CHAPTER 2

Literature review

2.1 Fish meal and fish oil as feed components

Fish meal is used as high-protein feed source and fish oil as feed additive in aquaculture. Fish meal and fish oil are produced mainly from harvesting stocks of small, fast reproducing fish (e.g., anchovies, small sardines and menhaden). In 2012 about 35 per cent of world fish meal production was obtained from fisheries by-products (frames, off-cuts and offal) from the industrial processing of both wild catches and farmed fish. In 2007 the largest producer of fishmeal was Peru (1.4 million tons) followed by China (1.0 million tons) and Chile (0.7 million tons). Other important producers were Thailand, the United States of America, Japan, Denmark, Norway and Iceland (Tacon *et al.*, 2011).

Fish meal prices increased significantly from 2006 to 2013, peaking at USD 1747/t in 2013. Since then, there has been a slight decline, but prices remained high. The average fishmeal price is projected to decrease in the near future: in 2025 it is expected to be 14% lower in nominal terms and 30% in real terms as compared to the base period. The price ratio between fish meal and oilseed meal is expected to increase due to a strong preference for fish meal at certain stages of animal rearing, in particular for some species raised in aquaculture. This difference in price ratio will be accentuated in El Niño years as fishmeal supplies might become very limited. Since fish oil prices are starting from very high levels, a 3% decline in nominal terms and 21% in real terms is expected by 2025 compared to the 2013-15 period. The popularity of the Omega-3 fatty acids in human diets and the continuous growth of the aquaculture sector have contributed to an increase in the fish to vegetable oil price ratio since 2012. It is assumed this high ratio will be maintained over a medium-term period and magnified in years when El Niño occurs (FAO, 2016).

Patterns in the use of fish meal and fish oil have changed in time due to the growth and evolution of the world aquaculture industry. On a global basis, in 2008 (the most recent published estimate), the aquaculture sector consumed 60.8 percent of global fish meal production (3.72 million tons) and 73.8 percent of global fish oil production 0.78 million tons (Tacon *et al.,* 2011). In contrast 2002, the poultry and pork industries each used nearly 26 percent and 22 percent respectively of the available fish meal, while aquaculture consumed only 46 percent of the global fish meal supply and 81 percent of the global fish oil supply (Tacon *et al.*, 2006). The total use of fishmeal by the aquaculture sector is expected to decrease in the long term in favour of plant-based materials. It has gone down from 4.23 million tons in 2005 to 3.72 million tons in 2008 (or 12.8 percent of total aqua-feeds by weight), and is expected to decrease to 3.49 million tons by 2020 (at an estimated 4.9 per cent of total aqua-feeds by weight) (Tacon *et al.,* 2011). These trends reflect that fish meal is being used by industry as a strategic ingredient fed in stages of the growth cycle where its unique nutritional properties can give the best results or in places where price is less critical (Shepherd and Jackson, 2013). The most commonly used alternative to fish meal is that of soy bean meal. Price time series of both products show that use of fish meal is being reduced in less critical areas such as grower feeds, but remains in the more critical and less price-sensitive areas of hatchery and brood-stock feeds (Jackson and Shepherd, 2012). Fish meal is valuable not only for the quantity but also the quality of its protein. By this is meant that the amino acids which make up the protein are present in just the right balance for animal or human nutrition. The amino acid composition of typical samples of herring meal and white fish meal are showed in Table 1

Table 1 The amino acid balance of herring meal and white fish meal

Amino acid	herring meal g/100 g protein	white fish meal g/100 g protein
lysine	7·7	6·9
methionine	2·9	2·6
tryptophan	1·2	0·9
histidine	2·4	2·0
arginine	5·8	6·4
threonine	4·3	3·9
valine	5·4	4·5
isoleucine	4·5	3·7
leucine	7·5	6·5
phenylalanine	3·9	3·3
cystine	1·0	0·9
tyrosine	3·1	2·6
aspartic acid	9·1	8·5
serine	3·8	4·8
glutamic acid	12·8	12·8
proline	4·2	5·3
glycine	6·0	9.9
alanine	6·3	6·3

Source: Shepherd and Jackson, (2013)

2.2 Opportunities of replacing fish meal by plant proteins

Before 2006, many advances had been made in replacing portions of fish meal in aqua-feeds with alternative protein sources and the percentages of fishmeal in feeds for salmon, trout, sea bream and sea bass. The fish meal in the diet of these carnivorous species could be decreased by 25-50%, depending on species and life-history stage.

Similarly, the percentage of fish meal in feeds for omnivorous fish species also declined, especially in grow-out feeds. However, fish meal use by the aqua-feed sector continued to increase because of the growing aquaculture production and the required feed resources (Cabral *et al.*, 2011).

To be a viable alternative feedstuff to fish meal in aqua-feeds, a candidate ingredient must possess certain characteristics, including wide availability, competitive price, plus ease of handling, shipping, storage and use in feed production. Furthermore, it must possess certain nutritional characteristics, such as low levels of fiber, starch, especially non-soluble carbohydrates and anti-nutrients. Further necessary characteristics are a relatively high protein content, favorable amino acid profile, high nutrient digestibility and reasonable palatability. Although some plant-derived ingredients, such as soy protein concentrate (SPC) or wheat gluten, possess most of these characteristics, these alternative protein sources have been too expensive relative to the price of fish meal to be used in most aqua-feeds. It is likely that a combination of plant-derived feed ingredients will be required to replace fishmeal. In addition supplements, such as amino acids, flavorings and possibly exogenous enzymes, will be needed to produce aqua-feeds without fish meal that support growth rates necessary for the economic production of farmed fish. Several protein plants should have to be viable alternatives to fishmeal such as soybean meal, cottons seed, and pea/lupines (Cabral *et al.*, 2011)

Soybean: Soybean, *Glycine max* Linnaeus, is the leading oil seed crop produced globally. A large part of this production is used in the extraction of oil yielding a press cake of high protein quality. This is processed to yield a wide array of soybean products, such as soy flour, SBM, SPC and soy protein isolate (SPI) which have been evaluated in fish. Soybean meal has been the predominant form of soybean used and is available either as de-hulled (48% crude protein) or with hulls added (44% crude protein) (Lall, 1991). Soy products are regarded as economical and nutritious feedstuff with high crude protein content and a reasonably balanced amino acid profile. However, certain nutritional characteristics and the presence of several anti-nutritional factors merit discussion. Concentrations of the 10 essential amino acids (EAA) and tyrosine are generally lower in SBM than in fish meal with the exception of cystine, which is present at higher concentrations in SBM (Hansen, 2009). The EAA of concern are lysine, methionine and threonine that may be limiting in soy-based diets fed to aquatic animals. Concentrations of these EAA increase with processing of soy flakes to SPC and SPI and approach or exceed those found in fish meal. However, due to the processing costs involved, these products are not yet economical for large-scale use in aqua-feeds. Crude fat and ash concentrations of solvent extracted SBM and other soy products tend to be lower than those in fish meal, but carbohydrate concentrations tend to be higher. Lower ash and fat concentrations in soy products can be overcome by appropriate supplementation with mineral premixes and lipids, but high concentrations of carbohydrates remain an area of concern. Carbohydrates in soybeans are largely present as oligosaccharides such as sucrose, raffinose and stachyose. Sucrose is generally available to aquatic animals, but raffinose and stachyose are not digestible due to a lack of galactosidases that are necessary to metabolize these complex sugars. An added concern is the low availability of phosphorus and cationic minerals that are largely unavailable due to their being bound in or by phytic acid. The addition of the enzyme phytase to feeds has been shown to improve phosphorus and cationic mineral availability. There are differences in concentrations of vitamins between soy products and fishmeal. Vitamin availability data in fish are scarce and feeds are generally fortified with vitamins assuming minimal availability from feedstuff (Olsen, 2011).

2.3 Challenges associated with replacing fish meal by plant proteins

The first challenge is exploiting price relations. Agricultural product prices as well as fishmeal prices declined in late 2008, but they did not return to their pre-2007/07 levels. It remains to be seen if the pricing relationships between fishmeal and plant protein concentrates will adjust to favour plant proteins, or if demand for fishmeal will result in higher prices, driving a switch to higher plant protein concentrate use in aqua-feeds. Other plant-derived protein ingredients, such as lupine and rapeseed/canola protein concentrates, have been developed and researched as potential fishmeal substitutes, but there is no significant production of any alternative protein concentrate other than those from soy or wheat (Cabral *et al.*, 2011).

The second challenge associated with replacing fish meal with plant protein concentrates is associated with anti-nutritional compounds in plant proteins. Plant protein concentrates present a mixed picture concerning anti-nutrients (Francis *et al.*, 2001). Proteins produced from oilseeds, in general, contain more anti-nutrients of concern for fish than do proteins produced from grains. However, many are destroyed or inactivated by processes involved with product manufacture or during extrusion and pelleting. For example, soybean meal contains compounds that cause distal enteritis in the intestinal of salmonids, however, soy protein concentrate does not. The factor(s) in soybean meal responsible for enteritis is evidently removed or deactivated during the processing involved with extracting carbohydrates from soybean meal to make soy protein concentrate or soy isolates.

The final challenge associated with replacing fish meal with plant proteins is an increasing impact of aquaculture on the aquatic environment. As mentioned above, most plant protein ingredients contain non-protein fractions that are poorly digested, such as phytic acid, non-soluble carbohydrates and fibre. These materials just pass through the digestive tract of fish and are excreted as feces. In freshwater farming systems, these materials may stay in ponds or be discharged into streams or rivers in flow-through farming systems. In the marine environment, they pass through pens into surrounding waters. Nutritional strategies must be developed to minimize this potential problem, along the lines of strategies to lower phosphorus discharges from freshwater fish farms (Hardy, 2010).

2.4 Utilization of fishery by-products

Globally, ≈ 70 million tons of fish are processed by filleting, freezing, canning or curing. Most of these processes reveal by-products and waste. For example, in the fish filleting industry, the product yield is often about 30–50 %. Global production of tuna species was 4.76 million tons live weight in 2011 versus only ≈ 2000 million tons of canned tuna in product weight. Solid wastes or by-products generated by the tuna canning industry could be as high as 65 % of the original material, and this includes heads, bones, viscera, gills, dark muscle, belly flaps, and skin. The tuna loin industry reportedly generates about 50 % of raw material as solid wastes or by-products. Global production of farmed salmon was about 1.93 million tons in 2011; most of the fish are filleted, and some of these fillets are smoked before marketing. The fillet yield in salmon is quoted about 55 %. A large proportion of farmed tilapia (global production about 3.95 million tons in 2011) is marketed in filleted form, and the fillet yield in this species is about 30–37 %. Annual production of *Pangasius* exceeds a million tons, most of it going for distribution in filleted and frozen form. The fillet yield in this species is about 35 % (FAO, 2014)

Thus, fish processing generates considerable quantities of by-products and meat from most portions such as heads, frames, belly flaps, liver and roes. These contain high-quality proteins, lipids with long-chain omega-3 fatty acids, micronutrients (such as vitamin A, D, riboflavin, and niacin) and minerals (such as iron, zinc, selenium, and iodine). In many countries, fish processing establishments are small or medium-sized, and the amount of processing by-products generated may not be sufficient to justify running a fish meal plant. Producing silage from these by-products would be a convenient and relatively inexpensive way of preserving them. This is common practice in Norway, where silages from different farmed salmon slaughtering plants go to a centralized processing plant. The pooled silage is then processed into an oil and the aqueous phase is evaporated to a concentrated fish protein hydrolysate with a dry matter content of at least 42–44 % is used along with fish oil in feed for pigs, poultry and fish other than salmon. Some large fish-slaughter plants process byproducts using commercial enzymes to obtain hydrolysates and oil of very high quality (Tacon and Metian, 2015).

Fish processing by-products are highly perishable and, therefore, they need preserving as soon as they are produced. However, fish processing establishments in many developing countries are medium or small scale, and may not have facilities to preserve small volumes of by-products generated. Thus, investments (in terms of finance, infrastructure and human resources) in this area may not be profitable. Where the by-products are used for human consumption, they need to be handled and processed in compliance with systems based on good hygienic practice, good manufacturing practice and Hazard Analysis Critical Control Points (HACCP) safety management. Major challenges facing the fish gelatine industry, for example, are certification of the raw material, and the variable quality of the raw material with regard to parameters such as colour and odour. Moreover, fish gelatine is not able to compete with mammalian gelatine on price. The recovery yield of chitosan from shrimp waste is reportedly only 10 percent, and to produce good-quality chitosan, proper preservation of the shrimp waste is essential. In addition, the use of corrosive acid and alkaline conditions in its production requires specially adapted equipment and working conditions (FAO, 2014).

2.5 Protein hydrolysate

Protein hydrolysates are breakdown products of enzymatic conversion of proteins into smaller peptides. Generally, protein hydrolysates are small fragments of peptides that contain 2–20 amino acids. These protein hydrolysates are produced by the enzymatic hydrolysis of native proteins. Protein hydrolysis decreases the peptide size, and thereby making hydrolysates the most available amino acid source for various physiological functions of human body. Protein hydrolysates are used as readily available sources of protein for humans and animals due to their good functional properties (Neklyudov *et al.*, 2000a).

In recent years, several attempts have been made for utilization of the protein rich fish processing by-product wastes and underutilized fish proteins for the production of commercially valuable food ingredients. Fish protein hydrolysates with good nutritional composition, amino acid profile, and antioxidant activities have gained great attention of food scientists. Due to the presence of essential nutrients and bioactive components fish protein hydrolysates find various industrial applications (Chalamaiah *et al.*, 2012).

2.6 Fish Protein Hydrolysate (FPH)

Fish protein hydrolysates are the conversion waste products from seafood processing industries, which includes head, skin, trimmings, fins, frames, viscera and roes. In recent times the conversion of inexpensive fish processing by-products and underutilized fish proteins into valuable products became a great field of interest for food scientists all over the world. Currently, fish protein hydrolysates are considered the most important source of protein and bioactive peptides (Neklyudov *et al.*, 2000b).

2.6.1 Proximate composition of fish protein hydrolysates

Due to different protein levels in raw fish materials a wide range of protein content in fish protein hydrolysates 60% to 90% of total composition is reported (Choi *et al.,* 2009); (Dong *et al.*, 2008); (Khantaphant *et al.,* 2011). High protein content of fish protein hydrolysate demonstrates its potential use as protein supplements in human nutrition. Several studies reported the fat content for various fish protein hydrolysates were below 5% (Benjakul and Morrissey, 1997); (Abdul-Hamid *et al.,* 2002); (Dong *et al.,* 2008); (Bhaskar and Mahendrakar, 2008). The low fat content of fish protein hydrolysates results from the removal of lipids in the soluble protein fractions by centrifugation. Most of the studies demonstrated that protein hydrolysates from various fish proteins contain moisture below 10% (Abdul-Hamid *et al.,* 2002); (Bhaskar and Mahendrakar, 2008); (Chalamaiah *et al.*, 2010). The ash content of fish protein hydrolysates was reported to vary widely ranging from 0.45% to 27% of total composition (Benjakul and Morrissey, 1997); (Bhaskar and Mahendrakar, 2008); (Chalamaiah *et al.,* 2010).

2.6.2 Amino acid composition of fish protein hydrolysates

Amino acids are essential for synthesis of a wide variety of proteins with important functions including carriers of oxygen, vitamins, carbon dioxide, enzymes and structural proteins. The amino acid composition of fish protein hydrolysates is important because of the nutritional value and influence on the functional properties (dos Santos *et al.*, 2011). The variation in amino acid composition of different fish protein hydrolysates mainly depends on several factors such as raw material, enzyme source, and hydrolysis conditions (Klompong *et al.*, 2009). The amino acid composition of protein hydrolysates prepared from various fish processing waste proteins show Table 2.

Table 2 Amino acid composition of protein hydrolysates prepared from various fish processing waste proteins (%)

Amino acid	Capelin (*Mallotus villosus*)[1]	Pacific whiting (*Merluccius*)[2]	Herring (*Clupea harengus*)[3]	Nile tilapia (*Oreochromus niloticus*)[4]
Aspartic acid	9.89 ± 0.53	10.10	10.72	0.67
Threonine	4.56 ± 0.03	5.12	4.74	0.74
Glutamic acid	13.4 ± 0.03	13.80	15.87	1.52
Glycine	5.14 ± 0.01	7.88	7.59	2.98
TAlanine	6.00 ± 0.01	6.53	7.74	1.33
Valine	5.77 ± 0.01	4.72	4.34	0.56
Methionine	2.05 ± 0.01	3.02	4.94	0.48
Isoleucine	4.25 ± 0.04	4.30	3.15	0.35
Leucine	7.60 ± 0.01	7.16	8.12	0.77
Tyrosine	2.47 ± 0.01	3.50	2.64	0.40
Histidine	2.09 ± 0.02	2.10	1.22	0.64
Lysine	8.49 ± 0.06	8.33	8.46	0.54
Arginine	5.70 ± 0.02	7.29	7.06	2.76

[1] Source adapted from (Shahidi *et al.*, 1995)

[2] Source adapted from (Benjakul and Morrissey, 1997)

[3] Source adapted from (Liaset *et al.*, 2000)

[4] Source adapted from (Candido and Sgarbieri, 2003)

2.6.3 Potential applications of fish protein hydrolysates in aqua-feed

Protein hydrolysates generated from fish proteins are good nutritional supplements as bioactive compounds and can be easily absorbed and utilized for various metabolic activities (Nesse et al., 2011). Fish protein hydrolysates with good nutritional composition, amino acid profile, and antioxidant activities have gained great attention of food scientists. Due to the presence of essential nutrients and bioactive components in fish protein hydrolysates, these find various industrial applications. In many countries, traditional and commercial preparations of fish protein hydrolysates are currently used as health foods, functional foods and nutraceuticals (Chalamaiah *et al.*, 2012). Fish protein hydrolysates (FPH) have been successfully tested for incorporation into different food systems such as cereal products, fish and meat products, desserts and crackers etc. (Kristinsson and Rasco, 2000).

In recent years, several studies have investigated the effects of dietary hydrolysates protein on the growth of several fish species. In last decade, (Kotzamanis *et al.,* 2007) found that the incorporation of fish protein hydrolysates in the diet at a concentration of 10% effectively improved growth, survival and the intestinal development of sea bass larvae. The diets with low replacement rates for fishmeal by FPH (10% of total ingredients) produced improved growth of sea bass larvae compared to diets with higher replacement rates of 19%. A similar trend was observed by Cahu *et al.* (1999), who found that replacing 25% of fishmeal with a commercial FPH facilitated the onset of the adult mode of digestion in developing sea bass larvae, while replacement rates of 50% and 75% led to a reduction in larval growth. Moreover, fish hydrolysate was produced by enzymatic treatment and size-fractionated by ultrafiltration (UF). The permeate yield after ultrafiltration and the non-ultrafiltered fish hydrolysate were tested as feed ingredients using high plant protein diets. Fish meal was used in the control diet (FM). The feeding trial lasted for 60 days, and fish fed with 37 g kg^{-1} UF showed the best growth, feed efficiency, digestibility and protein utilization. (Zheng *et al.*, 2012). Recently, Kosravi *et al*. (2015) evaluated the supplemental effects of three different types of protein hydrolysates in a low fish meal (FM) diet on growth performance, feed utilization, intestinal morphology, innate immunity and disease resistance of juvenile red sea bream. A FM-based diet was used as a high fishmeal diet (HFM) and a low fish meal (LFM) diet was prepared by replacing 50% of FM by soy protein concentrate. Three other diets were prepared by supplementing shrimp, tilapia or krill hydrolysate to the LFM diet (designated as SH, TH and KH, respectively). At the end of the feeding trial, significantly ($P < 0.05$) higher growth performance was obtained in fish fed HFM and hydrolysate treated groups compared to those fed the LFM diet. Significant improvements in feed conversion and protein efficiency ratios were obtained in fish fed the hydrolysates compared to those fed the LFM diet. This growth improvement was associated with improved feed conversion and protein efficiency ratios.

2.7 Lysozyme Activities

Lysozyme is an important parameter in the immune defense of both invertebrate and vertebrate. Lysozyme is bactericidal, hydrolysing β- and linked glycoside bonds of bacterial cell wall peptidoglycans resulting in lysis. Although primarily associated with defence against Gram positive bacteria, Gram negative bacteria can also be lysed by this enzyme (Jollès and Jolles, 1984). Lysozyme is also known to be an opsonin and activate the complement system and phagocytes. It is present in mucus, lymphoid tissue, plasma and other body fluids of most fish species. Cod and several other marine species like haddock, pollack and wolffish show very little or no lysozyme activity in their tissues or body fluids. These species on the other hand show high chitinase activity in their plasma and various organs. *Chitinase* is a hydrolase, which may be involved in the defence against bacterial and fungal pathogens but such a role in immune defense of fish has still to be proven. Other natural lysins in fish serum, commonly detected by their spontaneous haemolytic effect on heterologous erythrocytes, are usually, but not always, attributed to the activation of the alternative pathway of the complement system (Saurabh and Sahoo, 2008).

Various immunomodulators have been reported to enhance the nonspecific immunity in fish, including killed bacteria and bacterial products (Anderson, 1992); (Kim *et al.,* 1999), chitin (Sakai, 1999); (Siwicki *et al.*, 1998), levamisole (Mulero *et al.,* 1998); (Kim *et al.,* 1999), β-glucans (Chen and Ainsworth, 1992)), certain vitamins ((Blazer, 1992), hormones (Kajita *et al.,* 1992); (Harris and Bird, 2000) and protein hydrolysates (Dalmo *et al.,* 1996); (Goldberg and Katz, 2007). Some immunostimulants may protect rainbow trout (*Oncorhyncbus mykiss*) against diseases such as furunculosis

(Siwicki *et al.,* 1998), and decrease the mortality of rainbow trout (Wahli *et al.,* 1998) and rockfish (Sebastes schlegeli) (Kim *et al.,* 1999). Peptides from fish protein hydrolysates (FPHs) may stimulate the activity of fish macrophages (Espelid et al., 1996); (Gildberg *et al.,* 1996). Both in vivo and in vitro experiments have shown that low molecular weight (MW) peptide fractions from FPHs stimulated oxidative burst and morphological cell reactions.

2.8 Fish meat quality

Fish meat is a source of high quality protein, vitamins, and essential minerals. Information on chemical composition of fish tissues is highly relevant for the standardization of food products based on nutritional criteria. The quality of fish carcass is a necessary factor to define the preparation process of the products and cuts (Reis Neto *et al.,* 2012). Further, information on the processing yield may be of great help for fish quality control and for the tracing system, resulting in a profit increase of the processing chain (Esmerini *et al.,* 2010). Studies on the effect of weight on yield, especially with regard to the presentation forms of the product to consumers (carcass yield) may greatly improve meat yields and profits.

Flesh quality is a complex set of characters involving intrinsic factors such as texture, chemical composition, color, fat content (Kaushik *et al.,* 1995) and is heavily influenced by extrinsic factors such as pre- and post-slaughter handling procedures (Johnston, 1999); (Gjedrem, 1997). In farmed fish, feeding with artificial diets provides a wide range of nutrients and this fact, not only determines fish growth rate but flesh composition, in particular the lipid content, which may be quantitatively and qualitatively modified (Izquierdo *et al.,* 2003). Concerning the organoleptic properties, a high content of fat in the farmed fish could lead to lower texture characteristics. Texture is also related to other factors, such as collagen content of the flesh and the muscle fibre size (Johnston, 1999). These are plastic processes, whose balance in growing fish depends on both intrinsic (genotype) (Johnston and McLay, 1997);(Johnston, 1999) and extrinsic factors such as temperature (Johnston *et al.,* 2006) photoperiod (Johnston, 1999) diet (Bjørnevik *et al.,* 2003) and ecological conditions (Johnston, 1999). Peritoneal fat can dramatically increase and affect filleting yield, both in the case of salmons and gilthead sea bream (Einen and Roem, 1997); (Company *et al.*, 1999)

2.9 Red tail catfish

Red tail catfish (H. *wyckioides*) belongs to the native fish fauna of many rivers in the Asian countries, particularly in Thailand, Laos, Cambodia, Myanmar and also in China (Kottelat, 1998). Its meat gain a high appreciation among the fish consumers, thus its marketable price is scoring higher than for some other kinds of fish such as the Nile tilapia (*Oreochromis* sp.), walking catfish (*Clarias* sp.) and others (Tippayadara *et al.*, 2016). In Thailand, red-tail catfish is a popular species cultured in cages to attain marketable sizes. The fish also does well across a broad water chemistry and temperature range, and eats a wide variety of prepared, frozen and live foods, which makes it become an excellent candidate species for intensive aquaculture (Hee and Rainboth, 1999). Although some techniques in rearing red-tail catfish have been developed yet the problem of low growth rate is a major hindrance in increasing its production (De Silva, 2016). Protein is the most important component in feeds, not only because of its high economic cost and high requirements in carnivorous fish diets but also because growth promoters are associated with some protein sources (Wilson, 2002). Protein requirement of channel catfish range between 32-36 % (NRC, 2011).

Recently, many research project have been conducted to find out the cultivation characteristic of this fish species. (Jiwyam and Nithikulworawong, 2014) have studied stocking densities of 25, 50, 100, 200, and 400 fish/m^3. At the end of 56 days, the mean fish weights among the stocking densities of 25 and 50 fish/m^3 were significantly lower than those of the 100, 200, and 400 fish/m^3 density. The specific growth rates and final mean weights amongst fish reared in higher stocking densities of 100, 200, and 200 fish/m^3 were higher than those of the low stocking densities of 25 and 50 fish/m$^{3.}$ Dietary protein requirement of juvenile Asian red-tailed catfish has proved by (Deng et al., 2011) with six isocaloric semi-purified diets were formulated to contain graded levels of protein (240, 290, 340, 390, 440, and 490 g/kg). At the end of trial, feed intake increased ($P<0.05$) steadily with increasing dietary protein levels, but no difference ($P>0.05$) was observed among fish fed diets containing 390, 440, and 490 g/kg protein. Specific growth rate (SGR) of fish fed diet containing 440 g/kg protein was higher

(P<0.05) than that of fish fed diets containing 240, 290, 340, and 390 g/kg protein, but not different (P>0.05) from that of fish fed diet containing 490 g/kg protein. Feed conversion ratio decreased (P<0.05) steadily with increasing dietary protein level up to 440 g/kg, whereas protein efficiency ratio in fish fed diet containing 440 g/kg protein was higher (P<0.05) as compared to fish fed diets containing 240, 290 and 340 g/kg protein. Regarding feeding intensity for fingerling and juvenile red tail catfish studies ranging from T1 (2%), T2 (3%), T3 (4%, control), and T4 (5%) showed a rate of 5% body live weight per day for fingerling fish to be the most appropriate rate for the (Tippayadara *et al.*, 2016). However, there are no available studies concerning the replacement of fish meal by fish protein hydrolysate in diet of juvenile red tail catfish (*H. wyckioides*).