EFFECT OF DIFFERENT DIETARY LIPID SOURCES ON GROWTH, TISSUE FATTY ACID COMPOSITION AND MUSCLES QUALITY OF AFRICAN CATFISH
(Clarias gariepinus)

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by

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Ahmed
Penang, Malaysia

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LIST OF ABBREVIATION

CLO	Crude liver oil
CNO	Coconut oil
CO	Canola oil
CPO	Crude palm oil
CY	Carcass yield
DHA	Docosahexaenoic acid
EAA	Essential amino acids
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
FAO	Food and Agriculture Organization
FBW	Final body weight
FCR	Feed conversion ratio
FO	Fish oil
FY	Fillet yield
HIS	Hepatosomatic index
HUFA	High unsaturated fatty acid
IPF	Interaperitonea fat
LO	Linseed oil
MAD	Malondialdehyde
MCFA	Medium chain fatty acid
MCT	Medium chain triglyceride
MPOB	Malysian Palm Oil Board
MPOPC	Malaysian Palm Oil Promotion Council
MUFA	Monounsaturated fatty acids
NAFED	National Agency for Export Development
NPN	Non protein efficiency ratio
PER	Protein efficiency ratio
PFAD	Palm fatty acid distillates
PO	Poultry oil
PUFA	Poly unsaturated fatty acid
RBDPO	Refined, bleached and deodorized palm olein

RO	Rapeseed oil
SFA	Saturated fatty acids
SFO	Sunflower oil
SGR	Specific growth ratio
SO	Soybean oil
TBARS	Thiobabituric acid reactive substances
USFA	Unsaturated fatty acids
VSI	Viscerosomatic index
WG	Weight gain

KESAN SUMBER YANG BERLAINA KE ATAS PERTUMBUHAN, KANDUNGAN ASID LEMAK DALAM TISU KUALITI OTOT IKAN KELI AFRIKA *(Clarias gariepinus)*

ABSTRAK

Kajian ini dijalankan untuk menguji kesan penggantian sepenuhnya dan penggantian separa minyak ikan (FO) dengan minyak kelapa sawit mentah (CPO) dan minyak kelapa (CNO) ke atas prestasi pertumbuhan, penggunaan pemakanan, kandungan proksimat dan kandungan asid lemak di dalam otot dan hati ikan keli Africa (*Clarias gariepinus*). Ini diikuti dengan kajian kesan penyimpanan beku ke atas kualiti otot ikan setelah diberi pemakanan uji selama 12 minggu. Lima diet praktikal berisonitrogen (35% protein kasar) dan isotenaga (19.73 kJ g^{-1}) dirumuskan untuk mengandungi 8% minyak ikan (FO, Diet 1), minyak sawit mentah (CPO, Diet 2), minyak kelapa (CNO, Diet 3), 1:1 minyak ikan ke minyak sawit mentah (FO/CPO, Diet 4) dan 1:1 minyak ikan ke minyak kelapa (FO/CNO, Diet 5). Setiap diet diberi kepada dua replikat kumpulan ikan yang mengandungi 90 ekor ikan, pada berat awal 6±0.05 gm yang ditempatkan dalam tangki simen sehingga ikan membesar sehingga saiz pasaran sebanyak ≥ 180 gm. Ikan yang diberikan Diet 2 yang mengandunig 100% CPO memberi kadar pertumbuhan yang paling baik berbanding dengan kumpulan ikan yang dibesarkan dengan diet lain manakala ikan yang diberi makan FO/CNO mempunyai nisbah kecekapan protein yang tertinggi. Nisbah penggunaan makanan (FCR), kadar kemandirian, indeks biologi, jumlah bersih dan pengeluaran tidak menunjukkan perbezaan yang signifikan di antara kumpulan ikan-ikan dalam kajian ini. Penggunaan minyak tumbuhan menunjukkan kesan yang ketara pada minggu ke 4, 8 dan 12 kajian. Isi ikan yang dibesarkan dengan diet CPO dan CNO mempunyai kandungan protein yang paling tinggi manakala isi ikan yang dibesarkan

dengan FO pula mempunyai kandungan lemak yang paling tinggi. Secara amnya, kandungan asid lemak yang terdapat di dalam isi ikan-ikan yang dikaji mencerminkan kandungan asid lemak yang terdapat di dalam diet uji. Di dalam otot segar, hanya titisan cair selepas pembekuan adalah paling rendah secara signifikan ($P<0.05$) bagi ikan yang diberi pemakanan FO manakala ikan yang memakan pemakanan FO/CPO mempunyai nilai yang paling tinggi. Ikan yang diberi Diet CNO (Diet 5) mempunyai isi yang paling lembut. Penggunaan minyak tumbuhan mengurangkan kadar pengoksidaan lemak secara signifikan. Hanya bau dan tahap kekunyahan dipengaruhi oleh dengan penggantian ini dan kesemua nilai warna berubah secara signifikan ($P<0.05$) kecuali nilai kekuningan. Penyimpanan beku selama 3 bulan dan 6 bulan meningkatkan nilai titisan cair selepas pembekuan. Peningkatan masa penyimpanan beku menghapuskan perbezaan dalam nilai kekerasan dan pH. Pengoksidaan lemak meningkat dengan tempoh penyimpanan tetapi ikan yang diberi pemakanan CPO dan CNO mempunyai nilai terendah secara signifikan. Penilaian sensory menunjukkan kesan terhad penggantian minyak ikan ke atas bau, warna dan berjus. Kandungan proksimat dipengaruhi secara signifikan dengan tempoh penyimpanan. Sepanjang kajian penyimpanan beku, kandungan asid lemak di dalam otot hanya berubah dengan sedikit. Kajian ini menunjukkan bahawa CPO dan juga CNO boleh digunakan sebagai lemak alternative di dalam diet ikan keli Afrika untuk meningkatkan kadar pertumbuhan dan penggunaan pemakanan. Secara am, sumber-sumber lemak ini tidak mempunyai kesan yang negative ke atas kualiti otot kerana kedua-dua lipid ini mengurangkan pengoksidaan otot secara signifikan.

EFFECT OF DIFFERENT DIETARY LIPID SOURCES ON GROWTH, TISSUE FATTY ACID COMPOSITION AND MUSCLES QUALITY OF AFRICAN CATFISH *(Clarias gariepinus)*

ABSTRACT

This study was carried out to investigate the effects of total and partial replacement of fish oil (FO) with crude palm oil (CPO) and coconut oil (CNO) on growth performance, feed utilization, proximate composition and fatty acid composition of muscles and liver of African catfish (*Clarias gariepinus)*. This was followed by an investigation on the effect of frozen storage on the muscle quality of fish raised on the respective test diets after 12 weeks. Five isonitrogenous (35% crude protein) and isoenergetic (19.73 kJ g^{-1}) practical diets were formulated to contain 8% of fish oil (FO, Diet 1), crude palm oil (CPO, Diet 2), coconut oil (CNO, Diet 3), 1:1 fish oil to crude palm oil (FO/CPO, Diet 4) and 1:1 fish oil to coconut oil (FO/CNO, Diet 5). Each diet was given to duplicate groups of 90 fish with an initial weight of 6 ±0.05 gm in cement tanks until they reached the marketable size of ≥ 180 gm. Fish fed Diet 2 containing 100% CPO gave the best growth performance compared to the other treatments while those fed FO/CNO had the highest protein efficiency ratio (PER). There were no significant differences ($P>0.05$) for feed conversion ratio (FCR), survival rate, biological indices and yield production among all the fish fed the experimental diets. The plant based oil diets affected the muscle composition significantly at Weeks 4, 8 and 12 of the feeding trial, respectively. The muscles of fish fed CPO and CNO had the highest protein content while, the highest deposition of lipid was in fish fed FO diet. In general, the depositions of fatty acids in the muscles reflected the fatty acid composition of the experimental diets. In the fresh muscle, only post thaw drip was significantly lowest ($P<0.05$) for fish fed with

FO while those on the FO/CPO diet had the highest amount of leaky liquid and those on the CNO Diet (Diet 5) had the softest muscles. Inclusion of dietary plant based oils reduced the lipid oxidation significantly. Only odour and chewiness attributes were affected by this replacement and all the colour values showed significant differences ($P<0.05$) except for the yellowness value. Frozen storage for 3 and 6 months showed, a parallel increase in post thaw drip with storage time. Increasing storage time, eliminated the differences in hardness and pH values. Lipid oxidation increased with storage time but, fish fed CPO and CNO had the lowest significant value. The sensory evaluation showed limited effect of this replacement, in odour, colour and juiciness attributes. The proximate composition was significantly affected by increase the storage time. Throughout storage time, only limited changes in fatty acids composition of the muscles were detected. This study demonstrated that, CPO and to a lesser extent CNO can be used as an alternative lipid source in African catfish diets to improve growth and feed utilization. In general both these lipid sources did not have a negative effect on muscle quality, since both of them decreased the muscles oxidation significantly.

CHAPTER 1

INTRODUCTION

1.1 Background

Aquaculture has grown significantly during the past half-century. This development was quite rapid compared to all other animal food-producing sectors. The aquaculture has grown at an average rate of 8.8 percent per year since 1970, compared with only 1.2 percent for fisheries capture and 2.8 percent for terrestrial farmed meat production systems over the same time (FAO, 2007). In addition to that, there is a notable increase in the numbers of aquaculture species from 54 to 246 by 2003 (Tacon et al., 2006). Most of world production of aquaculture in 2004 came from fresh water with 56.5% , which exceeded the production of mariculture (36.0%) and brackish water (7.4%) production together (FAO, 2007). In many countries, African catfish *Clarias gariepinus* is considered as one of the important fresh water species in aquaculture sector.

African catfish *Clarias gariepinus* has many good attributes that made it an appropriate fish for aquaculture, and since the 1970's, it has been considered to hold great promise for fish farming in Africa (Graaf and Janssen, 1996). This species, having a high growth rate, consume a varied range of feedstuffs, surviving in poorly oxygenated waters, being very resistant to handling and stress (Graaf and Janssen, 1996; Pillay and Kutty, 2005). In addition to that, *Clarias gariepinus* can inhabit all kinds of water bodies (ACE, 1995), this feature makes it widely distributed in aquaculture in Africa from the Nile to West Africa and from Algeria to Southern Africa, and also existing in West-Asia (Israel, Syria and South of Turkey) (Graaf and

Janssen, 1996). A good growth of this specie can be achieved by feeding a diet which meets all its nutritional requirements.

The numerous studies of dietary lipid in fish nutrition do not mean that it is more important than the other nutrients (protein, carbohydrate, vitamins and minerals). The understanding of some reactions are still in the early stages like; anabolic and catabolic pathway for some PUFA, enzymology, and PUFA molecular biology and genetics (Sargent et al., 2008). In general, lipids can play four main functions in fish nutrition; provide energy, supply fish with essential fatty acids, serve as structural compounds in cell membrane and has regulatory functions (Webster and Lim, 2002). Fish oil (FO), has been use as dietary lipid in aqua feed for a long period of long time. This oil is extracted from small marine pelagic fish e.g., capelin, herring, sand eel, mackerel, anchovy and sardine (Sargent et al., 2008).

Nowadays, the demand of fish oil in aquaculture is increasing, and has grown rapidly for the last two decades (8.8 percent per year since 1970) (FAO, 2007), and on the other hand, there is little improvement in the fishing production (only 1.2 percent for fisheries capture) (FAO, 2007). This makes fish oil supply uncertain and there is variation in the price of fish oil. Hence, the researchers are looking for new sources of lipid. The big production of vegetable oils gives them a preference to substitute fish oil in fish diet.

The results of most of these studies have been encouraging. Rosenlund et al. (2001) tested lipids sources (rapeseed, soybean, linseed, palm and poultry oil) and replacing fish oil with those lipids without any negative effect on the growth. Caballero et al. (2002) emphasized that, growth was not affected when fish oil was replaced with either (SO, CPO and RO) in rainbow trout fed for 64 days. In some other studies, some vegetable oils showed better growth performance compared with the fish fed with fish oil (Ng et al., 2003 and Bahurmiz &Ng, 2007). But generally, using vegetable oils as dietary lipid in fish nutrition reduces the amount of HUFA like EPA and DHA (Francis et al., 2006). The high production of crude palm oil, low price and a good content of anti-oxidation substance will make it a good component to replace fish oil in aquaculture.

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CPO has been identified as an important dietary lipid in aqua feed since the mid-1990s (Ng, 2002). This oil has high production (43.11 million tons) (MPOB,

2008), it has a balance between the saturated and unsaturated fatty acids. On the other hand, the high content of carotenoids and vitamin E especially, tocotrinol and tocopherol (Sundram et al., 2003) makes it the richest known source of these compounds. All these attributes make CPO more stable against lipid oxidation.
Many fish species showed good results when dietary fish oil replaced by dietary palm oil. Catfish is one of such species (Legendre et al., 1995; Ng et al., 2000; Lim et al., 2001; Ng et al., 2003; Ng et al., 2004a). Red hybrid tilapia showed the same encouraging results too (Ng et al., 2001; Ng and Chong, 2004; Bahurmiz and Ng, 2007). Similarly, encouraging results have also been shown in cold water fish such as Atlantic salmon (Torstensen et al., 2000; Rosenlund et al., 2001; Bell et al., 2002; Ng et al., 2004b) and rainbow trout (Caballero et al., 2002; Fonseca-Madrigal et al., 2005). The other vegetable oil used in this study was coconut oil (CNO).

CNO is an edible oil which is extracted from the copra of coconut palm tree (Hertrampf and Piedad-Pascual, 2000). The palm is most common in the tropics, where the world copra production is about 5.5 million MT a year, and oil quantity that is extracted from copra flesh range from 63% to 68% (Arroyo, 1974). The main characteristic of this oil is its high content of saturated fatty acid and appreciable mount of medium chain fatty acid (MCFA).

Using MCFA as sources of energy has been investigated mainly in terrestrial animals (Aurousseau et al., 1989; Benevenga et al., 1989; Bozzolo et al., 1993). A few studies have been carried out using MCFA as dietary lipid in fish nutrition. Studies by Nematipour et al. (1990); Mustafa et al. (1991); Nakagawa and Kimura (1993) showed, good results about using medium chain triglyceride (MCT) in commercial diets of ayu. However the growth of channel catfish, Nile tilapia, carp and red tilapia were reduced when MCT was used as a dietary lipid (Stickney and Andrews, 1972; Takeuchi et al., 1983; Shikata et al., 1994; Rabfianni et al., 2004) respectively. Compound diets of CNO proved to be an efficient lipid source for first-feeding African catfish larvae compared with unsaturated oils such as peanut, cotton or fish oils (Legendre et al., 1995). Marine fish, especially the juvenile red drum revealed very good utilization of CNO (Craig and Gatlin, 1995). A good replacement of fish oil with vegetable oils must not reduce the quality characteristics of fish muscles.

The available results of effect replacement dietary fish oil with vegetable oil on the muscle quality of farmed fish are often contradictory (Regost et al., 2003a; Røra et al., 2003).

The dietary composition of fish diet is considered as one of external factors that can affect the muscle quality of the fish (Shearer, 1994). On the other hand, not all fish species can respond to this change in the diet (Bell et al., 2001; Røra et al., 2003; Ng & Bahurmiz 2009). In many previous studies, dietary lipid (source and level %) showed obvious qualitative and quantitative changes in the muscle fatty acids composition (Ng et al., 2003; Piedecausa et al., 2007 and Morais et al., 2006, respectively). Study the effect of dietary lipid on the muscle oxidation cannot be ignored in studies of this nature.

The effects of lipid oxidation on the muscle are not restricted only on; off odour, discoloration, but also include; reduction of the muscle capacity to hold the water, texture, nutritional value, protein change and at the end reduce the shelf life (Santos-Yop, 1996: Nielsen & Jessen, 2007). The changes become more clear when the muscles have high level of lipid especially, *n-3* series (Sargent et al., 1993; Ng et al., 2004a; Regost et al., 2004). Exposing muscles to light, oxygen or frozen storage especially, for long time are some of the factors that lead to increase in the lipid oxidation (Nielsen & Jessen, 2007).

Freezing is to convert the water from liquid form to solid stage by reducing the temperature (Zaritzky, 2006). This method is considered as one of the best means to preserve food because it keeps the product for long time without serious changes in the quality characteristics compared with the fresh ones (Berry et al., 2008). This is absolutely correct if the freezing done in good conditions (very low temperature and without fluctuation in the temperature during the storage time). Nevertheless, some deterioration can happen in this condition especially if the food is stored for long time (Zaritzky, 2008). This is because; freezing cannot inhibit the microbial, enzymatic and chemical activity completely especially, at the commercial storage (Nesvadba, 2008). These changes include protein denaturation, water loss, off odour and lipid oxidation (Nielsen and Jessen, 2007).

The fatty acid composition of the chosen oils (crude palm and coconut oils) may form a positive effect on maintaining or improving the muscle quality since the level of PUFA is low and the limited content < 1% of HUFA. In addition the quantity of anti-oxidation substances mainly, vitamin E and caroteniods in CPO influence the quality of muscle upon storage. Further, the high content of SFA (92.83%) in coconut oil will reduce the potentiality of oxidation to occur in the muscle which at the end can extend the preservation time and protect muscle quality.

Although studies have been carried out previously on the use of CPO in catfish fingerling diets, no reports are available for the use of coconut oil and crude palm oil for the production of marketable fish.

1.2 Research objectives

The main objective is to investigate the potential of substituting fish oil with crude palm oil and coconut oil of the African catfish *Clarias gariepinus.*

To achieve this goal, the study was split to the following objectives:

1. To evaluate the effects of crude palm oil and coconut oil on growth performance, feed utilization, body indices, yield, proximate composition and fatty acids composition of marketable size of African catfish muscles.
2. To measure the effects of feeding African catfish with dietary lipid FO, CPO, CNO, the combination of fish oil with crude palm oil (FO/CPO) and fish oil with coconut oil (FO/CNO) on quality parameters (post-thaw drip, hardness, colour, sensory and oxidation) of the fresh muscles.
3. To investigate the quality and nutritional stability of the muscles of the African catfish after being subjected to prolonged frozen storage.

CHAPTER 2

LITERATURE REVIEW

2.1 Aquaculture

There are many definitions for the term 'aquaculture' but here I refer to the definition of the United Nations (UN) Food and Agricultural Organization (FAO) defines it as:

> The farming of aquatic organisms including fish, prawns, Molluscs and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production such as regular stocking, feeding, protection from predators etc. Farming also implies individual or corporate ownership of the stock being cultivated. (FAO, 1997).

Aquaculture has a long history that dates back to 4000 years ago. Its publication also started in Asia, ancient Egypt and central Europe (Ackefors, 1994). However, this sector was regarded as a major source of protein only in the last four decades (Pillay and Kutty, 2005), when it was realized that the landings from many wild fisheries were in excess or close to sustainable levels (De Silva and Anderson, 1995). It is widely known that aquaculture plays a vital role in supplying the world with distinct share of aquatic animals and plant productions. Table 2.1 shows the comparison between the global yield of fisheries and aquaculture production for several years.

At the beginning of 1990s, Asia provided 83 % of the world's total of aquaculture and by the year of 1999, it had risen to 89 %. Most of the increase was attributed to China which shared 50 % of the world's total aquaculture production in 1990 and 68 % in 1999 (Brugere and Ridler, 2004). In many Asian countries, fresh water aquaculture production has contributed to high total aquatic production and it is dominated by finfish. In Southeast Asia (Indonesia, Malaysia, Thailand, Philippines, Singapore, Vietnam, Cambodia, Laos, Brunei Darussalam), fresh water fish dominate aquaculture production and account for 29 % of the total production (Ali, 2001). In 1995, world production of Clariidae exceeded 0.2 million metric tons which made it the second most important group of farmed catfish in the world (Hecht *et al*., 1997).

Table 2.1 World fisheries and aquaculture production.

Production	2000	2001	2002	2003	2004	2005[1]
	Million tonnes					
Inland						
Capture	8.8	8.9	8.8	9.0	9.2	9.6
Aquaculture	21.2	22.5	23.9	25.4	27.2	28.9
Total inland	30.0	31.4	32.7	34.4	36.4	38.5
Marine						
Capture	86.8	84.2	84.5	81.5	85.8	84.2
Aquaculture	14.3	15.4	16.5	17.3	18.3	18.9
Total marine	101.1	99.6	101.0	98.8	104.1	103.1
Total capture	95.6	93.1	93.3	90.5	95.0	93.8
Total aquaculture	35.5	37.9	40.4	42.7	45.5	47.8
Total world fisheries	131.1	131.0	133.7	133.2	140.5	141.6

Note: Excluding aquatic plants.
[1]Preliminary estimate.
Source: FAO (2007).

2.2 African catfish *Clarias gariepinus*

2.2.1 Taxonomy, Habitat and Distribution

African catfish (*Clarias gariepinus)* with its tolerant attributes to the aquaculture environment become the second most important group of farmed fish in the world (FAO, 1983). This fish was introduced into Asian countries such as The Republic of Korea, China, Taiwan, Philippine, Cambodia, Laos, India, Thailand, Malaysia and Indonesia in the late 1980s (FAO, 2002).

Myers *et al*. (2006) gave the taxonomy of African catfish as follows:

Kindom Animalia

Phylum Chordata

Subphylum Vertebrata

Class Actionopterygii

Subclass Neopterygii

Order Siluriformes

Suborder Siluroidae

Family Clariidae

Genus *Clarias*

Species *Clarias gariepinus*

African catfish have been widely introduced around the world. They are found as far south as South Africa and north into northern Africa. They have also been introduced in Europe, the Middle East and in parts of Asia. They migrate within streams and rivers (Teugels, 1986).

Clarias gariepinus live in a variety of freshwater environments, including quiet waters like lakes, ponds and pools. They are also very prominent in flowing rivers, rapids and around dams. They are very adaptive to extreme environmental conditions and can live in pH range of 6.5-8.0. They are able to live in very turbid waters and can tolerate temperatures of 8-35 ºC. Their optimal temperature for growth is 28-30 ºC (Teugels, 1986). They are bottom dwellers and do most of their feeding there. They are also obligate air breathers, which mean that they do spend some time on the surface. This species can live in very poorly oxygenated waters and is one of the last species to live in such an uninhabitable place (Pienaar, 1968). They are also able to secrete mucus to prevent drying and are able to burrow in the muddy substrate of a drying body of water (Skelton, 1995).

2.2.2 Morphology and feeding habits

African catfish *C. gariepinus* has a long cylindrical body with fairly long dorsal and anal fins. The dorsal fin contains from 61 to 80 soft rays and the anal fin has 45-65 soft rays. It has strong pectoral fins with spines that are serrated on the outer side (Teugels, 1986). The head is large depressed and heavily boned, with quite large and subterminal mouth and small eyes (Skelton, 1993). The skin colour on the dorsal part is dark grey or black and cream coloured on the ventral part. When fully grown, this species can reach up to 1.7 meters including the tail and can weigh up to 59 kg. In the wild, this species prefers to breed in the summers after the rainy season where vast numbers migrate to lay their eggs on vegetation on verges of rivers and lakes (Skelton, 1993). African catfish, with its omnivorous feeding behaviour, is not specific in its food requirements. The species is known to feed on wide range of food; insects, plankton, snails, crabs, shrimp, and other invertebrates. They are also able to eat dead animals including birds, reptiles, amphibians, small mammals, other fishes, eggs, and plant matter such as fruits and seeds. This species may also hunt smaller fish. They are also referred to as sharptooth catfish because of their fine, pointed bands of teeth (Skelton, 1993).

2.2.3 Culture

This species tolerates a wide range of environmental condition such as; living in low level of oxygen, survival even in marsh, salty and toxic gases environment, endurance of several times higher level of ammonia compared with most other species, stocking in high density, eating wide range of foodstuff (Horváth et al., 2002). This species is well known in Africa, the first attempts of culturists to use this fish in aquaculture sector were in 1950_s by Dutch researchers (Horváth et al., 2002). In addition to many qualities listed above which makes *Clarias gariepinus* a good candidate in aquaculture, it is easy to propagate, reacts well to hormone treatment, and to rapidly develop eggs. It can be spawned several times a year. All these characteristics, as well as the low costs of production and boneless meat favoured by many consumers (Horváth et al., 2002), encourage raising this fish under artificial conditions. The rapid growth of this species in both natural and aquaculture condition has also been noted (Hogendoorn, 1983).

2.2.4 Nutritional requirements

The increase in fish demand and insufficiency of the fisheries sector to face this increased demand has contributed to the fast growth of the aquaculture sector. Hence, aquaculturists have focused on the means to increase yield combined with rapid growth by using semi-intensive and intensive culture, which rely partly or totally on artificial feed respectively (Lovell, 1989).

The high price of manufactured diet makes diet the most costly item in aquacultyre (Tacon, 1987). Many factors can affect the energy requirements of fishes such as; physical, biochemical activities, water temperature, salinity, body size, age, density, reproductive, and stress factors. For example, protein requirements increase when water temperature and salinity increase (Zeitoun *et al.*, 1973; Bech, 1987). All animals, including fish require nutrients in different quantities to survive, grow, be active and healthy.

Proteins, lipids and carbohydrates are requested in high quantity; while vitamins and minerals are needed in small quantity (Webster and Lim, 2002). The functions of these nutrients are shown below;

- Synthesize new protein and replace the old one, creation of (hormones, enzymes);
- Sources of energy and essential fatty acids;
- Regulatory functions that ensure good, normal and healthy growth; and
- Support the body skeleton (Webster and Lim, 2002: Wilson, 2008).

On this basis, it becomes very clear how feed (nutritional and costly) is very important to the fish and farmers respectively. Many experiments have been conducted to understand the nutritional requirements of the fish. Studying the effect of using a wide range of new ingredients as sources (protein, lipid and the energy requirement) of fish diet at different levels allowed the culturists at the end to know the nutritional requirements for optimum growth for many species (Guillou et al., 1995; Regost et al., 2003;Montrro et al., 2005). This way ensures a good growth and less operational expenditures by minimizing feed wastage.

Generally, the differences among fish species in nutritional requirement are not vast and are often identified as requirement of warm water or cold water fish, finfish or crustacean, carnivorous or omnivorous and marine or fresh water fish. Therefore, when nutritional requirement are not available for one species, the data obtained from other fish species could also be applied. Haylor (1989) summarized the nutritional requirement of African catfish as presented in Table 2.2.

Table 2.2 Nutritional data of African catfish (fry, fingerling and adult).

Nutritional requirement (%)	African catfish		
	Fry	Fingerling	Adult
Crude protein	50	40	40
Crude lipid	9.5	10	10
Carbohydrate	20	30	30

2.3 Biochemistry of lipid and fatty acids in relation to fish

2.3.1 Lipids

Lipids are often deposited in the flesh, liver and in some species around the intestines (Love, 1997). The term "lipid" does not have a precise definition. It is difficult to provide definition for this class of substance. The early definitions relied on the criterion, whether the substance is soluble in water or soluble in organic solvents; benzene or chloroform (deMan, 1999). This definition has faced some problems, since there are substances called monoglycerides, which are undoubtedly lipids, but soluble in water more than in inorganic solvents (deMan, 1999).

Lipids consist of numerous chemical compounds, including monoglycerides, diglycerides, triglycerides, phosphatides, cerebrosides, sterols, terpenes, fatty alcohols and fatty acids. Also there are several substances associated with lipids such as the following:

- Fat-soluble vitamins
- Pigments
- Hydrocarbons
- Oxidation products
- Trace metals
- Water

These substances play vital roles. For example, the fat-soluble vitamins, sterols and phospholipids are of nutritional importance; the free fatty acids are an index for degree of hydrolysis of triglycerides; and the presence of peroxides, aldehydes, and ketones are indicative of the amount of oxidative deterioration that has taken place in the fat (Aurand, et al., 1987). Dietary lipid plays important roles in energy supply, source of essential fatty acid, cell membrane structure, and work as vehicle to carry the fat-soluble vitamins A, D and K and spring action on dietary protein (Watanabe, 1982).

2.3.2 Fatty acids

Fatty acids constitute the main component of phospholipids, triglycerides, diglycerides, monoglycerides and sterol esters. Fatty acids can be defined as carboxylic acids with long-chain hydrocarbon side groups, which consist of elements such as carbon, hydrogen and oxygen. Classifying the fatty acids into groups relied on several criteria: the length of chain, degree of unsaturation and the position of double bonds. The following equation clarifies this classification:

C x:y (n-z) e.g. C18:3*n-3*

Where:

C: carbon atom.

x: number of carbon atoms.

y: number of double bonds.

n: is often replaced by ω, omega, in popular jargon.

z: first double bond from the non-carboxyl (COOH) end.

According to this criteria the fatty acids can be saturated (SFA, no double bond), monounsaturated (MUFA, one double bond), polyunsaturated (PUFA, two or more double bonds) or highly unsaturated fatty acids (HUFA, more than 4 double bonds) (Lobb and Chow, 2008).

2.3.3 Essential fatty acids

EFAs are those types of fatty acids that cannot be synthesized by body or can be synthesized but in insufficient amounts. So for this reason, each species of fish may have its own specific essential fatty acid requirement. Generally, the cold-water fish require highly unsaturated fatty acids of the *n-3* class of lipids, while warm-water fish require HUFA from either the *n-3* or *n-6* classes, or a mixture of both (Webster and Lim, 2002). Some scientists consider Linoleic acid (C18:2*n-6*) and its relatives as essential because without such acids animals will die (Gurr, 1984), the vital role of C18:2*n-6* and linolenic acid (18:3*n-3*) are used as the base for elongate and desaturate process therefore, they are considered as EFAs especially for those fish which have ability to perform this process and vice versa (Bell, 1998). Not all the fish species have the ability to carry out this process. The quantity and quality of EFAs requirement can be affected by some factors like: the dietary lipid level; and the stage of development of the fish (Izquierdo, 1996 as cited by Rargent et al., 2008). This relation was very clear with red sea bream and yellowtail fingerlings where increase in the dietary lipid level leads to increase the requirement of n-3 HUFA (Rargent et al., 2008). In contrast, the relation did not work with other species like larval of gilthead sea bream (Salhi etal., 1999). The ability of fresh water fish to perform the elongation and desaturation process is higher than marine fish. Fresh water fish with vegetable oil has an adequate amount of C18, especially C18:2n-6 and C18:3n-3 are always allowed to synthesize fatty acids (DHA, EPA and ARA) through this phenomenon (Webster and Lim, 2002).

2.3.4 Fatty acid requirements

Requirements of EFAs of fish differ most of time according to fish species and also water temperature. In general, the requirement of EFAs for freshwater are either dietary linoleic acid, 18:2*n-6* and /or linolenic acid, 18:3*n-3* or both, whereas stenohaline marine fish require dietary HUFA; eicosapentaenoic acid (EPA), 20:5*n-3* and/or docosahexaenoic acid (DHA), 22:6*n-3* (Watanabe, 1982; NRC, 1993). Among the freshwater species, the ayu, channel catfish, coho salmon and rainbow trout require 18:3*n-3* or EPA and/or DHA. Chum

Salmon, common carp and Japanese eel require an equal mixture of 18:2*n-6* and 18:3*n-3* (NRC, 1993). In respect of temperature, cold-water fish have a greater demand for *n-3* PUFA than warm-water fish (Steffens, 1989; Stickney and Hardy, 1989). Supply of fish with adequate amount of their EFAs requirement will prevent all the common deficiency symptoms and will ensure rapid growth, good feed utilization and satisfactory reproductive performance (Léger, 1980). While some fish fed with less than 0.5% of 18:3n-3 show a decline on growth, erosion of caudal fin and shock syndrome caused by physical irritation (Castell et al., 1972). On the other side, the excessive amount of EFAs affected negatively on the growth (NRC, 1993).

2.3.5 Main functions of lipids

There are many studies that investigated the roles of dietary lipid in fish nutrition (Cowey and Sargent, 1977: Watanabe, 1982: Stickney and Hardy, 1989). Table 2.3 below illustrates the main function of lipids.

Table 2.3 The main function of lipid in fish nutrition

The function	An example
1. Lipids liberate energy in the form of ATP via mitochondria β-oxidation.	1 g lipid liberates 9 cal GE. 1g protein liberates 5.65 cal GE. 1 g carbohydrate liberates 4.1 cal GE.
2. Lipids play a structure and role of cellular Membrane.	C16:0, C18: 1*n-9*, C20:5*n-3* and C22: 6*n-3*.
3. They supply fish with the EFAs	C18:2*n-6*, C18:3*n-3*, C20:5*n-3* and C22:6*n-3*.
4. Lipids provide fish with soluble vitamins.	K, A, D and E.
5. Lipids make fish diet more palatable.	Fish oil.

2.4 Lipids sources in aqua feed

Marine oil can be divided into: fish oil, fish liver oil and marine mammal oil (Hertrampf and Piedad-Pascual, 2000). The high content of HUFA especially C20:*5n-3* FA and C22:6*n-3* FA is the major characteristic of fish oil. For many years, fish oil has been derived from industrial fisheries e.g. capelin, herring, sand eel, anchovy and sardine (Sargent et al., 2002). Fish oil has been used in the food industry, to feed terrestrial and aquatic animals and many industrial products (Hertrampf and Piedad-Pascual, 2000).

Many factors can influence the composition of single fish oil, such as the catching season, fishing ground, geographical location and processing. (Hertrampf and Piedad-Pascual, 2000).The Aquafeeds demand of fish oil continues to increase and has already reached 87% of the global supply of fish oil (Tacon, et al., 2006). In the last 50 years, the aquaculture sector has increased rapidly, though at the beginning, the total global aquaculture production increased over 85-fold from 638, 577 tonnes in 1950 to 54, 785, 841 tonnes in 2003 (Tacon et al., 2006). The sector is growing at an average compound rate of 8.8 % per year since 1950, compared with 3.0 % per year for total capture-fisheries landings (FAO, 2007). Secondly, the number of farmed species and countries which joined this sector increased. In the past, only 54 species from 42 countries were reported but, by 2003 the number of farmed species had increased to 246, and number of countries increased to 164 (Tacon et al., 2006). In the light of these facts, aquaculture industry must reduce the dependency of fish oil in fish diet for the successful expansion of this sector by using alternative oils and plant oils are good candidates.

2.4.1 Crude palm oil

Crude palm oil is extracting from the outside part (mesocrap) of palm tree (Elaeis guineensis) fruits. The high production of this oil (43.11 million tons) exceeds all vegetable and animal oils (Table 2.4). Malaysia is said to be the first producer of this crop with 41.12% of the total world production (MPOB, 2008). Besides, the chemical composition of this oil gives a desirable attribute like its stabilization against the oxidation. The high content of SFA (around 50%) and low in HUFA (Table 2.5) plays a high role to protect the muscle quality against the oxidation (Chong, 1993). In addition to this, the present minor unique compounds in crude palm oil such as; carotenoids, tocopherol, sterols, aliphatic alcohols (Table 2.6) although, represent less than 1%, but, they have a vital role to protect the oil from oxidation (Chong, 1993: Hertramf and Piedad-Pascual, 2000). In the light of these facts, the potential of this oil to reduce pressure on fish oil demand is big. The long run effect is to sustain expansion on aquaculture sector.

Table 2.4 World Production Of 17 Oils & Fats ('000 Tonnes)

Oil/fat	production
Palm Oil	43,118
Palm Kernel O	4,989
Soyabean Oil	37,164
Cottonseed Oil	5,029
Groundnut Oil	4,445
Sunflower Oil	10,687
Rapeseed Oil	19,847
Corn Oil	2,408
Coconut Oil	3,130
Olive Oil	3,081
Castor Oil	603
Sesame Oil	803
Linseed Oil	643
Total vegetable oils	135,947
Butter	7,123
Tallow	8,585
Fish Oil	1,076
Lard	7,740
Total animal oils	24,524

Source : (MPOB, 2008)

Table 2.5 Fatty acid composition (% of total fatty acids) of crude palm oil.

Fatty acids	%	Fatty acids	%
C12:0	0.2	C18:4*n-3*	ND
C14:0	1.1	C20:0	0.3
C16:0	42.8	Total saturates	48.2
C16:1	0.1	Total monoenes	41.2
C18:0	3.8	Total PUFA	10.6
C18:1	41.1	Total *n-3* PUFA	0.3
C18:2*n-6*	10.3	Total *n-6* PUFA	10.3
C18:3*n-3*	0.3	*n-3*: *n-6*	0.03

[1] ND= non-detectable (< 0.01 g/100 g fatty acids).
Source: Ng et al. (2003).

Table 2.6 Minor constituents of Malaysian crude palm oil.

Component	%	mg/kg
Carotenoids		
α-carotene	36.2	
β- carotene	54.4	500 - 700
γ-carotene	3.3	
Lycopene	3.8	
Xanthophylls	2.2	
Vitamin E		
α -tocopherol	28	
α -tocotrienol	29	500 - 800
γ - tocotrienol	28	
δ - tocotrienol	14	
Sterols		
Cholesterol	4	
Campesterol	21	300
Stigmasterols	21	
β-sitosterol	63	
Phosphatides		500 - 1000
Total alcohols		
Triterpenic alcohol	80	800
Alipahtic alcohol	20	

Source: Sundram et al. (2003).

The incorporation of palm oil in fish diet began in mid-1990s (Ng, 2002) when great efforts were exerted by many researchers to use this oil and its derivatives as alternative to dietary fish oil in fish diets. The results of these studies encouraged the use of this oil and its derivatives in fish diet up to 100% instead fish oil.

Catfish is one of the species which show good response towards using dietary palm oil in their diet by improved growth and feed utilization. Legendre et al. (1995) found that, using palm oil to feed the fry of African catfish *Heterobranchus longifilis* gave highest growth performance compared to cod liver oil as a sole lipid source. Study by Ng et al. (2000) confirmed that, replacement of up to 90% of fish oil with palm oil on tropical bagrid catfish (*Mytus nemurus*) did not affect the growth. Lim et al. (2001) concluded that, using palm oil at least 8% in African catfish *Clarias* *gariepinus* improved growth performance and dietary protein retention. In previous study *Clarias gariepinus* was fed with 10% semi-purified dietary crude palm oil and led to significantly higher final body weight, weight gain and feed utilization efficiency compared with fish fed fish oil (Ng et al., 2003). In other studies, Ng et al. (2004a) found that, gradual replacement of fish oil with palm fatty acid distillate gave better growth in African catfish. Ochang et al. (2007) concluded that, partial or total replacement of cod-liver oil with crude palm had no significant effect on final body weight (FBW), weight gain (WG), feed conversion ratio (FCR) and specific growth ratio (SGR) of African catfish *Clarias gariepinus*. With other species (red hybrid tilapia), Ng et al. (2001) reported that, the palm-origin oils; CPO, crude palm kernel oil (CPKO) and PFAD can replace up to 100%, 100% and 50%, respectively of dietary fish oil (cod liver oil, CLO) without affecting the fish growth. Ng and Chong (2004) found that, after 5 months of feeding Red hybrid tilapia with dietary CPO solely or in combination with FO and linseed oil (LO) didn't show any significant differences with remaining diets (100% fish oil, 100% soybean oil) on growth parameters . Red hybrid tilapia showed similar results but, in separate study carried out by (Bahurmiz and Ng, 2007). Where they used dietary CPO and its two derivatives palm fatty acid distillates (PFAD) and refined bleached and deodorized palm olein (RBDPO) to replace FO.

Similar encouraging results have been shown in cold water fish such as Atlantic salmon (Torstensen et al., 2000; Rosenlund et al., 2001; Bell et al., 2002; Ng et al., 2004b) and rainbow trout (Caballero et al., 2002; Fonseca-Madrigal et al., 2005).

With respect to flesh quality, Rosenlund et al. (2001) did not observe significant differences in fillet quality, in terms of texture and colour of Atlantic salmon fed diets in which at least 50% of fish oil was replaced by different oils including CPO, rapeseed (RO), LO, soybean oil (SO) or poultry oil (PO). Ng and Chong (2004) observed that, the filets of tilapia fed with dietary CPO showed higher stability against oxidation during frozen storage condition compared with the fish fed the remaining diets, while quality parameters such as holding capacity, muscle texture and proximate composition were not significantly different. Sensory evaluation showed the same results except for juiciness attribute. Ng and Bahurimz (2009) reported that, the results of red tilapia fed with alternative lipids (palm oil and two of its derivatives) did not significantly influence some quality parameters like; thaw drip and texture of fresh fillet. However, oxidative stability of fillet lipids was significantly affected. Fillets of fish fed palm oil-based diets exhibited significantly lower levels of lipid oxidation than those of fish fed the FO diet. With the exception of fillet hardness, seven other sensory attributes did not differ significantly among treatments.

2.4.2 Coconut oil

Coconut oil is edible oil which is extracted from copra (the dried meat or kernel of the coconut) of coconut palm family *Arecacea,* species *Cocos nucifera* (Hertrampf and Piedad-Pascual, 2000). The palm is most common in the tropics. Approximately, this tree begins the first production of fruits after six years of planting and can be harvested throughout the year (Hertrampf and Piedad-Pascual, 2000). In addition, CNO is used in; foods, soap and cosmetics (Hertrampf and Piedad-Pascual, 2000). World copra production is about 5.5 million MT a year. About75 % of the production comes from, Philippines (43%), Indonesia (24%) and India (7%) (Hertrampf and Piedad-Pascual, 2000).

The quantity of oil that can be extracted from copra flesh, range from 63% to 68% (Arroyo, 1974). Saturated fatty acids form the highest percentage (91.6%) of the total fatty acid composition of coconut oil (Table 2.7) and, MCFA is an appreciable part of this percentage in coconut oil. MCFA is defined as the fatty acids that have a carbon atoms range from 6- to 12. The other natural sources of MCFA are milk fat and palm kernel oils (Gurr and Harwood, 1991). MCFA are also available as MCT,which are commercial products prepared from coconut oil and consisting of mixed triacylglycerols containing mainly C8:0 and C10:0 (Bach and Babayan, 1982). Lauric acid (C12:0) is one of the MCFA present in CNO in high amount. This fatty acid additive function inside the human body lies in its conversion to "monolauin" which act as antibacterial and anti-protozoa used by human body to destroy lipid coated viruses such as HIV, herpes, influenza, various pathogenic bacteria and protozoa such as Giardia lamblia (Enig, 2002, 2003; Leduc, 2002).

Table 2.7 fatty acid composition of coconut oil (% by weight of the total fatty acids).

Fatty acid	%	Fatty acid	%
C8:0	7.77	C18:*2n-6*	1.65
C10:0	6.40	Total SFA	92.46
C12:0	48.94	Total MUFA	5.89
C14:0	18.36	Total PUFA	1.65
C16:0	8.18	*n-3*	0
C18:0	2.82	*n-6*	1.65
C18:1n-9	5.60		

Source: Rabfianni et al. (2004).

Using MCFA as source of energy has been investigated mainly in terrestrial animals (Aurousseau et al., 1989; Benevenga et al., 1989; Bozzolo et al., 1993). On the other hand, this group of fatty acids have been used currently in clinical human nutrition through enteral or parenteral administration (Bach and Babayan, 1982; Ulrich et al., 1996).