



Aquaterra[®], A Novel
Source of Omega-3,
Delivers Aquaculture
Improved Production with
Unique Fatty Acid Profile

Successful results of industrial scale trials



Applied Research on the Use of Aquaterra®, a New Source of Omega-3 for Use in Salmon Feed.

Successful results of industrial-scale trials

Sergio Silva¹, Pablo Berner², Malcolm Devine³, Benita Boettner²

¹ BeClever SPA, sergiosilvapalma@gmail.com

² Nuseed Global Management, pablo.berner@nuseed.com; benita.boettner@nuseed.com

³ Nuseed Research and Development, malcolm.devine@nuseed.com;

ABSTRACT

Diet is a major factor in the health, growth, and quality of farmed salmon. In particular, the long chain polyunsaturated fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are essential nutrients in salmon feed. These nutrients are historically sourced from ocean-caught fish; however, the ocean cannot provide enough of these nutrients to sustainably supply the rapidly growing aquaculture industry. As a result, the aquaculture industry needs alternative sources of Omega-3 nutrients. This article describes Aquaterra® Omega-3 oil, a novel source of DHA+ EPA delivered via canola at fish oil equivalency (9-11% DHA+EPA); Aquaterra® is also an excellent source of alpha linolenic acid (ALA), important for achieving a desirable ω -6/ ω -3 ratio. Previous tank trials in Norway demonstrated the efficacy of Aquaterra® Omega-3 oil as a partial replacement for fish oil in aquaculture feed.

Three commercial scale trials were conducted in Chile in 2018-19. The results indicate inclusion of Aquaterra® Omega-3 oil in aquaculture feed delivers a favorable FCR compared to the control diet, while maintaining fish growth and feed intake. This oil contributes to marked improvement in FIFO and FFDR, reducing dependence on marine ingredients and addressing sustainability goals of the industry. The Aquaterra® Omega-3 oil diet led to a significant increase in the total level of Omega-3 fatty acids in the fillets of fish at harvest and resulted in a more favorable ω -6/ ω -3 ratio. Survivability of fish fed Aquaterra Omega-3 oil diets improved, measured in lower fish mortality of 1.49-1.90%. Aquaterra® Omega-3 oil is confirmed as an excellent land-based complement to traditional marine ingredients to meet the nutritional requirements of salmon farmed for human consumption.

Introduction

All feeds manufactured for use in salmonid aquaculture contain marine ingredients. Although inputs of marine origin are renewable resources, there are limits to the quantities the world's oceans can supply. Whereas aquaculture has delivered an impressive increase in fish for human consumption, wild-catch fisheries have maintained static production levels since the late 1980s. Thus, the shortage of raw marine materials challenges the economic and environmental sustainability of fisheries and aquaculture (FAO, 2018).

According to SalmonChile (2020), 84% of salmonids produced in Chile in 2018 meet at least one of these certification standards: Best Aquaculture Practices, GlobalGap, or Aquaculture Stewardship Council (ASC). These standards specify criteria on including marine origin inputs in the feed. Global Salmon Initiative member companies represent the world's major salmon producers in Chile, Norway, North America, and other regions. Member companies have achieved ASC certification for 273 farms with 82 in process; 60% of the production is certified and these companies have a commitment to reach 100% (GSI, 2020). These standards include requirements on the incorporation of inputs of marine origin into feed, which results in sustainability

indicators such as Fish In: Fish Out (FIFO) and Forage Fish Dependency Ratio (FFDR).

As the availability of marine origin oil declines, it needs to be replaced by other lipid sources. This has drastically influenced the formulation of fish feed, leading to higher incorporation of plant-derived and animal by-products, where approved, replacing marine ingredients (Skretting, 2014; Marine Harvest, 2016).

According to Ytrestøyl and Åsgård (2015), the replacement of marine oils with plant-based sources has been increasing in Norway, with marine oil content declining from 24% in 1990 to 10.9% in 2013. Mowi's annual report for 2018 indicates their use of fish oil in salmon diets was 10% (Mowi, 2019). The Chilean average marine oil incorporation was 7% in 2017. (Marine Harvest, 2018).

Importance of Omega-3 fatty acids for human and fish nutrition

Consumers are increasingly focused on food functionality. Salmonids are frequently cited as a source of long-chain polyunsaturated fatty acids, and the health benefits these confer.

Thus, EPA (20:5n-3) and DHA (22:6n-3) are considered fatty acids of interest in animal and human nutrition.

A decline in EPA and DHA content in salmon produced in Norway (Sissener et al., 2016) and Scotland (Sprague, 2016) has been reported, and although there are no reports from Chile, everything seems to indicate the same trend. However, farmed salmon thus far still offers more EPA+DHA for human consumption than most other fish species and more than any land animals (Sprague et al., 2016).

In human nutrition, both EPA and DHA are considered to have beneficial effects in neurodegenerative diseases (Martínez et al., 2018), cardiovascular health (Balk and Lichtenstein, 2017; Punia et al., 2019), hypertension (Colussi et al., 2016), inflammation (Layé et al., 2018) and cancer (D'Eliseo and Velotti, 2016). DHA, in particular, has a positive influence on fluidity and permeability of cell membranes (Stillwell and Wassall, 2003). DHA is a predominant fatty acid in the central nervous system and retina and plays an essential role in brain development (Singh, 2005).

The composition of dietary fatty acids can also affect many aspects of fish growth and health. According to Roberts (2012), nutritionally compromised diets often increase a species' susceptibility to infectious diseases. In this regard, there is now growing concern about the effect that low availability of Omega-3 fatty acids could have on the ability of fish to survive disease or environmental challenges.

The minimum long-chain Omega-3 fatty acid requirement in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) has been estimated at 1% (Hardy 2001, Storebakken 2001, Rosenlund et al. 2016, Sissener et al. 2016). However, another long-term experiment with Atlantic salmon grown in sea cages showed that 1% of EPA + DHA in the feed was insufficient under adverse environmental conditions. Salmon fed 1% EPA + DHA or lower had significantly higher mortality rates than salmon fed 1.7% (Ruyter et al., 2016).

Another seawater trial with Atlantic salmon from 150 g to 5,000 g tested two fish stocks with feed containing 1.6% or 2.6% EPA+DHA. In this case the population fed with the low Omega-3 diet had a higher frequency of melanin spots in the fillets (28.2% vs 21.5%), possibly because of the role that fatty acid balance plays in the inflammatory process. The authors concluded that EPA+DHA levels of 1.6% and higher in fish feed appear to be sufficient under certain conditions, although there are also indications that higher levels may be positive in some disease situations (Sissener et al., 2016).

A study with Atlantic salmon suggests that low dietary EPA and DHA may interrupt the barrier function of fish skin, due to changes in the phospholipid profile (Cheng et al., 2018). On the other hand, dietary DHA supplementation is related to the capacity to keep the normal structure and improve the deficiency symptoms in the intestinal epithelium, such as swollen enterocytes and vacuolization (Bou et al., 2017). In a study conducted with Atlantic salmon in freshwater (around 50-190 g), it was concluded that EPA appears likely dispensable and even detrimental when provided in excess. The majority of EPA from the diet is catabolized or converted into DHA. Little-to-no DHA is catabolized, and the DHA from the diet is efficiently deposited in the tissues (Emery, 2015). According to Bou (2017), results from Atlantic salmon reared in

Analyte		Standard Canola	Aquaterra® Omega-3
Palmitic	C16:0	3.9	4.3
Stearic	C18:0	1.6	2.7
Oleic	C18:1n-9c	63.6	42.3
Linoleic	C18:2n-6c	13.1	7.2
α -linolenic acid (ALA)	C18:3n-3	10.3	20.2
Stearidonic acid (SDA)	C18:4n-3	0.0	2.3
eicosapentaenoic acid (EPA)	C20:5n-3	0.0	0.4
Docosapentaenoic acid (DPA)	C22:5n-3	0.0	0.9
docosahexaenoic acid (DHA)	C22:6n-3	0.0	9.2
Sum: EPA+DPA+DHA		0.0	10.5
Total Omega-3		10.4	33.5
Total Omega-6		11.3	7.8
Omega-6/ Omega-3		1.1	0.23
Total Saturated Fatty Acids		6.7	8.3
Total Monounsaturated Fatty Acids		68.9	46.9
Total Polyunsaturated Fatty Acids		23.5	41.3

Table 1. Percentage of major fatty acids contained in conventional canola oil and Aquaterra® Omega-3 oil. Minor fatty acids omitted from the table.

seawater (around 50 g – 400 g) suggested that dietary EPA was converted to DHA in group diets lacking DHA with very limited retro conversion of DHA to EPA. That background suggests that a low ratio EPA: DHA in the diet could be beneficial.

The aquaculture industry needs new sources of oils for fish nutrition. These sources must provide the essential Omega-3 fatty acids to meet the nutritional requirements of the fish, to meet the challenges of fish farming. These fatty acids should also be delivered at a certain level to maintain the appropriate composition in the salmon fillet as a source of healthy lipids for human consumption.

Faced with this need, Nuseed - in collaboration with the Commonwealth Scientific and Industrial Research Organization (CSIRO) and the Grains Research and Development Corporation (GRDC) - applied advanced plant genetics to develop Aquaterra®, a land-based Omega -3 oil delivering the nutritional benefits of microalgae via canola to benefit the aquaculture industry.

Aquaterra® Omega-3 oil contains significant levels of DHA and other long-chain polyunsaturated fatty acids. The oil also contains high levels of alpha linolenic acid (ALA, 18:3n-3) and has a much lower ω -6/ ω -3 ratio in comparison with conventional canola oil (see Table 1). The unique combination of high DHA, high ALA and low ω -6/ ω -3 ratio are the major attributes of Aquaterra® Omega-3 oil.

Previous Research with Aquaterra® Omega-3 Oil

The Norwegian aquaculture industry is interested in investigating novel sources of fatty acids for fish nutrition for the sustainable growth of the industry. In 2016 the Norwegian Institute of Food, Fisheries and Aquaculture (NOFIMA) conducted a research study to validate the safety and effectiveness of Aquaterra® Omega-3 oil as an ingredient in salmon feed, this study was funded by the Norwegian Seafood Research Fund (NHF).

The objectives of the study were: (1) to evaluate the partial replacement of fish oil with Aquaterra® Omega-3 oil in fry diets, from 1 g to 20 g in freshwater; (2) to evaluate the effect on the replacement of conventional canola oil by Aquaterra® Omega-3 oil in salmon diets from 450 g to 1,500 g in seawater; and (3) to

determine feed safety in the use of Aquaterra® Omega-3 oil by analyzing the oil for pollutants and unwanted substances.

Growth and survival were excellent in all diets, in both freshwater and seawater. The replacement of fish oil by Aquaterra® Omega-3 oil in the freshwater trial had no adverse effect on salmon growth and survival (Ruyter et al., 2019). In the seawater phase, in which Aquaterra® Omega-3 oil increasingly replaced conventional canola oil, growth and survival were also excellent. In this trial the content of DHA and ALA in the fillets increased as the inclusion rate of Aquaterra® Omega-3 oil increased. The EPA content of Aquaterra® Omega-3 oil is relatively low (0.5%) compared to fish oil. However, the EPA content in the fillet also increased, suggesting the fish had the physiological capacity to produce EPA from ALA. This was confirmed by a separate experiment, which showed that some of the ALA in the diet was converted to EPA, thus generating a compensatory effect in favor of Aquaterra® Omega-3 oil (Ruyter, unpublished results). Concerning fish health, the hepatosomatic and cardio-somatic index did not differ, nor were there any differences in biological markers (expression of genes and enzymes) associated with health status.

The study also showed there were no significant levels of pesticides or organic pollutants in the oil. More recent independent analyses

Trial	Trial 1		Trial 2		Trial 3	
	Control	Aquaterra® Omega-3	Control	Aquaterra® Omega-3	Control	Aquaterra® Omega-3
Initial weight (g)	1.623	1.585	1.240	1.134	1.158	1.131
Gained weight (g)	3.703	3.680	4.994	4.889	5.593	5.552
SGR	0,48	0,48	0,52	0,56	0,81	0,83
SFR	0,62	0,61	0,72	0,76	1,07	1,12
FCRb	1,28	1,28	1,40	1,37	1,37	1,35
FCRe	1,37	1,34	1,60	1,52	1,43	1,40
TGC/GF ₃	(*)	(*)	1,92	2,01	2,52	2,51
Survival	93,61	95,1	89,28	91,18	88,86	90,73

Table 2. Summary of production performance in three industrial-scale trials. SGR - Specific Growth Rate; SFR - Specific feed rate; FCRb - Biological feed conversion ratio; FCRe - Economic feed conversion ratio; TGC/GF₃ - Thermal growth coefficient. (*) Not available.

Analyte	Lipid Composition g/100 g NQC					
	Trial 1 n=40		Trial 2 n=3		Trial 3 n=25	
	Control	Aquaterra® Omega-3	Control	Aquaterra® Omega-3	Control	Aquaterra® Omega-3
Oleic, 18:1n-9	5,10	5,5*	6,66	6,66	6,69	6,94
ALA, 18:3n-3	0,59	0,75*	0,80	1,18*	0,86	1,07*
EPA, 20:5n-3	0,24	0,23*	0,46	0,37*	0,57	0,55
DHA, 22:6n-3	0,30	0,35*	0,51	0,67*	0,72	0,79*
EPA+DHA	0,55	0,58	0,97	1,04	1,29	1,35
Total ω-6	2,00	2,10*	2,58	2,48	3,78	3,71
Total ω-3	1,30	1,50*	2,15	2,49*	2,56	2,79*
ω-6/ω-3	1,48	1,40*	1,22	1,00*	1,48	1,33
Saturated	1,70	1,80	2,52	2,39	3,36	3,34
Monounsaturated	6,0	6,4	8,02	7,93	8,11	8,42
Polyunsaturated	3,3	3,7*	4,73	4,98	6,34	6,49
Total Lipids	11,0	11,8*	15,3	15,3	17,8	18,3

Table 3. Comparison of lipid and fatty acid content of fillets (NQC) of fish raised on the experimental diets, for each trial. * indicates statistically significant difference with a 95% confidence level.

of Aquaterra® Omega-3 oil have confirmed these results.

Commercial Scale Trials with Aquaterra® Omega-3 Oil

In 2017 Nuseed initiated collaboration with the Chilean salmon industry to confirm the feasibility of using Aquaterra® Omega-3 oil — already validated by Nofima — according to each company's criteria. The effort brought together the expertise of Nuseed, feed producers, fish producers, processing plants, analytical laboratories, and scientific advisors. The objective was evaluating Aquaterra® Omega-3 oil's performance in Chile's challenging production conditions, on a commercial scale.

The trials involved five leading companies among Chilean salmon and feed producers, with a total population of 2,650,000 salmon in trials spread over three fish farms, two in the tenth region and one in the eleventh region.

The main design aspects of the on-farm trials are detailed on the next page.

Materials and Methods

Three feeding tests were carried out on populations of Atlantic

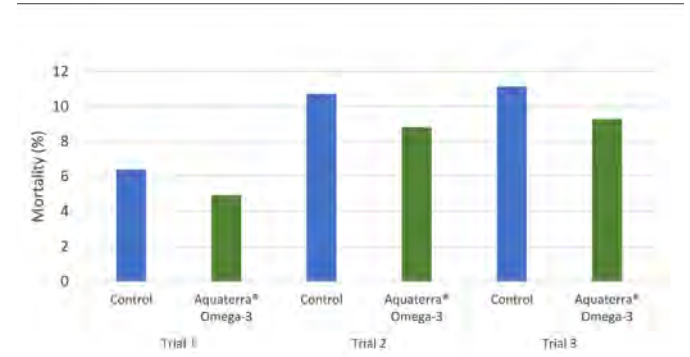


Fig. 1. Mortality Rate Comparison, consolidated in the processing plant.

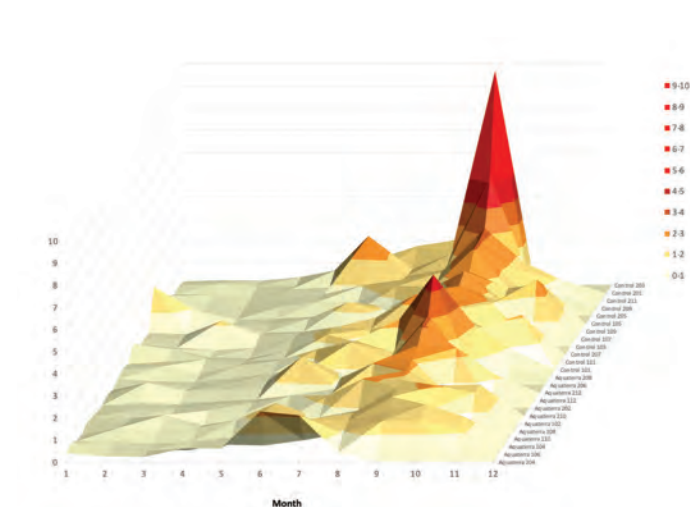


Fig. 2. Cage-to-cage analysis of the evolution of the monthly and cumulative mortality rate (Trial 2)

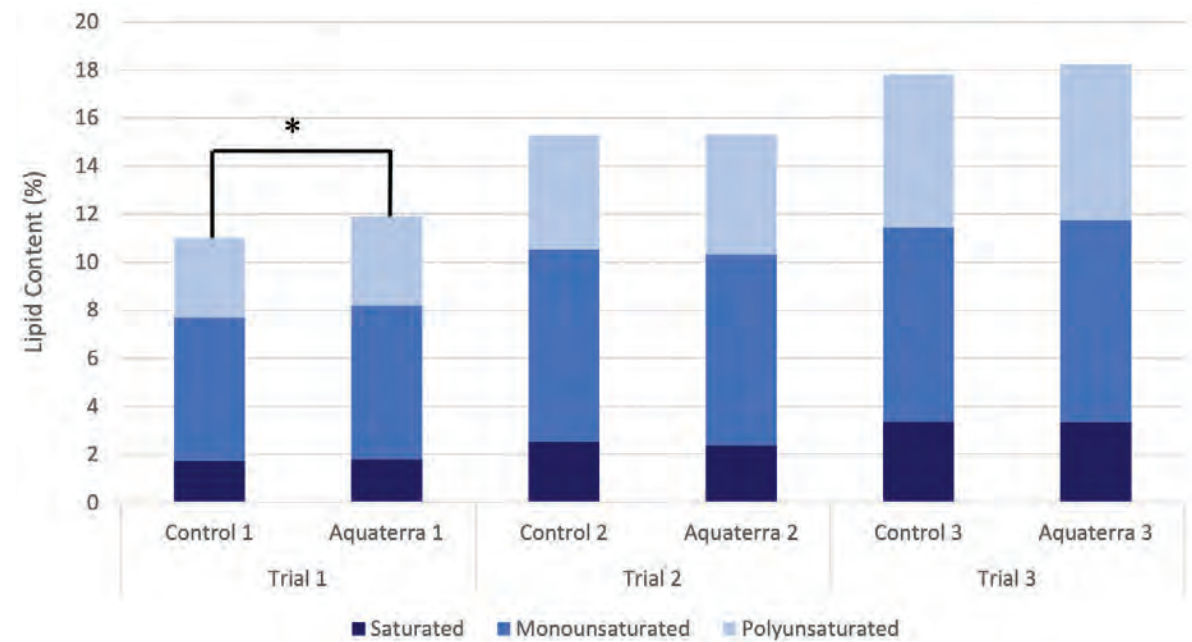


Fig. 3 Saturated, monounsaturated and polyunsaturated fatty acid content of fillets from the three trials. * indicates statistically significant difference with 95% confidence level.

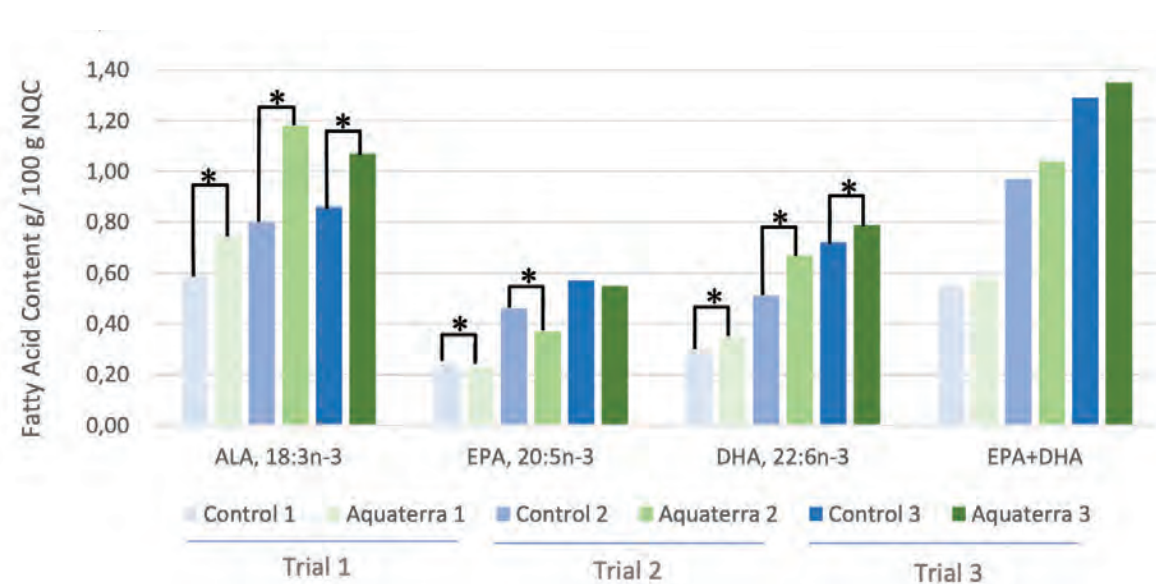


Fig. 4. Comparison of fillet content of the main fatty acids of interest. * indicates statistically significant difference with 95% confidence level.

salmon, with an equivalent number of cages at each site. Trial 1 consisted of 16 cages with approximately 40,000 fish in each cage, with an initial fish weight of approximately 1.6 kg. Trial 2 included 24 cages with about 45,000 fish in each cage and an initial weight of approximately 1.2 kg. Trial 3 included 18 cages with approximately 50,000 fish and an initial weight of approximately 150 g.

The standard formulation used by each feed company was established as the control diet. Incorporation of Aquaterra® Omega-3 oil varied between 3 and 7% of the feed, with a replacement of fish oil with Aquaterra® Omega-3 oil between 30 and 60%. The EPA+DHA content in both the control and Aquaterra® Omega-3 oil diets ranged from 1.7 to 2.0%.

Feed Conversion Ratio (FCR), Specific Growth Rate (SGR), and Survival Rate were recorded as the most relevant productive aspects to evaluate growth.

At harvest, analysis of the NQC fatty acid profile (GC) and lipid content (Soxhlet) were performed by SGS Chile, while the counterpart portion was evaluated by Tracelab for analysis of astaxanthin content (HPLC) and color expression (Salmofan™). Both labs are accredited and expert in their respective fields.

In addition, in Trial 2 a sensory panel composed of 8 people, selected and trained according to strict criteria established in ISO 8586, carried out an organoleptic evaluation in Dictuc's aroma and flavor center (Santiago, Chile).

This trial also included processing plant quality reports associated with production batches from the experimental cages.

As sustainability indicators, the Fish in: Fish out (FIFO) ratio used in the Best Aquaculture Practices Certification Standard (BAP) and the Forage Fish Dependency Ratio for Oil (FFDRo) used in the Aquaculture Stewardship Council standard were used.

Statistical Methodology

The data were evaluated using Software StatgraphicsTM. In the case of simple comparisons Student's T or Wilcoxon test was used. For multiple comparisons ANOVA and Tukey's test were applied to establish differences.

Results

Productive Performance & Survivability

As can be seen in Table 2, there were no significant differences in productive performance between the control and Aquaterra® Omega-3 oil diets. There was a difference, however, in the level of mortality, which influenced the economic conversion factor (FCRe). In Trials 1, 2 and 3 there was 1.49%, 1.90% and 1.87% less mortality in the total consolidated cages fed with Aquaterra® Omega-3 oil compared to the control diets (Fig. 1). In terms of biomass gain, this represents an increase of 44.6 tons, 77.8 tons and 19 tons, at the three sites respectively with the Aquaterra® Omega-3 oil diet compared to the control.

Trial 2 included more data, allowing for deeper analysis of the

results. This trial suffered several environmental stresses, including low oxygen concentration, an amebiasis outbreak and SRS, resulting in a high mortality situation. Mortality increased with both diets over time but was lower in the case of the fish on the Aquaterra® Omega-3 oil diet. To establish whether this result was attributable to a single cage, mortality in each individual cage was examined (Fig. 2). The Aquaterra® Omega-3 oil cages had higher resistance to environmental stresses, resulting in lower mortality and higher biomass compared to the control.

Lipid composition

The composition of the main fatty acids of interest (EPA, DHA, and ALA), and the proportion of saturated, monounsaturated and polyunsaturated fatty acids in the NQC, were determined (Table 3). The data are expressed as g/100 g of NQC.

In addition, the sum of EPA+DHA and ratios of interest, such as the proportion of ω -3 and ω -6 fatty acids, are included, both for fish and human health.

There were no differences in the content of saturated, monounsaturated and polyunsaturated fatty acids in the fillets of fish at harvest in the three trials, apart from an increase in polyunsaturated fatty acids in fish on the Aquaterra® Omega-3 oil diet in Trial 1 (Table 3 and Figure 3).

The level of DHA was higher in fish fed the Aquaterra® Omega-3 oil diets in all trials, with statistically significant differences. The EPA level was slightly lower in the fish fed the Aquaterra® Omega-3 oil diets in Trials 1 and 2. However, in the full sea cycle trial (Trial 3), there was no significant difference in the EPA level between diets. Furthermore, there were no differences in

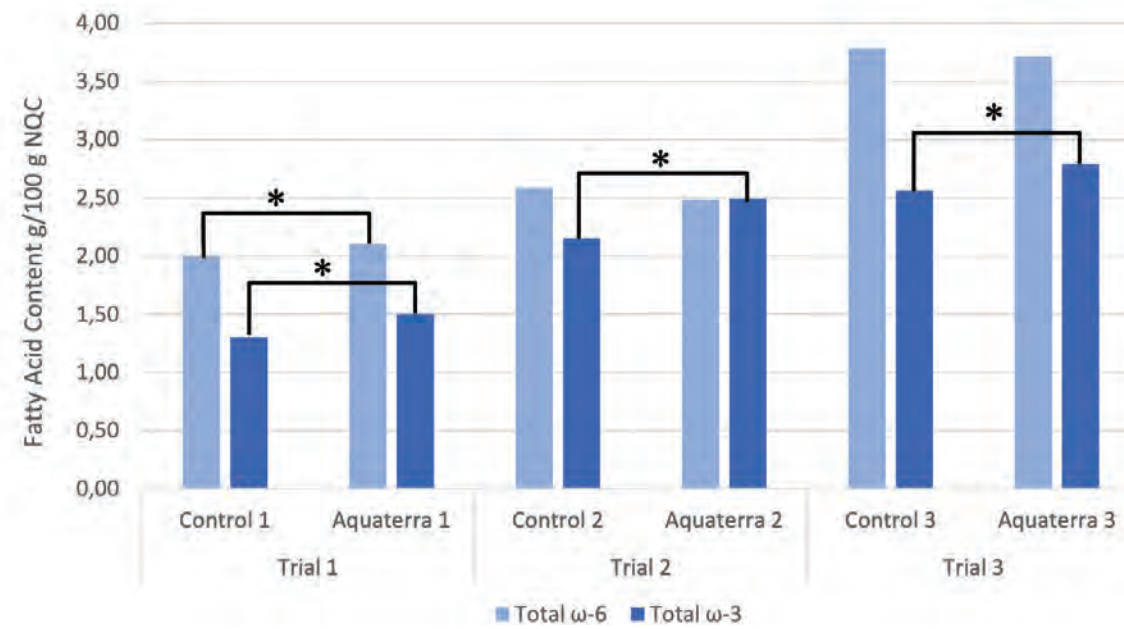


Fig. 5. ω -6 and ω -3 content of fillets from fish fed the two diets. * indicates statistically significant difference with 95% confidence level.

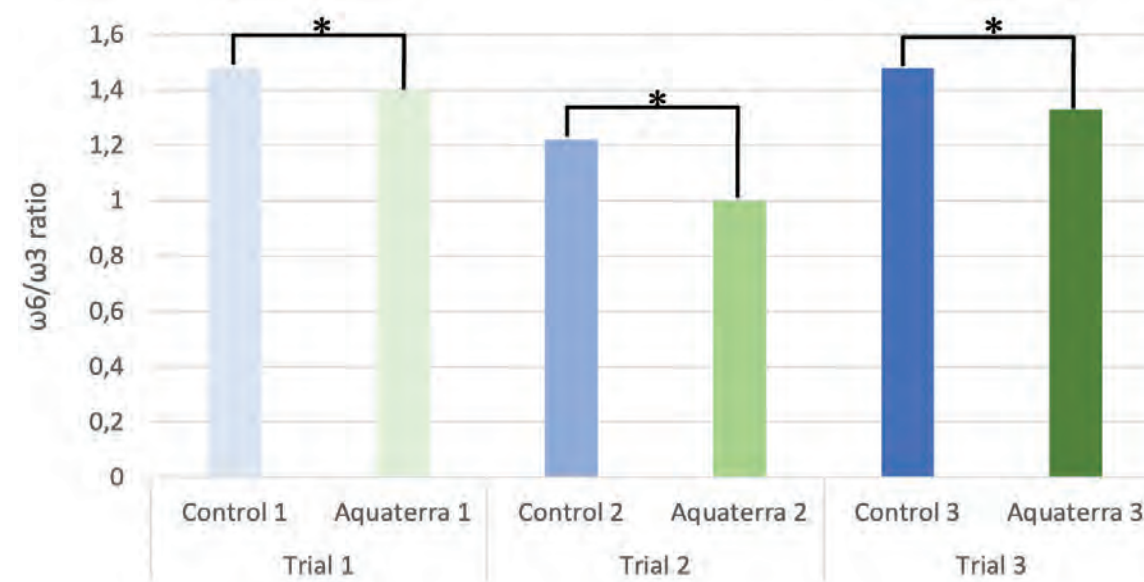


Fig. 6. ω -6/ ω -3 ratio in fillets raised on the Aquaterra® Omega-3 oil or control diets. * indicates statistically significant difference with 95% confidence level.

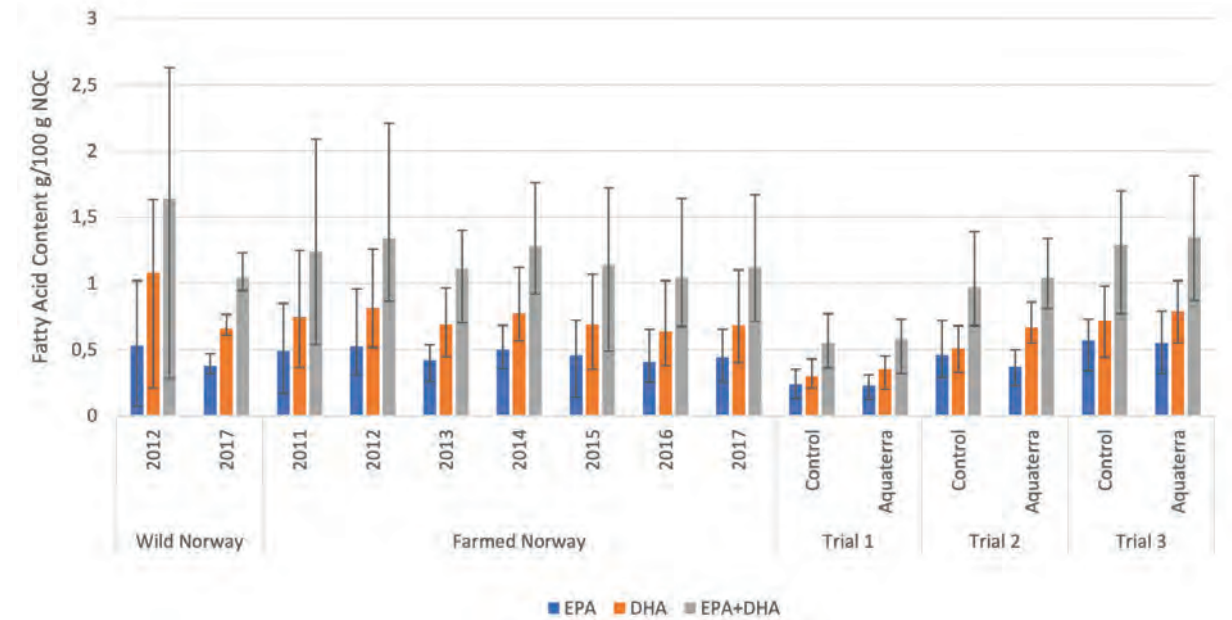


Fig. 7. Comparison of EPA and DHA content from the trials reported here with values obtained in the monitoring conducted by the Institute of Marine Research of Norway, which includes wild and farmed salmon.

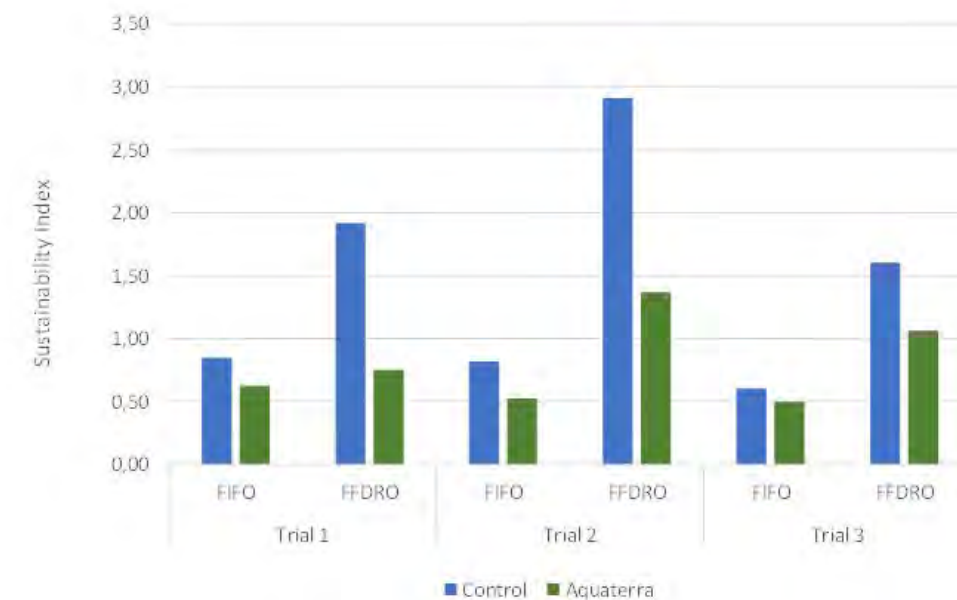


Fig. 8. Comparison of FIFO (BAP) and FFDRo (ASC) sustainability indicators.



terms, with a median of 10.5% in both cases (n=130). For the trim D fillet yield, the median was 60.7% in both dietary treatments.

Astaxanthin Content and Fillet Color

There were no significant differences in astaxanthin content between the control diet and the Aquaterra® Omega-3 oil diet, in any of the trials, with a range between 6.0 to 7.2 ppm. As for the expression of color, Trial 3 was the only test utilizing an Aquaterra® Omega-3 oil diet for the entire cycle, from sea entry to harvest. The color results in this trial were excellent for both diets, although with more readings in Salmofan categories 25 and 26 in fish fed the Aquaterra® Omega-3 oil diet.

Sustainability Indicators

As shown in Figure 8, the sustainability indicators improved markedly with the use of Aquaterra® Omega-3 oil. In all cases, the difference between the Aquaterra® and control diets is accentuated by the difference in the FCR_e and by the effect of the higher survival rate of fish fed the Aquaterra® diet (Table 2).

Organoleptic Analysis

There was no difference in the organoleptic aroma, taste and texture analysis of samples of raw salmon between those fed the Aquaterra® Omega-3 oil diet and the control diet. This is clear in the almost perfect overlap of the radial graph for fish fed the two diets (Fig. 9).

Conclusions

The following conclusions can be drawn from the trials evaluating the performance of Aquaterra® Omega-3 oil under commercial scale Chilean conditions:

Weight gain was excellent in all three trials, with no differences between the two diets. However, the inclusion of Aquaterra® Omega-3 oil consistently reduced total mortality in all three trials, resulting in an improved FCR_e. According to the literature, the DHA+EPA content of the feed may influence the survival rate of fish growing under stress conditions in commercial production (Sissener et al., 2016). The consistent lower mortality rate in all the populations fed with Aquaterra® Omega-3 oil diets in the trials may be due to the unique fatty acid profile and ω -6/ ω -3 ratio.

The major difference in fatty acid content of fillets from fish was an increase in DHA and ALA content in fish on the Aquaterra® Omega-3 oil diet compared to the control, reflecting the high DHA and ALA content of Aquaterra® Omega-3 oil. The Aquaterra® Omega-3 oil diet led to a significant increase in the total level Omega-3 fatty acids and, in this way, resulted in a more favorable ω -6/ ω -3 ratio. Interestingly in Trial 1, although there was more EPA in fillets of fish on the control diet, the difference was minimal, only 19 mg/100 g of fillet. In the full-cycle Trial 3, the EPA level in the fillets was equivalent, with no significant statistical difference. This indicates that despite the low EPA level of Aquaterra® Omega-3 oil it is possible to obtain a fillet EPA profile equal to that in a typical diet. That suggests that some of the ALA in the diet may be converted to EPA, in agreement with previous results obtained by Ruyter (unpublished results).

A comparison of the EPA+DHA content in fish from these trials

with wild and farmed salmon originating in Norway shows that Aquaterra® Omega-3 oil contributes to the achievement of a healthy nutritional composition of the fillet. With an appropriate nutritional strategy, it is feasible to achieve EPA+DHA levels comparable to those obtained in farmed and wild salmon.

The use of Aquaterra® Omega-3 oil did not impact the deposition of astaxanthin in the fillets. There was a higher number of fillets scoring Salmofan categories 25 and 26 from fish fed the Aquaterra® Omega-3 oil diet in Trial 3, with no differences in Trials 1 and 2. As for sensory analysis, no adverse effects were detected on the taste, aroma, or texture (to the palate) of fish raised on a diet including Aquaterra® Omega-3 oil.

Finally, incorporation of Aquaterra® Omega-3 oil contributed to a marked improvement in FIFO and FFDR_o in the three trials, reducing dependence on marine ingredients while addressing the sustainability goals of the industry.

These industry trials confirm Aquaterra® Omega-3 oil as a safe and effective partial replacement of fish oil that will contribute to the sustainable development of the industry and satisfy the nutritional requirements of commercially raised salmon. Aquaterra® Omega-3 oil provides a unique fatty acid profile that leads to improved nutritional status of the fish and maintains the characteristic composition of fatty acids in the fillet that confers the identity of a healthy product typical of salmon and valued by the consumer market.

BIBLIOGRAPHICAL REFERENCES

Balk, E. M. and Lichtenstein, A. H. (2017). Omega-3 fatty acids and cardiovascular disease: Summary of the 2016 agency of healthcare research and quality evidence review. *Nutrients* 9(8). doi: 10.3390/nu9080865.

Bou, M. et al. (2017). Requirements of n-3 very long-chain PUFA in Atlantic salmon (*Salmo salar* L): effects of different dietary levels of EPA and DHA on fish performance and tissue composition and integrity. *Br. J. Nutr.* 117, 30-47.

Cheng, K. et al. (2018). Reduced dietary levels of EPA and DHA have a major impact on the composition of skin membrane lipids in Atlantic salmon (*Salmo salar* L.). *J. Agric. Food Chem.* 66. 10.1021/acs.jafc.8b02886.

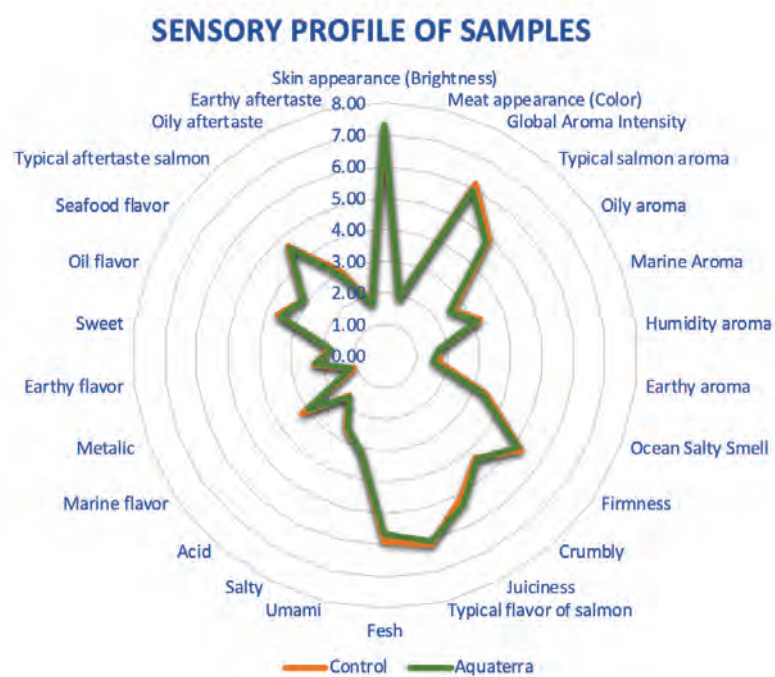
Colussi, G. et al. (2016). Omega-3 polyunsaturated fatty acids in blood pressure control and essential hypertension. In: *Update on Essential Hypertension*. Ed. L. Salazar-Sanchez, IntechOpen, doi: 10.5772/63501.

D'Eliseo, D. and Velotti, F. (2016). Omega-3 fatty acids and cancer cell cytotoxicity: Implications for multi-targeted cancer therapy. *J. Clin. Med.* 5(2) 15. doi: 10.3390/jcm5020015.

EFSA (2009). Labelling reference intake values for n-3 and n-6 polyunsaturated fatty acids. *EFSA J.* 1176, 1-11.

Emery, J.A. et al. (2016). Uncoupling EPA and DHA in fish nutrition: dietary demand is limited in Atlantic salmon and effectively met by DHA alone. *Lipids* 51, 399-412. <https://doi.org/10.1007/s11745-016-4136-y>.

FAO (2018). *El estado mundial de la pesca y la acuicultura 2018*. FAO. Roma.: Licencia: CC BY-NC-SA 3.0 IGO., p. 250. Available at:



This is reflected in a significant decrease in the ω -6/ ω -3 ratio in all trials (Fig. 6).

In addition, a comparison of the EPA and DHA content of fillets (measured in NQC) from the three trials was made with levels typically found in wild Norwegian salmon and salmon farmed in Norway. It is interesting to note that the diets used in the three trials represent low, intermediate and high levels of EPA+DHA when compared with the historical Norwegian diets (Fig. 7), with the diet in Trial 3 resulting in the highest levels of EPA+DHA in the fillets.

It can be concluded that the fillets produced in these trials constitute an important source of EPA and DHA for human consumption. According to the recommendation of the European Food Safety Authority (EFSA, 2009), a 250 mg/d intake of Omega-3 long chain fatty acids (EPA plus DHA) is recommended, which is in accordance with the available evidence on the relationship between intake of these fatty acids and cardiovascular health in healthy populations. In this regard, a 150 g portion of fish from Trials 1, 2 and 3 would cover the requirement for 3.5, 6.2 and 8.1 days, respectively.

Finished Product Quality

Yield in processing plant

In Trial 2, it was possible to track fish to the processing plant to evaluate performance in head-on eviscerated product and Trim D fillet. The processing plant had access to the performance sampling data that is usually developed as a control method, isolating the fish corresponding to diet-fed cages (control and Aquaterra® Omega-3 oil).

There was no difference in the weight of the viscera in percentage

Fig. 9 Summary of organoleptic results (fresh salmon from Trial 2), shown in an intensity graph by attribute. Intensity 0 = the attribute is not perceived; 9 = maximum intensity of the attribute.

the sum of EPA+DHA between the two diets in any trial (Fig. 4). This may be a consequence of the ALA to EPA conversion previously observed in the 2016 NOFIMA study. Additionally, there was a significant increase in the ALA fillet content in fish fed the Aquaterra® Omega-3 oil diet in all three trials (Fig. 4).

The level of total Omega-3 fatty acids was significantly higher in fish fed the Aquaterra® Omega-3 oil diet in all three trials (Fig. 5).

<ftp://ftp.fao.org/docrep/fao/007/y5600s/y5600s00.pdf>.

GSI (2020). Section Find a Farm. Available at <http://asc.force.com/Certificates/>. Last access April 23, 2020.

Hardy, W (2001). Rainbow Trout, *Oncorhynchus mykiss*. Chapter 14. In: Nutrient requirements and feeding of finfish for aquaculture. Eds: Webster, C. and Lim, C. Cabi Publishing.

Layé, S. et al. (2018). Anti-inflammatory effects of Omega-3 fatty acids in the brain: Physiological mechanisms and relevance to pharmacology. *Pharmacol. Rev.* 70(1) 12–38. doi: 10.1124/pr.117.014092.

Marine Harvest (2016). Salmon farming industry handbook 2016 p. 94. Available at: <http://www.marineharvest.no/globalassets/investors/handbook/2016-salmon-industry-handbook-final.pdf>.

Marine Harvest (2018). Salmon Farming Industry handbook 2018. Available at <https://mowi.com/wp-content/uploads/2019/04/2018-salmon-industry-handbook-1.pdf>.

Martínez, E. et al. (2018). Protective effect of Omega-3 fatty acids EPA and DHA in the neurodegenerative disease. In *Bioactive molecules in food*. Eds: Mérillon J. and Ramawat, K.G. Springer. pp. 1–17. doi: 10.1007/978-3-319-78030-6.

Mowi (2019). Salmon Farming Industry Handbook 2019. Available at <https://corpsite.azureedge.net/corpsite/wp-content/uploads/2019/06/Salmon-Industry-Handbook-2019.pdf>.

Punia, S. et al. (2019). Omega 3-metabolism, absorption, bioavailability and health benefits—A review, *PharmaNutrition*, 10, 100162. doi: 10.1016/j.phanu.2019.100162.

Roberts, R. J. (2012). *Fish Pathology*. Fourth Edition. Wiley-Blackwell. Oxford.

Rosenlund, G. et al. (2016). Atlantic salmon require long-chain n-3 fatty acids for optimal growth throughout the seawater period. *J. Nutr. Sci.* 5, p. e19. doi: 10.1017/jns.2016.10.

Ruyter, B. et al. (2016). Langtidseffekter av lave Omega-3-nivåer i

fôr på fiskens helse - fhf.no.02.06.2016.Nofima, p. 52. Available at: <https://www.fhf.no/prosjektdetaljer/?projectNumber=900957>.

Ruyter, B. et al. 2019. n-3 Canola oil effectively replaces fish oil as a new safe dietary source of DHA in feed for juvenile Atlantic salmon. *Br. J. Nutr.* 122, 1329–1345.

SalmonChile (2020). Informe de sustentabilidad Gestión 2018. Available at https://www.salmonchile.cl/sustentabilidad_2018/.

Singh, M. (2005). Essential fatty acids, DHA human brain. *Indian J. Pediatr.* 72(3), 239–242. doi: 10.1007/BF02859265.

Sissener, N. H. et al. (2016). Long-term feeding of Atlantic salmon in seawater with low dietary long-chain n-3 fatty acids affects tissue status of the brain, retina and erythrocytes. *Brit. J. Nutr.* 115, 1919–1929. doi: 10.1017/S0007114516000945.

Sissener, N. et al. (2016). Oppdatering av utredningen: Effekter av endret fettstoffsammensetning i fôr til laks relatert til fiskens helse, velferd og robusthet. Report published by NIFES and Nofima (Norway).

Skretting (2014). Reporte de Sustentabilidad 2014, p. 34. Available at: <http://www.skrettingguidelines.com/readimage.aspx?asset=3291>.

Sprague, M. et al. (2016). Impact of sustainable feeds on Omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006–2015. *Sci. Rep.*, 6, 1–9. doi: 10.1038/srep21892.

Stillwell, W. and Wassall, S. R. (2003). Docosahexaenoic acid: Membrane properties of a unique fatty acid. *Chem. Phys. Lipids.* 126, 1–27. doi: 10.1016/S0009-3084(03)00101-4.

Storebakken, T. (2001). Atlantic salmon, *Salmo salar*. Chapter 6. In: Nutrient requirements and feeding of finfish for aquaculture. Eds: Webster, C. and Lim, C. Cabi Publishing.

Ytrestøl, T. et al. (2015). Utilisation of feed resources in production of Atlantic salmon (*Salmo salar*) in Norway. *Aquaculture* 448, 365–374. doi: 10.1016/j.aquaculture.2015.06.023.

