STUDIES ON LIPID NUTRITION AND THE EXPRESSION OF GENES FOR DESATURASE AND ELONGASE ENZYMES IN SNAKEHEAD, *Channa striata* (BLOCH, 1793) FINGERLINGS

MOHAMMED ALIYU PAIKO

UNIVERSITI SAINS MALAYSIA

2011

STUDIES ON LIPID NUTRITION AND THE EXPRESSION OF GENES FOR DESATURASE AND ELONGASE ENZYMES IN SNAKEHEAD, *Channa striata* (BLOCH, 1793) FINGERLINGS

by

MOHAMMED ALIYU PAIKO

Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

July 2011

ACKNOWLEDGEMENTS

All gratitude is to Allah, the ALL knowing, The most High; without Who's permission, nothing is possible. Alhamdu Lillah!

My sincere appreciation goes to Professor Roshada Hashim, under whose guidance and supervision, and who as well offered constructive, academic criticisms, enabling this study to be completed in record time. I am also highly appreciative of the role which Associate Professor Alexander Chong Shu-Chien played in guiding me through the biotechnology studies, in addition to use of his research facilities. Both scholars offered me numerous opportunities, directing me to explore and discover numerous of my potentials during my PhD studentship. May Allah reward them.

Funding for most of my research was provided by the USM-UPEN PERAK Satellite Biotechnology Project, which I wish to acknowledge with gratitude. Funding was also provided by Universiti Sains Malaysia, under the Postgraduate Students Research grant (USM-RU-PRGS Account number 100/PBIOLOGI/841019), also graciously acknowledged. The USM Postgraduate students Fellowship and travel schemes also supported me financially, enabling me to fully concentrate on my research, as well as travel internationally to present my research findings at International conferences.

I salute the courage of my wife; Aishetu Asabe Bello for her understanding, my Children; AbdulAzeez, Fateema-Nana and Umayra for their patience, well as ALL my lab colleagues and friends. Thank you indeed.

Finally, ALL members of staff (academic and non-academic) in USM, particularly those in the School of Biological sciences and IPS, are warmly acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AA	Amino acids
ADC	Apparent digestibility coefficient
ArA	Arachidonic acid
bp	Base pairs
BSA	Bovine serum albumin
C18	18-Carbon
cDNA	Complementary DNA
CoA	Co-enzyme A
CPO	Crude palm oil
CT	Thresh hold cycle
CY	Carcass yield
DHA	Docosahexaenoic acid
DNA	Deoxyribose nucleic acid
DO	Dissolved Oxygen
EFA	Essential fatty acids
EPA	EicosaPentaenioc acid
FA	Fatty acids

FAD Fatty acyl desaturase

FAE Fatty acyl elongase

FAME Fatty acid methyl ester

FAO Food and Agricultural Organization of the United Nations

FBW Final body weight

FCR Feed conversion ratio

FI Feed intake

FID Flame ionization detector

FM Fishmeal

FO Fish oil

GC Gas chromatography

GDP Gross domestic product

GE Gross energy

HSI Hepatosomatic index

HUFA Highly unsaturated fatty acids

IPF Intraperitoneal fat

LA Linoleic acid

LnA Linolenic acid

LO Linseed oil

MUFA Monounsaturated fatty acids

NFE Nitrogen free extract

n-3 Omega 3 series fatty acids

n-6 Omega 6 series fatty acids

PCB Poly chlorinated bio-phenyls

PCR Polymerase chain reaction

PER Protein efficiency ratio

PFAD Palm fatty acid distillate

PI Protein intake

PUFA Polyunsaturated fatty acids

qRT Quantitative Real Time

RNA Ribonucleic acid

SBO Soy bean oil

SD Standard deviation

SFA Saturated fatty acids

SGR Specific growth rate

UV Ultra violet

VO Vegetable oils

VSI Viscerosomatic index

wk week

WG Weight gain

LIST OF SYMBOLS

α Alpha particles

β Beta particles

λ Lambda particles;in wavelengths

μ micro; One millionth of a liter, gram, mole

% Percentage; A fraction of 100

KAJIAN KE ATAS NUTRISI LIPID DAN EKSPRESI GEN UNTUK ENZIM DESATURASE DAN ELONGASE DALAM IKAN HARUAN, *Channa striata* (BLOCH, 1793) JUVENIL

ABSTRAK

Kajian ini mengkaji nutrisi lipid dalam *C. striata* juvenil, melalui siri ujian pemakanan, termasuk penilaian nisbah lipid/protein dan asid lemak (FA), serta saringan beberapa tisu bagi ungkapan gen untuk desaturase acyl lemak (FAD) dan enzim lemak acyl elongase (FAE). Kajian-kajian ini memberikan gambaran tentang metabolisme lipid dalam spesis; menunjukkan bahawa ikan haruan juvenil memerlukan nisbah 65/450 g kg^{-1} lipid/protein, dengan diet yang membekalkan kandungan tenaga kasar 18.5 kJ g^{-1}. Tisu yang dikaji mendedahkan bahawa gen FAD dan FAE lebih banyak diekspresikan di dalam hati dan otak berbanding otot, buah pinggang, kulit, usus, testis dan ovari. Dalam kajian selanjutnya, diet yang diberikan kepada spesies ini digantikan secara berperingkat dengan minyak sawit mentah (CPO) dan sulingan asid lemak sawit (PFAD), kedua-duanya secara relatifnya lebih murah daripada minyak ikan (FO) dan banyak terdapat di Malaysia dan Indonesia. Keputusan mendedahkan bahawa prestasi pertumbuhan dan kecekapan makanan bertambah baik ($P<0.05$) dengan gantian minyak sayuran(VO) yang meningkat dalam diet sehingga 50% daripada minyak ikan (FO), tambahan diet VO selanjutnya mengakibatkan penurunan tidak signifikan. Ekspresi gen sebagai tindak balas kepada pemakanan VO mendedahkan bahawa FAE adalah dikawal selia dalam kumpulan 50CPO dan 50PFAD berbanding diet kawalan, manakala FAD hanya dikawal selia dalam kumpulan 50CPO sewajarnya. Komposisi

badan keseluruhan lipid, FA dan asid amino (AA) telah dibandingkan antara *C. striata* juvenil yang diternak dan yang liar, keputusan menunjukkan tidak banyak perbezaan dalam kandungan asid arakidonik (ArA), glysin, asid aspartik dan glutamik, serta AA esential. Ini memberikan informasi penting mengenai kesesuaian bagi penggunaan ikan liar dan ikan yang dipelihara dalam bidang farmaseutikal. Keputusan yang diperoleh di dalam tesis ini memberikan maklumat berguna tentang bagaimana metabolisme lipid dalam ikan air tawar adalah dipengaruhi oleh pengambilan diet VO. Ia juga menunjukkan tisu di mana aktiviti-aktiviti enzim FAD dan FAE dinyatakan, pada masa yang sama juga mendedahkan bukti menarik menjelaskan peraturan enzim ini apabila diet yang mengandungi VO seperti CPO dan PFAD diberi makan kepada spesies *C. striata.* Ini adalah penting bagi pembangunan berterusan, makanan kos rendah untuk akuakultur komersial spesies air tawar; dan sumber yang perlu bagi pertumbuhan berterusan industri.

STUDIES ON LIPID NUTRITION AND THE EXPRESSION OF GENES FOR DESATURASE AND ELONGASE ENZYMES IN SNAKEHEAD, *Channa striata* (BLOCH, 1793) FINGERLINGS

ABSTRACT

This study examined lipid nutrition in *C. striata* fingerlings, through a series of experimental feeding trials, including; the evaluation of lipid/protein ratio and fatty acid (FA) requirements, as well as the screening of some of its tissues for the expression of genes for fatty acyl desaturase (FAD) and fatty acyl elongase (FAE) enzymes. These preliminary studies provided some insight about lipid metabolism in the species; suggesting that snakehead fingerling requires 65/450 g kg^{-1} lipid/protein ratio, provided in a diet supplying a GE content of 18.5 kJ g^{-1}. The tissues screened revealed that FAD and FAE genes are all expressed more in the liver and brain than in the muscle, kidney, skin, intestine, testis and ovary. In further experimental trials, dietary FO in feeds for the species was incrementally substituted with crude palm oil (CPO) and palm fatty acid distillate (PFAD), both of which are relatively cheaper than FO and abundant in Malaysia and Indonesia. Results revealed that growth performance and feed efficiency improved ($P<0.05$) with increasing vegetable oils (VO) in the diet up till 50% of FO was substituted, above which additional dietary VO resulted in non-significant decline. The expression of genes in response to dietary VO revealed that FAE was up-regulated in the 50CPO and 50PFAD groups compared to the control, whereas FAD was only up-regulated in the 50CPO group accordingly. The whole body composition of lipids, FA and amino acids (AA) was compared between

reared and wild *C. striata* juvenile, the result revealing no much difference in the contents of arachidonic acid (ArA), glycine, aspartic and glutamic acids, as well as all other essential AA respectively. This provided an important highlight regarding the suitability for use of both the wild and the reared fish for their pharmaceutical values accordingly. The results presented in this thesis provide useful information on how lipid metabolism in freshwater fish is affected by the intake of dietary VO. It also shows the tissues in which FAD and FAE enzyme activities are noted, while also revealing compelling evidence explaining the regulation of these enzymes when diets containing VO like CPO and PFAD are fed to *C. striata* species. This is important for the development of sustainable, low-cost feed for commercial aquaculture of warm freshwater species; a resource which is necessary for the continued growth of the industry.

CHAPTER 1

INTRODUCTION

1.1 Background

From an activity which began primarily as an Asian freshwater food production system, aquaculture is now widespread and is practiced on all the continents of the world, within all types of aquatic environments and involving a diverse range of aquatic plant and animal species. Furthermore, aquaculture activity has now developed to become fully automated, involving large-scale commercial and industrial productions of high quality aquatic species which are traded on locally communal, regional, national and across international boundaries, even though as a matter of fact, it began as an activity which was principally family based, non-commercial and a small scaled production system (Subasinghe et al. 2009).

Aquaculture, a term now widely used in the last two centuries to refer to all the types of culture of aquatic animals and plants in the freshwater, brackish water and marine water environments (Pillay and Kutty, 2004), continues to be the fastest growing animal-food production sector in the world, with per capita supply from aquaculture increasing from 0.7 kg in 1970 to 7.8 kg in 2009, representing a mean annual growth rate of about 6.9%. Production volume from aquaculture also grew consistently to about 51.7 million metric tones (valued at US$78.8 billion) in 2006, from less than 1 million tonnes produced in the early 1950s, representing an average annual growth of about 7%. The production volume in 2006 represented 47% of the total world food fish supply, 58% by quantity (or 48% by value) of which were farmed fish from the freshwater environment (FAO 2009).

In the effort towards global elimination of hunger and malnutrition, aquaculture plays a significant role by supplying fish and other aquatic products rich in essential fatty acids, protein, vitamins and minerals. Through improvements in income generation, provision of employment opportunities and contributing to the increase in returns of resource use, aquaculture also makes significant contributions to national development. Food and agricultural organization (FAO) reports show that twelve million full-time employment positions were directly created by aquaculture in Asia in 2004 (FAO 2006) and it also significantly contributed to the national gross domestic product (GDP) in many developing countries of Latin America and Asia (FAO 2006).

By supplying aquatic fish products from local and commercial fish farming and via the supply of food purchased with foreign exchange, aquaculture contributes to the quantity of global food production. Similarly with respect to food availability, aquaculture also contributes to food quality through the provision of nutritious and energetic aquatic food products which are high in protein, essential fatty acids and other vital nutrients (Subasinghe et al. 2009). The health benefits accompanying the consumption of fish products in the prevention of heart-related diseases, for pregnant and lactating women, infants and pre-school children are particularly important. Aquaculture contributes in this respect, to nutritional well being of society. The understanding of the health benefits associated with fish products has helped to substantially encourage its increased consumption, especially in the developed nations, although not as much in the developing countries. Generally through the promotion of fish consumption, the fisheries and aquaculture products contributed about 12% of the total protein consumed by humans in the year 2002 (FAO 2006) and 15.7% of global population's intake of animal protein and 6.1% of all protein consumed in 2007 (FAO 2010).

However, despite all the advantages accompanying the rapid expansion of aquaculture, it is also associated with the heavy reliance on added artificial feeds, because the development of aquaculture has made it mandatory that fish are stocked and grown at densities that cannot be maintained on naturally available food. Globally, over 220 species of finfish and shellfish are cultured. The species cultured are varied including; giant clams which obtain most of their nourishment from symbiotic algae, mussels which filter plankton from water, carps that feed largely by grazing on plants and salmon which rely on preying on smaller fish. Traditionally, the aquaculture of these species involves enclosing them in secure systems such as ponds or floating cages/pens, where they are raised under suitable conditions, protected from predators and competitors and most times fed artificial feeds and medicated with drugs. As the intensity of an aquaculture operation increases, fish are confined at higher densities, supplied with all nutritional requirements and managed more heavily. Marine fish and species such as salmon which migrate between fresh and salt water are reared in floating net cages near the shore, with all their nourishments supplied by formulated feeds. Carp, catfish and other freshwater finfish on the other hand, are mostly grown in ponds, sometimes integrated with other agricultural productions. Farming of crustaceans is dominated by shrimp, grown in coastal ponds. As a result of this diversity, the farming of crustaceans, marine species, as well as freshwater finfish therefore, vary greatly in intensity and in the reliance on formulated artificial feeds

(Naylor et al 2000).

Some of the most important ingredients used in formulating artificial fish feed are fishmeal (FM) and fish oil (FO) both from marine fisheries (Naylor et al 2000), supplying essential amino acids (especially methionine and lysine) deficient in vegetable protein sources as well as essential FA (particularly, EPA and DHA) not found in VO. FM and FO are also good sources of metabolic energy for fish (De Siva and Anderson 1995). The global demand for these raw materials (FM and FO) for aquaculture has increased consistently despite the static global production levels and it is feared that their supply may not be able to meet demand in the near future (FAO 2007). According to Tacon (2007), aquaculture accounted for the consumption of 3.06 million tonnes (or 56%) of global FM and about 0.78 million tonnes (or 87%) of global FO produced in the year 2006. Using estimates of the latest consumption level, Pike and Barlow (2003) earlier predicted that by year 2010 aquaculture will account for 98% of the total global FO consumption. Similarly, Tacon et al. (2006) estimated that aquaculture consumed 87% of the global FO supplies in 2005 and predicted that the global consumption in the near future would hover at about 88% of global supplies; FAO (2010) statistics confirmed that consumption of FO by aquaculture accounted for 85% of global productions. Turchini et al. (2009) on the other hand reported that the global supply of FO in the last 25 years was about 1.5 million tonnes per annum. The demand for FO for direct human consumption is also currently responsible for boosting its market price. Early in the year 2008, the price of FO soared to an all time record of US$1,700 per tonne compared to US$715/tonne a year earlier (FAO 2009), raising further concern regarding the sustainability of the dependence of aquaculture on the finite resource.

Meanwhile, the report on the review of the state of marine fishery resources confirmed that the maximum capture fishery potential from the world's oceans has probably been reached (NRC 1999). It is therefore expected that the increasing demand for fish for direct human consumption would be reliant on the continued growth of the aquaculture industry in the coming years (FAO 2009). Added to this, is the challenge of the continued increase in the prices of FM and FO, which could undermine many aquaculture enterprises (Tacon 1998), as feed accounts for the largest cost in commercial aquaculture (Naylor et al. 2000).

Moreover, the trend of global fish consumption in the last 2 decades has generally tallied with the increase in global food supply, with the global per capita fish consumption and food supply reaching 16.4 kg per annum in the year 2005 (FAO 2009). This trend follows the growth in human population, which has also increased steadily from 6 billion in the year 2000 to 6.7 billion in 2009 and is expected to continue increasing (US census bureau 2010). Aquaculture continues to play an increasing role in satisfying the demand for human consumption of fish and fishery products, as the major increases in the supply of fish consumed in the last few years originated from aquaculture (the mean contribution of aquaculture to per capita fish available for human consumption rose from 14% in 1986 to 47% in 2006) (FAO 2009).

Among cultured freshwater fish, snakehead species is a potential candidate for large scale commercial aquaculture, especially in Asia. However, its seeds supply (fry and fingerlings) are still dependent on collections from natural spawning grounds in the wild (Hossain et al. 2008), whereas currently in south-east Asia, the species is raised almost exclusively feeding on raw fish (or on home-made feed containing marine trash fish) (FAO 2009), a practice which is unsustainable. There is however, the possibility for the large scale, commercial rearing of the fish with compounded feed pellets (Qin and Fast, 1998).

Snakehead fish is a carnivorous air-breather and a valuable food fish in Asia (Wee 1982) which is indigenous to many tropical countries, as well as a valuable source of protein throughout the Asia pacific region (Mohsin and Ambak 1983). It is a predaceous fish that resides in swamps, slow-flowing streams and in crevices near riverbanks in Southern China. In taxonomy, it belongs to the family *Channidae* (Qasim 1966) and its scientific name is *Channa striata* (Bloch, 1793) (see Plate 1.1).

Plate 1.1: Lateral view of *Channa striata* fingerling; showing the eye, skin color and fin features as well as the stream-lined shape of the fish. Source: Aliyu-Paiko's Personal research pictures

The total capture data of this species is on the decline, with FAO (2010) fish species statistics showing that the total global capture production reduced from 68,375 to 46,750 tonnes from 2004 to 2008 (with Thailand and Philippines as the major global producers, accounting for over 85%). In the same report however, the aquaculture of the species is reported to be on the increase globally (increasing from 5,448 to 14,031 tonnes between year 2003 and 2008).

Snakehead (called "*haruan*" in Malaysia) is cultivated in India, Pakistan and Thailand and is probably the main food fish in Thailand, Indo-China and Malaysia because of its firm, white and practically boneless flesh which has an agreeable flavour (Hossain et al. 2008). In addition to being a reliable source of protein, *C. striata* extract is also important for its putative effects on wound healing (Baie and Sheikh, 2000), its use by patients in the post-surgery period to promote wound healing; *haruan* is good for soup (especially the skin, which is sold separately; Davidson, 1975) eaten by patients during the post-surgery period to promote wound healing (Mat Jais et al., 1997), the claim that it is rejuvenating and for reducing pain (Zakaria et al. 2004). Similarly, the anti-inflammatory properties of the extract (Somchit et al. 2004), which were attributed to its unusually high content of arachidonic acid and of all the amino acids (especially glycine, aspartic and glutamic acids) necessary for wound healing (Zuraini et al. 2006), are also well established.

However, FAO (2010) statistics on *C. striata* species indicated that the total landing of the fish from the wild has steadily declined. Whereas Hossain et al. (2008) asserted that the decline in wild *C. striata* in Bangladesh was due to the destruction of their natural breeding habitats due to human activities like road construction and over-fishing, Wee (1981) contended that the decline in wild snakehead population in Thailand was due to overfishing and destruction of their spawning grounds with pollutants from manufacturing industries while Lam and Lai (1998) blamed the disappearance of wild snakeheads in Hong Kong on their dwindling habitats due to pollution and urbanization. The aquaculture of the species globally however, is reported by FAO (2010) to be on the increase (as is the case with most other cultured fish).

1.2 Statement of the problem

The continued growth in aquaculture production would ultimately depend on the improvement in feed efficiency by fish, in terms of utilizing cheap, sustainable ingredients (especially through the reduction in the use of FM and FO in fish feeds) (Naylor et al. 2000) and increasing the number, as well as improving the efficiency of rearing those fish species which are suitable for commercial production (FAO 2009). The continued growth in the number of fish species groups for aquaculture has however, recently been lower than during the last 2 decades, but is still substantial, with new potential species being continuously introduced for aquaculture (FAO 2006). On a very large scale, research effort in aquaculture is currently focused on the search for suitable, sustainable alternatives to replace FM and FO in diets for fish (Tacon 2004; Webster et al. 1999; Al-Owafeir and Belal 1996; Varghese and Oommen 2000; Lim et al. 2001; Ng et al. 2001, 2003, 2004; 2007; Bell et al. 2002), with vegetable sources showing greater potentials.

1.3 Objectives of the research

The principal objective of this research was to study lipid nutrition in *C. striata* (Bloch, 1793) fingerlings (ranging in size between 3-5 g), with a view to understand its nutrient requirements, to enable the development of a low-cost feed. The objective was however, broken into measurable ones as follows; Measurable objectives;

1. To determine the optimum lipid/protein ratio and fatty acid (FA) utilization for higher survival, good growth and higher feed efficiency in *C. striata* (Bloch, 1793) fingerlings.

2. To evaluate the replacement of FO in the diet for *C. striata* (Bloch, 1793) fingerlings with relatively cheaper, sustainable alternatives of vegetable origin, such as crude palm oil (CPO) and palm fatty acid distillate (PFAD)

3. To measure the expression of the activities of desaturase and elongase enzymes in different tissues of *C. striata* (Bloch, 1793) fingerlings and evaluate the effects of substituting dietary FO with CPO and PFAD on the expression of these enzymes, using PCR techniques.

4. To evaluate the differences in the body lipid content and fatty acids composition between reared and wild *C. striata* juveniles.

The proper understanding of the lipid nutrition in this species as outlined in the above mentioned objectives would ultimately assist in the development of a low-cost feed, which is necessary for the large-scale commercial aquaculture of *C. striata*.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In this chapter, a comprehensive review of the relevant literature of the research area is provided, to justify the need for the studies to be carried out. Chapter 2 begins with an overview of the major nutrients (proteins, lipids and carbohydrates) in fish feed, their sources in the natural environment of fish and the role which each nutrient plays in fish metabolism. Subsequently, the role which artificial feed plays in global aquaculture, the annual production and consumption are also reviewed in greater details. The chapter continues with an evaluation of the importance of fishmeal (FM) and fish oil (FO) as the most important feed ingredients in aquaculture and the reasons for their continued use. Finally, the chapter concludes with a complete review of alternative sources of dietary lipids to replace FO in aquaculture, the need for them and the advantage and disadvantage of each.

2.2 Major feed nutrients and their roles in fish metabolism

The major nutrients in fish feed are proteins (together with the constituent amino acids), lipids (consisting of the different classes and types of FA), carbohydrates (simple and complex sugars), vitamins (water and fat soluble) and minerals. Each of these nutrients serves different metabolic purposes and is required in different proportions by fish based on species, seasons, health/nutritional status, culture conditions and other vital considerations. A review of the three major groups is as follows;

2.2.1 Proteins and amino acids

Proteins are complex, organic compounds (polymers), composed of many amino acids (monomers) linked together through peptide bonds and cross-linked between chains by sulfhydryl and hydrogen bonds, as well as Van-der-Waals forces. According to Pillay and Kutty (2005), animals generally rely on dietary protein as their main source of nitrogen and essential amino acids (AA); with reports by several authors indicating that fish generally demonstrate a requirement for a higher level of dietary protein than terrestrial farmed vertebrates (Bowen 1987; Cowey 1994, 1995). The reason for this high protein requirement by fish is quite often attributed to fish having high apparent protein needs, since their basal energy requirements are lower than those of terrestrial animals due to their aquatic mode of life (Kaushik and Seiliez 2010).

During the initial feeding of fry, their requirements for protein are highest and decrease as the fish increases in size. Young fish require about half of their diet as proteins for maximum growth (Bowen 1987), which is much higher than the requirements of terrestrial animals. This is because most of the wet weight gain in lean fish is in the form of muscle tissue, unlike in terrestrial animals where the deposition is considerably of both fat and protein (Robinson and Li 1999).

Several factors influence fish requirements for protein, among which are; water temperature, body size, stocking density, dissolved oxygen levels and the presence of toxins. The decline in water temperature also leads to the decline in fish body temperature and consequently, the reduction in metabolic rates (Pillay and Kutty 2005).

Dietary protein is a major factor affecting growth performance in fish and also one of the most important sources of energy, because it plays a vital role in growth and tissues development of fish species (Kim and Lee 2004). It also directly affects fish weight gain (Sheng and He 1994), because it is one of the major constituents of cells and tissues. High protein diets have also been suggested to promote good growth rates and feed utilization, without causing excessive accumulation of lipid in the liver (Jobling et al. 1991; Dos Santos et al. 1993).

Dietary protein has a tremendous effect on the cost of feed (Miller et al. 2005) as its cost is far higher than lipids and carbohydrates (Lovell 1989; McGoogan and Gatling III 1999). In feed formulation, optimizing protein and energy levels in the diet not only promote growth and minimize nitrogenous output, but also reduces the cost of feed. When excess protein is present in the diet, some of it would be utilized for energy production, which is undesirable because it raises the cost of protein relative to energy and also results in increased nitrogen excretion (Ruohonen et al. 1999; Jahan et al. 2002). Therefore, the amount of protein included in the diet of fish is a vital consideration, to promote feed efficiency and growth performance.

2.2.2 Lipids and fatty acids

By definition, lipid refers to a large and heterogeneous group of substances classified together based on their high solubility in non-polar solvents or as they relate to such compounds. Most of the lipids found in eukaryotes could be categorized into three major classes (derived from acetyl-CoA) as follows; 1) straight chain fatty acids

(FA); 2) branched, 3) cyclic, and other specialized FA and polyprenoid compounds, including carotenoids and sterols and their derivatives (Leaver et al. 2008). More simply, lipids generally refer to a group of fat-soluble compounds found in the tissues of plants and animals and which could be broadly classified as fats, phospholipids, sphingomyelins, waxes and sterols (Pillay and Kutty 2005). According to Webster and Lim (2002), these organic compounds liberate approximately 9.4 kcal of gross energy (GE) g^{-1}, producing the highest amount of energy in terms of kcal g^{-1} compared to carbohydrates (4.1 kcal of GE g^{-1} and proteins (5.6 kcal of GE g^{-1}). This makes dietary lipids in most species, as the major dietary non-protein energy sources to be well utilized both at the digestive level and at the post-absorptive level (Sargent et al. 2002).

Lipids and their constituent FA are, together with proteins, the major organic constituents of fish. Lipids also play major roles as sources of metabolic energy for growth, including reproduction and movement, including migration (Tocher 2003). Lipid is an energy-dense nutrient and is readily metabolized by fish (NRC 1993). Dietary lipid is relatively well digested and exerts a greater sparing effect on protein than dietary carbohydrate, thus playing a definite role during metabolism in fish, for better feed utilization; especially in fry and fingerling which require high energy intake for rapid growth (Ellis and Reigh 1991; Raj et al. 2007). Dietary lipids thus provide essential FA for normal growth and development of cells and tissues (Sargent et al. 1989), as improvement in growth and feed utilization by fish was reported to be due to the protein-sparing effect of dietary lipids (DeSilva et al. 1991; Chaiyapechara et al. 2003). Webster and Lim (2002) summarized lipids as essential nutrients in fish diets for the roles they play in four major body functions, which are; the provision of metabolic energy, the provision of essential FA, their roles as structural components, as well as their regulatory functions. Henderson and Sargent (1985) reported the preferential use of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) for energy production in the mitochondrial systems of fish, whereas polyunsaturated fatty acids (PUFA) are known to be essential for the growth of healthy fish (Turchini et al. 2009), since they are important components of all cell membranes (Tocher et al. 2003). The other benefit of adding lipids to fish diets is that; it increases feed palatability and assists in reducing dust. It also improves pellet stability during feed manufacture, transportation and storage (Steffens 1989). Higher lipid concentration in feed pellets also contributes to its stability in water (Chaiyapechara et al. 2003).

Dietary lipids consist of 2 series of essential fatty acids (EFA), namely; the n-6 series, derived from linoleic acid (LA) and the n-3 series from linolenic acid (LnA). Since both series cannot be synthesized by fish *de novo*, they must therefore, be supplied in the diet (Steffens 1989; Trautwein 2001). The requirements for these EFA in fish vary among species due to their feeding habitats; its been established that marine and cold water fish require greater amount of the n-3 series than the n-6, whereas the warm freshwater fish require more of the n-6 series of FA than the n-3 or have demonstrated requirement for both (Kanazawa 1985). Consequently, many studies on EFA requirements have shown that this requirement can differ from species to species, with *Channa striata* demonstrating a requirement for both n-3 and n-6 FA for maximum growth (Kanazawa 1985).

As a result of the above therefore, in freshwater fish, EFA requirements are met by supplying 18:3n-3 and/or 18:2n-6 in the diet, although better growth performance could be achieved by supplying the n-3 highly unsaturated fatty acids (HUFA), namely, 20:5n-3 or eicosapentaenoic acid (EPA) and 22:6n-3 or docosahexaenoic acid (DHA) (Kanazawa 1985). On the other hand for marine fish, EPA and especially, DHA are regarded as EFA due to their requirement for good growth and the ability of most marine species studied so far to barely be able to convert 18:3n-3 to EPA and ultimately, to DHA (Sargent et al. 1995, 2002). This metabolic insufficiency was identified to be due to the relative deficiency in one of the two enzymes in the conversion pathway from 18:3n-3 to EPA; which is the C18 to C20 elongase multienzyme complex (Ghioni et al. 1999) or the Δ5-fatty acid desaturase (Tocher and Ghioni, 1999). However, the deficiency in one or both of these enzymes means that, in addition to blocking the conversion of 18:3n-3 to EPA, there could also be a similar inability to convert 18:2n-6 to 20:4n-6 or arachidonic acid (ArA) (Bell and Sargent 2003).

In aquaculture, high dietary lipid level have been used to augment the fish supply of energy because it has been implicated to interact with dietary protein to effect growth performance (Miller et al. 2005). The supplementation of dietary lipid as the non-protein energy source has been reported to be generally more effective than carbohydrates in many species and is currently the trend in fish feed production (Schuchardt et al. 2008). There are also reports of improvement in feed conversion ratio (FCR) values and higher nitrogen and phosphorous retention in fish when diets of higher lipid levels are fed (Hillestad et al. 1998; Hemre and Sadnes 1999). Interestingly, the reported apparent digestibility coefficient (ADC) for lipids in the literature appear to be mostly greater than 80% regardless of fish species being cultured, the level of dietary lipid or due to other environmental considerations (Hua and Bureau 2009). Furthermore, it is well established by many authors that the FA profile of fish tissues is directly related to the dietary composition (Watanabe 1982; Caballero et al. 2002). According to Robin et al. (2003), incorporation of FA into tissues is also modulated by various metabolic factors, such that the final tissues composition depends upon the initial FA content, cumulative intake of dietary FA, the growth rate and duration. The changes in FA profile of fish tissues following changes in dietary FA composition also vary among fillet lipid classes and between tissues (Trushenski et al. 2008a, b).

An understanding of the lipid requirements of each species is therefore important, to ensure that the FA in the diet is included in the right proportions during the formulation of efficient feeds, to maximize growth performance and minimize excess tissues energy deposition.

2.2.3 Carbohydrates

Carbohydrates are also complex organic compounds (polymers), composed of carbon, hydrogen and oxygen. Carbohydrates are the most abundant and relatively available sources of metabolic energy in animals, and a major class of nutrients besides proteins and lipids. Plants are a major source because they store their energy in the form of carbohydrates; in contrast to animals, which store excess energy as lipids. Carbohydrates are the cheapest sources of energy and as such its inclusion in fish diets could lead to a reduction in feed costs. Unfortunately, most fish (especially carnivorous species) have limited natural access to carbohydrates and are better adapted to utilize proteins and lipids than carbohydrates at both digestive and metabolic levels (Wilson 1994). Carbohydrates could be broadly categorized into three main groups; monosaccharides, oligosaccharides and polysaccharides (Webster and Lim 2002).

In some fish species, the supply of carbohydrates as dietary non-protein energy greatly reduces the catabolism of protein and lipids for energy. Additionally, the synthesis of various biologically important compounds such as ribonucleic acids (RNA) and deoxyribonucleic acids (DNA), is usually derived from carbohydrates (NRC 1993). Furthermore, in addition to being used as a source of energy, carbohydrates play the physical role of acting as binders in the formulation of pellets and also used for the texturing of manufactured feeds (Pillay and Kutty 2005). As earlier mentioned, dietary carbohydrates in some fish exerts a sparing effect on dietary protein, but generally, carbohydrates have relatively lower digestibility in fish (Steffens 1989).

The utilization of carbohydrates by fish depends on several factors; the feeding habit of the species, the natural habitat and water temperature. The last 2 mentioned factors may be the reason why some warm-water fish have the ability to utilize more carbohydrates than cold-water and marine fish (NRC 1993). Some fish species appear to show good growth results than others with increase in the level of dietary carbohydrate inclusion. Degani and Viola (1987) demonstrated that the specific growth rate of European eel increased with more carbohydrate in the diet, while Jantrarotai et al. (1994) showed that hybrid catfish *C. macrocephalus* and *C. gariepinus* are capable of utilizing carbohydrates and could tolerate up to 50 % carbohydrate in their diets. This was higher than values reported for channel catfish (28 %) (Anderson et al. 1984) and *Tilapia zilii* (40 %) (El Sayed and Garling 1988). However, the protein-sparing effects of carbohydrates remain highly controversial (Wilson 1994; Hemre et al. 2002; Stone 2003), such that largely, the recommended level of inclusion of digestible carbohydrates in fish diets is species-dependent. Moreover, even when digestible carbohydrates were made available in the diets, the metabolic utilization of absorbed glucose was limited in most fish (Moon 2001; Panserat et al. 2002), such that the net energy supply was reduced (Bureau 1997; Hemre et al. 2002). This observation however, differs between species (Furuichi and Yone 1982; Panserat et al. 2000; Shiau and Lin 2001).

In *C. striata* fingerlings, inclusion of dietary carbohydrates was reported to play a positive role in influencing growth performance (Arockiaraj et al. 1999). This may be related to the observation of Chakrabarti et al. (1995), demonstrating similar activity among α-amylase and lipase enzymes in the intestines and other sections of the gut and liver of the species.

Generally, carbohydrate utilization in fish has been reported to be relatively higher in herbivorous and omnivorous warm, freshwater fish species than in carnivorous species in cold marine, brackish and freshwater environments (Wilson 1994). Aside from the level of dietary inclusion, how efficiently carbohydrates in the diet are utilized by fish has also been associated with such factors as botanical origin, complexity of the molecule and technological treatments applied (Wilson 1994; Stone 2003; Krogdahl et al. 2005).

2.3 The role of artificial feed in aquaculture

As earlier stated, the rapid expansion of Aquaculture is accompanied with the heavy reliance on added feeds, since fish are stocked and grown at densities that cannot be supported by natural food; and the most dominant ingredients for making fish feed are FM and FO, both from marine fisheries (Naylor et al 2000). According to FAO (2007), 44.8 % (or 28.2 million tons) of the world aquaculture production in the year 2005 (including aquatic plants), estimated at 62.96 million tons depended on the direct application of feeds, either as single ingredient, home-made feed or as industrially-manufactured compound aqua-feeds (FAO 2009).

The major consumers by quantity of feed are herbivorous and omnivorous fish species; an estimated 23.13 million tons of compound aquafeed was produced in 2005, with about 42 % of this consumed by carps. However, in Southeast Asia, farmers are reported to still raise some freshwater fish (for example snakeheads and marble goby) and marine fish (grouper and Asian seabass) almost exclusively on raw fish (FAO 2009). These data show that artificial feed application in aquaculture is essential for the continued growth and development of the industry.

2.4 Fishmeal and fish oil and their use in aquaculture

FM refers to the brown flour obtained after cooking, pressing, drying and milling (collectively termed 'reducing or reduction') of fresh, raw fish and fish trimmings. FM is made from about any type of seafood, but is generally manufactured from wild-caught, small, bony/oily marine fish which are usually deemed unsuitable for direct human consumption (FIN 2006). FM as an industry began in the 19th century when surplus catches of herring were processed for oil, and was used in tanning, soap production and for other industrial purposes (FAO 1986).

Historically, the majority of the global FM produced was used to feed domestic livestock such as chickens and pigs and to a limited extent, in the production of pharmaceutics and fertilizers. However, with the rapid increase in aquaculture production since the 1970s, an increasing quantity of FM is now diverted for use in aquafeeds (Pike and Barlow 2003). The use of FO in feeds for aquaculture has also increased to become a key source of metabolic energy and EFA (Tacon 2004; Anon. 2002).

Many reasons are responsible for the preferable use of FM and FO in the diet for farmed animals (including fish). These include (but are not limited to):

1. FM and FO are feed ingredients from natural sources, very rich in protein and containing all essential AA, minerals and the essential marine oils (made up predominantly of n-3 FA). Total protein in FM could be as high as 70 % or higher.

2. They are highly digestible by farm animals, leading to increased growth and reduced wastage of feed.

3. These ingredients have been reported to be of major benefits to animal health, including improved immunity against diseases, higher survival and growth rates and reduction in deformities of farmed animals.

4. FM and FO also increase feed appeal, thus encouraging farmed fish to locate

feed thereby increasing consumption and reducing wastage (FAO 1986).

According to the report of FAO (2009), global FM production in recent decades has stabilized at about 6 million metric tonnes (product grade); on the other hand, Turchini (2009) contended that the annual global production of FO has not exceeded 1.5 million metric tonnes in the last 25 years, generally hovering at about 1 million tonnes per annum in the last 5 years. Using estimates, Tacon (2006) showed that Aquaculture currently consumes approximately 87 % of global FO production while New (2002) showed that aquaculture has the theoretical potential to utilize the total global FO production by the year 2010, assuming the current supply level and the growth in aquaculture continue steadily. Therefore, the major challenge facing the aquafeed industry is to find sustainable alternative feed resources to FM and FO, the current supply level not being able to sustain the growth of aquaculture forecasted in the near future.

2.5 Alternatives to fish oil in aquaculture

In the search for sustainable alternatives to replace FO which is deemed to be urgently required in aquaculture, Caballero et al. (2002), Turchini et al. (2003), Ng et al. (2003) and Bell and Waagbø (2008) all unanimously agree that oils from vegetable sources are the prime candidates. Sargent et al. (2002) demonstrated however, that vegetable oils (VO) are devoid in the n-3 HUFA abundant in FO, even though VO are also abundant sources of short chain PUFA like LA and LnA. In the diet of fish, similar to that of all vertebrates, PUFA are essential, although their requirements vary with species, age, physiological state of fish and other important factors (Tocher 2003). These FA (n-3

PUFA) are known to be essential for the growth of healthy fish (Turchini et al. 2009), as they constitute important components of all cell membranes (Tocher 2003). However, the current popular strategy adopted in the aquafeed industry is to partially or fully substitute FO with suitable VO, depending on the species and size of fish targeted or to use finishing diets containing marine FO (containing abundant level of n-3 FA), after rearing fish for long periods feeding on VO diets (rich in n-6 FA) (Ng 2002; Bell et al. 2001). Moreover, substitution of marine FO with VO has also been reported as an effective strategy to reduce (rinsing) the levels of dioxins and dioxin-like PCBs and organo-chlorinated pesticides in fish feeds (Bell et al. 2005; Berntssen et al. 2005, 2007). This is because marine FO has been implicated as one of the potential sources of these toxic substances, which are dangerous to consumers (WHO1999; Jacobs et al. 2002; Karl et al. 2003). Most of the potential sources of these hazardous and toxic substances have been or are in the process of being banned in most developed countries (EurActiv 2001; Turchini et al. 2009).

Many different VO are currently in use in aquaculture to partially or fully replace FO; the principal ones are discussed in subsequent sections.

2.5.1 Vegetable oils

The following VO are popularly used in aquaculture, to partially or fully substitute FO in the diets for different species;

Among the VO, crude palm oil (CPO) and soya bean oil (SBO) are the most abundantly produced oils in the world. However, unlike the production of FO which has remained static for over 30 years, the production of CPO and SBO has increased rapidly (Turchini et al. 2009). CPO (over 80 % of which is produced in Malaysia and Indonesia) is an abundant source of SFA (especially 16:0) and MUFA (principally 18:1n-9) (Ng et al. 2007), all of which are good sources of metabolic energy for fish (Bell et al. 2002). CPO has been used to successfully substitute a significant portion of dietary FO without any negative effects on growth performance, feed efficiency and body indices in many farmed species including; climbing perch (Varghese and Oommen 2000), rainbow trout (Fonseca-Madrigal et al. 2005) and warm water fish such as catfish (Legendre et al. 1995; Ng et al. 2003; Bahurmiz and Ng 2007; Viegas and Contreras 1994; Al Owafeir and Belal 1996).

SBO, currently the second most abundantly produced VO in the world (Basiron 2007; Turchini et al. 2009) is rich in n-6 FA (especially 18:2n-6) which are crucial for freshwater fish (Kanazawa 1985; Chou and Shiau 1996). The use of SBO in diets for several salmonid species has generally resulted in the fish showing comparable growth performance to those fed a FO-based diet (Caballero et al. 2002; Ruyter et al. 2006).

Other important VO used in Aquaculture include LO, currently the richest and readily available vegetable source of n-3 FA, made up of 55 % by weight of LnA (18:3n-3) (Bell et al. 2004). As the precursor for n-3 HUFA biosynthesis, LnA is also one of the favoured substrates for β-oxidation in fish and mammals (Bell et al. 2004). The beneficial effect of LnA in cardiovascular diseases and in some forms of cancer in humans has also been documented (Sinclair et al. 2002).

Other commonly used VO includes Canola/rapeseed oil, cottonseed oil, sunflower oil and corn oil. However, the use of each individual VO in Aquaculture is limited to a number of factors; the most prominent being their FA compositions, availability in desired quantities, presence of anti-nutrients, market price etc (Turchini et al. 2009). Therefore, to achieve the best results in the substitution of dietary FO in aquaculture, Bell (2006) and Turchini et al. (2009) opined that the VO most suited to substitute FO for any given species should contain high amount of MUFA, contain similar level of SFA to that in the fish being fed (as energy sources) and low in C18 PUFA, especially 18:2n-6 because it is poorly oxidised and difficult to remove using finishing diets.

2.5.1(a) Palm oil: Production, unique characteristics and potentials for use in aquaculture

Palm oil (PO or crude palm oil, CPO) refers to the oil which is extracted from the fruits of Oil Palm tree (*Elaeis guineensis*), a resource which originated from west and central Africa, where in the countries from which it originated, the oil was widely used in a variety of ways for quite a long time. The palm tree was introduced to Malaysia by the British during their occupation of Malaysia in 1910, but from the 1960s, a major oil palm plantation scheme was introduced by the Malaysian government with the main aim of poverty eradication. Indonesia and Malaysia are currently the world's largest producers of CPO (USDA 2010), supplying about 90% of the globally produced CPO in the year 2009.

Oil is extracted from the fruit of the palm, made up of two parts; the interior part (endocarp) or the kernel, and the surrounding exterior, a fleshy mesocarp (Plate 2.1). The mesocarp is made up of 49% crude palm oil (CPO), while the kernel contains about 50% crude palm kernel oil (Cyberlipid Center 2005). In other words from the