STUDIES ON THE USE OF FERMENTED FISH SILAGE IN DIETS FOR JUVENILE TILAPIA (*Oreochromis niloticus*) AND CATFISH (*Clarias gariepinus*)

OYEDAPO ADEWALE FAGBENRO

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

INSTITUTE OF AQUACULTURE, UNIVERSITY OF STIRLING,

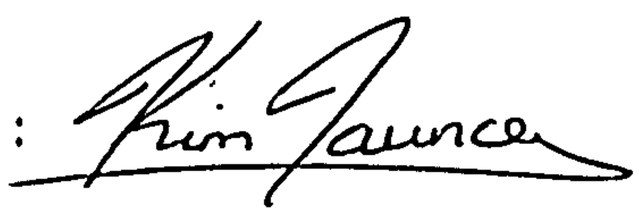
STIRLING, SCOTLAND

1994

DECLARATION

This thesis has been composed by the candidate. Except where specifically acknowledged, the work described has been conducted independently and has not been submitted for any other degree.

Signature of candidate : 

Signature of supervisor : 

Date : 20/10/94

DEDICATION

This thesis is dedicated to my wife, OMOTUNDE,

my daughter, MOYOSOREOLUWALORIMILOPOLOPOATINIGBAGBOGBO

and my son, MOFOPEFOLUWANITORITIOSEUNTIANUREDUROLAILAI.

ACKNOWLEDGEMENTS

"...I can no answer make but thanks,

and thanks, and ever thanks..."

from "The Winters Tale"

by William Shakespeare

Firstly, a lot of thanks go to a special friend of my family, who wishes to remain anonymous.

I hereby express profound gratitude to Dr. KIM JAUNCEY for his supervision, Dr. FIONA McLAREN and Mr. ALAN PORTER for assistance with analytical work, Dr. N.J. DIX and Dr. STUART MILLAR for assistance with microbiological work, Dr. CLIVE FOX for assistance with enzyme assays, Dr. RALF KRUEGER for assistance with histological work, and Mr. KEITH RANSON and Dr. GRAHAM HAYLOR for breeding the African clariid catfish.

I am grateful for funding received from the ASSOCIATION of COMMONWEALTH UNIVERSITIES (London) through the provision of a Commonwealth Academic Staff Scholarship under which this study was made possible. I also thank The BRITISH COUNCIL (Lagos, London, Edinburgh and Manchester) for the efficient way with which they tended my interests.

Finally, I thank Mr. DONALDSON for the donation of sugar beet molasses from INTERNATIONAL MOLASSES LTD., Grangemouth.

ABSTRACT

Fermented silage was prepared from a mixture of minced tilapias (***Oreochromis spp.***), different carbohydrate substrates (molasses, corn flour, tapioca flour) and ***Lactobacillus plantarum*** as inoculum, incubated anaerobically for 30 days at 5°-35°C. The pH and protein solubilization were temperature-dependent, and the source of carbohydrate substrate did not affect non-protein nitrogen (NPN) content or proximate composition of tilapia silage. During storage at 30°C for 180 days, NPN content increased and there was 8-11% loss of tryptophan. Moist diets containing tilapia silage (stored up to 60 days) were fed to ***Clarias gariepinus*** and differences in growth and protein utilization were demonstrated, but there were no effects on body composition. Partial replacement of fish meal with co-dried tilapia silage and soybean flour blend (FS:SBF) in dry diets supported growth and protein utilization similar to the control treatment. Fish growth and protein utilization were reduced with total replacement of fish meal. Apparent protein digestibility decreased with increasing dietary level of co-dried FS:SBF. Carcass composition was not affected and morphological defects were not observed. Co-dried tilapia silage blended with soybean meal, poultry by-product meal, hydrolysed feather meal or meat and bone meal (FS:SBM, FS:PBM, FS:HFM, FS:MBM) (providing 50% of the dietary protein) in dry diets fed to ***Oreochromis niloticus*** and ***Clarias gariepinus*** gave differences in growth, protein utilization and digestibility, and apparent energy digestibility. Carcass composition was not affected by silage blend and histological examination of exocrine pancreas, liver and intestine tissues did not show any lesions suggestive of nutritional imbalance. Haematocrit, haemoglobin content and mean cell haemoglobin concentration values showed no differences among the treatments. The results indicated that fermented tilapia silage is a suitable protein supplement in moist or dry diets for ***Oreochromis niloticus*** and ***Clarias gariepinus***, without affecting feed efficiency, fish growth or health.

CONTENTS

Page

ACKNOWLEDGEMENTS i

ABSTRACT ii

CONTENTS iii

TABLES vii

FIGURES ix

ABBREVIATIONS x

CHAPTER 1 : GENERAL INTRODUCTION 1

1.1 Aquaculture in Africa 2
1.2 Aquaculture in Nigeria 3
1.3 Aquaculture feeds and feedstuffs 5
Fish meal
Fish silage
1.4 Tilapias as substrate for silage production . . . 10
1.5 Objectives 11

CHAPTER 2 : LITERATURE REVIEW 12

2.1 Silage 13
Principles of ensilage
Microbiology of ensilage

2.2 Fish silage production 23
Fish substrates
Acid preservation process
Fermentation process
Liquefaction (Autolysis)
Storage stability
Chemical quality
Dried fish silage products

2.3 Utilization of fish silage 38
Aquaculture diets
Nutritional physiology and pathological effects

2.4 Tilapia and catfish feed requirements 43

2.5 Water quality requirements for tilapia and catfish culture . 45

CHAPTER 3 : PREPARATION AND STORAGE OF FERMENTED TILAPIA SILAGE . . 46

3.1 Introduction 46
Objectives

3.2 Materials and Methods 48
Fermentation substrates
Preparation of tilapia silage
Analytical methods
Bacteriological methods

3.3 Results and Discussion 51
Effects of incubation temperature
Effects of source of carbohydrate
Effects of prolonged storage

CHAPTER 4 : PRESERVATION AND STABILITY OF FERMENTED TILAPIA SILAGE . . 61

4.1 Introduction 61
Objectives

4.2 Materials and Methods 64
Fermentation substrates
Preparation of tilapia silage
Analytical methods
Bacteriological methods

4.3 Results and Discussion 68
Raw tilapia silage
Effects of preheating fish substrate
Effects of salt addition
Effects of potato extracts
Effects of formalin
Effects of ginger extracts

CHAPTER 5 : PROPERTIES OF FERMENTED TILAPIA SILAGE DIETS . . . 84

5.1 Introduction 84
Objectives

5.2 Materials and Methods 86
Preparation of tilapia silage
Preparation of tilapia silage diets
Water stability and protein leaching
Protein digestibility trial
Gastric evacuation time
Analytical methods
Statistical analysis

5.3 Results and Discussion 90
Effects of binders on water stability and protein leaching
Effects of binders on protein digestibility
Effects of binders on gastric evacuation time

CHAPTER 6 : EVALUATION OF FERMENTED TILAPIA SILAGE IN MOIST FISH DIETS . 94

6.1 Introduction 94
Objectives

6.2 Materials and Methods 96
Experimental fish
Experimental systems
Fermented tilapia silage
Experimental diets
Analytical methods
Growth trial
Protein digestibility trial
Carcass analysis
Statistical methods

6.3 Results and Discussion 103
Experimental diets
Growth response and Feed conversion
Protein utilization and digestibility
Carcass composition
Pathological effects

CHAPTER 7 : EVALUATION OF DIFFERENT LEVELS OF DRIED TILAPIA SILAGE IN DRY FISH DIETS 111

7.1 Introduction 111
Objectives

7.2 Materials and Methods 113
Experimental fish
Experimental systems
Fermented tilapia silage
Experimental diets
Analytical methods
Growth trials
Protein digestibility trials
Carcass analysis
Statistical methods

7.3 Result and Discussions 117
Experimental diets
Growth response and Feed conversion
Protein utilization and digestibility
Carcass composition
Histological effects
Haematological effects

CHAPTER 8 : EVALUATION OF DIFFERENT TYPES OF DRIED TILAPIA SILAGE IN DRY FISH DIETS 138

8.1 Introduction 138
Objectives

8.2 Materials and Methods 140
Experimental systems
Fermented tilapia silage
Experimental diets
Analytical methods

Growth and Protein digestibility trials
Carcass analysis
Statistical methods

8.3 Results and Discussion 149
Experimental diets
Growth response and Protein utilization
Carcass composition
Histological and haematological effects

CHAPTER 9 : ECONOMICS OF FISH SILAGE PRODUCTION AND UTILIZATION . . 161

9.1 Fish silage production 161
9.2 Fish silage utilization 163
9.3 Model of fish silage production costs in Nigeria . . . 166
Liquid silage
Dried fermented silage products

CHAPTER 10 : CONCLUSIONS 171

REFERENCES 174

ADDITIONAL REFERENCES 193

APPENDICES 197

TABLES

Table 2.1 : Classification of lactic acid bacteria important in silage.

Table 2.2 : Classification of silage additives.

Table 2.3 : A summary of the recommended nutrient requirements for ***O. niloticus*** and ***C. gariepinus***.

Table 2.4 : A summary of the recommended water quality requirements for ***O. niloticus*** and ***C. gariepinus***.

Table 3.1 : Average pH and NPN of tilapia silage fermented at different temperatures.

Table 3.2 : pH of fermented tilapia silages prepared using different carbohydrate substrates.

Table 3.3 : NPN and proximate composition of fermented tilapia silages prepared using different carbohydrate substrates.

Table 3.4 : Average pH, nutrient and proximate composition of tilapia silage during fermentation and storage for 180 days at 30°C.

Table 4.1 : Changes in pH and chemical characteristics of "raw" tilapia silage fermented at 30°C.

Table 4.2 : Proximate composition and gross energy content of minced tilapia and fermented tilapia silage.

Table 4.3 : Amino acid composition of minced tilapia and fermented tilapia silage.

Table 4.4 : Changes in pH and NPN of cooked tilapia silage fermented at 30°C.

Table 4.5 : Changes in pH and NPN of salted tilapia silage fermented at 30°C.

Table 4.6 : Changes in pH and characteristics of PE-treated tilapia silage fermented at 30°C.

Table 4.7 : Changes in pH and characteristics of GE-treated tilapia silage fermented at 30°C.

Table 4.8 : Changes in pH and characteristics of formalin-treated tilapia silage fermented at 30°C.

Table 5.1: Ingredient composition of the basal diets.

Table 5.2: Formulation of moist tilapia silage diets.

Table 5.3 : Water stability and protein leaching in moist tilapia silage diets.

Table 5.4 : Apparent protein digestibility and gastric evacuation time in ***O. niloticus*** fed moist tilapia silage diets.

Table 6.1 : Formulation of the binder meal.

Table 6.2 : Proximate composition and gross energy content of catfish diets, and properties of their respective wet silages.

Table 6.3 : Summary of growth and protein utilization by ***C. gariepinus*** fed moist diets containing stored fermented tilapia silage.

Table 6.4 : Body composition of ***C. gariepinus*** fed moist diets containing stored fermented tilapia silage.

Table 7.1 : Proximate composition, gross energy content and amino acid composition of co-dried tilapia silage:soybean flour blend and fish meal.

Table 7.2 : Formulation of dry diets for ***O. niloticus***.

Table 7.3 : Formulation of dry diets for ***C. gariepinus***.

Table 7.4 : Properties, proximate composition and gross energy content of tilapia diets.

Table 7.5 : Properties, proximate composition and gross energy content of catfish diets.

Table 7.6 : Amino acid composition of tilapia diets.

Table 7.7 : Amino acid composition of catfish diets.

Table 7.8 : Summary of growth performance and protein utilization by ***O. niloticus*** fed diets containing increasing levels of co-dried FS:SBF blend.

Table 7.9 : Summary of growth performance and protein utilization by ***C. gariepinus*** fed diets containing increasing levels of co-dried FS:SBF blend.

Table 7.10 : Carcass composition of ***O. niloticus*** fed diets containing increasing levels of co-dried FS:SBF blend.

Table 7.11 : Carcass composition of ***C. gariepinus*** fed diets containing increasing levels of co-dried FS:SBF blend.

Table 7.12 : Blood parameters of ***O. niloticus*** and ***C. gariepinus*** fed increasing dietary levels of co-dried FS:SBF blend.

Table 8.1 : Proximate composition and gross energy content of co-dried tilapia silage: filler blends.

Table 8.2 : Amino acid composition of co-dried tilapia silage:filler blends.

Table 8.3 : Formulation of dry diets for ***O. niloticus***.

Table 8.4 : Formulation of dry diets for ***C. gariepinus***.

Table 8.5 : Properties, proximate composition and gross energy content of tilapia diets.

Table 8.6 : Properties, proximate composition and gross energy content of catfish diets.

Table 8.7 : Amino acid composition of tilapia diets.

Table 8.8 : Amino acid composition of catfish diets.

Table 8.9 : Summary of growth performance and protein utilization by ***O. niloticus*** fed diets containing different co-dried tilapia silage:filler blends.

Table 8.10 : Summary of growth performance and protein utilization by ***C. gariepinus*** fed diets containing different co-dried tilapia silage:filler blends.

Table 8.11 : Carcass composition of ***O. niloticus*** fed diets containing different co-dried tilapia silage:filler blends.

Table 8.12 : Carcass composition of ***C. gariepinus*** fed diets containing different co-dried tilapia silage:filler blends.

Table 8.13 : Blood parameters of ***O. niloticus*** fed diets containing different co-dried tilapia silage:filler blends.

Table 8.14 : Blood parameters of ***C. gariepinus*** fed diets containing different co-dried tilapia silage:filler blends.

Table 9.1 : Production costs for producing fermented and acid silages from 1 tonne of fish wastes.

Table 9.2 : Cost of production of 1 tonne of fermented fish silage products.

Table 9.3 : Comparison of price/tonne of dried silage products and other animal protein feedstuffs used in aquaculture.

FIGURES

Figure 2.1 :Summary of pathways of carbohydrate fermentation by lactic acid bacteria.

Figure 6.1 : Growth of ***C. gariepinus*** fed moist diets containing stored tilapia silage.

Figure 7.1 : Growth of ***O. niloticus*** fed diets containing increasing levels of co-dried FS:SBF blend.

Figure 7.2 : Growth of ***C. gariepinus*** fed diets containing increasing levels of co-dried FS:SBF blend.

Figure 7.3 : Cross section of ***C. gariepinus*** liver from C-100 treatment showing hypertrophic hepatocytes arranged in large foci.

Figure 7.4 : Cross section of ***C. gariepinus*** liver from C-100 treatment showing hypertrophic hepatocytes scattered in the liver parenchyma.

Figure 7.5 : ***C. gariepinus*** liver showing different glycogen concentration of hypertrophic and normal hepatocytes.

Figure 7.6 : High power view of hepatocytes with hyaline droplets in their cytoplasm.

Figure 8.1 : Growth of ***O. niloticus*** fed diets containing different co-dried tilapia silage: filler blends.

Figure 8.2 : Growth of ***C. gariepinus*** fed diets containing different co-dried tilapia silage: filler blends.

ABBREVIATIONS

ADC	- apparent digestibility coefficient
ADCP	- Aquaculture Development and Coordination Programme
ADG	- average daily weight gain
AOAC	- Association of Official Analytical Chemists
AV	- Anisidine value
BHA	- butylated hydroxyanisole
BHT	- butylated hydroxytoluene
BV	- biological value
CHO	- total carbohydrates
CMC	- carboxymethyl cellulose
DM	- dry matter
EAA	- essential amino acids
FCR	- feed conversion ratio
FFA	- free fatty acids
FM	- fish meal
GE	- ginger extracts
GET	- gastric evacuation time
GG	- guar gum
H&E	- haematoxylin and eosin
HFM	- hydrolysed feather meal
HSI	- hepatosomatic index
LDM	- loss of dry matter
MBM	- meat and bone meal
MWG	- mean weight gain
NH_3-N	- ammonia nitrogen
NPN	- non-protein nitrogen
NPU	- net protein utilization
PA	- proteolytic activity
PAS	- periodic acid Schiff
PBM	- poultry by-product meal
PE	- potato extracts
PER	- protein efficiency ratio
pK_a	- water activity
PPV	- protein productive value
PV	- peroxide value
RD	- reference diet
SBF	- soybean flour
SBM	- soybean meal
SGR	- specific growth rate
TA	- titrable acidity
TBA	- thiobarbituric acid
TCA	- trichloroacetic acid
TKN	- total Kjeldahl nitrogen
TMA	- trimethylamine
TMA-N	- trimethylamine nitrogen
TMAO	- trimethylamine oxide
TMAO-N	- trimethylamine oxide nitrogen
TRS	- Torry Research Station
TS	- tilapia silage
TVN	- total volatile nitrogen
v/w	- volume/weight
WG	- wheat gluten
w/w	- weight/weight

CHAPTER 1 : GENERAL INTRODUCTION

"...If people feed fish,

then fish will feed people..."

Robert Stickney (1993)

Fish supplies over 50% of the total animal protein consumed in developing countries, and less so in developed countries (FAO,1991). The over-exploitation of fish resources and the ever increasing protein demand by the world population have posed problems to the fish supply from natural waters. Faced with a supply constraint, attention has now been drawn to aquaculture as a means to combat protein malnutrition in the developing countries.

Aquaculture has been practised for many centuries in various parts of the world and it has still not reached the limits of its development. In many countries, aquaculture research has focused on optimizing the production process for highly valued commodities. One of the most important problems in aquaculture is the development of practical diets for the rearing of larval and juvenile organisms in order to increase growth and survival rates. The development of better feeds and their more efficient use have resulted in the production of larger fish for which there is not only a higher demand, but which also commands a premium price.

1.1 AQUACULTURE IN AFRICA

Although a bas relief traced the history of aquaculture in Africa to 2500 BC in Egypt (MAAR *et al.*,1966), very few African countries have a background in fish culture. Pond fish culture was first tried in Kenya in 1924 and was later introduced to other countries after World War II (HUISMAN,1986; JACKSON,1988). Ever since, tilapias, clarlid catfishes and the exotic carp have been the predominant fish produced (BALARIN & HATTON,1979; VANDEN BOSCCHE & BERNACSEK,1990).

Over 82% of the world aquaculture production is attributed to Asia and Oceania while Africa's contribution is 0.1% (FAO,1991). Even less than 10% of Africa's total domestic fish production is supplied through aquaculture (SATIA,1989). Presently, freshwater, brackishwater and coastal marine environments contribute 97%, 1%, and 2%, respectively, of the total aquacultural production in Africa (SATIA,1989); of which 97.5% are finfish, 0.6% crustaceans and 1.9% molluscs. Marine and brackishwater fish culture as well as mollusc and crustacean culture are recent; and marine algae are not cultured.

Aquaculture production in sub-Saharan Africa is predominantly rural and oriented towards meeting the nutritional needs of the farmer and the extended family; and diversify their activities and incomes. Only recently has aquaculture been seen as likely to meet the shortfalls in fish supply and to reduce fish imports in some countries (Nigeria, Kenya, Zambia,

Zimbabwe, Cote d'Ivoire) where commercial aquaculture has started (HUISMAN,1986; KUTTY,1986).

1.2 AQUACULTURE IN NIGERIA

Artisanal fishermen and fishing communities in Nigeria had for generations practised traditional methods of fish culture in tidal pools and floodplains (DADA,1975; SAGUA,1976). These were extensive polyculture systems which do not fall strictly under the modern definition of fish culture, that is, "production under controlled conditions", and presently they do not play any significant role in the national economy.

The first attempt at fish farming was in 1951 at a small experimental station in Onikan (Lagos State) and various ***Tilapia*** species were used (LONGHURST,1961). Modern pond culture started with the establishment of a pilot fish farm (20 ha) in Panyam (Plateau State) for rearing the common carp, ***Cyprinus carpio*** (OLANIYAN,1961; AJAYI,1971), following the disappointing results with tilapias. Although the first years of Panyam fish farm's existence were hardly satisfactory, with production averaging less than 800 $kg.ha^{-1}.yr^{-1}$, the trials nevertheless generated sufficient interest that more fish farms were established by regional governments.

Presently, small-scale farms comprise a large proportion of aquaculture ventures ranging from homestead sandcrete ponds (25-40 m^2) operated by individual farmer or family to small earthen ponds (0.02-0.2 ha)

operated as part-time or off-season occupation by communities, institutions, associations or cooperative societies (ANYANWU *et al.*,1989). There are over 3,000 homestead sandcrete ponds, about 2,000 small earthen ponds and 60 commercial farms (>3ha) in operation (SATIA, 1990). Although the available water surface suitable for aquaculture was estimated as 483,406 ha (ITA *et al.*,1985), the total area of production units is 1,000 ha (SATIA,1990) with a yield of 5,500-7,750 MT.yr^{-1} (FAO/IFAD, 1987; FAO,1991).

Both indigenous and introduced species are cultivated in ponds, reservoirs and cages. Tilapias (***Oreochromis***, ***Sarotherodon***, and ***Tilapia*** spp). and clariid catfishes (***Clarias*** and ***Heterobranchus*** spp.) are the most widely cultured fish in tropical Africa (VANDEN BOSCCHE & BERNACSEK,1990) and are suited to low-technology farming systems in many other developing countries because of their fast growth rate, efficient use of natural aquatic foods, propensity to consume a variety of supplementary feeds, omnivorous food habits, resistance to disease and handling, ease of reproduction in captivity, and tolerance to wide ranges of environmental conditions.

Ironically, the preferred indigenous species such as the brackishwater catfish (***Chrisichthys nigrodigitatus***), the Nile perch (***Lates niloticus***) and bony tongue (***Heterotis niloticus***) are also the most difficult to breed. As fish hatcheries are yet in an early state of development in Nigeria, fish seeds are in short supply and a flourishing market for fish seeds collected

in the wild exists (FAGBENRO,1987). Among the introduced species, the common carp (***Cyprinus carpio*** ex. Austria/Israel,1954) has shown satisfactory performance but with a major drawback, that of a complex reproduction necessitating specialized hatchery techniques. Other exotic fishes such as the channel catfish (***Ictalurus punctatus***) and largemouth bass (***Micropterus salmonides***) (both ex.USA,1976) do not seem to have established in freshwater farms and reservoirs. The fate of the platy (***Xiphophorus maculatus***) is not known but top minnow (***Poecilia reticulata***) and goldfish (***Carrasius auratus***) introduced from the UK in 1972 for teaching purposes have been successfully established.

1.3 AQUACULTURE FEEDS AND FEEDSTUFFS

Aquaculture has developed from traditional extensive systems to semi-intensive and intensive systems, basically by the increase of stocking density to maximise the utilization of culture space. As this has exceeded the "natural" carrying capacity, inputs are required to maintain the fish growth and yield at harvest. Feed is a major constituent of culture inputs. The higher the culture technology, the more the culture system relies on exogenous feed supply, and natural supplies become less significant. Several factors are therefore considered such that the feeds are nutritionally adequate, physically acceptable, practically applicable and economically feasible.

The dependence on artificial feeds (as a result of intensive practices) has become necessary with commercialization of aquaculture. In many African countries, the existing animal feed manufacturers produce feeds mainly for livestock and poultry production. Due to limited demand and high cost of feedstuffs, specific feed industries to support aquaculture have not generally been developed. Feeds account for over 50% of operational cost in intensive aquaculture and protein is the most expensive component of artificial feeds (GRANT,1989).

1.3.1 Fish meal

There is a high and competitive demand for high quality protein concentrates in poultry, livestock and fish feed formulation. Fish meal, valued for its amino acid balance and unidentified growth factors, is widely used and plays a role in improving productivity and product quality of feeds. It provides the major protein source in most dry commercial aquaculture feeds and presently accounts for about 12% (approximately 6.5 million MT) of the worlds production (PIKE,1990) and is expected to double (to about 25%) by year 2000 AD. Fish meal production was 12% less in 1990 than in 1989 (IAFFM,1990, 1991) as fish stocks used for fish meal reduction appear to be in a worldwide decline (NEW,1991). If the present trend continues with the growing fish feed industry, a stagnating supply of fish meal will present sobering economic implications for aquaculture.

Until suitable alternative sources of protein are found or other animal feeds begin to rely less on fish meal, the cost of raising fish can be expected to increase significantly. In many countries, fish meal is expensive because local production falls short of demand in most cases, and it is imported thereby placing strong limitations on animal protein production. Fluctuations in cost, availability and quantity of fish meal have prompted the search for alternative sources of protein for aquaculture diets. Because feed costs constitute a high proportion (up to 70% in fry diets) of total fish production costs, the use of low-cost alternate sources of protein in fish feeds is an alternative approach to reduce production costs.

Many agro-industrial and food processing wastes/by-products of plant and animal origin have been considered as possible replacements for fish meal in animal feeds. Unlike plant residues which frequently contain anti-nutritional factors and lack some essential amino acids (hence they require elaborate and expensive processing to render them suitable for animal feeding), animal by-products do not contain toxic compounds, and hence require little processing to preserve and upgrade their nutritive value (TACON & JACKSON,1985). Large quantities of animal by-products such as abattoir wastes, poultry processing wastes, shrimp heads, fish cannery wastes and leather tannery wastes abound in Nigeria and they create disposal problems for their respective industries (BALOGUN,1990). Current disposal practices include burial, municipal garbage disposal and dumping in fields or streams. Recycling such

wastes into animal protein feedstuffs using low-level (artisanal) technologies will encourage and enhance aquaculture production.

1.3.2 Fish silage

The use of fishery by-products in animal and aquaculture feeds has received much attention worldwide and the two most important techniques (other than the direct production of rendered dry meals) used to preserve/upgrade their nutritional value are:

(a) ensilling through chemical acidification (acid-preserved silage) or microbial fermentation (fermented fish silage), and

(b) protein hydrolysis using selected exogenous enzymes (protein hydrolysate).

Both procedures rely on producing unfavourable conditions for putrefactive microorganisms, but conducive conditions for proteases (low pH required in the silage; high temperature required in the hydrolysate).

Fish silage production is useful along coastal fishing villages where the available fishery products are inadequate for economic fish meal production (REILLY,1985). The potential substrates available for fish silage production include:

(a) low-value mixed fish species generated from by-catch of shrimp fisheries which are usually thrown overboard at sea to create space for holding shrimps (KOMPIANG *et al.*,1980a),

(b) small-sized pelagic fishes caught in substantial quantities in the industrial fisheries (TREVINO *et al.*,1982),

(c) fish entrails (heads, bones, viscera, fins, tails) produced in large quantities during filleting process and in fish canneries (GREEN *et al.*,1988),

(d) stunted and undersized (unmarketable) tilapias and other similar species produced as a result of overpopulation caused by the prolific breeding habits of cultured tilapias (FOLTZ *et al.*,1982),

(e) fish cultured in waste-fed (manured) ponds which become unmarketable because of beliefs and attitudes associated with religion and hygiene (EDWARDS,1980), and

(f) unsold fish from markets during seasons of glut (REILLY,1985).

Efficient utilization of such alternative feedstuffs requires complete information on their chemical characteristics and nutritional value. Proximate composition analyses and digestibility coefficients of feedstuffs provide essential data for formulating animal feeds on a least-cost basis. Feeding trials conducted with test animals provide evaluations of nutritional quality and suitability of the least-cost feeds. The history, production and nutritional value of fish silage in animal diets is well documented in the literature but only recently has attention been focused on its possible use in fish diets.

Acid silage has been fed to carps, salmonids, eels, catfish, sea bass and tilapias with satisfactory results but few comparable results are available

for fish fed fermented silage. Preliminary studies however indicated that fermented silage is nutritionally equivalent to fish meal in diets for common carp, ***Cyprinus carpio*** (DJAJASEWAKA & DJAJADIREDJA,1980) and the clarild catfish, ***Clarias batrachus*** (WEE *et al.*,1986).

1.4 TILAPIAS AS SUBSTRATE FOR SILAGE PRODUCTION

Under semi-intensive and extensive pond culture systems, tilapias show early maturation and prolific breeding, resulting in stunted growth; and because of their small sizes and bony feature, they have a low consumer appeal (MOSES,1983). Whereas Asian communities accept small sizes of fish, Africans have strong preference for large table fish (BALARIN,1984) and other socio-economic factors such as beliefs and attitudes, associated with religion and disease effect, affect acceptability of tilapias/fish cultured in waste-fed (manured) ponds.

Large quantities of cichlids are landed from freshwaters of Africa in short periods and often glut the market, consequently much remains unsold and spoils as a result of poor handling and processing (SHIMANG,1992). These surplus unmarketable tilapias could be economically recycled for animal feeding through ensiling. The preparation of acid silage in Nigeria has involved different fish by-products as substrates which include clupeids - ***Pellonula afzeluisi, Sierrathrissa leonensis*** (AJAYI,1985), ***Clupea harengus*** offals (OLOGHOBO & BALOGUN,1988; OLOGHOBO *et al.*,1988) and mackerel - ***Scomber scomber*** offals (BALOGUN & OYEYEMI,1986;

ADEJUMO,1987). To date, no fermented silage production and feeding trials have been conducted.

1.5 Objectives

Given the decreasing supply and increasing cost of both plant and animal protein, alternative sources of nutritious but inexpensive proteins for animal feeds need to be developed. These may be provided from by-products of commercial fishery or fish processing operations. As fish meal production is capital and energy intensive, consequently it becomes necessary to identify a new technology by which fish products can be preserved/rendered.

Lactic acid fermented products offer greater scope for low-cost fish preservation and offer scope for the use of underutilized fish species (STROM,1992). Hence, the present study was conducted to assess the method of preserving fish by biological fermentation and evaluate use of such fermented products in fish feeds. The objectives of this study therefore were to investigate the nutrient quality and storage properties of fermented silage prepared from whole tilapias, and the nutritional value of the fermented tilapia silage as a protein supplement in moist and dry diets for juveniles of all-male Nile tilapia (***Oreochromis niloticus*** LINNAEUS) and the African clariid catfish (***Clarias gariepinus*** BURCHELL).

CHAPTER 2 : LITERATURE REVIEW

"...An honest tale speeds best,

being plainly told..."

from "King Richard III"
by William Shakespeare

Fermentation is one of the oldest and most important methods of preparing foods. Food fermentations are complex chemical transformations of organic substances brought about by the catalytic action of enzymes, either native to the substance or elaborated by a few species of microorganism. Microorganisms ferment foods to obtain the energy and growth requirements for their metabolism. Generally, foods provide a good medium for growth because they contain the nutrients (proteins, lipids, carbohydrates, vitamins, minerals) and other nutritive requirements for growth of microbes.

Microorganisms are ubiquitous but a pure culture of any microbial species rarely occurs naturally, hence growth associations (bacteria, yeasts, moulds) generally occur in fermenting foods. The rates of growth of the various species require that favourable environmental conditions be established in the product to be fermented. These conditions must be favourable to the desired organism but at the same time unfavourable for the many other microorganisms whose growth may result in off-flavours, spoilage, and even toxicity/pathogenicity to the consumer.

Fermented foods may be classified into products that essentially undergo an acidic, alcoholic, mould or enzymatic fermentation (PEDERSON,1978). Important acid products include fermented milk, most cheeses, sausages and vinegars; alcoholic products include beers and wines; and mould-fermented products include certain cheeses and many oriental foods. Enzyme products include meats, fish sauces, teas and possibly coffee, vanilla and cocoa. In nearly all foods, several microorganisms play a role in the fermentation.

2.1 SILAGE

2.1.1 Principles of ensilage

The word "silage" is derived from the process of storing chopped green forage in a silo. The subsequent fermentation yields acetic and formic acids which act as preservatives. In the 1920s, Dr. A.I. Virtanen made the process more reliable by adding inorganic acid to lower the pH since many crops do not have enough starch or sugar for natural fermentation to yield acids. Acid addition causes an instantaneous stop in the respiratory processes in the plants, preventing loss of organic carbon from the material, notably sugars. Acid-tolerant bacteria surviving in the acidified grass silage slowly convert sugars into lactic acid and thus keep the pH low. Residual sugars in the grass also contribute to preservation by repressing the production of deaminating enzymes in the bacteria and thus prevent ammonia formation from amino acids.

RAA *et al.*(1983) attributed the lack of success in preserving forage grass by natural fermentation to low levels of fermentable sugars in the raw material. Therefore, molasses, potato and sugar beet have been added as sugar sources to stimulate lactic acid bacterial fermentation and produce stable (pH<4.5) silages. Since plants have low population of lactic acid bacteria, combined sugar addition with starter cultures of such bacteria was used with acceptable results. The same principle is followed when applying natural fermentation for preserving fish (VAN VEEN & STEINKRAUS,1970).

Fish silage and fish protein hydrolysates are liquefied products obtained from whole fish or fish offal by enzymatic activity under accelerated conditions (pH <4.0 and temperatures >25°C) of digestion. Usually, the term "fish silage" is associated with the product of autolysis by endogenous proteases (pepsin, cathepsins) and lipases, mainly from the fish viscera (RAA & GILDBERG,1982), while "fish hydrolysates" is associated with the product formed by the addition of exogenous proteases (ficin, bromelain, papain) to fish protein (MACKIE,1982). RAA & GILDBERG (1982) traced the earliest production of fish silage to the 1920s in Sweden, a procedure which was later introduced all over Europe, Asia, the Americas and recently Africa (LEE,1990).

Traditional fermented fish products are popular in southeast Asia and as commonly applied, the term "fermented fish" covers two categories of product (ADAMS *et al.*,1987) namely:

(a) fish-salt formulations, e.g. fish sauce; and

(b) fish-salt-carbohydrate mixtures, e.g. *burong-isda* in the Phillippines and *pla-ra* in Thailand.

The first category depends primarily on autolytic processes while the second category involves enzymatic processes in which a lactic acid fermentation occurs. Fish silage has a potential application as a means to utilize waste or surplus fish which are not used directly as human food. Lactic acid fermented products offer considerable scope for the use of underutilized fish species as well as offers scope for low-cost fish preservation. The fact that small quantities of raw materials may be available, or their supply irregular or geographically dispersed, make their use suited for small-scale production.

The first objective in preservation by natural fermentation is the achievement of anaerobic conditions, under which lactic acid bacteria dominate. This occurs by storing substrates in sealed containers thereby preventing re-entry, and circulation, of air. Where oxygen is in contact for any period of time, aerobic microbial activity occurs and the substrates decay to a useless and frequently toxic product, often as a result of autoxidation (RAA,1980).

The second objective is to discourage the activity of clostridia, which thrive under anaerobic conditions. Their growth is undesirable because they produce butyric acid and degrade amino acids to a variety of products which are of poor nutritional value. The commonest way of

inhibiting clostridial growth is by lactic acid fermentation. The lactic acid produced decreases the pH to a level (about 4.0) at which clostridial development is inhibited.

The antimicrobial activity of lactic acid, like other weak organic acids (such as formic and propionic acids), is due to the relatively high pK_a values. At pH below their pK_a values, the acids remain as undissociated molecules and hence pass freely through the plasma membrane of the microbes. Once inside the relatively high pH environment of the cytosol, these acid molecules dissociate and hence lower the pH accordingly, killing the cell (RADLER,1975). The rate of lactic acid production depends on the initial lactic acid bacteria population and substrate availability (McDONALD,1981), which in turn is influenced by the degree of physical comminution (mincing, chopping). Finely chopped substrates are more readily preserved by acid than coarsely fragmented materials (STANTON & YEOH,1977). The comminution of fish is essential as coarse pieces of fish coupled with inadequate stirring of the liquefied product may result in pockets of undigested raw materials; which sink to the bottom of the fermentation bin or are suspended in the aqueous portion, and may be discarded along with sediments (bones), resulting in loss of nutrients.

2.1.2 Microbiology of ensilage

Lactic acid bacteria (lactobacilli) are micro-aerophilic, gram-positive, non-spore forming microbes which ferment sugars (mainly glucose and

fructose) to a mixture of acids, but predominantly lactic acid. They are facultative, that is, able to grow both in the presence and absence of oxygen. They belong to four genera namely, ***Lactobacillus***, ***Pediococcus***, ***Streptococcus***, and ***Leuconostocs***, and are classified into two groups namely homolactics (homofermentative) and heterolactics (heterofermentative)(Table 2.1) based on their physiological activity (Figure 2.1; PERDERSON,1971). The homolactics produce two moles of lactic acid per mole of glucose while the heterolactics produce only one mole of lactic acid together with ethanol and carbon dioxide. A concentration of lactic acid bacteria of 10^7 cells.g^{-1} after 24 h causes a decrease of the pH to 4.5. The final acid content is about 40 g.kg^{-1}. This acid production is much higher than it is in herb silages, and is due to the buffering effect of proteins and amino acids which are present in very high concentrations in fish silage (HAN-CHING *et al.*,1992).

Lactobacilli also inhibit the growth of other microorganisms during fermentation (WIRAHADIKUSUMAH *et al.*,1971). Delayed sealing of the silage container (fermentation bin) usually results in a reduction in the amount of lactic acid produced and frequently, a normally dominant lactic acid bacterial fermentation is replaced by a clostridial one. The presence of oxygen encourages the development of yeasts (LEVIN *et al.*,1989) and also causes an increase in the population of gram-negative bacteria.

Table 2.1 : Classification of lactic acid bacteria important in silage.

Bacteria	Natural habitat
Homolactics	
Lactobacillus lactis	milk and milk products
L. bulgaricus	"
L. helveticus	"
L. casei	"
Streptococcus lactis	"
S. diacetilactis	"
S. acidophilus	intestinal tracts and membranes of animals and humans
S. faecalis	"
S. salivarius	"
S. pyogenes	"
L. plantarum	intact and rotten plants
L. delbruckii	"
S. lactis	"
Heterolactics	
L. brevis	milk and milk products
L. fermentum	intact and rotten proteins
Leuconostoc mesenteroides	"
Bifidobacterium bifidum	intestinal tracts and membranes of animals and humans

source : SCHLEGEL (1986)

(a) Fermentation of glucose and fructose by homofermentative lactic acid bacteria

$C_6H_{12}O_6$ + 2 ADP → 2 CH_3-CHOH-COOH + 2 ATP
(glucose) (lactic acid)
(fructose)

(b) Fermentation of pentoses by homofermentative and heterofermentative lactic acid bacteria

$C_5H_{10}O_5$ + 2 ADP → CH_3-CHOH-COOH + CH_3-COOH + ATP
(xylose) (lactic acid) (acetic acid)
(arabinose)

(c) Fermentation of glucose and fructose by heterofermentative lactic acid bacteria

$C_6H_{12}O_6$ + ADP → CCH_3-CHOH-COOH + C_2H_5OH + CO_2 + ATP
(glucose) (lactic acid) (ethanol)

$3C_6H_{12}O_6$ + H_2O + 2 ADP → CCH_3-CHOH-COOH + 2 $C_6H_{14}O_6$ + CH_3-COOH + CO_2 + 2 ATP
(fructose) (lactic acid) (acetic acid)

$C_6H_{12}O_6$ + 2 $C_6H_{12}O_6$ + H_2O + 2 ADP → CCH_3-CHOH-COOH + 2 $C_6H_{14}O_6$ + CH_3-COOH + CO_2 + 2 ATP
(glucose) (fructose) (lactic acid) (acetic acid)

Figure 2.1 : Summary of pathways of carbohydrate fermentation by lactic acid bacteria.

The main hazard associated with proteinaceous foods such as fermented fish is from the growth of food poisoning bacteria and the production of biogenic amines (histamine, putrescine, cadavarine, spermine and spermidine) from the decarboxylation of free amino acids (RITCHIE & MACKIE,1978), which may be produced in amounts sufficient to cause poisoning in fishes (EITENMILLER *et al.*,1982). Of particular concern with unheated foods under anaerobic conditions is the growth of and toxin production by ***Clostridium botulinum*** (OWENS & MENDOZA, 1985). The botulinum toxin is easily destroyed by cooking but is very stable in salty and acidic environments (HUSS & RYE-PEDERSON,1980).

The importance of yeasts in aerobic deterioration of silages is well known. LEVIN *et al.*(1989) reported that yeast population of more than 10^5 organisms.g^{-1} silage are prone to deterioration. The yeasts involved are mainly pellicle yeasts belonging to the genera ***Hansenula, Candida, Saccharomyces, Pichia*** and the sediment yeast, ***Torulopsis.*** The initiation of aerobic deterioration by yeasts is followed by a second group of microflora consisting of proteolytic bacteria, streptomyces and moulds. The most common mould species isolated from deteriorated silages belong to the genera ***Monascus, Mucor, Geotrichum, Byssochlamys, Aspergillus, Penicillium*** and ***Fusarium*** (McDONALD,1981).

Frequent opening of the bin changes the environment from anaerobic to aerobic. The amount of air exchange during the storage period in the bin has a marked influence on the composition of the final product and

on the losses of nutrients (ESPE,1987). The most effective method of preventing aerobic deterioration is to ensure that silage is immediately used or stored when opened (BATISTA,1987). The treatment of substrates with additives prior to ensiling also influences the aerobic stability of silages. The original objectives in using silage additives were to ensure that lactobacilli dominated the fermentation and to improve the nutritional value of the silage.

McDONALD (1981) classified silage additives into four categories (Table 2.2) namely;

(a) fermentation stimulants,

(b) fermentation inhibitors,

(c) aerobic deterioration inhibitors, and

(d) silage nutrient enhancers.

He noted that chemical additives are unlikely to completely prevent the aerobic deterioration of silages and can only have a delaying effect on microbial activity. The best methods of prevention lie in efficient management techniques which reduce the aerobic exposure of silages to a minimum.

Table 2.2 : Classification of silage additives.

Silage additives					
Fermentation stimulants		Fermentation inhibitors		Anaerobic deterioration inhibitors	Nutrients[1]
Bacterial cultures	Carbohydrate sources	Acids	Others		
Lactic acid bacteria	Glucose Sucrose Molasses Cereals Whey Beet pulp Citrus pulp Potatoes Cellulases	Mineral acids Formic acid Acetic acid Lactic acid Benzoic acid Acrylic acid Glycollic acid Sulphamic acid Citric acid Sorbic acid	Formaldehyde Paraformaldehyde Sodium nitrite Sulphur dioxide Sodium metabisulphite Ammonium bisulphate Sodium chloride Antibiotics Carbon dioxide Carbon bisulphide Hexamethylenetetramine Bronopol Sodium hydroxide	Propionic acid Caproic acid Sorbic acid Pimaricin Ammonia	Urea Ammonia Biuret Minerals

[1] most substances listed under carbohydrate sources can also be listed under nutrients.

source : McDONALD (1981)

2.2 FISH SILAGE PRODUCTION

2.2.1 Fish substrates

The substrate for fish silage production are usually fish offals (head, bone, gut, fins, liver, roe), unsold whole fish (undersized, low-value, by-catch, spoilt), and shrimp processing wastes (BERTULLO,1984; OCKERMAN,1992). Fish viscera are less favoured as the oil content is likely to be high due to the presence of liver (JOHNSEN & SKREDE,1981). In order to avoid an oily taint in the flesh of terrestrial animals, GOHL (1981) recommended using whole lean fish. Even then, JAYAWARDENA *et al.*(1980a) and VAN WYK & HEYDENRYCH (1985) noted that fresh fish produced better silage than fish in which spoilage had set in, because of the risk from bacterial toxins (e.g. ***Clostridium botulinum***), formation of biogenic amines and products of oxidative rancidity.

Various fish species (lean or fatty, marine or freshwater, temperate or tropical) have been used as substrates but the inclusion of toxic species, such as puffer fish (***Sphaeroides maculatus***), posed a hinderance to the use of low-value (by-catch) fish in the Indo-Pacific region (DISNEY & JAMES,1980). Fish frames and shrimp processing wastes are less preferred because of the buffering of the acid by calcium from fish frames and large deposits of undigested fractions (chitin) produced, which require large volume of acid to neutralize (TREVINO *et al*,1982).

Two traditional methods used for fish silage production are acid preservation and microbial fermentation methods. For either method, the raw materials must be chopped and minced into pieces (5-10 mm). VERBURG & FREEMAN (1984) described a process that does not involve mincing of fish substrates. Providing the substrates are not putrid, a malty odour develops during storage and the process is virtually odourless. It varies between brown and grey viscous suspension, depending on the substrate and degree of autolysis (DISNEY & JAMES,1980).

2.2.2 Acid preservation process

The choice of preservative lies between a single inorganic acid, or inorganic acid mixture, and organic acid or organic/inorganic acid mixture. The acids lower the pH below 4.0 and prevent growth of bacteria and fungi, as well as killing fish parasites and their eggs. When added to whole fish, 3-4% formic acid is adequate (NORMAN *et al.*,1979) but the amount depends on the quantity of bones and scales present in the substrate (which may neutralize the acid). Organic acids are more expensive than the common inorganic acids, they are weakly acidic and hence do not require neutralization before being used as animal feed ingredient (TATTERSON,1982; LO *et al.*,1993a). In addition, organic acids have fungicidal properties, of which propionic acid was reported to be the most effective (RAA *et al.*,1983). However, DISNEY & JAMES (1980) warned that propionic acid alone should not be used because it is generally more expensive than formic acid.

An important consideration in the choice of acid mixture is that the growth of pathogenic bacteria, such as ***Clostridium botulinum*** and ***Salmonella*** spp. and other spoilage bacteria should be prevented at pH 3.5. Inorganic acids alone are no longer widely used as preservatives because handling of such acids is hazardous, the neutralization is laborious, the resultant high salt level from the neutralization is undesirable in animal nutrition, and corrosion of ensilage equipment is more severe at the pH 2.0 of a stable inorganic acid silage (BATISTA, 1987). The same principle is applied to acid preservation of non-fish substrates such as zooplankton (STEFFENS & SPANGENBERG,1985), casein (ASGARD & AUSTRENG,1985a), blood (ASGARD & AUSTRENG,1986) and artemia (ABELIN *et al.*,1991).

2.2.3 Fermentation process

Preservation of fish products is accomplished by the production of lactic acid by lactobacilli as a result of anaerobic fermentation of sugars at temperatures > 20°C while inhibiting growth of competing fermentive bacteria. The antagonistic properties of lactobacilli are attributable to lowering of pH (TRAMER,1966), production of hydrogen peroxide (PRICE & LEE,1970; DAHIYA & SPECK,1978) and production of antibiotics (LINDGREN & CLEVSTROM,1978). Also, certain viruses are inactivated by lactic acid bacteria during fermentation (GILBERT *et al.*,1983).

Access to free sugar is essential for the growth of lactic acid bacteria and this is a limiting nutrient in fish products (JAMES *et al.*,1977; LINDGREN & REFAI,1984), thus its preservation requires the addition of large quantities of fermentable carbohydrates. Apart from being the energy source for bacterial growth (ADAMS *et al.*,1987; TWIDDY *et al.*,1987), carbohydrates may also serve as natural sources of lactobacilli, unlike in acid silage where they are used to absorb moisture and facilitate drying (DISNEY *et al.*,1978).

Carbohydrates that have been used to produce stable silages include rice bran, wheat middlings, potatoes, whey, tapioca meal, malt, oat meal, lactose, molasses, maize, starch and citrus meal. The stability of the silage produced depends on the fish to carbohydrate ratio, with or without addition of a starter. When molasses is used, at least 10% (w/w) addition is required (ROA,1965; KOMPIANG *et al.*,1980b); but with cereal meals a small proportion of malt (as the source of maltose) is needed to convert starch to sugar. A summary of optimum levels of carbohydrates and inoculum used in lactic acid bacterial fermentation of fish products is presented in Appendix 1.

The conversion of carbohydrates to lactic acid takes place in three stages. Initially, the starch of the carbohydrate source is hydrolysed to maltose by α and ß amylase (present in the malt). Maltose molecules thus formed are further broken down to glucose by maltase. The last step is the conversion of glucose to lactic acid by lactobacilli. The

reaction is temperature-dependent and consequently the rate of pH decline depends on incubation temperature.

It is essential to avoid the development of heterofermentative bacteria (which often predominate early during fermentation) because it results in the production of ammonia (NH_3) gas which may cause significant increase in volume of fish silage products in the fermentation bin (STANTON & YEOH,1977). This does not only present a practical problem, but represents non-efficient acid production from the sugar as well as protein-nitrogen loss. The development of NH_3-producing bacteria can be suppressed by the addition of 5% NaCl (sodium chloride)(STANTON & YEOH,1977).

The various sources, combinations and proportions of the different substrates (fish, carbohydrate, starter culture, salt, water) for fermented silage coupled with the various conditions (incubation temperature, pH, duration) of fermentation and storage make the standardization of quality for lactic acid fermentation process impossible (HASSAN & HEATH, 1987). They also affect the nutrient quality and hygiene of the fish silage (KRISHNASWAMY *et al.*,1965; JAMES *et al.*,1977; ALI *et al.*,1984).

2.2.4 Liquefaction (Autolysis)

The preserved fish substrate gradually liquefies because the endogenous proteases and lipases catalyse the degradation of proteins (into

peptides and amino acids) and fats (into free fatty acids, diglycerides and monoglycerides, and glycerol), respectively. This activity strongly determines the characteristics of the liquid fish silage produced. According to WHEATON & LAWSON (1985) the enzymes may originate from four sources namely:

(a) viscera and digestive organs,

(b) muscle tissue,

(c) plants, and

(d) microorganisms.

Information on the specific origin of proteolytic enzymes responsible for autolysis in fish silage is conflicting. Proteases from prey fish, stomach, intestine (GILDBERG & RAA,1980), muscle (SIEBERT & SCHMITT,1965) and bacteria (LISTON,1965) were found to contribute to the total proteolytic activity in fish silages. BACKHOFF (1976), GILDBERG & RAA (1979) and JAYAWARDENA & POULTER (1980) established that the enzymes mainly responsible for liquefaction were those of the gut, skin and other parts of the fish rather than those of the flesh. Usually, it is attributed to gut enzymes, and in acid products (low pH) to pepsin (RAA & GILDBERG, 1976; AKSNES,1988).

Autolysis, indicated by liquefaction, has been measured by a decline in viscosity of fish silage (TATTERSON & WINDSOR,1974) or by an increase in the volume of the aqueous phase after centrifugation (BACKHOFF,1976; GILDBERG & RAA,1977). As autolysis progresses, a protein fraction

resistant to enzymatic digestion remains. RAA & GILDBERG (1976) suggested that muscle proteins cross-linked by disulphide bonds in the amino acids may account for the structural resistance of the sediment proteins to enzymatic attack, and which may have been stabilized by hydrophobic association and hydrogen bonds (HALL *et al.*,1985a).

Liquefaction during silage production is usually completed within 7 days at 23°-25°C (RAA & GILDBERG,1982) but VERBURG & FREEMAN (1984) liquefied catfish offals within 2 h by combined heating of the raw materials at 50°C and continuous agitation of the silage container for 30-60 min. Liquefaction rate is influenced by the activity of digestive enzymes, proximate composition of raw materials (moisture, protein, lipid, ash), pH, temperature, and type and level (%) of preservative acid(s) incorporated (RAA *et al.*,1983).

Starter cultures have been used to lower pH and increase liquefaction rate. ROA (1965) ensiled a mixture of minced herring and 10% molasses with ***L. plantarum***. JAMES *et al.*(1977) added ***L. plantarum*** to a mixture of minced silver bellies (***Leiognathus*** spp.), jewfish (***Pseudociaena*** spp.), sole (***Cynoglossus semifasciatus***), and carbohydrate, and made them into a slurry with 30% by weight of water which attained a low pH of 4.0-4.5 within 72 h; LINDGREN & PLEJE (1983) used ***Pediococcus acidilactiti*** and obtained pH <4.5 within 30 h at 20°-24°C, while VAN WYK & HEYDENRYCH (1985) used eight different cultures of lactobacilli and pH dropped within 48 h at 34°C. Other starter cultures that have proven successful include

L. brevis and ***P. cerevisiae*** (STANTON & YEOH,1977), ***L. acidophilus*** (TIBBETTS *et al.*,1981) and ***Streptococcus lactis*** (DHATEMWA,1989).

The protein in fish silage becomes solubilized after a week at 23°-30°C (JAMES *et al.*,1977). During liquefaction, proteins are converted to short-chain peptides, a portion of which may be further hydrolysed to free amino acids. The rate of autolysis and yield of soluble protein (non-protein nitrogen, NPN) are higher in silage prepared from fish viscera because fish gut enzymes are mainly responsible for autolysis (AKSNES, 1988), and because autolysis is temperature dependent, LINDGREN & PLEJE (1983) and STONE & HARDY (1986) opined that the fish could be pasteurized (90°C for 30 min) before fermentation or acidification.

As autolysis continues, the percentage of nitrogen as free amino acids increases while that present as polypeptides decreases (STONE & HARDY, 1986). Further degradation of free amino acids gives ammonia which causes loss of essential amino acids (tryptophan, phenylalanine, tyrosine and arginine). BERG (1985) suggested that formaldehyde could be added to fermented silage in order to reduce the amino acid degradation but HOLE & OINES (1991) warned of its toxicity to fish. HAARD *et al.*(1985) and HUSAIN & OFFER (1987) recommended that the addition of formaldehyde at 10 $L.tonne^{-1}$ silage will stop proteolysis and lipid oxidation. The ammonia and amines produced as a result of protein degradation bring about an increase in pH thereby lowering the storage potential (LINDGREN & PLEJE,1983; HAALAND *et al.*,1990).

2.2.5 Storage stability

Fish silage separates into three phases during storage : fish oil at the top, highly soluble protein and minerals in the middle layer and partially soluble materials and bones at the bottom. Proper mixing and stirring are essential to reduce oxidation of the lipid fraction and to maintain the homogeneity of the product. Fish silage is stable provided an adequate level of antioxidant is added to prevent lipid rancidity and provided autolysis is halted. BERG (1985) claimed that in fermented silage stored for several months, the lactic acid bacteria serve as an antioxidant.

Apart from a slight dilution effect of the preservative acid, no differences were found in proximate composition and amino acid profiles of various fish (substrates) and their respective products (acid and fermented silages, fish protein hydrolysates) stored at 23°-30°C for 35 days (TATTERSON,1982; HASSAN, 1982; RITCHIE & MACKIE,1982). JAMES *et al.* (1977) observed that free amino acids in both acid and fermented silages decreased slightly after storage for 6 months. The amino acids most affected were lysine, methionine, cystine, tryptophan, tyrosine, glutamic acid and proline.

The loss of tryptophan in stored fish silage is common, particularly at high temperature (30°C) (HAALAND & NJAA,1989a). Under acidic conditions, tryptophan is unstable and is rapidly lost. Histidine may also be limiting in fish silage prepared from partly spoiled fish and stored for long periods

(DISNEY *et al.*,1978). STONE & HARDY (1986) found that portions of free phenylalanine, arginine and glutamic acids were degraded with a loss of over 9% of the amino nitrogen as ammonia over a 6-week storage period, but no degradation products of tyrosine were detected.

During aerobic storage, spoilage of fermented food products is initiated by yeasts, which assimilate lactic acid, and is continued by proteolytic bacteria and moulds (BECK,1978; McDONALD,1981). These obligate aerobic microbes are capable of growth at relatively low pH utilizing carbohydrates and proteins. The fungistatic effects of butyric and propionic acids are well established (BECK,1978) but the effect of propionic acid on yeasts is limited. Sorbic acid and benzoic acid are used as antifungal agents in fermented or acidulated foods (BAIRD-PARKER,1980). Sorbic acid has a growth inhibiting effect on yeasts and moulds, but benzoic acid is mainly effective against moulds. JENSEN & JORGENSEN (1975) suggested that potassium sorbate was better since it is more soluble than sorbic acid. ESPE (1987) and LEVIN *et al.*(1989) used 0.3% and 0.1% (w/w) potassium sorbate with good results in herring and hake silages.

Fermented silage maintains a good nutrient value during long-term storage and rancidity is prevented even during drying, with lactic acid serving as the antioxidant (RAA *et al.*,1983). RAA & GILDBERG (1982) noted that fermented silage can be stored for at least a month at 30°C without any appreciable loss of nutrients. Despite this, ammonia

formation in fermented silage is considerably higher than in a corresponding acid silage. This implies that the depression of deamination by glucose is not absolute, but essential amino acids are hardly degraded since nutrient content was unaffected.

2.2.6 Chemical quality

The quality of fish silage depends on the quality of the raw materials, and age and storage conditions of the silage. Generally, there are no accepted chemical or biological quality parameters but changes in pH was suggested as a good indication of quality deterioration in fish silage (POULTER *et al*,1980) while NPN was reported as the best means of assessing autolysis (BACKHOFF,1976). PEDERSEN (1987) suggested that the following parameters would be suitable:

(a) total volatile nitrogen (TVN),

(b) trimethylamine nitrogen (TMA-N),

(c) trimethylamine oxide nitrogen (TMAO-N),

(d) peroxide value (PV),

(e) anisidine value (AV),

(f) thiobarbituric acid (TBA),

(g) biogenic amines (histamine, putrescine, cadavarine, spermine, spermidine),

(h) total number of viable bacteria less than $10^5.g^{-1}$, and

(i) total number of viable fungi less than $5{,}000.g^{-1}$.

Acceptable limits for the chemical parameters have not been decided because there is inadequate reliable information for them to be defined (HOLE & OINES,1991). Based on knowledge from fish meal processing, the quality of raw fish being processed greatly affects the quality of the product. Total volatile nitrogen (TVN) consists mainly of trimethylamine (TMA) and ammonia (NH_3). TMA originates from bacterial decomposition of trimethylamine oxide (TMAO) and analyzing for it may be used as a criterion for freshness of the raw material. HAALAND & NJAA (1989b) noted that the TVN of fish silage increases with storage even in silage made from fresh raw fish, and hence concluded that TVN is of limited value. The TMA of fish silage changes little after ensiling, and TMA/TMA+TMAO is therefore a better indicator of freshness of raw fish. According to STANTON & YEOH (1977), both pH and lactic acid content are useful indicators of successful fermentation of fish and fishery products.

Irrespective of the quality of raw material used, pH remains the factor that has the greatest influence on chemical composition and nutritional value of fish silage, particularly during liquefaction. Hence, STONE *et al.* (1989) opined that acid-stabilized fish silage (pH 2.0) as opposed to conventional silage (pH 4.0), could improve the nutritional value of fish silage as an animal feedstuff because it would produce high proportion of intact proteins, polypeptides and free amino acids after prolonged storage (42 days maximum).

Most freshwater or marine fishes and crustaceans contain thiaminase, an enzyme which degrades thiamine (vitamin B_1) and hence their silages may lack the vitamin, characterized by damage to the central nervous system. According to ANGLESEA & JACKSON (1985), ensiling fish containing thiaminase does not inactivate the enzyme immediately, but after extended storage, the thiaminase activity decreases below levels which can be reliably estimated. The presence of thiaminase in fish silage may not be a problem if the silage is mixed with a dry meal to form moist pellets. If the dry meal contains a thiamine supplement, this will therefore remain intact until the feed is eaten by the fish. Thiaminase becomes completely inactivated after 5 min at 82°C (GNAEDINGER & KRZECZKOWSKI,1966) hence RAA *et al.*(1983) recommended that the silage should be heated. Heating also safeguards against the spread of possible acid-resistant virus and makes oil removal easier. Thiamine is also decomposed by physical and chemical conditions such as intensive light, heavy metals, sulphite and some carbohydrates. It is heat stable under acid conditions but labile under neutral and alkaline conditions (EVANS,1975; ARNOLD,1978).

The limits of microbial indicators have been set, somewhat arbitrarily, at the same levels as the upper limits of microorganisms in raw milk (HOLE & OINES,1991). Some pathogenic bacteria (***Clostridium botulinum*** type E, ***Vibrio anguillarum***, ***Aeromonas salmonicida***, ***Renibacterium salmonirarum***, ***Yersinia ruckeri***, ***Streptococcus***, ***Salmonella*** spp.) are effectively destroyed in acid and fermented silages (BYLUND & WIKLUND,

1987; SMAIL *et al.*,1990) but fish silages at low pH are not completely protected against fungal growth. The aflatoxin- producing fungus, ***Aspergillus flavus***, is able to grow in the surface lipid of fish silage (MACKIE *et al.*,1971). When propionic acid is added to silage at 2% level, the growth of this and other fungi is prevented (STROM *et al.*,1980). In order to obtain successful fermentation and stable fish silage, HASSAN & HEATH (1987) advised that it was important to start with fish and carbohydrate with low mould counts.

2.2.7 Dried silage products

Liquid fish silage is viscous, bulky and difficult to transport, stir or store; and can only be fed to pigs directly. There are no solids present to make into presscake, hence water removal by evaporation is necessary. Because of the low solids concentration, fish silage is difficult to dry alone. Several methods of removing or reducing the water content of silages include spray drying, vacuum evaporation or drum drying (JENSEN & SCHMIDTSDORFF,1977; HARDY *et al.*,1983). Alternatively, a filler can be added and then dried together after which the co-dried product can be used as protein supplement for poultry or fish. Co-drying is a process whereby a small percentage of dry products are added to the wet silage to adsorb the solubilized proteins.

Under tropical conditions, the mixture of fish silage and filler material is sun-dried but the drying rate depends on the ambient climatic

conditions. Though simple and cheap, the effectiveness of sun-drying depends on relative humidity and may leave a high moisture content (45-50%) in the product and hence increases the susceptibility to fungal attack and possibility of contamination by animal parasites through faecal droppings. Subsequent deterioration depletes the nutrients and may be toxic for animal feeding. Alternatively, a solar dryer or kiln may be used for drying to a moisture content below 10%, but a compromise has to be achieved in terms of drying rate, cost and the desired moisture content of the product.

According to HARDY *et al.*(1983), co-drying the silage-filler mixture in conventional fish meal drying equipment prevents foaming (a feature of liquid fish silage) and facilitates drying by providing particles on which the silage can be adsorbed. This creates the possibility of customizing the nutritional and economic value of the dry fish silage diet by varying the combination and proportion of feedstuffs. DISNEY *et al.*(1978) cautioned that fish silage-carbohydrate products can only be fed to animals when mixed with other feedstuffs but not as a complete diet. STONE *et al.*(1984) successfully dried a blend of fish silage, canola meal and wheat bran, and when milled, the resulting dry product resembled fish meal and was incorporated as 50% of a fish diet formulation. It was later pelleted without difficulty.

Co-dried acid silages have been used to replace fish meal in diets for poultry with satisfactory results (DISNEY *et al.*,1978; OLOGHOBO &

BALOGUN,1988; OLOGHOBO *et al.*,1988). As a feed resource, fish silage contains the same nutrient levels as the fish substrate from which it is prepared (JACKSON *et al.*,1984a; ASGARD & AUSTRENG,1985b); oily fish such as sprat, herring and mackerel produce silages with higher oil levels than whiting, saithe or haddock. The protein and oil content of the dried product is easily altered by the type and amount of filler material used. Various filler materials that have been used include rice bran, maize flour, taploca flour, soybean flour, wheat middlings, whey, potato flour, taploca meal and soybean-feather meal mixture (DISNEY & JAMES,1980). The choice is determined by cost and local availability (DISNEY *et al.*, 1978). JAYAWARDENA *et al.*(1980b) recommended that good-quality rice bran (containing >10% protein) should be used at an optimum ratio of 1:3 (rice bran:fish silage).

2.3 UTILIZATION OF FISH SILAGE IN AQUACULTURE DIETS

2.3.1 Aquaculture diets

Fermented silage has been used as a feed supplement for various livestock and poultry animals (BROWN & SUMNER,1985; HASSAN & HEATH, 1986; LOPEZ,1990) and results have generally shown that it has good nutritional quality. The biological value of its protein was also comparable with that of fish meal protein (KOMPIANG *et al.*,1980b; TIBBETTS *et al.*,1981). However, only recently has its potential in aquaculture diets been recognised and hence few studies have

assessed their suitability (WEE *et al.*,1986). Contrastingly, acid silage has been fed as dietary protein for various fishes and shrimps (AFFANDI,1986; LALL,1987; GONCALVES *et al.*,1989; LUIS & BATISTA,1990).

Generally, fish silage has been compared with fish meal and its suitability (or otherwise) assessed by fish growth responses, protein utilization and digestibility. Feeding experiments have indicated that fish silage has good replacement value when it is partly substituted for fish meal in livestock and poultry diets (LALL,1991). However, conflicting results have been reported on fish silage as fish meal replacer (either partially or totally) in aquaculture diets. For example, while DJAJASEWAKA & DJAJADIREDJA (1980) obtained superior feed value for fish silage over fish meal diets, inferior values were reported by HARDY *et al.* (1983) and LUIS & BATISTA (1990). STONE *et al.*(1989), GONCALVES *et al.*(1989) and LALL (1991) found that fish silage produced similar growth performance to that obtained using fish meal. KOMPIANG *et al.*(1980a, b) and ALI *et al.*(1984) concluded that microbial fermentation offers an advantage over acid-preservation in that when the fish silage was fed to animals, it posed fewer nutritional problems and did not require neutralization before use.

YONE *et al.*(1986a) and HOSSAIN *et al.*(1987) found that fermented scrap meal (from mackerel waste) improved growth and feed efficiency in red sea bream, ***Chrysophrys major,*** and its performance was comparable to a white fish meal-based control diet. Similarly, WEE *et al.*(1986) found

no significant differences in daily weight gain, % weight gain, specific growth rate, and food conversion efficiencies among catfish, ***Clarias batrachus***, fed moist fermented tilapia silage diets and commercial pelleted feed. EDWARDS *et al.*(1987) also concluded that catfish, ***C. macrocephalus***, and snakehead, ***Channa striata***, grew well on fermented tilapia silage-based feed. MANIKANDAVELU *et al.*(1992) reported a considerably higher growth of carp, ***Cyprinus carpio***, fed fermented silage-based diet over a control fish meal-based diet; which suggest that the fish meal sample used in their study was of poor quality.

Poor performance of fish silage in aquaculture diets has been attributed to various reasons which include the following:

(a) poor water stability and rapid leaching of nutrients,

(b) type and quantity of acidulents used for preservation,

(c) reduced palatability and inhibitory effect of acid on digestive enzymes of aquaculture species,

(d) specific threshold limits of acid diet by individual fish species,

(e) high levels of hydrolysed protein (peptides, free amino acids),

(f) limiting amino acids and other nutrient deficiencies,

(g) interaction of acidulent with either protein or amino acids,

(h) effects of toxic products from lipid hydrolysis, and

(i) bitter flavour of certain polypeptides (formed during

proteolysis) which may affect the acceptability of the product.

2.3.2 Nutritional physiology and pathological effects

Apart from the effects of feeding fish silage-based diets on growth performance of aquaculture species, particular attention has also been paid to the possible pathological effects of feeding fish with silage-based diets following a report by ASGARD (1981) that salmon (***Salmo salar***) showed small internal inflammations. Ultrastructural cytology of hepatocytes of eels (***Anguilla anguilla***) fed fish silage diets (AFFANDI & BIAGIANTI,1987) revealed few structural modifications in the distribution of endoplasmic reticulum and mitochondria, which were benign and characteristic of nutritional imbalance.

AUSTRENG & ASGARD (1986) reported that fish silage produced using propionic acid or its combination with either formic acid or sulphuric acids were not palatable to Atlantic salmon (***S. salar***), but rainbow trout (***S. gairdneri***) fed similar diets neither showed food aversion nor growth depression. RUNGRUANGSAK & UTNE (1981) observed that formic and sulphuric acids caused a reduction in protease activity and growth depression particularly at the higher level of incorporation into the diet but these effects were not apparent in fish fed hydrochloric acid-preserved fish silage.

JACKSON *et al.*(1984b) observed histopathological defects (vacuolation, swelling and degranulation of eosiphilic granule cells) in peripheral tissues of the gut of salmon (***S. salar***) and they related these to the absence of an antioxidant in the fish silage used in the diet and the consequent formation of fatty acid oxidation products. The exact nature of the toxin were not ascertained but ELLIS (1982) suggested that such cellular changes are often associated with immune responses in fish.

ADEJUMO (1987) and LIE *et al.*(1988) found no metabolic disorders or reduced health in salmon, based on haematological tests and analyses of serum constituents, after feeding co-dried acid silage diets to tilapia (***Oreochromis niloticus***) and salmon (***S. salar***), respectively. Similarly, YONE *et al.*(1986b) and HOSSAIN *et al.*(1988) found no effect of feeding mould-fermented scrap meals on proximate and fatty acid composition of muscle and liver, haematological characteristics, and chemical components in plasma of red sea bream (***Chrysophyrys major***).

WEE *et al.*(1986) reported some mortality as well as occurrence of scoliosis and lordosis (vertebral column curvature) in catfish, ***Clarias batrachus***, fed acid or fermented silage diets but surprisingly did not consider these deformities as diet related. TACON (1985) noted that vertebral column curvature in fishes is due to dietary tryptophan deficiency which is characteristic of fish silage. These deformities were not reported for ***O. niloticus*** fed fish silage diets (HERNANDEZ,1983; ADEJUMO,1987; PHROMKUNTHONG & CHETHANON,1987).

2.4 TILAPIA AND CATFISH FEED REQUIREMENTS

Fish growth rates are determined by the combined effects of food quantity and quality. The quantity of food consumed is regulated through appetite to satisfy nutrient and energy requirements of fish. Efficient production and growth of fish in intensive aquaculture depends on the feeding of complete feeds at levels not exceeding the dietary needs.

Comparisons of results of nutrient requirements of fish is complicated by the experimental conditions including species, size and age of fish, stocking density, nutrient source, non-nutrient constituents of feed, and variations in abiotic factors (WILSON,1989). Although many studies have assessed the dietary protein requirements of warmwater fishes, most were conducted with fry or juveniles and over short periods (NRC,1983). Little information is available on the dietary protein requirements from the juvenile through large adult stages, a period when most of the feed costs are incurred during intensive culture (JAUNCEY & ROSS,1982). However, there is a general trend towards decreasing dietary protein requirement with age of fish (NRC,1983). A summary of the recommended protein levels required for optimum/maximum growth of intensively cultured ***Oreochromis niloticus*** and ***Clarias gariepinus*** is presented in Table 2.3.

Table 2.3 : A summary of the recommended nutrient requirements for ***O. niloticus*** and ***C. gariepinus***.

	O. niloticus[1]	***C. gariepinus***[2]
Crude protein (%)	30-47	38-42
Crude lipid (%)	6-10	>8
Carbohydrate (%)	25	-
Crude fibre (%)	8-10	-
Digestible energy (kJ/g)	NA	12
Essential fatty acids	0.5-1% 18:2n-6 or 1% 20:4n-6	-
Amino acids[3] (% dietary protein)		
Arginine	4.20	4.3
Histidine	1.72	1.5
Isoleucine	3.11	2.6
Leucine	3.39	3.5
Lysine	5.12	5.0
Methionine Cystine	2.68 (3.21)*	- (3.3)*
Phenylalanine Tyrosine	3.75 (5.54)**	- (5.0)**
Threonine	3.75	2.0
Tryptophan	1.00	0.5
Valine	2.80	3.0

[1] sources : SANTIAGO & LOVELL (1988), DeSILVA *et al.*(1989)

[2] sources : UYS (1989), HAYLOR (1992)

[3] no values are available on the amino acid requirements for ***C. gariepinus***, hence values for channel catfish, ***Ictalurus punctatus*** (WILSON,1989) are cited, since there is little difference in overall requirements between both species.

* methionine + cystine

** phenylalanine + tyrosine

2.5 WATER QUALITY REQUIREMENTS FOR TILAPIA AND CATFISH CULTURE

Tilapias and clariid catfishes are tolerant of a wide range of environmental fluctuations, which are characteristic of their natural habitats. Under semi-intensive or intensive culture, their water quality requirements for optimal production are not fully known but the recommended water quality requirements of ***O. niloticus*** and ***C. gariepinus*** are presented in Table 2.4.

Table 2.4 : A summary of the recommended water quality requirements for ***O. niloticus*** and ***C. gariepinus***.

	O. niloticus[1]	***C. gariepinus***[2]
Temperature (°C)	20-35	26-33
pH	5-11	6.5-8
DO_2 ($mg.L^{-1}$)	>3.0	>3.0
NH_3 ($mg.L^{-1}$)	>2.4 (pH=7.3-7.5)	>2.5 (pH=7)
CO_2 ($mg.L^{-1}$)	>72.6	<15 ppm
NH_4^+ (ppm)	-	<8.8 (pH=7)
NO_3^- (ppm)	-	<250
NO_2^- (ppm)	-	<0.25
Salinity (ppt)	13.5-29	0-2.5

[1] BALARIN & HATTON (1979)

[2] VIVEEN *et al.*(1985), BRITZ (1988)

CHAPTER 3 : PREPARATION AND STORAGE OF FERMENTED TILAPIA SILAGE

The experiments described in this chapter have been published in

Bioresource Technology, 46 (1993): 207-211.

Animal Feed Science and Technology, 45 (1994): 167-176.

3.1 INTRODUCTION

The chemical composition, quality and nutritive value of fish silage vary markedly depending on the source and freshness of raw materials and environmental conditions. Therefore, optimum conditions need to be established for each source. At present, acceptable analytical methods and quality criteria for fish silage have not been established but several chemical tests used as criteria for freshness of substrate for fish meal production were proposed by HARDY & MASUMOTO (1990). Generally, pH and NPN content are accepted and used as indicators for fish silage quality (HARDY & MASUMOTO,1989; BATISTA *et al.*,1989).

Most studies on the preparation and properties of fish silages have involved acid-preserved silages. Information is limited on the nutritional quality and value of fermented silage prepared using warmwater fish, which constitutes an important source of substrate for fish silage manufacture, particularly in the tropics (DISNEY *et al.*,1978). The fact that

only small quantities of fish wastes may be available, their supply irregular or geographically dispersed constitutes a limitation to the use of fish silage. The practical solution to this is to prepare and store the fish silage as the substrate becomes available. Significant changes that occur during storage of fish silage are autolysis of the tissues and release of ammonia (ESPE *et al.*,1989).

3.1.1. Objectives

Most fish and fishery by-products are unstable when fresh and under various storage conditions. Rapid deterioration of fresh fish is due to autolysis and microbial spoilage. The objectives of this study were to investigate both the basic features and some chemical quality parameters of fermented tilapia silage which could be used to evaluate its inclusion in fish diets. Tilapias were used as substrate in this study to provide a low-oil fish that would be representative of some of the underutilized species and would provide material that was representative of waste fish from artisanal fisheries or aquaculture operations in developing countries. This study investigates the:

(a) effect of incubation temperature on the fermentation of minced tilapia,

(b) effect of carbohydrate source on the fermentation of minced tilapia,

(c) changes that occur in chemical quality of fermented tilapia silage during prolonged storage.

3.2 MATERIALS AND METHODS

3.2.1 Fermentation substrates

Mixed-sex tilapias (***Oreochromis spp.***, <100 g) culled as a result of routine husbandry techniques in the Tropical Aquarium were used as protein substrate. They were kept frozen (-20°C) and later thawed at room temperature before use. Alternative carbohydrates used for fish silage preparations were sugar beet molasses (INTERNATIONAL MOLASSES Ltd., Grangemouth), corn flour or taploca flour (TESCO STORES, Stirling). A pre-fermented starter, prepared with freeze-dried cultures of ***Lactobacillus plantarum*** (NCIMB 11974, NCIMB Ltd., Aberdeen), was inoculated into molasses for large quantity production, and incubated until it gave 10^7 bacteria.g^{-1} molasses. ***L. plantarum*** was used as inoculum because it is homofermentative and grows at the desired temperatures (5°-35°C).

3.2.2 Preparation of tilapia silage

Ungutted partly-thawed tilapias were chopped and minced using a HOBART A-200 mincer and passed through a die plate with 3 mm-diameter holes. Minced tilapia was distributed into three 1-kg batches (A, B, C). Batch A was mixed with 150 g of molasses and 50 mL of inoculum inside 5-L plastic containers and sealed. The mixture was divided into three portions and each was incubated at 5°, 20° or 35°C for 30 days. Batch B was divided into three portions and to each portion

was added 150 g of molasses, corn flour or taploca flour, and 50 mL of inoculum. The mixtures were incubated in sealed containers at 30°C, representing ambient tropical temperature, for 30 days. Batch C was similarly mixed with 150 g of molasses and 50 mL of pre-fermented starter culture was and the mixture was fermented at 30°C for 7 days, and stored at 30°C for 180 days. After fermentation for the desired period, the fish silages were heated to 90°C in a temperature-controlled water bath and maintained for 30 min to halt autolysis.

No antioxidants were added to the substrates before or after ensiling because of the low fat content of tilapias (TRS,1989). Triplicate 30 g samples were taken from the substrate and silages at the start of the experiment and after 2, 4, 7, 15, 30, 60, 90 and 180 days. Samples were kept in resealable polyethylene bags (115 x 90 mm) and stored at -20°C prior to analysis. The tilapia silages were swirled daily during the first 14 days of storage, and on sampling days. In order to prevent mould growth, the surface of silages and inner walls of the containers were sprayed with 1% (volume/weight, v/w) potassium sorbate solution (6 g in 20 mL water) after each sampling (LEVIN *et al.*,1989).

3.2.3 Analytical methods

(a) Proximate composition and Free fatty acid content

Moisture content was determined by oven-drying at 105°C for 24 h; lipid content by extracting the residue with 40°-60°C petroleum ether for 8 h;

ash content by ignition at 550°C for 24 h; total nitrogen content by the Kjeldahl method using the TECATOR-KJELTEC system 1003 unit and crude protein estimated as N x 6.25 (AOAC,1990). Tryptophan content was determined colorimetrically (FISCHL,1960) after hydrolysis in 4.2 $mol.L^{-1}$ NaOH. The free fatty acid content was determined after extraction with methanol/chloroform mixture (PEARSON,1971).

(b) pH

Distilled water was added to 5 g samples of tilapia silages and made to 50 mL, and each mixture was centrifuged at 8000 $rev.min^{-1}$ for 10 min in an MSE Minor S centrifuge. The pH of the supernatant was determined.

(c) Protein autolysis

The tilapia silages and minced tilapia were analysed for non-protein nitrogen (NPN) by the trichloroacetic acid (TCA) precipitation technique (BACKHOFF,1976) thus: to 5 g of sample, 10 mL of 20% (w/v) TCA was added and after homogenization, the sample was filtered and the nitrogen content of the filtrate was determined by the Kjeldahl method.

3.2.4 Bacteriological methods

Lactic acid bacteria were enumerated, using the Miles Misra technique on de Man Rogosa Sharpe Agar (MRS)(Oxoid Ltd., Basingstoke). After incubation at 30°C for 24 h, catalase negative, gram-positive, opaque, white colonies were counted as lactic acid bacteria.

3.3 RESULTS AND DISCUSSION

3.3.1 Effect of incubation temperature on fermentation

Incubation temperature had a pronounced effect on the rate of hydrolysis of fish protein as indicated by both pH and NPN content. There was a rapid pH decline within 48 h at 35°C and 96 h at 20°C while at 5°C pH did not fall below 5.5 within 14 days (Table 3.1) after which the silage putrefied and was therefore discarded. The pH remained at about 3.9 and 4.1 in the silages at 35°C and 20°C respectively, for up to 30 days of incubation (Table 3.1). The rapid pH decline in the silages incubated at 20° and 35°C indicated a suitable development of lactobacilli.

LINDGREN & PLEJE (1983) obtained pH <4.5 within 30 h during the fermentation of Baltic herring (***Clupea harengus***) at 24°C, and temperature reduction to 12°C caused a delay in pH decline. Desirable preservative pHs (<4.5) usually occur within 48-72 h of fermentation (RAA *et al.*,1983) and the rapid establishment of lactobacilli is important for a decrease in redox potential and pH (RAA,1980). A low redox potential prevents growth of aerobic bacteria, associated with spoilage, included in the intestinal flora of ungutted fish during fermentation (SHEWAN,1977).

Table 3.1 : Average pH and NPN (g.100g^{-1} TKN) of tilapia silage fermented at different temperatures.

Fermentation period (days)	5°C		20°C		35°C	
	pH	NPN	pH	NPN	pH	NPN
0	6.5	15.7	6.5	15.8	6.5	15.8
2	6.2	17.3	4.8	18.7	4.4	20.3
4	5.9	19.5	4.5	22.4	4.2	23.6
7	5.5	20.9	4.3	28.3	4.0	30.5
15	5.5	21.4	4.2	35.9	3.9	37.1
30	-	-	4.1	45.1	3.8	48.8

The 35°C silage started to liquefy within 24 h while at 20°C, liquefaction occurred after 48 h. At 5°C, the tilapia silage was "porridge-like". The change in consistency of tilapia silage with incubation temperature closely reflected the changes in soluble nitrogen content. Prior to fermentation, about 16% of TKN was TCA-soluble and NPN rapidly attained a peak of 45-50% of TKN in 30 days at 20°C and 35°C; but at 5°C, the increase was slower and reached a lower peak after 15 days (Table 3.1). This phenomenon is consistent with results of LINDGREN & PLEJE (1983) who found a peak protein solubilization (>60%) in herring silage at 24°C and a lower peak level (40-45%) at 12°C after 28 days fermentation. LO *et al.*(1993b) reported that at 22°C, the NPN of acid-preserved (0.75% formic acid + 0.75% citric acid) salmon silage was about 60% of TKN, while at 5°C, it decreased to about 35% of TKN.

Fermented tilapia silage can therefore be produced over a wide range of temperatures (20°-35°C) which includes common ambient temperature in tropical countries (28°-32°C). This represents an important practical application of using the fermentation technique for preserving fish as it would save costs of procuring the equipment needed to keep temperature at optimum. Also, a stable silage would be produced at 30°-35°C which corresponds to the optimum range for lactobacilli (McDONALD,1981).

3.3.2 Effect of carbohydrate source on fermentation

After incubation for 7 days, a desirable and stable pH <4.5 was attained. Molasses gave a more rapid pH decline than either corn flour or tapioca flour (Table 3.2). The slow initial pH decline was similarly reported by WEE *et al.*(1986) and could possibly be as a result of high proportion of unhydrolysed starch in corn and tapioca flours, a phenomenon which VAN WYK & HEYDENRYCH (1985) considered as limiting and a risk to the stability of silages. Hence, the addition of an amylolytic enzyme source may be necessary so that starch in corn and tapioca flour can be hydrolysed to sugars, suitable carbohydrate substrates for fermentation (ADAMS *et al.*,1987; TWIDDY *et al.*,1987).

The amount of fermentable carbohydrate needed depends on the choice of carbohydrate and fish species used as substrate. The amount of carbohydrate added usually varies between 10% and 50% of total weight of silage components and earlier experiments (ROA,1965; STANTON & YEOH,1977; LINDGREN & PLEJE,1983) have shown that mixtures of fish and carbohydrate in a 1:1 ratio will produce stable silages. At this ratio however, the volume of carbohydrate required could prove uneconomical.

Table 3.2 : pH of fermented tilapia silages prepared using different carbohydrate substrates.

Fermentation period (days)	Molasses	Corn flour	Tapioca flour
0	6.7	6.7	6.7
2	4.5	4.6	4.9
4	4.3	4.5	4.8
7	4.1	4.4	4.5
15	3.9	4.3	4.2
30	3.9	4.0	4.1

Fermented tilapia silage was incubated for 30 days without deterioration in nutritional characteristics and the source of carbohydrate did not affect the NPN content (Table 3.3). The high NPN content of the fermented tilapia silage was normal, since similar values were reported for fermented Baltic herring (***Clupea harengus***) silage stored for 32 days (LINDGREN & PLEJE,1983). However, BATISTA *et al.*(1989) found lower NPN values (<30%) in fermented snipefish (***Macrorhamphosus*** spp.) silage over the same period of storage. HASSAN & HEATH (1987) reported that water soluble-N content increased from 58.7% to 71.8% and 77.5% in ensiled white perch plus 5% lactose after storage for 35 days at 23°-25°C and 37°C, respectively.

Table 3.3 : NPN ($g.100g^{-1}$ TKN) and proximate composition ($g.100g^{-1}$ DM) of fermented tilapia silages prepared using different carbohydrate substrates.

	Molasses	Corn flour	Tapioca flour
NPN	45.83	54.18	47.06
Dry matter (DM)	30.55±1.14	35.54±1.15	35.11±1.08
Crude protein	42.35±2.50	43.07±1.79	40.44±1.76
Crude lipid	10.63±1.53	16.54±1.28	13.47±1.30
Ash	15.55±1.67	11.92±1.25	12.30±0.85

All the carbohydrates tested as fermentation substrates were suitable but molasses was a superior source for silage stability. The need to minimize costs in developing countries indicates that the carbohydrate source should be based on indigenous staple foods such as rice, cassava or corn. The use of molasses is particularly appropriate for tropical regions where it is frequently available as an industrial by-product. Moreover, corn flour and tapioca flour have competitive uses in human diets and may prove uneconomical. In addition, DHATEMWA (1989) also warned of a risk of cyanide poisoning if the proper variety of cassava was not used.

3.3.3 Effect of prolonged storage on silage stability

There were no signs of bacterial or fungal spoilage at any time but a rise in pH was observed. The pH stabilized at 4.0 up to 60 days after which it increased gradually to 4.9 after 180 days at 30°C (Table 3.4). The stability in pH might be due to the buffering action of amino acid and other salts in the product or the partial neutralization of the acid by calcium from bones and scales in tilapia silage sediments. During storage, autolysis affects the buffering capacity of the material and increases pH (SINELL,1980) which lowers the storage potential. However, it is not known if these changes could affect availability of protein in fermented silage for nutritional metabolism in farmed fish species.

Proximate composition of the silage during fermentation showed little change (Table 3.4). A slight variation in moisture, crude protein and ash contents occurred, but the crude lipid content apparently increased. An apparently increasing lipid content could be accounted for by the fact that lactic acid is soluble in ether and hence extractable with ether during lipid determination (STETCHER *et al.*,1968). The free fatty acid content increased with storage up to a maximum of 3.48 (% oleic acid) after 180 days (Table 3.4) as a result of lipolysis. This is considered low compared with increases of 2-6% and 6-18% during prolonged storage for sprat (***Sprattus sprattus***) silage (JACKSON *et al.*,1984a; AUSTRENG & GJEFSEN,1981, respectively).

Table 3.4 : Average pH, nutrient and proximate composition (g.100g^{-1} DM) of tilapia silage during fermentation and storage for 180 days at 30°C.

Days	Fermentation		Storage					
	0	7	0	15	30	60	90	180
Dry matter	32.25	33.65	32.20	32.25	30.55	29.90	28.55	28.40
Crude protein	41.26	41.39	42.57	42.50	42.35	43.32	42.83	42.95
Crude lipid	9.77	11.74	9.25	9.30	10.63	10.54	14.36	14.45
Ash	15.04	15.30	15.16	15.19	15.55	15.38	13.49	13.32
Tryptophan[1]	18.0	16.5	16.5	16.5	16.0	15.0	15.0	15.0
pH	6.5	4.1	4.0	4.0	4.0	4.0	4.6	4.9
NPN[2]	15.8	28.5	29.3	37.6	40.2	48.5	50.6	51.4
FFA[3]	1.21	1.37	1.65	2.18	2.54	2.91	3.03	3.48

[1] mg.g^{-1} protein

[2] NPN = non-protein nitrogen (g.100g^{-1} TKN)

[3] FFA = free fatty acid (% oleic acid).

A sharp fall (>8% difference) occurred in the tryptophan content of tilapia silage within 7 days of fermentation. Tryptophan content showed a further decrease (11%) after 30 days storage and the extent of tryptophan loss levelled off as the length of storage of the fish silage progressed up to 180 days (Table 3.4). A similar trend of superior tryptophan level before fermentation was reported by VAN WYK & HEYDENRYCH (1985). Tryptophan is labile under acid conditions and has a low water solubility, particularly in the free form (NIELSEN *et al.*,1985), and hence precipitates. This was confirmed by HALL *et al.*(1985a, b) and ESPE *et al.*(1991) who found higher tryptophan contents in the undigested sediments than in the aqueous phase in fish silage.

BACKHOFF (1976) reported that 25% of tryptophan in herring silage was lost during the first 17 days of storage whereas at 15°C and 30°C, losses of 10% and 33% occurred respectively, in the same period; while in cod (***Gadus morhua***) silage, 20% was lost after 54 days at 30°C. JENSEN & SCHMIDTSDORFF (1977) found that tryptophan was affected by pH and duration of acid treatment. KOMPIANG *et al.*(1980b) reported nearly 60% loss in sprat (***Sprattus sprattus***) silage stored for 24 weeks at 20°C, and concluded that tryptophan loss in fish silage is dependent on both temperature and storage. Generally, loss of amino acids in fermented silages has been attributed either to their interaction with sugars in the unutilized molasses (JAMES *et al.*,1977) or to their use as nitrogen source by some microbes (JONSSON *et al.*,1983).

Depending on storage temperature, up to 70% of the protein nitrogen in acid silage becomes TCA-soluble. Similarly, protein digestion occurs in fermented silage during storage but it is significantly lower than for acid silage. For example, BATISTA *et al.*(1989) reported a gradual protein solubilization in fermented snipefish (***Macrorhamphosus spp.***) silage which attained 25% of TKN after 30 days, much lower than >60% of TKN observed in comparable acid silage. They attributed this to the adsorption of enzymes and proteins by carbohydrates which prevented their interaction. However they did not observe this effect of carbohydrates in fermented blue whiting (***Micromesistius poutassou***) silages in which soluble nitrogen after 30 days storage were 64.8-68.7% of TKN, these values being similar to those of its acid silages. Similarly, LINDGREN & PLEJE (1983) reported that >55% of TKN was TCA-soluble after 8 days fermentation of Baltic herring (***Clupea harengus***) at 24°C, then slower thereafter reaching >60% of TKN. The patterns are similar but differences are probably due to variations in enzymatic activity from one species to another. Thus, it appears that autolysis is more limited in fermented tilapia silage.

CHAPTER 4 : PRESERVATION AND STABILITY OF FERMENTED TILAPIA SILAGE

The experiments described in this chapter have been published in

Food Chemistry, 48 (1993): 331-335.

Food Chemistry, 50 (1994): 383-388.

4.1 INTRODUCTION

Hydrolysis of protein and lipids contribute to the deterioration of unpreserved fish products. During ensilage and storage of fish, liquefaction occurs mainly by endogenous proteolytic enzyme activity and yields high content of soluble peptides, free amino acids and ammonia (BATISTA *et al.*,1989; DONG *et al.*,1993). The nutritional quality of fish silage can be improved by inhibiting enzyme activity or by limiting the degree of proteolysis. Protease inhibitors from plants have successfully suppressed proteolysis in minced fish (LANIER *et al.*,1981; GOWDA & KARUNASAGAR,1985). Some varieties of potato (***Solanum tuberosum***) contain inhibitors for carboxypeptidases, chymotrypsin, trypsin, and cathepsins (RYAN *et al.*,1974; BUSSE & BELITZ,1976; PEARCE *et al.*,1982).

Formaldehyde inhibited protein hydrolysis and lipid rancidity in acid silage (HAARD *et al.*,1985; HUSAIN & OFFER,1987) but proved toxic to

some livestock animals. As an alternative, it was suggested that cooking the fish substrate prior to ensiling (WOOD *et al.*,1985) or addition of common salt (NaCl)(GILDBERG *et al.*,1984) would halt autolysis and yield a stable fish silage. Termination of the ensiling process after 3-7 days improved weight gains, protein efficiency ratio (PER), biological value (BV) and net protein utilization (NPU) when silage diets were fed to mink (SKREDE,1981), sheep (OFFER & HUSAIN,1987), salmonids (LALL,1991) and rats (ESPE *et al.*,1992a).

High level of unsaturated lipids in fish silage makes it susceptible to oxidation and consequent formation of toxic products which have caused reduced growth performance reported in some fish silage nutritional trials. RAA & GILDBERG (1982) suggested that lipid oxidation in fish silage could be checked by antioxidants such as ethoxyquin, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). These synthetic antioxidants are expensive, furthermore they are slowly metabolized in fish muscle (LOVAAS,1989), hence they are prohibited in many meat and fish products. A variety of natural antioxidants from vegetable extracts or spices may offer cheap alternatives (CHIPAULT *et al.*,1952; PRATT & WATTS,1964; BISHOV & HENICK,1978; BYUN *et al.*,1986a, b; LARSON,1988). Ginger (***Zingiber officinale***) possesses strong antioxidative properties (LEE *et al.*,1986; JITOE *et al.*,1992) and minimised lipid oxidation in fish oils (BYUN *et al.*,1986a).

4.1.1 Objectives

Feeding trials have generally indicated that fish silage has good nutritive value when partly substituted for fish meal in livestock and poultry feeds (LOPEZ,1990; BIGUERAS-BENITEZ & NACORDA,1992). Acceptable diets for various cultivated fish species have included fish silage but further improvements in its nutritional quality are needed. This study investigates the effects of:

(a) inhibiting autolysis in the silage by cooking minced fish before fermentation,

(b) adding 5% salt (sodium chloride, NaCl) on the stability of tilapia silage,

(c) extract of potato tubers as protease inhibitors in preserving tilapia silage,

(d) formalin as an agent to inhibit autolysis of fermented tilapia silage,

(e) extract of ginger rhizomes as lipid antioxidant in tilapia silage.

4.2 MATERIALS AND METHODS

4.2.1 Fermentation substrates

Mixed-sex tilapias were used as fish substrate. In one treatment, minced tilapias were steamed for 30 min at 90°C, while in another, salt (NaCl) (SIGMA CHEMICAL Co., Dorset) was added, prior to fermentation. Molasses was used as carbohydrate source and ***L. plantarum*** starter culture, prepared as described in **Chapter 3.2.1**, was used as inoculum. Ginger rhizomes and potato tubers (TESCO STORES, Stirling) were separately peeled, sliced, crushed and soaked in water (2 $L.kg^{-1}$), and homogenised. The homogenate of ginger was used as antioxidant without further purification while the water soluble fraction of potato was used as extract for protease inhibitor. Formalin (FISONS SCIENTIFIC EQUIPMENT, Loughborough) was used as an additive to wet silage.

4.2.2 Preparation of tilapia silage

Minced tilapias were distributed into six 1-kg batches (D, E, F, G, H, I). Batch D was used as the raw treatment. Prior to fermentation, Batch E was steamed for 30 min at 90°C and used as the preheated treatment while 5% NaCl (w/w) was added to Batch F and used as the salted treatment. Each batch was mixed with 150 g of molasses and 50 mL of inoculum inside 5-L plastic buckets and sealed air-tight. After fermentation for 72 h, potato extract (PE) was added to Batch G at 5%

(v/w), formalin to Batch H at 5 $mL.kg^{-1}$, and ginger extract (GE) to Batch I at 5 $mL.kg^{-1}$. The silage mixtures were incubated and handled as described in **Chapter 3.2.2.** Triplicate 30 g samples were taken from all silages at the start and after 2, 4, 7, 15 and 30 days. Samples were stored at -20°C prior to analysis.

4.2.3 Analytical methods

(a) Proximate composition

Moisture, crude protein, crude lipid and ash contents were determined as described in **Chapter 3.2.3.**

(b) pH and Lactic acid content

pH of silage was determined as described in **Chapter 3.2.3** using a digital pH meter. Lactic acid content, estimated as titrable acidity (TA) was determined by the indicator method (PEDERSON & ALBURY,1969) thus: 10 mL of supernatant (obtained from pH determination) was titrated against 0.1 $mol.L^{-1}$ sodium hydroxide (NaOH) using phenolphthalein as indicator. Acidity was expressed as % lactic acid using to the formula:

$$\% \text{ Lactic acid} = \frac{(\text{vol. of NaOH})(\text{N of NaOH})(\text{eq. wt. of lactic acid}) \times 10^2}{10^3 \times \text{wt. of sample(g)}}$$

(c) Total carbohydrate

Oven-dried tilapia silage samples were hydrolysed in 98-100% formic acid for 24 h at 110°C. Total carbohydrate (CHO) was determined by the

phenol-sulphuric acid procedure (anthrone reaction, DUBOIS *et al.*,1956) using glucose as standard.

(d) Protein autolysis

Tilapia silages and minced tilapia were analysed for non-protein nitrogen (NPN) using the trichloroacetic acid (TCA) precipitation technique (BACKHOFF,1976), ammonia nitrogen (NH_3-N) and total volatile nitrogen (TVN) using the micro-diffusion technique (CONWAY,1968).

(e) Amino acid analyses

The amino acid composition was determined in an acid hydrolysate (6 $mol.L^{-1}$ hydrochloric acid (HCl) under reflux for 24 h at 110°C) of the sample using an automatic amino acid analyzer (LKB 4151 ALPHA-PLUS, LKB BIOCHROM Ltd., Cambridge). Tryptophan content was determined colorimetrically (FISCHL,1960) after hydrolysis in 4.2 $mol.L^{-1}$ NaOH.

(f) Free fatty acid content and Lipid rancidity

The free fatty acid (FFA) content was determined after extraction with methanol/chloroform mixture (PEARSON,1971). Peroxide (PV) and thiobarbituric acid (TBA) contents were determined using distillation methods (PEARSON,1971) and used to estimate rancidity.

(g) Gross energy content

Minced tilapia and tilapia silage were oven-dried at 80°C for 12 h and ground in a coffee grinder; 1 g of which was used for gross energy assay

by combustion in a bomb calorimeter (GALLENKAMP & Co. Ltd., Loughborough).

(h) Protease activity

Protease activity (PA) was estimated as the release of TCA-soluble Folin positive material from haemoglobin at pH 4.4 (pepsin activity) using tyrosine as a standard (GILDBERG & RAA,1983). PA was expressed as μmol tyrosine equivalent.h^{-1} at 25°C.

4.2.4 Bacteriological methods

Lactic acid bacteria were enumerated on de Man Rogosa Sharpe Agar (MRS) using the methods described in **Chapter 3.2.4.**

4.3 RESULTS AND DISCUSSION

4.3.1 Raw tilapia silage

The pH decreased to 3.9 after 30 days, and was accompanied by an increase in TA and decrease in total CHO values (Table 4.1). Storage of fish above freezing point without denaturation of proteolytic enzymes causes rapid liquefaction of the protein which is evident in the changes in TCA-soluble substances. Initially, about 16.0% of TKN was TCA-soluble but with time, there was a gradual increase in protein solubilization (Table 4.1) which attained a peak of 45.8% after 30 days. This value is lower than that usually obtained in acid silages (BATISTA,1987), and is due to the adsorption of enzymes and proteins by the carbohydrates, thereby preventing their interaction (RAA *et al.*,1983).

NPN and/or ninhydrin-positive materials are useful chemical criteria for fish silage (ESPE *et al.*,1989) since the most obvious changes that occur during ensilage are autolysis of tissues and release of ammonia. Prior to fermentation, minced tilapia had 9.2 mg TVN.g TKN^{-1} and 3.9 mg NH_3-N.g TKN^{-1}; both of which increased slightly during storage (Table 4.1). The low TVN value indicated a fresh fish substrate was used. Production of TVN (mainly NH_3-N) is greatly influenced by storage temperature and may be a result of autolytic or microbial deaminases in fish (LINDGREN & PLEJE, 1983; JONSSON *et al.*,1983; HAALAND & NJAA,1988,1989a).

Table 4.1 : Changes in pH and chemical characteristics of "raw" tilapia silage fermented at 30°C.

Fermentation period (days)	pH	TA	CHO	NPN	TVN	NH_3N	TBA	PV	FFA	PA
0	6.7	0.25	50.2	16.0	13.2	7.8	22.2	142.5	1.21	1.17
2	4.5	0.29	35.7	19.6	14.5	8.6	19.5	105.3	ND	ND
4	4.3	0.40	24.9	21.7	15.1	10.8	17.7	93.1	ND	ND
7	4.1	0.78	15.3	28.5	17.3	12.3	16.1	82.5	1.37	1.91
15	3.9	1.04	7.7	36.8	18.9	13.7	15.6	63.2	1.81	4.95
30	3.9	1.38	4.1	45.8	26.6	20.8	13.0	39.0	2.36	6.58

TA = titrable acidity (%)
CHO = total carbohydrates ($\mu g.g^{-1}$ dry weight)
NPN = non protein nitrogen ($g.100g^{-1}$ TKN)
TVN = total volatile nitrogen ($g.100g^{-1}$ TKN)
NH_3N = ammonia nitrogen ($g.100g^{-1}$ TKN)
TBA = thiobarbituric acid ($milliequivalent.kg^{-1}$ lipid)
PV = peroxide value ($milliequivalent.kg^{-1}$ lipid)
FFA = free fatty acid (as oleic acid, %)
PA = protease activity (μmol tyrosine $equivalent.h^{-1}$ at 25°)
ND = not determined

Changes in FFA, TBA and peroxide values are shown in Table 4.1. The initial low TBA values changed little with time. The TBA value might arise from the residual lipid. The reduction of the initially high peroxide value may have been due to the destruction of hydroperoxides.

Proximate composition of the tilapia silage and minced tilapia (Table 4.2) showed little variation after 30 days. Similarly, reports on the chemical analyses did not show any obvious differences between other fish substrates and their silages (HASSAN,1982; AJAYI,1985; BROWN & SUMNER,1985; ESPE,1987). Moisture content in tilapia silage was lower than in minced tilapia. A slight increase in dry matter was similarly reported by JACKSON *et al.*(1984a) and ESPE *et al.*(1989). ESPE *et al.* (1989) suggested that it was due to binding of water during proteolysis while VAN WYK & HEYDENRYCH (1985) attributed it to the evaporation of carbon dioxide and ethanol as a result of yeast fermentation. HASSAN & HEATH (1987) attributed the lower moisture content to condensation on the inside of the fermentation bin and loss during sampling.

Lipid content of tilapia silage was higher than that of minced tilapia (Table 4.2) and could be related to extraction of lactic acid along with ether during lipid content determination as STECHER *et al.*(1968) reported that lactic acid is soluble in ether. Tilapia being a low-fat fish generates low lipid (<4%) silage. This does not pose potential problem of rancidity during storage, compared with 6-18% from sprat (***Sprattus sprattus***) silage (AUSTRENG & GJEFSEN,1981; HALL & LEDWARD,1986).

Table 4.2 : Proximate composition (g.100g^{-1} DM) and gross energy content of minced tilapia and fermented tilapia silage.

	Minced tilapia	Tilapia silage
Dry matter (DM)	26.27±1.08	30.55±1.14
Crude protein	62.85±3.40	42.35±2.50
Crude lipid	7.08±1.45	10.63±1.53
Ash	18.03±1.88	15.55±1.67
Gross energy (kcal.g^{-1} DM)	4.69±0.21	5.28±0.30

Tilapia silage contained 42.35±2.5% protein while minced tilapia contained 62.85±3.4% protein. The lower protein content of tilapia silage was due to the addition of molasses and slight dilution effect by the acid produced (JAMES *et al.*,1977). Apparent protein contents of the tilapia silage ranged from 12.98-13.70% of dry matter, indicating that four to five times more fish silage than fish meal would be required in feeds, for equivalent protein contents. Amino acid analysis showed a slight difference (marginal loss) between the total sum of amino acids in minced tilapia and tilapia silage (Table 4.3).

Table 4.3 : Amino acid composition (EAA, g.100g^{-1} protein) of minced tilapia and fermented tilapia silage.

	Minced tilapia	Tilapia silage
Arginine	6.82	6.02
Histidine	2.66	2.86
Isoleucine	3.80	3.84
Leucine	7.35	6.09
Lysine	5.95	6.39
Methionine	2.05	2.14
Cystine[1]	1.93	1.06
Phenylalanine	3.07	3.22
Tyrosine[1]	2.45	2.79
Threonine	3.70	3.79
Tryptophan	1.80	1.64
Valine	4.78	3.65
Total EAA	41.98	39.64

[1] non-essential amino acids

4.3.2 Effect of preheating on fermentation of minced tilapia

Within 48 h of fermentation, cooked silage showed a slower pH decline compared with raw silage (Table 4.4) and did not give a lower pH even after incubation for 30 days at 30°C; while raw silage liquefied between 48 and 72 h, cooked silage was "porridge-like" up to 30 days. This agrees with earlier reports that the prevention of liquefaction in fish silages is a normal phenomenon when the fish substrate is heated before ensiling (WOOD *et al.*,1985; BATISTA,1987) because at 60°C and above, enzyme activity would have been destroyed (STRASDINE *et al.*,1988).

In raw silage, NPN increased from 16.0% to 45.8% within the 30 days of incubation while in cooked silage, it increased to 17.6% after 7 days and later decreased to 17.1% after 30 days (Table 4.4). BATISTA (1987) and ESPE *et al.*(1992a) reported a similar trend in fermented whiting (***Merlangius merlangus***) and acid capelin (***Mallotus villosus***) silages, respectively. In acid silages, NPN may rise up to 70-90% depending on the incubation/storage temperature (TATTERSON,1982; BATISTA,1987). Such increase in NPN is associated with the change in consistency. The cooked silage retained a dense consistency (less liquefaction) for a longer period which indicated that protein solubilization is essentially an enzymatic process. Heating accelerates the rate of proteolysis, but temperatures below 60°C are required to maintain enzyme activity (STRASDINE *et al.*,1988).

Table 4.4 : Changes in pH and NPN of cooked tilapia silage fermented at 30°C.

Fermentation period (days)	pH	NPN
0	6.7	16.6
2	5.4	16.8
4	4.6	17.2
7	4.4	17.6
15	4.2	17.4
30	4.2	17.1

NPN = non protein nitrogen (g.100g^{-1} TKN)

It is pertinent to note that during storage, NPN values above 70% were reported in silages prepared from coldwater fishes irrespective of ensiling method (TATTERSON & WINDSOR,1974), whereas lower values of 40-50% were always recorded for silages prepared from warmwater fishes (HALL *et al.*,1985a). The reasons for this are yet to be established but it is unlikely that these differences are due to enzyme activation in coldwater fishes as the temperature of optimum activity for enzymes from coldwater and warmwater fishes is similar (RAA & GILDBERG,1982).

4.3.3 Effect of added salt (5% NaCl) on fermentation

The addition of 5% NaCl to silage mixture did not improve pH decline (Table 4.5) compared with the raw silage (Table 4.1). Visual inspection of consistency of the salted silage indicated that an inhibition of autolysis occurred as slight liquefaction was noticeable after incubation for 7 days at 30°C. The delay in pH decline in the salted silage within 48 h were due to partial inhibitory effect of salt (NaCl) on the growth of ***L. plantarum*** and proteolytic activity as suggested by GILDBERG *et al.* (1984).

PEDERSON (1979) reported that 3.5% NaCl or more was detrimental to growth of all bacteria in sauerkraut because it reduces the availability of soluble nutrients such as amino acids. Some lactobacilli strains degrade amino acids (JONSSON *et al.*,1983) and produce ammonia. Apart from suppressing these ammonia-producing microbes (SUBASINGHE *et al.*,1990), salt has also been used to inhibit hydrolysis in acid silage (STANTON & YEOH,1977) and fish sauce (OREJANA & LISTON, 1982; GILDBERG *et al.*,1984). Salt concentration >5% inhibited the activity of digestive proteases, particularly pepsins, which are active under acid conditions (STANTON & YEOH,1977; OREJANA & LISTON,1982); hence reduction of salt level is important to improve autolysis.

Table 4.5 : Changes in pH and NPN of salted tilapia silage fermented at 30°C.

Fermentation period (days)	pH	NPN
0	6.7	16.6
2	5.5	17.5
4	4.8	18.6
7	4.5	19.8
15	4.3	20.4
30	4.3	20.7

NPN = non protein nitrogen ($g.100g^{-1}$ TKN)

4.3.4 Effect of potato extract on protease activity in tilapia silage

The NPN content of the PE-treated silage is presented in Table 4.6 and it shows little differences (P>0.05) between the raw (Table 4.1) and PE-treated silages. This suggests that the solubilization of protein was not affected by potato extract. This observation agrees with MAKINODAN *et al.*(1975) who also found no effect of potato extract on protein hydrolysis in white croaker. However, AKSNES (1989) reported that protease inhibitors from potato extract inhibited protein hydrolysis in minced capelin (***Mallotus villosus***) by decreasing the access to free amino acids (arginine, tyrosine and lysine), thus suppressing the medium for microbial (spoilage bacteria) growth.

This may not apply to fermented fish silages because fermentation involves lactic acid bacteria, which utilize carbohydrates, (preferably, rather than amino acids), as nutrients for growth (RAA,1980; VAN WYK & HEYDENRYCH,1985). Moreover, because of the acidic medium of fish silages, pepsin is presumably the main enzyme involved in fermentation (OREJANA & LISTON,1982); it seems therefore that protease inhibitors from potato extract do not affect peptic activity, similarly reported by AKSNES (1989) in stored capelin.

Table 4.6 : Changes in pH and characteristics of PE-treated tilapia silage fermented at 30°C.

Fermentation period (days)	pH	NPN	PA
0	6.6	16.3	1.20
2	4.5	20.0	ND
4	4.3	21.5	ND
7	4.1	28.9	1.91
15	4.0	37.4	1.43
30	3.9	46.2	1.40

NPN = non protein nitrogen (g.100g^{-1} TKN)
PA = protease activity (µmol tyrosine equivalent.h^{-1} at 25°C)
ND = not determined

4.3.5 Effect of ginger extract on lipid oxidation in tilapia silage

Changes in FFA, PV and TBA values of GE-treated tilapia silage during incubation are shown in Table 4.7. The increase in FFA content of both raw (Table 4.1) and GE-treated tilapia silages were marginal and identical, suggesting that GE addition did not affect lipid hydrolysis (production of FFA). Initially, there was a decrease in PV values of raw and GE-treated silages, and as expected, the decrease in PV value was further sustained in raw silage because fermentation under anaerobic conditions limits oxygen availability.

With the addition of ginger extract after fermentation for 72 h, there was a rapid development of hydroperoxides in the lipid of the GE-treated silage which reached PV value of 162 by day 15, after which it dropped to 137 by day 30. This probably reflected the degradation of part of the hydroperoxides to form secondary breakdown products such as aldehydes as suggested by JACKSON *et al.*(1984a), and according to LABUZA (1971), the addition of an antioxidant to an actively oxidising system cannot destroy peroxides or their breakdown products or ameliorate their destructive effects but may prevent further build up of these reactive species.

There was a decrease in TBA value in raw silage; and the decrease was faster ($P<0.05$) with the addition of ginger extract (Table 4.7). As such, there being no oxidation changes during incubation, ginger extract thus

proved to be effective as an antioxidant for tilapia silage. The potency of ginger extract is dependent on pH, with maximum values at pH 5 (LEE & AHN,1985). The pH 3.9 attained in GE-treated silage (Table 4.7) is considered optimum and was therefore ideal for its antioxidative effectiveness. Thus the stability of tilapia silage determined by TBA values was improved by the addition of ginger extract.

Table 4.7 : Changes in pH and characteristics of GE-treated tilapia silage fermented at 30°C.

Fermentation period (days)	pH	TBA	PV	FFA
0	6.7	22.5	143.2	1.20
2	4.4	19.7	106.0	ND
4	4.2	16.2	128.8	ND
7	4.0	14.8	156.4	1.29
15	3.9	11.9	162.7	1.61
30	4.0	9.1	137.5	2.57

TBA = thiobarbituric acid (milliequivalent.kg^{-1} lipid)
PV = peroxide value (milliequivalent.kg^{-1} lipid)
FFA = free fatty acid (as oleic acid, %)
ND = not determined

There being no established values associated with rancid/lipid quality in fish silages, it is safe to say that ginger extract was effective in preventing lipid oxidation in tilapia silage. Since tilapia cannot be considered as a fatty fish (TRS,1989), the extent of lipid protection that ginger extract can provide for fish silages is unknown. Further studies need to be conducted using fatty fish, such as mackerel, in order to assess the full potential of ginger extract as an antioxidant.

4.3.6 Effect of formalin on protein hydrolysis

Soluble protein content of raw tilapia silage increased as reflected by a high percentage of NPN (Table 4.1), indicating a high degree of protein hydrolysis, presumably to peptides and free amino acids during storage. Addition of formalin resulted in a significant ($P<0.05$) lowering of the soluble nitrogen content to 22.5% NPN over the same incubation period (Table 4.8). This signifies that proteolysis was inhibited, possibly by affecting protease activity as suggested by HUSAIN & OFFER (1987) after similar occurrence with formalin-treated formic acid whiting silage kept for 10 days. Addition of formalin to formic acid cod silage after liquefaction (48 h) also halted proteolysis leaving only 20-30% of TKN as NPN after 36 days (HAARD *et al.*,1985).

When autolysis is allowed to continue, the resultant free amino acids are further degraded to ammonia, its production being higher in fermented silage than in an acid silage (BATISTA,1987). Despite reduced protein solubilization in formalin-treated silage, NH_3-N production increased markedly ($P<0.05$) up to 15 days of storage and declined slightly afterwards (Table 4.8). This does not refute the inhibition of protein hydrolysis by formalin as it would be expected that degraded products would affect the buffering capacity of the silage and increase pH (SINELL,1980), but this was not the case with pH in this treatment.

Table 4.8 : Changes in pH and characteristics of formalin-treated tilapia silage fermented at 30°C.

Fermentation period (days)	pH	NPN	NH_3N
0	6.7	16.2	7.6
2	4.5	19.8	8.4
4	4.3	20.5	12.3
7	4.3	20.8	18.8
15	4.3	21.7	28.5
30	4.3	22.5	24.2

NPN = non protein nitrogen (g.100g^{-1} TKN)
NH_3N = ammonia nitrogen (g.100g^{-1} TKN)

Although the evidence of decarboxylation or deamination of amino acids by ***L. plantarum*** is conflicting (MEYER,1965; JONSSON *et al.*,1983), it is unlikely that the NH_3-N was derived from amino acids because no differences between the total amino acid content of minced tilapia and tilapia silage (Table 4.3) were found, as also reported by KOMPIANG *et al.*(1980b). The NH_3-N could have been formed by hydrolysis of amide nitrogen, degradation of nucleic bases or oxidation of amines by bacterial aminooxidases (HASSAN & HEATH,1987).

The general effect of formalin on protein has been reviewed by BARRY *et al.*(1973). Formalin forms methylene cross linkages between proteins, at the ε-amino group of lysine. The linkages in the resultant methyl compounds are hydrolysed under the acid-pepsin conditions and the proteins become liberated. The level of formalin added should be

closely related to the protein content of the ensiled material (MACKIE, 1971). If the level is too high, irreversible bonding occurs and lysine is destroyed; and if the level is too low, it would be possible for a clostridial fermentation to occur. The latter is undesirable in lactic acid fermented fish silages. However, the level used in this study was within the safe range recommended for acid silages (HAARD *et al.*,1985; HUSSAIN & OFFER,1987).

CHAPTER 5 : PROPERTIES OF FERMENTED TILAPIA SILAGE DIETS

The experiments described in this chapter will be published in

Aquaculture Engineering, 14 (1995): in press.

5.1 INTRODUCTION

The potential of fish silage in aquaculture lies mainly as a substitute for fish waste and fish meal in moist and dry feeds, respectively. The incorporation of fish silage reduces fish meal levels, raises moisture content and improves firmness of pellets. Moist feed containing fish silage requires binders to achieve proper integrity of pellets (WOOD,1980a). The use of binders is important in the manufacture of moist feeds and experimental diets in aquaculture, in order to improve water stability, aid prehension, thus increasing feed efficiency and reducing wastage and fouling of water systems (HARDY, 1989; HUANG,1989). It also increases the cohesion of faeces, making their collection for digestibility studies more reliable (WOOD,1980b).

LALL (1991) recommended that 1% of pregelatinized starch would be required for every 10% moisture in fish silage feeds to produce acceptable pellets. However, cost and availability may pose problems. Natural binders commonly used in fish diets include alginates, starches,

gums, gelatin and agar. Generally, they represent dietary fibre or filler material and it is assumed they have no nutritional value, but the real effects on digestion in fish are not fully understood. Binders can have detrimental effects on various digestive processes. For example alginates and guar gum depressed protein digestibility of moist and dry feeds in rainbow trout (ROSELUND & UTNE,1981; STOREBAKKEN,1985).

5.1.1 Objectives

Fish nutritionists have generally studied the physiological responses in diet evaluation while the physical aspects of the diet have often been neglected. HEINEN (1981) compared 11 pellet binders added at 3% to a trout feed and measured water stability, but the effects of feeding the pellets to fish were not determined. This study evaluates the:

(a) effects of carboxymethyl cellulose, guar gum or wheat gluten as binder on water stability and protein leaching in moist tilapia silage based diets,

(b) effects of the binders on apparent dry matter, protein and lipid digestibility; and gastric evacuation time in ***Oreochromis niloticus*** fed with moist tilapia silage diets.

5.2 MATERIALS AND METHODS

5.2.1 Preparation of tilapia silage

Mixed-sex tilapias, molasses and ***Lactobacillus plantarum*** starter culture were used to prepare silages as described in **Chapter 3.2.1**. Three batches (J, K, L) of silage mixture were incubated at 30°C for 7 days, and used to prepare moist diets for ***O. niloticus*** as described later.

5.2.2 Preparation of tilapia silage diets

Three moist diets (TS-CMC, TS-GG, TS-WG) were formulated using tilapia silage as protein source (Table 5.1). Carboxymethyl cellulose (CMC), guar gum or wheat gluten (SIGMA CHEMICALS Co. Ltd., Dorset) was added as alternative binder to the basal diet at 3% (Table 5.2). Dry ingredients were milled to approximately 250 µm, mixed for 10 min in a HOBART A-200 mixer until a homogenous paste was obtained. A mixture of cod liver oil and corn oil (1:1) was gradually added to the mixture and mixed for another 5 min. Subsequently, the tilapia silage was added to the dry mixture and blended until it became a dough-like paste which was then extruded through a HOBART A-200 pellet mill with 3 mm-diameter die into strands. The strands were broken to 5 mm-long pellets, packed in plastic bags and frozen (-20°C) immediately until fed.

Table 5.1. Ingredient composition of the basal diets.

	g.100g^{-1} dry weight
Fish meal	12
Tilapia silage	50
Dextrin	30
Corn oil	2
Cod liver oil	2
Mineral mix[1]	2
Vitamin mix[2]	1
Chromic III oxide	1

[1] g.kg^{-1}: $MgSO_4.7H_2O$, 5.10; NaCl, 2.40; KCl, 2.00; $FeSO_4.7H_2O$, 1.00; $ZnSO_4.H_2O$, 0.22; $CuSO_4.5H_2O$, 0.0314; $MnSO_4.4H_2O$, 0.1015; $CoSO_4.7H_2O$, 0.0191; $CaIO_3.6H_2O$, 0.0118; $CrCl_3.6H_2O$, 0.0051.

[2] mg.kg^{-1}: thiamine HCl, 50; riboflavin, 50; calcium pantothenate, 100; niacin, 200; pyridoxine HCl, 40; biotin, 6; folic acid, 15; cyanocobalamin, 0.1; inositol, 200; ascorbic acid, 1000; choline chloride, 4000; menadione, 40; α-tocopherol acetate, 400; para-amino benzoic acid, 50; vitamin A acetate, 2000 IU; vitamin D_3, 1000 IU; dilutant, α-cellulose.

Table 5.2. Formulation (g.100g^{-1} dry weight) of moist tilapia silage diets.

	TS-CMC	TS-GG	TS-WG
Basal diet	97	97	97
CMC	3	0	0
Guar gum	0	3	0
Wheat gluten	0	0	3

5.2.3 Water stability and protein leaching

Triplicate 50 g samples of pre-screened pellet crumbles of each diet were placed on a sieve and slowly immersed in a 40-L glass aquarium containing deionized water at 27°C for 10 min. The sieves were removed and the crumbles allowed to drain for 1 min, oven-dried at 105°C for 2 h, cooled in a desiccator and reweighed. Water stability was calculated as the percentage difference in sample weight after re-weighing and expressed as percentage loss of dry matter (% LDM). Leaching of total protein was determined by the Kjeldahl method (AOAC,1990) and expressed on a percentage remaining basis as follows:

$$\frac{\text{g protein remaining/g pellet remaining}}{\text{g protein nutrient/g initial pellet}} \times 10^2$$

5.2.4 Protein digestibility trial

The diets were fed to all-male ***O. niloticus*** (mean weight, 48.9±3.5 g) at 5% bw.day^{-1}, twice daily for 15 days. Faeces from 20 fish fed each diet were collected by anal extrusion, pooled and analysed for protein (AOAC,1990) and chromic oxide using the acid digestion method (FURUKAWA & TSUKAHARA,1966). Apparent digestibility coefficient (ADC) was calculated according to AUSTRENG & REFSTIE (1979) formula:

$$ADC = 10^2 \times (a-b)/a$$

where: a = protein in feed/chromic III oxide in feed;

b = protein in faeces/chromic III oxide in faeces.

5.2.5 Gastric evacuation time

Gastric evacuation time was determined using the serial/sequential slaughter method modified by SHIAU *et al.*(1988) from HILTON *et al.* (1981). Tilapias that were used for the digestibility study were starved for 24 h to obtain standard clearance of the stomach, and thereafter fed to satiation with the diets without chromic III oxide. Six fish were randomly taken, anaesthetized with 60-7- $mg.L^{-1}$ benzocaine (ROSS & GEDDES, 1979), and dissected at hourly intervals. Serial slaughter was continued until almost all food was completely evacuated from the stomach. The stomachs were removed from the surrounding tissues and weighed individually. The feed-digesta contents of each stomach were then removed, weighed and the moisture content of the ingesta determined.

5.2.6 Analytical methods

The proximate composition, pH and protein autolysis were determined as described in **Chapter 3.2.3.**

5.2.7 Statistical analysis

Data were subjected to the one-way analysis of variance (ANOVA) test and means differences were determined using Duncan's new multiple range test (ZAR,1984). Standard error was calculated to identify the range of mean values.

5.3 RESULTS AND DISCUSSION

5.3.1 Effect of binders on water stability and protein leaching

Over 90% of the initial dry weight of the pellets were recovered after immersion in water for 10 min regardless of the binder incorporated (Table 5.3). In general, data on water stability of the pellets show that high physical stability of the pellets was maintained. The differences in physical stability of the pellets probably reflects the respective viscosity of the binders. Leaching of total protein content was very low (<3%) in all treatments (Table 5.3).

Table 5.3 : Water stability and protein leaching in moist tilapia silage diets.

	TS-CMC	TS-GG	TS-WG
Water stability (% LDM)	8.04a	7.95a	8.23a
Protein leaching (% total N)	2.64a	2.68a	2.75a

a, b - values in the same row with similar letter are not significantly different (P=0.05).

The delivery of essential nutrients is the primary purpose of any animal ration and the loss of nutrients due to leaching being the most important in aquaculture feeds. The high physical stability of moist fermented tilapia silage pellets were effective for nutrient retention and did prevent losses due to leaching (Table 5.3). The advantage of using binders in moist fish silage feeds is obvious as feed consistency is improved and

wastage reduced (VIOLA *et al.*,1986), thereby ensuring optimum feed uptake (WOOD,1980a, b). The binding capacity of sugar beet molasses used in tilapia silage preparation may also have contributed to the pellet stability, in addition to high pelletability of other feedstuffs (WOOD, 1987).

5.3.2 Effect of binders on gastric evacuation time in *O. niloticus*

Based on hourly determinations, it was apparent that gastric transit time was unaffected by the binder used (Table 5.4). As the rate of passage of food is influenced by several factors such as temperature, meal size, fish size, diet (FANGE & GROVE,1979), it is difficult to interpret to what extent it is directly influenced by binders. Low concentration of binders probably explain the lack of difference. Guar gum has been reported to delay stomach emptying time in rainbow trout, ***Salmo gairdneri*** (STOREBAKKEN,1985) only at higher concentrations (4%, 8%), attributed to the increased viscosity. CMC and guar gum are water-soluble and they give highly viscous solution when dissolved, which should slow diffusion and mixing.

Table 5.4 : Apparent protein digestibility and gastric evacuation time in ***O. niloticus*** fed moist tilapia silage diets.

	TS-CMC	TS-GG	TS-WG
$ADC_{protein}$	88.1a	78.3b	87.0a
Gastric evacuation time (h)	6-8	7-9	6-8

a, b - values in the same row with similar letter are not significantly different (P=0.05).

5.3.3 Effect of binders on protein digestibility by *O. niloticus*

The results for apparent protein digestibility of ***O. niloticus*** fed moist tilapia silage diets are presented in Table 5.4. Fish fed with diets containing CMC or wheat gluten as alternative binder had similar $ADC_{protein}$ and were superior ($P<0.05$) to those fed diets in which guar gum was used as binder. Such difference probably reflects the viscosity and gelling properties of individual binders. Furthermore, high digestibility response with CMC is attributed to its low inclusion level and agrees with similar observations made by SHIAU *et al.*(1988) with ***O. niloticus***.

Unlike guar gum, wheat gluten does not gel during mixing of feedstuffs prior to pelleting (HARDY,1989), and hence should not prevent nutrients becoming available for digestion. Gelling during mixing prior to pelleting renders the nutrients unavailable. The interaction of the test binders with molasses (also a binder) in tilapia silage may have interfered with feed digestion (VENS-CAPPELL,1984). STOREBAKKEN (1985) similarly reported negative effects of guar gum on digestibility of dry matter, protein or lipid in rainbow trout. By contrast, VIOLA *et al.*(1986) found no effect on ***O. niloticus*** growth.

CHAPTER 6 : EVALUATION OF FERMENTED TILAPIA SILAGE IN MOIST FISH DIETS

The experiments described in this chapter have been published in

Bioresource Technology, 48 (1994): 43-48.

6.1 INTRODUCTION

The desire to produce animal protein foods has led to the investigation of alternative technologies that use biological wastes. Recycling these wastes into new animal protein products via animal husbandry opens interesting perspectives. For example, some of the costs of producing livestock, poultry or fish could be lowered using fish silage as a nutrient source for such animals. A major goal in fish nutrition is to formulate diets which give fast growth, optimal health and product quality at the lowest cost. Lactic acid fermentation of fish by-products minimises energy requirements and provides suitable material for inclusion in fish feeds, but the product obtained is frequently degraded.

The nutritional value of a given feed or feedstuff depends on its nutrient composition, amount consumed, the extent to which the component nutrients are destroyed, lost or degraded during preparation or processing, palatability and bioavailability. Hydrolysis of protein and lipids contribute to the deterioration of ensiled fish products. Moist pellets

prepared by blending wet silage with dry binder meal were accepted by salmonids and produced good growth (ASGARD & AUSTRENG,1981); and therefore warrants further investigation. The use of autolysed protein from acid silage as a feedstuff for rat, poultry and fish have been studied (JACKSON *et al.*,1984b; LALL,1991; ESPE *et al.*,1992a, b, c), but information on the use of autolysed protein from fermented silage in fish diets is limited.

6.1.1 Objectives

Further to an earlier study on the storage properties of lactic acid fermented tilapia silage (**Chapter 3**), this study evaluates the use of autolysed protein from tilapia silage in moist diets for juvenile catfish, ***Clarias gariepinus***. The effects on growth, feed conversion, protein utilization and digestibility, and body (fillet) composition were investigated.

6.2 MATERIALS AND METHODS

6.2.1 Experimental fish

Juveniles of ***C. gariepinus,*** maintained in the Tropical Aquarium were acclimated to the experimental system for 7 days preceding the start of growth trials and fed commercial trout pellets (EWOS-BAKER OMEGA 3, 49% protein) to satiation. Satiation was reached within 10-15 min of the onset of feeding.

6.2.2 Experimental systems

(a) Growth trials

Catfish were held in a thermo-regulated water recycling system (Appendix 2) which comprised twelve 1-m^2 (400-L) rectangular fibreglass tanks fitted with lids. Water flowed from a header tank to the tanks at 2 $L.min^{-1}$, and drained through central stand pipes into a series of settling tanks. Water was pumped up to a tank containing gravels, used as biological filter, and flowed to the header tank which was aerated using air stones. Individual tanks were also aerated to maintain >90% oxygen saturation. A 3-kw thermostatically-controlled immersion heater was fitted into the header tank to maintain water temperature at 27±1°C. Water losses due to evaporation were replaced by a continuous fresh water input at 0.15 $L.min^{-1}$. About 25% of total water volume was replaced weekly with preheated fresh water to avoid the accumulation of

excretory products. Prior to the replacement, water samples were taken to determine pH, total ammonia, total nitrite and dissolved oxygen (DO_2) concentration (Appendix 3).

(b) Protein digestibility trials

An independent system, whose design is similar to the system used for the growth trials, was used and it comprised twelve 60-L cylindrical polypropylene tanks fitted with lids. At the bottom of each tank was a settling column to which was attached a control valve.

6.2.3 Fermented tilapia silage

Four batches of minced tilapias were used (M, N, O, P). Prior to fermentation, batch M was steamed for 30 min at 90°C and used as substrate for the unautolysed (control) treatment. Each batch was mixed with 15% molasses and 5% inoculum (w/w) inside 5-L plastic buckets and sealed. The mixtures were incubated at 30°C for 7 days. This temperature represented the optimum under which fermented fish might be stored, as well as being the optimum for the growth of ***Lactobacillus plantarum*** strains (McDONALD,1981). The tilapia silages were stirred daily to obtain a proper blend and were heated to 90°C for 30 min to halt autolysis. Silages prepared from batches N, O and P were stored at 30°C for 15, 30 and 60 days, respectively.

6.2.4 Experimental diets

Four moist diets (CS-0, CS-15, CS-30, CS-60) were formulated to contain 40% protein and 4.86 kcal.g^{-1} gross energy in dry matter for catfish. The diets contained tilapia silages prepared from Batches M, N, O, P, respectively. Chromic III oxide was incorporated as an internal marker for determination of apparent protein digestibility coefficient. Dry ingredients in the binder meal, based on the proportion given in Table 6.1, were milled to approximately 250 μm and mixed for 10 min inside the bowl of a HOBART A-200 industrial food mixer (HOBART Co. Ltd., London). Cod liver oil was gradually added to the mixture and mixed for another 5 min. An equal weight of the tilapia silage was added to the mixture and blended. The homogenous paste was then cold-extruded under pressure into strands through 3 mm-die plates in a HOBART A-200 pellet mill and subsequently broken into 5 mm-long pellets and frozen (-20°C) immediately until fed.

6.2.5 Analytical methods

Moisture, crude protein, crude lipid, crude fibre and ash contents of diets were determined according to AOAC (1990). The pH and non-protein nitrogen (NPN) content of wet silages were determined as described in **Chapter 3.2.3**, and are presented in Table 6.2.

Table 6.1 : Formulation of the binder meal.

	$g.100g^{-1}$ dry weight
Fish meal (74.9% protein)	40
Soybean meal (47.5% protein)	20
Corn starch	20
Fish oil	10
Mineral mix[1]	4
Vitamin mix[2]	2
Carboxymethyl cellulose	4

[1] $g.kg^{-1}$: $MgSO_4.7H_2O$, 5.10; NaCl, 2.40; KCl, 2.00; $FeSO_4.7H_2O$, 1.00; $ZnSO_4.H_2O$, 0.22; $CuSO_4.5H_2O$, 0.0314; $MnSO_4.4H_2O$, 0.1015; $CoSO_4.7H_2O$, 0.0191; $CaIO_3.6H_2O$, 0.0118; $CrCl_3.6H_2O$, 0.0051.

[2] $mg.kg^{-1}$: thiamine HCl, 50; riboflavin, 50; calcium pantothenate, 100; niacin, 200; pyridoxine HCl, 40; biotin, 6; folic acid, 15; cyanocobalamin, 0.1; inositol, 200; ascorbic acid, 1000; choline chloride, 4000; menadione, 40; α-tocopherol acetate, 400; para-amino benzoic acid, 50; vitamin A acetate, 2000 IU; vitamin D_3, 1000 IU; dilutant, α-cellulose.

Table 6.2 : Proximate composition ($g.100g^{-1}$ DM) and gross energy content of catfish diets[1], and properties of their respective wet silages.

	CS-0	CS-15	CS-30	CS-60
Moisture	34.25	34.31	34.28	34.50
Crude protein	39.33	38.82	39.19	38.35
Crude lipid	8.58	8.52	8.60	8.56
Crude fibre	3.53	3.61	3.57	3.55
Ash	10.54	10.45	10.37	10.60
Gross energy[2]($kcal.g^{-1}$ DM)	4.58	4.57	4.58	4.56
pH	4.0	4.0	4.0	4.0
NPN ($g.100g^{-1}$ TKN)	17.2	37.6	40.2	48.5

[1] all values represent mean of three replicates.

[2] calculated from the dietary composition (4.1 $kcal.g^{-1}$ for carbohydrate, 9.5 $kcal.g^{-1}$ for lipid, 5.6 $kcal.g^{-1}$ for protein).

6.2.6 Growth trial

(a) Stocking

Juvenile ***C. gariepinus*** weighing 15-16.5 g were acclimated for 7 days and fed to satiation with a commercial trout pellet. They were later sorted into four groups of 60 ***C. gariepinus*** (mean weight, 18.5±1.3 g) representing the diet treatments, and were randomly distributed into triplicate tanks (20 fish per tank) within the experimental system. The diets were assigned randomly to the tanks and each group was fed at 5% body weight per day in two equal portions at 09.00-09.30h and 16.30-17.00h for 70 days. This amount was close to maximum daily ration for both fishes according to the level of dietary intake during acclimation.

(b) Sampling procedure

Catfish were weighed individually at the start of growth trials (day 0). On day 14, 28, 42 and 56, ten fish were taken randomly from each tank; anaesthetized with 60-70 $mg.L^{-1}$ benzocaine (ROSS & GEDDES,1979); batch-weighed; revived in fresh water and returned to their respective tanks. For the final sampling (day 70), all fish were weighed individually.

(c) Growth and Feed utilization data analyses

(i) Mean weight gain (MWG,g)

$$MWG = W_t - W_o$$

(ii) Average daily weight gain (ADG,$g.day^{-1}$)

$$ADG = W_t - W_o/t$$

(iii) Specific growth rate (SGR,%.day^{-1})

$$SGR = 10^2 \times (\ln W_t - \ln W_o)/t$$

where: W_t and W_o = final and initial fish weight; t = feeding period.

(iv) Feed conversion ratio (FCR)

FCR = dry weight of feed (g)/wet weight gain by fish (g)

(v) Protein efficiency ratio (PER)

PER = wet weight gain by fish (g)/protein intake (g)

(vi) Protein productive value (PPV,%)

$$PPV = 10^2 \times (\text{protein gain/protein intake})$$

6.2.7 Protein digestibility trial

Ten catfish were stocked per tank (three tanks per treatment), acclimated for 7 days and fed the experimental diets to satiation. The frequency of feeding was maintained at 5% body weight per day, twice daily from 09.00-09.30h and 16.30-17.00h for 30 days. Faeces from fish fed each diet were collected at the bottom of a settling column in 150 mL conical flasks, pooled and oven-dried to constant weight at 105°C. The chromic III oxide content of diets and faeces was determined in triplicate 50-100 mg portions of moisture-free samples using the wet acid digestion method (FURUKAWA & TSUKAHARA,1966). Protein and gross energy content of faeces were determined using AOAC (1990) methods. Apparent digestibility coefficient (ADC) of protein was calculated using AUSTRENG & REFSTIE (1979) formula as follows.

$$ADC = 10^2 \times (a-b)/a$$

where: a = protein in feed/chromic III oxide in feed;

b = protein in faeces/chromic III oxide in faeces.

6.2.8 Carcass analysis

(a) Carcass composition

Nine fish taken at the start and three fish per tank taken at the end of the growth trials, were homogenised and analysed for moisture, protein, lipid and ash contents (AOAC,1990).

(b) Hepatosomatic index

Six fish taken at the start and six fish taken from each treatment at the end of the growth trial were anaesthetized with benzocaine and weighed individually. Their liver were removed and weighed individually.

Hepatosomatic index (HSI) was calculated as:

$$HSI = (\text{liver weight/body weight}) \times 10^2$$

6.2.9 Statistical methods

Statistical comparisons of the results were made by one-way analysis of variance (ANOVA). All % data were square root arc-sine transformed prior to analysis. Differences in mean values were determined using Duncan's new multiple range test at 5% probability. Standard error was calculated to identify the range of mean values (ZAR,1984) using Statgraphics Software (Statistical Graphics Corporation).

6.3. RESULTS AND DISCUSSION

6.3.1 Experimental diets

The pH of the wet silages was constant at 4.0. The chemical properties of the silages have been reported in an earlier study (**Chapter 3**). Autolysis (NPN content) of the wet silages increased with storage time and reached a peak of 48.5% after storage for 60 days. The NPN levels were consistent with values recorded by various workers who used other fishes as substrates (LINDGREN & PLEJE,1983; BATISTA *et al.*,1989).

Few variation occurred between moisture, protein, lipid, fibre and ash contents of the silage diets (Table 6.2). Fish became accustomed to the diets within the first week. At all feeding periods, the quantities of feed presented were consumed within 10 min. During the first week, fish mortality was less than 10% of the initial number in any of the treatments and was not diet related, rather it was largely due to handling and anesthesia. Dead fish were replaced with fish of similar size. Thereafter, no mortality occurred.

6.3.2 Growth response and Feed conversion

The summary of growth performance by catfish is presented in Table 6.3. The best growth response (MWG, ADG, SGR) was obtained in fish fed with CS-15 diets in which tilapia silage stored for 15 days was incorporated. This was followed by a progressive decline in growth for fish in CS-0, CS-30 and CS-60 diet treatments, but these were not significantly different ($P>0.05$). A plot of growth of fish versus time supported these results (Figure 6.1). With respect to feed conversion (FCR) values, CS-0, CS-15 and CS-30 diet treatments showed no difference ($P>0.05$) among themselves, but CS-15 was superior ($P<0.05$) to CS-60 diet treatment.

Good growth and protein utilization was similarly reported by WEE *et al.* (1986) for catfish, ***Clarias batrachus***, fed diets containing autolysed protein from 8-week old tilapia silage, but this resulted in decreased growth, poor feed conversion and high mortality when fed to ***Clarias macrocephalus*** or snakehead, ***Channa striata*** (EDWARDS *et al.*,1987). AOE *et al.*(1974) and WOOD *et al.*(1985) reported that autolysed proteins in hydrolysates were inferior to whole protein in sustaining growth of young carp, ***Cyprinus carpio***. This was partly attributed to the lack of stomachs in carp (IWAI,1969). The inability to utilize free amino acid diets efficiently has also been reported with another cichlid, ***Tilapia zillii*** (MAZID *et al.*,1978).

Dietary proteins are absorbed from the gut mainly as peptides and free amino acids. During protein digestion, the rate of uptake of peptides and amino acids is in good balance with their metabolic use, but when pre-digested proteins are ingested, the rate of uptake may exceed the anabolic capacity of the animal and more amino acids are therefore catabolized, and may eventually lead to lower utilization of the dietary protein for protein synthesis (YAMADA,1982; ASH & McLEAN,1989).

In light of this, diet CS-15 may have been utilized more efficiently because the available free amino acids had not reached the overload limit, and absorption was prolonged as digestion progressed resulting in more efficient conversion of dietary protein for fish flesh. Conversely, the CS-60 diets may have been inferior to CS-0 diet because they contained a higher proportion of amino acids which exceeded the capacity of the liver to synthesize proteins. It is evident from the results that autolytic activity in fish silage should be restricted in order to preserve nutritional quality.

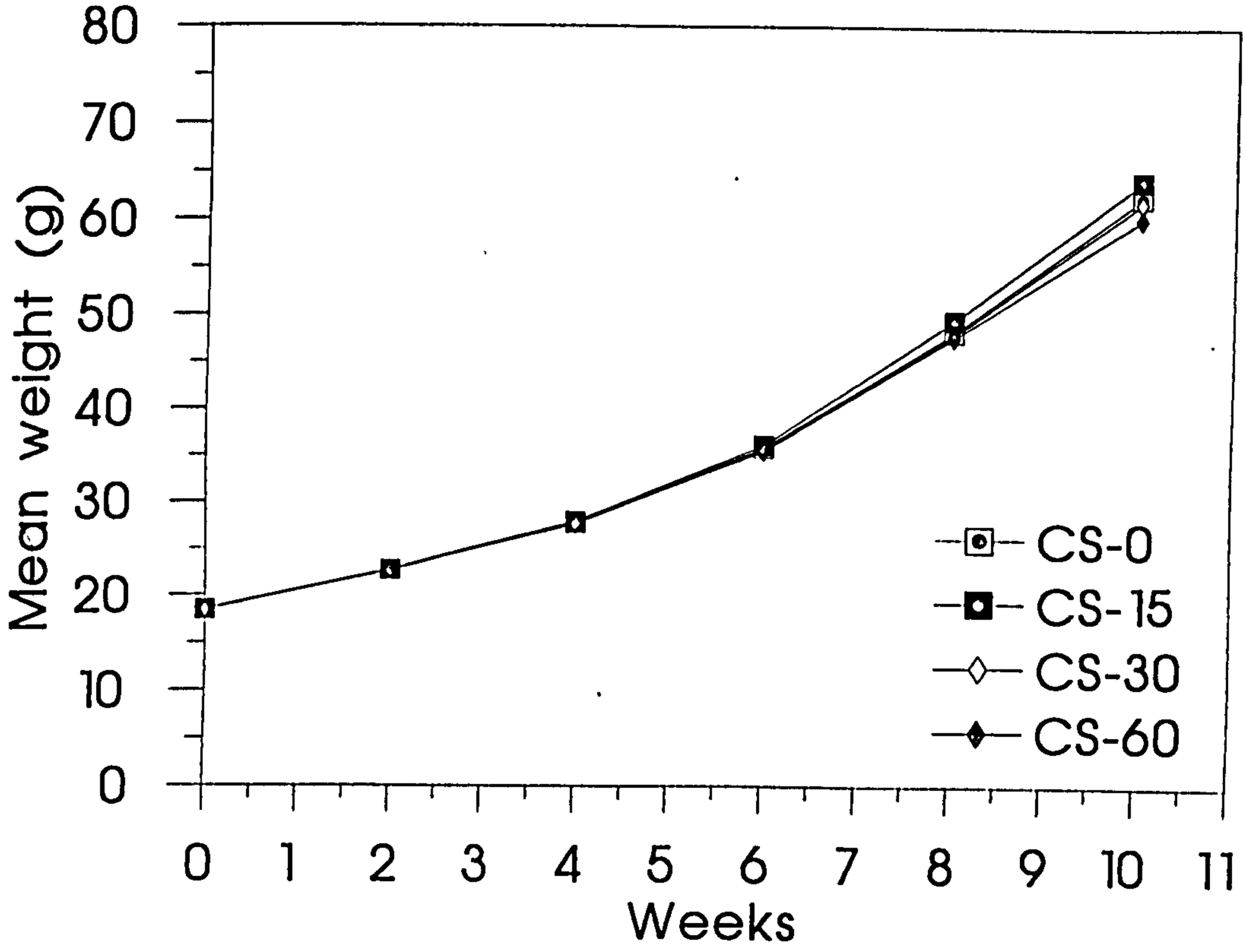


Figure 6.1 : Growth of ***C. gariepinus*** fed moist diets containing stored tilapia silage.

Table 6.3 : Summary of growth performance and protein utilization[1] by ***C. gariepinus*** fed moist diets containing stored fermented tilapia silage.

	Initial wt.[2] (g)	Final wt.[2] (g)	MWG[2] (g)	ADG[2] ($g.day^{-1}$)	SGR[2] ($\%.day^{-1}$)	FCR	PER	PPV (%)	$ADC_{protein}$ (%)
CS-0	18.5	62.4	43.9	0.63	1.74	2.44ab	1.06a	18.80ab	72.9a
CS-15	18.5	63.5	45.7	0.65	1.76	2.37a	1.09a	19.09a	71.6a
CS-30	18.5	61.9	43.4	0.62	1.73	2.46ab	1.05a	18.12ab	72.5a
CS-60	18.5	59.8	41.7	0.60	1.69	2.55b	1.02a	17.91b	70.4a
SEM[3]	0.08	1.24	1.17	0.02	0.02	0.03	0.01	0.22	-

[1] all values represent mean of three replicates.

[2] no significant differences were found among treatment means ($P>0.05$)

[3] pooled standard error calculated from residual mean square in the ANOVA test.

6.3.3 Protein utilization and digestibility

PER and PPV followed a similar trend as growth performance and feed conversion, respectively. Generally, protein utilization by catfish was efficient in all treatments. This may be due to the high (>80%) protein digestibility of diets (Table 6.3). In fact, the protein of CS-15 diets was better utilized than the unautolysed protein in CS-0 diet. The influence/ presence of pre-digested protein in the silage diets may also have contributed to the good growth and protein utilization, though less apparent with increasing dietary levels of autolysed protein. It therefore follows that a good amino acid/protein availability resulted in an improved absorption.

Catfish that consumed the diets containing either autolysed or whole protein had high and similar digestibility coefficients ($P<0.05$) and there was no trend shown with increasing dietary levels of autolysed protein (Table 6.3). Since protein digestibility of these stored silages were also similar, it appears that silage stored for 15-30 days are suitable dietary protein sources for catfish. This high protein digestibility agrees with 66.16-75.85% reported by WEE *et al.*(1986) and EDWARDS *et al.*(1987) when 8-week old fermented tilapia silage was fed to catfishes.

The use of fermented fish silage has been justified for poultry and livestock (TIBBETTS *et al.*,1981; BROWN & SUMNER,1985; HASSAN & HEATH, 1986; 1987) and for fish, it gave satisfactory results (WEE *et al.*,1986). The

present study with moist diets containing autolysed protein from fermented tilapia silage stored for 15-60 days, showed good growth performance and protein utilization comparable to the control (intact protein) diet. Overall performance indicated that dietary protein, part of which is autolysed, is beneficial to catfish juveniles. This suggests that some pre-digested protein in the diet is advantageous, but there would be a limit over which the fish would have difficulties in absorbing the protein for synthetic purposes.

6.3.4 Carcass composition

Moisture content decreased while the protein and lipid contents were higher ($P<0.05$) than the respective initial contents (Table 6.4). Although autolysed protein from the CS-15 diet was more efficiently utilized than intact protein in the CS-0 diet, the difference did not affect body (fillet) nutrient composition. In fact, differences in the nutrient deposition in fish muscle (fillet) among the diet treatments (Table 6.4) were not significant ($P>0.05$). Usually, when the level of amino acids exceeds the capacity of the liver to synthesize proteins, excess amino acids may be degraded or transformed to glycogen or lipid (NOSE,1989). No corresponding increase in body lipid content occurred with increased dietary level of autolysed protein. This may be explained by the fact that ***C. gariepinus*** does not store fat in the muscle (DEGANI,1988; DEGANI *et al.*,1989).

Table 6.4 : Body composition (% dry weight basis) of ***C. gariepinus*** fed moist diets containing stored fermented tilapia silage.

	Dry matter	Crude protein	Crude lipid	HSI[2]
Initial	20.35	70.02	6.73	1.78
CS-0	21.55	77.73	8.63	1.83
CS-15	21.35	77.80	8.95	1.79
CS-30	21.16	77.64	8.46	1.89
CS-60	21.19	77.87	8.82	1.85

[1] all values represent mean of three replicates.

[2] HSI = hepatosomatic index (%)

6.3.5 Pathological effects

No morphological deformities were observed in both ***C. gariepinus*** fed any of the dried fish silage diets. In particular, there were no signs of back deformities, contrary to the observation of WEE *et al.*(1986) in ***C. batrachus***. There were no differences ($P>0.05$) in hepatosomatic index in catfish fed increasing levels of dietary autolysed protein from fish silage or the control diet (Table 6.4). Although some livers were pale in colour, this was not diet related.

CHAPTER 7 : EVALUATION OF DIFFERENT LEVELS OF DRIED TILAPIA SILAGE IN DRY FISH DIETS

The experiments in this chapter have been/will be published in

Aquatic Living Resources, 7 (1994): 79-85.

Aquatic Living Resources, 8 (1995): in press.

7.1 INTRODUCTION

Alternative protein sources in fish feeds are principally derived from animal processing by-products or from oil seed residues remaining after oil removal. Collectively, they may account for up to 50% of the total protein component within a commercial feed, the rest being supplied by fish meal protein. Products made from fish hydrolysates include fish silage, liquified fish, and their concentrated, dried, or co-dried derivatives; their nutritional quality varies depending on the production steps during manufacture (STONE & HARDY,1989; STONE *et al.*,1989). They may also have price and nutritional advantages over competing alternative dietary protein sources (HARDY & MASUMOTO,1990).

Utilizing high-moisture products such as fish silage presents many difficulties. The high cost of transportation, the extremely labile nature of the product, by microbial and enzymatic degradation, and the absence of or limited access to drying or refrigeration facilities close to the

production site precludes the economical use of such products. According to DISNEY & JAMES (1980), these problems might be resolved by adding dry feedstuffs to the ensiled fish and co-drying, thereby creating an "added value" feed ingredient.

7.1.1 Objectives

Further to the previous *in vitro* studies on the nutritional properties of fermented tilapia silage, this study evaluates wet tilapia silage co-dried with soybean flour (FS:SBF) as partial or total replacement for fish meal in dry diets for ***O. niloticus*** and ***C. gariepinus***, with regards to growth performance, feed conversion, protein utilization and digestibility, carcass composition and histopathological effects.

7.2 MATERIALS AND METHODS

7.2.1 Experimental fish

Juveniles of all-male ***O. niloticus*** (hormone-induced sex reversal) and ***C. gariepinus***, maintained in the Tropical Aquarium were acclimated to the experimental system for 7 days preceding the start of growth trials and fed commercial trout pellets (EWOS-BAKER OMEGA 3, 49% crude protein) to satiation (10-15 min of the onset of feeding).

7.2.2 Experimental systems

The experimental systems used for growth and digestibility trials are as described in **Chapter 6.2.2.** Water samples were taken weekly to determine pH, total ammonia, total nitrite and dissolved oxygen (DO_2) concentration (Appendix 3).

7.2.3 Fermented fish silage

Fermented tilapia silage was prepared as described in **Chapter 3.2.1** using the pre-fermented ***Lactobacillus plantarum*** starter culture, molasses and minced tilapia mixture (2:5:100), and incubated anaerobically at 30°C inside tightly-sealed 5-L plastic containers for 7 days. The tilapia silage was stirred daily to obtain a proper blend and was heated to 90°C for 30 min to halt autolysis; and then stored at 30°C for 30 days

before use so that the low pH could kill any pathogens present. The pH was monitored every week and ranged between 4.0 and 4.3. The fish silage was blended with soybean flour (1:1, dry weight basis) and the mixture oven-dried at 45°C for 48 h. Samples of the co-dried FS:SBF blend were milled to <0.25 mm; and analysed for proximate and amino acid composition (Table 7.1).

Table 7.1 : Proximate composition (g.100g^{-1}), gross energy content and amino acid composition (g.100g^{-1} protein) of co-dried tilapia silage:soybean flour blend and fish meal.

	FS:SBF	Fish meal
Dry matter	94.39	91.27
Crude protein	56.08	74.85
Crude lipid	5.61	10.42
Ash	9.06	11.54
Gross energy (kcal.g^{-1} DM)	4.33	4.90
Amino acids		
Arginine	6.87	7.48
Histidine	2.75	2.54
Isoleucine	3.63	3.47
Leucine	6.57	6.66
Lysine	6.04	6.54
Methionine	2.47	2.55
Cystine	1.48	1.36
Phenylalanine	3.40	3.32
Tyrosine	2.34	2.88
Threonine	4.15	4.23
Tryptophan	0.80	1.07
Valine	4.38	4.14

7.2.4 Experimental diets

(a) Formulation

Five dry diets were formulated for ***O. niloticus*** and ***C. gariepinus*** in which co-dried FS:SBF blend was incorporated as protein source, replacing 0% (control, T-0,C-0), 25% (T-25,C-25), 50% (T-50,C-50), 75% (T-75,C-75) or 100% (T-100,C-100) of fish meal protein (Tables 7.2 and 7.3). Chromic III oxide was added as an internal marker for determination of apparent protein digestibility coefficient. The diets contained 30% protein, 3.82 kcal.g^{-1} gross energy and 40% protein, 4.30 kcal.g^{-1} gross energy in the dry matter, respectively (Tables 7.4 and 7.5).

(b) Preparation

Co-dried FS:SBF blend and the basic ingredients were finely ground (<0.25 mm) mixed thoroughly based on the proportion given in Tables 7.2 and 7.3, inside the bowl of a HOBART A-200 industrial food mixer (HOBART Co. Ltd., London). The process was repeated with the addition of a mixture of cod liver oil and corn oil (1:1), and warm water (30% of the total ingredient weight) until the binder had been primed. The homogenous paste was then cold-extruded under pressure into noodles through 3 mm die plates in a HOBART A-200 pellet mill, dried at 45°C for 16 h (to <10% moisture), and subsequently broken into small pellet size (<3 mm). Dry pellets were kept in sealed polyethylene bags and stored at -20°C until they were thawed shortly before feeding.

(c) Properties

Distilled water was added to 5 g samples of milled diets and made to 50 mL, and the mixture was centrifuged at 8000 rev.min^{-1} for 10 min using an MSE Minor S centrifuge. The supernatant was used for pH determination. The diets were tested for water stability thus; triplicate 50 g samples of pre-screened pellet crumbles of each diet were placed on a sieve and slowly immersed in a 40-L glass aquarium containing deionized water at 27°C for 10 min. The sieves were removed and the crumbles allowed to drain for 1 min, oven-dried at 105°C for 2 h, cooled in a desiccator and reweighed. Water stability was calculated and expressed as the percentage loss of dry matter (% LDM) in sample weight.

7.2.5 Analytical methods

(a) Proximate composition and gross energy content

Moisture, protein, lipid, fibre and ash content of the diets were determined by AOAC (1990) methods. Gross energy content of the diets was determined using a bomb calorimeter. Proximate composition and energy content of the diets are presented in Tables 7.4 and 7.5.

(b) Amino acid composition

Triplicate samples of dietary protein sources and diets were hydrolysed with 6 mol.L^{-1} HCl for 24 h at 110°C *in vacuo* and used for amino acid content determination using the LKB 4151 ALPHA-PLUS amino acid

analyzer. Tryptophan content was determined colorimetrically after hydrolysis in 4.2 mol.L^{-1} NaOH (FISCHL,1960). The amino acid composition of the diets are presented in Tables 7.6 and 7.7.

7.2.6 Growth trials

Five groups of 90 ***O. niloticus*** (mean weight, 8.2±0.1 g) and five groups of 90 ***C. gariepinus*** (mean weight, 10.8±0.3 g) representing the diet treatments were randomly distributed into triplicate tanks (30 fish per tank) within the experimental system. The diets were assigned randomly to the tanks and each group was fed at 4% body weight per day in two equal portions at 09.00-09.30h and 16.30-17.00h for 70 days. Fish were weighed individually at the start and end of the growth trials. On day 14, 28, 42 and 56, ten fish per tank were taken randomly using a fine-mesh hand net; anaesthetized with 60-70 mg.L^{-1} benzocaine (ROSS & GEDDES, 1979); batch-weighed and returned to their respective tanks. Growth responses (MWG, ADG, SGR), feed conversion (FCR) and protein utilization (PER, PPV) were evaluated as described in **Chapter 6.2.6**.

Table 7.2 : Formulation (g.100g^{-1} DM) of dry diets for ***O. niloticus***.

	Tilapia diets				
	T-0	T-25	T-50	T-75	T-100
Fish meal	40.0	30.0	20.0	10.0	0.0
FS:SBF	0.0	13.4	26.8	40.1	53.5
Corn starch	35.0	32.5	30.0	27.5	25.0
Cellulose flour	13.0	12.1	11.3	10.4	9.5
Common ingrdients[1]	12.0	12.0	12.0	12.0	12.0

Table 7.3 : Formulation (g.100g^{-1} DM) of dry diets for ***C. gariepinus***.

	Catfish diets				
	C-0	C-25	C-50	C-75	C-100
Fish meal	53.5	40.0	27.0	13.5	0.0
FS:SBF	0.0	17.9	35.7	53.5	71.5
Corn starch	25.0	22.5	20.0	17.5	15.0
Cellulose flour	9.5	7.7	5.3	3.5	1.5
Common ingrdients[1]	12.0	12.0	12.0	12.0	12.0

[1] cod liver oil, 3%; corn oil, 3%; carboxymethyl cellulose, 2%; mineral mix[a], 2%; vitamin mix[b], 1%; chromic III oxide, 1%.

[a] g.kg^{-1}: $MgSO_4.7H_2O$, 5.10; NaCl, 2.40; KCl, 2.00; $FeSO_4.7H_2O$, 1.00; $ZnSO_4.H_2O$, 0.22; $CuSO_4.5H_2O$, 0.0314; $MnSO_4.4H_2O$, 0.1015; $CoSO_4.7H_2O$, 0.0191; $CaIO_3.6H_2O$, 0.0118; $CrCl_3.6H_2O$, 0.0051.

[b] mg.kg^{-1}: thiamine HCl, 50; riboflavin, 50; calcium pantothenate, 100; niacin, 200; pyridoxine HCl, 40; biotin, 6; folic acid, 15; cyanocobalamin, 0.1; inositol, 200; ascorbic acid, 1000; choline chloride, 4000; menadione, 40; α-tocopherol acetate, 400; para-amino benzoic acid, 50; vitamin A acetate, 2000 IU; vitamin D_3, 1000 IU; dilutant, α-cellulose.

Table 7.4 : Properties, proximate composition (g.100g^{-1} DM) and gross energy content (kcal.g^{-1} DM) of tilapia diets[1].

	T-0	T-25	T-50	T-75	T-100
Moisture	7.64	7.83	7.78	7.81	7.75
Crude protein	29.63	29.90	29.85	29.99	29.79
Crude lipid	7.40	7.47	7.52	7.48	7.50
Crude fibre	3.50	3.56	3.58	3.60	3.62
Ash	9.01	9.18	9.45	9.50	9.53
Gross energy	3.92	3.91	3.90	3.88	3.87
pH	6.3	6.3	6.5	6.4	6.5
Water stability (% LDM)	5.4	5.3	5.5	5.4	5.6

[1] all values represent mean of three replicates.

Table 7.5 : Properties, proximate composition (g.100g^{-1} DM) and gross energy content (kcal.g^{-1} DM) of catfish diets[1].

	C-0	C-25	C-50	C-75	C-100
Moisture	7.49	7.60	7.62	7.65	7.58
Crude protein	39.14	39.39	39.07	38.93	39.47
Crude lipid	8.48	8.52	8.56	8.60	8.54
Crude fibre	3.52	3.57	3.58	3.61	3.64
Ash	10.47	10.42	10.63	10.66	10.70
Gross energy	4.18	4.19	4.22	4.23	4.24
pH	6.3	6.5	6.5	6.4	6.5
Water stability (% LDM)	5.5	5.2	5.3	5.5	5.6

[1] all values represent mean of three replicates.

Table 7.6 : Amino acid composition (g.100g^{-1} protein) of tilapia diets.

	T-0	T-25	T-50	T-75	T-100
Arginine	6.91	6.82	6.73	6.67	6.65
Histidine	2.58	2.56	2.60	2.58	2.60
Isoleucine	4.71	4.64	4.55	4.67	3.47
Leucine	8.36	8.16	7.92	7.66	7.48
Lysine	6.91	6.82	6.57	6.32	6.10
Methionine Cystine[1]	2.16 1.55	2.18 1.50	1.92 1.54	1.72 1.53	1.51 1.61
Phenylalanine Tyrosine[1]	4.65 3.33	4.54 3.30	4.49 3.24	4.50 3.13	4.47 3.05
Threonine	4.20	4.10	4.01	3.89	3.82
Tryptophan	1.29	1.31	1.38	1.44	1.48
Valine	5.65	5.41	5.10	4.72	4.40

Table 7.7 : Amino acid composition (g.100g^{-1} protein) of catfish diets.

	C-0	C-25	C-50	C-75	C-100
Arginine	6.84	6.68	6.69	6.66	6.50
Histidine	2.52	2.48	2.52	2.51	2.50
Isoleucine	4.64	4.57	4.53	4.45	4.32
Leucine	7.92	7.70	7.52	7.32	6.98
Lysine	6.89	6.78	6.59	6.37	6.04
Methionine Cystine[1]	2.15 1.47	2.09 1.43	1.89 1.47	1.70 1.50	1.45 1.50
Phenylalanine Tyrosine[1]	4.47 3.23	4.40 3.13	4.41 3.09	4.37 3.00	4.29 2.91
Threonine	4.08	4.01	3.94	3.83	3.68
Tryptophan	1.27	1.31	1.37	1.45	1.45
Valine	5.52	5.30	5.00	4.64	4.24

[1] cystine and tyrosine are dispensable but spare requirements for methionine and phenylalanine, respectively.

7.2.7 Digestibility trials

The procedure used for the digestibility trials are as described in **Chapter 6.2.7.** ADC of protein and energy were evaluated using AUSTRENG & REFSTIE (1979) and PAGE & ANDREWS (1973) formulae as follows:

$$ADC_{protein} = 10^2 \times (a - b)/a$$

$$ADC_{energy} = E_d - (E_f \times I_d/I_f)$$

where : a = protein in feed/chromic III oxide in feed;

b = protein in faeces/chromic III oxide in faeces.

E_d = gross energy of diet; E_f = gross energy of faeces;

I_d = chromic III oxide in feed; I_f = chromic III oxide in faeces.

7.2.8 Carcass analysis

(a) Carcass composition

Nine fish taken at the start and three fish per tank taken at the end of the growth trials, were homogenised and analysed for moisture, protein, lipid and ash contents (AOAC,1990).

(b) Hepatosomatic index

Six fish taken at the start and six fish taken from each treatment at the end of the growth trials were anaesthetized with benzocaine and weighed individually. Their livers were removed and weighed individually, and used to calculate the hepatosomatic index (HSI) as follows:

$$HSI = (\text{liver weight/body weight}) \times 10^2$$

(c) Histological and haematological examination

Six fish taken at the start and six fish taken from each treatment at the end of the growth trials were anaesthetized with benzocaine and weighed individually. Blood was collected in heparinized centrifuge tubes from the caudal vessels to determine the haematocrit and haemoglobin content using the microhaematocrit tube and cyanomethaemoglobin method (SVOBODOVA *et al.*,1991), and the mean cell haemoglobin concentration (MCHC) was calculated.

Tissues from liver, pancreas and intestine were fixed in a neutral 1:10 formalin solution, dehydrated in graded ethanol series, cleared with xylene and blocked in paraffin. The blocks were sectioned at 5 μm, placed on glass slides and stained with haematoxylin and eosin (H & E) or periodic acid Schiff (PAS) and haematoxylin, and examined under a light microscope.

7.2.9 Statistical methods

Statistical comparisons of the results were made by one-way analysis of variance (ANOVA). All % data were square root arc-sine transformed prior to analysis. Differences in mean values were determined using Duncan's new multiple range test at 5% probability. Standard error was calculated to identify the range of mean values (ZAR,1984) using Statgraphics Software (Statistical Graphics Corporation).

7.3. RESULTS AND DISCUSSION

7.3.1 Experimental diets

The pH of all experimental diets were 6.3-6.5 and their stability in water was high, indicated by low % LDM (Tables 7.4 and 7.5). Few variations occurred in the moisture, protein, lipid and fibre contents of diets. Fish became accustomed to the diets within the first week of growth trials. Low mortality (<10%) occurred during the acclimation period and was replaced with fish of similar size. No mortality occurred throughout the duration of either tilapia or catfish growth trials.

The near neutral pH of tilapia silage based-diets (Tables 7.4 and 7.5) does not pose any problem to either tilapia or catfish because much lower pH occurs in their stomachs, pH 2 in ***O. niloticus*** (NAWAB,1987) and pH 4 in ***C. gariepinus*** (UYS,1989). ADEJUMO (1987) recorded a lower pH of 5.25 and 5.55 with co-dried maize:acid silage and cassava:acid silage-based diets, respectively. Water stability of all diets was suitable for fast and competitive feeders such as ***O. niloticus*** and ***C. gariepinus*** as it ensured optimum feed uptake before disintegration of pellets. High fish survival was due to conducive water quality conditions in the experimental systems, which were within tolerance range stipulated by for ***O. niloticus*** (BALARIN & HATTON,1979) and ***C. gariepinus*** (VIVEEN *et al.*,1992).

7.3.2 Growth response and Feed conversion

The summary of fish growth responses and feed conversion are presented in Tables 7.8, 7.9. Fish growth responses and feed conversion efficiencies in the T-100 and C-100 treatments were significantly lower ($P<0.05$) while in fish fed with other silage diets, growth improved as the inclusion level of co-dried FS:SBF blend increased up to 50% fish meal replacement. A plot of growth of fish versus time supported these results (Figures 7.1, 7.2).

With the exception of the T-100 treatment, MWG and ADG of ***O. niloticus*** were not significantly different ($P>0.05$). Differences in SGR values were however significant ($P<0.05$), while in ***C. gariepinus***, the MWG, ADG and SGR values showed significant differences. FCR values of 1.33-1.54 and 1.42-1.50 were obtained in all treatments by tilapia and catfish, respectively, with significant differences ($P<0.05$).

Growth responses by fish in treatments with fish meal replacement up to 75% were better than in the control treatment (Tables 7.8 and 7.9) which suggest that co-dried FS:SBF blend can partially replace fish meal as dietary protein in dry diets for ***O. niloticus*** and ***C. gariepinus***; while total replacement was inferior to the control. This agrees with reports that total replacement of fish meal by dried acid silage were accepted but gave poor growth responses in ***O. niloticus*** (HERNANDEZ, 1983; ADEJUMO, 1987). Lower growth responses by fish in both T-100 and C-100 treatments were

probably due to non-inclusion of fish meal in the diets causing a reduced palatability of diets or appetite.

Replacement of fish meal with fish silage has had variable successes. With acid silage, fish growth has often been reduced in direct proportion to the % dietary inclusion of silage (HARDY *et al.*,1984; PHROMKUNTHONG & CHETANON,1987; LAPIE & BIGUERAS-BENITEZ,1992). Poor growth performances of various fishes fed with fish silage-based diets have been ascribed to acidity of diets, high proportion of free amino acids and hydrolysed proteins (BROMLEY & SMART,1981; JACKSON *et al.*,1984b; HARDY *et al.*,1984; WOOD *et al.*,1985; LIE *et al.*,1988; GONCALVES *et al.*, 1989; STONE *et al.*,1989). Acidity is reported to reduce diet acceptance and affects protease activity in fish guts (RUNGRUANGSAK & UTNE,1981), and a high level of free amino acids may act as an appetite depressant (LALL,1991). HARDY *et al.*(1984) suggested that liquefaction of silage should be restricted if higher levels of dried fish silage were to be used in fish diets. In this study, pH of all diets was near neutral (Table 7.2) and fermentation was restricted to 7 days which ensured limited proteolysis.

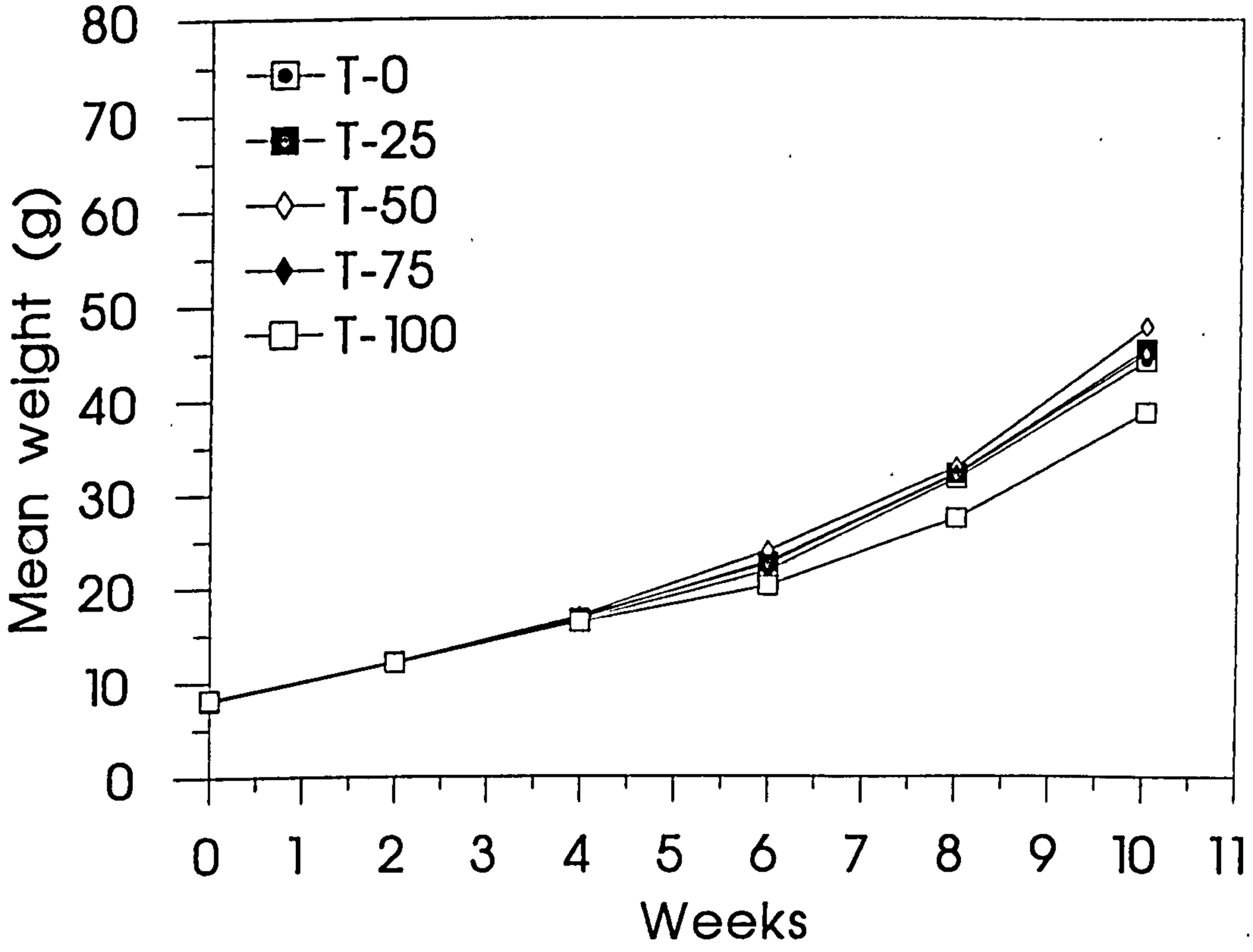


Figure 7.1 : Growth of ***O. niloticus*** fed diets containing increasing levels of co-dried FS:SBF blend.

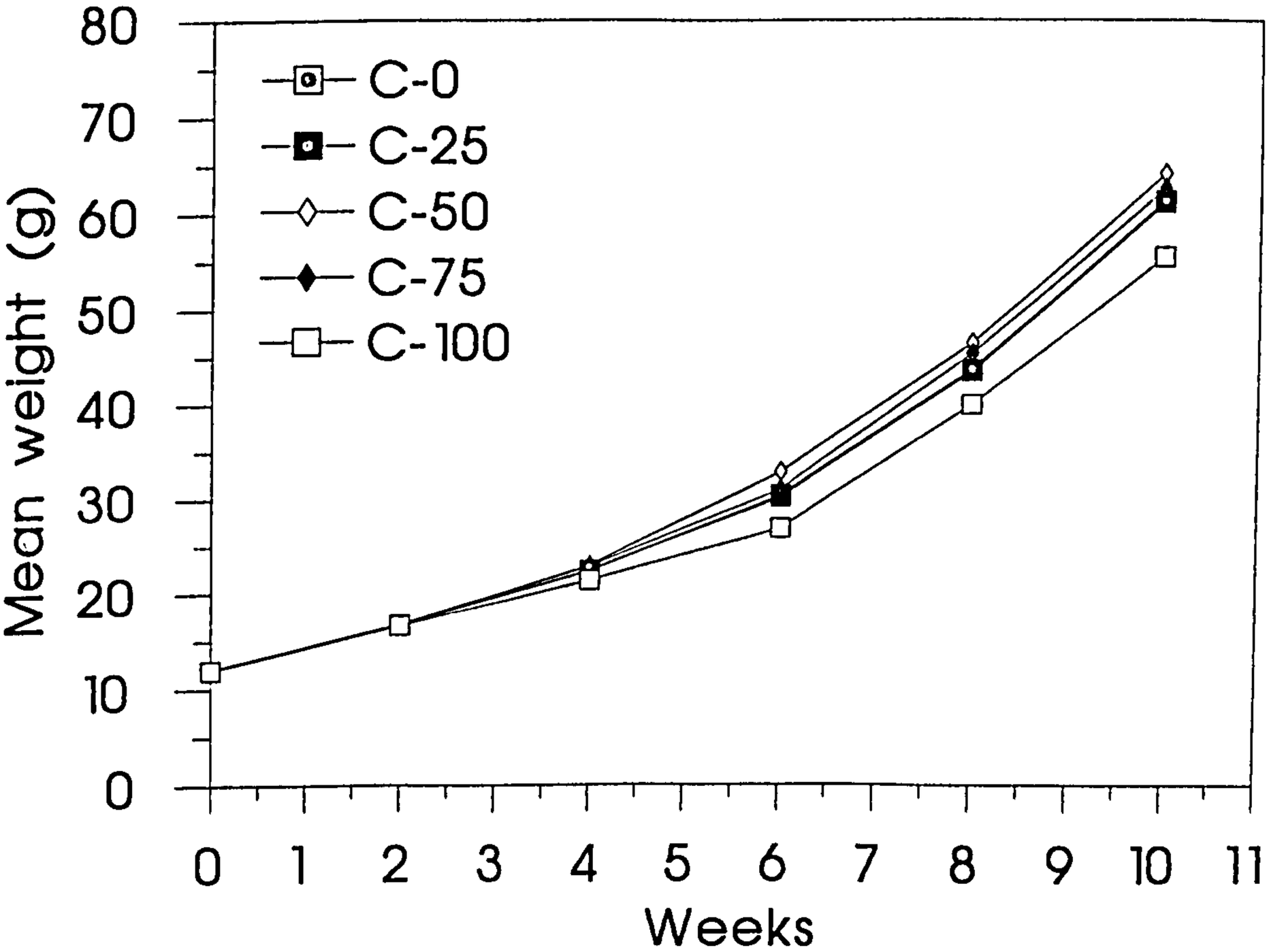


Figure 7.2 : Growth of ***C. gariepinus*** fed diets containing increasing levels of co-dried FS:SBF blend.

Table 7.8 : Summary of growth performance and protein utilization[1] by ***O. niloticus*** fed diets containing increasing levels of co-dried FS:SBF blend.

	Initial wt.[2] (g)	Final wt. (g)	MWG (g)	ADG ($g.day^{-1}$)	SGR ($\%.day^{-1}$)	FCR	PER	PPV (%)	$ADC_{protein}$ (%)	ADC_{energy} (%)
T-0	8.2	44.3	36.1a	0.52a	2.41a	1.40a	2.41a	35.74a	82.7ab	72.7a
T-25	8.0	45.6	37.6a	0.54a	2.48ab	1.37a	2.45a	36.45a	83.9bc	71.9ab
T-50	8.1	47.9	39.8a	0.57a	2.54b	1.33a	2.52a	37.38a	88.9bc	70.7ab
T-75	8.2	45.1	37.0a	0.53a	2.44ab	1.39a	2.40a	35.87a	85.4ac	70.3ab
T-100	8.2	38.8	30.6b	0.44b	2.22c	1.54b	2.18b	32.42b	78.0a	63.7b
SEM[3]	0.12	1.20	1.11	0.02	0.02	0.02	0.04	0.56	1.12	1.34

[1] all values represent mean of three replicates.

[2] no significant differences were found among treatment means ($P>0.05$)

[3] pooled standard error calculated from residual mean square in the ANOVA test.

Table 7.9 : Summary of growth performance and protein utilization[1] by ***C. gariepinus*** fed diets containing increasing levels of co-dried FS:SBF blend.

	Initial wt.[2] (g)	Final wt. (g)	MWG (g)	ADG ($g.day^{-1}$)	SGR ($\%.day^{-1}$)	FCR	PER	PPV (%)	$ADC_{protein}$ (%)	ADC_{energy} (%)
C-0	12.0	61.2	49.2b	0.70b	2.33b	1.43ab	1.80b	30.13ab	82.5b	79.7b
C-25	12.0	61.4	49.5bc	0.71b	2.34bc	1.42ab	1.79b	30.33ab	85.4b	77.6b
C-50	12.1	64.2	52.1c	0.74b	2.38c	1.42a	1.81b	30.26ab	88.0c	75.5b
C-75	12.1	62.7	50.6bc	0.72b	2.35bc	1.42a	1.81b	30.76b	84.7bc	74.1ab
C-100	12.0	55.6	43.6a	0.62a	2.19a	1.50b	1.68a	28.82a	80.2a	72.6a
SEM[3]	0.07	0.66	0.60	0.01	0.01	0.02	0.37	0.37	0.99	1.64

[1] all values represent mean of three replicates.

[2] no significant differences were found among treatment means ($P>0.05$)

[3] pooled standard error calculated from residual mean square in the ANOVA test.

7.3.3 Protein utilization and digestibility

The results of protein utilization, expressed as PER and PPV values, are presented in Tables 7.8 and 7.9. Except for T-100 and C-100 treatments, PER and PPV values obtained for both ***O. niloticus*** and ***C. gariepinus*** in all treatments were not significantly different ($P>0.05$). $ADC_{protein}$ values (Tables 7.8 and 7.9) were high for both experimental fishes and improved with increasing dietary levels of co-dried FS:SBF blend. Only the $ADC_{protein}$ values from the T-100 and C-100 treatments were lower than those in the control treatments.

The lack of significant difference in PER and PPV values by experimental fish fed with the dried silage-based diets (except the fish meal-free diets), compared with fish fed fish meal (control) diets (Tables 7.8 and 7.9) indicates that co-dried FS:SBF blend can replace up to 75% of fish meal protein in fish diets without affecting fish growth performance, feed conversion or protein utilization. The lower $ADC_{protein}$ of T-100 and C-100 silage protein may have produced a tendency towards the poorer performance of fish.

WILSON *et al.*(1984) attributed reduced protein utilization by channel catfish (***Ictalurus punctatus***) fed offal-based silage diets to the marginal or slight deficiency levels of histidine, isoleucine and total aromatic amino acids in the diets. Considering that all diets used in the present study met or exceeded the amino acid requirements of both fish

species, stipulated by SANTIAGO & LOVELL (1988) and UYS (1989), lower utilization of protein of the T-100 and C-100 diets could have been caused by factors other than specific amino acid deficiency. As fish silage contains NPN, a diet containing fish silage as the sole protein source means less protein 'amino acids' are available for digestion.

The availability and biological value of amino acids from silages may however be less than indicated due to the loss of some amino acids as a result of the Maillard reaction (reactions between α-amino groups and sugar aldehyde groups). Fish silage contains free amino acids and thus becomes susceptible to the Maillard reaction which causes reductions in biological activity of lysine and other amino acids, particularly during co-drying with a feedstuff which has a high carbohydrate content (BALTES,1982). The silage might also have contained some residual sugar after liquefaction/fermentation (KOMPIANG *et al.*,1980b) and this could have enhanced the formation of Maillard reaction products (KIES,1981). Such loss of amino acids might have also contributed to the poor protein utilization and digestibility, and eventually a low carcass protein and poor growth, obtained in fish fed T-100 and C-100 diets.

Lower $ADC_{protein}$ values reported for salmonids fed dried acid silages (HARDY *et al.*,1983;1984) were attributed to high levels of free amino acids and peptides resulting from proteolysis which interfered with protein absorption. Such loss of dietary protein is detrimental to efficient protein utilization. It is unlikely that this was the case in this study because

degradation of protein in fermented tilapia silage is minimal, particularly if fermentation is terminated after 7 days. Nevertheless, $ADC_{protein}$ was comparable to those of dried acid silages used as dietary protein for ***O. niloticus*** (HERNANDEZ,1983; BIGUERAS-BENITEZ *et al.*,1992) which had $ADC_{protein}$ values >90%.

In this study, the digestibility by experimental fish of the proteins in the silage diets was high, and the co-dried FS:SBF blend had a good amino acid profile that is comparable to that of fish meal; therefore it is possible to substitute fish meal components in the diets to the extent observed presently without deleterious effects. Up to 75%, fish fed with the substituted diets performed better than the control diets. It could be that at this inclusion level, fish silage was metabolized better.

YONE *et al.*(1986) and HOSSAIN *et al.*(1987) found that fermented scrap meal (from mackerel waste) improved the growth and feed efficiency in red sea bream, ***Chrysophrys major,*** to levels comparable to fish fed white fish meal-based control diet. Similarly, WEE *et al.*(1986) found no significant differences in ADG, SGR and FCR among catfish, ***Clarias batrachus*** fed moist fermented tilapia silage diets and commercial pelleted feed. MANIKANDAVELU *et al.*(1992) reported a considerably higher growth of carp, ***Cyprinus carpio,*** fed fermented fish silage based diet over a control diet based on fish meal. The data presented in this study with ***O. niloticus*** and ***C. gariepinus*** are in agreement with the findings of the above reports.

7.3.4 Carcass composition

The carcass composition and hepatosomatic index of ***O. niloticus*** and ***C. gariepinus*** at the beginning and end of the growth trials are presented in Tables 7.10 and 7.11. Fish fed either the control or dried tilapia silage diets had higher carcass protein and lipid contents than the initial carcass. The differences in final composition of fish fed with the dried tilapia silage diets were however not significant ($P>0.05$). The higher level of carcass lipid in fish fed dried tilapia silage diets may be due to the occurrence of reductions in their moisture contents (Tables 7.10 and 7.11). There were no differences in hepatosomatic index in tilapia or catfish fed increasing levels of dried fish silage and control diet.

7.3.5 Histological effects

No morphological deformities were observed in either ***O. niloticus*** or ***C. gariepinus*** fed any of the dried fish silage diets. In particular, there were no signs of back deformities (usually associated with tryptophan deficiency in fish silages), contrary to the observation of WEE *et al.*(1986) in ***C. batrachus***. Fishes have a dietary tryptophan requirement of 0.5-1.0% (WILSON,1989); hence with the diet containing co-dried FS:SBF blend contributing as much as 50% of the total protein, tryptophan would still meet the minimum dietary requirement. No histological changes were observed in the exocrine pancreas and intestine tissues but the livers from catfish fed C-100 diet showed some structural changes.

Table 7.10 : Carcass composition of ***O. niloticus***[1] fed diets containing increasing levels of co-dried FS:SBF blend.

	Moisture	Crude protein	Crude lipid	Ash	HSI[2]
Initial	79.65	12.32	4.06	3.92	1.35
T-0	76.80	14.35	5.21	3.56	1.68
T-25	76.57	14.44	5.25	3.53	1.78
T-50	76.62	14.42	5.34	3.60	1.65
T-75	76.45	14.48	5.38	3.59	1.68
T-100	76.25	14.34	5.50	3.55	1.72

[1] all values represent mean of three replicates.

[2] hepatosomatic index

Table 7.11 : Carcass composition of ***C. gariepinus***[1] fed diets containing increasing levels of co-dried FS:SBF blend.

	Moisture	Crude protein	Crude lipid	Ash	HSI[2]
Initial	78.60	13.87	5.11	2.69	1.49
C-0	75.61	16.22	5.93	2.48	2.00
C-25	75.48	16.36	6.00	2.46	1.89
C-50	75.46	16.19	6.06	2.49	2.04
C-75	75.49	16.38	6.13	2.47	1.99
C-100	75.40	16.40	6.23	2.48	2.05

[1] all values represent mean of three replicates.

[2] hepatosomatic index

A loss of hepatocyte uniformity was detected, due to polymorphic hepatocyte swelling. Affected cells were concentrated in large foci (Figure 7.3) or scattered in the hepatic parenchyma (Figure 7.4). Compared with neighbouring hepatocytes, the glycogen content of these cells was lower, as detected by histochemical PAS assay (Figure 7.5). Their cytoplasm contained higher amounts of lipids, but fatty degeneration of hepatocytes was not observed. The cytoplasmic organelles were concentrated near the hepatocytes cell border. Their cytoplasm was free from lipofuszin or ceroid granules and the nuclei of hypertrophic liver cells showed no sign of degeneration in form of karyorhexis, lysis or pycnosis. The number of swollen hepatocytes was low, indicating a mild effect of the liver metabolic activity. In one liver sample, abnormal hyaline eosinophilic droplets occurred in the cytoplasm of some liver cells (Figure 7.6), probably these droplets represent an excessive accumulation of proteineous substances, thereby supporting the hypothesis of exceeding liver protein anabolic capacity.

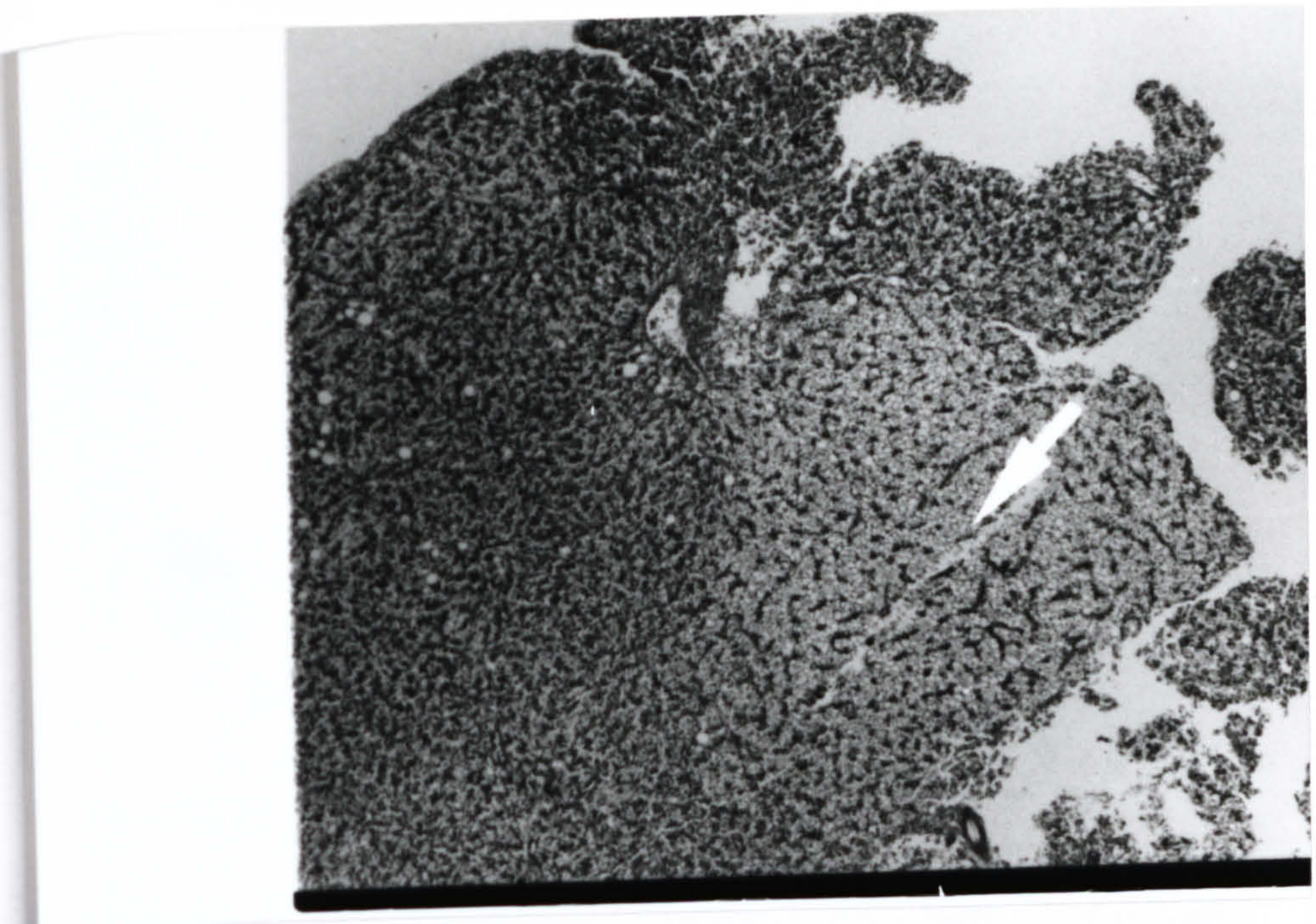


Figure 7.3 : Cross section of catfish liver from C-100 group showing hypertrophic hepatocytes arranged in large foci (arrowed)(HE staining, x 100).

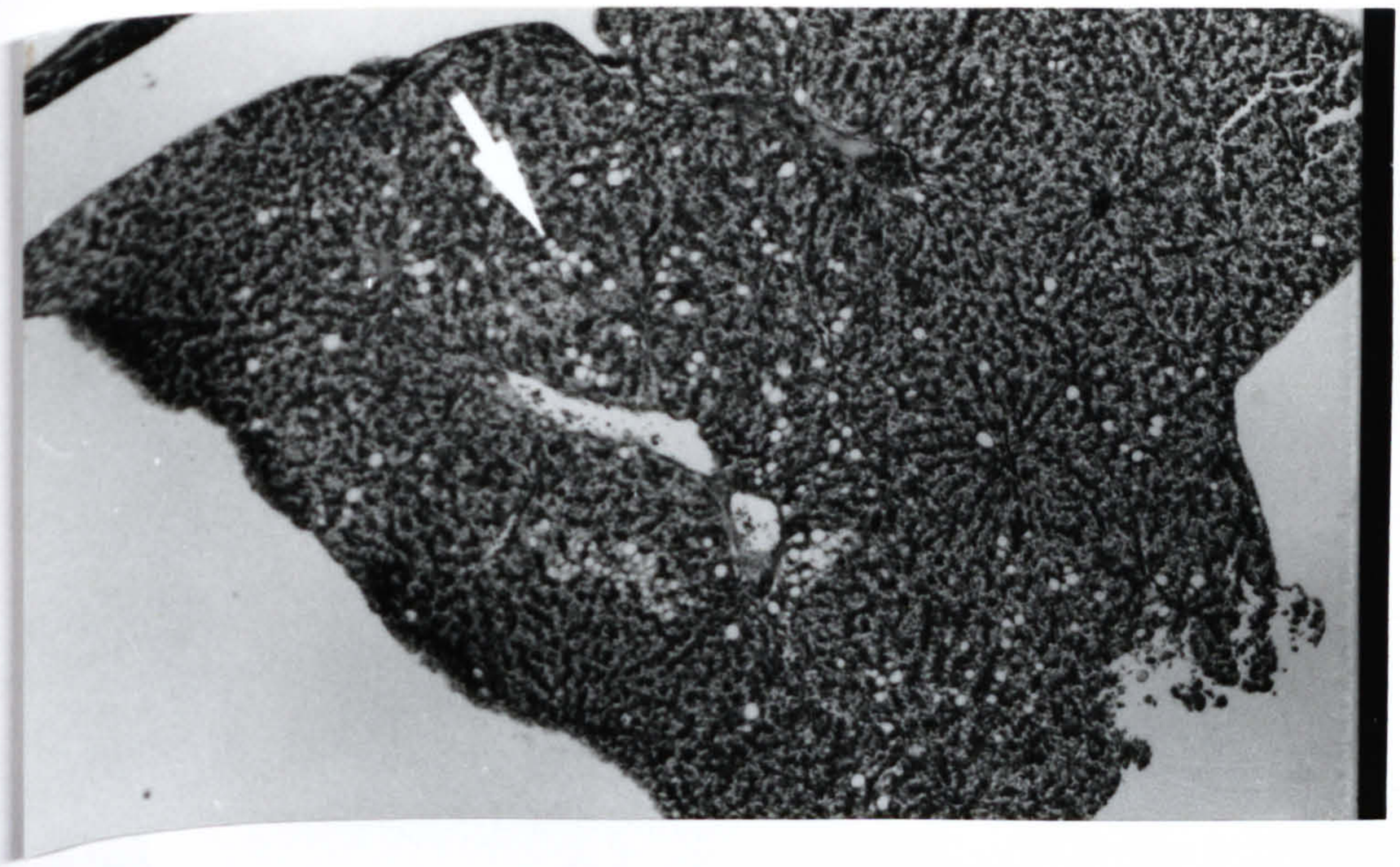


Figure 7.4 : Cross section of catfish liver from C-100 group showing hypertrophic hepatocytes scattered in the liver parenchyma (arrowed)(HE staining, x 100).

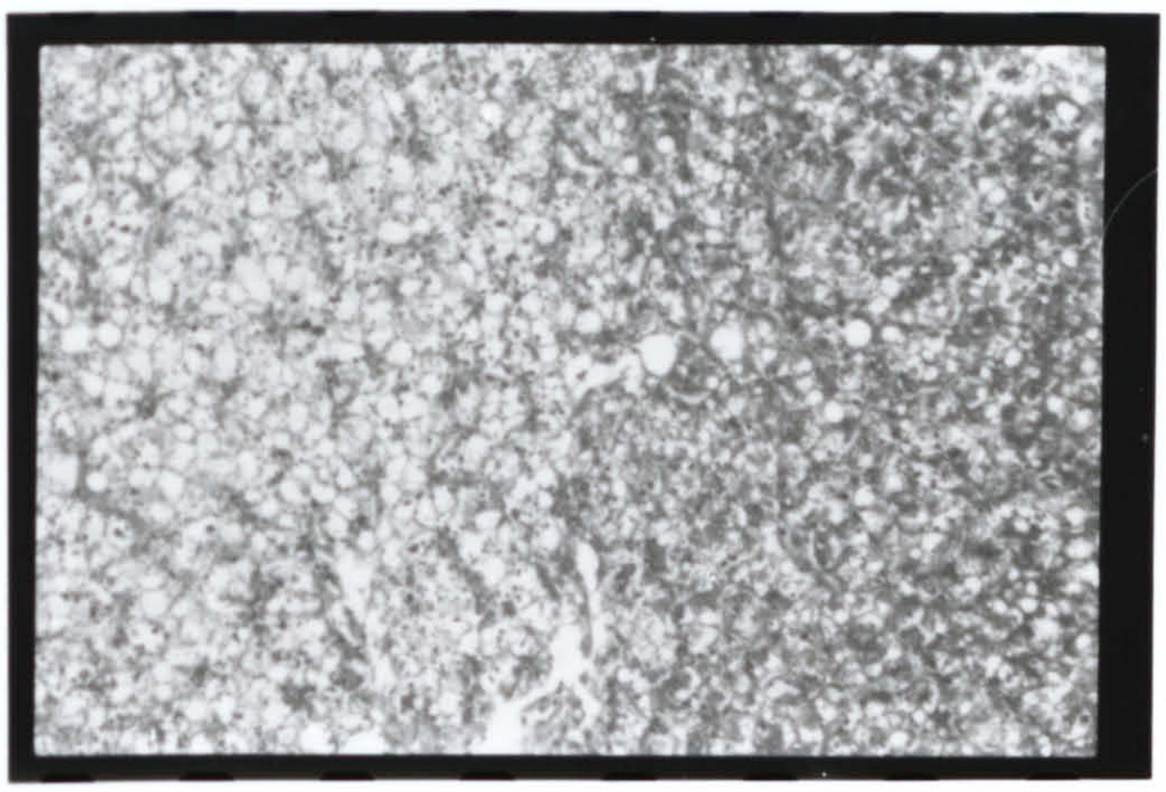


Figure 7.5 : Catfish liver showing different glycogen concentration of hypertrophic (left) and normal (right) hepatocytes (PAS-H staining, x 250).

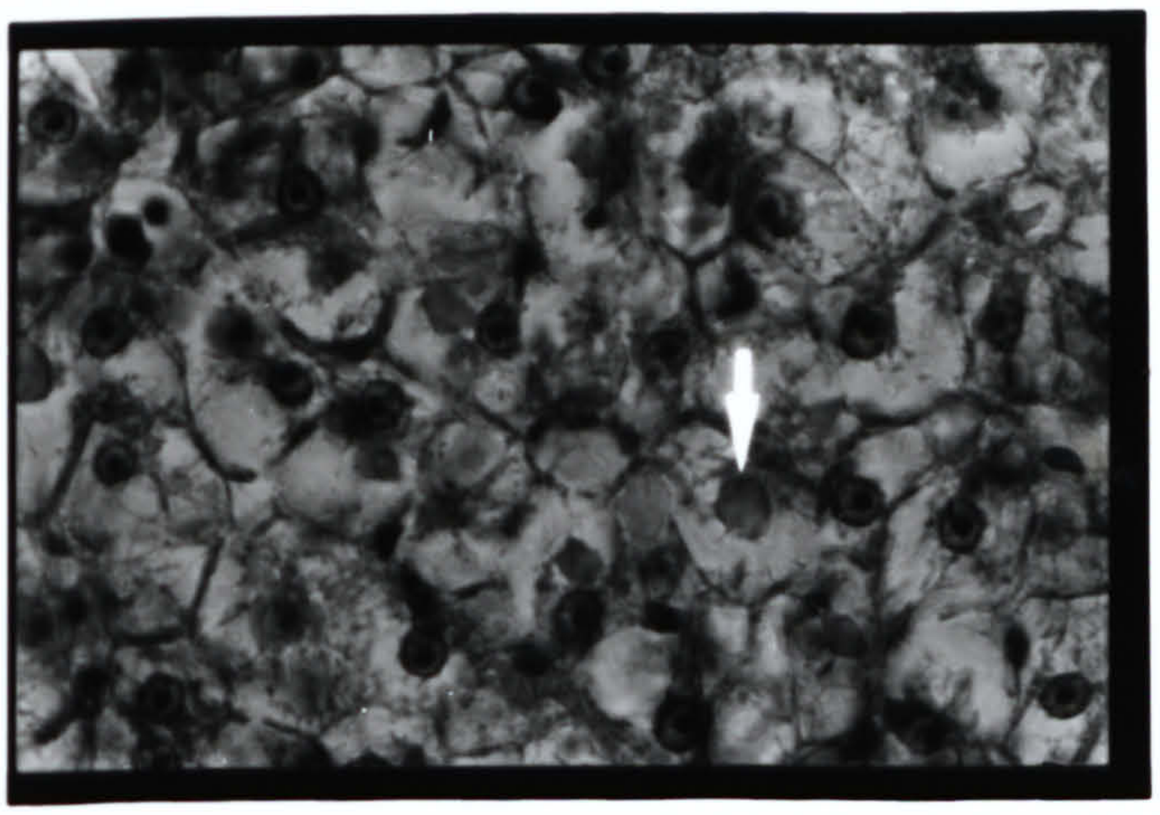


Figure 7.6 : High power view of hepatocytes with hyaline droplets (arrowed) in their cytoplasm (HE staining, x 1000).

7.3.6 Haematological effects

Tilapia and catfish fed T-100 diet and C-100 diet, respectively, had lower ($P<0.05$) haematocrit and haemoglobin contents than catfish fed other diets (Table 7.12), but MCHC values showed no differences among treatments.

Table 7.12 : Blood parameters of ***O. niloticus*** and ***C. gariepinus***[1] fed diets containing increasing levels of co-dried FS:SBF blend.

	Haematocrit (Hc, %)	Haemoglobin (Hb, g.100ml^{-1})	MCHC[2] (%)
Initial (tilapia)	23.1	6.2	26.84
T-0	31.5	8.5	26.98
T-100	25.9	7.1	27.41
Initial (catfish)	22.8	6.3	27.63
C-0	31.9	8.3	26.02
C-100	27.2	7.4	27.21

[1] all values represent mean of three replicates.

[2] mean cell haemoglobin concentration = (Hb/Hc) x 10^2

CHAPTER 8 : EVALUATION OF DIFFERENT TYPES OF DRIED TILAPIA SILAGE IN DRY FISH DIETS

The experiments described in this chapter will be published in

Israeli Journal of Aquaculture, 46 (1994): in press.

Bioresource Technology, 50 (1995): in press.

8.1 INTRODUCTION

Intensive fish culture relies almost entirely on the use of complete feeds. There has been little interest, however, in the commercial production of complete feeds for low value fish. This is mainly attributable to the high cost of the feed in relation to the low market value of such species. Since feed costs account for up to 60% of the total operating costs, intensive fish culture is often restricted, for economic reasons, to those species which command a higher market price. Feed ingredients are selected mainly on the basis of cost, chemical composition, nutritional value, availability and physical properties (HARDY,1989).

Efficient utilization of feedstuffs requires information on their chemical characteristics and nutritional value. One of the most important aspects in evaluating biological effectiveness of a feedstuff is the determination of its digestibility, being the ability of the animal to digest and absorb the nutrients fed. Proximate composition and digestibility of feedstuffs are

essential for feed formulation on a least-cost basis; hence provide evaluations of nutritional quality and suitability of feeds (TACON,1990). Aquaculture nutrition research typically uses long-term experimental treatments, and food utilization efficiencies are statistically evaluated; after which the suitability of a formulated diet is assessed with regards to significant differences in rates of growth and survival. Growth differences in fish evoked by different dietary regimens are the ultimate expression of differences in:

(a) food acceptance and food intake,

(b) food digestion and absorption in the gut,

(c) assimilation and metabolism of absorbed nutrients in fish tissues, and

(d) presence of toxins or antinutrients in the feedstuffs.

8.1.1 Objectives

Further to the study in the preceding chapter, this study evaluates the growth response, feed conversion, protein utilization and digestibility by ***Oreochromis niloticus*** and ***Clarias gariepinus*** fed dry diets containing fermented tilapia silages, co-dried with different filler materials (soybean meal, poultry by-product meal, hydrolysed feather meal or meat and bone meal).

8.2 MATERIALS AND METHODS

8.2.1 Experimental systems

The experimental systems used for growth and digestibility trials are as described in **Chapter 6.2.2.** Water samples were taken weekly to determine pH, total ammonia, total nitrite and dissolved oxygen (DO_2) concentration (Appendix 3).

8.2.2 Fermented tilapia silage

Fermented tilapia silage was prepared as described in **Chapter 7.2.3** using the pre-fermented ***Lactobacillus plantarum*** starter culture, molasses and minced tilapia mixture (2:5:100), and incubated anaerobically at 30°C inside tightly sealed 5-L plastic containers for 7 days. The wet tilapia silage was blended (1:1, dry weight basis) with soybean meal (SBM), poultry by-product meal (PBM), hydrolysed feather meal (HFM) or meat and bone meal (MBM)(CHETTLES Ltd., Nottingham) and oven-dried in an oven at 45°C for 48 h. The proximate composition, gross energy content and amino acid composition of the co-dried tilapia silage:filler blends are presented in Tables 8.1 and 8.2, respectively.

Table 8.1 : Proximate composition (g.100g^{-1}) and gross energy content of co-dried tilapia silage:filler blends.

	Dry matter	Crude protein	Crude lipid	Ash	Gross energy (kcal.g^{-1} DM)
FS:SBM	91.5	49.8	7.1	11.6	4.30
FS:PBM	96.3	58.5	12.4	16.2	4.68
FS:HFM	91.7	68.0	6.8	9.7	4.95
FS:MBM	94.8	54.6	10.5	23.5	4.28

FS:SBM = fish silage:soybean meal;
FS:PBM = fish silage:poultry by-product meal;
FS:HFM = fish silage:hydrolysed feather meal;
FS:MBM = fish silage:meat and bone meal.

Table 8.2 : Amino acid composition (g.100g^{-1} protein) of co-dried tilapia silage:filler blends.

	FS:SBM	FS:PBM	FS:HFM	FS:MBM
Arginine	6.40	6.22	7.16	6.48
Histidine	2.62	2.29	2.01	2.39
Isoleucine	4.20	3.94	4.32	3.55
Leucine	6.70	6.46	7.13	6.09
Lysine	6.19	5.67	4.56	6.07
Methionine	1.65	1.97	1.40	1.72
Cystine	1.36	1.31	2.44	1.02
Phenylalanine	3.98	3.18	3.41	3.30
Tyrosine	2.88	2.20	2.76	2.18
Threonine	3.75	3.55	4.24	3.53
Tryptophan	1.53	1.22	1.13	1.12
Valine	4.08	4.26	5.64	4.26

8.2.3 Experimental diets

Four dry isocaloric diets were formulated for tilapia and catfish to contain 30% and 40% protein in dry matter, respectively. Each of the different co-dried tilapia silage:filler blends was incorporated as protein supplement, supplying 50% of the dietary protein in the dry matter (Tables 8.3 and 8.4). A control diet (T-RD) containing fish meal and soybean meal as the protein source were also formulated for tilapia while a commercial trout diet (FULMAR FEEDS, Glasgow) was used as a reference diet (C-RD) for the catfish, the protein source being a combination of fish meal, poultry meal and feather meal. The diets were prepared as described in **Chapter 7.2.4** and were stored at -20°C in air-tight sealed polyethylene bags. Small portions of each diet were thawed prior to daily feeding.

8.2.4 Analytical methods

Moisture, protein, lipid, fibre, ash and gross energy content of triplicate samples of diets were determined using AOAC (1990) methods. Amino acid composition of diets were determined as described in **Chapter 7.2.5.** The proximate composition, energy content, properties and amino acid composition of the diets are presented in Tables 8.5, 8.6, 8.7 and 8.8.

Table 8.3 : Formulation (g.100g^{-1} DM) of dry diets for ***O. niloticus***.

	Tilapia diets				
	T-SBM	T-PBM	T-HFM	T-MBM	T-RD
Fish meal	10.0	10.0	10.0	10.0	15.0
Soybean meal	15.0	15.0	15.0	15.0	35.0
FS:SBM	30.0	-	-	-	-
FS:PBM	-	25.5	-	-	-
FS:HFM	-	-	22.0	-	-
FS:MBM	-	-	-	27.5	-
Corn meal	23.0	26.0	28.0	26.0	28.0
Cellulose flour	12.0	13.5	15.0	11.5	12.0
Common ingredients[1]	10.0	10.0	10.0	10.0	10.0

[1] cod liver oil, 2.5%; corn oil, 2.5%; carboxymethyl cellulose, 2%; mineral mix[a], 2%; vitamin mix[b], 1%; chromic III oxide, 1%.

[a] g.kg^{-1}: $MgSO_4.7H_2O$, 5.10; NaCl, 2.40; KCl, 2.00; $FeSO_4.7H_2O$, 1.00; $ZnSO_4.H_2O$, 0.22; $CuSO_4.5H_2O$, 0.0314; $MnSO_4.4H_2O$, 0.1015; $CoSO_4.7H_2O$, 0.0191; $CaIO_3.6H_2O$, 0.0118; $CrCl_3.6H_2O$, 0.0051.

[b] mg.kg^{-1}: thiamine HCl, 50; riboflavin, 50; calcium pantothenate, 100; niacin, 200; pyridoxine HCl, 40; biotin, 6; folic acid, 15; cyanocobalamin, 0.1; inositol, 200; ascorbic acid, 1000; choline chloride, 4000; menadione, 40; α-tocopherol acetate, 400; para-amino benzoic acid, 50; vitamin A acetate, 2000 IU; vitamin D_3, 1000 IU; dilutant, α-cellulose.

Table 8.4 : Formulation (g.100g^{-1} DM) of dry diets for ***C. gariepinus***.

	Catfish diets			
	C-SBM	C-PBM	C-HFM	C-MBM
Fish meal	15.0	15.0	15.0	15.0
Soybean meal	20.0	20.0	20.0	20.0
FS:SBM	40.0	-	-	-
FS:PBM	-	34.5	-	-
FS:HFM	-	-	29.5	-
FS:MBM	-	-	-	36.5
Corn meal	13.0	16.0	20.0	17.0
Cellulose flour	2.0	4.5	5.5	1.5
Common ingredients[1]	10.0	10.0	10.0	10.0

[1] cod liver oil, 2.5%; corn oil, 2.5%; carboxymethyl cellulose, 2%; mineral mix[a], 2%; vitamin mix[b], 1%; chromic III oxide, 1%.

[a] g.kg^{-1}: $MgSO_4.7H_2O$, 5.10; NaCl, 2.40; KCl, 2.00; $FeSO_4.7H_2O$, 1.00; $ZnSO_4.H_2O$, 0.22; $CuSO_4.5H_2O$, 0.0314; $MnSO_4.4H_2O$, 0.1015; $CoSO_4.7H_2O$, 0.0191; $CaIO_3.6H_2O$, 0.0118; $CrCl_3.6H_2O$, 0.0051.

[b] mg.kg^{-1}: thiamine HCl, 50; riboflavin, 50; calcium pantothenate, 100; niacin, 200; pyridoxine HCl, 40; biotin, 6; folic acid, 15; cyanocobalamin, 0.1; inositol, 200; ascorbic acid, 1000; choline chloride, 4000; menadione, 40; α-tocopherol acetate, 400; para-amino benzoic acid, 50; vitamin A acetate, 2000 IU; vitamin D_3, 1000 IU; dilutant, α-cellulose.

Table 8.5 : Properties, proximate composition (g.100g^{-1} DM) and gross energy content (kcal.g^{-1} DM) of tilapia diets.

	T-SBM	T-PBM	T-HFM	T-MBM	T-RD
Moisture	8.25	8.09	8.20	8.14	8.18
Crude protein	30.37	29.79	29.91	29.85	30.16
Crude lipid	7.38	7.90	7.25	7.44	7.13
Crude fibre	3.51	3.26	3.18	3.56	3.05
Ash	6.29	6.35	6.10	6.53	6.15
Gross energy	3.82	3.84	3.81	3.82	3.83
pH	6.5	6.5	6.3	6.6	6.4
Water stability (% LDM)	5.6	5.4	5.2	5.5	5.6

[1] all values represent mean of three replicates.

Table 8.6 : Properties, proximate composition (g.100g^{-1} DM) and gross energy content (kcal.g^{-1} DM) of catfish diets.

	C-SBM	C-PBM	C-HFM	C-MBM	C-RD
Moisture	8.40	8.60	8.28	8.34	8.60
Crude protein	39.71	39.99	39.47	39.59	40.30
Crude lipid	7.8	8.54	7.83	7.92	12.0
Crude fibre	4.5	4.47	4.28	4.85	4.6
Ash	9.5	9.36	9.43	10.07	9.6
Gross energy	4.30	4.31	4.32	4.30	4.41
pH	6.3	6.5	6.4	6.4	6.6
Water stability (% LDM)	5.2	5.6	5.3	5.6	5.4

[1] all values represent mean of three replicates.

Table 8.7 : Amino acid composition (g.100g^{-1} protein) of tilapia diets.

	T-SBM	T-PBM	T-HFM	T-MBM	T-RD
Arginine	6.7	6.6	7.6	6.7	6.9
Histidine	2.6	2.4	2.3	2.5	2.6
Isoleucine	4.5	4.4	4.9	4.2	4.7
Leucine	7.9	7.8	8.7	7.6	8.4
Lysine	6.5	4.6	5.6	6.4	6.9
Methionine	2.0	2.1	1.8	2.0	2.2
Cystine	1.5	1.5	2.3	1.3	1.6
Phenylalanine	4.4	4.1	4.4	4.1	4.7
Tyrosine	3.2	2.9	3.3	2.8	3.3
Threonine	4.0	3.9	4.6	3.9	4.2
Tryptophan	1.4	1.2	1.2	1.2	1.3
Valine	5.0	5.1	6.3	5.1	5.7

Table 8.8 : Amino acid composition (g.100g^{-1} protein) of catfish diets.

	C-SBM	C-PBM	C-HFM	C-MBM	C-RD
Arginine	6.8	6.6	7.7	6.7	6.8
Histidine	2.6	2.4	2.3	2.4	2.5
Isoleucine	4.5	4.4	4.9	4.2	4.6
Leucine	7.6	7.5	8.4	7.2	7.9
Lysine	6.7	6.3	5.8	6.5	6.9
Methionine	2.0	2.1	1.8	2.0	2.2
Cystine	1.5	1.5	2.3	1.3	1.5
Phenylalanine	4.3	4.0	4.3	4.0	4.5
Tyrosine	3.2	2.8	3.2	2.8	3.2
Threonine	4.0	3.9	4.5	3.8	4.1
Tryptophan	1.4	1.2	1.2	1.2	1.3
Valine	5.0	5.1	6.4	5.0	5.5

8.2.5 Growth and digestibility trials

Juveniles of ***O. niloticus*** and ***C. gariepinus***, each taken from the same hatching were fed trout pellets (EWOS-BAKER OMEGA 3, 49% protein) to satiation during a 7-day acclimation period as described in **Chapter 7.2.6**. Growth and digestibility trials were conducted as described in **Chapter 7.2.6** and **7.2.7**. using the different co-dried fish silage:filler blends as dietary treatments. Growth and feed utilization data were analysed as described in **Chapter 6.2.6**. Apparent protein and energy digestibility were evaluated as described in **Chapter 7.2.7**.

8.2.6 Carcass analysis

(a) Carcass composition

Nine fish taken at the start and three fish per tank taken at the end of the growth trials, were homogenised and analysed for moisture, protein, lipid and ash contents (AOAC,1990).

(b) Hepatosomatic index

Six fish taken at the start and six fish taken from each treatment at the end of the growth trials were anaesthetized with 60-70 $mg.L^{-1}$ benzocaine (ROSS & GEDDES,1979), and weighed individually. Their liver were removed and weighed individually. Hepatosomatic index (HSI) was calculated as follows:

$$HSI = (\text{liver weight/body weight}) \times 10^2$$

(c) Histological and haematological examination

Six fish taken at the start and six fish taken from each treatment at the end of the growth trials were anaesthetized with benzocaine and weighed individually. The methods used for histological and haematological examinations of fish are as described in **Chapter 7.2.8.**

8.2.9 Statistical methods

Statistical comparisons of the results were made by one-way analysis of variance (ANOVA). All % data were square root arc-sine transformed prior to analysis. Differences in mean values were determined using Duncan's new multiple range test at 5% probability. Standard error was calculated to identify the range of mean values (ZAR,1984) using Statgraphics Software (Statistical Graphics Corporation).

8.3 RESULTS AND DISCUSSION

8.3.1 Experimental diets

The pH of all experimental diets were 6.3-6.5 and their stability in water was high, indicated by low % LDM (Tables 8.5 and 8.6). Water stability of all diets was suitable for fast and competitive feeders such as ***O. niloticus*** and ***C. gariepinus*** as it ensured optimum feed uptake before disintegration of pellets. Few variations occurred between moisture, protein, lipid and fibre contents of diets. Fish became accustomed to the diets within the first week of growth trials. Feed consumption was not affected by the filler material used, which might have been due to the presence of free amino acids and peptides acting as attractants. Nitrogenous compounds from fish wastes/by-products may be feed attractants, promoting consumption and consequently growth (MEYERS,1987; DANIEL & BAYER,1989; MUNDHEIM & OPSTVEDT,1993).

Low mortality (<10%) occurred during the acclimation period and was replaced with fish of similar size. No mortality occurred throughout the duration of either tilapia or catfish growth trials. High fish survival was due to conducive water quality conditions in the experimental systems, which were within tolerance range for both ***O. niloticus*** (BALARIN & HATTON, 1979) and ***C. gariepinus*** (VIVEEN *et al.*,1985).

8.3.2 Growth performance and Protein utilization

The growth responses by ***O. niloticus*** and ***C. gariepinus***, expressed as MWG, ADG and SGR are presented in Tables 8.9 and 8.10, respectively. Generally, the control (T-RD) and reference (C-RD) diet treatments yielded numerically, but not statistically significant, increases in both weight gains and feed efficiency compared with the silage diets. A plot of growth of fish versus time supported these results (Figures 8.1, 8.2). These growth performance values showed significant differences ($P<0.05$) among the treatments and the lowest values occurred in T-HFM and C-HFM treatments. For ***C. gariepinus***, FCR values obtained in the C-RD treatment were better ($P<0.05$) than in other treatments (Table 8.10), while ***O. niloticus*** in all the treatments showed no significant differences ($P>0.05$)(Table 8.9). A similar trend occurred with the PER values obtained for both experimental fishes, but PPV values showed significant differences ($P<0.05$) among the treatments.

No differences occurred in the $ADC_{protein}$ for tilapias but with catfish, the values were lower ($P<0.05$). Differences in ADC_{energy} values among the treatments were significant ($P<0.05$) with the values obtained from both T-SBM and C-SBM treatments being the lowest.

Table 8.9 : Summary of growth performance and protein utilization[1] by ***O. niloticus*** fed diets containing different co-dried tilapia silage:filler blends.

	Initial wt.[2] (g)	Final wt. (g)	MWG (g)	ADG ($g.day^{-1}$)	SGR ($\%.day^{-1}$)	FCR	PER	PPV (%)	$ADC_{protein}$ (%)	ADC_{energy} (%)
T-SBM	8.2	36.3	28.1ab	0.40ab	2.13bc	1.74a	1.90a	28.13ab	83.6a	76.9a
T-PBM	8.2	37.0	28.8ab	0.41ab	2.15ab	1.74a	1.94a	29.07ab	86.6a	83.4b
T-HFM	8.6	35.2	26.6b	0.38c	2.01c	1.77a	1.89a	27.55b	80.7a	84.7b
T-MBM	8.0	37.6	29.6ab	0.42ab	2.20ab	1.70a	1.97a	29.28ab	84.9a	81.1ab
T-RD	8.1	39.8	31.7a	0.45a	2.27a	1.64a	2.02a	30.38a	84.1a	80.2ab
SEM[3]	0.12	1.13	1.05	0.01	0.03	0.03	0.04	0.59	1.55	1.25

[1] all values represent mean of three replicates.

[2] no significant differences were found among treatment means ($P>0.05$)

[3] pooled standard error calculated from residual mean square in the ANOVA test.

Table 8.10 : Summary of growth performance and protein utilization[1] by ***C. gariepinus*** fed diets containing different co-dried tilapia silage:filler blends.

	Initial wt.[2] (g)	Final wt. (g)	MWG (g)	ADG ($g.day^{-1}$)	SGR ($\%.day^{-1}$)	FCR	PER	PPV (%)	$ADC_{protein}$ (%)	ADC_{energy} (%)
C-SBM	11.5	59.7	48.2bc	0.69bc	2.35bc	1.58b	1.60b	24.64c	85.4a	74.5b
C-PBM	12.1	64.2	52.1b	0.74b	2.38b	1.59b	1.58b	26.30b	86.7a	80.1a
C-HFM	11.7	57.7	46.0c	0.66c	2.28c	1.56b	1.60b	25.09bc	79.4b	78.7ab
C-MBM	12.1	62.8	50.7b	0.72bc	2.36bc	1.59b	1.59b	26.23b	87.2a	79.7a
C-RD	11.6	70.8	59.2a	0.85a	2.59a	1.45a	1.71a	29.92a	-	-
SEM[3]	0.09	1.14	1.07	0.02	0.02	0.02	0.02	0.33	0.96	1.03

[1] all values represent mean of three replicates.

[2] no significant differences were found among treatment means ($P>0.05$)

[3] pooled standard error calculated from residual mean square in the ANOVA test.

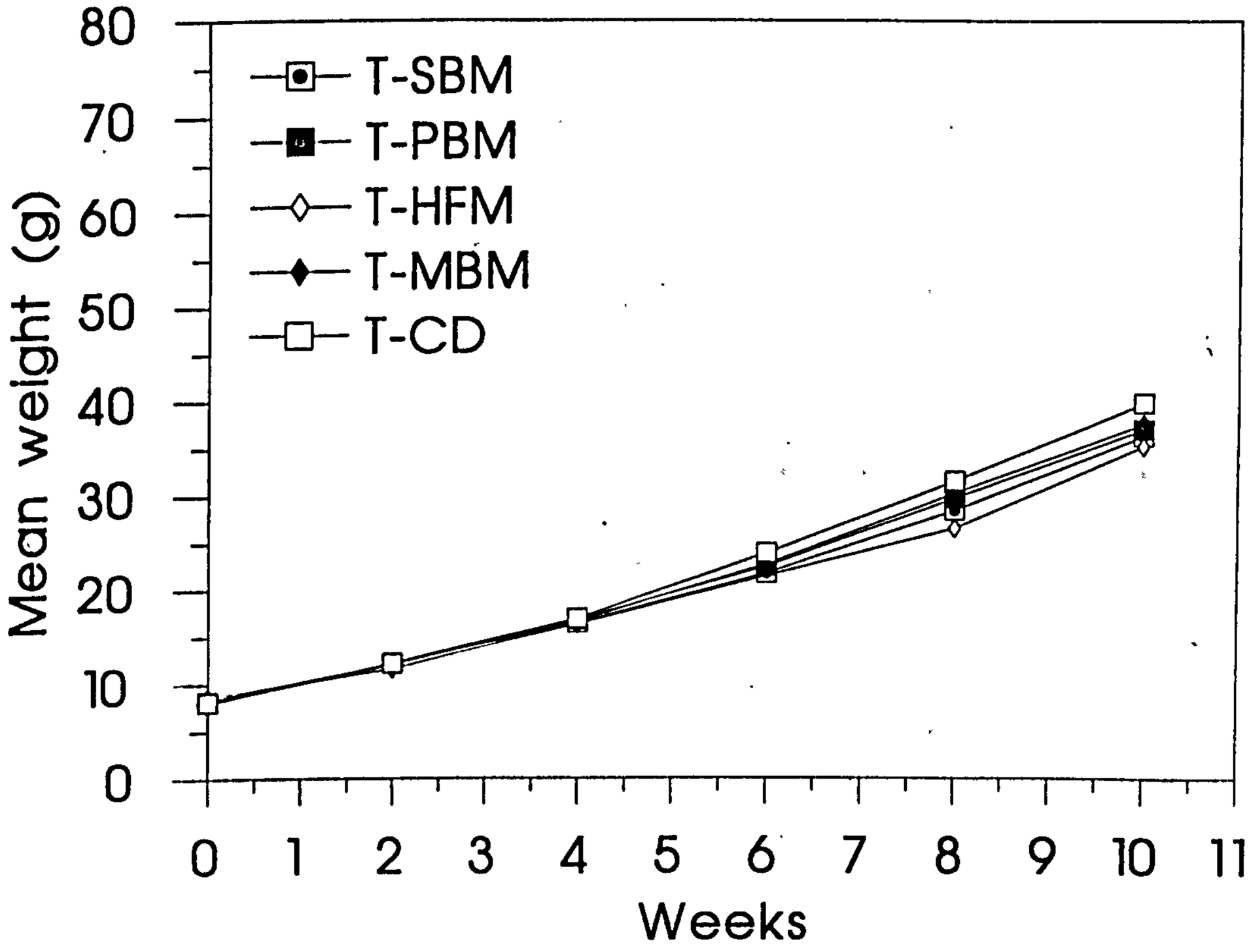


Figure 8.1 : Growth of ***O. niloticus*** fed diets containing different co-dried tilapia silage:filler blends.

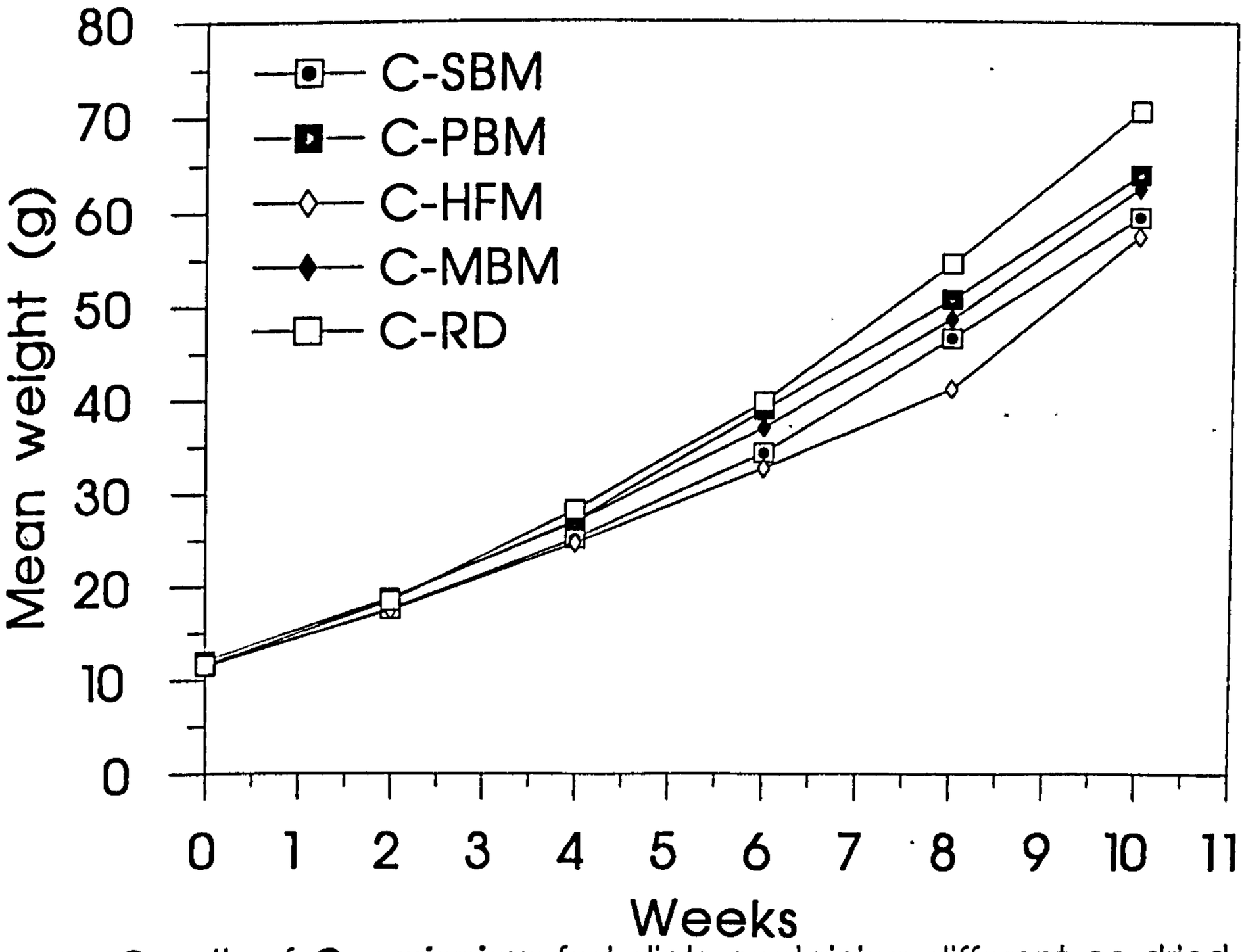


Figure 8.2 : Growth of ***C. gariepinus*** fed diets containing different co-dried tilapia silage:filler blends.

The relatively poor growth performance in the T-HFM and C-HFM treatments may be attributed to many factors. Hydrolysed feather meal has a lower nutrient digestibility for fish and its amino acid profile is inadequate, being deficient in methionine, lysine and histidine (NRC, 1983). However, this was not reflected in the amino acid composition of the experimental diets which indicated that the requirements of ***O. niloticus*** and ***C. gariepinus*** were exceeded (Tables 8.9 and 8.10). This implies that the poor performance of fish fed diets containing FS:HFM may have been caused by factors other than specific amino acid deficiency.

Apart from co-dried fish-silage products being sources of protein, they also provide dietary energy (DISNEY *et al.*,1978). The differences in performance of fish among treatments probably represents the corresponding differences in apparent digestibility of protein and/or energy component of the filler used in the silage blend. It was apparent from $ADC_{protein}$ and ADC_{energy} values (Tables 8.9 and 8.10), that the protein and energy content of FS:HFM and FS:SBM, respectively, were less digestible to catfish than other blends which reduced their nutritional quality and resulted in relatively poor performance compared with fish in the other treatments.

Poor digestibility of protein causes poor protein utilization, hence the differences in growth probably represent the corresponding difference in apparent digestibility of protein of the filler material used in the silage

blend or the differences in intake due to palatability of diets. While hydrolysed feather meal is considered as an inferior source of protein for warmwater fish because of its poor digestibility and amino acid profile (TACON,1993), soybean meal has a good amino acid profile and its protein is digestible by tilapia and catfish (HANLEY,1987; SHIAU *et al.*, 1987; BALOGUN & OLOGHOBO,1989), but it has a lower digestible energy than fish meal.

HARDY *et al.*(1984) found that growth rates and feed efficiencies were compromised in rainbow trout, ***Salmo gairdneri***, which were fed dry diets containing acid silage in which a mixture of soybean-feather meals was used as filler, when compared with trout fed diets containing liquefied fish or fish meal as protein sources. They attributed this, among other factors, to lower digestible protein and energy of the filler. Addition of oil to the present diet formulations to increase the energy content might correct this anomaly and improve weight gains but care must be taken not to compromise water stability of the diets. Similarly, KLING *et al.*(1994) reported that Atlantic salmon, ***Salmo salar***, fed diets containing co-dried blends of acid ensiled dogfish processing waste and soybean meal were significantly smaller than those receiving diets containing dried blends of silage and poultry meal or fish meal; but they found no differences in feed conversions among the treatments.

Fish by-products vary considerably in nutrient composition and nutritive value. Variations are due to differences in types of fish used, parts of fish

used, season of capture, and processing methods (OCKERMAN,1992). Protein content of co-dried tilapia silages in this study ranged from 49.8-68.0% of dry matter, and the amino acid profile was comparable with that of commercially prepared fish meal. This suggests that they could be incorporated into fish diets as the sole protein source. Various reasons may explain the good feed utilization values obtained in the present study including:

(a) the diets were nutritionally adequate, particularly in that the essential amino acid content, and the protein contents were highly digestible;

(b) there were minimal loss of nutrients because the diets were water stable and rapidly consumed;

(c) both tilapia and catfish are known for efficient conversion of feed into fish biomass; and

(d) the presence of rapidly assimilated pre-digested proteins.

Acid-ensiled fish and fish by-products co-dried with protein feedstuffs, used as protein sources in moist or dry aquaculture diets, were reported to have nutritional value comparable with that of commercial fish meal but there have been no studies evaluating the suitability of dried fermented silage in dry aquaculture diets. In this study, co-dried fermented tilapia silage was suitable as a protein source in dry diets for ***O. niloticus*** (omnivore) and ***C. gariepinus*** (carnivore).

The results show that a well-balanced dry feed based on co-dried fermented silage is as efficient in supporting growth as a dry fish meal-based diet. The fact that co-dried fermented silage protein provided 50% of the total dietary protein means that feed costs in aquaculture production can be significantly reduced, if sufficient waste/trash fish are available. Hydrolysed protein from fermented silage can be preserved by blending and co-drying with soybean meal, poultry by-product meal, hydrolysed feather meal or meat and bone meal and each blend has a high protein content and good amino acid profile. The blends had no adverse effects on the consistency of the diet, diet acceptance by fish, nutrient and energy digestibility, as well as the growth, quality and health of fish.

8.3.3 Carcass composition

Carcass composition and hepatosomatic index of ***O. niloticus*** and ***C. gariepinus*** at the begining and end of the growth trials are presented in Tables 8.11 and 8.12. Carcass composition of both fishes was unaffected by silage blend and there were no effects of feeding silage diets on hepatosomatic index.

Table 8.11 : Carcass composition of ***O. niloticus*** fed diets containing different co-dried tilapia silage:filler blends.

	Moisture	Crude protein	Crude lipid	Ash	HSI[2]
Initial	78.23	12.87	4.20	4.02	1.48
T-SBM	76.89	14.38	5.11	3.47	1.69
T-PBM	76.41	14.55	5.29	3.52	1.68
T-HFM	76.84	14.09	4.93	3.84	1.75
T-MBM	76.55	14.43	5.06	3.80	1.62
T-RD	76.03	14.60	5.58	3.61	1.67

[1] all values represent mean of three replicates.

[2] hepatosomatic index.

Table 8.12 : Carcass composition of ***C. gariepinus*** fed diets containing different co-dried tilapia silage:filler blends.

	Moisture	Crude protein	Crude lipid	Ash	HSI[2]
Initial	77.54	14.09	4.86	2.97	1.72
C-SBM	76.09	15.40	5.20	2.61	1.85
C-PBM	75.80	16.22	5.27	2.53	1.89
C-HFM	76.32	15.59	5.08	2.41	1.92
C-MBM	75.58	16.07	5.26	2.58	1.87
C-RD	74.43	16.90	6.10	2.40	1.94

[1] all values represent mean of three replicates.

[2] hepatosomatic index.

8.3.4 Histological and haematological effects

Histological examination of exocrine pancreas, liver and intestine tissues did not show any lesions suggestive of nutritional imbalance. The intestines of catfish fed C-HFM diet had reduced mucus lining. Haematocrit and haemoglobin content as well as the mean cell haemoglobin concentration values showed no significant differences among the silage treatments (Tables 8.13 and 8.14). Thus, fish growth in the silage treatments was not compromised by the health of the fish.

ADEJUMO (1987) and LIE *et al.*(1988) found no metabolic disorders or reduced health, based on haematological tests and analyses of serum constituents, in ***O. niloticus*** or ***Salmo salar*** fed diets containing co-dried acid silage. Similarly, YONE *et al.*(1986b) and HOSSAIN *et al.*(1988) found no effects of feeding red sea bream (***Chrysophyrys major***) with mould-fermented scrap meals on proximate and fatty acid composition of muscle and liver, haematological characteristics, and chemical components in plasma.

Table 8.13 : Blood parameters of ***O. niloticus***[1] fed diets containing different co-dried tilapia silage:filler blends.

	Haematocrit (Hc, %)	Haemoglobin (Hb, mg.100ml^{-1})	MCHC[2] (%)
Initial	29.6	8.0	27.03
T-SBM	31.7	8.9	28.08
T-PBM	29.3	8.5	29.01
T-HFM	28.5	8.2	28.77
T-MBM	29.4	8.8	29.93
T-RD	28.8	8.7	30.21

[1] all values represent mean of three replicates. haematocrit (%)

[2] mean cell haemoglobin concentration = (Hb/Hc) x 10^2

Table 8.14 : Blood parameters of ***C. gariepinus***[1] fed diets containing different co-dried tilapia silage:filler blends.

	Haematocrit (Hc, %)	Haemoglobin (Hb, mg.100ml^{-1})	MCHC[2] (%)
Initial	31.9	8.5	26.65
C-SBM	30.8	8.6	27.92
C-PBM	31.5	8.4	26.67
C-HFM	32.1	8.9	27.73
C-MBM	30.4	8.4	27.63
C-RD	32.4	9.1	28.09

[1] all values represent mean of three replicates. haematocrit (%)

[2] mean cell haemoglobin concentration = (Hb/Hc) x 10^2

CHAPTER 9 : ECONOMICS OF FISH SILAGE PRODUCTION AND UTILIZATION

"To what purpose is this waste"

St. Matthew, 26:8

The BIBLE (KJV)

9.1 ECONOMICS OF FISH SILAGE PRODUCTION

The rationale for fish silage production assumes the use of waste fish, which should be available at low cost. Production also depends on access to other raw materials and continuity in processing. In some countries, waste fish is converted into fish meal but the scale of operation and capital costs involved restrict such a processing method. A flexible, low-cost method capable of handling regular but variable quantities of waste fish at relatively low levels of output is often preferred. Ensiling of waste fish therefore offers a good alternative, even to prevent losses due to rapid spoilage and insect infestation under non-chilled conditions (BROWN & SUMNER,1985).

Ensiling requires less capital outlay and the scale of operation is easily adjusted to the supply of raw materials (POTTER *et al.*,1978) and is considered attractive in small fishing communities or scattered fish landing areas remote from fish meal factories. It is also adaptable for use

on board fishing vessels where by-catch and fish viscera occur in large quantities during fishing/shrimping operations. Since the equipment required for fish silage production and storage is simple and relatively cheap, its production can be economical either on a small or large scale (RAA *et al.*,1983) and therefore much cheaper than either freezing or conversion to fish meal (ANDERSON,1989). AAGARD *et al.*(1980) and BALOGUN & OYEYEMI (1986) noted that producing acid silage on a pilot scale gave a more cost-efficient product than fish meal produced on a similar scale.

DISNEY & JAMES (1980) and ARASON *et al.*(1990) concluded that the simplicity of fish silage production, low capital investment, energy saving and environmental benefit, altogether favour fish silage as being economically suitable for commercial application. KOMPIANG *et al.* (1980a) and ASGARD & AUSTRENG (1981) noted that the cost benefits of fish silage has two perspectives: its application as a feedstuff in animal (including fish) feeds and its potential as a means of using fish wastes to reduce possible environmental pollution. It has also been used as fertilizer for vegetables (JANGAARD,1987; GAO *et al.*,1992; LO *et al.*,1993b).

Apart from the type, source and cost of raw materials used in fish silage production, the bulky liquid product makes transportation and storage uneconomical. Fish silage has been dried in a drum dryer and successfully used in fish diets (HARDY *et al.*,1984), but drum drying proved uneconomical. Co-drying fish silage with an added small percentage of

other dry commodities (filler material or feedstuffs) alleviates both problems, but the choice of drying method and type of filler also determine the economic benefits of using fish silage for animal feeding.

9.2 ECONOMICS OF FISH SILAGE UTILIZATION

Fish feeding constitutes one of the most important factors affecting the production and economic results of aquaculture. A major objective in fish nutrition is the formulation of diets which give fast growth and optimal fish health and product quality at the lowest possible cost. According to URBAN & PRUDER (1991) the benefits derived from least cost formulation in aquaculture diets relies on improved reliability of the food supply, predictability of diet composition and reduced feed cost.

Experimental feeds or supplements can only be applied to commercial aquaculture production if an expectation for increased profit exists. Economic models are therefore usually developed from experimentally derived values for feeding cost, growth rate, growth efficiency and mortality. SHANG (1981) identified five main factors which determine the profitability of using a particular diet, namely (a) revenue, (b) variable cost, (c) the fish yield, (d) desired production time, and (e) the fixed cost.

The primary economic criterion for selecting diet/ingredient however remains the need to maximise profit, because reducing food cost may

decrease conversion efficiencies, growth rate or increased mortality, resulting in decreased profit (URBAN & PRUDER, 1991). Feeding cost is a main component of variable cost and is a function of (a) price and proportion of the diet components, (b) quantity of food required by the cultivated animal/fish, (c) labour cost of feeding the diets, and (d) other costs necessary to produce or obtain the diet.

Feeding cost accounts for up to 40% of total cost of aquaculture production (SHANG,1981) and reports of low food costs may not necessarily correspond to low feeding cost. The economic viability of fish silage production, being location and time specific, needs local study; hence it is impossible to generalise on the economics of artisanal production of silage-based feeds. However, considering that there is no large capital expenditure, the savings on unit feed costs achieved by small-scale on-farm fish silage-based feed production would be considerable. Also, existing labour on a small farm may be able to absorb the extra work load of making feeds, keeping operational costs low. Farm-made compound feeds provide a potentially cheaper alternative to the purchase of commercially manufactured products, where they are locally available.

An economic analysis of results from feeding silage diets to tilapia and catfish in this study was not conducted taking cognisance of the fact that there is a considerable difference between the laboratory experiments described in the preceding chapters and the operation of

a commercially viable enterprise. The pertinent question therefore remains as - is it economical to feed diets containing dried fermented fish silage compared with conventional prepared diets?

The economics of fish waste or fish silage utilization depends on local conditions and are dependent on several factors such as: (a) amount and continuity of available fish wastes, (b) sanitary/septic quality, (c) nutrient quality, (d) handling, (e) transportation, (f) storage and (g) removal or reduction of moisture (drying). While it may be possible to obtain market prices for the conventional ingredients, that of fish silage is not easily assessed because of differences in availability, technology and scale of production.

The fact that co-dried fermented silage can provide 50% of the total dietary protein means that the cost of production can be significantly reduced, if sufficient low cost trash fish being available. The use of dried fish silage diets could mean a lower price of fish and therefore is particularly useful for tropical developing countries. The results of this study cannot be directly extrapolated for commercial evaluation of inclusion of fermented silage in fish diets without a detailed cost analysis; and do not necessarily prove that silage can be used as an economical supplement for commercial fish production. The feeding cost method represents an improvement on providing no economic analysis or evaluating food cost alone, but it remains inadequate as it merely provides basic information required for the next step - that silage be

tested in commercial-scale trials to see whether or not profitability is improved.

9.3 MODEL OF FISH SILAGE PRODUCTION COSTS IN NIGERIA

This evaluation is based on the supply of fish wastes at Igbokoda Industrial Fishing Terminal (Ondo State, Nigeria) where 250 kg of waste fish (mainly croakers and mackerels) are generated per day for 200 days per year. The production cost of liquid fish silage and dried fish silage products are computed from current market prices of materials, labour and transport service in Nigeria obtained from the Nigerian Office of Statistics (1993). The cost of computation was based on the assumption that no capital investment was incurred except that of purchasing the silage containers.

9.3.1 Liquid silage

The cost of producing liquid silage from 1 tonne of fish wastes is presented in Table 9.1, and it shows that lactic acid fermented silage is about 25% cheaper than its corresponding acid (2.5% formic acid + 2.5% hydrochloric acid) silage.

Table 9.1 : Production costs[1] for producing fermented and acid silages from 1 tonne of fish wastes.

	Fermented silage	Acid silage
Plastic containers (10 nos x 250-L)	2,000	2,000
Fish wastes (1 tonne mixed species)	2,000	2,000
Formic acid (250 L)	-	1,000
Hydrochloric acid (250 L)	-	750
Molasses[2]	100	-
Transport	450	450
Labour	1,500	1,500
TOTAL	N 6,050 £ 151.25	N 7,800 £ 195

[1] all prices are quoted in Naira (Nigerian currency)
Current exchange rate: £ = N40 or $ = 25

[2] obtainable free of charge but commercially collected volumes are priced.

sources : Nigerian Office of Statistics (December 1993),
Central Bank of Nigeria (July 1994)

9.3.2 Dried fermented silage products

The cost of producing dried fermented fish silage products (liquid silage blended and co-dried with various protein feedstuffs) is presented in Table 9.2. It shows that the average cost is about half of the market price of 1 tonne of herring meal. The cost of production of dried fish silage products could not be compared with that of fish meal because there is no commercial fish meal production in Nigeria. However the cost of unit of protein of the dried silage products were compared with that

of commercial fish meals available in Nigeria. The cost of dried silage products per unit of protein is 22-53% lower than that of fish meal (Table 9.2).

These values were used to compute for the feeding trials in this study, and it showed that fish silage diets were 25-33% cheaper than the commercial fish feeds in Nigeria. Thus, in terms of production costs, fish silage is preferable than fish meal because of reduced overhead costs resulting from lower capital investment. However, it must be noted that it was not established whether fish silage or commercial feed production as a whole costs more, because no data are available for commercial feed preparation expenses. In terms of protein content, the liquid fish silages were not competitive because of the high water content, but the dried silage products were nutritionally equivalent to fish meal. The dried products contain the energy component of fish feeds which must be added to fish meal at additional cost.

A comparison of cost per unit of protein of dried silage products in this study with commercially available animal protein feedstuffs in Nigeria is presented in Table 9.3 and shows that the silage products were the least cost protein.

Table 9.2 : Cost[1] of production of 1 tonne of fermented fish silage products.

Dried silage	Liquid silage	Filler material	Labour & Transport	TOTAL	% protein	Cost/unit protein	
						N	£
FS:SBM	6,000	1,750	2,000	9,750	49.8	195.78	4.89
FS:SBF	6,000	2,250	2,000	10,250	56.1	182.71	4.57
FS:PBM	6,000	1,500	2,000	9,500	58.5	162.39	4.06
FS:HFM	6,000	1,250	2,000	9,250	68.0	136.03	3.40
FS:MBM	6,000	1,000	2,000	9,000	54.6	164.84	4.12
Herring meal	-	-	-	20,000	72	277.78	6.94
White fish meal	-	-	-	16,250	65	250.00	6.25

[1] prices are quoted in Naira (Nigerian currency)

FS:SBM = fish silage:soybean meal;
FS:SBF = fish silage:soybean flour;
FS:PBM = fish silage:poultry by-product meal;
FS:HFM = fish silage:hydrolysed feather meal;
FS:MBM = fish silage:meat and bone meal.

Table 9.3 : Comparison of price[1]/tonne of dried silage products and other animal protein feedstuffs used in aquaculture.

	Price ($)		Country	Reference
	Tonne	Unit protein		
Fermented silage	360-410	6.59-7.83	Nigeria	This study
Fermented silage	480	10.67	Norway	STROM & RAA (1992)
Acid silage	594-644	10.80-11.71	Nigeria	BALOGUN & OYEYEMI (1986)
Acid silage	602	10.95	UK	MOIR (1987)
Herring meal	800	11.11	Nigeria	BALOGUN (1990)
White fish meal	650	10.00	Nigeria	BALOGUN (1990)
Shrimp meal	525	9.81	Nigeria	BALOGUN (1990)

[1] prices are quoted in US $

CHAPTER 10 : CONCLUSIONS

"The stone the builders rejected

has become the capstone"

St. Matthew, 21:42

The BIBLE (NIV)

Fishery products are collected from a wide variety of species found in the aquatic environment and usually only the most desirable and easiest to obtain portion of the carcass is salvaged for human food. This leaves a large percentage of the tissue, much of which is high in protein and nutritionally desirable, as by-product. Many other species are often unintentionally caught when fishing for food species and these 'trash fish' can also be processed into useful by-products such as silage.

Tilapia silage products were stable during fermentation and storage. The pH did not vary appreciably within the desired range (4.0-4.5) and the rate of autolysis was influenced by incubation temperature, addition of salt, preheating and aerobic exposure. Degradation of proteins was low in fermented tilapia silage which retained similar amino acid profile to that of minced tilapia, thus suggesting its potential as a good protein source. Lipid rancidity was not apparent during storage.

Improvements in the fermentation technique for large-scale production and long-term storage would require psychrotrophic lactic acid

fermenters, a protease inhibitor for the autolytic process in fish and an economically acceptable yeast inhibitor when aerobic storage is intended. An alternative would be to pasteurize the fish before fermentation in order to destroy endogenous autolytic enzymes. The fermentation process with the addition of molasses and lactobacilli proved to be a reliable technique for preserving protein from fish wastes/ by-products under tropical conditions.

The growth performance of tilapia or catfish was not impaired by inclusion of up to 40 or 53.3% of the dietary dry matter, respectively (equivalent to 50% of the dietary protein) as co-dried fermented tilapia silage. The low values of protein utilization in this study could be attributed to the feeding activity of the experimental fishes coupled with the use of restrictive or fixed feeding regimes that would lead to over-estimation of the intake.

According to the results of the feeding trials, the inclusion of co-dried fermented fish silage in fish diets can be recommended at levels up to 50%. Although the results of this study suggest that the quality of protein in co-dried fermented silage is adequate for ***O. niloticus*** and ***C. gariepinus***, this conclusion must be interpreted cautiously as it should not/cannot be used as the sole source of dietary protein.

The major cost involved in the preparation of co-dried fish silage is that of drying, and unless the drying procedure can be made energy

effective, its use may not be justified. The economic viability of the drying procedure would also be enhanced if large quantities of waste fish are available for ensilage, and on a continuous basis.

In conclusion, co-dried fermented tilapia silages are suitable as protein supplement for fish diets. Further studies with a longer rearing period and to investigate possible histopathological effects on fish tissues as well as the economic feasibility of large-scale production of fermented tilapia silage from waste-grown tilapias are needed to confirm these results.

REFERENCES

"Summa epistlae, gaudes, gaudete"

Bengel, 1850 A.D.

AAGARD, T., DISNEY, J.G., JAYAWARDENA, K.M. & POULTER, R.G.(1980) Studies on the preparation of fish silage. IV Economics of production. *Bulletin of the Fisheries Research Station, Sri Lanka,* ***30***:37-39.

ABELIN, P., TACKAERT, W. & SORGELOOS, P.(1991) Ensiled artemia biomass: a promising and practical feed for penaeid postlarvae. pp.125-127, In: *Larvi '91 -Fish and crustacean larviculture symposium* (P. Lavens, P. Sorgeloos, E. Jaspers & F. Ollevier,eds.). European Aquaculture Society, Special Publication No.15, Gent, Belgium.

ADAMS, M.R., COOKE, R.D. & TWIDDY, D.R.(1987) Fermentation parameters involved in the production of lactic acid preserved fish-glucose substrates. *International Journal of Food Science and Technology,* ***22***:105-114.

ADEJUMO, T.O.(1987) Effect of feeding fish silage (from mackerel offals) on the growth and haematological parameters of *Oreochromis niloticus* (Trewavas) fingerlings. M.Sc. Thesis, University of Ibadan, Ibadan, Nigeria. 111pp.

AFFANDI, R.(1986) Utilization of silage as food in eel culture. *Vie Milieu,* ***36***:316-317.

AFFANDI, R. & BIAGIANTI, S.(1987) A study of the liver of eels kept in captivity: disturbances induced in hepatocytes by artificial diets. *Aquaculture,* ***67***:226-228.

AJAYI, S.S.(1971) Panyam fish farm and the introduction of the European carp (*Cyprinus carpio*). *Nigerian Field,* ***36***:23-31.

AJAYI, O.A.(1985) Utilization of clupeids for fish silage production: a preliminary biochemical and nutritional evaluation. M.Sc. Thesis, University of Ibadan, Ibadan, Nigeria.

AKSNES, A.(1988) Location of enzymes responsible for autolysis in bulk-stored capelin (*Mallotus villosus*). *Journal of Science of Food and Agriculture,* ***44***:263-271.

AKSNES, A.(1989) Effect of proteolytic inhibitors from potato on the quality of stored herring. *Journal of Science of Food and Agriculture,* ***49***:225-234.

ALI, M.T., HINDU, I.N. & MAKENE, J.(1984) Biological and chemical silage. FAO/GCP/INT/391/DEN. FAO, Rome. 22pp.

ANDERSON, K.E.(1989) The ensilage of capelin for fish feed as an alternative to freezing- an economic analysis. *Canadian Translation of Fisheries and Aquatic Science* No.5266 rev. National Resources Council, Ottawa. 127pp.

ANGLESEA, J.D. & JACKSON, A.J.(1985) Thiaminase activity in fish silage and moist fish feed. *Animal Feed Science and Technology,* ***13***:39-46.

ANYANWU, P., EZENWA, B. & UZUKWU, P.(1989) Fish culture in homestead tanks in Nigeria : practices, problems and prospects. *Aquabyte,* ***2***(3):8-10.

AOAC (Association of Official Analytical Chemists)(1990) Official methods of analysis. Association of Official Analytical Chemists, Washington, DC., USA. 13th edition. 1094pp.

AOE, H., IKEDA, K. & SAITO, T.(1974) Nutrition of protein in young carp. II. Nutritive value of protein hydrolysates. *Bulletin of the Japanese Society of Scientific Fisheries,* ***40***:375-379.

ARASON, S., THORRODDSSON, G. & VALDIMARSSON, G.(1990) The production of silage from waste and industrial fish. pp.79-85, In: *Making profits out of seafood wastes: Proceedings of the International Conference on Fish by-products* (S. Keller,ed.). Alaska Sea Grant Coll. Program, Anchorage, Alaska, USA.

ARNOLD, R.G.(1978) Thiamine degradation chemistry. pp.749-753, In: *Encyclopedia of food science* (M.S. Peterson & A.H. Johnson, eds.). AVI Publishing Co., Inc., Westport, Conn., USA.

ASGARD, T.(1981) Acid preservation could make offals a source of income. *Fish Farmer,* ***4***:145-156.

ASGARD, T. & AUSTRENG, E.(1981) Fish silage for salmonids: a cheap way of utilizing waste as feed. *Feedstuffs,* ***52***:22-24.

ASGARD, T. & AUSTRENG, E.(1985a) Casein silage as feed for salmonids. *Aquaculture,* ***48***:233-252.

ASGARD, T. & AUSTRENG, E.(1985b) Dogfish offal, ensiled or frozen, as feed for salmonids. *Aquaculture,* ***49***:289-305.

ASGARD, T. & AUSTRENG, E.(1986) Blood, ensiled or frozen, as feed for salmonids. *Aquaculture,* ***55***:263-284.

ASH, R. & McLEAN, E.(1989) Protein digestion and absorption in intact proteins. pp.49-69, In: Protein and amino acid nutrition in fish, Animal Nutrition and Health Series, Roche, Switzerland.

AUSTRENG, E. & ASGARD, T.(1986) Fish silage and its use. pp.218-229, In: *Trends and problems in aquaculture development, Proceedings of the Second International Conference of Aquafarming* (E. Grimali & H. Rosenthal,eds.). "Aquacoltura '84", Verona, Italy.

AUSTRENG, E. & GJEFSEN, T.(1981) Fish oils with different contents of free fatty acids in diets for rainbow trout fingerlings and salmon parr. *Aquaculture,* ***25***:173-183.

AUSTRENG, E. & REFSTIE, T.(1979) Effect of varying dietary protein level in different families of rainbow trout. *Aquaculture,* ***18***:145-156.

BACKHOFF, H.P.(1976) Some chemical changes in fish silage. *Journal of Food Technology,* ***11***:353-363.

BAIRD-PARKER, A.C.(1980) Organic acids. pp.126-135, In: *Microbial ecology of foods* (J.H. Silliker,ed.). Academic Press, London.

BALARIN, J.D. (1984) Towards an integrated approach to tilapia farming in Africa. *The Courier, **85***:82-84.

BALARIN, J.D. & HATTON, J.P.(1979) Tilapia: a guide to their biology and culture in Africa. University of Stirling, Stirling, Scotland. 174pp.

BALOGUN, A.M.(1990) Feeding fish with alternative feed formulations and resources: prospects, problems and implications. pp.669-697, In: *Proceedings of the National Workshop on Alternative Feed Formulations of Livestock Feeds in Nigeria* (G.M. Babatunde,ed.). Economic Affairs Office of The Presidency, Lagos. Nigeria.

BALOGUN, A.M. & OLOGHOBO, A.D.(1989) Growth performance and nutrient utilization of *Clarias garlepinus* (Burchell) fed raw and cooked soybean diets. *Aquaculture, **76***: 119-126.

BALOGUN, A.M. & OYEYEMI, I.E.(1986) Cost analysis of a small scale fish silage production from cannery wastes in Nigeria. *Journal of West African Fisheries, **2***:66-77.

BALTES, W.(1982) Chemical changes in food by the Maillard reaction. *Food Chemistry, **9***: 59-73.

BARRY, T.N., FENNESSY, P.F. & DUNCAN, S.J.(1973) Effect of formaldehyde treatment on the chemical composition and nutritive value of silage. *New Zealand Journal of Agricultural Research, **16***:64.

BATISTA, I.(1987) Fish silage: preparation and uses. pp.227-248, In: *Nutrition in Marine Aquaculture* (A. Bruno,ed.). FAO/UNDP/ MEDRAP, Tunis, Tunisia.

BATISTA, I., NUNES, M.L., MENDES, R. & NUNES, M.C.(1989) Preparation, characterization and quality control of different kinds of fish silage. NATO Project (Technology of Fish Feed Production) Technical Report, INIP, Lisbon. 21pp.

BECK, T.(1978) The microbiology of silage fermentation. pp.61-115, In: *Fermentation of silage-a review* (M.E. McCullough,ed.). National Feed Ingredient Association, Iowa, USA.

BERG, H.(1985) Trials with Ensi-Ferm, heat treated, lactic acid bacteria fermented fish silage. Finnish Fur Animal Laboratory, Vasa. Manus, Sweden.

BERTULLO, E.(1984) Use of aquatic animal products for silage production. *Revista Latinoamericana de Tecnologia de Alimentos Pesqueras, **1***:27-31.

BIGUERAS-BENITEZ, C.M. & NACORDA, H.M.E.(1992) Evaluation of fish silage as protein source for poultry. pp.148-154, In: *Proceedings of IPFC Workshop on fish silage* (D. James,ed.). *FAO Fisheries Report* No.470. FAO, Rome.

BIGUERAS-BENITEZ, C.M., SERRANO, A.E. & LAPIE, L.(1992) Preparation, chemical evaluation and measurement of digestibility of fish silage in *Oreochromis niloticus*. Paper presented at the Third Asian Fisheries Forum, 26-30 October 1992. Asian Fisheries Society, Singapore (in press).

BISHOV, S.J. & HENICK, A.S.(1978) Natural antioxidants. pp.536-539, In, *Encyclopedia of Food Science* (M.S. Pederson & A.H. Johnson, eds.), AVI Publishing Co.,Inc. Westport, Connecticut.

BRITZ, P.J.(1988) Environmental requirements for the hatchery rearing of African sharptooth catfish, *Clarias gariepinus* (Pisces:Clariidae) larvae and juveniles. M.Sc. Thesis, Rhodes University, Grahamstown, South Africa.

BROMLEY, P.J. & SMART G.(1981) The effects of the major food categories on growth composition and food conversion in rainbow trout (*Salmo gairdneri* RICHARDSON). *Aquaculture,* ***23***:325-336.

BROWN, N. & SUMNER, J.(1985) Fish silage. pp.404-413, In: Spoilage of Tropical Fish and Product Development (A. Reilly,ed.). *FAO Fisheries Report* No.317 Supplement. FAO, Rome.

BUSSE, T. & BELITZ, H-D.(1976) Hemmung von kathepsinen aus forellenmuskel und aus rindermilz durch proteinaseinhibitoren der kartoffel. *Zeitschrift fuer Lebensmittel-Untersuchung -Forschung,* ***162***:357-364.

BYLUND, G. & WIKLUND, T.(1987) Microbiological tests on fish silage for occurrence and survival of fish pathogens. *Soumen Kalankasvattaja* (Finland's Fish Breeder), May 1987.

BYUN, H-S., KIM, S-B., PARK, Y-H. & YOON, H-D.(1986a) Antioxidative effect of ginger extracts on fish oil. *Bulletin of the Korean Fisheries Society,* ***19***:327-332.

BYUN, H-S., KIM, S-B., PARK, Y-H. & YOON, H-D.(1986b) Antioxidative effect of onion and mustard powder extracts on fish oil. *Bulletin of the Korean Fisheries Society,* ***19***:453-458.

CHIPAULT, J.R., MIZUNO, G.R., HAWKINS, J.M. & LUNDBERG, W.O.(1952) The antioxidant properties of natural spices. *Food Research,* ***17***:46.

CONWAY, E.J.(1968) Microdiffusion analysis and volumetric error. Crosley, Lockwood & Sons,London. 467pp.

DADA, B.F.(1975) Present status and prospects for aquaculture in Nigeria. pp.79-85, In: *FAO/CIFA Symposium on Aquaculture in Africa.* CIFA/T4, FAO, Rome.

DAHIYA, R.S. & SPECK, M.L.(1978) Hydrogen peroxide formation and its effect on *Staphylococcus aureus. Journal of Dairy Science,* ***51***:1568-1572.

DANIEL, P.C. & BAYER, R.C.(1989) Fish by-products as chemo-attractant substrates for the American lobster (*Homarus americanus*): concentration, quality and release characteristics. *Fisheries Research,* ***7***: 367-383.

DEGANI, G.(1988) Body composition of African catfish (*Clarias gariepinus*) at different ages. *Israeli Journal of Aquaculture,* ***40***:118-121.

DEGANI, G., BEN-ZVI, Y. & LEVANON, D.(1989) The effects of different protein levels and temperatures on feed utilization, growth and body composition of *Clarias gariepinus* (Burchell 1822). *Aquaculture,* ***76***:293-301.

DeSILVA, S.S., GUNASEKERA, R.M. & ATAPATTU, D.(1989) The dietary protein requirements of young tilapia and an evaluation of the least cost dietary protein levels. *Aquaculture,* ***80***:271-284.

DHATEMWA, C.M.(1989) Simple methods of processing fish for animal feeds. pp.203-206, In: *Proceedings of the FAO Expert Consultation on Fish Technology in Africa,* (A. Tall,ed.) *FAO Fisheries Report* No.400 Supplement. FAO, Rome.

DICKSON, M.W.(1991) The development of tilapia feeds based on locally available materials in Zambia. Ph.D. Thesis, University of Stirling, Stirling, Scotland. 200pp.

DISNEY, J.G. & JAMES, D.(eds.)(1980) *Fish silage production and its use.* *FAO Fisheries Report* No.230. FAO, Rome. 105pp.

DISNEY, J.G., HOFFMAN, A., OLLEY, J., CLUCAS, I.J., BARRANCO, A. & FRANCIS, B.J.(1978) Development of fish silage/carbohydrate animal feed for use in the tropics. *Tropical Science,* ***20***:129-144.

DJAJASEWAKA, H. & DJAJADIREDJA, R.(1980) Fish silage as a feed for freshwater fish. pp.74-76, In, *Fish silage production and its use* (J.G. Disney & D. James, eds.). *FAO Fisheries Report* No.230. FAO, Rome.

DONG, F.M., FAIRGRIEVE, W.T., SKONBERG, D.I. & RASCO, B.A.(1993) Preparation and nutrient analysis of lactic acid bacterial ensiled salmon viscera. *Aquaculture,* ***109***:351-366.

DUBOIS, M., GILLES, K.A., HAMILTON, J.M., REBERS, P.A. & SMITH, F.(1956) Colorimetric determination of sugars and related substances. *Analytical Chemistry,* ***28***:350-356.

EDWARDS, P.(1980) A review of recycling organic wastes into fish, with emphasis on the tropics. *Aquaculture,* ***21***:261-279.

EDWARDS, P., POLPRASERT, C. & WEE, K.L.(1987) Use of ensiled septage-raised tilapia as feed ingredient for carnivorous fish production. pp.164-183, In: *Resource Recovery and Health Aspects of Sanitation.* AIT Research Report No.205, Environmental Sanitation Information Center, Asian Institute of Technology (AIT), Bangkok, Thailand.

EITENMILLER, R.R., ORR, J.H. & WALLIS, W.W.(1982) Histamine formation in fish: microbiological and biochemical conditions. pp.39-50, In: *Chemistry and biochemistry of marine food products* (R.E. Martin, G.F. Flick, C.E. Hebard & D.R. Ward,eds.). AVI. Westport, Connecticut, USA.

ELLIS, A.E.(1982) Differences between the immune mechanisms of fish and higher vertebrates. pp.1-23, In: *Microbial diseases of fish* (R.J. Roberts,ed.). Academic Press Inc., London.

ESPE, M.(1987) Formic acid silage from herring. A study of the storage stability of the protein measured chemically and nutritionally. Cand. Sci. Thesis, University in Bergen. 95pp.

ESPE, M., RAA, J. & NJAA, L.R.(1989) Nutritional value of stored fish silages as protein source for young rats. *Journal of Science of Food and Agriculture,* ***49***:259-270.

ESPE, M., HAALAND, H. & NJAA, L.R.(1991) A note on the amino acid distribution in fish silage layers. *Fiskeridirektoratet Skrifter, Serie Ernaering,* ***IV***(2):127-132.

ESPE, M., HAALAND, H. & NJAA, L.R.(1992b) Substitution of fish silage protein and a free amino acid mixture for fish meal protein in a chicken diet. *Journal of Science of Food and Agriculture,* ***58***:315-319.

ESPE, M., HAALAND, H. & NJAA, L.R.(1992c) Autolysed fish silage as a feed ingredient for atlantic salmon (*Salmo salar*). *Comparative Biochemistry and Physiology,* ***103A***:369-372.

ESPE, M., HAALAND, H., NJAA, L. & RAA, J.(1992a) Growth of young rats on diets based on fish silage with different degrees on hydrolysis. *Food Chemistry,* ***44***:195-200.

EVANS, C.W.(ed.)(1975) Thiaminase and their effects on animals. In: *Vitamins and Hormones,* ***33***: 467pp.

FAGBENRO, O.A.(1987) A review of biological and economical principles underlying commercial fish culture production in Nigeria. *Journal of West African Fisheries,* ***3***:171-177.

FANGE, R. & GROVE, D.(1979) Digestion. pp.161-260, In, Bioenergetics and growth (D.J. Randall & J.R. Brett,eds.), Fish Physiology, Vol.8. Academic Press, New York.

FAO (Food and Agriculture Organization)(1991) Aquaculture production (1986-1989). *FAO Fisheries Circular* No.815 Rev.3. FAO, Rome. 141pp.

FAO/IFAD (1987) Nigeria: Small-scale Fisheries Development Project. Preparation Report, Annex 2: Freshwater aquaculture development. Report of the FAO/IFAD Cooperative Programme, Investment Centre No.77/87. IF-NIR 23. FAO, Rome.

FISCHL, J.(1960) Quantitative colorimetric determination of tryptophan. *Journal of Biological Chemistry,* ***235***:999-1001.

FOLTZ, J.W., GIBSON, J.M. & WINDELL, J.T.(1982) Evaluation of tilapia meal for fish diets. *Progressive Fish-Culturist,* ***44***:8-11.

FURUKAWA, A. & TSUKAHARA, H.(1966) On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. *Bulletin of the Japanese Society of Scientific Fisheries,* ***32***:502-504.

GAO, Y., LO, K.V. & LIAO, P.H.(1992) Utilization of salmon farm mortalities-fish silage. *Bioresource Technology,* ***41***:123-127.

GILDBERG, A. & RAA, J.(1977) Properties of a propionic acid/formic acid preserved silage of cod viscera. *Journal of Science of Food and Agriculture,* ***28***:647-653.

GILDBERG, A. & RAA, J.(1979) Solubility and enzymatic solubilization of muscle and skin of capelin (*Mallotus vilosus*) at different pH and temperature. *Comparative Biochemistry and Physiology,* ***63B***:309-314.

GILDBERG, A. & RAA, J.(1980) Tissue degradation and belly bursting in capelin. pp.255-258, In: *Advances in Fish Science and Technology* (J.J. Connell,ed.), Fishing News Books, London.

GILDBERG, A. & RAA, J.(1983) Purification and characterization of pepsins from the Arctic fish capelin (*Mallotus villosus*). *Comparative Biochemistry and Physiology,* ***75A***:337-342.

GILDBERG, A., ESPEJO-HERMES, J. & OREJANA, F.M.(1984) Acceleration of autolysis during fish sauce fermentation by adding acid and reducing the salt content. *Journal of Science of Food and Agriculture,* ***35***:1363-1369.

GILDBERT, J.P., WOOLEY, P.E., SHOTTS Jr., E.B. & DICKENS, J.A.(1983) Viricidal effects of *Lactobacillus* and yeast fermentation. *Applied Environmental Microbiology,* ***46***: 452-458.

GIURCA, R. & LEVIN, R.E.(1992) Optimization of the lactic acid fermentation of hydrolyzed cod gurry with molasses. *Journal of Food Biochemistry,* ***16***:83-97.

GNAEDINGER, R.H. & KRZECZKOWSKI, R.A.(1966) Heat inactivation of thiaminase in whole fish. *Commercial Fisheries Reviews,* ***28***(8):11.

GOHL, B.(1981) Tropical Feeds.
FAO Animal Production and Health Series No.12. FAO, Rome. 529pp.

GONCALVES, J.F., SANTOS, S., PEREIRA, V.S., BAPTISTA, I. & COIMBRA, J.(1989) Use of fish silage as ingredient for eel fingerlings. *Aquaculture,* ***80***:135-146.

GOWDA, H.S.V. & KARUNASAGAR, I.(1985) Effect of protease inhibitors from *Adananthera pavonia* on the biochemical and microbiological quality of fishes. *Journal of Science of Food and Agriculture,* ***36***:1113-1119.

GRANT, J.(1989) Fish meal: what is it doing to the price of feed?
Canadian Aquaculture, ***5***:64-68.

GREEN, S., WISEMAN, J. & COLE, D.J.A.(1988) Examination of stability, and its effect on the nutritive value, of fish silage in diets for growing pigs.
Animal Feed Science and Technology, ***21***:43-56.

HAALAND, H. & NJAA, L.R.(1988) Ammonia (NH_3) and total volatile nitrogen (TVN) in preserved and unpreserved stored, whole fish.
Journal of Science of Food and Agriculture, ***44***:335-342.

HAALAND, H. & NJAA, L.R.(1989a) Effect of temperature on the autolysis of capelin silages stored for one year.
Fiskeridirektoratet Skrifter, Serie Ernaering, ***II***(7): 219-226.

HAALAND, H. & NJAA, L.R.(1989b) Total volatile nitrogen-a quality criterion for fish silage? *Aquaculture,* ***79***:311-316.

HAALAND, H., ESPE, M., NJAA, L.R. & MYKLESTAD, H.(1990) Chemical composition and variation in some parameters during storage of 8 acid silages prepared from capelin. *Fiskeridirektoratet Skrifter, Serie Ernaering,* ***III***(2):59-74.

HAARD, N.F., KARIEL, N., HERZBERG, G., FELTHAM, L.A.W. & WINTER, K.(1985) Stabilization of protein and oil in fish silage for use as a ruminant feed supplement.
Journal of Science of Food and Agriculture, ***36***:229-241.

HALL, G.M. & LEDWARD, D.A.(1986) Silage from tropical fish. 3. Lipid behaviour.
Journal of Food Technology, ***21***:45-54.

HALL, G.M., LEDWARD, D.A. & LAWRIE, R.A.(1985b) Silage from tropical fish.
2. Undigested fraction. *Journal of Food Technology,* ***20***:573-580.

HALL, G.M., KEEBLE, D., LEDWARD, D.A. & LAWRIE, R.A.(1985a) Silage from tropical fish.
1. Proteolysis. *Journal of Food Technology,* ***20***:561-572.

HAN-CHING, L., IN, T., MAUGUIN, S. & MESCLE, J.-F.(1992) Application of lactic acid fermentation. pp.193-211, In: Fish processing technology. (G.M. Hall,ed.). Blackie Academic, London.

HANLEY, F.(1987) The digestibility of foodstuffs and the effects of feeding selectivity on digestibility determination in tilapia, *Oreochromis niloticus*. *Aquaculture,* ***66***:163-179.

HARDY, R.W.(1989) Diet preparation. pp.475-548, In: Fish nutrition (2nd edition) (J.E. Halver,ed.). Academic Press, Inc., London.

HARDY, R.W. & MASUMOTO, T.(1990) Specifications for marine by-products for aquaculture. pp.109-120, In: *Making profits out of seafood wastes: Proceedings of the International Conference on Fish By-products* (S. Keller,ed.). Alaska Sea Grant Coll. Programme, Anchorage, Alaska, USA.

HARDY, R.W., SHEARER, K.D. & SPINELLI, J.(1984) The nutritional properties of co-dried fish silage in rainbow trout (*Salmo gairdneri*) dry diets. *Aquaculture,* ***38***:35-44.

HARDY, R.W., SHEARER, K.D., STONE, F.E. & WIEG, D.H.(1983) Fish silage in aquaculture diets. *Journal of the World Mariculture Society,* ***14***:695-703.

HASSAN, T.E.(1982) Fish silage produced by microbial fermentation. Ph.D. Thesis, University of Maryland, Maryland, USA. 182pp.

HASSAN, T.E. & HEATH, J.L.(1986) Biological fermentation of fish waste for potential use in animal and poultry feeds. *Agricultural Wastes,* ***15***:1-15.

HASSAN, T.E. & HEATH, J.L.(1987) Chemical and nutritive characteristics of fish silage produced by biological fermentation. *Biological Wastes,* ***20***:187-201.

HAYLOR, G.S.(1992) African catfish hatchery manual. Stirling Aquaculture, University of Stirling, Scotland. 86pp.

HEINEN, J.M.(1981) Evaluation of some binding agents for crustacean diets. *Progressive Fish-Culturist,* ***43***:142-145.

HERNANDEZ, J.Z.(1983) Evaluation of fish silage in the formulation of practical diets for tilapia (*Oreochromis niloticus* TREWAVAS). M.Sc. Thesis, University of Stirling, Stirling, Scotland. 43pp.

HILTON, J.W., CHO, C.Y. & SLINGER, S.J.(1981) Effect of extrusion processing and steam pelleting diets on pellet durability, pellet water absorption, and the physiological response of rainbow trout (*Salmo gairdneri* R.). *Aquaculture,* ***25***: 185-194.

HOLE, R. & OINES, S.(1991) Can fish silage be used as a nutritional solution to reduce aquaculture waste when considering the slaughtering quality of fish? pp.103-109, In: *Nutritional strategies and aquaculture waste: Proceedings of the First International Symposium on Nutritional Strategies in Management of Aquaculture Waste* (C.B. Cowey & C.Y. Cho,eds.). University of Guelph, Ontario, Canada.

HOSSAIN, M.A., FURUICHI, M. & YONE, Y.(1987) Effects of scrap meals fermented with fungi on growth of red sea bream and feed efficiency. *Nippon Suisan Gakkaishi,* ***53***:1669-1671.

HOSSAIN, M.A., FURUICHI, M. & YONE, Y.(1988) Proximate and fatty acid composition of liver, hematological characteristics, and chemical components in plasma of red sea bream fed on scrap meals fermented with molds. *Nippon Suisan Gakkaishi,* ***54***:1385-1389.

HUANG, H-J.(1989) Aquaculture feed binders. pp.316-318, In, *Proceedings of the Peoples Republic of China Aquaculture and Feed Workshop* (D.E. Akiyama,ed.). American Soybean Association, Singapore.

HUISMAN, E.A.(1986) Current status and role of aquaculture with special reference to the African region. pp.11-22, In: *Aquaculture Research in the African Region* (E.A. Huisman,ed.). Pudoc, Wageningen, The Netherlands.

HUSAIN, R.A.K. & OFFER, N.W.(1987) Effect of formaldehyde treatment on the degradation of acid-preserved fish silage protein in vitro. *Animal Feed Science and Technology, **16**:297-304.*

HUSS, H.H. & RYE PEDERSON, E.(1980) The stability of *Clostridium botulinum* type E toxin in salt and low acid environment. *Journal of Food Technology, **15**:619-627.*

IAFMM (International Association of Fish Meal Manufacturers)(1990) World fish meal production down. *Feedstuffs, **62**:7.*

IAFMM (International Association of Fish Meal Manufacturers)(1991) World production of fish meal drops. *Feedstuffs, **63**:18.*

ITA, E.O., SADO, E.K., BALOGUN, J.K., PANDOGARI, A. & IBITOYE, B.(1985) Inventory survey of Nigeria inland waters and their fishery resources. 1. A preliminary checklist of inland water bodies in Nigeria. *Kainji Lake Research Institute Technical Report Series* No.14. KLRI, New Bussa, Nigeria.

IWAI, T.(1969) Fine structure of the gut epithelial cells of larval and juvenile carp during absorption of fat and protein. *Archives of Histology and Cytology/Nihon Soshikigalu Kiroku, **30**:183-199*

JACKSON, P.B.N.(1988) Aquaculture in Africa. pp.459-480, In: *Biology and ecology of African freshwater fishes* (C. Leveque, M.M. Bruton & G.W. Ssentongo,eds.). ORSTOM, France.

JACKSON, A.J., KERR, A.K. & BULLOCK, A.M.(1984b) Fish silage as a dietary ingredient for salmon. II. Preliminary growth findings and nutritional pathology. *Aquaculture, **40**:283-291.*

JACKSON, A.J., KERR, A.K. & COWEY C.B.(1984a) Fish silage as a dietary ingredient for salmon. I. Nutritional and storage characteristics. *Aquaculture, **38**:211-220.*

JAMES, M.A., IYER, K.M. & NAIR, M.R.(1977) Comparative study of fish ensilage prepared by microbial fermentation and formic acid silage. pp.273-275, In: *Handling, processing and marketing of tropical fish.* Tropical Products Institute, London.

JANGAARD, P.M.(1987) Fish silage: a review and some recent developments. pp.8-33, In: *Proceedings of Fish silage Workshop,* University of St. Anne, Church Point, Nova Scotia. General Education Series No.7, Department of Fisheries and Oceans, Halifax, Nova Scotia, Canada.

JAUNCEY, K. & ROSS, B.(1982) A guide to tilapia feeds and feeding. Institute of Aquaculture, University of Stirling, Stirling.111pp.

JAYAWARDENA, K.M. & POULTER, R.G.(1980) Studies on the preparation of fish silage. II. Rate of liquefaction in different parts of silver belly. *Bulletin of the Fisheries Research Station, Sri Lanka, **30**:25-31.*

JAYAWARDENA, K.M., GUNERAINE, Q., VILLADSEN; A. & POULTER, R.G.(1980b) Studies on the preparation of fish silage. III. Dried silage products. *Bulletin of the Fisheries Research Station, Sri Lanka,* ***30***:33-36.

JAYAWARDENA, K.M., VILLADSEN, A., GUNERAINE, Q. AAGARD, J. & POULTER, G.(1980a) Studies on the preparation of fish silage. I. Effect of quality of raw material and type of acid. *Bulletin of the Fisheries Research Station, Sri Lanka,* ***30***:17-23.

JENSEN, J. & SCHMIDTSDORFF, W.(1977) Fish silage, low fat and soluble fish protein production. pp.23-26, In: *Symposium on the production of fish meal.* International Association of Fish Meal Manufacturers, Hertfords., UK.

JENSEN, P.M. & JORGENSEN, G.(1975) Production and use of fish silage for mink. Beretn. Statens Husdybrugsford (427):79pp. *Chemical Abstracts,* ***83***:386.

JITOE, A., MASUDA, T., TENGAH, I.G.P., SUPRAPTA, D.N., GARA, I.W. & NAKATANI, N.(1992) Antioxidant activity of ginger extracts and analysis of the c o n t a i n e d curcuminoids. *Journal of Agriculture and Food Chemistry,* ***40***:1337-1340.

JOHNSEN, F. & SKREDE, A.(1981) Evaluation of fish viscera silage as a feed resource: chemical characteristics. *Acta Agriculturae Scandinavica,* ***31***:21-28.

JONSSON, S., CLAUSEN, E. & RAA, J.(1983) Amino acid degradation by a *Lactobacillus plantarum* strain from fish. *Systematic Applied Microbiology,* ***4***:148-154.

KIES, C.(1981) Bioavailability: a factor in protein quality. *Journal of Agriculture and Food Chemistry,* ***29***:435-440.

KLING, L.J., HUNTER, J.A., GOLDHOR, S.H. & GIURCA, R.A.(1994) Hydrolyzed dogfish processing waste as a feed ingredient for Atlantic salmon diets. In: World Aquaculture Society Conference Proceedings, WAS, New Orleans, USA. pp.210.

KOMPIANG, I.P., ARIFUDIN, R. & RAA, J.(1980a) Nutritional value of ensilaged by-catch fish from Indonesian shrimp trawlers. pp.349-351, In: *Advances in Fish Science and Technology* (J.J. Connell,ed.). Fishing News Books Ltd., Surrey, UK.

KOMPIANG, I.P., YUSHADI, S. & CRESSWELL, D.C.(1980b) Microbial fish silage: chemical composition, fermentation characteristics and nutritional value. pp.38-43, In: Fish silage production and its use (J. G. Disney & D. James,eds.). *FAO Fisheries Report* No.230. FAO, Rome.

KRISHNASWAMY, M.A., KODOL, S.B. & REVANKAR, G.D.(1965) Nutritional evaluation of an ensiled product from fish. *Canadian Journal of Biochemistry,* ***43***:1879-1883.

KUTTY, M.N.(1986) Aquaculture development and training in Africa. pp.23-29, In, *Aquaculture Research in the African Region* (E.A. Huisman,ed.), Pudoc, Wageningen, The Netherlands.

LABUZA, T.P.(1971) Kinetics of lipid oxidation in foods. *CRC Critical Reviews in Food Technology,* ***2***:355-405.

LALL, S.P.(1987) Fish silage as feed for salmon and trout. pp.56-63, In: *Proceedings of Fish silage Workshop,* University of St. Anne, Church Point, Nova Scotia. General Education Series No.7, Department of Fisheries and Oceans, Halifax, Nova Scotia, Canada.

LALL, S.P.(1991) Nutritional value of fish silage in salmonid diets. pp.63-74, In: *Fish Silage workshop, Bulletin of the Aquaculture Association of Canada,* No.91-1 (R.G. Ackman & J. O'Dor,eds.). Aquaculture Association of Canada.

LANIER, T.C., LIN, T.S., HAMANN, D.D. & THOMAS, F.B.(1981) Effects of alkaline protease in minced fish on texture of heat-processed gels. *Journal of Food Science,* ***46***:1643-1645.

LAPIE, L.P. & BIGUERAS-BENITEZ, C.M.(1992) Feeding studies on tilapia (*Oreochromis niloticus*) using fish silage. pp.165-177, In: (D. James,ed.) *FAO Fisheries Report* No.470, Suppl. FAO, Rome.

LARSON, R.A.(1988) The antioxidants of higher plants. *Phytochemistry,* ***27***:969-978.

LEE, C.H.(1990) Fish fermentation technology-a review. pp.1-13, In: *Post harvest technology, preservation and quality of fish in south-east Asia* (P.J.A. Reilly, R.W.H. Parry & L.E. Barile,eds.). International Foundation for Science (IFS), Stockholm, Sweden.

LEE, I. & AHN, S.(1985) The antioxidant activity of gingerol. *Korean Journal of Food Science and Technology,* ***17***:55-59.

LEE, Y.B., KIM, Y.S. & ASHMORE, C.R.(1986) Antioxidant property in ginger rhizome and its application to meat products. *Journal of Food Science,* ***51***:20-23.

LEVIN, R.E., WITKOWSKI, R., MEIRONG, Y. & GOLDHOR, S.(1989) Preparation of fish silage with phosphoric acid and potassium sorbate. *Journal of Food Biochemistry,* ***12***:253-259.

LIE, O., WAAGBO, R. & SANDNES, K.(1988) Growth and chemical composition of adult Atlantic salmon (*Salmo salar*) fed dry and silage-based diets. *Aquaculture,* ***69***:343-353.

LINDGREN, S.E. & CLEVSTROM, G.M.(1978) Antibacterial activity of lactic acid bacteria. *Swedish Journal of Agricultural Research,* ***8***:61-73.

LINDGREN, S.E. & PLEJE, M.(1983) Silage fermentation of fish or fish waste product with lactic acid bacteria. *Journal of Science of Food and Agriculture,* ***34***:1057-1067.

LINDGREN, S.E. & REFAI, O.(1984) Amylolytic lactic acid bacteria in fish silage. *Journal of Applied Bacteriology,* ***57***:221-228.

LISTON, J.(1965) Bacteriological enzymes and their role in the deteriorative changes in fish. pp.53-57, In: *The technology of fish utilization,* FAO International Symposium, Husum (R. Kreuzer,ed.). Fishing News Books Ltd., London, UK.

LO, K.V., LIAO, P.H. & GAO, Y.(1993a) Effect of temperature on silage production from salmon farm mortalities. *Bioresource Technology,* ***44***:33-37.

LO, K.V., LIAO, P.H., BULLOCK, C. & JONES, Y.(1993b) Silage production from salmon farm mortalities. *Aquaculture Engineering,* ***12***:37-45.

LONGHURST, A.R.(1961) Report on the Fisheries of Nigeria. Federal Fisheries Services, Ministry of Economic Development, Lagos.

LOPEZ, C.S.(1990) Microbial ensilage of trash fish for animal feeds. pp.189-192, In: *Post-harvest technology, preservation and quality of fish in south-east Asia* (P.J.A. Reilly, R.W.H. Parry & L.E. Barile,eds.). International Foundation for Science (IFS), Stockholm, Sweden.

LOVAAS, E.(1989) A new antioxidant for improved feed quality. pp.665-671, In: *Aquaculture, a biotechnology in progress* (N. De Pauw, E. Jaspers, H. Ackefors, N. Wilkins,eds.). European Aquaculture Society, Belgium.

LUIS, O.J. & BATISTA, I.(1990) The influence of protein quality and lipid composition from two Portuguese fish meals on growth of early juveniles of *Palaemon serratus* (Crustacea;Decapoda).
Boletim Instituto Nacional de Investigacao das Pescas, No.15, pp.5-14.

MAAR, A., MORTIMER, M.A.E. & VAN DER LINGEN, I.(1966) Fish culture in Central East Africa. FAO/UN, Rome. 158pp.

MACKIE, P.(1971) The biochemistry of silage.
John Wiley & Sons, New York.

MACKIE, I.M.(1982) General review of fish protein hydrolysates.
Animal Feed Science and Technology, ***7***:113-124.

MACKIE, I.M., HARDY, R. & HOBBS, G.(eds.)(1971) Fermented fish products.
FAO Fisheries Report No.100. FAO, Rome. 59pp.

MAKINODAN, Y., TOYOHARA, H. & NIWA, E.(1985) Implication of muscle alkaline proteinase in the textural degradation of fish meat gel.
Journal of Food Science, ***50***:1351-1355.

MANIKANDAVELU, D., NEETHISELVAN, S., JAGATHEESAN, G. & SUNDARSANAN, K.(1992) Use of fermented ensilage based diet in enhancing the growth of common carp (*Cyprinus carpio* var. *communis*). *Fish Technology,* ***29***:111-113.

MAZID, M.A., TANAKA, Y., KATAYAMA, T., SIMPSON, K.L. & CHICHESTER, C.O. (1978) Metabolism of amino acids in aquatic animals. III. Indispensable amino acids for *Tilapia zillii*. *Bulletin of the Japanese Society for Scientific Fisheries,* ***44***: 739-742.

McDONALD, P.(ed.)(1981) The biochemistry of silage.
Wiley and Sons, Chichester.

MEYER, V.(1965) Marinades. In, *Fish as food* (G. Borgstom,ed.),
Academic Press, New York.

MEYERS, S.P.(1987) Aquaculture feeds and chemoattractants.
Infofish Marketing Digest, ***1/87***: 35-37.

MOIR, A.J.G.(1987) An assessment of the economics of using partially dried silage as a feed for aquaculture. M.Sc. Thesis, University of Stirling, Scotland. 52pp.

MOSES, B.S.(1983) Introduction to tropical fisheries.
Ibadan University Press, Ibadan, Nigeria. 117pp.

MUNDHEIM, H. & OPSTVEDT, J.(1993) Growth rate of Atlantic salmon (*Salmo salar*) fed fish meal with varying content of water soluble protein. pp.847-855, In: *Fish nutrition in practice* (S.J. Kaushik & P. Luquet,eds.). INRA, France.

NAWAB, A-R. A-H.(1987) Studies on food intake, digestion and growth of *Oreochromis niloticus*. Ph.D. Thesis, University of Stirling, Stirling, Scotland. 275pp.

NEW, M.B.(1991) Turn of the millenium aquaculture. *World Aquaculture,* ***22***:28-49.

NIELSEN, H.K., WECK, D.D., FINOT, P.A., LIARDON, R. & HURRELL, R.F.(1985) Stability of tryptophan during food processing and storage. I. Comparative losses of tryptophan, lysine and methionine in different model systems. *British Journal of Nutrition,* ***53***:281-292.

NORMAN, G.A., SILVERSIDE, D., HECTOR, D.A. & FRANCIS, S.(1979) The acid treatment of animal by-products. *Tropical Science,* ***21***:221-230.

NOSE, T.(1989) Protein and amino acid nutrition in fish. pp.13-44, In: Protein and amino acid nutrition in fish, Animal Nutrition and Health Series, Roche, Switzerland.

NRC (National Research Council)(1983) Nutrient requirements of warmwater fishes and shellfishes. National Academy Press, Washington, D.C. 102pp.

OCKERMAN, H.W.(1992) Fishery by-products. pp.155-192, In: Fish processing technology. (G.M. Hall,ed.). Blackie Academic, London.

OFFER, N.W. & HUSAIN, R.A.K.(1987) Fish silage as a protein supplement for early-weaned calves. *Animal Feed Science and Technology,* ***17***:165-177.

OLANIYAN, C.I.O.(1961) On the introduction of the common carp, *Cyprinus carpio,* into Nigerian waters and its possible effect on the hydrography of the region. *Great Britain Journal of West African Science Association,* ***7***:79-92.

OLOGHOBO, A.D. & BALOGUN, A.M.(1988) The value of dried fish silage for pullets and the layer hen. *Nigerian Journal of Animal Production,* ***14***:67-76.

OLOGHOBO, A.D., BALOGUN, A.M. & BOLARINWA, B.B.(1988) The replacement value of fish silage for fish meal in practical broiler rations. *Biological Wastes,* ***25***:117-125.

OREJANA, F.M. & LISTON, J.(1982) Agents of proteolysis and its inhibition in patis (fish sauce) fermentation. *Journal of Food Science,* ***47***:198-203.

OWENS, J.D. & MENDOZA, L.S.(1985) Enzymically hydrolysed and bacterially fermented fishery products. *Journal of Food Technology,* ***20***:273-293.

PAGE, J.W. & ANDREWS, J.W.(1973) Interactions of dietary levels of protein and energy on channel catfish (*Ictalurus punctatus*). *Journal of Nutrition,* ***103***: 1339-1346.

PEARCE, G., SY, L., RUSSELL, C., RYAN, C.A. & HASS, G.M.(1982) Isolation and characterization from potato tubers of two polypeptide inhibitors of serine proteases. *Archives of Biochemistry and Biophysics,* ***213***:456-462.

PEARSON, D.(1971) The chemical analysis of foods. 6th edition, J. & A. Churchill, London.

PEDERSEN, O.(1987) Fish silage and quality, product specifications and quality standard. I and II. *Norsk Fiskeoppdrett,* ***12***(2):74-76.

PEDERSON, C.S.(ed.)(1971) Microbiology of food fermentation. AVI Publishing Co., Inc., Westport, Conn., USA.

PEDERSON, C.S.(1978) Food fermentations. pp.311-314, In: *Encyclopedia of Food Science* (M.S. Petterson & A.H. Johnson,eds.). AVI Publishing Co., Inc., Westport, Conn., USA.

PEDERSON, C.S.(1979) Fermented vegetable products. pp.153- 209, In: *Microbiology of Food fermentation*. 2nd edition, AVI Publishing Co., Inc., Westport, Conn., USA.

PEDERSON, C.S. & ALBURY, M.N.(1969) The sauerkraut fermentation. New York State Agricultural Experimental Station Technical Bulletin No.824. 84pp.

PHROMKUNTHONG, W. & CHETHANON, S.(1987) Replacement of fish meal with silage for feeding tilapia (*Sarotherodon niloticus*). *Songklanakarin Journal of Science and Technology, **9***:215-223.

PIKE, I.H.(1990) Fish meal and oil in farm feeds. *Fish Farming International, **17***:64.

POTTER, D.P., TATTERSON, I.N. & WIGNALL, J.(1978) Fish by-products: fish meal and fish silage. *Process Biochemistry, **13***:22-25.

POULTER, R.G., JAYAWARDENA, K.M., GANEGODA, P. & RANAWEERA, K.N.P. (1980) Studies on fish silage in Sri Lanka: a summary. pp.64-68, In: *Fish silage production and its use* (J.G. Disney & D. James,eds.). *FAO Fisheries Report* No.230. FAO, Rome.

PRATT, D.E. & WATTS, B.M.(1964) The antioxidant activity of vegetable extracts. *Journal of Food Science, **29***:27.

PRICE, R.J. & LEE, J.S.(1970) Inhibition of *Pseudomonas* spp. by H_2O_2 producing lactobacilli. *Journal of Milk and Food Technology, **33***:13-17.

RAA, J.(1980) Biochemistry of microbial fish spoilage and preservation by lactic acid bacteria and added acid. pp.3-16, In: *Global Impacts in Applied Microbiology* (S.O. Emejuaiwe, O. Ogunbi & S.O. Sanni,eds.). Academic Press, London.

RAA, J. & GILDBERG, A.(1976) Autolysis and proteolytic activity of cod viscera. *Journal of Food Technology, **11***:619-628.

RAA, J. & GILDBERG, A.(1982) Fish silage: a review. *CRC Critical Review in Food Science and Nutrition, **16***:383-419.

RAA, J., GILDBERG, A. & STROM, T.(1983) Silage production- theory and practice. pp.117-132, In: *Upgrading wastes for feeds and food* (D.A. Ledward, A.J. Taylor & R.A. Lawrie,eds.). Butterworths, London.

RADLER, F.(1975) The metabolism of organic acids by lactic acid bacteria. pp.25-26, In: *Lactic acid bacteria in beverages and food* (J.G. Carr, C.V. Cutting & G.C. Whiting,eds.). Academic Press, London.

REILLY, A.(ed.)(1985) Spoilage of tropical fish and product development. *FAO Fisheries report* No.317 Supplement. FAO, Rome. 474pp.

RITCHIE, A.H. & MACKIE, I.M.(1982) Preparation of fish protein hydrolysates. *Animal Feed Science and Technology, **7***:125-133.

ROA, P.D.(1965) Ensilage of fish by microbial fermentation. *Fish News International,* ***4***:283-286.

ROSELUND, G. & UTNE, F.(1981) Bindermidler i vatfor til fisk. Fiskeridirektoratets Vitamininstitutt, Bergen, Norway. Papporter og Oversikter, No.13 A/B. 56pp.

ROSS, L.G. & GEDDES, J.A.(1979) Sedation of warmwater fish species in aquaculture research. *Aquaculture,* ***16***:183-186.

RUNGRUANGSAK, K. & UTNE, F.(1981) Effect of different acidified wet feeds on protease activities in the digestive tract and on the growth rate of rainbow trout (*Salmo gairdneri* Richardson). *Aquaculture,* ***22***:67-79.

RYAN, C.A., HASS, G.M. & KUHN, R.W.(1974) Purification and properties of a carboxy-peptidase inhibitor from potatoes. *Journal of Biological Chemistry,* ***249***:5495-5499.

SAGUA, V.O.(1976) Aquaculture and fishery development in Nigeria: a review. pp.1-34, In: *Proceedings of the Agricultural Society of Nigeria Conference.* ASN, Ile-Ife, Nigeria.

SANTIAGO, C.B. & LOVELL, R.T.(1988) Amino acid requirements for growth of Nile tilapia. *Journal of Nutrition,* ***118***:1540-1546.

SATIA, B.P.(1989) A regional survey of the aquaculture sector in Africa south of the Sahara. ADCP/REP/89/36. FAO/UNDP, Rome. 60pp.

SATIA, B.P.(1990) National reviews for aquaculture development in Africa. 29. Nigeria. *FAO Fisheries Circular* No.770.29. FAO, Rome, 193pp.

SCHLEGEL, H.G.(1986) General microbiology. Cambridge University Press, Uk. 587pp.

SHANG, Y.C.(1981) Aquaculture economics: basic concepts and methods of analysis. Westview Press, Colorado, USA. 153pp.

SHEWAN, J.M.(1977) The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. pp.51-66, In: *Handling, processing and marketing of tropical fish.* Tropical Products Institute, London.

SHIAU, S.-Y., CHUNG, J.L. & SUN, C.L.(1987) Inclusion of soybean meal in tilapia (*Oreochromis niloticus* X *O. aureus*) diets at two protein levels. *Aquaculture,* ***65***:251-261.

SHIAU, S.-Y., YU, H.L., HWA, S., CHEN, S.Y. & HSU, S.I.(1988) The influence of carboxy-methyl cellulose on growth, digestion, gastric emptying time and body composition of tilapia. *Aquaculture,* ***70***:345-354.

SHIMANG, G.N.(1992) Post harvest losses in inland fisheries in Nigeria. pp.78-83, In: *Proceedings of the symposium on post-harvest fish technology. CIFA Technical Paper* No.19. FAO, Rome.

SIEBERT, G. & SCHMITT, A.(1965) Fish tissue enzymes and their role in the deteriorative changes in fish. pp.47-52, In: *The technology of fish utilization,* FAO International Symposium, Husum. (R. Kreuzer,ed.). Fishing News Books Ltd., London.

SINELL, H.J.(1980) Interacting factors affecting mixed populations. pp.221-231, In: *Microbial ecology of foods* Volume 1 (J.H. Silliker, ed.). Academic Press, London.

SKREDE, A.(1981) Utilization of fish and animal by-products in mink nutrition. VI. The digestibility of amino acids in fish viscera products and the effects of feeding on growth, fur characteristics, and reproduction. *Acta Agriculturae Scandinavica,* ***31***:171-178.

SMAIL, D.A., HUNTLY, P.J. & MUNRO, A.L.S.(1990) Fate of four fish pathogens after exposure to an ensiling mixture containing fish farm mortalities. *International Council for the Exploitation of the Sea (ICES) Mariculture Committee Paper* CM 1990/F:50. 7pp.

STANTON, W.R. & YEOH, Q.L.(1977) The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. pp.3-16, In: *Handling, processing and marketing of tropical fish*. Tropical Products Institute, London.

STECHER, G.B., WINDHOLZ, M. LEAHY, S. BOLTON, D. & EATON, L.(eds.) (1968) Merck Index. pp.604-605. Merck & Co., Inc. Raltamy, New Jersey, USA.

STEFFENS, W. & SPANGENBERG, M.(1985) Using zooplankton silage for fry production. *Zeitschrift fuer die Binnenfischerei der DDR,* ***32***(7):203-207.

STONE, F.E. & HARDY, R.W.(1986) Nutritional value of acid stabilized silage and liquefied fish protein. *Journal of Science of Food and Agriculture,* ***37***:797-803.

STONE, F.E. & HARDY, R.W.(1989) Plasma amino acid changes in rainbow trout (*Salmo gairdneri*) fed freeze-dried fish silage, liquefied fish and fish meal. pp.419-426, In: *Proceedings of the Aquaculture International Congress.* Vancouver, B.C., Canada.

STONE, F.E., HARDY, R.W. & SPINELLI, J.(1984) Autolysis of phytic acid and protein in canola meal (*Brassica* spp.), wheat bran (*Triticum* spp.) and fish silage blends. *Journal of Science of Food and Agriculture,* ***35***:513-519.

STONE, F.E., HARDY, R.W., SHEARER, K.D. & SCOTT, T.M.(1989) Utilization of fish silage by rainbow trout (*Salmo gairdneri*). *Aquaculture,* ***76***:109-118.

STOREBAKKEN, T.(1985) Binders in fish feeds. 1. Effect of alginate and guar gum on growth, digestibility, feed uptake and passage through the gastro-intestinal tract of rainbow trout. *Aquaculture,* ***47***:11-26.

STRASDINE, G.A., BEAMES, R.M., FISHER, L.J. & JONES, Y.M.(1988) An assessment of ensiling the processing wastes from dogfish to produce a protein feed for monogastric animals. *Canadian Journal of Animal Science,* ***68***:873-880.

STROM, T.(1992) Ensiling of fish by lactic acid bacterial fermentation for feed production. pp.142-147, In: *Proceedings of IPFC Workshop on fish silage* (D. James,ed.). *FAO Fisheries Report* No.470. FAO, Rome.

STROM, T., GILDBERG, A., STORMO, B. & RAA, J.(1980) Fish silage: why not use propionic acid? pp.352-355, In: *Advances in Fish Science and Technology,* Jubilee Conference of the Tory Research Station, Aberdeen, Scotland (J.J. Connell,ed.). Fishing News Books Ltd., Surrey, England.

SUBASINGHE, S., MOHIDEEN, M.S. & VIDANAPATHIRANA, S.(1990) Microbiological and biochemical changes in *Amblygaster sirm* during high-salt fermentation. pp.29-303, In: *Post-harvest technology, preservation and quality of fish in southeast Asia* (P.J.A. Reilly, R.W.H. Parry & L.E. Barile,eds.). International Foundation for Science (IFS), Stockholm, Sweden.

SVOBODOVA, Z., PRAVDA, D. & PALACKOVA, J.(1991) Unified methods of haematological examination of fish. Research Institute of Fish Culture and Hydrobiology, Vodany, Czechoslovakia. 31pp.

TACON, A.G.J.(1985) Nutritional fish pathology. ADCP/REP/85. UNDP/FAO, Rome. 22pp.

TACON, A.G.J.(1990) Standard methods for the nutrition and feeding of farmed fish and shrimp. Volume 1 : The essential nutrients. Argent Laboratories Press, Redmond, Washington. 117pp.

TACON, A.G.J.(1993) Feed ingredients for warmwater fish : fish meal and other processed feedstuffs. *FAO Fisheries Circular* No.856. FAO, Rome. 64pp.

TACON, A.G.J. & JACKSON, A.J.(1985) Utilization of conventional and unconventional protein sources in practical feeds. pp.119-145, In: *Nutrition and Feeding of Fish* (C.B. Cowey, A.M. Mackie & J.G. Bell,eds.). Academic Press, London.

TATTERSON, I.N.(1982) Fish silage- preparation, properties and uses. *Animal Feed Science and Technology, 7*:153-159.

TATTERSON, I.N. & WINDSOR, M.L.(1974) Fish silage. *Journal of Science of Food and Agriculture, 25*:369-379.

TIBBETTS, G.W., SEERLY, R.W., McCAMPBELL, H.C. & VEZEY, S.A.(1981) An evaluation of an ensiled waste fish product in swine diets. *Journal of Animal Science, 52*:93-100.

TRAMER, J.(1966) Inhibiting effect of *Lactobacillus acidophilus*. *Nature, 211*:204-205.

TREVINO, J.E., YOUNG, R.H., UVALLE, A., CREAN, K., MACHIN, D.H. & LEAL, E.H. (1982) Fish silage from by-catch. pp.103-105, In: *Fish by-catch from the sea*. IDRC, Ottawa, Canada.

TRS (Torry Research Station)(1989) Yield and nutritional value of the commercially more important fish species. pp.173-175, In: *FAO Fisheries Technical Paper* No.309. FAO, Rome. 187pp.

TWIDDY, D.R., CROSS, S.J. & COOKE, R.D.(1987) Parameters involved in the production of lactic acid preserved fish-starch substrate combinations. *International Journal of Food Science and Technology, 22*:115-121.

URBAN, E.R. & PRUDER, G.D.(1991) A method of economic comparisons for aquaculture diet development. *Aquaculture, 99*:127-142.

UYS, W.(1989) Aspects of the nutritional physiology and dietary requirements of juvenile and adult sharptooth catfish *Clarias gariepinus* (Pisces:Clariidae). Ph.D. Thesis, Rhodes University, South Africa. 190pp.

VANDEN BOSSCHE, J.-P. & BERNACSEK, G.M.(1990) Source book for the inland fishery resources of Africa:2. *CIFA Technical Paper* No.18.2. FAO, Rome. 411pp.

VAN VEEN, A.G. & STEINKRAUS, K.H.(1970) Nutritive value and wholesomeness of fermented foods. *Food Chemistry, 18*:576-578.

VAN WYK, H.J. & HEYDENRYCH, C.M.S.(1985) The production of naturally fermented fish silage using various lactobacilli and different carbohydrate sources. *Journal of Science of Food and Agriculture,* ***36***:1093-1103.

VENS-CAPPELL, B.(1984) The effects of extrusion and pelletting of feed for trout on the digestibility of protein, amino acid and energy and on feed conversion. *Aquaculture Engineering,* ***3***:71-89.

VERBURG, G.B. & FREEMAN, D.W.(1984) On-farm production of liquefied catfish protein. *Aquaculture,* ***41***:171-179.

VIOLA, S., GUR, N. & ZOHAR, G.(1986) Effects of pelleting temperature, binders and basic grains on water-stability of pellets and on growth of tilapia. *Bamidgeh,* ***37***:19-26.

VIVEEN, W., RICHTER, C., VAN OORDT, P., JANSSEN, J. & HUIMAN, E.(1985) Practical manual for the culture of the African catfish (*Clarias gariepinus*). Directorate General for International Technical Cooperation, The Hague, The Netherlands, 94pp.

WEE, K.L., KERDCHUEN, N. & EDWARDS, P.(1986) Use of waste grown tilapia silage as feed for *Clarias batrachus* L. *Journal of Aquaculture in the Tropics,* ***1***:127-137.

WHEATON, F.W. & LAWSON, T.B.(1985) Processing aquatic food products. John Wiley and Sons Inc., New York. 518pp.

WILSON, R.P.(1989) Protein and amino acid requirements of fishes. pp.51-76, In: *Progress in Fish Nutrition* (S.Y. Shiau,ed.). Marine Food Science Series No.9. National Taiwan Ocean University, Keelung, Taiwan.

WILSON, R.P., FREEMAN, D.W. & POE, E.W.(1984) Three types of catfish offal meals for channel catfish fingerlings. *Progressive Fish-Culturist,* ***46***:126-132.

WIRAHADIKUSUMAH, S.(1968) Preventing *Clostridium botulinum* Type E poisoning and fat rancidity by silage fermentation. *Lantbrukshoegskolans Annaler,* ***34***:551-589.

WIRAHADIKUSUMAH, S., RAJALA, O., LINDGREN, S. & NILSSON, R.(1971) Antimicrobial properties of lactic acid bacteria isolated from fish silage. *Swedish Journal of Agricultural Research,* ***1***:225-227.

WOOD, J.F.(1980a) The preparation of water-stable fish feeds. 1. Methodology of feed preparation - a brief resume. *Tropical Science,* ***22***:351-356.

WOOD, J.F.(1980b) The preparation of water-stable fish feeds. 2. The potential for fish silage as a feed ingredient. *Tropical Science,* ***22***:357-360.

WOOD, J.F.(1987) The functional properties of feed raw materials and their effect on the production and quality of feed pellets. *Animal Feed Science and Technology,* ***18***:1-17.

WOOD, J.F., CAPPER, B.S. & NICOLAIDES, L.(1985) Preparation and evaluation of diets containing fish silage, cooked fish preserved with formic acid and low-temperature-dried fish meal as protein sources for mirror carp (*Cyprinus carpio*). *Aquaculture,* ***44***:27-40.

YAMADA, S.(1982) Plasma amino acid changes in *Tilapia nilotica* fed a casein and a corresponding free amino acid diet.
Bulletin of the Japanese Society of Scientific Fisheries, ***48***:1783-1787.

YONE, Y., HOSSAIN, M.A., FURUICHI, M. & KATO, F.(1986a) Effect of fermented and fermented-resteamed scrap meals on growth and feed efficiency of red sea bream. *Bulletin of the Japanese Society of Scientific Fisheries,* ***52***:549-552.

YONE, Y., HOSSAIN, M.A., FURUICHI, M. & KATO, F.(1986b) Effect of fermented scrap meal on proximate compositions of muscle and liver, hematological characteristics, and chemical components in blood plasma of red sea bream. *Bulletin of the Japanese Society of Scientific Fisheries,* ***52***:1461-1464.

ZAR, J.H.(1984) Biostatistical analysis.
Prentice-Hall, Englewood Cliffs, NJ, USA. 383pp.

ADDITIONAL REFERENCES

"Quod ali cibus est aliis fiat acre venenum"

Lucretius, 95-55 B.C.

ADAMS, M.R., COOKE, R.D. & RATTAGOOL, P.(1985) Fermented fish products of S.E. Asia. *Tropical Science*, ***25***:61-73.

ADCP (Aquaculture Development and Coordination Programme)(1983) Fish Feeds and feeding in developing countries - an interim report on the ADCP Feed Development Programme. ADCP/REP/83/18. UNDP/FAO, Rome, 97pp.

ANDERSON, J., JACKSON, A.J., MATTY, A.J. & CAPPER, B.S.(1984) Effects of dietary carbohydrate and fibre on the tilapia *Oreochromis niloticus* (Linn.). *Aquaculture*, ***37***:303-314.

ARNESEN, P., BRATTAS, L.E., OLLI, J. & KROGDAHL, A.(1989) Soybean carbohydrates appear to restrict the utilization of nutrients by Atlantic salmon (*Salmo salar*). pp.273-280, In: *Proceedings of the Third International Symposium on Feeding and Nutrition in Fish*, Toba, Japan.

BATTERHAM, E.S., GORMAN, T.B.S. & CHVOJKA, R.(1983) Nutritional value and mercury content of fish silage for growing pigs. *Animal Feed Science and Technology*, ***9***:169-179.

BOLSEN, K.K. & HEIDKER, J.I.(1985) Silage additives USA. Chalcombe Publications, USA.

BOONYARATPALIN, M.(1982) Fermented trash fish as possible feeds for fish under culture. FAO Field Document.. pp.111-112, In: Report of the 2nd Advisory Committee Meeting of Network of Aquaculture Centres in Asia (NACA). FAO/UNDP NACA Project. Bangkok, Thailand.

CHINABUT, S., LIMSUWAN, C. & KITSAWAT, P.(1991) Histology of the walking catfish, *Clarias batrachus*. National Inland Fisheries Institute, Bangkok, Thailand.

COOKE, R.D., TWIDDY, D.R. & REILLY, P.J.(1987) Lactic acid fermentation as low-cost means of food preservation in tropical countries. *Microbiology Reviews*, ***46***:369-379.

DANIELEWSKI, S.(1980) Freshwater fish farming demonstration and development in Nigeria. Final Report Project NIR/75/059.

DISNEY, J.G., TATTERSON, I.N. & OLLEY, J.(1977) Recent developments in fish silage. pp.231-240, In: Handling, processing and marketing of tropical fish. Tropical Products Research Institute, London.

DUNCAN, D.B.(1955) Multiple range and multiple F tests. *Biometrics*, ***11***: 1-42.

DYER, J., FRENCH, H.V. & SNOW, J.M.(1950) Proteins in fish muscle. *Journal of the Fisheries Research Board of Canada*, ***7***: 585-593.

EDWARDS, P.(1985) Use of terrestrial vegetation and aquatic macrophytes in aquaculture. Paper presented at the Bellagio Symposium on Detritus in Aquaculture (August 1985). 22pp.

ESPE, M. (1990) Ensilasje er godt son for, men kvalitetskriteriene ma vaere strenge. *Norsk Fiskeoppdrett, **15***(9):36-39.

FAO (Food and Agriculture Organization)(1965) Investigation of the possibility of brackishwater fish culture in the Niger Delta. Report to the Government of Nigeria. Expanded Programme of Technical Assistance (EPTA/FAO), Rome.

FATIMA, R. & QADRI, R.B.(1987) Studies on the preparation of fish silage. *Tropical Science, **27***:1-8.

FERGUSON, K.A., HEMSLEY, J.A. & REIS, P.J.(1967) Nutrition and wool growth - the effect of protecting dietary protein from microbial degradation in the rumen. *Australian Journal of Science, **30***:215-217.

HASTINGS, W.H.(1964) Fish feed processing research. *Feedstuffs, **36***(21):13.

HEPHER, B.(1968) A modification of Hasting's method for the determination of water stability of fish-feed pellets. *EIFAC Technical Paper* No.9. FAO, Rome.

HILTON, J.W. & SLINGER, S.J.(1983) Effect of wheat bran replacement of wheat middlings in extrusion processed (floating) diets on the growth of juvenile rainbow trout (*Salmo gairdneri*). *Aquaculture, **35***:201-210.

HUIT, N.B.(1986) Evaluation of fish silage prepared with molasses and *Sargassum spp.* M.Sc. Thesis, UPV College of Fisheries, Philippines.

JANGAARD, P.M.(1991) Fish silage in Norway: current activities. pp.28-53, In: *Fish Silage Workshop, Bulletin of the Aquaculture Association of Canada,* No.91-1 (R.G. Ackman & J. O'Dor,eds.). Aquaculture Association of Canada.

JAYARAM, M.G. & SHETTY, H.P.C.(1981) Formulation, processing and water stability of two new pelleted feeds. *Aquaculture, **23***:355-359.

JOHNSON, R.J., BROWN, N., EASON, P. & SUMNER, J.(1985) The nutritional quality of two types of fish silage for broiler chickens. *Journal of Science of Food and Agriculture, **36***:1051-1056.

KARMAS, E. & LAUBER, E.(1987) Novel products from underutilized fish using combined processing technology. *Journal of Food Science, **52***:7-9.

KAUSHIK, S.J.(1989) Use of alternative protein sources for intensive rearing of carnivorous fishes. pp.181-200, In: *Progress in Fish Nutrition* (S.-Y. Shiau,ed.) Marine Food Science Series No.9, National Taiwan Ocean University, Keelung, Taiwan.

KROGDAHL, A.(1985) Fish viscera silage as a protein source for poultry. *Acta Agriculturae Scandinavica, **35***:3-32.

LUQUET, P.(1991) Nutrient requirement of tilapia, *Oreochromis* spp. pp.169-179, In: *Handbook of nutrient requirements of finfish* (R.P. Wilson,ed.). C.R.C. Press Inc., Florida, USA.

MARIOGHAE, I.E.(1987) The biology and culture of shrimps and prawns. pp.63-64, In: *Nigerian Institute for Oceanography and Marine Research Annual Report.* NIOMR, Lagos, Nigeria.

MERINO, A.P.(1985) A comparison of the chemical composition of acid preserved and fermented tilapia silages. M.Sc. Thesis, University of Stirling, Scotland. 37pp.

MESCHKAT, A.(1967) The status of warmwater fish culture in Africa. *FAO Fisheries Report* No.44(2):88-108. FAO, Rome.

MILLER, E.L.(1967) Determination of tryptophan content of feeding stuffs with particular reference to cereals. *Journal of Science of Food and Agriculture,* ***18***:381-385.

NEW, M.B.(1987) Feed and feeding of fish and shrimp. FAO/ADCP/Rep/87/26. UNDP/FAO, Rome. 275pp.

NILSON, R.(1970) Preventing the deterioration of protein sources and products. pp.353-356, In: *Evaluation of novel protein products* (A.E. Bender, R.H. Kihlberg, B. Lofqvist & L. Munck,eds.). Pergamon Press Ltd., Oxford, UK.

NOAILLAC-DEPEYRE, J. & GAS, N.(1974) Fat absorption by the enterocytes of the carp (*Cyprinus carpio* L.). *Cell Tissue Research,* ***155***:353-365.

NONOTTE, L., NONOTTE, G. & LERAY, G.(1986) Morphological changes in the middle intestine of the rainbow trout, *Salmo gairdneri,* induced by a hyperosmotic environment. *Cell Tissue Research,* ***243***:619-628.

OFFER, N.W., EVANS, R.A. & AXFORD, R.F.E.(1971) The protection of dietary protein by formaldehyde treatment and its effect on the composition of duodenal digesta in sheep. *Proceedings of the Nutrition Society,* ***30***:42A.

OLYMPIA, M., VALENZUELA, A.G. & TAKANO, M.(1990) Burong bangus, a traditional fermented fishery product in the Philippines. pp.67-76, In: *Post harvest technology, preservation and quality of fish in southeast Asia* (P.J.A. Reilly, R.W.H. Parry & L.E. Barile,eds.). International Foundation for Science (IFS), Stockholm, Sweden.

PETERS, G.(1982) The effect of stress on the stomach of the European eel, *Anguilla anguilla* L. *Journal of Fish Biology,* ***21***:497-512.

RAGUNATH, M.R. & McCURDY, A.R.(1987) Autolysis-resistant sediment in fish silage. *Biological Wastes,* ***20***:227-239.

ROSS, L.G. & ROSS, B.(1984) Anaesthetic and sedative techniques for fish. Institute of Aquaculture, University of Stirling, Stirling. 35pp.

SAISITHI, P., KASEMSARN, B., LISTON, J. & DOLLAR, A.M.(1966) Microbiology and chemistry of fermented fish. *Journal of Food Science,* ***31***:105-110.

SEGNER, H. & JUARIO, J.V.(1986) Histological observations on the rearing of milkfish, *Chanos chanos,* fry using different diets. *Journal of Applied Ichthyology,* ***2***:162-173.

SIVALINGAM, S.(1972) Fish culture possibilities around Lagos Lagoon and results of recent trials. *Federal Fisheries Service Occasional Paper* No.3. 21pp.

SOUANE, M.(1987) Fish fermentation technology in Senegal. Paper presented at the United Nations University Workshop on Fish Fermentation Technology, June 22-26, Seoul, Korea.

STIRLING, H.P.(1985) Chemical and biological methods of water analysis for aquaculturists. Institute of Aquaculture, University of Stirling, Stirling. 119pp.

STORCH, V., JUARIO, J.V. & PASCUAL, F.P.(1986) Early effects of nutritional stress on the liver of milkfish, *Chanos chanos* (FORSSKAL) and on the hepatopancreas of the tiger prawn, *Penaeus monodon* (FABRICUS). *Aquaculture,* ***36***:229-236.

STRADMEYER, L., METCALF, N.B. & THORPE, J.E.(1988) Effect of food pellet shape and texture on the feeding response of juvenile Atlantic salmon. *Aquaculture,* ***73***:351-379.

STROM, T. & EGGUM, O.(1981) Nutritional value of fish viscera silage. *Journal of Science of Food and Agriculture,* ***32***:115-120.

TABACHEK, J.L.(1988)The effect of food particle size on the growth and food efficiency of Arctic charr (*Salvelinus alpinus*). *Aquaculture,* ***71***:319-330.

TACON, A.G.J., JAUNCEY, K., FALAYE, A., PANTHA, M., MAC GOWAN, I. & STAFFORD, E.A.(1983) The use of meat and bone meal, hydrolysed feather meal and soybean meal in practical fry and fingerling diets for *Oreochromis niloticus.* pp.356-365, In: *Proceedings of the First International Symposium on Tilapia in Aquaculture* (L. Fishelson & Z. Yaron,eds.). Tel Aviv University, Israel.

TANG, T-P.(1986) An investigation of the use of acid-preserved "mullet" and mutton silage in moist rations for rainbow trout (*Salmo gairdneri* RICHARDSON). M.Sc. Thesis, university of Stirling, Scotland. 41pp.

VANBELLE, M., ARNOULD, J.M. & JOSSART, J.M.(1978) Effect of formaldehyde treatment of silage upon ruminal activity, digestion, and nitrogen retention by sheep. pp.629-639, In, *Constraints to grass growth and grassland output,* Merebeke, Belgium.

VATANA, P. & DEL ROSARIO, R.R.(1983) Biochemical changes in fermented rice-shrimp, *Macrobrachium idella* mixture: changes in protein functions. *Food Chemistry,* ***12***:33-43.

WALTER, H.E.(1984) Proteinases : methods with haemoglobin, casein and azocoll as substrates. pp.270-277, In: *Methods of enzymatic analysis* Volume V. (H.U. Bergmeyer,ed.). Verlag Chemie, Weinheim.

WANKOWSKI, J.W.J. & THORPE, J.E.(1979) The role of food particle size on the growth and juvenile Atlantic salmon (*Salmo salar*). *Journal of Fish Biology,* ***14***:351-379.

WILLEMSE, J.J., MARKUS-SILVIS, L. & KETTING, G.H.(1984) Morphological effects of stress in cultured elvers, *Anguilla anguilla* (L.). *Aquaculture,* ***36***:193-201.

WIRAHADIKUSUMAH, S.(1968) Preventing *Clostridium botulinum* Type E poisoning and fat rancidity by silage fermentation. *Lantbrukshoegskolans Annaler,* ***34***:551-589.

ZUBERI, R., FATIMA, R., SHAMSHAD, S.I. & QADRI, R.B.(1992) Preparation of fish silage by microbial fermentation. pp.155-164, In: *Proceedings of IPFC Workshop on fish silage* (D. James,ed.). *FAO Fisheries Report* No.470. FAO, Rome.

APPENDIX

The experiments described in this study have been published in

FAGBENRO, O.A. & JAUNCEY, K.(1993) Chemical and nutritional quality of raw, cooked and salted fish silages.
Food Chemistry, 48 (3): 331-335.

FAGBENRO, O.A. & JAUNCEY, K.(1993) Chemical and nutritional quality of stored fermented fish (tilapia) silage.
Bioresource Technology, 46 (3): 207-211.

FAGBENRO, O.A. & JAUNCEY, K.(1994) Chemical and nutritional quality of dried fermented fish silage and their nutritive value for tilapia (*Oreochromis niloticus*).
Animal Feed Science and Technology, 45 (2): 167-176.

FAGBENRO, O.A. & JAUNCEY, K.(1994) Chemical and nutritional quality of fermented fish silage containing potato extracts, formalin or ginger extracts.
Food Chemistry, 50 (4): 383-388.

FAGBENRO, O.A. & JAUNCEY, K.(1994) Growth and protein utilization by juvenile catfish (*Clarias gariepinus*) fed moist diets containing autolysed protein from stored lactic acid fermented fish silage.
Bioresource Technology, 48 (1): 43-48.

FAGBENRO, O.A., JAUNCEY, K. & HAYLOR, G.S.(1994) Nutritive value of diets containing dried lactic acid fermented fish silage and soybean meal for juvenile *Oreochromis niloticus* and *Clarias gariepinus*.
Aquatic Living Resources, 7 (2): 79-85.

FAGBENRO, O.A. (1994) Evaluation of dried fermented fish silage as feedstuff in diets for *Oreochromis niloticus*.
Isareli Journal of Aquaculture, 46 (3/4): in press.

FAGBENRO, O.A. & JAUNCEY, K.(1995) Water stability, nutrient leaching and nutritional properties of moist fermented fish silage diets.
Aquaculture Engineering, 14 (1): in press.

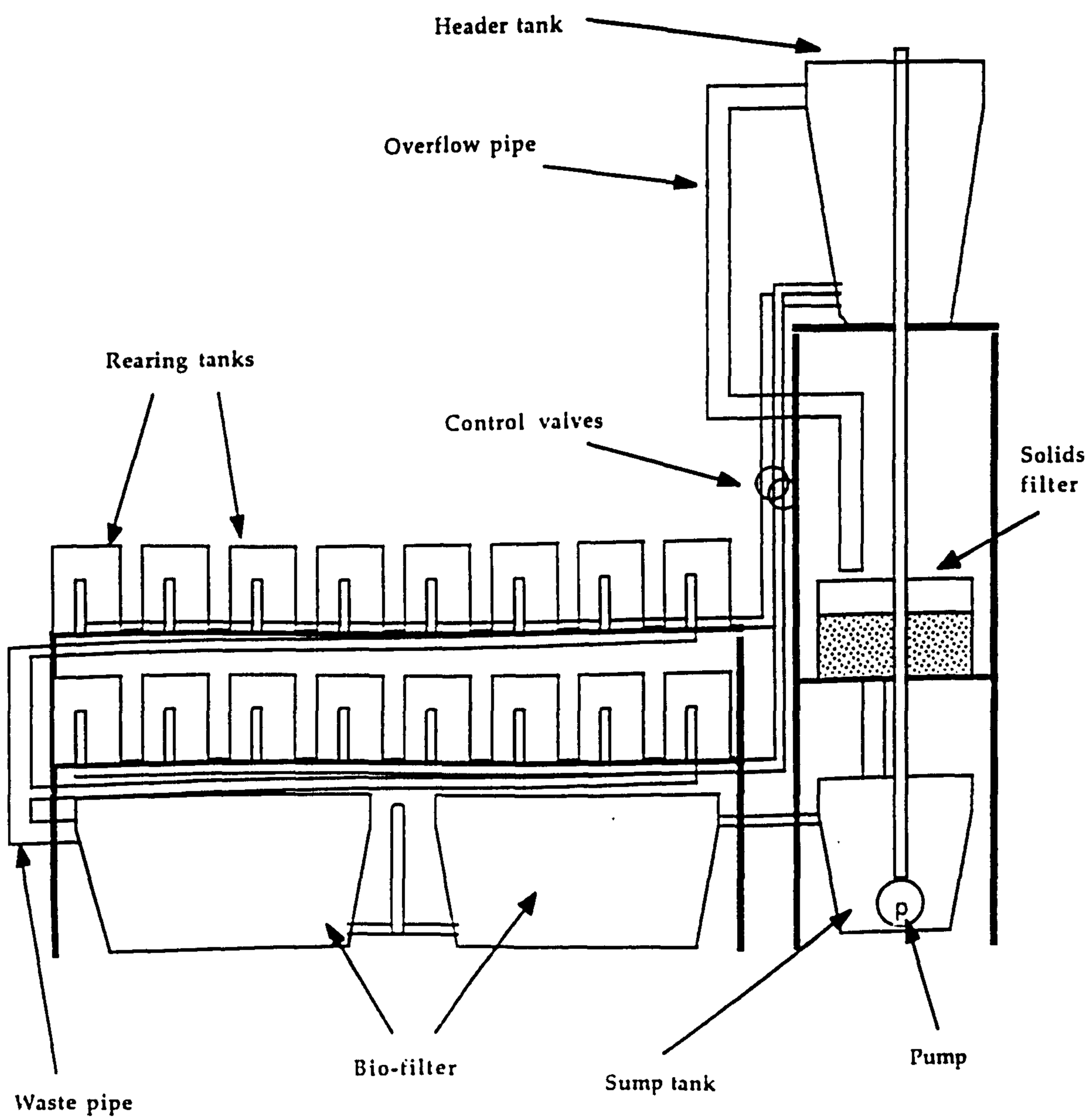
FAGBENRO, O.A. & JAUNCEY, K.(1995) Growth and protein utilization by juvenile catfish (*Clarias gariepinus*) fed dry diets containing co-dried lactic-acid fermented fish-silages and protein feedstuffs.
Bioresource Technology, 50 (2): in press.

FAGBENRO, O.A., JAUNCEY, K. & KRUEGER, R.(1995) Nutritive value of dried lactic acid fermented fish silage and soybean meal in dry diets for juvenile catfish, *Clarias gariepinus* (BURCHELL,1822).
Aquatic Living Resources, 8 (1): in press.

Appendix 1 : Optimum level of carbohydrates and inoculum used in lactic acid fermentation of fish products.

Substrate (fish)	Carbohydrate source (w/w)	Inoculum (v/w)	References
Fish by-catch	5% dried molasses + 30% corn	5% ***Lactobacillus acidophilus***	TIBBETTS *et al.*(1981)
Herring	10% molasses + 5% barley + 5% oats	10% ***L. plantarum***	LINDGREN & PLEJE (1983)
Hake	5% refined sugar + 5% whey powder	5% ***L. plantarum***	VAN WYK & HEYDENRYCH (1985)
Filleting wastes	12-15% molasses	5% ***L. plantarum***	BROWN & SUMNER (1985)
Tilapia (***Oreochromis niloticus***)	20% molasses 20% cassava	2% ***L. casei*** 2% ***L. casei***	WEE *et al.*(1986)
White perch	5% lactose	1% ***L. plantarum***	HASSAN & HEATH (1986,1987)
Trout	3% lactose	1% ***L. plantarum***	HASSAN & HEATH (1987)
Offals & guts of perch (***Lates niloticus***)	25% cassava + 12.5% sugar cane	1.25% ***L. plantarum*** + 1.25% ***Streptococcus lactis***	DHATEMWA (1989)
Tilapias	10% molasses	***L. plantarum***	DICKSON (1991)
Hydrolysed cod gurry	7.5% molasses	1% ***L. plantarum***	GIURCA & LEVIN (1992)
Salmon viscera	5% molasses 3% dextrose	0.8% ***S. faecium*** + 0.2% ***L. plantarum***	DONG *et al.*(1993)

Appendix 2 : Diagram (design) of the experimental system used for growth and digestibility trials.



Appendix 3 : Water quality in the experimental system during tilapia and catfish growth trials.

Water quality parameter	Range
pH	6.9-7.2
Temperature	27-28°C
Dissolved oxygen concentration (DO_2)	6.8-8 $mg.l^{-1}$
Total ammonia	0.098-0.125 $mg.l^{-1}$
Total nitrite	0.029-0.033 $mg.l^{-1}$