious effects of raw soybean. Rackis (1972) observed that only 30-50% of the growth inhibition effect and almost all of the pancreatic hypertrophy were due to trypsin inhibitor in rats. Earlier, Gertler et al (1967) observed only a minor role of trypsin inhibitor in the growth inhibition. Similarly, Saxena et al (1963) recorded pancreatic hypertrophy with diets containing a soybean fraction devoid of

antitryptic activity. Kakade et al (1973) resolved this by their observation that selective removal of trypsin inhibitor did not only improve protein efficiency ratio but also reduced pancreas size.

High trypsin inhibitor activity in inadequately heat treated soybean meal reduced protein and energy digestibility of soybean meal in rainbow trout (Sandholm et al., 1979; Smith et al., 1980) growth rate in carp (Viola et al., 1983) and channel catfish (Robinson et al., 1981). No pancreatic hypertrophy was observed in the channel catfish fingerlings fed for 10 weeks. The effect of the inhibitor on trypsin is enzymatic. As in Fig 2.1, the inhibitor mimics trypsin and gets attached to valuable substrate like peptides according to the lock and key hypothesis of enzyme activity (Roberts, 1980), thereby increasing non-digestion of protein. It also stimulates pancreatic oversecretion of enzymes containing cystine and consequently triggers methionine deficiency, all symptomised as growth retardation (Vohra and Kratzer, 1991).

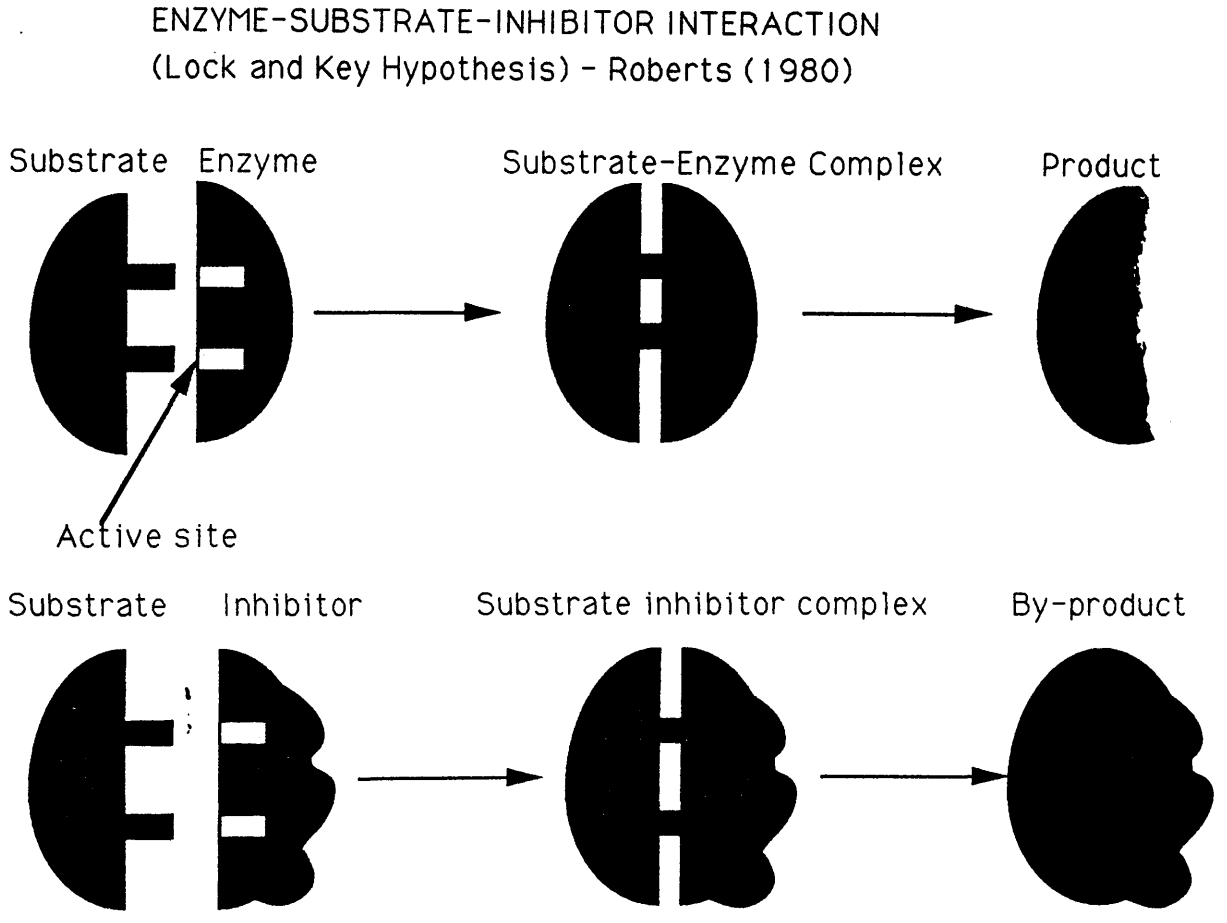


Fig. 2.2.1: Enzyme - Substrate - Inhibitor Interaction

2.2.2 Phytic Acid

Differences in zinc availability between animal and plant protein have been attributed to the phytate content of the latter as addition of phytic acid to animal protein decreased zinc availability in animals (O'Dell and Savage, 1960; Oberleas et al., 1962). Phytic acid forms complexes with raw soybean protein which chelates zinc and other essential minerals to cause mineral deficiencies (Rackis, 1965).

This reduces zinc bioavailability. Zinc bioavailability could be improved upon by zinc supplementation to keep the phytate : zinc ratio at less than 10 (Lo et al., 1981). Animals fed raw soybean diets adequately supplemented with minerals and vitamins will exhibit growth, protein efficiency or pancreatic hypertrophy unaffected by physiological effects associated with perosis, rachitogenicity and mineral deficiencies (Rackis, 1965).

Zinc requirement of channel catfish has been found to be 20 $mgkg^{-1}$ using purified diets (Gatlin and Wilson, 1983) or 88$mgkg^{-1}$ diet in practical diets (Lovell, 1977) or even higher at 150 $mgkg^{-1}$ (Gatlin and Wilson, 1984). These discrepancies are due to the fact that practical diets usually contain feedstuffs high in phytic acid (Nelson, 1968).

Supplementation of rainbow trout soybean meal based diets with zinc was considered necessary (Wekell et al., 1983), as soybean meal has apparent binding potential with zinc (Spinelli et al., 1982). However, Spinelli (1983) observed that reduced growth in rainbow trout fed diets containing phytic acid was due to reduction in protein availability rather than alteration in the bioavailability of zinc, iron or copper. This observation was probably due to a favourable phytate : mineral ratio. The phytate is a product of mineral salts - phytic acid reaction, when the mineral component is bonded and rendered unavailable (Chelation). The phytic acid is inositol hexaphosphate with the chemical structure below.

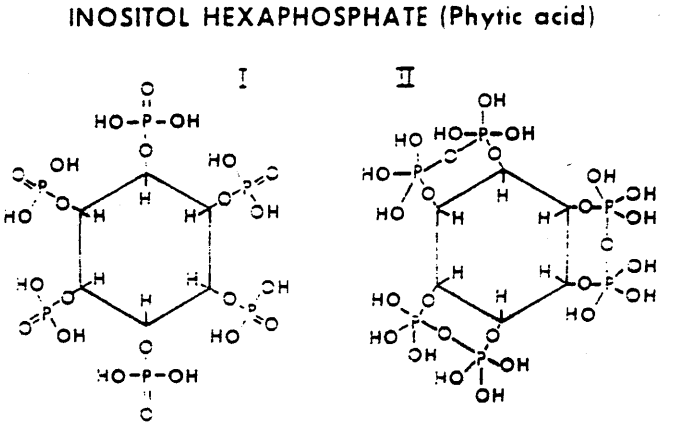


Fig. 2.2.2: Structure of Phytic Acid

2.2.3 Soybean Lectin (Haemagglutinin)

Soybean lectin has been studied using purified lectin. It neither caused rapid weight loss nor death when fed at high dietary levels but slow growth rate. Unlike kidney bean lectin, haemagglutinin does not induce acute toxic responses and as such body reserves of lipid, glycogen or protein were not depleted (Grant, 1989). However, it binds with the epithelium of the small intestine where it induces an antibody response . It is absorbed into circulation at a much slower rate than kidney bean lectin (van Kempen, 1993). Haemagglutinin has no effect on stomach bearing fish as it is inactivated by pepsin (NRC, 1983).

2.2.4 Miscellaneous Anti-nutritional Factors in Soybean

Other physiological effects caused by raw soybean include thyroid enlargement (goitrogenicity), reduction in fat absorption and metabolisable energy, decrease in transamidase activity, increased oxygen consumption and lower glycogen content (Rackis, 1965). A number of thermostable ANF like estrogen and saponin do not pose danger to animals. Estrogen will only inhibit growth when fed at relatively high levels. Saponins do not exert deleterious effects on animals in the presence of dietary proteins as the enzyme-inhibiting property is abolished (Rackis, 1965).

2.3 Poultry Products and By-products in Aquaculture Feeds

Certain nutrient deficiencies and antinutritional factors presence in plant proteins have seemingly made animal protein utilisation in aquaculture feeds indispensable, hence the need to search for lower cost animal proteins that are readily available. Apart from fishmeal, other animal proteins in use in aquaculture feeds are animal by-products, poultry by-products, meat and bone meal including poultry meat meal, blood meal, feather meal, milk by-products, gelatin and wet fish products including silage (Halver,1989). In view of widespread poultry farming practice in the world, which makes available poultry products and by-products, there is a relatively large potential for use of these as cheaper and more readily available sources of lower cost animal protein than fish meal.

2.3.1 Nutritive Value of Poultry Products and By-products

Poultry by-product meal has 58.7-62.8% crude protein, 13.1-14.1% ether extract, 2.3-2.4% crude fibre and 15.7-16.8% ash. Hydrolysed feather meal is very high in protein, but low in lipid and ash. They both have good essential amino acid profile as in Table 2.5 (NRC, 1983). Refinements have been made to enrich PBM resulting in a meal of protein content 68-72% and ash content of 8-11% (Fowler, 1991). Poultry meat meal is 63.5 % protein, 12.0% lipid and 16.3% ash and it is

high in all essential amino acids except methionine and histidine that are 1.7% and 1.6% (percent protein). However this compares favourably with the 0.6% methionine in soybean.

Table 2.5 Proximate Composition of Selected Poultry Products and By-products.

Proximate Composition (% as fed)	Poultry By-product Meal (Int. Feed No.5-03-798)	Hydrolysed Feather Meal (Int. Feed No. 5-03-795)
Moisture	7.0	7.0
Protein	58.7	84.9
Lipid	13.1	2.9
Ash	15.7	3.5
EAA (% Dry Matter)		
Arginine	3.8 (6.6)	7.1 (8.4)
Histidine	1.0 (1.7)	1.0 (1.2)
Isoleucine	2.4 (4.1)	4.1 (4.8)
Leucine	4.0 (6.8)	6.9 (8.1)
Lysine	2.9 (4.9)	2.3 (2.7)
Metthionine	1.1 (1.9)	0.6 (0.7)
Cystein*	0.9 (1.5)	3.2 (3.8)
Phenylalanine	1.8 (3.1)	3.1 (3.7)
Tyrosine*	0.9 (1.5)	2.3 (2.7)
Threonine	1.9 (3.2)	4.0 (4.7)
Tryptophan	0.5 (0.9)	0.5 (0.6)
Valine	2.9 (4.9)	6.5 (7.7)

NRC(1993)

Data in parenthesis represent EAA (% Protein)

* Non-EAA

2.3.2 Utilisation of Poultry Products and By-products in Aquaculture Feeds

Poultry by-product meal (PBM) has been used to partially replace fishmeal in the diets chinook salmon (Westgate, 1979; Fowler, 1981a,b, 1982), coho salmon (Higgs et al., 1979), rainbow trout (Tiews et al., 1979; Koops et al., 1982) and European eels (Gallagher and Degani, 1978). Full-fat poultry by-product meal was observed to perform eaqually well as herring fishmeal in the diets of coho salmon (*Oncorhynchus kisutch*) while complete substitution of herring fishmeal with defatted PBM retarded growth, lowered food conversion and protein efficiency (Higgs, et al 1979). PBM was also observed to replace 50% fishmeal in diets of chinook salmon without compromising performance (Fowler, 1982). He postulated replacement of fish meal at above 50% with refined poultry by-product meal in salmon diets without compromising growth. Steffens (1994) achieved complete substitution of fish meal with poultry by-product meal in rainbow trout diets. Meat and bone meal was found to successfully substitute 40% of fishmeal in a 50% (crude protein) diet of sea breams (*Sparus aurata*) (Davies, et al 1993). Successful partial and complete substitution of fishmeal with a combination of meat and bone meal, and blood meal by varying their inclusion levels in a 36% crude protein diet of *Oreochromis mossambicus* has been reported (Davies, et al 1989).

2.4 Antinutritional Factors and Toxic Substances in Poultry Products and By-products

Poultry by-product meal like other animal feedstuffs do not habour much in the way of naturally occuring toxicants . Only avian and fish eggs and certain kinds of shellfish and amphibians have been identified with toxicants of animal tissue origin (Liener, 1974). However, the presence of other toxic substances has been traced to intentional and inadvertent source of contamination. The use of additives in the food processing industry, the use of certain chemicals for animal and public health reasons and the consumption of plant foodstuffs by animals have led to the presence of a wide range of toxic substances in animal feedstuffs (Liener, 1974). Those related to poultry products and by-products are further discussed below:

2.4.1 Naturally Occuring

i Poultry Eggs

Avian egg whites have been found to contain the following substances : Ovomucoid and ovoinhibitors which are protease inhibitors with ovomucoid targeting trypsin; avidin - a glycoprotein, binds biotin (a vitamin B) and ovotransferrin binds metallic ions and rendering them unavailable, though it has been observed to inhibit

bacterial growth in its free form. The egg yolk is a rich source of cholesterol which when in excess could lead to coronary dysfuntion in most animals excluding fish.

ii Poultry Meat and Meat Products

Poultry meat lipid is high in cholesterol. Processed meat, particularly broiled and grilled meat products, contain polycyclic hydrocarbons like benzpyrenes and benzanthracenes which are carcinogenic. These are normally generated from the incomplete combustion of lipid and present in sooth. High intake of liver could lead to hypervitaminosis as a result of excess of vitamin A (Liener, 1974).

2.4.2 Exogenous Sources

i Additives

The inclusion of additives to meat products, including poultry to preserve and improve their market value has led to inadvertent introduction of toxic substances into animal foodstuffs. Of the additives commonly used, negative effects have been reported with pesticides and antibiotics. Until the 1970's, the use of DDT and methyl mercury containing pesticides was widespread and their non-biodegradability was later found to be a problem. Similarly, there has

been general use of tetracycline and penicillin in meat and animal feed industry in Argentina, U.S.A. and Canada and hypersensitivity to these in humans has been reported (Liener, 1974).

ii Contaminants

These are toxic substances that find their way into animal foodstuffs by accident. Public and animal health concern has led to the use of pesticide in the environment. Pesticide residues are picked up by non-target organisms including poultry. These include non-biodegradable pesticides and pesticide residue. The threat of radioactivity has led to the presence of radioactive debris (radionuclides) in the environment, from nuclear fission. These include strontium-90 in bone and milk, cesium-137 in muscle, liver and spleen, iodine-131 in milk and sodium-22. The radioactivity of these have been associated with structural malformation and physiological dysfunction (Liener, 1974).

iii Toxic Substances in Plants

It is interesting to note that most toxic substances in plant feedstuffs are present in animal foodstuffs as well, particularly poultry. This has been brought about by their food chain link. The meat of quail was once found to be poisonous because of its ingestion of toxic plant feedstuffs. Similarly, poultry feeds on plants, some of these contain

stable toxic substances that show up later in poultry meat, products and by-products (Liener, 1974).

2.5 Utilisation of Soybean Flour - Poultry Meat Meal Blends in Aquaculture Feeds

Mohsen and Lovell (1990) tested some animal proteins to replace soybean meal in channel catfish diets. It was observed that fish meal and meat and bone meal replaced soybean meal successfully as their levels increased in the diets. This was not possible with blood meal. Presently, commercial catfish diets contain 5-10% fishmeal. The bulk of the balance comes from soybean meal. In view of high cost of fishmeal, replacing it with lower cost animal proteins would be economical (Mohsen and Lovell, 1990).

2.6 Tilapia and Catfish Feeds and Nutrient Requirements

Lovell(1990) reported that fish generally require higher dietary protein levels than land animals and commercial feeds contain 25-45% protein. Hence, high protein feedstuffs like fishmeal, animal products, by-product meals and oilseed meals make up to 60% or more in commercial feeds.

Fish, as ectotherms, do not need to maintain constant body temperature as required by endothermic land animals and consequently have lower maintenance energy requirements than land animals. The heat increment associated with the assimilation of ingested food is equally lower in fish. Dietary energy sources in fish are primarily proteins and triglycerides. Warmwater fish digest carbohydrate energy well while cold water fishes do so poorly (Lovell, 1990).

The performance of a feed is therefore dependent on its ability to supply adequate protein and energy required by the fish. This could be in terms of gross protein and energy, digestible protein and energy and most preferably metabolisable protein and energy. However, it is less complicated, more reliable and more generally applicable to characterise feed quality in terms of its gross energy - protein ratio as digestibility and metabolism are conspecific, vary also according to fish growth stage or physiology, feed composition and environmental condition. Mangalik (1986) observed that while protein and energy requirements of fish changes with size, the protein - energy ratio changes only slightly.

Though feed with an adequate supply of protein and energy will meet the recovery and maintenance energy demands of fish, it is desirable for such feed to supply essential minerals and vitamins to

meet their structural, physiological and metabolic demands by the fish. 23 minerals have been found essential in animals. Seven of these are macro-elements, viz; calcium, chloride, magnesium, phosphorus, potassium, sodium and sulphur; and sixteen trace elements, viz; aluminium, arsenic, cobalt, chromium, copper, fluorine, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium and zinc. Nine of these have been found essential in fishes (Davis and Gatlin , 1991).

Requirements for these in fish are met from the environment, feedstuffs and mineral premix supplements. Lovell (1990) observed that 15 vitamins are essential in fish feed. However, all fishes do not require all 15 of the vitamins. Requirements for these are meet from feedstuffs and vitamin premix supplement. NRC (1993) has made a concise summary of nutrient requirements of fishes. Excerpts for tilapia and channel catfish (closely related to Clariid catfish) are given in the Table 2.6.

Table 2.6: Nutrient requirements of tilapia and catfish grower diets.

NUTRIENT	TILAPIA		CATFISH	
Energy-Digestible ($kcalkg^{-1}$ diet)	3,000		3,000	
% Crude Protein (Digestible)	28 - 32%		28 - 32%	
Amino acids	% Protein	% Diet	% Protein	% Diet
Arginine	4.2	1.2	4.3	1.2
Histidine	1.7	0.5	1.5	0.4
Isoleucine	3.1	0.9	2.6	0.7
Leucine	3.4	1.0	3.5	0.9
Lysine	5.1	1.4	5.1	1.4
Methionine + Cystine	3.2	0.9	2.3	0.6
Phenylalanine + Tyrosine	5.7	1.6	5.0	1.4
Threonine	3.6	1.1	2.0	0.6
Tryptophan	1.0	0.3	0.5	0.1
Valine	2.8	0.8	3.0	0.8

Table 2.6 Cont'd

NUTRIENT	Diet. Level (mgkg-^{1} diet)	T I L A P I A	C A T F I S H
n-3 Fatty acids (%)	-	-	0.5 - 1.0
n-6 Fatty acids (%)	-	0.5 - 1.0	-
Potassium (KCl)	500	NT	*
Sodium (NaCl)	600	NT	*
Calcium ($CaHPO_4.2H_2O$)	7278	*	*
Magnesium ($MgSO_4.7H_2O$)	1275	500 mgkg^{-1}	0.5
Phosphorus ($CaHPO_4.2H_2O$)	see calcium	0.5%	0.5%
Zinc ($ZnSO_4.7H_2O$)	55	20 mgkg^{-1}	20.0 mgkg^{-1}
Iron ($FeSO_4.7H_2O$)	250	NT	30.0 mgkg^{-1}
Copper ($CuSO_4.5H_2O$)	8	*	5.0 mgkg^{-1}
Manganese ($MnSO_4.4H_2O$)	25	*	2.4 mgkg^{-1}
Selenium	-	NT	0.3 mgkg^{-1}
Chlorine (KCl,NaCl& $CrCl_3$)	see sodium, potassium & Chromium	NT	*
Iodine ($CaIO_3.6H_2O$)	3	NT	1.1 (est.)
Chromium ($CrCl_3.6H_2O$)	1	-	-
Cobalt ($CoSO_4.7H_2O$)	5		

Chemical formula in parenthesis depicts dietary inclusion form

Table 2.6 Cont'd

NUTRIENT	Diet. Level (mgkg-1 premix)	TILAPIA	CATFISH
Vitamin A	1000	NT	1,000-2,000 $IUkg^{-1}$
Vitamin D	4	NT	500.0 $IUkg^{-1}$
Vitamin E	7000	50 $IUkg^{-1}$	50.0 $IUkg^{-1}$
Vitamin K	1500	NT	*
Thiamin	4250	NT	1.0 $mgkg^{-1}$
Riboflavin	3000	6 $mgkg^{-1}$	9.0 $mgkg^{-1}$
Vitamin B_6	1250	NT	3.0 $mgkg^{-1}$
Vitamin B_{12}	1.25	**	*
Vitamin C	37500	50 $mgkg^{-1}$	25.0-50.0 $mgkg^{-1}$
Biotin	90	NT	*
Choline	74050	NT	400.0 $mgkg^{-1}$
Folic acid	1000	NT	1.5 $mgkg^{-1}$
Niacin	12500	NT	14.0 $mgkg^{-1}$
Inositol	25000	NT	**
Pantotheinic acid	5250	10 $mgkg^{-1}$	15.0 $mgkg^{-1}$

Chemical forms of the vitamins are: Vitamin A, retinol all trans palmitate (type VII); vitamin D, cholecalciferol; vitamin E, dl alpha tocopherol acetate; vitamin K, menadione sodium bisulphite; vitamin C, L ascorbic acid; vitamin B_1, thiamine hydrochloride; vitamin B_2, riboflavin; vitamin B_6, pyridoxine hydrochloride; pantothenic acid, calcium dl pantothenate; niacin, niacinamide; biotin, biotin; folic acid, folic acid; vitamin B_{12}, cyanocobalamin; choline, choline chloride and inositol; myoinositol.

* Required in diet but quantity not determined
** No dietary requirement demonstrated experimentally
NT Not tested

Source: NRC (1993)

2.8 Hypotheses

These are experiment specific. The null hypotheses in line with the objectives are:

i There is no difference in the digestibility of soybean flour, poultry meat meal and wheat flour in *Oreochromis niloticus* and *Clarias gariepinus.*

ii The digestibility, apparent amino acid availability and wastes generation potential of soybean flour - poultry meat meal blend based diets with 0.5% and 1.0% chromic oxide levels show no differences in *O.niloticus* and *C.gariepinus* with blends and Cr_2O_3 levels.

iii The performance of all soybean flour - poultry meat meal blend (100:00; 25:75; 50:50 and 75:25) based diets and the soybean flour based diets fed to *O.niloticus* and *C.gariepinus* is the same, i.e. blending has no effect.

iv Replacement of fish meal at 25%, 50% and 75% with the above blends creates no performance differences with the diets fed to *O. niloticus* and *C. gariepinus,* i.e. replacement is ineffective.

v There is no difference in the performance of the blend based diets when each of them is supplemented with 0.5% dl- methionine and fed to *O. niloticus* and *C. gariepinus.*

vi Mineral premix reconstitution by individual and joint removal of calcium, phosphorus, magnesium and zinc does not change the performance of the best blend based diet in *O. niloticus* and *C. gariepinus.*

vii There is no difference in the performance of raw and autoclaved soybean flour - poultry meat meal based diets fed to *O. niloticus* and *C. gariepinus,* i.e. autoclaving does not affect utilisation of the diets

CHAPTER THREE

GENERAL MATERIALS & METHODS

3.1 Experimental Systems

Two experimental systems were used for this research, viz: Digestibility System and Feeding Trial System as described below:

3.1.1 Digestibility System

As in Fig. 3.1, this system consists of 12 units of ten litres capacity each. Each unit has a fish holding bucket, glued to a funnel shaped lower part onto which a rubber tubing tap is fixed. The bucket part holds the fish while the funnel shaped part is a faecal matter collection chamber separated from the upper part with a mesh. In each unit is a 50W heater to keep the desired temperature of 25-28°C and an air stone to aerate the unit. All units are supplied with water from a header tank thermo-regulated at 25-28°C. All units are on a work-top below which are sewage tanks to receive sewage and waste water from the units. The system is a static water type.

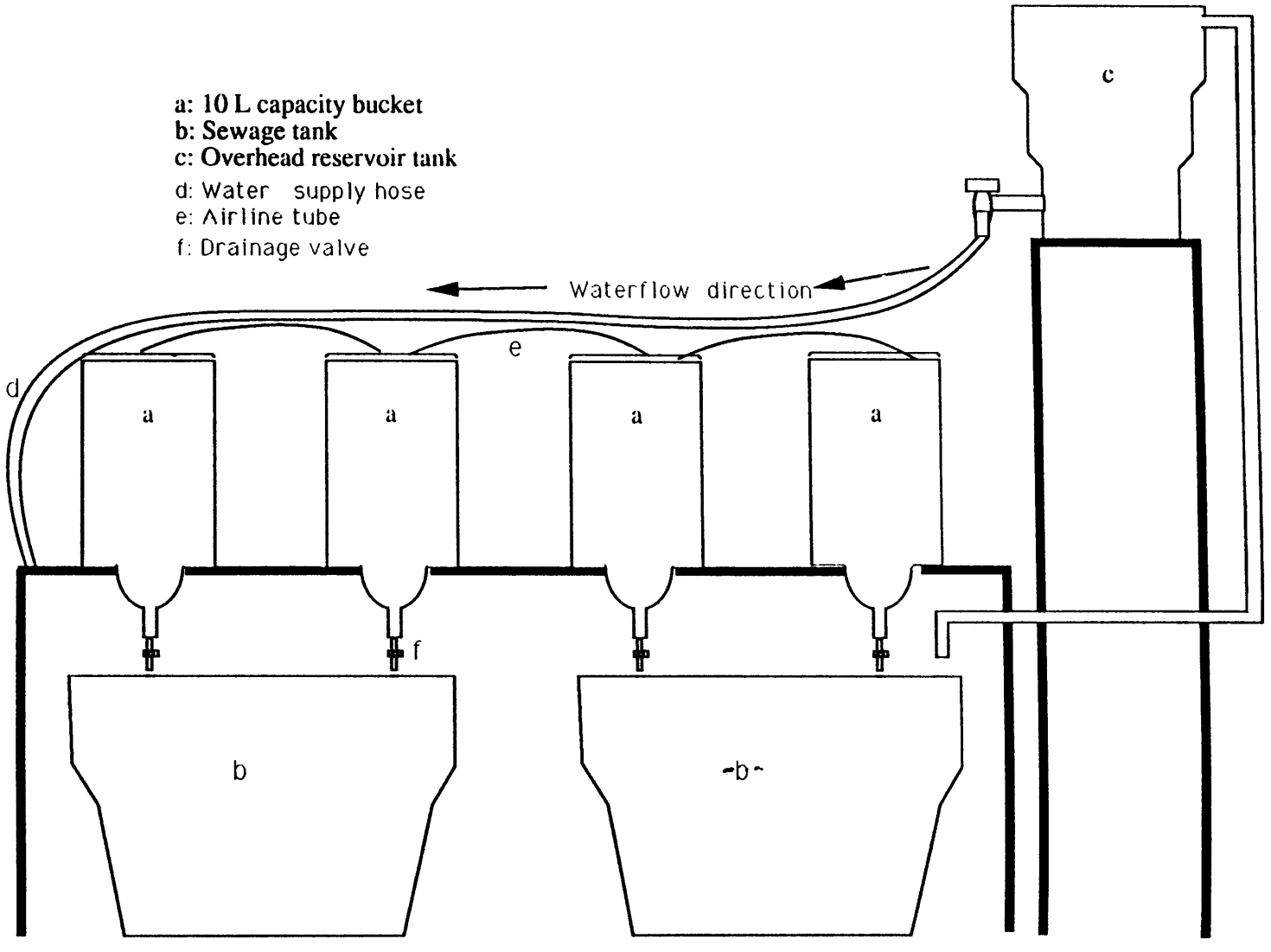
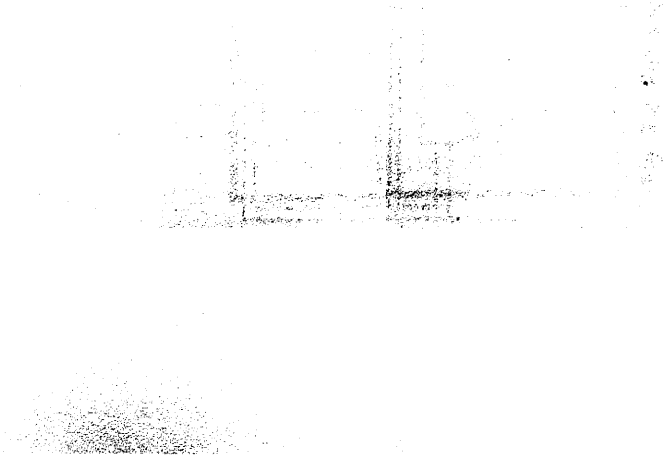


Fig. 3.1: Digestibility System.

3.1.2 Feeding Trial System

As depicted in Fig. 3.2, this system is recirculatory. It consists of 24 tank units of 50 litres capacity each. These are connected to a plumbing system that supplies water continuously. Fitted internally to each unit is an overflow and drain pipe, onto which a screen is fixed. This maintains water level without letting out fish. The units are mounted on a metallic framework below which are tanks to receive waste water. In these are bio-rings and bio-filters to filter waste water. These tanks are connected to a clean water collecting sump from which used water is pumped overhead to a header tank where it is further treated and recirculated. The heating system is central to the building, maintaining temperature at 25-28°C. The system itself was thermoregulated by its own 3KW heater.



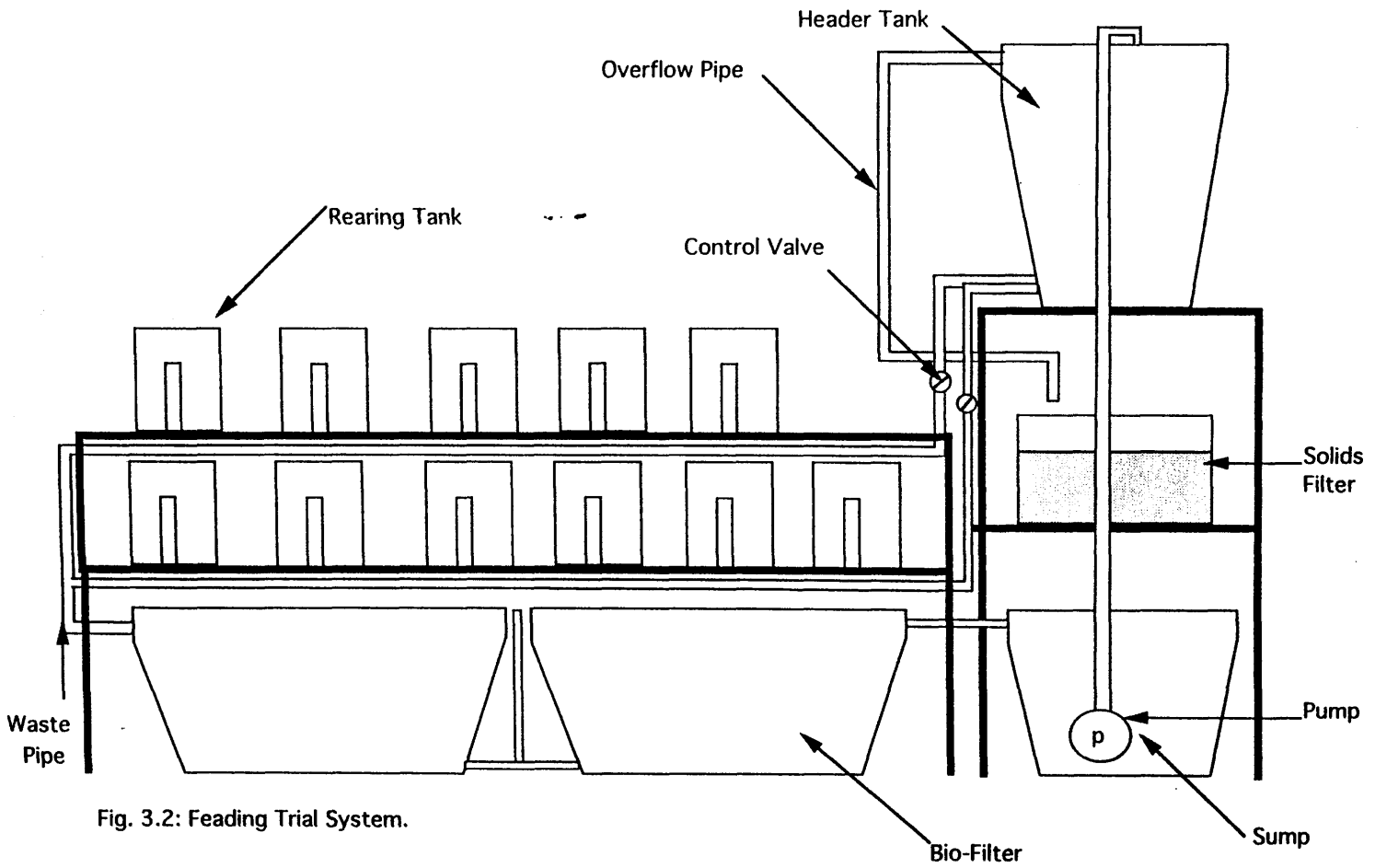


Fig. 3.2: Feading Trial System.

3.2 Experimental Designs

Completely randomised block and factorial designs were the two designs adopted for this research depending on the number of treatment variables.

3.2.1 Completely Randomised Block Design

This was used in cases of one treatment variable as in chapters 4, 6 and 9 where ingredient incorporation, soybean flour:poultry meat meal blending and reconstitution of mineral premixes were the respective variables.

3.2.2 Factorial Design

This was used in cases of two treatment variables as in chapters 5, 7, 8 and 10. Blends and chromic oxide levels were the two variables in chapter 5 , blends and levels of fish meal substitution were the two variables in chapters 7, blend and levels of methionine supplemented were the two variables in chapter 8 while blends and heat treatment were the two variables in chapter 10.

3.3 Experimental Fishes

Two tropical fish species were used for this research, viz; *Oreochromis niloticus* and *Clarias gariepinus*. They were bred and reared in the tropical aquarium and hatchery complex of the institute where this research was carried out as described below.

3.3.1 Breeding and Rearing of *Oreochromis niloticus*

Two methods were adopted in the breeding of *Oreochromis niloticus*. The simplest being recovery of naturally fertilised eggs from the mouth of the fish for further incubation using an incubation system. The other method involves stripping the eggs from a gravid broodstock female and the sperm from a broodstock male. Gamates were mixed using a dry or wet method. Eggs were then fertilised and the mixture ready for incubation. Incubation takes place and in about 2-3 days, the larvae hatched out. The larvae absorbed their yolk in 2-3 days after which they were transferred to a rearing/nursery tank where they were fed exogenously starting with hatched artemia till they were a month old. They were further reared for 1-2 months on commercial trout diet (crude protein-40%; lipid-7%) from Ewos U.K.ltd.

3.3.2 Breeding and Rearing of *Clarias gariepinus*

Breeding of catfish was carried out artificially by hypophysation (induced breeding). A plump gravid female *C. gariepinus* was selected and anaesthesised in 1 mlL^{-1} benzocaine solution (10g benzocaine in 100ml 95% ethanol). It was then injected with lutenising hormone releasing hormone (LHRHa) carried in vehicle solution (Bovine serum albumin, Sigma Chemical Co. U.K. ltd) containing pimozide. Dosage rate was $2.0cm^3kg^{-1}$ of the hormone solution was adequate to induce ovulation. A 1cm or 2cm syringe and No. 18 needle were used to inject the hormone into the fish hypodermally behind the base of the pectoral fin. The fish was returned to the tank and was ready for stripping in 18 hours.

The female was then stripped of her eggs into a bowl or basin. A mature male was sacrificed to obtain its milt. This was mixed with the eggs of the female to fertilise. The mixture was then incubated in a wire mesh tray on an incubation stand for 28-30 hours to hatch. Larvae absorbed their yolk in 2-3 days and were then transferred to rearing/nursery tank where they were reared for 2-3 months to the desired sizes according to Haylor (1993).

3.4 Experimental Diets

This involved diet formulation and preparation as detailed below:

3.4.1 Diet Formulation

Two specifications were set for all the diets in all the experiments in addition to those specific for each experiment. Diets were isocaloric (4 kcalg-1) and isonitrogenous (38%) to give an energy : protein ratio of 110 : 1. Equational method (Halver, 1989) was adopted for formulation to calculate the inclusion level of each ingredient to meet the desired specifications. Fishmeal, soybean and poultry meat meal were used as protein supplements, wheat flour as a carbohydrate source, soy oil as a lipid source to reconstitute the diets to meet the specifications and all were treated as variable ingredients. Fixed ingredients were mineral and vitamin premixes as nutrient supplements, carboxymethyl cellulose as binder and chromic oxide as digestibility indicator.

3.4.2 Diet Preparation

Both fixed and variable ingredients were mixed at computed levels thoroughly using a Hobert mixer. Water was added at 30-50% v/w to give a pelletable mixture. The steam conditioned California Pellet Mill (CL2 model, San Francisco, California) was used to pellet the diets. An appropriate die was used to give pellets of desired sizes of 1mm or 2mm. The pellets were then dried by convection at 60°C overnight in a drying cabinet, packed in black polythene bags, sealed and stored at -30°C until required.

3.5 Experimental Operation

This could be divided into routine and non-routine management. The routine management for the feeding trial was feeding the fish daily, monitoring water quality fortnightly and cleaning the system regularly (fish husbandry). Checking the system for leaks and malfunctions constituted the non - routine management. In addition to these, faecal matter was collected daily for subsequent analysis in the digestibility studies.

3.5.1 Fish Husbandry

When fish greater than 5g were used, they were fed at 4% body weight day^{-1}, twice daily while those less than 5g were fed at 6% body weight day^{-1}, thrice daily for 56 days except one day in a week in accordance with Jauncey and Ross (1982). Fish were stocked at either 10 or 15 per 50 litre tank in either duplicate or triplicate per treatment depending on the facilities available and the experimental design. Fishes were weighed individually at the beginning and end of every feeding trial, and bulk - weighed fortnightly in between to adjust feeding rate.

Weighing individually involved anaesthetizing a fish with $1mlL^{-1}$ v/v concentration 2-phenoxy-ethanol in water for 3-5 minutes. The fish was

then wiped dry with a tissue paper and weighed on a top-loading Mettler balance. Fish from one tank were anaesthetized at a time and quickly weighed individually within a time limit of 3-5 minutes as already described or all weighed together after drying off surface water in the case of bulk-weighing. The digestibility studies of feedstuffs and diets for juvenile fish that could not be conducted in the conventional digestibility system were conducted using the feeding trial system. Fish were stocked at 10 per tank and weighed only at the beginning to calculate feeding rate. They were fed at 10:30 every morning. Faecal matter was collected once a day at 10:00 by siphoning from the bottom of each unit.

Digestibility of feedstuffs and diets for fingerlings was conducted in the set-up digestibility system where fishes were stocked at 5 per tank. In all cases they were fed once daily for three weeks after a week acclimation to the diets. At 12:00 every day, diets were placed on a feeding dish using a feeding pipe to direct the diets into the dish. Fish were allowed to feed for one hour after which the feeding dish was removed and diet not eaten flushed out and clean water added to maintain the water level. At 11:00 every morning, faecal matter was collected by draining from the collection chamber below. Clean water was again added as before.

3.5.2 Water Quality Management

Fortnightly water parameters such as temperature, dissolved oxygen, pH, NH_3-N, NO_2-N, NO_3-N, calcium hardness and total hardness were measured as follows:

i Temperature

Temperature of the water in the feeding trial system was thermo-regulated with a 3 KW heater measured with a mercury bulb thermometer. The tropical aquarium central heating system provided a constant warm temperatrure environment of 25-28°C readable from a digital display unit.

ii Dissolved Oxygen (DO)

Water samples from the systems were taken fortnightly for DO analysis in the Water Quality Laboratory using an oxygen meter - YSI model 57 from YSI inc., Ohio,USA. Calibration of the meter and its use were according to the YSI operating instructions and measurement expressed in mgl^{-1}

iii Ammonia Nitrogen (NH_3-N)

This was measured using ammonia SeaTest kit from Aquarium Systems inc., Sarrebourg, France. The direction for use on the kit was followed. Measurement of NH_3-N was read in mgl^{-1}.

iv Nitrite Nitrogen (NO_2-N)

This was similarly measured as for ammonia except for the use of nitrite SeaTest Kit instead and NO_2-N expressed in mgl^{-1}.

v Nitrate Nitrogen (NO_3-N)

The procedure was the same as for NO_2-N except for the use of nitrate SeaTest kit and NO_3-N measured in mgl^{-1}.

vi Calcium Hardness (Ca-Hardness)

Water samples were taken to the laboratory. 100ml was placed in a conical flask and a calcium hardness tablet of eriochrome black-T was added in addition to 1ml of 0.4M sodium hydroxide. The mixture was then stirred with a magnetic stirrer to dissolve the tablet. The solution was titrated against the disodium salt of ethylene diamine tetra-acetic acid (Na_2EDTA) to give a purple colour end point. The

titre value was multiplied by ten to give the Ca-Hardness in mgl^{-1}. This method was according to Stirling (1985).

vii Total Hardness

100ml of water sample was measured into a conical flask, to which 2ml of ammonia buffer was added together with a total hardness tablet. The same procedure was followed as in Ca-Hardness to give a blue end point. Similarly, the titre value was multiplied by ten to give the total hardness in mgl^{-1}. This method was according to Stirling (1985).

3.6 Experimental Analyses

This comprised chemical analysis and biological evaluation.

3.6.1 Chemical Analysis

General chemical analyses carried out were chromic oxide and proximate analyses of feedstuffs, diets and carcass, as well as the energy levels in the feedstuffs and diets. These were moisture, protein, lipid, ash, carbohydrate, crude fibre, nitrogen free extracts and energy using methods after AOAC (1990) as detailed below.

i Chromic Oxide Analysis

The method of Furukawa and Tsukahara (1966) was employed. It involved acid digestion of 50mg sample with 5ml conc. nitric acid followed by 3ml perchloric acid with digest turning from green to yellow. Digest was diluted to 100ml and absorbance read spectrophotometrically at 350nm. Percentage Cr_2O_3 was calculated thus:

$$\text{Weight of } Cr_2O_3 \text{ in sample} = \frac{\text{Absorbance} - 0.0032}{0.2089}$$

$$\%Cr_2O_3 \text{ in Sample} = \frac{\text{Weight of } Cr_2O_3 \text{ (100)}}{\text{Sample weight (g)}}$$

i Moisture

It is a gravimetric measurement of water in the feedstuffs, diets and carcass - expressed as a percentage of the initial sample weight.

ii Protein

The micro-kjeldahl method according to AOAC (1990) was used for the determination in duplicate as follows. 100-200mg sample was digested in concentrated sulphuric acid and ammonia from the digest was released when reacted with 40% sodium hydroxide and distilled, trapped in 2% boric acid and quantified by titration against 0.2M hydrochloric acid - both distillation and titration were automated

using Kjeltec Auto 1030 Analyser and run according to operation manual from the manufacturer. % protein was calculated using the titre values for the blank and samples as follows;

$$\% \text{ protein} = \frac{(\text{sample titre} - \text{blank titre}) \times 0.2 \times 14.007 \times 6.25 \times 100}{\text{Sample weight (approx. 200mg)}}$$

iii Lipid

The method was that of solvent extraction using soxhlet extractor. 1g sample was weighed into a thimble and corked with cotton. 50ml petroleum-ether (40-60°C) was added to a pre-weighed cup. Both thimble and cup units were coupled to the Soxtec System 1043 Extractor and run according to the specifications of the operation manual from the manufacturer. Extracted lipid in the cup weighed and expressed as a percentage of the original sample.

iv Crude Fibre

lg of defatted sample in a pre-weighed scintaglass crucible was used for crude fibre determination using acid-base hydrolysis. The crucible was fitted to the Fibertec 1020 and run according to the manufacturer's operating instructions. Hydrolysed and oven-dried sample was later ashed in the muffle furnace at 550°C and crude fibre in the defatted sample expressed as a percentage of the original undefatted sample.

v Ash Content

This measured the total inorganic matter by incineration. 1g sample was weighed into a pre-weighed crucible and incinerated overnight at 450°C. The increase in the final weight of crucible after incineration represented the ash and was expressed as percentage of the original sample.

vi Total Carbohydrate

This summed crude fibre and nitrogen free extract. It was determined by the difference method (Watts, 1975) after non reproducible results were obtained from an adaptation of the Dubois method (phenol-sulphuric acid method).

vii Total Energy

This was determined either directly or calorimetrically using adiabatic autobomb calorimeter or calculated from carbohydrate, protein and lipid contents.

a) Bomb Calorimetry

Approximately 1g sample was molded into a tablet using briquette press and reweigh. The calorimeter bucket was filled with water at 21-23°C. Benzoic acid table was coupled in a bomb and pressurised with oxygen and combusted inside the Gallenkamp Bomb Calorimeter following the procedures in the operation manual. The temperature rise was substituted into the equation below to evaluate energy. The energy value determined from such a standard of known energy checked the caliberation accuracy. Sample combustion then took place and its energy similarly evaluated.

$$\text{Energy } (kcalg^{-1}) = \frac{(\text{Temperature difference} \times 10.82) - 0.0896}{\text{Sample weight (Xg)} \times 4.18}$$

b) Calculated Gross Energy

Using multiplier factors of 4.1, 5.4 and 9.5 $kcalg^{-1}$ for carbohydrate, protein and lipid respectively according to Jobling (1983). Carbohydrate , protein and lipid energy contents were summed up to give the total or gross sample energy.

viii **Nitrogen Free Extract**

This measured the amount of acid/alkali soluble carbohydrate. This was calculated as difference of total carbohydrate and crude fibre.

3.6.2 Biological Evaluation

Biological parameters measured were according to Maynard et al.(1979) , Bondi (1989) and Halver (1989) and expressed thus:

i **Specific Growth Rate (SGR)** Brown (1957);

$$SGR = \frac{Ln(\text{Mean final weight}) - Ln(\text{Mean initial weight})\ (g) \times 100}{\text{Time (days)}}$$

ii **Food Conversion Ratio (FCR)**

Computed as;

$$FCR = \frac{\text{Weight of food fed(g dry weight)}}{\text{Weight gain of fish(g wet weight)}}$$

iii **Protein Efficiency Ratio (PER)** Osborne, et al.(1919)

Calculated as;

$$PER = \frac{\text{Weight gain of fish (g wet weight)}}{\text{Crude protein fed (g)}}$$

iv **Net Protein Utilisation (NPU)** Bender & Miller,1953 and Miller & Bender,1955)

It is expressed as Apparent NPU (ANPU) and computed as;

$$\text{App. NPU (\%)} = \frac{\text{Carcass protein gain (g) x 100}}{\text{Protein fed(g)}}$$

v Mortality (Mort.)

Calculated as ;

$$\text{Mort.(\%)} = \frac{\text{Number of dead fishes x 100}}{\text{Number of fishes stocked}}$$

vi Digestibility

Nutrient and feedstuff digestibility values were evaluated according to Maynard et al(1979) and Bondi (1987) as stated thus:

$$\text{ADC (\%) of nutrients} = 100 - \left(100 \frac{Cr_2O_3 \text{ in diets}}{Cr_2O_3 \text{ in faeces}} \times \frac{\text{nutrient in faeces}}{\text{nutrient in diet}}\right)$$

Apparent digestibility coefficients of feedstuffs were calculated according to Cho, et al., (1985) as given below:

$$\text{Test ingredient(ADC)} = \frac{\text{ADC of test diet - 0.7 ADC of ref.diet}}{\text{0.3 ADC of test diet}}$$

3.7 Statistical Analysis

Results of carcass composition, the evaluation parameters of the diets and other data were subjected to one way Analysis of Variance (ANOVA) using Turkey's test (Steele and Torrie,1960), often at 5% and seldomly at 10% probability level. Multifactor Analysis of Variance (MANOVA) was used to check significance of factors and their interactions in experiments with factorial design. Multiple parameter means comparison of treatments was according to Duncan multiple

range test (1955). Simple regression by product moment and correlation were used to relate diet and carcass essential amino acids and mineral composition (Zar, 1984).

Percentage data were transformed by arc-sine transformation (Zar,1984) prior to the ANOVA and reversed afterwards. All statistics were executed using the statgraphics (version 5.0) and minitab (version 10.1) packages.

CHAPTER FOUR

NUTRITIVE VALUE OF FEEDSTUFFS

4.1 Biochemical Evaluation of Soybean Flour.

4.1.2 Introduction

Soybean absolute suitability in animal feeds including aquaculture species as a replacer of fish meal has been bedeviled by some draw-backs including antinutritional factors (ANFs) explained in 2.2 (Liener, 1980).. ANFs that have caused noticeable physiological and anatomical defects in fish are trypsin inhibitors and phytates .

Trypsin inhibitor inhibits the enzymatic action of trypsin and heightens methionine deficiency by pancreatic oversecretion of cystine, symptomised by tissue hypertrophy, rachitogenicity, etc. in animals. Phytic acid chelates minerals like phosphorus, calcium, magnesium, zinc, iron, copper etc to form phytate, renders them unavailable and its toxicity is symptomised by structural malformation and physiological inefficiency. It has been reported to cause 8-10% reduction in growth of fish and high mortality after 90 days feeding (Spinelli, 1978).

The physiological effects of saponin, estrogen and allergens on aquatic animals have not been known (Lim and Akiyama, 1989) though saponin was reported to elicit haemolytic activity in farm animals (Gestetner et al. 1971; Liener, 1980). 1-3% of the protein of defatted soybean is haemaglutinnin which binds carbohydrate to

elicit toxicity in animals (Jaffe, 1980). However, it has been found to have an insignificant contribution to the poor nutritive value of raw soybeans (Turner and Liener, 1975). In fact, it is inactivated by the pepsin of stomach bearing fishes (NRC, 1983).

Fortunately, the ANFs of economic concern are heat labile and therefore readily destroyed through heat processing. However, heat processing has its own disadvantage. It reduces protein availability and solubility. The browning reaction (Maillard reaction) that takes place renders unavailable much of the lysine and could trigger lysine deficiency in fish with high lysine requirement.

Though, commercial soybean products currently in use in fish diets have been toasted either at 177°C for 10 minutes or 105°C for 20 minutes (Lovell, 1990), there is still the use of raw soybean in research diets. Therefore, there still exist the aforementioned flaws of rawness and undertoasting, toasting and overtoasting of soybean products. Evaluating the levels of these ANFs and effects of processing on nutritive value of soybean have become appropriate and conventional preambles in any investigation on soybean product utilisation in fish diets.

4.1.3 Materials and Methods

Feedstuffs assayed were: Soybean flour (raw, dehulled and solvent extracted soybean flour from Sigma Chemical Supply Company ltd, U.S.A; dehulled , solvent extracted and autoclaved soybean flour at 131°C at 15 pounds per square inch for 20 minutes and commercial soybean flour supplied by Dr Viv Crampton of Ewos UK ltd.), fishmeal, poultry meat meal and wheat flour. Proximate composition was determined for all feedstuffs while protein solubility index, trypsin inhibitor activity assay, determination of phytic acid and available lysine were conducted for soybean products. Proximate composition was done as in 3.6.1.

i Protein Solubility Index (PSI) of Soybean Products

Several methods are in use in the determination of the adequacy of heat treatment of soybean meal. These include urease activity (Caskey and Knap, 1944; Bird et al., 1947) trypsin inhibitor activity (Kakade et. al., 1974; Hammerstrand, et. al., 1981), formaldehyde titration (Almquist and Maurer (1953), cresol red dye-binding (Olomucki and Bornstein, 1960), orange G dye-binding (Moran et al, 1963), fluorescence test (Hsu et. al., 1949), Coomassie Blue (Kratzer, 1990) and protein solubility index (Vohra and Kratzer, 1991).

Araba and Dale (1990) have demonstrated clearly the superiority of protein solubility index (PSI) over most others. Urease activity destruction precedes that of lysine unavailability such that it drops to zero when lysine is still available. Araba and Dale (1990) observed a better relationship between chick response and overprocessing evaluated using PSI than cresol red dye binding or urease activity.

The protein solubility index method (Araba and Dale, 1990) was therefore adopted for acertaining the protein quality of raw and toasted soybean products. It involves measuring the solubility of soybean in a weak alkali solution (0.2% potassium hydroxide) and soluble protein is measured using the Kjeldahl method. The amount of soluble protein is expressed as a percentage of total protein.

ii Available Lysine

The A.O.A.C. (1990) method for assaying available lysine was used which is the same as that of conventional amino acid analysis. The method takes advantage of the ability of free - amino group of lysine in protein to bind with 1-fluoro-2,4-dinitrobenzene (DNFB) part of which has been bound by carbohydrate during toasting (Maillard reaction) and considered unavailable or destroyed. Upon acid hydrolysis, stable DNFB retained its lysine group while that unavailable was hydrolysed and detectable by the amino acid analyzer. The difference was considered as available lysine.

iii Trypsin Inhibitor Activity

The improved method of Hammerstrand et al. (1981) over the standard AACC (American Association of Cereal Chemists) method which in itself was an improvement over Kakade et al.(1969, 1974) was employed here. All the methods share the same principle of determining trypsin inhibitor in soybean products based on the tryptic hydrolysis of a synthetic substrate, benzoyl-DL-arginine-p-nitroanilide hydrochloride (BAPA). Three soybean flour samples, viz: Raw, dehulled and solvent extracted soybean flour; autoclaved, dehulled and solvent extracted soybean flour, and toasted commercial soybean flour were assayed. There was a difference in synthetic trypsin activity (as depicted in absorbance at 410nm) of a standard BAPA solution (without inhibition) and that of soybean sample plus BAPA solution (with inhibition) after a correction for sample effect using sample blank. This was attributed to trypsin inhibitor activity in the sample. The trypsin inhibitor activity was quantified using the formula below:

$$\text{TI, mg/g of sample} = \frac{\text{differential absorbance}}{0.019 \times 1{,}000} \times \text{dilution factor}$$

A dilution factor of 1 in 60 was used. Choice of dilution factor provides for differences in the sensitivity of substrates. The differential absorbance was first converted to trypsin inhibitor unit (TIU). One TIU represented 0.01 increase in absorbance.

iv Phytic Acid Determination

A modification of the McCance-Widowson method for determining phytic acid according to Wheeler and Ferrel (1971) was adopted. Three soybean flour samples, viz: Raw, dehulled and solvent extracted soybean flour; autoclaved, dehulled and solvent extracted soybean flour, and toasted commercial soybean flour were analysed. The phytate was extracted with trichloroacetic acid and precipitated as ferric salt. The iron concentration was determined colorimetrically at 480nm and extrapolated from a calibration curve using iron standard. The phytate phosphorus content was calculated from the iron content assuming a constant 4 Fe:6 P molecular ratio in the precipitate.

4.1.4 Results and Discussion

i Proximate analysis

Table 4.1 presents the proximate composition of all feedstuffs. Highest moisture value of 13.1% was recorded for wheat flour and lowest for soybean oil. Fishmeal recorded the highest protein value of 66.4% and no protein was detected in soybean oil. Soybean oil was approximately 100% lipid while dehulled and raw soybean flour had the lowest lipid of 0.4%. Ash highest value of 16.3% was recorded in poultry meat meal while that of carbohydrate of 38.4% came from wheat flour. Highest gross and digestible energy values of 8 and 9 and 2.7 and 3.7 $kcalg^{-1}$ were recorded for soybean oil and wheat four respectively.

This table depicts clearly that fishmeal, poultry meat meal and the soybean flour product all have above 20% protein and according to NRC (1993) should be used as protein supplements in fish diets, soybean oil has 100% oil and act as a good source of lipid while wheat flour which is low in both protein and lipid but high in carbohydrate will better serve as a good carbohydrate source. A diet of any desired specification can easily be formulated from these feedstuffs.

Table 4.1 Percentage proximate composition of feedstuffs.

Proximate composition (%)	FM	PMM	SF	SF*	SF**	SF***	SO	WF
Moist.	10.1	5.1	4.4	4.7	8.6	10.5	-	13.1
Prot.	66.4	63.5	50.5	50.9	50.6	46.9	-	10.3
Lipid	8.3	12.0	0.4	0.6	0.5	1.7	100	1.3
Ash	14.9	16.3	6.3	6.1	6.8	6.7	-	1.3
CHO	0.3	3.1	38.4	37.7	33.5	34.2	-	74.0
Crude Fibre	0.3	0.8	2.7	2.9	3.2	5.3	-	1.9
NFE	T	2.3	35.7	34.8	30.3	28.9	-	72.1
Energy-analysed ($kcalg^{-1}$)	5.2	5.0	-	-	-	4.4	NA	3.7
Energy($kcalg^{-1}$)*	4.4	4.7	4.3	4.4	4.2	4.2	9.0	3.7
Digestible Energy($kcalg^{-1}$)**	3.5	3.7	2.7	2.7	2.6	2.6	8.0	2.7

T, Trace
NA, Not analysed
CHO, Carbohydrate
* = Calculated according to Jobling (1983).
** = Calculated according to New (1987).
FM,Fish meal; PMM,poultry meat meal; SF, dehulled and raw soybean flour; SF*, dehulled ana autoclaved soybean flour; SF**, dehulled and toasted soybean flour; SF***, toasted soybean flour- commercial; SO, soybean oil; WF, wheat flour.

ii Soybean Quality Analyses

The protein solubility index (PSI), trypsin inhibitor (TI) activity level and phytate phosphorus content of the soybean products differed significantly with the products (P>0.05). The PSI, TI activity and phytate phosphorus level were highest in the raw soybean flour and lowest in the commercial soybean flour while those of autoclaved soybean flour were intermediate between the two as shown in Table 4.2. This shows that heat processing reduces the protein solubility level, ANF activity and available lysine. Kakade et al (1974) recorded trend of trypsin inhibitor activity reduction in soybean products with increase in heat treatment while a similar trend was observed for phytate phosphorus in heat treated wheat flour (Wheeler and Ferrel, 1971).

Table 4.2 Level of trypsin inhibitor (TI) activity, phytate phosphorus and available lysine in the soybean flour (SF) used in experimental diets

SF type	(TIU /mg SF)	Phytate-P (mg/100g sample)	Available lysine (as % protein)	PSI(%)
Raw SF	91.3 (96.9-110.9)*	789.7	4.3	90.8
Autoclaved SF	39.8	405.3	4.2	82.4
Commercial SF	7.3 (7.6-10.9)*	357.2	4.1	73.8

TIU=Trypsin inhibitor unit; P=Phosphorus; PSI=Protein solubility index

*Kakade et al. (1974).

4.2 Digestibility of Soybean Flour, Poultry Meat Meal and Wheat Flour in *Oreochromis niloticus* and *Clarias gariepinus*

4.2.1 Introduction

A basic step in the choice of a feedstuff in the formulation of a good diet is knowledge of its digestibility. This has been necessitated by the quest for the development of low-cost diets using agricultural by-products in fish culture (De Silva, et al., 1990). Most of these are non-conventional and will therefore demand characterisation by biological and chemical evaluation. Digestibility is also an indicator of potentially available energy and nutrients for maintenance, growth and reproduction of the animal and a measure of indigestible nutrients that account for major portion of wastes generated from aquaculture operation (Cho, 1991). The digestibility coefficients of feedstuffs in a diet are largely unaffected by biotic and abiotic factors, and additives.(Cho and Kaushik, 1990). This has made possible the development of a mathematical and predictive model for the digestibility of innumerable diet formulations possible from such feedstuffs.

Several methods have been developed for feedstuff digestibility investigation. Cho et al., (1985) replaced 30% of a reference diet with the test ingredient. De Silva et al., (1990) found that mixing 15-20% of

the ingredient (leaf meal) in a reference diet would be more desirable in determining the digestibilty of leaf meal in *Oreochromis aureus*. However, their results showed very high apparent digestibility coefficients (ADC) for test diets with 10 and 20% inclusion levels of the test ingredient. There were cases at 10% where this gave higher ADC of test diet than the reference diet signifying >100% digestibility which is theoretically impossible. Values obtained at 20% and above guaranteed <100% ADC values.

This controversy requires further investigation into the conventional digestibility study method. As a result of the foregoing, 30% inclusion levels of ingredients, viz; soybean flour, poultry meat meal and wheat flour into a reference diet containing fish meal and wheat flour to give the test diet were adopted for this investigation. The study is intended to basically characterise the feedstuffs in terms of their macro-nutrient digestibility.

4.2.2 Materials and Methods

Four reference diets (4 $kcalg^{-1}$ energy and 38% crude protein) containing fish meal as protein supplement and wheat flour were formulated. Soybean flour (conventionally used commercial grade), poultry meat meal and wheat flour were used to individually replace 30% of each of three of the reference diets to give three test diets as shown in Table 4.4. The proximate composition of the ingredients are presented in Table 4.3. These four diets were fed to *Oreochromis niloticus* (M±S.E.=21.6±0.6g) and *Clarias gariepinus* (M±S.E.= 50.0±1.8g) at 2% body weight per day at 11:00 Hr for four weeks.The first week for acclimation and faecal collection was effected for the remaining three weeks.

Waste was siphoned out at 13:00 and faecal collection made at 10:00 before next feeding. Fish were stocked at 10 per 50L tank in a recirculatory system having water flow rate reduced to 0.5L per minute circulating from the top. Water quality parameters were monitored as described in 3.5.2: Temperature, 26-27°C; dissolved oxygen (DO), 4.4-6.2 mgL^{-1}; pH, 6.0-6.5; NH_3-N, 0.2-0.6 mgL^{-1}; NO_2-N, 0.2 mgL^{-1}; NO_3-N, 10-20 mgL^{-1}; Ca-hardness, 51-62 mgL^{-1} and total hardness, 59-71 mgL^{-1} .

Proximate analyses of feedstuffs, diets and faecal samples were carried out according to A.O.A.C (1990) explained in 3.6.1. Evaluation parameters measured were apparent digestibility coefficients of nutrients and feedstuffs as in 3.6.2. Leaching effect was compensated for using a multiplier factor of nutrient ADC in fish stripped of faeces divided by that obtained from the draining method of the Guelph system. Data statistical analysis was done as in 3.7.

Table 4.3 Proximate composition (% as fed) of feed ingredients

Proximate composition	Ingredients			
	Fish Meal	Soybean flour	Poultry meat meal	Wheat flour
Moisture	10.1	10.2	5.1	13.1
Protein	66.4	46.9	63.5	10.3
Lipid	8.4	1.7	12.0	1.2
Ash	14.9	6.7	16.3	1.3
EAA*(% protein)				
Arginine	4.3	5.6	5.6	1.4
Histidine	1.8	1.9	1.6	0.9
Isoleucine	2.8	3.3	2.8	1.2
leucine	5.7	6.1	5.8	2.6
Lysine	5.9	4.5	4.8	1.3
Methionine	2.0	0.6	1.7	0.4
Cystine**	0.5	0.7	0.7	0.4
Phenylalanine	3.0	3.7	3.4	1.8
Tyrosine**	2.5	2.7	2.5	0.6
Threonine	3.3	3.1	3.3	1.2
Valine	3.4	3.2	3.5	1.5

*EAA=Essential amino acids.
** Non-EAA

Table 4.4 Ingredients and proximate composition of reference and test diets used in feedstuff digestibility study.

Diets	Ref.Diet (RF)	Test Diet (RF+SF)	Test Diet (RF+PMM)	Test Diet (RF+WF)
Fish meal	52.4	36.7	36.7	36.7
Soybean flour	-	30	-	-
Poultry meat meal	-	-	30	-
Wheat flour	31.9	22.3	22.3	52.3
Soybean oil	7.8	5.5	5.5	5.5
Vitamin premix[1]	2.0	1.4	1.4	1.4
Mineral premix[2]	4.0	2.8	2.8	2.8
Binder (CMC)*	1.5	1.1	1.1	1.1
Cr_2O_3	0.5	0.4	0.4	0.4
Proximate composition				
Moisture	8.5	7.6	6.2	6.3
Protein	38.2	40.2	44.4	31.7
Lipid	13.4	10.9	13.0	11.0
Ash	9.5	8.5	10.9	7.7
Energy($kcalg^{-1}$)	4.6	4.6	4.7	4.6

* CMC = Carboxymethyl cellulose

[1,2]Constituents of mineral and vitamin premixes are as in Table 2.5.

4.2.3 Results

i Experiment 1 *Oreochromis niloticus*

The total dry matter (TDM), protein, lipid and ash apparent digestibility coefficients (ADCs) of the reference diet and test diets are presented in Table 4.5 The dry matter, protein, and lipid ADCs were high in test diets containing SF, PMM and WF in decreasing order while that of ash was lowest. These, except ash, were almost as digestible as the reference diet. Infact the protein of soybean was more digestible. Nutrient ADCs of SF, PMM and WF as shown in Fig. 4.1 confirm this observation as they were very high for all the nutrients evaluated and that of soybean protein was >100%.

Table 4.5 Total dry matter, protein, lipid and ash digestibilty coefficients of a mixture of reference diet and test diets fed to *Oreochromis niloticus*

Diets	Total dry matter digestibility	Protein digestibility	Lipid digestibility	Ash digestibility
Ref.diet(RD)	96.7±0.3	85.0±1.2	97.0±1.4	46.15±0.1
RD+30%SF	96.3±0.3	87.4±0.0	96.3±0.9	43.45±1.1
RD+30%PMM	95.3±0.0	84.9±0.9	95.6±0.3	44.20±0.0
RD+30%WF	93.7±0.0	80.7±1.0	93.1±2.2	43.40±1.3

Data expressed as Mean ± Standard Error of duplicate values

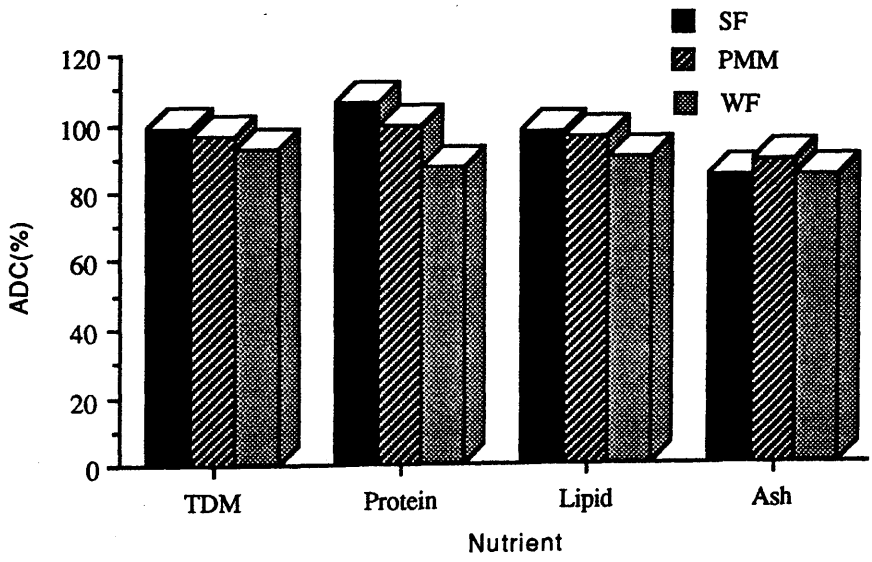


Fig.4.1: Digestibility of commercial grade soybean flour (SF), poultry meat meal (PMM) and wheat flour (WF) in *Oreochromis niloticus*

ii Experiment 2 *Clarias gariepinus*

Table 4.6 depicts the dry matter, protein, lipid and ash apparent digestibility coefficients (ADCs) of the reference diet, test diets and the feedstuffs. The dry matter, protein and lipid ADCs were high in test diets containing SF, PMM and WF. Dry matter and protein ADCs were highest in PMM test diet while lipid was highest in SF test diet and ash was lowest. These were almost digestible as the reference diet. Infact the dry matter was more digestible. Fig. 4.2 depicts that very high ADCs of SF, PMM and WF were recorded for all the nutrients evaluated and those of dry matter were >100%.

Table 4.6 Total dry matter, protein, lipid and ash digestibilty coefficients of a mixture of reference diet and test diets fed to *Clarias gariepinus*

Diets	Total dry matter digestibility	Protein digestibility	Lipid digestibility	Ash digestibility
Ref.diet(RD)	93.3±0.0	90.4±0.0	91.1±0.0	49.20±0.0
RD+30%SF	91.2±0.5	84.2±0.0	93.1±0.2	49.10±0.4
RD+30%PMM	95.8±0.0	85.4±0.1	88.3±2.3	49.40±0.0
RD+30%WF	92.3±0.2	81.9±0.6	87.0±0.1	49.15±0.1

Data expressed as Mean ± Standard Error of duplicate values

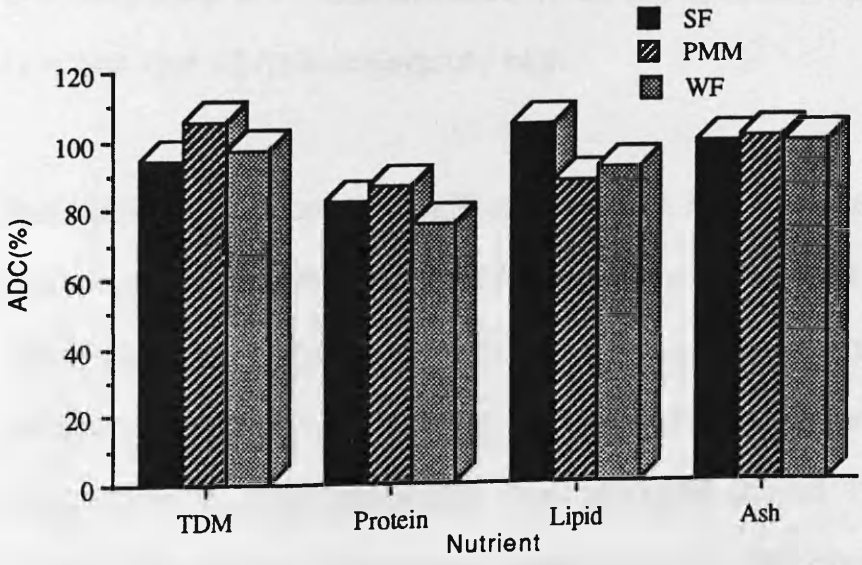


Fig.4.2: Digestibility of commercial grade soybean flour (SF), poultry meat meal and wheat flour in *Clarias gariepinus*

4.4.4 Discussion

Very high nutrient digestibilities of the feedstuffs were observed in this investigation. The choice of fish meal as the sole protein supplement in the reference diet gave high dry matter, protein, lipid and ash apparent digestibility coefficients. However, test diets containing 30% replacement of reference diet with the feedstuffs also gave high nutrient ADCs insignificantly different from those of the reference diet. Consequently, the feedstuff ADCs which are derivatives of reference and test diet ADCs were equally high.

The >100% ADC recorded for SF protein in *O. niloticus* was not unconnected with the fact that it has been reported to be equally, or more, digestible than fish meal in some fishes. The protein of dehulled, solvent-extracted soybean meal containing 49% crude protein was reported to be 85% digestible in channel catfish (Lovell, 1977), rainbow trout (Smith, 1976) and tilapia (Popma, 1982) and that these ADC were equal to or higher than those for whole fish meal protein (Lovell, 1990). The high dry matter PMM ADC in *C gariepinus* was as a result of higher dry matter ADC for the test diet containing PMM than the reference diet. De Silva et al.,(1990) recorded high dry matter and protein ADCs in *Oreochromis aureus* fed leaf meal with even higher ADCs for dry matter in night faecal collection and protein in daytime faecal collection at 10% level of replacement of the reference diet with the test feedstuff.

The excessively high feedstuff digestibilty observed in this investigation was attributed to reference diet type and level of replacement by the feedstuffs in the test diets. The conventional use of fish meal which lacks absolute digestibility superiority over other high quality feedstuffs like dehulled, toasted and solvent extracted soybean meal needs to be reconsidered. 30% replacement level is, from all indication, inadequate as all test diets were almost as digestible as the reference diet including that containing wheat flour. This level was probably insignificant to effect any change.

A significantly high level of inclusion that would make the test diet share insignificantly different nutritive value with the feedstuffs rather than reference diet may be more appropriate. The feedstuff ADC results from this investigation were more representative of fish meal ADC in the reference diet than those of SF, PMM and WF in the test diets. These postulations are not conclusive until after further investigation. It is recommended that the level of reference diet that will not alter the ingredient nutrient level significantly but be acceptable to the fish, be established, incorporated in the ingredient and used as test diet in future investigations. In vitro screening could be carried out to confirm the findings.

CHAPTER FIVE

DIGESTIBILITY, APPARENT AMINO ACID AVAILABILITY OF SOYBEAN FLOUR-POULTRY MEAT MEAL BLEND BASED DIETS FOR *OREOCHROMIS NILOTICUS* AND *CLARIAS GARIEPINUS*.

5.1 Introduction

Digestibility estimations have been a major focus in aquaculture nutrition for assessing ingredient or diet quality. However, variability still exists on the analytical method and evaluation procedure used for such estimation and an aspect of this is the use of internal or external markers (Bowen, 1978; De Silva and Perera, 1983; Tacon and Rodrigues, 1984). Higher digestibility values were recorded with the use of an internal marker (crude fibre) than an external one (Cr_2O_3) in *Oreochromis aureus* (De Silva et al, 1990).

However, the use of internal marker particularly chromic oxide has been the convention. Common levels of this marker are 0.5% and 1.0%. Comparison of two levels is uncommon leading to paucity of information on the effect of Cr_2O_3 levels on digestibility values generally. Though, this marker is biochemically inert (Maynard et al. 1979 and Bondi, 1987) but their suspected physical inactivity calls for caution in the choice of level in a digestibility study. Consequently, varying Cr_2O_3 levels (0.5% and 1.0%) were used in this experiment to establish the ideal marker level for optimum nutrient digestibility and availability in *O.niloticus* and *C.gariepinus* fed diets containing three SF:PMM blends as protein supplements. As a preamble, the water stability of the diets was evaluated taking into cognisance its role in diet disintegration and the subsequent leaching effect that often leads to over-estimation of the digestibility of heavily leached nutrient.

5.2 Fingerlings

5.2.1 Materials and Methods

i Experimental Design

Two seperate experiments were conducted to evaluate the digestibility of soybean flour (SF) - poultry meat meal (PMM) blend based diets in fingerling *Oreochromis niloticus* and *Clarias gariepinus*. For each experiment, a 2 x 3 factorial design was used and it involved 12 10-L tanks. Each treatment was duplicated. Fingerlings of *Oreochromis niloticus* (M ± S.E.M.= 22.1 ± 0.4g) and *Clarias gariepinus* (M ± S.E.M.= 23.3 ± 0.6g) were stocked at 5 per tank in a still water digestibility system. The system itself was supplied with water from an overhead tank thermoregulated at 28°C. Two randomly distributed units received the same treatment.

ii Diets, Feeding and Fish Husbandry

Six isocaloric and isonitrogenous diets (Diets I - VI) were formulated on the basis of two factors, viz ; 0.5% and 1.0% levels of Cr_2O_3 , and 25:75, 50:50 and 75:25 soybean flour (SF) : poultry meat meal (PMM) blends based diets. Table 5.1 shows the proximate analysis of the diets used. Soybean flour used was of commercial grade with a 73.63% protein

solubility index as analysed according to Araba and Dale (1990). High quality poultry meat meal was supplied by Chettles UK Ltd, Nottingham. Feedstuffs were mixed using Hobart Mixer and pelleted using California Pellet Mill (Model CL2) equipped with steam conditioner. Pelleted diets were dried by convection at 60°C overnight. After cooling diets were packed in sealed black polyethylene bags and stored at -30°C until required.

Initially fish were fed for one week to acclimate them to the diet, the system and free their guts from pre - experimental diet. They were fed for three weeks to collect sufficient faecal matter for analysis . Feed was given at 11.00 at 2% body weight. The fishes were allowed to feed for one hour after which feed remnants were flushed out. Faecal matter was collected at 10.00 the following morning, weighed and oven-dried overnight at 105°C prior to proximate and Cr_2O_3 analyses as in 3.6.1.

iii Experimental Analysis

Feedstuffs, diets and faecal matter were analysed for proximate composition in duplicate according to AOAC (1990) methods stated in 3.6.1. Water stability of diets was investigated by two methods, viz: Net immersion method, Immersing 5g of each diet in the water of the system for 30 minutes, 1, 2 and 3 hours; and Beaker method, leaving

diet in a beaker of water with occasional and regular stirring for the same time durations. Cr_2O_3 , apparent digestibilty coefficients (ADC) of the diets were estimated as in 3.6.1. Leaching effect was compensated for using a multiplier factor of nutrient ADC in fish stripped of faeces divided by that obtained from the draining method of the Guelph system. The apparent amino acid availability (AAAA) of the diets were derived from the formula for ADC and statistical analysis done as in 3.7.

Table 5.1 Level of inclusion of ingredients in the soybean flour - poultry meat meal blend based diets of *Oreochromis niloticus* and the proximate composition of the diets.

	Diets					
	25:75	SF:PMM Blend	50:50	SF:PMM Blend	75:25	SF:PMM Blend
Ingredients	I (0.5)	II (1.0)	III (0.5)	IV (1.0)	V (0.5)	VI (1.0)
Soybean flour	14.8	14.8	32.5	32.5	54.0	54.0
Poultry meat meal	44.5	44.5	32.5	32.5	18.0	18.0
Wheat flour	27.4	27.4	20.8	20.8	12.8	12.8
Soybean oil	5.2	5.2	6.2	6.2	7.3	7.3
Vitamin premix[1]	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix[2]	4.0	4.0	4.0	4.0	4.0	4.0
CMC(Binder)*	1.5	1.0	1.5	1.0	1.5	1.0
Cr_2O_3(Marker)	0.5	1.0	0.5	1.0	0.5	1.0
Proximate composition (% as fed)						
Moisture	5.2	5.8	6.1	7.5	7.4	10.2
Protein	39.1	39.4	39.2	38.5	38.8	38.3
Lipid	10.7	10.9	10.4	10.2	10.0	9.6
Ash	12.0	11.8	10.9	10.9	9.9	9.7
Energy $kcalg^{-1}$	4.5	4.5	4.5	4.4	4.4	4.4

* CMC = Carboxymethyl cellulose
Figures in parentheses represent the level of Cr_2O_3 (%) in the diets
SF, Soybean flour; PMM, Poultry meat meal.

5.2.2 Results and Discussion

i Diets Water Stability

Figs. 5.1a and b show the water stability of selected diets representing 25:75; 50:50 and 75:25 soybean flour (SF) : poultry meat meal (PMM) inclusion levels i.e diets I, III and V respectively. Diet V had higher stability than diets III and I in that order implying that diets with more SF were more water stable than those with more PMM. This trend was observed with the two methods adopted here. However, higher values were obtained with the method of stirring the diets in a beaker of water than immersing in the water of the experimental system. More than 80% and 60% of any of the diets were water stable after 3 hours with the immersion in the system water and stirring in a beaker of water methods respectively.

The higher carbohydrate content of SF than PMM provided additional binding and consequently better stability. The occasional stirrling of diet in a beaker of water caused more attrition and abrasion than recorded with net immersed diets in the water of the system.

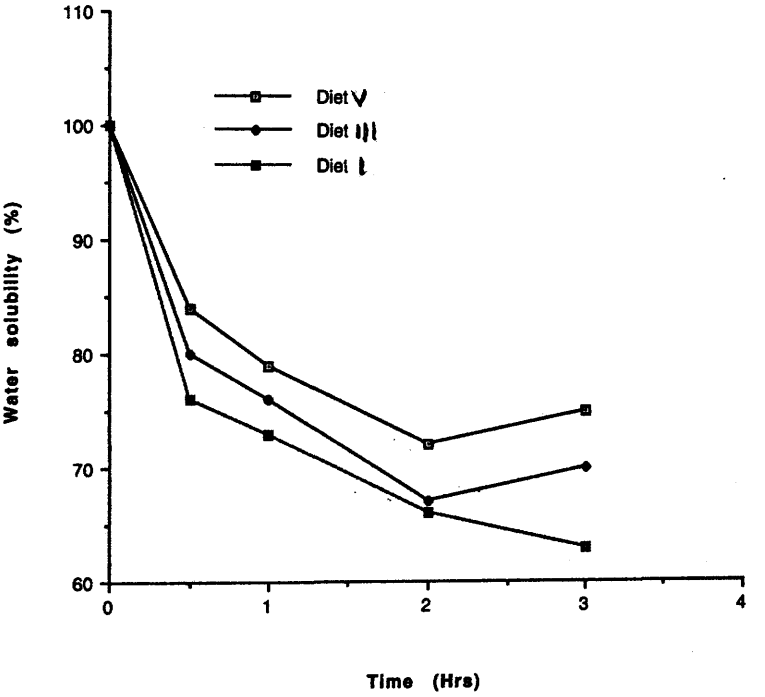


Fig.5.1a: Water stability of SF:PMM blend based diets - Beaker method.

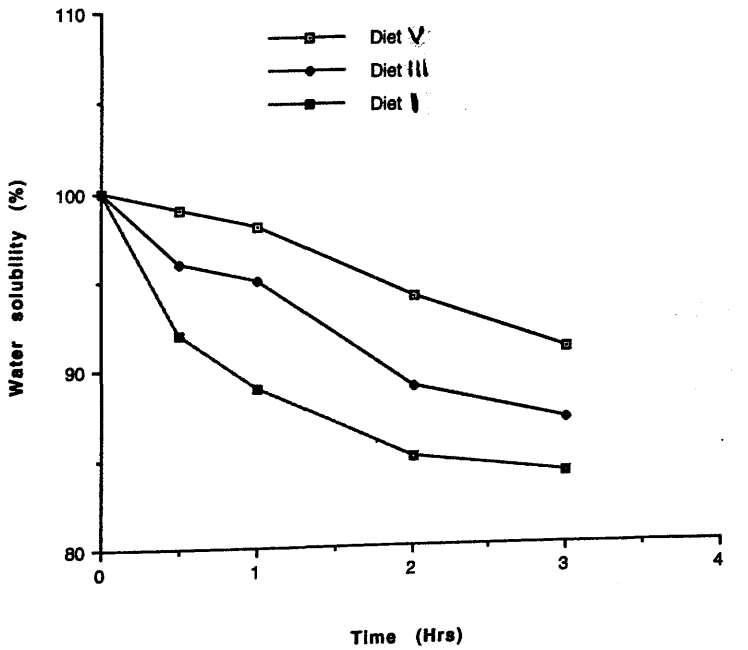


Fig.5.1b: Water stability of SF:PMM blend based diets - Net immersion method.

ii Experiment 1: Digestibility, Amino Acid Availability of SF-PMM Blend Based Diets in *Oreochromis niloticus* Fingerlings

(a) Nutrient Digestibility

Fig. 5.2 presents the dry matter (DM) protein (PR), lipid (LP) and ash digestibility coefficients of the six diets. Lipid digestion was highest of the three nutrients. Significantly higher values were obtained for diets containing 0.5% Cr_2O_3 than 1.0% Cr_2O_3 for protein and ash in most cases. The high lipid digestibiity coefficients for all the diets may be attributed to high lipase activity in fish generally (Sargent, et al 1989; De Silva and Anderson, 1995). Protein digestibilty was moderately higher and lower for diets containing 0.5 and 1.0 % Cr_2O_3 respectively. The increased level of Cr_2O_3 lowered digestibility in the latter, in implied agreement with De Silva et al. (1990) who observed that ingredients with Cr_2O_3 (external marker) had lower digestibilities than those with crude fibre (internal marker) in *Oreochromis aureus.*

Lovell (1990) reported that the protein of dehulled solvent extracted soybean meal was 85% digestible, equal to or higher than that of whole fish meal protein in channel catfish. Protein of the 75:25 SF:PMM blend based diet was better digestible than of 25:75 and 50:50 SF:PMM. The herbivorous feeding habit of tilapias (Reed et al, 1969) could be responsible for this trend of observation. This requires confirmation through further study.

Low ash ADCs relative to those of protein and lipid were observed here. This might be due to poor enzymatic digestion of ash, as digestion of inorganic matter is largely due to stomach acid (Lovell 1990). Very low ash digestibilty values have been reported for soybean meal and poultry by-products in rainbow trout (Alexis, et al 1988). The general effect of Cr_2O_3 level on ash ADC pattern that ash of diets containing 1.0% Cr_2O_3 were more digestible than those with 0.5% even after the correction for Cr_2O_3 presence in the ash. This reversed trend may need to be further investigated.

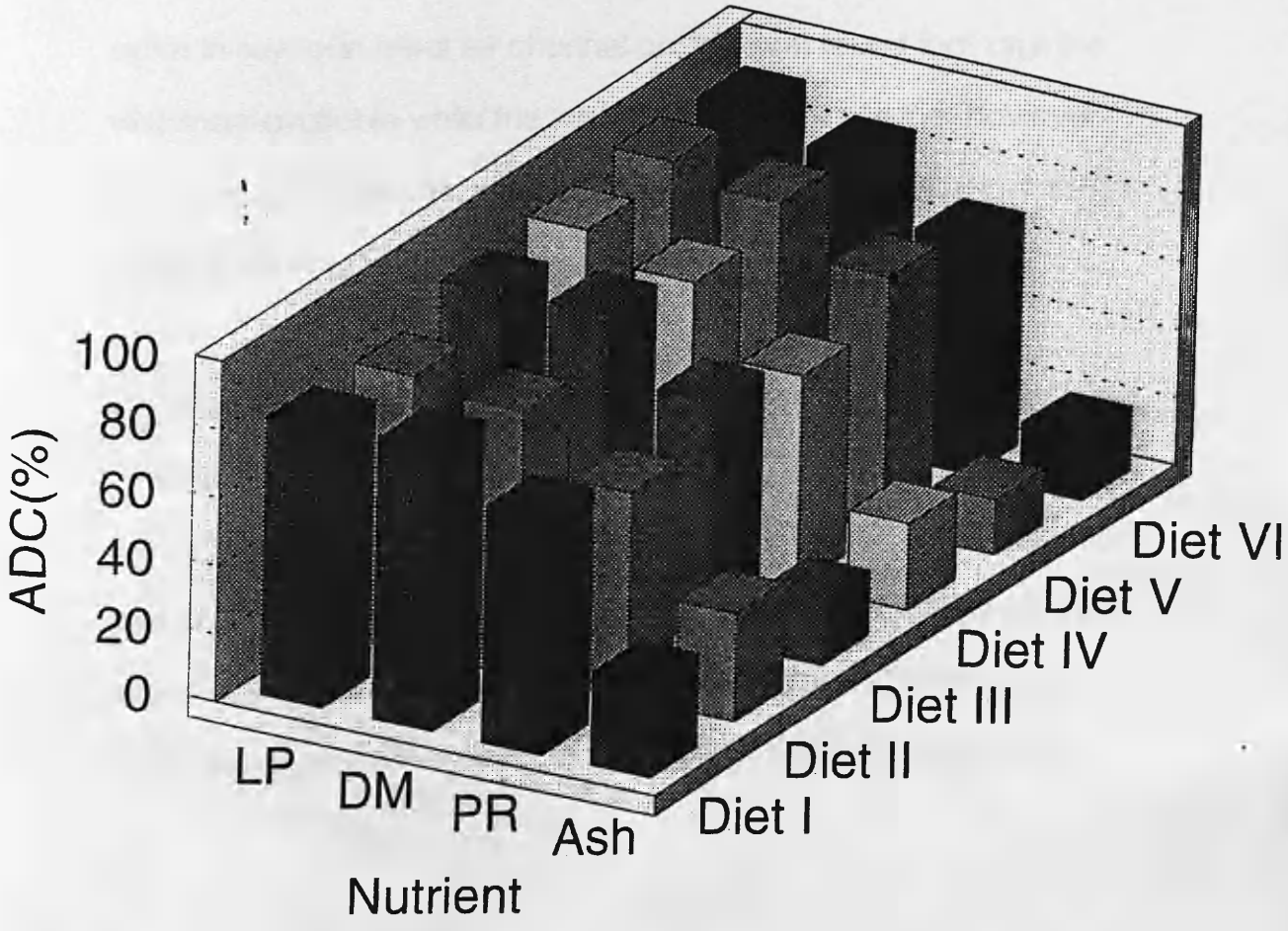


Fig.5.2: Nutrient digestibility of SF-PMM blend based diets fed to *Oreochromis niloticus* fingerlgs.

(b) Nutrient Availability

Table 5.2 shows the availability of 17 amino acids analysed. The overall pattern of apparent amino acid availability corresponds to that of protein digestibility. There was no significant difference in amino acid availability between the blending ratios of SF:PMM in the diets ($p>0.05$). Overall apparent amino acid availability was significantly higher in diets containing 0.5% than 1.0% Cr_2O_3 ($p<0.05$). Methionine had the highest availability while the least available was cystine . Wilson , et al (1981) investigated the availability of amino acids in soybean meal for channel catfish and found that arginine was most available while the least available was glycine. However, they presented the availability of methionine + cystine in combination, these have been seperated in this investigation.

Table 5.3 relates the amino acids in the diets to the requirement data of Santiago and Lovell (1988) to establish the limiting amino acids. It shows that methionine was the first limiting amino acid while Leucine was in excess. Coincidentally, methionine had the highest availability. Whether deficiency of an amino acid could possibly influence its apparent availability may need to be experimentally investigated.

Table 5.2 Amino acid (% protein) availability of soybean flour - poultry meat meal blend based diets (Diets I-VI) in *Oreochromis niloticus* fingerlings

	Diets					
EAA*	I	II	III	IV	V	VI
Arg	76.19±1.61	67.32±3.01	83.10±0.51	66.48±1.51	82.93±1.67	75.57±0.23
His	74.59±1.73	67.69±1.12	82.74±0.78	69.16±3.66	84.57±0.13	72.32±0.24
Iso	72.94±1.29	62.39±3.60	80.03±1.14	62.77±0.62	80.96±3.40	71.03±1.61
Leu	77.14±2.28	63.79±2.98	79.85±0.90	63.06±1.53	83.31±1.04	70.57±0.96
Lys	77.85±1.46	72.65±1.80	84.09±0.88	70.73±0.80	85.73±0.59	75.11±0.59
Met	89.43±1.23	85.03±1.21	91.50±0.25	69.79±2.82	93.17±0.27	89.41±0.47
Phe	74.64±2.09	62.13±3.25	79.91±1.29	64.33±4.26	82.05±1.66	71.53±0.59
Thr	71.51±1.73	56.29±3.58	77.48±1.25	58.52±0.28	80.56±0.95	68.03±2.42
Val	72.28±2.20	60.14±3.39	76.67±1.26	61.26±0.23	76.67±0.59	67.29±1.04
Non-EAA*						
Ala	76.66±1.30	72.72±2.33	77.09±1.45	64.91±0.88	78.17±2.27	65.77±8.64
Asp	73.17±1.03	57.02±3.09	80.11±1.49	62.49±0.54	74.14±0.48	72.75±2.15
Cys	83.42±2.07	65.02±2.73	82.23±2.08	57.96±0.00	85.12±2.64	69.95±3.27
Glu	78.59±2.60	67.60±0.06	82.76±0.88	66.34±0.81	83.75±4.16	75.94±0.16
Gly	79.99±2.02	75.76±3.25	82.13±1.24	68.86±2.14	79.01±6.27	72.86±1.50
Pro	78.79±1.99	72.94±2.93	83.16±0.25	67.92±2.78	84.28±1.90	73.64±0.00
Ser	72.58±1.92	57.81±3.71	79.21±1.45	60.52±1.14	84.07±2.25	70.64±1.64
Tyr	74.39±1.50	64.73±6.36	77.81±1.11	57.64±1.12	80.31±2.62	70.32±0.71
M±S.E.M.	74.95±6.97^{ab}	66.53±7.54^{a}	81.17±3.55^{c}	64.28±4.24^{a}	82.28±4.24^{c}	72.40±4.78^{ab}

*EAA, essential amino acid
Data on the same row carrying same superscript are insignificantly different (p>0.05)

Table 5.3 Essential amino acids (% protein) in soybean flour - poultry meat meal blend based diets and the requirements of *Oreochromis niloticus.*

	D		I	E	T	S	
Amino acids	I(25:75)		III(50:50)		V(75:25)		Requirement*
Arg	3.47	(82.6%)	3.46	(82.4%)	3.53	(84.1%)	4.20
His	1.78	(103.5%)	1.83	(106.4%)	1.88	(109.3%)	1.72
Iso	3.10	(99.7%)	3.02	(97.1%)	3.11	(100.0%)	3.11
Leu	5.95	(175.5%)	5.86	(172.9%)	5.86	(172.9%)	3.39
Lys	4.83	(94.3%)	4.86	(94.9%)	4.86	(94.9%)	5.12
Met	1.46	(54.5%)	1.40	(52.2%)	1.15	(42.9%)	2.68
Phe	3.60	(96.0%)	3.55	(94.7%)	3.65	(97.3%)	3.75
Thr	3.38	(90.1%)	3.20	(85.3%)	3.19	(85.1%)	3.75
Val	3.66	(130.7%)	3.63	(131.4%)	3.56	(127.1%)	2.80

Figures in parenthesisis relates amino acid requirements to amino acids present in the diets
* Source (Santiago and Lovell, 1988)

ii Experiment 2: Digestibility and Amino Acid Availability of Soybean Flour-Poultry Meat Meal Blend Based Diets in *Clarias gariepinus* Fingerlings

(a) Nutrient Digestibility

Fig. 5.3 presents the dry matter, protein, lipid and ash digestibility coefficients of the six diets. Similar, to the observation in *O.niloticus,* lipid digestion was highest of the three nutrients. The digestibility of dry matter and lipid did not reflect the level of Cr_2O_3 in the diets. Significantly higher values were obtained for diets containing 0.5% Cr_2O_3 than 1.0% Cr_2O_3 for protein and ash in most cases.

However, protein of 75:25 SF:PMM blend based diet which was best digestible than 25:75 and 50:50 SF:PMM in *O.niloticus* was not. *C.gariepinus* seemed to digest efficiently proteins of both SF and PMM hence the better protein ADCs of 50:50 SF:PMM than any other. The omnivorous feeding habit which tends towards carnivory (Reed et al, 1969) could be responsible. This is not conclusive as it will need to be further investigated. Pattern of ash digestibility was also similar to that of *O.niloticus.*

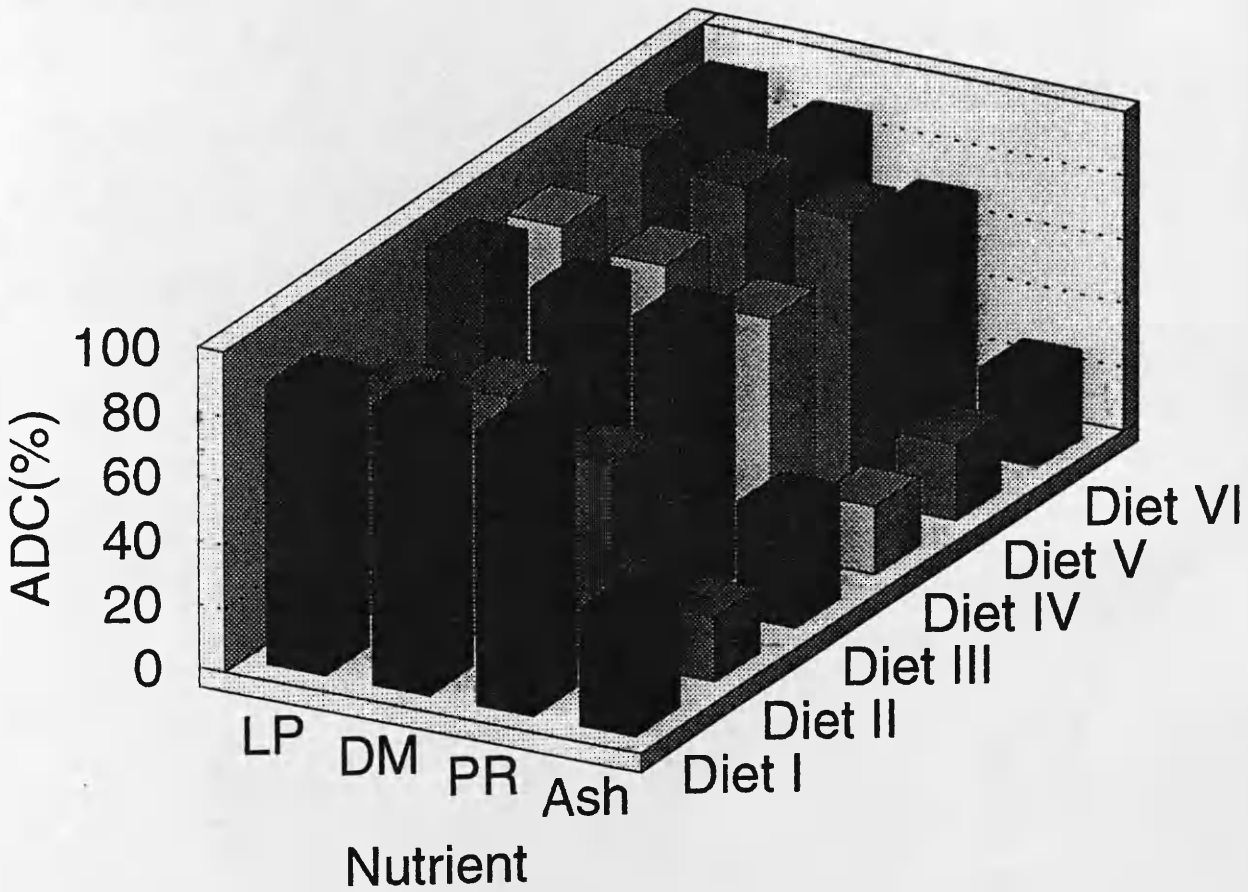


Fig.5.3: Nutrient digestibility of SF-PMM blend based diets fed to *Clarias gariepinus* fingerlings.

(b) Nutrient Availability

Table 5.4 shows the availability of 17 amino acids analysed. The overall pattern of apparent amino acid availability corresponds to that of protein digestibility and similar to that of *O.niloticus*. There was no significant difference in amino acid availability between the blending ratios of SF:PMM in the diets ($p>0.05$).

Table 5.5 relates the amino acids in the diets to the requirements data of NRC (1983) to establish the limiting amino acids. It shows that methionine was the first limiting amino acid while Leucine was in excess following the same trend as in *O.niloticus*.

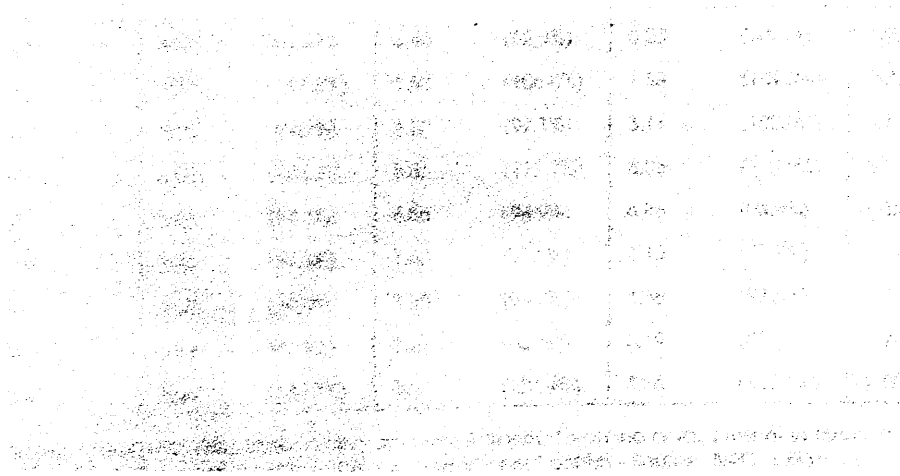


Table 5.4 Amino acid (% protein) availability of soybean flour - poultry meat meal blend based diets (Diets I-VI) fed to *Clarias gariepinus* fingerlings

	Diets					
EAA	I	II	III	IV	V	VI
Arg	86.28±0.65	45.89±5.76	86.99±0.25	62.87±1.73	90.65±0.46	82.12±0.05
His	89.12±0.49	60.98±1.08	89.06±0.30	70.05±0.16	91.25±0.49	69.85±0.21
Iso	85.42±0.56	44.59±5.27	85.22±0.64	63.94±1.15	89.71±0.40	64.73±1.36
Leu	87.04±0.93	55.69±3.71	86.67±0.42	67.32±0.77	89.04±0.33	67.60±0.81
Lys	89.97±0.44	64.56±2.34	89.31±0.34	71.23±0.23	91.56±0.38	77.74±0.45
Met	92.62±0.44	72.14±2.36	93.15±0.08	82.26±0.18	93.90±0.03	78.56±0.25
Phe	87.23±0.74	57.89±3.41	86.79±0.59	66.83±1.16	91.75±2.06	68.22±0.43
Thr	85.83±1.30	52.34±4.02	85.50±0.55	64.92±0.00	89.70±0.25	67.71±1.74
Val	85.29±0.89	45.57±5.34	84.49±0.59	64.47±3.68	89.12±0.56	64.27±0.83
Non-EAA						
Ala	89.04±0.40	60.94±3.39	87.47±0.55	67.31±1.02	89.29±0.76	67.28±5.80
Asp	87.54±0.34	54.10±3.24	87.89±0.42	67.59±1.29	90.01±0.13	69.94±1.58
Cys	83.92±0.53	44.12±2.01	87.44±1.07	67.45±1.63	90.94±0.09	67.22±2.68
Glu	90.03±0.78	61.79±0.05	89.67±0.32	71.20±1.03	92.35±0.18	72.50±0.13
Gly	89.35±0.73	69.77±4.03	88.04±0.54	69.42±2.00	90.13±0.34	71.93±1.21
Pro	88.32±0.77	60.94±4.29	88.85±0.14	66.64±1.51	90.66±0.23	71.62±0.00
Ser	86.28±0.72	55.65±4.05	85.90±0.72	66.76±0.93	90.02±0.38	68.71±1.15
Tyr	89.00±0.39	61.65±0.00	89.70±0.40	70.89±1.24	92.17±0.20	73.01±0.41
M±S.E.	$87.94±2.00^{b}$	$58.62±7.50^{a}$	$88.00±1.14^{cd}$	$68.41±1.84^{a}$	$90.82±1.05^{d}$	$73.01±2.33^{bc}$

Data on the same row carrying same superscript are insignificantly different (p>0.05)

Table 5.5 Essential amino acids (% protein) in soybean flour - poultry meat meal blend based diets and their requirements of *Clarias gariepinus*.

	Diets					
Amino acids	I(25:75)		III(50:50)		V(75:25)		*
Arg	3.47	(82.6%)	3.46	(82.4%)	3.53	(84.1%)	4.20
His	1.78	(103.5%)	1.83	(106.4%)	1.88	(109.3%)	1.72
Iso	3.10	(99.7%)	3.02	(97.1%)	3.11	(100.0%)	3.11
Leu	5.95	(175.5%)	5.86	(172.9%)	5.86	(172.9%)	3.39
Lys	4.83	(94.3%)	4.86	(94.9%)	4.86	(94.9%)	5.12
Met	1.46	(54.5%)	1.40	(52.2%)	1.15	(42.9%)	2.68
Phe	3.60	(96.0%)	3.55	(94.7%)	3.65	(97.3%)	3.75
Thr	3.38	(90.1%)	3.20	(85.3%)	3.19	(85.1%)	3.75
Val	3.66	(130.7%)	3.63	(131.4%)	3.56	(127.1%)	2.80

Figures in parenthesisis relates amino acid requirements to amino acids present in the diets
* Requirement level of a related species - the channel catfish - Source (NRC, 1983)

5.3 Juveniles

5.3.1 Materials and Methods

Two parallel experiments were conducted to evaluate the digestibility of soybean flour (SF) - poultry meat meal (PMM) blend based diets in juvenile *Oreochromis niloticus* and *Clarias gariepinus*. For each experiment, a 2 x 3 factorial design was used and it involved 12 50-L tanks per experiment. Each treatment was duplicated. Juveniles of *Oreochromis niloticus* (M ± S.E.M.= 55.3 ± 0.9g) and *Clarias gariepinus* (M ± S.E.M.= 52.9 ± 2.0g) were stocked at 10 per tank in a recirculatory feeding trial system. Two randomly distributed units received the same treatment. Diets, preparation, feeding of fish was same as in 5.2. Experimental analyses,feedstuffs, diets and faecal matter were carried out as in 3.6.1. Digestibility coefficients and availability of nutrients were measured as in 3.6.2 and statistical analyses were carried out as in 3.7.

5.3.2 Results and Discusion

i Experiment 1: Digestibility and Amino Acid Availability of Soybean Flour-Poultry Meat Meal Blend Based Diets in *Oreochromis niloticus* Juveniles

(a) Nutrient Digestibility

The dry matter, protein, lipid and ash apparent digestibility coefficients (ADCs) of the six diets are depicted in Fig.5.4. Lipid digestion was highest. However, it was not significantly affected by the levels of the marker, either 0.5% Cr_2O_3 or 1.0% Cr_2O_3 ($P>0.05$). Dry matter had markedly higher ADCs in the diets with 0.5% Cr_2O_3 than those with 1.0%, while the reverse was the case for protein and ash ADCs ($P<0.05$).

The high lipid digestibility coefficients for all the diets may be attributed to high lipase activity in fish generally (Sargent, Henderson and Tocher, 1989; De Silva and Anderson, 1994). Protein digestibility was moderately high and low for diets containing 1.0 and 0.5% Cr_2O_3 respectively. The increased level of Cr_2O_3 decreased dry matter digestibility, increased protein digestibility and seemed to increase ash digestibility. This implies that certain nutrient digestibility could increase with increase in Cr_2O_3 from 0.5% to 1.0%. This could be due to the

ability of the juvenile *O.niloticus* to tolerate the marker better than the fingerlings as a result of its maturity.

Protein of 75:25 SF:PMM blend based diet (diets V and VI) was more digestible than 25:75 SF:PMM (diets I and II) and 50:50 SF:PMM (diets III and IV) in agreement with the observation in the fingerling and that of Lovell, et al (1990). Effect of Cr_2O_3 level on ash ADC pattern was in agreement with that of protein as diets containing 1.0% Cr_2O_3 were morer digestible than those of 0.5%.

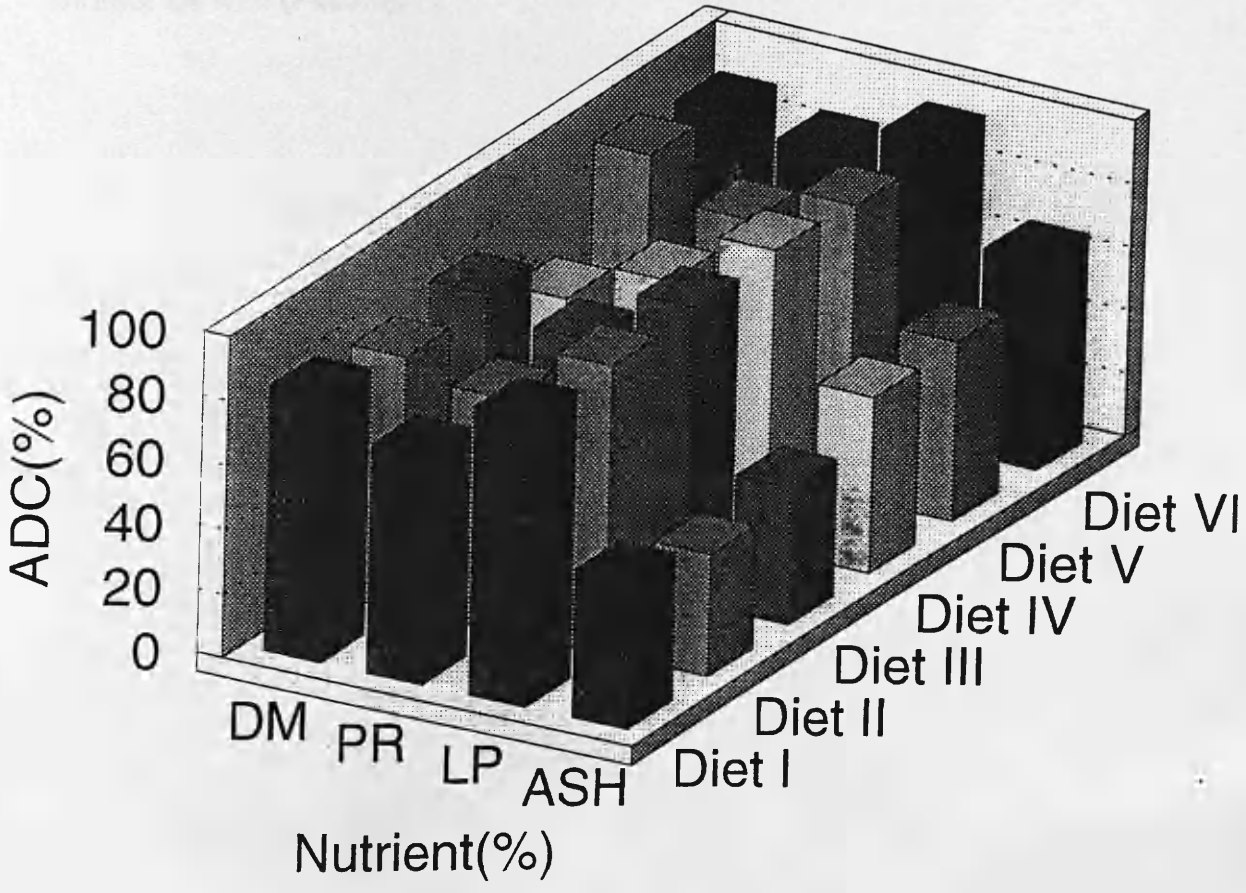


Fig.5.4: Nutrient digestibility of SF-PMM blend based diets fed to *Oreochromis niloticus* juveniles.

(b) Nutrient Availability

The availability of 17 amino acids analysed are shown in Table 5.6. The overall pattern of apparent amino acid availability corresponds to that of protein digestibility. There was significant difference in amino acid availability between the blending ratios of SF:PMM in the diets ($p<0.05$). Average AAAA of 75:25 SF:PMM blend based diets was higher than those of 25:75 SF:PMM and 50:50 SF:PMM. There was significant variation in the individual amino acid availability and the marker as well ($P<0.05$).

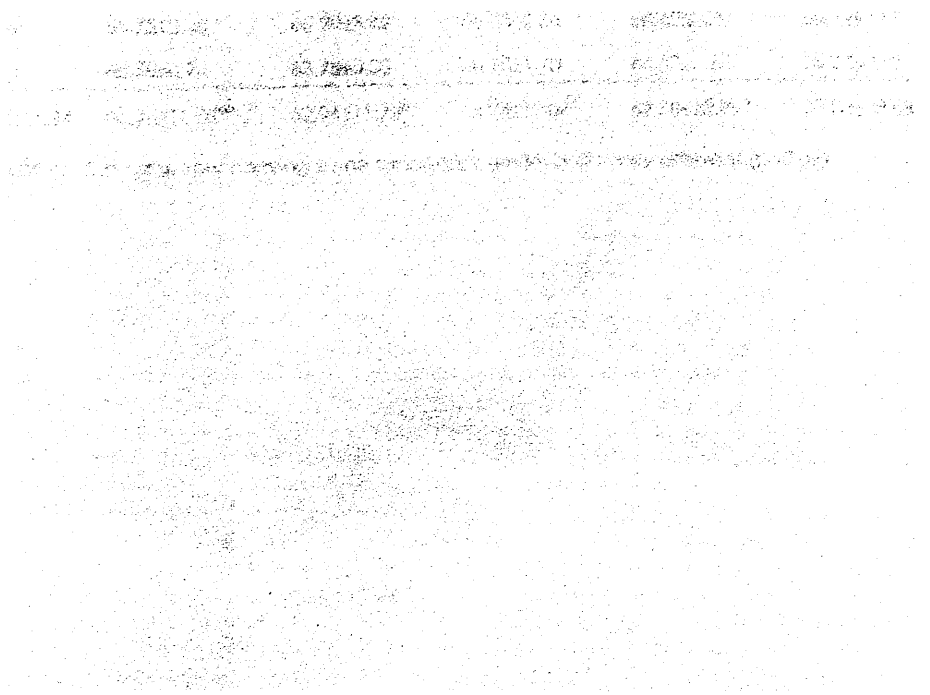


Table 5.6 Amino acid (% protein) availability of soybean flour - poultry meat meal blend based diets (Diets I-VI) in juvenile *Oreochromis niloticus*

	Diets					
EAA	I	II	III	IV	V	VI
Arg	65.84±2.30	61.89±3.51	67.88±0.97	65.44±1.76	76.53±1.57	77.42±0.21
His	83.61±1.11	77.36±0.78	79.56±0.93	77.46±1.99	79.90±1.48	83.00±0.14
Iso	68.91±1.49	66.73±3.20	69.68±1.73	67.30±0.74	73.91±0.75	78.22±1.21
Leu	70.71±2.92	68.83±2.56	70.19±1.34	67.55±1.10	71.02±2.90	69.28±1.00
Lys	82.40±1.17	76.33±1.55	77.34±1.25	72.28±1.94	72.19±1.22	77.17±0.55
Met	87.01±1.51	86.50±1.10	82.18±0.54	80.57±0.64	95.97±0.32	87.80±0.46
Phe	68.39±2.60	65.71±2.94	69.10±1.98	67.09±2.01	70.97±0.00	69.13±0.65
Thr	61.45±5.95	58.32±3.42	63.90±2.01	58.58±1.76	65.15±1.11	61.51±2.92
Val	51.00±3.88	62.43±3.74	38.82±3.29	40.07±0.00	67.37±1.23	65.34±1.11
Non-EAA						
Ala	56.76±2.41	54.62±3.87	57.25±2.71	56.49±2.74	55.67±7.92	46.61±13.34
Asp	68.87±1.19	63.52±2.26	67.80±2.41	62.99±1.26	73.33±0.45	72.22±2.15
Cys	50.10±2.22	46.70±4.16	49.72±5.88	48.42±2.25	63.81±3.12	62.10±4.12
Glu	74.31±3.13	70.65±0.05	68.07±1.63	62.54±3.60	78.34±0.58	77.57±0.15
Gly	43.96±5.64	43.32±7.60	42.28±4.03	42.05±4.27	47.08±1.46	45.54±3.00
Pro	61.18±3.65	43.07±6.19	56.07±0.64	66.62±2.58	57.72±3.51	60.03±0.00
Ser	64.75±2.46	60.29±3.49	66.03±2.36	60.62±0.39	69.22±0.89	65.06±1.66
Tyr	68.78±0.74	63.78±0.00	66.15±1.70	64.43±1.74	75.24±0.45	71.51±0.67
M±SE.M	66.35±11.82^{ab}	62.94±11.73^{b}	64.24±12.04^{a}	62.38±10.95^{a}	70.20±10.88^{ab}	68.82±11.54^{ab}

Data on the same row carrying same superscript are insignificantly different (p>0.05)

ii Experiment 2: Digestibility of Soybean Flour-Poultry Meat Meal Blend Based Diets in *Clarias gariepinus* Juveniles.

(a) Nutrient Digestibility

Fig. 5.5 presents the dry matter, protein, lipid and ash digestibility coefficients of the six diets. Lipid digestion was highest of the three nutrients and dry matter. Only ash digestibility reflected the level of Cr_2O_3 in the diets. Significantly higher values were obtained for diets containing 0.5% Cr_2O_3 than 1.0% Cr_2O_3. ($P<0.05$).

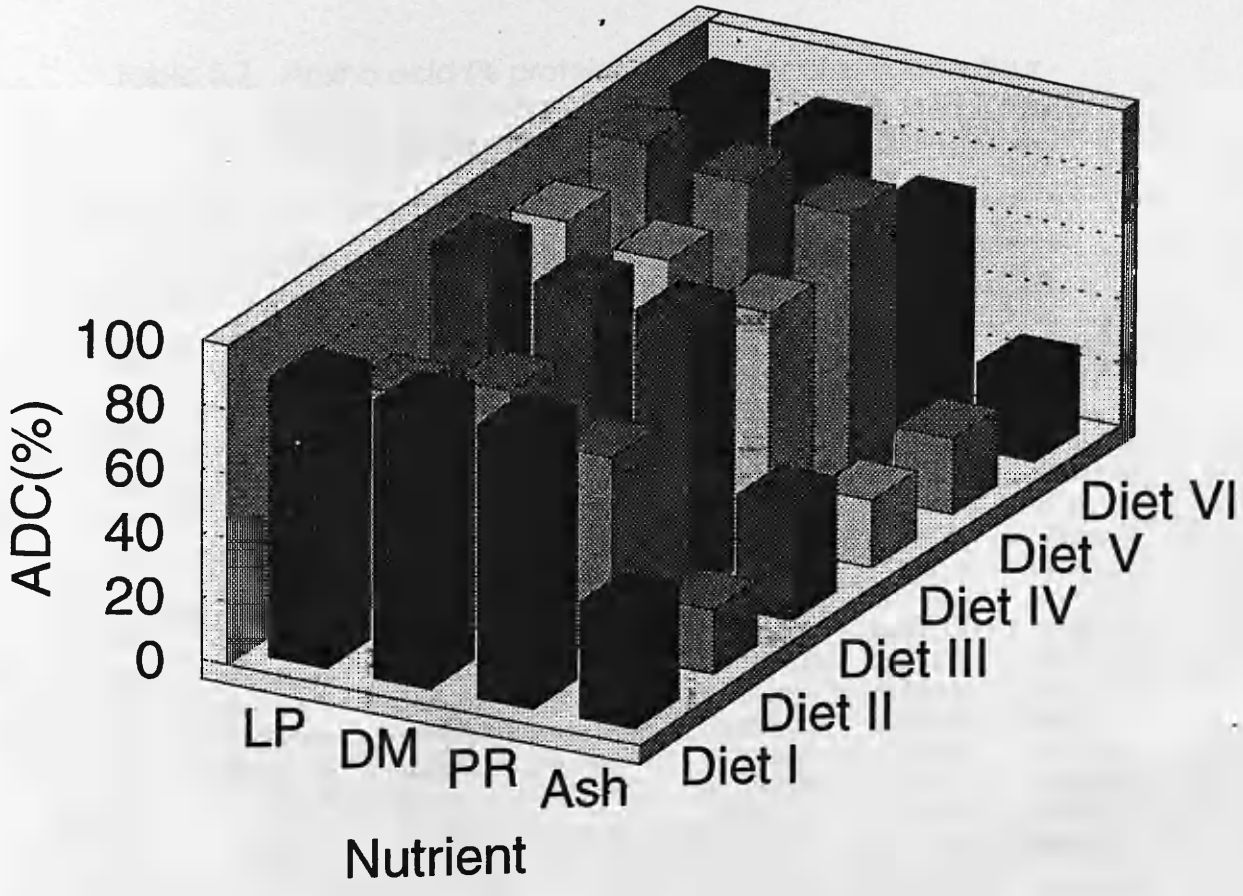


Fig.5.5: Nutrient digestibility of SF-PMM blend based diets fed to *Clarias gariepinus* juveniles.

(b) Nutrient Availability

Table 5.7 presents the availability of 17 amino acids analysed. The overall pattern of apparent amino acid availability corresponds to that of protein digestibility. Overall apparent amino acid availability was not significantly higher in diets containing 0.5% than 1.0% Cr_2O_3 ($p>0.05$). Similarly, there was no significant difference in the amino acid availability between the blending ratios of SF:PMM in the diets ($p>0.05$). This could be due to the ability of the more mature juvenile *C. gariepinus* to tolerate the marker better than the fingerlings.

Table 5.7 Amino acid (% protein) availability of soybean flour - poultry meat meal blend based diets (Diets I-VI) fed to *Clarias gariepinus* juveniles

	Diets					
EAA	I	II	III	IV	V	VI
Arg	77.51±9.72	72.56±2.47	79.73±6.10	76.82±4.91	90.04±10.01	90.98±8.02
His	91.97±1.22	84.94±3.39	87.35±3.35	85.14±6.63	87.81±6.04	91.17±4.43
Iso	85.27±3.17	82.54±2.68	86.20±0.81	83.28±2.20	91.46±2.33	96.77±0.01
Leu	87.73±4.66	85.36±2.16	87.05±0.62	83.79±0.37	88.08±2.25	85.93±0.23
Lys	90.53±1.44	83.86±1.56	84.97±1.24	79.41±1.99	79.31±1.48	84.78±0.56
Met	95.71±1.67	95.14±1.20	90.39±0.59	88.62±0.71	93.92±5.81	89.18±0.54
Phe	84.86±4.24	86.44±4.31	90.97±6.00	88.32±5.72	93.56±8.85	91.09±7.76
Thr	76.32±8.22	72.36±3.45	79.30±1.63	72.70±1.39	80.87±2.26	76.33±2.79
Val	63.33±5.51	77.47±3.80	49.39±5.28	50.94±1.14	85.62±0.35	83.07±3.27
Non-EAA						
Ala	70.46±3.76	67.76±4.06	71.04±2.58	70.10±2.64	69.05±9.07	57.77±15.92
Asp	85.50±2.41	78.82±2.39	84.14±2.07	78.18±0.71	91.03±1.55	90.26±1.68
Cys	62.20±3.44	57.93±4.54	61.84±6.87	60.24±2.35	79.37±3.30	77.55±1.34
Glu	92.26±4.89	87.69±1.03	84.48±1.09	77.65±1.13	97.24±1.80	96.28±0.88
Gly	54.60±7.59	53.71±8.85	52.45±4.44	52.17±4.74	58.45±2.43	56.51±3.12
Pro	75.92±3.70	53.42±7.09	69.60±0.02	82.71±4.12	71.68±5.14	74.52±0.82
Ser	80.39±3.93	74.82±3.51	81.95±2.03	75.24±0.35	85.53±1.50	80.38±1.68
Tyr	85.32±1.93	79.12±0.94	82.04±1.13	79.94±3.10	93.34±1.66	88.69±0.21
M±S.E.M.	79.99±11.70[a]	76.11±12.07[a]	77.82±12.68[a]	75.60±11.39[a]	84.49±10.87[a]	83.01±11.69[a]

Data on the same row carrying same superscript are insignificantly different ($p>0.05$)

CHAPTER SIX

SOYBEAN FLOUR-POULTRY MEAT MEAL BLENDS AS DIETARY PROTEIN SOURCES IN PRACTICAL DIETS OF *OREOCHROMIS NILOTICUS* and *CLARIAS GARIEPINUS*

6.1 Introduction

Generally, high dietary levels of plant proteins or complete substitution of animal proteins has resulted in poor growth and feed efficiency in fish for reasons aforementioned (Jackson et al.,1982; Viola et al.,1983; Dabrowski et al.,1989; Lim,1992). There is therefore limited use of plant proteins in aquaculture feeds. Much improvement is required when a plant protein use in a fish diet is desirable. Soybean could be enriched by supplementing it with nutrients that are deficient in their simple or compound forms.

Blending with feedstuffs that are rich in such nutrients could be promising.It has been reported that soybean nutrient imbalance (particularly EAA) could be improved by the incorporation of low cost animal protein such as poultry meat meal - this approach has been found to be economical in catfish diet (Mohsen and Lovell, 1990). Past experiments have established the successful utilisation of poultry by-product meal (PBM) in fish diets (Fowler,1981a,b; 1982; 1991; Steffens,1994). Therefore, this study was designed to investigate the nutritional improvement of soybean flour by the incorporation of poultry meat meal as blend in the practical diets of *Oreochromis niloticus* and *Clarias gariepinus*.

6.2 Materials and Methods

Oreochromis niloticus (M±S.E = 18.2±0.5g) and *Clarias gariepinus* (M±S.E.=8.2±0.5g) fingerlings were stocked in duplicate (10 fish/ tank) in 50-L tanks and fed four diets containing soybean flour(SF) - poultry meat meal(PMM) blends by weight, viz; reference diet (100:00 SF:PMM), diet I (75:25 SF:PMM), diet II (50:50 SF:PMM) and diet III (25:75 SF:PMM), at 4% body weight per day, twice daily at 09:30 and 17:00 for eight weeks. Fish were bulk- weighed fortnightly and feeding rates adjusted accordingly. The tanks were cleaned twice a week by siphoning off bottom wastes in addition to the self cleaning mechanism of the recirculatory system. Water flow rate was maintained at 2L min^{-1} per tank.

Water quality parameters were monitored fortnightly as in 3.5.2 and recorded thus: Temperature, 26±0.0 °C; dissolved oxygen (DO), 5.2±0.3 mgL^{-1}; pH, 6.6±0.1; NH_3-N, 0.3±0.1 mgL^{-1}; NO_2^--N, 0.2±0.0 mgL^{-1}; NO_3^--N, 12.5±2.5 mgL^{-1}; Ca-hardness, 61.8±3.3 mgL^{-1} and total hardness, 77.3± 1.5 mgL^{-1}.

The formulation and proximate composition of the experimental diets are shown in Table 6.1 Soybean flour used was of commercial grade with a 73.63% protein solubility index as analysed according to Araba and Dale (1991). Poultry meat meal was supplied by Chettles UK ltd, Nottingham. Pellets of 3mm diameter 5mm long were prepared using

a California Pellet Mill (model CL2) equipped with a steam conditioner and subsequently dried by convection at 60°C overnight. After cooling, diets were packed in polythene bags and stored at -30°C.

Chemical analyses of feedstuffs, diets and carcass were performed according to AOAC (1990) as described in 3.6.1. Eight specimens were used for the initial carcass analysis while four specimens per tank were processed for the final carcass analysis per species. Data were analysed statistically according to the procedure in 3.7

Table 6.1 Percentage composition and proximate nutrient content of diets fed to *Oreochromis niloticus* and *Clarias gariepinus*

Ingredients	Reference Diet (100:00)	Diet I (75:25)	Diet II (50:50)	Diet III (25:75)
Soybean flour	80.5	54.0	32.5	14.8
Poultry meat meal	-	18.0	32.5	44.5
Wheat flour	2.8	12.8	20.8	27.4
Soy oil	8.7	7.3	6.2	5.2
Vitamin premix[1]	2.0	2.0	2.0	2.0
Mineral premix[2]	4.0	4.0	4.0	4.0
Binder(CMC)[3]	2.0	2.0	2.0	2.0
Proximate analysis(% as fed)				
Moisture	7.6	6.3	5.7	5.3
Crude protein	38.9	38.1	38.8	38.3
Lipid	10.2	10.3	10.8	11.1
Crude fibre	4.7	3.8	2.8	2.6
Ash	8.4	9.5	10.8	11.1
Gross energy ($Kcalg^{-1}$) -as calculated	4.5	4.5	4.5	4.5

[3] CMC (Carboxymethyl cellulose)
Figures in parenthesis represent blending ratio of soybean flour and poultry meat meal.
Constituents of vitamin and mineral premixes are as in Table 2.6

6.3 Results

Experiment 1 - *Oreochromis niloticus*

No significant differences were observed in the mean initial weights (8.2±0.5g) and mean final weights (23.1±1.5g) of *O. niloticus* while a trend of significant variation was observed in their SGR, FCR, PER and ANPU among the diets ($P<0.10$). Diet II was best utilised with favourable SGR , FCR and PER. Conversely, reference diet was recorded poor FCR, SGR, PER and ANPU. Mortality was only recorded for the reference diet and this was insignificantly ($P>0.05$) (Table 6.2). Diets I and III produced growth responses between those of diet II and the reference as depicted in Fig. 6.1

Table 6.2 Evaluation of soybean flour-poultry meat meal blend utilisation in practical diets of *O.niloticus* fed for 56 days.

Parameter.	Reference (100:00)	Diet I (75:25)	Diet II (50:50)	Diet III (25:75)	± Pooled S.E.M.
Mean initial weight(g)	8.3 ± 0.6^{a}	8.2 ± 0.6^{a}	8.2 ± 0.2^{a}	8.1 ± 0.5^{a}	± 0.5
Mean Final Weight(g)	20.0 ± 1.8^{a}	23.7 ± 0.1^{a}	25.0 ± 2.0^{a}	23.6 ± 1.1^{a}	± 1.5
SGR (% Day^{-1})	1.6^{a}	1.9^{ab}	2.00^{b}	1.9^{ab}	±0.00
FCR	1.9 ± 0.1^{b}	1.6 ± 0.1^{a}	1.5 ± 0.1^{a}	1.6 ± 0.0^{a}	± 0.1
PER	1.5 ± 0.1^{a}	1.8 ± 0.2^{b}	1.9 ± 0.1^{b}	1.8 ± 0.0^{b}	± 0.1
ANPU (%)	23.5 ± 0.0^{a}	27.5 ± 0.1^{ab}	29.5 ± 0.0^{b}	30.0 ± 0.0^{b}	± 0.0
Mortality(%)	2.6 ± 2.6^{a}	0.0^{a}	0.0^{a}	0.0^{a}	± 0.7

Figures in the same row carrying the same superscript are statistically insignificant ($p>0.10$)
Figures in parenthesis are the blending ratios of the blend constituents (soybean flour: Poultry meat meal).

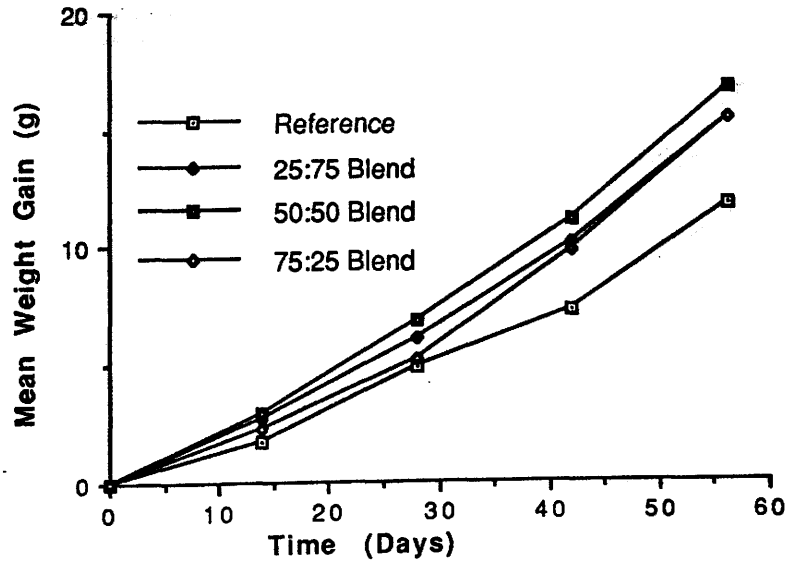


Fig. 6.1: Growth response of *Oreochromis niloticus* fed SF-PMM blend based diets.

Table 6.3 shows the amino acid composition of the diets vis-a-vis their requirements in *O.niloticus* as established by Santiago and Lovell (1988). Methionine + cystine was observed to be the first limiting amino acid followed by phenylalanine + tyrosine, threonine and lysine in that order. There was excess of leucine in all the diets. Increasing PMM incorporation increased methionine and reduced leucine in the diets. Carcass composition of the fingerlings fed the four diets is shown in Table 6.4. Protein, lipid and ash varied significantly with the diets ($P<0.05$). Protein content was highest in fingerlings fed diet III and lowest in fingerlings fed diet I. Fingerlings fed diet III had the highest lipid content while the lowest lipid and ash values were recorded in fishes fed the reference diet. There was a general increase in carcass lipid and ash with increasing poultry meat meal in the diets.

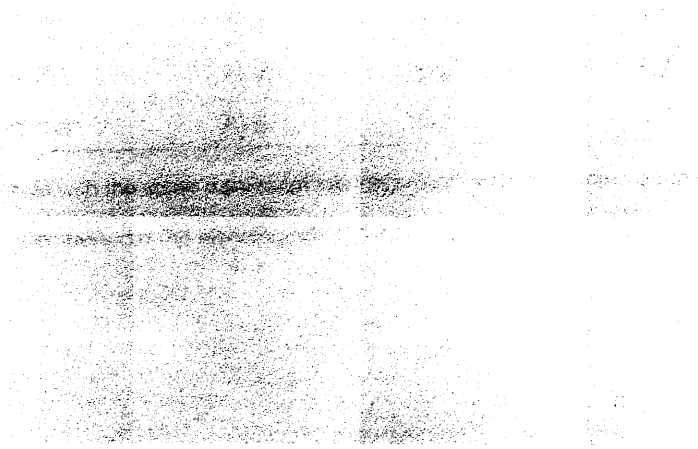


Table 6.3 Amino acid composition of soybean flour- poultry meat meal blend based diets fed to fingerlings of *Oreochromis niloticus* and their requirements.

Amino acid (% protein)	Reference Diet(100:00)	Diet I (75:25)	Diet II (50:50)	Diet III (25:75)	*
Arg	5.6(133.3)	5.3(126.2)	5.2(123.8)	5.0(119.0)	4.2
His	2.1(123.5)	2.0(117.6)	1.8(105.9)	1.8(105.8)	1.7
Iso	3.3(106.5)	3.1(100.0)	2.9(93.5)	2.8(90.3)	3.1
Leu	5.9(174.6)	5.6(164.7)	5.6(164.7)	5.5(161.8)	3.4
Lys	4.6(90.2)	5.0(98.0)	4.7(92.2)	5.1(100.0)	5.1
Met+Cys	1.1(34.4)	1.3(40.6)	1.4(43.8)	1.5(46.9)	3.2
Phe+Tyr	5.2(94.5)	4.8(87.3)	4.5(81.8)	4.2(76.4)	5.5
Thr	3.1(81.6)	3.2(84.2)	3.3(84.2)	3.4(89.5)	3.8
Val	3.4(121.4)	3.4(121.4)	3.5(125.0)	3.7(132.1)	2.8

Figures in parenthesis represent amino acid content in the diets expressed as percentages of their requirements.

* Essential amino acid requirements - Source : Santiago and Lovell (1988).

Table 6.4 Carcass composition of *Oreochromis niloticus* fed soybean flour-poultry meat meal blends based practical diets for 56 days

Diet	Moisture	Protein	Lipid	Ash
Reference(100:00)	74.1^{a}	15.9^{ab}	5.6^{a}	3.6^{a}
Diet I(75:25)	73.4^{a}	15.4^{a}	6.5^{ab}	4.2^{ab}
Diet II(50:50)	70.6^{a}	16.0^{b}	7.3^{ab}	4.5^{b}
Diet III(75:25)	71.7^{a}	16.2^{b}	8.2^{b}	4.6^{b}
Initial sample	70.5	15.3	8.7	3.4

Column figures with the same superscript are insignificantly different fom each other ($p>0.05$)

Figures in parenthesis are the blending ratios of the blend constituents soybean flour : poultry meat meal).

Experiment 2 - *Clarias gariepinus*

No significant differences were observed in the mean initial weight (19.4±1.8g) and the mean final weight (69.2±7.7g) of *C. gariepinus* fingerlings (P>0.05) (Table 6.5). A trend was observed in the performance of fingerlings fed the diets. Diet recorded the best SGR, PER and ANPU and lowest FCR values. The reverse was the case for fingerlings fed reference diet as they had the poorest FCR, SGR, PER and ANPU, and these varied significantly with the diets (P<0.10). Diet I had better FCR and PER than diet III except for ANPU. Insignificant mortality was recorded only in diets I and III (P>0.05). Diets I and III showed a performance intermediate between the reference diet and diet II shown in Fig. 6.2.

Table 6.5 Evaluation of soybean flour-poultry meat meal blend utilisation in practical diets of *Clarias gariepinus* fed for 56 days.

Parameter.	Reference (100:00)	Diet I (75:25)	Diet II (50:50)	Diet III (25:75)	±S.E.M
Mean initial weight(g)	19.6±2.9^{a}	19.8±0.6^{a}	20.3±1.5^{a}	17.8±1.5^{a}	± 1.8
Mean Final Weight(g)	51.1±1.5^{a}	70.4±7.6^{a}	93.2±5.7^{a}	65.2±12.2^{a}	± 7.7
SGR (% Day^{-1})	1.7^{a}	2.3^{ab}	2.7^{b}	2.3^{ab}	± 0.0
FCR	1.6±0.2^{a}	1.2±0.1^{a}	1.0±0.0^{a}	1.3±0.2^{a}	± 0.1
PER	1.8±0.2^{a}	2.4±0.1^{a}	2.8±0.0^{a}	2.2±0.3^{a}	± 0.2
ANPU (%)	31.5±0.1^{a}	40.0±0.0^{a}	47.5±0.0^{a}	40.4±0.4^{a}	± 0.2
Mortality(%)	0.0^{a}	2.6^{a}	0.0^{a}	2.6^{a}	±0.00

Data on the same row with the same superscript are statistically insignificant (p>0.10)
Figures in parenthesis are the blending ratios of the blends constituents (SF:PMM).

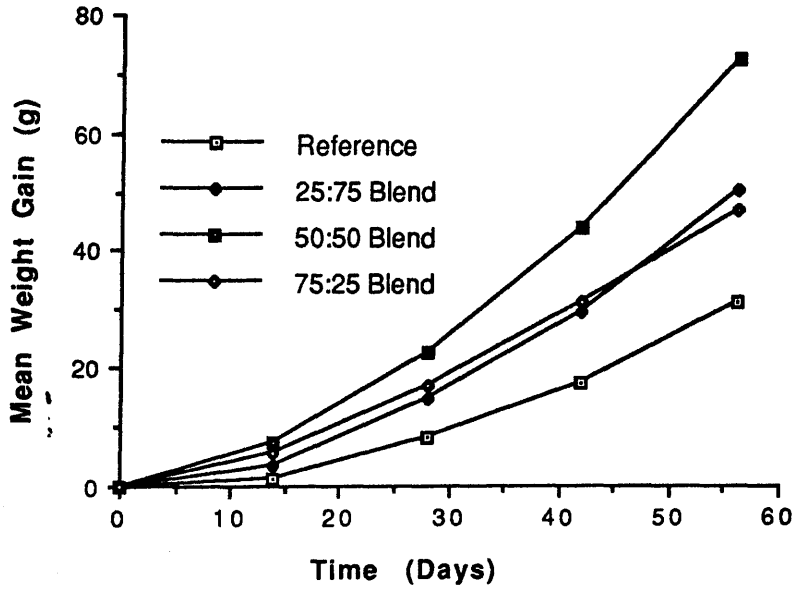


Fig. 6.2: Growth response of *Clarias gariepinus* fed SF-PMM blend based diets.

The amino acid composition of the diets as in Table 6.6 shows that methionine + cysteine was the first limiting amino acid, followed by phenylalanine + tyrosine and lysine in that order. There was excess of leucine in all the diets and increasing the level of PMM in the diets increased methionine + cysteine but decreased phenylalanine + tyrosine and leucine. No significant differences were observed in the carcass proximate composition of *C. gariepinus* fed the diets ($P>0.05$).

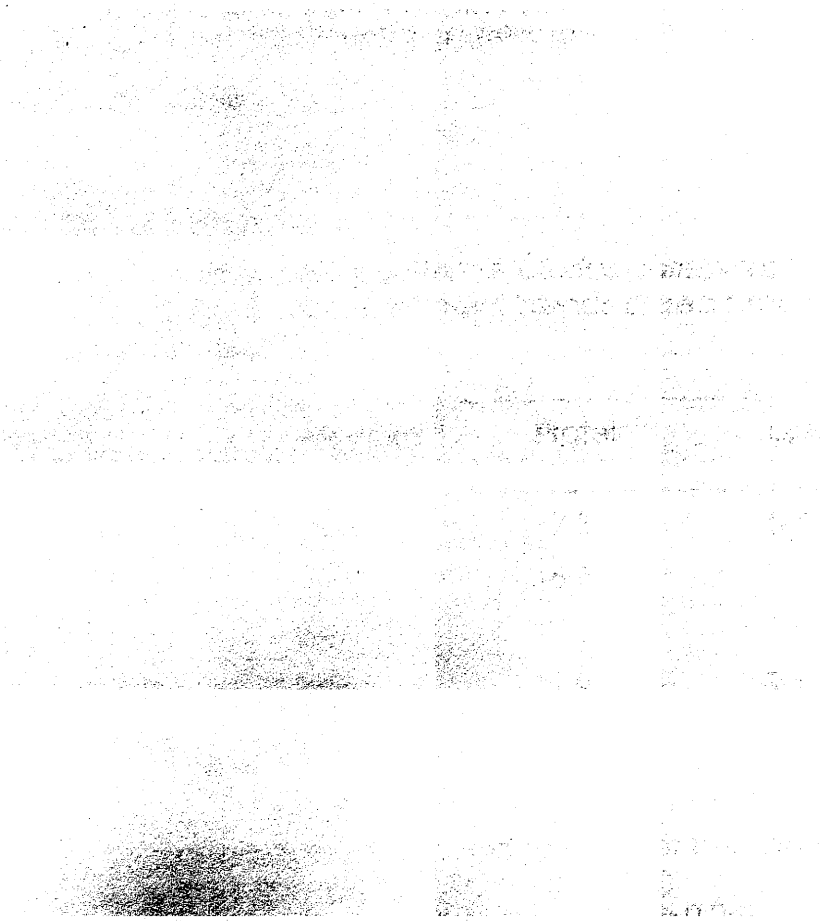


Table 6.6 Amino acid composition of soybean flour-poultry meat meal blend based diets fed to fingerlings of *Clarias gariepinus* for 56 days presented against its requirements.

Amino acid	Reference diet(100:00)	Diet I	Diet II	Diet III	*
Arg	5.6(130.2)	5.3(123.3)	5.2(120.9)	5.0(116.3)	4.3
His	2.1(140.0)	2.0(133.3)	1.80(120.0)	1.80(120.0)	1.5
Iso	3.3(126.9)	3.1(119.2)	2.9(111.2)	2.8(107.7)	2.6
Leu	5.9(168.6)	5.6(160.0)	5.6(160.0)	5.5(157.1)	3.5
Lys	4.6(90.2)	5.0(98.0)	4.7(92.2)	5.1(100.0)	5.1
Met+Cys	1.1(47.8)	1.3(56.5)	1.4(60.9)	1.5(65.2)	2.3
Phe+Tyr	5.2(104.0)	4.8(96.0)	4.5(90.0)	4.2(84.0)	5.0
Thr	3.1(155.0)	3.2(160.0)	3.2(160.0)	3.4(170.0)	2.0
Val	3.4(113.3)	3.4(113.3)	3.5(116.7)	3.7(123.3)	3.0

Figures in parenthesis represent amino acid content in the diets expressed a percentages of their requirements in a related species - the channel catfish.

* Source: NRC (1983).

Table 6.7 Carcass composition of *Clarias gariepinus* fed soybean flour-poultry meat meal blends based practical diets for 56 days

Diet	Moisture^{NS}	Protein^{NS}	Lipid^{NS}	Ash^{NS}
Reference(100:00)	69.9	17.3	8.0	3.5
Diet I(75:25)	71.3	16.9	7.4	3.7
Diet II(50:50)	68.8	17.1	9.2	3.6
Diet III(75:25)	69.6	17.9	9.1	3.9
Initial sample	68.0	16.4	6.2	3.1

Figures in parenthesis are the blending ratios of the blends constituents (soybean flour:poultry meat meal).
NS= Not significant with respect to the diets (P>0.05)

6.4 Discussion

The highest mean final weight, SGR, PER, and lowest FCR values recorded for fishes fed diet II in both experiments suggests the superiority of 50:50 blend of soybean flour and poultry meat meal over the other blends for the culture of *O.niloticus* and *C.gariepinus*. The improved performace of diet II was due to improved essential amino acids (EAA) balance , Sadiku and Jauncey (in Press) have shown that SF is higher in all the essential amino acids than PMM except threonine, valine, methionine and lysine. Methionine was identified as the first limiting amino acid as shown in Tables 6.3 and 6.6, the risk of its deficiency could be reduced by the incorporation of PMM in the diets.

Problems of lysine unavailability and sulphur containing amino acid destruction through toasting of SF (Snyder and Kwon, 1987; Goihl, 1987) could therefore be ameliorated by the incorporation of PMM. A good blend of SF and PMM as in diet II gave an improved amino acid balance. Higher level of inclusion of soybean flour as in the reference diet and diet I increase the risk of methionine deficiency and that of PMM as in diet III increases the risk of Phenylalanine deficiency. This could militate against high inclusion levels of both SF and PMM in an ideal SF:PMM blend based diet of *O.niloticus* and *C.gariepinus*.

The level of leucine which when present in excess would be toxic to fish (Hughes, et al 1984; Robinson, et al 1984, Tacon, 1992) and observed to have caused scoliosis, deformed opercula, scale deformities, scale loss and spongiosis of epidermal cells in *Onycorynchus mykiss* (Cho and Cowey,1991) was in excess in all the diets. Though there is paucity of information on the toxicity of this amino acid in *O.niloticus* and *C. gariepinus*, absence of symptoms of deficiency and toxicity in the two experiments indicated that their excessive levels were not critical to the fish over this duration. Inclusion of PMM was beneficial as it reduced leucine in the diets.

Increasing order of carcass lipid and ash from reference diet to diet III in *O.niloticus* with increased inclusion levels of PMM rather than SF was due to higher levels of lipid and ash in diets with high PMM (Sadiku and Jauncey , in Press). This was not the trend in *C.gariepinus* which could tolerate higher inclusion level of this in its diet than *O.niloticus*, as catfishes requires more lipid than tilapia (NRC, 1983). In fact, tilapia has been documented to perform better on low lipid diets (Jauncey and Ross, 1982; Jauncey, 1993).

In conclusion, there is nutritional improvement of soybean meal with the incoporation of PMM (animal protein). Whether this is ecomonically viable as postulated by Fowler (1991), needs to be further investigated.

CHAPTER SEVEN

SUBSTITUTION OF FISHMEAL WITH SOYBEAN FLOUR - POULTRY MEAT MEAL BLENDS IN PRACTICAL DIETS FOR *OREOCHROMIS NILOTICUS* AND *CLARIAS GARIEPINUS*

7.1 Introduction

Replacement of fish meal has been one of the current fish meal research needs (Chamberlain, 1993) and replacement of fish meal with vegetable proteins (partially or totally) has been the goal of aquaculture (Wee and Wang, 1987;Lim and Dominy, 1989; Ng and Wee, 1989; Shiau et al. 1990).

Lovell (1990) and Lim (1992) documented reported variable successes on utilisation of soybean in aquaculture feeds. Highest level of replacement of fish meal meal with soybean meal without any supplementation in tilapia diets was found to be 50% in a diet of 25% crude protein (Viola and Arieli, 1983).High level of dietary soybean meal or complete substitution of animal proteins resulted in poor growth and feed efficiency of fish (Jackson et al. 1982; Dabrowska et al.1989) hence their indispensability. This could be due to increase in critical state of methionine and available lysine with increase in replacement level. Incorporation of low cost animal protein (rich in methionine and available lysine) is therefore a necessity for nutritional improvement of soybean and its subsequent use to replace fishmeal.

Therefore, the objectives of this study are to ameliorate amino acid imbalance of SF by PMM incorporation as blends, establish the ideal levels of incorporation of PMM in SF and replacement of fishmeal in practical diets for *O.niloticus* and *C. gariepinus.*

7.2 Materials and Methods

i Experimental fish, fish husbandry and fish diets

Ten isocaloric and isonitrogenous diets - Control Diet (0% replacement of fish meal), Diets I-IX - of 25:75; 50:50; 75:25 soybean flour (SF)-poultry meat meal (PMM) blends replacing fish meal at 25%, 50% and 75%, were fed to fingerlings of *Oreochromis niloticus* (M±S.E = 7.1±0.2g) and *Clarias gariepinus* (M±S.E = 10.2±0.8g) at 4% body weight per day at 09:30 and 17:00 Hrs for 56 days in two seperate experiments as experiments 1 and 2 respectively. Fish were bulk-weighed fortnightly and feeding rates adjusted accordingly.

Deffated, toasted and dehulled SF (Type II) obtained from Sigma Chemical Co. ltd, U.S.A. was used for experiment 1 while defatted and toasted SF supplied by Dr Viv Crampton of Ewos U.K. ltd was used for experiment 2. High quality PMM was supplied by Chettles UK ltd, Nottingham. The formulation and proximate composition of the experimental diets are shown in Table 7.1. 3mm pellets were prepared using California Pellet Mill (model CL2) equipped with a steam conditioner and subsequently dried by convection at 60°C overnight. After cooling, diets were packed in sealed black plastic bags and stored (-30°C) until required.

ii Experimental system

A 3 X 3 factorial design plus two controls was adopted. Fishes were stocked in duplicate into twenty 50 L tanks at 15 fish/ tank for *O.niloticus* and 10 fish/tank for *C.gariepinus*. The tanks were regularly cleaned by siphoning off waste. Water flow was maintained at 2L min^{-1} per tank and water quality parameters were monitored fortnightly using methods described in 3.5.2 and recorded as follows: Temperature, 26-27°C and 27°C; dissolved oxygen (DO), 4.5-6.0 and 5.5-6.7 mgL^{-1}; pH, 6.0 and 6.0; NH_3-N, 0.4-1.0 and 0.6-1.0 mgL^{-1}; NO_2-N, 0.2 and 0.2 mgL^{-1}; NO_3-N, 20 and 20 mgL^{-1}; Ca-hardness, 88-173 and 49-77 mgL^{-1} and total hardness, 123-210 and 69-105 mgL^{-1} for experiments 1 and 2 respectively.

iii Experimental and statistical analyses

Chemical analysis of feedstuffs, diets and carcass (initial and final) was performed according to AOAC (1990) following the procedures in 3.6.1. Eight specimens of fingerlings were taken for initial carcass analysis while four from each tank were used for final carcass analysis. Biological parameters measured according to the description in 3.6.2 and data analysed statistically according to procedures in 3.7

Table 7.1 *Feedstuffs inclusion levels and proximate analysis of soybean flour-poultry meat meal blend substituted fish meal based diets fed to Oreochromis niloticus for 56 days*

		D	I	E	T	S				
Ingredients	Control	I	II	III	IV	V	VI	VII	VIII	IX
SBF *	-	3.4	6.9	10.6	6.8	14.2	22.3	10.4	22.1	35.3
PMM *	-	10.1	20.7	31.9	6.8	14.2	22.3	3.5	7.4	11.8
Fish meal	52.4	40.3	27.6	14.2	40.9	28.5	14.9	41.6	29.4	15.7
Wheat flour	31.9	31.1	30.3	29.5	30.0	28.0	25.9	28.9	25.6	21.9
Soybean oil	7.8	7.2	6.6	5.9	7.4	7.0	6.6	7.7	7.5	7.4
Vit.premix[1]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Min.premix[2]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Binder(CMC)*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Indicator	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Proximate composition										
Moisture	9.5	8.9	8.4	7.8	9.0	8.5	7.9	9.0	8.5	7.9
Protein	37.6	38.1	38.1	36.1	38.6	38.3	37.7	38.8	38.1	36.8
Lipid	10.6	9.3	9.9	9.9	10.9	10.6	10.2	10.9	10.5	10.4
Ash	10.5	9.5	10.3	10.1	10.6	10.6	11.2	11.2	10.4	9.7
Energy ($Kcalg^{-1}$)	4.3	4.3	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4

Data on the same column carrying the same superscript are statistically insignificant from each other ($p>0.05$)
Constituents of vitamin and mineral premix are as presented in Table 2.5

7.3 Results and Discussion

7.3.1 Experiment 1 - *Oreochromis niloticus*

i Nutrient Utilisation

Mean initial weights and the mean final weight of fishes fed different diets did not differ significantly ($P>0.05$). The SGR, FCR and PER differences between the diets were significant ($p<0.05$) while those of ANPU and mortality were insignificant ($p>0.05$) as depicted in Table 7.2. MFW, SGR, FCR, PER, ANPU differed significantly with replacement levels ($p<0.05$) but not blending ratios of soybean flour - poultry meat meal blends in the diets. The interactions of substitution and blending were insignificant in all cases ($p>0.05$). Diet VII was the best utilised of the ten diets. It had the lowest FCR, highest SGR and PER while diet III had the poorest nutrient utilisation with highest FCR and lowest SGR, PER.

Figs. 7.1a, b and c show that the control diet gave the best growth response in 25:75 and 50:50 blends while that of 25% substitution was best of all replacement levels in 75:25 blends. Poorest growth responses were observed with 75% replacement in 25:75 and 75:25 blends while that of 50% replacement was poorest in 50:50 blend.

Table 7.2. Utilisation of soybean flour - poultry meat meal blend substituted fish meal based diets by *Oreochromis niloticus* fed for 56 days

Diet	SF:PMM	%FM	MIW (g)	MFW (g)	SGR(%d^{-1})	FCR	PER	ANPU (%)	S.(%)
Contr	-	100	6.7±0.1^{a}	23.3±1.0^{a}	2.2^{bc}	1.35±0.07^{ab}	2.14±0.11^{bc}	36.47±0.04^{a}	98.31±1.70^{a}
I	25:75	75	7.1±0.3^{a}	22.1±0.7^{a}	2.0^{abc}	1.50±0.01^{abcd}	1.91±0.02^{abc}	33.80±0.01^{a}	93.33±0.00^{a}
II	25:75	50	7.3±0.2^{a}	21.9±0.3^{a}	1.9^{ab}	1.57±0.04^{bcd}	1.83±0.05^{ab}	33.71±0.02^{a}	100.00±0.00^{a}
III	25:75	25	7.1±0.1^{a}	20.0±0.9^{a}	1.8^{a}	1.68±0.03^{d}	1.71±0.03^{a}	29.20±0.02^{a}	96.55±3.45^{a}
IV	50:50	75	7.1±0.1^{a}	23.4±0.2^{a}	2.1^{abc}	1.41±0.02^{abc}	2.03±0.03^{abc}	33.29±0.06^{a}	92.82±7.19^{a}
V	50:50	50	6.9±0.3^{a}	21.2±1.3^{a}	2.0^{abc}	1.53±0.02^{abcd}	1.88±0.02^{abc}	32.61±0.00^{a}	98.31±1.70^{a}
VI	50:50	25	7.2±0.1^{a}	22.5±0.3^{a}	2.0^{abc}	1.48±0.01^{abcd}	1.94±0.05^{abc}	33.78±0.02^{a}	98.52±1.70^{a}
VII	75:25	75	7.1±0.2^{a}	24.8±1.9^{a}	2.3^{c}	1.32±0.07^{a}	2.18±0.11^{c}	35.10±0.01^{a}	92.82±7.19^{a}
VIII	75:25	50	7.0±0.2^{a}	22.0±0.4^{a}	2.1^{abc}	1.48±0.03^{abcd}	1.93±0.04^{abc}	33.87±0.00^{a}	83.47±0.20^{a}
IX	75:25	25	7.6±0.0^{a}	21.6±0.1^{a}	1.8^{a}	1.66±0.09^{d}	1.73±0.09^{a}	32.26±0.01^{a}	98.31±1.70^{a}
S.E.M.			±0.2	±0.9	±0.0	±0.04	±0.06	±0.02	±

FM, fishmeal; MIW, mean initial weight; MIF, mean final weight; SGR, specific growth rate; FCR, food conversion ratio; PER, protein efficiency ratio; ANPU, apparent net protein utilisation; S., Survival; d, day.

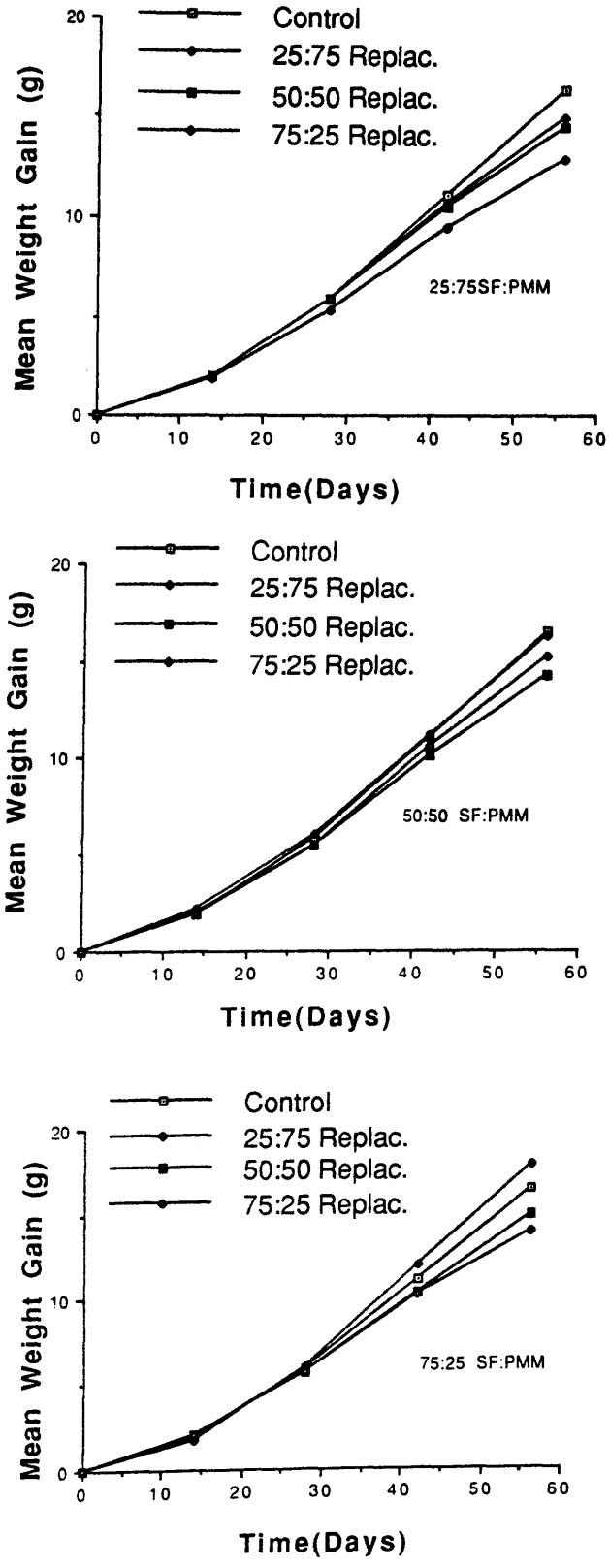


Fig.7.1: Growth responses of *Oreochromis niloticus* fed FM based diets with SF:PMM blend (a,25:75; b,50:50; and c,75:25) substitution of FM.

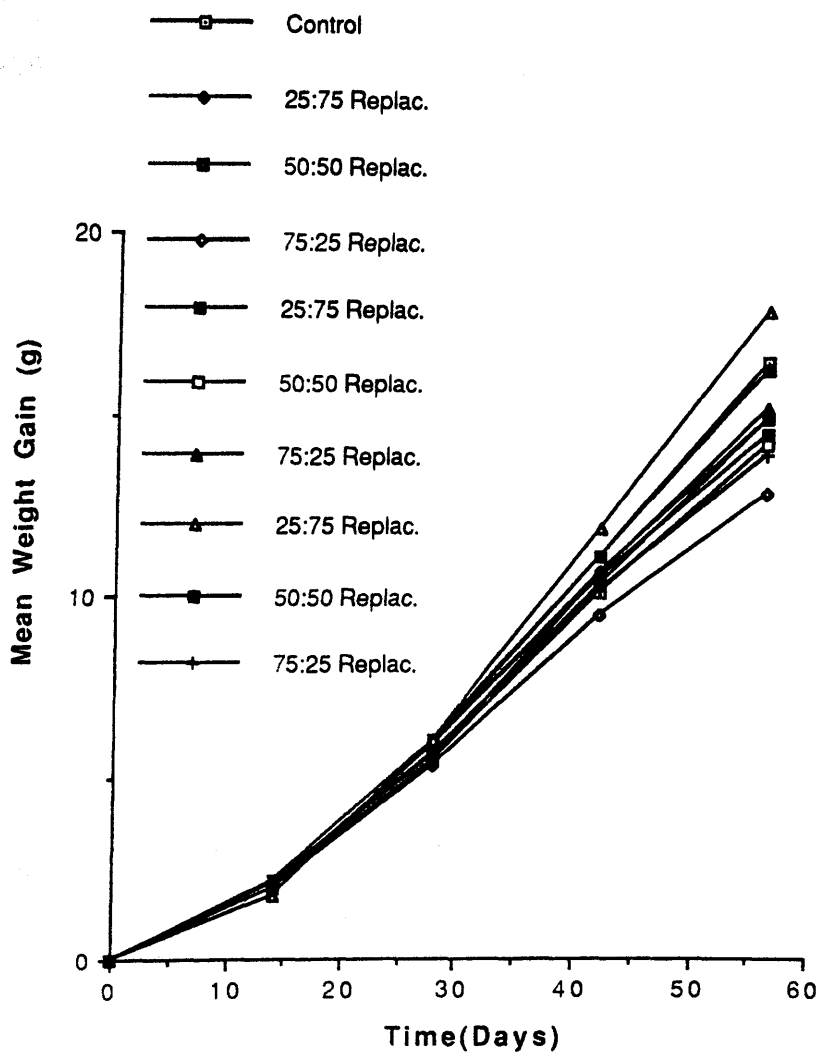


Fig.7.2: Combined growth responses of *Oreochromis niloticus* fed FM based diets with SF:PMM blend substitution of FM

Table 7.3 shows that there was deficiency of essential amino acids (EAAs) in all the diets. Though there was excess of leucine and deficiency of methionine in all the diets, there was improvement of amino acid imbalance with the incorporation of poultry meat meal (PMM) in soybean flour (SF) as blends generally. Methionine level increased while that of leucine decreased with increase in PMM and decrease in SF. Higher substitution level of fish meal also caused reduction of methionine and lysine, and increase in other essential amino acids.

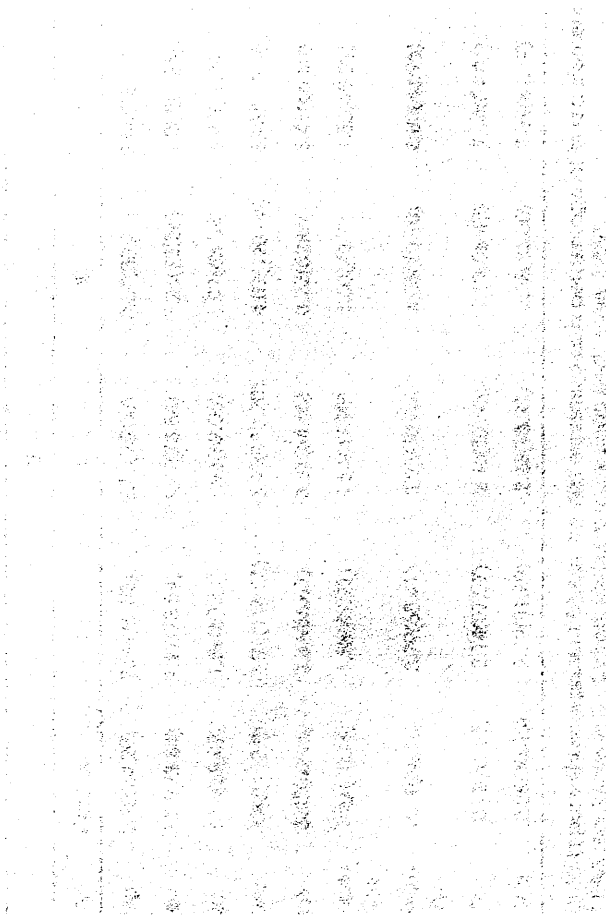


Table 7.3 Essential amino acid (% protein) (EAA) composition of soybean flour-poultry meat meal blend substituted fish meal based diets fed to *Oreochromis niloticus* for 56 days presented against its requirements.

			D	I	E	T	S				
EAA	Control	I	II	III	IV	V	VI	VII	VIII	IX	*
Arg	2.70(64.29)	2.29(69.52)	3.15(75.00)	3.39(80.71)	2.94(70.00)	3.20(76.19)	3.49(83.10)	2.97(70.71)	3.27(77.86)	3.61(85.95)	4.20
His	1.27(73.84)	1.27(73.84)	1.27(73.84)	1.26(73.26)	1.28(74.26)	1.29(75.00)	1.31(76.16)	1.30(75.58)	1.33(77.33)	1.37(79.65)	1.72
Iso	1.85(59.49)	1.89(60.77)	1.93(62.06)	1.97(63.34)	1.90(61.09)	1.97(63.34)	2.04(65.59)	1.93(62.06)	2.02(64.95)	2.12(68.17)	3.11
Leu	3.83(112.9)	3.91(114.53)	3.99(117.70)	4.07(120.06)	3.93(115.93)	4.05(119.47)	4.12(121.53)	3.97(117.11)	4.12(121.53)	4.30(126.84)	3.39
Lys	3.47(67.76)	3.39(66.62)	3.31(64.65)	3.22(62.89)	3.41(66.60)	3.35(65.42)	3.28(64.06)	3.44(67.19)	3.39(66.21)	3.35(65.43)	5.12
Met+ Cys	1.61(50.16)	1.58(49.22)	1.54(47.98)	1.50(46.73)	1.55(48.29)	1.49(46.42)	1.43(44.55)	1.53(47.66)	1.44(44.86)	1.33(40.50)	3.21
Phe+ Tyr	3.61(65.16)	3.79(68.41)	3.92(70.76)	4.05(73.10)	3.82(68.95)	3.99(72.02)	4.18(75.45)	3.87(69.86)	4.09(73.83)	4.34(78.34)	5.54
Thr	2.12(56.53)	2.14(57.07)	2.16(57.60)	2.19(58.40)	2.14(57.07)	2.18(58.13)	2.22(59.20)	2.16(57.60)	2.20(58.67)	2.26(60.27)	3.75
Val	2.25(80.36)	2.29(81.79)	2.33(83.21)	2.38(85.00)	2.29(81.79)	2.34(83.57)	2.39(85.36)	2.30(82.14)	2.35(83.93)	2.41(86.07)	2.80

Data in parenthesis represent EAA in the diet expressed as a percentage of its requirement by *O.niloticus*
* EAA requirements by *O.niloticus* (Source: Santiago and Lovell, 1989)

ii Carcass composition

Moisture and ash differed insignificantly with the diets ($p>0.05$) while those of protein and lipid were significant ($p<0.05$) as depicted in Table 7.4. Carcass protein was highest in diets II and IX, and lowest in diet VII. Highest carcass lipid was observed in fishes fed diet I while the lowest was in those fed diet IX. Dietary nutrient did not significantly affect nutrient deposition in the carcass ($p>0.05$).

Table 7.4 Carcass composition of *Oreochromis niloticus* fed soybean flour - poultry meat meal blend substituted fish meal based diets for 56 days

Diet	SF:PMM	%FM	Moisture	Protein	Lipid	Ash
Control	-	100	71.2^{a}	16.3^{ab}	7.3^{cde}	4.8^{a}
I	25:75	75	70.1^{a}	16.7^{ab}	8.2^{e}	4.5^{a}
II	25:75	50	70.5^{a}	17.1^{b}	7.2^{cd}	4.3^{a}
III	25:75	25	70.6^{a}	16.1^{ab}	8.1^{de}	4.8^{a}
IV	50:50	75	70.7^{a}	16.4^{ab}	7.0^{c}	4.7^{a}
V	50:50	50	72.3^{a}	16.4^{ab}	5.6^{b}	5.0^{a}
VI	50:50	25	71.5^{a}	16.5^{ab}	7.1^{cd}	4.5^{a}
VII	75:25	75	70.9^{a}	15.6^{a}	7.7^{cde}	4.5^{a}
VIII	75:25	50	70.6^{a}	16.5^{ab}	7.2^{cd}	4.6^{a}
IX	75:25	25	71.6^{a}	17.1^{b}	4.7^{a}	4.6^{a}
Initial sample			71.8	14.8	8.3	3.7

Data on the same column carrying the same superscript are statistically insignificant from each other ($p>0.05$).

iii Discussion

Replacement of fish meal at 25% with soybean flour-poultry meat meal blend of 75% soybean flour was found to be most appropriate as replacement above and blending below these levels inhibited performance generally. This is in agreement with ealier reports. Jackson (1982) observed that 25% inclusion of soybean in place of fish meal gave equally a good growth response of tilapia as the control i.e without replacement. Similarly , at suboptimal and optimal protein levels of 24% and 32% respectively, soybean meal could replace fish meal at 30% but better at suboptimal level (Shiau et al.1987,1990).

Diet VII did better than the control diet despite its disadvantaged amino acid balance over the control diet and diets I-VI. It was better in amino acid balance than diets VIII and IX with respect to the critical amino acids - methionine, leucine and lysine. The superiority of this diet could not be attributed to better amino acid balance. It could be due to factors other than those investigated here, possibly the feeding behaviour of tilapia. In fact, tilapia has earlier been documented to grow better on methionine and available lysine deficient soybean meal based diet than on fishmeal that has better amino acid balance, thereby putting to rest the indispensability of fish meal in tilapia diets (Rumsey, 1993).

Amino acid requirements of tilapia as contained in NRC (1983) and Tacon(1990) were not met in any of the diets except leucine that was in excess. Higher inclusion levels of SF decreased methionine and increased other amino acids which when in excess could be detrimental to the fish. Excess of certain EAAs like leucine have been reported to exert toxic effect in fish (Hughes, et al, 1984; Robinson et al, 1984). Infact, Cho *et al.* (1991) reported toxicity of leucine in rainbow trout (*Oncorhynchus mykiss*) when present in excess of 13.4%. Although, there was excess of leucine in these diets, no structural deformity characteristic of leucine toxicity was observed.

Diet VII was higher in soybean flour than poultry meat meal. This suggests a marginal level of incorporation of poultry meat meal in soybean flour to give optimum performance of the diets in *O.niloticus*. Poultry by-product meal on its own has been postulated to successfully replace fishmeal at >50% level without compromising performance in chinook salmon (Fowler,1991). Improvement of soybean flour was therefore achieved by the incorporation of poultry meat meal. It was evident that addition of poultry meat meal ameliorated methionine and available lysine deficiency, and reduced leucine, and the risk of its toxicity. This did not affect carcass deposition of nutrients, as dietary nutrients related insignificantly with carcass nutrients. The difference in carcass nutrient was primarily due to nutrient metabolism in the fish. A blend higher in soybean flour than poultry meat meal could therefore be considered as a suitable alternative source of protein to partially replace fish meal.

7.3.2 Experiment 2 - *Clarias gariepinus*

i Nutrient Utilisation

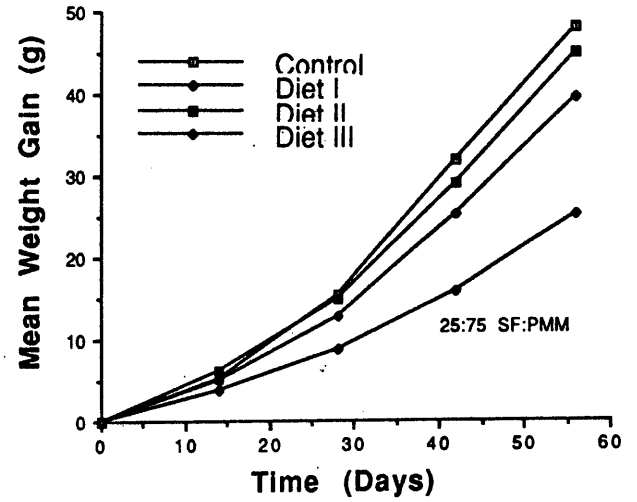
Mean initial weights did not differ significantly (P>0.05). The mean final weight(MFW) of Diet V -50(50:50), was highest while that of Diet III-75(25:75) was lowest, other diets were intermediate. The MFW and the SGR differences between the diets were significant ($p<0.05$) while those of FCR, PER, ANPU and mortality were insignificant ($p>0.05$) as depicted in Table 7.5. SGR, FCR, PER, ANPU differed significantly with replacement levels ($p<0.05$). The blending ratios of soybean flour - poultry meat meal blends of the diets had an insignificant effect on the nutrient utilisation of the diets ($p>0.05$). The interactions of replacement and blending were insignificant in all cases ($p>0.05$)

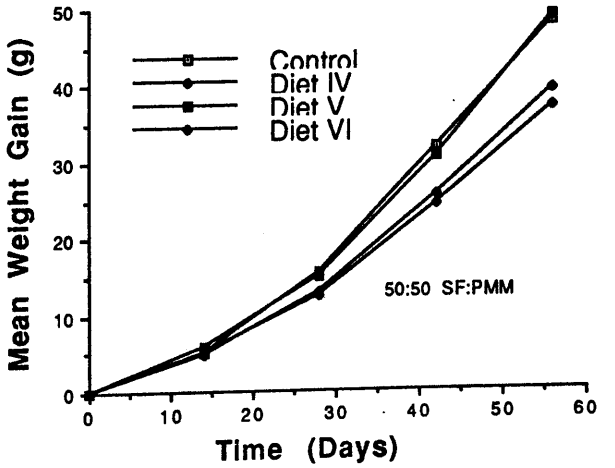
The control diet was the best utilised of the ten diets. It had lowest FCR, highest SGR, PER and ANPU, followed by diet V, while diet III had the poorest nutrient utilisation with highest FCR and lowest SGR, PER and ANPU. Diet V recorded the highest MFW. There was 100 percent survival of fish fed diets I-V while low mortality was recorded in others. Figs. 7.2a, b and c show that control diet gave the best growth response in all cases except diet V (50 percent replacement with 50:50 blend) that performed well. Poorest growth responses were observed with 75% replacement with 25:75 blend (diet III), 25 percent replacement with 50:50 blend (diet IV) and 75% replacement with 75:25 blend (diet IX) as in Figs. 1a, b and c respectively.

Table 7.5 Utilisation of soybean flour - poultry meat meal blend substituted fish meal based diets by *Clarias gariepinus* fed for 56 days

Diet	SF:PMM	%FM	MIW	MFW	SGR	FCR	PER	ANPU	MORT.
Cont	-	100	9.6 ± 0.9^{a}	58.1 ± 10.01^{ab}	3.3^{b}	0.91 ± 0.00^{a}	2.93 ± 0.05^{b}	55.08 ± 0.03^{a}	5.3 ± 5.3^{a}
I	25:75	75	10.4 ± 1.4^{a}	50.5 ± 7.3^{ab}	2.8^{ab}	1.03 ± 0.02^{a}	2.57 ± 0.04^{a}	47.99 ± 0.00^{a}	0.0^{a}
II	25:75	50	11.6 ± 0.1^{a}	57.1 ± 4.3^{ab}	2.8^{ab}	1.03 ± 0.06^{a}	2.64 ± 0.07^{a}	47.88 ± 0.10^{a}	0.0^{a}
III	25:75	25	8.8 ± 0.7^{a}	34.3 ± 2.5^{a}	2.4^{a}	1.21 ± 0.14^{b}	2.27 ± 0.32^{a}	38.20 ± 0.60^{a}	0.0^{a}
IV	50:50	75	9.8 ± 0.7^{a}	46.9 ± 0.5^{ab}	2.8^{ab}	1.04 ± 0.08^{a}	2.52 ± 0.19^{a}	41.39 ± 0.51^{a}	0.0^{a}
V	50:50	50	11.3 ± 0.3^{a}	60.9 ± 2.7^{b}	3.0^{ab}	0.94 ± 0.05^{a}	2.81 ± 0.14^{a}	49.05 ± 0.01^{a}	0.0^{a}
VI	50:50	25	9.3 ± 0.6^{a}	48.8 ± 2.6^{ab}	3.0^{ab}	0.98 ± 0.03^{a}	2.72 ± 0.09^{a}	45.80 ± 0.24^{a}	2.6 ± 2.6^{a}
VII	75:25	75	10.7 ± 0.5^{a}	48.8 ± 7.8^{ab}	2.7^{ab}	1.12 ± 0.09^{a}	2.32 ± 0.19^{a}	41.59 ± 0.56^{a}	0.0^{a}
VIII	75:25	50	10.1 ± 1.5^{a}	44.6 ± 8.4^{ab}	2.6^{ab}	1.17 ± 0.05^{a}	2.27 ± 0.09^{a}	39.18 ± 0.54^{a}	2.6 ± 2.6^{a}
IX	75:25	25	10.4 ± 0.4^{a}	43.5 ± 2.9^{ab}	2.5^{ab}	1.17 ± 0.10^{a}	2.36 ± 0.20^{a}	44.68 ± 0.16^{a}	2.6 ± 2.6^{a}
S.E.M			±0.8	±5.8		±0.07	±0.16	±0.27	±1.3

Data on the same column carrying different superscripts are significantly different from each other ($p<0.05$)





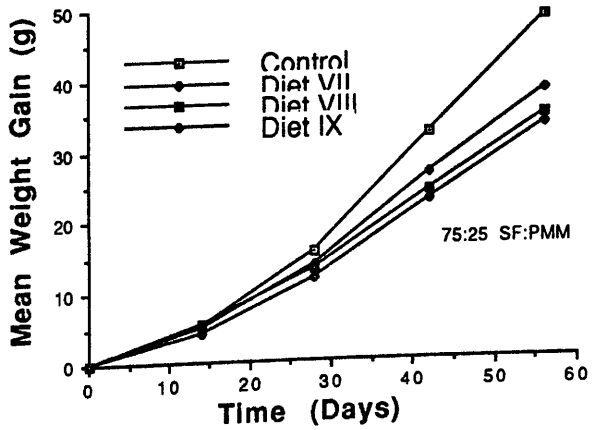


Fig.7.3: Growth responses of *Clarias gariepinus* fed FM based diets with SF:PMM blend (a,25:75; b,50:50; and c,75:25) substitution of FM.

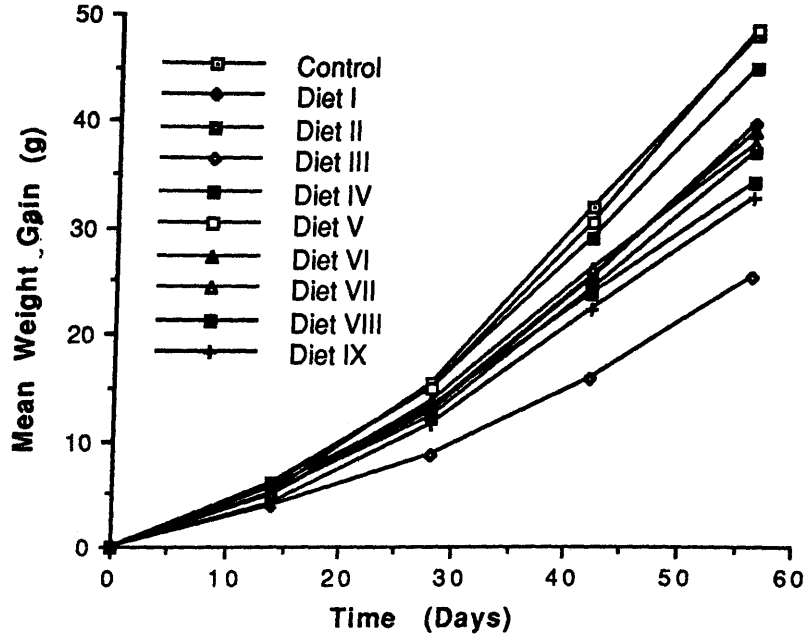


Fig.7.4: Combined growth response of *Clarias gariepinus* fed FM based diets with SF:PMM blend substitution of FM

There was essential amino acid imbalance in all the diets. Only requirements for leucine and threonine were met as shown in Table 7.6. There was excess of leucine and deficiency of methionine as the first limiting amino acid. Highest methionine and lowest leucine were recorded in Control Diet while the converse was the case in Diet IX. Increase in poultry meat meal increased methionine and reduced leucine. Increase in fish meal replacement reduced methionine and increased leucine.

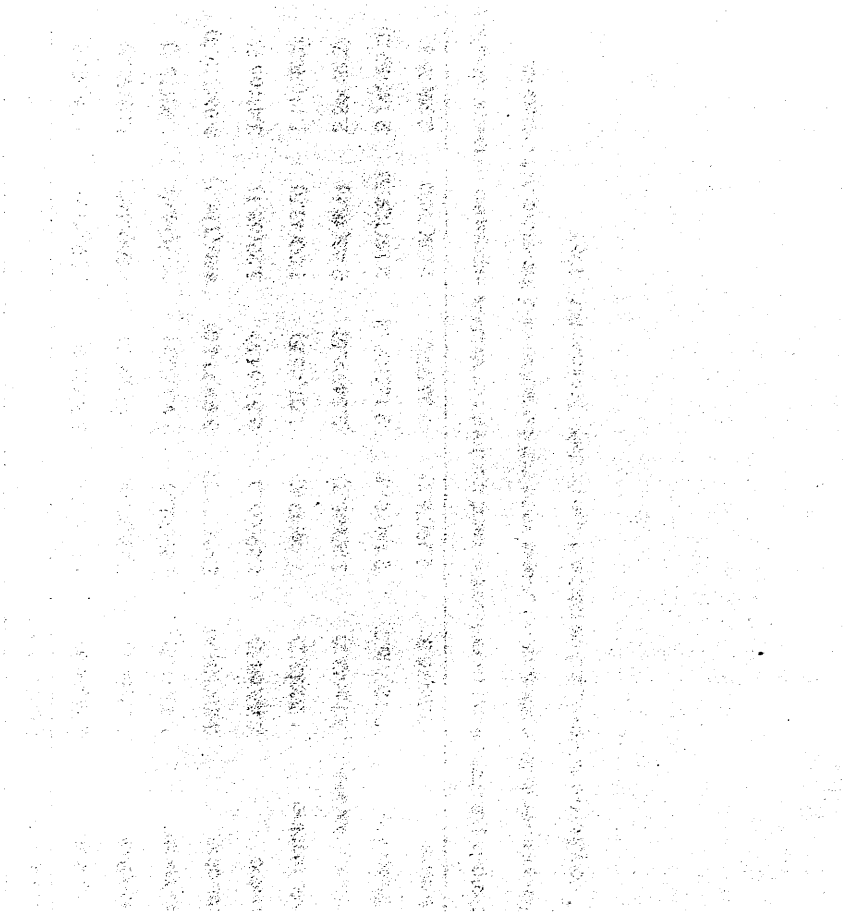


Table 7.6 Amino acid composition (% protein) of Control Diet, Diets I-IX fed to *Clarias gariepinus* fingerlings for 56 days in relation to their requirements.

			D	I	E	T	S				
Amino acid	Control Diet	I	II	III	IV	V	VI	VII	VIII	IX	*
Arginine	2.70(64.3)	2.92(69.5)	3.15(75.0)	3.39(80.7)	2.94(70.0)	3.20(76.2)	3.49(83.1)	2.97(70.7)	3.27(77.9)	3.61(86.0)	4.2
Histidine	1.27(84.7)	1.27(84.7)	1.27(84.7)	1.26(84.0)	1.28(85.3)	1.29(86.0)	1.31(87.3)	1.30(86.7)	1.33(88.7)	1.37(91.3)	1.5
Isoleucine	1.85(71.2)	1.89(72.7)	1.93(74.2)	1.97(75.8)	1.90(73.1)	1.97(75.8)	2.04(78.5)	1.93(74.2)	2.02(77.7)	2.12(81.5)	2.6
Leucine	3.83(109.4)	3.91(111.7)	3.99(114.0)	4.07(116.3)	3.93(112.3)	4.05(115.7)	4.12(117.7)	3.97(113.4)	4.12(117.7)	4.30(122.9)	3.5
Lysine	3.47(68.0)	3.39(66.5)	3.31(64.9)	3.22(63.1)	3.41(66.9)	3.35(65.7)	3.28(64.3)	3.44(67.5)	3.39(66.5)	3.35(65.7)	5.1
Methionine	1.20(52.2)	1.14(49.6)	1.07(46.5)	1.00(43.5)	1.11(48.3)	1.02(44.3)	0.92(40.0)	1.09(47.4)	0.96(41.7)	0.82(35.7)	2.3
Phenylalanine	2.16(43.2)	2.25(45.0)	2.34(46.8)	2.43(48.6)	2.26(45.2)	2.37(47.4)	2.49(49.8)	2.28(45.6)	2.42(48.2)	2.56(51.2)	5.0
Threonine	2.12(106.0)	2.14(107.0)	2.16(108.0)	2.19(109.5)	2.14(107.0)	2.18(109.0)	2.22(111.0)	2.16(108.0)	2.20(110.0)	2.26(113.0)	2.0
Valine	2.25(75.0)	2.29(76.3)	2.33(77.7)	2.38(79.3)	2.29(76.3)	2.34(78.0)	2.39(79.7)	2.30(76.7)	2.35(78.3)	2.41(80.3)	3.0

Data in parenthesis represent amino acid content of the diets expressed as percentages of their requirements in a related species - the channel catfish.

Requirements for cystine and tyrosine were not available and therefore not included.

* Requirements expressed as percentage protein (Source: NRC,1983

ii Carcass Composition

Table 7.7 shows that carcass composition of the fishes fed the ten diets differed insignificantly with diet ($p>0.05$)

Table 7.7 Carcass composition of *C.gariepinus* fed soybean flour - poultry meat meal blends substituted fishmeal based diets

Diet	SF:PMM	%FM	Moisture ^{NS}	Protein ^{NS}	Lipid ^{NS}	Ash ^{NS}
Contr.	-	100	71.0	18.2	7.4	3.6
I	25:75	75	67.4	17.4	9.0	3.6
II	25:75	50	68.6	18.1	6.9	3.8
III	25:75	25	71.4	16.5	6.8	3.5
IV	50:50	75	69.6	16.1	8.8	3.4
V	50:50	50	70.8	17.1	7.9	3.5
VI	50:50	25	66.8	16.6	9.1	3.3
VII	75:25	75	63.7	17.2	9.4	3.5
VIII	75:25	50	63.5	16.8	9.8	4.1
IX	75:25	25	69.5	18.1	7.7	3.9
Initial sample			76.0	15.3	5.0	2.9

NS = Not significantly different along the columns ($p>0.05$)

iii Discussion

Diet V did equally well as the control diet. Diet V was a 50:50 blend of soybean flour and poultry meat meal. This suggests an equal level of incorporation of poultry meat meal in soybean flour to give optimum nutrient balance and good performance of the diets. It was evident that addition of poultry meat meal ameliorated methionine and available lysine deficiency, and reduced leucine. In terms of amino acid balance, only the superiority of control diet could be attributed to better amino acid balance as it had the highest methionine and the least leucine. That of diet V could be due to factors other than those investigated here, possibly due to improved palatability and reduction in antinutritional factors that demand further investigation.

Conversely, higher inclusion level of SF increased other amino acids which when in excess could be detrimental to the fish as aforementioned. Performance of fishes fed diet V equals that of the control diet suggesting diet V as the best alternative to fishmeal protein diet i.e a 50:50 blend of SF:PMM replacing fish meal at 50%. The rest of the discussion regarding risk of excess leucine toxicity is similar to that of *O.niloticus*.

The superiority of diet V not withstanding, favourable SGR, FCR, PER, ANPU and high survival rate made them suitable for the intensive culture of grower *C.gariepinus*.

CHAPTER EIGHT

SUPPLEMENTATION OF SOYBEAN FLOUR-POULTRY MEAT MEAL BLEND BASED DIETS WITH DL-METHIONINE TO IMPROVE NUTRIENT UTILISATION IN *OREOCHROMIS NILOTICUS* AND *CLARIAS GARIEPINUS*.

8.1 Introduction

Several investigations into the effects of supplementing synthetic amino acids to diets with amino acid deficiencies have been conducted. The results , to date, have been inconsistent sometimes showing improvements when the single most limiting amino acid is supplemented and sometimes only when two or more deficient amino acids are added. Some authors have argued that naturally rich sources of a deficient amino acid are superior to supplements.

Individual supplementaion of methionine, lysine, histidine or leucine to the diet of rainbow trout was observed not to improve the growth rate of the fish but collective supplementation did (Rumsey and Ketola, 1975). Fordiani and Ketola (1980) recorded improved growth in rainbow trout fed a methionine supplemented commercial soybean diet, but growth was not improved when the soybean was reheated. Viola et al. (1982) , Tacon et al. (1984) and Murai et al. (1986) recorded improved growth in carp, *Oreochromis niloticus* and channel catfish respectively when they were fed soybean containing diets with methionine supplementation.

A major factor militating against successful utilisation of synthetic amino acid supplemented to diet appears to be premature absorption. This makes it unavailable when jointly required with the other amino acids by the fish. This joint availability is evaluated as

balance of essential amino acids in the plasma (BEAAP). Premature absorption reduces BEAAP value of amino acid supplemented diets. This low BEAAP value has been found responsible for the reduced growth consistently observed when fish silage was substituted for fish meal in salmon diets (Hardy, 1991).

The use of complementary feedstuffs, in terms of aimno acid profile, in fish diet formulation to meet nutrient requirements could therefore be the most effective way of overcoming amino acid deficiency. The amino acids of such diets are concurrently available to fish thereby giving them a high BEAAP value. For example, a combination of menhaden and soybean protein will be better than either of them alone in eel's diet as their critically limiting amino acids are complementary (Lovell,1990). Other lower cost animal proteins than fishmeal, such as poultry meat meal, are richer in methionine and cystine than soybean. Blending of such materials with soybean in fish diets should be beneficial.

The present experiment was designed to investigate the effects of blending methionine rich but lower cost, animal protein (poultry meat meal) with methionine deficient soybean flour and the supplementation of these blends with dl-methionine on the performance of the Nile tilapia (*Oreochromis niloticus*) and the African catfish (*Clarias gariepinus*).

8.2 Materials and Methods

Two parallel experiments were conducted using 24 50L tanks. *Oreochromis niloticus* (M±S.E = 1.85±0.05g) and *Clarias gariepinus* (M±S.E. = 3.25±0.07g) fingerlings were stocked in duplicate (15 fish/ tank). They were fed six soybean flour(SF) - poultry meat meal blend (25:75; 50:50; 75:25) based diets with or without dl- methionine supplementation (diets I-VI) at 6% body weight per day, thrice daily at 10:00 , 14:00 and 18:00 for eight weeks. Fishes were bulk weighed every 2 weeks and feeding rates adjusted accordingly. A 2x3 factorial design was adopted for the experiments

The tanks were regularly cleaned by siphoning off accumulated wastes. Water flow was maintained at 2L min^{-1} per tank and water quality parameters were monitored fortnightly using the methods described in 3.5.2 and got the following range of values: Temperature, 26-27°C; dissolved oxygen (DO), 6.0-6.6 mgL^{-1}; pH, 6.0; NH_3-N, 0.4-1.0 mgL^{-1}; NO_2^--N, 0.2 mgL^{-1}; NO_3^--N, 20 mgL^{-1}; Ca-hardness, 63-99 mgL^{-1} and total hardness, 84-118 mgL^{-1}.

The proximate composition of the experimental diets is shown in Table 8.1. 1mm pellets were prepared using a California Pellet Mill (model CL2) equipped with a steam conditioner and subsequently dried by convection at 60°C overnight. After cooling, diets were packed in sealed black polythene bags and stored (-30°C) until required.

Chemical analysis of feedstuffs, diets and carcass (initial and final) was performed according to AOAC (1990) accoding to the procedures in 3.6.1. Eight specimens for each species were used for the initial carcass analysis while four specimens per tank were processed for the final carcass analysis.

Heparinized blood samples - using ammonium salt of heparin, were collected in eppendorf test tubes as described by Svobodova, et al (1991). A 1:1 mixture of blood plama sample:10% 5-sulphosalicyclic acid solution were refrigerated for 30 minutes and then centrifuged for 10 minutes at 23,000 r.p.m. using Beckman Ultra Centrifuge, Palo Alto, California. The supernatant was saved for amino acid analysis. Alpha-plus Amino Acid Analyser (LKB Bichrom Ltd, Cambridge) was used for amino acid analysis of the feedstuffs, diets, carcass and the blood plasma. Biological parameters measured as described in 3.6.2. Statistical analysis was carried out as described in 3.7

Table 8.1 Inclusion levels of feedstuffs in diet and proximate composition of dl-methionine supplemented soybean flour-poultry meat meal blend based practical diets.

	Diets					
Feedstuffs	I	II	III	IV	V	VI
Soybean flour	53.4	54.0	32.5	32.5	14.8	14.8
Poultry meat meal	17.8	18.0	32.5	32.5	44.5	44.5
Wheat flour	13.5	12.8	20.8	20.8	27.4	27.4
Soybean oil	7.4	7.3	6.2	6.2	5.2	5.2
Vitamin premix	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix	4.0	4.0	4.0	4.0	4.0	4.0
Binder (CMC)	1.5	2.0	1.5	2.0	1.5	2.0
DL-Methionine	0.5	-	0.5	-	0.5	-
Proximate analysis as fed (%)						
Moisture	4.3	5.5	4.4	4.0	2.7	1.3
Protein	39.0	39.2	39.4	38.9	39.9	40.5
Lipid	10.2	10.3	10.5	10.9	11.3	11.2
Carbohydrate*	36.6	35.3	34.8	35.2	35.0	35.2
Ash	9.9	9.7	10.9	11.0	11.1	11.8
Energy ($Kcalg^{-1}$)	4.6	4.5	4.6	4.6	4.7	4.7

* Calculated by difference

8.3 Results

Experiment 1 - *Oreochromis niloticus*

Table 8.2 shows the biological evaluation parameters of *Oreochromis niloticus* fed the six experimental diets with or without Dl-methionine supplementation. It was observed that there was no significant difference in nutrient utilisation by *O. niloticus* for all the diets ($p>0.05$). It is noteworthy that there was a trend of fish performance as those fed the diets with methionine consistently had better nutrient utilisation than those fed the diets without methionine except the SGR for diets III and IV only. Favourable weight gain, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilisation (ANPU) were recorded. Fig. 8.1 showed no difference in the growth response of fishes fed the diets.

Table 8.2 Effects of dl- methionine supplementation of soybean flour - poultry meat meal blend based diets by *Oreochromis niloticus* fed for 56 days.

	Diets						
	I	II	III	IV	V	VI	± S.E.M.
MIW *	1.77±0.04	2.08±0.00	1.78±0.07	1.87±0.20	1.76±0.01	1.83±0.08	±0.09
MFW *	15.46±1.16	15.52±2.11	15.89±1.88	16.79±2.66	15.89±1.01	15.05±0.41	±1.71
SGR *	3.87	3.58	3.90	3.92	3.93	3.76	±0.00
FCR *	0.98±0.05	1.07±0.10	1.00±0.04	1.02±0.02	1.03±0.02	1.10±0.01	±0.05
PER *	2.57±0.13	2.33±0.23	2.48±0.10	2.46±0.05	2.41±0.05	2.24±0.01	±0.12
ANPU*	41.99±0.04	40.84±0.15	40.28±0.03	38.86±0.01	38.56±0.00	35.41±0.00	±0.04
MORT*	12.58±0.01	6.67±0.00	6.67±0.00	1.70±1.70	1.70±1.70	6.67±0.00	

NS, not significantly different with the diets ($p>0.05$)

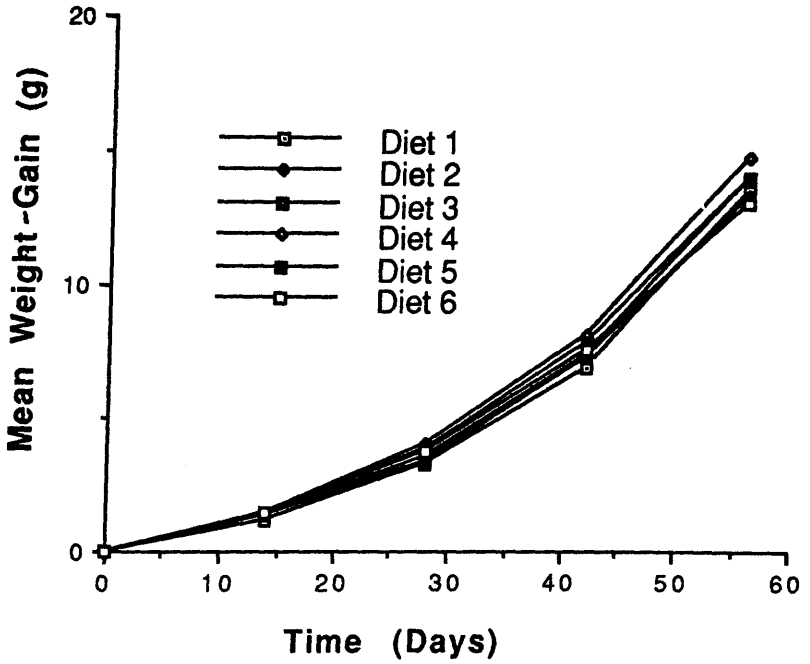


Fig.8.1: Growth response of *Oreochromis niloticus* fed dl-methionine supplemented SF-PMM blend based diets.

The comparative presentation of the EAA profiles of the diets and their requirements given in Table 8.3. The critically limiting amino acid was methionine while there was an excess of leucine. Diets with methionine supplementation had higher methionine content than those without. Diets with higher SF had less methionine and more leucine than those with PMM.

Table 8.3 *Oreochromis niloticus* requirements for essential amino acids contained in soybean flour-poultry meat meal blend based diets with or without dl-methionine supplementation.

	Diets						
	I	II	III	IV	V	VI	*
Arg	5.6	5.8	5.5	5.4	5.0	5.2	4.2
His	2.0	2.0	1.9	1.8	1.7	1.8	1.7
Iso	3.0	3.1	3.0	3.0	2.7	2.8	3.1
Leu	6.1	6.2	6.0	6.0	5.6	5.8	3.4
Lys	4.9	5.0	4.7	4.7	4.5	4.7	5.1
Met+Cys	2.2	1.6	2.5	1.8	2.4	1.9	3.2
Phe+Tyr	5.9	6.0	5.5	5.4	5.0	5.1	5.5
Thr	3.2	3.3	3.2	3.2	3.1	3.2	3.8
Val	3.4	3.4	3.4	3.4	3.3	3.4	2.8

* Requirement level for *Oreochromis niloticus* (Santiago and Lovell, 1988)

The EAA profiles of carcasses of fish fed diets I-VI showed no significant difference with the diets (Table 8.4). However, carcass EAA appeared to reflect the dietary EAA pattern. Methionine and histidine were low in both the diets and carcasses while arginine, lysine and leucine were high also in both diets and carcasses. Fig. 8.2 shows a significant positive correlation between dietary and carcass EAA ($P<0.05$). Fig. 8.3 presents the EAA pattern in the blood plasma of *O. niloticus* and its relationship with time after feeding. Diets without methionine supplementation (diet II and VI) were observed to have the highest level of methionine with a peak at six hours after feeding, beyond this was a fall to a very low level.

Table 8.4 Carcass essential amino acid composition of *Oreochromis niloticus* fed dl-methionine supplemented soybean flour-poultry meat meal blend based diets for 56 days.

EAA (%protein)	Diets					
	I	II	III	IV	V	VI
Arg*	4.62	4.40	4.29	4.62	4.39	4.34
His*	1.87	1.77	1.72	1.85	1.73	1.73
Iso*	3.18	3.01	3.03	3.25	3.18	3.08
Ieu*	5.38	4.84	5.12	5.49	5.09	5.13
lys*	5.60	5.02	5.39	5.73	5.37	5.39
Met*	1.33	1.40	1.64	1.68	1.55	1.63
Phe*	3.05	2.85	2.89	3.07	2.88	2.90
Thr*	3.24	2.93	2.92	3.30	3.08	3.10
Val*	3.67	3.38	3.62	3.99	3.50	3.55

*Data in the same row carrying the same superscript differ insignificantl from each other ($P>0.05$)

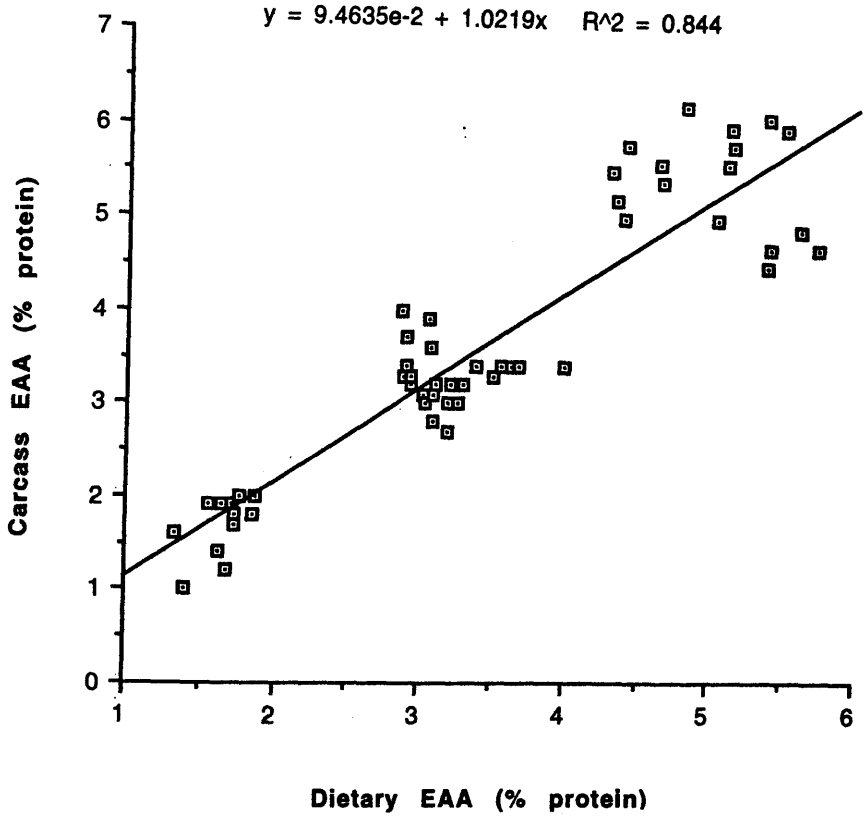


Fig.8.2: Relationship between dietary and *Oreochromis niloticus* carcass essential amino acids using data from Tables 8.3 and 8.4.

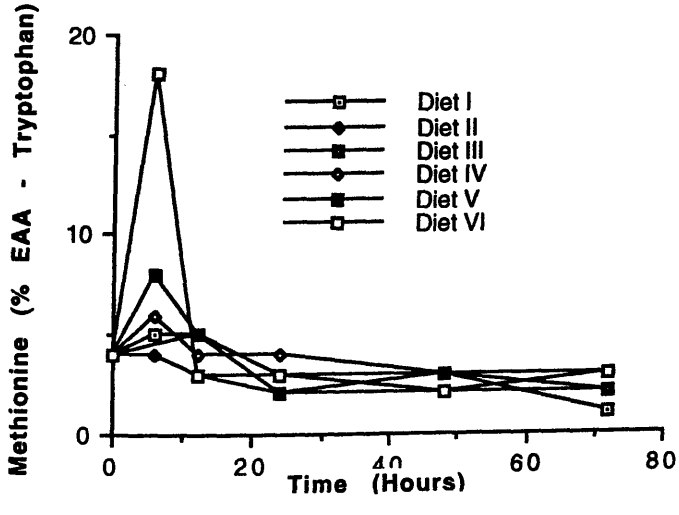


Fig. 8.3: Methionine level in the plasma of *Oreochromis niloticus* fed dl-methionine supplemented and unsupplemented SF-PMM blend based diets.

Histologically, there was fat deposition in the liver tissue and vacuolation of hepatocytes which became pronounced with increased lipid content as a result of increased incorporation of poultry meat meal in the diet were evident. These were so pronounced in the 75:25 SF:PMM that they could have been pathological at this level (Plates 8.1a, b, c and d).

However, certain degree of liver vacuolation is characteristic of farmed fish (Turnbull, Personal Communication). This could be responsible for the insignificant difference in the mortality of fish fed these diets.

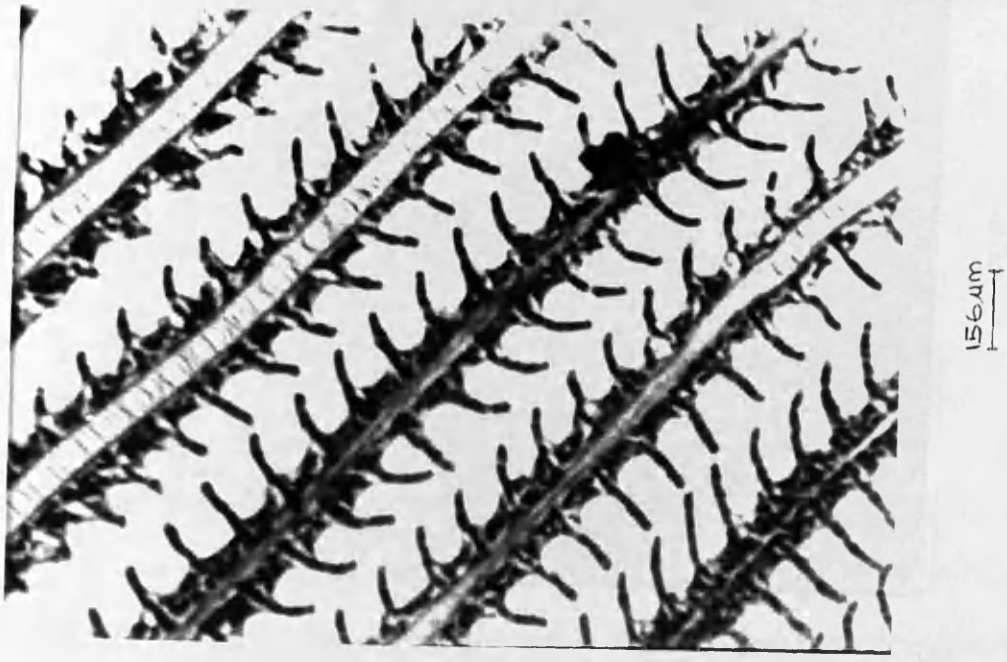


Plate 8.1a: Normal gill of *Oreochromis niloticus* fed SF-PMM blend based diets containing low PMM or high SF.

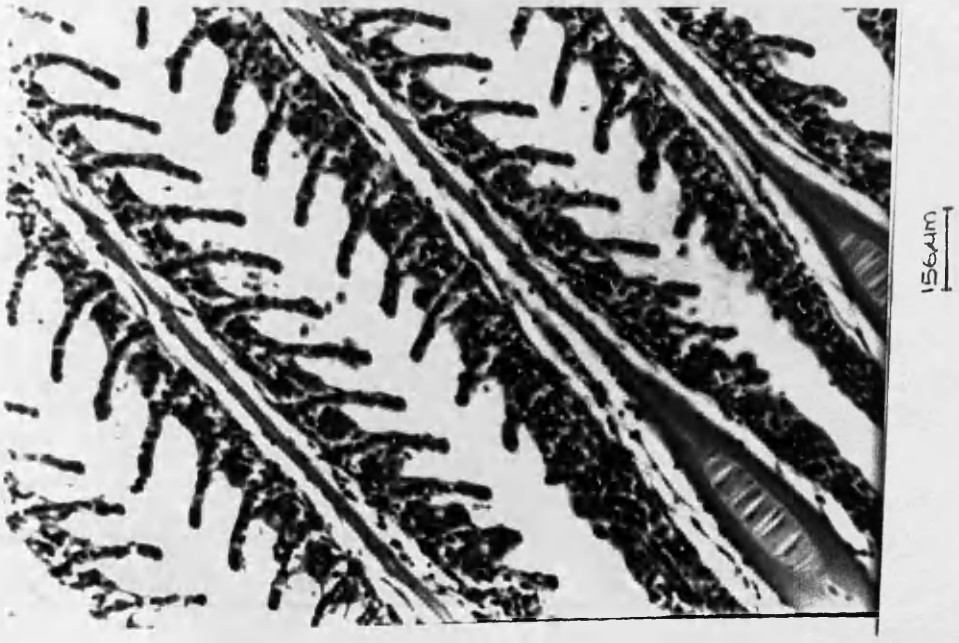


Plate 8.1b: Gill of *Oreochromis niloticus* fed SF-PMM blend based diets containing high PMM or low SF, showing hypertrophy (1° gill lamella)

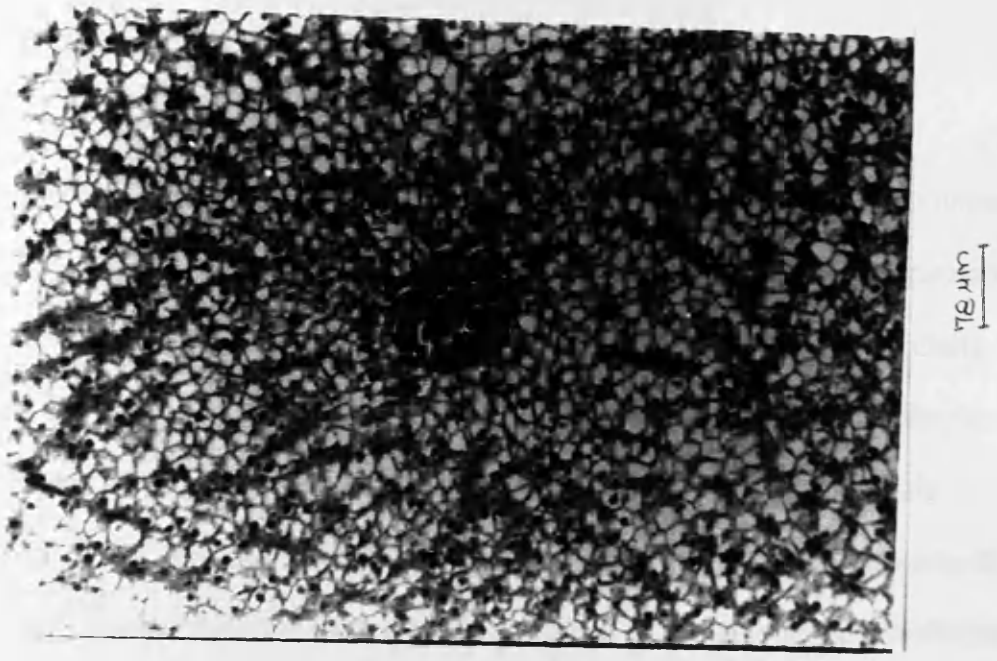


Plate 8.1c: Normal liver with slight vacoulation of the hepatocytes in *Oreochromis niloticus* fed dl-methionine supplemented and unsupplemented SF-PMM blend based diets containing low PMM. See exocrine pancreas at the centre

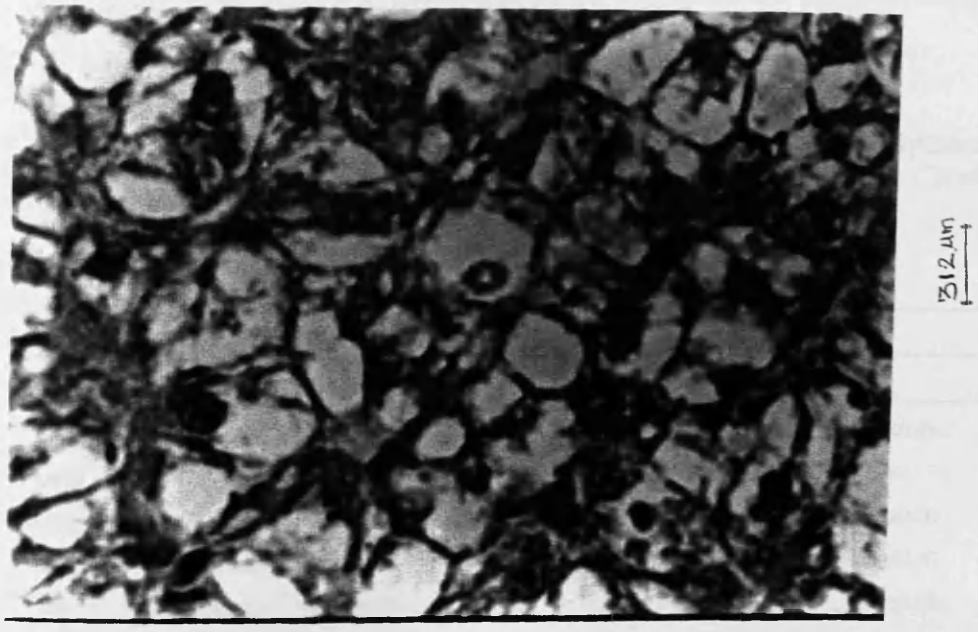


Plate 8.1d: Liver with pronounced vacoulation of the hepatocytes in *Oreochromis niloticus* fed dl-methionine supplemented and unsupplemented SF-PMM blend based diets containing high PMM.

Experiment 2 - *Clarias gariepinus*

Favourable weight gain, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilisation (ANPU) were recorded in *C. gariepinus* fed the six diets (Table 8.5). There was no significant difference in nutrient utilisation with or without dl-methionine supplementation for all the diets ($p>0.05$). However, fish fed diets containing methionine consistently had better nutrient utilisation than those fed diets without methionine supplementation. Similarly, a trend of best average nutrient utilisation was observed in 50:50 SF:PMM blend except for SGR. There was no marked difference in the growth response of fish fed the various diets (Fig. 8.4).

Table 8.5 Effects of dl- methionine supplementation of soybean flour - poultry meat meal blend based diets on *Clarias gariepinus* fed for 56 days.

	Diets						
	I	II	III	IV	V	VI	± S.E.M.
MIW (NS*)	3.32±0.12	3.28±0.26	3.24±0.23	3.40±0.17	3.01±0.14	3.26±0.26	±0.20
MFW "	32.10±1.30	28.70±3.27	35.89±3.60	32.72±3.39	35.66±2.43	33.74±7.75	±4.14
SGR "	4.06±0.00	3.86±0.01	4.29±0.01	4.04±0.00	4.42±0.00	4.13±0.00	±0.00
FCR "	0.95±0.06	0.98±0.09	0.86±0.04	0.95±0.05	0.90±0.04	1.00±0.10	±0.06
PER "	2.64±0.15	2.55±0.23	2.88±0.15	2.66±0.14	2.76±0.12	2.49±0.25	±0.18
ANPU "	47.25±0.11	46.94±0.43	55.72±0.18	42.69±0.14	46.48±0.00	35.93±0.41	±0.21
MORT. "	9.74±0.03	18.01±3.10	20.01±0.00	13.33±0.00	1.70±1.70	1.70±1.70	±1.14

NS, not significantly different with the diets ($p>0.05$)

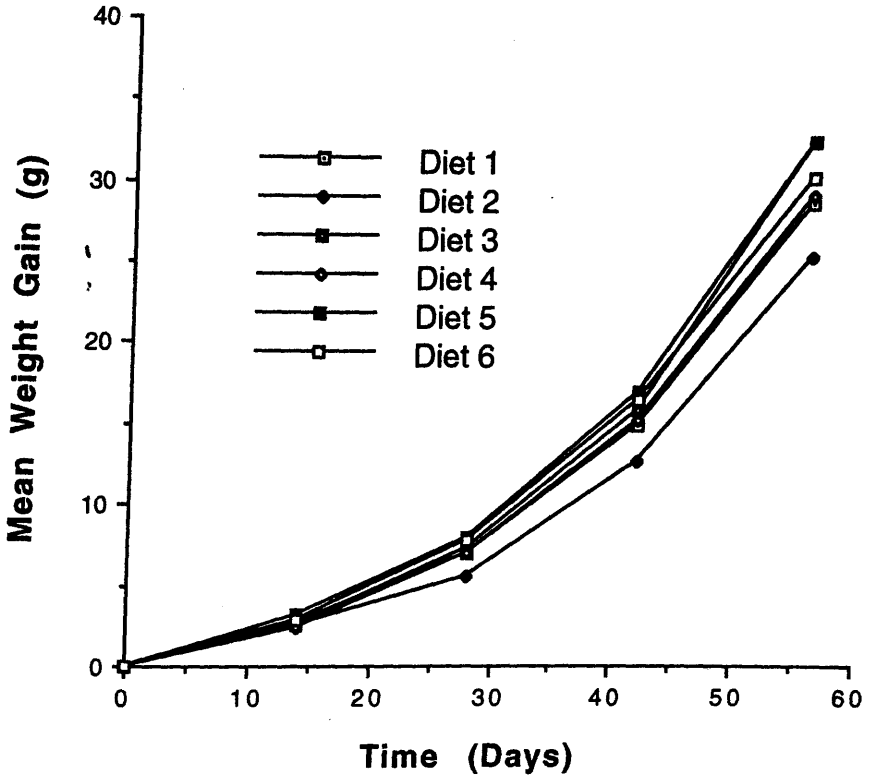


Fig.8.4: Growth response of *Clarias gariepinus* fed dl-methionine supplemented and unsupplemented SF-PMM blend based diets.

Table 8.6 shows a comparative presentation of the EAA profiles of the diets and their requirement by a closely related species (channel catfish). The most limiting amino acids were methionine and phenylalanine while there was superabundance of leucine. Diets with methionine supplementation had higher methionine content than those without. Diets with higher SF had less methionine and more leucine and phenylalanine than those with PMM.

Table 8.6 *Clarias gariepinus* requirements of essential amino acids contained in soybean flour-poultry meat meal blend based diets with or without dl-methionine supplementation.

	Diets						
	I	II	III	IV	V	VI	*
Arg	5.6	5.8	5.5	5.4	5.0	5.2	4.3
His	2.0	2.0	1.9	1.8	1.7	1.8	1.5
Iso	3.0	3.1	3.0	3.0	2.7	2.8	2.6
Leu	6.1	6.2	6.0	6.0	5.6	5.8	3.5
Lys	4.9	5.0	4.7	4.7	4.5	4.7	5.1
Met	1.6	1.0	1.9	1.2	1.9	1.4	2.3
Phe	3.9	4.0	3.7	3.6	3.3	3.4	5.0
Thr	3.2	3.3	3.2	3.2	3.1	3.2	2.0
Val	3.4	3.4	3.4	3.4	3.3	3.4	3.0

* Requirement level of a related species - channel catfish (NRC, 1983)
Requirements for cystine and tyrosine not available and therefore not included

The EAA profiles of the carcasses of fish fed diets I-VI is presented in Table 8.7. There was no significant difference in carcass EAAs with the diets ($p>0.05$). Dietary EAA pattern was similar to that of the Carcass EAAs. Methionine and histidine were low both the diets and carcass while arginine, lysine and leucine were high also in both diet and carcass. A significant positive correlation was observed between the dietary and carcass EAA as depicted in Fig. 8.4 ($P<0.05$). The methionine in the blood plasma of *C. gariepinus* and its relationship with time after feeding are depicted in Fig. 8.5. It was observed that diets without methionine supplementation (diet IV) had the highest level of methionine at six hours after feeding when levels peaked and declined sharply afterwards.

Table 8.7 Carcass essential amino acid composition of *Clarias gariepinus* fed dl-methionine supplemented soybean flour-poultry meat meal blend based diets for 56 days.

	Diets					
	I	II	III	IV	V	VI
Arg*	4.51	4.37	5.02	5.09	4.94	4.42
His*	1.92	1.84	1.97	2.14	1.93	1.19
Iso*	3.21	3.21	3.35	3.67	3.56	3.39
leu*	5.45	5.33	5.88	6.14	5.93	5.69
lys*	6.00	5.73	6.45	6.60	6.24	6.15
Met*	1.54	1.53	1.31	1.46	1.79	1.47
Phe*	3.12	3.04	3.38	3.49	3.40	3.17
Thr*	3.20	3.16	3.49	3.67	3.49	3.29
Val*	3.63	3.55	3.95	4.15	4.00	3.84

*Data in the same row carrying the same superscript differ insignificantly from each other ($P>0.05$)

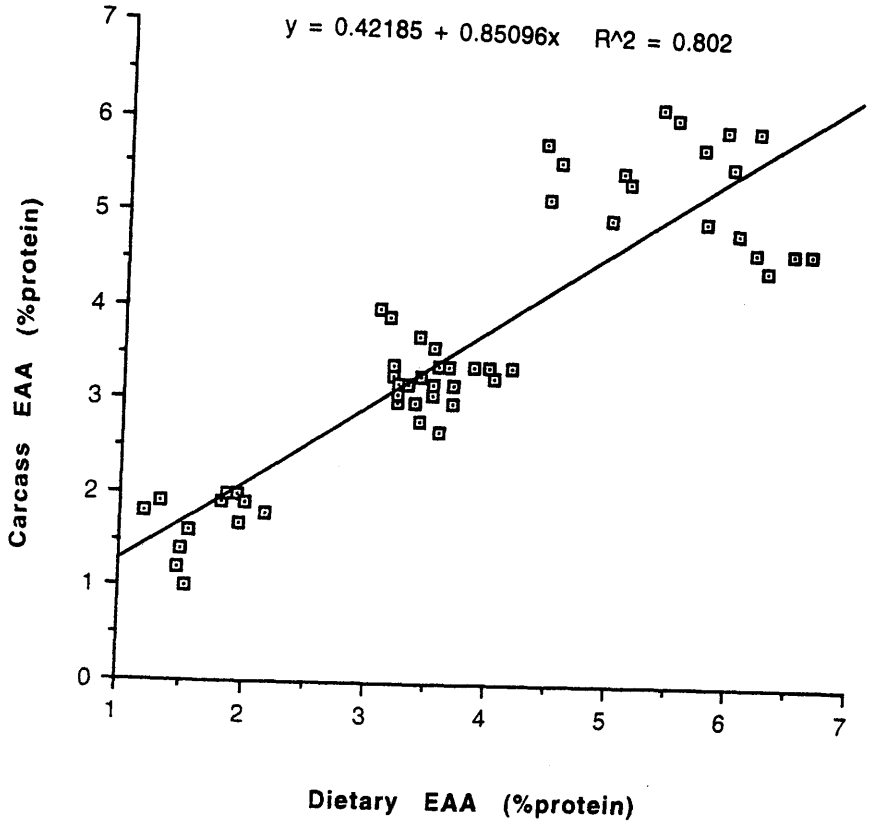


Fig.8.5: Relationship between dietary and *Clarias gariepinus* carcass essential amino acids using data from Tables 8.6 and 8.7.

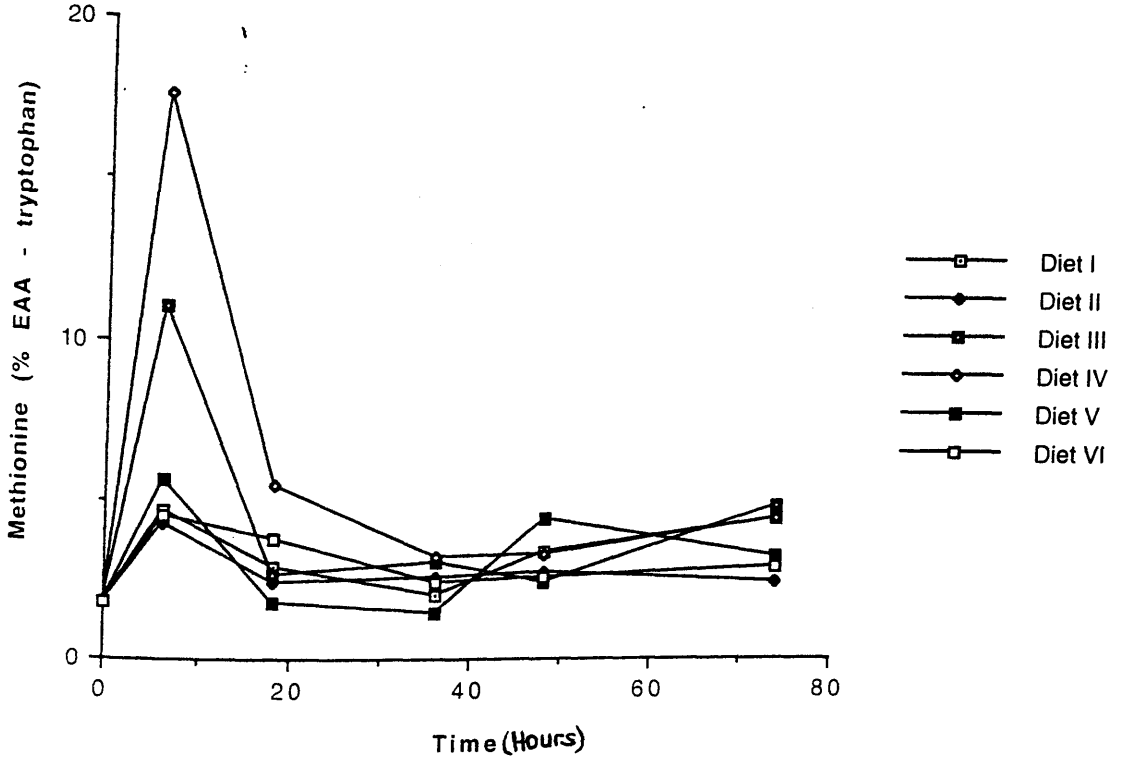


Fig. 8.6: Methionine level in the plasma of *Clarias gariepinus* fed dl-methionine supplemented and unsupplemented SF-PMM blend based diets.

Supplementation of the diets with dl- methionine did not adversely affect the histology of the gills and liver. However, fat deposition in the liver tissue and vacuolation of hepatocytes which became pronounced with increased lipid content as a result of increased incorporation of poultry meat meal in the diet were evident. Diets with 75:25 SF:PMM showed a pronounced and uneven vacuolation that could be pathological (Plates 8.2a, b, c and d).

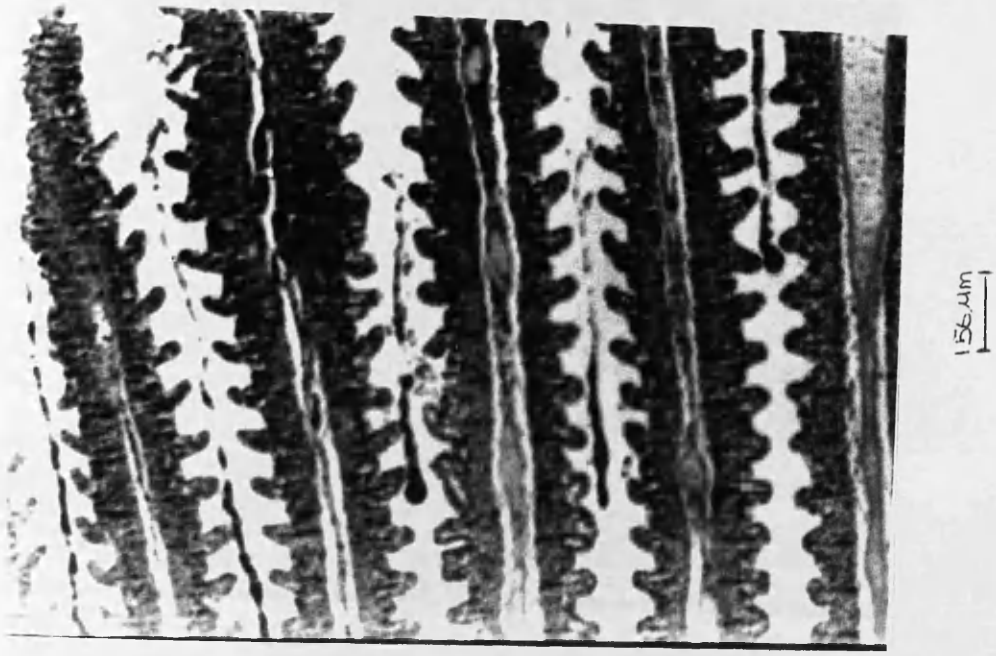


Plate 8.2a: Gill lamellae of *Clarias gariepinus* fed dl-methionine supplemented and unsupplemented SF-PMM blend based diets containing low PMM.

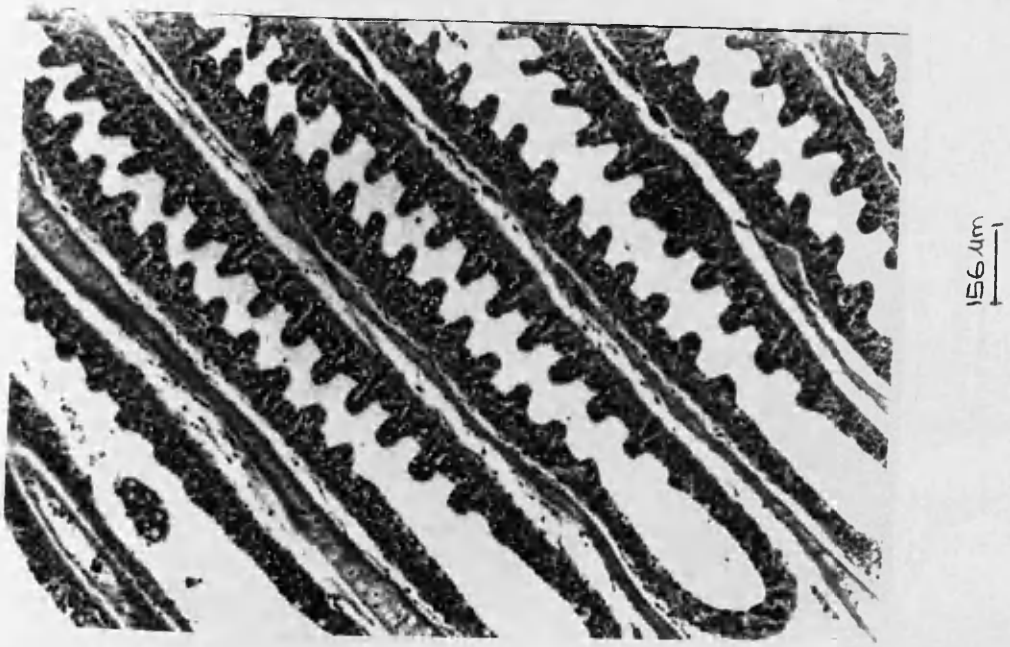


Plate 8.2b: Gill lamellae of *Clarias gariepinus* fed dl-methionine supplemented and unsupplemented SF-PMM blend based diets containing high PMM.

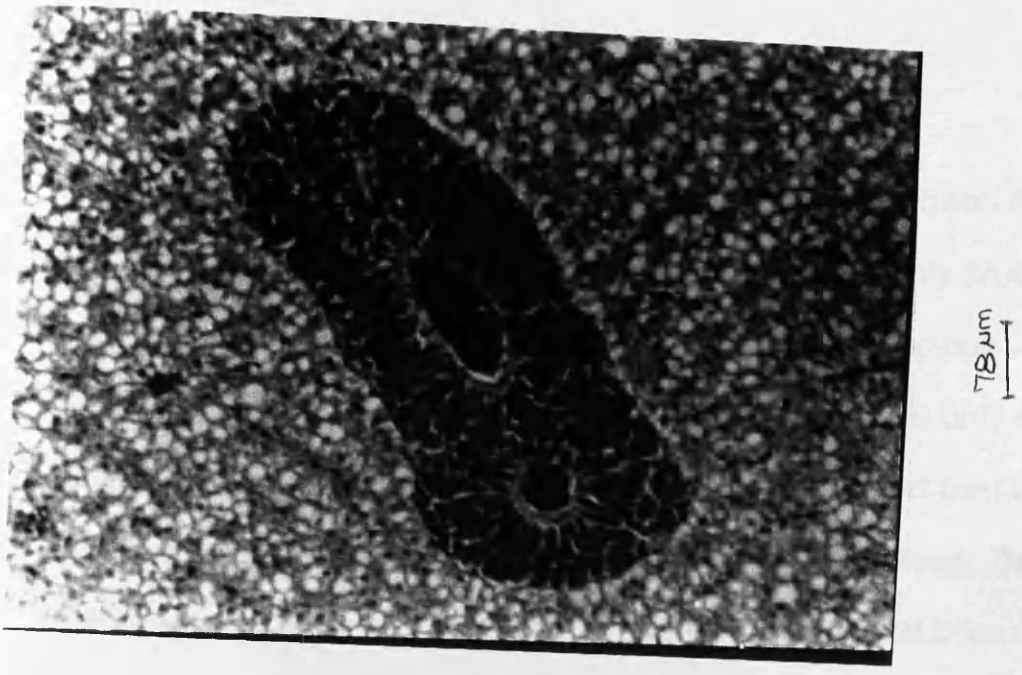


Plate 8.2c: Normal liver with slight vacuolation of the hapatocytes in *Clarias gariepinus* fed SF-PMM blend based diets containing low PMM. Notice exocrine pancreas at the centre

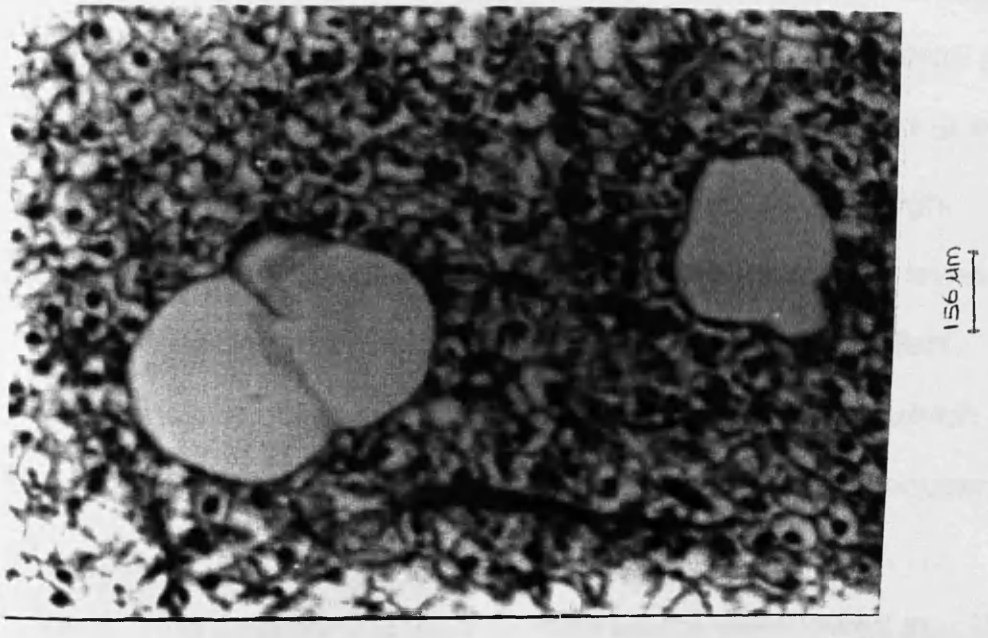


Plate 8.2d: Liver showing pronounced vacuolation and lipid deposition in the hepatocytes of *Clarias gariepinus* fed SF-PMM blend based diets containing high PMM.

8.4 Discussion

Sadiku and Jauncey (in press) investigated the use of soybean flour (SF) - poultry meat meal (PMM) blends as sources of dietary protein in practical diets for *Oreochromis niloticus* and *Clarias gariepinus*. Significant differences were observed in the SGR, FCR, PER and ANPU where diets containing the 50:50 SF:PMM blend performed best of four diets containing 100:00; 25:75; 50:50 and 75:25 SF:PMM blends. This difference was investigated in the current experiment and it was found that methionine supplementation sligtly improved the performance of these diets.

Table 4.1 shows that PMM is richer in methionine than SF while the latter is richer in phenylalanine in agreement with NRC (1983). PMM blending with SF greatly improved the methionine content of the diets as this increased with increase in PMM in the diets. Although methionine levels in all diets were below the requirement levels for both fishes and phenylalanine for only *C. gariepinus*, all diets performed equally well as a result of PMM incorporation which probably raised the methionine level above the critical requirement.

Diets I and II containing 25:75 SF:PMM blends were lowest in methionine while the 75:25 SF:PMM blend (diet V and VI) was highest and conversely for phenylalanine. Better performance of 50:50

SF:PMM than any other in an earlier feeding trial, the trend of which was similarly observed in this investigation, could be attributed to amino acid balance of the diets. Increase in PMM incorporation in the diet raised the level of certain essential amino acids (lysine, methionine, threonine and valine) while others decreased and a good balance needs to be maintained.

For *C. gariepinus,* a good balance of methionine and phenylalanine justifies the 50:50 blending of SF and PMM as 75:25 and 25:75 SF:PMM aggravates methionine and phenylalanine deficiency respectively. The phenylalanine requirement of *O. niloticus* was met in some of the diets while the level in others was not critical, better performance of 50:50 SF:PMM could be attributed to overall essential amino acid balance of the diets. Hardy (1991) observed poorer growth of salmon fed a fish silage based diet than when fed fishmeal diet as a result of better balance of essential amino acid in the plasma (BEAAP) of the latter.

The lower methionine peak recorded for the latter at six hours was as a result of premature absorption of the free amino acid in the diets including the supplemented synthetic methionine. Premature absorption of EAAs has been found to be related to the proportion of peptides and free amino acids in the diet . In addition, premature absorption and metabolisation of certain EAAs such as methionine ,

arginine, isoleucine, leucine and valine occurs in the first three to six hours (Hardy, 1991). Much of the methionine in the supplemented diets would have disappeared at six hours and beyond, thereby giving them the lower level recorded.This trend suggested no differential utilisation of dietary amino acids in the tissue.

The carcass EAA pattern reflected that of the diets. Tissue amino acid patterns of a fish species have been reported to reflect dietary amino requirements of such fish and that the rate of carcass deposition can be equated to the dietary requirement (Jauncey, et al, 1983). Bowen (1980) and Cowey and Tacon (1981) postulated the existence of correlation between the pattern of EAAs in the tissue and that of the dietary requirement. Since favourable perfomance of the fish was observed with all the diets, EAAs levels in the diets were not critically low where deficient.

Histologically, incorporation of poultry meat meal was detrimental to the general well-being of both fishes. Evenly distributed fat deposits and vacuolated hepatocytes of fishes fed low level of PMM were characteristic of cultured fish in intensive culture.

CHAPTER NINE

EFFECT OF MINERAL PREMIX RECONSTITUTION OF SOYBEAN FLOUR-POULTRY MEAT MEAL BLEND BASED DIETS IN *OREOCHROMIS NILOTICUS* AND *CLARIAS GARIEPINUS*

9.1 Introduction

The use of mineral premixes in fish diets has become a tradition in intensive fish feed production. The need for a premix is as a result of insufficient supply of minerals in the diets, from feedstuffs, to meet the mineral requirements of the fish.

These minerals are of structual and physiological importance to the fish as; structural components of hard-tissue matrices like bone, rays, teeth, scales and in soft tissue nutrients like protein, phospholipid, nucleic acids and metalloproteins. They are also cofactors/activators of enzymes, soluble minerals in osmoregulation, acid-base balance (buffer) and in production of membrane potential (Davis and Gatlin , 1991).

Unlike terrestial animals that rely entirly on a dietary source of minerals, fish also derive minerals from their immediate environment. What is included in the diet is to compensate for the negative balance of its environmental supply of minerals and requirements. Marine fishes will require less of a dietary source of minerals than the freshwater fishes. Several studies have been conducted to establish appropriate requirement levels of some of these minerals in tilapia and catfish. NRC (1993) gave the requirements as follows: phosphorus, 0.5%; magnesium, 500mg/kg; zinc, 20mg/kg for *O.niloticus* and phosporus,

0.45%; magnesium, 400mg/kg; manganese, 2.4mg/kg; zinc, 20mg/kg for channel catfish (a related fish to *C.gariepinus*).

For economic considerations and nutritional variability, inclusion levels of these minerals in diets do not always comply with requirement data . Such data are generated from purified diets instead of practical ones (Gatlin and Wilson, 1984). The interactions of these minerals in fish diets with respect to their utilisation by fish have not been accounted for in such nutrient requirements studies. Some minerals have been found to spare each other. Dabrowska et al. (1989) reported the sparing effect of magnesium on calcium, phosphorus and zinc. Calcium was reported to inhibit phosphorus (Davis and Gatlin III, 1991).

In view of these interactions, it would be necessary to investigate the effect of the presence or absence of certain minerals in conventionally used mineral premixes and performance of fishes fed diets with such reconstituted premixes. This study is aimed towards developing economically and nutritionally viable mineral premixes for the soybean flour-poultry meat meal blend based diets used in this study.

9.2 Materials and Methods

Oreochromis niloticus (M±S.E = 3.95±0.09g) and *Clarias gariepinus* (M±S.E. = 1.06±0.02g) fingerlings were stocked in duplicate (10 fish/ tank) in 50-L tanks and fed five 50:50 soybean flour(SF)-poultry meat meal blend based diets - Diet I , Diet II (- calcium and phosphorus), Diet III (- magnesium), Diet IV (-zinc) and Diet V (- Ca, P, Mg and Zn) at 6% body weight per day, thrice daily at 10:00 , 14:00 and 18:00 for eight weeks. Fish were bulk-weighed every 2 weeks and feeding rates adjusted accordingly.

The tanks were regularly cleaned by siphoning off wastes. Water flow was maintained at 2L min^{-1} per tank and water quality parameters were monitored fortnightly according to the methods described in 3.5.2 and recorded thus: Temperature, 26-27°C; dissolved oxygen(DO), 4.5-5.5mgL^{-1}; pH, 5.0-6.5; NH_3-N, 0.2-1.0mgL^{-1}; NO_2^--N, 0.2mgL^{-1}; NO_3^--N, 20mgL^{-1}; Ca-hardness, 36-77mgL^{-1} and total hardness, 44-103 mgL^{-1}.

The formulation and proximate composition of the experimental diets are shown in Table 9.1. 1mm pellets were prepared using a California Pellet Mill (model CL2) equipped with a steam conditioner and subsequently dried by convection at 60°C overnight. After cooling, diets were packed in sealed black polythene bags and stored (-30°C) until required.

Chemical analysis of feedstuffs, diets and carcass (initial and final) was performed according to AOAC (1990) described in 3.6.1. Eight specimens for each species were used for the initial carcass analysis while four specimens per tank were processed for the final carcass analysis. Calcium, magnesium and phosphorus were determined using Atomic Absorption Spectrophotometer (AAS) and phosphorus was spectrophotometrically analysed according to (Allen, 1940) Histological examination of whole fingerling trunk cross section and those of gill, bone and liver were carried out before and after the experiment respectively. Procedures in 3.6.2 were used for evaluating biological parameters. Data were analysed as described in 3.7

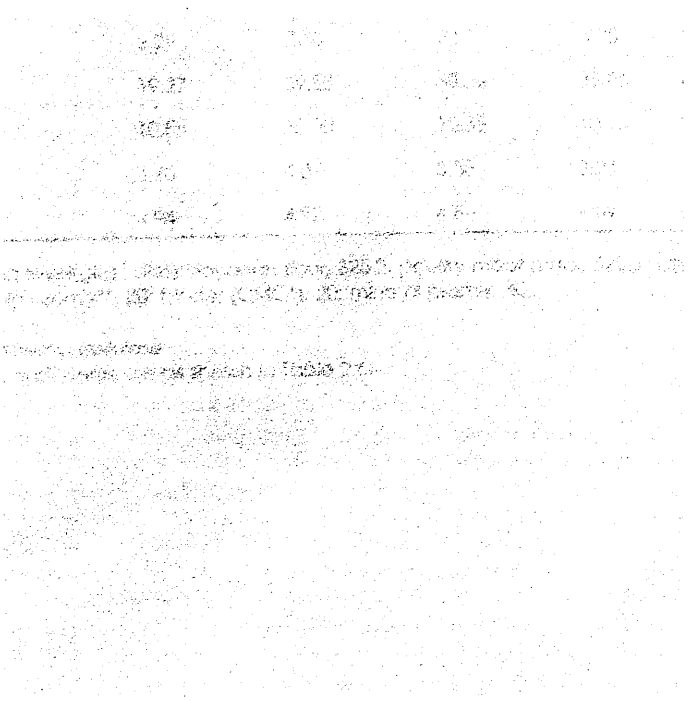


Table 9.1 Mineral premix composition as reconstituted in 50:50 soybean flour:poultry meat meal blend based diets

	Diets				
Mineral(gkg^{-1} premix)	I	II(-Ca&P)	III(-Mg)	IV(-Zn)	V (Ca,P,Mg&Zn)
$CaHPO_4.2H_2O$	727.78	-	834.13	731.80	-
$MgSO_4.7H_2O$	127.50	468.37	-	128.21	-
$ZnSO_4.7H_2O$	5.50	20.20	6.30	-	-
NaCl	60.00	220.41	68.77	60.33	430.96
KCl	50.00	183.67	57.31	50.28	359.14
$FeSO_4.7H_2O$	25.00	91.84	28.65	25.14	179.57
$MnSO_4.4H_2O$	2.54	9.32	2.91	2.53	18.23
$CuSO_4.5H_2O$	0.79	2.88	0.90	0.79	5.64
$CoSO_4.7H_2O$	0.48	1.75	0.55	0.48	3.43
$CaIO_3.6H_2O$	0.30	1.08	0.34	0.30	2.12
$CrCl_3.6H_2O$	0.13	0.47	0.15	0.13	0.92
Mineral composition (gkg^{-1} diet)					
Ca	20.95	15.64	19.82	17.85	15.04
Mg	23.72	33.66	19.82	21.82	17.81
P	0.60	0.53	0.62	0.53	0.33
Zn	0.16	0.27	0.14	0.08	0.10
Proximate composition (% as fed)					
Moisture	2.29	2.70	3.01	3.28	2.01
Protein	39.27	39.28	38.52	38.86	40.18
Lipid	10.56	10.50	10.32	10.15	9.96
Ash	3.70	4.07	3.55	3.23	3.88
Energy ($kcalg^{-1}$)	4.94	4.92	4.89	4.89	4.92

Ingredient inclusion level(gkg^{-1} diet): Soybean flour, 325.2; poultry meat meal, 325.2; wheat flour, 208.0; soy oil, 61.6; vitamin premix**, 20; binder (CMC*), 20; mineral premix, 40.

* CMC = Carboxymethyl cellulose
**Vitamin premix constituents are as shown in Table 2.6.

9.3 Results and Discussion

9.3.1 Experiment 1 - *Oreochromis niloticus*

Table 9.2 shows that there was no significant difference in the mean final weight (MFW), specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilisation (ANPU) and mortality (P>0.05). The requirements for calcium according to Robinson *et al.*(1984), Mg and Zn according to NRC (1993) were met in all diets. Individual or collective withdrawal of these minerals did not distort this balance. The variation between the diets in terms of nutrient utilisation was therefore insignificant (p>0.05). Similarly, there were no marked variation in the growth responses of the fish to the diets as shown in Fig. 9.1

Table 9.2 Nutrient utilisation of *Oreochromis niloticus* fed 50:50 soybean flour-poultry meat meal blend based diets for 56 day with reconstituted mineral premix

	Diets				
Parameters	I	II	III	IV	V
MIW *	3.94±0.47	4.02±0.08	4.09±0.11	3.88±0.03	3.85±0.59
MFW *	21.37±0.59	18.19±2.71	21.05±1.70	18.67±0.77	17.73±2.33
SGR(%/day)*	2.90±0.02	2.69±0.24	2.92±0.18	2.82±0.06	2.74±0.05
FCR *	1.62±0.08	1.62±0.13	1.50±0.11	1.59±0.07	1.65±0.05
PER *	1.60±0.04	1.58±0.14	1.74±0.13	1.63±0.08	1.51±0.04
ANPU(%) *	25.16±5.25	27.90±1.29	30.63±4.16	27.85±0.99	26.32±0.46
MORT.(%) *	0.00	0.00	0.00	0.00	0.00

* Row data differences from means comparison are insignificant (P>0.05).

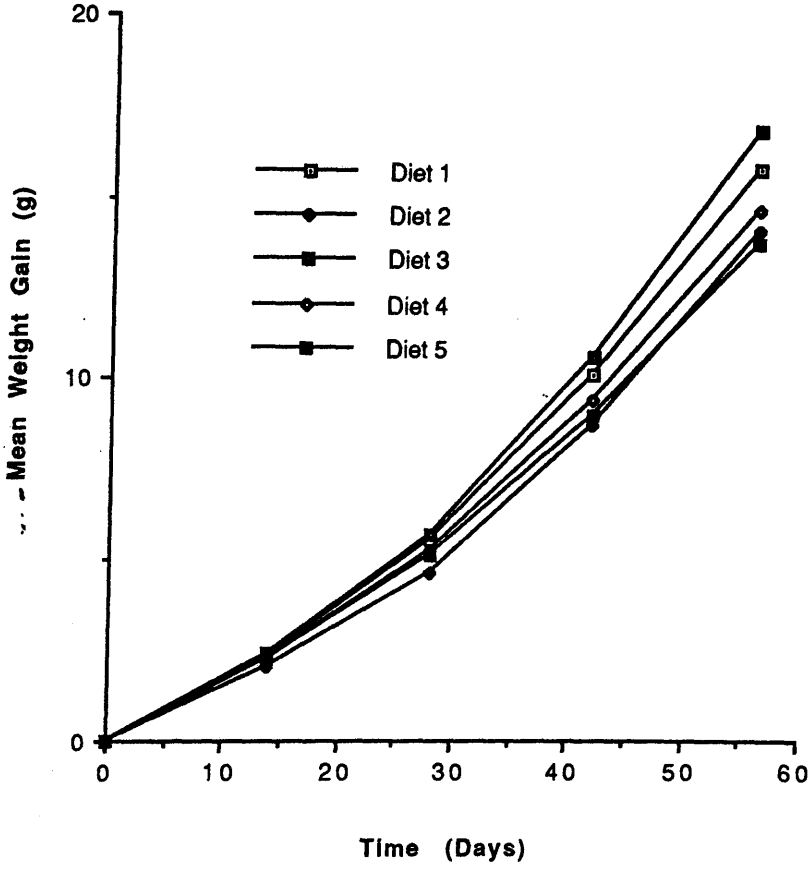


Fig.9.1: Growth response of *Oreochromis niloticus* fed mineral reconstituted SF-PMM blend based diets.

Carcass composition (moisture, protein, lipid and ash) of fishes fed the diets differed insignificantly ($p>0.05$). However, carcass phosphorus differed significantly with the diets ($p<0.10$) as in Table 9.3. Highest carcass phosphorus was observed in fishes fed diet I while the lowest was in those fed diet V. The insignificant difference in carcass mineral deposition could be attributed to adequate supply of minerals in the diets as aforementioned. However, phosphorus requirement was not met in diet V. Its deficiency was annulled by an excess of magnesium which has been reported to spare phosphorus (Dabrowska et al.1989). Carcass deposition of phosphorus was highest in diet I and lowest in diet V.This corresponded to the dietary inclusion level of phosphorus implying a relationship between the dietary phosphorus and its carcass deposit. There were no histopathological symptoms observed

Table 9.3 Carcass proximate and mineral composition of *Oreochromis niloticus* fed soybean flour-poultry meat meal blend based diet with reconstituted mineral premix for 56 days

	Diets				
Carcass composition (% wet wt.)	I	II	III	IV	V
Moisture *	69.60±1.06	69.30±0.84	69.04±1.68	70.17±0.18	68.32±0.17
Protein *	16.63±0.63	16.98±0.45	17.00±0.98	16.63±0.11	16.00±0.14
Lipid *	8.78±0.14	9.04±0.30	8.83±0.83	11.33±4.44	9.79±0.04
Ash *	3.96±0.25	3.83±0.77	4.15±0.06	3.84±0.39	4.09±0.30
Carcass mineral composition ($mgkg^{-1}$ dry wt.)					
Ca	$23.78±0.37^{b}$	$24.86±0.13^{a}$	$27.15±7.04^{a}$	$21.13±2.12^{a}$	$16.44±0.28^{a}$
Mg	$0.59±0.02^{a}$	$0.59±0.01^{a}$	$0.50±0.10^{a}$	$0.40±0.20^{a}$	$0.40±0.01^{a}$
P	$0.81±0.02^{b}$	$0.73±0.03^{b}$	$0.67±0.04^{ab}$	$0.69±0.03^{ab}$	$0.55±0.04^{a}$
Zn	$0.09±0.01^{a}$	$0.09±0.00^{a}$	$0.09±0.01^{a}$	$0.09±0.01^{a}$	$0.09±0.01^{a}$

* = Data along the row not significantly different from each other ($P>0.05$).
Figures in the same row carrying the same superscript are not significantly different ($p>0.10$).

Fig. 9.2 shows a positve strong correlation between dietary phosphorus and carcass phosphorus ($p<0.05$), as increase in dietary phosphorus led to an increase in carcass phosphorus. This correlation was not significant in other minerals. The strong positive correlation between dietary phosphorus and its carcass deposition indicated that there was increase in the carcass deposition of phosphorus with its increase in the diets. This trend was observed to correspond to increase in dietary magnesium which did not increase proportionately in the carcass suggesting a preferential utilisation of magnesium to meet other physiological demands to spare phosphorus for its mobilisation towards tissue formation (Davis and Gatlin, 1991).

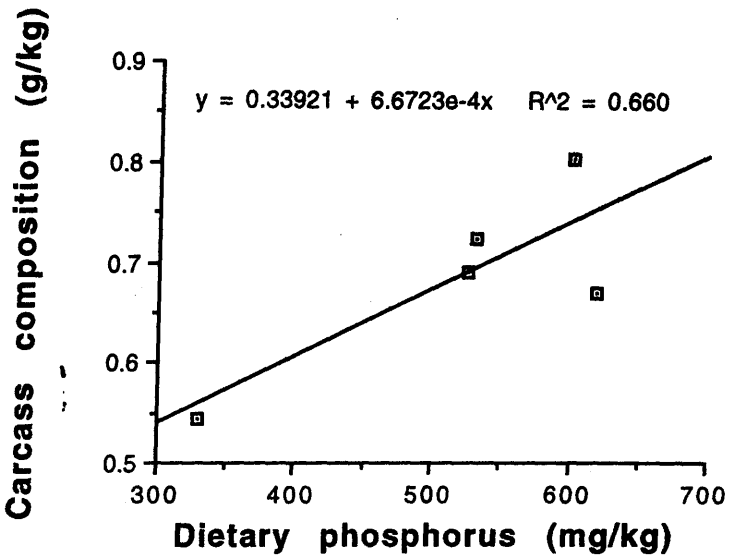


Fig.9.2: Relationship between dietary and carcass phosphorus of *Oreochromis niloticus* fed mineral reconstituted SF-PMM blend based diets.

Table 8.1 shows that phosphorus was in excess in all the diets except diet V. In view of the environmental impact of phosphorus in aquaculture operations, care should be taken against oversuppplementation of dietary phosphorus. Phosphorus dietary supplementation should be minimised and this will in turn reduce feed cost (Davis and Gatlin, 1991). In diets of *O.niloticus* as in this experiment, it has been observed that phosphorus supplementation in a mineral premix with adequate magnesium may not be necessary as magnesium spares phosphorus. Magnesium level in such premix could be increased in the diets to spare phosphorus. This suggests undersupplementation of phosphorus in a diet with high level of magnesium to make a low pollution diet.

Histological examination of the fish showed no differences in spinal cord, kidney and liver: No difference in the level of ossification of the vertebral column; the interstitial lymphoid tissues of the kidney were normal for tilapia species and exocrine pancreas combined with some fat deposits were observed and these are not uncommon with fishes in intensive culture (Plates 9.1a, b, and c respectively).

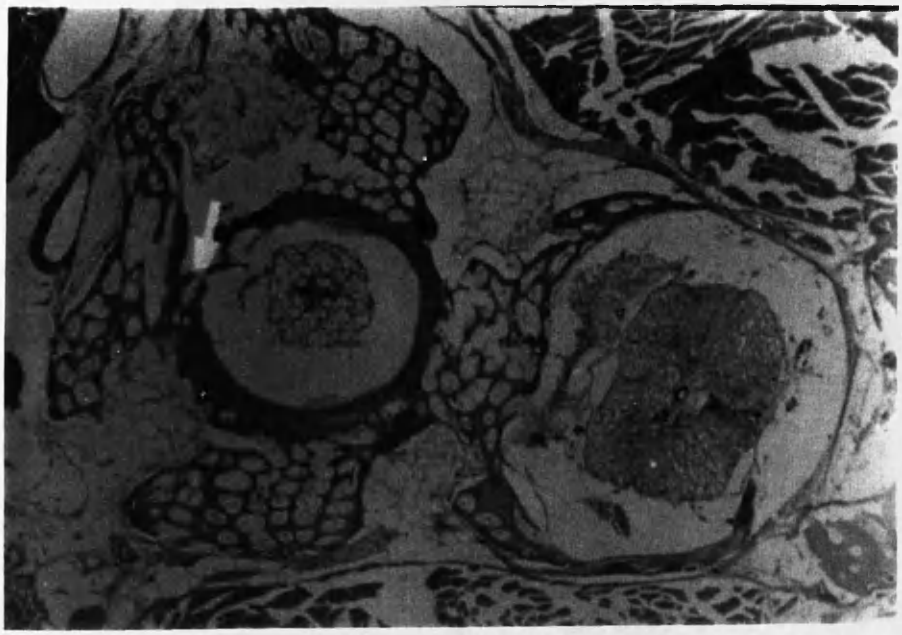


Plate 9.1a: Vertebral column of *Oreochromis niloticus* fed mineral reconstituted SF-PMM blend based diets

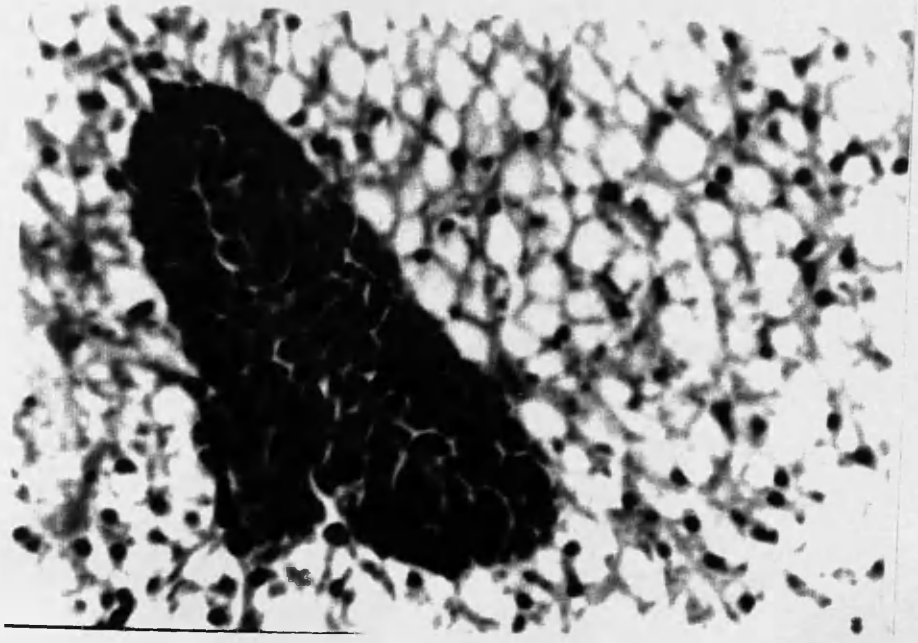


Plate 9.1b: Liver of *Oreochromis niloticus* fed mineral reconstituted SF:PMM blend based diets. Notice the exocrine pancreas to the left

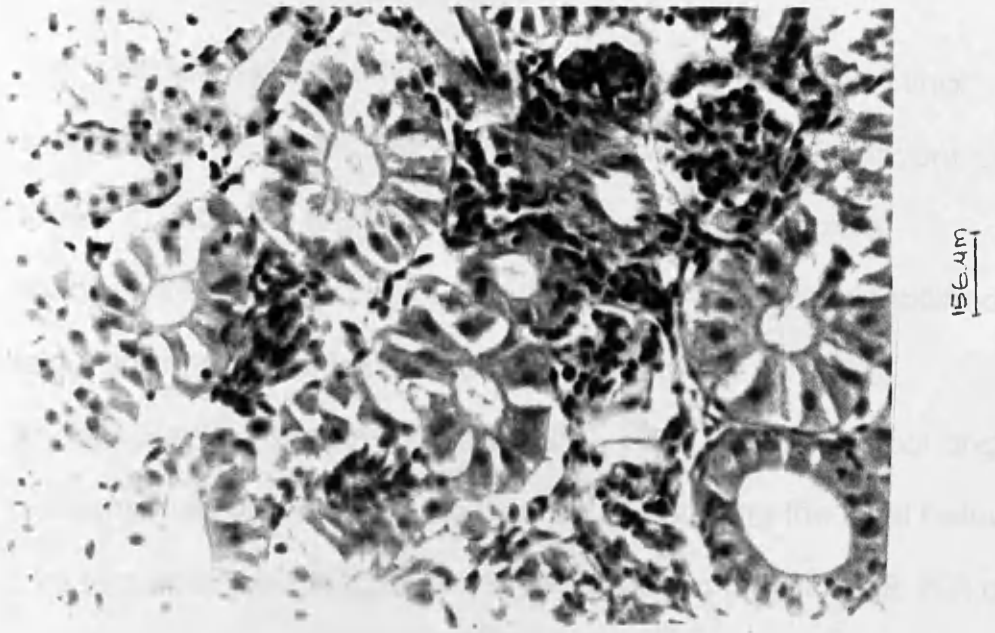


Plate 9.1c: Kidney of *Oreochromis niloticus* fed mineral reconstituted SF-PMM blend based diets.

9.3.2 Experiment 2 - *Clarias gariepinus*

The variation between the diets with respect to their mean final weight, SGR, FCR, PER and ANPU of the fishes were insignificant as shown in Table 9.4 (p.>0.05). The dietary supply of calcium, magnesium and phosphorus in these diets satisfied the established requirements for catfish, viz; calcium (Andrews et al., 1973), phosphorus, magnesium and zinc (NRC, 1993). Their individual and collective withdrawal from the premix did not bring the level below that required. The utilisation of nutrients in terms of SGR, FCR, PER and ANPU remained insignificantly different amongst the diets (p>0.05). Fig. 9.3 depicts similar growth responses by the fish to the diets.

Table 9.4 Nutrient utilisation of *Clarias gariepinus* fed 50:50 soybean flour-poultr meat meal blend based diets with reconstituted mineral premix for 56 days

	Diets				
Parameter	I	II	III	IV	V
MIW *	1.11±0.03	1.14±0.04	1.02±0.06	1.02±0.04	1.01±0.01
MFW *	8.85±0.49	9.78±0.40	9.07±1.42	9.40±1.00	9.27±0.88
SGR(%/day)*	3.71±0.13	3.85±0.01	3.91±0.39	4.05±0.00	3.80±0.42
FCR *	0.90±0.06	0.80±0.01	0.91±0.10	0.89±0.01	0.92±0.05
PER *	2.73±0.01	3.19±0.04	2.88±0.30	2.89±0.04	2.72±0.16
ANPU(%) *	42.29±3.07	50.14±0.18	45.86±2.02	47.21±1.74	43.64±3.71
MORT.(%) *	5.00	10.00	0.00	5.00	10.00

* = Row data means comparison not significantly different (P>0.05)

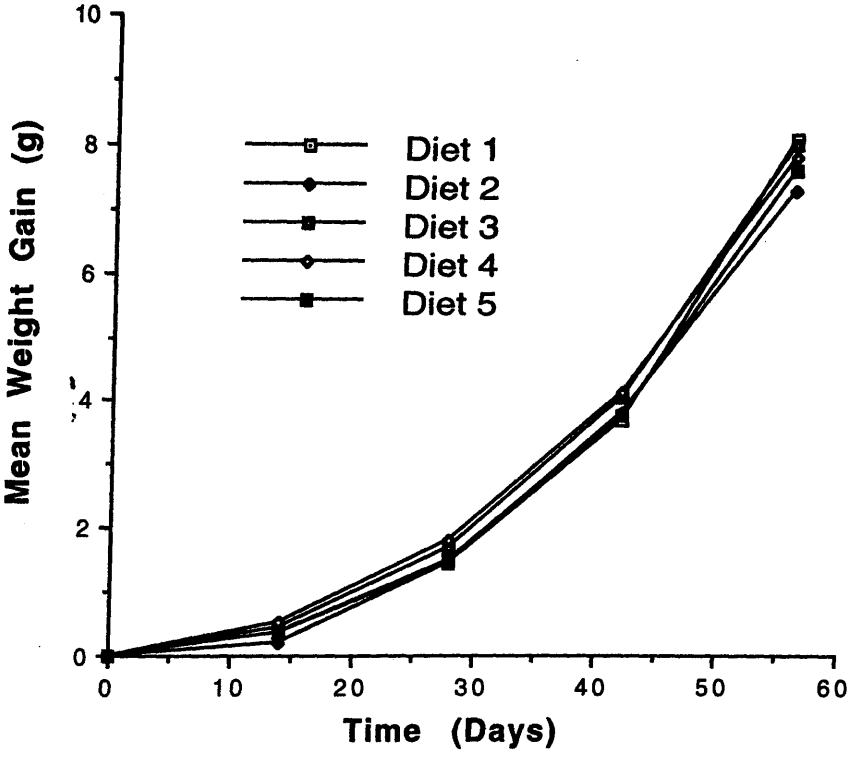


Fig.9.3: Growth response of *Clarias gariepinus* fed SF-PMM blend based diets.

Table 9.5 shows that moisture, protein, lipid and ash also differed insignificantly with diet ($p>0.05$). However, carcass calcium and phosporus differed significantly with diet ($p<0.10$). Highest and lowest carcass calcium were recorded in fishes fed Diets I and V respectively while carcass phosohorus was highest in fish fed Diets I and III, and lowest in those fed Diet V. There were no histopathological symptoms evident in fish fed the various diets.

Table 9.5 Carcass proximate and mineral composition of *Clarias gariepinus* fed soybean flour-poultry meat meal blend based diet with reconstituted mineral premix for 56 days

	Diets				
Carcass Composition (% wet weight)	I	II	III	IV	V
Moisture *	74.50±0.40	74.38±0.01	74.13±0.20	72.90±0.04	74.68±0.03
Protein *	15.67±0.09	15.64±0.12	15.57±0.78	16.13±0.33	15.87±0.41
Lipid *	5.64±0.09	5.87±0.08	5.84±0.08	6.78±0.10	5.46±0.21
Ash *	2.88±0.51	3.39±0.18	2.61±0.74	3.46±0.41	3.15±0.04
Mineral composition (mg/kg dry wt.)					
Ca	18.97±0.38^{b}	15.54±0.63^{ab}	18.16±0.68^{ab}	16.84±1.50^{ab}	14.69±0.28^{a}
Mg	0.59±0.01^{a}	0.60±0.01^{a}	0.69±0.09^{a}	0.59±0.01^{a}	0.60±0.01^{a}
P	0.72±0.05^{b}	0.58±0.03^{a}	0.73±0.03^{b}	0.58±0.04^{a}	0.60±0.02^{ab}
Zn	0.08±0.00^{a}	0.08±0.00^{a}	0.09±0.00^{a}	0.08±0.00^{a}	0.07±0.00^{a}

* = Not significant with respect to the diets ($P>0.05$).

Figures in the same row carrying the same superscript not significantly different ($p>0.10$)

Magnesium levels in the diets were in excess of requirements, hence its possible sparing effect on phosphorus. Davis and Gatlin (1991) reported that the functions of calcium and phosphorus are primarily stuctural - mobilisation towards the formation of the cephalo-nuchal crest. This could be responsible for the differences observed in carcass calcium and phosphorus deposition with the diets. Carcass calcium and phosphorus were high in fishes fed diets I and III, and lowest in diet V because of their high and low levels in the diets respectively, depicting a relationship between dietary calcium and phosphorus and their carcass deposition. Fig 9.4 shows that dietary calcium had strong positive correlation with carcass calcium ($p<0.05$) while dietary phosphorus correlated insignificantly with carcass phosphorus ($p>0.05$). High levels of calcium in the diets led to high calcium levels in the carcass. Correlation of dietary and carcass mineral showed that only calcium had positive and significant correlation ($p<0.05$). The requirement for calcium was quite high. This was probably not unconnected with the structural function of calcium in the formation of skeleton and bony structures which demanded more of calcium than any other mineral. The main target site for dietary calcium in *C. gariepinus* was the bony structure while that of phosphorus could be physiological.

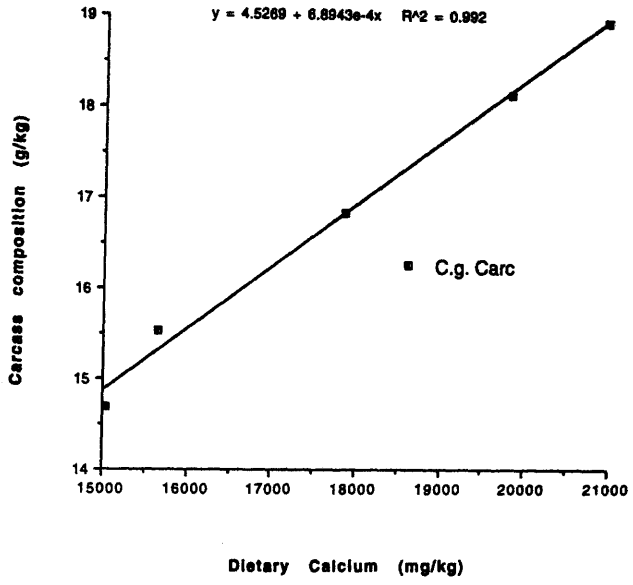


Fig.9.4a: Relationship between dietary and carcass phosphorus of *Clarias gariepinus* fed SF-PMM blend based diets.

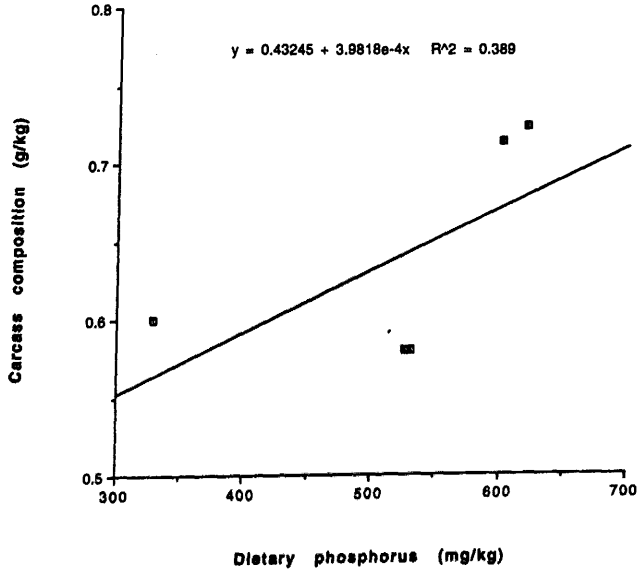
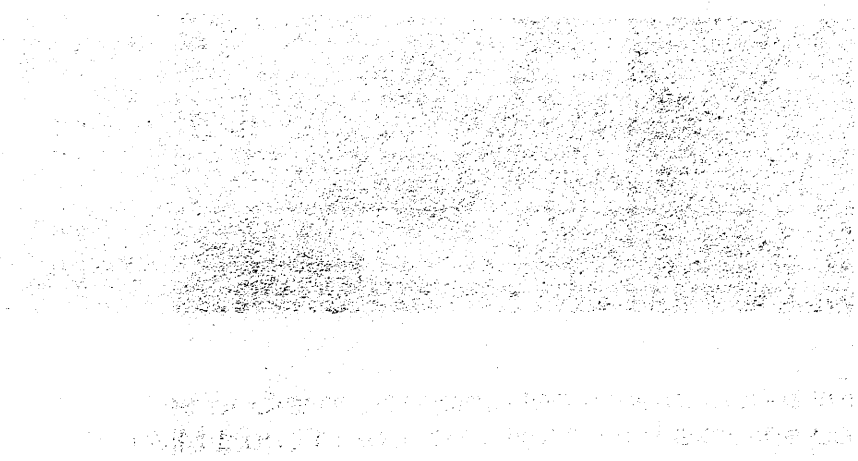


Fig.9.4b: Relationship between dietary and carcass calcium of *Clarias gariepinus* fed SF-PMM blend based diets.

The magnesium sparing effect on phosphorus might not be dominant over the inhibitory effect of calcium on phosphorus as supplementation of calcium in the basal diet appeared to inhibit phosphorus bioavailability (Davis, 1990). It was evident that calcium and phosphorus supplementation would be necessary in catfish diets.

The vertebral column, liver and kidney did not show any histopathological symptom. Reconstitution of minerals in the diets did not create any marked difference in vertebral column development. Liver tissue showed the exocrine pancreas and a few fat deposit in the hepatocytes , this was normal with farmed fishes. Well developed interstitial lymphoid tissue were observed in the Kidney (Plates 9.2a, b and c)



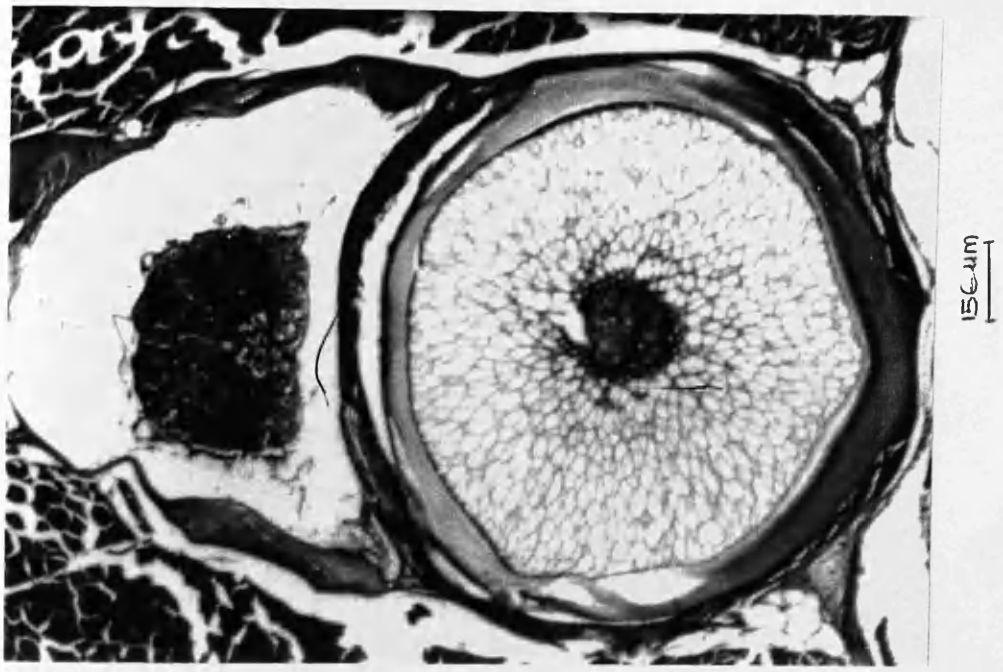


Plate 9.2a: Vertebral column of *Clarias gariepinus* fed mineral reconstituted SF-PMM blend based diets.

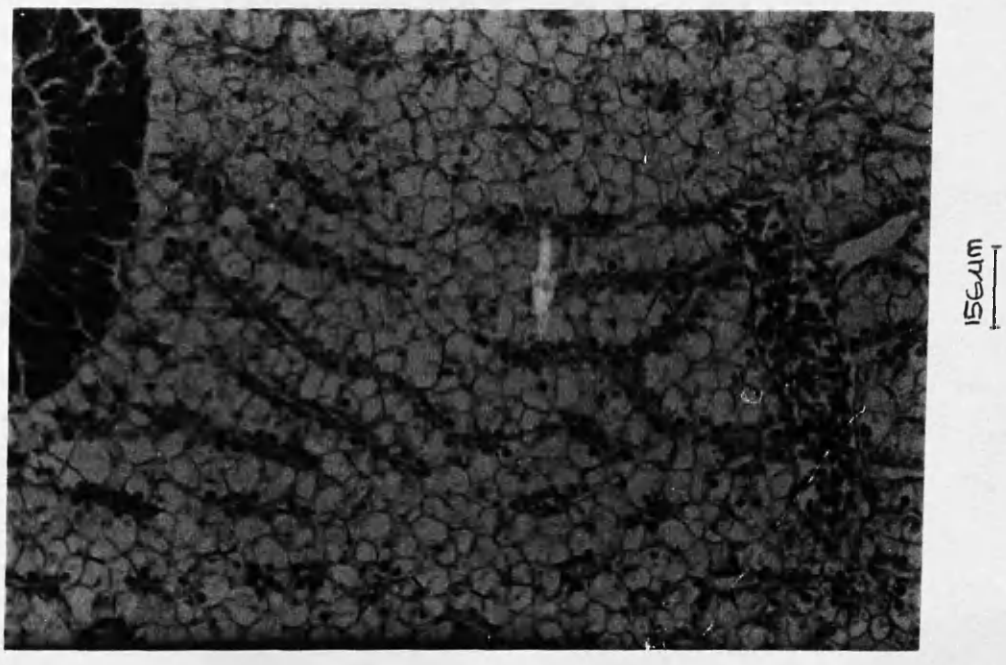


Plate 9.2b: Liver of *Clarias gariepinus* fed mineral reconstituted SF-PMM blend based diets. Notice the exocrine pancreas to the left.

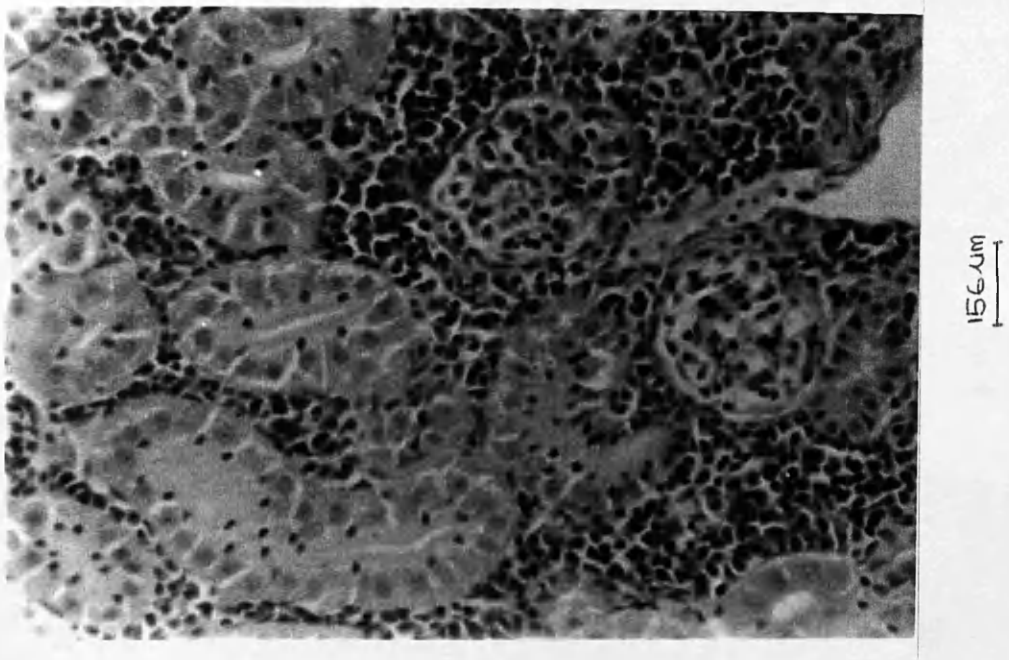


Plate 9.2c: Kidney of *Clarias gariepinus* fed mineral reconstituted SF-PMM blend based diets. Notice the well developed interstitial tissue.

CHAPTER TEN

EFFECTS OF DETOXIFICATION BY AUTOCLAVING RAW SOYBEAN FLOUR AND BLENDING WITH POULTRY MEAT MEAL ON THEIR UTILISATION IN PRACTICAL DIETS OF *OREOCHROMIS NILOTICUS* AND *CLARIAS GARIEPINUS*.

10.1 Introduction

With respect to economics, availability and nutritional value, soybean meal appears to be the most promising vegetable protein source for animal feeds but not without its inherent flaws (Lim, 1992). Notable among these are its relative inefficiency as recovery energy source for tissue growth and repair, and gonadal development when incorporated at high level in fish diets. These have been attributed to nutrient deficiency and imbalance such as minerals and amino-acids, presence of anti-nutritional factors (ANFs) and toxic substances in plant feedstuffs, diet stability in water and palatability (Lim and Dominy, 1991).

Natural nutrient sources incorporated to provide the deficient nutrient at the right time has been suggested. Some lower cost animal proteins - than fish meal, are higher in methionine than soybean are lower in the ANFs. These are poultry by-product meal, blood meal, shrimp meal, meat meal, crab meal, gelatin, e.t.c. (NRC, 1983). Processing of raw plant protein to remove ANFs and toxic substances would also be beneficial and it has become area of focus of aquaculture nutrition (Chamberlain, 1993). Processing of raw plant feedstuffs like soaking in water, drying , heating etc. have been found capable of reducing the ANFs. Heat treatment is an effective way of removing most of the ANFs (Tacon, 1982; Lovell, 1990). Feedding heat

treated soybean meal to channel catfish diet improved its growth response (Wilson and Poe, 1985). Processing of SF through processing and nutrient enrichment by the incorporation of natural nutrient sources has been proposed as possible remedies to the flaws associated with SF utilisation in aquaculture feeds. Results from such experimental evaluation have recorded relative successes.

There is also paucity of information on the use raw SF blended with PMM and autoclaved SF blended with PMM in aquaculture feeds. The aim of this investigation is therefore, to evaluate the effects of such processing and nuitrient enrichment on diet utilisation by *O.niloticus* and *C.gariepinus*.

10.2 Materials and Methods

i Experimental fish, fish husbandry and fish diets

Eight isocaloric (4.2 $kcalg^{-1}$) and isonitrogenous (38%) diets - Diets I-VIII 25:75; 50:50; 75:25; 100:00 soybean flour - poultry meat meal blends, using raw and autoclaved soybean flour were fed to fingerlings of *Oreochromis niloticus* (M±S.E. = 2.78±0.03) and *Clarias gariepinus* (M±S.E = 14.73±0.13g) at 4% body weight per day at 10:00 and 18:00 for eight weeks in two seperate experiments - Experiments 1 and 2 respectively. Fishes were bulk-weighed every 2 weeks and feeding rates adjusted accordingly.

Defatted, dehulled and raw soybean flour (Type I) was obtained from Sigma Chemical Co. ltd, U.S.A. Half of this was autoclaved at 121°C at 15 psi for 20 minutes. Table 10.1 depicts the varying levels of anti-nutritional factors (trypsin inhibitor and phytic acid) and available lysine in raw and autoclaved soybean flour used in both experiments. High quality poultry meat meal was supplied by Chettles UK ltd, Nottingham. The formulation and proximate composition of the diets are shown in Table 10.2. 2mm pellets were prepared using a California Pellet Mill (model CL2) equipped with a steam conditioner while diets containing raw soybean were pelleted using Hobart A200 Mixer to prevent the heat effect of steam conditioning. Diets were

subsequently dried by convection at 40°C overnight and packed in sealed black plastic bags and stored (-30°C) until required.

ii Experimental system

A 2 X 4 factorial design using twenty-four 50L tanks was adopted for each experiment. Fingerlings were stocked at 15 fish per tank in triplicate per treatment. The tanks were regularly cleaned by siphoning off accumulated waste. Water flow was maintained at 2L min^{-1} per tank. Water quality parameters were monitored as described in 3.5.2 and recorded thus: Temperature, 26-27 °C and 25-27 °C; dissolved oxygen (DO), 4.4-6.2 and 5.1-6.0 mgL^{-1}; pH, 6.0-6.5 and 6.0-7.0; NH_3-N, 0.2-0.6 and 0.2-0.6 mgL^{-1}; NO_2-N, 0.2 and 0.2 mgL^{-1}; NO_3-N, 10-20 and 10-20 mgL^{-1}; Ca-hardness, 51-62 and 46-57 mgL^{-1} and total hardness, 59-71 and 66-93 mgL^{-1} for experiment 1 and 2 respectively.

iii Experimental analysis

Chemical analysis of feedstuffs, diets and carcass (initial and final) was performed according to AOAC (1990) following the procedures in 3.6.1. Eight specimens of fingerlings were taken for initial carcass analysis while four from each tank were used for final carcass analysis. Trypsin inhibitor activity assay in soybean flour was according to Hammerstrand et al. (1981) described in 4.1.3. Biological parameters

measured according to the methods in 3.6.2. Data were analysed statistically as described in 3.7.

Hepatosomatic index (HSI) and gonadosomatic index (GSI) were calculated using the formula below:

$$HSI\ (\%) = \frac{\text{Liver Weight(g) } 100}{\text{Fish body Weight(g)}}$$

$$GSI\ (\%) = \frac{\text{Gonad Weight(g) } 100}{\text{Fish Body Weight(g)}}$$

Table 10.1 Level of trypsin inhibitor (TI) activity, phytate phosphorus and available lysine in the soybean flour (SF) used in experimental diets

SF type	TI activity (TIU /mg SF)	Phytate phosphorus (mg/100g sample)	Available lysine (as % protein)
Raw SF	91.3	789.7	4.3
Autoclaved SF	39.8	405.3	4.2
Commercial SF	7.3	357.2	4.1

Table 10.2 Ingredients and proximate composition of raw and autoclaved SF-PMM blend based diets.

Diets	I	II	III	IV	V	VI	VII	VIII
Ingredients								
Soybean flour	14.5	31.2	50.3	72.6	14.5	31.0	50.0	71.9
Poultry meat meal	43.6	31.2	16.8	-	43.6	31.0	16.6	-
Wheat flour	29.0	24.5	19.3	13.2	29.2	24.9	20.0	14.2
Soybean oil	4.8	5.2	5.7	6.3	4.7	5.1	5.4	5.9
Mineral premix[1]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin premix[2]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Binder (CMC)[3]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Proximate composition (% as fed)								
Moisture	2.2	2.2	3.4	2.5	2.6	4.7	4.2	3.6
Protein	38.6	39.4	38.5	39.5	39.0	37.0	37.7	38.5
Lipid	8.9	6.5	4.0	3.2	9.5	7.8	5.5	3.8
Ash	11.3	10.6	9.2	8.2	11.3	10.3	9.2	.2
Energy ($kcalg^{-1}$)	4.5	4.4	4.3	4.4	4.6	4.4	4.3	4.3

[1,2] Constituents are as presented in Table 2.6
[3]CMC, Carboxymethyl cellulose

10.3 Results

10.3.1 Experiment 1 - *Oreochromis niloticus*

i Nutrient Utilisation

Table 10.3 presents the response of *O.niloticus* fingerlings fed raw and autoclaved soybean flour (SF) - poultry meat meal (PMM) blend based diets. It was observed that diets containing autoclaved soybean flour did better than those with raw soybean flour at the same incorporation level. Similarly, diets containing high inclusion levels of PMM performed better than those with soybean flour. There was significant variation in the mean final weight (MFW), specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilisation (ANPU) of fishes fed the different diets ($P<0.05$). Mortality variation with the diets was insignificant ($P>0.05$).

Diet V containing 25:75 SF:PMM with autoclaved SF did better than those of any other blending ratio with or without autoclaving. It gave gave highest MFW, SGR, PER and ANPU, and lowest FCR values. Poorest nutrient utilisation was observed in fish fed raw soybean flour based diet without PMM (diets IV) with lowest MFW, SGR, PER and ANPU, and highest FCR values. The interraction of autoclaving and

blending was insignicant as diets with more PMM did better than those with less and diets with autoclaved SF did better than those with raw and PMM and SF effects were independent of each other ($P>0.05$). Fig. 10.1 shows that fish fed diet V had the highest growth response while those fed diets IV and VIII recorded the least growth response.

Table 10.3 Effects of autoclaving of soybean flour and incorporation of poultry meat meal on utilisation of soybean flour-poultry meat meal blend based diets fed to *O.niloticus* fingerlings for 56 days

Diets	MIW	MFW	SGR	FCR	PER	ANPU	MORT.
I	2.7±0.0^{a}	10.9±1.0^{ab}	2.5b^{c}	1.7±0.1^{abc}	1.5±0.1^{bc}	21.1±0.0^{bc}	1.7±1.7^{a}
II	2.8±0.1^{a}	10.1±0.4^{ab}	2.3a^{bc}	1.9±0.1^{bcd}	1.3±0.0^{ab}	18.2±0.0^{ab}	0.8±0.8^{a}
III	2.7±0.0^{a}	8.6±0.4^{a}	2.1^{ab}	2.2±0.1^{cd}	1.2±0.1^{ab}	16.3±0.0^{ab}	3.2±3.2^{a}
IV	2.8±0.1^{a}	8.6±0.1^{a}	2.0^{a}	2.3±0.1^{d}	1.1±0.0^{a}	15.0±0.0^{a}	0.8±0.8^{a}
V	2.7±0.2^{a}	15.1±1.2^{c}	3.1^{d}	1.3±0.1^{a}	2.0±0.1^{d}	27.6±0.0^{d}	0.0±0.0^{a}
VI	2.8±0.1^{a}	12.5±0.8b^{c}	2.7^{cd}	1.6±0.1^{ab}	1.7±0.1^{cd}	24.7±0.0^{cd}	4.4±1.2^{a}
VII	3.0±0.1^{a}	10.3±0.6a^{b}	2.2^{ab}	1.9±0.1^{bcd}	1.4±0.1^{abc}	18.6±0.0^{ab}	0.8±0.8^{a}
VIII	2.8±0.1^{a}	8.5±0.5^{a}	2.0^{a}	2.2±0.1^{cd}	1.2±0.1^{ab}	16.4±0.0^{ab}	0.8±0.8^{a}
±S.E.M.	±0.1	±0.7	±0.0	±0.1	±0.1	±0.0	±1.1

Data in the same coulmn carrying the same superscript are insignificantly different from each other ($P>0.05$).

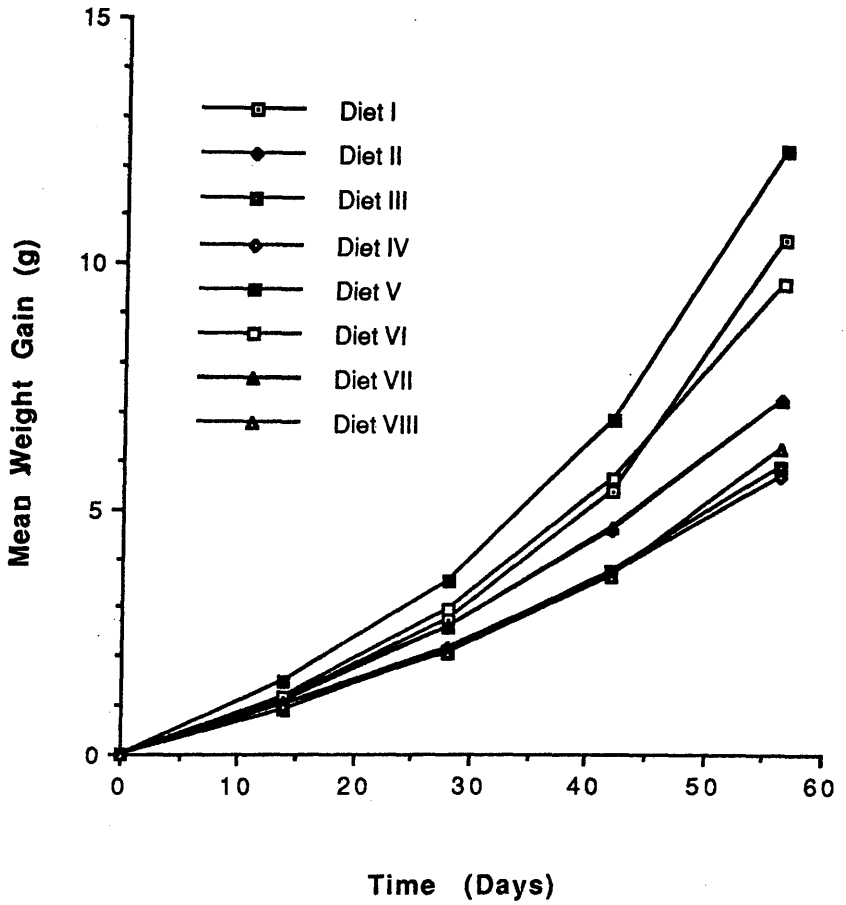


Fig. 10.1: Growth response of *Oreochromis niloticus* fed raw and toasted SF:PMM blend based diets.

Table 10.4 shows that there was no variation in gonadosomatic or hepatosomatic indices of fishes fed these diets with different trypsin inhibitor and available lysine levels. There was no noticeable effect of these treatments on the reproductive state of the fishes. There seemed to be significant and unpatterned individual diet to diet variation, average GSI and HSI of fishes fed diets containing raw soybean flour differed insignificantly with those fed diets containing autoclaved soybean ($P>0.05$). However, sex differences resulted in significant variation in GSI and HSI ($P<0.05$). Female *O.niloticus* had higher GSI than males and conversely for HSI.

Table 10.4 Gonadosomatic and hepatosomatic indices of *O.niloticus* fed raw and autoclaved soybean flour-poultry meat meal blend based diets for 56 days .

	Male		Female	
Diet	GSI	HSI	GSI	HSI
I	1.1	0.7	1.9	0.8
II	1.3	0.6	3.3	0.6
III	0.5	0.7	3.7	1.5
IV	1.0	0.8	3.6	0.5
Average	0.9^a	0.7^a	3.2^b	0.9^a
V	0.5	0.7	4.6	0.5
VI	0.4	0.8	3.4	0.4
VII	0.6	0.5	3.5	0.6
VIII	1.7	0.8	3.2	0.4
Average	0.8^a	0.7^a	3.7^b	0.5^a

Data in the same row carrying the same superscirpt are significantly different ($P>0.05$)

ii Carcass composition

There was significant variation in the moisture, lipid and ash contents in carcasses of fish fed diets containing different levels of raw and toasted SF, and PMM as shown in Table 10.5 ($P<0.05$). The highest moisture content was recorded in carcass of fishes fed diets III and IV while the least was with fishes fed diet V. Fish fed diet V had the highest carcass lipid content while those fed diet IV had the least.

Table 10.5 Carcass composition of *O.niloticus* fed raw and autclaved soybean flour-poultry meat meal blend based diets for 56 days

Diets	Moisture	Protein	Lipid	Ash
I	73.9^{abc}	15.2^{a}	6.9^{c}	3.5^{ab}
II	74.4^{abc}	15.2^{a}	5.2^{b}	4.4^{b}
III	74.8^{bc}	15.4^{a}	5.1^{b}	4.1^{ab}
IV	76.1^{c}	15.2^{a}	3.5^{a}	4.3^{ab}
V	71.5^{a}	15.0^{a}	7.3^{c}	4.4^{ab}
VI	73.5^{ab}	15.4^{a}	6.6^{c}	4.0^{ab}
VII	75.1^{bc}	15.2^{a}	4.9^{b}	4.0^{ab}
VIII	75.0^{bc}	15.5^{a}	5.1^{b}	3.4^{a}
±S.E.M	±0.0	±0.0	±0.0	±0.0
Initial carcass	66.6	18.6	8.0	4.9

Data in the same column carrying the different superscripts are significantly different from each other ($P<0.05$)

iii Histology

Examinaton of the fish fed the various diets showed hypertrophy of the primary gill lamellae in fish fed diets containing raw soybean flour (Plate 10.1a and b). There was hepatocytes vacuolation and lipid deposition in the liver of fish fed all the diets. However, these were not severe enough to be pathological even with a high inclusion level of poultry meat meal at 25:75 SF:PMM as vacuoles were small and evenly distributed considered normal in farmed fish fed high protein - energy diets (Plate 10.2a and b)

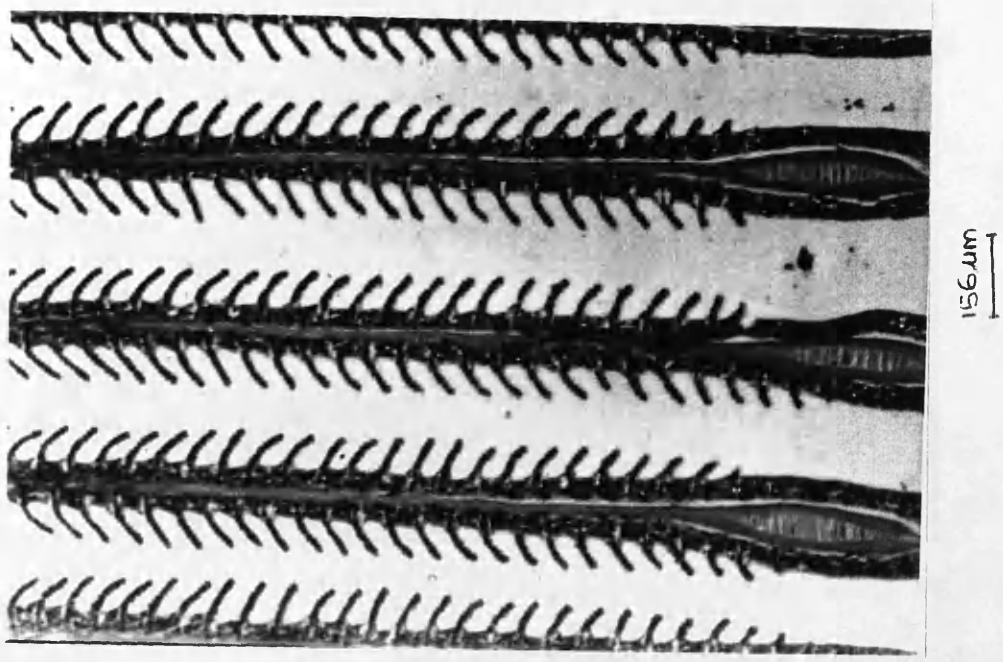


Plate 10.1a: Normal gill of *Oreochromis niloticus* fed SF-PMM blend based diets containing autoclaved SF.

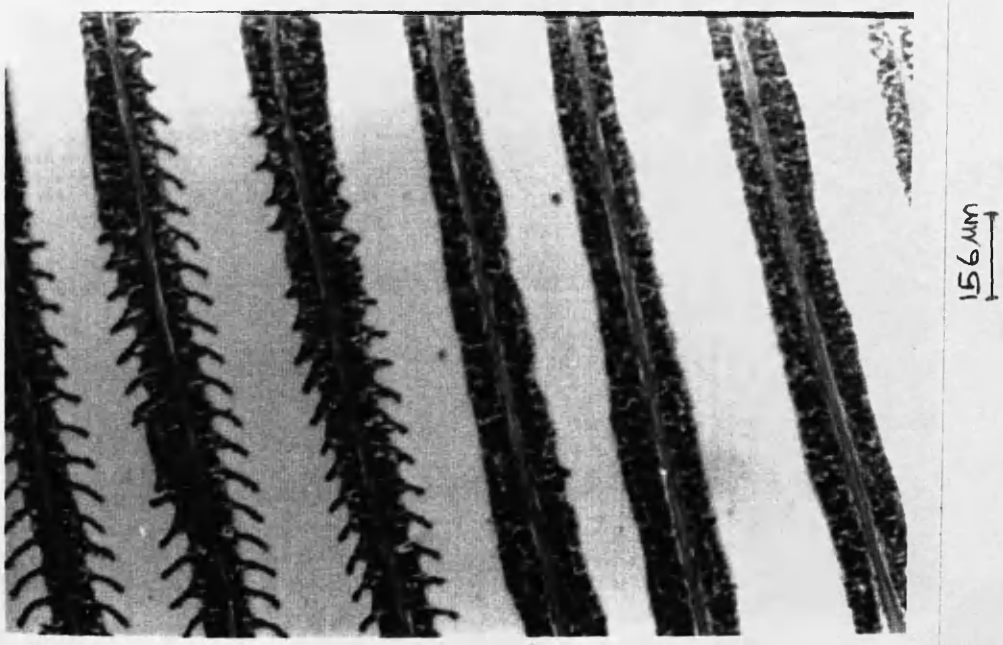


Plate 10.1b: Gill of *Oreochromis niloticus* fed SF-PMM blend based diets containing raw SF. Notice hypertrophy of the lamellae.

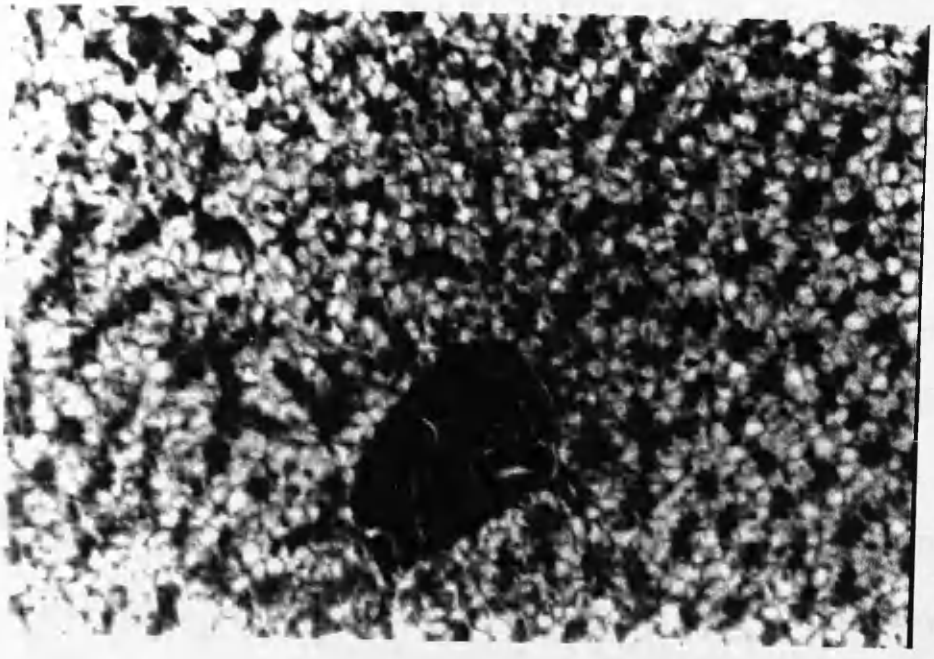


Plate 10.2a: Liver of Oreochromis niloticus fed SF-PMM blend based diets containing autoclaved SF showing relatively pronounced vacuolation of the hepatocytes. See exocrine pancreas at the centre.

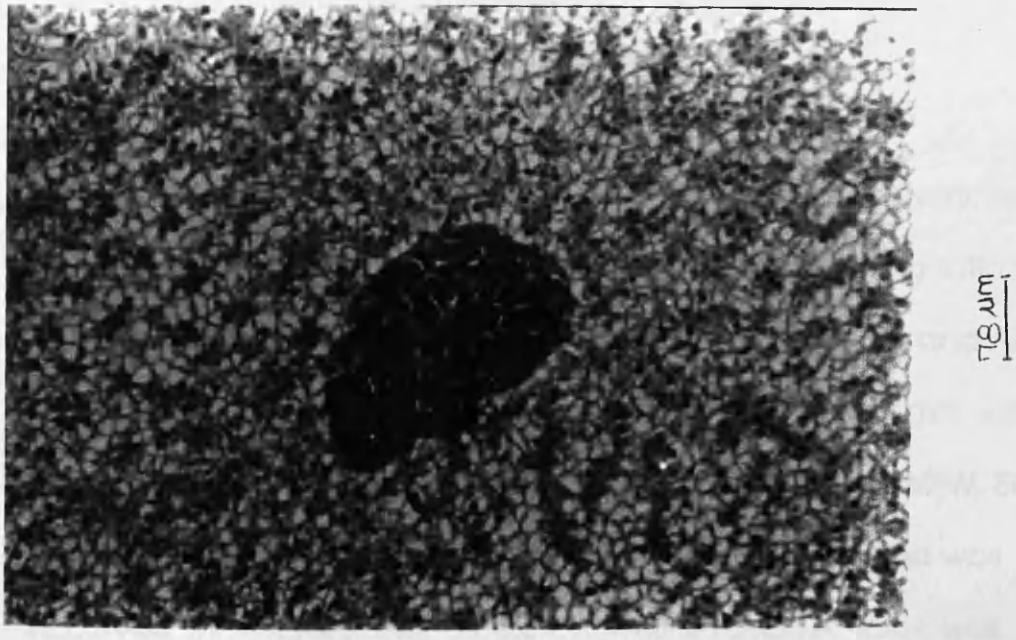


Plate 10.2b: Liver of *Oreochromis niloticus* fed SF-PMM blend based diets containing raw SF showing slight vacuolation of the hepatocytes. Notice the exocrine pancreas at the central lower half.

10.3.2 Experiment 2 - *Clarias gariepinus*

i Nutrient Utilisation

Diets containing high inclusion levels of PMM performed better than those with soybean flour. Similarly, It was observed that diets containing autoclaved soybean flour did better than those with raw soybean flour at the same incorporation level as shown in Table 10.6. There was significant variation in the mean final weight (MFW), specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilisation (ANPU) of fishes fed the different diets ($P<0.05$). The reproductive state of the fishes and mortality were unaffected significantly by variation in the diets ($P>0.05$).

Diet V and VI containing 25:75 and 50:50 SF:PMM respectively, with autoclaved SF did better than those of any other blending ratio with or without autoclaving. They gave highest MFW, SGR, PER and ANPU, and lowest FCR values. Fish fed raw soybean flour based diet without PMM (diets IV) had poorest nutrient utilisation with lowest MFW, SGR, PER and ANPU, and highest FCR values. The same response was observed with fish fed diet VIII except that better PER value was recorded with fish fed this diet. Insignicant interraction was recorded between autoclaving and blending as diets with more PMM did

better than those with less and diets with autoclaved SF did better than those with raw. PMM and SF effects were independent of each other (P>0.05). Fishes fed diet V and VI had the highest growth response while those fed diets IV and VIII recorded the poorest growth response as shown in Fig. 10.2.

Table 10.6 Effects of autoclaving of soybean flour and incorporation of poultry meat meal on utilisation of soybean flour-poultry meat meal blend based diets fed to *C.gariepinus* fingerlings for 56 days

Diets	MIW	MFW	SGR	FCR	PER	ANPU	MORT.
I	14.6±0.3^{a}	56.9±3.6^{ab}	2.4^{d}	1.3±0.0^{ab}	2.0±0.1^{cd}	31.2±0.0^{c}	1.7±0.0^{a}
II	15.0±0.6^{a}	48.7±0.9^{bc}	2.1^{cd}	1.5±0.1^{abc}	1.7±0.1^{c}	20.2±0.0^{b}	0.0±0.0^{a}
III	14.8±0.5^{a}	43.2±1.7^{ab}	1.9^{bc}	1.6±0.0^{bc}	1.6±0.0^{bc}	18.5±0.0^{b}	3.00±0.8^{a}
IV	14.8±0.3^{a}	34.9±1.1^{a}	1.5^{a}	2.1±0.1^{d}	1.2±0.1^{a}	10.4±0.0^{a}	0.8±0.8^{a}
V	14.7±0.4^{a}	57.7±3.3^{c}	2.4^{d}	1.3±0.1^{a}	2.1±0.1^{d}	32.2±0.1^{c}	0.0±0.0^{a}
VI	14.4±0.3^{a}	56.3±3.2^{c}	2.4^{d}	1.3±0.1^{a}	2.1±0.1^{d}	33.3±0.1^{c}	0.8±0.8^{a}
VII	15.3±0.4^{a}	41.5±1.3^{ab}	1.8^{ab}	1.8±0.1^{cd}	1.5±0.1^{abc}	15.0±0.1^{ab}	0.8±0.8^{a}
VIII	14.4±0.3^{a}	35.4±1.7^{a}	1.6^{a}	2.0±0.1^{d}	1.3±0.1^{ab}	10.7±0.0^{a}	1.5±1.5^{a}
±S.E.M.	±0.4	±2.2	±0.0	±0.1	±0.1	±0.0	±0.6

Data on the same column carrying different superscript are significantly different from each other (P<0.05).

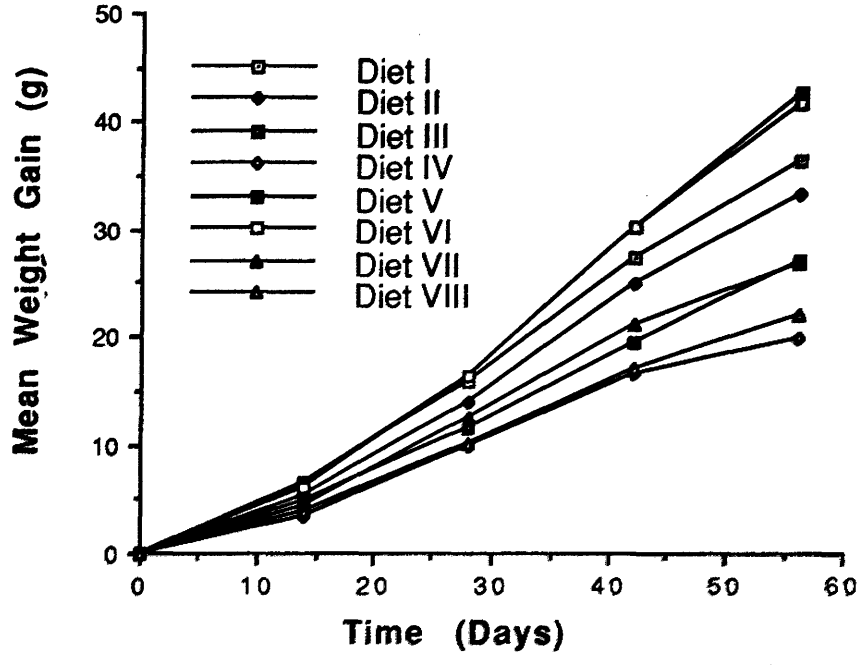


Fig. 10.2: Growth response of *Clarias gariepinus* fed raw and autoclaved SF:PMM blend based diets.

There were no marked differences in the average gonadosomatic and hepatosomatic indices of fish fed the diets containing raw and autoclaved soybean flour as depicted in Table 10.7 ($P>0.05$). There seemed to be unpatterned variation amongst diets. Sex differences resulted into significant variation in GSI and HSI ($P<0.05$). Female *C.gariepinus* had higher GSI than males and conversely for HSI.

Table 10.7 Gonadosomatic and hepatosomatic indices of *C.gariepinus* fed raw and autoclaved soybean flour-poultry meat meal blend based diets for 56 days .

	Male		Female	
Diet	GSI	HSI	GSI	HSI
I	0.4	1.3	1.1	0.4
II	0.5	0.7	0.5	1.2
III	0.1	0.9	0.3	0.6
IV	0.3	1.2	2.4	0.9
Average	0.3^a	1.0^b	1.1^b	0.8^b
V	0.4	1.7	0.6	0.7
VI	0.5	1.3	0.3	1.4
VII	0.3	0.9	0.4	0.7
VIII	0.3	1.7	3.4	0.8
Average	0.4^a	1.4^b	1.2^b	0.9^b

Data in the same row carrying the same superscirpt are significantly different ($P>0.05$)

No marked differences were observed in the histogenesis of testis and the ovary of fishes fed diets containing raw and autoclaved soybean flour. Plates 10.3a and b show the testis sections of seminferous tubules with lumen containing different stages of spermatogenic cells with no marked difference with the diets. The Ovary contains oogenic cells at different stages of development with oogonia and oocytes reflecting no signs of differences in the diets (Plate 10.4a and b)

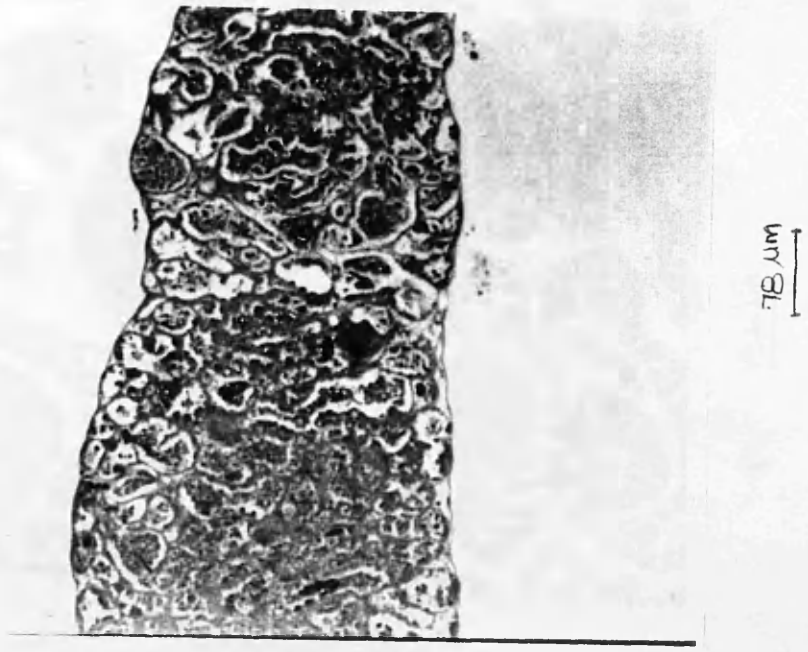


Plate 10.3a: Testis of *C.gariepinus* fed diets containing autoclaved soybean flour. Notice lumen of seminiferous tubules containing spermatogenic cells as well as empty ones.

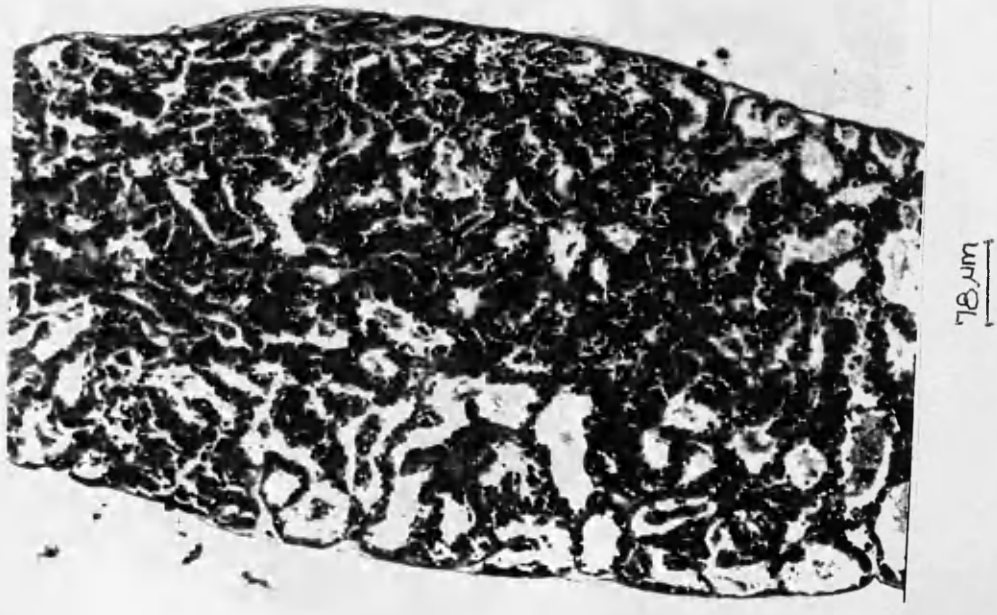


Plate 10.3b: Testis of *C.gariepinus* fed diets containing raw soybean flour. Notice empty lumen of seminiferous tubules and others containing spermatogenic cells.

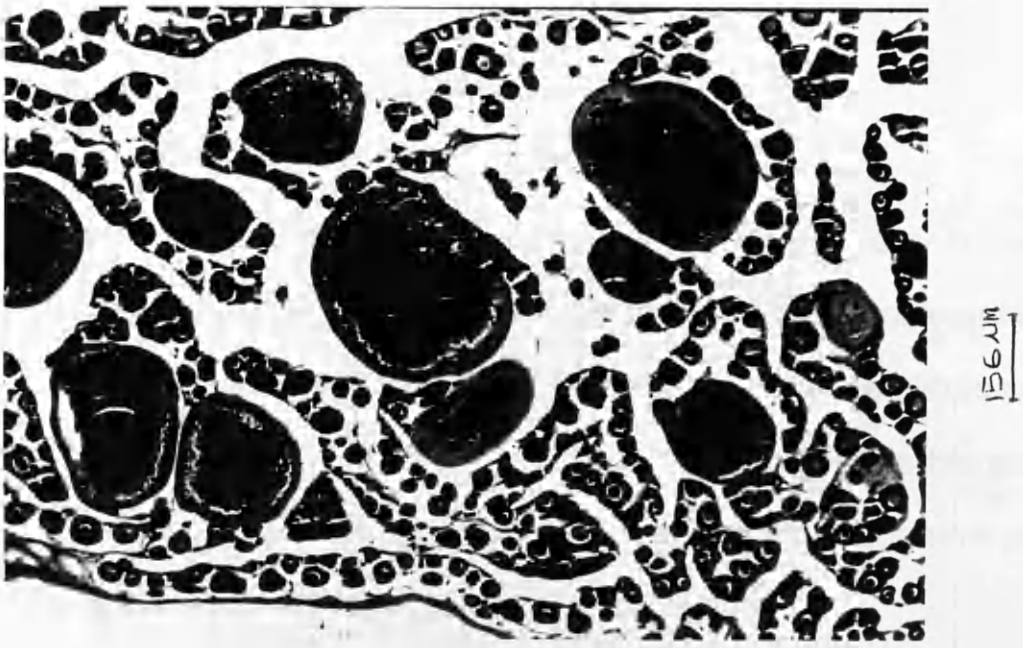


Plate 10.4a: Ovary of *C.gariepinus* fed diets containing autoclaved soybean flour. Notice oogenic cells at different stages.

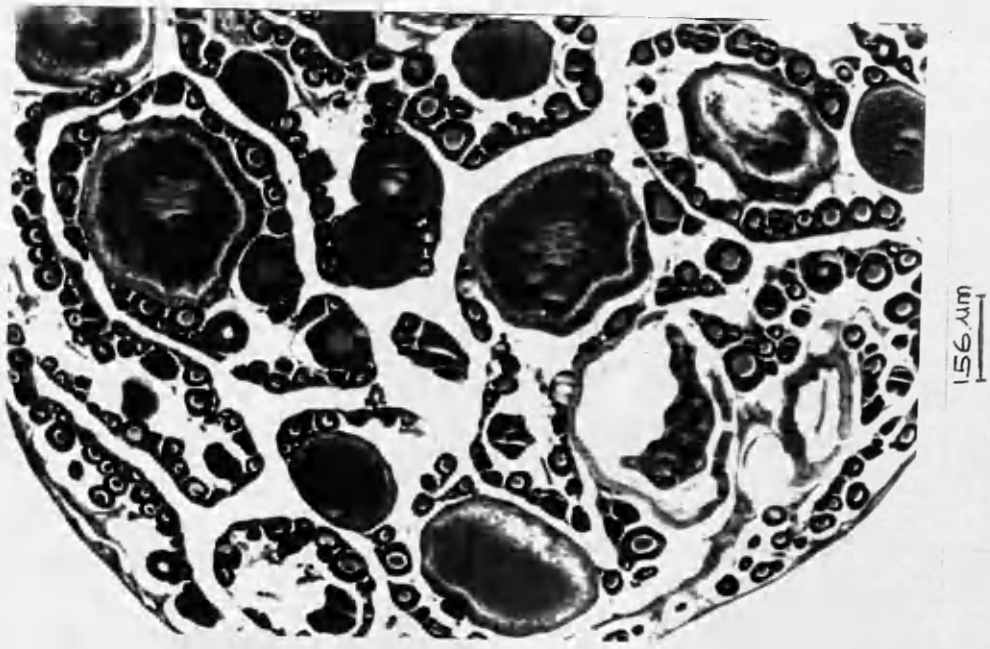


Plate 10.4b: Ovary of *C.gariepinus* fed diets containing raw soybean flour. Notice oogenic cells at different stages.

ii Carcass composition

Table 10.8 shows that there was significant variation only in the carcass moisture content of fish fed diets containing different levels of raw and toasted SF, and PMM ($P<0.05$). The highest moisture content was recorded in carcass of fish fed diets III and IV while the least was with fish fed diet VIII. Carcass protein, lipid and ash contents differed insignificantly with the diets ($P>0.05$).

Table 10.8 Carcass composition of *C.gariepinus* fed raw and autclaved soybean flour-poultry meat meal blend based diets for 56 days

Diets	Moisture	Protein	Lipid	Ash
I	71.0^{ab}	17.1^{a}	6.2^{a}	4.0^{a}
II	71.4^{abc}	16.0^{a}	6.6^{a}	4.6^{a}
III	72.4^{bc}	16.3^{a}	5.0^{a}	4.0^{a}
IV	72.7^{bc}	16.3^{a}	4.2^{a}	4.1^{a}
V	69.9^{a}	16.9^{a}	6.1^{a}	3.9^{a}
VI	70.2^{a}	16.6^{a}	8.0^{a}	3.7^{a}
VII	71.3^{abc}	15.9^{a}	6.0^{a}	3.3^{a}
VIII	72.8^{c}	15.7^{a}	4.7^{a}	4.2^{a}
±S.E.M	±0.0	±0.0	±0.0	±0.0
Initial carcass	70.5	17.0	26.7	10.7

iii Histology

Histopathological examinaton of the fishes fed the diets showed hypertrophy of the primary gill lamellae in fishes fed the diets

containing raw soybean flour (Plate 10.5a and b). There was hepatocytes vacuolation and lipid deposition in the liver of fish fed all the diets. However, these were not observed to be pathological even with high inclusion level of poultry meat meal at 25:75 SF:PMM as vacuoles were small and evenly distributed considered normal in farmed fish fed high protein - energy diets (Plate 10.6a and b)

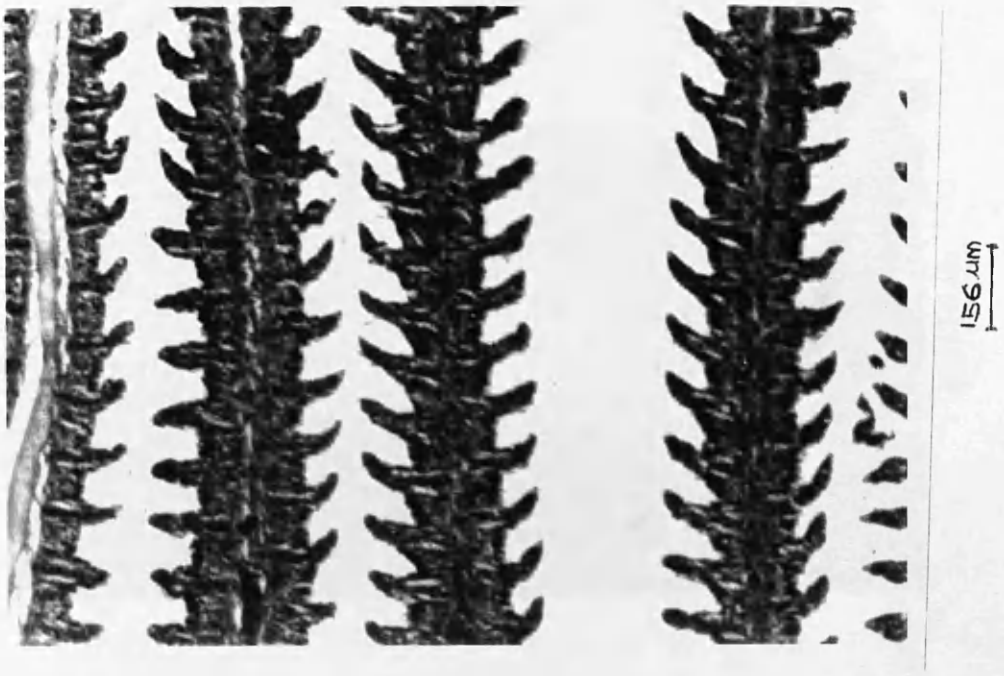


Plate 10.5a: Gills of Clarias gariepinus fed SF-PMM blend based diets containing autoclaved SF.

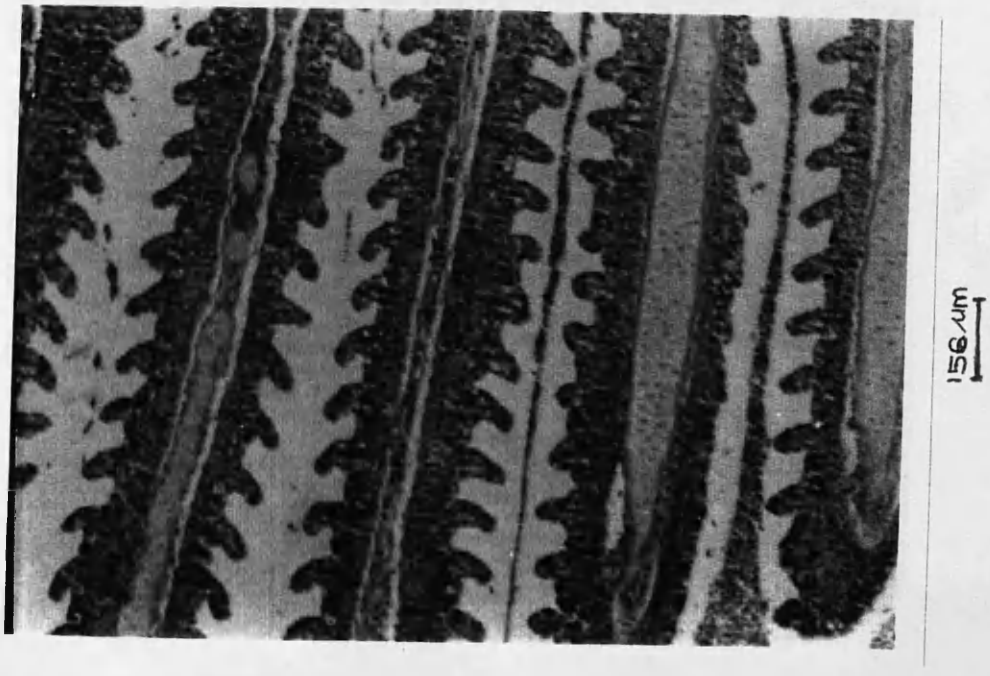


Plate 10.5b: Gills of *Clarias gariepinus* fed SF-PMM blend based diets containing raw SF showing hypertrophy of the lamellae.

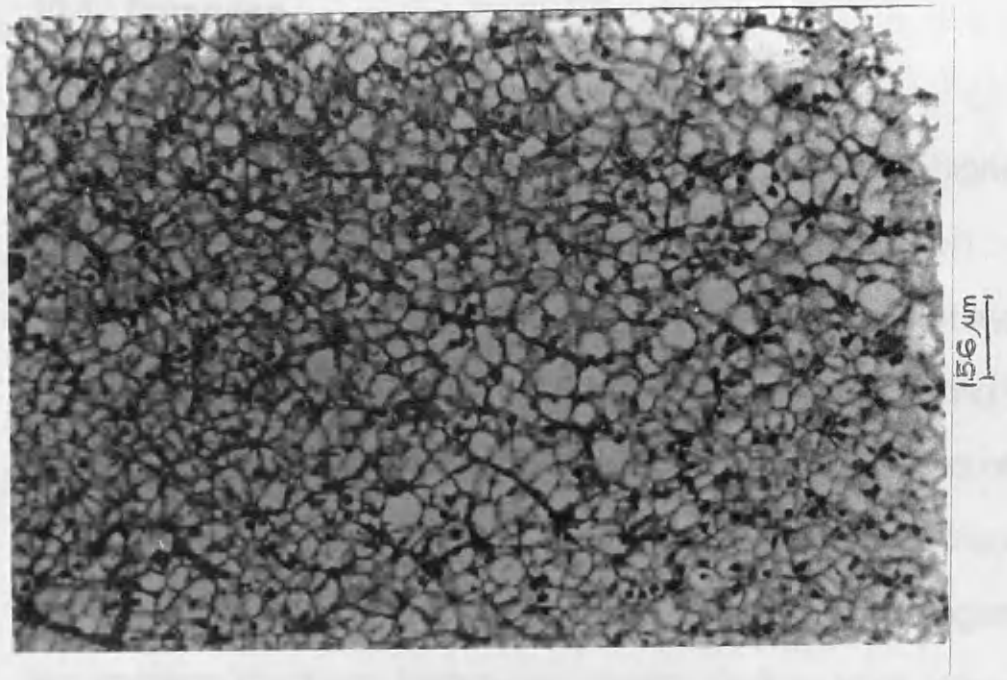


Plate 10.6a: Liver of *Clarias gariepinus* fed SF-PMM blend based diets containing autoclaved SF showing relatively pronounced vacuolation of the hepatocytes.

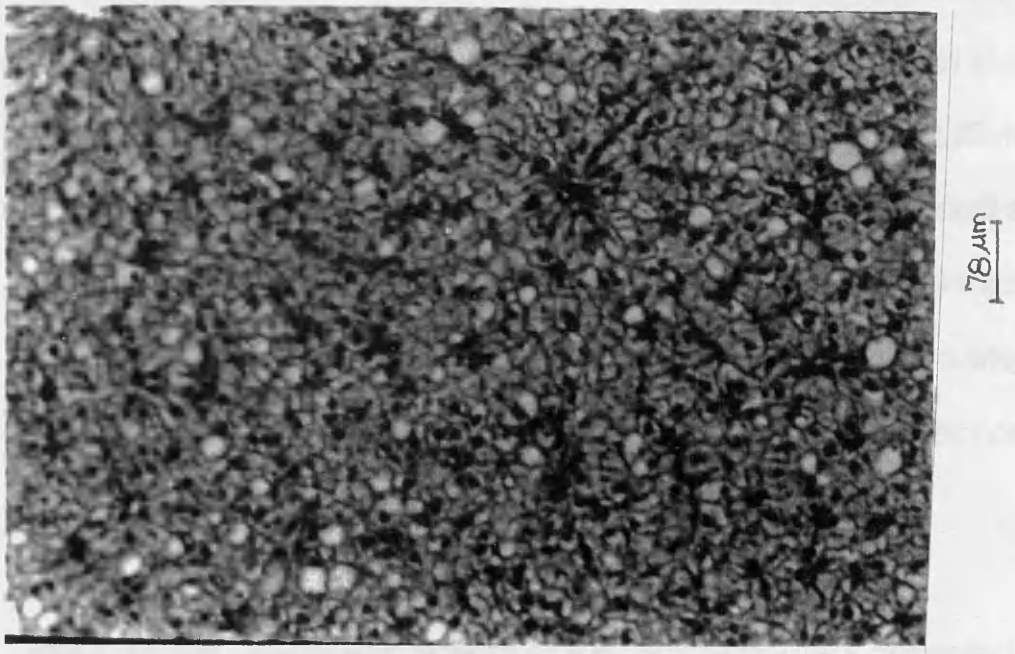


Plate 10.6b: Liver of *Clarias gariepinus* fed SF-PMM blend based diets containing raw SF showing slight vacuolation of the hepatocytes.

10.4 Discussion

Poorer nutrient utilisation by both fish fed diets containing higher levels of incorporation of raw soybean flour was evident. They had poorer SGR, FCR, PER and ANPU. This was presumably as a result of trypsin inhibitor (TI) activity that increased in the diets with increasing levels of raw soybean (diets I-IV). Table 10.1 shows that raw SF had about three times the TI of autoclaved SF. Growth retardation and poor protein efficiency ratio were observed in channel catfish fingerlings fed soybean meal with high trypsin inhibitor activity level (Wilson and Poe, 1985) and carp (Viola et al 1983).

The graded incorporation of PMM in the diets had double effects on TI activity. It reduced the levels of TI activity to residual level in diets containing raw soybean flour and high levels of PMM as in diets I and II where its inclusion level was above 30%. PMM is also a good source of lysine. This compensated for lysine rendered unavailable through autoclaving of soybean. It thereby improved growth and nutrient utilisation, as well as counterbalancing any deleterious effect of unavailable lysine in diets with raw soybean and PMM.

Much of the deleterious effects of raw soybean meal observed in this investigation was due to TI activity as it has been documented to account for 30-50% of the growth inhibitory effect of raw soybean

meal fed to rat (Kakade et al. 1973). Though the level of TI activity in diets containing autoclaved soybean flour as compared to commercial soybean flour depicted inadequate heating, TI has been reduced to residual levels incapable of eliciting significant growth depression and poor nutrient utilisation, particularly in a high protein diet. Rackis et al. (1975) observed maximum nutrient utilisation in rats fed soybean meal where 79-87 % of the inhibitors were inactivated.

The reproductive performances (GSI and HSI) of both fishes were unaffected by differences in TI activity level in different diets and hence the similarity in the gonadodal development of fish fed diets containing both raw and autoclaved SF. Santiago et al(1988) reported that feeding *Leucaena* leaf meal to *O.niloticus* did not markedly affect the GSI of the male and female Nile tilapia. Sex seemed to account more for a marked difference in GSI and HSI than TI activity as females had higher GSI while males had higher HSI. The lower average lipid level in fingerlings fed diets containing raw SF than those with autoclaved SF could be attributed to trypsin inhibition as it has been reported to result into tissue loss of lipid.

CHAPTER ELEVEN

AQUACULTURE NUTRITION AND WASTE GENERATION POTENTIAL OF AQUACULTURE OPERATION

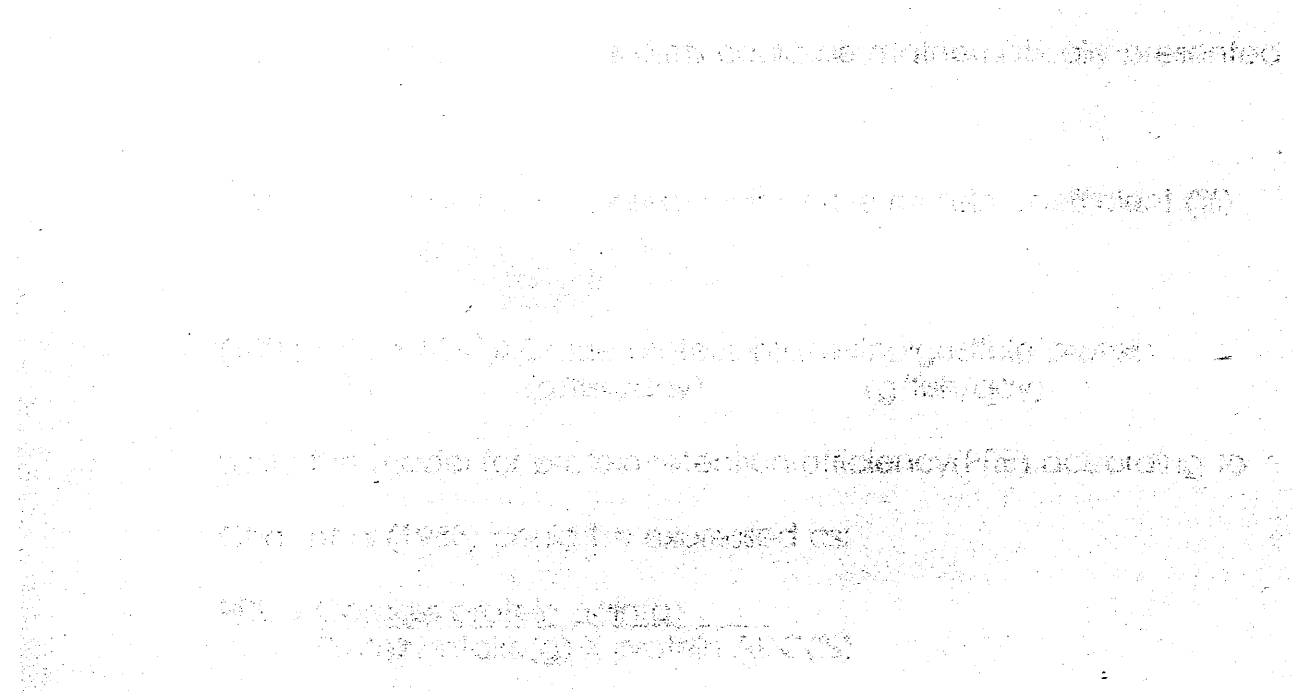
11.1 Introduction

Nutritional approaches to quantifying solid and soluble wastes from aquacultural operations have been proposed. These are based on the importance of digestibility as an indicator of potentially available energy and nutrients for growth, maintenance and reproduction of the animal as well as the levels of indigestible nutrients (solid wastes output) which account for the major portion of aquaculture wastes (Cho, 1993). Cho postulated that it is possible to determine the proportions of nutrients retained in fish and excreted as solid wastes from the digestibility of feed, comparative carcass analysis and measurements of feed intake and growth. This would be helpful in developing predictive models for waste estimation in aquaculture operations. Biological and chemical methods of quantifying waste output in effluent are use. The biological method seems to offer promise.

Cho et al. (1991) reported that there was agreement in results from both methods for nitrogen waste output in brown trout culture. However, the chemical method was inappropriate for phosphorus waste output which was excessively high. Similarly they observed that the use of fixed ADC and NRE (Nutrient retention efficiecy) for estimating waste output for different ingredients and diet formulations was equally misleading. Digestibility and nutrient utilisation studies of an ingredient or a diet formulation are therefore necessary preambles

to predicting its waste generation potential and subsequent modelling.

It is quite uncommon to see environmental impact assessment of feeding trials quantifying waste from such experiments in numerical terms. The farthest ever gone is evaluating the digestibility (often) and metabolisability (seldom) of the diets used leaving the end user of such findings to narrow them down to their specifications without a worked example. This is a case study serving as a worked example on the application of such important parameters, viz; digestibility, nutrient utilisation and nutrient retention.



11.2 Materials and Methods

Materials and methods required are more of software than hardware in this study. Results from the nutrient digestibility experiment of three soybean flour (SF) - poultry meat meal (PMM) blend based diets with 0.5% and 1.0% levels of Cr_2O_3 in both *Oreochromis niloticus* and *Clarias gariepinus* (Tables 5.3 and 5.6; Figs. 5.2 and 5.3) were used in conjunction with data from feeding trial experiments (Tables 6.2, 6.4 6.5 and 6.7) of the fish species on the diets to predict the waste generation potential and nutrient retention efficiency of rearing *O.niloticus* and *C.gariepinus* fingerlings on these diets.

The model for faecal nitrogen waste output (indigestible crude protein) estimation for the diets could be mathematically presented as:

100-protein ADC(%)=Apparent indigestible protein coefficient (%)

or

(100-protein ADC)XCrude protein intake=Indigestible protein
(%) (g/fish/day) (g/fish/day)

while the model for protein retention efficiency(PRE) according to Cho, *et al* (1985) could be expressed as:

$$PRE = \frac{\text{Carcass protein gain(g)}}{\text{Protein intake(g) X protein ADC(\%)}}$$

iv Statistical Analysis.

Data were analysed statistically according to the procedures in 3.7

11.3 Results and Discussion

Figs. 5.1 and 5.2 revealed that protein digestibility of the diets differed significantly from each other for both *O.niloticus* and *C.gariepinus* ($p<0.05$). Diet III (50:50 SF:PMM) had the highest protein ADC, followed by diets V(75:25 SF:PMM) and I (25:75 SF:PMM) in that order - all with 0.5% Cr_2O_3. They generated lower faecal nitrogen wastes than diets II, IV and VI with 1.0% Cr_2O_3 as shown in Table 11.1a and b. This was probably due to their high level of the marker as it has been observed that marker type influences digestibility (De Silva et al, 1990; Sadiku and Jauncey, 1995).

Table 11.2a and b depict the protein retention effeciency (PRE) derived from carcass protein gain , ADC and protein intake data. Carcass protein gain, protein intake and apparent net protein utilisation (ANPU) data of Sadiku and Jauncey (in press) from a previous feeding trial using the same diets were used in this prediction. In *O.niloticus*, there was insignificant difference in protein retention effeciency of the diets ($p>0.05$). This was in agreement with the finding of the feeding trial where the protein utilisation parameters also varied insignificantly with the diets ($p>0.05$). Though the protein ADC was found to differ significantly with the diets, it was not adequate to change this finding. Conversely, there was significant difference in the protein retention efficiency of the diets ($p>0.05$) in

C.gariepinus. Higher protein retention efficiency was observed in diets with 1.0% than 0.5% Cr_2O_3. This was due to the higher protein ADC in the latter while they shared the same protein intake.

There seems to be agreement in the trend of nitrogen waste generation potential of the diets in both species. However, opposing trends were observed in their nutrient retention efficiency in both species. This was probably due to the fact that the theoretical assumption of the three diets having similar protein intake level does not hold in practice if both species were fed diets originally containing 0.5% and 1.0% Cr_2O_3 levels. Protein intake could have been higher at the 0.5% level and this could have resulted into better protein efficiency by *C.gariepinus* fed diet with 0.5% Cr_2O_3 than those with 1.0% .

Although, there is relative validity in this predictive modelling, to achieve absolutely validity, it is strongly recommended that feeding trials and digestibility study diets should be the same in all respects. The experimental subjects and conditions for both experiments should be very similar. More work needs to be done to expand the scope of this investigation towards developing comprehensive nutritional models for quantifying waste output generation in aquaculture operations for most of the commercially available ingredients and aquaculture species. The faecal nitrogen waste output and nutrient

retention of several aquaculture species fed diets of diffferent ingredients , feed combinations and nutrients other than those investigated here should be evaluated.

Table 11.1a. Daily individual protein intake and digestion in *Oreochromis niloticus* fingerlings fed soybean flour - poultry meat meal blend based diets

Diets	Diet ration (g protein/fish/day)*	Protein ADC(%)	Indigestible protein (g/fish/day)**
I(25:75)	0.16^{a}	$69.88b^{bc}$	0.05^{ab}
II(25:75)	0.16^{a}	60.78^{a}	0.06^{b}
III(50:50)	0.16^{a}	70.75^{c}	0.05^{ab}
IV(50:50)	0.16^{a}	62.07^{a}	0.06^{b}
V(75:25)	0.15^{c}	74.60^{d}	0.04^{a}
VI(75:25)	0.15^{a}	68.50^{b}	0.05^{ab}

Table 11.1b Daily individual protein intake (*) and digestion in *Clarias gariepinus* fingerlings fed soybean flour - poultry meat meal blend based diets

Diets	Diet ration (g protein/fish/day)*	Protein ADC(%)	Indigestible protein (g/fish/day)**
I(25:75)	0.39^{a}	86.76^{c}	0.05^{a}
II(25:75)	0.39^{a}	62.44^{a}	0.15^{b}
III(50:50)	0.47^{c}	88.06^{c}	0.06^{a}
IV(50:50)	0.47^{b}	73.69^{b}	0.13^{b}
V(75:25)	0.38^{c}	87.87^{c}	0.05^{a}
VI(75:25)	0.38^{c}	70.25^{b}	0.11^{b}

Figures in parenthesis represents the blending ratios of soybean flour:poultry meat meal.
*Imported data from previous 56 days feeding trial by the experimenter as a case study for predicting waste generation potential and nutrient retention of the same diets in *O.niloticus* and *C.gariepinus*

**Calculated values predicting indigestible protein and PRE

Figures in the same column with the same superscripts are insignifcantly different ($p>0.05$)

Table 11.2a Individual fish carcass protein and retention in *Oreochromis niloticus* fingerlings fed soybean flour - poultry meat meal based diets

Diet	Carcass protein (g/fish/day)*	ANPU(%)*	Protein retention efficiency **
I(25:75)	0.05[a]	27.50[a]	0.42[a]
II(25:75)	0.05[a]	27.50[a]	0.48[a]
III(50:50)	0.05[a]	29.50[a]	0.40[a]
IV(50:50)	0.05[a]	29.50[a]	0.45[a]
V(75:25)	0.05[a]	30.00[a]	0.40[a]
VI(75:25)	0.05[a]	30.00[a]	0.44[a]

Table 11.2b Individual fish carcass protein and retention in *Clarias gariepinus* fingerlings fed soybean flour - poultry meat meal based diets

Diet	Carcass protein (g/fish/day)*	ANPU(%)*	Protein retention efficiency **
I(25:75)	0.16[a]	40.00[a]	0.46[ab]
II(25:75)	0.16[a]	40.00[a]	0.64b[b]
III(50:50)	0.23[b]	47.50[a]	0.55[ab]
IV(50:50)	0.23[b]	47.50[a]	0.65[b]
V(75:25)	0.16[a]	40.00[a]	0.40[a]
VI(75:25)	0.16[a]	40.00[a]	0.58[ab]

Figures in parenthesis represents the blending ratios of soybean flour:poultry meat meal.

*Imported data from previous 56 days feeding trial by the experimenter as a case study for predicting waste generation potential and nutrient retention of the same diets in *O.niloticus* and *C.gariepinus*

**Calculated values predicting indigestible protein and PRE

Figures in the same column with the same superscripts are insignifcantly different (p>0:05)

CHAPTER TWELVE

GENERAL DISCUSSION

The nutritional studies in this research are intended to investigate the nutritional improvement of soybean flour by the incorporation of low cost animal protein - poultry meat meal (PMM) as blends, their utilisation either as the sole protein supplement and their role as replacers of fishmeal in practical diets of *Oreochromis niloticus* and *Clarias gariepinus*. In addition, improvement of such diets through essential amino acid profile enrichment by supplementation and reconstitution of their minerals were studied. To achieve these aims, several feeding trials were conducted to establish which of 25:75; 50:50 and 75:25 SF:PMM blends would be optimum and at which of 25%, 50 and 75% replacement level of fishmeal. Effects of dl-methionine supplementation and reconstitution of minerals of significant nutritional importance such as calcium, phosphorus, magnesium and zinc were examined. Digestibility studies of these blends were conducted to evaluate their quality in terms of nutrients availability and utilisation.

There has been no problem free utilisation of soybean in aquaculture diets. Lovell (1990) and Lim (1992) documented reported variable successes on the utilisation of soybean in aquaculture feeds. Replacement of fishmeal meal with soybean meal without any supplementation in tilapia diets was successful up to 50% in a diet of 25% crude protein (Viola and Arieli, 1983). High level of dietary soybean meal or complete substitution of fish meal resulted in poor

growth and feed efficiency of fish (Jackson et al. 1982; Dabrowska et al.1989) hence the seeming fishmeal indispensability. However, rising cost and uncertain availability have made it mandatory to reduce the fishmeal content of fish diets (Shiau et al.1987; Dong et al, 1993). This has resulted again in another search - a close substitute to fishmeal, of lower cost and more readily available animal protein.

Poultry by-product meal (PBM) has been reported as a successful replacer of fish meal (Higgs, 1979; Fowler, 1981a,b; 1982; 1991; Steffens,1994). Blending with soybean meal has been postulated to result in economic improvement of soybean meal based diets (Mohsen and Lovell, 1990). A readily available poultry product - poultry meat meal (PMM) was therefore blended with soybean flour (SF) at 25:75; 50:50 and 75:25 (SF:PMM) as sole protein source or partial replacer of fishmeal in the diets of *Oreochromis niloticus* and *Clarias gariepinus*. A good blend of SF and PMM as in diet II (50:50) gave an improved amino acid balance.

Feeding trials in Chapter Six show high levels of inclusion of soybean flour as in the reference diet and diet I (75:25) increased the risk of methionine deficiency and leucine excessiveness in both *O.niloticus* and *C. gariepinus* while that of PMM as in diet III (25:75) increased the risk of phenylalanine deficiency in *C. gariepinus* (Tables 6.3. and 6.6).

Excesses of certain EAAs like leucine have been reported to exert toxic effects in fish (Hughes, Rumsey and Nesheim, 1984; Robinson, Poe and Wilson, 1984). Infact, Cho *et al.* (1991) reported toxicity of leucine in rainbow trout (*Oncorhynchus. mykiss*) when present in excess of 13.4% i.e 113.4% of the requirement level. This could militate against high inclusion levels of SF and PMM in an ideal SF:PMM blend based diet for *O.niloticus* and *C.gariepinus*.

Fishmeal replacement potential of SF was also improved by the incorporation of PMM into SF as blends. A 25% replacement of fishmeal using 75:25 SF:PMM blend based diets (diet VII) was found most suitable for the intensive culture for *O.niloticus* (Table 7.2). Diet VII was higher in soybean flour than poultry meat meal. This suggests a marginal level of incorporation of poultry meat meal in soybean flour to give optimum performance of the diets in *O. niloticus*. In *C. gariepinus*, diet V did equally well as the control diet. Diet V was a 50:50 blend of soybean flour and poultry meat meal replacing 25% fishmeal (Table 7.5). This suggests an equal level of incorporation of poultry meat meal in soybean flour to give optimum nutrient balance and good performance of the fish on the diets. Poultry by-product meal on its own has been postulated to successfully replace fish meal at >50% level without compromising performance of chinook salmon (Fowler, 1991). Blending with SF was found beneficial for the intensive culture of both *O.niloticus* and *C.gariepinus* as favourable

performance parameters were observed in both experiments reported in Chapter Seven.

In terms of amino acid balance, methionine was the first limiting EAA in the diets. Improvement of soybean flour based diet was therefore achievable by the incorporation of methionine-rich poultry meat meal. It was evident that addition of poultry meat meal ameliorated methionine and available lysine deficiency, and reduced leucine, and the risk of its toxicity. The better performance of diet VII than the control is not absolutely in agreement with the speculations that poor nutrient utilisation and growth response were linked to methionine defficiency. Only the superiority of the control diet in *C. gariepinus* could be attributed to better methionine level, as it had the highest methionine and the least leucine, but that diet V, lower in methionine than diets VIII, IX and X, was its closest rival put this to further question. Performance of the blends in partial replacement of fishmeal could not therefore be linked to methionine. It could be due to factors not studied in these experiments and therefore worthy of further investigation.

Despite the marked differences in performance of both fish with different replacement and blending levels, values for nutrient utilisation parameters were favourable for all diets depicting the possibilty of replacing fish meal up to 75% with any of the blends

successfully. Improvement of fish meal replacement levels with soybean flour was therefore achieved by the incorporation of poultry meat meal in both fish. It is evident from the foregoing that partial replacement of fishmeal with any of the blends does not require EAA supplementation, but utilisation of blends higher in either SF or PMM as sole source of protein require EAA supplementation, particularly methionine. An attempt was therefore made to supplement the diet with methionine from natural and synthetic sources.

Poultry Meat Meal is a good natural source of methionine and improved diet methionine level when blended. In addition, supplementation of crystalline methionine was investigated in feeding trials in Chapter Eight. Methionine supplementation was beneficial to SF:PMM blends utilisation in the diets of *O.niloticus* and *C.gariepinus*. 50:50 SF:PMM blend with 0.5% methionine supplementation was best utilised by both species. The phenylalanine requirement of *O. niloticus* was met in some of the diets while the level in others was not critical, that of methionine was greatly improved. The good performance of 50:50 SF:PMM could be attributed to overall essential amino acid balance of the diets . In *C. gariepinus,* a good balance of methionine and phenylalanine justifies the 50:50 blending of SF and PMM as 75:75 and 25:75 SF:PMM aggravates methionine and phenylalanine deficiency respectively. Viola et al. (1982) , Tacon et al. (1984) and Murai et al. (1986) recorded improved growth in carp, *Oreochromis*

niloticus and channel catfish respectively when fed soybean containing diets with methionine supplementation.

Blood plasma methionine dynamics showed highest methionine peaks in fishes fed diets without methionine supplementation at six hours after feeding. The lower methionine peak recorded for the diets with methionine supplementation at six hours may have been a result of premature absorption of the free amino acids in the diets including the supplemented synthetic methionine which has been reported to be related to the proportion of peptides and free amino acids in the diet . In addition, premature absorption and metabolism of certain EAAs such as methionine , arginine, isoleucine, leucine and valine occurs in the first three to six hours (Hardy, 1991). Much of the methionine in the supplemented diets would have been metabolised at six hours and beyond, thereby giving them the lower level recorded. This was not found to adversely affect nutrient utilisation in contrast to an earlier finding with poorer growth of salmon fed fish silage diets than with fishmeal diets. This was as a result of better balance of essential amino acid in the plasma (BEAAP) in fishmeal without premature absorption of EAAs (Hardy, 1991).

Feeding trials in Chapter Nine depict that mineral reconstitution of diets based on 50:50 SF:PMM blend (optimum blend) for both *O.niloticus* and *C.gariepinus* did not markedly affect nutrient utilisation

and growth response. However, tissue deposition of some minerals related significantly with their levels in the diets. In *O.niloticus*, carcass deposition of phosphorus was highest in diet I (+Ca, P, Mg and Zn) and lowest in diet V (-Ca, P, Mg and Zn) and this reflected the dietary inclusion level of phosphorus with a strong positive correlation. Carcass calcium and phosphorus were high in *C.gariepinus* fed diets I and III (-Mg), and lowest in diet V because of their high and low levels in the diets respectively (Tables 9.3 and 9.5). Only dietary calcium had strong positive correlation with carcass calcium ($p<0.05$). This may be due to the structural requirement of calcium by *C.gariepinus*.

There was oversupplementation of phosphorus in the diets for *O.niloticus*. Its dietary supplementation should be minimised and this will in turn reduce feed cost (Davis and Gatlin , 1991) and protect the fish evironment from phosphorus pollution. supplementation of Phosphorus in the mineral premix is not a dear necessity as it could be spared by magnesium. However, in *C.gariepinus*, it was evident that calcium and phosphorus supplementation would be necessary as supplementation of calcium in the basal diet appeared to inhibit phosphorus bioavailability (Davis, 1990).

Detoxification of soybean flour (SF) through heat processing enhanced SF:PMM blend based diets utilisation by *O.niloticus* and

C.gariepinus as reported in Chapter Ten. It reduced the level of TI activity to residual level in diets containing autoclaved soybean flour (diets V-VIII) . Incorporation of PMM had similar effect, particularly in diets I - III (containing raw SF). PMM is also a good source of lysine. This compensated for lysine loss through autoclaving of soybean in diets V-VII. Heat processing of soybean meal was observed to enhance growth and protein efficiency ratio in channel catfish with reduction in trypsin inhibitor activity level (Wilson and Poe, 1985).

Lastly, developing environmentally friendly diets is increasingly becoming a policy by feed manufacturers to promote the market value of their products. Such diets are highly digestible, metabolisable and generating less waste in aquaculture operation. To achieve this, careful selection of ingredients of high nutrient digestibility and utilisation is desirable. Feedstuffs used in this research are fishmeal, defatted soybean flour, poultry meat meal and wheat flour. Chapter Four presents the nutritive value of the feedstuffs indicating their digestibilities. SF:PMM blend based diets, availability of amino acids in the blend based diets were studied and reported in Chapter Five. High digestibility values were obtained for the dry matter, protein, lipid and ash of these feedstuffs. Diets formulated from these using SF:PMM blends similarly had high nutrient digestibilities in both fish species - particularly protein and lipid, thereby generating little nitrogenous waste. Diets used in this research could therefore be considered to be environmentally friendly, of good quality and market value.

CHAPTER THIRTEEN

CONCLUSION AND RECOMMENDATION

In conclusion, the following objectives have been achieved:

i Differences were observed in dry matter, protein lipid and ash digestibility in *O.niloticus* and *C.gariepinus*. ADC of dry matter, lipid and ash were high while those of ash for soybean flour (SF), poultry meat meal (PMM) and wheat flour (WF) were relatively lower in *O.niloticus*. In *C.gariepinus*, ADCs of dry matter, lipid and ash were high while those of protein for the three ingredients were relatively lower.

ii There were differences observed in nutrient digestibilities, apparent amino acid availabilities and nitrogenous waste generation potential of SF:PMM blend based diets in *O.niloticus* and *C.gariepinus*. Lipid digestibility was highest while ash was lowest for both fishes. Except for ash, nutrient digestibilities were highest in diets with 0.5% Cr_2O_3 than with 1.0% with less nitrogenous waste in the former than the later.

iii There was nutritional improvement of soybean flour with the incorporation of PMM as blends and that 50:50 SF:PMM was found to be optimum for the intensive culture of *O.niloticus* and *C.gariepinus*.

iv There was successful 75% replacement of fishmeal with any of the blends, though 25:75 SF:PMM replacing 25% was best for *O.niloticus* and 50:50 SF:PMM replacing 25% was best for *C.gariepinus*.

v Methionine supplementation of the blend based diets did marginally improve nutrient utilisation - particularly the methionine supply from PMM to SF.

vi Mineral premix reconstitution did not markedly alter nutrient utilisation of the optimum blend by both species. However, it was suggested that phosphorus could be saved in the diets of *O.niloticus* as it appeared to be spared by magnesium.

vii Heat processed SF had residual level of trypsin inhibitor or phytate phosphorus. Blending SF with PMM increased methionine and reduced leucine in SF:PMM blend based diets. Therefore autoclaving and blending enhanced SF utilisation in the diets of both species.

Finally, other low cost animal proteins and complementary plant proteins should be similarly studied to develop a variety of animal-animal protein, plant-animal protein and plant-plant protein blends as suitable alternatives to fishmeal in the diets of aquaculture species.

As a postscript, the CHNSO method of nitrogen dermination was tested and found suitable and recommended for protein determination, particularly when dealing with microsample size. It requires only 5mg sample. Results of protein content of some feedstuffs using CHNSO Analyser 2400 model and micro-kjeldahl technique are presented in Appendix 1.

CHAPTER FOURTEEN

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CHAPTER FIFTEEN

APPENDICES

Appendix 1: Comparison of the Micro-kjeldahl and CHNSO Methods of Protein Determination of the Feedstuffs.

Feedstuffs	CHNSO	Microkjeldahl
Fishmeal	70.8[a]	74.2[a]
Poultry Meat Meal	64.7[a]	66.0[a]
Soybean Flour (Toasted)	49.7[a]	53.5[a]
Soybean Flour (Raw)	52.1[a]	55.9[a]
Wheat Flour	9.2[a]	11.0[a]

Data in the same row carrying the same superscript are differed insignificantly (P>0.05).

Appendix 2: List of Conference Papers and Publications from the Research.

1 Substitution of fishmeal with soybean flour-poultry meat meal blends in practical diets of *Oreochromis niloticus*. Oral Presentation at the *25th Anniversary Conference (Silver Jubilee) of the World Aquaculture Society*, Marriott Hotel, New Orleans, Louisiana, USA. 14-18 January, 1994.

2 Digestibility, amino acids availabilty and waste generation potential of soybean flour-poultry meat meal blends based diets for Oreochromis niloticus (Trewavas). Poster presentation at the *Aquaculture and Water Resources Management Conference*, Institute of Aquaculture, University of Stirling, Stirling, UK. 21-25 June, 1994.

3 Supplementation of soybean flour-poultry meat meal blend based diets with dl-methionine to improve nutrient utilisation in *Oreochromis niloticus* and *Clarias gariepinus*. Poster presentation at *Aqua Nor and Aquaculture Europe '95 - European Aquaculture Society Confernce*, Trondheim - Norway. 9-12 August, 1995.

4 Digestibility, amino acids availabilty and waste generation potential of soybean flour-poultry meat meal blends based diets for *Oreochromis niloticus* fingerlings - accepted for publication in *Aquaculture Research*

5 Digestibility, amino acids availabilty and waste generation potential of soybean flour-poultry meat meal blends based diets for *Clarias gariepinus* fingerlings - accepted for publication in *Journal of Applied Aquaculture*.

6 Soybean flour-poultry meat meal blend as dietary protein source in practical diets of *Oreochromis niloticus* and *Clarias gariepinus* - accepted for publication in *Asian Fisheries Science*.