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EDITORIAL

This proceeding contains papers presented at the International Conference on Animal Nutrition, organized by the Department of Fisheries and Watershed Management, KNUST, Kumasi Ghana on 8-9th August, 2016.

The proceeding primarily provides participants with detailed information on the scientific contents of the meeting. Additionally, it can be used by participants and others as a publication reflecting the present state of agro-by-products used in animal feed production in the West African sub-region.

Although, not peer-reviewed, the manuscripts have passes through a limited editing process in order to improve, where needed compliance with the editors scientific and technical guidelines.

The editorial committee of the conference expresses it profound gratitude to the authors who submitted abstracts and followed it up with full manuscripts which have published in this proceedings.

Kumasi, 13th December 2016.

Table of Contents

DIETARY EFFECT OF SODIUM CHLORIDE (NACL) ON GROWTH AND NUTRIENT UTILIZATION OF THE AFRICAN CATFISH, *Clarias gariepinus*, FINGERLINGS

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Abstract
This study was conducted to determine the dietary effects of sodium chloride (NaCl) on the growth and nutrient utilization of the African *Clarias gariepinus*. Four practical diets containing different levels of NaCl (T1, 0%; T2, 1%; T3, 2% and T4, 4%) were fed to *C. gariepinus* fingerlings (N=100; mean weight, 36.7-41.5 g), at 5% body weight for 70 days. The weight gain of catfish was highest in T2 diet treatment (89.35±14.78) and lowest in T4 diet trewatment (98.95±0.35). Catfish survival and specific growth rate, as well as feed efficiency and protein intake showed no significant differences ($p>0.05$) among dietary treatments. Therefore, in order to make this experimental trial complete and useful to the aquaculture industry (for profit maximization and production of high quality fish), an inclusion level of 4% NaCl is recommended to improve growth and nutrient utilization in *C. gariepinus.*

Introduction

All forms of aquatic animals require inorganic elements, or minerals, for their normal life processes. Fishes are able to absorb some inorganic element from their diets and external environment in both fresh and sea water. Many essential elements are required in such small quantities which are difficult to formulate in the diet. The most commonly used measure of nutritional status is the level of trace elements, which is a range of tissue levels compatible with optimum growth and function. The level of mineral intake influences tissues concentration which causes a gradual decline in the function of an organ until clinical and deficiency occur.

Although most of the naturally occurring mineral elements are found in animal tissues, many of these are present because they are constituents of animal food and may not have essential function in the animal metabolism. The term essential mineral element is restricted to a mineral element which has a metabolic role in the body. Before an element can be classified as essential, it is generally considered necessary to prove that purified diets lacking the element cause deficiency symptoms in animals and that those symptoms can be eradicated or prevented by adding the element to the diets. Most research on mineral nutrition has been carried out in this way, but some of the mineral elements required by animals for normal health and growth are needed in such minute amounts that the construction of deficient diets is often difficult to achieve in such studies, but also to ensure that animals do not obtain the element under investigation from cages, troughs, attendants, or dust in the atmosphere.

The minerals, such as Na, K, and Cl have primarily electro-chemical functions, which are concerned with the maintenance of acid-base balance and osmotic control of water distribution within the

body. The quantitative dietary requirements for Na, K, and Cl depend on the amounts required for growth and reproduction and that which is unavoidably lost by fish through gut, kidney and by passive diffusion across the gills and general body surface. Freshwater fishes remain dependent on an adequate supply of minerals as there is a continuous efflux of ions from the body (Cowey and Sargent, 1979). Diets supplemented with various levels of NaCl have been widely used in the fish farming industry, mainly for seawater adaptation. Nutritional effects of such diets are not fully understood, with different assessments resulting from the differences in diets preparation methods, salt content, nutrient balance and feeding levels. Thus the objective of this study was to determine the effects of dietary inclusion of NaCl on survival, growth performance, nutrient utilization and carcass composition of *Clarias gariepinus* fingerlings.

Materials and Methods

There were four duplicated treatments, each representing different inclusion level of NaCl: 0% (control), 1% (treatment 2), 2% (treatment 3), 4% (treatment 4) in diets 1, 2, 3 and 4, respectively. Four experimental diets were formulated to provide 40% crude protein. All dietary ingredients were weighed using a top balance (Meter Toledo, PB8001 London). The ingredient was then grounded to small particle size. Ingredient including vitamin/premix and sodium chloride were thoroughly mixed in a Hobart A-200T pelleting and mixing machine (Hobart LTD London, England) to obtain a homogenous mass, cassava starch was added as binder. The resulted mash was then pressed without steam through a mixer with 4.0mm die attracted to the Hobart pelleting machine. Diets were immediately sun-dried for two days to prevent molten and mucus growth. After drying, the diets were stored in the laboratory at room temperature prior to feeding. Proximate composition (moisture, crude lipid, crude protein, crude fibre, total ash) of the diets was carried out according to AOAC (2000) methods. A total of 100 *C. gariepinus* fingerlings were stocked in circular transparent plastic containers containing 15 litres of water during a 5-day acclimation period. Ten catfish fingerlings were weighed and stocked in each plastic container. The water in containers was changed and the fish were sampled every other weeks to measure growth parameters. Water quality parameters (temperature, dissolved oxygen level and pH values) were monitored daily using a thermometer, dissolved oxygen meter and a pH meter, respectively.

Table 1: Ingredients composition of experimental diets (g/100g dry matter)

Ingredients	Diets			
	T1	T2	T3	T4
Fish meal (65% CP)	25	25	25	25
Soya bean (42% CP)	35	35	35	35
Maize (9.5% CP)	15	14	13	11
Sodium chloride	0	1	2	4
Blood meal (85% CP)	10	10	10	10
Fish oil	6	6	6	6
Vegetable oil	4	4	4	4
Vitamin-mineral Mix	3	3	3	3
Starch (Binder)	2	2	2	2

The fish were hand-fed twice daily (8:00 - 9:00am and, 5:00 - 6:00pm) according to their body weight (5% BW) for 70 days. Fish mortality (%) was monitored daily and recorded. At the end of the feeding trial, proximate composition of five experimental fish in each treatment was carried out according to AOAC (2000) methods. Fish growth indices were estimated from fortnightly measurements made using the following formulas:

Weight Gain: Difference between the initial weight and final weight gain

Mean Weight Gain=Mean Final Weight-Mean Initial Weight

Total Weight Gain= Final Average Weight-Initial

#####Average Weight

Percentage Weight Gain (PWG): This was calculated as: $PWG = \frac{\text{Total weight gained}}{\text{Initial weight}} \times 100$

Daily Growth Rate (DGR): This was calculated as: $DGR = \frac{\text{Weight gain (g)}}{\text{T (days)}}$

Average Daily Growth Rate (ADGR): This was calculated as: $ADG = \frac{\text{Final weight} \quad \text{Initial weight}}{\text{No of days}}$

Specific Growth Rate (SGR): This was calculated from the relationship of the different in the weight gain fish within the experimental period. $SGR\ (\%) = \frac{\text{Log W2} \quad \text{Log W1}}{\text{T}} \times 100$

Where W2= final body weight, W1= initial body weight of fish, T = duration of study in days

Feed Conversion Ratio (FCR): From the weight gained and feed consumed by each group of fish, the feed conversion ratio (FCR) was calculated using the following expression. FCR = Feed intake/Net weight gain

Protein Intake (PI): This was calculated as PI = feed intake x % crude protein in the diet.

All data obtained were presented as means ± SE and analyzed by one way analysis of variance (ANOVA) test using the SPSS Software (2004 version, Chicago Illinois, US).

Results

Water temperature ranged from 25.1 to 27.0°C, dissolved oxygen ranged from 6.7 to 9.6 mg/L and pH ranged from 7.0 to 9.3 as seen in Fig 1.Fish mortality was low (< 10%) throughout the feeding trial with values of 10%, 10%, 15% and 10% for treatment 1,2,3 and 4 respectively. Table 2 shows the proximate

composition of the experimental diets. Moisture composition ranged between 5.07 and 8.53 g/100g, catfish in the control treatment had the lowest value followed by Diets T4 (4% NaCl), T3 (2% NaCl) and T2 (1%NaCl). Diet T2 has the lowest ash content while Diet T4 had the highest value. Diet T1 had the highest lipid content while Diet T4 had the lowest lipid content. Diet T3 had the lowest crude protein content while Diet T4 had the highest value. Growth performance and nutrient utilization indices of *Clarias gariepinus* fed varying inclusion levels of NaCl are shown in Table 3. There were significant differences ($p<0.05$) in weight over the 70 day feeding trial. Catfish fed with the control diet had a lower weight gain than catfish fed with the test diets containing NaCl. Fish fed 1% (NaCl) supplemented diet (T2) had the highest percentage weight gain while fish fed with Diet T4 had the highest specific growth rate (SGR). Feed conversion ratio (FCR) gave the highest value in catfish fed with diet T3 and the lowest value when fed with diet T4.

Table 2: Proximate composition of the experimental diets (mg/100g DM)

	T1	T2	T3	T4
Moisture	3.91	4.63	4.94	5.37
Ash	12.96	12.13	14.02	14.23
Lipid	21.43	24.69	24.59	26.27
Protein	39.95	42.04	41.24	38.9
Crude fiber	4.45	3.52	4.13	3.46

Table 3: Growth and nutrient utilization of *Clarias gariepinus* fed varying dietary inclusion levels of NaCl

	T1	T2	T3	T4
Weight Gain (g)	79.45 ± 28.92^{c}	89.35 ± 14.78^{b}	89.75 ± 40.52^{a}	98.95 ± 0.35^{d}
Average Daily Growth (g)	1.14 ± 0.42^{a}	1.28 ± 0.21^{a}	3.37 ± 0.58^{a}	1.42 ± 0.01^{a}
Food Conversion Ratio	3.32 ± 0.78^{b}	3.13 ± 0.43^{c}	3.37 ± 1.20^{b}	2.89 ± 0.13^{a}
Percentage weight gain (%)	213.21 ± 27.05^{d}	260.13 ± 96.39^{c}	221.00 ± 107.48^{a}	205.58 ± 41.71^{b}
Specific Growth Rate (%/fish)	0.71 ± 0.06^{a}	0.72 ± 0.85^{a}	0.71 ± 0.21^{a}	0.80 ± 0.06^{a}
Protein Intake (g)	100.66 ± 13.46^{b}	115.81 ± 3.81^{b}	114.77 ± 11.09^{b}	111.08 ± 5.64^{b}
Survival (%)	85.00 ± 7.07^{a}	85.00 ± 7.07^{a}	100.00 ± 0.00^{a}	95.00 ± 7.07^{a}

Discussion

The varying inclusions levels of sodium chloride and the effects on the growth performance and nutrient utilization of *C. gariepinus* fingerlings were studied for 70days. The experimental diets were isonitrogenous hence they contained approximately the same crude protein level. Lovell (1989) noted that the optimum level of protein in feed for growth of intensively cultured American channel catfish is 25-45%, while Balogun (1990) recommended 37.50% crude protein for *C. gariepinus* fingerlings. The dietary protein level used for this study is within 38-42. The protein intake of all the fish in the dietary treatments were similar ($p>0.05$). This showed that NaCl did not have any effects on protein intake of the

experimental fish. The difference in percentage weight gain could be attributed to the different NaCl levels used in the diets. The weight gain in Treatment 4 (4% NaCl) was the highest among the other NaCl levels used. This agrees with Machiels and Henken (1987) who showed that the highest weight gain were found when NaCl content in feed is >4%.

From to the results (Table 3), the highest weight gain 98.95+ 0.35(g) was recorded in Treatment 4 (with 4% of NaCl) followed by treatment 3 (3% NaCl). There were no differences ($p<0.05$) within the treatment used. This may be attributed to the high NaCl contents in the Treatment 4.The decrease in weight gain of treatments 1and 2 (0% and 2% NaCl, respectively), could be as a result of non-acceptance of the particular diet. In treatment 1 (0% NaCl), slow growth rate was recorded. This might be due to low acceptability of the treatment by the fish during the period of experiment.

Haylor (1992) deduced that specific growth rate can vary significantly when measured over successive short interval from first feeding. Similarly, SGR at lower stocking density was significantly greater than specific growth rate at high stocking density. The specific growth rate was highest in fish fed with treatment 1 and 3 i.e. (0% and 2% NaCl). Hogendoorn (1979) reported an increase in SGR as feeding intake increased. The qualities of the various treatments used were further substantiated by the variation in their final mean weight among various fish. The lower feed conversion ratio in treatment 4 has significant difference in the weight gain. This may be due to increase in food consumption bringing a proportional increase in growth, however there was significant difference among the treatments ($p<0.05$).

Mean weight gain/fish was also affected by the protein levels of the diets supplied to the test fish. This is justified by the levels of significant difference that occurred within the four NaCl level diets. Treatments 1 and 2 have significant difference, from treatments 3 and 4 ($p<0.05$) This experiment showed that mortality was not high throughout the experimental period, (survival was about 9%) .This result may be attributed to little or no competition for food among the fingerlings as there was enough space to move about for food. The result recorded for the nutrients utilized as shown by FCR and feed efficiency (FE) indicated that treatment 2 had the highest protein intake while treatment 1(0% NaCl) had the least protein intake. The inverse relationship occurring between the feed conversion ratio and protein levels is similar to the one reported by Jauncey (1982) for *Sarotherodon mossambicus.*

Carcass Composition of the Experimental Fish

The moisture composition ranged between 5.07 and 8.53, the control has the lowest value of (5.07) followed by T4 (4% of salt), 5.09, T3 (2% salt), 5.58, and T2 (1% NaCl) has the highest value of 8.53%. Salt as a hygroscopic substance might have caused the increase in the moisture content of the samples. Sample T2 has the lowest ash content of 12.40% and T4 has the highest value of (15.05). The increase in the ash content value might be due to the addition of salt in the diets which was assimilated and deposited in fish tissue. The control has the highest lipid content which may suggest the fact that the addition of salt might have reduced the lipid content of the rest samples other than the control. i.e. the more the addition of salt the lower the lipid content of the fish sample. Treatment T3 had the lowest protein content of 61.62% and treatment T4 had the highest of 65.82%. NaCl may have reduced the values but the value increased with additional salt in the diet.

Conclusion

This study shows that *C. gariepinus* increased in weight gain and feed efficiency as the dietary level of NaCl increased. Hence, a 4% dietary inclusion level of NaCl is beneficial in order to achieve good growth in the culture of *C. gariepinus*.

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USE OF AGRO BY-PRODUCTS IN ANIMAL FEEDS: LEAST COST & LEAST RISK

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Abstract

What is fed to animals intended for human consumption raises important public health concerns. These concerns arise not only from what is fed to animals but also from gaps in regulations and systems intended to ensure the safety of feed and the food supply. A variety of substances, including many waste materials from the agriculture, food, and rendering industries, are “recycled” into feed for food-producing animals. Some of these ingredients used in feed, particularly those from animal and mixed sources, may result in

unwanted feed contaminants or have other unintended consequences. Animal feed plays an important role in the cycling of dioxin, arsenic, pathogens, antibiotic-resistant bacteria, prions, and other substances of public health concern. In this report we have highlighted areas where further public health investigation is warranted, and have also identified some of the barriers to tracing the connections between animal feed and public health, in particular, the lack of effective surveillance systems. It is vital for public health professionals to better understand the feed industry, feed ingredients, and the current regulatory framework, in order to begin to address the public health risks associated with current animal feed practices.

A wide range of agro by-products is allowed in the manufacture of animal feeds which are sourced from the under listed:

a. Plant sources

- Grains (corn, sorghum, wheat)
- Oilseed meals and cakes (soybean, cottonseed, canola, sunflower seed)
- Grain by-products (distillers grains, brewer's yeast, corn gluten meal)
- Fruit and fruit by-products (dried citrus pulp, apple pulp)
- Molasses and sugar
- Alfalfa products
- Miscellaneous plant products (banana/plantain peels, coffee hulls, cocoa bean pods)

b. Animal sources

- By-products of slaughtered animals (meat and bone meal, hydrolyzed feather meal, blood meal, tannery wastes, animal carcasses)
- Fisheries by-products (fishmeal, **fish oil**, shrimp head meal)
- Animal wastes (dried ruminant or swine wastes, dried poultry litter)

Cutting Costs: Use of Waste as Feed

Feed remains one of the top costs for farmers, accounting for about 50-75% of total livestock production expenditures (Muirhead, 2003). Therefore, the use of less costly ingredients that still meet the nutritional requirements of the animal is another major trend in the animal feed and animal production industries. The commercial animal feed industry "was born out of the needs of grain, oilseed and meat processors to find an economical and safe way to dispose of their waste by-products" (Muirhead, 2003). The industry refers to the practice as "recycling." For this reason, the rendering industry, which deals with the large quantities of animal products/parts not suitable for use as human food, is closely associated with the animal feed industry.

The use of animal waste (manure or a mixture of manure, urine, and litter) as a feed ingredient is one type of "recycling" practice that has accompanied the shift to concentrated animal feeding operations. Whereas manure from animals was traditionally used to fertilize locally grown crops, the manure output from these concentrated operations overwhelms the capacity of local croplands to absorb it. The bulk and weight of animal waste generally makes transporting it not economical, and its use in feed is considered by practitioners to be a viable alternative to disposal in a landfill. The use of recycled animal waste for feed has been recognized and deliberately incorporated into animal feed for many years.

Increasing Demand for Meat and Aquaculture Products Fuels Demand for Feed

The rapid growth in world production and consumption of meat and meat products, called has fueled feed demand. Feed and feed ingredients, unlike meat, can easily be stored and shipped over long distances. Feed for aquaculture production is one of the most rapidly growing sectors, since aquaculture production has risen about 9.2%/annum globally since 1970, fueling the demand for feed, compared to growth rates of 1.4% for capture fisheries and 2.8% for terrestrial farmed meat production systems (FAO, 2002).

Feedstuffs of Public Health Interest

Some ingredients of particular interest from a public health perspective are listed below.

1. Specified Risk Materials (SRMs)

SRMs are materials known to harbor the highest concentrations of prions, the agents that are believed to cause bovine spongiform encephalopathy, or BSE, commonly known as mad cow disease. Different scientists, countries, and agencies define SRMs differently, and the definition may depend on the age and/or species of the animal in question and/or the BSE risk status of the country of origin of the animal.

2. Mammalian and Poultry Protein (poultry litter, blood, animal wastes, animal carcasses)

A number of rendered animal by-products are used in feed instead of listing specific ingredients, products from aquatic animals, and products from both food-producing and non-food-producing animals. The rendering processes used by most plants would not eliminate BSE infectivity. In most cases, the process would reduce the infectivity of the raw materials (if it were present). There is evidence that blood (e.g., from sheep) can carry the agent that causes transmissible spongiform encephalopathies (TSEs). One BSE-related concern with poultry litter is that it can contain poultry feed that has spilled onto the litter, and poultry feed may legally contain ingredients such as bovine meat and bone meal that are prohibited from use in ruminant feed. Other public health issues (e.g., microbial pathogens, drug residues) may be raised by the use of these ingredients as well.

3. Antimicrobials

Antimicrobials are added to feed, primarily at sub-therapeutic levels. Such uses are alleged to help compensate for crowded conditions present in intensive production systems (National Research Council, 1999, pp. 4, 28), and to promote growth. Globally, there is widespread use and misuse of antibiotics to control diseases in aquaculture species (Garrett et al., 1997; Hernández-Serrano, 2005). In many Southeast Asian countries, antimicrobial use is unregulated and involves antimicrobials not permitted in the U.S. (e.g., nitrofurans and chloramphenicol) (Choo, 2001). Chloramphenicol, a potent antimicrobial linked to aplastic anemia in humans, has been detected in imported shrimp and crayfish from Asia. Aquaculture production is rapidly worldwide, yet only four antimicrobials are presently approved and available for use in aquaculture in the U.S. The lack of approved antimicrobials puts pressure on some producers to use unapproved products. Some countries have made efforts to reduce the use of antimicrobials in feed.

4. Animal Fats

Up to 8% of animal and fish feed can be fat. The annual production of animal fats (white and yellow tallow, greases, and poultry fat) is estimated to be 3.6 billion pounds of inedible tallow, 3 billion pounds

of grease, and 1.4 billion pounds of recycled fat. These are largely by-products and waste from rendering and meat-processing plants. Animal fats have been recognized as the greatest potential source of contamination by dioxin-like compounds, and considered it a "high-priority risk management intervention" to interrupt the cycle of dioxin-like compounds (DLCs) through forage, animal feed, and food-producing animals (including fish). Governments, in collaboration with the animal production industry, identify means to achieve the reduction or elimination of DLC-containing animal fat as a component of animal feed."

5. Arsenic

Arsenic is added to poultry and swine feed to promote growth, improve feed efficiency, improve pigmentation, and other uses. Arsenic can contaminate poultry and poultry litter/waste, ultimately increasing levels of arsenic in the environment and possibly increasing exposures to arsenic among consumers of chicken. Cattle given feeds containing poultry litter had elevated levels of arsenic in edible muscle tissue (Westig et al., 1981). According to a recent estimate, based on an analysis of arsenic in chicken liver, people consuming large amounts of chicken can ingest a sizable proportion of the tolerable daily intake of arsenic established by WHO (Lasky et al., 2004). An estimated 75% of the arsenic in litter is readily soluble in water (Rutherford, 2003). When the poultry litter is applied to agricultural fields, the arsenic is released into the environment and may result in increased levels of arsenic in surface and groundwater, as well as increased uptake by plants (Rutherford, 2003).

6. Minerals and Mineral Mixes

Minerals and mineral mixes and premixes used in animal feed can contain contaminants such as dioxin and various heavy metals. Some mineral mixes and premixes are by-products or co-products of industrial metal production and can become contaminated. For example, mineral mixes containing zinc oxide obtained from brass production have been found to have high levels of dioxin contamination. Hazardous wastes are sometimes recycled as nutritional supplements in animal feed preparations. For example, zinc oxide reclaimed from emission control dust from electric arc furnaces is a listed hazardous waste. Minerals may also contain heavy metal contaminants such as lead, arsenic, cadmium, and mercury. Several mineral products used as feed ingredients, and the "typical" levels of these contaminants in mineral feed ingredients. Lead is considered only "moderately toxic" and the maximum tolerance in complete feed is 30 ppm

FISHERY WASTES MANAGEMENT IN NIGERIA: THE AQUAFEED OPTION

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Abstract

Large quantities of fishes are landed from marine and freshwaters in Nigeria in short periods and often glut the market, consequently much remain unsold and spoil due to poor handling and processing. Large quantities of fishery wastes are thus generated and abound in coastal region of Nigeria, where they create disposal problems. Current disposal practices include burial, municipal garbage disposal and dumping in fields or streams. Fishery wastes represent oversupply to immediate market demands due to improper preservation. They comprise low-value fishes, waste fish, carcass and by-products from filleting and deboning processes. An alternative fishery wastes disposal is the recycling into fish meal or silage for use as aquaculture feed supplements. Presently, their contribution to aquafeeds is based on crushing sun-dried wastes, milled to powder and blended with various carbohydrates feedstuffs to produce farm-made aquafeeds. The processing is currently undertaken on cottage level. Fishery wastes meal and fishery wastes silage (fermented) were evaluated as substitutes/replacements for commercial herring meal in a standard/reference diet. Growth and carcass composition catfish fed the test diets were similar. Fishery wastes could be economically utilized to produce acceptable high-protein feedstuffs (meal, silage) for aquafeeds. Fermented fish silage has been used as feed supplement for various aquaculture fishes and they possess good nutritional quality, and the biological value of its protein was comparable with that of fish meal protein. The production of meal and silage appears an attractive way of utilizing fishery wastes, and will reduce the foreign currency drain presently involved in importing herring fish meal.

Keywords: Fishery wastes, fish meal, fermented fish silage, aquaculture feeds.

Introduction

Feeding in aquaculture constitutes an important factor affecting the production and economic results of aquaculture. Herring fish meal is the conventional protein source in aquaculture feeds (aquafeeds) and has become expensive and scarce; particularly in developing countries (Tacon, 1993). Hence, a strong economic incentive supports exploring alternative protein feedstuffs and supplements in aquafeeds. Waste materials and underutilized fishes from fisheries operations have been used to produce protein supplements in aquafeeds. The benefits derived from least cost development of aquafeeds relies on improved reliability of the feedstuff supply, predictability of diet composition and reduced feed cost (Urban & Pruder, 1991); more so that feeds or supplements can only be applied to commercial aquaculture production if an expectation for increased profit exists.

Fisheries industries in Nigerian generate large amounts of wastes which create disposal problems and represent non-utilization of valuable protein sources (Obe et al., 2015. There is the need to develop new and productive techniques for the management of fishery wastes, particularly in the use as high protein feedstuff for their inclusion in aquafeeds. Other than rendering fishery wastes into direct dry meals, ensilation techniques used to produce silage is recognized as a means of reusing these wastes and managing such resources towards recycling, reuse packages (Brown & Sumner, 1985), Fish silage is a liquid product direct from acid addition or biological fermentation of whole or parts of fish to which no materials are added other than acid, and in which liquefaction of the fish mass is carried out by enzymes present in the fish. Fish silage has thus become of interest as protein feedstuff as substitute for fish meal in the aquafeed industry.

In Nigeria, lactic acid fermentation has been the preferred method (Fagbenro, 1996) using lactic acid starter cultures (molasses) plus cassava starch as energy source in a 1:1 (wastes: starch) ratio. This study provides information needed to facilitate low-cost production of dry meal or silage from fishery wastes for potential use in aquafeeds.

Preparation and Properties of Fishery Wastes Products

Fishery wastes products (whole fish, heads, and viscera) were derived from low oil fishes, obtained from commercial fish processing operators in coastal regions of southern Nigeria; and separated into two batches, A. and B. Waste materials in Batch A were converted into fishery wastes meal (FWM) using the conventional cottage dry fish meal industry practice in Nigeria. The wastes were washed in water and sun-dried at ambient temperature (27oC) for seven days; crushed, milled to fine meal (< 250 μm) and stored at ambient temperature in mini farm silos. They were later be blended with other feedstuffs to produce aquafeeds (Obe et al., 2015).

Waste materials in Batch B were converted into fishery wastes silage (FWS) using lactic acid fermentation methods. They were chopped and minced and passed through a die plate with 3mm-diameter holes. Molasses was mixed with the minced fish (150 g molasses/kg fish mince), and pre-fermented starter culture of Lactobacillus plantarum was used to inoculate the mixture (50 ml inoculum/kg fish mince), and incubated anaerobically in sealed containers for seven days at ambient temperature for 25-30oC. The fermented silage was heated to 90oC in a temperature-controlled water bath for 30 minutes to halt autolysis

(Fagbenro et al., 1997). Liquid fish silage thus produced was blended with cassava starch and co-extruded while heating. This formed an intermeshing matrix of proteins and starch which become gelatinized, giving an effective bonding and thus good feed stability. An advantage of this process is that it incorporates liquid fishery wastes silage as an ingredient in the feed mixture without the need/cost of pre-drying.

FWM and FWS were analyzed for proximate composition using standard methods (AOAC, 2000) while the essential amino acid (EAA) composition was determined in acid hydrolysates. Tryptophan content was determined after alkali hydrolysis (Fischl, 1960; Miller, 1967). Gross energy content was determined using an adiabatic bomb calorimeter. Proximate and EAA compositions and gross energy content of FWM and FWS (table 1) were similar except for crude lipid content which was lower ($P > 0.05$) in FWS. This is attributed to loss of volatile lipids/oils during the co-drying process. Values for the EAA composition of both fishery wastes products were also similar ($P > 0.05$) except for tryptophan content. This is expected in silage products since they are produced under acid conditions ($pH < 4.5$) during fermentation via protein autolysis, which destroy tryptophan (Miller, 1967).

Table 1: Proximate and essential amino acid (EAA) compositions of fishery wastes products

(g/100g DM)	FWM	FWS
Moisture	6.3	6.8
Crude protein	61.1	58.9
Crude lipid	10.4[a]	5.6[b]
Ash	11.5	9.1
Gross energy (kcal/100g DM)	4.90	4.83
EAA composition (g/100g protein)		
Arginine	7.18	6.87
Histidine	2.54	2.75
Isoleucine	3.47	3.63
Leucine	6.66	6.57
Lysine	6.54	6.40
Methionine	2.55	2.47
Phenylalanine	3.32	3.40
Threonine	4.23	4.15
Tryptophan	1.07[a]	0.80[b]
Valine	4.14	4.38

Mean values in a row with different superscripts differ significantly ($P < 0.05$), Students' t-test (Zar, 1984).

Development and Nutritional Evaluation of Fishery Wastes-Based Aquafeeds

Feed formulation and preparation

Three dry isoproteic and isocaloric practical diets (pelleted) were formulated (table 2) containing Herring fish meal (HFM), FWM or FWS as protein sources and prepared following the formulation recommended for cultivated fishes (Fagbenro & Adebayo, 2005). The control diet (HFM-D) contained 275 g/kg herring

fish meal providing 50% protein in diet, which was totally replaced with FWM or FWS in test diets, FWM-D and FWS-D, respectively (table 2). The ingredient composition of the fishery wastes diets are also presented in table 2. All ingredients were milled to small particle sizes (< 250µm), mixed, extruded and passed through a die size of 2 mm to obtain strands which were oven-dried for 24 h; which were later broken into 2 mm sizes and stored in plastic containers at -20oC prior to use. The diets were analyzed for proximate and EAA compositions (section 2). The nutrient composition of the diets is presented in table 3 while EAA composition is presented in table 4. Few variations occurred in proximate composition of the diets. No apparent differences were shown in EAA composition of the diets and the EAA requirements of African catfish were met (table 4) (Fagbenro et al., 2000).

Table 2: Ingredient composition (g/100g DM) of experimental diets

	HFM-D	FWM-D	FWS-D
Fish meal (Herring)	275	0	0
Fishery wastes meal	0	330	0
Fishery wastes silage	0	0	355
Soybean meal	445	445	445
Cellulose (non-nutritive)	160	105	80
Corn starch	30	30	30
Corn oil: Fish oil (1:1)	60	60	60
Vitamin-Mineral mix. [1]	30	30	30

[1] DSM Nutritional Products Limited, Basle, Switzerland.

Table 3: Nutrient content (g/100g DM) of experimental diets

Diets	Moisture	Protein	Lipid	Fibre	Ash	Gross energy[1]
HFM-D	7.5	40.7	8.5	3.5	10.5	4.18
FWM-D	7.1	40.9	8.3	3.4	10.3	4.24
FWS-D	6.9	40.8	7.5	3.6	9.5	4.22

[1] kcal/g dry matter (DM)

Table 4: Essential amino acid composition (g/100g protein) of test diets

	FWM-D	FWS-D	Catfish requirements*
Arginine	6.91	6.84	4.45
Histidine	2.58	2.52	2.15
Isoleucine	4.71	4.64	3.06
Leucine	8.36	7.92	5.22
Lysine	6.91	6.89	5.70
Methionine	2.16	2.15	1.83
Phenylalanine	4.65	4.47	2.73
Threonine	4.20	4.08	3.16
Tryptophan	1.48	1.25	0.71
Valine	5.65	5.52	2.90

*(Fagbenro et al., 2000

Fish feeding/growth trial

Apparently healthy same-sibling fingerlings of the African catfish (Clarias gariepinus) produced by hormone-induced artificial fertilization method on a commercial farm with mean weight of 20 g were acclimated to experimental conditions for seven days and fed commercial catfish feeds. Groups of 20 catfish fingerlings (mean weight, 12.1 g) were stocked into indoor 60-litre capacity cylindrical plastic tanks; each filled with 20 litres of aerated water. Each diet was fed to catfish in triplicate tanks, to apparent satiation (equivalent to 5% fish body weight) twice daily (09.00h and 16.00h) for 70 days. Fish mortality was monitored daily, total fish weight in each tank was determined at weekly intervals, and the amount of diet was adjusted according to the new fish weights. Initial and weekly mean weights were recorded per diet treatment. Growth response and feed utilization indices were determined as:

Weight gain = Final weight of fish - Initial weight of fish

$$\text{Percentage weight gain} = \frac{\text{Mean weight gain}}{\text{Mean weight initial}} \times 100$$

$$\text{Specific growth rate (SGR, \% per day)} = \frac{\text{LogeW2} - \text{LogeW1}}{T2 - T1} \times 100$$

Where: W2 = wt. of fish at time T2 (final), W1 = wt. of fish at time T1 (initial)

Protein efficiency ratio (PER) = Weight gain/Protein intake

Feed conversion ratio (FCR) = Total feed consumed/Weight gain

Catfish fingerlings became accustomed to the diets within the first week of feeding trial, and no catfish mortalities occurred during the 70-day growth/feeding trial. The summary of catfish growth responses and feed utilization are presented in table 5. Final weight and mean weight gain of catfish fed with test diets were similar ($P > 0.05$) to those fed with the control diet. Weight gain, average daily gain and FCR values

were also similar (P > 0.05) to those fed the control diet. With respect to PER values, diet treatments showed no difference (P > 0.05).

Table 5: Growth and feed utilization of catfish fed with experimental diets.

	HFM-D	FWM-D	FWS-D
Initial weight (g)	12.1	12.1	12.1
Final weight (g)	62.7	61.2	58.6
Weight gain (g)	50.6[a]	49.1[ab]	46.5[b]
ADG (g/day)	0.72[a]	0.70[a]	0.67[b]
SGR (%/day)	2.35	2.33	2.19
FCR	1.42	1.43	1.50
PER	1.81	1.80	1.78
Survival (%)	100	100	100

Values in a row with different superscripts are significantly different (P<0.05), ANOVA test (AOAC, 2000).

Good growth and protein utilization were similarly reported for Asian catfish, *Clarias batrachus*, (Wee et al., 1986) fed with diets containing autolysed protein from tilapia wastes silage; while for another Asian catfish, *C. macrocephalus*, or snakehead, *Channa striata*, there were decreased growth, poor feed conversion and high mortality (Edwards et al., 1987). This was attributed to their inability to utilize free amino acid present in silage-based diets efficiently. All the diets used in this study met or exceeded the essential amino acid requirements of the African catfish stipulated by Fagbenro et al. (2000). Several fishery wastes after ensilation or fermentation have been evaluated/used as protein sources in aquaculture diets, which include tilapia wastes (Fagbenro et al., 1997), clam wastes (Goodrich et al., 1984), viscera silage (Fagbenro & Fasakin, 1986), and shrimp head wastes silage (Fagbenro & Bello-Olusoji, 1997).

In many of these studies, the waste meals could not replace more than 50% of fish meal protein. Higher replacement levels led to poor palatability, poor nutrient utilization, poor growth, poor digestibility pathological lesions and/or poor reproductive performance. Yone et al. (1986a) and Hossain et al. (1987) found that fermented scrap meal from mackerel wastes improved growth performance and feed efficiency in red sea bream (*Chrysophrys major*). Manikandavelu et al. (1992) reported a considerably higher growth of common carp (*Cyprinus carpio*) fed with fermented fish silage-based diet over a control diet based on commercial fish meal. The inclusion of fishery wastes has been justified in monogastric animal feeds (Hassan & Heath, 1986), giving good growth in poultry (Hassan & Heath, 1986; Johnson et al., 1986) and pigs (Tibbetts et al., 1981).

Water quality parameters

Water temperature and dissolved oxygen (DO) were measured daily using a combined digital YSI dissolved oxygen meter (YSI Model 57 YSFI; Yellow Springs, Ohio); pH was monitored weekly using a pH meter (Metler Toledo-320, Jenway, UK). Initial temperature, pH, and DO concentration of the water before stocking were 27.2oC, and 7.6 mg/L respectively. The water quality parameters during the growth/feeding trial were water flow, 1 L/minute; pH, 6.6 - 7.5; temperature, 27.2 - 28.1oC; DO concentration, 7.6-8.5 mg/L; and were within the tolerable limits recommended for growth and culture of the African catfish (Viveen et al., 1985).

Carcass analyses

Five catfish taken at the start and three catfish per tank taken at the end of the growth trials were homogenized and analyzed for proximate analysis (AOAC, 2000). Another set of five catfish taken at the start and five catfish from taken from each treatment at the end of the growth/feeding trials were anaesthetized in 2.5 ml quinaldine/L of water and weighed individually. Their livers were removed and weighed individually and used to calculate the hepatosomatic index (HSI) as: HSI = (liver weight/body weight) x 102. Carcass composition and HSI of African catfish at the beginning and end of the growth trial are shown in table 6. Fishes had higher carcass protein and lipid contents at the end than the initial fish and the differences were not significant. Similar observations were reported in red sea bream fed with diets containing fermented scrap meal (Yone et al., 1986b). There were no differences in HSI.

Table 6: Carcass composition of African catfish fed with experimental diets.

		HFM-D	FWM-D	FWS-D
	Initial			
Moisture	78.6	75.60	75.49	75.48
Crude protein	13.87	16.22	16.40	16.36
Crude lipid	5.11	6.23	6.13	6.06
Total ash	2.69	2.48^{b}	2.69^{a}	2.46^{b}
Hepatosomatic index	1.49	2.00	2.04	2.05

Values in a row with different superscripts are significantly different (P<0.05), ANOVA test (Zar, 1984).

Nutrient digestibility trials

Apparent digestibility coefficient (ADC) of crude protein, crude lipid and gross energy in the experimental diets for C. gariepinus was determined (Fagbenro, 2001) as follows: ten fish were stocked into 20-litre cylindrical plastic tanks supplied with aerated water and acclimated for seven days. Each diet was assigned to duplicate tanks and fish were fed to apparent satiation twice daily (8.30–9.00h and 16.0–16.30h) for 14 days. On day 15, faeces were collected from each anaesthetized fish eight hours after feeding using the dissection method (Fagbenro, 2001). Dry matter, crude protein and crude lipid contents were analyzed in triplicate samples of diets and faeces (AOAC, 2000) and gross energy content was determined by bomb calorimetry. Digestibility was determined using the acid insoluble ash method (Halver et al., 1993). African catfish fed with the experimental diets had high and similar digestibility coefficients (P <0.05) as

presented in table 7. These agree with the values reported by Wee et al. (1986) and Edwards et al. (1987) for Asian catfishes, C. batrachus and C. macrocephalus fed with fermented tilapia silage.

Table 7: Apparent digestibility (%) of nutrients in diets fed to African catfish.

	HFM-D	FWM-D	FWS=D
Dry matter digestibility	88.0	84.7	85.4
Protein digestibility	82.5	80.2	81.1
Lipid digestibility	71.6	72.0	72.6
Energy digestibility	79.2[a]	75.8[a]	70.8[b]

All values represent means of three replicates.

Mean values with different superscripts are significantly different (P<0.05), ANOVA test (Zar, 1984).

Haematological and Histological examinations

Five catfish taken at the start and three catfish per tank taken 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 cyanomethaemoblobin method (Svobodova et al., 1994), and the mean cell haemoglobin concentration (MCHC) was calculated. Table 8 shows that African catfish fed with fishery wastes silage-based diets had lower (P <0.05) haematocrit and haemoglobin contents, and values obtained were however within the range stipulated for the African catfish (Svobodova et al., 1994). MCHC values calculated were not different among the diet treatments, similarly reported for red sea bream fed with scrap meal silage-based diets (Yone et al., 1986b).

Table 8: Haematological features of African catfish fed with experimental diets.

		HFM-D	FWM-D	FWS-D
	Initial			
Haematocrit (Hc,%)	22.8	31.9[a]	31.5[a]	25.9[b]
Haemoglobin (Hb, g/100ml)	6.3	8.3[a]	8.5[a]	7.1[b]
[1]MCHC (%)	27.63	26.02	26.98	27.41

All values represent means of three replicates.

Mean values in a row with different superscripts are significantly different (P<0.05), ANOVA test (zar, 1984).[1] Mean Cell Haemoglobin Concentration = (Hb/Hc) x 102

Tissues from liver 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 5um, placed in glass slides and stained with haematoxylin and eosin, and examined under a light microscope. Histological examination was done as described by Chinabut et al. (1991). No morphological deformities were observed in African catfish fed any of the diets. In particular, there were no signs of back deformities, usually associated with tryptophan deficiency in fish silages (Tacon, 1992), and was contrary to the observation of in Asian catfish (Wee et al., 1986.

Costs of production of protein feedstuffs from Fishery Wastes

Economic analyses were calculated using profit index (PI) and incidence of cost (IC) models (Shang, 1981) as: PI = value of fish/cost of feed; IC = cost of feed/kg of fish produced. According to Urban and Pruder (1991), the primary economic criterion for selecting diet/ingredient however remains the need to maximize profit, because reducing food cost may decrease conversion efficiencies, growth rate or increased mortally, resulting in decreased profit. The economic viability of fishery wastes meal or fishery wastes silage production, being location and time specific, needs local study; hence it is impossible to generalize on the economics of farm-made production of fishery wastes-based feeds. The existing labour on a small farm may be able to absorb the extra work load of making feeds, thereby 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 (Brown & Sumner, 1985). The pertinent question therefore remains as – is it economical to feed diets containing co-dried fermented fishery wastes silage compared with conventional prepared diets?

The economics of fishery wastes meal or fishery wastes 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 fishery wastes meal or fishery wastes silage is not easily assessed because of differences in availability, technology and scale of production. The fact that fishery wastes meal or silage can provide 50% of total dietary protein means that the cost of production can be significantly reduced, if sufficient low cost fishery wastes are available. The use of fishery waste-based diets could mean a lower price of cultivated fish and therefore is particularly useful for tropical developing countries. This cannot be directly extrapolated for commercial evaluation of inclusion of fishery wastes in aquafeeds. Without a detailed cost analysis; it may not be proven that fishery wastes can be economical supplements for commercial aquaculture production.

Conclusions

Fishery wastes meal and fishery wastes silage supported comparative growth and feed conversion; and can be used by African catfish as a dietary protein source. Carcass composition of catfish showed similar contents, similarly reported when diets containing citric acid ensiled viscera wastes and fermented shrimp head wastes silage were fed to African catfish (Fagbenro & Fasakin, 1986; Fagbenro & Bello-Olusoji, 1997; Nwanna et al., 2000) without affecting catfish growth performance, feed conversion or protein utilization. However, Wilson et al. (1984) attributed reduced protein utilization by American channel catfish (Ictalurus punctatus) fed offal-based silage diets to marginal or slight deficiency levels of histidine, isoleucine and total aromatic amino acids in their dies. The production of fishery wastes meal or silage reflects a potential alternative to the challenge of waste management for the fishery industry. Fishery wastes, which are thus unfit for human consumption, can be recycled into farm-made aquafeeds through cultivated fish which are acceptable in human foods (Brown & Sumner, 1985). The production of dry meal and co-dried silage appears an attractive way of utilizing fishery wastes, and will reduce the foreign

currency drain presently involved in importing fish meal. However, fish meal production should not overshadow the benefit of direct human consumption of low value fishes in the fight against malnutrition.

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HEPATOPROTECTIVE AND STRESS - REDUCING EFFECTS OF DIETARY *MORINGA OLEIFERA* EXTRACT AGAINST *AEROMONAS HYDROPHILA* INFECTION AND TRANSPORTATION-INDUCED STRESS IN AFRICAN CATFISH *CLARIAS GARIEPINUS* (BURCHELL, 1822) FINGERLINGS

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Abstract

The main aim of the present study was to assess the hepatoprotective and stress-reducing effect of *Moringa oleifera* extract against *Aeromonas hydrophila* infection and transportation- induced stress in African catfish, *Clarias gariepinus* fingerlings. Fish were fed diets representing different supplementation levels of *M. oleifera* leaf extract. The graded levels of *M. oleifera* leaf extract were 0.00g (control), 0.05g, 0.10g, 0.15g, 0.20g, 0.25g per 100g for each diet. After six weeks of the feeding trial, fish previously fed each experimental diet were exposed to pathogenic strain of *A. hydrophila* at a concentration of 9.3×10^5 CFU /mL. After bath exposure, fish from each dietary treatment were placed into the aquaria culture system. They were fed their respective diets at 5% body weight twice daily, and mortality was monitored for the remaining 4 weeks of the feeding trial. After the feeding trial, fish previously fed each experimental diet were kept in plastic tanks for a 2-hour journey. Blood and liver samples were collected for hepatocellular assessments (Aspartate transaminase (AST), Alanine transaminase (ALT), Lactate dehydrogenase (LDH) and Malate dehydrogenase (MDH) tests) and histological examination. Results showed that the increases of the AST, ALT, LDH, MDH and hepato-histological insults induced by stressors were significantly reduced ($P < 0.05$) by supplementing the fish with *M. oleifera* leaf extract in the diets. Based on the result of this study, a dose of 0.10g/100g dietary Moringa leaf supplementation was sufficient as hepatoprotective and stress reducing agent in *C. gariepinus* fingerlings.

Keywords: Hepatoprotective, Stress - reducing, *Moringa oleifera*, *Aeromonas hydrophila,* African Catfish, *Clarias gariepinus*

Introduction

Stress and stress-related diseases are currently a much discussed topic in animal including fish husbandry and research (Guo, 2016). Stress management is therefore becoming subjects of growing interest for an increasing number of aquaculture fish species (Conte, 2004). In the aquatic environment, fish are unavoidably exposed to wide ranges of stimuli associated with environmental stress and pathological challenges (Xie *et al,* 2008). Stress responses provide the animal with an ability to cope in the short-term during exposure to the encounter and increase its chance of survival under adverse conditions (Rapatsa and Moyo, 2014; Liu *et al,* 2015). Environmental variables, particularly nutrition, are ultimately important in affecting fish in time of stress (Barton and Iwama, 2005). Most compounds absorbed by the intestine pass through the liver, which enables it to regulate the level of many metabolites in the blood (Good, 2004). Liver injury is often instigated by the bioactivation of complex reactions involving chemically reactive metabolites, which have the ability to interact with cellular macromolecules such as proteins, lipids and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage and oxidative stress (Larrey, 2000).

Pathogenic organism can cause diseases in fish, for instance bacteria are unavoidable in fish because the fish body is made of pure protein with fatty materials which are good substrates for bacterial growth (Okaeme and Ibiwoye, 2001). Again, water in which fish develop is a favourable medium for bacterial growth (Subasinghe, 2005). Farmed fish frequently encounter and tolerate poor environmental conditions, which are well below the considered optimal (Adewolu and Adeoti, 2010). Fish undergoes physiological stress response consequent to handling and transportation procedures, such stress reduce the capacity of fish, hindering their ability to perform essential functions (Subashinge, 2005; Welker *et al*, 2007). Stressors in aquaculture are unavoidable and cause many harmful effects. Strategies to attenuate them should be considered. The use of plants extracts in aquaculture has increased rapidly for the prevention of diseases and also to avoid the indiscriminate use of antibiotics, which can lead to the development of resistant strains of pathogenic microbes (Chatterjee *et al,* 2006; Kaleeswaran *et al*, 2011). Phytogenic products and extracts are cheaper, non-toxic and biodegradable alternative to antibiotics.

The *Moringa oleifera* tree is a single genus family of shrubs and trees cultivated across the whole of the tropical belt and used for a variety of purposes (Jahn, 1996; Becker, 2003). Verdcourt (1993) stated that almost every part of the plant is of value for food and it is probably the most popular plant in ECHO's seedbank of underutilized tropical crops. Moringa (drumstick, horse-radish) belongs to the moringaceae family, there are thirteen species of Moringa trees in the family moringaceae and *Moringa oleifera* is the

most widely cultivated species (Ojiako, 2014). Different parts of Moringa have shown great antioxidant activity (Anwar *et al*, 2007) as well as immunomodulatory function in animals (Ojiako, 2014). It can be recognized by the compound pinnate leaves, and the long narrow angular fruits containing large wind seed. *Moringa oleifera* contains antioxidants which can inactivate damaging free radicals produced through normal cellular activity and from various stresses (Makanjuola *et al*, 2013; Rapatsa and Moyo, 2014). Traditionally, the leaves, fruits, flowers, and immature pods of this tree are edible (Ojiako, 2014). The leaves, in particular, have been found to contain phenolics and flavonoids which have various biological activities, including antioxidant, anticarcinogenic, immunomodulatory, antidiabetic and hepatoprotective functions and the regulation of thyroid status in human and animals (Hussain *et al*, 2014).

The African catfish, *Clarias gariepinus* is the most important fish species cultured in Nigeria; it grows rapidly, it is disease and stress resistant, sturdy and highly productive in polyculture with many other fish species (Hammed *et al.,* 2015). This species has shown considerable potential as a fish suitable for use in intensive aquaculture (Adebayo, 2016). *C. gariepinus* production is considered to be the fastest growing segment of the Nigeria aquaculture industry over the past decade (FAO, 2014). More investors are entering catfish *C. gariepinus* farming in Nigeria as there exists a large unmet demand and market prices of catfish *C. gariepinus* which are more than those of other species (Fagbenro *et al.,* 1992). The African catfish (*C. gariepinus*) is the leading aquaculture species in Nigeria (FAO, 2014). The aim of the present study was to evaluate the hepatoprective and stress-reducing effects of dietary *Moringa oleifera* extract against *A. hydrophila* infections and transportation-induced stress in African catfish, *Clarias gariepinus*,

Materials and Methods

Extraction of *Moringa oleifera* Leaf

The leaves of *M. oleifera* were collected from a farm settlement at Ijare, Ondo State, Nigeria. It was identified and authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. The leaves were destalked, washed and dried in the shade. *M. oleifera* leaves were ground with pestle and mortar, leaves were then extracted according to the modified method of Makanjuola *et al* (2013) as follows. Five hundred grams of the powdered leaf were soaked in 1.5 liter of warm water (60^{o}C). Each solution was allowed to stand for 24 hours, after which it was sieved with a

muslin cloth and filtered using No 1 Whatman filter paper. The filtrate were collected in a beaker and concentrated with the aid of rotary evaporator (Resona, Germany).

Preparation of Experimental Diets

The feed ingredients were purchased at Adebom Feedmill, Ondo road, Akure, Ondo State, Nigeria. Six isonitrogenous and isocaloric diets were formulated to meet the requirements of 40% crude protein (Table 1) for *C. gariepinus* fingerlings (National Research Council, 2011) using feed formulation software (WinFeed soft 2.0, USA). All dietary ingredients were weighed with a weighing top balance (Metler Toledo, PB8001 London). The ingredients were then ground to a small particle size (approximately 20 µg). Ingredients including *Moringa oleifera* extract, vitamin and mineral premix were thoroughly mixed in a Hobbart A-200T mixing machine (Hobbart Ltd London England) to obtain a homogenous mass. Alginate, *Laminaria digitata* (IGV GmbH, Germany®) was added as binder. The resultant mash was pressed without steam through a mincer using 2mm diameter die attached to the Hobbart pelleting machine. Diets were immediately air - dried, after drying the diets were broken up, sieved and stored in air-tight transparent plastic containers, labeled and stored until feeding. Standard and official methods (AOAC, 2010) were used to perform the proximate analyses of feed of fish in the study.

Proximate Analyses of Moringa leaf and experimental feed

Standard and official methods (AOAC, 2010) were used to perform the proximate analyses of Moringa leaf and feed of fish in the study. Formulated feed were blended to a homogeneous mince using a meat grinder (Binatone, UK) with a 4 mm diameter orifice plate. A sub-sample of Moringa leaf extract and feed were taken and stored for estimation of dry matter which was determined after drying in the oven (Gallenkamp, UK) at 105°C for 24 h. The remaining homogenates were dried in the oven and used for all subsequent analyses. Ash content was calculated by weight loss after incineration in a muffle furnace (Carbolite, UK) for 12 h at 550ºC. A Parr bomb calorimeter was used to calculate gross energy content, this method measures energy content by combustion under an atmosphere of compressed oxygen with benzoic acid as a standard. The Kjeldahl technique was used to measure crude protein. In this technique, the nitrogen (N) content was determined and multiplied by a conversion factor of 6.25.

Table 1: Composition of the experimental diet (g/100g) containing dietary *Moringa Oleifera* for African catfish, *Clarias gariepinus* fingerlings

	MLSC0	MLSC5	MLSC10	MLSC15	MLSC20	MLSC25
Fish meal (68 % CP)	23.50	23.50	23.50	23.50	23.50	23.50
GNC (48 % CP)	29.00	29.00	29.00	29.00	29.00	29.00
Soybean meal (42 % CP)	20.50	20.50	20.50	20.50	20.50	20.50
Yellow maize	10.50	10.50	10.50	10.50	10.50	10.50
Vegetable oil	7.00	7.00	7.00	7.00	7.00	7.00
Rice Bran	5.50	5.45	5.40	5.35	5.30	5.25
Alginate	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin Mineral mix	2.00	2.00	2.00	2.00	2.00	2.00
Moringa leaf extract	0.00	0.05	0.10	0.15	0.20	0.25

Proximate composition of experimental diets fed to *Clarias gariepinus* (% dry matter basis)

Crude protein	39.98	40.06	40.09	40.20	40.19	40.22
Lipid	10.05	10.23	10.15	10.41	10.42	10.45
Crude fibre	5.63	5.72	6.01	6.31	6.94	6.69
Ash	8.93	8.33	8.54	9.06	9.13	9.74
Dry matter	92.37	92.19	91.06	90.14	90.18	90.07
Nitrogen-free extract (NFE)	27.78	27.85	26.27	24.21	23.50	22.90
Gross Energy (kJ/g)	15.81	15.79	15.88	15.93	15.96	16.04

Composition of vitamin-mineral mix (Aquamix) (quantity/kg), Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6 mcg; Calcium; Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450 L- lysine, 10 g; Selenium, 50 ppm.

Experimental fish and feeding trial

C. gariepinus fingerlings were obtained from the Hatchery unit of the Department of Fisheries and Aquaculture Hatchery, Federal University of Technology Akure, prior to the feeding trial. Fish were graded by size and groups of 15 fish of 10.00 ± 0.05 g per replicate for *C. gariepinus* were stocked into glass tanks of 60cm ×45cm×45cm dimension. A commercial diet, Nutreco ® (35% crude protein) was fed to all fish during a 2- week conditioning period. Each experimental diet was fed to six replicate groups of fish for 70days. All groups were fed their respective diets at the same fixed rate (initially 5% of body weight per day). This rate was adjusted each week. Fish were fed by 0900-1000 and 1700-1800h GMT, for 7 days each week. Dissolved oxygen was monitored using HANNA 98103SE (HANNA instruments, Rhode Island). Temperature and pH were monitored using YSI-IODO 700 Digital probe (IFI Olsztyn, Poland).

Physico-chemical water parameters: Dissolved oxygen was monitored using HANNA 98103SE (HANNA instruments, Rhode Island). Temperature and pH were monitored using YSI-IODO 700 Digital probe (IFI Olsztyn, Poland).

Aeromonas hydrophila **challenge and transportation –induced stress**

After six weeks of the feeding trial, fish previously fed each experimental diet were exposed to pathogenic strain of *Aeromonas hydrophila* (MPSTR 2143, mildly pathogenic strain, Animal care Laboratory, Ogere). This isolate was grown in brain-heart infusion broth (EM Science, Darmstadt, Germany) in a shaking bath at 27ºC overnight the Department of Microbiology, FUTA. The concentration of bacterial suspension was determined by the serial plate count method and diluted to 9.3×10^5 CFU (colony forming unit)/mL in fresh well water as described by Li (2005). Fish from each dietary treatment was immersed in the bacterial suspension for 5 hours. After bath exposure, fish from each dietary treatment was placed into the aquaria culture system. Fish were fed their respective diets at 5% body weight twice daily for the remaining 4 weeks of the feeding trial. At the end of the feeding trial, 15 fish previously fed each experimental diet from each treatment were kept in plastic tanks for a 2-hour journey. The liver samples were collected immediately after transportation for 2 hours from fish for further analyses.

Assessment of hepatocellular damage

Hepatocellular stress activities were determined by Aspartate transaminase (AST), Alanine transaminase (ALT), Lactate dehydrogenase (LDH) and Malate dehydrogenase (MDH) tests according to the procedure

of Hardy and Sullivan (2003). The livers of 3 fish from each treatment were removed by dissection and weighed. The tissue was homogenized with chilled 0.25 M sucrose solution in a glass tube using a mechanical tissue homogenizer. The tube was continuously kept in ice to avoid heating. The homogenate was then centrifuged (5000x g for 10 minutes at 40^0C) in a cooling centrifuge machine and stored at -20ºC till use.

Aspartate transaminase (AST) and Alanine transaminase (ALT) were measured by the estimation of oxaloacetate and pyruvate released in a spectrophotometer at 540nm and the results were read on the calibrated graph respectively.

Lactate dehydrogenase (LDH) and Malate dehydrogenase (MDH) activities were measured by the change in optical density (OD) at 340 nm for 5min. The supernatant was used directly as an LDH and MDH source in the kinetic study. LDH and MDH activities were determined following the oxidation of NADH at 340 nm in a circulating thermobath at 25ºC. The reaction mixture was contained in a total volume of 1 ml, 50 mM Imidazol, 1 mM KCN buffer pH 7.4 at 25ºC, 0.13 mM of NADH and different concentrations of pyruvate for LDH saturation plots. Substrate saturation plots for oxalacetate were determined for MDH by the oxidation of NADH at 340 nm.

Histopathological examination

The liver were collected and fixed in Davidson's freshwater fixative by 24h then rinsed and put into 70% ethanol until dehydrated in graduated ethanol 50–100%, cleared in xylene, and embedded in paraffin. Sections of 5 lm thickness were prepared, stained with haematoxylin and eosin (H&E) dye. Photomicrographs were taken with the aid of Olympus digital camera (Olympus, UK) at 50 µm. Tissue sections were compared after examination under the microscope, for significant differences in the morphology of the tissues.

Statistical analysis

This experiment was designed with a completely randomised design (CRD) to test for significant differences in the mean of treatments. The data were expressed as mean ± standard deviation (SD).The differences between mean of treatments were considered significant at $P < 0.05$ by one way analysis of variance (ANOVA) using Statistica® software. Follow–up procedures were performed where significant differences occurred in the means using Tukey test.

Results

Effects of *M. oleifera* leaf extract on hepatocellular damage indicators

Significantly higher alanine transferase, aspartate transferase, lactate dehydrogenase and malate dehydrogenase ($P< 0.05$) was recorded in fish fed the control diet compared with other dietary treatments. In the liver tissue the increases of the Aspartate transaminase (AST), Alanine transaminase (ALT), Lactate dehydrogenase (LDH) and Malate dehydrogenase (MDH) induced by *A. hydrophila* infections and transportation-induced stress were significantly inhibited ($P < 0.05$) by supplementing the fish with 0.10g, 0.15g, 0.20g, 0.25g per 100g *M. oleifera* leaf extract in the diets (Table 2).

Table 2: Effects of *M. oleifera* leaf extract on hepatocellular damage indicators in experimental fish

	MLSC0	MLSC5	MLSC10	MLSC15	MLSC20	MLSC25
AST (µM)	60.28 ± 2.05^{d}	47.51 ± 1.70^{c}	30.35 ± 1.09^{ab}	28.65 ± 1.17^{a}	30.02 ± 1.20^{a}	34.04 ± 0.82^{b}
ALT (µM)	51.53 ± 1.58^{e}	27.16 ± 1.70^{d}	17.78 ± 1.24^{ab}	16.61 ± 1.25^{a}	19.26 ± 1.69^{c}	19.18 ± 1.03^{bc}
LDH (µM)	1.33 ± 0.06^{e}	1.12 ± 0.05^{c}	0.85 ± 0.02^{a}	0.94 ± 0.05^{b}	1.28 ± 0.01^{d}	1.35 ± 0.13^{e}
MDH (nM)	6.13 ± 0.05^{d}	3.12 ± 0.16^{c}	1.84 ± 0.03^{a}	1.73 ± 0.04^{a}	2.12 ± 0.15^{b}	2.26 ± 0.12^{b}

^{a,b,c,d,e,f} values in each row with different superscripts are significantly different ($P < 0.05$) using ANOVA Post Hoc (Tukey test) (mean values ± SD, mean of fish from 3 replicate tanks). ALT, Alanine transferase; AST, Aspartate transferase; LDH, Lactate dehydrogenase and MDH, Malate dehydrogenase.

Histology of the liver of *Clarias gariepinus* fed the experimental diets

Histology of the liver of *C. gariepinus* fed the experimental diets is shown in Figure 1A-E. In fish fed the MLSC0 diet, disorganised sinusoids (arrow), less nuclei and highly vacuolated cytoplasm (circles) was observed (Figure 1A). Liver of fish fed the MLSC5 diet showed erosion of the hepatocytes. However, fish fed MLSC10 to MLSC25 diets showed more nucleated hepatocytes (arrow) (Figure 1C-F).

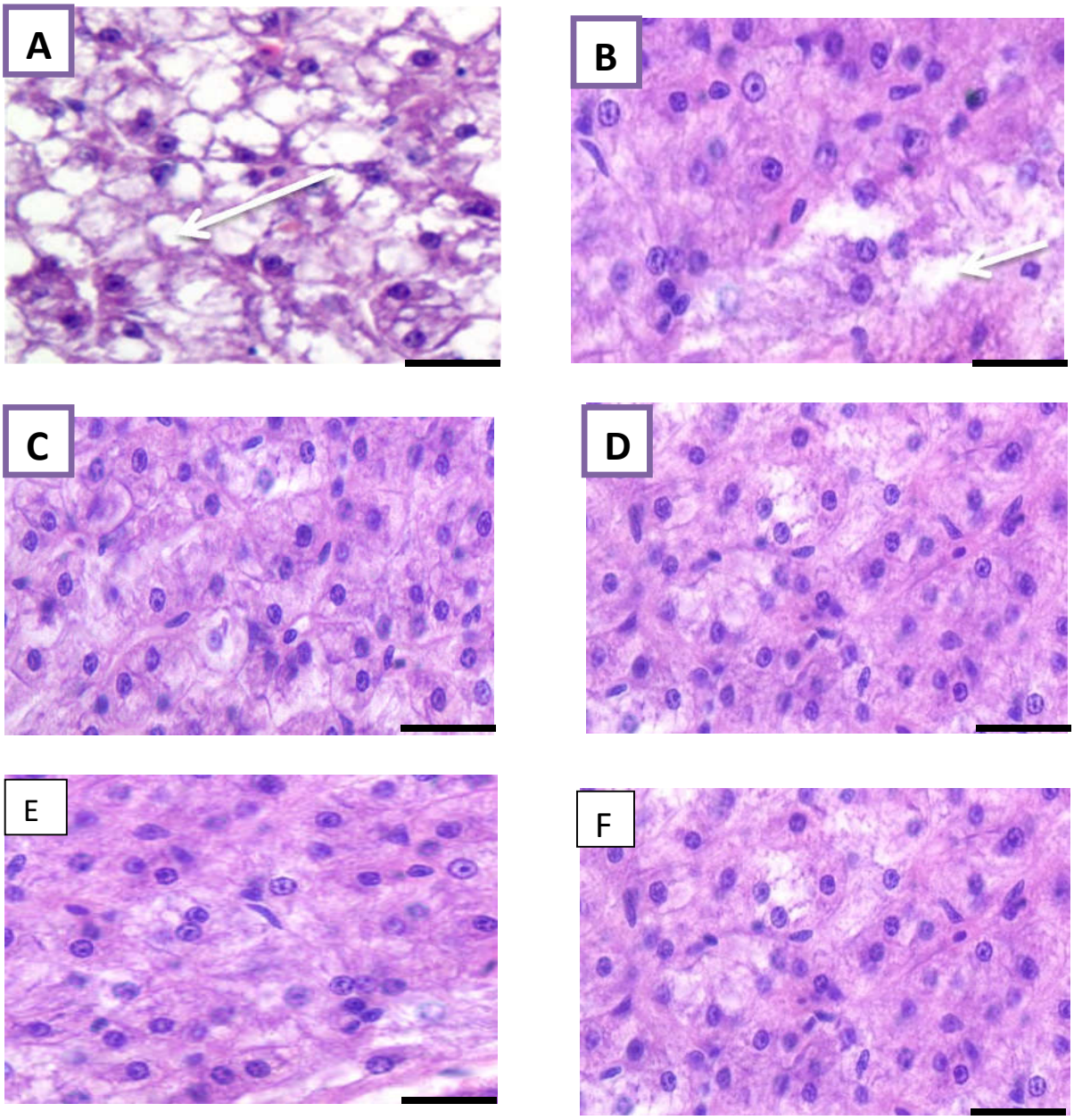

Figure 6**(A)** Liver of fish fed the MLSC0 diet showing disorganised sinusoids (arrow), less nuclei and highly vacuolated cytoplasm (circles). **(B)** Liver of fish fed the MLSC5 diet showing erosion of the hepatocytes and vacuolated cytoplasm (arrow) **(C)** Liver of fish fed MLSC10 diet showing more nucleated hepatocytes (arrow). **(D)** Liver with hepatocytes in fish fed MLSC15 diet showing nucleated hepatocytes. **(E)** Liver of fish fed MLSC20 diet showing nucleated hepatocytes. **(F)** Liver of fish fed MLSC25 diet showing nucleated hepatocytes (scale bar = 50 µm).

Discussion

Significantly elevated activities of cellular enzymes AST, ALT, LDH and MDH observed in fish in the control group exposed to *Aeromonas hydrophila* and transportation-induced stress indicated that stressors caused liver injury in the present study. Stressors like transportation, stocking density and pathogenic stress have been reported to cause hepatocellular damage in Nile tilapia by increasing the activities of cellular enzymes AST and ALT (Tekle and Sahu, 2015). Soosean *et al* (2010) also reported that increase in the activity of cellular enzymes (AST and ALT) is an indicator of cellular damage in stressed fish. In the present study, amino-transferase activities were found highest in the control group compared to the other dietary groups. The higher activity of AST and ALT indicates the mobilization of aspartate and alanine via gluconeogenesis for glucose production to cope with stress (Barton and Iwama, 2005). Elevated level of transaminase activity during stress would lead to increase feeding of ketoacids into TCA cycle, thereby affecting oxidative metabolism (Tekle and Sahu, 2015). Moringa leaf supplementation significantly reduced the activities of AST and ALT suggesting that Moringa leaf protected the membrane integrity of the liver cells against stressors. Cao *et al*, (2016) stated that an important mechanism of the hepatoprotective effects may be related to an antioxidant capacity to scavenge reactive oxygen species. Hence, as there was less cellular activity in the Moringa supplemented groups, it can be inferred that addition of Moringa plant extracts reduced stress and improve growth and health of fish in the present study.

Fish fed the control diet in this study showed higher LDH activity than fish fed the Moringa supplemented diets. Generally, LDH and MDH activities increases in stress condition (Barton and Iwama, 2005). Significantly lower LDH and MDH activities in the Moringa treated groups suggested that there was stress mitigating effect of Moringa on the liver of fish in the current study. This is in agreement with the findings of Tekel and Sahu, (2015), which reported that the MDH activity in *O. niloticus* fingerlings subjected to pathogenic stress was higher in the control than fish treated with *M. oleifera* flower. Therefore, the lower LDH and MDH activity in *C. gariepinus* fed dietary Moringa leaf supplemented diets showed that Moringa has the ability to ameliorate the effects of stressors used in the present study. Furthermore, various histopathological changes were noticed in the liver of *C. gariepinus* fed the control and MLSC5 diets which were not observed in fish fed the other Moringa supplemented diets (Figure 1). These changes were as follows: the cord-like parenchymal structures of the liver were lost, resulting in disorganised sinusoids and highly vacuolated cytoplasm with loss of nuclei. Highly vacuolated cytoplasm, deformed sinusoids and vacoulation in the hepatocytes were observed as signs of physiological dysfunction in unhealthy *Cyprinus carpio* (Venkatesen *et al,* 2012). The enterocytes of *C. gariepinus* showed apparently

normal structures in fish fed MLSC10 and MLSC15 diets. Fish fed MLSC10, MLSC15 and MLSC20C diets did not show any apparent histopathological changes in the liver, as the histology of the liver of fish in these dietary treatments showed normal hepatocytes, numerous nuclei and cytoplasmic organelles suggesting that the inclusion of Moringa in fish fed MLSC10, MLSC15 and MLSC20 diets had positive effects on the liver of tilapia in this study.

This results of the present study is in agreement with many studies that reported the role of plant extracts in stimulating the immune system by modulating the activity of metabolic and antioxidative stress enzymes. For example, Kaleeswaran *et al*, (2011) reported positive effects of *Cynodon dactylon* (L.) on the innate immunity and disease resistance of Indian major carp, *Catla catla*. Tekle and Sahu (2015) reported the ameliorative effects of Moringa flower on *O. niloticus* subjected to *Aeromonas hydrophila* induced stress. *M. oleifera* plant has been widely reported to contain constituents such as nitrile, glycosides and quercetin (Ojiako, 2014) which are believed to be responsible for enhancing hepatoprotection, immunity against oxidative stress and microbial diseases. Therefore the presence of potent antioxidants in Moringa supplemented diets was helpful in reducing the negative effects of stressors in *C. gariepinus*. Hammed *et al,* (2015) also reported that the presence of potent antioxidants in Moringa leaf can be correlated with increase in antibody production which helps in the survival and recovery of fish during stressful periods.

Conclusion

In the present study, supplementation of Moringa leaf at the dose of 1.00g/kg in the diet was sufficient to induce hepatoprotective and ameliorative effects against stress -induced liver damage in fish in a dose dependent manner. The hepatoprotective action of Moringa leaf was probably related with its eliminating free radical, maintaining the integrity of the hepatocyte membrane and increasing the antioxidant enzyme activities, inhibiting ROS damage. Supplementation of Moringa leaf may potentially be used as a hepatoprotective and stress reducing agent for improved fish performance and health especially during stress periods

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GUSTATION AND GROWTH PERFORMANCE OF NILE TILAPIA, *OREOCHROMIS NILOTICUS* FED VARYING LEVELS OF DIETARY AFRICAN BASIL, *OCIMUM GRATISSIMUM* LEAF SUPPLEMENTATION

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Abstract

The gustation and growth performance of Nile tilapia*, Oreochromis niloticus* fed African basil, *Ocimum gratissimum* leaf meal supplemented diet was assessed in the present study. *O. niloticus* of the initial weight of 11.85 ± 0.24 was evaluated over a 56 days period. Five experimental diets were formulated at 0 mg/g (control), 20 mg/g, 40 mg/g, 60 mg/g and 80 mg/g inclusion levels of *O. gratissimum*. African basil leaf was treated by soaking in water for 72 hours and sun-dried. All diets were isonitrogenous with each treatment having three replicates. Fish fed the 80mg/g *O. gratissimum* leaf meal recorded the best growth performance in body weight gain and specific growth rate (SGR). Statistically, there was significant increase in growth and nutritional performance of fish in this study with increasing inclusion levels of *O. gratissimum* ($P<0.05$). There was no adverse effect of *O. gratissimum* supplementation on the hepatosomatic and intestinosomatic index of fish in this study. There was also significant increase in the gustation of fish with increasing *O. gratissimum* supplementation ($P<0.05$). This could be attributed to the supplementation of *O. gratissimum.* Therefore, the present study suggests that *O. gratissimum* leaf meal may be supplemented up to 80 mg/g level to increase gustation and growth performance of *O. niloticus.*

Keywords: Gustation, African basil leaf, *Ocimum gratissimum*, growth performance, Nile tilapia, *Oreochromis niloticus*

Introduction

Aquaculture is a growing industry and its role in ensuring consistent supply of aquatic species for human benefit cannot be overstated. However, the growth in aquaculture has caused major challenges; one of these challenges is the production of practical feeds for the farming of fish (Globefish, 2014). The main objectives in diet formulation for aquaculture are to satisfy all known nutrient requirements for growth of

the species; minimize feed cost and while using ingredients that will result in products that are readily utilized to minimize wastage (Tacon and Mertian, 2009). Apart from the nutritionally balanced formulations, palatability and attractiveness are other important factors that determine the success of the feed manufacture; they promote ingestion leading to utilization of available nutrients (FAO, 2012). Diet palatability and attractiveness would help to reduce the time that fish spend approaching the feed and reduce deterioration of rearing pond environments from overloaded nutrient input (Fournier, 2012). In animal production system, the primary concern is maintaining a desirable level of output; this can be achieved by reducing feed loss while attaining optimum growth.

In focusing on palatability and good growth performance in fish, many attractive molecules likely to be involved in olfaction and gustation should be identified. Therefore, the use of some natural additive like African basil leaf *(Ocimum gratissimum)* can be vital. The addition of this can aid feed acceptability, digestion, immune development and fish growth (Gbadamosi and Salako, 2014). African basil leaf which is botanically known as *Ocimum gratissimum*, comes from a family known as Lamiaceae; a home grown shrub used mainly as spices for cooking delicacies due to its unique aromatic taste and smell. It is a tropical plant species that could be of a great value to aquaculture nutrition and health management (Charis, 2000). Most plants that belong to family Lamiaceae are good feed attractants which can be used in practical diets for aquatic animals (Kanda et al*,* 1971). The plant leaves are recommended for use in treating many human diseases such as digestive disorder, lowering glucose level in blood and of sedative effect in man (Almeida et al, 2007). The use of this plant product is not popular in livestock production in many countries like Nigeria probably because of the limited information about the nutritional properties which can have beneficial effects on many farm animals. *Ocimum gratissimum* is a very good source of all of nutrients, minerals and vitamins such as omega-3 fatty acid, vitamin K, iron, calcium, vitamin A and vitamin C (Daniel et al, 2011). Basil leaves come complete with a collection of antioxidants and other wonderful phytonutrients (Almeida and Alviano, 2007). Harada (1992) found that spices were highly effective in attraction for fish and shellfish with special reference to strong effect for basil. Basils improve foods palatability through their aroma, increase digestibility and impact some medical function when consumed by man or animals as part of their food (Rutherford- Fortunati, 2013). The Nile tilapia, *Oreochromis niloticus* is a deep-bodied fish with cycloid scales, silver in colour with olive/grey/black body bars and often flushes red during the breeding season (Picker and Griffiths 2011). It is considered as an excellent species for aquaculture in tropical and subtropical regions, as a result of the high tolerance to handling, stress situations and critical conditions, tolerance to sub optimal water quality, tolerance to high stocking densities, and its fast growth (El-Sayed, 1998). Nile tilapia is also acceptable in term of tastes and

preferences because of the white flesh, neutral taste and firm texture this is why it is often called the 'aquatic chicken' (Fitzsimmons, 2008). The aim of the present study was to study the effects of dietary *Ocimmum gratissimum* on the gustation and growth performance of Nile tilapia, *Oreochromis niloticus*.

Materials and Methods

Experimental diets

Five isonitrogenous diets containing 35% crude protein were formulated for Nile tilapia, *Oreochromis niloticus* fingerlings in an eight-week trial experiment (Table 1). African basil leaf, *Ocimum gratissimum* was used as supplement at 0mg, 20mg, 40mg, 60mg and 80mg in diet 1,2,3,4 and 5 respectively. Identification of African basil leaves was done at the department of Crops, Soil and Pest Management where they were identified as *Ocimum gratissimum.* African basil leaf was treated by soaking in water for 72 hours and sun-dried. All dietary ingredients were first milled to small particle size. The dry ingredients were thoroughly mixed by adding hot water until a consistent dough resulted. The dough was then pelleted using Hobart A- 200 pelleting machine with a 2.0mm die. After pelleting, the diets were sun dried immediately for a week to avoid mould formation and later broken mechanically into small sizes and packed in dry, air tight small containers (labeled) prior to use. Starch was used as binder. A sample of each diet was taken for proximate analysis according to the method of AOAC (1990).

Table 1: Composition of the experimental diet in g/100g dry matter containing various inclusion level of basil leaves for *Oreochromis niloticus*

INGREDIENTS	T1 (control)	T2	T3	T4	T5
Fishmeal (72%)	22.5	22.5	22.5	22.5	22.5
Soybean meal (48)	22.5	22.5	22.5	22.5	22.5
Maize (10%)	45.0	45.0	45.0	45.0	45.0
Vegetable oil	6.0	6.0	6.0	6.0	6.0
Vitamin	2.00	2.00	2.00	2.00	2.00
Starch	2.00	2.00	2.00	2.00	2.00
Basil leaf (mg/g)	0.00	20.00	40.00	60.00	80.00

Vitamins supplied mg/100g diet supplied by Vitafeed: thiamine (B1) 2.5mg: riboflavin (B2), 2.5mg pyridoxine 2.0mg: pantothenic acid, 5.0mg: inositol, 3mg: biotin, 0.3mg: folic acid, 0.75mg para- amino benzonic, 2.5mg: chlorine, 200mg; niacin, 10.0mg cynobalamin (B_{12}), 10.0mg: menadoxine (k), 2.0mg.

Experimental System And Fish

Oreochromis niloticus with average weight of 11.85±0.24 g were obtained from the hatchery of Fisheries and Aquaculture Teaching and Research Farm, FUTA, Akure, Ondo state. The fish were distributed randomly into plastic tanks (40cm x 30cm x 35cm) at twelve fish per tank. Each treatment was in replicate groups of fish. They were acclimated for seven days while being fed commercial feed diet. The post-fingerlings were not fed for 24 hours before they started on the experimental diet to maintain a uniform stomach condition of the fish and to induce their appetite for the commencement of the feeding trial. During the feeding trial, fish were fed to satiation with their respective diets twice daily between 9:00 am and 5:00 pm. Feed were administered bit by bit to check the rate at which the fish picked the feeds. The weight of the fish in each tank was taken and recorded every week. Their agility (activeness) was checked at each feeding period and water changed regularly to ensure the water was conducive for growth and survival of the fish.

Carcass analyses of experimental fish

Carcass analyses of experimental fish before and after experiment were analysed according to AOAC (1990) for moisture content, fat, fibre and ash.

Performance evaluation

Fish performances during the experiment were based on productivity indices on growth performance and nutrient utilization efficiencies as described by Fasakin *et al*. (1997) as follows;

Daily weight gain (g/fish/day) = (final weight (FW) - Initial weight (IW)

Feed Intake (FI) = feed consumed (g $fish^{-1}$)

Specific growth rate (SGR) = $(\log_e Wt - \log_e W_i)/ T \times 100$

Where Wt = Final weight (g), W_i = Initial weight (g) and T = rearing periods (days),

Feed conversion ratio (FCR) = dry weight of feed (g)/ fish weight gain (g),

Protein efficiency ratio (PER) = fish weight gain (g)/ protein fed (g).

Hepatosomatic index

At the end of feeding trials, fish were removed from each treatment for hepatosomatic index according to Gbadamosi and Salako (2014). Fish were randomly picked from each tank and dissected using a dissecting kit: each organ was carefully traced and cut out. The organ was placed on a petri dish and weighed (considering the weight of the petri dish). Two fishes from each treatment were separated for liver samples. The percentage relative organ weight was calculated as HSI % = Liver weight / Somatic weight x 100

Intestinosomatic index

At the end of experimental period, fish were also removed for the intestinosomatic index. Fish were randomly picked from each tank and dissected using a dissecting kit: each organ was carefully traced and cut out. The intestine was placed on a petri dish and weighed (considering the weight of the petri dish). Two fishes from each treatment were separated for intestine samples. The percentage relative organ weight was calculated as follows: ISI % = Intestine weight/ Somatic weight x 100.

Gustation

Gustation was determined following the procedure of the bioassay described by Sveinsson and Hara (1990). Fish were transported to the research farm and kept in rectangular-shaped experimental tanks containing up to 30 litres level of water. The fish were fed *ad libitum* twice a day. Pellets were dropped into the tanks one by one with an interval of 10-15 minutes. In each treatment, several parameters were registered, which are; the treatment tank, number of grasps at the pellet, retention time of the pellet after the first grasp and the total retention time of all grasps; whether the pellet was swallowed or finally rejected by the fish. The pellets containing different treatments were offered to the fish at random sequence. Pellets rejected or not swallowed were removed from the aquarium immediately after the end of each time.

Data analyses

Biological data obtained were subjected to one-way analysis of variance (ANOVA). Where means were significantly different, they were compared with Duncan's multiple range test using SPSS statistical software 16. Surface plot of the gustation was done with the aid of mini tab version 17.0.

Results

Proximate Composition of Experimental Diets

The proximate composition of the experimental diets as shown in Table 2 showed that the experimental diets used in this experiment were isonitrogenous.

Table 2: Proximate Composition of Experimental Diets Used in feeding *Oreochromis niloticus* (% dry matter)

INGREDIENTS	T1 (control)	T2	T3	T4	T5
Moisture content	4.67	7.00	6.00	4.00	5.33
Ash	10.00	12.00	8.00	6.00	6.00
Crude protein	35.70	35.5	35.53	36.05	36.38
Fat	8.00	6.00	8.00	12.00	10.00
Fibre	0.5	1.0	1.5	1.0	1.5
NFE	41.13	41.65	40.97	40.95	40.92
African basil mg/g	0.00	20.00	40.00	60.00	80.00

[1]NFE, Nitrogren Free Extract.

Whole Body Composition of Experimental Fish

The result of the carcass analysis of Nile tilapia at the beginning and the end of the experimental period was shown in Table 3. There was significant difference ($P < 0.05$) between the initial and the final body composition of fish used during the experiment with respect to moisture, crude protein, crude lipid and ash content. For crude protein, the highest was recorded for fish fed 80mg/g of basil leaf supplementation. However, there was no significant difference ($P > 0.05$) in the protein content of fish fed diet T4 and T5 (60mg/g and 80mg/g respectively) and fish fed with the control diet and T1 (20mg/g). There was significant difference ($P< 0.05$) in the crude protein of control diet and T2, T3, T4 and T5. The highest crude lipid was recorded for fish fed 60mg/g of basil leaf supplementation. There was no significant difference ($P>0.05$) in lipid content of the fish fed with different inclusion levels of basil leaf supplementation.

Table 3: Carcass analyses of Nile Tilapia (% dry matter) Fed with Experimental Diets

	INITIAL	T1	T2	T3	T4	T5
MC	9.10±0.57^{b}	3.34±0.34^{a}	4.50±0.83^{a}	4.34±0.67^{a}	3.67±0.34^{a}	3.64±0.97^{a}
Ash	13.00±1.00^{b}	13.00 ±1.00^{b}	11.00±1.00^{a}	13.00±1.00^{b}	12.00±0.00^{ab}	14.00±0.00^{c}
Fat	7.90±0.10^{a}	11.00±1.00^{b}	11.00 ±1.00^{b}	2.00±0.00^{b}	13.00±1.00^{b}	11.00±1.00^{b}
CP	56.73±0.73^{a}	56.70±0.35^{a}	62.74±0.96^{b}	64.23±0.18^{bc}	64.84±0.26^{c}	65.28±0.35^{c}
NFE	23.55±0.78^{d}	15.97±0.02^{c}	10.76±0.13^{b}	6.44±0.16^{a}	6.50±0.93^{a}	6.08±0.32^{a}

Figures in each row having the same superscripts are not significantly different (P> 0.05)

Growth Performance and Nutrient Utilization of Nile Tilapia with the Experimental Diets

The growth performance and nutrient utilization of *Oreochromis niloticus* fed the experimental diets that contain different inclusion levels of *Ocimum gratissimum* (Table 4). The result showed differences in the mean weight, specific growth rate and feed conversion ratio of Nile tilapia fingerlings fed with the experimental diets. Fish fed 80 mg/g African basil leaf showed the highest percentage weight gain and specific growth rate of 8.15±0.07 and 0.95±0.00 respectively. The feed conversion ratio among in the treatments showed significance difference (P<0.05). However, the best feed conversion ratio (FCR) was recorded in fish fed 20mg/g *O. gratissimum* leaf meal, which was significantly different from other dietary treatments (P<0.05). Statistically, there was significantly increased growth and nutritional performance of fish in this study with increasing inclusion levels of *O. gratissimum* (P<0.05). Mortality was observed during the feeding trials, however there was no significant differences (P>0.05) in the mortality of fish during the experimental period.

Table 4: Growth Performance and histometrics of *O. niloticus* fed the experimental diets (Mean ± SE)

PARAMETERS	D1 (control)	D2	D3	D4	D5
IW (g fish^{-1} tank^{-1})	11.80±0.14^{a}	11.75±0.07^{a}	7.10±0.65^{a}	11.90±0.28^{a}	11.7±0.14^{a}
FW (g fish^{-1} tank^{-1})	17.30±1.13^{a}	18.80±0.57^{ab}	19.45±0.21^{b}	19.40±0.57^{b}	19.85±0.21^{b}
WG (g)	5.50±1.27^{a}	7.05±0.49^{ab}	7.35±0.64^{ab}	7.50±0.85^{ab}	8.15±0.07^{b}
FI (g fish^{-1}day^{-1})	9.75±0.21^{ab}	8.73±1.18^{a}	10.00±1.89^{ab}	9.15±0.12^{ab}	11.93±0.84^{b}
SGR	0.67±0.14^{a}	0.85±0.06^{ab}	0.85±0.09^{ab}	0.88±0.11^{ab}	0.95±0.00^{b}
PSR	91.54±1.05^{a}	93.67±O.84^{a}	94.09±1.14^{a}	98.12±1.46^{a}	96.54±1.74^{a}
FCR	1.82±0.38^{b}	1.21±0.04^{a}	1.36±0.13^{ab}	1.23±0.12^{a}	1.47±0.12^{ab}
HSI	1.33±0.50^{a}	1.11±0.02^{a}	1.06±0.49^{a}	1.70±0.44^{a}	1.94±0.25^{a}
ISI	3.11±0.28^{ab}	2.34±0.67^{a}	3.67±0.08^{b}	3.39±0.57^{ab}	3.99±0.38^{b}

Figures in each row having the same superscript are significantly different ($P<0.05$)

IW = Initial Weight, FW = Final Weight Gain, WG = Weight Gain FI = Feed Intake SGR == Specific Growth Rate, FCR = Feed Conversion Ratio, HIS = Hepatosomatic Index, ISI = Intestinosomatic Index.

Gustation

The results of the gustation in each treatment show that the highest number of grasps is recorded in treatment five (T5) and the highest time of grasps is recorded in treatment four (T4). The lowest number of grasps and time of grasps is recorded in treatment two (T2) one (T1) respectively. The number of grasps ranges from 12.88 to 15.62 while the time of grasps ranges from 24.00 to 28.94 (Figure 1).

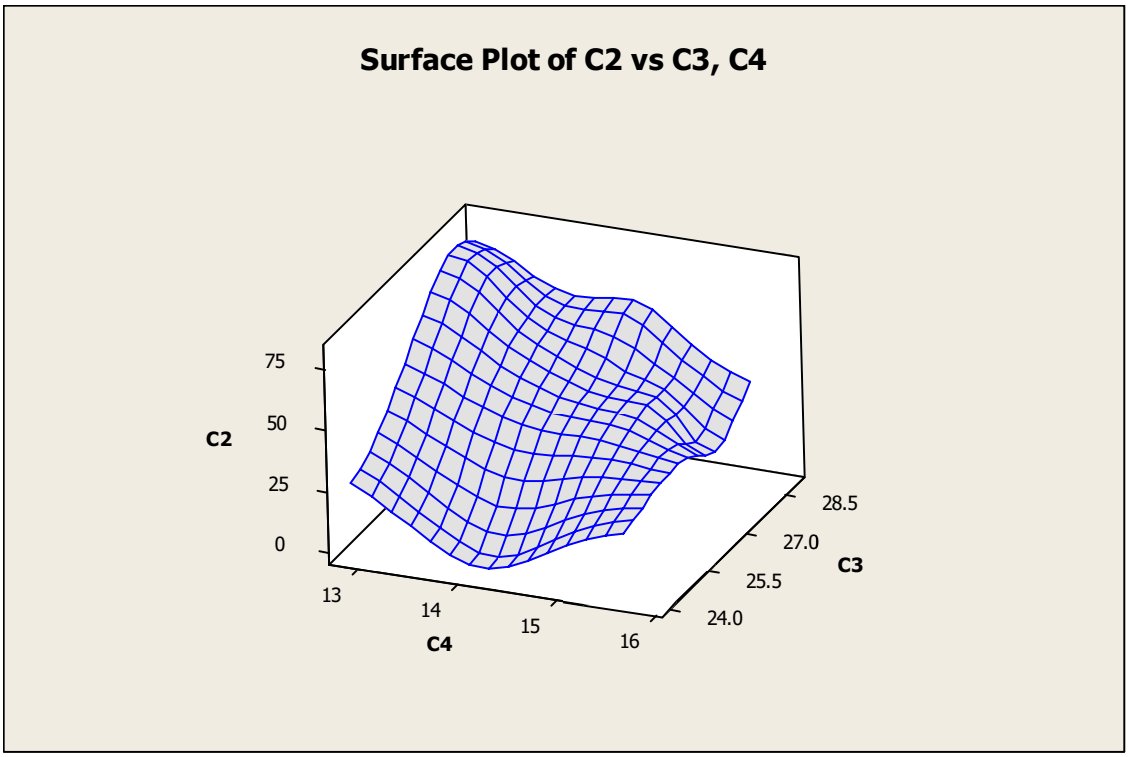

Fig. 1: Surface plot of the gustation of *Oreochromis niloticus* fed experimental diets showing the number and time of grasps.

Where C2= Dietary basil leaf supplementation levels, C3 =Time of grasps and C4= Number of grasps.

Discussion

The present study showed a significant improvement of fish growth, feed utilization and gustation activities when fish fed diets containing basil leaf were compared with the control. The beneficial effects of the inclusion of basil leaf meal on fish growth is associated with higher protein retention, antioxidant and antimicrobial activities due to its phenolic and aromatic compounds (Gultierrez et al., 2008). The results indicated that basil has some ability to increase the growth performance of Nile tilapia fed *O. gratissimum.* African basil contains 0.17% oleanolic acid and small amount of ursolic acid (El-Dakar, 2015). It contains active compounds such as planteose, mucilage, polysaccharides and fixed oil that consists of linoleic acid (50%), linolenic acid (22%), oleic acid (15%) as well as 8% unsaturated fatty acids (Gultierrez et al., 2008). Rutherford-Fortunati (2013) reported that the phytochemical screening of basil revealed the presence of glycoside, gums, mucilage, proteins, amino acid, tannins, phenolic compound, triterpenoids, steroids, sterols, saponins, flavones and flavonoids in it. Essential oil composition and phenolics have been well reviewed by Makri and Kintzios (2008). The result on the growth performance and nutrient utilization of Nile tilapia in this study supports the findings of Amirkhani

and Firouzbakhsh (2013) that basil leaves extract improves growth and specific growth rate and lowers feed conversion ratio of common carp at 4% and 8% inclusion levels in fish diets. The weight gain and specific growth rate of Nile tilapia in this study increase with increase in the inclusion levels of *Ocimum gratissimum,* but the feed conversion ratio (FCR) is significantly different by basil-supplemented diets. This is in concordance with the work of Gbadamosi and Salako, which reported that growth performance and gustation increased with increasing levels of *O. gratissum* in the diet of *Clarias gariepinus* fingerlings.

Positive effects of *O. gratissum* on growth and feed utilization can be explained in two ways; first that olfactory feed ingredients like spices (basil) enhance growth through their ability to act as feeding enhancers (El Dakar et al., 2008 and Ahmad and Abdel-Tawwab, 2011). This can be seen in the feed intake (FI) of Nile tilapia in this study which increases with increase in the inclusion levels of basil (*Ocimum gratissimum).* The second explanation may be because it contains vital compounds like aromatic oils, essential fatty acids, vitamins, minerals which may have important effects on growth (Azeez, 2008),.

The proximate body composition of fish fed with experimental diets in this study showed a significant increase in fish protein content which is in line with Abdelhamid and Soliman (2012) results where they reported that using dried leaves of guava and camphor trees in tilapia diets significantly improved fish carcass composition (protein, ether extract, energy content). In general, adding spices and medicinal herbs such as basil, garlic, onions, marjoram, caraway, fennel, anise, licorice, black seeds and fenugreek to fish diets results in improvement of protein digestibility and energy retention (Sakr, 2003, El-Dakar et al, 2004 and El-Dakar et al., 2008).

The apparent gustation coefficients represented by the values of number of grasps, time of grasps and retention time in *Oreochromis niloticus* fed basil leaf meal was higher than those fed with the control diets. In concordance with this result, Gbadamosi and Salako (2014) concluded that African basil appealed to the taste of fish by improving their gustation. Furthermore, in the current study no apparent histometric alterations were observed in the liver and intestines of fish fed the African basil leaf supplemented diets compared with the control diet, suggesting that it has no negative effect on growth performance and health of fish.

Conclusion

Generally, this study showed that dietary *O. gratissimum* can be used up to 80mg/g into the feed of *Oreochromis niloticus*, without adverse effect on the growth performance and health of the fish compared to the control. African basil appealed to the taste of fish by improving their gustation and it has no negative

effect on growth performance of fish fed with inclusion level ranging from 20mg/g to 80mg/g during the experimental period.

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EFFECT OF PALM KERNEL CAKE AS A REPLACEMENT FOR SOYBEAN MEAL ON THE GROWTH PERFORMANCE AND FATTY ACID PROFILE OF AFRICAN CATFISH, *CLARIAS GARIEPINUS*

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Abstract

The effect of dietary palm kernel cake (PKC) on the growth performance and fatty acid profile of African catfish, *Clarias gariepinus* was assessed in a 56 day feeding trial. A total of two hundred fingerlings of *C. gariepinus* were used for the experiment. Five varying levels of palm kernel cake were used to replace soybean meal at 0%, 5%, 10%, 15% and 20% in treatment T1, T2, T3, T4, and T5 respectively. A treatment contained three replicates of 15 fishes stocked in a rectangular tank of 40 x 35 x 30 (cm^3). At the end of the experiment, growth performance and nutrient utilization parameters such as the specific growth rate, feed conversion ratio and protein efficiency ratio were significantly better in fish fed the control and 5% diets compared with other dietary treatments ($P<0.05$). There was no significant difference in the survival rate between T1 and T2. Fish from each treatment was also analyzed to determine some essential fatty acid content. The result showed that linolenic acid was higher in the control and lowest in treatment five. However, eicosapentaenoic (EPA), docosapentaenoic (DPA) and docosahexaenoic (DHA) acids were highest in fish fed 20% palm kernel cake. The study showed that fish fed with 5% replacement of soybean meal with palm kernel cake had comparably good nutritional performance and fatty acid profile with the control diet. Therefore soybean meal can be replaced with 5% palm kernel cake without affecting the growth of *C. gariepinus* while enhancing the fatty acid quality of the fish.

Keywords: Palm kernel cake, replacement, growth performance, fatty acid profile, African catfish, *Clarias gariepinus*

Introduction

Fish play an important role in the protein supply of the world. The global demand for fish and fish products is increasing as a result of increasing global populations (Delgado et al., 1999: Globefish, 2013). Furthermore, there is increasing public awareness of the health benefits of consuming fish, in particular, but not exclusively due to the high levels of n-3 highly unsaturated fatty acids (HUFA) present in fish flesh (Simopoulous, 2008; FAO, 2010). The aquaculture sector is emerging as a vibrant and significant

production sector for high protein food. Global production of food from aquaculture, including finfishes, crustaceans, molluscs for human consumption, was reported to reach 52.5 million tonnes in 2008 (FAO, 2010). Aquaculture will continue to be relevant in the future as a source of protein. However, the growth in aquaculture has caused major challenges; one of these challenges is the production of practical diets for the farming of fish and shellfish. Increased fish farming has resulted into increased production of aqua feed which depends heavily on fishmeal and soybean meal as the main source of protein, because of its well-balanced nutrients (Hussein, 2012).

Aquaculture depends on the common input ingredients such as fishmeal, soybean, fish oil and wheat, for which it competes in the marketplace with the animal husbandry sector as well as with direct human consumption. Furthermore, many of the key ingredients traditionally used in formulating feed for commercial or on-farm aquaculture feeds are internationally traded commodities. The reduction of these conventional feed stuff will therefore be important to reduce feed costs and avoid competition with other users (FAO, 2009). Soybean meal is the most extensively used ingredient among the plant protein sources considered in aquafeeds (Lovell, 1988; NRC, 2011). It is also the most evaluated substitute for fish meal as a result of its availability, consistent quality, high protein content and relatively well-balanced amino acid profile compared with other plant protein sources, stable price and supply (Bhosale et al, 2010). Therefore, it has become imperative to assess different protein sources as cheaper and appropriate replacement for fish meal and soybean meal in aqua feed, because of the increasing demand for these conventional ingredients (Azaza et al, 2008).

The African oil palm (*Elaeis guineensis* Jacq.) is a native of West Africa; mainly distributed in Nigeria, Sierra Leone, Liberia, the Ivory Coast, Ghana and Cameroon and the equatorial regions of the Republic of Congo and Zaire (Ecocrop, 2011). Palm kernel meal is an important feed ingredient and the by-product of the oil palm *Elaeis guineensis*. This palm tree is cultivated for its oils rich in highly saturated vegetable fats: the palm oil, extracted from the fruit flesh; and the palm kernel oil, extracted from the fruit kernel (Jegede et al, 1998). Palm kernel cake (PKC) is an agro by-product that is being produced locally; it is easily available and affordable. Its potentials should therefore be exploited fully for sustainable aquaculture production in Nigeria. *C. gariepinus*, is a species of catfish which belong to the family *Clariidae,* the air breathing catfishes (Idodo-Umeh, 2003). They are predominantly cultured in Nigeria because of their rapid growth, resistance to diseases, its highly productive characteristics in poly culture with possibility of other fish species and its predatory nature in controlling some fish species such as tilapias and fathead minnows (FAO, 2014). Therefore, this study sought to address the scarcity of data on

the effect of partial replacement of conventional protein feedstuff like soybean meal with an agro by-product like palm kernel cake (PKC) on the growth, health and fatty acid profile of African catfish, *Clarias gariepinus*.

Materials and methods

The experiment was carried out at Federal University of Technology Teaching and Research farm of the Department of Fisheries and Aquaculture Technology for the period of 56 days. The control had 0% inclusion of palm kernel cake, the graded level of palm kernel cake used were 0.00g/kg (control), 5%, 10%, 15% and 20% replacement of soybean meal in treatment 1, 2,3,4,5 denoted as TI, T2, T3, T4 and T5 respectively.

Experimental procedure

The feed ingredients were purchased at Dotan feedmill Lafe in Akure. Palm kernel cake used was obtained from Turracum Palm oil Farm, Ikoro-Ekiti, Ekiti State, Nigeria. Fingerlings used for the experiment were purchased at the Teaching and Research farm of the Fisheries and Aquaculture Department, Federal University of Technology, Akure, Ondo-State, Nigeria. Two hundred pieces of fish with initial weight of 6.20 ± 0.02 were acclimatized in the experimental tank (45 x 30 x 35cm^3) for one week on the farm during which the fish were fed with Durante 3mm, commercial feed before the feeding trial. After acclimatization, the fish were randomly re-distributed in the experimental tanks using an electronic digital scale (KERN 770, max. 220 g, d= 0.0001g) in which fifteen fingerlings were allocated into a rectangular plastic tanks and the treatment were replicated in triplicates. The water used was sourced on the university farms. Throughout the experimental period, water parameters like temperature (27.5-29.5ºC) was recorded with a thermometer, dissolved oxygen (4.5- 4.8mgL-1) and pH (7.3-8.0) were determined with a pH meter and DO meter (Hannah H198106 model)

Experimental diets

The feed ingredients were purchased from Dotan feed mill in Akure. The ingredients used were fishmeal, soybean meal, maize, soybean oil, vitamin premix, starch, di calcium phosphate (DCP), lysine and methionine. Formulated. Five experimental diet were formulated to meet the requirements of 35% crude protein (Table 1) for *C. gariepinus* fingerlings (National Research Council, 2010) in which diet 1 contained 0% of palm kernel cake and serve as control and diet 2,3,4 and 5 contained 5%, 10%, 15%, and 20% replacement of soybean meal with PKC respectively. The ingredient was weighed according to their calculated weight, mixed thoroughly with hot water to form homogenized dough, and then each diet was

pelletized to 2mm size using a locally made manual pelletizer. The pelleted feeds were sun dried for 3 days and the feeds were stored in an air tight container before feeding of fish. Diets were immediately air - dried, sieved and stored in air-tight transparent plastic containers, labeled and stored until feeding. Fish were fed by 0900-1000 and 1700-1800h GMT, for 7 days each week. Growth was monitored weekly by batch weighing of fish from each tank. Standard and official methods (AOAC, 1995) were used to perform the proximate and carcass analyses of feed of fish in the study.

Crude lipid analysis and measurement: Crude lipid was measured by the chloroform-methanol 2:1 extraction method using an Agilent 6890N gas chromatograph (split / splitless injection method, 70 eV EI) (Wallborn, Germany) interfaced directly with an Agilent 5975 mass spectrometer. For calculations of the absolute amount of individual fatty acids, Heptadecanoic acid (17:0) methyl ester was used as internal standard.

Table 1: Feed ingredients composition in g/kg dry matter containing the inclusion level of PKC for *Clarias gariepinus*

	T1 (g/kg) (0%)	T2 (g/kg) (5%)	T3 (g/kg) (10%)	T4 (g/kg) (15%)	T5 (g/kg) (20%)
Fishmeal	200	200	200	200	200
Soybean meal	455	432.25	409.50	386.75	364
PKC	0	22.75	45.50	68.25	91.00
Maize	175	175	175	175	175
Sunflower oil	90	90	90	90	90
Vitamin premix	20	20	20	20	20
Starch	20	20	20	20	20
D C P	20	20	20	20	20
Lysine	10	10	10	10	10
Methionine	10	10	10	10	10

Vitamin Premix content **:** Vitamin A 4,000,000IU, Vitamin D3 800,000IU, Vitamin K3 1,600mg, Vitamin B1 4,000mg, Vitamin B2 3,000mg, Vitamin B6 3,800mg, Vitamin B12 3mcg, Nicotonic acid 18,000mg, Pantothenic acid 8,000mg, Folic acid 800mg, Biotin 100mcg, Choline chloride 120,000mg, Iron 8,000mg, Copper 800mg, Manganese 6,000mg, Zinc 8,000mg, Iodine 400mg, Selenium 40mcg, Vitamin C(coated) 60,000mg, Inositol 10,000mg, Cobalt 150mg, Lysine 10,000mg, Methionine 10,000mg and Antioxidant 25,000mg.

Evaluation of growth performance and nutrient utilisation

Growth performance and feed nutrient utilisation of fish were determined in terms of final weight gain (WG),daily feed intake, survival (%), specific growth rate (SGR). Feed conversion ratio (FCR) and protein utilization ratio (PER)

These growth responses were calculated as

Daily weight gain (g/fish/day) =feed consumed (g)/ (final body weight (BW) (g))/days

Feed conversion ratio (FCR) =feed consumed (g)/ (final BW (g)-initial BW (g))

Specific growth rate (%) = (Ln (final BW (g)-Ln (initial BW (g))/days*100

Protein efficiency ratio=fish weight gain (g)/protein fed (g).

Statistical analyses: The data were expressed as mean ± standard deviation (SD).The differences between mean of treatments were considered significant at $P \leq 0.05$ by one way analysis of variance

(ANOVA) using Statistica 16® software. Follow–up procedures were performed where significant differences occurred in the means using Tukey test.

Results

Proximate composition of experimental diets

The proximate composition of the experimental feeds is shown in Table 2. The moisture content was between 6.69% and 8.63%. The crude protein content increased with increasing levels of soybean meal with the highest recorded in TI. The highest fat contents were obtained in treatment three (T3) as 14.15%, while the lowest values were obtained in treatment four (T4) as 12.14%.

Table 2: Proximate composition of experimental diets (% dry weight)

PARAMETERS	T1	T2	T3	T4	T5
Moisture content	6.69	7.68	8.98	8.56	8.63
Ash content	6.45	5.59	5.15	5.07	5.03
Lipid	12.75	13.72	14.15	13.05	12.14
Crude fibre	5.74	6.08	6.14	6.34	6.48
Crude protein	35.00	34.70	34.30	34.20	34.05
Nitrogen-free extract	33.37	32.23	31.28	32.78	33.67
Gross energy (MJ/kg)	18.74	17.95	17.93	18.04	18.01

Nitrogen free extract was calculated as 100-(crude protein + ash + crude fibre + ether)

Carcass composition of experimental fish

The initial and final proximate analyses (% dry weight) of the experimental fish are presented in Table 3. In the present, it was found that T3 had the highest moisture content of (6.74%) and was followed closely by treatment four (T4) with (6.48%) while the lowest moisture content was observed in treatment one (T1) and treatment two (T2) which were both 5.57%. However, the highest crude protein of 58.79% was recorded in treatment three (T3) while the lowest value was recorded for treatment one (T1) as 52.73%. The fat content was highest in T1 (17.35%) followed closely by T2 (17.14%) and the lowest was observed in T4 (9.18%).

Table 3: Proximate composition (% dry weight) of the carcass of *Clarias gariepinus* fed the experimental diet containing varying inclusions levels of PKC

Parameters	Sample	Final Sample of Fish				
	Initial %	T1	T2	T3	T4	T5
Moisture	10.06	5.57±0.17^{a}	5.57±0.23^{a}	6.74±0.05^{c}	6.48±0.27^{bc}	5.86±0.11^{ab}
Protein	32.10	52.73±0.88^{a}	56.05±0.86^{b}	58.79±0.00^{c}	56.26±0.79^{b}	54.06±0.77^{ab}
Fat	16.74	17.35±0.08^{c}	17.14±0.12^{c}	12.34±0.62^{b}	9.18±0.11^{a}	9.7±0.18^{a}
Ash	15.72	15.79±0.14^{a}	15.56±0.23^{a}	17.51±0.24^{b}	21.42±0.27^{d}	20.3±0.46^{c}
NFE	25.39	8.57±0.12^{bc}	5.68±0.52^{a}	4.63±0.90^{a}	6.67±0.37^{ab}	10.09±0.60^{c}

^{a,b,c} values in each row with different superscripts are significantly different ($P < 0.05$) using ANOVA Post Hoc (Tukey test) (mean values ± SD, mean of fish from 3 replicate tanks).

The fatty acid profile of the experimental fish fed the experimental diets

The result of the fatty acid profile analysis is presented in Figure 1. It showed that the essential fatty acid, linolenic acid was higher in treatment one (T1) and lower in treatment five T5. The Arachidionic acid was higher in treatment one (T1) and treatment five (T5). However, the docosapentaenoic (DPA) acid content was highest in treatment five (T5) as shown in Figure 1.

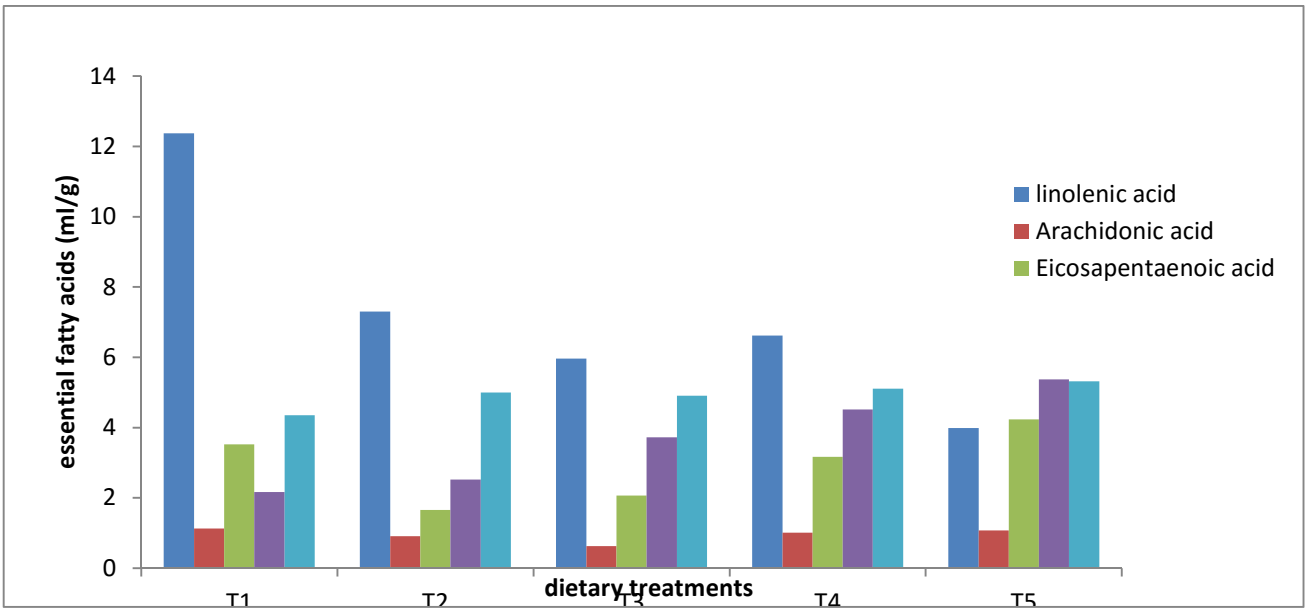

Fig. 1 Fatty acid profile of *C .gariepinus* fed with the experimental diets

Growth performance and nutrient utilization of *Clarias gariepinus* fed the experimental diet

The results of the growth performance and nutrient utilization of *Clarias gariepinus* fingerlings fed varying PKC as shown in Table 3. In the feeding trial fish fed diet the control diet had the highest percentage weight gain and highest specific growth rate of 1.61 followed by those fed 5% PKC replacement in T2. However, there was no significant difference in the SGR and FCR between fish in T2 and the control. The fish fed with 20% PKC diet in T5 had the lowest weight gained. Protein efficiency ratio also showed significance difference ($P<0.05$) with the highest recorded in T1 while the lowest protein efficiency ratio were T5. Mortality was not recorded in the control (T1) while the highest mortality was recorded in T4 and T5 as 83.34%.

Table 3 Growth parameters of *C. gariepinus* fed the experimental diets (mean ± SD)

PARAMETERS	T1 (control)	T2	T3	T4	T5
Initial weight (g)	6.18 ± 0.01^{a}	6.16 ± 0.02^{a}	6.16 ± 0.03^{a}	6.10 ± 0.09^{a}	6.18 ± 0.07^{a}
Final weight (g)	45.25 ± 0.41^{c}	44.36 ± 0.22^{c}	42.93 ± 0.40^{b}	42.30 ± 0.20^{b}	41.03 ± 0.09^{a}
Weight gain (g)	39.07 ± 0.42^{c}	38.20 ± 0.20^{c}	36.77 ± 0.37^{b}	36.20 ± 0.29^{b}	34.85 ± 0.03^{a}
Feed Intake (g)	60.78 ± 0.48^{a}	61.18 ± 1.42^{a}	61.43 ± 2.40^{b}	61.73 ± 0.81^{ab}	$72.51\pm.1.00^{a}$
SGR %	1.61 ± 0.05^{c}	1.51 ± 0.02^{c}	1.33 ± 0.05^{b}	1.26 ± 0.08^{b}	1.04 ± 0.01^{a}
PER %	16.35 ± 0.37^{b}	13.63 ± 1.55^{b}	7.09 ± 0.89^{a}	8.98 ± 1.11^{a}	6.75 ± 0.45^{a}
FCR %	1.60 ± 0.00^{a}	1.61 ± 0.20^{a}	1.67 ± 0.06^{b}	1.71 ± 0.02^{b}	2.08 ± 0.02^{c}
Survival Rate %	100 ± 0^{c}	93.34 ± 6.67^{c}	85.34 ± 6.67^{a}	83.34 ± 3.34^{ab}	83.34 ± 3.34^{ab}

^{a,b,c} values in each row with the different superscript are significantly different ($p < 0.05$) by using ANOVA post hoc (Tukey test) (mean values ± SD, n = 3) (where n is the number of replicate tanks).

Weight of fish fed with experimental diets for 8 weeks

The weight of fish used during the experiment is presented in Figure 2. This graph showed that there were significant differences in the growth of the fish in each treatment tank, the fish in each treatment started with the same weight and after the third week there were differences in the weight. It was observed that treatment one (T1) without PKC inclusion had the highest weight and the treatment two (T2) with the inclusion of PKC compared well with treatment one (T1) in which the final growth rate was almost the same and there were no significant differences in treatment one and treatment two, while Treatment five (T5) had lowest weight gained.

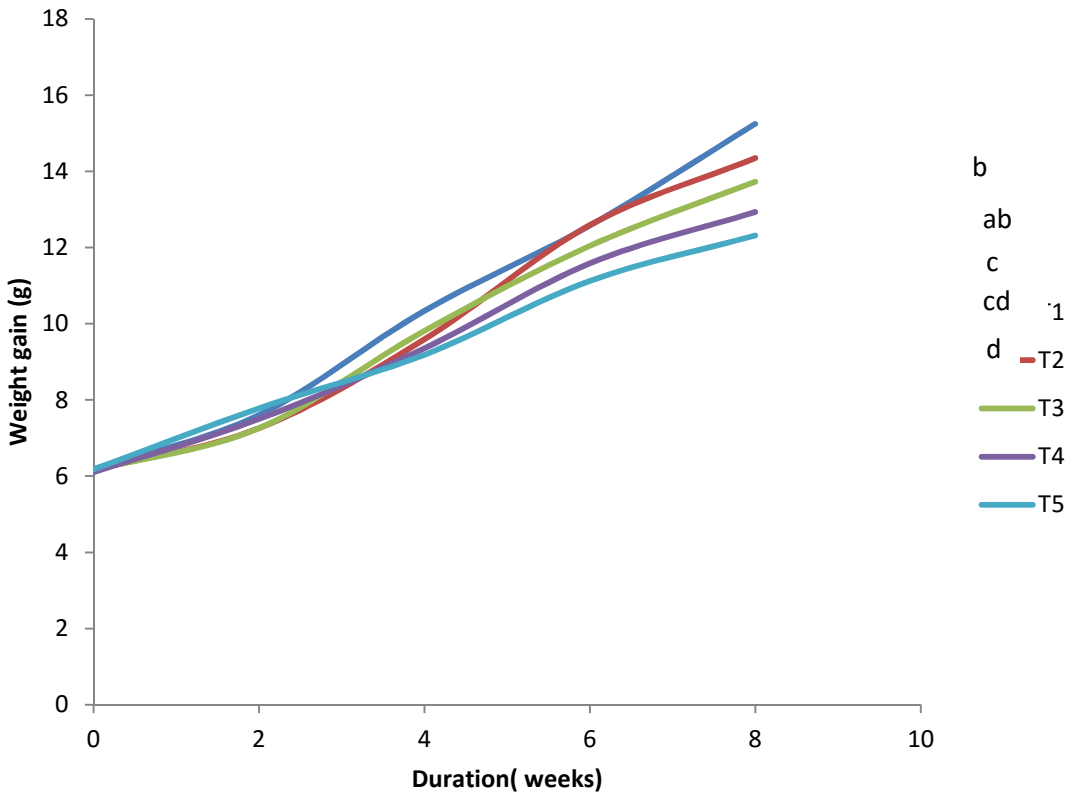

Figure 2: Weight of the fish fed with experimental diets for 8 weeks

[a,b,c,d] values in each row with the different superscript are significantly different ($p < 0.05$) by using ANOVA post hoc (Tukey test) (mean values ± SD, n = 3) (where n is the number of replicate tanks).

Discussion

The proximate composition of the experimental diets revealed that the crude protein ranges from 34.05 in treatment five to 35.00 in treatment one. This range is suitable for *C. gariepinus* growth for commercial fish. According to Hassan, (2001) the protein requirements for *C. gariepinus* range from 30-45% of dry diets. The result of the proximate analysis also revealed that the ash content was highest in the control and lipid ranges from 12.14% in treatment five to 14.15% in treatment three. This result is in line with that of Simopoulus (2008), who reported that in general, 10-20% of lipid in most freshwater fish diets gives optimal growth without producing an excessively fatty fish.

The growth performance of *C. gariepinus* fingerlings fed with the experimental diets showed that there were no significant differences ($P>0.05$) in the final weight of fish in T1 and T2. Weight gain and specific growth rate of the fish increased with decreasing percentage inclusion levels of PKC. The highest growth performance was recorded in the control followed closely by T2 which had 5% inclusion of PKC. In agreement with this result, Sotolu (2010) reported that the mean weight gained and the specific growth rate of *C. gariepinus* fed diets with different vegetable oils on catfish increased with increasing levels of lipid in the diets. The results of the present study showed that the amount of feed consumed by fish fed the PKC based diet was not significantly different from those fed the fish meal diet suggesting that PKC was well accepted by the fish used in this experiment. Similarly, previous studies on the inclusion of PKC in the diet of African catfish reported that *O.niloticus* has no difficulty with the palatability and acceptability of microalgae in its diets (Oliveira et al., 1998; Ng et al., 2002). However, Adikwu, (1997) reported that Nile tilapia fed diets where palm kernel meal replaced 30 to 90% of fish meal had a lower final weight than fish fed the control diet and a protein efficiency ratio that declined with increasing dietary palm kernel content, though feed efficiency was higher for the palm kernel-based diets use of palm kernel meal in fish diets, it was then concluded that the use of PKC in aquafeed is limited by its low protein content, amino acid deficiencies and presence of antinutritional factors (non-starch polysaccharides). Despite these limitations, there have been encouraging results in tilapia and catfish, with fish growing well with dietary levels up to 20% and even higher (Ng, 2004).

Survival was high in fish fed the 5% PKC and similar to those fed the control diets in the present study, suggesting that 5% PKC was well tolerated without any negative effects on fish health. This result is in agreement with other animal models where PKC was used to replace soybean meal. For instance Silva et al (2005), Boateng et al (2008) and Bello et al (2011) reported that the total inclusion of PKC meal did not have any negative effects on the growth, survival and health of broilers, pig and west African dwarf

goats respectively. The fatty acid profile was influenced by the inclusion of PKC in the diet. This result is in agreement with previous studies on fatty acid nutrition of tilapia, which reported that the dietary source and composition of fatty acids determines the fatty acid composition of the fish body (Lim et al, 2010; Sotolu, 2010).

The fatty acid composition of diets showed that that linolenic (LA), arachidionic (ARA), eicosapentaenoic (EPA), docosapentaenoic (DPA) and docosahexaenoic (DHA) acid are found among all the diets. Among the acid, LA (18:2n-6) appear at highest concentration in in T1 and lowest in T5. ARA (20:4n-6) was highest in control diet (T1) followed closely by T5. EPA (20:5n-3) was highest in T5 and the lowest was occurred in T2. DPA (22:5n-3) was detected as the highest T5 and the lowest in T1 which means that it increases with increasing PKC. DHA (22:6n-3) was also detected as the highest in T5 and lowest in T1.This is in agreement with Sotolu (2010) which stated that hepatic eicosapentaenoic and docosahexaenoic acids levels increases with increasing vegetable oil in the diet of African catfish. The composition of HUFA like 20:5n-3 and 22: 6n-3 in Nile tilapia and catfish is reported to be dependent on the fatty acid composition of the diets (Simopoulos, 2008; Lim et al, 2010). Likewise in the present study, the rate of production of 20:5n-3 in fish at the end of the feeding was reflected by the composition of n-3 PUFA like 20:5n-3 in the diet. These changes might influence the nutritional quality of the final fish product for the human consumer and offer a vehicle through which these n-3 fatty acids may be available to the final consumer of the fish (Sargent, 2002; Babalola and Apata, 2011). However, fish fed the control diet had a higher total n-6 polyunsaturated fatty acid and lower n-3/n-6 ratio than fish fed the PKC diets. This may be attributed to high levels of the 18:2n-6 in the in the soybean meal diet (Sargent et al, 2002: Menoyo et al, 2007).

Conclusion

Palm kernel cake (PKC) is considered as an acceptable source of nutrients such as protein and lipid and other bioactive compounds for fish. The result showed that fish that were fed with 5% PKC inclusion recorded growth performance and nutrient utilization which compared favourably with the control. However, fish fed diets with more than 5% PKC had a lower final weight than fish fed the control diet and a protein efficiency ratio that declined with increasing dietary palm kernel content in the diet. Results from this study therefore suggest that dietary PKC is capable of replacing soybean up to 5% in feeds for African catfish, as a dietary source of protein and can provide similar growth performance and fatty acid profile to soybean meal, without compromising the health of the fish.

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EFFECT OF DIETARY INCLUSION OF GRADED LEVEL OF DIFFERENTLY TIMED DRY HEAT TREATED *Jatropha curcas* SEEDMEAL AS PROTEIN SOURCE ON THE GROWTH AND ECONOMIC PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus*) JUVENILES.

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Abstract
The effect of dietary inclusion of differently timed dry heat treated *Jatropha curcass* on the growth and economic performance of Nile tilapia (*Oreochromis niloticus*) was evaluated in a 56-day feeding trial. Five isonitrogenous and isolipidic dietary treatments (35% crude protein and 10% crude lipid) were made consisting of soybean meal (control) which was replaced by 5-minute and 10-minute toasted *Jatropha curcass* seedmeal at 20 and 40% to make other four test diets. 225 Juveniles of *Oreochromis niloticus* were acclimatized for a week, weighed and allotted into five dietary treatments. Each treatment was replicated three times with fifteen fish per replicate. Fish were fed 5% body weight on two equal proportions per day for 56 days. Growth data were collected on two-week interval basis. Growth and economic performance was evaluated using weight gain (g); specific growth rate (%/day); feed conversion ratio (FCR) and protein efficiency ratio, net return, gross margin and benefit-cost ratio as indices. The results from the study indicated that there was significant difference ($p<0.05$) in the growth and economic performance parameters among the fish exposed to different dietary treatments. However there was no significant variations ($p>0.05$) in the different growth and economic performance parameters of fish fed CTR and fish fed D520T.
Keywords: Gross margin, net return, feed ingredient, tilapia, growth

Introduction

Soybean meal is a conventional plant protein source in fish feeds that has the capability to replace up to 50% of the fish meal (Storebakken *et al.* 2000). However, its sustainable use as fishmeal replacer is restricted because of the various uses to which soybean is put by human. This consequently led to its hike in price (Azaza *et al*., 2009). There is, therefore, need to look for cheaper plant protein source which can replace soybean meal in fish diets (Yue and Zhou, 2008). *Jatropha curcas* seedmeal has nutrient density that is comparable to any other protein sources. It has a good amino acid profile with its essential amino acids level

(except lysine) higher than soybean meal (Kumar *et al.* 2010). Studies on the use of *Jatropha* seedmeal are; Kumar et al., (2009) on rainbow trout diets; Kumar et al. (2010) on carp diet; Fakunle et al., (2013) and Alatise et al., (2014) on Clarias gariepinus diet. There exist paucity of information on the use of the seedmeal as dietary protein source in Nile Tilapia diets especially report on the use of dry heat treatment to reduce anti-nutritional factor in the seedmeal and economic analysis of feeding the diets containing Jatropha curcas seedmeal in Nile Tilapia diets. Thus, this study therefore examines the effect of dietary inclusion of graded level of differently timed dry heat treated Jatropha curcas seedmeal as protein source on the growth and economic performance of Nile tilapia (Oreochromis niloticus) juveniles.

Materials and Methods

Collection and processing of *Jatropha curcas* seed

Three kilograms of mature seed *Jatropha curcas* were sourced from neighboring village within Ilora, Oyo town. The seed were stored with polyethene bags at room. Before the commencement of the experiment, the ***Jatropha curcas*** were decoated to obtain the seeds. The seeds were divided into two parts, the first part was roasted for 5 minutes and the second part was roasted for 10 minutes so as to eliminate the possible effect of anti-nutritional factor that could be present in the kernel. These seed meal and some other basic feed ingredients were then taken to the laboratory for proximate composition analyses following the procedure of AOAC (1990).

Table 1: Proximate Composition of the some Feed Ingredients

Parameter	Fish meal	Soybean Meal	5-Min Toasted JCM	10-min Toasted JCM	Maize
Moisture	9.75	10.70	3.80	3.80	10.48
Crude Protein	72.4	38.74	33.96	34.72	9.87
Crude Lipid	10.45	30.68	39.03	39.43	4.28
Crude Fibre	-	5.10	13.11	10.16	5.78
Ash	8.32	4.48	6.09	4.25	6.73
*NFE	-	10.30	4.01	7.64	62.35

*Nitrogen Free Extract

JCM *Jatropha curcas meal*

Diet formulation and preparation

Based on proximate composition of the basic feed ingredients in table1, five experimental diets were formulated consisting of a control diet which is made up of soybean meal and 4 test diets replacing soybean meal at a rate of 20% and 40%by 5minute and 10 minute toasted *Jatropha curcas* seed meal. The ingredients were manually mixed together in the laboratory before the addition of vitamin premix. Fish oil was later added to the dry ingredient and mixed thoroughly. Warm water was added to the premixed ingredients and homogenized until a dough-like paste was formed. The dough was passed through an improvised manual pelleting machine to produce 2mm pellet size. The moist pellets were sun-dried to a constant weight and kept in air tight containers. The gross composition of the experimental diets is given in

Table 2: Gross composition (g/100g) of experimental diets containing differently timed dry heat treated *Jatropha curcass* seedmeal fed to *Oreochromis niloticus*

Ingredients	CTR	D520T	D540T	D1020T	D1040T
Fishmeal	20.83	20.83	20.83	20.83	20.83
Soybean Meal	42.10	33.68	25.62	33.68	25.62
5-Min Toasted	-	9.42	18.85	-	-
10-Min Toasted	-	-	-	9.21	18.43
Corn	10.00	10.00	10.00	10.00	10.00
Fish Premix	5.00	5.00	5.00	5.00	5.00
Fish Oil	2.50	2.50	2.50	2.50	2.50
Starch	19.57	18.59	17.20	18.78	17.62
Total	100.00	100.00	100.00	100.00	100.00

Specification: each kg contains: Vitamin A , 4,000,000IU; Vitamin B, 800,000IU; Vitamin E, 40,000IU, Vitamin K_3, 1,600mg; Vitamin B_1, 4,000mg; Vitamin B_2, 3,000mg; Vitamin B_6, 3,800mg, Vitamin B_{12} 3 mcg; Nicotinic Acid 18000mg. Pantothenic Acid 8,000mg; Folic Acid 800mg Biotin, 100 mcg Choline Chloride 120,000mg; Iron 8,000mg; Copper 800mg; Manganese,6,000mg; Zinc 8,000mg; Iodine 400mg;; Selenium, 40 mcg,vit C Coated 60,000mg,Inositol 10,000mg; Cobalt,150m, Lysine 10,000mg; Methionine 10,000 mg, Antioxidant 25,000mg manufactured by Bi-mix Brrand, Corporate head office/factory: 1, Odo-Olowu Street, Ijesatedo, Lagos, Nigeria.

Experimental Design

A completely randomized design was adopted with three replicates per treatment. Each replicate had 15 fish and were grouped fed. Juveniles of ***Oreochromis niloticus*** (tilapia) were purchased from Masopa fish farm along Alakia road Ibadan Oyo state, Nigeria. The fish were allowed to acclimatize for 15 days. During this period, they were fed on commercial diet. Prior to the commencement of the feeding trial, all fish were starved for 24 hours. This practice was to prepare the gastrointestinal tract for the experimental diet while at the same time to increase the appetite of the fish.

Feeding Trial

The feeding trial was conducted in the wet laboratory of Federal College of Animal Health and Production Technology, Ibadan. The experimental system contained a set of 15 rectangular plastic tanks each with a capacity of 55 liters of water. 225 *Oreochromis niloticus* (tilapia) juveniles with initial weight of 15.19±0.02 were used for the experiment. All fish were fed twice daily at a fixed feeding rate of 5% of their body weight per day. Feeding was generally done in the morning at 09:00 am and 05:00 pm in the evening. Periodic weighing was done at two weeks interval and the feed adjusted as required. Growth performance indices were estimated using the procedures of Jimoh and Aroyehun (2011). Water temperature and dissolved oxygen were measured using a combined digital YSI dissolved oxygen meter (YSI Model 57, Yellow Spring Ohio); pH was monitored weekly using pH meter (Mettler Toledo – 320, Jenway UK. Bionomic Analysis was done following the methods explained in Faturoti, (1989); Abu *et al.* (2010); Boateng *et al.*, (2014) and Jimoh *et al* (2015); Straight line method of depreciation was used to evaluate the cost of Aquaria tanks with the following properties.

Cost of Plastic Tanks	₦57,000
No of years (life Span)	5 yrs
Savage value	10% of Cost Price

Statistical Analysis

Data obtained from the experiment was expressed in mean ±SD and it was subjected to one way Analysis of Variance (ANOVA) using SPSS 16.0 version. Where the ANOVA reveals significant difference ($P<0.05$) Duncan multiple range test was used to compare differences among individual treatment means.

Results

Table 3 reveals the proximate composition, calculated amino acid and fatty acid profile of the experimental diets. The diets met the optimal dietary protein and lipid requirement of tilapia (Luquet, 1991).

Table 3: Proximate Composition (g/100g Dry Matter) of Experimental Diets Containing Graded Levels of Differently Timed Dry Heat Treated *Jatropha curcass* Seedmeal fed to *Oreochromis niloticus*

Parameters	CTR	D520T	D540T	D1020T	D1040T
Moisture	9.95±0.17^{a}	10.06±0.12^{a}	9.70±0.17^{a}	10.16±0.16^{a}	9.94±0.25^{a}
Crude Protein	35.17±0.05^{a}	35.18±0.08^{a}	35.18±0.01^{a}	35.17±0.01^{a}	35.16±0.01^{a}
Crude Lipid	10.49±0.24^{a}	10.66±0.31^{a}	10.38±0.06^{a}	10.38±0.01^{a}	10.38±0.01^{a}
Crude Fibre	11.92±0.14^{c}	12.40±0.49^{bc}	12.60±0.11^{b}	13.16±0.16^{a}	13.27±0.40^{a}
Ash	6.44±0.01^{b}	6.50±0.03^{ab}	6.52±0.03^{a}	6.37±0.06^{c}	6.19±0.02^{d}
NFE	26.04±0.1^{a}	25.20±0.39^{bc}	25.62±0.25^{ab}	24.75±0.35^{c}	25.06±0.33^{bc}
Energy	4.00±0.03^{a}	4.02±0.03^{ab}	4.01±0.01^{ab}	3.97±0.02^{b}	3.99±0.01^{bc}

Calculated Amino Acid Profile of Experimental Diets					
Arginine%	2.5	2.3	2.1	2.3	2.1
Histidine%	0.8	0.8	0.7	0.8	0.7
Isoleucine%	1.6	1.4	1.3	1.4	1.3
Leucine%	2.6	2.3	2.1	2.3	2.1
Lysine%	2.4	2.2	2.0	2.2	2.0
Methionine%	0.7	0.7	0.6	0.7	0.6
M+C%	1.2	1.1	1.0	1.1	1.0
Phenylalanine%	1.5	1.3	1.2	1.3	1.2
P+T%	2.6	2.3	2.1	2.3	2.1
Threonine%	1.5	1.4	1.3	1.4	1.3
Tryptophan%	0.4	0.4	0.3	0.4	0.3
Valine%	1.7	1.5	1.4	1.5	1.4

Calculated Fatty Acid Profile of Experimental Diets					
LOA (18:2n-6)%	5.1	4.2	3.3	4.2	3.3
LNA (18:3n-3)%	0.8	0.6	0.5	0.6	0.5
ARA (20:4n-6)%	0.1	0.1	0.1	0.1	0.1
EPA (20:5n-3)%	0.3	0.3	0.3	0.3	0.3
DHA (22:6n-3)%	0.7	0.7	0.7	0.7	0.7
Total n-3%	1.8	1.7	1.6	1.7	1.6
Total n-6%	5.2	4.2	3.4	4.2	3.4
n3:n6	0.4	0.4	0.5	0.4	0.5

LOA: Linoleic Acid; LNA: Linolenic Acid; ARA: Arachidonic Acid; EPA: Eicosapentanoic Acid; DHA Docosahexanoic Acid.

Fatty acid and amino acid profiles of the experimental diets were calculated using a program (excel) developed by NACA (2008)

Table 4 reveals the growth performance of *Oreochromis niloticus* fed diets containing graded levels of differently timed dry heat treated *Jatropha curcass* seedmeal. The weight gain of fish fed CTR was the highest which was significantly different ($p<0.05$) from the weight gain of fish fed other dietary treatments except the fish fed D520T. Fish fed diet D1040T had the lowest weight gain which was not significantly different ($p>0.05$) from the weight gain of fish fed D1020T. No significant variation ($p>0.05$) existed among the weight gain of fish fed D520T, D540T and D1020T.

Table 4: Growth Performance of *Oreochromis niloticus* Fed Diets Containing Graded Levels of Differently Timed Dry Heat Treated *Jatropha curcass* Seedmeal

Parameters	CTR	D520T	D540T	D1020T	D1040T
Initial Weight	15.19 ± 0.02^{a}	15.14 ± 0.01^{a}	15.17 ± 0.01^{a}	15.15 ± 0.01^{a}	15.19 ± 0.01^{a}
Final Weight	21.71 ± 0.45^{a}	21.08 ± 0.17^{ab}	20.77 ± 0.65^{b}	20.39 ± 0.54^{bc}	19.75 ± 0.37^{c}
Weight Gain	6.52 ± 0.45^{a}	5.94 ± 0.16^{ab}	5.60 ± 0.65^{b}	5.24 ± 0.54^{bc}	4.56 ± 0.37^{c}
%WG	42.92 ± 2.97^{a}	39.23 ± 1.01^{ab}	36.92 ± 4.30^{b}	34.59 ± 3.44^{bc}	30.02 ± 2.44^{c}
SGR	0.64 ± 0.36^{a}	0.59 ± 0.02^{ab}	0.56 ± 0.06^{b}	0.53 ± 0.05^{bc}	0.47 ± 0.03^{c}
Food Fed	7.71±0.54	7.05±0.13	6.77±0.67	6.52±0.02	5.87±0.53
FCR	1.18 ± 0.25^{c}	1.19 ± 0.02^{c}	1.21 ± 0.0^{bc}	1.24 ± 0.02^{b}	1.29 ± 0.02^{a}
Protein Fed	2.70±0.19	1.21±0.02	2.37±0.24	2.28±0.26	2.05±0.19
PER	2.41 ± 0.05^{a}	2.41 ± 0.05^{a}	2.36 ± 0.04^{ab}	2.30 ± 0.04^{b}	2.22 ± 0.04^{c}
Survival	88.00±1.73	91.00±10.15	93.33±6.51	82.33±4.04	83.33±15.28

Row means with different superscripts are significantly different ($p<0.05$) from each other

[1] Mean weight gain= final mean weight –initial mean weight

[2] Percentage weight gain= [final weight-initial weight_/initial weight] X 100

[3] Specific growth rate= [In final weight-In initial weight] X 100
[4] Feed conversion ratio=dry weight of feed fed /Weight gain (g)
[5] Protein efficiency ratio=fish body weight (g)/ Protein fed
[6] Percentage survival = {(total number of fish- mortality)/total number of fish] X 100

Similarly trends of results as observed for weight gain was also as observed for final weight, %weight gain and SGR, and a reverse trend was also observed for FCR. The PER of fish exposed to diet CTR was the highest which was not significantly different ($p>0.05$) from the PER of fish fed D520T and 540T. The PER of fish exposed to diet D1040T was the lowest. There was no significant difference ($p>0.05$) in the PER of fish fed diet D540T and diet D1020T.
Figure 4.1 shows the Growth Curve of *Oreochromis niloticus* fed diets containing graded levels of *Jatropha curcas* for 56 days.

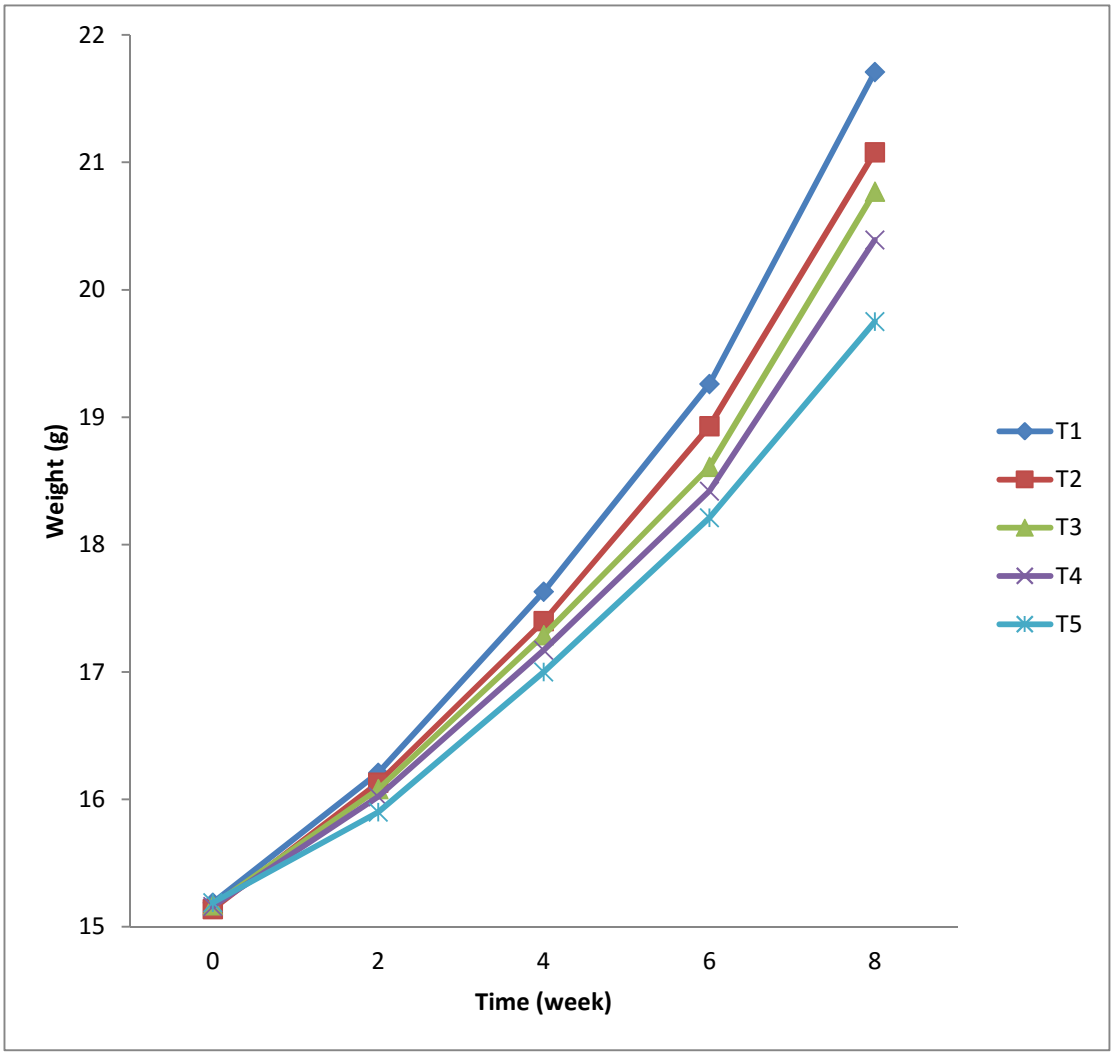

Figure 1: Growth Curve of *Oreochromis niloticus* fed diets containing graded levels of *Jatropha carcass* for 56 days

Cost of production of 1kg diet

Table 5 shows the cost (₦) of producing 1kg of each experimental diet containing differently timed dry heat treated *Jatropha curcas* fed to *Oreochromis niloticus*. There was reduction trend in the cost of producing the diet with increasing inclusion of *Jatropha curcas* when compared with control.

Table 5: Cost of production (₦) of the experimental diets containing differently timed dry heat treated *Jatropha curcas* seedmeal fed to *Oreochromis niloticus*

	Price/kg	CTR	D520T	D540T	D1020T	D1040T
Fishmeal	680	141.64	141.64	141.64	141.64	141.64
Soybean	155	65.26	52.20	39.71	52.20	39.71
5-min Toasted	68	-	6.41	12.82	-	-
10-min toasted	68	-	-	-	6.26	12.53
Cornmeal	72	7.20	7.20	7.20	7.20	7.20
Fish Premix	700	35.00	35.00	35.00	35.00	35.00
Fish Oil	600	15.00	15.00	15.00	15.00	15.00
Starch	50	9.78	9.29	8.60	9.39	8.81
Total		**273.88**	**266.74**	**259.97**	**266.70**	**259.90**

Table 6 shows the economic analysis of producing *Oreochromis niloticus* with diets containing differently timed dry heat treated *Jatropha curcas* seedmeal. The incidence of cost, which is the cost of producing 1kg Tilapia fish, was highest using diet D1040T and lowest using diet D520T. There existed significant variation ($p<0.05$) in the incidence of cost of fish produced using different dietary treatments. However, there was no significant different ($p>0.05$) in the incidence of cost of producing 1kg tilapia fish with diet D1040T, D1020T and CTR. The profit index and gross profit of the produced with D540T was the highest which is not significantly different ($p>0.05$) from profit index and gross profit of fish produced by using diet CTR and D520T.

The net return of producing tilapia fish with diet CTR was the highest which is not significantly different ($p>0.05$) from the net return of fish produced with diets D520T and D540T. Fish fed diet D1040T had the lowest net return. There was no significant variations ($p>0.05$) in the net returns of fish fed D540T and D1020T.

Table 6: Economic Analysis of Producing *Oreochromis niloticus* With Diets Containing Differently Timed Dry Heat Treated *Jatropha curcas* Seedmeal.

Parameters	CTR	D520T	D540T	D1020T	D1040T
Output Biomass (kg)	21.71±0.45^{a}	20.39±0.57^{ab}	19.75±0.37^{b}	21.08±0.17^{bc}	20.77±0.6
Cost of Feeding (x10^{3})	2.11±0.15^{a}	1.88±0.0.36^{ab}	1.76±0.17^{bc}	1.74±0.0.20^{bc}	1.53±0.00
Incidence of cost ( )	324.10±6.89^{ab}	316.53±4.08^{b}	314.57±5.20^{b}	331.60±5.55^{a}	334.40±5.
Value of fish ( )	3.91±0.27^{a}	3.57±0.96^{ab}	3.36±0.39^{b}	3.14±3.15^{bc}	2.73±0.22
Profit index ( )	1.85±0.40^{ab}	1.89±0.25^{a}	1.91±0.30^{a}	1.81±0.26^{bc}	1.79±0.31
Gross profit ( )	275.90±6.89^{ab}	283.47±4.10^{a}	285.43±5.20^{a}	268.40±5.55^{b}	265.60±5.
Total Variable Cost(x10^{3})	2.34±0.15^{a}	1.96±0.20^{ab}	1.75±0.14^{b}	2.10±0.34^{bc}	1.98±0.18
*Total Fixed Cost(x10^{3})	1.71	1.71	1.71	1.71	1.71
Total Cost(x10^{3})	4.05±0.15^{a}	3.67±0.20^{ab}	3.46±0.14^{bc}	3.81±0.35^{bc}	3.69±0.18
Total Revenue (x10^{3})	13.03±0.27^{a}	12.23±0.32^{ab}	11.85±0.22^{b}	12.65±0.10^{bc}	11.33±0.5
Gross margin(x10^{3})	10.80±0..42	10.40±0.23	10.69±0.41	10.60±0.00	10.40±1.5
Net return (x10^{3})	8.98±0.13^{a}	8.56±0.12^{a}	8.39±0.09^{ab}	8.83±0.07^{bc}	8.77±0.21
Cost of Juveniles: 15		1kg Tilapia=₦600		1 USD=₦365	
Cost of Aquaria tank				57,000	
Less 10% Savage Value				5,700	
Depreciation				10,260	
Value of Aquaria Tank (2 months)				1,710	

Table 7 shows the profitability analysis of producing *Oreochromis niloticus* with diets containing differently timed dry heat treated *Jatropha curcas* seedmeal. Benefit cost ratio (BCR) of producing fish with D540T was the highest which is not significantly different ($p>0.05$) from the BCR of fish produced with other dietary treatments except D520T and D1020T. BCR of fish produced by using diet D1040T was the lowest.

Table 7: Profitability Analysis of Producing *Oreochromis niloticus* with Diets Containing Differently Timed Dry Heat Treated *Jatropha curcas* seedmeal.

Parameters	CTR	D520T	D540T	D1020T	D1040T
Benefit Cost Ratio	3.22±0.60^{b}	3.32±0.15^{ab}	3.38±0.51^{a}	3.33±0.95^{ab}	3.43±0.70^{c}
Gross Ratio	0.31±0.10^{a}	0.30±0.00^{ab}	0.29±0.06^{b}	0.30±0.10^{ab}	0.29±0.06^{b}
Expense Structure Ratio	0.42±0.15^{c}	0.45±0.00^{bc}	0.46±0.21^{ab}	0.46±0.25^{ab}	0.50±0.25^{a}
Rate of Return	2.22±0.60^{b}	2.32±0.15^{ab}	2.38±0.51^{a}	2.33±0.95^{ab}	2.43±0.70^{a}

Rate of return of producing Nile tilapia diets with different dietary treatments follow the same trends of results as accounted for BCR. Gross ratio (GR) of fish with CTR was the highest but it was not significantly different from the GR of fish produced by using diets D520T AND D1020T. The expense structure ratio (ESR) followed reverse trends of results.

Discussion

It appears from the results of growth performance processing time and levels of inclusion of *J. curcas* had impact on the growth performance of Nile tilapia. The growth performance of tilapia fed diets containing lower timed heat treated *J. curcas* were comparable to that of control even at higher replacement level with soybean using FCR and PER as indices of assessment. These results are in consonance with the work of Workagegn *et al.* (2013) for Nile Tilapia juveniles fed the same seedmeal replacing soybean meal that the growth performance of heat treated *Jatropha carcass* kernel meal was comparable to that of control at 10% replacement level. The results, however, differ from what Kumar *et al.* (2010) reported that inclusion of cooked Jatropha seedmeal in the diet of carp was possible at levels higher than 50% in the diet of fish without compromising their growth performance. Plausible reasons might be the different heat treatments applied more so that the seedmeal was completely detoxified of the phorbol esters (PEs) which is a toxic compound in the seedmeal (Kumar *et al.* 2010). Makkar *et al* (2012) reported that detoxified *J. carcass* seedmeal could replace more than 50% of fishmeal in the fish diets. Although, Davies and Gouveia (2008) reported that thermal processing of raw pea seedmeal, dry heat treatment in particular (180ºC:30 min), led to a greatly improved feed utilization and consequent better growth performances, various factors could imply the relatively poor growth performance recorded at higher processing time and inclusion level recorded in this study, one of which could be incomplete inactivation of the anti-nutrients in the seedmeal, the principal of it being PEs by the heat treatment applied which consequently led to poor digestibility of protein (Kumar *et al*, 2009). Kumar *et al.* (2010) reported that antinutrients in *J.curcass* seedmeal especially the major toxic components called phorbol esters restricts its use in fish feed.

This incidence of cost analysis of feeding *J. curcas* seedmeal to *Oreochromis niloticus* showed the impact of the cost of feed on the variable cost of fish production since maintenance and sustenance of aquaculture depends on its economic viability and relative profitability (Adeparusi and Balogun 1999). A profit index above 1 showed that it is profitable to feed the fish with the diet (Jimoh *et al*., 2013). Gross profit remains the primary interest on most capital investment, the gross profit margin and loan repayments where applicable form the basis after operational evaluation (Faturoti, 1989). Gross margin was reported to be a good measure of profitability (Olagunju *et al;* 2007). The experiment showed that it is profitable to replace soybean meal with differently timed dry heat treated *J. curcas* seedmeal using gross profit as an index of assessment. This result agrees with the finding of Fagbenro *et al* (2001), Abu *et al* (2010) and Jimoh *et al.* (2012) that feeding fish with cheaper and lesser known feed ingredients left some profit margin. Although the economic implication of using the different dietary treatments might not be well appreciated since the margin might be too small, it will be much explicit when the magnitude of total cost and expected revenue of its large scale operation is critically and objectively considered (Faturoti, 1989). Adeparusi and Balogun (1999) reported profit margin as increasing when fish meal was replaced by roasted pigeon peal meal in a diet fed to *Clarias gariepinus*. Jimoh (2004) also reported an increase in profit margin in the production of Tilapia up to 30% of soybean meal with jackbean meal. The reduced gross profit on tilapia produced by diet D1040T was due to lower growth rate of growth of fish. This result is in agreement with the report of Jimoh (2004) that profit margin reduced with jackbean replacement level beyond 30%. This implies that feeding fish with differently timed dry heat treated *J. curcas* seedmeal had minimum impact on variable cost of production hence the profit recorded.

The experiment showed that the average total variable and total cost of producing *Oreochromis niloticus* with the diets was met or covered by the total revenue realized from the sale tilapia and left a positive gross margin and net returns in all the dietary treatment groups. Positive gross margin and net returns indicate that it is profitable to feed *Oreochromis niloticus* differently timed dry heat treated *J.curcass* seedmeal. A result similar to what was reported in Jimoh *et al* (2015) when soybean meal was replaced by watermelon seedmeal. Boateng *et al* (2013) reported a positive operating profit when all variable cost of production of all male tilapia was covered by the gross revenue as an indicator of profitability of all male tilapian aquaculture enterprise in Ghana. Jimoh et al (2014) reported similar trends of results on incidence of cost analysis when *Clarias gariepinus* was fed diets containing *Chrysophyllum albidum* seedmeal. The ESR value reported in this study is lower than the value reported by Adebayo and Daramola,(2013). The rate of return recorded in this study is higher than what Boateng *et al* , (2014) reported on return on investment for all male tilapia farming in Ghana. Adebayo and Daramola (2013) reported ROR value of 0.62 for catfish production

Conclusion and Recommendation

It is evident from the present study that soybean meal could be replaced up to 40% by incompletely detoxified *Jatropha curcass* seedmeal at lower dry heat processing time without compromising the growth performance of tilapia fish

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MARKET AVAILABILITY OF OILSEED PROTEIN MEALS IN GHANA

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Abstract

Oilseed meals have been recommended as potential replacers of fishmeal in animal feeds especially in aquaculture feeds. This study sought to assess the market availability of some oilseed meals (groundnut meal, palm kernel cake, soybean meal, groundnut husk, copra meal and cottonseed meal) by identifying their sources. An attempt was also made to assess the production levels of the selected oilseed meals. Data was gathered mainly from processors of the selected oilseeds. Geographic locations of processing sites were taken using a GPS device to develop a distribution map. Ninety-one (91) sources of oilseed meals were identified across the country. Groundnut cake was abundant in the Northern savannah zone, accounting for 65.84 % of the quantity produced annually. Production of palm kernel cake covered the Eastern, Western, Central, Ashanti and Greater-Accra Regions. Seventy-nine percent (79.7%) of copra meal was traced from the southern part of the Western region. Soybean meal was largely produced in the Ashanti and Brong-Ahafo regions. Production of soybean meal ranked high with 51% of the total oilseed meal production whereas palm kernel cake contributed 25% of the amount. Estimated production quantities of the oilseed meals ranged between approximately 9,000MT and 66,000MT. These figures suggest that the investigated oilseed meals and or by-products are available in large quantities (>1000 MT) for animal feed production.

Keywords: Oilseed meals, animal feed, market availability

Introduction

Aquaculture is a fast growing sector in the area of food production worldwide. It is anticipated to contribute more than half of the global fish consumption by 2030 (NACA/FAO, 2000) therefore playing a significant role in scaling up food security while improving the livelihoods of many across the world, especially in developing countries. In Sub-Saharan Africa, fish is the cheapest and primary nutritional source of protein (Ben and Heck, 2005). African's aquaculture production in 2012 was 2.23% of the world's total (FAO, 2014). The projected world population of 8.5 billion people by 2030 (UN-DESA, 2008) implies an increase in the demand of fish. In order to meet its demand, Africa is expected to expand

its food production by 300% (Cocker, 2014). In order for African aquaculture to meet the high demand for fish due to its increasing population, Cocker (2014) recommends a shift from extensive to more intensive systems of food production to sustain its growth. Gabriel et al. (2007) asserted that fish feeds will play a substantial role in intensification of aquaculture. Despite its importance, fish feed technology and management is recognized as one of the least developed sectors in aquaculture in most developing countries (FAO, 2003).

Feed ingredients are the raw materials incorporated into a diet to formulate fish feed and are used in both commercial and farm-made feeds. Protein is regarded as the most expensive component in aquaculture feeds with fishmeal being the conventional source of protein in fish diets. Pike (2005) estimated that aquaculture utilised approximately 46 percent of the total fishmeal produced globally in 2002 as against piggery (24 percent) and poultry (22 percent).

Tacon and Hasan (2007) indicated that the dependence on fishmeal in aqua feeds as a source of high quality animal protein places a huge constraint on the animal feed industry. New (2002) advised that if new protein sources are not sought, there will be a keen competition for available fishmeal due to influence from market forces which will resultantly decrease production to feed manufacturers and consequently fish farmers.

New (2002) recommended that the alternative sources should be able to meet the nutritional requirements of cultured fishes, be economic and also able to sustain the intensification of aquaculture. Plant protein sources constitute a good portion of aquaculture feeds for fishes low on the trophic level and rank second after fishmeal as dietary protein source for high trophic fishes (Tacon et. al., 2011). Agbo et.al., (2011) stated that oilseeds and their by-products most often form majority of the dietary protein within feeds mainly because of their relatively high protein content and low cost. Oilseeds have very high oil content and are usually processed to obtain edible oils and/ or biodiesels these include rapeseed, canola, coconut, soybean, sesame, palm kernel, shea, etc. After extraction of the oil which is mostly the main product, the agro by-product becomes a potential raw material in manufacturing animal feeds. Oilseeds are the most studied ingredient sources with potential to replace fishmeal partially or totally (Tacon et. al., 2009). Cocker (2014) highlighted availability of ingredients as one of the vital factors to be considered in choosing ingredients for feed preparation. A lot of countries are endowed with diverse agro-industrial by-products which may not have human nutritional value yet have the potential to replace fishmeal in animal feeds. The emergent aqua feed industry in Ghana will therefore fuel the demand for fish feed ingredients in the form of agro-industrial by-products like soybean meal, maize, rice bran, wheat bran, cottonseed

cake; hence enlarging the crop sub-sector of agriculture which contributed 61.3% to the agricultural gross domestic product (GDP) of Ghana's economy (SRID, 2013). This study aimed at identifying sources of groundnut meal, groundnut husk, cottonseed meal, soybean meal, palm kernel cake and copra meal and to assess their production levels in Ghana.

Methodology

The study was conducted in Ghana located in West Africa at latitude 4º 44'N and 11º 11'N and longitude 3º 11' W and 1º 11'E and has a total land area of 238,539 square kilometers. With a population growth rate of 2.4% per annum, Ghana's population was 25.37 million in 2012. Ghana is divided into five major agro-ecological zones according to the Statistics Research and Information Directorate (SRID), 2013. The agro-ecological areas of Ghana were grouped into five main zones for data collection namely the Rain Forest, Deciduous Forest, Transitional Zone, Coastal Savanna and Northern Savanna (Guinea and Sudan Savanna). The agro-ecological zones are distinguished by natural vegetation and largely influenced by climate and soil characteristics.

Semi-structured questionnaires were used as a survey instrument to obtain information from respondents. Using a non-probability sampling method; the snowballing approach was employed and data was collected from a variety of primary and secondary sources. The primary source of data through questionnaire administration was from oilseed processors. The geographic location of all data collection sites were taken with the aid of a GPS device with an accuracy of 3.0m and a distribution map of identified sources was generated.

Annual production was estimated using cumulative calculations and represented as graphs and tables using Microsoft Excel.

Results

Sources of Oilseed Protein Meals

The study concluded with 91 sources of oilseed protein meals being identified across the country though it is suspected that there are many more processors especially small-scale operators. All the feed ingredients investigated except groundnut meal were regarded as agro-industrial by-products. The oilseed protein meals were found to be dominant in the agro-ecological zones where their respective oilseed crops are cultivated (Table 1). Cotton on the other hand, though cultivated in the northern savannah zones of Ghana, had no records of a processing mill that produces cottonseed oil and meal. However,

communication with some feed ingredients traders revealed that cottonseed meal is imported from Burkina Faso and Cote D'Ivoire. Groundnut cake was abundant in the Northern savannah zone with the Northern Region accounting for 65.84 % of the quantity. Production of palm kernel cake covered the Eastern, Western, Central, Ashanti and Greater-Accra Regions located within the rain forest, deciduous forest and coastal savannah agro-ecological zones. Medium to large scale producers were concentrated in the Eastern and Western regions and these Regions produced 57.65% and 32% of the total palm kernel cake respectively; while the Ashanti and Greater-Accra regions produced 8.4% and 2% correspondingly. Seventy-nine percent (79.7%) of copra meal was traced from the southern part of the Western region. A processing mill in Techiman located within the Transitional zone accounted for 76.9% of total soybean meal production. Two of the companies interviewed import soybean from Argentina while a copra meal producer in the Ashanti Region imports dried coconut from Cote D'Ivoire to supplement what is bought from the Western region. Figure 1 is a distribution map showing the sources of the various oilseed meals in Ghana.

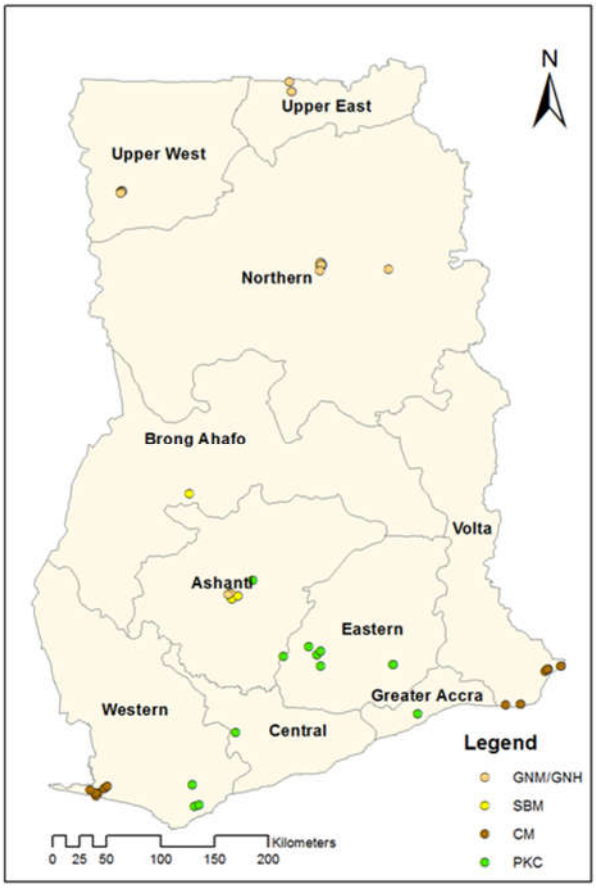

Figure 1. Distribution of Oilseed Meal Sources in Ghana

Table 1.Sources and Agro-ecological Zones of Oilseed meals in Ghana

Feed Ingredient	Source	
Oilseed Meal	**Agro-ecological Zone(s)**	**Town(s)**
Groundnut meal and Groundnut husk	Northern Savannah and Deciduous Forest	Navrongo, Kanvilli, Tagrayiri, Savelugu, Jisonaayili, Kambali, Duori, Wappani, Kpaguri, Zagyuri, Paga, Sang, Wa, Kumasi-Aboabo
Palm kernel cake	Rain Forest, Deciduous Forest, Coastal Savannah	Kwae, Juaben, Kade, Koforidua, Mpohor, Akyem Abaam, Akyem Brenase, Pretea, Kade Damang, Aboabo, Twifo-Praso, Adum Banso, Kumasi Roman Hill, Boadi, Atonsu Agogo , Ashiaman
Copra meal	Coastal Savannah, Deciduous Forest and Rain forest	Boadi**,** Atonsu Agogo, Ezinlibo, Allowuley, Bonyere, Kabenla Suazo, Nawule, Ohiamadwen, Sosuazo, Tikobo Number 1, Atorkor, Denu Nugorlikope, Fuveme, Agbozume; Ajevikope, Bayikor, Dzaglame, Vidzakope
Soybean meal	Deciduous Forest, Transitional Zone, Northern Savannah	Atonsu Agogo , Boadi, Kanvilli, Boankra, Abountem, Techiman
Cottonseed meal	-	Burkina Faso*

Local Production of Oilseed Meals

The production of oilseed meals in Ghana is done on small to large-scale. Processing of groundnut and coconut oils is predominately done on small scale (≤ 1 tonne/day), while soybean is processed on medium (2-30 tonnes/day) to large scale (>30 tonnes/day). Palm kernel processing is operated on all levels thus small, medium and large scales. An estimate of over 146,145 tonnes of the selected oilseed meals was produced in 2014/2015**.** Production of soybean meal ranked high with 51% of the total quantity whereas PKC contributed 25% of the amount. The largest palm kernel mill (Ghana Oil Palm Development Company) has a production capacity of 60 tonnes per day. The production of soybean meal varied significantly among processors as the highest daily production recorded was 150 tonnes and the least was 4 tonnes. Groundnut husk, a by-product obtained from the preliminary stages of groundnut processing such as roasting and winnowing in the production groundnut paste and or oil was mostly disposed off; making it difficult for processors to track its quantity. A ratio of 1:4 was used in estimating the quantity of groundnut husk produced from raw unshelled groundnut. No figure was estimated for the quantity of cottonseed meal produced locally as there was no evidence of a cottonseed processing mill or industry.

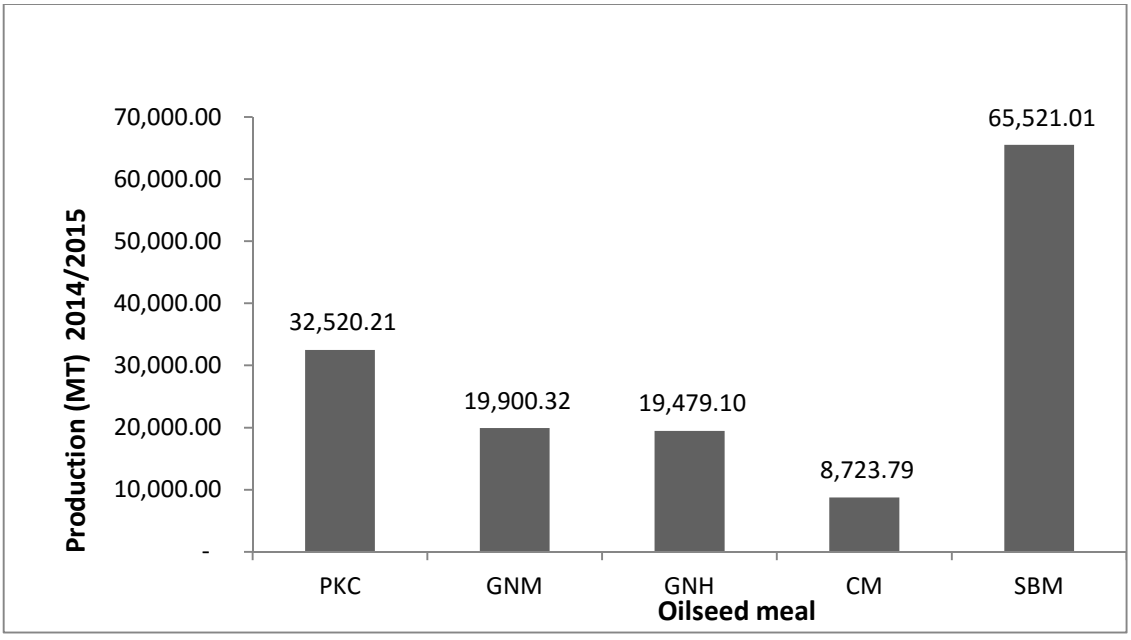

Figure 2.4 Production of the selected oilseed meals in Ghana (2014/2015)

Discussion

Sources of Oilseed Protein Meals (Availability)

Availability of oilseed meals varied from place to place. Oilseed meals were available in the agro-ecological zones where their respective oilseed crops are mostly cultivated with the exception of cottonseed meal and soybean meal. Groundnut for instance is grown in all the agro-ecological zones though bulk of the production almost 85% of the area under groundnut cultivation in Ghana emanates from the Guinea Savanna and Sudan Savanna zones in the Northern regions (Monyo and Laxmipathi, 2014). Soybean and cotton are widely grown in the three Northern regions of Ghana (USAID, 2012; Scholtes *et al.,* 2011) within the Northern (Guinea and Sudan) savannah zone. There have been futile attempts by some processors to organise soybean producers making them resort to importing from countries like Brazil, Argentina and the United States. USAID (2012) reported that roughly half of the soy used in Ghana in 2010 was imported from Argentina and Brazil as soybean meal or soybean oil.

An interview with a member of the Cotton Development Board revealed that though cotton is widely cultivated in Northern Ghana especially in the Upper West Region, no processing mill has been established. Furthermore, cottonseeds are exported to neighbouring countries mainly Burkina Faso where they are further processed into cottonseed oil and cake (pers.com 2015). Scholtes *et al.,* (2011) recommended that since there is no evidence of cotton by-product utilization, there should be some emphasis on promoting the potential of the sub-sector. It was predicted that processing cottonseed after ginning would provide income generation in Ghana and yield high returns for the economy. Nzema Dstricts of the Western region are predominantly coconut farmers, with over 30,000 ha, i.e about 80% of Ghana's coconut crop (Kyiamah, 2007)

Though over ninety sources were identified, there could be other oilseed processors that were not located and it is expected that most of them will be small scale operators. Dadzawa (2012) identified three other sources of oilseed by-products namely groundnut cake and husk, soybean meal and palm kernel cake in the Ashanti Region. This gives a premise in the use of oilseed by-products in fish feed as there are abundant sources across the country.

Local Production of Oilseed Meals

Soybean meal ranked as the highest locally produced oilseed meal in 2014/2015, a similar scenario observed in the global market (OECD, 2015). This may be largely due to the production capacity of large scale processor which produces 150 tonnes a day and employs the solvent method of extraction in its operation. Also soybean cultivation in Ghana has seen a significant growth the early 2000's due to numerous interventions to improve the production of the crop. Soybean production in Ghana has increased

from about 1,000 T in 1979 (Obirikorang et al., 2014) to 151709 MT in 2013 (SRID, 2013). There is an anticipated strong demand for protein meal which will likely drive further expansion of oilseed production worldwide. (OECD, 2015) proposed that this will result in a high contribution of the meal component to the overall oilseed yield and additionally support the expansion of soybean production in countries like Brazil. SRID (2013) recorded net surpluses of 116,580 MT for groundnut and 77,083 MT for soybean and total exports of 50 MT for both commodities. This presupposes that groundnut and soybean which are the key raw materials for processing are highly abundance and or available. However, groundnut's growth rate has over the years (1985–2007) declined in yield (0.7%) while increasing in production at 6.1% (Monyo and Laxmipathi, 2014). Soybean also dominated the protein meal market globally with a share of approximately 68% of the traded volumes in 2014 (USDA-FAS, 2015). This can be attributed to the high production of soybean compared to other oilseed crops since 1995 (Tacon et al., 2011). In a study by Dadzawa (2012) it was reported that groundnut husk accounted for more than half of the oilseed by-products used by fish farmers as supplementary feeds or as an ingredient in local feed formulation. By and large, a similar trend in rankings of the production of the selected oilseeds meals were also observed in the global production estimates; copra meal was the least produced followed by groundnut meal, palm kernel cake and the most produced oilseed worldwide was soybean meal (FAS/USDA, 2016).

Conclusions

The study reveals various sources where oilseed meals; groundnut meal, palm kernel cake, soybean meal, groundnut husk, and copra meal are produced in Ghana. However, particular meals were found to be dominant in the growing areas of the respective oilseed. No source of cottonseed meal was identified. Substantial quantities of the mentioned oilseed meals are produced above 8,000 tonnes annually. This implies that their usage in animal feeds will be sustainable seeing that these are produced in large quantities. On the contrary, the use of groundnut meal as an ingredient in the manufacturing of animal feed will not be sustainable as this will compromise food security. It was also realized that the main competitor for soybean meal is the poultry sector.

Recommendations

There are several investment opportunities in cottonseed and feed manufacturing sub-sectors to be harnessed in Ghana. The Cotton Development Board should liaise with investors to establish cottonseed processing mills in order to encourage the domestic production of cottonseed meal as it has a huge economic potential for the country. Ghana should tap into by-product production/ oilseed processing to

reap extra profit. Many other small scale producers are yet to be identified, however for Ghana's production of oilseed meals to reach international markets, there is the need for more mechanical equipment/ systems for production. There is also a huge potential in meeting market demand for oilseed meal in the animal feed sectors of the country and beyond. The Ministry of Food and Agriculture (MoFA) should facilitate the cultivation and processing of oilseed crops to increase their by-product utilization and boost the animal feed sectors in Ghana.

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GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF Clarias gariepinus JUVENILES FED FARM-MADE FEED

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Abstract

Clarias gariepinus juveniles (mean weight 35.77±1.2g) were stocked into twelve rectangular aquarium tanks at ten fish per tank and subjected to four treatments with each treatment replicated thrice. Three farm-made feeds were formulated at 40% CP, designated as J1, J2 and J3 being toasted soybean with poultry feed and bovine blood meal blend, cracked-and-cooked soybean with poultry feed and bovine blood meal blend and autoclaved soybean with poultry feed and bovine blood meal blend respectively. Coppens® aquafeed of 3mm diameter grains' size was used as Juveniles' Reference Diet (JRD). Fishes were fed these diets for a period of seventy days during which the growth response, nutrient utilization and economic performance were evaluated. The result showed that JRD had the best growth and nutrient utilization with SGR of 1.094±0.02, FCR of 1.219±0.03 and PER of 1.027±0.03, followed by J1 with SGR of 0.795±0.02, FCR of 1.877±0.06 and PER of 0.666±0.02, then J2 with SGR of 0.673±0.06, FCR of 2.337±0.26 and PER of 0.540±0.07, while the least values (SGR: 0.59±0.05, FCR: 2.749±0.31 and PER: 0.459±0.05) were recorded in J3. However, in the economic evaluation, the best performance in terms of Profit Index (PI) and Incidence of Cost (IC), were recorded in J1 (1.96, 0.41), followed by JRD (1.64, 0.49). For profit maximization, the toasted soymeal blend (J1), which had the best economic performance overall, with growth and nutrient utilization indices next to JRD, is recommended for feeding Clarias gariepinus juveniles.

Keywords: Clarias gariepinus juveniles, Farm-made, Growth, Nutrient utilization, Economic performance.

Introduction

Fishmeal had been the main source of animal protein in the aqua feeds industry because it has high protein content, supplies the appropriate amount of amino acids, it is highly palatable and possesses many other good qualities (Gatlin et al., 2007). However, due to its high demand, its cost is steadily increasing bringing about low returns to investment; and there is also general a scarcity of good quality fishmeal. Hence the need to explore other less expensive plant and animal protein sources to develop low cost farm-made feeds for the aquaculture feed industry. This paper reports the use of farm-made feeds in feeding juveniles of the African catfish, Clarias gariepinus.

The African clariid catfish, Clarias gariepinus (Burchell 1822) is one of the most commonly cultivated fish species in Nigeria. Fagbenro and Adebayo (2005) reported that about 35, 570metric tonne of fish was produced in Nigeria in year 2000 and was based mainly on tilapias and catfishes. Fagbenro, et al. (2005) and Fasakin et al. (2006) also reported that tilapias and catfishes are suited to low-technology farming

system because of their many qualities which include: ability to consume supplementary feed and natural aquatic food, resistance to diseases, fast growth rate, palatability etc.

Farm-made feeds can be defined as feeds in pellet or other forms, consisting of one or more locally available natural and / or artificial feedstuffs, put together in various proportions or ratio, and produced for the exclusive use of a particular farming activity (New et al., 1993). The formulae of farm-made feeds used by farmers or researchers for various cultivated species are diverse with farmers and researchers trying out various combinations and substitution in order to arrive at a feed that will bring about the best result in terms of production and economic gain.

Materials and Methods

Experimental Diets

Soybean was purchased from the Oba market, Akure, distributed into three treatment batches and processed in Federal University of Technology, Akure (F.U.T.A.) Fish Farm using three heat processing methods which include: toasting, crack-and-cook and autoclaving.

Toasting: This was carried out by putting sand in a heavy metallic frying pan to a depth of 40 cm and allowed to warm up on kerosene stove to 550C. At 550C the seeds were added and toasted for 20mins.

Cracking-and-cooking: Soybean was cracked into 2-5 coarse pieces/seed using a milling machine, the shaft was blown off, and the soybean was cooked for 45 minutes, oven-dried for 24 hours at 400C and kept for further use.

Autoclaving: This was done by steam-cooking the soybean at 15 Per Square Inch (PSI) in an autoclave for 15 minutes. It was oven-dried for 24hours at 400C and kept until use.

Fresh cattle blood was collected from the abattoir and processed to blood meal while broiler starter was purchased from a feedstuff market in Akure.,

Three farm-made feeds were formulated for the juveniles of Clarias gariepinus at 40% CP (Tables 1 & 2). The formulations were designated as juveniles-feed 1, 2, 3 (J1, J2 and J3, representing toasted soybean blend (TSB), cracked-and-cooked soybean blend (CSB) and autoclaved soybean blend (ASB) respectively). The feeds were pelleted using a Hobart A120 pelleting machine with a die size of 3mm, sundried at 36oC and kept till use. Coppens® aquafeed of 3mm diameter grains size was used as Juveniles Reference Diet (JRD).

Table 1: Farm – Made Feeds Design

J1	BS:TSB:BBM	1:1:0.50
J2	BS:CSB:BBM	1:1:0.48
J3	BS:ASB:BBM	1:1:0.50
	African catfish requirements*	40% protein, 10%lipid, 18.5 MJ/KG

* VanWeerd (1995)

Where: J1-J3=Farm-made feeds/ experimental juveniles diets: J1= toasted soybean meal blend,

J2= cracked-and-cooked soybean meal blend and J3=autoclaved soybean meal blend

TSB = Toasted soybean meal, CSB = Crack-and-cook soybean meal

ASB = Autoclaved soybean meal, BS = Broiler starter, BBM = Bovine blood meal

Experimental Set-up

Clarias gariepinus juveniles (mean weight 35.77±1.2) were procured and stocked in 12 rectangular glass tanks of dimension 75 x 45 x 30 cm each at 10 fish / tank. The fish were acclimated to experimental conditions for seven days before the commencement of the experiment. The treatments were replicated thrice and the experiment lasted for 70 days. The feed were fed to the fish at 5% of their body weight twice daily at 8.00-9.00h and 15.00-16.00h. Uneaten feed and feaces were siphoned out using a siphoning pipe two hours after feeding to minimize pollution.

During the experimental period, the fish were weighed in batches fortnightly, the weight recorded and the feed adjusted accordingly. Fish mortality was monitored and recorded daily.

Water quality parameters (temperature and dissolved oxygen (DO)) were measured fortnightly using combined digital DO and temperature meter (YSI model 57) while portable pH meter (Knick

Table 2: Ingredient Composition of Juveniles Diet (g/Kg)

	REFERENCE DIET (JRD)	FARM – MADE FEEDS J1	J2	J3
Toasted soybean meal (TSB)		400	-	-
Cracked-and-cooked soybean meal(CSB)		-	403	-
Autoclaved soybean meal(ASB)		-	-	400
Bovine blood meal(BBM)		200	194	200
Broiler starter(BS)		400	403	400
Total		1000	1000	1000
[1]Vit./Min. premix		50	50	50
Corn Starch		100	100	100

[1]Each kg contains: Vit. A: 4,000,000IU; Vit. B: 800,000IU; Vit. E: 16,000mg; Vit. K_3: 800mg; Vit. B_1: 600mg; Vit. B_2: 2,000mg; Vit. B_6: 1,600mg; Vit. B_{12}: 8mg; Niacin: 16,000mg; Caplan: 4,000mg; Folic Acid: 400mg; Biotin: 40mg; Antioxidant: 40,000mg; Chlorine chloride: 120,000mg; Manganese: 32,000mg; Iron: 16,000mg; Zinc: 24,000mg; Copper: 32,000mg; Iodine: 320mg; Cobalt: 120mg; Selenium: 800mg manufactured by DSM Nutritional products Europe Limited, Basle, Switzerland.

Portamess pH meter, Model 912) was used to measure pH. The water was changed weekly during the experimental period in order to minimize pollution.

Growth Performance Evaluation

Diet performance during the study was based on productivity indices of growth performance and nutrient utilization efficiency according to Steffens (1989) as follows:

i Weight gain = final weight of fish (W_2)-Initial weight (W_1)

ii Specific growth rate (SGR) = $\frac{\text{Log}_e \text{ final weight} - \text{Log}_e \text{ initial weight} \times 100}{\text{Rearing period (Days)}}$

iii Protein efficiency ratio (PER) = $\frac{\text{fish weight gain (g)}}{\text{Protein consumed (g)}}$

iv Feed conversion ratio (FCR) = $\frac{\text{weight of feed (g)}}{\text{Fish weight gain (g)}}$

Proximate analyses of ingredients, feeds and fish

Proximate analyses of the ingredients, feeds and that of the experimental fish before and after the experiment were determined in Fisheries and Aquaculture Technology (F.A.T.)Department Nutrition Laboratory, F.U.T.A. using the procedures described in AOAC (2000) to determine the moisture, crude protein (CP), lipid, ash, and crude fibre. Gross energy content was determined in F.A.T. Department Nutrition Laboratory, F.U.T.A. using a Ballistic Bomb Calorimeter (Gallenkamp& Co Ltd, Loughborough, England).

Economic Performance Evaluation

In order to determine an economic evaluation of each treatment, the following information was used:

1. Feed Cost/cost of 1kg feed: Cost of preparation of feed + ingredient based cost
2. Feeding Cost: Feeding fish in 12 glass tanks by one man/day at 15.00/day

   For 70days = 1,050.00

   For each treatment = 1,050/4= 262.50
3. Fish cost: cost of fish juveniles + transportation

   30.00/fish juveniles, 120 x 30.00 = 3,600.00

   Transportation cost for 120 fingerlings = 500.00

   Fish cost = 3,600.00 + 500.00 = 4,100.00; 1,025.00/treatment.
4. Cost per kg fish = cost of feed consumed x 1000/ weight gain
5. Profit index (PI= value of fish/cost of feed) and
6. Incidence of cost (IC= Cost of feed/kg of fish produced) models were also used (Vincke, 1969).

Statistical Analysis

Data obtained were subjected to one way analysis of variance (ANOVA) test ($p<0.05$) to determine the treatment means in the growth data. Duncan's multiple range test was used to characterize and quantify the differences between treatments using SPSS version 20 for Windows.

Results

Proximate Composition of Experimental Diets

Table 3 gives the proximate composition of the diets fed to *Clarias gariepinus* juveniles. The diets were formulated at 40% CP to meet the requirement of *C. gariepinus* juveniles (van Weerd, 1995). There were no significant differences ($p>0.05$) in the crude protein, gross energy and NFE contents in all the diets but significant differences ($p<0.05$) existed in the moisture, ash, lipid and crude fibre contents. All the diets were isocaloric (18.48-18.57 MJ/kg) and isonitrogenous (39.59-40.20% CP).

Table 3: Proximate composition (g/100g dry matter) of Experimental diets fed to *Clarias gariepinus* juveniles.

Parameters	JRD	J1	J2	J3
Moisture	8.30[b]	8.75±0.27[a]	8.71±0.63[a]	9.04±0.46[a]
Ash	9.70[a]	8.97±0.55[b]	7.81±0.50[c]	8.96±0.37[b]
Lipid	12.00[a]	10.06±0.03[b]	9.91±0.28[b]	9.32±0.09[c]
Crude protein	40.00[a]	39.62±0.85[a]	40.20±0.82[a]	39.59±0.98[a]
Crude fibre	2.50[b]	4.81±0.29[a]	5.22±0.16[a]	4.77±0.37[a]
NFE	27.50[a]	27.91±1.19[a]	28.27±0.82[a]	28.44±0.74[a]
Energy (MJ/kg)	18.51[a]	18.54[a]	18.57[a]	18.48[a]

Values are means of triplicate samples except for JRD and energy values.

Means along the same row followed by the same superscripts are not significantly different ($p>0.05$).

Growth Performance and Nutrients Utilization of *Clarias gariepinus* juveniles fed farm-made feeds

The effect of feeding farm-made feeds using differently processed soybean meal blends on the growth performance and nutrient utilization of *Clarias gariepinus* juveniles is presented in Table 4. There were no significant differences between the initial weights of fish for each experimental diet which means that the weights were similar so there was no bias introduced from the initial weights. There were significant differences ($p<0.05$) in all the parameters determined except initial weight and percentage survival. The Juveniles' Reference diet (JRD) had the highest value and differed significantly ($p<0.05$) from the other diets for the final weight (FW), weight gain (WG), average daily weight gain (ADWG), specific growth rate (SGR) and protein efficiency ratio (PER), but had the lowest value in feed conversion ratio (FCR) and also was significantly different from the FCR of other diets. This was followed by J1, then J2 and J3 in that order. There were also significant differences between the values obtained for J1 and the remaining diets but there were no significant differences ($p>0.05$) between J2 and J3 except in FCR values.

Table 4: Growth performance and nutrient utilization of *Clarias gariepinus* juveniles fed farm-made feeds.

Parameters	JRD	J1	J2	J3
Initial wt (g)	356.70±1.41^{a}	357.90±1.74^{a}	357.97±0.31^{a}	358.13±1.12^{a}
Final wt (g)	767.23±10.79^{a}	624.40±8.36^{b}	573.87±25.32^{c}	541.57±21.49^{c}
Weight gain (g)	410.53±11.90^{a}	266.50±8.76^{b}	215.87±25.93^{c}	183.43±20.84^{c}
Survival (%)	100.00±00^{a}	100.00±00^{a}	100.00±00^{a}	100.00±00^{a}
Av. daily wt gain	5.86±0.06^{a}	3.81±0.12^{b}	3.08±0.37^{c}	2.62±0.30^{c}
SGR	1.094±0.02^{a}	0.795±0.02^{b}	0.673±0.06^{c}	0.59±0.05^{c}
PER	1.027±0.03^{a}	0.666±0.02^{b}	0.540±0.07^{c}	0.459±0.05^{c}
FCR	1.219±0.03^{d}	1.877±0.06^{c}	2.337±0.26^{b}	2.749±0.31^{a}

Values are means of triplicate samples.

Means along the same row followed by the same superscripts are not significantly different ($p>0.05$).

Carcass Analysis of Experimental Fish

The whole body composition of *Clarias gariepinus* juveniles fed farm-made feeds is given in Table 5. There were significant differences ($p<0.05$) between the initial and final body compositions of fish during the experiment with respect to moisture, crude protein (CP) and lipid, but no significant variations ($p>0.05$) between the ash contents. All final body compositions had CP values higher than the initial value, fish fed J1 had the highest, followed by the fish fed J3, next was fish fed J2, but there were no significant differences ($p>0.05$) in the CP of fish fed all the diets. The Ash, lipid and moisture contents of the experimental carcass were all lower than that of the initial.

Table 5: Carcass analysis of *Clarias gariepinus* juveniles fed farm-made feeds

Parameters	INITIAL	JRD	J1	J2	J3
Moisture	10.12±0.30^{a}	6.92±1.20^{b}	4.98±0.31^{c}	6.14±0.32^{b}	6.02±0.78^{b}
Ash	14.00±0.19^{a}	13.72±0.25^{a}	13.50±1.55^{a}	13.12±0.79^{a}	13.00±2.44^{a}
Lipid	17.21±0.10^{a}	16.06±1.23^{b}	14.01±0.92^{c}	14.10±1.65^{c}	14.07±1.05^{c}
Protein	55.90±0.70^{b}	62.60±2.86^{a}	67.05±2.00^{a}	66.20±2.52^{a}	66.60±3.47^{a}

Values are means of triplicate samples.

Means along the same row followed by same superscripts are not significantly different ($p>0.05$).

Water Quality Parameters

The results of water quality parameters monitored in the experiment are presented in Table 6. Temperature, dissolved oxygen and pH values between 2 weeks interval for JRD ranged from 24.20ºC to 26.60ºC, 4.86mg/l to 6.42mg/l and 7.19 to 7.89 respectively; those for J1 ranged from 24.30ºC to 26.60ºC, 5.04mg/l to 6.49mg/l and 7.11 to 7.82 respectively; those for J2 ranged from 24.30ºC to 26.60ºC, 4.94mg/l to 6.53mg/l and 7.26 to 7.65 respectively; while those for J3 ranged from 24.40ºC to 26.60ºC, 5.11mg/l to 6.47mg/l and 7.26 to 7.83 respectively. The values for each diet were all within the range suitable for warm water fish species.

Table 6: Water Quality Parameters for *Clarias gariepinus* juveniles fed farm-made feeds

Parameters	JRD	J1	J2	J3
Temp (^{O}C)	25.68±0.006[a]	25.69±0.03[a]	25.71±0.01[a]	25.73±0.02[a]
DO (mg/litre)	5.54±0.09[a]	5.64±0.06[a]	5.63±0.11[a]	5.57±0.07[a]
pH	7.57±0.07[a]	7.47±0.03[b]	7.45±0.03[b]	7.49±0.03[ab]

Means along the same row followed by same superscripts are not significantly different (p>0.05).

Economic Analysis for *Clarias gariepinus* juveniles fed farm-made feeds

The cost analysis for feeding *Clarias gariepinus* juveniles is presented in Table 7. The result shows that cost of 1kg feed and cost of feed fed is highest for JRD followed by J3 and J2 and lowest in J1, while the feeding cost and fish cost are uniform for all the diets. Cost per kg fish is highest in J3, followed by J2, JRD and J1 in that order. Value of fish in naira and quantity of fish produced in grammes followed the same trend with the highest values recorded in JRD, followed by J1, J2 and J3 respectively. The profit index (PI) ranged from 1.10 to 1.96 with the highest value recorded for J1 followed by JRD, J2 and J3 respectively while the trend of values for incidence of cost (IC) is in the reverse order of PI values.

Table 7: Economic evaluation of *Clarias gariepinus* juveniles fed farm-made feeds

	JRD ( )	J1 ( )	J2 ( )	J3 ( )
Toasted Soybean Meal		98.00	-	-
Crack and cook Soybean Meal		-	103.36	-
Autoclaved Soybean Meal		-	-	148.00
Bovine Blood Meal		20.00	19.40	20.00
Broiler Starter		40.00	40.30	40.00
Vit./Min. Mix		50.00	50.00	50.00
Starch		10.00	10.00	10.00
1.Cost of 1kg feed	400.00	218.00	223.06	268.00

2. Feeding cost	291.67	291.67	291.67	291.67
3. Fish cost	1,025.00	1,025.00	1,025.00	1,025.00
4.Cost per kg fish	487.18	409.01	516.58	730.48
5.Cost of feed fed	200.00	109.00	111.53	134.00
6.Value of fish	328.42	213.20	172.72	146.75
7.Quantity of fish produced(g)	410.53	266.50	215.90	183.44
8. Profit Index(PI)	1.64	1.96	1.55	1.10
9. Incidence of Cost (IC)	0.49	0.41	0.52	0.73

N.B.: Market price of 1kg of fresh unprocessed catfish, *Clariasgariepinus* is averagely 800.00

Market price of 1kg unprocessed soybean is 120.00; Broiler Starter (BS) is 100.00/kg

Bovine blood meal (BBM) is 100.00/kg, Vitamin/Mineral mix is 1,000.00/kg

Starch is 100.00/kg.

Discussion

The proximate composition of experimental diets fed to *Clarias gariepinus* juveniles show that the experimental diets have values which are closely related with crude protein values ranging between 39.59% and 40.20% as recommended for *Clarias gariepinus* juveniles (van Weerd, 1995). Therefore, no bias has been introduced through the feed formulation and any differences in performance evaluation cannot be ascribed to dietary composition.

Apart from the fish fed the reference diet, fish fed on toasted soybean blend (J1) had the best productivity indices among the experimental diets; all the productivity indices (FW, WG, ADWG, SGR, PER) followed the same trend while the trend for FCR was the reverse of the others. This indicates that toasting made the nutrients in the soybean to be biologically available and nutritionally acceptable to fish. This is in agreement with the work of Adeparusi and Jimoh (2002) that toasting was the best processing method in eliminating antinutritional factors of lima bean but in contrast to Nwanna *et al*. (2005) who found cooking as the best processing method closely followed by toasting in terms of highest numerical total phosphorus, MWG, SGR, FCR, ADG and mineral liberation on the effects of different treatments of dietary soybean meal and phytase on the growth and mineral deposition in *Clarias gariepinus*. The next experimental diet in terms of performance was the cracked-and-cooked soybean blend (J2). This is in contrast to the work of Fagbenro *et al.* (2007) who found cracking and cooking method as the best method in eliminating the toxic thermostable antinutritional factors present in jackbean seeds, with the jackbean being able to provide 20% of the total dietary protein in *Oreochromis niloticus* diet when cracked and cooked in distilled water and 30% of the total dietary protein when cracked and cooked in trona solution. The autoclaved soybean blend (J3) had the least performance among the experimental diets, this could be an indication that autoclaving only resulted in partial removal of the antinutrients and the residual effect of the antinutrients resulted in poor utilization of the diet, also Fulmer (1989) reported that moist heat treatment caused lysine destruction and protein denaturation at a much faster rate than dry heat treatment, hence the better performance of the toasted soybean blend than the autoclaved blend. However, most of its productivity indices values (FW, WG, ADWG, SGR and PER) except FCR were not significantly different

($P>0.05$) from that of J2 but significantly differed ($P<0.05$) from productivity indices (FW, WG, ADWG, SGR, PER and FCR) of fish fed on J1 and reference diet while there is also significant difference ($P<0.05$) between that of J1 and the reference diet.

Values obtained for growth and nutrient utilization is an indication that the fish utilized the nutrient in the farm- made feeds well. This good performance could be attributed to the combination of different feed ingredients, in this case broiler starter, soybean meal and blood meal. It was reported by Onwudike (1981) that the complete replacement of fish meal with a combination of groundnut cake and blood meal did not significantly reduce egg production in laying hens and compared favourably with an all – fishmeal diet, also, Laporte *et al*., 2009 and Udo *et al*., 2012 opined that animal proteins can easily be combined with other feed ingredients with complementary amino acid profiles in order to match the nutritional requirements of a wide range of farmed species. Soybean meal and blood meal are known to be very efficient protein supplements in animal nutrition, and broiler starter is a commercial diet readily available at a low cost compared to fish feed. Agbebi *et al*., 2009 found that fish meal can be replaced completely (100%) by blood meal with no adverse effects on growth, survival and feed conversion of *Clarias gariepinus* juvenile.

Though high productivity values were obtained in fish fed on farm-made feeds, superior growth performance and best nutrient utilization were obtained in fish fed the commercial (reference) diet. The reference diet is known to be a fish meal based diet therefore its superior performance may be attributed to the high quality of fish meal used in the feed formulation. However, fishmeal is scarce and expensive, also, the use of wild fish from capture fishery to feed farmed fish places direct pressure on fisheries resources.

In the result of economic evaluation of feeding farm-made feeds to juveniles of *Clarias gariepinus* (Table 7) JRD had the highest value for quantity of fish produced in grammes and value of fish produced in Naira. However, J1, J2 and J3 had better economic values in terms of cost of 1kg feed and cost of feed fed as costs of production were minimized. This agrees with Akegbejo-Samsons and Fasakin (2008) who suggested that cost reduction in fish feed can be implemented by replacing fish meal with a combination of two or three protein ingredients such as blood, shrimp and maggot meals in a compounded ration. J1 has slightly higher PI and lower IC than the fish fed JRD which shows that J1 performed better economically than JRD and that cost could be reduced by feeding juveniles of *Clarias gariepinus* with farm-made feed made with toasted soybean and this saved cost could mean rewarding returns to investment and a means of making aquaculture operations sustainable as feeding cost is the highest singular investment cost in fish farming being as high as 70-80% in many cases. This is in agreement with the work of Rana and Hasan (2013) who reported that when farm made feeds were used, feed cost dropped from 70-80% to below 60% of total production cost irrespective of intensity of stocking or species stocked. For profit maximization, J1 which has the best economic performance and productivity indices (FW, WG, ADWG, SGR, FCR and PER) next to JRD is recommended for the feeding of *Clarias gariepinus* juveniles.

Farm-made feeds facilitate the use of locally available agricultural products and wastes of agro-processing industries that would otherwise have limited use within the community. In this respect, their use in farm-made feeds has significant environmental advantages. Farm-made feeds are also potentially cheaper than commercial aquafeeds.

Conclusion and Recommendations

The result of this study shows the efficacy of using farm-made feeds for feeding juveniles of *Clarias gariepinus.* No dietary related mortality or morphological symptoms of nutrient deficiencies were observed in the fish fed on the farm made diets; the efficient economic performance is an indication that farm-made feeds should be greatly considered in feeding juveniles of the African clariid catfish, *Clarias gariepinus* For profit maximization, J1 which has the best economic performance overall, and productivity indices (FW, WG, ADWG, SGR, FCR and PER) next to JRD is recommended for the feeding of *Clarias gariepinus* juveniles.

It is recommended that toasted soybean meal (TSB) farm-made feed be used for feeding juveniles of *Clarias gariepinus* in order to reduce cost of fish production and reduce pressure on natural fish stock in the ocean. TSB could also be produced on a commercial scale and made available to fish farmers for a more economically viable production.

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EFFECT OF FISHMEAL REPLACEMENT WITH OILSEED MEALS ON POSTPRANDIAL AMMONIA AND PHOSPHORUS EXCRETION RATES OF THE NILE TILAPIA, *Oreochromis Niloticus*

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Abstract

This experiment was conducted to evaluate the effect of substituting fishmeal with relatively cheaper and readily-available oilseed meal mixtures on the postprandial total ammonia nitrogen (TAN) and dissolved phosphorus excretion rates of Nile tilapia adults. Four experimental diets were formulated for this study with a fishmeal-based diet serving as the control diet. The three test diets (Diets 1, 2 and 3) contained different inclusion levels of oilseed meal mixtures (groundnut, cottonseed, copra and soybean meals) collectively contributing to approximately 80% of total dietary protein. Each diet was randomly assigned in triplicates to experimental tanks each containing 15 adult tilapia with total bulk weight of ~1kg. Feed was administered at 2% of total fish weight in a single meal after which water was sampled from each tank at 3-hour intervals over 24 hours. The fish group fed oilseed-based Diet 3 recorded the lowest cumulative TAN excretion rate of 106.14±1.95 mg kg^{-1}. The highest cumulative TAN excretion of 162.31 ± 4.39 mg kg^{-1} was recorded in the fish groups that were fed the control diet. Accumulated dissolved phosphorus in the closed systems at the end of the 24-hour sampling period were recorded to be 127.15±4.16 mg kg^{-1} for the control diet, 45.11±7.78 mg kg^{-1} for Diet 1, 43.61±2.08 mg kg^{-1} for Diet 2 and 32.94±1.09 mg kg^{-1} for Diet 3. Overall, the study showed the potential of minimising metabolic waste output using oilseed meal mixtures as replacements to fishmeal in Nile tilapia diets.

Key Words: Postprandial metabolism; Nile tilapia; Fishmeal substitution; Oilseed meals

Introduction

Global aquaculture production has increased markedly over the last few decades. Present estimates put the production figure around 90 million tonnes (FAO, 2014). Same can be said for Ghana which has had its output from aquaculture production experience significant growth over the last few years. The marked increment in the country's aquaculture production from 1000 MT in 2003 to 12,475 MT in 2014 can largely be attributed to the emergence of large-scale cage aquaculture farms in the early 2000s within the lower strata of the Lake Volta and on the lower Volta River. The increasing trend in world fish production has also led to an intense use of artificial diets in fish production systems (Cho, 1993). In Ghana, the intensification of commercial cage aquaculture on the Volta River over the last decade and the use of traditional local feed mixtures combined with commercial aquafeeds by most fish pond farmers have been the catalyst for the upsurge in factory-formulated aquafeeds (Obirikorang *et al.*, 2015a).

The high cost of commercial aquafeeds has been identified as one of the most important issues that inhibit the development of commercial and non-commercial aquaculture in the sub-Saharan Africa including Ghana (Hecht, 2007). Owing to this and the steady decline in the use of fishmeal within compound aquafeeds (FAO, 2012), large number of plant products have been evaluated as potential protein sources in diets for fish including cottonseed, groundnut and soybean meals (Agbo, 2008; Obirikorang *et al*., 2015b), *Leucaena leucocephala* leaf meal (Amisah *et al*., 2009), pito mash, rice bran, and groundnut bran

(Abarike *et al*., 2012) and palm kernel and copra meal (Obirikorang, 2015b). Most of these studies have, however, focused on their effects on diets digestibility and on fish growth with very little focus on their effects on waste excretion. Although the aquaculture industry presently aims at improving the efficiency of feeds, reductions in the amounts of waste excretions into the culture water medium is also very important (Fournier *et al.*, 2003).

The levels of nitrogen (N) and essential amino acid (EAA) in aquafeeds and the efficiency with which they are digested and assimilated by the target species influence the amounts of nitrogenous wastes that are excreted into the environment (Rodehutscord *et al.*, 1994). With studies into alternative fish feed ingredients to substitute fishmeal, particularly those originating from oilseed meals becoming a prioritized area of research, it is worth noting that these meals tend to have reasonably high crude protein contents and their essential amino acid (EAA) profiles are often unbalanced with the limiting amino acids generally being lysine, methionine and threonine (Jauncey, 1998). In cases where single or multiple amino acids are limiting, the amino acid requirements of the cultured species are not satisfied leading to oxidation or conversion of AAs, and an increase in ammonia excretion (Médale *et al*., 1998; Robaina *et al*. 1999; Yiğit *et al*., 2005). Conversely, dietary EAA supplies that exceed the optimum requirements for tissue growth and maintenance cannot be stored in the fish and thus usually oxidised resulting in the production of nitrogenous waste products, of which ammonia is approximately 80-90% in fish (Kaushik and Cowey, 1991; Bureau, 2004). Appropriately balanced diets tailored to the nutritional requirements of the target species will thus significantly reduce the amounts of these nitrogenous excretions into the culture environment (Hasan, 2001). According to Cho and Bureau (1997), reductions in excreted dissolved wastes such as total ammonia-nitrogen (TAN) are key factors for the long-term sustainability of aquaculture globally. With the global concern of reducing water pollution, the reduction of phosphorus excretion by fish is becoming imperative for fish food industries (Rodehutscord *et al.*, 2000). Studies on replacing fishmeal with plant protein sources have often resulted in reductions in phosphorus excretion rates in fish without compromising growth performance (Ketola and Harland, 1993; Castro *et al*., 2011).

The present study was thus conducted to examine metabolic excretion rates following the ingestion of diets containing different oilseed meal matrices, with the ultimate aim of assessing the practicability of their use as raw materials in cost-effective diets for Nile tilapia. Specifically, this study investigated the effects of substituting fishmeal with combinations of different oilseed meals (soybean, groundnut, cottonseed and copra meals) in feeds for Nile tilapia (*Oreochromis niloticus*) with respect to postprandial ammonia and phosphorus excretion rates.

2. Methodology

2.1 Experimental Diets

Four diets were formulated out of which one served as the control diet, having fishmeal as the main protein source. The three test diets were formulated to contain different inclusion levels of oilseed meal mixtures (groundnut, cottonseed, copra and soybean meals) collectively contributing to approximately 80% of total dietary protein. Palm oil and wheat bran were added to the diets to serve as lipid and carbohydrate sources respectively.

All basal ingredients used in the feed formulation were finely-ground, sieved and weighed out according to the specified formulations (Table 1). Vitamin and mineral premixes were added to each diet and gelatinized cassava flour served as binder. Pelletization was carried out with a meat grinder fitted with a 2 mm die plate and subsequently oven-dried at 80°C for 24hours.

Table 1: Diet formulation (g.kg^{-1}) and proximate composition (% dwb) of reference and test diets

Ingredients	Control Diet	Diet 1	Diet 2	Diet 3
Fishmeal	384.00	70.50	92.60	22.00
Cottonseed meal	-	120.60	193.90	284.00
Soybean meal	-	235.10	33.00	102.70
Copra meal	-	370.10	50.10	78.00
Groundnut meal	-	36.00	10.36	179.70
Wheat bran	525.00	115.60	37.60	280.60
Starch (binder)	20.00	20.00	20.00	20.00
Palm oil	56.00	17.10	3.30	18.00
Vitamin and Mineral premix	15.00	15.00	15.00	15.00
Proximate Composition (%)				
Dry matter	91.17	91.15	91.33	93.36
Crude Protein	30.68	30.15	29.40	30.44
Crude lipid	11.75	11.71	11.95	11.17
Ash	9.17	7.44	7.86	5.44
NFE	34.44	36.79	36.28	40.03
Phosphorus	2.58	1.01	1.27	0.89
Gross energy (kJ/g)	17.80	18.07	17.90	18.48

2.2 Proximate Analysis of Feed

Proximate analysis of dry matter, ash and the macronutrients (crude protein and lipid) in the experimental diets were determined according to the procedures of the Association of Official Analytical Chemists (AOAC, 2005). Dry matter was determined by oven drying the feed samples at 105°C for 24 hours. Ash content was calculated from the weight loss after incinerating the feed samples in a muffle furnace at 550°C for 6 hours. Dietary crude protein was determined by the Kjeldahl method (Foss Kjeltec 2200). Crude lipid was determined following the method of Bligh and Dyer (1959). Dietary phosphorus was determined following the ammonium molybdate method outlined by Stirling (1985).

2.3 Experimental Units

The trial was conducted in a modified recirculating system (Cho and Slinger, 1979) consisting of twelve 150 L, cylindro–conical PETG thermoplastic tanks and a reservoir tank inside a 10.7m long shipping container. Water supply to each tank was maintained at a flow rate of 2.5 L min^{-1} by means of controlled taps connected to each tank. A relay-controlled porcelain 1.0kW porcelain heater (Jevi A/S, Denmark) submerged in the reservoir tank was used in maintaining the water temperature at approximately 25°C. The reservoir tank was also fitted with biofilters and an air pump (SIBO BV V-30, Nerthelands) to provide aeration. Dissolved oxygen concentration in each tank was kept above 70% concentration at all times by aerating with air stones connected to an external air pump (HIBLOW HP-40, Japan). A 12-hour illumination and 12 hour darkness was achieved by means of a 7W led lamp overhead each tank.

2.4 Fish Husbandry

All-male Nile Tilapia were obtained from the Aquaculture Research and Development centre (ARDEC) at Akosombo, Ghana and transported to the holding facility at the Faculty of Renewable Natural Resources' farm at Kwame Nkrumah University of Science and Technology, Ghana. At the start of the experiment all 12 experimental tanks were stocked with 15 individuals with bulk weight of ~1kg (mean weight 66.5±1.02g). The start of the TAN and phosphorus excretion trial was preceded with acclimating the fish to the experimental facility and feeding them with the experimental diets for 10 days. During this period, daily rations of the diets corresponding to 4% of the total fish body mass were divided in two equal portions, and administered to the fish at 10:00 and 15:00 h respectively.

2.5 Total Ammonia–Nitrogen (TAN) and Phosphorus Excretion Rates

This experiment was carried out in the week following the acclimating period. Food was withheld for 24 hours prior to water sampling to ensure that any food is completely removed from the guts of the fish. The experimental tanks were thoroughly cleaned to take out all faecal and organic material. Water flow to each tank was stopped throughout the 24-hour sampling period to ensure no water exchange. Dissolved oxygen levels in each tank were, however, kept at >70% at all times during the sampling period. The experimental diets were then administered at a rate of 2% of total fish weight in a single meal. Water samples for postprandial TAN and phosphorus analysis were taken at 3-hour intervals for 24 hours with an initial sampling carried out 3 hours prior to administering the dietary treatments. Approximately 20 ml of water samples were collected from each tank and immediately analysed for total ammonia nitrogen and phosphorus concentrations in duplicates. Total NH_3-N excretion rates for each treatment group were calculated using the ammonia and dissolved phosphorus produced in each experimental tank after each sampling period by the formula for a static system as described by Frisk *et. al* (2013):

$$TAN/P = \frac{(N_2 - N_1) \times V}{T \times M}$$

Where,
N_1: initial TAN/P concentrations in water (mgL^{-1})
N_2: final TAN/P concentrations in the water (mgL^{-1})
V: the water volume (l) in the tank during the sampling periods
T: the time (h) between the sampling periods
M: is the bulk mass of fish (kg)

2.5.1 Determination of Total Ammonia-nitrogen

The analysis of total ammonia-nitrogen in water samples collected from each experimental tank was carried out using a Wagtech 7100 photometer**.** The indophenol method was employed in the analysis of following standard procedures outlined by Eaton *et. al* (2005). This method allowed ammonia in the water to react with alkaline salicyclate in the presence of chlorine to form a green-blue indophenol complex, and the intensity of the colour produced was considered proportional to the ammonia-nitrogen concentration which was read at 640nm.

2.5.2 Determination of Dissolved Phosphorus

Phosphorus determination was based on the vanamolybdate method, whereby phosphates in the water samples reacted with ammonium molybdate in the presence of ammonium vanadate to form a yellow phosphovanadomolybdate. The resultant colour intensity which is proportional to the phosphate (PO_4) concentration was read at 490nm with a Wagtech 7100 photometer. Total phosphorus concentration was calculated by multiplying PO_4 values by a factor f 0.33.

2.3 Statistical Analysis

One-way analysis of variance (ANOVA) was used to test for the effects of the experimental diets on ammonia-nitrogen and phosphorus excretion rates of Nile tilapia. The Tukey multiple comparison test was further used to determine which treatments differed when significant effects of the diets were observed. Differences were considered significant at $p<0.05$. All graphs and statistical analyses were executed using SigmaPlot ver. 12.0 (Systat Software, Inc)

3. Results

3.1 Postprandial Total Ammonia-Nitrogen Excretion Rates

The accumulated total ammonia-nitrogen excretion rates of the fish groups fed the experimental diets were significantly different ($p=0.0075$) at the end of the 24-hour period. The fish group fed Diet 3 recorded the lowest daily TAN excretion rates of 106.14±1.95 mg kg^{-1}. The highest cumulative TAN excretion over the 24 hours period of 162.31 ± 4.39 mg kg^{-1} was recorded in the fish groups that were fed the fishmeal-based control diet. The cumulative TAN excretion rates of the fish groups fed Diets 1 and 2 were 128.54±1.46 mg kg^{-1} and 144.04±6.82 mg kg^{-1} respectively. Despite the significant differences in TAN excretion rates, excretion patterns were fairly similar among all dietary treatments with peak TAN excretion rates occurring 6 hours after meal ingestion. The Diet 2 group showed the highest mean peak excretion rate of 27.89 ±2.82mg kg^{-1} hr^{-1}, while the Control group showed the lowest peak TAN excretion rate of 17.32 ±0.57mg kg^{-1} hr^{-1} which was significantly lower ($p<0.05$) compared to groups fed Diets 2 and 3. However, the TAN excretion rate for the Control group did not differ significantly ($p>0.05$) from groups fed Diet 1.

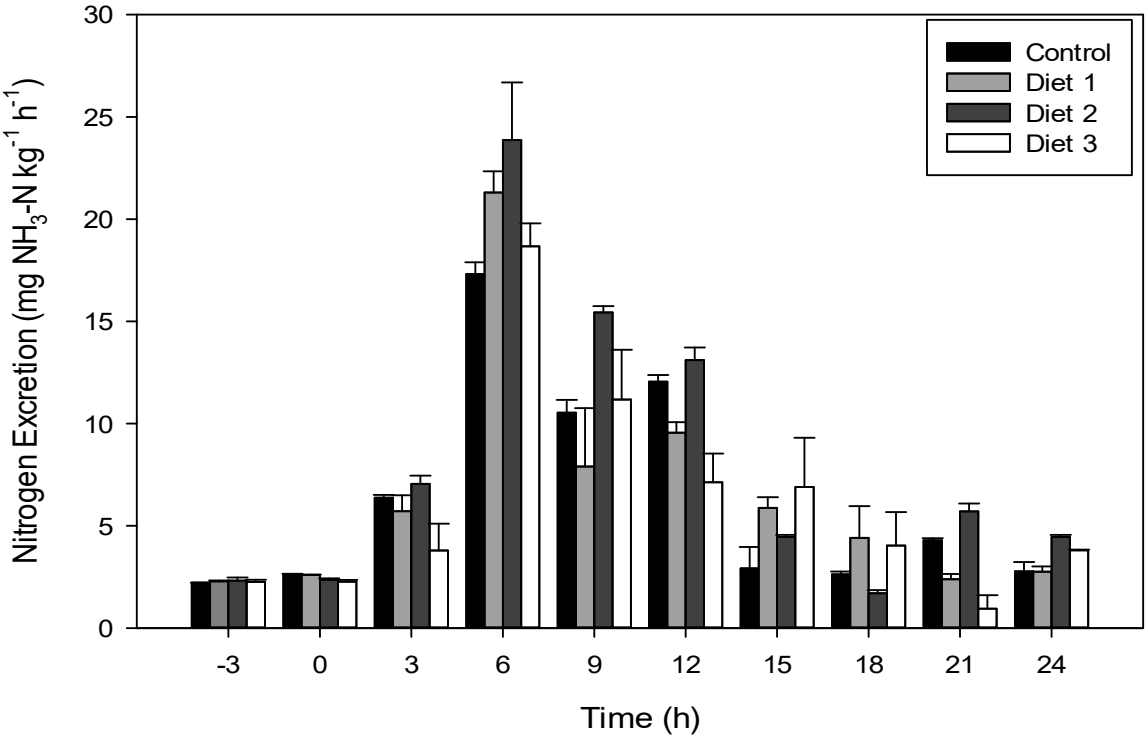


Fig.1: Means and ±SD of postprandial excretion rates in *O. niloticus* fed diets containing different plant protein matrices

The ammonia-nitrogen excretion rates were again plotted as percentages of consumed dietary nitrogen (N) over a 24-hour post-feeding period (Figure 2). Expressed as percentages of the N intake in the single administered meal, total ammonia excretion rates of the Control, Diet 1, Diet 2 and Diet 3 varied significantly ($p= 0.0008$) and were calculated to be 42.27, 33.47, 37.51 and 27.64±0.51% respectively.

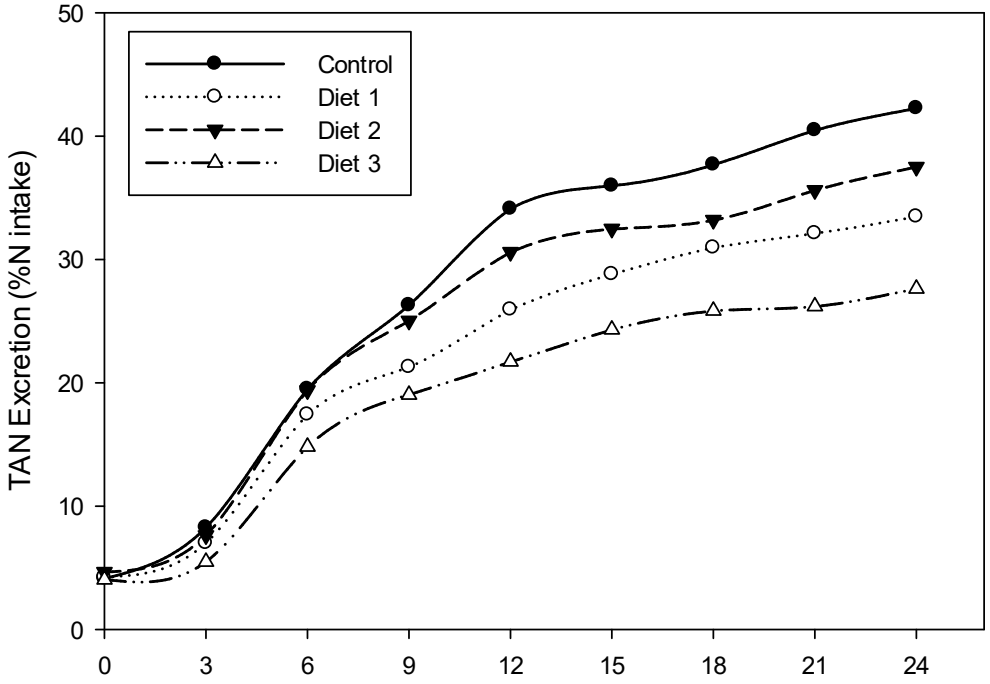
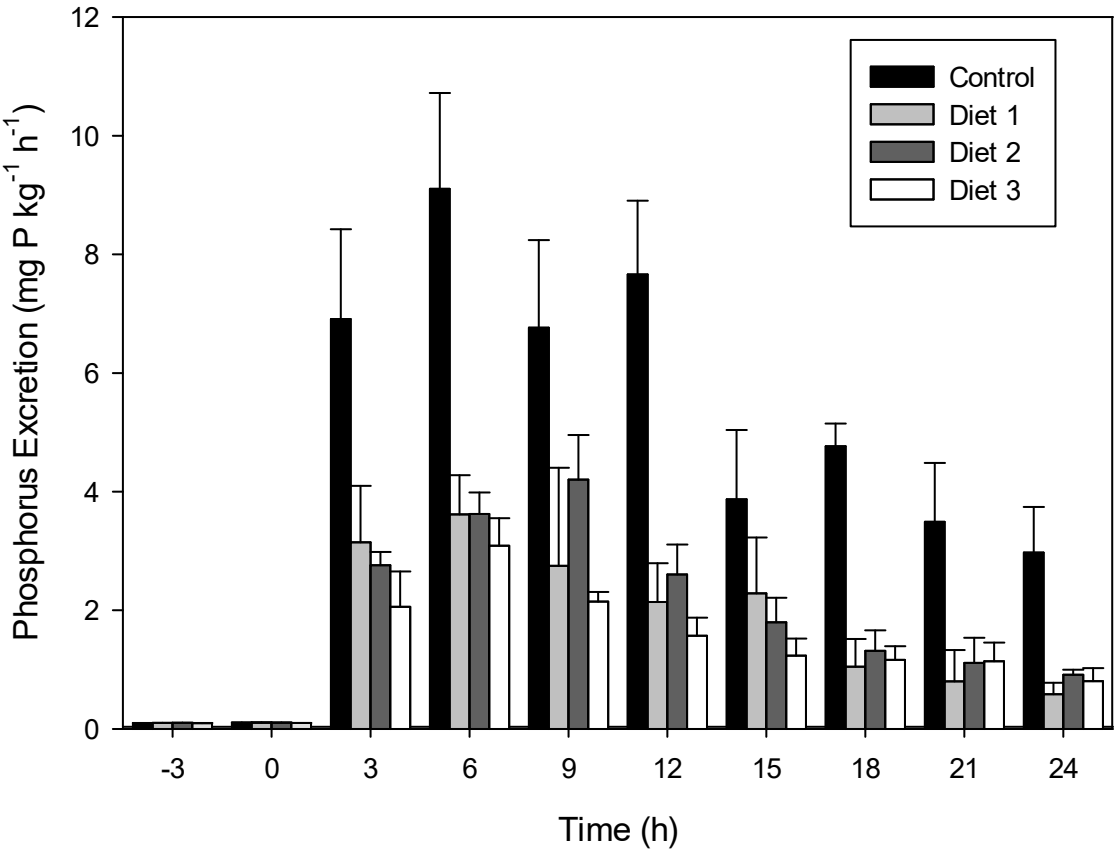


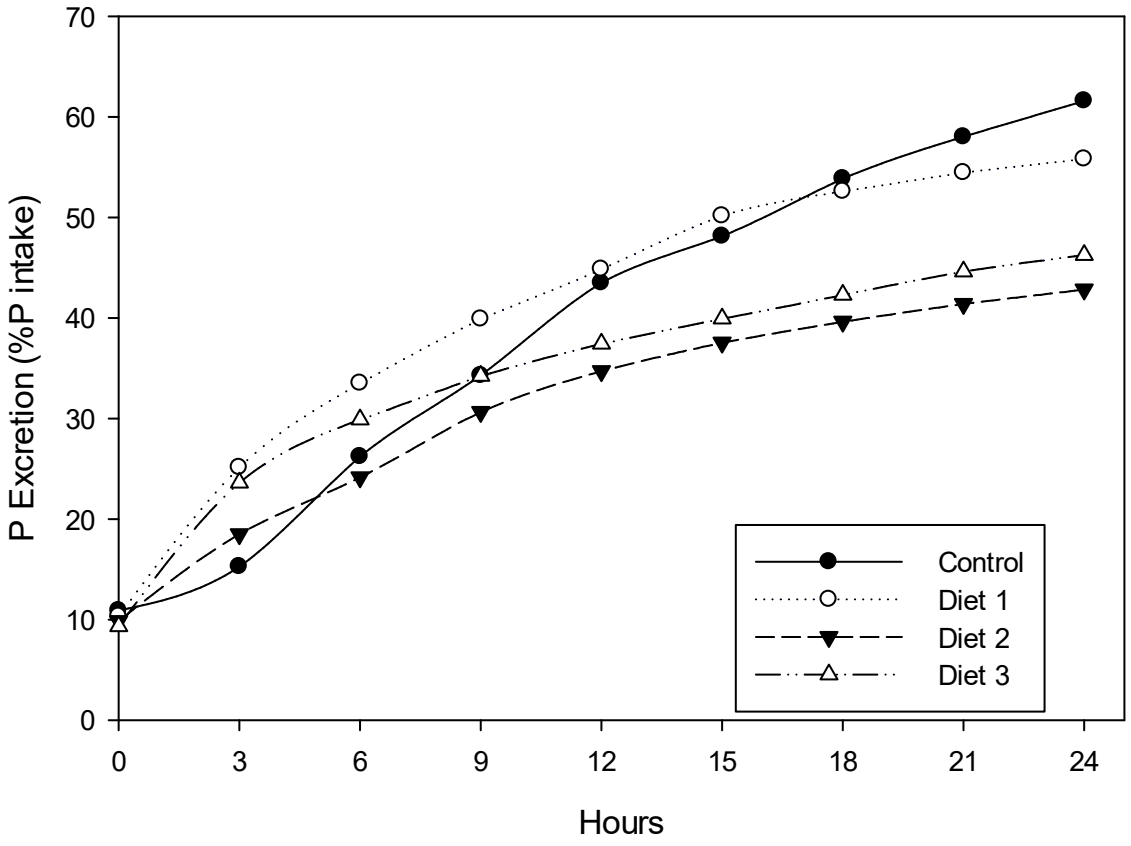
Fig.2: Cumulative TAN excretion rates by *O. niloticus* as a proportion of ingested dietary N

3.2 Postprandial Dissolved Phosphorus Excretion Rates

Pre-feeding dissolved phosphorus levels were similar in all the experimental tanks and varied between 0.09 and 0.11 mgL^{-1}. Phosphorus levels recorded in all the treatment tanks 3 hours post-feeding were significantly higher ($p<0.0001$) than the pre-feeding levels. The fishmeal-based control diet elicited the highest postprandial phosphorus excretion rates among the various dietary treatments. The additions of the oilseed meals resulted in significantly lower ($p<0.0001$) phosphorus excretion rates in the fish groups that were fed the plant-based diets (Figure 3). At the end of the 24-hour sampling period, accumulated dissolved phosphorus in the closed systems were recorded to be 127.15±4.16 mg kg-1 for the control diet, 45.11±7.78 mg kg-1 for Diet 1, 43.61±2.08 mg kg-1 for Diet 2 and 32.94±1.09 mg kg-1 for Diet 3. With the exception of Diet 2 that recorded peak phosphorus excretion 9 hours post-feeding, all the dietary groups recorded peak phosphorus excretion rates 6 hours post-feeding.



At the end of the 24-hour sampling period, the accumulated phosphorus in each closed system was plotted against the ingested phosphorus in their respective administered dietary treatment to estimate the proportion of ingested phosphorus that is excreted as dissolved phosphorus via fish urinary or brachial systems (Figure 4). After 24 hours, 61.60% of the ingested phosphorus in the control diet was excreted. Lower proportions were recorded for the oilseed meal diets with Diets 1, 2 and 3 recording 55.83, 42.84 and 46.26% respectively.



4.0 Discussion

4.1 Total Ammonia Nitrogen Excretion Rates

With the experimental diets being isonitrogenous, the amino acid composition of the diets may offer an explanation as to why inclusion of the oilseed meals resulted in significant reductions in TAN excretion rates. According to Cowey and Walton (1988), amino acid catabolism of ingested feeds can contribute to as much as 90% excreted nitrogen in fish. It is thus very probable that the recorded differences in TAN excretion rates could have been due to differences in amino acid profiles. Although imbalances or mismatch in availability of dietary essential amino acids (EAAs) may also elicit a facultative postprandial energy expenditure to maintain nutritional homeostasis (Fu and Xie, 2004), absorbed dietary amino acids in excess of those required for growth and maintenance in fish are catabolised and degraded resulting in the production of nitrogenous waste products, principally ammonia. Unlike fishmeal which meets all the EAA requirements of Nile tilapia, the oilseed meals used in this study are limiting in multiple EAAs (Agbo, 2008; Obirikorang, 2015b). This is possibly why the fishmeal based control diet elicited the highest post-feeding TAN excretion rates in Nile tilapia.

The recorded peak TAN excretion rates 6 hours after feed administration for all the experimental diets fed to the *O. niloticus* in this study, falls well within the ranges reported for Nile tilapia other fish species in other studies (Engin and Carter, 2001; Lam *et al*., 2008; Obirikorang, 2015b).

4.2 Phosphorus Excretion Rates

Digestibility and dietary quantities of phosphorus appeared to be the factors that affected the phosphorus excretion rates in the different dietary treatments. In terms of dietary levels, the fishmeal control diet had the highest phosphorus levels of 2.58%, which was more than twice the dietary phosphorus levels of any of the test diets. The phosphorus requirement of tilapia has been estimated to be around 0.5% (NRC, 1993) implying that phosphorus requirements of the target species in this study was exceeded. According to Bureau and Cho (1999) and Reid and Moccia (2007) the excess supply of dietary phosphorus will result in elevated excretion rates as soluble orthophosphate *via* the urinary or brachial pathways. It is thus very likely that the excessive dietary level relative to requirement of the control diet resulted in the significantly higher excretion rates in the fish groups fed that diet. Fishmeal is known to have very high levels of bioavailable phosphorus like most animal protein sources, such as meat and bone meals and its partial replacement vegetable sources tends to reduce dietary phosphorus levels as well as excretion rates in fish (Cromey *et al*., 2010).

Oilseed meal sources possess a large proportion of their phosphorus in the phytate form, which is unavailable for the fish because they do not possess the enzyme phytase to break down phosphorus in that form (NRC, 1993). The bio-available phosphorus in soybean meal has been reported to be between 29 and 54% (Wilson *et al*., 1982). Agbo (2008) reported phosphorus digestibility of 53% for cottonseed meal. It is thus very possible that besides the lower dietary levels of phosphorus in the oilseed diets relative to the fishmeal control diet, the non-availability of significant portions could have accounted for the lower phosphorus excretion rates in the fish groups fed the oilseed meal diets. According to Bureau and Cho (1999), approximately two-thirds of the phosphorus in plant ingredients is present as phytate-phosphorus, with the remaining portion present as inorganic salts or other compounds that are bioavailable to fish. It is also possible that the high replacement of fishmeal with the oilseed mixtures resulted in diets with low digestibility which in turn could have resulted in low dissolved phosphorus as was observed in this study. Indigestible phosphorus such as phytate-bound phosphorus is egested in faeces (Bureau and Cho, 1999; Reid, 2007) and this is usually not recorded in the water like metabolic phosphorus that is mostly excreted mostly as phosphate via urine (Bureau, 2004).

Conclusions

Under the experimental conditions, the plant protein mixtures appear to be promising candidates as fishmeal replacers in Nile tilapia diets as far as reducing ammonia nitrogen and phosphorus excretion rates are concerned. The inclusions of the oilseed meals resulted in as much as 25% and 35% reductions in dissolved phosphorus and total ammonia nitrogen excretion rates respectively relative to the fishmeal control diet.

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