OPTIMAL PROTEIN-LIPID LEVEL AND REPLACEMENT OF FISH MEAL WITH PLANT PROTEIN SOURCES IN FEEDS FORMULATED FOR *Pangasianodon hypophthalmus* (Sauvage, 1878)

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by

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TAHAP PROTEIN-LIPID YANG OPTIMAL DAN PENGGANTIAN SERBUK IKAN DENGAN SUMBER PROTEIN TUMBUHAN DI DALAM MAKANAN FORMULA BAGI *Pangasianodon hypophthalmus* (Sauvage, 1878)

Oleh

PREEDA PHUMEE

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ABBREVIATIONS

AA	**amino acid**
ArA	**arachidonic acid**
BBI	**Bowman-Birk inhibitor**
CMC	**carboxymethylcellulose**
CO	**corn oil**
CP	**crude protein**
DHA	**docosahexaenoic acid**
DFI	**daily feed intake**
DM	**dry matter**
EAA	**essential amino acid**
EAAI	**essential amino acids index**
EPA	**eicosapentaenoic acid**
FA	**fatty acid**
FAME	**fatty acid methyl esters**
FAO	**food and agriculture organization**
FBW	**final mean body weight**
FCR	**feed conversion ratio**
FID	**flame ionization detection**
FM	**fish meal**
FO	**fish oil**
GC	**gas chromatography**
GE	**gross energy**
HSI	**hepatosomatic index**
HPLC	**high pressure liquid chromatography**

IPF	**intraperitoneal fat**
LA	**linoleic acid**
LnA	**linolenic acid**
MUFA	**monounsaturated fatty acid**
mmt	**million tones**
NEAA	**non-essential amino acids**
NFE	**nitrogen free extract**
NPU	**net protein utilization**
NSPs	**non-starch polysaccharides**
OA	**oleic acid**
PA	**palmitic acid**
PER	**protein efficiency ratio**
PI	**Protein intake**
PPV	**protein productive value**
PUFA	**polyunsaturated fatty acid**
RGR	**relative growth rate**
SBA	**soybean agglutinin**
SBM	**soybean meal**
SBO	**soybean oil**
SFA	**saturated fatty acids**
SGR	**specific growth rate**
SP	***Spirulina***
SPC	**Soy protein concentrate**
SPI	**soy protein isolate**
TEAA	**total essential amino acids**

TNEAA	**total non-essential amino acids**
TI	**trypsin inhibitors**
TUI	**trypsin untis inhibited**
VSI	**viscerosomatic index**
WG	**weight gain**

TAHAP PROTEIN-LIPID YANG OPTIMAL DAN PENGGANTIAN SERBUK IKAN DENGAN SUMBER PROTEIN TUMBUHAN DI DALAM MAKANAN FORMULA BAGI *Pangasianodon hypophthalmus* (Sauvage, 1878)

ABSTRAK

Kajian ini dijalankan untuk mengetahui keperluan protein serta kualiti nutrisi kacang soya dan *Spirulina* (*Spiruluna platensis*) gred haiwan terhadap patin sutchi, *Pangasianodon hypohpthalmus.* Terdapat tiga eksperimen berbeza telah dijalankan di dalam kajian ini. Eksperimen yang pertama dijalankan bagi mengetahui nisbah protein dan lemak di dalam diet yang sesuai bagi ikan ini dan diikuti dengan eksperimen bagi menentukan tahap penggantian kacang soya dan *Spirulina* yang optimum untuk menggantikan serbuk ikan. Di dalam eksperimen yang pertama, kumpulan yang mengandungi sepuluh ekor ikan (3.69 ±0.18 g) dalam triplikat diberi makan dua kali sehari untuk lapan minggu. Terdapat lapan diet berbeza di mana setiap satu diet mengandungi kandungan tenaga yang sama (isoenergetik) yang terdiri daripada kombinasi empat peringkat protein berbeza (25%, 30%, 35% dan 40%) dan dua peringkat lemak berbeza (6% dan 12%). Sebagai keputusan, pertumbuhan ikan dipengaruhi oleh kandungan nisbah protein dan lemak. Di dalam peringkat protein yang sama, peningkatan peringkat lemak memberikan kesan kepada peningkatan pertumbuhan dan penggunaan makanan. Kandungan protein meningkat manakala kandungan lemak menurun di dalam badan apabila meningkatnya tahap kandungan protein di dalam diet. Keputusan menunjukkan bahawa terdapat kesan penyimpanan protein apabila ikan diberi diet yang mengandungi 30% protein dan 12% lemak. Ia juga menunjukkan peningkatan terhadap pertumbuhan dan penggunaan makanan berbanding ikan yang diberikan makanan yang mengandungi 40% protein protein dan 12% lemak. Di dalam eksperimen yang kedua, terdapat 15 ekor ikan per kumpulan (6.10 ± 0.16 g), lapan kumpulan kesemuanya di mana setiap kumpulan terdiri daripada triplikat. Terdapat lapan diet di mana setiap satunya adalah isonitrogenus (30% CP) dan isoenergetik (18MJ kg^{-1}) yang mengandungi 0-100% kandungan protein daripada serbuk kacang soya Argentina (SBM) menggantikan serbuk ikan, dan diberikan kepada ikan selama dua belas minggu. Pertumbuhan ikan, penggunaan makanan, penghadaman

kandungan kering dan protein menurun dengan meningkatnya kandungan serbuk kacang soya di dalam diet. HSI dan VSI meningkat dengan meningkatnya kandungan serbuk kacang soya di dalam diet. Kandungan asid lemak bagi otot ikan pula mencerminkan kandungan asid lemak di dalam diet yang diberikan. Asid palmitik, oleik dan linoleik menunjukkan antara kandungan asid lemak yang tertinggi di dalam otot ikan. Jumlah nilai n-6 PUFA di dalam otot ikan meningkat dengan meningkatnya kandungan serbuk kacang soya. Keputusan menunjukkan bahawa serbuk kacang soya boleh menggantikan sehingga 45% protein serbuk ikan di dalam diet juvenil ikan patin sutchi tanpa sebarang kesan buruk terhadap pertumbuhan, penggunaan makanan dan komposisi badan ikan. Di dalam eksperimen yang terakhir, terdapat sepuluh diet yang isonitrogenus (30% CP) dan isoenergetik (19 MJ kg^{-1}) diformulasi untuk menghasilkan diet yang mengandungi 55% protein daripada serbuk ikan dan 45% protein daripada kacang soya (diet berserbuk ikan yang tinggi) atau 40% protein daripada serbuk ikan dan 60% protein kacang soya (diet berserbuk ikan yang rendah). Di dalam setiap peringkat kandungan serbuk ikan/ serbuk kacang soya, *Spirulina* digantikan dengan 0% (SO), 15% (S15), 30% (S30), 45% (S45) dan 60% (S60) protein kacang soya. Setiap diet diberikan kepada kumpulan triplikat selama 12 minggu. Peningkatan tahap kandungan *Spirulina* di dalam diet meningkatkan pertumbuhan dan penggunaan makanan oleh ikan yang diberikan diet berserbuk ikan yang rendah tetapi tiada kesan terhadap komposisi otot ikan. Keputusan eksperimen menunjukkan penambahan *Spirulina* bersama dengan serbuk kacang soya Argentina mampu memberikan alternatif sumber protein yang baik di dalam diet juvenil patin sutchi.

OPTIMAL PROTEIN-LIPID LEVEL AND REPLACEMENT OF FISH MEAL WITH PLANT PROTEIN SOURCES IN FEEDS FORMULATED FOR *Pangasianodon hypophthalmus* (Sauvage, 1878)

ABSTRACT

The present investigation was carried out to evaluate protein requirement and the nutritional quality of soybean and animal grade *Spirulina* (*Spirulina platensis*) on sutchi catfish, *Pangasianodon hypohpthalmus*. A series of three experiments were carried out. Firstly, to determine the optimal dietary protein to lipid ratio followed by the determination of the optimum replacement level of fish meal with soybean meal and finally the evaluation of the nutritional quality of a combination of soybean meal and *Spirulina* to replace fish meal. In the first study, triplicate groups of ten fish (3.69 ± 0.18 g/fish) were fed twice daily for eight weeks with each of the eight isoenergetic diets comprising a combination of four protein levels (25%, 30%, 35%, or 40%) and two lipid levels (6% or 12%), respectively. The results showed that, the growth performance of fish was affected by dietary protein and lipid ratio. At the same protein level, increased dietary lipid levels resulted in increased growth and feed utilization. Body protein content increased while body lipid content decreased with increase in dietary protein levels. The results revealed the presence of a protein-sparing effect of lipid as fish fed on 30% protein and 12% lipid diet had growth and feed utilization comparable to those fed on 40% protein and 12% lipid diet. In the second experiment, the triplicate groups of 15 fish (6.10 ± 0.16 g/fish) were fed eight isonitrogenous (30% CP) and isoenergetic (18MJ kg^{-1}) diets which contained 0% - 100% dietary protein from Argentine soybean meal (SBM) in replacement of fish meal, for twelve weeks. Growth performance, feed utilization, dry matter and protein digestibility worsened with the increase in soybean meal. The hepatosomatic index (HSI) and viscerosomatic index (VSI) increased with increasing soybean meal in the diet. The fatty acid content of fish muscle mirrored fatty acid content of tested diets which were used in this study. Palmitic acid, oleic acid and linoleic acid represented the high value among all fish muscle. The total n-6 PUFA value in fish muscle increased with increasing soybean meal level. Results showed that soybean meal can replace up to 45% of fish meal protein in the diets of juvenile sutchi catfish without any adverse effect on growth, feed utilization and body composition. In the final study, ten isonitrogenous (30% CP) and isoenergetic (19 MJ kg^{-1}) diets were formulated to provide either 55% fish meal protein and 45% soybean protein diets (high-fish meal based diets) or 40% fish meal protein and 60% soybean protein diets (low-fish meal based diets). Within each fish meal /soybean meal level, *Spirulina* substituted 0% (S0), 15% (S15), 30% (S30), 45% (S45) and 60% (S60) of soybean protein. Each diet was fed to three replicate groups of fish for 12 weeks. Increasing *Spirulina* levels improved growth performance and feed utilization of fish fed low-fish meal based diets but had no effect on fish muscle composition. The results of the study showed that the inclusion of *Spirulina* integrated with an Argentine soybean could be a good alternative protein source in diet for juvenile sutchi catfish.

CHAPTER 1

INTRODUCTION

1.1 Background of Aquaculture

Aquaculture remains the fastest growing segment of global food production in the last three decades, especially in developing countries. The growth of aquaculture is encouraged by the ever-increasing demand for fish because of the expanding world population and the limited supply of wild fish. Aquaculture accounts over 30% of commercial fish and shellfish production annually (FAO, 2009). In 2006, the world consumed 110.4 million metric tonnes (mmt) of fish, about 51.7 mmt (46.8%) of which originated from aquaculture (FAO, 2009). It is estimated that by 2020, aquaculture will produce over 68 mmt, accounting for approximately 40% of global seafood production. It is also predicted that in 2030 aquaculture would be expected to produce an additional 28.8 mmt – providing a total of 80.5 mmt overall (FAO, 2009).

Aquaculture sector is still highly dependent on marine captured fisheries as a source for its dietary protein, especially fish meal. Obviously, the growth of aquaculture sector is also accompanies by the need for the supply of feed to meet the demand. Additionally, there has been a growing trend towards the increased use of compounded feeds for farmed fish. Total compounded fish feed production in 2003 was 19.5 mmt (Tacon *et al.*, 2006). This production was increased to between 20.2 and 22.7 mmt in 2006. (Tacon & Metian, 2008) and in 2010, the predicted production volume is 37.2 mmt (Hardy, 2006).

Generally, fish meal is one of the main ingredients in compounded fish feeds and fish meal consumption by the aquaculture sector is expected to increase. In

2003, aquaculture sector consumed a total of 2.94 mmt of fish meal (Tacon *et al.*, 2006) and this amount increased to 3.72 mmt in 2006 constituting 68.2% of total fish meal production (Tacon & Metian, 2008).

Due to the increasing cost of fish meal, aquaculture sector is thus facing particular challenges in identifying substitutes for fish meal of lower cost source of protein in order to maintain both the quality and quantity of compound fish feed. Cost of fish feed accounts for more than 50% of the operating costs of production (Sehagal & Toor, 1991), therefore, it is of great importance to culturists to improve cost effective fish feed. Aquaculture nutritionists are thus currently challenged to formulate diets which meet the nutritional requirements, minimize costs, limit environmental impacts and also enhance product quality. The general approach to reduce feed cost are (1) replacing expensive animal protein source (fish meal) with relatively less expensive plant protein sources, (2) improving the utilization of feed by diet composition and/or feeding management strategies.

The search for protein sources to replace fish meal in compounded fish feed has effects on the continued growth and sustainability of aquaculture production. Fisheries by-catch is one of the potential sources of protein. Products such as fish protein concentrate, fish hydrolysate and fish silage have received relatively high considerations (Hardy *et al.*, 2005; Li *et al.*, 2004).

Currently, the various plant feedstuffs traditionally used as protein sources include oilseed, legumes and cereal grains (Heikkinen *et al.*, 2006; Shafaeipour *et al.*, 2008). However, they often lack of essential amino acids, rich in complex carbohydrates and contain anti-nutritional compounds (Francis *et al.*, 2001). Soybean meal has been demonstrated as an effective protein source for omnivorous and herbivorous fish species (Boonyaratpalin *et al.*, 1998; Carter & Hauler, 2000; Mulumpwa & Kang'ombe, 2009). However, the inclusion of soybean meal in fish feed is limited, especially in carnivorous fish feed because soybean contains low levels of lysine and methionine, high fibre content and anti-nutritional factors such as protease inhibitors, lectins, phytic acid, saponin, phytoestrogen, antivitamin and allergens (Francis *et al.*, 2001).

Several methods used to remove or inactivate anti-nutritional effects of plant proteins sources have been suggested, including; processing treatments, enzyme (phytase) incorporation, combination with other protein sources and genetically improvement (Barrows *et al.*, 2008; Biswas *et al.*, 2007).

Sutchi catfish (*Pangasianodon hypophthalmus*), commonly known as “basa”, “ca tra”, “Swai”, “Thai pangus”, “Vietnamese catfish” and “Pangasius catfish”, is an important species for aquaculture in Asian countries because of its fast growth and tolerance to low water quality (Phan *et al.*, 2009). Sutchi catfish is a freshwater fish species with omnivorous feeding behaviours. The fish is distributed in Southeast Asia: Mekong, Chao Phraya, and the Mekong basins and was introduced into river basins for aquaculture (Roberts & Vidtayanon, 1991).

Culture of Pangasius catfish started in the late 1990s and has rapidly developed in recent years along the Mekong River Delta. The three typical culture systems for Pangasius are cage, earthen pond and fence culture within these three aquaculture systems, the production of pond culture has increased rapidly (Phuong *et al.*, 2007). Vietnam is known as the major producer of Pangasius in the Asian region (Phuong & Oanh, 2010). In 2005, Vietnam supplied 89% of the world production while Malaysia had a share of 1% of the world production. The major markets for

Pangasius fish are the European Union, Russia, Southeast Asia and United States (Corsin, 2005).

In general, feed type used in Pangasius culture varies according to farm size. The large-scale farms tending to favour the manufactured feed pellets or commercial diet whereas small-scale operations often use farm-made feed, which trash fish is the major component and contains high level of moisture (Corsin, 2005). However, the constraints of trash fish usage in farm-made feed are the quality and availability of fish, which deliver on site in decomposed form and increase in the price. Hence it led to the promotion of use of formulated diet under certain condition (Hung *et al.*, 2007).

Feed cost is an important parameter which affects profitability in aquaculture investments, as it usually constitutes 65 to 70% of total operating costs. Therefore, the selection of appropriate feeds is of primary concern to farmers (Phu & Hien, 2003).

1.2 Problem Statement

General approaches are proposed to reduce feed cost for sustainable aquaculture. These include optimized feeding strategy, development of low-cost feed and improved nutritional value of feed ingredients. In this study, soybean meal was used to substitute fish meal and the improvement of the nutritional value of soybean based diet by incorporating *Spirulina* for sutchi catfish were considered.

Protein is a major factor affecting growth of fish and also an important source of energy. It has a tremendous effect on the cost of feed. Generally, fish meal is the main protein source in compounded fish feeds and fish meal consumption by the aquaculture sector is increasing day by day. The price of fish meal has risen because of the static production and coupled with increasing demand. It is becoming uneconomical for use in fish feeds. There is need to look for alternative protein sources. One such possible alternative protein source is soybean meal due to its ready market availability and cost.

A number of studies had been reported that soybean meal can be used as an ingredient of fish feed for many species. The levels of soybean meal that can be inclusion into the diets is variable based on species of fish, age, fish meal quality and soy product. Previous studies reported that the inclusion of soybean meal in fish feed at high level depresses growth and feed utilization of fish (Mulumpwa & Kang'ombe, 2009; Zhou *et al.*, 2005). This is because soybean has an anti-nutritional factors, such as trypsin inhibitors, which affect enzyme activity (Alarcón *et al.*, 2001; Tibaldi *et al.*, 2006). The mixture of vegetable origin proteins is considered as substitute for fish meal in fish feed especially in diets for omnivorous fish species (Hansen *et al.*, 2007; Lee *et al.*, 2002a). *Spirulina* is a good source of protein, of preferable quality: high digestibility, high level of protein and essential amino acids (Belay *et al.*, 1996; Clément *et al.*, 1967). Furthermore, soybean contains low levels of lysine and methionine, whereas *Spirulina* has higher level of these two amino acids comparison to soybean used in the present study. The mixture of soybean and *Spirulina* is assumed to be suitable protein source to replace fish meal in the diet for sutchi catfish.

This research is divided into three main studies, purposely designed to determine the effects of soybean based diet on growth performance, feed utilization and flesh quality of juvenile sutchi catfish. The first phase of these studies looked at the optimum protein and lipid ratio in the diet for juvenile sutchi catfish. The second phase involved the replacement of fish meal with soybean meal. The last phase was about the improvement of soybean based diet nutritional by the inclusion of *Spirulina*.

1.3 Research Objectives

The main objective of the study was to develop a low-cost feed for *Pangasianodon hypophthalmus* (Sauvage, 1878)

Measurable objectives:

1. To determine the optimum protein lipid ratio for growth of juvenile sutchi catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878).
2. To evaluate the effects of replacing fish meal with soybean meal in formulated diets for juvenile sutchi catfish (*P. hypophthalmus,* Sauvage, 1878).
3. To improve the nutritional value of soybean based diet by mixing with *Spirulina* for juvenile sutchi catfish (*P. hypophthalmus*, Sauvage, 1878).

CHAPTER 2

LITERATURE REVIEW

2.1 Aquaculture Challenges

The aquaculture sector has increased rapidly in the last three decades. The rapid growth has been due to a combination of increasing demand because of world population growth and static production in the wild capture fishery (FAO, 2009). Aquaculture continues to be the fastest growing sector. FAO (2009) reported that aquaculture production was less than 1 mmt in the early 1950s and rose to 51.7 mmt in 2006. The sector increased the supply of food fish from 0.7 kg per capita in 1970 to 7.8 kg per capita in 2006. The Asia-Pacific region is the dominant aquaculture producer.

The world production of capture fisheries has been relatively stable. World capture - production rose from 93.2 mmt in 2002 to 94.6 mmt in 2004. Then slightly declined to 94.2 mmt in 2005 and to 92.0 mmt in 2006 (FAO, 2009). Meanwhile, human consumption of food fish increased from 100.7 mmt in 2002 to 110.4 mmt in 2006. Aquaculture accounted for 47% of that consumption (FAO, 2009). Therefore, production from fisheries will not be able to meet global demand for fish from an expending world population. Hence, aquaculture is considered important to maintain fish consumption at current levels. Since aquaculture is still growing, the supply of aquafeeds must also grow to meet demand. Aquafeeds will continue to grow from around 22 mmt in 2005 to around 32 mmt in 2012 (Jackson, 2007).

Fish meal has been relied on as the major protein source in fish feed because it is very high in protein (can be higher than 70%), essential amino acids, essential fatty acids and minerals (Gatlin III *et al.*, 2007). Fish meal has been reported to offer major benefits to fish health, including improvements in the immune system, higher survival and growth and a reduced incidence of deformities. In addition, it has highly digestible protein which leads to increased growth and less wastage of feed and is considered to increase the appeal of feed. This encourages high consumption of fish meal in aquafeeds. Aquaculture has consumed increasing amounts of fish meal, rising from 45 % in 2002 to 57% in 2006 (Jackson, 2007).

Global fish meal production in the first quarter of 2009 was 433 000 tonnes. Overall fish meal production in 2009 is likely to be in line with 2008 production, or slightly lower (Josupeit, 2009). It is expected that this static production coupled with increasing demand for fish meal will increase its price. The price of fish meal has risen dramatically since 2006, to levels higher than USD 1000 per tonne (Figure 2.1) (Josupeit, 2009).

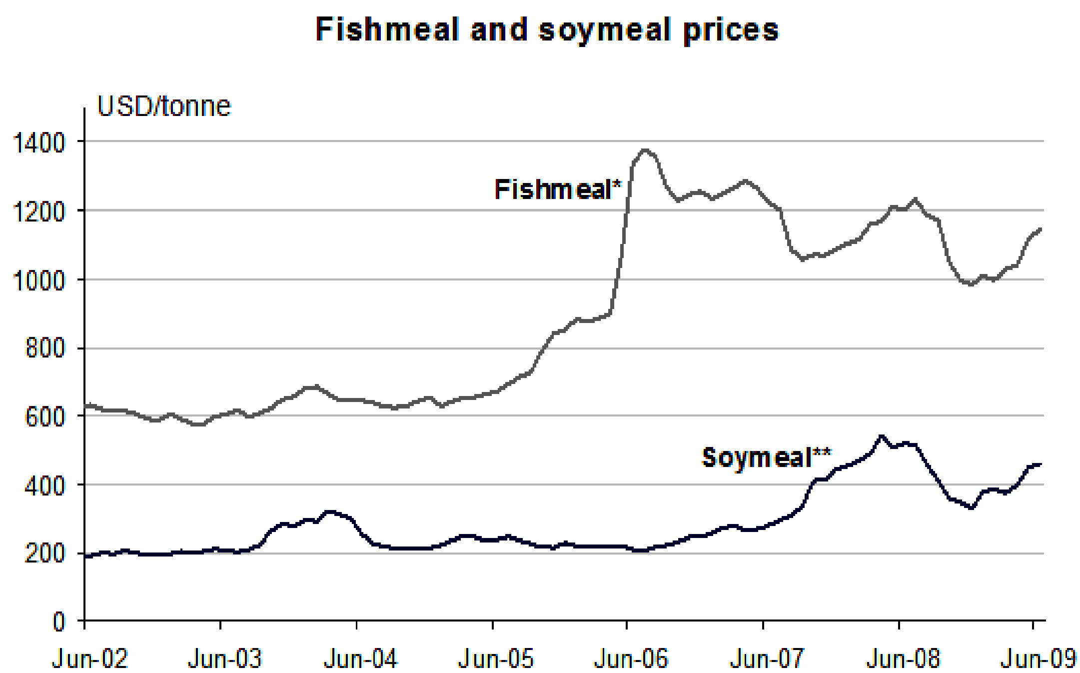

Figure 2.1 Prices of fish meal and soybean meal
Source: Fish meal market report –August 2009 (Josupeit, 2009).

2.1.1 Feeds Costs and Solution

Aquaculture production costs are rising because of the increasing of price of fish meal. Generally, feed costs account for more than 50% of variable operating costs of an aquaculture operation, and protein is the most expensive dietary component in fish feed (Bright *et al.*, 2005). One way that the aquaculture sector can become sustainable is to reduce feed costs. Approaches to reduce feed costs include using mixed feeding schedules and changed formulations. The commonest approach is to replace fish meal, the most expensive ingredient, by cheaper protein sources, and to decrease dietary protein by increasing non-protein energy (De Silva, 2006).

Fish meal replacement by less expensive sources of protein is one approach to reduce feed costs. The development of fish feeds has involved strong efforts to define and develop cost-effective protein sources that can, at least partly, substitute for expensive high-quality fish meal (Albrektsen *et al.*, 2006; Lim & Lee, 2009; Refstie *et al.*, 2001). Fish meal can be replaced with protein derived from a range of non-fish sources such as by-products from animal processing, oil seed plants, zooplankton, micro-algae or even insects and bacteria (Ai *et al.*, 2006; Atack *et al.*, 1979; Tadesse *et al.*, 2003). Although alternative protein sources have high protein content, they may be deficient in one or more essential amino acids. For example, animal by-product meals have low amount of lysine, isoleucine and methionine (El-Sayed, 1998; Millamena, 2002), soybean meal is low in methionine (Hernández *et al.*, 2007), and wheat gluten is low in lysine (Hansen *et al.*, 2007). Microalgae, in particular, is seen as a very promising alternative for fish meal because it has a very high protein content, and may also be rich in omega 3 fatty acids and easy to grow in large quantities (Harel *et al.*, 2002; Yaşar & Şevket, 2006).

In addition, inclusion of non-protein energy, like lipids and carbohydrates, in diets at optimum levels can minimize the amount of protein used for energy, and it can contribute to reduced feed costs. Protein is the most expensive feed component, but optimum dietary energy may be more important because a deficiency can result in protein used to meet energy requirement rather than for protein synthesis. Thus, incorporation of dietary non-protein energy can reduce the oxidation of protein to energy, and hence increase the use of dietary protein for growth (Salhi *et al.*, 2004). However, excess energy leads to poor feed utilization, suppressed fish growth, and increased deposition of carcass lipid (Ai *et al.*, 2004; Wang *et al.*, 2006b). Juvenile red drum fed a high-energy diet had lower weight gain related to reduced nutrient intake especially of protein and also had high fat deposition in the peritoneal cavity, and reduced ammonia production (McGoogan & Gatlin III, 1999). Fish fed high energy diets showed a reduction in glutaminase activity, that enzyme releases ammonia while catabolizing glutamine to glutamate, which would explain the lower ammonia production (McGoogan & Gatlin III, 1999). Dietary protein has gradually been replaced by lipid to reduce the catabolic loss of ingested protein (Kim & Lee, 2005; Lee *et al.*, 2002b; Miller *et al.*, 2005).

2.2. Sutchi Catfish

2.2.1 Biology, Morphology and Classification

Sutchi catfish, *Pangasianodon hypophthalmus,* was formerly known as the irisdescent shark, *Pangasius hypophthalmus.* It is also known as the Siamese shark, swai (Thai), striped catfish, panga, Thai pangus (Bangladesh), and ca tra (Vietnam). This species is found in Southeast Asia in the Mekong basin as well as the Chao Phraya River, and is heavily cultivated for food. It has also been introduced into other river basins as a food source, and is commonly kept as a hobby (Roberts & Vidtayanon, 1991).

Sutchi catfish is omnivorous, feeding on fish and crustaceans as well as on vegetable debris. It is a freshwater fish that native to a tropical climate, preferring pH of 6.5-7.5, water hardness of 2.0-29.0 dGH and temperature of 22-26°C. This species is a migratory fish that moves upstream to spawn during the flood season when water levels are high and returns downstream to seek rearing habitats when water levels recede (Rainboth, 1996).

This species has scale less skin with a long slender body and a dark gray or black dorsal fin which has one hard fin ray and six soft fin rays. An adipose fin is situated between the dorsal and caudal fin. The juveniles have a black stripe along the lateral line and a second long black stripe below the lateral line (Figure 2.2) (Rainboth, 1996). However, the adults are uniformly grey with a dark stripe on the middle of the anal fin and in each caudal lobe, and small gill rakers regularly interspersed with larger ones. The mouth is located in a low position with two pairs of barbells, of which the longer pair is on the upper jaw (maxillary barbell) and the shorter pair is on the lower jaw (mandibulary barbell) (Kottelat, 2001).

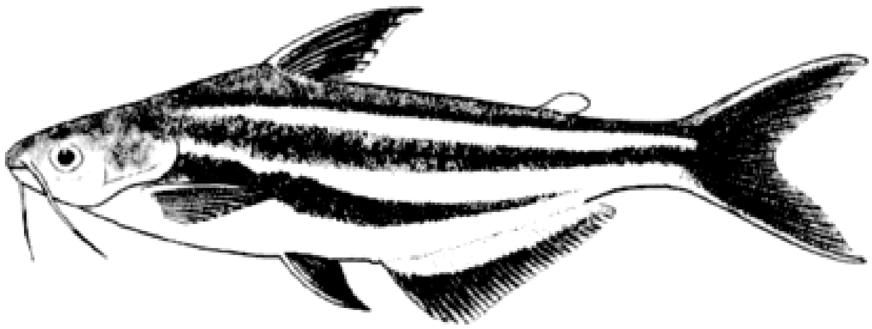

Figure 2.2 *Pangasianodon hypophthalmus* (Sauvage, 1878)

Source: modified from Rainboth (1996)

Scientific classification

Kingdom	Animalia
Phylum	Chordata
Super-class	Osteichthyes
Class	Actinopterygii (ray-fined fishes)
Sour-class	Neopterygii
Infra-class	Teleostei
Super-order	Ostariophysi
Order	Siluriformes (catfish)
Family	Pangasiidae (shark catfishes)
Genus	*Pangasianodon*
Species	*hypophthalmus*

Scientific name *Pangasianodon hypophthalmus* (Sauvage, 1878)

Synonym: *Helicophagus hypophthalmus*, Sauvage 1878,

Pangasius hypopthalmus, Sauvage, 1878,

Pangasius sutchi, Fowler, 1937

2.2.2 Aquaculture

The culture of *Pangasius* catfish has developed rapidly in recent years. This fish is popular because of its fast growth rates and high productivity. It is cultured in many Southeast Asian countries, such as Vietnam, Thailand, Bangladesh and Malaysia (Amin *et al.*, 2005; Hien *et al.*, 2010). The two main culture systems used are cage and earthen pond culture (Phu & Hien, 2003). The most valuable species are sutchi catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878), and basa, *Pangasius bocourti* (Sauvage, 1880) (Więcaszek *et al.*, 2009). The products are mainly exported as fillets to European and American markets. Vietnam is the main exporter, followed by China and Thailand. In the first eight months of 2009, Vietnam had exported 334,000 tonnes (Josupeit, 2009).

The ability to raise fish at a high density to maximize production is being developed in *Pangasius* culture. Stocking density is important for fish production. Understocking fails to make the maximum possible use of space, and overstocking may result in stress, leading to enhanced energy requirements that contribute to reduced growth and feed utilization (Rahman *et al.*, 2006). An effect of density on growth had been reported in many fishes. High density leads to lower growth and feed utilization, and reduced disease resistance in Nile tilapia (Yi *et al.*, 1996). Rahman *et al*. (2004) studied the effect of density on growth of *P. hypophthalmus* in earth ponds. Fish were fed diets containing 30% protein for 120 days. The results showed that the most suitable density with respect to growth performance and profits was 100 fish/decimal (approximate 2.5 fish/m^2, 1 decimal= 40.46 m^2).

Stocking density affects on the yield, such that high stocking density results in higher yield per unit of production costs. A density of 150 fish m^{-3} has been reported to produce the best production and economics (Rahman *et al.*, 2006). The optimal stocking density will depend on capital costs, fish growth, market price and the size of fish to maximize production (Merino *et al.*, 2007).

2.2.3 Feed

Many kind of commercial diet have been produced since the rapid development of *Pangasius* catfish culture, although the use of pellets is still limited. The cage culture system has used 96 % of homemade feeds (or farm-made feeds) (Phu & Hien, 2003). Feed cost is an important factor affecting the profit of production. The average feed costs typically comprise more than 80% of the total variable production costs in this industry, varying from 73.6 % on farms using farm-made feed to 92.5 % on those using manufactured pellets (Phuong *et al.*, 2007). The fish feed type used in *Pangasius* culture varies according to farm size. Large-scale farms tend to favour the commercial diets whereas small-scale operations often use farm-made diets (Corsin, 2005).

2.2.3.1 Homemade Feed

The minimization of feed costs leads to more profits. Thus most farmers used farm-made feeds for *Pangasius* catfish culture. Although pellets or commercial feeds have been introduced to fish farmers, the proportion of pellets used is very low compared to farm-made feeds. Because of the cost of pellets is higher than farm-made feeds which were made from locally available and cheap ingredients. Pellets have been supplemented at the beginning of crops when fish are small (Phu & Hien, 2003).

Feeding is divided into two stages. The first stage uses feed containing high levels of protein and minerals. The second stage (last three months) uses high concentrations of carbohydrates to fatten fish. Hence, farmers can change the ingredients to reduce the costs of feed. Farm-made feeds contain 15-25% protein.

Farm-made feed has been improved. The former practise was to use agricultural by-products (rice bran and broken rice), vegetables (water spinach, squash, carrots, etc.), and trash fish in the proportions 40, 45, and 15%, respectively. Later, the main ingredients were changed to trash marine fish (30 to 40%), and rice bran (60 to 70%) depending on the size of fish and the investment capacity of the farmers. Recently, vitamins and minerals have been supplemented to farm-made feed to improved nutritional balance (Phuong & Oanh, 2010). However, farm-made feed contributes to high fat deposition in the abdomen of fish which reduces the quality and production of the fillets, while failing to meet the requirements for low fat products (Phu & Hien, 2003).

2.2.3.2 Commercial Feed

Although fish fed commercial feed or pellets gave better feed conversion ratio than those fed farm-made feed, the cost for one kilogram of fish produced is higher than for farm-made feed. Hence, pellets have not been as acceptable to fish farmers. If more attention is paid to product quality, then pellets would be expected to replace farm-made feeds for *Pangasius* catfish culture because farm-made feed contributes to the low quality of fillets (Phu & Hien, 2003).

2.3 Nutritional Requirements

Increased understanding of the information of the nutritional requirements of fish has been encouraged by the development of the aquaculture industry, which is dependent on artificial feeds. Since the aquaculture industry has continued to grow, the study of nutritional requirements is increasingly important (Cowey & Cho, 1993). Fish feeds are currently being developed to improve growth, feed utilization and health of fish. Formulated diets have to contain balanced and good nutrition which are necessary for healthy growth. The nutritional requirements of fish need to meet the formulations of high quality diets that promote growth and feed utilization whilst minimizing waste and thus contribute to sustainable aquaculture. Those are the main considerations for reducing feed costs (Oliva-Teles, 2000). Moreover, the effect of fish nutrition on the quality of fish flesh, including colour and appearance, smell and taste, texture, shelf life and nutritional quality must also be concerned (Lie, 2001).

Further, consumers are becoming more concerned with how fish are produced, and what types of feed ingredients are used. Authorities have an increased focus on food safety and the traceability of production from egg to plate. The need for improved knowledge of fish nutrition is therefore great (Lie, 2001).

2.3.1 Proteins

Studies of nutritional requirements focus on protein since it is the principle dietary component for the growth and health of fish. Furthermore, it is the most expensive nutrient in commercial diets (Abidi & Khan, 2007). The optimum dietary protein level is influenced by several factors including; the protein to energy ratio, digestibility and quality of protein and the amount of non-protein energy presence in the diet (Bright *et al.*, 2005; Guimarães *et al.*, 2008; Saavedra *et al.*, 2009).

The optimal protein requirement for growth has been estimated for some *Pangasius* catfish. Jongyotha *et al*. (2003) reported that 35% protein was optimal for juvenile (average weight 24.3g) snail eater (*Pangasius conchophilus*, Robert and Vidthayanon, 1991). Protein requirements for *P. bocourti* and *P. hypohpthalmus* were in the range of 12-13 and 11-12 g/kg/day, respectively (Hung *et al*., 1998). Chuapoehuk and Pothisoong (1985) fed *P. hypophthalmus* fry (average body weight 0.2 g) with diets containing 20-50% protein for 60 days in circular concrete tanks. The results indicated that diets containing 25% protein produced optimum growth. The estimates of optimal dietary protein levels probably vary due to differences in the size of fish, temperature, stocking density, amount of non-protein energy in the diet, and the quality of protein sources used in the trials.

2.3.2 Lipids

Dietary lipids serve as an important source of energy and essential fatty acids that are needed for normal growth and development. Lipids contain more energy per unit weight than dietary protein and carbohydrate. Lipids can increase feed palatability, reduce dust and improve stability of pellets during transportation and storage. Further, high lipid diets can reduced water pollution (Chaiyapechara *et al*., 2003)

In general, the diets containing 10-20 % lipid gave optimal fish growth (De Silva & Anderson, 1995). The current trend is to increase the amount of lipid in diets to spare proteins, improve feed utilization and minimize the amount of waste (Kim & Lee, 2005; Vergara *et al*., 1999). However, excess dietary lipid may result in excessive fat deposition in the visceral cavity and flesh which would affect product quality and storage (Hemre & Sandnes, 1999; Hossain *et al*., 2005; Tocher *et al*., 2003). The fatty acid requirements of fish need to be considered as the fatty acid composition of the dietary lipid has an influence on tissue fatty acid composition (Blanchard *et al.*, 2008; Lee, 2001).

Lipids were considered a source of essential fatty acids. Lately, studies on lipid requirements of fish appear to focus on the requirements for polyunsaturated fatty acids (PUFAs). They have been recognized as an important part of human nutrition. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been particularly noted for their therapeutic effects (Bell *et al.*, 2002).

Information on lipid requirements of *Pangasius* catfish has been scarce. Some studies have reported energy requirements. Hung *et al.*(1998) reported that energy requirements were 128 kJ kg^{-1} day^{-1} for the maintenance of *P. bocourti* and 92 kJ kg^{-1} day^{-1} for *P. hypophthalmus*.

2.3.3 Carbohydrates

Carbohydrates are the least expensive source of dietary energy, and are derived from plant sources, such as grains, legumes and oilseed. Plant carbohydrates are classified as energy reserve polysaccharides or as structural polysaccharides (non-starch polysaccharides; NSPs). Starch is the predominant energy reserve carbohydrate, and is referred to as digestible carbohydrate, while NSPs are referred to as indigestible carbohydrate (Stone, 2003).

It has been demonstrated that fish do not require carbohydrates. If fish feed does not provided carbohydrates, then other compounds, such as protein and lipids, are catabolized for energy, and for various biologically compounds usually derived from carbohydrates (Wilson, 1994). Thus, it is important to provide the appropriate amount of carbohydrates in fish feed to reduce protein and lipid levels in the diets which may lead to a reduction in feed costs for fish production.

However, the ability of fish to utilize carbohydrates as an energy source varies with respect to fish species, dietary inclusion level, feed intake, complexity of the molecule and the technological treatment applied (Stone, 2003; Wilson, 1994). Warm-water fish can use greater amounts of dietary carbohydrate than cold-water and marine fish (Wilson, 1994). Carbohydrate, mainly starch, is the main source of energy. Based on the findings of many researchers, the recommended inclusion of starch is up to 20% for carnivorous fish, 40% for warm-water omnivorous fish (Stone, 2003) and 42 % for juvenile white sae bream (Sá *et al.*, 2008). For herbivorous, such as Nile tilapia, a diet containing 60% cassava starch did not reduce growth (Wee & Ng, 1986). Cacot (1994 cited by Hung *et al*. 2003) reported that *P. bocourti* and *P. hypophthalmus* fed with a moist paste of dry pellets containing a large amount of carbohydrates-rich feedstuffs, which can be 60-80% starch. Hung *et al*. (2003) studied the utilization of starch in fingerlings of *P. bocourti* and *P. hypophthalmus*. Fish were fed a starch intake ranging from 0 to 40 g kg^{-1} day^{-1}. The results clearly showed that maximum growth occurred for fish fed diets containing 60% and 20% starch for *P. bocourti* and *P. hypophthalmus*, respectively.

2.4 Proteins and Amino Acids

2.4.1 Introduction

The word protein derives from the Greek word *proteus,* meaning first. Proteins are macromolecules that contain 50-55% carbon, 21.5-23.5% oxygen, 15.5-18 % nitrogen, 6.5-7.5 % hydrogen, and small amounts of phosphorus and sulphur (Jobling, 1994). Proteins were the first substances to be recognized as a vital part of living tissue, making up about 65 – 75 % of the dry weight of most animal cells (Wilson, 2002).

Proteins are assembled from amino acids. About 20 amino acids are commonly found in most proteins. Proteins are linear polymers of amino acids formed by peptide bonds between the carboxyl group of one amino acid and the amino group of the next (Figure 2.3). The amino acid composition of proteins determines their quality and value as a feed constituent (Moran *et al.*, 1994).

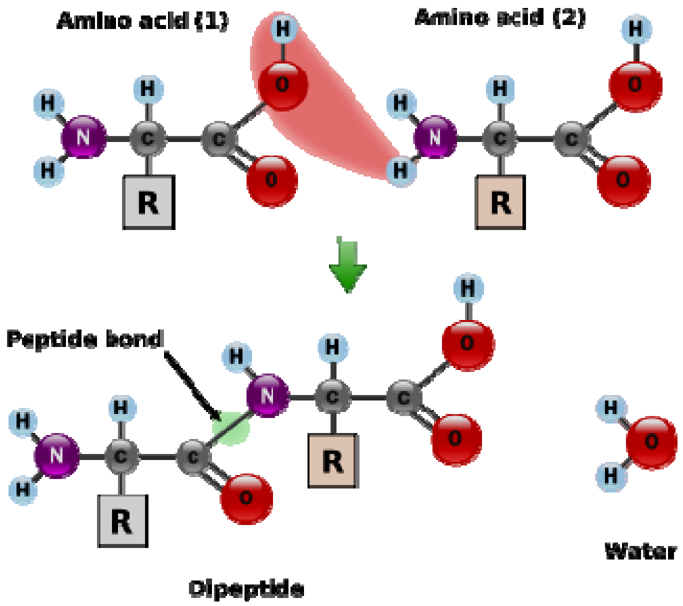

Figure 2.3 A peptide bond which links two amino acids

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