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Saturated fat and cholesterol in Atlantic salmon (*Salmo Salar* L.) feeds are important for fish performance, fillet quality and colour.

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Abstract:

Reduced marine ingredients in feeds for farmed Atlantic salmon has resulted in reduced saturated fatty acids (SFA) and cholesterol (CHOL) in the feed. The consequences of this were investigated in a feeding trial with five different levels of SFA, ranging from 10-28 % of total fatty acids (TFA) in the feed. With a low fish meal and fish oil content, all these diets had a low basal level of CHOL (<500 mg/kg). Furthermore, the 10 % SFA and the 25 % SFA diets were also made with added CHOL (>2100 mg/kg), giving a factorial design with high/low SFA and high/low CHOL, in addition to the regression design with SFA. The results showed the biggest impact by low SFA levels, such as reduced growth coupled with increased feed intake and feed conversion ratio (FCR), possibly related to energy spent on endogenous production of SFA. A similar tendency on growth and FCR was also seen for CHOL, but less pronounced. Low SFA (10% of TFA) also had a negative impact on fillet

quality, resulting in increased liquid loss after freezing and thawing. Low SFA (10% of TFA) and low CHOL (<500 mg/kg) in combination reduced muscle firmness, while both also had a negative impact on fillet colour and astaxanthin content. While neither SFA nor cholesterol are essential nutrients for salmon, as these can be produced endogenously, the current results show that levels should not be as low as 10% SFA of TFA or <500 mg/kg CHOL, which would compromise fish performance and fillet quality.

Keywords:

Salmo salar, saturated fat, cholesterol, astaxanthin, fillet colour, fillet quality

1. Introduction:

Feeds for farmed Atlantic salmon (*Salmo salar* L., referred to as salmon for the remainder of the paper) are formulated with reduced fish oil (FO) and increased plant oil compared to 20 years ago (Aas et al., 2022). Consequences of this shift including reduction in n-3 long-chain polyunsaturated fatty acids (LC-PUFA) and increase in fatty acids (FA) typically rich in plant oils, such as 18:1n-9 and 18:2n-6 have been studied extensively (Sissener, 2018). However, this shift also brings about changes in the dietary lipid composition that have received far less attention; namely reduced levels of saturated fatty acids (SFA) and cholesterol (CHOL) in the feed. A possible reason for this is that SFA and CHOL are not considered essential nutrients for salmon, as the fish are able to produce them endogenously. However, salmon are adapted to a natural diet high in these nutrients. It is not known if salmon can produce enough when dietary levels are dramatically reduced, or if this production would divert too much energy away from growth or have other negative consequences for the fish.

Cholesterol is an essential component of cell membranes modulating membrane fluidity, for cell division and growth, and as a precursor of bile acids, vitamin D and steroid hormone synthesis. Research on low CHOL diets for fish has been limited, but the biomarkers of CHOL biosynthesis pathway is upregulated in liver of salmon fed a plant-based diet (Leaver et al., 2008). Some studies focusing on dietary CHOL supplementation have been performed in salmon, however CHOL levels have often been high in both treatment groups (Bjerkeng et al., 1999; Ignatz et al., 2022; Kortner et al., 2014), due to the basal diet containing more marine ingredients than current commercial formulations in Norway. Positive growth effect of adding CHOL to fish diets with low basal levels of CHOL have been observed in some cases but not others (Deng et al., 2014; Krogdahl et al., 2020; Sissener et al., 2017a; Zhu et al., 2014; Zhu et

al., 2018), probably depending on the fish species, CHOL levels and the other feed ingredients used. In mammals, *de novo* synthesis is the main source of CHOL and this synthesis is promoted by dietary SFA (Gu and Yin, 2020; Lin et al., 1992), but it is not known if this is also the case in salmon.

A FO-based diet would generally provide 22–28 % of fatty acids as SFA, while current commercial Norwegian grow-out feeds for salmon have a mean level of 16.6% SFA (range 15.1-19.4%) (Sele et al., 2023). The percentage of SFA in polar lipids (cell membranes) is kept quite constant despite large variation in the feed (Sissener, 2018), reflecting the important structural role of 16:0 in membrane phospholipids (Sargent et al., 2002). However, a low level of SFA in the feed will largely be reflected in the fillet, which mainly contains neutral lipids (Bell et al., 2002; Sissener, 2018; Sissener et al., 2017b; Torstensen et al., 2000), raising the question of how this may affect fillet quality. Lutfi et al. (2023) observed a higher liquid loss from fillet of fish that received low FO in the feed. This can be assumed to be an effect of low SFA rather than low EPA and DHA, as the same was described in fish given high DHA from genetically modified rapeseed oil with very low SFA in the feed (Ruyter et al., 2022). In another study, fillet firmness was reduced in salmon fed rapeseed oil (10.2 % dietary SFA), compared to soybean oil and two types of FO (Regost et al., 2004).

The red / pink colour of salmon muscle is caused by retention of the pigment astaxanthin, and is among the most important quality criteria for consumers (Alfnes et al., 2006). Improved fillet colour in fish fed diets based on FOs compared to vegetable oils has been observed (Regost et al., 2004), and some studies have shown that the dietary cholesterol content can affect pigmentation of the fillet (Chimsung et al., 2014; Sissener et al., 2017a). As astaxanthin

is among the more expensive feed ingredients, factors potentially affecting its retention in muscle are of high interest for the aquaculture industry.

The aim of the current study was to investigate if there is a minimum level of SFA in the feed required for maintaining good fish performance and fillet quality, and to investigate potential interactions with dietary CHOL on these parameters.

2. Materials and methods:

2.1. Feeds and experimental design:

The diets were produced at the Skretting Aquaculture Innovation (AI) feed technology plant (Stavanger, Norway). Five diets were formulated to contain 10, 15, 20, 25 and 30% of their total FA as SFA, respectively, and were named SFA10, SFA15, SFA20, SFA25 and SFA30 according to their formulated level of SFA (Table 1). Additionally, extra batches of the SFA10 and the SFA25 diets were made with added CHOL (91% purity, Carbogen Amcis, Nederland), resulting in a total of seven diets. These diets were named SFA10C and SFA25C. Hence, the experimental design included both a regression for graded dietary SFA, as well as a factorial design with high/low SFA and high/low CHOL.

The diets were made using mostly commercially relevant ingredients. However, as FO has a considerable content of SFA, no FO was used, and EPA and DHA were rather supplied by Veramaris algae oil (Veramaris®, AX Delft, Netherlands) as well as some Croda oil (marine concentrate of EPA and DHA, Incromea™ E1050, Croda Pharma, UK) for the SFA10 diets. Veramaris oil contained a small amount of SFA, which meant that SFA could not be reduced all the way to 10% using this as the only source of EPA and DHA. The gradient of dietary SFA

was made by gradually exchanging palm oil (high in SFA) with a combination of rapeseed oil and camelina oil. Wheat was removed in diets where cholesterol was added, other than that the protein mix was the same in all diets, including 5 % fish meal of the total feed recipe. The diets were formulated to meet all known dietary requirements of salmon.

Three feed batches were produced, due to the need for increased pellet size as the fish grew; 4 mm pellets of all 7 diets, and 6 mm and 9 mm pellets of the four diets that were used for the second phase of the experiment (SFA10, SFA10C, SFA25 and SFA25C). The 6 and 9 mm pellets were similar to the 4 mm pellets, although with slight decrease in protein and increase in lipid content, according to common practice in commercial diets due to reduced protein requirement as the fish gets bigger. Four of the diets (4 mm pellets only) have already been described in a published paper focusing on post-prandial kinetics after 7 weeks of feeding and mineral digestibility and retention (Fang et al., 2025). However, also these diets are included in the current manuscript for consistency.

2.2. Fish trial:

The trial was performed at Skretting Al Lerang Research station (Strand, Norway) and conducted according to the guidelines of the Norwegian State Commission for Laboratory Animals. The National food safety authorities approved the protocol (identification number: FOTS ID 15387). The fish stock used were all-female Atlantic salmon (Benchmark Genetics, Stofnfiskur, Iceland, fertilized 26.03.2020), that had been vaccinated with Alpha Ject micro 6 approximately 3 months before the trial. The fish trial was divided into two phases, where the fish were fed 7 different experimental diets in the first phase, while in the second phase 4 experimental diets were used. Fish were acclimatized for 14 days in circular tanks (\emptyset 1m,

450L), fed a Skretting commercial feed (Spirit supreme plus 75/70A, 3 mm pellets, 45% protein, 25.2% fat, 8.3% starch, 1.7% fiber, 8% moisture 70 ppm astaxanthin). After the two-week acclimation period, all fish were weighed and sorted and feeding with the 7 experimental diets to triplicate tanks (Figure 1) was commenced two days later, 90 fish were stocked in each tank at the onset of the trial. Tanks were supplied with running sea water (flow-through system) at 12 °C and the fish were exposed to 24 h continuous light throughout the trial. Average water quality parameters were 28.6 ppt salinity and 107% O₂ saturation in the first period of the trial, and 34 ppt salinity and 104% O₂ saturation in the second. Fish were fed to satiation, with an estimated overfeeding of 10% to ensure that the growth potential of the diets was fully explored, using automatic feeders (Hølland Teknologi AS, Sandnes, Norway). Fish were fed three meals per day, each lasting two hours (07:30-09:30, 12:00-14:00, 18:00-20:00). Feed collection was conducted 30 min after each meal finished (third meal in 1 m tanks was collected the day after) to monitor feed intake. Standard husbandry procedures at the station were used.

For the second part of the trial focusing on only four diet groups (SFA10, SFA10C, SFA25, SFA25C), remaining fish from the first phase were pit-tagged (RFID Solutions AS, Stavanger, Norway) directly after the 12-weeks sampling. Circular tanks (∅ 3 m, 7000L) were used, starting with 102 fish per tank and ending with 79 fish per tank. Of these fish, 58 were from the “correct” original diet groups, from which data are reported in this paper, while the remaining 44 fish per tank were from the other dietary groups and were only kept in the experimental tanks to ensure optimal stocking density throughout the trial. The four different diets were fed to duplicate tanks during this period of the trial. Feeding was conducted as above, but with two meals per day (7:30-09:30 and 12:00-14:00). 6mm pellets were used from 12 weeks to 25 weeks, and 9 mm pellets from 25 to 38 weeks.

2.3. Samplings

At the start of the trial all fish were weighed (164.7 g, SD 20.5). After ~7 weeks of feeding, all fish were weighed again to calculate feed- and growth performance parameters. The main samplings were conducted after 12 weeks of feeding for all seven diet groups, and after 38 weeks (9 months) of feeding for the four diet groups used in the second part of the trial. After 25 weeks, pellet size was changed, and all fish were weighed to assess feed- and growth parameters. Additionally, whole fish were sampled and homogenized along with weighing at 7 weeks.

The sampled fish were euthanised with an overdose of anaesthetic (Tricaine Pharmaq, 0.2 g/l). Before samplings, fish were starved for 24 h. At the 12-weeks sampling, all fish were weighed and fork length was measured (144-150 fish per diet group). Homogenate of pooled muscle samples (Norwegian quality cut) were made using fillets from 6 fish per tank, for analyses of fatty acids, total lipid, cholesterol and astaxanthin. Weight of gutted fish was registered for 6 fish per tank, and used to calculate yield (weight of gutted fish in % of total weight). Homogenized samples of whole fish were made from 5 fish per tank (pooled samples).

Fish used in the second part of the trial (week 7 to week 38), were individually weighted in week 25 (when pellet size was changed from 6 to 9mm) and at the final sampling. Due to PIT-tagging, these data were also used to calculate weight gain and growth rate for individual fish. Fish with a growth rate of <40% of the average growth rate in the population were excluded; this was the case for two fish in week 12 to 25 (group SFA10C and SFA25C) and three fish in week 25 to 38 (groups SFA10, SFA25, SFA25C). At the final sampling in week 38, only fish that

had been fed the same diet from the start of the trial were sampled for analysis and evaluation. At the final sampling, NQC (left side) from 20 individual fish were sampled per tank; and homogenized individually. For the assessment of liquid loss, three pieces of fillet ($50 \text{ g} \pm 10 \text{ g}$) were cut just anterior of the NQC area. These were weighted and placed in a pre-weighted zip lock bag with pad to soak up excess moisture. The fillet pieces were kept on ice during the sampling of each tank, and frozen at -20°C afterwards. The Digital SalmoFan™ (Salmon App version 2.1.1, DSM-Firmenich-, Switzerland) was used to measure pigmentation in 20 fish per tank (40 per diet group) and gives a score between 20 (light red) to 34 (dark red). Each fillet was scanned 6 times (different spots within the NQC), and an average of the scans was used as the value of the individual fillet. Further measurement of the fillet colour was done using the Minolta Chroma Meter (CR-400 Minolta, Konica Minolta Sensing Inc., Japan) at six locations on the fillet (labelled A-F, see figure 2), on 20 fish per tank (40 per diet group, same fish as used for SalmoFan). This produces L^* , a^* , and b^* -values, representing the lightness (higher L^* -value means a lighter colour), redness (higher a^* value means a redder colour) and yellowness (higher b^* value means a more yellow colour).

2.4. Feed analyses

Feed analysis of proximate composition was performed by Skretting AI Laboratory (Stavanger, Norway). Total nitrogen was determined using the Kjeldahl method and crude protein calculated as $\text{Nx}6.25$ (NMKL 2003). Total fat was measured by NMR (NMKL, 2014). Moisture was measured gravimetrically after drying at 103°C for 24h (NMKL 1991) and ash after flame combustion of the sample at 550°C for 16-18h (NMKL 1991). Fatty acid analysis of feeds was performed following the exact same method as described in Sissener et al. (2016) only with a

different gas chromatograph (Scion 436 GC with CP-8400 autosampler, Scion Instruments, Livingstone, UK), equipped with a PTV split/splitless injector (70°C for 2 min, 30°C/min to 150°C, 4.0°C/min to 225°C and held for 4.58 min). All samples were integrated using the software Chromeleon® version 7.2 (Thermo Scientific Dionex). Cholesterol was analysed by HPLC method by Masterlab (Boxmeer, Netherlarosenlnds).

2.5. Fillet and whole fish; proximate, fatty acid (FA), cholesterol and astaxanthin analyses

Nitrogen was measured with the nitrogen analyser Vario Macro Cube, according to AOAC official methods of analysis (AOAC, 1995) and protein calculated as N x 6.25. Total fat was measured gravimetrically after acid hydrolysis and extraction with diethyl ether, and fatty acids by GLC (gas liquid chromatography) as described previously (Lie and Lambertsen, 1991; Torstensen, B.E. et al., 2004). Analyses of cholesterol was performed as described by (Laakso, 2005). Fillet astaxanthin was determined using an accredited normal phase HPLC method, with detection at 470 nm, and then quantified using an external calibration curve (Ørnsrud et al., 2004).

2.6. Liquid loss and muscle firmness.

After being frozen for ~2 months, fillet cutlet-samples were thawed overnight at 12°C. After thawing, samples were dried off briefly with the pad before both the fillet piece and the bag with pad was weighed. Afterwards, samples were placed back in the freezer and were later sent to Nofima for testing muscle firmness.

Instrumental texture analysis for muscle firmness was performed using a Texture Analyzer TA.XTplus100C (Stable Micro Systems Ltd., Surrey, UK) equipped with a 30 kg load cell and a flat-ended cylindrical probe (\varnothing 0.5"; type P/0.5) at a travel speed of 1 mm/s. Analysis was performed on one sample per fish, which had been thawed at 0 to 2°C for 24 hours, on the cutlet surface in the longitudinal direction of the fish, in the center of the most dorsal loin. Results are presented as the work (N x sec) required to reach 70% of sample thickness.

2.7. Calculations:

Specific growth rate (SGR) % = $[\text{Ln}(\text{Final weight}) - \text{Ln}(\text{Initial weight})] \times 100/\text{days}$

Condition factor (CF) = $(\text{bw}/\text{fl}^3) \times 100$ (bw = body weight, fl = fork length)

Feed conversion ratio (FCR) = $\text{total feed intake}/(\text{final biomass} - \text{initial biomass})$

Nutrient retention = $[(\text{final biomass} \times \text{final nutrient concentration}) - (\text{initial biomass} \times \text{initial nutrient concentration})] \times 100/(\text{Total feed intake} \times \text{nutrient concentration in feed})$

2.8. Statistics

Statistical analysis was conducted using the software Statistica (Version 13; Statsoft, Tulsa, OK, USA) and GraphPad Prism (Version 10.4.1, Dotmatics, Boston, MA, USA). When five different levels of SFA were fed, regression analyses were run on these groups (not including the diet groups with added CHOL). For each response parameter, the simplest model (first order polynomial/linear regression) was used unless second order polynomial was a significantly better fit ($p < 0.05$). For the factorial design (diet groups SFA10, SFA10C, SFA25

and SFA25C), two-way ANOVA was used to investigate effect of high/low SFA and high/low CHOL as the predicting factors were used. Please bear in mind that whenever there are significant interaction effects, significant main effects must be interpreted with caution, as these are not independent. T-tests were used in such cases to examine the effect of one factor within each level of the other. For parameters measured on individual fish (as opposed to measured for each tank/on pooled samples for each tank), a nested ANOVA was first used to test for potential tank effects. P-values < 0.05 are considered statistically significant, but slightly higher p-values may be discussed as trends (with weaker confidence).

3. Results:

3.1 Diets

The proximate composition was very similar between all diets within each pellet batch (Table 1). The values for SFA in the different diets were close to formulated levels and gave a good range of 10-28 % SFA of total FAs. CHOL was <500 mg/kg for all low CHOL diets, and >2100 mg/kg for high CHOL diets. For the diets without added CHOL, the CHOL level was consistently slightly higher in the SFA10 diet, probably due to some small amount of CHOL being present in the Croda oil used. EPA and DHA were similar across all diets within each pellet batch, while there were slight gradients in both total n-6, total n-3 (due to a gradient in 18:3n-3) and total MUFA in the opposite direction to SFA. Fatty acid digestibility for the diet groups SFA10, SFA10C, SFA25 and SFA25C have been published in another paper from this trial (Fang et al., 2025), showing a slight negative effect of high dietary SFA on digestibility of most FAs, most pronounced for the digestibility of SFAs themselves.

3.2 Fish performance

Weight and CF-data both from the 7 and 12-weeks samplings fit a second order polynomial model where these parameters first increased with increasing dietary SFA, before decreasing again (Figure 3, Table 2, Supplementary table 1 and Supplementary Figure 1), indicating that SFA levels both low and high end of the tested range impacted negatively on weight and CF. At 7 weeks, feed intake increased linearly with increasing dietary SFA, while the same was the case for FCR in December.

Both at 7 and 12 weeks, the two-way ANOVA showed significant interaction effects between SFA and CHOL on fish weight and CF, both being lower in fish where dietary SFA and CHOL were simultaneously low (SFA10 group) (Table 2). Yield (gutted weight as % of total weight) was not affected by dietary SFA or CHOL.

In the final 6 months of the trial (week 12 to week 38), significant effects were seen on most fish performance parameters (Table 3). While weight data for all fish at 12 weeks is given in Table 2, weights at the same time point for fish that were part of the second phase of the trial are included in Table 3, to be able to follow the performance of these fish. At 12 weeks, weights of these fish were significantly reduced both by low SFA, low CHOL and their interaction, meaning that fish fed low levels of both these factors were the smallest. However, in the period from week 12 to 25, there seemed to be some compensatory growth in the SFA10 group, resulting in the numerically highest SGR and weight gain with significant effects of SFA*CHOL ($p < 0.001$). Nevertheless, weights in week 25 were significantly lower for low SFA groups compared to high SFA groups. Then, in the period from week 25 to 38, reduced growth from low dietary SFA was seen, and final weights were significantly lower in the low SFA

groups. When it comes to CHOL, effects on growth were less clear than for SFA, with tendencies or significant effects at some time points, mostly evident as interaction effects with SFA, but without a consistent pattern. At the final sampling, there was a tendency ($p=0.09$) for increased final weights of fish fed additional CHOL, with a similar tendency of improved growth in the period leading up to the final sampling. For CF, there was a highly significant interaction effect between SFA and CHOL, where fish fed low levels of both had lower CF.

The reduced growth of low SFA treatments happened despite an increased feed intake in these groups, also causing an increased FCR (Figure 4). There were also similar tendencies for feed intake and FCR for the low CHOL treatments, while feed intake was the only parameter that was close to a significant interaction effect.

Few mortalities were registered during the trial (4 fish in total, data not shown), with no pattern according to diet group.

3.3 Retention of 16:0 and cholesterol

Apparent retention of the SFA palmitic acid (16:0) increased with decreasing dietary SFA (Figure 5), being ~130 % in the SFA10 groups (95 % confidence interval; 111- 146 % for these two groups), ~106 % in the SFA15 group, and seemingly stable around 69-82% for the other groups (SFA20 -30). This means that an average of 28.4 g of 16:0 was produced by the biomass of fish in each tank fed the SFA10 diets during this period of the experiment (and probably even more, as digestibility would not be 100 %). For CHOL, average retention in the dietary groups without added CHOL in the diet was 352 % compared to 43 % for the groups with added dietary CHOL. The 95% confidence intervals show clear differences between the high and low groups, and that the low group is well above the 100 %-mark,

showing high endogenous production. In each tank fed the low CHOL diets, the estimated total amount of CHOL in the fish biomass had increased by 5 g more than what had been provided through the diet.

3.4 Muscle composition

At 12 weeks, there were no differences between the dietary groups in cholesterol content in fillet, while crude lipid and total fatty acids in fillet both fit a second order polynomial similar to the weight and FCR data (Table 4, Supplementary Table 2, Supplementary Figure 1). There were also major differences in the fatty acid profile in the fillet (Table 4, Supplementary Table 2, Supplementary Figure 1). Most of these reflected differences in the respective diets. Sum SFA in fillet ranged from 12.5 to 23.8% of total fatty acids (TFA), compared to 10.5 to 28.3% in the feeds. Hence, the three groups with lowest dietary SFA, exhibited higher relative levels in fillet compared to feeds, while the opposite was the case for the four highest dietary SFA groups. Other FA classes in the fillet closely resembled the dietary composition, while DHA stood out with higher levels in fillets compared to feeds in all dietary groups and the highest level seen in the SFA30 group. Fillet DHA increased with increasing dietary SFA (Table 4, Supplementary Table 2, Supplementary Figure 1), despite being similar in all feeds. Dietary CHOL had an impact on fillet levels on 18:2n-6, 20:4n-6, sum n-6 and 18:3n-3, which all seemed to be slightly higher in the fillet with added dietary CHOL.

At the end of the trial, similar results were seen on fillet FA composition (Table 5). Most FAs in fillet were strongly affected by high/low SFA, but with most of the differences reflecting dietary composition. Total SFA in fillet was once again higher than in diets in the low SFA groups, and lower than the diets in high SFA groups. DHA was higher in high SFA groups,

without a clear explanation in the dietary levels. Dietary CHOL had significant impact on the fillet levels of 16:0, sum SFA and 20:4n-6 (all higher with high CHOL), while there were also significant interaction effects for several FAs, some of which may be due to minor unintended variations between diets.

The muscle concentrations of cholesterol increased from week 12 to week 38, but still with no differences between diet groups (Table 5). The much higher variation observed in cholesterol levels in week 38 compared to week 12 is probably due to individual samples being analyzed at this time point, rather than pooled samples. Furthermore, there were no differences between dietary treatments in total FAs in fillet, crude lipid level or dry matter (Table 5). Fillet protein concentration was neither affected by SFA nor CHOL individually, but had a significant interaction effect, where the highest fillet protein levels were seen in fish fed low SFA with added CHOL and high SFA without added CHOL.

3.5 Fillet colour

Fillet astaxanthin was affected by dietary CHOL in week 12 (Table 4), and by both SFA and CHOL in interaction in week 38 (Figure 6). The 2-way ANOVA showed a stronger main effect of CHOL compared to SFA, but these results must be treated with caution when the interaction is also significant, as the main effects are then not independent. However, the t-tests used to follow up showed significant effects of CHOL, within each SFA level ($p < 0.0001$ and $p = 0.037$ for low and high SFA respectively), while the effect of SFA was only significant at low CHOL ($p < 0.0001$) and not at high CHOL ($p = 0.27$). Fish size and fillet lipid content were attempted to be included in the statistical model to see if these factors could explain some of the differences seen, but neither of them had a significant impact.

Supporting the astaxanthin data, similar effects were seen both for Digital SalmoFan™ and the A-value measured by Minolta (Table 6). For Digital SalmoFan™, there were highly significant effects of both SFA, CHOL and an interaction effect. Both low SFA and low CHOL reduced the score, while the strongest effect was seen when both these dietary factors were low at the same time. Results from the Minolta Chroma Meter from section D, which is equivalent to the NQC, are shown in detail in Table 6. Lightness (L-value) did not differ across groups, while redness (a*-value) and yellowness (b*-value) increased with increasing dietary SFA and CHOL. Results from the other five sections were similar (not shown in detail), but with most sections (B, C, E, F) showing a significant effect of SFA and significant interaction effect for the L-value, with diet group SFA10 standing out with the highest values.

Overall, fillets from the SFA10 group were lighter in colour with less red and less yellow, while the SFA25C group were on the other side of the spectrum. The SFA10C and the SFA25 groups were similar to each other, and more similar to the SFA25C group than to the SFA10 group, which stood out the most from the rest.

3.6 Fillet quality

Liquid loss after freezing and thawing was significantly affected by SFA, with a higher loss of liquid from the fillet in the low SFA groups (Figure 7), but no effect of CHOL. Fillet firmness, measured as the force required to break the surface of the fillet, showed a significant interaction effect between dietary SFA and CHOL, as both being low (diet SFA10) seemingly caused a softer fillet (Figure 7).

4. Discussion:

Due to not being essential nutrients, reductions of SFA and CHOL in salmon feeds have received limited attention. This study is unique in terms of exploring the long-term effects on fish performance, fillet quality and colour of salmon fed very low levels of these nutrients (yet realistic for future feed formulations).

4.1 Effect of SFA on performance

Overall, low dietary SFA had a negative effect on fish performance. Initially, this effect appeared to be related to feed intake, however, the negative effect on growth persisted throughout the trial, even when feed intake was higher in the low SFA groups. Among the many feeding trials where FO has been replaced with various plant oils in feeds for salmon, the general result is no effect on growth, provided that the feeds contain sufficient EPA and DHA. While there are some exceptions showing a negative effect on growth with high SFA (Menoyo et al., 2003; Mock et al., 2021), especially in fish reared at cold water temperature (4.2°C) (Karalazos et al., 2007), negative effects on growth by reducing SFA have not been shown previously. This is likely due to a lack of trials using SFA levels as low as in the current trial over an extended period of time. Performance was not affected in salmon fed a rapeseed oil diet with 11.9% SFA for 17 weeks (Bell et al., 2001), or a linseed oil diet with 10.5% SFA for 40 weeks (Bell et al., 2004). Both trials were run at ambient temperature (range 5-17°C). Notably, the current trial was run at a constant temperature of 12°C, to avoid differences in digestibility complicating the interpretation of results. Still, reduced digestibility may have caused the reduced growth at the high end of the SFA range (>25% SFA), and this point would likely be shifted down in a trial run at a lower temperature (Ng et

al., 2004). Hence, seasonally tailored diets with higher SFA at elevated water temperatures may be useful (Mock et al., 2021). The current result of reduced performance in salmon fed 10% SFA long-term is a novel finding, but the validity may be related to water temperature.

4.2 Endogenous production of SFA and CHOL

The increased FCR/ less efficient feed utilization in the 10% SFA groups indicate that the fish are spending energy on endogenous production of SFA, supported by the retention data showing higher accumulation of palmitic acid (16:0) in fish compared to what was eaten (retention >100%), support this. We expect the endogenous production of 16:0 to continue throughout the trial or even increase as tissue levels are being gradually depleted. Previous studies have also indicated *de novo* production of SFA in salmon when dietary intake is low (Mock et al., 2021; Sissener et al., 2017b). While calculated retention values were higher for CHOL than 16:0, the latter is found in the fish body in much higher amounts (~20-fold higher at 12 weeks), hence the absolute amounts produced would be higher for 16:0. As production of 16:0 is also slightly more energy-consuming (Carta et al., 2017; Jones, 1997), it makes sense that low SFA has the most impact on fish growth in the current trial.

4.3 Effect of CHOL on performance

The negative effect on performance from low CHOL was less clear than for low SFA, and mostly seemed to be linked to an interaction with low SFA. The low dietary CHOL in the current study may not have been sufficiently low to cause significant effects at most sampling points, potentially CHOL levels were borderline of what the salmon could handle, hence only trends on growth and FCR. In mammals, *de novo* synthesis of CHOL is promoted by dietary SFA (Gu and Yin, 2020; Lin et al., 1992), which might explain why negative effects of low dietary CHOL on performance are first seen as interaction effects with low dietary

SFA. No dietary effects on muscle cholesterol levels indicate that endogenous synthesis was sufficient to maintain tissue levels in our trial.

Regarding previous studies on added CHOL and fish performance, the positive effect seen in some studies could be attributed to the hypocholesterolemic effect of antinutrients present in plant protein sources that are not sufficiently refined (Deng et al., 2014). Most studies in salmon had higher CHOL than current Norwegian commercial feeds in the basal formulation and reported no effects on performance of CHOL addition (Bjerkeng et al., 1999; Ignatz et al., 2022; Kortner et al., 2014). Of studies conducted with lower levels, dietary CHOL ranging from 867–3153 mg kg⁻¹ (Sissener et al., 2017a) or 700 to 4450 mg kg⁻¹ (Krogdahl et al., 2020) did not affect growth in salmon, while lower levels have not been tested in salmon. Positive effect of added CHOL was seen on growth in Rainbow trout, when levels were <100 mg/kg vs ~1200 mg/kg (Hong et al., 2024), supporting that lower levels than used in the current trial may have given more pronounced negative effects on fish performance.

4.4 Fillet composition

Fatty acid profile in the fillet was clearly affected by the diets with a pronounced difference in fillet SFA between the high and low SFA diet groups, while proximate composition remained similar. Total SFA in fillet in the low SFA groups decreased throughout the trial, being ~12.6 % after 12-weeks feeding to ~11.3% after 38 weeks. Compared to previous feeding trials in salmon, fillet SFA levels at the end of the trial were lower in our study. For instance, salmon fed a rapeseed oil diet with 11.9 % SFA for 17 weeks had muscle SFA of 14.8 % (Bell et al., 2001), while salmon fed a linseed oil diet with 10.5% SFA for 40 weeks had final muscle SFA of 12.7 % (Bell et al., 2004). This is probably due to a combination of two factors; duration of the trial and fish size/ muscle lipid content at the end of the trial.

Changes in muscle FA composition in salmon is known to roughly follow a dilution model (Jobling, 2004), and stabilizing muscle FA profile according to diet can require up to a 7-fold increase in body weight (Rosenlund et al., 2016). Muscle lipid content generally increases with fish size, and consequently storage lipids (TAG) will constitute an increasing proportion compared to phospholipids, which would reduce SFA (Sissener, 2018; Torstensen et al., 2004).

Some of the apparent effects on muscle FA profile seemed to be related to minor dietary variations. However, DHA in muscle was significantly lower in low SFA groups at 5.8 % of TFA compared to high SFA groups at 6.2 /6.3 %. While there was also a difference in the diets in the same direction (low SFA diets 5.2 /5.3 % DHA and high SFA diets 5.4 /5.5 %), this dietary difference appears too minor to fully explain the difference seen in muscle. An “omega-3 sparing effect” of SFA has been suggested in other fish species (Turchini et al., 2011) and salmon (Codabaccus et al., 2012), and a data synthesis study using data from salmonids fed various lipid sources found that dietary SFA was positively correlated to DHA muscle storage (Colombo et al., 2018). The proposed mechanism is related to use of SFA for oxidation, sparing n-3 FAs from this fate (Codabaccus et al., 2012; Turchini et al., 2011), but could also be related to reduced digestibility of SFA in high SFA diets, hence a higher proportion of FAs taken up would be n-3. When taking into account that feed intake was higher in the low SFA groups, meaning that total intake of DHA would also have been higher, our data seem to support a positive effect of dietary SFA on DHA deposition in salmon.

4.5 Fillet quality

The difference in fillet SFA is the most likely cause of the differences in liquid loss after freezing and thawing, due to a difference in melting point. Lutfi et al. (2023) found

decreasing liquid loss from 4.2 to 2.8 % in fillets from salmon fed increasing FO inclusion at the expense of rapeseed oil, with increasing SFA from 12.6 to 17.0 %. Rørå et al. (2003) also found a lower value of water loss in fresh muscle of fish fed a diet with higher levels of SFA (FO vs. soybean diet, 26 vs. 21.1 % muscular SFA). In salmon fed diets balanced in SFA, increased DHA seemed to cause increased liquid loss, assessed by manual scoring (Ruyter et al., 2022), however the differences in muscle DHA (>2-fold) were much higher than in our study. Liquid loss in all our groups from our trial was higher than previous studies (Kousoulaki et al., 2016; Lutfi et al., 2023), but this could be related to temperature during storage. In contrast to liquid loss that was only affected by dietary SFA, fillet firmness was reduced by low dietary SFA and low dietary CHOL in combination. Firm structure of fish fillet is considered to be high quality (Wang et al., 2024). In line with our results, fillet firmness was lower in salmon fed rapeseed oil (10.2 % dietary SFA of TFA, 13.4 % SFA in muscle) compared to soybean or FO (17-25 % SFA in muscle) (Regost et al., 2004). In that study, dietary CHOL was not analyzed but was probably relatively high due to a high fish meal content of the feeds. The effects on muscle firmness in our trial, which were measured after a second thawing event, may have been largely determined by the effect of SFA on liquid loss, which was measured after the first thawing event. Freezing and thawing of fish negatively affects liquid loss and texture, where a reduced liquid loss is reflected in improved muscle texture (Chan et al., 2022). An important point to keep in mind here, is that the final weights of our fish were only 2.5 – 2.8 kg, hence even more severe effects on muscle FA composition and consequently also on muscle quality parameters may have been seen if the fish were continued on these diets until the standard commercial harvest size of ~5 kg.

4.6 Fillet colour

Redness of the fillet, due to astaxanthin deposition, is another important aspect of quality and especially how the product is perceived by consumers. Astaxanthin is a costly feed ingredient, and feed producers are reporting that the amount added to the feed to achieve similar redness of the fillet has increased drastically in recent years (Ytrestøyl et al., 2024). Further, it is unclear if low astaxanthin status affects fish health, as astaxanthin is a potent antioxidant (Goto et al., 2001), has anti-inflammatory effects (Ohgami et al., 2003) and have shown positive effects on the immune system (Jyonouchi et al., 1991). The current results very clearly point to low CHOL and low SFA negatively impacting flesh colour, especially when both are simultaneously low. Although astaxanthin and SalmoFan scores both had a significant interaction effect, complicating the interpretation of the main effects (low SFA or low CHOL alone), the measured redness from Minolta showed highly significant effects of both factors, indicating that both each factor and their interaction are of importance here.

Being a hydrophobic compound, the intestinal absorption of astaxanthin can be modulated by other lipid molecules. Some studies have shown a positive effect on fillet astaxanthin from FO-based diets compared to plant-oil based diets (Regost et al., 2004; Rørå et al., 2005; Sissener et al., 2016; Waagbø et al., 2013), however without pinpointing the exact lipid components causing this effect. A complicating factor here is that several fatty acids and lipid components tend to co-vary, for instance EPA+DHA, SFA and CHOL are generally all higher in FO-based diets. Positive effects of FO have often been ascribed to EPA+DHA, and Ytrestøyl et al. (2023) did indeed show that EPA and especially DHA affected pigmentation positively also in a trial with diets without fish meal /FO (hence no co-variation with SFA and CHOL). Dietary CHOL is often not analyzed in trials focusing on oil replacement, but in trials focusing specifically on sterols in salmonids, positive effects of dietary CHOL on astaxanthin absorption / pigmentation were shown (Chimsung et al., 2014; Jin et al., 2024; Sissener et

al., 2017a). Results in the literature on how SFA affects pigmentation appear to be mixed. For instance, Olsen et al. (2005) found that salmon fed with lard (30% SFA) had higher blood astaxanthin after feeding compared to salmon fed herring oil (19% SFA), concluding on a positive effect of either SFA or MUFA on utilization of dietary pigment. On the other hand, algal carotenoid had higher digestibility in salmon fed 20% SFA (canola oil + FO) compared to 30 or 44% (poultry oil or tallow, respectively, + FO) (Courtot et al., 2022). This discrepancy could be due to other dietary factors/ nutrient interaction or environmental factors, as SFA has been shown to be negative for digestibility of astaxanthin only at low temperatures (Sigholt et al., 2008). Variation in which particular SFA is being increased in the diet, or the lack of considering dietary CHOL and its interaction with SFA might also explain some of the different results between trials. The current results of low dietary SFA and CHOL in combination reducing fillet pigmentation have not been reported previously and are of high relevance for the formulation of future salmon diets, especially if EPA+DHA sources other than fish meal and FO are being utilized.

4.7 Implications for the industry

Currently, commercially used levels of SFA and CHOL in Norway are higher than the lower groups used in this study (Sele et al., 2023). However, these nutrients are mostly provided by the FO and fish meal fractions of the feed, which might decrease if novel sources of EPA+DHA increase in availability /production volume as predicted (Glencross et al., 2024). Hence, it is increasingly important to know what the minimum limits of these nutrients are to ensure optimal performance, fish health and product quality. Relevant novel sources of EPA+DHA are algal oils or transgenic canola and camelina, all of which have been successfully used in salmonid feeds (Betancor et al., 2015; Ruyter et al., 2019; Santigosa et

al., 2020). Neither algal oils or plant oils contain CHOL, and transgenic canola contains as little as 7.4% SFA (Ruyter et al., 2019). Long-term trials in the sea-water phase have been conducted with all of these sources to demonstrate their ability to replace FO as the main EPA+DHA source, however land-animal products or palm oil was used to balance SFA (Hatlen et al., 2022; Leyton et al., 2024; Tocher et al., 2024). While land-animal products are commonly used for salmon farming in Canada, Australia and Chile, providing both SFA and CHOL, these ingredients have so far been avoided in Norway due to concerns of consumer acceptance. Considering the importance of these nutrients for salmon performance and fillet quality, as demonstrated in the current trial, it seems wasteful not to use such ingredients, which can be locally sourced and are not competing with human food.

Conclusion:

In conclusion 10 % SFA of dietary FAs appears to be insufficient for optimal performance and fillet quality in Atlantic salmon reared at 12°C, compared to 24 % SFA. Based on the first phase of the trial including five levels of SFA, a minimum of 15 % SFA should be recommended to not compromise growth performance, but we are lacking data to give a minimum recommendation of SFA for fillet quality. The data showed endogenous production of both SFA (16:0) and CHOL when dietary levels were low, possibly explaining effects on performance. Furthermore, the data indicate that supplemental CHOL can to some extent compensate for low SFA. Low SFA causes high liquid loss from the fillet after freezing and thawing, while low SFA and low CHOL in combination reduce fillet firmness and colour, indicating that CHOL should be added to basal diets containing <500 mg/kg.

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Author contributions, CRediT:

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Table 1. Formulation and analyses of the experimental feeds, including 4 mm pellets used from the start of the trial to week 12, 6 mm pellets used from week 12 to 25, and 9 mm pellets used from week 25 to 38.

	SFA10	SFA10C	SFA15	SFA20	SFA25	SFA25C	SFA30
Ingredients (g kg⁻¹), 4 mm pellets:							
Soy protein concentrate	300.0	300.0	300.0	300.0	300.0	300.0	300.0
Wheat gluten	166.0	166.0	166.0	166.0	166.0	166.0	166.0
Rapeseed oil	164.9	164.9	152.0	123.0	93.9	93.9	65.1
Pea protein concentrate	72.0	72.0	72.0	72.0	72.0	72.0	72.0
Wheat	69.0	67.0	69.0	69.0	69.0	67.0	69.0
Fish meal	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Vitamin and mineral premix	47.5	47.5	47.5	47.5	47.5	47.5	47.5
Camelina Oil	41.0	41.0	27.1	24.3	21.5	21.5	18.7
Sunflower meal	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Faba bean dehulled	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Guar meal	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Croda oil	15.5	15.5	-	-	-	-	-
Veramaris oil	11.9	11.9	30.7	30.7	30.7	30.7	30.7
Palm oil	-	-	23.5	55.4	87.1	87.1	118.8
Yttrium premix	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Water	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Astaxanthin	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Cholesterol	-	2.2	-	-	-	2.2	-
Analyzed composition of selected nutrients, 4 mm pellets:							
Cholesterol, mg kg⁻¹	509	2307	242	226	234	2384	217
Σ SFA, % of TFA	10.3	10.4	14.0	19.3	24.0	23.7	28.3
16:0, % of TFA	6.5	6.6	10.1	14.9	19.2	19.0	23.2
18:0, % of TFA	2.2	2.2	2.2	2.6	3.0	3.0	3.3
Σ MUFA, % of TFA	50.4	50.2	48.6	46.0	43.4	43.6	41.2
18:1n-9, % of TFA	42.9	42.9	42.6	40.8	38.9	39.1	37.4
Σ n-6, % of TFA	19.9	20.1	19.0	17.8	16.6	16.7	15.7
18:2n-6, % of TFA	19.0	19.2	18.1	16.9	15.8	15.9	14.9
20:4n-6, % of TFA	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Σ n-3, % of TFA	18.3	18.2	17.0	15.6	14.3	14.3	13.1
18:3n-3, % of TFA	10.3	10.3	8.6	7.2	5.9	6.0	4.7
EPA, % of TFA	2.0	2.0	2.4	2.4	2.4	2.4	2.4
DHA, % of TFA	5.2	5.2	5.3	5.3	5.3	5.3	5.3
EPA + DHA, % of TFA	7.2	7.1	7.7	7.7	7.8	7.7	7.7
n-6/n-3	1.1	1.1	1.1	1.1	1.2	1.2	1.2
Lipid, g kg ⁻¹	268	271	274	274	277	275	274
Protein, g kg ⁻¹	452	459	453	461	461	459	444
Ash, g kg ⁻¹	46	44	45	45	47	44	45
Moisture, g kg ⁻¹	73	67	71	67	67	73	74
Ingredients (g kg⁻¹), 6 mm pellets:							
Soy protein concentrate	280.0	280.0	-	-	280.0	280.0	-
Rapeseed oil	168.2	168.2	-	-	90.0	90.0	-
Wheat gluten	120.0	120.0	-	-	120.0	120.0	-
Palm oil	-	-	-	-	98.6	98.6	-
Camelina Oil	60.1	60.1	-	-	37.9	37.9	-
Wheat	60.0	57.8	-	-	60.0	57.9	-
Sunflower meal	52.3	52.3	-	-	52.3	52.3	-
Pea protein concentrate	50.4	50.4	-	-	50.4	50.4	-
Faba bean dehulled	50.0	50.0	-	-	50.0	50.0	-

Fish meal	50.0	50.0	-	-	50.0	50.0	-
Vitamin and mineral premix	46.7	46.7	-	-	46.7	46.7	-
Guar meal	20.0	20.0	-	-	20.0	20.0	-
Veramaris oil	17.8	17.8	-	-	31.3	31.3	-
Croda oil	11.8	11.8	-	-	-	-	-
Water	11.3	11.3	-	-	11.3	11.3	-
Yttrium premix	1.0	1.0	-	-	1.0	1.0	-
Astaxanthin	0.5	0.5	-	-	0.5	0.5	-
Cholesterol	-	2.2	-	-	-	2.2	-

Analyzed composition of selected nutrients, 6 mm pellets:

Cholesterol, mg kg⁻¹	370	2116	-	-	296	2174	-
Σ SFA, % of TFA	10.5	10.4	-	-	23.7	24.2	-
16:0, % of TFA	6.6	6.5	-	-	18.9	19.3	-
18:0, % of TFA	2.2	2.2	-	-	3.0	3.0	-
Σ MUFA, % of TFA	49.7	49.6	-	-	43.6	43.1	-
18:1n-9, % of TFA	41.4	41.4	-	-	38.0	37.6	-
Σ n-6, % of TFA	19.5	19.6	-	-	16.3	16.1	-
18:2n-6, % of TFA	18.6	18.7	-	-	15.5	15.3	-
20:4n-6, % of TFA	0.4	0.4	-	-	0.2	0.2	-
Σ n-3, % of TFA	19.0	19.2	-	-	15.2	15.1	-
18:3n-3, % of TFA	11.1	11.3	-	-	6.9	6.7	-
EPA, % of TFA	2.2	2.2	-	-	2.6	2.6	-
DHA, % of TFA	4.9	4.9	-	-	4.7	4.8	-
EPA +DHA, % of TFA	7.1	7.0	-	-	7.3	7.4	-
n-6/n-3	1.0	1.0	-	-	1.1	1.1	-
Lipid, g kg ⁻¹	292	290	-	-	294	296	-
Protein, g kg ⁻¹	421	418	-	-	410	413	-
Ash, g kg ⁻¹	44	44	-	-	45	44	-
Moisture, g kg ⁻¹	68	69	-	-	73	70	-

Ingredients (g kg⁻¹, 9 mm pellets:

Soy protein concentrate	258.3	258.3	-	-	258.3	258.3	-
Rapeseed oil	203.2	203.2	-	-	78.6	78.6	-
Wheat gluten	144.9	144.9	-	-	144.9	144.9	-
Palm oil	-	-	-	-	131.3	131.3	-
Wheat	71.2	69	-	-	71.2	69	-
Camelina Oil	65	65	-	-	55.4	55.4	-
Faba bean dehulled	60	60	-	-	60	60	-
Vitamin and mineral premix	44.5	44.5	-	-	44.5	44.5	-
Fish meal	50	50	-	-	50	50	-
Sunflower meal	40	40	-	-	40	40	-
Veramaris oil	26.7	26.7	-	-	39.2	39.2	-
Guar meal	20	20	-	-	20	20	-
Croda oil	9.8	9.8	-	-	-	-	-
Water	5	5	-	-	5	5	-
Yttrium premix	1	1	-	-	1	1	-
Astaxanthin	0.5	0.5	-	-	0.5	0.5	-
Cholesterol	-	2.2	-	-	-	2.2	-

Analyzed composition of selected nutrients, 9 mm pellets:

Cholesterol, mg kg⁻¹	419	2142	-	-	321	2279	-
Σ SFA, % of TFA	10.3	10.4	-	-	25.8	26.4	-
16:0, % of TFA	6.7	6.7	-	-	20.9	21.5	-
18:0, % of TFA	2.1	2.1	-	-	3.1	3.1	-
Σ MUFA, % of TFA	50.6	50.4	-	-	41.7	41.2	-

18:1n-9, % of TFA	42.7	42.4	-	-	36.0	35.7	-
Σ n-6, % of TFA	19.0	19.0	-	-	15.6	15.4	-
18:2n-6, % of TFA	18.0	17.9	-	-	14.6	14.4	-
20:4n-6, % of TFA	0.3	0.3	-	-	0.3	0.3	-
Σ n-3, % of TFA	18.9	19.0	-	-	15.3	15.4	-
18:3n-3, % of TFA	11.0	11.0	-	-	7.1	7.1	-
EPA, % of TFA	1.9	1.9	-	-	2.0	2.0	-
DHA, % of TFA	5.2	5.3	-	-	5.4	5.5	-
EPA + DHA, % of TFA	7.1	7.2	-	-	7.4	7.5	-
n-6/n-3	1.0	1.0	-	-	1.0	1.0	-
Lipid, g kg ⁻¹	327	331	-	-	336	331	-
Protein, g kg ⁻¹	391	393	-	-	384	381	-
Ash, g kg ⁻¹	44	43	-	-	42	45	-
Dry matter, g kg ⁻¹	65	67	-	-	66	68	-

SFA; Saturated fatty acids (FA), TFA; total FA, EPA; eicosapentaenoic acid, DHA; docosahexaenoic acid, MUFA; monounsaturated FA.

Table 2. Fish performance during the first 12 weeks of the trial, when all 7 diets were fed to triplicate tanks of Atlantic salmon. SGR, feed intake and FCR are given per tank (n=3), and separated for the two periods from start to week 7 and week 7-12. Length is given as fork length. The column “Reg.” shows the results from the regression analyses, with “1st” for data that fit a first order polynomial (linear regression) with a slope significantly different from zero, and “2nd” for data where a second order polynomial is a significantly better fit than the first order polynomial (complete results from the regression analyses can be found in the supplementary material; table 1, 2 and figure 1). The three columns to the right show the results from two-way ANOVA with high/low SFA and high/low CHOL as the categorical factors (only data from groups SFA10, SFA10C, SFA25 and SFA25C are included) SGR, Feed intake and FCR are calculated per tank (n=3 per diet group). Week 7: weight, length and CF based on data from 163-165 fish per diet group. Week 12: weight, length and CF based on data from 144-150 fish per diet group. Yield (weight of gutted fish in % of total weight) is based on data from 6 fish per tank (18 fish per diet group).

	SFA10	SFA10C	SFA15	SFA20	SFA25	SFA25C	SFA30	Reg.	ANOVA SFA	ANOVA CHOL	Inter- action
<i>Initial weight:</i>											
Weight, g	163.8 ± 20.5	166.2 ± 19.6	165.2 ± 20.1	164.2 ± 21.5	163.8 ± 20.2	165.7 ± 21.1	163.7 ± 20.3	n.s.	n.s.	n.s.	n.s.
<i>7-weeks sampling (after 50 feeding days):</i>											
Weight, g	332.5 ± 43.3	364.4 ± 50.3	365.3 ± 53.2	354.7 ± 48.9	364.0 ± 49.8	362.5 ± 53.3	357.2 ± 44.2	2nd	n.s.	p=0.0005	p=0.0008
SGR	1.43 ± 0.04	1.58 ± 0.12	1.60 ± 0.15	1.54 ± 0.05	1.60 ± 0.13	1.58 ± 0.02	1.57 ± 0.05	n.s.	n.s., p=0.13	n.s.	n.s., p=0.14
Length	30.6 ± 3.8	31.1 ± 1.4	31.1 ± 1.5	30.6 ± 1.4	30.9 ± 1.6	30.9 ± 1.5	30.8 ± 1.3	n.s.	n.s.	n.s.	n.s.
CF	1.18 ± 0.12	1.22 ± 0.14	1.21 ± 0.06	1.24 ± 0.16	1.24 ± 0.16	1.22 ± 0.08	1.22 ± 0.09	2nd	n.s.	p=0.02	p=0.04
Feed intake	1.15 ± 0.02	1.22 ± 0.08	1.23 ± 0.06	1.19 ± 0.02	1.25 ± 0.05	1.22 ± 0.02	1.24 ± 0.04	1st	n.s., p=0.10	n.s.	n.s., p=0.11
FCR	0.81 ± 0.01	0.77 ± 0.01	0.77 ± 0.03	0.77 ± 0.02	0.78 ± 0.03	0.77 ± 0.00	0.79 ± 0.00	n.s.	n.s.	n.s., p=0.11	n.s.
<i>12-weeks sampling (total 83-84 feeding days):</i>											
Weight, g	487.9 ± 63.4	528.7 ± 86.5	537.9 ± 81.5	525.6 ± 76.2	543.0 ± 76.1	533.1 ± 76.9	512.7 ± 62.6	2nd	p<0.0001	p=0.01	p<0.0001
SGR	1.15 ± 0.02	1.10 ± 0.09	1.16 ± 0.08	1.19 ± 0.04	1.22 ± 0.05	1.15 ± 0.05	1.10 ± 0.04	n.s.	n.s., p=0.12	n.s.	n.s.
Length	33.8 ± 1.6	34.3 ± 1.7	34.5 ± 1.6	34.1 ± 1.6	34.4 ± 1.6	34.5 ± 2.6	34.3 ± 2.5	1st	p=0.0007	p=0.03	n.s.
CF	1.26 ± 0.09	1.31 ± 0.20	1.31 ± 0.07	1.32 ± 0.10	1.32 ± 0.08	1.29 ± 0.07	1.28 ± 0.13	2nd	n.s., p=0.06	n.s.	p<0.0001
Yield%	88.8 ± 0.7	88.5 ± 1.1	89.5 ± 1.1	89.4 ± 1.0	88.6 ± 1.0	88.5 ± 0.6	90.1 ± 1.3	n.s.	n.s.	n.s.	n.s.

Feed intake	1.05 ± 0.03	1.01 ± 0.08	1.06 ± 0.06	1.07 ± 0.03	1.12 ± 0.04	1.07 ± 0.02	1.05 ± 0.04	n.s.	n.s., p=0.053	n.s.	n.s.
FCR	0.91 ± 0.02	0.92 ± 0.03	0.92 ± 0.01	0.91 ± 0.03	0.92 ± 0.02	0.93 ± 0.02	0.95 ± 0.02	1st	n.s.	n.s.	n.s.

Initial weight: 164.7 g. Unit feed intake = %/day. SGR; Specific growth rate, CF; condition factor, FCR; feed conversion ratio, n.s.; not significant.

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Table 3. Fish performance of Atlantic salmon fed four diets with high/low SFA and CHOL in a factorial design, from week 12 to 38. Results are given as mean and standard deviation, and statistical results from a two-way ANOVA with high/low SFA and high/low CHOL as the categorical factors. Weight data are based on all fish in this part of the trial (110-116 fish per diet group), and SGR and weight gain in % is also calculated for all fish individually (due to PIT-tag). Length is given as fork length. Feed intake and FCR are given for the entire period from week 12 to 38 and are calculated per tank (n=2 per diet group). Length, CF and yield in week 38 are based on 40 fish per diet group.

	SFA10	SFA10C	SFA25	SFA25C	ANOVA SFA	ANOVA Chol	Interaction
Weight, week 12, g	486 ± 64	526 ± 82	539 ± 80	529 ± 72	p<0.0001	p=0.03	p=0.0003
SGR, week 12-25	1.10 ± 0.14	1.02 ± 0.16	1.04 ± 0.15	1.09 ± 0.14	n.s.	n.s., p=0.17	p<0.0001
Gain %, week 12-25	170 ± 32	150 ± 34	155 ± 31	165 ± 31	n.s.	n.s., p=0.13	p<0.0001
Weight, week 25, g	1315 ± 236	1313 ± 240	1364 ± 229	1401 ± 221	p=0.002	n.s.	n.s.
SGR, week 25-38	0.72 ± 0.13	0.76 ± 0.15	0.76 ± 0.15	0.77 ± 0.11	p=0.03	n.s., p=0.07	n.s.
Gain %, week 25-38	97 ± 23	104 ± 28	105 ± 26	106 ± 21	p=0.04	n.s., p=0.08	n.s., p=0.12
Weight, week 38, g	2563 ± 434	2650 ± 522	2774 ± 505	2842 ± 460	p<0.0001	n.s., p=0.09	n.s.
Length, week 38, cm	57.6 ± 6.4	55.8 ± 2.0	56.9 ± 2.5	57.9 ± 4.6	n.s.	n.s.	n.s., p=0.06
CF, week 38	1.46 ± 0.28	1.59 ± 0.10	1.57 ± 0.08	1.53 ± 0.20	n.s.	n.s.	p=0.006
Yield %, week 38	87.2 ± 1.3	87.3 ± 5.2	88.2 ± 1.3	87.8 ± 1.2	n.s., p=0.13	n.s.	n.s.

SGR; Specific growth rate, CF; condition factor. Yield = gutted weight as % of total weight.

Table 4. Results on muscle FA profile (% of total fatty acids), total FAs, crude lipid, cholesterol and astaxanthin, after 12 weeks of feeding the 7 experimental diets. Analyzed on pooled samples per tank (n=3 per diet group), each pooled sample consisting of NQC from 6 fish. The column “Reg.” shows the results from the regression analyses, with “1st” for data that fit a first order polynomial (linear regression) with a slope significantly different from zero, and “2nd” for data where a second order polynomial is a significantly better fit than the first order polynomial (complete results from the regression analyses can be found in the supplementary material; table 1, 2 and figure 1). The three columns to the right show the results from two-way ANOVA with high/low SFA and high/low CHOL as the categorical factors (only data from groups SFA10, SFA10C, SFA25 and SFA25C are included). All numbers are given as mean \pm SD.

	SFA10	SFA10C	SFA15	SFA20	SFA25	SFA25C	SFA30	Reg.	SFA	CHOL	Inter-action
16:0	8.6 \pm 0.0	8.5 \pm 0.0	10.9 \pm 0.3	13.9 \pm 0.1	16.4 \pm 0.1	16.5 \pm 0.2	18.9 \pm 0.1	1st	p<0.0001	n.s.	n.s.
18:0	2.7 \pm 0.0	2.7 \pm 0.1	2.8 \pm 0.1	3.1 \pm 0.0	3.3 \pm 0.0	3.3 \pm 0.1	3.4 \pm 0.1	1st	p<0.0001	n.s.	n.s.
sum SFA	12.7 \pm 0.1	12.5 \pm 0.3	15.2 \pm 0.1	18.3 \pm 0.2	21.1 \pm 0.1	21.2 \pm 0.2	23.8 \pm 0.2	1st	p<0.0001	n.s.	n.s.
18:1n-9	40.3 \pm 0.1	40.5 \pm 0.5	40.7 \pm 0.2	39.9 \pm 0.2	38.8 \pm 0.2	38.7 \pm 0.1	37.7 \pm 0.1	2nd	p<0.0001	n.s.	n.s.
sum MUFA	49.4 \pm 0.1	49.3 \pm 0.4	48.5 \pm 0.1	47.2 \pm 0.2	45.9 \pm 0.1	45.9 \pm 0.1	44.4 \pm 0.1	2nd	p<0.0001	n.s.	n.s.
18:2n-6	16.0 \pm 0.1	16.2 \pm 0.1	15.3 \pm 0.1	14.7 \pm 0.2	13.9 \pm 0.1	14.0 \pm 0.1	13.4 \pm 0.2	1st	p<0.0001	p=0.04	n.s.
20:4n-6	0.9 \pm 0.0	1.0 \pm 0.0	0.8 \pm 0.1	0.8 \pm 0.0	0.8 \pm 0.0	0.8 \pm 0.0	0.7 \pm 0.8	1st	p<0.0001	p=0.004	n.s.
Sum n-6	18.9 \pm 0.1	19.2 \pm 0.1	18.2 \pm 0.1	17.5 \pm 0.2	16.7 \pm 0.1	16.7 \pm 0.1	16.0 \pm 0.2	1st	p<0.0001	p=0.001	p=0.01
18:3n-3	7.6 \pm 0.1	7.9 \pm 0.1	6.5 \pm 0.1	5.7 \pm 0.1	4.8 \pm 0.1	4.9 \pm 0.0	4.0 \pm 0.1	2nd	p<0.0001	p=0.04	n.s.
20:5n-3 (EPA)	1.7 \pm 0.0	1.7 \pm 0.1	1.9 \pm 0.1	1.9 \pm 0.1	1.8 \pm 0.0	1.9 \pm 0.1	1.9 \pm 0.1	n.s.	p=0.01	n.s., p=0.08	n.s., p=0.08
22:6n-3 (DHA)	6.5 \pm 0.0	6.4 \pm 0.3	6.5 \pm 0.1	6.5 \pm 0.2	6.6 \pm 0.2	6.5 \pm 0.1	7.0 \pm 0.1	2nd	n.s.	n.s.	n.s.
EPA+DHA	8.2 \pm 0.1	8.0 \pm 0.5	8.4 \pm 0.1	8.4 \pm 0.1	8.5 \pm 0.2	8.4 \pm 0.1	8.9 \pm 0.1	1st	n.s., p=0.07	n.s.	n.s.
Sum n-3	17.8 \pm 0.0	17.8 \pm 0.4	16.7 \pm 0.2	15.8 \pm 0.1	15.0 \pm 0.2	14.9 \pm 0.1	14. \pm 0.2	2nd	p<0.0001	n.s.	n.s.
n-6/n-3	1.1 \pm 0.0	1.1 \pm 0.0	1.1 \pm 0.0	1.1 \pm 0.0	1.1 \pm 0.0	1.1 \pm 0.0	1.1 \pm 0.0	n.s.	n.s.	n.s.	n.s.
Total FA g kg⁻¹	92.2 \pm 1.3	94.2 \pm 13.5	97.7 \pm 5.6	94.3 \pm 1.2	95.4 \pm 2.4	97.9 \pm 3.5	89.9 \pm 4.5	2nd	n.s.	n.s.	n.s.
Crude lipid, g kg⁻¹	105 \pm 0	107 \pm 14	106 \pm 5	108 \pm 3	110 \pm 2	112 \pm 6	101 \pm 4	2nd	n.s.	n.s.	n.s.
Chol, mg kg⁻¹	344 \pm 97	361 \pm 71	340 \pm 26	414 \pm 43	368 \pm 87	374 \pm 24	326 \pm 6	n.s.	n.s.	n.s.	n.s.
Asta, mg kg⁻¹	1.35 \pm 0.07	1.80 \pm 0.26	1.27 \pm 0.06	1.50 \pm 0.17	1.50 \pm 0.10	1.67 \pm 0.06	1.40 \pm 0.17	n.s.	n.s.	p=0.01	n.s. (p=0.18)

Table 5. Muscle FA composition muscle, proximate composition and cholesterol at the end of the trial (week 38). Data are presented as mean \pm SD. For fatty acids, protein and dry matter, samples from 5 individual fish per tank have been analyzed, while samples from 10 individual fish per tank were analyzed for cholesterol and 20 individual fish per tank were analyzed for crude fat. Statistical results are from two-way ANOVA with high/low SFA and high/low CHOL as the categorical factors.

	SFA10	SFA10C	SFA25	SFA25C	ANOVA-SFA	ANOVA-chol	Inter-action
16:0	7.9 \pm 0.2	7.8 \pm 0.2	17.5 \pm 0.3	18.3 \pm 0.4	p<0.0001	p=0.001	p<0.0001
18:0	2.5 \pm 0.1	2.4 \pm 0.1	3.2 \pm 0.1	3.2 \pm 0.2	p<0.0001	n.s.	n.s.
Sum SFA	11.4 \pm 0.3	11.2 \pm 0.2	21.8 \pm 0.3	22.6 \pm 0.4	p<0.0001	p=0.003	p<0.0001
18:1n-9	42.0 \pm 0.4	42.1 \pm 0.2	38.5 \pm 0.4	38.0 \pm 0.4	p<0.0001	n.s.	p=0.01
Sum MUFA	50.5 \pm 0.5	50.5 \pm 0.4	45.3 \pm 0.4	44.8 \pm 0.4	p<0.0001	n.s.	n.s.
18:2n-6	16.1 \pm 0.2	16.4 \pm 0.2	13.7 \pm 0.1	13.5 \pm 0.2	p<0.0001	n.s.	p=0.0002
20:4n-6	1.2 \pm 0.1	1.2 \pm 0.1	0.9 \pm 0.0	1.0 \pm 0.0	p<0.0001	p=0.03	n.s.
Sum n-6	19.4 \pm 0.2	19.6 \pm 0.1	16.6 \pm 0.1	16.4 \pm 0.2	p<0.0001	n.s.	p=0.0004
18:3n-3	8.9 \pm 0.2	9.1 \pm 0.2	5.8 \pm 0.1	5.8 \pm 0.2	p<0.0001	n.s.	p=0.03
20:5n-3 (EPA)	1.5 \pm 0.1	1.5 \pm 0.1	1.6 \pm 0.1	1.6 \pm 0.1	p=0.001	n.s.	p=0.02
22:6n-3 (DHA)	5.8 \pm 0.3	5.8 \pm 0.3	6.3 \pm 0.2	6.2 \pm 0.2	p<0.0001	n.s.	n.s.
EPA+DHA	7.3 \pm 0.4	7.3 \pm 0.2	7.9 \pm 0.2	7.8 \pm 0.2	p<0.0001	n.s.	n.s.
Sum n-3	17.8 \pm 0.5	17.9 \pm 0.4	15.3 \pm 0.3	15.1 \pm 0.3	p<0.0001	n.s.	n.s.
n-6/n-3	1.1 \pm 0.3	1.1 \pm 0.0	1.1 \pm 0.0	1.1 \pm 0.3	n.s.	n.s.	n.s.
Total FA, g kg⁻¹	154.2 \pm	145.0 \pm	150.5 \pm	154.5 \pm	n.s.	n.s.	n.s.
Protein, g kg⁻¹	200 \pm 12	203 \pm 5	209 \pm 6	199 \pm 7	n.s.	n.s.	p=0.02
Lipid, g kg⁻¹	157 \pm 29	154 \pm 18	159 \pm 21	157 \pm 20	n.s.	n.s.	n.s.
Dry matter, g kg⁻¹	369 \pm 25	365 \pm 12	374 \pm 17	373 \pm 18	n.s.	n.s.	n.s.
Chol, mg kg⁻¹	456 \pm 205	437 \pm 161	440 \pm 99	431 \pm 132	n.s.	n.s.	n.s.

Table 6. Fillet colour, measured by Digital SalmoFan and Minolta. Minolta from section D of the muscle. Data are presented as mean \pm SD. Statistical results are from two-way ANOVA with high/low SFA and high/low CHOL as the categorical factors. The four diet groups are SFA10; containing 10% saturated fat (SFA) as % of total fatty acids, SFA10C; 10 % SFA and added cholesterol (CHOL), SFA25; 25 % SFA and SFA25C; 25 % SFA and added CHOL.

	SFA10	SFA10C	SFA25	SFA25C	ANOVA SFA	ANOVA Chol	Inter- action
Digital SalmoFan™	23.6 \pm 1.1	24.8 \pm 1.0	25.0 \pm 1.0	25.2 \pm 0.9	p<0.0001	p<0.0001	p=0.002
Fillet lightness, L*- value	40.5 \pm 2.0	40.0 \pm 2.1	39.8 \pm 2.0	39.9 \pm 2.4	n.s.	n.s.	n.s.
Fillet redness, a*- value	9.9 \pm 1.4	11.0 \pm 1.5	11.1 \pm 1.4	11.5 \pm 1.4	p=0.0005	p=0.001	n.s.
Fillet yellowness, b*- value	7.8 \pm 1.5	8.5 \pm 1.5	8.6 \pm 1.3	9.1 \pm 1.2	p=0.002	p=0.01	n.s.

Author contributions, CRediT:

Conceptualization: N.H.S, I.S, G.R., B.R.; Data curation: N.H.S, I.S.; Formal analysis: N.H.S, T.L.; Funding acquisition: N.H.S, I.S, G.R., B.R. Ø.S., A.P.J.P.; Investigation: N.H.S, I.S, G.R., B.R. Ø.S., A.P.J.P.; Methodology: N.H.S, I.S, G.R.; Project administration: B.R, N.H.S.; Resources; I.S, G.R., B.R, N.H.S, T.L.; Validation: N.H.S., I.S., Ø.S.; Visualization; N.H.S.; Writing: N.H.S.; Writing – review and editing: all coauthors.

Journal Pre-proof

Dear Editor,

I confirm that none of the authors have any conflict of interest.

Best regards,

Nini H. Sissener

Figure texts:

Figure 1. Overview of the experimental design. The trial started with 7 diets (5 levels of SFA, and two of these also with added CHOL) fed to triplicate tanks. Fish were weighed after 7 weeks to assess performance, before extensive sampling from all diet groups after 12 weeks. After this sampling, only four diet groups were continued (SFA10, SFA10C, SFA25, SFA25C) in duplicate tanks and fed 6 mm pellets. After 25 weeks, all fish were weighed, and pellet size was changed from 6 to 9 mm pellet. The final sampling was conducted after 38 weeks (9 months) of feeding.

Figure 2. Sections of the fillet for measurements with Minolta Chrome Meter.

Figure 3. Fish weights (after 12 weeks) related to dietary saturated fatty acids (SFA). Fish weights fitted (the dots show all individual fish weights) with a second order polynomial function, including 95% confidence interval of the curve. The two diet groups fed additional cholesterol are not included in this analysis/graph.

Figure 4. Retention of 16:0 and cholesterol. Apparent retention (gain of nutrient in % of the amount of nutrient eaten) in the feeding period from week 7-12 for the saturated fatty acid 16:0 and cholesterol (CHOL). Retention of 16:0 is shown for the 5 diet groups with increasing level of SFA (the two with additional CHOL are not included), with a fitted curve (second order polynomial function) including 95% confidence interval of the curve. For CHOL, the five diet groups with no added CHOL (lowCHOL) and the two groups with added CHOL (highCHOL) are compared, with the figure showing mean and the 95% confidence interval for the mean.

Figure 5. Feed intake and feed utilization. The figure shows feed intake (unit: % of body weight/day) and feed conversion ratio, in the second phase of the trial from week 12 to 38. Data are shown as mean \pm standard deviation. The four diet groups are SFA10; containing 10% saturated fat (SFA) as % of total fatty acids, SFA10C; 10% SFA and added cholesterol (CHOL), SFA25; 25% SFA and SFA25C; 25% SFA and added CHOL.

Figure 6. Fillet astaxanthin after 38 weeks. Data are based on 20 individual fish per tank (40 per diet group), and presented as mean \pm SD. Statistical results are from two-way ANOVA with high/low SFA and high/low CHOL as the categorical factors. The four diet groups are SFA10; containing 10% saturated fat (SFA) as % of total fatty acids, SFA10C; 10% SFA and added cholesterol (CHOL), SFA25; 25% SFA and SFA25C; 25% SFA and added CHOL.

Figure 7. Liquid loss and muscle firmness. Data are presented as mean \pm SD. Statistical results are from two-way ANOVA with high/low SFA and high/low CHOL as the categorical factors. The four diet groups are SFA10; containing 10% saturated fat (SFA) as % of total fatty acids, SFA10C; 10% SFA and added cholesterol (CHOL), SFA25; 25% SFA and SFA25C; 25% SFA and added CHOL.

Highlights:

- Low dietary saturated fatty acids (SFA,10 % of FA) reduce Atlantic salmon growth
- Added dietary cholesterol (CHOL) can partially mitigate this effect
- Both SFA and CHOL are produced endogenously when dietary levels are low
- Low SFA increases liquid loss from fillet after freezing
- Low SFA and CHOL in combination reduce fillet colour and firmness

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Experimental time line:

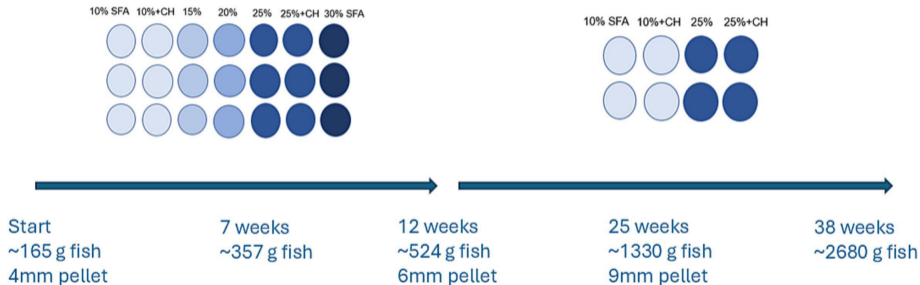


Figure 1

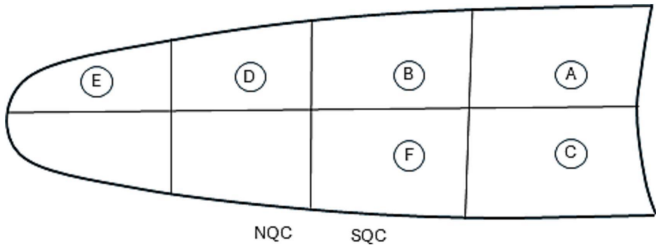


Figure 2

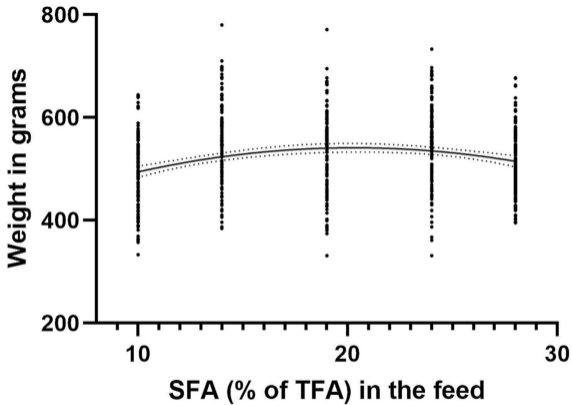
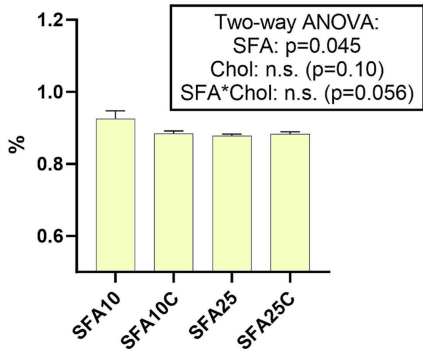


Figure 3

Feed intake



Feed conversion ratio

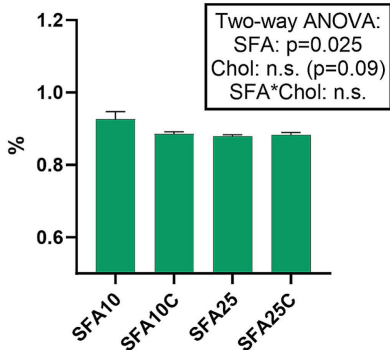
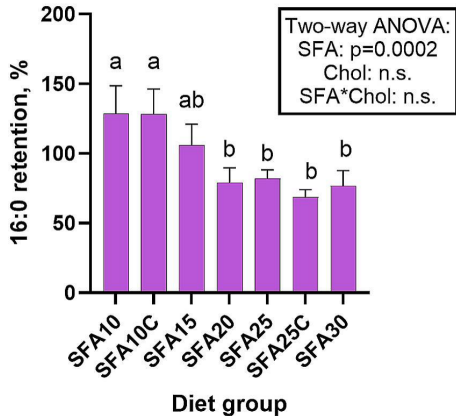


Figure 4

16:0 retention



CHOL retention with 95% C.I.

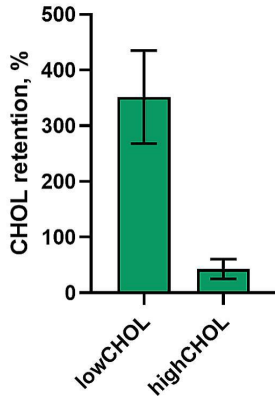
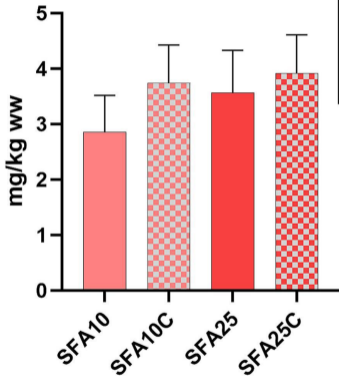


Figure 5

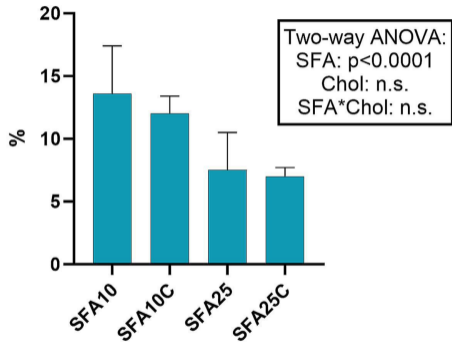
Fillet astaxanthin



Two-way ANOVA:
SFA: $p=0.0001$
CHOL: $p<0.0001$
SFA*CHOL: $p=0.016$

Figure 6

Liquid loss after freezing and thawing



Muscle firmness, distal measurement

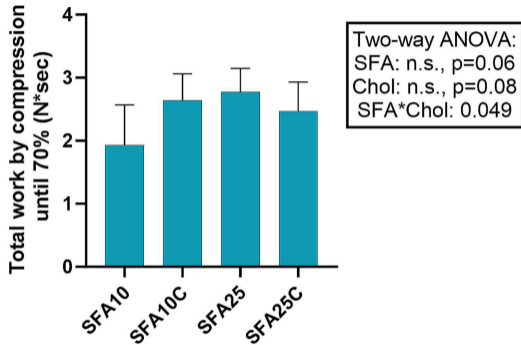


Figure 7