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Values from waste

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Auðlindir og afurðir

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Report summary

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Titill / Title	**Values from waste / Hliðarafurðir í verðmæti**		
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Ágrip á íslensku:	Skýrslan lýsir fyrstu niðurstöðum verkefnis tækniyfirfærsla til þróunar og nýsköpunar við framleiðslu virðisaukandi afurða úr vannýttum hliðarafurðum fiskvinnslu á þremur mismunandi svæðum þ.e. Norður Íslandi, Norður Noregi og Norðvestur Rússlandi. Verkefnið var sameiginlegt átak rannsókna- og þróunaraðila auk fiskiðnaðar á svæðunum. Skýrslan gefur innsýn í magn ónýttra afurða á svæðinu. Auk þess er fjallað um nýtingu þriggja ónýttra hráefna, blóðs, svilja og augna, og mögulega nýtingu þeirra sem lífvirkra efna í sér fóður fyrir fisk auk annara nota.		
Lykilorð á íslensku:	*Auk afurðir, fiskblóð, svil, fiskaugu*		
Summary in English:	The report describes first results of work on technology transfer for development and innovation for production of value added products from underutilized by-products of fish production and processing in three different areas i.e. Northern Iceland, Northern Norway and North Western Russia. The project is a joint effort of research and development entities and fish processing industries in the above mentioned areas. The report gives an overview on availability of underutilized by-products in the area. In addition, possible ways of utilizing three different by products, fish blood, fish testes and fish eye compounds, and how they might be used as bioactive compounds into speciality feeds for aquaculture and other possible products.		
English keywords:	*By products, fish blood, fish testes and fish eye compounds*		

Contents

Report to NORDREGIO:

Project: Values from waste No A15016

Development of fish feed based on unused raw materials from fish processing in the Nordic region.

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1 Background

There is a large international attention on environmental and sustainable management and development of fishery resources and industrial fish farming. The Nordic industry has traditionally been a supplier of raw material for traditional food processing, or export of unprocessed raw material to other countries. Historically, by-products from the industry has a low value, or in many cases no value at all. During the last years there has been a growing interest in utilization of this resource. High-tech products and tailor made food/feed for humans and animals have a huge potential, and are only at its infancy in Nordic countries.

By-products products of fish production may include blood, heads, eyes, tongues, livers, testes, roe, cut-offs, skin, bones, backs, guts and swim bladder. Currently, much of the by-products that is being taken care of is conserved by ensilage, and processed for further use as gross animal feed ingredients where the value creation is relatively low. Nevertheless, the level of utilization of raw materials may be increased, and more advanced products developed, and contribute to value creation in the fisheries and aquaculture industry. Many seafood producers welcome the increased focus on the by-products utilization, recognizing the possibilities for value added production and alternative use of by-products and waste. This will not only increase the economy of the sea food industry, but also strengthen the society and increase establishment of high tech jobs in Nordic countries. Material, such as blood and testes of fish processing, may be used in the development of functional feed for fish and warm blooded animals. This may increase utilization of by-products and waste of the fish processing in fisheries and aquaculture, add value to the industry production, and reduce waste of potentially high value feed ingredients.

In general, fish products are low in calories and a very healthy food. New technology has made it possible to explore the therapeutic importance of fish-based diet on cardiovascular diseases, neurodegenerative diseases, radicals-mediated diseases, and cancer. Fish have a positive impact on human health and development. This is related to the fact that fish products, in addition to their high nutritional value, contain biochemical ingredients that may have bioactive properties. Bioactive peptides have shown various biological activities including antihypertensive, antibacterial, anticoagulant, anti-inflammatory, and antioxidant activities, and may be a potential material for biomedical and food industries. The unique properties of marine proteins give them a role as functional ingredients in food. Bioactive peptides may contain 3–20 amino acid residues and their activities are based on their amino acid composition and sequence (Khora 2013).

Different ingredients of by-products could be integrated into, and form so-called functional feed for fish. A definition of functional food for humans could go equally well for other animals and fish; “A functional food is a food given an additional function (often one related to health-promotion or disease prevention) by adding new ingredients or more of existing ingredients”. The general category of functional foods includes processed food, or foods fortified with health-promoting additives. Functional foods are part of the continuum of products that individuals may consume to increase their health and/or contribute to reducing their disease burden. Functional food may be a natural or processed food that contains known biologically-active compounds which in defined quantitative and qualitative amounts provides a clinically proven and documented health benefit, and thus, an important source in the prevention, management and treatment of chronic diseases of the modern age (Functional Foods and Chronic Diseases 2011). A large proportion of these functional feed ingredients may be derived from by-products that occur under traditional fish production and aquaculture.

The composition of the by-products is complex and may be conserved and processed in various ways. The most important is the separation of oils and proteins. Most of the raw material are utilized for low price products and very little are utilized in the higher paying markets for example, as dietary supplements, cosmetics or pharmaceuticals market. The majority of by-products is used for meal, ensilage and feed for fur-bearing animals. Only 10% is used for more high-value products, such as human food, health food and biochemical.

Similar to blood from warm-blooded animals, blood from fish may be a valuable product in future. Technology for utilization of blood from warm-blooded animals as food and feed ingredients are already developed. The blood is separated in red hemoglobin and a colourless plasma and sold separately to various food applications. Dried blood from warm blooded animals is well known in the

feed/food market. It has a good protein composition with favourable amino acids. As with warm-blooded animals, plasma of fish blood may be used in the food industry as water binders and as an gelating ingredient in the feed/food. Haemoglobin meal, is the red part of the separated blood. It may be used as iron enrichment in food like bread, blood sausages, black pudding etc. Traditional blood products from warm blooded animals has to be dried at high temperature. The proteins denaturize and often get a bitter taste. It has been suggested that the pet food industry might be willing to pay a significantly higher price for dried fish blood as compared to traditional dried blood products (RUBIN report 167). If it is possible to separate plasma from red blood cells, the salmon blood may be used in different, more advanced fish products, whereas the red phase may be used as an iron enrichment in dietary supplements. In Scandinavia, approximately 15 000 tons of plasma of blood of warm-blooded animals are produced per year (2008). The price is around 10 GBP/kg for frozen plasma with a dry weight content of 10%. The red phase, hemoglobin meal, of food quality are sold (2008) for around 10 GBP/kg, but then with a dry weight content of 90% (Rubin report 167).

Special products created from resources such as fish blood and testes may be high value ingredients for use in fish or animal feed to strengthen their stress resistance, disease resistance and improve growth and development. The point is that testes may be used directly at different concentrations, probably small amounts is sufficient. Marine protein and fat are favourable and may replace a significant volume of the existing feed products/ingredients used for pets and pigs, but is scarcely established in the market yet. During early life, pig juveniles feed on milk with high digestibility. Gradually, during the weaning period the milk is being replaced by commercial dry feed. Weaning is a very critical period during the early life of many animals. At this time the digestive and immune system is immature. Often a combination of environmental influences and inferior quality of the feed make the animal juveniles more susceptible to diseases around weaning. In general, the absorption of energy and nutrients tends to be low. This may increase the stress level, affect the immune system negatively, reduce growth, and have large impact on general development in juveniles. The weaning feed for fish, pets and piglets should provide normal development and increased growth in early life of animals.

The market for feed and feed ingredients to different animals is large, for example the market for feed to pets and pig juveniles is large, around 100.000-120.000 tons (Rubin report 167). In swine production 38.000 tons of wet feed mixture (2006) were used (Mattilsynet, 2007). The market for fish feed ingredients in aquaculture is increasing. An increasing volume of fish feed ingredients are coming from soya and other land plants. Because of this, oil as well as other raw material, isolated from farmed salmon and trout, will contain less omega-3 fatty acids than wild fish. This is related to the fact that a large portion of the feed ingredients, that was originally of marine origin, is replaced by raw material from vegetables.

Very little experience and knowledge exists on use of fish blood and testes. In agriculture diets for individual animals is normally set up to optimize milk or meat production. Use of concentrates, a high energy, protein, vitamin and mineral rich feed, is essential for the production. Norway imports around 900 thousand tons of soya every year (2013), approximately 360 thousand tons, mostly as concentrate, is used in aquaculture every year. Because of this, the industry has for a long time been looking for new resources to substitute the imported proteins, vitamins, carbohydrates and minerals.

1.1 General overview of Norwegian seafood industry

Categories of by-products

According to the Norwegian Seafood Research Fund – FHF (Fiskeri og havbruksnæringens forskningsfond) the specific definitions may be used for the different *raw material basis and should apply to wild and farmed fish, shellfish, and mollusks harvested in Norwegian waters, or processed in Norway.*

The by-products are divided into groups depending on origin and on further processing. By-products may be processed according to specific hygienic rules and regulations, and may then be used for human consumption and/or feed for animals. Products according to the by-product regulations (e.g. ensilage, transport without refrigeration to fish meals factory etc.) is termed a by-product. By-products are divided into category II and category III. By-products shall not be used for human consumption.

Total raw material and by-products/by-products in Norway and Iceland

In the present study, public available statistics are used to provide an overview of the fish industry and aquaculture in Norway, where the main sources are the **Directorate of Fisheries – www.fiskeridir.no/statistikk/akvakultur**, Statistisk Sentral Byrå (SSB), Norway and the Norwegian Seafood Council, Analyse av marint restråstoff 2013. Ref Kontali/ SINTEF report A26097, see also references.

The Norwegian sea food industry produces more than 867.000 tons of by-products (Table 1). By-products from aquaculture and fisheries constitute an important value-added resource in Norway. A large proportion of this material is utilized. In salmon and in herring production, most of the by-products is taken care of, for example for salmon around 86% is utilized, whereas in fish industry only 33 % (in 2013) is used (see Table 1).

In Iceland the total production of by-products from the sea food industry is 379.000 tons (Table 2). Of this quantity about 52.000 tons are presently utilized (Arson, 2016. Personal communication).

The total volume of harvested salmon and trout in Norway in 2013 was approximately 1.217.600 tons, which provided 333.200 tons of by-products. Volume of different white fish species, mostly cod, haddock, and saithe, landed in Norway in 2013 was about 900.000 tons. Norwegian vessels landed about 775 000 tons, and foreign vessels around 125.000 tons. Total raw material, rest raw material/by products and utilization in 2013 in different sectors in Norway is shown in Table 1 (Kontali/ SINTEF 2013). Landings of small volumes of other species are included.

Table 1. Total raw material (fish and shellfish) and by-products/waste after processing, and percentages of utilization listed by species in 2013 in Norway. Numbers are given in tons x1000.

	Aquaculture Salmon and trout	**Pelagic, herring, capelin, mackerel**	**White fish, cod, saithe, haddock**	**Shellfish**	**Total**
Total fish (tons)	1 301	965	775	25	3066
By-products available (tons)	350	178	340	12	867
By-products in % of Total fish	26	18	44	48	28
By-products utilized (tons)	289	178	113	5	585
By-products utilized (%) by sector	86	100	33	1	67
By-products utilized (tons)	61	0	227	7	282

In excess of 230.000 tons of by products from the white fish sector is not utilized. This is partly related to lack of efforts in developing new methods and technological solutions on board the vessels for taking care of the by-products. However, also low prices of by-products may explain the low interest amongst fishermen to bring this ashore. In the aquaculture sector, most of the by-products are utilized, except for the fish blood which are treated as part of the process water from the salmon slaughterhouses.

Among all sectors, the white fish sector has the largest amount (230.000 tons) of non-utilized raw materials. Approximately 33 % being of the by-products from Norwegian vessels are utilized. Open sea fleet exploits only 7 %. The coastal fleet utilizes around 60%. In the pelagic sector, almost all by-products are utilized (Table 1.).

According to Kontali /SINTEF (2013), by-products from the Norwegian sea food industry are distributed on different material such as heads, guts, liver, blood from aquaculture (around 36.000 tons) roe and testes (around 7.000 tons each), shells of shrimps and crabs, backs and cut-offs.

Table 2. Total raw material (fish and shellfish) and by-products/waste after processing, and percentages of utilization listed by species in 2013 in Iceland. Numbers are given in tons x1000.

	Aquaculture Salmon and trout	**Pelagic, herring, capelin, mackerel**	**White fish, cod, saithe, haddock**	**Shellfish**	**Total**
Total fish (tons)	10	660	500	30	1200
By-products available (tons)	2	62	300	15	379
By-products in % of Total fish	20	9	60	50	31
By-products utilized (tons)	0	62	250	15	327
By-products utilized (%) by sector	0	100	83	100	86
By-products Unutilized (tons)	2	0	50	0	52

1.2 Fish blood products

In Norway, Iceland and Russia, there are large slaughterhouses for farmed fish, mainly salmonids. In salmon and trout, blood (Figure 1) is around 3.5 and 4.0% (of live-weight) of the fish. This is available and an interesting raw material for further processing and development. However, with the present slaughtering technology, it is only possible collect up to two percent of the fish's weight as blood.

Figure 1. Salmon blood at a slaughter house (Kirkenes Processing AS) in Norway.

With an annual production of farmed fish in Norway of 1.300.000 tons (2015), about 26.000 tons (2%) of salmon blood may be available. Salmon blood contains approximately 10 - 12,5% protein and 0,8% fat with a high content of omega-3 fatty acids (Kjølås, F.H. and Storrø 2005). Blood plasma separated from salmon blood coagulate rather quickly after bleeding. Separate blood plasma of salmon blood

gives weaker gels by heating compared with plasma from warm-blooded animals, however the red phase is forming strong gels. Previous projects have reported large difficulties in collecting and drying fish blood as properties of fish blood was very different as compared to blood of warm blooded animals. Vital Marine, in collaboration with Marine Harvest and Core Competence, did not manage to separate blood plasma and hemoglobin in salmon blood and concluded that utilization of salmon blood must be based on gently dried whole blood (RUBIN-report 151, 188). Large scale separation of salmon blood in plasma and red phase have not been conducted, and methods for collection and separation of blood components are not developed. Thus, plasma and hemoglobin products of salmon, have not been tested by the feed/food industry.

There are two possible ways to approach the problem of collecting fish blood at slaughter houses, namely “dry bleeding” during bleeding and slaughtering of the fish, or by separating fish blood component from the process water after the bleeding. However, the latter may impose several problems related to different types of “pollution” of the process water, e.g. salt, fish faces and fish scales. When using a dry bleeding method at the slaughterhouses, it is possible to collect the blood of salmon during slaughtering. Blood from whitefish from fishery are also a potential raw material, but it would be a difficult task to find methods and equipment for this purpose. However, future prizes on this resource may be incentives to develop methods and equipment that will make it economically feasible to collect blood from wild fish. The collection and processing of salmon blood is a complex task as the blood coagulates even at low temperatures. The addition of an anticoagulant solution to the blood immediately after bleeding may prevent this. The blood can then be fixated or separated in plasma and blood cells, for example by using membrane technology or centrifugation equipment, or by dry, or freezing the blood or plasma within coagulation starts. The coagulation time may be increased by lowering the temperature of the fish. This will extend the time allowed for bleeding and improve the efficacy of bleeding, especially in the summer months with higher seawater temperatures. Trials have shown that under temperature near 0^{o}C, the blood will coagulate within approximately 33 minutes, while at 10^{o}C the blood will coagulate within 10 minutes (Tobiassen et al. 2015). A somewhat longer time before coagulation of blood during slaughtering process was reported by Olsen et al. (2006), with up to ca 60 minutes at temperatures close to zero.

A Norwegian company, SeaSide AS, 6200 Stranda, has developed a salmon slaughter production line based on dry bleeding and individual handling of each salmon during the bleeding process. During the bleeding the fish is positioned head down, and the bled blood is pumped to a separate tank. Minimum bleeding-time is set to 4 minutes. This system may be a new possibility for a profitable collection of fish blood from fish slaughter houses in Nordic countries.

Pre-treatment according to protocol, cooling and storing under controlled environment is very important. For fish blood, it is very important that coagulation is avoided after slaughtering. Chilling the fish before slaughtering and maintain very low temperature after the fish is bled will probably improve the fish fillet quality, increase the bleeding and postpone the coagulation time up to one hour (Olsen et al 2006). Future work need to have focus on developing technology at traditional salmon/trout slaughter houses to facilitate collection of fish blood, avoid coagulation, and to separate plasma and blood cells.

1.3 Cod testes

Cod testes contains several interesting components, such as DNA/nucleotides, phospholipids and positive nuclear proteins. Proteins from cod testes may stimulate the immune system and represent a large value as functional fish feed ingredient (Khora, 2013). Nucleotides are the building blocks in DNA, and are used in the pharmaceutical industry and may be a valuable feed ingredient (Fehringer et al. 2014). Phospholipids in cod testes contain high amounts of the polyunsaturated fatty acids, DHA and EPA.

Nuclear proteins are bound to DNA in the nucleus, and have a particularly high percentage of positive amino acids. The increase in uses of land plant ingredients in fish feed, that have low, or no content of particularly two essential amino acids, taurine and arginine. In general, this has made the amino acid profile of today's commercial diets less favourable compared to diets based on fish products. These are abundant in marine fish testes and make testes an even more valuable ingredient in new feed products with higher content of taurine and arginine.

Other methods for use in separation of functional feed/food ingredients should be further explored, for example membrane technology, and a method (Figure 2) developed by Kristinsson and presented by Hultin et al. (2005) that have shown that fish proteins can be solved at very high or low pH values. This new method has several advantages, compared with hydrolysis and extraction processes. The high or low pH value gives the protein a powerful charge and causes disintegration. Based on this, the fat content is less, and results in better oxidative stability and less odour in the end products. A further advantage is that unwanted items such as bones, microorganisms, cholesterol, and membrane lipids (phospholipids) are removed by the first centrifugation. Work is in progress to commercialize this promising process of isolating valuable materials and ingredients of both cold-and warm-water fishes (Geirsdottir 2005). Whether this method could be modified for use in utilization of testes and fish blood is not presently known, but should be elucidated a future project.

Ongoing developmental work aims to stabilize the proteins from changes in functional properties as well as to find economic/effective ways to stabilize the protein to avoid oxidation changes during processing and in the end product. However, protein isolates based on fish by products is a relatively new product and applications are under development.

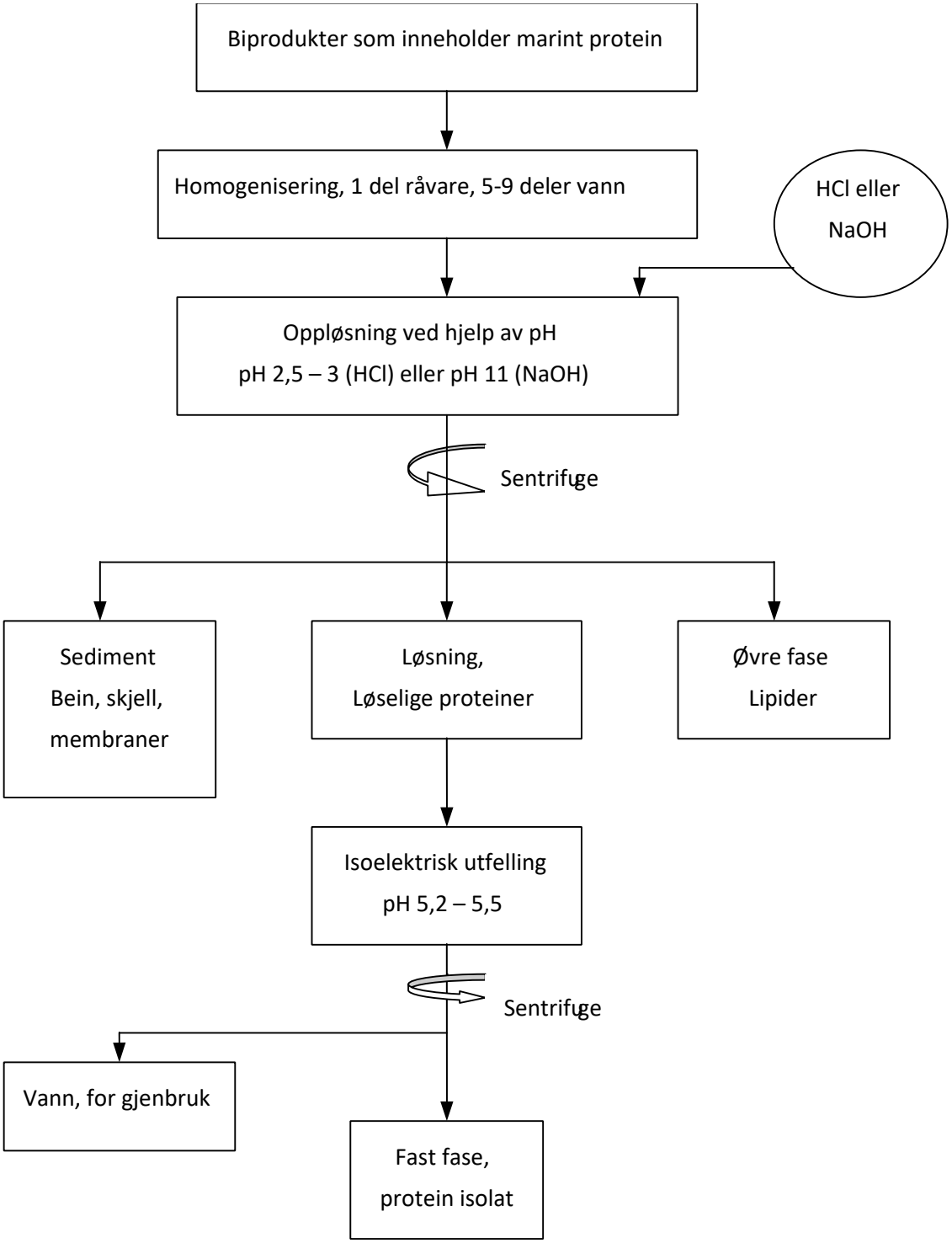

Figure 2. Production of protein isolate (Hultin et al 2005 after Geirsdottir 2005).

The high content of phospholipids in many marine organisms has received great attention in the recent years. For example, BioSea Management AS, Tromsø has patented a technology to manufacture marine phospholipids from marine products. Eximo AS, Tromsø, is manufacturing marine phospholipids for own use in special feed products, PhosphoNorse and AgloNorse Extra.

Further work into feed ingredients of testes should focus on collection, storage and possessing of cod testes to ensure the quality throughout the whole production process. This could include a collaboration between the present consortium, different companies and R&D institutions to investigate and develop special feed ingredients as mentioned in the present report.

2 Project activities

2.1 Utilization of by-products for special feed ingredient/products

The partners of the present project have found that in Nordic countries and Russia there is a growing interest amongst the ingredient industry to increase the utilization of by-products in production. The potential for increasing the utilization rate and value creation on by-products is large. This is especially true for by-products that has a very low utilization, e.g. testes, or no utilization, e.g. fish blood that are the two main focus areas of this project. In Russia there is also an interest in utilizing very specific bioactivity related to fish eye lenses. Tree different sources of material may be processed to high value products;

1. Salmon blood from slaughter houses in aquaculture
2. Testes from fish industry (cod fishery and herring) in Norway and Iceland
3. Fish eye compounds to arrest early cell division in fish and mussel eggs

The coastal fishery fleet has a large potential for increasing the value creation of by-products. Similar to roe from cod, testes, and ingredients of testes, may be a high priced product, that is also available in large volumes during fishery in the cod spawning season in Norway, Iceland and Russia.

An interesting segment for use of testes and blood may be the development and extraction of selected ingredients to be used in functional and specialized feed to various species, both in agriculture and aquaculture. For example, this may be used in start and weaning feed for different fish species, such as salmon, halibut, and lumpsucker. In a case study, we will in particular focus on development of feed to be used at certain vulnerable developmental stages of fish. A new innovative feed that strengthen growth and the general health status through improved immune capacity of fish before any stressful events during production may be of special interest, and probably obtain a high price in the market.

This goes for start feeding of fish when the growth potential is very high. Reduced growth in this phase of the fish life cycle cannot be restored in later stages. It is also well known that there is a reduced growth, often disease outbreaks, and mortality, during the first weeks after transferring newly smoltified salmon from land based freshwater aquaculture systems to sea water based cages. The change in environment, from a protected and stable environment in land tanks to more unstable surroundings in sea water cages is a stressful experience for the fish. This new feed concept idea could help in producing a more robust fish and that will improve the ability of fish to withstand and cope with these stressful influences. Moreover, at fish farms in cases with outbreak of diseases amongst their fish, the fish often have low appetite, resulting in little or no intake of medicine feed. A new functional and specialized feed with high palatability would increase the fish farmer's ability to treat diseased fish, using this new feed could be a tasty and super vector for different medicines.

Nevertheless, based on these raw materials the consortium will consider development of functional food for warm blooded animals such as functional weaning feed for pets and pigs. But this will be at a later stage, and based on our experience of functional fish feed production.

During the project period we planned to follow stepwise implementation of the different tasks (see table 2)

1. Collection of raw material (fish testes, fish eyes and fish blood) from traditional fish industry in Lofoten and Iceland. This will include trials with pre-treatment of the raw material, including cooling, freezing or drying.
2. Make new feed based with different recipes, using different levels of ingredients of both blood and testes. This will include mixing with commercial fish feed of testes/blood ingredients at different levels.
3. Testing the ingredient in start feeding fish trials. This will include testing this feed with different levels of the experimental ingredients in comparison with commercial feed without these ingredients.
4. Parallel working on possible technical solutions to collect blood from salmon slaughter houses.
5. Establish contact with new partners within fish processing, and feed production.

Table 3. Time table for different tasks.

Tasks	2014*	2015	2016
Initial investigations on waste/by-products - status in Norway for potential new products	4	1,2,3,4	1,2,3
Meetings –work shops	1	1,4	2,3,4
New ingredients in fish feed - collection of cod testes in Lofoten and Iceland		2	1
Pilot scale feed production		3, 4	
Pilot scale trials with salmon smolt using fish feed based on testes			1,2,3
Parallel investigations on fish (salmon) blood, how to process into new fish feed ingredient	4	1,2,3,4	1, 2, 3, 4
New partners		2, 3, 4	

2.2 Cod testes collected in Bakkafjordur, Iceland and Lofoten, Norway.

The raw material (testes from cod) was collected at peak spawning from the fish processing industry, L. Berg, Svolvær, Lofoten and from Toppfiskur in Bakkafjordur, Iceland in March 2016. The fish was delivered by coastal fishing boats to the processors. Fish were slaughtered and processed along a production line. Among the gut content, only the roe was selected for human consumption. The rest of the gut content, including the testes, was lead to a container to further processing and use in bulk as raw material for animal feed. The cod testes for the present project was selected at the production line and put into Styrofoam boxes and transported to the processing room. The cod testes were filled into plastic bags, 2-3 kg and vacuumed and sealed (Figure 3). Immediately after, the testes were frozen

at minus 20, and transported to Bodø by boat 3 hours and thereafter put into a freezer at minus 20 C. Testes (7 kg) was sent by air to Iceland (Matis) for further analyses of quality and biochemical composition and comparison to testes from cod caught in Icelandic water.

Figure 3. Cod testes collected at L.Berg Svolvær, Lofoten, packed and stored in vacuum in plastic bags.

2.3 Collection and processing of blood

An agreement was reached with the aquaculture farm of Haukamýri, Húsavík to get the serum obtained from the slaughter of Arctic charr. We went to the area on March 29 and washed and prepared two thousand liter tanks. When slaughtering, the containers where the fish are bled were only half filled with water so blood would not be wasted. This was also done to get the most of the blood per unit volume of water. Usually icy water is poured continually through the containers while the fish is bled. We used 660 liters tubs and 300 kg of fish slaughtered into each tub. After slaughtering, the fish was heaved from the tubs and the serum pumped into aforementioned thousand liter tanks (Figure 4).

Figure 4. The fish removed from the blood water (bleeding tank).

Eimskip comes daily to the Haukamýri farm to export products to the market. The serum filled containers for the experiment got a ride with the truck to Akureyri.

Skimming of blood

The emulsion tank used in the project was borrowed from Iceprótein ehf. Sauðárkrókur. The first task was for the students to learn how it functions.

The first test runs were with water. The air pump seemed to function fine as well as the scrapers which scrape the foam from the surface of the liquid in the tank. In the next experiment with the tank, ten liters of blood from pigs was brought from food processing company Norðlenska ehf. This experimental blood generated a lot of foam without the introduction of flocculent. The foam was easily scraped off. This experiment revealed that it would be necessary to find a good way to capture the foam where it was very extensive in volume.

When skimming started with the fish blood, it proved difficult to achieve foaming in the emulsion tank, although all pumps in the machine were more efficient than when the pre-studies were made with pig blood. This suggests that there is a substantial difference between fish blood and pig blood. We could not let the solution in the tank foam well enough to be able to skim so it was tried to stir the blood water. For this we used a drill with an attached whip that would normally be used to mix paint or concrete. With this approach, it was possible to whip the serum so that foam formed. The foam was skimmed and gave 6.75 kg of product. The product was placed in a refrigerator, then frozen and sent to Matis in Reykjavík, where it was freeze-dried and will be used in an experiment feed for fish. Half a liter was kept and used for analysis of water content and nutrients.

Another dose of fish blood was received on the 24th of May, approximately 1000 liters. The serum was treated much like as in the previous experiment. Now it was however time to experiment with the flocculent. It was decided, in accordance with Dr. Rannveig Björnsdóttir, that it would be best to solve 2 g of flocculent in water and then pour the solution into the emulsion tank. It did not appear to dissolve despite attempts to stir. Finally, it was decided to remove the flocculent from the water (very slimy, floating on water) and add 2 g straight into the tank. This was slightly better and less slimy. That day we received help from some workers at the University of Akureyri. The serum was stirred vigorously and it was possible to skim fairly large amount of foam. Foam was collected in large barrels where the aim was to reach the highest possible quantity.

2.4 Analyses of testes and blood water

The testes from Iceland were frozen without vacuum and sent to Matís for analysis (Table 4 and Table 5)

Table 4. Nutrient composition of cod testes.

	Iceland	Norway
	%	%
As is		
Water	84,7	85,0
DM	15,3	15,0
Protein	13,0	12,5
Lipid	2,0	2,3
Ash	1,7	1,8
In DM		
Protein	85,4	83,6
Lipid	13,0	15,4
Ash	11,3	12,0

Table 5. Fatty acid composition of cod testes compared with herring oil.

FA % of FA Met. Esters	Iceland	Norway	Herring Atlantic
SAF	24,9	23,5	24,1
MUFA	28,3	26,8	56,4
PUFA	46	46,9	18
unknown	0,8	2,8	
EPA + DHA	40,1	41,4	16,4
Total omega 3	44,8	45,6	14,1

The fish blood was collected as blood water from Haukamyrargil Arctic char fish farm in Húsavík, northern Iceland. This is the way the farmer bleeds his fish in their processing. The material was very deluted (blood/water ratio). The blood water was first skimmed to collect blood protein using a commercial skimmer for protein collection at the University of Akureyri, Iceland. The skimmed blood

protein was then sent to Matís, Reykjavík for freeze drying. This method turned out to be extremely laborious, therefore, the main part of the skimmed blood protein was spray dried at IceProtein in Saudárkrókur, northern Iceland.

By the chemical composition, it appears that the skimmed blood water is a mix of plasma and blood cells (Table 3).

Table 6. Nutrient composition of Arctic char blood.

	Whole blood	Plasma	Cells	Skimmed blood freeze dried
	%	%	%	%
As is:				
Water	86,4	92,7	78,7	3
DM	13,6	7,3	21,3	97
Protein	10,5	4,2	18,6	70
Lipid	2,1	1,4	0,4	10
Ash	0,9	0,9	1,0	10
Rest	0,1	0,8	1,4	8
In DM:				
Protein	77,0	58,0	87,1	72
Lipid	15,3	19,0	1,8	10
Ash	6,8	12,4	4,7	10
Rest	0,9	10,5	6,4	8

2.5 Formulation of diets with testes and skimmed blood water

Based on nutritive value of cod testes and skimmed Arctic charr blood protein and nutrient requirement of young salmonid fish for start feeding, Table 2 and Table 3 show that both these raw materials can make significant contribution to start feed diets for salmonid fishes.

Table 7. Formulation of diet with maximum inclusion of cod testes.

N°	Name	Share	Min	Max
5100014	Cod testes FD	36,848		
7	WHEAT	16,000	8,50	16,00
1140	FISH OIL	12,119		
194	SOYA 47 DTI	10,269	5,00	15,00
519	MONOCA-PHOSPHATE	6,441		
3080	SPC 64	5,000	5,00	10,00
130	CORNGLMEAL 60 cp	5,000	5,00	10,00
700001	FM MEAL710/125	3,855		
173	WHEATGLuten mealG	3,000	3,00	10,00
2150	Premix Laxa	1,000	1,00	
475	METHIONIN	0,468		
		100,000		

Table 8. Formulation of diet with maximum inclusion of skimmed blood protein.

N°	Name	Share	Min	Max
5100013	Skimmed Blood Water FD	29,129		
700001	FM MEAL710/125	20,800		
1140	FISH OIL	11,692		
7	WHEAT	9,853	8,50	16,00
130	CORNGLMEAL 60 cp	9,700	9,70	9,70
194	SOYA 47 DTI	5,000	5,00	15,00
3080	SPC 64	5,000	5,00	10,00
519	MONOCA-PHOSPHATE	3,496		
173	WHEATGLuten mealG	3,000	3,00	10,00
480	LYSINE-HCl	1,117		
2150	Premix Laxa	1,000	1,00	
475	METHIONIN	0,213		
		100,000		

2.6 Testing of diets made with testes and skimmed blood water

Trials are planned to test out the effect of testes and skimmed blood protein in start feeding trials on salmonid fish. Different inclusions of the test raw materials will be tested. The effect of the test raw materials will be evaluated based on: survival, growth, chemical composition of the fish, and samples collected for measurement of immune response and well-being in a future project.

2.7 Collection and testing of fish eye compounds to arrest early cell division in fish and mussel eggs

Fish eyes as by-products from the fish- and aquaculture industry may have characteristics that can be utilized in treatments of animal and human diseases. In this part of the project, as an attempt to develop a model for further testing, we did preliminary tests on the effects on cell division in fish eggs and mussel eggs using fish eye ingredients.

Due to the nature of their work, fishermen easily develop wounds on their hands during work at sea. A well-known remedy for the treatment of such wounds was the eye of *Sebastes marinus* that was squeezed and smeared on the wounds. The healing process of the wound was accelerated, and it terminated quickly. This is a well-known story amongst fishermen in Norway. Nevertheless, there are no scientific work in this field that confirm these and other possible effects of fish eyes and fish eye compounds on human and animal health.

A picture of the fish eye lens is shown in 5, illustrating the main components of the fish eye lens. The epithelium of the fish eye lens covers its frontal semisphere and is made of a monostratal flat layer of cells. Its main functions are trophic and protective. Epithelial cells at the frontal surface of the lens are flattened and closely attached to each other. Here, practically, there are no mitotic cell divisions. The epithelial reproductive (generative) zone is located between frontal and backward (posterior) semispheres of the lens where the lens fibers are continuously generated. The epithelial cells in this zone are smaller in size, have enhanced mitotic activity and acquire a prismatic form. Fibers of cortex lens have nucleus.

We collected eyes from two very common marine species, *S. marinus* and *Gadus morhua*. The eyes were carefully dissected from the head of the fishes. Thereafter, we dissected the lens from the rest of the eyes. In the further work we used the whole lens, and also the central zone of the lens where there are no mitoses.

For an experimental model we selected fish eggs and mussel eggs that are easily obtained from aquaria of fish/mussel in culture. The cell division of newly fertilized fish eggs of many fish and mussel species are easy to follow under a dissecting microscope during the first cell divisions. We used newly fertilized of eggs of cichlidae, *Archocentrus nigrofasciatum* (Cichlasoma), and mussel, *Asolene spixi*. During the work we discovered that the shell of eggs mussels was covered with a thick layer of mucus. We tried to release the eggs from the mucosa, but this was a very difficult task. In a future work we need to find a way to get the mucus separated from the egg shell, or select an other species as model, e.g. eggs of blue mussels, *Mytilus edulis* and/or sea urchins, *Strongylocentrotus droebachiensis*.

Methods

Eyes of fish *(G. morhua* and *S. marinus*) was sampled from wild caught fish, at a fishery survey, and frozen at minus 20 C, and kept at this temperature until used in further experiments. Later on the lenses were dissected from the eye and dried before added to the developing eggs. The effect on cell division of fish and mussel eggs were tested at early cell division, i.e. at 2, 4, 8, and 16 cell stages. Three to five lenses were placed in the vessel with a water volume of 100 ml for one hour. Thereafter, developed fish and mussel eggs were added to the vessel. The exposure of lens of fish eyes to developing fish and mussel eggs were terminated after 2-3 hours' experiment. Temperature of water in aquarium and in the vessel was 20 C.

Results

The soluble fractions of the lens of cod G. morhua and S. marinus can inhibit mitotic activity cells division of fish and mussel eggs.

The cell division in eggs of *Archocentrus nigrofasciatum* (Cichlasoma) stopped in 48% of the cases. The cell division in eggs of mussels, *Asolene spixi* stopped in 10% of the cases. We suggest that there are antimitotic substances in the epithelial zone (that has no mitosis) and as in cortex, which has fibers with nucleus.

Discussion

Our data is consistent with data of other authors, that have shown earlier that properties of the soluble fractions of the lens of freshwater fishes eyes are able to suppress mitotic activity in the generative zones of the lens of freshwater fish eyes and other species of lower vertebrates (for example, common frogs) (Nikiforov – Nikishin, 2001). Further, it has been shown earlier that there may be a suppression of mitotic activity in the epithelium of the lens in amphibians. This has also been shown in experiments where fish eyes lense have been implanted in the anterior chamber of the eye of common frogs *Rana*

temporaria. These experiments also indicated the presence of keilon - like substances in the lens. (Nikiforov – Nikishin, 2001).

Conclusion

The soluble fractions of the lens of cod *G. morhua* and S. *marinus* can inhibit mitotic activity cells division of fish and mussel eggs. Inhibition of mitotic activity in tissues with increased proliferative activity can find application in medicine (suppression of cell divisions in malignant tumors). It gives the opportunity to use the lenses of fish as valuable biological material.

In future work, we need to use a more appropriate model objects such as development eggs *Strongylocentrotus droebachiensis* and *Mytilus edulis.* To confirm our preliminary, but promising results, we will do experiments with lenses of cod and salmon eyes implantation under the skin of the fish, to see if it is possible to stop the mitoses in the cambial layer of the skin.

Further, when large scale production will be launched, an automated system for collecting fish eyes has to be developed and implemented at fish industry sites: for example, it may be possible to use equipment similar to the device used for extruding stones from cherries.

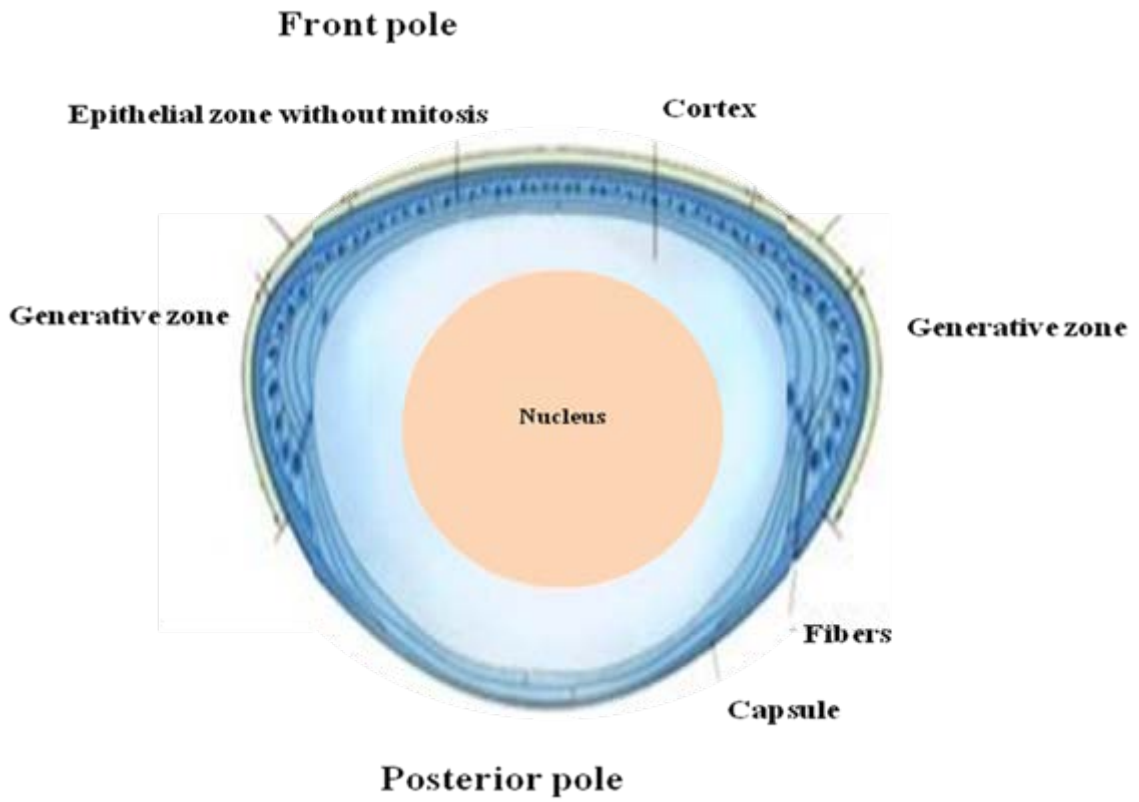

Figure 5. Lens of fish eye (the general scheme).

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