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Product quality, fish welfare and salmon louse infestation of Atlantic salmon and rainbow trout fed a diet supplemented with fermented soybean and macro algae (*Saccharina* latissima)

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Master of Science in Aquaculture

Acknowledgement

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Abstract

Aquaculture is an expanding industry that is expected to grow furthermore (FAO, 2020). The expansion leads to a demand of sustainable feed ingredients that are limited today. Atlantic salmon (*Salmo salar L.*) and rainbow trout (*Oncorhynchus mykiss*) fed with commercial control feed or the same feed with inclusion of fermented soybean and macro algae, *Saccharina latissima* (test feed, macro algae) were farmed at commercial farming locations at the Norwegian West coast for 13 and 6 months, respectively. The effect of feed on product quality, fish welfare and salmon louse infestation were investigated. Results of this trial showed improved growth of salmon fed test feed, resulting in a 19% higher body weight at harvest (6.6 kg vs. 5.5 kg). The body weight of the rainbow trout fed test feed was 6.5% lower compared with the control feed (3.1 kg vs. 3.4 kg). The test feed had no positive effect on fillet colour or texture but gaping and scale loss were lower in salmon. Salmon fed with control feed or feed with inclusion of macro algae had similar development in biometric traits. There was similar development in salmon louse number in Atlantic salmon, while in rainbow trout there was a trend showing lower salmon louse number in fish fed with test feed compared to fish fed with control feed.

Sammendrag

Akvakultur er en ekspanderende næring som forventes å vokse ytterligere (FAO, 2020). Utvidelsen fører til en etterspørsel etter bærekraftige fôrråvarer som er begrenset i dag. Atlantisk laks (Salmo salar L.) og regnbueørret (Oncorhychus mykiss) fôret med kommersielt kontrollfôr eller samme fôr med tilskudd av fermentert soyabønner og makroalger, Saccharina latissima (testfôr, makroalger) ble oppdrettet på kommersielle oppdrettsanlegg langs den norske vestlandskysten i henholdsvis 13 og 6 måneder. Effekten av fôr på produktkvalitet, fiskevelferd og luseinfestasjon ble undersøkt. Resultatene fra dette forsøket viste forbedret vekst av laks fôret med testfôr, noe som resulterte i 19% høyere rundvekt ved slakt (6.6 kg vs. 5.5 kg). Rundvekten til regnbueørreten fôret med testfôr var 6.5% lavere sammenlignet med kontrollfôr (3.1 kg vs. 3.4 kg). Testfôret hadde ingen positiv effekt på filetfarge eller tekstur, men filetspalting og skjelltap var lavere i laks. Laks fôret med kontrollfôr eller testfôr med tilskudd av makroalger hadde tilsvarende utvikling i biometriske trekk. Det var tilsvarende utvikling i lusetall hos atlantisk laks, mens det i regnbueørret var en trend som viste lavere lusetall hos fisk fôret med testfôr sammenlignet med fisk fôret med kontrollfôr.

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1. Introduction

The global human population is expected to be 9.7 billion in 2050. At the same time fish intake on world basis have been increasing, from 9 kg annual consumption per capita in 1961 to 20.3kg live weight in 2017 in the world. The total fish consumption has increased with 3.1% annually from 1961 and until 2017, while annual population growth rate was 1.6% (FAO, 2020). Because of the growth in global aquaculture there is a further need for novel feed ingredients, that won't compete with human diet and can improve fish health, product quality and fish welfare**.** The aim of this study is to examinate effects of dietary supplementation of sugar kelp (*Saccharina latissima*) in Atlantic salmon (*Salmo salar L.*) and rainbow trout (*Oncorhynchus mykiss*).

2. Theoretical background

The growing global human population and fish intake demands growth in aquaculture. Fish production from fishery has reached the limit, and the last 30 years the growth in fish consumption have been covered by aquaculture (figure 1). Although white fish, such as tilapia and carps are the most produced fish in aquaculture, Atlantic salmon and rainbow trout are high-value fish products that are highly demanded in the world food market (FAO, 2020).

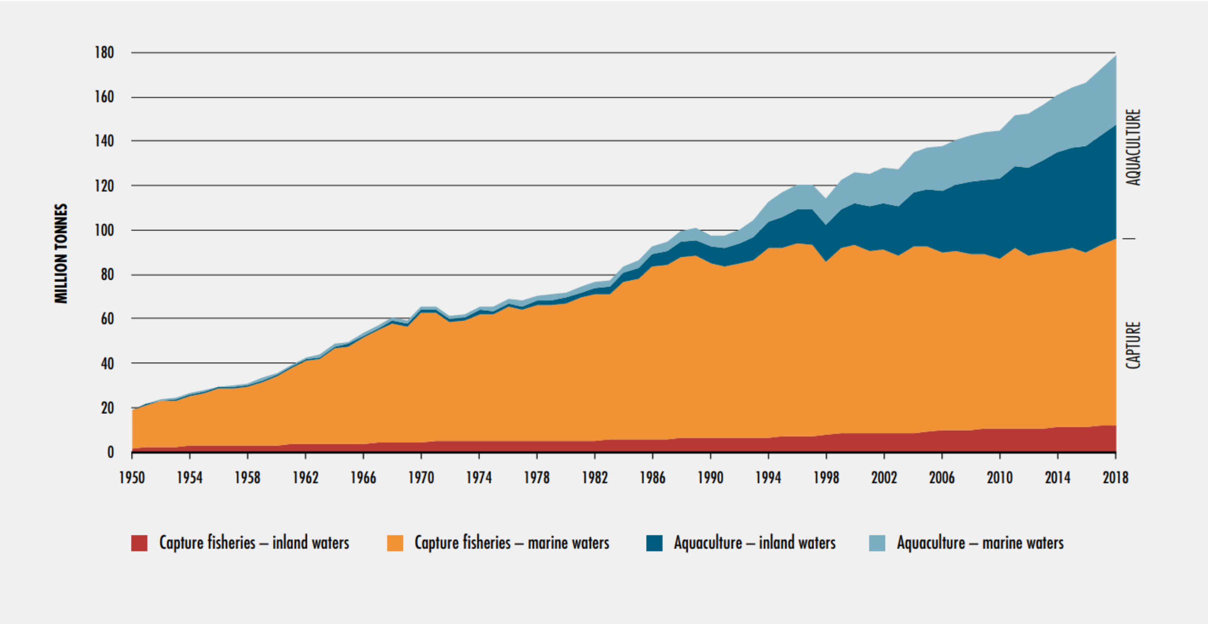

Figure 1 Development in world capture fisheries and aquaculture production the last 70 years (FAO, 2020).

The natural salmonid diet is high in marine ingredients, but the growth in aquaculture has forced producers to find new feed ingredients, as marine ingredients have become a limited resource. In the past 30 years major part of marine feed ingredients in Norwegian aquaculture have been replaced with plant ingredients (Ytrestøyl et al., 2015), mainly soybean protein. The increased proportion of plant materials in fish feed are shown to have negative effect on fish health (Baeverfjord & Krogdahl, 1996), e.g., soybean may increase inflammation in skin mucus (Djordjevic et al., 2021). Marine ingredients are high in marine fat and minerals, that traditionally were not consumed by humans. The plant feed materials lack some of these elements and have led to change in the chemical composition of fish products (Esaiassen et al., 2022). In addition, plant production for feed is directly competing with human food production in the land area resources.

2.1. Fish feed ingredients

Norwegian aquaculture is a growing industry where the demand of fish feed is high. Demand in marine feed ingredients have led to a replacement with plant feed ingredients. Content of marine feed ingredients used in Norwegian aquaculture has decreased from around 90% in 1990 to approximately 30% in 2010's (Aas et al., 2019). The replacement is unfortunate for the growing world population, as food production need to be increased. Replacement of marine feed is also causing changes in nutritional compositions of fish products (Jakobsen & Smith, 2017; Sprague et al., 2016). By dietary inclusion of novel feed ingredients, the nutritional content may be manipulated to fish products richer in omega-3-fatty acids and minerals (Kousoulaki et al., 2021). Replacement of fish oil with plant oil gives less desirable omage-3-fatty acids in fish products of Atlantic salmon and rainbow trout. Because the fatty acid profile in fish muscle reflects the dietary fatty acid profile, replacement of fish oil with plant oil gives less desirable omega-3-fatty acids in fish products of Atlantic salmon and rainbow trout (Esaiassen et al., 2022). Marine feeds are rich in minerals and fatty acids that are important for both fish and human health (Shahidi & Ambigaipalan, 2015).

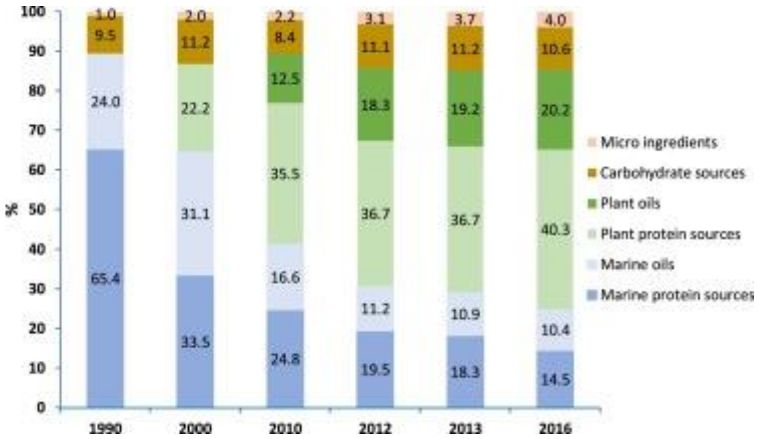

Figure 2 Ingredient sources (% in feed) in Norwegian salmon feed (Aas et al., 2019).

2.2. Sugar kelp (*Saccharina latissima*)

Sugar kelp (*Saccharina latissima*) is a marine brown algae of the family *Laminariaceae* that is found in the northern hemisphere, along Atlantic and Pacific Ocean (Paulino et al., 2016). Methods for cultivating this sugar kelp have been developed and today it is farmed in Norway, Scotland, Faroe Islands and USA (Boderskov et al., 2021; Kim et al., 2015). Brown algae in general are high in minerals, polysaccharides, vitamins and polyunsaturated fatty acids (Gupta & Abu-Ghannam, 2011). The protein in brown algae has low digestibility in monogastric animals, but by fermenting the algae polysaccharides and non-protein nitrogen may be transformed into available protein (Øverland et al., 2019). The inclusion of sugar kelp in fish diet can increase levels of minerals in fish products and contribute with health benefits (Granby et al., 2020; Øverland et al., 2019).

Sugar kelp is an algae-specie high in iodine (Øverland et al., 2019). Iodine deficiency is a great human health issue, it is important for a well-functioning thyroid gland. The lack of iodine, especially in pregnant women, may impact brain development, growth, and metabolism (Nerhus et al., 2018; Zimmermann, 2012). Fish products are good sources of iodine, although the iodine content varies significantly between fish species (Nerhus et al., 2018). Addition of macro algae in fish feed rich in plant ingredients may increase the iodine content in fish product (Granby et al., 2020).

In addition, in vitro trials have shown antimicrobial effects of green, red, and brown algae in fish and shrimp (Gupta et al., 2010; Vatsos & Rebours, 2015). Algae extracts have a potential to be used as drugs on aquaculture pathogens in the future (Noorjahan et al., 2022).

Figure 3 Sugar kelp (Saccharina latissima). Photo: ©Tatum – stock.adobe.com

2.3. Flesh quality

Atlantic salmon and rainbow trout are two high-value species on the commercial market. The high price leads to a quality expectation of these fish products (Landazuri-Tveteraas et al., 2021). Fillet colour (Heia et al., 2011), fillet texture, gaping and fat content are important quality traits of Atlantic salmon and rainbow trout (Sigurgisladottir et al., 1997).

The red-orange fillet colour of Atlantic salmon and rainbow trout is a sought quality parameter for consumers (Yagiz et al., 2009). The fillet colour comes from several carotenoids, where astaxanthin and canthaxanthin are the main pigments. Wild salmonids receives the pigments from crustaceans through the food chain, in aquaculture astaxanthin is added to the fish feed (Ytrestøyl et al., 2021). In general rainbow trout have a more intense red-orange flesh colour compared to Atlantic salmon.

Fillet texture is an important quality parameter due to processing of the fillets. Fillets with soft texture will fall apart during processing (Torgersen et al., 2014). This leads to degradation of fillet quality and reduce the economic value of the fish (Moreno et al., 2012). Softness of fillets are caused by degradation of connective tissue, mainly collagen (Moreno et al., 2016; Torgersen et al., 2014). Fillet gaping appears as slits of holes between muscle segments (Ofstad et al., 2006) . The loss of connective tissue between muscle segments develops gradually post-mortem (Ashton et al., 2010).

2.4. Fish welfare

Fish welfare is an important aspect of sustainable aquaculture, as Norwegian aquaculture is today experiencing several issues regarding fish welfare and health (Sommerset et al., 2021). Atlantic salmon and rainbow trout production are intensive production systems, and are vulnerable towards viral, bacterial, and parasitic infections. The awareness towards fish health and fish welfare is high, as fish health and fish welfare have direct impact on production output and product quality (Hvas et al., 2021; Noble et al., 2018).

2.4.1. Scale loss

The main reason for scale and mucus loss is fish handling, mechanical delousing and parasite infections (Gismervik et al., 2017; Powell, 2021). Loss of scale and mucus can expose fish to pathogenic infections (Brydges et al., 2009; Conte, 2004) and stress in the osmoregulatory system, which can lead to increased mortality (Stien et al., 2013).

2.4.2. Intestinal, cardiac and hepatic fat accumulation

Accumulation of fat around internal organs is a welfare indicator in fish. The fat comes from intensive production with diet high in fat. The accumulation of fat can indicate disfunction of internal organs and lead to reduced fish health (Pettersen et al., 2014).

2.5. Salmon louse

Salmon louse (*Lepeophtheirus salmonis*) is a major challenge in Norwegian aquaculture of Atlantic salmon and rainbow trout (Lekang et al., 2016; Sommerset et al., 2021). Moreover, salmon louse from aquaculture may infect wild stock during migration from lakes to the sea (Kristoffersen et al., 2018; Liu et al., 2011). The Norwegian fish health report for 2021 states that high mortality is the biggest challenge in the Norwegian industry today; averaging 15% in the sea phase for both salmon and rainbow trout (Sommerset et al., 2021). Mortality causes, besides from viral and bacterial infections, are damages from fish handling. Fish handling is required during transporting, fish sorting and during delousing. Salmon louse has developed immunity towards several chemical treatments (Coates et al., 2021), and today fresh water-treatment, thermal and mechanical louse removing methods are mostly used (Andrews & Horsberg, 2021; Overton et al., 2019). Norwegian aquaculture laws requires delousing when there are above 0.5 sexual mature salmon louse per fish (Lovdata, 2012). The most used method for delicing today is mechanical delicing with fresh water. All delousing methods requires fish handling; therefore, it is important to find sustainable ways to prevent salmon louse larvae to grow. The salmon louse infestation is also a noticeable economical cost in the industry (Costello, 2009).

Salmon louse infection causes damage on the fish skin, which can lead to scale loss, bleeding in epidermis and changes in epidermal mucus (Easy & Ross, 2009; Jónsdóttir et al., 1992). Fish scale and epidermis is a part of fish's osmotic regulation, thus when disrupted it can cause a disbalance in the osmotic regulation. As every infection, salmon louse cause stress that impacts growth rate and fish welfare, and can cause fish death as a stress result (Easy & Ross, 2009). When salmon louse cannot be sufficiently removed, an emergency slaughter must be performed. This leads to a lower economic income from the fish production.

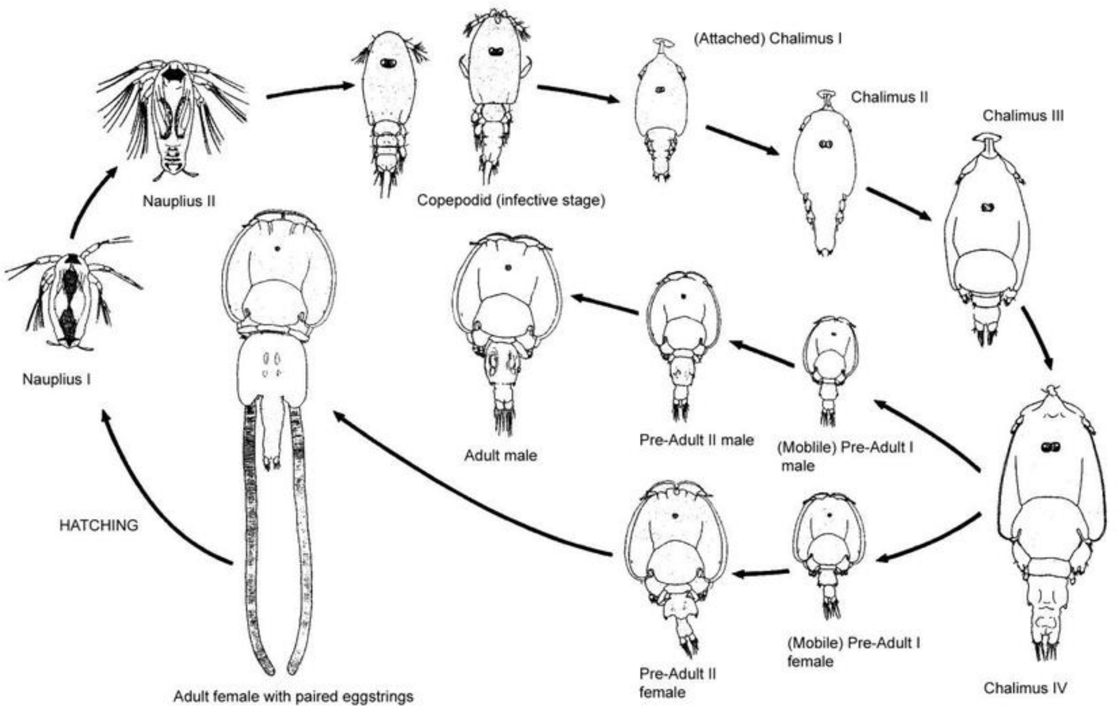

Figure 4 Life stafe of salmon louse (Lepeophtheirus salmonis) showing mobile pre-adult, attached chalimus I and sexual mature female stage(Whelan, 2022).

3. Materials and methods

3.1. Fish feed

Fish were fed a commercial control feed or test feed with inclusion of macro algae (*Saccharina latissima*). The test feed had the same composition as the control feed, except that 4% of soybean meal was replaced with EP-2299ng produced by European Protein. EP2299ng is a product containing soybean fermented with 4% macro algae sugar kelp (*Saccharina latissima*).

The fish used were Atlantic salmon (*Salmo salar L.*) and rainbow trout (*Oncorhynchus mykiss*). The feeds were formulated to similarly, suitable for each specie. Salmon were fed with four similar feeds with varying pellet sizes; 4.5 mm, 6 mm and two different feeds of 9 mm (table 1). Crude fat content of the salmon feed ranged from 24.2% to 30.8% in control feed and 25.7% to 28.3% in test feed. Rainbow trout were fed five different feeds and four different pellets sizes (table 2). The feed composition for the different pellets sizes was the same during the growing period, with adjustment in protein-fat ratio regarding fish size.

Table 1 Proximate composition of the commercial control feed and test feed with inclusion of macro algae Saccharina latissima fed to Atlantic salmon.

	Atlantic salmon							
Diet	**Control**	**Control**	**Control**	**Control**	**Test**	**Test**	**Test**	**Test**
Size (mm)	**4.5**	**6**	**9A**	**9B**	**4.5**	**6**	**9A**	**9B**
Dry matter, %	93.0	93.3	93.4	93.8	93.1	93.4	93.5	93.3
Ash, %	6.3	7.2	7.3	7.0	6.7	7.2	7.4	7.3
Crude fat, %	24.2	25.5	27.6	30.8	25.7	25.9	28.3	28.3
Starch, %	11.0	10.5	11.5	11.7	10.3	9.8	10.6	12.0
Energy, MJ/kg	23.9	23.9	24.2	24.0	23.6	24.0	24.4	24.0
Astaxanthin, %	16.5	11.6	38.5	23.5	14.8	28.2	44.3	39.0

Table 2 Proximate composition of the commercial control feed and test feed with inclusion of macro algae Saccharina latissima fed to rainbow trout.

	Rainbow trout									
Diet	**Control**	**Control**	**Control**	**Control**	**Control**	**Test**	**Test**	**Test**	**Test**	**Test**
Size (mm)	**3**	**4.5**	**6**	**9A**	**9B**	**3**	**4.5**	**6**	**9A**	**9B**
Dry matter, %	93.3	93.4	94.2	93.2	92.4	93.1	93.8	92.6	93.0	93.0
Ash, %	8.1	7.2	7.7	7.6	7.7	7.7	7.6	7.4	7.3	7.6
Crude fat, %	25.7	24.7	21.3	29.2	24.8	24.8	25.5	25.6	28.7	27.5
Starch, %	10.0	10.5	9.6	12.3	9.9	10.0	10.4	12.1	12.1	10.3
Energy, MJ/kg	23.4	23.3	24.1	23.7	23.6	23.4	23.8	23.5	24.0	23.9
Astaxanthin, %	47.0	33.4	51.2	37.7	30.1	36.7	31.4	44.4	55.1	44.7

3.2. Fish materials

Atlantic salmon and rainbow trout were farmed at 4 different commercial fish farms in Western Norway. Atlantic salmon were farmed at Krigsholmen and Litle Lunnøy in Austevoll municipality (figure 5). There was in total eight sea cages of Atlantic salmon, four cages were fed with control feed and four cages were fed with test feed. There was two control cages and two test cages at each location; Krigsholmen and Litle Lunnøy.

Rainbow trout were farmed at Storlia and Hatlem Øst in Hyllestad municipality (figure 5). There was in total 4 sea cages of rainbow trout, 2 fed with commercial control feed and 2 cages fed with test feed with inclusion of macro algae. Three fish groups were farmed at Hatlem Ø location; one control group and two test groups. The last control group was placed at Storlia (figure 5).

Figure 5 Map showing location of Atlantic salmon (red dot) and rainbow trout (blue dot) farm (Kartverket, 2022) .

3.3. Biometric traits, salmon louse counting and fish sampling

Body weight and length were measured approximately once a month throughout the growing period in sea, in total 12 times of Atlantic salmon and 7 times of rainbow trout (table 3 and 4). Salmon louses were counted simultaneously. The counting included attached, mobile and sexual mature female salmon louse (*Lepeophteirus salmonis*). Fish were anaesthetised during measurement and louse counting.

There was three samplings of Atlantic salmon and three samplings of rainbow trout. In April 2021 there was a sampling from Krigsholmen and Litle Lunnøy (table 3 and 4). There was a final sampling of fish from Litle Lunnøy in August 2021 and from Krigsholmen in January 2022. High sea water temperatures resulted in faster biomass growth than expected at Litle Lunnøy and the fish were slaughtered in August. From August 2021 and until January 2022 the trial with Atlantic salmon was continued at Krigsholmen only. And due to sea louse infestation, two cages from Krigsholmen were slaughtered in October. Because of weather condition at the slaughtering point, fish sampling was not possible.

Rainbow trout were sampled in June, September, and December 2021 (table 4).

Table 3 Fish sampling. louse counting and biometric traits measurement of Atlantic salmon.

Location	Krigsholmen				Litle Lunnøy			
Diet	Control Cage 1	Control Cage 3	Test Cage 2	Test Cage 4	Control Cage 1	Control Cage 3	Test Cage 2	Test Cage 4
15.12.2020	🔵	🔵	🔵	🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵
20.01.2021	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵
04.03.2021	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵
29.04.2021	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵
30.04.2021	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
28.05.2021	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵
29.06.2021	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵
06.08.2021	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵
16.08.2021					🔴	🔴	🔴	🔴
19.08.2021					⚫ 🔵			⚫ 🔵
14.09.2021	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵				
19.10.2021		⚫ 🔵		⚫ 🔵				
25.11.2021		⚫ 🔵		⚫ 🔵				
19.01.2022		🔴		🔴				
21.01.2022		⚫ 🔵		⚫ 🔵				

🔴 Fish sampling

🔵 Lice counting

⚫ Biometric traits measurment

Table 4 Fish sampling. louse counting and biometric traits measurement of rainbow trout.

Diet	Control Cage 5	Control Cage 1	Test Cage 2	Test Cage 3
01.06.2021	⚫ 🔵 🔴	⚫ 🔵 🔴	⚫ 🔵 🔴	⚫ 🔵 🔴
02.07.2021	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵
03.08.2021	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵
16.09.2021	⚫ 🔵 🔴	⚫ 🔵 🔴	⚫ 🔵 🔴	⚫ 🔵 🔴
18.10.2021	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵
17.11.2021	⚫ 🔵	⚫ 🔵	⚫ 🔵	⚫ 🔵
07.12.2021	⚫ 🔵 🔴	⚫ 🔵 🔴	⚫ 🔵 🔴	⚫ 🔵 🔴

🔴 Fish sampling

🔵 Lice counting

⚫ Biometric traits measurment

Fish sampling

The fish was slaughtered at the production site and intestine was removed. The intestines were placed in plastic bags for further analysis, and the fish were placed on ice in Styrofoam boxes. Fish materials were transported to the Norwegian university of Life Science in Ås for further analysis.

Intestine were analysed one day after slaughter; fat accumulation around viscera, heart and liver were scored. Livers and hearts were weighted individually. The gutted fish were stored on ice in a refrigerated room and analysed one week post harvesting.

At the time of quality analysis fish were manually filleted. There was performed visual quality scoring; fillet colour, fillet texture and gaping. Biometric traits were measured; length, slaughter weight and fillet weight.

3.3.1. Fat accumulation

The liver colour was scored from 1 to 5 (figure 6a) described by Mørkøre et. al (2020), where 1 is pale, 3 is normal liver and 5 is dark red/brown. Visceral fat was scored by the visibility of pyloric caeca (figure 6b) from 1 to 5, where 1 is fully visible pyloric caeca and 5 is no visible pyloric caeca.

Figure 6 Visual scoring of liver colour (a) and visceral fat according to visibility of pyloric caeca (Mørkøre et al., 2020).

Fat accumulation on the heart surface was scored from 0 to 3, where 0 is no visible fat and 3 is where most of the heart is covered by fat (figure 7).

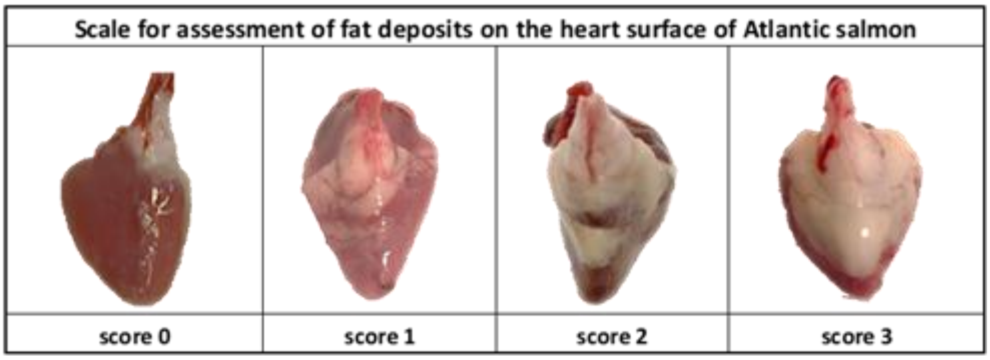

Figure 7 Visual scoring scale of fat deposition on the heart surface of Atlantic salmon (NMBU, Unpublished).

3.3.2. Quality analysis

Biometric traits were length, gutted weight and fillet weight. The analysed quality traits were fillet texture, fillet colour and gaping.

Texture was visually scored by adding a pressure of 1 kg on a point in the dorsal part of the fillet, just anterior to the dorsal fin. The texture was scored from 1 to 4. Where 1 is no visible mark after removing the pressure and 4 is severe holes in the pressure point.

Visual analysis of fillet gaping was performed by sliding a hand underneath the fillet to expose possible gaps. Gaping was scored from 0 to 3, where 0 is no gaping, 1 is up to five gaps, 2 is up to 10 gaps and 3 is over 10 gaps in the fillet. Fillet colour were visually analysed under standard light conditions in a Salmon colour box from Skretting using SalmoFan^{TM} fillet colour scale from DSM (figure 8).

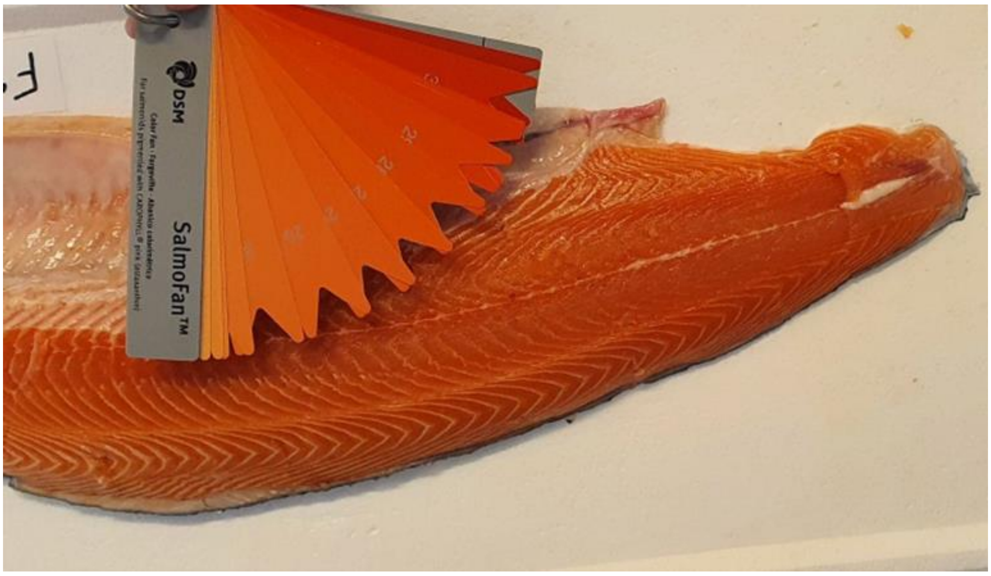

Figure 8 Visual scoring of fillet colour of rainbow trout with SalmoFanTM (DSM)

3.4. Operational welfare indicators (OWIs)

Welfare analyses were based on images of whole gutted fish.

Scale loss was determined according to an OWI scale (Kolarevic et al., 2018), where the score 0: no scale loss, 1: individual scale loss, 2: small areas of scale loss and 3: severe area of scale loss (figure 9). The left side of the fish were scored, divided into seven areas: head to dorsal fin, dorsal fin, Norwegian quality cut respectively dorsal and ventral, and tail (figure 10). The total scale loss was a mean score from the seven areas.

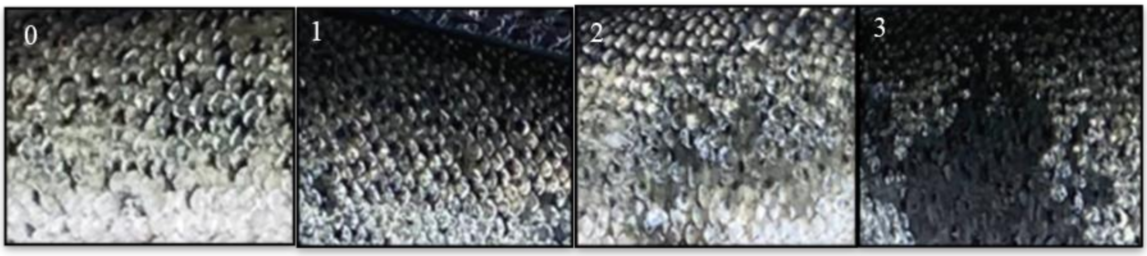

Figure 9 Welfare score of scale loss of rainbow trout (Oncorhynchus mykiss) (Formanowicz & Sumeng, 2022).

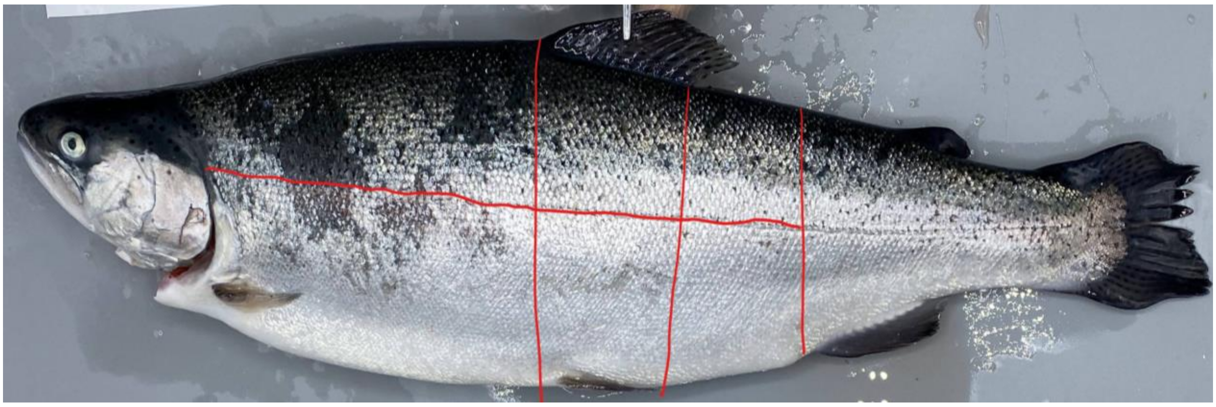

Figure 10 Matrix for evaluation of scale loss of rainbow trout (Oncorhynchus mykiss).

3.5. Calculations

$$Condition\ factor\ (CF): \frac{Body\ weight\ (g)}{Body\ length (cm)^3} x\ 100$$

$$Gutted\ yield: \frac{Gutted\ body\ weight\ (g)}{Body\ weight\ (g)} x\ 100\%$$

$$Fillet\ yield: \frac{Fillet\ weight\ (g)}{Body\ weight\ (g)} x\ 100\ \%$$

$$Hepatosomatic\ index\ (HSI): \frac{Liver\ weight\ (g)}{Body\ weight\ (g)} x\ 100\%$$

$$Cardio\ somatic\ index\ (CSI): \frac{Heart\ weight\ (g)}{Body\ weight\ (g)} x\ 100\%$$

3.6. Statistics

Statistical analyses ANOVA were performed using the SAS software package (SAS Institute, Cary, NC, USA; version 9.4). Significant differences among means of dietary treatment were ranked by pdiff and Duncans multiple range test. Data were corrected for systematic effects of gender and body weight, when unbalanced, and for farming location. The level of significance was set to 5% ($P \leq 0.05$).

4. Results

4.1. Biometric traits

Development in body weight of Atlantic salmon (figure 11) fed with control or test feed was similar from December 2020 and until August 6th in 2021. In January, the salmon fed with test feed had a significantly higher body weight (P≤0.05) compared to fish fed with control feed, respectively 6616±148 and 5542±227 grams in mean body weight. Fish farmed at Litle Lunnøy had significant higher (P≤0.05) mean body weight compared to fish farmed at Krigsholmen from December 2020 until August 2021, when trial at Litle Lunnøy was ended. The exception was on August 6th, when only fish from Litle Lunnøy were weighted.

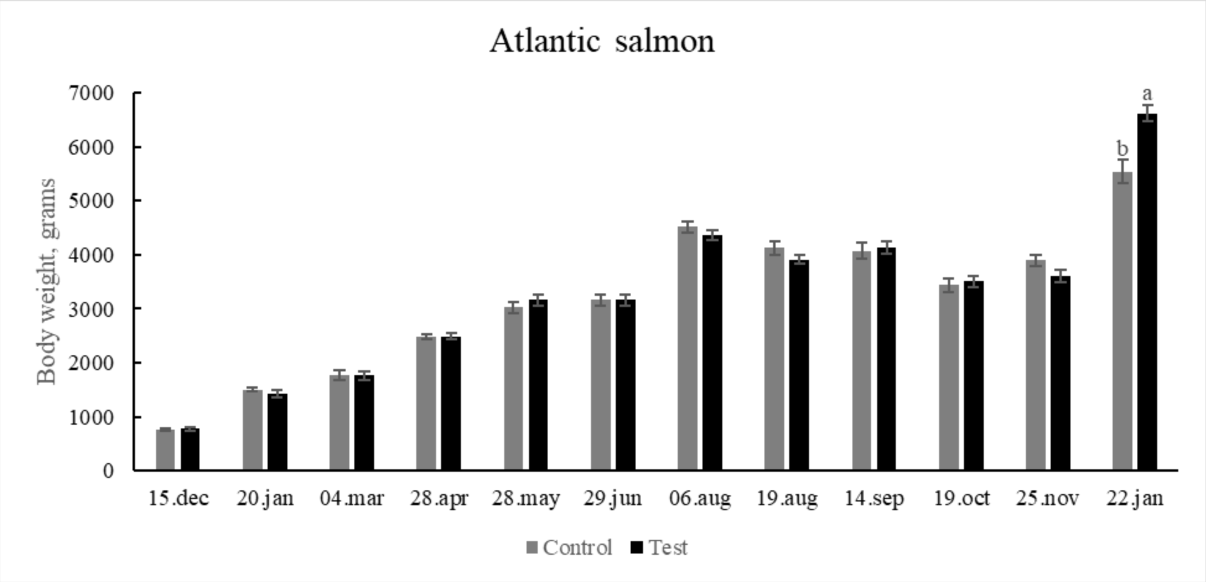

Figure 11 Development in body weight in Atlantic salmon fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima). Different letters above error bars indicate significant effect of diet (P≤0.05) within sampling point.

The body weight of the rainbow trout (figure 12) fed with control feed and test feed increased respectively from 457±16 and 485±19 grams in June 2021 to 3357±48 and 3151±44 grams in December the same year. The control and the test fish had similar body weight increases in June and July. From August and until the end of the experiment in December rainbow trout fed with control feed had a significantly higher body weight compared to fish fed with test feed (P≤0.05).

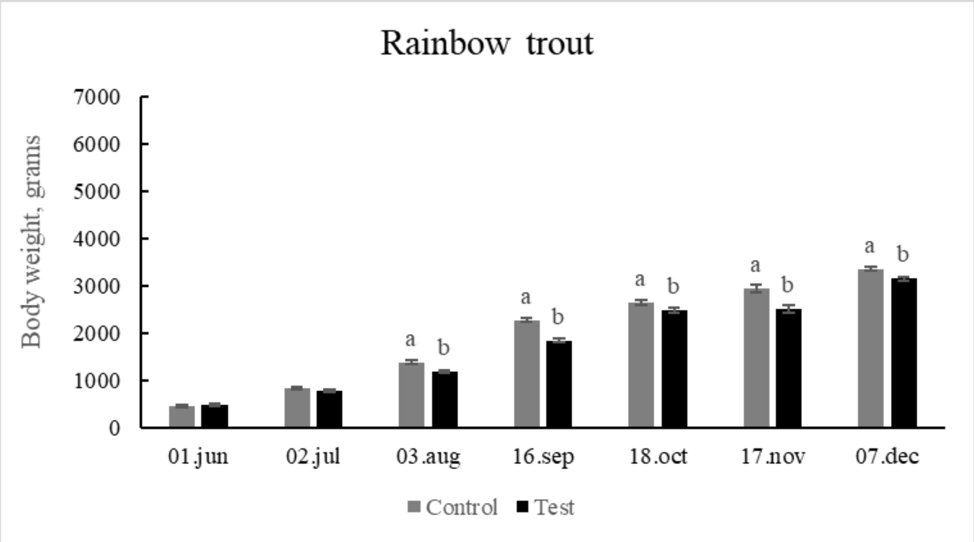

Figure 12 Development in body weight in rainbow trout fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima). Different letters above error bars indicate significant effect of diet (P≤0.05) within sampling point.

Condition factor

Fish fed with test feed had significant higher ($P \leq 0.05$) condition factor compared to fish fed with control feed in April 2021, on August 19th 2021 and in January 2022 (figure 13). In November 2021 fish fed with control feed had significant higher ($P \leq 0.05$) condition factor compared to fish fed with test feed. For the rest of the period development in condition factor was similar in both fish groups. Fish farmed at Litle Lunnøy had significant higher ($P \leq 0.05$) CF compared to fish farmed at Krigsholmen from December 2020 until June 2021, except in March 2021 when CF was similar at both farming locations. On August 19th condition factor at both farming location were similar, with higher condtion factor at Litle Lunnøy ($P=0.07$).

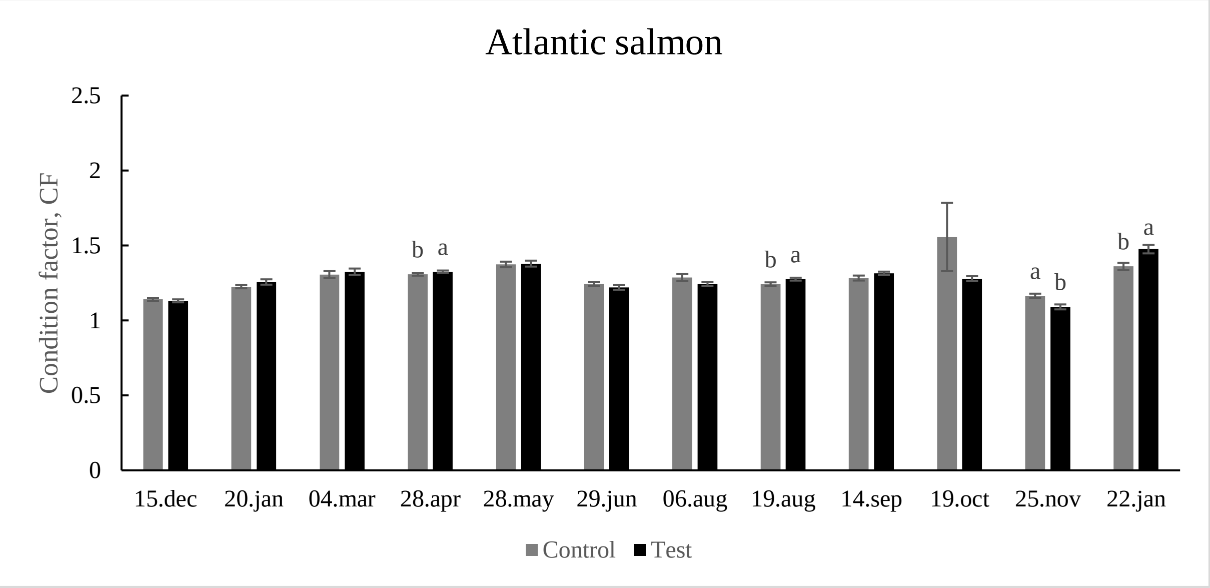

Figure 13 Development in condition factor (CF) in Atlantic salmon fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima). Different letters above error bars indicate significant effect of diet (P≤0.05) within sampling point.

The condition factor of rainbow trout showed no significant difference between fish fed with control feed and fish fed with test feed in June, July and August 2021 (figure 14). In September, October and November the same year fish fed with control feed had significant higher ($P \leq 0.05$) condition factor compared to fish fed with test feed ($P \leq 0.05$). The final sampling showed no significant difference in condition factor between the two groups, and the mean value was respectively 2.1 ±0.0 and 2.0±0.0 for the control and test group.

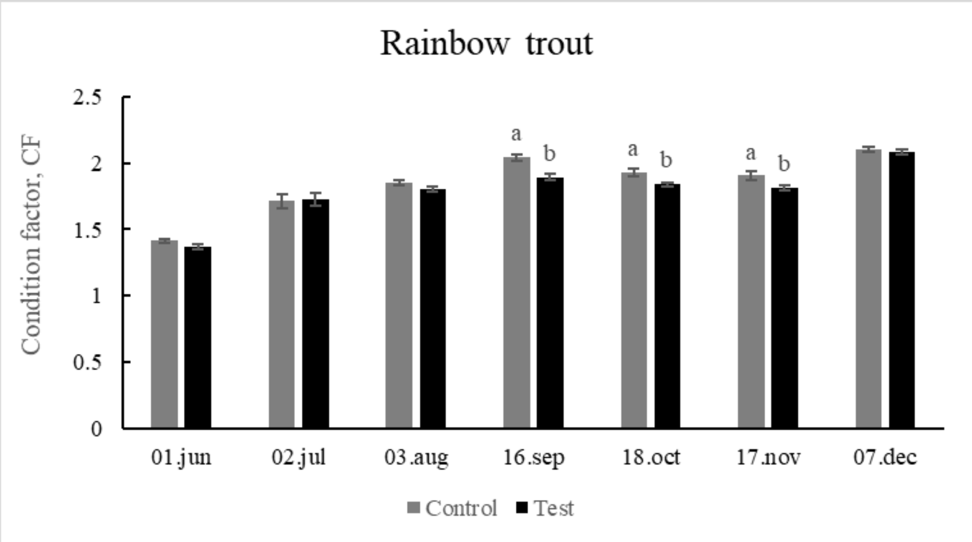

Figure 14 Development in condition factor (CF) in rainbow trout fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima). Different letters above error bars indicate significant effect of diet (P≤0.05) within sampling point.

Development in gutted weight, gutted yield, fillet weight and fillet yield of Atlantic salmon (table 5) showed similar results at all three samplings dates for fish fed with control feed and fish fed with test feed.

Table 5 Development of gutted weight, gutted yield, fillet weight and fillet yield in Atlantic salmon fed with commercial control feed and test feed with addition of macro algae (Saccharina latissima).

		Atlantic salmon		
		30.apr	**16.aug**	**19.jan**
Gutted weight, grams	**Control**	2021 ± 140	4079 ± 203	4828 ± 572
	Test	2092 ± 155	3606 ± 191	5031 ± 398
Gutted yield, %	**Control**	87.3 ± 0.7	90.4 ± 0.3	88.5 ± 0.7
	Test	88.1 ± 0.3	89.0 ± 0.4	87.2 ± 0.4
Fillet weight, grams	**Control**	733 ± 52	1445 ± 76	3484 ± 446
	Test	759 ± 61	1292 ± 65	3530 ± 274
Fillet yield, %	**Control**	64.3 ± 0.7	64.2 ± 0.7	63.3 ± 0.8
	Test	64.3 ± 0.5	64.0 ± 0.9	61.2 ± 1.0

Development in gutted weight of rainbow trout (table 6) showed similar results for fish fed with control feed and fish fed with test feed at sampling in June and December. In September fish fed with control feed had significant ($P \leq 0.05$) higher gutted weight compared to fish fed with test feed. The mean gutted weight in grams were 1928±102 and 1631±65 for fish fed with control feed and fish fed with test feed respectively (table 6).

Development in fillet weight of rainbow trout showed similar results for fish fed with control feed and fish fed with test feed at sampling in June and December. In September fish fed with control feed had significantly ($P \leq 0.05$) higher fillet weight compared to fish fed with test feed.

Rainbow trout fed with control feed and test feed had similar development in fillet yield and gutted yield at all three samplings times (table 6).

Table 6 Development of gutted weight, gutted yield, fillet weight and fillet yield in rainbow trout fed with commercial control feed and test feed with addition of macro algae (Saccharina latissima). Different letters indicate significant effect of diet (P≤0.05) within sampling point.

		Rainbow trout		
		01.jun	**16.sep**	**07.dec**
Gutted weight, grams	**Control**	351 ± 37	1928 ± 102 [a]	3099 ± 115
	Test	387 ± 34	1631 ± 65 [b]	3040 ± 151
Gutted yield, %	**Control**	87.9 ± 0.9	86.4 ± 0.5	84.0 ± 0.6
	Test	89.1 ± 0.6	86.1 ± 0.5	84.9 ± 0.5
Fillet weight, grams	**Control**	118 ± 15	730 ± 39 [a]	1140 ± 38
	Test	129 ± 14	609 ± 26 [b]	1158 ± 64
Fillet yield, %	**Control**	59.6 ± 1.9	65.3 ± 0.7	62 ± 1.5
	Test	60.4 ± 2.8	63.7 ± 0.7	64 ± 0.7

4.2. Quality traits

Development in fillet colour of Atlantic salmon (figure 15) show similar SalmoFan score in April and January. Fish fed with control feed had a significantly (P≤0.05) higher SalmoFan score in August compared to fish fed with test feed. SalmoFan score was respectively 25.5±0.2 and 24.7±0.2 for fish fed with control feed and fish fed with test feed.

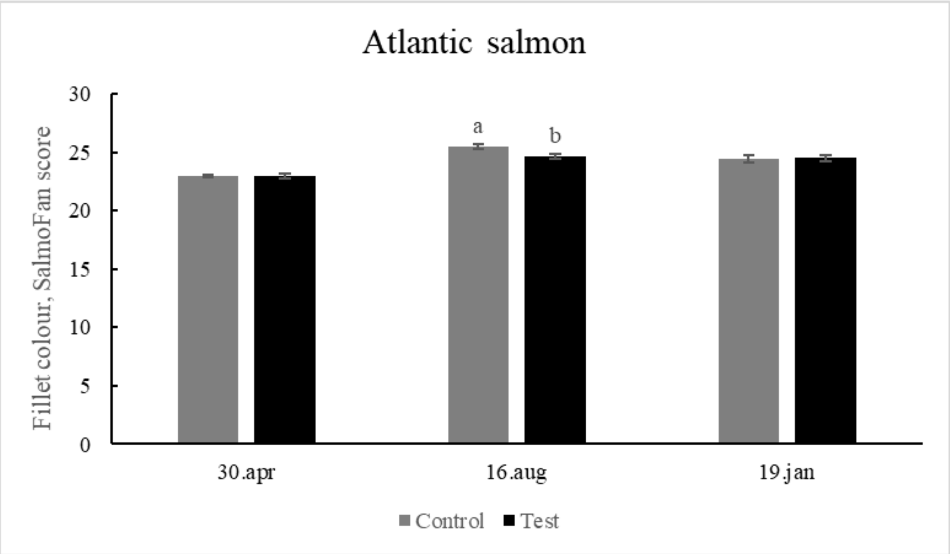

Figure 15 Development in fillet colour of Atlantic salmon fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima). Different letters above error bars indicate significant effect of diet (P≤0.05) within sampling point.

Rainbow trout fed with control feed and test feed had similar development in fillet colour at all three samplings dates (figure 16).

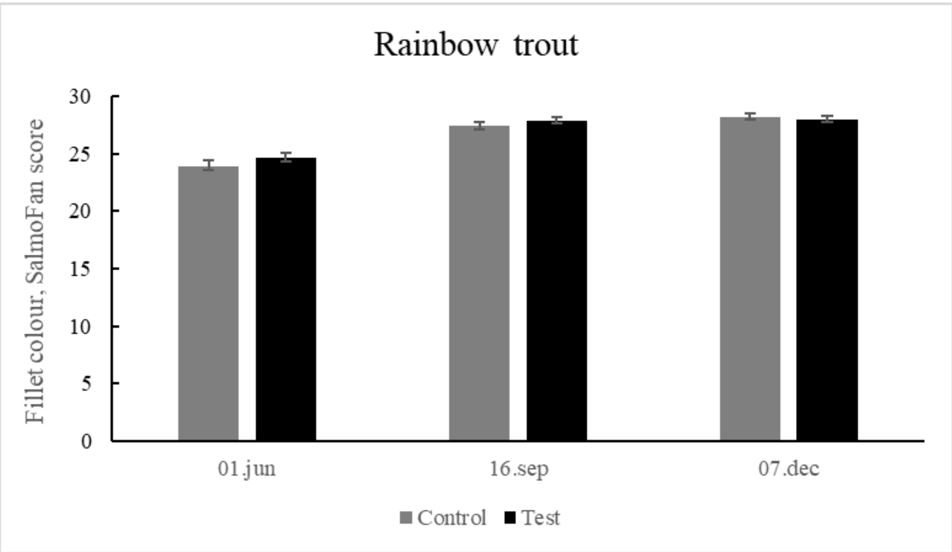

Figure 16 Development in fillet colour of rainbow trout) fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima).

Development in fillet gaping of Atlantic salmon showed similar development in April and August (table 7). Salmon fed with control feed had significantly higher mean fillet gaping score (P≤0.05) compared to fish fed with test feed in January.

Table 7 Development in fillet gaping in Atlantic salmon fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima). Different letters indicate significant effect of diet (P≤0.05) within sampling point.

Atlantic salmon

		30.apr		16.aug		19.jan	
	Diet	**Mean ±**	**S.E.**	**Mean ±**	**S.E.**	**Mean ±**	**S.E.**
Fillet gaping	**Control**	0.06 ±	0.04	0.84 ±	0.23	0.63 ±	0.26 [a]
	Test	0.16 ±	0.06	0.77 ±	0.23	0.00 ±	0 [b]

Development in fillet gaping of rainbow trout showed similar results for fish fed with control feed and fish feed with test feed at samplings in April, August, and January (table 8).

Table 8 Development in fillet gaping in rainbow trout fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima).

Rainbow trout

		01.jun		16.sep		07.dec	
	Diet	**Mean ±**	**S.E.**	**Mean ±**	**S.E.**	**Mean ±**	**S.E.**
Fillet gaping	**Control**	0.50 ±	0.20	0.00 ±	0.00	0.00 ±	0.00
	Test	0.38 ±	0.16	0.00 ±	0.00	0.30 ±	0.15

Development in fillet texture in Atlantic salmon showed similar texture in April and January (figure 17). Salmon fed with test feed had significant higher (P≤0.05) mean texture score (softer fillets) in August compared to fish fed with control feed. Texture score was respectively 1.2±0.1 and 1.5±0.1 for fish fed with control feed and for fish fed with test feed.

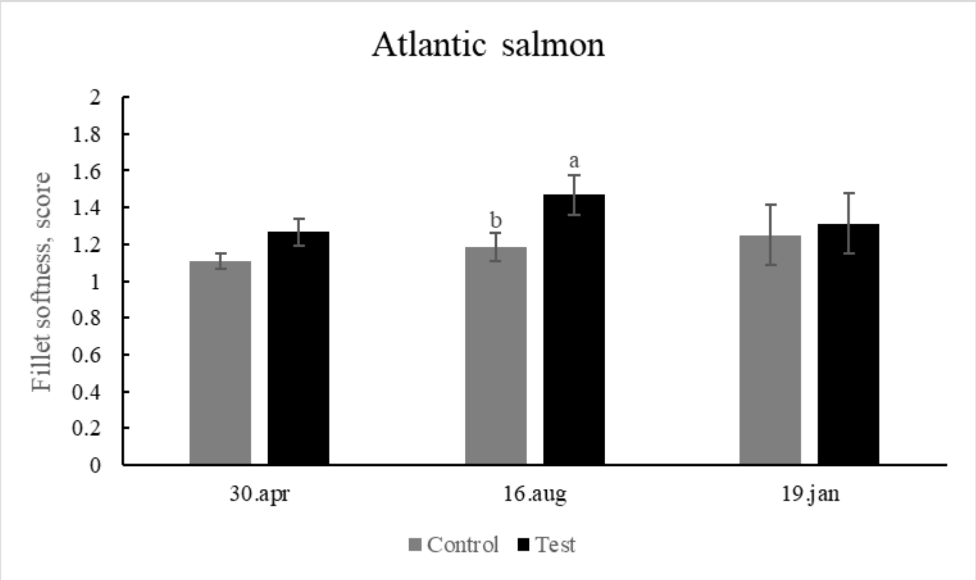

Figure 17 Development in fillet softness (score 1-4) in Atlantic salmon fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima). Different letters above error bars indicate significant effect of diet (P≤0.05) within sampling point.

Rainbow trout fed with control feed and test feed had similar development in fillet texture at all three samplings dates (figure 18).

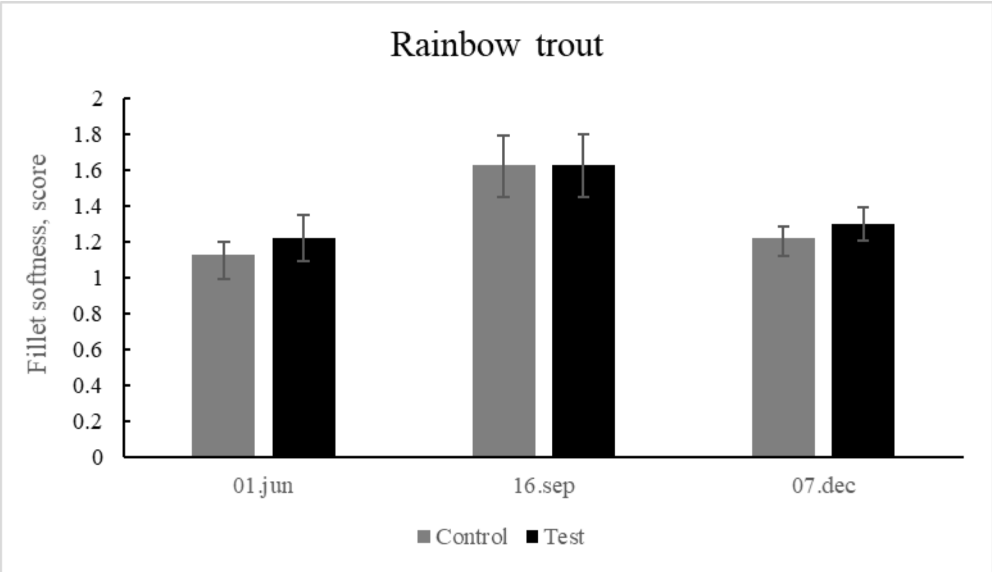

Figure 18 Development in fillet softness (score 1-4) in rainbow trout fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima).

4.3. Fish welfare

Development of fat accumulation around viscera and heart of Atlantic salmon (table 9) showed similar mean score at all three samplings times. There was no difference in liver score of salmon at sampling in April and January, but in August fish fed with test fish had significantly darker liver ($P \leq 0.05$) compared to fish fed with control feed; fish fed with control feed had a mean liver score of 2.8±0.2 and fish fed with test feed have a mean liver score 3.3±0.2 (table 9). Fish fed with test feed had significantly higher hepatosomatic index compared to fish fed with control feed in April. In August and January there was similar development in hepatosomatic index in both fish groups. Results for cardio somatic index (CSI) showed significantly higher CSI in fish fed with control feed in August, while in April and January fish fed with control feed and fish fed with test feed had similar development in CSI.

Table 9 Development in visceral (score 1-4), cardiac fat accumulation (score 0-3), liver colour (score 1-5) and hepatosomatic and cardio somatic index in Atlantic salmon fed with commercial control feed and test feed with addition of macro algae (Saccharina latissima). Different letters indicate significant effect of diet ($P \leq 0.05$) within sampling point.

Atlantic salmon

		30.apr	16.aug	19.jan
	Diet	**Mean ± S.E.**	**Mean ± S.E.**	**Mean ± S.E.**
Viscera	**Control**	2.77 ± 0.09	3.16 ± 0.17	2.88 ± 0.13
	Test	2.80 ± 0.10	3.16 ± 0.13	2.38 ± 0.18
Liver	**Control**	2.47 ± 0.10	2.84 ± 0.17 [b]	2.50 ± 0.27
	Test	2.73 ± 0.08	3.28 ± 0.17 [a]	2.00 ± 0.33
Heart	**Control**	0.13 ± 0.06	1.09 ± 0.18	0.50 ± 0.27
	Test	0.27 ± 0.09	0.81 ± 0.17	0.63 ± 0.18
HSI, %	**Control**	1.19 ± 0.02 [b]	0.88 ± 0.02	1.37 ± 0.04
	Test	1.27 ± 0.02 [a]	0.92 ± 0.05	1.37 ± 0.04
CSI, %	**Control**	0.14 ± 0.00	0.13 ± 0.00 [a]	0.12 ± 0.00
	Test	0.14 ± 0.01	0.10 ± 0.01 [b]	0.14 ± 0.01

Fat accumulation around liver and heart in rainbow trout (table 10) showed similar mean scores at all three samplings times. Results of viscera fat accumulation showed similar amount of fat in June and September, but at the final sampling in December fish fed with control feed had significantly (P≤0.05) higher fat content around viscera compared to fish fed with test feed.

Development in hepasomatic (HSI) and cardiosomatic (CSI) index was similar at all three samplings times in rainbow trout (table 10).

Table 10 Development in visceral (score 1-4), cardiac fat accumulation (score 0-3), liver colour (score 1-5) and hepatosomatic and cardio somatic index in rainbow trout fed with commercial control feed and test feed with addition of macro algae (Saccharina latissima). Different letters indicate significant effect of diet (P≤0.05) within sampling point.

Rainbow trout

		01.jun	16.sep	07.dec
	Diet	**Mean ± S.E.**	**Mean ± S.E.**	**Mean ± S.E.**
Viscera	**Control**	1.78 ± 0.15	3.59 ± 0.09	3.19 ± 0.13 [a]
	Test	1.69 ± 0.15	3.43 ± 0.10	2.73 ± 0.15 [b]
Liver	**Control**	2.94 ± 0.16	2.94 ± 0.15	2.72 ± 0.18
	Test	2.75 ± 0.12	2.75 ± 0.14	2.70 ± 0.16
Heart	**Control**	0.13 ± 0.13	0.23 ± 0.17	0.38 ± 0.14
	Test	0.00 ± 0.00	0.00 ± 0.00	0.27 ± 0.19
HSI, %	**Control**	0.90 ± 0.11	1.32 ± 0.06	1.21 ± 0.03
	Test	0.75 ± 0.08	1.33 ± 0.03	1.28 ± 0.05
CSI, %	**Control**	0.16 ± 0.02	0.09 ± 0.00	0.09 ± 0.00
	Test	0.14 ± 0.01	0.09 ± 0.00	0.09 ± 0.00

Atlantic salmon fed with control feed had significantly (P≤0.05) more scale loss compared to fish fed with test feed in April and January. In August there was similar results in scale loss in both fish groups (figure 19).

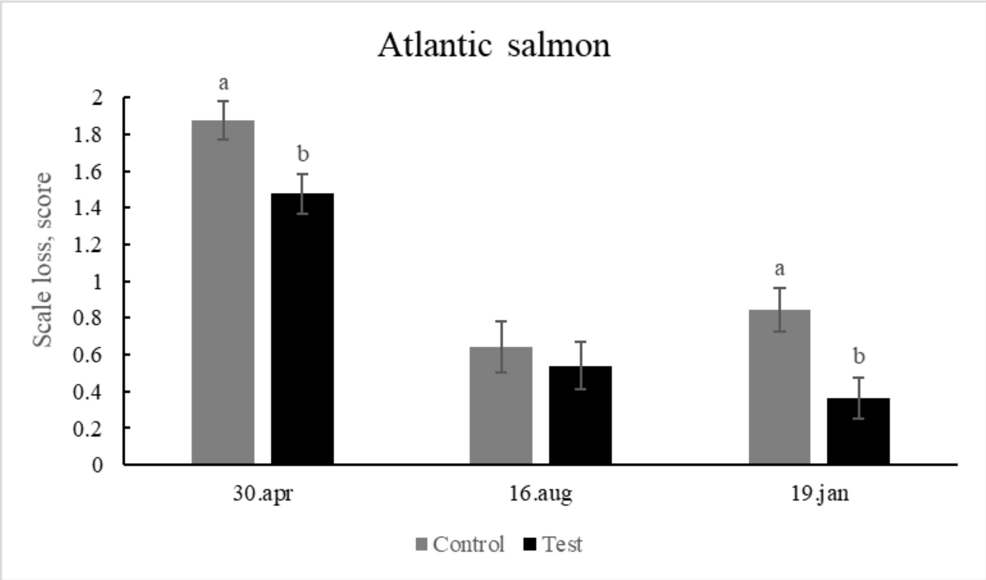

Figure 19 Development in scale loss in Atlantic salmon fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima). Different letters above error bars indicate significant effect of diet (P≤0.05) within sampling point.

Development in scale loss of rainbow trout showed similar results at all three samplings times in fish fed with control feed and test feed. Fish fed with control feed had a mean scale loss of 1.5±0.2, 1.5±0.1 and 0.4±0.1 in April, August and January. The respective results for fish fed with test feed was1.4±0.2, 1.4±0.1 and 0.4± 0.1 (figure 20).

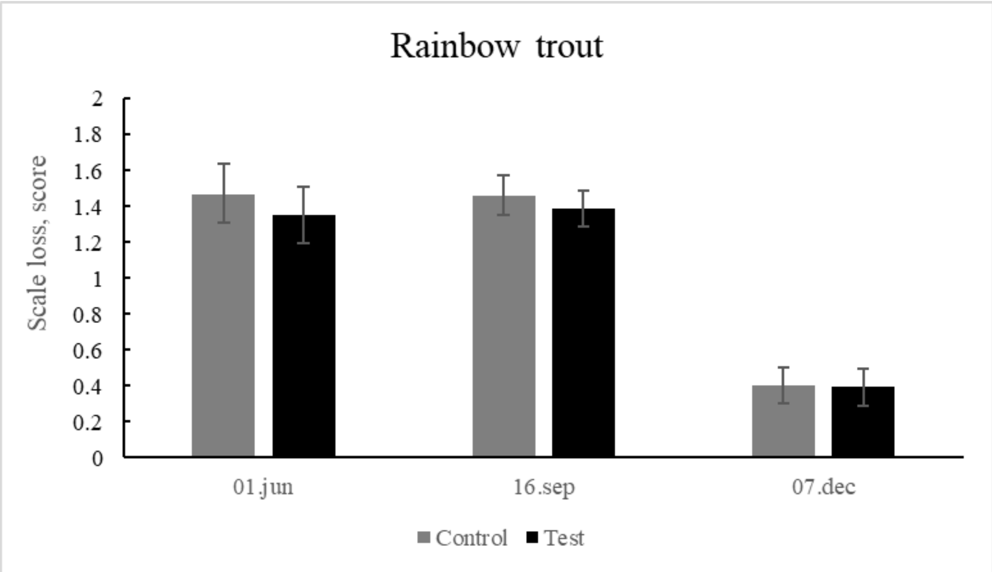

Figure 20 Development in scale loss in rainbow trout fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima).

4.4. Salmon louse

Development in mobile salmon louse on Atlantic salmon showed similar results in December, January, March, August, September, October, and November (table 11). In April fish fed with test feed had significantly more ($P \leq 0.05$) mobile salmon louse compared to fish fed with control feed. In May, June, August, and January fish fed with control feed had significantly more mobile salmon louse compared to fish fed with test feed. There was significant effect ($P \leq 0.05$) of farming location on mobile salmon louse in period from January 2021 to May 2021 and on August 19th 2021.

Atlantic salmon fed with test feed had significant more ($P \leq 0.05$) attached salmon louse in April 28th, compared to fish fed with control feed. The mean attached salmon louse number was respectively 0.70±0.04 and 0.38±0.06. On June 29th, October 19th and January 22nd fish fed with control feed had a significant more attached salmon louse compared to fish fed with test feed. For the rest of the period there was similar development in mean attached salmon louse in fish fed with control feed and fish feed with test feed. There was a significant effect ($P \leq 0.05$) of farming location on attached salmon louse in December 2020, April 2021, and May 2021.

Salmon fed with test feed had significant more ($P \leq 0.05$) sexual mature salmon louse on December 15th, August 6th, September 14th and October 19th compared to fish fed with control feed. On June 29th, August 19th and January 22nd fish fed with control feed had significant more sexual mature salmon louse compared to fish fed with test feed. For the rest of the period there was similar development in mean number of sexual mature salmon louse in those two feed groups. There was a significant effect of farming location on sexual mature salmon louse in January 2021, March 2021, April 2021, June 2021 and August 19th 2021.

Table 11 Development in mobile, attached, and sexual mature salmon louse on Atlantic salmon fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima). Different letters indicate significant effect of diet (P≤0.05) within sampling point.

	Mobile		Attached		Sexual mature	
Date	**Control**	**Test**	**Control**	**Test**	**Control**	**Test**
15.dec	0.50 ± 0.06	0.61 ± 0.07	0.02 ± 0.01	0.03 ± 0.01	0.20 ± 0.04 b	0.44 ± 0.06 a
20.jan	1.36 ± 0.11	1.57 ± 0.15	0.12 ± 0.03	0.14 ± 0.04	0.14 ± 0.03	0.12 ± 0.03
04.mar	3.36 ± 0.33	3.34 ± 0.29	0.03 ± 0.02	0.01 ± 0.01	0.66 ± 0.09	0.71 ± 0.09
28.apr	1.37 ± 0.09 b	1.79 ± 0.21 a	0.38 ± 0.04 b	0.70 ± 0.06 a	0.12 ± 0.02	0.09 ± 0.02
28.may	8.05 ± 0.39 a	7.04 ± 0.41 b	2.94 ± 0.22	2.97 ± 0.28	0.48 ± 0.10	0.41 ± 0.08
29.jun	1.78 ± 0.14 a	0.79 ± 0.09 b	0.48 ± 0.06 a	0.29 ± 0.05 b	2.38 ± 0.25 a	0.73 ± 0.12 b
06.aug	2.16 ± 0.16	2.59 ± 0.19	0.01 ± 0.01	0.06 ± 0.03	1.08 ± 0.13 b	1.58 ± 0.18 a
19.aug	2.81 ± 0.17 a	2.36 ± 0.12 b	0.11 ± 0.05	0.09 ± 0.03	1.81 ± 0.16 a	1.40 ± 0.12 b
14.sep	7.74 ± 0.38	7.21 ± 0.32	0.08 ± 0.05	0.02 ± 0.02	6.10 ± 0.39 b	7.54 ± 0.44 a
19.oct	9.58 ± 0.72	10.03 ± 0.60	0.40 ± 0.13 a	0.00 ± 0.00 b	4.30 ± 0.37 b	7.73 ± 0.52 a
25.nov	0.30 ± 0.09	0.13 ± 0.06	0.00 ± 0.00	0.03 ± 0.03	0.10 ± 0.06	0.00 ± 0.00
22.jan	2.19 ± 0.21 a	1.62 ± 0.19 b	0.35 ± 0.08 a	0.06 ± 0.04 b	1.13 ± 0.14 a	0.30 ± 0.07 b

Development in number of mobile salmon louse on rainbow trout showed similar results over the whole growing period except from December 7th (table 12). The results showed significantly higher ($P \leq 0.05$) number of mobile salmon louse on fish fed with test feed in December 7th, compared to fish fed with control feed.

The results showed similar development in mean number of attached salmon louse in rainbow trout at all louses counting points except from October 18th and December 7th. In October 18th fish fed with control feed had significantly higher ($P \leq 0.05$) number of attached salmon louse compared to fish fed with test feed. In December 7th fish fed with test feed had significantly higher number of attached salmon louse compared to fish fed with control feed.

There was similar development in mean number of sexual mature salmon louse in rainbow trout at all counting times except of October 18th. On October 18th fish fed with control feed had significant more ($P \leq 0.05$) sexual mature salmon louse compared to fish fed with test feed (table 12).

Table 12 Development in mobile, attached, and sexual mature salmon louse in rainbow trout fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima). Different letters indicate significant effect of diet ($P \leq 0.05$) within sampling point.

	Mobile		Attached		Sexual mature	
Date	Control	Test	Control	Test	Control	Test
01.jun	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
02.jul	0.85 ± 0.13	0.29 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.24 ± 0.05	0.04 ± 0.02
03.aug	1.09 ± 0.11	1.00 ± 0.12	0.01 ± 0.01	0.00 ± 0.00	0.57 ± 0.08	0.36 ± 0.06
16.sep	2.84 ± 0.25	2.06 ± 0.16	0.16 ± 0.05	0.13 ± 0.04	0.52 ± 0.10	0.44 ± 0.07
18.oct	4.48 ± 0.38	4.41 ± 0.38	4.39 ± 0.21 a	3.58 ± 0.18 b	1.38 ± 0.13 a	0.77 ± 0.08 b
17.nov	0.86 ± 0.14	0.18 ± 0.04	3.98 ± 0.31	3.75 ± 0.30	0.31 ± 0.09	0.14 ± 0.04
07.dec	12.10 ± 0.23 b	13.03 ± 0.20 a	2.27 ± 0.10 b	3.26 ± 0.14 a	2.07 ± 0.12	1.96 ± 0.10

Development in total number of salmon louse on Atlantic salmon (figure 21) showed significantly more (P≤0.05) salmon louse on fish fed with test feed compared to fish fed with control feed in December 2020, April 2021, August 6th 2021 and October 2021. In June 2021, August 19th 2021 and January 2022 fish fed with control feed had significant more (P≤0.05) salmon louse compared to fish fed with test feed. For the rest of the on-growing period total number of salmon louse was similar in these two fish groups.

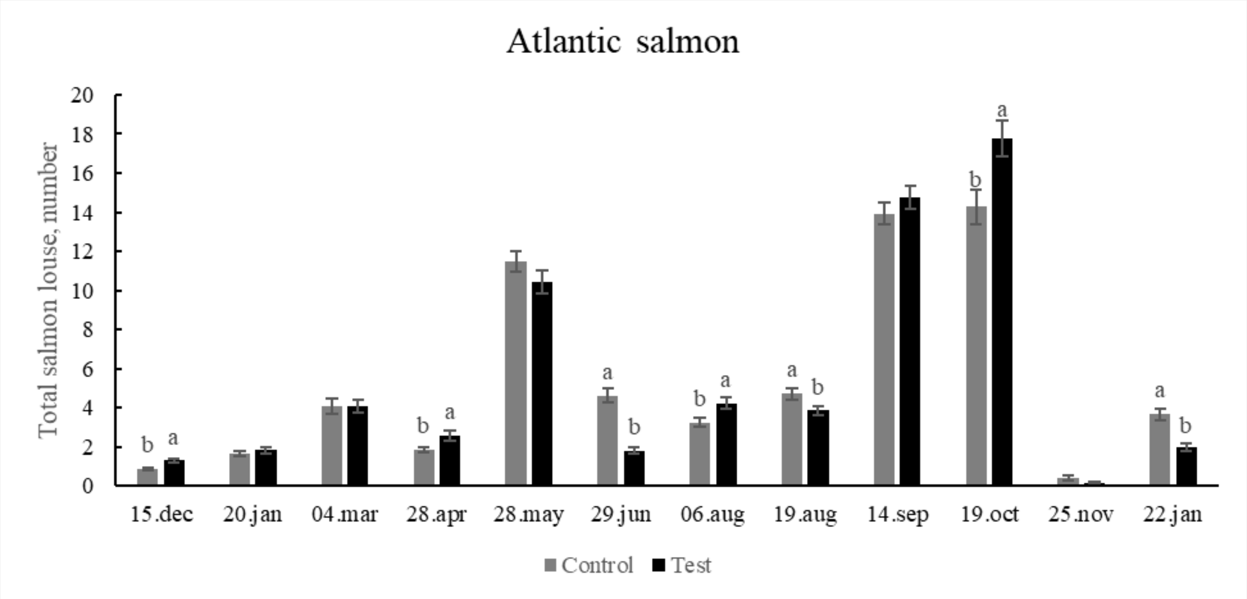

Figure 21 Development in total mean number of salmon louse in Atlantic salmon fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima) Different letters above error bars indicate significant effect of diet (P≤0.05) within sampling point.

Development in total number of salmon louse on rainbow trout showed similar results for fish fed with control feed and test feed from June 2021 until September 2021 (figure 20). In October and November 2021 fish fed with control feed had significantly more ($P \leq 0.05$) salmon louse compared to fish fed with test feed. At the final louse counting fish fed with test feed had significant more ($P \leq 0.05$) total salmon louse compared to fish fed with control feed.

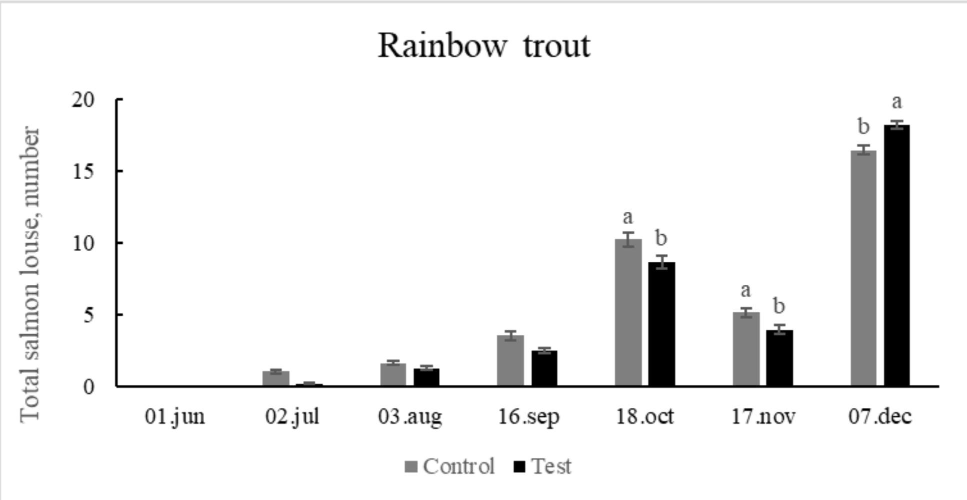

Figure 22 Development in total number of salmon louse in rainbow trout fed with commercial control feed or commercial feed with inclusion of macro algae (Saccharina latissima). Different letters above error bars indicate significant effect of diet (P≤0.05) within sampling point.

5. Discussion

5.1. Biometric traits

Body weight

The body weight of the Atlantic salmon (figure 11) showed a similar development for both feed groups throughout the growing period, except the last weighting before harvesting, where fish fed with test feed were 19% heavier compared to fish fed with control feed (6.6 kg vs. 5.5 kg). The results indicate no effect of feed with inclusion of macro algae in development of body weight of Atlantic salmon during the early farming phase, but a significantly enhanced weight increase towards harvesting. The growth rate differed between the farming locations.

Although results were adjusted for systematic effects of farming location in the statistical model, it is possible that environmental effects interrupted the interpretation of dietary effects.

It is well known that sea temperature affects the growth rate of salmon (Austreng et al., 1987). However, in the present study it is expected that other factors than temperature cause the growth differences.

The results for rainbow trout (figure 12) showed that fish fed with test feed had a consistent lower body weight compared to fish fed with control feed during the last four months prior harvesting. The results thus show that rainbow trout and salmon responded differently to inclusion of macro algae inclusion in the feed. Similar result was observed in fish trial with rainbow trout performed by Granby et al. (2020) with approximately 10 times higher sugar kelp inclusion (4% dry weight of the feed). When the inclusion level was 1% and 2% of sugar kelp, body weight was similar for fish fed with control feed, 1% macro algae inclusion and 2% macro algae inclusion. In addition, Granby et al. (2020) used a sugar kelp meal, while in the present trial sugar kelp was fermented with soybean meal. This may indicate that the sugar kelp inclusion should be used carefully in the future trials to eliminate negative effect on body weight in rainbow trout. It is however possible, that the rainbow trout responded negatively to the fermented soybean used in the present study.

Condition factor

At three measurement times, April, August and January, Atlantic salmon fed with test feed had significantly higher CF compared to fish fed with control feed. In November fish fed with control feed had higher CF compared to fish fed with test feed. At the remaining measurement times the development in CF was similar in both diet groups (figure 13). The condition factor development varied throughout the trial, but for most of the time fish fed with control feed and test feed have similar development in CF. Because of the inconsistent variation between fish groups, it is difficult to conclude although the results indicate enhanced voluminous body shape by supplementing the diet with fermented soybean with inclusion of sugar kelp at three sampling points, including harvesting.

Rainbow trout fed with control feed had significantly higher CF compared to fish fed with test feed in September, October, and November (figure 14). Hence, the condition factor showed a trend where fish fed with control feed had higher CF compared to fish fed with test diet.

The results for body weight were also significantly higher for fish fed with control feed at the same sampling points. For the rest of the growing period CF development were similar for both diet groups.

Rainbow trout had a higher CF compared to Atlantic salmon; this is expected since there is a difference in body shape of these two farmed species. Atlantic salmon has more slim body shape, while rainbow trout has shorter, more compact body shape.

Gutted weight, gutted yield, fillet weight and fillet yield

The results for production traits gutted weight, gutted yield, fillet weight and fillet weight in Atlantic salmon showed no effect of diet (table 5). The results for rainbow trout were similar except in September (table 6), where fish fed with control feed had significantly higher gutted weight and fillet weight compared to fish fed with test feed. There were similar results in all four traits for the rest of the observation time.

5.2. Quality traits

Fillet colour

The fillet colour in Atlantic salmon fed control feed was 23.0±0.2, 25.5±0.2 and 24.4±0.3 in respectively April, August, and January. Mean fillet colour in Atlantic salmon fed feed with inclusion of macro algae was 24.7±0.2 in August, in April and January the fillet colour in test fish was similar compared to fish fed with control feed (figure 15). The increase in mean Salmofan colour score from April to August and January was expected, as inclusion of astaxanthin in the fish feed increased in the final growth period.

The development in fillet colour of rainbow trout was similar in June, September, and December (figure 16). The increase in SalmonFan score was similar compared to Atlantic salmon.

The results showed no clear trend in effect of macro algae inclusion in Atlantic salmon and rainbow trout diet, although test feed resulted in paler colour of salmon sampled in August.

Fillet gaping

There were similar results in fillet gaping in Atlantic salmon in April and August, but in January fish fed with control feed had significant more fillet gaping compared to fish fed with test feed. The results showed low (0.63±0.26) fillet gaping in fish fed with control feed in January, while fish fed with test feed had mean value of 0.0 fillet gaping score (table 7). Rainbow trout had similar low fillet gaping score in both feed groups at all three samplings times (table 8). In general, both fish species had low occurrence of fillet gaping, but in Atlantic salmon fish fed with control feed tended to have more filet gaping.

Fillet texture

Atlantic salmon fed with test feed had significant softer fillet texture compared to fish fed with control feed in August (figure 17). In April and January, the fillet texture was similar in fish fed with both diets. Similar quality development was seen in fillet colour (figure 15).

There was no significant effect of diet in fillet texture of rainbow trout during all three samplings times (figure 18).

5.3. Fish welfare

Fat accumulation and organ index

In general, the development in fat accumulation in Atlantic salmon was similar for both diet groups (table 9). Only in August fish fed with test feed had significantly darker livers compared to fish fed with control feed. This may indicate less fat accumulation in the liver of the test fish in August.

In rainbow trout the fat accumulation was similar in both diet groups, except visceral fat in December. Fish fed with control feed had significantly paler liver compared to fish fed with test feed in December.

The feed composition of control feed and test feed were similar, with the similar amount and source of fat in both diet groups. Hence the higher score for visual fat accumulation around the viscera of rainbow trout and the paler livers in salmon indicate that test ingredients reduced the lipid deposition.

Scale loss

Atlantic salmon fed with control feed had significant higher scale loss compared to fish fed with test feed in April and January. The scale loss was highest in April for both diet groups (figure 19).

Rainbow trout fed with control feed and rainbow trout fed with test feed had similar development in scale lose during the trial. The scale lose was similar in June and September, whereas the final analysis in December showed lowest scale loss in both diet groups (figure 20).

Both species had most scale loss during spring in the early on-growing stage. This can indicate that smaller fish are more vulnerable forwards scale loss in early sea-phase. Sissener et al. (2021) studied the effect of differences in sea water during transition from fresh water to sea water without previous acclimatisation. The results showed that fish in the coldest water were more vulnerable towards loss of scale and mucus, and wounds formation during the first 6 weeks after transfer.

Fish welfares were scored by visual analysis from pictures. The pictures were taken during quality analysis one week after slaughter. Slaughter, bleeding and gutting of fish can cause scale loss. Preferable pictures for welfare should be taken before any fish handling at sea site and storage. Scale loss analysis is visual and thus subjective. To eliminate subjective bias of the analysis and fish handling, digital analysis with use of artificial intelligence (AI) and/or underwater cameras should be considered in future studies.

5.4. Salmon louse

Mobile salmon louse

During the trial period of Atlantic salmon, the number of mobile salmon louse varied in both diet groups, with the highest number of mobile salmon louse counted in October (9.6-10). While the lowest number was registered in November (0.1-0.3). The differences in mobile salmon louse between October and November may be a result of successful delousing and emergency slaughter of two sea cages. The trend showed similar results for the two diet groups at most louse counting times. Salmon fed the test diet had significantly lower amount of mobile salmon louse in May, June, August (19^{th}) and January. In April fish fed with test feed had significant ($P \leq 0.05$) more mobile salmon louse compared to fish fed with control feed (table 11).

Development of mobile salmon louse in rainbow trout (table 12), showed a trend of higher number of mobile salmon louse in fish fed with control feed compared to fish fed with test feed. However, last salmon louse counting in December showed significant higher number of mobile salmon louse in fish fed with test feed compared to fish fed with control feed.

Attached salmon louse

The number of attached salmon louse in Atlantic salmon was similar at most louse counting times, except in April, June, October and January. In April fish fed with test feed had significant higher number of attached salmon louse compared to fish fed with control feed, while in June, October and January fish fed with control feed had significant higher number of attached salmon louse compared to fish fed with test feed.

Rainbow trout had low numbers of attached salmon louse from June to September (table 12). From October there was observed a rise in number of attached salmon louse in both diet groups. Fish fed with control feed had significant higher number of attached salmon louse compared to fish fed with test feed in October. In November there was a similar development in number of salmon louse, while in December fish fed with test feed had significant higher numbers of attached salmon louse compared to fish fed with control feed, respectively 3.3 and 2.3 attached salmon louse per fish.

Sexual mature salmon louse

Sexual mature salmon louse determines the delousing process (Lovdata, 2012). In general numbers of sexual mature salmon louse were high from June until the end of the experiment, except in November. The low number of salmon louse may be caused of the emergency slaughter of two sea cages at Krigsholmen. In December 2020, on August 6th, September and October fish fed with test feed had significant higher number of sexual mature salmon louse compared to fish fed with control feed. While in June, on August 19th and in January fish fed with control feed had significant higher number of sexual mature salmon louse.

Number of sexual mature salmon louse observed on rainbow trout were similar during the trial period except in October. Where fish fed with control feed had significant higher number of sexual mature salmon louse compared to fish fed with test feed (table 12). The number of sexual mature exceeded 0.5 in August, September, October and December.

Total number of salmon louse

Development in total mean number of salmon louse in Atlantic varied throughout the trial period. In December, April, August 6th and October fish fed with test feed had higher mean salmon louse infestation compared to fish fed with control feed (figure 21). While in June, August 19th and January fish fed with control feed had significantly higher mean louse number. For the rest of the trial period the development in mean salmon louse was similar in both diet groups. The results may indicate no effect of diet on salmon louse number. The reason for significant effect may be caused by other factors, e.g. location of the sea cage at the sea location.

Development in total number of salmon louse in rainbow trout showed similar development in the period from June to September, with a trend of more salmon louse in fish fed with control feed. In October and November fish fed with control feed had significantly more salmon louse infestation compared to fish fed with test feed. At last louse counting the trend changed, and fish fed with test feed had significantly more salmon louse compared to fish fed with control feed. The result may indicate that diet with inclusion of macro algae may influence number of salmon louse to a certain number of salmon louse. From November to December the total number of salmon louse in rainbow trout were more than tripled (respectively 5 and 4 in control fish and test fish in November to 16 and 18 in December).

The trial was performed at commercial farming location which decreased the possibility to control equal treatments of all fish in the trial. E.g., delousing could have been performed just before lice counting and biometric trait measurements. This can lead to false, low salmon louse number and decrease in body weight due to starvation and stress factors for the fish.

6. Conclusion

The main finding of the macro algae inclusion was:

- Development in biometric traits of Atlantic salmon fed with control feed and test feed with inclusion of macro algae was similar for most of the traits during the trial period.
- Improved growth of salmon during the period before harvesting, resulting in a 19% higher body weight at harvest (6.6 kg vs. 5.5 kg). Reduced growth of rainbow trout during the last five months before harvesting, resulting in 6.5% lower body weight (3.1 kg vs. 3.4 kg).
- Higher condition factor of salmon at three out of 12 sampling points and lower at one sampling point. Lower condition factor of rainbow trout at three out of seven sampling points.
- A tendency towards less fat accumulation
- No positive effect on fillet colour or texture, but less gaping in salmon
- Less scale loss in salmon, but no effect on scale loss in rainbow trout
- No consistent effect on sea louse infestation, although periods with lower salmon louse numbers

7. Appendix

7.1. Scale loss

Table 13 Development in scale loss in Atlantic salmon fed with commercial feed or commercial feed with inclusion of macro algae (Saccharina latissima). Different letters above error bars indicate significant effect of diet (P≤0.05) within sampling point.

Atlantic salmon

		30.apr	16.aug	19.jan
	Diet	**Mean ± S.E.**	**Mean ± S.E.**	**Mean ± S.E.**
Dorsal 1	Control	1.9 ± 0.1 [b]	0.8 ± 0.3 [a]	1.1 ± 0.3
	Test	1.3 ± 0.2 [a]	0.4 ± 0.2 [b]	0.3 ± 0.2
Dorsal 2	Control	2.0 ± 0.2	0.7 ± 0.3	0.9 ± 0.3 [a]
	Test	1.7 ± 0.2	0.3 ± 0.2	0.0 ± 0.0 [b]
Dorsal 3	Control	2.3 ± 0.2 [a]	0.9 ± 0.3	1.0 ± 0.3
	Test	1.8 ± 0.2 [b]	0.7 ± 0.2	0.5 ± 0.3
Ventral 1	Control	1.8 ± 0.1 [a]	0.3 ± 0.1	0.3 ± 0.2
	Test	1.1 ± 0.1 [b]	0.2 ± 0.1	0.4 ± 0.2
Ventral 2	Control	1.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.2
	Test	1.2 ± 0.1	0.2 ± 0.2	0.5 ± 0.2
Ventral 3	Control	1.2 ± 0.2	0.2 ± 0.1	0.6 ± 0.2
	Test	0.9 ± 0.2	0.4 ± 0.1	0.4 ± 0.2
Tail	Control	2.6 ± 0.1	1.4 ± 0.2	1.7 ± 0.4
	Test	2.5 ± 0.1	1.5 ± 0.3	0.6 ± 0.3
Total	Control	1.9 ± 0.1	0.6 ± 0.1	0.8 ± 0.1 [a]
	Test	1.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1 [b]

Table 14 Development in scale loss in rainbow trout fed with commercial feed or commercial feed with inclusion of macro algae (Saccharina latissima).

Rainbow trout		30.apr			16.aug			19.jan		
	Diet	**Mean**	**±**	**S.E.**	**Mean**	**±**	**S.E.**	**Mean**	**±**	**S.E.**
Dorsal 1	Control	1.9	±	0.3	2.4	±	0.2	0.8	±	0.2
	Test	1.6	±	0.3	2.4	±	0.1	1.0	±	0.3
Dorsal 2	Control	1.5	±	0.3	1.9	±	0.2	0.6	±	0.2
	Test	1.3	±	0.3	2.1	±	0.2	0.7	±	0.2
Dorsal 3	Control	1.6	±	0.3	1.6	±	0.3	0.4	±	0.2
	Test	1.4	±	0.3	1.8	±	0.2	0.8	±	0.2
Ventral 1	Control	1.3	±	0.2	1.3	±	0.2	0.3	±	0.2
	Test	1.5	±	0.2	1.0	±	0.2	0.1	±	0.1
Ventral 2	Control	1.1	±	0.3	0.5	±	0.2	0.1	±	0.1
	Test	1.0	±	0.2	0.2	±	0.1	0.0	±	0.1
Ventral 3	Control	0.8	±	0.3	0.3	±	0.1	0.1	±	0.1
	Test	0.6	±	0.2	0.2	±	0.1	0.0	±	0.1
Tail	Control	2.2	±	0.2	2.1	±	0.1	0.5	±	0.2
	Test	2.0	±	0.2	2.0	±	0.2	0.1	±	0.1
Total	Control	1.5	±	0.2	1.5	±	0.1	0.4	±	0.1
	Test	1.4	±	0.2	1.4	±	0.1	0.4	±	0.1

7.2. Sexual mature salmon louse in Atlantic salmon

Table 15 Development in mean number of sexual mature salmon louse in Atlantic salmon fed with commercial feed or commercial feed with inclusion of macro algae (Saccharina latissima) cage-wise at sea site Krigsholmen and Litle Lunnøy in Western Norway.

Location	**Krigsholmen**				**Litle Ludnøy**			
Diet	**Control 1**	**Control 2**	**Test 1**	**Test 2**	**Control 1**	**Control 2**	**Test 1**	**Test 2**
dec.20	0.6	0.3	0.2	0.5	0.4	0.09	0.5	0.3
jan.21	0.2	0.0	0.0	0.1	0.1	0.23	0.2	
mar.21	0.5	0.3	0.4	0.4	0.6	1.30	1.1	1.0
apr.21	0.0	0.0	0.0	0.0	0.2	0.20	0.2	0.1
may.21	0.8	0.4	0.6	0.4	0.5	0.20	0.4	0.3
jun.21	1.6	0.8	0.0	0.9	6.6	0.48	1.8	0.3
6th aug.21	N/A	N/A	N/A	N/A	0.3	1.90	1.7	1.5
aug.21	2.5	2.7	2.6	2.9	1.5	N/A	N/A	0.1
sep.21	7.1	5.1	9.1	6.0	N/A	N/A	N/A	N/A
okt.21	N/A	4.3	N/A	7.7	N/A	N/A	N/A	N/A
nov.21	N/A	0.1	N/A	0.0	N/A	N/A	N/A	N/A
jan.22	N/A	1.1	N/A	0.3	N/A	N/A	N/A	N/A

7.3. Nephrocalsinosis

Figure 23 Rainbow trout with nephrocalsinosis in kidney.

Figure 24 Rainbow trout with a regular kidney.

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