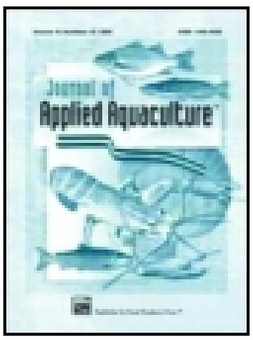

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Sudden increased mortality in large seemingly healthy farmed Atlantic salmon (*Salmo salar* L.) was associated with environmental and dietary changes

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ABSTRACT

Mortality of seemingly healthy farmed Atlantic salmon is a large problem in Norwegian aquaculture, and has been linked to infectious and noninfectious cardiovascular diseases. In this study, an event of sudden mortality of seemingly healthy farmed salmon during the winter period in northern Norway is reported. The experimental fish reared in net-pens were fed two dietary treatments; control and test experimental diets in duplicates. An increased mortality of 6% and 10% was only observed within the two net-pens receiving the test diets. The moribund fish had significantly higher lipid content in the liver, altered liver fatty acid composition, and increased levels of alanine aminotransferase and alkaline phosphatase in the blood plasma compared to non-dying fish. Instant and significant reduction in mortality was observed when the fish fed the test diet were starved. The observed mortality was associated with dietary and environmental changes. Possible mechanism for the increased mortality is discussed.

KEYWORDS

Atlantic salmon; mortality; liver fat; dietary changes; PRV; recruitment of fat cells

Introduction

Mortality of seemingly healthy farmed Atlantic salmon (*Salmo salar* L.) is frequently observed in Norwegian salmonid aquaculture and has been primarily linked both to infectious and noninfectious cardiovascular diseases (Dalum et al. 2017; Hjeltnes et al. 2018; Poppe, Taksdal, and Bergtun 2007). Cardiomyopathy syndrome (CMS) and heart and skeletal muscle inflammation (HSMI) are widespread, contagious, and fatal cardiovascular diseases in Norwegian salmon farming (Hjeltnes et al. 2018), and are associated with piscine myocarditis virus (PMCV) and piscine reovirus (PRV), respectively (Haugland et al. 2011; Løvoll et al. 2010; Palacios et al. 2010). PRV seems to be ubiquitous among farmed salmon in Norway (Løvoll et al. 2012), whereas PMCV seems to be more associated with clinical CMS outbreaks (Wiik-Nielsen et al. 2012). CMS and HSMI may affect and cause mortality among large and seemingly well-fit salmon, and is a great economic burden for the farming industry. In many cases, however, cardiovascular and

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circulatory failure are also observed without viruses present and may represent a significant proportion of the nonspecific mortality in fish farms (Dalum et al. 2017; Tørud and Hillestad 2004). The agents and causes to increased mortality in aquaculture are complex and outbreaks of many disease conditions occur as results of intricate interactions between environmental conditions, metabolic distress, nutritional imbalances, and the presence of pathogens, combined with effects of site management and handling stress (Contessi et al. 2006; Crane and Hyatt 2011; Wheatley et al. 1995).

Photoperiod and temperature are important environmental cues in fish, changing dramatically with the season, particularly at high latitudes. Several studies show that seasonal changes may alter the utilization of lipids for energy production or storage (Mørkøre and Rørvik, 2001; Aksnes, Gjerde, and Roald 1986; Nordgarden et al. 2003a, 2003b). The late summer and autumn period is characterized by optimal sea temperatures for growth and the duration of daylight becomes noticeably shorter. For salmon, this period is associated with increased somatic growth and high lipid deposition (Mørkøre and Rørvik, 2001; Dessen et al. 2017; Rørvik et al. 2018). The winter period is generally associated with reduced feed intake, growth, and feed utilization (Mørkøre and Rørvik, 2001), which correlates with low temperatures and little daylight. Studies also reported a decline or stagnation in the level of muscle fat in salmon during this period (Mørkøre and Rørvik, 2001; Nordgarden et al. 2003a). The dietary lipid level and inclusion of vegetable oils (VO) in salmonid diets have increased gradually over the recent years (Torrissen et al. 2011; Turchini, Torstensen, and Ng 2009). Previous studies have shown that increased dietary energy level, VO inclusion, and temperature can alter lipid metabolism, hepatic fat content, and fatty acid (FA) composition in fish (Leaver et al. 2008; Martinez-Rubio et al. 2013a, 2013b; Ruyter et al. 2006). In many cases of infectious and noninfectious heart diseases in salmon farming, few specific etiologies related to seasonal changes and nutritional aspects seem to have been identified or described.

The present study was a part of a large-scale project evaluating the effects of seasonal dietary changes in protein-to-lipid ratio on growth, feed utilization, and quality-related parameters during the growth-out phase in the sea. During this trial, an unexpected event of sudden mortality of seemingly healthy farmed salmon during the winter period was observed. During the period of increased mortality, samples were secured to investigate possible causes for the mortality. Chemical analyzes of liver lipid content, FA composition and blood parameters of moribund and live salmon were conducted. In addition, all fish that were sampled were screened for detection of a range of RNA viruses that are known to infect salmonids in this area. The results of these analyses are linked to seasonal and dietary-induced changes.

Material and methods

The research reported in this study was approved by The Norwegian Directorate of Fisheries, allowance T-H-8 and T-H-19, and was carried out in accordance with national guidelines, laws, and the animal welfare act. Fish were treated as production fish up to the point of tissue sampling which was only conducted *postmortem (*according to regulation FOR-2015-06-18-761).

The present trial was carried out at NOFIMA large-scale research & development (R&D) facility in collocation with Nordlaks Oppdrett AS, at the sea site Dypingen (Kvernsundet, Bjarkøy, Troms, Northern Norway). The R&D facility consisted of four net-pens (130 m circumference cages) and a feed barge with an automated feeding system. Atlantic salmon yearling smolt (S1) (AquaGen strain, AquaGen AS, Hemne, Norway) vaccinated with Pentium Forte Plus (Novartis Animal Vaccines Ltd, Essex, UK) and transferred to sea on the 3 of May 2015 were used in the study. The mean body weight at sea transfer was 148 g with a mean number of 99 263 salmons per net-pen (SEM = 355). Two diet series (test and control) were formulated and produced by BioMar AS (Myre, Norway) according to target dietary protein and lipid contents defined by Nofima. Net-pen 2 and 3 were fed the test series, whereas pen 1 and 4 were fed the control series. The pens receiving the same dietary treatment were placed diagonally in relation to each other and on the opposite sides of the site (to randomize the localization). The experimental diets were produced as 3.5, 5, 7 and 10 mm pellets. Crude protein and lipid content in all pellet sizes were assessed by on-line near-infrared (NIR) analyses by BioMar and dietary levels of fat and protein used are illustrated in Figure 1(a,b) respectively. The test diet series was designed to have a low fat and a high protein content, whereas the control series was designed to have generally higher lipid and a lower protein content. The salmon was exposed to ambient environmental conditions (Figure 2(a)).

On the 22nd of January 2016, a sudden and marked increase in mortality among large seemingly healthy fish in the two test pens (pen 2 and 3) was observed (Figure 2(b)). The local veterinary service (Vesterålen Fiskehelsetjeneste AS, Sortland, Norway) performed an inspection of the site on 25th of January 2016, whereas Nofima carried out samplings at the site on the 3rd and 17th of February 2016. On the 3rd of February, five fish with normal appearance and swimming behavior (defined as normal not dying individuals) were sampled from each of the four experimental pens. In addition, five moribund (dying) fish were sampled from the test pens 2 and 3 with the increased mortality. Moribund fish were alive and had a seemingly normal outer appearance, but were collected from the dead fish collection net (bottom of the pen) and had lethargic behavior. During this sampling, the color appearance of the liver (scoring scale from 0 to 5; pale/yellow – normal/brown) was evaluated and pieces of liver were stored at −20°C prior to analysis of fat content and FA

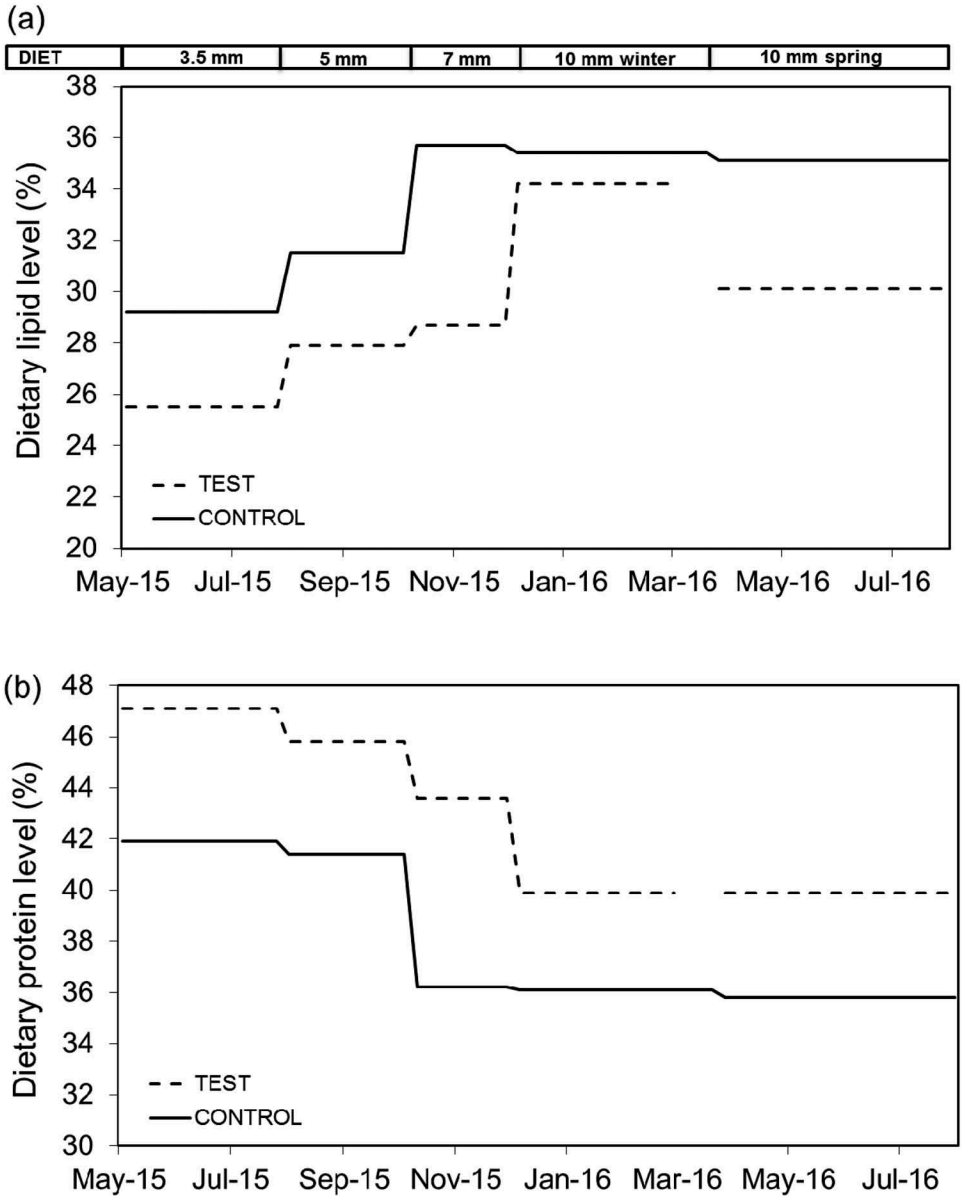

Figure 1. The weighted mean dietary lipid (a) and protein level (b) of the control and test experimental diet series used during the production cycle (May 2015 to July 2016). The values for protein and lipid levels are based on the mean Near Infrared (NIR) analysis of each batch, weighted for the total amount of feed produced in each batch of the particular pellet size.

composition as described by Rørvik et al. (2018). On the 17th of February, 10 normal and 10 moribund fish were sampled from both test pens, and blood was drawn from the caudal vein using vacuum tubes with ethylene diamine-tetra-acetic acid (EDTA). All sampled fish were sedated (Benzoak vet® containing benzocaine of 200 mg/ml, dosage at 2 ml x 10 L^{-1} as recommended, ACD

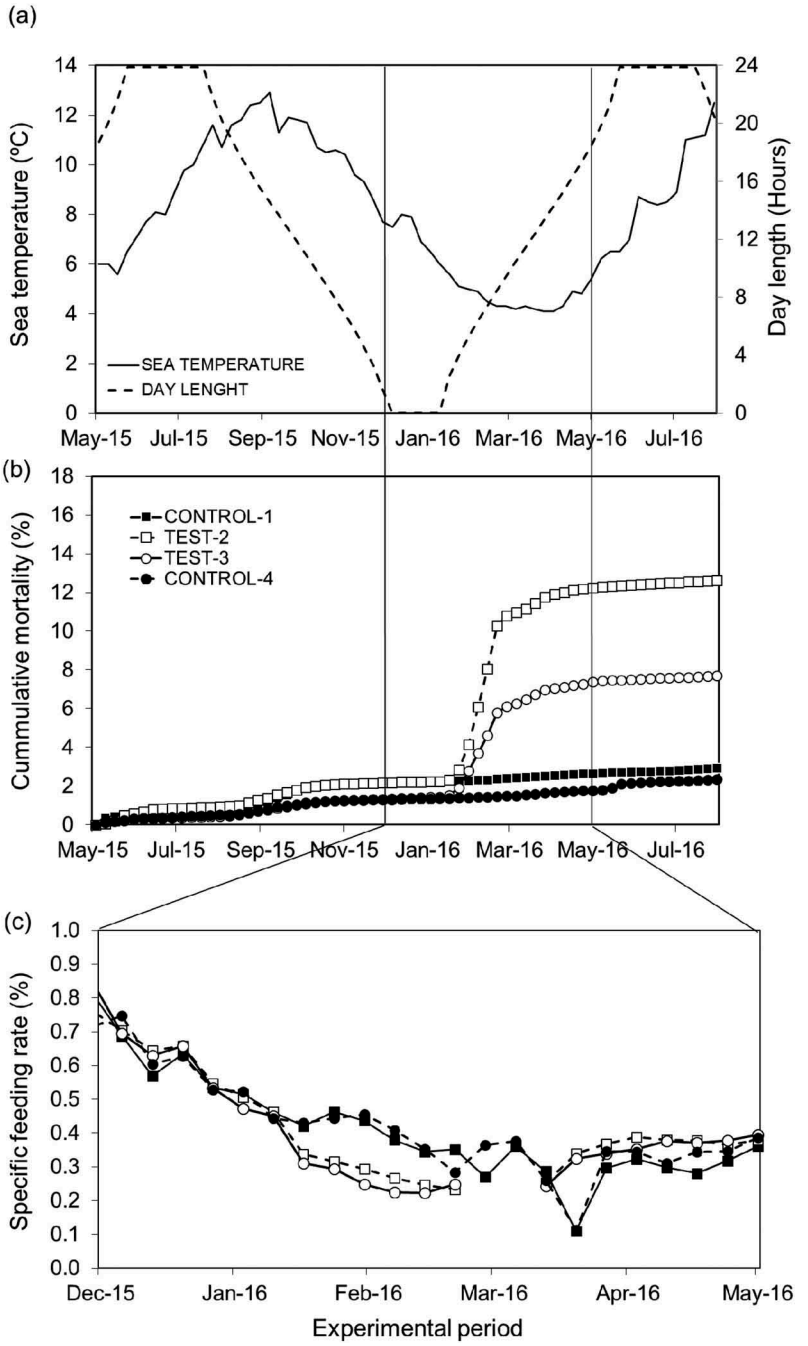

Figure 2. Ambient daily sea temperature (°C, y-axis), day length (hours of daylight, z-axis) (a) and weekly cumulative mortality during the production cycle for the control and test pens (b) during the production cycle (May 2015 to July 2016). The specific feeding rate (%) for the control and test pens from December 2015 to May 2016 (c).

Pharmaceuticals AS, Leknes, Norway) and killed by a blow to the head, before blood was taken and different organs were dissected. Plasma was separated by centrifugation, stored on dry ice and then sent to the Central Laboratory at the Norwegian University of Life Sciences (Oslo, Norway) for analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP) based on the methods described by Tietz (1995). In addition to the mentioned samplings, fish were routinely sampled from all experimental pens at several occasions for analysis of biometric parameters (visceral somatic index referred to as VSI and visceral fat score) and muscle fat content as described by Dessen et al. (2018). The specific feeding rate (SFR), condition factor and organ indices were also calculated as described in the mentioned study.

Different tissues (liver, kidney and hearts) were collected for histopathological examination (fixed in formalin) and RT-PCR analysis (fixed in RNA *later*® Thermo Fisher Scientific, Massachusetts, USA) during the veterinary inspection, and these analyses were conducted by the National Veterinary Institute of Norway, NVI (Harstad, Norway). In addition, heart tissue from all fish sampled on the 3 of February was also evaluated by histopathological analysis (blinded scoring of lesions in the heart, epicardium and ventricle) and RT-PCR analysis by Fish Vet Group (Oslo, Norway) and PatoGen (Ålesund, Norway), respectively. The samples were screened by RT-PCR analysis for the detection of a range of RNA viruses that are known to infect salmonids in this area; PRV, infectious salmon anemia virus (ISAV), salmonid alphavirus (SAV), PMCV and infectious pancreatic necrosis virus (IPNV) using primers and probes as described elsewhere (Hodneland and Endresen 2006; Jonstrup et al. 2013; Liu et al. 2008; Løvoll et al. 2010; Ørpetveit et al. 2010; Palacios et al. 2010; Snow et al. 2006). Since the increased mortality was only observed within the dietary test group, the test diet used in this period was also screened for toxins and abnormalities in the content of nutrients and FA composition (standard procedures and analysis conducted by BioMar). However, no toxins or abnormalities in the content of nutrient were detected in the test diet. The FA composition of the test and control 10 mm winter diets used is shown in Table 1.

The moribund fish from the test pens were defined as the Moribund-T group, whereas the live fish with normal behavior from the test and control pens were defined as the Normal-T and Normal-C group, respectively (n = 10). Statistical analysis was performed using the General Linear Model (GLM) procedure in the SAS 9.4 computer software (SAS Institute Inc., Cary, NC, USA). The effect of diet was tested using the normal fish from the test and control group. If no significant differences were detected, the normal fish were pooled and tested against the moribund fish to test the effect of health status (dying vs. not dying). In cases where significant differences in diet were detected, the normal and moribund fish from the test group were tested against each other. Non-parametric data (scores) were

Table 1. Fatty acid composition (% of total) of the 10 mm winter test and control diets used prior to the increased mortality among the test group.

10 mm winter diet	Control	Test
14:0	1.9	2.4
16:0	7.9	8.0
18:0	2.6	2.2
Σ SFA[a]	14.0	14.0
16:1n-7	1.6	1.9
18:1n-9	40.0	38.0
18:1n-7	5.4	4.6
20:1n-9	2.9	3.8
Σ MUFA[b]	52.8	52.2
18:2n-6	16.3	14.0
20:4n-6	0.2	0.2
Σ n-6[c]	16.8	14.7
18:3n-3	5.8	5.9
20:5n-3	4.4	5.5
22:5n-3	0.4	0.6
22:6n-3	2.6	3.5
Σ n-3[d]	13.5	15.7
n-3/n-6	0.8	1.1

[a]Includes 15:0, 17:0, 20:0, 22:0, and 24:0.
[b]Includes 14:1n-5, 16:1n-5, 17:1n-7, 22:1n-7, 22:1n-11, 22:1n-9, and 24:1 n-9.
[c]Includes 16:2n-6, 18:3n-6, and 20:2n-6.
[d]Includes 16:2n-3 and 20:4n-3.

tested using the non-parametric Kruskal–Wallis test and Pearson product-moment correlation coefficient was used to describe the association between two variables. The level of significance was chosen at $P \leq 0.05$. The results are presented as mean ± standard error of mean (SEM), unless stated otherwise.

Results

The increase in acute mortality on the 22nd of January was only observed in the two pens fed the test diet series. The mortality increased with about 4% and 6% within test-pens 2 and 3, respectively, versus 0.1% for the controls during January, February, and March (Figure 2(b)). Prior to the increased mortality, the lipid content in the test diet series was elevated from 28.7% to 34.2% in early December 2015, whereas the lipid content in the control series was gradually increased to such high dietary fat during the autumn (Figure 1(a)). The dietary shift for the test group was done after a period with substantial somatic growth and deposition of lipids in the muscle for both dietary groups. On the 7th of January, both groups had accumulated about the same level of fat in the muscle (Figure 3). However, the VSI was significantly ($P < .05$) lower in the test than in the control group on this date (Figure 4).

ISAV, SAV, PMCV, or IPNV were not detected by the RT-PCRs analyses. However, PRV (average Ct-value of 22.3) was found in all fish sampled on the 3rd of February. Hence, no significant differences in Ct-PRV values

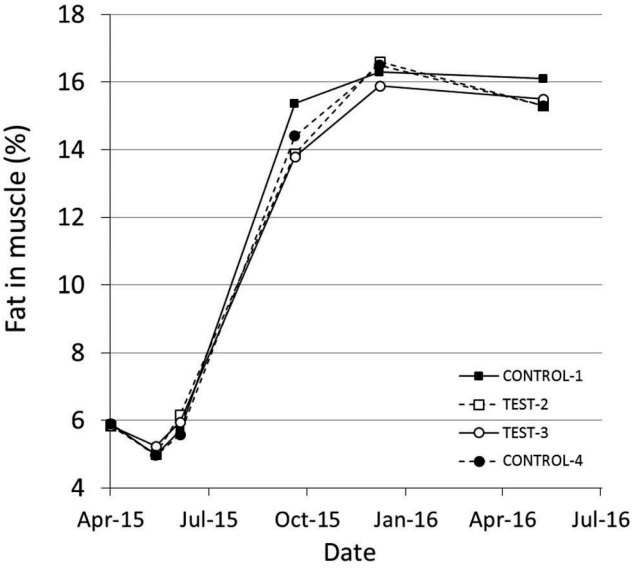

Figure 3. Percentage change of muscle fat content during the experimental period (May 2015 – June 2016) for the control and test group. Samplings were conducted in May, June, and October 2015, in addition to January and June 2016. Values are shown as pooled means of the pens.

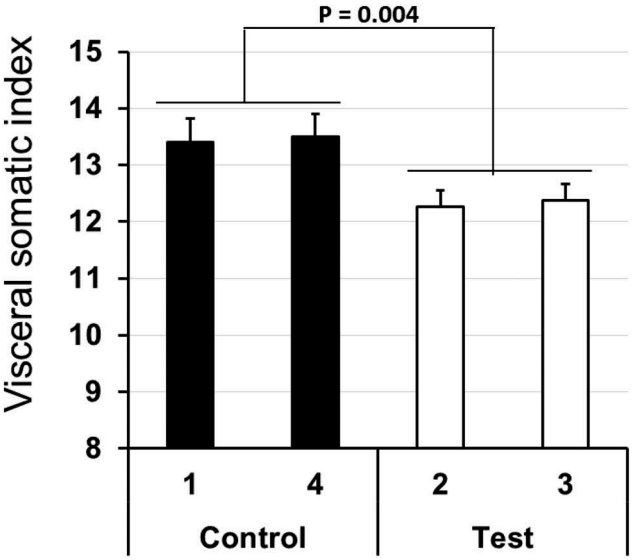

Figure 4. Visceral somatic index (a) and visceral fat score (b) of Atlantic salmon fed control and test dietary series sampled January 7, 2016. Values are shown as pen means ± SEM, n = 10.

between the dietary groups nor any differences between normal and moribund fish in the test pens were detected. The gross evaluation of all moribund fish often revealed pale hearts with blood-filled atrium. Liver was regularly yellowish and pale, and swollen spleen was often observed. Feed matter was found in the gastrointestinal tract among most of the moribund

fish. The histopathological findings from moribund fish sampled during the veterinarian inspection showed mild to moderate myocarditis and epicarditis in the heart, in addition to moderate degrees of hemorrhagic necrosis in the liver. The majority of the hearts sampled from both normal and moribund fish on the 3rd of February had normal histopathological appearance. However, some hearts within all groups displayed mild changes that can be associated with CMS or mild HSMI. This was mainly indicated by diffuse infiltration of inflammatory cells in the epicardium and minor inflammatory changes in the compact layer of the ventricle. The histopathological score was overall numerically higher for the fish sampled in the test pens (both moribund and normal live fish), but no significant differences were detected between moribund and normal fish groups, or between the dietary groups.

There were no significant differences in biometrics between the groups of fish sampled on the 3rd of February (Table 2). However, the Moribund-Test group had a significantly ($P < .05$) higher liver lipid content compared to the other groups (Figure 5). In addition, the Moribund-Test group had numerically higher hepato-somatic index (HSI) and a significantly lower liver color score (more pale/yellow) than the Normal-T group (Table 2). A significant ($P < .001$) overall negative correlation was found between liver fat content and liver color score ($r = -0.66$, $n = 30$). Regarding the blood samples, significantly ($P < .05$) higher levels of ALT and AP in plasma were observed among the moribund compared to the normal fish within the pens with high mortality (Figure 6(a,b)). A similar pattern was observed for AST, although no significant differences were detected. The analysis of the FA profile in the liver showed that moribund fish had significantly ($P < .05$) higher levels of palmitoleic acid (16:1 *n*-7), vaccenic acid (18:1 *n*-7) and oleic acid (18:1 *n*-9), irrespective of dietary treatment (Table 3). In addition, the Δ^9 desaturate index (Δ^9 DI) was 1.3 folds higher ($P < .05$) in moribund versus the normal fish groups (Table 3).

Due to an exponential increase in mortality, the feeding of the two test pens was stopped on the 15th of February to test the potential effect of starvation on mortality rates. After 5 days, a marked reduction in daily mortality was observed and on the 25th of February, the mortality leveled

Table 2. Biometric data of Atlantic salmon fed the two experimental diets and presenting differences in health status. (Mean values with their standard errors; $n = 10$).

Dietary treatment	Control	Test	Test	*P*-value	
Health status	Normal	Normal	Moribund	Diet	Health status
Body weight, g	2533 ± 227	2501 ± 152	2511 ± 151	ns	ns
Gutted weight, g	2201 ± 192	2211 ± 129	2145 ± 104	ns	ns
Length, cm	57.6 ± 1.6	58.3 ± 1.0	57.6 ± 1.0	ns	ns
CF	1.30 ± 0.04	1.25 ± 0.03	1.31 ± 0.04	ns	ns
CFg	1.13 ± 0.03	1.11 ± 0.02	1.12 ± 0.02	ns	ns
HSI	1.49 ± 0.08	1.48 ± 0.05	1.56 ± 0.09	ns	ns
Liver score, 0 – 5	1.7 ± 0.1	2.3 ± 0.3	1.3 ± 0.2	ns	0.001

CF; condition factor, CFg; condition factor gutted, HSI: hepatic-somatic index.

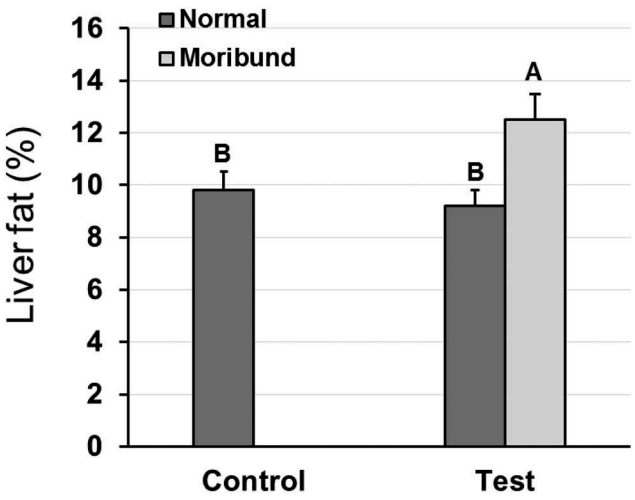

Figure 5. Liver fat content of Atlantic salmon sampled February 3, 2016. Normal-C; normal fish from control pens, Normal-T; normal fish from the test pens, Moribund-T; diseased/moribund fish from the test pens. Values are shown as means ± SEM, n = 10. Significant differences ($P < .05$) are indicated by different subscript letters on the bars.

off to normal levels (Figure 7). On the 12th of March, the feeding was resumed using a diet with lower lipid content (30% fat, Figure 1(a)). No increase in mortality was observed after re-feeding and during the latter stages of the production (Figure 2(b)). Figure 2(c) shows that daily specific feed intake did not differ between the test and control groups during the first 6 weeks after the dietary change. However, the daily specific feed intake in the test group dropped during the period of acute mortality and recovered after the starvation period. All net-pens were harvested during August 2016.

Discussion

The described results were obtained during a large-scale study that was initially designed to evaluate the effects of diets diverging in protein-to-lipid ratio on growth, feed utilization, and quality-related parameters. Previous studies show that lipid dense diets may improve feed utilization, especially at low temperatures (Bendiksen et al., 2003; Hillestad et al. 1998; Karalazos et al. 2007). Thus, the lipid level in the 10 mm winter test diet was increased so that it contained both a high protein and lipid level. The test group switched to another diet during early December, when there was nearly no daylight (polar night) and the water temperature was 8°C. Both experimental groups showed a substantial increase in weight and fat deposition prior to winter, which is consistent with previous observations of growth and lipid storage in farmed salmon during late summer and autumn (Mørkøre and Rørvik, 2001; Alne et al. 2011; Dessen et al. 2017). The muscle fat content did not increase after January, which may indicate that the

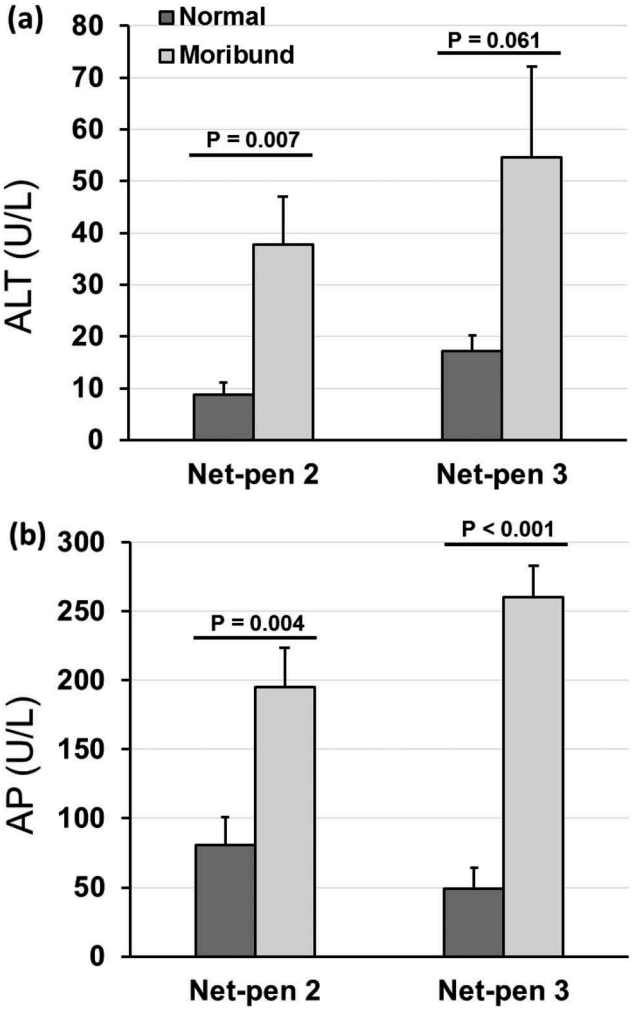

Figure 6. Alanine aminotransferase (a) and alkaline phosphatase (b) and aspartate aminotransferase (c) in plasma of Atlantic salmon sampled February 17, 2016. Normal-C; normal fish from control pens, Normal-T; normal fish from the test pens, Moribund-T; diseased/moribund fish from the test pens. Values are shown as means ± SEM, n = 10. Significant differences are indicated over the bars.

possibility and/or ability to store fat have reached an upper limit relative to this point of time.

The liver of the moribund fish had a higher lipid content and percentages of the FA 16:1 *n*-7, 18:1 *n*-7, and 18:1 *n*-9 compared to the other groups during the time of mortality. The increase in hepatic lipid content and the aforementioned FAs, together with slightly lower percentages of saturated FAs (particularly C18:0) may indicate an up-regulated Δ^9-desaturase hepatic activity. This assumption is strengthened by the significant increase in Δ^9 desaturase index among the moribund fish. In salmon, lipid dense diets with increased inclusion of vegetable oils at low water temperatures have shown to

Table 3. Fatty acid composition (% of total) in the liver of Atlantic salmon fed the two experimental diets and presenting differences in health status. (Mean values with their standard errors; n = 10).

Dietary treatment	Control	Test	Test	*P*-value	
Health status	Normal	Normal	Moribund	Diet	Health status
14:0	1.0 ± 0.12	1.2 ± 0.14	1.4 ± 0.03	ns	ns
16:0	7.7 ± 0.30	7.3 ± 0.19	6.6 ± 0.29	ns	ns
18:0	3.9 ± 0.14	3.7 ± 0.09	3.5 ± 0.15	ns	ns
Σ SFA[a]	13.4 ± 0.31	13.0 ± 0.25	12.4 ± 0.39	ns	ns
16:1n-9	0.0 ± 0.00	0.1 ± 0.04	0.1 ± 0.05	0.025	ns
16:1n-7	**1.7 ± 0.07**	**1.9 ± 0.13**	**2.4 ± 0.13**	**ns**	**0.001**
18:1n-9	**36.0 ± 0.97**	**35.8 ± 1.65**	**40.6 ± 0.92**	**ns**	**0.001**
18:1n-7	**2.8 ± 0.04**	**2.9 ± 0.09**	**3.2 ± 0.06**	**ns**	**0.009**
20:1n-9	4.7 ± 0.13	4.7 ± 0.13	5.0 ± 0.20	ns	ns
Σ MUFA[b]	48.5 ± 1.23	49.1 ± 1.66	54.9 ± 1.24	ns	0.006
18:2n-6	10.5 ± 0.21	9.5 ± 0.33	9.8 ± 0.23	0.023	ns
20:4n-6	0.8 ± 0.08	0.9 ± 0.09	0.6 ± 0.04	ns	0.027
Σ n-6[c]	11.4 ± 0.18	11.3 ± 0.25	10.6 ± 0.21	ns	0.051
18:3n-3	3.2 ± 0.12	3.3 ± 0.10	3.4 ± 0.08	ns	ns
18:4n-3	2.0 ± 0.05	1.6 ± 0.23	1.9 ± 0.05	ns	ns
20:5n-3	4.9 ± 0.32	4.1 ± 0.30	3.0 ± 0.17	0.031	0.010
22:5n-3	1.4 ± 0.08	1.5 ± 0.16	1.1 ± 0.09	ns	0.023
22:6n-3	11.1 ± 0.71	12.2 ± 1.46	8.2 ± 0.71	ns	0.011
Σ n-3[d]	23.6 ± 1.02	23.6 ± 1.74	18.9 ± 0.93	ns	0.02
Δ^9 DI[e]	**3.3 ± 0.18**	**3.5 ± 0.21**	**4.3 ± 0.24**	**ns**	**0.008**

[a]Includes 15:0, 17:0, 20:0, 22:0, and 24:0.
[b]Includes 14:1n-5, 16:1n-5, 17:1n-7, 20:1n-11, 22:1n-7, 22:1n-11, 22:1n-9 and 24:1 n-9.
[c]Includes 16:2n-6, 18:3n-6, 20:2n-6, and 20:3n-6.
[d]Includes 16:2n-3, 20:4n-3, and 20:3n-3.
[e]Δ^9 DI = (16:1n-7 + 18:1n-9)/(16:0 + 18:0)

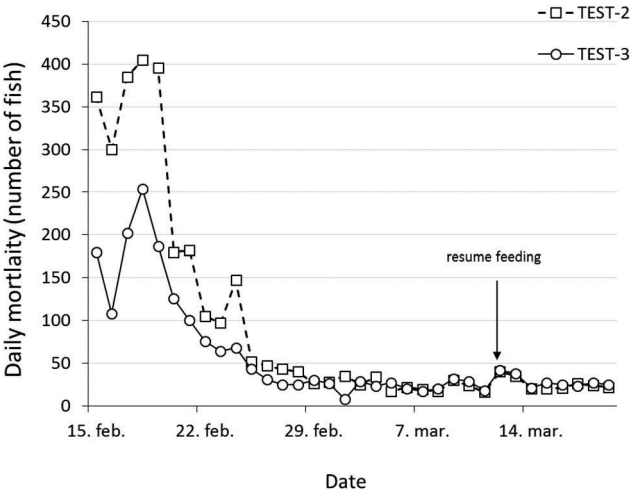

Figure 7. Daily mortality in test-pens 2 and 3 from starvation February 15 and until the end of March 2016. Feeding was resumed on March 12, 2016.

increase liver lipid content (Karalazos et al. 2007). Ruyter et al. (2006) found higher lipid content and percentages of monounsaturated FAs, especially 18:1 *n*-9, in the liver and intestine of salmon kept at 5°C compared with salmon

reared at 12°C, suggesting a higher Δ^9 desaturase activity. In the present study, salmon was switched to a diet with higher lipid content (similar vegetable oil inclusion as the control) when the water temperature was 8°C, and when the water temperature was reduced to 5°C, the mortality started. Thus, the dietary alterations together with the environmental conditions in the present study may have increased the Δ^9 desaturase activity. However, it may also indicate a reduced capacity of the liver to secret fat as very low-density lipoproteins. These factors may consequently explain the increased levels of lipid and monounsaturated FAs in the liver. The moribund fish did also have significantly higher levels of ALT and AP in plasma than previously reported in healthy salmon (Sandnes, Lie, and Waagbø 1988). AST, AP, and ALT have been used as a useful diagnostic tool to detect liver and kidney disturbances in salmonids (Racicot, Gaudet, and Leray 1975; Sandnes, Lie, and Waagbø 1988). In mammals, increased levels of AST, AP, and ALT are associated with impaired liver function, liver tissue damage, and necrosis (Giannini et al. 1999; Oguz et al. 2013). Hence, the increased ALT and AP may also be a sign of organ failure due to moribund state.

The presence of PRV was detected in all groups, in addition to some sings of histopathological changes that can resemble HSMI. However, it has been observed that PRV can be present in high titers without causing mortality or marked lesion in the heart (Garseth et al. 2013). Diets with increased lipid content have previously been shown to increase inflammatory responses, heart lesions, hepatic fat content, and signs of hepatic steatosis in salmon experimentally challenged with Atlantic salmon reovirus (ASRV) (Martinez-Rubio et al. 2013a). The presence of PRV and the clinical signs of the moribund fish may have reduced the ability to resist the long-term inflammation associated with PRV and/or HSMI among the test group. However, this theory is weakened by the detection of the same PRV virus load and similar histopathological heart scores in both normal and moribund fish. A significant and instant reduction in mortality was observed when the fish within the test group were starved. Starvation is known to increase β-oxidation in rodents (Osmundsen, Bremer, and Pedersen 1991), and may have altered the metabolic state of the fish. Reduced dietary energy is known to increase the resistance toward HSMI and CMS in Atlantic salmon (Martinez-Rubio et al. 2014, 2012). In addition, observation from the salmon industry indicates that starvation may reduce the mortality of large salmon associated with CMS (Personal communication 2014).

During the present trail, it was observed that the VSI was significantly lower among the test group compared to the control group prior to the acute mortality. This seems to indicate a lower visceral fat deposition in the test group. In mammals, when the dietary energy intake exceeds the amount of energy being expended, the excess of energy is normally stored in the form of triglycerides in white adipose tissue. Adipose tissue achieves the safe storage

of lipids by increasing the recruitment of new adipocytes (hyperplasia) and/or the expansion of the existent ones (hypertrophy) (Otto and Lane 2005). However, the expandability capacity of adipose tissue has a limit, and sustained energy overload may lead to the deposition of lipids in non-adipose tissues, leading to lipotoxicity and inflammation (Carobbio, Pellegrinelli, and Vidal-Puig 2017; Solinas, Borén, and Dulloo 2015; Unger et al. 2010). Salmonids normally store lipids in visceral adipose tissue and intramuscularly, whereas very little is stored in the liver and heart (Sheridan 1994; Weil, Lefèvre, and Bugeon 2013). The understanding of factors orchestrating salmon fat distribution and adipocyte recruitment is scarce. However, the development of mesenchymal stem cells isolated from Atlantic salmon white adipose tissue is characterized (Todorčević et al. 2010; Vegusdal et al. 2003). The capacity to recruit adipocytes in humans is increased during childhood and remains constant during adulthood regardless of total body fat content (Knittle et al. 1979; Spalding et al. 2008). Whether this is the case for salmon remains to be elucidated. Nonetheless, the results from the present study suggest that the use of a low-fat diet during a period where salmon is known to increase the amount of visceral adipose tissue may have impaired the recruitment capacity of adipocytes. Thus, the capacity of this fish to accommodate the excess of energy when suddenly increasing the dietary lipid content would be challenged. As a result, the excess of energy would be stored in other organs, such as liver and heart, contributing to a low-grade inflammatory state and an increased risk of cardiometabolic diseases. Considering that the salmon liver contributes to about only 1–1.5% of the body mass, this organ might be affected quickly by overload of fat. To investigate this in more detail, histological evaluation of the liver for micro- and macrovesicular steatosis (fat staining of liver) and gene expression should be conducted in future research. However, the observation of that feed matter was found in the gastrointestinal tract among most of the moribund fish indicates that the fish dies suddenly.

To summarize, the increase in mortality in the present study might be due to an interaction between the nutritionally induced metabolic changes and challenging environmental conditions. Risk factors such as increased liver fat, and increased blood plasma levels of ALT and AP were identified among the moribund dying fish. It is suggested that the increase in mortality may be related to reductions in the fat storage capacity among the test group. The mortality can also be associated with the presence of PRV and reduced ability to resist long-term inflammation associated with PRV and/or HSMI due to the impaired haptic function. These suggested hypotheses/causes need to be further investigated, tested, and verified.

Practically, the study shows that liver status is important to follow-up during the production and that starvation can be a tool to stop unforeseen instantaneous mortality associated with reduced metabolic state.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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