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Benefit and drawbacks of fish meal substitution in aquaculture diets

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Dedicated to my family

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1. Introduction

1.1 Expanding demand for aquaculture

1.1.1 Worldwide increasing consumption of fish and the benefits of fish meat

Aquaculture is one of the fastest growing food producing sectors throughout the world. The growth of aquaculture and global consumption of farmed fish have been increasing for the last several decades.

Food fish consumption shows a rapid and massive growth. In per capita it grew from 9.0 kg in 1961 to 20.2 kg in 2015, an average rate of about 1.5 % per year. The estimates for further growth was about 20.3 kg in 2016 and 20.5 kg in 2017. This growth has been fuelled not only by the growth of the global population but also by change in habits, as the food the average annual increase of the global food fish consumption has been twice the rate of population growth between 1961 and 2016 (FAO 2018). The expansion in consumption has been driven by the combination of many factors, including reduced wastage, better utilization and growing demand. It is also linked to the population growth and urbanisation. In 2015, fish provided almost 20 % of average per capita intake of animal protein for about 3.2 billion people. Population in developing countries have a higher part of fish protein in their diets compare to in developed countries (FAO 2018). The world fish consumption was projected to reach 20.3 kg in 2016 and 21.5 kg in 2030. Per capita fish consumption will increase in all regions except Africa, the highest growth rates are projected for Latin Amerika, Asia and Oceania (FAO 2018). Fish meat has several positive effects, including low saturated fats, carbohydrates and cholesterol, wide range of essential micronutrients and various vitamins and minerals. In addition, fish plays a central and increasing role effective in the nutritional security of poor and vulnerable populations around the globe (World Bank 2013).

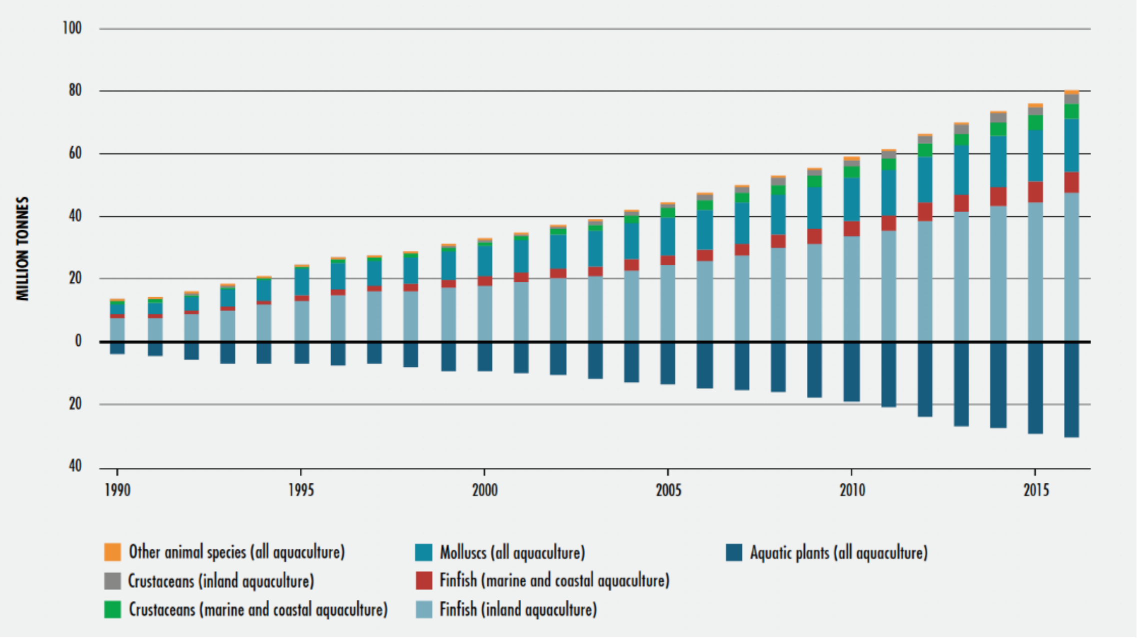


Figure 1: **World aquaculture production of food fish and aquatic plants, 1990-2016** (FAO 2018).

1.1.2 Fisheries production

The global fisheries production reached up to 174.0 million tonnes (Mt) and from which 90.4 Mt comes from capture and 83.6 Mt comes from aquaculture (FAO 2016, FAO - Food and Agriculture Organization of the United Nations 2017). About 88 % (over 151 million tonnes) was utilized for direct human consumption and the other 12 % used for non-food purposes like fish meal (FM) or fish oil (FO) (FAO 2018). In the last several years the total aquaculture production increase contrasted with the total capture fisheries production, which has experienced a decline (Fig. 1 and Fig. 2).

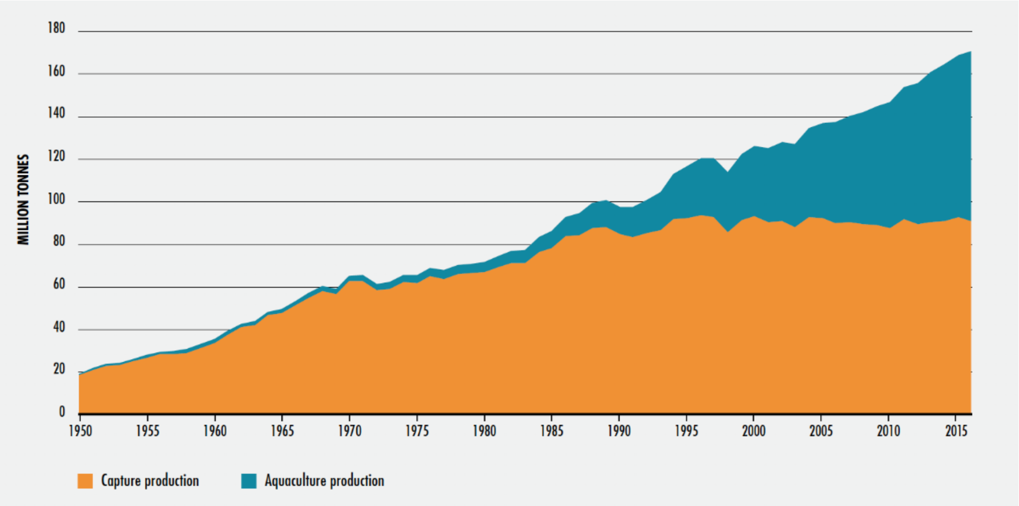


Figure 2: **World capture fisheries and aquaculture production (FAO 2018).**

The global total capture fisheries totalled 90.9 Mt according to the FAO capture database in 2016, this shows a decrease in comparison to the two previous years.

The global catch from inland waters in 2016 showed an increase of 2.0 % over the previous year, and 10.5 % in comparison to the 2005–2014 average (FAO 2018).

1.1.3 Rise of aquaculture

Conversely, the other part of the fish production, aquaculture is growing faster than other food production sectors in the last decades (FAO 2018, Fig.3). The rapid expansion of the global aquaculture production shows that in the past three decades the annual growth rate of the global aquaculture production is more than 8 %. It grew from 5.2 Mt in 1981 to 62.7 Mt in 2011 (World Bank 2013). Whereas the average annual growth declined to 5.8 % during the period 2000-2016, there were some countries in Africa and Asia where double-digit growth was observed between 2006 to 2010 (FAO 2018).

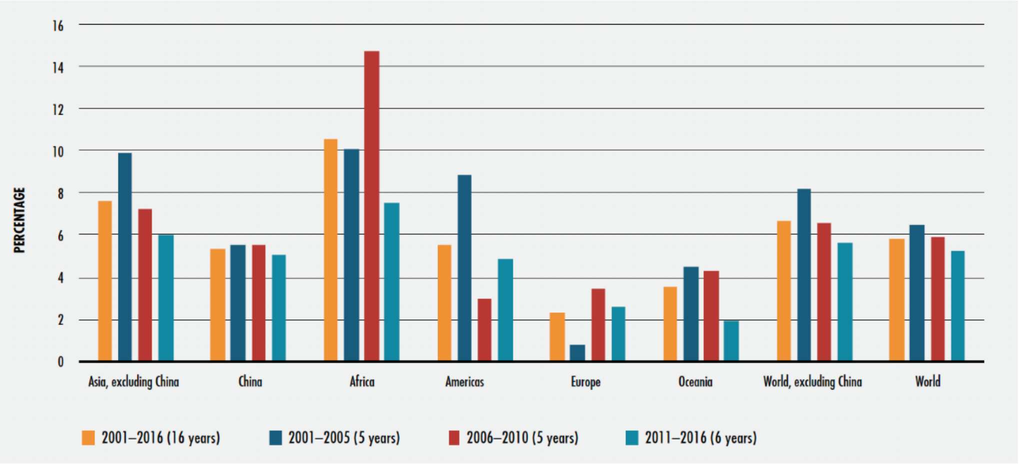


Figure 3: **Average annual growth rate of aquaculture production by volume (excluding aquatic plants)(FAO 2018).**

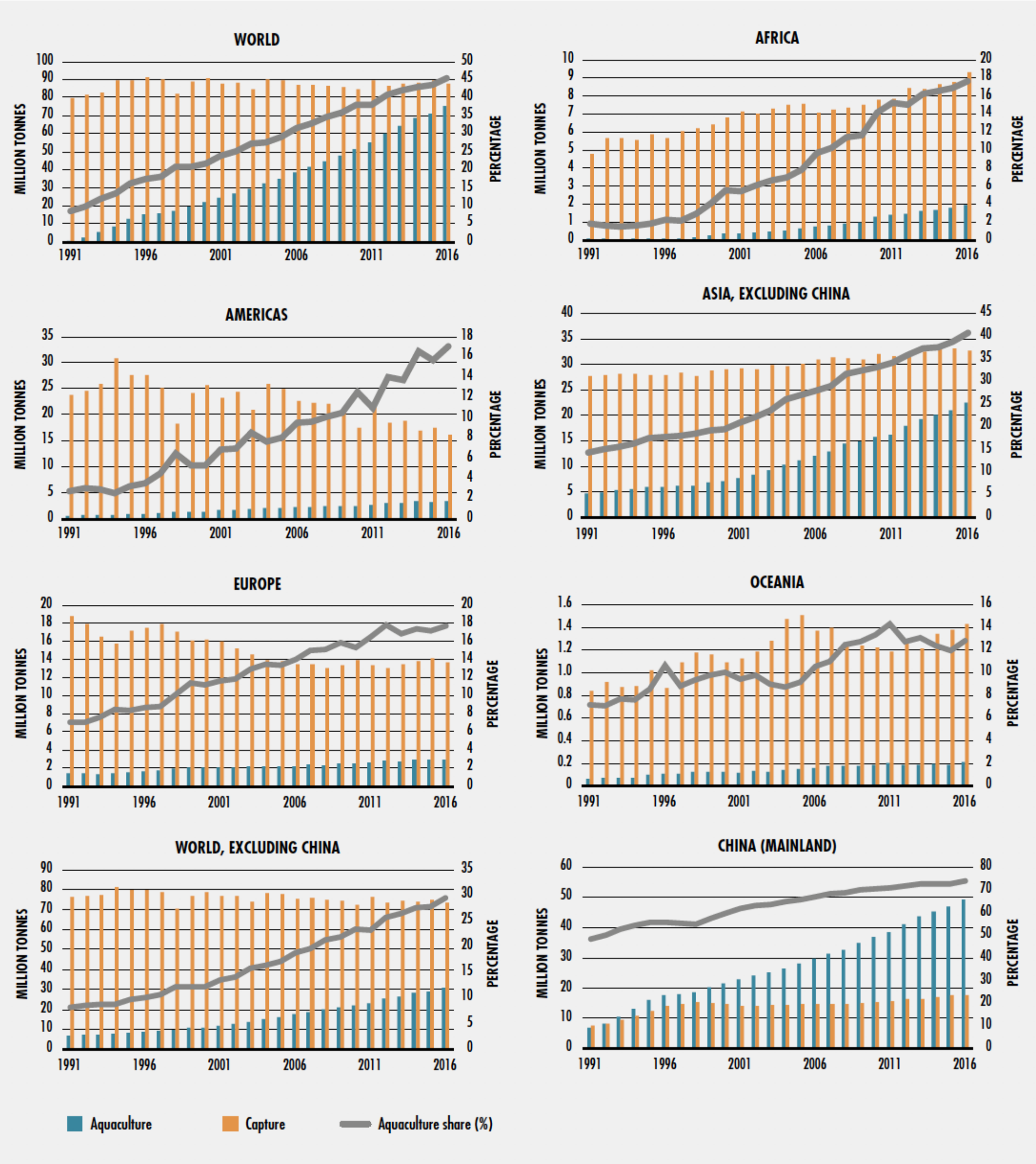


Figure 4: **Aquaculture contribution to total fish production (excluding aquatic plants) (FAO 2018).**

Consequently, the contribution of aquaculture to the global fish production has grown continuously. It has risen from 25.7 % in 2000 to 46.8 % in 2016 (Fig. 4).

Inland aquaculture has also expanded, from 5.9 % in 2000 to 64.2 % in 2016.

The global aquaculture production in 2016 was 110.2 Mt, the value estimated at USD 243.5 billion. The total production included 80.0 Mt of food fish, 30.1 Mt of aquatic plants (mostly seaweeds and microalgae) and 37 900 tonnes of non-food products (as ornamental shells and pearls) (FAO 2018).

China is a major influence on the global fish markets. In 2011, Asia produced 88 % of the world aquaculture production by volume and-, China alone accounted for 62 %. Thanks to the rapid growth in the fish production, the shares of China in the global fish production grew from 7 % in 1961 to 35 % in 2011. Aquaculture represents now more than 70 % of the total fish produced in China (World Bank 2013).

1.2 Aquaculture is largely dependent on fishmeal

1.2.1 Importance of fish meal

Until recently, FM was the chief protein source in fish feed for several reasons. It is considered the most nutritious and most digestible ingredients for farmed fish feeds (FAO 2018). FM is rich in protein content, has excellent essential amino acid (EAA) profile and nutrient digestibility, lack anti-nutritional factors (ANFs), is palatable and easily available.

Fish meals is primarily made from small pelagic fish, which after drying and grinding offers a cheap and suitable protein source consisting of whole fish, fish trimmings or fish by-products, which is often wasted. It includes a number of different species of mackerel, sardine and anchovy. Therefore, the production of fishmeal and fish oil is dependent on the catches of these species. Consequently, fishmeal production peaked at 30 Mt in 1994 and has showed a varying but overall declining trend since then. In 2016, it was down to less than 15 Mt, as a result the reduced catches of anchovy (FAO 2018).

In 2030, it is estimated that fishmeal production will be 19 % higher than in 2016, (reaching an estimated production of 5.3 Mt) because of the significant increasing of the world food fish consumption. It is expected that about 54 % of the growth will come from improved use of fish waste, cuttings and trimmings obtained from the fish processing (FAO 2018).

1.2.2 Price of fish meal and problem with supplies

Fishmeal is considered to be most expensive protein source in fish feed (Tacon 1993) and several previous studies have reported that the price of the fish feed is a decisive factor in the fish industry. Coyle et al. (2004) reported that feed costs account for more than 50 % of total production costs in aquaculture because of the use of the expensive protein source, fish meal. According to Tan & Dominy (1997) the feed costs accounts about 60 % of the total farm production costs.

Therefore, the continued growth of the fish industry depends largely on the production of sustainable and cost-effective feeds.

In late 2016 and early 2017, fish meal and fish oil prices started to decrease, thanks to the sufficient catches of small pelagic fish in Europe and the normalization of the climate in South America, following an abatement of El Nino. Due to the steady and growing demand, the prices of fish meal and fish oil are expected to increase again (FAO 2018). However, this is considered a momentary phenomenon and the trend overtime is opposite: several factors are contributing to the increasing prices of fishmeal in nominal terms. This includes the growing demand for fish products, population growth and the potential decline in capture fisheries production as a result of policy measures in China. Consequently, the slowdown in Chinese fisheries and aquaculture production will result in higher prices not only in China but throughout, the world (FAO 2018). The price of fishmeal and fish oil has increased significantly to over United States dollar (US$) 1600 and US $900-1800 per metric ton (FAO 2012). Relatively 4-5 tons of whole fish are required to produce 1 ton of dry fishmeal. The total world production of fishmeal was 4,445,000 tonnes in 2016 (IFFO Fishmeal and Fish Oil Statistical Yearbooks 2016) and FM doubled in price in recent years (Pavan Kumar et al. 2014).

The price of fishmeal is expected to rise again and could be about 20 % higher than in 2016 (FAO 2018). According to another study the prices between 2010 and 2030 period are expected to rise by 90 % for fish meal and 70 % for fish oil (World Bank 2013). Moreover, as a result of the high demand for fish for human consumption together with the higher prices it is estimated that the average price of internationally traded fish will increase by 25 % in 2030, compared to the prices in 2016. In addition, the price of the fishmeal and fish oil are expected to continue this upward trend by 2030.

However, the availability of the resources of FM and fish oil has seen a decrease overtime. This is mainly because of the reduced catch comes from capture fishery and the resulting increase in costs of fishmeal and fish oil.

Moreover, there is an environmental burden resulting from the exploitation of fish stocks in order to sustain the fish farming industry.

The production of fishmeal and fish oil cannot keep up with the growing aquaculture industry. For this reason, there is a need for optimal protein and lipid sources to support the growth of the aquaculture production and many fish nutritionists have concentrated their efforts to find alternative protein sources to substitute fish meal in the diet. These alternatives need to replace the nutritional profile of fishmeal without compromising growth rates, while remaining available at a reasonable price in the general market.

1.3 Digestion of teleost fish

Digestion is a process where ingested materials are reduced to small size molecules suitable for absorption through the gut wall and into the blood stream.

The digestive track of teleost fish displays a high level of variability between species (Kim 2017). It is divided in five parts, the oesophagus, the stomach when present (a number of fish lineage have lost this organ) (Le et al. 2019), the pyloric caeca (all three sometimes referred to collectively as the foregut), the midgut and the hindgut (Fig.5). Most of the initial break down of the food takes place in the foregut, involving mechanical and acidic process as well as enzymes. The midgut is usually the longest part of the gut and contains enzymes secreted by the pancreas, from the intestinal wall and the bile from the liver, which renders the milieu alkaline. The enzymes digest all three groups of the food, including proteins, lipids and carbohydrates, which includes hydrolysis of proteins to polypeptide chains or to amino acids and digestion of carbohydrates to sugar molecules and lipids to glycerol and fatty acids. The hindgut starts posterior from the midgut with increased diameter and ends at the anus, it is where most of the adsorption of nutrients take place (Moraes und De Almeida 2019).

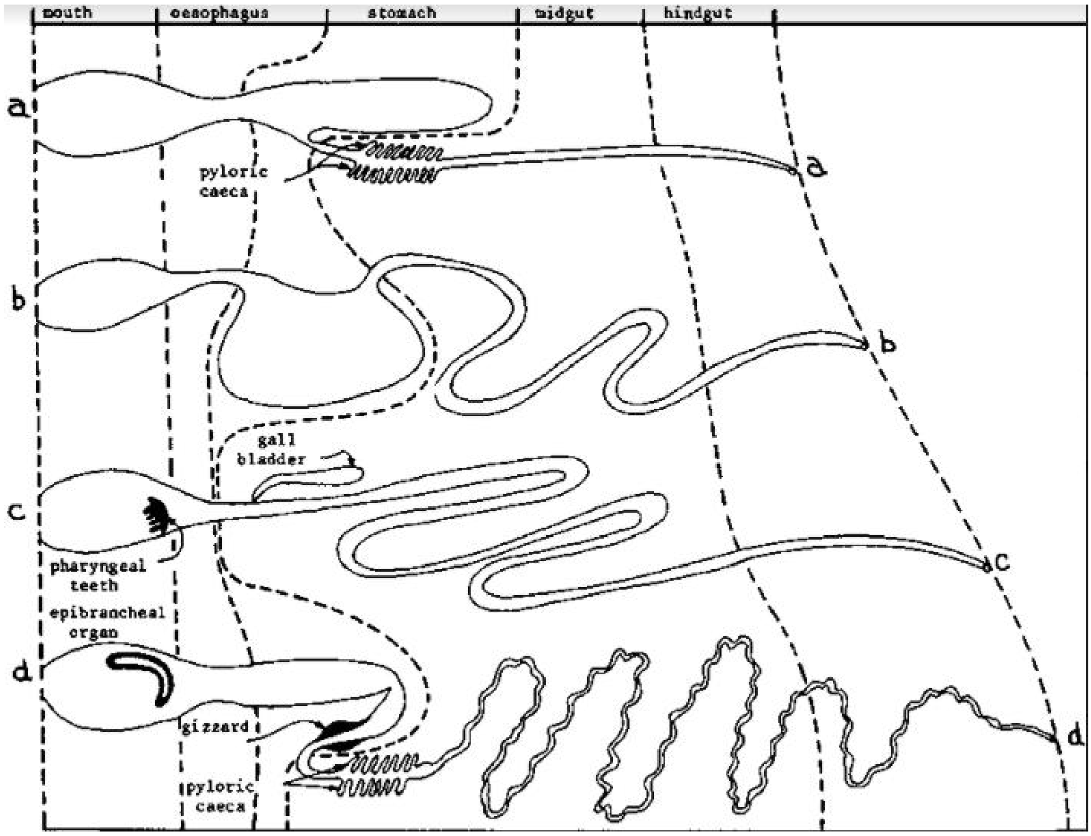


Figure 5: **Digestive track of teleost fish** (Smith 1980)**.**

In fish, such as cyprinids, gobidids or blennies which lack a stomach and pylorus, the foregut consist of the oesophagus and an intestine anterior, in which opens to the bile duct and the midgut connects directly to the oesophagus.

Stomachless fish are most commonly herbivores and omnivores, while carnivores fish have stomach in which peptic digestion takes place (Le et al. 2019).

In general, fish only limited amounts of carbohydratases (Castillo und Gatlin 2015) and are therefore not naturally well suited for the digestion of carbohydrates, notably starch and cellulose (Krogdahl et al. 2005). Carnivorous fish like Atlantic salmon or Japanese yellowtail are particularly poor in this regard, as can be expected from the fact that most of the carbohydrates in the diet originates from plant material.

Correspondingly, herbivorous fish usually exhibit higher levels of carbohydrase activity than carnivore or omnivore fish, although this remains limited.

One important factor is that the majority of fish species are carnivorous and, although omnivorous and herbivorous fish all have relatively low protein requirements, about 25 % to 35 % of their diet, it is particularly marked in carnivorous fish that have higher dietary protein requirements, ranging from 40 % to 55 % of the diet (Wilson R.P. 2002, Jobling 2011). Most of the protein digestion takes place in the stomach (Moraes und De Almeida 2019) although it continues in the intestine as well. Because of the poor utilization of carbohydrates, protein is a very important energy source for carnivorous species. Their diets consume about 50 % protein, which is used very efficiency, because energy is catabolized through the excretion from the waste nitrogen. It is often the most expensive source in the manufactured aquaculture diets. Moreover, animal proteins tend to have a better balance of amino acids that matches of the fish better and therefore have a higher nutritional values compared to plant proteins (Moraes und De Almeida 2019).

1.4 Evaluating alternatives sources of nutrients

There are many criteria for considering alternative protein sources for FM include nutrient profile, palatability, digestibility, anti-nutritional factors, availability and price. That means that the alternative sources of proteins should be both economical and sustainable.

Digestibility is an important factor for alternative protein sources. Proteins are made of amino acids and if they lack the proper balance of essential amino acids, it can cause an imbalanced protein profile and would have lower digestibility and nutritional value**.** Early research by De Silva & Perera Singh demonstrated that the proper utilization of nutrients and energy from feed ingredients depend largely on the extent of their digestibility (De Silva und Perera 1985).

Opinions are divided in the question about replacing FM and many previous reports did not recommend replacing fish meal in the diets (De Francesco et al. 2004, Engin und Carter 2005, Bonaldo et al. 2006).

1.4.1 Vegetal sources

Plant feedstuffs are the major dietary protein sources for the artificial diets fed to omnivorous and herbivorous fish and are second to FM in diets for carnivorous species (Tacon und Metian

2009). Several alternative protein sources of plant origin have been investigated, such as soybean meal (SBM), rapeseed/canola meal, cottonseed meal, Spirulina, distilled dried grains with solubles (DDGS) and it has been possible to increase their level of incorporation in the diets even further.

Plant proteins are commonly regarded as the best source to replace fish meal, even if there are some disagreements regarding their incorporation in the fish diet as replacement for fish meal. Notably, it has been argued that: plant ingredients can have ANFs, and can be deficient in certain EAA, have low nutrient digestibility, lesser nutrient bio-availability and palatability because of high level of non-soluble carbohydrates consisting of fibre and starch.

On the other hand, several studies have reported that partial replacement of FM with plant protein in fish diets did not result in negative effect on the animal's performances while lowering the production cost significantly. Results from several studies have also demonstrated that feeding animals with plant protein did not affect the performance of the animals (Merrifield et al. 2010, Sheikhzadeh et al. 2012, Kpundeh et al. 2015, Guo et al. 2016, Li et al. 2016)

It is very important that the alternative sources must be able to supply adequate non-essential and essential amino acids required for the growth of the fish. Furthermore, most plant protein sources contain anti-nutritional factors which can affect growth, nutrient utilisation and fish welfare in general (Francis et al. 2001).

1.4.2 Other animal protein sources

In addition to plant material, many animal by-products have been used as ingredients in fish diets to compensate for the short supplies and increasing cost of fish meal. Animal origin protein sources such as meat meal, meat and bone meal, poultry by-product meal, feather meal, blood meal, insect protein and earthworm have high potential as alternatives to FM in aqua feeds as they have similar amino acid profile and competitive prices.

However, in contrast to plant feedstuffs, the availability of animal by-products in aquaculture is highly variable depending on the region. For instance, in Australia the use of several animal products causes an increased risk of disease transmission (due to bovine spongiform encephalopathy). In contrast to this the use of animal products in the European Union is very strictly regulated and largely prevents their use in animal feeds (Klinger und Naylor 2012). Moreover, while animal by-products have good palatability and contains no anti-nutritional factors, poultry by-products, meat meal and meat and bone meal have high ash contents and high saturated fat levels and this limits their use in aqua feeds (Oliva-Teles et al. 2015).

1.4.3 Mixing animal and plant protein sources

Many studies have shown that mixing different animal by-products and plant protein sources is more efficient and effective in enriching the nutritional profile of diets. It could prevent nutritional deficiencies, result in a more complete and balanced diet than at single source of protein and ensure a proper supply of essential nutrients in fish protein (Lee und Bai 1997, Bae et al. 2012a, Nates und J 2015, Wang et al. 2016). The concentrated mixture of animal and plant protein sources such as soybean meal (SBM), poultry by-product meal (PBM), blood meal (BM), leather meal (LM), and feather meal (FEM) have already been used as fish meal replacements in the diets of many freshwater fish species (Engin und Carter 2005, Jo et al. 2017, Njue et al. 2018).

As the previous research has demonstrated there are many alternative sources for substituting the expensive fish meal, mainly from plant and animal origin. The present diploma thesis presents an overview regarding the benefits and drawbacks of using alternative sources of nutrients in fish feed.

2 Plant based protein sources for substituting fish meal in the aquatic diet

2.1 General information

Intensive and semi-intensive fish farming relies on the distribution of aqua feed to provide nutrition to the fish (or, in the case of semi-intensive fish farming, to supplement the natural productivity of the fish ponds). Traditionally, the major ingredient in this artificial feed has been animal proteins in the form of fishmeal, generally processed fish products improper for human consumption (fish offal or bones or by-catch).

The demand for fishmeal is increasing worldwide, following the increase in volumes produced by the aquaculture industry. However, the supply of fishmeal is limited and unreliable, resulting in highly variable and rising costs. Moreover, there is a concern about the pressure on wild fisheries that could be exerted by the need to provide ever increasing volumes of fishmeal. Hence it is important to find alternative sources of proteins and lipids, which are economical and sustainable (Gatlin et al. 2007).

Over the years, several components have been investigated and evaluated for use as ingredients in aquaculture feed (Enami 2002, 2003a, 2003b) such as their digestibility, palatability and nutrient utilization (Gomes et al. 1993, 1995, Glencross et al. 2007). In particular plant based materials have received sustained scrutiny as these are easily available and have reasonable price compared to fish meal. Several studies have suggested that replacement with plant proteins did not affect the performance of the animals (Merrifield et al. 2010, Sheikhzadeh et al. 2012, Kpundeh et al. 2015, Guo et al. 2016, Li et al. 2016).

2.2 Effects of plant proteins in fish

Plant protein sources are regarded as the most promising source to replace fish meal as they are a reliable and sustainable source of proteins. However, other aspects of plant proteins have hindered their adoption: Plant ingredients can have ANFs, they are often deficient in certain EAA, their nutrient can have limited digestibility, bio-availability and palatability because of excessive degrees of non-soluble carbohydrates consisting of fibre and starch.

However, several microorganisms, like yeast, bacteria and fungi have been shown to be effective at reducing the effects of anti-nutrients in plant material or adding of essential protein and amino acids (Daniel 2018).

2.2.1 Nutrient digestibility and utilization

The determination of digestibility of ingredients in a diet is a very important for evaluating the effective use of an ingredient for fish and shrimp species (Allan et al. 2000).
Furthermore, the activity of digestive enzymes such as of protease, amylase and lipase is also important to estimate the digestibility of feed ingredients (Zhang et al. 2014).
Previous reports have documented that plant protein in the fish diet affect the digestibility of nutrients by changing the digestive enzyme activity, digestion and absorption capacity of animal (Alarcón et al. 1999, Fontaínhas-Fernandes et al. 1999, Chong et al. 2002, Gaylord et al. 2004, Santigosa et al. 2008, 2011a, 2011b, Richard et al. 2011, Li et al. 2016).

This ANF content within plant sources can hinder the digestion as can excessive levels of fibre or the changes intestinal microflora resulting from feeding plant proteins. ANFs result in a decrease in growth performance and feed efficiency (Olvera-Novoa et al. 2002) and affect digestive enzyme activity, digestion and absorption capacity of animals (Alarcón et al. 1999).

The choice of plant ingredients is essential due to the variation in the digestibility of different plant sources. A study with striped catfish (*Pangasianodon hypophthalmus*) demonstrates that the apparent protein digestibility of different plant ingredients like soybean meal, maize meal rice and sweet potato were not the same (Da et al. 2013).

A study from Fontainhas-Fernandes et al. reported that Nile tilapia fed with plant protein sources had lower digestion than fish meal based diet fed fish group (Fontaínhas-Fernandes et al. 1999).
The activity of digestive and absorptive enzymes was reportedly lower in grass carp (*Ctenopharyngodon idella*) feeding with high-level of plant protein, although this problem could be allivated using supplementation with lysine and methionine (Jiang et al. 2016).

Santigosa found that Sea bream (*Sparus aurata*) fed with plant protein sources showed reduced digestive activity. However, a compensation mechanism was reported, associated with increased relative intestinal length and upregulation of the activity of trypsin, resulting in the growth rates similar to that of the groups fed the fish meal diets (Santigosa et al. 2008).

2.2.2 Bio-availability and utilization of micronutrients

Several reports have suggested that fish fed with plant protein have lowered concentration for several types of vitamins, including riboflavin, niacin, panthotetic acid and vitamin B12 (Bell und Waagbø 2008).

Vitamins are essential for the right metabolism of animals. According to Hansen plant protein sources have low B vitamins availability compared to animal proteins, and need to be supplemented with additional B vitamins in the diet (Hansen et al. 2015).

Similarly, plant-based diets are often low in Phosporus bioavailability (Goda et al. 2007). About 75 % of the Phosporus from plant ingredients comes from phytate-phosphorus, which has low digestibility in fish because they generally lack the enzyme phytase (Lall 1991). However, several techniques have been suggested to alleviate these issues, for example addition of meat and bone meal (MBM) at 7 % to plant based diet improved phosphorus absorption in Nile Tilapia (Suloma et al. 2013). In addition, supplementation of microbial phytase to the diet notably increased Phosphorus absorption in plant-based diet (Liebert und Portz 2005).

Organic acids have a positive effect on the digestion of animals and can improve phosphorus absorption in the small intestine (Ravindran und Kornegay 1993) and lower the pH in the stomach, which results in the increased pepsin activity and protein digestion in animals (Mroz et al. 2000).

In addition, supplementation with citric acid and fatty acid (FA) to the plant-based diet enhances the bioavailability and retention of certain minerals, such as Ca, Mg, Na, K, Zn and Mn (Sugiura et al. 2000, Sarker et al. 2012).

Similarly, according to Weng and Chen (Weng und Chen 2010) the fermentation of plant ingredients may improve the micro-nutrients (Vitamin A and B).

2.2.3 Biochemical composition in fish

As one might expect, the nutritional composition of the fish´ diet can change the biochemical composition of aquatic animals (Zhou und Yue 2010).

For example, in Atlantic cod excess supplementation of the diet with plant proteins has been correlated with decreased liver size, plasma triacylglycerol concentration (TAG) and lipid productive value (LPV).

The study of tissue transcriptomes (mRNA expression) provides further information about the effects of the different the dietary ingredients in the fish diet. The effects of replacing FM and FO with plant ingredients on the gene expression in fish has been widely studied (Leaver et al. 2008, Panserat et al. 2008, 2009, Torstensen et al. 2008, Geay et al. 2011, Tacchi et al. 2012). According to Panserat et al. the replacement of both FM and FO with plant ingredients in the diet of juvenile rainbow trout induced changes in the hepatic expression of genes involved in glucose metabolism and nucleic acid and in cell proliferation and apoptosis (Panserat et al. 2009)(Tacchi et al. 2012).

Moreover, plant protein sources in the diet have been suggested to have a hypocholesterolemic effect and leads to reduced growth. This can be explained by the fact that high amounts of plant ingredients contain negligible amount of cholesterol (Yun et al. 2011). Feeding juvenile rainbow trout with completely plant-based diets results decreased growth compared to trout were fed with marine ingredients based diet (Francesco et al. 2004).

In contrast, some studies have suggested that fish can be fed with plant-based protein without affecting the biochemical compositions. Rodiles has established that 30 % fish meal replacement with plant protein sources like SBM , soybean protein concentrates and wheat gluten meal did not affect the proximate composition of muscle, fatty acid profile and plasma - , hepatic-, and muscular metabolites parameter in Senegalese sole (Rodiles et al. 2015).

Atlantic code (*Gadus morhua*) fed with high plant proteins did not display any effect on the whole body, liver, muscle proximate compositions or blood parameters (Hansen et al. 2007). Plant-based protein had not compromised the body composition of crab by replacing fish meal at rate 64 % with cottonseed, after Jiang et al. (2013).

Reports says that plant protein often lack of lysine and methionine, two essential amino acids, which are important for growth (Nutr. Requir. Fish 1993) and are required for the biosynthesis of carnitine and energy metabolism in fish (Tanphaichitr et al. 1971).

2.2.4 Flesh quality

Previous authors have reported, that dietary plant proteins lower the flesh quality of fish (Alami-Durante et al. 2010, Valente et al. 2016), even though other reports have suggested that feeding plant proteins does not affect flesh quality in fish. In Atlantic salmon (*Salmo salar*) and gilthead sea bream feeding with high level of plant proteins was reported not to have any detrimental effects on the texture properties and flesh quality (Johnsen et al. 2011, Matos et al. 2012, 2014). Cabral et al. (2013) has found fish meal replaced by plant protein sources up to 75 % has not affected the flesh quality of Senegalese sole (*Solea senegalenses*).

L-Carnitine plays a role in the growth promotion of animals, reduces fat accumulation in fish tissues by increasing the lipid oxidation for using the energy from lipids (Harpaz 2005, Ozorio 2009). It was documented that high levels of plant proteins with L-Carnitine addition had growth promoting effect and decreased the ratio of intraperitoneal fat and the whole lipid body contents of silver perch (*Bidyanus bidyanus*), which results a better flesh quality (Yang et al. 2012). Hence, supplementing of L-Carnitine is feasible during increasing the plant protein content in the diet, this may spare the lysine and methionine, which are important for the fish.

2.2.5 Immune and stress parameters

Several studies have reported that increasing plant proteins in the diet of some carnivorous fish can disturb the fish immune system because of the ANFs content (Hardy 2010).

Ferrara reported that replacement fish meal with 40 % SBM induced inflammatory reaction in the gut of sharp snout sea bream (*Diplodus puntazzo*) (Ferrara et al. 2015). Atlantic salmon fed with SBM results enteritis in distal intestine (Baeverfjord und Krogdahl 1996). Another study documented that rainbow trout fed with plant-based diet had lowered cell survival and turnover (Overturf et al. 2012).

In contradiction with the previous findings, some studies suggested that fish meal can be replaced with plant proteins without affecting the immune performance of animals. Hansen reflected that Atlantic cod fed with plant based diets up to 44 % does not affect the intestinal or liver function.

Taurine as an amino acid, has a role in the immunoregulation and detoxication (Motawi et al. 2007, Gülyaşar et al. 2010). Supplementation of dietary plant protein with taurine has been shown to improve the immunity and decrease the ammonia levels in yellow catfish (*Pylodictis olivaris*) (Li et al. 2016).

Similarly, a recent study showed that rainbow trout fed with microalgae in the diet improved the immune and stress parameters (Sheikhzadeh et al. 2012).

2.3 Soybean meal in the aquatic diet

Plant-based products, such as soybean (*Glycine max*), are one of the most popular alternatives in aquaculture (Nordrum et al. 2000, Li und Robinson 2015).

Moreover, soybean has several positive effect on fish: it is highly palatable (Sugiura et al. 1998, Refstie et al. 2000, Watanabe 2002), it has high protein content and balanced amino acid profile (Gatlin et al. 2007, Nutr. Requir. Fish Shrimp 2011).

Soybean meal represents the most balanced profile of all plant proteins, having more than 90 % digestibility in freshwater omnivorous fish species. SBM after oil extraction, includes 44-48 % crude protein (NRC 1993).

There are several soybean products, which are excellent sources of plant protein such as full-fat SBM, hexane-extracted SBM, soy protein concentrates and soy protein isolates. However, even though full-fat and defatted SBM are good sources of digestible protein, they are low in lysine and methionine, high in ANFs and include low digestible non-starch polysaccharides (Moniruzzaman et al. 2020).

In addition, soybean includes anti-nutritional factors which have negative effects on digestion (Iwashita et al. 2008, Nutr. Requir. Fish Shrimp 2011, Teng et al. 2012) and can also cause enteritis (Refstie et al. 2000, Heikkinen et al. 2006, Krogdahl et al. 2015). Alongside these anti-nutritional factors, soybeans also have high levels of carbohydrates (Gordon 1993, Gatlin et al. 2007), which are not well digested, in particular by carnivorous fish species (Jobling 2011).

However, there are several known ways to eliminate or decrease the anti-nutritional factors in soybean.

Lectins and proteinase inhibitors can be decreased by heating during the feed-extrusion process (Barrows et al. 2007, Krogdahl et al. 2010, Bakke und A.M. 2011). Alcohol extraction decreases saponins, sterols and oligosaccharides (Krogdahl et al. 2010). Furthermore, fermentation or bioprocessing has been shown to eliminate or reduce several anti-nutritional factors as well (Hong et al. 2004, Refstie et al. 2005, Yamamoto et al. 2010, 2012).

2.3.1 Experimental diet

According to the result of Vorhees (J. M. Voorhees, M. E. Barnes 2019), bioprocessed soybean meal (BSM) can replace at least 80 % of the fish meal in adult rainbow trout. Adult rainbow trout have been investigated for a 125-day experiment fed BSM replacing 60 % or 80 % of dietary fish meal. At the end of the trial, there were no significant differences in growth parameters, percent mortality, intestinal morphology, splenosomatic-, viscerosomatic-, and hepatosomatic index among the experimental diets. The overall results of this study is similar to the previous experiments feeding SBM to rainbow trout (Yamamoto et al. 2010, 2012, Barnes et al. 2012, 2013, 2014, 2015, Bruce et al. 2017, 2018). As reported by Yamamoto, replacing 100 % of fish meal with fermented SBM has similar results as 80 %, while Barnes et al found the maximum substitution of fishmeal without detrimental effect to be 70 % (Yamamoto et al. 2010, 2012, Barnes et al. 2012, 2013, 2014, 2015). In contrast with previous studies, according to Moniruzzaman et al. only 30 % of the fish meal could be replaced with fermented protein concentrate without negative effect (Moniruzzaman et al. 2020).

As a conclusion, this study indicates that at least 80 % of the dietary fish meal can be replaced by BSM in the diets of adult rainbow trout. There is no information about the suitability of dietary SBM extends additional during the trout life cycle, spawning or prior. Further research is needed to complete if the BSM can replace 100 % of the FM in adult rainbow trout diets.

SBM has some beneficial effects on other fish species as well. Total and partial replacement of FM in African catfish (*Clarias gariepinus*) with SBM at 75 % and 100 % results in higher final body weight and specific growth rate (SGR), but no significant differences from the control (Goda et al. 2007).

SBM can be one of the most promising plant protein ingredients for catfish species as well. As the research of Shukla et al. (2018) established, liver catfish (*Heteropneustes fossilis*) fed with SBM up to 100 % replaced fishmeal, the fingerlings show maximum growth and the highest condition factor (K) compare with other plant based diets and the control diet.

In addition, studies have reported that fish meal can be successfully replaced by SBM in Pacific white shrimp (*Litopenaeus vannamei*).

2.3.2 Combination of SBM and corn gluten meal (CGM) in aquaculture diet

CGM is recovered from the wet milling process for the isolation of the starch, germ, protein and fibre components of corn and constitutes a good source of protein, containing at least 63 % protein and low fat (Fig. 6).
On the other hand, CGM also contains some ANFs and limited amino acids (Pereira und Oliva-Teles 2003, Moniruzzaman et al. 2020).
The mixture of SBM and CGM could increase the FM replacement level for aquatic animals (Lugner 2006).

Another study with turbot (*Scophthalmus maximus*) suggests that the FM level could be reduced by 30 % with a combined use of CGM-SBM with supplementation some elements as lysine, taurine and monocalcium phosphate (Sevgili et al. 2015). The nutrient composition of the whole body, fillet, viscera and liver were not affected significantly, however, whole body lipid levels were significantly lower in fish on 45 % and 60 % FM replacement levels than those on 100 % FM.

Ten diets were formulated for the experiment (Table 1), a high-protein fish meal (HFM) as a positive control and a low-protein fish meal (LFM) as a negative control. Eight experimental diets were prepared, consisting of four kinds of bioprocessed protein concentrates (BPCs) as a replacement of fish meal (BPC-A, -B, -C and -D) each at 30 % and 50 % FM replacement levels, where BPC-A was a solid-state fermented mixture of soybean and corn gluten meals;

BPC-B was pre-treated acid-hydrolysed BPC-A; BPC-C and BPC-D were BPC-A + 2 % shrimp soluble extract (SSE) and BPC-B + 2 % SSE. SBM and CGM was mixed and fermented by *Bacillus* spp. at pasteurization temperature, followed by a solid-state fermentation that allowed the mixed material goes to form into the solid state with addition of water.

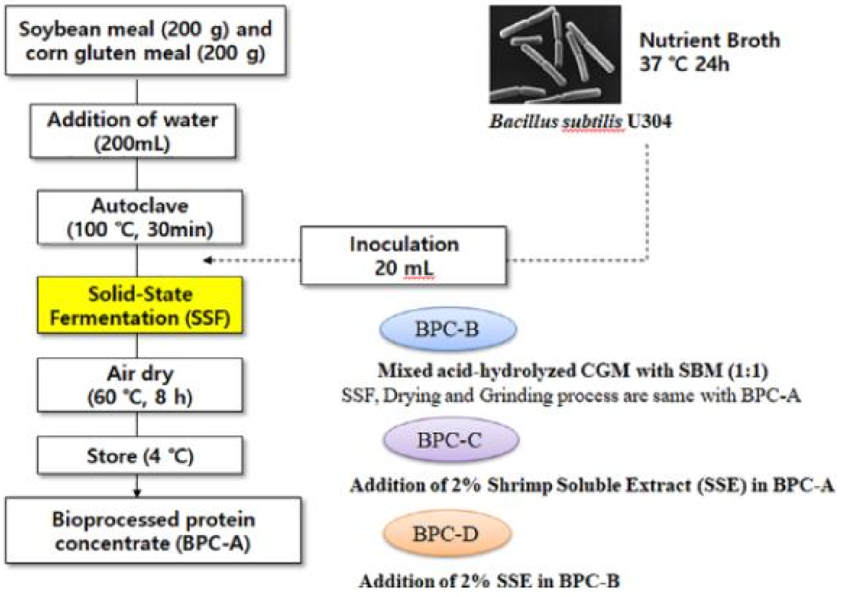
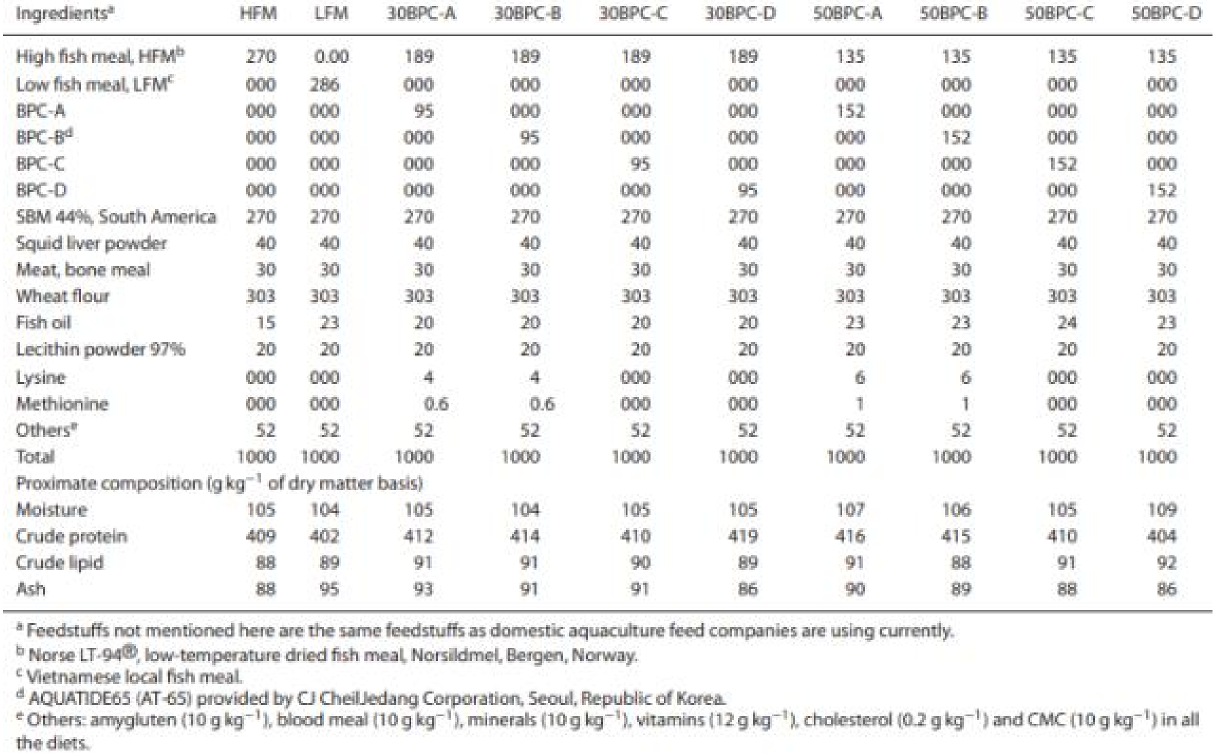


Figure 6: **Production processes of BPCs from SBM and CGM** (Moniruzzaman et al. 2020).

Table 1: **Composition of the experimental diets in juvenile Pacific white shrimp** (Moniruzzaman et al. 2020).



Ingredients[a]	HFM	LFM	30BPC-A	30BPC-B	30BPC-C	30BPC-D	50BPC-A	50BPC-B	50BPC-C	50BPC-D
High fish meal, HFM[b]	270	0.00	189	189	189	189	135	135	135	135
Low fish meal, LFM[c]	000	286	000	000	000	000	000	000	000	000
BPC-A	000	000	95	000	000	000	152	000	000	000
BPC-B[d]	000	000	000	95	000	000	000	152	000	000
BPC-C	000	000	000	000	95	000	000	000	152	000
BPC-D	000	000	000	000	000	95	000	000	000	152
SBM 44%, South America	270	270	270	270	270	270	270	270	270	270
Squid liver powder	40	40	40	40	40	40	40	40	40	40
Meat, bone meal	30	30	30	30	30	30	30	30	30	30
Wheat flour	303	303	303	303	303	303	303	303	303	303
Fish oil	15	23	20	20	20	20	23	23	24	23
Lecithin powder 97%	20	20	20	20	20	20	20	20	20	20
Lysine	000	000	4	4	000	000	6	6	000	000
Methionine	000	000	0.6	0.6	000	000	1	1	000	000
Others[e]	52	52	52	52	52	52	52	52	52	52
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Proximate composition (g kg^{-1} of dry matter basis)										
Moisture	105	104	105	104	105	105	107	106	105	109
Crude protein	409	402	412	414	410	419	416	415	410	404
Crude lipid	88	89	91	91	90	89	91	88	91	92
Ash	88	95	93	91	91	86	90	89	88	86

[a] Feedstuffs not mentioned here are the same feedstuffs as domestic aquaculture feed companies are using currently.
[b] Norse LT-94®, low-temperature dried fish meal, Norsildmel, Bergen, Norway.
[c] Vietnamese local fish meal.
[d] AQUATIDE65 (AT-65) provided by CJ CheilJedang Corporation, Seoul, Republic of Korea.
[e] Others: amygluten (10 g kg^{-1}), blood meal (10 g kg^{-1}), minerals (10 g kg^{-1}), vitamins (12 g kg^{-1}), cholesterol (0.2 g kg^{-1}) and CMC (10 g kg^{-1}) in all the diets.

Shrimps were fed one of the diets four times a day, at a level of 4 % of wet body weight in the first 4 weeks and 3 % in the second 4 weeks (Moniruzzaman et al. 2020) . The total weight was determined every 2 weeks. At the end of the feeding trial, all shrimp were weighed and calculated the growth parameters such as weight gain (WG), feed efficiency, SGR and digestive enzymes (lipase, amylase, protease) activities.

At the end of the trial, among the several experimental feeding diets, the BPC-B, BPC-C and BPC-D diets at 30 % of FM replacement were the most effective and were associated with significantly higher weight gain and specific growth grain. Nonetheless, shrimp fed the HFM, LFM, BPC-B, BPC-C and BPC-D diets showed no significant differences of WG and SGR at 30 % of FM replacement level.

Other feeding studies were conducted to determine the optimal replacement rate of FM at the pacific white shrimp and it was found that 20 % of FM (Table 2) could successfully replace with fermented SBM (Shao et al. 2019), while another study suggested that it was possible to fully replace of FM by SBM under pond conditions (Amaya et al. 2007). Conversely, Qiu und Davis have reported that a plant-based spray-dried fermented biomass can only replace up to 50 % of FM (Qiu und Davis 2018). According to the present study, the 50 % FM replacement level is not successful in the diet of pacific white shrimp, may be because of the high level of ANFs in the BPCs such as saponin and other ANFs.

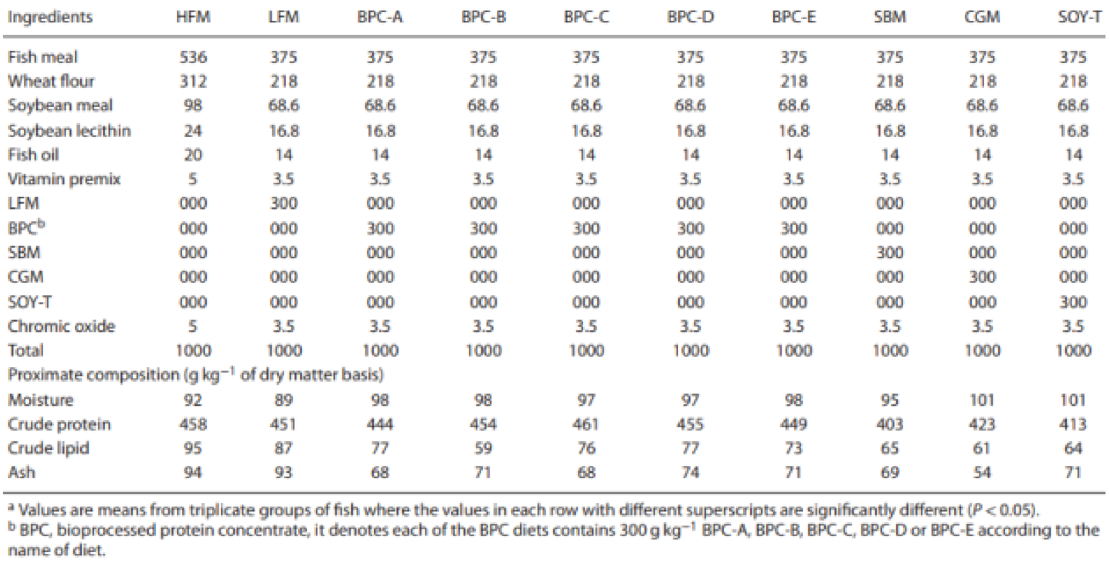
Similarly, pre-treated acid-hydrolysed ingredients could partially replace FM in the diet of the juvenile pacific white shrimp thanks to the fermentation process. Indeed, result shows that shrimp fed the BPC-D diet at 30 % FM replacement showed an improved health status compared to the other diets. Moreover, the study also showed that the addition of amino acids such as methionine and lysine in BPC cannot replace 30 % of FM.

This may indicate that the acid hydrolysis is an important step to remove ANFs in the diet ingredients and without it, the 30 % FM replacement with BPC can be attainable only if we add 2 % SSE in BPC.

Acid hydrolysis is an important process, which can be widely used to treat lignocellulosic materials to obtain sugars (monosaccharide). Protic acid is used to catalyse the cleavage of a chemical bond with the addition of the elements of water. Compared with other methods, acid hydrolysis brings higher sugar yield and good reproducible (Chen 2015).

Although the results show that the pre-treated acid-hydrolysed BPC with supplementation of crystalline amino acids (lysine and methionine) and pre-treated acid-hydrolysed BPC with the high values of digestive enzyme, the BPC-B and BPC-D diets, and HFM showed better achievement over the other diets in the present study (Moniruzzaman et al. 2020).

Table 2: **Comparison the experimental diets for digestibility evaluation in juvenile Pacific white shrimp** *(Moniruzzaman et al. 2020)*.



Ingredients	HFM	LFM	BPC-A	BPC-B	BPC-C	BPC-D	BPC-E	SBM	CGM	SOY-T
Fish meal	536	375	375	375	375	375	375	375	375	375
Wheat flour	312	218	218	218	218	218	218	218	218	218
Soybean meal	98	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6
Soybean lecithin	24	16.8	16.8	16.8	16.8	16.8	16.8	16.8	16.8	16.8
Fish oil	20	14	14	14	14	14	14	14	14	14
Vitamin premix	5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
LFM	000	300	000	000	000	000	000	000	000	000
BPC[b]	000	000	300	300	300	300	300	000	000	000
SBM	000	000	000	000	000	000	000	300	000	000
CGM	000	000	000	000	000	000	000	000	300	000
SOY-T	000	000	000	000	000	000	000	000	000	300
Chromic oxide	5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Proximate composition (g kg^{-1} of dry matter basis)										
Moisture	92	89	98	98	97	97	98	95	101	101
Crude protein	458	451	444	454	461	455	449	403	423	413
Crude lipid	95	87	77	59	76	77	73	65	61	64
Ash	94	93	68	71	68	74	71	69	54	71

[a] Values are means from triplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).
[b] BPC, bioprocessed protein concentrate, it denotes each of the BPC diets contains 300 g kg^{-1} BPC-A, BPC-B, BPC-C, BPC-D or BPC-E according to the name of diet.

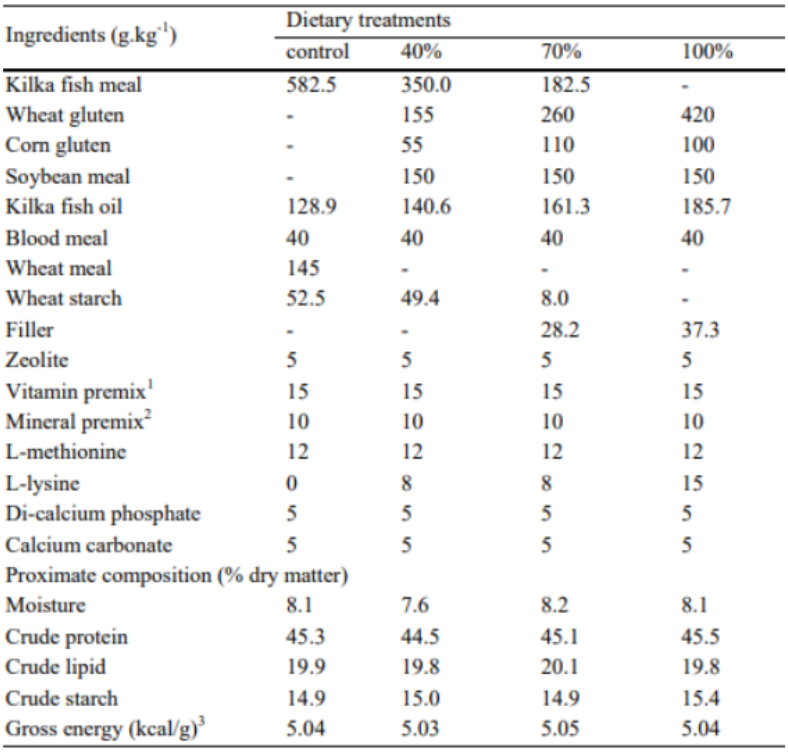
supplementation of 2 % SSE has a better digestibility than other plant protein-based diets. The enzyme activity determined that all the experimental diets are well digested by shrimp, whereas

As a conclusion, this research demonstrated that partial replacement of high-protein FM with high quality fermented protein concentrates from plant sources is successful in the diet of pacific white shrimp. Shrimps fed with BPC-B, BPC-C and BPC-D diets at 30 % FM replacement level as well as BPC-D diet at 50 % FM replacement showed significantly higher growth performance than the other experimental diets.

2.3.3 SBM with addition of wheat gluten and corn gluten in fish diet

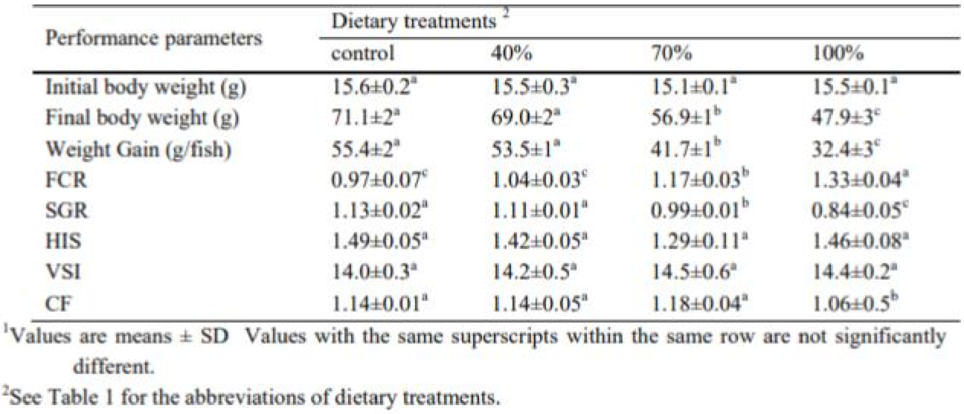
Diets of rainbow trout were prepared in which fishmeal (from Kilka, *Clupeonella* sp.) was replaced at levels of 40 %, 70 % and 100 % with experimental plant sources (wheat gluten, CGM and SBM) to reach similar levels of proteins, lipid and energy (Table 3). Results from this feeding experiment have suggested that incorporation had no detectable effects up to 40 %. However, the group for which fish meal was replaced with 70 % and 100 % plant protein resulted in decrease WG, SGR, and DGR (daily growth rate) and increased feed conversion ratio (FCR). The condition factor was the lowest in fish diet complete replacement of fish meal with plant sources. Similarly, serum total immunoglobulin and alternative complement activity were unchanged at 40 % inclusion level but were degraded when more vegetal proteins were incorporated (Jalili et al. 2013, Table 4).

Table 3: **Composition and ingredients of experimental diet** *(Jalili et al. 2013)***.**



Ingredients (g.kg^{-1})	Dietary treatments			
	control	40%	70%	100%
Kilka fish meal	582.5	350.0	182.5	-
Wheat gluten	-	155	260	420
Corn gluten	-	55	110	100
Soybean meal	-	150	150	150
Kilka fish oil	128.9	140.6	161.3	185.7
Blood meal	40	40	40	40
Wheat meal	145	-	-	-
Wheat starch	52.5	49.4	8.0	-
Filler	-	-	28.2	37.3
Zeolite	5	5	5	5
Vitamin premix^{1}	15	15	15	15
Mineral premix^{2}	10	10	10	10
L-methionine	12	12	12	12
L-lysine	0	8	8	15
Di-calcium phosphate	5	5	5	5
Calcium carbonate	5	5	5	5
Proximate composition (% dry matter)				
Moisture	8.1	7.6	8.2	8.1
Crude protein	45.3	44.5	45.1	45.5
Crude lipid	19.9	19.8	20.1	19.8
Crude starch	14.9	15.0	14.9	15.4
Gross energy (kcal/g)^{3}	5.04	5.03	5.05	5.04

Table 4: ***Growth parameters of rainbow trout fed with experimental diets*** *(Jalili et al. 2013)***.**



Performance parameters	Dietary treatments ^{2}			
	control	40%	70%	100%
Initial body weight (g)	15.6±0.2^{a}	15.5±0.3^{a}	15.1±0.1^{a}	15.5±0.1^{a}
Final body weight (g)	71.1±2^{a}	69.0±2^{a}	56.9±1^{b}	47.9±3^{c}
Weight Gain (g/fish)	55.4±2^{a}	53.5±1^{a}	41.7±1^{b}	32.4±3^{c}
FCR	0.97±0.07^{c}	1.04±0.03^{c}	1.17±0.03^{b}	1.33±0.04^{a}
SGR	1.13±0.02^{a}	1.11±0.01^{a}	0.99±0.01^{b}	0.84±0.05^{c}
HIS	1.49±0.05^{a}	1.42±0.05^{a}	1.29±0.11^{a}	1.46±0.08^{a}
VSI	14.0±0.3^{a}	14.2±0.5^{a}	14.5±0.6^{a}	14.4±0.2^{a}
CF	1.14±0.01^{a}	1.14±0.05^{a}	1.18±0.04^{a}	1.06±0.5^{b}

[1] Values are means ± SD Values with the same superscripts within the same row are not significantly different.

[2] See Table 1 for the abbreviations of dietary treatments.

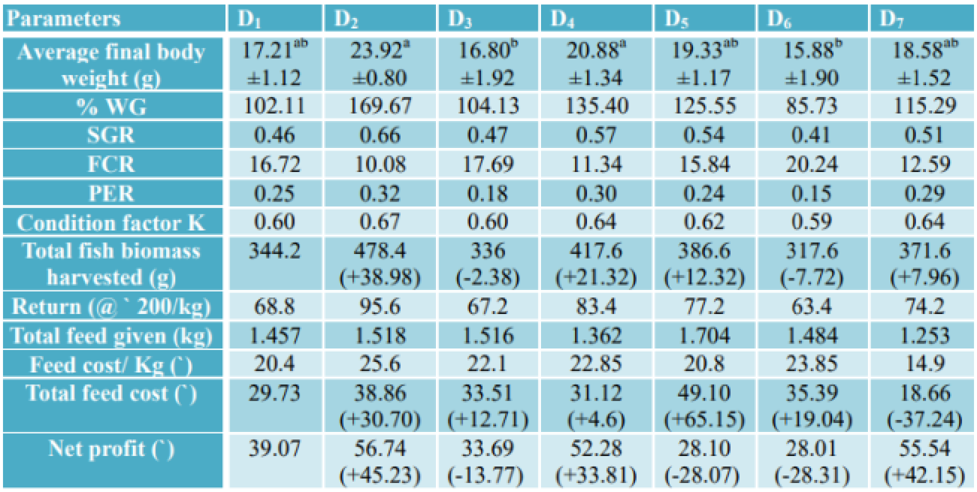
These results are consistent with those reported by Bell and Torstensen where substituting 30 % and 35 % fish meal with wheat gluten based plant ingredients did not adversely affect fish growth in Atlantic salmon (*Salmo salar*) and Atlantic halibut (Storebakken et al. 2000, Gordon Bell et al. 2002, Torstensen et al. 2004, Helland und Grisdale-Helland 2006). It is also in accordance with the results that substitution of 50 % of fishmeal with corn gluten in Atlantic salmon had no detrimental effects (Mente et al. 2003). Although it appears that inclusion of higher level of plant ingredients in salmonids diets has adverse effects on fish performance (Francesco et al. 2004, Gaylord et al. 2006, Palmegiano et al. 2006, Drew et al. 2007).

2.3.4 SBM with groundnut in fish diet

Groundnut meal (GNM) is highly palatable for fish, it also has better binding properties for pelleting compared to soybean (Lovell 1998) and has valuable vitamin B, E and K content.
The drawback of groundnut meal is that they can be the deficient of several essential amino acids including methionine, cysteine and lysine. This amino acid quality can be improved either enrichment with the deficient amino acids (Friedman 1996, Eyo und A.A 1998, Eyo und Olatunde 1998) or the addition of the other protein sources (Ovie und Ovie 2010).

Seven experimental diets were formulated for liver catfish (*Heteropnuestes fossilis)*, where the control diet was based on fish meal, another diet was traditional feed what is used for carp farming and five diets were formulated by partially and fully replacement of FM with SBM and GNM (Shukla et al. 2018) .

Table 5: **Growth parameters and economic evaluation at the end of the experiment** (Shukla et al 2018).



Parameters	D$_1$	D$_2$	D$_3$	D$_4$	D$_5$	D$_6$	D$_7$
Average final body weight (g)	17.21^{ab} ±1.12	23.92^{a} ±0.80	16.80^{b} ±1.92	20.88^{a} ±1.34	19.33^{ab} ±1.17	15.88^{b} ±1.90	18.58^{ab} ±1.52
% WG	102.11	169.67	104.13	135.40	125.55	85.73	115.29
SGR	0.46	0.66	0.47	0.57	0.54	0.41	0.51
FCR	16.72	10.08	17.69	11.34	15.84	20.24	12.59
PER	0.25	0.32	0.18	0.30	0.24	0.15	0.29
Condition factor K	0.60	0.67	0.60	0.64	0.62	0.59	0.64
Total fish biomass harvested (g)	344.2	478.4 (+38.98)	336 (-2.38)	417.6 (+21.32)	386.6 (+12.32)	317.6 (-7.72)	371.6 (+7.96)
Return (@ ` 200/kg)	68.8	95.6	67.2	83.4	77.2	63.4	74.2
Total feed given (kg)	1.457	1.518	1.516	1.362	1.704	1.484	1.253
Feed cost/ Kg (`)	20.4	25.6	22.1	22.85	20.8	23.85	14.9
Total feed cost (`)	29.73	38.86 (+30.70)	33.51 (+12.71)	31.12 (+4.6)	49.10 (+65.15)	35.39 (+19.04)	18.66 (-37.24)
Net profit (`)	39.07	56.74 (+45.23)	33.69 (-13.77)	52.28 (+33.81)	28.10 (-28.07)	28.01 (-28.31)	55.54 (+42.15)

The average body weight, % WG, SGR and protein efficiency ratio (PER) at the end of the culture period, were observed to be highest in diet with 100 % replaced with SBM and lowest in diet with the mixture of SBM and GNM for 100% to replace fish meal (Table 5). Groundnut meal shows acceptability up to 50 % in diet in place of fish meal. When it is replaced at 100 % the fish meal, the growth parameters get deteriorated. According to (Nguyen et al. 2009), the combination of groundnut meal with some other plant protein ingredients can result in growth enhancement. In contracts with above mentioned research, the study cannot confirm it, because the diet with 100 % replace fish meal having combination of SBM and GNM does not give encouraging results.

The low feed utilization, feed intake and growth performance of the diet with 50 % replaced fish meal with GNM and the diet with mixture of SBM and GNM for 100 % replaced fish meal might be because of the deficiency of sulphur containing amino acids such as lysine, methionine and cysteine (Jauncey 1998) moreover the deficient of vitamin B12 and calcium in groundnut meal.

The experiment documented the maximum growth of fingerlings of liver catfish (*H. fossilis)* in diet with 100 % replaced fish meal with SBM, followed by diet with 50 % replaced fish meal with SBM, and the diet with 50 % replaced fish meal with GNM.

The present study demonstrates the acceptability of the SBM at 50 % and 100 % level by *H. fossilis* without any deteriorating effect (Shukla et al. 2018).
The diet containing 50% of GNM in place of fish meal performed better than diet containing 100 % groundnut. Similar results were observed in the study of (Jackson et al. 1982). He documented in a study for Tilapia species, that groundnut cake can replace up to 25 % of fish meal, but at higher levels the growth rate decreases.

Studies in channel catfish (*Ictalurus punctatus*) have shown that a complete replacement of fish meal by SBM or some other ingredients like cotton seed meal or meat with bone meal did not results in growth impairment or reduced feed efficiency (Reigh 1999, Li et al. 2000). Furthermore, Nyirenda used SBM as an only protein source in the diets of Oreochromis Karongae and observed higher growth rate in term of SGR and fish yield (Nyirenda, J., Mwabumba, M. et al. 2000).

The condition factor (K) of the fish shows physical and biological characteristics and fluctuations by interaction among the feeding condition, parasitic infection and physiological factors (Cren 1951) with assessment of the fish condition (Lambert und Dutil 1997) based on weight at a given length (indicating energy reserves in fish). In the above mentioned study the maximum value of ´K´ was recorded in diet with fully replace fish meal with SBM followed by diet with 50 % replace fish meal with SBM and the traditional carp diet. Diets with different inclusion levels of GNM ended up at the end list. The values of `K´ in the diet 100 % and 50 % replace fish meal with SBM, and the traditional diet were higher than the control which reflects that fish fed with diet containing SBM replaced fish meal and diet without fish meal were more potent than fish fed with the control diet.

As a conclusion the present study clearly documents that 100 % of replacement of fish meal with SBM in catfish diet is a balanced high quality diet.
Hence it can be concluded, that SBM can be one of the most promising plant protein ingredients for catfish species.

2.4 Canola/Rapeseed in the aquatic diet

The name of canola and rapeseed refers to plants from the genus *Brassica* in particular, *Brassica napus* and *Brassica rapa* that are farmed throughout the world (Table 6). Canola/ rapeseed meal has relatively higher protein content compared to other oilseed meals, except SBM. The amino acid balance of canola protein makes it one of the best available commercial protein sources according to Friedman (1996), moreover its price is quiet economical, it is about half of the cost of fish meal per kg of protein.

The minimum crude protein content for Canadian canola meal is 36 %. Canola meal has a good amino acid profile for the animal feeding. Like the most plant protein based aquatic diet, canola meal has limited in lysine content, but its methionine and cysteine levels are high. The crude fibre content is higher than the SBM, because the canola hulls stay with the meal. The carbohydrate content of the canola meal is quite complex and results in a significant contribution to a digestible energy. However, these carbohydrates are protected by the cells walls, so their effect is modest during digestion (Slominski und Campbell 1990, Bell 1993). Canola is a good essential minerals source, especially selenium and phosphorus, compared to other vegetable origin oilseed meals. About the vitamin content of the canola meal there is a limited information sources, but it seems to be rich in thiamine, riboflavin, choline, biotin and folic acid.

The use of canola/rapeseed meal has been found no to have significant adverse and negative effects on the weight, size, carcass quality, taste or other physiological features of the animals.

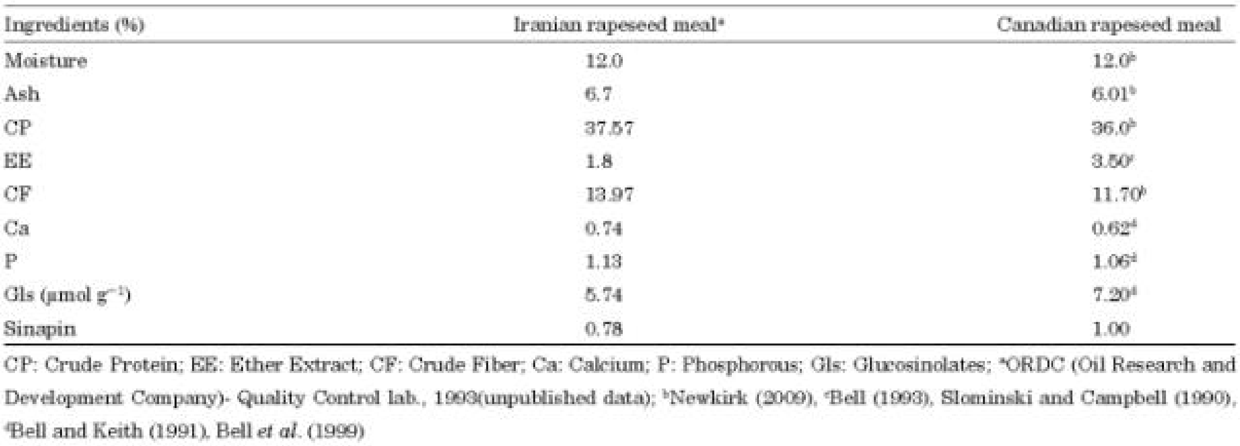
Table 6: **The world`s top canola/ rapeseed producers (tones) by countries in 2010** (Enami 2011a**).**

Rank	Country	Production
1	Canada	12642900
2	China	12102010
3	India	5833000
4	Germany	5154700
5	France	4719053
6	Ukraine	2872800
7	Poland	2105840
8	UK	1973030
9	Australia	1615000
10	Czech Republic	1048943
11	Russia	752200
12	Romania	673033
13	USA	660334
14	Hungary	651500
15	Denmark	629200
16	Belarus	513959
17	Slovakia	424444
18	Iran	390000
19	Pakistan	390000
20	Lithuania	330200
	WORLD	57856158

Source: Faostat.fao.org (2010)

The drawback factors for using canola meal in the aquatic diet are the anti-nutritional ingredients such as glucosinolates (GLs), phenolic compounds (tannins and sinapine), phytate and relatively high fibre content, includes 14.5 % cellulose, 5 % hemicellulose and 8.3 % lignin. Canola contains small amounts of heat labile (glucosinolates) and heat stable (phytic acid, phenolic compounds, tannins and fibre) anti-nutritional factors. The traditionally rapeseed meal includes 120-150 mikromol/g of total glucosinolates**.** The cultivation and use of canola/rapeseed meal by different aquaculture farms has shown a progressive trend in Iran and other countries in recent years. As the supplied table shows, the Iranian and Canadian canola meals contain approximately only 7.2 mikromol/g (Enami 2011a, Table 7) .

Table 7: **Comparison the compounds of the Iranian and Canadian rapeseed meal** (Enami 2011a**).**



Ingredients (%)	Iranian rapeseed meal[a]	Canadian rapeseed meal
Moisture	12.0	12.0[b]
Ash	6.7	6.01[b]
CP	37.57	36.0[b]
EE	1.8	3.50[c]
CF	13.97	11.70[b]
Ca	0.74	0.62[d]
P	1.13	1.06[d]
Gls (μmol g^{-1})	5.74	7.20[d]
Sinapin	0.78	1.00

CP: Crude Protein; EE: Ether Extract; CF: Crude Fiber; Ca: Calcium; P: Phosphorous; Gls: Glucosinolates; [a]ORDC (Oil Research and Development Company)- Quality Control lab., 1993(unpublished data); [b]Newkirk (2009), [c]Bell (1993), Slominski and Campbell (1990), [d]Bell and Keith (1991), Bell *et al.* (1999)

There are several different types of glucosinolates and each of these products has a negative effect on the animal. They have adverse effects on the thyroid hormone production and liver function (Hardy und Sullivan 1983) . Furthermore, besides the toxic effect of the glucosinolate, its bitter taste leads to a reduced feed intake for several animals (Bell 1993, R. 1994). This can be avoided by limiting the amount of canola meal in the diet as dietary inclusion of canola meal below 30 % results in glucosinolate content 2.650 mikromol/kg (Higgs et al. 1983).

There are many others components in canola meal, which may have anti-nutrient effects (Bell 1993).

Tannins and sinapine do not appear to have negative effects like the anti-nutritional factors, but they reduce appetence of the feed and potentially impact the feed intake (Clandidnin.D.R. 1961). Although according to Qiao and Classen 2003 (Qiao und Classen 2003), the bitter taste of sinapine in the canola meal does not affect the feed intake or growth rate.

Finally, the canola meal`s energy and protein content are too low to be widely used as a feed ingredient in the aquaculture (Enami 2011b).

Despite these limitations, it is important to note that the nutritional value of the canola/rapeseed meal depends on the oil extraction method and process.

There are several factors which can affect the nutritional quality and value of canola meal: the residual oil content of the meal, the levels of glucosinolates and their products and the level of heat imparted during oil extraction.

The protein quality can similarly be influenced by the processing steps of the production. Remarkably, excess heating during oil extraction processes can result reduced digestibility of protein and some amino acids, like lysine.

Plant breeders in Iran have worked on improvement of quality of the plant to reduce both erucic acid and glucosinolates content. They achieved to reduce the glucosinolates content up to 77.3 % and enchanced the rapeseed production area with 20 and 26 times.

Canola Protein Concentrate (CPC) by aqueous extraction of protein (Mwachireya et al. 1999, Thiessen et al. 2004) has the same crude protein levels as fish meal and a higher methionine and lysine levels compared to corn gluten and SBM. This extraction process results in the complete removal of phytate, and only includes extremely low levels of glucosinolates in a CPC and allow a higher level of fish meal replacement in aqua feeds. Research by Thiessen has suggested that CPC can replace up to 75 % of fish meal without any adverse effects in growth or feed efficiency (Thiessen et al. 2004).

Canola meal is commonly used in the aquaculture diets for both fish and shrimp species, such as trout, carp, tilapia, bass and sea bream.

Hardy and Sullivan (Hardy und Sullivan 1983) have reported that replacement with canola at 20 % did not affect the weight gain of rainbow trout (*Oncorhynchus mykiss*) while reducing feed costs were reduced by US$ 18.7 per metric ton. A negative effect, was the impairment of the thyroid function. Dietary rapeseed can induce thyroid hypertrophy, reduced levels of intrathyroidal iodothyronines, depressed plasma-protein-bound iodide or thyroxine titers, and histopathological changes in thyroid (Higgs D.A., J.R. Markert, D.W.MacQuarrie 1979) moreover, thyroid hormone synthesis and peripheral monodeiodination of thyroxine (T4) to 3.5,3'-triiodo-e-thyronine (T3)(Yurkowski et al. 1978, Higgs D.A., J.R. Markert, D.W.MacQuarrie 1979, Higgs et al. 1982). According to Hardy and Sullivan rapeseed meal may comprise up to 22 % of the diet for rainbow trout (Yurkowski et al. 1978), without reducing growth or plasma T4 concentrations, although thyroid hypertrophy or hyperplasia may result at higher levels of substitution.

2.4.1 Canola meal for shrimp

A study by Lim (Lim et al. 1997) was conducted on using canola /rapeseed meal for shrimp diets. High-fibre canola meal and low-fibre canola meal were used for juvenile pacific white shrimp (*Penaeus vannamei*). At the end of the study one or more fibre-reduced and solvent-extracted canola protein products were found to represent cost-effective replacement for fish meal protein. Another experiment improved the nutritional value of canola meal by addition of enzymes in diets for juvenile giant tiger prawn (*Penaeus monodon*). As a result, the live weight gain was higher and showed better FCR for the low- fibre canola meal with added enzyme diets than the other experimental feeds (Buchanan et al. 1997).

A trial diet with soybean and canola meal promises for pacific white shrimp was also successful for a long term period and in clear water (*Litopenaeus vannamei*)(Suárez et al. 2009).

Moreover, adding extruded canola meal in the diet for blue shrimp (*Litopenaeus stylirostris*) results in no significant effect on performance (Cruz-Suarez et al. 2001).

2.4.2 Canola meal for fish

Canola meal has been used already for over 20 years as a feed ingredient in salmon and trout diets.

Canola meal is used in salmonid diets at up to 20 % inclusion levels but it is possible further replacement of fish meal in the diet for highly valued species (Newkirk 2009). None of the experimental diet trials using canola meal resulted in any significant adverse effect on animal performance or on other factors, furthermore, it can reduce the total cost of the diets. Replacing SBM about 31 % of the diet with canola meal for channel catfish without adversely affecting growth or other performance, is also a benefit of the canola meal (Lim 1998).

According to Erdugan and Olmez, (Erdogan und Olmez 2009), low-level canola incorporation content of canola in the diet of angel fish (*Pterophyllum scalare*) can result in higher weigh gain and adding of cellulose enzymes in different ratios to the diet, does not lead to any adverse effects in growth parameters and digestibility.

It has been documented, that protein from canola meal can replace up to 10 % of the fish meal protein (Viegas et al. 2008) in the diet of Nile tilapia without adverse effects, although, this is likely an underestimate and other studies have suggested that the tolerable levels of canola meal can be higher. For example, in another experiment, Nile tilapia were fed diets containing 24.7 % CPC, for replacing fish meal, SBM and CGM (Borgeson et al. 2006) As a result, the fish group receiving the CPC diet have been grown significantly faster (2.29 g/ day) than the control diets group (1.79 g /day).

Moreover, research by Thiessen et al (Thiessen et al. 2004) recorded that CPC can replace up to 75 % of fish meal without any adversely affects in growth or feed efficiency.

Canola meal is a potential plant protein based source to replace substantial levels of fish meal in diets for carnivorous fish (rainbow trout)(Shafaeipour et al. 2008). Study performed by Safari and Boldaji (O. Safari und F.Boldaji. 1985) documented that canola meal up to 40 % in the diet of rainbow trout to substitute fishmeal is more effective because of the improvement of the growth performance compared to 40 % SBM. High levels of fibre, alone or together with phytate influences negatively the digestibility of canola meal products for rainbow trout.

As a conclusion it is recommended to add some amounts of canola meal to fishmeal in the diet to increase the diet mass which leads to decreasing the feed intake by animals.
The secondary plant compounds such as glucosinolates and tannins can be eliminated or reduced by new processing technologies to increase the nutritional properties of canola meal.
Because of the low energy and protein content of the canola it is not widely used as a feed ingredient in the aquaculture, but the processed form or the Canola Protein Concentrate could play an important role in the substitution of fishmeal in the aquatic animals.

2.5 Cottonseed in aquaculture diets

Cottonseed, *Gossypium hirsutum* Linnaeus is one of the leading plant protein source (third after soybean and rapeseed) worldwide (Gatlin et al. 2007) and, while more expensive than other vegetal protein sources, it is still available at lower cost than animal protein (Lovell 1989). Cottonseed meal (CSM) can be recovered from cottonseed using either, mechanical or solvent extraction.

Mechanical extraction is a traditional method of cottonseed oil extraction using a technology such as a hydraulic press or screw press. The resulting cake is dried and processed into large pellets. It is not a very efficient method and up to 20 % of the seed oil may remain in the pressed cake (Bailey's Ind. Oil Fat Prod. 2005). At the solvent extraction, the oil is extracted solvent alone in contrast with the mechanical extraction. The end product is heated to eliminate the solvent and then prepared into meal.

Nutritionally, cottonseed meals contain high levels of proteins (Forster LA 1995) which make them highly palatable and well digested by most aquatic animals (Li und Robinson 1995).

CSM is a partial replacement of SBM and it has been generally used at low levels in aquaculture. The presence of anti-nutritional factors like phytic acid, gossypol and cyclopropenoid acid limits the usage of the cottonseed in the aquaculture diets. Furthermore, the relative costs of CSM compared with other protein sources and the higher nutritive value of soybean meal recommend for using low levels of cottonseed meal in the aqua feed (Li und Robinson 2006).

Several research observed gossypol content in the CSM which is described as an anti-nutritional factor. It has high impact on the growth, carcass composition and pathological changes in the fish.

Furthermore, CSM is relatively low in some essential amino acids, such as lysine, cysteine and methionine.

2.5.1 Anti-nutritional factors in the CSM

Phytates works as strong chelators and mold protein-phytic acid complexes, which are available in 70 % of the phosphorus in feedstuffs of plant origin. These may reduce the bioavaibility of protein (Spinelli et al. 1983) and minerals, such as zinc, manganese, copper, molybdenum, calcium, magnesium, and iron (RR 1977).

Cyclopropenoic fatty acids (CFAs) in the diet cause lesions, increase glycogen deposition and elevate saturated fatty acid concentration in the liver in rainbow trout (Struthers et al. 1975a, 1975b). Furthermore, CFAs have been associated with hepatomas in rainbow trout (Hendricks et al. 1980) and act as are potent carcinogen, in particular in the presence of aflatoxins (Wales und Sinnhuber 1972).

Dietary CFAs changes the activity of many liver enzymes (Eisele et al. 1983) which may explain the accumulation of the saturated fatty acids in the liver of fish (Roehm et al. 1970).

Gossypol is a fat-soluble, natural polyphenol and yellow compound of cotton plants. It is toxic to fish (Herman 1970, Rinchard et al. 2000) and terrestrial animals (Colin-Negrete et al. 1996) although it has several positive properties, contains anti-parasitic, anti-bacterial, and antioxidant factors (Bickford et al. 1954, Margalith 1967, Montamat et al. 1982, Wichmann et al. 1982, Heidrich et al. 1983).

CSM contains gossypol, in an amount that varies depending on the cotton species and some environmental factors (Boatner et al. 1949, Cherry et al. 1978).

Gossypol can accumulate in various tissues in aquatic animals, the highest concentration is in the liver and the lowest in the muscle. It has been reported that Pacific white shrimp fed diets containing CSM at 500 mg/kg or more caused light yellow-greenish colouration (Lim 1996).

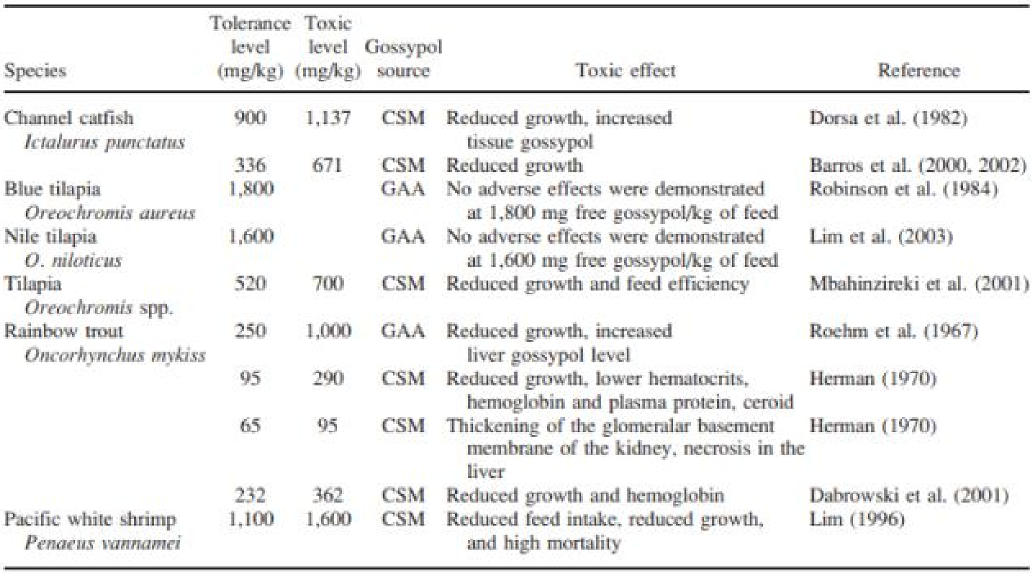
Gossypol exists in two forms, either in bound or in free from. The bound form, in which gossypol is bound with free amino groups of protein, has little significance to animals because it passes unabsorbed through the gastrointestinal tract (Evans 1985, Thacker und Kirkwood 2017). In contrast, the free form, is highly toxic for animals (Ogunji 2004) and is associated with reduced feed consumption, growth, hematocrits, hemoglobin, and reproductive capacity, and histological changes in various tissues and organs and rarely death.

Gossypol has been shown some detrimental effects on the reproduction capacity of mammals by affecting the reproductive tissues or gonadal and pituitary hormone secretion (Randel et al. 1992). A study from Dabrowski et al. (2001) found decreased sperm concentration in rainbow trout as dietary free gossypol increased from 0 mg/kg to 463 mg/kg. The sperm motility and fertilization rate stayed unaffected after 258 days of feeding.

A 3-year study summarized the effects of dietary gossypol on reproduction performance of rainbow trout and observed that long-term feeding of high levels of gossypol reduces sperm and egg quality. After the first year the fish showed reduced sperm concentration, egg weight and quality of feeding diets including total gossypol of 7.037–9.549 mg/kg compared with diets having lower gossypol levels. In the second year the reproductive performances were reduced (sperm and egg quality and progeny growth) (Lee und Dabrowski 2002). The effect of gossypol and its maximum tolerance levels in multiple aquatic species have been reviewed by Li and Robinson in 2006 (Table 8).

Consequently, the Commission of the European Communities limits the amount of free gossypol in feed materials (with the exception of cottonseed, cottonseed meal and cottonseed cakes) at 20 mg/kg which amount has no adverse effects on growth and nutrient digestibility. The limit for the cottonseed and cottonseed meal are 5.000 mg/kg and 1.200 mg/kg (Anon 2009).

Table 8: **Tolerance and toxicity of free gossypol from cottonseed meal to fish and shrimp** (use of cottonseed meal in the aquatic animal diet)(Li and Robinson 2006).



Species	Tolerance level (mg/kg)	Toxic level (mg/kg)	Gossypol source	Toxic effect	Reference
Channel catfish *Ictalurus punctatus*	900	1,137	CSM	Reduced growth, increased tissue gossypol	Dorsa et al. (1982)
	336	671	CSM	Reduced growth	Barros et al. (2000, 2002)
Blue tilapia *Oreochromis aureus*	1,800		GAA	No adverse effects were demonstrated at 1,800 mg free gossypol/kg of feed	Robinson et al. (1984)
Nile tilapia *O. niloticus*	1,600		GAA	No adverse effects were demonstrated at 1,600 mg free gossypol/kg of feed	Lim et al. (2003)
Tilapia *Oreochromis* spp.	520	700	CSM	Reduced growth and feed efficiency	Mbahinzireki et al. (2001)
Rainbow trout *Oncorhynchus mykiss*	250	1,000	GAA	Reduced growth, increased liver gossypol level	Roehm et al. (1967)
	95	290	CSM	Reduced growth, lower hematocrits, hemoglobin and plasma protein, ceroid	Herman (1970)
	65	95	CSM	Thickening of the glomeralar basement membrane of the kidney, necrosis in the liver	Herman (1970)
	232	362	CSM	Reduced growth and hemoglobin	Dabrowski et al. (2001)
Pacific white shrimp *Penaeus vannamei*	1,100	1,600	CSM	Reduced feed intake, reduced growth, and high mortality	Lim (1996)

Toxic systemic effects include reduced haematocrit, haemoglobin, reproduction capacity as well as results lesions in some organs like in the liver, kidney, spleen and gonads but the mechanism behind the nutrient digestibility are unknown (Anon 2009). In rainbow trout, it has been shown a dietary level of 0.01 % free gossypol can cause liver damage. Similarly, Barros (Barros et al. 2000) observed that 55 % CSM in the diet of catfish can cause pancreas and liver necrosis. Moreover, inclusion at 0.03 % gossypol suppressed the growth in the rainbow trout (Gatlin et al. 2007). Similar growth depression was documented in channel catfish fed a diet including more than 900 mg free gossypol/kg (Dorsa et al. 1982). According to Makinde, gossypol can cause haemolytic anaemia at high concentration besides having detrimental effects on growth and feed efficiency parameters (Makinde et al. 1997).

On the other hand, multiple studies have reported that inclusion of gossypol in the diet has no significant effect on liver glycogen deposition (Herman 1970, Barros et al. 2000, Evans et al. 2010) and some other fish species, like Nile tilapia can tolerate the high gossypol level in the diet without any adverse effect (Lovell 1998).

There are some methods, with which the gossypol can be partially or totally inactivated: by heat treatment (Herkelman et al. 1991, Bollini und Campion 1999, ElMaki et al. 2007), by soaking (Rani und Hira 1993, Duhan et al. 2002, ElMaki et al. 2007) by fermentation (Marfo et al. 1990, Antai und Nkwelang 1998) or supplementation of diets with enzymes hydrolysing this specific anti-nutrients (Southern et al. 1990, Sandberg und Svanberg 1991, Cheng und Hardy 2002, Portz und Liebert 2004, Sajjadi und Carter 2004) amino acids (Li und Robinson 1998, Fagbenro 1999) or minerals (Jones 1987, Martin 1990, El-Saidy und Gaber 2004). During these processes free gossypol binds to cottonseed protein, resulting in bound gossypol, which is no more toxic. Unluckily, this binding process reduces the protein quality because the free gossypol bond to amino acids, especially to lysine (Kuiken und Lyman 1948, Baliga und Lyman 1957, Conkerton et al. 1957).

Some experiments show promising results about the supplementation of iron as a ferrous sulphat to the cottonseed meal diet. The free gossypol bond to the iron to make a strong complex and as a result the gossypol become non-toxic. Iron has been used to bind with the toxic free gossypol and thereby reduce the toxicity at single-stomach animals (Jones 1987, Martin 1990, Sealey et al. 1997, Barros et al. 2002). El-Saidy and Gaber have documented improved growth, feed utilization and blood parameters for fish fed with CSM diet including 1:1 iron in free gossypol in contrast CSM diet without iron supplementation (El-Saidy und Gaber 2004). However, the ferrous sulfate to free gossypol at 1:1 weight ratio does not appear to be effective in reducing the toxicity to channel catfish (Barros et al. 2000, 2002). Another approaches, to minimize the gossypol level, is the addition of lysine and iron to the solvent extracted cottonseed meal is a good option. According to several studies the supplementation of lysine and iron to the CSM diet reduces the gossypol toxicities in fish (Robinson 1991, Robinson und Li 1994, Dabrowski et al. 2000, Rinchard et al. 2000, Lee et al. 2006).

Notably acetone can also decrease the gossypol and aflatoxin levels in the cottonseed meal and improved crude protein and amino acid digestibility (Cheng und Hardy 2002).

Although, CSM is digested relatively well by fish and crustaceans, it is less digestible than SBM and is severely deficient in several amino acids, including lysine, isoleucine, methionine and cysteine. This deficiency is these essential amino acids may be compensated by the use of a combination of animal and plant protein sources or by adding of synthetic amino acids (Robinson und Li 1994).

2.5.2 CSM effects on the growth

Despite the presence of these antinutritional factors and its lower nutritional values compared to that of SBM (Barros et al. 2002), it has some other advantages, such as being associated with lower FCR. This performance might be due to its higher fibre quality.

Gui`s studies on crucian carp (*Cearassius auratus gibelio*) reported that using cottonseed meal hydrolysate (CMH) caused positive effect on growth and feed utilization (Gui et al. 2010).

According to Mbahinzireki, CSM can partially replace FM in the feed of tilapia up to 50 % of the diet (Mbahinzireki et al. 2001). However, these can also be associated with reduced farming performances and El-Sayed reported that juvenile Nile tilapia fed a diet including 65 % CSM displayed a 24 % reduction in weight gain and a 35 % reduction in feed efficiency compared with the control diet (fish meal based) (El-Sayed 1990). Similarly, Ofojekwu and Ejike documented that Nile tilapia fed with cottonseed cake diet results much lower weight gain and feed efficiency compared with the fishmeal based diet (Ofojekwu und Ejike 1984).

Lee et al (Lee et al. 2002) suggested that CSM can be used as a substitute for fish meal, by the incorporation of at least 15 % in diet. The high dietary supplementation of CSM up to 58,8/100 g or the complete substitution for FM in the diet has not adverse effects on growth of rainbow trout, brood stock and catfish (Lee et al. 2006). Luo has found that the fish fed with solvent extracted cottonseed meal at 100 % level shows significant lower weight gain, specific growth rate, feed conversion efficiency and protein efficiency ratio than fish fed with other diets (Luo et al. 2006).

According to Blom et al., CSM can be a suitable alternative for partial replacement for FM and SBM, with supplementary lysine content (Blom et al. 2001).
Moreover, (Lim und Lee 2009) concluded that the mixture of cottonseed with iron and phosphorus supplementation can be replaced up to 40 % fishmeal protein in diets for olive flounder (*Paralichthys olivaceus)*. They suggested that 30 % of fishmeal protein replacement by CSM with some iron and phosphorus supplementation can be used for commercial. Furthermore, the supplementation of iron and phosphorus can also increase the inclusion of the CSM for fishmeal replacement for marine fish species.

Generally, CSM represents an economical alternative protein sources in aquatic animal feeds because it is relatively highly palatable to most aquatic animals. Although its use in the aquaculture largely depend on the animal`s tolerance to gossypol, lysine and methionine requirements.
There is evidence that CSM can be used in high levels in aquatic animal feeds, but its use has limited to about 10 % to 15 % in the aquatic diet. Hence, it is recommended that low level of CSM can be included in the aqua feeds. Supplementation of lysine and required iron level would increase the maximum inclusion level of CSM. Moreover, it is advised to use solvent extracted CSM than pure CSM because solvent extraction decreases gossypol level while increasing the protein level.
However, more research is required in this aspect to know more about the effects of CSM in the fish diet.

2.6 Algae in the aquaculture diet

Algae represent a very diverse group of promising feedstuff. The amino acid profile of several algae match that of fishmeal and contain all essential amino acids, although the protein content shows significant differences among the species (8-50 %). Furthermore, almost all algae are rich in polyunsaturated fatty acid (n-3 LC-PUFA) and act as a good feed supplement for neutralising intestinal inflammation and also aid in the preparation of the feed pellet. Multiple studies have focused on the utilisation of algae as a potential ingredient for aqua feed development. The dose and the aquatic species influences the effect of algal inclusion in feed.

According to several studies, high inclusion level of algae in aqua feed has an adverse effect on fish growth and feed efficiency. This effect may be caused by the presence of anti-nutritional compounds, such as lectins, tannins, phytic acid, protease and amylase inhibitors in algae. In contrast, used at low inclusion level, algae are very useful ingredients for the aqua feed (Kaladharan und Jaseera 2019).

2.6.1 Spirulina in the aquatic diet

Spirulina (*Arthospira*) algae is a fast-growing, photosynthetic, filamentous blue-green microalga (Cyanobacteria). There are two common spirulina species which are used for diets due to the nutritional value; *Arthrospira maxima* and *Arthrospira plantesis*. These grow naturally in the Central Africa region around Lake Chad, Niger, East Africa near the Great Rift Valley and Mexico, where is used as a human food source (Habib et al. 2008) and contained in the diets of many fish, like Tilapia (Khallaf und Alne-na-ei 1987).
Normally, spirulina is produced with continuous-harvesting technology that support the exponential growth. The culturing costs depend on the technology and materials were used.

Spirulina represent a possible alternative protein source for cultured fish among the algae ingredients. Spirulina is a balanced ingredient for using in fish feeds (Table 9). Its nutritional profiles are well-documented. It has high protein content, between 41-63 % by dry weight. Cyanobacteria of the genus *Spirulina* contain all 10 amino acids considered essential for fish in suitable level, with the exception of methionine, cysteine and lysine that are only present at sub-optimal levels. These well-balanced amino acid content can make Spirulina as a potential replacer of fish meal in aqua feed (Hanel et al. 2007).

In addition, spirulina contains vitamins, essential fatty acids (MM et al. 2006), antioxidant pigments, such as carotenoids (Richmond 1986, Volkmann et al. 2008) and has antimicrobial activity (Richmond 1986, El-Sheekh et al. 2014) and anticancer properties (Dasgupta et al. 2001).

Table 9: **Nutritional content of spirulina compared to fish meal** (Mosha 2019)**.**

	Spirulina	**Brown fish meal**
Proximate composition (g100g^{-1})		
Dry matter	82,2	92,28
Crude protein	61,3	67,44
Ash	6,90	12,76
Lipid	5,50	10,52
Crude fiber	3	1,46
Essential amino acids (g100g^{-1})		
Arginine	4,10	3,67
Lysine	3,10	1,55
Isoleucine	2,6	3,02
Leucine	4,7	4,84
Histidine	1	5,05
Methionine	1,37	1,87
Phenylalanine	2,5	2,69
Threonine	2,7	2,71
Tryptophan	1,2	0,72
Valine	0,3	3,41

Compared to fishmeal, spirulina has high amounts of polyunsaturated fatty acids (PUFA), furthermore, the mineral and vitamin content are also notable (Allen 2016).

Due to the lack of cellulose in the cell wall, the digestibility is high (Olvera-Novoa et al. 1998). It was reported that some fish species such as Nile tilapia showed increased digestibility and nutrients uptake through the gut fed with spirulina in their diets (Moriarty 1973).

Other positive effects have also been documented, such as increased feed utilization, physiological activity, stress response and disease resistance (Mustafa und Nakagawa 1995). Spirulina as a FM replacer has additional benefits like improving feed intake, growth and survival rate, boosting the immune system, improving fillet colour, firmness and reduction of the feeding costs (Watanuki et al. 2006, Ibrahem et al. 2013, Yeganeh et al. 2015, Allen 2016)**.** Spirulina has also been correlated to an increased production of extra cellular enzymes like protease and lipase in red swordtail (James et al. 2006), attributed to the activation of protein synthesis and somatic growth which reduces the fish mortality.

Spirulina improves the fish reproduction as well (Lu und Takeuchi 2004). It was documented increased egg quality and quantity at the Nile Tilapia fed with Spirulina algae as a main feed.

Similar results have been conducted at goldfish (*Carassius auratus*) (James et al. 2006) swordtail (*Xiphophorous hellerii*)(James et al. 2006) and bassa (*Pangasius bocourti*) (Meng-Umphan 2009).

Furthermore, spirulina can be produced by low-cost technologies and can be marketed as dry powders and several studies have been conducted in both finfish and shellfish using dried spirulina powders as a supplement in the diets with successful results (Braune 1990, Zeinhom 2004).

In finfish culture, Ayyappan et al clearly demonstrated better growth rate by feeding spirulina meals for carps (Ayyappan et al. 1991). According to Nadeesha and Guroy the body weight gains of fish increased with the level of less than 25 % algae in the fish diet (Nandeesha et al. 2001, Güroy et al. 2012). Rainbow trout showed the highest weight gain when FM was replaced with 7.5 % Spirulina algae (Teimouri et al. 2013).

Similarly, the usefulness of the spirulina for replacing up to 40 % of FM protein has been reported in Nile tilapia diets (Olvera-Novoa et al. 1998). Tilapia have been feed with a diet which contains 43 % spirulina, shows better FCR than CGM control feed, in addition without adversely affect the growth and feed intake. Similarly, silver seabream (*Pagrus auratus)* fed with 50 % of spirulina in the diet, did not suffer negative growth effects compared to the control diet (FM)(El-Sheekh et al. 2014).

In addition, good results have been reported in shellfish culture, such as white shrimp (*Litopenaeusschmitti*) (Jaime-Ceballos et al. 2005), in pacific white shrimp (*Litopenaeus vannamei* (Hanel et al. 2007) and green tiger prawn (*Penaeus semisulcatus*) (Ghaeni et al. 2011). The giant fresh water prawn (*Macro brachium rosenbergii*) feed a diet supplemented with spirulina at 5-20 % of the diet significantly improved growth, survival and feed utilization. Fish meal partial replacement with spirulina has also been evaluated in juvenile Pacific white shrimp (*Litopenaeus vannamei*) with positive result (Hanel et al. 2007).

Ornamental fish such as Guppy fish fed diets with algae cells have also shown positive growth performance (Dernekbasi et al. 2010).

However, other studies have found that high spirulina inclusion results in depressed growth rate. Sharma de Panta and El-Sayed documented that Spirulina addition above 30 % in the diet decrease the fish growth (El-Sayed 1994, Sharma und Panta 2012). According to Olvera-Novoa, there is a significant difference in the growth rates if the diets containing 10 % and 20 % spirulina protein or 40 %. As a result, 40 % spirulina in the diet causes halved growth rates than the diets with 10 % and 20 % algae protein (Olvera-Novoa et al. 1998).

The reasons for the variable results to be the difference of spirulina concentration, source, raw or dried, fish species and other conditions.

As a conclusion, due to the nutritional composition and the relative digestibility of spirulina algae, it should be a viable alternative protein source in the aqua feed. Furthermore, for the effective fish growth performance, quality of fillets, immune system modulation and low feed costs, the additional level of spirulina should be less than 20 % in the fish diet. More research is needed for the high inclusion level of spirulina up to 50 % to be evaluating its effectiveness.

2.6.2 Thraustochytrids

Thraustochytrids, are non-photosynthetic microalgae and represents one of the richest source of PUFA on the market. They are osmo-heterotrophic monocentric fungi with more than 30 species. They are classified under kingdom Heterokonta and class Labyrinthulomycetes.

Approximately, Thraustochytrids produce total fat of about 10-50 % of biomass and 30-70 % of docosahexaenoic acid (DHA) under non optimised condition. This omega-3-fatty acid is required for optimal growth and fish development, furthermore it is relevant for the development of neural tissues such as brain and retina.

The production capability for biomass, total lipids and DHA content fluctuates wide largely, influenced by medium composition, incubation temperature, pH, culture age and seawater concentration.

According to several studies on fish, Thraustochytrid oil can be used as a sufficient alternative to fish oil.

The addition of dried marine microalgae (*Schizochytrium*) in the diet of channel catfish (*Ictalurus punctatus*), at low concentration about at 1.0-1.5 %, results in an improved weight gain with feed efficiency ratio (Kaladharan und Jaseera 2019).

Another study showed that spray-dried biomass of *Schizochytrium* sp. included at 5 % in the diet of Atlantic salmon successfully replaced fish oil without any adverse effect on fish growth rate and FCR, dietary protein, energy digestibility and flesh quality. Similar fish oil replacement study was made with Nile tilapia and reflected significant improved weight gain, feed conversion ratio, protein efficiency ratio and higher content of DHA in the fillet lipids.

Similarly, it has also been reported that feeding of Pacific white shrimp (*L. vannamei*) larvae with *Schizochytrium* meal (4 %) can improve the growth performance.

Consequently, several countries around the world are producing omega-3 rich oils from Thraustochytrids as a possible improved and alternative sources of PUFA.

It has been concluded that heterotrophic microalgae like *Schizochytrium* sp. are promising aqua cultural feedstuff aquaculture species due to their nutritional quality and ability.

The mass cultivation technology of *Schizochytrium* is fully developed and can be produced commercially instead of fish oil in the near future.

2.7 Distiller`s dried grains with soluble (DDGS) in the aquatic diet

DDGS is the major by-product of ethanol production and used as a high-protein animal feed. It is a dried by-product leftover from the fermentation of corn by enzymes and yeast producing ethanol and carbon dioxide. It is characterised by a high energy content, medium protein quantity, digestible fibres and accessible phosphorus which makes it a sustainable fish feed with a high nutritional value. Furthermore, its lack known anti-nutritional factors (Erickson et al. 2012). The apparent digestibility coefficient of DDGS of protein was 94.42 % and of dry matter 76.23 % (Sandor et al. 2016).

The availability of DDGS as a feed ingredient is increasing with the increase of biofuel industry (Liu 2011, Brown et al. 2016). DDGS, have several benefits like suitable protein content (26-33 %), fat content (9-14 %) and phosphorus content (7-10 %) for the aquatic animal feeds and increased global production. They include high quantity of non-starch polysaccharides but it does not contain anti-nutritional factors like trypsin inhibitor, glucosinolates and erucic acid ,that are usually present in most of the plant ingredients and has low level of phytate (Overland et al. 2013, Brown et al. 2016).

The usefulness of the DDGS for aqua feed has been tested only in few species such as rainbow trout (*Oncorhynchus mykiss*) (Cheng und Hardy 2004, Overland et al. 2013, Welker et al. 2014) channel catfish (*Ictalurus punctatus*) (Lim et al. 2009, Li et al. 2011b), and Nile tilapia (*Oreochromis niloticus*) (Schaeffer et al. 2009, 2012, Li et al. 2011a, Khalil et al. 2013). The digestibility of DDGS has been determined on C. *Carpio* and was 94.42 % for the apparent digestibility coefficient of protein and 76.23 % for dry matter (Sandor et al. 2016).

2.7.1 Experimental diet

The nutritional profile of the corn-based DDGS diet was the follows: crude protein 27 %, crude fat 9 %, crude fiber 10 %, crude ash 4-6 %, starch 3 % and P content 7-10 g/kg. The essential amino acid content, such as lysine, methionine, tryptophan and threonine were at low concentrations according to the nutritional demand of the fish (Jobling 2011). Synthetic methionine and lysine was added to the diet to balance the essential amino acid ratio.

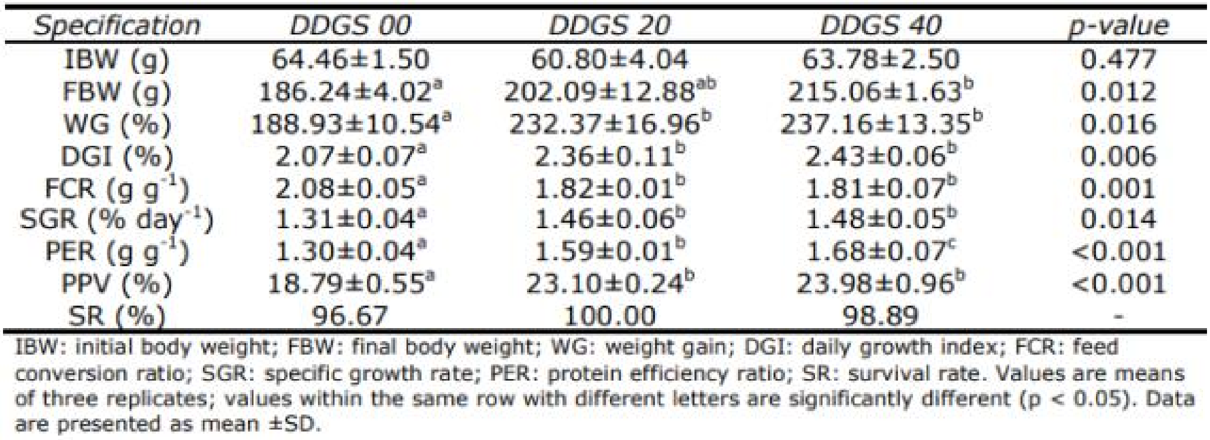
Three isonitrogenous (about 35 %) and isolipidic (about 6 %) diets were prepared in which DDGS was set in three different levels: 0 % as DDGS 00, 20 % as DDGS 20 and 40 % as DDGS 40, and all diets were based on terrestrial plants and industrial by-products, without fish meal.

Hemp seed oil was used as a lipid source due to its optimal fatty acid ratio and beneficial effects on fish health (Da Porto et al. 2012).

At the end of the feeding period (12 weeks), all fish were starved for 24 hours and the body weight was measured (Lines und Spence 2012) Five fish were randomly selected and over-anesthetized (Matuk 1987). Blood samples were taken to measure biometrical indices like condition factor, hepatosomatic index, viscerosomatic index and taken sample from the liver, muscle, head kidney and mid-gut.

After the 12-week trial, statistical differences were observed in terms of feed conversion parameters and growth. Common carp reflected significant benefits for groups fed with DDGS inclusion experimental feeds compared to the control in diet WG, daily growth index (DGI), and SGR (Table 10). There were not detected significant differences between DDGS 20 and DDGS 40, although DDGS 40 showed slightly higher values most of the parameters (Révész et al. 2019).

Table 10: **Measured parameters of Cyprinus carpio juveniles** (Révész et al. 2019)**.**



Specification	*DDGS 00*	*DDGS 20*	*DDGS 40*	*p-value*
IBW (g)	64.46±1.50	60.80±4.04	63.78±2.50	0.477
FBW (g)	186.24±4.02^{a}	202.09±12.88^{ab}	215.06±1.63^{b}	0.012
WG (%)	188.93±10.54^{a}	232.37±16.96^{b}	237.16±13.35^{b}	0.016
DGI (%)	2.07±0.07^{a}	2.36±0.11^{b}	2.43±0.06^{b}	0.006
FCR (g g^{-1})	2.08±0.05^{a}	1.82±0.01^{b}	1.81±0.07^{b}	0.001
SGR (% day^{-1})	1.31±0.04^{a}	1.46±0.06^{b}	1.48±0.05^{b}	0.014
PER (g g^{-1})	1.30±0.04^{a}	1.59±0.01^{b}	1.68±0.07^{c}	<0.001
PPV (%)	18.79±0.55^{a}	23.10±0.24^{b}	23.98±0.96^{b}	<0.001
SR (%)	96.67	100.00	98.89	-

IBW: initial body weight; FBW: final body weight; WG: weight gain; DGI: daily growth index; FCR: feed conversion ratio; SGR: specific growth rate; PER: protein efficiency ratio; SR: survival rate. Values are means of three replicates; values within the same row with different letters are significantly different ($p < 0.05$). Data are presented as mean ±SD.

Crude protein content was higher (< 0.05) in the DDGS fed groups compared to the control group. At higher DDGS level the crude fat content increased while the crude ash level decreased. The experimental diet did not show adverse effect on gut health, only some generic differences were found in the histological session.

The present study documents that the replacement of SBM and corn grain with DDGS had beneficial effects on the growth performance of *C. carpio*. DDGS diet is an easily digestible and usable feedstuff for Common carp. The protein efficiency ratio and protein productivity values are relatively good for *C. carpio* using DDGS up to 40 % in the diet, comparable with the results with other plant sources (Hasan et al. 1997).

Moreover, there were no histological differences, like enteritis features, epithelium thickness in the intestines between the experimental groups in contrast with the group fed SBM (Urán et al. 2008).

The other beneficial effects of DDGS is the absence of ANFs. The plasma biochemical parameters are important factors for reflecting injury, but in this study these parameters showed no significant differences between the experimental groups.

The activity of ALT is the main indicator of liver damage. In the current study, the plasma ALT levels were relatively low, indicating that DDGS diet does not have adverse effect on liver in *C. carpio*.

DDGS is a suitable feed ingredient for omnivorous fish, because of its appropriate protein content (Brown et al. 2016). The diet of Channel catfish can include DDGS up to 40 %, or 70 % with supplemented lysine without affecting growth performance and feed utilization (Webster et al. 1992) (Révész et al. 2020).

According to the current study, the DDGS at high inclusion level (up to 40 %) represents an appropriate dietary protein and fat source for *C. carpio* without negative consequences on the growth and the health.

The decrement of liver fat deposition is remarkable after a three-months feeding period using the diet with 40 % of DDGS at the common carp.

Another study reported the digestibility of corn DDGS in common carp juveniles at two temperatures, 20 Celsius degrees (°C) and 30 °C (Révész et al. 2020).

Fish were fed under different temperature regimes with or without DDGS to assess the interaction between the water temperature and nutrient digestibility of corn DDGS.

The biggest drawback of using DDGS in fish feed is the variability of the nutrient concentration among the DDGS sources (Liu 2011). DDGS in corn has some xanthophylls content and this can lead to yellow pigmentation of fish fillets (Lim et al. 2011). The replacement of fishmeal by corn DDGS can cause depressed growth performance of Nile tilapia (Coyle et al. 2004, Salama et al. 2011).

According to Watanable et al. (Watanabe et al. 1996) the optimum range of water temperature for the growth and nutrient utilization in Common carp is between 20-25 °C. Moreover, these authors reported a direct effect on dry matter and protein digestibility by increasing the water temperature (20-30 °C). In contrast to the digestibility of phosphorus, where the water temperature does not influence the digestibility of phosphorus.

As a result, the present study reflected that 30 % DDGS in the diet of common carp did not affect growth performance and feed efficiency. Several studies reported promising results about the use of moderate (15-30 %) inclusion level of DDGS in channel catfish, tilapia and rainbow trout (Webster et al. 1991, 1993, Cheng und Hardy 2004, Coyle et al. 2004).

In conclusion, this study suggested that corn DDGS constitutes a suitable ingredient for use in common carp diet at least up to 30 %. Furthermore, corn DDGS includes digestible phosphorus source, which can reduce the need for supplemental inorganic phosphorus.
For example, corn DDGS will reduce the diet costs and also the reduce of quantities of phosphorus by excreted from animals.

Overall, corn DDGS promises to have a high potential substitution in the diets for Common carp and further study need to be known the maximum inclusion level.
To summarize, DDGS are high-quality and cheap and they can enhance the development of aquaculture and improve the profitability.

2.8 Mixing of plant and animal protein sources for replace fish meal in the aquaculture diet

Alone, most single plant protein or animal by-products might be lacking some important nutrient to be completely substituted fish meal in the aqua feed.
Animal by-products are less expensive and have similar amino acid profile compared to fish meal. On the other hand, they are deficient in several essential amino acids which reduces the protein digestibility and may impair growth rate at high inclusion level in the diet (Damusaru et al. 2019). Moreover, plant-based protein sources may contain anti-nutritional factors and have sometimes unbalanced amino acid profiles and low protein content (Carter und Hauler 2000). These disadvantages can affect the growth performance and nutrient utilization in fish (Wilson und Poe 1985, Hernández-Infante et al. 1998).

Combining different plant protein sources and animal by-products in aquaculture diet is more effective and efficient to enrich the optimal nutritional profile of fish because it allows for a more completed and balanced than any other single source of protein (Lee und Bai 1997, Bae et al. 2012b, S. und K. 2015, Wang et al. 2017). It has been documented that the combination of animal and plant protein sources alongside supplementation with amino acids may prevent nutritional deficiencies that could affect fish performance and ensure proper supply of essential nutrients (Lunger et al. 2007, S. und K. 2015, Herath et al. 2016). Previous studies have also reported that the concentrated mixture of animal and plant protein sources including SBM, PBM, BM, LM, and FEM have been used as a fish meal replacers in the diet of several freshwater fish species (Engin und Carter 2005, Kasper et al. 2007, J.G. et al. 2016). According to Hernandez and Roman (Hernández und Roman 2016) and Jo et al 2016 (Jo et al. 2017) substituting fish meal with a mixture of SBM, PBM, BM, and FEM can balance the amino acid requirements in the diet, resulting in improved feed sustainability and palatability as well as reduced production cost and environmental effects (Damusaru et al. 2019).

3 Animal-based protein as an alternative for substitution fish meal in the aquaculture diet

3.1 Animal By-products in the aqua feed

Currently, around 20 % of the global fish meal production is supplied through the use of fishery by-products and 10 % through the use of aquaculture by-products, in particular reuse of part of the fish unfit for human consumption (Ytrestøyl et al. 2015, Jackson und Newton 2016, FAO 2018). A major advantage is that the FM produced through this method will be almost identical to the one obtained from more traditional sources of (Hua et al. 2019). However, the exact composition can vary based on the type of raw material and the manufacturing processes, as well as on the nutrient composition of the fish species and which portion of the fish is used (fillets compared to the head, viscera, skin, blood or bones).

Several studies have reported on the successful fish meal from fishery and aquaculture by-products in aqua feeds (Ytrestøyl et al. 2015, Jackson und Newton 2016, FAO 2018, Froehlich et al. 2018). For example, tuna by-products at the inclusion rate of 15.8-21.4 % can replace up to 25-30 % of the protein in the diet of spotted rose snapper (*Lutjanus guttatus*) without affecting growth performance (Hernández et al. 2014). Similarly, the substitution rate of tuna by-product meal in the diet of Korean rockfish (*Sebastes schlegeli*) can be replaced about 75 % of fish meal if the dietary inclusion rate of 58.1 %.

However, the recycling of fish in this fashion could present some risk of transmission of pathogens if the fish had been infected and outbreak have been tracked to raw fish used as food for other fish (Mitchum und Sherman 1981, Inglis et al. 1993). Moreover, because the growth of aquaculture outpaces that of fisheries, this protein source will soon reach its limits and is unlikely to keep up the rapid growth of the aquaculture industry.

Another aquatic source protein is krill. Krill meal has several benefits in fish farming due to its high protein content. It is used as a feed additive in aquaculture diets and as a growth enhancer in fish and shrimp. Krill meal contains approximately 60 % protein with well-balanced amino acids, 25 % lipids, omega-3-fatty acids and astaxanthin (Ashish et al. 2020) and its inclusion improves he yield alongside fillet quality and the fish health.

Animal by-products such as poultry or fish offal, meat and bone meal (MBM) or blood meal (BM) have also been successfully used in aqua feeds. By-products are viable alternative protein sources to conventional fish meal because they are more economical and sustainable protein origin (Kim et al. 2018).

PBM has a potential to substitute fish meal because it has been shown to be well digested by several fish species (Bureau et al. 1999, Rawles et al. 2006, Yang et al. 2006). Its relatively high protein content and lower price compared to fish meal, makes PBM a promising alternative protein source in the feed of carnivorous fish species like rainbow trout (Shapawi et al. 2007). The effect of replacing fish meal with PBM has been investigated for several fish species, including Chinook salmon (*Oncorhynchus tshawytscha*) (Fowler 1991), Nile tilapia and rainbow trout. However, according to Fagbenro and Bello-Olusoji poultry waste can replace only up to approximately 40 % of the dietary fishmeal without adversely effects on growth performance and feed utilization (Fagbenro und Bello-Olusoji 1997). In rainbow trout, inclusion of PBM at levels above 50 % has been linked to increased body lipid content of fish (Steffens 1994, Bureau et al. 1999, EL-Haroun et al. 2009).

Another animal by-product of interest is MBM. It contains high protein levels with a well-balanced profile of amino acids and well digestible minerals like phosphorus and calcium. It has the advantage of-not containing anti-nutritional factors (Suloma et al. 2013). Several studies have investigated the inclusion of MBM at multiple replacement levels in different fish species. Moderate fishmeal substitution levels for olive flounder or rainbow trout range from 20 % to 45 %, in contrast with more omnivorous species such as the African catfish or Nile tilapia for which the replacement levels can reach up to 75 % and 100 % with no ill-effects (El-Sayed 1998, Goda et al. 2007). The major disadvantages of MBM are considered to be the high ash content because of the presence of bone and other organic matter (Bureau et al. 1999).

BM from slaughterhouse waste is also a potential protein sources for replace FM in the aquaculture diet (Tacon et al. 2011). It includes high quality proteins, namely lysine and histidine, it has high digestibility (80-99 %). Further, special forms of iron, like haemiron may support the oxidation of feed components (Bureau et al. 1999, Millamena 2002, El-Haroun und

Bureau 2007). According to Agbebi et al FM can be totally substituted with BM with no adverse effect on growth and feed conversion ratio at juvenile catfish (*Clarias gariepinus*) (Agbebi O T 2009, Ashish et al. 2020).

3.1.1 Experimental diet

A 12 weeks' diet experiment was conducted on African catfish (*Clarias gariepinus*) to evaluate the effects of partial replacement of FM with two kinds of fermented animal by-product: fish offal´s silage (FOS) and poultry offal`s silage (POS), fermented with *Lactobacillus acidophilus* which facilitates nutrient utilization by breaking down proteins as amino acids and peptides (Table 11 and 12). This leads to a better performance, incidence of cost and profit index (PI) (El-Sayed et al. 2020) on the growth performance, microbiological and organoleptic fish quality. In addition, the profit index was calculated by comparing the value of the fish and the cost of production, based on the difference in cost between these ingredients. Five diets were formulated by partial substitution dietary FM at 20 % and 40 % with fermented FOS as well 20 % and 40 % POS and the control diet with 100 % FM.

Offal have a high protein and lipid content (Table 11), low carbohydrate content and lack of ANFs but on the other hand they have some drawbacks including the presence of indigestible particles, microbial contaminations and high moisture (Cruz-Suárez et al., 2007; Samaddar and Kaviraj, 2014).

Table 11: **Composition of fermented fish offal`s silage and poultry offal`s silage** (El-Sayed et al. 2020)**.**

Nutrient %	**fish offal`s silage**	**poultry offal´s silage**
Moisture	76.4+/-0.8	66.3+/-0.4
Crude Protein	35.2+/-0.7	41.7+/-0.7
Ether Extract	7.9+/-1.3	24.8+/-1.1
Ash	8.6+/-0.2	6.3+/-0.3

Table 12: **Dietary compositions of experimental diets** (El-Sayed et al. 2020).

Ingredients %	**Diet 1 (control)**	**Diet 2 (20 % FOS)**	**Diet 3 (40% FOS)**	**Diet 4 (20% POS)**	**Diet 5 (40% POS)**
Poultry meal (66 %)	19,40	19,7	20,3	19,5	20
Fish meal (61.6 %)	10	8	6	8	6
Poultry offal`s silage (41.7 %)	0	0	0	2	4
Fish offal`s silage (35.2 %)	0	2	4	0	0
Ground Y. Corn (8.7 %)	21,1	19,8	18,8	19,7	19
Soybean meal (41.6 %)	26,5	27,6	28,1	27,7	28
Wheat middling (17.1 %)	15	14,6	14,5	15	15
Oil mix	6	6,1	6,1	6,1	6
Premix (Vit. &Min.)	0,3	0,3	0,3	0,3	0,3
Common salt	0,3	0,3	0,3	0,3	0,3
Mono calcium phosphate	1,4	1,6	1,6	1,4	1,4
Crude protein (%)	34,3+/-0,7	34,3+/-0,4	34,3+/- 0,4	34,3+/- 0,4	34,3+/-1,6
Digestible energy	2950 Kcal DE/kg	2950 Kcal DE/ kg	2950 Kcal DE/ kg	2950 Kcal DE/kg	2950 Kcal DE/kg

Table 13: **Growth performance parameters of the experimental diets for African catfish** (El-Sayed et al. 2020)**.**

Parameter	**Diet 1 (control**	**Diet 2 (20 % FOS)**	**Diet 3 (40 % FOS)**	**Diet 4 (20 % POS)**	**Diet 5 (40 % POS)**
Initial Wt. (g)	299,7+/-1,3	304,7+/-4,2	303+/-1,1	301+/-1,3	299,7+/-1,6
Final Wt. (g)	440,3+/-4,6	389,7+/-1,6	374,7+/-1,3	385,3+/-3,6	410,7+/-3,1
Weight Gain (g)	140,6+/-3,3	85+/-4	64,6+/-1,3	84,3+/-7,2	111+/-2,1
Feed intake (g)	245,8+/-2,7	167,2+/-7	136,3+/-1,3	149,2+/-6,5	246,9+/-8
Feed Conversation Ratio	1,75+/-0,1	2+/-0,1	2,1+/-0,1	1,77+/-0,1	2,2+/-0,1
Protein efficiency ratio	1,67+/-0,01	1,48	1,38	1,58+/-0,03	1,24+/-0,06

At the end of the feeding trial the growth performance of fish was compared between the diet groups and the results showed significant differences in the final average body weight of fish (FW) and WG between the groups (Table 13). The highest FW after the control diet was recorded the fifth group with 40 % POS while both 20 % FOS and 20 % POS groups showed same data. The lowest value was found in the group supplemented with 40 % FOS. Diet with 40 % POS recorded the highest WG, but less than the control diet.

After calculation of the PI, the third group (40 % FOS) showed the highest PI while the control group the lowest PI, suggesting that this replacement was an economically viable strategy in commercial farms. However, significant differences in taste and texture were recorded between the groups. In this context the diet with 40 % POS and 40 % FOS showed the lowest value in opposite with the control diet and the group with 20 % POS. The smell of the different groups also presented perceptible differences, suggesting an effect on the organoleptic qualities of the final product.

Microbiological examination resulted in significant differences in the number of total bacteria among the groups. The diet with 40 % FOS supplemented the highest value, followed by the group with 40 % POS and the control group. The lowest value was found in the group with 20 % POS and 20 % FOS.

This data showed that POS and FOS have high protein level and are competitive with other conventional dietary protein sources. The group with 20 % POS recorded the highest FCR, PER, best IC and PI comparing the other groups in the feeding trial, except the control group. As a conclusion it appears that POS might be a profitable and sustainable protein sources in aquaculture diets. It can effectively substitute fish meal in African catfish diets till the substitution level of 20 % without negatively effecting the growth and microbial qualities.

However, this substitution also resulted in alterations in the organoleptic qualities of the finished product which could plausibly hinder the sale and the consumption of the fish. More research will be necessary to investigate this critically important aspect.

3.2 Insect protein in aqua culture

As a result of the short life cycle of the insects and ability of growing on a wide range organic waste with high productivity and relatively good nutritional profiles, insect meal as a suitable aqua feed ingredient is increasing significance in many countries (IPIFF 2018, Berggren et al. 2019). In addition, the amino acid contents of insect proteins make them a suitable candidate for the replacement of fishmeal (Animal Breeding und HAS 2018). Moreover, protein production from insects and insect larvae meets the needs of sustainable development because large amount of protein with excellent biological value can be produced in a small area.

According to the latest EU regulation (Regulation (EU) No 2017/893), only certain feed of animal origin may be used to feed insects and insect larvae. Notably, this regulation prohibits the use of organic manure as feed of animal origin. Nonetheless, in some African countries, the `recycling` of poultry manure is for feed purposes for rearing insect larvae. However, it should be borne in mind that insect larvae, when reared on poultry manure, may carry certain pathogenic bacteria, such as *Salmonella* sp. Although *Salmonella* sp. can be killed by heat treatment before use for animal feed, there is a risk of rancidity due to the generally high fat content of insect larvae (Animal Breeding und HAS 2018).

The global market of edible insects is growing rapidly, expected to expand from 1 billion dollar by 2019 to reach 8 billion dollar by 2030 (E.M. 2019, Ltd. 2019). The use of insects and their larvae as a feed component is currently only allowed by the European Union for companion animals and fish, but the obtained protein from them, in the form of protein hydrolysate is allowed for all farm animals.

Based on a detailed toxicological study of insects that they can be used as feed, the EFSA in 2015 classified the following species as safe for direct use and protein extraction: black fly (*Hermetie illucens*), house fly *(Musca domestica*), common flea beetle (*Tenebrio molitor*), beetle (*Alphitobius diaperinus),* house cricket (*Acheta domesticus*), striped cricket (*Gryllodes sigillatus*), ground cricket (*Gryllus assimilis*). These seven species are non-pathogenic, non-vectors of pathogens, and non- invasive (Hua et al. 2019).

In countries outside the European Union and United States, many other insect species and their larvae are also used to feed farm animals. For example, the silkworm (*Bombynx mori*) or locusts, of which the red losuct (*Nomadacris septemfaciata*) is mainly used for animal feed in Africa and Asia (Animal Breeding und HAS 2018).

The crude protein content of these insects ranges from 40 % to 63 % although it can be up to 83 % in defatted insect meal (Makkar et al. 2014). The amino acid profile varies between species and the crude lipid content of insects and ranges from 8.5 % to 36 % (Barroso et al. 2014, Henry et al. 2015). Comparing insect meal with FM shows that insect meal contains negligible amounts of eicosapentaenoic (EDA) and docosahexaenoic acid (DHA) and diverse level of omega-3 and omega-6 fatty acid level (Hua et al. 2019).

Depending on the husbandry technology and the organic waste used for feeding, the house fly larvae have a protein content of 42-62 % with an excellent amino acid composition and an extremely good apparent digestibility of amino acids up to 91 % (Pretorius 2011).

Meanwhile, the crude protein content of silkworms is 47-63 %, depending on the technology, feeding and processing, which can be increased up to 80 % by degreasing (Khan et al. 2016).

It has also been reported that the non-defatted silkworm pupae using at 30 % in the diet of common carp could replace fish meal without any adverse effect on the texture, flavour or colour of the fish (Nandeesha et al. 1990).

Furthermore, the material used to feed insects or insect larvae has a significant effect on the nutrient content, including the crude protein of the produced insect meal (Oonincx et al. 2015). Lipid quality, EDA and DHA content can be manipulated by feeding insects (Sealey et al. 2011). Vitamin and mineral level also depend on the substrate of feeding materials (Henry et al. 2015). The carbohydrates level of the insects is low. The nutritive value of insect meal can be achieved with the combination of nutrient sources to improve the fatty acid contents, palatability and even the digestibility (Henry et al. 2015).

During the use of insects and insect larvae, a number of concerns has been raised regarding the potential of these organism to act as vectors for pathogenic microorganism. Indeed, insects or insect larvae may naturally be infected with insect-specific microorganism that are harmful to animals, including humans, however due to their high tissue specificity, it is generally considered that they do not pose a real risk to either farm animals or humans consuming the animal product (Eilenberg et al. 2015).

Insect meal can be used successful for 100 % replacement of FM (even for carnivorous fish) according to the recent studies. For example, using black soldier fly larvae in the dietary inclusion rate of 14.75 % replaced 100 % of the FM in the diet of Atlantic salmon (Belghit et al. 2019).

Another successful dietary replacement was reporting at the rainbow trout by using yellow mealworm at level from 5-25 % improved the growth and achieved the 100 % replacement of FM (Rema et al. 2019).

Some studies reported that insect meal can affect the sensory profile of the fish fillets of the Atlantic salmon (Belghit et al. 2019) and rainbow trout (Borgogno et al. 2017).

Another conflicting result exists regarding the price of the insect meal. Insect meal is not a price-competitive material for aqua feed (IPIFF 2018). An experiment with the European sea bass *(Dicentrarchus labrax*) demonstrated that the using of yellow mealworm into the aqua feed resulted in increased feed prices (Arru et al. 2019). Moreover, the global production of insect meal is increasing and the price of it expected to be competitive with the FM by 2023 (W. und B. 2016).

Based on these results, it appears that if supply of insect meal at a competitive price can be secured, the use of insect meal in aqua feed is a promising alternative for FM.

3.3 Earthworm in the fish diet

Earthworms are a group of terrestrial invertebrate within the order Opisthopora. Earthworms have a high protein content (chemical analysis showed the chemical composition of their body tissues was composed of 60-70 % protein, 7-10 % fat, 8-20 % carbohydrate and 2-3 % minerals) and they represent a good source of proteins (Tacon et al. 1983, Sogbesan und Ugwumba 2008, Guerrero R.D 2009). Earthworms can provide not only essential amino acids but also – fats, vitamins and mineral content and for this reason, have been used in the diet of pigs, rabbit and fish species (Stafford und Tacon 1985, J.R. 1986, Mason, W,T., Rothamann, R.W and Deguinme 1992). Due to their high reproductive rate and biomass production of earthworm species, they are highly practical for worm meal production and are recommended for processing dried and pulverized form for feed preparation. The practices are currently on the rise and, in Japan, there are currently over 3.000 vermicomposting plants aimed at producing earthworm for fish feed, in particular for the farming of eel (*Anguilla japonica*). However, there are many earthworm species, often endemic to specific regions, and multiple species are known not to be suitable for aquaculture production because they contain antinutritional factors. In contrast, *Eisenia fetida* is considered among the most suitable. Moreover, unprocessed earthworm meals only have a limited shelf-life; processing can also increase the digestibility of the chitinous elements (Musyoka et al. 2019). Generally, more research is needed to improve the use of earthworm meals while overcoming limitations to its application, including scaling-up production to a more intensive scale.

3.3.1 Experimental diet

Trial was performed in African catfish *(Clarias Gariepinus*) fingerlings to compare diets that incorporate a mixture of earthworm and fish meal at different ratio as protein source (Omeru, E. D. AND Solomon 2016).

The experimental diet included artificial fish meal as sole protein source (control diet – group A), 70 % earthworm meal and 30 % FM (group B) or 50 % earthworm meal and 50 % FM (group C). In addition, other ingredients such us groundnut cake (20 g), cornflower (20 g), rice barn (20 g), eggs (70 g) and brewer`s yeast (30 g) were added to complement the proteins.

The fingerlings were fed with 4 % of their body weight twice a day for twelve weeks long and the best growth performance and nutrient utilization was reported from the fish group fed 56 % level of whole earthworm (Fig. 7 and 8).

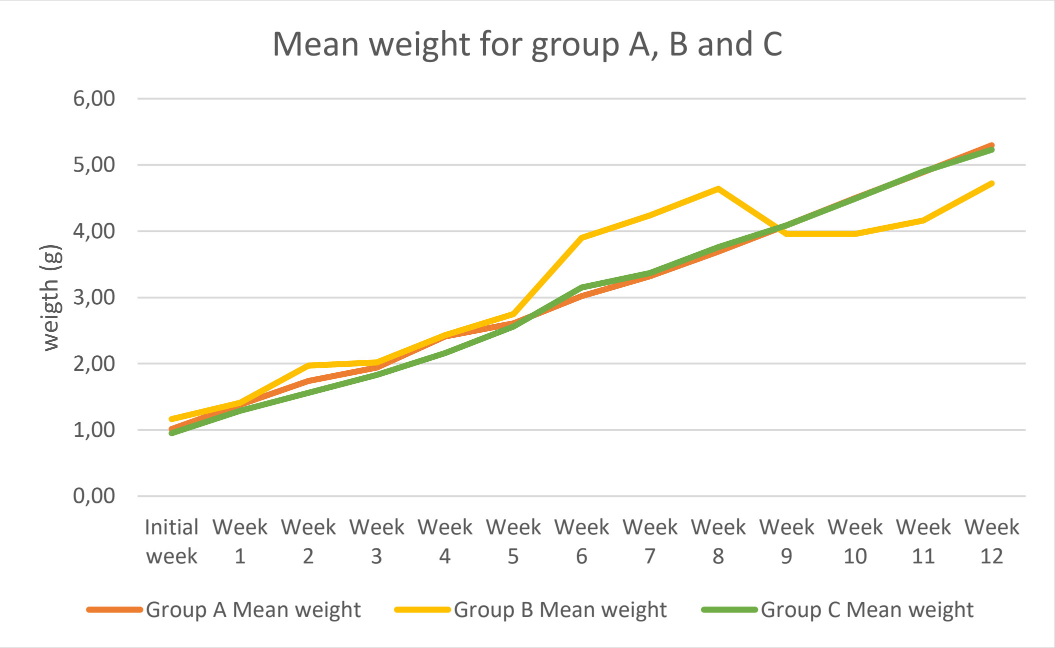


Figure 7: **Mean weight for the three experimental diet** (Omeru, E. D. AND Solomon 2016)**.**

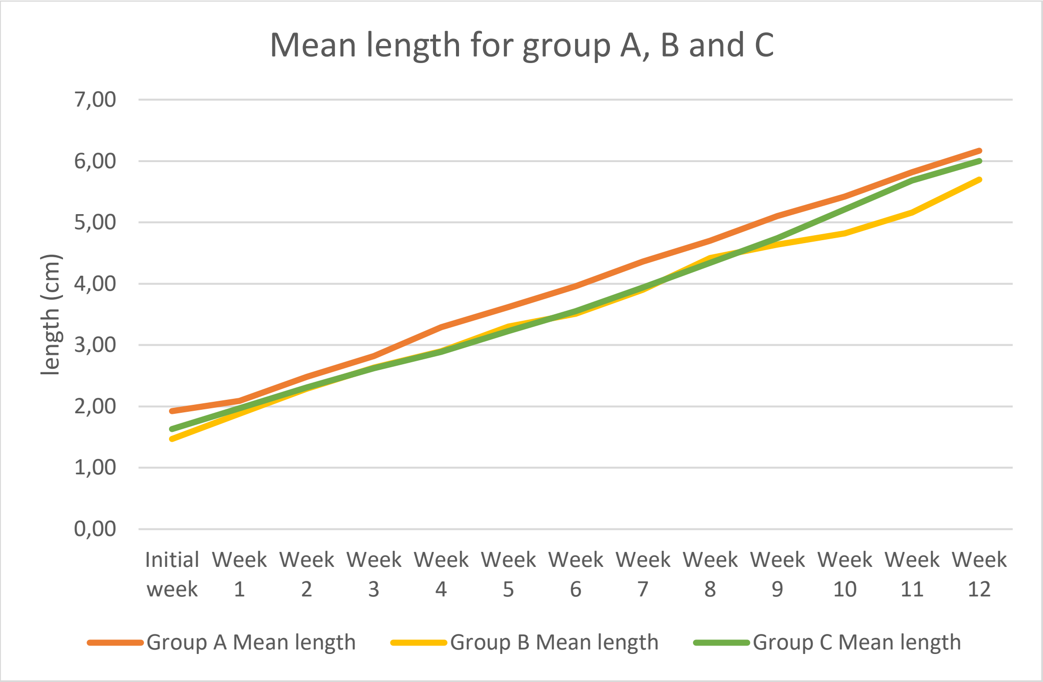


Figure 8: **Mean length for the three experimental diet** (Omeru, E. D. AND Solomon 2016)**.**

According to the result of the present study the inclusion of whole earthworm meal in the diet of African sharp tooth catfish is beneficial; not only is it cheaper and more sustainable, it is linked to an increased growth and survival of the fish.

Discussion

Currently, a major source of proteins in the diet of aquaculture remains fishmeal acquired largely from the wild. However, there is a strong need to replace this expensive and limited resource with more stable and more sustainable sources. Consequently, many alternatives have been suggested. However, each one of these alternatives is different regarding the balance of the various nutrients it contains and some of them have been associated with anti-nutritional factors. Moreover, a notable group of nutrients are polyunsaturated fatty acids (PUFAs). These PUFA are famously a major health benefit associated with the consumption of fish and aquatic products but they are also not synthetized de novo in significant numbers by aquatic animals and, for them too, must largely be acquired through the diet. However, many possible sources of alternative proteins and nutrients only have low levels of PUFAs.

Among the most commonly suggested alternatives, soybean meals are a popular source of sustainable proteins of vegetal origin and are considered sustainable. They have a balanced protein profile and have already been widely adopted in both humans and animal diets. Their use has raised some concerns, however, because soybean is known to contain some anti-nutritional factors, notably phytoestrogens that have been suspected to have deleterious effects on the sexual development and reproduction of fish and other aquaculture animals. However, such effects have not been confirmed through experimentation. Moreover, SBM can also contain lectins and proteinase inhibitors, although these can be removed from the diet during processing. Finally, one major issue with soybean is that it requires the use of farming and the considerable increase in the use of soybean in the last several decades (15 times increase since the 1950ies) has required vast amount of new agricultural lands and has led to deforestation worldwide. Therefore, this resource might not necessarily be as sustainable as one would think.

Canola/ rapeseed meal has a high protein content with good amino acid profile and it makes rapeseed meal a popular commercial protein sources for the animal feeding. On the other hand, canola meal brings its own set of limitations such as the presence of glucosinolates, high fibre content of low digestibility and anti-nutritional ingredients, which limit its maximum acceptable amount in aquatic diets. Furthermore, other components like tannin and sinapine can reduce the appetence of feed and impact the feed intake. With a special aqueous extraction process, it is possible to remove drawback factors of canola meal and it can result in higher level of replacement fish meal.

Cottonseed meal can be produced from cottonseed by special extractions. It has a high protein content but because of the presence of the anti-nutritional factors and their relatively expensive price compared to other protein sources, its usage is limited in the aquaculture diets. The high gossypol level of cottonseed is toxic for animals and affects negatively the feed consumption, growth and the reproduction capacity. Supplementation of lysine and iron could increase the inclusion level of cottonseed in the aquatic diet.

Algae, such as Spirulina in the aquaculture diet represent a promising feedstuff, containing all essential amino acids, essential fatty acids and vitamins. Or particular interest is that algae are a major source of PUFAs in the food chain and their inclusion would likely strengthen the health benefits associated with the consumption of fish. High supplementation level of algae in the aqua feed may have adverse effect on fish growth and fish efficiency because of the presence of anti-nutritional compounds. Using Spirulina at low level to replacing fish meal have several beneficial effects like positive effects on the growth performance, as well as increased fillets quality and boosting the immune system.

Distiller`s dried grains is characterised as a major by-product of ethanol production with high energy content, medium protein quantity and digestible fibres. It is a cheap sustainable fish feed with high nutritional value and lack of anti-nutritional factors.

Among the animal based protein sources, animal by-products, insect protein and earthworm represent interesting substitutes of fish meal. By-products such as fish offal´s silage or poultry offal´s silage are economical potential protein sources for the aquaculture diet, but they have been associated with degraded organoleptic qualities. On the other hand, because of the short reproduction circle, high productivity and good nutritional profiles, insect meal is a suitable candidate for the replacement of fish meal. However, insect meal may affect the sensory profile of the fish fillets and because of its expensive price, it is not considered a price-competitive protein sources.

Earthworms have high protein content, include fats, vitamins and mineral content and due to its high reproductive rate, earthworm meal is a good protein source for substituting fish meal.

However, not all species are equally well suited and, indeed, several species of earthworms have been shown to harbour anti-nutritional compounds (Musyoka et al. 2019). This suggests that some non-endemic species might have to be introduced for farming, which is problematic. Moreover, scaling-up of earthworm production to industrial scale, as would be required to contribute to aquaculture production to a significant extent, is still technically difficult.

As seen above each of the various alternatives have its own characteristics, both advantages that warrant them being considered as a replacement source and limitations that hinder their applications. Another aspect is that the price or availability of the various foodstuff is likely to fluctuates over time and to vary from regions to regions. By-products of agriculture products, for example, being more easily available in regions that already harbour a large land-based agriculture industry. Moreover, none of the alternatives, with the exception of by-products from fisheries and aquaculture, which are intrinsically limited in term of available volume, perfectly matches the composition of the fishmeal they aimed to replace. In addition, even when the nutrients are present in the diet, their digestibility and bioavailability might be more limited than that of fishmeal: Aquatic animals and their digestive systems have evolved to take maximum advantage of their natural diets and, notably, their digestive enzymes are unable to extract nutrients as efficiently from other sources. This later aspect means that the precise balance of the nutrients will have to be further adapted to the species of the animals being farmed, in order to match the specific requirements of the farmed species not just in term of composition but more in term of bioavailable nutrients.

For this reason, it is likely that fishmeal will not be replaced by a single alternative but rather by multiple sources each with its own composition, in order to match the requirement of the farmed species more accurately. In particular, it is likely that most of these nutrients will need to be complemented with some source rich in PUFAs as these are required dietary complements that are only present at significant levels in a limited number of foodstuffs. Combining different food sources would also be a way to keep any anti-nutritional factor in any individual ingredient at a low level. In addition, these foodstuffs might likely be subjected to pre-processing before hands to make them more suitable for the animals being farmed.

In this context, it might be tempting to resort to genetically modified organisms (GMOs) to ensure that these alternative food source do contain some elusive nutrients. However, this approach would likely prove unpopular due to the public suspicions regarding such GMOs. Similarly, some foodstuff such as blood meal and poultry offal`s silage (POS) might also seem distasteful to the consumers. Moreover, and perhaps more importantly, some foodstuff might result in change in the taste, smell or texture of the final products and result in making them less appealing to the consumers. Only limited research has been performed on this subject but some replacement meals like fish offal´s silage and POS have been shown in the past to be associated with degraded organoleptic qualities, which would represent a severe limitation to their adoption.

Beside these technical aspects, because of the importance of food cost in aquaculture settings, the availability of these ingredients on a large scale must also be stable and their price should not be too high nor should it fluctuates too widely. Moreover, because their price must remain low, their production should not be in competition with other land use.

In this context, more research is going to be required to optimise the composition of the diets and match them more precisely to the various farmed species and even to the precise life stage of the species being farmed, as both the requirements and digestive systems of animals vary over the course of their lives. This task is made more daunting considering that new foodstuffs are continuously being offered and that, on the other side of the coin, the aquaculture industry is continuously being wideners with new species being domesticated that had not previously been farmed. In addition, more research might help discover or develop new sustainable sources for these foodstuff.

Summary

The continuous increase in human population has made continued access to sustainable sources of food, in particular nutrient-rich and high-quality proteins, an area of concern. At the same time, aquaculture has been the fastest growing food sector worldwide and the annual growth rate of global fish consumption increased twice as the population growth (FAO 2018, Hua et al. 2019).

Traditionally for most farmed fish, fish meal derived from small pelagic fish has been the main dietary protein source. Notably, proteins are an important part of the fish diet, especially considering that the fish digestive system is ill-equipped to digest carbohydrates and that fish rely on gluconeogenesis more than carbohydrates as source of energy. Unfortunately, the availability of the fish meal and fish oil from forage fish has been continuously decreasing over the last twenty years and many resources have been overexploited and are unable to sustain the rapid expansion of the aquaculture (Hua et al. 2019). The total production of aqua feed for the aquaculture species is expected to increase by 75 % from 49.7 million tonnes in 2015 to 87.1 million tonnes in 2025 (Tacon und Methian 2015). Therefore, it is estimated to answer to this new demand, an additional 37.4 million tonnes of aqua feeds will be required by 2025. Because of the increased price and restricted availability of fish meal alternative protein sources are desperately needed.

So far, plant-based protein ingredients have been the most commonly used sources of replacement for this fish meal in studies. Plant protein sources are easily available and they have a lower market price compared to fish meal. However, the presence of anti-nutritional factors limits their application in aqua feed. Many ingredients such as soybean meal are already commonly substituted for fish meal and some other plants like rapeseed meal, cottonseed, algae and distiller`s dried grains all have advantages as protein sources and might be applied in the future.

In addition, that animal-based protein sources such as earthworm, insects or animal by-products are not only cheap sources comparatively but are nutritionally enriched.

Therefore, developing and optimizing alternative sources of protein play an important role for the future in the aquaculture industry.

Zusammenfassung

Der kontinuierliche Anstieg der menschlichen Bevölkerung hat den kontinuierlichen Zugang zu nachhaltigen Nahrungsquellen, insbesondere zu nährstoffreichen und hochwertigen Proteinen zu einem Anliegen gemacht. Gleichzeitig ist die Aquakultur der weltweit am schnellsten wachsende Lebensmittelsektor und die jährliche Wachstumsrate des globalen Fischkonsums stieg doppelt so schnell an, wie das Bevölkerungswachstum (FAO 2018, Hua et al. 2019).

Traditionell ist für die meisten Zuchtfische Fischmehl aus kleinen pelagischen Fischen die Hauptproteinquelle in der Ernährung gewesen. Vor allem Proteine sind ein wichtiger Bestandteil der Fischnahrung, insbesondere, wenn man bedenkt, dass das Verdauungssystem von Fischen nicht in der Lage ist Kohlenhydrate zu verdauen. Zudem sind Fische mehr auf Glukoneogenese als auf Kohlenhydrate als Energiequelle angewiesen. Leider hat die Verfügbarkeit von Fischmehl und Fischöl aus Futterfischen in den letzten zwanzig Jahren kontinuierlich abgenommen und viele Ressourcen wurden übernutzt und sind nicht in der Lage, die schnelle Expansion der Aquakultur zu unterstützen (Hua et al. 2019). Es wird erwartet, dass die Gesamtproduktion von Aquafutter für die Aquakulturarten um 75 % von 49,7 Millionen Tonnen im Jahr 2015 auf 87,1 Millionen Tonnen im Jahr 2025 ansteigen wird (Tacon und Methian 2015). Daher wird geschätzt, dass zur Deckung dieser neuen Nachfrage bis 2025 zusätzlich 37,4 Millionen Tonnen Aquafutter benötigt werden. Aufgrund des gestiegenen Preises und der eingeschränkten Verfügbarkeit von Fischmehl werden dringend alternative Proteinquellen benötigt.

Bisher wurden in Studien vor allem pflanzliche Eiweißbestandteile als Ersatz für dieses Fischmehl verwendet. Pflanzliche Proteinquellen sind leicht verfügbar und haben einen niedrigeren Marktpreis im Vergleich zu Fischmehl. Allerdings schränkt das Vorhandensein von antinutritiven Faktoren ihre Anwendung im Aquafutter ein. Viele Inhaltsstoffe wie Sojabohnenmehl werden bereits häufig als Ersatz für Fischmehl verwendet und einige andere Pflanzen wie Raps Mehl, Baumwollsamen, Algen und getrocknetes Getreide von Brennereien haben alle Vorteile als Proteinquellen und könnten in Zukunft eingesetzt werden.

Hinzu kommt, dass tierische Proteinquellen wie Regenwurm, Insekten oder tierische Nebenprodukte nicht nur vergleichsweise billige Quellen, sondern auch ernährungsphysiologisch angereichert sind.

Daher spielt die Entwicklung und Optimierung alternativer Proteinquellen eine wichtige Rolle für die Zukunft in der Aquakulturindustrie.

Abbreviation

Anti-nutritional factors	ANFs
Bioprocessed protein concentrates	BPCs
Bioprocessed soybean meal	BSM
Blood meal	BM
Canola Protein Concentrate	CPC
Celsius degree	°C
Condition factor	K
Corn gluten meal	CGM
Cottonseed meal hydrolysate	CHM
Cottonseed meal	CSM
Cyclopropenoic fatty acids	CFAs
Daily growth index	DGI
Daily growth rate	DGR
Distilled dried grains with solubles	DDGS
Docosahexaenoic acid	DHA
Docosahexaenoic acid	DHA
Eicosapentaenoic acid	EDA
Essential amino acid	EAA
Fatty acid	FA
Feather meal	FM
Feed conversion ratio	FCR

Final body weight	FW
Fish meal	FM
Fish offal`s silage	FOS
Fish oil	FO
Glucosinolates	GLs
Groundnut meal	GNM
Genetically modified organisms	GMOs
High-protein fish meal	HFM
Leather meal	LM
Lipid productive value	LPV
Low-protein fish meal	LFM
Meat and bone meal	MBM
Million tonnes	MT
Polyunsaturated fatty acid	PUFA
Poultry by-product meal	PBM
Poultry offal`s silage	POS
Profit index	PI
Protein efficiency ratio	PER
Shrimp soluble extract	SSE
Soybean meal	SBM
Specific growth rate	SGR
Triacylglycerol concentration	TAG

United States dollar	US$
Weight gain	WG

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Content of figures

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