THE NUTRITIVE VALUE OF CORN PROTEIN CONCENTRATE AS A FISH MEAL REPLACEMENT IN THE FEEDS OF RED HYBRID TILAPIA, *Oreochromis* sp.

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ABBREVIATIONS

ADC	Apparent digestibility coefficient
ANF	Anti-nutritional factors
CF	Condition factor
CGM	Corn gluten meal
CPC	Corn protein concentrate
DBL	Dried basil leaves
FAO	Food and Agricultural Organization
FBW	Final body weight
FCR	Feed conversion ratio
FFSB	Extruded full-fat soybean
FI	Feed intake
FM	Fish meal
GSI	Gonadosomatic index
HSI	Hepatosomatic index
IBW	Initial body weight
IPF	Intraperitoneal fat
NEAA	Non-essential amino acids
PER	Protein efficiency ratio
SBM	Soybean meal
SGR	Survival growth rate
SPC	Soy protein concentrate
VSI	Viscerasomatic index
WG	Weight gain

NILAI NUTRISI PROTEIN PEKAT JAGUNG SEBAGAI PENGGANTI SERBUK IKAN DALAM DIET IKAN TILAPIA HIBRID MERAH, *Oreochromis* sp.

ABSTRAK

Tujuan kajian ini dijalankan adalah untuk menyiasat kesan prestasi pertumbuhan dan nisbah kecekapan makanan terhadap ikan tilapia hibrid merah, *Oreochromis* sp., yang diberi diet mengandungi protein pekat jagung (CPC) sebagai pengganti serbuk ikan (FM). Dalam eksperimen pertama, lima isonitrogenus (35% protein) dan isolipidik (10% lemak) diet dengan campuran peratusan yang berbeza iaitu 0, 25, 50, 75 atau 100% telah dirumuskan dan diberi dua kali sehari kepada tiga replikasi tilapia (berat badan awal: 10.33 $\pm$0.02 g) selama 63 hari. Berat badan akhir (FBW) yang tinggi relatifnya (56.37 – 62.72 g) telah didapati pada pemakanan yang mengandungi CPC sehingga 50%. Bagi diet CPC75 dan CPC100, FBW telah mengurang dengan ketara, iaitu masing-masing pada 45.68 dan 16.8 g (P<0.05). Nisbah Penukaran Makanan (FCR) dan Pengambilan Makanan (FI) antara diet tidak menunjukkan perbezaan yang ketara kecuali diet CPC100 (P>0.05). Bagi komposisi seluruh badan, peratusan protein mentah dalam badan tilapia menunjukkan trend yang semakin mengurang secara beransur-ansur dalam pemakanan tilapia diet yang mengandungi CPC yang semakin tinggi secara peratusan. Bagi peratusan lemak dalam badan tilapia pula, ikan-ikan tidak memberi perbezaan yang ketara. Pekali Penghadaman Jelas Protein (ADCP) menunjukkan peningkatan dari 80.63 ke 84.65 % apabila diberikan diet yang berprotein pekat jagung dari diet kawalan sehinnga CPC75. Pengurangan yang ketara telah didapati pada diet CPC100, iaitu

pada 73.65%. Bagi kesan perubahan warna pada kulit dan fillet, tilapia yang diberi CPC100 telah menunjukkan nilai ketara yang tertinggi dalam nilai-b (masing-masing pada 7.89 dan 3.66) berbandingan dengan pemakanan diet yang lain. Bagi jumlah karotenoid dalam fillet, nilai ketara yang terendah dan tertinggi didapati masing-masing pada tilapia yang diberi diet kawalan (0.0162 mg/kg) dan CPC100 (0.0353 mg/kg). Dalam eksperimen kedua, tujuh isonitrogenus (35% protein) dan isolipidik (10% lemak) dengan campuran CPC telah dicampurkan dengan 25% protein pekat soya (SPC) untuk meningkatkan kualiti keseluruhan profil nutrisi dan juga ditambah dengan 0.5% Betaine-HCl dan 2% daun selasih kering (DBL) secara alternatif sebagai makanan penarik (FA) yang dapat meningkatkan kesedapan dan FI terhadap ikan tilapia hibrid merah. Tilapia telah dikaji secara tiga replikasi (berat badan awal: 7.30 $\pm$ 0.02 g) dan diberi makanan dua kali sehari selama 56 hari. Kajian tersebut telah menunjukkan campuran 25% SPC ke dalam diet dapat meningkatkan prestasi pertumbuhan dan nisbah kecekapan makanan apabila membuat perbandingan dengan diet yang hanya mengandungi CPC dalam peratusan bahan tumbuhan yang sama dalam diet. Tilapia yang diberi diet C-SPC50 dan C-SPC50A (75% bahan tumbuhan) telah menunjukkan peratusan Keuntungan Berat (WG) yang lebih baik masing-masing pada 528.91% dan 594.89% jika dibanding dengan tilapia yang diberi diet CPC75 (263.82%). Penghasilan yang sama juga boleh dibuktikan dalam perbandingan antara CPC100, C-SPC75 dan C-SPC75A. Tambahan FA dalam diet juga menunjukkan peningkatan dalam Kadar Pertumbuhan Tertentu (SGR), WG, FCR dan FI terhadap tilapia berbanding dengan tilapia yang diberi diet yang tidak mengandungi FA. Peratusan komposisi seluruh badan protein dan lemak antara diet-diet tidak menunjukkan perbezaan yang ketara ($P>0.05$) kecuali diet CPC100. Dalam kedua-dua kajian, tiada perbezaan struktur secara histologi yang didapati dalam perut tilapia hibrid merah yang diberi diet CPC. Kesimpulannya, FM dalam diet tilapia dapat digantikan dengan CPC sehingga mencapai 50% tanpa memberi kesan negatif dalam kesan prestasi pertumbuhan dan nisbah kecekapan makanan. Untuk meningkatkan penggantian FM dengan bahan tumbuhan, 50% CPC boleh dicampurkan dengan 25% SPC dan tambahan 0.5% betaine-HCl dan 2% DBL untuk mencapai penggantian sebanyak 75% dalam pemakanan tilapia tanpa membawa kesan negatif.

THE NUTRITIVE VALUE OF CORN PROTEIN CONCENTRATE AS A FISH MEAL REPLACEMENT IN THE FEEDS OF RED HYBRID TILAPIA, *Oreochromis* sp.

ABSTRACT

The objective of this study is to evaluate the effect of fish meal (FM) replacement with corn protein concentrate (CPC) on growth performance and nutrient utilization of red hybrid tilapia, *Oreochromis* sp.. In the first experiment, five isonitrogenous (35% crude protein) and isolipidic (10% lipid) diets with five different inclusion levels of CPC at 0, 25, 50, 75 or 100% were formulated and fed to triplicate groups of tilapia (initial weight of 10.33 $\pm$ 0.02 g) twice daily for 63 days. Relatively high final body weight (FBW) (56.37 – 62.72 g) was obtained in treatments up to 50% CPC inclusion level. For CPC75 and CPC100, final body weight (FBW) (45.68 and 16.8 g respectively) were significantly ($P<0.05$) reduced. With the exception of CPC100 diet, there were no significant differences ($P>0.05$) in feed conversion ratio (FCR) and feed intake (FI) among all the treatments. For the whole body composition, there was a gradual decreasing trend shown in crude protein level with increasing CPC inclusion levels. Lipid content in whole body was not significantly different. Apparent digestibility coefficient of protein (ADCP) was gradually increasing from 80.63 to 84.65% in the treatments up to 75% CPC inclusion level. However, ADCP at CPC100 was significantly reduced to 73.65%. For skin and muscle coloration, there was significantly higher colouration in terms of *b*-values shown on the 100% CPC fed fish (7.89 and 3.66 respectively) as compared to other treatments. The total carotenoid content in muscle was significantly the lowest and highest in fish fed the control (0.0162 mg/kg) and CPC100 (0.0353 mg/kg) diet, respectively. In the second experiment, seven isonitrogeneous (35% crude protein) and isolipidic (10%) with CPC inclusion diets were alternatively mixed with 25% of soy protein concentrate (SPC) to improve overall nutrient plant protein quality; and supplemented alternatively with 0.5% betaine-HCl and 2% dried basil leaves (DBL) as feeding attractants (FA) to enhance the palatability and FI of the red hybrid tilapia. The tilapia were tested in triplicate groups (initial weight of 7.30 ± 0.02 g) and fed twice daily for 56 days. Diets with 25% of SPC tended to enhance the growth performance and feed utilization efficiency of tilapia compared to fish fed the solely added CPC diet at the same percentage of plant protein ingredients (PPI) in the diet. Tilapia fed with diets C-SPC50 and C-SPC50A (75% PPI added) were shown better % weight gain (WG) at 528.91% and 594.89%, respectively, as compared to WG of tilapia fed with CPC75 (263.82%). Similar outcomes also can be proved at the diets among CPC100, C-SPC75 and C-SPC75A. Addition of both FA into the diets showed better specific growth rate (SGR), WG, FCR and FI as compared to the diets without supplementation of feeding attractants. With the exception of CPC100, no significant differences found on whole body protein and whole body lipid among all the treatments. In both experiments, no histological changes were observed in the gut of the red hybrid tilapia fed with CPC. In conclusion, the results showed that CPC can be a potential alternative PPI to replace FM up to 50% in tilapia diet without negatively affect growth performance and nutrient utilization. However, to replace FM with more PPI, 25% of SPC with supplementation of 0.5% betaine-HCl and 2% of DBL can be mixed into 50% of CPC diets to make the substitution level up to 75% in tilapia diet without causing any adverse effect.

CHAPTER 1

INTRODUCTION

Aquaculture has expanded enormously and is now the fastest growing worldwide food industry, with a global production at an average annual rate of 6.2 percent in the period 2000-2012 (9.5 percent in 1990-2000) from 32.4 million to 66.6 million tonnes (FAO, 2014). In 2009, the proportion of fisheries production had increased to 70% (about 66 million tonnes) where harvested wild fish were used for human consumption and another 30% (about 23 million tonnes) were used for non-food purposes such as fish oil, fish meal or used directly as fish feed and as pet food (Olsen & Hasan, 2012). El-Ebiary (2005) stated that it is estimated that majority of the worldwide human consumption of fish will be provided from aquaculture by the year 2030. Thus, fish production in aquaculture is increasing at an exponential rate due to a growing worldwide demand.

In Malaysia, similar to other Asian and Southeastern Asian nations, fish and other seafood are crucial daily diets of people and depend on these as their main source of animal protein (Othman, 2010). Moreover, Malaysia, which is surrounded by the sea, had led to the opportunity for the local people to have adequate and cheap production of fish food. Based on Department of Fisheries Malaysia (DFM) (2012), fisheries sector in Malaysia, including marine capture, inland capture and aquaculture, had produced over 1.8 million tonnes of fish in 2012. Fisheries production including capture fisheries had increased 6.86% and 12.98% in terms of quantity and value as compared to year 2011. Moreover, according to FAO (2014), Malaysia was the top 15 ranking as the major marine fish production country from 2011 (1.37 million tonnes) to 2012 (1.47 million tonnes).

To overcome the increasing world demand for protein sources, the increase in production of farmed fish species has to be practiced. Tilapia, after carp, the second most important farmed freshwater omnivorous cichlids are widely cultured over 100 tropical and sub-tropical countries in various culture systems (Ng & Romano, 2013). According to FAO (2013), world production of tilapia was 3.96 metric tonnes in 2011 and was estimated to reach 4.21 metric tonnnes in 2012 (6% growth). Among the various species of tilapia including *Oreochromis* sp., *Tilapia* sp. and *Sarotherodon* sp., with Nile tilapia (*O. niloticus*) is the most common farmed tilapia species with global aquaculture production of 2.79 million tonnes per year in 2011 (FAO, 2013).

Currently, the production of feeds for farmed aquatic animals rely heavily on fish meal (FM) and fish oil (FO) (Lim *et al.*, 2008; Palmegiano *et al.*, 2006). Tacon and Metian (2008) stated that utilization of FM in aquaculture in 1999 was over 2 million tonnes and will be increased to over 4 million tonnes by 2015. Hence, it can be predicted that FM and FO will decrease in availability and increase in cost due to competition within the aquafeeds and terrestrial feeds sector (El-Ebiary, 2005; Palmegiano *et al.*, 2006; Zhao *et al.*, 2009). Currently, fish meal and fish oil that are used in commercial tilapia feeds at between 0-20% and 0-10%, respectively, are depending on countries and cultural systems, with terrestrial-based protein, lipid and carbohydrates being used in higher proportion (Tacon & Metian, 2008; Ng & Romano, 2013).

FM is the most crucial dietary protein source in compounded diet for many important farmed species due to its easily digestible and balance essential amino acids composition. However, FM is costly and limited in supply which had led to many researches on other alternative protein sources to replace FM in the diets. The high demand for FM had been concerned that it may be not ethically correct to harvest fish for use in aquaculture feed as it can be consumed by human directly (Tacon & Metian, 2008; Olsen & Hasan, 2012).

Recently, this issue has been forcing the aquaculture feed industry to carry out a large number of studies in order to formulate less expensive and more readily available ingredients especially alternative plant protein sources, to partially or completely substitute FM, including rice protein concentrate (Palmegiano, 2006; Sanchez-Lozano, 2009), corn gluten meal (CGM) (Wu *et al.,* 1995; Regost *et al.*, 1999; Wu *et al.,* 2000a; Wu *et al.*, 2000b; El-Ebiary, 2005), soybean meal (SBM) (Furuya *et al.*, 2004; Lim *et al.*, 2008), soybean protein concentrate (SPC) (Chatzifotis *et al.*, 2008; Zhao *et al.*, 2009; Salze *et al.*, 2010; Freitas *et al.*, 2011), potato protein concentrate (Tusche *et al.,* 2011), rapeseed protein concentrate (Slawski *et al.*, 2011) and pea protein concentrate (Sanchez-Lozano *et al.,* 2009).

Among the alternative plant source ingredients, SBM is widely used, because SBM is easily available and economical protein source with high digestible protein and good amino acid profile. Currently, SBM is the most commonly used fish meal substitute, however, generally this contains less crude protein (42% - 50%) than fish meal (approximately 72%) (Freitas *et al.,* 2011). Moreover, Francis *et al.* (2001) stated that plant protein sources contain anti-nutritional factors (ANF), which can be another potential obstacle that needs to be overcome to successfully replace FM. In a review of ANF by Francis *et al.* (2001), saponin, tannins, phorbol esters and gossypol were believed to be the ones most likely to be in sufficiently high dietary concentrations to pose growth/health problems to fish. SBM has several anti-nutritional factors (ANF) that include saponins, urease activity, trypsin inhibitor, glycinin, β-conglycinin, lectins, and oligosaccharides which can lower fish growth or compromise their health (Borgeson *et al.*, 2006; Chen *et al.*, 2011; Sorensen *et al.*, 2011).

Although the use of dietary SBM often results in lower growth rates for tilapia (El-Ebiary, 2005; Goda *et al.*, 2007), the use of dietary SBM is still often a cheaper option (Davis et al. 2010). Nevertheless, SPC has been proposed as an alternative to SBM since SPC contains higher crude protein levels (of 65% to 67%) as well as having less ANF (Freitas *et al.*, 2011). However, similar to SBM, there are problems with the imbalanced amino acid profile with SPC which is characterized by high tryptophan and the essential amino acid (EAA) lysine, while low in the EAA methionine. This latter EAA is the first limiting amino acid source for fish (Freitas *et al.*, 2011; Zhao *et al.*, 2009). However, Zhao *et al.* (2009) found that methionine supplementation and an increased feeding frequency of six times per day were required for *O. niloticus* to achieve comparable growth rates to those fed FM-based diets. It was suggested that both these were necessary for tilapia to obtain their optimal methionine requirements (Zhao *et al.*, 2009).

Although the plant proteins are closely matched to FM, especially SBM and SPC, the amino acids proportion is often significantly different from each other. Therefore, supplementation of essential amino acids in the diet, such as arginine, cystine, lysine, threonine and methionine needs to be provided to restore these requirements in fish (Regost *et al.*, 1999; Zhao *et al.*, 2009; Freitas *et al.*, 2011). Based on Furuya *et al.* (2004), all plant protein sources, especially, SBM can fully replace FM in tilapia diet, without causing any adverse effect on growth performances, composition and carcass yield if essential amino acid are supplemented (lysine, methionine and threonine). However, it should be noted that both Furuya *et al.* (2004) and Zhao *et al.* (2009) suggested that free amino acids are less efficient than bound amino acid in terms of nutrient utilization efficiency as free amino acid easily pass through the stomach more rapidly.

The other protein source that appears to be a potential ingredient to substitute FM or as a blend to SPC in tilapia feed is corn protein concentrate (CPC). Compared to CGM and other plant protein sources, CPC contains higher crude protein levels of about 70-75% and less than 0.5% fat, which is comparable to fish meal in terms of protein levels (Philips and Sternberg, 1979). CPC is low in lysine and tryptophan, yet rich in sulfur amino acids, which include methionine and cystine (Philips and Sternberg, 1979) and can complement the amino acid profile of SPC. Despite the potential use of CPC in fish diets, to date, no studies have examined the feasibility of CPC in tilapia diets.

With the continued rise in the FM price and shortage, an alternative source is needed to replace FM in tilapia diets. Hence, the present study has been designed to replace FM with CPC in tilapia feed at various inclusion levels. In the first experiment, FM was replaced by CPC and the aim of the experiment is to determine the growth performances and feed utilization efficiency of red hybrid tilapia fed with different replacement level of CPC in fish meal based diets. Moreover, as the reference from results of the first experiment, a second experiment was conducted to improve the diet by replacing FM with CPC at higher inclusion level or completely replacement by adding feeding attractants. The objective of the second experiment was to improve the feed intake of red hybrid tilapia and consequently to enhance the growth performance by feeding the fish with a mixture of dietary CPC and SPC, with and without adding feeding attractants. Both experiments were conducted to determine the effects of increasing dietary CPC on body composition, colouration, body organ indices and gut histology.

CHAPTER 2

LITERATURE REVIEW

2.1 Tilapia production in global and Malaysia

Tilapia is a freshwater species native to Africa, has been subsequently introduced worldwide since the 20th century (especially Nile tilapia) and is now cultured in over 100 tropical and subtropical countries, including Malaysia (Eknath & Hulata, 2009; Ng & Romano, 2013). Mozambique tilapia (*Oreochromis mossambicus*) was the first tilapia species introduced to Asia in the 1940s. However, Nile tilapia (*O. niloticus*) was largely produced and had replaced Mozambique tilapia in terms of aquaculture production due to its better growth performances in culture system (Ng & Romano, 2013). Tilapia, also known as 'aquatic chicken' was culture enormously in aquaculture industry. Its popularity is attributed to their fast growth, high adaptability to crowded conditions, disease resistance, high flesh quality, high marketability, ability to readily reproduce in captivity and ability to accept low cost terrestrial-based diets (Borgeson *et al.*, 2006; Ng & Romano, 2013).

Globally, after carps, tilapia culture is one of the fastest growing aquaculture sectors yielding over 3.9 million tonnes per year in 2011 (FAO, 2013). According to Figure 2.1, Nile tilapia (2.8 million tonnes per year in 2011) was the largest amount of tilapia produced in aquaculture as compared to other tilapia species from year 2000 to 2011. Based on FAO (2012), production of tilapia is widely distributed especially 72 % in Asia (particularly in China and Southeast Asia), 19% in Africa and 9% in America. The expanding of tilapia production is now seen as the potential whitefish consumption in Asia, America and Africa for domestic consumption (FAO, 2014).

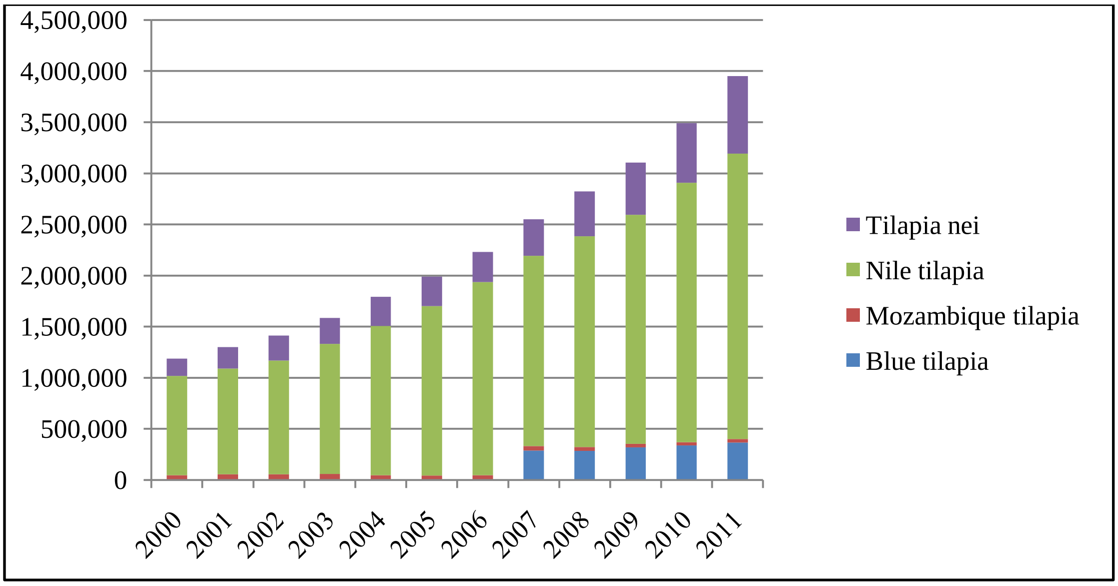

Figure 2.1 Annual productions globally from year 2000-2011 (metric tons) of Blue tilapia (*Oreochromis aureus*), Mozambique tilapia (*Oreochromis mossambicus*), Nile tilapia (*Oreochromis niloticus*) and Tilapia nei (*Oreochromis* sp.). Bar chart based on the values from Food and Agriculture Organization (FAO, 2013).

In Malaysia, red tilapia (*Oreochromis* sp.) production increased from 8,214 tonnes in 1998 to 20,061 tonnes in 2003, and is the most widely produced tilapia species constituting approximately 90% (Hamzah *et al.*, 2011). In 2012, freshwater aquaculture in Malaysia had contributed a total of 51,555 tonnes valued at RM 372.43 million of tilapia including red and black tilapia (DFM, 2012). Both tilapia productions had showed an increase of 20.49% and 23.35%, respectively when compared to 42,786.23 tonnes valued at RM 301.92 million in 2011. Due to the high popularity of this species, considerable effort has been made to genetically improve tilapia species to produce a higher quality fish at a lower cost, such as the GIFT strained tilapia (Genetically Improved Farmed Tilapia, *O. niloticus*) (Mamun *et al.*, 2007; Teoh *et al.*, 2011).

2.2 Protein requirement for Nile tilapia

Generally, protein is the most expensive component in tilapia feed. This nitrogenous complex substance consists of basic amino acids (AA) units. The amino acids can be divided into essential amino acids (EAA) and non-essential amino acids (NEAA). The amino acid profiles of feed differ significantly among the variety of protein sources.

The protein requirement of tilapia has been studied extensively at different life stages with different tilapia species (Santiago & Lovell, 1988; Larumbe-Moran *et al.*, 2010). Several studies had been done to determine the protein requirement of Nile tilapia (*O. niloticus*) (Santiago & Lovell, 1988; Furuya *et al.*, 2004; Larumbe-Moran *et al.*, 2010). Furthermore, requirement level of specific amino acids such as methionine, cystine and lysine in tilapia were studied as well (Gaye-Siessegger *et al.*, 2007; Nguyen & Davis, 2009).

There are several factors that can affect the protein requirements for tilapia, which include the different species, different size, protein source, energy content, water quality, culture condition, feeding rate and densities of natural food (Ng & Romano, 2013). For example, Ng and Romano (2013) have pointed out that tilapia larvae have a higher protein requirement of 35-50%, which decreases with increasing fish size (juvenile: 30-40% and adult: 20-30%) for optimum growth performances.

There are 10 essential amino acids that required by tilapia, including arginine, lysine, histidine, methionine, threonine, valine, leucine, isoleucine, pheylalanine, and tryptophan (Santiago & Lovell, 1988). Table 2.1 showed the proportion level of amino acid levels required by *O. niloticus*. FM served as the best protein sources and excellent amino acid profile for the tilapia comparing to other plant protein sources (El-Sayed, 1999.).

Moreover, there were studies comparing the efficiency of utilizing synthetic amino acids and natural protein bound form. Gaye-Siessegger *et al.* (2007) stated that tilapia is poor in using synthetic amino acid due to uptake rates of free amino acids into plasma is faster than natural protein-bound form. Also, Ng *et al.* (1996) found that white sturgeon significantly better in utilizing intact protein compared to free amino acids. The poor utilization of free amino acids may be due to faster excretion in warm water species (tilapia and carps) and increased catabolic rates of absorbed amino acids in plasma (Ng *et al.*, 1996).

Table 2.1: Amino acid requirements for Nile tilapia (*Oreochromis niloticus*) (modified from Santiago & Lovell, 1988**)**

Amino acid	Percentage of the protein
Lysine	5.12
Arginine	4.20
Histidine	1.72
Threonine	3.75
Valine	2.80
Leucine	3.39
Isoleucine	3.11
Methionine*	2.68
Phenylalanine **	3.75
Tryptophan	1.00

*Cystine included as 0.54% of the protein

**Tyrosine included as 1.79% of the protein

2.3 Plant protein sources

The majority of studies investigating fish meal and oil substitutes have focused primarily on protein since this is often the most expensive ingredient in the diet (accounting for approximately 35 to 40 % of the dietary content, depending on the species) (Nguyen & Davis, 2009).

Indeed, many studies have investigated traditional plant sources to replace fish meal such as SBM and cottonseed meal (El-Ebiary, 2005; Guo *et al*. 2011; El-Saidy and Saad, 2001), while other plant ingredients that had been extensively studied were rice protein concentrate (Palmegiano *et al.*, 2006), potato protein concentrate (Tusche, 2011; Tusche, 2013), CGM (Regost *et al.*, 1999; El-Ebiary, 2005), SPC (Day & Gonzalez, 2000; Freitas *et al*., 2011), rapeseed protein concentrate (Slawski *et al.*, 2012), cacoa husks (Pouomogne *et al*. 1997), mucuna seeds (Siddhuraju & Becker, 2003), fungi-degraded date pits (Belal, 2008) or rice wine residue (Vechklang *et al*. 2011). Although many of these plant-based sources have been met with some success, for this industry to continue expanding, it is imperative to identify more readily available ingredients for designing less costly practical diets.

There are two ingredients that appear to have great potential in replacing traditional protein sources to tilapia feeds (*e.g.* FM, SBM), namely CPC and SPC.

2.3.1 Corn protein concentrate

Corn is highly produced in the United States and can be directly consumed by human (Jao *et al.*, 1985). Aside from their direct consumption, corn can be processed into starch, oil, corn gluten meal, corn protein concentrate (CPC), corn gluten feed and syrup via wet milling (Jao *et al.*, 1985; Wu *et al.*, 1995).

In the case of CPC, this is produced from the dried protein fraction of the corn originating from the endosperm after removal of the majority of the non-protein components by enzymatic solubilization of protein streams which gained from wet milling process and during this process, sulfur amino acids, such as methionine and cystine, become more concentrated (Figure 2.2). Due to their high protein content (at 70-75 %), good AA profile, especially methionine (2.26 % of protein) and lysine (6.66 % of protein) (Table 2.2) as well as being a good binder of water and fat which provides greater feed stability, this is currently being used in the pet food industry, *e.g.* Empyreal75® (Phillips & Sternberg, 1979). However, interestingly, there is limited published information regarding the use of CPC in aquatic animal feeds (Phillips and Sternberg, 1979), and none for the potential use in tilapia feeds.

However, there was a study had been conducted on CPC (Empyreal 75®, Cargill Corn Milling, USA) replacing fish meal in shrimp diets fed on Pacific white shrimp (*Litopanaeus vannamei*). Further review on how CPC affects the growth performance and cost effective of Pacific white shrimp will be at Section 2.4.

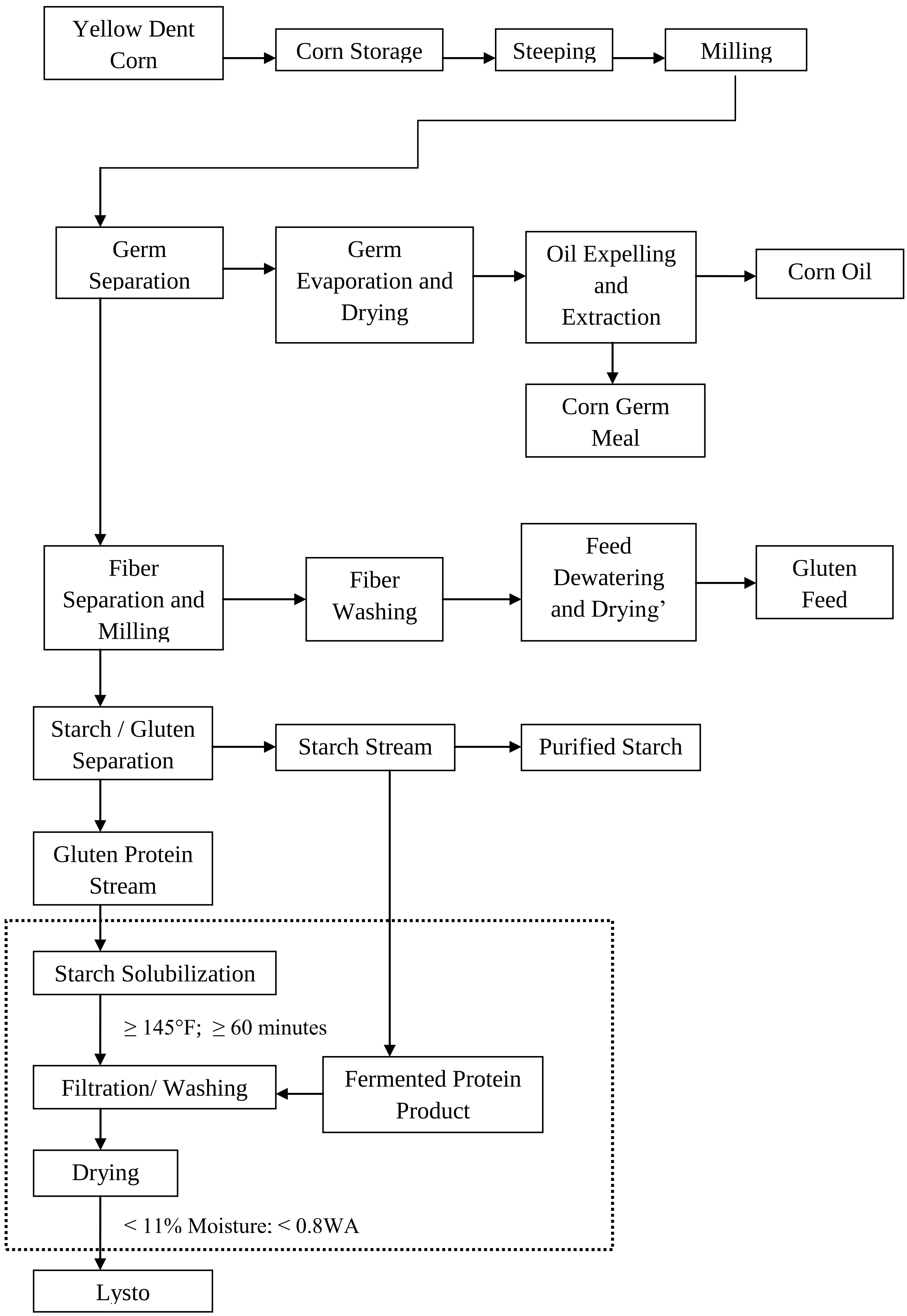

Figure 2.2 The process flow (including wet milling) of corn protein concentrate, Lysto™ (figure provided by Cargill Corn Milling)

Table 2.2 had showed the composition of amino acid profile present in Lysto^{TM} (Cargill Corn Milling, Cargill, Inc., Blair, NE, USA). Lysine is the limiting factor of amino acid component in corn. However, lysine in Lysto^{TM} was supplemented and had been improved to 6.66 % of protein in the ingredient which had fulfilled the basic requirement of tilapia in the diets (Table 2.1).

Table 2.2 Amino acid profiles of corn protein concentrate (Lysto^{TM}) provided by Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.

Amino acid profile	**Percent protein**
Aspartic acid	5.24
Glutamic acid	19.69
Serine	4.09
Glycine	2.45
Histidine	1.80
Threonine	2.80
Arginine	2.84
Alanine	8.04
Proline	8.36
Tyrosine	4.72
Valine	4.22
Methionine	2.26
Isoleucine	3.77
Leucine	15.35
Phenylalanine	5.66
Lysine	6.66
Cystine	1.58
Tryptophan	0.48

2.3.2 Soy protein concentrate

Soybean protein concentrate (SPC) is one of the soybean products obtained by treating defatted soy flakes to aqueous alcohol extraction or enzyme degradation. However, the most commonly used method is via alcohol extraction, which normally removes anti-nutritional factors (ANF) and soluble carbohydrates (Peisker, 2001; USSEC, 2008) (Table 2.3). This is important since ANF common to soybeans can cause intestinal or digestive tract damage in some animal species, especially to young poultry and marine species (Francis *et al*., 2001). Also, further modifications of aqueous alcohol in the mixture, temperature and time of processing may further lower the ANF level, which is especially preferred in aquafeeds (USSEC, 2008).

Table 2.3 Proximate analysis and anti-nutritional factors level of soybeans, soybean meal and soy protein concentrate alcohol extracted (modified from Peisker, 2001).

	Soybeans	Soybean meal	Soy protein concentrate alcohol extracted
Moisture (%)	10-12	10-12	7
Crude Protein (%)	35.5	42-50	65
Fat (%)	19	1-1.5	1
Ash (%)	4.7	5.5-6	6
Anti-nutritional factors (ANF) level			
Urease activity (pH-rise)	2.0	0.05-0.5	<0.05
Trypsin inhibitor, mg/g	45-50	1-8	2
Glycinin (ppm.)	180.000	66.000	<100 (< 3 Soycomil)
β-conglycinin (ppm.)	>60.000	16.000	<10
Lectins (ppm.)	3.500	10-200	<1
Oligosaccharides (%)	14	15	3 (Raffinose 0.2-0.3; Stachyose 2-3)
Saponins (%)	0.5	0.6	0

Due to the lower ANF level and a higher crude protein value (of 65-67%), SPC providing a nutrient dense ingredient within aquatic feeds. Thus, SPC is in high demand as an alternative to both fishmeal and soybean meal for the diets of both marine and freshwater aquaculture species (Hansen *et al.*, 2007; USSEC, 2008; Zhao *et al.*, 2009; Freitas *et al.*, 2011). It is estimated that the demand for SPC in aquaculture feeds is projected to exceed 2.8 million tonnes by 2020 (FAO, 2012).

Table 2.4 Amino acid profile of fish meal and soy protein concentrate in dry matter basis (g /kg of protein) (modified from Zhao *et al*., 2009).

Amino acid profile	g/kg of protein	
	FM	SPC
Threonine	34	32
Valine	59	61
Methionine	20	16
Cystine	20	24
Isoleucine	47	44
Leucine	84	87
Phenylalanine	58	61
Tyrosine	56	54
Lysine	55	55
Histidine	29	32
Arginine	57	59
Tryptophan	9	9

2.3.3 Other plant protein sources

There are many other plant protein sources that had been studied to lower the dependency of FM in the fish diets. Plant protein sources that are commonly used include SBM, wheat gluten meal, corn gluten meal (CGM), rapeseed/canola meal, cottonseed meal, sunflower seed meal, groundnut/peanut meal, mustard oil cake, lupin kernel meal and broad bean meal (FAO, 2012). The substitution level of plant protein sources to replace FM can be varying widely which depends upon the species and species group of the fish that fed with it (Table 2.5).

SBM is the most common source of plant protein used in aquafeeds. SBM can be utilized in aquafeed up to 60% due to its lower cost, high protein content (about 40%) and good amino acid profile. Based on FAO (2012), SBM had been consumed in aquaculture sectors at about 6.8 million tonnes (23.2%) out of 29.3 million tonnes of total compound aquafeed productions in 2008. Zhou and Yue (2011) had showed high digestibility in tilapia fed with SBM diet (about 90%). Although SBM is good in protein and amino acid profile, SBM is deficient in sulphur essential amino acid of methionine and cysteine which does not meet the requirement of tilapia for optimal growth (Furuya *et al*., 2004; El-Ebiary, 2005). However, supplementation of L-lysine and DL-methionine into the diet had fulfilled the amino acid requirement of tilapia and resulted in better growth performance and feed utilization efficiency (Furuya *et al*., 2004; Goda *et al*., 2007). Moreover, present of anti-nutritional factors in SBM such as saponin (eg. galacto-oligosaccharides, stachyose and verbascose) can restrict the growth of fish.

Besides SBM, rapeseed or canola meal is another commonly used plant ingredients in the diets of tilapia which can be included up to 40% in replacing FM. At the point of nutritional profile, rapeseed meal is comparable to FM. Rapeseed meals have relatively high protein content (about 60 %), low level of antinutritional factors and balance amino acid profile which reflects the requirements of fish. However, higher inclusion of rapeseed meal in diet appears to be unfavourable due to diet taste resulting in reduced feed intake and restricted growth. Ng and Romano (2013) had reviewed that Mozambique tilapia fed with rapeseed meal ranging between 25 and 50% can give similar growth and feed utilization efficiency to tilapia fed with SBM. They also stated that growth and feeding efficiency of tilapia can be reduced due to the present of glucosinolate in rapeseed meal.

Other plant proteins such as CGM, lupin, peas, rice and barley are increasingly used in aquaculture feeds. The selections of plant protein are still based on the local market availability and cost. Moreover, it also needs to depend on the nutritional profiles including protein level, lipid level and anti-nutrient content.

Table 2.5 Plant protein sources usage for major aquaculture species and species groups (modified from FAO, 2012).

Plant protein sources	Inclusion level in compound aquafeed (%)
Soybean meal	3-60
Wheat gluten meal	2-13
Corn gluten meal	2-40
Rapeseed/ canola meal	2-40
Cottonseed meal	1-24
Groundnut/ peanut meal	≈30
Mustard oil cake	≈10
Lupin kernel meal	5-30
Sunflower seed meal	5-9
Canola protein concentrate	10-15
Broad bean meal	5-8
Field pea meal	3-10

2.4 Plant proteins in fish feeds

There have been several studies investigating the effects of soy protein concentrate (SPC) on different fish species, yet none on the utilization of corn protein concentrates (CPC) on tilapia feed. Therefore, SPC will be discussed using the available literature, while corn gluten meal (CGM) will instead be discussed since the amino acid available in CGM is similar to CPC.

Previous studies have been conducted on replacing fish meal with SPC for several fish species throughout the world (Day & Gonzalez, 2000; Peisker, 2001; Hansen *et al.*, 2007; Zhao *et al.*, 2009; Ngandzali *et al.*, 2010; Salze *et* al., 2010; Freitas *et al.*, 2011). Firstly, Zhao *et al.* (2009) studied the effects of totally replacing fishmeal with SPC with or without methionine supplementation by increasing feeding frequency in Nile tilapia (*Oreochromis niloticus* GIF strain). The feeding frequencies employed were fish meal twice per day and soymeal protein concentrates 2 times per day with methionine hydroxy analogue supplementations (MHA) (SPCM2), and 6 times per day with and without MHA supplementations (SPC6 and SPCM6, respectively). The results showed that both the FM and SPCM6 diets gave a high SGR of 6.27 and 6.37 %/day, respectively, whereas the SPC6 diet gave a SGR of 6.07 %/day which was significantly less than the SPCM6 treatment. Furthermore, fish in the SPCM6 treatment group showed a high protein efficiency ratio (PER) at 2.65 and lower feed conversion ratio (FCR) at 0.97, which was not significantly different from FM group. However, the PER was reduced to 2.57 while the FCR was significantly increased to 1.02 for those in the SPC6 treatment. Zhao *et al.* (2009) suggested that the level of dietary methionine and feeding frequency can, in turn, affect the methionine intake, which likely led to the FM and SPCM6 treatments providing the highest SGR. Lastly, Zhao *et al.* (2009) also pointed out that productive protein value (PPV) could be enhanced by increasing the feeding frequency and methionine supplementation (SPCM6 = 0.41) since the PPV values between the FM and SPCM6 groups were not significantly different.

On the other hand, Ngandzali *et al.* (2010) studied the effect of partial replacement of FM by SPC on the growth performance, body composition, feed utilization, nutrition digestibility and phosphorus discharge of juvenile black sea bream (*Acanthopagrus schlegelii*). In this study, SPC diets were formulated at 0, 8, 16, 24, 32 or 40% to partially replace fish meal in which all diets were supplemented with phytase at 2000 phytase activity U kg^{-1}, with the exception of control diet. The reason phytase was added was because SPC can contain elevated levels of phytic acid, which is an antinutritional factor that may negatively affect protein digestibility and intestinal mucosa (Francis *et al.*, 2001; Riche *et al.*, 2001). Ngandzali *et al.* (2010) reported that the survival rates of the fish ranged between 97.3% and 98.8%, and were not significantly different with each other. Moreover, as the dietary SPC inclusion level increased from 0 (46.73 g/fish) to 16 % (52.32 g/fish), the feed intake (FI) increased, however, at a SPC inclusion level of 24 to 40 %, the FI decreased to 48.08 and 47.85 g/fish, respectively. For the hepatostomatic index (HSI), whole body protein, phosphorous (P) content and proximate analysis of the dorsal muscle was not significantly different among fish fed the experimental diets (Table 2.6). With the exception of the fish fed the diets with 16 % SPC having a significantly lower P content than those fed the 24 % SPC diet, no significant difference in the dorsal muscle P content was detected. However, the protein content in the liver evaluated was the highest for those fed the 0% SPC (control diet) compared to the diet formulated with 24% SPC and higher (Table 2.4). Nevertheless, Ngandzali *et al.* (2010), found that the partial replacement of fish meal for SPC to levels as high as 40%

did not significantly affect either the growth performance or feed utilization of the tilapia when 2000 U kg^{-1} of phytase was supplemented. Further research directions were suggested, such as examining the optimal dietary phytase inclusion levels as well as measuring the immune response and physiological status of the digestive tract for fish fed high SPC diets.

Table 2.6 Effect of soy protein concentrate dietary supplemented with phytase on proximate analysis of whole body, dorsal muscle and liver composition of juvenile black sea bream (g kg^{-1} dry matter) (modified from Ngandzali *et al.*, 2010).

	Diet (substitution level %)					
	0	8	16	24	32	40
Whole body composition						
Moisture	650.2 ±13.0^{b}	655.6 ±3.8^{b}	664.9 ±9.0^{ab}	651.9 ±5.0^{b}	676.4 ±19.1^{ab}	689.4 ±11.0^{a}
Protein	219.6 ±2.2	210.3 ±5.6	214.3 ±4.1	214.0 ±4.2	209.1 ±10.2	205.0 ±1.1
Lipid	122.9 ±3.0^{a}	116.5 ±3.7^{ab}	111.5 ±6.0^{ab}	117.5 ±2.2^{ab}	109.7 ±8.8^{ab}	104.9 ±2.7^{b}
Ash	49.1 ±1.7	48.9 ±1.9	48.7 ±3.1	48.9 ±2.2	46.4 ±1.3	48.6 ±2.2
Phosphorus	24.5 ±2.0	24.2 ±3.1	27.0 ±4.5	27.6 ±2.5	23.0 ±3.2	23.6 ±2.0
Dorsal muscle composition						
Moisture	745.9 ±3.0	752.7 ±9.0	749.0 ±9.0	744.8 ±4.0	754.2 ±1.2	754.2 ±9.0
Protein	214.5 ±4.0	206.8 ±5.0	210.0 ±8.0	211.7 ±3.0	203.4 ±9.0	204.1 ±8.0
Lipid	33.9 ±3.0	31.3 ±3.0	33.3 ±3.0	35.5 ±4.0	33.7 ±3.0	31.2 ±1.0
Ash	3.6 ±0.1	3.7 ±0.0	6.4 ±0.6	3.6 ±0.0	3.7 ±0.0	3.5 ±0.0
Phosphorus	3.1 ±0.2^{ab}	3.2 ±0.1^{ab}	3.1 ±0.1^{a}	3.3 ±0.4^{b}	3.2 ±0.1^{ab}	3.2 ±0.3^{ab}
Liver composition						
Moisture	580.7 ±4.2	577.8 ±3.7	575.9 ±2.2	573.1 ±1.9	570.8 ±3.3	579.4 ±2.5
Protein	137.2 ±4.0^{a}	119.7 ±7.0^{ab}	120.2 ±9.0^{ab}	113/2 ±6.0^{b}	113.9 ±7.0^{b}	115.8 ±9.0^{b}
Lipid	91.5 ±17	107.9 ±22	94.3 ±36	114.7 ±9.0	119.9 ±25	100.9 ±16
Ash	9.4 ±0.4	8.9 ±0.5	9.1 ±0.3	9.3 ±0.6	9.5 ±0.2	9.5 ±0.3

Data presented as mean ± SD (n=3); values with different superscripts within the same row indicate significant differences ($P < 0.05$).

A mixture of a plant protein based diet was studied by Hansen *et al.* (2007) to replace FM at 0, 25, 50, 75 and 100% for a high protein mixture of 14% SBM, 36% SPC and 50% wheat gluten meal in the diets of the Atlantic cod (*Gadus morhua* L.). At a total FM replacement of 100%, DL-methionine and L-lysine was added to fulfill the essential amino acids minimum requirement for the Atlantic cod. Based on the results, SGR significantly decreased while the FCR increased at the 75 and 100 % dietary plant inclusion levels. Moreover, the ratio of essential amino acids to non-essential amino acids decreased linearly from 2.12 in the FM diet to 1.75 in the 100% replaced diet since the AA profile of the diets reflected that of the ingredients. However, the apparent digestibility coefficient (ADC) of dry matter, crude protein and crude fat were not significantly different among the diets, although the ADC of starch was significantly lower as the inclusion of plant protein increased. Furthermore, with the exception of the 100% plant protein, the HSI was not significantly affected in the range of 25 to 75 % dietary plant protein inclusion levels. Thus, Hansen *et al.* (2007) stated that there was a great potential for the use of plant protein in cod diets at a level up to 50%, since no adverse effect in growth or feed utilization were detected in this study.

Regost *et al.* (1999) investigated the effects of partial or a total replacement of FM by corn gluten meal (CGM) in the diets for turbot (*Psetta maxima*) at 0 (control), 20, 40 and 57 % CGM. Since CGM is deficient in arginine and lysine, the 57% CGM diet was tested with or without these supplementations. Based on the results, 20% CGM contained diet gave comparable growth performances to the turbot group that fed with 100% FM (Regost *et al.*, 1999). Furthermore, turbot fed with the 20% CGM diet had the lowest feed efficiency rates (1.14%) and the highest PER (2.22%) within the dietary treatments except the control diet. Interestingly, despite high digestibility of plant protein, including CGM, the 40 and 57 % CGM diets resulted in significantly lower digestibility for various fish (El-Saidy & Gaber, 2008; Tram *et al.*, 2010; Zhou & Yue, 2011). Therefore, Regost *et al.* (1999), suggested this may have been due to the quality of CGM (*i.e.* bigger particle size), error in feces collection or the acidity of CGM. However, it is worthy to note that the supplementation of both crystalline lysine and arginine led to increased levels of these essential amino acids (EAA) within both the plasma and muscle indicating that EAA supplementation can be effectively used to balance the dietary AA profile.

Goda *et al.*, 2007 conducted a study on completely replacing FM with either, SBM, extruded full-fat soybean (FFSB) or CGM in Nile Tilapia (*O. niloticus*) and tilapia galilae (*Sarothrodon galilaeus*) with supplementation of L-lysine and DL-methionine (Goda *et al.*, 2007). *O. niloticus* showed significantly higher final body weight (FBW) (at 323.6g), weight gain (293.0g/17 weeks) and SGR (1.97% per day) in the control diet compared to all plant protein-based diets. Moreover, the CGM diet led to the lowest growth performance in this fish species (FBW: 266.0g, FI: 424.4g/fish/17 weeks, WG: 232.4g/17 weeks and SGR: 1/73% per day) (Goda *et al.*, 2007). On the other hand, for *S. galilaeus*, the results showed that dietary SBM led to the highest growth performance in terms of FBW, weight gain and SGR, while the FFSB diets led to significantly lower growth performance. However, for both species, the FCR was significantly lowest when fed with the SBM diet, while the significantly highest FCR were showed on those fed the FFSB diet. Meanwhile, the feed utilization parameters of protein productive value (PPV), fat retention (FR) and energy retention (ER) were significantly different between both species. For *O. niloticus*, the CGM diet led to significantly higher PPV, FR and ER than all other diets, although on the other hand, the protein intake, fat intake and gross energy intake for those fed with the CGM was significantly lowest. However, for *S. galilaeus*, the significantly highest PPV and ER for those fed the SBM diet, while the significantly highest FR was when fed the CGM diet. For the intake parameters, those fed the SBM diet led to the significantly highest protein and gross energy intake, the significantly highest fat intake were showed on the fish fed with the FFSB diet. Goda *et al.* (2007) suggested that both SBM and FFSB supplemented with L-lysine and DL-methionine can completely replace dietary FM for *O. niloticus* while SBM diet can be comparable to FM in terms of growth and feed utilization for *S. galilaeus*.

In addition, there was a study conducted by Rhodes *et al.* (2014) , using corn protein concentrate (CPC) to replace fish meal in the diet of Pacific white shrimp (*Litopenaeus vannamei*). The CPC used was provided by Cargill Corn Milling Company of Empyreal75® where the lysine content in the ingredient was still insufficient to meet the requirement of tilapia. Four isonitrogenous (36% protein) and isolipidic (10% lipid) contained 15% of fish meal which was replaced by CPC in four different graded levels of 0, 4, 8 and 12%. The lipid content of the diets were significantly higher than the formulated value which may confound the results as increasing trend was found with reductions in fish meal.

The results showed no differences in survival, FCR and mean final weight (from 17.2g to 20.5g) among the treatments. The feed costs per unit of production was calculated on these four tested diets and they found that CPC can help to reduce the feed cost without significantly reducing the yield production, also no adverse effect on growth performances and feed utilization efficiency found on the white shrimps (Table 2.7). Thus, CPC was proposed to use as an alternative protein source in commercial shrimp feed.

Table 2.7 Growth performances of Pacific white shrimp after 16 weeks of culture in a 0.1 hectare pond. Average initial weight was 0.023 ± 0.002g (modified from Rhodes *et al.,* 2014).

	Final weight (g)	Survival (%)	FCR	Production value ($)	Feed cost ($)/pond	Feed $/kg shrimp
CPC0	20.5	64.9	1.38	2106.72	791.41	1.60[a]
CPC4	17.5	77.6	1.34	1808.40	715.69	1.39[ab]
CPC8	17.2	83.6	1.27	1844.05	651.31	1.20[b]
CPC12	18.7	75.9	1.29	2018.08	598.16	1.11[b]
SE	0.5289	2.3024	0.03487	65.2261	4.0777	0.0369
P- value	0.2112	0.1423	0.6898	0.3727	<0.0001	0.0049

2.5 Feed Attractants

There are large numbers of feed additives that can be added in the feed to attract the fish to consume the feed pellets and therefore enhance the growth performance. The feed additives include binders, preservatives (antioxidants, antimicrobial components) and feeding stimulants (derivates of amino acids, betaine). Other feed additives such as exogeneous enzymes, prebiotics, probiotics, hormones and pigments that may also influence the quality of diets.

Plants are safer and cheaper natural sources. Several studies had been conducted in utilizing plant sources as feed attractants to improve the quality of diets such as medicinal plants, herbs, spices (garlic, onion) and aromatic plants. These ingredients were only added in small amount; however, they contributed towards the odor and flavor of the diets due to the present of volatile and fixed oils (El-Dakar *et al*., 2008). Moreover, substitution of these additives in feeds can minimize the use of chemicals throughout the global trend to go back to more natural ingredients.