

**REPLACEMENT OF FISHMEAL WITH SHRIMP
WASTE SILAGE IN DIETS OF AFRICAN CAT FISH
CLARIAS GARIEPINUS (BURCHELL, 1822)
FINGERLINGS.**

BY



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**FEDERAL UNIVERSITY OF TECHNOLOGY
AKURE, ONDO STATE, NIGERIA**

August 2003

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**APROJECT REPORT SUMMITTED IN PARTIAL
FULFILMENT OF THE AWARD OF MASTER OF
TECHNOLOGY (M,TECH) IN FISHERIES**

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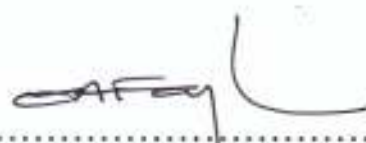
**THE DEPARTMENT OF FISHERIES AND WILDLIFE
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AKURE, ONDO STATE, NIGERIA**

August 2003



CERTIFICATION

I certify that this study was carried out by IHIMEKPEN A. FRIDAY, a Master of Technology (M.Tech) student of the Department of Fisheries and wildlife, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Ondo State.

 5/9/03

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DEDICATION

This project is dedicated to Almighty God who spared
My life and gave me strength and ability to complete this
project.



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ABSTRACT

Five (5kg) of raw mixed shrimp waste was fermented with 250ml of *Lactobacillus plantarum*, using 750g of cane molasses as carbohydrate source. After incubation for 14 days, a desirable and stable pH of 4.39 was attained, the product was co-dried with 15% (750g) hydrolysed feather meal. The blends were evaluated as protein providing 58.90% crude protein. The dried shrimp waste silage meal were incorporated as protein supplement into pelleted diets for catfish *Clarias gariepinus* fingerlings (12.05 ± 0.03 g) at 0%, 5%, 10%, 20%, 30% and 40% (diets 1 – 6) as replacement levels for brown fishmeal (72% protein). The effects of the diets of the shrimp waste silage (diets 1-6) on the growth and economic performance of the catfish fingerlings were evaluated. Results showed that catfish fed diets 1 – 6 (0 – 40%) showed no significant difference ($P > 0.05$) in food conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and apparent digestibility of nutrient (ADC protein) but there were significant difference ($P < 0.05$) in protein productive value (PPV) among the treatments. However, the fingerlings fed diets 1 – 5 (0-30%) showed no significant difference ($P > 0.05$) in mean weight gain but differed significantly ($P < 0.05$) with the mean weight gain of the fish fed diet 6 (40%).



The result showed that the best profit margin would be realized by feeding the catfish with 30% (diet 5) fermented shrimp waste silage meal. It was concluded that dried fermented shrimp waste silage meal is suitable and has a great potential feedstuff in African catfish diets up to 30% replacement for the highly expensive fishmeal.

One of the problems currently facing livestock (including fish) production in Nigeria is the inavailability of alternative feed ingredients that will encourage the production of cheap fish and livestock feeds (Fatureti and Iwezue, 1993).

Fish meal is the preferred dietary protein source for many farmed fish and shrimp species, and it is valued for its amino acid balance, vitamin content, palatability and unidentified growth factors (Tacon, 1993). However, its production is capital and energy – intensive, hence it is not manufactured in many developing countries. It is imported only at a prohibitive cost which is uneconomical to feed manufacturers. Periodic scarcity and high prices of fishmeal have therefore encouraged the evaluation of alternative protein feed stuffs (Fagbenro, 1994).

Oil seeds and pulses constitute a readily available source of dietary protein for use within aquafeed but their use is usually restricted by the presence of endogenous anti-nutritional factors or anti-nutrients (Hendricks and Bailey, 1989, Krogdahl, 1989). Furthermore, most of the conventional protein and energy feedstuffs in aquafeed have limited supplies and their prices have forced fish nutritionists and fish farmers to consider alternative sources. For these reasons there is need to evaluate unconventional animal

protein sources such as shrimp waste which are less expensive and are not useful to man as direct food.

Nigeria is naturally endowed with abundant quantities of shrimps on her continental shelf with a maximum potential yield estimated at 3,500-4000 metric tonnes (Olaniyi, 1999). About 1/3 of shrimp is made up of the head and the digestive system which are mostly discarded as waste, hence over 1,167 metric tonnes of shrimp waste could be produced annually from processing (Ajayi and Adetayo, 1982).

It is note worthy that vast amount of shrimp waste are produced in Nigeria by the export-oriented shrimp processing industry from harvest through various processing operations (Balogun and Akegbejo-Samsons, 1992). The increase in shrimp production without the corresponding development of technology of utilizing it's waste has resulted in waste collection, disposal and pollution problems .

Sun-drying or cooking followed by coarse milling are the most common techniques for shrimp waste meal production. However, there are limitations. Cooking requires excessive use of firewood or other scarce fuels, while sun-drying is frequently carried out under unhygienic conditions, leading to meals with high microbial loading (Barratt and

Montano, 1986, Hall and De-Silva, 1994, Fagbenro and Bello-Olusoji, 1997).

Shrimp waste (mainly the heads) has a lot of chitin, a protein carbohydrate complex that can only be digested by chitinase, an enzyme that is not available in culturable fishes. However, fermentation will render the chitin in shrimp waste fairly digestible (De-Silva and George, 1994). The resulting liquid silage could be co-dried with fillers (dry protein or carbohydrate feedstuffs) to aid drying but the choice is determined by the cost, the fish species and local availability (Lopez, 1990).

1.1 JUSTIFICATION:

Fish meal is a conventional animal protein source and is very expensive (₦150,000/tonne) and scarce. When available, it may be of poor quality, hence there is need to source and evaluate other under-utilized animal protein sources, some of which are shrimp parts which are usually discarded as waste, for there is no alternative use for these. Their disposal usually create environmental problems. Shrimp waste also contain high protein contents and unidentified growth factors both of which are suitable as potential protein feed stuffs in fish diets.

1.2 GENERAL OBJECTIVE:

The general objectives of this study is to evaluate the nutritional and economic value of fermented mixed shrimp waste silage meal (SWSM) as a dietary protein source and as replacement for fish meal in fish feed formulation.

1.3 SPECIFIC OBJECTIVES:

The specific objectives of this study are :

- to determine the proximate composition of fermented mixed shrimp waste silage meal

- to evaluate the effect of replacing fish meal at 0%, 5%, 10%, 30% and 40% with fermented mixed shrimp waste meal in the diets of *Clarias gariepinus* fingerlings.

- to evaluate the digestibility (protein) of the experimental diets.

- to estimate the economic benefits of feeding the experimental diets (0% 5%,10%,20% 30% and 40%, diets 1-6) to *C. gariepinus*.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 SHRIMP WASTE MEAL

Most of the shrimps found in Nigerian territorial waters are mainly *Paeneus notialis*, *Paeneus kerathurus*, *Parapeneopsis atlantica*, *Parapenaeopsis longirostris* etc. The southern pink shrimp, *Paeneus notialis*, is the most abundant and commercial species often constituting as much as 90% of the decapod landings (Ajayi and Adetayo, 1982, FAO, 2001).

The shrimp fishery in Nigeria is mainly export-oriented with a potential revenue of about 1.6 million U.S Dollar per annum (Balogun and Akegbejo-Samsons, 1992, Isebor, 1999). This may be the reason while most shrimp trawlers in Nigeria prefers to export the product rather than selling them at the local market.

Furthermore, for the product (shrimps) to be accepted in the international market, shrimp must be processed to meet international quality standard and this processing involves the removal of the head, the telson and the exoskeleton. As these shrimps are processed for export, considerable volumes of waste are generated. These wastes constitutes an environmental

hazard through their decomposition when they are not properly disposed. Instead of allowing these wastes to decompose, they can be processed into a product of high nutritional value for fish and livestock.

The shrimp wastes can be collected, chilled and stored prior to processing or processed directly by cooking and drying to give a stable product that can be used as a livestock or fish feed (De-Silva and George 1994). Feeds made from whole shrimp waste (heads, meat, viscera and hulls) tend to have a high fibre content because of the presence of chitin and a low protein content. Heating and water removal are important in the processing of shrimp waste into meal, but Meyers (1986), Table 1, suggested that a processing method which increases or retains the protein contents, eliminates heating and removes chitin would appear to be beneficial.

The intensity of heat used in drying the waste may significantly affect the nutritional qualities of the final meal.

Table I: Protein and chitin levels of various shrimp meals

Forms	% Crude Protein	% Chitin	%Corrected Protein
Dehydrated	37.3	20.6	28.0
Sun dried	51.7	9.0	47.8
Shrimp heads	58.2	11.1	53.5
Shrimp hulls	45.9	54.2	22.8

Source: Meyers (1986)

Fagbenro (1990) working on the apparent digestibility of crude protein and gross energy in some plant and animal based feed stuffs by *Clarias isheriensis* observed that although shrimp meal contain chitinous materials, the dry matter digestibility was high, possibly because of its attribute as a feed attractant.

Well processed shrimp head meal have an amino acid profile comparable to that of soybean (Meyers, 1986) and in addition comprises an alternative form of proteinous feed stuff in areas where ingredients may be in relatively short supply. Barratt and Montano, (1986), Table 2, also reported similarity in amino acid profile of shrimp head meal with fish meal (Menhaden)

Table 2: Amino acid composition of fishmeal and shrimphead meal

Amino acid	Fishmeal (Menhaden)	Shrimphead meal
Alanine	5.97	7.56
Arginine	5.98	6.70
Aspartic acid	8.76	9.07
Cystine	0.70	2.35
Glutamic acid	12.20	13.60
Glycine	6.58	6.57
Histidine	2.52	2.23
Isoleucine	4.28	6.20
Leucine	7.10	9.20
Lysin	7.66	9.20
Methionine	2.89	1.65
Phenylalanine	3.92	4.60
Proline	4.52	3.31
Serine	3.55	3.49
Threonine	3.85	4.22
Tryptophan	1.16	0.63
Tyrosine	3.25	3.64
Valine	4.98	6.77

Source: Barratt and Montano (1986)

2.2 FISH MEAL:

Of the world's production of 70 million tonnes of fish, about one third (1/3)

is not used for direct human consumption and may be considered as raw material for fishery by-products (Barlow and Windsor, 1984).

The most important by-products are fishmeal production, more than 90% of which are produced from oily species of fish such as anchovy, capeline and menhaden while less than 10% is being produced from white fish offal such as cod and haddock. Fishmeal is the predominant product as 95% of all raw material not used for direct human consumption is processed into fishmeal because it is a stable, high protein concentrate which maybe transported around the world without deterioration, whereas fish itself is highly perishable.

Historically, fishmeal has provided the major portion of protein in most dry commercial aquatic feeds because the protein derived from fishmeal has been shown in research and practical production to be used efficiently (Rumsey, 1993).

2.2.1 COMPOSITION OF FISHMEAL:

Fish meal contains 65-72% crude protein, 9.6% lipid, 24.25% ash, 0.7% fibre and 0.45% of other constituents for white and brown fish respectively (Ayina *et al*, 1993 and Fasakin *et al* 1998). Mineral component include iodine, manganese, iron, calcium and phosphorus. Heavy metals and other elements in fishmeal include aluminium, flouride,



lead, mercury, molybdenum and antimony. Vitamins like Folic, B₁₂, Choline and Biotin are also present (Barlow and Windsor, 1984). It is also known to contain unknown growth factor (U.G.F). This facilitates growth in animals, including fish.

Fishmeal has digestibility of about 80 to 95%. It has total digestible nutrient (T.D.N) value of 65% for white fish meal and TDN of 78.5% for herring meal, the metabolisable energy of fish meal (white fish) is also found to be 2,860 kcal/kg (Fasakin and Balogun, 1998). However, the production of fish meal is not likely to increase in the nearest future as fish stocks used for fish meal production appear to be in worldwide decline (Harvey 1991, New 1991). Production in the world's primary producing countries were 12% less in 1990 than 1989, by the year 2001, it was predicted that fishmeal production will probably decline by another 5% (Starkey, 1990). Hence, the success of aquaculture most likely will depend on the fishfeed manufacturing industries reducing its dependence on fishmeal.

2.3 SILAGE:

Silage is the material produced by fermentation of crops (fish, leaves, shrimp etc) of high moisture content (Windsor and Tatterson 1974). Ensilage is the name given to the process. Fermentation process is controlled either by encouraging anaerobic lactic fermentation by bacteria on a carbohydrate source or by direct addition of a weak acid solution.

Silage (fish, shrimps etc) prepared by using both organic or inorganic acid must be neutralised before it can be fed to animals. However, a fermented silage has the advantage because acids are very expensive, difficult to obtain and dangerous to store, while acid are not needed in organic fermentation as the acids is generated during fermentation through the process of lactic acid fermentation (De-Silva and George, 1994). An energy source and inoculum of lactic acid bacteria are needed to start the fermentation process. Energy source like sugar must be added because bacteria needs it to hasten fermentation process. A good silage is expected to change from semi-solid state to liquid state in 2 – 3 days as the pH is reduced and the enzymes breaks down the

protein. If the liquid and solid (sediment) parts are separated by centrifugation, the liquid represents about 60 – 70% (wet weight) of the silage. The liquid contains about half the protein and the rest remaining with the sediment (De-Silva and George, 1994).

Liquid silage can be co-dried with dried feedstuff (filler) materials such as carbohydrates, cereals and crop residues or animal by-product (such as poultry by-product meal, hydrolyzed feather meal, meat and bone meal). According to Hady *et al* (1983) co-drying the silage with filler mixture facilitates drying by providing particles on which the silage can be absorbed, creates the possibilities of customising the nutritional and economic values of the dry product by varying the combination and proportion of ingredients with which the eventual diet is formulated. The protein and oil content of the dried product is easily altered by the type and amount of filter used, (Fagbenro and Jauncey, 1998).

Water removal can be accomplished only by evaporation (Hardy, 1983). The intensity of heat used in drying the wet silage may significantly affect nutritional qualities of the final meal.

2.4 SHRIMP WASTE SILAGE:

Shrimp waste silage is a material made from shrimp waste (mainly head) to which acid is added and in which liquefaction is carried out by enzymes naturally present in the shrimp. Shrimp waste silage can also be produced by bacterial fermentation, initiated by mixing shrimp waste with a fermentable sugar or *Lactobacillus* which favours the growth of lactic acid bacteria (De-Silva and George, 1994).

Shrimp waste silage is a liquid and a method of removing or reducing the water content of shrimp silage is co-drying (Fox et al, 1994). Co-drying is a process whereby a small percentage of dried products such as hydrolysed feather meal, bone meal, soybean meal etc are added to the wet silage to absorb the solubilized protein and some of the moisture (Fagbenro and Bello-Olusoji, 1996). De-Silva and George, 1994 observed that cassava allowed to ferment for 3-4 days when added to shrimp waste silage has been found to produce an excellent silage.

Faturoti and Iwezue (1993) reported that nutritionally a 20% - 30% shrimp waste silage substitution level for fish meal in the diet of *C. gariepinus* proved beneficial by performing better than other tested treatments. Fox et al (1993) reported that shrimp head silage meal containing diets performed significantly better in terms of final individual

weight, conversion ratio and production than fish meal diets fed to juvenile *Penaeus notialis* and there was increase in protein level of 44.44% (without ensilation) to 51.2% of shrimp silage and high chitin level of 14.3% (without ensilation) and 8.8% of shrimp silage, (Fox *et al* 1993). Hamzat (1997) reported that nutritionally and economically, a 20% shrimp waste silage substitution level for fishmeal in the diet of *C. gariepinus* performed better than other diets. Fagbenro and Bello-Olusoji (1997) reported a desirable and stable pH < 4.5 after fermentation of shrimp head for 7 days, Hamzat 1997, after inoculation of shrimp head wastes with cassava for 14 days reported a pH of 4.52, pH of < 4.5, 4.0-4.5 were reported by Fagbenro, 1996 and Kompiang *et al* 1979 after fermentation for 7 and 3 days respectively.

Crude protein of 14.10% (wet basis) and 69.00% (dried basis) of shrimp head silage was reported by Infofish marketing digest, No. 4/86 (Table3).

Table 3: Proximate composition of shrimp head silage

Components	% Wet basis	5% Dry basis
Protein	14.10	69.00
Fat	1.40	6.80
Ash	3.50	17.10
Calcium	1.08	5.29
Phosphorus	0.30	1.47
Moisture	81.00	7.00

Source: Infofish Marketing Digest, No. 4/86

Fagbenro and Bello-Olusoji (1997) reported crude protein of 50.6 ± 2.5 for minced shrimp heads, 41.8 ± 1.8 for fermented shrimp head silage (cassava starch) and 42.7 ± 2.1 for fermented shrimp head silage (cane mollasses). Hamzat, (1997) reported crude protein content of fermented shrimp head silage meal as 55.10. Apparent digestibility coefficient (%) (protein) of solar-dried shrimp head meal and fermented shrimp head silage meals as reported by Fagbenro and Bello-Olusoji (1997) are as follows: Solar-dried shrimp head meal 76.2%, fermented shrimp head silage: hydrolysed feather meal blend 79.5%. Fermented shrimp head silage: poultry by-product meal blend 75.7% fermented shrimp head silage: soybean meal blend 77.3%.

2.5 FISH SILAGE:

Fish silage is a liquid product made from whole or part of fish to which acid is added or produced by bacterial fermentation with a fermentable sugar which favours the growth of lactic acid bacteria (Raa, 1982) and in which liquefaction is carried out by enzymes naturally present in the fish (Windsor, 1974). Stone et al (1984) co-ensiled fish protein waste and canola meal/wheat bran mixture (90: 10w/v) and reported an



improvement in the nutritional properties of the blends as well as a change in its physical properties which facilitated its preparation as a conventional feed stuff.

Fish ensilation helps to hydrolyse all the intact proteins to soluble peptide fragments and free amino acids, Raa and Guildberg (1982). Ensilation of fish farm mortalities or fishery wastes/by-products represents a simple, practical and economic process of trash fish utilization for the manufacture of animal feeds and crop fertilizers (Ockerman, 1992, Lo *et al*, 1993, Fagbenro and Jauncey, 1994).

Balogun and Oyeyemi (1986) have shown that co-dried fish silage based feeds provide an economic return better than that obtained with dried fish meal based feeds on a small scale production. It has also be demonstrated by Disney *et al* 1978 that fish silage blended with fillers can replace fish meal in poultry diets, but because poultry has lower quantitative requirements for dietary protein than fish, energy of fibrous feedstuffs, (maize, rice bran, rice straw, wheat offal, cassava meal etc) have been used as fillers, Lopez (1990). However, Fagbenro and Jauncey (1994) stated that since fish is higher in protein than chicken, the formulation of fish feed necessitates the inclusion of high protein feed stuffs (feather meal, poultry by-product meal, soybean meal etc) as filler for wet silage.

Furthermore, feeding experiment with fermented fish silage invariably showed that the nutritional quality is good, with the biological value of the protein being similar to that of skimmed milk powder (Krishnaswamy *et al* 1965). Feed conversion efficiency and daily live weight gain of pigs on cereal diets with fish silage supplement has been shown to equal that with fish meal (Cameron, 1962) and is significantly better than on a diet based on soya protein (Batterham and Gorman, 1980).

In the diets for carp, fish silage has been shown to be equally good source of protein and even better if the fish was boiled prior to ensiling (Djajasewaka and Djajadiredja, 1980). Fagbenro and Jauncey (1994) reported that *Clarias gariepinus* and *Oreochromis niloticus* fed with 50% fish meal replaced with fermented Tilapia silage (Table 4) diets gave the best growth rates, feed conversion ratio, protein utilization and digestibility than other tested diets. They also reported that growth, feed and protein efficiencies were lowest with fish fed with fish meal-free diets while all other silage diets supported growth performance and nutrient utilization similar to those fed with the control diet.

Table 4: Proximate composition of minced Tilapia and Tilapia Silage

	Tilapia silage	Minced tilapia
Dry matter	30.55	26.27
Crude protein	42.35	62.85
Crude lipid	10.63	7.08
Ash	15.55	18.03

Source: Fagbenro and Jauncey 1994

2.6 ACID PRESERVATION OF FISH/SHRIMP SILAGE

To preserve green plants, with a mixture of hydrochloric acid and sulphuric acid as recommended by Vitarian in the 1920s, it is naturally sufficient to lower the pH to about 4.0 but to preserve fish or shrimp by the same acids, the pH has to be below 2 (Edwin, 1940). The preservation of green forage at pH 4 by adding inorganic acids according to Raa and Gildberg (1982) is not entirely due to the low pH but sugar present in the green forage contribute to preservation by repressing the production of deaminating enzymes in those bacteria which survive at pH 4, thereby preventing an increase in pH.

In addition, sugar favours the growth of acid tolerant lactic bacteria which produce sufficient acid to maintain the low pH. Such preservation mechanisms are not sufficient in acidified fish and shrimp because they contain very low level of free sugar and fish/shrimp has a high buffering

capacity compared with green forage, due to high levels of proteins and minerals, so that more acid is needed to produce a stable fish/shrimp silage than forage silage (Raa and Gildberg 1982). According to the empirical formula developed by Edwin, (1940) $(Ca \times 0.14 + b \times 0.9)$ (Of 14N acid per 100kg of raw material)>. The quantity of acid required to lower pH to 2 in fish/shrimp homogenate depends on the concentration of protein and ash minerals in the raw material

a = % Crude protein of the wet weigh and

b = % Ash of wet weight.

Raa and Gildberg (1982) noted that the empirical formula was based on work with the "A.I. virtaren acid that is (mixture of sulphuric acid and hydrochlonic acid with a normality of 14). The formula implies that 9 litres of 14N inorganic acid is needed to preserve 100kg of the most bony fish with very low ash and protein contents. These quantities correspond to 6.3kg (3.4L) and 2.81kg (1.5L) of concentrated sulphuric acid.

Finn (1953) observed that the quantities of sulphuric acid calculated by Edwin's formula will give pH 1.5 to 1.8, when an equilibrium between fish and the acid has set in which will occur after 24 to 48 hours of proper grinding and mixing. When equilibrium has set in, the pH of the silage must

be tested (Finn, 1953). If the pH is higher than 2.0, more sulphuric acid has to be added so as to reduce the pH to 2.0. This is calculated with another formula (Edwin, 1940)) $S = 0.3 * S^* (pH-2)$. Where S = litre of sulphuric acid (50%) being added/100kg of fish silage.

$S^* =$ Litre of sulphuric acid (50%) added originally per 100kg raw material.

pH is the pH value, found on testing

For example, if originally 5.5 litres of sulphuric acid (50%) per 100kg raw materials were used and produced a pH value of 2.8 instead of 2.0 then

$S = 0.3 * 5.5 * (2.8 - 2) = 1.3$ which means that 1.3 litre of sulphuric acid (50%) must be mixed with the original 5.5. litres to produce a pH value of 2.0.

Furthermore, before mineral acid silage can be fed to animals it must be neutralised. Olssum, (1942) stated that addition of 37g of calcium carbonate per kilogramme silage can be used as feed about 3 hours after the addition of lime. Fish acidified with formic acid alone are contrary to sulphuric acid silage of a good keeping quality, even if the formic acid (23.5N) in his experiment and established formulae for the calculation of quantity of formic acid (90%) to be used at various periods, he added that 1litre of formic acid (85%). On the basis of Olssum's formulae, and the

maximum contents of ash for type of fish concerned, it is possible to calculate the quantity of organic acid required to ensure preservation. Finn, (1953) reported that organic acids are more expensive than inorganic ones. For example, in 1982, the price of one litre of formic acid (85%) is about 8 times higher than the price of 1 litre of sulphuric acid free of arsenic(50%). The difference in price as observed by Raa and Gildberg, (1982) is partly counter balanced by the higher efficiency of the organic acids, and it is not necessary to neutralize a silage produced with organic acids.

To reduce the cost of preservation, it may be a recommended practice to use a mixture of inorganic and organic acids, using the cheap inorganic acid to lower the pH sufficiently for organic acid to become anti microbial, Finn, 1953. Lo et al (1993) used citric acid alone and a combination of citric, formic and propionic acid to produced fish silage from salmon farm mortalities, reported that the pH of 2% (N/W) citric acid silage not only had greater increase in pH, but also remained higher than that of 3% (N/W) citric acid silage by day 30. The silage using 3% (V/W) formic acid, a mixture of formic acid and propionic acid maintained stability. The 3% (V/W) formic acid gave the lowest pH value. Based on his result, he concluded that though citric acid could be used for ensiling without the addition of other acids. However, in order to achieve stability, a larger volume in citric acid

alone would be needed than if it were mixed with other acids. The 3% (V/W) formic acid silage had a pH value of 3.3, while that of the mixture 1% (V/W) formic acid and 1% (V/W) propionic acid was 3.9. This indicated that in practice, 1% (V/W) formic and 1% (V/W) propionic acid was enough for retaining complete stability of the silage. A combination of propionic acid and formic acid silage was less susceptible to microbial deterioration than the silage with formic acid alone (Raa and Gildberg 1982). The formic acid/citric acid also gave a lower pH value than the formic and propionic acid silage.

2.7 PRESERVATION OF FISH AND SHRIMP SILAGE BY LACTIC ACID BACTERIA

On a media containing sugar, lactic acid bacteria produce large amount of lactic acid which decreases the pH and this render the medium unsuitable for the growth of most other micro-organisms. Growth of lactic acid bacteria produce antibiotics which contribute to their preservative action (Lindergrem and Clerston, 1979). These bacteria according to Roa, (1965) not only prevent microbial spoilage of food or feeds but also add flavour to the products and may prevent rancidification and other chemical reaction which causes quality reduction.

Fish and fishery products are rich in protein and lipids but have a very low level of free sugar (Fructose, glucose, lactose) available for fermentation by bacteria. The most significant source of energy for growth of bacteria in fish is the pool of free amino acids which increases as a result of proteolysis of the fish (Raa and Gildberg (1982) while spoilage bacteria utilize amino acids as a source of energy and thus produce ammonia, lactic acid through bacteria has limited ability to decompose amino acid except for arginine if glucose is absent (Johnson, 1979). In the presence of fermentable sugars, growth of lactic acid bacteria is enhanced even when the environment is also rich in amino acids. This is because glucose in particular represses the production of deaminating enzymes in competing spoilage bacteria (Raa and Gildberg 1982). Also the spoilage bacteria ferment glucose and produce acids if the environment is anaerobic. These fermentable sugars contribute to pH reduction at initial stages of fermentation and this render the environment less suitable for their own growth but as pH falls, the acid tolerant lactic acid bacteria becomes predominant, Faturoti and Iwezue (1993), Hamzat, (1997).

Furthermore, a relatively high quantity of carbohydrate must be added to the fish in order to ensure a successful preservation (Nelson and Rydin, 1963). Addition of 20kg of dry mixture of malt and oatmeals to 100kg of fresh herring will produce a good silage..



Other raw materials that can be used in lactic acid fermentation include fermented rice, cooked rice, cassava, potatoes or molasses (De-Silva and George, 1994). The sugar in these sources are in the form of starch which must be broken down into simple sugar for use by bacteria.

2.7.1 LACTO BACILLUS SPECIES:

Lactobacillus species are widely distributed in nature existing in both parasitic and saprophytic state including the human oral cavity and human and animal fecal matter when lactose and dextrin diets are consumed and in wide variety of dairy products (Joklik and Smith, 1972). Lactobacilli are gram-positive, non-sporing, generally non-motile rods varying from short to rather long and slender forms of uniform width 0.6 to 0.9 μ m in diameter after forming chain. Growth is obtainable in three to four days on blood agar or on Ragosa's and other lactobacillus selective agar media acidified to pH 5.0, Sims, (1970).

Most lactobacilli produce only lactic acid as a fermentation end-product and are termed homofermentative. They can reduce the pH of glucose broth to 3.0 in two to four days (Joklik and Smith, 1972). Lactobacillus media can be purchased from any reputable chemical and scientific company in Nigeria.

2.8 ESSENTIAL NUTRIENTS IN FISH FEED

2.8.1 PROTEIN AND AMINO ACID:

Protein is essential in the diet of all fishes as a source of amino acids, which are the building blocks for flesh, enzymes, eggs, milt, antibodies and some hormones. Protein can be used as a source of energy but this is wasteful and should be avoided Dupree and Huner, (1984). A liberal supply of protein of good quality must be available to fish throughout life. The quantity of protein required varies with fish species and age. Weight gain of fish is essentially linear with protein content in the feed.

Dupree and Huner, (1984) noted that 20 to 40% protein content in the diet of fish was essentially directly proportional on fish growth but 50% protein content in the diet did not improve growth so it is important not to feed more protein than can be used by fish because no benefit is realised in growth, the excess can be damaging to the fish, and the cost of the protein is high.

Quantitative requirement of the 10 amino acids essentials in the diet of five (5) species of fish and the chicks are shown in Table 5 (Santiago and Lovell, 1988, and Fagbenro and Jauncey, 1994). The protein component in the diet supplies these amino acid are Tyrosine, Cystine, Glycine, Alanine, Aspartic acid, Glutamic acid and Proline. However, the first two of these may have a sparing function – Tyrosine can spare Phenylalanine and Cystine

can spare methionine - thus reducing the quantity of Phenylalamine and Methionine required in the diet.

Table 5: Amino acid requirements (as percent of protein in the diet) of several fishes and the chick

Amino Acid	Channel Catfish	Common Carp	<i>O. niloticus</i>	Chick	<i>C. gariepinus</i> a
Arginine	4.3	4.2	4.20	5.6	4.3
Histidine	1.5	2.0	1.72	1.4	1.5
Isoleucine	2.6	2.3	3.11	3.3	2.6
Leucine	3.5	3.4	3.39	5.6	3.5
Lysine	5.0	5.7	5.12	4.7	5.0
Methionine	2.3	3.1	2.68	3.3	2.3
Phenylalanine	5.0	6.5	3.75	5.6	5.0
Threonine	2.0	3.9	3.75	3.1	2.0
Tryptophan	0.5	0.8	1.00	0.9	0.5
Valine	3.0	3.6	2.80	3.4	3.0

Source: Santiago and Lovell (1988)

a = Fagbenro and Jauncey, 1994

Table 6 shown the amino acid content of unfermented oven dried shrimp head meal and whole pond-reared juvenile and wild adult *P. Monodon* muscle as presented by Fox *et al* (1994).

Table 6: Amino acid content of unfermented oven dried shrimp head meal and whole pond-reared juvenile and wild adult *P. Monodon* muscle

Amino acid	Unfermented oven dried shrimp head meal	Pond-reared <i>P. monodon</i> Juvenile muscle	Wild adult <i>P. Monodon</i>
Arginine	19.6	15.58	18.69
Histidine	5.9	4.84	4.31
Isoleucine	11.5	8.67	8.78
Leucine	20.4	14.91	14.91
Lysine	19.9	14.76	15.35
Methionine	2.0 + Cystine	7.56	6.61
Phenylalanine	74.0 + Tyrosine	15.88	14.53
Threonine	13.2	7.72	7.28
Tryptophan			
Valine	14.9	10.07	9.50
Alanine	23.5		
Tyrosine	12.0		
Glycine	23.0		
Glutamic	46.2		
Aspartic	33.4		
Proline	18.3		
Cystine	1.9		

Source: Fox *et al*, 1994

2.8.1.1 EVALUATION OF PROTEIN:

Quantity of protein obtained from a diet depends on the amount of the food and on the protein content of food. Foods of a low protein content may provide useful amounts of protein if consumed in sufficient quantity.

In order to test changes in amino acid availability, the use of Biological test using the animal is necessary (Church and Pond, 1988). Poor

digestibility tends to be depressed as the concentration of dietary carbohydrate increases (Rychly and Spannoff, 1979).

Apart from chemically measuring amino acid and their availability within feed protein, there are many biologically methods of evaluating protein quality namely:

(a) **SPECIFIC GROWTH RATE (SGR)**

$$= \frac{100 (\text{Log}_e \text{ final body weight} - \text{Log}_e \text{ initial body weight})}{\text{Rearing period days}}$$

(b) **FOOD CONVERSION RATION (FCR)**

$$\text{FCR} = \frac{\text{Feed fed (Dry wt)}}{\text{Fish wt gain (fresh wt.g)}}$$

(c) **FOOD EFFICIENCY (FC)**

$$\text{FC} = \frac{\text{Wt gain (fresh wt.g)}}{\text{Food fed (Dry wt g)}}$$

(d) **PROTEIN EFFICIENCY RATIO (PER)**

$$\text{PER} = \frac{\text{Wt gain (Fresh wt.g)}}{\text{Protein Fed (g)}}$$

(e) **PROTEIN PRODUCTIVE VALUE**

$$\text{PPV} = \frac{\text{Protein gain}}{\text{Protein fed}} \times \frac{100}{1}$$

(f) **PROTEIN FED**

$$= \text{Crude protein} \times \text{Quantity of Feed Fed.}$$

2.8.2 LIPID:

Lipids are made up of fats and oils. Fats and oils may differ considerably in composition, depending on their origin, animal depot fats, ruminant milk fats, marine oil and vegetable oils, the latter are divided into Fruit coat fats and seedfats. Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) occur in major amounts in the fish from deep waters – cod, mackerel, tuna, sword fish, sardines and herring.

ESSENTIAL FATTY ACIDS:

Lipids are important sources of metabolic energy (ATP). They are the most energy rich of all classes of feed nutrition with a gross value of 9.5k cal/g (Tacon, 1993). It could be used to spare the more valuable protein for growth. Free fatty acids derived from triglycerides (Fats and oils) are the major aerobic fuel source for energy metabolism of fish muscle.

Lipids are essential components of all cellular and sub-cellular membranes. Lipid classes that are involved include the polyunsaturated fatty acid containing phospholipids and sterolesters. They serve as biological carriers for the absorption of the fat soluble vitamins A,D,E and K. They are required

for optimal lipid transport bound to phospholipids as emulsifying agents and are precursors in feed prostagland hormones (NRC, 1980).

It has been shown that some warm water fish required n-6 or a mixture of n-3 and n-6 fatty acids while marine fishes, who spend much of their lives at low water temperature, require n-3 fatty acids. Warm water fish seem to require Omega-3 fatty acid than cold water fishes (Lovell, 1984). There is evidence that salinity of the water may affect fatty acid requirements (Tacon, 1993).

Finally, from a feed technological view point, lipids acts as lubricants for the passage of feed through pellet die, as substances which reduces the dust of feed and play a role in the palatability of the feed.

2.8.3 CARBOHYDRATE AND ENERGY:

Carbohydrates function as precursors for various metabolites such as non-essential amino acids and fatty acids. It is included as starch in fish feeds, primarily for its binding characteristics. Fish can use carbohydrates, but no dietary requirement has been established (Dupree and Huner, 1984). Metabolic energy which is essential in fish for growth, reproduction comes from protein, fats and carbohydrates through the process of oxidation or respiration.



The amount of energy required by fish depends on the species, age, sex, water – temperature, activity and water quality.

2.8.4 FIBRE:

The fibre content of food is define as the residue remaining after acid and alkaline digestion. Some carbohydrate components of fibre have been found to have beneficial health effects and attention has been shifted to what is now known as dietary fibre which is usually 2 to 16 times greater than the crude fibre contents. Fibre is defined as plant material which is not degraded by the endogenous secretions of the human digestive tract, their glycosidic linkage are resistant to digestion (NRC,1983).

Fish tolerate up to 8% fibre but higher amount depress growth. It improves gastric evacuation time, and form bulk of feed. Excess fibre reduces nutrient intakes and increases faecal waste production. Highly digestible feed ingredients should be used thereby limiting the fibre content of diet.

2.8.5 MINERALS:

Besides the organically bound elements (Hydrogen, Carbon, Nitrogen and oxygen), dietary need for about 22 minerals has been established in animals (Church and Pond, 1988), including fishes and shrimps.

Minerals are classified into two types according to their concentration in the animal body. Those required in large quantities are the macro/major minerals while the micro/trace minerals are required in small quantities.

Trace elements are usually present in amount up to 50ppm. The major minerals are calcium, phosphorus, magnesium, sodium, bicarbonate, potassium, chlorine and sulphur. The trace elements are iron, iodine, manganese, copper, cobalt, zinc, selenium, molybdenum, flourine, aluminium, nickel, vanadium, silicon and chromium aluminium, nickel, and chromium are non-nutritive, non toxic elements (Deman, 1984).

The general functions of minerals can be summarised as follows:

Minerals are essential constituents of skeletal structures such as bones and teeth. They are important in the maintenance of osmotic pressures and thus regulate the exchange of water and solute within the animal body. They are structural constituents of soft tissues. They also function in the transmission of nerve impulses and muscle contraction. Minerals play a vital role in the acid base equilibrium of the body and thus regulate the pH of the blood and other body fluid. Minerals serve as essential components, for many enzymes, vitamins, hormones and respiratory pigments or as co factors in metabolism and enzymes activators.



2.8.6 VITAMINS:

Vitamins are minor components of food that play an essential role in human nutrition (Deman, 1984). They are organic substance present in minute amounts, measured in milligram and microgram per 100g of natural food. They are essential for controlling metabolic process and when absent from the diet cause deficiency disorders seen as growth and metabolic defects and in other detectable clinical signs and symptoms (Kilgour, 1987).

Two water-soluble vitamins are required in larger quantities. They function other than as coenzyme and are referred to as the macro vitamins. These are vitamin C (Ascorbic acid/myo-inositol) and choline. Vitamin A,D and K are the fat soluble. The fat soluble vitamins are absorbed from the gastro-intestinal tract in the presence of fat and can be stored within the fat reserves of the body whenever dietary intake exceeds metabolic demands. Storage may increase with dietary intake to the extent that a toxic condition hyper-vitaminosis may occur.

The water soluble vitamins are not stored in appreciable quantities in the body. Body storage is rapidly depleted in the absence of regular dietary supply. This may lead to vitamins toxicities.

2.9 ECONOMIC IMPORTANCE OF FISH FEED:

Feed formulation and diet evaluation should be based on production cost i.e. least cost per unit of product produced (Hardy, 1980). The least expensive diet is not always the best in term of cost per unit weight gain or cost per egg, in the case of broodfish.

Economic evaluation of feed is a very important aspect of fish formulation. Hasting (1970) gave an equation that shows the variables explaining a numerical evaluation of fish production as:

$$W_t = F(T+Sr+Wo+H+Fa+Fq+Fm+FF+D+Wpt.....)$$

Where W_t = Weight at given time

T = Time

FF = Feeding frequency

D = Disease and parasitism

Fq = Feed quality

FM = Feeding method

Wo = Stocking size

H = Water temperature

Wp = Water parameters

Sr = Stocking rate

Fa = Feed amount

There were no co-efficient to determine which feed will be most profitable. Based on the economic importance of feed, Crampton (1985) recommended the following as the fish consideration in formulating feed:

- (a) The market value of the fish when finally ready for sale and
- (b) The fixed or non-feed costs involved in producing the fish
- (c) The value of fish varies with sizes and species.

Increased growth rate or survival would have a very marked effect on profitability. These two criteria are the basis upon which the farmer judge competing feeds. The value of feed bears a direct relationship with the value of fish; he is concerned with obtaining maximum feed efficiency. This makes growth rate important in helping to reduce the contribution of feed to fixed costs.

Crampton, (1985) stated that the maintenance of an aquaculture industry depends on its continuing economic viability and its relative profitability.

These in turn depend on how well the productivity of aquaculture can be sustained utilising natural factors and on market factors. Market factors are reflected in prices paid for inputs used in aquaculture and the price(s) obtained for its product(s). Maintaining economic viability or profitability

of production of a species is one of the ways of sustaining aquaculture. This is very important once it is realised that aquaculture has an important role to play in the process of achieving sustainable development. This is evident in that deficiency of animal protein for human consumption exists in many developing countries, Aquaculture can therefore help to reduce deficiency and the incidence of poverty.

2.10 FAMILY CLARIIDAE:

The family Clariidae is divided into two genera – Clarias and Heterobranchus. Genus Heterobranchus has the rayed dorsal fin followed by an adipose fin while Genus Clarias has a single rayed dorsal fin extending almost to the tail. Both genera have four pairs of barbels on the flattened, strongly-depressed head.

2.10.1 THE AFRICAN CATFISH CLARIAS GARIOPINUS:

The two most important clarias species in aquaculture are *C. batrachus* and *C. gariepinus* Haylor, (1991). In Nigeria, the production of the clarias species is next to that of the tilapiines (Hogendoorn, 1980). This shows that *Clarias gariepinus* is a highly promising aquaculture candidate

for the African region because of the species excellent adaptation to the climate of these regions, and Nigeria in particular.

Clarias gariepinus is a large clariid catfish with a long head which is about 1/3 of the total length. It has a scale-less shiny skin which is darkly pigmented in the dorsal and light lateral parts of the body (Viveen *et al*, 1985). *Clarias* are good fish to be raised in areas where high heat and long dry season spells are found. *Clarias* species are good to eat, easy to keep and can be used in ponds in a number of ways because of the following characteristics. A number of these characteristics are directly or indirectly related to the air-breathing capacity of *Clarias* species.

- (1) Excellent adaptation to the climate
- (2) High growth rate and very efficient feed conversion
- (3) Ability to mature and remain gravid throughout the year in captivity
- (4) Acceptance of relatively cheap feeds
- (5) Ability to support high population densities under culturable conditions.
- (6) Disease resistance and
- (7) Consumer acceptance.

2.10.2 FEEDING AND NUTRITION OF CLARIAS GARIEPINUS:

Several types of feeds are used to rear this catfish in Africa. These include various kinds of pellets, chicken grower pellets, trout pellets and formulated catfish pellets. Experiments carried out by Hogendoorn (1980, 1981, Ayinla, 1988) showed that initial feeding with commercial trout starter granules, leads to excellent results, the fingerlings attained an individual body weight of 10g in 7 – 8 weeks after yolk sac absorption at 30°C.

Nutritionally studies have focused on the formulation of a specific diet for *C. gariepinus*. Ayinla (1988) reported that the crude protein percentage of 40% will give good growth while Faturoti *et al* (1986) used five rations compound containing 31,34,37 and 40 dietary crude protein levels in feeding the juveniles of *Clarias lazera*. The best growth response was achieved at 40% crude protein deposition.

Non-pelletised feeds such as maize bran, chicken offal, cocoa podhusk, maize, brewery waste, heat treated and cooked soybeans, condemned wheat, wheat bran, groundnut cake, palm kernel cake, blood, bone meal and poultry and poultry by-product meal, have also been used in feeding the catfish.

Pellets have also been made using combinations of ingredients such as groundnut cake, PKC, cotton seed cake, blood meal, maize, soybean meal,



fish meal, rice bran and brewer's waste (Balogun and Ologhobo, 1989, Haylor, 1990).

Feed conversion ratios (FCR) are as varied as the feeds and vary between 1:6 and 1:1 under pond culture conditions and 1:1.2 under ultra-high density culture conditions (Uys, 1989)

A lipid content of 8 - 10% is recommended for African catfish (Uys, 1989), however, this must be adjusted in order to attain the required energy level of 12kg.g-1 digestible energy. No information is available on the dietary vitamin and mineral requirements of *C. gariepinus*. The levels of inclusion recommended by Robinson (1984) for channel catfish are commonly used. It is also recommended that mineral supplements should only be added to such diets which contain less than 20% ingredients of animal origin (e.g. fish meal or carcass meal), (Uys, 1989).

From an age of six weeks, the dietary requirements of *C. gariepinus* do not seem to change (Uys' 1989) except that the required daily ratio decreases with size. As the fish grow, their relative food consumption rates decrease from approximately 10% body weight day 1 - 4 weeks old to 20% of body weight (10 weeks and older). Similarly, growth rates decrease from



14% to 2% of body weight and feed conversion ratio (FCR) increase from 0.7 to 1.2.

Hogendoorn (1981) proposed a size and temperature related model for the estimation of daily ratio for *C. gariepinus*.

2.11 WATER PARAMETERS IN CATFISH CULTURE:

Many fish feeding experiments have been conducted in controlled environments to prevent interaction of environmental effects such as natural food organisms, temperature and water quality, with the nutrient variable being studied. Studies are conducted in aquaria/tanks placed indoor to allowed accurate collection and analysis of data in the hatcheries. Anyanwu (1988), recommended that on daily basis, dissolved oxygen, ammonium nitrate, carbon dioxide, pH and suspended solids should be checked.

(i) Temperature:

Temperature is the most important environmental factors affecting the equilibrium and rates of chemical reactions, growth rates, nutritional requirements, and the enzymatic and chemical composition of cells. Temperature has been found to affect the growth of *Clarias gariepinus* (Veveen *et al*, 1985). Temperature is by far the most pervasive of all environment stresses disturbing the

maintenance of homeostasis in a living system. Poikilotherms e.g. fish cannot adapt their internal temperature to the environmental temperature. A number of fishes are eurythermal, capable of being tolerant to wide fluctuations in ambient temperature. In poikilothermic animals, temperature has an over ruling effect in all metabolic rates including development (Boyd, 1986). 25 - 27°C was reported by Viveen *et al* (1985) as optimum. Anyanwu (1988) reported temperature of between 25 - 30°C while Akinyemi (1988) reported temperature of between 22 – 35°C as optimum for catfishes.

(ii) **pH:**

Acid and alkaline death points are 4 and 11 respectively. Anyanwu (1988). Waters with pH values between 6.5 and 9.0 at day break are most suitable for fish production. During periods of photosynthesis, carbon dioxide is rapidly removed. These results in an increase in carbonate concentration leading to high pH. The hydrolysis of carbonate causes the pH to rise hence pH is directly proportional to the carbonate concentration. When the carbonate is precipitated, it limit the rise in pH. pH of between 6.5 - 8.0 and 6.5 - 9.0 was reported by Viveen *et al* (1985) and Anyanwu (1988)

respectively. PH of 6.5 - 9.0 was also reported by Akinyemi (1988) and Boyd, 1979 respectively as optimum for fish growth.

(iii) **Dissolved Oxygen:**

Oxygen is one of the most critical factors for survival of fish and for a successful fish farming (Fish production). Oxygen is a gas present in air but fish breathes oxygen in water through its gills as dissolved oxygen, i.e oxygen -in-solution. Fishes use the oxygen to oxidize food to produce energy.

Broadly speaking, the oxygen consumption of fish increases as the food intake, its activity increases. However, when expressed as a percentage of fixed fish weight, oxygen consumption decreases as the fish grows (i.e. 1 kilogram of large fish requires less oxygen than 1 kilogram of small fish). Viveen *et al* (1985) reported that at eggs, larvae to fry, fingerlings and Adult stages, oxygen concentration closed to saturation is desirable for *Clarias gariepinus* but catfish with full grown arborescent organ can survive low dissolved oxygen content as low as 0 - 33ppm (Viveen *et al*, 1985). Dissolved oxygen of 3 - 8mg/l and 5mg/l were reported by Anyanwu, (1988) and Boyd, (1979) respectively.

Finally, N_2 less than 102%, salinity less than 15ppt, CO_2 up to 40 - 45ppm, NH_3 up to 0.1ppt, NO_2 up to 10 - 15ppm are desirable for catfish (Viveen et al, 1985) production.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 SAMPLE COLLECTION AND PROCESSING:

Shrimp waste (comprising mainly heads of *Penaeus notialis*, *Parapenaeopsis atlantica*, *Parapenaeus longirostris*, *Penaeopsis miersi* and *Penaeus kerathurus*) were collected from Inter-continental fishing company (ICF), Lagos and transported in ice block to Federal University of Technology, Akure, Fisheries and wild life laboratory.

Five (5) kilogrammes of the sample were weighed out and thoroughly washed with hot water before blended into paste. 250ml of *Lactobacillus plantarum* purchased from Finlab, Lagos and 750g of cane molasses were added to the paste and allowed to undergo anaerobic fermentation for 14 days in an air tight polyethene bag after which the wet product was co-dried with 15% hydrolysed feather meal (750g) in the oven at 48°C for five (5) days.

The pH of the silage dropped from 7.10 on the first day to 4.39 on the fourteen day. The dried product was then grinded into powder form and kept in the freezer (-20°C) until the diet were formulated.

3.2 EXPERIMENTAL DIETS:

Six (6) isonitrogenous diets (40% crude protein) were formulated to contain increasing levels of dried biologically fermented shrimp waste silage meal at 0% (control), 5%, 10%, 20%, 30% and 40% (i.e. diet 1 - 6), Table 8.

Other ingredients were purchased from Boar Farm Limited, Lagos. The ingredients were thoroughly mixed and prepared cassava starch and hot water were added and mixed until it became a dough like paste after which each diet mixture were extruded through a 2-min die mincer of Hobart A - 200T pelleting machine. The pellet were oven dried at 60°C for 4 days and kept in the freezer (-20°C) for future use.

Table 7: INGREDIENTS AND PROXIMATE COMPOSITION OF EXPERIMENTAL DIETS

Ingredients and Percentage Inclusion

Ingredients (g/100g/dm)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Fish meal (72%)	36.11	34.30	32.50	28.28	25.28	21.69
Soybean meal (45.4)	30	30	30	30	30	30
SWSM (58.96)	0	2.12	4.43	8.86	13.29	17.72
Cassava flour	23.89	23.58	23.07	22.25	21.43	20.61
Codliver + vegetable oil (1:1)	5	5	5	5	5	5
Vita-mineral premix	3	3	3	3	3	3
Starch	2	2	2	2	2	2
Percentage inclusion (%)	0	5	10	20	30	40

PROXIMATE COMPOSITION (G/100/G/DM) OF EXPERIMENTAL DIETS						
	1	2	3	4	5	6
Moisture	9.82	9.75	9.79	9.77	9.73	9.74
Crude protein	40.37	40.12	40.18	40.42	40.20	40.40
Ether extract	13.89	14.37	14.27	14.24	14.40	14.49
Crude fibre	4.37	4.59	4.56	4.60	4.72	4.70
Ash	13.98	13.84	13.99	14.14	14.20	14.24
NFE	17.57	17.33	17.21	16.83	16.73	16.43
*Gross energy (kcal/g/g)	436.42	438.25	437.33	436.66	436.6	437.09

*Gross Energy (kcal/g) is based on physiological values of 5 kcal/g protein, 9 kcal/g lipid and 4 kcal/g carbohydrate
DM: Dry matter.



3.3 EXPERIMENTAL PROCEDURE:

Clarias gariepinus fingerlings (mean weight of 12.05 ± 0.03) were procured from Femi Fish Farm, Akure and transported in oxygenated polyculture bag to FUTA Nutrition Laboratory. The fingerlings were starved and acclimatised for 72 hours. The feeding trials were conducted in 12 glass tanks (40x90x40cm). Water supply was from Akin Deko Hall (FUTA Hostel). Aeration was facilitated by the use of Charles Austen pump (Model CAPEX 1.2xNO.D.J.12499,PCX-002/3). Stale water was drained out of the tanks every other day to maintain relatively uniform physico-chemical water parameters and preventing fouling.

Twenty (20) fingerlings per tank of *Clarias gariepinus* with mean weight of 12.05 ± 0.03 g with two replicate per treatment were used. The fingerlings were fed at 5% of their body weight thrice daily for 84 days (12 weeks). Weekly weighing of the fish was done and feed offered adjusted accordingly. At the start and termination of the feeding trials, five (5) fish per treatment were killed by decapitation. The fish were homogenized with 30ml of deionised water and kept in the freezer (-20°C) until analysis could be done.

Faecal samples were collected both by pressing the stomach of the experimental fish six hours after been fed and also by siphoning with a 5mm tubing pooled from each tank to remove the faeces. The sample were sun-dried and kept in freezer for digestibility studies. Mortality were monitored daily and recorded.

3.4 WATER ANALYSIS:

The physiochemical parameters determined were:

i) Water Temperature (O°C):

This was done using Temperature meter (Jenway model No. 9071), made in the United Kingdom.

ii) Dissolved Oxygen (D0₂):

This was done using D0₂ meter (Jenway 9071) made in UK.

iii) pH

This was determined using Toledo 320 pH meter made in UK.

3.5 ANALYTICAL PROCEDURE:

Proximate analysis was conducted in triplicate samples of diets and experimental fish. The proximate analysis of the diets and fish were based on dry weight basis. Proximate analysis of the fish was by

randomly selecting five (5) fingerlings from each tank for analysis, as described by AOAC (1990).

MOISTURE CONTENT:

This was determined after oven-drying a known weight of the fish and the diet samples separately at 105°C. Weight of the samples were checked at interval for 2 days (24 hrs) until a constant weight was observed. Samples were cooled in dessicator before weighing.

Crude Protein:

The crude protein was determined using the micro-kjeldahl distillation procedures as described by A.O.A.C. (1990) using Gallenkamp equipment.

The percentage crude protein was calculated by multiplying the total nitrogen by a factor of 6.25.

$$\text{Total Nitrogen (N)} = \frac{\text{Vol. Of acid} \times \text{molarity} \times 0.014 \times \text{diluting factor}}{\text{Weight of sample used}}$$

$$\% \text{ Crude Protein} = \text{N} \times 6.25$$

Lipid:

The fat content was determined by subjecting the sample to a continuous extraction with petroleum ether using Gallenkamp soxhlet equipment as described by A.O.A.C. (1990). The ether residue was the residues obtained from evaporation of the solvent.

Crude Fibre:

Crude fibre was determined as loss on ignition of dried lipid-free residues after digestion with 1.25% H₂SO₄ and 1.25% NaOH.

$$\% \text{ Crude fibre} = \frac{\text{Wt of Residues} - \text{Wt of Ash} \times 100}{\text{Wt of sample}}$$

Ash Content:

This was determined by subjecting the already oven dried samples with known weight to ignition in a muffle furnace (Gallenkamp brand) at 550°C for 5 hours after which samples were cooled in dessicator and weighed.

$$\% \text{ Ash} = \frac{\text{Wt of Ash}}{\text{Wt of sample}} \times 100$$

Nitrogen Free Extract (NFE):

This is a measure of carbohydrate available in the diet and fish respectively. It was determined by subtraction method

$$\% \text{ NFE} = 100 - (\% \text{moisture} + \% \text{ Crude Protein} + \% \text{ Crude Lipid} + \% \text{ Crude fibre} + \% \text{ Ash})$$

3.6 GROWTH AND PROTEIN UTILIZATION PERFORMANCE

Biological Analysis as describe by Olevera Et Al (1990)

- (i) Average weight gain (g):

$$\text{Final body weight} - \text{Initial body weight}$$

- (ii) Percentage weight gain (%): $\frac{\text{Final body wt} - \text{Initial body wt}}{100} \times$

Initial body wt.

- (iii) Specific Growth Rate (SGR% / day)

$$\frac{\text{Loge (Final body Wt} - \text{Initial body Wt)} \times 100}{\text{Time (days)}}$$

- (iv) Food Conversion Ratio (FCR):

$$\frac{\text{Total dry feed fed}}{\text{Total Wet weight gain}}$$

- (v) Protein Efficiency Ratio (PER):

$$\frac{\text{Wet Weight Gain}}{\text{Protein Fed}}$$

- (vi) Protein Intake (Fed):

$$\% \text{ Crude Protein} \times \text{Quantity of Feed fed}$$

- (vii) Protein Productive Value (PPV):

$$\frac{\text{Protein gain} \times 100}{\text{Protein fed}}$$

3.7 DIGESTIBILITY TEST:

Experimental feed and fecal samples were collected from fish fed the experimental diets. Digestibility test was determined using Acid In Soluble Ash method (AIA) as describe by Halver et al (1993).

25ml of 10% HCL were added to ash content of the feed and feaces of fish and it was boiled gently over a low flame for 5 minutes after which it was filter through ashless filter and washed with hot distilled water. The residue from the filters were then returned into crucible and ignited until it was carbon free and was weighed.

$$\% \text{ AIA In Soluble Ash (AIA): } \frac{\text{Wt of Ash} - \text{Wt of AIA}}{\text{Wt of Ash}} \times 100$$

The value obtained from AIA, were used as indicator in the calculation of digestibility coefficient.

Apperent Protein Digestibility :

$$100 - 100 \frac{(\% \text{ AIA in Feed})}{(\% \text{ AIA in Feaces})} \times \frac{(\% \text{ Nutrient in Feaces})}{(\% \text{ Nutrient in Feed})}$$

3.8 HEPATOSOMATIC INDEX (HSI)

At the end of the feeding trials, 2 fish per tank were randomly taken from each tank (12 tanks) and weighed individually, dissected and their livers removed and weighed individually. Hepatosomatic Index was calculated as:

$$\text{HSI (\%)} = \frac{\text{Liver Weight}}{\text{Somatic Weight}} \times 100$$

3.9 STATISTICAL ANALYSIS:

Statistical comparison of growth performance, protein utilization values, Hepatosomatic index were made using one way Analysis of variance (ANOVA). Duncan's multiple range test (Duncan, 1955) was used to compared differences among individual means.

3.10 COST EVALUATION:

Cost evaluation of experimental diets were based on the current prices of the ingredients bought from Boar Farms Limited, Lagos and the price of shrimp waste was calculated at thirty thousand naira (N30,000) per tonne at N30.00 per kilogramme being the cost of labour, transportation, oven drying and milling.



CHAPTER FOUR

4.0 RESULTS

Fish in all the experimental units became accustomed to the diets within the first week of acclimation.

4.1 SHRIMP WASTE SILAGE MEAL (SWSM):

Table 8 shows the proximate composition of the silage.

Table 8: PROXIMATE COMPOSITION OF FERMENTED SHRIMP WASTE SILAGE MEAL (SWSM)

Parmeter	% Composition
Crude protein	58.96
Moisture	10.58
Ash	21.87
Fibre	3.35
Lipid	3.61
NFE	1.63

4.2 EXPERIMENTAL DIETS:

Table 8 shows the ingredients and proximate analysis of the experimental diets and the result shows that the diets were iso-nitrogenous.

4.3 WATER PARAMETER:

Table 9 and fig. 1,2 and 3 shows the result of water parameter monitored during the experiment. The temperature ranged between 22.38 ± 0.12 and 22.50 ± 0.41 , while Dissolved oxygen (DO_2) and pH values

ranged between 4.24 ± 0.19 to 4.58 ± 0.42 and 6.37 ± 0.12 and 6.45 ± 0.19 respectively.

Table 9: Mean water quality parameters measured during the experimental period

Tanks	Temperature (°C)	D.O (Mg/l)	pH
1	22.46 ± 0.20	4.4 ± 0.21	6.45 ± 0.19
2	22.38 ± 0.12	4.50 ± 0.13	6.42 ± 0.14
3	22.44 ± 0.14	4.24 ± 0.19	6.38 ± 0.21
4	22.44 ± 0.10	4.58 ± 0.42	6.37 ± 0.12
5	22.50 ± 0.41	4.57 ± 0.22	6.38 ± 0.12
6	22.47 ± 0.27	4.46 ± 0.09	6.37 ± 0.09

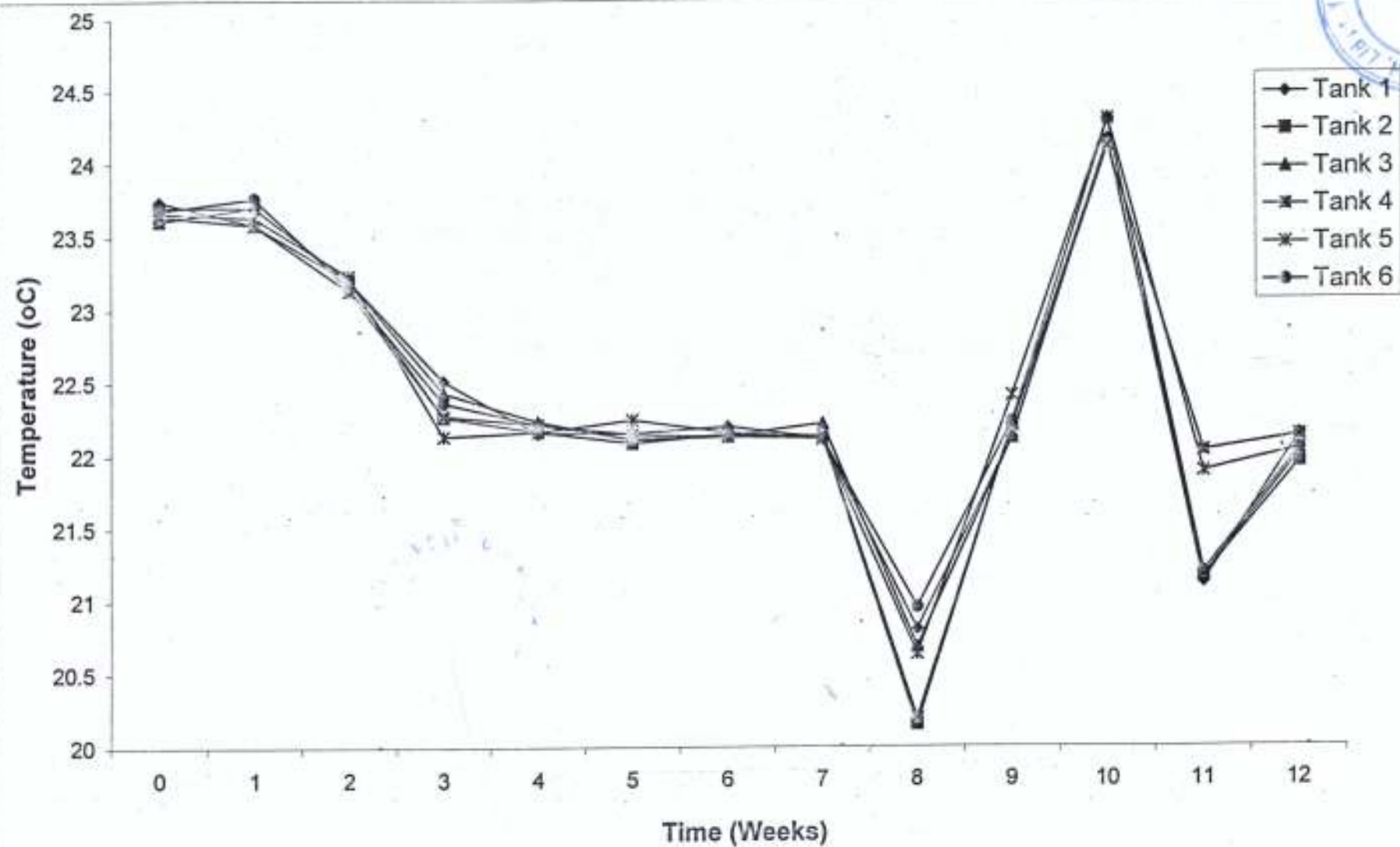


Fig 2: Mean Temperature Values Recorded During the Culture Period

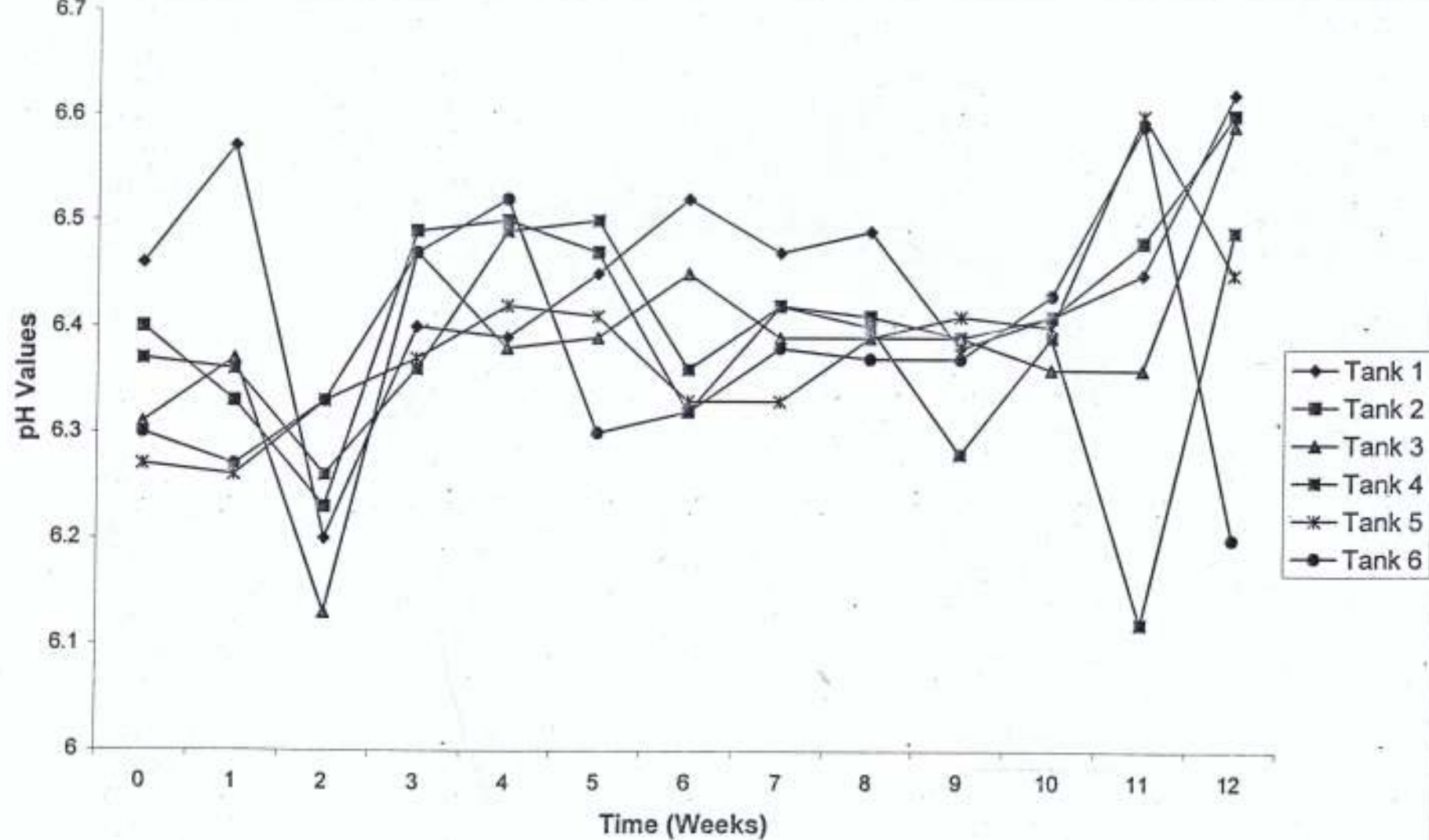


Fig. 3: Mean pH Values Recorded During The Culture Period

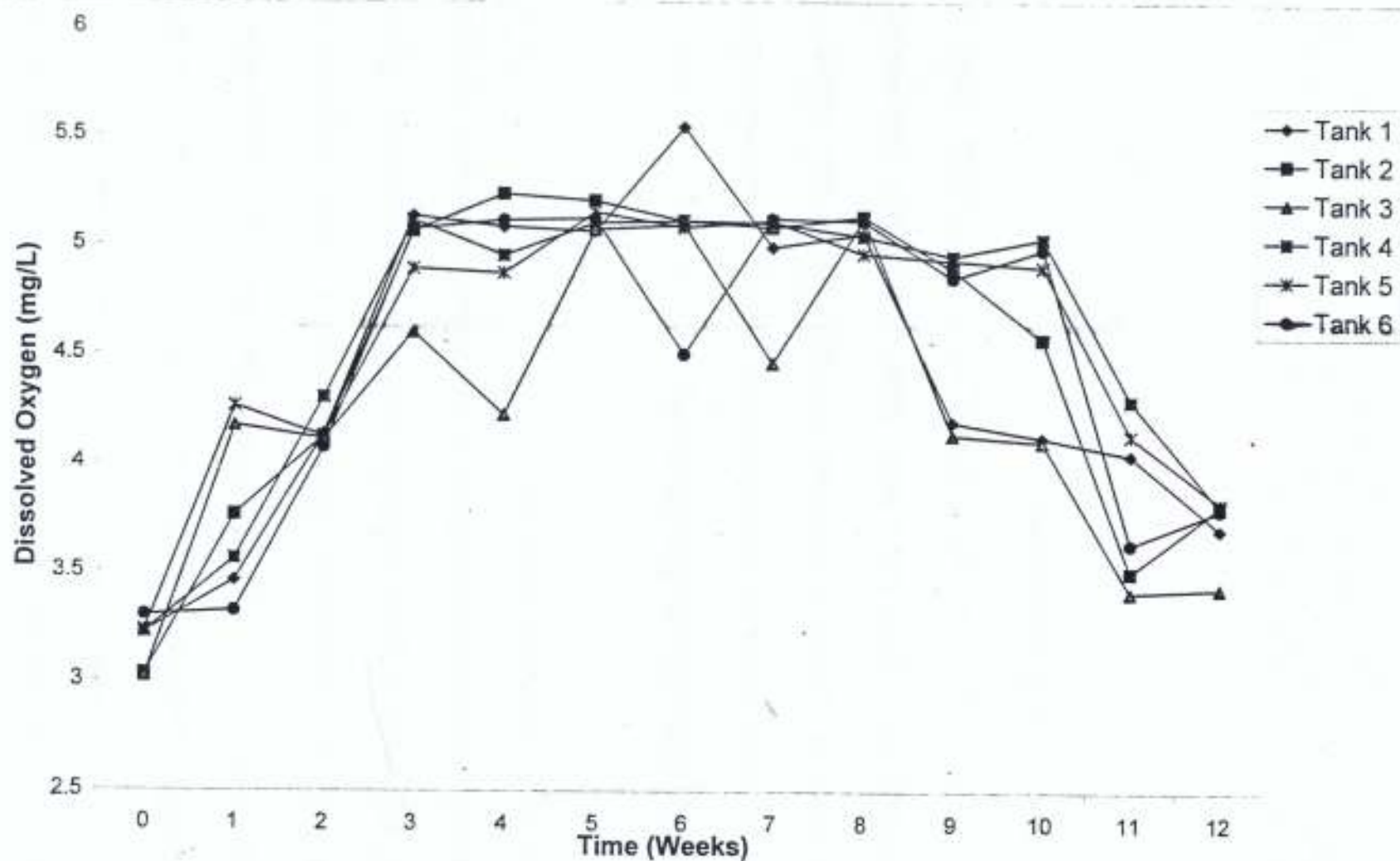


Fig. 4: Mean Dissolved Oxygen Recorded During The Culture Period

4.4 GROWTH RESPONSE:

Summary of the result of the growth performance of *Clarias gariepinus* fingerlings fed the experimental diets are shown in Table 10 and fig 2.

Generally, Treatment I (control) (i.e feed with fish meal as main protein source) was superior to other treatments which have shrimp waste silage but however, Treatment2 (5%), Treatment 3 (10%), Treatment 4 (20%) and Treatment 5 (30%) exhibited similar growth responses. There were no significant difference ($P>0.05$) in the values recorded for specific growth rate (SGR) within the treatments.

4.5 FOOD CONVERSION RATIO (FCR):

The calculated values for Food Conversion ratio are presented in table 10. There were no significant difference ($P>0.05$) between the treatments, however, Treatment 4 (20%) had a better value of 5.72 ± 0.10 followed by Treatment I (0%) and Treatment 5 (30%) which had similar value of 5.73 ± 0.01 and 5.73 ± 0.08 respectively.

4.6 PROTEIN UTILIZATION:

Protein efficiency Ratio (PER): There were no significant difference ($P>0.05$) between the treatments in the values for protein efficiency ratio as shown in Table 10, however, Treatment, 2, 3 and 5 had a better value of 0.44 ± 0.01 each while Treatment I and 6 had value of 0.43 ± 0 and 0.42 ± 0 respectively.

4.7 HEPATOSOMATIC INDEX (HSI):

There were no significant difference ($P>0.05$) between the treatments in values of Hepatosomatic index (HSI) recorded as shown in table 10.

4.8 SURVIVAL:

There were no significant variation ($P>0.05$) in the number of survival after the experiment as shown in table 10.



Table 10: HEPATOSOMATIC INDEX, GROWTH AND PROTEIN UTILIZATION OF CLARIAS GARIEPINUS FINGERLINGS FED SWSM DIETS FOR 84 DAYS

Parameters	TREATMENTS					
	1(%)	2(5%)	3(10%)	4(20%)	5(30%)	6(40%)
Initial mean weight (g)	12.03±0.12	12.04±0.01	12.11±0.01	12.08±0.03	12.03±0.01	12.03±0.01
Final mean weight (g)	28.62±0.49	27.68±0.51	27.56±0.14	27.45±0.14	27.10±0.06	26.49±0.01
Mean weight gain (g)	16.59±0.5a	15.64±0.50a	15.45±0.15a	15.38±0.22a	15.07±0.07a	14.46±0.01b
Weight gain (%)	137.97±4.33a	129.87±4.39b	127.59±35b	127.81±1.58b	125.27±0.69b	120.20±0.1c
Feed fed (g)	94.95±0.12	90.54±1.06	88.82±0.47	87.85±0.20	86.33±0.86	83.87±0.49
Survival (%)	95±50a	90±10a	85±50a	90±00a	95±50a	85±50a
S.G.R. (%) / day	1.03±0.02a	0.99±0.03a	0.91±0.08a	0.99±0.01a	0.96±00a	0.94±00a
FCR	5.73±0.01a	5.80±0.13a	5.75±0.03a	5.72±0.10a	5.73±0.08a	5.81±0.04a
PER	0.43±00a	0.44±0.01a	0.44±0.01b	0.43±0.0a	0.44±0.1a	0.42±00a
PPV	8.79±0.01a	6.67±0.08a	9.50±0.05c	9.41±0.03c	7.26±0.07abc	6.88±0.05a
HSI (%)	1.50±0.04a	1.52±0.04a	1.49±0.03	1.48±0.02a	1.51±0.03a	1.49±0.03a
ADC protein	83.68a	82.87a	80.67a	80.46a	80.04a	79.69a

Key: SGR = Specific Growth rate
 FCR = Food Conversion ratio
 PER = Protein Efficiency ration
 PPV = Protein Productive value
 HSI% = Hepatosomatic Index
 ADC = Apparent digestibility

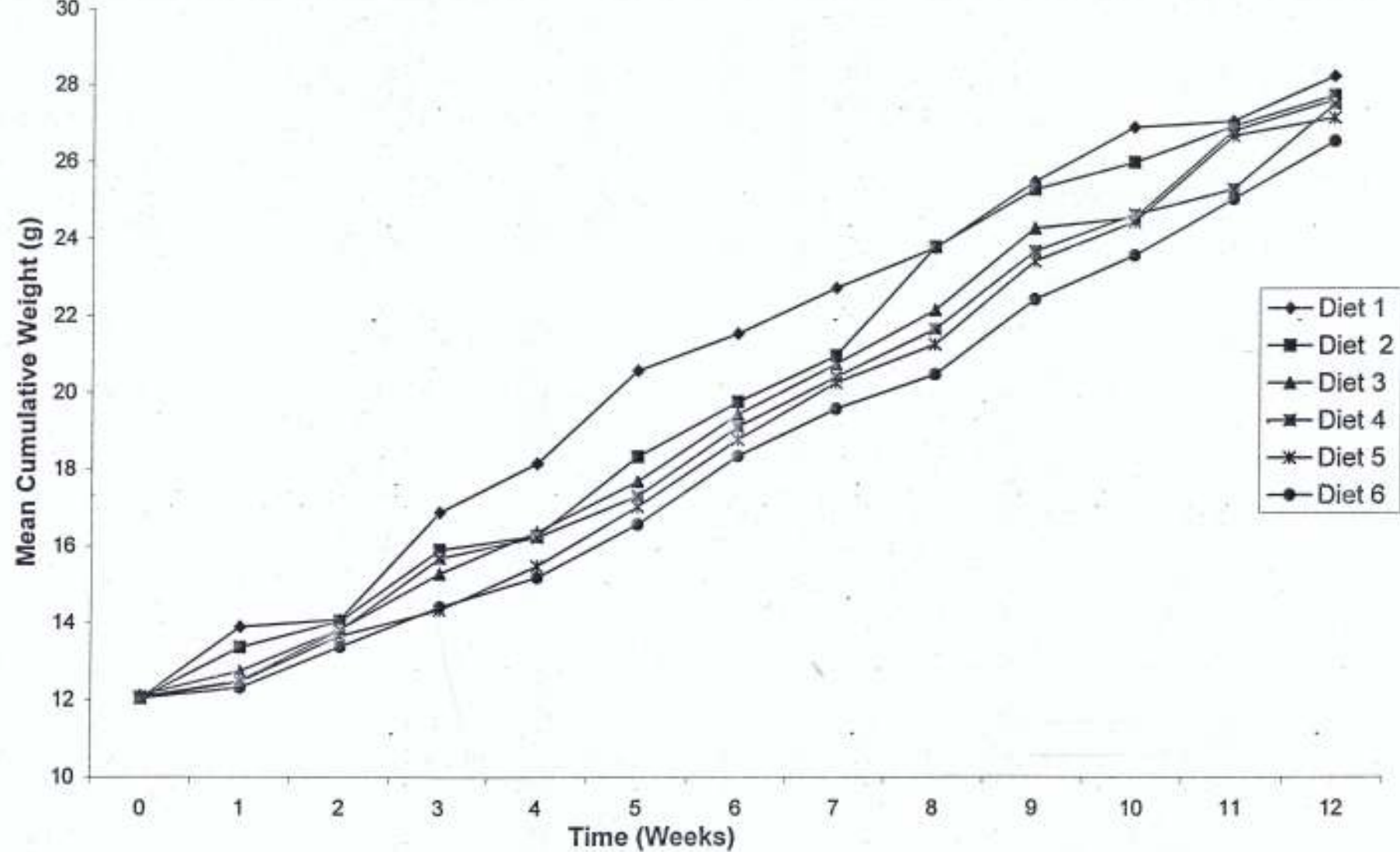


Fig. 1: Mean Cumulative Weight Increases of *Clarias gariepinus* Fed Experimental Diets For Twelve Weeks

4.9 PROXIMATE COMPOSITION OF THE CARCASS OF FISH FED (BEFORE AND AFTER) THE EXPERIMENT DIETS

Table 11: Proximate composition of the carcass of the fingerlings fed (Before and after the experimental diets)

Sample	Ash	Moisture	Crude Protein	Lipid	CHO
Initial to	13.13	11.73	66.75	2.34	4.05
Control T1 (%)	12.10	12.76	70.12	2.90	2.01
T2 (5%)	11.71	11.82	69.17	3.87	3.43
T3 (10%)	11.13	11.38	70.14	3.96	3.39
T4 (20%)	11.51	11.52	70.10	3.22	3.65
T5 (30%)	11.88	11.31	69.27	3.85	3.69
T6 (40%)	11.43	12.17	69.08	3.83	3.49

4.10 DIGESTIBILITY TEST (ACID INSOLUBLE ASH):

The crude protein in the feeds ranged between 40.12 to 40.42 while in faeces, it ranged between 8.20 to 13.86.

AIA in the feeds and faeces increased with inclusion of shrimp waste meal. The digestibility for diet 1 (control) was however the highest with least record for diet 6.

Table 12: DIGESTIBILITY TEST (AIA)

	Diet 1(%)	Diet 2(5%)	Diet 3(10%)	Diet 4(20%)	Diet 5(30)	Diet6(40%)
Nutrient (C,P) in feeds	40.37	40.12	40.18	40.42	40.20	40.40
Nutrient (C,P) in faeces	8.20	10.81	12.82	13.27	13.44	13.86
% AIA in feeds	0.45	0.56	0.63	0.75	0.92	1.16
% AIA in faeces	0.56	0.88	1.04	1.26	1.54	1.96
Digestibility (%)	83.68	82.87	80.67	80.46	80.04	79.69

4.11 COST EFFECTIVENESS:

The cost analysis is shown in table 13. Of the six(6) dietary treatments, treatment 6 (40%) appeared to be most cost effective. It has the highest profit index and the lowest incidence of cost but did not give the best result as compared with treatment 1 – 5 (0% - 30%).

Table 13: Feed ingredients cost

		T R E A T M E N T S					
		1	2	3	4	5	6
Feed Fed(g)		94.95	90.54	88.82	87.85	86.33	83.87
Ingredient	Cost/(N)						
Fish meal (72%)	120	4.11	3.73	3.46	2.98	2.62	2.18
Cassava flour	20	0.45	0.43	0.42	0.42	0.41	0.40
Soybean meal	40	1.14	1.09	1.07	0.05	1.04	1.01
SWSM	30	0	0.0058	0.12	0.23	0.34	0.45
Vita-mineral premix	500	1.43	1.36	1.33	1.32	1.30	1.26
Cod-liver oil	100/500ml	1.58	1.51	1.48	1.46	1.44	1.26
Vegetable oil	80 / litre	0.172	0.164	0.161	0.160	0.160	0.138
Starch	20	0.038	0.036	0.036	0.035	0.35	0.033
Milling and pelleting	10	0.950	0.905	0.888	0.879	0.863	0.839
Transportation	10	0.95	0.91	0.89	0.88	0.86	0.84
Cost of feed/g		10.820	10.141	9.855	9.44	9.068	8.410



Table 14: Cost analysis

Treatments	1	2	3	4	5	6
Cost of feed (fed) (₦)	10.820	10.141	9.855	9.414	9.068	8.410
Value of fish (₦)	4.15	3.91	3.86	3.84	3.77	3.62
Weight of fish (kg)	0.0166	0.0156	0.0155	0.0154	0.0151	0.0145
Incidence of cost	651.81	650.06	635.81	611.30	600.53	580.00
Profit index	0.383	0.386	0.392	0.408	0.416	0.430

$$\text{Incidence of Cost} = \frac{\text{Cost of feed fed}}{\text{Kg of fish produced}}$$

$$\text{Profit Index} = \frac{\text{Value of fish}}{\text{Cost of feed fed.}}$$

CHAPTER FIVE

DISCUSSION

The shrimp waste used for the silage production are made up of mix species but mainly the heads of *Peaneus notialis*. This confirmed the earlier findings by Ajayi and Adetayo (1982) , F.A.O (2001) that commercial shrimp species often landed by Nigerian fishing companies are made up of about 90% *Paeneus notialis*.

This present study on the shrimp waste silage has demonstrated that shrimp/fish can be successfully preserved as silage for feed production by fermentation method that is the addition of *Lactobacillus plantarum* with a carbohydrate source(s) such as cane mollasses or cassava.

Successful fermentation of fish and shrimp has also been reported by other workers (Disney et al 1978), Faturoti and Iwezue 1993, Fagbenro and Jauncey 1994, Fagbenro and Bello-Olusoji 1997, Fox *et al*, 1994 and Hamzat, 1997) De-Silva and George (1994), Lo *et al* (1992) worked on acid silage.

After incubation for 14 days, a desirable and stable pH of 4.3 was achieved and this value is lower than pH 4.52 achieved by Hamzat, (1997) within the same period. Several workers have recommended addition of a starter culture to increase the rate of liquefaction. James *et al* (1976) cited

by Hamzat (1997) have ensiled silver bellies (*Leiognathus spp*, Jewfish (*Pseudociaena spp*) and sole fish *Cynoglossus semifassiatius*) with 15% by weight of commercial mollasses and *Lactobacillus plantarum* and made into slurry with 30% by weight of water and attained a low pH 4.0 – 4.5 within 72 hours. Fagbenro (1996) also fermented shrimp heads with 5% (^w/_w) *Lactobacillus plantarum* as inoculum and obtainable a pH 4.5 after incubation for 7 days. Roa (1965) has also ensiled minced herring with 10% mollasses after inoculation with *Loctobacillus plantarum*. However, Kampiang et al 1979 demonstrated that fresh frozen fish from the by-catch of fish/shrimp trawlers, after thawing with running water could be preserved by natural anaerobic fermentation with mollasses without the addition of any starter culture and also attained a low pH of 4.0 – 4.5 within 3 days. Rattagool et al (1976) cited by Hamzat 1997 reported that no starter culture was required for successful fermentation of trash fish and attained a pH of 4.25 within 7 days. Therefore, it would appear that a starter culture may not be necessary for rapid fermentation.

The value of the proximate composition of the fermented shrimpwaste silage meal for crude protein was 58.96% (Table 7) and this compares favourably with the value of 58.2% (Meyers 1986), 69.00% (Info Fish

Marketing Digest, No.4/86, 41.8% (Fagbenro and Bello-Olusoji, 1997), 55.10% (Hamzat, 1997).

The fermented mixed shrimp waste silage meal (SMSM) was used to replace fish meal in six (6) graded diets of 0%, 5%, 10%, 20%, 30% and 40%. In spite of the iso-nitrogenous nature of the feeds, the fish still exhibited a great disparity in their growth responses, however, there was no noticeable signs of nutritional deficiency.

Growth response of fish in all the diets fed the fermented mixed shrimp waste silage meal were inferior to the control diet (Table 10). The growth depression of fish tend to be more pronounced with increasing dietary level of fermented shrimp waste silage meal inclusion and this reveals the superiority of fish meal over shrimp waste silage meal as a protein source in fish feed.

This study reveals the inadequacy of a 40% replacement of fish meal with fermented mixed shrimp waste silage meal in the diet of *Clarias gariepinus* fingerlings. However, poor growth performance in diet 6 (40%) agrees with earlier work by Faturoti and Iwezue (1993) recommendation of 20 – 30% replacement level and higher than 20% recommended by Hamzat, 1997, Hardy *et al* 1983. However, Hardy *et al* 1983 attributed his lower



inclusion level to ensilation with acid which increased the acidity of the diets.

Similarity in Food Conversion Ratio (FCR) Specific Growth Rate (SGR) and Protein Efficiency Ratio (PER) (Table 10) may be due to a variety of feeding stimulants (such as amino acids and nucleotides) contained in shrimp waste which enhance the value of the diet considerable in terms of its attractiveness to various aquatic species. Also fermentation process reduces the ash and chitin content of shrimp head thereby increasing its nutrient availability for cultivated aquatic animal. Fox et al (1994), after formic acid ensilation of shrimp heads reported a similar phenomenon of lower chitin and ash. However, Diet 6 (40%) showed a significant difference in percentage mean weight gain from Diet 1 – 5 (0-30%). There was also significant difference in Protein Productivity Value (PPV) within the treatment.

During the feeding trial, mortalities were recorded but there was no significant difference in percentage survival (Table 10) of the fish in all diets. The mortality recorded were at the initial stage of the experiment and may be attributed to initial handling stress during weighing and daily routine management.

The experimental fish in the tanks at the end of the feeding trial appears to be morphologically normal. Hamzat, (1997), Fagbenro and Bello-Olusoji, (1997) also recorded no significant difference in fish mortalities in their earlier works.

The hepatosomatic index and carcass composition were unaffected by the inclusion of fermented shrimp waste silage meal in diet (Table 10). Although, there tend to be a decrease in apparent digestibility of nutrient (ADC protein) as the inclusion of shrimp waste silage meal increases (Table 12) however, there was no significant difference in the apparent digestibility of nutrient (ADC protein). This agrees with earlier findings by Hamzat (1997), De-Silva and George (1994), Fagbenro and Bello-Olusoji (1997) that fermentation or ensilation of shrimp wastes will render the chitin fairly digestible since feedstuffs which are poorly digested would be of limited nutritional value to an animal. Digestibility values reported in this study suggest that shrimp waste silage meals represent an alternative form of protein feedstuff in aquaculture feeds and also provide basic information for further work using pelleted fish feeds containing a fermented shrimp head silage as protein feed stuff which will result in lower costs of fish feed for aquaculture production.

It is noteworthy, that the experiment was carried out in a non-recirculatory system. However, values obtained for physiochemical parameters (Table 9) fell within the acceptable levels of the characteristics of water that could support optimal growth of fish (Boyd, 1986, Viveen *et al* 1985, Anyanwu, 1988, Haylor, 1990). The non-recirculatory nature of the water may have effect on the growth of the fish, despite the regular replacement of the water and aeration in the culture tanks, though *Clarias* species are known to be handy and can survive under harsh environmental conditions, but good and healthy environmental condition will enhance its growth.

Analysis of the cost effectiveness showed a decrease in total cost as the level of fish meal decrease (Table 13 and 14); this could reduce cost of production. However, diet 6 (40%) was least cost diet though did not give the best result in terms of feed utilization and other tested parameters as compared with diets 1 – 5 (0-30%), so replacement of fish meal with shrimp waste silage meal up to 30% will be more economical in terms of cost, increased growth rate and protein utilization.

CHAPTER SIX

CONCLUSION

In the overall assessment, though fish meal is rich and nutritive, shrimp waste silage meal ((SWSM) can be used to partially substitute it.

With the various levels of fermented shrimp waste silage meal (0,5,10,20,30 and 40%) replacement of fish meal, it was observed that 30% level substitution gave the best and economic results. Finally, it is an indication that feed costs in aquaculture production can be significantly reduced with the availability of shrimp waste. Shrimp waste fermented and co-dried with protein filler can be preserved and used as a protein supplement up to 30% which will result in least costs for aquaculture production.



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APPENDIX

Results Showing Mean Body Weight (GM)

Week	T1			T2			T3			T4			T5			T6		
	R1	R2	AV	R1	R2	AV	R1	R2	AV	R1	R2	AV	R1	R2	AV	R1	R2	AV
0	12.01	12.04	12.03	12.03	12.05	12.04	12.10	12.12	12.11	12.05	12.10	12.08	12.04	12.02	13.03	12.02	12.04	12.03
1	13.85	13.90	13.88	13.40	13.30	13.35	12.98	12.48	12.73	12.42	12.52	12.47	12.40	12.49	12.45	12.36	12.54	12.3
2	14.02	14.12	14.07	14.00	14.05	14.03	13.80	13.84	13.82	13.78	13.80	13.79	13.70	13.59	13.65	13.30	13.41	13.36
3	16.58	17.16	16.87	16.27	15.51	15.89	13.26	15.28	15.27	15.52	15.82	15.67	14.58	14.08	14.33	14.23	14.56	14.40
4	18.08	18.19	18.14	16.50	16.01	16.26	16.37	16.35	16.36	16.40	16.03	16.22	15.92	15.02	15.47	15.14	15.20	15.17
5	20.28	20.81	20.55	18.93	17.68	18.31	17.73	17.58	17.66	17.21	17.34	17.28	17.57	16.44	17.01	16.43	16.67	16.55
6	21.48	21.53	21.51	20.01	19.45	19.73	19.51	19.30	19.41	19.12	19.04	19.08	19.26	18.21	18.75	18.10	18.53	18.32
7	22.66	22.71	22.69	21.08	20.78	20.93	20.90	20.52	20.71	20.43	20.32	20.38	20.40	20.06	20.23	19.28	19.81	19.55
8	23.69	23.75	23.72	23.28	24.20	23.74	22.31	21.90	22.11	21.65	21.59	21.62	21.33	21.10	21.22	20.65	20.23	20.44
9	25.99	24.89	25.44	24.57	25.90	25.24	24.62	23.84	24.23	23.54	23.74	23.64	23.45	23.30	23.38	22.36	22.42	22.39
10	27.23	26.48	26.86	26.42	25.90	12.95	24.94	25.10	24.52	24.10	25.10	24.60	24.56	24.26	24.41	23.45	23.62	23.54
11	28.14	27.19	27.67	27.01	26.74	26.88	26.83	26.68	26.76	26.58	26.91	25.25	26.83	26.43	26.63	24.40	25.58	24.99
12	29.10	28.13	28.62	28.18	27.17	27.68	27.70	27.42	27.56	27.64	27.26	27.45	27.04	27.16	27.10	26.48	26.50	26.49

**ANALYSES OF VARIANCE (ANOVA) OF AVERAGE
DAILY WEIGHT GAIN**

Diets	A	B	Tr+Total	Tr+Means
1	0.203	0.192	0.395	0.198
2	0.192	0.18	0.372	0.186
3	0.186	0.182	0.368	0.184
4	0.186	0.180	0.366	0.183
5	0.179	0.180	0.359	0.180
6	0.172	0.172	0.344	1.771

$$Cf = \frac{(2.204)^2}{12}$$

$$\begin{aligned} Ss \text{ total} &= 0.203^2 + \dots + 0.172^2 - Cf \\ &= 0.4057 - 0.4048 \\ &= 0.0009 \end{aligned}$$

$$\begin{aligned} Ss \text{ trts} &= \frac{0.395^2 + \dots + 0.344^2}{2} - Cf \\ &= 0.4055 - 0.4048 \\ &= 0.0007 \end{aligned}$$

$$\begin{aligned} Ss \text{ Error} &= 0.0009 - 0.0007 \\ &= 0.0002 \end{aligned}$$

ANOVA TABLE

S.V	d.f	Ss	Mss	Fcal	Ftab(0.05)
Trts	5	0.0007	0.0001	0	4.39 ^{ns}
Error	6	0.0002	0.0000		
Total	11	0.0009			

n^s = Not significant (P>0.05)

Analysis of Variance (ANOVA) of Average Percentage Weight Gain

Diets	A	B	Tr+Total	Tr+Means
1	142.30	133.64	275.94	137.97
2	134.25	125.48	259.73	129.87
3	128.93	126.24	255.17	127.59
4	129.38	126.23	255.61	127.81
5	124.58	125.96	250.54	125.27
6	120.30	120.10	240.4	120.2

$$Cf = \frac{(1537.39)^2}{12}$$

$$= \underline{196964.00}$$

$$\begin{aligned} Ss \text{ total} &= 142.30^2 + \dots + 120.10^2 - Cf \\ &= 1973922.1 - 196964.00 \\ &= \underline{428.11} \end{aligned}$$

$$\begin{aligned} Ss \text{ trts} &= \frac{275.94^2 + \dots + 240.4^2}{2} - Cf \\ &= 197306.60 - 196964.00 \\ &= \underline{342.6} \end{aligned}$$

$$\begin{aligned} Ss \text{ Error} &= 428.11 - 342.6 \\ &= \underline{85.51} \end{aligned}$$

Anova Table

S.V	d.f	Ss	Mss	Fcal	Ftab(0.05)
Trts	5	342.6	68.52	4.81	4.39*
Error	6	85.51	14.25		
Total	11	428.11			

* = Significant ($P > 0.05$).

Analysis of Variance (ANOVA) of Specific Growth Rate (SGR %)

Diets	A	B	Tr+Total	Tr+Means
1	1.05	1.01	2.06	1.03
2	1.01	0.96	1.97	0.99
3	0.83	0.98	1.81	0.91
4	0.99	0.98	1.97	0.99
5	0.96	0.96	1.92	0.96
6	0.94	0.94	1.88	0.94

$$\begin{aligned}Cf &= \frac{(11.61)^2}{12} \\&= \underline{11.23}\end{aligned}$$

$$\begin{aligned}Ss \text{ total} &= 1.05^2 + \dots + 0.94^2 - Cf \\&= 11.26 - 11.23 \\&= \underline{0.03}\end{aligned}$$

$$\begin{aligned}Ss \text{ trts} &= \frac{2.06^2 + \dots + 1.88^2}{2} - Cf \\&= 11.25 - 11.23 \\&= \underline{0.02}\end{aligned}$$

$$\begin{aligned}Ss \text{ Error} &= 0.03 - 0.02 \\&= \underline{0.01}\end{aligned}$$

Anova Table

S.V	d.f	Ss	Mss	Fcal	Ftab(0.05)
Trts	5	0.02	0.004	2	4.39 ^{ns}
Error	6	0.01	0.002		
Total	11	0.03			

n^s = Not significant difference (P>0.05)



Analysis of Variance (ANOVA) of the Feed Conversion Ratio (FCR)

Diets	A	B	Tr+Total	Tr+Means
1	5.57	5.89	11.46	5.73
2	5.67	5.92	11.59	5.80
3	5.72	5.77	11.49	5.75
4	5.62	5.81	11.43	5.72
5	5.81	5.65	11.46	5.73
6	5.77	5.84	11.61	5.81

$$Cf = \frac{(69.04)^2}{12}$$

$$= 466.25$$

$$Ss \text{ total} = 5.57^2 + \dots + 5.84^2 - Cf$$

$$= 397.34 - 397.21$$

$$= 0.13$$

$$Ss \text{ trts} = \frac{11.46^2 + \dots + 11.61^2}{2} - Cf$$

$$= 397.22 - 397.2$$

$$= 0.01$$

$$Ss \text{ Error} = Ss \text{ total} - Ss \text{ trt}$$

$$= 0.13 - 0.01$$

$$= 0.12$$

Anova Table

S.V	d.f	Ss	Mss	Fcal	Ftab(0.05)
Trts	5	0.01	0.002	0.1	4.39 ^{ns}
Error	6	0.12	0.02		
Total	11	0.13			

n^s = Not significant (P>0.05)

Analysis of Variance (ANOVA) of Average Weight Gain

Diets	A	B	Tr+Total	Tr+Means
1	17.09	16.09	33.18	16.59
2	16.15	15.12	31.27	15.64
3	15.6	15.3	30.9	15.45
4	15.59	15.16	30.75	15.38
5	15.0	15.14	30.14	15.07
6	14.46	14.46	28.92	14.46

$$Cf = \frac{(185.16)^2}{12}$$

$$= \underline{2857.02}$$

$$\begin{aligned} Ss \text{ total} &= 17.09^2 + \dots 14.46^2 - Cf \\ &= 2863.12 - 2857.02 \\ &= \underline{6.1} \end{aligned}$$

$$Ss \text{ trts} = \frac{33.18^2 + \dots 28.92^2}{2} - Cf$$

$$= 2861.94 - 2857.02$$

$$= \underline{4.92}$$

$$\begin{aligned} Ss \text{ Error} &= 6.1 - 4.92 \\ &= \underline{1.18} \end{aligned}$$

Anova Table

S.V	d.f	Ss	Mss	Fcal	Ftab(0.05)
Trts	5	4.92	0.984	4.99	4.39*
Error	6	1.18	0.197		
Total	11	6.10			

* = significant ($P > 0.05$).

Analysis of Variance (ANOVA) of Protein Efficiency Ratio (PER)

Diets	A	B	Tr+Total	Tr+Means
1	0.43	0.43	0.86	0.43
2	0.43	0.44	0.87	0.44
3	0.43	0.44	0.87	0.44
4	0.43	0.43	0.86	0.43
5	0.43	0.44	0.87	0.44
6	0.42	0.42	0.84	0.42

$$Cf = \frac{(5.17)^2}{12}$$

$$= \underline{2.2274}$$

$$Ss \text{ total} = 0.43^2 + \dots + 0.42^2 - Cf$$

$$= 2.2279 - 2.2274$$

$$= \underline{0.0005}$$

$$Ss \text{ trts} = \frac{0.86^2 + \dots + 0.84^2}{2} - Cf$$

$$= 2.2278 - 2.2274$$

$$= \underline{0.0004}$$

$$Ss \text{ Error} = 0.0005 - 0.0004$$

$$= \underline{0.0001}$$

Anova Table

S.V	d.f	Ss	Mss	Fcal	Ftab(0.05)
Trts	5	0.0004	0.00008		4.39 ^{ns}
Error	6	0.0001	0.00002		
Total	11	0.0005			

$$n^s = \text{Not significant (P>0.05).}$$

Analysis of Variance (ANOVA) of Protein Productive Value (PPV)

Diets	A	B	Tr+Total	Tr+Means
1	8.78	8.80	17.58	8.79
2	6.59	6.74	13.33	6.67
3	9.45	9.55	19.0	9.5
4	9.46	9.41	18.87	9.44
5	7.19	7.33	14.52	7.26
6	6.92	6.83	13.75	6.88

$$Cf = \frac{97.05^2}{12}$$

$$= \underline{784.89}$$

$$Ss \text{ total} = 8.78^2 + \dots 6.83^2 - Cf$$

$$Ss \text{ total} = 801.89 - 784.89$$

$$= \underline{17}$$

$$Ss \text{ trts} = \frac{17.58^2 + \dots 13.75^2}{6} - Cf$$

$$= 801.86 - 784.89$$

$$= \underline{16.97}$$

$$Ss \text{ Error} = 17 - 16.97$$

$$= \underline{0.03}$$

Anova Table

S.V	d.f	Ss	Mss	Fcal	Ftab(0.05)
Trts	5	16.97	3.39	678	4.39*
Error	6	0.03	0.005		
Total	11				

* = Significant difference ($P > 0.05$).

Analysis of Variance (ANOVA) of Protein Intake

Diets	A	B	Tr+Total	Tr+Means
1	38.38	38.28	76.66	38.33
2	36.75	35.90	72.65	36.33
3	35.88	35.50	71.38	35.69
4	35.43	35.59	71.02	35.51
5	34.53	35.05	69.58	34.79
6	33.69	34.09	67.78	33.89

$$Cf = \frac{(429.07)^2}{12}$$

$$= 15341.76$$

$$Ss \text{ total} = 38.38^2 + \dots + 34.09^2 - Cf$$

$$= 15365.28 - 15341.76$$

$$= 23.52$$

$$Ss \text{ trts} = \frac{76.66^2 + \dots + 67.78^2}{2} - Cf$$

$$= 15364.61 - 15341.76$$

$$= 22.86$$

$$Ss \text{ Error} = 23.52 - 22.86$$

$$= 0.66$$



Anova Table

S.V	d.f	Ss	Mss	Fcal	Ftab(0.05)
Trts	5	22.86	4.57	41.55	4.39*
Error	6	0.66	0.11		
Total	11	23.52			

* = Significant ($P > 0.05$).

Analysis of Variance (ANOVA) of Survival Rate

Diets	A	B	Tr+Total	Tr+Means
1	90	100	190	95
2	100	80	180	90
3	90	80	170	85
4	90	90	180	90
5	100	90	190	95
6	80	90	170	85

$$Cf = \frac{(1080)^2}{12}$$

$$= \underline{97200}$$

$$Ss \text{ total} = 90^2 + \dots + 90^2 - Cf$$

$$= 97800 - 97200$$

$$= \underline{600}$$

$$Ss \text{ trts} = \frac{190^2}{2} + \dots + \frac{170^2}{2} - Cf$$

$$= 97400 - 97200$$

$$= \underline{200}$$

$$Ss \text{ Error} = Ss \text{ total} - Ss \text{ trt}$$

$$= 600 - 200$$

$$= \underline{400}$$

Anova Table

S.V	d.f	Ss	Mss	Fcal	Ftab(0.05)
Trts	5	200	40	0.60	4.39 ^{ns}
Error	6	400	66.67		
Total	11	600			

n^s = No significant difference (P>0.05).

Analysis of Variance (ANOVA) of Hepatosomatic Index (HSI %)

Diets	A	B	Tr+Total	Tr+Means
1	1.46	1.54	3.0	1.5
2	1.48	1.56	3.04	1.52
3	1.50	1.47	2.97	1.49
4	1.46	1.50	2.96	1.48
5	1.53	1.48	3.01	1.51
6	1.46	1.51	2.97	1.49

$$Cf = \frac{(17.95)^2}{12}$$

$$= 26.8502$$

$$Ss \text{ total} = 1.46^2 + \dots + 1.5^2 - Cf$$

$$= 26.8627 - 26.8502$$

$$= 0.0125$$

$$Ss \text{ trts} = \frac{3.0^2 + \dots + 2.97^2}{2} - Cf$$

$$= 26.8505 - 26.8502$$

$$= 0.0003$$

$$SS \text{ Error} = 0.0125 - 0.0003$$

$$= 0.0122$$



Anova Table

S.V	d.f	Ss	Mss	Fcal	Ftab(0.05)
Trts	5	0.0003	0.0006	0.03	4.39 ^{ns}
Error	6	0.0122	0.0020		
Total	11	0.0125			

n^s = There is no significant difference