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Fillet quality of Atlantic salmon (*Salmo salar*) as affected by dietary and environmental treatment

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FILLET QUALITY OF ATLANTIC SALMON (*SALMO SALAR*) AS AFFECTED BY DIETARY AND ENVIRONMENTAL TREATMENT

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ABSTRACT

Fillet quality of Atlantic salmon (*Salmo salar* L) is crucial for both salmon producers and consumers. There are endogenous and exogenous factors that affect fillet quality. This thesis mainly focuses on exogenous factors: diet and environment, studied in a two-year-long experimental trail.

Dietary high protein-to-lipid (P/L) ratio (Test diet) and low protein-to-lipid (P/L) ratio diet (Control diet) were fed to Atlantic salmon reared in sea net pens (Flemma) to investigate dietary influence on biometric traits and fillet quality. Effect of rearing environments was studied in salmon reared in commercial sized sea net pens located on the Norwegian west coast (Flemma) or in small research tanks on land (Sunndalsøra). All fish were reared in seawater and the feed used in the environmental study was the Control diet.

Major fillet quality parameters analyzed include fillet gaping, fillet color, myocommata and myomere's area, width and color (L^* , a^* and b^* values), drip loss and fillet texture. Fillet analyses were determined post-rigor (fresh fillets). Additionally, drip loss and texture were analyzed after frozen storage (thawing at 4°C and 20°C).

Salmon reared in sea net pens (Flemma) have significantly higher body weight, body length, fillet yield, fillet color, brighter and wider myocommata, improved firmness, slimmer body shape (lower condition factor, CF), as well as lower fillet gaping and less drip loss. Feeding salmon high P/L diet significantly increased fillet color score and myomere's area, but significantly decreased myocommata's lightness (L^* value). Frozen storage resulted in decreased firmness and increased drip loss, with a significantly higher drip loss from fillets thawed at 4°C compared with 20 °C. The effect of frozen storage and thawing conditions showed the same pattern for the dietary groups and rearing environment.

To summarize, fillet quality of farmed Atlantic salmon is improved by feeding high protein-to-lipid (P/L) diet and rearing in large sea net pens. Effects of rearing environment were more pronounced than effects of dietary treatment.

Key words: Atlantic salmon, isoenergetic diet, environment, protein-to-lipid (P/L) ratio, product quality, fillet quality, fillet yield, color, myocommata, myomere, drip loss, texture, gaping.

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LIST OF ABBREVIATIONS:

- P/L: Protein-to-Lipid ratio
- FM: Fish Meal
- FO: Fish Oil
- VIS/NIS: Visible and Near Infrared
- HSMI: Heart-Skeletal Muscle Inflammation
- PD: Pancreatic Disease
- CMS: Cardiomyopathy Syndrome
- DP: Digestible Protein
- DE: Digestible Energy
- DL: Digestible Lipid
- DP/DE: Digestible Protein/Digestible Energy
- EPA: Eicosapentaenoic Acid
- DHA: Docosahexaenoic Acid
- PUFA: Poly Unsaturated Fatty Acid
- USD: US Dollars
- FAO: Food and Agriculture Organization
- FCR: Feed Conversion Ratio
- FI: Feed Intake
- CF: Condition Factor
- NFE: Nitrogen Free Extract
- NQC: Norwegian Quality Cut
- ADC: Apparent Digestible Coefficient
- ATP: Adenosine Triphosphate
- HSI: Hepatosomatic Index
- SGR: Specific Growth Rate
- SEM: Standard Error of Mean
- ANOVA: Analysis of Variance

1. INTRODUCTION

Aquaculture is one of the fastest growing industries worldwide, among which, global aquaculture production of Atlantic salmon (*Salmo salar* L) has increased rapidly from 1,460,000 tons in 2010 to over 2,240,000 tons in 2016 (Food 2018). For Norway, Atlantic salmon aquaculture industry could date back to the early 1970s (Asche and Bjørndal 2011). According to FAO 2020 Fisheries & Aquaculture report, Norway has been the second largest world marine and coastal aquaculture producer of finfish during 2003 to 2018, with 1.4 million tons production, only 0.1 million tons less than China (Tacon 2020).

Within aquaculture, farmed Atlantic salmon contributes to over 90 percent of the total global farmed salmon market-share. Norway contributes to more than half of overall global salmon market (Iversen, Asche et al. 2020)), and at present (2020), Norwegian aquaculture sector of salmonids is the largest worldwide (Tacon 2020).

For example, farmed salmonids account for only 4% in total aquaculture volume but worth three times in economic value, 13% of total production value (Asche and Bjørndal 2011). In Norway's perspective specifically, it stands out as one of the most profitable and cutting-edge technological industries in its national fiscal revenue. In 2018, Norwegian seafood export industry peaked at a record-high revenue value worth over 12 billion USD (Bergesen and Tveterås 2019).

However, there are still many challenges for Norwegian salmon industry. For example, aquaculture species diversity is relatively low (mainly rely on Atlantic salmon) and the sustainability of raw feed material supply is still under heated discussion since marine-catch resources are scarce and the sustainability is questioned. The intensive dependence on fish oil and fish meal in traditional salmon farming was against sustainability (Deutsch, Gräslund et al. 2007, Tacon and Metian 2008). A great majority of fishery resources worldwide have been fully exploited or already been overexploited (Brander 2007). Alternative lipid and protein source from discards and by-products in production for human-food consumption could potentially save up to three times of forage fish capture (Ytrestøyl, Aas et al. 2015).

Fillet quality plays an essential role in the commercial world. Quality grading of Atlantic salmon is based on visual properties, thus three different quality classes: superior, ordinary and production are defined based on Norwegian industrial standards including body size, deformities, external blemishes (Misimi, Erikson et al. 2008, Sture, Øye et al. 2016). "Production class" salmon is not allowed for export (Misimi, Erikson et al. 2008) and growth rate has been the main market-price determinant (Analyse 2014). For consumers, flesh color and fillet firmness are also important indications for salmon fillet quality. Hence it is important to obtain high quality salmon fillets to succeed profitably by means of dietary and environmental optimization.

Several other rising problems related to fish diseases, such as pancreatic disease (PD), heart and skeletal muscle inflammation (HSMI) disease have introduced high mortality rate (up to 63%) in salmon farming, which subsequently cause poor quality fillet at slaughter site (Heuch, Bjørn et al. 2005, Jansen, Bang Jensen et al. 2017). Besides, sea lice infestation has increasing over past few decades in salmonid aquaculture due to fast development of salmon farming, which further leads to a higher resistance against pharmaceutical delousing-treatment in Atlantic salmon (Aaen, Helgesen et al. 2015). As a resolution, using non-pharmaceutical treatment such as mechanical or thermal sea lice removal becomes more than prevalent and popular (Lekang, Salas-Bringas et al. 2016). But in return, it causes higher stress level and mortality rate for treated salmon, hence, physical treatment somehow downgrades fish welfare and production parameters (Erikson, Gansel et al. 2016). Some studies revealed that high dietary P/L ratio helped to improve growth rate (Dessen, Weihe et al. 2017), survival rate during naturally occurred PD outbreaks (Dessen, Mørkøre et al. 2019) and production qualities (slaughter yield and muscle thickness) in Atlantic salmon (Weihe, Dessen et al. 2019). Site management by environmental alteration could also help to improve parasite resistance (Bui, Oppedal et al. 2013) and animal welfare/behavior (Glaropoulos, Stien et al. 2019) thus to improve fillet quality in Atlantic salmon.

It is therefore urgent to figure out the best way, in terms of dietary and environmental modification in order to optimize fillet quality in Atlantic salmon.

To reiterate, dietary effects are important for fillet quality in Atlantic salmon. For example previous research have shown that dietary inclusion of Antarctic krill meal (Mørkøre, Moreno et al. 2020), microalgae and organic-mineral meal (Kousoulaki, Mørkøre et al. 2016) and glutamate supplemented meal (Larsson, Koppang et al. 2014) could enhance growth performance and fillet quality in Atlantic salmon. However, there is limited knowledge regarding effects of dietary protein/lipid (P/L) ratio on fillet quality of Atlantic salmon. Secondly, rearing environment is also fundamental for salmonids' performance. Effects of environmental factors including salinity, pH, water temperature, natural currents, photoperiod have not been studied with regard to fillet quality of salmon.

To summarize, it is of great importance to study effects of diet and rearing environment on fillet quality of Atlantic salmon.

2. OBJECTIVES

The overall objective of this thesis is to investigate environmental and dietary effects on fillet quality of farmed Atlantic salmon.

The specific objectives are:

- Study effects of rearing environment on fillet quality of Atlantic salmon.

- Study effects of different protein-to-lipid (P/L) ratio diet on fillet quality parameters of Atlantic salmon in order to optimize production profit in Atlantic salmon farming industry.

3. THEORETICAL BACKGROUND

Atlantic salmon fillet quality consists of three main perspectives: physical quality, nutritional quality and sensory quality. This chapter gives an overall information about how Atlantic salmon fillet quality parameters are affected by these two exogenous factors shown in Figure 3.1: dietary and environmental treatment.

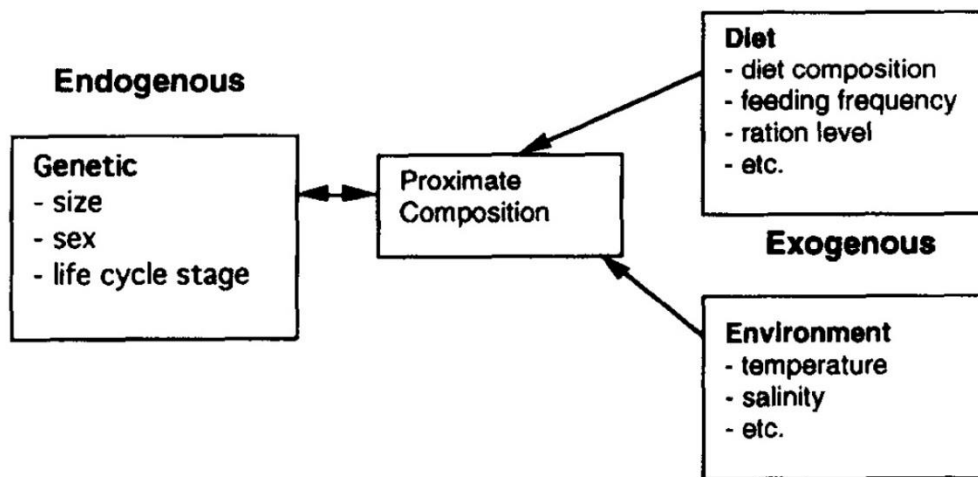


Figure 3.1, Endogenous and exogenous factors affect fish quality/proximate composition (Shearer 1994).

First and foremost, fillet quality is significantly dependent on feed materials (Thomassen 2007). Dietary composition somehow determine salmon fillet composition (Rasmussen 2001) even though studies have documented other non-dietary factors such as genetic background, rearing condition, sex/sexual maturation and life cycle stage (Dunajski 1980, Shearer 1994, Gjedrem 1997) play a role in salmonoids fillet quality too.

Atlantic salmon is regarded as a fatty fish usually having 60g/kg to 220g/kg fat content and an average of 150g/kg to 160g/kg standard fat level in Norwegian Quality Cut (NQC) (Rørå, Kvåle et al. 1998). Atlantic salmon were normally fed on fishmeal (FM) and fish oil (FO) as main protein and energy source (Green and Authority 2016), but from 1990 to 2013, the inclusion level of FM and FO has decreased from around 90% (65% and 24%, respectively) to 30% (18% and 11%, respectively), due to sustainable and production reasons (Ytrestøyl, Aas et al. 2015). An example of

commercial Atlantic salmon feed ingredients and chemical composition in 2012 is shown in Table 3.1, Table 3.2.

Table 3.1, Feed ingredients used in Norwegian salmon feed in 2012. Data are reported by EWOS, BioMar and Skretting (Ytrestøyl, Aas et al. 2015).

| | | Feed ingredient | Total amount used (tonnes) | % inclusion (of total diet) |
|--------------------|-----------------|---|----------------------------|-----------------------------|
| Plant ingredients | Protein sources | Soy protein concentrate | 346,730 | 21.3 |
| | | Sunflower expeller | 97,354 | 6.0 |
| | | Wheat gluten | 97,137 | 5.8 |
| | | Fava beans | 30,753 | 1.9 |
| | | Pea protein | 12,936 | 0.8 |
| | Oil sources | Maize gluten | 12,509 | 0.8 |
| | | Horse beans | 4442 | 0.3 |
| | | Rapeseed oil | 298,991 | 18.3 |
| | Starch sources | Wheat | 161,432 | 9.9 |
| | | Pea | 16,466 | 1.0 |
| | | Tapioca | 3396 | 0.2 |
| Marine ingredients | Protein sources | Fish meal | 317,241 | 19.5 |
| | Oil | Fish oil | 182,579 | 11.2 |
| Microingredients | | Pigments, vitamins, minerals, amino acids | 50,715 | 3.1 |

Table 3.2, Estimated average feed composition, total energy and total nutrient used in Norwegian salmon feed production in 2012 (Ytrestøyl, Aas et al. 2015).

| | Average composition of Norwegian salmon feed in 2012 (% or MJ/kg) ^a | Total amount of nutrients used in Norwegian salmon feed 2012 (tonnes or GJ) ^b | Nutrients from marine ingredients (tonnes or GJ) ^c | Nutrients from plant ingredients (tonnes or GJ) ^d |
|--------------------|--|--|---|--|
| Dry matter | 93.8 | 1,528,961 | 469,233 | 1,009,013 |
| Energy | 24.5 | 39,930,108 | 13,519,644 | 26,365,196 |
| Protein (N × 6.25) | 35.5 | 578,994 | 212,469 | 364,615 |
| Lipid | 32.5 | 529,904 | 212,940 | 316,964 |
| EPA | 1.5 | 24,903 | 24,903 | 0 |
| DHA | 1.1 | 18,106 | 18,106 | 0 |
| Phosphorus | 0.9 | 15,011 | 6747 | 4645 |

Average dry matter content in feed ingredients was 93.4%, and the same average dry matter content was assumed for feed.

a

Calculated from all ingredients used in 2012 and their chemical composition, reported by the three largest Norwegian feed companies (BioMar, Ewos and Skretting).

b

Calculated from average composition and the total of 1,451,908 tonnes of feeds used in 2012 (Akvafakta, http://akvafakta.fhl.no/fhl_statistikk/SRL/2013/Akvafakta%2013-01.pdf).

c

Fraction of nutrient of marine origin in the feed ingredients multiplied by the total amount of nutrient used in feed in 2012.

d

Fraction of nutrient of plant origin in the feed ingredients multiplied by the total amount of nutrient used in feed in 2012.

To conclude, estimated average composition of Norwegian salmon feed are: dry matter 93.8%, energy 24.5 MJ/kg, protein (N*6.25):35.5%, lipid 32.5%, EPA 1.5%, DHA 1.1% and phosphorus 0.9% (Ytrestøyl, Aas et al. 2015).

Besides, originally, Atlantic salmon as a carnivore fish in nature feed on 35-39% fat source and 30-35% protein source where protein-to-lipid (P/L) ratio is below 1:1.

Dietary lipid content not only affects nutritional quality, but also sensory quality, in terms of texture, taste and flavor (Rørå, Kvåle et al. 1998). Fat is deposited in myocommata that is rich in connective tissue. Excessive fat deposition between the myomeres affects the perceived red color of salmon fillets (Christiansen, Struksnæs et al. 1995). Nutritionally, dietary fat content greatly contributes to whole body and edible part's lipid content since dietary fatty acid profile leads to correspondingly fatty acid profile in salmon tissues. Fillet with proportionally high lipid content could be beneficial for smoking while lean fish fillets are popular when sold as fresh or frozen owing to a higher fillet yield (Wathne 1995). On the other hand, though high body lipid accumulation induces low slaughter yield as a result of elevated visceral weight relative to whole body weight (Lie 2001). However, protein content tends to be more stable in spite of dietary protein or amino acid composition compared to dietary lipid's influence on whole body and muscle adiposity regulation.

In contemporary salmon industry, energy dense diet with high lipid content is widely used in salmon farming since it generally improve feed utilization and growth rate (Torrissen, Olsen et al. 2011). On the contrary, fish fed with lean diet (P/L >1) would significantly reduce lipid deposition in muscle and viscera hence increasing feed intake, growth, weight gain, nutrient retention and better biometric traits of salmon (Dessen, Weihe et al. 2017). Moreover, energy dense diet tend to induce pancreatitis, pancreatic injury and overwhelming oxidative tension (Yan, Li et al. 2006). As a result, functional feed with high P/L ratio is used for viral disease treatment, which is supposed to have clinical application such as preventive medication, immune stimulation and anti-inflammation actions (Dessen, Mørkøre et al. 2019). Dessen (Dessen, Mørkøre et al. 2019) found that farmed Atlantic salmon naturally affected by pancreatic disease (PD) fed on high P/L ratio (47% protein, 24% fat, P/L:2) diet had significantly higher survival rate, quality parameter, lower mortality rate, lower prevalence of runts, and higher tolerance to PD outbreak. However, there is still not enough knowledge or literature review behind such high P/L feed's dietary effects among histopathology, mortality and quality parameters in farmed salmonid fish. But one thing for sure, there is positive correlation between high intake of lipid-rich energy dense diet and metabolic disease prevalence such as heart-skeletal muscle inflammation (HSMI), pancreatic disease (PD) and cardiomyopathy syndrome (CMS) (Weihe, Dessen et al. 2018).

Like in many other major salmonids-exporter countries, Atlantic salmon farming in Norway gradually altering to high P/L ratio diet at finishing phase when feed

utilization is considered substantial through lifecycle, which would promote slaughter yield and fillet quality. Interestingly, some studies also found high dietary P/L supply with balanced amino acid profile could be beneficial in enhancing feed utilization, muscle growth, protein deposition and yield production in Atlantic salmon (Bureau, Kaushik et al. 2003, Karalazos, Bendiksen et al. 2007). As a consequence, it could be practical to understand dietary effects of, especially between traditional commercial feed (P/L ratio<1) and lean feed (P/L ratio>1) in fillet quality of Atlantic salmon.

Environmental factors such as several infectious or non-infectious disease outbreak could occur under certain rearing conditions, reasons like nutritional shortcomings, pathogenic organisms, metabolic distress together with poor site management and inappropriate handling stress would also have impacts on quality-related biometric characteristics (Contessi, Volpatti et al. 2006, Crane and Hyatt 2011). For instance, apart from seawater temperature and photoperiod, smolt-type and the time of sea-transfer could determine seasonal growth and lipid deposition in Atlantic salmon (Johnsen, Hagen et al. 2011). Several studies have shown seasonal environmental changes significantly affect feed utilization and growth rate in Atlantic salmon (Oppedal, Taranger et al. 2003).

3.1. Effects of feed

A great number of exogenous factors, mainly categorized into environmental and dietary, have been revealed to determine fillet quality in cultured Atlantic salmon. In farmed salmonid species, body protein content is dependent on size while lipid content is influenced by life-cycle stage and dietary energy intake (Jobling 2001). However, lipid content in salmon feed has been increased gradually regardless of season since lipid is relatively cheaper energy source compared to protein and salmonids itself have great capability to utilize lipid source in energy dense diet for better growth and muscle gain (Azevedo, Leeson et al. 2004). Nevertheless, small size post-smolts require much more dietary protein content than larger salmon during fast-growing out phase (Storebakken 2002). To summarize, it is important to take into consideration of fish size and life cycle in seawater stage when rationing protein-to-lipid (P/L) ratio to realize optimal growth and health.

3.1.1. Dietary protein

The basic protein and amino acid requirement for salmonids to maintain healthy growth have been published (NRC 2011). Protein is the major organic matters in fish tissue made up to 65%~75% on dry-weight basis (Wilson 2003). Dietary protein is obtained by fish to either build new proteins especially during fast growth and reproduction period or to compensate proteolysis (protein maintenance). In feed cost perspective, protein source has been more expensive than lipid source if used as energy source. As a result, it leads to a trend in salmon farming industry to replace

protein content with lipid content (Torrissen, Olsen et al. 2011). That is the reason why in today's salmonids diet, protein inclusion level has been lower compared to traditional salmonids feed.

3.1.2. Dietary lipid

In Atlantic salmon, one special trait is to withstand periodic lipid/energy deficiency and ability to replenish or restore lipid/energy deposition once fed with sufficient dietary lipid and protein source without significant negative influence. In other words, salmon go through lipostatic mechanism to regulate lipid level as a way to achieve compensatory growth (Won and Borski 2013). Whole body lipid content is balanced between fish's metabolic energy demand and dietary energy input (Shearer 1994). For salmonids, carcass lipid composition reflects dietary lipid distribution (Bell, Ghioni et al. 1994). It is known that poly-unsaturated-fatty-acids (PUFAs: n-3, n-6) are helpful to avoid cardiovascular disease and other chronic disease, thus it is worthwhile to take consideration of PUFA-rich salmonids. However, salmonids with higher PUFA content show a lower acceptable taste and texture after freeze storage than salmon with rich short-chain saturated fatty acid profile due to oxidation (Shearer 2001).

Body size, dietary lipid inclusion level and feed ration alter lipid deposition in whole body, visceral cavity and muscle (regarded as main lipid storage area) in salmonids (Rasmussen 2001).

3.1.3. Dietary protein to dietary lipid ratio

Some studies have investigated effect of different dietary protein-to-lipid ratio among lipid deposition mainly in visceral cavity and muscle in salmon. In spring and early summertime when sea water temperature is low, no significant difference in fat content or growth rate is seen between salmon fed with high and low P/L ratio feed (Dessen, Weihe et al. 2017). Other than P/L ratio, fish size could also be a causative factor. Some studies indicate there is steady occurrence of impaired growth when fish fed with low P/L diet compared to high P/L diet. Dietary protein-to-lipid ratio could be related to digestible protein to digestible energy: DP/DE ratio, which has been widely emphasized in salmonids' feed production industry. In a previous study, several different DP/DE ratio diets (14.1g/MJ; 16.41g/MJ; 18.8 g/MJ; 21.91g/MJ) were fed to small and medium sized Atlantic salmon with initial weight of 1.0 kg and 2.5 kg, respectively. In general, both smaller and larger sized fish fed with DP/DE at 14.1g/MJ level showed least optimal growth rate, feed conversion ratio (FCR) and nitrogen/energy retention compared to any other DP/DE ratio diets (Einen and Roem 1997).

Specifically, for smaller salmonids with initial weight at 1.0kg, DP/DE at 18.8g/MJ seems the best for optimal growth when for larger fish with initial weight at 2.5kg, DP/DE at 16.4g/MJ is optimal. This may indicate that DP/DE ratio is supposed to be

formulated dependent on fish size/energy requirement to optimize growth rate and production quality. In addition, in smaller fish, carcass yield is positively correlated to DP/DE ratio. Overall, fish fed with DP/DE ratio at 21.91g/MJ have significantly higher protein content, but significantly lower body lipid content compared to similar sized fish fed with any other diets. In summary, Atlantic salmon with initial weight around 1~2.5kg need approximate DP/DE:19 g/MJ ratio diet while fish with initial weight around 2.5~2.5kg require about DP/DE:16~17 g/MJ ratio diet (Einen and Roem 1997).

3.2. Effects of environment

Environmental conditions also influence fillet quality. In high latitudes country like Norway where significantly different daylength is observed throughout the year, environmental factors involving photoperiod and water temperature are supposed to be considered rather critical in fish growth. Generally, lipid utilization or retention is related to seasonal water temperature and daylength/photoperiod fluctuation (Mørkøre and Rørvik 2001, Nordgarden, Ørnsrud et al. 2003). During late summer/early autumn period, when seawater is relatively warm compared to the rest of year cycle, relatively higher somatic growth and lipid accumulation/deposition phenomenon are observed (Mørkøre and Rørvik 2001).

3.2.1. Temperature

Water temperature is vital in almost every aspect of salmonid life stage (Armstrong and Schindler 2013). Slight ambient water temperature could significantly effect salmonid growth rate, fish behavior, disease resistibility, mortality rate and of course, biometric quality (Sullivan, Martin et al. 2000). During late summer and autumn period, water temperature is optimal for salmonids to reduce stress level and maintain high biometric criteria (Richter and Kolmes 2005). During this time, high somatic growth and high lipid deposition is accompanied with increased feed intake and feed utilization (Mørkøre and Rørvik 2001).

Temperature influences growth rate, disease resistance and mortality primarily by two aspects. On the one hand, temperature is vital for salmonids to maintain metabolism rate and feed conversion ratio since they are poikilotherm species. On the other hand, salmonids can only withstand a small range of lethal temperature as is shown below in Table 3.3 (Wehrly, Wang et al. 2007).

Table 3.3, Lower and upper critical temperature range, incipient lethal temperature and thermal tolerance for 10 different salmonids species (Elliott 1994).

| Species | Lower critical range (° C) | Upper critical range (° C) | Upper incipient lethal temperature | Thermal tolerance (° C ²) |
|-------------------------------|----------------------------|----------------------------|------------------------------------|---------------------------------------|
| <i>Salmo salar</i> | 0–7 | 22–33 | 27.8 | 708 |
| <i>S. trutta</i> | 0–4 | 20–30 | 24.7 | 583 |
| <i>Salvelinus alpinus</i> | 0 | 20–27 | | |
| <i>S. fontinalis</i> | 0–7 | 20–29 | 25.3 | 625 |
| <i>Oncorhynchus gorbuscha</i> | | 21–28 | 23.9 | 450 |
| <i>O. keta</i> | 0–7 | 22–28 | 23.8 | 468 |
| <i>O. nerka</i> | 0–7 | 22–28 | 24.4 | 505 |
| <i>O. kisutch</i> | 0–6 | 23–28 | 25.0 | 528 |
| <i>O. tshawytscha</i> | 0–7 | 22–28 | 25.1 | 529 |
| <i>O. mykiss</i> | 0–9 | 19–30 | 26.2 | |

Accordingly, optimal ambient temperature for Atlantic salmon is around 13°C (Handeland, Arnesen et al. 2003). Some studies found indications that temperature over 20°C induce higher prevalence of melanin production (Larsen, Austbø et al. 2013). In general, sea water temperature in western Norwegian fjords (latitude: 58°N -78°N) can vary from $0.5 \pm 2^\circ\text{C}$ to $11 \pm 2^\circ\text{C}$ (Ljungström, Claireaux et al. 2020). Taking Ekkilsøy (63°03'N/7°35'E, one of the rearing sites belong to Marine Harvest research station in Norwegian west coast) for example, seawater temperature peaks at 15°C in late August but with an average temperature at 9.8°C, shown in Figure 3.2.

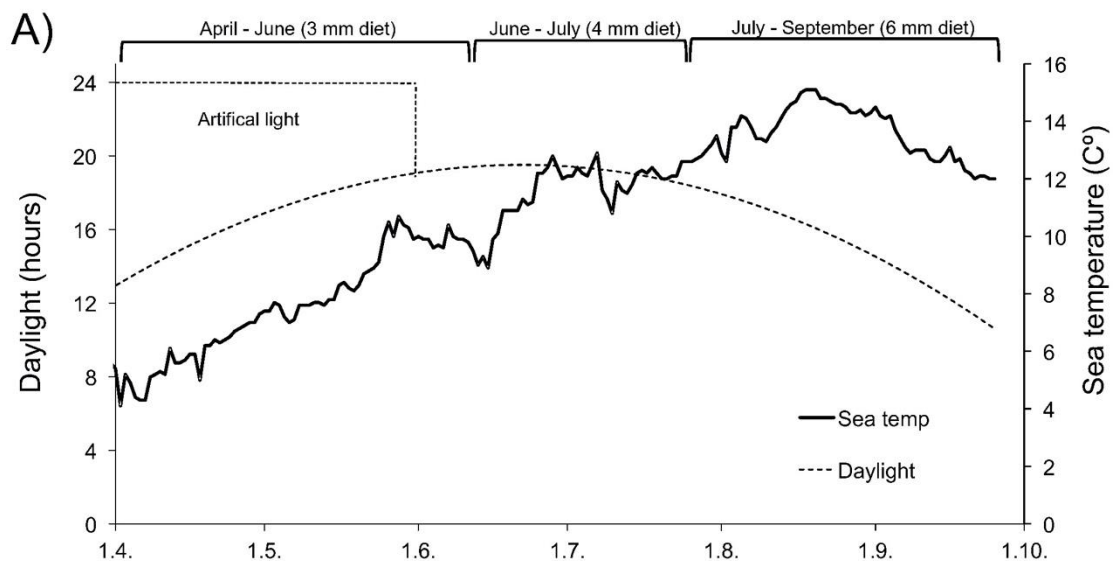


Figure 3.2, Seawater temperature (°C) and daylength(hours) during 04/2012~09/2012 in west coast of Norway(63°03'N/7°35'E) (Dessen, Weihe et al. 2017).

The average temperature in late summer and early autumn time, farmed salmon deposit more fat in whole-body and muscle owing to higher feed intake (Nordgarden, Oppedal et al. 2003, Rørvik, Dessen et al. 2018), hence achieving increased condition factor (CF) and weight gain. On the other hand, during late autumn and winter time, when seawater temperature is low and day length shortened, salmon tend to store

more adipose and tend to have comparable lower feed intake, fat deposition, growth rate, condition factor (Brett, Shelbourn et al. 1969, Alne, Oehme et al. 2011). It is assumed if salmonids fed with isoenergetic sufficient diet with relatively high protein-to-lipid (P/L) ratio would reduce fat deposition in body, muscle and visceral cavity thus enhancing feed intake and growth especially prior to high fat accumulation time in autumn (Jobling, Larsen et al. 2002).

3.2.2. Salinity

Temperature and salinity have quite a few complex interactions when it comes to metabolic rate since the previous one affects thermal regulation when the latter one affects osmoregulation. Both of them are energy consuming. Salinity is regarded as one of the key factors reported to influence fish growth especially in aspects of egg fertilization, incubation, embryogenesis, larval growth, feed intake and feed conversion ratio (FCR) (Boeuf and Payan 2001). Thus, it is thoughtful to also look at interactive correlation between salinity, fish growth and biometric traits in Atlantic salmon. For example, rainbow trout (*Salmo gairdneri*) juveniles reared in higher salinity condition claimed to have significantly ($P < 0.05$) higher mortality rate than lower salinity level (Zeitoun, Halver et al. 1973).

3.2.3. Exercise

Atlantic salmon reared in a raceway with consistent current have higher muscle mass, bigger hypertrophied white fibers and larger amount of stored glycogen in muscle compared to fish reared in tanks (Totland, Kryvi et al. 1987). In other words, salmonids tend to grow faster and utilize feed more efficiently when reared in flowing currents than in standing water (Jobling, Baardvik et al. 1993). Specific growth rate (SGR), feed intake (FI) and susceptibility to fin damage are improved (Jørgensen and Jobling 1993). As a consequence, it is more common to attain superior-quality fish in raceway condition.

3.3. Fillet quality parameters

Atlantic salmon fillet quality can be affected by many factors during pre-mortem and post-mortem phase. There are several biometric characteristics that are able to define salmon fillets' quality parameters, such quality indicators are listed as follow: lipid content/composition/distribution among fillet, texture (firmness, elasticity and integrity), color intensity/distribution, gapping, drip loss (Sigurgisladottir, ØTorrissen et al. 1997) and muscle segment formation.

Soft texture, high volume of drip loss, pale or unevenly distributed color, fatty fillet, terrible gapping and melanin spots are general downgrading factors in salmon market (Färber 2017). Among those, coloration and dis-coloration have received most

attention (Shahidi and Brown 1998). While through life cycle, feed intake, disease and environmental factors influence fillet quality traits (Thomassen 2007).

3.3.1. External appearance

The external appearance of fish morphology represents first impression of fish quality (Waagbø, Sandnes et al. 1993). Condition factor ($\text{weight}/\text{length}^3$) is used to show fish's body condition and thickness/leanness. In addition, carcass/slaughter yield and fillet yield are representative as well. In order to standardize quality assessment, color and fat content are measured in designated area in belly flip while texture measurement is conducted in dorsal fillet part. Gaping score is assessed from overall fillet area. Every measurement should not vary from different fish individuals.

3.3.2. Texture

Texture is one of the most important quality criteria in fish. Consumers tend to prefer fish with firm texture with considerable amount of connective tissue and muscle. Texture could be represented with gaping, firmness/consistency and juiciness. Different storage method influences flesh texture. Frozen storage slows protein deterioration caused by enzymatic activity and lipid oxidation compared to fridge-storage. But slow freezing could also be problematic for texture due to slow intracellular crystallization and more scattering between muscle segments.

Gaping score is widely estimated and it is correlated with fish size (Borderías and Sánchez-Alonso 2011), growth rate and environmental conditions. Even in the same fish, gaping score could vary in different part of the flesh. From anterior end to posterior end, ventral part to dorsal part, less gaping score is discovered. Gaping happens when connective tissue between muscle segments breaks. In sea net pens, rearing environment and dietary input have impacts on flesh texture: firmness and gaping score (Mørkøre 2008).

Gaping/slits can be costly and troublesome since it not only makes fillet difficult to be mechanically processed (skinned, smoked or/and sliced) but also impairs market price due to unfavorable appearance. Gaping seems not to be effectively affected by thawing method, freezing rate or storage time-period (Love 1988).

3.3.3. Color

It is common practice to measure flesh color as a way to represent fish quality in commercial market. Liver color, skin color, fillet color and discoloration spots are also widely assessed as quality-related indicators. For example, visceral fat was assessed visually, scoring from 1~5 in which different numbers indicate the degree of visceral lipid deposition. Below is scale (Figure 3.3) standardized for liver color(A) and visceral fat (B) assessment.



Figure 3.3, Scale for assessment of visual liver color (A) and visceral fat index according to visibility of pyloric caeca in Atlantic salmon (B, from 1-5, “clearly visible” to “not visible”) (Mørkøre, Moreno et al. 2020).

The redness/pinkish coloration in white muscle mainly come from astaxanthin whose level varies from season to season. Astaxanthin (carotenoid) as a pigmentation source is added as one of Atlantic salmon feed ingredients to improve fillet coloration (Quevedo, Aguilera et al. 2010). As an antioxidant, astaxanthin is also vital for immune-system and reproduction functionality.

Astaxanthin gradually accumulate and deposit in flesh until fish go through sexual maturation. Besides, a relatively higher growth rate during autumn would lead to poorer pigmentation in flesh (Rørvik, Ytrestøyl et al. 2010). So, it is strategic to harvest market size salmon before they are sexually matured when fillet color is the highest during the year. Below (Figure 3.4) is SalmonFan™ usually used to measure Atlantic salmon flesh color.



Figure 3.4, SalmonFan™ for fillet color assessment in Atlantic salmon (DSM, Nutritional Products Ltd., Basel Switzerland).

3.3.4. Drip loss

Liquid holding capacity, or so-called liquid loss, is a great matter in consumers market. Firstly, liquid loss causes direct financial loss for fish farmers/producers since fish lose weight/yield. Secondly, liquid loss is unfavorable for consumers and much liquid could induce bacterial reproduction thus bringing up with food-contamination concerns.

Liquid loss is affected by different freezing rates. Fish frozen at a fast speed tend to form rapid ice nucleation within intracellular space which create smaller ice-crystals and have less structural destruction on flesh (Petzold and Aguilera 2009). However, liquid loss formed during thawing process is still a complicated and comprehensive progress where further studies are needed (Zhu, Ramaswamy et al. 2004).

3.3.5. Myocommata and image analysis

Salmon flesh is made up of muscle fiber and connective tissue, where muscle fiber made up from muscle proteins while connective tissue (myocommata) made up from collagen, matrix and lipid.

Lipid content in edible part of fish are important to food scientists owing to three perspectives: sensations after cooking, health benefits and off-flavor after frozen storage (Hall 2012). Atlantic salmon subjected to moderate starvation prior to slaughter develop less lipid oxidation, off-flavor and off-smell.

According to Folkestad, fat content and pigment concentration can be determined on live whole fish or fillet by digital photography (Folkestad, Wold et al. 2008). Lipid level is analyzed and predicted by digital image analysis. Since belly flap claimed to have high adipocytes concentration level thus the highest fat percentage (Einen, Waagan et al. 1998). It is convincible to scan belly flap cutlet inside PhotoFish box (AKVAgrou, Bryne, Norway) to get digital images for further image analysis by using ImageJ. Lipid content is variable during seasonal period, during individuals under different and even same environmental conditions (Bell, McEvoy et al. 1998). These variables could cause problems in salmon process industry (Rørå, Kvåle et al. 1998).

The dietary and environmental effects on a series of biometric traits, nutrient retention and fillet quality parameters were evaluated.

4. MATERIAL AND METHODS

This experiment was designed to reveal dietary and environmental effects on fillet quality of Atlantic salmon (*Salmo salar* L).

The experiment lasted for seven-month, started from 05/2019 and terminated in 11/2019. The rearing locations were Flemma that on the Norwegian west coast (commercial sized sea net pens) and Sunndalsøra that is located on its eastern side along the same fjord (small research tanks on land), marked with white dot shown in Figure 4.1.

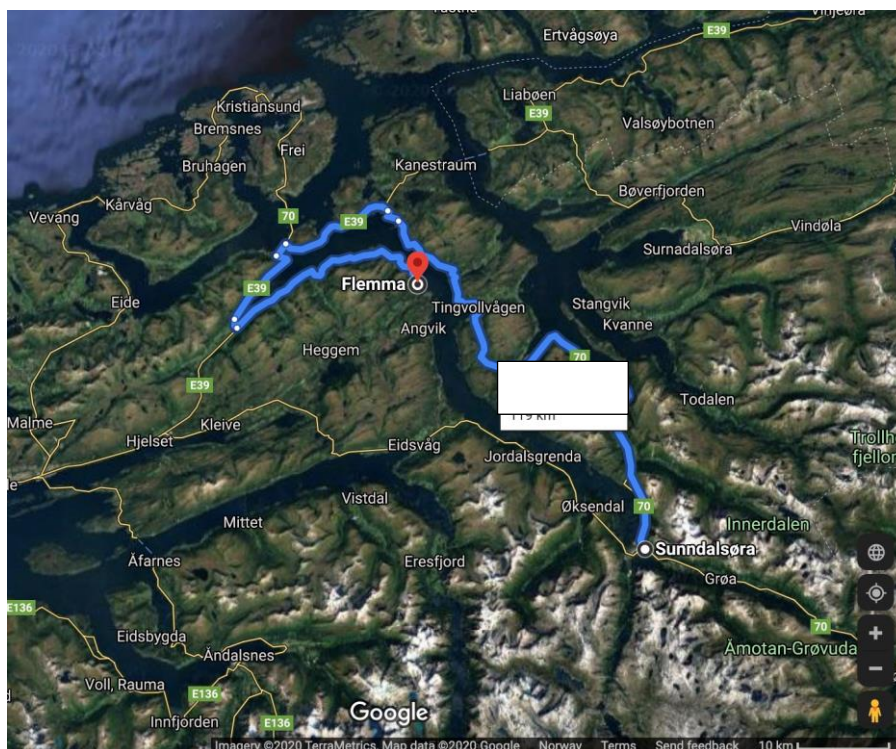


Figure 4.1, Geological location of the two different rearing environments. Red pin stands for the location of Flemma and the southeastern area near Grøa is where Sunndalsøra belongs to. Flemma and Sunndalsøra belong to a same fjord.

4.1. Dietary treatment and feeding scheme

The fish in sea net-pen in Flemma and tanks in Sunndalsøra were fed a commercial diet (control diet) (6 tanks), while the fish in sea net-pen in addition were fed a lean diet (test diet) (2 net pens per diet). The lean diet is modified to include a relatively lower lipid content but a higher protein content, whereas control diet is a commonly used commercial diet.

Test diets' feed formulation used in this experiment is close to the one used in Dessen's experimental trial (Dessen, Weihe et al. 2017). Two isoenergetic diets differing in protein/lipid (P/L) ratio with similar digestible energy were fed to fish from the same smolt producer (Lerøy, Belsvik, Norway). Calculated digestible protein (DP) is 335g/kg (low P/L) and 379g/kg (high P/L), digestible energy (DE) is 22.1 MJ/kg (low P/L) and 21.8 MJ/kg (high P/L). Therefore, estimated DP/DE ratio for Control (low P/L) and Test (high P/L) is 15.2 g MJ/kg and 17.4 g MJ/kg, respectively.

In general, the high P/L ratio diet have significantly higher protein, but lower fat level compared to low P/L ratio diet. The ingredients as well as macronutrients such as vitamins/mineral premix and astaxanthin used in Dessen's experiment is shown in Table 4.1. Regarding the salmon used in this thesis is approaching slaughter size (average 4~5kg body weight), so the pellet size in 6mm is relevant. Same for approximate chemical compositions of two experimental diets, detailed information is shown in Table 4.2.

Table 4.1, Formulation (g/kg) in experimental diets (Dessen, Weihe et al. 2017).

| Pellet size | 3 mm | | 4 mm | | 6 mm | |
|---|------|-----|------|-----|------|-----|
| | LP | HP | LP | HP | LP | HP |
| <i>Formulation, (g kg⁻¹)</i> | | | | | | |
| Micro ingredients^a | 25 | 25 | 25 | 25 | 15 | 15 |
| Wheat | 119 | 105 | 138 | 100 | 140 | 125 |
| Wheat gluten | 20 | 58 | 20 | 63 | 28 | 69 |
| Soy protein concentrate | 38 | 26 | 15 | 61 | 56 | 45 |
| Fish meal | 492 | 531 | 520 | 511 | 387 | 425 |
| Krill meal | 55 | 55 | 15 | 15 | - | - |
| Porcine blood meal | - | - | - | - | 45 | 30 |
| Fish oil | 110 | 95 | 127 | 116 | 151 | 136 |
| Rapeseed oil | 110 | 95 | 127 | 116 | 151 | 136 |
| Pigment^b (mg kg⁻¹) | 50 | 50 | 50 | 50 | 50 | 50 |

a
Vitamin and mineral premixes.

b
Astaxanthin.

Table 4.2, Approximate chemical compositions (g/kg) of the experimental diets (Dessen, Weihe et al. 2017). Pellet size of 6mm is regarded as relevant for this

experimental trial.

| Pellet size | 3 mm | | 4 mm | | 6 mm | |
|--|-------|------|------|------|------|------|
| Diet code | LP | HP | LP | HP | LP | HP |
| <i>Chemical composition, (g kg⁻¹)</i> | | | | | | |
| Crude protein ($N \times 6.25$) | 444 | 483 | 413 | 452 | 390 | 441 |
| Crude lipid | 286 | 260 | 328 | 285 | 347 | 316 |
| Ash | 89 | 94 | 85 | 90 | 55 | 58 |
| Water | 71 | 73 | 64 | 79 | 62 | 62 |
| Crude fiber | 1.6 | 1.2 | 0.8 | 0.7 | 1.1 | 1.0 |
| Total starch | 73 | 73 | 77 | 69 | 101 | 88 |
| NFE ^a | 108.4 | 88.8 | 109 | 93 | 145 | 122 |
| Gross energy, (MJ kg ⁻¹) | 23.8 | 23.3 | 24.4 | 23.4 | 25.2 | 24.9 |
| Crude protein/lipid ratio | 1.55 | 1.86 | 1.26 | 1.59 | 1.12 | 1.40 |
| <i>Digestibility calculations^b</i> | | | | | | |
| Calculated DP, (g kg ⁻¹) | 382 | 415 | 355 | 389 | 335 | 379 |
| Calculated DE, (MJ kg ⁻¹) | 20.6 | 20.3 | 21.5 | 20.6 | 22.1 | 21.8 |
| Estimated DP/DE ratio (g MJ kg ⁻¹) | 18.5 | 20.5 | 16.5 | 18.9 | 15.2 | 17.4 |

- a: NFE= Nitrogen free extracts=1000 - (protein + lipids + ash + fiber + water)
- b: the amounts of digestible protein and digestible energy were estimated based on gross energy content of 23.7 MJ/kg (protein), 39.5 MJ/kg (lipids) and 17.2 MJ/kg(carbohydrate). The apparent digestible coefficients (ADCs) used is 0.86 and 0.94, for protein and lipids respectively (Einen and Roem 1997); 0.50 for NFE (Arnesen and Krogdahl 1993).

4.2. Experimental design and fish material

Post-smolts from the same smolt producer were separated into sea net pens at Flemma or on land tanks in Sunndalsøra. Smoltification process was regarded as completed by conducting seawater challenge test developed by Clarke (Clarke, Saunders et al. 1996), when plasma osmolality, chloride content and gill Na⁺, K⁺-ATP activity were tested before seawater transportation. Detailed fish feeding regime and their rearing environment is shown in Table 4.3. Random 5 fish from 6 tanks (30 fish in total) and 33 fish from 2 different large sea net pens (66 fish in total) were selected for analysis.

Table 4.3, Feeding regime in different rearing environments.

| 09-12-2019 | 09-12-20192 | 09-12-20193 | 09-12-20194 | 09-12-20195 | 09-12-20196 | 14/11/2019 | 21-11-2019 | 14-11-2019 | 21-11-20197 |
|-------------|-------------|-------------|-------------|-------------|-------------|------------|------------|------------|-------------|
| sunndalsøra | sunndalsøra | sunndalsøra | sunndalsøra | sunndalsøra | sunndalsøra | flemma | flemma | flemma | flemma |
| control | control | control | control | control | control | control | control | test | test |
| tank 101 | tank 103 | tank105 | tank107 | tank109 | tank 111 | tank 7 | tank 7 | tank 110 | tank 10 |
| 5 | 5 | 5 | 5 | 5 | 5 | 8 | 16 | 17 | 16 |
| | | | | | | tank107 | | | |
| | | | | | | 9 | | | |

4.3. On-site sampling and slaughter

The research was conducted within the Norwegian guidelines, Norwegian national laws and animal welfare behavior rules & regulations. Fish were well fed and treated as production fish until harvesting. Before harvesting, salmon were starved for 3~4 days. Selected salmon were weighed in bulk at the end of the experimental trial. All fish were anesthetized on-site with MS-222 (Metacaine 0.1g/L, Alpharma, UK) and killed by a blow to the head, gill arched cut and bled out in big containers filled with seawater (3~4 fish per box at the maximum). Salmon's body length and body weight were recorded individually before being gutted.

Fish packed in plastic bags were transported to NOFIMA, Ås in ice-filled-insulated Styrofoam-salmon-boxes within 24 hours straight after slaughter. Quality analysis such as flesh texture, flesh color, gaping was evaluated and recorded when fillets were fresh, one week after harvesting. Duplicate sample from same individual fish were packed and stored in a freezer room (-20°C) for 3 months in order to study the effects of frozen storage on texture and drip loss. Thawing was performed at 4°C and 20°C, respectively. Thawing was performed on stainless steel flat-surface under designated temperature (4°C and 20°C respectively) until the temperature reached 6~8°C since it is regarded as common temperature to measure flesh texture.

In fresh fillet, texture analysis is conducted in anterior part above lateral line shown in figure 4.2. In thawed fillet after freeze-storage, texture analysis is conducted in anterior part but slightly former compared to the location that was being conducted in fresh fillet (figure 4.2). Texture is measured in the anterior area, on the line where muscle segment changes its direction above lateral line, about the middle point from anterior dorsal fin-end to the head posterior-end.

The total collected and sampled fish number: n=96 (Sunndalsøra: n=30; 5 fish/tank*6tank, Flemma with control diet: n=33 fish, Flemma with test diet: n=33 fish).

4.4. Quality analysis

Individual fillet is divided and sliced into individual parts for analyses and quality parameters measurement. Dissecting method is shown in Figure 4.2:

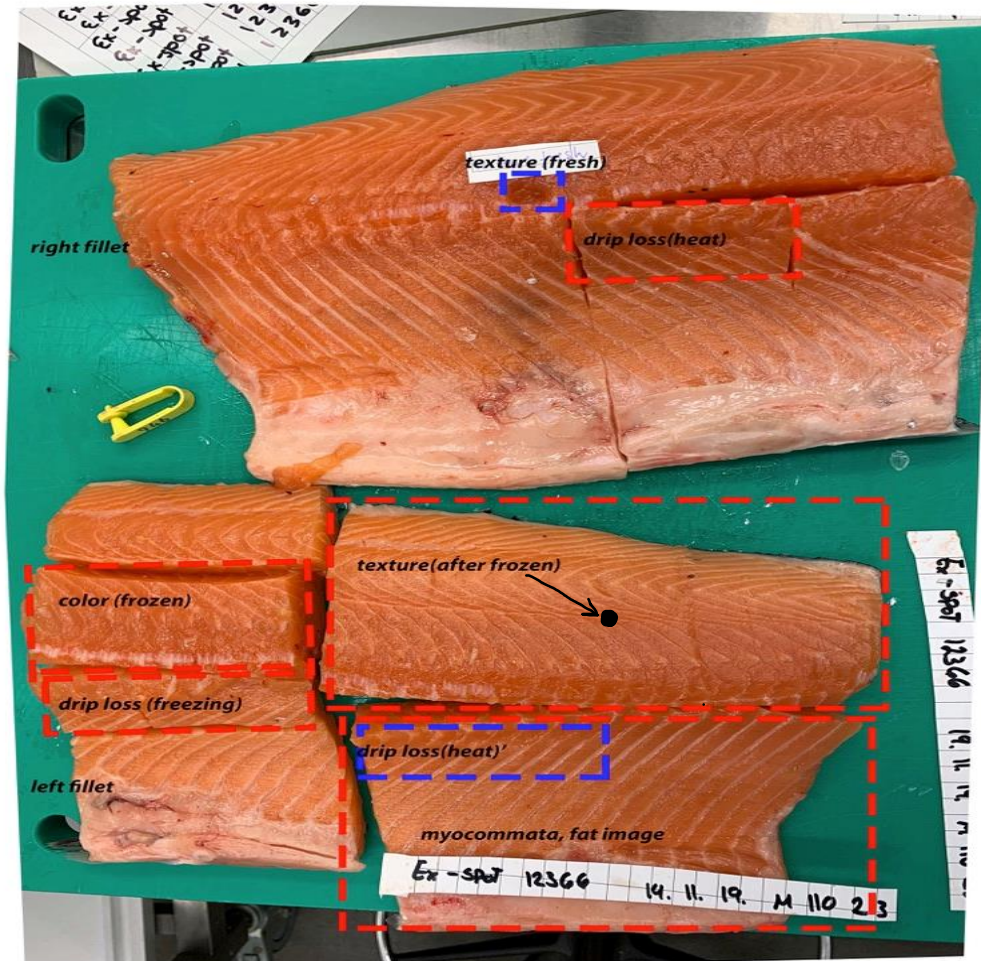


Figure 4.2, Standardized dissection image on both sides of fillet. Top shows the right fillet side while the lower shows fillet of the left side. If both sides of fillet are available, samples are collected as above otherwise from the same fillet regardless left or right side.

In general, samples are taken from both sides of fillet, however, sometimes when only one side of fillets is available, drip loss (heat) piece is sampled from modified location in the same fillet side. As is shown above, drip loss (heat)' in blue dotted line is the alternative for drip loss (heat) when only left fillet is available.

Drip loss pieces (freezing/heat) have a standard size: 6cm*3cm. Drip loss (freezing) and drip loss (heat) are in mirror-image-formation on both sides of fillet to minimize individual errors.

Initial sample collecting time were: 14th of November, 21st of November and 9th of December. Sample collecting were kept on the same day then packed and stored in freezer room. Texture analysis was done on 14th of February. Drip loss measurement was done on 24th of June.

4.5. Myocommata and myomere image analysis

Myocommata or so-called connective tissue between muscle segment is made up of lipid, collagen and matrix. It has a high lipid inclusion level, so it has been used as a way to measure fat content in flesh apart from other lipid analyses.

The fillets were photographed inside PhotoFish light-proof aluminum box equipped with a digital camera under a built-in internal light source setting. A calibration card, QPcard 101(QPcard AB, Gothenburg, Sweden, 142mm x 40mm.) with standardized white, grey and black patches, is set aside fillet cutlets to calibrate lightness and white balance shown in Figure 4.3.

By converting R, G, B values to CIE L*, a* and b*, it is possible to estimate lipid content, to analyze both myomere and myocommata's width and color parameters.

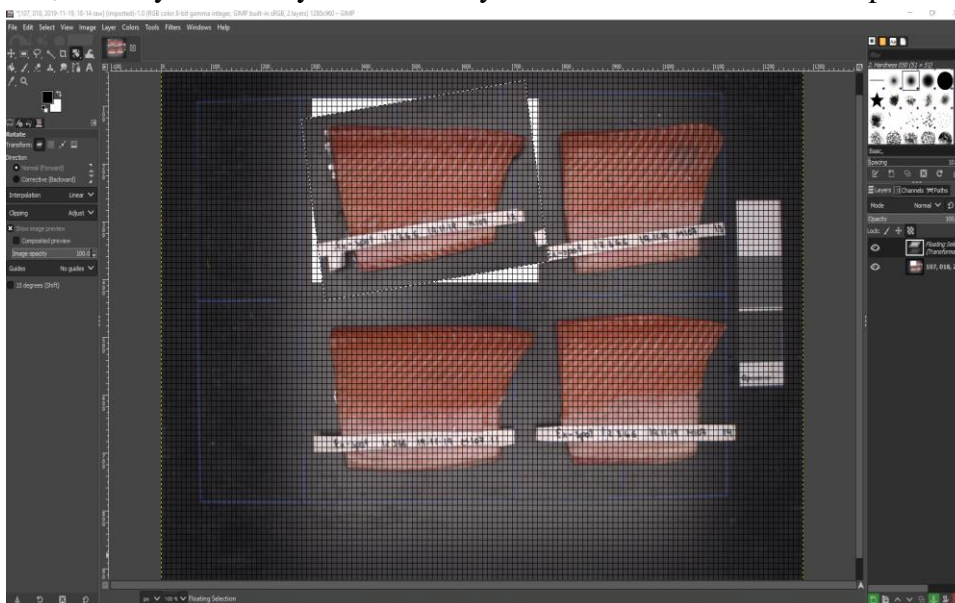


Figure 4.3, Using GIMP (GNU Image Manipulation Program, GPLv3+, Copyright © 2003-2011) to rotate lateral line until horizontal transverse septum is parallel with horizontal grid.

With the help of grid in Figure 4.3, it is applicable to rotate selected belly flap to horizontal level which would increase accuracy while measuring myocommata's area and width.

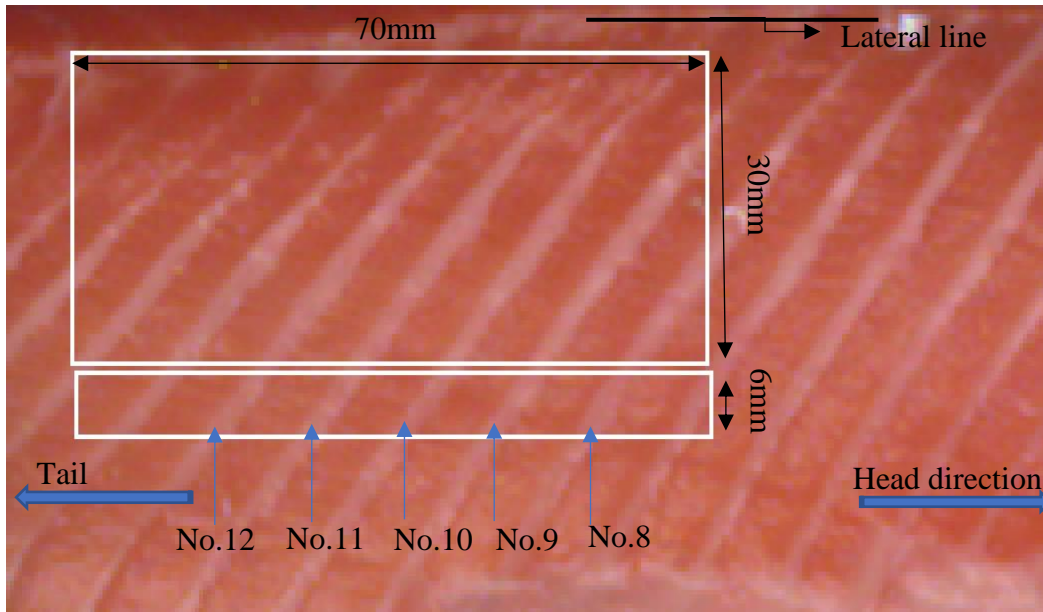


Figure 4.4, Image analysis of RGB values, width of five definitive myocommata and myomere (No.8, No.9, No.10, No.11 and No.12).

In Figure 4.4, the measurement area is taken approximately 30mm beneath lateral line, with 6mm in height and 70mm in width (14pixel*163pixel). Five myocommata from anterior part numbered 8 to 12 are included. Smaller rectangle is cropped out and measured individually to assure accuracy. Then using ImageJ software to conduct RGB value measurement, area measurement and width measurement applied on individual myocommata and myomere consecutively. Mean values are recorded manually in excel then further converted into CIE L*, a* and b* values.

4.6. Color measurement

Color is an important factor when it comes to customer choice and profit margins. These fish samples were evaluated using SalmoFanTM (Figure 3.4) score ranges from 20-34.

4.7. Texture measurement

Texture analysis was performed using a texture instrument TA-XT2 (SMS, Stable Micro System Ltd, Surrey, England) by pressing a flat ended cylinder (12.5mm diameter, type P/0.5) at a steady speed of 1 mm/s into the muscle until it reached 70% of fillet thickness. Firmness is positively correlated with breaking force. Breaking force (N) (Fb, Newton) is the force needed to puncture the fillet surface, which was recorded in the computer system. Core temperature in every fillet was also measured and recorded in graphs so as to remove the wrong data. The ideal core temperature shall be close to 2°C~8°C and shall not measure firmness while fillets were still

frozen or hard since that overestimated firmness. It was practical to measure texture once fillet surface temperature reached 2°C. Because during thawing process temperature rises rather quickly after 2~3°C (shown in Figure 4.5 and Figure 4.6).



Figure 4.5, Fillets thawing on stainless steel surface (room temperature is about 6-8°C) during texture measurement.



Figure 4.6, Texture measuring point marked in black dots, which were taken from middle of crossing line where muscle segments change their direction. Crossing line is designated to start from anterior dorsal fin end to fillet's another end.

4.8. Drip loss measurement

Drip loss is measured after freeze storage for approximate seven months. It was measured on 24/06/2020 and 25/06/2020 due to Covid-19 outbreak. Two collected cutlets from every fish individual (referred to drip loss(freezing) and drip loss(heat) respectively) are thawed in two different ways to see different temperature and different thawing method's effect on frozen flesh.

One way is to take the frozen cutlets named "drip loss (heat)" into fridge room with room temperature steady at 4°C the day before measurement. Before cutlet transportation, initial weight of every cutlet (W1) is weighed and recorded manually in Excel. After 17 hours of thawing under 4°C condition, half-thawed cutlets are taken to room temperature at 20°C for 2 hours. Again, before transportation, initial weight (W2) is measured and recorded manually. Thawing and measuring are conducted simultaneously within 3 hours. In every measurement, drip loss is not removed/absorbed with paper tissue, instead, by tearing scale before measurement to save time and ensure accuracy. After thawing, final weight (W3) is recorded.

Another way is to take the frozen cutlet on the same day as measuring. Cutlet named with "drip loss(freezing)" are taken from -20°C freezer room directly to room temperature at 20°C to thaw for 1 hour. Before thawing and after thawing, individual weights are measured and recorded manually, in which initial weight marked as W4 and final weight as W5.

4.9. Calculations:

Condition factor (CF): $(\text{body weight(g)}) / (\text{fish body length(cm)})^3 * 100$.

Slaughter/carcass yield: $(\text{gutted body weight(g)}) / (\text{whole body weight(g)}) * 100$.

Fillet yield: $(\text{fillet weight(g)}) / (\text{whole body weight(g)}) * 100$.

Hepatosomatic index (HSI%): $(\text{liver weight(g)}) / (\text{whole body weight(g)}) * 100$.

Drip loss: $(\text{Weight of frozen cutlet(g)} - \text{weight of thawed cutlet(g)} (4^\circ\text{C}, 20^\circ\text{C})) / (\text{weight of frozen cutlet(g)}) * 100$.

4.10. Data statistical analysis

The experimental trial was conducted using randomized block design and all data were analyzed in ANOVA procedure cooperated with SAS program (SAS university edition, Oracle VM VirtualBox Manager 6.0.14, © 2007-2019 Oracle Corporation, USA) and R Studio (RStudio 1.3.959, © 2009-2020 RStudio, PBC).

Diet and environment were defined as class variables. Sea net pens and tanks were regarded as experimental variables. Significant differences between biometric traits were tested by one-way ANOVA. Homogeneity of Variance was tested by Bartlett's test, if $P > 0.05$, it means there is no different variance between the two populations, so further T-test would be valid. Correlation between two quality parameters were tested by Pearson's correlation coefficient. All results are presented as mean \pm SEM.

Difference is set as significant at level of 5% ($P < 0.05$), and if P value is between $0.05 < P < 0.1$, it is assumed there is a trend. Otherwise stated separately.

5. RESULTS

This chapter talks about how rearing environment and diet affect biometric traits and fillet quality characteristics. The first part illustrates the general biometric traits of the fish. The second part demonstrates various quality parameters in salmon fillet.

For fish reared in Sunndalsøra and Flemma fed with control diet, the average body weight, body length, gutted weight are, 3142g and 4423g, 58cm and 71cm, 2719g and 3900g, shown in Table 5.1. All these three major indicators are significantly different from each other under environmental treatments ($P < 0.05$). The carcass and fillet yield relative to body weight are, 87.0% and 88.1%, 62.6% and 63.6%, respectively. Carcass yield shows no significant difference ($P > 0.05$) while fillet yield shows significant difference ($P < 0.05$) between the two rearing environments. The condition factors are 1.6 and 1.2 respectively, which shows significant difference between rearing environments ($P < 0.05$).

Table 5.1, Biometric traits and liver color of Atlantic salmon (*Salmo salar* L) reared in different environments: Sunndalsøra (inland tanks) or Flemma (sea net pens) fed with control diet. Results are presented as means \pm SEM and significant differences between environmental treatments are indicated by different superscripts.

| Rearing site | Sunndalsøra | Flemma | P-value |
|------------------------------------|------------------------------|------------------------------|-------------|
| Body weight, g | 3142 \pm 166 ^a | 4423 \pm 29 ^b | $P < 0.001$ |
| Body length, cm | 58.2 \pm 0.9 ^a | 70.7 \pm 0.2 ^b | $P < 0.001$ |
| Gutted weight, g | 2719 \pm 154 ^a | 3900 \pm 22 ^b | $P < 0.001$ |
| Fillet weight, g | 1970 \pm 108 | 2791 \pm 33 | 0.34 |
| Liver weight, g | 36.2 \pm 2.3 ^a | 42.4 \pm 1.5 ^b | 0.021 |
| Liver color, score | 3.5 \pm 0.1 ^a | 3.0 \pm 0.1 ^b | 0.0099 |
| Condition factor (CF) ¹ | 1.6 \pm 0.0 ^a | 1.2 \pm 0.1 ^b | $P < 0.001$ |
| Carcass yield, % ² | 87.0 \pm 0.8 | 88.1 \pm 0.5 | 0.16 |
| Fillet yield, % ³ | 62.6 \pm 0.9 ^a | 63.6 \pm 0.6 ^b | 0.013 |
| HSI% ⁴ | 1.14 \pm 0.04 ^a | 0.96 \pm 0.03 ^b | $P < 0.001$ |

1. Condition factor, CF= (body weight, g)/ (body length, cm)³*100;

2. Carcass yield= (gutted weight, g)/ (body weight, g) *100;

3. Fillet yield= (fillet weight, g)/ (body weight, g) *100;

4. HSI%= (liver weight, g)/ (body weight, g) *100;

For fish reared in Flemma fed with either control diet or test diet, the average body weight, body length, gutted weight are, 4423g and 4510g, 71cm and 72cm, 3900g and 3959g, shown in Table 5.2. All these three major indicators are not significantly different from each other except for body length ($P < 0.05$). It is assumable that by further reducing lipid inclusion in isoenergetic diets, improved growth response could be recognized. The carcass and fillet yield relative to body weight are, 88.1% and 87.8%, 63.6% and 64.6%, in which dietary treatments showed no significant difference ($P > 0.05$). The condition factor is 1.2 for both dietary treatments ($P > 0.05$).

Table 5.2, Biometric traits and liver color of Atlantic salmon (*Salmo salar* L) reared in Flemma (sea net pens) fed with different diets differing in dietary P/L ratio. Results are presented as means \pm SEM and significant differences between dietary treatments are indicated by different superscripts.

| Diets | Control | Test | P-value |
|------------------------------------|-----------------------------|-----------------------------|---------|
| Body weight, g | 4423 \pm 29 | 4510 \pm 39 | 0.080 |
| Body length, cm | 70.7 \pm 0.2 ^a | 72.1 \pm 0.3 ^b | 0.00017 |
| Gutted weight, g | 3900 \pm 22 | 3959 \pm 35 | 0.17 |
| Fillet weight, g | 2791 \pm 33 ^a | 2886 \pm 27 ^b | 0.029 |
| Liver weight, g | 42.4 \pm 1.5 | 43.1 \pm 1.9 | 0.77 |
| Liver color, score | 3.0 \pm 0.1 | 3.1 \pm 0.1 | 0.37 |
| Condition factor (CF) ¹ | 1.2 \pm 0.0 | 1.2 \pm 0.0 | 0.93 |
| Carcass yield, % ² | 88.1 \pm 0.5 | 87.8 \pm 0.2 | 0.51 |
| Fillet yield, % ³ | 63.6 \pm 0.6 | 64.6 \pm 0.7 | 0.23 |
| HSI% ⁴ | 0.96 \pm 0.03 | 0.96 \pm 0.04 | 0.97 |

1. Condition factor, CF= (body weight, g)/ (body length, cm)³*100;
2. Carcass yield= (gutted weight, g)/ (body weight, g) *100;
3. Fillet yield= (fillet weight, g)/ (body weight, g) *100;
4. HSI%= (liver weight, g)/ (body weight, g) *100;

5.1. Fillet gaping

As is shown in Figure 5.1, there is no significant difference in gaping between dietary treatment ($P > 0.05$) while there is significant difference in gaping between environmental treatment ($P < 0.05$). Gaping is significantly lower (score=0.2 \pm 0.1) in fillets of salmon reared in sea net pens compared to fillets of salmon reared in tanks (score=1.6 \pm 0.2).

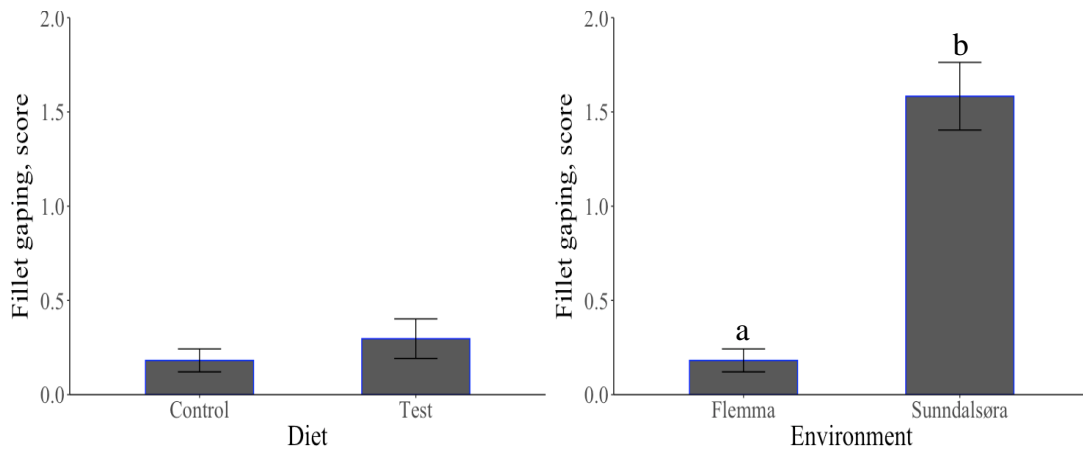


Figure 5.1, Gaping score of Atlantic salmon (*Salmo salar* L) as affected by diet (left) and environment (right). Dietary effects were studied in salmon fed low P/L ratio (control) or high P/L ratio (test) reared in sea net pens (Flemma). Environmental effects were studied in salmon fed low P/L ratio (control) diet reared in sea net pens (Flemma) or small tanks inland (Sunndalsøra). Results are presented as means \pm SEM and significant differences are indicated by different letters above the columns ($P < 0.05$).

5.2. Fillet color

Salmon fillet color was read and recorded according to color fan SalmoFan™ (DSM). Scores ranged from 22-25 for the three groups shown in Figure 5.2. All three groups are significantly different from each other. Salmon fed test diet reared in sea net pens have the highest fillet color score (score= 24.5 ± 0.1). In sea net pens, salmon fed with test diet (score= 24.5 ± 0.1) have significantly higher color score than salmon fed with control diet (score= 23.9 ± 0.1). For salmon fed with control diet, salmon reared in sea net pens (score= 23.9 ± 0.1) have significantly higher color score than salmon reared in tanks (score= 22.5 ± 0.2).

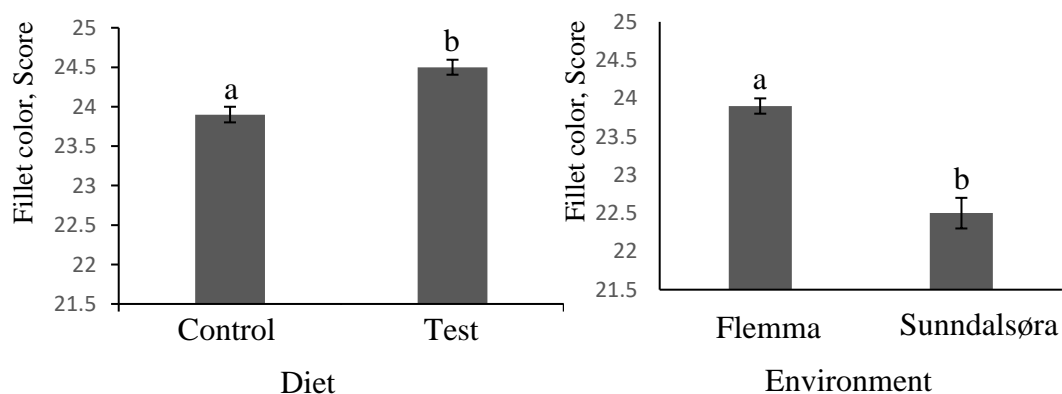


Figure 5.2, Fillet color score of Atlantic salmon (*Salmo salar* L) as affected by diet (left) and environment (right). Dietary effects were studied in salmon fed low P/L ratio (control) or high P/L ratio (test) reared in sea net pens (Flemma). Environmental effects were studied in salmon fed low P/L ratio (control) diet reared in sea net pens (Flemma) or small tanks inland (Sunndalsøra). Results are

presented as means \pm SEM and significant differences are indicated by different letters above the columns ($P < 0.05$).

5.3. Hepatic somatic index (HSI%)

In Figure 5.3, HSI% showed no significant difference for Atlantic salmon fed with diets differing in P/L ratios ($P = 0.97$). However, salmon reared in tanks (HSI% = 1.14 ± 0.04) have significantly higher HSI% compared to salmon reared in sea net pens (HSI% = 0.96 ± 0.03).

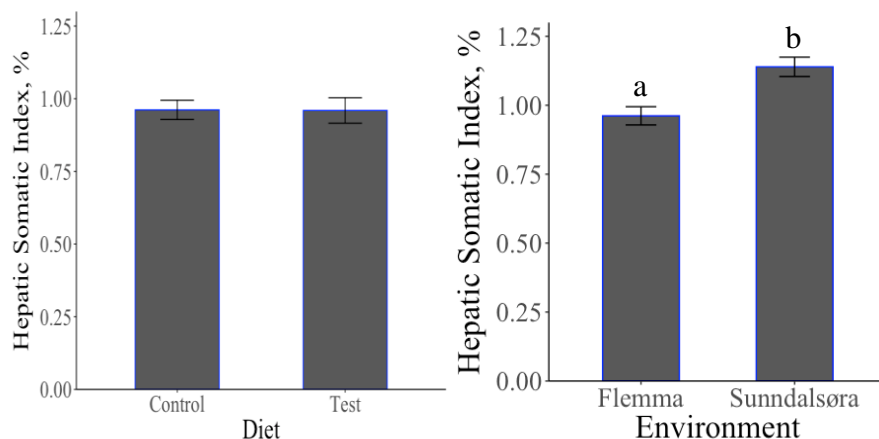


Figure 5.3, Hepatic somatic index (HSI%) of Atlantic salmon (*Salmo salar* L) as affected by diet (left) and environment (right). Dietary effects were studied in salmon fed low P/L ratio (control) or high P/L ratio (test) reared in sea net pens (Flemma). Environmental effects were studied in salmon fed low P/L ratio (control) diet reared in sea net pens (Flemma) or small tanks inland (Sunndalsøra). Results are presented as means \pm SEM and significant differences are indicated by different letters above the columns ($P < 0.05$).

5.4. Myocommata area

For Atlantic salmon fed with different diet, myocommata area in the belly flap show no significant difference between the dietary treatments (Figure 5.4). However, salmon reared in sea net pens show significant greater myocommata area (area = $14.6 \pm 0.7 \text{ mm}^2$) than salmon reared in tanks (area = $9.0 \pm 0.4 \text{ mm}^2$).

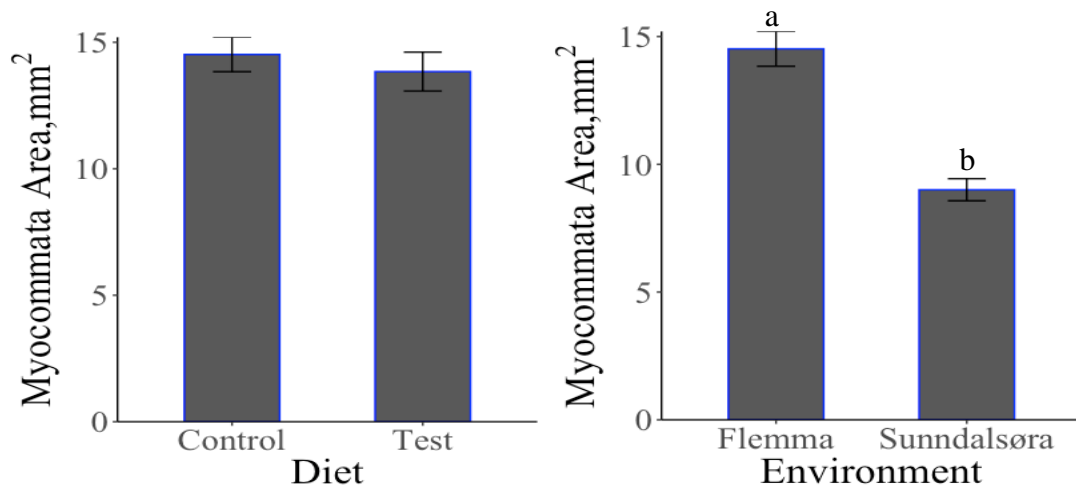


Figure 5.4, Myocommata area of Atlantic salmon (*Salmo salar* L) as affected by diet (left) and environment (right). Dietary effects were studied in salmon fed low P/L ratio (control) or high P/L ratio (test) reared in sea net pens (Flemma). Environmental effects were studied in salmon fed low P/L ratio (control) diet reared in sea net pens (Flemma) or small tanks inland (Sunndalsøra). Results are presented as means \pm SEM and significant differences are indicated by different letters above the columns ($P < 0.05$).

5.5. Myocommata width

There is no significant difference found in myocommata width between dietary treatments ($P > 0.05$). However, between environmental treatments, myocommata width was significantly wider in salmon reared in sea net pens (width = 1.6 ± 0.1 mm) than salmon reared in tanks (width = 1.1 ± 0.1 mm) shown in Figure 5.5.

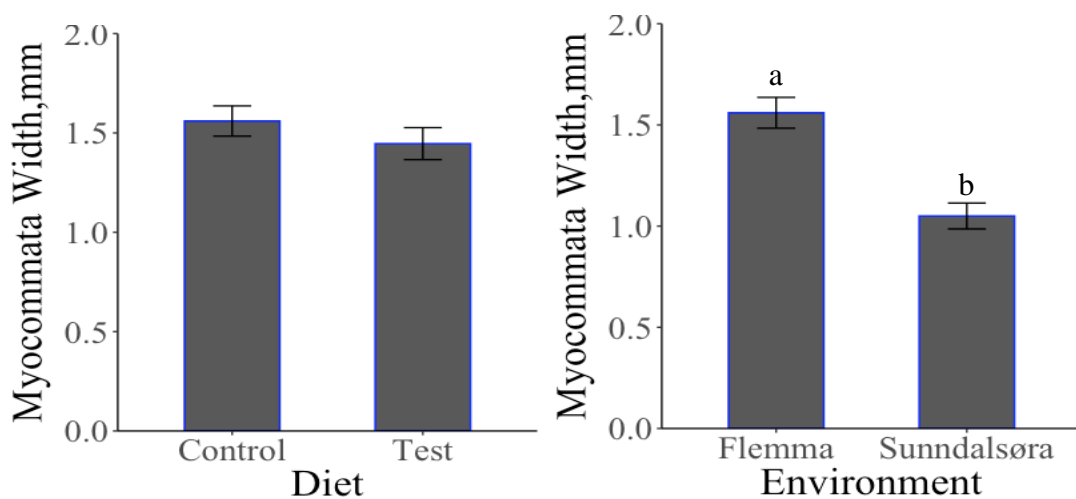


Figure 5.5, Myocommata width of Atlantic salmon (*Salmo salar* L) as affected by diet (left) and environment (right). Dietary effects were studied in salmon fed low P/L ratio (control) or high P/L ratio (test) reared in sea net pens (Flemma). Environmental effects were studied in salmon fed low P/L ratio

(control) diet reared in sea net pens (Flemma) or small tanks inland (Sunndalsøra). Results are presented as means \pm SEM and significant differences are indicated by different letters above the columns ($P < 0.05$).

5.6. Myocommata's L*, a* and b* value

In the perspective of myocommata lightness (L* value), salmon fed with control diet have significant lighter appearance (L*value=58.8 \pm 0.8) than salmon fed with test diet (L*value=55.1 \pm 1.0). Between environmental treatments, salmon reared in sea net pens have significant lighter appearance (L*value=58.8 \pm 0.8) than salmon reared in tanks (L*value=57.2 \pm 0.7). Salmon fed with control diet and reared in sea net pens have the lightest myocommata area shown in Figure 5.6.

In the perspective of myocommata redness (a* value), there is no significant difference between dietary treatments. However, redness (a* value) of myocommata of salmon reared in sea net pens show significantly higher redness (a*value=23.2 \pm 0.4) than salmon reared in tanks (a*value=21.2 \pm 0.5).

In the perspective of myocommata yellowness (b* value), no significant difference in myocommata yellowness (b* value) was found. However, significantly higher yellowness (b* value) has been found in salmon reared in tanks (b*value=14.8 \pm 0.5) than salmon reared in sea net pens (b*value=13.6 \pm 0.4), shown in Figure 5.6.

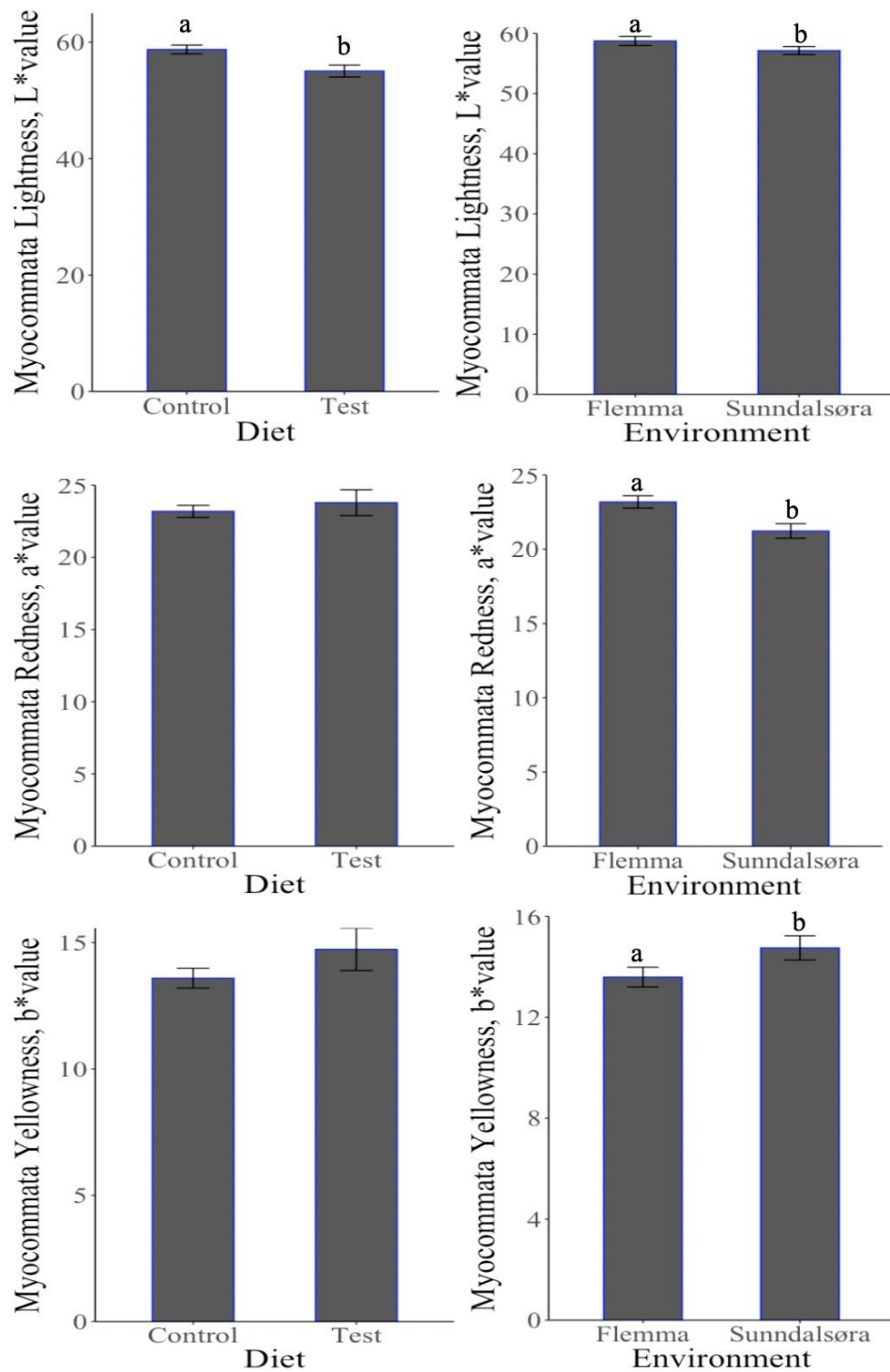


Figure 5.6, Myocommata's L*, a* and b* values of Atlantic salmon (*Salmo salar* L) as affected by diet (left) and environment (right). Dietary effects were studied in salmon fed low P/L ratio (control) or high P/L ratio (test) reared in sea net pens (Flemma). Environmental effects were studied in salmon fed low P/L ratio (control) diet reared in sea net pens (Flemma) or small tanks inland (Sunndalsøra). Results are presented as means \pm SEM and significant differences are indicated by different letters above the columns ($P < 0.05$).

5.7. Myomere area

In Figure 5.7, significant differences found in both dietary and environmental treated salmon. Salmon fed with test diet have significantly greater myomere area (area= 40.4 ± 0.9) than salmon fed with control diet (area= 35.3 ± 1.5). Salmon reared in sea net pens have significantly greater myomere area than salmon reared in tanks (area= 33.8 ± 1.3), shown in Figure 5.7.

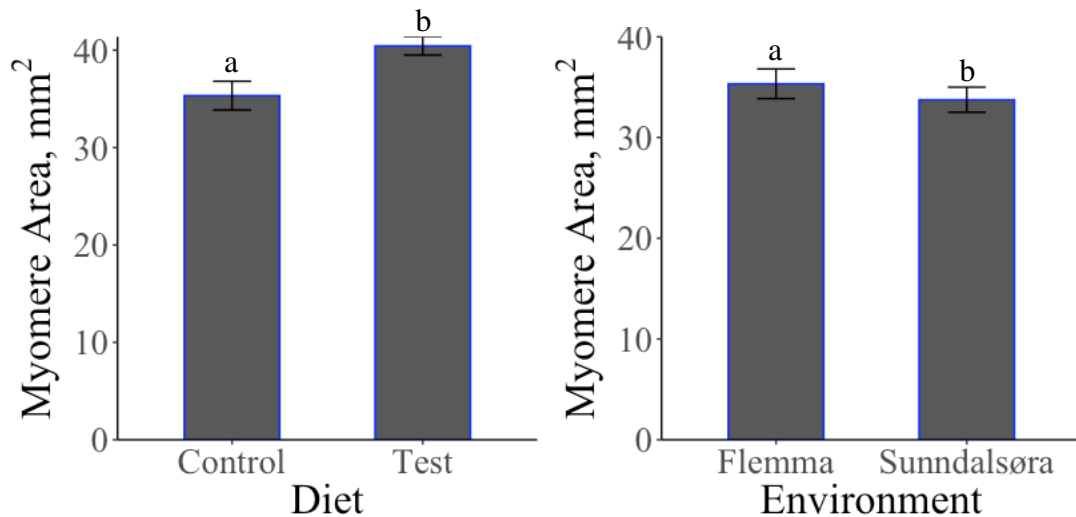


Figure 5.7, Myomere area of Atlantic salmon (*Salmo salar* L) as affected by diet (left) and environment (right). Dietary effects were studied in salmon fed low P/L ratio (control) or high P/L ratio (test) reared in sea net pens (Flemma). Environmental effects were studied in salmon fed low P/L ratio (control) diet reared in sea net pens (Flemma) or small tanks inland (Sunndalsøra). Results are presented as means \pm SEM and significant differences are indicated by different letters above the columns ($P < 0.05$).

5.8. Myomere width

No significant difference in myomere width was found between the different diets, shown in Figure 5.8. But salmon reared in sea net pens show significant wider myomere (width= 4.7 ± 0.1) than salmon reared in tanks (width= 4.7 ± 0.2).

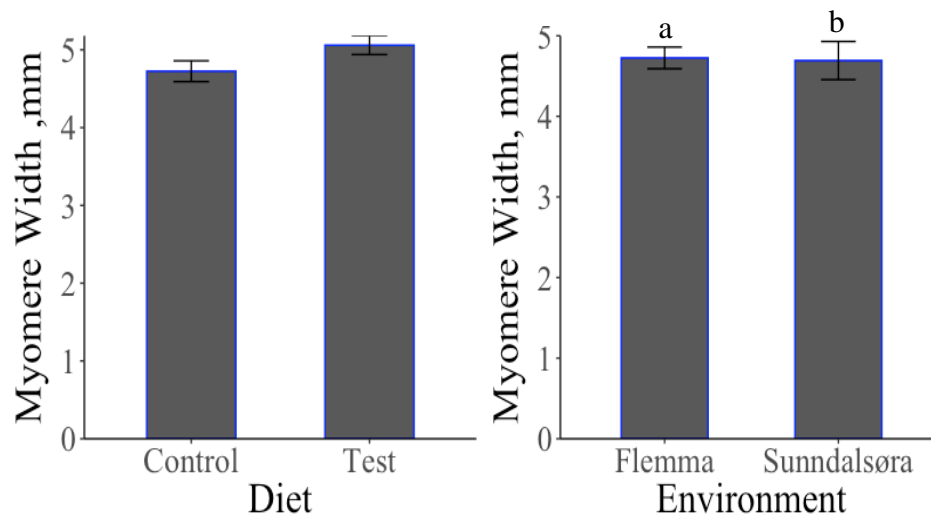


Figure 5.8, Myomere width of Atlantic salmon (*Salmo salar* L.) as affected by diet (left) and environment (right). Dietary effects were studied in salmon fed low P/L ratio (control) or high P/L ratio (test) reared in sea net pens (Flemma). Environmental effects were studied in salmon fed low P/L ratio (control) diet reared in sea net pens (Flemma) or small tanks inland (Sunndalsøra). Results are presented as means \pm SEM and significant differences are indicated by different letters above the columns ($P < 0.05$).

5.9. Myomere's L*, a* and b* value

No significant difference of myomere's L*, a* or b* value was found between dietary treatments, shown in Figure 5.9. Conversely, salmon reared in sea net pens show both higher redness (a*value= 28.6 ± 0.5) and yellowness (b*value= 23.8 ± 0.5) than salmon reared in tanks (a*value= 23.5 ± 0.7 , b*value= 20.0 ± 0.7 , respectively). Significantly higher lightness was found in salmon reared in tanks (L*value= 52.9 ± 0.8) than salmon reared in sea net pens (L*value= 51.1 ± 0.5).

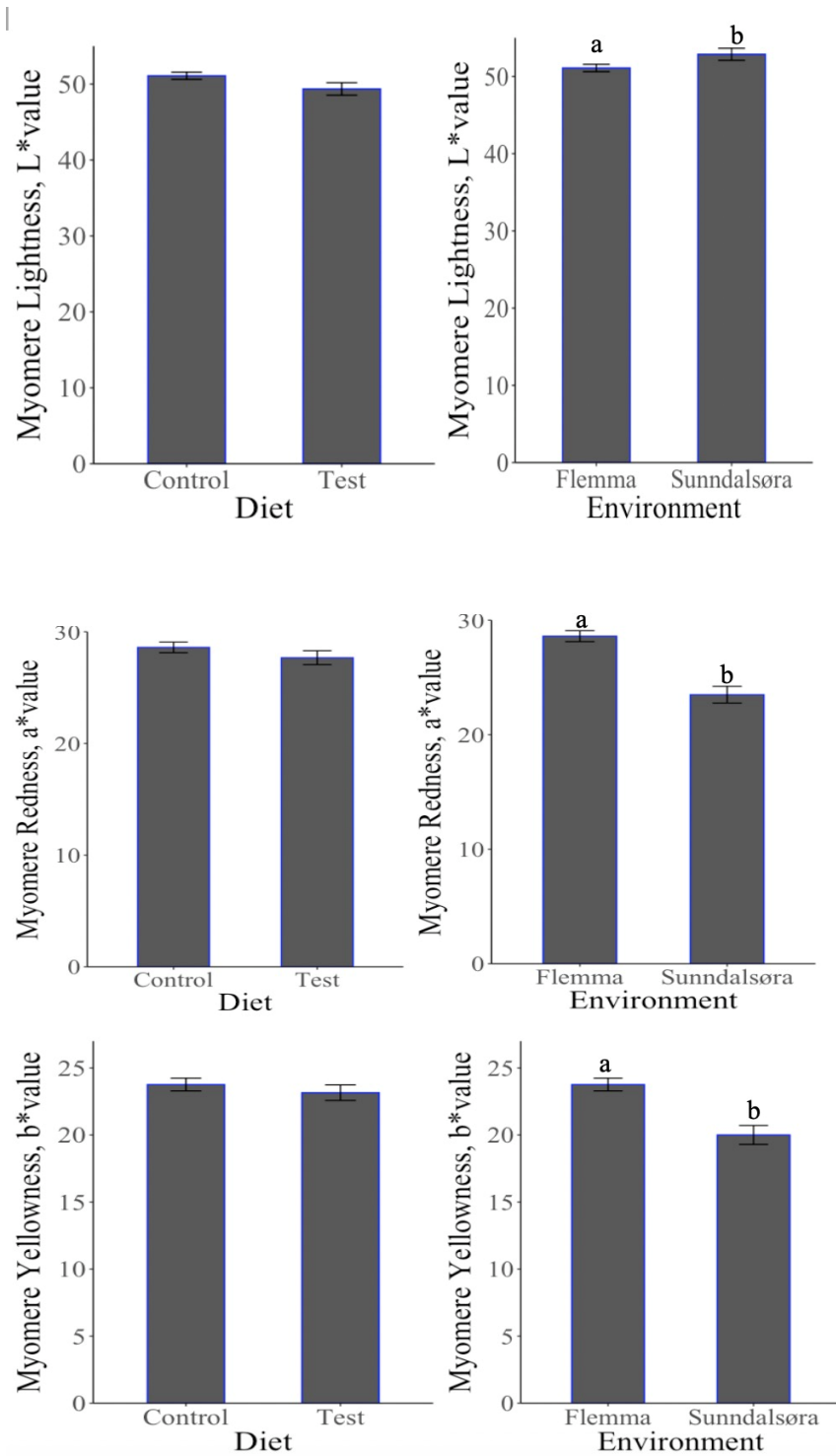


Figure 5.9, Myomere's L*, a* and b* values of Atlantic salmon (*Salmo salar* L.) as affected by diet (left) and environment (right). Dietary effects were studied in salmon fed low P/L ratio (control) or high P/L ratio (test) reared in sea net pens (Flemma). Environmental effects were studied in salmon fed low P/L ratio (control) diet reared in sea net pens (Flemma) or small tanks inland (Sunndalsøra). Results are presented as means \pm SEM and significant differences are indicated by different letters above the columns (P<0.05).

5.10. Texture

Texture was measured while fillets were fresh (post rigor) and after 3-month frozen-storage (-20°C). Frozen fillets were thawed under room temperature until core temperature reached around $2\sim 8^{\circ}\text{C}$.

No significant difference was found in salmon reared in sea net pens fed with control and test diet (Figure 5.10). However, fresh fillets showed significantly higher texture results compared to the same fillets after frozen-storage regardless of dietary treatment ($N_{\text{Fresh/Control}}=12.7\pm 0.4 > N_{\text{Freeze/Control}}=8.7\pm 0.5$; $N_{\text{Fresh/Test}}=12.0\pm 0.3 > N_{\text{Freeze/Test}}=9.0\pm 0.7$) and environmental treatments ($N_{\text{Fresh/Flemma}}=12.7\pm 0.4 > N_{\text{Freeze/Flemma}}=8.7\pm 0.5$; $N_{\text{Fresh/Sunndalsøra}}=7.3\pm 0.2 > N_{\text{Freeze/Sunndalsøra}}=5.8\pm 0.4$).

As is shown in Figure 5.10, salmon reared in sea net pens show significant firmer texture ($N_{\text{Flemma}}=8.7\pm 0.5$ and 12.7 ± 0.4 , after frozen-storage and in-fresh, respectively) than salmon reared in tanks ($N_{\text{Sunndalsøra}}=5.8\pm 0.4$ and 7.3 ± 0.2 , after frozen-storage and in-fresh, respectively).

Likewise, it is shown in Figure 5.10, between environmental treatments, significantly firmer texture was found in fresh fillets ($N_{\text{Fresh}}=12.7\pm 0.4$ and 7.3 ± 0.2 , reared in Flemma and Sunndalsøra, respectively) than fillets after frozen-storage ($N_{\text{Freeze}}=8.7\pm 0.5$ and 5.8 ± 0.4 , reared in Flemma and Sunndalsøra, respectively).

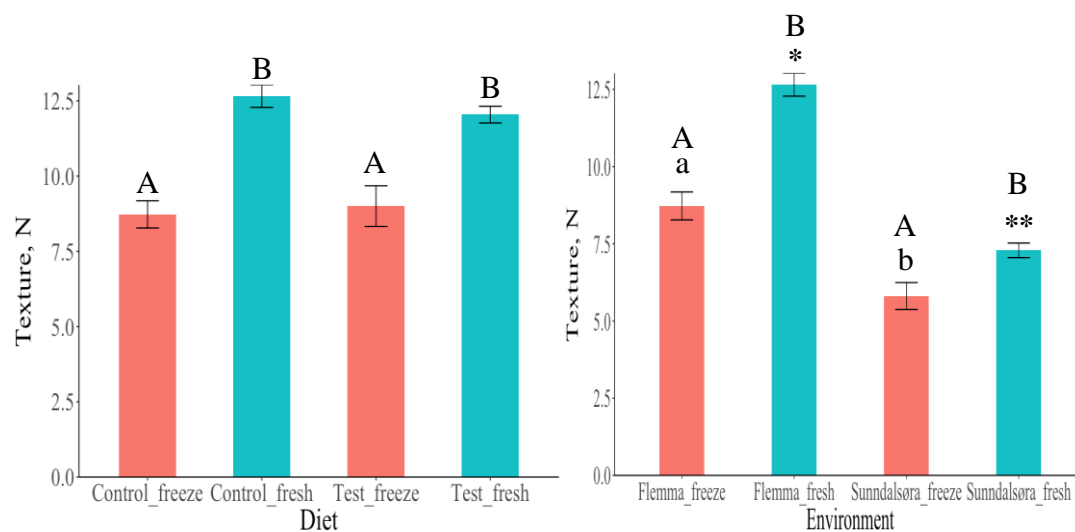


Figure 5.10, Texture of Atlantic salmon (*Salmo salar* L) as affected by diet (left) and environment (right). Dietary effects were studied in salmon fed low P/L ratio (control) or high P/L ratio (test) reared in sea net pens (Flemma). Environmental effects were studied in salmon fed low P/L ratio (control) diet reared in sea net pens (Flemma) or small tanks inland (Sunndalsøra). Results are presented as means \pm SEM and significant differences are indicated by small/capital letters and asterisk (a and b (between orange columns), A and B (between orange and blue columns), * and ** (between blue columns)).

5.11. Drip loss

Drip loss was measured in thawed frozen-fillets after 7-month frozen-storage. Frozen fillets were thawed in two different methods. One group fillets were firstly thawed at 4°C for 17hrs then transported to room temperature (20°C). Another group fillets were thawed directly at room temperature (20°C). Therefore, it was regarded to have two variants in dietary treatment and environmental treatment.

Salmon reared in sea net pens, significantly higher drip loss was found in salmon fed with low P/L diet (drip loss% =1.9±0.1) than fish fed with high P/L diet (drip loss% =1.6±0.1) when thawed in 20°C. Consistently, salmon fillets thawed under 4°C (drip loss% =3.0±0.2 and 2.9±0.2, fed low P/L and high P/L diet, respectively) have significantly higher drip loss than salmon fillets thawed under 20°C (drip loss% =1.9±0.1 and 1.6±0.1, fed control and test diet, respectively)(Figure 5.11).

Fillets from salmon reared in tanks (drip loss% =4.4±0.3) have significant higher drip loss than fillets from salmon reared in sea net pens (drip loss% =3.0±0.2) while thawed under 4°C. Likewise, when thawed under 20°C, fillets of salmon reared in tanks (drip loss% =3.8±0.2) have significant higher drip loss than fillets of salmon reared in sea net pens (drip loss% =1.9±0.1).

Comparing two different thawing temperatures within sea net pens (Flemma), salmon fillets thawed under 20°C (drip loss% =1.93±0.11) had significant less drip loss compared to salmon fillets thawed under 4°C (drip loss% =3.0±0.2, $P=5.96 \times 10^{-6} < 0.05$). On the contrary, comparing two different thawing temperatures within tanks (Sunndalsøra), salmon fillets thawed under 20°C (drip loss% =1.9±0.1) did not show significant different drip loss than salmon fillets thawed under 4°C (drip loss% =3.0±0.2, $P=0.12 > 0.05$) (Figure 5.11).

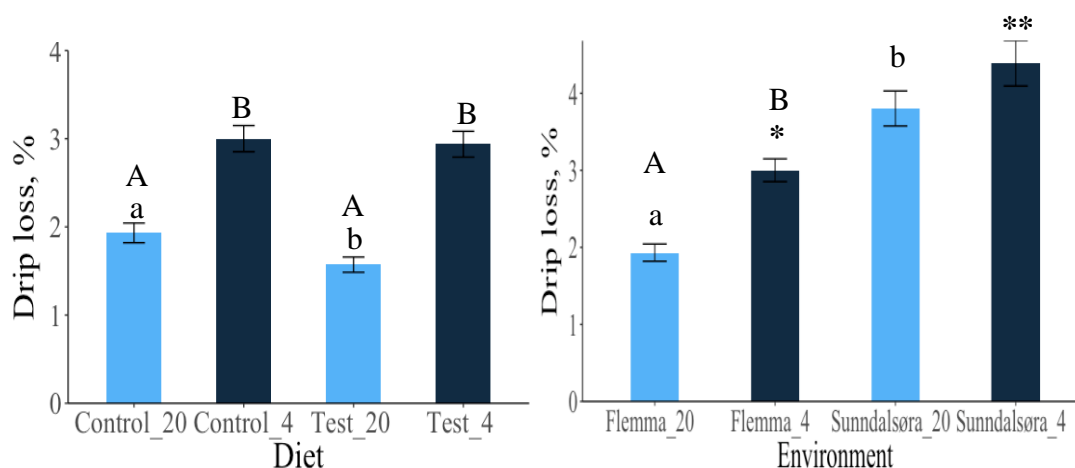


Figure 5.11, Liquid loss of Atlantic salmon (*Salmo salar* L.) as affected by diet (left) and environment (right). Dietary effects were studied in salmon fed low P/L ratio (Control) or high P/L ratio (Test) reared in sea net pens (Flemma). Environmental effects were studied in salmon fed low P/L ratio (Control) diet reared in sea net pens (Flemma) or small tanks inland

(Sunndalsøra). Results are presented as means \pm SEM and significant differences are indicated by small/capital letters and asterisk (a and b (between light blue columns), A and B (between light blue and dark blue columns), * and ** (between dark blue columns)).

Table 5.3, Fillet quality parameters of Atlantic salmon (*Salmo salar* L) from dietarily and environmentally treated groups. Results are presented as means \pm SEM and significant differences are indicated by superscripts (a and b (horizontal comparison), A and B (vertical comparison)).

| | Diet | | | Environment | | |
|----------------------------------|------------------------------|------------------------------|---------|-------------------------------|------------------------------|---------|
| | Control | Test | P-value | Flemma | Sunndalsøra | P-value |
| Fillet gaping score | 0.2 \pm 0.1 | 0.3 \pm 0.1 | 0.34 | 0.2 \pm 0.1 ^a | 1.6 \pm 0.2 ^b | P<0.05 |
| Fillet color score | 23.9 \pm 0.1 ^a | 24.5 \pm 0.1 ^b | P<0.05 | 23.9 \pm 0.1 ^a | 22.5 \pm 0.2 ^b | P<0.05 |
| HSI, % | 0.96 \pm 0.03 | 0.96 \pm 0.04 | 0.97 | 0.96 \pm 0.03 ^a | 1.14 \pm 0.04 ^b | P<0.05 |
| Myocommata area, mm ² | 14.6 \pm 0.7 | 13.8 \pm 0.8 | 0.52 | 14.6 \pm 0.7 ^a | 9.0 \pm 0.4 ^b | P<0.05 |
| Myocommata width, mm | 1.6 \pm 0.1 | 1.5 \pm 0.1 | 0.32 | 1.6 \pm 0.1 ^a | 1.1 \pm 0.1 ^b | P<0.05 |
| Myocommata L*value | 58.8 \pm 0.8 ^a | 55.1 \pm 1.0 ^b | P<0.05 | 58.8 \pm 0.8 ^a | 57.2 \pm 0.7 ^b | P<0.05 |
| Myocommata a*value | 23.2 \pm 0.4 | 23.8 \pm 0.9 | 0.56 | 23.2 \pm 0.4 ^a | 21.2 \pm 0.5 ^b | P<0.05 |
| Myocommata b*value | 13.6 \pm 0.4 | 14.7 \pm 0.8 | 0.24 | 13.6 \pm 0.4 ^a | 14.8 \pm 0.5 ^b | P<0.05 |
| Myomere area, mm ² | 35.3 \pm 1.5 ^a | 40.4 \pm 0.9 ^b | P<0.05 | 35.3 \pm 1.5 ^a | 33.8 \pm 1.3 ^b | P<0.05 |
| Myomere width, mm | 4.7 \pm 0.1 | 5.1 \pm 0.1 | 0.075 | 4.7 \pm 0.1 ^a | 4.7 \pm 0.2 ^b | P<0.05 |
| Myomere L*value | 51.1 \pm 0.5 | 49.4 \pm 0.8 | 0.087 | 51.1 \pm 0.5 ^a | 52.9 \pm 0.8 ^b | P<0.05 |
| Myomere a*value | 28.6 \pm 0.5 | 27.7 \pm 0.6 | 0.26 | 28.6 \pm 0.5 ^a | 23.5 \pm 0.7 ^b | P<0.05 |
| Myomere b*value | 23.8 \pm 0.5 | 23.2 \pm 0.6 | 0.43 | 23.8 \pm 0.5 ^a | 20.0 \pm 0.7 ^b | P<0.05 |
| Drip loss at 4°C, % | 3.0 \pm 0.2 ^A | 2.9 \pm 0.2 ^A | 0.76 | 3.0 \pm 0.2 ^{a/A} | 4.4 \pm 0.3 ^b | P<0.05 |
| Drip loss at 20°C, % | 1.9 \pm 0.1 ^{a/B} | 1.6 \pm 0.1 ^{b/B} | P<0.05 | 1.9 \pm 0.1 ^{a/B} | 3.8 \pm 0.2 ^b | P<0.05 |
| Texture fresh, N | 12.7 \pm 0.4 ^A | 12.0 \pm 0.3 ^A | 0.20 | 12.7 \pm 0.4 ^{a/A} | 7.3 \pm 0.2 ^{b/A} | P<0.05 |
| Texture freeze, N | 8.7 \pm 0.5 ^B | 9.0 \pm 0.7 ^B | 0.74 | 8.7 \pm 0.5 ^{a/B} | 5.8 \pm 0.4 ^{b/B} | P<0.05 |

- Correlations (Pearson correlation coefficient) among the quality parameters are presented in Appendix.

6. DISCUSSION

In this experimental trial, two main variants are: diet and environment. Control diet is common commercial diet regarded as “low protein, high fat” diet while test diet is specially formulated as “high protein, low fat” “lean” diet. Environmental changes are considered as either rearing in big sea net pens (Flemma) or rearing in small tanks (Sunndalsøra). The results revealed that environmental treatment have significant effect on production quality in Atlantic salmon. In this experimental trial, salmon reared in sea net pens have generally lower fillet gaping score, higher fillet color score, higher myocommata and myomere’ area, width and coloration, lower liquid loss and firmer texture, compared with salmon reared in small tanks on land.

The environmental effects were more pronounced than dietary effects on myocommata and myomere’s area and width may owe to significantly different salmon sizes selected from two rearing environment. In other words, the significant different myocommata and myomere’s area were positively correlated to different salmon sizes, in this experiment salmon sizes have not been corrected in statistical analyses. This might be a potential error needs consideration.

Salmon reared in sea net-pens showed a higher homogeneity compared to salmon reared in tanks which was preferred during further processing, such as automatic gutting and filleting. However, this phenomenon was due to intentional size-selection in harvest site.

In general, feed intake and growth rate are relatively low in first one or two months after transferring to seawater (Oehme, Grammes et al. 2010, Alne, Oehme et al. 2011). However, feed intake and growth rate would come back to normal level after certain adaptation period (Usher, Talbot et al. 1991, Jobling, Andreassen et al. 2002). Because after seawater transfer, post-smolt needed time to adapt to a whole new living condition, consisting of different osmosis, salinity, water temperature, different photoperiod, etc. Within which, temperature and photoperiod have been proven to have significant influences on salmonids’ growth rate (Brett 1979, Austreng, Storebakken et al. 1987, Forsberg 1995, Boeuf and Le Bail 1999). As a result, in Norwegian salmon farming industry, artificial light ($10\text{W}/\text{m}^2$) is constantly applied during late autumn/winter period to avoid sexual maturation in order to ensure good production quality (Taranger, Haux et al. 1998). However, environmental effects on salmon fillet quality still need further and deeper investigation.

On the other hand, fillet quality in farmed salmon varied throughout the year cycle. For example, fillet gaping prevalence in farmed Scottish salmon industry is much worse in spring and summer time than in autumn and winter time (Lavety, Afolabi et al. 1988). In addition, Mørkøre also found in farmed Atlantic salmon post-smolts regardless being transferred after 9 month (0+salmon) or after 16 month (1+salmon), fillet gaping was usually highest during spring/summer time (Mørkøre and Rørvik 2001). Besides, firmness in fillets is negatively correlated to salmon growth rate. Softening of fish flesh was often found during autumn and early winter when fast

growth usually happened (Ando 1999, Mørkøre and Rørvik 2001). Furthermore, gaping is positively correlated to poor texture according to Bremner (Bremner 1999). That is to say, softer salmon fillets with lower breaking strength have higher gaping score.

In this study however, in salmon fed control diet reared in sea net pens, a slightly negative linear correlation was found between gaping and fillet texture after frozen-storage ($R=-0.45$; $P<0.05$). For salmon reared also in sea net pens but fed test diet, a weaker negative correlation was found between gaping and breaking force after frozen-storage ($R=-0.22$; $P>0.05$). No correlation was found between gaping and breaking force in salmon fed control diet reared in tanks. These illustrate fillets with higher firmness/breaking strength have significantly lower gaping scores, which was in accordance with Bremner's study (Bremner 1999). Besides, this study also illustrated dietary treatment has no significant effect on fillet gaping score while environmental treatment did have. Salmon reared in tanks had the highest gaping score (1.6 ± 0.2) compared to salmon reared in sea net pens (0.2 ± 0.1 , $P<0.05$).

Salmon reared in sea net pens fed test diet, a slightly negative correlation between breaking strength (after frozen-storage) and drip loss (thawed under 20°C) was found ($R=-0.52$; $P<0.05$). However, salmon fed control diet but reared in tanks, a slightly negative correlation between breaking strength (in fresh fillets) and drip loss (under 20°C) was also found ($R=-0.36$; $P<0.05$). This implied that firmer fillets with increased breaking-strength, their liquid holding capacity significantly increased thus having significant less drip loss when thawed under 20°C temperature.

Overall more or less negative correlations ($R<0$) was found between drip loss (whether being thawed under 4°C or 20°C) and breaking strength (whether measured in fresh fillets or in thawed fillets after frozen storage). And it was documented that in fillets after frozen-storage, like under -20°C super-chilling condition, a higher drip loss was always found due to the mechanism of increasing myofiber breakage and lower myofiber contraction capability. As a result, a better frozen-storage technique with less ice-crystal formation shall be further investigated and applied in salmon process industry in order to guarantee high quality product (Bahuaud, Mørkøre et al. 2008).

In the perspective of fillet texture parameter, firmness did change significantly after frozen-storage under -20°C for 3-month ($P<0.05$). In every group of salmon treated with frozen-storage under -20°C for 3-month, breaking strength measured in fresh fillets were all significant higher compared to the same salmon measured after 3-month frozen-storage ($P<0.05$). Numerically, fresh fillets from salmon fed control diet reared in sea net pens have the highest breaking strength ($N=12.7\pm 0.4$). Fresh fillet from salmon fed test diet reared in sea net pens have the second highest breaking strength ($N=12.0\pm 0.3$). But the difference of breaking strength between these two

dietary treatments was not significant ($P=0.20$). The firmness is downgrading might owe to breakage of myofiber and deterioration of other connective tissues in the fillets after intra and extracellular ice-crystals formation. Besides, several studies found freezing process is comparatively more influential compared to thawing process and thawing method in alteration of quality parameters in Atlantic salmon (Zhu, Ramaswamy et al. 2004, Alizadeh, Chapleau et al. 2007, Bahuaud, Mørkøre et al. 2008).

In environmental treatments, in both fresh fillet and thawed fillet (after 3-month frozen-storage), salmon reared in sea net pens have significantly higher breaking strength (8.7 ± 0.5 and 12.7 ± 0.4 , after frozen-storage and in fresh, respectively) compared to fish reared in tanks (5.8 ± 0.4 and 7.3 ± 0.2 , after frozen-storage and in fresh, respectively, $P_{\text{Environment_Freeze-storage}} < 0.05$; $P_{\text{Environment_Fresh}} < 0.05$). Likewise, fresh fillets (12.7 ± 0.4 and 7.3 ± 0.2 , in sea net pens and tanks, respectively) have significantly higher breaking strength compared to thawed-frozen-fillets (8.7 ± 0.5 and 5.8 ± 0.4 , in sea net pens and tanks, respectively) (after 3-month frozen-storage) ($P_{\text{Flemma}} < 0.05$, $P_{\text{Sundalsøra}} < 0.05$).

In the perspective of drip loss, different thawing temperatures as well as different thawing methods have crucial influence on Atlantic salmon's texture, color and drip loss contents (Zhu, Ramaswamy et al. 2004). Freezing speed prior to thawing would induce differences on fillet liquid-holding-capacity (drip loss content), however no significantly different color or texture changes was observed (Zhu, Ramaswamy et al. 2004).

In this study, results are on the opposite side between dietary and environmental treatments.

In dietary treated groups, significantly higher drip loss was found in fish thawed under 4°C (3.0 ± 0.2 and 2.9 ± 0.2 , fed with control and test diet, respectively) than thawing under 20°C (1.9 ± 0.1 and 1.6 ± 0.1 , fed with control and test diet, respectively). $P_{\text{Control}} < 0.05$; $P_{\text{Test}} < 0.05$). When comparing two different dietary treatments, significantly lower drip loss was found in fish fed with test diet (1.6 ± 0.1) compared to fish fed with control diet (1.9 ± 0.1), however, only when thawed under 20°C ($P < 0.05$). This indicates that slower and longer thawing process would somehow induce higher degree of myofiber and tissue breakage thus further impaired liquid-holding-capacity of intra and extracellular segments compared to fast thawing process (Kaale, Eikevik et al. 2013, Kaale, Eikevik et al. 2014). Apart from thawing process, high protein-to-lipid (P/L) ratio diet improved salmon fillet's firmness which leads to significant less drip loss compared to salmon fed control diet ($P < 0.05$).

In environmentally treated groups, salmon reared in sea net pens (1.9 ± 0.1) have significantly lower drip loss compared to salmon reared in tanks (3.8 ± 0.2) when salmon fillets thawed under 20°C ($P < 0.05$). Similarly, when thawed under 4°C , salmon reared in sea net pens (3.0 ± 0.2) also have significantly lower drip loss compared to salmon reared in tanks (4.4 ± 0.3 , $P < 0.05$). This indicate salmon reared in

sea net pens have significantly better muscle structure hence increasing liquid holding capacity (Bahuaud, Mørkøre et al. 2010). When taking different thawing temperature (4°C and 20°C, respectively) as variable factor, significantly lower drip loss was found in salmon thawed under 20°C (1.9 ± 0.1) compared to salmon thawed under 4°C (3.0 ± 0.2) while both reared in sea net pens ($P < 0.05$). No significant difference of drip loss was found in salmon reared in tanks when thawed under different temperatures. Numerically, salmon fed test diet and reared in sea net pens have the least drip loss (1.6 ± 0.1) followed by salmon fed control diet and reared in sea net pens (1.9 ± 0.1 , $P < 0.05$). In general, salmon reared in sea net pens have significant less drip loss than salmon reared in tanks. To summarize, the difference of drip loss under two different thawing temperature might be due to different thawing time and muscle structure alteration, etc. Nevertheless, further research is needed to elucidate the mechanism behind how drip loss is affected by rearing environment since thawing process is an intricate process.

In the perspective of fillet coloration, salmon fed test diet and reared in sea net pens have significantly higher fillet color score than salmon fed control diet and reared in sea net pens ($P < 0.05$). The least coloration was found in salmon fed control diet and reared in tanks. Both dietary and environmental treatment have significant influences on fillet coloration in Atlantic salmon. Thus, in order to obtain optimal fillet coloration, higher P/L diet as well as seawater environment shall be considered and optimally applied in salmon farming industry.

In some studies, salmon fed different dietary protein/lipid ratio diet do not have significant different growth rate when reared under warm water temperature at 11°C or under lower water temperature at 4°C (Karalazos, Bendiksen et al. 2007, Karalazos, Bendiksen et al. 2011). However, if salmon fed a low dietary protein/lipid P/L ratio but with sufficient energy for necessary growth, low dietary protein/lipid P/L diet showed no negative effect on salmon growth and feed utilization with a protein sparing phenomenon had been recognized (Einen and Roem 1997, Bendiksen, Berg et al. 2003, Azevedo, Leeson et al. 2004, Azevedo, Leeson et al. 2004). In addition, water temperature plays a significant role for salmon to obtain good growth rate, good nutrient retention and feed utilization (Bendiksen and Jobling 2003, Ng, Sigholt et al. 2004, Ruyter, Moya-Falcón et al. 2006).

When reared in low water temperature around 4°C, salmonids are able to grow efficiently when fed low DP/DL ratio diet (Einen and Roem 1997, Azevedo, Leeson et al. 2004, Solberg 2004, Karalazos, Bendiksen et al. 2007) while in warm water conditions, salmonids preferably grow better when fed higher P/L ratio diets (Bendiksen, Berg et al. 2003). In other studies, temperature is found highly and positively correlated to feed intake in in-season Atlantic salmon post-smolts (Dessen, Weihe et al. 2017). From the period July to September, the feed intake is significant higher compared to the period from April to July.

It's worth further research to determine effects of even lower dietary P/L ratio diet on growth rate, feed utilization and chemical composition of Atlantic salmon during summertime (high temperature).

7. CONCLUSION

Fillet quality of farmed Atlantic salmon is improved by feeding high protein-to-lipid (P/L) diet and rearing in sea water environment. Concise results are summarized as follow:

- High P/L diet significantly increased body length by 2% and fillet weight by 3.4%. However, no significant difference was seen on other biometric traits or liver color in this experimental trial.
- Atlantic salmon reared in sea net pens (Flemma) have significantly higher body weight, body length, gutted weight, liver weight and fillet yield but significantly lower liver score, condition factor (CF) and HSI%.
- Atlantic salmon reared in sea net pens (Flemma) consistently and significantly improved fillet quality parameters while high dietary P/L did not enhance fillet quality parameters comparably.
- Atlantic salmon fed high P/L diet significantly increased fillet color score and myomere's area, but significantly decreased myocommata's lightness (L^* value).
- Atlantic salmon fed high P/L diet have significant lower drip loss when fillets thawed under 20°C, but no other significant difference was found in drip loss when salmon fillets thawed under 4°C.
- No significant difference was found in fillet firmness in salmon fed low P/L diet and high P/L diet.
- Atlantic salmon reared in sea net pens (Flemma) have significant lower fillet gaping score, lower drip loss whether thawed under 4°C or 20°C, but significant higher fillet color score and higher fillet firmness in both fresh fillets and after frozen-storage fillets.
- Atlantic salmon reared in sea net pens (Flemma) significantly increased myocommata's area, width, lightness (L^* value), redness (a^* value), myomere's area, width, redness (a^* value), yellowness (b^* value) but significantly decreased myocommata's yellowness (b^* value) and myomere's lightness(L^* value).
- The significant lower myocommata and myomere's area, width is positively correlated to salmon size, in which salmon size has not been correlated in myocommata and myomere's area/width statistical analyses. This might be the potential error need to be considered.

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APPENDIX

Pearson correlation coefficient

Table 1, Pearson correlation coefficient among fillet color, drip loss (at 4°C and 20°C), gaping, texture (fresh and after frozen storage), myocommata area, myocommata width, myomere area and myomere width of Atlantic salmon (*Salmo salar* L.) fed with low P/L(control) diet, reared in sea net pens (Flemma). *P<0.05, **P<0.001, ***P<0.0001. Only if P<0.05, correlation coefficient value is regarded as relevant.

| | Fillet color | Driploss, 4°C | Drip loss,20°C | Gaping | Texture-fresh | Texture-frozen | Myocommata area | Myocommata width | Myomere area | Myomere width |
|------------------|--------------|---------------|----------------|--------|---------------|----------------|-----------------|------------------|--------------|---------------|
| Fillet color | | 0.14 | -0.02 | -0.25 | 0.16 | 0.26 | 0.28 | 0.32 | 0.10 | 0.07 |
| Drip loss,4°C | | | 0.06 | -0.01 | 0.01 | 0.14 | -0.12 | 0.32 | -0.16 | 0.30 |
| Drip loss,20°C | | | | 0.27 | -0.31 | -0.25 | 0.14 | 0.19 | 0.46 | 0.35 |
| Gaping | | | | | 0.15 | -0.45* | 0.07 | 0.07 | 0.11 | 0.09 |
| Texture-fresh | | | | | | 0.15 | 0.32 | 0.03 | 0.11 | -0.18 |
| Texture-frozen | | | | | | | -0.21 | -0.43 | 0.10 | 0.20 |
| Myocommata area | | | | | | | | 0.80* | 0.61* | 0.31 |
| Myocommata width | | | | | | | | | 0.18 | 0.11 |
| Myomere area | | | | | | | | | | 0.75* |
| Myomere width | | | | | | | | | | |

Table 2, Pearson correlation coefficient among fillet color, drip loss (at 4°C and 20°C), gaping, texture (fresh and after frozen storage), myocommata area, myocommata width, myomere area and myomere

width of Atlantic salmon (*Salmo salar* L.) fed with high P/L (test) diet, reared in sea net pens (Flemma). *P<0.05, **P<0.001, ***P<0.0001. Only if P<0.05, correlation coefficient value is regarded as relevant.

| | Fillet color | Driploss, 4°C | Drip loss,20°C | Gaping | Texture-fresh | Texture-frozen | Myocommata area | Myocommata width | Myomere area | Myomere width |
|------------------|--------------|---------------|----------------|--------|---------------|----------------|-----------------|------------------|--------------|---------------|
| Fillet color | | 0.23 | 0.00 | -0.17 | -0.09 | 0.04 | -0.23 | -0.26 | -0.42 | -0.14 |
| Drip loss,4°C | | | 0.17 | -0.14 | -0.11 | -0.03 | -0.37 | -0.37 | -0.25 | 0.06 |
| Drip loss,20°C | | | | -0.18 | -0.06 | -0.52* | -0.10 | 0.01 | 0.24 | 0.16 |
| Gaping | | | | | 0.12 | -0.22 | -0.34 | -0.42 | -0.37 | -0.39 |
| Texture-fresh | | | | | | 0.10 | 0.38 | 0.46 | 0.22 | 0.05 |
| Texture-frozen | | | | | | | 0.55* | 0.44 | -0.19 | -0.44 |
| Myocommata area | | | | | | | | 0.97*** | -0.06 | -0.50 |
| Myocommata width | | | | | | | | | 0.02 | -0.40 |
| Myomere area | | | | | | | | | | 0.65* |
| Myomere width | | | | | | | | | | |

Table 3, Pearson correlation coefficient among fillet color, drip loss (at 4°C and 20°C), gaping, texture (fresh and after frozen), myocommata area, myocommata width, myomere area and myomere width of Atlantic salmon (*Salmo salar* L.) fed with low P/L (control) diet, reared in inland tanks (Sunddalsøra). *P<0.05, **P<0.001, ***P<0.0001. Only if P<0.05, correlation coefficient value is regarded as relevant.

| | Fillet color | Driploss, 4°C | Drip loss,20°C | Gaping | Texture-fresh | Texture-frozen | Myocommata area | Myocommata width | Myomere area | Myomere width |
|------------------|--------------|---------------|----------------|--------|---------------|----------------|-----------------|------------------|--------------|---------------|
| Fillet color | | | | | | | | | | |
| Driploss, 4°C | | | | | | | | | | |
| Drip loss,20°C | | | | | | | | | | |
| Gaping | | | | | | | | | | |
| Texture-fresh | | | | | | | | | | |
| Texture-frozen | | | | | | | | | | |
| Myocommata area | | | | | | | | | | |
| Myocommata width | | | | | | | | | | |
| Myomere area | | | | | | | | | | |
| Myomere width | | | | | | | | | | |

| | | | | | | | | | |
|---------------------|------|-------|-------|--------|-------|-------|-------|--------|--------|
| Fillet color | 0.24 | -0.13 | 0.05 | 0.02 | 0.06 | 0.00 | 0.16 | 0.06 | 0.09 |
| Drip loss,4°C | | 0.31 | -0.08 | 0.11 | -0.19 | 0.36 | 0.43 | -0.01 | -0.30 |
| Drip loss,20°C | | | 0.16 | -0.36* | -0.17 | 0.36 | 0.03 | 0.19 | -0.15 |
| Gaping | | | | -0.13 | -0.04 | -0.08 | -0.22 | 0.15 | -0.11 |
| Texture-fresh | | | | | 0.46* | -0.05 | 0.08 | -0.28 | -0.14 |
| Texture- frozen | | | | | | 0.26 | 0.05 | -0.03 | -0.06 |
| Myocommata area | | | | | | | 0.65* | -0.07 | -0.35 |
| Myocommata width | | | | | | | | -0.59* | -0.59* |
| Myomere area | | | | | | | | | 0.65* |
| Myomere width | | | | | | | | | |



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