THE EFFECTS OF DIETARY LIPID LEVEL AND SOURCE ON THE GROWTH AND BODY COMPOSITION OF THE MALAYSIAN MAHSEER, *Tor tambroides*

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AA	Arachidonic acid
AGR	Accumulative growth rate
AOAC	Association of Official Analytic Chemists
ATP	Adenosine triphosphate
CF	Condition factor
CMC	Carboxymethyl cellulose
DGR	Daily growth rate
DHA	Docosahexaenoic acid
EFA	Essential fatty acis
EPA	Eicosapentaenoic acid
FAO	Food and Agricultural Organization
FCR	Feed conversion ratio
FER	Feed effenciency ratio
GC	Gas chromatography
HSI	Hepatosomatic index
HUFA	Highly unsaturated fatty acids
IPF	Intra-peritoral fat
IUCN	International Union for Conservation of Nature and Natural Resources
LA	Linoleic acid
MUFA	Monounsaturated fatty acids
NAP3	Third National Agriculture Policy of Malaysia
NPU	Net protein utilization
PER	Protein efficiency ratio
PUFA	Polyunsaturated fatty acids
SGR	Specific growth rate
USD	United State Dollar
VSI	Viserosomatic Index

KAJIAN TENTANG KESAN TAHAP LIPID DAN JENIS LIPID TERHADAP TUMBESARAN DAN KOMPOSISI BADAN IKAN KELAH, *Tor tambroides.*

ABSTRAK

Ikan kelah, *Tor tambroides* merupakan ikan yang mempunyai nilai yang tinggi di negara ini. Ikan ini terkenal di kalangan penggemar sukan memancing dan juga dihidangkan sebagai makanan istimewa di kebanyakan bahagian di negara ini. Ikan kelah mempunyai harga pasaran yang tinggi, namun hasil kajian tentang nutrisi, terutamanya nutrisi lipid adalah amat terhad. Kajian ini dijalankan untuk menilai tahap lipid yang paling sesuai dalam makanan ikan ini. Kajian berikutnya pula adalah untuk melihat kesan jenis-jenis lipid terhadap tumbesaran dan juga komposisi badan ikan kelah.

Kajian selama 13 minggu telah dijalankan ke atas anak ikan kelah dengan memberi enam jenis makanan berbeza dengan tahap lipid yang berbeza (0%, 3%, 6%, 9%, 12% atau 15% tambahan lipid). Ikan telah diagihkan kepada tiga replikat untuk setiap jenis permakanan. Ikan diternak di dalam akuarium kaca dan diberi makan sehingga mencukupi. Ikan yang diberi makanan yang mengandungi 0% tambahan lipid menunjukan pertumbuhan yang lebih rendah berbanding tumbesaran ikan yang diberi makanan yang ditambah lipid. Pertambahan tahap lipid dalam makanan dari 3% ke 15% tidak memberi kesan terhadap pertumbuhan. Komposisi lipid badan ikan dan index intraperitoneal (IPF) meningkat dengan penambahan tahap lipid dalam makanan. Data ini menunjukan bahawa ikan kelah memerlukan tahap lipid yang rendah (3%-6%) di dalam makanan.

Kajian lanjutan juga dijalankan dengan menggunakan tujuh jenis pemakanan ikan, iaitu minyak hati kod (CLO), minyak bunga matahari (SFO), minyak kanola (CO), minyak kelapa sawit (PO), lemak ayam (PF) dan dua jenis campuran minyak CLO dan SFO yang masing-masing mempunyai nisbah omega-3 : omega-6 adalah 0.6 (M1) dan 0.3 (M2). Ikan yang diberi makan SFO, PO, PF dan M2 menunjukkan kadar partumbuhan yang lebih tinggi ($P<0.05$) berbanding dengan CO dengan PF dan M2 memberi kadar pertumbuhan yang terbaik. Kadar pertumbuhan yang lebih tinggi dalam ikan yang diberi makan SFO dan M2 berbanding dengan CLO dan M1 menunjukan bahawa kehadiran omega-3 di dalam makanan menjejaskan pertumbuhan ikan. Profil asid lemak di dalam hati dan otot adalah sama dengan profil jenis lipid dan ikan yang diberi makan CLO mempunyai kandungan omega-3 yang tertinggi. Ikan kelah juga menunjukkan penerimaan terhadap lemak ayam (PF) dan minyak kelapa sawit (PO) tanpa menjejaskan pertumbuhan dan berpotensi menjadi jenis lemak yang digunakan dalam makanan ikan kelah.

THE EFFECTS OF DIETARY LIPID LEVEL AND SOURCE ON THE GROWTH AND BODY COMPOSITION OF THE MALAYSIAN MAHSEER, *Tor tambroides*

ABSTRACT

The Malaysian mahseer, *Tor tambroides* is regarded as a highly valued fish in the country. Popular among sport fishing enthusiast and serve as a delicacy in many parts of this country, the Malaysian mahseer can fetch a very high price in the market, there is very limited studies on the nutrition, especially lipid studies, on the fish. The present study was conducted to determine the best lipid level inclusion in the fish's diet and also a follow up study on the effect of different lipid source on the growth and body composition of the Malaysian mahseer.

A 13-week feeding trial was conducted using Malaysian mahseer fingerlings fed six different diets with different lipid level (0%, 3%, 6%, 9%, 12% or 15% added lipid). The fish was distributed into triplicate each treatment in a series of glass tank and was fed close to apparent satiation. Fish fed diets with 0% added lipid showed lower growth compared to fish fed diets with lipid added, and increase in lipid level from 3% to 15% added lipid however did not affect growth. Body lipid composition and intraperitoneal index (IPF) increase with dietary lipid level. The data showed the Malaysian mahseer requires low lipid level (3% - 6%) in their diet.

A follow up study was done using seven different treatments comprising of cod liver oil (CLO), sunflower oil (SFO), canola oil (CO), palm oil (PO), poultry fat (PF) and two different oil mixes of CLO and SFO with omega-3 : omega-6 ratio of 0.6 (M1) and 0.3 (M2). Fish fed SFO, PO, PF and M2 showed significantly higher

(P<0.05) growth rate compared to CO with PF and M2 giving the best growth performance. Higher growth rate in fish fed SFO and M2 compared to CLO and M1 indicated that higher omega-3 in the diet depresses growth of this fish. Fatty acid profile of the liver and muscle mirrored the lipid source with CLO fed fish having the highest omega-3 content. The Malaysian mahseer also showed acceptance towards poultry fat and palm oil without compromising growth, and is a potentially cheaper lipid source for the Malaysian mahseer feeds.

1.0 INTRODUCTION

1.1 GENERAL INTRODUCTION

Mahseer are freshwater fish in the genus of *Tor*, which inhibits fast and clear water streams and are naturally distributed from the trans-Himalayan region to south-east Asia (which includes Peninsular Malaysia and Borneo). The Malaysian mahseer (*Tor tambroides*) similar to its counter parts from other region are also highly valued in term of food and sport fishing (Ng, 2004). In many parts of the country, the Malaysian mahseer are considered a delicacy and have a very high market value where in the state of Sarawak, the Malaysian mahseer (*Tor tambroides*) can fetch from Malaysia Ringgit 160 to 240 for 1 kg (Nguyen *et al.*, 2005). Due to over fishing and also mainly caused by habitat destruction, resulting in depletion of natural stocks and contributing to decrease of the mahseer population and the mahseer have been regarded as endangered in many parts of the world (Rahman *et al.*, 2005; Bazaz and Keshavanath, 1993; Nguyen *et al.*, 2005). Thus, conservation efforts have been made amongst researchers, developers, conservationist and government all across Asia to increase and save the population of the mahseer in their natural habitats. The success in breading several species of mahseer have been recorded, such as *Tor putitora* (Ayub *et al.*, 2007; Baidya *et al.*, 2007) and in Malaysia, the success of breeding for the first time, two major mahseer species in the Tarat Inland Fisheries Station, Serian, Sarawak through artificial propagation (Ingram *et al.*, 2005). The success leads to further conservation effort in maintaining wild stocks and also increasing the potential of the Malaysian mahseer as an aquaculture species (Ingram *et al.*, 2007). Information and studies regarding nutrition especially lipid nutrition of the Malaysian mahseer have been very limited and understanding the nutrient requirement of the fish will prove crucial especially for aquaculture purposes.

Fish nutrition are important aspects in improving growth performance and health of fish, thus lipid nutrition are equally important as other nutrient. Lipid plays a huge role in the development of fish and optimum requirement of lipid in the fish diet is essential for performance and also to maintain cost efficiency. High lipid inclusion in the diet may result in increasing lipid deposition which causes potential problem towards the flavor and flesh quality of fish (Lim *et al*., 2001), excessive inclusion of lipid in the diets will also increase unnecessary production cost. Lipid in scarcity however have been proven to have an negative effects in many fish species, for example the channel catfish fed insufficient amount of lipid cause poorer weight gain and feed efficiency values (Stowell and Gatlin, 1992). Lipid nutrition also includes fatty acid requirement which are important and varies between fish species and is influence by many factors. Cold water marine species such as the pacific salmon have dietary requirements especially for omega-3 fatty acids (Grant *et al.,* 2008) meanwhile warm water fishes such as Nile tilapia thrive better in higher omega-6 diets (Lim *et al*., 2008).

Fish oil is a major dietary lipid source used in many commercial fish feeds and besides providing energy and essential fatty acids, it also helps improve palatability and appearance of the feed (Ng *et al.,* 2003). Global fish oil productions however have declined since recent decades and researchers are looking out for potential replacement in the production of commercial aquaculture feeds. Several lipid sources have been identified as a potential replacement, such as vegetable oil (Grant *et al.,* 2008; Tocher *et al*., 2003), animal fat (Shapawi *et al.,* 2007; Suhabadra *et al.,* 2006) and also palm oil. Ng *et al*. (2001) have reported the potential of palm oil to be used in hybrid tilapia feeds and Bell *et al.* (2001) also reported that no different in growth rate of Atlantic salmon between fish fed diets containing palm oil and the commercially use fish oil. Although cost efficiency is the main reason for alternatives lipid sources research, physical effects and essential fatty acids are some of the issue that cannot be taken lightly in determining the best lipid source for aquafeeds.

The present study was carried out on the Malaysian mahseer fingerlings to determine the effects of lipid level growth performance and body composition as well as determining lipid level requirement. A follow up study was also carried out to see the effects of various lipid sources with different fatty acid profiles and determining the best lipid source for the Malaysian mahseer.

1.2 OBJECTIVES

1. To determine lipid level requirement and the effect of increasing lipid level on the growth performance and body composition of the Malaysian mahseer, *Tor tambroides*.
2. To investigate the effects of various lipid sources with various fatty acid profiles towards the growth and body composition of the Malaysian mahseer.
3. To determine the best lipid source or sources to suit the lipid requirement of the Malaysian mahseer fingerlings.

2.0 LITERATURE REVIEW

2.1 Aquaculture

2.1.1 Introduction and History of Aquaculture

Aquaculture can be described as farming or cultivation of freshwater and marine organisms with the sole purpose of consumption and production. Aquaculture have evolved and developed rapidly throughout the millennia and becoming one of the fastest-growing food production activities in the world (FAO,2009).Until the year 2000 there have been reports of more than 220 species of finfish and shellfish farmed (Naylor *et al*., 2001). It has been reported that aquaculture actually started in China around 4000 years ago during the Tang Dynasty where the major carp species where the main aquaculture products (Lin and Peter, 1991; Li *et al*., 1987) meanwhile marine fish farming was believed to have started in Indonesia around 1400 A.D and in the Philippines around 1700 A.D when young milkfish were trapped in coastal pond at high tides (De Silva and Anderson, 1995). In Japan, the aquaculture of salmonid species have been claim to have started since the 1800 (Nose, 1985).

Fish are one of the main protein sources and throughout the millennium overfishing have been a main contribution towards the rapid reduction of fish stocks in the wild. Due to the rapidly increasing human population in the world which expanded beyond 6 billion, demands in fish as protein source also increase. Munilkumar and Nandeesha (2007) reported that 90% of the Northern India population are fish consumers and demands have been increasingly heavy but the gap between supply and demand still exists. With almost 50% of Japanese main source of animal protein comes from fish, increasing demands and limiting supplies of domestic supplies was also recorded in Japan (Nose, 1985). As world demands increase, aquaculture have been one of the main alternatives to tackle the problem of depleting fish supplies in the wild and also to help fulfill rising fish demands in the world.

Aquaculture also provides work opportunities, helping to increase the economic status of local communities especially in poor developed areas. Dempster and Sanchez-Jerez (2008) reported the plan to expand aquaculture industry by the European Union to increase seafood supplies, reduced seafood trade deficits and to provide more job opportunities in European countries, which shows the seriousness of even the highly developed countries towards aquaculture. Contribution of the aquaculture industry to income and employment can be expected to increase substantially in the long term (Shang, 1985) and with better techniques and scientific research, a bigger scale in the industry will be achieved in the near future.

Benefits of aquaculture towards the environment have been stated by many (Stotz, 2000; Rahman *et al*., 2005) especially in the role to help counter the problem of overfishing wild stocks. Stotz (2000) have documented the replacement of scallop fisheries with aquaculture species after the industry collapsed due to overfishing. The aquaculture of many species also helps in conservation and reduces the impact of overfishing towards the environment and dependency towards wild stocks can be reduced thus benefiting the survival of aquatic species.

2.1.2 Aquaculture in Malaysia

Aquaculture in Malaysia started in the 1920's with small scale extensive polyculture of various carp species and was more developed in the 1930's to the 1940's with the introduction of shrimp and blood cockles culture. Aquaculture in Malaysia continues to increase from extensive to semi-extensive culture and with the introduction of new species and new method, by the early 1990's aquaculture in Malaysia has moved into intensive commercial aquaculture with high stocking density and supplementary feedings, plus the establishment of government and private hatcheries and feed mills. The industry has been developing and has been increasing for 10% in the last 5 years, and is now a lucrative and self sustaining industry in the country (FAO, 2009).

Realizing the potential and benefits of such a lucrative industry, the Malaysian government has form the Third National Agriculture Policy of Malaysia (NAP3, 1998-2010) to ensure the contribution towards the country remains strong. The seriousness of the government towards developing the industry can be seen in the building of several research facilities and increasing funds towards the research and development of aquaculture in the country. Several government and private agencies have been set up to educate and train young pioneers and local farmers to be involve in a more intensive industry, with the aid of technology and scientific research.

Most of the freshwater aquaculture production are marketed for domestic consumption, these species includes catfish, tilapia, freshwater prawn, Chinese carps and river carps, where prices depends on supplies and demands. Many marine fishes such as seabass, grouper, crabs, tiger prawns and shrimp are produce for export market such as to countries like Singapore, Taiwan, Hong Kong, EU, Japan, USA and Australia. The value of export market in 2003 alone was estimated at about USD 100 million (FAO, 2009). Aquaculture industry can be one of the leading economy contributors to the country if the government continue to educate the farmers and public towards the application of scientific research and method without neglecting the environmental issue regarding aquaculture.

2.2 Malaysian mahseer, *Tor tambroides*

2.2.1. Introduction of the Malaysian mahseer

Regarded as the king of the river (Dinesh and Nandeesha, 2007), the mahseer have been a much sought after fish by anglers, food enthusiasts and hobbyist alike (Ingram *et al*., 2005). The mahseer are large cyprinids that inhabit rapids and fast flowing streams. The mahseer are mainly omnivorous, feeding primarily animal matters (Rainboth, 1991) and the Malaysian mahseer, *T. tambroides* exclusively has been known to feed on the fruits fallen into the river (Siraj *et al*., 2007). Mohsin and Ambak (1992) reported that the fish specifically fed on the 'Ara' fruit which fall into the river. The mahseer which conceived of almost 20 different species is distributed mainly in the Asian region, ranging from Afghanistan, Pakistan, across the southern Asia to Thailand, Malaysia and Indonesia (Siraj *et al.*, 2007). The Mahseer have suffered major depletion of natural stocks due to rapid development, environmental perturbation, overfishing and habitat destruction (Bhatt *et al*., 2004; Rahman *et al*., 2005; Ingram *et al*., 2005).

The Malaysian mahseer are also called "ikan kelah" in the peninsular, in east Malaysia they are called "ikan empurau" and "ikan semah" by the local Ibans in Sarawak, while the Dusun in Sabah refer to them as "ikan pelian" (Ng, 2004). Malaysian mahseer are highly valued in Malaysia, where price can be higher than USD25 per kg and regarded as one of the most expensive freshwater food fish in the country (Ng *et al*., 2007). There have been 3 species recorded so far in Malaysia as reported by Ng (2004), are *Tor tambroides*, *Tor tambra* and *Tor duoronensis*.

Plate 2.1 Picture of a Malaysian mahseer, *Tor tambroides*
(Source : www.itto-pulongtau.com)

2.2.2 Taxonomy and Description

Misra (1959) classified this genus as:

Phylum : Vertebrata

Subphylum : Craniata

Superclass: Gnathostomata

Series : Pisces

Class : Teleostomi

Subclass : Actinopterygii

Order : Cypriniformes

Division : Cyprini

Suborder : Cyprinoidei

Family : Cyprinidae

Genus : *Tor*

Species : *Tor tambroides* (Bleeker,1854)

Desai (2003) described a true mahseers as having a similar characteristic as carp with big scales, fleshy lips continuous at angles of the mouth with an interrupted fold or groove across the lower jaw, two pairs of big barbells, lateral-line scales ranging from 22-28, and length of head equal or greater than the depth of body.

Regarded as the largest species in the cyprinidae family where size can reach up to 250cm (Mohsin and Ambak, 1992), the Malaysian mahseer (*Tor tambroides*) however can be differentiated by its most distinctive features which are pointed snout as viewed from the dorsal, black coloration at the edge of individual scale and the present of fleshy median lobe as viewed from the central side of the fish (Siraj *et al*., 2007). Siraj *et al*. (2007) also mention of having a streamlined body, cylindrical body, powerful muscular tail and hypertrophied lips, which are important characteristic of the fish to live in fast flowing streams.

2.2.3 Geographical Distribution

Mahseer are unique fast flowing, clear waters dweller which can only be found naturally within Asia (Ng, 2004). Their thrive for cool, pristine and clean water has cause them to inhibit fast flowing streams and river in the uplands. Bhatt *et al*. (2004) mention that the Himalayan mahseer (*Tor putitora*) occurs naturally in rivers and streams and were then introduce into lakes. Rai *et al*. (2007) mention that the mahseer inhabit a moderately warm and cold climatic region of tropical highlands and although considered cold-water fishes the mahseer such as *T. khudree* can tolerate temperatures up to 35˚C (Dinesh and Nandeesha, 2007).

In Malaysia, the mahseer can only be found in rural inland areas of peninsular and Borneo. In the peninsular, there have been reports from Sungai Tahan, Taman Negara in Pahang, Malaysia (Tan, 2002), Nenggiri River in the state of Kelantan (Ambak *et al.*, 2007), Perak and Negeri Sembilan. In east Malaysia, Esa *et al*. (2007) reports of distribution in Ulu Limbang, Ba’Kelalan, Bario, Layar and Ulu Baleh in Sarawak. In Sabah meanwhile, reports have been found in Keningau, Liwagu and Tawau. The Mahseer mainly inhibits the highlands of Borneo where there is still availability of cool, rapid and clear water.

2.2.4 Population and Status

Populations of mahseer around the world have been declining in recent years. Rapid development, deforestation, pollution, over exploitation of natural stock and other human related harms has been the major factor of the depleting population of mahseer, worldwide. In India, both major mahseer, *T. putitora* and *T. tor* have been listed as Vulnerable by IUCN (2003). *Tor tor* was reported to be decreasing in population due to various human impacts and also over fishing by fishermen who harvest them during critical migration period (Islam, 2003). Bhatt *et al.* (2004) reported that mahseer population have vanished from some areas in India and declined rapidly. In Bangladesh, the mahseer (*Tor putitora*) is considered endangered (Rahman *et al*., 2005) and rapidly disappearing from the natural environment (Islam, 2003).

The population of the Malaysian mahseer has also experiencing depopulation which is contributed by uncontrolled development in within and around the area (Ambak *et al*., 2007, Ingram *et al*., 2007). Jalal *et al* (2005) considered the Malaysian mahseer as endangered after a recent catch statistic which showed a decline in population.

2.2.5 Achievements and Conservation

A breakthrough in breeding the *Tor tambroides* was achieved for the first time at the Indigenous Fish Research and Production Centre, Sarawak, Malaysia (Ingram *et al.,* 2005). The species was artificially propagated and have yield success after a three year effort done by the centre with collaboration with Deakin University headed by Prof. Sena De Silva. The success has breathed new life into the effort of conservation and also open a potential commercialization of this species.

2.2.6 Economical Potential

Mahseer around the world have been known for its value both culturally and economically. There were many reports indicated the potential of mahseer for aquaculture, such as the Himalayan golden mahseer (*Tor putitora*) having many qualities which makes it a good species for aquaculture (Mohan and Basade, 2005 ; Lakra *et al*., 2006), and *Tor tor* was also reported by Islam (2003) as a promising candidate for coldwater aquaculture. Rahman *et al*.(2007) reported the polyculture of mahseer with other indigenous major carp species has yielded success where by total productivity of the indigenous carp was increased without any negative effects. Keshavanath *et al*. (2002) reported the potential of *Tor khudree* for aquaculture in India, taking the facts that suitability of method, feeding and environment of farming must be determined to yield the best production.

The Malaysian mahseer*, T. tambroides* have also been highly rated by many as a great candidate for the aquaculture industry (Nguyen *et al*.,2005, Ingram *et al.*,2005, Ng *et al.,* 2007) , due to its high market value and the recent achievement in breeding. The success of propagation breeding of this species has helped to boost and provide footing for the development of a large scale production of this locally (Ingram *et al*., 2007). The product of the aquaculture can be also use to replenish the wild stock with the consideration of preserving the genetic aspects of the indigenous species. Aquaculture of the mahseer will no doubt provide work opportunities and increase economic status of the local community especially in the rural part of the country. Ingram *et al*. (2005) also have reported that the ability of the Malaysian mahseer to be pond reared, which increase a potential rural aquaculture industry in the country using either pond culture or open cage culture systems.

2.3 Nutrition in Fish

2.3.1 Nutrient Requirement in Fish

The understanding of nutrient requirement in fish has been a very important aspect in fish cultivation. Essential amount of information regarding the needs of various nutrients in the fish diet will help to increase production, health and control cost of production by optimizing nutrient needs of fish. Protein and lipid have been one of the most studied aspects in fish's nutrition requirement. Proteins are essential for fish survival as they help to promote growth and generate muscle tissues, the understanding of optimal protein requirement especially the essential amino acids requirement is undoubtedly important. Lipid helps to increase palatability of fish diets and fish require an essential amount of fatty acids from lipid to help in physiology metabolism and lipid are also essential energy source for fish. The role of carbohydrate has been unclear in fish, although species such as salmon showed poor ability to digest and utilize carbohydrate (Conwey and Sargent, 1979). Carbohydrate is a well known energy source for terrestrial animal and also human being, but fish cannot seems to utilize carbohydrate as effectively as certain land animals but Shiau (1997) has reported that a number of warmwater fishes especially tilapia have shown ability to utilize carbohydrates. Vitamins and minerals are both important nutrients in fish diet and insufficient amount of both can cause vitamin and minerals deficiency problems, retard growth and also affects the mortality of fish (Cowey and Sargent, 1979). All aspects of nutrition in fish are essential for the survival and performance of fish, optimal amount and satisfying the requirement of all the nutrient may help in improving fish husbandry in captivity.

2.3.2 Protein

Proteins are essential nutrient especially for the development of growth and tissues of fish and soft tissues makes up to almost 80% of fish muscle and organ. Fish consume protein to obtain subunits of protein, called amino acids which are important in protein tissue synthesis.

Amino acids can be categorized as indispensable or essential and dispensable or nonessential acid amino. Indispensible amino acids are essential amino acids that have to be incorporated in the diets of animals as this type of amino acids cannot be synthesized in sufficient quantities for growth of animal. Dispensable amino acids can be synthesize in animal and are not essential in the diets of animal. Fish amino acid requirement can be variable from one another depending of species and requires an essential amount of amino acids and nonspecific nitrogen throughout life. Regular intakes of protein to meet the amino acids requirement in fish are vital to help build protein especially during growth and reproduction or to maintain protein in the body of fish. Cowey and Sargent (1979) listed the amino acid requirement of certain species of fish and the rat (Table 2.1).

Table 2.1 Amino Acid Requirement of Certain Species of Fish and of the Rat

(Values are grams per 100 g of dry diet.)

Amino acid	Chinnok Salmon	Japanese Eel	Carp	Rat
Arginine	2.4	1.7	1.6	0.2
Histidine	0.7	0.8	0.8	0.4
Isoleucine	0.9	1.5	0.9	0.5
Leucine	1.6	2.0	1.3	0.9
Lysine	2.0	2.0	2.2	1.0
Methionine	1.6	1.9	1.2	0.6
Phenylalanine	2.1	2.2	2.5	0.9
Threonine	0.9	1.5	1.5	0.5
Trytophan	0.2	0.4	0.3	0.2
Valine	1.3	1.5	1.4	0.4

Source : Cowey and Sargent (1979)

Protein requirement is an important aspect when formulating feeds for a particular species and protein requirement can be defined as the minimum or optimum requirement of protein for the best growth performance. Protein requirement of fish are much higher than that of other vertebrates and decrease as fish reaches the stages of maturity. Protein requirements can vary depending on various factors such as species, fish size, age, feeding rate, diet composition, water temperature and stocking density. Fish fry initial feeding requires high protein but will decrease as the fish grow and determining protein requirements in different species and even at different stages of fish maturation is important to control cost price of diet formulation and also for the environment of culture. Excessive protein provided in diet will not be harmful for fish, however the increase of nitrogen excretion can pollute the water. Protein source are also considered expensive and the inclusion of excessive protein in diet is a waste. Inadequate protein in the diet however will have negative effects on growth and loss of weight, so the optimum proportion of protein in diet is essential to both maintain performance and also cost of the aquaculture production.

Reducing cost and increase feed quality have always been a struggle for aquafeeds producers especially in term of protein source. Fishmeal has always been used as a major protein component in aquafeeds because it is an excellent source of protein with balanced amount of essential amino acids, nutrient, lipid and vitamins for fish. Fishmeal inclusion in animal feeds also helps to increase feed efficiency, growth, feed palatability and also enhance nutrient absorption (Miles and Chapman, 2006). However, the downside is that fishmeal is becoming more expensive, supplies of total global fish meal production has remained static over the past quarter century (FAO, 2006) and demands are increasing, with Southeast Asian and East Asian being the highest fishmeal importing countries which accounted for more than half the total imports in the world (FAO, 1996). Due to these reasons, researchers have been looking for other alternatives such as in the form of poultry-base-meal and vegetable-base-meal as replacements. Although these alternative protein components might lack the benefits of fishmeal such as essential amino acids content (Subhadra *et al*., 2006), researchers and aquafeeds producers alike are looking for ways to incorporate and reduce dependency on fishmeal thus reducing aquafeeds production cost.

2.3.3 Lipid

Generally lipid which are common names given to fat soluble compounds found in plants and animals are important energy sources essential for development and growth by generating ATP as a form of metabolic energy. When in excessive amount, lipid will be stored as solid fat in the body, such as the adipose fat in the gut of fish. Lipids are as important as other nutrient for fish especially in marine fishes which have limitation in converting dietary carbohydrates into energy. Lipid can be classified as triacylglycerols, phospholipids, sphingolipids, wax esters and sterols. Triacyglycerols are primary energy deposits of animals and normally used as storage during inadequate food and energy intake. Fish has a special ability to metabolize these compounds readily which helps in survival of fish under long term period of deprivation. This can be observed in salmons during its migratory journey going upstream to spawn. Other classes of lipid are also essential for fish, where some acts as components for hormones function, tissue compounds and for other physiological function.

Fatty acids which is the components of lipid that can be in the form of a straight chain or branch chain, as in the cases of fish, the lipid consist of numerous unsaturated double bonds. Fishes requires essential amount of particular fatty acids or generally known as essential fatty acids (EFA). Most lipid studies done on fishes have been emphasizing on EFA requirement and energy requirement.

There are many factors that can effects EFA requirement in different types of fish, such as environmental factors (salinity and temperature), effects of diets and seasonal variation. EFA requirement of fish in different habitat salinity differs to one another, marine fish is reportedly requires higher linoleic omega-3 polyunsaturated fatty acids (PUFA) compared to freshwater which adapt better to high omega-6

PUFA in their diets (Sargent *et al.,* 2002). Studies also showed that marine fish contained higher ratio of omega-3 to omega-6 PUFA compared to fresh water fish. Besides salinity, it is reported as well that cold water fishes such as salmon requires more omega-3 PUFA (Grant *et al.,* 2008) compared to those of warm water species.

Dietary lipid plays a big role in effecting fatty acid composition of fish. When dietary lipid contained high certain amount of fatty acids, the fish that feeds on will most probably exhibit an almost similar profile to the diet, as such high omega-3 PUFA in diet will cause the body composition or other parts of the fish to accumulate high amount of the omega-3 PUFA, although higher or lower omega-3/omega-6 will effects growth and survival of fishes differently (Sargent *et al.,* 2002). Some fish express seasonal changes in the fatty acid profile, for example the herring showed a different values in total lipid and iodine content at a certain period of month in a year.

2.3.4 Lipid and Fatty Acid Requirement in Fish

Dietary lipid requirement vary between species, stage of life, habitat, feeding habit and more (Biswas *et al.,* 2009). Understanding the optimal lipid requirement is important to yield optimal growth by the same time optimizing the usage of lipid incorporation in the feeds. Too much or too little lipid inclusion may affects growth and survival of fish, for example the tilapia optimal lipid level requirement is 12% and beyond that causes a reduce in feed efficiency (Chou & Shiau, 1996). Du *et al.,* (2008) emphasize on the important of lipid level evaluation as within limits, increasing level will not only improve feed utilization but also protect against the metabolism of protein as energy especially in carnivorous species. Most marine fish require high levels of lipid for growth and survival (Morais *et al*., 2001), this could be due to the low temperature and the ability of marine fish to utilize lipid for energy better than those of warm water fishes. Table 2.2 shows the statistics of optimum lipid requirement in several species of marine and freshwater fish.

Table 2.2 Optimum lipid requirement of Certain Species of Fish (Values are % lipid in Diet)*.

	Scientific Name	Optimum Lipid Requirement (%)
Marine species		
Pasific Bluefin Tuna	*Thunnus orientalis*	17.9
Grouper	*Epinephelus malabaricus*	9
Cobia	*Rachycentron canadum*	5.8
Freshwater species		
Bagrid Catfish	*Psedobagrus fulvidraco*	19
Hybrid Tilapia	*Oreochromis niloticus* × *Oreochromis aureus*	12
Chinese Longsnout Catfish	*Leiocassis longirostris*	14.3
Gibel carp	*Carassius auratus gibelio*	14.1

* Data compiled from Biswas *et al*. (2009), Kim and Lee (2005), Chou *et al*.(2001), Chou and Shiau (1996), Lin and Shiau (2003) and Pei *et al*.(2004).

Detailed information has been limited regarding of optimum lipid requirement in many species (Halver, 2009), and studies have been more focusing on the essential fatty acids requirement especially regarding fish oil replacement in the diets. Freshwater fish are known to have the ability to convert 18-carbon of both omega-3 and omega-6 series to a more highly unsaturated PUFA and marine in contrast do not have that capability (Glencross, 2009). The limitation and difference plays an important role in EFA requirement in various species of fish. Numerous studies have conclude that marine fish have a greater requirement for omega-3 fatty acids, while EFA requirement of some freshwater species can be satisfied by mixture of omega-6 and omega-6. Marine species like the salmon and red sea bream has a dietary requirement of high omega-3 fatty acids (Grant *et al.,* 2008). Glencross (2009) reported that red sea bream fed high omega-3 HUFA showed rapid growth and good feed conversion and the supply of 22:6n-3 (DHA) is especially essential for marine fish larvae. Other species such as turbot also exhibit better growth under high omega-3 PUFA than omega-6. Juvenile fat cod requires sufficient amount of 20:5n-3 (EPA) and DHA for normal growth (Lee and Cho, 2009). High requirement of omega-3 fatty acids among marine species can be associated with their carnivorous nature; while omnivorous have the ability to utilize short chain fatty acids and lowering their dependency on omega-3 HUFA. In certain species, the increase of omega-3 fatty acids might result in growth depression such as the tilapia (Lim *et al.*, 2008; Kanazawa *et al*., 1980; Huang *et al.,* 1998). Similar growth depression was also observe in African catfish and channel catfish (Ng *et al.,* 2001). Common carp, *Cyprinus carpio* required both omega-3 and omega-6 with requirement more towards 18-carbon PUFA (Halver, 2009). Du *et al*. (2008) reported that herbivorous grass carp has a low capacity to utilize high dietary lipid and excess HUFA content will result in adverse effect on growth performance. Satisfying EFA requirement in all species are important aspects of fish nutrition as failing to meet the requirement will cause EFA deficiency symptoms in the fish. Poor growth rate and high mortality are one of the sign of EFA deficiency and rainbow trout showed caudal fin erosion and shock syndrome (Glencross, 2009). Sinnhuber (1969) reported skin disorder in salmon fed lipid free diet, and EFA deficiency in tilapia can suppress immune function and increase the susceptibility of fish to infectious pathogens (Lim *et al.*, 2008).

2.4 Lipid Source for Aquaculture Feeds

Lipid source have been highly regarded as an important component in aquaculture, by not only supplementing essential nutrient but also helps increase acceptability and stability to the feed pellet. There are many source of lipid used in the industry today, ranging from fish oil, animal fat to vegetable base lipid. Generally, fish oil is a major dietary lipid source used in many aquaculture feeds (Ng *et al*., 2001) but due to the rising price and diminishing stocks in the wild, many researchers have turn to vegetable base oil and animal fats as cheaper substitutes. EFA deficiency and harmful components may still be an issue regarding the use of vegetable oils and animal fats but researchers are finding methods to implement the use of this cheap lipid source and reduce the usage of fish oil for the benefits of the industry.

2.4.1 Fish Oil

Fish oil is a major lipid source used in feeds of many aquaculture species such as cod (Morkore, 2006), salmon (Bell *et al*., 2001), tilapia (Ng *et al*., 2001) and other species. Fish like herring, mackerel, anchovies have been used to extract fish oil in the market, are rich and practically unique source of highly unsaturated fatty acids (HUFA) especially the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which also known to be health beneficial for human consumers (Bell *et al.*, 2001). Fish oil have been a preferred choice for its acceptability, nutrition value and Ng *et al.* (2001) also reported that fish oil helps to improve palatability and appearance of the diets. Fish meal has been reported to increase feed efficiency, growth, digestibility and absorption in many species of fish (Rinchard *et al*., 2007, Lee *et al.,* 1967). Fish oil has also been reported to help in cell and membrane structure development, EFA in fish oil are also necessary for larval development, growth, reproduction and also assist the immune system in fish where Sinnhuber (1969) reported that fish oil such as cod liver oil contained around 40 types of fatty acids compared to 8 to 10 types in vegetable oil.

Due to increasing demands and over dependant on the fish oil over the years, price have been increasing and demands could exceed supplies within decades (Bahurmiz & Ng, 2007). The declining supplies of fish like anchovies and herrings which are major fish oil suppliers are also contributed by over exploitation and climate changes over the past few years. Tacon & Metian (2008) have reported that aquaculture alone has consumed around 68.2 % of fish meal and 88.5 % of fish oil in 2006. Fish oil is still mostly used for aquafeeds for farmed carnivorous fish. Grant *et al*., (2008) also reported a state of decline in global fish stock which means decreasing stock for fish oil production. Besides high cost and limiting supplies of fish oil, there have been a report of a possible accumulation of dioxin and dioxin-like components in fish oil (Xue *et al*., 2006) which might be harmful for human consumption. Fish oil is still considered an important aquafeeds component, although it is predicted that there will be a declining trend of fish oil inclusion level in feed production on the long term as industries are looking into cheaper and more abundant alternatives.

2.4.2 Vegetable Oil

Vegetable oil holds a promising cost-effective partial replacement for fish oil (Grant *et al*., 2008). Rinchard *et al*,. (2007) considered vegetable oils as an excellent substitute for fish oil especially in freshwater aquaculture feeds because of the ability of freshwater fish to convert dietary linoleic (18:2*n*-6) and linolenic (18:3*n*-3) acids which are found abundantly in vegetable oil, into arachidonic acid (AA, 20:4*n*-6), EPA(20:5*n*-3) and DHA (22:6*n*-3) which are essential fatty acids for development in fish. Vegetable oil such as olive oil, soya bean oil, sunflower oil and corn oil contained high omega-6 PUFA especially of the linoleic acids. Canola oil is an example of a few vegetable that contained a balance amount of omega-6 and omega-3, which are also abundant in source (Grant *et al.*, 2008, Bakhshish *et al.*, 1984, Bakhshish *et al*., 1988).

Although cheap and almost unlimited, vegetable oil lacks numerous highly unsaturated fatty acids such as AA which can affect health and physiology of fish and even human consumers. Vegetable oil lacking HUFA might not be suitable especially for carnivorous marine species but can be used successfully as lipid source for most freshwater species as this species are capable to elongate 18 carbon fatty acids into HUFA such as LA and AA (Turchini *et al*., 2006). Regost *et al*.(2003) reported the effect of soybean and linseed oil significantly on the quality of turbot flesh particularly odor, color and texture compared to those fed fish oil. High content of PUFA, especially in vegetable oil fed fish, might also increase lipid peroxidation (Ng *et al*., 2001) which an effect of reducing freshness and shelf-life of seafood products and there has been a profound impact on the fatty acids composition of rainbow trout tissue with an increase in 18:2*n*-6 and 18:3*n*-3 and decrease in DHA and EPA (Rinchard *et al*., 2007). Recent studies conduct by Welker and Congleton (2004) showed that omega-6 fatty acids which are richly found in vegetable oil enhance the cortisol response to stress leading to an increase peroxidative damage to fish tissue. Most marine fish exhibit poorer growth rate under vegetable fed regime (Yildiz & Sener, 2004; Lee *et al*, 1967). Still, vegetable oil is a