EFFECTS OF REPLACEMENT OF FISHMEAL WITH *MORINGA OLEIFERA* SEED MEAL ON GROWTH PERFORMANCE OF CATFISH

(*Clarias gariepinus*)

BY

OMOLOUNNU OMOYEMI KEHINDE

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CERTIFICATION

This is to certify that the experiment reported here was conducted by:

MR. OMOLOUNNU OMOYEMI KEHINDE

The report has been read and approved having met the requirements of the Department of Fisheries and Aquaculture, Faculty of Agriculture, Federal University Oye- Ekiti, for the award of Bachelor of fisheries and aquaculture (B. fish).

....

DR. T .O BABALOLA **DATE**

PROJECT SUPERVISOR

....

DR. T .O BABALOLA **DATE**

HEAD OF DEPARTMENT

....

EXTERNAL EXAMINER **DATE**

DEDICATION

This project is dedicated to Almighty God for the grace given unto me to have come, saw and conquered in this land. To my parent, families, relatives, friends. I dedicate this project to everyone that has added positively to my life in one way or the other throughout my stay in FUOYE.

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ABSTRACT

The effects of *Moringa oleifera* seed meal (MSM) on the growth performance and haematological indices of *Clarias gariepinus* fingerlings were investigated for a period of eight (8) weeks. Five iso-nitrogenous (40% crude protein) diets were formulated under completely randomized experiment in which MSM replaced fish meal (FM) at 0% (T1), 25% (T2), 50% (T3), 75% (T4) and 100% (T5) inclusion levels. Catfish fingerlings (8.35 ± 0.06 g) stocked at 10 fish per 20-litre tank in three replicates were fed (5% body weight) twice daily at 8.00 hrs to 9.00 hrs and 17.00 hrs to 18.00 hrs for 8 weeks. Growth performance indices showed that weight gain (WG) (g), growth rate (GR), specific growth rate (SGR), mean growth rate (MGR) and percentage weight gain of fish fed 0 and 25% Moringa seed meal inclusion level was significantly different ($P<0.05$) from fish fed with 50, 75 and 100% inclusion level. Food utilization indices showed that fish fed with control (0%) and 25% diets consumed significantly ($P<0.05$) more feed (17.47 ±1.28 g) than fish fed with 50, 75 and 100% MSM (6.31± 0.58). Also, food conversion ratio (FCR) and Daily weight gain (DWG) were significantly different ($P<0.05$) among the treatment with 0 and 25% not significantly different

There was no significant difference ($p>0.05$) in the hematological values except for heamoglobin and MCH with less significant differences. PCV was highest (36.00%) in fish fed diet Diet 5 while the lowest (30.67%) was recorded in Diet 3.

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CHAPTER ONE

INTRODUCTION

Since farming of aquatic animals in Nigeria was broadly adopted and improved, the major constraints have been the high cost of feed. Feed alone accounted for between 60-70% of the running expense involved in the operation of fish farm as an enterprise (Gabriel *et al.*2007). Fish meal is the major ingredient in commercial fish feeds, especially those used for rearing carnivorous species (Nwanna *et al.*, 2002), because it has balanced amino acid profile which makes it suitable as animal protein in fish nutrition. However, it is scarce and expensive, resulting in over 65% of the operating costs of aquaculture (Nwanna, 2002, Luo *et al.*, 2004). The Production of aqua feeds with minimum amount of fish meal (FM) is a necessity to reduce feed costs and more importantly, to cope with the declined catches and ecological pressure on wild fish stocks (Naylor *et al.*, 2009; FAO 2010). It was speculated that aquaculture sector alone will consume equivalent of about 23.8 million metric tons (mmt) of fish or 87% of non-Food fish in 2006 (Mgbenka *et al., 2013*). For 2015, the production range for China is between 1.5 and 1.8 MMT, and the same is expected for 2016. It is estimated that by 2020 the figure will rise to about 50 % (World Fish Center, 2010). In 2011, the non-food uses of world fisheries were 23.2 mmt out of a total fish production of 154 mmt (Mgbenka *et all 2013*). Hardy (2000) discovered that the proportion of global fishmeal production used in fish feeds increased from 10 to 35% since 1985.

As public awareness about the health benefits of fish consumption continues to increase, the global demand for aquatic foods is also expected to continue to rise (Gina, 2009). Furthermore, the world's population is expected to grow by more than 30 % by 2050, resulting in an estimated 2.3 billion more mouths to feed, with the major growth expected in the developing

countries where fish is the primary source of protein (UN, 2010). Aquaculture is recognized as the only way to meet these increasing demands for aquatic foods (Allan, 2004). According to Food and Agriculture Organization (FAO 2014), fish meal, despite its potentials and importance in aqua feed is relatively scarce and expensive; the need to replace it with other viable protein sources should be considered because of the following reason;

- Fish meal production from whole fish competes with the general demand of fish for human consumption.
- To cut down exorbitant cost of fish feed as a result of inclusion of fish meal as part of feedstuff (feed takes 60% cost of production)

Because most of the conventional plant/animal protein sources are in great demand for human consumption, there is a need to examine other unconventional feedstuff products. Plant protein sources such as soybean meal, cottonseed meal, groundnut meal, sunflower seed, and rapeseed have been investigated for fish diets (Ogunji 2004; El-sayed 1999; El-sayed and Tacon 1997). In agriculture, the use of Moringa in feed production is known especially for poultry feeds (Du *et al.*, 2007; Oduro *et al.*, 2008; Olugbemi *et al.*, 2010; Zanuet *et al.*, 2012). Earlier studies have shown that *M. oleifera* is a promising protein source for use in diet of Tilapia (Richter *et al.*, 2003). Moringa leaves are readily eaten by cattle, sheep, goats, pigs, chickens and rabbits. It can also be used as food for herbivorous fish species. Several studies demonstrate that significant proportions of traditional fodder can be replaced with moringa leaf. A study in Fiji reports significant weight gain over traditional fodder when 50% of fodder contained Moringa (Aregheore, 2002).Analysis of Moringa showed that its leaves, kernel and the fat-free kernel meals contain 26.4 %, 36.7 % and 61.4 % of crude protein, respectively. (*Egwui, et al., 2013*).

Moringa oleifera hold considerable potential for becoming animal and fish feed ingredients because of its high nutritional quality. However, there is paucity of information regarding the utilization of Moringa seed meal in fish feed. (Olumuji *et al.*, 2014, Anwar *et al.*, (2003).

1.2. OBJECTIVES

The general objective of this research is to determine the Utilization of different replacement levels of moringa seed meal as protein sources in the diet of *Clarias gariepinus.*

Determine the effects of dietary levels of *Moringa oleifera* seed meal that could replace fish meal and to check the effect on growth performance parameters of *Clarias gariepinus*

Also to determine the effects of replacing fish meal with Moringa seed Meal on growth performance of *C. gariepinus*. Then, establish the optimal substitution level for the optimal performance catfish *C. gariepinus.*

CHAPTER TWO

LITERATURE REVIEW

2.1. *Moringa oleifera* plant, its composition and nutritive value

Moringa oleifera (fam. Moringaceae) is a widely cultivated species originated from north-west India that can be exploited both in irrigated and in dry conditions and that presents a high potential due to its multiplicity of uses in food, cosmetics, medicinal and other industrial applications (Lorenzi and Matos., 2002; Silva and Kerr., 1999; Correa, 1998; Matos., 1998; Ramachandran et al., 1980). Moringa plant is native to the sub-Himalayan regions of Northwest India (Foidl *et al*, 2001; Isaac, 2012). The plant now thrives in many countries of Africa, Arabia, South East Asia, the Pacific and Caribbean Islands as well as South America, producing flowers and fruits at all seasons (Isaac, 2012). Moringa seeds contain pterygospermin, a potent antibiotic and fungicide effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* In the Philippines, due to their high iron content, moringa leaves are used in the treatment of aenemia. Moringa roots and bark are used in cardiac and circulatory problems (Orwa *et al.,* 2009). Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolic (Anwar *et al.*, 2007) mature seeds yield 32-40% edible oil called ben oil from its high concentration of behenic acid. The seed contain hypotensive activity, strong antioxidant activity and chelating proper against arsenic toxicity (Arabshashi *et al.*, 2009; Ghasi *et al*; 2000 Mehta *et al.*, 2003). This tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, Vitamin C, and carotenoids suitable for utilization in many of the so called "developing" regions

of the world where under nourishment is a major concern (Tsaknis *et al.*, 1999). Moringa plant is an exceptionally nutritious tree with a variety of potential uses (Fahey, 2005)

Apart from the medicinal uses, *Moringa oleifera* is a good source of vitamins (A and E), amino acids and low level of anti-nutritional compounds (Yang *et al.*, 2006). *Moringa oleifera* boosts the immune systems (Jayavardhanan *et al.*, 1994; Fuglier, 1999; Olugbemi *et al.*, 2010a). Nutritional value of its leaves has been recently reviewed and revealed it to be a good source of protein, iron, calcium, vitamins A and C when compared with other plant foods such as cassava, amaranth, carrot and mango (Ferreira *et al.*, 2008). The oil extracted from its seeds (known as ben or behen oil due to the high behenic acid content) has a 38 - 40 % yield and can be used as a food, a cosmetic and a lubricant (Banerji et al., 2009). *Moringa oleifera* leaves are rich source of proteins (31.5%) but contain less carbohydrates (13.5%) and lipids (2.5%). The amino acid profile shows a well balance composition. The highest amino acid percent is that of Glycin (9%), the lowest is that of sulfuramino acids, Methionine and Cystein (average 0.4%). (Foild *et al.* 2001)

The leaves of *Moringa* contain more ascorbic acid (average 250 mg 100 g^{-1} fresh matter) than orange and lemon. *M. oleifera* tree is rich in iron, pctassium, calcium, zinc, magnesium, and produces man with useful vitamins. The beta-carotene found in *M. oleifera* is a precursor of retinol. The medicinal potential cum antimicrobial activities of *Moringa oleifera* is tremendous (Foild *et al.* 2001) Amino acids, fatty acids, minerals and vitamins are essential in animal feed. Such nutrients are used for osmotic adjustment; activate enzymes, hormones and other organic molecules that enhance growth, function and maintenance of life process (Anjorin *et al*, 2010). Nutritional composition of the plant plays a significant role in nutritional, medicinal and therapeutic values (AlKharusi *et al*, 2009). The root, leaf, bark, fruit, flowers, gum, seed and seed oil have been utilized for various ills in the autochthonous medicine (Fozia *et al.* 2012).

Moringa was found to have a group of unique compounds containing sugar and rhamnose, which are uncommon sugar-modified glucosinolates (Fahey *et al.,* 2001; Fahey, 2005; Amaglo *et al.*, 2010). These compounds were reported to demonstrate certain chemo-preventive activity, by inducing apoptosis (Brunelliet *et al.*, 2010). Johnson (2005) observed that the leaf, seed and fruits of *M. oleifera* are naturally rich sources of vitamins and minerals. *M. oleifera* leaves parts have been shown to contain the following water soluble vitamins; 2.6 mg of vitamin B1(thiamine), 20.5 mg of vitamin B2 (riboflavin), 8.2 mg of vitamin B3 (nicotinic acid) and 220 mg of vitamin C Zaku *et al.,* 2015). Moringa tree has in, recent times, been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe and caroteroids suitable for utilization in many developing regions of the world where under nourishment is a major concern (Oduro *et al.*, 2008).

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concentration of behenic acid. The seed contain hypotensive activity, strong antioxidant activity and chelating proper against arsenic toxicity (Arabshashi *et al.*, 2009; Ghasi *et al*; 2000 Mehta *et al.*, 2003).This tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, Vitamin C, and carotenoids suitable for utilization in many of the so called "developing" regions of the world where under nourishment is a major concern (Tsaknis *et al.,* 1999). Moringa plant is an exceptionally nutritious tree with a variety of potential uses (Fahey, 2005)

Table 1: Chemical composition of *Moringa oleifera* seed meal

Nutrient	Composition
Dry matter	90.40%
Soluble carbohydrate	25.80%
GE Kcal/g	5.79%
Crude protein	25.37%
Crude fat	14.16%
Crude fiber	30.64%
Mineral matter	4.03%

Table 2: Mineral composition of *M. oleifera* seed meal

Minerals	**Values**
Calcium (mg/100 g)	68.765
Magnesium (mg/100 g)	130.23
Phosphorus (mg/100 g)	438.23
Sodium (mg/100 g)	7.345
Iron (mg/100 g)	4.265
Copper (mg/100 g)	0.235
Zinc (mg/100 g)	2.280
Cretinine (microgram)	11.425
Arsenic (microgram)	0.00
Iodine (microgram)	1.87
Manganese (microgram)	1.56
Selenium (microgram)	2.045
Fluoride (microgram)	0.00

Source: International Journal of Fisheries and Aquatic Studies 2015

Table 3: Content of vitamins B-complex and ascorbic acid in *M. oleifera* (Concentration (mg/100g)

Thiamine	0.38
Riboflavin, B2	0.13
Niacine, B3	4.69
Pyridoxin, B6	2.3
Cynocobalamin, B12	3.6
Ascorbic acid, C	4.31

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2:1:1 Protein and Amino Acid composition of Moringa

Makkar and Becker (1997) worked extensively on the chemical composition of *M. oleifera* parts they reported that moringa leaves; the kernel and the fat free kernel meals contain 26.4%, 36.7% and 61.4% of crude protein, respectively. This has placed Moringa plant parts as potential protein source. Defatted seed cake is free of most plant secondary metabolites such as tannins, saponins, alkaloids, inhibitors of trypsin and amylase, lectin and cyanogenic glucosides, but contains glucosinolates (Makkar and Becker, 1997). Moringa seed meal has high essential amino acid contents, except for lysine, threonine and valine which are present in lower levels (Makkar and Becker, 1999; Francis, *et al.,* 2005). The high methionine and cysteine contents are close to those of human and cow milk and chicken eggs (Oliveira, *et al., 1999*). Analysis revealed that moringa can be a good source of essential amino acids which are all contained in appreciable quantities (Ram, 1994), and can serve as equally good source of amino acids for man and livestock (Anhwange, *et al.*, 2004).

2.1.2 Oil compositions

Moringa oleifera is widely grown in the tropics; quality of oil is almost similar to olive oil (Mohammed, *et al.,* 2003). The oil content of seeds ranges between 30-40%, oil is edible and rich in monounsaturated fatty acids (Tsakinis, *et al.,* 1998). *Moringa oleifera* oil improved the oxidative stability of vegetable oils and butter oil (Anwar, *et al.* 2007 ;). Moringa kernel contains over 40% by weight of oil – the fatty acid composition is said to be similar to that of olive oil. The oil yield is greater than sunflower (2.0 $t.ha^{-1}$) or peanut (0.5 $t.ha^{-1}$). It contains all eight (8) of the essential amino acids found in meat products. The seed oil contains 3% palmitic acid, 7.4% stearic acid, 8.6% behenic acid and 65.7% of oleic acid among other fatty acids (Egwui, *et al.,* 2013). The oil extracted from *M. oleifera* seeds is regarded as having a good commercial interest due to its physical, chemical and pharmacological characteristics (Fahey, 2005). The seed oil contains all the fatty acids contained in olive oil, except linoleic and was used as its acceptable substitute (Morton, 1991). The fatty acids found in the crude moringa oil were (with the respective percentage): palmitic 16:0 (9.61%), palmitoleic 16:1 (2.78%), estearic 18:0 (8.70%), oleic 18:1 (66.26%), 9-nonadecenoic 19:1 (2.52%), arachidic 20:0 (3.59%), behenic 22:0 (5.39%) and lignoceric 24:0 (1.17%).The major saturated fatty acids present in the seeds are palmitic, stearic, arachidic and benic acids (Abdulkarim *et al.,* 2005). The moringa seed oil is high (80.4%)in polyunsaturated fatty acid (Anwar and Rashid, 2007; Ogbunugafor *et al.*, 2011). The characteristics of *Moringa oleifera* seed oil can be highly desirable especially with the current trend of replacing polyunsaturated vegetable oils with those containing high amounts of monounsaturated acids (Corbett, 2003). The fatty acid composition of Moringa oil is quite similar to olive oil in its contents of C18:1 and C18:0 (Anwar and Bhanger, 2003), which

explains its potential as an edible oil. High oleic acid vegetable oils have been reported to be very stable even in highly demanding applications like frying (Warner and Knowlton, 1997).

2.2. Energy and Protein value of *Moringa oleifera*

Bridgemohan (2008) identified 'drumstick vegetable' tree or *Moringa oleifera* Lam a potential high protein and energy crop in Trinidad and Tobago. Moringa has been demonstrated as an energy crop (Lalas and Tsaknis 2002), with crude protein, organic matter (OM) and fibre contents higher than soya bean (>40%) or rape seed (Soliva *et al.*, 2005). The role of legume forage and seeds as a source of both energy and protein in livestock feeds has been well reviewed (Tothill 1986). Fats or oils and carbohydrates are sources of non- protein energy. Moringa is a non-leguminous multipurpose tree. Its leaves contain between 257.00 to 261.00 g kg^{-1}DM crude protein (Sultana *et al.*, 2014) and negligible amounts of anti-nutritive compound (Nouala *et al.*, 2006; Ogbe and Affiku, 2011; Aye and Adegun, 2013; Mendieta-Araica *et al.*, 2011). Moringa foliage dried meal, on the other hand, contains 214.80 to 216.20 g CP kg^{-1}DM, 268.30 to 310.29 g ADF kg^{-1}DM and 347.1 to 381.8 g NDF kg^{-1}DM (Sultana et al., 2014)

2.3. Factors Affecting use of *Moringa oleifera*

Moringa leaves are free from antinutrients except for saponins and phenols. The concentration of phenol is much below the toxic threshold levels for animals (Makkar and Becker, 1997) and saponins were inactive as far as haemolytic properties are concerned. In addition to the antinutrients listed above, alkaloids are also present in kernel meals (root-bark have been found to have two alkaloids, moringine and moringinine; moringinine is known to stimulate cardiac activity, raise blood-pressure, act on sympathetic nerve-endings as well as smooth muscles all over the body, and depress the sympathetic motor fibres of vessels in large doses only.

Glucosinolates, lectins and alkaloids which form the major ant nutrient substances in Moringa seed meal could be easily removed by water extraction (Makkar and Becker, 1999). However, this method has the disadvantage of also removing some soluble nutrients. Solid state fermentation of the seed meal using *Rhizopusoligosporus* sp. could be considered as this mould has been found to degrade glucosinolates in defatted rapeseed meal (Bau *et al.*, 1994). Anti-nutritional effects include interference with digestion by binding to proteins or minerals. Tannins also reduce the absorption of vitamin B12 (Vikas Kumar *et, al* 2012). When present in high quantity in fish diet; saponins are highly toxic to fish, with the detergent action of saponins causing damage to the respiratory epithelium of gills and inhibition of active transport of nutrients (Vikas Kumar *et, al* 2012). Long term consumption of glucosinolates leads to thyroid dysfunction affect metabolism and growth in fish (Vikas Kumar *et, al* 2012). Saponins increase the digestibility of carbohydrate rich foods by detergent-like activity that reduces viscosity, preventing the normal obstructing action of such foods in the intestine

(a) Human consumption of *M. oleifera*

M. oleifera leaves are highly nutritious. The young leaves are edible and are commonly cooked and eaten like spinach or used to make soups and salads. The leaves can be consumed either in raw, cooked or dried over a screen for several days and ground into a fine powder that can be added to almost any food as a nutrient supplement (Makkar and Becker, 1996), Nutrition content of a plant plays an essential function in medicinal, nutritional, and therapeutic properties (Al Kharusi *et al.*, 2009). The leaves can be eaten fresh cooked or stored as dried powder for several months the pods, when young can be cooked; eaten like beans (National Research Council, 2006). The health benefits of Moringa to man include relief from stomach disorders, allergies and edema. The antioxidant power of moringa aids in liver protection, diabetes, eye protection,

cardiovascular health, bone health, uroliathiasis, wound healing, healthy hair and skin. It is rich in antibacterial and antifungal properties that help to fight various infections, including herpes. (National Research Council, 2006).

It is rich in phytonutrients, which are effective for preventing various medical conditions such as cancer, neurodegenerative diseases, bronchial asthma sickle cell disease, nephrotoxicity, high cholesterol, high blood pressure, anemia, and obesity, as well as helping to build a strong immune system. (National Research Council, 2006).

(b) Industrial uses of *M. oleifera* oil

The oil content of dehulled seed (kernel) is approximately 42%. The oil is brilliant yellow. It is used as a lubricant for fine machinery such as timepieces because it has little tendency to deteriorate and become rancid and sticky (Ramachandran *et al.,* 1980). It is also useful as vegetable cooking and frying oil. The oil is known for its capacity to absorb and retain volatile substances and is therefore valuable in the perfume industry for stabilizing scents. The free fatty acid content varies from 0.5 to 3%. The seed oil of Moringa contains approximately 13% saturated fatty acids and 82% unsaturated fatty acids (Ferrao and Mendez, 1970). It has a particularly high level of oleic acid (70%). Other vegetable oils normally contain only about 40% oleic acid. Many companies across the world manufacturing various products of Moringa leaves such as Moringa tea, Moringa Tablets, Moringa capsules, Moringa leaf Powder, Moringa Soaps and Moringa face wash. Some beverages are also available in market prepared by Moringa leaves. So it is necessary to hygienically drying and processing of Moringa leaves for further uses (Mishra *et al.,* 2012)

2.4 Dietary protein requirements of *C. gariepinus*

Protein is the most expensive component in supplementary fish feed (Fagbenro *et al.*, 1992). Dietary protein is used by fish for growth; energy and body maintenance (Kausshik and Medale, 1994). Generally speaking, fish meal constitutes the main protein source (some 40-60%), relinquished only when a protein-rich alternative is included, mostly of vegetable origin (e.g. groundnut cake, soybean meal) (Chuapoehuk, 1987; Balogun and Ologhobo, 1989). Fish has the highest level of easily metabolisable high quality protein, fats, vitamins, calcium, iron and essential amino acids when compared to other sources of animal protein such as poultry and beef (Ayoola, 2010).

Results from various studies confirm that 40% protein as the requirement for *C. gariepinus* and also showed that increasing the dietary protein content of the fish to 45% did not have any statistical advantage.

Clarias gariepinus is generally considered to be one of the most important tropical catfish species for aquaculture. It has an almost Pan-African distribution, ranging from the Nile to West Africa and from Algeria to Southern Africa. They also occur in Asia Minor (Israel, Syria and South of Turkey). *C. gariepinus* at various geographical locations bears different names. It iscalled *C. lazera*in Northern and Central Africa, *C. senegalensis* in East Africa, *C. masambicus*in West Africa and *C. gariepinus*in South Africa (Viveen et al, 1985).

2.5 Amino Acid Requirement of Catfish

Various efforts have been made to establish the crude protein and amino acid requirement of C. *gariepinus*. Ayinla (1988) recommended 35 and 40% crude protein (CP) for raising table size and brood stock respectively. Of the 10 essential amino acids (EAAs) required by fresh water fish species, only three EAAs have been studied, these are arginine, methionine and lysine. In order to formulate and compound aqua feeds that will meet the nutrient requirements of the catfish. The first need is to supply the indispensable amino acid requirement of the animal, and secondly to supply dispensable amino acids or sufficient amino nitrogen to enable their synthesis (Macartney, 1996).

Table 4: Amino acid content g/100g protein of *Moringa oleifera*

Aino Acid	Root	Leaves	seeds
Alanine	3.36	3.43	3.23
Arginine	1.74	1.88	8.06
Aspartate	6.01	6.86	6.14
Cysteine	2.42	2.05	2.02
Glutamate	13.53	15.14	14.76
Glycine	4.6	5.15	5
Histidine	1.91	1.9	2.01
Isoleucine	1.84	2.33	4.35
Leucine	5.02	5.22	5.27
Lysine	3.62	3.6	3.24
Methionine	0.76	0.95	0.97
Phenylalanine	3.98	4.26	4.53
Serine	3.61	4.2	4.25
Threonine	3.94	4.38	3.22
Tyrosine	2.43	2.2	2.33
Valine	3.03	3.36	3.09

Source:http://moringafacts.net/moringa-amino-acid-content

CHAPTER THREE

MATERIALS AND METHODS

3.1. Processing and proximate analysis of Moringa

Moringa seeds (5 kg) (*Moringa oleifera* Lam.) were collected from a Moringa farm in Federal Housing Estate Ogbomoso Road Oyo, Oyo State, Nigeria and were manually dehaulled and dried for two days to reduce its moisture content. The seeds were thoroughly screened to remove dirt and other foreign substance present. Thereafter, the seeds were taken through extraction process with manual hand oil extractor using Methanol as organic solvent. At the end of extraction process, Moringa seed cake and oil were collected and properly stored. The cake collected was therefore ground and analyzed for proximate composition according to AOAC (2000).

3.2. Fish diet formulation and processing

The feed ingredients were Fish meal (FM), Moringa seed cake (MSM) Wheat offal, Groundnut cake (GNC), Maize (M), Soya bean cake (SBC) Starch(S), Vegetable oil (VO) Methionine (M),Premix (P), Salt (S) Chromic oxide (CO). Five iso nitrogeneous (40% protein) rations were formulated as shown in Table 3:1. Diet 1 was the control and it contained fishmeal, soybean meal and groundnut cake as the main source of protein and supplemental methionine source respectively Diet 2, 3, 4 and 5 had fish meal portion replaced by fat free *M. oleifera* at 25%, 50%, 75% and 100% respectively. No lysine was present in all of these Diets all had methionine included at 1.0 level.

The experimental diets were prepared by weighing different quantities of feedstuffs required based on the feed formulation prepared and moringa seed meal was included at 25%,

50%, 75% and 100% respectively. The dry ingredients were mixed with pregelatinized starch and oil and the resulting moist dough was pelleted using a locally assembled hand pelletizer through a 2- mm die. The moist pellets were then sun dried on a raised platform and stored in cool dry place until used. The fish were fed daily the assigned diets (triplicated for each diet) at 5% body weight and fed two times daily for a period of 8 weeks after acclimatization.

Table 5. Percentage composition (%) of the experimental diets.

INGREDIENT	DIET 1 (0%MSM)	DIET 2 (25%MSM)	DIET 3 (50%MSM)	DIET 4 (75%MSM)	DIET 5 (100%MSM)
Fish meal (72%)	**25.00**	**18.75**	**12.5**	**6.25**	**-**
Moringa seed meal (75%)	**-**	**6.00**	**12.00**	**18.00**	**24.00**
Soybean meal (44%)	**20.75**	**21.00**	**21.00**	**22.64**	**18.00**
Groundnut Cake (44%)	**20.00**	**20.00**	**20.00**	**18.00**	**21.89**
Maize/corn	**15.00**	**12.00**	**12.66**	**14.00**	**14.00**
Wheat offal	**12.00**	**15.00**	**14.59**	**13.86**	**14.86**
Methionine	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
Vitamin /min premix	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
Salt	**0.50**	**0.50**	**0.50**	**0.5**	**0.50**
Starch	**2.00**	**2.00**	**2.00**	**2.00**	**2.00**
Vitamin. C	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**
Vegetable oil	**2.00**	**2.00**	**2.00**	**2.00**	**2.00**
Chromic oxide	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**
TOTAL	**100**	**100**	**100**	**100**	**100**

3.3. Experimental design and feeding trials

One hundred and fifty fingerlings of *Clarias gariepinus* were obtained from a private fish farm in usin- Ekiti, Ekiti state. The fish (mean body weight 8.35 ± 0.06 g) were randomly allotted into 15 rectangular tanks with 10 fish in each tank. Each of the five treatments was replicated in triplicate. The fish were acclimatized for two weeks and fed at 5% body weight twice daily (Okoye *et al.,* 2001) with a formulated diet of 40% crude protein. Prior to the commencement of the experiment, all fish were starved for 24 h to eliminate variation in weight due to residue food in the gut and at the same time to increase the appetite of the fish (Madu and Akilo, 2001). The fish initial weight (IW) ranged from 8.35 to 8.41 g, mean weight (MW) 8.35 g were weighed with weighing balance.

The feeding trial begins after 24 hours starvation of the fish and the experimental diet was introduced to the fish at varying level of moringa inclusion; 0%, 25%, 50%, 75%, 100%. The 0% diet contain no moringa seed meal but fish meal, 25% diet contain only 25% of moringa meal inclusion, 50% diet contain only 50% Moringa seed meal, 75% diet contain 75% of moringa seed meal inclusion, 100% diet contain 100% of moringa meal inclusion. The fish were fed twice daily at 5% of their body weight for a period of 8 weeks and feed quantity was adjusted in accordance with their body weight. Each fish was weighed using weighing balance on weekly basis.

3.4. Determination of fish growth and performance

Growth performance was expressed as the mean weights gain (MWG), Mean weight Gain (RWG), Specific Growth Rate (SGR) and Protein Efficiency Ratio (PER), Feed conversion Ratio (FCR). The calculation formulas are as follows:

Protein efficiency ratio (PER)

$$PER = \frac{\text{Weight gain (g)}}{\text{Crude protein fed}}$$

Feed conversion ratio (FCR)

$$FCR = \frac{\text{Feed intake} \times 100}{\text{Body weight gain}}$$

Specific Growth Rate

$$SGR = \frac{\log_e(\text{final weight}) - \log_e(\text{initial weight})}{\text{Time period [days]}}$$

Mean weight gain (MWG)

MWG = Final weight (g) of fish - Initial weight (g) of fish.

Daily growth rate (DGR)

$$DGR = \frac{\text{Final weight} - \text{Initial weight}}{\text{Number of day}}$$

3.5. Sample collection

At the end of feeding trial, fish blood samples were collected with eparinized bottles. Blood samples were obtained from the caudal vein of fish from each tank. Blood for serum analysis were collected into bottles (EDTA Bottle)

3.5.1. Fish Haematological profile

Immediately after sampling, blood smear were prepared, red blood and white blood cell count were carried out using standard haematological techniques (Dacie and Lewis 2001). Fifty μL haematocrit tubes was filled with blood samples, after centrifugation (7200 rpm for 10 min) of each blood sample, packed cell volume (PCV) was determined by the Wintrobe and Westergreen

method as described by Blaxhall and Daisley (1973). Haemoglobin levels (Hb in grams per deciliters) were obtained by the cyanomethaemoglobin spectrometric method (Dorafshan *et al.*, 2008). The blood indices including mean corpuscular volume (MCV in femtoliters), mean corpuscular heamoglobin (MCH in pictograms per cell), and mean corpuscular haemoglobin concentration (MCHC in grams per decilitre) were calculated according to the following formulars (Dacie and Lewis 2001):

$$\text{MCV (fl)} = \frac{\text{PCV (\%)}}{\text{RBC } (10^{-6}\mu l^{-1})}$$

$$\text{MCH (pg)} = \frac{[\text{Hb } (gdl^{-1})]}{\text{RBC } (10^{-6}\mu l^{-1})}$$

$$\text{MCHC } (gdl^{-1}) = \frac{[\text{Hb } (gdl^{-1})]}{\text{PCV (\%)}}$$

3.6. Statistical analysis

Each of the five dietary treatments was assigned to three plastic tanks in a completely randomized design. Weight gain, feed intake, FCR, SGR and haematological and serum biochemical parameters of fish were subjected to one-way Analysis of Variance (ANOVA). When significant differences among treatments were found ($p< 0.05$), Duncan's multiple range test (Duncan, 1955) was used to compare the treatment means using the software SPSS 15.0

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Growth response, nutrient utilization and survival parameters of *Clarias gariepinus* fingerlings fed Varying levels of *Moringa oleifera* seed meal diet

The result obtained for the growth response, nutrient utilization and survival parameters of fish fed *M. oleifera* based diet during juvenile stage are shown in Table 4 below. The fish fed 25% *M. oleifera* seed meal diet gained 9.60g, while the fish fed control diet (0% Moringa seed) gained 10.75g. The values obtained for the fish fed control diet and 25% *M. olifera* seed meal diet were not significantly different ($P > 0.05$) but were significantly different ($P < 0.05$) when compared with fish fed 50, 75, and 100% *M. oleifera* seed meal diets. The best growth result (Final body weight and SGR) were of fish fed 0% and 25%. Specific Growth Rate (SGR) of fish fed with 50%, 75% and 100% were significantly lower than those fed with 0% and 25% diets. There was no significant difference ($P > 0.05$) in the feed conversion ratio (FCR) in the fish fed control diet, and 25% *M. oleifera* seed meal diet but there was a significant difference when compared with the fish fed the diets containing 50, 75 and 100% *M. oleifera* seed meal. The highest value of 2.02 for protein efficiency ratio (PER) was observed in fish fed diet containing 0% *M. oleifera* seed meal and lowest value of 0.46 was recorded in fish fed diet containing 100% *M. oleifera* seed meal. Mortality rate (MR) was highest in fish fed with 25% *M.oleifera* seed meal based diet. Fish mortality exhibited no significant correlation with increase in *M.* oleifera seed meal in the diets formulated.

Table 6. Growth performances of *Clarias gariepinus* fed with different levels of defatted moringa seed meal for 56 days.

PARAMETERS	DIET 1 (0%MSM)	DIET 2 (25%MSM)	DIET 3 (50%MSM)	DIET 4 (75%MSM)	DIET 5 (100%MSM)
MIW (g)	8.35 ± 0.87^{a}	8.38 ± 0.00^{a}	8.36 ± 0.10^{a}	8.41 ± 0.01^{a}	8.39 ± 0.03^{a}
MFW(g)	19.1 ± 1.37^{b}	17.98 ± 0.90^{b}	11.20 ± 0.07^{a}	10.58 ± 0.32^{a}	10.31 ± 0.3^{a}
BWG(g)	10.75 ± 1.29^{b}	9.60 ± 0.90^{b}	2.84 ± 0.55^{a}	2.17 ± 0.32^{a}	1.92 ± 0.60^{a}
FI (g)	18.82 ± 0.90^{d}	16.11 ± 1.65^{c}	7.77 ± 0.39^{b}	5.95 ± 0.59^{a}	4.84 ± 0.76^{a}
FCR	1.76 ± 0.19^{a}	1.74 ± 0.17^{a}	2.74 ± 0.18^{b}	2.75 ± 0.14^{b}	2.53 ± 0.55^{b}
PER	2.02 ± 1.39^{b}	1.84 ± 0.60^{b}	0.55 ± 0.90^{a}	0.53 ± 0.80^{a}	0.46 ± 0.75^{a}
MORTALITY (%)	26.67 ± 37.86^{a}	30.00 ± 30.00^{a}	3.33 ± 5.77^{a}	16.67 ± 20.82^{a}	10.00 ± 0.00^{a}
SGR	1.48 ± 0.45^{c}	1.36 ± 0.05^{c}	0.52 ± 0.00^{b}	0.41 ± 0.03^{ab}	0.37 ± 0.00^{a}
DWG (g)	0.19 ± 0.03^{b}	0.17 ± 0.02^{b}	0.05 ± 0.00^{a}	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}

Values are means ± SD. Means in the same row having different superscripts are significantly different ($P < 0.05$), while values in same row with same superscript are not significantly different ($P > 0.05$). MIW=Mean Initial Weight, MFW=Mean Final Weight, TWG=Total Weight Gain, FI = Feed Intake, FCR= Feed Conversion Ratio, PER= Protein Efficiency Ratio, SGR=Specific Growth Rate, DWG=Daily Weight Gain.

Significant differences ($P<0.05$) exist in Mean final weight (MFW), Feed conversion ratio (FCR), Body weight gain (BWG), Protein efficiency ratio (PER), Specific growth rate (SGR), Daily weight gain (DWG) with control and 25% Moringa inclusion level not significantly different from each other. Specific growth rate ranged from 0.63 and 0.16 with lower values in 50, 75 and 100% Moringa inclusion level.

Specific growth rate SGR of fish fed with diet 25% MSM inclusion level was significantly different ($P<0.05$) from the fish fed diet with 100% MSM inclusion level but not significantly different ($P>0.05$) from fish fed 0% inclusion level. Feed intake of fish fed 75%

MSM inclusion level was not significantly different (P>0.05) from fish fed 100% MSM but significantly different (P<0.05) from the fish fed with other diets (0, 25, and 50%).

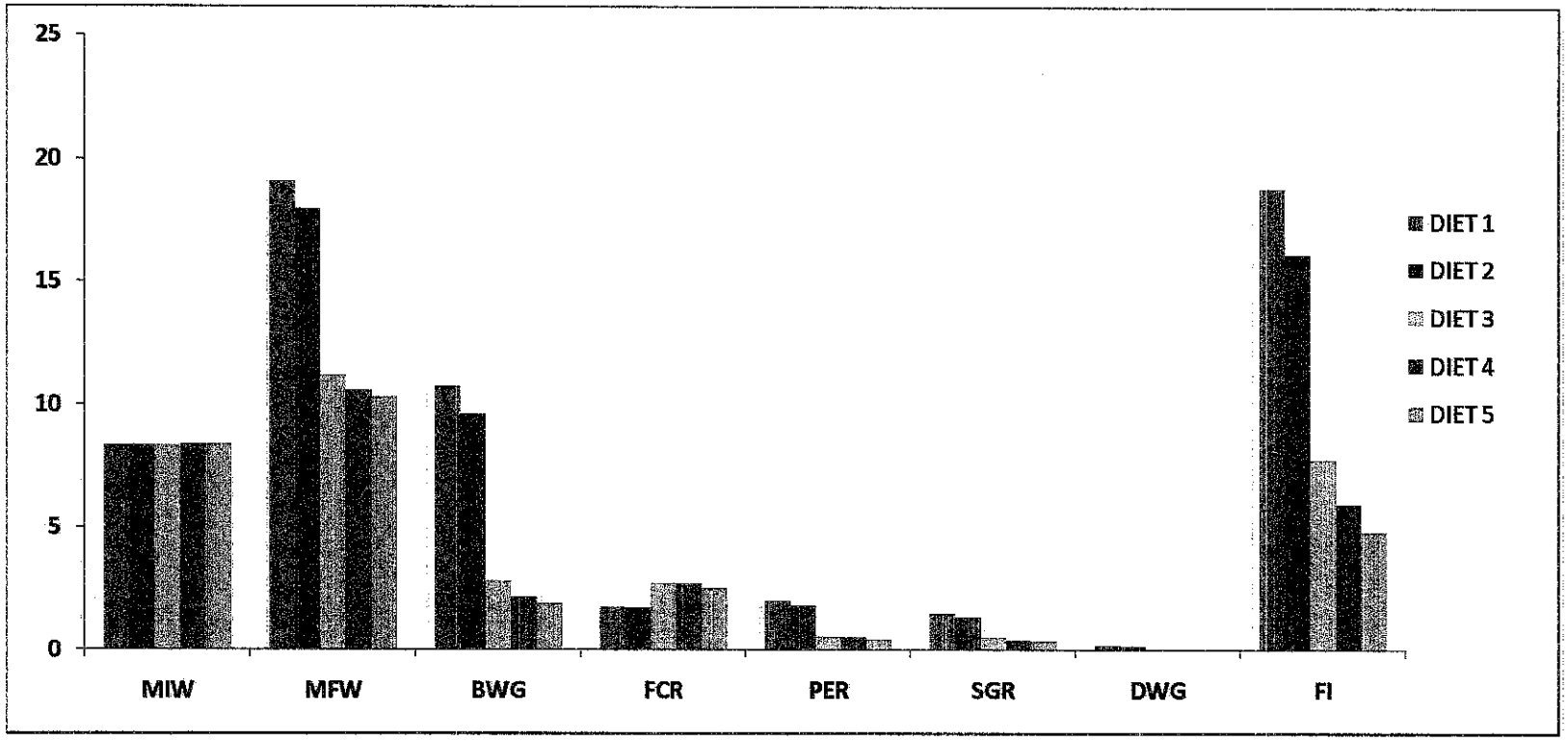

FIGURE 1: Growth parameters indicators of *C. gariepinus* fingerlings fed different levels of *Moringa oleifera* seed meal diet for 58 days

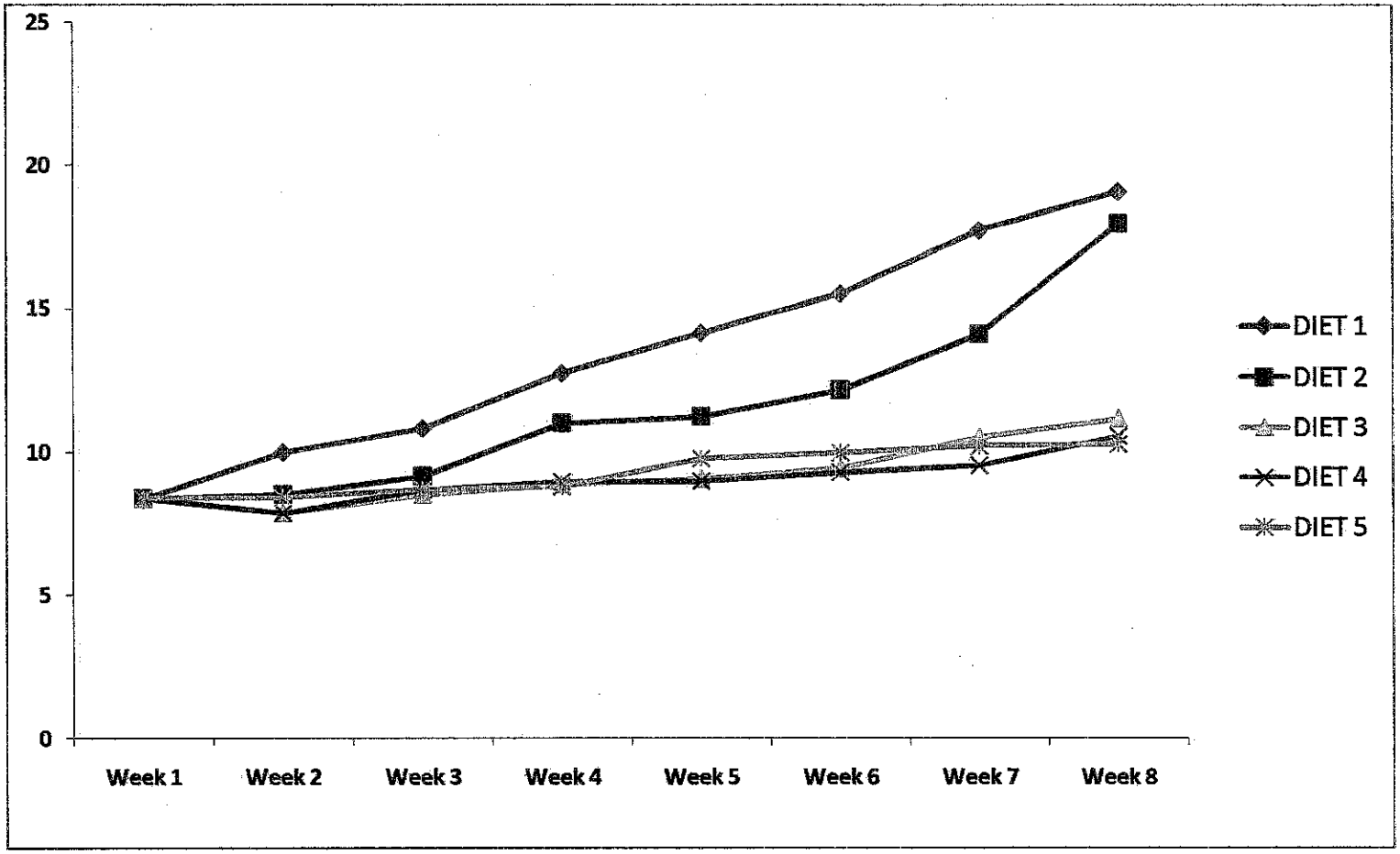

FIGURE 2: Growth pattern of *gariepinus* fingerlings fed different levels of *Moringa oleifera* seed meal diet for 58 days Vertical line is the mean weight in gram (g) and the horizontal line is the periods (weeks).

4.2. Haematological indices of *Clarias gariepinus* fingerlings fed different levels of *Moringa oleifera* seed meal diet.

The haematological Parameters of *C. gariepinus* fed *M. oleifera* based diets are presented in Table 5. The red blood cell count (RBC), Packed cell volume; (PCV), white blood cell (WBC), MCH, mean corpuscular haemoglobin observed in this study were not significantly different ($P>0.05$) among the treatments. The hemoglobin concentrations of fish fed 0, 50, 75 and 100% MSM in experiment were significantly different ($P<0.05$) with relatively close range of differences and totally and significantly different ($P<0.05$) from fish fed with 25% MSM. There were no significant different ($P>0.05$) in the MCV of fish fed 0, 75 and 100% MSM inclusion level but significantly different ($P<0.05$) from fish fed with 25% diet. It was also observed that the fish fed 50% MSM have higher MCV value than the control (0% MSM). There was no significant difference ($P>0.05$) in the value of WBC but the values decrease as the level of MSM increased in the diets.

Table 7. Haematological indices of *Clarias gariepinus* fingerlings fed different levels of *Moringa oleifera* seed meal diet.

Diets*	PCV (%)	Hb (gdL^{-1})	RBC ($\times$ 10-6μL^{-1})	MCV ($\times 10^{-6}$ fl)	MCH ($\times 10^{-6}$ pg cell)	MCHC (gdl^{-1})	WBC ($\times 10^{3}\mu L$)
1	33.00^{a}	6.16^{b}	3.13^{a}	12.62^{ab}	1.55^{ab}	0.15^{a}	20.37^{a}
2	32.67^{a}	4.40^{a}	4.96^{a}	6.66^{a}	1.15^{a}	0.14^{a}	35.93^{a}
3	30.67^{a}	5.20^{ab}	2.51^{a}	17.47^{b}	3.02^{b}	0.17^{a}	29.22^{a}
4	33.50^{a}	6.00^{b}	2.97^{a}	11.27^{ab}	2.04^{ab}	0.18^{a}	17.80^{a}
5	36.00^{a}	5.03^{ab}	4.07^{a}	8.33^{ab}	1.25^{a}	0.14^{a}	36.10^{a}

Diets: 1 = Control feed, 2 =25% Moringa seed meal, 3 = 50% Moringa Seed meal, 4 = 75% Moringa seed meal 5 = 100% Moringa seed meal.

PCV, Packed cell volume; WBC, white blood cell; RBC, red blood cell; Hb, haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

Values in the same column followed by the same letter are not significantly different at $p > 0.05$

4.3 Growth performances and haematological parameters of *Clarias gariepinus* fed different levels of Moringa seed meal

The growth and nutrient utilization by the fish decreased as *M. oleifera* seed meal increased in the diets. This observation may be as a result of persistent increase in the substitution levels of fish meal with *M. oleifera* seed meal in the diets which could retard growth as reported by Richter *et al* (2003), and also supported by *Afung et al* 2001 which showed that higher substitution of *M. oleifera* leaf meal with fish meal had an impact on lowering the growth performance due to the presence of anti-nutrients such as phenol, tannins, phylates and saponins. The results of the growth response observed in this study might probably be an indication that the parameters were influenced by the replacement levels of fishmeal meal with MSM. Results showed that de-fatted MSM could replace fish meal up to 25% replacement level without any negative influence on the growth, beyond which growth was significantly depressed. The decrease in the growth rate could also be due to reduction in level of protein and essential amino acids (supplied by fish meal) in the diet having higher substitution levels of fish meal with *M. oleifera* seed meal. The result of the specific growth rate (SGR) could be due to differences in the *M. oleifera* seed meal substitution levels which decreased considerably from 50% to 100% substitution levels in the diets.

Lower feed intake recorded in this study as MSM inclusion increased above 25% in the diet. This might be due to lowering palatability of diets containing higher levels of inclusion of *M. oleifera* seed meal. This could result from the presence of tannin in MSM. Van Egmund *et al.* (1990) and Fasasi *et al.* (2003) observed that tannins interfere with digestion by displaying anti-trypsin and antiamylase activity, forming complexes with vitamin B12 and interfering with the bioavailability of proteins. Azaza *et al.* (2009) reported that the presence of 2.4% tannin in faba

beans (*Vicia faba* L. var. minuta) might be responsible for low palatability and consequently low feed intake in Nile tilapia.

Protein efficiency ratio (PER) was high in fish fed with 25% *M. oleifera* seed meal diet, which was not statistically significant different ($P > 0.05$) from value of 0% *M. oleifera* seed meal diet . However, control diet recorded the highest value of growth rate in the experiment. This value was not statistically significantly different ($P > 0.05$) from values of 25% *M. oleifera* seed meal diet. These results seem to have direct link with palatability of the diet which causes reduced feed intake as the inclusion of *M. oleifera* seed meal increases in the fish diet. (Faturoti, 1989).

Fish fed with 25% *M. oleifera* meal diet showed better feed conversion ratio (FCR). However, there was a decrease across the treatments. The reason for this observation might be due to presence of some anti-nutritional factors present in high concentration in *M. olifera* seed meal as reported by Osabor *et al.*2009 *and* Obasa *et al.2013* that; the anti-nutritional factors content in MSM were rather high compared to some other plant based nonconventional feed ingredients like African breadfruit (*Treculia africana*) (Osabor *et al.* 2009) and mango (*Mangifera indica*) seed (Obasa *et al.* 2013). Moreover, a decreasing trend was observed in the PCV of fish fed different levels of Moringa seed meal and was attributed to the presence of some anti-metabolites such as tannin and phenol in *M. oleifera* seed meal. Dienye *et al.*, (2014).

The physiological state resulting from different levels of MSM in diets was clearly reflected by the absence of significant differences ($p>0.05$) in the red blood cell count (RBC) and packed cell volume (PCV) observed in this study. This is in line with the observation of Obasa *et al.* (2013), feeding the African catfish with fermented African breadfruit (T. africana) seed meal based diets.

Likewise, Brucka-Jastrzebska and Protasowicki (2005) subjected common carp *(Cyprinus carpio)* to cadmium and nickel exposure for a prolonged period.

The absence of significant difference in the WBC values among the treatments probably signified that Moringa seed meal even at high level of inclusion in diet was not toxic to *C. gariepinus* juvenile and did not have any influence on its immune status. Allen (1994) observed increased WBC (leucocytes) counts in *Oreochromis aureus*. Maheswaran *et al.* (2008) also observed increase in WBC when *Clarias batrachus* (L.) were exposed to mercuric chloride. Douglas and Jane (2010) demonstrated that the amount has implication in immune responses and the ability of the animal to fight infection. High WBC count is usually associated with microbial infection or the circulating system (Oyawoye and Ogunkunle, 1998).

The values of Hb, MCV and MCH were significantly different among dietary groups; fish fed diet 2 (25% MSM) had the lowest values for the same heamatological traits. In this study, the consumption of Moringa seed meal may not impact negative effect on the defensive function of WBC.

The Hb concentration value of fish fed diets 1, 3 and 5 were similar and showed that feeding these Moringa seed meal levels to C. gariepinus exert no significant negative influence on Hb concentrations. The Hb concentrations and PCV are basic values revealing the degree of anaemia while the MCHC is a useful index of the average Hb concentration of the red cells (Swash and Mason, 1984). In the present study, C. gariepinus could be considered to be adequately haemoglobinized as none of the haematological parameters measured are out of the range considered normal for fish (Sandnes et al., 1988).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The *Moringa* plant is one of the cheapest close substitutes being a plant feed stuff, it has a nutrient profile that is close to what is obtainable in fishmeal and it can also be made available in quantity that can support the aquaculture industry. The results obtained from this study showed that *M. oleifera* seed meal could be substituted with fish meal up to 25% level in *Clarias gariepinus* diets without any negative effects. Fish fed 25% MSM inclusion level produces the same result as that raised without substitution and having no adverse toxicological effect on the fish as revealed through the haematological indices, fish feed can be produced at a relatively cheaper cost and as thus profit of fish farmers can be increased. All the performance parameters measured; weight gain, feed intake, feed/ gain ratio, protein efficiency ratio, were compared.

The implication of these findings is that *Moringa oleifera* seed meal can be used to replace fish meal up to 25% level. This will lead to a good reduction in exorbitant cost of feed production in Aquaculture. It is therefore important that fish farmers who still rely solely on fish meal as the only viable protein source in fish feed production, to consider and adopt the use of Moringa seed meal as supplement to complement fish meal in fish diet. This may lead to reasonable increase in their income level and make aquaculture operation worthwhile. Combination of seed and leaf meals in desired proportion might result in obtaining a plant-based protein source that could favorably replace fishmeal in fish feeds. The inclusion of *M. oleifera* as a major protein concentrates (50 to 100% inclusion levels) in the diets of *C. gariepinus* significantly reduced growth rate, feed consumption, feed/gain ratio, protein efficiency ratio and specific growth rate.

5.2 Recommendation

Given the nutritional potential of the seed (*Moringa oleifera*), I will recommend that:

Research effort should be geared towards establishment of *M. oleifera* plantation in Nigeria.

Research efforts should also be geared towards development of strain that will be low in anti-nutritional factors present in the seed.

Careful control of processing conditions is essential to prevent both functional and nutritional damage to the protein that may possibly result from excessive heat treatment.

Research efforts should be geared towards the study of the biochemical aspect of oil extraction process as it relates to M. oleifera seed and its utilization for *Clarias gariepinus* diets.

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