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Alternative Protein Sources for Aquaculture Feeds

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Abstract: With a global seafood production of about 51.7 million ton in 2006 and an annual growth rate of 6.9% from 1970-2006, aquaculture is one of the fastest growing sectors in the food industry. Feed represents 40-70% of operating costs for aquaculture operations. Fish diets typically contain between 20 and 55% crude protein, depending on the fish species. High quantities of fish meal are commonly used in these feeds to supply fish with essential proteins, amino acids and fatty acids. However, during the last 3 years prices for fish meal have remained above \$1000 ton^{-1} and have even approached \$2000 ton^{-1} in 2010. Increasing expenses and potential declining supplies of fish meal have led scientists to search for less expensive but compatible alternative protein sources for fish feed; most of which are based on animal or plant protein sources. The objective of this study is to review some alternative protein sources including animal byproducts (e.g., poultry byproducts), fishery byproducts, bacterial proteins, plant proteins (e.g., soybean meal, distillers dried grains with solubles and others). The problems and advantages of these alternatives (i.e., nutritive, levels of inclusion and their acceptability values in fish diets) will be discussed.

Key words: Aquaculture, DDGS, extrusion, fish feed, fish meal replacement, nutrition, processing, alternative proteins

INTRODUCTION

Due to health awareness and population growth, seafood consumption has been increasing for years. In 2009, global fish production was estimated at 142 million ton (metric ton) of which aquaculture produced approximately 52 million ton. World production of aquaculture with an annual growth rate of around 7%, makes it one of the fastest growing animal food-producing sectors. Aquaculture products, composed of fish, crustaceans, mollusks, amphibians (such as frogs) and reptiles (such as turtles) have been steadily increasing whereas capture fisheries remained relatively stable at an estimated 90 million ton (FAO, 2009a, b). Depending on the species raised and specific feed requirements, aquaculture may actually increase the exploitation of endangered wild fishery stocks. For example, carnivorous fish require large amounts of fish meal and fish oil in their feeds which come from wild-caught fish. Fish production using little or no fish meal can reduce environmental problems (Naylor *et al*., 1998). Historically, dried fish has been used as an animal feed and fertilizer for thousands of years. Initially, fish meal was a byproduct of fish oil production but became a high protein feed ingredient in poultry and pig diets since the 1950s. Now-a-days, livestock diets contain considerable portions of soybean meal and corn, at least in the USA but in many countries fish meal is still used as the main protein source for animal diets (Hardy and Tacon, 2002). Fish meal has become the most important and cost-effective protein for commercial aquaculture feed. It provides fish with high quality protein, essential fatty acids, trace minerals (USB, 2008) has high palatability (Li *et al*., 2006) and promotes optimum growth performance (Gomes *et al*., 1995). Fish generally require between 25-55% protein in their diets, depending on the species and maturity (NRC, 1993).

For example, salmonids have the highest protein demand, needing 40-50% (or even higher) levels for fry (Hardy, 1996) whereas Nile tilapia (*Oreochromis niloticus*) typically require 30-45% dietary protein for optimum performance (Al Hafedh *et al*., 1999; Abdel-Tawwab *et al*., 2010); channel catfish (*Ictalurus punctatus*) require 30-35%, depending on the protein source and age of fish (Reis *et al*., 1989). Variation in dietary protein for optimal growth can also be caused by hygiene, environmental conditions and stocking density (Abdel-Tawwab *et al*., 2010). Amino acids are the determining factor to meet the metabolic demands of fish (Guimaraes *et al*., 2008). Deficiencies in Essential Amino Acids (EAA) can lead to inferior protein utilization and result in reduced growth

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and decreased feed efficiency ratios (Anderson *et al*., 1992). Salmon diets usually contain about 40-60% fish meal (Gillund and Myhr, 2010) while tilapia diets contain 20-30% (or less) fish meal (El-Saidy and Gaber, 2002; Hernandez *et al*., 2010). Different dietary protein sources vary in their nutritional and biological values and differ in digestibility and amino acid composition (Anderson *et al*., 1992).

Protein is not the only important dietary component, however lipids, fibers, carbohydrates and antinutritional factors are also essential to proper nutrition. Fish require certain amounts of dietary lipids as sources of energy and essential fatty acids. The amount of lipid needed varies between species. For example, optimal levels for juvenile grouper (*Epinephelus malabaricus*) are about 9% (Lin and Shiau, 2003) both juvenile hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*) (Chou and Shiau, 1996) and juvenile European sea bass (*Dicentrarchus labrax*) require about 12% lipids to achieve maximum growth (Peres and Oliva-Teles, 1999). Rainbow trout (*Oncorhynchus mykiss*) in contrast, require at least 16% lipids (Lee and Putnam, 1973). Generally, salmonids grow well on diets containing up to 20% lipid (Jackson *et al*., 1984). An imbalance in digestible energy-to-Crude Protein (CP) ratio, excessive fat deposition (NRC, 1993; Peres and Oliva-Teles, 1999; Regost *et al*., 2001) and growth depression (Lin and Shiau, 2003) may result from feeding too high amounts of dietary lipid if the lipid level is lower than needed, however some fish such as rainbow trout can convert protein into fat (Lee and Putnam, 1973). Fibers are indigestible and nutritionally unavailable carbohydrate components in animal and plant sources and are commonly used as fillers or binders in fish feed (Dias *et al*., 1998). Fish can tolerate up to a certain amount of dietary fiber in their feed which is generally >10%, before it begins to affect growth rate (Anderson *et al*., 1984; Del Carmen Gonzalez-Pena *et al*., 2002).

There are no specific carbohydrate requirements for fish. Fish can utilize protein, fat and other components in their feed if no carbohydrates are present. It is recommended to add digestible carbohydrates to fish diets (NRC, 1993) since, certain species show reduced growth without them. For marine or coldwater fish concentrations of up to 20% carbohydrates seem to be optimal whereas fresh or warm water fish require concentrations >20%. Additionally, carbohydrates are the least expensive source of dietary energy (Wilson, 1994). Antinutritional Factors (ANF) such as trypsin inhibitors, hemagglutinating agents, phytic acids, gossypols, cyclopropenoic fatty acids, glucosinolates, erucic acids, alkaloids and thiaminases are substances found in plant sources that can affect fish performance can reduce digestibility or even prove toxic to fish (NRC, 1993). Fish meals are classified by the type of fish or the species of origin. Commonly used fish for fish meals include herring, menhaden and anchovy and others. They vary in their amino acid profiles and proximate compositions (USB, 2008). For the USA market, menhaden is the major fish source used for fish meal (Ingredients101, 2010a). Sinc, the 1980's intensive farming of salmon and trout has led to greater use of fish meal in fish diets.

Consequently, by 2008 nearly 60% of global fish meal supplies were utilized in aquaculture whereas use in pig and poultry feeds decreased to 31 and 10%, respectively (Jackson, 2009). Almost 70% of global fish meal has been fed to salmon, trout and shrimp, despite the fact that these species account for only 7% of global aquaculture production (Hardy, 2000). This is due to their need for high protein and high energy feeds.

Fish meal production was recorded at 2.6 million ton in 2008 with a decrease of 100,000 ton compared to 2007 (FAO, 2009a). In 2006, >77% of world fish production was used for human consumption whereas about 33% was used for fish meal and fish oil manufacture (FAO, 2009a). Overall, feeding costs generally represent the highest amount of total operating expenses of an aquaculture operation even up to 70% in some instances (Metts *et al*., 2007; Thompson *et al*., 2008). In Mediterranean aquaculture between 1.5 and 2.5 kg of feed are needed to produce 1 kg of fish; this makes up around 45% of production expenses (Martinez-Llorens *et al*., 2008). This is true particularly for carnivorous species such as salmonid diets (Meyers, 1994) because protein is the costliest component in aquaculture diets (Nguyen *et al*., 2009).

Based on increasing expenses, limited sources of fish meal, increasing demands and overfishing of wild stocks, numerous studies on plant protein sources, bacterial protein meal, fishery and animal byproducts have been conducted to examine the feasibility of replacing fish meal partially or even totally, in diets for different fish species. In addition, plant protein sources are relatively easy to obtain and their cost per unit is generally lower compared to fish meal (The FishSite, 2010). In 2004/2005, the average price of fish meal containing 60% protein was \$630 ton^{-1}, compared to \$876 ton^{-1} in 2008/2009 whereas the average price from October, 2009 to July, 2010 increased from \$1120-1516 ton^{-1} for the US east coast (USDA, 2010a). Previous studies have shown that fish meal can be effectively replaced by alternative protein sources (Kaushik *et al*., 1995; El-Sayed, 1998; Goda *et al*., 2007). Investigations for fish meal replacement are often for a single alternative protein source and can result in a high percentage of substitution but seldom equivalent values for growth performance, survival rate and specific weight gain. Essential amino acid composition, particularly lysine and methionine are lower in plant and animal sources than in fish meal and often have to be supplemented to achieve growth rates and weight gain of equal values. Various studies of total replacements for fish meal have also been accomplished using mixtures of alternative protein sources. In the majority of these cases, at least one portion was an animal protein source such as poultry byproduct meal, meat and bone meal, feather meal or blood meal (Wang *et al*., 2010a).

An important aspect of any substitute is the nutritional value of the alternative protein source. Rendered animal byproduct meals such as meat and bone meal, blood meal, feather meal and poultry byproduct meal are initially waste products from livestock and poultry production. They have been used for decades in salmonid feeds, however inclusion has been limited due to poor digestibility and large variations in quality. In addition, the nutritional quality is affected by the raw material composition, freshness and processing conditions (Bureau *et al*., 1999). Although, protein content and quality from alternative protein sources is often inferior to fish meal, high substitution levels through proper preprocessing and supplementation with EAA are possible and are already applied in many commercial fish diets.

The objective of this study is to review some potential alternative protein sources for fish meal. These include fish meal from fishery discard, fish processing byproducts, fish silage from processing wastes, meat and bone meal, blood meal, poultry-by products, feather meal, bacterial protein meal, soybean meal, soy protein concentrate, soy protein isolate, corn gluten meal, corn gluten feed, distillers dried grains with solubles, alfalfa meal, cottonseed meal, rapeseed meal, lupin seed meal, pea, lentil, chickpea, navy bean, pinto bean and black bean meal. Discussion includes processing, nutritive values, inclusion levels in previous fish feeding trials, availabilities and prices of these protein sources.

MATERIALS AND METHODS

Fishery products:

Fish meal: Fish meal is mostly produced from whole pelagic (i.e., surface-dwelling fish not bottom-dwelling) fish (e.g., anchovy, herring, mackerel, menhaden, sardine, tuna) or seafood processing byproducts such as processing waste, viscera and other unused parts of fish (Hardy and Tacon, 2002; Yano *et al*., 2008) that have little or no use for human consumption. Fish meal is generally light brown in color and is produced by cooking, pressing, drying and milling fresh raw fish and fish trimmings. It can be sold as four types; high quality meal, which is usually used in small-scale aquaculture units such as trout farms or marine species, low temperature meal which is highly digestible and used in salmon production, prime meal and fair/average quality meal which has lower protein content and is used for pig and poultry feeds (FIN, 2009).

Fish meal made of anchovy, herring, mackerel, menhaden or sardine can often have Crude Protein (CP) content between 67.4-89.6% (dry basis, db), 7.3-15.7% (db) crude fat, 0.5-1.1% (db) crude fiber, 11.2-22.3% (db) ash, 4.6-6.9% (db) lysine and 1.7-2.7% (db) methionine (Table 1 and 2).

Fish meal is mixed with other ingredients in fish feed (e.g., trout diets contain about 20-30% fish meal whereas catfish feed is predominantly based on soybean meal (50-55%) and corn (25-35%) and often contains only a small portion of fish meal) (Hammoumi *et al*., 1998).

Fish waste, fishery byproducts and/or discarded fish: Because of limited availability of wild caught fish and rising prices, feeding expenses can account for 40-80% of production costs (Rawles *et al*., 2010). Consequently, whole fish used in fish meal processing may be limited in the near future. Approximately half of processed seafood is discarded as waste material (Yano *et al*., 2008). Moreover, unintended by-catch such as other fish, mammals, turtles and birds are non-target organisms that are accidently captured and then discarded back into the

Table 1: Proximate composition for various fish meals (%, db)*

Fish meal type	Crude protien (%)	Crude fat (%)	Crude fiber (%)	NDF (%)	ADF (%)	ASH (%)	References
Anchovy	71.2-89.6	10.0-11.4	0.5-1.1	NA	NA	12.4-19.2	Anderson *et al*. (1993), NRC (2001), Hardy *et al*. (2005), Batal and Dale (2009) and Kop and Korkut (2010)
Herring	77.4-86.0	9.1-15.7	0.7-1.1	NA	NA	11.2-15.5	Anderspn *et al*. (1993), NRC (2001) and Batal and Dale (2009)
Mackerel	71.2	10.3	NA	NA	NA	15.0	Alibaba Group (2010)
Menhaden	67.4-70.4	10.4-11.1	0.8-1.1	NA	NA	20.4-22.3	Anderspn *et al*. (1993), NRC (2001) and Batal and Dale (2009)
Sardine	67.4-73.3	7.7-8.3	1.1	NA	NA	12.8-17.2	Mesomya *et al*.(2002), Harnandez *et al*. (2008) and Batal and Dale (2009)
Tune	57.0-64.4	7.3	0.9-5.4	NA	NA	23.5-26.9	NRC (2001) and Batal and Dale (2009)

*All data converted to a dry matter basis. NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; NA: Not Available

Table 2: Essential amino acid profiles for various fish meals (%, db)

Fishmeal type	Arg (%)	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Phe (%)	Thr (%)	Trp (%)	Val (%)	References
Anchovy	3.7-4.6	1.6-2.5	2.8-4.0	5.4-6.3	4.6-6.2	2.1	2.6-3.4	2.8-3.2	0.3-0.8	3.4-4.1	Anderson *et al.* (1993), NRC (1998), Glencross *et al.* (2005) and Batal and Dale (2009)
Herring	4.3-6.6	1.4-2.1	3.1-4.4	5.2-7.3	5.8-6.9	1.7-2.7	2.7-3.7	1.7-3.3	0.8-1.2	3.7-6.1	Anderson *et al.* (1993), NRC (1998) and Batal and Dale (2009)
Mackerel	4.0-4.2	2.9-3.1	2.8-2.9	5.1	5.6	1.9	2.7-2.8	2.9	0.7	3.4-3.5	Kim and Easter (2001)
Menhaden	4.0-5.9	1.5-1.9	2.8-3.6	4.8-5.6	5.0-6.1	1.9-2.4	2.7-2.9	2.8-3.2	0.4-0.7	3.3-4.3	Anderson *et al.* (1993), NRC (1998) and Hernandez *et al.* (2008)
Sardine	2.9-5.5	1.3-2.4	2.8-3.6	4.1-5.2	4.8-6.4	1.9-2.2	2.2-2.9	2.8-3.0	0.5	3.2-3.6	Hernandez *et al.* (2008) Batal and Dale (2009)
Tune	3.4-3.7	1.9	2.6	4.1	4.2-4.4	1.6	2.3-2.7	2.5-2.7	0.6-0.8	3.0	NRC (1993) and Batal and Dale (2009)

*All data converted to a dry matter basis

Table 3: Proximate composition for various animal byproducts (%, db)*

Animal byproducts	Crude protein	Crude fat	Crude fiber	NDF	ADF	Ash	References
	-----------------	-----------------	(%)	-----------------	-----------------		
Fish meal (fish waste)	56.5-80.0	5.2-34.4	0.07	NA	NA	6.5-26.8	Millamena (2002), Li *et al.* (2004), Hardy *et al.* (2005) and Goddard *et al.* (2008)
Fish silage (fish waste)	31.6-56.5	5.9-7.5	6.8-8.3	NA	NA	11.4-23.5	Hammoumi *et al.* (1998), Hossain *et al.* (1997) and Geron *et al.* (2007)
MBM	47.3-54.3	7.2-10.3	2.5-2.6	34.9	6	31.1-41.3	NRC (1993, 1998), Allan *et al.* (2000) and Guimaraes *et al.* (2008)
BM	80.0-98.8	0.0-1.7	0.0-1.1	14.8	26.4	1.0-3.1	NRC (1998), Allan *et al.* (2000), Bureau *et al.* (2000), Fasakin *et al.* (2005) and Martinez-Llorens *et al.* (2008)
PBM	58.0-64.2	12.0-17.1	2.1-2.5	0.0-2.5	NA	15.6-22.5	NRC (1993), Fasakin *et al.* (2005) and Guimaraes *et al.* (2008)
FEM	89.6-92.4	3.3-11.2	1.3-3.3	1.5	17.6	3.0-4.2	NRC (1993), Allan *et al.* (2000), Batal and Dale (2009) and Ingredients101 (2010d)
BPM	67.0-68.1	8.1-10.4	NA	NA	NA	6.2-8.1	Skrede *et al.* (1998), Storebakken *et al.* (2004) and Aas *et al.* (2006 a, b)

*All data were converted to on a dry matter basis; MBM: Meat and Bone Meal; BM: Blood Meal; PBM: Poultry byproduct Meal; FEM: Feather Meal; BPM: Bacterial Protein Meal; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; NA: Not Available

Table 4: Essential amino acid profile of various animal protien sources (%, db)*

Animal byproducts	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	References
	----------	----------	----------	----------	----------	(%)	----------	----------	----------	----------	
Fish meal (fish waste)	3.8-6.1	1.0-1.9	2.0-4.1	3.1-6.5	2.0-5.2	1.0-2.3	2.2-3.6	2.2-4.0	NA	2.3-4.1	Li *et al.* (2004)
Fish silage (fish waste)	1.9-3.0	1.0-1.4	1.2-3.2	2.4-3.5	2.4-3.3	1.9-2.2	1.3-2.2	1.6-2.1	0.2-0.4	1.4-3.0	Borghesi *et al.* (2008)
MBM	3.2-4.3	0.6-1.2	1.1-1.8	2.3-3.5	2.3-3.5	0.5-1.1	1.5-1.9	1.2-2.1	0.2-0.3	1.8-2.6	NRC (1993), Allan *et al.* (2000) and Guimaraes *et al.* (2008)
BM	2.4-4.0	3.0-6.0	0.8-1.0	9.8-11.6	6.9-8.0	0.7-1.5	5.4-6.3	3.1-5.4	0.8-1.7	5.2-8.2	NRC (1993), Allan *et al.* (2000), Kats *et al.* (1994), Sauvant (2004) and Ingredients101 (2010b)
PBM	3.7-4.6	1.2-1.5	2.2-2.7	4.2-4.6	2.4-5.0	1.0-1.4	1.7-2.5	1.0-2.5	0.4-0.5	2.5-3.3	NRC (1993), Guimaraes *et al.* (2008) and Batal and Dale (2009)
FEM	5.7-6.7	0.5-1.1	3.9-4.9	6.8-8.0	1.8-2.9	0.6-0.7	4.1-4.6	4.0-4.7	0.4-0.6	5.6-7.3	NRC (1993), Allan *et al.* (2000), Sauvant (2004) and Guimaraes *et al.* (2008)
BPM	3.9-4.5	1.2-1.6	2.5-3.4	4.4-5.5	3.1-4.3	1.5-2.1	2.3-3.1	2.5-3.4	0.8-1.5	3.2-4.3	Skreda *et al.* (1998) and Aas *et al.* (2007)

*All data were converted to on a dry matter basis; MBM: Meat and Bone Meal; BM: Blood Meal; PBM: Poultry Byproduct Meal; FEM: Feather Meal; BPM: Bacterial Protein Meal

sea because they are not needed (Harrington *et al.*, 2005). Since, this negatively affects the environment, processing of fish waste to fish meal represents is a promising alternative method of waste utilization. However, fish waste varies highly in its physical nature and proximate composition. For example, fish waste consisting of intestines has high lipid but low protein contents (Yano *et al.*, 2008). The raw waste materials vary in nutritional values, depending on the size of fish and may require special preservation to maintain quality (Goddard *et al.*, 2008). Depending on the raw waste used, fish meal based on by-catch or fish waste may contain between 56.5-80.0% (db) CP, 5.2-34.4% (db) crude fat, 0.07% (db) crude fiber, 6.5-26.8% (db) ash, 2.0-5.2% (db) lysine and 1.0-2.3% (db) methionine (Table 3 and 4). Generally, seafood waste is only available during the fishing season often from poorly accessible areas where it is difficult to process. Sometimes, it can be preserved as press cake meal (Hardy *et al.*, 2005). In seafood processing plants, up to 50% of the processed fish can result in waste material (Ferraz de Arruda *et al.*, 2007). Fish waste is composed of body parts that are not consumable by humans and can include heads, fins, viscera, scales and under-sized fish (Pagarkar *et al*., 2006; Yano *et al*., 2008). Large quantities of fish waste resulting from inefficient processing can result in a high environmental burden. To avoid increasing pollution due to waste disposal problems, this low-cost material could be used as fish feed. Previous studies have reported that fish meals produced from discarded fishery waste and processing byproducts can be suitable as a total or partial replacement for commercial fish meal for rainbow trout (Hardy *et al*., 1983, 2005; Stone *et al*., 1989), tilapia (Goddard *et al*., 2008; Chitmanat *et al*., 2009) and red drum (*Sciaenops ocellatus*) (Li *et al*., 2004; Whiteman and Gatlin, 2005). Hardy *et al*. (2005) suggested that limiting factors such as chitin and bones should be removed and diets should be supplemented with amino acids and minerals to successfully use higher inclusion levels of these meals in commercial rainbow trout diets.

Fish silage: Another alternative method to use fish waste can be the production of fish silage. Compared to fish meal, the production of fermented fish silage requires less technology and cost and is relatively easy to produce (Fagbenro *et al*., 1997; Ferraz de Arruda *et al*., 2007). On the other hand, transportation costs can be high due to high water content (Rustand, 2003). Fish silage is a liquid or semi-liquid product that can be preserved using microbial fermentation (Borghesi *et al*., 2008). Fish silage is made of low-value whole fish or fish parts that are ensiled by fermentation using lactic acid bacteria that either exist naturally in the fish waste or are inoculated as starter cultures. Enzymes can also be added to the starter cultures (Tatterson and Windsor, 1974) or enzymes can be used as a sole source of liquefaction (Borghesi *et al*., 2008). Another common way to liquefy fish waste is by adding 2-3% formic acid and storing at room temperature for several days until pepsins and other acid proteases in the fish tissue have dissolved the raw material. A pH of 3-4 generally indicates that the silage is well preserved (Rustand, 2003). Fish silage contains typically high amounts of unsaturated lipids that may be oxidized and form toxic products causing cellular changes.

Jackson *et al*. (1984) suggested adding antioxidants to increase storage life. Fish silage generally contains 31.6-56.5% (db) CP, 5.9-7.5% (db) crude fat, 6.8-8.3% (db) crude fiber, 11.4-23.5% (db) ash, 2.4-3.3% (db) lysine and 1.9-2.2% (db) methionine (Table 3 and 4). The lower protein in fish silage compared to fish meal is due to the liquefaction which can convert the proteins to polypeptides and free amino acids (Tatterson and Windsor, 1974). Traditionally, fish silage has been used as a dietary ingredient for poultry and swine feed (Ferraz de Arruda *et al*., 2007). Crop residues and/or crop byproducts can be used as fillers in the fish silage to achieve better drying characteristics (Fagbenro and Jauncey, 1994) during preparation of fish diets. Studies have reported that fish silage can be successfully integrated into diets for rainbow trout (Stone *et al*., 1989), Atlantic salmon (*Salmo salar)* (Lie *et al*., 1998), juvenile Nile tilapia (Fagbenro and Jauncey, 1994), juvenile pacu (*Piriactus mesapotamicus)* (Vidoiti *et al*., 2002) and juvenile catfish (Fagbenro *et al*., 1997) as a highly digestible fish meal alternative. Neutralized, dried fish silage replaced fish meal in trout diets but resulted in lower growth rate, although it did not affect final weight or palatability. The authors hypothesized that the inferior growth rate was caused by less intact protein and higher portion of free amino acids (Stone *et al*., 1989).

Animal protein sources

Meat and Bone Meal (MBM): High amounts of meat and bone meal are steadily produced in the USA and other industrialized countries. In fact, the meat industry produces most of the organic waste in the food processing sector (Banks and Wang, 2006). MBM is a product of animal rendering and is produced from animal waste tissues that are not used for human foods. It consists of trimmings, bones, viscera, undigested feed, blood, heads, hooves, hides and/or dead livestock (Shirley and Parsons, 2001). The variety of raw materials often results in large variations in chemical composition and protein quality. MBM has been primarily used as a high protein supplement in animal diets for many years. But due to the Bovine Spongiform Encephalopathy (BSE) crisis in 1990's, MBM has been prohibited for use in ruminant feeds in many countries.

Moreover, particularly in the European Union, MBM is hardly used in traditional feed markets any more (Garcia and Rosentrater, 2008). Depending on the source, CP content is around 47.3-54.3% (db), crude fat is 7.2-10.3% (db), crude fiber is 2.5- 2.6% (db) and ash is 31.1-41.3% (db). Lysine content (2.5-3.5% db) and methionine (0.5-1.5% db) are lower than fish meal (Table 3 and 4). In 2004/05, the average price for MBM for the Central USA was \$181 ton^{-1}, compared to \$356 ton^{-1} in 2008/2009 (USDA, 2010a).

For years MBM has been fed to salmonids (Bureau *et al*., 2000). MBM is commonly included in commercial fish diets but generally at levels not >20%. In experimental studies, various results were reported for diverse species. For example for Nile tilapia fingerlings, 40% MBM was used in combination with 36% wheat bran and 10% corn starch and totally replaced fish meal in a 30% CP diet; results indicated no significant differences in weight gain, specific growth rate or survival rate (El-Sayed, 1998). In diets for rainbow trout, MBM at 24% inclusion replaced fish meal up to 32% in a 48% CP diet (Bureau *et al*., 2000). For juvenile hybrid striped bass (*Morone chrysops* x *M. saxatilis*), MBM could be used as the primary protein source at 45% inclusion level in a 44% CP diet (Bharadwaj *et al*., 2002). Webster *et al*. (2000) concluded that juvenile sunshine bass fed a diet of 35% soybean meal and 35% meat and bone meal resulted in higher weight gain, specific growth rate and survival than the control diet which contained 30% fish meal. For juvenile Japanese flounder (*Paralichthys olivaceus*), 18% MBM replaced 20% fish meal in a 48% CP diet (Kikuchi *et al*., 1997) without compromising growth rate or weight gain. When using MBM, reduced growth may be due to deficiencies in EAA such as methionine, lysine and isoleucine. Furthermore, MBM may exhibit lower digestibilities (Millamena, 2002). In studies with Nile tilapia, diets formulated with MBM resulted in the lowest apparent protein digestibility (78.4%) compared to fish meal which was 88.6% (Guimaraes *et al*., 2008).

Blood Meal (BM): Blood meal is made of blood from animal processing plants and dried after the whole blood has been centrifuged to remove foreign material (Ingredients101, 2010b). Spray, ring or flash-drying systems can be used to process the blood and to eliminate pathogens (Martinez-Llorens *et al*., 2008). It is a high-quality protein product with CP levels varying between 80.0-98.8% (db), 0.0-1.7% (db) crude fat, 0.0-1.1% (db) crude fiber, excess concentrations of lysine (6.9-8.0%, db) and low concentrations of methionine (0.7-1.5%, db) (Table 3 and 4). Regarding essential amino acid content, BM is deficient in isoleucine but has excess histidine, leucine, phenylalanine, threonine and valine compared to fish meal (Table 2 and 4). These EAA imbalances can lead to negative effects in fish performance. On the other hand, enrichment in isoleucine can balance these adverse effects (Yamamoto *et al*., 2004). In June 2009, ruminant BM prices were about \$785 ton^{-1} and in June, 2010 about \$930 ton^{-1} (The Jacobsen Price Guide, 2010). Similar to meat and bone meal, ruminant BM has generally been avoided in animal and fish diets to protect against Bovine Spongiform Encephalopathy (BSE) (Fasakin *et al*., 2005). Porcine BM, however is commonly used in ruminant diets. High dietary inclusion levels of BM have not been effective in growth performance for most fish species, considering the fact that small inclusion levels of BM have generally been substituted for fish meal. For Nile tilapia fingerlings, 10% inclusion of BM, constituting 31% protein of the CP in the diet, resulted in satisfactory production and average weight gain (Otubusin, 1987). In a 47% CP diet for juvenile and growing gilthead sea bream (*Sparus aurata*), BM could be included between 5 and 15% for fish meal without affecting growth performance and survival rate (Martinez-Llorens *et al*., 2008). Several studies have been conducted with rainbow trout diets incorporating 5% BM (Satoh *et al*., 2003), 6-12% BM (El-Haroun *et al*., 2009) and up to 23% BM without affecting growth performance and survival; the latter diet contained 23% blood powder as the primary protein source in a 47% CP diet, replacing 65% of the herring meal (Luzier *et al*., 1995). BM at 8% inclusion in combination with meat meal (32% inclusion level) replaced 80% of fish meal in a 45% CP diet for juvenile grouper (*Epinephelus coioides*) with similar growth rates and weight gain as the fish meal-based control diet (Millamena, 2002). Prices for pork and other non-ruminant BM are more expensive than ruminant blood because there are limited amounts available.

Poultry Byproduct Meal (PBM): Poultry production is one of the fastest growing agricultural sectors (Zielinska *et al*., 2007) and produces increasing quantities of wastes and byproducts. Slaughterhouse wastes can be recycled as a potential source of protein in fish diets (Kherrati *et al*., 1998). Poultry byproducts consist of feet, heads, undeveloped eggs, gizzards and viscera (FAO, 2010a). Depending on the source, PBM can contain approximately 58.0-64.2% (db) CP, 12.0-17.1% (db) crude fat, 2.1-2.5% (db) crude fiber and 15.6-22.5% (db) ash (Table 3). Regarding the EAA profile, values are similar to the EAA of fish meal except for methionine which is lower at 1.1-1.4% (db). Lysine content varies between 3.1-5.0% (db) (Table 4). Processing conditions affect the quality of PBM, its protein and amino acid availability and digestibility (Thompson *et al*., 2008).

PBM has been used in commercial diets for a variety of fish species such as sunshine bass and hybrid striped bass (Rawles *et al*., 2009, 2010). PBM has been evaluated as a potential feed ingredient for juvenile male hybrid tilapia where 67% of the fish meal could be replaced with 30% PBM in a 35% CP diet (Fasakin *et al*., 2005). In Nile tilapia fingerling diets, PBM at 47 and 27% inclusion levels with other non-fish meal sources totally replaced fish meal in 30 and 34% CP diets, respectively without differences in growth or weight gain (El-Sayed, 1998; Hernandez *et al*., 2010). It has also been examined in rainbow trout diets where 37 and 40% fish meal was replaced with 20% PBM and 30% PBM in 45% and 50% CP diets, respectively (Sevgili and Erturk, 2004; El-Haroun *et al*., 2009). PBM combined with feather meal at 27% inclusion and supplemented with methionine and lysine could substitute for 50% fish meal in practical

51% CP diets for rainbow trout without compromising weight gain or specific growth rate (Steffens, 1994). Using high-quality PBM at 30% inclusion level allowed a total replacement of fish meal in diets for hybrid striped bass in a 37% CP diet combined with soybean meal (32%), wheat (20%) and corn meal (12%) (Pine *et al*., 2008) as well as 35% PBM with soybean meal (26%), wheat (15%) and cottonseed meal (5%), supplemented with lysine and methionine in a 43% CP diet (Rawles *et al*., 2009).

Feather meal: Feathers are byproducts of broiler, Turkey and poultry processing operations. If they are left unprocessed, they are not digestible. Processing of feather meal is conducted by steam hydrolysis under high pressures and temperatures followed by drying and grinding (Chandler, 2009). Feather meal is commonly fed to monogastric and ruminant animals (FAO, 2010b) and has proximate concentrations of 89.6-92.4% (db) CP, 3.3-11.2% (db) crude fat, 1.3-3.3% (db) crude fiber and 3.0-4.2% (db) ash. Feather meal has 1.8-2.9% (db) lysine and 0.6-0.7% (db) methionine which is lower than fish meal. It has excess phenylalanine, threonine and valine (Table 3 and 4). The average price for feather meal in 2004/05 in Arizona was \$268 ton^{-1} and in 2008/09, it was \$503 ton^{-1} (USDA, 2010a).

Feather meal has been used for several years in aquafeeds, however poor digestibility and variations in quality have limited its use (Bureau *et al*., 2000). For Nile tilapia, poultry feather meal has one of the lowest apparent protein digestibilities among animal products such as PBM and fish meal (Guimaraes *et al*., 2008). Recently, improved processing conditions have resulted in relatively higher digestibilities (Bureau *et al*., 1999). In 54-57% CP diets for juvenile rainbow trout, feather meal could be included up to 15% without affecting growth, feed efficiency, nitrogen or energy gains for the same level of fish meal (Bureau *et al*., 2000). Other studies have reported partial replacement of dietary fish meal with feather meal up to 20% for Indian major carp fry (*Labeo rohita*) in a 35% CP diet compensating for 50% of the fish meal (Hasan *et al*., 1997) and up to 10% for Nile tilapia fry in a 25% CP diet replacing 66% of the fish meal (Bishop *et al*., 1995) without compromising growth and feed utilization.

Bacterial Protein Meal (BPM): Bacterial protein meal is produced by fermentation for protein synthesis with bacteria cultures consisting of *Methylococcus capsulatus*, *Alcaligenes acidovorans*, *Bacillus brevis* and *Bacillus firmus* often using methane as an energy and carbon source and ammonia as a nitrogen source (Skrede *et al*., 1998; Aas *et al*., 2006a). In this aerobic process, bacterial biomass is continuously harvested, short-time heat treated to obtain a sterile product, centrifuged then spray-dried. BPM generally contains about 67.0-68.1% (db) CP, 8.1-10.4% (db) crude fat and 6.2-8.1% (db) ash (Table 3); crude fiber has not been reported. Compared to fish meal, BPM contains similar levels of EAA such as a lysine content of 3.1-4.3% (db) and methionine of 1.5-2.1% (db) whereas tryptophan is lower at 0.8-1.5% (db) (Table 4). BPM has been investigated in diets for Atlantic salmon fry where at least 19% BPM compensated for up to 29% fish meal in a 48% CP diet (Storebakken *et al*., 2004). In other studies, up to 36% BPM inclusion levels were substituted for 46% of the fish meal in a 51% CP diet (Aas *et al*., 2006a). For rainbow trout, up to 31% fish meal could be replaced with 15% BPM inclusion in a 40% CP diet (Overland *et al*., 2006). In other experiments, 27% inclusion of BPM in a 50% CP diet could replace 36% of the fish meal (Aas *et al*., 2006b). In contrast, the incorporation of BPM in diets for Atlantic halibut (*Hippoglossus hippoglossus*) was only successful at 9% inclusion levels, compensating for 12% of fish meal in a 49% CP diet (Aas *et al*., 2007).

RESULTS AND DISCUSSION

Plant protein sources: Leguminous crops include peas and beans of which soybean has been the most investigated and used plant protein source in aquafeeds (Davis *et al*., 2002). Studies with other plant protein sources including corn products, cottonseed meal, alfalfa meal and canola, amongst others in fish feeds have been promising. Advantages of plant protein sources can include lower price, greater availability and improved consistency of composition. Compared to fish meal, though they vary in their nutritional and biological values and generally have lower palatability. Deficiencies of EAA often cause decreases in growth performance and feed efficiency ratios (Anderson *et al*., 1995). Locations, seasonal changes, growth conditions, agricultural practices as well as variations between individual plants can affect the nutritional composition of plant materials (Harnly *et al*., 2009). Another limiting factor to using plant-derived proteins is the presence of ANF (anti nutritional factors) or toxicants that may be present as protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, alkaloids, tannins, cyanogens and glucosinolates (Murray *et al*., 2010). ANF can adversely affect digestion, absorption and physiological utilization of protein and amino acids and can limit the palatability and the nutritive utilization of protein (Burel *et al*., 1998; Murray *et al*., 2010). Numerous ANF can be inactivated or reduced by heat treatment (Francis *et al*., 2001), dehulling, germination and other processing steps (Bau *et al*., 1997; El-Adawy, 2002; Kuo *et al*., 2004). Corn products are very promising due to the absence of ANF and high availability, although there are some nutritional limitations.

Soybean Meal (SBM): Soybean meal is produced by removing oil from whole soybeans, toasting the flakes then grinding into meal (AgMRC, 2010). Nutrient composition commonly ranges from 46.9-51.2% (db) CP, 1.5-4.7% (db) crude fat, 7.1-8.4% (db) crude fiber and 6.1-7.4% (db) ash. In addition, lysine content is generally 2.8-4.0% (db) and methionine is 0.5-0.9% (db) both of these concentrations are lower than found in fish meal (Table 4 and 5). In aquafeeds, SBM is the most commonly used plant protein because of its high availability, nutritional value, consistent composition and reasonable price (Refstie *et al*., 2000; Thompson *et al*., 2008). Factors which can limit the value of SBM in fish feed include Antinutritional Factors (ANF) such as protease inhibitors, trypsin inhibitors, phytates, lectins, phytic acid, saponins, phytoestrogens, antivitamins and allergens. Heat treatment can reduce the trypsin inhibitors but it has to be conducted with care due to possible reduction of nutritional value caused by overheating which can lead to the destruction of lysine, denaturation of protein and thus reduction in protein quality and digestibility (Francis *et al*., 2001; Barrows *et al*., 2007). Global SBM production has increased steadily over the last several decades and was estimated at 152.2 million ton for 2008/2009, compared to a production of 90.1 million ton in 1996/1997 (USDA, 2010b). Average SBM price for 2004/05 was $205 ton^{-1}, compared to an average price of $365 ton^{-1} in 2008/09 (USDA, 2010a). Several studies have reported that the total replacement of fish meal with SBM has resulted in low palatability, reduction of nutrient digestibility and reduced growth (Thompson *et al*., 2007) and should be supplemented with methionine and/or lysine. Goda *et al*. (2007) concluded that dry extruded SBM and extruded full-fat soybean supplemented with lysine and methionine could effectively be used as the main source of dietary protein in a 28% CP diet fed to Nile tilapia fingerlings. In the same study, tilapia galilae (*Sarotherodon galilaeus*) fingerlings showed higher growth rate, weight gain and feed intake when fed the extruded SBM diet compared to the fish meal control diet. Furthermore, they assumed that high growth performance and feed utilization could be

Table 5: Proximate composition for various plant protien sources (%, db)*

Plant protien sources	Crude protien	Crude fat	Crude fiber	NDF	ADF	Ash	References
	(%)						
SBM	46.9-51.2	1.5-4.7	7.1-8.4	12.6-13.5	8.8	6.1-7.4	Cervantes-Prahm and Stein (2008), Abimorad *et al*. (2008), Guimaraes *et al*. (2008), Glencross *et al*. (2005), Caine *et al*. (2008) and Liu *et al*. (2009)
SPC	59.0-74.5	0.3-5.4	3.1	10.3	NA	5.5-7.9	Glencross *et al*. (2005), Tibbetts *et al*. (2006), Cervantes-Prahm and Stein (2008) and Cruz-Suarez *et al*. (2009)
SPI	88.5-92.6	0.1-4.8	0.4	NA	NA	4.7-6.5	Glencross *et al*. (2005), Tibbetts *et al*. (2006) and Cruz-Suarez *et al*. (2009)
CGM	66.7-74.7	4.8-9.5	0.8-2.4	10.4-12.6	5.6-6.9	1.1-2.2	Tibbetts *et al*. (2006), Abimorad *et al*. (2008), Guimaraes *et al*. (2008) and Ingredients101 (2010e)
CGF	18.0-23.8	3.5-3.9	7.5-8.0	33.8-45.0	8.8-13.0	6.1-6.8	NRC (2001), Sauvant (2004), Myer and Hersom (2008) and Ingredients101 (2010f)
DDGS	26.8-33.7	3.5-12.8	5.4-10.6	25.0-51.3	8.0-23.6	2.0-9.8	NRC (2001), Rosentrater and Muthukumarappan (2006) and Stein *et al*. (2006)
CSM	33.4-47.0	1.6-4.5	13.0-28.6	23.9-35.3	17.5-24.6	5.7-7.8	Cheng and Hardy (2002), Sauvant (2004), Guimaraes *et al*. (2008) and Ingredients101 (2010c)
Alfaalfa meal	18.0-19.2	2.7	25.7-28.8	41.6-48.0	32.8-36.8	10.4-11	NRC (2001), Ali *et al*. (2003) and Fekete *et al*. (2004)
RSM/Canola	33.7-43.1	4.8	11.5-12.4	28.3-29.8	19.6-20.5	6.5-7.9	Sosulski and Zadernowski (1981), Burel *et al*. (2000), NRC (2001), Sauvant (2004) and Caine *et al*. (2008)
Lupin meal	34.1-48.2	5.5-6.2	11.6-19.0	21.6-30.7	16.2-27.6	2.8-4.9	Allan *et al*. (2000), Booth *et al*. (2001), Mariscal-Landin *et al*. (2002), Sujak *et al*. (2006) and Pisarikova *et al*. (2008)
Pea meal	22.4-30.7	1.1-2.6	4.4-11.8	13.3-21.3	6.9-14.5	3.0-3.9	Mariscal-Landin *et al*. (2002), Sauvant (2004), Caine *et al*. (2008) and Khattab *et al*. (2009)
Lentil meal	25.9-33.1	1.0-3.6	1.2-7.5	8.1-9.0	5.4-6.0	2.6-4.3	Candela *et al*. (1997), Bednar *et al*. (2001), Wang and Daun (2006), Wang *et al*. (2009) and El-Adaway *et al*. (2003)
Chickpea meal	21.0-24.0	4.7-5.2	3.9-9.1	10.9-16.9	5.8-13.4	2.7-3.6	Abreu and Bruno-Soares (1998), Booth *et al*. (2001) and Iqbal *et al*. (2006)
Navy bean	22.8-30.4	1.6	5.6-8.1	22.2-25.8	6.7-9.9	3.5-5.6	Meiners *et al*. (1976), Paduano *et al*. (1995), Gyori *et al*. (1998), Bednar *et al*. (2001), Hoover and Ratnayake (2002) and Feedstuff (2010)
Pinto bean	21.6-22.4	0.8-1.0	5.0-7.4	NA	6.7	3.8-5.0	Meineres *et al*. (1976), Sotelo *et al*. (1995) and Wang *et al*. (2010b)
Black bean	23.2-25.9	1.6-3.6	3.3-3.5	NA	NA	4.2-5.0	Berrios *et al*. (1999), Bednar *et al*. (2001), Siddiq *et al*. (2010) and Wang *et al*. (2010b)

*All data were reported on a dry matter basis; SBM: Soybean Meal; SPC: Soy Protien Concentrate; SPI: Soy Protien Isolate; CGM: Corn Gluten Meal; CGF: Corn Gluten Feed; DDGS: Distillers Dried Grains with Solubles; CSM: Cottonseed Meal; RSM: Rapeseed Meal; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; NA: Not Available ascribed to inactivation of heat-labile ANF in SBM via extrusion. Other studies also concluded that dehulled solvent-extracted and expeller-pressed SBM at 64 and 68% inclusion levels, respectively could totally replace fish meal in commercial diets containing 32% protein for juvenile tilapia without methionine or lysine supplementation and without affecting weight gain, survival rate or palatability (Nguyen *et al*., 2009). In studies of Southern catfish (*Silurus meridionalis*) 39% of the fish meal could be replaced with 23% SBM inclusion level without adversely affecting specific growth rate in a 48% CP diet; the researcher determined that reduced growth in diets containing high levels of SBM were due to imbalances of EAA such as methionine and could be improved by supplementation of methionine (Ai and Xie, 2005). Much research has been conducted for rainbow trout where SBM supplemented with or without lysine and methionine could replace 20-50% of the fish meal without compromising growth performance or weight gain (Oliva-Teles *et al*., 1994; Refstie *et al*., 2000). Barrows *et al*. (2007) came to similar conclusions when determining that extrusion of solvent-extracted SBM resulted in higher weight gain and feed intake when fed to rainbow trout. Dehulled and solvent-extracted SBM at 27% inclusion level supplemented with methionine replaced at least 33% of fish meal in a 37% CP Atlantic salmon diet (Carter and Hauler, 2000). Compared to rainbow trout, Atlantic salmon seems to be more sensitive to ANF in defatted SBM; in contrast to trout, they were not able to grow at a similar rate when fed diets with defatted SBM substituted for 37% of the dietary protein compared to when fed fish meal-based diets (Refstie *et al*., 2000). Defatting, dehulling, solvent-extracting, expeller pressing or fermenting can be applied to SBM to remove ANF and/or to improve the nutritive value. Investigations on SBM processed with or without amino acids resulted in successful total replacement of fish meal in diets for commercially important species such as Nile tilapia (Furuya *et al*., 2004; Ngyuen *et al*., 2009).

Soy Protein Concentrate (SPC): Soy protein concentrate is one of the three major soy protein products that is used in aquafeeds (Wang and Johnson, 2001). SPC is derived from dehulled, solvent-extracted, defatted flakes which are then extracted with ethanol or other acids so that soluble carbohydrates and various ANF are removed (Lusas and Riaz, 1995; USSEC, 2010). With a CP of 59.0-74.5% (db), it has a CP content similar to fish meal. Crude fat is 0.3-5.4% (db), crude fiber is 3.1% (db) and ash is 5.5-7.9% (db). SPC is low in lysine with levels of 2.8-4.7% (db); methionine is low also ranging from 0.9-1.0% (db), thus these are limiting amino acids (Table 4 and 5). SPC has been used to replace fish meal in feed for salmonids (Carter and Hauler, 2000). Studies at 58% SPC inclusion level supplemented with lysine, methionine and taurine in a 46% CP diet showed that yellowtail (*Seriola quinqueradiata*) juveniles had comparable growth to fish fed a fish meal-based diet (Takagi *et al*., 2008). SPC at 49% inclusion level substituted up to 75% fish meal in 46% CP diets for juvenile cobia (*Rachycentron canadum*) without negatively affecting weight gain or growth rate (Salze *et al*., 2010). Furthermore, it was also possible to totally replace fish meal for juvenile rainbow trout with methionine supplemented SPC at 62% inclusion level in a 46% CP diet without reducing growth or nutrient utilization (Kaushik *et al*., 1995).

Soy Protein Isolate (SPI): Soy protein isolate is the purest commercially available soy protein product and has the highest protein content of all soy products. It is made from SPC by further processing to remove insoluble fiber using alkaline extraction and soluble sugars via acid precipitation (Wang and Johnson, 2001; Swain *et al*., 2004). Crude protein content is 88.5-92.6% (db), crude fat is 0.1-4.8% (db), crude fiber is <0.4% (db) and ash is 4.7-6.5. Lysine is 4.5-5.7% (db) and methionine is 1.1-1.3% (db) (Table 5 and 6). In recent years, SPE has been gaining attention in aquafeeds. In 45% CP diets for juvenile cobia, SPI at 23% inclusion level substituted for 41% of fish meal and resulted in higher growth rate and weight gain compared to the fish meal-based control diet (Lunger *et al*., 2007).

On the other hand, when fed to post-juvenile Chinook salmon (*Oncorhynchus tshawytscha*) at 15 and 30% inclusion levels, digestibility of SPI could not be validated due to poor palatability (Hajen *et al*., 1993).

Corn Gluten Meal (CGM): Corn gluten meal is a coproduct of corn wet-mill processing which separates corn into starch, germ, protein and fiber fractions. It is the product remaining after the extraction of starch from the corn kernel (Goda *et al*., 2007) and consists predominantly of gluten (Sauvant, 2004). CGM has traditionally been used as cattle, swine and poultry feed (Wu *et al*., 1996). Crude protein content is high at 66.7-74.7% (db), crude fat is 4.8-9.5% (db), crude fiber is 0.8-2.4% (db) and ash is 1.1-2.2% (db). CGM is low in lysine, ranging from 1.0-2.1% (db) and low in methionine, ranging from 0.9-1.8% (db) (Table 5 and 6). CGM does not contain ANF. Average prices for CGM (containing 60% CP), increased from \$299-\$539 ton^{-1} from 2004/05 to 2008/09 for the Midwestern USA (USDA, 2010a). Several studies have examined CGM as a protein substitute in diets for fish. Nile tilapia have been fed up to 18% CGM inclusion

Table 6: Essential amino acid profiles of various plant protien sources (%, db)

Plant protien sources	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	References
	(%)										
SBM	3.2-4.2	1.2-1.4	2.0-2.3	3.7-4.4	2.8-4.0	0.5-0.9	2.3-2.4	1.7-2.4	0.5-0.6	2.2-2.5	Glencross *et al.* (2005), Guimaraes *et al.* (2008) and Cervantes-Prahm and Stein (2008)
SPC	4.5-6.4	1.5-2.0	2.6-3.7	4.7-5.9	2.8-4.7	0.9-1.0	3.0-3.6	2.5-3.1	0.9-1.0	2.7-3.8	NRC (1998), Glencross *et al.* (2005) and Cervantes-Prahm and Stein (2008)
SPI	6.7-7.5	2.2-2.4	3.8-4.6	6.8-7.2	4.5-5.7	1.1-1.3	4.5-4.7	3.1-3.6	1.1	4.0-4.6	NRC (1998) and Glencross *et al.* (2005)
CGM	1.9-2.2	1.1-1.4	2.0-2.8	9.7-11.2	1.0-2.1	0.9-1.8	3.3-4.4	1.7-2.3	0.3-0.5	2.4-3.4	NRC (1993), Guimaraes *et al.* (2008) and Abernorad *et al.* (2008)
CGF	0.9-1.2	0.6-0.7	0.6-0.7	1.6-2.2	0.6-0.7	0.3-0.5	0.7-0.8	0.7-0.8	0.1-0.2	0.9-1.1	Schroeder (1997), NRC (1998) and Sauvant (2004)
DDGS	0.9-2.2	0.6-1.0	0.9-1.5	2.4-4.0	0.5-1.1	0.5-0.8	1.3-1.7	0.8-1.3	0.2-0.3	1.3-1.8	NRC (1993), Stein *et al.* (2006) and Rosentrater and Muthukumarappan (2006)
CSM	0.9-5.7	0.5-1.4	1.2-2.4	1.0-2.8	1.0-2.2	0.5-1.1	1.6-3.3	0.9-1.7	0.3-0.6	1.4-2.9	NRC (1998), Cheng and Hardy (2002) and Guimaraes *et al.* (2008)
Alfaalfa meal	0.6-1.1	0.3-0.5	0.7-1.1	1.2-1.6	0.6-1.0	0.3-0.4	0.7-1.1	0.6-0.9	0.3-0.5	0.9-1.3	NRC (1998), Ali *et al.* (2003) and Batal and Dale (2009)
RSM/Canola	1.1-2.5	0.5-1.2	0.8-1.8	1.2-2.9	1.2-2.5	0.4-0.8	0.7-1.6	0.9-1.8	0.3-0.5	1.0-2.2	NRC (1993), Fan and Sauer (1995) and Sauvant (2004)
Lupin meal	3.8-4.4	0.9-1.2	1.4-1.7	2.3-2.8	1.4-2.1	0.2-0.4	1.3-1.4	1.3-1.5	0.3	1.3-1.6	NRC (1998), Allen *et al.* (2000), Booth *et al.* (2001) and Pisarikova *et al.* (2008)
Pea meal	1.5-2.5	0.5-0.6	0.9-1.1	1.5-1.7	1.5-1.7	0.2-0.3	1.0-1.1	0.8-0.9	0.2	1.0-1.2	NRC (1998), Allen *et al.* (2000), Booth *et al.* (2001) and Sauvant (2004)
Lentil meal	2.3	0.9	1.1	2.1	1.6-1.9	0.2	1.4	0.9	0.2	1.4	NRC (1998)
Chickpea meal	1.5-2.0	0.2-1.1	0.8-1.1	1.1-2.2	1.4-1.8	0.2-1.0	1.1-1.5	0.7-1.0	0.2	0.8-1.3	Allan *et al.* (2000), Booth *et al.* (2001), Sauvant (2004) and Brenes *et al.* (2008)
Navy bean	6.9	7.1	2.1	6.5	6.0	1.1	3.9	3.4	NA	3.3	Gyori *et al.* (1998)
Pinto bean	6.4	2.8	4.4	8.1	6.9	1.1	5.4	4.0	0.9	5.0	Savoie
Black bean	5.5-6.7	1.9-2.9	4.1-4.3	7.7-8.6	6.7-7.1	2.0-2.7	5.1-5.8	3.1-4.4	1.4-1.5	4.9-5.1	Evans and Bandemer (1967) and Hernandez *et al.* (2008)

*All data were reported on a dry matter basis; SBM: Soybean Meal; SPC: Soy Protien Concentrate; SPI: Soy Protien Isolate; CGM: Corn Gluten Meal; CGF: Corn Gluten Feed; DDGS: Distillers Dried Grains with Solubles; CSM: Cottonseed Meal; RSM: Rapeseed Meal

level in a 36% CP, non-fish meal diet (Wu *et al*., 1995). For turbot (*Psetta maximal*), CGM at 20% inclusion level substituted for 40% of the fish meal in a 49% CP diet (Regost *et al*., 1999). For gilthead sea bream juveniles, CGM replaced up to 30% of the fish meal, at a 16% inclusion level, in a 43% CP diet (Robaina *et al*., 1997) and 60% of the fish meal, at a 41% inclusion level, in a 48% CP diet (Pereira and Oliva-Teles, 2003). For Japanese flounder juveniles, 40% of the fish meal was replaced by 29% CGM and 11% potato starch in a 56% CP diet (Kikuchi, 1999).

Corn Gluten Feed (CGF): Corn gluten feed, similar to CGM is a coproduct of corn wet milling where the corn kernel is separated into starch, germ and fiber fractions (Wu *et al*., 1995). Compared to CGM, CGF also consists of bran, the steeping liquor and germ can be added as well (Sauvant, 2004). With a CP of 18.0-23.8% (db), CGF has a considerably lower CP content than CGM; it contains almost half the amount of crude fat of CGM (3.5-3.9%, db). Crude fiber is 7.5-8.0% (db) which is considerably higher than CGM (up to 10 times the amount) which is expected due to the corn bran added to the CGF. Ash is 6.1-6.8% (db) which is also lower than CGM. CGF is low in lysine (0.6-0.7%, db) as well as methionine (0.3-0.5%, db) (Table 5 and 6). The average price for CGF containing 21% protein for the Midwestern USA, increased from \$57 ton^{-1} in 2004/05 to \$85 ton^{-1} in 2008/2009 (USDA, 2010a). Traditionally, it has been used in livestock diets and pet foods (Robinson *et al*., 2001). CGF has also been used in aquafeeds. For example in a 35% CP diet for Nile tilapia fry, 42% CGF and 51% soy flour were used as the sole protein source (i.e., no fish meal), this diet resulted in good but significantly lower, weight gain and feed conversion ratio than the control diet which contained fish meal; protein efficiency ratio was not significantly different from the control diet (Wu *et al*., 1996).

Channel catfish fingerlings (*Ictalurus punctatus*) were able to utilize CGF at inclusion levels up to 50% without adverse effects on palatability, weight gain or feed efficiency in a 32% CP diet when compared to a soybean meal-based, non-fish meal diet (Robinson *et al*., 2001).

Distillers Dried Grains with Solubles (DDGS): Distillers dried grains with solubles is the major coproduct of ethanol production which is mainly from corn grain in the USA and consists of 26.8-33.7% (db) crude protein, 3.5-12.8% (db) crude fat, 5.4-10.6% (db) crude fiber and 2.0-9.8% (db) ash. It is low in lysine with levels of 0.5-1.1% (db) and low in methionine with levels of 0.5-0.8% (db) (Table 5 and 6). As a result of the fermentation process, higher amounts of most nutrients are available compared to corn including protein, fat, fiber and minerals. In fact, levels of these nutrients are concentrated by approximately 3 times that of raw corn (Jacques *et al*., 2003). DDGS does not contain ANF which are commonly present in many other plant protein sources (Lim *et al*., 2009). The average price for DDGS in Indiana, USA was \$84 ton^{-1} in 2004/05, \$129 ton^{-1} in 2008/09 and \$200 ton^{-1} in 2011 (USDA, 2010a). A continued increase in ethanol production due to changes in national energy policies will generate higher quantities of DDGS over time. Hence in the near future, prices are expected to decrease compared to SBM (Bals *et al*., 2006). Corn-based DDGS has historically been used for cattle (both beef and dairy) feed due to its high amounts of protein and fiber (Bals *et al*., 2006). Its use in fish diets has been limited, however due to low levels of EAA, particularly lysine and methionine (Table 6) as well as high fiber levels. However, compared to other protein sources such as SBM, DDGS is competitive on a per unit protein basis and is highly palatable to fish (Lim *et al*., 2009). Since the late 1940's, DDGS has been incorporated at low inclusion levels in many fish feeds (Thompson *et al*., 2008). In numerous studies, DDGS has been used as a potential protein substitute for species such as Nile tilapia where 35% DDGS in combination with 58% soy flour could be incorporated in a 40% CP diet, replacing fish meal without affecting growth performance or feed conversation ratios (Wu *et al*., 1996). Similar results for Nile tilapia were achieved where DDGS was included up to 20% in a 32% CP diet (Lim *et al*., 2007). In a 30% CP diet for hybrid tilapia, DDGS at a 30% inclusion level, combined with other non-fish meal sources, totally replaced fish meal without significant differences in weight gain, specific growth rate, feed conversation ratio or protein conversation ratio (Coyle *et al*., 2004). Several studies have also been conducted for channel catfish and have replaced SBM in combination with or without corn meal. DDGS could be integrated up to 30% in a 32% CP diet without impacting weight gain, body length, survival or organoleptic quality (Webster *et al*., 1993). DDGS inclusion levels between 30-40%, supplemented with lysine in 28 and 31% CP diets were feasible without adversely affecting fish performance (Robinson and Li, 2008; Lim *et al*., 2009). For rainbow trout levels up to 15 and 22.5%, DDGS were successfully incorporated without and with supplemental lysine and methionine, substituting for 50 and 75% of the fish meal, respectively without adverse effects on weight gain, feed conversation ratio or survival (Cheng and Hardy, 2004).

Cottonseed Meal (CSM): Cottonseed meal is a byproduct from the solvent extraction of oil from partially dehulled cottonseeds (Sauvant, 2004). It can be expeller or solvent-extracted of which the latter is the more commonly used method. Depending on the processing used, the fat content differs. CSM is a high protein supplement often used for cattle feed and has about 89% of the energy value of SBM with a protein content of nearly 44% (Ingredients101, 2010c). CSM generally contains around 33.4-44.7% (db) crude protein, 1.6-4.5% (db) crude fat, 13.0-28.6% (db) crude fiber and 5.7-7.8% (db) ash. Like many other plant protein sources, it is low in lysine and methionine compared to fish meal with concentrations of 1.0-2.2% (db) and 0.5-1.1% (db), respectively (Table 5 and 6).

On a protein basis, CSM is generally priced competitively with soybean meal and could reduce feeding expenses by 10-20% when replacing SBM (Robinson and Li, 2008). Prices for CSM were at \$139 ton^{-1} for 2004/05 and increased to \$281 ton^{-1} in 2008/09 (FAS, 2010).

CSM does contain several ANF substances, however including phytic acid, phytoestrogens, antivitamins and cyclopropenois acid (Francis *et al*., 2001) as well as the toxic pigment gossypol (Robinson and Li, 1994; Francis *et al*., 2001). In contrast to other non-ruminant animals such as poultry and swine, fish can tolerate higher amounts of gossypol (Cheng and Hardy, 2002). But limiting factors of CSM are the partial unavailability of lysine (Robinson and Li, 2008) and methionine as well as high concentrations of fiber (Cheng and Hardy, 2002). Thus, lysine and methionine are generally supplemented when using high concentrations of CSM in fish diets (Cheng and Hardy, 2002; Robinson and Li, 2008). CSM has been used in several fish feeding trials. In 29 and 32% CP diets for catfish, CSM supplemented with lysine could be included at levels of >30% to replace 50 and 60% SBM, respectively without adverse effects on fish performance (Robinson and Li, 1994, 2008). When adding iron at a ratio of 1:1 to free gossypol in 33% CP juvenile Nile tilapia diets supplemented with lysine and methionine, CSM at 67% inclusion level with other non-fish meal sources could totally replace fish meal without detrimental effects on growth performance and feed utilization (El-Saidy and Gaber, 2004). Studies in feed formulations for rainbow trout showed that CSM could be incorporated up to 10%, substituting for 60% of the fish meal in a 44% CP diet without significant differences in weight gain, feed conversion ratio or survival (Cheng and Hardy, 2002).

Alfalfa meal: Alfalfa is the most important and widely grown forage legume and is called the queen of forages because of its nutritional quality and high biomass production (Ali *et al*., 2003; Yang *et al*., 2008). It is a low cost, widely used animal feed (Yanar *et al*., 2008). Generally, CP is 18.0-19.2% (db), crude fat is 2.7% (db), crude fiber is 25.7-28.8% (db) and ash is 10.4-11.0% (db). It is low in lysine and methionine with 0.6-1.0% (db) and 0.3-0.4% (db), respectively when compared to fish meal (Table 5 and 6). The average price for dehydrated alfalfa meal in 2004/05 was \$142 ton^{-1} and rose to \$260 ton^{-1} in 2008/09 (USDA, 2010a). Several studies have examined the use of alfalfa meal in Nile tilapia diets as a substitute for fish meal. Ali *et al.* (2003) concluded that alfalfa meal could be included only up to 5% in a 36% CP diet without reducing growth performance. The researchers assumed that high fiber levels and ANF were the reasons for growth depression and poor nutrient utilization. Likewise, Yousif *et al.* (1994) did not recommend the use of crude alfalfa for Nile tilapia diets, due to growth depression and decreasing feed utilization efficiency at inclusion levels between 5-30% in 41% CP diets. The researcher assumed that the presence of ANF and imbalances in the amino acid profile were responsible for these unwanted effects. ANF of alfalfa meal can include protease inhibitors, saponins, phytoestrogens and antivitamins (Francis *et al.*, 2001). On the other hand, purified alfalfa leaf protein concentrate could be included up to 26% in a 45% CP diet for tilapia, replacing 35% of the fish meal without negative growth performance (Olvera *et al.*, 1990).

Canola/Rapeseed Meal (RSM): Rapeseed belongs to the genus Brassica and includes several species such as *Brassica napus*, *Brassica campestris* and *Brassica juncea* these are all closely related and similar in appearance. Traditional varieties of rapeseed contain high amounts of erucic acid whereas newer Canadian varieties (known as canola) contain lower values that were achieved by genetic modification. Therefore, canola and rapeseed are two different species (Shahidi, 1990). Furthermore, canola is a registered trademark of the Canadian Canola Association and refers to cultivars containing <2% erucic acid (Raymer, 2002). In 2008/09, canola meal had a global production of 30.8 million ton and was the second largest protein meal produced after SBM (which yielded a global production of 151.6 million ton) (USDA, 2010c). The average world price for canola meal reached a value of \$195 ton^{-1} in 2008/09, compared to \$131 ton^{-1} in 2004/05 (FAS, 2010). Canola/Rapeseed (*Brassica napus*) meal is a byproduct obtained from solvent extraction of rapeseed cake which remains after processing rapeseed to remove the oil (Lardy and Kerley, 1994). With a CP content of 33.7-43.1% (db), it is one of the most widely used protein sources in animal feed and it is also used as a fertilizer (Peterson and Hustrulid, 1998). Crude fat is 4.8% (db), crude fiber is 11.5-12.4% (db) most of which is derived from the hulls (Bell, 1984) and ash is 6.5-7.9% (db). As with many other plant protein sources, it is low in lysine (0.6-1.0%, db) and low in methionine (0.3-0.4%, db) (Table 5 and 6). RSM does contain ANF such as tannins, sinapin and phytic acid that limit inclusion rates in fish diets. Processing strategies such as dehulling and thermal treatment can reduce the ANF (Burel *et al.*, 2000). Davies *et al.* (1990) concluded that RSM could be included up to 15 in 38% CP tilapia (*Oreochromis mossambicus*, Peters) feeds and could replace SBM without reducing growth performance. Studies on the inclusion of 30% dehulled, solvent-extracted and 30% dehulled, heat-treated RSM in combination with fish meal in practical diets for rainbow trout and turbot have shown that protein digestibility was similar to high quality fish meal whereas digestibility for turbot was significantly lower without thermal treatment for the 46% CP diet for rainbow trout, 28% of fish meal replacement was possible whereas for the 52% CP turbot diet, 30% fish meal substitution was shown to be possible (Burel *et al.*, 2000). On the other hand, both expeller-extracted and solvent-extracted canola meal could be included up to 60% in 30% CP diets for juvenile red sea bream (*Pagrus auratus*, Paulin) substituting for 50% fish meal without adverse effects on growth rate, feed performance or protein utilization (Glencross *et al.*, 2004).

Lupin Seed Meal (LSM): Lupin consists of different species (Sirtori *et al.*, 2004) are used as ingredients in livestock feeds. The main species commonly used in fish feeds are *Lupinus angustifolius*, *Lupinus albus* and *Lupinus luteus* (De la Higuera *et al.*, 1988; Burel *et al.*, 1998; Glencross *et al.*, 2005). Lupin is a protein-rich legume (CP from 34.1-48.2%, db) with a high oil content (crude fat from 5.5-6.2%, db) and high crude fiber (11.6-19.0%, db).

Ash is 2.8-4.9% (db), lysine is 1.4-2.1% (db) and methionine is low at 0.2-0.4% (db) (Table 5 and 6). Whole LSM contains approximately 16% (db) crude fiber whereas dehulled LSM has only around 3.7% (db) (Chien and Chiu, 2003). Depending on the species, CP of dehulled extruded LSM is approximately 42-55% (db), compared to 32-38% (db) for whole LSM (Burel *et al.*, 1998; Glencross *et al.*, 2005; Burel *et al.*, 2000; Chien and Chiu, 2003). Lysine and methionine content for dehulled and whole lupin is similar (Allan *et al.*, 2000; Glencross *et al.*, 2005; Glencross *et al.*, 2007). Disadvantages of whole LSM are high fiber contents and antinutrients such as a-galactosides, trypsin inhibitor and inositol phosphates (Martinez-Villaluenga *et al.*, 2006). However, ANF are mainly alkaloids that affect palatability and can be washed out using water (Robaina *et al.*, 1995). Dehulling increases the nutritive value of LSM by reducing the fiber content (Davis *et al.*, 2002; Chien and Chiu, 2003), increasing the

CP content and decreasing the amount of carbohydrates without affecting the lipid level (Glencross *et al*., 2007). Nonetheless, Hughes (1988) determined that the nutritional value of lupin is not dependent on heat treatment when used as a replacement for full fat SBM in rainbow trout fingerling diets. The researchers concluded that when lupin was included up to 40% in the diets, weight gain was equal to and digestibility was higher than the diet using full fat SBM as the primary protein source. Other studies on rainbow trout diets likewise reported that heating LSM (*Lupinus albus*) did not improve nutritional quality of the diets. Supplemented with lysine and methionine, crude LSM at 44% inclusion level replaced fish meal partially (up to 30%) in a 45% CP diet without detrimental effects on weight gain, protein efficiency or digestibility (De la Higuera *et al*., 1988). Examining dehulled LSM (*Lupinus angustifolius*) at 40% inclusion levels in 44% CP diets for juvenile rainbow trout showed that 49% of the fish meal could be replaced without significantly affecting growth performance or nutrient utilization (Farhangi and Carter, 2001). Burel *et al*. (1998) concluded that depending on the crop between 50 and 70% extruded lupin (*Lupinus albus*) could be incorporated in 40 and 44% CP diets, replacing >50 and 76% of the fish meal, respectively without significant differences in growth performance. In 56% CP diets for juvenile gilthead sea bream, LSM (*Lupinus angustifolius*) was included up to 30% without compromising weight gain, feed efficiency or protein efficiency ratio, replacing up to 30% of the total dietary protein. However, the researchers concluded that LSM level should not exceed 20% of total protein to avoid liver lipid deposition (Robaina *et al*., 1997).

Pea meal: Field pea (*Pisum sativum*) has long been used as a protein source in livestock feeds (Davies *et al*., 2002). Peas contain about 22.4-30.7% (db) CP, 1.1-2.6% (db) crude fat, 4.4-11.8% (db) crude fiber and 3.0-3.9% (db) db ash. Starch content is often near 50% (db). Lysine is about 1.5-1.7% (db) and methionine is very low from 0.2-0.3% (db) (Table 5 and 6). Extruded field pea has a protein content of 26% (db) and crude fat of 1.9% (db) (Adamidou *et al*., 2009). Air classification can concentrate the starch and protein fractions (Boye *et al*., 2010). Pea protein concentrates which are produced by air separation after dehulling can yield a protein content of nearly 49% (db) (Carter and Hauler, 2000), lysine of 6.2% (db) and methionine of 0.9% (db) (Sanchez-Lozano *et al*., 2009). The average price for dry peas was \$249 ton^{-1} in 2009, compared to \$150 ton^{-1} in 2004 (USDA, 2010d). Pea meal contains a variety of ANF such as protease inhibitors, phytic acid, polyphenols, tannins, cyanogens and lectins (Francis *et al*., 2001). And given that the protein content of peas is relatively low and the starch content is high (Burel *et al*., 2000), certain species (such as salmonids) have shown limited digestibility for peas. Heat treatment with (e.g., extrusion) has led to more successful use in fish feed ingredients, though. In addition, dehulling can result in increased digestibility due to the decrease in fiber (Burel *et al*., 2000). Extruded pea protein concentrate at inclusion levels of 20 and 27% replaced 20 and 33%, respectively of the fish meal in Atlantic salmon parr diets without detrimental effects on growth performance (Carter and Hauler, 2000; Overland *et al*., 2009). Inclusion of 25% dehulled peas and 20% air-classified pea protein replaced up to 78 and 61% soybean meal, respectively in 53% CP diets for rainbow trout diets without significantly affecting feed intake and growth performance (Thiessen *et al*., 2003). Pea-derived meal at 40% inclusion replaced 18% fish meal in 48% CP European sea bass fingerling diets without statistically significant differences in final weight or specific growth rate (Gouveia and Davies, 1998). In nutrient digestibility studies, extruded field peas could be included at 30% in 44% CP diets for European sea bass and replaced up to 10% of the fish meal without significant differences in protein, fat, starch or energy digestibility (Adamidou *et al*., 2009).

For 44% CP juvenile gilthead sea bream diets, up to 20% fish meal was replaced with 35% dehulled, defibered, extruded pea meal and 37% infrared radiation-treated ground whole pea, respectively without compromising fish performance (Pereira and Oliva-Teles, 2002). In 30% CP diets for juvenile milkfish (*Chanos chanos* Forsskal), ground peas at 10% inclusion level substituted for 20% SBM without adverse effects on weight gain, survival or body composition (Borlongan *et al*., 2003).

Lentil meal: Lentils are important leguminous seeds for many developing countries (Salunkhe and Kadam, 1989) and *Lens culinaris* are the most used and most studied lentil species (Rozan *et al*., 2001). They contain 25.9-33.1% (db) CP, 1.0-3.6% (db) crude fat, 1.2-7.5% (db) crude fiber, 2.6-4.3% (db) ash and are low in lysine (1.6-1.9%, db) and methionine (0.2%, db) (Table 5 and 6). Average prices for lentils in 2009 were \$573 ton^{-1}, compared to \$328 ton^{-1} in 2004 (USDA, 2010e). Like other legumes, lentils contain ANF such as trypsin inhibitors, tannins, polyphenols and phytates that limit their utilization (Vidal-Valverde *et al*., 1994). Dehulling and cooking lentils can result in significantly increased protein and starch levels and decrease tannins. Furthermore, cooking reduces phytic acid levels (Vidal-Valverde *et al*., 1994; Wang *et al*., 2009), germination lowers trypsin inhibitor activity, tannins and phytic acid and soaking decreases phytates and tannins. It is suggested to use >1 method to reduce ANF most effectively (Vidal-Valverde *et al*., 1994). Unfortunately, animal feeding trials with lentils have been limited. The only published study found on use of lentils in fish diets was on extruded red lentil (*Lens culinaris* L.) meal at 30% inclusion, integrated into a 45% CP diets for juvenile rainbow trout. These diets resulted in significantly lower weight gain, specific growth rate and digestibility values than the control diet when replacing 30% of the fish meal content. The researchers assumed that the lower growth rate was a result of the lower methionine content in the diet (Yagci *et al*., 2009). Likewise, feeding studies for rats (a monogastric animal) including whole lentil meal or dehulled lentil meal as the sole protein source resulted in significantly reduced growth and weight gain (Cuadrado *et al*., 2002).

Chickpea meal: Chickpeas (*Cicer arietinum* L.) also known as garbanzo beans have mainly been produced for human food. Crude protein is 21.0-24.0% (db), crude fat is 4.7-5.2% (db), crude fiber is 3.9-9.1% (db) and ash is 2.7-3.6% (db). Lysine and methionine are both low at 1.4-1.8% (db) and 0.2-1.0% (db), respectively (Table 5 and 6). Crude protein of dehulled or extruded chickpeas have been shown to be around 24 and 28% db, respectively and crude fat for both is approximately 5% db (Booth *et al*., 2001; Adamidou *et al*., 2009). The average wholesale price for chickpeas in 2008/09 for Idaho, USA was \$901 ton^{-1}, compared to \$856 ton^{-1} in 2004/05 (USDA, 2010f). Chickpeas contain several ANF including trypsin inhibitors, alpha-galactosides, saponins and tannins (Nestares *et al*., 1996; Salgado *et al*., 2001). Cooking or heat treating chickpeas can significantly decrease trypsin inhibitor activity levels (El-Adawy, 2002; Wang *et al*., 2010b). Studies on chickpeas in fish diets are limited but some research has been done with other animals including pigs (Mustafa *et al*., 2000), rats (Nestares *et al*., 1996; Rubio *et al*., 1998) and chickens (Christodoulou *et al*., 2006; Brenes *et al*., 2008). Diets with extruded chickpeas were shown to result in improved weight gain in growing chickens (Brenes *et al*., 2008).

In digestibility studies for European sea bass, extruded chickpea meal could be included up to 30% in a 44% CP diet without adversely affecting protein, fat, starch or energy digestibility (Adamidou *et al*., 2009). Booth *et al*. (2001) suggested that chickpeas (*Cicer arietinum*-cv. Desi.) could be dehulled and refined to improve energy digestibility for use in juvenile silver perch diets. Furthermore, they concluded that dehulling yielded >80% reduction in ADF and >10% increase in protein although, it did not improve protein digestibility.

Common beans: Common bean (*Phaseolus vulgaris* L.) is a plant species which contains a wide range of genetic varieties such as navy, pinto and black beans. They are primarily used as human food and have low levels of fat and high levels of proteins, complex carbohydrates, vitamins and minerals (Anton *et al*., 2008). They are rich in lysine but deficient in methionine and contain ANF such as phytates, trypsin and chymotrypsin inhibitors (Reddy *et al*., 1985). Various ANF can be inactivated by proper heat treatment (Van Der Poel, 1990). Common beans have hardly been investigated as substitutes for fish meal and information about digestibility for fish diets is rare or not available.

However based on moderately high protein content, a lysine concentration similar to that of fish meal and relatively high availability, common beans could be a potential aquafeed ingredient when processed appropriately. On the other hand, high price, generally >\$600 ton^{-1} in the USA must be considered a potential limiting factor. In one of the few studies, extruded common bean at a 30% inclusion level has been investigated in 45% CP juvenile rainbow trout diets. There were no significant differences for weight gain, specific growth rate or digestibility among the fish fed the control diet when substituting for 30% of the fish meal (Yagci *et al*., 2009).

Navy (white) beans: Navy beans are widely produced for human food. Crude protein content is generally between 22.8-30.4% (db), crude fat is around 1.6% (db), crude fiber is 5.6-8.1% (db) and ash is approximately 3.5-5.6% (db). Lysine is around 6.0% (db) (which is similar to fish meal) whereas methionine is low at 1.1% (db) (Table 5 and 6). Cooked freeze-dried navy bean powder contains approximately 25% (db) crude protein, 5.2% (db) crude fiber and 2% (db) crude fat (Thompson *et al*., 2009).

The average wholesale price for dry navy beans in 2008/09 for Michigan, USA was \$871 ton^{-1}, compared to \$653 ton^{-1} in 2004/05 (USDA, 2010f). Navy beans contain high amounts of trypsin inhibitors, phytic acids (Anton *et al*., 2008; Martin-Cabrejas *et al*., 2009), chymotrypsin inhibitors (Martin-Cabrejas *et al*., 2009), lectins and protease inhibitors (Paduano *et al*., 1995; Martin-Cabrejas *et al*., 2009). After heat treatment, levels of trypsin inhibitors and phytic acids can be significantly reduced (Anton *et al*., 2008).

For other monogastric animals such as rats, poor growth has been observed when fed navy beans. On the other hand, growth improved after heat treatment (Kakade and Evans, 1966). When fed to sheep, navy beans were only suitable at low levels after heat treatment (Paduano *et al*., 1995).

Pinto beans: Crude protein concentration of pinto beans is generally between 21.6-22.4% (db), crude fat is 0.8-1.0% (db), crude fiber is 5.0-7.4% (db) and ash is 3.5-3.8% (db) (Table 5). With lysine of approximately 6.9% (db), it appears to be an adequate source for lysine but it is low in methionine, at around 1.1% (db) when compared to fish meal. The average wholesale price for dry pinto beans in 2008/09 for Colorado, USA was \$914 ton^{-1}, compared to \$782 ton^{-1} in 2004/05 (USDA, 2010f). As for other pulses, pinto beans contain trypsin inhibitors, phytic acids, tannins and oligosaccharides that limit protein and carbohydrate utilization. Cooking can significantly decrease trypsin inhibitor activity, tannins and sucrose (Wang *et al*., 2010b).

Black beans: Black beans have a crude protein content between 23.6-25.9% (db), crude fat of 1.6-3.3% (db) and ash of 4.2-4.7% (db) (Table 5). Again, cooking or heat treatment can significantly decrease the ANF (Wang *et al*., 2010b). Cooked freeze dried black bean powder contains approximately 27% (db) protein, 2% (db) crude fat and 5% (db) crude fiber (Thompson *et al*., 2009). The average wholesale price for dry black beans in 2008/09 for Michigan, USA was \$1030 ton^{-1}, compared to \$583 ton^{-1} in 2004/05 (USDA, 2010f).

CONCLUSION

Traditionally, commercial aquafeeds have been based on fish meal due to its many advantages, including high protein content, high digestibility, essential amino acid profile, fatty acid profile, minerals, vitamins and palatability. These characteristics make it very challenging to find less expensive alternatives to fish meal without affecting fish performance and fillet quality. Large amounts of unwanted nutrients such as fiber, ANF and other carbohydrates can limit inclusion levels of many plant protein sources even though the protein content might be adequate. Processing prior to use in the feed can enhance protein availability by reducing or inactivating heat-labile ANF such as trypsin inhibitors. Additionally, dehulling can reduce high levels of fiber and enhance the protein content. Furthermore, many plant protein sources lack EAA such as lysine and methionine but this can be compensated for by supplementation of these components. Limiting factors for animal byproducts also include cost-intensive processing as well as deficiencies or excesses of various EAA. Variations in nutrient composition and protein quality exists in both animal and plant protein sources, based on inconsistencies of the raw materials, seasonal changes, growth conditions, cultivation practices and differences in cultivars or species used, etc.

Since, fishery byproducts, particularly fish meals produced from fishery discard and processing waste are initially fish products, they appear to be the most adequate fish meal replacers. However, differences in quality have to be considered and depending on the source, removal of structural components such as chitin and bones may have to be accomplished to improve the nutritional value. In addition, the limited availability has to be considered. Nevertheless, other promising alternative protein sources could include animal byproducts such as meat and bone meal and poultry byproduct meal. The advantages of animal products include sufficient amounts of lysine and/or methionine, few ANF, sufficient quantities in the marketplace and substantially lower prices than fish meal. Potential plant protein sources can include soybean meal, soy protein concentrate, soy protein isolate, distillers dried grain with solubles, cottonseed meal, rapeseed/canola meal and lupin seed meal all of which can yield high fish meal replacement levels but they may require lysine and/or methionine supplementation. Due to relatively low prices and no ANF (which are commonly present in many plant sources), distillers dried grain with solubles and cottonseed meal appear to be attractive alternatives, particularly to higher priced SBM. On the other hand, common beans, corn gluten meal, chickpeas and lentils appear to be too high priced to be competitive with fish meal. Depending on the location of an aquaculture facility a sustainable strategy would be to use locally available plant sources in combination with animal protein sources such as rendered products, depending on what is available.

RECOMMENDATIONS

When using plant protein sources such as soybean, pea or lentil, it is recommended to dehull and to heat treat these materials to reduce fiber content, enhance protein content and remove ANF. The use of alternative plant or animal protein sources is still in its infancy. More research, particularly commercially-focused studies examining feed acceptability, digestibility, fillet quality and impacts on entire production costs have to be conducted to successfully replace fish meal completely. Promising solutions may be found with combinations of protein sources where one portion is animal based and the other is a plant based. Factors that should be considered are the regionality of products that may therefore be less expensive and more available (e.g., rendered products and crops that are planted regionally). These factors may not only contribute to more cost-efficient commercial fish production but may also lead to better protection of the environment, since transportation can be reduced. Additionally, this approach may add new jobs since, production and processing could be conducted regionally. Thus, the intention this effort is to use fish meal as an enhancing ingredient not as a primary protein source in aquafeeds. Depletion of oceans can be minimized and diversity of fish species can be protected of such strategies are adopted.

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