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

Aquaculture has sustained a global growth, and is expected to increasingly fill the shortfall in aquatic food products resulting from static or declining capture fisheries. In Norway, farming of Atlantic salmon is a nationally important industry, with a total production of approximately one million metric tons in 2011. This corresponds to a doubling of the production over the last ten years and more than 60 percent of total Norwegian seafood exports.

Norwegian farmed salmon is, in general, of good quality, but impairments such as soft texture occur. Insufficient flesh firmness causes financial loss because the fillets are damaged during machine filleting and slicing, and because soft fillets are rejected by the consumers. The occurrence of soft flesh seems to vary; being more pronounced during periods when the fish has a high weight gain. It is crucial to get a better understanding of how to avoid or prevent soft muscle texture by determining the underlying biological factors involved. This would lead to increased number of salmons having the correct fillet quality, reduced numbers of complaints from the market due to soft salmon flesh, subsequent financial losses and damaged reputation of the salmon aquaculture industry.

The tasks of the current project were twofold: First: to determine the underlying molecular and morphological basis of salmon fillets with varying texture properties – ranging from soft to firm Second: to determine possible dietary impacts on regulation of muscle growth, oxidative stress and texture. Regarding dietary impacts, the main interest was amino acids, as the knowledge on the impact of dietary fatty acids has improved significantly in the last years.

Research tools

Novel, advanced tools were developed and implemented in the project. These include tools using biotechnology, histomorphometry, enzyme analyses, metabolomics and proteomics. It was also developed and implemented micro-array chip technology with targeted genes related to fillet quality. Analyses were performed using intact muscle from salmon with varying texture due to inherent genetic characteristics and/or dietary treatment. Additional gene expressions were studied in salmon muscle stem cells grown in a medium with varying nutritional composition. Studies using muscle stem cells in fish nutrition research are novel and provided new and supplementary insights compared to traditional feeding trials. Analyses of connective tissue of salmon muscle with varying firmness were determined by several different methods, and improved protocols were developed. New methods were developed and adjusted for analysing protein degrading enzymes (calpain/calpastatin) in salmon muscle and their relationship with fillet texture was determined. Muscle structure characteristics (histology) were studied by image analyses, using program developed in the project and also by new colouring techniques (immunohistochemistry) for identifying potential markers associated with muscle texture. Finally, new protocols were developed for analysing particular proteins (proteomics, 2D DIGE) and metabolite fingerprinting and profiling (metabolomics, H NMR) of firm and soft salmon muscle. The latter methods are promising, but further development is needed before they can be implemented in the texture research of farmed salmon.

In-vivo and *in-vitro* studies

Specific feeding trials (*in-vivo* studies) were performed within the project and also cell culture studies were established in the laboratory (*in-vitro* studies). Additionally, pronounced cooperation was established with other on-going research projects, that gave us access to fish material and also results obtained in those projects; hence our results on texture and molecular/structural characteristic could be related to e.g. production related parameters. These projects were financed by FHF, NRC and also

industrial stakeholders – in particular the Norwegian breeding company SalmoBreed AS. This cooperative approach was both mutual beneficial, cost efficient and greatly enhanced the knowledge regarding molecular and histomorphometric factors determining texture variations of salmon muscle.

Project group
The project group was interdisciplinary and international. The research partners were Nofima, Norwegian University of Life Sciences, Sintef, Havforskningsinstituttet, Sweedish University of Agricultural Sciences, Instituto de Ciencia y Tecnología de Alimentos y Nutrición (Spain). It was also established a close dialogue and partly direct involvement with industrial stakeholders throughout the value chain (feed producers, salmon farmers, processing industry, breeding).

Results

Soft versus firm muscle. Salmons representing a wide range in texture properties were analysed by gene expression, histology, enzyme, connective tissue and gross compositional analyses. The results showed that the nutritional metabolism was different in salmons with soft flesh being characterized by enhanced utilisation of proteins for energy production and a more pronounced anaerobic/carbohydrate metabolism. Hence it was concluded that fillet firmness seems to depend largely on the metabolic properties of the muscles; both aerobic metabolism using lipids as fuel, and rapid removal of damaged proteins, appear to result in a firmer flesh. Salmon with soft texture had less stable connective tissue proteins. No correlations were observed between soft muscle and activity of protein degrading enzymes. The histological analyses revealed a broad range of new insights where the most significant results were related to the accumulation of glycogen inside the muscle cells. In humans and non-aquatic animals it is shown that abnormally high glycogen levels in the muscle may be due to nutritional imbalance, or possibly genetic defects.

Genetics. Analyses of large number of Atlantic salmon belonging to different families showed that a significant genetic variation in muscle texture, implying that flesh firmness can be improved through selective breeding. A comparison of selected ("farmed") and un-selected ("wild") salmon reared together and under the same rearing conditions showed that the selected salmon grew significantly faster they had a lower degree of early and unwanted sexual maturation, whereas the fillet fat content was similar between the fish groups. The texture of the selected salmon was softer compared to the un-selected salmon. The softer texture of the selected salmon was correlated to lower connective tissue stability and less mineralisation of the tissue. These results suggest that improved firmness can be achieved both through selective breeding and through improved nutrition, where the diets are supplemented with nutrients that ensure proper mineralisation and connective tissue stabilisation.

Nutrition. Dietary amino acid composition significantly affected the texture. In particular we also detected beneficial effects by increasing the levels of the amino acid glutamate and/or arginine to a diet resembling commercial salmon feed. In particular the glutamate supplementation was beneficial to firmness of the muscle and also to the stability of the firmness during prolonged ice-storage or frozen storage. Gene expression analyses revealed a generally improved immune status and improved nutritional metabolism in the salmon that were given diets with the supplemented amino acids. Glutamate supplementation also gave smaller, leaner and healthier livers, less muscle degradation and improved connective tissue stability. Salmon given glutamate supplemented feed also showed improved ability to cope with stress during slaughter handling. Muscle cell studies revealed that the positive effects of glutamate supplementation could be related to a stimulated expression of proteins that are known to strengthen the muscle tissue.

Vitamin E supplementation to a standard salmon diet significantly improved firmness whereas only minor texture effects were observed for salmons given feed with different lipid levels or lipid sources of varying freshness quality. However, rancid dietary oils decreased the robustness to acute stress and also resulted in unpleasant sensory properties of the final product. By summing up, the *in-vivo* and *in-vitro* studies it is reasonable to conclude that muscle structure of salmon can be improved through dietary optimisation. Furthermore, muscle texture seems to be sensitive to the robustness status of the living salmon, implying that a holistic approach is required in order to fully understand and improve firmness of salmon fillets. The above mentioned results are already considered by the industry and hypothesis' based on the results from the project are already being tested in new experiments.

Results from the project have contributed to a broader and deeper understanding of factors that influence texture properties in farmed Atlantic salmon. The results show that both selective breeding and in particular dietary optimization may reduce the number of salmon with unwanted flesh quality. Research tools developed in the project are already implemented in new projects

Communication

Dissemination of the results from the project has been given priority. Because the salmon farming and processing industry is requesting information about factors that can reduce the problem with soft texture, we have emphasized to immediately communicate relevant results through information channels that are available to the industry, including web-based channels. In the project period we have had a close dialog with FHF who have provided valuable advises and assistance. Results from the project have also been communicated at scientific meetings and one book chapter and five scientific papers are already published. Two scientific publications are under review and 10 additional scientific papers are in preparation (most of them will be submitted this year). Results from the projects were part of one PhD thesis (MØ Gaarder, UMB), and will be part of one additional PhD thesis (submission mid-April, T. Larsson, UMB). Six students have defended their master thesis based on results from the project, and additionally three will defend their thesis in 2012 and two in 2013. In our opinion, the extensive cooperation with other adjacent projects, high student and industry involvement, and the interdisciplinary approach of the project has resulted in a high and cost efficient knowledge production, of value to both industry and the scientific community.