GROWTH PERFORMANCE OF *Clarias gariepinus* JUVENILES FED PROCESSED ALMOND (*Terminalia catappa* LINNAEUS) KERNEL MEAL

BY

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ABSTRACT

Increasing cost of feedstuff due to competition between livestock and human necessitates research into Low-Cost Unconventional Feedstuffs (LCUF) for profitable fish farming. Almond kernel has been reported to be nutrient-rich feedstuff for livestock; however information on the potential of almond (*Terminalia catappa*) as a LCUF has not been well documented in aquaculture. Therefore, the use of almond kernel meal as a replacement for soybean meal in the diet of *Clarias gariepinus* was investigated.

Chemical compositions of Soaked almond kernel meal (SOAM), Boiled almond kernel meal (BOAM), Roasted almond kernel meal (ROAM), Mechanically extracted almond kernel meal (MEAM), Solvent (petroleum spirit) extracted almond kernel meal (SEAM) and Raw almond kernel meal (RWAM) were determined using standard procedures. Crude protein digestibility of the meals was conducted using *C. gariepinus* juveniles (n = 420, 9.15±0.08 g). Two meals with highest crude protein digestibility were used for nutrient utilisation and growth studies. Each of these meals was used to replace soybean meal at 0.0, 25.0, 50.0, 75.0 and 100.0% in formulating isonitrogenous (40% crude protein) diets. The diets were fed to *C. gariepinus* (n = 600, 12.02±0.04 g) twice daily at 3.0% body weight for 105 days. Mean weight gain (MWG), Specific growth rate (SGR) and Feed conversion ratio (FCR) were calculated. Blood (5mL) was sampled from the fish for Packed Cell Volume (PCV), White Blood Cell (WBC), Plasma protein and albumin determination using standard procedures. Histopathological evaluations of the liver and kidney were carried out using standard procedures. Data were subjected to descriptive statistics and ANOVA at $\acute{\alpha}_{0.05}$.

Crude protein values significantly varied from 24.1% in BOAM to 35.4% in SEAM. Crude fibre ranged from 3.3% (ROAM) to 3.8% (SEAM). Phosphorus (0.5%) and calcium (0.3%) were highest in ROAM, while lowest values of phosphorus (0.2%) and calcium (0.2%) were in RWAM. Phytate ranged from 0.04% (ROAM) to 0.09% (RWAM), while tannin ranged from 0.03% (ROAM) to 0.07% (RWAM). Fish fed ROAM had highest crude protein digestibility (89.0%) followed by MEAM (88.9%) while the least value (87.1%) was recorded in fish fed SEAM. High MWG (69.0±2.9g; 68.5±1.5g), SGR (1.82%; 1.81%) and least FCR (1.54±0.05; 1.53±0.03) were obtained in fish fed 75.0% ROAM and MEAM, respectively, while the lowest values of MWG (27.0±0.9g), SGR (1.12%) and highest FCR (2.1±0.2) were obtained in fish fed 100.0% ROAM. Elevated PCV

(28.0%) was obtained in fish fed 50.0% MEAM inclusion, while least PCV (23.0%) was obtained in fish fed 100.0% ROAM. The WBC significantly varied from 10.3±0.05x10^6/µl to 11.8±0.01x10^6/µl in fish fed 0.0% MEAM and 100.0% ROAM, respectively. Fish fed 50.0% MEAM had significantly higher plasma protein (4.8±0.1g/dL) and albumin (2.9±0.1g/dL), while significantly lower levels of plasma protein (3.4±0.01g/dL) and albumin (1.8±0.1g/dL) were obtained at 100.0% ROAM. Vascular fibrosis of the liver and tubular necrosis of the kidney were observed in fish fed ROAM and MEAM at inclusion levels above 75.0%.

Roasted almond kernel meal could replace soybean meal up to 75.0% in *Clarias gariepinus* diet. Growth depression and health impairment could result from replacement beyond this level.

Keywords: *Terminalia catappa*, Soybean meal, *Clarias gariepinus*, Crude protein digestibility.
Word count: 500

CERTIFICATION

I certify that this work was carried out by Mr. Kalu Okorie ELEZUO in the Department of Aquaculture and Fisheries Management, Faculty of Agriculture and Forestry, University of Ibadan, Nigeria.

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DEDICATION

This work is dedicated to the loving memory of my parents, Late Ambassador Chief and Elder (Mrs.) Agwu, Okorie Elezuo who were great sources of inspiration and encouragement to me in my educational pursuit but passed on few months to this glorious moment. May God grant their souls eternal rest, Amen.

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CHAPTER ONE

1.0 INTRODUCTION

Aquaculture is the cultivation of aquatic organisms, which include fish, mollusks, crustaceans, amphibians and algae in enclosed water bodies. Although its origin dates back to 475 BC, fish farming started in Nigeria in the 1950s (Omitoyin, 2007). Fish contributes over 40% of total dietary protein consumption of Nigerians and is a staple source of protein of the average Nigerian diet (Ajani et al., 2011). It is relatively cheaper than most other types of animal protein (Eyo et al., 2003.). It is also a rich source of essential amino acids (EAAs) such as lysine, leucine, valine, tryptophan, histidine and methionine suitable for complementing high carbohydrate diet (Falaye, 2013).

Fish is a very rich source of Omega-3 Poly-Unsaturated Fatty Acids (PUFAs) known to be useful in preventing cardiovascular disorder and cancer (AHA, 2002). Recent studies revealed that regular consumption of fish with Omega-3 fatty acids may lower the risk of age related macular degeneration which causes vision impairment (Bressier et al., 1988; Michels and Kurz-Levin, 2009). Furthermore, fish contains water soluble vitamin (B complex), fat soluble vitamin A and D and minerals such as iodine, iron, phosphorous and calcium which have positive effects on human health. Fish oil significantly lowers blood pressure (AHA, 2002). Fish play a major role in both the diet and the economy of the rural poor in the developing countries (FAO, 1994).

FAO (2010) states that 925 million people are undernourished and nearly all of the undernourished populace are in developing countries. To prevent malnutrition, there is need to increase protein supply particularly of animal origin in the diet of the Nigerian populace. Recent trends all over the world, including Nigeria, point to a decline in fish production from capture fisheries. This is an indication that fish stocks have been exploited at full capacity or even over exploited. Fish farming therefore remains the only viable option for increasing fish production to meet the protein need of Nigerians and the world at large.

Aquaculture has gained more popularity in recent years than it was decades ago in terms of yield nationally and globally. Yields have increased by 10% per annum throughout the past decades

(FAO, 1994). This accounts for 30% of the world's food fish production. As fish farming continues to expand, it has been noted that certain constraints are impeding the realization of the desire to solve the malnutrition problem in Nigeria. The major constraints are lack of sufficient good quality fish seed and inavailability of cost effective nutritive fish feeds (Falaye, 1998).

Feed is estimated to account for more than 60-70% of total cost of fish production (Falaye, 1992). Fish meal has been utilized as a main protein component in balanced diet for fish (Bekibele, 2005), and is the single most expensive fish feed ingredient which makes up over 50% of the total cost of feed (Ekelemu et al., 2000). Fishmeal is especially rich in the essential amino acids (EAAs) and is highly digestible for fish (Falaye, 1998). However, it is mostly imported, its price is exorbitant thus this makes the cost of finished fish feed to be high. In addition, the competition for fish and fish products by man and the livestock industry necessitated a search for alternative source of protein that is cheap, of good quality and not competed for like fish.

Soya bean meal has been successfully used to partly replace fishmeal in aqua feed to produce cheaper fish feed in Nigeria. Recently, due to the increased alternative use of soya beans as food for man coupled with its competitive use by livestock feed industries and its export potentials; it has become expensive (Ekelemu et al., 2000). This has led to fish nutritionists searching for other protein sources that can replace soybean meal and/or partly replace fishmeal to produce cost effective fish feed. Almond kernel meal has great potentials of filling this gap.

Tropical almond (*Terminalia catappa* Linnaeus) also known as Indian almond is a large tropical tree in the leadwood tree family, Combretaceae. Humans have spread the tree widely and the native range is uncertain. It has been naturalized in a broad belt extending from Africa to Northern Australia and New Guinea through South-East Asia and into the Indian sub continent. More recently, it has been introduced to parts of America (Wikipedia, 2010). It grows to 35 metres tall, with horizontal branches. The nut (kernel) is edible. They are dry-season decidous, before falling they turn pinkish-reddish or yellow-brown. The flowers are monocious, with distinct male and female flowers on the same tree (Burkill, 1998). The fruit is a drupe, 5-7 cm long and 3-5.5 cm broad, green at first, then yellow and finally red when ripe, containing a single seed.

Global production of almonds is around 1.7 million tones, with a low of 1 million tones in 1995 and a peak of 1.85 million tones in 2002 (Molody, 2008). Major (commercial) producers are the USA, Spain, Syria, Italy, Iran and Morocco. Nigeria produces about 0.1 million tones annually (Annongu et al., 2006). *Terminalia catappa* is widely grown in tropical regions of the world as an ornamental tree, grown for the deep shade its large leaves provide. The fruit is edible and tastes slightly acidic. The wood is red, solid and has high water resistance (Wikipedia, 2010).

According to Burkill (1988), tropical almond is a widely growing shade plant in Nigeria and other higher rainfall areas of West Africa. The tree is more of an ornamental plant than a feed or food source but provides seasonal fruit that may be useful for human consumption. The plant fruits two times in a year and it is estimated that only about 20% of the fruit is consumed, the remaining 80% being wasted (Burkill, 1988).

Nutritionally, almond contains about 26% carbohydrates; therefore, it can be made into flour for cakes and cookies for low carbohydrate diets or for patients suffering from diabetes. Almond flour is gluten-free and therefore a popular ingredient in place of wheat flour for gluten-sensitive people and people with wheat allergies and celiac disease. Almonds are a rich source of protein and vitamin E, containing 24mg/100g. They are also rich in monounsaturated fat, a "good" fat responsible for lowering cholesterol (White, 2006). Some of the health benefits of almonds include, improved movement of food through the colon and prevention of cancer among others (Davis and Iwahashi, 2001). Due to the chemical richness of the leaves, they are used in different traditional medicines. For instance, in Taiwan they are used to treat liver diseases. In Suriname, a tea made from the leaves is prescribed against dysentery and diarrhea (Molody, 2008). Keeping the leaves in an aquarium is said to lower the pH and heavy metal content of the water. It is also believed that it helps prevent fungus forming on the eggs of fish (Chukwuma, 2015).

1.1 Statement of Problem

Nigeria has excellent fish culture potential because of her abundant water resource. However, aquaculture has not made much progress due to high cost of feed (Eyo et al., 2003). According to Falaye et al. (2014), aquaculture development in Nigeria is limited by inadequate supply of fish feed stuffs, particularly fish meal which is scarce and expensive. This has led to fish nutritionist

searching for alternative source of protein which is cheap and of good quality. Recently, the concern raised about the negative impact of fish meal production on global fish stocks has increased this interest (Tacon, 1994). Soya bean meal has been successfully used for sometime now to partly replace fish meal to produce cheaper fish feed in Nigeria. Recently, due to the increased alternative use of soya beans as food for man coupled with it's competitive use by livestock feed industry and it's growing export potentials, it has become expensive (Ekelemu et al., 2000). This has necessitated the search for other protein sources that can replace soya been meal to achieve cheaper and good quality fish feed.

1.2 Justification

A wide variety of plants, especially legumes, have been assessed either as alternative to fishmeal or soybean meal, most of which are of limited relevance in human nutrition. Among all plant sources, soybean (*Glysine max*) has received the most attention as dietary plant protein source in livestock feed. Soya bean has high protein and fat content and contains a good mix of assential amino acids. However, it contains anti-nutritional factors, which have to be removed or reduced before feeding to monogastric animals such as fish. The extensive use of soya bean in livestock feed is limited by decline in its production and the various uses it is put to in human nutrition including soy flour, soymilk and infant formula (FAO, 2001). In addition, there is a rise in the export potential of soybeans. These factors have caused soya beans to become expensive. In recent times livestock and fish nutritionists are in search of feed ingredient(s) that can replace soybean meal to produce cheaper livestock and fish feed. Burgos et al. (2002) evaluated velvet bean (*Mucuna pruriens*) meal as replacement for soya bean in diets of dual-purpose cows. Flores et al. (2002) examined the growth of pigs fed diets with Mucuna bean flour versus soya bean meal. Corazon et al. (1988) worked on reproductive performance and growth of Nile tilapia (*Oreochromis niloticus*) broodstock fed diet containing *Leucaena leucocephala* leaf meal. Osuigwe et al. (2005) studied some haematological changes in hybrid catfish (*Heterobranchus longifilis x Clarias gariepinus*) fed different dietary levels of raw and boiled jackbean (*Canavalia ensiformis*) seed meal. Sotolu (2008) used *Leucaena leucophala* seed meal to replace soybean meal in the diet of *Clarias gariepinus*. Olasunkanmi (2011) worked on the utilization and biochemical effects of *Mucuna utilis* meal as a replacement for soya bean meal in the diet of *Clarias gariepinus*.

According to United States Department of Agriculture (USDA, 2008), Nigeria's soybean output of 450,000MT in 2007/2008 is much lower than domestic demand. This caused the price of soybean meal to double within eight months to a peak of ₦130, 000 per metric tonne in August 2008. Lolade (2015) reported that Nigeria's soybean demand is estimated at 2.2 million metric tonnes while the country produces about 550,000 tonnes annually, leaving a demand gap of 1.65 million tonnes annually. Therefore, a suitable replacement is required to reduce the demand on soya bean meal. Tropical almond kernel meal is a possible alternative.

Tropical almond (*Terminalia catappa* L.*)* is a widely growing plant in West Africa (Burkill, 1988). The tree is more of an ornamental plant used as sunshade and for land scaping than food but provides fruits that are consumed by man especially children. It is readily and locally available. The fruit is a good source of nutrients; the kernel contains about 24.5% crude protein, 36.0% ether extract and 6.0% ash (Olapade and Kargbo, 2015). This makes the kernel meal a potential alternative protein concentrate to soybean meal in aqua feed. However, there is dearth of documentation on the potential of tropical almond (*Terminalia catappa*) kernel meal as a low cost unconventional fish feedstuff. Therefore, the use of tropical almond kernel meal as a replacement for soybean meal in the diet of *Clarias gariepinus* was investigated.

The output of this work will provide a cost effective feed, which will in turn reduce the cost of fish production and improve the health status of the Nigerian populace through enhanced animal protein intake. *Clarias gariepinus* was chosen for this study because of its economic importance in Nigeria, hardiness, ability to withstand handling stress and good response to feed.

1.3 Main Aim/ Objective

The main aim/objective of this research is to study the potentials and effects of replacing soya bean meal with processed tropical almond (*Terminalia catappa*) kernel meal in *Clarias gariepinus* diet.

1.4 Specific Objectives

The specific objectives of this study include:

1. to evaluate the effects of different processing methods on the chemical composition of tropical almond kernel and determine the best processing method of tropical almond kernel for optimum digestion and utilization by *Clarias gariepinus*.
2. to assess the growth performance and nutrient utilization of *C. gariepinus* fed processed tropical almond kernel meal - based rations.
3. to assess the haematology, serum biochemistry and histopathological profiles of *C. gariepinus* fed processed tropical almond kernel meal -based rations.
4. to estimate the economic benefit of replacing soya bean meal with tropical almond kernel meal in the diet of *C. gariepinus.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Use of Unconventional Plant Materials as Protein Sources in Animal Feed

Feedstuffs of plant origin are as a whole lower in protein content when compared with those of animal origin. In addition, the presence of high amounts of carbohydrate, fibre and other organic molecules (anti-nutrients) such as glucosides, phytates and cyclopropenes in these sources present the nutritionist with problems that are generally not encountered with sources of animal origin (D' Mello, 2000; Soetan, 2008). The nutrient requirements of fishes are such that they offer less flexibility in diet formulation than do those of most land animals. First of all, several species of fish selected for culture are almost pure carnivores or omnivores, requiring a diet of high protein content. These fish have very poor utilization of carbohydrate as an energy source, and some evidence is developing that the inclusion of certain types of carbohydrates in the diet is, in fact, detrimental (Orire and Sadiku, 2014). Hence, it has been recently reported that soya bean meal extracted with alcohol performed better in rations than unextracted meal. This improvement has been attributed to the removal of low molecular weight carbohydrate by the alcohol.

Nevertheless, a wide variety of plants especially legumes have been assessed either as alternative to fish meal or soya bean meal most of which are of limited relevance in human nutrition. Faturoti and Akinbote (1986) recorded good level of economic performance when fish meal was substituted with 20% cassava peel in the diet of tilapia. Oresegun and Alegbeleye (2001) suggested addition of 0.2% methionine with 20% inlusion of cassava peel in the diet of tilapia. Corazon et al. (1988) worked on reproductive performance and growth of Nile tilapia (*Oreochromis niloticus*) broodstock fed diet containing *Leucaena leucocephala* leaf meal. Keembiyehetty and De Silva (1993) examined the performance of juvenile *Oreochromis niloticus* reared on diets containing cowpea, *Vigna catiang* and blackgram, *Phaseolus mungo* seed. Alegbeleye et al. (2001) worked on the use of bambara groundnut (*Vigna subterranea*) meal in the diet of *Heteroclarias* fingerlings. Alegbeleye (2005) examined the growth performance and haematological profiles of *Oreochromis niloticus* fingerlings fed differently processed cottonseed (*Gossypium hirsute*) meal. Adeyemo (2005) studied the haematological and histopathological effects of cassava mill effluent on *Clarias gariepinus*. Ogunji et al. (2005) worked on the effects of different processing methods of pigeon pea (*Cajanus cajan*) on the haematology of African catfish (*Clarias gariepinus*) larvae. Falaye and

Fregene (2005) reported that 50% replacement of fish meal with soya bean meal in the diet of *Macrobrachium vollenhovenii* gave the best growth performance. Nweke and Ugwumba (2005) reported that duckweed, *Lemna paucicostata* was a suitable protein source for tilapia diet up to 50% inclusion level. Koyeme et al. (2006) reported that water hyacinth, *Eichhornia crassipes* was also a suitable protein source for catfish fingerlings. Sotolu (2008) used *Leucaena leucophala* seed meal to replace soybean meal in the diet of *Clarias gariepinus* and got best growth performance at 20% inclusion. Olasunkanmi (2011) worked on the utilization and biochemical effects of *Mucuna utilis* meal containg 27.48-29.23% crude protein as a replacement for soya bean meal in the diet of *Clarias gariepinus*. Adesina (2014) used sunflower (*Helianthus annuus*) seed meal to replace soybean meal in *Clarias gariepinus* diet and got positive results at 20% to 40% inclusion.

2.2 Tropical Almond (*Terminalia catappa* L.)

Terminalia catappa commonly known as Almond, Tropical almond, Indian almond, Sea almond and Umbrella tree is a large tropical tree in the leadwood tree family, Combretaceae (Burkill, 1988).

2.2.1 Taxonomy of Tropical Almond (*Terminalia catappa* L.)

Kingdom: **Plantae**
Division: **Magnoliophyta**
Class: **Magnoliopsida**
Order: **Myrtales**
Family: **Combretacaea**
Genus: ***Terminalia***
Species: ***Terminalia catappa* L.**

2.2.2 Occurrence and Geographical Distribution of Tropical Almond (*Terminalia catappa*)

Humans have spread tropical almond widely and the native range is uncertain. It has long been naturalized in a broad belt extending from Africa to Northern Australia and New Guinea through Southeast Asia into the Indian subcontinent. More recently, it has been introduced to parts of the

Americas (Wikipedia, 2010). According to Olapade and Kargbo (2012), tropical almond originated from tropical Asia, India, the Malay Peninsula, Taiwan, Burma but thrives well in other tropical regions of the world including Nigeria.

2.3 Aspect of the Biology of Tropical Almond (*Terminalia catappa*)

2.3.1 Description of Tropical Almond (*Terminalia catappa*)

Tropical almond grows to 35 metres tall, with an upright, symmetrical crown and horizontal branches. It has corky, light fruit that is dispersed by water. The nut (kernel) within the fruit is edible when fully ripe. As the tree gets older, its crown becomes more flattened to form a spreading, vase shape. Its branches are distinctively arranged in tiers. The leaves are large, 15-25 centimetres long and 10-14 centimetres broad, avoid, glossy dark green and leathering. They are dry-season deciduous; before falling, they turn pinkish- reddish or yellow- brown, due to pigments such as violaxanthin (Wikipedia, 2010).

2.3.2 Reproduction of Tropical Almond (*Terminalia catappa*)

Almond flowers are monoecious, with distinct male and female flowers on the same tree. Both are one centimeter in diameter, white to greenish, inconspicuous with no petals, they are produced on auxiliary or terminal spikes. The fruit is a drupe 5-7 centimetres long and 3-5.5 centimetres broad, green at first, then yellow and finally red when ripe, containing a single seed (Morton, 1985).

2.4 Availability/Production of Tropical Almond (*Terminalia catappa*)

Global production of almond is around 1.7 million tons, with a low of 1 million tons in 1995 and a peak of 1.85 million tons in 2002 (Molody, 2008). According to the same author, major producers are USA, Spain, Syria, Italy, Iran, Morocco, Algeria, Tunisia and Greece. Nigeria produces about 0.1 million tons annually (Annongu et al., 2006). Tropical almond has a kernel yield estimated at about 5kg per tree per year and 10kg per tree per year for selected genetic stock grown on high quality sites (Traditional tree, 2006).

2.5 Uses of Tropical Almond (*Terminalia catappa*)

2.5.1 Culinary Uses

The almond is often eaten on its own, raw or toasted. It is also a component of various dishes. According to Traditional tree (2006), almonds are available in many forms, butter, almond milk and almond oil. Along with other nuts, sweet almond can be sprinkled over deserts, particularly ice cream based dishes. Sweat almonds are used in pastries, cookies and cakes. They are also used to make almond butter, a spread similar to peanut butter. The young developing fruit of the almond tree (green almonds) can be eaten completely, when they are still green and fleshy. The fruit is somewhat sour. Nevertheless, it is a popular snack in parts of the Middle East, eaten dipped in salt to balance the sour taste (Morton, 1985).

In Italy, sweet almonds are the base for almond macaroons, a common dessert. In Puglia and Sicily, almond paste is used to make small soft cakes, often decorated with jam or chocolate. In Greece, ground blanched almonds are used as the base material in a great variety of desserts. Because of their white colour, most are traditionally considered "wedding sweets" and are served at wedding banquets (Wikipedia, 2010). In addition, a soft drink known as "soumada" is made from almonds in various regions.

In Morocco, almond in the form of almond paste are the main ingredients in several desserts. Whole fried almonds are also used in sweet tajines. A drink made from almond mixed with milk is served in important ceremonies, such as weddings and can also be ordered in some cafes (Wikipedia, 2010).

Almonds can be processed into a milk substitute called almond milk. Raw, blanched and lightly toasted almonds all work well for different production techniques, some of which are very similar to that of soymilk and some of which use no heat resulting in "raw milk". Almond oil, obtained from the dried kernel is good for application to the skin as an emollient, and has been traditionally used to lubricate the skin during a massage session (Traditional Tree, 2006).The same author stated that, it is a mild, lightweight oil that can be used as a substitute for olive oil. Almond oil is also used as a wood conditioner of certain woodwind instruments, such as the oboe and clarinet.

2.5.2 Nutrition and Health Benefits of Tropical Almond (*Terminalia catappa*)

Burkill (1988) reported the nutrient composition of almond kernel as follows:

Protein	23.1%
Oil	51%
Crude fibre	9.3%

Ekop and Eddy (2005) gave the composition of almond kernel as follows:

Moisture	25%
Ash	4.50%
Lipid	9.20%
Protein	22.05%
Fibre	3.00%
Carbohydrate	61.25%
Calorie	416.00kcal

According to Wikipedia, (2010), all parts of the plant (leaves, bark, roots, fruit and wood) are used in traditional medicine, such as in dysentery, dressing of rheumatic joints, treating coughs, and asthma. The fruit may be helpful in treatment of leprosy, headaches and in reducing travel nausea. Leaves have been used to get rid of intestinal parasites; treat eye problems, rheumatism, wounds and stop bleeding during teeth extraction. Almond has strong antibacterial properties and works against Gram positive and Gram negative micro-organism. The leaves have been shown to protect against acute liver injury caused by some hepatotoxicants.

In Nigeria, fallen leaves are used as an herb to treat liver diseases. The leaves also have potential in the management of sickle cell disorders. They equally have antioxidant as well as anti-clastogenic properties. Research suggests that moderate consumption of the seed kernel may be useful in the treatment of men with sexual dysfunction, primarily from premature ejaculation (Chukwuma, 2015). The various extracts of leaves and bark of the plant have also been reported to be anticancer, antioxidant, anti-Human Immuno-deficiency virus (HIV) reverse transcriptase, and anti-inflammatory (Wikipedia, 2010) .

2.6 The Use of Tropical Almond (*Terminalia catappa*) as Feed Stuff.

A few works have been done on the use of almond kernel as feed ingredient. Burkill, (1988) stated the proximate composition of almond kernel as follows: 23.1 % crude protein, 51-65% sweet flavoured oil, 9.3% crude fibre. Adejumo, (2005) examined the utilization of Indian almond (husk/kernel) meal as a replacement for wheat offal in broiler finisher rations. The result showed that *Terminalia catappa* husk and kernel meal can be safely incorporated into poultry finisher rations up to 15% inclusion level without adverse effects. Annongu et al. (2006) studied the potential of using almond fruit waste as an alternative feedstuff for livestock using cockerels. The result showed that treated almond fruit waste improved feed intake, body weight gain and feed conversion ratio even better than the reference diet. Olapade and Kargbo (2015) worked on growth and heamatological responses of *Clarias gariepinus* juveniles to varying inclusions of *Terminalia catappa* seed meal based diets and got positive results at 50% substitution of the seed meal for fishmeal.

2.7 Water Quality Parameters in Catfish Culture.

Water quality affects all the phases of life of fish. Both water quality and quantity have the potential to significantly affect fish metabolism, survival and growth. It is therefore pertinent to ensure good water quality for optimum fish growth, survival and yield. Nutrition experiments are mainly conducted in controlled environment to prevent interaction of environmental effects such as natural food organisms, temperature and other water quality parameters with the variable being studied. Such experiments are conducted in tanks placed indoor to allow accurate collection of data in the laboratories.

2.7.1 Temperature

Temperature is a fundamental parameter in all aquatic environments. It is the dominant regulator of nearly all physio-chemical cycles and consequently of metabolism and fish pond productivity. It determines the amount of dissolved oxygen in the water which in turn affect how well the fish feed and whether they stay alive or not. Tropical fish require water with a temperature range of 25 – 32°C to grow and reproduce (Boyd, 1979; Omitoyin, 2007). Below or above this optimum range,

they do not feed well and they become stunted. Variation in temperature regime can occasion disease outbreak, which will eventually lead to mortality.

2.7.2 Dissolved Oxygen (DO)

The dissolved oxygen content of the pond water is one of the most critical factors in fish culture. Fish require adequate dissolved oxygen in the water. It must not be less than 4mg/litre for fish to survive and grow well (FAO, 1993a). Low dissolved oxygen (less than 3mg/litre) affects fish adversely. Retarded growth occurs; fish may cease to feed and becomes more susceptible to disease infestation. Below a certain critical value, fish mortality occurs (Boyd, 1979).

Generally, the oxygen consumption of fish increases as the food intake and its activity increases. However, when expressed as a percentage of fixed fish weight, oxygen consumption decreases as the fish grows (Viveen et al., 1985). The same author reported that at larvae to fry, fingerlings and juvenile stages, oxygen concentration close to saturation is desirable for catfish but adults with full grown arborescent organ can survive low dissolved oxygen.

2.7.3 pH

pH is a measure of acidity or alkalinity of water. Water with a pH less than 7 is acidic, above 7 is alkaline while 7 is neutral. Very low and very high pHs have negative effects on fish production. Fish may survive but will not grow and reproduce at normal rate. Water with a pH value of about 6.5 to 9 is the best for fish culture (Alabaster and Lloyd, 1980). Above pH of 11, fish mortality may occur.

Water pH also has a significant influence on the toxic action of a number of substances such as ammonia, hydrogen sulphide and heavy metals on fish (FAO, 1993a). During periods of photosynthesis, carbon dioxide is rapidly removed. This results in an increase in carbonate concentration leading to high pH. The hydrolysis of carbonate causes the pH to rise; hence, pH is directly proportional to the carbonate concentration.

2.7.4 Ammonia

Ammonia is a nitrogenous waste produced by either the pond organisms (including fish) or decomposition of organic matter. High concentration of ammonia affects fish growth and can be prevented by good pond management. Even in small quantity, ammonia has been known to stress fish and has been responsible for more un-explained fish kill in fish culture than any other water quality parameter (Alabaster and Lloyd, 1980). Ammonia is the major end product in the breakdown of protein in fish. Ammonia is also produced in the pond from bacterial decomposition of organic matter. The presence of ammonia in water is indicative of pollution. Acute effects of ammonia include loss of equilibrium, increased oxygen uptake, convulsion, coma and mortality. The sub-lethal effects of ammonia are reduced growth, changes in liver, kidney and gills (Boyd, 1979). For catfish culture, ammonia level in ponds should be maintained at less than 0.05 ppm (Viveen et al., 1985).

2.7.5 Nitrite and Nitrate

Nitrite and nitrate are products of decomposition of organic matter. High concentration of nitrite in ponds may occur when the weather is relatively cold during the harmathan period of the dry season. It occurs when uneaten feed accumulates in the pond and is not completely broken down. According to Viveen et al. (1985), nitrite concentration in catfish ponds should not exceed 0.25 ppm.

When catfish is exposed to lethal nitrite concentration, the nitrite enters the fish blood through the gills and combines with the heamoglobin to form methaemogobin. This compound causes the toxic disease known as "brown blood disease". Nitrite concentration in ponds can be reduced by flushing the pond and adding fresh water to the pond. Table salt (NaCl) can also be used to control nitrite toxicity in tanks. It is distributed in the tank at a rate to give a ratio of chloride to nitrite of 3:1 or greater (Boyd, 1979).

Nitrate is relatively non toxic to fish. Fish can tolerate nitrate to several hundred mg/litre. It serves as a nutrient used by phytoplankton for primary productivity in the pond. However, excess levels in the pond could lead to algal blooms.

2.7.6 Water Hardness and Total Alkalinity

Alkalinity refers to amount of carbonates (CO_3^{2-}) and bicarbonates (HCO_3^-) in the water and total hardness refers to the concentration of calcium (Ca) and magnesium (Mg), expressed in milligram per litre (mg/l) of $CaCO_3$. Alkalinity measures the amounts of CO_3^{2-} and HCO_3^-in water. Because calcium and magnesium bond with carbonates and bicarbonates, alkalinity and water hardness are closely related and produce similar measured levels in ponds (Ajani et al., 2011). Alkalinity is also a measure of the buffering capacity of water. It provides stability to the pond environment. Some salts, mainly bicarbonate in water act as buffers. They can combine with acids (hydrogen ions) to prevent a change in pH and so the water remains near neutral or slightly alkaline. According to FAO (1993a), water with alkalinity and hardness of 50- 200mg/litre is the most productive for catfish. This alkalinity range provides a good buffering effect to pH swings that occur in ponds. Water of low alkalinity is poorly buffered against pH change. When total hardness is between 50 – 200 mg/litre, growth of plankton and fish is favoured. Total alkalinity and hardness can be increased by liming the pond.

2.7.7 Carbon Dioxide (CO_2)

While aquaculturists are rightly concerned with maintaining adequate concentration of dissolved oxygen (DO), knowledge of the 'flip-side' of the oxygen equation is also important. The primary sources of CO_2 in fish ponds are respiration by fish and other living organisms in the pond and decomposition of organic matter in the pond. During the day when the sun is shining, dissolved oxygen (DO) is supplied to the pond water by photosynthesis of phytoplankton and other aquatic plants in the pond. During the night, photosynthesis cease, and the algae, sediment and fish consume oxygen, producing the fluctuating pattern of DO. The daily pattern of CO_2 concentration is generally opposite that of DO. During the day, algae take up CO_2 that is free in the water and CO_2 concentration is therefore lowest (often 0mg/l) during the late afternoon, when DO is highest. During the night, the respiration of pond organisms produces CO_2, which accumulates to maximum (often 10 – 15mg/l) at dawn. CO_2 concentrations are highest when DO concentrations are lowest. Therefore, dawn is a critical time for evaluating pond water quality. If environmental carbon (IV) oxide concentrations are high, fish will have difficulty reducing internal CO_2 concentrations, resulting in accumulation in fish blood. This inhibits the ability of haemoglobin to carry oxygen

causing the fish to feel stress similar to suffocation. According to Onuoha and Elezuo (2013), the density of the algae bloom has an important effect on the magnitude of daily fluctuations of oxygen and CO_2. Oxygen and carbon dioxide concentrations in ponds with a light algae bloom will not fluctuate much between early morning and late afternoon, whereas ponds with thick, dense algae bloom will have more extreme fluctuations. Carbon dioxide problems are therefore more likely as the thickness of the bloom increases.

2.7.8 Salinity

Salinity is the total concentration of all dissolved ions in the water. Each fish species has an optimal salinity range. This range allows the fish to efficiently regulate their internal body fluid composition of ions and water. If the salinity is too high, the fish will start to lose water to the environment (exosmosis). As freshwater fish are not physiologically adapted to osmo-regulate within saline water, decreased growth and survival may occur under this condition. Fish species differ in their osmotic pressure requirement; therefore, the optimum salinity for fish culture differs to some extent with species.

According to Boyd (1979), freshwater fish thrive in water with salinity level below 1 ppt. Fish is very sensitive to sudden changes in salinity, even for those species that are more tolerant to wider salinity range. Therefore, if any change in salinity of the culture system is to be carried out, it should be done gradually over several days. Small fish and fry of most fish species are more susceptible to sudden changes in salinity than adult fish. Before an aquaculture project commences, salinity of the water source should be tested. Since salinity tolerance vary amongst species, it is therefore important to choose an aquaculture species best suited to salinity of the water source (particularly for coastal areas).

2.8 Anti-Nutritional Factors (Anfs) in Plant Feedstuffs.

Anti-nutritional factors are compounds which act to reduce nutrient utilization and or feed intake (Osagie, 1998). They have a shielding effect on the ingredients, thereby preventing them from being digested and hence unavailable to the fish.

Anti-nutritional factors(ANFs) can also be defined as those substances generated in natural food stuffs by the normal metabolism of species and by different mechanisms (e.g. inactivation of some nutrients, diminution of the digestive process, or metabolic utilization of feed) which exert effects contrary to optimum nutrition (Cheeke and Shull, 1985; Soetan, 2008). Being an ANF is not an intrinsic characteristic of a compound but depends upon the digestive process of the ingesting animal. For example, trypsin inhibitors, which are ANFs for monogastric animals, do not exert adverse effects in ruminants because they are degraded in the rumen (Cheeke and Shull, 1985). Anti-nutritional factors diminish animal productivity but may also cause toxicity during periods of scarcity or confinement when the feed rich in these substances is consumed by animals in large quantities.

ANFs can be classified into two groups according to their heat resistance. Heat liable and heat stable ANFs. Heat liable ANFs are the ANFs that can be inactivated by heat application while heat stable are those that cannot be inactivated by heat application.

2.8.1 Saponins:

Saponins are a heterogeneous group of naturally occurring foam-producing triterpene or steroidal glycosides that occur in a wide range of plants, including pulses and oil seeds such as kidney bean, chickpea, soybean, groundnut, lupin and sunflower (Liener, 1989). It has been reported that saponins can affect animal performance and metabolism in a number of ways as follows: erythrocyte haemolysis, reduction of blood and liver cholesterol, depression of growth rate, testabloat (ruminants), inhibition of smooth muscle activity, enzyme inhibition and reduction in nutrient absorption (Liener, 1989). Saponins have also been reported to alter cell wall permeability and therefore produce some toxic effects when ingested (Johnson et al., 1986). Saponins have been shown to bind to the cells of the small intestine thereby affecting the absorption of nutrients across the intestinal wall (Johnson et al., 1986). When large quantities of feedstuffs containing saponin is fed to monogastric animals such as fish, it causes diarrhea and vomiting. It also causes haemolysis (breakage of red blood cells).

2.8.2 Cyanogenic Glycosides

They are nitriles bound in glycosidic form with an aromatic or aliphatic aglycones which liberate hydrogen cyanide (HCN), sugar and aldehyde or ketones when treated with dilute acid or appropriate hydrolytic enzymes. They are also referred to as Cynogens. Cynogens have a wide distribution among certain grasses, root crops suh as cassava, fruit kernels and some legumes such as linseed, lima bean and kidney bean. Some cultivars of *Phaseolus lunatus* (lima bean) contain a cyanogenic glycoside called phaseolutanin from which HCN is liberated due to enzyme action, especially when tissues are broken down by grinding or chewing or under damp conditions (D'Mello, 2000). Hydrolysis occurs rapidly when the ground meal is cooked in water and most of the liberated HCN is lost by volatilization. HCN is very toxic at low concentration to animals. HCN can cause dysfunction of the central nervous system, respiratory failure and cardiac arrest (Osuntokun, 1972). Cyanogenic glucoside on hydrolysis yields toxic Hydrocyanic acid (HCN). The cyanide ions inhibit several enzyme systems; depress growth through interference with certain essential amino acids and utilization of associated nutrients. They also cause acute toxicity, neuropathy and death (Osuntokun, 1972). According to D'Mello, (2000), Hydrogen cyanide inhibits respiratory enzyme cytochrome oxidase. When cytochrome is blocked, ATP formation ceases and the tissue suffers energy deprivation and death follows rapidly. Hydrogen cyanide rapidly reacts with iron and copper ion in the blood, leading to the combination of cyanide with haemoglobin to form cyanoheamoglobin, which is not an oxygen carrier.

2.8.3 Alkaloids

Alkaloids cause gastrointestinal and neurological disorders (Aletor, 1993). The glycoalkaloids, solanine and chaconine present in potato and *Solanum spp*. are haemolytically active and toxic to fungi and humans (Saito et al., 1990). Some of the toxicological manifestations of potato glyco-alkaloids involve gastrointestinal upsets and neurological disorders, especially in doses in excess of 20 mg/100 g sample. Coumarins, which are constituents of forage, have been associated with the so-called bleeding disease in cattle consuming spoiled or putrid sweet clover. It is believed that cinnamic acid or its derivatives are the precursors of coumarin and that when plant tissues containing derivatives are disrupted by mastication, freezing, drying or microbial spoilage,

coumarin is transformed to the haemorrhagic factor, Dicoumarol. Dicoumarol depresses blood platelet concentration and by implication reduces clotting time (Aletor, 1993).

2.8.4 Tannins

Tannins may form a less digestive complex with dietary proteins and may bind and inhibit the endogenous protein, such as digestive enzymes (Liener, 1989). Both the protein precipitation and incorporation of tannin phenolic into the precipitate increase with increase in molecular size of tannins. Tannins have been found to interfere with digestion by displaying anti-trypsin and anti-amylase activity. Tannins also have the ability to complex with vitamin B (Liener, 1989). Tannins cause decreased feed consumption in animals, bind dietary protein and digestive enzymes to form complexes that are not readily digestible (Aletor, 1993). They also cause decreased palatability and reduced growth rate (Roeder, 1995). Other adverse nutritional effects of tannins have been reported to include intestinal damage, interference with iron absorption and the possibility of tannins producing a carcinogenic effect (Roeder, 1995).

2.8.5 Trypsin / Protease Inhibitors

Protease inhibitors are widely distributed within the plant kingdom, including the seeds of most cultivated legumes. Protease inhibitors have the ability to inhibit the activity of proteolytic enzymes within the gastrointestinal tract of animals (Liener and Kakade, 1980). Trypsin inhibitor and chymotrypsin inhibitor are protease inhibitors occurring in raw legume seeds. Protease inhibitors are the most commonly encountered class of antinutritional factors of plant origin. These inhibitors have been reported to be partly responsible for the growth-retarding property of raw legumes. The retardation has been attributed to inhibition of protein digestion but there is evidence that pancreatic hyper activity, resulting in increased production of trypsin and chymotrypsin with consequent loss of cystine and methionine is also involved (McDonald et al., 1987). Trypsin inhibitors have been implicated in reducing protein digestibility and in pancreatic hypertrophy (Liener, 1989). They cause pancreatic enlargement and growth depression. Trypsin inhibitors are polypeptides that form well characterized stable complexes with trypsin on a one-to-one molar ratio, obstructing the

enzymatic action (Carlini and Udedibie, 1997). Protease inhibitors are inactivated by heat especially moist heat, because of even distribution of heat (Liener and Kakade, 1980).

2.8.6 Lectins (Phytohaemagglutinins)

Phytohaemagglutinins or Lectins are glycoproteins widely distributed in legumes and some oil seeds (including soybean) which possess an affinity for specific sugar molecules and are characterized by their ability to combine with carbohydrate membrane receptors (Pusztai, 1989). Lectins have the capability to directly bind to the intestinal mucosa, interacting with the enterocytes and interfering with the absorption and transportation of nutrients (particularly carbohydrates) during digestion and causing epithelial lesions within the intestine. Although lectins are usually reported as being heat labile, their stability varies between plant species, many lectins being resistant to inactivation by dry heat and requiring the presence of moisture for more complete destruction (Pusztai, 1989). According to Hendricks et al. (1990), soya bean lectins otherwise known as soya bean agglutinin (SBA) have bean shown to be able to bind extensively to the intestinal surfaces, particularly in distal part of the small intestine of Atlantic salmon and may contribute to the toxic effects of full fat soybean and soybean products in diets for Salmonids. Haemagluttinins are proteins known for agglutinating red blood cells. They depress animal growth by interfering with the digestion and absorption of nutrients in the gastrointestinal tract.

2.8.7 Phytic Acid (Phytate)

Phytic acid occurs naturally throughout the plant kingdom and is present in considerable quantities within many of the major legumes and oilseeds. This includes soybean, rapeseed and cotton seed. About 62-73% and 46-73% of the total phosphorus within cereal grains and legume seeds respectively are in the form of organically bound phytin phosphorus. As phytic acid accumulates in storage sites in seeds, other minerals apparently chelates to it forming the complex salt phytate (Spinelli et al., 1983). The major part of the phosphorus contained within phytic acid is largely unavailable to mongastric animals such as fish, due to the absence of the enzyme phytase within the digestive tract of monogastric animals (Spinelli et al., 1983). According to the same author, phytic acid acts as a strong chelator, forming protein and mineral-phytic acid complexes; the net result

being reduced protein and mineral bioavailability. The same authors reported that phytic acid chelate metal ions such as calcium, magnesium, zinc, copper, iron and molybdenum to form insoluble complexes that are not readily absorbed from gastrointestinal tract. Phytic acid also inhibits the action of gastrointestinal tyrosinase, trypsin, pepsin, lipase and amylase (Liener, 1989). Phytates bind minerals like calcium, iron, magnesium and zinc and make them unavailable to consumers (Nelson et al., 1968).

2.8.8 Oxalates

Oxalates affect calcium and magnesium metabolism and react with proteins to form complexes, which have an inhibitory effect in peptic digestion. However, ruminants unlike monogastric animals can ingest considerable amounts of high-oxalate plants without adverse effects, due principally to microbial decomposition in the rumen (Oke, 1969). Oxalic acid binds calcium and forms calcium oxalate, which is insoluble. Calcium oxalate adversely affects the absorption and utilization of calcium in the animal body. Oxalates, like phytates, bind minerals like calcium and magnesium and interfere with their metabolism. They also cause muscular weakness and paralysis. Oxalates also cause gastrointestinal tract irritation, blockage of the renal tubules by calcium oxalate crystals and hypocalcaemia (Oke, 1969). Jones (1981) reported that oxalates cause nephrotic lesions in the kidney. Oxalate, phytate and tannins are anti-nutrients, which could be toxic when consumed in an unprocessed food (Ojiako and Igwe, 2008). Too much of soluble oxalate in the body prevents the absorption of soluble calcium ions as the oxalate binds the calcium ions to form insoluble calciumoxalate complexes. As a result of this, people with the tendency to form kidney stones are advised to avoid oxalate-rich foods (Adeniyi et al., 2009).

2.8.9 Gossypols

Gossypols are reported to cause animal and human toxicity and high incidence of irreversible testicular damage. Dietary gossypol can also bring about increased requirement for lysine and iron, which it can chelate (Aletor, 1993). At the physiological level, gossypol reduces oxygen availability in the blood. Skutches (1974) showed that proteins were reduced by approximately 20% in pigs fed 0.06% free gossypol in the diet. Other physiological abnormalities include hypertrophy and dilution

of heart muscles and changes in electrocardiogram. Dietary free gossypol of up to 0.02- 0.03% has been reported to cause death in growing pigs while poultry can tolerate fairly high dietary levels (Aletor and Onibi, 1990). This same author noted that gossypol is rapidly deposited in the eggs and has been implicated in egg yolk discoloration.

2.9 Reducing the Effects of Anti-Nutritional Factors in Plant Feedstuffs

Plant phytochemicals exhibit diverse pharmacological and biochemical actions when ingested by animals and man (Amadi et al., 2006; Soetan, 2008). Most of the toxic and anti-nutrient effects of these compounds in plants could be removed by several processing methods such as soaking, germination, boiling, autoclaving, fermentation, genetic manipulation and other processing methods (Soetan, 2008). The toxic effects of oxalate, phytate and tannins could be avoided, provided the plant food is cooked before consumption (Enechi and Odonwodu, 2003). Some of the processing methods that could reduce the levels of ANFs and / or reduce their effects are discussed below.

2.9.1 Sundrying

Sundrying is ommonly used in the tropics to process food. This treatment can be effective for the removal of ANFs in leaf meals, grains and legumes (Falaye et al., 2014). According to Oyelese (1994), sundrying of cassava leaves can reduce hydrogen cyanide concentration by 90%.

2.9.2 Thermal Method

Heat treatment is an effective method of improving plant products through the inactivation of most of the heat liable ANFs. Visitpanich et al. (1985) reported total elimination of adverse effects of trypsin inhibitor in pig fed pigeon pea heated to 124^{o} C for 15 minutes. However, over heating of soya bean causes harmful effect on animal's growth performance. This is most probably due to reduced lysine availability. Boiling reduced the phytic acid to zinc molar ratio for yellow yam and cocoyam. Boiling or roasting reduced the levels of cyanoglucosides in sweet potato, yellow yam and cocoyam. Roasting greatly lowered the level of trypsin inhibitor activity compared to boiling (Enechi and Odonwodu, 2003).

2.9.3 Water Treatment

Acording to Sotolu (2008), cold-water treatment such as soaking in water and fermentation have been found to be very useful in reducing antinutritional factors in leucaena. Washing with water and soaking the leaves and seeds of leucaena lowered their mimosine contents. Prolonged soaking in water with temperature of 30^{o} C was most effective in removing almost all the mimosine in leucaena leaves. Soaking and fermentation produce enzymes that break down complexes to release free tannins. The free tannins leached out. Cooking and fermentation also broke down tannin enzyme and protein-tannin complexes and released free tannins, which subsequently leached out of the products.

2.9.4 Use of Feed Supplements

The use of feed supplements provide a good means of improving plant feedstuffs. Ferric sulphate and polyethylene glyol have been utilized to complex with mimosine and tannin respectively, with significant improvements on the growth of poultry birds fed leucaena based ration (D'Mello and Acamovic, 1982). Methionine supplementation was used in improving cassava leaf meal for poultry birds and swine (Ravindram, 1990). Methionine and lysine have been used to improve soya bean meal in catfish diet. Phytase has also been used to minimize the effect of phytate in plant feedstuffs in fish feed (Nwanna and Schwarz, 2007; Nwanna et al., 2007; Orisasona, 2014).

Other methods used in improving plant feedstuffs include, use of remen microbes, isoelectrical precipitation, use of improved cultivars, oil extraction, germination and sprouting (Sotolu, 2008; Ijeh and Elezuo 2008; Adesina, 2014).

2.10 The Use of Haematological Parameters in Fish Health Management

Heamatology is the science of studying the anatomical, physiological and pathological aspects of blood. Haematological profile has for long been used in clinical and pathological diagnosis in human and livestock (Ayoola, 2011). Svobodova et al. (1991) stated that ichthyo-haematology would be useful in the appraisal of suitability of feeds, fish condition, toxic effect of substances and diagnosis of diseases. According to the same authour, haematological parameters are important in analyzing the health status of animals exposed to toxicants. Osuigwe et al. (2005) reported that the

application of haematological indices has become a valuable tool for fishery biologist in assessing the health of fish and monitoring stress response. Blood parameters such as packed cell volume (PCV), red blood cell (RBC), Haemoglobin concentration (Hb), White blood cell (WBC) and plasma biochemical constituents are used to assess the health status of fish. Decrease in packed cell volume, haemoglobin and red blood cell count are indicators of reduced fish activity and is unhealthy for the fish (Adeyemo, 2005; Osuigwe et al., 2005). The reduction in packed cell volume and haemoglobin are possible reason for aneamia. The same author stated that the most common haematological variables measured during stress are red and white blood cells count, haemoglobin content, hematocrit value and red blood cell indices. Fish heamatological parameters are often determined as indices of their health status (Oshode et al., 2008). However, Klontz (1963) stated that the history of applying heamatological methods as diagnostic aids in episodes of diseases in confined and free-living populations of fish is quite inadequate. The major reason for the insufficiency, as compared with mammalian medicine is the variability of data. Fish are subject to many environmental influences, which alter the healthy hemogram, that is, the baseline data for cellular and plasma components (Klontz, 1963). Terry et al. (2000) in consonant with Klontz (1963) reported that heamatology could serve as aids in fish disease diagnosis but stated that caution should be applied in doing so. Terry et al. (2000) also stated that there are no “normal” values because of the responsiveness of blood vascular system to external stimuli. He suggested that the most appropriate approach is to establish the basedline data for the fish in a specific situation and monitor the population for changes in the heamatological profile. He concluded that if good techniques are applied and interpretations are guarded, heamotological methods have value in fish health management.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample Collection and Preparation

Tropical almond fruits for this study were manually picked from tropical almond trees at Nnamdi Azikiwe hall, University of Ibadan, Ibadan, Nigeria. They were sundried for four days and de-hulled manually to get the kernels.

3.2 Processing of Almond Kernels into Meals

Six kilograms of raw almond kernels were sundried for two to three days. A portion of it was milled with a Thomas Wiley milling machine (Model K 925) and designated as raw almond kernel meal (RWAM). Another portion was boiled in water at 100^{o} C and atmospheric pressure for 30 minutes at a proportion of 1:1.5 (kernel to water) w/v ratio (Ijeh and Elezuo, 2008; Orisasona, 2014). The boiled kernels were drained, sundried for three days, milled with a Thomas Wiley milling machine (Model K 925) and designated as boiled almond kernel meal (BOAM). Another portion was soaked in water at room temperature for 24 hours at a proportion of 1:2 (kernel/water) w/v ratio (Ijeh and Elezuo, 2008). The water was drained and changed at 12-hour interval. After soaking, the kernels were drained, sundried for three days, milled and designated as soaked almond kernel meal (SOAM). A fourth portion was put in a stainless steel pan, placed over a Stuart Scientific electric cooker (model SH 1) and roasted at 80^{o} C for 4 hours until the kernel coat became dark brown (Sotolu, 2008; Adesina, 2014) and emitted a characteristic aroma similar to that of roasted melon . The roasted kernels were milled with a Thomas Wiley milling machine (Model K 925) and designated as roasted almond kernel meal (ROAM). Another portion was ground with a Thomas Wiley milling machine (Model K 925) and de-oiled in a soxhlet apparatus containing petroleum spirit (boiling point range 60^{o}-80^{o} Celcius) for 6-12 hrs. It was put in stainless steel tray, air-dried for 4 days and sun-dried for one day for complete removal of residual solvent (Bello et al., 2012; Falaye et al., 2016) and designated as solvent extracted almond kernel meal (SEAM). The last portion was sundried for two to three days, milled and loaded into the receptacle of a locally made mechanical screw press and pressed for 24 hours. Oil was collected in a compartment at the base of the screw press, removed through a tap and stored in a bottle. The resultant cake was manually broken into fine particles and designated as mechanically extracted almond kernel meal (MEAM).

3.3 Amino Acid Analysis of Tropical Almond (*Terminalia catappa*) Kernel Meal.

A potion of raw almond kernel meal was subjected to amino acid analysis. Amino acid was extracted from the meal following the modified method of AOAC (2006). The pulverized sample was dried to constant weight in the laboratory. Ten grammes of the sample was weighed into a 250ml conical flask. The sample was defatted by solvent extraction using 30 ml of petroleum spirit with soxhlet apparatus equipped with thimble. The sample was hydrolysed three times for complete hydrolysis. The amino acid content of the sample was recovered by extracting with 30 ml dichloro methane three times before concentrating to 1.0 ml. The concentrated extract was derivatised for volatility and subjected to gas chromatography analysis. The analyses were carried out at the Livestock Research Laboratory of the Institute of Agricultural Research and Training, Moor plantation, Ibadan. The amino acids were read from the chromatogram with enhanced integrator.

3.4 Chemical Analysis of Raw and Differently Processed Tropical Almond Kernel Meals.

The raw and differently processed tropical almond kernel meals were subjected to chemical analysis following the standard method of analysis described by the Association of Official Analytical Chemist (AOAC), (2005). Parameters analyzed were crude protein, crude fat (ether extract), crude fibre, ash, moisture, Nitrogen free extract, anti-nutritional factors and minerals. All the analyses were done in triplicates.

3.4.1 Determination of Crude Protein

Crude protein was determined by the routine semi-micro kjeldahl procedure/technique. This consisted of three techniques of analysis namely digestion, distillation and titration. The percentage crude protein was calculated by multiplying the total nitrogen by a factor of 6.25

Total Nitrogen (N) is given by:

$$N = \frac{\text{Vol of acid} \times \text{molarity} \times 0.01 \times \text{diluting factor}}{\text{Weight of sample used}}$$

% crude protein = N x 6.25 (AOAC, 2005).

3.4.2 Determination of Crude Fat

Fat content was determined by subjecting the sample to a continuous extraction with petroleum ether using Gallenkampt soxhlet equipment as described by AOAC (2005). The ether residue was the residue obtained from evaporation of the solvent.

3.4.3 Determination of Crude Fibre

Each sample was defatted with petroleum ether using soxhlet extraction system. 2g of defatted sample was weighed into 600ml beaker and 100ml trichloroacetic acid added for digestion. The mixture was boiled and refluxed for 40 minutes. The filtered concentrate was washed six times with hot distilled water and once with methylated spirit. The residue was placed in porcelain crucible and ashed in a Gallenkamp muffle furnace at 600^{o}C for 5 hours. The ash was cooled in a desiccator and weighed. The percentage crude fibre content was calculated as follows:

Crude fibre content (%) = $W_1 - W_2 / W_1 \times 100$

Where W_1 = Initial weight of sample (g)

W_2 = final weight of sample (g)

3.4.4 Determination of Dry Matter and Moisture

Two grams of the sample was weighed into a previously weighed crucible. The crucible plus sample taken was then transferred into the oven set at 100^{0}C to dry to a constant weight for 24 hours overnight. At the end, the crucible plus sample was removed from the oven and transferred to a dessicator, cooled for ten minutes and weighed. Percentage dry matter and moisture were calculated as follows:

$$\% \text{ Dry matter (\% DM)} = \frac{W_3 - W_0}{W_1 - W_0} \times 100$$

$$\% \text{ Moisture} = \frac{W_1 - W_3}{W_1 - W_0} \times 100$$

Or % Moisture = 100-% DM

Where, W_0 = weight of empty crucible

W_1 = weight of crucible plus sample (before oven drying)

W_3 = weight of crucible plus oven-dried sample (AOAC, 2005).

3.4.5 Determination of Ash

Two grams of the sample was weighed into a porcelain crucible. This was transferred into the muffle furnace set at 550^{0}C and left for about 4 hours. About this time it had turned to white ash. The crucible and its content were cooled to about 100^{0}C in air, then room temperature in a dessicator and weighed. The percentage ash was calculated from the formula below:

$$\text{Ash content (\%)} = \frac{\text{wt. of ash}}{\text{Original wt. of sample}} \times 100$$

(AOAC, 2005).

3.4.6 Calculation of Nitrogen-Free Extract

The Nitrogen-free extract (NFE) was determined by difference. This was done by subtracting sum of (% Moisture + % crude protein + % Ether Extract + % crude fibre+% Ash) from 100 i.e (100- (% M + % CP + % EE + % CF + % Ash).

3.4.7 Determination of Mineral Composition

The mineral composition of the raw and differently processed almond kernel meals, experimental diets and fish carcass were analyzed after ashing following the standard method of AOAC (2005). Sodium, potassium and calcium were determined by means of flame photometer (Jenway, model PFP 7) while magnesium, iron and phosphorus were determined by means of Atomic Absorption Spectrophotometer (Buck UK, model Accusys 211).

3.4.8 Determination of Anti-Nutritional Factors

The raw and differently processed tropical almond kernel meals were subjected to anti-nutritional factor analysis. Tannin was determined according to the method of Swain (1979), oxalate was determined following the method of Pons and Hoffpauir (1957). Trypsin inhibitor was determined following the casein digestion method of Kakade et al. (1974). Phytate was determined as described by Maga (1983).

3.4.8.1 Determination of Phytate (Phytic acid)

Phytate was determined using the procedure described by Maga (1983). 100mls of 2% concentrated hydrochloric acid was used to soak 2g of each sample in 250ml conical flask for 3 hours. The extract was filtered through a double layer of hardened filter paper. 50mls of each filtrate was placed in 250mls beaker and 100mls of distilled water added to give proper acidity. 100mls of 0.3% ammonium thiocyanate solution was then added into each solution as indicator, the solution was then titrated with standard iron (ii) chloride solution containing 0.00195g iron per ml. The end point showed slightly brownish yellow colour which persisted for 5 minutes. The percentage phytic acid was calculated using the formula:

% Phytic acid = {X x 1.19 x 100/2} x 3.55

Where X = Titre value x 0.00195.

3.5 Digestibility Study

A 28-day digestibility feeding trial was conducted to study the utilization of raw and differently processed tropical almond kernel meals in the diet of *Clarias gariepinus*.

3.6 Formulation and Preparation of Diets for Digestibility Study

Seven isonitrogenous diets of 40% crude protein level as recommended by Faturoti (2000) were formulated with each of the raw and differently processed tropical almond kernel meal (including a control diet without tropical almond kernel meal) using Pearson square method. Apart from the reference diet (control), which lacked almod kernel meal, the other six experimental diets consisted of boiled almond kernel meal (BOAM), soaked almond kernel meal (SOAM), roasted almond kernel meal (ROAM), mechanically extracted almond kernel meal (MEAM), solvent extracted almond kernel meal, (SEAM) and raw almond kernel meal (RWAM) respectively. These meals were mixed homogenously with other ingredients namely fish meal, soybean meal, yellow maize, wheat offal, vitamin and mineral premixes, palm oil, dicalcium phosphate, table salt, cassava starch and chromic oxide marker (Table 1).

Table 1: Ingredient composition of the experimental diets for digestibility study

Ingredient (g/100g DM)	RFRD	BOAM	SOAM	ROAM	MEAM	SEAM	RWAM
Fish meal	26.72	26.72	26.72	26.72	26.72	26.72	26.72
Soya bean meal	39.16	26.72	26.72	26.72	26.72	26.72	26.72
Almond kernel meal	-	35.90	25.88	25.16	22.14	17.34	26.72
Yellow maize	13.06	1.33	6.34	6.70	8.21	10.61	5.92
Wheat bran	13.06	1.33	6.34	6.70	8.21	10.61	5.92
Palm oil	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Cassava starch	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Dicalcium phosphate	1.75	1.75	1.75	1.75	1.75	1.75	1.75
Vitamin/mineral premix	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Table salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Crude Protein (%)	40	40	40	40	40	40	40

RFRD: Reference diet (control); BOAM: Boiled almond kernel meal diet; SOAM: Soaked almond kernel meal diet; ROAM: Roasted almond kernel meal diet; MEAM: Mechanically extracted almond kernel meal diet; SEAM: Solvent extracted almond kernel meal diet; RWAM: Raw almond kernel meal diet; DM: Dry matter

Each ingredient was ground into fine powder, carefully measured and mixed homogenously with other ingredients using hot water. The final mixture (dough) was made into pellets using a local pelleting machine of 2mm die. The feeds were sufficiently sun-dried (for three days), to moisture content of less than ten percent, packed in a polyethylene bag, labeled appropriately and stored in airtight plastic tank in the laboratory.

3.7 Proximate Analysis of the Experimental Diets and Fish

Each of the seven experimental diets was subjected to chemical analysis following the procedure of AOAC (2005) as previously described. Proximate analysis was also carried out on a sample of the experimental fish at the beginning of the experiment. Similarly, at the end of the experiment, ten fish from each treatment were sacrificed and the carcass subjected to proximate analysis as previously described (AOAC, 2005).

3.8 Experimental Design and Procedure

A digestibility study was set up using a Completely Randomized Design (CRD). The experiment was set up in Aquaculture and Fisheries Management laboratory, University of Ibadan, Ibadan, Oyo State, Nigeria. 63-litre rectangular plastic tanks filled with 48 litres of clean water were used for the experiment. 20 *Clarias gariepinus* juveniles (mean weight 9.15±0.07g) were randomly stocked in each tank in triplicate per treatment (that is 21 units in all). The experimental fish were acclimatized for 14 days prior to the commencement of the experiment. The experiment lasted for four weeks between December 2012 and January 2013. The fish were fed to apparent satiation, twice daily (between 08.00- 09.00 hours and 16.00-17.00 hours). Uneaten feed particles were siphoned out of each tank (an hour after feeding) daily with a rubber tube. Fish faeces were collected from each tank daily (before morning feeding) as from a week from the day of commencement of the experiment by siphoning with a rubber tube. The siphoned faeces were filtere with filter paper. The faeces were pooled for each treatment, dried and stored for analysis. Fish mortality in each tank was recorded and used to calculate survival rate for each treatment. The culture water was changed twice weekly with fresh water throughout the experimental period to prevent fouling of the culture water. The length and weight measurements of the fish were done forthnightly. Water quality parameters including temperature, dissolved oxygen and pH were measured forthnightly throughout the period of the experiment.

3.9 Calculation of Growth, Feed Digestibility and Nutrient Utilization Indices

The experimental fish in the seven treatments were weighed weekly throughout the period of the 28-day trial. The effects of various treatments (diets) on growth, feed digestibility and nutrient utilization were evaluated as follows:

3.9.1 Mean Weight Gain (MWG).

MWG = $(W_2 - W_1)$ g

Where, W_1 = initial mean weight of fish at the beginning of the experiment

W_2 = final mean weight of fish at the end of the experiment

3.9.2 Specific Growth Rate (SGR)

$$SGR\ (\%) = \frac{Log_e\ W_2 - Log_e\ W_1}{T} \times 100$$

Where: Log_e = natural logarithm.

W_1 = Initial weight (g) of fish at the beginning of the experiment

W_2 = final weight (g) of fish at the end of the experiment

T = culture period/experimental period in days

3.9.3 Percentage Weight Gain (PWG): This was calculated using the relationship:

PWG (%) = Mean weigth gain / Initial mean weight X 100

3.9.4 Condition Factor (K): Condition factor was calculated using the formula:

$K = W/L^3 \times 100$

Where: W = weight of fish; L = Length of fish

3.9.5 Feed Conversion Ratio (FCR): This was calculated using the formula:

$$FCR = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

3.9.6 Gross Feed Conversion Efficieny (GFCE): This was calculated as a percentage of the reciprocal of feed conversion ratio as follows:

GFCE = 1/FCR X 100

3.9.7 Nitrogen Metabolism (Nm): This was calculated following the method of Bender and Miller (1963) cited in Sotolu (2008) as follows:

$$Nm = \frac{0.549 \times (W_1 + W_2)t}{2}$$

Where W_1 = initial mean weight of fish

W_2 = final mean weight of fish

t = experimental period (in days)

0.549 = metabolism factor/constant

3.9.8 Net Protein Utilization (NPU): NPU was calculated according to the method of Bender and Miller (1963) cited in Sotolu (2008) as follows:

$$NPU = \frac{N_2 - N_1 + Nm}{Nd}$$

Where: N_1 = Nitrogen content of fish before experiment

N_2= nitrogen content of fish at the end of the experiment

Nm= nitrogen metabolism

Nd = nitrogen in experimental diet

Nitrogen content = Protein content / 6.25

3.9.9 Determination of Apparent Digestibility Co-efficient (ADC)

At the beginning of the experiment, the fish in each experimental tank were starved for a day to empty their stomach and to stimulate their appetite. The formulated diets (treatments) were adjusted to accommodate 0.5% chromic oxide (Cr_2O_3) (Table 1). Fish were fed as described under section 3.8. Uneaten feeds were siphoned out of the tanks an hour after feeding in order to prevent contamination of faecal materials with uneaten feed. Fish faeces were collected by siphoning them out of the tanks using a small rubber hose. They were filtered using a filter paper. The faecal

materials were pooled for each treatment, dried and stored for analysis. Samples of experimental feed and faeces were analyzed for protein, energy, dry matter and chromic oxide by the wet acid digestion method of Furukawa and Tsukahara (1966). Samples were digested with concentrated nitric acid and oxidation of chromic oxide with 70% perchloric acid. 50 mg of the sample was put in a kjedahl flask. 5 ml of concentratd nitric acid was added to the flask and the mixture was gently boiled for 20 minutes. The boiled sample was cooled and 3ml of 70% perchloric acid was added to the flask. The resultant mixture was gently heated for another 10 minutes until the solution was then put inside a 100 ml volumetric flask and diluted to 100ml with distilled water. The absorbance of the solution was determined by means of Spectrophotometer (Model, Uvikon 810) at 350 nm. Percentage chromic oxide content was calculated using the following models:

Weight of chromic oxide in sample = Absorbent – 0.0032 / 0.2089

Chromic oxide (%) = (Weight of chromic oxide/ Weight of sample) x 100

Apparent Digestibility Co-efficient of crude protein, dry matter and gross energy in the experimental diets were calculated according to Nwanna (2003) as follows:

$$ADC = 10^2 - \frac{[10^2 \text{ X } (Id \text{ X } Nf)]}{If \text{ x } Nd}$$

Where, Id = % chromic oxide in the diet

If = % chromic oxide in faeces

Nf = Nutrient in fish faeces

Nd = Nutrient in the diet

3.9.10 Survival Rate (SR)

$$S\,R\,(\%) = \frac{\text{initial number of fish stocked} - \text{mortality}}{\text{Initial number of fish stocked}} \times 100$$

3.10 Analysis of Water Quality Parameters

The physico- chemical water quality parameters of the culture media including temperature (^{0}C), dissolved oxygen (mg/litre) and pH were measured weekly using Horiba U-22 XD Multi-parameter water quality checker.

3.11 Formulation and Preparation of Roasted Almond Kernel Meal (ROAM) and Mechanically Extracted Almond Kernel Meal (MEAM) Based Diets

Five isonitrogenous diets containing 40% crude protein (Faturoti, 2000) were formulated using ROAM and MEAM at different inclusion levels of 0.0%, 25.0%, 50.0%, 75.0% and 100.0% in replacement of soya bean meal respectively with 0.0% being the control diet (Tables 2 and 3 respectively). Pearson square method was used in formulating the diets. Other ingredients used in compounding the diets were, fishmeal (72% crude protein), yellow maize, wheat offal, fish premix, palm oil, dicalcium phosphate, table salt and cassava starch. All the ingredients were separately ground and measured according to the formulation. They were thoroughly mixed together to ensure homogeneity and pelletized using local manual pelleting machine of 2mm die. The pelleted feeds were sun- dried properly and packed in separate airtight polyethylene bags. They were stored safely in the experimental site.

3.12 Experimental Set-Up for the Replacement of Soya Bean Meal (SBM) with Graded Levels of ROAM and MEAM in *C. gariepinus* Diet.

20 *Clarias gariepinus* juveniles (average weight of 12.03±0.01 g) were stocked in each 63 litres plastic tank and replicated randomly thrice per treatment. The fish were acclimatized for seven days after which the feeding trial commenced. The fish were fed twice daily between 08:00- 09:30 hours and 16:00- 17:30 hours for 15 weeks at 3% of their body weight. The culture water was changed every three days to maintain the water quality at optimum level. Total cleaning of the tanks was done every six days. The fish were weighed forth-nightly using electronic weighing scale (Tesco Electronic scale, Model: EK4150-STR). Fish mortality in each tank was recorded and used to calculate survival rate for each treatment. Water temperature, pH, dissolved oxygen, ammonia and nitrite were measured at the commencement of the experiment and forth nightly, throughout the period of the experiment.

Table 2: Ingredient composition of roasted almond kernel meal (ROAM) based diets at 0% to 100% inclusion levels for *C. gariepinus* juveniles.

Ingredient (g/100g DM)	Diet				
	1(0%)	2(25%)	3(50%)	4(75%)	5(100%)
Fish meal (72%)	30.95	30.95	30.95	30.95	30.95
Soya bean meal (44%)	30.95	26.59	20.72	12.47	-
Almond kernel meal (28.58%)	-	8.86	20.72	37.41	60.01
Yellow maize (10%)	15.18	12.93	9.93	5.71	0.65
Wheat offal (17%)	15.18	12.93	9.93	5.71	0.65
Palm oil	1.00	1.00	1.00	1.00	1.00
Cassava starch	2.50	2.50	2.50	2.50	2.50
Table salt	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	1.75	1.75	1.75	1.75	1.75
Vitamin/mineral premix	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100
Crude protein (%)	40	40	40	40	40

Legend:

Diet 1 = Control diet (0 % ROAM inclusion)

Diet 2 = 25 % ROAM inclusion

Diet 3 = 50 % ROAM inclusion

Diet 4 = 75 % ROAM inclusion

Diet 5 = 100 % ROAM inclusion

Table 3: Ingredient composition of mechanically extracted almond kernel meal (MEAM) based diets at 0% to 100% inclusion levels for *C. gariepinus* juveniles.

Ingredient (g/100g DM)	Diet				
	1(0%)	2(25%)	3(50%)	4(75%)	5(100%)
Fish meal (72%)	30.95	30.95	30.95	30.95	30.95
Soya bean meal (44%)	30.95	26.09	19.83	11.53	-
Almond kernel meal (30.64%)	-	8.70	19.83	34.59	55.11
Yellow maize (10%)	15.18	13.26	10.82	7.59	3.10
Wheat offal (17%)	15.18	13.26	10.82	7.59	3.10
Palm oil	1.00	1.00	1.00	1.00	1.00
Cassava starch	2.50	2.50	2.50	2.50	2.50
Table salt	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	1.75	1.75	1.75	1.75	1.75
Vitamin/mineral premix	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100
Crude protein (%)	40	40	40	40	40

Legend:

Diet 1 = Control diet (0% MEAM inclusion)

Diet 2 = 25 % MEAM inclusion

Diet 3 = 50 % MEAM inclusion

Diet 4 = 75 % MEAM inclusion

Diet 5 = 100 % MEAM inclusion

3.13 Determination of Survival Rate, Growth and Nutrient Utilization Indices of *Clarias gariepinus* Juveniles Fed Graded Levels of ROAM and MEAM Based Diets

Mean weight gain, percentage weight gain, specific growth rate and condition factor were determined as previously described in section 3.9. Also feed conversion ratio, gross feed conversion efficiency, nitrogen metabolism, net protein utilization and survival rate were determined as previously described (section 3.9).

3.13.1 Determination of Hepatosomatic Index

Six (6) specimens from each treatment were used for this study at the end of the feeding trial. Their body weights were taken, the fish were dissected, their liver were removed and weighed corresponding to their body weight. Hepatosomatic index (HSI) was calculated using the relation:

$$HSI = \frac{\text{liver weight}}{\text{Total body weight}} \times 100$$

3.14 Assessment of Water Quality Parameters.

Water temperature, dissolved oxygen, pH, ammonia and nitrite were measured at the beginning of the experiment after which they were measured fortnightly throughout the period of the experiment. Water temperature, dissolved oxygen and pH were measured using Horiba U-22 XD multi-parameter water quality checker while ammonia and nitrite were measured using Fresh water aquaculture test kit (Model AQ-2, Code 3633-03, Lamotte U. S. A).

3.15 Haematological Assessment

Samples of the experimental fish were tranquilized with 150mg/l of Tricaine Methane Sulphonate for blood collection (Wagner et al., 1997). Blood samples (5mL) were collected at the commencement and termination of the feeding trial by taking blood from the caudal artery using 2ml plastic syringe and needle. These were put into labeled bottles containing lithium heparin as anti-coagulant. The blood samples were analyzed at the Analytical Research Laboratory of the Department of Haematology, Faculty of Veterinary Medicine, University of Ibadan. Haematological parameters which included packed cell volume (PCV), heamoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular haemoglobin

concentration (MCHC) were determined by standard haematological procedures as described by Schalm et al. (1975) and Kelly (1979).

3.16 Packed Cell Volume (PCV)

Pre- heparinised sample bottles were filled with 2ml of blood from the experimental fish and sealed immediately with plasticine. The sample bottles were centrifuged for 5 minutes using a micro haematocrit centrifuge. PCV was then read using the haematocrit reader (Schalm et al., 1975; Kelly, 1979).

3.17 Haemoglobin Concentration (Hb)

About 0.02ml of well mixed blood was added to 4ml of Drabkins solution (potassium ferricyanide, 200 mg Potassium cyanide, 50 mg Potassium dihydrogen phosphate) as described by Kelly (1979). The entire mixture was then made up to 1 litre with distilled water and pH adjusted to neutral (pH = 7.0). The mixture was allowed to stay for about 10 minutes and the haemoglobin was then read photometrically by comparing with a cyanomethaemoglobin standard at 625nm.

3.18 Red Blood Cell and White Blood Cell Counts

Red and white blood cells counts were done using Newbauer haemocytometer as described by Kelly (1979). Red blood cell count was determined by diluting 1:200 of blood sample with Dacies fluid (99ml of 3% aqueous solution of sodium citrate and 1 ml of 40% formaldehyde). White blood cell count was determined by diluting 1:200 of blood sample with 3% aqueous solution of acetic acid and gentian violet added to the mixture later. The mixtures were allowed to settle for 2 minutes, mounted on microscope, and counted.

3.19 Mean Corpuscular Haemoglobin Concentration (MCHC)

Mean corpuscular haemoglobin concentration was calculated using the formular:

$$MCHC = \frac{\text{Heamoglobin concentration}}{\text{PCV (\%)}} \times 100$$

3.20 Serum Biochemical Analysis

Total plasma protein was determined by the biuret method (Reinhold, 1953). Plasma sodium, glucose, chloride and potassium concentrations were determined by using the flame photometer (Falaye et al., 1999). Plasma albumin was determined by the bromocresol method (Doamas et al., 1971), while globulin was determined as described by Coles (1986). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined as described by Toro and Ackermann (1975).

3.21 Histopathological Analysis

At the end of the feeding trial, six fish specimen from each treatment were sacrificed. Their livers, intestines and kidneys were removed and observed for gross lesions. Samples of each of the excised organs were placed in Bouin's fluid for 6 hours, and fixed in 10% buffered formalin and processed through the conventional paraffin embedding technique (Sainte-Marie, 1962; Falaye et al., 1999). Thin sections were mounted on clean glass slides, and stained with haematoxylin and eosin (H & E) for histopathological examination under a light microscope.

3.22 Economic Evaluation of the Diets

Economic evaluation parameters for the diets were calculated following the method of Vincke (1969) cited in Nwanna (2003) as follows:

$$\text{Profit index (PI)} = \frac{\text{value of fish produced}}{\text{Cost of feed used in production}}$$

$$\text{Incidence of Cost (IC)} = \frac{\text{cost of feed used in production}}{\text{fish weight gain}}$$

Economic analysis was based on the following:

1. A major assumption was that all other operating costs for fish production remained the same for all the dietary treatments. Thus, cost of feed was the only economic criterion (expenditure) considered in this study.
2. Cost of feed was based on the prevailing market prices of the feed ingredients as at the time of purchase (that is time of commencement of the experiment)

3. Value of fish produced (cost of fish cropped) depends on the selling price of fish per kilogram (₦ 500/kg) in the markets around Ibadan as at the end of the experiment
4. Cost of producing almond kernel meals depended on the cost of gathering the fruits, de-hulling the fruits and processing the kernels.
5. Total weights of fish produced were obtained from the total weight of fish recovered at the end of the feeding trial.

3.23 Statistical Analysis

Data resulting from the experiment were subjected to one- way analysis of variance (ANOVA) test using Statistical Package for the Social Sciences (SPSS) software. Determination of significant mean differences among individual means was done (at P = 0.05) using Fisher's least significant difference (LSD).

CHAPTER FOUR

5.0 RESULTS

4.1 Amino Acid Profile of Tropical Almond Kernel Meal

The results of amino acid analysis of raw tropical almond kernel meal are shown in Table 4. Glutamate was highest 17.36 g/100g, followed by Arginine, an essential amino acid (13.79 g/100g) while methionine was the least (1.02 g/100g).

4.2 Chemical Composition of Differently Processed Almond Kernel Meals.

The proximate compositions of raw and differently processed tropical almond kernel meals are presented in Table 5 while Table 6 shows mineral and anti-nutritional factors' composition of raw and differently processed tropical almond kernel meals.

All the six methods used in processing tropical almond kernel produced different results of proximate composition. Boiled almond kernel meal (BOAM) yielded the lowest crude protein value of 24.07% followed by soaked almond kernel meal (SOAM) 28.16% while solvent extracted almond kenrnel meal (SEAM) yielded the highest crude protein value of 35.38%, followed by mechanically extracted almond kernel meal (MEAM) and raw almond kernel meal (RWAM, control).

Crude fat content of RWAM was the highest, followed by ROAM while the least was SEAM followed by MEAM. Crude fibre content had a narrow range of 3.33% to 3.79%. SEAM had the highest value followed by RWAM then SOAM while ROAM had the least value. There was a significant difference ($p<0.05$) among the crude fibre values. However, there was no significant difference ($p>0.05$) between the crude fibre values of SEAM and RWAM. Value for ash was highest in SEAM (4.87%) followed by RWAM (4.28%), ROAM (3.95) and MEAM (3.76%) while SOAM had the least value (2.78%) followed by BOAM (3.31%). Moisture content was lowest in ROAM (4.85%) followed by RWAM (5.81%) then MEAM (8.52%) while BOAM had the highest value (13.65) followed by SOAM (13.18%).

Table 4: Amino acid profile of raw tropical almond kernel meal.

Amino Acid	Amount (g/100g)
Glycine	4.01
Alanine	4.36
Serine	5.53
Proline	1.22
*Valine	5.37
*Threonine	5.53
*Isoleucine	4.26
*Leucine	7.37
Aspartate	9.12
*Lysine	6.60
*Methionine	1.02
Glutamate	17.36
*Phenylalanine	4.55
*Histidine	2.48
*Arginine	13.79
Tyrosine	3.01
Cystine	2.13

*Essential amino acid

Source: Field data

Table 5: Proximate composition of raw and differently processed tropical almond kernel meals (Means ± SEM)

Parameters (%)	Processing treatments					
	BOAM	SOAM	ROAM	MEAM	SEAM	RWAM
Crude protein	24.07±0.08^{a}	28.16±0.06^{b}	28.58±0.11^{c}	30.64±0.07^{d}	35.38±0.06^{e}	28.73±0.05^{c}
Crude fibre	3.58±0.01^{c}	3.65±0.01^{d}	3.33±0.01^{a}	3.49±0.02^{b}	3.79±0.01^{e}	3.75±0.01^{e}
Crude fat	20.86±0.01^{d}	18.38±0.02^{c}	29.86±0.02^{e}	7.28±0.02^{b}	1.86±0.02^{a}	30.27±0.02^{e}
Ash	3.31±0.02^{b}	2.78±0.01^{a}	3.95±0.01^{d}	3.76±0.01^{c}	4.87±0.01^{f}	4.28±0.02^{e}
Moisture	13.65±0.01^{f}	13.18±0.02^{e}	4.85±0.02^{a}	8.52±0.02^{c}	8.86±0.02^{d}	5.81±0.01^{b}
Nitrogen-free extract	34.78±0.10^{d}	34.01±0.09^{c}	29.18±0.11^{b}	46.15±0.07^{f}	45.24±0.06^{e}	28.16±0.41^{a}
*Gross energy	4.33±0.02^{bc}	4.30±0.01^{bc}	4.43±0.02^{ab}	3.32±0.02^{d}	3.32±0.02^{d}	4.87±0.02^{a}

Mean values in each row with similar superscripts are not significantly different ($p>0.05$).

*Unit of Gross Energy not % but Kcal/g

Legend:

BOAM = Boiled almond kernel meal

SOAM = Soaked almond kernel meal

ROAM = Roasted almond kernel meal

MEAM = Mechanically extracted almond kernel meal

SEAM= Solvent extracted almond kernel meal

RWAM = Raw almond kernel meal

Table 6: Mineral and anti-nutritional factors (ANFs) composition of raw and differently processed tropical almond kernel meals. Means ± SEM

Minerals and ANFs (%)	Processing treatments					
	BOAM	SOAM	ROAM	MEAM	SEAM	ROAM
Sodium	0.052±0.00^{c}	0.064±0.00^{d}	0.045±0.00^{b}	0.035±0.00^{a}	0.091±0.00^{f}	0.078±0.00^{e}
Potassium	0.256±0.01^{b}	0.255±0.00^{b}	0.367±0.00^{c}	0.260±0.00^{b}	0.235±0.00^{a}	0.261±0.00^{b}
Calcium	0.26±0.01^{cd}	0.23±0.01^{bc}	0.29±0.02^{d}	0.27±0.01^{cd}	0.22±0.01^{b}	0.20±0.01^{a}
Magnesium	0.252±0.00^{a}	0.272±0.00^{a}	0.269±0.02^{a}	0.264±0.00^{a}	0.329±0.00^{c}	0.299±0.00^{b}
Phosphorus	0.33±0.01^{c}	0.22±0.01^{a}	0.50±0.01^{e}	0.42±0.01^{d}	0.27±0.01^{b}	0.21±0.01^{a}
Iron	0.031±0.00^{ab}	0.024±0.00^{a}	0.035±0.00^{ab}	0.046±0.00^{bc}	0.027±0.00^{a}	0.059±0.00^{c}
Oxalate	0.026±0.00^{c}	0.029±0.00^{c}	0.013±0.00^{a}	0.021±0.00^{b}	0.040±0.00^{d}	0.051±0.00^{e}
Phytate	0.052±0.00^{c}	0.065±0.00^{d}	0.035±0.00^{a}	0.043±0.00^{b}	0.074±0.00^{e}	0.092±0.00^{f}
Tannin	0.036±0.00^{c}	0.050±0.00^{d}	0.026±0.00^{a}	0.032±0.00^{b}	0.052±0.00^{d}	0.065±0.00^{e}
*Trypsin inhibitor (TIU/mg)	5.66±0.02^{c}	7.17±0.02^{d}	0.01±0.00^{a}	3.09±0.01^{b}	13.90±0.02^{e}	22.47±0.03^{f}

Mean values in each row with similar superscripts are not significantly different ($p>0.05$).

* Unit of Trypsin inhibitor is TIU/mg

Nitrogen-free extract (NFE) ranged between 28.16% and 46.15%. The highest value was observed in MEAM, followed by SEAM while RWAM had the least value. There were significant differences ($p<0.05$) among the treatments' NFE values. Gross energy values ranged between 3.32 kcal/g and 4.87 kcal/g with the highest value observed in RWAM while the least value was observed in SEAM.

Sodium values ranged between 0.035% and 0.091 % while potassium ranged between 0.226% and 0.367%. Calcium was highest in ROAM and least in RWAM. Magnesium was highest in SEAM and least in BOAM. Phosphorus was highest in ROAM (0.50%) followed by MEAM (0.42%) and least in RWAM (0.21%). Phosphorus content of SOAM and RWAM were similar ($P>0.05$). There were significant differences ($p<0.05$) in the mineral content of the differently processed almond kernel meals.

Oxalate was highest in RWAM followed by SEAM and least in ROAM followed by MEAM. Phytate was highest in RWAM (0.092%) and least in ROAM (0.035%) followed by BOAM (0.052%). Tannin ranged between 0.026% and 0.065% while trypsin inhibitor ranged between 0.01 and 22.47 TIU/mg.

4.2.1 Proximate and Gross Energy Composition of Experimental Diets for Digestibility Study

Proximate and gross energy compositions of the seven experimental diets used for digestibility study are presented in Table 7. Crude protein content of the experimental diets ranged between 40.03 to 40.24%. Crude fat was highest in RWAM diet followed by ROAM diet and least in SEAM diet. Crude fibre ranged between 2.45% and 3.34%. Ash content was highest in ROAM diet followed by MEAM diet and least in BOAM diet. Moisture content of the diets ranged between 5.04% and 6.23%. NFE was highest in MEAM diet, followed by SEAM diet and least in SOAM diet. Gross Energy ranged between 3,478.67 kcal/kg and 3,698.00 kcal /kg.

Table 7: Proximate and gross energy contents (kcal/kg) of differently processed tropical almond kernel meal based diets fed to *Clarias gariepinus* juveniles during digestibility study. Means ± SEM

Diet	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	Moisture (%)	NFE (%)	Gross energy [Kcal/kg]
RFRD	40.10±0.01^{a}	5.95±0.02^{ab}	2.98±0.02^{a}	13.20±0.04^{a}	5.14±0.01^{a}	32.63±0.02^{b}	3,504.00±2.89^{c}
BOAM	40.24±0.02^{a}	5.06±0.02^{ab}	2.45±0.01^{a}	12.85±0.02^{a}	6.23±0.03^{a}	33.17±0.02^{b}	3,478.67±1.45^{a}
SOAM	40.08±0.01^{a}	6.86±0.01^{b}	2.95±0.01^{a}	13.01±0.01^{a}	5.85±0.02^{a}	31.25±0.01^{ab}	3,601.00±2.89^{e}
ROAM	40.05±0.02^{a}	6.92±0.01^{b}	3.30±0.01^{a}	12.87±0.01^{a}	5.04±0.02^{a}	31.82±0.02^{ab}	3,665.00±2.89^{f}
MEAM	40.23±0.10^{a}	4.93±0.02^{ab}	2.56±0.02^{a}	13.09±0.01^{a}	5.47±0.02^{a}	33.72±0.10^{b}	3,487.00±2.31^{b}
SEAM	40.03±0.09^{a}	4.71±0.02^{a}	2.81±0.01^{a}	13.21±0.01^{a}	5.66±0.02^{a}	33.58±0.09^{b}	3,529.33±2.40^{d}
RWAM	40.18±0.01^{a}	6.99±0.02^{b}	3.34±0.01^{a}	13.04±0.01^{a}	5.64±0.02^{a}	30.81±0.01^{a}	3,698.00±3.46^{f}

Mean values in each column with similar superscripts are not significantly different ($p>0.05$)

Legend:

RFRD: Reference diet (Control)

BOAM: Boiled almond kernel meal based diet

SOAM: Soaked almond kernel meal based diet

ROAM: Roasted almond kernel meal based diet

MEAM: Mechanically extracted almond kernel meal based diet

SEAM: Solvent extracted almond kernel meal based diet

RWAM: Raw almond kernel meal based diet

NFE = Nitrogen-free extract

4.3 Carcass Proximate and Mineral Composition of *Clarias gariepinus* Juveniles Fed Differently Processed Tropical Almond Kernel Meal Based Diets during Digestibility Study

The proximate composition of *Clarias gariepinus* juveniles fed differently processed tropical almond kernel meal based diets during digestibility study is presented in Table 8 while the mineral composition is presented in Table 9. Initial crude protein of the fish carcass before commencement of the experiment was 48.41%. At the end of the experiment, it increased in all the treatments. There was a significant difference ($p<0.05$) between the initial crude protein value and the final values. There were also significant differences among the treatment's crude protein values. Similarly, there was a significant difference between the initial crude fat value of the experimental fish carcass and the final values. There were also significant differences ($p<0.05$) among the treatments' crude fat values. The same trend was observed for ash content of the experimental fish carcass. In contrast, nitrogen- free extract (NFE) of the initial fish carcass was significantly higher ($p<0.05$) than the treatments' final NFE values.

Table 9 shows that there were significant differences ($p<0.05$) in sodium values between the dietary treatments and the initial value ($p<0.05$) and between carcass of fish fed the reference diet and fish fed the other diets. Fish fed BOAM was significantly higher ($p<0.05$) in potassium values than initial sample, RFRD, ROAM, MEAM, SEAM and RWAM. No significant difference ($p>0.05$) was observed in potassium values between carcass of fish fed BOAM and fish fed SOAM and between carcass of fish fed ROAM and fish fed RWAM. Significant differences ($p<0.05$) were observed for carcass calcium, phosphorus and iron values among the treatments.

4.4 Growth, Nutrient Utilization and Digestibility Indices of *Clarias gariepinus* Juveniles Fed Differently Processed Tropical Almond Kernel Meal Based Diets.

There were significant variations in some of the growth and nutrient utilization indices considered (Table 10). Mean weight gain (MWG) was highest in fish fed ROAM, followed by fish fed MEAM, then fish fed BOAM and least in fish fed SOAM. A detailed mean weight change of experimental fish was shown in Figure 1. Specific growth rate (SGR) was highest in fish fed ROAM and least in fish fed SOAM. There were significant differences ($p<0.05$) among

Table 8: Proximate composition of *Clarias gariepinus* juveniles fed differently processed tropical almond kernel meal based diets during digestibility study. Means ± SEM

Treatments	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	Moisture (%)	NFE (%)
Initial	48.41±0.04^{a}	6.84± 0.01^{a}	0.01±0.00^{a}	5.16±0.03^{a}	6.20±0.01^{e}	33.39±0.07^{g}
RFRD	61.82±0.04^{d}	12.52±0.01^{d}	0.02±0.01^{a}	8.63±0.01^{b}	5.18±0.01^{a}	11.83±0.07^{e}
BOAM	62.13±0.10^{e}	12.96±0.01^{ef}	0.10±0.01^{c}	9.25±0.01^{f}	5.52±0.01^{c}	10.04±0.03^{c}
SOAM	58.84±0.14^{b}	12.84±0.01^{e}	0.09±0.01^{c}	8.76±0.01^{c}	6.17±0.02^{e}	13.30±0.14^{f}
ROAM	62.46±0.08^{f}	13.02±0.01^{ef}	0.01±0.01^{a}	9.20±0.01^{e}	5.61±0.01^{d}	9.70±0.02^{b}
MEAM	63.68±0.06^{g}	12.22±0.01^{c}	0.06±0.01^{b}	9.29±0.01^{f}	6.37±0.01^{f}	8.38±0.01^{a}
SEAM	62.36±.07^{ef}	11.93±0.01^{b}	0.12±0.01^{d}	8.90±0.02^{d}	6.34±0.02^{f}	10.35±0.03^{d}
RWAM	60.13±0.08^{c}	13.13±0.01^{f}	0.04±0.01^{b}	9.42±0.01^{g}	5.31±0.02^{b}	11.97±0.03^{e}

Mean values in each column with similar superscripts are not significantly different ($p>0.05$)

Legend: RFRD: Reference diet (control); BOAM: Boiled almond kernel meal based diet; SOAM: Soaked almond kernel meal based diet; ROAM: Roasted almond kernel meal based diet; MEAM: Mechanically extracted almond kernel meal based diet; SEAM: Solvent extracted almond kernel meal based diet; RWAM: Raw almond kernel meal based diet; NFE = Nitrogen-free extract

Table 9: Mineral composition of *Clarias gariepinus* juvenile fed differently processed tropical almond kernel meal based diets during digestibility study. MEAN ± SEM

Treatments	Minerals (mg/g)					
	Sodium	Potassium	Magnessium	Calcium	Phosphorus	Iron
Initial sample	8.90 ± 0.02^{g}	10.50 ± 0.01^{f}	3.70 ± 0.01^{d}	22.26 ± 0.02^{a}	15.30 ± 0.02^{a}	4.70 ± 0.01^{f}
RFRD	11.50 ± 0.01^{e}	18.40 ± 0.02^{b}	5.40 ± 0.01^{c}	27.00 ± 0.02^{b}	28.10± 0.02^{bc}	6.10 ± 0.01^{e}
BOAM	14.50 ± 0.02^{a}	19.50 ± 0.01^{a}	7.40 ± 0.01^{a}	32.80 ± 0.03^{c}	29.70± 0.03^{c}	7.90 ± 0.01^{a}
SOAM	13.80 ± 0.01^{b}	19.10 ± 0.02^{a}	6.70 ± 0.01^{b}	31.40 ± 0.02^{bc}	29.10± 0.02^{c}	6.90 ± 0.01^{c}
ROAM	13.40 ± 0.01^{bc}	17.80 ± 0.01^{c}	6.30 ± 0.01^{b}	28.80 ± 0.02^{b}	28.50± 0.03^{bc}	7.20 ± 0.01^{bc}
MEAM	12.60 ± 0.01^{d}	16.50 ± 0.01^{d}	5.60 ± 0.01^{c}	30.20 ± 0.03^{bc}	27.30± 0.02^{b}	5.80 ± 0.01^{e}
SEAM	10.90 ± 0.01^{f}	15.90 ± 0.01^{e}	6.60 ± 0.01^{b}	29.20 ± 0.02^{b}	28.50± 0.02^{bc}	7.50 ± 0.01^{b}
RWAM	13.10 ± 0.01^{c}	17.50 ± 0.01^{c}	6.30 ± 0.01^{b}	30.80 ± 0.03^{bc}	29.10± 0.02^{c}	6.50 ± 0.01^{d}

Means with same letters along the column are not significantly different at p=0.05

Legend: RFRD: Reference diet (control); BOAM: Boiled almond kernel meal based diet; SOAM: Soaked almond kernel meal based diet; ROAM: Roasted almond kernel meal based diet; MEAM: Mechanically extracted almond kernel meal based diet; SEAM: Solvent extracted almond kernel meal based diet; RWAM: Raw almond kernel meal based diet.

Table 10: Nutrient utilization and growth performance indices of *Clarias gariepinus* juveniles fed differently processed almond kernel meal based diets for 28 days (Digestibility study). Means ± SEM

Parameters	Experimental diets						
	RFRD	BOAM	SOAM	ROAM	MEAM	SEAM	RWAM
Initial mean wt. (g)	9.08 ± 0.03 [a]	9.17 ± 0.39 [a]	9.07 ± 0.11 [a]	9.21 ± 0.15 [a]	9.20 ± 0.30 [a]	9.10 ± 0.33 [a]	9.22 ± 0.23 [a]
Final mean wt. (g)	14.80 ± 0.72 [a]	15.80 ± 1.05 [a]	13.92 ± 0.21 [a]	18.12 ± 1.30 [b]	15.86 ± 0.89 [a]	14.13 ± 0.86 [a]	15.04 ± 0.85 [a]
Mean wt. gain (g)	5.71 ± 0.72 [ab]	6.64±0.66 [abc]	4.85±0.10 [a]	8.90±1.30 [c]	6.66±0.60 [abc]	5.03±0.61 [a]	5.83±0.72 [ab]
SGR (%)	1.74±0.19 [c]	1.95±0.22 [ab]	1.53±0.06 [b]	2.42±0.27 [a]	1.95±0.12 [ab]	1.57±0.17 [c]	1.75±0.16 [c]
PWG (%)	62.90±8.00 [b]	72.06±4.21 [c]	53.52±0.61 [a]	96.65±14.17 [c]	72.17±4.00 [c]	55.06±5.63 [a]	63.12±7.11 [b]
Mean feed intake (g)	8.29±0.45 [a]	8.53±0.13 [a]	7.56±0.07 [a]	8.64±0.46 [a]	8.16±0.58 [a]	7.84±0.55 [a]	8.50±0.80 [a]
FCR	1.45±0.14 [a]	1.28±0.15 [b]	1.56±0.09 [a]	0.97±0.16 [b]	1.23±0.07 [b]	1.56±0.14 [a]	1.46±0.08 [a]
GFCE (%)	68.97±6.94 [b]	78.13±9.15 [b]	64.10±3.47 [b]	103.09±8.08 [a]	81.30±3.99 [b]	64.10±5.45 [b]	68.49±3.34 [b]
Nitrogen metabolism	183.54±5.55 [ab]	191.92±11.05 [ab]	176.67±2.45 [a]	210.09±10.06 [b]	192.64±9.18 [ab]	178.52±8.82 [a]	186.46±7.86 [ab]
NPU (%)	28.94±0.86 [a]	30.15±1.72 [a]	27.81±0.38 [a]	30.74±1.57 [a]	30.31±1.43 [a]	28.22±1.38 [a]	29.29±1.22 [a]
Survival rate (%)	98.33±1.67 [a]	98.33±1.67 [a]	98.33±1.67 [a]	100.00±0.00 [a]	98.33±1.67 [a]	98.33±1.67 [a]	98.33±1.67 [a]
Condition factor	0.64±0.01 [a]	0.66±0.06 [a]	0.62±0.02 [a]	0.65±0.04 [a]	0.66±0.09 [a]	0.61±0.06 [a]	0.65±0.05 [a]

Mean values in each row with similar superscripts are not significantly different ($p>0.05$).

Legend: RFRD: Reference diet (control); BOAM: Boiled almond kernel meal based diet; SOAM: Soaked almond kernel meal based diet; ROAM: Roasted almond kernel meal based diet; MEAM: Mechanically extracted almond kernel meal based diet; SEAM: Solvent extracted almond kernel meal based diet; RWAM: Raw almond kernel meal based diet; SGR= specific growth rate, PWG= percentage weight gain, FCR= feed conversion ratio, GFCE= gross feed conversion efficiency, NPU= net protein utilization, wt = weight

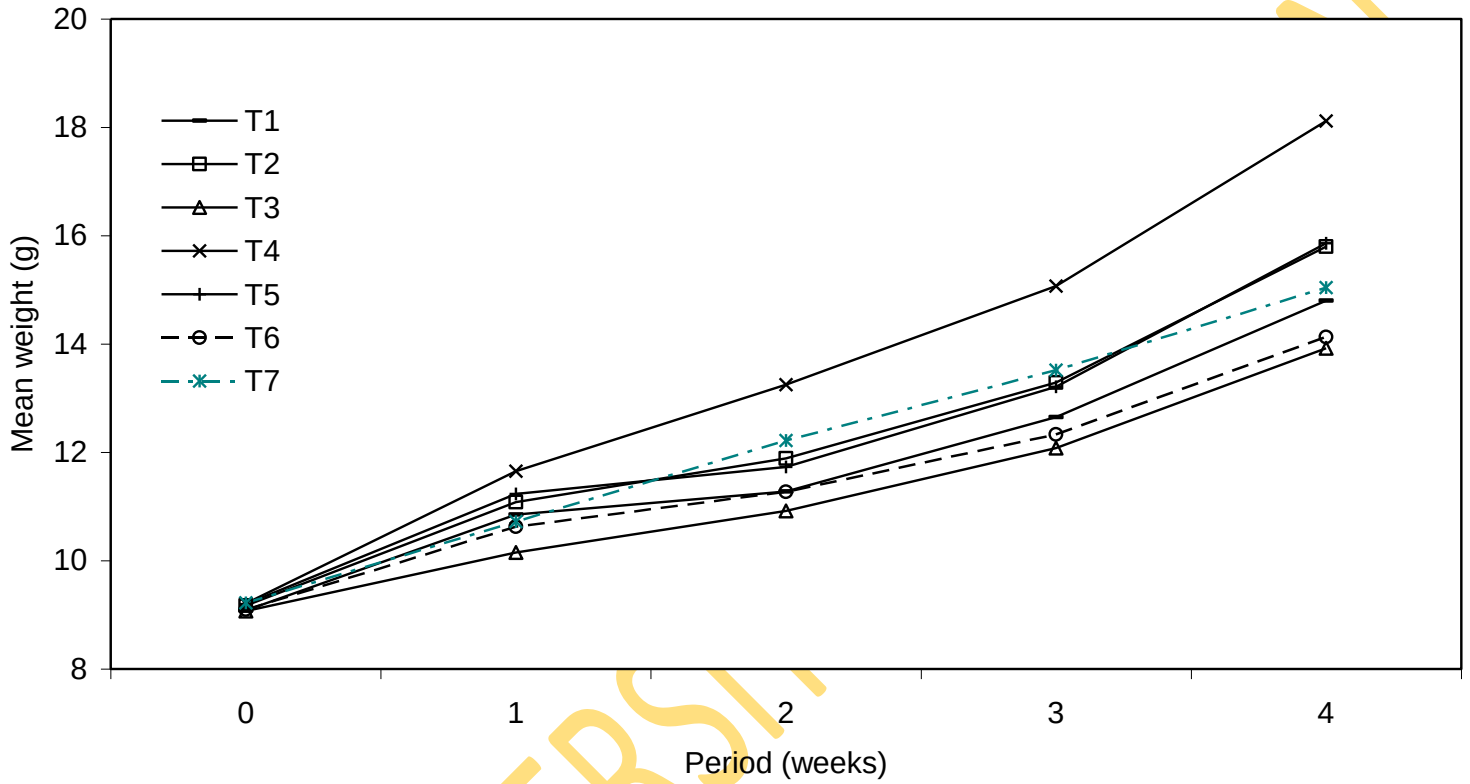

Figure 1: Mean weekly weight changes of *Clarias gariepinus* juveniles fed raw and differently processed tropical almond kernel meal based diets during digestibility study

Legend:

T1= RFRD; T2= BOAM; T3= SOAM; T4= ROAM; T5= MEAM; T6= SEAM; T7= RWAM

the SGR values of the treatments. Survival rate ranged between 98.33% and 100%. There was no significant difference ($p>0.05$) in the survival rate of fish amongst the dietary treatments.

Fish fed SOAM consumed the least amount of feed followed by fish fed MEAM while fish fed ROAM consumed the highest amount of feed. There was no significant difference ($p>0.05$) in the mean feed intake values. Feed conversion ratio (FCR) was least in fish fed ROAM followed by fish fed MEAM, then fish fed BOAM and highest in fish fed SEAM and SOAM. There were significant differences ($p<0.05$) in the FCR values. Fish fed ROAM had the highest Nitrogen metabolism (Nm) value, followed by fish fed MEAM while fish fed SOAM had the least value. Net protein utilization (NPU) was highest in fish fed ROAM and least in fish fed SOAM. There was no significant difference ($p>0.05$) in the NPU values.

Apparent digestibility co-efficient (ADC) of crude protein was highest in ROAM (89.03%), followed by RFRD (88.95%) and MEAM (88.92%) (Table 11). Fish fed SEAM had the least ADC of crude protein (87.06%). There were significant differences ($p<0.05$) in the ADC of crude protein values amongst the treatments. Apparent digestibility co-efficient of gross energy was highest in fish fed ROAM and least in fish fed RFRD (control diet). There were significant differences ($p<0.05$) among the treatments' ADC of gross energy values. Similar trend was observed for apparent digestibility co-efficient of dry matter with significant differences ($p<0.05$) among the treatments. Fish fed MEAM and ROAM had the highest ADC of dry matter value while fish fed SEAM had the least value.

4.5 Results of Water Quality Parameters Assessed during Digestibility Study

Results of water quality parameters monitored during digestibility experiment are presented in Table 12. Initial pH of the culture media was 6.95 while final treatments' values ranged from 6.85 to 6.99. Initial dissolved oxygen content of the culture media was 5.19 mg/l while final values ranged between 4.27 mg/l and 4.78 mg/l. Water temperature was 27.95 ^{o}C for the initial value. This fell in all the treatments by the end of the study to a narrow range of 25.00 ^{o}C to 25.10 ^{o}C.

Table 11: Digestibility indices of nutrients by *Clarias gariepinus* juveniles fed processed tropical almond kernel meal based diets for 28 days .Means ± SEM

Digestibility indices (%)	Experimental diets						
	RFRD	BOAM	SOAM	ROAM	MEAM	SEAM	RWAM
ADC of crude protein	88.95±0.06^{d}	88.40±0.06^{c}	87.59±0.06^{b}	89.03±0.05^{d}	88.92±0.18^{d}	87.06±0.06^{a}	87.70±0.06^{b}
ADC of gross energy	86.80±0.02^{a}	86.91±0.01^{b}	87.42±0.01^{c}	88.51±0.02^{g}	88.19±0.01^{f}	87.49±0.01^{d}	88.08±0.03^{e}
ADC of dry matter	72.27±0.00^{c}	72.60±0.02^{e}	72.49±0.00^{d}	74.54±0.01^{f}	74.54±0.00^{f}	71.84±0.01^{a}	72.03±0.01^{b}

Mean values in each row with similar superscripts are not significantly different (p>0.05).

Legend: RFRD: Reference diet (control); BOAM: Boiled almond kernel meal based diet; SOAM: Soaked almond kernel meal based diet; ROAM: Roasted almond kernel meal based diet; MEAM: Mechanically extracted almond kernel meal based diet; SEAM: Solvent extracted almond kernel meal based diet; RWAM: Raw almond kernel meal based diet; ADC: Apparent digestibility Co-efficient

Table 12: Initial and final treatment values of water quality parameters monitored during digestibility study. Means ± SEM

Treatments	Water quality parameters		
	pH	Dissolved oxygen (mg/L)	Temperature (^{o}C)
Initial values	6.95±0.00	5.19±0.01	27.95±0.00
RFRD (Control)	6.99±0.01	4.78±0.01	25.00±0.03
BOAM	6.85±0.02	4.62±0.01	25.00±0.03
SOAM	6.93±0.01	4.27±0.01	25.05±0.00
ROAM	6.85±0.02	4.47±0.01	25.05±0.03
MEAM	6.95±0.01	4.65±0.02	25.00±0.00
SEAM	6.85±0.01	4.42±0.01	25.05±0.03
RWAM	6.91±0.01	4.60±0.01	25.10±0.03

Legend: RFRD: Reference diet (control); BOAM: Boiled almond kernel meal based diet; SOAM: Soaked almond kernel meal based diet; ROAM: Roasted almond kernel meal based diet; MEAM: Mechanically extracted almond kernel meal based diet; SEAM: Solvent extracted almond kernel meal based diet; RWAM: Raw almond kernel meal based diet.

4.6 Proximate Composition of Roasted Almond Kernel Meal (ROAM) and Mechanically Extracted Almond Kernel Meal (MEAM) Based Diets.

Proximate composition and gross energy content of ROAM and MEAM based diets are shown in Tables 13 and 14 respectively. ROAM and MEAM were used to replace Soya bean meal (SBM) at inclusion levels of 0%, 25%, 50%, 75% and 100% each.

Gross energy of ROAM based diets were 3,802.00 kcal/kg, 3,803.00 kcal/kg, 3,809.00 kcal/kg, 3,899.00 kcal/kg and 3,995.00 kcal/kg in diets 1, 2, 3, 4 and 5 respectively (Table 13). Calorie/protein ratio ranged between 94.86 and 99.83. Gross Energy content of MEAM based diets ranged between 3,802.00 kcal/kg and 3,914.00 kcal/kg while Calorie/protein ratio ranged between 94.87 and 97.68 (Table 14).

Crude protein content of ROAM based diets ranged between 39.96% and 40.10 %. There was no significant difference ($p>0.05$) in the crude protein content of the diets. For MEAM based diets, crude protein content ranged between 39.98% and 40.08%. There was no significant difference ($p>0.05$) in the crude protein content of the diets.

Crude fat content of ROAM based diets varied significantly ($P<0.05$) with a range between 4.07% and 20.59%. Diet 2 (25%) had the least fat content while diet 5 (100%) had the highest value. Crude fat content of MEAM based diets significantly ($P<0.05$) varied with a range between 4.18% and 10.04%. Diet 1 (0%, control) had the least value (4.18%) while diet 5 (100%) had the highest value (10.04%).

Crude fibre content was highest (2.98%) in diet 5 (100% ROAM inclusion) and least (2.51%) in diet 2 (25% ROAM inclusion). For MEAM based diets crude fibre was highest (2.78%) in diet 4 (75% inclusion) and least (2.55%) in control diet (0% inclusion). There was no significant difference ($p>0.05$) in the crude fibre content of ROAM and MEAM based diets.

Ash content ranged between 11.68% and 13.25% for ROAM based diets. For MEAM based diets ash content was highest (13.25%) in control diet and least (12.37%) in diet 5 (100% inclusion).

Table 13: Proximate composition and gross energy contents of ROAM based diets at inclusion levels of 0% to 100%. Means ± SEM

Parameters	Diet				
	1(0%) (Control)	2 (25%)	3 (50%)	4 (75%)	5 (100%)
Crude protein (%)	40.08±0.02^{a}	39.96±0.06^{a}	40.10±0.06^{a}	40.06±0.02^{a}	40.02±0.03^{a}
Crude fat (%)	4.18±0.01^{a}	4.07±0.01^{a}	6.38±0.02^{b}	10.40±0.04^{c}	20.59±0.02^{d}
Crude fibre (%)	2.55±0.01^{a}	2.51±0.05^{a}	2.67±0.03^{a}	2.71±0.06^{a}	2.98±0.02^{a}
Ash (%)	13.25±0.01^{b}	13.31±0.01^{b}	13.21±0.01^{b}	13.19±0.01^{b}	11.68±0.03^{a}
Moisture (%)	4.84±0.01^{a}	4.79±0.01^{a}	4.90±0.00^{a}	4.88±0.02^{a}	5.20±0.01^{a}
Nitrogen-free extract (%)	35.11±0.03^{d}	35.38±0.05^{e}	34.74±0.04^{c}	28.76±0.11^{b}	19.53±0.07^{a}
Gross Energy (Kcal /kg)	3,802.00±8.00^{a}	3,803.00±10.50^{a}	3,809.00±10.00^{a}	3,899.00±2.50^{b}	3,995.00±4.00^{c}
Calorie/protein ratio	94.86±0.25^{a}	95.17±0.14^{a}	94.99±0.38^{a}	97.33±0.02^{b}	99.83±0.03^{c}

Mean values in each row with similar superscripts are not significantly different (p>0.05).

Table 14: Proximate composition and gross energy contents of MEAM based diets at inclusion levels of 0% to 100%. Means ± SEM

Parameters	Diet				
	1(0%) (Control)	2 (25%)	3 (50%)	4 (75%)	5 (100%)
Crude protein (%)	40.08±0.02^{a}	40.06±0.01^{a}	39.98±0.03^{a}	40.04±0.01^{a}	40.07±0.02^{a}
Crude fat (%)	4.18±0.01^{a}	4.19±0.01^{a}	5.46±0.01^{b}	8.57±0.02^{c}	10.04±0.01^{d}
Crude fibre (%)	2.55±0.01^{a}	2.59±0.01^{a}	2.66±0.01^{a}	2.78±0.01^{a}	2.69±0.01^{a}
Ash (%)	13.25±0.01^{b}	13.03±0.02^{b}	12.59±0.01^{a}	13.05±0.01^{b}	12.37±0.02^{a}
Moisture (%)	4.84±0.01^{a}	5.47±0.01^{b}	6.03±0.01^{c}	6.19±0.01^{c}	5.92±0.01^{bc}
Nitrogen-free extract (%)	35.11±0.03^{e}	34.67±0.02^{d}	33.29±0.03^{c}	29.37±0.02^{b}	28.92±0.03^{a}
Gross Energy (Kcal /kg)	3,802.00±8.00^{a}	3,810.00±5.00^{a}	3,898.00±1.00^{b}	3,905.00±5.00^{b}	3,914.00±3.00^{b}
Calorie/protein ratio	94.86±0.25^{a}	95.11±0.10^{a}	97.50±0.05^{b}	97.53±0.10^{b}	97.68±0.12^{b}

Mean values in each row with similar superscripts are not significantly different (p>0.05).

Nitrogen free extract (NFE) was highest in diet 2 (25% ROAM inclusion) and least in diet 5 (100% ROAM inclusion). For MEAM based diets, NFE ranged between 28.92% in diet 5 (100% inclusion) and 35.11% in diet 1 (control).

4.7 Proximate and Mineral Composition of *Clarias gariepinus* Juveniles' Carcass Before and After Feeding with ROAM and MEAM Based Diets.

Initial crude protein value of the experimental fish was 50.28% and it increased in all the final treatments' values as shown in Table 15 and 16 for ROAM and MEAM based dietary treatments respectively. Carcass of fish fed diet 4 (75% ROAM) had the highest crude protein content while carcass of fish fed diet 5 (100% ROAM) had the least value. There were significant differences ($p<0.05$) among the treatments' carcass crude protein values. For MEAM based diets, fish fed diet 2 (25% MEAM) had the highest crude protein content while fish fed diet 5 had the least value. Crude fat content of initial fish carcass was 4.12%. This increased in all the final values to a range of 5.84% to 6.25% and 5.85% to 6.18% for ROAM and MEAM based diets respectively. Initial fibre content of fish was 0.01%. This increased in all the final treatment values to a narrow range of 0.03% to 0.09% and 0.03% to 0.08% in carcass of fish fed ROAM and MEAM based diets respectively.. Initial ash content was 7.20%. This increased in all the final treatment values to a range of 10.51% to 10.95% and 11.37% to 11.90% in carcass of fish fed ROAM and MEAM based diets respectively. Nitrogen free extract (NFE) was 31.62% in the initial fish sample. This reduced in all the final treatment values in carcass of fish fed ROAM and MEAM based diets. There were significant differences ($p<0.05$) among the treatments' NFE values.

For ROAM based diets, initial carcass sodium was 2.40 mg/g (Table 17). This significantly increased at the end of the experiment to a range between 3.60mg/g and 4.40mg/g. Initial carcass magnesium value was 1.90mg/g. This also significantly increased at the end of the experiment to a range between 3.50mg/g and 4.30mg/g. Similar trend was observed for carcass potassium, phosphorus, calcium and iron contents. For MEAM based diets, treatments' final magnesium, potassium, phosphorus, calcium and iron content ranged from 2.50 to 3.50mg/g, 10.90 to 12.90mg/g, 21.00 to 22.20mg/g, 28.70 to 29.70mg/g and 3.19 to 3.36mg/g respectively (Table 18).

Table 15: Proximate composition of *Clarias gariepinus* juveniles before and after feeding with ROAM based diets at 0% to 100% inclusion levels. Means ± SEM

Parameters (%)	Initial	Diet 1 0% (Control)	Diet 2 25%	Diet 3 50%	Diet 4 75%	Diet 5 100%
Crude protein	50.28±0.11^{a}	66.97±0.06^{e}	65.55±0.05^{d}	64.89±0.03^{c}	66.95±0.10^{e}	57.26±0.10^{b}
Crude fat	4.12±0.01^{a}	5.79±0.02^{b}	6.25±0.02^{d}	6.04±0.02^{c}	5.84±0.02^{b}	6.07±0.03^{c}
Crude fibre	0.01±0.00^{a}	0.03±0.00^{a}	0.08±0.01^{a}	0.05±0.01^{a}	0.09±0.01^{a}	0.05±0.02^{a}
Ash	7.20±0.02^{a}	10.85±0.02^{d}	10.95±0.02^{e}	10.87±0.02^{d}	10.65±0.02^{c}	10.51±0.03^{b}
Moisture	6.79±0.02^{e}	5.17±0.02^{cd}	5.13±0.01^{c}	5.04±0.02^{b}	5.19±0.02^{d}	4.55±0.02^{a}
Nitrogen-free extract	31.62±0.16^{e}	10.38±0.77^{a}	12.06±0.01^{bc}	13.13±0.09^{c}	11.44±0.17^{ab}	21.58±0.07^{d}

Mean values in each row with similar superscripts are not significantly different ($p>0.05$).

Table 16: Carcass composition of *Clarias gariepinus* juveniles fed varying inclusion levels (0% to 100%) of MEAM based diets. Means ± SEM.

Parameter (%)		Experimental Diet's final values				
	Initial value	1(0%) Control	2(25%)	3(50%)	4(75%)	5(100%)
Crude protein	50.28±0.11^{a}	67.01±0.01^{c}	70.28±0.02^{e}	65.61±0.01^{b}	67.89±0.01^{d}	65.48±0.02^{b}
Crude fat	4.12±0.01^{a}	5.85±0.01^{b}	6.18±0.01^{f}	6.14±0.01^{e}	6.08±0.01^{d}	6.05±0.01^{c}
Crude fibre	0.01±0.00^{a}	0.03±0.01^{a}	0.03±0.01^{a}	0.06±0.01^{a}	0.05±0.01^{a}	0.08±0.01^{a}
Ash	7.20±0.02^{a}	11.90±0.01^{f}	11.37±0.01^{b}	11.53±0.01^{c}	11.78±0.01^{d}	11.85±0.01^{e}
Moisture	6.79±0.02^{f}	5.09±0.01^{c}	5.31±0.01^{e}	4.96±0.02^{b}	5.23±0.01^{d}	4.89±0.01^{a}
Nitrogen-free extract	31.62±0.16^{e}	10.13±0.01^{c}	6.84±0.02^{a}	11.71±0.03^{d}	8.98±0.01^{b}	11.67±0.02^{d}

Mean values in each row with similar superscripts are not significantly different ($p>0.05$).

Table 17: Mineral composition of *Clarias gariepinus* juveniles before and after feeding with ROAM based diets at 0% to 100% inclusion levels. Means ± SEM.

Minerals (mg/g)	Initial value	Diet 1 0% (Control)	Diet 2 25%	Diet 3 50%	Diet 4 75%	Diet 5 100%
Sodium	2.40±0.01^{a}	3.60±0.01^{b}	4.00±0.02^{bc}	3.70±0.01^{b}	4.20±0.02^{bc}	4.40±0.01^{c}
Magnesium	1.90±0.01^{a}	3.50±0.02^{b}	3.90±0.02^{bc}	3.60±0.01^{b}	4.10±0.02^{bc}	4.30±0.01^{c}
Potassium	9.00±0.02^{a}	13.00±0.02^{e}	12.40±0.02^{d}	11.20±0.01^{c}	10.60±0.01^{b}	11.10±0.01^{c}
Phosphorus	11.30±0.02^{a}	22.30±0.02^{c}	20.80±0.03^{b}	20.40±0.01^{b}	21.90±0.03^{c}	21.80±0.03^{c}
Calcium	17.60±0.02^{a}	29.10±0.03^{c}	28.50±0.02^{b}	29.70±0.03^{d}	29.80±0.03^{d}	28.50±0.02^{b}
Iron	1.96±0.01^{a}	3.17±0.01^{b}	3.30±0.01^{d}	3.15±0.01^{b}	3.24±0.01^{c}	3.14±0.01^{b}

Mean values in each row with similar superscripts are not significantly different ($p>0.05$).

Table 18: Mineral composition of *Clarias gariepinus* juveniles fed MEAM based diets at inclusion levels of 0% to 100%. Means ± SEM

Mineral (mg/g)		Experimental diet				
	Initial value	1(0%) (Control)	2(25%)	3(50%)	4(75%)	5(100%)
Sodium	2.40±0.01^{a}	3.70±0.01^{bc}	3.80±0.01^{c}	3.40±0.01^{b}	4.20±0.01^{d}	4.00±0.01^{cd}
Magnessium	1.90±0.01^{a}	3.40±0.01^{c}	2.80±0.01^{b}	2.50±0.01^{b}	3.50±0.01^{c}	2.80±0.01^{b}
Potassium	9.00±0.02^{a}	12.90±0.01^{d}	12.00±0.01^{c}	11.70±0.01^{c}	12.60±0.01^{d}	10.90±0.01^{b}
Phosphorus	11.30±0.02^{a}	22.20±0.03^{c}	21.30±0.01^{b}	21.00±0.03^{b}	21.60±0.01^{b}	21.40±0.03^{b}
Calcium	17.60±0.02^{a}	29.00±0.02^{b}	29.40±0.03^{c}	28.80±0.03^{b}	29.70±0.03^{c}	28.70±0.02^{b}
Iron	1.96±0.01^{a}	3.19±0.01^{b}	3.19±0.01^{b}	3.36±0.01^{d}	3.19±0.01^{b}	3.23±0.01^{c}

Mean values in each row with similar superscripts are not significantly different ($p>0.05$).

4.8 Growth and Nutrient Utilization Indices of *Clarias gariepinus* Juveniles Fed Graded Levels of ROAM and MEAM Based Diets at 0% to 100% Inclusion Levels for 105 Days.

The growth performance and nutrient utilization indices of *C. gariepinus* fed graded levels of ROAM and MEAM based diets are presented in Tables 19 and 20 respectively. For ROAM based diets, Mean weight gain (MWG) was highest (69.03 g) in fish fed diet 4 (75% inclusion) followed by fish fed diet 3 (65.51 g) then fish fed diet 2 (61.17 g) and least (27.02 g) in fish fed diet 5 (100% inclusion) followed by fish fed control diet. Specific growth rate (SGR) was highest in fish fed diet 4 followed by diet 3 then diet 2 and least in fish fed diet 5. There were significant differences ($p<0.05$) among the treatments' SGR values. Percentage weight gain (Relative growth rate) was highest in fish fed diet 4, followed by diet 3 and least in fish fed diet 5 followed by fish fed control diet. There were significant differences ($p<0.05$) in percentage weight gain values among the treatments. Survival rate ranged between 61.67 % and 90.00%. Hepatosomatic index was highest in fish fed diet 4, followed by fish fed diet 2 and diet 3 while fish fed diet 5 had the least value. Results of mean fortnight weight changes of *C. gariepinus* fed ROAM and MEAM based diets are shown in Figures 2 and 3 respectively.

Mean feed intake (MFI) significantly varied between 57.91g in fish fed diet 5 (100% ROAM inclusion) being the least and 106.02 g in fish fed diet 4 being the highest. Feed conversion ratio (FCR) was least in fish fed diet 4 followed by diet 3 and the control diet while the highest value was recorded in fish fed diet 5. There was no significant difference ($p>0.05$) in FCR values of dietary treatments 1 to 4 whereas there was a significant difference ($p<0.05$) between FCR value of dietary treatment 5 (100% ROAM diet) and the other treatments' FCR values. Nitrogen metabolism (Nm) was highest in fish fed diet 4, followed by diet 3 and least in fish fed diet 5. The same trend was observed for net protein utilization (NPU).

For MEAM based diets, Mean weight gain was highest in fish fed diet 4 (75% inclusion) followed by fish fed diet 3 and least (51.55 g) in fish fed diet 5 (100% inclusion). Specific growth rate (SGR) was highest in fish fed diet 4 followed by diet 3 and least in fish fed diet 5. There were significant differences ($p<0.05$) among the treatments' SGR values. Percentage weight gain (Relative growth rate) was highest in fish fed diet 4 followed by diet 3 then diet 2

Table 19: Growth performance and nutrient utilization indices of *Clarias gariepinus* juveniles fed ROAM based diets at inclusion levels of 0% to 100% for 105 days. Means ± SEM

Growth parameters	Diet				
	1(0%)	2 (25%)	3 (50%)	4 (75%)	5 (100%)
Initial mean weight (g)	12.03±0.06^{a}	12.03±0.07^{a}	12.02±0.08^{a}	12.02±0.08^{a}	12.03±0.07^{a}
Final mean weight (g)	67.13±2.28^{b}	73.21±2.92^{bc}	77.53±2.37^{cd}	81.05±2.82^{d}	39.06±0.99^{a}
Mean weight gain (g)	55.10±2.24^{b}	61.17±2.89^{bc}	65.51±2.37^{cd}	69.03±2.90^{d}	27.02±0.92^{a}
Specific growth rate (%)	1.64±0.05^{b}	1.72±0.04^{ab}	1.76±0.03^{a}	1.82±0.06^{a}	1.12±0.07^{c}
Percentage weight gain (%)	457.78±17.29^{b}	508.29±22.91^{bc}	545.24±20.37^{cd}	574.87±28.52^{d}	224.50±6.41^{a}
Survival rate (%)	88.33±1.67^{b}	90.00±0.00^{b}	86.67±1.67^{b}	86.67±1.67^{b}	61.67±3.33^{a}
Condition factor	0.65±0.03^{b}	0.68±0.02^{b}	0.65±0.01^{b}	0.63±0.01^{b}	0.56±0.01^{a}
Hepatosomatic index	1.00±0.05^{a}	1.03±0.05^{a}	1.03±0.04^{a}	1.04±0.06^{a}	0.97±0.04^{a}
Total feed intake (g)	1504.06±24.97^{b}	1736.71±33.16^{c}	1758.49±31.29^{c}	1838.21±54.00^{c}	714.97±54.16^{a}
Mean feed intake (g)	85.25±3.06^{b}	96.49±1.84^{c}	101.49±1.96^{cd}	106.02±1.49^{d}	57.91±2.40^{a}
Feed conversion ratio	1.55±0.05^{a}	1.58±0.05^{a}	1.55±0.03^{a}	1.54±0.05^{a}	2.14±0.06^{b}
GFCE (%)	64.52±3.61^{b}	63.29±2.08^{b}	64.52±1.31^{b}	65.36±2.28^{b}	46.73±2.12^{a}
Nitrogen metabolism	2281.69±66.80^{b}	2456.83±84.99^{bc}	2580.86±68.13^{cd}	2682.33±78.65^{d}	1472.50±30.53^{a}
Net protein utilization (%)	356.37±10.42^{b}	384.86±13.30^{bc}	402.37±10.61^{cd}	418.88±12.27^{d}	230.26±4.77^{a}

Mean values in each row with similar superscripts are not significantly different (p>0.05).

GFCE = Gross feed conversion efficiency

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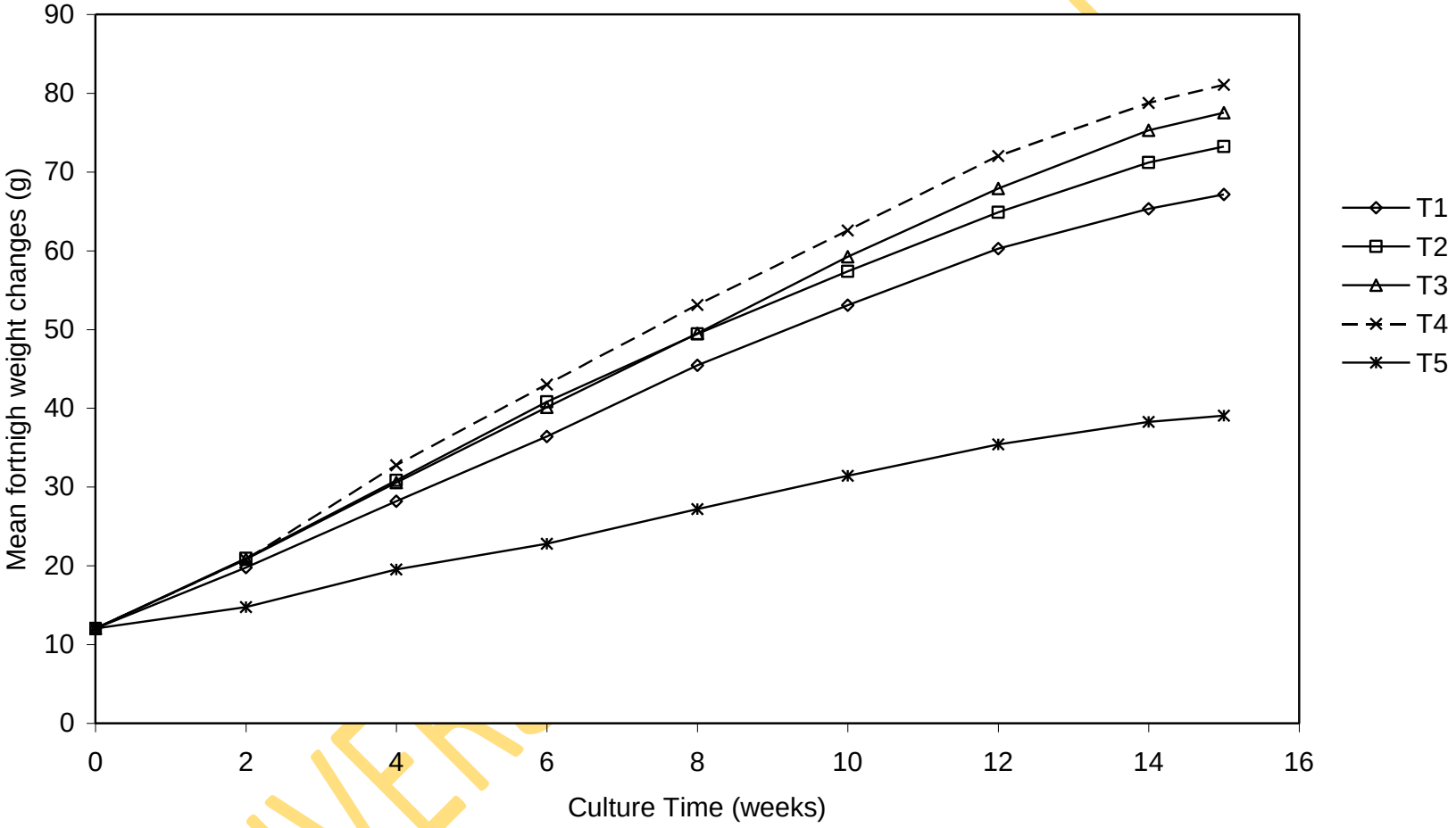


Figure 2: Mean fortnight weight changes of *Clarias gariepinus* juveniles fed varying inclusion levels of ROAM diets for 105 days

Legend: T1= Diet 1 (0%, control); T2= Diet 2 (25%); T3= Diet 3 (50%); T4= Diet 4 (75%); T5= Diet 5 (100%)

Table 20: Growth performance and nutrient utilization indices of *Clarias gariepinus* juveniles fed MEAM based diets at inclusion levels of 0% to 100%. Means ± SEM

Growth parameters	Diet				
	1(0%, control)	2 (25%)	3 (50%)	4 (75%)	5 (100%)
Initial mean weight (g)	12.02±0.07 [a]	12.03±0.08 [a]	12.03±0.06 [a]	12.02±0.08 [a]	12.04±0.04 [a]
Final mean weight (g)	67.07±2.76 [bc]	71.82±4.15 [abc]	75.12±4.38 [ab]	80.47±1.59 [a]	63.57±1.09 [c]
Mean weight gain (g)	55.05±2.70[ab]	59.79±4.23[abc]	63.09±4.44[bc]	68.45±1.58[c]	51.55±1.08[a]
Specific growth rate (%)	1.64±0.02[cd]	1.70±0.08[cd]	1.74±0.03[a]	1.81±0.02[a]	1.58±0.02[d]
Percentage weight gain (%)	453.25±15.15 [b]	497.39±38.44 [c]	524.62±49.30[d]	569.70±13.35 [e]	429.02±8.99[a]
Survival rate (%)	88.33±1.67[a]	86.67±1.67 [a]	86.67±3.33 [a]	86.67±1.67 [a]	88.33±1.67 [a]
Condition factor	0.643±0.02[a]	0.640±0.01 [a]	0.640±0.02 [a]	0.646±0.03 [a]	0.620±0.01 [a]
Hepatosomatic index	1.06±0.16 [a]	1.02±0.15 [a]	1.01±0.06 [a]	1.05±0.37 [a]	1.03±0.15 [a]
Total feed intake (g)	1510.71±10.61 [c]	1604.75±79.88 [bc]	1682.75±62.60[ab]	1812.34±11.6 [a]	1509.33±24.84 [c]
Mean feed intake (g)	85.57±1.69 [bc]	92.55±3.97 [abc]	97.62±7.29 [ab]	104.61±1.33 [a]	85.48±0.85 [c]
Feed conversion ratio	1.55±0.04 [a]	1.55±0.08 [a]	1.55±0.02 [a]	1.53±0.03 [a]	1.66±0.02 [b]
GFCE (%)	64.33±1.72 [a]	64.60±3.23 [a]	64.63±0.88 [a]	65.36±1.37 [a]	60.24±0.55 [b]
Nitrogen metabolism	2279.38±81.55[ab]	2416.96±117.31[abc]	2511.98±124.70[bc]	2665.70±46.31[c]	2178.60±31.66[a]
Net protein utilization (%)	356.01±12.72[ab]	376.38±18.24[abc]	392.88±19.48[bc]	416.31±7.22[c]	340.26±4.94[a]

Mean values in each row with similar superscripts are not significantly different ($p>0.05$).

GFCE = Gross feed conversion efficiency

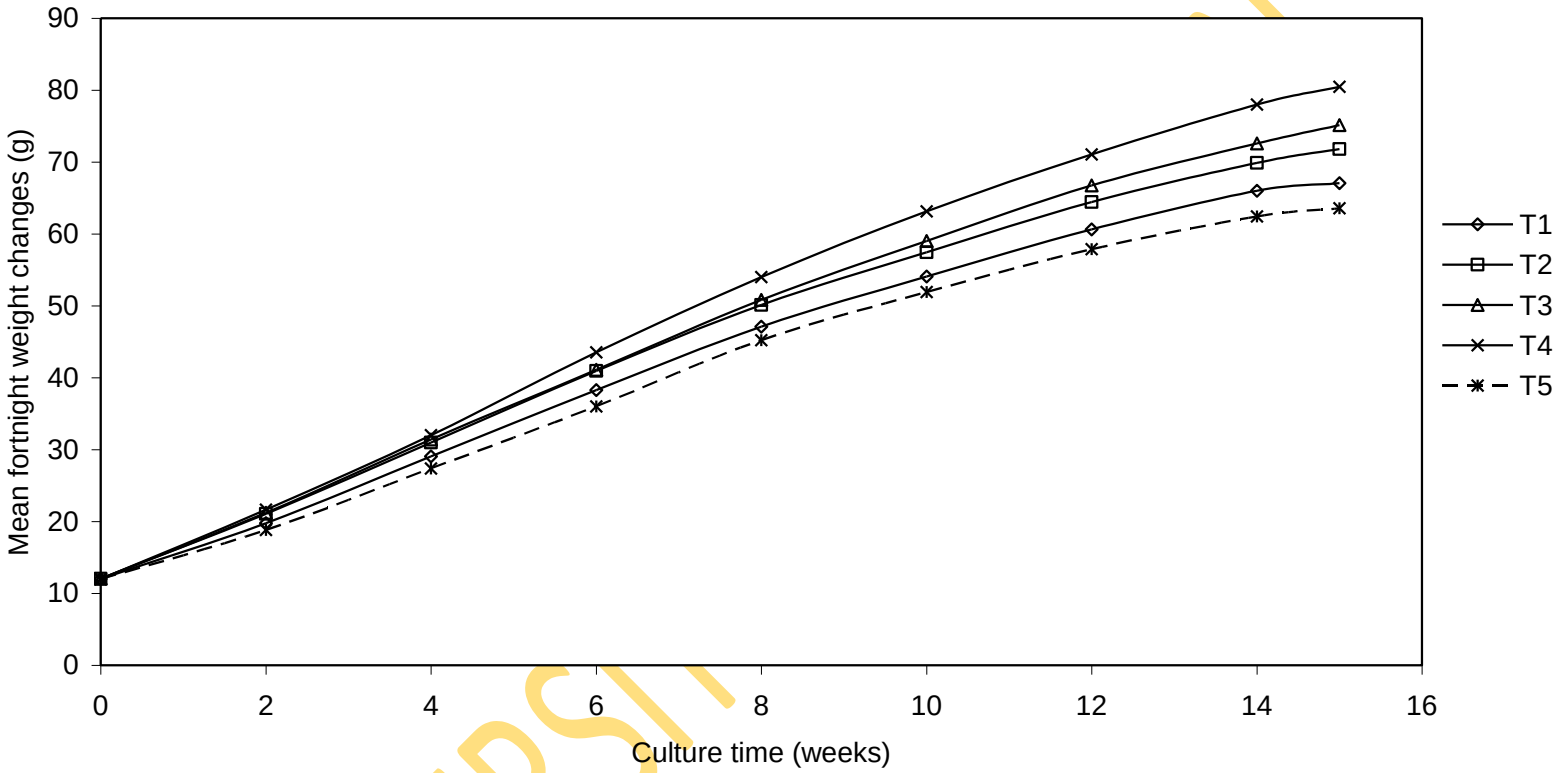


Figure 3: Mean fortnight weight changes of *Clarias gariepinus* juveniles fed varying inclusion levels of MEAM based diets for 105 days. Legend: T1= Diet 1 (control); T2= Diet 2 (25%); T3= Diet 3 (50%); T4= Diet 4 (75%); T5= Diet 5 (100%)

and least in fish fed diet 5. There were significant differences ($p<0.05$) in percentage weight gain values among the treatments. Survival rate ranged between 86.67 % and 88.33%. There was no significant difference ($p>0.05$) in the survival rates. Mean feed intake (MFI) for MEAM based diets significantly varied between 85.48 g for fish fed diet 5 (100% inclusion) being the least and 104.61g for fish fed diet 4 (75% inclusion) being the highest. Feed conversion ratio (FCR) was least in fish fed diet 4 and highest in fish fed diet 5. There was no significant difference ($p>0.05$) in FCR values among dietary treatments 1 to 4 but there was a significant difference ($p<0.05$) between the FCR value of treatment 5 (100% MEAM) and the other dietary treatments' FCR values. Net protein utilization (NPU) was highest in fish fed diet 4 and least in fish fed diet 5. There were significant differences ($p<0.05$) in the treatments' NPU values.

4.9 Haematological and Serum Bio-Chemical Assessment of Fish Fed Graded Levels of ROAM and MEAM Based Diets.

Initial packed cell volume (PCV) of experimental fish was 21.00 %. For ROAM based diets, PCV values significantly increased ($p<0.05$) in all the treatments with the highest value (27.15%) observed in fish fed diet 3 followed by 26.00% observed in fish fed diet 4 and the least value (23.00%) observed in fish fed diet 5 (Table 21). Initial Heamoglobin concentration (Hb) was 7.20 g/dL. This increased in all the treatments' final values with the highest value (8.70 g/dL) observed in fish fed diet 3 (50% ROAM inclusion), followed by 8.60 g/dl observed in fish fed diet 4 while the least value (7.40 g/dL) was observed in fish fed diet 5. Initial red blood cell count (RBC) was 1.69 $x10^6$ /mL. This increased in all the final treatments' values to a range of 2.74 to 3.83 $x10^6$/mL. There was a significant difference ($p<0.05$) in the treatments' RBC values. Initial white blood cell count was 7.90 $x10^6$/µL. This increased in all the treatments' final values to a range between 10.40 and 11.80 $x10^6$/µL. Total plasma protein content of fish fed ROAM based diets exhibited an irregular pattern. Initial total plasma protein was 4.30 g/dL (Table 22). This increased to 4.40 g/dL in fish fed diet 4, 4.50 g/dL in fish fed control diet, 4.70g/dL in fish fed diet 3 and reduced to 3.40 g/dL in fish fed diet 5 whereas it remained the same (4.30g/dL) in fish fed diet 2. Albumin content of the experimental fish blood also exhibited an irregular pattern with the initial value of 2.70 g/dL which increased to 2.80 g/dL in treatment 3 (50% ROAM inclusion) but reduced to 2.50 g/dL, 2.60 g/dL and 1.80 g/dL in treatments 2, 4 and 5 respectively. There was no significant difference ($p>0.05$) between treatments 1 to 4 albumin

Table 21: Haematological indices of *Clarias gariepinus* juveniles fed ROAM based diets at inclusion levels of 0% to 100% for 105 days. Means ± SEM

Parameters	Initial value	Diet 1 0% (Control)	Diet 2 25%	Diet 3 50%	Diet 4 75%	Diet 5 100%
PCV (%)	21.00±1.00^{a}	25.00±1.00^{bc}	24.00±1.00^{bc}	27.15±0.00^{d}	26.00±1.00^{cd}	23.00±0.00^{ab}
Hb (g / dL)	7.20±0.10^{a}	8.30±0.10^{b}	8.30±0.10^{b}	8.70±0.10^{c}	8.60±0.10^{bc}	7.40±0.10^{a}
RBC (x10^{6} /mL)	1.69±0.01^{a}	3.41±0.02^{c}	3.40±0.02^{c}	3.83±0.02^{d}	3.83±0.02^{d}	2.74±0.01^{b}
WBC (x10^{6} /µL)	7.90±0.02^{a}	10.40±0.01^{b}	10.50±0.02^{b}	10.45±0.01^{b}	10.48±0.01^{b}	11.80±0.01^{c}
Platelet (x10^{4}/mL)	20.70±0.01^{b}	24.80±0.02^{c}	24.60±0.01^{c}	31.80±0.03^{e}	27.60±0.02^{d}	20.40±0.03^{a}
MCHC (g/dL)	34.29±1.01^{a}	33.20±1.05^{a}	34.58±1.10^{a}	32.04±0.98^{a}	33.08±0.08^{a}	32.17±1.0^{a}

Mean values in each row with similar superscripts are not significantly different (p>0.05).

Legend: PCV = packed cell volume; Hb = heamoglobin concentration; RBC = red blood cell; WBC = white blood cell; MCHC = mean corpuscular heamoglobin concentration

Table 22: Serum biochemical indices of *Clarias gariepinus* juveniles fed ROAM diets at inclusion levels of 0% to 100% for 105 days. Means ± SEM

Parameters	Initial	Diet 1 0% (Control)	Diet 2 25%	Diet 3 50%	Diet 4 75%	Diet 5 100%
Total protein (g/dL)	4.30±0.10^{b}	4.50±0.10^{b}	4.30±0.10^{b}	4.70±0.05^{b}	4.40±0.10^{b}	3.40±0.01^{a}
Albumin (g/dL)	2.70±0.10^{b}	2.70±0.10^{b}	2.50±0.05^{b}	2.80±0.10^{b}	2.60±0.05^{b}	1.80±0.10^{a}
Globulin (g/dL)	1.60±0.10^{a}	1.80±0.10^{a}	1.80±0.10^{a}	1.90±0.10^{a}	1.80±0.00^{a}	1.60±0.10^{a}
A: G ratio	1.69±0.17^{b}	1.50±0.03^{b}	1.40±0.11^{b}	1.47±0.05^{b}	1.44±0.03^{b}	1.13±0.13^{a}
AST (U/L)	24.90±1.00^{a}	32.50±1.00^{b}	32.90±2.00^{b}	33.60±2.00^{b}	33.40±1.00^{b}	53.50±1.00^{c}
ALT (U/L)	31.50±1.00^{a}	55.00±2.00^{b}	55.50±1.00^{b}	54.90±1.00^{b}	55.40±0.50^{b}	72.00±0.50^{c}
Glucose (mg/dL)	298.00±2.00^{a}	326.00±4.00^{c}	307.00±3.00^{ab}	308.00±2.00^{ab}	315.00 ±5.00^{bc}	361.00 ±5.00^{d}
Potassium (mmol/L)	5.19±0.01^{e}	4.32±0.01^{c}	4.21±0.01^{b}	3.54±0.02^{a}	4.46±0.02^{d}	4.50±0.01^{d}
Sodium (mmol/L)	135.80 ±0.10^{a}	137.10±0.10^{b}	135.90 ±0.10^{a}	136.80 ±0.20^{b}	135.90 ±0.10^{a}	135.80±0.00^{a}
Chloride (mmol/L)	105.20 ±0.20^{a}	112.50±0.30^{c}	112.90 ±0.20^{c}	114.00±0.40^{d}	111.50 ±0.10^{b}	114.10±0.10^{d}

Mean values in each row with similar superscripts are not significantly different ($p>0.05$).

Legend: A: G = albumin: globulin; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase

values and the initial value but there was a significant difference ($p<0.05$) between treatment 5 and the other treatments' albumin values. Initial aspartate aminotrasferase (AST) value was 24.90 U/L. Final AST values ranged between 32.50 U/L and 53.50 U/L. Initial alanine aminotransferase (ALT) was 31.50 U/L with an irregular pattern exhibited by fish fed ROAM based diets. Fish fed diet 5 had the highest final ALT value (72.00 U/L) while fish fed Diet 3 had the least value (54.90 U/L). Final Plasma glucose ranged from 308.00 mmol/L to 361.00 mmol/L; potassium ranged from 3.54 mmol/L to 4.50 mmol/L; soduim from 135.80 mmol/L to 137.10 mmol/L while final chloride values ranged from 111.50 mmol/L to 114.10 mmol/L.

For MEAM based diets, initial PCV value was 21.00%. This increased in all the treatments with the highest value observed in fish fed diet 3 while the least value was observed in fish fed diet 2 and diet 5 (Table 23). Initial heamoglobin concentration (Hb) was 7.20 g/dL. This increased in all the treatments' final values with a range of 8.40 to 8.98 g/dL. Initial red blood cell count (RBC) was 1.69 ($x10^6$ /mL). This increased in all the final treatments' values to a range of 2.56 to 3.93 $x10^6$ /mL. There was a significant difference ($p<0.05$) in the treatments' RBC values. The fish white blood cell count before the commencement of the experiment was 7.90 $x10^6$/µL. This increased in all the treatments' final values to a range of 10.30 to 11.38 $x10^6$ /µL.

Results of serum biochemistry of *Clarias gariepinus* fed mechanically extracted almond kernel meal based diets are shown in Table 24. Total plasma protein and albumin exhibited irregular pattern. Globulin ranged between 1.60 and 1.90 g/dl. Initial AST value was 24.90 U/L. Final AST values ranged between 32.70 U/L and 48.00 U/L. Initial ALT value was 31.50 U/L with an irregular pattern displayed. Fish fed diet 5 had the highest final ALT value while fish fed diet 3 had the least value. Final Plasma glucose ranged between 298.00 and 358.00 mmol/L. Final plasma Potassium ranged between 2.50 and 3.97 mmol/L; Soduim ranged between 132.80 and 140.80 mmol/L while final Chloride values ranged from 109.30 to 115.90 mmol/L

Table 23: Haematological indices of *Clarias gariepinus* juveniles fed MEAM based diets at inclusion levels of 0% to 100% for 105 days. Means ± SEM

Parameters	Initial	Diet				
		1(0%)	2 (25%)	3 (50%)	4 (75%)	5 (100%)
PCV (%)	21.00±1.00[a]	26.00±1.00[a]	25.00±1.00[a]	28.00±1.00[a]	26.00±2.00[a]	25.00±1.00[a]
Hb (g / dL)	7.20±0.10[a]	8.50±0.10[bc]	8.50±0.10[bc]	8.98±0.10[c]	8.70±0.10[bc]	8.40±0.10[b]
RBC ($x10^6$ /mL)	1.69±0.01[a]	3.63±0.01[c]	2.56±0.01[b]	3.93±0.01[d]	3.93±0.01[d]	2.57±0.01[b]
WBC ($x10^6$ /µL)	7.90±0.02[a]	10.30±0.05[b]	10.35±0.05[b]	10.30±0.01[b]	10.40±0.01[b]	11.38±0.01[c]
Platelet ($x10^4$)	20.70±10.00[a]	32.20±10.00[c]	32.00±10.00[c]	33.81±5.00[d]	33.80±20.00[d]	26.60±5.00[b]
MCHC (g/dL)	34.29±1.01[a]	32.69±0.69[a]	34.00±1.03[a]	32.07±1.01[a]	33.46±0.06[a]	33.60±0.30[a]

Mean values in each row with similar superscripts are not significantly different ($p>0.05$).

Legend: PCV = packed cell volume; Hb = heamoglobin concentration; RBC = red blood cell count; WBC = white blood cell count; MCHC = mean corpuscular heamoglobin concentration

Table 24: Serum biochemical indices of *Clarias gariepinus* juveniles fed MEAM based diets at inclusion levels of 0% to 100% for 105 days. Means ± SEM

Parameters		diets				
	Initial	1(0%) Control	2 (25%)	3 (50%)	4 (75%)	5 (100%)
Total protein (g/dL)	4.30±0.10 ^{b}	4.00±0.10^{a}	4.00±0.10^{a}	4.80±0.10^{c}	4.70±0.10^{b}	4.00±0.00^{a}
Albumin (g/dL)	2.70±0.10 ^{c}	2.30±0.10^{b}	2.40±0.10^{b}	2.90±0.10^{c}	2.80±0.10^{c}	2.25±0.10^{a}
Globulin (g/dL)	1.60±0.10 ^{a}	1.70±0.10 ^{a}	1.60±0.10 ^{a}	1.90±0.10 ^{a}	1.90±0.00 ^{a}	1.73±0.10^{a}
A:G ratio	1.69±0.17 ^{a}	1.35±0.03 ^{b}	1.50±0.14 ^{b}	1.54±0.14 ^{b}	1.48±0.06 ^{b}	1.30±0.00 ^{b}
AST (UL)	24.90±1.00 ^{a}	32.70±1.00^{b}	33.20±1.00 ^{b}	33.80±1.00^{b}	33.60±1.00^{b}	48.00±1.00^{c}
ALT (UL)	31.50±1.00 ^{a}	55.20±1.00^{b}	55.50±1.00^{b}	54.80±1.00^{b}	55.00±1.00^{b}	62.10±1.00^{c}
Glucose (mmol/L)	298.00±2.00 ^{a}	318.00 ±2.00^{b}	319.00 ±2.00^{b}	295.00 ±2.00^{a}	298.00±1.00 ^{a}	358.00±1.00^{c}
Potassium (mmol/L)	5.19±0.01 ^{a}	3.07±0.01^{c}	2.58±0.01^{e}	2.98±0.01^{d}	3.97±0.01^{b}	2.50±0.01^{f}
Sodium (mmol/L)	135.80 ±0.10 ^{d}	132.80 ±0.10^{e}	140.80 ±0.10^{a}	137.90 ±0.10^{b}	136.00 ±0.10^{d}	137.20±0.10^{c}
Chloride (mmol/L)	105.20±0.20 ^{e}	109.30 ±0.20^{d}	115.90 ±0.10^{a}	113.40 ±0.10^{b}	110.50 ±0.20^{c}	110.90±0.40^{c}

Mean values in each row with similar superscripts are not significantly different ($p>0.05$).

Legend: A: G = albumin: globulin; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase

4.10 Histopathological Profile of *Clarias gariepinus* Juveniles Fed Graded Levels of ROAM and MEAM Based Diets.

Results of histopathological analysis of the liver, kidney and intestine sections of fish fed ROAM based diets are presented in Table 25 and Plates 1 to 15. There was no marked histological change in liver of fish under treatments 1 to 4 as mild/moderate periportal inflammation was common to all of them. However, liver section of fish fed diet 5 had a marked histological change from the control as they had some lesions including severe periportal inflammation, microvesicular steatosis, multifocal areas of necrosis, vascular fibrosis and focal area of fibrous nodule. There was no lesion observed in the kidney sections of fish fed diets 1, 2, 3 and 4 but kidney sections of fish fed diet 5 had some lesions. Mild infiltration of the mucosa and submucosa by inflammatory cell was observed in the intestine sections of fish under treatments 1 to 4 whereas intestine sections of fish fed diet 5 (100% ROAM) had severe infiltration of the mucosa and submucosa by inflammatory cell.

Results of histopathological analysis of fish fed MEAM based diets are presented in Table 26 and Plates 16-30. There was no marked histological change in liver of fish fed control diet and those fed diets 2, 3 and 4. They all had no lesion except for moderate / mild peiportal inflammation. However, liver section of fish fed diet 5 had a marked histological change from the control as they had macrovesicular steatosis, focal area of vacuolar degeneration and vascular fibrosis in addition to moderate peiportal inflammation. Kidney sections of fish fed 0% to 75% MEAM based diets had no lesion while kidney sections of fish fed 100% MEAM based diet had tubular necrosis; focal area of lymphoid nodule and fibrosis of the wall of collecting tubules and some blood vessels. No lesion/ulceration was observed in the intestine sections of fish fed diets 1 to 4 except for mild infiltration of the mucosa and submucosa by inflammatory cell while intestine sections of fish fed diet 5 (100% MEAM) had severe infiltration of the mucosa and submucosa by inflammatory cell.

Table 25: Summary of results of histopathological examination of *Clarias gariepinus* juveniles fed ROAM-based diets at inclusion levels of 0 % to100 % for 105 days.

Dietary Treatment	observation made on the organs		
	Liver	Kidney	Intestine
Diet1 (0%) Control	No lesion except for mild periportal inflammation	No lesion	Mild infiltration of the mucosa and submucosa by inflammatory cell
Diet2 (25%)	No lesion except for moderate periportal inflammation	No lesion	Mild infiltration of the mucosa and submucosa by inflammatory cell
Diet3 (50%)	No lesion except for mild periportal inflammation	No lesion	Mild infiltration of the mucosa and submucosa by inflammatory cell
Diet4 (75%)	No lesion except for mild Periportal inflammation	No lesion	Mild infiltration of the mucosa and submucosa by inflammatory cell
Diet5 (100%)	microvesicular steatosis; severe peri-portal inflammation; multifocal areas of necrosis; vascular fibrosis; focal area of fibrous nodule	Tubular necrosis; necrosis of glo-meruli; vascular fibrosis; fibrous thickening of wall of the vessels and collecting ducts; desquamation of epithelial cells of collecting duct	Severe infiltration of the mucosa and submucosa by inflammatory cell

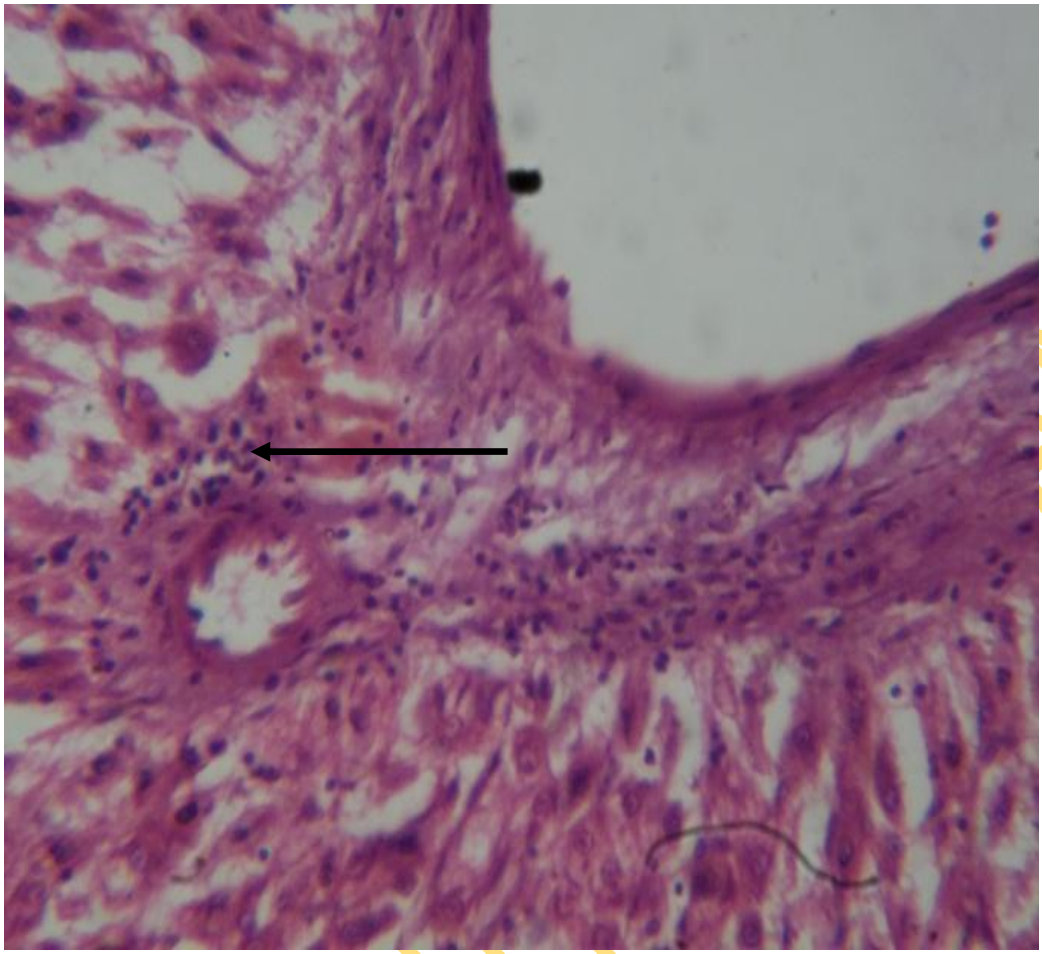
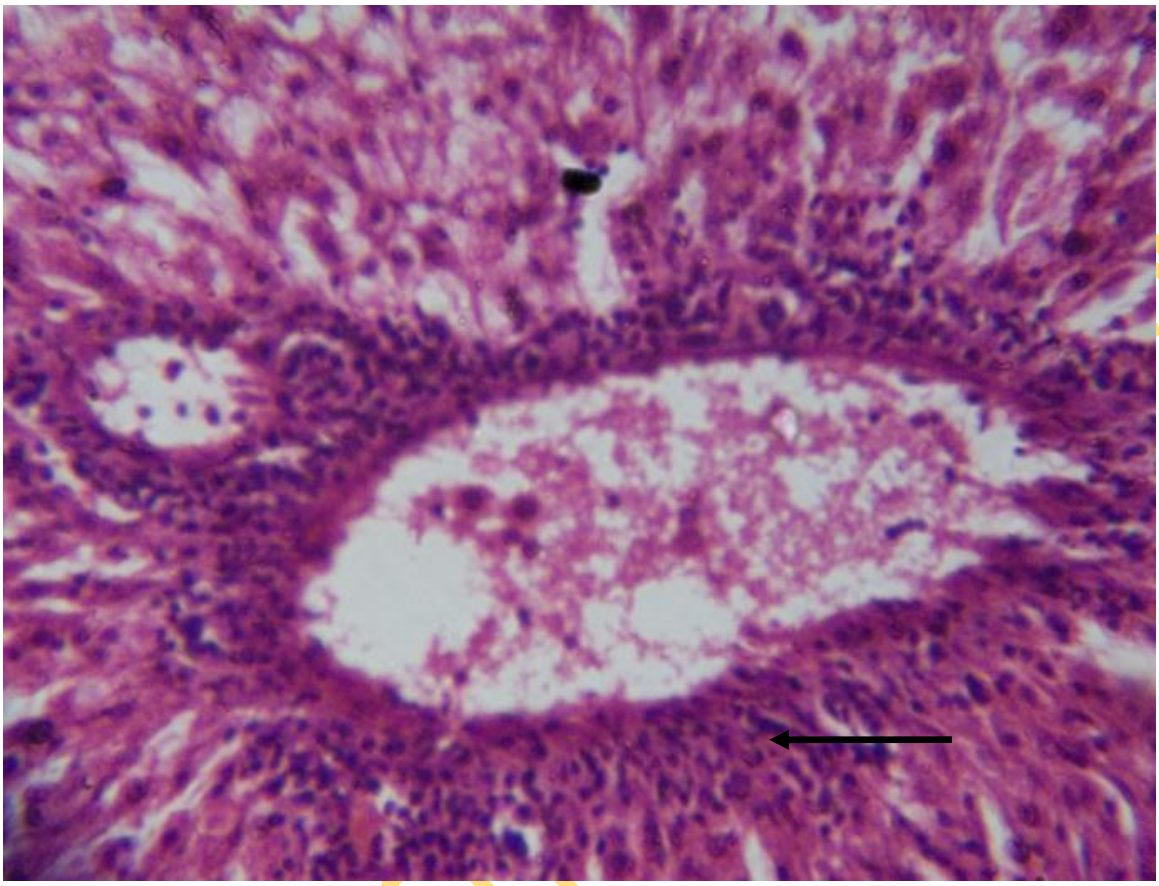


Plate 1: Histology of liver of a catfish fed diet containing 0% ROAM (diet 1, control) showing no lesion except for mild periportal inflammation (black arrow). X400



Portal tract

Plate 2: Histology of liver of a catfish fed diet containing 25% ROAM (diet 2) showing no lesion except for moderate periportal inflammation (black arrow) X400

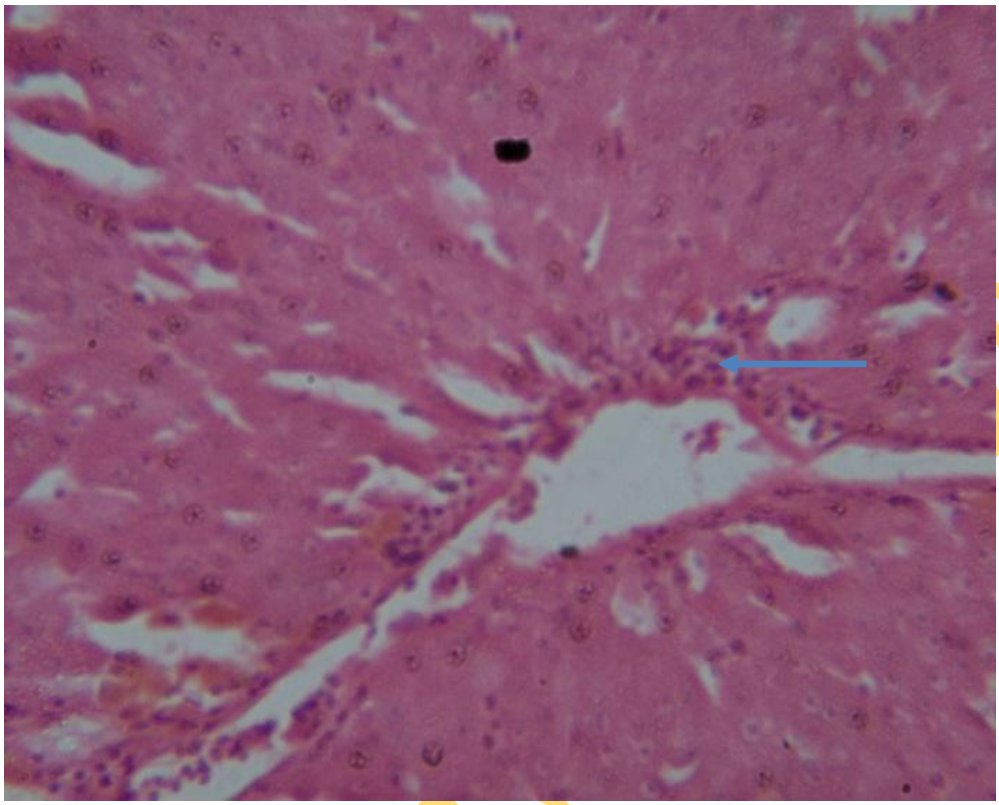


Plate 3: Histology of liver of a catfish fed diet containing 50% ROAM (diet 3) showing no lesion except for mild periportal inflammation (blue arrow). X400

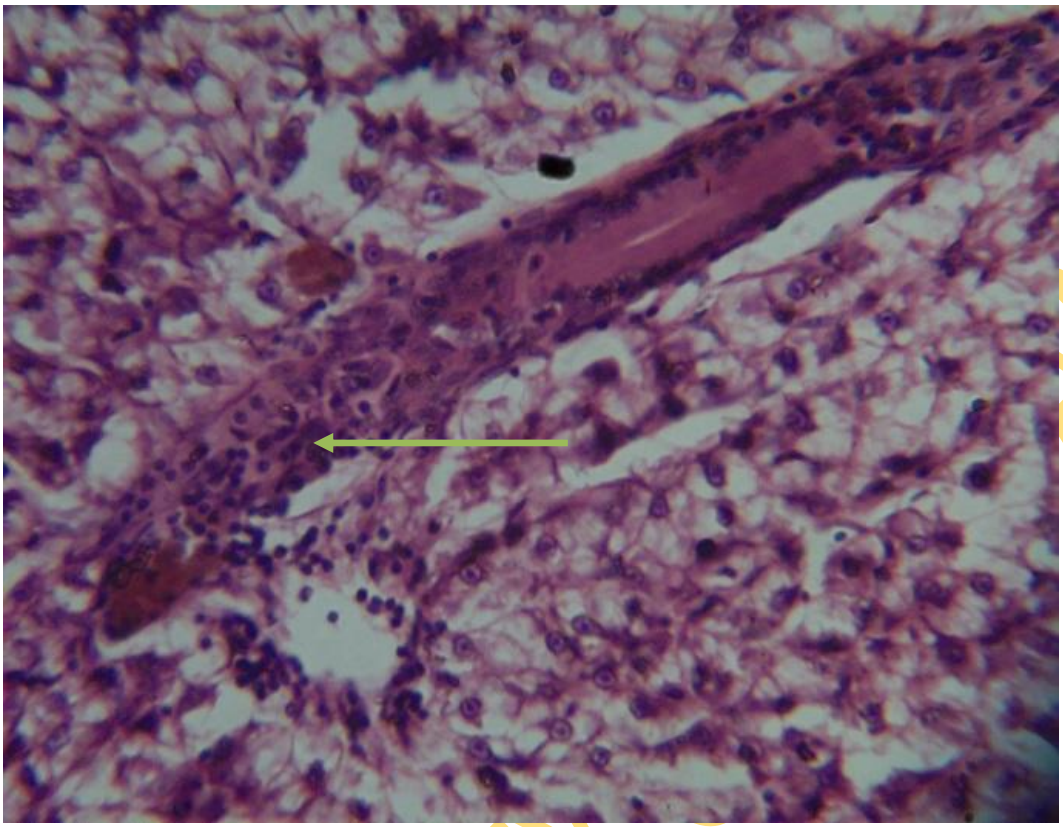
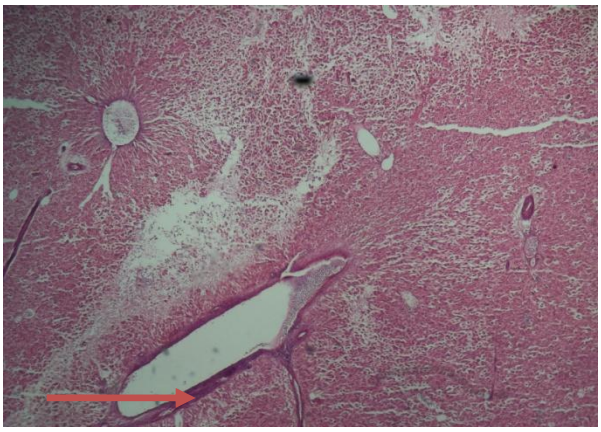
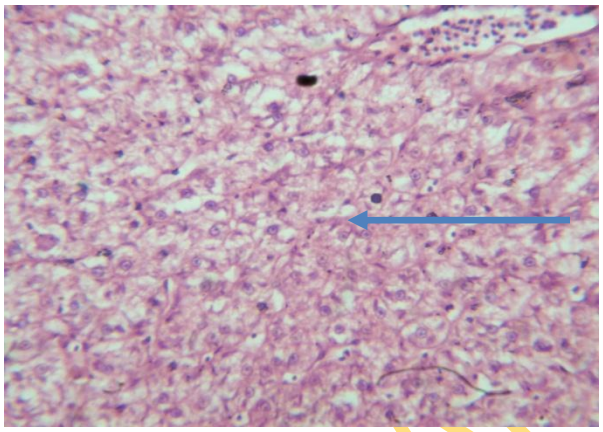


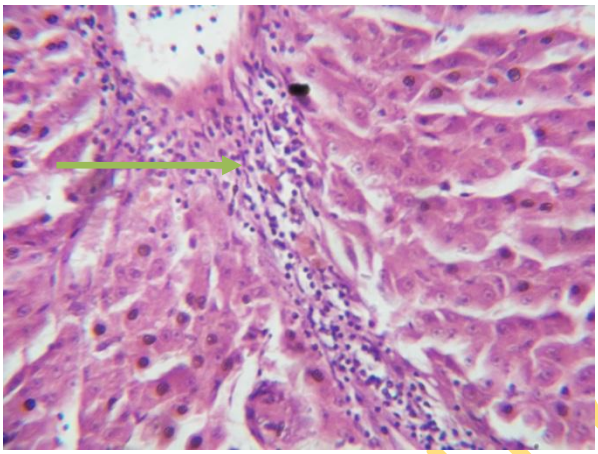
Plate 4: Histology of liver of a catfish fed diet containing 75% ROAM (diet 4) showing no lesion except for mild periportal inflammation (green arrow). X400



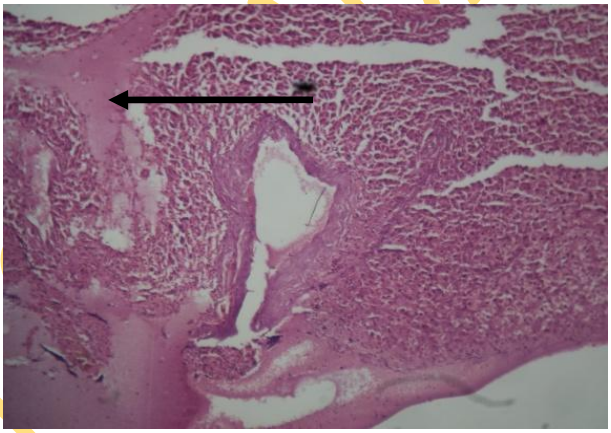
(a)



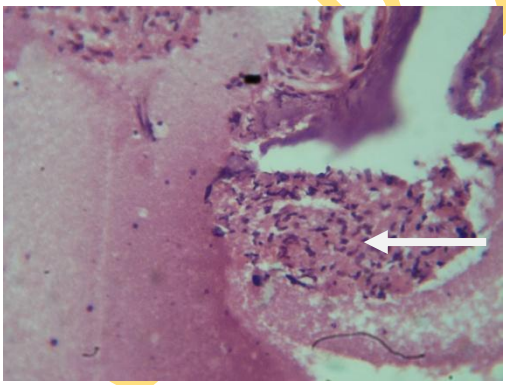
(b)



(c) Hepatocytes



(d)



(e)

Plate 5: Histology of liver of a catfish fed diet containing 100% ROAM (diet 5) showing (a): vascular fibrosis (red arrow), (b) disseminated microvesicular steatosis (blue arrow), (c) severe periportal inflammation (green arrow), (d) multifocal areas of necrosis (black arrow) and (e) focal area of fibrous nodule (white arrow). X400

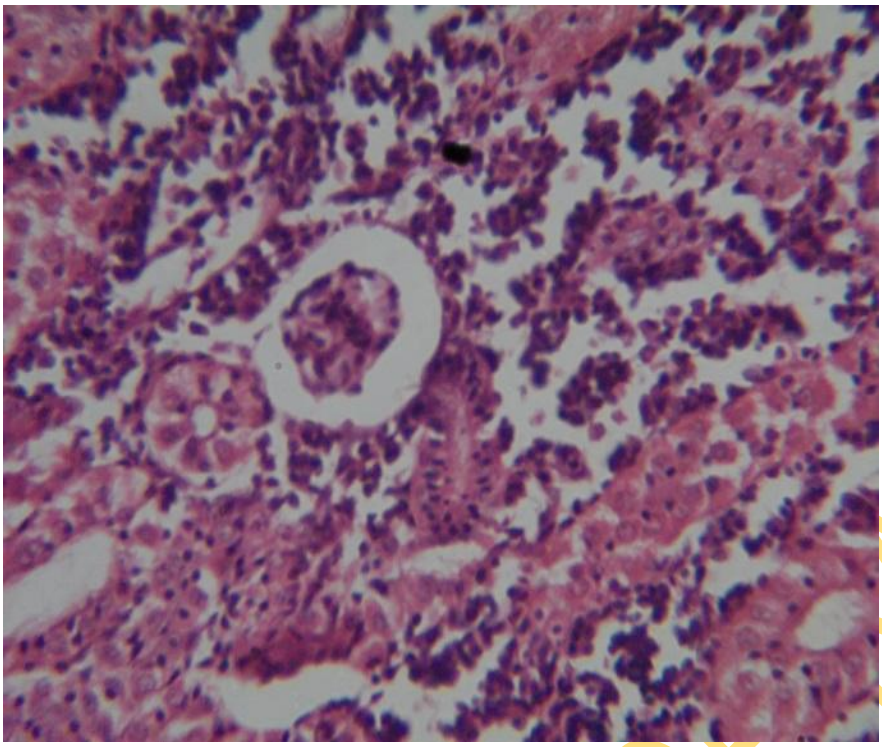


Plate 6: Histology of kidney of a catfish fed diet containing 0% ROAM (diet 1, control) showing no visible lesion. X400

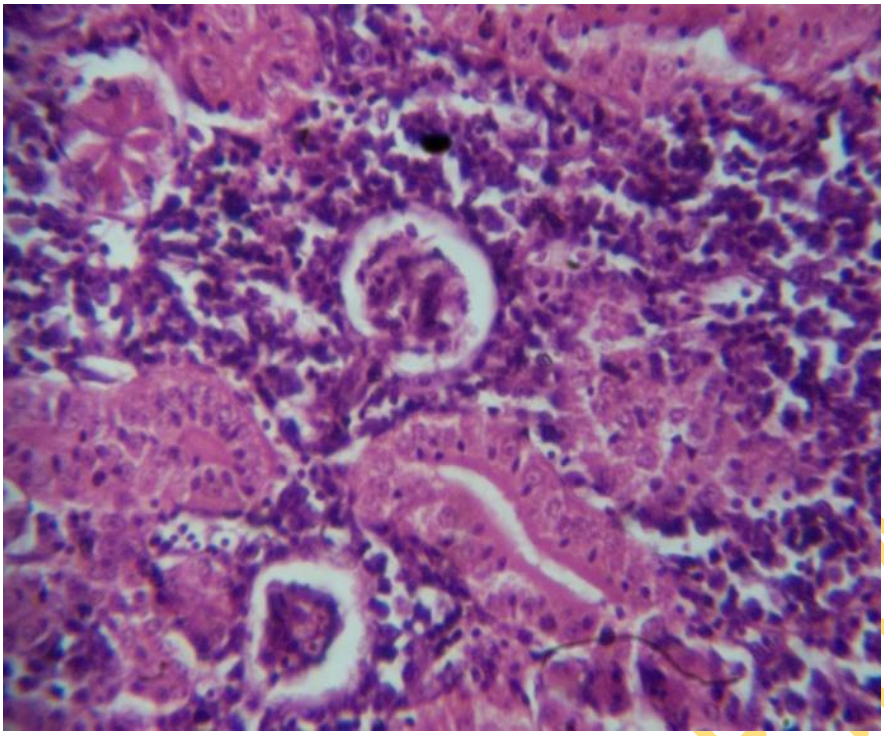


Plate 7: Histology of kidney of a catfish fed diet containing 25% ROAM (diet 2) showing no visible lesion. X400

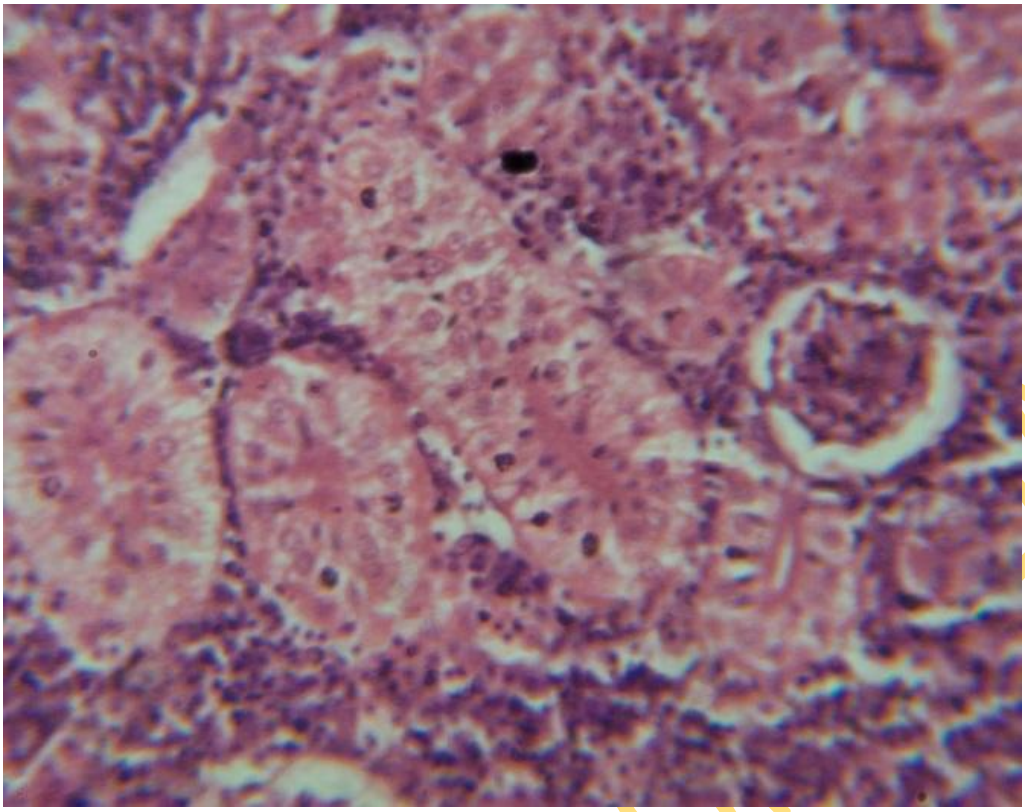


Plate 8: Histology of kidney of a catfish fed diet containing 50% ROAM (diet 3) showing no visible lesion. X400

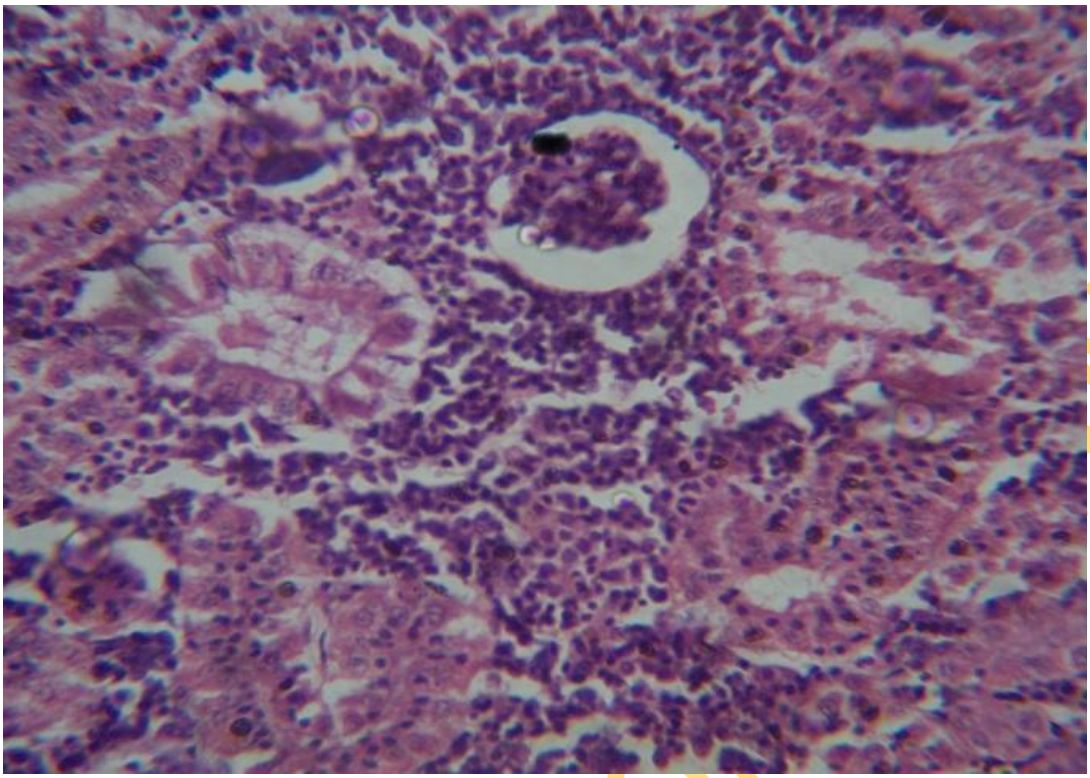
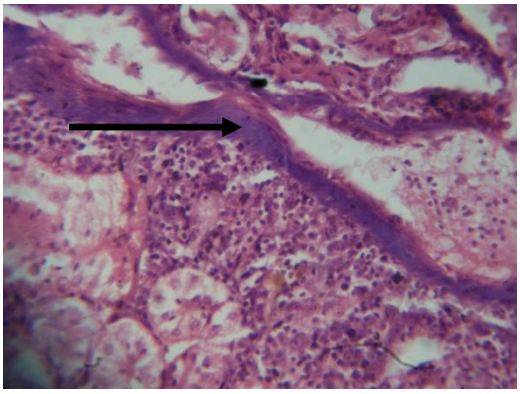
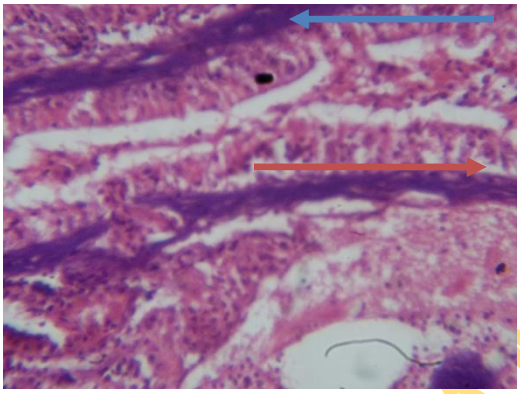


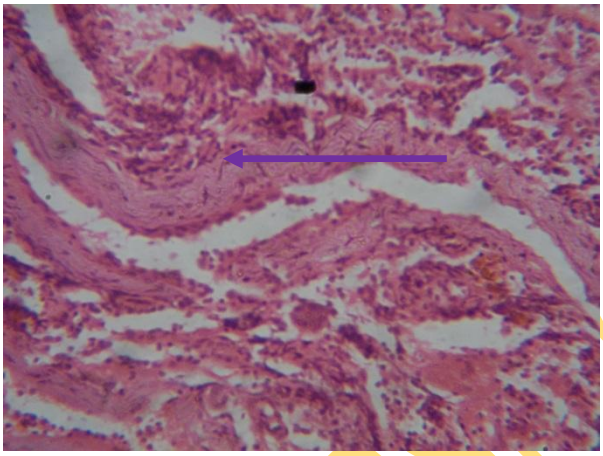
Plate 9: Histology of kidney of a catfish fed diet containing 75% ROAM (diet 4) showing no visible lesion. X400



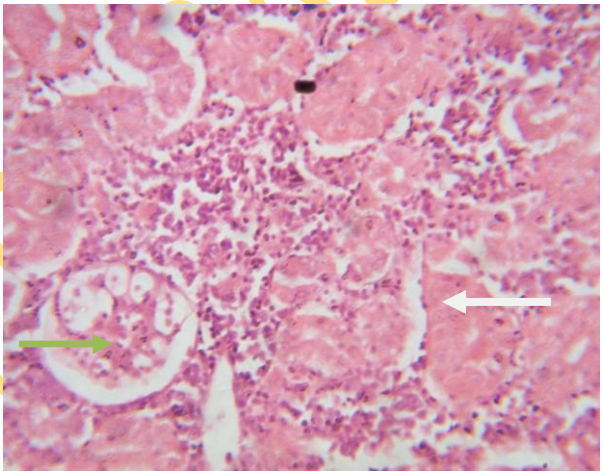
(a)



(b)



(c)



(d)

Plates 10 (a), (b), (c), and (d): Histology of kidney of a catfish fed diet containing 100% ROAM (diet 5) showing: fibrous thickening of the wall of the vessels (black arrow, 10 a) as well as collecting ducts (blue arrow, 10 b); desquamation of the epithelial cells of the collecting duct (red arrow, 10 b); vascular fibrosis (purple arrow, 10c); tubular necrosis (white arrow, 10 d) and necrosis of glomeruli (green arrow, 10 d). X400

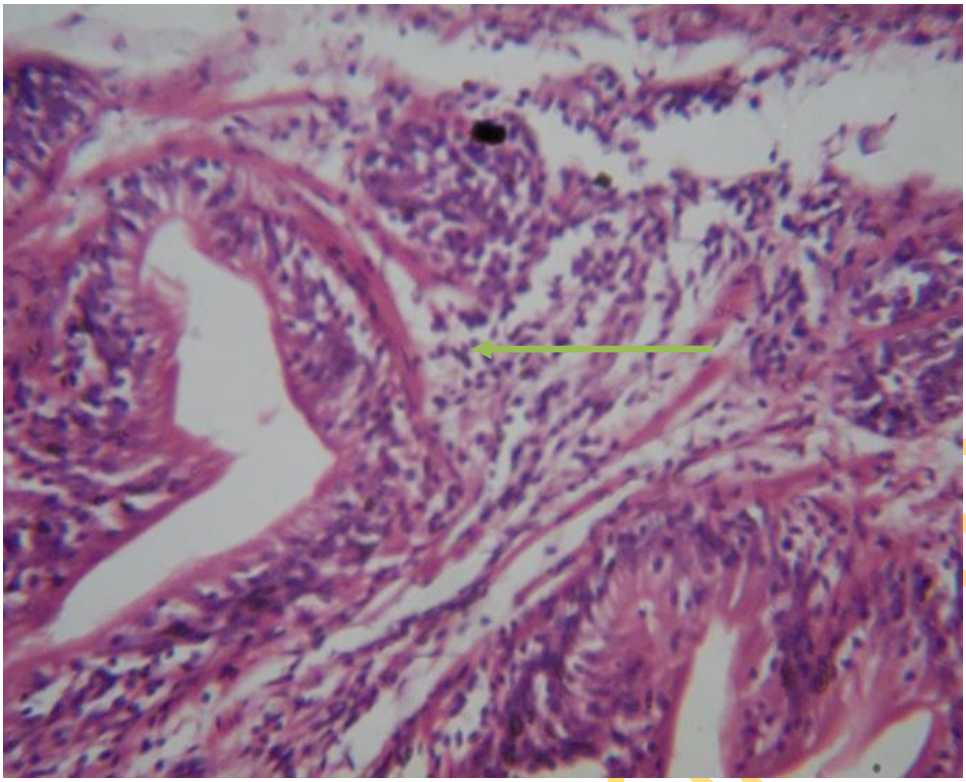


Plate 11: Histology of intestine of a catfish fed diet containing 0% ROAM (diet 1, control) showing no lesion except for mild infiltration of the mucosa and sub mucosa by inflammatory cell (green arrow). X400

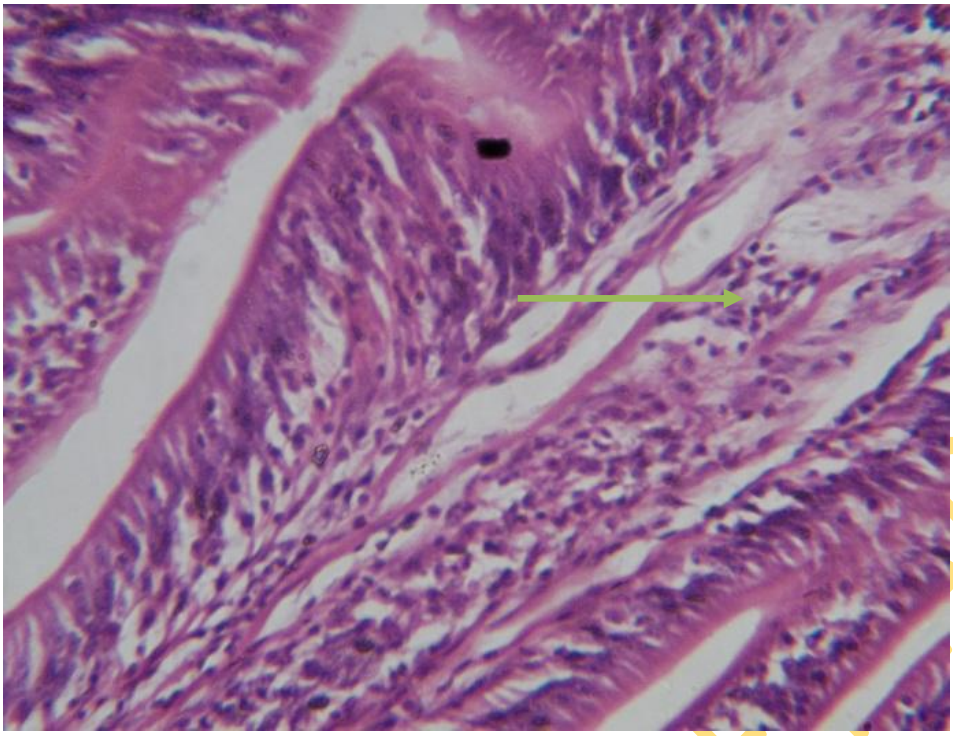


Plate 12: Histology of intestine of a catfish fed diet containing 25% ROAM (diet 2) showing no lesion except for mild infiltration of the mucosa and sub mucosa by inflammatory cell (green arrow). X400

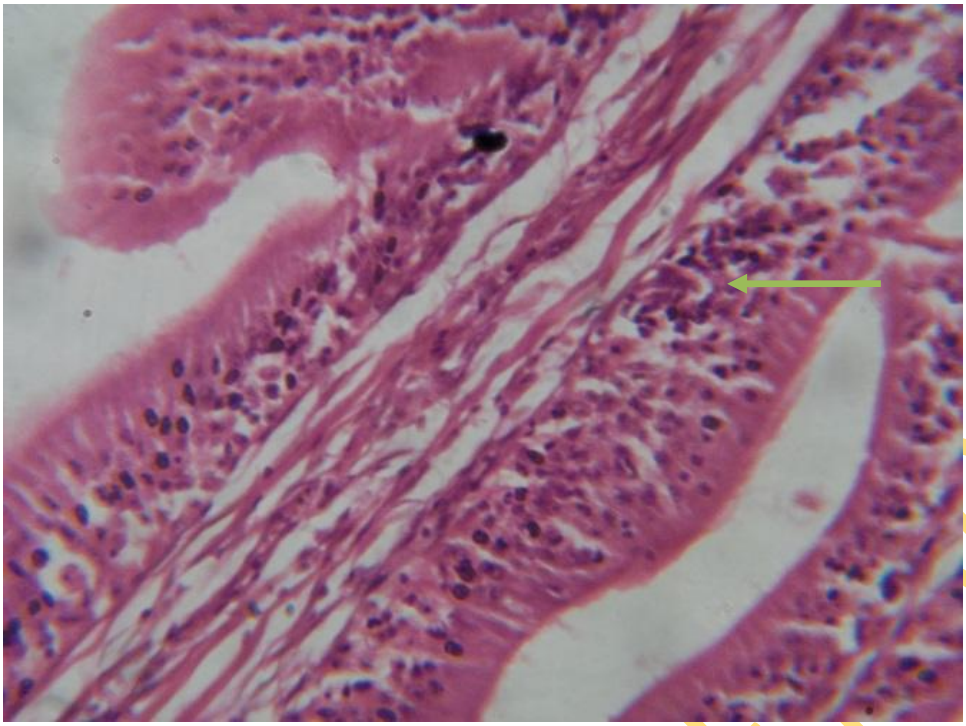


Plate 13: Histology of intestine of a catfish fed diet containing 50% ROAM (diet 3) showing showing no lesion except for mild infiltration of the mucosa and sub mucosa by inflammatory cell (green arrow). X400

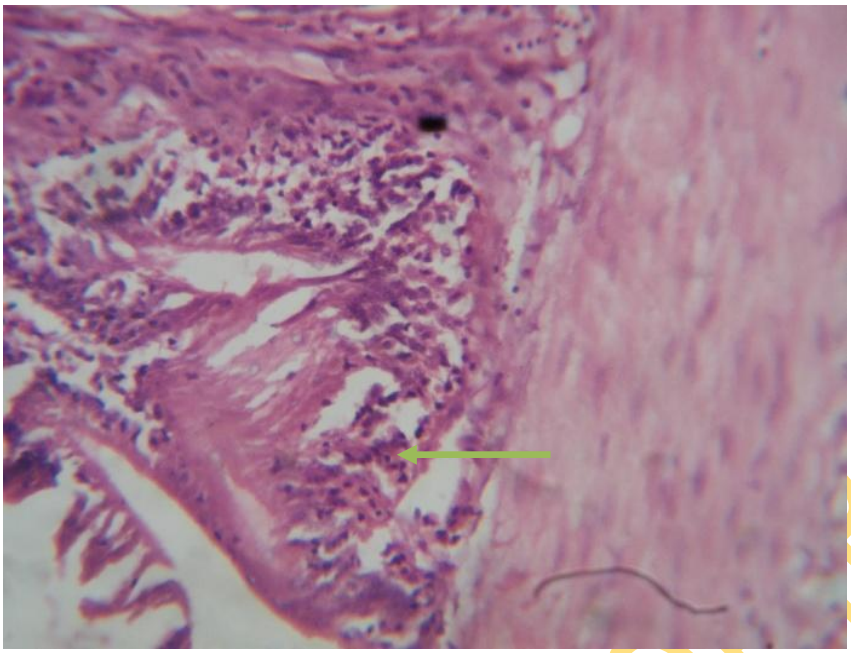


Plate 14: Histology of intestine of a catfish fed diet containing 75% ROAM (diet 4) showing no lesion except for mild infiltration of the mucosa and sub mucosa by inflammatory cell (green arrow). X400

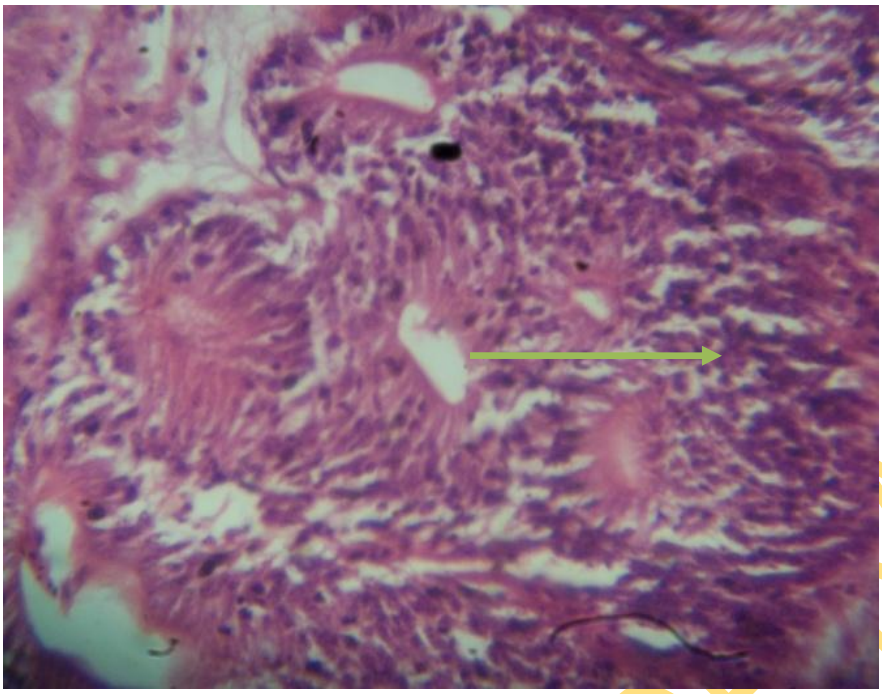


Plate 15: Histology of intestine of a catfish fed diet containing 100% ROAM (diet 5) showing severe infiltration of the mucosa and sub mucosa by inflammatory cell (green arrow). X400

Table 26: Summary of results of histopathological examination of *Clarias gariepinus* juveniles fed MEAM-based diets at inclusion levels of 0 % to100 % for 105 days.

Dietary Treatment	observation made on the organs		
	Liver	Kidney	Intestine
Diet1 (0%) Control	No lesion except for mild periportal inflammation	No lesion	Mild infiltration of the mucosa and submucosa by inflammatory cell
Diet 2 (25%)	No lesion except for mild periportal inflammation	No lesion	Mild infiltration of the mucosa and submucosa by inflammatory cell
Diet 3 (50%)	No lesion except for moderate periportal inflammation	No lesion	Mild infiltration of the mucosa and submucosa by inflammatory cell
Diet 4 (75%)	No lesion except for moderate Periportal inflammation	No lesion	Mild infiltration of the mucosa and submucosa by inflammatory cell
Diet 5 (100%)	disseminated macrovesicular steatosis; moderate periportal inflammation; focal area of vacuolar degeneration; vascular fibrosis	Tubular necrosis; focal area of lymphoid nodule; fibrosis of the wall of collecting tubules and some blood vessels	Severe infiltration of the mucosa and submucosa by inflammatory cell

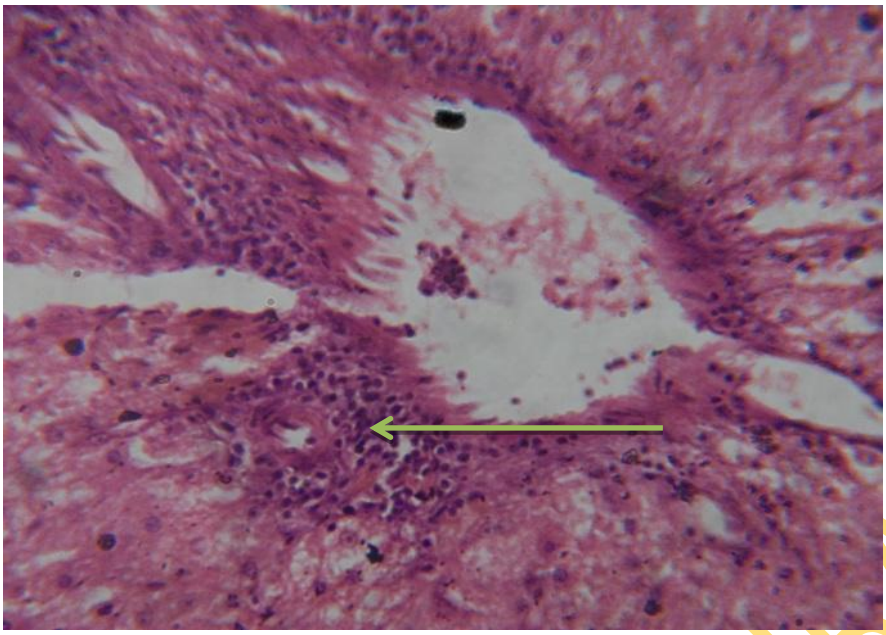


Plate 16: Histology of liver of a catfish fed diet containing 0% MEAM (diet 1, control) showing no lesion except for mild periportal inflammation (green arrow). X400

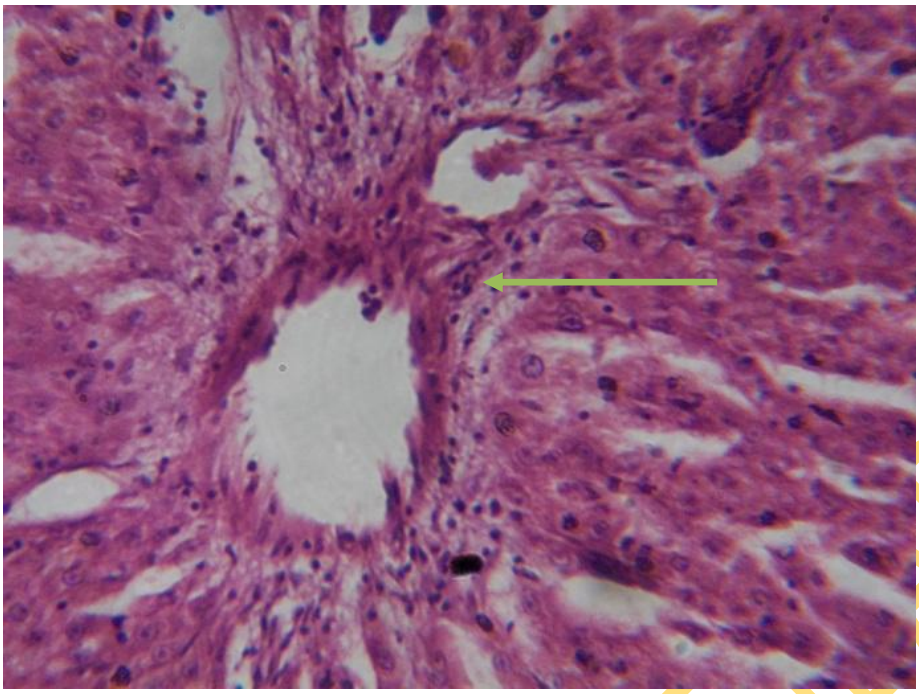


Plate 17: Histology of liver of a catfish fed diet containing 25% MEAM (diet 2) showing no lesion except for mild periportal inflammation (green arrow) X400

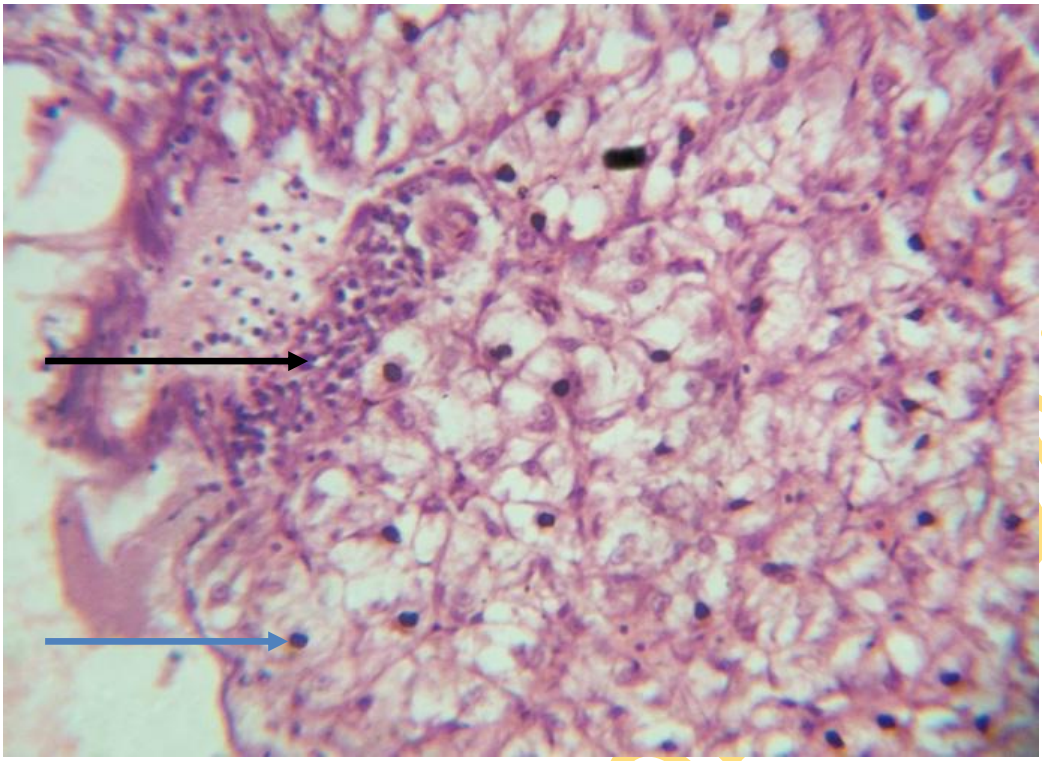


Plate 18: Histology of liver of a catfish fed diet containing 50% MEAM (diet 3) showing moderate periportal inflammation (black arrow). The hepatocytes are undergoing cell division as evidenced by mitotic figures (blue arrow). X400.

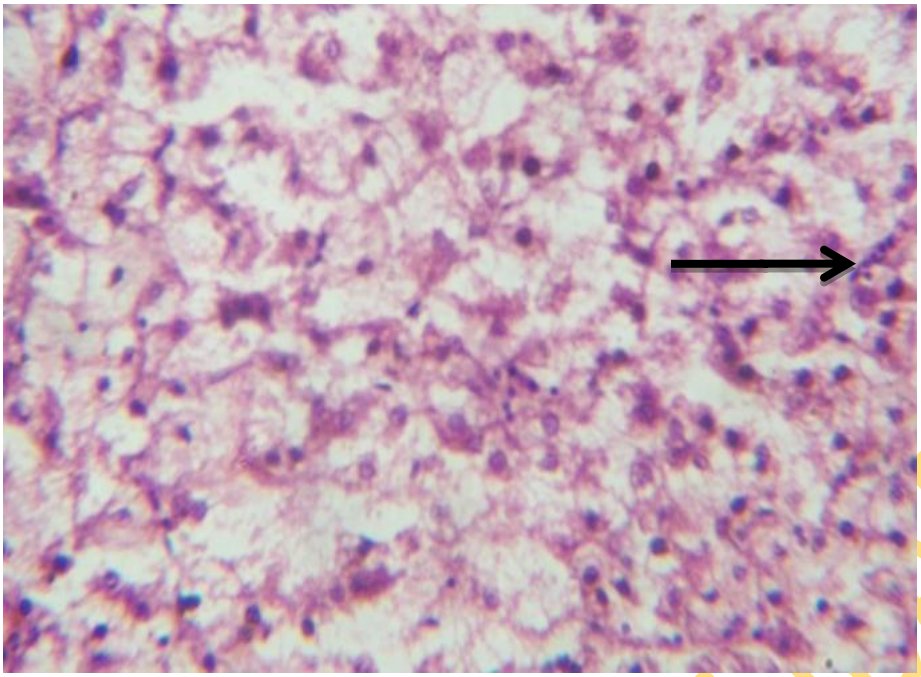
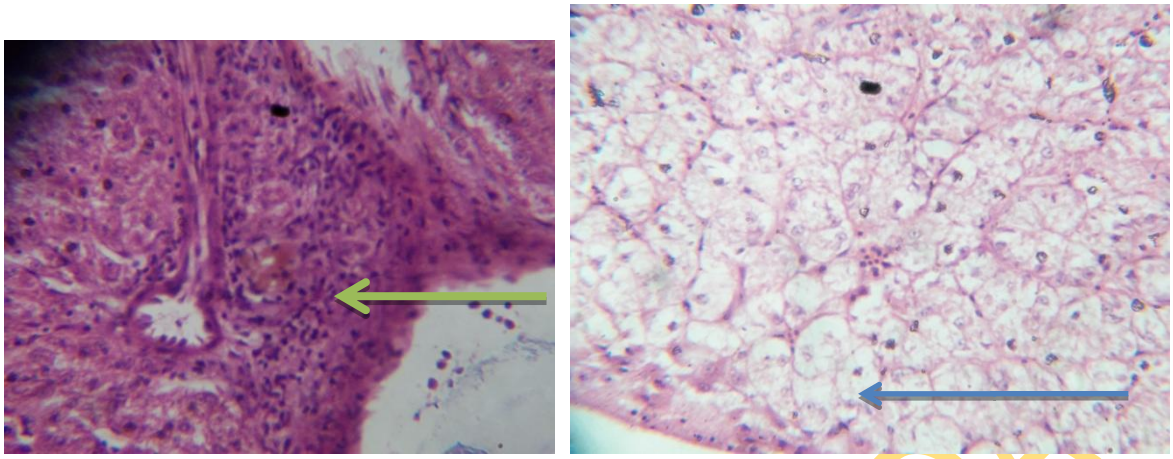
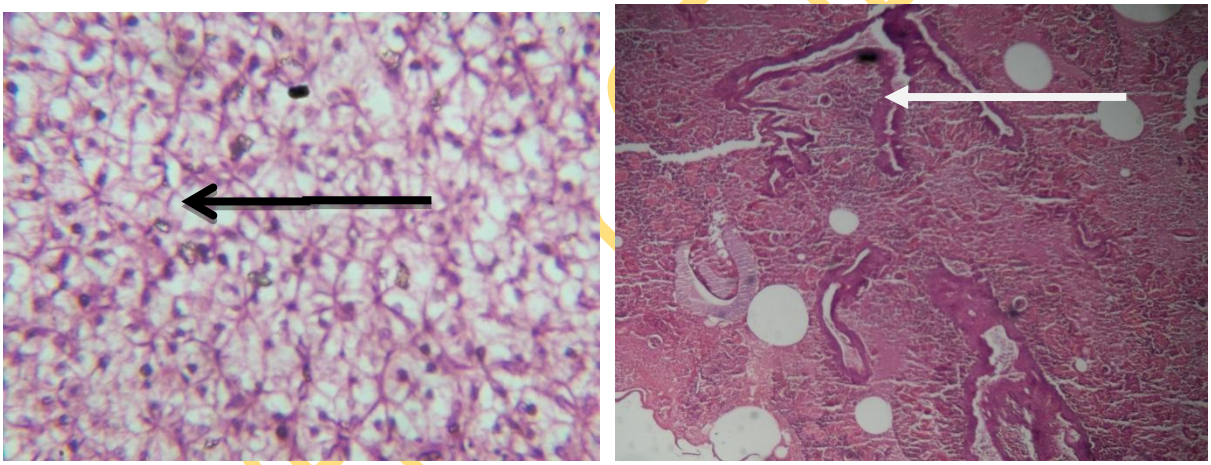


Plate 19: Histology of liver of a catfish fed diet containing 75% MEAM (diet 4) showing moderate periportal inflammation (black arrow). X400



(a) (b)



(c) (d)

Plate 20: Histology of liver of a catfish fed diet containing 100% MEAM (diet 5) showing: (a) moderate periportal inflammation (green arrow); (b) focal area of vacuolar degeneration (blue arrow); (c) disseminated macro-vesicular steatosis (fatty degeneration) (black arrow); (d) vascular fibrosis (white arrow). X400

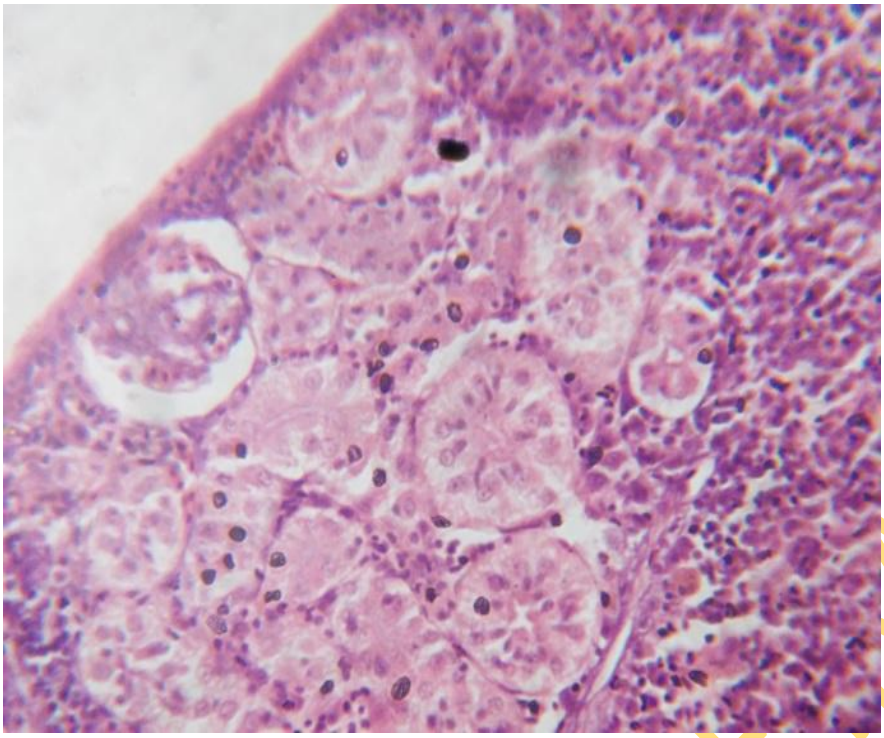


Plate 21: Histology of kidney of a catfish fed diet containing 0% MEAM (diet 1, control) showing no visible lesion. X400

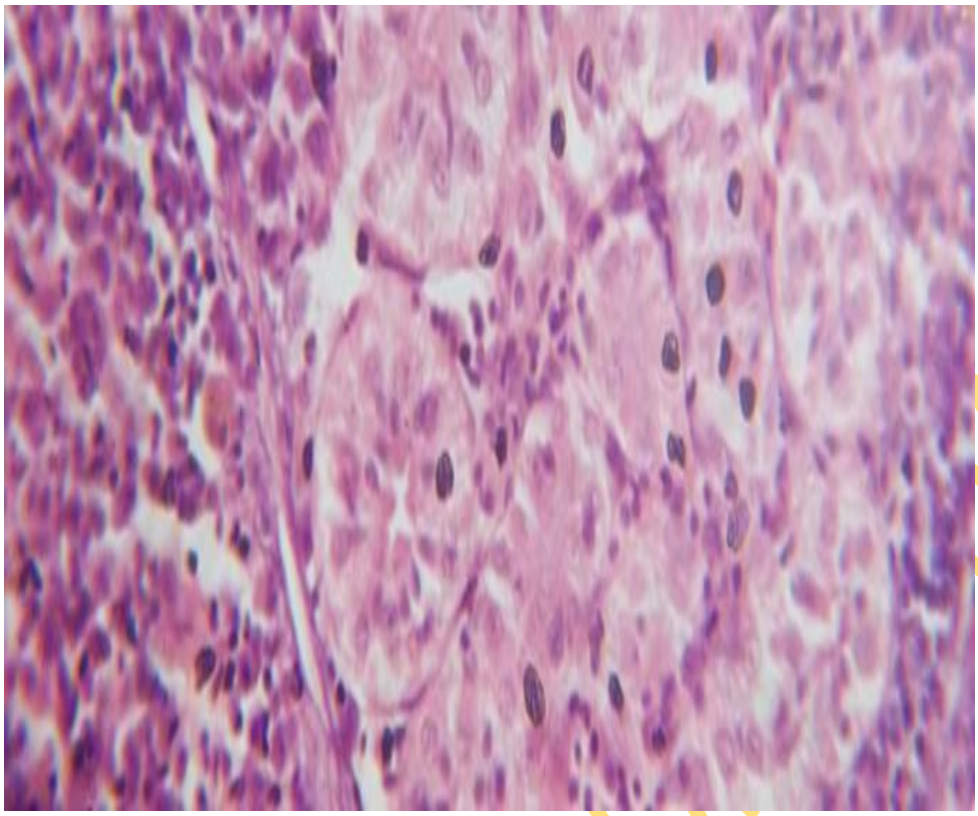


Plate 22: Histology of kidney of a catfish fed diet containing 25% MEAM (diet 2) showing no visible lesion. X400

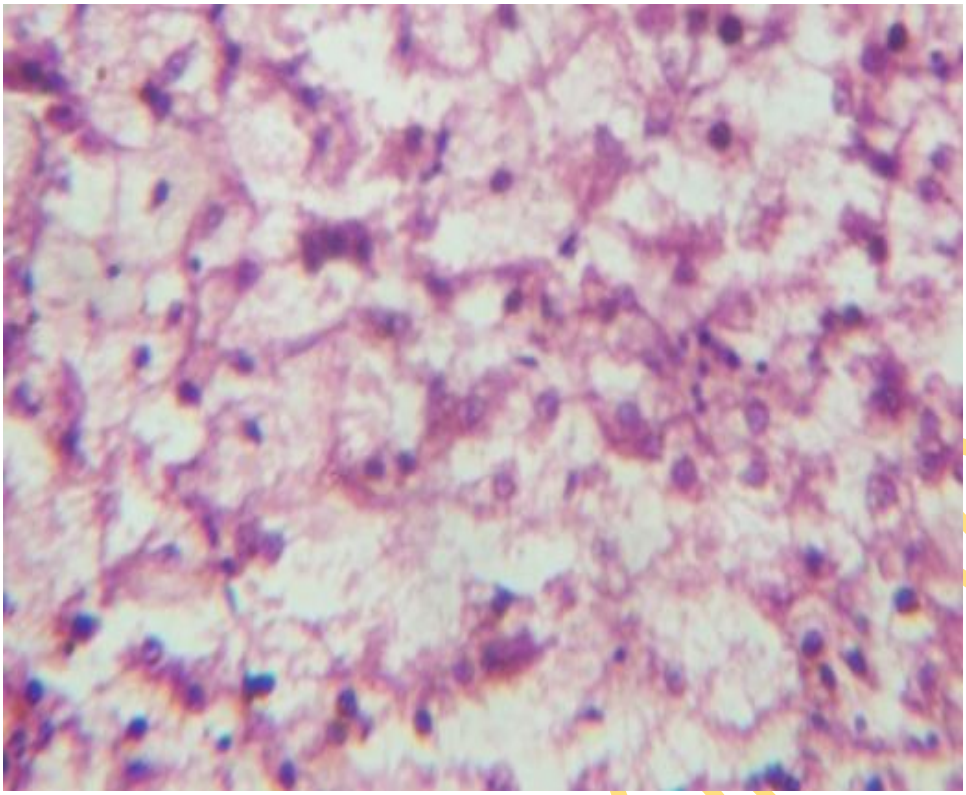


Plate 23: Histology of kidney of a catfish fed diet containing 50% MEAM (diet 3) showing no visible lesion. X400

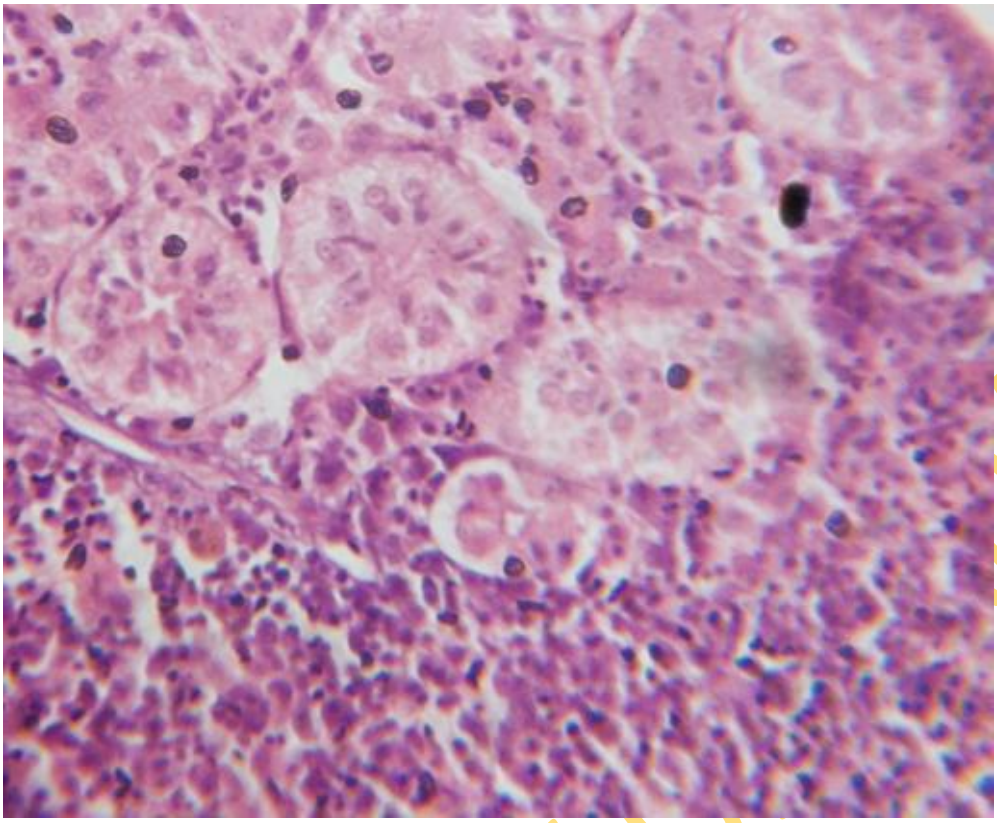
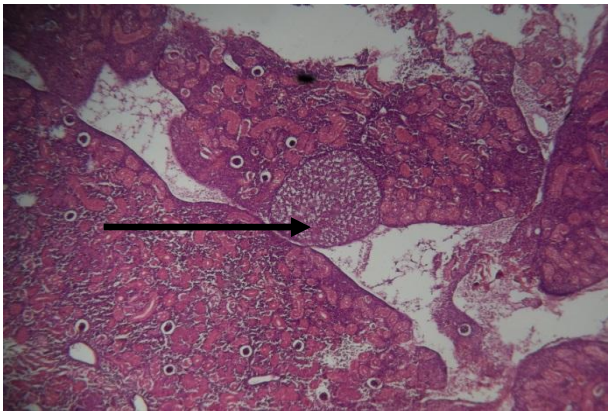
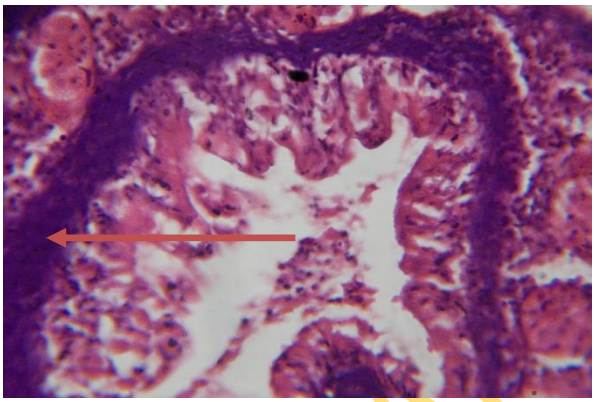


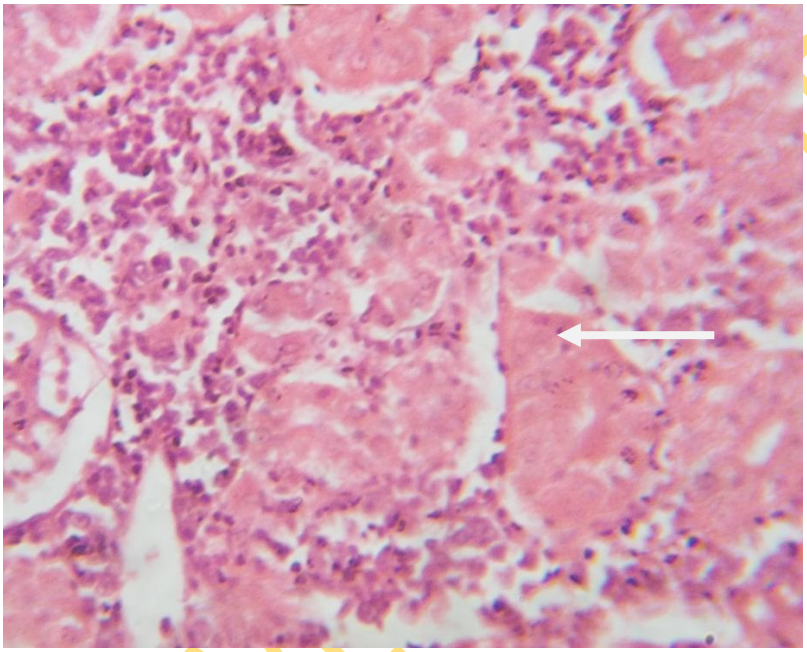
Plate 24: Histology of kidney of a catfish fed diet containing 75% MEAM (diet 4) showing no visible lesion. X400



(a)



(b)



(c)

Plate 25: Histology of kidney of a catfish fed diet containing 100% MEAM (diet 5) showing: (a) a focal area of lymphoid nodule (black arrow); (b) fibrosis of the wall of collecting tubules and some blood vessels (red arrow); (c) tubular necrosis (white arow). X400

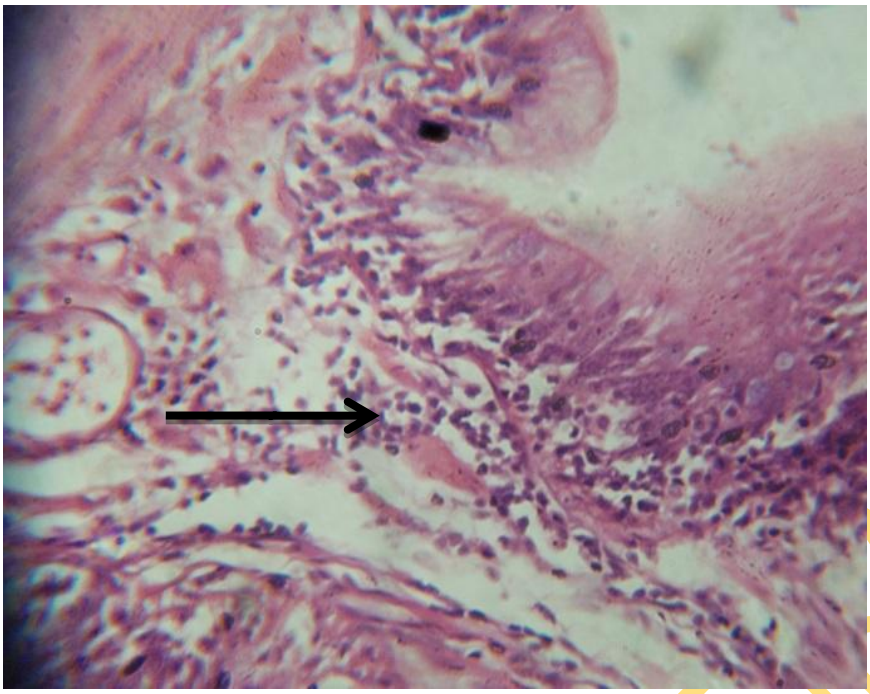


Plate 26: Histology of intestine of a catfish fed diet containing 0% MEAM (diet 1, control) showing no lesion except for mild infiltration of the mucosa and the submucosa by inflammatory cell (black arrow). X400

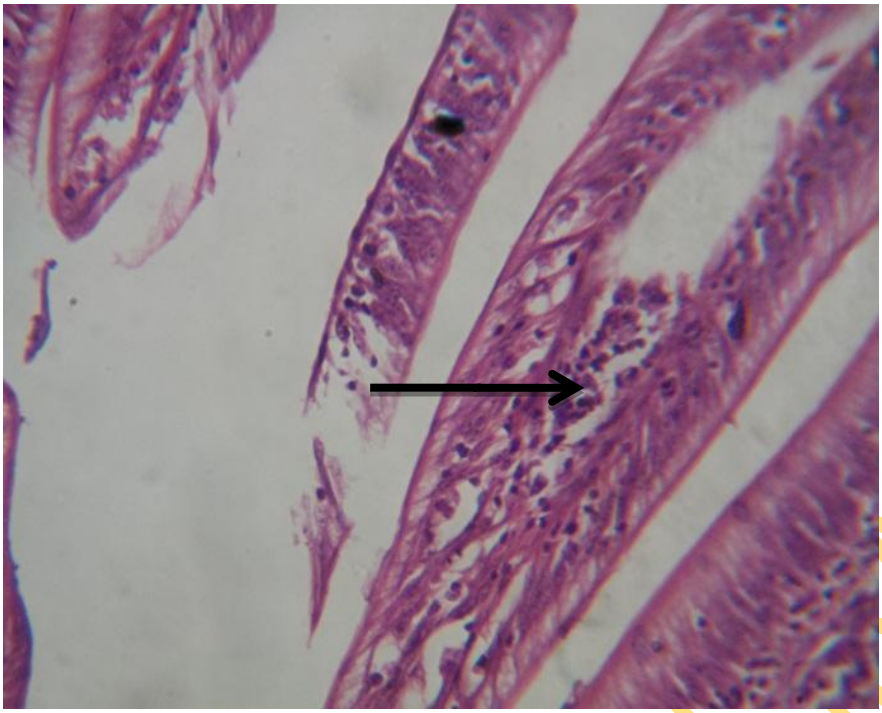


Plate 27: Histology of intestine of a catfish fed diet containing 25% MEAM (diet 2) showing no lesion except for mild infiltration of the mucosa and the submucosa by inflammatory cell (black arrow). X400

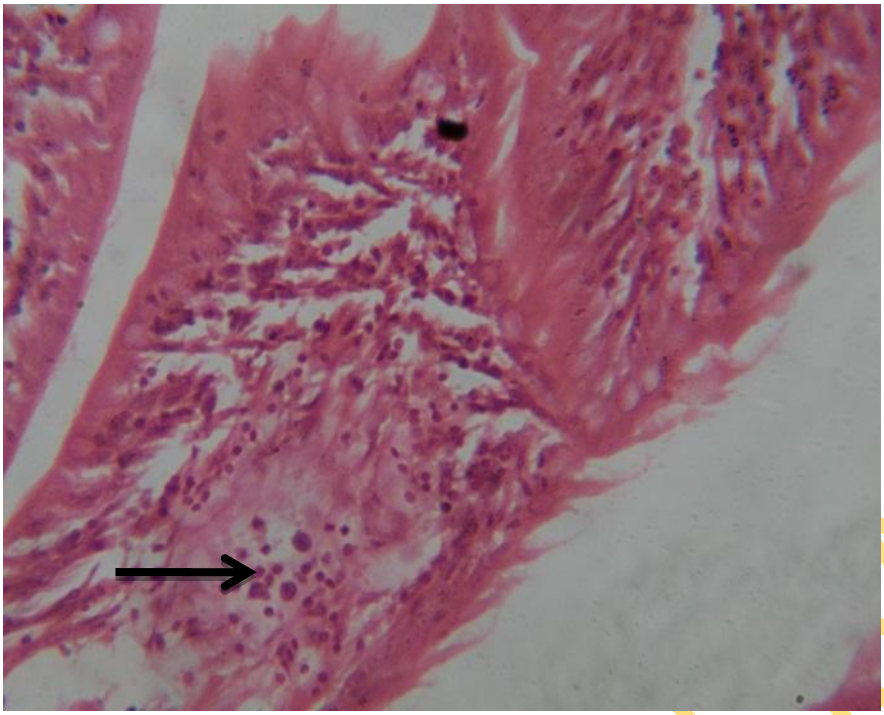


Plate 28: Histology of intestine of a catfish fed diet containing 50% MEAM (diet 3) showing no lesion except for mild infiltration of the mucosa and submucosa by inflammatory cell (black arrow). X400

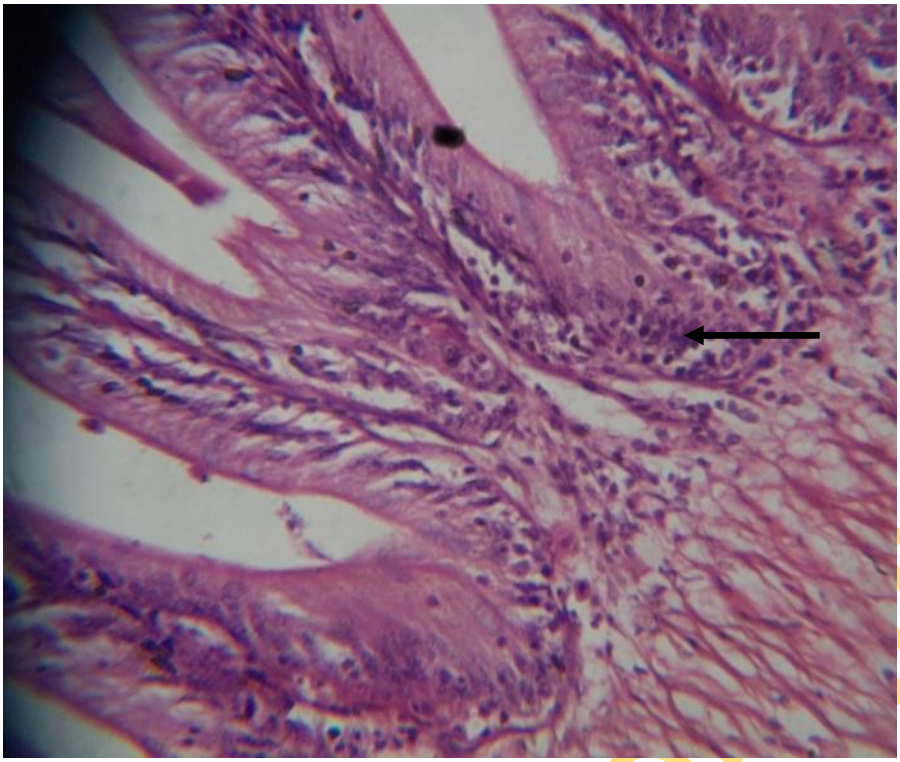


Plate 29: Histology of intestine of a catfish fed diet containing 75% MEAM (diet 4) showing mild infiltration of the mucosa and submucosa by inflammatory cell (black arrow). X400

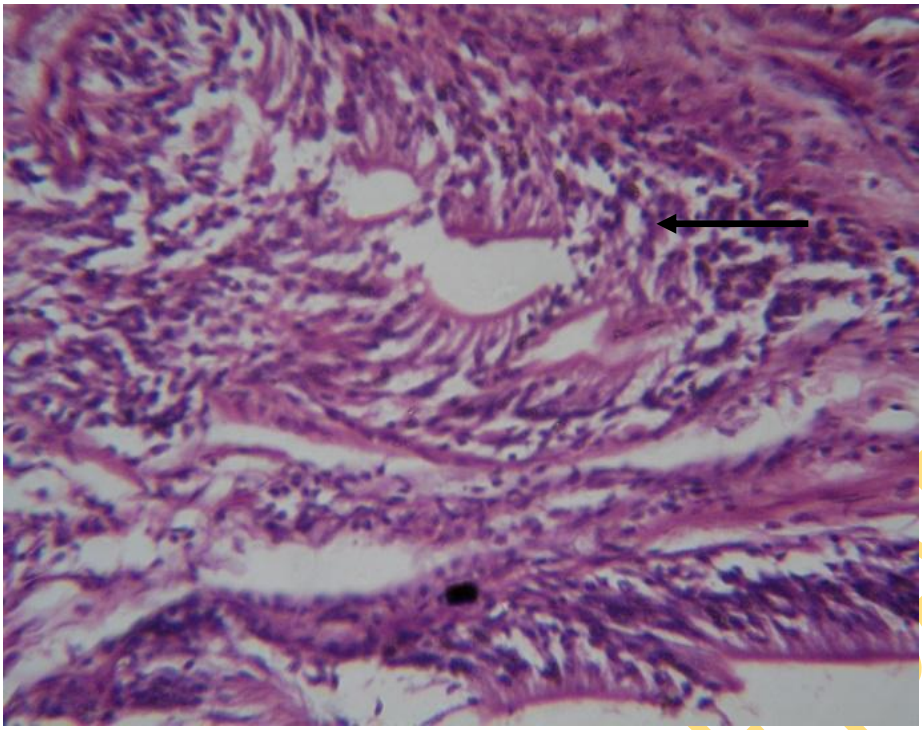


Plate 30: Histology of intestine of a catfish fed diet containing 100% MEAM (diet 5) showing severe infiltration of the mucosa and submucosa by inflammatory cell (black arrow). X400

4.11 Water Quality Parameters Monitored During Feeding Trial Using Graded Levels of ROAM and MEAM Based Diets.

Results of water quality parameters monitored during feeding trial using graded levels of ROAM based diets are presented in Table 27. Degree of acidity or alkalinity (pH) was highest in the culture media of fish fed diet 3 (7.25) followed by culture media of fish fed diet 5 (7.15) and least in the culture media of fish fed diet 2 (6.65) while the initial value was 6.96. Dissolved oxygen content varied among the culture media of fish fed gaded levels of ROAM diets. It was highest in the culture media of fish fed diet 2 (5.25mg/l), followed by the culture media of fish fed diet 3 (5.00) and least in the culture media of fish fed diet 5 (4.50mg/l) while the initial value was 5.87 mg/l. Initial water temperature was 28.45 ^{o}C. This fell in all the treatments by the end of the study to a narrow range of 25.40 ^{o}C to 25.83 ^{o}C. Initial ammonia content of the culture media was 0.00 mg/l. This increased in all the traetments' culture media to a range of 0.02 to 0.04 mg/l. Initial nitrite value was 0.00 mg/l. This increased in all treatments culture media to a narrow range of 0.01 to 0.03 mg/l.

Results of water quality parameters monitored during feeding trial using graded levels of MEAM based diets are presented in Table 28. pH was highest in the culture media of fish fed diet 2 (6.85) followed by culture media of fish fed diet 5 (6.80) and least in the culture media of fish fed diet 4 (6.55) while the initial value was 6.96. Dissolved oxygen content varied among the culture media of fish fed graded levels of MEAM based diets. It was highest (5.24mg/l) in the culture media of fish fed diet 2 followed by the culture media of fish fed diet 5 (5.20 mg/l) and least in the culture media of fish fed diet 4 (4.60mg/l) while the initial value stood at 5.87 mg/l. Initial water temperature was 28.45 ^{o}C. This fell in all the treatments by the end of the study to a narrow range of 25.40 ^{o}C to 25.87 ^{o}C. Initial ammonia value was 0.00mg/l. This increased in all the treatments' culture media to a range of 0.02 to 0.03 mg/l. Initial nitrite level of the culture water was 0.00 mg/l. This increased in all the treatments to a narrow range of 0.01 to 0.02 mg/l.

Table 27: Initial and final values of water quality parameters obtained during feeding trial with graded levels of ROAM based diets. Means ± SEM

Treatment	Water quality parameter				
	pH	Dissolved oxygen (mg/L)	Temperature (^{o}C)	Ammonia (mg/L)	Nitrite (mg/L)
Initial values	6.96±0.01	5.87±0.01	28.45±0.01	0.00±0.00	0.00±0.00
Diet 1 (0%, Control)	6.90±0.01	4.65±0.01	25.83±0.17	0.02±0.00	0.01±0.00
Diet 2 (25%)	6.65±0.01	5.25±0.03	25.77±0.15	0.02±0.00	0.01±0.00
Diet 3 (50%)	7.25±0.00	5.00±0.13	25.60±0.10	0.03±0.00	0.02±0.00
Diet 4 (75%)	6.75±0.01	4.70±0.06	25.80±0.15	0.03±0.01	0.02±0.00
Diet 5 (100%)	7.15±0.01	4.50±0.08	25.40±0.06	0.04±0.01	0.03±0.01

Table 28: Initial and final values of water quality parameters obtained during feeding trial with graded levels of MEAM based diets. Means ± SEM

Treatments	Water quality parameters				
	pH	Dissolved Oxygen (mg/L)	Temperature (°C)	Ammonia (mg/L)	Nitrite (mg/L)
Initial values	6.96±0.01	5.87±0.01	28.45±0.01	0.00±0.00	0.00±0.00
Diet 1 (Control)	6.75±0.01	4.70±0.01	25.60±0.06	0.02±0.00	0.01±0.00
Diet 2	6.85±0.01	5.24±0.01	25.57±0.03	0.02±0.00	0.01±0.00
Diet 3	6.50±0.12	4.70±0.06	25.40±0.06	0.03±0.01	0.02±0.00
Diet 4	6.55±0.01	4.60±0.06	25.70±0.06	0.03±0.01	0.02±0.00
Diet 5	6.80±0.06	5.20±0.01	25.87±0.03	0.02±0.00	0.01±0.00

4.12 Economic Evaluation Indices of Replacing Soya Bean Meal with Grdaded Levels of ROAM and MEAM in the Diet of *Clarias gariepinus* Juveniles Fed for 105 Days

Results of cost analysis of formulating roasted almond kernel meal diets at 0% to 100% inclusion levels are shown in Table 29 while economic evaluation indices of replacing soya bean meal with 0% to 100% inclusions of ROAM in the diet of *C. gariepinus* juveniles are shown in Table 30. Control diet had the highest cost (₦236.60 per kg). The cost of the diets reduced in all other treatments in a regular pattern with diet 5 (₦220.20/kg) being the least cost feed. Diet 4 produced fish with the highest value followed by diet 3 while the least value of fish was produced by diet 5. Diet 4 produced fish with the least Incidence of cost, followed by fish fed diet 3 while diet 5 produced fish with the highest incidence of cost. Fish fed diet 4 had the highest Profit index while diet 5 produced fish with the least Profit index.

Results of cost analysis of formulating mechanically extracted almond kernel meal diets at 0% to 100% inclusion levels are shown in Table 31 while economic evaluation indices of replacing soya bean meal with 0% to 100% inclusions of MEAM in the diet of *C. gariepinus* are shown in Table 32. Control diet had the highest cost. The cost of the diets reduced progressively in a regular pattern with diet 5 (₦ 223.70/kg) being the least cost feed. Diet 4 produced fish with the highest value followed by diet 3 while fish with the least value was produced by diet 5. Diet 4 produced fish with the least incidence of cost, followed by Diet 3 while diet 5 produced fish with the highest incidence of cost. Fish fed diet 4 had the highest profit index value while diet 1 produced fish with the lowest value of profit index. However, there was no significant difference ($p>0.05$) in the profit index values.

Table 29: Cost analysis of 0% to 100% ROAM based diets fed to *Clarias gariepinus* juveniles for 105 days.

Feed Ingredients	Cost of ingredient (N/kg)	Dietary Treatment				
		1 (0%, Control.)	2 (25%)	3 (50%)	4 (75%)	5 (100%)
Fish meal	500.00	(30.95) 15.48	(30.95) 15.48	(30.95) 15.48	(30.95) 15.48	(30.95) 15.48
Soya bean meal	120.00	(30.95) 3.71	(26.59) 3.19	(20.72) 2.49	(12.47) 1.50	- -
Almond kernel meal	69.98	- -	(8.86) 0.62	(20.72) 1.45	(37.41) 2.62	(60.01) 4.20
Yellow maize	110.00	(15.18) 1.67	(12.93) 1.42	(9.93) 1.09	(5.71) 0.63	(0.65) 0.07
Wheat offal	36.00	(15.18) 0.55	(12.93) 0.47	(9.93) 0.36	(5.71) 0.21	(0.65) 0.02
Palm oil	250.00	(1.00) 0.25	(1.00) 0.25	(1.00) 0.25	(1.00) 0.25	(1.00) 0.25
Cassava starch	140.00	(2.50) 0.35	(2.50) 0.35	(2.50) 0.35	(2.50) 0.35	(2.50) 0.35
Table salt	50.00	(0.50) 0.03	(0.50) 0.03	(0.50) 0.03	(0.50) 0.03	(0.50) 0.03
Dicalcium phosphate	240.00	(1.75) 0.42	(1.75) 0.42	(1.75) 0.42	(1.75) 0.42	(1.75) 0.42
Fish Premix	600.00	(2.00) 1.20	(2.00) 1.20	(2.00) 1.20	(2.00) 1.20	(2.00) 1.20
Cost of feed (₦/100g feed)		23.66	23.43	23.12	22.69	22.02
Cost of feed per kg (₦)		236.60	234.30	231.20	226.90.	220.20

Values of ingredient composition are indicated in parentheses. Costs of feed ingredients are prices of ingredient per kilogram as at the beginning of the experiment (March, 2013). Exchange rate: N 157.00: 1USD.

Table 30: Economic evaluation indices of replacing soya bean meal with 0% to 100% inclusions of ROAM in the diet of *Clarias gariepinus* juveniles. Means ± SEM

Parameter	Diet				
	1 (0%)	2 (25%)	3 (50%)	4 (75%)	5 (100%)
Total feed intake (g)	1504.06±24.97	1736.71±33.16	1758.49±31.29	1838.21±54.00	714.97±54.16
Total weight of fish (g)	1187.33±7.00	1317.67±9.20	1334.33±9.50	1406.67±8.00	483.00±7.20
Total weight gain (g)	946.67±6.24	1077.00±8.30	1103.00±8.86	1166.33±7.20	242.33±6.45
Cost of feed per kg (₦)	236.60±0.00	234.30±0.00	231.20±0.00	226.90.±0.00	220.20±0.00
Total Cost of feed used in production (N)	355.86±5.91^{b}	406.91±7.77^{c}	406.56± 7.23^{c}	417.09±12.25^{c}	157.44±11.93^{a}
Total value of fish produced (N)	593.67±3.12^{b}	658.84.50±2.99^{bc}	667.17±2.20^{bc}	703.34±3.84^{c}	241.50±1.09^{a}
Incidence of cost	0.376±0.03^{a}	0.378±0.01^{a}	0.369±0.01^{a}	0.358±0.01^{a}	0.650±0.01^{b}
Profit index	1.67±0.05^{b}	1.62±0.04^{b}	1.64±0.03^{b}	1.69±0.05^{b}	1.53±0.08^{a}

Mean values in each row with similar superscripts are not significantly different (p>0.05).

Table 31: Cost analysis of MEAM based diets fed to *Clarias gariepinus* juveniles for 105 days.

Feed Ingredients	Cost of ingredient (N/kg)	Dietary Treatments				
		1 (0%)	2 (25%)	3 (50%)	4 (75%)	5 (100%)
Fish meal	500.00	(30.95) 15.48	(30.95) 15.48	(30.95) 15.48	(30.95) 15.48	(30.95) 15.48
Soya bean meal	120.00	(30.95) 3.71	(26.09) 3.13	(19.83) 2.38	(11.53) 1.38	- -
Almond kernel meal	76.00	- -	(8.70) 0.66	(19.83) 1.51	(34.59) 2.63	(55.11) 4.19
Yellow maize	110.00	(15.18) 1.67	(13.26) 1.46	(10.82) 1.19	(7.59) 0.83	(3.10) 0.34
Wheat offal	36.00	(15.18) 0.55	(13.26) 0.48	(10.82) 0.39	(7.59) 0.27	(3.10) 0.11
Palm oil	250.00	(1.00) 0.25	(1.00) 0.25	(1.00) 0.25	(1.00) 0.25	(1.00) 0.25
Cassava starch	140.00	(2.50) 0.35	(2.50) 0.35	(2.50) 0.35	(2.50) 0.35	(2.50) 0.35
Table salt	50.00	(0.50) 0.03	(0.50) 0.03	(0.50) 0.03	(0.50) 0.03	(0.50) 0.03
Dicalcium phosphate	240.00	(1.75) 0.42	(1.75) 0.42	(1.75) 0.42	(1.75) 0.42	(1.75) 0.42
Fish Premix	600.00	(2.00) 1.20	(2.00) 1.20	(2.00) 1.20	(2.00) 1.20	(2.00) 1.20
Cost of feed (N/100g feed)		23.66	23.46	23.20	22.84	22.37
Cost of feed per kg (N)		236.60	234.60	232.00	228.40	223.70

Values of ingredient composition are indicated in parentheses. Costs of feed ingredients are prices of ingredient per kilogram as at the beginning of the experiment (March, 2013). Exchange rate: N 157.00: 1USD.

Table 32: Economic evaluation indices of replacing soya bean meal with 0% to 100% inclusions of MEAM in the diet of *Clarias gariepinus* juveniles. (Means ± SEM).

Parameters	Experimental diets				
	1(0%) control	2(25%)	3 (50%)	4 (75%)	5 (100%)
Total feed intake (g)	1510.71±10.61	1604.75±79.88	1682.75±62.60	1812.34±11.6	1509.33±24.84
Final total weight (g)	1183.00±7.10	1247.00±8.20	1296.67±9.10	1394.33±8.00	1122.67±7.20
Total weight gain (g)	942.67±6.40	1006.33±7.50	1056.00±8.70	1154.00±7.20	882.33±6.80
Cost of feed per kg (N)	236.60±0.00	234.60±0.00	232.00±0.00	228.40±0.00	223.70±0.00
Total Cost of feed used In Production (N)	357.43±2.51^{b}	376.47±8.67^{bc}	390.40±14.39^{bc}	413.94±2.39^{c}	337.64±2.97^{a}
Total value of fish produced (N)	591.50±12.10^{ab}	623.50±16.62^{bc}	648.34±17.28^{bc}	697.17±14.51^{c}	561.34±9.68^{a}
Incidence of cost	0.379±0.01^{a}	0.374±0.02^{a}	0.370±0.01^{a}	0.359±0.01^{a}	0.383±0.01^{a}
Profit index	1.65±0.04^{a}	1.66±0.07^{a}	1.66±0.02^{a}	1.68±0.01^{a}	1.66±0.04^{a}

Mean values in each row with similar superscripts are not significantly different (p>0.05).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Amino Acid Profile of Tropical Almond Kernel.

Result of the present study showed that Tropical almond kernel has a good amino acid profile comprising of both essential and non-essential amino acids. Glutamate had the highest value while methionine was the least. This is consistent with the work of Muhammad and Oloyede (2009) which reported glutamate and methionine as the highest and least values for raw almond kernel meal respectively. The values of the essential amino acids – isoleucine (4.26g/100g), leucine (7.37g/100g), lysine (6.60g/100g), methionine (1.02g/100g) and histidine (2.48g/100g) recorded in the present study are comparable to the values 4.51g/100g, .07g/100g, 6.66g/100g, 1.23g/100g and 2.38g/100g reported for isoleucine, leucine, lysine, methhionine and histidine respectively by Muhammad and Oloyede, (2009) for raw almond kernel meal.

5.2 Chemical Composition of Raw and Differently Processed Tropical Almond Kernel Meal

5.2.1 Effects of Different Processing Methods on the Proximate and Mineral Composition of Tropical Almond Kernel Meal.

Processing of tropical almond kernel meal using different methods produced variation in their chemical composition, digestibility and nutrient utilization by the experimental fish. This observation agrees with the findings of Olukunle (1996), Oyelese (1994) and Omitoyin (2005). They had earlier observed that processing of the test feedstuffs (sesame seed, cassava peels and poultry by-products respectively) produced variations in their chemical composition and digestibility of nutrients.

Results of the present study showed that solvent extracted almond kernel meal (SEAM) had the highest crude protein content (35.38%), followed by mechanically-extracted almond kernel meal (MEAM) (30.64%) while boiled almond kernel meal (BOAM) had the least value of 24.07%. The high crude protein content of SEAM could be attributed to the concentration of the remaining compounds (nutrients) after extraction of the oil component. This is consistent with the report of Adesina (2014) who attributed high protein content of solvent extracted sunflower

seed meal to the concentration of all inorganic solvent-soluble components after the separation of the organic solvent-soluble potion of the meal. The lower crude protein content of boiled almond kernel meal could be attributed to leaching and/ or breakdown of protein during boiling. This observation agrees with the reports of Giami and Ikpimi (1992) which reported reduction in the protein content of sunflower seed meal processed by boiling. It is also consistent with the report of Iyayi and Egharevba, (1998) who reported a reduction in crude protein content of boiled *Mucuna utilis* seeds compared to the raw seeds.

Raw almond kernel meal had the highest fat content followed by roasted almond kernel meal while solvent extracted tropical almond kernel meal had the lowest crude fat content. The crude fat content of raw and differently processed almond kernel meals in the present study (1.86 to 30.27%) are lower than the value of 36.0% reported by Olapade and Kargbo (2012) for raw almond kernel meal.

Crude fibre contents of raw and differently processed tropical almond kernel meal samples had a narrow range of 3.33% to 3.79% and were lower than those of Falaye et al., (2014) for lima bean (4.77-6.87) and Adesina (2014) (14.19%-23.94%) for processed sunflower seed meals. Ash content was highest (4.87%) and the least (2.78%) in the solvent extracted and soaked almond kernel meals respectively. This is similar to those obtained by Falaye et al. (2014) (3.65-4.36%) for processed lima bean. Moisture content was least in roasted almond kernel meal (4.85%) and highest in boiled almond kernel meal (13.65%). The low moisture content of roasted almond kernel meal could be attributed to the direct removal of moisture by roasting.

Nitrogen free-extract (NFE) content of the differently processed tropical almond kernel meals ranged from 28.16 to 46.15% with the least and highest values recorded for raw and mechanically extracted almond kernel meals respectively. The high nitrogen-free extract content of the differently processed tropical almond kernel meals is an indication that they can also serve as an energy source in aqua-feed. Solvent-extracted tropical almond kernel meal had the least gross energy value while raw tropical almond kernel meal had the highest value followed by roasted almond kernel meal. There were significant differences ($p<0.05$) in the gross energy content of the raw and differently processed meals. The high gross energy content of raw and

roasted almond kernel meals could be attributed to the high crude fat content of these two meals compared to the other meals.

All the processing methods resulted in an increase in the calcium and phosphorus content of tropical almond kernel meal. This is similar to the report of Sotolu (2008) who observed increase in the calcium content of roasted and soaked Leucaena seed compared to the raw (sundried) one. Solvent extraction resulted in an increase in Magnesium and sodium content of tropical almond kernel (compared to the raw meal) while the other processing methods slightly reduced these minerals. This is in agreement with the report of Falaye et al. (2014) which observed reduction in Magnesium content of Lima bean seed processed by toasting, soaking, boiling and autoclaving. The high magnesium and sodium content of SEAM could be attributed to the concentration of the remaining nutrients (including some minerals) after extraction of the oil component. Roasting significantly ($p<0.05$) increased potassium content of tropical almond kernel meal while the other processing methods caused a slight reduction in potassium values. All the processing methods caused a reduction in iron content of tropical almond kernel meal. Generally, the differently processed almond kernel meals had a good mineral profile.

5.2.2 Effects of Different Processing Methods on the Level of Anti-Nutritional Factors in Processed Tropical Almond Kernel Meals.

Apart from trypsin inhibitor, the levels of antinutritional factors in tropical almond kernel meals in this study were generally low. Oxalate was in the range of 0.013 to 0.026%, phytate 0.035 to 0.092% and tannin 0.026 to 0.065%. This is relatively low compare to that of Adesina (2014) with the ranges 0.11 to 0.18% for oxalate, 0.11 to 0.16% for phytate and 0.21 to 0.45% for tannin for differently processed sunflower seed meals. It is also relatively low compared to that of Falaye et al., (2014) with ranges 0.62 to 1.09% for oxalate, 0.73 to 1.04% for phytate and 0.044 to 0.09% for tannin for differently processed lima bean meals.

All the processing methods employed in this study reduced the levels of anti-nutritional factors namely oxalate, phytate, tannin and trypsin inhibitor present in raw tropical almond kernel.

Tannin content reduced from 0.065% for the raw kernel meal to 0.052% for the solvent extracted kernel meal, 0.050 for the soaked kernel meal, 0.036% for the boiled kernel meal, 0.032% for the mechanically extracted kernel meal and 0.026 for the roasted kernel meal. The reduction in the level of tannin resulting from the various processing methods employed in this study is in line with the recommended methods for removal of condensed tannins from seeds which includes dehulling, autoclaving, heat treatment (boiling and roasting) and fermentation (Griffiths 1991). Oxalate has been reported to reduce the physiological value of calcium in seeds (Johnson et al., 1979). For this study, oxalate significantly reduced from 0.051% in the raw kernel meal to 0.040 for solvent extracted kernel meal, 0.0290% for the soaked kernel meal, 0.0257% for the boiled kernel meal, 0.021% for mechanically-extracted kernel meal and 0.013% for the roasted kernel meal.

Phytic acid occurs naturally throughout the plant kingdom and is present in considerable quantities within many of the major legume and oil seeds. Spinelli et al. (1983) reported that about 62-73% and 46-73% of the total phosphorus within cereal grains and legume seeds are in the form of organically bound phytin phosphorus respectively. The major part of the phosphorus contained within phytic acid is largely unavailable to monogastric animals due to the absence of the enzyme phytase within the digestive tract of monogastric animals. Phytic acid acts as a strong chelator, forming protein and mineral-phytic acid complexes; the net result being reduced protein and mineral bioavailability (Spinelli et al., 1983).

The different processing methods used in this study reduced phytate from 0.092% for the raw meal to 0.074% for solvent extracted meal, 0.065% for the soaked meal, 0.052% for the boiled meal, 0.043% for mechanically extracted meal and 0.035% for the roasted meal being the least. This is in line with the reports of Iyayi and Egharevba (1998) and Falaye et al. (2014) who recorded reduction in phytate content of processed *Mucuna utilis* seeds and lima bean meal respectively. It also agrees with the report of Hossain and Jauncey, (1990) cited in Adesina (2014) which stated that heat treatment reduced the phytic acid content of linseed and sesame meals by up to 72% and 74% respectively.

5.3. Proximate Composition of Experimental Diets Used for Digestibility Study

The crude protein content of all the experimental diets fell within the range 40.03 to 40.24%. There was no significant difference (P>0.05) in the crude protein content of the experimental diets. Crude fat content varied significantly (p<0.05) among the dietary treatments with a range of 4.71 to 6.99%. Raw Almond Kernel Meal Diet (RWAM) had the highest value (6.99%) while Solvent Extracted Almond Kernel Meal Diet (SEAM) had the least value (4.71%). The crude fat content of the experimental diets obtained in this study (4.71 to 6.99%) compares well with that of Sotolu and Sule (2011) (4.21 to 7.14%) for water hyacinth meal based diets fed to *Clarias gariepinus*. Crude fibre content ranged between 2.45 and 3.34%. RWAM had the highest value while boiled almond kernel meal diet (BOAM) had the least value. The crude fibre content of the experimental diets in this study (2.45 to 3.34%) are similar to 2.87 - 3.06% and 3.50 to 4.28% reported by Adesina (2014) and Agbebi *et al*.(2012) for sunflower seed meal diets and biscuit waste based diets fed to *Clarias gariepinus* respectively. Ash content ranged between 12.85% and 13.20%. The ash content of the experimental diets in this study compares well with that of Sotolu (2008) (10.36 to 11.41% for processed Leucaena meal diets. Generally, the diets formulated with the differently processed almond kernel meals had comparable nutrient content-crude protein, crude fat, crude fibre, ash and nitrogen-free extract. The observed differences for some of the nutrients could be attributed to the differences in the proximate composition of the differently processed kernel meals used to formulate the different diets.

Moisture content of the experimental diets had a narrow range (5.18 to 6.34%). The control diet (RFRD) had the least moisture content (5.18%) while SEAM had the highest value (6.34%). The values of moisture content obtained in this study (5.18 to 6.34%) fell within the recommended level (<10%) for catfish diet (Tacon, 1987; Ajani et al., 2011). Nitrogen-free extract (NFE) ranged between 30.81 to 33.58%. SEAM had the highest value (33.58%) while RWAM had the least value. The range of NFE values obtained in this study (30.81 to 33.58%) are similar to the values 28.75 to 35.94% reported by Agbabiaka et al. (2013) for tiger nut based diets and 21.29 to 34.64 % reported by Ovie et al., (2012) for baker's yeast based diets. It is however higher than the values 27.12 to 29.36 reported by Falaye and Oloruntuyi (1998) for plantain peel meal based diet, 19.58 to 22.94% reported by Bello et al. (2012) for walnut leaf and onion bulb residues based diets, 19.32 to 25.21 reported by Ochang et al. (2007) for palm oil based diets. The

difference in the NFE values could be attributed to differences in the ingredient composition of the diets.

Gross energy content of the experimental diets ranged between 3,478.67 and 3,698 kcal/kg. The highest value was observed in RWAM while the least value was observed in BOAM. The gross energy content observed in the present study (3478.67 to 3698.00 kcal/kg) are comparable to that of Sotolu (2008) (3001.58 to 3132.21 kcal/kg) observed for differently processed Leucaena seed meal based diets but slightly higher than 3,478.67 to 3,698 kcal/kg reported by Sotolu and Faturoti (2009) for soaked Leucaena seed meal based diets. The difference in the energy values could be because of differences in the ingredient composition of the diets.

5.4 Carcass Composition of *Clarias gariepinus* Juveniles Fed Differently Processed Tropical Almond Kernel Meal Based Diets.

The crude protein content of the initial fish sample was 48.41% while the final carcass protein values were significantly higher ($p<0.05$) and ranged between 60.13 and 63.68%. Mechanically etracted almond kernel meal diet (MEAM) had the highest value (63.68%) while RWAM had the least value (60.13%). There was a significant difference ($p<0.05$) among the treatments. Increased body protein level recorded in all the treatments indicates that the diets supported the growth of fish. It also indicates that the protein requirement of *Clarias gariepinus* juveniles for growth and body maintenance was satisfied. The higher body protein deposition and increased weight gain is indicative of the adequacy of the protein content of the raw and differently processed almond kernel meal based diets. This observation is consistent with the findings of Bello et al. (2012) which reported higher carcass crude protein content at the end of the feeding trial compared to that before the feeding trial. Similar observations were also made by Nweke and Ugwumba (2005) and Ochang et al. (2007).

Crude fat content of the fish before commencement of the experiment was 6.84%. This increased in all the treatments with fish fed RWAM having the highest value (13.13%) while the least value (11.93%) was observed in fish fed SEAM. There was a significant difference ($p<0.05$) between the final treatment values and the initial value. There was also a significant difference

($p<0.05$) among the treatments' crude fat content. This could be linked to the difference in the crude fat content of the diets.

Crude fiber content of the initial fish carcass was 0.01%. This increased in all but one treatment (RWAM). Fish fed SEAM had the highest final treatment value (0.12%) while fish fed RWAM had the least value (0.01%). There were significant differences ($p<0.05$) among the final treatments' mean values and between the final treatments' values and the initial value.

Ash content of the initial fish carcass was 5.16%. This increased in all the treatments at the end of the experiment. Fish fed RWAM had the highest value (9.42%) while fish fed RFRD (control) had the least value (8.63%). There was a significant difference ($p<0.05$) between the initial value and the treatments' values. There was also significant difference ($p<0.05$) among the treatments' ash values. The increase in *Clarias gariepinus* carcass ash content at the end of the experiment observed in this study is consistent with the repot of Bello et al. (2012) which also reported increase in *C. gariepinus* juveniles' ash content after feeding them with walnut leaf and onion bulb residues based diets. It also agrees with the report of Agbebi et al. (2012) which recorded increase in ash content of fingerlings of the same species after feeding them with biscuit waste based diets. The higher body ash deposition is indicative of the adequacy of the raw and differently processed almond kernel meal based diets in meeting the nutrient requirement of the fish including protein. This observation is consistent with the findings of Nwanna et al. (2014b) which recorded higher carcass ash content for higher levels of crude protein in the diet of *Clarias gariepinus.*

Moisture content of fish carcass ranged between 5.18 and 6.37%. Fish fed control diet (RFRD) had the least value 5.18% while fish fed MEAM had the highest value 6.37%. The carcass moisture content obtained in this study (range, 5.18 to 6.38%) is similar to 4.87 to 6.16% reported by Adesina (2014) and 5.44 to 8.91% reported by Ochang et al. (2007).

Initial Nitrogen-free extract (NFE) was 33.39%. This reduced significantly ($p<0.05$) in all the final treatment values. Fish fed soaked almond kernel meal (SOAM) diet had the highest value 13.30% while fish fed MEAM had the least value (8.38%). There was a significant difference ($p<0.05$) among the treatments' NFE values. The reduction in NFE values in all the treatments

observed in the present study is in consonant with the reports of Sotolu and Faturoti (2009) and Bello et al. (2012) who reported reductions in fish carcass NFE values at the end of the feeding experiment. The reduction in nitrogen-free extract content of fish fed differently processed almond kernel meal at the end of the experiment could be associated with increase in protein content of the fish carcass at the end of the experiment for all the diatery treatment.

5.5 Growth, Nutrient Utilization and Digestibility Indices of *C. gariepinus* Juveniles Fed Differently Processed Tropical Almond Kernel Meal Based Diets

5.5.1 Growth Performance of *C. gariepinus* Juveniles Fed Differently Processed Tropical Almond Kernel Meal Based Diets.

According to Eyo and Olatunde (2003), a feedstuff may present an excellent chemical composition, and be of less value unless it can be digested and utilized for growth by the target species. All the dietary treatments exhibited weight gain indicating that the experimental fish were able to digest the feed and converted the protein in the feed to extra flesh.

There were variations in the values of the mean weight gain of fish fed differently processed tropical almond kernel meal based diets because of the extent of digestibility of the diets. This observation is consistent with the report of Nwanna (2003) which reported significant mean weight gain in experimental fish fed diets with higher digestibility values.

Highest (2.42%/day) and least (1.53%/day) values of specific growth rate (SGR) were recorded for roasted almond kernel meal diet (ROAM) and soaked almond kernel meal diet (SOAM) respectively. There were significant differences ($P<0.05$) among the treatments' SGR values. The SGR values recorded in this study are higher than 0.49 to 0.62% recorded for *C. gariepinus* fed differently processed sunflower seed meal diets (Falaye et al., 2016). Percentage weight gain (relative growth rate) followed the same pattern with ROAM having the highest value followed by MEAM while SOAM had the least value.

Condition factor (K) represents the wellbeing or fullness of fish. The K values obtained in this study for differently processed tropical almond kernel meal based diets had a narrow range of

0.61 to 0.66. There were no significant differences (P>0.05) among the treatments. This indicates that all the differently processed almond kernel meal based diets had positive effects on the fish well-being comparable to the control diet.

Fish fed differently processed tropical almond kernel meal diets in this study showed high survival rate ranging from 98.33 to 100%. This is higher than that reported by Falaye et al. (2014) for *C. gariepinus* fed differently processed lima bean based diets (93.33- 96.66%). However, there was no significant difference (P>0.05) among the treatments' survival rate. The generally high survival rate of the experimental fish in all the treatments suggests that the differently processed almond kernel meal based diets supported fish growth and survival.

5.5.2 Nutrient Utilization Indices of *Clarias gariepiuus* Juveniles Fed Differently Processed Tropical Almond Kernel Meal Diets.

Fish fed ROAM consumed the largest amount of feed (172.90 g) while those fed SOAM consumed the least amount (148.61g). The highest feed intake by fish fed ROAM could be attributed to the effect of heat treatment (roasting) in reducing the levels of anti nutritional factors present in tropical almond kernel. The best (lowest) feed conversion ratio (FCR) was observed in fish fed ROAM followed by those fed MEAM while the highest value was observed in fish fed SEAM and SOAM. This indicates a superior level of utilization of ROAM and MEAM diets by the experimental fish. FAO (1993 b) documented that the lower the FCR the better the feed utilization. The FCR values 0.97 to 1.56 reported in this study are better than those reported by Ihimekpen (2003) (5.72 to 5.81) for *Clarias gariepinus* fingerlings fed shrimp waste based diets, Keremah and Green (2005) (4.91 to 9.25) for hybrid cat fish fingerlings fed fish offal based diets and konyeme et al. (2005) (3.57 to 3.95) for *C. gariepinus* fingerlings fed water hyacinth based diets. The lower FCR for ROAM and MEAM indicates better feed utilization by fish fed these diets and is justified by better growth performance exhibited by fish fed these diets. The better nutrient utilization and growth performance exhibited by fish fed processed almond kernel meal especially ROAM and MEAM based diets could therefore be attributed to improved nutritive value (lower levels of anti-nutritional factors, better digestibility indices) of roasted and mechanically extracted almond kernel meals.

Nitrogen metabolism (Nm) ranged between (176.67 and 210.09) with the highest value observed in fish fed ROAM while the least value was observed in fish fed SOAM. There was no significant difference ($p>0.05$) between Nm value of control diet (RFRD) and the other dietary treatments. The Nm values obtained in this study are lower than those reported by Falaye et al. (2016) (1843.29 to 2155.50). The differences in the nitrogen metabolism values could be as a result of differences in the other nutrient utilization indices and digestibility co-efficients of the different experimental diets.

Net protein utilization (NPU) was highest in fish fed ROAM (30.74%) followed by those fed MEAM (30.31%). Fish fed SOAM had the least NPU value of 27.81%. The NPU range (27.81 to 30.74%) obtained in this study is higher than the range 7.02 to 7.23% reported by Sotolu (2008) for the same species fed differently processed leuceana seed meal diets. NPU had been associated with superiority of feed and its protein quality (Steffens, 1989).

5.5.3 Digestibility Indices of Differently Processed Tropical Almond Kernel Meal Based Diets by *Clarias gariepinus* Juveniles.

Apparent digestibility co-efficient (ADC) of crude protein recorded in this study significantly varied ($P<0.05$) among the dietary treatments. Fish fed ROAM had the best ADC of crude protein value (89.03%) followed by fish fed RFRD (control, without almond kernel meal) (88.95%) and MEAM (88.92%) while fish fed SEAM had the least value (87.06%). The best ADC of crude protein observed in ROAM could be attributed to the effect of heat treatment in reducing the anti-nutritional factors in the raw kernel thereby making the nutrients more available to the fish. This is consistent with the lower levels of anti nutritional factors recorded for roasted almond kernel meal compared to the raw meal and the meals obtained by other processing methods (Table 6). The ADC of crude protein obtained in this study is similar to that reported by Falaye et al. (2016) (80.69 to 85.91%). The generally high ADC of crude protein values observed in all the treatments indicate that the experimental fish properly utilized the protein in all the experimental diets. This could be attributed to the generally low levels of crude

fibre (Table 5) and low levels of anti nutritional factors (Table 6) in raw and differently processed almond kernel meals.

There was significant variation ($P<0.05$) in the values of apparent digestibility co-efficient of dry matter (ADCdm) obtained in this study. Fish fed SEAM had the least value (71.87%) while fish fed MEAM and ROAM had the highest value (74.54%). This observation is in agreement with the report of Falaye et al. (2016) which had fish fed mechanically-extracted sunflower seed meal diet having the highest ADCdm compared to the other processing methods. ADCdm is mainly used to describe how efficient feeds or individual feed ingredients are being digested and how much of their nutrient is biologically available to fish for growth and maintenance (Fagbenro, 2003). Degani et al. (1997) maintained that apparent digestibility of dry matter provides a better estimate of the quantity of digestible materials in the feed rather than individual nutrients. The fairly high ADCdm values recorded in all the dietary treatments indicated good diet acceptance and utilization by *Clarias gariepinus*.

Apparent digestibility co-efficient of gross energy ADCGE was generally high in all the treatments and varied significantly ($P<0.05$) among the treatments. ADCGE was highest in ROAM, followed by MEAM while control diet (RFRD) had the least value. The ADCGE values recorded in this study are higher than the values reported by Nwanna (2003) (78.7 to 80.5%) and Falaye et al (2014) (58.19 to 82.05%) for fermented shrimp head waste meal based diets and lima bean meal based diets fed to *Clarias gariepinus* respectively. The fairly high ADCGE values recorded for all the diets suggest that the amount of energy ingested and digested were the required quantity by *Clarias gariepinus* for optimum conversion of feed into flesh. Fagbenro (1996) stated that digestibility of nutrients and energy contents in feedstuffs could be used to assess the suitability and nutritive value of feedstuffs or diets in fish rather than looking at growth responses to the test diets.

From the present study, roasted almond kernel meal diet had the best growth performance indices (MWG, ADG, PWG and SGR) and apparent digestibility co-efficients of nutrients, followed by mechanically extracted almond kernel meal diet.

5.6 Physico-Chemical Water Quality Parameters Monitored During Digestibility Study. Water quality parameters of the experimental tanks (Dissolved oxygen, pH and temperature) were measured forth nightly and maintained within the range recommended by Viven et al. (1985) and Omitoyin (2007). The pH of the experimental setup varied from the initial value and among the treatments though with a narrow range (6.85 to 6.99). The optimum pH range for most fish species is between 6.5 and 8.5 (Boyd, 1979; Viveen et al., 1985). Branson (1993) stated that most fish species would die at pH above 10 as sub-lethal effects such as gill damage can occur. All the pH values were within the optimum range for warm-water fish species (Viveen et al., 1985). This indicates that the pH of the experimental units were adequate for the experimental fish throughout the experimental period.

The final dissolved oxygen (D.O) values in all the dietary treatments (range, 4.27 to 4.78 mg/l) were lower than the initial D.O level (5.1 mg/l). Dissolved oxygen concentration is one of the most critical water quality parameters in fish culture (Boyd 1979). Swingle (1996) stated that farmed fish would die if exposed to D.O of less than 3mg/l for long period. Low levels of dissolved oxygen lead to reduced feed intake, impaired growth and reproduction as well as increased susceptibility to parasites and diseases (Dupree and Hunner 1984). The D.O values obtained in this study all fell within the optimum range recommended for catfish culture (Viveen et al., 1985).This indicates that the environmental conditions for the fish throughout the experimental period were adequate in terms of dissolved oxygen level.

The final values of temperature had very narrow range of 25.00^{o}C to 25.10^{o}C and were lower than the initial value (27.95^{o}C). Temperature is a dominant parameter affecting nearly all physiological cycles in water and has an inverse relationship with dissolved oxygen (Boyd and Lichtkoppler 1979). Above or below the optimum range for temperature, fish do not feed well, may become stunted and have increased susceptibility to diseases (Lichtkoppler 1979; FAO, 1993a). All the temperature values obtained in this study fell within the optimum range, 24^{o}C to 31^{o}C recommended for *Clarias gariepinus* (Viveen *et al*; 1985).

5.7 Replacement of Soya Bean Meal with Graded Levels of ROAM and MEAM in *Clarias gariepinus* Diet.

5.7.1 Proximate Composition and Gross Energy Contents of Graded Levels of ROAM-Based Diets Fed to *Clarias gariepinus*.

The crude protein content of all the five formulated ROAM diets fell within the range of 39.96 to 40.10% although aimed at 40%. Such variation often exists during the process of chemical analysis of experimental diets as observed in the works of Oyelese (1994); Omitoyin (1995); Alegbeleye (2005)-and Falaye et al. (2014). This can be attributed to the fact that each of the diets had different ingredient composition.

Crude fat content of the experimental diets varied significantly ($P<0.05$) with a range between 4.07 to 20.59 %. 25% ROAM inclusion (Treatment 2) had a crude fat value sligthly lower than that of the control diet (treatment 1). There was a regular increase in the values of crude fat from 4.07 % for 25 % ROAM inclusion, to 6.38 % for 50 % ROAM inclusion (treatment 3), 10.4 % for 75 % ROAM inclusion (treatment 4) to the highest value of 20.59 % for 100 % ROAM inclusion (treatment 5). This is in agreement with the work of Fagbenro et al. (2010) which reported a directly proportional increase in crude fat values with increase in inclusion levels of sunflower and sesame seed meals in the diets of *Clarias gariepinus*.

Crude fibre values ranged between 2.51 and 2.98% with an irregular pattern from 0% to 100% ROAM inclusion levels. This observation is in line with the work of Fagbenro et al. (2010) which observed an irregular pattern of crude fibre in sunflower seed meal based diets. From this study, the crude fibre values of all the ROAM diets were relatively low (2.51 to 2.98 %) and fell within the recommended level (3% maximum) for juveniles of omnivorous fish species (Tacon, 1994; Ajani et al., 2011).

Ash content ranged between 11.68 % and 13.31 % with no regular pattern. The ash content obtained in this study is higher than that reported by Sotolu and Faturoti (2009) with a range of 7.03 to 9.62% and with no regular pattern among the treatments. It is also higher than the values reported by Bekibele (2005) with a range between 9.49 and 12.01 % with no regular pattern among the treatments.

Moisture content ranged between 4.79 and 5.20 %. This range is lower than the range of between 6.78 and 8.10 % reported by Bello et al. (2012) for walnut leaf and onion bulb residues based diets. Nitrogen free extract (NFE) varied significantly (P<0.05) among the treatments. 25% ROAM inclusion had the highest value while 100 % ROAM inclusion had the least value. NFE values in this study followed an irregular pattern. This observation is in agreement with the work of Fagbenro et al. (2010) who also reported an irregular pattern of NFE values at varying inclusion levels of sunflower seed meal in the diet of *C. gariepinus*.

Gross energy (G.E) content of graded levels of ROAM diets was least in the control (0% ROAM) diet and increased progressively with increasing inclusion levels of ROAM with 100% ROAM diet having the highest value (3,995.00 kcal /kg). This differed significantly (P<0.05) from the values of the other dietary treatments. The progressive increase in the energy content of the diets with increase in inclusion levels of ROAM could be linked with the corresponding progressive increase in the crude fat content of the diets with increase in the inclusion levels of ROAM The range of G.E obtained in this study (3802.0 to 3995.00 kcal/kg) is higher than the range (2889.85 to 3086.00 kcal/kg) reported by Sotolu and Faturoti (2009) for graded levels of processed (soaked) *Leucaena leucocephala* seed meal based diets fed to *Clarias gariepinus* fingerlings.

5.7.2 Proximate Composition and Gross Energy Contents of Graded Levels of MEAM Based Diets Fed to *Clarias gariepinus* Juveniles.

The crude protein content of the five formulated MEAM diets were within the range 39.98 to 40.16 %. There was no significant difference (p>0.05) in the crude protein content of the experimental diets. Crude fat content of the experimental diets varied significantly (P<0.05). Treatment 1(control) had the least crude fat value of 4.18%. This increased progressively among the treatments to the highest value 10.45% observed in diet 5 (100% MEAM inclusion). This is consistent with the report of Fagbenro et al. (2010) which observed a directly proportional increase in crude fat values with increase in inclusion level of sunflower and sesame seed meals in the diets of *Clarias gariepinus*.

Crude fibre value ranged between 2.55% and 2.78%. There was no significant difference (P>0.05) among the treatments' crude fibre values. The crude fibre values obtained in the present study displayed an irregular pattern from treatment 1 to treatment 5. According to Fagbenro and Arowosoge (1991), dietary fibre had been linked to causing low protein digestibility and bio-availability. However, the crude fibre contents of all the diets were relatively low (2.55 to 2.78%). Crude fibre content of the experimental diets obtained in this study are lower than the values 6.21% to 6.73% reported by Osuigwe et al. (2005). All the crude fibre values obtained for MEAM based diets fell within the recommended level (3% maximim) for omnivorous fish species (Tacon, 1987).

The control diet had the highest ash content followed by diet 4 (75% MEAM diet) while diet 5 (100 MEAM diet) had the least ash content. There were significant differences (P<0.05) among the treatments mean values. The ash content of the experimental diets recorded in this study are slightly lower than the values 13.84- 14.24% reported by Nwanna (2003) for fermented shrimp head waste based diets but higher than the values 9.49-12.01% reported by Bekibele (2005) for mucuna beans based diets.

Moisture content exhibited an irregular pattern with the highest value (6.19%) observed in diet 4 (75% MEAM diet) while the least value (4.84%) was observed in the control diet. The moisture content recorded for the experimental diets (4.84 to 6.19%) is similar to 4.84 to 5.66% reported by Sotolu and Faturoti (2009) for Leuceana seed meal-based diets. The moisture content of the experimental diets fell within the desirable range (<10%) for catfish feed (Tacon, 1987; Onuoha and Elezuo, 2013). Nitrogen-free extract (NFE) was highest (35.11%) in the control diet. This reduced progressively in a regular pattern to the least value of 28.92% recorded for diet 5 (100% inclusion). The NFE values obtained in this study are lower than the values of 43.25 to 45.27% reported by Bekibele 2005 but higher than the values of 19.68 to 22.98 reported by Keremah and green 2005.

Gross energy (G.E) contents of graded levels of MEAM diet was least (3,802.00kcal/kg) in the control diet. This increased progressively in a regular pattern with increasing inclusion levels of MEAM, with diet 5 (100% inclusion) having the highest value (3,914.00 kcal/kg). The gross energy values of the experimental diets recorded in this study (range, 3,802.00 to 3,914.00

kcal/kg) are higher than the values 2,889.85 to 3086.00 kcal/kg reported by Sotolu and Faturoti (2009) but less than the range 516.18 to 4,564.52 kcal/kg reported by Ochang et al. (2007).

5.8 Proximate Composition of *Clarias gariepinus* Juveniles Fed with ROAM and MEAM Based Diets

5.8.1 Initial and Final Carcass Proximate Composition of *Clarias gariepinus* juveniles Fed Graded Levels of ROAM Diets

The crude protein content of the initial fish sample before the commencement of the experiment was 50.28% while the final values were significantly higher ($P<0.05$) and ranged between 57.26 to 66.97%. There was significant difference ($P<0.05$) among the treatments. This observation indicated higher protein utilization and retention by fish in all the dietary treatments. Sotolu and Faturoti (2009) observed that final fish carcass composition showed higher crude protein content in *C. gariepinus* fed processed Leuceana seed meal diets and attributed it to different utilization levels of the diets. This observation indicates that there was protein synthesis and increased tissue production. Similar observation was made by Nweke and Ugwumba (2005) and Bello et al., (2012). Although all the diets produced higher values of carcass protein than the initial value, the margin was narrow at 100% ROAM inclusion indicating a lower level of utilization of the feed at this inclusion level compared to 25%, 50% and 75% inclusion levels. This observation is consistent with that of Alegbeleye (2005) who reported that effective utilization of bambara groundnut at varying degrees was responsible for the various levels of carcass protein content of Heteroclarias fingerlings.

Crude fat content of the initial fish sample was 4.12%. This increased in all the treatments with fish fed 50% ROAM diet having the highest value (6.25%) while fish fed control diet had the least value (5.79%). The values exhibited an irregular pattern from 0% to 100% ROAM inclusion levels. Bekibele (2005) similarly reported increased values of crude fat in the final carcass sample of *C. gariepinus* fed graded levels of Mucuna bean meal-based diets.

Crude fibre content of the initial fish sample was 0.01% while final carcass content significantly ($P<0.05$) increased with the highest value (0.085%) observed in fish fed 75% ROAM diet and

the least final value (0.030%) observed in fish fed control diet. This is consistent with the reports of Nweke and Ugwumba (2005) and Olasunkanmi (2011) who reported significantly higher final values of crude fibre in all the treatments for *Clarias gariepinus* fed graded levels of duckweed meal diets and differently processed mucuna meals based diets respectively.

Ash content of the initial fish sample (7.195%) was significantly ($P<0.05$) less than the final values in all the treatments. The final ash content of the fish carcass had a narrow range of 10.51 to 10.95%. The higher final carcass ash values obtained at the end of the experiment is in line with the report of Bello et al. (2012) which observed increase in final carcass ash values for *C. gariepinus* juveniles fed walnut leaf and onion bulb residues based diets compared to the initial value. The fact that the final values of ash content were higher than the initial value indicated that ROAM diets were well utilized by the experimental fish.

The moisture content of initial fish sample was 6.79 %. This reduced significantly ($P<0.05$) in all the treatments to a range of 4.54% to 5.19%. The variation in the moisture content of the final fish carcass followed no regular pattern as the carcass of fish fed 75% ROAM diet had the highest value while those fed 100% ROAM diet had the least value. The reduction in final carcass moisture content observed in this study is consistent with the reports of Ibiyo et al. (2005) and Fagbenro et al. (2010), who at different studies observed lower values for final carcass moisture content compared to the initial value.

Nitrogen-free extract (NFE) in the initial fish carcass was 31.62%. This reduced significantly ($P<0.05$) to a range of 10.38 to 21.58% in the final carcass value among the treatments. This observation agrees with that of Sotolu (2008) who recorded lower final carcass NFE values compared to the initial value.

5.8.2 Initial and Final Carcass Proximate Composition of *Clarias gariepinus* Juveniles Fed Graded Levels of MEAM Based Diets.

The crude protein content of the initial fish sample before the commencement of the experiment was 50.28%. The final crude protein values were significantly ($P<0.05$) higher and ranged

between 65.48 and 70.28%. There were significant differences (P<0.05) among the treatments except in treatments 5 and 3 where there was no significant difference (P>0.05) between the two treatment means. This observation indicated higher protein utilization and retention by fish in all the dietary treatments. Adesina et al. (2013) observed that final fish carcass composition showed higher crude protein content in *C. gariepinus* juveniles fed graded levels of boiled sunflower seed meal-based diets and attributed it to different utilization levels of the diets. The significant increase in crude protein content of the fish carcass at the end of the experiment indicates that there was protein synthesis and increased tissue production.

Crude fat content of the initial fish sample was 4.12%. This increased significantly (P<0.05) in all final treatment values with a range of 5.85 and 6.18%. There were significant differences (P<0.05) among the treatment mean values. The final crude fat content of the experimental fish exhibited an irregular pattern among the treatments. The increase in final crude fat content of the experimental fish observed in the present study is consistent with the reports of Bello et al. (2012) and Falaye et al. (2016) which also reported increase in final crude fat content of the experimental fish fed walnut leaf and onion bulb residues-based diets and differently processed sunflower seed meal-based diets respectively. Jimoh et al. (2014) also reported an increase in the final carcass fat content in all but one treatment value for *C. gariepinus* fed *Chrysophyllum albidum* based diets.

Crude fibre content of the initial fish sample was 0.01% while final carcass crude fibre content significantly (P<0.05) increased with the highest value (0.08%) observed in fish fed diet 5(100% MEAM diet) and the least value (0.03%) observed in fish fed control diet. The range of final carcass crude fibre obtained in this study are less than the values 1.21 to 6.43%, 1.06 to 1.32% and 0.15 to 1.43% reported by Nweke and Ugwumba (2005), Adesina et al. (2013) and Falaye et al. (2014) respectively for *Clarias gariepinus* juveniles.

Ash content of the initial fish sample was significantly (P<0.05) lower than the final values in all the treatments. The final ash content of the experimental fish ranged between 11.37% and 11.90%. There were significant differences (P<0.05) in the final values of ash among the treatments. The final ash values observed in this study is higher than the values of 3.73 to 4.69%

and 7.43 to 8.22% reported by Ochang et al. (2007) and Sotolu and Sule (2011) for *Clarias gariepinus* fed diets with graded levels of palm oil and water hyacinth meal based diets respectively. The fact that the final values of ash content were higher than the initial value indicates that MEAM diets were well utilized by the experimental fish.

The moisture content of initial fish sample was 6.79%. This reduced in all the treatments. The final moisture content ranged between 4.89% and 5.31%. There was a significant difference ($P<0.05$) between the initial value and the final treatments' values. There were also significant differences ($P<0.05$) among the treatments' final moisture values. The variation in the moisture content of the final fish carcass followed no regular pattern as the carcass of fish fed diet 2 (25% inclusion) had the highest moisture content (5.31%) followed by fish fed diet 4 (75% inclusion) while fish fed diet 5 (100% inclusion level) had the least value (4.89%). The final treatment carcass misture content observed in the present study is less than the values of 5.71 to 8.91% reported by Ochang et al. (2007) and 17.49 to 21.93% reported by Sotolu and Sule (2011). The reduction in final carcass moisture content observed in this study is consistent with the report of Ibiyo et al. (2005) which observed lower values for final carcass moisture content compared to the initial value.

5.9 Growth and Nutrient Utilization Indices of *Clarias gariepinus* Juveniles Fed Graded Levels of ROAM and MEAM Based Diets

5.9.1 Growth Performance Indices of *Clarias gariepinus* Juveniles Fed Graded Levels of ROAM Diets.

The occurrence of weight gain at all inclusion levels of ROAM diets observed in this study indicates that the experimental fish responded positively to all the experimental diets and that the protein contents of the experimental diets adequately enhanced growth of the fish (Fagbenro and Arowosoge , 1991). Mean final weight and mean weight gain exhibited a similar trend at the end of the experiment. There were significant variations ($P<0.05$) in the mean weight gain among the treatments. The control (0% ROAM) diet had a mean weight gain of 55.10 g, which progressively increased, with higher inclusion levels of ROAM up to 75% inclusion, which had the highest mean weight gain (69.03 g). At 100 % ROAM inclusion the mean weight gain fell to 27.02 g which was the least. Mean weight gain and specific growth rate are usually considered as

the most important measurement of productivity of diets (Omitoyin and Faturoti, 2000). The highest MWG (69.03 g) obtained at 75% ROAM inclusion level was significantly higher ($P<0.05$) than the MWG of fish fed the control diet (55.10 g). The decrease in MWG of fish at 100% inclusion level of ROAM could be attributed to low feed intake and low feed utilization. This trend is in agreement with the report of Bekibele (2005) who observed an increasing trend in MWG of *Clarias gariepinus* fed mucuna bean meal diet as the inclusion levels increased up to a certain limit. Jackson et al. (1982) reported good growth in tilapia (*Sarotherodon mossambicus*) fed 35.2% sunflower seed meal replacing 50% of the fish meal protein. Olukunle and Falaye (1998) also reported that 25% sesame seed cake incorporation supported weight gain in *Clarias gariepinus* similar to diets containing 100% fishmeal.

Specific Growth Rate (SGR) was highest in treatment 4 (75% ROAM diet) and least in treatment 5 (100% ROAM diet). There were significant differences ($P<0.05$) among the treatments' SGR values. The values of SGR obtained in this study (1.12 to 1.82%) are higher than the values 1.00 to 1.59% reported by Falaye et al. (2014) for *C. gariepinus* fed differently processed lima bean based diets. This suggests that almond kernel meal supports faster growth of *C. gariepinus* than lima bean meal.

A direct relationship was observed among mean weight gain, percentage weight gain (relative growth rate) and specific growth rate in the overall growth pattern of fish in this study. This observation is common in nutrition experiments (Faturoti 1989; Alegbeleye et al., 2001; Nwanna, 2003; Falaye et al., 2014) indicating that any of these parameters could be adequately used to describe the growth pattern of fish.

Condition factor (k) had a narrow range (0.56 to 0.68). Treatment 5 (100% inclusion level) had the least value while fish fed 25% ROAM diet had the highest value. There was no regular pattern in the variation of K values among the treatments. The K values obtained in this study indicate that the diets were utilized for growth and well being of the experimental fish since they fell within 0.5 and 1.0 (Lagler, 1956).

Apart from treatment 5 (100% ROAM diet) which had the least survival rate (61.67 %), other treatments had a fair survival rate which ranged from 86.67 % to 90.00 %. There was no significant difference (P>0.05) in the survival rate of treatments 1 (control) to 4 (75% inclusion). However, survival rate of treatments 1 to 4 significantly differed (P<0.05) from that of treatment 5. The significantly lower survival rate of fish fed 100% ROAM diet could be attributed to starvation due to low feed intake as it was observed that fish fed this diet had significantly low total feed intake (714.97 g) compared to others (range, 1504.06 g to 1751.69 g).

5.9.2 Nutrient Utilization Indices of *Clarias gariepinus* Juveniles Fed Graded Levels of ROAM Diets.

Total feed intake (TFI) was highest (1751.69 g) in treatment 4 (75% inclusion level) and least (714.97 g) in treatment 5. The highest TFI value observed in fish fed 75% ROAM diet suggests that this diet was most palatable and acceptable to the fish. The progressive increase of total feed intake from 0% to 75% inclusion shows that the experimental fish accepted ROAM diets even more than the control diet. Mean feed intake (MFI) followed a similar trend like TFI with the highest value (106.02 g) observed in treatment 4 (75% ROAM diet) and least value (57.91g) observed in treatment 5(100% inclusion). The very low TFI and MFI observed in fish fed 100% ROAM diet could be linked to the high crude fat content of the diet (20.59%, Table 14) which probably resulted into poor palatability and acceptability of the diet. Feed palatability and acceptability on the other hand are affected by a number of factors including the appearance of the feed, taste of the feed and acceptability of the individual feedstuffs that make up the feed (NRC 1973; Tacon 1987).

Feed conversion ratio (FCR) was best (least value) in treatment 4 (75% ROAM diet) and poorest (highest value) in treatment 5(100 ROAM diet). There were no significant differences (P>0.05) in the FCR values of treatment 1 (control) to treatment 4 whereas there was a significant difference (p<0.05) between FCR value of treatment 5 (100% ROAM) and the other treatments' FCR values. The least FCR value recorded for fish fed 75% ROAM diet indicates a superior level of utilization of ROAM diet than the control diet. The FCR values obtained in this study (1.57 to 3.30) are lower than the values (2.81 to 6.05) reported by Olasunkanmi and Omitoyin (2011); (4.91 to 9.25) reported by Keremah and Green (2005) and (1.95 to 6.94) reported by

Nweke and Ugwumba (2005). FAO (1993b) documented that the lower the FCR the better the feed utilization by the fish. In this study, the lowest FCR value observed in treatment 4 indicates better feed utilization by the fish at 75% ROAM inclusion and this accounted for better growth performance of fish fed 75% ROAM diet among the other diets. FCR significantly ($P<0.05$) dropped to the poorest level (highest value) in fish fed 100% ROAM diet indicating that inclusion of roasted almond kernel meal beyond 75% caused significantly poor feed utilization and conversion to fish flesh.

Nitrogen metabolism (Nm) had treatment 4 and 5 having the highest and least values respectively. There were significant differences ($P<0.05$) among the treatments. There was progressive increase in the value of Nm from treatment 1(control) to treatment 4(75% inclusion) after which Nm fell significantly to the lowest value in treatment 5 (100 ROAM diet). The Nm values recorded in this study (range, 1472.54 to 2682.32) are similar to that reported by Adesina et al. (2013) (range, 1550.94 to 2742.75).

Net protein utilization (NPU) varied significantly ($P<0.05$) among the treatments. The highest NPU value (418.88%) was recorded in treatment 4 (75% inclusion level) while the least (230.26%) was recorded in treatment 5 (100% ROAM diet). NPU is a factor of the quality, digestibility and utilization of the protein fed to the fish (Falaye et al., 1999 a). Inclusion of roasted almond kernel meal at 75% level resulted into a better NPU compared to the control and the other treatments. This observation is in consonant with the report of Bekibele (2005) who recorded highest NPU values at 30% -50% inclusion level of Mucuna bean meal in *C. gariepinus* diet.

5.9.3 Growth Performance Indices of *Clarias gariepinus* Juveniles Fed Graded Levels of MEAM Diets.

There were significant variations ($P<0.05$) in the mean weight gain among MEAM based dietary treatments. The control (0% MEAM) diet had a mean weight gain of 55.05 g, which progressively increased, with higher inclusion levels of MEAM up to 75% inclusion after which growth was depressed at 100% MEAM inclusion. Similar trend was observed for both

percentage weight gain and specific growth rate with both indices increasing as inclusion levels of MEAM increased up to 75%, after which both indices reduced to the lowest values at 100% MEAM inclusion. Olukunle and Falaye (1998) reported that 25% sesame seed cake inclusion supported weight gain in *C. gariepinus* similar to that of 100% fish meal diet. Similar trend was observed by Adesina et al. (2013) which reported increase in mean weight gain, percentage weight gain and specific growth rate at 20% inclusion level of boiled sunflower seed meal-based diet fed to *Clarias gariepinus* juveniles. Olapade and Kargbo (2015) reported higher values of mean weight gain and specific growth rate at 50% inclusion level of almond kernel meal (in replacement of fish meal) in *Clarias gariepinus* diet. The higher optimum level of almond kernel meal inclusion in the present study (compared to sesame seed cake and boiled sunflower seed meal) could be due to the relatively low levels of anti-nutritional factors present in almond kernel. This is further supported by the fact that almond kernel can safely be eaten raw (Burkill 1988; Wikipedia 2010).

Condition factor (k) range between 0.620 and 0.646. The highest value was observed in treatment 4, while the least value was observed in treatment 5. There were no significant differences ($P>0.05$) among the treatments' K values. This indicates that mechanically-extracted almond kernel meal diets did not negatively affect the wellbeing of the experimental fish. The K values (0.620 to 0.646) obtained in this study are higher than the values 0.49 to 0.57 reported by Adesina (2014). Condition factor is used to assess the robustness and general well-being of fish (Fulton 1904 cited by Nash et al., 2006).

Survival rate had a narrow range between 88.33 and 88.67%. Treatment 1 (control) and treatment 5 had the least value (88.33%) while treatments 2, 3 and 4 had 88.67%. There was no significant difference ($P>0.05$) among the treatments. The fairly high survival rate of the experimental fish for all the treatments implies that mechanically-extracted almond kernel meal diets were well digested and utilized and as such supported survival of the experimental fish.

5.9.4 Nutrient Utilization Indices of *C. gariepinus* Juveniles Fed Graded Levels of MEAM Diets

Mean Feed Intake (MFI) was highest in treatment 4 (75% MEAM) while the least value (85.48g) was observed in treatment 5 (100% MEAM). There were significant differences ($P<0.05$) among the treatments' MFI values. Feed conversion ratio (FCR) had a range of 1.57 to 1.71. The best FCR (least value) was observed in treatment 4 (75% MEAM inclusion) while the poorest value was observed in treatment 5 (100% MEAM inclusion). Feed conversion ratio measures the ability of fish to convert feed to flesh (FAO, 1993b). Putting it in another way FCR measures the utilization of feed and its conversion to flesh by fish. The FCR values obtained in this study (range 1.53 to 1.66) are better than the values 3.46 to 4.94 reported by Ochang et al. (2007).

Nitrogen metabolism (Nm) and net protein utilization (NPU) followed similar pattern with both parameters increasing with higher MEAM inclusion levels up to 75%. At 100% inclusion level, the values of both parameters reduced to the least among all the treatments. Nm and NPU are useful tools in expressing protein utilization (Falaye et al., 1999 a). The results of Nm and NPU obtained in this study indicate that mechanically- extracted almond kernel meal-based diets were well digested and utilized better than the control diet up to 75% inclusion level, beyond which the experimental fish could no longer digest and utilize the diet effectively. This was further buttressed by the growth performance indices-MWG, PWG and SGR, which had highest values at 75% inclusion and lowest values at 100% inclusion levels respectively.

5.10 Heamatological Profile of Fish Fed ROAM and MEAM Based Diets

5.10.1 Haematological Indices of *Clarias gariepinus* Juveniles Fed Graded Levels of Roasted Almond Kernel Meal Diets.

Haematological parameters are important in analyzing the health status of animals (Ayoola, 2011). Improvement in the haematological parameters at the end of the experiment indicated positive contribution of roasted almond kernel meals' protein in blood formation and improving the overall wellbeing of the fish. Many protein-rich diets have been observed to improve blood formation in animals fed such diets (Tambuwal et al., 2002).

Packed cell volume (PCV) and Hemoglobin count (Hb) have been suggested as tests that can be carried out on fish to check their health status (Etim et al., 1998). Packed cell volume increased from the initial value of 21.00% to final values ranging from 23.00% to 27.15%. This is consistent with Koizhuev (1964) who stated that fish packed cell volume ranged between 20% and 35%. Olasunkanmi (2011) reported higher final values of PCV than the initial value in *Clarias gariepinus* fed mucuna bean meal diets. In contrast, Sotolu and Faturoti (2009) reported lower PCV values for *Clarias gariepinus* fed leuceana seed meal at inclusion level above 40%. They associated this to the probable residual effect of mimosine present in the seeds after processing. Treatment 5(100% ROAM diet) had the least PCV value which was significantly lower ($P<0.05$) than that of other dietary treatments. Osuigwe et al. (2005) ascertained that the reduction in values of PCV was due to the presence of toxic substances in the diet of fish. The reduced PCV value of fish fed 100% ROAM diet could be attributed to the residual effect of anti- nutritional factors present in the kernel after processing and probably due to the very low feed intake by fish fed 100% ROAM diet. This is consistent with the report of Adeyemo (2005) which also attributed reduced PCV to loss of appetite by the experimental fish. It can therefore be inferred from this study that replacement of soybean meal with roasted almond kernel meal beyond 75% inclusion level could adversely affect the haematology of fish hence compromising its health status.

Haemoglobin (Hb) count increased from an initial value of 7.2 g/dl to final values, which ranged from 7.4g/dl to 8.7g/dl. There were significant differences ($p<0.05$) between the initial Hb value and the final values and among the treatments' final values. This coincides with the report of Orisasona (2014) which reported higher final treatments' Hb values than the initial value for *Clarias gariepinus* fed graded levels of boiled lima bean based diets. Physiologically, haemoglobin is crucial to fish survival because of its important role in oxygen carrying capacity of blood (Etim et al., 1998). The same author reported that certain physiological stresses elevate haemoglobin levels in fish while very low Hb values could be because of anaemia. Red blood cells (RBC) increased from an initial value of $1.69x10^6$/mL to final values, which ranged from $2.74x\ 10^6$/mL to $3.83\ x10^6$/mL. These values are higher than the values 1.07 to $1.43\ x10^6\ mm^{-3}$ reported by Osuigwe et al. (2005). In this study, effects of ROAM based diets on the haematological parameter of *Clarias gariepinus* includes, increased level of RBC, haemoglobin

count and PCV values. This observation indicates that ROAM diets were well utilized in red blood cell synthesis (erythropoiesis) to replace the older ones as the fish grew. It was also observed that PCV, Hb and RBC followed similar pattern with treatment 5 having the least values among the treatments. This suggests that 100% inclusion of ROAM in the diet of *C. gariepinus* may result into impairment of the fish heamatology and health status.

White blood cell count (WBC) had an initial value of $7.90x10^6$/µl which increased significantly ($P<0.05$) in all the final values ranging from 10.40 to $11.80x10^6$/µl. Unlike PCV, Hb and RBC, WBC values exhibited an irregular pattern. The WBC values obtained in this study are higher than the values $2.70x10^3$/µl to $4.80x10^3$/µl reported by Ochang et al. (2007). This could be because of differences in culture environment, species, and size of fish used. The significantly high WBC value (above the reference range) observed in fish fed diet 5 (100% ROAM diet) could be linked to stress imposed on the fish by the likely higher levels of anti-nutrients present in the diet.

Platelet count exhibited an irregular pattern with the initial value at $207.00x10^9$/L, which reduced to $204.00x10^9$/L observed in treatment 5 (100% ROAM diet). Fish fed the other diets had significantly ($P< 0.05$) higher platelet values with the highest value ($318.00x\ 10^9$ /L) observed in treatment 3(50% ROAM diet). The range of platelet count $204.00\ x\ 10^9$ /L to $318.00x\ 10^9$ /L observed in this study are within the normal range for platelet count (150-400 $x10^9$/L) (Xiaoyun et al., 2009). This indicates that roasted almond kernel meal diets did not affect the platelet count of the experimental fish negatively.

Mean corpuscular hemoglobin concentration (MCHC) had an initial value of 34.29 g/dl, which reduced in all the final values to a range of 32.04 to 33.20 g/dl. Schaim et al. (1975) reported the normal range of MCHC as 32 to 36g/dl. The same author stated that high MCHC could be associated with anemia and liver diseases while low levels could be as a result of little iron in the body or gastrointestinal tract tumors. All the MCHC values obtained in the present study were within the normall range.

5.10.2 Serum Biochemical Indices of *C. gariepinus* Juveniles Fed Graded Levels of ROAM-Based Diets.

Total plasma protein content of the experimental fish blood exhibited an irregular pattern with a range between 3.40g/dl and 4.70g/dl. The highest value was observed in treatment 3 while the least was observed in treatment 5 (100% ROAM diet). The observed reduction in the total plasma protein observed in fish fed 100% ROAM diet could be as a result of malnutrition due to low feed intake by fish fed this diet as it was observed that fish fed diet 5 (100% ROAM) had significantly low feed intake. Plasma albumin content followed an irregular trend with the initial value of 2.7g/dl while the final treatment values ranged between 1.80 g/dl in treatment 5 to 2.80g/dl in treatment 3. Albumin is a component of plasma protein. Similar to total plasma protein, the significantly lower ($p>0.05$) serum albumin observed in treatment 5 (100% ROAM) could be as a result of malnutrition caused by low feed intake.

Initial plasma globulin content was 1.6g/dl, which increased slightly in the treatments to the highest value (1.9g/dl) in treatment 3 and reduced to 1.8g/dl and 1.6g/dl in treatments 4 and 5 respectively. There was no significant difference ($P>0.05$) in the values of globulin recorded in this study. The final globulin values (1.6 to 1.9 g/dl) obtained in this study are similar to the final globulin value 1.84 to 1.99g/dl reported by Falaye et al. (1999 b) for Indian carp fed soybean milk residue based diets.

Initial albumin: globulin ratio was 1.69, which reduced in all the treatments except in treatment 3, which had the highest value (1.76). There was a significant difference ($P<0.05$) between the albumin/globulin ratio value of treatment 5 and the other treatments. The range of albumin: globulin ratio obtained in this study (1.13 to 1.76) was close to the normal range 1.2 to 1.5 (Rambhaskar and Srinivasa-Rao, 1986). The same author noted that elevated albumin/globulin ratio is of no clinical significance.

Alanine aminotransferase (ALT) is a blood plasma enzyme that functions as a link between carbohydrate and protein metabolism by catalyzing the inter-conversion of strategic organic compounds to pyruvic acid and alanine to glutamic acid (Tiwari and Sing, 2004). Final ALT values 54.90 to 72.00 U/L in treatments 1 to 5 were significantly higher ($P<0.05$) than the initial

value (31.50 U/L). Higher values in treatments 1 to 5 suggests that ALT in the experimental fish were efficient in utilizing amino acids in ROAM diets for metabolism. A similar observation was made by Adesina (2014) who reported higher final values of ALT in all the dietary treatments compared to the initial value. However Pincus et al., (2011) stated the reference range for ALT as 7-56 U/L. This same author stated that elevated ALT could be associated with liver damage due to a number of factors including, toxins, hepatitis, and necrosis etcetera. It was observed that the ALT values of treatments 1-4 were within the reference range while that of treatment 5 was higher than the range. The significantly high value of ALT observed in fish fed diet 5 could therefore be associated with liver damage due to the presence of anti-nutritional factors, which were probably higher at 100% ROAM inclusion.

Aspartate aminotrasferase (AST) is another plasma enzyme which plays a similar role as ALT. Both ALT and AST act as a link between carbohydrate and protein metabolism (Adesina, 2014). AST has been used clinically as a marker for liver health in particular and an animal's health in broader sense (Adesina, 2014). Initial AST value was 24.90 U/L. Fish fed control diet had AST value of 32.50 U/L. There were no significant differences in the final AST values among treatments 1 to 4 whereas treatment 5's AST value significantly differed from the other treatments. The significantly higher AST value exhibited by fish fed diet is an indicator that fish fed this diet (100% ROAM diet) had impaired liver health.

The initial blood glucose value was 298mg/dl. This increased significantly ($P< 0.05$) in the final values for all the treatments. The highest final value of blood glucose (361.00 mmol/l) was observed in treatment 5 (100% ROAM diet) while the least value (307.0 mmol/l) was observed in treatment 2 (25% ROAM diet). There were significant differences ($P<0.05$) among the treatments and between the treatments' values and the initial value. Elevated blood glucose (hyperglycemia) has been attributed to tumors and stress (Orisasona 2014). The highest blood glucose value observed in treatment 5 (100% inclusion) could be associated with stress due to the presence of anti nutritional factors in the diet which was probably higher at 100% inclusion level.

Serum potassium (k^+) has been clinically used to diagnose kidney and heart problems. High serum potassium (hyperkalemia) has been associated with kidney failure and red blood cell destruction while low serum potassium (hypokalemia) has been linked with renal artery stenosis (Pincus and Abraham, 2011).The initial serum potassium was 5.19 mmol/L. This significantly ($P<0.05$) reduced in the final values for all the treatments with a range of 3.54 and 4.50 mmol/L. There were significant differences ($P<0.05$) among the treatments. The final serum potassium values 3.54 to 4.5 mmol/l obtained in this study are within the normal range 3.5-5.2 mmol/l reported by Pincus and Abraham, (2011). This suggests that roasted tropical almond kernel meal diets had positive effects on the experimental fish in maintaining their kidney and heart in good health.

Serum sodium was lowest, 135.8 mmol/l in the initial fish sample and treatment 5 and increased in the other treatments' final values. There were significant differences ($P<0.05$) in the final serum sodium values among the treatments. However, there was no significant difference ($P>0.05$) between treatments 3 and 1 (control). Blood sodium represents a balance between the sodium (Na^+) and water in the food and drinks consumed and the amount in urine. High serum sodium (hypernatremia) may be due to kidney and liver diseases among others, while low serum sodium (hyponatremia) can be associated with liver and kidney failure (Pincus et al, 2011). All the blood sodium values obtained in this study fell within the normal range 135-145 mmol/l (Pincus et al, 2011; Pincus and Abraham, 2011).

5.10.3 Haematological Indices of *Clarias gariepinus* Juveniles Fed Graded Levels of Mechanically –Extracted Almond Kernel Meal Diets.

The application of haematological indices has become a valuable tool for fishery biologist in assessing the health of fish and monitoring stress response (Fagbenro and Adeparusi, 2003). Improvement in the haematological profile of the experimental fish at the end of the experiment indicated the beneficial effect of mechanically –extracted almond kernel meal diets in blood formation and improving the overall health of the experimental fish.

Initial packed cell volume (PCV) value was 21.00%, which increased in all the treatments. The least final treatment value (25.00%) was observed in treatments 2 and 5 while the highest value 28.00% was observed in treatment 3. Higher final treatments' PCV observed in the present study for MEAM based diets concurs with the reports of Orisasona (2014) which reported higher final values of PCV than the initial value in *Clarias gariepinus* fed graded levels of boiled lima bean meal based diet. The PCV values obtained in this study (21.0-28.0%) are within the normal fish PCV range (20% to 35%) reported by Koizhuev (1964).

Haemoglobin count (Hb) was initially 7.2g/dl in the fish before the commencement of the experiment and increase in all final treatment values. The highest (8.98 g/dl) Hb count was observed in fish fed diet 3(50% inclusion). There were significant differences ($P<0.05$) among the treatments. The range of Hb values obtained in the present study (8.40 to 8.98g/dl) is similar to the values of 8.17 and 8.43g/dl reported by Sotolu and Faturoti (2009) for *Clarias gariepinus* fingerlings fed varying inclusions of Leucaena seed meal based diets. They are however lower than the values 10.7 to 12.6% reported by Nwanna et al. (2014a) for the same species fed plantain peels based diets.

Red blood cell (RBC) increased from an initial value of 1.69 $x10^6$/mL to final treatment values which ranged from 2.56 to 3.63 x 10^6/mL. The final treatments' RBC values obtained in the present study are similar to the values 2.60 to 3.80$x10^6$/ul reported by Omitoyin (2006) but higher than the values 1.40 to 2.27$x10^6$/ul reported by Ochang et al. (2007). The increase in final red blood cell count and heamoglobin count compared to the initial value observed in this study, indicates that MEAM based diets were well utilized by the experimental fish in the synthesis of haemoglobin and red blood cells.

White blood cell count (WBC) had an initial value of 7.90 x 10^6/µl which increased significantly ($P<0.05$) in all the final values ranging from 10.30 to 11.38 x 10^6/µl. The highest value was observed in treatment 5 while the least value was observed in fish fed control diet. There was a significant difference ($P<0.05$) between WBC value of treatment 5 and the other treatments whereas there were no significant differences ($P>0.05$) among the WBC values of treatments 1 to 4. The treatments' WBC values obtained in this study are comparable to the values 9.20 to 11.50 x 10^6/µl reported by Omitoyin (2006).

White blood cells play an important role in the immune system of living organisms (Etim et al., 1998; Ajani, 2006). Higher final treatment values of white blood cell (within the normal range) by *Clarias gariepinus* in this study indicates that the fish were able to utilize mechanically-extracted tropical almond kernel meal diets in producing more white blood cells to boost its immune system. This is in agreement with the report of Adesina (2014) which reported higher final WBC values for *C. gariepinus* juveniles fed graded levels of boiled and mechanically-extracted sunflower seed meal diets than the initial value. However, Orisasona (2014) linked elevated WBC in fish to stress. The significantly high WBC value obtained in fish fed diet 5 (above the normal range) could therefore be associated with stress due to the presence of anti-nutritional factors, which may be higher at 100% MEAM inclusion level.

Initial platelet count was 207.00 x 10^9/L which increased significantly ($P<0.05$) in all treatments. The highest platelet count (338.0 x 10^9/L) was observed in treatment 4 (75% MEAM diet) while the lowest value (266.0 x10^9/L) was observed in fish fed control diet. Xiaoyun et al. (2009) stated that low platelet count can be caused by disordes in the bone marrow or exposure to toxic chemicals while high platelets count can be caused by gastro intestinal cancer and inflammatory conditions. The same author stated that temporary high values of platelet count can result after physical activity or exertion. The platelets counts (266.0 to 338.0 x 10^9/L) obtained in this study were all within the normal range (150-400 x 10^9/L) (Xiaoyun et al., 2009).

Mean Corpuscular Haemoglobin Concentration (MCHC) had an initial value of 34.29 g/dl, which reduced in all the treatments. The range of MCHC obtained in the present study (32.07 to 34.29 g/dl) is similar to 32.0 to 33.0 g/dl reported by Adesina (2014), 33.0- 33.33g/dl reported by Omitoyin (2006) and 30.17 to 34.20 g/l reported by Ochang et al. (2007). All the MCHC values obtained in the present study were within the normal range of between 32 and 36g/dl (Schaim et al., 1975). There were no significant differences ($P>0.05$) among the MCHC values. This and the fact that all the MCHC values were within the normal range indicate that mechanically extracted almond kernel meal based diets did not negatively affect the experimental fish mean corpuscular heamoglobin concentration.

5.10.4 Serum Biochemical Indices of *C. gariepinus* Juveniles Fed Graded Levels of MEAM Diets

Total plasma protein (TPP) content of the experimental fish blood exhibited an irregular pattern with an initial value of 4.3g/dl and treatment values, which ranged between 4.0 and 4.8g/dl. Fish fed control diet (treatment 1) had plasma protein of 4.00g/dl. Fish fed diet 3 and diet 4 had a significantly higher ($p<0.05$) total plasma protein than fish fed the control diet. The range of total plasma protein 4.0 to 4.8g/dl obtained in this study is higher than the values 2.30 to 3.77 reported by Adesina (2014) for *C. gariepinus* fed graded levels of boiled sunflower seed meal based diets but less than the values 63.0 to 69.0 g/dl reported by Agbabiaka et al. (2013) for *C. gariepinus* fed tigernut based diet. Hrubec et al. (2001) reported that plasma protein increased with age and size of fish. The increase in total plasma protein observed in treatments 3 and 4 could be attributed to increased size (higher mean weight) of fish under these diatery treatments. This is in line with the report of Falaye et al. (1999) which reported higher total plasma protein for the treatment that had the highest growth rate for India carp fed soybean milk residue based diets.

Similarly, plasma albumin content followed an irregular trend with the initial value of 2.70 g/dl while the treatment values ranged between 2.25 and 2.90 g/dl. Treatment 1 (control) had plasma albumin value of 2.30g/dl. There was no significant difference between the plasma albumin value of treatment 2 and control while treatments 3 and 4 had significantly higher ($p<0.05$) plasma albumin. Plasma albumin is a component of plasma potein, therefore the higher values observed for treatment 3 and 4 correlates with higher plasma protein observed for these two treatments. This is consistent with the report of Falaye et al. (1999) which reported higher plasma albumin for the treatment that had the highest growth rate for India carp fed soybean milk residue based diets.

Initial plasma globulin content was 1.60g/dl. Fish fed contol diet had 1.70g/dl. Plasma globulin is a component of plasma protein. High values of plasma globulin have been linked to acute infection and inflammatory diseases (Pincus and Abraham 2011). However, there were no significant differences ($P>0.05$) among the treatments and between the treatment values and the initial value. This indicates that mechanically extracted almond kernel meal had no net effect on the plasma globulin of the experimental fish. The plasma globulin values (1.73 to 1.9g/dl)

obtained in this study are similar to the values 1.84 to 1.99 g/dl reported by Falaye et al., (1999) for India carp fed soybean milk residue based diets.

Initial albumin: globulin ratio was 1.69. This reduced in all final treatment values with a range between of 1.30 and1.54. The range of albumin /globulin ratio obtained in this study are within the normal range of 1.2 to 1.5 (Pincus and Abraham 2011). The same author reported that elevated albumin/globulin ratio is of no clinical significance. There were no significant differences (P>0.05) among the treatments'A:G ratios which implies that mechanically extracted almond kernel meal diets did not have any effect on the experimental fish A:G ratio.

Alanine aminotransferase (ALT) is a blood plasma enzyme that functions as a link between carbohydrate and protein metabolism by catalyzing the inter-conversion of strategic organic pyruvic acid and alanine to glutamic acid (Tiwari and Singh 2004). Initial ALT value was 31.50 U/L. Fish fed control diet had ALT value of 55.20 U/L. There was no significant difference (p> 0.05) in the ALT values of treatments 1 to 4 while there was a significant difference (p< 0.05) between ALT value of treatment 5 and the other treatments. Pincus et al. (2011) stated that the normal range for ALT is 7 to 56 U/L. The ALT values of fish fed diets 1 to 4 were all within the normal range while that of diet 5 was higher than the normal range. The significantly high ALT value observed in fish fed diet 5 (100% MEAM) could be associated with impaired liver health of fish fed this diet. Similar trend was observed for Aspartate aminotransferase (AST) with fish fed control diet having a value of 32.70 U/L while fish fed diet 5 had significantly higher value.

The initial blood glucose value was 298.00 mmol/L. This increased in all treatments final values except in treatment 4, which had the same value as the initial value. Fish fed control diet had glucose level of 318.00mmo/L. There was no significant difference between glucose value of treatment 1 (control) and treatment 2 (25% MEAM diet) while treatments 3 and 4 had glucose values which were significantly lower (p<0.05) than that of control. On the other hand, treatment 5 had a significantly higher (p<0.05) glucose value than the control. Ojolicks et al. (1995) associated high blood glucose to an increased depletion of liver glycogen in period of high physical activity and stress. The significantly high glucose value observed in fish fed diet 5

(100% MEAM) could therefore be linked to stress associated with the presence of substances antagonistic to the utililization of some nutrients. These substances were probably higher at 100% MEAM inclusion level.

The initial serum potassium was 5.19 mmol/l which significantly ($P<0.05$) reduced in all the treatments. Treatment 1 (control) had serum potassium value of 3.07mmol/L while the highest and lowest values were observed in fish fed diet 4 and diet 5 respectively. Pincuss and Abraham (2011) reported the normal range of serum potassium as 3.7 to 5.2mmol/L. All the serum potassium values obtained in the present study fell within the normal range. This implies that mechanically extracted almond kernel meal based diets did not negatively affect the serum potassium of the experimental fish.

Blood chloride was lowest (105.2mmo/l) in the initial fish sample and increased in all the treatments. Fish fed control diet had serum chloride value of 109.3mmo/L. There were no significant differences ($P<0.05$) among the treatments. The chloride values obtained in this study, 109.3 to 115.9 mmol/l are close to the normal range 98-108mmol/l reported by Pincus and Abraham (2011). This and the fact that there were no significant differences ($p>0.05$) in the serum chloride value of fish fed control diet and the other diets imply that mechanically extracted almond kernel meal based diets did not affect the serum chloride of the experimental fish negatively.

5.11 Histopathological Profile of *Clarias gariepinus* Juveniles Fed Graded Levels of ROAM and MEAM Based Diets

5.11.1 Histopathological Observation on *C. gariepinus* Juveniles Fed Graded Levels of ROAM Diets.

There was no marked histological changes in liver of fish fed control diet and those fed diets 2, 3 and 4, as they all had no lesion except for moderate / mild peiportal inflammation. However, liver section of fish fed diet 5 had a marked histological change from the control as they had microvesicular steatosis, severe periportal inflammation, multifocal areas of necrosis, vascular fibrosis and focal area of fibrous nodule. These observations are similar to that of Adesina (2014)

who reported mild to moderate vacuolations of the hepatocytes, diffuse vacuolations, severe diffuse vacualations, very severe diffuse vacuolation, very severe vacuoular degeneration, severe diffuse fatty infiltration et cetera, in fish fed graded levels of sunflower seed meal based diets. Omitoyin et al. (2006) and Olasunkanmi (2011) observed similar trend in *Clarias gariepinus* exposed to lindane and graded levels of processed mucuna bean meal based diets, respectively. The poorest histopathological state of liver observed in treatment 5 (100% ROAM diet) could be attributed to the effects of anti-nutritional factors present in tropical almond kernel which were probably higher at this inclusion level. The liver and kidney have been identified as the sites that are mostly affected by toxic substances in human and animals and various clinical signs have been linked with liver detoxifying and kidney removing these toxicants (Benjamin, 2009).

There was no histopathological changes observed in the kidney of fish fed control diet and diets 2, 3 and 4. However, fish fed diet 5-showed marked histological changes from the control diet. These included tubular necrosis, necrosis of glomeruli, vascular fibrosis, fibrous thickening of wall of the vessels and collecting ducts; desquamation of epithelial cells of collecting duct. Olasunkanmi (2011) and Adesina (2014) reported similar observation in *Clarias gariepinus* and linked the histological changes in the kidney with ingestion of a high percentage of the test ingredients, which imposed stress on the organ above its physiological capacity to adapt. There was no histopathological change observed in the intestine sections of fish fed control diet and diets 2 to 4 as mild infiltration of the mucosa and sub mucosa by inflammatory cell was common to them. However, fish fed diet 5 had severe infiltration of the mucosa and submucosa by inflammatory cells.

5.11.2 Histological Observation in *C. gariepinus* Fed Graded Levels of MEAM Based Diets.

There were no marked histological changes in liver of the fish fed control diet and those fed diets 2, 3 and 4, as they all had no lesion except for moderate / mild peiportal inflammation. However, liver section of fish fed diet 5 had a marked histological change from the control as they had macrovesicular steatosis, focal area of vacuolar degeneration and vascular fibrosis in addition to moderate peiportal inflammation. This observation is similar to that of Adesina (2014) who reported mild to moderate vacoulations of the hepatocytes, diffuse to very severe vacuolation and severe diffuse fatty infiltration in the liver of fish fed sunflower seed meal diets. Olasunkami, (2011) also observed a similar trend in *C. gariepinus* fed processed mucuna bean

meal diets. The liver and kidney have been identified as the sites that are mostly affected by toxic substances in human and other animals and various clinical signs have been linked with liver detoxifying and kidney removing these toxic substances (Pincus et al., 2011). There was no lesion observed in the kidney of fish under treatments 1(control), 2 (25% MEAM), 3 (50% MEAM) and 4 (75% MEAM). However, kidney sections of fish fed 100% MEAM based diet had tubular necrosis, focal area of lymphoid nodule and fibrosis of the wall of collecting tubules and some blood vessels. Sections of the intestine of fish under treatments 1 (control) to 4 did not show any histological change as mild/modeate infiltration of the mucosa and sub mucosa by inflammatory cell was common to them. However, intestine sections of fish fed diet 5 (100% MEAM) had severe infiltration of the mucosa and sub mucosa by inflammatory cell.

5.12 Water Quality Parameters Monitored During the Study.

For ROAM based diets, initial and final values of pH ranged between 6.65 and 7.25. Initial values of dissolved oxygen was 5.87mg/l. This fell in final treatment values with the least value 4.5mg/l observed in treatment 5. Initial temperature value was 28.45^{o}C, which reduced in all the final treatment values to the least 25.4^{o}C observed in treatment 5. Initial and final values of the water quality parameters monitored in this study including ammonia and nitrite levels all fell within the recommended ranges for catfish culture (Boyd, 1979; Viveen *et al*., 1985). This revealed that ROAM does not have any deleterious effect on the water quality of the culture media of fish.

For MEAM based diets, initial and final values of pH ranged between 6.54 and 6.96. Initial value of dissolved oxygen was 5.87 mg/l, which reduced in all the final treatment values with a range of 4.6 to 5.24 mg/l. Initial water temperature value was 28.45^{0}C. Final water temperature reduced in all the treatments with a narrow range of 25.40^{0}C to 25.87^{0}C. Initial and final values of water quality parameters monitored in this study including ammonia and nitrite levels, all fell within the optimum ranges recommended for catfish culture (Boyd, 1979; FAO, 1993a). This revealed that MEAM does not have any harmful effect on the culture tanks' water quality.

5.13 Economic Evaluation of Replacing Soya Bean Meal with Roasted Almond Kernel Meal in the Diet of *Clarias gariepinus*

The replacement of soya bean meal with roasted tropical almond kernel meal in the present study reduced the cost of feed production, which indicates a cheaper and more cost effective non-conventional feed ingredient relative to soya bean meal. Thus, the farmer will benefit economically through the utilization of the cheaper roasted tropical almond kernel meal.

The total cost of producing control diet was N236.60/kg, which reduced progressively to the least cost in diet 5. Highest values of total feed intake, total weight of fish produced and total weight gain were observed in fish fed 75% ROAM diet. This was earlier attributed to the fact that roasted almond kernel meal was better utilized at 75% inclusion level than at any other level. Value of fish produced (in naira) followed a similar trend as total feed intake, total weight of fish produced and total weight gain. In this study, the highest fish weight gain at 75% inclusion level produced the highest value of fish. This is consistent with the report of Nwanna et al. (2014a) which reported that the dietary treatment with the highest weight gain also produced fish with the highest value for *Clarias gariepinus* fed plantain peel based diets.

Treatment 5(100% ROAM) had the highest incidence of cost while treatment 4 (75% ROAM diet) had the least (best) incidence of cost. There was no significant difference ($P>0.05$) among treatments 1 to 4 incidence of cost values but treatment 5 had an incidence of cost value which was significantly higher ($P<0.05$) than the rest. Profit index was highest (best) at 75% ROAM inclusion level while it was lowest at 100% ROAM inclusion level. The highest profit index at 75% inclusion level indicates that profit was generated from fish fed 75% ROAM diet more than any other diet including the control diet. In the present study, though 100% ROAM diet was the cheapest diet, it produced fish of lower value compared to the other diets. 75% ROAM diet was therefore the most cost–effective feed in this study. This finding further buttresses the fact that 75% ROAM diet was better utilized.

5.14 Economic Evaluation of Replacing Soya Bean Meal with Mechanically-Extracted Almond Kernel Meal in the Diet of *Clarias gariepinus*.

The substitution of soya bean meal with mechanically-extracted almond kernel meal in the present study reduced the cost of feed production. The cost of producing MEAM based diet reduced progressively across the treatments to the least cost ₦223.70/kg for 100% MEAM diet. Highest values of total feed intake (TFI), total weight of fish produced and total weight gain were obtained in treatment 4 (75% MEAM diet). The highest total weight gain of fish obtained at 75% MEAM inclusion level led to the highest value of fish produced.

Diet 4 (75% MEAM diet) had the lowest (best) incidence of cost while 100% MEAM diet had the highest incidence of cost. Profit index was highest (best) in treatment 4 while the least value was observed in treatment 1. However, there was no significant difference ($P>0.05$) in the profit index values. The best incidence of cost and profit index at treatment 4 (75% MEAM) indicates that more profit was generated from fish fed this diet. Diet 4 (75% MEAM diet) was therefore the most cost effective diet. This observation is in contrast to Sotolu (2008) and Adesina et al. (2013) which reported poorer economic returns at higher (above 20%) inclusion levels of their test ingredients. This could be as a result of higher levels of anti-nutritional factors such as mimosine and phytate respectively in their test ingredients which resulted into much reduced growth and value of fish produced beyond 20% inclusion levels.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

This study was designed to investigate the effect of boiling, soaking in water, roasting, mechanical-extraction and solvent extraction on the chemical composition of tropical almond kernel meal and its nutritional values to *Clarias gariepinus* juveniles. Mechanical extraction and solvent extraction resulted into higher crude protein content than the raw tropical almond kernel meal while the other processing methods resulted in reduction in crude protein content. Solvent extracted almond kernel meal (SEAM) had the highest crude protein content 35.38% while boiled almond kernel meal (BOAM) had the least value (24.07%). Raw almond kernel meal (RWAM) had the highest crude fat content while SEAM had the least. SEAM had the highest crude fibre content (3.79%) while BOAM had the least (3.33%). Ash content was highest (4.87%) in SEAM and least (2.78%) in soaked almond kernel meal (SOAM).

There were reductions in the phytate, oxalate, tannin and trypsin inhibitor contents of raw tropical almond kernel meal as a result of processing. Fish fed ROAM diet produced the highest mean weight gain (MWG) (8.9g) and percentage weight gain (PWG) (85.79%) while the least values for both parameters were recorded in fish fed SOAM diet. ROAM diet also showed superior value for pecific growth rate (SGR), while the least value was observed in fish fed SOAM diet. Fish fed ROAM diet equally produced the best feed conversion ratio (FCR) while fish fed SEAM and SOAM diets had the poorest FCR. Among the differently processed tropical almond kernel meals, Apparent digestibility co-efficient (ADC) of crude protein was highest in ROAM diet (89.03%) followed by MEAM diet (88.92%) and least (87.06%) in SEAM diet. Similarly, ADC of gross energy was highest (88.51%) in ROAM diet, followed by MEAM diet (88.19%) and least (86.8%) in RWAM diet.

ROAM diet had the best digestibility co-efficient of nutrients and best growth performance indices followed by MEAM diet. These two meals were subsequently used to replace soya bean meal at 0, 25, 50, 75 and 100% in formulating *Clarias gariepinus* diet used for further growth studies. 75% ROAM diet produced fish with the highest MWG (69.03g), PWG (574.87%) and

SGR (1.82%) while 100% ROAM diet produced fish with the least MWG (27.02%), PWG (224.5%) and SGR (1.12%). In addition, 75% ROAM dietary treatment had the highest total feed intake (TFI) (1838.21g), Nitrogen metabolism (Nm) (2682.32) and best FCR (1.54) while 100% ROAM dietary treatment had the least MFI (57.91g), TFI (6716g), Nm (1472.54) and poorest FCR (2.14).

Similarly, 75% MEAM dietary treatment had the highest MWG (68.45g), PWG (569.697) and SGR (1.81) while fish fed 100% MEAM diet had the least MWG (51.55g), PWG (429.02%) and SGR(1.58). 75% MEAM dietary treatment also had the highest TFI (1812.34g), MFI (104.61g), Nm (2665.69) and the best FCR (1.53) while 100% MEAM dietary treatment had the least TFI (1509.33g), MFI (85.48g), Nm (2178.59), and poorest FCR(1.66).

Heamatological and serum biochemical analyses of the experimental fish blood indicated that apart from diet 5, the experimental fish tolerated ROAM diet to an appreciable extent as they showed improvement in the various observed heamatological parameters up to 75% inclusion level of ROAM. Fish fed MEAM diets also showed appreciable improvement in their harmatology and serum biochemistry. 75% ROAM and MEAM diets which had the best growth performance, nutrient utilization and economic returns and had no marked negative health implication on the fish tends to be the bench mark for both ROAM and MEAM inclusion levels in *Clarias gariepinus* diet.

Histological examination of the intestine, liver and kidney of the experimental fish showed changes with treatment 5 (100% ROAM and 100% MEAM) in each case having poor histopathological profiles.

Economical replacement of soya bean meal with ROAM and MEAM up to 75% in *C. gariepinus* diet is feasible as profit index was highest in fish fed 75% ROAM and 75% MEAM diets. 75% ROAM diet had the best growth performance and was the most cost effective. Beyond 75% inclusion level growth performance and economic evaluation indices reduced for both ROAM and MEAM based diets. It is therefore not healthy and economical to substitute soya bean meal with ROAM or MEAM beyond 75% inclusion level for profitable and sustainable aquaculture.

In this study, water quality parameters monitored (pH, DO, temperature, ammonia and nitrite) in the course of the experiments were observed to be within the optimum ranges recommended for catfish culture.

6.2 Conclusion

This study showed that processing (boiling, roasting, soaking, mechanical and solvent extraction) ultimately reduced the levels of anti- nutritional factors (tannin, phytate, oxalate and trypsin inhibitor) present in raw tropical almond kernel. Roasted almond kernel meal was the best digested and utilized meal and can successfully replace up to 75% of soya bean meal in *Clarias gariepinus* diet when growth, economic benefits and health implications are considered. Fish fed diet above 75% replacement level showed some negative health implications. In addition, this study has shown that mechanically-extracted almond kernel meal can equally replace up to 75% of soya bean meal in *C. gariepinus* diet without compromising growth, economic benefits and health status of the fish. Substitution beyond this level caused growth and health depression to the fish.

6.3 Recommendations

Underutilized kernels, oil seeds and nuts are valuable potential sources of both protein and energy in aqua feeds. Tropical almond kernel used in this study has great potential for utilization in the diet of catfish. However, it cannot presently be used as a total substitute for soya bean meal but as a partial replacement. The problem of palatability and bio-safety at 100% inclusion level should be solved. Results from this study provide baseline information for further investigation.

Further research is needed into a combination of processing methods to further reduce or completely eliminate the effect of phytate, oxalate, tannin and other anti- nutritional factors in tropical almond kernel with minimal damage to its nutrient composition. This will go a long way in improving the suitability of tropical almond kernel meal possibly as a total replacement for soya bean meal in *C. gariepinus* diet.

Research efforts should be geared towards getting improved/ mechanized method of de-hulling (de-shelling) tropical almond fruit to get the kernel. This necessitates multi- disciplinary research collaborations among agricultural engineers and agronomist/horticulturist in developing a cracking machine for almond fruit.

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APPENDIX 1

Plate 31: Tropical almond fruits

Appendix 2

Plate 32: Dried almond fruits ready for de-shelling

Appendix 3

Plate 33: Tropical almond kernel