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Skin health and product quality of Atlantic Salmon (*Salmo salar* L.) fed increased LC-PUFAs from micro algae (*Schizochytrium sp*.)

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Acknowledgements

This thesis was written in collaboration with Cermaq, Nofima and NMBU, investigating effects of increased omega 3 in salmon feed with the inclusion of algae oil.

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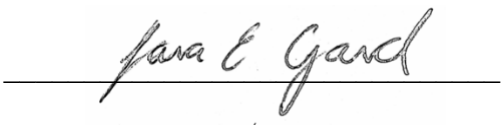

Sara Eldal Gard

Abstract

The Atlantic salmon is a valuable protein source that can increase food security for the growing world population. However, there is an urgency of novel sustainable ingredients to support production growth in the Aquaculture industry. Micro algae have the potential to replace fish oil in Atlantic salmon feed. It is a viable source, rich in n-3 LC PUFA. The heterotrophic micro algae studied in this thesis, *Schizochytrium sp*. is sustainable and efficient to cultivate. The micro algae has high EPA (C20:5n-3) and DHA (C22:6n-3) content and studies show diets with *Schizochytrium sp.* result in improved EPA+DHA retention efficiency. This study investigated the effects of dietary modifications on skin health, product quality (fillet pigmentation) and nutritional quality of Atlantic Salmon. The three test diets consisted of control diet (7.5% EPA+DHA from fish oil, FO), resembling commercial feed, test diet 1 containing increased levels of n-3 LC-PUFA from marine sources (10% EPA+DHA, FO) and test diet 2 with increased levels of n-3 LC-PUFA supplemented with *Schizochytrium sp.* (algae oil, AO) (5% FO+5% AO). The test feeds were given to the Atlantic salmon in artic farming environment in Hammerfest, Finnmark in triplicate commercial sea-cages from October 22nd 2022. The sampling period for this thesis were from November 2022 and April 2023. The result showed an overall positive development on bodyweight for all diet groups, indicating no negative effects of micro algae inclusion. Biometric traits were also similar for the dietary groups and no dietary effect were observed on skin health or fillet pigmentation. The chemical composition of the fillets showed increased level of oleic acid, linoleic acid and DHA in salmon fed diet supplemented with micro algae. Diet with increased n-3 LC PUFA had the highest level of EPA in the salmon fillet. Astaxanthin was not affected by the modified diets, but had a positive development during the experimental period.

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Introduction

The global production of salmonids surpassed 2.6 million tons in 2020 (Shahbandeh, 2022). Of that year, the Norwegian production of Atlantic salmon accounted for half of the global salmon supply, namely 1.4 million tons. Intensive Atlantic salmon farming has within two decades, become Norway's second largest export commodity, next to oil- and gas production and was valued at 72.5 billion NOK in 2019 (Steinset, 2020).

The product quality is a crucial factor affecting the costumer´s willingness to pay (WTP), and therefore influences the value and supply demand of the aquaculture industry. Hence, product quality parameters such as, muscle texture, skin- and flesh quality alter the economic value of the Norwegian salmon (Alfnes et al., 2006; Steine et al., 2005). Furthermore, research suggests that animal welfare impacts the product quality (Van De Vis et al., 2003; Grimsrud et al., 2013). The salmon skin is a vital protection organ for the fish with several functions such as osmoregulation (Boutin et al., 2013). Poor skin health is adverse for animal welfare and causes quality downgrading, which has economic consequences. Nutritional quality is a parameter closely linked the salmons skin health and the product quality (Waagbø et al., 1993; Noble et al., 2018).

Nutritional quality impact the individual´s resilience and tolerance to stressors related to rearing activities (Boutin et al., 2013; Bou et al., 2017; Huyben et al., 2023). The feed composition alters the nutritional value of the salmon which ultimately affects the consumer´s valuation of the product (Colombo et al., 2022). Salmonids are commonly known as a major source of omega-3 fatty acids. However, as a consequence of the terrestrial feed evolution, the levels of the important and unique marine oils have decreased (Sprague et al., 2016). The Aquaculture industry´s ability to meet growing seafood demand while simultaneously produce sustainably is depending on the development of novel feed ingredients that contain marine oil and -protein properties (FAO, 2020). Among other nutritionally appropriate novel ingredients, microalgae are considered as a viable ingredient. The single-cell organism can increase the level of digestible EPA, DHA and added nutritional benefits, in addition to contributing to sustainable farming as microalgae cultivation requires low land area and nutritional input (Lenihan-Geels et al., 2013; Colombo et al., 2022).

2. Aim of study

The aim of the study was to investigate effects of increased dietary levels of PUFA n-3 from algae oil on skin health, appearance of muscle and fatty acid composition of the muscle.

2.1 Hypothesis

1. LC-PUFA inclusion from algae oil will reduce amount of scale loss and skin haemorrhage.

2. LC-PUFA inclusion from algae oil improve red pigmentation of the salmon muscle.
2.1 and increase favourable fatty acids (EPA, C20:5n-3 and DHA, C22:6n-3)

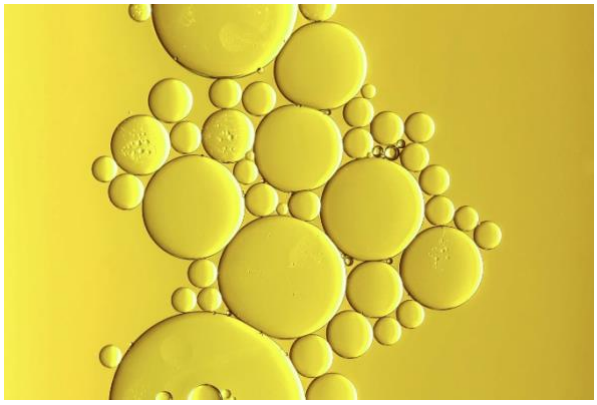

Figure 1. Algae oil. Available from: https://www.skretting.com/en/news-and-stories/aquaculture-pioneer-atlantic-sapphire-announces-the-inclusion-of-algal-omega-3-feed-ingredients/ (Accessed: 23.03.23)

3. Theoretical background

3.1 Animal welfare and efforts to improve it

The definition of fish welfare can be defined by the same standards as livestock. Broom (1986) defines animal welfare by the animal's perception of its ability to cope with its environment. The teleost fish is equipped with intricate sensory organs adapted to specific environment parameters such as, temperature, oxygen, and salinity (Pitcher, 1986). Moreover, the physical and mental state of the animal is a product of internal and environmental stimuli (e.g., genetical traits, nutritional value of the feed ingredients, environmental surroundings, and handling procedures).

The industry handbook Fishwell (Noble et al., 2018) was developed as a standardized tool to ensure fish welfare and production success in commercial farming. Equipped with the knowledge of the welfare parameters, the farmer could recognize adverse signs in early phases and make necessary changes to ensure proper animal welfare. Since fish inhibit a large variation of sensory perceptions, both internal and external, the welfare indicators (WIs) are divided into operational welfare indicators (OWIs) (covers both animal-based and environment-based indicators) and Laboratory welfare indicators (LABWIs). OWIs functions as a hands-on tool for the farmer to use directly on the rearing facility. Animal welfare is a combination the of the individuals internal and external complexity and thus LABWIs needs to be seen in combination with OWIs (Noble et al., 2018). Moreover, biometric traits are intertwined with animal welfare. Fillet weight (about 60-70% of total body weight) and condition factor (normally around 1.2-1.4) are both welfare indicators and economically important (Mørkøre et al., 2020).

Epidermal problems are valuable OWIs and easy to detect. They can indicate serious welfare concerns depending on type, severity, frequency of injury and the potential threat of pathogens in the environment (Noble et al., 2018). For this thesis, evaluating the epidermal damage with the use of the OWI: scale loss and skin haemorrhage can help evaluate the relationship between nutrition and skin health.

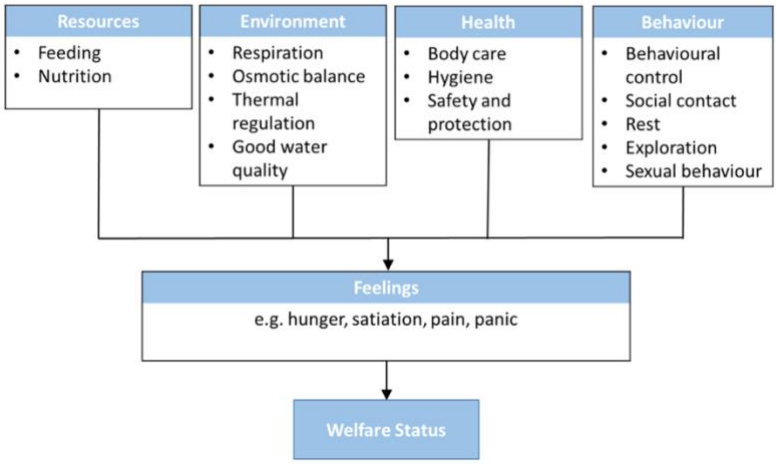

Figure 2. A categorical overview of the welfare needs of salmon. The status the physiological requirements affects their mental state and consequently the welfare status of the animal. Adapted from "Mellor, D. J., Patterson-Kane, E. & Stafford, K. J. (2009) The Sciences of Animal Welfare. John Wiley & Sons Ltd, Oxford, UK, 212 pp. Available from: (Noble et al., 2018). (Accessed: 09.02.23)

3.2 Basic fish immune system

The skin of the fish is a part of the innate immune system, and the initial physical barrier to protect against exogenous pathogens and parasites (Fletcher & Grant, 1969; Tort et al., 2003). Thus, a vital organ of protection, and a metabolically active tissue that have functions with communication, sensory perception, locomotion, and osmoregulation (Marshall & Bellamy, 2010). The skin epithelium has a stratified cellular sheet structure covered in mucus. Keratocyte- and mucus cells are two cell types with structural and protective functions, respectively (Karlsen et al., 2012). The mucosa-associated lymphoid tissue (MALT) defence system of teleost fish is a critical component in their immune system because of their direct contact with the aquatic environment (Ángeles, 2012). MALT contains B cells and immunoglobins provide cellular and humoral defences against microbes and stressors, contributing to homeostasis in the fish (Fletcher & Grant, 1969).

Complex bacterial communities populate the mucus and share species of microbiota with the aquatic environment, including opportunistic pathogens (Boutin et al., 2013). The mucus production in the epithelium increases as a physiological response to stressors, which indicates that the general immunologic state of the skin is altered (Tort et al., 2003). This is supported by Minniti et al. (2017), where rapid change in the mucosal microbiota was seen in Atlantic salmon within 24h after being subjected to the stressful event of repeated netting, including 30 seconds air exposure each netting. Furthermore, several studies suggest mucosal

dysbiosis after stressful events, commonly induced by rearing practises such as sorting, netting and transport, resulting in decreased immunity status for the fish (Tort et al., 2003; Boutin et al., 2013; Minniti et al., 2017). Results from Boutin et al. (2013) indicated that microbiota dysbiosis is a triggering factor for opportunistic infections in fish epithelium. Incidents of parasitosis are an economic liability for the industry, and consequently disables industry growth. Nonetheless, there is only a limited number of studies focusing on the immune responses and the bacterial community of the fish skin-mucosa.

3.2.1 Epidermal damage

The layer under the epidermis, namely dermis consists of nerve cells, blood vessels and scales (Takle et al., 2015). The Atlantic salmon have cycloid scales, which have an outer layer of calcium covering connective tissue in the inner layer. The scales are arranged structurally underneath the epidermis to protect underlying tissue (Duguid et al., 2018). After surpassing the initial defence barrier of the salmon, exogenous pathogens can infiltrate and increase the risk of disease and mortality of the host. Therefore, skin damage as scale loss and wounds are detrimental for several reasons. Firstly, epidermal damage compromise osmoregulation and behavioural need of protection, ultimately resulting in discomfort and adverse perception of its environment (Rose et al., 2012; Noble et al., 2018). Secondly, studies show the large network of nociceptors in the epidermal layer, suggesting sensations of painful stimuli. However, the extent of the fish's perception of pain needs further studies (Rose et al., 2012). In addition to decreased animal welfare, epidermal damage such as winter ulcer poses economic loss and decreases the economic value of product quality by downgrading of fillet quality (Løvoll et al., 2009).

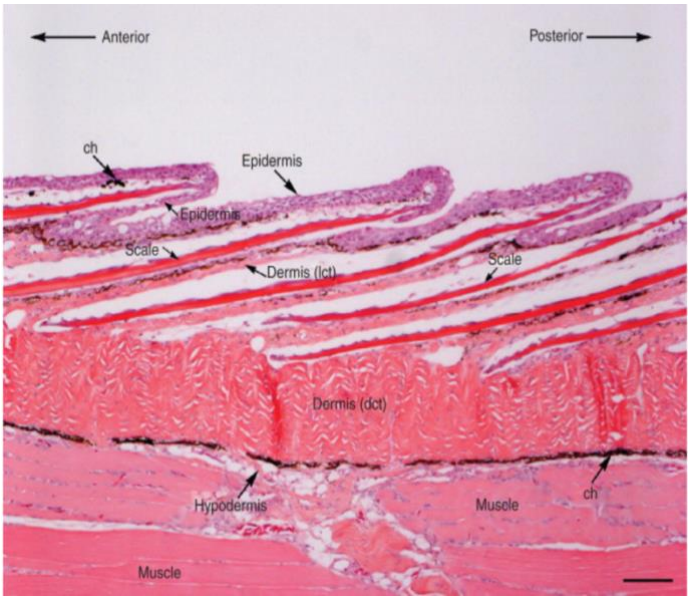

Figure 3. Histological section of skin layers of Oncorhynchus mykiss, showing overlapping scales. The scales lie in pockets of loose connective tissue of the stratum spongiosum of the dermis. The dense connective tissue of the stratum compactum of the dermis lies beneath the scales, separated from the skeletal muscle by a thin hypodermis comprised of adipose tissue and an upper layer of chromatophores.

Scale = 100 µm.

Available from: (Elliott, 2011). (Accessed: 24.03.23)

3.3 Product quality: muscle pigmentation

Product quality can be divided into different categories: nutritional, technological, hygienic, sensorics and technical quality. The initial product appearance (sensory quality) is the most crucial aspect in terms of economic importance because it affects the consumer´s decision to purchase (Alfnes et al., 2006; Steine et al., 2005).

The red colour of salmonid muscle is characteristic for the species. There are individual differences of degree of redness (see fig. 4), which has economic consequences as the muscle colour is closely related to the market value of the product (Alfnes et al., 2006; Grimsrud et al., 2013). Carotenoid concentrations of about 6 mg/kg give a sufficient red pigmentation for the consumer market (Torrissen et al., 1989). Steine et al., (2005) proved stronger WTP for highly pigmented salmon fillet. With the use of Salmofan^{TM} the study proved fillets under R23 value was difficult to sell. Astaxanthin and canthaxanthin are carotenoids with beneficial properties, including vitamin A and antioxidant (Torrissen et al., 1989). Carotenoids are involved in salmonid pigmentation of skin and muscle in addition to having vital physiological functions throughout the life cycle of the Atlantic salmon, such as sexual maturation and stress responses (Bjerkeng et al., 1992; Aas et a., 2019). Carotenoids are obtained through the salmon´s natural marine diet of crustaceans, invertebrates, and smaller fish species. The prey cannot either synthesise carotenoids themselves but obtain the pigment from organisms in the first trophic level, namely microalgae (Bjerkeng et al., 1992).

Extracting carotenoid directly from the primary source is suggested more sustainable for the environment and wild fish stocks (Colombo et al., 2022; Veramaris, 2023). Farmed Atlantic salmon obtain their carotenoid requirement through pelleted feed. The limited wild stocks of krill and shrimp have directed the industry to utilize synthesized astaxanthin and canthaxanthin (Hardy & Lee, 2010). Although astaxanthin is the most physiological suited for salmon to absorb and deposit in the flesh, a combination with canthaxanthin give a higher total carotenoid deposition (Torrissen et al., 1989). Additionally, there are individual differences in pigment absorption and deposition in the salmon flesh, factors such as fish size, growth rate, sexual maturation, and genetic background.

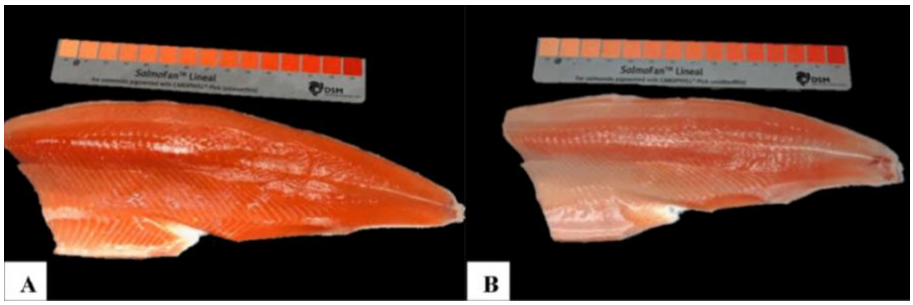

Figure 4. Comparative example of colour range of salmon fillet. Example A scores highest pigmentation and has highest market value. Redness pigmentation evaluation with SalmoFanTM. Available from: https://www.frdc.com.au/project/2014-248 (Accessed: 24.03.23)

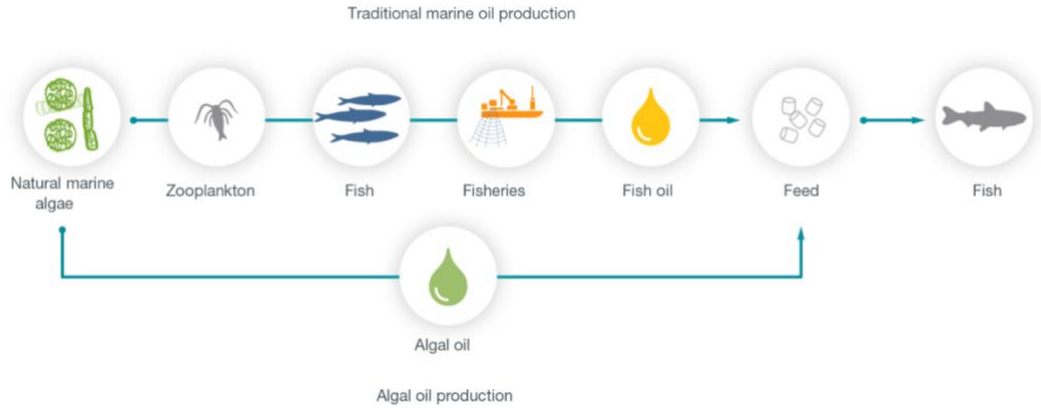

Figure 5. Schematic picture skipping steps in the feed chain to extract carotenoids directly form microalgae. Available from: https://www.skretting.com/en/Innovation/our-innovations/ingredient-innovation/omega-3-from-marine-algae--an-innovative-solution-for-increased-protein-production/ *(Accessed 30.03.23)*

3.4 Nutrition and salmon feed evolution

Whilst the global capture fisheries have plateaued at 85–95 million tons captures per year, the global demand of marine resources is increasing (FAO, 2020). Terrestrial resources are more available and economic beneficial compared to aquatic resources. Replacing most of the marine protein and oils in the aquafeed with plant materials have allowed the aquaculture industry to grow despite the scarcity of marine sources. Colombo et al. (2022) termed the rapid terrestrial shift of aquafeeds "Aquafeed 2.0". Consequently, the aquaculture industry is currently relying on terrestrial feedstuffs. However, agricultural crops are in competing with livestock- and human consumption. Moreover, cultivation of crops transcripts to deforestation, areal footprint, use of freshwater, pesticide and fertiliser, and nutrient water pollution (Troell et al., 2014; Ytrestøyl, et al., 2019; Colombo et al., 2022). Hence, overuse of terrestrial resources and lack of sustainable management weakens the aquacultures' ability to contribute to sustainable food production (Troell et al., 2014). There is a need for "Aquafeed 3.0" (Colombo et al. 2022). A new transition to ensure sustainable development in the aquafeed industry, that utilise circular bioeconomy and elevate resource resilience by introducing novel feed ingredients.

Nutrition is fundamental for essential growth, maintaining physiological functions and good animal welfare (Noble et al., 2018). From 1990 to 2013, the marine sourced ingredients in salmon feed went from 90% to ~30%, respectively. Accordingly, the use of plant ingredients increased to compose 70% of the gross feed composition (Ytrestøyl et al., 2019). Moreover, the "Aquafeed 2.0" transition to terrestrial ingredients presents challenges for the carnivore Atlantic salmon. Carnivorous fish species are metabolically adapted to high protein (>40%) and -fat diets (>15%) and have low carbohydrate tolerance (Francis et al., 2001; Colombo et al., 2022). As the current aquafeed diets have higher carbohydrate and lower protein and omega-3 (n-3) content, the carnivore Atlantic salmon faces physiological challenges such as reduced digestibility, gut enteritis, and elevated immune responses to stress, which increases the susceptibility to pathogens (Francis et al., 2001; Ytrestøyl et al., 2019).

Ingredient sources (% of the feed) 1990-2013

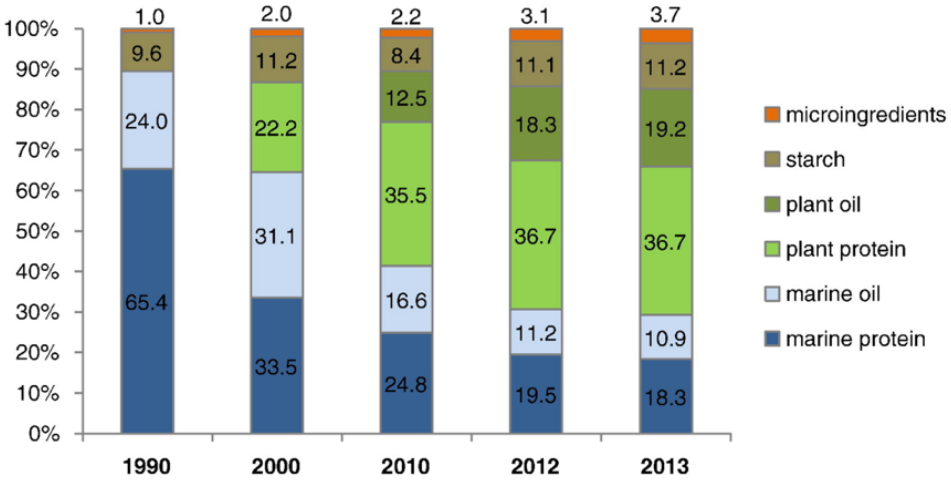

Figure 6. Salmon feed ingredient evolution from 1990 to 2013. Available from (Ytrestøyl et al, 2019). (Accessed 31.03.23)

3.4.1 Essential fatty acids in the teleost fishes

The long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA,) EPA (20:5n-3) and DHA (22:6n-3) are incorporated in major fundamental physiological roles in the Atlantic salmon, such as cellular structures, immunity, inflammation and metabolic functions (Hardy & Lee, 2010; Horn et al., 2019). The LC n-3 PUFAs are obtained through the diet and several factors (e.g., life stage, endogenous processes, genetic background and exothermic factors) can influence digestibility and deposition of PUFAs (Horn et al., 2019). The Atlantic salmon is evolutionarily adapted to biosynthesize n-3 LC-PUFA and needs the valuable lipids throughout its life cycle (Zheng et al., 2005). Studies suggest the salmon have limited capacity to biosynthesis n-3 PUFAs from precursor dietary fatty acids found in vegetable lipids, such as oleic acid (18:1n-9), linoleic acid (18:2n-6) or α-linolenic acid (18:3n-3; ALA) (Henderson & Tocher, 1987; Bell et al., 1997; Tocher et al., 2000).

Atlantic salmon is known to be a rich source of EPA and DHA, two essential fatty acids (EFA) uniquely beneficial to human health. However, with the "Aquafeeds 2.0" evolution, EFA are largely substituted by sunflower-seed, soy- and rapeseed oil, with a fatty acid profile lacking EPA and DHA. Consequently, the fatty acid composition in salmon muscle is altered (Miller et al., 2008; Hardy & Lee, 2010). Reports of decreased omega-3 levels in Norwegian, Scottish and Chile farmed Atlantic salmon suggests having to double the present portion size to obtain the same levels of EPA and DHA as in 2006 (Ytrestøyl et al.,

2019; Sprague et al., 2016). A study on dietary effects of *Schizochytrium sp.* diet suggested increased retention of n-3 LC-PUFA when combined with increased levels of rapeseed- and camelina oil (Kousoulaki et al., 2016).

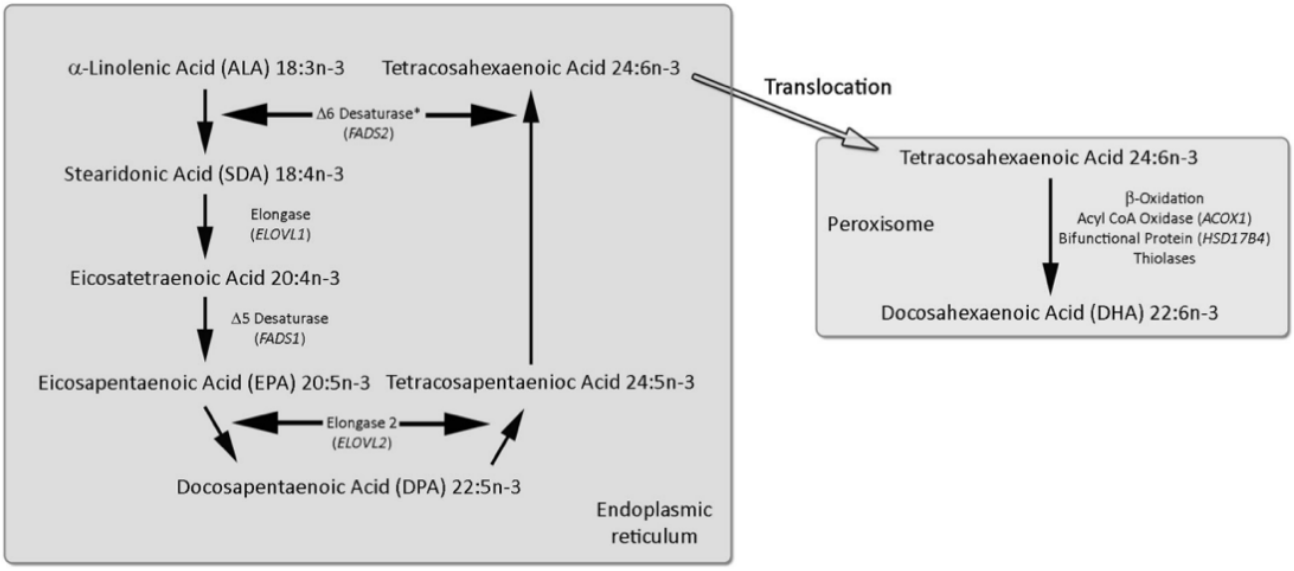

Figure 7. Synthesis of EPA, DPA and DHA from ALA. PUFAs are synthesized from ALA by a progressive series of enzymatic desaturation and chainelongation steps, in the endoplasmic reticulum. In the final stage tetracosahexaenoic acid (24:6n-3) is translocated to the peroxisome and is shorted by one cycle of the β-oxidation pathway to form DHA (22:6n-3). (Dyall, 2015). (Accessed: 20.05.23)

3.5 Algae oil

Many classes of single-cell organisms including yeast, bacteria and microalgae have been investigated as a potential aquafeed ingredient in the past decade. They have the potential to provide the aquaculture industry with rich LC-PUFA nutrients in addition to having added benefits, such as astaxanthin and beta glucans (Miller et al., 2008). By selecting specific strains of microalgae to optimise nutritional qualities, it is possible to increase fish health and product quality (Posten & Feng Chen 2016; Colombo et al., 2022). The major classes of microalgae that are of interest for potential aquafeed sources are *Thraustochytrids*, *Diatoms* and *Chlorophyceae* (Miller et al., 2008; Colombo et al., 2022). The microalgae investigated in this study is *Schizochytrium sp*., a species in the *Thraustochytrid*s family. The microalgae class is a heterotrophic marine photosynthetic primary producer of n-3 PUFA and display favourable lipid profile for aquafeed (Miller et al., 2008). The majority of PUFA synthesising microalgae can only produce either DHA or EPA, but *Schizochytrium sp*. is a

species of microalgae containing both PUFAs, exceeding 50% DHA + EPA according to Veramaris (2023).

3.5.1 Cultivation for aquafeeds

Stated by Posten & Feng Chen (2016), the photosynthesis-based plants are a cornerstone of the bioeconomy concept. Mass cultivation of single-cell organisms are relatively new, but has potential to achieve sustainable cultivation, as microalgae only requires sunlight and carbon dioxide to grow (Colombo et al., 2022). Advances in technologies have led to automatic production of microalgae in open or closed reactors with reliable growth thorough the different seasons. However, mass cultivating microalgae and processing to valued products still face economical and logistical challenges. Protein and lipid ingredients from by-products, capture fisheries and soy production used in aquafeed are in the price range of US$2 per kg, whereas current cost of production of general single-cell ingredients are high and can vary between US$4 to US$300 per kg (Posten & Feng Chen 2016; Ruiz et al., 2022). This price difference challenges microalgae cultivation in the position to substitute current aquafeed ingredients. However, interest in the biofuel-, and chemical industry have made technological developments in commercial single-cell cultivating (Posten & Feng Chen 2016). New technology and methods for up-scaling in terms of technical challenges and economic feasibility will provide a market for commercial use of sustainable microalgae (Colombo et al., 2022).

Single cell organisms can grow under extreme conditions on non-agriculture land area and up-cycle nutrients from industrial waste and by-products (Colombo et al., 2022). Veramaris cultivate in phototrophic conditions. *Schizochytrium sp.* is blended with sugar, precisely dextrose from corn, wheat, and beets. The two materials (microalgae and sugar) go through a fermentation process where the algae cells grow rapidly and convert the dextrose to omega-3 fatty acids capsuled in oil vesicles. Further on, the cell wall is ruptured in the downstream process, and the oil vesicles are separated from the aqueous phase. The residual water is removed from the oil by centrifugation and the result is a highly concentrated algal oil and a liquid co-product, which can be converted into a protein source for agriculture farming or into biogas for electricity production. And the final product, algal oil provides EPA and DHA to the aquaculture industry.

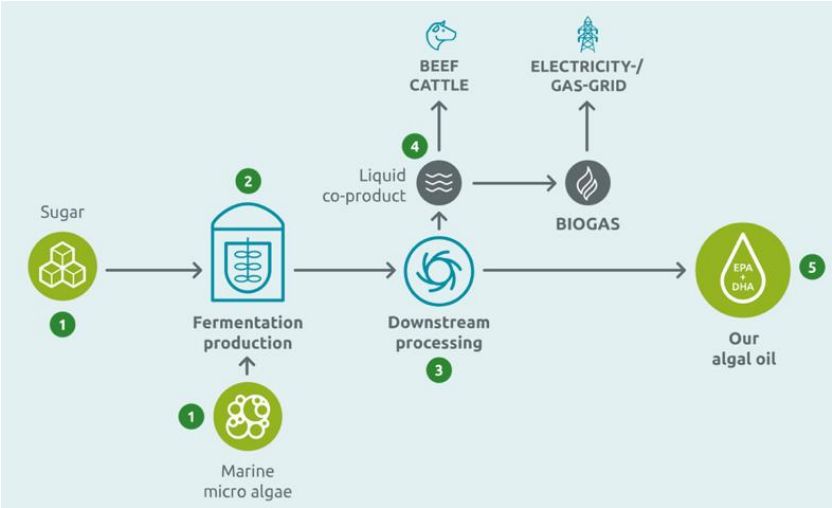

Figure 8. Illustration of Veramaris algal oil production showing which materials goes into the fermentation process and which co-products can be extracted from the downstream processing. Available from: https://www.veramaris.com/what-we-do-detail.html (Accessed: 23.04.23)

4. Material and Method

4.1 Trial design

The study is an ongoing feed trial in collaboration with Cermaq, Nofima and NMBU. The feed trial began October 22nd 2022, and scope of this thesis includes methods and data acquired from 1. mid sampling, November 22 nd 2022 and the 2. mid sampling, April 4th 2023.

Atlantic salmon (*Salmo salar* L.) were farmed in Cermaq´s commercial arctic location in Slettnesfjorden, Hammerfest in Finnmark. The average start weight of the fish was 200g ± 15%, and ± 5% weight deviation between the cages. The feed trial was designed for a large-scale production, with a total of nine cages containing 100 000 individuals per cage. Triplicates of the control and test feeds were given by assigning to selected cages by randomisation (see table 1 for diet-assigned net pen). The cages receive the same diet throughout the whole life cycle.

Table 1. Dietary codes given to the test subject of this trial, the Atlantic salmon (Salmo salar L.*) and the assigned net pen number to each diet in triplicates.*

Feed code	Net pen number
Control	1, 4, 8
Test 1	3, 5, 7
Test 2	2, 6, 9

4.2 Test feed composition

The three experimental diets were produced by Biomar. They had different oil content and composition, but with similar gross composition known to meet nutritional needs. The control diet was a commercial diet formulated with 7.5% total EPA & DHA originating from fish oil (FO). The two test diets had 2.5% increased levels of omega-3 (EPA+DHA). Test diet 1 had 10% total EPA & DHA (fish oil, FO) and Test diet 2 had 10% total – EPA & DHA, where 50% of the EPA & DHA was sourced from algae oil (AO) (*Schizochytrium sp.*). Pellet size and composition were adjusted to and named 200-500-1000 which correlated to the fish size (e.g., 200 for 200g fish, 500 for 500g fish etc.) (appendix table 2).

Table 2. Overview of the experimental diets.

	Total EPA + DHA, %	Fish oil, %	Algae oil, %
Control	7.5	7.5	
Diet 1	10	10	
Diet 2	10	5	5

Table 3. Gross composition of commercial feed and test feed: diet 1 and diet 2 with inclusion of micro algae (Schizochytrium sp.) of size 500.

Size: 500	Fat, %	Protein, %	Ash, %	Water, %	EPA + DHA, %
Control	31.2	42.1	5.5	5.3	7.7
Diet 1	31.4	41.7	5.4	5.3	10.1
Diet 2	31.4	42	5.5	5.3	9.7

Table 4. Gross composition of commercial feed and test feed: diet 1 and diet 2 with inclusion of micro algae (Schizochytrium sp.) of size 1000.

Size: 1000	Fat, %	Protein, %	Ash, %	Water, %	EPA + DHA, %
Control	34.3	38	5	5.4	7.8
Diet 1	34.0	37.8	5	5.2	11.4
Diet 2	35.3	37.5	4.7	5.1	10.6

4.3 Sampling procedures

Daily registrations of environmental parameters such as water temperature, salinity and oxygen were measured at site standard measuring depth (appendix table 1). Daily feed regime, fish behavioural parameters and appetite were also registered. Two representative (~200g) feed samples from each diet were taken at start of the trial, mid sampling, and end of the trial.

4.3.1 Biometric traits

Individual weight and length of 50 fish per cage, and bulk weight (200/cage) were sampled at the start of the study (October), the first (November) and second (April) (mid) sampling. From each cage, 10 fish were sampled for analyses (n=90 in total), where each individual was given a unique sample number (date and fish identification).

Welfare scores (severity of scale loss and occurrence of skin haemorrhage) were scored on production site after slaughter, in addition to and histology samples of the skin. The whole gutted fish with ID number were transported in Styrofoam boxes on ice, to Nofima, Ås for analysing of welfare and quality traits (scale loss, skin haemorrhage, SalmoFan^{TM}).

4.3.2 Fishwell operational welfare indicators (OWIs) – skin health

Photo evaluation method were used to assess OWIs. Pictures of the right side of the fish were taken in standardized light box. The right side were divided into three sections for scoring: anterior dorsal fin, posterior dorsal fin and under the lateral line (ventral) (figure 10). Nofima´s Fishwell OWI´s were used to determine grade of scale loss and skin haemorrhage, (see figure 9) (Noble et al., 2018). The scoring system for scale loss was: 0: no scale loss, 1: individual scale loss, 2: small areas of scale loss, 3: severe area of scale loss, while the scoring system for skin haemorrhage was: 0: no skin haemorrhage, 1: minor haemorrhaging, 2: large area of haemorrhaging, 3: significant bleeding.

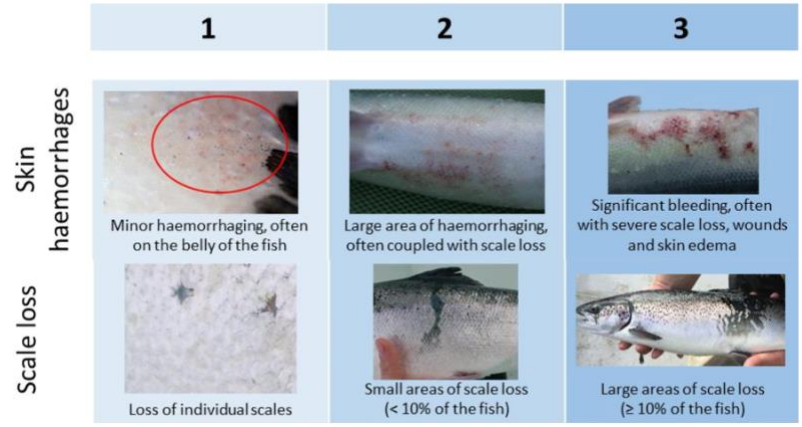

Figure 9. Operational Welfare Indicators (OWIs) for skin haemorrhages and scale loss used for evaluating skin health. The WIs scoring system is simplified and ranges from 0-3. Available from: (Nobel et al., 2020). (Accessed: 09.02.23)

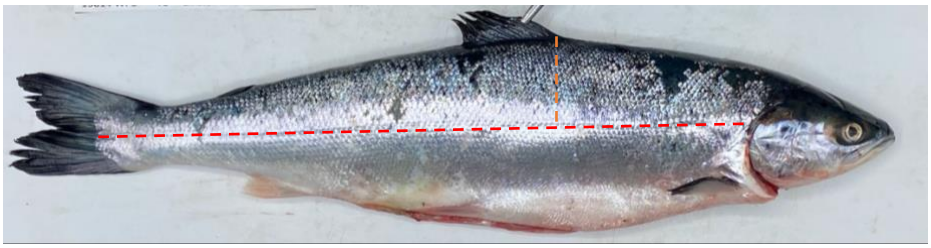

Figure 10. Schematic picture of the areas (anterior dorsal fin, posterior dorsal fin, and ventral area) used for epidermal welfare scoring of scale loss and skin haemorrhage.

4.3.3 Analysis of fillet colour

The cutlet between the posterior end of the dorsal fin and the anus (Norwegian quality cuts, NQC) were taken from the right side of the fish and placed in colour box with standardised light conditions before evaluating fillet colour using Salmofan^{TM} by DSM (dsm, 2023) (figure 11).

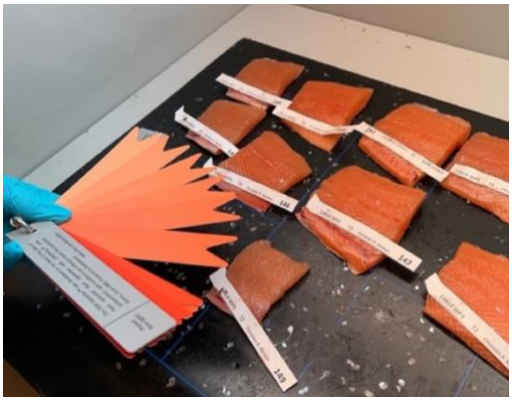

Figure 11. Visual scoring of fillet colour using Salmofan^{TM}.

4.3.4 Histology of dermis

The histology samples were taken at production site at Slettnesfjorden in Hammerfest. The skin samples were taken in the NQC area without cutting into the muscle (figure 12). Then, the skin samples were placed in formalin and transported to the NMBU veterinary institute for data processing. Pictures of the dermis was taken.

The pictures of the dermis were analysed with the NDP.view2 Plus image viewing software U12388-01 (version 2.9.29) by Hamamatsu.

The dermis thickness was analysed on 6 individuals per net pen from the November 2022 sampling. The mean dermis thickness (µm) was measured by extracting measurements from five representative dermis sites of each sampled fish (figure 13). The observations were done by making annotations of the dermis thickness in the image viewing program per fish and then calculating the mean value (thickness, µm) of those five annotations.

Figure 12. Picture of the NQC area on Atlantic salmon (Salmo salar L.*) where the skin histology was sampled to analyse the dermis thickness (µm).*

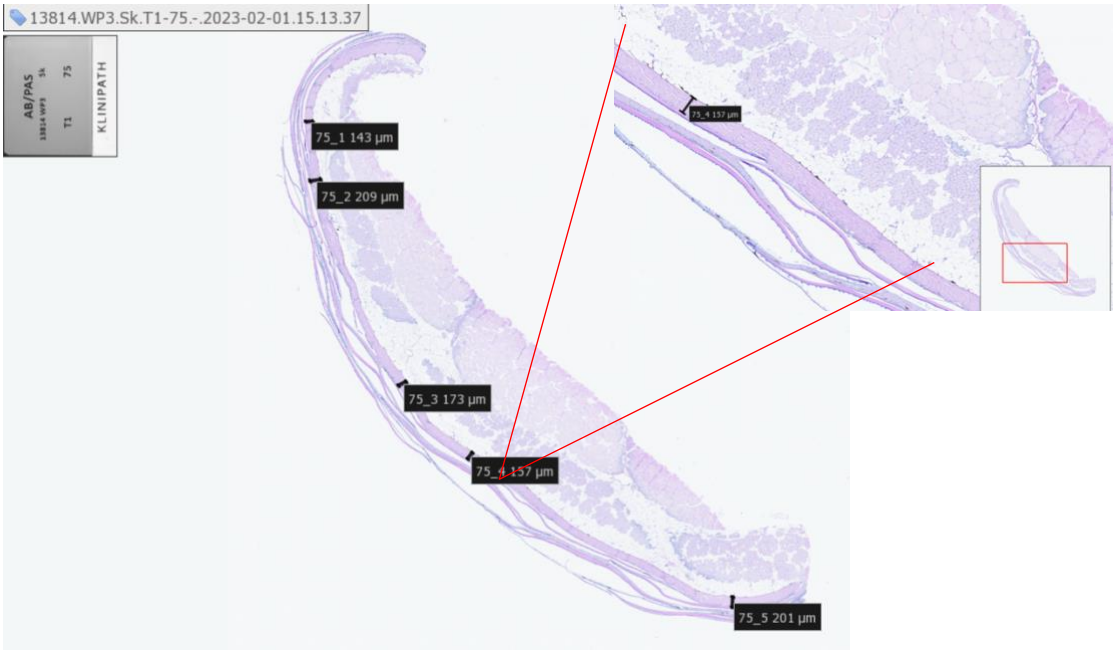

Figure 13. Screenshot of the image viewing tool NDP.view2 Plus, software U12388-01 (version 2.9.29). Picture show the five annotations used to calculate mean value of the dermis thickness (µm) on Atlantic salmon (Salmo salar L.*).*

4.3.5 Chemical analyses

NQCs from the fish in each net pens were homogenized and pooled prior to being analysed by LabTek, NMBU Ås for total fat, fatty acid composition and astaxanthin. Soxtec™ 8000 Extraction system in combination with Foss Hydrotec™ 8000 Hydrolysis system was used to determine the total fat. The fatty acids as fatty acid methyl esters (FAME) were analyzed with the Trace GC Ultra with auto injector (Thermo Scientific). The quantity of astaxanthin was measured with an HPCL method, with Ultimate 3000 UHPLC with UV detector (Thermo Scientific).

4.4 Data treatment

SAS (statistical analysis software, version 9.4) was used to perform the statistical analyses with ANOVA the dietary effect on biometric parameters, operational welfare indicators, skin parameters and fillet colour. The level of significance was set to 95% ($p \leq 0.05$). GLM procedure with The GLM Procedure least squares means was used to rank significant differences between means of diets. Data was corrected for systematic effects of gender when significant. Figures were performed in Microsoft Excel version 16.75.2 using the SAS data.

4.5 Calculations

$$Condition\ factor\ (CF): \frac{Body\ weight\ (g)}{Body\ length\ (cm)^3} x100$$

$$Slaughter\ yield: \frac{Carcass\ weight\ (g)}{Body\ weight\ (g)} x100$$

$$Fillet\ yield: \frac{Fillet\ weight\ (g)}{Body\ weight\ (g)} x100$$

5. Results

The study evaluated how modified diets with increased n-3 LC PUFA and with supplementation of the novel ingredient micro algae affected skin health, muscle colour and chemical composition of *Salmo salar* L. The results are presented in four parts. The first part presents biometric traits of the fish, the second describes the skin health in line with the OWI´s and histology of dermis. The third part describes quality traits of fillet pigmentation, and the fourth and final part presents the chemical analysis.

5.1 Biometric traits

5.1.1 Body weight and length

The mean body weight (BW) of the fish fed with control diet exhibited an average value of 518.3±15.8g at the initial sampling conducted in November 2022. Subsequently, these fish displayed an increased mean BW 1188.2±43g during the second sampling conducted in April 2023. Fish that were fed diet 1 demonstrated an average BW of 505.3±17.8g in November, which increased to 1176.3±57.6g on average in April. Similarly, fish fed test diet 2 group exhibited an initial mean BW of 505.5±18.9g in November, which reached 1130.4±57.4g on average by April. There was no significant dietary effect on the BW development (p=0.89) (table 5).

The length of the fish had similar range for all dietary groups within each sampling time in November 2022 and April 2023 (table 5). The different diets had no significant effect on length development (p=0.69).

5.1.2 Economic traits

The average fillet weight of fish fed the control diet increased from 176.4±5.4g to 374.48±13.9g from November 2022 to April 2023, respectively. Fish that were fed diet 1 demonstrated an average fillet weight of 169.7±6.1g in November and increased to 376±19.9g on average in April. Similarly, fish fed test diet 2 group exhibited an initial mean fillet weight of 170.9±7g which increased to 376±19.9g on average in April. There was no dietary effect on development of fillet weight (p=0.88) (table 5).

The mean fillet yield (FY) of the fish fed control diet exhibited a decrease from 75.8% to 73.3% from November 2022 to April 2023. Fish that were fed diet 1 demonstrated an

average FY of 75.6% in November, which decreased to 74.1% on average in April. Similarly, fish fed with test diet 2 group exhibited an initial mean FY of 75.3% in November and subsequently decreased to 73.1% on average by April. There was no dietary effect on fillet yield (p=0.5). The condition factor (CF) of the fish sampled in November 2022 and April 2023 did not differ significantly between the fish fed the different diets of increased n-3 LC PUFA and micro algae supplementation (table 5).

Table 5. Biometric traits of Atlantic salmon (Salmo salar L.*) fed either a control diet with 7.5% EPA and DHA from fish oil (FO), the same diet with 10% EPA and DHA from FO (Diet 1) or a diet with 5% EPA and DHA from FO and algae oil (Diet 2), respectively (n=10 per net pen, triplicate net pens per diet). Results are presented as means ± standard error for fish samples in November 2022 and April 2023, and different superscripts within the same row indicate significant differences between diets and sampling time (p<0.05).*

	November 2022			**April 2023**		
n=50	**Control**	**Diet 1**	**Diet 2**	**Control**	**Diet 1**	**Diet 2**
Body weight, g	518.3±15.8^{b}	505.3±17.8^{b}	505.5±18.9^{b}	1188.2±43^{a}	1176.3±57.6^{a}	1130.4±57.4^{a}
Length, cm	35.4±0.3^{b}	35±0.4^{b}	35±0.5^{b}	45.6±0.4^{a}	45.3±0.5^{a}	45.4±0.6^{a}
Fillet, g	176.4±5.4^{b}	169.7±6.1^{b}	170.9±7.0^{b}	374.48±13.9^{a}	376±19.9^{a}	357.2±19.3^{a}
Condition Factor[1]	1.0±0	1.0±0	1.0±0	1.0±0	1.0±0	1.0±0
Fillet yield[2], %	75.8±0.6^{a}	75.6±0.6^{a}	75.3±1^{a}	73.3±0.3^{b}	74.1±0.5^{b}	73.1±0.7^{b}

1. Condition factor, (CF) = (body weight, g)/ (body length, cm)^{3}*100;
2. Fillet yield = (fillet weight, g)/ (body weight, g) *100;

5.2 Animal welfare

5.2.1 OWI: Skin haemorrhage

Skin haemorrhage ranged between OWI score 0.7-1.2 (figure 14). There was no significant effect of diet (p= 0.29) on skin haemorrhage. However, the sampling time had significant effect on haemorrhage (p=0.003) for the control group. The amount of skin haemorrhage decreased from November 2022 to April 2023 on the fish fed the control diet and diet 1 with increased marine n-3 LC PUFA.

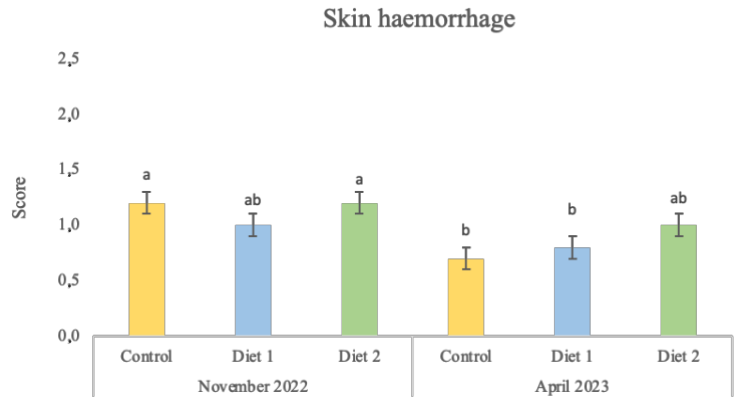

Figure 14. Total score of OWIs for skin haemorrhage of Atlantic salmon (Salmo salar L.*) fed eighter a control diet with 7.5% EPA and DHA from fish oil (FO), the same diet with 10% EPA and DHA from FO (Diet 1) or a diet with 5% EPA and DHA from FO and algae oil (Diet 2), respectively. Results are presented as mean ± standard error for fish sampled in November 2022 and April 2023, and different superscripts above the error bas indicate significant differences between diets and sampling time (p<0.05).*

5.2.2 OWI: Scale loss

Total scale loss ranged from 3.3-5.5 (figure 15a). The different diets had no significant effect (p=0.16) on the total amount of scale loss. However, sampling time had a significant effect on the scale loss for all the dietary groups (p<0.0001). Total scale loss on the dorsal part ranged from 2.4-3.6 (figure 15b). The different diets did not have significant difference on the total scale loss on the dorsal part of the fish either (p=0.21), but there was a significant effect of sampling time (p<0.0001). The total scale loss on ventral part ranged from 1-1.9 (figure 15c). Similarly, the different diets did not have effect on scale loss on the ventral (p=0.15), but there was significance difference of sampling time (p<0.0001) on amount of scale loss.

The total scale loss on anterior side ranged from 0.9-1.9 (figure 16a). The diet had no significant effect (p=0.29) on amount of scale loss. Sampling times showed a significant effect (p<0.0001) on the amount of scale loss on the anterior side. Scale loss on the posterior side ranged from 1.4-1.7 (figure 16b) and exhibit no significant difference due to dietary treatment (p=0.35) or sampling time (p=0.33) on the amount of scale loss on the ventral side.

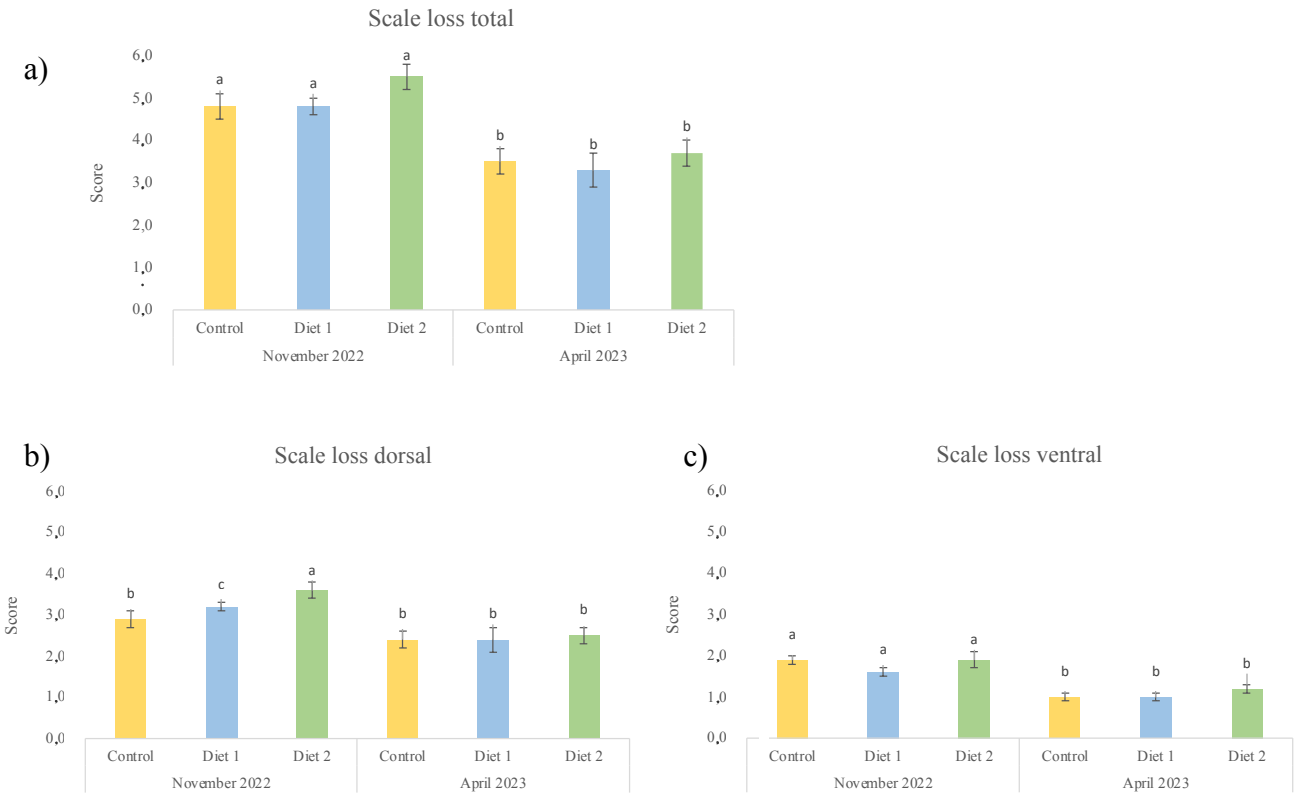

Figure 15. Total score of OWIs for scale loss of Atlantic salmon (Salmo salar L.*) fed eighter a control diet with 7.5% EPA and DHA from fish oil (FO), the same diet with 10% EPA and DHA from FO (Diet 1) or a diet with 5% EPA and DHA from FO and algae oil (Diet 2), respectively. Results are presented as overall degree of each OWI score (range: 1-3) in November 2022 and April 2023, the different superscripts above the error bas indicate significant differences between diets and sampling time ($p<0.05$). 15 a) illustrate the degree of total scale loss of the whole side of the fish, b) illustrate the degree of total scale loss on dorsal part, c) illustrate total degree of scale loss on ventral part. Red line in figure 10 separate 15b and 15c.*

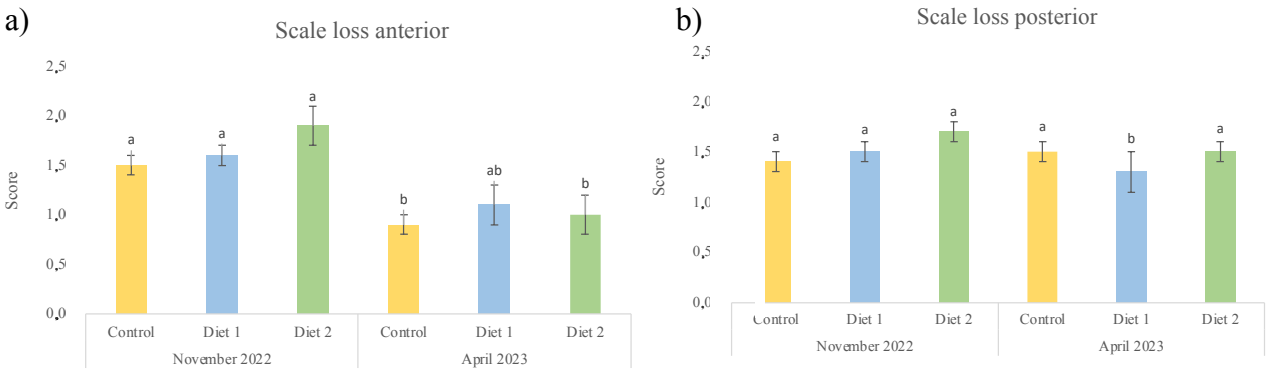

Figure 16. Total score of OWIs for scale loss on anterior and posterior side on the dorsal part of Atlantic salmon (Salmo salar L.*) fed eighter a control diet with 7.5% EPA and DHA from fish oil (FO), the same diet with 10% EPA and DHA from FO (Diet 1) or a diet with 5% EPA and DHA from FO and algae oil (Diet 2), respectively. Results are presented as overall degree of each OWI score in November 2022 and April 2023. The different superscripts above the error bas indicate significant differences between diets and sampling time ($p<0.05$). 16 a) shows scale loss on anterior side. b) shows scale loss on posterior side. Orange line in figure 10 separate the anterior and posterior side.*

5.2.3 Histology – Dermis thickness (µm)

The mean dermis thickness (µm) ranged from 163.7µm-171.8µm (figure 17). Neither diet nor sampling time had significant effect on the dermal thickness with the significance level of $p<0.05$.

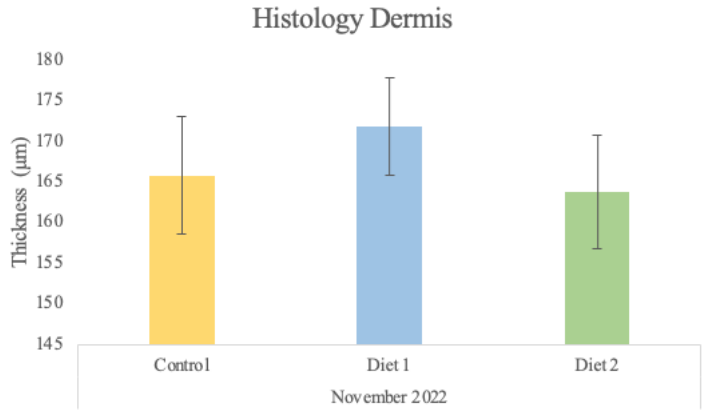

Figure 17. Figure illustrates dermis thickness(µm) of Atlantic salmon (Salmo salar L.*), fed eighter a control diet with 7.5% EPA and DHA from fish oil (FO), the same diet with 10% EPA and DHA from FO (Diet 1) or a diet with 5% EPA and DHA from FO and algae oil (Diet 2), respectively. The results are presented as mean dermis thickness (µm) ± standard error for fish sampled in November 2022, there was no significant differences between diets and sampling time ($p<0.05$).*

5.3 Fillet colour

The SalmoFan score ranged from 21.1-23.9 on average (figure 18). The different diets had no significant effect on the fillet colour ($p=0.35$). However, different sampling times showed a significant effect ($p<0.0001$) in the fillet colour of all dietary groups. The colour intensity was significantly higher in the April sampling compared to November.

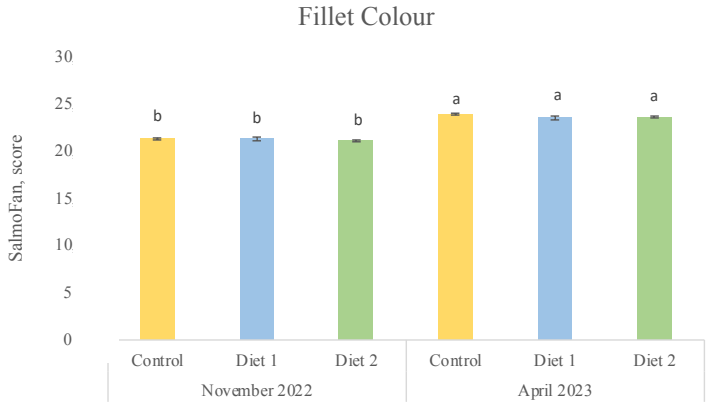

Figure 18. The figure shows the results of SalmoFan score of NQC fillets from Atlantic salmon (Salmo salar L.*) fed eighter a control diet with 7.5% EPA and DHA from fish oil (FO), the same diet with 10% EPA and DHA from FO (Diet 1) or a diet with 5% EPA and DHA from FO and algae oil (Diet 2), respectively. Results are presented as mean ± standard error for fish sampled in November 2022 and April 2023, and different superscripts above the error bas indicate significant differences between diets and sampling time ($p<0.05$).*

5.4 Chemical Analysis

The total amount of fatty acids were not significantly affected by diet or sampling time (table 6). The diet ($p=0.0032$) and sampling time ($p<0.0001$) had a significant effect on C18:1n-9 level in the fish fed the different diets (table 6). The diet ($p<0.0001$) and sampling time ($p<0.0001$) had significant effect on C18:2n-6 level of the fish fed different diets (table 6). Additionally, the sampling time and diet had significant effect ($p=0.0114$) on the level of C18:2n-6. Diet 2 exhibited the highest level in both sampling times. Only the sampling time ($p<0.0001$) had significant effect on level of C18:3n-3 in the fish fed the different diets (table 6).

The diet ($p<0.0001$) and sampling time ($p=0.0005$) had significant effect on the level of EPA (C20:5n-3) in the fish fed different diets (table 6). Diet 1 exhibited the highest level of EPA followed by control diet and then diet 2. The diet ($p<0.0001$) and sampling time ($p=0.03$) had significant effect on the level of DHA (C22:6n-3) in the fish fed different diets (table 6). Diet 2 exhibited the highest level of DHA, followed by control diet and then diet 1.

Astaxanthin (mg/kg) ranged from 2mg/kg 3.5mg/kg on average (table 6). The sampling time ($p<0.0001$) had a significant effect on level of astaxanthin in the fish fed the different diets. Diet did not have a significant effect on level of astaxanthin.

Table 6. Chemical analysis of Atlantic salmon (Salmo salar L.*) NQC fed either a control diet with 7.5% EPA and DHA from fish oil (FO), the same diet with 10% EPA and DHA from FO (Diet 1) or a diet with 5% EPA and DHA from FO and algae oil (Diet 2), respectively. Results are presented as g/kg ± standard error for fish samples in November 2022 and April 2023, and different superscripts within the same row indicate significant differences between diets and sampling time (P<0.05).*

	November 2022			**April 2023**		
%	**Control**	**Diet 1**	**Diet 2**	**Control**	**Diet 1**	**Diet 2**
Total fat	9.2±0.3	8.8±2	8.6±1.6	8.9±1	8.9±2.1	8.5±1.9
C18:1n-9	29.4±0.5^{b}	29.9±0.8^{b}	31.8±0.6^{a}	26.8±1^{c}	26±1^{c}	28.6±2^{c}
C18:2n-6	10.8±0.1^{c}	11±0.3^{c}	11.7±0.1^{b}	11.8±0^{b}	11.7±0.2^{b}	12.1±0.2^{a}
C18:3n-3	4.5±0^{b}	4.5±0.2^{b}	4.7±0.3^{b}	14.1±1.2^{a}	14±1.8^{a}	12±1.3^{a}
C20:5n-3	3.9±0.2^{ab}	4.1±0.3^{a}	2.7±0.1^{c}	2.9±0.1^{c}	4.3±0.3^{a}	2.2±0^{d}
C22:6n-3	7.6±0.1^{b}	7.4±0.4^{b}	9.3±0.6^{a}	7.2±0.2^{b}	6.4±0.7^{c}	9.7±0.8^{a}
Astaxanthin, mg/kg	2±0.1^{b}	2.2±0.5^{b}	2±0.2^{b}	3.5±0.2^{a}	3.2±0.4^{a}	3.4±0.2^{a}

6. Discussion

This thesis studied the effect on skin health, muscle quality and fatty acid composition in response to diets with increased marine omega-3 (DHA and EPA) and supplemented with algae oil inclusion. Diet 1 had increased omega-3 levels, and diet 2 had increased omega-3 levels with a 50% supplementation of the omega-3 sourced from the micro-algae *Schizochytrium sp*. The control diet was a commercial diet, resembling industry standard diet. The different diets compare the effects of novel ingredients with a commercial diet to detect potential benefits of using micro algae as a feed ingredient.

The result of this study indicates limited influence of dietary effect on skin health and product quality. Importantly, the dietary effects on status of skin health and fillet quality needs to be viewed holistically because external (e.g., rearing conditions) and internal (e.g., genetically predisposition to the ingredients used) could influence the results of this study.

6.1 Biometric traits

The control diet, test diets 1 with increased n-3 LC PUFA and test diet 2 n-3 LC PUFA including micro algae supplementation had similar development throughout both sampling periods. The similar growth development of control diet, diet 1 and diet 2 effects confirm that inclusion of *Schizochytrium sp*. can be incorporated in Atlantic salmon feed without negative effects on growth. Nonetheless, the results did not give significant effect between the different diets on the biometric traits: body weight, length, or fillet weight (table 5).
Fillet yield is the percentage of fillet relative to the total body weight. As the fillet is the edible part and most valuable part of the fish, it is an important index for the industry. The FY for all diets was above 70% (figure 5). Although diet 2 with supplemented *Schizochytrium sp.* did not have a significant effect on fillet yield, Kousoulaki et al., 2015 and 2016 proved increased carcass yield, resulting in increased amount of fillets.

The condition factor is an index reflecting the physical condition and relative fitness of the individual. The CF normally range between 1.2-1.4 (Mørkøre et al., 2020). The CF index was 1.0 for all diet groups in both samplings, which was relatively low compared to what would be expected of the fish with that weight and length (figure 5). Several factors can influence the CF, such as season, sex, sexual maturation stage, feeding, muscular development (Getso et

al., 2017). The results indicate no dietary effect with increased marine PUFAs and inclusion of micro algae in FY or CF during the two sampling times in November2022 and April 2023.

6.2 Skin health

6.2.1 Operational welfare indicator: Skin hemorrhage

Skin haemorrhage is a result of physical trauma to the skin causing bleeding in the abdominal region. External factors, such as handling and transporting can cause skin haemorrhage. The diets with increased levels of n-3 LC-PUFAs or inclusion of micro algae did not show any effect on amount of skin haemorrhage in the Atlantic salmon. However, the instances of skin haemorrhage decreased from November 2022 to April 2023 (figure 14). The sea water transfer trigger a stress response in the smolt which can reduce mucus layer and increase occurrence of scale loss and wounds (Sissener et al., 2021). However, external factors are the main cause of skin bleeding, but future studies could focus on whether micro algae could have on skin resilience and immune response.

6.2.2 Operational welfare indicator: Scale loss

Figure 15 shows overall degree of the total OWI scale loss on the whole, dorsal, and ventral part of the fish for each diet group. The fish sampled in November 2022 exhibited the highest score for total scale loss (figure 15a). Most of the fish had OWI score 2: small areas of the fish (<10% of the fish) (Noble et al., 2018). The results showed similar development of total scale loss for all control diet, diet with increased n-3 LC-PUFAs and diet with increased n-3 LC- PUFAs including micro algae supplementation. The total amount of scale loss in all areas decreased significantly from November 2022 to April 2023 (figure 15a, b, c). The diet with increased n-3 PUFA supplemented with micro algae exhibited the highest value of total scale loss on the dorsal area in the November 2022 sampling, compared to control diet and diet 1 (figure 15b). Furthermore, there was higher frequency of scale loss on the dorsal part of the fish compared to the ventral part (figure 15b and 15c). It should be investigated if these results are caused by a specific external factor, such as netting the fish or using the landing net to catch the fish, to eliminate such occurrences.

Figure 16 shows an overall degree of the total OWI scale loss score on the anterior and posterior side of the dorsal part of the fish for each diet group. The Atlantic salmon fed with control diet, diet 1 and diet 2 lost similar amount of scale loss during the two samplings. The

results showed no significant difference on scale loss between the diet groups. These results are similar to the study by Sissener et al. (2021), who tested if dietary modification in the freshwater stage would make the smolt more robust and increase survival in the sea water.

The scale loss score was evaluated by visual analysis after the fish had been transported to Nofima Ås, where the picture were taken. Handling of the fish (packaging activity, transporting the fish wiping blood and water residue off the skin) can cause additional scale loss and therefore have an impact on perceived amount of scale loss. The pictures taken for visual analysis used to score OWIs should preferably be taken at sampling site to eliminate source of error.

6.2.3 Histology of dermis

The histology data was only collected from the November sampling. The results show mean dermis thickness (µm) in declining order: control (171.8µm), diet 1 (165.7µm), diet 2 (163.7µm) (figure 17). The test diets: control, diet 1 with increased n-3 LC PUFA and diet 2 with increased n-3 LC PUFA and micro algae supplementation showed no significant difference on dermis thickness between the diet groups or sampling time.

6.3 Product quality trait: Fillet colour

The fillet colour is a major quality parameter for the consumer. Salmon fillets with a strong red pigmentation are more desired and are considered to be fresher and have higher quality by the consumer (Alfnes et al., 2006). The visual measurement tool, SalmoFan™ is important for the industry and give measurements based on the overall impression of the muscle. However, the visual tool can suffer subjective measurement based on the evaluation by different people. In this thesis, Dr. Mørkøre performed all SalmoFan™ measurements to ensure professional and predictable measurements.

The Atlantic salmon fed control diet, diet 1 and diet 2 showed similar development of red pigmentation, measured with SalmoFan™, but the April 2023 results were significantly different from the November 2022. The SalmoFan score in November 2022 sampling ranged from 21.1 to 23.9 and the April 2023 sampling ranged from 23.6 to 23.9. Steine et al. (2005) studied consumer-based preference on the fillet colours redness, and proved higher

willingness to pay for highly pigmented salmon fillets where fillets under colour score 23 was more difficult to sell.

6.4 Chemical analysis

The chemical analysis was pooled from the NQCs (figure 6). The Atlantic salmon fed control diet and the modified diets: diet 1 with increased n-3 LC PUFAs and diet 2 with increased n-3 PUFAs supplemented with *Schizochytrium sp.* had the same development of total fatty acid content. The fatty acids found in plant sources (C18:1n-9, C18:2n-6 and C18:3n-3) had an overall higher content than the marine fatty acids (C20:5n-3 and C22:6n-3) for all test diets, except from C18:3n-3 in the November 2022 sampling.

There was a dietary effect of oleic acid (C18:1n-9) level in the Atlantic salmon fed the different diets. Diet 2 had the highest amount of oleic acid, and control diet had the lowest amount of oleic acid in the November 2022 sampling. The amount of oleic acid evened out in the April 2023 sampling where all diets had similar amount of the fatty acid. There was a dietary effect on percent linoleic acid (C18:2n-6) in the Atlantic salmon fed different diets. Diet 2 had the highest amount of the fatty acid in both sampling times. Control diet and diet 1 exhibited increased precent linoleic acid from November 2022 to April 2023. There was no dietary effect on the percentage of α-linolenic acid (C18:3n-3) in the Atlantic salmon fed different diets. The amount of α-linolenic acid exhibited similar development through the different sampling times.

There was an influence of the different diets on percent EPA (C20:5n-3) in the Atlantic salmon fed different diets. The control diet and diet 2 decreased in level of EPA from November 2022 to April 2023 sampling, while diet 1increased. There was a dietary effect on percent DHA (C22:6n-3) in the Atlantic salmon fed different diets. Diet 2 had a positive development and exhibited the highest overall amount of DHA in both the November and April sampling. The control diet had similar level of DHA in both sampling times, while diet 1 decreased in level of DHA. The astaxanthin amount increased from November 2022 to April 2023 for all dietary groups.

7. Conclusion

The study investigated if dietary modifications of dietary EPA and DHA level and origin given to Atlantic salmon could affect skin health, fillet quality and chemical composition. Neither the level (7.5-10%) not the origin (fish oil or algae oil, *Schizochytrium sp*.) had a significant effect on skin haemorrhage, scale loss, dermis thickness or fillet colour.

In conclusion, LC-PUFA inclusion from algae oil did not reduce amount of scale loss or skin haemorrhage, or improve red pigmentation of the salmon muscle. All diets showed a similar development of biological traits during the experimental period, indicating no negative effects of micro algae inclusion on growth. There was a dietary effect on oleic acid, linoleic acid, EPA and DHA. Inclusion of LC-PUFA from micro algae oil gave the highest levels of oleic acid, linoleic acid and DHA compared to the commercial diet and only with increased marine n-3 LC PUFA. Hence, increase the levels of DHA (C22:6-n3) The diet with increased omega-3 gave the highest level of EPA (C20:5n-3). Astaxanthin was not affected by the modified diets but increased from November 2022 to April 2023.

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Appendix

Table 1. Weekly production data of the mean temperature and oxygen from sea water transfer, the November 2022 and April 2023 sampling.

Mean temperature and oxygen from the beginning

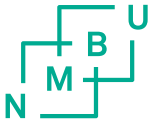
Location: Slettnesfjord, Hammerfest

Year	Week	Mean-temp. [°C]	Oxygen(5m) mean [mg/l]
2022	29	9.1	11.86
	30	10.5	11.04
	31	11	10.41
	32	10.2	11.57
	33	10.3	10.77
	34	10.7	9.76
	35	10.3	9.48
	36	9.9	9.76
	37	9.4	9.72
Start of trial	38	9.3	9.78
	39	8.7	9.78
	40	8.5	9.72
	41	8.2	10.59
	42	8.2	10.26
	43	8.2	10.26
	44	8.1	10.4
	45	7.8	10.42
	46	7.6	10.33
	47	7.1	10.08
	48	6.7	10.25
	49	6.3	10.86
	50	5.7	10.82
	51	5.5	10.75
	52	5.3	10.7
2023	1	5.3	11.09

	2	5.3	11.48
	3	5.2	11.68
	4	4.9	12
	5	4.8	11.85
	6	4.7	11.9
	7	4.5	11.75
	8	4.4	12.44
	9	4.04	12.66
	10	3.97	12.23
	11	3.92	12.44
	12	3.97	12.28
	13	3.62	12.35
	14	4.14	12.76
	15	4.14	12.89
	16	4.13	13.15
	17	4.12	13.33

Table 2. Production data of the average weight of each net pen containing the Atlantic salmon (Salmo salar L.*) in the feed trial and when the fish was given a new pellet size. The yellow colour indicates control diet, the blue diet 1 and green diet 2.*

	Pellet size		Average weight (g)	
	November 2022	April 2023	November 2022	April 2023
Netpen1	200	1000	523	1187
Netpen4	500	1000	516	1207
Netpen8	500	500	576	1218
Netpen3	200	1000	467	1018
Netpen5	500	1000	506	1213
Netpen7	500	500	563	1243
Netpen2	200	500	518	1211
Netpen6	200	500	570	1206
Netpen9	500	500	584	1288



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