

Today's and tomorrow's feed ingredients in Norwegian aquaculture

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<i>Summary:</i> <p>During the last decade, the production of Atlantic salmon has increased from around 900,000 tons worldwide in 2000 to more than 1,500,000 tons today, an increase of almost 70%. Norway is the main producer of Atlantic salmon. However, the growth in the salmon industry has raised concerns about the environmental impacts of fish farming. The consumer demands documentation of a safe and environmentally sustainable production of salmon. Feed is a major input factor in salmon production and sustainability of the salmon production is very often a discussion about use of feed ingredients. Traditionally, fish meal and fish oil were the most important ingredients in salmon feeds. Growth in the salmon production is made possible by an extensive use of alternative feed ingredients. The purpose of this report is to evaluate strength, weakness, opportunities and threats of the most important ingredient resources used in salmon feed today and those that may be used in the future. Alternative plant ingredients, microbial ingredients, animal by-products and marine resources are discussed. It is expected that plant ingredients will be even more important in future diets and that use of microbial ingredients will increase. Therefore, it is important with extensive research on production technologies for alternative ingredients, and for technology to upgrade plant and microbial ingredients to increase the use in future diets. There is an urgent need to explore alternative sources of EPA and DHA resources to meet the need for these limited nutrients in salmonid diets.</p>		

Preface

The growth in the salmon industry has raised concerns about the environmental impacts of salmon farming. Increasing consumer awareness on sustainability and food safety puts pressure on the aquaculture industry to document that the production of salmon is safe and environmentally sustainable. Feed is a major input factor in salmon production. Sustainability of the salmon production is a discussion about the use of feed ingredients. A main argument used against sustainability of salmon production is the dependency of fish meal and fish oil and the effect this may have on wild fish stocks. The salmon industry has switched to use of more plant ingredients in the formulations as the relative cost of the limited marine ingredients has increased during the past decade. It is therefore important with knowledge about the use of present ingredients, potential ingredients for the future, and availability of the ingredients on the world market. Knowledge about the use of ingredients in the Norwegian salmon production can also be used to estimate potential environmental impacts related to the nutrient flow. The purpose of this report is therefore to give an analysis of strengths, weaknesses, opportunities and threats (SWOT) for the major feed ingredients used in the Norwegian salmon industry today and for ingredients that potentially may be used in the future.

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1 Introduction

The total production of farmed salmonids in Norway in 2010 was approximately 944,000 tons Atlantic salmon and 55,700 tons rainbow trout (Fiskeridirektoratet, 2011), a production that have more than doubled since the year 2000. In total 1,365,225 tons of feed were used in 2010, however, this figure also includes feed for other species such as Atlantic cod (21,240 tons in 2010). The steadily increasing global production of farmed fish and shellfish has resulted in higher pressure on the limited and highly valuable ingredient resources such as fish meal and fish oil. Consequently, the ingredient composition in Norwegian salmon aquaculture industry has changed over the past decade towards use of plant based ingredients (Table 1).

Table 1 Use of plant ingredients vs. fish ingredients over the past 20 years in Norwegian aquaculture (% used of total feed sold from three feed companies).*

	1990	2000	2010
Fish meal	63.8	37.5	25.6
Plant protein (various sources)	0	15.4	36.9
Starch (mainly wheat)	10.3	10.9	9.4
Fish oil	23.4	30.7	17.0
Plant oil	0	0.0	12.0

*Microingredients such as vitamins, minerals and amino acids are excluded

Increasing competition and peaking ingredient prices of major resources used in salmon farming is calling for a review of resources being used today and potential resources that will be used in near future. A vast amount of experiments are carried out over the last two decades in evaluating potential alternatives to fish meal and fish oils in diets for salmonids. The main focus has been on ingredients derived from either plant origin (Thomassen and Røsjø, 1989; Rosenlund, *et al.*, 2001; Gatlin, *et al.*, 2007; Hemre, *et al.*, 2009; Gunstone, 2011), terrestrial animal origin (Bureau, *et al.*, 1999; Turchini, *et al.*, 2009), Krill, Amphipods and Copepods (Olsen, *et al.*, 2010) or single cell organisms from bacterial meal (Øverland, *et al.*, 2010), yeast and algae (Skrede, *et al.*, 2011). The current plant protein ingredients being used by the Norwegian aquaculture industry include soybean meal, sunflower meal, pea protein concentrate, beans, wheat gluten and corn gluten. In near future the alternatives also include canola, lupins and distillers dried grains with solubles (DDGS). The plant oil used at present is mainly rapeseed oil (low erucic acid). However, depending on the cost, small amounts of palm oil and soybean-oil may be used. The search for new very long chain (VLC) n-3 fatty acid containing oils as alternatives to fish oil is urgent, and many are suggested, but no immediate solution is seen.

2 World production of marine and plant resources used in production of salmonids

2.1 Fish meal and fish oil

The aquaculture industry in Norway has traditionally been dependent on feed ingredients from marine sources; fish meal and fish oil. Between 1999 and 2008, the amount of the marine catches that was processed into fish meal and fish oil decreased from 27 to 22%. Of the world's total fishery production in 2008, including freshwater and aquaculture, 81% was used for human consumption, 14% for fish meal and fish oil production and 5% was used for other purposes (FAO, 2010). The global capture fisheries production has been relatively stable at around 80 million tons in the last decade. In 2010 the Norwegian salmon feed industry consumed 257,167 tons of fish meal and 165,277 tons of fish oil from reduction fisheries, and in addition 68,292 tons of fish meal and 53,396 tons of oil produced from trimmings and silage was used. This adds up to 544,132 tons of marine ingredients, which is 41.4% of the total amount of ingredients used in salmon feeds. A considerable part of both fish oil (52%) and fish meal (47%) used in 2010 was of North Atlantic origin. The fish species used in production of meals and oils used by the Norwegian aquaculture feed producers in 2010 are listed in Table 2.

Total annual worldwide production of fish meal has been relatively stable during the last 20-30 years, varying between 4.57 million tons in 1977 to 7.48 million tons in 1994, with a mean of 6.07 million tons (Tacon, *et al.*, 2006). In 2009 the fish meal industry estimated the production to be 4.83 million tons, according to IFFO (2011). The global fish oil production has fluctuated significantly, with a peak at around 1.6 million tons at the end of the 1990'ties. Since 2005, the production of fish oil has been steadily declining, and is currently less than a million tons (Silva, *et al.*, 2010).

Table 2 Marine species used for production of fish meal and fish oil used in Norwegian salmon feed production in 2010, tons of fish meal and fish oil.

Marine species used in Norwegian salmon feed in 2010			
	Fish meal	Fish oil	sum
	(tons)	(tons)	(tons)
Anchoveta	81,832	24,655	106,487
Blue whiting	22,007	2,223	24,230
Sprat (brisling)	21,492	45,731	67,223
Norway pout	14,753	4,508	19,261
Atlantic herring - Norwegian spring-spawning	10,828	8,581	19,408
Atlantic herring - North Sea	11,243	12,699	23,942
Atlantic herring - Icelandic summer-spawning	7,166	7,479	14,645
Capelin	20,777	2,466	23,243
Sandeel	41,882	24,913	66,795
Atlantic mackerel	3,420	4,129	7,549
Chilean jack mackerel	4,805	0	4,805
Boar fish	11,886	0	11,886
Gulf menhaden	0	20,922	20,922
Other/unknown species	5,077	6,970	12,047
Sum from reduction fisheries	257,167	165,277	422,445
Meal and oil from trimmings/silage	68,292	53,396	121,687
Total amount	325,459	218,673	544,132

2.2 Plant ingredients

The use of ingredients in feed for salmonids is determined by the nutritional quality of the ingredient and availability on the world market, which often dictates the price. It is also important that the ingredient is easy to store and handle. Ingredients are roughly categorized into protein, oil and carbohydrate rich ingredients according to the proximate chemical composition.

Oilseeds are primarily grown for the extraction of oil, yielding a press cake high in protein that can be used as protein ingredients. Soybean meal was in 2009 the major oilseed crop (Fig. 1), with a global production of 211 million tons (Soystats, 2010). Oilseeds are mainly grown in USA, Brazil and Argentina (Fig. 2). The world production of grains amounted to 2230.9 mill tons in 2009/2010 (USDA, 2011). The major cereals produced in the world are corn and wheat (Fig. 3). Pulses are defined as the edible seed of legumes, such as pea, bean, lentil and chickpea, and usually exclude those that are used for the extraction of oil (such as soybeans). The world production of these pulses was 40.5 mill tons in 2007 (PulseCanada, 2007). The most important pulses for human consumption and aquaculture feed ingredients are shown in Figure 4. The major producing countries in the world are Canada (36% of production), USA (10%) and China (10%). In Europe, France is the biggest supplier of pulses with 6% of the world production. Lupins are other grain legumes that have gained interest for the aquaculture sector. The global production of lupins amounted to approximately 1 mill tons in 2009 (FAOSTAT, 2009). Australia is the main supplier with approximately 70% of the world production.

The main plant oil production has since 2005 been palm oil, with a current production of more than 40 million tons per year. Of the major oilseeds, soybean oil production amounted to 37.7, rapeseed oil to 19.4 and sunflower oil to 10.1 million tons in 2007-2008 (Gunstone, 2011).

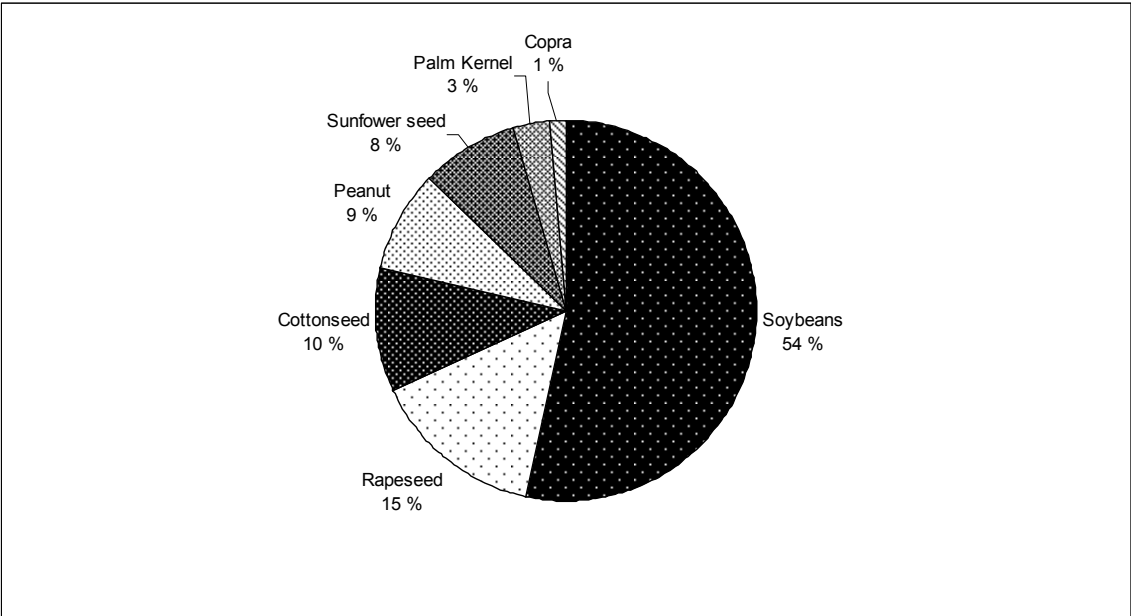


Figure 1 The world oil seed production in 2009 (Soystats, 2010)

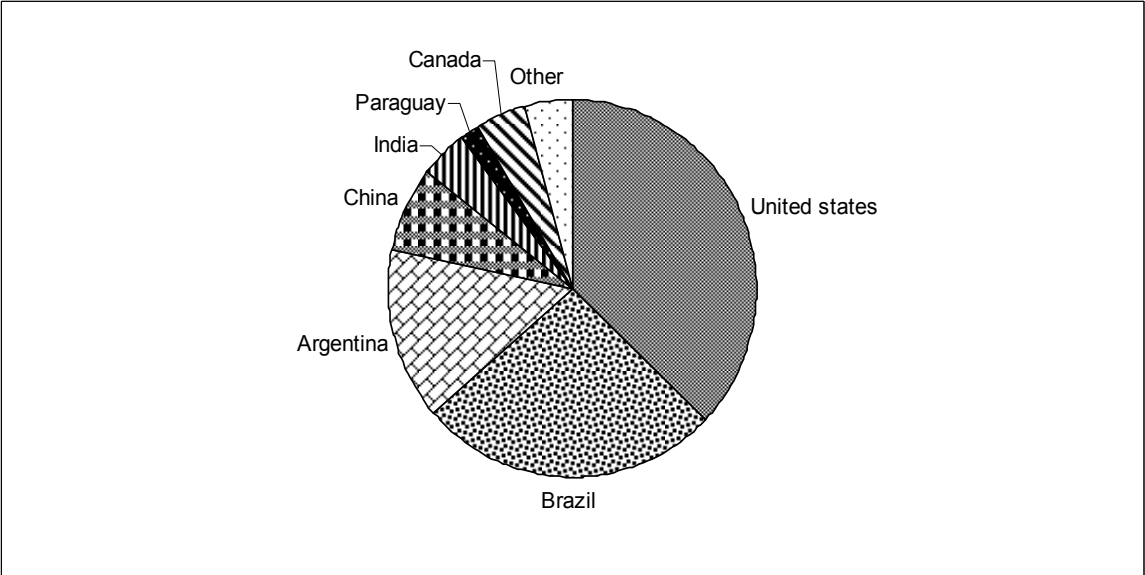


Figure 2 The main world oil seed producing countries

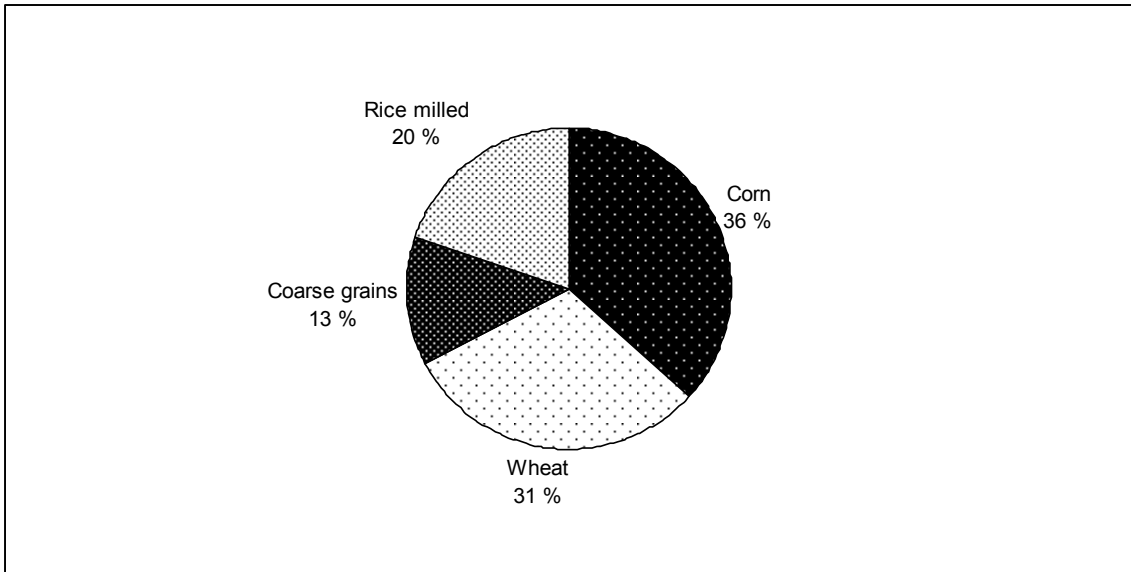


Figure 3 The world production of wheat, corn, rice and coarse grains. Coarse grains include sorghum, barley, oats, rye, millet and mixed grains.

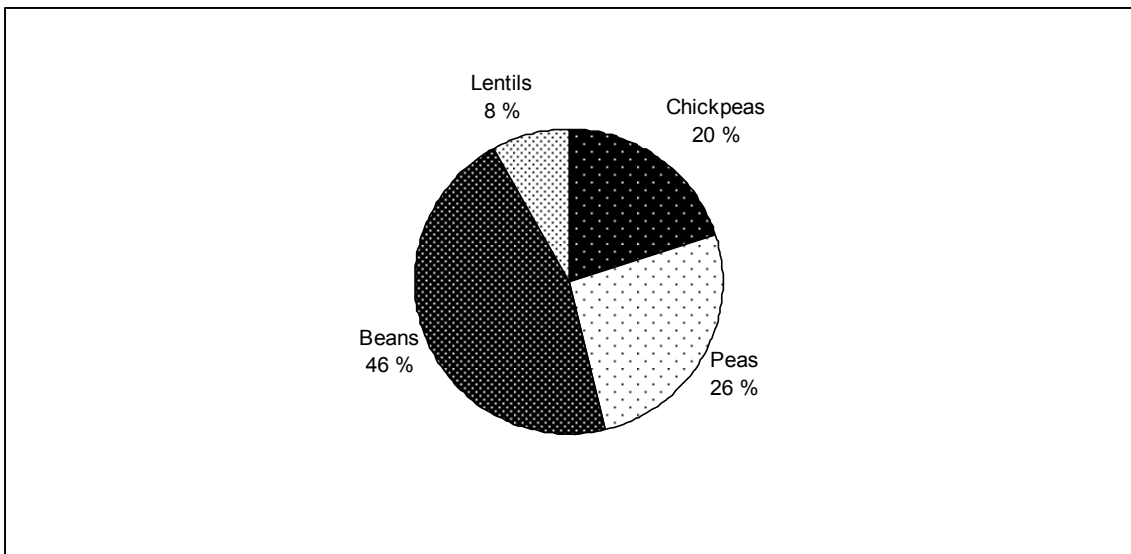


Figure 4 The world production of pulses (PulseCanada, 2007)

3 Marine ingredients

Fish meal is considered the 'gold standard' ingredient and ideal source of protein in feeds for salmonids. Fish meal has a high protein content ranging from 51-72% depending on the source (NRC, 1993), or from 70-74% in fish meals produced from species common in reduction fisheries in the North Atlantic (Fiskeriforskning-SSF, 2004). It has a high nutrient digestibility, an excellent amino acid profile, high palatability stimulating high feed intake and an overall lack of anti-nutrients (Gatlin, *et al.*, 2007). Fish meal is also an important source of elements such as selenium. Fish meal is a rich source of this element, and with the present feed act it is not allowed added to the feed. The main limitation for use of this resource is the limited supply and increasing cost, as well as consumers concerns about the sustainability of the fishery industry. These are the driving factors for the increased use of alternative protein ingredients in diets for salmonids.

Similarly, fish oil is considered as the optimal oil for salmonid species. Fish oil is the key provider of the essential and health beneficial very long chained (VLC) n-3 fatty acids not found in plant oils. As indicated in Table 3, the content of EPA (eicosapentaenoic acid, 20:5 n-3) and DHA (docosahexaenoic acid, 22:6 n-3) varies considerably, not only between species but also within the species. Peruvian anchovy (*Engraulis ringens*) can contain anywhere from about 10 to more than 20% of EPA and 10 – 15% DHA. The fatty acid of this oil is also characterized by high percentages of palmitic acid (C 16:0), C 16:1 n-7 and oleic acid (C 18:1 n-9). Capelin oil (*Mallotus* spp.) generally contains less of EPA (5-10%) and DHA (about 5%). Herring oil (*Clupea harengus*) contains about 5-15% EPA and 2-10% DHA. Notably, both these oils contain high levels of the zooplankton derived 20:1 n-9 and 22:1 n-11 (cetoleic acid). Menhaden oil (*Brevoortia* spp.) contains about 10-15% EPA and 5-15% DHA.

Table 3 Fatty acid composition of fish oils commonly used in salmonid production.

Fatty acids	Anchovy	Herring	Capelin	Menhaden
14:0	6.5-9.0	4.6-8.4	6.2-7.0	7.2-12.1
16:0	17.0-19.4	10.1-18.6	10.0	15.3-25.6
18:0	4.2	1.4	1.2	4.2
16:1	9.0- 3.0	6.2-12.0	10.0-14.3	9.3-15.8
18:1 n-9	10.0-22.0	6.2-12.0	14.0-15.0	8.3-13.8
20:1	0.9-1.0	7.3-25.2	17.0	n.d.-1.0
22:1 n-11	1.0-2.1	6.9-30.6	15.4	n.d.-1.4
18.2 n-6	2.8	0.1-0.6	0.7	0.7-2.8
18.3 n-3	1.8	n.d.-2.0	0.2	0.8-2.3
20:5 n-3	7.6-22.0	3.9-15.2	6.1-8.0	11.1-16.3
22:5 n-3	1.6-2.0	0.8	0.6	2.0
22:6 n-3	9.0-12.7	2.0-7.8	3.7-6.0	4.6-13.8

Source: De Silva et al. (2011)

3.1 Production of fish meal and oil in Norway

The major part of fish meals used in Norwegian aquaculture feeds come from fisheries in Northern Europe. Species used for production of fish meal and fish oil have varied considerably over the years (Fig. 5), due to variation in stocks and regulations of fisheries. During the years from 1965 to 1985 abundant catches were landed for the fish meal and fish oil industry. Herring and mackerel were important species in the start of this period, while capelin was the dominating species from around 1970 to 1984-85. As larger quantities of mackerel and herring were used for human consumption, less of the catches were available for reduction to meal and oil. From 1970 and onward, also species like blue whiting, sand eel and Norway pout were caught. From 1995, the relative importance of the category “Other” has increased. This category includes trimmings from fish for human consumption.

The use of by-products in fish meal production is increasing also world wide. According to IFFO, the amount of ingredients coming from by-products has reached over 25% of global production, and 22% of Norwegian production (Chamberlain, 2011).

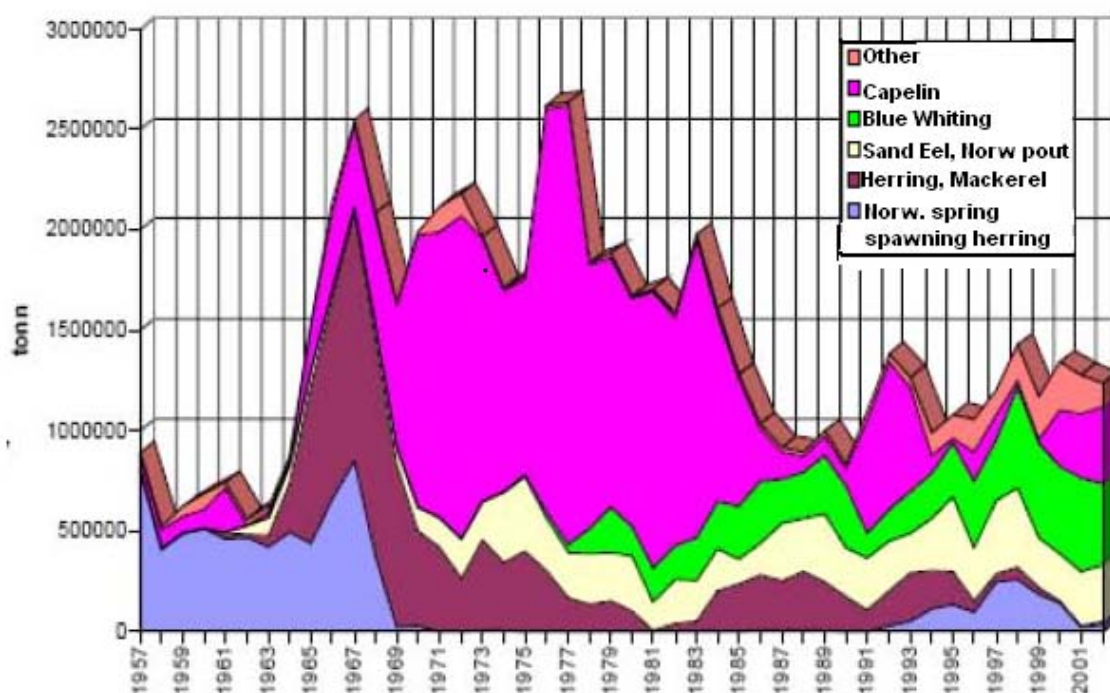


Figure 5 Fish species used for production of fish meal and oil in Norway (1957-2002) (Fiskeriforskning-SSF, 2004).

3.2 Marine by-products

The total global capture fisheries and aquaculture production added up to 142.3 million tons in 2008, and 115.1 million tons of this was used directly for human consumption (FAO, 2010). Of the remaining 27.2 million tons for non-food uses, 20.8 million tons was reduced to fish meal and fish oil. The remaining 6.4 million tons represents a resource that is used for different purposes, also directly as feed in aquaculture, for livestock and fur animals. Trimmings and by-products from fisheries are included in fish meal and fish oil production, and according to IFFO (Chamberlain, 2011) the world average by-product inclusion in the production was 25% in 2008. The by-product coefficient varied from 14% in Chilean fisheries to 100% in Canada; to some extent reflecting the typical fisheries in the different countries. However, not all by-products from fisheries are utilized, thus there is a potential to improve the utilization of by-products from fisheries.

In Norwegian fisheries the total amount of by-products from fisheries and aquaculture was 914,000 tons in 2010, and 716,000 tons, i.e. 78% of total, was utilized for different purposes (RUBIN, 2011). The main categories of by-products in Norwegian fisheries come from 1) Herring and mackerel, 2) Gadoids, and 3) Aquaculture. All by-products from herring and mackerel fisheries and from aquaculture are utilized, while there still is a potential for more use of by-products from Gadoid fisheries. By-catches from fisheries also represent a resource. The largest part of by-products is used for fish meal (260,000 tons) and silage production (278,700 tons). Fish meals and oils from by-products from capture fisheries are

used in aquaculture feeds, but there are some restrictions in use of by-products from aquaculture.

3.3 Efforts to improve sustainability of fisheries

A prerequisite for using fish meal and oil in aquaculture feed should be that the actual species and stocks harvested are managed and utilized in a sustainable way (FAO, 2011b,a; Torstensen, *et al.*, 2011). In order to ensure the sustainability of fisheries, different systems have been introduced at different levels, like The United Nations Convention on the Law of the sea, The FAO Code of Conduct for Responsible Fisheries, The United Nations Fish Stocks Agreement, and also different regional organizations (FAO, 2011b). There is also a number of independent organizations working with fish stock assessments and giving advice, e.g. FAO (UN Food and Agriculture Organization), that publish comprehensive statistics and information in order to provide politicians and other decision makers with facts. Research on fish stock assessments, management of stocks, and advising total allowable catch (TAC) for actual fish species, is carried out by governmental institutes as well as international non-governmental organizations. Examples of such organizations are ICES (The International Council for the Exploration of the sea), IMARPE (Peru – Institute of Fisheries Research) and IFOP (Chile – Institute of Fisheries Research). The maximum sustainable yield (MSY) is the theoretical largest amount of fish that can be harvested from a stock over time without reduction in population size. This is the management tool that EU has committed to reach within 2015 for all commercially harvested fish stocks. ICES is implementing this tool in their advices.

Advices from ICES are the basis for fisheries management in the EU, Iceland and Norway, but national marine research institutes are also advising catch quotas and management of fish stocks for the national fisheries. There are examples where political fisheries authorities allow higher catches than recommended by ICES and other independent institutions, and also examples of the opposite.

Private standards and certification schemes are developed to contribute to sustainability and responsible fisheries management (FAO, 2011b). The international fish meal and fish oil organization (IFFO), who represent the fish meal and fish oil producers, have developed their IFFO-RS standard for Responsible Sourcing of raw materials (IFFO, 2010), and an increasing number of production plants are certified in this system. In Norway there are two approved plants, in Denmark there are three and in Iceland nine, while there are more than 50 in Peru.

Marine Stewardship Council (MSC) is an independent, global, non-profit organization with certification and ecolabelling programs for fisheries and sustainable seafood (<http://www.msc.org/>). The MSC set science based standards, and the certification process is performed by an accredited third party in order to ensure independence. At present, there are 133 certified fisheries in the MSC program, among them a number of mackerel and herring fisheries in the North Atlantic. Moreover, 129 fisheries are under evaluation. A problem might be that different fisheries on the same stocks are certified independently. An example is mackerel fisheries, where several nations have fisheries and national quotas are

set. This year the sum of quotas was 307,000 tons higher than the total recommended quota from ICES, even though many of the mackerel fisheries involved have a MSC certification.

Evaluation of sustainability of fisheries in different countries is a diverse and challenging task. Mondoux, et al., (2008) have designed a system for ranking maritime countries in term of the sustainability of their fisheries, based on a number of parameters. Peru, Norway and Chile, all nations with important reduction fisheries, are ranked among the top 10. One of the parameters used was the relative use of fish meal for aquaculture, assuming that use of fish meal for feed is a threat to marine ecosystems where small pelagic species are key prey species (Alder and Pauly, 2006), and that use of fish meal in feed removes a source of cheap protein from poor people (Pauly and Alder, 2005). The claim that using fish for feed is contributing to poverty and hindering the poor and undernourished population access to valuable proteins has been addressed by the FAO. According to Wijkström (2009) the present practice with use of fish meal in feed does not affect poverty or nutritional status in most of the world. In Asia there may be some effects, partly positive and partly negative.

3.4 Management of fish stocks used in salmon feeds

The fluctuation in fish stocks over time, underscore the importance of proper monitoring and management of important fish species for both human consumption and for fish meal and oil production. Good management also involves collaboration among countries and sound scientific methods to carry out the assessment.

Anchoveta

Anchoveta is the most important species for fish meal and oil production in the world. It is harvested along the coast of Peru, and is considered fully exploited, in periods over-exploited, by the FAO (FAO, 2010). Stock size is affected by different factors, e.g. El Niño, and may show significant fluctuations. It is difficult to find reliable information on stocks and quota assessment.

Blue whiting

Blue whiting is a widely distributed species in the North Atlantic. It is spawning west of the British Isles, and main nursing area is probably the Norwegian Sea. Recent studies indicate that there may be more than one stock. From 1998-99 there was a rapid increase in catches of this species, with a peak in 2003-2004. Catches and Spawning Stock Biomass (SSB) have decreased markedly since, and are now at a critical low level. The spawning stock is expected to be low in the coming years, due to low recruitment the last years. The EU, Norway, Iceland and the Faeroe islands are collaborating about the management of the stock, and have agreed on a long term management strategy, with quotas in agreement with ICES advice.

European sprat

The European sprat is mainly found in the North Sea and Skagerak. Stocks have varied from a peak around 1975 to a minimum in 1986. According to ICES, the state of the stock is

unknown because the available information is inadequate to assess the size and reproduction. However, catches during the later years seems not to have caused problems for the stock (Havforskningsinstituttet, 2011a).

Norway pout

Low recruitment during some years caused a critically low spawning stock in the period 2004-2006. Better recruitment has increased the spawning stock. The pout is short lived, has large variation in recruitment, and is exposed to varying degree of pressure from predators, and therefore long term prognoses are difficult to give. According to ICES, the available information is inadequate to evaluate stock trends relative to risk, and therefore the state of the stock is unknown. The recommendation for 2011 is zero catches.

Atlantic herring

The Norwegian spring spawning herring stock has full reproductive capacity and harvesting is sustainable. The present spawning stock consists of a number of strong year classes, and is expected to decrease during the coming years (Havforskningsinstituttet, 2011c). The North Sea herring has had lower recruitment after the good year class in 2000, but the stock is considered to have full reproductive capacity and to be harvested at a sustainable level. ICES is considering the stock to have low productivity. The Icelandic summer spawning herring has had a declining spawning stock biomass for the last years. A high *Ichthyophonus* infection has been observed in the stock since 2008, and this has probably caused additional mortality, although strong year classes was seen before this infection. The infection seems to be abating, and new advices from ICES will build on the newest surveys in autumn 2011. There is no formal management plan for this stock.

Capelin

Capelin in the Barents Sea is considered to have full reproductive capacity. Recommended quota also take into consideration variations in predation from other species, e.g. cod and herring (Havforskningsinstituttet, 2009). Icelandic capelin is found off Iceland and Jan Mayen, and the management goal for the stock is to obtain the targeted spawning biomass. Recommendations for quota from Icelandic authorities are calculated in a way that is not approved by the ICES, and therefore the ICES recommend a more careful quota assessment (Mayen, 2011).

Sand eel

Status for Sand eel stocks in the Norwegian Economic zone was considered (by Norwegian fisheries authorities) worse than expected by ICES, and Norwegian authorities closed the fisheries in 2009. However, there was a Sand eel fishery in the EU zone, and fish meal from sand eel was on the market. Until 2010, ICES regarded sand eel in the North Sea as one stock, but from 2011 the sand eel in The North sea, Shetland and Skagerak will be regarded as seven different stocks, and managed separately (Havforskningsinstituttet, 2011d).

Atlantic mackerel

According to ICES there is a risk that the mackerel stock is harvested in a not-sustainable way. However, the stock is considered to have full reproductive capacity. Some restrictions for fisheries are recommended (Havforskningsinstituttet, 2011b)

3.5 SWOT marine resources

Strengths

Fish meal and fish oil, as feed ingredients for farmed fish have obvious advantages. The nutrient content is favorable and the nutrient availability is normally high. If well managed, fish stocks can be harvested yearly as a self renewing source of nutrients (FAO, 2011b). Recently, FAO published guiding principles for the use of wild fish in aquaculture (FAO, 2011a) in order to promote sustainable use of wild fish stocks as feed resources in aquaculture. Assuming that these guidelines are implemented and that fish meal and fish oil are produced only from certified 'responsible managed' fish stocks, the aquaculture sector will be more sustainable. The major part of fish meals and fish oils used in Norwegian fish feeds come from fisheries in Northern Europe, and transport costs are lower than for ingredients purchased from other regions in the world. By-products from Norwegian fisheries and aquaculture production is to a large extent (78%) utilized for different purposes (RUBIN, 2011). The largest part of by-products from captured fisheries is used for fish meal (57%) and silage production (27%).

Weaknesses

Fish meal and fish oils are limited resources, and the use of fish to feed fish is debated. Some are concerned about the ethical aspect and suggest that fish should be used directly for human consumption. FAO addresses this topic in a recent report (FAO, 2009). Even though the conclusion of the report was that the practice of using fish as feed is viable provided sustainable management of reduction fisheries, these facts can be difficult to communicate to the public. The several different certification schemes for fisheries have all been debated, all have their weaknesses. There is at present no such scheme that is accepted as superior in order to ensure sustainability.

Opportunities

Today, significant amounts of fish meal and oil come from trimmings and by-product from catches that primarily go to human consumption. It is possible to increase the amount of fish meal and fish oil from these sources, and contribute to a better utilization of marine resources. Fish meals may vary in quality according to raw material freshness, fish species and drying technology, but the major quantities of fish meals produced in Norway are gently dried (LT quality). Similarly, fish oils may vary significantly in raw material freshness (oxidative stability) and fatty acid composition depending on fish species. It is still possible to improve and standardize processing of catches in a way that allows for optimal nutrient utilization of the fish meals and oils. High quality fish meal is well utilized and special qualities of fish meal may facilitate the same level of fish performance with lower dietary levels of fish meal. (Kousoulaki, *et al.*, 2009). This represents a great opportunity for better utilization of

the fish meal resource, in combination with plant ingredients. In the future, fish meal may be used as a functional ingredient with amino acids or other substances with a biological effect; and as a palatable ingredient, stimulating feed intake in plant based diets.

Fish oils, or the lipid part of fish meal, are at present the sole source for the essential VLC n-3 fatty acids and is therefore still needed in fish diets in order to secure good growth, health and survival (Ruyter, *et al.*, 2000a, b, c). However, the capacity for conversion of 18:3n-3 from plant oils to EPA and DHA and the gene expression of the Δ 5- and Δ 6- desaturase activities are depressed when salmon is fed high dietary levels of fish oils, while plant oils increase the capacities (Ruyter, *et al.*, 2003; Moya-Falcón, *et al.*, 2005; Kjær, *et al.*, 2008). These results demonstrate a potential to improve the contents of VLC n-3 fatty acids in the product by optimizing the oil combinations in fish diets. The dilution of EPA and DHA in the muscle when plant oils are fed to salmon may be reduced by a more strategic use of fish oil in the feed. Fish oil should be included in the diet when the retention of the lipid is greatest at falling photo period in the autumn (Alne, *et al.*, 2011), or in finishing diets (Bell, *et al.*, 2004). In the future, the salmonid aquaculture may be capable of becoming a net producer of VLC n-3 fatty acids (Crampton, *et al.*, 2010; Bendiksen, *et al.*, 2011; Sanden, *et al.*, 2011; Turchini, *et al.*, 2011). Net production of VLC n-3 may be achieved with use of plant oils in combination with breeding programs for selection of fish with higher capability for conversion of 18:3 n-3 (ALA) to 22:6 n-3 (DHA). The level of the VLC n-3 fatty acids in fish flesh is considered important for obtaining high nutritional quality of farmed fish products. In spite of reduced total content of VLC n-3 fatty acids in salmon fed high levels of plant oils it is important to inform the consumers that the product is still supplying an amount of these fatty acids that promotes positive health effects compared to meat from terrestrial farmed animals.

Threats

Climate changes and diseases may affect fish stocks, and thus the amount and species of fish available for meal and oil production. The national fish stock management programs are designed to adjust to variation caused by external factors like climate, but still stocks of short lived fishes may be temporarily over-fished. Content of environmental pollutants like persistent organic pollutants (POPs) and heavy metals, are well known in marine fish species, especially in long lived carnivorous species, however, techniques are being developed to remove contaminants from oil (Oterhals and Nygård, 2008; Oterhals and Berntssen, 2010; Oterhals, 2011). Major industrial accidents may cause serious contamination locally and damage important species for fish meal and oil production. For oils obtained from farmed fish by-products a significant use of plant oils in fish diet may reduce the value of these oils as the levels of EPA and DHA will be less.

3.6 Marine ingredients from lower trophic levels

Zooplankton is marine sources from a lower trophic level with large standing biomass (Olsen, 2011; Torrissen, *et al.*, 2011). Based on the annual production of *Calanus finmarchicus* in the Norwegian sea, Torrissen, *et al.* (2011) estimated that harvesting 1% of the annual production of this species alone could yield 2-3.5 million tons of marine oils and protein. This resource will however, most likely not be used in near future. There is still a

need to further develop the catching and processing technology to make these resources economically and practically feasible (Torrissen, *et al.*, 2011). Other shrimp like crustaceans, the Antarctic krill, is harvested commercially and different products are on the market. The most abundant species of krill, *Euphausia superba*, is found in Antarctic waters. The standing biomass is estimated to vary between 125-750 million tons (Nicol and Endo, 1997), with a potential biomass production of more than 100 million tons per year assuming a life span of 6 years (Olsen, 2011). The catch quota for 2011/2012 is 5.61 million tons, however, the landings are approximately 200,000 tons. The Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) is regulating the fisheries of krill.

Fresh krill contain approximately 20% dry matter (DM). The DM contains approximately 60-78% crude protein, 7-26% crude lipid and 12-17% ash (Hansen, 2011). The proximate composition (DM, crude protein, crude fat and ash) of the krill meal is similar to that of fish meal, though crude protein content is somewhat lower and fat level slightly higher. The chemical content varies according to sex, age, season and area of harvest. The amino acids profile of the krill meal is almost identical to that of fish meal. The amino acid composition is shown in Table 4. The lipid fraction of krill meal is characterized by a high proportion of polar lipids, and high content of polyunsaturated fatty acids (PUFA), sterols (mainly cholesterol) and astaxanthin esters.

Based on the nutrient composition, krill is a promising alternative to fish meal in aqua diets. Partial substitution of fish meal with krill meal have shown improved growth rate in Chinook salmon (25% krill meal) (Anderson, *et al.*, 1997) and Atlantic salmon (Olsen, *et al.*, 2006). However, full replacement of fish meal with krill meal has shown a negative effect on growth performance. This is mainly attributed to the exoskeleton of krill that contains chitin. Several experiments have shown that chitin depress growth (Olsen, *et al.*, 2006; Yoshitomi, *et al.*, 2006; 2007; Hansen, *et al.*, 2010) and have a negative impact on lipid digestibility and amino acids (Hansen, *et al.*, 2010). Removing the exoskeleton from krill (deshelling) before processing will therefore improve nutrient digestibility and growth performance and allows greater inclusion levels in fish feed (Yoshitomi, *et al.*, 2006; Yoshitomi, *et al.*, 2007; Hansen, *et al.*, 2010). Meal made from deshelled krill can fully replace fish meal without negative effects on growth rate in Atlantic salmon (Hansen, *et al.*, 2010) and rainbow trout (Yoshitomi, *et al.*, 2007). Full substitution of fish meal with (whole) krill meal in diets for Atlantic cod did not affect growth performance (Moren *et al.*, 2006). Krill in the diet fed to Atlantic cod gave a whither color of the muscle and a more reddish skin color (Karlsen, *et al.*, 2006) suggesting that krill may improve the product quality in cod.

Table 4 Amino acids composition of krill meal in comparison to amino acid requirement of some species of fish (% of CP)

% of crude protein	Amino acids content		
	Krill meal ¹	Krill meal ²	Krill Hydrolysate ³
Agrinine	4.8	5.6	7
Histidine	1.6	2.2	2.8
Isoleucine	4.1	4.6	5
Leucine	6.3	7.2	8.3
Lysine	6.2	6.9	6.9
Methionine+cysteine	3.4	3.9	4.3
Phenylalanine+tyrosine	6.3	7.7	7.7
Threonine	4	7.2	5
Tryptophan	ND	0.8	1.5
Valine	4.2	4.7	5.5

¹ Hansen (2011)

² Storebakken (1988)

³ Sunotama (2006)

ND = not determined

Lipid is the key driver for the variation in chemical content of krill, and is mainly determined by the reproductive status of female krill. Krill oils composition can vary significantly, but are often containing high levels of phospholipids (60-80%), which can contain up to 15-20% EPA and 10-15% DHA (Phleger, *et al.*, 1998). It is yet to be determined if krill oils, rich in phospholipids giving special physical properties such as melting point, polarity etc., are suitable for the extruded processing involved in aquafeed production. At present also, the krill oil produced from the limited catches, are solely aimed at human consumption and at high prices. In a longer time perspective, such oils with their high contents of the valuable VLC n-3 fatty acids, may pose as important oil resources for future salmon farming. Krill is, however, also at the base of the ocean food chain, and concerns have been voiced on how overfishing or concentrated fishing in local regions may undermine the food chain and devastate marine life (Hill, *et al.*, 2006).

Limiting factors for use of krill in European fish feeds are high levels of fluoride, copper and cadmium that exceed the upper limits set by the EU (Commission dir. 2002/32/EC). The uptake and deposition of fluoride from krill depend on the hardness of the water (Moren, *et al.*, 2007; Hansen, *et al.*, 2011b). Consequently, fish in fresh water accumulates more fluoride than fish reared in salt water. Fluoride content in meals is mainly found in the exoskeleton and is therefore reduced if the krill is deshelled before processing (Yoshitomi, *et al.*, 2007; Hansen, *et al.*, 2010). Though, deshelling will not reduce the content of copper and cadmium.

Krill products (meals, hydrolysates and extracts) have feeding stimulatory effects and can be used to enhance feed intake. The greatest palatability effect is reported at the start of the experiment and it is gradually diminishing during the course of the experiment with salmonids (Olsen, *et al.*, 2006; Rungruangsak-Torrissen, 2007; Hansen, *et al.*, 2010; 2011a). In line with these results Oikawa and March (1997) demonstrated improved feed intake and growth performance in rainbow trout fed diets mainly based on plant ingredients. Krill therefore seem

to have a great potential as a feeding attractant in modern diets based on high inclusion of plant ingredients. The palatability has been associated with the content of glycine, betaine, arginine, proline, glucosamine and nucleotides (Shimizu, *et al.*, 1990; Carr, *et al.*, 1996).

Krill also contains high levels of astaxanthin, the main pigment found in wild Atlantic salmon and rainbow trout (Schiedt, *et al.*, 1981; 1986). The concentration of astaxanthin is ranging between 50-260 mg/kg krill meal and between 727-1080 mg/kg in krill oil depending on season, sex and maturation status (Clarke, 1980; Storebakken, 1988). The astaxanthin in krill is mainly present as diesters (76-90%) while unesterified astaxanthin is found in minor amounts (Maoka, *et al.*, 1985; Storebakken, 1988; Aas, *et al.*, 1998; Grynbaum, *et al.*, 2005; Albrektsen, *et al.*, 2006). The astaxanthin diesters from krill are more stable during feed processing. A loss of only 1% was observed during extrusion processing, which is low compared to free astaxanthin from Carophyll Pink that had 5% loss under the same processing conditions (Albrektsen, *et al.*, 2006). Esterified astaxanthin have a lower utilization than free astaxanthin (Torrissen and Brækkan, 1979; Storebakken, *et al.*, 1987). The digestibility of astaxanthin from krill was estimated to 53% and did not differ from the commercially produced synthetic produced Carophyll Pink (Albrektsen, *et al.*, 2006). However, retention of free astaxanthin from Carophyll Pink was reported to be higher than esterified astaxanthin from krill meal in Atlantic salmon (Albrektsen, *et al.*, 2006). In line with these results, Roncarati, *et al.* (2011) showed highest deposition of astaxanthin in fish fed free astaxanthin in the diet compared to rainbow trout fed krill meal in the diet (both diets contained 90 mg/kg astaxanthin). In the latter experiment, trout fed krill meal diet also had a paler pink-red colour on the SalmoFan scale compared to those fed free astaxanthin diet. Aas, *et al.* (1998) reported lower retention of astaxanthin diets supplemented with krill meal in rainbow trout, while no significant differences were found in Atlantic salmon. Astaxanthin retention was 10.2 and 5.5% in rainbow trout and Atlantic salmon, respectively, which is within the range reported in the literature for these two species. The latter experiment used Carophyll Pink as the pigment source in the control diet. In contrast, Mori, *et al.* (1989) reported that krill oil astaxanthin diesters had the same utilization as Carophyll Pink fed to juvenile coho salmon (*Oncorhynchus kisutch*). Also Suontama, *et al.* (2007) reported no significant differences in muscle astaxanthin concentration and visual colour characteristics (L*A*B*-values, Hunterlab) when 40% of dietary protein was replaced with Antarctic krill meal. In the latter experiment the control diet was supplemented with Carophyll Pink (64 mg/kg) and the total carotenoid concentration of the krill diet was 68 mg/kg, 40 mg from Carophyll Pink and 28 mg from the krill meal.

3.7 SWOT marine ingredients from lower trophic level

Strengths

Ingredients from lower trophic levels, such as zooplankton and krill, have a favorable nutrient composition and the nutrient availability is high if the ingredients are well processed. Krill is also a palatable ingredient, ensuring a high feed intake in fish. At present, krill have the greatest potential as an ingredient because the catching and processing technology have come further than that of smaller zooplankton. A precautionary catch limit managed by CCAMLR is ensuring that krill can be harvested yearly as a self renewing resource. Marine

resources from the Antarctica are generally low in environmental pollutants such as POP's and PCBs.

Weaknesses

There is still a need to further develop the catching and processing technology to make smaller zoo-plankton and North Atlantic krill economically and practically feasible (Torrissen, *et al.*, 2011). In order to maintain a high nutritional quality, zooplankton and krill need to be processed immediately. High proteolytic activity is causing fast degradation and deterioration of the nutritional value of zooplankton. Besides, the shell fraction should be removed if krill is included at higher levels in the diet. There is a concern about high levels of fluoride, copper and cadmium, exceeding the upper limits set by the EU (Moren, *et al.*, 2006), though no negative effect have been reported on fish health. Deposition of fluoride is affected by water hardness while copper and cadmium need to be controlled by other means. Shell fraction from the krill reduce the digestibility of fat and amino acids resulting in depressed growth rate (Hansen, 2011). Use of marine resources from the Antarctic is also debated. There are concerns that harvesting plankton from this vulnerable environment is a threat for the ecosystem in the ocean. Krill is at the base of aquatic food webs and represent important food resources for whales, penguins and seals.

Opportunities

Today the harvested amount of krill is approximately 200 000 tons, while the catch quota is set at 5.61 mill tons. It is therefore possible to increase the harvest of krill within safe limits. Processing technology is improving, and knowledge on safe use of krill is also increasing, in order to make krill safer to use in fish feed. The high palatability of krill makes it an ideal feeding attractant in plant based diets that may suffer from low palatability. The lipid part of krill is high in the essential VLC n-3 fatty acids and may as such represent an immediate available resource if prices could be lowered.

Threats

Increased temperature and melting of the icebergs is a major threat for the reproduction of krill in Antarctica. The high price of krill products may also in the future represent a threat for use in fish feed.

4 Plant ingredients

Plant ingredients are global commodities produced in large quantities and the abundant supply offers a great opportunity also in fish feed. The nutrient composition in plant ingredients varies among species and also among genotypes of the same species as well as growth conditions and processing. The main challenges associated with replacement of fish meal with plant protein ingredients is the lower levels of proteins, high levels of carbohydrates, unfavourable amino acid (Table 5) profiles and mineral contents and the presence of anti-nutritional factors (Table 6) in plant ingredients (Gatlin, *et al.*, 2007; Hemre, *et al.*, 2009).

Table 5 Amino acid composition in some commonly used plant protein ingredients compared to fish meal

g (16 g N) ⁻¹	Fish meal ^a	Soybean ^b	Soy protein concentrate ^c	Rapeseed ^d	Sunflower ^e	Pea ^f	Lupin ^g
Arginine	5.4	6.7	6.4	2.1	3.6	8.2	11.2
Histidine	2	2.4	2.5	1	1	2.7	1.8
Isoleucine	3.6	4	4.1	1.4	2.1	4.5	3.9
Leucine	6.3	6.7	6.6	2.6	3	7.5	7.7
Lysine	6.6	5.1	5.5	2.1	0.7	7.4	4.9
Methionine	2.5	1.1	1.2	0.7	0.8	0.9	0.5
Phenylalanine	3.5	4.6	4.5	1.4	2.2	4.9	3.8
Threonine	3.9	3.7	3.5	1.6	1.7	3.7	4.0
Tryptophan	1	1.5	1.3	0.4	0.6	0.9	0.7
Valine	4.1	4.1	4.1	1.8	2.3	4.8	3.5

^a Low-temperature dried fish meal (Romarheim *et al.*, 2005).

^b Hexane-extracted and toasted soybean meal with hulls (Romarheim *et al.*, 2005).

^c ADM, Netherland.

^d Defatted rapeseed meal (Hertrampf and Piedad-Pascual, 2000).

^e Defatted and dehulled sunflower meal (Hertrampf and Piedad-Pascual, 2000).

^f Pea protein concentrate, 350 g kg⁻¹ CP (Øverland *et al.*, 2009).

^g White lupin (Hertrampf and Piedad-Pascual, 2000).

Unfavourable amino acid composition and imbalanced nutrient composition can be balanced by combining ingredients from different origin and use of additives such as amino acids, vitamins and minerals. A greater concern may be that use of plant proteins also results in greater content of indigestible carbohydrates, diluting the energy concentration as well as the digestibility of energy in the diet. Anti-nutritional factors may also have negative impact on fish health and reduce utilization of nutrients. Protein digestibility and bioavailability of cysteine and other heat sensitive amino acids, such as lysine, arginine and others, may also be reduced by excessive heating (Draganovic, 2006; Morken, *et al.*, 2011a) in order to remove extraction solvent (e.g. hexane) after oil extraction (Aslaksen, *et al.*, 2007). Most of the plant ingredients used such as soybean meal, canola, sunflower meal, and corn are

grown as oil crops. The protein fraction used in animal feed is the leftover from oil extraction. The nutritional value is thus affected by processing steps carried out in order to remove oil.

Table 6 Undesirable components reducing nutrient utilization.

	Heat stable	Heat labile
Grains		
Wheat/wheat gluten	Phytate, NSP phenols	Amylase inhibitor
Corn / corn gluten	Phytate, NSP phenols	
Oilseed		
Soybeans	Saponins, tannins, phytate, NSP, alkaloids, cyanogens, phytoestrogens, antivitamin, phytosterols	Proteaseinhibitor, lectins
Rapeseed	Tannins, phytate, Non-starch polysaccharides (NSP), phenols, glucosinolates	Proteaseinhibitor
Cottonseed	Gossypol	
Sunflower	Tannins, phytate, NSP, phenols	Protease inhibitor, arginase inhibitor
Pulses		
Lupins	Alkaloids, phytates	Protease inhibitor, lectins
Peas	Saponins, tannins, phytate, NSP, alkaloids, cyanogens	Protease inhibitor, lectins
Beans	Saponins, tannins, phytate, NSP, alkaloids, cyanogens, polyphenols, phytoestrogens, antivitamin, phytosterols	Proteaseinhibitor, lectins, amylase and lipase inhibitor

Four major types of plant oils are produced: Palm, Soybean, Rapeseed and Sunflower oil, with 42.4, 37.7, 19.4 and 10.1 million tons pr year, respectively, in 2007-2008, according to Gunstone (2011). The main concerns related to using plant oils in fish feed are the lack of VLC n-3 fatty acids, and the high content, in some oils, of saturated fatty acids. The fatty acid compositions of the plant oils commented on below are shown in table 7.

Table 7 Fatty acid composition of some selected plant oils as compared to capelin oil.

Fatty acid	Soybean oil	Rapeseed oil	Sunflower oil	Corn oil	Palm oil	Capelin oil
14:0		0.2				6.2-7.0
16:0	7-12	2.8-5.9	3-10	8-19	44	10.0
18:0	2-5	1.0-2.4	1-10	0.5-4	4	1.2
16:1	0.5	0.1-0.6	1.0	0.5		10.0-14.3
18:1 n-9	19-30	53.4-64.6	14-65	19-50	39	14.0-15.0
18:2 n-6	45-58	18.8-22.9	20-75	34-62	11	0.7
18:3 n-3	4-10	7.6-12.9	0.7	2		0.2
20:0		0.4-0.6				n.d.
20:1 n-9		0.7-1.6				17.0
20:4 n-6						0.2
20:5 n-3						6.1-8.0
22:1 n-9		0.2-0.8				15.4
22:5 n-3						0.6
22:6 n-3						3.7-6.0

Kim et al. (2010), Brown and Hart (2010), Gunstone (2011)

4.1 Soybeans

Soybeans are commonly used in feeds for salmonids. The chemical composition and nutritional value depends on degree of processing. Full fat soybean meal (SBM) contains about 42% protein and 21% fat. SBM is usually referred to as fat-extracted soybeans ground to a meal. Usually SBM is dehulled and have a protein content of 48-50% if no hulls are added back (Lusas and Riaz, 1995). Soy protein concentrate is made by removing carbohydrates (sugars) from dehulled and defatted soybeans and have a protein content ranging from 62-69%. Soy protein concentrate is the most extensively used plant ingredient in feeds for salmonids. In 2010 the inclusion level ranged between 17-22% of the commercial feed for salmonids in Norway.

The amino acid profile of soy protein meets most of the indispensable amino acid (IAA) requirements for fish (NRC, 1993). SBM is relatively high in cystine, though lysine, methionine, and threonine may be limiting in some SBM based diets (Gatlin, *et al.*, 2007) (Table 5). SBM contains a range of anti-nutritional factors (Table 6) that reduce the digestibility and overall growth performance, feed utilization and fish health (Krogdahl, *et al.*, 2010).

Use of solvent extracted SBM in diets for salmonids has been investigated in a number of studies. Most investigations report that inclusion of 20-30% solvent extracted SBM in the diet for salmonids result in reduced digestibility of lipid, concurrent with a negative effect on feed intake, growth performance and reduced feed efficiency (Rumsey, *et al.*, 1993; Olli and Krogdahl, 1994; 1995; Rumsey, *et al.*, 1995; Davies, *et al.*, 1997; Bureau, *et al.*, 1998; Refstie, *et al.*, 1998; Storebakken, *et al.*, 1998; Refstie, *et al.*, 1999; 2000; 2001). Partly these negative effects can be caused by bioactive compounds in the alcohol soluble fraction of SBM shown to cause morphological changes in the distal intestine (van den Ingh, *et al.*,

1991; 1996; Knudsen, *et al.*, 2007; Yamamoto, *et al.*, 2008) of several fish species such as rainbow trout (Bureau, *et al.*, 1998; Heikkinen, *et al.*, 2006; Romarheim, *et al.*, 2008; Yamamoto, *et al.*, 2008), Atlantic salmon (Baeverfjord and Krogdahl, 1996; Refstie, *et al.*, 2000; Krogdahl, *et al.*, 2003; 2005) and Atlantic cod (Olsen, *et al.*, 2007). Soya saponins are associated with the onset of morphological changes known as SBM induced enteritis in Atlantic salmon (Knudsen, *et al.*, 2007; 2008). The negative effects of SBM on fat and energy digestibility may be explained by a reduction in bile acids in the intestinal chyme (Romarheim, *et al.*, 2008; Yamamoto, *et al.*, 2008; Sørensen, *et al.*, 2011).

Soy protein concentrate (SPC) have low content of anti-nutritional factors, soluble carbohydrates and fiber (Bureau, *et al.*, 1998; Storebakken, *et al.*, 2000), and can therefore be used at higher inclusion levels for carnivore fish. The extraction also eliminates alcohol soluble components which cause bitter off-flavor compared to SBM (Morr and Ha, 1991). Phytic acid is not removed (Storebakken, *et al.*, 1998, 2000), but the content can be reduced by pretreating the soy protein concentrate with phytase (Denstadli, *et al.*, 2006; 2007). Experiments have shown that approximately 50% of SPC can replace fish meal without negative effect on growth performance in Atlantic salmon and rainbow trout (Olli and Krogdahl, 1994; Medale, *et al.*, 1998; Mambrini, *et al.*, 1999).

The fatty acid composition of common soybean oils is dominated by a high percentage of linoleic acid, C 18:2 n-6, (45-58%) and only a low percentage (4-10%) of linolenic acid, C 18 n-3, is found (See table 4). In addition, soybean oil has a significant content (19-30%) of the C 18 monounsaturated oleic acid and relatively low concentration of saturated fatty acids, mainly C16 palmitic acid (7-12%). Soybean oil has been used as a source of dietary lipids in a high number of fish feeding studies, with grading levels from 5-100% inclusion. In salmon, no reduction in weight gain has been observed, even with a 100% substitution of fish oil (Thomassen and Røsjø, 1989; Rosenlund, *et al.*, 2001; Grisdale-Helland, *et al.*, 2002; Torstensen, *et al.*, 2005; Ruyter, *et al.*, 2006). It should be noted, however, that in all these studies, fish meal was included as part of the diet. Little is known about possible influence on fish growth and health when fish oil is replaced with plant oil concomitantly with a replacement of fish meal with plant meal. A recent study, however, indicated that when high levels of fish meal and fish oil are replaced simultaneously, it may result in a metabolic imbalance in fish (Torstensen, *et al.*, 2011), pointing to the need for more research in this area.

A significant incorporation of linoleic acid in muscle due to the very high dietary content of this fatty acid, as exemplified in fig. 6 (Thomassen and Røsjø, 1989), and a concomitant severe reduction in the essential VLC n-3 fatty acids, resulting in a nutritionally unfavourable n-6/n-3 ratio, may have limited the use of soybean oil in salmonid diets.

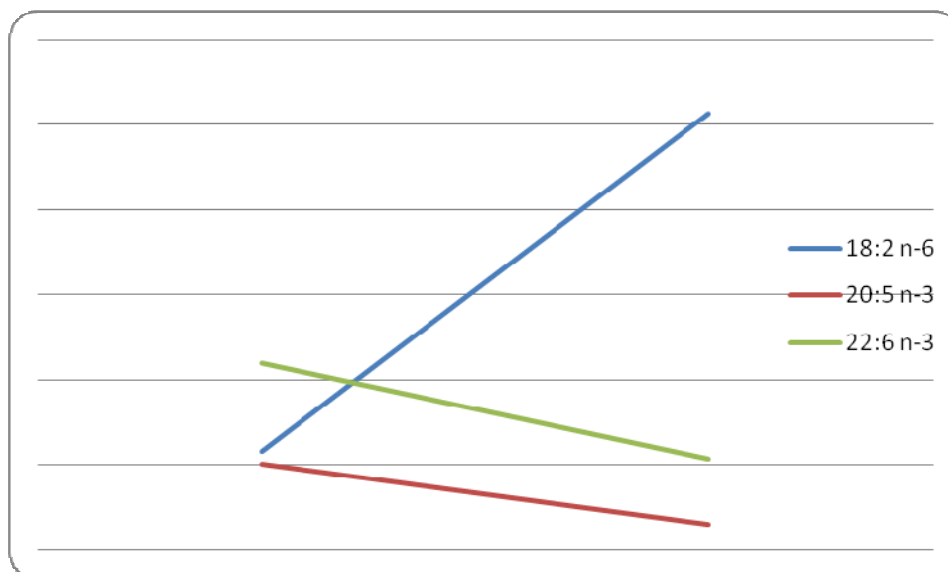


Figure 6 Fatty acid composition in muscle of salmon fed a diet added only soybean oil.

A major challenge for the commercial feed industry in Europe is that the cultivation of gene modified (GM) crops is increasing world wide. The major producing and exporting soy producers in the world, USA, Brazil and Argentina, are mainly producing GM soy. GM crops in the USA, Argentina and Brazil are comprising 91%, 99% and 71%, respectively, of the soybean production. The production of non-GM based animal feeds will be facing extra costs due to growing scarcity of raw materials and due to necessary adaptations in chain management, guarantees, and testing protocols for the maintenance of the non-GM status. The European consumers are in general more sceptical to the use of gene modified organisms (GMO) than American and Asian consumers. As long as safe use of products from fish and other production animals fed GM feed ingredients are a subject of controversy, the feed industry have to be careful about using such ingredients. Before GM products are allowed onto the market in the EU, they must have received regulatory approval by the EU. Food and feed applications of GM products are covered by EU Regulation 1829/2003/EC. Norway has, in line with the EU, a precautionary approach regarding use of GMO. According to the Norwegian feed act (Feed 2002-11-07 nr 1290: Act about feed ingredients), feed ingredients and feed additives made from GMO or that may contain GMO, is not allowed for trading or marketing without approval from the food safety authority. A temporarily permission is given for some corn and soy GMO products for use as feed ingredients, assumed that the feed is properly labelled. The feed industry is yet not using any of these ingredients.

Transgenic oilseed crops can be made to produce the major VLC n-3 fatty acids by the insertion of various genes encoding desaturases and/or elongases. The modification to introduce these fatty acids is complicated, and only low levels of DHA have been obtained (0.5-3.3%), as reviewed by Miller et al. (2010). For soybean oil, a content of 19.55% EPA and 3.3% DHA has been reported in 2007 (Damude and Kinney, 2008). Up until now, however, Europe and Norway have been reluctant to accept this new technology.

4.2 Rapeseed/canola

Oil seeds of the genus *Brassica* are grown as oil and protein crops with a world production of approximately 57.2 million tons in 2010 (<http://www.agricommodityprices.com>). The leading producers are the EU (20 million tons), China (12.8 million tons) and Canada (11 million tons) with about 77% of the world production. Canola and rapeseed are both names on the plants *Brassica napus* and *Brassica campestris*. Canola refers to Canadian varieties of rapeseed low in erucic acid (<2% in the oil fraction) and aliphatic glucosinolates (<30 $\mu\text{mol/g}$ glucosinolates / g of oil-free dry matter). Hilton and Slinger (1986) showed that feeding rainbow trout with traditional rapeseed caused thyroid hyperplasia and reduced plasma thyroxine concentration. Glucosinolates are turned into goitrogenic compounds interfering with normal thyroid function by the enzyme myrosinase. Myrosinase and glucosinolates are kept in separate compartments. However, damage to cells in the plant material is starting the process where the glucosinolate (thioglucoside) is transformed to the toxic secondary metabolites isothiocyanate and nitriles. The activity of myrosinase is reduced by heat treatment or toasting of canola after extraction of oil by hexane. Using the traditionally high erucic acid rapeseed oil in feed for salmon (Thomassen and Røsjø, 1989) showed no negative effect on growth rate as compared to a feed containing capelin oil, even at the highest inclusion (only rapeseed oil added). Erucic acid was, however, seen accumulating somewhat in the heart lipids, 6.5% and 3.7% in triacylglycerides and phospholipids, respectively, after 18 weeks of feeding. No increased mortality was observed. Most research on use of rapeseed in feeds for aquatic species has subsequently been conducted only with canola meals and oils.

Canola meals, resulting from oil extraction processes, contains about 3.5% crude oil, 35% crude protein, 6% ash and 12% crude fiber and 10% water. Compared to soy protein, canola protein is low in most essential amino acids, but the amino acid profile is similar. In comparison to fish meal, canola meal is limiting in lysine but have a high level of methionine and cystine. Because of a relatively high content of fiber and phytate, canola has a limited use for carnivore fish (Drew, *et al.*, 2007a). Anti-nutritional factors include phytic acid (3.1-3.7%, Higgs *et al.*, 1995), glucosinolates, phenolic compounds and soluble as well as insoluble fiber. The low erucic acid contents of canola along with low lipid contents in solvent extracted meals, is eliminating anti-nutritional effects from the oil component of these meals (NRC, 1993). Canola meal contains 15.5% cellulose, 5% hemicellulose and 8.3% lignin (Mwachireya, *et al.*, 1999). These anti-nutrients restrict the use of canola meals to levels of approximately 10% of the diet (Drew, *et al.*, 2007a). The nutritional value of canola can be improved by aqueous extraction of the protein removing the fibrous fraction, producing a canola protein concentrate (CPC) (Mwachireya, *et al.*, 1999; Thiessen, *et al.*, 2004). CPC contains approximately the same crude protein level as fish meal. In feeding experiment with rainbow trout, a reduction in growth and feed utilization was observed when CPC was included at 19 and 38% of the feed (Drew, *et al.*, 2007b).

Low erucic rapeseed oils, as the canola oil, is at present one of the most promising plant oils in use for salmon feed. As observed with the high erucic acid rapeseed oil, no negative effect on growth rate has been shown (Thomassen and Røsjø, 1989; Rosenlund, *et al.*, 2001), and the relatively low percentage of linoleic acid (about 20%) and medium percentage of linolenic acid (about 10%) results in a better n-6/n-3 ratio in salmon muscle than with most other plant oils. Importantly, these rapeseed oils also contain high levels of oleic acid (about 60%), and

low levels of saturated fatty acids (about 10%). Salmon, as a partly fresh water specie, has been shown to be able to produce VLC fatty acids from the C-18 PUFAs, and the good balance in rapeseed oils between the linoleic (18:2 n-6) and linolenic (18:3 n-3) acids, which are competing for the same enzymes, makes some own production of EPA and DHA possible. This has been demonstrated in feeding studies containing different dietary combinations of fish oils and rapeseed oils (Thomassen and Røsjø, 1989; Rosenlund, *et al.*, 2001; Kjær, *et al.*, 2008) as well as in primary cultures of liver cells (Moya-Falcón, *et al.*, 2005). The capacity of this process seems, however, not to be high enough to keep the muscle concentration of EPA and DHA at the same levels as seen when using pure fish oil diets (Torstensen, *et al.*, 2005). Also with rapeseed, GMO based oils containing EPA and DHA are produced (Thomassen, personal communication), but are still not on the market for use in aquaculture production.

4.3 Sunflower Meal and Oil

The world production of sunflower (*Helianthus annuus*) is 30 million tons per year. The main countries producing sunflower crop (67%) are Ukraine (6.5 million tons), Russia (5.5 million tons) and EU (6.7 million tons). Sunflower is grown in many countries around the world because of its high oil content and the ability of the plant to adapt to a variety of climates and soil conditions. The seeds contain about 74% kernel and 26% hulls (Lević, *et al.*, 1992) with an oil content ranging from 38-54%, fiber in the range 12-20% and protein content in the range 16-21%, respectively, depending on growing conditions and cultivars (Wan *et al.*, 1979). Sunflower meal is produced from the oil cake after oil extraction from dehulled sunflower seed. De-cortified solvent extracted meals have a chemical composition in the range: protein 45-55%, fiber 4-14%, oil 1-9% and ash 6-8% of the DM (NRC, 1993). Akande *et al.* (2011) have reported that the amino acid composition is dominated by glutamic acid. The first limiting indispensable amino acid is methionine. Among the indispensable amino acids, arginine has the highest content.

Because of the high fiber content only meals from decorticated seed can be used in aquaculture feeds. Meals that are lower in fiber and higher in protein can be produced if more of the seed hulls are removed before processing. Studies with rainbow trout have shown that up to 40% of the protein in the diet can be derived from sunflower meal without negative effects on growth, feed intake, feed efficiency (Stickney, *et al.*, 1996). Experiments with Atlantic salmon have shown that inclusion of sunflower meal up to 23% gave reduced digestibility of protein and all amino acids, while digestibility of lipid was improved (Aslaksen, *et al.*, 2007). Up to 27% inclusion of sunflower meal was used without negative effects on growth performance, feed utilization or body composition in post-smolt diets to Atlantic salmon (Gill, *et al.*, 2006). Sunflower meal can also be used as a bioactive ingredient in the battle against salmon louse. Diets fed to Atlantic salmon with sunflower meal included have shown a lice repelling effect (Refstie, *et al.*, 2010). Fish fed sunflower in the diet had a 27% less fish infested, and 42% fewer lice on the fish that was infested with lice.

Anti-nutritional factors in sunflower are first of all associated with the high content of fiber. The fiber composition has been characterized by Canibe, *et al.* (1999). Except for phenolic compounds, sunflower meal is low in anti-nutritional factors and allergenic compounds

(Frag and Daa, 1999). Phenolic compounds (the sum of chlorogenic acid, caffeic acid, derivative 1 and derivative 2) is ranging from 0.677-2.847 mg per gram dehulled partially defatted samples (Canibe, *et al.*, 1999). Chlorogenic acid is reported to function as an effective trypsin inhibitor (Kanto, 1989). Mild heating is destroying chlorogenic acid and improving the nutritive value of sunflower meal.

Sunflower oil is mainly exported from Argentina and Ukraine. Although it is the fourth most produced oil, sunflower oil production has not increased as much as that of the other three major plant oils. In addition to its regular high-linoleic variety, sunflower oil is available in high-oleic and mid-oleic varieties. None of these are GM crops (Gunstone, 2011). Sunflower oils belongs to the “high n-6” plant oil group, due to its dominating content of linoleic fatty acid (20-75%) and with hardly any linolenic acid (less than 1%). Sunflower oil also has a high content of the monounsaturated oleic acid (14-65%), and relatively low levels of saturated fatty acids. Sunflower oil has been fed to Atlantic salmon (Bell, *et al.*, 1993; Torstensen, *et al.*, 2000; Jutfelt, *et al.*, 2007). Jutfelt *et al.* (2007) reported similar growth rate in Atlantic salmon smolt fed a fish oil or a sunflower oil based diet, and a positive effect of sunflower oil diet on the rates of amino acid and free fatty acid intestinal uptake. Bell *et al.* (1993) also reported similar growth rate with sunflower oil diets, but this fish developed a marked cardiac histopathology, which was present also in fish oil fed fish, but in a less severe form.

4.4 Corn gluten meal

Corn gluten meal (CGM) is derived from corn grains and is a by-product of starch production (Hardy and Barrows, 2002). According to Gatlin *et al.* (2007) more than 400 products are produced from corn starch. Corn kernel is separated into bran, germ, gluten and starch, usually in a wet milling process followed by centrifugation of the starch-gluten slurry. The gluten protein is dried, refined and purified to contain a minimum protein content of 60%, but often it is 70-73% (Gatlin, *et al.*, 2007). Corn gluten has a low content of lysin (NRC, 1993). The protein digestibility of protein in Atlantic salmon diets containing 20.4% CGM was similar to the control diet with fish meal, with an apparent digestibility coefficients of 84.8 (Aslaksen, *et al.*, 2007). Mundheim *et al.* (2004) showed a linear reduction in protein digestibility, weight gain, specific growth rate and thermal growth coefficient when the level of protein from high quality fish meal was replaced with a mixture of soy and corn gluten (1:2) from 85.1% in 34.7%. On the contrary, Mente *et al.* (2003) showed that up to 50% CGM could be included without negative effect on growth in a short term study with small Atlantic salmon. Although CGM not contain harmful anti-nutritive factors, there are some reports showing that inclusion of more than 20% CGM may have a negative impact on flesh pigmentation (Mundheim, *et al.*, 2004). Corn gluten may contain 100-500 ppm of yellow xanthophyll carotenoids, mainly lutein and zeaxanthin that may reduce pigmentation. Skonberg *et al.* (1998) reported that rainbow trout fed with 22% CGM in the diet and without other carotenid supplementation had a higher yellow colour value compared to fish without CGM. In line with this, Saez *et al.* (2011) reported significantly lower astaxanthin concentration of the flesh in rainbow trout fed 19% CGM in the diet, compared to the control diet devoid of this ingredient. The latter authors did not detect lutein or zeaxanthin in the flesh of trout fed CGM. CGM has been used in Norwegian salmonid diets at inclusion levels of 5-11% of the diet. The main limitation in

use of CGM is the outspread of GMO and increasing challenges to guarantee non-GMO corn.

Corn oil is one of the “high n-6 fatty acid” oils and has been fed to a whole range of aquatic animals, but no report on use in salmon feed could be found. A study performed on Brown trout (*Salmo trutta*) did, however, not show any reduced growth upon exchanging 100% of fish oil by corn oil (Arzel, *et al.*, 1994).

4.5 Wheat gluten

The wheat gluten (WG) proteins are the major storage proteins that are deposited in the starchy endosperm cells of wheat. These proteins form a continuous matrix in the cells of the mature dry grain. When water is added to gluten protein, the proteins are brought together to form a continuous viscoelastic network to form dough. These viscoelastic properties of gluten are providing the baking properties of wheat in bread and processed foods. The WG is made by washing wheat flour with water until all the starch dissolves, leaving insoluble gluten as an elastic mass (Day, *et al.*, 2006). Commodities of WG contain 80-85% protein on a dry matter basis and varying amounts of starch, lipid and fibre. The content of lysine, arginine and methionine is low compared to fish meal. Feeding experiments have shown that WG has high digestibility in rainbow trout (Pfeffer, *et al.*, 1992; Sugiura, *et al.*, 1998) and Atlantic salmon (Sugiura, *et al.*, 1998; Storebakken, *et al.*, 2000). Several experiments have shown that WG can replace rather large proportion of fish meal in diets for salmonids, provided that the diets are supplemented with lysine, the first limiting amino acid in gluten (Pfeffer, *et al.*, 1995; Davies, *et al.*, 1997; Sugiura, *et al.*, 1998; Storebakken, *et al.*, 2000). WG is a highly digestible protein source (Pfeffer, *et al.*, 1995). The latter authors found an apparent digestibility of 99% for crude protein, when rainbow trout was fed a diet with 92.7% gluten and 1.45% lysine. The digestibility of protein from gluten was higher than those obtained for various hydrothermally treated plant protein sources (Pfeffer, *et al.*, 1995; Sugiura, *et al.*, 1998). Atlantic salmon diets with WG up to a level of 50% of dietary protein have shown improved protein digestibility (Storebakken, *et al.*, 2000). In the latter experiment, digestibility of fat and energy also tended to be improved up to an inclusion level of 25% of the protein. Moreover, no histopathology was observed. The high nutritional value of WG is associated with a mild processing preventing heat damages to the protein, reflected in a high digestibility of cysteine in diets containing WG (Storebakken, *et al.*, 2000) and the overall high protein digestibility of this product (Pfeffer, *et al.*, 1995; Davies, *et al.*, 1997; Sugiura, *et al.*, 1998; Robaina, *et al.*, 1999; Storebakken, *et al.*, 2000). Skonberg *et al.* (1998) reported that use of WG in diets for rainbow trout had no adverse effect on flavour or pigmentation of the fillets. WG is not only used in fish feed as a fish meal replacer. It also act as a pellet binder, improving the technical properties of feed (Draganovic, *et al.*, 2011).

4.6 Pulses

Pulses are the dry, edible seeds of legume plants. A recent review (Maskus, 2010) gave an overview over pulses grown around the world, their processing, functionality and application. Use of pulses in feed for salmonids is limited to field peas and faba beans. These will therefore be in focus.

Peas and beans have a rather similar proximate composition characterized by a high starch and low protein content. The chemical composition of pea dry matter varies with growth conditions and cultivars (Nikolopoulou, *et al.*, 2007). The seed dry matter is analyzed to contain protein ranging between 25-30%, fat ranging from 0.8-4%, ash ranging from 3.1-4.1%, starch in the range 33.0-47.5% and non starch polysaccharides (NSP) ranging from 14.4-18.0% (Nikolopoulou, *et al.*, 2007). The relatively high carbohydrate level may reduce the inclusion level of peas in diets for carnivore fish. Peas also contain heat-labile (trypsin/chymotrypsin inhibitors and lectins) and heat-stable (phytic acid, condensed tannins, saponins, antivitamin and the protein antigens legumin and vicilin) anti-nutritional factors which reduce their nutritional value to fish (Francis, *et al.*, 2001). Feeding 18% field peas to Atlantic salmon improved lipid digestibility and gave similar digestibility of organic matter and protein compared to a fish meal based control, and did not induce enteritis in the distal intestine, as seen when feeding SBM (Aslaksen, *et al.*, 2007).

Recent advances in processing technology have provided pea protein concentrates (PPC) with up to 55% protein on a dry matter basis (Wu and Nichols, 2005). The protein concentrate is more suitable as protein sources in aquaculture. Pea protein concentrate can be produced in a two step process in which dehulled peas are ground to small particle sizes in step one, followed by air classification which separates the particles based on differences in size and density (Vose, *et al.*, 1976; Wu and Nichols, 2005). The air-classification results in a protein rich fraction (<18 µm) with up to 55% protein and a starch rich fraction (>18 µm) (Wu and Nichols, 2005). Feeding up to 27% PPC has produced acceptable weight gain, feed intake, and feed conversion ratios in both Atlantic salmon (Carter and Hauler, 2000) and rainbow trout (Thiessen, *et al.*, 2003; 2004). Replacing fish meal with PPCs containing either 35% or 55% protein, in diets fed to Atlantic salmon gave no significant differences in weight gain or feed intake, but feed conversion rate tended to be lower fed the PPC 50% crude protein diet (Øverland, *et al.*, 2009). There were no differences in the digestibility of protein, fat, starch and most essential amino acids between the fish fed the fish meal control and the PPC 35% CP or PPC 50% CP diets, but the PPC diets gave lower energy digestibility. No histopathological changes were reported at 20% inclusion level. However, when PPC were included at 35% into the diet fed to Atlantic salmon, adverse effects were observed on growth rate, digestibility of fat and histopathological changes in the gastrointestinal tract (Penn, *et al.*, 2011).

4.7 Lupins

Lupins used in fish feed are harvested seeds of leguminous crops, and are considered to have a great potential in aquafeeds. Lupins are mainly grown for their protein value, and are considered to be valuable agricultural crops due to nitrogen fixation. The four important commercial species of lupins are the *L. Angustifolius* (Narrow-leafed Sweet Lupin), *L. albus* (White or Albus Lupin), *L. luteus* (Yellow lupins) and the *L. mutabilis* (Andean lupin). The main producing countries of lupins are Australia, accounting for approximately 70% of total production, followed by Belarus, Germany, Poland and Chile, with 8, 5, 4 and 3% of total production, respectively. The protein content varies between 36.5 – 56.7%, depending on cultivars and growing conditions (Glencross, *et al.*, 2008a). Yellow lupin and Andean lupin are reported to have the highest protein content (Glencross, *et al.*, 2008a; Glencross, *et al.*,

2011). The yellow lupin has a protein content of 400-450 g/kg dry matter for the whole seeds. The kernel meal of yellow lupin has a protein content of 530 g/kg DM, and further processing into protein concentrate may increase the protein content up to 750 g/kg (Glencross, *et al.*, 2006). Fat, ash and carbohydrate content in lupin kernel meals varies between 5.2-9.7%, 1.9-3.9% and 32.7-53.9%, respectively, among cultivars (Glencross, *et al.*, 2008a). The amino acid composition of lupins is characterized by a high content of arginine and glutamic acid, and deficiency of methionine and lysine. In addition, the amino acid composition varies among cultivars, and the yellow lupin has a higher content of methionine, cysteine and lysine compared to the narrow leafed lupin. Compared to other legumes, lupins have a low content of anti-nutritional factors, such as trypsin inhibitor, saponins and tannins (Serrano, 2011). Alkaloids are the predominant anti-nutritional factor in lupins and are limiting the use in animal feeds. The bitter wild lupins may contain from 5000 – 40 000 mg alkaloid per kilo (Pettersson, 2000). Alkaloids have a bitter taste that may cause reduced feed intake and are also toxic to animals at high levels. Modern seeds of lupin, developed by selective breeding by Australian plant breeders, have an alkaloid content lower than 0.6 g/kg DM. The modern genotypes contain from 30 – 150 mg/kg alkaloids (Pettersson, 1998). Recent research (Serrano, 2011) have shown that lupinine and sparteine, the main alkaloids found in yellow lupine and Andean lupin (Wink, *et al.*, 1995), reduced feed intake and growth performance when present at a level of 100 mg/kg diet.

Glencross, *et al.* (2011) investigated growth performance in rainbow trout (30 g) fed narrow leaf or yellow lupin meals at inclusion levels of 0, 100, 200, 300 or 400 g/kg. Growth performance was improved by either lupin varieties, and no growth reduction was observed at the highest inclusion level. However, a significant reduction in protein retention was observed at 400 g inclusion level for both lupin varieties. In comparison to SBM, lupin kernel meal is reported to have improved palatability and a higher growth performance in rainbow trout (Glencross, *et al.*, 2008b).

4.8 Palm oil

Palm oil is also gaining increased interest for use in the aquafeed industry. Global production of crude palm oil exceeded 43 million metric tons, and overtook soybean oil in 2005 as the most produced plant oil in the world (Ng and Gibon, 2010). Malaysia and Indonesia are the major producers. Various palm oil products exist as a result of refining and fractionation, and several have been used in feeding studies. A review is given by Ng and Gibon (2010). Several studies have been published where crude palm oil or refined palm oil have been replacing up to about 25% of fish oil in diets for Atlantic salmon and rainbow trout (Torstensen, *et al.*, 2000; Rosenlund, *et al.*, 2001; Bell, *et al.*, 2002; Ng, *et al.*, 2004; Miller, *et al.*, 2007), most reporting no negative effects on growth or feed conversion ratio. In one study, however, reduced apparent digestibility was seen when environmental water temperature was reduced to 8°C (Torstensen, *et al.*, 2000). Palm oil contains about 50% saturated, 40% monounsaturated and 10% linoleic acids. Both palmitic acid and especially oleic acid are known to be good substrates for mitochondrial beta-oxidation in Atlantic salmon, and consequently considered as positive for feed utilization.

4.9 Distillers dried grains with solubles

Distillers dried grains with solubles (DDGS) are by-products from ethanol production from the fermentation of dry milled whole grains. The starch portion of the kernel is converted to ethanol, while the remaining material – mainly fiber and protein – is sold as DDGS for livestockfeed. DDGS is a complex product with a dry matter crude protein content of 27% - 30% and carbohydrate content of 52%. The chemical composition varies among processing plants (Liu, 2008), and also among grains used in the process (Randall and Drew, 2010). Thus, DDGS is mainly used as feed for ruminants, and to lesser extent in feed for monogastric animals such as fish, poultry and pigs. Some recent research have investigated nutrient digestibility in fractionated DDGS from wheat (Randall and Drew, 2010). The latter researchers showed that sieving wheat DDGS increased CP from 371.6 g/kg in the original material to 432.4 g/kg, and reduced neutral detergent fibre from 271.2 to 215.5 g/kg and acid detergent fibre from 98.9 to 76.7 g/kg. Digestibility was improved for energy and dry matter, but not for crude protein. This latter research demonstrated that sieving is a mean to improve the nutritional value of DDGS in diets for carnivore fish. Biotechnologically upgraded barley protein concentrate (BPC) from ethanol production have also shown a potential as a feed ingredient for Atlantic salmon and rainbow trout (Burr, *et al.*, 2011; Morken, *et al.*, 2011a). On a dry matter basis, the BPC contain 58% protein, 13% lipid, 4% ash and 4% starch. The protein digestibility was estimated to 96% for Atlantic salmon (Burr, *et al.*, 2011). These fish experiments were carried out with a BPC test ingredient and is yet not commercial available.

4.10 Peanut meal and oil

Peanut meal available as a feed ingredient is derived from extracted peanuts not suitable for human consumption. Peanuts contain 35-40% oil before oil extraction carried out with a combination of pressing and extraction. The main fatty acids in peanut oil are 45% oleic acid, 30% linoleic acid and 10% palmitic acid. The global yearly production of peanut oil is about 4.5 million metric tons, with China, India and Nigeria as the main producing countries. Extracted peanut meal have variable chemical composition with an average content of protein, fat, fiber, and ash of 45.6, 2.5, 8.3, and 5.0% (Batal, *et al.*, 2005). Peanut protein is low in methionine and extremely low in lysine, but is high in Arginine. Peanuts can be a good source of protein and energy in fish feeds. Peanut meals tested in diets for warm water species has a high palatability and has a high protein digestibility. Heat-treated meals have no reported anti-nutritional properties that affect fish, though caution should be exercised in their use. Peanuts have a high susceptibility to contamination with the fungus, *Aspergillus flavus*, which produces aflatoxin. In spite of these positive characteristics, their use in fish feeds is limited maybe because of limited supply.

4.11 Carbohydrates (Binders)

Traditionally, carbohydrates are used to provide the targeted physical properties of extruded diets. A positive correlation is reported between amount of starch added to extruded fish feed and expansion, durability and hardness (Kraugerud, *et al.*, 2011). Starch sources with good binding and expansion properties are needed, because starch have to be kept to a minimum in diets for salmonids due to the low capacity of carnivorous fish to digest and metabolize

starch (Hemre, *et al.*, 2002). Expansion of the pellet is correlated with oil absorption (Sørensen, *et al.*, 2011) and is therefore an important measure in order to top coat the pellets with oil in post extrusion applications. The functional properties of starch are activated by gelatinization, a process that is mediated by free water and elevated temperature in the system. Use of pre-gelatinized starch improved pellet quality both in steam pelleting (John, 1987; Zimonja and Svihus, 2009) and extrusion (Sørensen, *et al.*, 2010) compared to native starch. The functional properties vary among starch sources (Sørensen, *et al.*, 2010; 2011). Potato starch result in higher physical quality measured in terms of expansion, hardness and durability compared to wheat starch or wheat (Sørensen, *et al.*, 2010). However, potato starch also gives a higher fat leaking than wheat (Sørensen, unpublished results). Pea and beans can be used as combined protein and starch sources due to their relatively high starch content. When functional properties of a starch rich pea fraction were compared to wheat (Sørensen, *et al.*, 2011), wheat gave a harder pellet, however, pea starch gave a more durable pellet. The two starch rich ingredients behaved differently during the process. Øverland *et al.*, (2009) did not observe differences in pellet quality when PPC (rich in starch) replaced fish meal in extruded diets for Atlantic salmon. Wheat of food grade quality is commonly used as a binder in extruded fish feed because of its excellent binding properties. In addition to wheat, also WG is used as a digestible binder in fish feed improving the physical quality of extruded fish feed (Draganovic, *et al.*, 2011). In addition to digestible starch rich sources and protein, there are numerous indigestible binders that could be used. These are commonly not used in extruded feed because they add cost to the formulation and dilute digestible energy.

4.12 SWOT plant ingredients

Strength

Plant ingredients are at present the most promising alternative protein and lipid sources in fish feed. In particular varieties of grain legumes, pulses and cereals have shown great potential as new protein and oil sources (Gatlin, *et al.*, 2007) because of their global availability and competitive pricing. These resources are consequently more sustainable in that they relieve the pressure on fish meal and fish oil. Use of these alternative ingredient resources has enabled the aquaculture industry to grow without using more fish meal and fish oil (Tacon and Metian, 2008). Based on this development, it is also projected that total use of fish meal in aquaculture feeds will decrease while the use of fish oil will remain stable (FAO, 2011a). The strength of some of the plant oils, such as rapeseed and soybean oils, are their low contents of saturated fatty acids making them well suited in salmon feed used at low environmental temperatures.

Weakness

The main drawbacks for using alternative plant protein sources in fish feeds is largely due to a low protein content and inadequacies in their amino acid composition (essential amino acid deficiencies), relatively high levels of some indigestible complex carbohydrate fractions and sugars. Also the presence of several anti-nutritional factors and low palatability can contribute to reduced nutritional value and limitations (Francis, *et al.*, 2001; Drew, *et al.*,

2007b). Histopathological changes in the gastrointestinal tract is observed when SBM is used in higher inclusion levels, higher than 20% in the diet of Atlantic salmon, and higher than 30% in diets for rainbow (Baeverfjord and Krogdahl, 1996), or when pea meal is used in higher concentration than 35% (Penn, *et al.*, 2011). There is a concern that use of modern diets with high inclusion level of plant ingredients are compromising fish health making the fish more exposed to diseases (Dale, *et al.*, 2009). Mycotoxins can also represent a challenge with use of more plant ingredients. The main and significant weakness of the plant oils available today is their lack of the VLC n-3 fatty acids found in fish oils. These fatty acids are shown to be essential to Atlantic salmon (Ruyter, *et al.*, 2000a,b,c) and Rainbow trout (Castell, *et al.*, 1972a; 1972b; 1972c). High levels of these VLC n-3 fatty acids stored in muscle lipids are also considered as important for the nutritional quality of the products. Further, some of the plant oils, as palm oils, have high percentages of saturated fatty acids resulting in digestibility problems at low water temperatures (Torstensen, *et al.*, 2000). The effects of plant oils on fish health and welfare is highly dependent on the type and level of both fish oil and plant oil in the diets. Adverse effects of plant oil inclusions have been described in stress resistance, immune parameters or histology of different tissues, in particular in relation to high inclusion levels of plant oils rich in n-6 fatty acids (Montero and Izquierdo, 2010).

Despite the abundant supply of plant ingredients with high nutritional quality, the aquaculture industry is faced with the opinion that some of these ingredients can also be used directly for human consumption. The human food market is becoming more and more significant, both in demand and pricing, and is it unclear if animal feeds can compete for plant oils such as soybean and corn oil supplies (Brown and Hart, 2010).

Opportunities

Advances in feed processing technology such as dehulling, fractioning (Drew, *et al.*, 2007b; Randall and Drew, 2010; Burr, *et al.*, 2011), extrusion cooking (Sørensen, *et al.*, 2002; 2005; Barrows, *et al.*, 2008; Morken, *et al.*, 2011a; 2011b), pre-enzyme treatment (Denstadli, *et al.*, 2006; 2007) or use of protein concentrates following extractions of non-starch polysaccharides, has resulted in a new generation of products applicable in fish feed formulations (Drew, *et al.*, 2007b; Gatlin, *et al.*, 2007). Also plant breeding are used to produce varieties low in anti-nutritional factors such as canola (Anderson-Hafermann, *et al.*, 1993) and lupins (Petterson, 2000), or glandless varieties of cotton seed (Lusas and Jividen, 1987). The emergence of the fuel ethanol and the bio-diesel industries has increased the total quantities of bio-fuels co-products. Recent technologies in the fuel-ethanol industry have allowed the industry to fractionate and upgrade the ingredients before it goes into fermentation or to fractionate the co-products after fermentation, which has resulted in by-products with potential to also be used as ingredients in fish feed (Barrows *et al.* 2008). GM soybean and rapeseed oils containing EPA and DHA seem to be a possible future resource to give salmon industry enough of these important compounds to secure the nutritional value of their products.

Threats

For salmon feed, the cost of ingredients accounts for approximately 75% of the production cost of formulated feed. Increasing commodity prices will therefore have a large impact on the production cost (FAO, 2010). The ingredient prices and feed prices are also associated with fluctuations – and overall rising energy and fuel prices, in addition to the supply and demand of the ingredient market. The non-GMO policy of Europe adds \$50-60 per tons of soybeans. In addition, the feed industry needs to have expensive quality programs to ensure non-GMO.

Both krill, algae and GMO-based plant oils, all containing EPA and DHA, may in a longer perspective have the possibility as resources for salmon industry. But production costs and focus on direct human use may hamper the development of these resources for the aquaculture industry. Also, if the non-acceptance of GMO-products in Norway is continued, the use of these oils in other countries, like Chile, may be a significant threat to our salmon production.

5 Microbial ingredients

Microbial ingredients from bacteria, yeast and microalgae are new ingredients that have a potential in diets for salmonids. Extensive research has been carried out to investigate the nutritional value of biomass produced from methane (Bioprotein) as a feed ingredient for Atlantic salmon, rainbow trout and Atlantic halibut (Storebakken, *et al.*, 2004; Berge, *et al.*, 2005; Aas, *et al.*, 2006a; 2006b; 2007; Øverland, *et al.*, 2010; Romarheim, *et al.*, 2011). Recent research has also shown that bacterial meal produced from natural gas can be used to prevent SBM-induced enteritis in Atlantic salmon (Romarheim, *et al.*, 2011). The chemical composition of bacterial biomass depends on factors such as substrate and conditions of fermentation, type of bacteria, and processing after fermentation. Spray-dried bacterial biomass has a proximate chemical composition similar to fish meal with approximately 96% dry matter, 70% crude protein, and 10% crude lipids. The lipids in bacterial meal are mainly phospholipids. Fatty acid composition is dominated by C 16:0 (49%) and C 16:1 n-7 (36%), as reported by Øverland *et al.* (2010).

Microalgae as a future ingredient resource have gained more interests lately. The chemical composition of microalgae varies depending on the species, and the potential as a feed ingredient varies accordingly (Skrede, *et al.*, 2011). Microalgae are natural food resources for certain fish species, and for zooplankton in the food chain. Nutrient digestibilities of the three microalgae species *Nannochloropsis oceanica*, *Phaeodactylum tricornutum* and *Isochrysis galbana*, were recently investigated with mink (Skrede, *et al.*, 2011). The protein digestibility, determined by linear regression, was 35.5%, 79.9% and 18.8%, respectively. Apparent protein digestibility of *Spirulina* algae was recently estimated to be 84.7% in Atlantic salmon (Burr, *et al.*, 2011). These results clearly demonstrated that some microalgae have a potential as a fish meal replacer in feeds for Atlantic salmon. Nutrient availability and utilization in the diet is, however, highly variable among various genus. The greatest potential for microalgae in the future may be as a producer of VLC n-3 PUFA. Biotechnological production of lipid-rich marine microorganisms with up to 47% lipids, rich in EPA and DHA is possible with fermentation technology. In a study evaluating DHA rich oil from a single cell microorganism, the thraustochytrid *Schizochytrium* sp. (Miller, *et al.*, 2007), replacement of fish oil with 100% Thraustochytrid oil in Atlantic salmon parr diet significantly increased the level of DHA in muscle tissue without any detrimental effect on growth. This thraustochytrid oil contained very high concentration of DHA (about 35%), and can thus also be used in blends with other oils to obtain a fatty acid profile closer to that of the natural diet of salmon.

Use of microbial ingredients is expected to increase in the future. Care need to be taken regarding the nutrient concentration and nutrient digestibility. Microalgae may contain toxins, and production of other microbial ingredients may assimilate undesirable constituents from the substrate on which it is growing.

5.1 SWOT microbial ingredients

Strength

Bacteria, yeasts and algae (microbial ingredients) can be produced under strictly controlled conditions. Depending on the organism, the proximate composition and amino acid pattern can be rather similar to fish meal (Øverland, *et al.*, 2010), and be sources for both protein and lipid. Recent research has also shown that bacterial meal can be used as a functional ingredient to prevent ingredient induced enteritis in salmon (Romarheim, *et al.*, 2011). Besides, microbial ingredients can be grown on substrates with minimum dependence on soil, water, and impact on climate conditions.

Weakness

For microbial ingredients a major challenge is bioavailability of nutrients. Many of the microalgae have rigid cell walls that impair protein digestibility of nutrients, and similar problems have been observed in both bacteria and yeast. Technology is therefore needed to improve the nutrient availability. The cost of some of these ingredients is also a main drawback. There is a high investment cost in the production technology. Research has also demonstrated the variation in nutritional quality of different sources microbial ingredients. Before use, each source of microbial ingredient requires thorough investigation to define the potential as a feed ingredient. All new ingredients in this group need development of a reliable large-scale production technology to be able to supply quantities of the ingredients that are commercially interesting to the salmon feed industry.

Opportunities

The greatest opportunity for microbial ingredient lies in the vast number of different organisms that potentially can be grown. Some of these microbial ingredients can be used to reduce waste problems in industrial production. One example is the zygomycetes *Rhizopus oryzae*, which is able to assimilate different sugars present in spent sulphite liquor, a waste product from the paper pulp industry with a high organic content. Marine microalgae have great potential to produce VLC n-3 fatty acids. The microbial ingredients are produced in closed and controlled systems with minimal risk to spread the organisms to the environment. Use of GMO to modify the fatty acid profile to a more favorable composition for the aquaculture industry should be of less risk to the environment and more acceptable than to grow GMO crops on the field.

Threats

There is an extensive need for research to investigate potential of microbial ingredients as feed ingredients, as well as production technologies to produce them. Large investments in production and processing facilities add costs to the ingredients. The products may be too expensive for the aquaculture industry.

6 Terrestrial animal by-products (protein and oils)

Animal by-products (ABPs) from terrestrial animals, such as bone, meat, skin and feathers, are resources that have a potential to be used in diets for fish. ABPs have been viewed as products with high variability in their chemical composition and low digestibility of the protein (Bureau, *et al.*, 1999; 2000). Nutritional quality of rendered animal protein ingredients is affected by composition, freshness of the raw materials, as well as cooking and drying conditions. Poppi *et al.* (2011) reported that manufacturing practices in the production of feather meal have been improved in Europe and North America, resulting in improved protein digestibility. However, apparent protein digestibility of feather meal has been reported to vary from 67 to 87% (Bureau, *et al.*, 1999; 2000; Sugiura, *et al.*, 2000; Cheng, *et al.*, 2004; Laporte, *et al.*, 2007; Davies, *et al.*, 2009; Laporte, *et al.*, 2009). Feather meal and poultry by-product meal is used as alternative protein sources in production of salmon in North and South Americas (Tacon, 2005; Poppi, *et al.*, 2011), but their use in Europe is currently prohibited because of fear of Bovine Spongiform Encephalopathy transmission. The European Union Animal by-products regulation only allows use of hydrolysates with molecular weight smaller than 10 kD from ruminants, as well as processed blood meal from non-ruminant animals (Mattilsynet, 2011b). The Norwegian aquaculture industry is currently not using ABPs because they fear the consumers' perception.

Poultry by-product can be a promising well balanced protein ingredient for carnivorous fish. Fat from poultry may, however, not be suitable to replace fish oil in diets for salmonids living in cold water, at least not in the coldest months of the year, because of high melting point (Turchini, *et al.*, 2009) and low content of n-3 fatty acids (Liu, *et al.*, 2004). Hence, poultry by-products should therefore be considered mostly as a source of protein. Poultry by-product meal (PBM) consists of ground rendered clean parts of the carcass, such as necks, feet, undeveloped eggs and intestines, exclusive feathers. PBM shows a greater variation in amino acid composition compared to fish meal. In general, PBM contains higher amounts of non-essential, and lower amount of essential amino acids, especially methionine and lysine, compared to fish meal. In average, the proportions of these two amino acids in PBM are about 30% lower than that of fish meal. This depends, however, on the quality and origin of the meal. The protein content and quality is lower when the meal has a higher content of connective tissues, such as bone and skin, whereas meals with high content of meat and viscera usually have a more favorable amino acid profile. By-products from ruminants contain more saturated fatty acids than by-products from poultry. Saturated fatty acids have a high melting point and are poorly digested by salmonids and encapsulate other nutrients making them poorly digestible (Austreng, *et al.*, 1980). World-wide about 9 million tons of fresh offal in the form of heads, feed and viscera are obtained annually (Hertrampf and Piedad-Pascual, 2000). The annual production of by-products from poultry and turkey production in Norway is 29 500 and 2 100 tons, respectively.

A few studies have been carried out with salmonids to investigate the performance of fish fed PBM, but no studies are publicly available with Atlantic salmon. Replacing 30% of the fish meal with PBM caused reduced growth in Chinook salmon (Fowler, 1991). In rainbow trout, 50% of the fish meal protein could be substituted by PBM without any growth reduction (Steffens, 1994). Full substitution, on the other hand, resulted in a significant growth

reduction. The growth reduction seen by Steffens (1994) in rainbow trout could be partly explained by methionine and lysine deficiency. Because of the high content of connective tissue, some essential amino acids, mainly methionine and lysine, are present in suboptimal proportions in PBM. An optimized combination of poultry by-products with different protein sources will partly compensate for amino acid deficiencies, when formulation is based on documented values for digestible essential amino acid composition in the ingredients. Furthermore, amino acid deficiencies that cannot be compensated by mixing ingredients can be corrected by use of synthetic amino acids (Steffens, 1994).

Large variation in apparent digestibility of protein (64-78%) was found between different batches of PBM in rainbow trout (Dong, *et al.*, 1993). This is mainly explained by origin and processing of the various types of poultry by-products. A rendering process involves one "sterilization" step, with high temperature for long time, resulting in reduction of digestibility of all amino acids, especial cysteine. As a mean to avoid Transmissible Spongiform Encephalopathies, the EU has set strict minimum limits for the heat treatment of ABPs (EC 1774/2002), which may limit the nutritional quality. Besides, digestibility of the protein is strongly affected by the drying of the meal, in particular the temperature at the end of the drying stage, when the water content is low (Opstvedt, *et al.*, 1984). By-products containing feathers require hydrolysis to be digestible, and the degree of hydrolysis is determining for their nutritional value. Thus, hydrolysis may improve the nutritional value.

Because of its low price and high nutritive value poultry by-products have been extensively used in salmon feeds in Chile and Canada the last years. In Chile alone, the inclusion of animal by-products, mainly PBM and hydrolyzed feather meal, have reached approximately 150,000 tons per year. This extensive use, however, has little publicly available scientific basis. In the EU and Norway, the inclusion of animal by-products, except non-ruminant blood meal and hydrolyzed proteins are prohibited, meaning that the European industry has an economic disadvantage. The main rationale for the ban on poultry by-products is the risk of contamination from ruminants. Since molecular techniques (PCR-based analyses) are now available that can discriminate between materials with different species origin (Fumiere, *et al.*, 2006; Santaclara, *et al.*, 2007), the ban on poultry products in animal feed, including fish feeds, may be raised within the EU.

6.1 Blood meal

Blood can be divided into plasma and red blood cells, 60 and 40%, respectively. Blood as feed ingredient commodity are sold as whole blood meal, haemoglobin meal and plasma powder. In Europe, only products of non-ruminant origin can be used in feeds. According to the European Animal Protein Association, the annual European production of blood meals (including full blood and haemoglobin meals) suitable for use in feeds are 40 000 tons of spray dried products and 50 000 tons of others. Spray dried plasma powder is commonly used in formulas for early weaned pigs and calves because it promotes passive immunity. For salmonid farming, full blood meal and hemoglobin meals have the greatest potential.

Blood meal has a high protein content and is a good lysine source (El-Haroun and Bureau, 2007). The protein content is typically 80% in full blood meal and as high as 95% in

hemoglobin meal. Blood meal is reported to prevent cataract in salmon, mediated by the high level of histidine (Breck, *et al.*, 2003). The low content of the essential amino acid isoleucine may limit the inclusion level of blood meal in salmon diets.

Spray dried blood meal have been reported to have excellent protein digestibility (~ 99%), whereas other drying methods may reduce digestibility to ~80% in rainbow trout (Bureau, *et al.*, 1999). EU regulations (EC 1774/2002) set minimum requirements for heat treatment of blood meal and other ABP meals, which may limit the possibilities of producing blood meal with optimal nutritional quality. Spray-dried blood meal is also reputed among fish feed manufacturers to have a positive technological (binding) effect in the production of fish feed, but this effect is also reduced by heat treatment leading to the denaturation of proteins.

Blood meals are high in iron, because of the high content of hemoglobin. The high iron content may be limiting the inclusion of blood meal in fish feed in order to avoid iron overload (Rørvik, *et al.*, 2003). Iron is a prooxidant and may therefore have a negative impact on the stability of the carotenoid pigment astaxanthin as well as lipid stability in fish feed.

6.2 SWOT animal by-products

Strengths

ABPs in particular from poultry, feather and blood-products are considered valuable ingredients around the world and are therefore extensively used in aquafeeds. The high protein content for example in blood meal leave an open space in the feed recipes and make this ingredient ideal in combination with cost efficient low-protein plant ingredients. The lysine content in blood meal is even higher than in fish meal, and can therefore counteract the low level of lysine in plant ingredients. It also has high histidine content, an amino acid needed to prevent cataract in salmon.

Weaknesses

Poultry by-products contain saturated fat, reducing energy digestibility, in particular in cold water. The protein quality of by-products can also be reduced by the processing of meals because extensive heat treatment is used for hygienic reasons as well as for drying. Feather meal may have low protein digestibility due to disulphide bonds. High iron content in blood meal limits inclusion because of oxidation of astaxanthin and/or overload of iron in the fish. Blood meal also has a low content of isoleucine that may give an imbalance in the branched chain amino acids. The content of ash is also high in some ABPs. In Europe, the volume of these by-products is rather low. Though, the limitation in using these ingredients is the present feed act and European ban.

Opportunities

The nutritional quality of ABPs can be improved by removing bones from the by-products. Low-temperature products may increase nutritional quality of feeds and at the same time give technical benefits that allow for higher inclusion of technically challenging ingredients, such as unicellular protein meals and some plant meals. If the feed manufacturing plant is

located near a poultry slaughter house, it is also possible to use fresh by-products in extruded feeds. Higher volumes of blood products can be available if blood from ruminants is re-introduced.

Threats

Europe is the most important market for Norwegian salmon. Because ABPs have not been used in animal feed for many years, the consumers may have negative reactions. It is also likely that the price of these ingredients will increase with increasing demand. The prime quality poultry by-products are used in pet food, a strong competitor to the salmon feed industry.

7 Other

7.1 Blue mussel

Blue mussel (*Mytilus edulis*) has repeatedly been suggested as a possible feed ingredient for fish, because of a favourable amino acid and fatty acid profile, as well as astaxanthin content. It is also seen as an important species in integrated multi-trophic aquaculture (Reid, *et al.*, 2010), because of its ability to filter feed and faecal particles from the water, and recycle waste into valuable nutrients. When blue mussels are grown with adequate nutrient supply, the growth rate may be very high, still individual variation in size is large at harvest. It is possible to sort the harvested mussels, and those under-sized for marketing may very well be used in feed. Blue mussel has been tested as an ingredient in diets for rainbow trout (Berge and Austreng, 1989), and also for other finfish like red sea bream (Kitamura, *et al.*, 1981) and Tiger puffer (Kikuchi and Furuta, 2009). The freeze dried blue mussel meat used in the recent study on Tiger puffer (Kikuchi and Furuta, 2009) provided very good growth, while whole blue mussel with shell seemed to have a slightly (non-significant) negative effect in rainbow trout (Berge and Austreng, 1989).

The blue mussel contains the important nutrients, while the shell fraction may be a dominating part of the product. The shell fraction varies with age, time of year and feeding status. In order to use blue mussel as a feed ingredient for salmonids, there is need for technology to separate meat and shell, and the mussels should preferably be harvested at a time when meat to shell ratio is high in order to obtain the highest yield. There is also a need for technology for careful drying of the meat. Large scale production and stability in availability of blue mussels may also be a challenge.

Blue mussels may contain toxins in periods when they feed on toxic algae. These alga toxins are dangerous to fish as well as to humans. The toxins are accumulated in the blue mussels, and depending on total level, several weeks are needed for detoxification (Sephton, *et al.*, 2007).

Strengths: Favourable nutrient content in meat fraction, palatability enhancer

Weaknesses: Lack of technology for large scale production and processing

Opportunities: Growing on waste or algae in the sea

Important species in integrated multi-trophic aquaculture

Threats: Carrier of algal toxins

7.2 Insect meal

Insect larvae may have a great potential as an alternative ingredient in feed for carnivore fish. The chemical composition of prepupa larvae varies with species, age, method of processing and the substrate maggot is produced on (St-Hilaire, *et al.*, 2007a, b; Aniebo and

Owen, 2010). Newton et al. (1977) reported that black soldier fly, *Hermetica illucens* (L.), contained approximately 40% protein and 30% fat on a dry matter basis. The protein, fat and ash content of black soldier fly prepupae reared on swine manure, was on a dry matter basis analyzed to be 43.6%, 33.1% and 15.5%, respectively (St-Hilaire, et al., 2007b). Housflye pupae commercially grown on cow manure had on a dry matter basis a protein, fat and ash content of 70.4%, 16.1% and 2.2% (St-Hilaire, et al., 2007b). The lipid composition is dominated by short and medium chained saturated fatty acids, but the amount and lipid composition can be manipulated by the substrate in the growth media (Sealey, et al., 2011). Black soldier flies fed 10% fish offal and 90% cow manure increased the lipid content by 43% compared to those fed only cow manure. The amount of long chain polyunsaturated omega 3 fatty acids (EPA, DHA and ALA) was also increased from less than 0.1% to 3% of the total lipids (Sealey, et al., 2011). Amino acid composition of black soldier fly prepupae and domestic house fly pupae was reported by St-Hilaire et al. (2007b). In comparison to an average quality fish meal, the content of arginine, histidine, isoleucin, leucine and lysine was slightly lower, while the content of methionine and valine was higher. Sealey et al., (2011) found that prepupa larvae from black soldier flies grown on 10% fish offal and 90% cow manure, could replace 50% of the fish meal in diets fed to rainbow trout without adverse affect on growth or sensory quality of rainbow trout fillets. In contrast, an earlier study with rainbow trout fed black soldier fly prepupa grown on swine manure gave a significant lower weigh gain and higher feed conversion ratio when 50% of fish meal was replaced with insect meal (St-Hilaire, et al., 2007b). A study with striped bass showed that full replacement of fish meal with insect meal gave lower growth rate and higher feed conversion ratio (Dabramo and Papadoyianis, 2009). The studies with rainbow trout and striped bass clearly indicate a great potential for use of insect meal from prepupa in feed for fish.

Strengths: favourable nutrient content, grown on animal manure or other waste, direct conversion of waste to valuable nutrients

Weaknesses: lack of large scale production technology, more knowledge is needed about practical use in carnivore fish diets

Opportunities: no need of land or sea areas for production, no limitation in resources for production. Unique way to recycle VLC n-3 fatty acids from fish offals with poor quality not suitable for other purposes. The growth media can be used as fertilizer

Threats: the hygienic quality need to be carefully evaluated to avoid contamination with harmful bacteria

8 Genetically modified organisms

For two decades now, GMO has been grown and each year more agricultural land is planted with GMO crops. The purpose with gene modification is to transfer genes expressing wanted traits into a host. Genetic engineering has generated plants with an innate resistance to pests, tolerance to herbicides and draught, changes in nutrient content, product quality and technical properties. Despite the power of this technology, there has been a strong resistance to the introduction of GMOs in agriculture and for consumer food products in Europe (Vergragt and Brown, 2008). According to these latter authors, the public objections is based on many issues such as a concerns about the risk assessment, the ethics and equity issues, power relations and the mistrust of technocrats and public authorities. Consequently, Europe has the world's strictest system for approval of GMOs. A GM plant ingredient can only be allowed onto the market if it can be documented, based on scientific data, that it is just as safe and healthy as a comparable conventional product (EFSA, 2008). Safety assessments include toxicity testing of the newly expressed proteins, potential occurrence of secondary effects, potential for horizontal gene transfer to other species, the potential allergic effects of newly inserted traits, and the role of the new food in the diet (Kuiper, *et al.*, 2001).

Most feeding trials with mouse and rats carried out to test safety of GM ingredients with improved agrotechnical traits, have shown no clinical effects or histopathological abnormalities (EFSA, 2008). A number of studies is also carried out to investigate growth performance, feed utilization, as well as health aspects when Atlantic salmon was fed GM soy (Roundup Ready[®]; GM-soy) or GM maize (MON810[®]Bt-maize) (Hemre, *et al.*, 2005; Sanden, *et al.*, 2005; 2006; Bakke-McKellep, *et al.*, 2007; Hemre, *et al.*, 2007; Sagstad, *et al.*, 2007; Bakke-McKellep, *et al.*, 2008; Sissener, *et al.*, 2011). Overall the results have shown that up to 130 g/kg Roundup Ready[®] GM-soy and up to 121 g/kg (MON810[®]Bt-maize) GM maize can be fed to Atlantic salmon without adverse effect on growth, feed utilization or health (Hemre, *et al.*, 2005; Sanden, *et al.*, 2006). Differences observed in body composition, gut histology and blood parameters was not associated with the moderate inclusion of the GM ingredients (Sanden, *et al.*, 2005; 2006; Bakke-McKellep, *et al.*, 2007; 2008). Some differences in organ somatic index (liver, spleen, head kidney) as well as changes in glucose transport between salmon fed GM and non-GM maize need to be addressed in future studies (Hemre, *et al.*, 2007). The safety feeding salmonids GM plant ingredients were recently discussed in the VKM report (Hemre, *et al.*, 2009) and Sissener *et al.* (2011). These reports conclude that GM commodities approved by the EU so far is safe to use. However, the safety assessment is challenged by a tremendous increase in new GMO varieties. Besides, approval of GMOs by the EU is not only based on safety assessment. Also environmental risk assessments, as well as ethics, in the use and production need to be documented by the producer (or applicant). According to the EEA agreement by EU member countries, Norway has to evaluate all GMO-notifications received by the EU. The notifications are evaluated according to the provisions in the Act of 2 April 1993 No 38 regarding the production and use of GMOs (Norwegian Gene Technology Act). The procedures for approval of GMOs in Norway are similar to the EU regarding the health and environmental risk assessments, but differs in that the Norwegian Gene Technology Act also require an assessment of the socio-economical impact as well as benefit to society (Myhr and Rosendal, 2009). Consequently, new GMOs

may not be approved in Norway despite that they are considered safe for human health as well as environment.

According to the EU legislations (1829/2003 and 1830/2003), GM containing feeds and products need to be labelled when GM material exceed 9 g/kg. The Norwegian food authorities have given a temporarily approval for use of GMO ingredients in fish feed (Mattilsynet, 2011a). Thus the Norwegian fish feed industry have the opportunity to use GM ingredients, but hesitate to use it because of the European consumer skepticism towards GMO.

9 Sustainable use of ingredients

The sustainability issue of alternative ingredients was left out from the SWOT mainly because there are at present no objective methods to measure sustainability of an ingredient. The growth in the salmon production has raised concerns about the environmental impacts of salmon farming and opinions regarding sustainability. The increasing consumer awareness about sustainability and food safety has forced the aquaculture industry to improve documentation of food safety and environmental impact of the production. Criteria's for sustainable production is however not clearly defined, and until now this discussion has mainly been focused on use of fish meal and fish oil. The former dependence of the aquaculture feed industry on fish meal and fish oil and the effect this may have on wild fish stocks is the main criticism against sustainability of salmon production. Several publications have therefore discussed the fish in : fish out ratio of salmon production. The main conflict of interest have been the different ways of calculating this ratio (Tacon and Metian, 2008; Naylor, *et al.*, 2009; Crampton, *et al.*, 2010; Bendiksen, *et al.*, 2011) and this ratio varies accordingly. Bendiksen *et al.* (2011) demonstrated that salmon became a net producer of fish protein and had a greater deposition of oil than consumed from high quality fish oil sources. This result may suggest that salmon production has become more sustainable by use of more plant ingredients in the diet. This is also supported by use of Life Cycle Analysis (LCA) to quantify the Ecological Footprint of Salmon feed (Buttle, *et al.*, 2011). According to LCA, ingredients from marine ecosystems have higher footprint than those from terrestrial systems, based on the assumptions that terrestrial monoculture crop systems are more productive per unit crop area. The LCA will however, not take into consideration negative impact of crop production on water consumption, deforestation, soil erosion, use of fertilizers (limiting P-resources), potential negative impact of growing GMO crops and use of pesticides. Marine resources are renewable assumed that the fish stocks are well managed, while plant ingredients require fertilizer that also contain nutrients from non-renewable sources (P). It is very likely that Atlantic salmon becomes a net producer also of fish oil in the future, assuming use of GMO ingredients high in EPA and DHA or microbial ingredients rich in these fatty acids. Still it may not be a more sustainable production if non-renewable input factors are used in the production of such ingredients.

Harvesting marine resources at a lower trophic level is considered to improve the eco-footprint (Buttle, *et al.*, 2011). However, major concerns are also raised regarding negative ecosystem impacts of harvesting krill. Krill is at the base of aquatic food webs and harvesting could reduce food resources for predators such as penguins, seals, and whales. The standing biomass of krill in the Antarctic is also vulnerable to climate changes. It is therefore a need to improve the knowledge about population dynamics of krill to ensure sustainable catch quotas (Suontama, *et al.*, 2007; Naylor, *et al.*, 2009). Diverging opinions were also revealed in a recent study assessing use of alternative feed ingredients to replace fish meal and oil (Gillund and Myhr, 2010). The main objective of this latter study was to 1) identify the key issues that need to be addressed when new ingredients are evaluated for salmonids, 2) to gather knowledge and perceptions among different actors in the Norwegian aquaculture industry, and 3) to identify uncertainties associated with use species from lower trophic levels, by-products and by-catch from fisheries and aquaculture, ABPs, plants, GM plants, nutritionally enhanced GM plants and products from microorganisms and GM

microorganisms. Participants from different interest groups within Norwegian salmon aquaculture were interviewed in the project. The participants in the project identified gaps of knowledge but they also had diverging opinions regarding these knowledge gaps. To a large extent their opinion was coloured by their area of expertise. This demonstrates the complexity in defining universal criteria's to be used in the discussion about sustainability of alternative ingredients.

10 Concluding remarks

The salmon diets have over the past ten years developed tremendously from fish meal based to multisource based. Fish meal and fish oil will still be important ingredients in salmonid diets, but at lower inclusion levels, perhaps more like functional ingredients. Plant ingredients are expected to be major ingredients in feed for salmonids for decades ahead. Because of the strict food safety and gene-technology acts in Europe, non-GMO ingredients from plants are used to replace fish meal and oils. ABPs, in particular from poultry have a great potential, but is still banned to be used in Europe. However, the aquaculture industry need to seek for new generation ingredients that are less attractive for direct human consumption, such as ABPs, zooplanktons, microbial ingredients and worms. For microbial ingredients like algae, bacteria and yeasts there is a need for reliable large-scale production systems to supply amounts that can make the products commercially interesting. Most of these products will also need processing to make the nutrients available for the fish, and a stable quality is required.

The consumer opinion is important for the reputation and credibility of the aquaculture industry. However, the consumer preferences and presumption of seafood is multi-faceted. Overall, consumers are concerned with sustainability of the food production, as well as food being healthy and cheap. A major challenge is how to evaluate sustainability. Replacing fish meal and fish oils with plant resources is considered as a sustainable alternative. However, critical voices are debating sustainability of using plant instead of fish resources and claim that also water footprint, soil erosion, use of fertilizer and deforestation need to be evaluated for feed crops.

A crucial question for the future is how to secure access to reliable sources of the VLC n-3 fatty acids EPA and DHA. For the near future there is a need to use all existing resources in the most efficient way, but there is also a need for new sources. Krill oil is already produced, but in too low quantities and with too high prices for the aquaculture industry. In a longer perspective the marine microalgae may become an important source. GM plants producing fat containing the VLC n-3 fatty acids may be ready for use in a relatively near future (5-10 years?). Selective breeding of fish that are able to utilize the available n-3 sources efficiently is also a tool for the future.

In order to maintain the competitive strength of Norwegian aquaculture there is a need for extensive research on alternative ingredients, on production and processing technology needed to allow efficient and acceptable use of these ingredients. Some of the actual ingredients, like GMO-crops and land ABPs are already in use in the salmon industry in other parts of the world and the fish produced are sold in Europe.

Suggestions for actions:

- Urgent need for reliable EPA/DHA resources.
 - Future promising ingredients like algae and yeast can be seen as combined protein and lipid sources. However, there is a gap in knowledge regarding production technology, bioavailability and general use of these ingredients

- Oils from GM plants will soon be on the market. More knowledge of the use in salmonid farming is needed, especially the influence of long term use on health and quality
- Krill oil consists mainly of phospholipids. The effects on feed processing, feed technical quality and digestibility are uncertain.
- Research on safe use of ABPs in Europe
- Research on better use of already existing and new marine sources/by-products to improve and stabilize both protein and oil qualities.
- Research on processing and upgrading of plant ingredients, how to concentrate nutrients and reduce anti-nutrients. Knowledge is needed on possible interactions and additive effects when several plant ingredients are used in large total amount of diet
- Research on upgrading and use of third generation ingredients such as by-products after bio-ethanol/diesel production. These by-products are not suitable for human consumption, but are used in terrestrial animal feeds.
- Research on the long term effects of a "modern" salmon diet with low or none fish meal and fish oil inclusion. Investigate the long term effects on fish health, productivity, quality and emissions to water.
- Nutrient requirements in fish are not well defined, and with "modern" low fish meal / fish oil diets it is even more necessary to know the requirements (amino acids, fatty acids, vitamins and minerals)

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