MANAGING TEXTURE QUALITY OF ATLANTIC SALMON THROUGH THE APPLICATION OF MOLECULAR AND MORPHOLOGIAL APPROACHES

1. Relevance

This proposal primarily addresses subprogram 2, **"**Strategic basic research" in the Fish Farming Program (Havbruksprogrammet), but is also relevant to goals given in subprogram 4, "Production of salmonids". The approach of the project is value chain oriented and multidisciplinary, focusing on enhanced perception of salmon by consumers and processing industry through improved fundamental knowledge on the impact of genetic and physiologic characteristics, rearing conditions, dietary composition and slaughter handling (ref. Fish Farming, actionplan 2009, section 4.1.2). This broad approach is facilitated through a close cooperation with other NRC, FHF and industrial financed projects. The proposal also coincides well with goals proposed by the Food(Mat)-Program on increased competitive ability regarding marked orientation for Norwegian food production. The project implies knowledge transfer between different stakeholders in the value chain. Novel analytical tools will be used, and advanced analytical methods developed in research on terrestrial husbandry animals, as well as in aquaculture, will be applied. Finally, the project focuses on parameters defined as research prioritised areas by FHF (The research fund of the fishery and farming industry) in "Action plan, salmon 2008" (section D, Fish Farming), and the program "Quality from gene to fork".

2. Scientific part

2.1. Background and State of the art

Growing awareness related to soft flesh and gaping has led to considerable financial losses to Norwegian salmon farmers. Fillets with soft texture or gaping have an unattractive appearance, the yield is decreased as the fillets are damaged during machine filleting and slicing. Soft texture also tend to correlate with other quality defects, such as reduced liquid holding capacity (LHC) and paler fillet colour. In the long-term, high frequency of deteriorated quality might also represent a threat to the good reputation of the farming industry as being producers of sustainable, superior quality products. Reliable statistics are missing on the severity of soft texture and gaping, but there are indications that the incidence show seasonal and geographical variation. Fillet texture is manifested through a complexity of events in the muscle with interactions to environmental factors in both the live fish and during the post-mortem period. The influence of carbohydrate dynamics was first discussed by Little (1965). Subsequently the correlation between soft texture and low ultimate pH, or rapidly declining pH post-mortem, has been ascribed to the impact of pH on structure and strength of the muscle proteins[1]. There is also evidence that soft texture and gaping coincide with high sarcoplasmic protein content, low level and degree of collagen cross-links [1], poor mineral status[2], and increased proteolytic activity[3]. Improved fundamental knowledge on factors impacting morphological characteristics and post-mortem degradation processes can help to narrow the biological variation and provide scientific based strategies that move population averages towards industrial and costumers' preferences.

Salmon farming routines have changed radically during the last decades. Essentially, farmers can now transfer smolts to sea throughout the whole year due to light and temperature manipulation in freshwater, facilitating year-round harvesting. Advanced farming practices and genetic selection for high growth have facilitated shorter production cycles. Research efforts to increase the raw material base for salmon feeds is mirrored in the commercial production. Dietary inclusion of plant raw materials have contributed to high degree of flexibility that is important for the sustainability of the industry. The impact of dietary plant ingredients on

growth and gut health is well documented, whereas the impact on muscle development and post-mortem degradation processes is scarce and fragmented. Stakeholders involved in salmon processing tend to infer that major causes to texture problems include plant supplemented feeds and explosive growth due to excessive focus on growth in the breeding programs

It is important to gain knowledge on the mechanisms that regulate protein and lipid breakdown in order to understand the underlying basis to texture deterioration. The activity of lysosomal and cytosolic enzymes have been associated with *post-mortem* softening of fish muscle. Degradation of myofibrils and intramuscular connective tissue (ICT) are probably caused by proteases such as cathepsins, as well as calcium-dependent proteases . In several fish species, cathepsins B, D and particularly L, are considered as enzymes playing an important role in *post-mortem* muscle softening. Recent results obtained by researchers in the project group, strongly suggested that different stimuli/stressors accelerate activity of different types of cathepsins. Also attention should be paid on interactions between dietary components and their impact on cellular differentiation and functions. Veterinarians have reported severe fat cell necrosis in salmon fillets coinciding with quality problems in their fieldwork. Furthermore, it is documented that several mammalian precursor cells can convert their differentiation towards that of adipocytes[4].

Inflammation is a complex reaction involving a number of cellular and molecular components. We have recently observed non-specific inflammatory conditions in salmon being rejected from the marked due to severe soft texture (Fig 1B). There are many conditions that cause inflammation, where poor diet is a commonly overlooked environmental cause. Obesity is known to lead to oxidative stress, increased inflammatory response and increased cell death in mammals. Attention has also been paid on the relationship between texture and fillet fat content and profile. The impact of total fillet fat content on texture is not studied in detail, but there are indications that high fat content (above 18- 20%) coincides with quality problems such as soft texture and decreased LHC during storage and processing. Dietary imbalances FA may alter the immunological status and stress resistance in fish. Although there are no consistent negative reports on deteriorated texture in salmon fed diets supplemented with plant oils, questions have been asked concerning the possibility of interactions between modified FA composition in the cellular membranes and the processes related to rigor mortis development and subsequent muscle breakdown. For example faster rigor development and ATP depletion post-mortem is documented in fish fed dietary plant oils. Decreased ability to cope with harvesting stress was proposed as a main reason to the accelerated degradation rate in fish fed plant oils. Significant interaction between nutritional status and the response to handling stress was also documented in a recently published study with salmon starved for 2 or 35d before slaughter[5].

The highly unsaturated FA (HUFAs, such as EPA and DHA) are vital constituents of cell membrane structure and function. However, these HUFAs are highly susceptible to attack by oxygen and other organic radicals. Resultant damage to HUFAs in membrane phospholipids can have deleterious consequences for cell membrane structure and function, with potential pathological effects on cells and tissues[6]. Also the mitochondrial membrane constitutes are very sensitive target for peroxidation, which could result in decreased FA oxidation rates. We recently showed that the mitochondria in liver, muscle and white adipose tissue of Atlantic salmon indeed are very sensitive targets for peroxidation, resulting in reduced FA β-oxidation capacity and cytochrome *c* (Cyt c) oxidase activity. A higher superoxide dismutase activity was also found in the dietary groups with increased incidence of oxidative stress an addition to increased caspase 3 activity, indicating increased apoptosis. In the proposed project we will test whether there is a link between lipid load, FA composition, oxidative stress and soft texture in Atlantic salmon, using both *in-vitro* and *in-vivo* approaches.

Research findings support the hypothesis about the relationship between high growth rate and soft texture, although some studies have failed to confirm this relationship. According to Folkestad et al. (2008), it is particularly salmon with high growth during the immediate

period prior to slaughtering that are predisposed to develop soft texture. Furthermore we have found that starvation produces increased firmness. Fish muscle cellularity reflects the balance between muscle growth of existing fibres (hypertrophy) and recruitment of new fibres (hyperplasia). Soft texture is found to coincide with large muscle fibre diameters[7] although the relationship is not unambiguous. Kiessling et al. (1991) stated that rapid growth favours volume increase of existing fibres in rainbow trout, and Christiansen et al. (1992) suggested that deteriorated texture of fast grown salmon is a result of rapid increase in muscle mass and delayed expansion of the myoseptal packing. However, the relationship between texture deterioration and accelerated growth and/or low muscle pH does not always hold, so that other factors are also involved. Seasonal acclimatisation may involve responses to environmental factors that affect the cellular metabolism, which in turn may affect the muscle microstructure and texture. There is a substantial need for principal knowledge about the impact of growth rate on muscle fibre growth pattern and morphology, development of extracellular matrix (ECM), and the balance between various cell compartments, also in relation to seasonality.

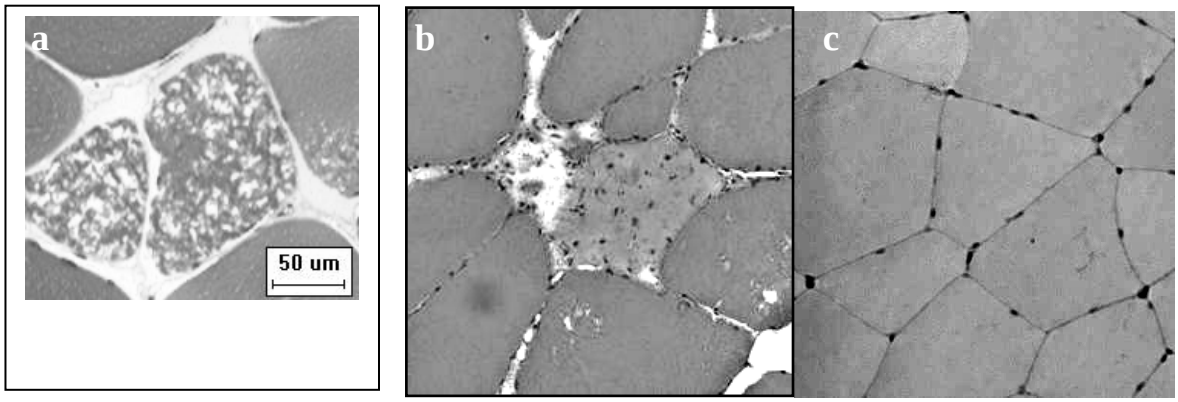

Figure 1. Muscle cell with severe damages from salmon with soft texture (a and b), and normal cell structure (c)

Collagens and proteoglycans (PGs) are major components of ECM. Recently we observed a correlation of r = 0.85 between fillet firmness and hydroxy proline (172179/S40), thus supporting several studies demonstrating that collagen content, organisation and strength significantly influence texture in fish and other animals. Collagen fibril formation and function are largely regulated by PGs, such as decorin[8] . Moreover, researchers within the project group have observed that the composition of GAGs and PGs in bovine muscles correlate with textural properties. PGs are also important in binding and preservation of growth factors and cytokines which contribute to the regulation of protease activity. Taylor et al. (2002) documented that breaks in cytoskeleton and connective tissue are important texture determinants, and that loss of attachments between myofibres parallel salmon muscle softening. In line, Ofstad et al. (2006) suggested that ECM degradation is a main cause to *post-mortem* softening of fish muscle, and that that differences in sulfation degree of GAGs are involved. However, little is known regarding the processes involved in this breakdown, as well as the contribution and effect of various types of PGs and GAGs. Different GAGs have recently been demonstrated in ECM of cod ICT: heparin sulphate (HS), chondroitin sulphate (CS), dermatan sulphate (DS) and keratin sulphate (KS)[9]. Further, ECM of spotted wolfish, a species with low propensity for gaping, had a different GAG composition and sulphation degree than ECM of cod. In a pilot study using immunohistochemistry revealed different expression of GAG structures were found in ECM in areas showing splits in cod muscle [9].

Progress has been made revealing underlying knowledge about biological processes that contribute to the delivery of consistent texture quality. Proteomics is a powerful tool that has been used in meat science for several years. Several reports have demonstrated an increase in solubilisation of actin [10] and different actin fragments during post mortem storage, of which

some correlate with texture. Using proteomics a correlation between increase in actin solubilisation and increased abundance of several small heat shock proteins have been unravelled in fish and mammalian muscle by researchers in Nofima Marin and Food, respectively.Through the application of tools of functional genomics and proteomics, and novel morphological examination techniques of live fibre development, and of macro and ultra-structural traits, it is possible to gain a deeper insight into these processes and their interaction with physiological, genetic, environmental and nutritional factors. Knowledge gained from these approaches can be beneficial in defining and optimising management systems for quality, providing assurance of fillet texture and in tailoring quality to suit market needs.

2.2. Principal objective - See application

2.3. Research challenges, research approach and methods

Progress has been made revealing factors influencing texture variations in salmon fillets, still underlying biological causes are to a large extent unexplained. The high interaction between the various quality attributes in salmon further complicates the possibility to extract the absolute significance of each single quality parameter. The scarce basic insight regarding factors determining texture variations, and the complex nature of the texture phenomenon, requires a broad and multidisciplinary approach to reveal causatives at molecular and structural levels.

Task 1. Determine underlying molecular and morphological basis of salmon fillets with a broad range in texture properties.

Profiling of salmon fillets with a broad variation in texture properties will be performed using fish with documented mechanical properties. The main test populations will be obtained from project A: “Firmer fillets” (financed by FHF), and B: ”New techniques to achieve more cost efficient selective breeding for improved consumer acceptance of aquaculture products (NRC173490/130). In both projects, salmon will be analysed mechanically in 2008, and the results obtained as well as muscle and blood samples will be available for the proposed project for in depth analyses of selected individuals, also facilitating correlation analyses to unravel underlying causatives for texture variations using a multivariate statistical approach.

Project A (**Study A**) consists of several sub-projects where commercially reared salmon with a substantial variation in origin will be studied: different strains, 0+ and 1+ salmon, diets, seasons and geographic locations along the Norwegian coast. The salmon has documented production history that can be linked to the texture properties. The main sub-project that will be utilised is entitled: “Global characterisation of factors influencing texture in farmed salmon using a multivariate approach”. This study is performed in connection with a large joint experiment in the projects "Integrated and dynamic production of farmed salmon in sea" (SIP179481/I30) and "Effect of bioactive fatty acids on survival (IPN/PD), growth and feed conversion for 1+ and 0+ farmed salmon" (BIP174215/S40), where the main aim is to reveal dietary and environmental impact on production efficiency, physiological conditions and health. Analyses performed in the SIP and BIP projects include morphological characterisation, and determination of the heart (HSMI) and digestive tract conditions. Salmon will be sampled regularly during six months, starting May 2008. The FHF project finances texture analyses and screening of proteolytic activity (catepsins) and identification and expression of genes involved using microarray in combination with real time PCR, micro structure analyses for determination of cellular irregularities in salmon with soft texture, and NMR for determination of macrostructural characteristics in selected individuals. The proposed study will enable an extension of the molecular and structural examinations, including improved insight regarding the relationship between texture and fish health and vascularisation.

In project B (**Study B**), individually PIT-tagged salmon belonging to 100 families are followed throughout the production phase in seawater by regular recording of weight and length, and non-invasive fillet fat and colour determination using VIS/NIR technology. In this experiment, results obtained Sept 2007 showed significant variations between families in texture and LHC. Composition, texture, gaping and LHC will be analysed in 10-15 salmon per family Autumn 2008 when the fish are expected to average 4kg. The present project will select three families with respectively firm, medium and soft texture (9 families in total) for determination of underlying molecular and structural variables associated with the texture variations. Structural and gene expression analyses will be performed in cooperation with the project "Increasing basic knowledge on muscle abnormalities in intensive production systems". Establishing a collaboration with the aforementioned studies (A and B), will enable a cost efficient study of the relationship between texture and properties and genetic background, environmental conditions, physiological conditions, growth history, dietary composition, and health status. The proposed project thus enables that texture results can be related with other production and quality characteristics using multivariate statistics to extract the importance of underlying factors for texture variations.

Task 2. Determine the impact of dietary fat level and fatty acid composition on regulation of muscle and fat cell growth, oxidative stress and texture.

This part focuses on gained basic knowledge of mechanisms involved in regulation of muscle- and fat cell growth in salmon fillets, and whether the relationship between the amount of muscle- and fat cells are influenced by quality- type- and level of dietary lipids. Furthermore, we wish to elucidate how lipid overload in salmon muscle affects oxidative stress, and in turn muscle necrosis and soft texture. Based on results from a screening *in-vitro* study where a broad range of lipids will be tested (**Study C**), certain lipid resources will be selected and tested *in-vivo* in a four months feeding trial using salmon with high and low fat content (**Study D**).

Unspecialised precursor cells (Mesenchymal stem cells) can develop to muscle, fat and bone cells. We have demonstrated that salmon adipocytes and muscle cells can develop from precursor cells isolated from visceral adipose tissue and muscle tissue, respectively. Recently, we demonstrated that precursor cells found in the muscle can be differentiated into osteoblasts (bone cells), when given certain stimuli. Differentiation of precursor cells to mature specialised cells is an important process during tissue growth. In the proposed project (Study C), we will test if unspecialised precursor cells in salmon muscle can turn into adipocytes rather than muscle fibres upon exposure to an "adipogenic" signal, like high lipid levels. Responses to exposure to a multitude of FA levels will also be tested at different temperatures in the *in vitro* screening study. Knowledge about these mechanisms in Atlantic salmon is absent. An in-*vivo* study will also be carried out (Study D) using adult salmon with high and low fillet fat content (<12% and above 18%) selected from a larger population using NIR determination on live fish . Diets will be lipid sources based on results obtained in the *in-vitro* study. Salmon will be sampled for analyses every four weeks during a four mounts trial. At Nofima Marin's research station at Averøy, an optimal period for our approach is Feb to end of May using 0+-salmon. During this period temperatures drops to <4°C in March, thereafter the temperature and photoperiod increase, triggering a significant compensatory growth period lasting until the end of May. PIT-tagging the fish facilitates individual recording of growth, thus the trial will provide combined information on texture in relation to dietary composition, fillet fat level, growth, environmental temperature and interactions between the factors. At the experimental termination, the ability to cope with confinement stress will be tested. Thorough analyses will be performed, partly designed based on results obtained in Study A and B.

Research facilities and analyses

Nofima Marine's laboratory has experience in cultivation of primary cells isolated from Atlantic salmon, and techniques for differentiation of salmon muscle cells and adipocytes from precursor cells have been established (myosatellites, preadipocytes). Recently we also differentiated myoblasts to osteoblasts (Study C). Feeding experiment will be performed in seawater at the research facilities at Averøy (Study D).
Salmon with a high texture variation will be selected for in depth analyses according to thresholds given in a database including mechanical analyses of Norwegian salmon since 1995. Fillet gaping, LHC, dry matter, chemical fat and protein content, and FA composition will be analysed according to standard methods. Additionally, fat and colour analyses will be determined using image analyses and VIS/NIR (Study A, B, D). In Study D, membrane lipid classes will be separated by high pressure thin layer chromatography (HPTLC). Microarrays developed for salmonids will be used to screen muscle samples to identify genes that are involved in inflammation and apoptosis in individuals with extreme texture properties. Additionally, texture results will be related to activity of superoxide dismutase, caspase 3 and Cyt c-oxidase in muscle. Study C: Determine the capacity of salmon muscle precursor cells (myosatelites) to proliferate and differentiate when added different types, qualities and quantities of lipid to the culture media. Their capacity to convert differentiation towards that of adipocytes will also be analysed. We are able to assess the cell proliferation by immuno-cytochemical detection of PCNA (proliferating cell nuclear antigen), and morphological analyses of muscle and adipose cells will be performed. These methods involve general ultra-structural studies by light and electron microscopy. Different staining techniques as well as immunocytochemical methods will give us the opportunity to characterise the cell types. The gene expression of specific muscle (myo D, α-actinin, myosin) and adipose (PPARγ, MTP) tissue markers will be characterised. Vitamin D deficiency can cause bone fragility and muscle weakness. 1,25-dihydroxy vitamin D level will be determined using HPTLC.
Matforsk AS – Nofima Mat: Compared to classical protein studies, proteomics make it possible to study "new" proteins and protein interactions that influence fillet texture and contribute to softening. Proteom analyses of both sarcoplasmic proteins and structural proteins will be extracted and separated by 2-dimensional gel electrophoresis. Data will be analysed using both uni- and multivariate statistical tools and selected proteins will be identified using Maldi-TOF/TOF mass spectrometry. Histochemistry with different staining methods (Haematoxylin eosin, Alcian Blue added electrolytes) and monoclonal antibodies against different GAG epitopes will be used to outline the connective tissue architecture and ECM composition with respect to textural properties. Identification of connective tissue PG's at RNA level of importance for textural properties and structure will be performed by real-time PCR with probes already designed for detection of connective tissue PG's in salmon. (Study A, B, D)
Norwegain Universtiy of life Sciences, IHA. Professor M. Thomassen will together with the post doc student, employed in the project, be responsible for analyses of degrading enzymes, with emphasis on calpain and cathepsin. The position as a post doc will be offered PhD Diane Bahuaud, who will continue the work established during her PhD in close cooperation with Matforsk AS – Nofima Mat. She will focus on analysing samples obtained in Study A and B.
The Norwegian School of Veterinary Science: Samples will be collected for morphological evaluation of inflammatory components in the pathogenesis and for evaluation of inflammatory responses in reparatory reactions. The expression of MHC class II has proved to be valuable in such investigations. In collaboration with other laboratories, we seek to develop additional inflammatory markers and markers for leucocyte populations. Such markers will be implemented as soon as they are available. Both muscle and organs will be investigated, aiming at identifying possible pathological changes which may have been neglected in previous investigations, which mainly have concentrated on muscle. Combined with the other investigations, we can define the changes on a broader context, filling in more information to the gaps in our knowledge on this costly condition (Study A, B, D).

Institute of Marine Research. Quantification of muscle fibre size (area, diameter) and shape (circularity, symmetry, convex deficiency, curvature statistics) will be determined using image analysis software developed by Dr. LH Stien. This software also detects membrane breakage and the number and area of intracellular caves. The method utilises edge detection techniques to find cell borders. The results of this analysis have shown high correlations with visual analyses in the project "Atlantic salmon – our most important raw material for food production".
SINTEF Fisheries and Aquaculture. 1H Magnetic Resonance Imaging (MRI) has been used in 'soft flesh' pre-projects to study longitudal and transversal fillet cross-sections. The method gives a macroscopic overview of the fillet as well as a map of the fat distribution in the muscle, appearing as in the myocommata. When soft-fleshed salmon fillets are studied, the images showed two distinctive features: (a) the soft (gelly-like) band along the fillet was a markedly defined area with a clearly different structure than the adjacent, normal looking muscle, (b) the typical 'V'-shaped fat stripes (myocommata) in salmon fillets were nearly missing (chemical analysis confirmed low fat content in the soft band). These phenomena were more pronounced in fish defined as more soft. In the current project, we will accordingly use MRI to describe the extent of softness and to compare the MR images (macroscopic level) with the results of fillet subsamples excised for other analyses described herein (microscopic level).
Swedish Agricultural University, SLU's laboratory has broad experience with different analyses of lipid- and quality parameters in raw and processed product of both fish muscle and meat. Different analyses on minor lipid components as well as oxidation parameters for both lipid and protein oxidation will be conducted and correlated to texture parameters. We have already applied various useful methods including oxidation products by GCMS headspace, TBARS, carbonyl content and protein degradation by gel-electrophoresis, in a collaboration with Nofima Marine and Food, within the project "Innovative and attractive fish dishes". Cholesterol oxidation will be used to create a holistic view of the ongoing oxidation processes. The obtained data will be correlated to FA composition in feed and muscle, as well as to texture data with a multivariate statistical approach.
Instituto del Frío (CSIC), Madrid: The group from CSIC has experience in determining the quality of different fractions in muscle fish collagen depending on e.g. physiological and processing conditions, and its relation with textural changes. Samples with different variables will be analyzed to study the molecular and morphological connective tissue and its principal component collagen properties. Methods to be used will be structural and biochemical analyses. For structural, SEM/optical microscopy and DSC analyses will be performed. From a biochemical point of view, amino acid composition, and glycosylation degree will be performed on acid soluble collagen. Specifically for cross- linking degree, molecular weight distribution will be determined by either SDS-PAGE or size exclusion chromatography and FTIR analyses.

Task 3. Educate one post-doc student
A 18 month post-doc period is proposed for Diane Bahuaud who is a PhD student at the University of Life Sciences (IHA). Bahuaud will defend her thesis Q4 2008. Her main focus is proteolytic activity and microstructural changes in relation to texture of salmon fillets. In the proposed project she continue focusing on degrading enzymes with emphasis on catepsins and microstructure in salmon muscle. Study A and B will be her main test populations.

2.4. Progress plan

	2009				2010				2011			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
A, B, C, D Analyses	x	x	x	x	x	x	x	x	x	x		
C, cell studies				x	x	x	x					
D, feeding trial									x	x		

Post doc	x	x	x	x	x	x						
Meeting reference group			x				x				x	
Project meeting	x		x		x		x		x		x	
Statistical data treatment		x	x	x	x	x	x	x	x	x	x	
Publication				x	x	x	x	x	x	x	x	x

2.5. Budget – see application

2.6. Project organisation and management

A three year project is proposed for the period 1/1-2009 to 31/12-2011. Partners directly involved in the project are: Nofima Marine, Nofima Food, University of Life Sciences (IHA; Post-doc), The Norwegian School of Veterinary Science, Sintef, Institute of Marine Research, Swedish University of Agricultural Sciences and Instituto del Frío, Spain. An administration board will be established with one member from each participant in the project, with Dr. T. Mørkøre from Nofima Marine as project manager. Additionally a Reference group will be established composed by members representing the farmed salmon industry (see section 4). The project will be closely co-ordinated with the national NRC-SIP project "Integrated and dynamic production of farmed salmon in sea" (project leader, PL: Prof. K-A. Rørvik, 1799481/I30), the NRC-BIP project "Effect of bioactive fatty acids on survival (IPN/PD), growth and feed conversion for 1+ and 0+ farmed salmon" (PL Prof. K-A. Rørvik; 174215/S40), the NRC project “The impact of pancreas disease (PD) on flesh quality of Atlantic salmon” (PL: Dr T. Mørkøre 172179/S40), the NRC project “Increasing basic knowledge on muscle abnormalities in intensive production systems” (PL: Prof II AMB Rørå), and the NRC-SIP project ”New techniques to achieve more cost efficient selective breeding for improved consumer acceptance of aquaculture products (PL: Dr K. Kolstad, 173490/130).

Partners and researchers involved in the project.

Nofima Marine (former Akvaforsk and Fiskeriforskning). Dr. T. Mørkøre (project manager) and Dr B. Ruyter will be responsible researchers. Dr. Mørkøre is a researcher within the field farmed fish quality and nutrition, being an experienced researcher on texture properties in farmed fish. Dr. Ruyters’ main research field is within lipids in farmed fish diets, and she has been responsible for establishing methodology for cultivation of primary cells isolated from Atlantic salmon. Both researchers have positions at Norwegian University of Life Sciences, Department of animal and aquaculture sciences (IHA).

Matforsk AS-Nofima Food (former Matforsk and Norconserv AS) is a leading research centre in the understanding of food quality and has a long tradition of research collaboration with Nofima A-F. The researchers that will be involved in the proposed project, Dr K. Hollung, Dr. K. Hannesson, Dr. E. Veiseth and Dr. M. E. Pedersen, have comprehensive skills and experience in proteomics and analysing connective tissues in foods, respectively and have several scientific papers within their research fields.

Norwegian University of Life Sciences: Professor M. Thomassen is the leader of the product quality research group at (IHA), covering a broad competence of meat and fish meat quality. Research performed during the last 5 years in a NRC-SUP Program ”Atlantic salmon- our most important raw material for food production: Knowledge basis for increased pre-rigor processing in Norway” has generated knowledge about treatment of salmon and effects on post mortem degradation. This project has been a close collaboration with Nofima Marin and Nofima Mat, and at present two PhD students work on studies of the degradation enzymes calpain and cathepsin. The NRC-SUP program ends this year, and so we hope that a funding of the present application will make a continuation of the fruitful and important scientific collaboration.

The Norwegian School of Veterinary Science (NVH): Dr. E.O. Koppang, VMD, dr.med.vet., is Associated Professor at Section of Anatomy and Pathology, Institute of Basic Sciences and

Aquatic Medicine, NVH. His main field of research is on inflammatory conditions in fishes. Koppang is project leader for histological analyses in the NRC financed project "InNovacc". and our laboratory has long experience with the assessment of inflammatory reactions
Institute of Marine Research, Animal welfare group: Dr LH Stien is an expert in image analyses. Dr Stien has developed programs for automatic analysis/ measurement systems for various quality related characteristics, including software for counting and describing muscle cell morphology.
SINTEF Fisheries and Aquaculture (SFA): Dr U. Erikson is responsible for the strategic activity area 'Harvesting and postharvesting processing' at SFA. He has experience from both research and consulting services related to water quality, fish stress, slaughter, processing, chilling and market quality. Also, he has been involved in different projects related to measuring techniques for assessment of stress and product quality (including a recent strategic research program on the relationship between salting and flesh structure evaluated partly by NMR and MRI). Dr. E. Veliyulin, also at SFA, will also be involved in the MRI experimental part of the project.
Swedish University of Agricultural Sciences (SLU): Dr S. Sampels and Dr J. Pickova have comprehensive skills regarding effects of dietary lipid on quality related specifically to degradation of lipid components post-mortem in fish and mammals. SLU has advanced equipments and experience suitable for this project, including analyses of lipid and protein oxidation products. The collaborations with SLU will be a strengthening of the already established cooperation with Nofima Marin on impacts of dietary lipids on production efficiency, physiological responses and fillet quality.
Instituto del Frío (CSIC), Madrid. Professor J. Borderías will the responsible researcher at CSIC, in cooperation with Dr P. Montero and Dr. C. Gomez. The research group has extensive experience in fish collagen studies and its relation with physiological and processing factors their impacts on muscle changes. The collaboration in this project will strengthen the relation with the rest of groups. The team of CSIC has the opportunity facilities to study cultured and processed salmons under controlled conditions.

3. Perspectives and strategic anchoring

3.1. Strategic anchoring

This project is well anchored in Nofima's strategic plan and assessed as a prioritized field of research. Similarly the proposed project covers research areas considered as relevant and important by the collaborating partners in the project.

3.2. Social relevance

Soft fillets, gaping and impaired ability to retain liquid seriously reduce consumer perception and negatively affect technological quality – resulting in negative market responses and product downgrading. The Government is aiming at increasing the market and consumer orientation. The proposed project contributes to fulfilling these governmental aims. Results from the project can contribute to better economical sustainability in salmon farming in that improved understanding of fundamental causes for fillet softening and gaping will increase the ability to tailor make salmon fillets of predictable and enhanced quality according to market demands.

3.3. – 3.5 Environmental perspective, Ethical aspects, Gender equality perspectives

The project will not have any negative environmental impact. Assuring normal muscle development is an issue that can be considered as important from an ethical point of view. The project group has approximately equal gender composition.

4. Communication

Knowledge transfer to industrial stakeholders from the proposed project will be ensured through a Reference Group (RG) consisting of 5 key persons representing each of the three largest feed manufactures, salmon producers and the salmon processing industry to ensure a close cooperation with the national and international industry (contact has been established). The RG and the project group will meet yearly for discussions, knowledge transfer and the RG will give advises regarding targeted communication and industrial application of the scientific results. Results from the project will be published in peer-reviewed international scientific journal with high impact factor, national and international popular scientific journals, as well as at national and international scientific and popular scientific conferences. In total the project is anticipated to directly support material for at least 10 per reviewed scientific papers.

References

1. Lie, X., Bickerdike, R., Lindsay, E., Campbell, P., Nickell, D., Dingwall, A., Johnston, I., 2005. Food Chem. 53, 6844-6850.
2. Mørkøre, T., Austreng, E., 2004. Aquaculture 230, 439-455.
3. Bahuaud, D., Mørkøre, T., Langsrud, Ø., Sinnes, K., Veiseth, E., Ofstad, R., Thomassen, M.S., 2008. Food Chemistry doi:10.1016/j.foodchem.2008.03.075.
4. Schiller P.C., D'Ippolito, G., Brambilla R., Roos B.A., Howard G.A., 2001. J. Biol. Chem. 276:14133-14138.
5. Mørkøre, T., T., Mazo, P.I., Tahirovic, V., Einen, O., 2008. Aquaculture 277, 231-238.
6. Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. Academic Press, San Diego, CA, USA, pp. 181-257.
7. Johnston, I.A., Bickerdike, R., Li, X.J., Dingwall, A., Nickel, D., Alderson, R., Campbell, P., 2007. Aquaculture 265, 148-155.
8. Danielson K.G., Baribault H., Holmes D.F., Graham H., Kadler K.E., Iozzo R.V., 1997. J. Cell Biol. 136: 729-743.
9. Tingbø M.G., 2007. Thesis. Univ. of Oslo.
10. Kwasiborski, A., Sayd, T., Chambon, C., Sante-Lhoutellier, V., Rocha, D., Terlouw, C., 2008. Meat Sci. in press.

References relevant for the analytical approaches

Bailey A.J., Light N.D., 1989. Eds: Bailey A.J. and Light, N.D., Elsevier Applied Sciences, London UK .

Folkestad, A., Rørvik, K-A., Kolstad, K., Mørkøre, T., 2008. Aquaculture Research 39, 329-332.

Hollung K., Veiseth, E., Jia, X., Færgestad, E.M.,Hildrum, K.I., 2007. Meat science 77, 97-104.

Jia, X., Therkildsen M., Hildrum, K.I., Hollung, K., Bendixen, E., 2006. Proteomics 6, 936-44.

Jørgensen S.M., Afanasyev S., Krasnov A., 2008. BMC Genomics 9:179.

Kiessling, A., Espe, M., Ruohonen, K., Mørkøre, T., 2004. Aquaculture 236, 645-657.

Kjær, M.A., Todorčević, M., Torstensen, B., Vegusdal, A., Ruyter, B., 2008. Lipids accepted.

Kjærsgård, I.V.H., Nørrelykke, M.R., Jessen F., 2006. Proteomics 6: 1606-1618.

Krasnov, A., Koskinen, H., Pehkonen, P., Rexroad C.E., Afanasyev, S., Mölsä, H., 2005. BMC Genomics 6:3

Love, R.M., 1988. Farrand Press, London.

Martinsen, T.S., Mydland, L.T., Takle, H., van Nes, S., Thomassen, M.S., Mørkøre, T., 2007. Symposium on Fish Nutrition & Feeding, Istanbul, Turkey.

Montero, P., Borderias, J., 1989. Lebensm. Untersuch.Forschung 189, 530–533.

Mørkøre, T., 2002. Dr. Sci. thesis. 2002:07. Dr. Sci. thesis, UMB, Ås, Norway. ISBN 82-575-0488-2.146 pp

Mørkøre, T., 2008. FHF report.

Ofstad, R., Olsen, R.L., Taylor, R., Hannesson, K.O., 2006. *Lwt – Food Sci. Technol.* 39, 2006, pp. 1143–1154.

Pedersen M.E., Kolset, S.O., Sørensen T., Eggen K.H., 1999. J. Agric. Food Chem. 47:1445-1452.

Pedersen M.E., Kulseth M.A., Kolset, S.O., Velleman, S., Eggen K.H., 2001. J. Muscle Foods 12:2001-2017.

Petrosillo, G., Ruggiero, F.M., Pistolese, M., Paradies, G., 2001. FEBS Letters 509, 435-438.

Ruyter, B., Andersen, Ø., Dehli, A., Farrants, A.K.Ö., Gjøen, T., Thomassen, M.S., 1997. Biochem. Biophys. Acta. 1348, 331-338.

Ruyter, B., Røsjø, C., Måsøval. K., Einen,O., Thomassen, M.S., 2000. Fish Physiol. Biochem.23, 151-158.

Ruyter, B., Thomassen, M.S., 1999. Lipids. 34, 1167-1176.

Tingbø M.G., Kolset S.O., Ofstad, R., Enersen G., Hannesson, K.O., 2005. Comp. Biochem. Physiol. 140B: 349-357.

Tingbø M.G., Kolset S.O., Ofstad, R., Enersen G., Hannesson, K.O. 2006. Comp. Biochem. Physiol. 143B: 441-452.

Todorčević, M., Kjær, M.A., Djaković, N., Vegusdal, A., Torstensen, B.E., Ruyter, B., 2008, Submitted.

Vegusdal, A., Østbye, T.K., Tran, T.N., Ruyter, B., 2004. Lipids 39, 649-658.
Vegusdal, A., Sundvold, H., Gjøen, T., Ruyter, B., 2003. Lipids 38, 289-296.
Verrez-Bagnis, V. et al., *Electrophoresis,* 22, 1539, 2001.