SOME ASPECTS OF UTILIZATION OF SOYABEAN MEAL BY THE YOUNG MUDFISH CLARIAS ANGUILLARIS LINNAEUS

BY

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DEDICATION

To My Father

To My Mother of Blessed Memory

To My Wife and Children

DECLARATION

I hereby declare that this thesis is my own work and has not been presented in any form for another qualification at any other University or Institution. Information derived from the published or unpublished works of others has been duly acknowledged in this work.

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CERTIFICATION

This thesis titled Some aspects of Utilization of Soyabean meal by the young mudfish *Clarias anguillaris* Linnaeus meets the regulations governing the award of the degree of Doctor of Philosophy, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Series of experiments were carried out using standard methods on some aspects of utilization of soyabean meal by the young mudfish, Clarias anguillaris. The results of the first experiment which was carried out to compare the chemical composition and nutritive value of soyabean meal with those of other plant protein sources shows that soyabean meal was the richest in protein, fat and indispensable amino acids although the level of methionine was found to be low.

Two experiments were conducted to study the effect of dietary supplements of methionine on the growth and food utilization of C. anguillaris fingerlingS. It was found that C. anguillaris fingerlings could utilize crystalline L- and D,L-methionine in soyabean diets effectively for growth.

In the next experiment, soyabean meal were processed and incorporated in the diet of C. anguillaris fingerlings. The fingerlings were ted diets containing raw soyabean meal, soyabean residue (after milk extraction), traditionally roasted soyabean meal and soyabean meal roasted at 100°C for 15 minutes, 30 minutes, 1 hour and 2 hours.

It was found that soyabean meal roasted at 100°C for 30 minutes was superior at promoting the growth of mudfish fingerlings compared with the other soyabean treatments.

An experiment was also conducted to test the effect of different dietary lysine levels on growth of fingerlings C.anguillaris fed D,L-methionine enriched soyabean diets. It was found that the mean weight gain, specific growth rate (SGR), feed conversion efficiency (FCE), protein efficiency ratio (PER), and apparent net protein utilization (NPU), increased linearly with increase in the level of added L-lysine in the diet.

The experiment to determine the level of replacement of soyabean meal with fish meal in diets of C. anguillaris juveniles showed that 100% level of fish meal gave the the highest weight gain although the highest PER, FCE and NPU was obtained at 90% fish meal inclusion. The best PER was obtained at 50% level.

In another experiment three hundred and twenty C. anguillaris fingerlings were distributed into 16 hapas and fed different levels of cow blood meal to substitute soyabean meal in the diet at

0, 10, 25, 50, 75, 90 and 100% levels. The results showed that the experimented diet compounded with 50% blood meal and 50% soyabean meal was highest in growth values while the poorest growth was recorded in the fingerlings fed diets containing 100% blood meal.

In the final experiment four hundred and eighty mudfish fingerlings were fed experimental diets containing isonitrogenous mixture of fish meal, soyabean meal, blood meal and groundnut cake. The best growth rate was obtained in fingerlings fed fish meal diets while those fed a mixture of soyabean meal and fish meal diets gave growth performance which was not significantly different from that of the control ration containing fish meal,, The diet mixture containing blood meal and fish meal was better utilized by the fingerlings than mixture of blood meal and soyabean meal.

Based on the result of experiments reported above it could be concluded that well processed soyabean meal can be adequately utilized to promote growth in the young mudfish C. anguillaris.

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1.0 GENERAL INTRODUCTION

Fish culture is gradually gaining prominence in Nigeria because of the growing deficit in fish production from capture fisheries (Bayagbona, 1983). With the low per capita consumption of animal protein caused by escalating cost and dwindling supply of meat, poultry and game, fish is considered the best option due to its relatively low cost. In recent times however, fish supply has continued to decline following the ban on fish importation and the low domestic fish production (Uboma et al., 1981; Ita, 1982; Tobor, 1985; Talabi, 1986). Consequently, intensification of fish culture has been identified by various workers as the only foreseeable short-term solution to inadequate fish supply to the nation (Sagua, 1976; Ita, 1980, 1989; Eyo, 1983). In an attempt to raise the standard of fish culture in Nigeria, the Federal Government has through the Directorate of Food, Roads and Rural Infrastructure (DFRRI) in 1987 funded all State Fisheries Departments as well as fisheries Research

Institutes in the area of fish seed production (Anon, 1989). This has resulted in the availability of Clarias sp. and Tilapia sp. seeds in some states of the federation. Increased production of fish seeds demand provision of suitable feeds to grow the fish to marketable size within a reasonably short period.

The highest proportional cost of a fish culture system is expended on fish feeds accounting for over two-thirds of the variable cost of a fish culture operation (Lovell, 1981a; Niamat and Jafri, 1984; Akiyama, 1988). The rapid expansion and success of commercial fish culture therefore depends largely upon the availability of good quality and cheap feeds (Hastings, 1976; Miller, 1976; Harris, 1980; Lovell, 1981a; UNDP/FAO, 1983)

Fish feeds were developed on the concept of "fish eat fish" which is why fish meal is often a major and traditional component of fish feeds (Akiyama, 1988). With the high cost and limited supply of fish meal, research efforts should be directed towards obtaining alternative

sources of protein for fish feed. Plant protein sources are commonly used in animal feeds, but depending on the animal being fed some of these plant protein sources are inadequate because their amino acid profile are often insufficient to support maximum growth of the animal (Mahnken et al., 1980). Therefore these plant protein sources must of necessity be supplemented with animal protein in order to bring the amino acid to a balance. Among the plant protein sources, soyabean has potentials in fish feeds because it has one of the best amino acid profile of all protein-rich plant feedstuffs for meeting the essential amino acid requirement of fish (Harris, 1980; Lovell, 1980a; Akiyama, 1988).

Previous studies on the utilization of soyabean meal by fish, gave conflicting results owing to the suboptimal methionine content (Andrews and Page, 1974; Runsey and Ketola, 1975; Steffens and Albrecht, 1976; Murai et al., 1982, 1986), and the presence of antinutritional factors in the bean (Rackis, 1974; Spinelli, 1980: Liener, 1981; Viola et al., 1983). Report on

such studies with tropical fish have been scarce. No studies have been reported on the utilization of soyabean meal by Clarias anguillaris (UNDP/FAO, 1983). It is in an attempt to develop suitable cost effective artificial feeds using soyabean meal, that this study was initiated.

1.1 Aims and Objective

The general aim of this study was to monitor the response of C. anguillaris fingerlings fed artificial feeds of various levels of soyabean meal as one of the protein sources. On the basis of this, identify the best formulation(s) that would support maximum growth of the young mudfish, C. anguillaris.

The specific objectives were as follows:

(i) To update information on the nutrient levels of the commonly used feed ingredients for fish culture in Nigeria.

(ii) To identify the level of synthetic L and D, L - methionine that must be added to the diet to ensure optimum utilization of soyabean meal.

(iii) To determine the level of added synthetic L - lysine that would improve the utilization of D, L - methionine enriched soyabean diet.

(iv) To determine the extent of replacement of soyabean meal with fish meal and blood meal in isocaloric diets that would support maximum growth of the fingerlings.

(v) To identify the best heat treatment of soyabean meal that would guarantee maximum utilization of soyabean diet by the fingerlings.

(vi) To determine the best substitution level for soyabean meal, fish meal, blood meal and groundnut cake in isonitrogenous diets that would promote the growth of the fingerlings.

CHAPTER TWO

LITERATURE REVIEW

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2.0.0 LITERATURE REVIEW

Aquaculture practice world wide can be divided into two broad categories - extensive and intensive. In extensive aquaculture, fish are stocked in often large water bodies without any attempt at supplemental feeding. Intensive aquaculture requires raising fish in a relatively small confinement e.g. ponds, cages, raceways and concrete tanks, at high stocking density and food supplied to the fish in the form of artificial diets.

The practice of supplemental feeding of fish in ponds has been in widespread use in Africa since 1950's (Miller, 1976). However, nutrition and feed technology has experienced underdevelopment because few efforts have been made to formulate feeds and evaluate them under controlled condition (Miller, 1976). Furthermore, the absence of traditional fish husbandry and the poor economic status of most rural farmers are possible reasons why artificial feeding was not widely accepted (FAO, 1967).

Artificial feeding of fish have the benefits of promoting faster growth, allowing high stocking density especially in polyculture trials, greater

production in terms of yield per hectare and enabling fish farmer through feeding of fish to have regular contact with his fish thus permitting early detection of disease problems (Miller, 1976). However, artificial feeding of fish are known to pose peculiar problems for both the fish farmer as well as the feed manufacturer. Such problens include rapid nutrient loss through normal deteriorative processes, leaching of feed by water unless quickly consumed and disintegration of feed aggregates into their nutrient components thus losing their original nutritional properties (UNDP/FAO, 1983). Therefore a successful artificial feed should not only meet the nutritional requirements of the fish, it should also have good water stability and acceptance by the feeding animal.

For clear understanding of the role played by feeds in fish nutrition it is pertinent to examine the nutritional requirements of most of the fish species so far studied.

2.1.0 Nutritional requirement of fish

Studies on the nutritional requirement of fish have been conducted mainly on exotic species

such as salmon, rainbow trout, carp and channel catfish (Cowey, 1978; Castell et al., 1972; Robinson and Lovell, 1978). Only very limited studies have been carried out on tilapia. Although the African catfish is regarded as a promising candidate for Aquaculture (Hogendoorn, et al., 1983), knowledge of the nutrient requirements of these species is restricted (Machiels and Henken, 1987). Halver (1976) observed that any balanced formula for fish diets must include an energy source plus sufficient indispensable amino acids, essential fatty acids and minerals to support life and promote growth.

Essentially fish have a requirement for protein, amino acids, lipids, essential fatty acids, carbohydrates, vitamins, minerals and sufficient energy to grow properly (Lovell, 1979).

2.1.1 Protein requirements

Proteins are needed to provide energy, to supply amino acids and to meet the requirements for functional proteins - enzymes and hormones (Ensminger and Olentine, 1978).

The protein requirements of fish varies from as low as 16% to as high as 60% but the total

protein content for optimal results depend on the quality of the proteins used (the lower the quality, the higher the required protein content), the live stages of the fishes, the water temperature, the feeding level and techniques and the genotype of the fish (EIFAC, 1971). Hastings (1976) also reported the existence of a relationship between the protein requirement and energy requirement at a given water temperature, size and age of fish and environmental stresses such as stocking density, low dissolved oxygen supply and the presence of toxicants.

Dupree and Sneed (1966) observed the effect of temperature on the protein requirement of channel catfish (Ictalurus punctatus). They reported that channel catfish showed optimal growth at 20°C on a 35 per cent protein diet whereas at 25°C they grew best on a 40 per cent protein diet. Similarly, striped bars (Morone saxatilus) fingerlings were found to require 47 per cent protein at 20°C and about 55 per cent at 24°C (Millikin, 1983). In an earlier study, Delong et al (1958) demonstrated that Chinook Salmon (Oncorhynchus tshawytzcha) fingerlings attained

optimum growth with a food containing 40 per cent protein at 8.3°C, whereas the same fish in water of 14°C attained optimum growth with rations containing 55 per cent protein. At the lower water temperature, rations containing more than 40% protein produce stress due to an excess of ammonia released from the gills (NRC, 1977). The requirement of the African catfish, Clarias gariepinus with respect to crude protein and metabolizable energy at increased water temperature were comparable to those of other omnivorous fish species studied. Thus at 24°C the optimal crude protein intake per unit metabolizable energy intake with respect to growth rate was about 25.4mg KJ^{-1} (about 106.3mg $KCal^{-1}$) and at 29°C this amount was about 34.7mg KJ^{-1} (about 145.4mg $KCal^{-1}$) (Heneken et al, 1986). Thus fish at higher temperatures should be fed diets containing a higher protein to energy ratio than fish at low temperatures. Similarly, Degani et al (1989) maintained Clarias gariepinus fingerlings with an initial weight of 10 - 12g at three temperatures: 23, 25 and 27°C and six experimental diets one

of 25%, two of 30%, two of 35% and one of 40% protein by dry weight. They observed that 27°C was a better temperature for growth and protein gain of C. gariepinus than 25°C or 23°C. The best growth rate and protein gain were found in fish fed a high protein diet (40% protein) rather than a low protein diet (25%, 30%, 35%). Hogendoorn et al (1983) also found that the optimum temperature for small C. gariepinus (between 0.5 and 5g) was 30°C and for large fish (125g) it was 25°C. However, the coldwater fish rainbow trout appear to behave differently among the fish so far studied. This follows the observation that rainbow trout fed practical diets containing 35, 40 and 45 per cent crude protein at temperatures ranging from 9 to 18°C showed no apparent differences in protein requirement (NRC, 1981). Since increased feed consumption occurred at the lower protein diets when rainbow trout was reared at higher temperatures it was thought probable that this was able to meet higher protein requirement at elevated water temperature (NRC, 1981).

The effect of dietary protein requirements on the age or size of fish showed a general decrease in protein requirement with increasing

fish age or size. For example salmonids require about 50 per cent protein during the initial feeding stage of fry and 40 per cent protein after 6 to 8 weeks with 35 per cent protein for yearlings (NRC, 1973). Similarly, channel catfish fry require a minimum of 40 per cent protein decreasing to 30 - 36 per cent for fingerlings and 25 - 30 per cent protein for fish weighing more than 114g (NRC, 1977; Andrews, 1977). Andrews (1977) further demonstrated that the higher protein concentration in the two ranges produce better growth of channel catfish fingerlings and juveniles whereas the lower protein concentration provide better protein conversion. Balarin and Haller (1982) have reported that tilapia of less than 1g require 35 - 50 per cent protein, 1 - 5g fish require 30 - 40 per cent protein, 5 - 25g fish require 25 - 30 per cent protein and tilapia weighing more than 25g require 20 - 25 per cent protein. Similarly, Carp (Cyprinus carpio) and eel (Anguilla japonica) showed a high protein requirement for young fish. The protein requirement decreased with age (Halver, 1976).

Lovell (1979) found that channel catfish of small size (10 - 20g) require 9 - 10g of high quality protein per kg of fish per day for maximum growth when fed a nutritionally balanced diet. As channel catfish approach harvestable size (0.5 kg) the daily protein requirement decreases to 7.5 - 8.0g per kg. If channel catfish fingerlings will consume, or are fed, at the rate of 3 per cent of their weight per day, a 33 per cent protein ration will provide all of their protein requirement (10g of protein per 30g of feed per kg of fish). However, if fed at the rate of 2.5 per cent of their weight per day, they would need a 40 per cent protein diet. In the absence of adequate protein in the diet, there is a rapid reduction or cessation of growth, or a loss of weight due to the withdrawing of proteins from certain less vital tissues to maintain the functions of more vital ones (Wilson, 1984; Lovell, 1980a). Alternatively, if too much protein is supplied by the diet, that which is not utilised to make new proteins will be broken down to produce energy (Wilson, 1984). In general warm water fish require 33 - 36% protein in the diet (Lovell, 1980a).

Another factor which alters the protein requirement of fish is the experimental methodology. It has heen shown for Channel catfish that crude protein requirement was 25 - 30 per cent in experiments conducted in earthenware ponds while the fish requirement for protein increased to 32 - 45 per cent with increased stocking density due to a decrease in the contribution of natural food protein (Robinson and Lovell, 1984). Studies in aquaria and other controlled environments have indicated that the protein requirement ranges from 25 - 36 per cent depending on fish size (Robinson and Lovell, 1984).

(i) Qualitative Amino Acids requirement

Fish, like other animals do not have a true protein requirement but have a requirement for a well balanced mixture of indispensable and dispensable amino acids (Wilson, 1985; Wilson and Halver, 1986). All the fish species so far studied require the same ten indispensable amino acids for growth (Cowey and Sergent, 1972; Mertz, 1972). These amino acids are arginine,

histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. These indispensable amino acids must be in the diet and must be present in nearly balanced amounts to furnish an acceptable amino acid pattern before fish flesh can be formed (Halver, 1976). Fish fed diets devoid of any one indispensable amino acid failed to grow, whereas those fed diets devoid of dispensable amino acids grew, as well as control fish, fed a diet that included all amino acids in the reference protein (Halver, 1957).

(ii) Determination of Quantitative Amino acids requirement

Amino acid requirement of fish could be determined quantitatively by any of the following methods:

(a) The basic method developed by Halver et al (1957). The procedure involves feeding graded levels of one amino acid at a time in a test diet containing either all crystalline amino acids or a mixture of casein, gelatin, and crystalline amino acids formulated so that the amino acids

profile is identical to whole chicken egg protein except the amino acid being tested (Mertz, 1972).

(b) The semipurified diet involves using an unbalanced protein as the major source of intact amino acids such as zein or corn gluten which are deficient in certain amino acids (Wilson, 1985).

(c) The practical type diet involves normal feedstuffs to furnish the bulk of the amino acids in the test diet. These may be formulated with a fixed amount of the desired protein level and the remaining amount of the protein equivalent is made up of crystalline amino acids (Wilson and Halver, 1986).

(iii) Quantitative amino acid requirement

The quantity of amino acids required by fish so far documented is summarised below. In general more work has been done on the requirement of fish for tryptophan, methionine, phenylalanine, isoleucine and lysine than other indispensable amino acids.

Arginine

Studies so far conducted on the Arginine requirement of fish indicate that Salmon have the highest requirement of 6% (Klein and Halver, 1970) while carp and the Japanese eel have a requirement of 4.3 and 4.5% respectively (Nose, 1979). The rainbow trout and tilapia have a requirement for less than 3 to 4% arginine per dietary protein (Wilson, 1985).

Histidine

Among the fish species so far studied, histidine requirement seem to be highest in carp and Japanese eel, 2 - 10% (Nose, 1979). It is lowest in the Salmon, 1.8% (Klein and Halver, 1970) and channel catfish, 1.5% (Wilson et al, 1980).

Threonine

The Japanese eel and Carp have a requirement for 4.0% and 3.9% threonine respectively (Nose, 1979). Chinook salmon have a requirement for 2.2% threonine (Delong et al, 1962) and channel catfish have the lowest threonine requirement of 2.0% per dietary protein (Wilson et al, 1978).

Valine

The valine requirement appears to be highest in the Japanese eel among all the fish so far reported 4% (Nose, 1979). Salmon, Carp and catfish have 3.2% (Chance et al, 1964), 3.6% (Nose, 1979) and 3.0% (Wilson et al, 1980) respectively. The valine requirement of lake trout varied between 2.6 - 3.3% dietary protein (Hughes et al, 1983).

Leucine

The leucine requirement values varies from 5.3% in the Japanese eel to 3.3% dietary protein in the common carp (Nose,1979). Values for salmon, catfish and lake trout are 3.9% (Chance et al, 1964), 3.5% (Wilson et al, 1980) 3.5 - 4.6% (Hughes et al, 1983) respectively. The latter values are recalculated based on the nitrogen content of the test diet.

Isoleucine

The isoleucine requirement of fish exhibit excellent agreement of about 2.0 - 2.6% among the fish studies except the Japanese eel which has a value of 4% (Nose, 1979). Common carp

has a value of 2.5% (Nose, 1979) while the values for Salmon, catfish and lake trout are 2.2% (Chance, et al., 1964), 2.6% (Wilson et al., 1980) and 1.54 - 2.6% (Hughes et al., 1983) respectively. The low values for lake trout was explained by the compositions of the test diet which was formulated to meet the required levels of amino acids for a 35% protein diet (Hughes et al., 1983). Thus when the total protein requirement of rainbow trout is calculated based on the apparent nitrogen content of the test diets a much lower dietary protein level is obtained but when the total protein level is recalculated based on the nitrogen content of the diet, the requirement value is within the range reported for other species.

Phenylalanine

Both phenylalanine and tyrosine are aromatic amino acids. The indispensable amino acid phenylalanine is readily converted to the dispensable tyrosine in fish, therefore in order to determine the total aromatic amino acid requirement (Phenylalanine plus tyrosine) the dietary requirement for phenylalanine is determined either in the absence of tyrosine or with test diets containing very low levels of tyrosine (Wilson and

Halver, 1986). Based on such determinations the phenylalanine requirement for carp and Japanese eel in the presence of 0% tyrosine is 6.5% and 5.8% of dietary protein respectively (Nose, 1979). Values for salmon and channel catfish are 5.1% (0.4% tyrosine)(Chance et al., 1964) and 5.0% (0% tyrosine) (Robinson et al., 1980) respectively. The latter authors also reported that tyrosine can replace or spare about 50% of the phenylalanine requirement of channel catfish.

Tryptophan

Apart from being a constituent of protein, tryptophan also functions as a precursor of serotonin and nicotinic acid and as a regulator of polysome function and carbohydrate metabolism (Wiltman, 1976; Sidransky et al., 1981).

Tryptophan requirement values was found to be 5% for Salmon (Halver, 1965) and rainbow trout (Walton et al., 1984). The common carp required 0.8% tryptophan while that of Japanese eel is 1.1% (Nose, 1979).

Wilson et al. (1978) reported that Channel catfish fed a tryptophan - deficient diet (0.05%) for eight weeks had significantly poorer weight gain and feed conversion efficiency than individuals fed diets containing as low as 0.12% tryptophan (0.5% dietary protein).

The dietary tryptophan requirment of fishes are generally lower than of terrestial animals. This has been ascribed to the inability of the fish to convert tryptophan to niacin thus reducing the metabolic need of tryptophan in fishes compared with terrestial animals (Wilson et al., 1978).

Tryptophan deficiency in rainbow trout resulted in several anatomical abnormalities such as hyperemia, scoliosis (twisted spines) and abnormal deposition of calcium both in the bony plates surrounding the notochord and the kidney (Kloppel and Post, 1975). Poston and Rumsey (1983) also reported the occurrence of caudal fin erosion, cataracts and short gill opercula in tryptophan deficient rainbow trout. Tryptophan deficiency has been reported to cause scoliosis and lordosis in sockeye salmon but not in chinook salmon

(Halver and Shanks, 1960). In general tryptophan deficiency symptoms has only been described in rainbow trout, sockeye salmon, brook trout and channel catfish (Millikin, 1982). The deficiency signs has not been reported in other species (Wilson, 1985).

Methionine

The dietary methionine requirements for fish have been shown to depend upon dietary cystine concentration similar to that of terrestial animals (NRC, 1983). Methionine has a sparing action with cystine. Cystine is a dispensable amino acid in that it can be synthesized by the fish from methionine. Wilson and Halver (1986) explained that when a fish is fed a diet containing methionine without cystine, a portion of the methionine is used for protein synthesis and a portion is converted to cystine for incorporation into protein. If cystine is included in the diet, it reduces the amount of dietary methionine needed, thus fish have a total sulphur requirement rather than a specific methionine requirement.

Studies on the methionine requirement of fish shows that chinook salmon (Halver et al., 1959) and Gilthead bream (Luquet and Sabaut, 1974) have a requirement for 4.0% in the presence of 1% cystine for the salmon, while the Japanese eel (Nose, 1979) and tilapia (Jackson and Capper, 1982) have a requirement for 3.2% methionine in the presence of 0% and o.7% dietary cystine respectively. Channel catfish and rainbow trout require the least at 2.3% (Harding et al., 1977) and 2.2% (Walton et al., 1982) respectively.

Methionine deficiency have been studied in several species. Page (1978) fed rainbow trout fingerlings with diet containing different levels of methionine and cystine. After 12 weeks, trout fingerlings fed 0.6% methionine plus 0.3 or 0.45% cystine did not develop any cataracts whereas those fed lower dietary methionine content (0.3 or 0.45%) combined with dietary cystine content of 0.4 to 0.6% produced varying degrees of cataracts in the rainbow trout. Poston et al.(1977) also observed cataracts in rainbow trouts fed diets containing isolated soyabean protein. Rainbow trout fingerlings

were fed diets deficient in methionine (0.36% methionine) containing soyabean protein isolate as the sole protein source in a 40% protein diet. After 8 weeks the trout fingerlings showed only initial signs of opacification of sub-capsular areas. After 16 weeks, all the trout fed 0.36% dietary methionine developed bilateral cataracts while non of the individuals fed the control diet containing 1.2% dietary methionine had cataracts. Walton et al. (1982) and Rumsey et al. (1983) have also observed cataract in methionine deficient rainbow trout. However, cataractogenesis was absent in methionine deficient diets fed to channel catfish. This was attributed to the relatively short duration of the study (8 weeks) and the slightly larger size of the catfish (mean weight,7.7g) compared with rainbow trout (mean weight, 5g).

Studies on the utilization of several dietary sulphur compounds in channel catfish showed that D.L - methionine was utilized as effectively as L - methionine by channel catfish (Robinson et al., 1978). Methionine hydroxy analogue was only 26%

as effective in promoting growth as L-methionine. No significant growth response was observed when either taurine or inorganic sulfate was added to the basal diet (Robinson et al., 1978). Similarly, Kim et al. (1984) has demonstrated that D-methionine can replace L-methionine on an equal basis in rainbow trout. Insufficient methionine in Salmom diet resulted in reductions in sulfhydryl group concentrations and a rapid decrease in the synthesis of lens glutathione during formation of most cataracts were reported by Poston et al. (1977). The authors feld that lens glutathione possibly protects the lens sulfhydryl groups from oxidation.

Lysine

Lysine is a basic amino acid with an α - amino group and an E - amino group. While the α - amino group is normally bound in peptide linkage, the E - amino group must be free and reactive otherwise the lysine, although chemically measurable, will not be biologically available (Cowey, 1978). During the processing of feedstuff proteins, the E - amino group of lysine may react with non-protein molecules

present in feedstuff to form additional compounds that render the lysine biologically unavailable (Cowey and Sergent, 1972).

The lysine requirement of salmon, eel, catfish and gilthead bream are about 5% of dietary protein while the rainbow trout fry require 6.0% (Ketola, 1980).

Excessive dietary lysine in the presence of marginal or adequate dietary arginine concentrations did not depress growth or feed efficiency of channel catfish nor did excessive arginine depress growth or feed efficiency of channel catfish in the presence of marginal dietary lysine concentrations (Robinson et al., 1981a) in contrast to lysine - arginine antagonism reported for terrestial animals by Maynard and Loosli (1969). The lysine requirement for tilapia was 4.1% of dietary protein (Jackson and Capper, 1982). This value is lower than those of other species reported above and may be associated with low growth rate observed and/or low digestibility or utilization of the test diet (Wilson, 1985).

Lysine deficiency may lead to depressed rates of collagen formation since Hydroxylysine is a constituent of collagen in fish species (Millikin, 1982). Lysine deficiency is also implicated in the formation of fin rot in rainbow trout (Ketola, 1979). Supplementation of lysine, arginine, histidine, isoleucine, threonine, tryptophan and valine to lysine - deficient corn gluten meal diet resulted in improved survival, increased growth and prevention of severe caudal fin erosion. At the same time removal of lysine from the amino acid mixture in the corn gluten meal supplemented diet increase mortality, reduced growth and resulted in caudal fin erosion of rainbow trout (Millikin, 1982).

The great reactivity of the epsilon amino group of lysine with aldehydes in sugars forming Maillard type compounds may mislead the investigator if diet formulations are based solely upon analysis of diet ingredients of finished feeds for indispensable amino acids content (Halver, 1976). Obviously biological availability of these reactive amino acids is

necessary and simple amino acid analyzer values do not reflect either the optically active form of the amino acid or the biological availability of it to the fish fed (Halver, 1976).

2.1.2 Fish energy requirement

Energy needs of fish are less than those of warm-blooded animals because fish being poikilothermic (Cold-blooded animals) do not have to expend energy in order to maintain body temperature (Lovell, 1979). By not having to regulate body temperature, more energy is available to the fish for growth, actvity and reproduction (Ensminger and Olentine, 1978). Fish require less energy for muscular activity to maintain their position in water than do animals on land (Tucker, 1969). Fish also lose less energy in protein catabolism and excretion of nitrogenous wastes as ammonia instead of as urea or uric acid (Goldstein and Forster, 1970). Lovell (1979) reported that the optimum amount of metabolisable energy for each gram of protein in the ration is approximately 14 - 16 KCal for poultry and 15 - 24 KCal for swine. This compares

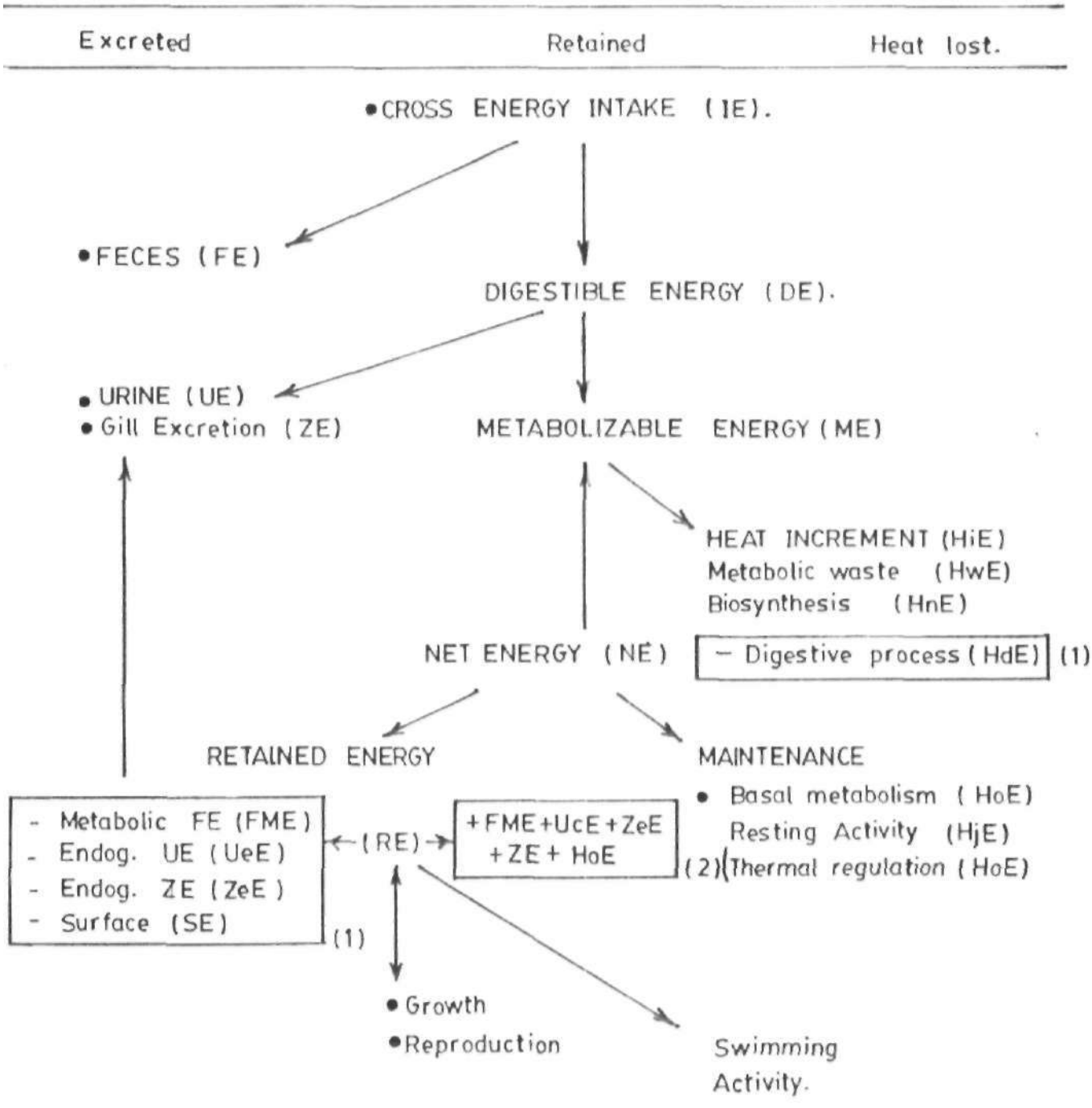
with values of 6 - 10 Kcal per gram of protein which have been found adequate for fish in experimental rations.

The relationship between the protein content and energy content of diet has been examined for several fish species. Lovell (1979) reported that when diets fed to channel catfish contained high percentage of protein (42% and above) and very low amounts of non protein energy (< 1.5 Kcal per gram of diet) growth was suppressed. When the protein level was reduced to 36% and the non protein energy level remains the same, growth increased. When the non protein energy in either the 42% or the 36% protein diets was increased, growth improved. He concluded that even though fish utilize protein for energy more efficiently than farm animals, when too many calories in a fish diet comes from protein the efficiency of utilization of the ration is suppressed. Lee and Putnam (1973) observed the response of rainbow trout to varying protein/ energy ratios in a test diet. The workers reported that the level of dietary protein which support maximum growth of rainbow trout depends

on the dietary energy. The rainbow trout required 160 and 130mg protein per Kcal at 3.0 and 3.7 Kcal/g diet respectively while at 4.4 Kcal/g diet 91mg protein per Kcal were needed for maximum growth. In general certain trends have been observed in most of the fish so far studied. An increase in the ratio of digestible energy: protein led to an increase deposition of lipid in the fish; an increase in energy level resulted in improved feed conversion efficiency; protein efficiency was negatively correlated with the ratio of dietary protein to energy. Starch or dexrin up to a level of 25% was effective as energy source, fish eat to meet their energy requirement (Cowey and Sergent, 1979). Figure 1.1 shows the schematic representation of energy losses in fish as food is digested and metabolished.

2.1.3 Carbohydrate requirement

Carbohydrates are incorportated in fish feed because they are the cheapest sources of dietary energy available. Dietary carbohydrates are sugars and fibre. Phillips et al. (1948) suggested that feeding trout with high levels of carbohydrates might result in poor health, in particular deleterious changes in the liver and eventual



• Can be measured directly

1) Body origin

2) Continuous replenishment of body loss.

Fig. 1.1. Schematic Representation of Energy losses in fish as food is digested and metabolised. (Adapted from Cho and Kaushik, 1985)

increased mortality. They suggested a safe maximum content of 12% of digestible carbohydrates in the diet of brook trout. Edwards et al.(1977) fed ten different families of rainbow trout for 24 weeks on each of three diets similar in protein and energy content but differing in the percentage of metabolizable energy as carbohydrate. They reported that fish growth was better on diets containing 17 and 25% of their metabolizable energy as carbohydrate than on that containing 38%. Fish fed on the 38% diet had lost condition by the end of 24 week experimental period. Similarly, Austreng et al.(1977) using three diets similar in protein and energy content but had different percentages of metabolizable energy present as carbohydrate (17, 25 and 38%) found that utilization of dietary energy and protein was poorer in fish fed high levels of carbohydrates but health of all fish appeared good at the end of the 24 weeks observation.

The rate of utilization of carbohydrates by common carp, red sea bream and yellow tail was compared by Furuichi and Yone (1980). They showed that growth was retarded in common carp

fed 40% dextrin, red sea bream fed 30% dextrin diet and yellow tail fed diets containing over 20% dextrin. Thus common carp and red sea bream appear to utilise carbohydrate better than yellow tail. However, the utilization of carbohydrate in fish diet appear to differ with complexity of the carbohydrate. The relative efficiency of utilization of carbohydrate was found to be as follows: Monosocharides are best used followed in order by disaccharides, simple polysaccharide, dextrins, cook starches and raw starches in brook trout (Phillips, 1948) and rainbow trout (Singh and Nose, 1967; Smith, 1971). Buhler and Halver (1961) also reported that the relative growth rates of chinook salmon fingerlings fed 20% carbohydrates were as follows: glucose > sucrose > fructose > maltose > dextrin > potato starch > galactose.

Furuichi and Yone (1981) investigated the availabilities of α - starch, dextrin and glucose in the nutrition of carp, Cyprinus carpio and red sea bream, Chrysophrys major by comparing the growth and feed efficiency and the changes in rates of absorption (per cent absorption

per hour) and in blood sugar levels after the administration of carbohydrate. They reported that the growth and feed efficiency of carp were highest when fed with the α - starch diet, followed by the dextrin and glucose diet in that order. Red sea bream did not show any significant difference in growth for the various carbohydrates sources, but showed higher feed conversion efficiency when fed with the α - starch diet than with the dextrin and glucose diets. The percentage absorption within the 2-hour period after the administration was highest in glucose, followed by dextrin and α - starch in decreasing order, but the absorptions after the period followed the reverse order. This difference was reflected in the change in blood sugar levels. These findings suggested that the availability of carbohydrate in fish nutrition may be influenced by the difference in per cent absorption before and after the 2-hour period at which time the plasma insulin showed the maximum level.

The extent of utilization of carbohydrates in fish is also a function of the type of fish being cultured. Carnivorous fish like the Atlantic

salmon, rainbow trout and the Japanese yellow tail are ill-equipped to handle significant quantities of raw carbohydrates in the diet, for example, gross energy in cooked cornstarch is about 40 per cent digested by rainbow trout (NRC, 1973) but approximately 80 per cent digested by channel catfish (NRC, 1977). Furthermore, carnivores (e.g. Salmonids) develop high levels of liver glycogen and suffer mortality when fed an excess, of carbohydrates (Phillips et al, 1948). On the other hand omnivores such as the common carp and the channel catfish are able to digest fair amount of carbohydrates in their diets. The grass carp, a herbivore susbsisted primarily on a vegetarian diet (Chow and Halver, 1980). In general warm water fish such as channel catfish, carps and tilapia utilise starch better than cold-water fish (Lovell, 1979).

2.1.4 Lipids and Essential Fatty acids requirement

Dietary lipids function as a ready source of energy for fish and in the provision of essential fatty acids which are needed for fish

growth and survival. Dietary lipids provide the vehicle for absorption of fat soluble vitamins and provide other compounds such as phospholipids and sterol esters which play a vital role in the structure of biological membranes at both the cellular and subcellular levels (NRC, 1983). Dietary lipids also provide the polyunsaturated fatty acids (PUFA) which are precursors for prostaglandins. Prostaglandins are a group of five carbon cyclic compounds which possess hormonal activities and are required in trace quantities. Prostaglandins were effective in inducing pigment dispersion in the skin of black goldfish (Abramowitz and Charvin, 1973).

In feed formulation, the optimal dietary lipid depend on whether the feed is raised for human consumption or are being hatchery cultured for release into natural waters. Wedemeyer et al. (1980) reported that since consumers require lean fish inclusion of lipid in the diet may not be necessary but may be essential in the latter case as energy reserve during acclimation to natural food. Lipids are useful components in diet formulation and are especially desirable for fry or fingerling feeds when higher intake is necessary

to promote rapid growth (Halver, 1976). For the dietary lipids to be effective in promoting growth they must be utilised. Lee and Sinnhuber (1972) reported that adequate amount of choline, methionine and tocopherol must be present in the diet for fish to utilise 20-30% of the dry diet ingredient as fat.

A major reason for supplementing the diets of cultured fish with lipids is to spare the oxidation of dietary protein as an energy source. At the same time a luxus of dietary lipid will result in excessive deposition of lipid within the fish and this may be unacceptable to the consumer (Cowey and Sergent, 1979). Therefore in order to determine the optimal dietary lipid concentrations for inclusion in fish feed consideration must be given to the protein sparing action of the feed i.e. the minimal dietary lipid concentration that maximizes dietary protein available for growth rather than for energy. Dupree (1969) demonstrated that channel catfish fingerlings (mean weight 1.25g) cultured at 26.7°C to 32.2°C and fed 35% protein diets grew faster on 8% dietary lipid either as beef tallow or

corn oil than on 4% of either dietary lipid sources. The dietary lipid concentration of 16% corn oil or beef tallow was found to reduce growth rate and protein deposition of channel catfish compared with 8% dietary lipid. He therefore concluded that maximum dietary protein sparing action occurred at less than 16% of either dietary sources but at more than 8% dietary lipid. Murray et al. (1977) also reported that channel catfish fingerlings (mean weight 0.5 and 1g at separate experiments) reared at 28°C grew faster when fed 35% protein and 12% lipid in the diet rather than 5% lipid combined with 25 or 35% protein or 12% lipid combined with 25% protein.

Similar minimal protein sparing action was also reported for other species. Reinitz et al., (1978) working with rainbow trout fingerlings (mean weight 2g) reported that successive increases in dietary lipid concentration of 7, 11, or 16% protein diets or 9, 15, or 21% lipid in 40% protein diets resulted in increased weight gain and improved feed conversion of the fingerlings within each dietary protein

concentration when reared at 11°C. Further studies (Reinitz and Hitzel, 1980) demonstrated that about 35% protein combined with either 23 or 27% dietary lipid provided better growth of rainbow trout fingerlings than 36% dietary protein combined with 14% dietary lipid. However, better growth rates of rainbow trout fingerlings was not produced by the two high lipid concentration diets than a 35% protein and 18% lipid diet. Similarly, Lee and Putnam (1973) reared rainbow trout fingerlings (initial mean weight 4.8g) for 18 weeks and reported better growth rates when fed 35% protein and 24% lipid than individuals fed 44 or 53% protein each combined with either 8, 16 or 24% lipid, indicating a minimal protein sparing action and an optimal protein to energy ratio of 73mg protein/Kcal. On the contrary Yu et al. (1977) studied the growth response of rainbow trout fingerlings (initial mean weight 5.4g) on three dietary protein concentrations 33, 39 and 44% combined with 22% lipid in each of three forms (22% herring oil, 14.6% herring oil plus 7.4% lard or 11% herring oil combined with 11% lard). They reported that better growth

was obtained in the rainbow trout fingerlings fed 44% protein and 22% lipid, regardless of the ratio of herring oil to lard when reared at 11.5°C over a 14 week period. Protein efficiency ratios (PER) and protein retention values (PRV) were similar regardless of dietary protein concentration and lipid source combinations fed to rainbow trout fingerlings.

The contradictory results of the above studies of the minimal dietary protein required (35% protein plus 24% lipid) for maximal growth of rainbow trout fingerlings can be partially explained by differences in dietary fibre concentrations. A low dietary fibre concentration (6.5%) was incorporated in diets containing 36, 44 or 53% protein plus 8% lipid (Lee and Putnam, 1973). However, in the study by Yu et al. (1977) dietary fibre concentrations were held constant at 11.2% in all diets. Therefore variable dietary fibre concentrations in the first study may have differentially affected the amino acid absorption rates or feed utilization (Millikin, 1982).

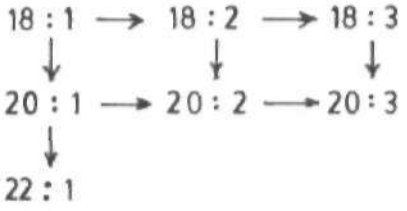
The essential fatty acid requirements have been demonstrated in several species of fish (Lee et al., 1967; Castell et al., 1972 a, b, c; Owen et al., 1975; Cowey et al., 1976; Kanazawa et al., 1980; Yamada et al., 1980). In most of these species it has been shown that fish tissues contain predominantly polyunsaturated fatty acids of the omega 3 series rather than the omega 6 series as obtained in terrestial animals (Kanazawa, 1985). One of the earliest experiments on the essentiality of polyunsaturated fatty acid in fish was conducted by Lee et al. (1967). These workers demonstrated that rainbow trout fingerlings fed diets containing 1% 18:3 w 3 as a supplement to 7.8% corn oil which contains linolenic acid, doubled their weight gain compared with individuals fed 10% corn oil as the sole dietary lipid source. Later Castell et al. (1972a,b,c) also reported that linolenic acid was superior to linoleic acid in supporting growth and feed conversion in rainbow trout. The essentiality of linolenic acid for the rainbow trout was further confirmed by Watanabe et al. (1974) who demonstrated that 1% methly linolenate plus 4%

methly laurate (C 12 - 0) provided maximal growth and prevention of fatty acid deficiency symptoms compared with rainbow trout fed 5% methly laurate. It was further observed that in this species as in mammals, dietary linoleate leads to elevated levels of 20 : 4 w 6 and 22 : 5 w 6 in the polar lipids of the liver while dietary linolenate similarly elevates tissue levels of 22 : 6 w 3 acids. On the other hand, dietary short chain saturated acids (e.g. laurate) elevate the levels of eicosatrienoic acid (20 : 3 w 9) in the body lipids of trout, an effect which is depressed by dietary linoleate and linolenate (Watanabe et al., 1974). Linolenic acid was also demonstrated to be essential in the Salmonids (Sinnuber, 1969).

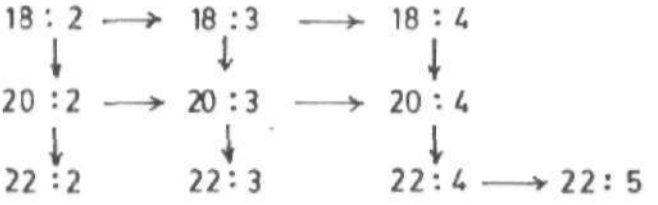
Turbot, Scophthalmus maximus a marine species appear to contrast with rainbow trout in their ability to elongate and further desaturate C 18 fatty acids at substantial rate. Turbot were fed isoenergetic diets containing one of three fats. These were hydrogenated coconut oil which exarcebates essential fatty acid deficiency in

mammals, cod liver oil which contains polyunsaturated fatty acids of the omega 3 series and corn oil which supplies mainly linoleic acid. At the end of 16 weeks, little conversion of linoleic to arachidonic occurred in the turbot fed the corn oil diet indicating that turbot have absolute requirement for polyunsaturated fatty acids (PUFA) of the omega 3 series as they cannot chain elongate and desaturate linolenic acid, 18 : 3 w 3 (Cowey et al., 1976). This observation was supported by other workers (Owen et al., 1975). It was further reported that turbot not only required the omega 3 fatty acids but specifically needed dietary 20 : 5 w 3 or 22 : 6 w 3 to satisfy its essential fatty acids requirement (Cowey et al., 1976). Mead and Kayama (1967) and Sinnuber (1969) have outlined the following pathway for the biosynthesis of polyunsaturated fatty acids in fish (Fig. 1.2). Vertical arrows show chain elongation reactions, and horizontal arrows show desaturation reaction.

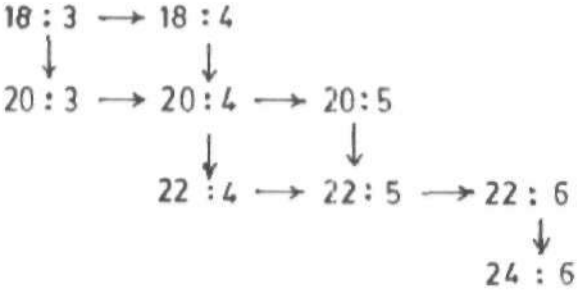
Fish species vary in their ability to elongate and desaturate 18 carbon fatty acids, to 20 - or 22 carbon fatty acids. Yamada et al. (1980)



Oleic Acid, Omega 9.



Linoleic Acid, Omega 6.



Linolenic Acid, Omega 3.

Fig. 1·2. Pathway for the biosynthesis of polyunsaturated fatty acids in fish.

administered labelled ^{14}C 18 : 3 w 3 to individuals of red sea bream, black sea bream, Mylio macrocephalus, Opaleye, Girelnigrican, striped mullet, Mugil cephalus and rainbow trout. They observed that only rainbow trout exhibited appreciable radio activity in 22 : 6 w 3 of body lipids. It was therefore concluded that marine species have limited ability to elongate and desaturate 18 : 3 w 3 resulting in dietary essentiality of eicosapentaenoic acid (20 : 5 w 3) or 22 : 6 w 3 and non essentiality of 18 : 3 w 3.

Tilapia zilli injected with labelled ^{14}C acetate converted it into palmitic (16 : 0), palmitoleic (16 : 1 w 7), stearic (18 : 0) and oleic (18 : 1 w 9) acids. There was scarcely any incorporation into linoleic (18 : 2 w 6), linolenic (18 : 3 w 3) eicosapentaenoic (20 : 5 w 3), and docosahexaenoic (22 : 6 w 3) acids. Thus unlike most fish species studied, Tilapia zilli fingerlings require 1% 18 : 2 w 6 or 20 : 4 w 6 fatty acids for optimal weight gain as opposed to fatty acid of the omega 3 series (Kanazawa et al., 1980). Since fish are incapable of "de - novo" synthesis of 18 : 2 w 6, 18 : 3 w 3, 20 : 5 w 3

and 22 : 6 w 3 fatty acids, dietary sources of these fatty acids are likely to be essential for normal growth and survival, (Kanazawa, 1985).

It could be said therefore that with the exception of tilapia and snakehead, fish generally require omega 3 fatty acids rather than omega 6 - fatty acids in contrast to terrestial animals which require omega 6 - fatty acids such as linoleic 18 : 2 w 6 and arachidonic 20 : 4 w 6. Kayama (1977) suggested that poikilothermic animals may have a requirement for omega 3 fatty acids because they generally have a lower body temperature than mammals (homothermic animals) and omega 3 fatty acids have a lower melting point than corresponding omega 6 - fatty acid of equal carbon chain. In other words the preference of fish for w 3 compared to w 6 polyunsaturated fatty acids reflects fundamentally the low temperature (generally lower than 20°C) of the majority of aquatic environment. Similarly, freshwater fish consume natural food that is partly terrestial in origin especially herbivores and omnivores hence freshwater fish have higher level of w 6 fatty acids in their tissues than

marine fish (Ackman, 1967). In the like manner, freshwater fish have higher levels of short chain w 3 polyunsaturates (e.g. 18 : 3 w 3) than marine fish (Gruger et al., 1964).

2.1.5 Minerals Requirement

Minerals are important as a constituent of bones and for the maintenance of skeletal structures and acid-base equilibrum in the fish. Minerals are also components of blood, enzymes and organic compounds in fish tissues and organs where they are indispensable for essential metabolic processes involving gas exchange and energy transactions (Halver, 1980). The following mineral elements are recognised as essential for body functions in fish: calcium, phosphorus, sodium, molybdenum, chlorine, magnesium, iron, potassium, selenium, iodine, manganese, copper, zinc, fluorine and chromium (Millikin, 1982). It is unlikely that fish have specific requirements for inorganic sulfur and cobalt when their needs for essential sulfur-bearing amino acids and cobalt - containing vitamin B_{12} are met (NRC, 1983).

(i) Calcium and phosphorus

Calcium and phosphorus are discussed together because they occur in the body combined with each other for the most part and because an inadequate supply of either limits the nutritive value of both (Halver, 1980). Apart from its structural functions in bones and scales, calcium is essential for the following physiological activities: blood clothing, muscle function, proper nerve impulse transmissions, osmoregulation, and for serving as a cofactor during various enzymatic processes (NRC, 1983). Phosphorus is a constituent of adenosine triphosphate (ATP), phospholipids, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and various coenzymes. Thus phosphorus is involved in energy transformations, cellular membrance permeability, genetic coding and general control of reproduction, growth, and serves as a buffer for the maintenance of normal pH in body fluids and cells (NRC, 1983). Much of the body calcium is found in the skeleton, and in tilapia calcium accounts for 19 to 24 per cent of the scales on dry weight basis (Garrod and Newell, 1958). It has also been reported that the amount of calcium

in the scales of tilapia, salmon and carp decreases with spawning and during starvation indicating that the minerals in scales are reabsorbed for physiological functions (Garrod and Newel, 1958; Yamada, 1958; Ichikawa, 1953).

Since freshwater fish drink very little water if any (Sinkiss, 1974) and marine fish swallow considerable quantities of sea water estimated at between 5 and 4 per cent of their body weight (Smith, 1930) drinking can provide a calcium intake for marine fish that equals roughly 40 to 52 per cent of that provided by the food (NRC, 1983). On the contrary, since freshwater fish drink little or no water they obtain almost no nutrients from drinking.

Studies on the significance of the dietary calcium-to-phosphorus ratios in fish nutrition has been complicated due to dietary factors such as magnesium and vitamin D or use of suboptimal calcium water concentrations in various studies (Millikin, 1982). However, Andrews et al. (1973) has reported that optimal growth and feed conversions occurred in channel catfish fingerings fed a 1.5 : 1 ratio of calcium to phosphorus.

Similary, Sakamoto and Yone (1973) observed optimal feed efficiency and serum inorganic phosphorus in red sea bream fed a 1 : 2 ratio of calcium to phosphorus.

Signs of calcium deficiency are difficult to produce due to low dietary requirement of this mineral element. For example no signs of deficiency was noticed when common carp and rainbow trout were reared in water containing 20mg calcium per liters and fed 0.03 per cent dietary calcium (Ogino and Takeda, 1976; 1978). Similarly, no signs of deficiency was detected when channel catfish were reared in water containing 14 mg per liter of calcium and fed a vitamin D adequate diet containing only 0.05 per cent calcium level. However depressed growth, poor appetite and poor feed conversion efficiency were observed in Japanese eel and red sea bream fed calcium deficient diets. The minimum calcium requirement for these species were for Japanese eel reared in water containing 19 ppm it was discovered to be 0.27 per cent (Arai et al., 1975) and 0.34 per cent of the diet for red sea bream, (Sakamoto and Yone, 1973).

Common carp fed phosphorus deficient diets had reduced blood phosphate levels, deformed backs (lordosis) abnormal calcification of ribs and soft rays of the pectoral fins and increase in carcass fat with decreased water content and increased gluconeogenic enzymes in the liver. (Ogino and Takeda, 1976). The minimum phosphorus requirement was 0.6 - 0.7 per cent of the diet. Lovell (1978) using purified diet with graded levels of highly available monobasic sodium phosphate, reported the minimum phosphorus requirement of channel catfish to be about 0.45 per cent of the diet. Phosphorus deficiency symptoms in channel catfish were reduced growth rate, reduced body content of calcium and phosphorus and loss of appetite. The Japanese eel was reported to require 0.29 per cent (Arai et al., 1975). Watanabe et al. (1980) reported the minimum phosphorus requirement for tilapia (Oreochromis niloticus) to be approximately 0.9 per cent.

Phosphorus in most plants is stored in the form of phytate which is almost unavailable nutritionally to most species of fish. Hence the availability of phosphorus to fish is often very

low in most plant products (NRC, 1983). Phosphorus in grains, soyabean and fish meal is 33 per cent, 35 per cent and 50 per cent available respectively whereas inorganic phosphorus from sodium, monocalcium or dicalcium phosphate is highly available (80 per cent) (Lovell, 1984). It was also observed that addition of 0.3 per cent phosphorus in the form of dicalcium phosphate to increase the non-phytate phosphorus in the diet of channel catfish from 0.22 per cent to 0.55 per cent, was adequate for maximum growth of the fish (Lovell, 1984). Therefore pond-raised channel catfish fed on all-plant diet require additional phosphorus in their ration for maximum growth. This observation is also applicable to many other freshwater fish species.

(ii) Magnesium

Magnesium is an essential constituent of bone in fish where it constitutes a little over 0.6 per cent of the ash content of bones compared with 30 per cent and 15 per cent for calcium and phosphorus respectively (Halver, 1980).

Magnesium deficiency symptoms have been studied in several species. The deficiency signs in channel catfish were poor growth, anorexia, sluggishness, high mortality, muscle flaccidity and depressed magnesium levels in the blood serum, bones and the entire body of the fish (Gatlin et al., 1982). Deficiency of magnesium in the diet of common carp and rainbow trout leads to loss of appetite, poor growth, sluggishness and convulsion followed by tetany including high mortality. Histological changes were also observed in muscle, pyloric caeca and gill filaments of trout fed magnesium - deficient diets (Halver, 1980).

The minimum dietary requirements of magnesium for channel catfish was established at 0.04 per cent of diet when fish are reared in water containing 1.6 ppm magnesium (Gatlin et al., 1982). In the rainbow trout the quantitative dietary requirement appeared to be between 0.025 and 0.07 per cent dietary magnesium when fish are reared in waters containing 1.2 and 3.1 ppm magnesium (Ogino et al., 1978; Knox et al., 1981).

Common carp reared in water containing 3.5 ppm magnesium require a minimum dietary magnesium of 0.04 per cent (Ogino and Chiou, 1976).

Although fish do not extract dissolved magnesium from water to meet dietary needs, natural foods as well as most artificial feed ingredients of both animal and vegetable origin are adequate sources of magnesium and deficiency had not been observed to date under ordinary rearing conditions (Halver, 1980).

(iii) Manganese

Manganese is a cofactor for the enzyme arginase and many other metabolic enzymes. Manganese is involved in the formation of bones and for the regeneration of erythrocyte (Halver, 1980). Ishak and Dollar (1968) demonstrated that tilapia (T. mossambica) reared in water containing 2.8 ppm manganese suffered poor growth, anorexia, loss of equilibrum, and mortality. When the diet was supplemented with 35.5 ppm magnesium, there was no benefit, nor was there any change when only the water was supplemented. However when both the diet and water were supplemented,

there was positive result. Ogino and Yang (1980) working with rainbow trout and common carp fed them with manganese deficient diets containing 4mg magnesium and observed depressed growth in both species and abnormal curvature of the backbone and malformation of the tail in the rainbow trout. The authors reported higher growth rate when the two species were fed with 12 to 13mg magnesium per kilogram of diet.

(iv) Iron

Dietary iron is essential for fish to maintain the normal haemoglobin level and deficiency was reported to cause hypochromic microcytic anemia in red sea bream (Sakamoto and Yone, 1978c) and common carp (Sakamoto and Yone, 1978b). Red sea bream was reported to require a minimum of 150mg iron per kilogram diet to prevent the occurrence of iron deficiency symptoms which included corpuscular diameter and low blood iron content (Sakamoto and Yone, 1978a). However, among the iron diets, ferrous and ferric chlorides were found to be more highly available than ferric citrate in the prevention of hypochromic microcytic anemia (Sakamoto and Yone, 1978c).

(v) Iodine

Iodine is a constituent of the hormone thyroxine and chinook salmon fed 0.1mg iodine per kilogram diet developed thyroid hyperplasia (goiter) and depressed growth whereas those fed 0.6 and 1.1mg iodine per kilogram diet in rearing water containing 0.2 ppb iodine attained maximum iodine storage (Woodall and LaRoche, 1964). The authors concluded that the minimum iodine requirement for chinook salmon was 1.6mg. Supplementation of diet with iodine was found to prevent goitre in brook trout (Gaylord and Marsh, 1912).

vi) Zinc

Dietary zinc is essential for the structure and function of insulin and deficiency in channel catfish led to depressed growth, loss of appetite, and reduced zinc and calcium in the bones (Gatlin and Wilson, 1983). Ogino and Yang (1978) demonstrated that rainbow trout fingerlings fed 1mg zinc per kilogram diet had poor growth and high mortality whereas those fed 5mg zinc per kilogram diet produced slow growth. Rainbow trout fingerlings fed 15 and 30mg zinc per kilogram diet had satisfactory growth. In a

later work (Ogino and Yang, 1979) the authors observed that common carp reared in water containing 10 ppb zinc needed 5 ppb zinc for maximum growth supporting a requirement for 15 to 30mg zinc per kilogram diet as for rainbow trout. Zinc deficiency symptoms in common carp were depressed growth, loss of appetite and high mortality with elevated concentration of iron and copper in the intestinal tract (Ogino and Yang, 1979).

(vii) Selenium

Selenium was found to be similar to vitamin E in that a deficiency of both caused muscular dystrophy in Atlantic salmon. This was prevented by sypplementing the diet with 0.1mg selenium per kilogram diet or 500 IU vitamin E per kilogram diet (Poston et al., 1976). Hilton et al. (1980) fed rainbow trout on diet containing graded levels of selenium in the diet from 0.07mg per kilogram dry diet up to 13mg per kilogram diet. The fish were reared in water containing 0.04 ppb selenium and fed 400 IU vitamin E per kilogram of diet. No selenium deficiency symptoms were observed even with fish fed the lowest selenium diet.

However since selenium is a component of glutathione peroxidase, the plasma glutathione activity showed an increase until the dietary selenium concentration of 0.15 to 0.38mg per kilogram was attained. Since the rainbow trout fed high selenium level (13 mg/kg) had depressed growth and high mortality it was concluded that dietary selenium concentration of 0.15 to 0.38mg per kilogram would be ideal for rainbow trout. Studies on selenium deficiency has been limited to a few cold water species No studies have been conducted in catfish, carp and other warmwater fishes (NRC, 1983).

(viii) Copper

Channel catfish fingerlings was found to have a dietary requirement of 1.5mg copper per kilogram diet (Murai et al., 1981). Ogino and Yang (1980) compared the growth responses of common carp fry fed 0.7mg copper per kilogram diet with rainbow trout fry fed similar concentration. They observed that the common carp fry suffered from depressed growth. This was corrected by supplementing the diet with 3mg

copper per kilogram diet, indicating that common carp require more than 0.7mg copper per kilogram diet. In contrast, rainbow trout fed 0.7mg copper per kilogram diet did not show any effect on growth. The authors then suggested that rainbow trout require less copper than the common carp.

(ix) Sodium, Potassium and Chloride Ion

Dietary deficiency of sodium, potassium and chloride ion have not been shown to give rise to deficiency symptoms although these elements are needed for several prcesses including; osmoregulation, acid-base balance, hydrochloric acid secretion in gastric juices, maintenance of membrane potentials and transmission of nerve impulses (NRC, 1983). Murray and Andrews (1979) could not find any significant difference in feed conversion efficiency and body moisture content of channel catfish fed 0.06 per cent sodium and 0.17 per cent chloride, although fish fed diets with added salt grew 6 to 8 per cent faster than those fed the basal diet. Thus channel catfish could tolerate up to 2 per cent salt in the diet with no adverse effect on growth.

2.1.6 Vitamin requirement

Vitamins are essential organic compounds needed for the growth and maintenance of fish. They are required in small amount and a continuous deficiency lead to disordered metabolism, disease and eventually death. Vitamin requirement in fish as well as their biochemical and physiological functions are generally similar to those of terrestial animals (NRC, 1977). The primary difference is that reaction rates of the enzymes involved differ in poikilothermic animals reared at different water temperatures (Halver, 1976). For some warm water fishes, intestinal synthesis by micro-organisms supplies the requirements for certain vitamins (NRC, 1983). Thus deficiency signs result only in those cases when antibiotics are fed along with a deficient diet.

Vitamin requiremtns are affected by the size age, stage of sexual maturity, and growth rate of fishes and by environmental factors and nutrient interrelationships (NRC, 1983). Generally, vitamins could be divided into two broad groups. Water soluble and fat soluble vitamins. The chemical

structure of the active forms of these vitamins are shown in Appendix 1.

Vitamin requirements are determined in the laboratory by feeding purified diets deficient in a specific vitamin. Test diets are available which can induce specific water soluble or fat-soluble vitamin deficiency symptoms in salmonids, ictalurids, cyprinids, eels and several marine fishes (Halver, 1976).

(i) Water Soluble Vitamins

The water-soluble vitamins are the vitamin B complex and vitamin C. The eight recognised members of the vitamin B complex are Thiamine, riboflavin, pyridoxine, pantothenic acid, niacin, biotin, folic acid and vitamin B12. The water-soluble essential nutritional factors choline, inositol and ascorbic acid are required in appreciable quantities in the diet and sometimes are not referred to as vitamins but as major dietary nutrients (Halver, 1980). Ascorbic acid is often excluded from this group (NRC, 1983).

(a) Thiamine

Thiamine (Vitamin B_1) is a complex nitrogenous base containing a pyrimidine ring joined to a thiazole ring first isolated from rice polishings in 1926 and synthesized in 1936 (Halver, 1980). Because of the presence of a hydroxyl group at the end of the side chain thiamine can form esters e.g. thiamine monophosphates, thiamine diphosphate (formerly thiamine pyrophosphates) and triphosphate (Appendix 1a) (McDonald et al.,. 1979).

Thiamine plays a role in the control of carbohydrate metabolism. The coenzyme Thiamine pyrophosphate is required for the decarboxylation of pyruvic acid and of alpha-ketoglutaric acid in the metabolism of carbohydrate (Halver, 1985), and for transketolation in the pentose phosphate shunt.

Murai and Andrews (1978a) have suggested that channel catfish fingerlings require a minimal dietary thiamine concentration of 1mg per kilogram dry diet to achieve maximal weight gain, feed efficiency and prevention of thiamine deficiency symptoms such as anorexia, poor growth,

dark colouration and high mortality. The rainbow trout was found to require less than 2mg per kilogram feed (Halver, 1985). This level was within the range of that reported for Turbot (Cowey et al., 1975). These authors have demonstrated that maximum growth rate of Turbot occurred when dietary thiamine concentration was greater than 0.6mg per kilogram dry diet.

The herbivores omnivores e.g. carp and channel catfish utilize dietary carbohydrate as an active energy source whereas many carnivorous fishes such as salmonids, turbot, tuna and sea bream metabolise carbohydrates less effectively (Hilton and Atkinson, 1982). Since thiamine requirement is related to carbohydrate intake in most of the animals studied, it was thought that the vitamin may be particularly important in certain carbohydrate consuming species of fish such as the grass fishes, the mullets and many others that eat phytoplankton or other plants (Halver, 1976). This information was found to be untrue due to confounding factors which make comparison between species and experiments difficult (NRC, 1983). Murai and Andrews (1978a)

stated those factors as methods of thiamine assessment, intestinal bacteria contributions and evolutionary responses to the presence of the enzyme thiaminase which is present in some raw and shell fish that destroys thiamine but could be inactivated by heating. Thiaminase acts by severing the pyrimidine and thiazole moieties of thiamine thereby rendering the vitamin unavailable or the vitamin may be made inactive by excessive heat treatment during feed mixing, compaction or extrusion (FAO, 1980). On the bases of this potential losses of thiamine in fish feed, Halver (1985) has recommended a thiamine content of 10 to 15mg per kilogram in most fish feeds. He suggested that lower levels may be used with impunity in channel catfish or carp diet preparations but carnivorous coldwater species need high levels for nutrient safety in fish production.

(b) Riboflavin

Riboflavin is a yellow-brown crystalline pigment which occur in the free form only in the eye, whey and urine, and was isolated in 1879 (Appendix 1b) (Halver, 1980).

Riboflavin is found in the tissue coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These coenzymes function as prosthetic groups of oxidation-reduction enzymes that are needed for the degradation of pyruvate, fatty acids and amino acids (NRC, 1983; Halver, 1985). Riboflavin along with pyridoxine is involved in the conversion of tryptophan to nicotinic acid and is important in the respiration of poorly vascularized tissues such as the cornea of the eye. It is also involved in the retinal pigment during light adaptation. Deficiency causes impaired vision and photophobia in experimental animals including fish (Halver, 1980).

Dietary essentiality of riboflavin has been reported for many species. Poston et al. (1977) fed riboflavin - deficient diets to rainbow trout fingerlings (initial mean weight 5.9g) and reported the development of bilateral corneal and lenticular lesions, fin necrosis, snout erosion and spinal deformation after 11 and 15 weeks of the experimental period. Takeuchi et al. (1980) also observed anorexia, poor growth, high mortality rate, leisions of fins and cataract in rainbow trout (initial mean weight 1.5g) fed riboflavin -

deficient diets for 8 weeks. Japanese eels fed riboflavin-deficient diets displayed poor growth, anorexia, haemorrhage of the fins and abdomen, including photophobia and lethargy (Arai et al., 1972). Similarly carp fingerlings fed riboflavin-deficient diet showed anorexia, poor growth, high mortality rate and hemorrhage of skin and fins (Takeuchi et al., 1980). In channed catfish, dwarfism (Murai and Andrews, 1978b) and Cataracts (Dupree, 1966) were also observed along with poor growth and anorexia in fish fed riboflavin - deficient diets for 8 weeks.

Dietary requirment of riboflavin may vary depending on the size of fish, the balance of other dietary ingredients, caloric density and the environmental conditions under which the fish is raised (Halver, 1980). For example rainbow trout (initial mean weight, 7g) required 12.2mg riboflavin per kilogram for maximum growth (Woodward, 1982) whereas larger rainbow trout fingerlings (initial mean weight 11.2g) required 3mg riboflavin per kilogram dry

diet as judged by the growth rate, food conversion efficiency and mean erythrocyte glutathione reductase activity coefficient or 12mg riboflavin per kilogram dry diet for maximal liver riboflavin content (Hughes et al., 1981). Stimulation of erythrocyte glutathione reductase activity has been found to be a sensitive biochemical measurement of riboflavin in fish (Hughes et al., 1981). Aoe et al. (1967a) found that common carp fingerlings (initial mean weight 1.5g) required 20mg riboflavin per kilogram dry diet over a 6 week experimental period whereas slightly larger carp fingerlings (initial mean weight 2.8g) required 10mg riboflavin per kilogram dry diet over 6 weeks. Channel catfish fingerlings was shown to require 9mg riboflavin per kilogram dry diet for maximal growth and 3mg riboflavin per kilogram dry diet to prevent the occurrence of dwarfism (Murai and Andrews, 1978b). In general trout requirements for riboflavin appear to be between 5 and 10mg Per kilogram dry diet whereas salmonids requirement is higher since maximum liver storage levels are not achieved until at least 10mg riboflavin per kilogram dry diet are fed (Halver, 1985).

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(c) Pyridoxine

Pyridoxine (Vitamin B_6) was first reported by Gyourgi in 1935 and synthesized in 1939 (Appendix 1f) (Halver, 1980). The three compounds pyridoxine, pyridoxal and pyridoxamine all have vitamin B_6 activity. Pyridoxine hydrochloride is the form in which vitamin B_6 is usually supplemented in feeds owing to its solubility in water and heat stability in acid or alkaline solution (Halver, 1980; Adams, 1978). Frye (1978) reported that pyridoxine hydrochloride is very stable in vitamin premixes although it may lose its stability if the premix also contains trace minerals. Slinger et al. (1979) found only 7 to 10 per cent losses in pyridoxine in pelleted or extruded feeds after 10 months of storage.

Pyridoxine, or the coenzymes pyridoxal phosphate and pyridoxamine phosphate are required in amino acid metabolism. Pyridoxine phosphate is a coenzyme in the transamination reactions in which an amino acid is converted to an α - keto acid and catabolized or an α - keto acid is converted to an amino acid. Pyridoxine phosphate is also

a coenzyme during the conversion of tryptophan to acetyl-CoA and pyruvate to cystine (NRC, 1983).

Dietary essentiality of pyridoxine has been reported for many species. Dupree (1966) observed nervous disorders, erratic swimming, opercle extension and tetany in channel catfish fingerlings fed pyridoxine deficient diets. Smith et al. (1974) studied the effect of pyridoxine deficient diet on rainbow trout fingerlings and observed the occurrence of hyperirritability, nervous disorders, erratic and rapid swimming, flexing of operculum, greenish-blue colouration and tetany just before death. Chinook salmon fed pyridoxine deficient rations were found to develop ataxia, oedema of peritoneal cavity, colourless serous fluid, blue-green colouration on dorsal surface and excessive flexing of operculum. Thus tetany, nervous disorder and greenish-blue discolouration appear to be common among the fish so far studied.

The dietary requirement of pyridoxine has also been studied. Yone (1975) reported a minimum requirement of 5 to 6mg pyridoxine per kilogram dry diet for maximal glutamic Oxaloacetic transaminase

activity and maximal glutamic pyruvate transaminase activity in the red sea bream. For maximum weight gain and pyridoxine liver content a minimum requirement of 2 to 5mg pyridoxine per kilogram dry diet has been recommended (Yone, 1975). Similarly, Adron et al. (1978) demonstrated that pyridoxine concentrations of 1.0mg per kilogram dry diet up to 30mg per kilogram had similar growth rates, whereas individuals fed 0.26 or 0.50mg pyridoxine per kilogram dry diet had reduced weight gain. These workers also observed that liver alanine amino transferase activity and muscle and liver aspartate aminotransferase activity increased with higher dietary pyridoxine concentrations up to 2.5mg per kilogram dry diet. They recommended a dietary pyridoxine concentration of 2.5mg per kilogram dry diet for maximal growth and liver aspartate aminotransferase activity of turbot. Andrews and Murai (1979) showed that channel catfish fingerlings require a minimum of 4.2mg pyridoxine per kilogram diet for maximum growth. The common carp was reported to require 5.4mg pyridoxine per kilogram dry diet for maximal growth rate and prevention of deficiency symptoms (Ogino, 1965). Removal of pyridoxine from the

diet of carp led to development of pyridoxine deficiency signs in 4 to 6 weeks. Rainbow trout and salmon finglings require between 5 to 10mg pyridoxine per kilogram diet. Marine fish appear to have a higher requirement when high protein diets are fed (Halver, 1982; Jurss, 1978). Since pyridoxine requirement depends on protein level, the onset of pyridoxine deficiency could be accelerated in carnivorous fish by feeding diets high in protein (Hardy et al., 1979).

(d) Vitamin B_{12} (Cyanocobalamin)

This is the antipernicious anemia factor which was found essential for the growth of chicken fed entirely on plant diet and was designated animal protein factor (APF) (Halver, 1980). Cyanocobalamin which is a large molecule that contains cobalt, is stable to mild heat in neutral solution but is rapidly destroyed by heating in dilute acid or alkali (Appendix 1h). The vitamin is necessary for normal growth, normal blood formation and healthy nervous system (Halver, 1982). Vitamin B_{12} together with folic acid is needed to provide single carbon units such as methyl group for the formation of DNA in hemopoietic

tissue. Vitamin B_{12} catalysis the formation of folic acid, folic acid coenzymes and deficiency of these vitamins accentuates the deficiency of the other; thus folic acid and cyanocobalamin have complementary roles in fish metabolism (Halver, 1982). John and Mahajam (1979) demonstrated that folic acid and vitamin B_{12} accelerated the development of a more pronounced anaemia in fish.

Chinook salmon fed vitamin B_{12} deficient diets for 16 weeks were reported to show growth retardation and reduced erythrocyte numbers (Halver, 1957) whereas channel catfish exhibited only reduced growth rate when fed vitamin B_{12} deficient diet for 36 weeks (Dupree, 1966). However, lower hematocrits was observed in channel catfish after 24 weeks (Limsuwan and Lovell, 1981). Kashiwada and Teshima (1966) did not report deficiency signs in common carp fed diets deficient in vitamin B_{12}. It was later demonstrated (Kashiwada et al., 1970) that the intestinal microbial synthesis of vitamin B_{12} in common carp was sufficient to meet the requirements of common carp for vitamin B_{12}. Similar observation has been demonstrated for tilapia (Lovall and Limsuwan, 1982).

The quantitative requirement of vitamin B_{12} in fish in the absence of folic acid has been tentatively set at 0.002 - 0.003 mg vitamin B_{12} per kg diet (Halver, 1972).

(e) Folic Acid (Folacin or Pteroylglutamic acid)

Folic acid (Appendix 1e) was synthesized in 1946 after it was found effective in curing metaloblastic anaemia in monkeys (Halver, 1980). Folic acid is stable to heat but deteriorates when exposed to sunlight or during prolonged storage especially at elevated temperatures (Frye, 1978).

Folic acid is essential for the synthesis of nucleic acids, DNA and RNA and thus is necessary for normal erythrocyte formation (NRC, 1983). As the biologically active coenzyme, formyltetrahydrofolic acid, it is requred for the transfer of single-carbon units (e.g. methyl groups) within rapidly metabolizing and dividing cells. Examples of this function are the formation of the amino acid methionine from homocysteine, serine from glycine, and the synthesis of choline from ethanolamine (Halver, 1982). Halver (1957) reported the occurrence of megaloblastic erythropoiesis, anorexia, anemia,

lethargy dark colouration and poor growth in chinook salmon fed folic acid deficient diets.

Smith and Halver (1969) also observed anaemia in Coho salmon fed folic acid - deficient diet. The anaemia was characterised by pale gills, anisocytosis and poikilocytosis of erythrocytes. The authors suggested that formation of blood cells in fish is very sensitive to folic acid deficiency because of the importance of folic acid in incorporation of nucleosides into deoxyribosenucleic acid. Arai et al. (1972) reported the occurrence of anorexia in Japanese eels fed folic acid deficient diet after 9 weeks; poor growth and dark colouration at 10 weeks whereas common carp fed a deficient diet for 16 weeks showed no deficiency signs with respect to growth, mortality, erythrocyte numbers or folic acid level of the hepatopancreas (Aoe et al., 1967c).

Quantitative folic acid requirement for young trout and salmon is 1 to 5mg per kilogram diet (McLaren et al., 1947).

(f) Pantothenic Acid

Pantothenic acid was synthesised in 1940 and may be considered as dihydroxydimethylbutyric acid bonded to /3-alanine (Halver, 1980). It is a white crystalline powder stable to autoclaving but labile to heat, hot alkali and hot acid (Appendix 1d).

Pantothenic acid functions as part of coenzyme A (CoA) in metabolic release of energy from all three energy providing nutrients - carbohydrates, fat and protein - by way of the tricarboxylic acid (TCA) cycle (Halver,1982). Thus pantothenic acid as a component of CoA is required for the oxidation and synthesis of fatty acids, oxidation of pyruvate and is involved as an acceptor and donor of acetate groups (acetylation reactions) such as the conversion of choline to acetylcholine and the conversion of oxaloacetic acid to citric acid (NRC, 1983). Dietary deficiency of pantothenic acid generally result in poor growth, anorexia, clubbed and exudate covered gills (Halver, 1982). Studies on channel catfish (Murai and Andrews, 1975) showed signs of anorexia in pantothenic acid deficient diet after 4 weeks of culture. After 8 weeks channel catfish developed clubbed gills and eroded skin,

lower jaws, fins and barbels. Arai et al. (1972) observed poor growth, skin lesions and abnormal swimming in the Japanese eel after 6 weeks on diet deficient in pantothenic acid. Poston and Page (1982) reported that pantothenic acid deficient trout shows signs of conglutinated mitochondria (clubbed mitochondria). These lesions are caused by anorexia within cellular energy transfer mechanism and may appear as vacuoles or hyaline bodies and eventually lead to necrosis (Hartroft, 1964; Rouiller, 1964). In general signs of pantothenic acid deficiency are manifested as a condition called dietary gill disease including clubbed, exudate covered gill lamellae, swollen operculum, fused gill filaments, abnormal swimming near the surface of the water, anorexia, poor feed conversion, loss of weight and high mortality within 8 - 10 weeks (Halver, 1982). Essentially dietary deficiency of pantothenic acid impairs the normal metabolism with mitochondrial-rich cells undergoing rapid mitosis and high energy expenditure. Thus organs involved in osmoregulation such as the gills and kidney tubules are most affected (Halver, 1982).

Pantothenic acid is usually added to feeds as either calcium d - pantothenate (92 per cent

activity) or calcium dl-pantothenate (46 per cent activity) because pure pantothenic acid is unstable, hygroscopic and viscous (NRC, 1983). Trout and Salmon require 10 - 20mg calcium pantothenate per kg of diet depending on the size of fish and diet composition (McLaren et al., 1947; Halver, 1972). Channel catfish fry require 250 mg/kg dry diet while the fingerlings require 10 mg/kg dry diet (Murai and Andrews, 1975; 1979). The higher pantothenic acid requirement of frys was associated with the possible micronutrient losses in small feed crumbles fed to fry compared with larger feed particles fed to fingerlings due to high surface to volume ratio in the small feed crumbles (Murai and Andrews, 1975).

(g) Niacin

Nicotinic acid synthesis took place in 1913 and in 1937 Niacin was postulated to be part of factor H for fish but the deficiency symptoms were not adequately described until 1947 (Halver, 1980).

Niacin in the form of niacinamide (Appendix 1c) is an essential component of two enzymes, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Both NAD and NADP

are involved as hydogen donors and acceptors in the release of energy from all three energy yielding nutrients - carbohydrate, fat and protein. The vitamin is required by all living cells and it is equally active either as nicotinic acid or as the amide (Halver, 1982). In many animals the amino acid tryptophan can be converted to nicotinic acid, so deficiency signs can only be induced by restricting dietary tryptophan (NRC, 1983). For brook trout and salmon (Poston and Combs, 1980) tryptophan is not efficiently converted to niacin therefore salmonids must obtain preformed niacin to meet their requirements. Signs of nutritional deficiency of niacin in trout and salmon are pellagralike, and include anorexia, reduced growth, poor feed conversion, a photosensitivity to sunburn, intestinal lesions, muscular weakness, spasms and increased mortality (McLaren et al., 1947). In catfish fed niacin-deficient - diets, deficiency symptoms observed after 6 to 8 weeks were skin and fin lesions, deformed jaws, anemia, exophthalmia and high mortality rates (Andrews and Murai, 1978). Arai et al., (1972) noticed reduced growth after 10 weeks in Japanese eel fed niacin - deficient - diets.

After 14 weeks the deficiency symptoms were ataxia, skin lesions, and dark colouration Aoe et al. (1967b) observed high mortality after 5 weeks in common carp fed niacin - deficient - diets.

Dietary requirement of niacin for maximum growth of common carp is 28mg niacin/kg dry diets (Aoe et al., 1967c) and 14.4mg niacin for channel catfish fingerlings (Andrews and Murai, 1978). Trout require at least 95mg of the vitamin per kg diet (Phillips and Brockway, 1957) while salmon may require up to twice that level (Halver, 1972). Halver (1972) reported that the presence of phenothiazine or antimetabolites such as pyridine - 3 - sulphuric acid and thioacetamide may increase the requirement for dietary niacin. Furthermore Poston (1969b) reported that niacin in excess of requirement inhibits growth.

(h) Biotin

Biotin was synthesized by workers of Merck and Company in 1943 and was variously called "CoenzymeR" and 'Vitamin H' (Appendix 1g) (Halver, 1980).

The enzyme is stable to heat and light but is destroyed by acids and alkali and by oxidising agents such as peroxides or permenganet. Biotin has been shown to be important in many reactions involving acetyl CoA Carboxylase in fish. Such reactions include the synthesis of fatty acids, oxidation of carbohydrates (Pyruvate carboxylase), synthesis of niacin and microsomal protein and formation of pancreatic amylase (Halver, 1982). Biotin deficiency sign in salmonids include anorexia, poor growth, and depressed liver acetyl CoA Carboxylase and Pyruvate carboxylase (Poston and Page, 1982). In the brook trout, biotin deficiency symptoms include pale coloured gills often with a mucuous coating (Poston and McCartney, 1974). In channel catfish depigmentation of the skin was observed after 14 weeks in fish fed Biotin-deficient diets. After 22 weeks there was significant reduction in the level of liver pyruvate carboxylase (Robinson and Lovell, 1978). Ogino et al. (1970) working on carp observed reduced growth rate between 8 to 12 weeks in

carp fed diets deficient in biotin. In Japanese eel fed a biotin deficient diet Arai et al. (1972) reported the occurrence of reduced growth, abnormal swimming behaviour and dark colouration after 8 weeks.

Quantitative biotin requirement have been studied for different species. Based on growth rates maximum liver storage of biotin and optimum feed conversion, young trout requires a minimum of 0.05 -0.25mg biotin per kg of diet (McLaren et al., 1947; Poston, 1976b; Castledine et al., 1978). Lake trout fingerlings required a minimum of 0.1mg biotin/ kg dry diet for optimal growth, and minimum of 0.5mg biotin/kg dry diet for optimum swimming stamina (Poston, 1976a). In channel catfish fingerlings, 8mg biotin per kg dry diet was found to enhance liver pyruvate decarboxylase activity (Robinson and Lovell, 1978). In brook trout fingerlings, 6mg biotin per kg dry diet was sufficient to increase acetyl CoA carboxylase and pyruvate decarboxylase activities (Poston and McCartney, 1974).

However, most diets containing fish meal, or a combination of fish and plant proteins will probably contain sufficient biotin for normal growth of young fish (Halver, 1982). The type and level of dietary fat can affect signs of biotin deficiency in salmonids (Halver, 1982). Feeding a partially hydrogenated soyabean oil obscured biotin deficiency signs in trout (Kitamura et al., 1967a; Poston and McCartney, 1974).

(i) Choline

Choline (Appendix 1k) has three functions: as a precursor to the neurotransmitter acetylcholine, as a methyl (CH_3) donor in metabolic reactions, and as a component in choline phosphoglycerides or phospholipids lecithin and sphingomyelin (NRC, 1983). Although the quantity of choline required in the diet is influenced by the level of methionine, folic acid and cyanocobalamin, choline is essential for growth, efficient conversion of food and prevention of fatty livers in trout (McLaren et al., 1947) and salmon (Halver, 1957).

The dietary requirement of Lake trout for choline was studied by Ketola (1976). Lake trout were fed an unsupplemented diet (30mg choline

per kilogram dry diet) their response were compared with those fed diets containing, equimolar supplements of aminoethanol, dimethylaminoethanol, methylaminoethanol, bethaine HCl and choline (equivalent to 2,600mg choline per kilogram dry diet). It was observed that lake trout fed unsupplemented diet and aminoethanol and bethaine supplements had reduced growth rate and high liver fat content. It was concluded that since bethaine a source of labile methyl groups did not affect growth or liver fat content, any metabolic function of choline in regulation of liver fat in lake trout is unrelated to transmethylation. The minimum level of choline required by trout fingerlings for optimal growth is no more than 1000mg per kilogram of purified diet (Ketola, 1976).

Channel catfish fed a choline - deficient diet developed hermorrhagic kidneys, intestine and enlarged livers (Dupree, 1966). Ogino et al. (1970) observed 10% higher lipid content after 30 days in common carp fed on a choline - deficient diet. An estimated minimum dietary choline - chloride concentration of 2000mg per kilogram was suggested.

(j) Inositol

Inositol sometimes called myoinositol (Appendix 1j) occurs as phospholipid lipositol in animal cells and it is present in nucleated erythrocytes (NRC, 1983). Inositol deficiency symptoms include poor feed digestibility and utilization, anorexia, reduced growth and distended abdomen (Halver, 1972). Aoe and Masuda (1967) reported that common carp require 4g inositol per kilogram dry diet for maximal weight gain, feed conversion and prevention of skin lesions. The requirement of inositol for the red sea bream was found to be between 550 and 900mg inositol per kilogram dry diet in direct proportion to dietary glucose concentrations of 10 to 40% (Yone, 1975). The tentative quantitative requirement for inositol is 250 to 300mg per kilogram of diet for young salmon and trout (McLaren et al., 1947).

(k) Ascorbic Acid

Although ascorbic acid has been known and feared since ancient times it was not until 1928 that the antisorbutic factor was identified (Appendix 1i) (Halver, 1980).

Most vertebrate animals are able to synthesize L-ascorbic acid (AA), but several species e.g. primates, guinea pigs, fish, bats, insects and some birds lack the ability to produce L-gulonolactone oxidase and are thus dependent upon a dietary source of vitamin C. Fish is unique among these animals in that they have a system of storing a chemically stable form of vitamin C which appears to allow metabolism of this compound differently from other vitamin C - requiring organisms (Tucker and Halver, 1984). The vitamin C include L-ascorbic acid, dehydro-ascorbic acid, and ascorbate - 2 - sulfate, and can be called vitamin C 1, dehydro C and vitamin C 2 for trivial nomenclature (Halver, 1985).

L-ascorbic acid is readily oxidized to the less biologically potent dehydoascorbic acid (Halver, 1980). Vitamin C is heat sensitive and is readily lost during processing and storage due to exposure to oxygen, heat, neutral or high pH and/or traces of metals (Halver, 1980). Hilton et al. (1977) found a 70 per cent loss of supplemental ascorbic acid during feed preparation and virtually complete destruction after seven weeks of storage.

Vitamin C is necessary for numerous hydroxylation reactions. The vitamin is required in the formation of hydroxyproline a constituent of collagen, which is the protein of capillaries, the fibrous tissues of scars, the matrix of bones and cartilage and the fibres of tendons. An ascorbic acid deficiency results in impaired collagen metabolism (NRC, 1983). Ascorbic acid plays a synergistic role with vitamin E as intracellular antioxidants and free radical traps and is also involved in the conversion of folic acid to folinic acid (Halver, 1980). Ascorbic acid also has a role in iron metabolism and detoxification of organic pollutants such as toxaphene and polychlorinated biphenyls during accumulation in the liver (Milliken, 1982). Thus the effects of organochlorine pesticides strychnine poisoning, PCEs and DDT, heavy metals and toxaphene contamination of feed all have a diminished effect when ascorbate nutrition is increased (Tucker and Halver, 1984).

The signs and symptoms of the deficiency of vitamin C in fish are reduced growth, anorexia

and lethargy, followed by structural deformation of skeleton and cartilage, haemorrhage and anemia acute lordosis and scoliosis and eventual dislocation of vertebrae (Halver et al., 1969). Wild fish consume insects, algae, plankton and other fish - all supplying dietary vitamin C (Tucker and Halver, 1984).

Poston (1967) reported that brook trout fingerlings fed ascorbic acid-deficient diets over a 34-week period developed scoliosis and/or lordosis, increased mortality rate and internal haemorrhaging. Similarly channel catfish experienced lordosis, scoliosis (and ultimately broken back) haemorrhage within the vertebral column and brittle vertebrae including reduced serum alkaline phosphatase activity (65% lower), lower vertebral collagen content (42% less on a dry basis) and less hydroxyproline in the collagen of scorbutic channel catfish (Wilson and Poe, 1973). Channel catfish fingerlings (initial weight 4 to 5g) fed ascorbic acid deficient diets had significant reductions in length and weight after 150 days.

There were also 10% reductions in backbone collagen and 16% in hydroxyproline concentration in collagen. In addition, 24% less force was required to cause permanent damage of vertebral centra (elastic limit) and failure of vertebral centra (rupture) occurred at 16% less force in channel catfish fed on supplemental ascorbic acid than in individuals fed 500mg ascorbic acid/kg dry diet (Hamilton et al., 1981). Arai et al. (1972) reported that Japanese eel fed ascorbic acid - deficient diet showed reduced growth after 10 weeks and haemorrhage in the head and fins after 14 weeks.

The dietary requirement for ascorbic acid by trout and salmon apparently depends upon several factors including fish size, growth rates, other dietary components and stress conditions under which fish may be reared (Halver, 1982). Halver et al. (1969) reported that rainbow trout (initial mean weight 0.3g) require 100mg ascorbic acid/kg dry diet and Coho salmon (initial mean weight 0.4g) require about 50mg ascorbic acid/kg dry diet based on

blood and anterior kidney ascorbic acid concentrations and growth rate. The minimum level of ascorbic acid in the blood of Coho salmon and rainbow trout necessary for normal growth and survival rate is 35mg ascorbic acid/g blood. Liver ascorbic acid levels below 30mg/g indicate an ascorbic acid deficiency in channel catfish (Lim and Lovell, 1978). Young and rapidly growing fish have been observed to require the highest amounts of L-ascorbic acid. Hilton et al. (1978) reported a lower requirement of ascorbic acid (40mg/kg dry diet) for larger rainbow trout (initial mean weight 6.7g) based upon growth rate, feed conversion, survival rate and serum iron levels. The authors also suggested that a liver ascorbate concentration of below 20mg per g indicated a dietary deficiency.

However, vitamin C requirements for fish cannot be easily defined. For example, a requirement for rapid growth of 100mg per kilogram dry diet under pristine environmental conditions may be increased to 5 to 10 fold

when the fish are exposed to environmental contaminants, physical or environmental stress or exposure to fish pathogens (Halver, 1985). In a classical experiment, (Halver, 1985) reported that mortality rates of channel catfish experimentally infected with a pathogen can be decreased when increased levels of dietary L-ascorbic acid are fed. Mortality rates were high in fish fed on ascorbic acid deficient diet but no mortality was recorded in all fish fed 10 to 20 times the apparent requirement for L-ascorbic acid. This was ascribed to the response of the antibody at high levels of L-ascorbic acid in the diet. Based on this observation, Halver (1985) has recommended that requirements of L-ascorbic acid should be increased to a minimum of 300mg per kilogram dry diet, and further increased to 1000mg per kilogram dry diet, when fish are exposed to environmental stressors. Since losses of ascorbic acid occur during processing and storage, Tucker and Halver (1984) have suggested the use of ascorbate - 2 - sulfate as the form of vitamin C supplementation in aquatic rations

instead of L-ascorbic acid because of its ready absorption from feeds as well as its stability.

(ii) Fat - Soluble Vitamins

(a) Vitamin A

Vitamin A was synthesized in the mid-thirties although the chemical structure of vitamin A (Appendix 11) and its relationship to Beta - carotene was shown by Von Euler in 1928 as cited by Halver (1980). Since then considerable work has been done on vitamin A and in recent time it has been shown that vitamin A occurs in two forms, vitamin A_1 (retinol) found in mammals and marine fishes, and vitamin A_2 (retinol$_2$), found in freshwater fishes (Lehninger, 1975). Vitamin A is needed in vertebrates for the regeneration of the light - sensitive compound rhodopsin in the retina. The vitamin is also involved in calcium transport across some membrane, in reproduction and embryonic development and in cellular and subcellular membrane integrity (NRC, 1983). Vitamin A is essential for normal structure and functions of eye and gill, through its involvement in the metabolism

of mucopolysaccharides and visual pigments, and for general maintenance of epithelial tissues of various physiological systems (Halver, 1982).

Vitamin A has been shown to be essential in the diet of fish species. Dupree (1966) reported that channel catfish fed 0.4 ppm β - carotene developed deficiency symptoms after two years such as exophthalmia, edema, and kidney haemorrhage. Poston et al. (1977) showed that pronounced eyeball protrusions and dermal depigmentation occurred in brook trout fed vitamin A deficient diets. Histopathological examination of the eyes of salmonids did not show any damaged lens in vitamin A deficient fish. Aoe et al. (1968) reported the occurrence of anorexia, faded body colour, fin and skin haemorrhages, exophthalmia and abnormal gill opercula between 8 to 16 weeks in common carp fed diets deficient in vitamin A.

The ability of the fish to convert carotenoids to vitamin A is dependent on water temperature. Cold water fish such as salmonids can utilize β - carotene at 12.4°C to 14°C but only small amounts of the β- carotene are hydrolysed at 9°C (Poston et al., 1977).

The dietary requirement of vitamin A for young trout is 2,500 - 5,000 international unit per kilogram of diet (Kitamura et al., 1967b). One international unit of vitamin A is defined as 0.30µg retinol (Halver, 1982).

Poston et al. (1966) showed that feeding high levels 2.2 million internations units of vitamin A (retinal palmitate) per kilogram of diet to trout at 8.3°C reduced growth and hematocrit, or packed cell volume (PCV) and caused necrosis of the caudal fin. Feeding up to 2.5 million international units of retinal palmitate at 12.4°C also reduced body fat and liver size of trout (Poston, 1971a). Increase in the protein content (Poston and Livingston, 1971) or methionine (Eckhert and Kemmeter, 1974) in diet of young trout reversed or ameliorated some of the toxic effects of hypervitaminosis A in fish fed low level of protein. Halver (1985) has recommended the level of vitamin A to be kept between 2000 and 4000 international units per kilogram diet.

(b) Vitamin D

Two main compounds having vitamin D activity are vitamin D_2 or ergocalciferol and vitamin D_3 or cholecalciferol (Appendix 1m) (NRC, 1983).

Vitamin D_3 is the precursor of the hormone, 1,25 - dihydroxy D_3 which is the biologically active form of the vitamin. It facilitates absorption of calcium ions from the gastrointestinal tract (Halver, 1985). Vitamin D_3 is at least three times as effective as vitamin D_2 in satisfying the requirements for vitamin D in trout (Barnett et al., 1979). One international unit of vitamin D is defined as the biological activity of 0.025 µg of cholecalciferol.

Qualitative cholecalciferol requirements for vitamin D have been studied for a few species. Lovell and Li (1978) fed channel catfish for 16 weeks in vitamin D-deficient diet and observed reduced weight gain, lower body ash (bone mineralization) lower body phosphorus and lower body calcium compared with controls. Barnett et al. (1979) compared the performance of rainbow trout fed two dietary concentrations 0% vitamin D_3 and

1,000 international unit of vitamin D_3 per kilogram diet. The authors observed decreased weight gain and feed efficiency in the rainbow trout fed 0% vitamin D_3. George et al. (1979) has reported slow growth, impairment of calcium homeostasis and the occurrence of tetany of the white skeletal muscle in rainbow trout fed vitamin D_3deficient diets. Poston (1969a) reported the occurrence of hypercalcemia and increased PCV but no difference in the rate of growth in fingerling trout fed 3.75 million international unit of vitamin D_3.

Vitamin D requirement has been estimated at 200 international units of vitamin D_3 per kilogram of diet (Halver, 1985). Five hundred to one thousand times this intake will result in hypercalcemia and some liver necrosis, therefore it is prudent to add about 2,000 international units of vitamin D per kilogram dry diet for fish feed preparation, but it is necessary to restrict excessive intake of fish liver oils, which contain large amounts of vitamin D, in order to prevent subclinical or clinical hypervitaminosis D.

(c) Vitamin E

Vitamin E is composed of class of compounds known as tocopherols the active form which was isolated, characterised and synthesised by Karrer as cited by Halver (1980) (Appendix 1n).

The most biologically active form of vitamin E is D - α tocopherol. Vitamin E acts as a lipid - soluble antioxidant and is involved in the protection of biological membrances containing phospholipids such as the erythrocyte, plasma membrane and microsomes (Halver, 1985). Susceptibility of these membrances to oxidative degradation and ultimately to disruption of cell function, varies directly with the level of polyunsaturated fatty acids in the phospholipids (Halver, 1982). Therefore the dietary requirement for the vitamin E depends upon the type and amount of polyunsaturated fatty acids in the diet and in the fish tissues as well as the level of other antioxidants and selenium which as a structural component of glutathione perioxidase also protects against peroxidative degradation (Halver, 1985).

Vitamin E deficiency symptoms was reported by Murai and Andrews (1974). After feeding channel

catfish for 16 weeks on α - tocopherol deficient diet containing oxidized menhaden oil, these authors reported the occurrence of reduced growth, muscular dystrophy, fatty livers, anemia, exudative diathesis and depigmentation.

Dietary supplementation of 25mg/kg α - tocopherol plus 125mg/kg ethoxyguin or 100mg/kg α - tocopherol alone prevented these symptoms even in the presence of menhaden oil. Poston et al. (1976) have stated the deficiency signs of vitamin E in trout and salmon to include impaired erythropoiesis, indicated by many immature irregularly shaped erythrocytes (poikilocytosis) of various sizes (anisocytosis), fragility and fragmentation of erythrocytes, extreme anemia, marked susceptibility to handling stress, high mortality, yellow to brown serous fluid in the body cavity (ascites) and increased content of body water (exudative diathesis).

Vitamin E has been shown to be important in the reproductive physiology of fishes. Halver (1985) reported that vitamin E measured as D - tocopherol were mobilised into developing ocytes in maturing rainbow trout females. He observed

the presence of tocopherol in the developing ova at least 3 months prior to spawning. No deficiencies in egg maturation, hatchability, or in yolk - sac absorption and fry development were observed and tocopherol levels were adequate until feeding of fry was initiated. The author therefore concluded that vitamin E supplementation must be focused upon early maturation of the female fish and is less critical during final maturation and spawning activity. An increase in the dietary level of linolenic acid in the diet of common carp resulted in an increase in the requirement for dietary α - tocopherol in order to prevent muscle dystrophy after 18 weeks of feeding (Watanabe et al., 1977). The Sekoke disease in common carp which is characterised by a mass loss of flesh on the back of the fish was found to be caused by feeding oxidized silk - worm pupae to common carp and was prevented by supplementing their diet with 500mg/kg of DC - α tocopherol acetate (Hashimoto et al., 1966).

Quantitative vitamin E requirements of fishes depend upon interaction of several factors, such as: dietary concentration of polyunsaturated

fatty acids, dietary selenium concentration, dietary concentrations of peroxidants and antioxidants, diet storage temperature and length of diet storage (Millikin, 1982). Watanabe et al. (1981) reported that rainbow trout fingerlings (initial mean weight 0.9g) fed a diet containing 15% pollock liver oil displayed general vitamin E signs such as aneroxia and reduced growth after 6 weeks. When diets were supplemented with 50mg α - tocopherol per kilogram dry diet the symptoms of vitamin E deficiency were prevented and growth rates were comparable with those fish fed 100, 300 or 500mg α - tocopherol per kilogram dry diet. Woodall et al. (1964) demonstrated that a dietary α - tocopherol level of 15 to 30mg plus 5% herring oil gave satisfactory growth of chinook salmon and prevented the occurrence of deficiency symptoms in this species. Hung et al. (1979) have reported the requirement of vitamin E acetate at 30 i.u. per kilogram diet in the presence of selenium for the salmonid. One international unit (i.u.) of vitamin E is defined as the biological activity of 1mg of

DL - α - tocopheryl acetate (Halver, 1982). Hung et al. (1981) reported that vitamin E deficiency was prevented in rainbow trout by feeding diets containing 24mg of α - tocopherol per kilogram dry diet combined with about 12% dietary lipid, which included 7.5% dietary unoxidized herring oil.

However, some natural tocopherols occur in fish fats, and larger amounts are present i vegetable oils such as corn oil or soyabean oil (Halver, 1985).

(d) Vitamin K

Vitamin K (Appendix 1o) was isolated by Dom from alfalfa and from fish meal in 1939 and was synthesised later that year (Halver, 1980). Vitamin K compounds are 2 methyl - 1, 4 - naptho-quinones, and are involved in electron transport and phosphorylation reactions of cellular metabolism (Brodie, 1961). Suttie (1980) showed that vitamin K is a required cofactor for a microsomal enzyme system that carboxylates glutamyl residues of precursors of blood clotting proteins to Y - carboxylglutamic acid residues in biologically - active proteins.

Therefore vitamin K deficiency results in the inability to synthesize proconvertin and prothrombin in the liver which are essential for forming blood clots (NRC, 1983).

Dietary supplementation of vitamin K was reported to be essential for channel catfish (Dupree, 1966). However, Murai and Andrews (1977) were unable to show a dietary requirement for vitamin K deficient diet for 30 weeks. The authors concluded that channel catfish have very low if any dietary requirement for vitamin K since individuals fed vitamin K deficient diets has similar weight gain, blood clotting times, prothrombin times and hematocrit values with individuals fed up to 1.2mg menadione sodium bisulfite per kilogram dry diet. Poston (1964) demonstrated the essentiality of vitamin K for the synthesis of plasma proteins (prothrombin and thromboplastin) which are essential in blood clotting. In a later experiment, Poston (1971b) reported that high levels of menadion sodium bisulfite (MSB), 2400mg per kilogram diet, did not adversely affect growth, survival and blood coagulation or PCV of fingerling trout blood.

The author recommended that ½ to 1mg of menaquinone equivalent of menadione sodium bisulfite (MSB) or menadione pyrimidinol (MPB) per kilogram of semipurified diet was sufficient to maintain normal coagulation and PCV of fingerling trout blood (Poston, 1976a).

In a recent experiment, Griffith (1984) showed that channel catfish injected with 100 to 300 parts per billion of crude aflatoxin developed haemorrhagic fins, pale livers and acute anemia. When these same fish were fed 50 to 100mg of menadione bisulfate per kilogram diet a rapid improvement in hemopoiesis was observed including a return to the normal hematocrit and red blood cell count within one week after the vitamin K supplementation. Fish fed the diet with 10mg vitamin K per kilogram diet and the aflatoxin contamination continued to die from anemia and blood loss through the haemorrhagic fins. This observation show that higher amounts of vitamin K may reduce the haemorrhages commonly encountered in aflatoxin intake. Since one symptom of acute aflatoxicosis

is the massive haemorrhagic effect encountered in trout, salmon and other fish susceptible to the fungal toxin, additional intake of vitamin K can ameliorate this haemorrhagic effect of aflatoxins in other fish besides the channel catfish (Halver, 1985).

2.2.0 Soyabean Production in Nigeria

Soyabean (Glycine max Linn : Merr.) belongs to the family Leguminosae and subfamily Papilionaceae (Graham, 1963). Soyabean is often referred to as a "golden bean", "miracle bean", "crop of the planet" or "God's sent golden bean" because of its excellent nutritive value (Singh, 1987). The crop originated in Northeast China and also in Southeast Asia where it has been cultivated for over 5000 years (Jackal and Singh, 1987). Soyabean was first cultivated in Africa by the French in Algeria in the 1800's before it came to Nigeria in 1908 (Ezedima, 1964; Jackal and Singh, 1987). Successful cultivation of the crop was not achieved until 1928 when the soyabean was tried in the northern parts of the country (Oyekan and Adedzwa, 1990). Commercial production

began in the 1940s with all the soyabean produced exported to Europe as other cash crops of the colonial era (Oyekan and Adedzwa, 1990). Research on the crop began in Nigeria in the 1950s at the Agricultural Research Station, Samaru, near Zaria (later the Institute for Agricultural Research, Ahmadu Bello University), and in the 1970s the International Institute of Tropical Agriculture (IITA) had began to collaborate with the Nigerian Scientists on Soyabean research (Lawani, 1989). As a result of the coordinated agronomic research, producive varieties of soyabean are now available in Nigeria which can yield 2,500 kg per hectare. Production has increased from 30,000 hectares in 1985 to 120,000 hectares in 1989 (Lawani, 1989). The main soyabean growing state are Benue, Niger and Kaduna. The commonest variety is the Malayan type (AERLS, 1985). The increased production of soyabean in Nigeria has rendered the crop easily available for human consumption and for incorporation into livestock feeds.

2.3.0 Nutritive Value of Soyabean

Soyabean is commonly known as an oil seed but judging from its composition it should actually pass for a protein seed. The average proximate composition of soyabean is 40% protein 21% oil, 34% carbohydrate and 5% ash (Kawamura, 1967).

2.3.1 Protein Quality of Soyabean meal

Soyabean meal is the highest quality (best balanced in essential amino acids) plant protein available (Lovell, 1980a).

The quality of diets made from soyabean protein depends on several factors: (a) amino acid composition; (b) presence of antinutritional factors; (c) digestibility; (d) overall composition of the diet and (e) nutrient requirements of organism involved (Ogundipe and Oguntunde, 1990).

Soyabean is unique among the plant - source proteins in that it is rich in lysine one of the essential amino acids often limiting in most feed ingredients. On the other hand, soyabean is limiting in methionine (Nose, 1978).

Since the protein of corn is deficient in lysine and tryptophan two amino acids which are abundant in soyabean meal protein, and soyabean meal is low in methionine and cystine which are abundant in corn meal, a proper combination of corn and soyabean meal result in performance superior to that occurring when either ingredient is fed singly (Baker et al., 1974).

Research on animal feeds have demonstrated that a deficiency in one amino acid is corrected by the addition of the synthetic form of the amino acid to the ration. However, when soyabean meal deficient in methionine was substituted isonitrogenously for menhaden meal, growth and feed effficiency of channel catfish were substantially reduced. Addition of free methionine, cystine or lysine, the most limited amino acids to these soyabean substituted diets did not enhance weight gain (Andrews and Page, 1974). It would appear that channed catfish cannot utilize synthetic amino acids for growth but this has been corrected by studies of Murai et al. (1982) who showed that channel catfish can utilize synthetic methionine added to soyabean meal. In a similar

studies with rainbow trout, Koops et al. (1976) were able to replace one quarter of fish meal with soyabean meal enriched with methionine. Dabrowska and Wojno (1977) also demonstrated that an addition of amino acids improved the utilization of feed mixtures containing soyabean meal and that rainbow trout can utilize soyabean protein enriched with cystine (1%) and tryptophan (0.5%) almost as well as protein from the fish meal. Fish fed with mixtures number 1 (control - containing 28% fish meal, 10% soyabean meal and no addition of cystine and tryptophan) and mixture number 6 (containing 17% fish meal, 26% soyabean and addition of cystine and tryptophan) shcwed the highest rate of growth. The lowest average weight gain was however, noted for fish fed with mixture number 2, characterised by a lower content of fish meal without additional amino acid. This result shows that feed containing high amount of soyabean meal with lower fish meal but enriched with synthetic cystine and tryptophan can be as well utilized as that containing higher fish meal but without cystine and tryptophan. However, Rumsey and Ketola (1975) obtained contrasting results.

When rainbow trout fingerlings were fed soyabean meal as the sole source of protein with the addition of methionine, lysine, histidine, and leucine individually and in several combinations growth did not improve, however, supplement of methionine, lysine, leucine, valine, histidine, threonine, tryptophan and tyrosine in a balance to simulate these levels for those of trout eggs significantly improved the growth. Murai et al. (1982) also reported improvement in weight gain and feed efficiency of carp when diet containing soyabean meal was supplemented with methionine alone. In a subsequent study Murai et al. (1986) demonstrated that carp fingerlings fed diets in which 75% of the fish meal was isonitrogenously replaced with methanol-treated or untreated soyabean flour achieved almost a 35% weight gain in 4 weeks if the test diets were supplemented with essetial amino acids to the level in a fish meal - torula yeast control diet. The addition of methionine to diets containing commercially processed soyabean flour was suggested as being the major factor responsible for the improved performance of fish

fed these diets. Thus most of the studies indicate elevation in nutritive value when soyabean meal is enriched with synthetic essential amino acids especially methionine.

On whether soyabean meal can successfully replace fish meal in the diet, experiments conducted with tilapia (Oreochromis niloticus X Oreochromis aureus) (Shian et al., 1987) showed that fish meal can be partially replaced by soyabean meal when the dietary level is below optimum level for growth (24%). At the optimum level of dietary protein (32%) replacement of 30% fish meal with soyabean significantly depressed growth and feed efficiency which were restored by addition of methionine to the level of the control diet. Similarly, Tacon et al. (1983) reported that supplementation of 0.8% D.L - methionine to a diet in which 75% of brown fish meal was replaced by soyabean meal improved the growth of Oreochromis niloticus . Viola and Arieli (1983) demonstrated that soyabean meal can be used to replace up to half of the fish meal in tilapia feeds having 25% crude protein content without requiring any supplementation.

With a 30% crude protein diet, Viola et al. (1982) found that isonitrogenous substitution of fish meal with 24% soyabean meal reduced the growth of tilapia (O. aureus X O. niloticus). When 2 - 3% dicalcium phosphate was added, the growth rate of fish increased significantly. Jackson et al. (1982) also observed a growth reduction of Sarotherodon mossambicus fed a diet in which 50% or more of the fish meal was replaced by soyabean meal. They attributed this to the low level of sulphur amino acid and the presence of other factors such as trypsin inhibitor and haemoglutinins. On the contrary, the poor performance of common carp fed a diet containing 45% soyabean meal was attributed to lack of phosphorus rather than sulphur amino acids, since the addition of 2% dibasic sodium phosphate to the soyabean meal diet improved its performance to a level obtained with the best commercial feed (Kim and Oh, 1985). Viola et al. (1986) however did not observe any effect on growth rate of carp when 18% soyabean meal was substituted for fish meal in 25% crude protein diet which reduced the total phosphorus content of the feed from 0.8% to 0.7%.

2.3.2 Soyabean carbohydrates

The carbohydrate portion of soyabean forms 35 - 40% of the raw bean and is made up of the crude fibre and nitrogen-free-extract fractions. The latter contain various oligosaccharides and polysaccharides. The insoluble carbohydrates consist of cellulose 4 per cent and hemicellulose 1.5 per cent (FAO, 1971). The starch content of soyabean is very small about 0.5% (Wilson et al., 1978) and thus most of the polysaccharides are acidic polysaccharides, arabinogalactan and cellulosic materials which are essentially non digestible. The more digestible carbohydrate components of soyabean meal are found in the oligosaccharide fraction (Potter and Potchanakorn, 1984).

On a dry matter basis soyabean contains sucrose 4.5 - 5.5%, starchyose 2.8 - 4.9% and raffinose 0.4 - 1.4% and soyabean meal contains 7.4 - 9.9% sucrose, 4.7 - 4.8% starchyose and 1.0 - 1.1% raffinose (Kawamura and Tada, 1967).

Carbohydrate are generally more cost effective as energy source compared to fat.

In general carnivorous species such as the Atlantic salmon and Japanese yellow tail are ill equipped to handle significant quantities of raw carbohydrates whereas the omnivores such as the common carp and the channel catfish are able to digest fair amount of carbohydrate in their diets (Chow and Halver, 1980). The herbivores e.g. grass carp subsists primarily on a vegetarian diet. Fish does not secrete cellulase and galactosidase (Chow and Halver, 1980). The inability of fish to secrete α-galactosidase may be partly responsible for the poor response of fish to dietary soyabean meal which contains significant level of the galactosidic oligosaccharides raffinose and starchyose. Chow and Halver (1980) reported that the nutritive value of soyabean meal will be enhanced if the bulk of this indigested starch is first transformed. They suggested that soaking the beans for 48 hours prior to processing for meal production will improve the nutritive value of soyabean meal for fish.

2.3.3 Soyabean lipids

Lipids are added in fish diets as a source of energy to spare some proteins for growth. However too much fat can be deleterious for the following reasons: It can cause an imbalance of protein and energy in the ration, it can lead to the production of fatty fish and it can pose problem during pelleting (Lovell, 1980b).

Soyabean oil is highly digestible and has a high degree of unsaturation containing about 85% unsaturated and 15% saturated fatty acid (Ogundipe and Oguntunde, 1990). The saturated fatty acids are mainly palmitic (16.0) 9% and stearic (18.0) 4%. The polyunsaturated fatty acids consists of Oleic (18.1) 24%, Linoleic (18.2) 54% and Linolenic (18.3) 8% (Nwar, 1985). The high linolenic acid content causes off-flavours through a reaction between oxygen and linolenic acids to form compounds such as 2 - n - pentylfuran (Smouse and Chang, 1967). This could be important during storage of feed containing soyabean oil when the use of antioxidants may become very essential.

The use of heat treated full fat soyabean is considered economical because of the possibility of using protein and fat on fish without extra investment in the purchase of separation equipment. Lovell (1980b) compared the growth response of rainbow trout fed practical trout diet containing 72 per cent heated full fat soyabean meal and 5 per cent fish mean with a control diet containing 25 per cent herring meal, 20 percent defatted soyabean meal and 5 per cent fish oil. The rainbow trout fed the full fat soyabean meal diets gained more weight than the control diets. Although the study did not aim at comparing the full fat soyabean with defatted soyabean meal, there was an improvement in protein quality through the use of heat treated full fat soyabean meal.

2.3.4 Minerals in Soyabean

Whole soyabean contains 0.28% calcium, 0.22% magnesium, 0.66% phosphorus, 1.7% potassium, Iron, manganese and zinc are 97 ppm, 28 ppm and 22 ppm respectively (FAO, 1971). Although soyabean is not a major mineral source, the

mineral content contributes to the overall mineral availability of the feed. However, whole soyabean contain phytic acid which can chelate divalent cations such as calcium, iron and zinc and thereby reduce their availability. Robinson (1984) reported that only 29, to 54 per cent of the phosphorus in soyabean meal is available to channel catfish. Since phytase which is necessary to release phosphorus from phytic acid complexes is not detected in the intestine of catfish (Robinson, 1984) the amount of nonphytin - bound phosphorus in soyabean may not be adequate to meet the dietary requirement of the mineral and therefore must be supplied as supplement to the feed. Spinelli (1980) studied the effect on rainbow trout using oregon moist pellet diet (OMP - diet) in which 50 per cent of the fish meal was substituted by dephytinized soyabean meal and observed growth values of 8 - 10 per cent better than the untreated soyabean meal. The growth rate of fish fed the untreated soyabean meal was 25 per cent lower than the control and mortality

was highest in the fish. Post mortem examination of the blood of the dead fish showed significantly lower than normal levels of zinc and iron. In another development, feeding purified (casein - based) diets containing 0.5 per cent of either calcium or sodium phytate resulted in a 10 per cent reduction in the growth rate of rainbow trout. This shows the binding effect of phytate and divalent cations by the formation of an insoluble protein-phytic acid - mineral complex causing reduced availability of zinc, copper and iron.

2.3.5 Soyabean Vitamins

Soyabean meal is not a rich source of vitamins. The dehulled meal contains riboflavin 3.1mg/kg, nicotinic acid 22.0mg/kg, pantothenic acid 14.5mg/kg, choline 2.7mg/kg, pyridoxine 8.0mg/kg, biotin 0.32mg/kg, folic acid 3.6mg/kg and alpha-tocopherol 3.3i.u./kg (Krider, 1973). Since soyabean is essentially devoid of vitamin B_{12}, supplements are essential when soyabean meal replaces commonly used animal by-product protein supplements in practical feed of such animals (Hill et al., 1944). However, in fish

feeds vitamin supplements are usually added to compensate for any deficiencies in the ingredients.

2.4.0 Processing of Soyabean

Studies have shown that different processing technique have profound effect on the growth of fish fed soyabean diets. Abel et al., (1984) subjected full-fat soyabeans to different treatments - thermally (118°C) or hydrothermally (90-95°C) either gently (0.5 or 15 min) or intensely (2.5 or 30 min). They were fed to carp along with the untreated samples. The available lysine was not affected by the heat treatment. The urease activity and the trypsin inhibitor which proved to be especially sensitive to moist heat were strongly reduced by both types of heat treatments at higher intensity. The head treated beans showed a higher oil extractability than the untreated beans. Even though all the experiment diets gave continuous weight increments up to the end of the feeding periods than the untreated beans, the more intensive treatment produced higher growth performance of the fish. However,

only 60 - 65% of the potential of the fish meal control diet was attained and a relatively higher body fat deposition observed. The distinctly better result obtained with carp fed on fish meal protein were attributed to the generally higher availability and/or the more balanced amino acid composition of the fish meal protein.

Viola et al. (1983) subjected soyabean meal to increasing degrees of beating in a cooker at 105°C for 10 min. (insufficient), 30 min. (proper) and 120 min. (overheated). Commercial soyabean meal in rations for carp was replaced by these experimental products on the basis of equal protein and fat contents. Synthetic methionine was included in all experimental rations. The authors observed reduced growth rates in carp fed the insufficiently heated soyabean meal whereas growth rates were equal for the properly heated and slightly overheated meals. Very significant improvement were however noted with the addition of lysine to the properly heated or slightly overheated meals, which showed that the limiting

factor for the growth of carp in properly processed commercial soyabean meals was not the residual antitrypsin, but inadequate lysine - a growth factor. Lysine availability is rather smaller in soyabean meals than yeast or cod muscle (Cowey et al., 1971), but this can vary with processing conditions (Dabrowski and Kozak, 1979).

Balogun and Ologhobo (1989) fed Clarias gariepinus fingerlings (mean weight 65g) on varying levels of raw and cooked soyabean meals in a practical fish diet and determined their growth performance and nutrient utilization. The trials were conducted in small plastic tanks. Groundnut cake was replaced in the control diet at 25%, 50%, 75% and 100% levels by raw and cooked soyabean meals to supply 40% protein in the final diets. The authors reported that food consumption of fish in all dietary treatments was similar but it did not result in similar body weight gain. Percentage weight gains and specific growth rate of fish fed varying levels of cooked soyabean meal were significantly superior to those of fish fed on raw soyabean meals.

It was therefore apparent that the growth - limiting effect of the raw soyabean meal was not a manifestation of a lower feed intake, suggesting the presence of certain toxic principles in the raw meals, not present in the processed (cooked) meal, which inhibited growth.

2.5.0 Antinutritional factors in Soyabean

Osborne and Mendel (1917) were the first scientists to demonstrate that heating of soyabean improved the nutritional qualities of the meal. In their classical experiment with rats, these workers reported that rats fed raw soyabeans as the sole source of protein failed to grow normally while those fed on soya cake meal (obtained from residue after the extraction of the oil) or on cooked soyabeans grew at a normal rate. These growth inhibitors were later identified as trypsin inhibitors because they interfere with the digestion of protein by inhibiting the enzyme trypsin (Kunitz, 1945; Birk et al., 1963). Liener (1981) and Weingartner (1987)

reported that trypsin inhibitors work by binding trypsin irreversibly thus making trypsin unavailable to perform its role in digestion. This causes the intestine to release cholecystokinin to stimulate the pancrease to produce more trypsin. This increase in the secretory activity of the pancrease causes the organ to enlarge. The amino acids present in the trypsin cannot be reabsorbed and thus are lost when the trypsin combines with the trypsin inhibitor. Liener (1981) has suggested that only animals whose pancrease weighs more than 0.3 per cent of total body weight will exhibit pancreatic hypertrophy when fed raw soyabeans. Trypsin inhibitors have been implicated in the growth inhibition of channel catfish (Robinson et al., 1981b) and rainbow trout (Sandholm et al., 1976). Dietary protein level have been reported to alter the effect of trypsin inhibitors in channel catfish and other animals thus increase in the protein level may diminish the effects of the inhibitor. In addition, occurrence of adaptation have been noticed with other animals i.e. older animals are not as sensitive to

trypsin inhibitors as younger animals (Robinson, 1984). Also dietary soyabean products was observed to decrease intestinal lipase in channel catfish (Robinson, 1984).

Weingartner (1987) reported the existence of at least five trypsin inhibitors in soyabeans with the Kunitz (Kunitz, 1945, 1947) and Bowman - Birk inhibitors (Birk, 1961) being the most studied. The composition of the two inhibitors in soyabean are Kunitz inhibitor 1.5 per cent (Freed and Ryan, 1978) and Bowman - Birk inhibitor 0.6 per cent (Gertler et al., 1967) Kunitz trypsin inhibitor is the most active having as much as 50 per cent of the total trypsin inhibitor activity (Rackis and Gumbmann, 1981). Soyabean trypsin inhibitor accounts for 6 - 10 per cent of the soyabean protein (Weingartner, 1987). Trypsin inhibitor has been reported to cause a decrease in the protein quality of soyabeans as a result of the low methionine content. The reason is that trypsin contains a large amount (15 - 22 per cent) of the sulphur amino acids methionine and cystine (Hwang et al., 1978). Thus when

raw soyabeans are used as feed, the small quantity of sulphur - containing amino acids ingested does not offset the large losses caused by trypsin inhibitor as it would if the food had large quantities of sulphur amino acids (Weingartner, 1987). In summary, raw soyabean not only inhibit growth and reduce protein digestibility but also depress metabolizable energy and fat absorption, cause pancreatic hypertrophy, stimulate hyper and hyposecretion of pancreatic enzymes, and reduce amino acid, vitamin and mineral availability (Rackis, 1974). Trypsin inhibitors are heat labile. The degree of deactivation of the toxin depends upon the applied temperature, duration of heating, particle size and moisture condition (UNDP/FAO, 1983). Atmospheric steaming (100°C) of raw defatted soya flakes for 15 minutes, inactivates about 95 per cent of the trypsin inhibitor (Weingartner, 1987). Steaming of whole soyabeans for 20 minutes partially inactivates the inhibitors and if the whole soyabean is adjusted to 20 per cent moisture, atmospheric steaming for 20 minutes will inactivate almost all the

trypsin inhibitor (Wolf and Cowan, 1971). Boiling whole soyabeans for 20 minutes was reported to inactivate most of the trypsin inhibitor (Nelson et al., 1976). Albrecht et al. (1966) and Nelson et al. (1976) demonstrated that if whole soyabeans are soaked overnight (to about 50-60 per cent moisture) only a 5 minutes blanching in boiling water is needed to inactivate the trypsin inhibitor. Similarly UNDP/FAO (1983) reported that overnight soaking or germination of the beans, while bringing about improvement in their nutritive values does not destroy the trpsin inhibitors.

Although heat treatment of raw soyabean inactivates the trypsin inhibitor and denatures the protein thereby making them more digestible, (Kakade et al., 1973), excessive heating may be undesirable because of the Maillard reaction causing the formation of unavailable sugar - amine complexes (Maynard et al., 1979) and the destruction of heat sensitive amino acids e.g. lysine and cystine (Smith and Circle, 1972).

Rumsey 1980) reported that feeding raw soyabeans to fish causes the gall-bladder to contract, increases excretion of bile acids, lowers intestinal proteolytic activity and affects methionine metabolism.

Some of the decrease in the efficiency of utilization of raw soyabeans have been associated with haemaglutinins (lectins) of the raw beans. Haemaglutinins as their name suggests agglutinate red blood cells and some are extremely toxic (Liener, 1976). They make up 1 - 3 per cent of the total protein in defatted soyabean flour (Liener and Rose, 1953). Haemaglutinins work by causing the release of lipases and amylases which are intestinal membrane - bound. These digestive enzymes are thus eliminated in the faeces, thereby causing a reduction in the digestive capabilities. These factors are easily destroyed by heat (Liener and Hill, 1953).

Urease is also found in large amounts in soyabeans. Although no deleterious effect of urease has been reported for fish, this enzyme

can degrade urea to form ammonia which is toxic to cattle (Weingartner, 1987). Urease is inactivated by heat although much heat is needed than trypsin inhibitor and haemaglutinins. Measurement of urease activity is regarded as an indirect method of determining the level of toasting/processing of the soyabean meal (Akiyama, 1988). Another antinutritional factor found in soyabean is phytic acid which is chemically hexametaphosphate of myoinositol. Phytic acid perse is not a problem but it binds to various essential elements such as protein, phosphorus, calcium, zinc and magnesium thereby reducing the nutritive value of feeds stuff (Robinson and Lovell, 1984). Whole soyabean contain 1 - 2 per cent phytic acid (Weingartner, 1987). The phosphorus bound to phytic acids are unavailable to monogastric animals including channel catfish because they don't have the enzyme phytase in their intestine which is necessary to release the phosphorus from phytic acid (Robinson, 1984).

Spinelli (1980) compared the growth rate of rainbow trout fed oregon moist pellet (OMP), and OMP type diets, in which 50% of the fish meal was substituted with undephytinized and dephytinized soyabean meal. He reported that feeding untreated soyabean meal resulted in 25 per cent reduction in growth when compared to the OMP control. Dephytinized soyabean meal gave growth values of about 8 - 10 per cent better than untreated soyabean meal. Total substitution with treated soyabean meal produced high mortality in the fish after about 90 days. On examination of the zinc, iron and copper contents of the blood of the carcass it was observed that zinc and iron were significantly lower than normal. Furthermore, feeding purified casein - based diets containing 0.5 per cent of either calcium or sodium phytate to rainbow trout resulted in a 10 per cent reduction in growth. Studies with channel catfish shows that only 29 to 54 per cent of the phosphorus in soyabean meals is available to the fish and up to 80 per cent of the available phosphorus are non-phytate phosphorus (Robinson, 1984). Since phytic acid does not

interfere with the bioavailability of minerals added to the rations (Forbes et al., 1979), levels of such minerals could be improved by supplementation.

CHAPTER THREE

GENERAL MATERIALS AND METHODS

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3.1.0 The Experimental Fish

Fish used for the study were mudfish Clarias anguillaris fingerlings and juveniles. The biology of the fish had been described by Reed et al. (1965) and recently by Madu (1989). Essentially the fish has an elongated body dorsally flattened head which terminate in a mouth surrounded with eight barbels. The body is about 3.1 to 3.8 times as long as the head. The nostrils are far apart and the pelvic fins are close to the snout. There are no spines in the dorsal and anal fins. The body is almost black at the back with white belly and greyish on the sides. One of the distinguishing features of this species is the possession of mostly pointed or granulated vomerine teeth forming a band not broader than the premaxillary teeth band (Figure 3.1).

Out of the ten species of Clarias described in West Africa, the two commonest species in Nigeria are C. anguillaris and C. gariepinus (Reed et al., 1965, Olatunde, 1983). The major differences between these species are shown in Table 3.1.

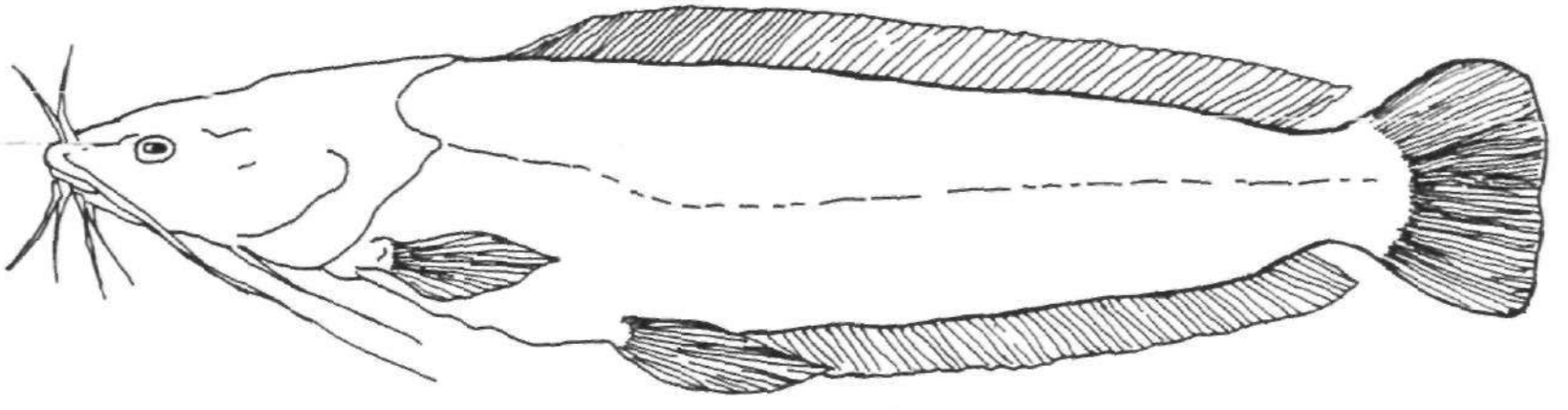


Fig. 3·1. Diagrammatic representation of a typical mud fish (x. 0.5)

Table 3.1. Main Differences between C. gariepinus (Buchell) and C. anguillaris (Linnaeus)

Structure	C. gariepinus	C. anguillaris
1. Pelvic fins	Midway between the tip of the snout and the root of the caudal fin or slightly close to the snout.	Nearer to the tip of the snout than to the root of the caudal fin.
2. Dorsal and anal fins	Dorsal fin has 62-82 rays. Anal fin has 50-65 rays.	Dorsal fin has 66-67 rays while anal fin has 52-61 rays.
3. Head and Body	The body is 3.0 to 3.5 times longer than the head.	The body is 3.1 to 3.8 times longer than the head.
4. Vomerine teeth	Mostly granular forming a band broader than the band of teeth on the premaxillary.	Mostly pointed and sub-conical forming a band not broader than that of the premaxillary.
5. Gill Rakers	Gill rakers are between 31-135 on the first fill arch.	Between 14-33 gill rakers on the first fill arch.
6. Colour	Blackish on the back, slightly yellowish on the belly.	Dark grey on the sides, black on the back and white at the belly.
7. Size	Grows to about 400mm long and a weight of 7 kg.	Grows almost to a metre length and weight of more than 7 kg.

Compiled from Reed et al. (1965); Holden and Reed (1972).

All samples of C. anguillaris fingerlings used for this work unless otherwise stated in the text, were obtained by induced breeding (hypophysation) of the brood stock in indoor hatchery of the National Institute for Freshwater Fisheries Research (NIFFR), New Bussa.

3.2.0 Experimental Facilities

All feeding experiments were conducted within the hatchery complex of NIFFR, New Bussa, using indoor mini flow-through plastic tanks and hapa cages suspended in outdoor concrete tanks.

3.2.1 Description of the Indoor Mini Flow-through System

Thirty circular plastic tanks each 0.25m deep and 0.55m in diameter were set up in the indoor hatchery. The tanks were layed in rows on three long tables each, 8m long, 0.7m wide and 1.2m high. Two water taps were installed close to each end of each table in such a way that the taps were slightly higher than the top of the plastic tanks. Each tank was covered with wire gauze of dimension 0.54 x 0.51m which prevented the fingerlings from

escaping after stocking. A long plastic tubing of 1cm diameter was connected at both ends to each pair of water taps with the entire length lying on the cover of the plastic tanks. At the centre of each tank the long tubing was cut into two portions. The loose ends were again joined together by a smaller tubing of 0.8cm diameter and 5cm length. On each small tubing were small openings of 0.3cm diameter to facilitate passage of water into the plastic tanks. To keep water in circulation a PVC pipe of 10cm length and 2cm diameter was suspended in each of the plastic tanks by iron rods. A short plastic tubing of 1cm diameter and 30cm length was passed into the suspended pipe through an opening 3cm from the top of the plastic tank. The tubing was inserted through the table into a common drain PVC pipe 9m long and 3cm diameter, hinged to the side of the table, (Plate 1).

Water for the system was pumped from the Kainji reservoir, a tributary of Kainji Lake, into a Biological filteration (Bio-filter) of gravel and sand. The biologically filtered water was pumped into an overhead storage tank which supplied



Plate 1 The indoor mini flow-through system.

water by gravity to the taps. On opening the taps, water flowed into the long plastic tubing from where it jets into each of the plastic tanks. Water was prevented from overflowing by the pipe which allowed excess water to drain out through the discharge plastic tubing. Water ceased to circulate when the water taps were closed.

3.2.2 Description of the Outdoor Hapa Cage System

Hapa nets were sewn into a total of 80 hapa cages of 1 x 1 x 1m with an opening at the top margin 30cm long. This opening was to facilitate stocking and removal of the fingerlings. The upper corners of each group of 9 hapas were tied to two long kuralon twines which were fastened at the opposite ends to iron rods. This was repeated on the other sides of the concrete tank (Plate 2). The hapa cages were anchored at the bottom corners to small concrete weights by four nylon twines 20cm long tied to the bottom corners of each hapa. This arrangement allowed the hapas to remain suspended in the concrete fish tank



Plate 2 Hapa cages suspended in outdoor concrete tanks.

measuring 10 x 10 x 2m with a 0.2m thick layer of mud at the bottom and having water of 1.0m deep. Water in the tank was fertilized with 5 kg chicken manure to ensure adequate supply of natural food throughout the duration of the experiment. The hapas were washed biweekly to remove uneaten food and algae growth which could block the hapas and prevent free flow of water within the system. Weak hapas were replaced with new ones whenever they were identified.

3.3.0 Chemical Analysis of Feeds and Feedstuffs

Methods of chemical analysis of the Association of Official Analytical Chemist (AOAC 1975, 1980) were used to determine the composition of the feeds and feedstuffs. The analysed chemical composition were moisture content, ash, lipid, crude protein, crude fibre, specific mineral elements and amino acids. Further analysis were carried out to determine the extent of digestibility of the feed and the protein solubility index. The particular methods of analysis are discussed as follows.

3.3.1 Determination of Moisture Content and Dry Matter

2g of each sample was accurately weighed out using a Sauter analytical balance into a petri dish nickel, with the sample spread as thinly as possible over the base of the dish. The dish and the contents were placed in a Gallenkamp hot box oven whose temperature was maintained at 105°C. The sample was dried for twenty four hours. After drying the sample was removed, cooled in a desiccator and weighed. The dish was returned to the oven redried for a further 30 minutes, removed, cooled and weighed. The drying was continued until a constant weight was reached. The moisture content was calculated from the weight loss of the sample.

Calculation

$$\frac{\text{Wt of dish with dried sample - Wt of dish}}{\text{Wt of dish with initial sample - Wt of dish}} \times 100$$

$$= \% \text{ Dry Matter.} \qquad \ldots\ldots\ldots\ldots \quad (1)$$

3.3.2 Ash Determination

The ash of a foodstuff is the inorganic residue remaining after the organic matter has been burnt away. The ash obtained is not necessarily of exactly the same composition as the mineral matter present in the original food

material as there may be losses due to volatilisation or some interaction between constituents (Pearson, 1976). The ash was determined according to AOAC (1980) as follows.

2g of sample was accurately weighed into a dry evaporating dish and charred over a low bunsen flame. After the sample had been fully charred, it was ashed at 550 - 570°C in a Carbolite furnace for 24 hours. After ashing the evaporating dish was removed and cooled in a desiccator and weighed. Sample was again placed in the furnace and reweighed every 30 minutes. When the weight of the ash was constant and no black particles remained in the evaporating dish, ashing was said to have been completed.

Calculation

$$\text{Per cent total ash} = \frac{X - Y \times 100}{Z} \quad \ldots . \quad (2)$$

Where X = Weight of evaporating dish + ash

Y = Weight of evaporating dish

Z = Weight of sample.

3.3.3 Determination of Lipid Content

The normal procedure for lipid determination is to extract the dried ground materials with light petroleum or n-hexane, evaporate off the

solvent and weigh the residue (Pearson, 1976). The determination of lipid content in this work required the use of any of the following two methods: The soxhlet continuous extraction method (AOAC, 1975) and the modified Bligh and Dyer technique (Hanson and Olley, 1963).

(i) The Soxhlet Continuous Extraction

5g of the sample powder was weighed into the thimble. Each end of the thimble was pluged with fat free cotton wool. The thimble and contents were placed in the central syphon portion or extractor of the soxhlet apparatus. A 250cm³ ground - glass - joint flask was weighed and 40cm³ of analytical grade diethyl ether and 40cm³ analytical grade petroleum ether poured into the flask. The flask was connected to the soxhlet syphon and condenser with the flask sitting in the heating mantle. The mantle was then connected to the electric supply and the heating was slowly increased until the solvent boiled. The vapour of the boiling solvent condensed in the soxhlet syphon were the sample contained and the lipid was dissolved. At a

certain level, the solvent with the dissolved lipid ran back into the 250cm³ ground-glass-joints flask. The heating and hence the extraction process was continued for about 8 hours. The mixed ether were distilled off and the flask and content were placed in the oven at 105°C for 3 hours. The flask was cooled in the desiccator and weighed. The flask and content were replaced in the oven for 30 minutes and reweighed. This was repeated until a constant weight was obtained.

Calculation of Result

The lipid content was calculated from the weight of material in the receiver flask.

$$\text{Per cent lipid} = \frac{X}{Y} \times 100 \quad . \quad . \quad . \quad (3)$$

Where X = Weight of lipid

Y = Weight of sample.

(ii) Modified Bligh and Dyer Technique Hanson & Olley (1963)

2g sample was weighed and transferred into a Warring blender. Chloroform (40cm³) and methanal (80cm³) were added and homogenised for 2 minutes.

Then an additional 40cm³ of chloroform was added and homogenised for half a minute. Finally 40cm³ of distilled water was added and the sample homogenised again for half a minute.

The content of the blender were transferred into a centrifuge tube and centrifuged for 15 minutes at 300 rpm. After centrifuging, the water phase at the top of the tube was removed. The methanol-lipid layer was collected into a small flask and evaporated in the oven.

Calculation of Result

The fat content was calculated as in formula 3 above from the weight of lipid in the flask.

3.3.4 Determination of crude fibre

The crude fibre is the organic residue which remains after the materials have been treated under standardised conditions with light petroleum, boiling dilute sulphuric acid, boiling dilute sodium hydroxide solution, dilute hydrochloric acid, alcohol and ether (Pearson, 1976). Crude fibre content was determined according to AOAC (1980).

The crude fibre content is commonly used as a measure of the nutrient value of feeds because the digestibility of crude fibre is low and feeds high in crude fibre content are of low nutritive value (Schanderl, 1970). It contains largely of cellulose and a little lignin.

Procedure

2g of sample was transferred to a soxhlet apparatus and extracted with petroleum ether and diethyl ether as above in section 3.3.3. The extracted sample was air dried and transferred to dry 1000cm³ conical flask. 200cm³ boiled 0.255 N sulphuric acid was added. The content of the flask was boiled gently for exactly half an hour with the flask rotated to mix the contents and remove particles from the sides.

A Buchner funnel fitted with a perforated plate covered with filter paper was prepared. Boiling water was poured into the funnel, and allowed to remain until the funnel was hot. The water was then drained by suction.

At the end of the half an hour boiling period, the acid mixture was allowed to stand for 1 minute and then poured immediately into a shallow layer of hot water under gentle suction in the prepared funnel. Suction was adjusted so that the bulk of the 200cm³ was completed within 10 minutes.

The insoluble matter was washed with boiling water until the washings were free from acid, then the insoluble matter was washed back into the original flask by means of a wash bottle containing 200cm³ 0.313 N sodium hydroxide solution measured at ordinary temperature and brought to boiling point. The boiling was continued for half an hour after which the mixture was allowed to stand for 1 minute and then filtered immediately through a Whatman No. 1 filter paper. All the insoluble materials were transferred to the filter paper and washed first with boiling water then with 1 per cent hydrochloric acid and finally with boiling water until free from acid. Then washing was continued twice with alcohol and three times with ether. The insoluble matter was

then transferred to a dry, weighed ashless filter paper and dried at 100°C to a constant weight. The paper and content were incinerated to an ash at a dull red heat.

Calculation of Result

The weight of the ash was subtracted from the increase of weight on the paper due to the insoluble material. The difference was reported as the fibre content.

$$\text{Per cent Crude fibre} = \frac{Y - X}{Z} \times 100 \quad \ldots(4)$$

Where X = Weight of the ashless filter paper

Y = Weight of ash after incinerating

Z = Weight of sample.

3.3.5 Determination of Crude Protein

In the quantitative determination of total nitrogen content of a protein the most reliable methods are the Kjeldahl or Dumas procedure (Lillevic, 1970).

The analysis of a compound for its protein content by the Kjeldahl method is based upon the determination of the amount of reduced nitrogen present i.e. its - NH_2 and = NH nitrogen. Ammonium compounds, urea, amino acids and more

complex breakdown products of protein will all be determined unless removed previously. For more routine purpose, the crude protein in the sample is then calculated by multiplying the total nitrogen by an empirical factor. It should be borne in mind however that such a figure for protein include some non-protein nitrogenous substances indicated above. Nitrogen in certain forms (e.g. nitrates, nitrites and nitroso compounds) is not estimated in the Kjeldahl process unless the procedure is modified (Pearson, 1976).

The method involves heating the sample with concentrated sulphuric acid. This converts the protein nitrogen into ammonium sulphate and is referred to as the digestion process. The second stage is the estimation of ammonia produced by neutralising the digest by distillation followed by titration with acid.

Procedure

Two procedures applied for the determination of the total Kjeldahl nitrogen were the semi automated method based on the use of the Technicon BD - 20 Block Digestor (AOAC, 1975), and the microkjeldahl method (AOAC, 1980).

(a) The Semi Automated Method

0.5g sample was accurately weighed on a filter paper and placed in the BD - digestor flask. A blank was set up by placing a known weight of filter paper in another flask. To these flasks were added 15cm³ concentrated sulphuric acid, 9g potassium sulphate and 0.42g Mercuric oxide. The BD - digestor was switched on in the fume cupboard and the flasks were heated for 2 hours at 410°C until a clear colourless solution was obtained. After heating the digests were allowed to cool to room temperature, after which they were transferred to a volumetric flask and made up to 250cm³ each with distilled water. From this stock, aliquot of 5cm³ were used for the distillation.

The Markham steam distiller was used for the distillation. 5cm³ of the made-up solution was pipetted into the distillation chamber and 10cm³ of 40% sodium hydroxide solution was added. The liberated ammonia was distilled into 10cm³ of 2% boric acid containing 2 to 3 drops of mixed indicator (Bromocresol blue and methyl red).

As ammonia was absorbed into boric acid, the colour of the solution changed from purple to green. The ammonium borate formed was titrated against standard 0.05 N Hydrochloric acid until the original purple colour was restored. The percentage crude protein in sample was then calculated.

Calculation

The percentage crude protein was calculated from the formula:

$$\% \text{ Crude Protein} = \frac{M \times 0.014 \times D \times T \times 6.25 \times 100}{W \times L} \quad \ldots (5)$$

Where:

M = Molarity of acid

0.014 = Nitrogen content per 100cm³

D = Dilution factor

6.25 = Protein conversion factor (1 Mg Nitrogen = 6.25 Mg protein)

W = Weight of sample

L = volume of liquor distillate.

(b) Microkjeldahl Method

0.5g of each sample was accurately weighed on a filter paper. The filter paper and sample were transferred to small Kjeldahl flasks. A

blank was prepared using only a filter paper. To another flask 0.165g of Glycine was added as standard. 10cm³ of concentrated sulphuric acid was added to each of the flasks. The flasks were warmed gently at first to minimise frothing then the heat was increased until the solution cleared. Boiling was continued for a further 30 minutes, after which the solutions Were allowed to cool. The contents of the flasks were transferred to the distillation apparatus. A receiving flask was set up containing approximately 25cm³ of boric acid and a few drops of screened methyl red. The digest was made alkaline by adding 40% sodium hydroxide solution. Care was taken to ensure that ammonia did not escape into the atmosphere. The ammonia was boiled over into the receiving flask and the contents of the receiving flask was titrated against 0.1 N sulphuric acid. This was repeated in all the samples.

The apparent protein content was Calculated as in formula (5) above.

Efficiency of Conversion of N to Ammonia

Calculated Nitrogen content of glycine sample = X (Glycine contains 18.67% N)

Analysed Nitrogen content of glycine sample calculated from data given above = Y

$$\% \text{ Recovery} = \frac{Y \times 100}{X} = Z\% \quad \ldots\ldots (6)$$

The apparent protein content was multiplied by $\frac{100}{Z}$ to get the crude protein of sample.

3.3.6 Determination of Mineral Elements

Mineral elements are the cations and the anions. Both groups of elements were analysed using the atomic absorption spectrophotometer (Semac Ltd Model ANA 80) and the varian spectrophotometer (Model 634) respectively (AOAC, 1980). Sodium and potassium were determined using the flame photometer (Jenway PFP7). Determination of calcium, iron, magnesium and manganese were done using atomic absorption. Phosphorus was determined with the varian spectrophotometer.

Determination of Calcium, Iron, Magnesium and Manganese

Procedure

250mg of sample was weighed out into a porcelain dish. The sample was heated carefully over a bunsen burner with the crucible cover until the smoke had ceased. Then the cover of the crucible was removed and the content roasted in a muffle furnace for 8 hours at 500°C. After ashing, the crucible was allowed to cool in a desiccator. The ash was extracted with 20% analytical grade hydrochloric acid. The solution was made up to 50cm³ with more 20% HCL.

A stock solution of the following elements were prepared. The elements were magnesium, potassium, manganese, sodium, calcium and iron. Each stock solution contained 1000 ppm of the element. The stock solutions were diluted to obtain solutions of 2, 4, 6, 8, 10 ppm of each element except magnesium. For magnesium the concentration was 0.2, 0.4, 0.6, 0.8, and 1.0 ppm due to the sensitivity of the magnesium lamp (see Appendix 2).

Instrumentation

The atomic absorption spectrophotometer (Semac Ltd Model ANA 80 manufactured by Tokyo Photoelectric Co. ltd) was used for the experiment. The instrument was put on for 30 minutes before the Acetylene gas knob was opened. The gas was ignited and the standard solutions were introduced at the Aspirator. The optical density was read at the wavelength of each element. If linear the equipment was confirmed to be working properly. The sample was then introduced and the concentration read at the galvanometer against distilled water.

Determination of Phosphorus using varian Spectrophotometer

The equipment used was varian spectrophotometer model 634 (Varian Techtron pty Ltd Switzerland).

Procedure

Ashing of sample was done as previously described. From the dry ash samples $5cm^3$ was taken and made up to $50cm^3$ with distilled water ($40cm^3$) and Baton solution ($5cm^3$). The mixture was left for about 15 minutes for colour to develop.

The colour was read at 385nm wavelength.

Preparation of Standard Solution

A stock solution of phosphorus was prepared by dissolving 0.2197g potassium hydrogen phosphate in a litre of distilled water. The stock phosphorus solution was diluted to obtain solutions of 1, 2, 3, 4, 5, ppm phosphorus (Appendix 2). 5cm³ of Baton reagent (see Appendix 3) was added and allowed to stand for 15 minutes. The optical density of the standard solutions and the unknown sample were read at 385nm wavelength. A calibration curve was plotted and the concentration of the unknown sample was extrapolated from the graph.

Calculation of the Result

The phosphorus content of feed material was calculated as follows:

$$\% \text{ Phosphorus in feed material} = \frac{\text{Mg P (from graph)} \times \text{Dilution factor} \times 100}{1{,}000{,}000 \times \text{Wt of sample}} \quad (7)$$

Determination of Sodium and Potassium

The instrument used for the determination of sodium and potassium was the flame photometer (Jenway model PFP7).

Procedure

The stock potassium solution was used to set the sensitivity control of the flame photometer to obtain a scale reading of just under 100 divisions. Distilled water was used to obtain a zero reading. The other standard solutions prepared as previously described in 3.3.6(i) were sprayed and the readings were noted. A calibration curve of concentration against scale reading was prepared for potassium.

After the sample readings were taken, the potassium solution was replaced with the sodium solution which had been diluted as in Appendix 2. The flame photometer was set with the stock sodium solution and distilled water to obtain a scale reading of 100 and zero respectively. The reading of the diluted standard solutions were taken and a calibration curve was prepared for sodium.

Preparation of Samples

2g dry sample was weighed into a 250cm³ conical flask and 25cm³ of 75% concentrated HCl was added. A small watch glass was placed on

top of the conical flask and the flask was boiled for 5 minutes and filtered hot into a separating funnel to separate out the fat. The aqueous layer was transferred to a 100cm³ volumetric flask and made up to mark. The solution was further diluted with distilled water until when aspirated the reading lay between the highest and the lowest of the standards. The concentrations of sodium and potassium of the sample were read off the calibration curves.

3.3.7 Amino acid Analysis

The samples were first of all weighed, defatted, hydrolysed and then evaporated in a rotary evaporator before they were loaded in the amino acid analyser (TSM).

(i) Determination of dry Weight

Three filter papers (No 1 - type) were accurately weighed on a chemical balance. About 0.5g of sample was added to the weighed filter paper. The sample was dried in the rotary evaporator. The weight of the dried sample and filter paper were noted. The difference between the weight of the filter paper with sample and

that of the filter paper gave the weight of the sample which was used for the analysis.

(ii) Defatting of the Samples

The weighed samples were carefully transferred into different lipid extraction thimbles. Each thimble was plugged with cotton wool (to prevent particles of the sample from floating) and dropped into 100cm³ soxhlet extractor. Both were connected to a round bottom flask which was 3/4 filled with petroleium spirit (60 - 80°C). The assembly was connected to Davids double surface condenser and heated with an electrothermal heater at 70°C. Extraction was allowed to continue for a minimum period of 6 hours after which the defatted samples were evaporated to dryness at 50°C under oxygen-free nitrogen.

The samples were divided into two parts, one for the determination of crude protein (nitrogen) and the other for hydrolysis.

(iii) Hydrolysis of the Samples

The defatted samples were accurately weighed and about 0.001 - 0.5g of each sample was transferred into an ampoule. 7cm³ of 6 N HCl were

added tothe ampoule and nitrogen gas (oxygen-free) was passed through the solution to expel the air for 20 minutes (this prevented the possible oxidation of some amino acids during hydrolysis). The ampoule was sealed and incubated at 100 - 110°C for 22 hours. At the end of incubation, the ampoule was broken at the neck and the content was filtered using a whatman filter paper No. 1.

(iv) Evaporation of the Sample Filterates

The filterate was transferred into 250cm³ round bottom flask of the rotary evaporator where it was evaporated to dryness at 40°C under suction. The residue was subsequently washed twice with 10cm³ of deionized water and evaporated to dryness each time. The residue was picked up in 3cm³ of 0.01 N HCl and preserved in refrigerator at-6°C ± 4°C, for loading into an amino acid analyser when required.

(v) Loading of Sample in Techniconsequential Multisample amino acid Analyzer (TSM)

The TSM is an automated instrument designed to separate, detect, and quatitate amino acids. The system can separate and analyze free acidic, neutral and basic amino acids from a protein

hydrolysate in $1^{1}/_{4}$ hours. The instrument provided accurate reproducible results without need of further operator attention until all analysis are completed. The TSM comprises independent modules, hydraulically and electrically inter-connected to form an integrated system. Each system is equipped with internal fault - detection and alarm device which constantly monitor system operation.

A known volume of hydrolysed samples and standard mixture were loaded respectively into thoroughly cleaned analytical cartidges, with the cartridges loaded with equal volume of internal standard solution (Norleucine) using a micropipette. The cartridges were then placed on a sample tray of the TSM sampler.

Two chromatrographic columns were packed with 75% resin slurry and mounted directly below TSM sampler. The front column was used for separation of the basic amino acid and the rear column for separation of the acidic and neutral amino acids. Buffer solutions were connected to the systems via a peristaltic valve.

(vi) Preparation of Buffers

(a) Buffer No. 1 (PH 3.25, 0.2 N Na+/0.1m Citrate/6% Methyl cellosolve). 400cm³ of stock solution was measured and poured into a 4 litre beaker equiped with stirrer. About 3,000cm³ of distilled water was added and agitated. 240cm³ Methyl cellosolve and 1.0cm³ thiodiglycol were added after which 6 N Hydrochloric acid was used to titrate the solution to PH 3.25. It was then transferred to a volumetric flask and made up to a total volume of 4 litres.

(b) Buffer No. 2 (PH 4.25, 0.2 N Na+/ 0.1m Citrate). This was prepared as buffer 1 except that methyl cellosolve was omitted and that it was titrated to PH 4.25.

(c) Buffer No. 3 (PH 5.25, 0.37 N Na+/ 0.1m Citrate). This buffer was also prepared in the same way as the second buffer except that 40.0g sodium chloride was added.

When the analyser was started, the buffers were delivered to the columns through the sampler, from two high-pressure pumps, and selected by the buffer - changing valve (the valve sequences the buffers required in the stepwise elution of the amino acids and sequences the flow of effluent from either column into the analytical system or to waste). The buffers washed the column, removing some of the amino acids bound to the resin by electrostatic forces. As the amino acids were eluted from the column, they were collected individually in an automatic fraction, carefully partitioned by the bubble system, into the analytical system. Here they were mixed with ninhydrin solution supplied through the proportioning pump. The reaction mixture now formed, passed through a capillary tube immersed in a 100°C water bath, where amino acids reacted with ninhydrin to produce a coloured product.

The developed effluent (reaction steam) was led through the colorimeter where the absorbance was monitored continuously as it passes over a sensitive photoelectric unit.

The signals from the colorimeter was finally fed into an automatic pen recorder which then ploted the intensity on moving linear chart paper, the speed of which was carefully preset. The type of amino acid was established on the basis of its position on the chromatogram, and it was quantitatively estimated on the basis of the area under each curve.

(vii) Method of Calculating amino acid value from Chromatogram peaks

The net height of individual peaks were readily measured. The half-height of the peak on the chart was located and the width of the peak at the half-height was accurately measured and recorded. The approximation of the area of each peak was obtained by multiplying the net height with the width at half-height.

The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$NE \simeq \frac{\text{Area of norleucine peak}}{\text{Area of each amino acid}} \quad \ldots (8)$$

From the norleucine equivalent above, a constant S was calculated for each amino acid in the standard mixture.

S std - NE std x Mol. Weight x μ MAA std. (9)

3.3.8 Digestibility Determination

The determination of feedstuffs digestibility involves measuring amount of the feedstuff ingested and subtracting that which is present in faeces following digestion (NRC, 1983). The method of Furukawa and Tsukahara (1966) was used for the determination of digestibility. The method involves the use of chromic oxide ($Cr_2 O_3$) as an indigestible marker. It is assumed that all the chromic oxide consumed will pass through the digestive tract and appear in the faeces. Analysis of the relative changes in chromic oxide percentage in the feed and faeces will represent the percentage of feed that was ingested (NRC, 1983).

Collection of Faeces

Faeces were collected weekly by manual stripping. This was done by pressing the fish belly gently until the faeces were expelled at the anus. The expelled faeces were collected and stored in the freezer at - 6 °C ± 4 °C. At the end of the experiment the fish were sacrificed and more faeces were removed at a position of the rectum close to the anus (Plate 3).



Plate 3 Extraction of faeces from the rectum for digestibility studies.

Faeces removed at this position was reported to give a better protein digestibility than those taken far away from the rectum (Austreng, 1978). The faeces were pooled with the ones earlier collected and analysed.

Procedure

0.05g of sample was weighed on a Whatman No. 1 filter paper and transferred with the filter paper to a dry 100cm³ Kjeldahl flask. 5cm³ of concentrated Nitric acid was added in such a manner that the particles that adhered on the inside of the flask were washed down. The flask was allowed to stand for 10 minutes after which it was heated on an electric heater for the sample to digest. When a white precipitate was obtained, the heater was turned off and the flask allowed to cool. 3cm³ perchloric acid was added to the digestion mixture and the flask reheated until the green colour changed to yellow, orange or red. The digestion was extended for 10 minutes to avoid change in colour from green to yellow due to insufficient oxidation of the content. The flask was allowed to cool slightly afterwards and 50cm³ of distilled water

was added. The flask was allowed to cool again before it was made up to 100cm³ with distilled water. The flask was allowed to stand for 10 minutes for the organic material to precipitate. The solution was transferred from the volumetric flask to the cuvex and the optical density was read at 350nm against distilled water.

Calculation

The standard curve obtain by the wet acid digestion technique was expressed by the following equation:

$$Y = 0.2089\ x + 0.0032 \quad \ldots\ldots (10)$$

Where Y is the optical density at 350nm and X is the chromic oxide content of the sample (Mg/100cm³).

The digestibility of the nutrient was expressed from the following formula:

Percentage nutrient =

$$100 - \left[100 \times \frac{(\%\ Cr_2O_3 \text{ in food})}{(\%\ Cr_2O_3 \text{ in faeces})} \times \frac{(\%\ \text{nutrient in faeces})}{(\text{nutrient in food})} \right] \quad \ldots\ldots (11)$$

3.3.9 Protein Solubility Index

The protein solubility index was determined according to the method of Dale et al. (1987).

0.2% potassium hydroxide (KOH) equivalent to 0.042 normal, pH 12.5 was used for the analysis. The KOH reagent was prepared by dissolving 2.360g of KOH in a volumetric flask with distilled water and diluting to 1000cm³.

Procedure

1.5g soyabean meal was placed in a 250cm³ beaker and 75cm³ KOH solution was added and allowed to stir for 20 minutes. After stirring, 50cm³ of the liquid was transferred to a centrifuge and centrifuged for 10 minutes. 15cm³ aliquot was taken for the determination of Kjeldahl protein. This 15cm³ aliquot was equivalent to 0.3g of original sample.

Calculation

$$\text{Per cent protein solubility} = \frac{\text{\% Protein in 0.3g portion of sample}}{\text{Crude protein of original sample}} \quad \ldots\ (12)$$

3.4.0 Physico-Chemical Analysis of Water

Water in the outdoor concrete tanks were analysed for their physico-chemical parameters to determine their suitability for the culture of the experimental fish. Generally, water samples



Plate 4 Domestic feed pelleter.

were taken weekly and the following parameters were determined in each case: dissolved oxygen, temperature and pH. The method used was based on the standard methods for the examination of water and waste-water (APHA, 1981).

3.4.1 Dissolved Oxygen (DO)

This was measured by applying the azide modification of the Winkler's method as described by APHA (1981). The water samples were taken using a TAC bottle. In the laboratory the water samples were analysed by adding manganous chloride and reagent II Winklers sodium hydroxide and sodium iodide. The bottle was stoppered carefully to exclude air bubbles and the water was shaken to permit complete reaction of the reagent with the oxygen present in the water sample. A golden brown precipitate indicated the presence of oxygen in the water. 1cm^3 of concentrated sulphuric acid was added to the precipitate to dissolve entirely. Finally the treated water samples were titrated against 0.025 N sodium thiosulphate using starch as an indicator.

3.4.2 Temperature

Temperature of water was measured in degree Celcius (°C) using the laboratory mercury in glass thermometer. The instrument was inserted at a depth of 0.05m from the surface of the water. The reading was taken after 2 minutes of insertion.

3.4.3 Hydrogen ion Concentration (pH)

The pH of the water was observed using colorimetric technique. The instrument used was the lovibond comparator. 10cm³ of the water was taken into one of the two glass tubes and placed directly into the control compartment of the comparator. Into the second glass tube 10cm³ of the water was taken and 10 drops of Bromothymol blue added. The tube was inserted into the second compartment. The colour of the sample was compared with the standard color and the pH was read.

3.5.0 Feedstuffs, Feeds and Feeding

The feedstuffs used in this study were of both plant and animal origin. The plant source feedstuffs were obtained from the Agricultural

production unit of the National Institute for Freshwater Fisheries Research (NIFFR) New Bussa. These were soyabean, groundnut seeds and maize seeds. Blood meal and fish meal were the animal proteins used in the study. Detailed description of the processing methods are available in the relevant chapters.

Feeds were prepared by grinding using the hammer mill (E.H. Bentall and Co. Ltd. Essex, England Model 11689). The feedstuffs were mixed into paste before pelleting either in the domestic pelleter with a 3mm die (Plate 4) or in the screw-type feed pelleter with a 3mm die (Plate 5). The pelleted feeds were dried in the sun or in the oven at 50 - 60°C.

Feeding was carried out twice daily morning and evening at a level which was equivalent to 3% or 5% of the biomass depending on the experiment being performed. Fish were weighed biweekly at which time their feed intake was altered to reflect the new weight. Cleaning of the tank and hapas was also carried out biweekly.

3.6.0 Acclimation of the Experimental Fish

The mudfish fingerlings used for the



Plate 4 Domestic feed pelleter.

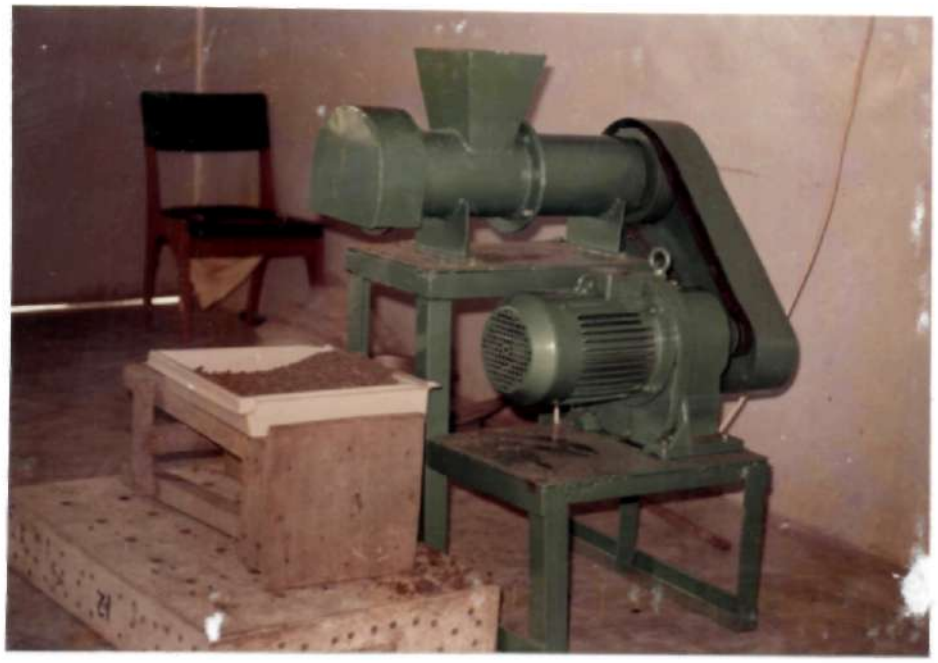


Plate 5 Screw type feed pelleting machine.

experiments were acclimated either in the mini flow-through tanks (for the indoor experiments) or the hapas (for the experiments conducted in concrete tanks). In the mini flow-through experiments, fish of a homogenous nature were stocked at the rate of 25 fingerlings per tank and acclimated for one week. During this period, the fingerlings were fed pelleted feeds for four days. The composition of the pelleted feeds is given in Table 3.2. The fish were kept off feed for 72 hours prior to the commencement of the experiment.

For the experiments conducted in hapas, fish were distributed into hapas at the rate of 25 fingerlings per hapa and allowed to acclimatise for one week. During this period fish were not fed artificial feeds but susbsisted on the natural food in the hapas.

3.7.0 Weighing of Fish during Experiment

Fish were weighed immediately after acclimation. Thereafter, weighing was done fortnightly. The final weight was taken at the termination of the experiment. Before

Table 3.2.

Composition of Standard Feeds fed to *C. anguillaris* during acclimation

Ingredient	% in diet
Fish meal	10
Soyabean meal	25
Groundnut cake	15
Blood meal	10
Maize	38
Vitamin/Mineral Premixes	2
Total	100

weighing, C. anguillaris fingerlings were carefully collected from each tank or hapa with a hand net of soft wire gauze 20cm diameter, 15cm deep; and 1mm mesh size. The fingerlings were transferred into a bowl 30cm in diameter and 20cm deep containing about 2 litres of pond water. The bowl was conveyed immediately to the laboratory. The fingerlings were removed from the bowl with the hand net and placed in a plastic perforated container 5cm in diameter and 4cm high which had been pre-weighed on a sauter analytical balance. The fingerlings were allowed to calm down before the weight was taken. The weight of the fish was determined by subtracting the weight of the container from the total weight. The fish were returned immediately to the tanks or hapas in a bowl of water.

3.8.0 Food Utilization Parameters

Formula of the food utilization parameters used were as follows:

Specific Growth Rate (SGR) =

$$\frac{\log_e W_2 - \log_e W_1}{T - t} \quad \text{(Brown, 1957)} \quad \ldots \quad (13)$$

Where W_1 is the initial weight (g at time t) and W_2 is the final weight at time T, and e is the base of natural logarithms.

Food Conversion Efficiency (FCE) =

$$\frac{\text{Weight gained by fish}}{\text{Weight of food consumed}} \times 100 \quad \ldots \quad (14)$$

(Halver, 1972)

Protein Efficiency Ration (PER)

$$\frac{\text{Weight gain}}{\text{Protein fed}} \quad \ldots \quad (15)$$

Where protein fed =

$$\frac{\text{\% protein in the diet} \times \text{total diet consumed}}{100} \quad (16)$$

(Osborne et al., 1919; Mazid et al., 1979)

Net Protein Utilization (NPU)

This was calculated as the relationship between protein gained in fish to protein consumed

$$\text{NPU} = \frac{\text{Fish protein gain}}{\text{Protein consumed}} \times 100 \quad \ldots \quad (17)$$

Where Protein gain = Final total body protein - initial total body protein

Protein consumed = Total dietary protein fed

(Dabrowski and Kozak, 1979; Mazid et al., 1979).

3.9.0 Statistical Analysis

At the end of the experiment all data were subjected to two-way analysis of variance (ANOVA) using a Tandy 4000 Lx computer (manufactured by radio shack USA). The means were compared by multiple range test (Duncan, 1955).

CHAPTER FOUR

CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF FEEDSTUFFS USED

CONTENTS

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4.1.0 INTRODUCTION

Feeding fish with artificial diets is a process of decision - making involving two important considerations. The first is using feedstuffs which are neither in common use nor aesthetically acceptable as human food. The second is the use of surplus foodstuffs whose nutritional losses will be compensated for by increased consumption and high nutritional - excellence of fish (Harris, 1980). Such decisions can be made on the basis of reliable information concerning the chemical composition of the different ingredients used in fish feeding (EIFAC, 1971).

Not until the pioneering work of Oyenuga (1968), little was documented on the chemical composition of feedstuffs grown in the tropics. Since then many workers have contributed towards the understanding of the chemical composition of these feedstuffs (Fetuga, 1972; Fetuga et al., 1973, 1974; Miller, 1976; Arowosoge, 1987; West et al., 1988). Such information would lead to more judicious use of some more commonly known sources and perhaps also step up the use of hitherto

neglected sources (Fetuga et al., 1974; Harris, 1980).

This study was aimed at upgrading and expanding our knowledge of the chemical composition and nutritive value of the commonly available feedstuffs in Nigeria. The information so obtained will be of value to feed manufacturers and aquaculturists in their choice of the best combinations of feedstuffs in the formulation and manufacture of fish feeds.

4.2.0 MATERIALS AND METHODS

4.2.1 Feedstuffs

The feedstuffs evaluated included white maize, yellow maize, guinea corn, palm kernel cake, cotton seed cake, mixed rice bran and husk, groundnut cake, blood meal, soyabean meal and fish meal. The fish meal were obtained from three species namely tilapia (Sarotherodon galilaeus) and the clupeids (Pellonula afzeluisi and Serrathrissa leonensis). These species are those commonly used for fish meal preparation in Kainji Lake area.

4.2.2 Sample Preparation

The sources of the various feedstuffs are described in Table 4.1. Maize, groundnut and soyabean were planted in the Kainji Lake basin by the production unit of the National Institute for Freshwater Fisheries Research (NIFFR), New Bussa. These feedstuffs were purchased from the unit and prepared as described below. In an attempt to eliminate expensive processing equipment and the need to look inwards for sourcing of raw materials, with the hope of improving indigenous technology, most of the feedstuffs used in this study were prepared by traditional methods using the locally available equipment.

(a) White Maize (WM)

White maize grains were finely ground in a hammer mill. Samples were stored in bottles before analysis.

(b) Yellow Maize (YM)

Yellow maize grains were similarly ground and stored in sample bottles prior to analysis.

(c) Guinea Corn (GC)

Guinea corn grains were purchased from New Bussa market and brought in

Table 4.1 Description of Feedstuffs

Common Name	Abbreviation	Scientific Name	Source
White Maize	WM	Zea mays Linn.	N.I.F.F.R. Production farm, New Bussa
Yellow Maize	YM	Zea mays Linn.	N.I.F.F.R. Production farm, New Bussa
Guinea Corn	GC	Sorghum sp. Linn.	New Bussa township Market
Palm Kernel Cake	PKC	Elaeis guineensis Tacq.	N.I.P.R.O.C Arondizogu, Imo State
Cotton Seed Cake	CSC	Gossypium sp. Linn.	Gusau Oil Mill, Gusau, Sokoto State
Rice bran/husk mixed	RBH	Oryza sativa Linn.	New Bussa Rice Mill
Groundnut Cake	GNC I	Arachis hypogaea Linn.	Gusau Oil Mill, Gusau, Sokoto State
Groundnut Cake	GNC II	Arachis hypogaea Linn.	N.I.F.F.R. Production farm, New Bussa
Blood Meal	BM	-	New Bussa township market abattoir
Raw Soyabean Meal	RSM	Glycine max Linn. Merrill	N.I.F.F.R Production farm, New Bussa
Mildly Roasted Soyabean meal	SM I	Glycine max Linn Merrill	N.I.F.F.R Production farm, New Bussa
Overheated Soyabean Meal	SM II	Glycine max Linn. Merrill	N.I.F.F.R. Production farm, New Bussa
Fish Meal (tilapia)	FM I	Sarotherodon galilaeus Trewavas	Captured by beach seining of N.E.P.A. reservoir, New Bussa
Fish Meal (Clupeid)	FM II	Pellonula afzeluisi Johnels	Captured by surface trawling of Kainji Lake at New Bussa
Fish Meal (Clupeid)	FM III	Sierrathrissa leonensis Thys van den Audenaerde	Captured by surface trawling of Kainji Lake at New Bussa

nylon bags to the feed mill section of the Institute. Samples were taken and milled before use.

(d) Cotton Seed Cake (CSC)

Cotton seed cake were purchased from Gusau Oil Mill Limited, Gusau, in Sokoto State. Samples were randomly taken and analysed.

(e) Palm Kernel Cake (PKC)

Palm kernel cake pellets were purchased from N I.P.R.O.C. Arondizuogu, Imo State and transported to the Institute in 100 kg jute bags. Samples were finely ground before analysis.

(f) Rice Bran and Husk (RBH)

A mixture of rice bran and husk was collected without cost from a local rice milling company in New Bussa. The machine was manufactured by Lewis C. Grant Ltd. Dysart, Scotland Model 1/20. The rice mill was a compact machine that threshed the husks, removed the brown rice coat and germ

together in a single operation to produce white rice. The brown rice coat and germ are known together as bran. The rice bran and husk are usually mixed together. Samples were taken at random from the mixture and analysed.

(g) Groundnut Cake (GNC I)

The groundnut cake were purchased in 100 kg jute bags from Gusau Oil Mill Ltd, Sokoto State. The cakes were produced after oil extraction by mechanical press. Samples were taken and finely ground and analysed.

(h) Groundnut Cake (GNC II)

The sample of groundnut cake was prepared traditionally as follows: 50 kg of raw groundnut was shelled manually and roasted on a hot pan over a fire with occasional stirring to prevent charring. After the groundnut had been thoroughly roasted, they were removed from the fire and allowed to cool in a bowl. The testa were removed by rubbing and then winnowed away.

The testa - free nuts were milled into a paste. The groundnut paste was allowed to disperse in a little cold water in a mortar. The oil was extracted by stirring the paste in the mortar with hot water. The cake was fried in groundnut oil to firm it and allowed to cool. Groundnut cake prepared this way is known locally as "Kulikuli". The cake was later ground and analysed.

(i) Blood Meal (BM)

Fresh cow blood was collected from the abattoir in New Bussa in a plastic bowl and brought to the feed mill section of the Institute. The scarlet blood was cooked in a big pot to harden up. The coagulated blood was dried in the sun and milled. The blood meal had a resemblance of charcoal dust.

(j) Raw Soyabean Meal (RSM)

The raw soyabeans were broken down to smaller pieces with a hammer mill

to remove the hull. The hulls were winnowed away. The mills were further reduced to smaller and fine particles with the hammer mill.

(k) Mildly Roasted Soyabean Meal (SBM I)

Samples of raw soyabeans were heat treated traditionally by roasting on a hot pan over a fire for about 10 minutes with occasional stirring. The heated soyabean were partially broken with the milling machine to remove the hull. The hulls were winnowed away. The dehulled soyabeans were finely ground and stored.

(l) Overheated Soyabean Meal (SBM II)

The raw soyabeans were roasted on a hot pan for about 20 minutes with occasional stirring until the beans were baked and just about to charr. The soyabeans were transferred to the milling machine and partially milled to break the beans and release the hulls. The hulls were expelled by winnowing. The dehulled soyabeans were finely ground and analysed.

(m) Fish Meal from whole tilapia (Sarotherodon galilaeus) (FM I)

Young tilapia were obtained by seining of the NEPA reservoir in New Bussa. The fish were transferred in a plastic bowl to the laboratory for processing. The fish were boiled in a big pot or drum, pressed in a muslin bag to partially remove water and oil and dried in the sun or gas oven before milling.

(n) Fish Meal from Clupeids (Pellonula afzeluisi) (FM II) and (Serrathrissa leonensis) (FM III)

Clupeids were prepared as described by Eyo (1988). Clupeids were caught with a trawl net of 3mm codend mesh size. Trawling was done with two boats each with 15 H.P. Yamaha outboard engine on Kainji Lake. The fish were immediately sundried on racks on chicken wire placed on wooden frame 5m long, 0.5m wide and 1 metre high for two to three days. After sun-drying the clupeids were ready for packing. Pellonula afzeluisi which is much larger than Serrathrissa leonensis (Otobo, 1977) were separated out and milled.

4.2.3 Chemical Analysis

Determination of moisture, crude protein, and crude fibre were carried out according to the AOAC (1975) methods. Ether extract was determined by the soxhlet method AOAC (1975). Calcium, magnesium, manganese and iron were determined according to the AOAC (1980) using Atomic Absorption spectrophotometer Sodium and potassium were determined by flame photometer AOAC (1980). Phosphorus was estimated using the varian spectrophometer (AOAC, 1980). The total amino acid composition was determined on an automatic model TSM - Technicon sequential multisample analyzer at the Department of Zoology, University of Jos, Jos, Plateau State.

4.3.0 RESULTS

4.3.1 Proximate Composition

Table 4.2 shows the mean values and standard deviation for the proximate composition of some of the available feedstuffs used in fish feed preparation.

(a) Dry Matter

GNC II recorded the highest mean

Table 4.2 Proximate composition of some available feedstuffs (% dry matter)*

Feedstuff **	Dry Matter	Crude Protein	Ether Extract	Crude Fibre	Total Ash	Nitrogen-Free Extract (NFE)
White Maize (WM)	91.37 ± 0.04	9.27 ± 0.68	5.01 ± 0.44	2.44 ± 0.38	1.74 ± 0.19	70.86 ± 3.05
Yellow Maize (YM)	90.42 ± 1.24	10.77 ± 3.71	3.56 ± 0.77	3.47 ± 1.22	1.94 ± 0.09	71.20 ± 2.93
Guinea Corn (GC)	88.84 ± 2.94	11.22 ± 2 18	2 48 ± 0.66	2.32 ± 0.22	1.77 ± 0.04	74.06 ± 7.12
Palm Kernel Cake (PKC)	87.79 ± 2.46	19.06 ± 4.34	7 57 ± 1.35	43.24 ± 6 62	5.54 ± 0 51	17.91 ± 8.66
Cotton Seed Cake (CSC)	90.51 ± 2.78	27 64 ±10 43	8.25 ± 2.31	31 89 ± 2 48	5.06 ± 0 49	26.08 ± 1.50
Rice Bran/Husk Mixed (RBH)	91 15 ± 1.69	6.88 ± 2.86	4.44 ± 2.51	40.23 ± 0 39	21.75 ± 2 83	8.68 ± 4.12
Groundnut Cake (GNC I)	91.03 ± 1 08	47.37 ± 0 65	13.23 ± 2.67	8.14 ± 1 13	6.34 ± 1.19	18 91 ± 9.52
Groundnut Cake (GNC II)	92.41 ± 1 61	40.59 ± 3 05	23 39 ± 3.90	6 03 ± 1 49	6.20 ± 1.18	19.01 ± 5.56
Blood Meal (BM)	88 17 ± 2.65	86.00 ± 4 70	0 67 ± 0 44	2 14 ± 0 01	5 20 ± 1.71	6.51 ± 8.27
Raw Soyabean Meal (RSM)	91.67 ± 0.57	40.70 ± 2.75	22 07 ± 0.83	6 30 ± 1 44	6.43 ± 0.56	16.62 ± 5.83
Soyabean Meal I (SBM I)	91.64 ± 0 48	46.21 ± 1.32	24.76 ± 0.54	4 70 ± 0 80	7.87 ± 2.73	17 97 ± 0.69
Soyabean Meal II (SBM II)	92.08 ± 0 34	48.13 ± 0.06	23 93 ± 1.73	4.12 ± 0 04	7.88 ± 1 30	20.65 ± 5.00
Fish Meal I (FM I)	89 19 ± 3.24	57 69 ± 2.05	1 81 ± 0 40	5.18 ± 3.46	33.55 ± 2.28	-
Fish Meal II (FM II)	90.22 ± 0.89	71.33 ± 3 16	7.97 ± 1.00	1.08 ± 1.10	20.22 ± 3 44	-
Fish Meal III (FM III)	90.23 ± 1.00	68.47 ± 1.37	8 02 ± 1 05	0 39 ± 0 08	17.81 ± 5.62	-

* See Table 4.1 for detailed description of feedstuffs.

** The values represent the mean and standard deviation of 3 samples received at the National Institute for Freshwater Fisheries Research New Bussa from 16 January 1988 to 30 April 1990.

values for the dry matter followed by SBM II, SBM I and RSM. The lowest mean values were recorded by PKC, BM and GC respectively. The other feedstuffs had mean dry matter values of 90 to 91%

(b) Crude Protein

The mean values for the crude protein ranged from 6.88 ± 2.86% in RBH to 86.00 ± 4.70% in BM. The mean values for the fish meals were high, 71.33 ± 3.16%, 68.47 ± 1.37% and 57.69 ± 2.05% for FM II, FM III and FM I respectively. The cereals recorded low crude protein values which were 11.22 ± 2.18%, 10.77 ± 3.71% and 9.27 ± 0.68% for GC, YM and WM respectively. The crude protein content of soyabean meals were 40.70 ± 2.75%, 46.21 ± 1.32% and 48.13 ± 0.06% for RSM, SBM I and SBM II respectively. The mean values for groundnut cake were 47.37 ± 0.65% and 40.59 ± 3.05% crude protein for

GNC I and GNC II respectively while those for PKC and CSC were 19.06 ± 4.34% and 27.60 ± 10.43% crude protein respectively. The CSC recorded the highest variation in crude protein among the samples analysed.

(c) Ether Extract

The mean values for the ether extract were highest in SBM I followed by SBM II. The lowest mean values were for BM followed by FM I and GC. GNC I and GNC II recorded mean values for the ether extract of 13.23 ± 2.67% and 23.39 ± 3.90% respectively. These values were higher than those of PKC (7.57 ± 1.35%) and CSC (8.25 ± 2.31%). The mean values for cereals were low 5.01 ± 0.44%, 3.56 ± 0.77% and 2.48 ± 0.66% for WM, YM and GC respectively.

(d) Crude Fibre

The highest mean values for the crude fibre were recorded in PKC (43.24 ± 6.62%) followed by RBH (40.23 ± 0.39%) and CSC (31.89 ± 2.48%). The lowest

crude fibres were recorded in FM III (0.39 ± 0.08%) and FM II (1.08 ± 1.10%). The mean values for the cereals were low 2.32 ± 0.22%, 3.47 ± 1.22% and 2.44 ± 0.38% for GC, YM and WM. Similarly, the mean values for the soyabean meals were also low 6.30 ± 1.44% for RSM, 4.70 ± 0.80% for SBM I, and 4.12 ± 0.04% for SBM II. Groundnut cake also recorded low crude fibre values of 6.03 ± 1.49% and 8.14 ± 1.13% for GNC II and GNC 1 respectively.

(e) Total Ash

The mean values for total ash ranged from 1.74 ± 0.19%, 1.94 ± 0.09% and 1.77 ± 0.04% for WM, YM and GC respectively. The total ash values for fish meals were high 33.55 ± 2.28%, 20.22 ± 3.44% and 17.81 ± 5.62% for FM I, FM II and FM III respectively. RBH also recorded high values of 21.75 ± 2.83%. The total ash values for the other feedstuffs were between 6 to 8%.

(f) Nitrogen Free Extract (NFE)

The mean values for NFE were highest in the cereals 74.06 ± 7.12%, 71.20 ± 2.93% and 70.86 ± 3.05% for GC, YM and WM respectively. The fish meals did not record any NFE. The values for BM and RBH were low 6.51 ± 8.27% and 8.68 ± 4.12% respectively. Soyabean meals had mean values for NFE of 16.62 ± 5.83%, 17.97 ± 0.69% and 20.65 ± 5.00% for RSM, SBM I and SBM II respectively. Groundnut cake also recorded moderate NFE values which were 18.91 ± 9.52% and 19.01 ± 5.56% while the mean values for PKC and NFE were 26.08 ± 1.50% and 17.91 ± 8.66% respectively.

4.3.2 Mineral Content

The mean values for the mineral content of feedstuffs are shown in Table 4.3.

(a) Sodium

The highest sodium content was recorded in BM 1.44 ± 0.10% while YM had the least mean value of 0.12 ± 0.13%. WM and GC had sodium content of

Table 4.3 Mineral content of feedstuffs (% dry matter)

Feedstuff*	Sodium	Calcium	Potassium	Phosphorus	Magnesium	Iron (ppm)	Manganese (ppm)
White Maize (WM)	0.24±0.04	0.15±0.07	0.42±0.03	0.22±0.11	0.07±0.02	1.50±0.12	0
Yellow Maize (YM)	0.12±0.13	0.19±0.21	0.30±0.13	0.28±0.04	0.09±0.04	1.80±0.15	1.10±0.07
Guinea Corn (GC)	0.16±0.10	0.08±0.09	0.30±0.08	0.28±0.04	0.11±0.02	1.50±0.14	0
Palm Kernel Cake (PKC)	0.22±0.15	0.44±0.11	0.23±0.04	0.53±0	0.28±0.02	1.60±0.57	1.20±0.04
Cotton Seed Cake (CSC)	0.20±0.11	0.46±0.25	1.10±0.14	0.49±0.09	0.30±0.01	1.80±0.10	0.20±0.02
Rice Bran/Husk Mixed (RBH)	0.50±0.14	0.33±0.45	1.00±0.85	0.62±0.11	0.31±0.04	2.60±0.38	0.75±0.07
Groundnut Cake (GNC I)	0.20±0.02	0.18±0.18	0.92±0.40	0.43±0.05	0.25±0.01	2.60±0.20	1.20±0.28
Groundnut Cake (GNC II)	0.20±0.10	0.22±0.27	0.74±0.71	0.37±0.13	0.22±0.02	1.80±0.15	0.20±0.02
Blood Meal (BM)	1.44±0.10	0.55±0.15	0.20±0.06	0.39±0.21	0.04±0.01	26.00±4.50	3.80±5.23
Raw Soyabean Meal (RSM)	0.16±0.01	0.41±0.11	1.52±0.11	0.35±0.07	0.19±0.01	2.80±0.12	3.20±1.50
Soyabean Meal I (SBM I)	0.14±0.03	0.43±0.16	1.58±0.14	0.49±0.02	0.20±0.03	2.50±0.15	3.90±0.60
Soyabean Meal II (SBM II)	0.14±0.03	0.44±0.11	1.63±0.24	0.76±0.01	0.20±0.01	2.30±0.16	3.20±0.50
Fish Meal I (FM I)	0.64±0.20	8.45±2.47	0.90±0.71	5.09±0.57	0.15±0.01	1.80±0.20	4.30±0.20
Fish Meal II (FM II)	0.88±0.15	3.56±0.21	0.90±0.82	2.24±0.42	0.10±0	1.60±0.25	0.40±0.20
Fish Meal III (FM III)	0.66±0.12	2.27±2.02	0.76±0.30	1.62±0.08	0.03±0.01	1.40±0.18	0.20±0.01

* See Table 4.1 for detailed description of feedstuffs.

0.24 ± 0.04 and 0.16 ± 0.10% respectively. The values for soyabean were low 0.16 ± 0.01% for RSM, 0.14 ± 0.03% for SBM I and 0.14 ± 0.03% for SBM II. Fish meal had sodium content of 0.64 ± 0.20 for FM I, 0.88 ± 0.15% for FM II and 0.66 ± 0.12% for FM III. The sodium content of other feedstuffs was lower than those of fish meal.

(b) Calcium

The calcium content was very high in the fish meals FM I (8.45 ± 2.47%), FM II (3.56 ± 0.21%) and FM III (2.27 ± 2.02%) and low in the cereals GC (0.08 ± 0.09%), YM (0.19 ± 0.21%) and WM (0.15 ± 0.07%). Soyabean meals also recorded low values for calcium RSM (0.41 ± 0.11%) SBM I (0.43 ± 0.16%) and SBM II (0.44 ± 0.11%). The calcium values for PKC and CSC were 0.44 ± 0.11% and 0.46 ± 0.25% respectively. GNC I, GNC II and RBM had calcium values of 0.18 ± 0.18%, 0.22 ± 0.27% and 0.33 ± 0.45% respectively. BM had 0.55 ± 0.15% calcium.

(c) Potassium

The highest potassium values were recorded in soyabean meals 1.52 ± 0.11%, 1.58 ± 0.14% and 1.63 ± 0.24% for RSM, SBM I and SBM II respectively. BM had the lowest mean values of 0.20 ± 0.06%. The mean potassium values for cereals was moderate 0.42 ± 0.03%, 0.30 ± 0.13% and 0.30 ± 0.08% for WM, YM and GC respectively. The fish meal also recorded moderate potassium values of 0.90 ± 0.71%, 0.90 ± 0.82% and 0.76 ± 0.30% for FM I, FM II and FM III respectively. The values for groundnut cake were 0.92 ± 0.40% and 0.74 ± 0.71% for GNC I and GNC II respectively. PKC, CSC and RBH had potassium values of 0.23 ± 0.04%, 1.10 ± 0.14% and 1.00 ± 0.85% respectively.

(d) Phosphorus

The values for phosphorus were high in fish meals 5.09 ± 0.57%, 2.24 ± 0.42% and 1.62 ± 0.08% for FM I, FM II and FM III respectively. Phosphorus

content of cereals was low 0.22 ± 0.11%, 0.28 ± 0.04% and 0.28 ± 0.04% for WM, YM and GC. The values for phosphorus in soyabean meal were also low. For RSM it was 0.35 ± 0.07%, SBM I recorded 0.49 ± 0.02%, and 0.76 ± 0.01% for SBM II. Phosphorus values for other feedstuffs were moderate

(e) Magnesium

The values for magnesium were generally low in all feedstuffs examined. The lowest value was obtained in FM III 0.03 ± 0.01% while RBH recorded the highest mean value of 0.31 ± 0.04%. The values for the scyabean meals were 0.19 ± 0.01% for RSM, 0.20 ± 0.03% for SBM I and 0.20 ± 0.01% for SBM II. BM values were 0.04 ± 0.01% whereas the cereals recorded 0.07 ± 0.02% for WM and 0.09 ± 0.04% for YM. The value for GC was 0.11 ± 0.02%. Magnesium values for PKC, CSC, GNC I and GNC II were 0.28 ± 0.02, 0.30 ± 0.01%, 0.25 ± 0.01% and 0.22 ± 0.02% respectively.

(f) Iron

The highest values for iron were recorded in BM, 26.00 ± 4.50 ppm, followed by the soyabean 2.80 ± 0.12 ppm, 2.50 ± 0.15 ppm and 2.30 ± 0.16 ppm for RSM, SBM I and SBM II respectively. The values for iron in groundnut cake were 2.60 ± 0.20 ppm and 1.80 ± 0.15 ppm for GNC I and GNC II respectively. The cereals had iron values of 1.50 ± 0.12 ppm, 1.80 ± 0.15 ppm and 1.50 ± 0.14 ppm for WM, YM and GC respectively. Values for the fish meal were 1.80 ± 0.20 ppm, 1.60 ± 0.25 ppm and 1.40 ± 0.18 ppm for FM I, II and III respectively. The values for other feedstuffs were about the same as those for fishmeal.

(g) Manganese

Manganese was not detected in WM and GC but in YM 1.10 ± 0.07 ppm was recorded. The highest value for magnesium was recorded in the fish meal FM I followed by BM 4.30 ± 0.20 and 3.80 ± 5.23 ppm

respectively. Soyabean also contained high magnesium 3.20 ± 1.50 ppm, 3.90 ± 0.60 ppm and 3.20 ± 0.50 ppm for RSM, SBM I and SBM II. The magnesium values for other feedstuffs were lower.

4.3.3 Amino Acid Composition

The amino acid composition of feedstuffs commonly used in fish feed is shown in Table 4.4. Considerable interest was focused on the indispensable amino acids because these amino acids are needed by the fish to maintain good health. They are arginine, histidine, lysine, valine, methionine, isoleucine, leucine, threonine and phenylalanine.

(a) Arginine

Arginine was very high in most of the feedstuffs except WM (2.82 g/16gN), GC (2.33 g/16gN) and BM (2.47 g/16gN). The highest value for arginine was recorded in GNC I (11.07 g/16gN), followed by GNC II and PKC (7.95 g/16gN) each.

Table 4.4 Amino acid composition of feedstuffs commonly used in fish feeds (g/16gN)

Amino Acid	White Maize (WM)	Yellow Maize (YM)	Guinea Corn (GC)	Palm Kernel Cake (PKC)	Cotton Seed Cake (CSC)	Ground-nut Cake (GNC I)	Ground-nut Cake (GNC II)	Blood Meal (BM)	Raw Soya-bean Meal (RSM)	Soya-bean Meal (SBM I)	Soya-bean Meal (SBM II)	Fish Meal (FM I)	Fish Meal (FM II)	Fish Meal (FM III)
Arginine	2.82	5.14	2.33	7.95	8.87	11.07	7.95	2.47	4.96	5.91	2.91	4.85	5.51	4.14
Histidine	2.46	2.70	2.11	2.24	2.91	2.85	3.99	6.04	3.52	2.44	2.55	3.23	4.28	2.94
Lysine	2.97	3.39	1.90	3.54	3.98	3.23	4.18	9.19	6.97	5.96	3.72	10.50	11.09	8.99
Valine	3.36	3.47	3.71	2.69	3.77	2.73	2.77	4.60	2.22	2.51	2.20	2.92	5.83	2.58
Methionine	1.43	1.67	0.85	1.23	1.37	0.61	0.87	1.57	1.23	0.96	1.06	3.11	2.21	2.38
Isoleucine	2.70	1.97	2.97	1.80	2.78	2.40	2.91	0.81	2.64	2.21	2.13	2.86	2.62	1.99
Leucine	7.99	7.89	14.14	3.91	6.52	6.73	5.15	10.28	5.13	5.52	4.42	6.76	8.31	4.84
Threonine	3.34	3.03	3.54	2.57	2.97	2.71	3.30	4.71	3.50	3.73	2.59	5.02	5.50	2.97
Tyrosine	1.66	2.28	1.90	1.12	3.07	3.36	3.41	2.16	2.56	2.82	2.21	2.76	3.91	2.85
Phenylalanine	2.99	3.46	5.18	3.95	5.97	4.37	5.20	7.52	5.04	4.62	4.35	4.36	5.50	2.97
Aspartic acid	5.90	2.11	6.45	6.40	6.28	9.82	10.30	8.72	7.97	8.74	8.02	8.84	12.53	8.32
Serine	3.43	3.87	5.06	9.50	4.84	5.00	9.99	9.50	9.45	5.57	6.79	7.26	6.43	7.35
Glutamic acid	12.16	11.52	17.07	17.20	21.77	10.74	22.29	8.40	15.73	15.49	14.02	15.07	18.58	12.69
Proline	5.05	6.11	6.74	2.79	3.42	3.47	4.26	4.61	5.61	4.67	3.56	6.25	4.75	5.03
Glycine	3.93	4.45	3.40	4.62	6.12	7.49	5.90	4.09	3.70	4.14	3.81	8.26	8.85	4.21
Alanine	3.94	3.82	5.19	2.74	3.23	2.84	3.62	6.81	3.09	3.71	2.72	6.53	8.26	4.96

* See Table 4.1 for detailed description of feedstuffs

(b) Histidine

Histidine was found to be high in BM (6.04 g/16gN), FM I (3.23 g/16gn), FM II (4.28 g/16gN), FM II (2.94 g/16gN) and GNC (3.99 g/16/gN). The values for cereals and soyabean were moderate.

(c) Lysine

Lysine was high in the animal protein sources namely BM (9.19 g/16gN), FM I (10.50 g/16gN), FM II (11.09 g/16gN) and FM III (8.99 g/16gN). Lysine was also high in RSM (6.97 g/16gN) and SBM I (5.96 g/16gN). Lysine was marginal in GNC I (3.23 g/16gN), YM (3.47 g/16gN), YM (3.47 g/16gN), PKC (3.54 g/16gN), CSC (3.98 g/16gN) and SBM II (3.72 g/16gN). WM and GC scored low lysine values of 2.97 and 1.90 g/16gN respectively.

(d) Valine

Valine was found in appreciable quantity in FM II and BM 5.83 and 4.60 g/16gN respectively. Valine was also high in the cereals WM (3.36 g/16gN), YM

(3.47 g/16gN) and GC (3.71 g/16gN). The level of valine in other feedstuffs was moderate.

(e) Methionine

The sulphur amino acid methionine was good enough in FM I (3 11 g/16gN), FM II (2.21 g/16gN) and FM III (2.38 g/16gN). Methionine values were very low in GC, GNC I, GNC II and SBM I and II; 0.85 g/16gN, 0.61 g/16gN, 0.87 g/16gN, 0.96 g/16gN and 1.06 g/16gN respectively. Other feedstuffs had higher methionine values.

(f) Isoleucine

An exceedingly low level of isoleucine was encountered in BM (0 81 g/16gN). Other feedstuffs had higher values. The highest values for isoleucine was recorded in GC (2.97 g/16gN) followed by GNC II (2.91 g/16gN).

(g) Leucine

Leucine was found ir considerable quantity in all the feedstuffs analysed. The highest leucine value was found in GC (14.14 g/16gN) followed by BM (10.28 g/16gN).

The lowest leucine value was obtained in PKC (3.91 g/16gN). Other feedstuffs had higher leucine values.

(h) Threonine

Threonine was appreciable in BM (4.71 g/16gN), FM I (5.02 g/16gN) FM II (5.50 g/16gN) and FM III (2.97 g/16gN). Moderate values were found in other feedstuffs. The lowest threonine value was found in PKC (2.57 g/16gN).

(i) Phenylalanine

Phenylalanine was highest in BM (7.52 g/16gN) and moderate in other feedstuffs except WM and FM III where low levels were recorded, 2.99 g/16gN and 2.97 g/16gN respectively.

(j) Dispensable Amino Acids

The dispensable amino acids analysed were Aspartic acid, Serine, Glutamic acid, Proline, Glycine and Alanine. Tyrosine could be described as dispensable depending on the level of phenylalanine. Of the dispensable amino acids glutamic acid was found in the highest concentration

in all the feedstuffs. The values for aspartic acid was also very high in some feedstuffs. The values for serine, proline, glycine and alanine were lower.

4.4.0 DISCUSSION

Maize (white and yellow) and guinea corn are the major energy sources in fish feeds. These feedstuffs were low in crude protein, ether extract, crude fibre and total ash but high in NFE. The high NFE coupled with low crude fibre has made these cereals very suitable as a ready source of available energy in fish feeds. The results of the chemical composition did not differ very much from those reported by Oyenuga (1968); Fetuga (1973, 1974); NRC (1983) and West et al. (1988). Although the mean values for the crude protein of guinea corn was higher than those of white and yellow maize, guinea corn was inferior in all but two indispensable amino acids leucine and phenylalanine. This has rendered the nutritive value of guinea corn inferior to those of white and yellow maize. Similar observation has been reported by Nelson et al. (1975) who attributed this inferiority to tannin content and poor amino acid availability in guinea corn.

The mean values for the crude protein content of palm kernel cake was comparable with that of Oyenuga (1968) (20.40%) and Fetuga et al. (1973) (18.70%) but lower than that of Miller (1976) (21.5%). The amino acid profile was within the range of those reported in the literature (NRC, 1983; Fetuga et al., 1974). The commonly deficient amino acids in oil seeds lysine and methionine were 3.54 g/16gN and 1 23 g/16gN respectively. These values did not differ very much from those reported by Fetuga et al. (1973), 3.82 g/16gN and 1.74 g/16gN respectively. With the mean crude fibre values of 43.24% the sample contained more crude fibre than those reported in the literature (Oyenuga, 1968; Fetuga et al., 1974). The recommended maximum level of crude fibre in fish diet is 8% (Dupree and Huner, 1984). The sample of palm kernel cake my not be suitable in fish feeds due to its high fibre content. Large amount of fibre in fish diet is not acceptable because it may dilute the nutrients, impair digestibility and decrease pellet quality (Dupree and Huner, 1984). Thus palm kernel cake has a limited nutrient value although it can be useful in a

mixed ration as a filler or binder (Miller, 1976).

The crude protein content of cotton seed cake was very variable. The mean values were lower than those reported by Oyenuga (1968) and Miller (1976); 44.8% and 47.3% respectively. The mean crude fibre values were higher than those reported by Oyenuga 1968) and Miller (1976). 9.9% and 9.6% respectively. The high fibre content and low crude protein was due to the high lint content of the original samples which were not decorticated before fat extraction. High fibre content in cotton seed cake could result in lowered digestibility of the proteins and hence reduce the extent of which the associated amino acids could be utilized (Ufodike and Matty, 1983). Although cotton seed cake had a fair amount of indispensable amino acids, it was limiting in lysine. The lysine level was lower than those of groundnut cake (GNC II) and soyabean meals (RSM and SBM I). This agrees with the observation of Oyenuga (1968) who reported lower lysine in cotton seed cake

than in soyabean and groundnut. However the composition of cotton seed was found to vary according to climate, soil, moisture and other factors (Oyenuga, 1968).

The crude protein values for the mixed rice bran and hust were lower than all other feedstuffs. The crude fibre values were high. Similar levels have been reported by Oyenuga (1968). Crude fibre is relatively undigested by fish (Akiyama, 1988). The high crude fibre values coupled with the poor crude protein content have made the mixed rice bran and husk of little nutritive value in fish feed. Oyenuga (1968) reported that rice husk had no nutritive value as it was high in crude fibre.

The groundnut cake industrially processed was higher in protein but lower in ether extract than the traditionally processed groundnut cake. This shows better efficiency in fat extraction by the industrial expeller process compared with the manual extraction techniques. There was, however, no apparent difference in the nutritive value of groundnut cake industrially processed and those processed traditionally as judged by their amino

acid profile. Indeed the traditionally processed groundnut cake was higher in histidine, lysine and methionine than those industrially processed. This could be attributed to excessive heat treatment during industrial processing causing some losses of these heat-labile amino acids.

Blood meal recorded the highest mean values for the crude protein and the lowest mean values for the ether extract than all the feedstuffs analysed. The values for the proximate composition did not differ very much from those reported by Oyenuga (1968) and Fetuga et al. (1974). They differed however from the values reported by Miller (1976). In spite of being high in protein content and some indispensable amino acids blood meal was low in methionine (1.57 g/16gN) and isoleucine (0.81 g/16gN). The isoleucine values for blood meal was the lowest amongst the feedstuffs analysed, while the leucine values were the highest (10.28 g/16gN). The isoleucine to leucine ratio was found to be more than 1 : 10. Fetuga et al. (1973) had reported that the isoleucine to leucine ratio of more than 1 : 10

was undesirable because it prevented the utilization of other amino acids in blood meal protein in feed of monogastric animals. Since Fetuga et al. (1973) worked on pigs and rats which are terrestial animals, it is not certain whether this generalisation could affect the utilization of blood meal by aquatic animals.

Soyabean meal was affected by heat treatment. Intensive traditional heat processing led to an increase in the dry matter, crude protein and NFE. The amino acids were also affected. Mild heat processing resulted in higher arginine values and lower values for other indispensable amino acids than the raw soyabean meal. Intensive heating further led to a reduction in the values for the indispenable amino acids. The low methionine values in all the samples analysed supports the observation that soyabean is limiting in methionine (Nose, 1978; Murai et al., 1982, 1986; NRC, 1983). Up to 50% of the lysine was lost through traditional heat processing of soyabean meal. The lysine availability has been reported to be affected by heat treatment (Smith and Circle, 1972; Viola et al., 1982).

These losses can be corrected in fish diet by the addition of synthetic amino acids (Rumsey and Ketola, 1975).

Fish meal had higher mean values for the crude protein and total ash than most other feedstuffs analysed. Tilapia was lower in crude protein and higher in total ash than the clupeids. Fish meal was high in calcium, phosphorus and potassium. It is therefore a good source of calcium phosphorus and all the other minerals required by fish (Lovell, 1981b). The amino acid composition of fish meals, showed optimal values for indispensable amino acids. Fish meals were rich in lysine and methionine, two indispensable amino acids in short supply in plant protein feedstuffs. The exceptionally well balanced amino acids distribution of fish meal has made it a traditional component of fish feeds (Akiyama, 1988).

4.5.0 CONCLUSION

The chemical composition of feedstuffs were generally close to those reported by earlier workers (Oyenuga, 1968; Fetuga, 1972; Fetuga et al., 1973, 1974; Miller, 1976, NRC, 1983; West et al.,

1988). However, slight differences were observed in a few instances. These variations may be due to the application of different processing methods, as well as environmental factors such as soil, climate, moisture etc. Further variations may result from the application of different analytical and hydrolytic techniques. These differences indicate the problems that may arise if feed formulations are based solely on literature values.

CHAPTER FIVE

THE EFFECT OF DIFFERENT METHODS OF SOYABEAN PROCESSING ON THE GROWTH AND FOOD UTILIZATION OF CLARIAS ANGUILLARIS FINGERLINGS.

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5.1.0 INTRODUCTION

The growing popularity of soyabean as a protein source in animal feeds is attributed to its high nutritive value and availability. Soyabean protein is considered the best plant protein source for meeting the essential amino acid requirement of fish (Akiyama, 1988). Soya protein is highly digestible to fish and the digestion coefficient are comparable or higher than fish meal protein (Akiyama, 1988). Currently, soyabean is relatively cheaper than other high protein feedstuffs.

In recognition of the key role of soyabeans in the development of aquaculture, through the production of animal feeds, soyabean has attracted a wide and diverse interests from food scientists, technologist, fish nutritionists and consumers. This has evolved research into how best this locally available plant feedstuff could be processed into fish feed. Although soyabean is highly nutritious, raw soyabean has been shown to possess antinutritional substances mainly trypsin inhibitors (Kunitz, 1945; Birk et al., 1963). These trypsin

inhibitors cause reduced growth and enlargement of the pancreas in fish fed raw soyabean diets (Rackis, 1974). Trypsin inhibitors are inactivated by heat (Rackis, 1974; Kakade et al., 1973). Studies on effect of soyabean processing methods on the growth of fish has not been given adequate attention in fish nutrition. Research in this area will bring into focus the real potentials of soyabean as an excellent protein source in fish diet. The specific objective of the study were:

(i) To determine the effect of heat treatment of soyabean meal on the growth and food utilization of Clarias anguillaris in an outdoor system.

(ii) To determine effect of heat treatment on the trypsin inhibitor level of soyabean.

(iii) To determine the effect of heat treatment on the nutritive value of soyabean.

5.2.0 MATERIALS AND METHODS

Raw soyabean supplied by the production unit of National Institute for Freshwater Fisheries Research (NIFFR) were subjected to seven different processing methods as follows:

Treatment (1) Raw soyabean (RS)

The raw soyabeans were partially ground in a hammer mill to ease removal of the hull when winnowed. The soyabeans were thereafter ground into powder.

Treatment (2) Soyabean Residue (SR)

Raw soyabean were parboiled for one and a half hours and then washed with water to remove the coat. The dehulled beans were milled into a paste. The soyabean paste was mixed with water and then sieved to extract the fat and milk. The residue was sundried and ground into powder with the hammer mill.

Treatment (3) Traditionally roasted soyabean meal (TRS)

Raw soyabeans were roasted in a frying pan with turning until they started burning. The

soyabeans were quickly removed and dehulled before grinding.

Treatment (4) Full Fat soyabean meal roasted for 15 minutes at 100°C (FS 15)

Raw soyabeans were ovendried for 15 minutes at 100°C. The beans were dehulled, winnowed and ground into powder with a hammer mill.

Treatment (5) Full Fat soyabean meal roasted for 30 minutes at 100°C (FS 30)

Raw soyabeans were ovendried for 30 minutes at 100°C. The roasted soyabean were dehulled, winnowed and ground into powder with a hammer mill.

Treatment (6) Full Fat soyabean mean roasted for 1 hour at 100°C (FS 1)

Raw soyabeans were ovendried for 1 hour at 100°C. The roasted beans were dehulled, winnowed and ground into powder with a hammer mill.

Treatment (7) Full Fat soyabean roasted for 2 hours at 100°C (FS 2)

Raw soyabeans were ovendried for 2 hours at 100°C. The roasted soyabeans were dehulled, winnowed and ground into powder before use.

Treatment (8) Control Experiment

Tilapia was collected from the reservoir, boiled and sundried. The dried fish were ground into powder to produce a fish meal.

Carbohydrate Source

Yellow maize was ground into powder and used as the major carbohydrate source.

Binder

Cod liver oil was used to bind the feed and to supply the essential fatty acids (FFA).

Premix

The vitamin and mineral premixes were supplied by poultry Grower Premix (M and B Embavit trade mark No. 9 see Appendix 6).

5.2.1 Feed and Feeding

Table 5.1 shows the ingredient composition of the diet fed to the mudfish. The feed mixtures were made into a dough with hotwater before pelleting in a kitchen hand pelleter. The pelleted feeds were sundried and fed to the fish.

Table 5.1. Composition of the experimental ration (g/100g)

Ingredients	RATION							
	1	2	3	4	5	6	7	8
Soyabean meal	79	79	77	77	78	76	76	-
Fish meal	-	-	-	-	-	-	-	50
Yellow maize	17	17	19	19	18	20	20	46
Cod liver oil +	2	2	2	2	2	2	2	2
Vitamin and mineral Premix+	2	2	2	2	2	2	2	2
Total	100	100	100	100	100	100	100	100

1 = Raw soyabean

2 = Soyabean residue

3 = Traditionally roasted soyabean meal

4 = Soyabean meal ovendried for 15 minutes at 100°C

5 = Soyabean meal ovendried for 30 minutes at 100°C

6 = Soyabean meal ovendried for 1 hour at 100°C

7 = Soyabean meal ovendried for 2 hours at 100°C

8 = Fish meal (control) diet

+ = Composition given in Appendix 6

A total of 16 hapas measuring 1m x 1m x 1m were suspended in an outdoor concrete tank measuring 10m x 10m x 2m. The experimental fish were collected from one of the outdoor concrete tanks and acclimated in the hapas for one week at which time they were fed uniform diets. After acclimation fish were starved for 24 hours before feeding with the experimental feeds. The initial weight of fish were taken and subsequent measurements were taken fortnightly. Feeding rate was at 5% biomass. Fish were fed twice daily morning and evening between 8.30 a.m. - 9.30 a.m. and 5.30 - 6.30 p.m. respectively.

5.2.2 Chemical Analysis

Chemical analysis of feed and fish samples was carried out in triplicate. Crude protein was determined by the Kjeldahl method, AOAC (1975). Fat was determined by the modified Bligh and Dyer method (Hanson and Olley, 1963).

Residual moisture was determined by oven-drying the feed for 24 hours at 105°C to a constant weight. Ash was determined by ignition in the furnace at 550°C for 24 hours (AOAC, 1975).

5.2.3 Water Analysis

Water samples were taken weekly for the determination of dissolved oxygen, temperature and pH (See section 3.4.0).

5.2.4 Protein solubility Index (PSI)

This was determined according to the method of Dale et al. (1987) (See section 3.3.9).

5.2.5 Food Utilization Parameters

The extent of utilization of each diet by the mudfish fingerlings was examined at the end of the experiment. The food utilization parameters determined were the specific growth rate (SGR), feed conversion efficiency (FCE), protein efficiency ratio (PER) and apparent net protein utilization (NPU). The formular of these parameters are shown in section 3.8.0.

5.2.6 Amino Acid Analysis

The amino acid analysis of the samples were carried out using a technicon amino acid analyser

at the Department of Zoology, University of Jos, Plateau State (See section 3.3.7).

5.2.7 Statistical Analysis

Statistical analysis of the data was carried out using the computer (See section 3.9.0).

5.2.8 Physico-chemical Parameters

The analysis of the physico-chemical parameters of water in the concrete tank was discussed in 3.4.0.

5.3.0 RESULTS

5.3.1 Proximate Composition of Feed Mixture

The proximate composition of the experimental diets is shown in Table 5.2. As far as possible all diets were formulated in such a way as to make them isonitrogenous (approx. 35% crude protein) and isocaloric. The analysed crude protein, however varied from 32.90% in the control fish meal diet to 35.93% in diet 3. All other diets contained a crude protein of approximately 35% except diet 2 compounded from soyabean residue after the milk had been extracted.

Table 5.2. Proximate composition of experimental diet (g/100g)

Component	R A T I O N							
	1	2	3	4	5	6	7	8
Moisture	8.15	7.68	7.79	8.10	7.88	7.64	7.15	8 57
Crude protein	35.77	33.27	35.93	35.03	35.03	35.27	35.88	32.90
Crude fat	9.11	8.50	9.20	9.50	10.00	9.60	9.80	7.00
Ash	9.30	5.30	9.10	9.82	8.58	7.65	9.64	15.88
Sub-totals	62.33	54.75	62.02	62.45	61.49	60.15	62.47	64.35
NFE	37.67	45.25	37.98	37.55	38.51	39.85	37.53	35.65
Dry matter	91.79	92.32	92.21	91.90	92.12	92.37	92.85	91.43

% Nitrogen free extract computed as 100 - sub-totals

The mixture gave a crude protein content of 33.27%. A similar trend was observed in the fat content. The ash content was highest in diet 8 (15.88%) and lowest in diet 2 (5.30%). Other mixtures had ash content ranging from 7.65% to 9.64%. The NFE varied from 35.65% in the control ration (Diet 8) to 45.25% in diet 2. The dry matter showed only slight variations from 91.43% in diet 8 to 92.85% in diet 7.

5.3.2 Physico-chemical Parameters

Data on the weekly physico-chemical parameters of the water in the fish tank are shown in Table 5.3. The pH was generally neutral throughout the duration of the experiment (7.0 - 7.8). The mean value was 7.48 ± 0.27. The temperature was slightly low (23.6°C) at the commencement of the experiment due to influence of harmattan but rose to 28.5°C at the end of the experiment. The mean temperature was 26.07 ± 1.92. The dissolved oxygen ranged between 4.0 and 6.95 with a mean of 5.50 ± 0.99. The above recorded physico-chemical parameters were considered adequate for the culture of tropical fish (See Appendix 4).

Table 5.3. Physico-chemical parameters of the concrete tank during the experimental period.

Date	Temperature °C	pH	Dissolved Oxygen (DO) mg/l
12/1/89	23.6	7.0	4.0
19/1/89	23.9	7.1	4.5
26/1/89	24.0	7.2	4.5
2/2/89	24.5	7.5	5.0
9/2/89	25.5	7.5	5.0
16/2/89	26.0	7.6	5.5
23/2/89	26.5	7.6	5.8
2/3/89	27.5	7.5	6.0
9/3/89	28.3	7.7	6.5
16/3/89	28.5	7.8	6.8
23/3/89	28.5	7.8	6.95
Mean	26.07	7.48	5.50
Standard Deviation	1.92	0.27	0.99

5.3.3 Gain in Weight

Table 5.4 shows the growth performance of C. anguillaris fingerlings fed experimental diets. There was no significant difference ($P>0.05$) in the initial mean weight of the fish. However, significant differences ($P<0.05$) were observed in the final weight of the fish taken at the end of the experiment indicating that the various feed mixtures had significant effects on the growth of the fish. The fish fed the fish meal control diets (Diet 8) recorded the highest mean weight gain of 2.21 ± 0.78g while those fed diet 1 gave the least weight gain of 0.75±0.23g. Among the fish fed soyabean diets the best growth rate was obtained in fish fed diet 5. This was followed by fish fed diet 4. Fish fed diets 6 and 7 gave lower growth rate of 1.51±0.63g and 0.82±0.57g respectively. The fish fed diet 2 gave a weight gain of 0.93 ± 1.14g.

5.3.4 Specific Growth Rate (SGR)

The SGR varied from 0.33 in diet 1 to 0.79 in diet 8. There was no significant difference

Table 5.4. Growth performance of Clarias anguillaris fingerlings fed experimental diets for 70 days

Feed No.	Initial Mean weight (g) ± SD	Final Mean weight (g) ± SD	Mean weight gain (g) ± SD	% Weight Gain (g)	Specific Growth Rate (SGR)	Feed Conversion Efficiency (FCE)	Protein Efficiency Ratio (PER)	Apparent Net protein Utilization % (NPU)
1	2.83 ±0.02	3.50^{a}±0.25	0.75^{a}±0.23	26.50^{a}	0.33^{a}	10.05^{a}	0.28^{a}	11.12^{a}
2	2.80 ±0	3.73^{a}±1.14	0.93^{a}±1.14	33.21^{b}	0.37^{b}	13.03^{b}	0.39^{b}	5.92^{b}
3	2.75 ±0.04	3.83^{a}±0.21	1.08^{a}±0.16	39.27^{c}	0.46^{c}	17.59^{c}	0.49^{c}	9.78^{c}
4	2.88 ±0.04	4.56^{b}±0.06	1.68^{b}±0.02	58.33^{d}	0.65^{d}	20.44^{d}	0.58^{d}	11.22^{a}
5	2.91 ±0.01	4.78^{b}±0.41	1.87^{b}±0.40	64.26^{e}	0.70^{e}	19.87^{d}	0.57^{d}	14.93^{d}
6	2.81 ±0.05	4.32^{b}±0.68	1.51^{b}±0.63	57.74^{d}	0.64^{f}	19.61^{d}	0.56^{d}	10.26^{c}
7	2.87 ±0.01	3.69^{a}±0.57	0.82^{a}±0.57	28.57^{a}	0.34^{a}	12.79^{b}	0.36^{e}	11.97^{a}
8	2.92 ±0.05 n.s	5.13^{c}±0.83 *	2.21^{c}±0.78 *	75.68^{f} *	0.79^{g} *	26.25^{e} *	0.80^{f} *	21.43^{e} *

Note: Figures followed by the same letters in each column are not significantly different (P>0.05)

SD = Standard Deviation

n.s = not significant

* = Significant at 0.05

between the SGR of fish fed diet 1 and those fed diet 7 (P>0.05). The highest SGR was observed in fish fed fish meal control diet (Diet 8) followed by those fed diet 5. The SGR of fish fed diets 2, 3, 4 and 6 were significantly different (P<0.05).

5.3.5 Feed Conversion Efficiency (FCE)

The control ration gave the best FCE of 26.25 which was expected due to high nutritive value of fish meal. The fish fed diet 1 gave the least FCE values of 10.05. Fish fed diet 4 gave FCE values of 20.44 which was higher than that of fish fed diet 5 (19.87). The FCE values of other soyabean meals were lower.

5.3.6 Protein Efficiency Ration (PER)

There was no significant difference (P>0.05) in the PER of fish fed diets 4, 5 and 6. There was significant difference (P<0.05) in the PER of fish fed the other soyabean diets. The PER of the control ration (Diet 8) which had the lowest protein content was significantly higher than the other treatment (P<0.05).

5.3.7 Apparent Net Protein Utilization (Apparent NPU)

The apparent NPU which measures the nutritive value of the feed was highest in the control fish meal ration (Diet 8) and lowest in fish fed diet 2. Fish fed diet 5 had apparent NPU of 14.93% which was greater than those of other fish fed soyabean meal. There were, however, no significant differences ($P>0.05$) between the apparent NPU of fish fed diets 1, 4 and 7.

5.3.8 Protein Solubility Index (PSI)

The data in Table 5.5 shows the percentage PSI of the soyabean meal. The least PSI of 49.9% was recorded in the soyabean residue followed by the traditionally roasted soyabean meal which scored 66.6%. The highest PSI was obtained from the raw soyabean meal.

5.3.9 Essential Amino Acid Content of Soyabean Meal (EAA)

The EAA content of the raw and roasted soyabean meal is shown in Table 5.7. The lysine content was lowest in the soyabean meal roasted at 100°C for 2 hrs and highest in the meal roasted for 30 minutes at 100°C. The raw soyabean meal

Table 5.5. Protein solubility index (per cent) of the processed soyabean meal

Parameters	Protein solubility index
Raw soyabean	90.3
Soyabean residue	49.9
Traditionally roasted soyabean meal	66.6
Soyabean meal ovendried for 15 minutes at 100°C	73 3
Soyabean meal ovendried for 30 minutes at 100°C	73.3
Soyabean meal ovendried for 1 hour at 100°C	70.5
Soyabean meal ovendried for 2 hours at 100°C	75.5

recorded a lysine content of 9.59 mg/16g N. This value was lower than that obtained for soyabean meal roasted for 30 minutes at 100°C which recorded 14.37 mg/16 g N, and higher than the soyabean residue which recorded 7.96 mg/16g N. Similar trend was observed in other essential amino acids analysed notably histidine, arginine, valine, methionine, isoleucine, leucine, threonine, phenylalanine. The values of methionine were generally very low.

5.4.0 DISCUSSION

In this study, fingerlings of C. anguillaris fed fish meal control diets produced higher mean weight gain than those fed soyabean diets (Table 5.4). This improved growth rate in fish fed fish meal diets have been observed by other workers (Spinelli et al., 1979; Dabrowski and Kozak, 1979; Abel et al., 1984). Among the soyabean diets, the mean weight gain was higher in C. anguillaris fingerlings fed soyabean diets which had been subjected to heat processing than fingerlings fed the unheated soyabean rations. The reason is that raw soyabean meal

Table 5.6. The correlation between protein solubility index (per cent), and trypsin inhibitor (mg/g meal) in soyabean meal*

Protein solubility	Trypsin inhibitor
90.2	21.0
87.7	12.3
79.1	3.1
83.2	2.2
74.9	2.1
71.8	1.0
58.5	0.5
38.0	0.1

* Data from Dale et al. (1987)

Table 5.7. Essential Amino Acid content of Processed soyabean meal (mg/16g N)

Amino Acids	Raw Soyabean Meal	Soyabean Residue+	Soyabean Meal toasted traditionally	Soyabean Meal Roasted at 100 °C for 15 minutes	Soyabean Meal Roasted at 100°C for 30 minutes	Soyabean Meal Roasted at 100°C for 1 hour	Soyabean Meal Roasted at 100°C for 2 hours
Lysine	9.59	7.96	9.42	7.63	14.37	9.77	5.96
Histidine	3.53	2.93	3.35	4.76	5.79	4.92	3.63
Arginine	15.14	8.34	11.20	12.96	21.12	14.30	9.18
Valine	4.4	4.76	6.23	4 64	8.43	6.79	5.68
Methionine	0.67	0.86	0.91	0.73	1.16	0.79	0.54
Isoleucine	4.80	5.46	4.29	5.23	6.63	5.53	3.80
Leucine	7.93	9.00	8.13	7.98	13.51	11.51	7.00
Threonine	4.10	4.70	3.86	4.45	6.77	5.63	3.34
Phenyl-alanine	4.14	4.94	5.31	5.64	9.88	7.25	4.98

* Residue after extraction of Soyabean milk

contain antinutritional factors which are inactivated by high temperature (Smith, 1977; Lovell, 1984; Robinson, 1984).

Among the antinutritional factors, trypsin inhibitors appear to be predominant (Kunitz, 1945; Birk et al., 1963). This inhibitor has bean implicated in causing pancreatic hypertrophy with excessive endogenous protein losses (Booth et al., 1960; Rackis, 1972). The protein solubility index is used to measure the trypsin inhibitor concentration because it seems to correlate better to the trypsin inhibitor concentration than the urease activity (Dale et al., 1987). The results obtained from this studies (Table 5.5) were compared with data of Dale et al. (1987) (Table 5.6), which indicate the correlation between the PSI and trypsin inhibitor in soyabean meal. In general, as the protein solubility index increased from 38.0 to 90.2%, the trypsin inhibitor increased proportionately from 0.1 mg/g meal to 21.0 mg/g meal. Further analysis of the result obtained indicates that except for the raw soyabean meal, the values

of the other soyabean meals fall within the trypsin inhibitor level of 1 - 3mg per g soyabean meal, which is considered good for aquaculture feeds (Akiyama, 1988). The optimal protein solubility index for aquaculture feeds was found to be 60 - 80 (Akiyama, 1988). This shows that the raw soyabean meal was of little nutritive value in fish feeds.

There was a progressive increase in the growth performance of C. anguillaris fed heated soyabean diets exposed to 15 minutes and 30 minutes at a temperature of 100°C, followed by a decline in growth rate among fingerlings fed soyabean diets heated at 100°C for 1 and 2 hours. This indicates that time of exposure of soyabean meal to heat treatment had a profound effect on the nutritive value as long processing time can lead to a decrease in amino acid availability (Dabrowski and Kozak, 1979). This observation was corroborated by the losses recorded in the essential amino acids content of soyabean meal when heated at 100°C for 1 and 2 hours (Table 5.7).

The high growth rate of fish fed soyabean diets roasted at 100°C for 30 minutes could be attributed to the high lysine value of the feed since a properly roasted soyabean meal is considered a fairly good source of lysine (Viola et al., 1983). Lysine is said to possess growth factor which has been confirmed by observations of energy and protein retentions of this amino acid (Viola et al., 1982).

The extremely poor growth rate of fish fed soyabean diets roasted at 100°C for 2 hours could be related to the low lysine values in the over heated soyabean meal which could probably be due to Maillard reaction (Maynard et al., 1979). This reaction had been implicated in causing the formation of unavailable sugar - amino complexes and the destruction of heat sensitive amino acids i.e. lysine and cystine (Smith and Circle, 1972; Maynard et al., 1979). The usual heat treatment encountered during traditional processing of soyabean meal appear to give less damage to the soyabean meal due to the short exposure time in the process. This was responsible for the better growth in fish

fed the traditionally processed soyabean diet compared with fingerlings fed overheated soyabean deits. The growth performance of fish fed the soyabean residue was poor due to the leaching of nutrients into the milk fraction during the extraction process.

5.5.0. CONCLUSION

Soyabean contains trypsin inhibitor which affect growth of C. anguillaris fed raw soyabean diets. Excessive heating of soyabean inactivates lysine thereby causing reduced growth rate of the fingerlings.

CHAPTER SIX

THE EFFECT OF SUPPLEMENTATION OF SOYABEAN DIET WITH L- AND D,L-METHIONINE ON THE GROWTH OF CLARIAS ANGUILLARIS FINGERLINGS.

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5.1.0 INTRODUCTION

Soyabean meal is generally regarded as one of the best sources of protein available for animal feeding (McDonald et al., 1979). In the United States and Canada soyabean meal has become the key feed among the protein supplements of plant origin and because of its high biological value this feed has now replaced most of the meat meal and fish meal which were in the past the main stay of protein quality in rations for non-herbivorous animals (Harris, 1980). Soyabean is one of the most abundant plant proteins available for fish feeds and considerable works have been devoted for its use in fish diets (Dabrowska and Wojno, 1977; Nose, 1978; Viola et al., 1983; Abel et al., 1984). Although the meal contains all the essential amino acids in its protein, the amount of methionine present is sub-optimal (McDonald et al., 1979; Nose, 1978).

One solution to the use in practical diets of proteins that are relatively deficient in one or more amino acids is to supplement the protein with appropriate amounts

of the amino acid in question (Cowey, 1978). Kaneko (1969) obtained positive results by feeding rainbow trout with feed containing 30% of soyabean meal enriched with methionine and lysine. Similarly, Koops et al. (1976) found that one quarter of fish meal can be replaced by soyabean meal enriched with methionine in the diets of rainbow trout. Soyabean meal has been supplemented with methionine in rainbow trout feeds with positive results (Steffens and Albrecht, 1976). Addition of methionine, lysine, histidine and leucine individually and in several combinations to the diets containing soyabean meal as the sole source of protein did not improve the growth of rainbow trout, however, supplement of methionine, lysine, leucine, valine, histidine, threonine, tryptophan and tyrosine in a diet to simulate these levels of those of trout eggs significantly improved the growth (Rumsey and Ketola, 1975. Murai et al. (1982) demonstrated that channel catfish can utilize synthetic methionine added to soyabean meal protein. The above results suggest that soyabean meal could be a major protein source in fish diet if the amino acid

balance is adjusted by the use of crystalline amino acids.

This study was undertaken to determine the best level of L- and D. L-methionine in heat treated full fat soyabean diet that would guarantee optimum utilization of soyabean protein by Clarias anguillaris fingerlings.

6.2.0 MATERIALS AND METHOD

The experiments were conducted in the Institute's hatchery complex using indoor flow-through tanks for the soyabean diet containing L-methionine. Hapas suspended in outdoor concrete tanks were used for the D. L-methionine supplemented diet experiments.

6.2.1 Indoor Experiment for L-methionine

A total of 360 mudfish, Clarias anguillaris fingerlings having mean weight of 17.20 ± 0.27g and measuring from 13.0 to 15.4cm with mean total length of 13.94 ± 0.74cm was used for the study. The fish were distributed into 18 plastic tanks 0.25m deep and 0.55m in diameter, (20 fish per tank) each tank maintaining approximately 30 litres of water.

Water in the plastic tank was kept in circulation by supply tube through which water passed into the tank and a discharge tube that conveyed the over flow into the drain. The experiment was conducted for 12 weeks between 3 June and 26 August 1988. The fish were acclimated in the indoor tank for one week before the commencement of the experiment (See section 3.6.0).

6.2.2 Outdoor Experiment for D, L-Methionine

Hapa nets constructed into 18 hapa cages with dimension 1 x 1 x 1 cubic metre were used for the outdoor experiment. 360 mudfish fingerlings having mean weight 1.0g and total length of 5 to 7cm were acclimated for one week in the outdoor concrete tank before they were stocked in the hapas (See section 3.6.0). The hapas were suspended in concrete tanks measuring 10m x 10m x 2m containing one metre deep water. The 14 weeks observation commenced from 8 October 1988 to 14 January 1989.

6.2.3 Feed and Feeding Levels

The raw soyabean was roasted on a heated pan to remove the trypsin inhibitor (Kunitz, 1945), and then milled after dehulling. The ground full fat soyabean meal was mixed with other ingredients shown in Table 6.1 and pelleted using the domestic pelleter. A total of six pelleted feeds were prepared. Pelleted feeds Nos. 1 and 6 were taken as controls (A and B). The control casein diet used for experiment 1 (DT6 i) was replaced by fish meal (DT6 ii) in experiment 2. 0.2%, 0.4%, 0.6% and 0.8% L- and D. L- methionine for experiments 1 and 2 respectively were added to feed Nos. 2, 3, 4 and 5 respectively and the diet made isonitrogenous by the addition of aspartic acid. Fish were fed twice daily at 9.00 a.m. and 4.00 p.m. with a total daily ration equal to 3% of their body weight. Measurements of fish weight and total length were taken fortnightly during which time the tanks and hapas were washed thoroughly.

6.2.4 Chemical Analysis

The crude protein was determined according to the AOAC (1975) methods.

Table 6.1. Composition of experimental diets (g/100g).

COMPONENT	DIET COMPOSITION						
	DT 1	DT 2	DT 3	DT 4	DT 5	DT 6(i)*	DT 6(ii)**
Soyabean meal	77	77	77	77	77	-	-
Casein	-	-	-	-	-	40	-
Fish meal	-	-	-	-	-	-	51
+ Methionine	-	0.2	0.4	0.6	0.8	-	-
Aspartic acid	1	0.8	0.6	0.4	0.2	3.5	-
++ Cod liver oil	2	2	2	2	2	2	2
Yellow maize	18	18	18	18	18	52.5	45
++ Vitamin and Mineral premix	2	2	2	2	2	2	2
Calculated crude protein %	36.68	36.68	36.68	36.68	36.68	36.68	35.30
Analysed crude protein %	35.82	34.95	35.3	34.85	35.40	35.75	34.55

+ L-Methionine was susbstituted for D,L - Methionine in the second experiment

++ The composition of Cod liver oil and vitamin /mineral premixes are shown in Appendix 6.

* Casein diet (control B) for the experimental fish in indoor flow-through tank fed L-Methionine.

** Fish meal diet (control B) for the experimental fish in outdoor hapas fed D, L-Methionine.

6.2.5 Food Utilization Parameters

The food utilization parameters used for the experiment were specific growth rate, feed conversion efficiency and protein efficiency ratio. Details on the application of these parameters are shown in section 3.8.0.

6.2.6 Physico-chemical Parameters

The analysis of water in the mini flow-through tanks and hapas for the physico-chemical parameters such as temperature, pH and oxygen were conducted weekly. Methods of determination of these parameters are shown in 3.4.0.

6.2.7 Statistical Analysis

Growth data were analysed statistically using the computer (Tandy 4000 Lx). Means were compared by the analysis of variance and the multiple range test (See section 3.9.0).

6.3.0 RESULTS

Table 6.2 shows the growth and food utilization of C. anguillaris fingerlings fed L-methionine supplemented soyabean diet. Diets 3, 4 and 5 were poorly utilised by the fish. No significant difference ($P>0.05$) was obtained

Table 6.2. Growth and food utilization of Clarias anguillaris fed soyabean diets containing different levels of L-Methionine for 12 weeks in indoor flow-through tanks.

	DIET DESIGNATION						
	DT 1	DT 2	DT 3	DT 4	DT 5	DT 6	SEM
Dietary Methionine (g/mg)	-	0.2	0.4	0.6	0.8	-	
Mean initial Weight (g)	17.10 ± 4.20	17.00 ± 4.86	17.00 ± 2.73	17.10 ± 2.52	17.70 ± 4.62	17.30 ± 1.00	0.10 n.s
Mean final weight (g)	19.90^{b} ± 3.15	29.59^{b} ± 2.50	21.00^{d} ± 4.10	21.26^{d} ± 3.21	22.67^{e} ± 1.68	35.70^{c} ± 2.17	2.56 *
% Weight gain	16.37^{b}	74.06^{d}	23.53^{d}	24.33^{e}	28.08^{e}	106.36^{c}	2.45 *
Specific Growth Rate (SGR)	0.18^{b}	0.66^{a}	0.25^{d}	0.26^{d}	0.29^{d}	0.86^{c}	0.11 *
Feed Conversion Efficiency (FCE)	6.07^{b}	21.85^{d}	7.44^{d}	8.20^{e}	7.58^{d}	25.32^{c}	3.47 *
Protein Efficiency Ratio (PER)	0.17^{b}	0.63^{a}	0.21^{d}	0.24^{d}	0.21^{d}	0.71^{c}	0.10 *

Note: Figures followed by the same letters in each row are not significantly different (P>0.05)

n.s. = not significant

* = significant at 0.05

in the percentage weight gain of fish fed diets 3 and 4. The best growth was obtained in fish fed casein diet (Diet 6) followed by those fed 0.2% L-methionine (Diet 2). Poor growth which was significantly different ($P<0.05$) was observed in fish fed the unsupplemented heat treated full fat soyabean diet (Diet 1) (Figure 6.1). In fish grown in hapas (Table 6.3) fingerlings fed diet 2 had higher mean weight gain which was statistically significant ($P<0.05$) compared with those fed diets 3, 4 and 5. Fingerlings fed (Diet 6) recorded the highest mean weight gain while the poorest was obtained among fingerlings fed diet 1 (Figure 6.2).

6.3.1 Specific Growth Rate (SGR)

The data in Table 6.2 shows that the SGR of fingerlings fed diet 2 was significantly higher ($P<0.05$) than those fed diets 3, 4 and 5. The control ration (Diet 6) gave the highest SGR while diet 1 recorded the least. In Table 6.3 similar trend in SGR was obtained as in Table 6.2. The SGR of fingerlings fed diets 1 and 3 were not statistically significant ($P>0.05$). Also the

Table 6.3. Growth and food utilization of Clarias anguillaris fingerlings fed soyabean diets containing different levels of D,L-Methionine for 14 weeks in outdoor hapas.

	D I E T D E S I G N A T I O N						
	DT 1	DT 2	DT 3	DT 4	DT 5	DT 6	SEM
Dietary Methionine (g/100g)	-	0.2	0.4	0.6	0.8	-	-
Mean initial weight (g)	1.03 ± 0.01	1.02 ± 0.01	1.03 ± 0.02	1.01 ± 0.0	1.00 ± 0	1.01 ± 0	0.01 n.s
Mean final weight (g)	3.83^{b} ± 0.44	4.25^{a} ± 0.65	3.70^{b} ± 0.33	3.05^{d} ± 0.35	3.00^{d} ± 0.55	5.04^{c} ± 0.54	0.31 *
% Weight gain	271.84^{b}	316.67^{a}	260.19^{b}	201.98^{d}	200.98^{d}	339.00^{c}	30.72 *
Specific Growth Rate (SGR)	1.34^{b}	1.46^{a}	1.31^{b}	1.13^{d}	1.12^{d}	1.64^{c}	0.08 *
Feed Conversion Efficiency (FCE)	28.51^{b}	30.13^{a}	24.63^{d}	26.29^{e}	25.22^{d}	41.59^{c}	2.58 *
Protein Efficiency Ratio (PER)	0.80^{b}	0.86^{a}	0.70^{d}	0.76^{e}	0.71^{d}	1.20^{c}	0.08 *

Note: Figures followed by the same letters in each row are not significantly different (P>0.05)

n.s = not significant

* = significant at 0.05

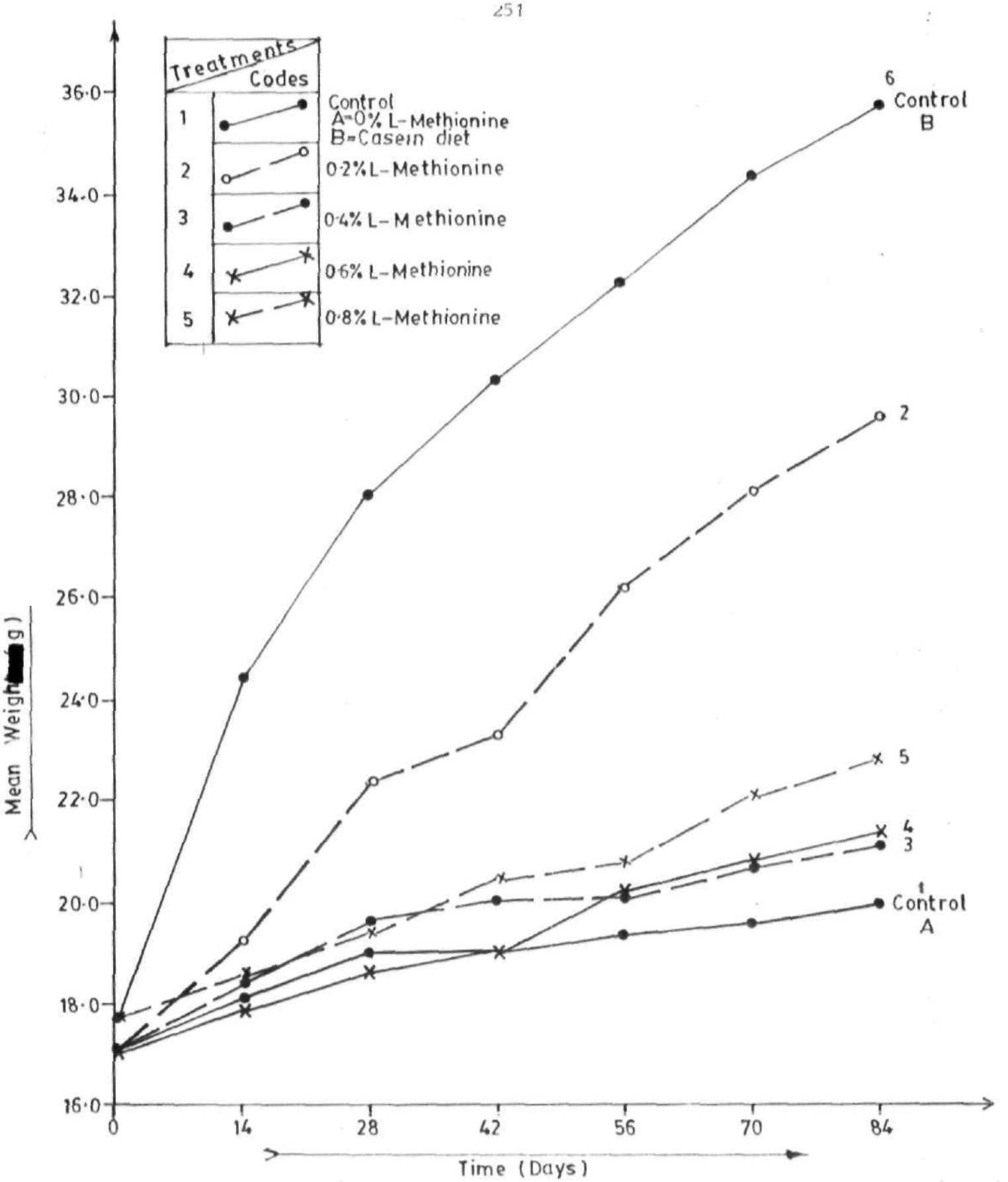


Fig. 6.1. Growth of Clarias anguillaris fingerlings fed soyabean diets containing various levels of L-methionine in indoor mini flow-through tanks.

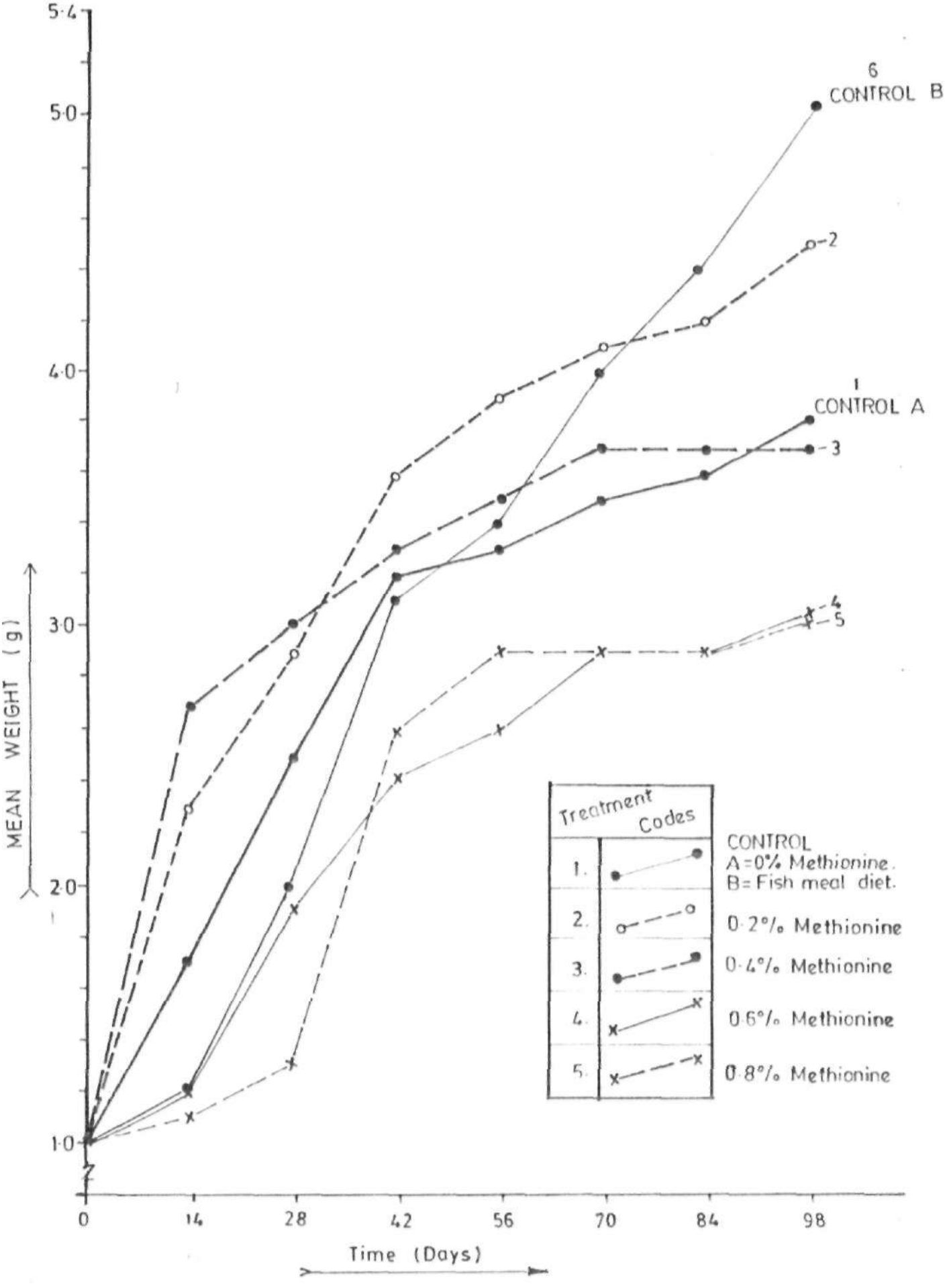


Fig. 6·2. Growth of Clarias anguillaris fingerlings fed soyabean diets containing various levels of D. L– methionine in outdoor hapas.

SGR of fingerlings fed diets 4 and 5 were not significantly different ($P>0.05$). The highest SGR was recorded in fingerlings fed diet 6 while the lowest was among those fed diet 1.

6.3.2 Feed Conversion Efficiency (FCE)

The best FCE in fish grown in tanks (Table 6.2) was recorded in fingerlings fed diet 6 followed by those fed diet 2. The latter was significantly higher ($P<0.05$) than those fed diets 3, 4 and 5. FCE of fingerlings fed diet 1 was the lowest.

In fingerlings grown in hapas (Table 6.3) the FCE was highest in fingerlings fed diet 6 followed by those fed diet 2. The latter was significantly different from those fed diets 3, 4 and 5 ($P>0.05$). The FCE of fingerlings fed diet 1 was the lowest.

6.3.3 Protein Efficiency Ratio (PER)

In fingerlings fed rations supplemented with L-methionine (Table 6.2), there was significant difference ($P<0.05$) between the PER of fish fed diet 2 and those fed diets 3, 4 and 5. Fingerlings fed diet 6 scored the

highest PER (0.71) while the lowest was obtained by fingerlings fed diet 1 (0.17).

Among the fingerlings fed D, L-methionine (Table 6.3), the highest PER was recorded by fish fed diet 6 (1.20) while the least (0.70) was obtained in fingerlings fed diet 3. Significant difference ($P<0.05$) was obtained in fingerlings fed the experimental diets except those fed diets 3 and 5 which did not show any significant difference ($P>0.05$) in the PER.

6.3.4 The Physico-chemical Parameters

Results of the physico-chemical parameters of the water in the mini flow-through tanks and hapas are recorded in Tables 6.4 and 6.5 respectively. In Table 6.4 the pH ranged from 7.3 to 7.6 with a mean pH of 7.45 ± 0.15. The dissolved oxygen varied from 6.0 to 8.0mg/l with a mean of 6.95 ± 0.59mg/l while the temperature was between 25.0 and 26.8°C with a mean of 25.85 ± 0.49. In Table 6.5 the pH ranged from 6.6 to 7.8 with a mean of 7.29 ± 0.35, while the dissolved oxygen varied between 7.2 and 16.0mg/l with a mean of 10.07 ± 3.59mg/l.

Table 6.4. Weekly record of the physico-chemical parameters of water in the mini flow-through tanks.

Date	pH	Dissolved Oxygen (DO) mg/l	Temperature °C
3/6/88	7.3	8.0	26.0
10/6/88	7.3	7.4	26.0
17/6/88	7.4	7.6	25.8
24/6/88	7.3	7.0	25.3
1/7/88	7.6	6.8	25.5
8/7/88	7.6	7.0	26.0
15/7/88	7.6	6.8	26.5
22/7/88	7.4	7.4	26.8
29/7/88	7.6	7.2	26.2
5/8/88	7.3	6.8	25.8
12/8/88	7.3	6.4	25.5
19/8/88	7.6	6.0	25.0
26/8/88	7.6	6.0	25.6
Mean	7.45	6.95	25.85
Standard Deviation	0.15	0.59	0.49

Table 6.5. Weekly record of the physico-chemical parameters of water in the outdoor hapas.

Date	pH	Dissolved Oxygen (DO) mg/l	Temperature °C
8/10/88	6.6	16.0	30.0
15/10/88	7.0	16.0	29.5
22/10/88	7.6	15.5	29.5
29/10/88	7.6	14.0	29.0
5/11/88	7.6	12.0	29.0
12/11/88	7.6	10.0	28.5
19/11/88	7.4	8.5	29.0
26/11/88	7.6	7.2	26.0
3/12/88	7.8	7.2	25.0
10/12/88	7.4	7.2	24.0
17/12/88	7.0	7.2	24.0
24/12/88	7.2	7.5	23 5
31/12/88	7.0	8.0	19.0
7/ 1 /89	7.0	7.5	19.5
14/ 1 /89	7.0	7.2	18.4
Mean	7.29	10.07	25.59
Standard Deviation	0.35	3.59	4.10

The temperature ranged between 18.4 and 30°C with a mean temperature of 25.59 ± 4.10°C. Except for the slightly low temperatures experienced at the last three weeks of the experiment conducted in hapas, the physico-chemical parameters of water in the two systems were adequate for the culture of C. anguillaris fingerlings (See Appendix 4).

6.4.0 DISCUSSION

Supplementation of heat - treated full fat soyabean diet with 0.2% cystalline methionine significantly increased the growth and food utilization of C. anguillaris fingerlings better than soyabean diets supplemented with 0.4, o.6 and 0.8% methionine. This was the case irrespective of whether the soyabean diet was supplemented with L- or D, L-methionine. The difference in the size of the fingerlings (initial mean weight 17.20g and 1.02g respectively) did not alter the fish requirement for 0.2% crystalline L- and D, L-methionine in the soyabean diet. This shows that C. anguillaris fingerlings can utilise L-methionine as effectively

as D, L-methionine for protein synthesis. Studies with channel catfish have shown that D, L-methionine is utilised as effectively as L-methionine (Robinson et al., 1978).

It is a well known fact that only the L-form of amino acids are usually utilised for protein synthesis. The D, L- form does not substitute for the L- form in most amino acids. Methionine differ from those amino acids that do not substitute the D, L- form for the L- form in that methionine is a straight chain amino acid while the other ones are branched chain. During the metabolism of branch chain amino acids the L- form is first transaminated forming a Keto acid which can then be decarboxylated and further metabolized or can be transaminated back to the amino acid (Freedland and Briggs, 1977). The D- form of the branch chain will also appear as the Keto derivatives after which it is subjected to action from two competing pathways, the one transaminating it to the L- form and then to protein or it could be decarboxylated and further degenerated. The rate of these two activities determines how much the D- amino

acid will necessarily be removed from potential protein synthesis (Freedland and Briggs, 1977). For methionine, the first metabolic step of the L- form is not transamination, the Keto derivative is not an intermediate and there is no pathway for the catabolism of the Keto derivative other than transamination. Therefore the Keto acids arising from action of D-methionine must be transaminated to the L- amino acid before they can be further metabolised (Freedland and Briggs, 1977). Thus D- methionine must appear as their L- forms and consequently will be available for protein synthesis.

Improved growth rate in fish fed methionine supplemented soyabean diet has been reported for other fish species. Murai et al. (1982) demonstrated improved growth rate in carp fed diets containing soyabean meal supplemented with methionine alone. Similarly, addition of methionine to soyabean flour diet was responsible for the improvement in weight gain and feed efficiency of carp fed soyabean flour diet (Murai et al., 1986). Kaneko (1969) experimenting with rainbow trout also obtained positive results

by feeding them with 30% of soyabean meal enriched with methionine and lysine.

However, Rumsey and Ketola (1975) did not improve the value of soyabean meal fed to rainbow trout by the addition of L-methionine, L- lysine, L- histidine and L- leucine individually and in several combinations. Similarly Andrews and Page (1974) reported that poor growth of channel catfish fingerlings fed diets containing soyabean meal was not improved by dietary supplements of methionine, cystine or lysine. The observations of these authors have been criticised in that they were based on supplementing diets that were not deficient in the particular amino acid diets that were not deficient in the particular amino acid requirement since all the quantitative amino acid requirement of channel catfish were only recently elaborated (NRC, 1983).

An increase in the level of crystalline L- and D, L-methionine beyond 0.2% in the soyabean diet caused a significant reduction ($P<0.05$) in the growth rate of C. anguillaris fingerlings corresponding to the relative amount of free

methionine in the diet (Figures 6.1 and 6.2). This observation has been reported for young carps fed amino acids test diets (Aoe et al., 1970).

The growth rate of catfish fed crystalline L-methionine in soyabean diets were significantly higher ($P>0.05$) than those fed the unsupplemented soyabean diets (control A) in the indoor flow-through tank (Figure 6.1). This confirms the observation that soyabean meal contain disproportionate levels of amino acids causing a reduction in growth rate which is corrected by the addition of amino acid (Harper et al., 1970).

The higher growth rate of fingerlings fed the unsupplemented soyabean diets compared with those fed higher (0.4, 0.6 and 0.8%) D, L-methionine levels in hapas (Figure 6.3) was attributed to the fact that fish fed unsupplemented soyabean diets could have obtained some of the methionine from the natural food in the hapas. This observation is particularly important in fish grown in well fertilized ponds. Unlike terrestial animals which may depend wholly on artificial diets, fish grown in ponds have access to exogenous natural foods

therefore some growth might be observed in unsupplemented soyabean diets. The availability of such nutrients will however depend on stocking density since high stocking density essential in intensive fish culture will cause a depletion in the natural source of methionine for the fish. However, 0.2% crystalline L- and D,L-methionine in heat treated full fat soyabean diet has been shown to give better growth and feed utilization than higher methionine levels. Supplementing the soyabean diet with crystalline methionine beyond the level essential for optimal growth is shown to inhibit the growth rate of the fish. This means that higher levels of methionine is toxic to mudfish.

The growth and food utilization indicators were better in the positive control ration than in the other diets. This demonstrates that the natural casein and fish meal diets were better utilized by mudfish than the supplemented crystalline amino acid rations. This has been observed for carp by Murai et al. (1987).

On the economic aspect, the current market prices for L- and D, L-methionine are ₦450 and ₦80 per kg respectively. Thus D, L-methionine is more than five times cheaper than L-methionine. The reason is that D, L- methionine is less expensive to synthesize than the pure L- form (Freedland and Briggs, 1977). Since D, L-methionine can substitute L-methionine, the use of D,L-methionine in synthetic diets or in dietary supplements is more economical than L-methionine.

6.5.0 CONCLUSION

C. anguillaris fingerlings can utilise full fat soyabean diet supplemented with L- and D, L-methionine for growth, provided the level of the crystalline methionine does not exceed 0.2% of the ration. Higher levels resulted in reduced growth rate of the fish. D, L-methionine should be preferred to L-methionine as a dietary supplement due to lower cost.

CHAPTER SEVEN

THE EFFECT OF DIFFERENT DIETARY LYSINE LEVELS ON GROWTH OF FINGERLINGS CLARIAS ANGUILLARIS FED D,L-METHIONINE ENRICHED SOYABEAN DIETS

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7.1.0 *INTRODUCTION*

Soyabean meal is a good source of essential amino acids and it is one of the very few plant sources rich in lysine (UNDP/FAO, 1983). However, studies have shown that overheating during soyabean processing might lead to a low availability of lysine (Dabrowski and Kozak, 1979; Viola et al., 1982).

When soyabean meal was substituted isonitrogenously for menhaden meal, growth and feed efficiency of channel catfish were substantially reduced. Addition of free methionine, cystine or lysine, the most limiting amino acids, to these soya-substituted diets did not enhance weight gain (Halver, 1980). On the contrary, Nose (1976) reported that enrichment of soyabean meal with methionine and lysine elevated the nutritive quality.

Rumsey and Ketola (1975) demonstrated that addition of methionine, lysine, histidine and leucine individually and in several combinations to the diets containing scyabean meal as the sole source of protein did not improve the growth

of rainbow trout, however, supplement of methionine, lysine, leucine, valine, histidine, threonine, tryptophan and tyrosine in a balance to simulate these levels for those of trout eggs significantly improved growth. These results suggest that the nutritive value of soyabean meal could be improved if the amino acid balance were adjusted with synthetic amino acids.

Since methionine is the limiting amino acid in soyabean meal (Murai et al., 1982, 1986; Steffens and Albrecht, 1976) and excessive heating may lead to low availability of lysine (Smith and Circle, 1972; Dabrowska and Kozak, 1979; Viola et al., 1982), this study was therefore undertaken to compare the growth performance and nutrient utilization of Clarias anguillaris fed different levels of synthetic lysine in heated soyabean meal enriched with crystalline D,L-methionine.

7.2.0 MATERIALS AND METHODS

7.2.1 Description of fish and the experiment

The experimental fish were reared in the indoor concrete tank of the hatchery complex with

Artemia until they were 1g. They were then transferred to the outdoor concrete tank and reared to the required weight of 4 to 5g using artificial diets with composition as shown in Table 3.2 . 600 fingerlings were transferred to twenty four flow through tanks at 25 fish per tank. The fingerlings were acclimated in the flow through tank for one week. The fish were kept off feed for the last 72 hours after which 20 fish were weighed and returned to each tank. The remaining fish were pooled and 10 fingerlings were sacrificed. The carcass were analysed for chemical composition.

7.2.2 Experimental Conditions

Throughout the period of the experiment, the dissolved oxygen was maintained at 5.74 ± 0.42mg/l. This was possible through the maintenance of a continuous flow of water in and out of the tank.

7.2.3 Feed and Feeding

Eight isonitrogenous and isocaloric diets were prepared with their composition as shown

in Table 7.1. Diets 1 through 7 contained soyabean meal which had been heat treated in the oven at 100°C for 30 minutes, dehulled and milled. This temperature and time were chosen because soyabean meal treated in this manner gave the best growth rate to mudfish fed heat treated soyabean diets in a previous experiment (See Chapter 5). Each of the soyabean diets was fortified with 0.2% D,L-methionine. The amount of D,L-methionine added was based on the report of a previous study which showed that 0.2% D,L-methionine was best utilised by C. anguillaris (See Chapter 6). The free amino acid levels in the diets were balanced by the addition of proportionate amount of glycine. The rations were made isocaloric by the addition of corn meal. The control ration (Diet 8) contained fish meal instead of soyabean meal. Vitamin and mineral premixes were supplied at 2% to each of the diets.

The ingredients were mechanically mixed (using a hand mixer) with hot water (approximately 80°C) and pelleted in a domestic pelleter. The pellets were oven dried at 50°C before feeding.

Table 7.1 Composition of the Experimental Diet (g/100g)

Ingredients g/100g	DIET DESIGNATION							
	DT1	DT2	DT3	DT4	DT5	DT6	DT7	DT8
Soyabean meal	75	75	75	75	75	75	75	-
Fish meal	-	-	-	-	-	-	-	64
D,L-Methionine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	-
L-Lysine	-	0.5	1.0	1.5	2.0	2.5	3.0	-
Glycine	3.0	2.5	2.0	1.5	1.0	0.5	-	-
*Cod Liver Oil	1.8	1.8	1.8	1.8	1.8	1.8	1.8	2.0
Yellow Maize	18	18	18	18	18	18	18	32
*Vitamin/Mineral Premix	2	2	2	2	2	2	2	2
Totals	100	100	100	100	100	100	100	100

* Composition as shown in Appendix 6

Fish were fed at 3% of their body weight twice daily at 9.00 a.m. and 4.00 p.m. Fish were weighed biweekly at the same time the quantity of feed was adjusted to the new weight. Water in the plastic tank was changed weekly, to get rid of uneaten food. The experiment was terminated after 8 weeks of observation.

7.2.4 Analytical Procedure

At the end of the feeding trials 8 fish were randomly taken from each tank and stunned by piercing the cranium with a needle. Three of the fish from each batch were cut open and the liver, heart and pancrease were excised, cleaned with cotton wool to remove blood and weighed. The remaining 5 fish from each tank were grouped into treatment (15 fish per treatment) and frozen in a domestic deep freezer at - 8°C ± 2°C for not more than one month. They were then homogenised and analysed for proximate composition. Crude protein, fat, fibre and ash were analysed according to the AOAC (1980) methods. Calcium and phosphorus were determined using the atomic absorption spectrophotometer (AOAC, 1980).

7.2.5 Growth and Food Utilization Parameters

The growth parameters monitored were weight gain, specific growth rate, feed conversion efficiency, protein efficiency ratio and apparent net protein utilization (See section 3.9.0)

7.2.6 Statistical Analysis

Data were analysed with the computer (Tandy 4000 Lx). Mean values were tested for significance by the analysis of variance (ANOVA) using the Duncan Multiple Range Test (See section 3.9.0).

7.2.7 Physico-chemical Parameters

Analysis of the physico-chemical parameters of water in the concrete tank was done weekly. The parameters were temperature, pH and dissolved oxygen. Details of each method are discussed in section 3.4.0.

7.3.0 RESULTS

7.3.1 Proximate Composition

Table 7.2 shows the proximate composition of the test diets. All diets were carefully

Table 7.2 **Proximate** Composition of the test and control diets (% dry matter)

	DIET DESIGNATION							
	DT1	DT2	DT3	DT4	DT5	DT6	DT7	DT8
Moisture	8.61	8.03	7.97	8.86	8.01	7.85	8.17	6.39
Crude protein	40.72	40.05	39.91	40.61	40.37	40.64	40.83	40.05
Crude fat	13.65	13.40	13.90	12.67	10.54	11.02	12.02	16.31
Crude fibre	3.59	4.33	3.64	3.80	3.86	4.62	4.20	1.30
Ash	4.99	4.80	4.95	5.03	5.88	5.86	5.50	20.90
Nitrogen free extracts (NFE)***	28.44	29.39	29.63	29.03	31.34	30.01	29.09	15.05
Calcium	0.72	0.62	0.61	0.72	0.61	0.68	0.65	6.28
Phosphorus	0.54	0.51	0.50	0.52	0.55	0.50	0.50	1.21

*** NFE was calculated as 100 - (Moisture + Crude Protein + Crude fat + Crude fibre + Ash).

formulated in such a manner as to make them isonitrogenous and isocaloric. The moisture content was about 8% in all the diets except diet 8 which had 6.39%. The crude protein approximated to 40% in all the rations. The values for crude fat lay between 11 to 14% in the treatments and 16.31% in the control ration (Diet 8). Crude fibre was about 5 - 6% in all the rations except diet 8 which had 20.90% crude fibre. The NFE values was between 28 - 31% in the diets and 15.05 in the control (Diet 8). Both calcium and phosphorus were low in diet 1 to 7 and high in diet 8.

7.3.2 Growth Performance

Table 6.3 shows the growth values of C. anguillaris fingerlings fed the experimental control diets. The initial mean weight of the fingerlings varied from 4.34 ± 0.01 to 5.81 ± 0.32g. There was no significant difference in the initial mean weight of fish fed diets 1 to 7 ($P>0.05$). Significant difference occurred between fish fed diet 1 and those fed other diets. The final mean weight also showed significant differences between means. Fingerlings fed diet 1

Table 7.3 Growth values of C. anguillaris fingerlings fed soyabean diets containing different levels of lysine

Feed No.	Initial Mean weight (g) ± SD	Final mean weight (g) ± SD	Mean weight gain (g) ± SD	Percentage weight gain	Quantity of feed fed	Specific Growth Rate (SGR)	Feed Conversion Efficiency (FCE)	Protein Efficiency Ratio (PER)	Apparent Net Protein utilization (NPU)
1	4.34 ±0.01	5.00^{a} ±0.03	0.66^{a} ±0.04	15.21^{a}	7.82^{a}	0.25^{a}	8.65^{a}	0.21^{a}	9.01^{a}
2	4.51 ±0.06	6.12^{ab} ±0.91	1.61^{ab} ±0.85	35.70^{ab}	8.17^{a}	0.55^{ab}	19.21^{ab}	0.48^{ab}	10.39^{a}
3	4.50 ±0.06	6.27^{b} ±0.38	1.77^{ab} ±0.44	39.33^{ab}	8.57^{a}	0.59^{b}	20.51^{b}	0.52^{b}	14.81^{ab}
4	4.58 ±0	6.59^{b} ±0.25	2.01^{bc} ±0.25	43.89^{b}	8.78^{a}	0.65^{b}	22.80^{b}	0.56^{b}	15.54^{b}
5	4.53 ±0.06	6.62^{b} ±0.33	2.09^{bc} ±0.28	46.14^{b}	8.70^{a}	0.68^{b}	23.97^{b}	0.60^{b}	18.40^{b}
6	4.66 ±0.04	6.75^{b} ±0.03	2.09^{bc} ±0.06	44.85^{b}	8.85^{a}	0.66^{b}	23.74^{b}	0.59^{b}	19.66^{b}
7	5.25 ±0.30	9.26^{c} ±0.15	4.01^{d} ±0.37	76.38^{b}	11.14^{b}	1.01^{bc}	36.84^{b}	0.91^{b}	26.82^{c}
8	5.81 ±0.32	14.55^{d} ±1.56	8.74^{e} ±1.87	150.43^{c}	15.79^{c}	1.64^{c}	55.69^{b}	1.39^{b}	36.55^{d}
	n.s	*	*	*	*	*	*	*	*

Note: Figures in the same column having the same subscript are not signficantly different (P>0.05)

SD = Standard Deviation

n.s = not significant

* = significant at 0.05

recorded the least final mean weight of 5.00g. This mean weight was not statistically significant ($P>0.05$) from those fed diet 2. There was no significant difference ($P>0.05$) in the final mean weight of fish fed diets 2, 3, 4, 5 and 6. However, significant difference was obtained between fish fed diets 7 and 8 ($P<0.05$).

The mean weight gain increased linearly from treatment 1 to 8. The lowest mean weight gain was recorded in fish fed diet 1 (0.66 ± 0.04g). Fish fed diets 1, 2 and 3 did not show any significant difference between means ($P>0.05$). Similarly, there were no significant differences ($P>0.05$) in fish fed diets 4, 5 and 6. The mean weight gain of fish fed diets 7 and 8 were significantly different ($P<0.05$). The lowest percentage weight gain was obtained in diet 1 while diet 8 gave the highest weight gain. Fish fed diet 7 recorded higher percentage weight gain than those fed other diets except those fed control diet 8.

7.3.3 Quantity of Feed fed

The quantity of feed fed to the fingerlings was lowest in diet 1 and highest in diet 8. There was no significant difference between the quantity of feeds in diet 1 to 6 ($P>0.05$). Significant difference was obtained between the quantity of feed in these diets and diet 7 and 8 ($P<0.05$). There was significant difference between diet 7 and 8 (Table 7.3).

7.3.4 Specific Growth Rate (SGR)

The SGR increased with higher levels of dietary lysine. Fish fed diet 1 recorded the lowest SGR (0.25). The highest SGR was obtained among fish fed diet 8 (1.64) The SGR of fish fed diets 1 and 2 was significantly different from those fed other diets ($P<0.05$). The SGR of fish fed diets 7 and 8 was also significantly different from those fed other diets ($P>0.05$) (Table 6.3).

7.3.5 Feed Conversion Efficiency (FCE)

The highest FCE was recorded among fish fed diet 8 (55.69). The lowest FCE was obtained in fish fed diet 1 (8.65). Fish fed diet 1 and 2 showed significant difference in FCE compared with those fed other diets ($P<0.05$).

7.3.6 Protein Efficiency Ratio (PER)

Fish fed diet 8 gave the highest PER which was significantly different from those fed diets 1 and 2 ($P<0.05$). No significant difference was obtained in fish fed the test diets except those fed diet 1 ($P>0.05$) (Table 7.3).

7.3.7 Apparent Net Protein Utilization (NPU)

The apparent NPU increases with increase in the level of dietary lysine. Fish fed diet 8 gave the highest NPU which was significantly different ($P<0.05$) from those fed other diets. Significant difference was obtained between fish fed diets 1, 2 and 3 and those fed other diets ($P<0.05$). Fish fed diet 7 was significantly different from those fed diet 8 ($P<0.05$) (Table 7.3).

7.3.8 Body Proximate Composition

The proximate composition of the carcass before and at the end of the experiment is shown in Table 7.4. The moisture content of fish fed diets 7 and 8 were the lowest. Fish fed diet 3 had the highest moisture while those fed other

Table 7.4 Body proximate composition of mudfish fingerlings before and at the end of the experiment (% dry matter)

Fish Feed No.	Moisture	Crude Protein	Crude fat*	Crude fibre*	Total Ash*	Calcium	Phosphorus
Initial	75.20	58.50	15.25	0.59	22.66	7.65	2.88
Final 1	74.83	62.17	16.37	0.36	20.10	6.48	2.88
2	73.69	62.66	13.96	0.41	22.92	7.54	3.20
3	75.00	64.41	9.58	0.66	25.36	8.13	3.91
4	73.21	64.81	13.50	0.62	19.42	5.30	2.41
5	74.33	65.93	11.45	0.63	21.98	7.23	2.94
6	72.45	66.49	9.68	0.14	21.66	6.41	3.26
7	71.62	69.45	10.27	0.70	19.10	5.77	2.63
8	71.01	73.14	9.41	0.17	17.28	5.70	2.44

diets had moderate moisture content. The highest crude protein was obtained in fish fed diet 8 and the lowest among those fed diet 1. The crude protein content of fish fed other diets was moderate. Fat content was very low in diets 3, 6 and 8 and high in diet 1. The fat content of other fish were not as high as those fed diet 1. The crude fibre content of the fish were generally very low. Fish fed diets 3, 4 and 5 produced carcass with the highest crude fibre. The crude fibre of those fed diet 8 were the least. The crude fibre of fish fed diets 1, 2, 6 and 7 were moderate. The total ash content was least among fish fed diet 8 and highest in fish fed diet 3. Fish fed other diets produced carcass of moderate ash content. The calcium values were about twice as high as the phosphorus values in fish for all treatments. The highest calcium and phosphorus values were obtained in fish fed diet 4. Fish fed diet 3 rations had the highest calcium and phosphorus in the carcass. The calcium and phosphorus values of fish fed other diets were lower than those fed diet 3.

Fish analysed before the experiment had higher moisture and lower crude protein than those analysed at the end. The crude fat, ash, crude fibre and total ash were moderate.

7.3.9 Organ analysis

The result of organ analysis (Table 7.5) showed differences in the percentage of the liver, heart and pancrease of the fingerlings in response to the level of lysine, methionine and glycine in diet. Fish fed diets 7 and 8 produced the lowest liver index of 1.08 ± 0.07% and 1.05 ± 0.06% respectively. The highest liver index of 1.35 ± 0.20% was obtained in fish fed diet 1. Fish fed other diets had lower liver index than those fed diet 1.

The heart index was highest in fish fed diet 7 (34 ± 0.09%) followed by fish fed diet 8 (0.30%). Fish fed diet 1 had heart index of 0.14%. The least heart index was recorded in fish fed diet 3 (0.09 ± 0.01%).

The pancrease index approximated to 0.1% in fish fed all the diets except diet 1. In fish fed diet 1, the pancreas index was only 0.02%.

Table 7.5 Percentage weight of liver, heart and pancreas of fish fed soyabean diets enriched with lysine, methionine and glycine

Fish Group	Liver index* Mean ± SD	Heart index* Mean ± SD	Pancreas index* Mean ± SD
1	1.35 ± 0.20	0.14 ± 0	0.02 ± 0
2	1.13 ± 0.07	0.15 ± 0.02	0.08 ± 0.01
3	1.28 ± 0.02	0.09 ± 0.01	0.10 ± 0.00
4	1.25 ± 0.02	0.10 ± 0	0.10 ± 0
5	1.22 ± 0.21	0.11 ± 0.02	0.11 ± 0.01
6	1.18 ± 0.01	0.15 ± 0.02	0.08 ± 0
7	1.08 ± 0.07	0.34 ± 0.09	0.07 ± 0
8	1.05 ± 0.06	0.30 ± 0	0.07 ± 0

* Organ weight expressed as percentage of body weight
SD = Standard Deviation

7.3.10 Physico-chemical Parameters

Table 6.6 shows the mean physico-chemical parameters of the water. The temperatures in the mini flow-through tanks varied from 25.0 to 26.5°C with a mean of 25.71 ± 0.83°C. The pH values ranged from 7.0 to 7.6.

The dissolved oxygen varied from 5.2 to 6.4 ppm. The results of the physico-chemical parameters were considered adequate for the fish (See Appendix 4).

7.4.0 DISCUSSION

The results in Table 7.3 indicate that addition of lysine to heated soyabean meal enriched with methionine led to an increase in the growth and food utilization of fingerling mudfish fed soyabean diets. Although soyabean meal is rich in lysine (UNDP/FAO, 1983) the usual heat processing needed to improve soyabean protein quality causes low availability of lysine in soyabean meal (Viola et al., 1982). The formation of sugar-amino complexes or Maillard reaction was responsible for the destruction of lysine (Maynard et al., 1979). The explanation is that lysine is a basic amino acid. In addition to

Table 7.6 Data of weekly physico-chemical parameters of water in the mini flow-through tanks

Date	Temperature °C	pH	Dissolved oxygen (DO) mg/l
10/10/89	26.5	7.3	5.2
17/10/89	25.3	7.6	6.4
24/10/89	26.3	7.3	5.3
31/10/89	26.8	7.0	5.5
7/11/89	25.0	7.6	6.0
14/11/89	26.5	7.3	5.3
21/11/89	24.5	7.6	6.0
28/11/89	25.5	7.6	6.0
5/12/89	25.0	7.6	6.0
Mean	25.71 ±	7.43 ±	5.74
Standard Deviation	0.83	0.22	0.42

the α - amino group normally bound in peptide linkage, it also contains a second, E - amino group. This E - group must be free and reactive otherwise the lysine, although chemically measurable will not be biologically available (Cowey, 1978). During the processing of feedstuff protein the E - amino group of lysine may react with non-protein molecules present in the feedstuff to form additional compounds that render lysine biologically unavailable (Cowey, 1978).

The result of this study is in agreement with those of Rumsey and Ketola (1975) and Keneko (1969) who demonstrated that soyabean meal enriched with methionine and lysine was a superior protein source to soyabean meal alone to rainbow trout. Meske et al. (1977) also achieved very good growth with grass carp fed soyabean diet supplemented with lysine and methionine.

The addition of up to 3% lysine did not give maximum growth to C. anguillaris fed methionine enriched soyabean diet, (Figure 7.1) neither did it give as much specific growth

rate, PER, FCE or apparent NPU to the fish as the control ration containing fish meal. This shows that in spite of the obvious benefits obtained by supplementation of soyabean meal with methionine and lysine, the nutritive value did not equal that of fish meal diet for mud fish. Similarly, Koops et al. (1976) working with rainbow trout also found that soyabean meal supplemented with amino acids methionine, lysine and glycine was a poor substitute to fish meal in the diet of rainbow trout.

The high growth rate obtained in fish fed 3% lysine compared with those fed lower lysine levels in the diet (Figure 7.1), may suggest a possible requirement of C. anguillaris for 3 per cent lysine in the diet. Koops et al. (1976) found that diets containing 3.8 - 4.3 per cent lysine in the protein fraction gave good growth to rainbow trout and suggested that the reported requirement of 5.0 per cent in the protein might be too high.

The liver index varied from 1.05% to 1.35% which was within the same range as those reported by Dabrowska and Wojno (1976) for rainbow trout

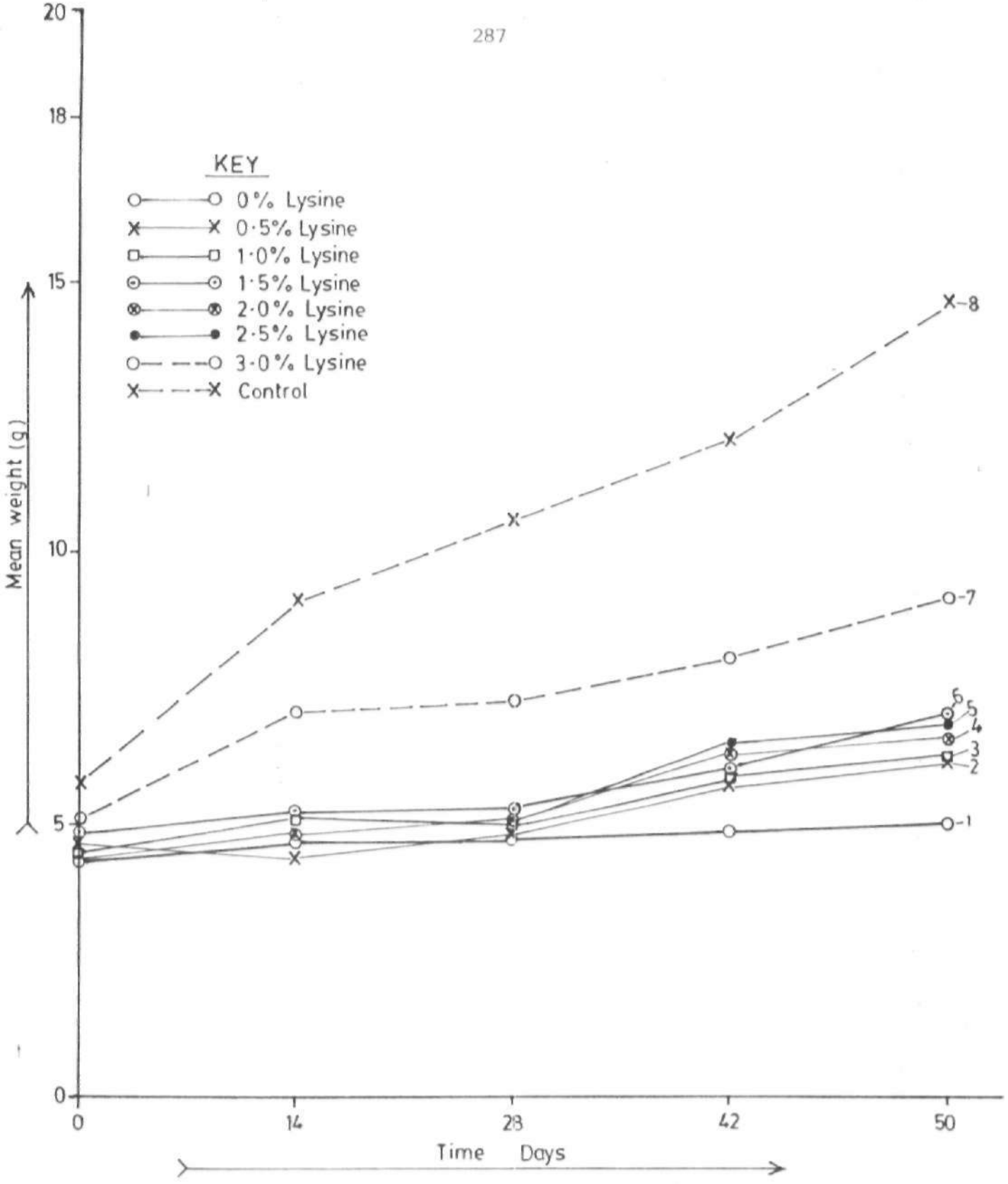


Fig. 7.1. Growth of C.anguillaris fingerlings fed different dietary levels of Lysine.

(1.07 to 1.39%) and Igbinosun and Roberts (1988) for the catfish Chrysichthys nigrodigitatus (1.06 to 1.30%). The heart index varied from 0.09% to 0.34%. This was higher than the range of 0.12 - 0.14% obtained with Chrysichthys nigrodigitatus (Igbinosun and Roberts, 1988). The pancreas index was 0.1 - 0.2%. In general fish fed diets containing high levels of lysine had bigger heart index, smaller liver index and moderate pancreas index compared with those fed the unsupplemented diets. Sotomi and Tanaka (1978) could not find any relationship between the relative weight of the liver of rainbow trout fed soyabean diets and that of trout fed commercial trout feeds. Liener (1981) has suggested that only animals whose pancreas weighs more than 0,3 percent of total body weight will exhibit pancreatic hypertrophy.

7.5.0 CONCLUSION

C. anguillaris could utilize synthetic amino acids for growth. Addition of 3% synthetic lysine in soyabean diet enriched with methionine significantly increased the growth and food utilization of C. anguillaris fingerlings. The

weight of the heart, liver and pancreas were affected by high levels of dietary lysine.

CHAPTER EIGHT

THE EVALUATION OF SOYABEAN MEAL AS REPLACEMENT FOR FISH MEAL IN THE DIET OF MUDFISH CLARIAS ANGUILLARIS JUVENILES

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8.1.0 INTRODUCTION

Fish meal is used extensively as a source of protein in fish feed because of its unrivalled nutritive value. Compared with other commercially used protein sources fish meal has the highest biological value, higher in digestible energy, rich in available lysine and methionine, minerals, vitamins and perhaps unidentified growth factors as required by fish (Lovell, 1981b). Although fish meal has been recognised as the best source of animal protein for most fish species, it is relatively expensive. As a result of the high cost of fish meal numerous attempts have been made to replace fish meal in fish feed with other less expensive protein sources (Gropp et al., 1976; Higgs et al., 1979; Jackson et al., 1982). Research on partial or total replacement of fish meal in fish feed with the less expensive soyabean meal have been carried out on a number of species (Dabrowski and Kozak, 1979; Murai et al., 1982; Tacon et al., 1983). Such studies have not been carried out on some of the locally available culturable fish species in Nigeria.

The objective of this study was to examine the effect of replacement of different levels of fish meal with soyabean meal in the diet of Clarias anguillaris juveniles.

8.2.0 MATERIALS AND METHODS

8.2.1 Feed Preparation

(i) Soyabean meal

Raw soyabean was toasted in the oven at 100°C for 30 minutes. The soyabean was dehulled after "exploding" in the mill and winnowed. The soyabean was finally ground into fine particles.

(ii) Fish meal

Fish meal was prepared by boiling and sundrying whole fresh tilapia. The dried fish was ground in a hammer mill and screened through a fine-meshed sieve.

(iii) Corn meal

Yellow maize was similarly ground into powder with the hammer mill.

The ingredients were mixed with cod liver oil and vitamin/mineral premixes to give seven rations containing 0%, 10%, 25%, 50%, 75%, 90% and 100% fish meal as the major protein source. The 100% fish meal ration was taken as the control diet. The composition of the rations is shown in Table 8.1. 70cm^3 of hot water (80°C) was added to each mixture to tenderise the starch component of the diet. The mixed ingredients were then pelleted into 3mm diameter with a domestic pelleter and dried in the oven at 50°C.

8.2.2 Experimental Fish and Feeding Methods

Juvenile mudfish Clarias anguillaris with mean weight of 23.8 ± 3.21g were collected by beach seining at Kigera reservoir in Kainji Lake area. Captured fish were maintained in the indoor flow-through tanks for 4 weeks on pelleted feeds (See Appendix 5). The experiment was set up in 21 hapas each measuring 1 x 1 x 1 metre suspended in one meter of water in an outdoor concrete tank with dimension 10 x 10 x 2 metres. Fish were transferred to the hapas and allowed to

Table 8.1. Composition of the experimental ration (g/100g)

Ingredients	RATION						
	1	2	3	4	5	6	7
Fish meal	0	6.5	16.3	32.5	48.7	58.5	65.0
Soyabean meal	65.0	58.5	48.7	32.5	16.3	6.5	0
Yellow maize	31.0	31.0	31.0	31.0	31.0	31.0	31.0
Cod liver oil*	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin/Mineral Premix*	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Total	100	100	100	100	100	100	100
% Fish meal in the protein fraction of ration	0%	10%	25%	50%	75%	90%	100%
% Soyabean meal in the protein fraction of ration	100%	90%	75%	50%	25%	10%	0%

* The composition of vitamin/mineral premixes and Cod liver oil are shown in Appendix 6.

acclimatise for one week. The experimental fish were starved for 72 hours before feeding (See section 3.6.0).

Fish were fed twice daily at 3% body weight. Each day's ration was divided into two equal portions and dispensed at 9.30 a.m. and 4.30 p.m. Feeding was carried out by manually dropping the feeds in the hapas through an opening 30cm wide at the upper edge of each hapa. Weight of fish were taken fortnightly to assess the growth rate and adjust the rations accordingly.

8.2.3 Proximate Analysis

Crude protein was analysed according to the AOAC method (1975). Crude fat was determined by the modified Bligh and Dyer method (Hanson and Olley, 1963). Ash was estimated by burning in a muffle furnace at 550°C for 24 hours and moisture content was measured by oven-drying at 105°C for 24 hours. Carbohydrates was estimated by difference method.

8.2.4 Feed Evaluation Parameters

At the end of the experiment the extent of utilization of each diet was evaluated using certain parameters. The feed evaluation parameters were the specific growth rate, the feed conversion efficiency, the protein efficiency ratio and apparent net protein utilization. These parameters are described in section 3.8.0.

8.2.5 Statistical analysis

The data obtained from the experiment were subjected to statistical analysis using the computer (Tandy 4000 Lx) (See section 3.9.0).

8.2.6 Determination of Physico-chemical Parameters

The physico-chemical parameters of the water in the hapas were analysed to determine the temperature, pH and dissolved oxygen concentration. The methods of analysis of these parameters are described in section 3.4.0.

8.3.0 RESULTS

8.3.1 Proximate Composition

Table 8.2 shows the proximate composition of the experimental diets. The all soyabean diet (Diet 1) contained the lowest crude protein (31.52%) and the highest crude protein level of 38.53% was recorded in the all fish meal diet (Diet 7). Crude fat was highest in diet 4 (10.48%) and lowest in diet 7 (7.00%). The ash content increased from 3.99% in diet 1 to 18.57% in diet 7. The Nitrogen Free Extract fraction was lowest in diet 7 (26.45%) and highest in diet 1 (44.71%).

The growth and food utilization of *C. anguillaris* fed the experimental diets are shown in Table 8.3.

8.3.2 Gain in Weight

The mean weight gain (32.46 ± 7.64g) was highest in fish fed 100% fish meal as the protein source (Diet 7) and lowest (9.31 ± 5.48g) in fish fed 10% fish meal along with 90% soyabean meal as the protein source (Diet 2). There was no significant difference ($P<0.05$) in the mean weight gain of fish

Table 8.2. **Proximate** composition of the experimental diets (g/100g)

Component	D I E T						
	1	2	3	4	5	6	7
Moisture	10.67	9.85	11.03	8.48	8.81	9.01	9.45
Crude protein	31.52	31.87	32.40	33.27	34.15	35.03	38.53
Crude fat	9.11	9.31	9.81	10.48	8.58	8.55	7.00
Ash	3.99	5.26	16.58	10.47	15.21	16.42	18.57
Sub-totals	55.29	56.29	69.82	62.70	66.75	69.01	73.55
NFE*	44.71	43.71	30.18	37.30	33.25	30.9	26.45
Dry matter	89.33	90.15	86.97	91.52	91.19	90.99	90.55

% Nitrogen Free Extract computed as 100 - subtotals *

Table 8.3. Growth and Food Utilization of Clarias anguillaris juveniles fed experimental diets for 84 days.

Feed No.	Initial mean wt (g) ± SD	Final mean wt (g) ± SD	Mean Weight gain (g) ± SD	% Weight gain (g)	Quantity of feed fed (g)	Specific Growth Rate (SGR)	Feed Conversion Efficiency (FCE)	Protein Efficiency Ratio (PER)	Apparent Net protein Utilization (NPU)
1	$22.73^{a}\pm1.05$	$32.28^{a}\pm2.62$	$9.55^{a}\pm1.57$	42.01^{a}	65.70^{a}	0.42^{a}	14.53^{a}	0.46^{a}	10.58^{a}
2	$21.00^{b}\pm1.70$	$30.31^{b}\pm3.78$	$9.31^{a}\pm5.48$	44.33^{a}	60.98^{b}	0.44^{a}	15.27^{a}	0.48^{a}	11.37^{a}
3	$23.69^{c}\pm2.74$	$41.12^{c}\pm2.52$	$17.43^{b}\pm0.22$	73.58^{b}	75.61^{c}	0.66^{b}	23.05^{b}	0.72^{b}	12.14^{a}
4	$24.26^{d}\pm2.56$	$51.37^{d}\pm5.40$	$27.11^{c}\pm2.84$	111.75^{c}	86.64^{d}	0.89^{c}	31.29^{c}	0.94^{c}	16.14^{b}
5	$21.47^{b}\pm0.71$	$45.69^{e}\pm2.55$	$24.22^{d}\pm1.84$	112.81^{c}	76.01^{c}	0.90^{c}	31.86^{c}	0.93^{c}	15.44^{b}
6	$24.69^{e}\pm0.84$	$54.92^{f}\pm1.02$	$30.23^{e}\pm11.05$	122.44^{d}	93.11^{e}	0.95^{c}	32.47^{c}	0.92^{c}	21.46^{c}
7	$28.85^{f}\pm6.55$ *	$61.31^{g}\pm1.42$ *	$32.46^{e}\pm7.64$ *	112.51^{c} *	103.45^{f} *	0.90^{c} *	31.38^{c} *	0.82^{c} *	16.83^{b} *

Note: Figures in the same column having the same subscripts are not significantly different (P>0.05)

SD = Standard Deviation

* = Significant at 0.05

fed diet 1 and diet 2. Similarly there was no significant difference ($P>0.05$) in the mean weight gain of fish diet 7 and diet 6. The percentage weight gain was similarly affected by the diet. The highest percentage weight gain was obtained in fingerlings fed diet 6 (122.44%) while fish fed diet 1 recorded the lowest percentage weight gain (42.31%). No significant difference ($P>0.05$) was observed in fingerlings fed diets 1 and 2, and also in those fed diets 4, 5 and 7. Fingerlings fed diets 3 and 6 were significantly different ($P<0.05$).

8.3.3 Specific Growth Rate (SGR)

There was a general increase in the SGR of the fish with increase in fish meal protein in the ration although diet 6 containing 9.0% fish meal protein and 10% soyabean meal protein recorded the highest SGR. There was no significant difference in the SGR of fish fed diets 4, 5, 6 and 7. The lowest SGR was observed in fish fed the soyabean meal protein (Diet 1) although the result was not significantly different ($P>0.05$) from fish fed the 90% soyabean meal combined with 10% fish meal as the protein source.

8.3.4 Food Conversion Efficiency (FCE)

The FCE also followed a similar trend with the SGR. The FCE increased from 14.53 in diet 1 to 32.47 in diet 6. There was no significant difference ($P>0.05$) in FCE of fish fed diets 4, 5, 6 and 7.

8.3.5 Protein Efficiency Ratio (PER)

The PER increased from 0.46 in diet 1 to 0.94 in diet 4. No significant difference was observed in PER of fish fed diets 4, 5, 6 and 7 ($P>0.05$). No significant difference was also observed in fish fed diets 1 and 2 ($P>0.05$). However fish fed diets 2, 3 and 4 were significantly different ($P<0.05$).

8.3.6 Apparent Net Protein Utilization (Apparent NPU)

The apparent NPU was lowest in diet 1 (10.58) and highest in diet 6 (21.46). There was no significant difference ($P>0.05$) in the apparent NPU of fingerlings fed diets 1, 2 and 3. Also the NPU of fish fed diets 4, 5 and 7 were not significantly different ($P>0.05$). Fingerlings fed diet 6 had a significantly higher NPU than those fed other diets ($P<0.05$).

8.3.7 Physico-chemical Parameters

The data in Table 8.4 shows the water quality parameters monitored in the concrete tank at weekly intervals throughout the duration of the experiment. The water temperature ranged from 25.40°C to 28.50°C with a mean of 27.06 ± 1.16°C while the dissolved oxygen ranged from 4.10 to 6.61 mg/litre, with a mean of 5.33 ± 1.01 mg/l. The pH stood within the neutral range 7.0 to 7.6 with a mean of 7.32 ± 0.21. The water quality was adequate for the growth of C. anguillaris (See Appendix 4).

8.4.0 DISCUSSION

The weight gain of C. anguillaris juveniles increased in response to higher percentage of fish meal in the diet with 100% fish meal protein diet recording the highest weight gain (Figure 8.1). This demonstrate that fish fed diets containing high fish meal components grow better than those fed high soyabean diet, (Dabrowski and Kozak, 1979; Abel et al., 1984). Although the 100% fish meal protein diet gave the best weight gain, it did not give the best SGR, PER or apparent NPU. The combination of 90% fish meal with 10% soyabean

Table 8.4. Water quality parameters of concrete tank during the experimental period.

Date	Water Temperature °C	Dissolved Oxygen mg/l	pH
22/3/89	28.00	4.10	7.20
29/3/89	28.30	4.25	7.10
5/4/89	28.50	4.21	7.00
12/4/89	28.05	4.22	7.50
19/4/89	27.50	5.01	7.60
26/4/89	27.20	5.25	7.40
3/5/89	27.50	5.18	7.30
10/5/89	26.51	6.17	7.00
17/5/89	25.45	6.25	7.20
24/5/89	25.52	6.61	7.50
31/5/89	25.40	6.52	7.40
7/6/89	26.05	6.45	7.50
Mean	27.06	5.33	7.32
Standard Deviation	1.16	1.01	0.21

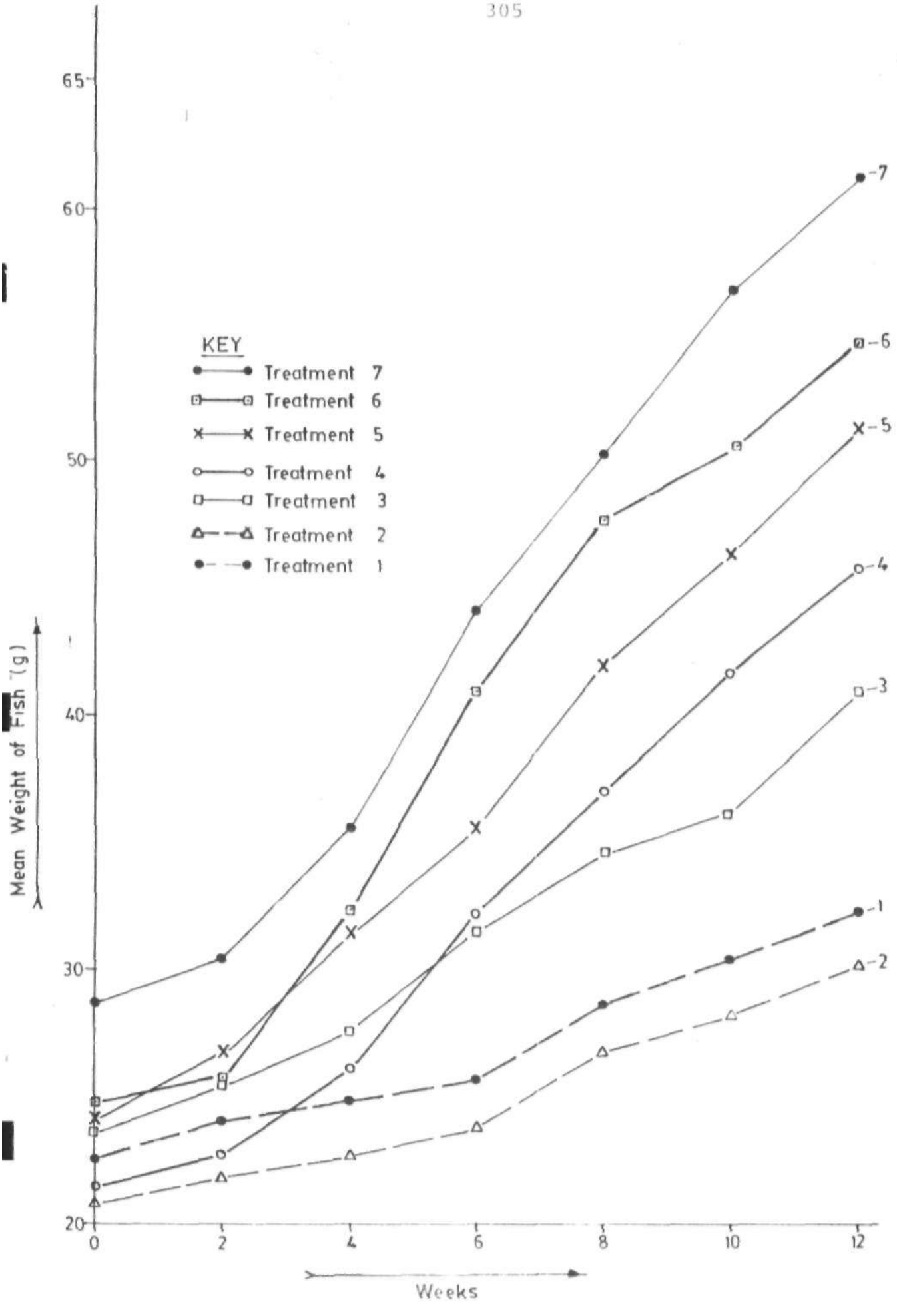


Fig. 8·1. Growth of Clarias anguillaris fingerlings fed seven experimental diets.

meal diet (Diet 6) which gave the best SGR, FCE and apparent NPU was better utilized by C. anguillaris juveniles than the 100% fish meal protein diet (Diet 7). The optimum growth of C. anguillaris juveniles fed 35.0% crude protein in diet as against other protein levels suggest a requirement of 35.0% crude protein by C. anguillaris juveniles. Since the NPU indicate the level of protein digestibility as well as the biological value of the diet (MacDonald et al., 1973); it follows that the addition of 10% soyabean meal to the ration was responsible for the high efficiency of utilization of the 90% fish meal replacement diet. Thus the quality of fish meal is enhanced by the addition of some levels of soyabean meal. This confirms the observation that experimental diets high in fish meal have not produced as good a growth rate as combination of fish meal and soyabean meal (Lovell, 1982).

The poor performance of C. anguillaris fed the soyabean diet as the only protein source could be attributed to imbalance in the amino acid pattern of the rations and the effect of

trypsin inhibitor which could still have been active even after the heat treatment of the raw soyabean.

With the growing scarcity of fish meal coupled with exhorbitant cost, the 50% level of replacement which attained more than 80% potential of the 100% fish meal protein with regard to weight gain would be the most cost effective. Cho et al. (1974) had similarly postulated the possibility of substituting 50% of fish meal with soyabean meal.

8.5.0 CONCLUSION

Although fish fed the 100% fish meal protein diet obtained the highest weight gain the 50% level of replacement which had a growth potential of 83% was regarded as the most cost effective. Based on the results of FCE, SGR and apparent NPU the protein requirement for the optimal growth of C. anguillaris juveniles is 35%.

CHAPTER NINE

THE EFFECT OF REPLACEMENT OF SOYABEAN MEAL WITH BLOOD MEAL ON THE GROWTH OF MUDFISH CLARIAS *ANGUILLARIS FINGERLINGS*

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9.1.0 INTRODUCTION

Soyabean meal is unique among the plant - source protein in that it is rich in the essential amino acids although the level of methionine is suboptimal (Nose, 1978). Supplementation of soyabean meal with animal proteins is necessary to bring the amino acid profile of the feed into proper balance.

Blood meal could be used to blend with soyabean meal in fish feed because it is high in protein although low in isoleucine (Harris, 1980). A combination of soyabean which is high in isoleucine with blood meal should meet the essential amino acid requirement. Studies have shown that blood meal can be well utilized by fish (Fowler and Banks, 1973; Dekimpe and Micha, 1974; Ufodike and Ugwuzor, 1985). Besides blood meal is usually discarded in slaughter houses and therefore costs very little.

The aim of this experiment was to determine the growth rate and food utilization of *Clarias anguillaris* fingerlings fed different

levels of blood meal and soyabean meal in an isocaloric diet. This experiment will enable fish nutritionists select the best combination of blood meal and soyabean meal in the ration that would give optimal growth of C. anguillaris in outdoor tanks.

9.2.0 MATERIALS AND METHODS

The experiment was conducted in the fish hatchery complex of NIFFR, New Bussa using one outdoor concrete tank measuring 10 x 10 x 2m. 320 C. anguillaris fingerlings having mean weight ranging from 2.86 ± 0.30 to 3.66 ± 0g were placed in 16 hapas measuring 1 x 1 x 1 metre suspended in the concrete tank.

9.2.1 Preparation of Protein Supplements

Fish Meal

Tilapia were collected from the reservoir and boiled for 10 minutes. The cooked fish were dried in the sun before grinding to powder in a hammer mill.

Blood Meal

Fresh cow blood was collected from the

abattoir and boiled for 20 minutes. The blood was dried in the sun and ground in the hammer mill.

Soyabean Meal

Raw soyabean were toasted for 30 minutes in the oven at 100°C to destroy the trypsin inhibitor and improve the nutritive value. The toasted soyabean were broken to smaller particles in a hammer mill to remove the hull and later ground into powder.

9.2.2 Feed and Feeding

The composition of the diet is shown in Table 9.1. The blood meal and full fat soyabean meal were mixed in different proportions to give 0%, 10%, 25%, 50%, 75%, 90%, 100%, blood meal in the protein fraction of the diet. The difference was made up with soyabean meal. Other ingredients shown in the table were mixed together in hot water to form a consistent dough. The dough was then pelleted using a domestic hand pelleter with 3mm die. The pellets were dried in the oven at a temperature of 50°C to evaporate the moisture before use.

A total of eight feed mixtures were prepared and Diet 8 containing fish meal as the only protein source was taken as control (Table 9.1).

Twenty-five Mudfish fingerlings were acclimated in each of 16 hapas for one week during which time the fish were not fed with prepared rations (See section 3.6.0).

Feeding with the experimental rations commenced after the initial weight of the fish were taken. Subsequent measurements were taken fortnightly at which time the feed weight was also adjusted accordingly. Fish were fed at 5% biomass per day, twice daily, 9.30 a.m. and 4.30 p.m. The hapas were thoroughly washed biweekly as the fish were being weighed to remove algae from the hapas and allow uninterrupted flow of water through the hapas. The experiment was monitored for 84 days.

9.2.3 Chemical Analysis

Crude protein was analysed according to AOAC (1975).

Table 9.1. Composition of experimental diets (g / 100g).

INGREDIENTS	DIET COMPOSITION							
	DT 1	DT 2	DT 3	DT 4	DT 5	DT 6	DT 7	DT 8
Soyabean meal	62	55.8	46.5	31	15.5	6.2	0	-
Fish meal	-	-	-	-	-	-	-	51
Blood meal	0	6.2	15.5	31	46.5	55.8	36.5	-
Yellow maize	34	34	34	34	34	34	59.5	45
Cod liver oil +	2	2	2	2	2	2	2	2
Vitamin/mineral Premix +	2	2	2	2	2	2	2	2
Total	100	100	100	100	100	100	100	100
Computed crude protein %	33.16	35.50	39.05	44.95	50.83	54.36	37.34	35.30
Analysed crude protein %	32.02	34.52	37.15	43.78	48.53	52.79	37.05	34.78

+ Composition as given in Appendix 6

9.2.4 Food Utilization Parameters

The food utilization parameters applied were specific growth rate, food conversion efficiency and protein efficiency ration. Details of the methods are given in section 3.8.0.

9.2.5 Physico-chemical Parameters

The dissolved oxygen (DO), temperature and pH of the water in the concrete tank were measured weekly using standard methods (See section 3.4.0).

9.2.6 Statistical Analysis

The analysis of data for statistical significance was carried out with the computer (Tandy 4000 Lx) using Duncan's multiple range test (See section 3.7.0).

9.3.0 RESULTS

9.3.1 Growth Performance

Table 9.2 shows the growth performance of C. anguillaris fingerlings fed various levels of blood meal in the soyabean diet. The initial mean weight varied between 2.86 ± 0.30g and 3.66 ± 0g.

Table 9.2. Growth performance of mudfish *fingerlings* fed blood meal diet for 84 days

Feed No.	Initial mean wt (g) ± SD	Final mean wt (g) ± SD	Mean weight gain (g) ± SD	% Weight gain (g)	Specific growth rate	Food Conversion efficiency	Protein efficiency Ratio	Mortality %
1	3.11 ± 1.08	5.71^{a} ± 0.13	2.60^{a} ± 1.22	83.60^{a}	0.76^{a}	9.42^{a}	0.33^{a}	2.5
2	3.54 ± 0.04	6.06^{a} ± 0.06	2.52^{a} ± 0.02	71.18^{a}	0.63^{a}	9.14^{a}	0.28^{a}	2.5
3	3.65 ± 0.05	9.21^{b} ± 3.52	5.56^{b} ± 3.61	152.32^{b}	1.02^{b}	20.27^{b}	0.59^{b}	2.5
4	3.66 ± 0	10.98^{c} ± 5.20	7.32^{b} ± 5.20	200.00^{b}	1.23^{c}	33.47^{c}	0.76^{c}	5.0
5	3.61 ± 0.05	8.67^{b} ± 0.61	5.06^{b} ± 0.66	140.16^{b}	1.04^{b}	31.60^{c}	0.62^{c}	5.0
6	3.60 ± 0.04	7.90^{b} ± 0.62	4.3^{a} ± 0.65	119.44^{a}	0.93^{b}	22.97^{b}	0.45^{a}	7.5
7	2.86 ± 0.30	5.72^{a} ± 0.45	2.86^{a} ± 1.56	100.00^{a}	0 82^{b}	12.35^{d}	0.32^{a}	10.0
8	3.23 ± 0.04	12.42^{d} ± 1.64	9.19^{c} ± 1 68	284.52^{c}	1.60^{d}	34.89^{c}	0.95^{e}	2.5
	n.s	*	*	*	*	*	*	

Note: Values in the same vertical line followed by the same letters are not significantly different (P>0.05)

SD = Standard Deviation
n.s = not significant
* = Significant at 0.05

There was no significant difference in the initial mean weight of the fingerlings ($P>0.05$), while in the final mean weight significant differences were obtained ($P<0.05$). This shows that the different feed mixtures had different effects on the growth of the fingerlings. These differences were manifested in the mean weight gain of the experimental fish. The least mean weight gain was observed in fish fed diet 2. There was no significant difference ($P>0.05$) in the mean weight gain of fish fed diet 1, 2 and 6. Similarly, no significant difference was obtained in fingerlings fed diets 3, 4 and 5. Fingerlings fed diet 8 had the highest mean weight gain (9.19 ± 1.68g) which was significantly different ($P<0.05$) from those fed other diets.

The percentage weight gain of the experimental fish was similarly affected by the diet intake. No significant difference ($P>0.05$) was obtained in the percentage weight gain of fingerlings fed diets 1, 2, 6 and 7. Also, no significant difference was observed in mudfish fingerlings fed diets 3, 4 and 5 ($P>0.05$).

Significant difference ($P<0.05$) was obtained in the percentage weight gain of fingerlings fed diet 8 which also scored the highest percentage weight gain (284%).

9.3.2 Specific Growth Rate (SGR)

The highest SGR was recorded in fish fed the control ration (diet 8) (1.60) followed by those fed diet 4 (1.23). The least SGR of 0.63 was recorded among fingerlings fed diet 2. This SGR was not significantly different ($P>0.05$) from that of fingerlings fed diet 1 (0.76). No significant difference was obtained in the SGR of mudfish fingerlings fed diets 5, 6 and 7 ($P>0.05$).

9.3.3 Feed Conversion Efficiency (FCE)

The FCE was generally low for C. anguillaris fingerlings fed diets 1 and 2 (9.42 and 9.14 respectively). The highest FCE was recorded in fingerlings fed the control ration (34.89). This was not significantly different from those of mudfish fingerlings fed diets 4 and 5. The FCE of mudfish fingerlings fed diets 3 and 6 were not significantly different ($P>0.05$).

The FCE of fingerlings fed diet 7 was significantly different from those fed diets 6 and 8 ($P<0.05$).

9.3.4 Protein Efficiency Ratio (PER)

The PER was lowest in fingerlings fed diet 2 although this was not significantly different ($P>0.05$) from those fed diets 1, 6 and 7. Mudfish fingerlings fed the control ration (diet 8) recorded the highest PER followed by those fed diet 4 and 5 respectively. The PER of fingerlings fed diet 3 were significantly different from those fed other diets ($P<0.05$).

9.3.5 Physico-chemical Parameters

Table 9.3 shows the water quality variables in the experimental outdoor tank. The water temperature ranged from 23.0°C to 28.7°C with a mean of 25.89 ± 2.10 while the pH ranged from 7.4 to 7.9 with a mean of 7.62 ± 0.17. The dissolved oxygen was between 5.0 to 6.9 mg/l. The physico-chemical parameters were considered suitable for the culture of C. anguillaris fingerlings (See Appendix 41).

9.3.6 Mortality

The percentage mortality was generally low

Table 9.3. Weekly water quality variables in the experimental outdoor tank.

Date	Temperature °C	pH	Dissolved Oxygen (DO) mg/l
21/1/89	23.0	7.5	5.0
28/1/89	23.5	7.4	5.0
4/2/89	24.1	7.4	5.7
11/2/89	24.2	7.5	5.9
18/2/89	25.3	7.7	5.7
25/2/89	25.5	7.5	5.8
4/3/89	26.3	7.6	5.2
11/3/89	27.2	7.7	5.5
18/3/89	28.5	7.8	6.0
25/3/89	28.7	7.9	6.8
1/4/89	28.3	7.8	6.9
Mean	25.89	7.62	5.77
Standard Deviation	2.10	± 0.17	0.63

(2.5%) for mudfish fingerlings fed the control diet and diets 1, 2 and 3; but rose in fish fed diets 4 to 6 (5 - 7.5%). The highest percentage mortality of 10% was recorded among fish fed diet 7 (containing 100% blood meal).

9.4.0 DISCUSSION

The results indicate an increase in the weight gain and food utilization by C. anquillaris fingerlings with rise in the percentage blood meal in the soyabean diet up to 50% blood meal. Beyond the 50% level of blood meal, a significant reduction in the growth performance of the fingerlings was observed (Figure 9.1). Compared with soyabean meal and indeed other protein sources, blood meal has more than 80% protein (Oyenuga, 1968; Miller, 1976; Raven and Walker, 1980). This is in contrast with soyabean meal which has 48% protein (Raven and Walker, 1980). This high protein level in blood meal was also reflected by the high crude protein level in the high blood meal rations. This shows that beyond 50% replacement of blood meal, the

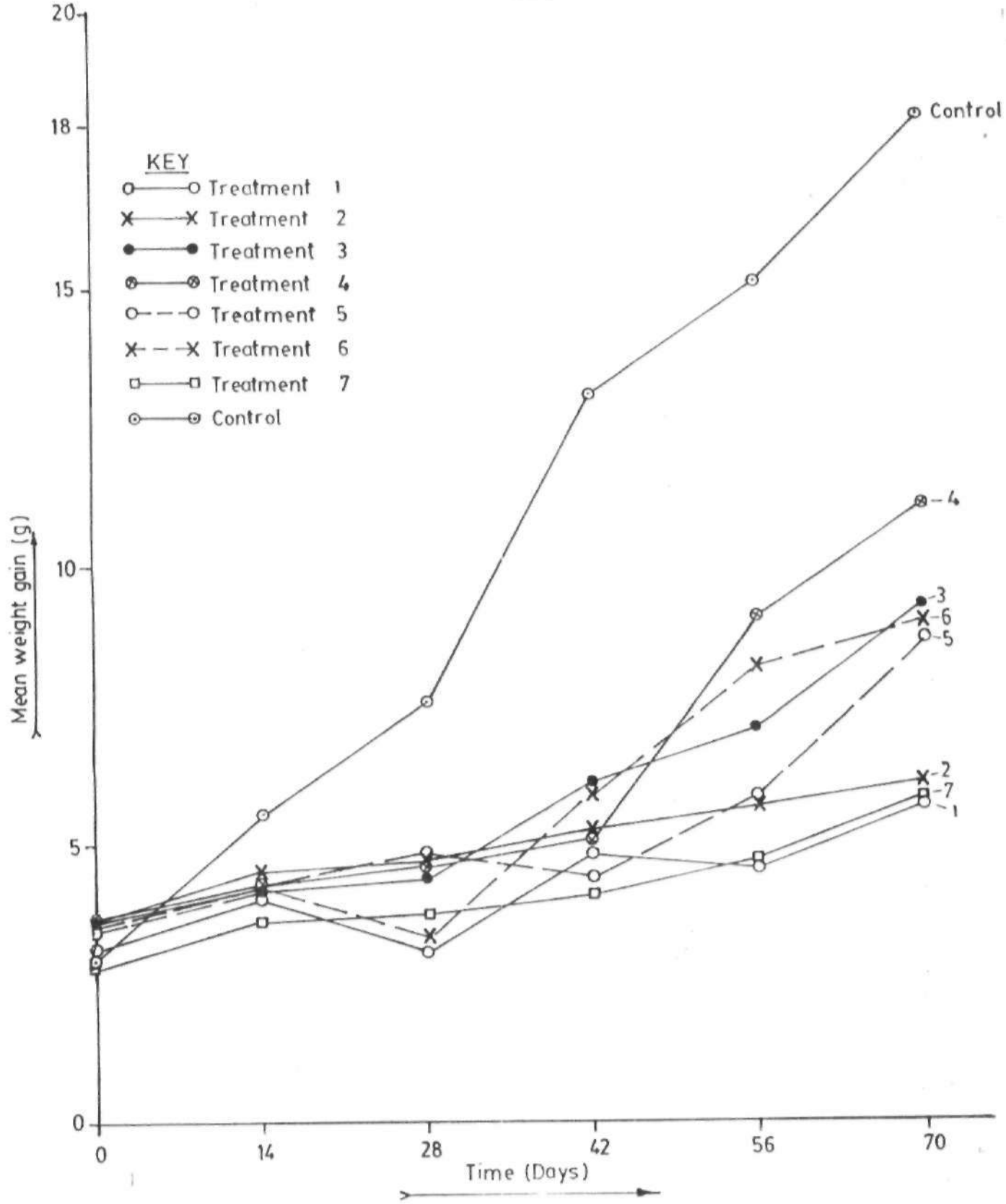


Fig. 9·1. Growth of C.anguillaris fingerlings fed different dietary levels of Soyabean meal and blood meal.

growth rate of the fingerlings was inversely proportional to the dietary blood meal level. This poor performance of fingerlings on high inclusion of blood meal in the diet could be traced to imbalance in the amino acid composition of blood meal. Blood meal is low in isoleucine (Harris, 1980), which could be responsible for the poor performance of C. anguillaris fed high blood meal diets. The protein content of the high blood meal diets were generally higher than the protein requirement of Clarias sp fingerlings 40% (Faturoti et al., 1986; Madu, 1989). The high protein content in the high blood meal diets could produce stress due to excess of ammonia released from the gills (Hastings, 1976). This could result in poor growth rate of the fingerlings.

The poor growth rate of fish fed the highest amount of blood meal was also observed in tilapia Oreochromis niloticus (Edochiem et al., 1985; Ufodike and Ugwuzor, 1985; Otubusin, 1987). Fowler and Banks (1973) also demonstrated that blood meal fed at 5.8% of the diet as a partial replacement for herring meal produced

increased fish growth, and as high as 11.6% of blood meal could be incorporated into diet with growth comparable to those of controls.

It was observed that fish fed the highest levels of blood meal recorded the highest mortality in the hapas. Whether this high mortality was caused by a nutritional defect in the diet was not clear. However, it has been reported that blood meal is unpalatable to most livestock hence it is not popular in protein supplement of livestock feeds (Cullison, 1979). This might further explain the poor growth rate of fish fed the high blood meal diets. However, inclusion of low levels of blood meal in soyabean diet will improve the growth rate of C. anguillaris without deleterious effect on the fish.

In general the combination of 50% blood meal and 50% soyabean diet gave the best mean weight gain, best specific growth rate, best feed conversion efficiency and protein efficiency ratio compared with all other treatments. This shows that the nutritive value of blood meal

is enhanced by the addition of an equal level of soyabean meal in the diet. Thus the combination of blood meal and soyabean meal has a higher effective biological value than soyabean meal alone (Harris, 1980).

9.5.0 CONCLUSION

C. anguillaris fingerlings could utilize up to 50% blood meal in the soyabean diet for growth. Beyond this level of substitution the growth of the fingerlings was reduced. Inclusion of high levels of blood meal in the diet of C. anguillaris should be avoided to prevent high mortality of fingerlings.

CHAPTER TEN

THE UTILIZATION OF FEED MIXTURES CONTAINING BLOOD MEAL, FISH MEAL, GROUNDNUT CAKE AND SOYABEAN MEAL BY FINGERLINGS MUDFISH CLARIAS ANGUILLARIS

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10.1.0 INTRODUCTION

The complete fish diets whether dry or moist contain high levels of protein, two to four times higher than those of warm blooded animals. They rely highly on fish meal to supply principal protein (Mertz, 1972; Nose, 1978). However fish meal is very scarce and when available are usually very expensive (Eyo, 1986). The source of dietary proteins must be changed with less emphasis on fish meal in order to reduce cost. The abundance and availability of proteins of plant origin has increased research emphasis on their potential utilization (Mahnken et al., 1980) in animal feeds.

Koops et al. (1976) reported that one quarter of fish meal could be replaced by soyabean meal enriched with methionine in the diet of rainbow trout . Tiews et al. (1976) also demonstrated that a quarter of fish meal protein could be susbstituted by isonitrogenous amounts of soyabean protein concentrate (hypro, methionine supplemented) or a mixture of meat and bone meal and blood meal in the diet of rainbow trout,

even if the remaining dietary protein comes from poultry products and feather meals. It has been demonstrated that diets containing 50% groundnut cake as the principal protein source can be used in complete feeding of carp (UNDP/FAO, 1983). These results indicate that plant and animal feedstuffs could be almost as effective in promoting growth in fish as fish meal.

The aim of this study was to determine the growth performance and nutrient utilization of Clarias anguillaris fingerlings fed isonitrogenous mixture of fish meal, soyabean meal, blood meal and groundnut cake in the diet.

10.2.0 MATERIALS AND METHODS

10.2.1 Description of Fish

The experimental fish were obtained by artificial reproduction as described earlier. The fry were reared with Artemia nauplii in the indoor hatchery until they were 1g. The fingerlings were transferred to the outdoor concrete tank and fed 40% crude protein diet containing high level of fish meal as the major protein source until they attained the weight of 13-14g.

600 fingerlings were randomly distributed into 24 hapas (25 fingerlings per hapa) and allowed to acclimatise for one week. During this period fish were not fed artificial diets but survived on the natural food in the hapas.

10.2.2 Diet

The composition of the eight experimental diets is shown in Table 10.1. The diets were formulated in such a way as to make them isonitrogenous with variable energy levels. Diets 1 and 5 containing soyabean and fish meal respectively as the protein sources were regarded as control diets. Diet 2 contained a mixture of soyabean meal and blood meal while in diet 3, soyabean meal and groundnut cake were the protein mixture. The protein sources in diet 6 was a mixture of blood meal and groundnut cake whilst in diet 7 it was blood meal and fish meal. A mixture of groundnut cake and fish meal were the protein components in diet 8. Variable levels of corn meal were added to balance the ration. Vitamin and mineral premixes as well as chromic oxide were added to each ration at 2g and 1g respectively. The ingedients were mixed in warm

Table 10.1 Composition of experimental diets (g/100g)

Ingredients	DIET DESIGNATION							
	DT1	DT2	DT3	DT4	DT5	DT6	DT7	DT8
Soyabean meal	74	27	41	34	-	-	-	-
Blood meal	-	27	-	-	-	30	25	-
Groundnut cake	-	-	41	-	-	30	-	38
Fish meal	-	-	-	34	64	-	25	38
Yellow Maize	23	43	15	29	33	37	47	21
Vitamin/Mineral Premix*	2	2	2	2	2	2	2	2
Chromic oxide	1	1	1	1	1	1	1	1
Total	100	100	100	100	100	100	100	100

* Composition of vitamin and mineral premix as shown in Appendix 6.

water of about 80°C with a hand mixer and pelleted with a domestic pelleter. Pellets were left to dry in the oven at 50°C.

10.2.3 Experimental Procedure

At the end of acclimation, 20 fingerlings were randomly selected and weighed into each hapa. The remaining 5 fingerlings were pooled. 15 fish were selected from this stock and frozen at - 8°C ± 2°C for a period of about one month before samples were analysed for body composition.

The test diets were introduced the following morning after the mean body weight of the fish has been determined. Feeding was carried out at 9.00 a.m. and 4.00 p.m. daily at a rate equivalent to 3% of the mean body weight of the fish. All the fish in each hapa were weighed biweekly. Faeces were collected by stripping at weekly intervals and stored in the domestic freezer. Faeces were pooled at the end of the feeding trial for digestibility determination.

At the end of the 56 days observation, the final mean body weights of the fish in the hapas were determined. Eight fish samples were randomly selected from each hapa and killed by stunning the cranium with a needle. The fish were transferred separately into capped 100cm³ bottles and frozen at 8°C ± 2°C for about one month. They were later homogenised and analysed for proximate composition.

10.2.4 Chemical Analysis

Crude protein, crude fat, crude fibre, ash and moisture were determined in fish and feed samples according to the AOAC (1980) methods. Analysis of calcium and phosphorus were carried out using the atomic absorption spectrophotometer (AOAC, 1980).

Feed conversion efficiency, specific growth rate, protein efficiency ratio and apparent net protein utilization were monitored as previously stated (Section 3.8.0). Protein

digestibility was determined on feed and faeces by the method of Furukawa and Tsukahara (1966).

10.2.5 Statistics

Analysis of data were done statistically using the computer (Tandy 4000 Lx). Values were tested for significance by the analysis of variance (ANOVA) and the Duncen's Multiple Range test (See section 3.9.0).

10.2.6 Physico-chemical Parameters

The physico-chemical parameters (temperature, pH and dissolved oxygen) of the water in the concrete tanks were analysed weekly using standard methods (See section 3.4.0).

10.3.0 RESULTS

10.3.1 Proximate Composition

Table 10.2 shows the proximate composition of the experimental diets. The diets were formulated in such a way as to make them isonitrogenous with variable calorie contents. The analysed composition shows residual moisture of about 9% in all the diets. The crude protein varied from 36.84% in diet 1 to 41.21% in diet 4. The fat content was highly variable, ranging from 2.84% in the control ration (Diet 5) to 15.43% in diet 1.

Table 10.2 Proximate composition of the test diet (% dry matter)

	DIET DESIGNATION							
	DT1	DT2	DT3	DT4	DT5	DT6	DT7	DT8
Moisture	8.79	8.88	8.61	9.87	8.74	9.09	8.88	9.09
Crude protein	36.84	40.09	37.39	41.21	40.70	40.06	40.35	37.16
Fat	15.43	10.56	13.66	7.58	2.84	13.55	7.98	8.12
Ash	4.69	3.65	5.33	16.38	26.31	4.77	12.48	15.48
Crude fibre	6.±0	4.17	7.22	9.40	3.12	3.17	2.37	5.65
Dry matter	91.21	91.12	91.39	90.13	91.26	90.91	91.12	90.91
Nitrogen Free Extract * **(NFE)**	28.15	32.65	27.79	15.56	18.29	29.36	27.94	24.50
Calcium	0.83	0.77	0.98	4.99	7.52	0.62	3.29	3.92
Phosphorus	0.50	0.33	0.50	1.71	2.81	0.44	1.67	1.67

* NFE was calculated as 100 - (Moisture + crude protein + crude fat + crude fibre + ash).

The total ash content was highest in the control ration (Diet 5) and lowest in diet 2, 26.31% and 3.65% respectively. The crude fibre varied from 2.37% in diet 7 to 9.40% in diet 4. The dry matter was about uniform approximating to 91% of the diet. The values for the NFE were between 15.56 and 32.65%. Calcium was low in diets 1, 3 and 6 and high in the others. The highest calcium content of 7.52% was in the control diet (Diet 5). Phosphorus was also highest in diet 5 (2.81%) while the least value was in diet 6 (0.44%).

10.3.2 Growth Performance

Table 10.3 shows the performance characteristics of C. anguillaris fingerlings fed isonitrogenous mixture of soyabean meal, blood meal, fish meal and groundnut cake. The initial mean weight of the fingerlings varied from 12.97 ± 0.23 to 13.99 ± 0.57g. There was no significant difference in the initial mean weight of the fingerlings ($P>0.05$). The final mean weight showed statistical significance between fish fed diets 1 and 2 ($P<0.05$).

Table 10.3 Growth performance characteristics of mudfish (Clarias anguillaris) fingerlings fed mixtures containing soyabean meal, blood meal, fish meal and groundnut cake.

Feed No.	Initial mean weight (g) ± SD	Final mean weight (g) ± SD	Mean weight gain (g)	Percentage weight gain	Quantity of feed fed	Specific Growth Rate (SGR)	Feed Conversion Efficiency (FCE)	Protein Efficiency Ratio (PER)	Apparent Net Protein utilization (NPU)
1	13.99 ±0.57	15.37^{a}±0.22	1.38^{a}±0.79	9.86^{a}	24.54^{a}	0.17^{a}	5.61^{a}	0.15^{a}	6.00^{a}
2	13.54 ± 1.00	16.25^{b}±0.16	2.71^{a}±0.85	20.01^{a}	24.32^{a}	0.33^{a}	11.24^{a}	0.28^{a}	7.21^{a}
3	13.20 ±0.94	19.05^{c}±0.35	5.85^{b}±0.59	44.32^{b}	25.35^{a}	0.66^{b}	23.07^{b}	0.60^{b}	11.77^{b}
4	13.34 ±1.20	22.73^{c}±1.31	9.39^{c}±2.50	70.39^{c}	29.01^{b}	0.93^{c}	32.15^{c}	0.78^{b}	18.66^{c}
5	12.97 ±0.23	23.15^{c}±0.28	10.18^{c}±0.06	78.49^{c}	28.38^{b}	1.04^{c}	36.06^{c}	0.89^{b}	21.55^{c}
6	13.90 ±0.29	21.33^{c}±1.67	7.43^{b}±1.96	53.45^{b}	27.70^{b}	0.77^{bc}	26.61^{b}	0.67^{b}	6.79^{a}
7	13.22 ±0.42	21.32^{c}±2.61	8.10^{b}±2.18	61.27^{b}	27.54^{ab}	0.85^{bc}	29.22^{bc}	0.73^{b}	12.59^{b}
8	13.73 ±1.20	21.80^{c}±1.99	8.07^{b}±0.78	58.78^{b}	29.67^{b}	0.83^{bc}	27.21^{bc}	0.72^{b}	14.72^{b}
	n.s	*	*	*	*	*	*	*	*

Note: Figures in the same column having the same subscript are not significantly different (P>0.05)

SD = Standard deviation

n.s = not significant

* = Significant at 0.05

There was no significant difference among fingerlings fed other diets (P>0.05).

The mean weight gain was lowest in fish fed diet 1 (1.38 ± 0.79g) and highest in fish fed diet 5 (10.18 ± 0.06g). No significant difference was noticed in fish fed diets 1 and 2 (P>0.05). Also fish fed diets 4 and 5 did not show any significant difference in weight gain (P>0.05). There was no significant difference in weight gain of fish fed diets 3, 6, 7 and 8, (P>0.05). The percentage weight gain was similarly affected by dietary treatment. The least percentage weight gain was observed in diet 1 (9.86%) and the highest in diet 5 (78.49%). Diet 1 and 2 did not show any significant difference (P>0.05). No significant difference was also noticed in fish fed diets 3, 6, 7 and 8, (P>0.05). Similarly fish fed diets 4 and 5 did not show any statistical significance difference (P>0.05).

10.3.3 Quantity of Feed Fed

The quantity of feed fed varied from 24.32g in diet 2 to 29.67g in diet 8. No significant difference was noticed in fish fed diets 1, 2 and 3 (P>0.05). Also, there was no significant

difference in fish fed diets 4, 5, 6, 7 and 8 ($P>0.05$).

10.3.4 Specific Growth Rate (SGR)

The SGR was affected by the types of feed fed. Fish fed diets 1 and 2 had SGR of 0.17 and 0.33 respectively. The SGR were significantly different from those fed diets 3 and 4 ($P<0.05$). Fish fed diets 4 and 5 did not show any significant difference in the SGR ($P>0.05$). Similarly, no significant difference was noticed in fish fed diets 6, 7 and 8 ($P>0.05$).

10.3.5 Feed Conversion Efficiency (FCE)

The mean values for the FCE varied with diet. Fish fed diet 1 did not differ significantly from those fed diet 2 ($P>0.05$) Whereas fish fed diet 3 was significantly different from those fed diet 4 ($P<0.05$). Fish fed diet 4 and 5 were not significantly different ($P>0.05$) Also fish fed diets 6, 7 and 8 did not show significant difference ($P>0.05$).

10.3.6 Protein Efficiency Ratio (PER)

The PER varied from 5.61 in diet 1 to 0.89 in diet 5. There was no significant difference in the PER of diet 1 and 2 ($P>0.05$) The

PER of the remaining diets were not significantly different from each other (P>0.05).

10.3.7 Apparent Net Protein Utilization (NPU)

The apparent NPU was affected by dietary protein mixtures. Fish fed diets 1, 2 and 6 had low NPU values which were not statistically significant (P>0.05). Fingerlings fed diets 3 and 4 showed significant differences in the NPU values (P<0.05). Similarly, NPU values for fish fed diets 5 and 6 were significantly different (P<0.05). Fish fed diets 7 and 8 did not show any significant differences in their NPU values (P>0.05).

10.3.8 Apparent Digestibility of Dietary Protein

The result of the apparent digestibility of dietary protein in mudfish fed isonitrogenous feed mixtures is shown in Figure 10.1 and Appendix 7. The best protein digestibility of 88.55% was obtained in the fish meal control diet (Diet 5) followed by diet 4. The digestibility of diet 7 and 8 were almost the same 57.74 and 58.53% respectively. So also was diet 3 and 6; 47.37 and 47.00% respectively. The worst protein

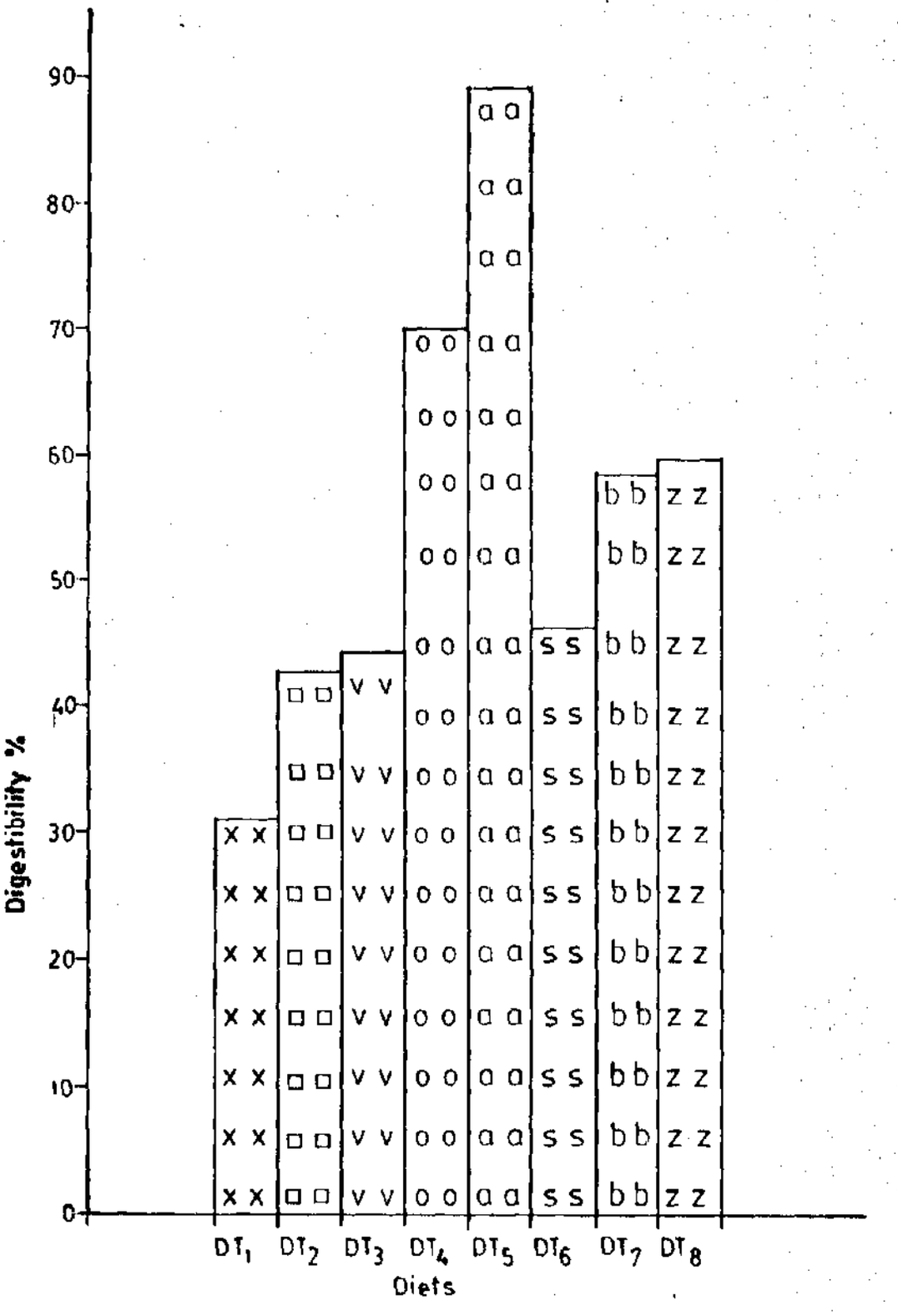


Fig. 10·1 Apparent digestibility of dietary protein in mudfish fingerlings fed isonitrogenous feed mxtures.

digestibility was in diet 1 (31.98%) followed by diet 2 (44.64%).

10.3.9 Body Proximate Composition

The proximate composition of C. anguillaris fingerlings before and at the end of the experiment is shown in Table 10.4. The moisture content was highest at the end of the experiment in fish fed diet 1 and lowest in fish fed diet 8. Fish fed other diets had moderate moisture content. Crude protein was highest in fish fed diet 5 (67.10%) and lowest among fish fed diet 1. The crude protein content of fish fed other diets was fair. The crude fat was lowest in fish fed diet 5 and highest among those fed diet 1. Others were moderate. Crude fibre was generally low in all the carcass analysed. Carcass of fish on diet 5 gave the highest values while those on diet 2 were lowest. Total ash was lowest in fish fed diet 8 and highest among fish fed diet 3. The total ash of the other fish were fair. Calcium and phosphorus were generally high. The calcium values varied from 4.18% in fish fed diet 4 to 6.73% in fish fed diet 3 while phosphorus ranged from 1.96% in the carcass of fish fed

Table 10.4 Body composition of Clarias anguillaris fingerlings before and at the end of the experiment (% dry matter)

Fish Fed Diet No.	Moisture (10 wt)	Crude Protein	Crude fat	Crude fibre	Total ash	Calcium	Phosphorus
Initial	75.94	58.33	15.67	0.98	20.17	6.38	3.67
Final							
1	74.81	60.54	20.02	0.84	17.81	5.20	2.18
2	74.70	61.22	16.61	0.62	17.32	6.37	2.85
3	72.48	62.73	17.60	0.65	19.06	6.73	2.83
4	71.74	66.02	17.19	0.86	15.93	4.18	1.96
5	71.39	67.10	15.04	1.28	16.61	6.12	2.35
6	72.35	61.05	19.09	0.80	19.40	6.72	3.31
7	72.40	63.41	17.27	0.76	15.55	5.16	2.79
8	69.41	63.80	18.92	0.96	15.06	5.28	2.17

diet 4 to 3.31% in those fed diet 6. The carcass value of fish before the experiment showed higher moisture content lower crude protein and higher total ash than those analysed at the end of the experiment. The crude fat, crude fibre, calcium and phosphorus were moderate.

10.3.10 Physico-chemical Parameters

Table 10.5 shows the physico-chemical parameters of water in the outdoor concrete tank measured at weekly interval. The mean temperature was 28.75 ± 2.02°C. The pH was between 7.5 and 7.6 with a mean of 7.58 ± 0.05 The dissolved oxygen varied from 6.4 to 8.8 with a mean dissolved oxygen of 7.38 ± 0.89 mg/l. The physico-chemical parameters were adequate for the growth of the fingerlings (See Appendix 4).

10.3.11 Economic Appraisal of Results

The data in Table 10.6 show the economy of weight gain (EWG) of the feed mixture. The data were computed from the prices of feedstuffs in Appendix 5. The lowest EWG was recorded in diet 6 followed by diets 7 and 3. Diet 1 recorded the highest EWG followed by diets 8 and 2. The EWG of diets 3, 4 and 5 were moderate.

Table 10.5 Weekly physico-chemical parameters of the water in the outdoor concrete tank

Date	Temperature °C	pH	Dissolved oxygen (DO) mg/l
13/10/89	30.50	7.60	8.80
20/10/89	31.50	7.60	8.50
27/10/89	29.50	7.50	7.50
3/11/89	28.50	7.60	6.80
10/11/89	29.00	7.60	6.60
17/11/89	29.00	7.50	6.80
24/11/89	27.00	7.60	7.60
7/12/89	25.00	7.60	6.40
Mean	28.75	7.58	7.38
Standard Deviation	2.02	0.05	0.89

Table 10.6 Economy of weight gain (EWG) of the feed mixture

Diet No.	Composition	Feed consumed wt (g)	Cost (K)	Body Weight gain (g)	EWG Cost Wt/gain x 103
1	SB. YM	24.54	0.08	1.38	57.97
2	SB. BM. YM	24.32	0.05	2.71	18.45
3	SB GC. YM	25.35	0.10	5.85	13.68
4	SB FM. YM	29.01	0.13	9.39	13.84
5	FM. YM	28.38	0.16	10.18	15.72
6	BM. GC YM	27.70	0.02	7.43	2.69
7	BM. FM YM	27.54	0.07	8.10	8.64
8	GC FM. YM	29.67	0.14	8.07	17.35

SB = Soyabean meal BM = Blood meal FM = Fish meal

GC = Groundnut cake YM = Yellow maize

10.4.0 DISCUSSION

The results in Table 10.3 and Figure 10.2 indicate that C. anguillaris fingerlings responded differently to isonitrogenous mixtures of blood meal, groundnut cake, soyabean meal and fish meal in the diet. Fingerlings fed soyabean meal alone as the only protein source were the poorest in mean weight gain, SGR, FCE, PER and apparent NPU. The poor utilization of the soyabean meal should be due to imbalance in the indispensable amino acids following suboptimal level of methionine (Steffens and Abrecht, 1976; NRC, 1983) and poor digestibility of soyabean meal by the mudfish. Igbinosun and Roberts (1988) have also observed poor digestibility of soyabean meal by the catfish Chrysichthys nigrodigitatus. Poor digestibility of soyabean meal was also reported for rainbow trout and plaice (Windell et al., 1974; Cowey et al., 1974).

The mixture of soyabean meal and blood meal also gave low growth rate. The mixture appeared to be well balanced in indispensable amino acids. The low isoleucine content of blood meal

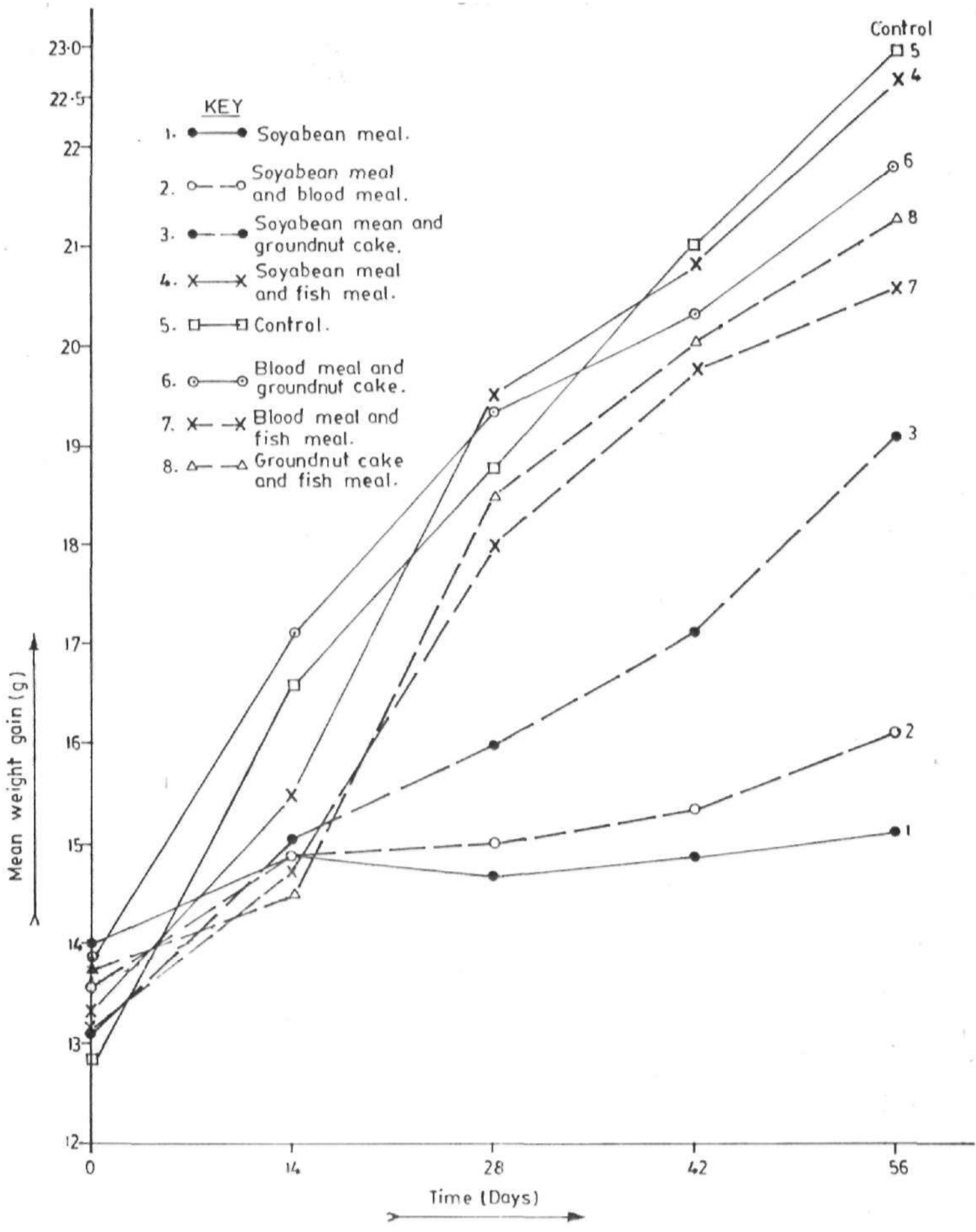


Fig. 10·2. Growth of C.anguillaris fingerlings fed isonitrogenous mixture of soyabean meal, fish meal, blood meal and groundnut cake.

was made up for by the high isoleucine in soybean meal. Both ingredients were high in lysine (a growth factor). The slow growth rate of fish fed the mixture could have been caused by the poor digestibility of the dietary protein resulting in low availability of the nutrients to the fingerlings.

The diets containing groundnut cake and soyabean meal gave growth performances which was significantly lower than that of the control ration containing fish meal. This could be traced to imbalance in the two plant protein sources. Both soyabean meal and groundnut cake have suboptimal levels of methionine (UNDP/FAO, 1983; Marris, 1980). Therefore a mixture of the two protein sources will further increase the deficiency of methionine in the diet. It has been shown that fish fed diets with low levels of methionine failed to grow properly (Halver, 1957). The poor apparent digestibility of the dietary protein also reduced the availability of the nutrients to the fish.

A mixture of soyabean meal and fish meal gave mean weight gain which was not significantly different from that of the control ration containing

fish meal This was attributed to the high nutritive value of the ration. The suboptimal methionine content of the soyabean meal was balanced by the high methionine content in fish meal. The mixture was highly digestible due to the high level of fish meal in the diet.

The diet containing blood meal and groundnut cake gave a significantly lower growth rate than the control ration due to the low isoleucine in blood meal and methionine in groundnut cake. The poor digestibility of the mixture also contributed to the poor growth of the fingerlings.

The diet mixture containing blood meal and fish meal was better utilized by mudfish fingerlings than mixture of blood meal and soyabean meal due to the high nutritive value of fish meal. The apparent protein digestibility of the mixture was also enhanced by the presence of fish meal.

The addition of fish meal to groundnut cake resulted in better growth rate to mudfish fingerlings than the addition of soyabean meal to groundnut cake. This should be due to the excellent nutritive value of fish meal.

The control ration containing fish meal as the only protein source (Diet 5) gave the highest performance in terms of mean weight gain, SGR, FCE, PER, apparent NPU and apparent digestibility of dietary protein than other diets. This shows that fish meal has the highest biological value and digestible energy than other commercially used protein sources (Lovell, 1981b). In general fingerlings fed diets containing high fish meal components grew better than those fed higher soyabean compositions. This has been observed by earlier workers (Dabrowski and Kozak, 1979; Abel et al., 1984).

The economy of weight gain (EWG) is used to describe the total financial cost in Kobo (k) of ingredients consumed per unit of body weight (Igbinosun, 1982). Thus ingredients of minimum EWG will give rise to the most economical feed. In Table 10.6 the mixture of blood meal and groundnut cake as well as blood meal and fish meal had the lowest EWG. The reason is that blood meal at the moment in Kainji area costs little. Therefore feedstuffs containing blood meal appear

economical. But the irregular supply of blood meal especially on a commercial scale, and the poor FCE of these diets are factors which do not render blood meal diets the first choice. Fish fed a mixture of groundnut cake and soyabean meal did not grow well but those fed soyabean meal and fish meal were not significantly different in mean weight gain, SGR, FCE, PER and apparent NPU than those fed control ration containing only fish meal. This appears to be the mose suitable diet for the fish. Thus the EWG should not be the only criteria for measuring the usefulness of a feedstuff in fish diet. Availability of the feedstuff and conversion efficiency of the diet should be of primary concern, since high conversion efficiency indicates not only adequate nutrient content and nutrient balance but food acceptability, availability and digestibility (Hastings, 1976). Feeds with high FCE and moderate EWG should be preferred to those with low FCE and low EWG.

10.5.0 CONCLUSION

Isonitrogenous mixture of soyabean meal and fish meal was the best in, mean weight gain, SGR, FCE, PER, apparent NPU and apparent digestibility of dietary protein to *C. anguillaris* fingerlings, than mixture of soyabean meal with blood meal and groundnut cake. The ration containing mixture of soyabean meal and fish meal should be prefered to the other mixtures.

CHAPTER ELEVEN

11.0.0 SUMMARY AND CONCLUSION

For any animal to express its full genetic potentials, the intake of adequate and well balanced feed is very necessary. This is particularly so in cultured fish. In C. anguillaris the attempt to develop a nutritious diet based on soyabean meal was sequel to the availability and high nutritive value of soyabean. Soyabean is widely grown and is very abundant. There is less competition with man for soyabean since the crop is grown mainly for livestock feeds. Comparison of the chemical composition and nutritive value of soyabean meal with those of other plant protein shows that soyabean meal was the richest in protein, fat and indispensable amino acids although the level of methionine was low. C. anguillaris fingerlings fed raw soyabean diets did not grow as well as expected from a protein source of such a high nutritive value. The following five factors in soyabean were responsible for the poor utilization of soyabean meal by the mudfish. The suboptimal level of methionine, the presence of antinutritional factors, especially trypsin inhibitor, the destruction of lysine during heat

treatment, the poor digestibility of soyabean protein and the palatability of the meal to C. anguillaris fingerlings.

The synthetic methionine appear in two forms, the L-methionine and the D,L-methionine. The D,L-methionine is less expensive than L-methionine because it is easier to synthesize than the pure L-form (Freedland and Briggs, 1977). Incorporation of different levels of synthetic L and D,L-methionine to soyabean diet for mudfish shows that C. anguillaris would utilize 0.2% L or D,L-methionine for growth. Higher levels were toxic to the fish. It was also established that C. anguillaris could utilize free amino acids for growth. The addition of 0.2% L or D,L-methionine also improved utilization of the soyabean diet. Since D,L-methionine, is less expensive than L-methionine addition of D,L-methionine to soyabean meal would be more cost effective.

One of the factors that militate against proper utilization of soyabean meal is the presence of antinutritional factors especially trypsin inhibitor. Trypsin inhibitor was reported by Rachis (1974) to cause depressed

growth and pancreatic hypertrophy in fish fed soyabean diets and was inactivated by heat treatment. Since C. anguillaris fingerlings fed raw soyabean diet did not grow properly, it was necessary to optimize the heat treatment in order to destroy the trypsin inhibitor contained in soyabean and improve the protein quality. Soyabean meal roasted for 30 minutes at 100°C gave the best growth performance and food utilization to C. anguillaris fingerlings. Over heating of the meal resulted in the reduction of the level of some indispensable amino acids. Lysine was most affected by heat treatment. The residue left after extraction of soyabean milk had low nutritive value and could not promote the growth of C. anguillaris fingerlings as well as the heat treated soyabean meal. The usual commercial toasting of soyabean meal was reported as 120°C for 20 minutes (Viola et al., 1983).

Heat treatment of soyabean meal caused destruction of lysine due to the formation of sugar - amino complexes or Maillard reaction (Maynard et al., 1979). The addition of different levels of synthetic L-lysine to the soyabean diet

enriched with methionine led to an increase in the growth rate of C. anguillaris in response to the added lysine. The maximum addition of 3% synthetic lysine resulted in a significantly higher growth rate than lower lysine levels.

The protein digestibility of soyabean meal verified in isonitrogenous mixtures of soyabean meal with blood meal, groundnut cake and fish meal, showed that soyabean meal was poorly digested by C. anguillaris fingerlings. Poor digestibility of soyabean meal was also reported for rainbow trout and plaice (Windell et al., 1974; Cowey et al., 1974) and the catfish Chrysichthys nigrodigitatus (Igbinosun and Roberts, 1988). The addition of other protein sources except blood meal significantly improved the digestibility of soyabean meal. The highest protein digestibility of soyabean meal was obtained in isonitrogenous mixture of soyabean meal and fish meal. The growth rate of mudfish fed this diet was not significantly different from those fed tha control diet of fish meal as the only protein source.

The often considerable quantity of uneaten feed recovered in the hapas and tanks when soyabean meal was fed as the only protein source to C. anguillaris fingerlings suggests that soyabean meal may not be very palatable to the mudfish. The palatability of soyabean meal was improved with the addition of fish meal. Similar observation was reported for Chinook Salmon (Akiyama, 1988). The identification of the substance in soyabean meal which reduces palatability in certain species of fish has not been established but is not believed to be trypsin inhibitor (Akiyama, 1988).

Information on the requirement of C. anguillaris for essential fatty acids and other nutrients are not currently available. Absence of information on nutritional requirements has greatly undermined the development of least cost formulations for mudfish. The least cost approach could only be used effectively if the nutritional requirements of the animal and the digestibility of the feedstuffs by the animal are known (Robinette, 1984).

The usually fast growth rate of C. anguillaris fed fish meal diets suggests that mudfish have the genetic potentials for growth under culture provided they are fed artificial diets of high nutritive value. Heat treated soyabean meal has potentials for improving the growth rate of mudfish if enriched with 0.2% synthetic methionine and 3% lysine. Feeding isonitrogenous mixture of soyabean meal and fish meal, or soyabean meal and groundnut cake was found to improve the growth of mudfish fingerlings.

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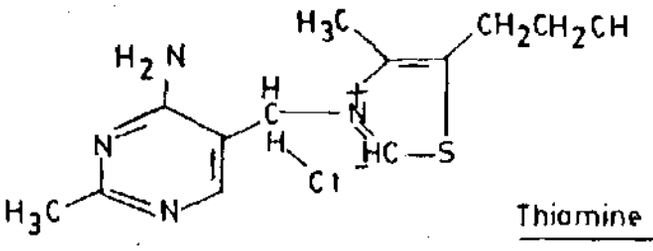
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Appendix 1

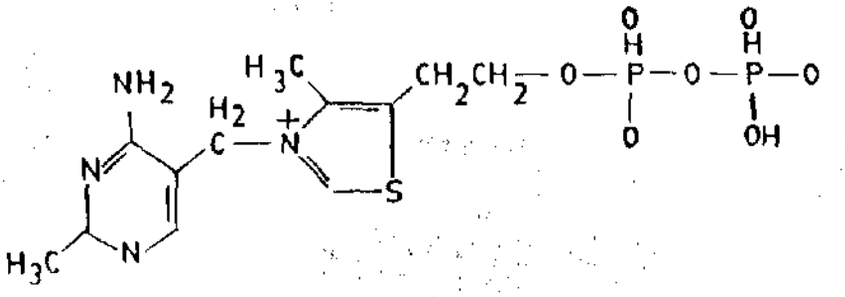
Chemical Structure of Vitamins.

(I) Water-Soluble vitamins

(a) Thiamine

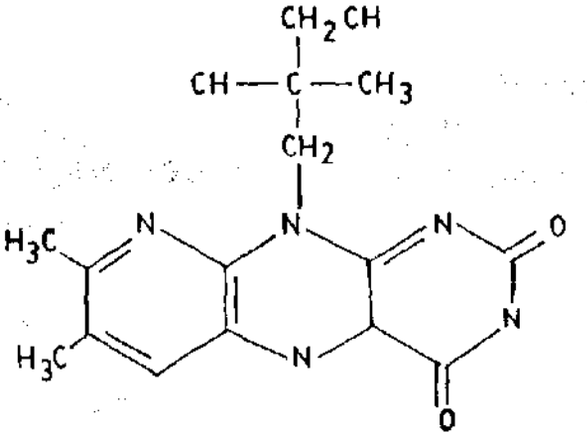


Thiamine hydrochloride



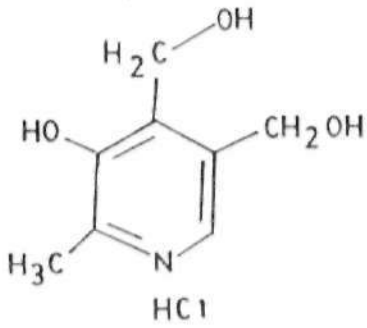
Thiamine pyrophosphate

(b) Riboflavin

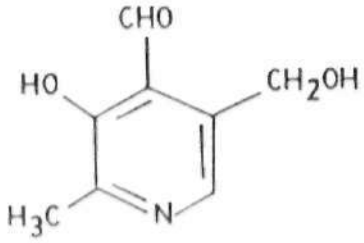


Riboflavin

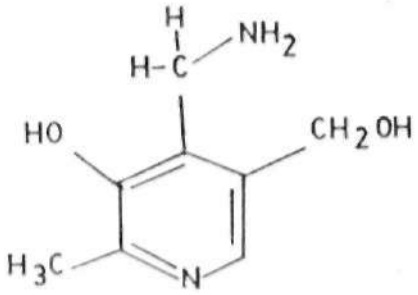
(f) Pyridoxine



Pyridoxine hydrochloride

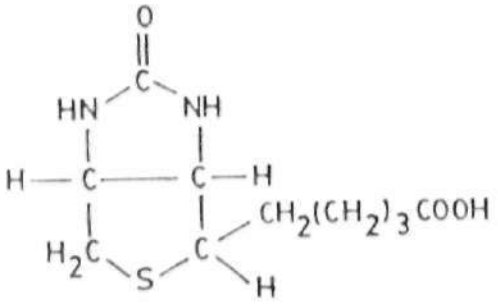


Pyridoxal



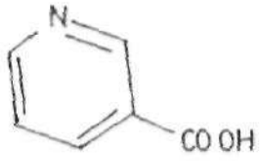
Pyridoxamine

(g) Biotin

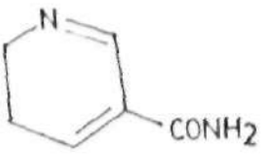


Biotin

(c) Niacin

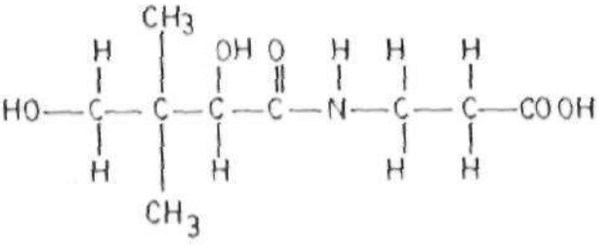


Nicotinic acid



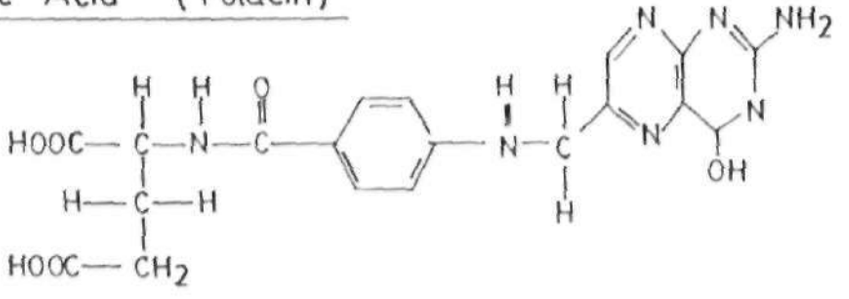
Niacinamide

(d) Pantothenic Acid



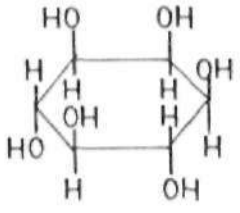
Pantothenic acid

(e) Folic Acid (Folacin)



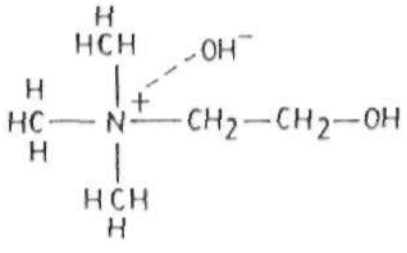
Folic acid

(j) Inositol



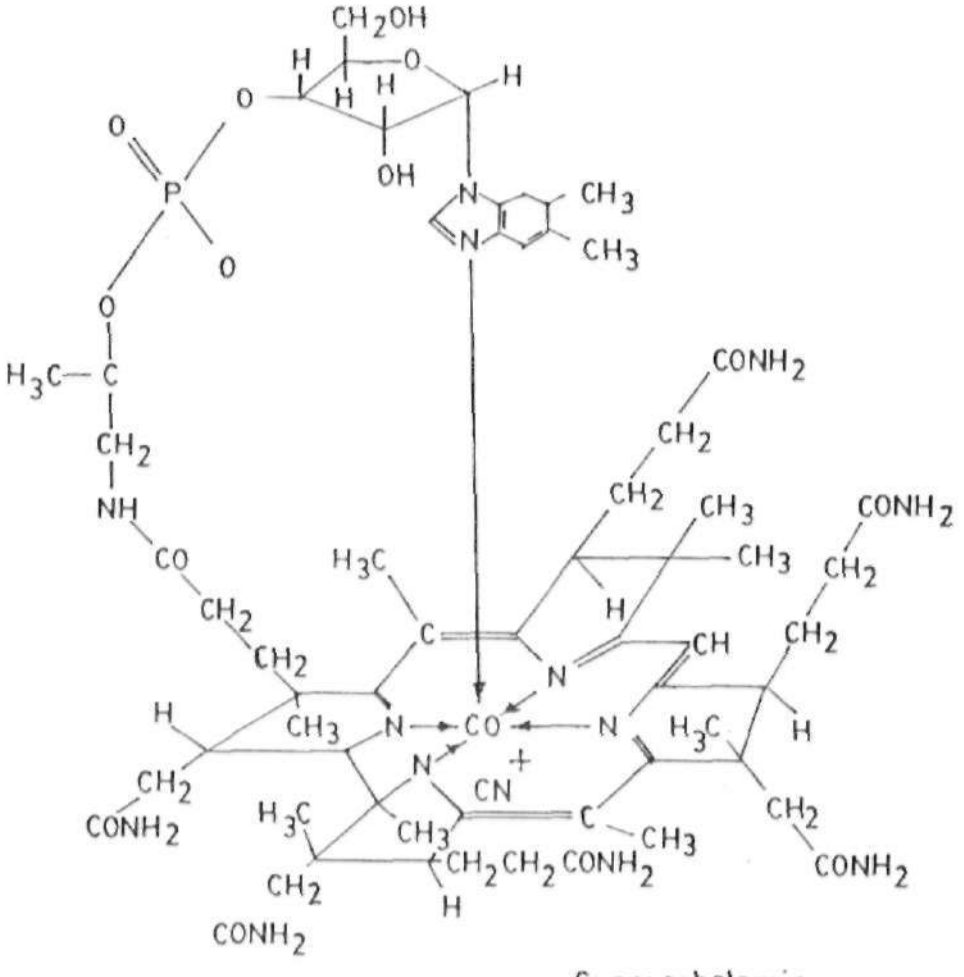
Inositol

(k) Choline



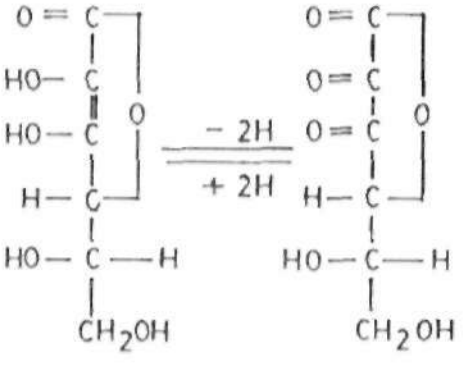
Choline

(h) Vitamin B 12



Cyanocobalamin

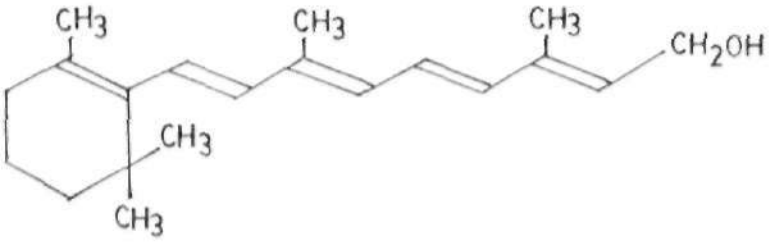
(i) Ascorbic Acid



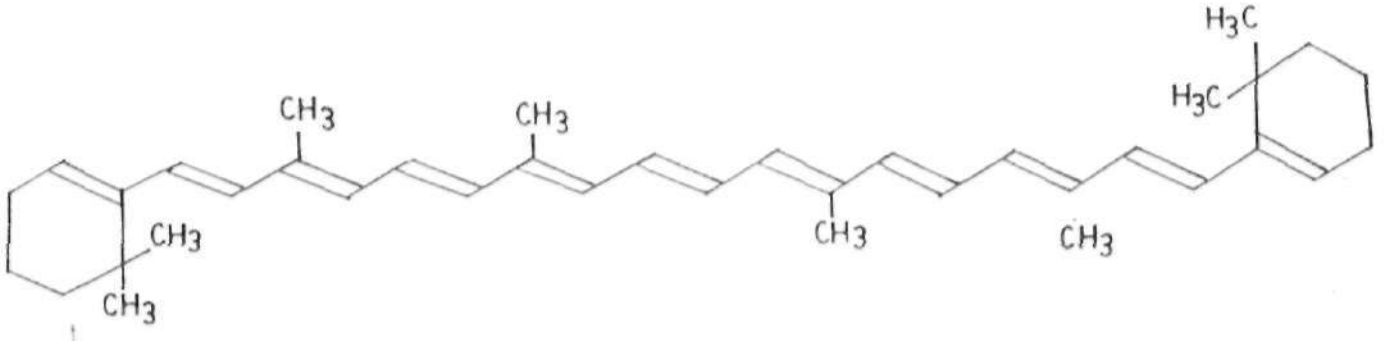
L-ascorbate Dehydroascobic acid

(II) Fat - Soluble Vitamins

(I) Vitamin A

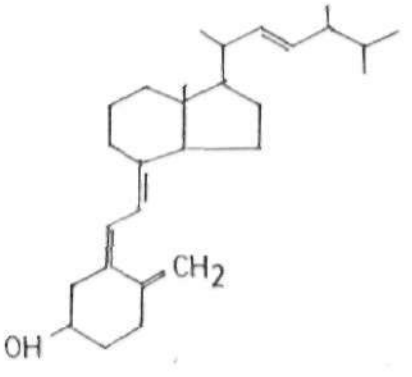


Vitamin A alcohol



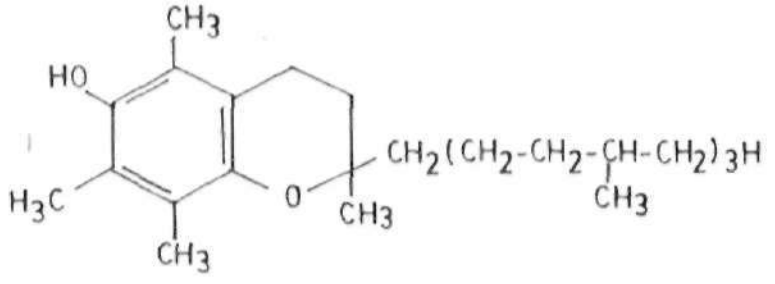
Beta - carotene

(m) Vitamin D



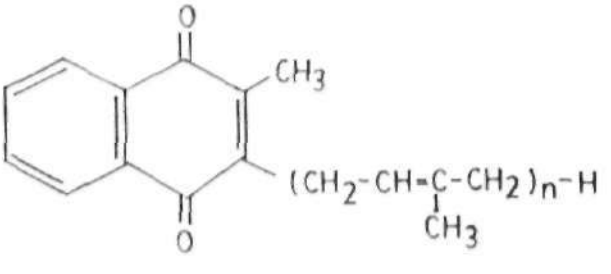
Vitamin D_2

(n) Vitamin E



Alpha – tocopherol

(o) Vitamin K



Vitamin K_2 series; n may equal 6, 7 or 9 isoprene units.

Appendix 2

Dilution of Stock Solution of Mineral Elements

The standard solutions of sodium, potassium, calcium, magnesium, iron and manganese were used for the analysis. The solutions were manufactured by Tokyo photoelectric Ltd, Japan. Each standard solution contained 1000 ppm of the element. The stock solutions were diluted to give 2, 4, 6, 8 and 10 ppm of each element except magnesium. For magnesium the solutions were further diluted to give 0.2, 0.4, 0.6, 0.8 and 1.0 ppm due to the sensitivity of the magnesium lamp.

Procedure

0.2cm^3 of the stock solution was taken in a graduated pipette and made up to 100cm^3 with distilled water in a volumetric flask. This dilution gave 2 ppm. Other dilutions were made as followes:

0.4cm^3 made up to 100cm^3 gave 4 ppm

0.6cm^3 made up to 100cm^3 gave 6 ppm

0.8cm^3 made up to 100cm^3 gave 8 ppm

1cm^3 made up to 100cm^3 gave 10 ppm.

Appendix 2 (Cont'd)

Magnesium

A further dilution was made as follows:
5.00cm³ of solution containing 2 ppm was made up to 50cm³ with distilled water to obtain 0.2ppm.
5.00cm³ of solution containing 4 ppm was made up to 50cm³ with distilled water to obtain 0.4ppm.
Similarly 5.00cm³ of 6 ppm made up to 50cm³ gave 0.6 ppm.
5.00cm³ of 8 ppm made up to 50cm³ gave 0.8 ppm.
5.00cm³ of 10 ppm made up to 50cm³ gave 1.0 ppm.

Phosphorus

1cm³ stock phosphorus solution contained 0.05mg phosphorus. 0, 1, 2, 3, 4 and 5cm³ of the stock phosphorus solution was pipetted into six 50cm³ volumetric flasks. The flasks were made up to mark with distilled

water. The solutions contained 0, 5, 100, 150, 200 and 250mg phosphorus respectively.

Appendix 3

Preparation of baton reagent (APHA, 1981)

Baton reagent is also known as ammonium vandomolybdate reagent and was prepared as follows:

40.0g ammonium molybdate was dissolved in 800cm³ of deionised water at 50°C. The solution was allowed to cool (Label A).

2g ammonium metavandate was dissolved in 600cm³ of boiling deionised water The mixture was allowed to cool and 280cm³ analytical grade concentrated nitric acid was slowly added with stirring. The flask was cooled again (Label B).

Solution A was added to solution B and the mixture was made up to 2 litres with deionised water. The solution was stored in the dark and used whenever necessary.

Appendix 4

Requirements of Warmwater fish for Temperature, pH and Dissolved oxygen*

(i) Temperature

The best temperature of most warmwater fishes is somewhere in the range 20°C - 32°C

(ii) pH

Most warmwater fishes prefer pH's of 6 to 9, pH's above 10 or below 5.5 are usually lethal.

(iii) Dissolved Oxygen

The optimum dissolved oxygen levels are above 5mg/l. Values between 3 and 5mg/l are stressfull. Oxygen concentrations below 2mg/l are often lethal depending on the size of the fish, water temperature and other water quality factors.

(iv) Special features of mudfish

Since the mudfish can practice aquatic as well as aerial respiration it has a

low aquatic oxygen threshold.

* Compiled from Dupree and Huner, 1984; Heneken et al., 1986

APPENDIX 5 Current Prices of some feed ingredients

Ingredient	Price per tonne
Fish meal	₦ 8,000.00
Soyabean	4,000.00
Groundnut cake	5,000.00
Blood meal	1,000.00
Maize	1,000.00
Guinea corn	1,200.00
Vitamin/Mineral Premix (broiler)	80,000.00

Appendix 6

Composition of Vitamin/Mineral premixes and Cod Liver Oil

A. Vitamin and Mineral premixes

Embavit No. 9 - Poultry Grower

May and Baker Nigeria Limited

(i) Vitamins

Ingredient	*Content per 100 grams*
Vitamin A	400,000 (I.U.)
Vitamin D_3	80,000 (I.U.)
Vitamin B_1	0.02
Vitamin B_2	0.16
Nicotinic Acid	0.8
Calcium Pantothenate	0.4
Vitamin B_{12}	0.02
Vitamin K_3	0.8
Vitamin E	0.8
Folic Acid	0.02
Choline chloride	0.1

(ii) Minerals

Cobalt	0.16
Copper	0.32
Iron	0.28
Iodine	0.032

Appedix 6 (Cont'd)

Minerals	Content per 100 grams
Manganese	2.56
Zinc	16.0
Selenium	0.0064
BHT	0.2

B. Cod Liver Oil (Seven seas brand)

Ingredient	Content per cm^3
Vitamin A	700 I.U
Vitamin D	80 I.U
Vitamin E	0.1

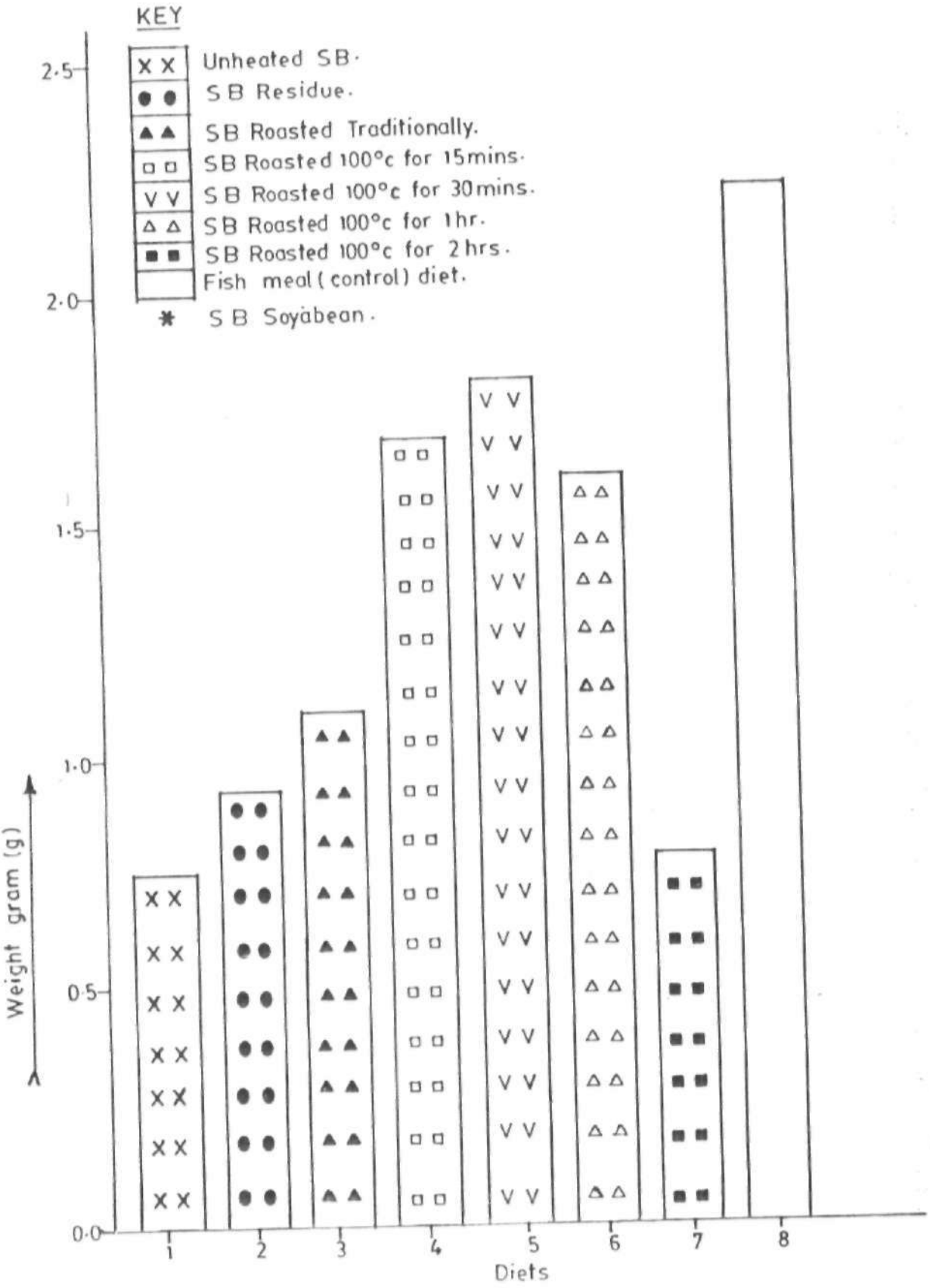
Appendix 7

Apparent Protein Digestibility of C. anguillaris fingerlings fed isonitrogenous mixtures of blood meal, fish meal, soyabean meal and groundnut cake.

Diet No	Digestibility
1	31.98
2	44.64
3	47 37
4	69 .82
5	88.55
6	47 .00
7	57 .74
8	58.53

Appendix 8

The mean weight gain value for C. anguillaris fingerlings fed processed soyabean diets.



Appendix 9

The mean weight gain values for C.anguillaris fingerlings fed soyabean meal, fish meal, blood meal and ground nut cake.

