Comparison of two functional feed strategies for optimization of Atlantic salmon health in the face of typical, real-world challenges to gill and skin integrity

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Abstract

Amoebic gill disease (AGD), proliferative gill disease (PGD) and skin disorders of various causes are major challenges affecting the marine salmonid farming industry negatively with implications for growth, welfare and survival. The aim of the present study was to evaluate the effectiveness of functional ingredients as tools for the non-medicinal mitigation of common gill diseases and skin disorders in Atlantic salmon, *Salmo salar*, farmed under real-world environmental conditions. In this study we tracked changes in skin and gill tissues over an extended period at a pilot-scale net-pen farm in Norway between September 2020 and July 2021 in salmon fed either a feed containing a seasonally tailored, multi-purpose, FI package (diet SC) or a feed with an FI package specifically targeting gill and skin health (Diet GSS). We can say that development of severe AGD, PGD and skin damage was avoided by salmon fed both feeds. Based on the results obtained in the trial it's fair to propose that the gill and skin health focused package (GSS) did not make a material difference to the health of the salmon relative to the default, seasonally adapted FI strategy (SC). On that basis, we would not recommend the proposed new strategy as a replacement or support for the current seasonal package used in today's grower feed.

1. Introduction

1.1 Functional ingredients

One of the strategies for improving the health and wellbeing of farmed salmon is to increase their robustness through the application of functional ingredients (FIs). Functional ingredients are non-medicinal components in the feed which can be either classical nutrients e.g. vitamin E or feed supplements e.g. purified yeast cell wall components, the consumption of which, is associated with improvements in health, wellbeing or quality. During a production cycle, farmed fish are subject to stressors that, amongst others, could be caused by: environmental factors e.g. changing water temperature; interference or handling e.g. grading, anti-parasite treatments, transport etc; and diseases. It is in these situations that feeds containing functional ingredients (functional feeds) can be used to support the immune system to secure fish health, welfare and good growth.

1.2 Gill health

The gills are continuously exposed to infectious agents and non-infectious factors of both biotic and abiotic character and these contribute to an emerging gill health problem in the salmon farming industry. Gill diseases and also other conditions associated with damage to the gills, are not only responsible for significant economic losses in the fish farming industry, but also from a fish welfare point of view, are of significant concern.

1.2.1 Amoebic gill disease

Amoebic gill disease (AGD) is a major challenge in the salmonid farming industry caused by *Paramoeba perurans*. Outbreaks of AGD have been reported worldwide in Atlantic salmon and in other fish species and this disease is mostly prevalent in areas with high salinity and water temperatures above 12°C. At a macroscopic level, *P.perurans* can cause grey/white raised mucoid patches to be formed on the gills due to excessive mucous production. As AGD develops further, the surface of the lesions undergoes several modifications including: epithelial hypertrophy and stratification coupled with mucous cell recruitment; the formation of interlamellar vesicles that may contain amoebas; and a decrease in the number of chloride cells.

1.2.2 Proliferative gill disease

Proliferative gill disease (PGD) is, alongside AGD, another major challenge for the industry. This disease also accounts for a large proportion of production cost during sea water production phase. Unlike AGD, PGD often presents itself as a multifactorial complex where interactions of numerous pathogens, microorganisms and environmental factors play a part in the severity of the development of the disease. Unlike AGD, PGD is more likely to affect larger fish during the second summer and autumn of their production cycle and can result in severe proliferative pathology with significant reduction in gill surface area and thickening of the secondary lamellae.

1.3 Skin health

Skin, alongside mucous secreted by goblet cells in the epidermis, represents the first defensive barrier. A breach in this barrier will cause the fish to struggle with osmoregulation and also be a portal of entry for pathogenic microorganisms, which under the right environmental conditions, may cause ulcers and wounds to develop. Bacteria typically involved in this process are *Moritella viscosa* and *Tencacibaculum spp*. Another emerging challenge that could impact the skin by removing scales and mucous is the implementation of new, mechanical and hydro-thermal tools for salmon louse (*Lepeophtheirus salmonis*) removal. Optimisation of skin integrity and healing through superior nutrition and / or the application of functional ingredients represents a way to limit the impact of those pathologies that rely on breaking the skin as a mode of entry to the animal.

1.4 Objectives

The objective of the present study was to establish whether, by comparison to feeds containing a seasonally changing functional ingredient package (Mowi's current strategy, Diet SC), feeds with a gill and skin health focused, functional ingredient package (Diet GSS) would be more effective as a tool to support salmon health. The pilot-scale farm at which the trial took place facilitated natural exposure to many of the gill and skin health challenges experienced in Norwegian salmon farming and as such represented an ideal opportunity to evaluate a new functional feed proposal under real world conditions.

2. Materials and Methods

2.1 Husbandry and feeding

The feeding trial was conducted at Mowi's Averøy Field Trials Station in Norway between September 2020 and July 2021. The Atlantic salmon, *Salmo salar*, used for the trial were obtained from Mowi ASA's facility in Slørdal, Norway. At the start of the trial and with a mean weight 120g, the fish were randomly distributed amongst 6 marine net pens (5x5x5m; 125 m^3) at an abundance of 120 fish per pen. The fish were given a period of 14 days acclimation to environmental conditions whilst fed a commercial feed (Mowi JUPITER 75) before the study started. Upon commencement of the 315 day feeding period, the fish were fed one of 2 feed programmes (Table 1) with 3 pens allocated to each feed. The salmon were fed in excess using a combination of automatic feeders and a waste feed collection system. A daily over-feed of 15% was targeted and the excess feed amount was confirmed gravimetrically on dry weight corrected, recovered waste feed before feed intake and feed conversion factor were calculated. Fish health and welfare were monitored daily following Mowi's standard procedures. Water temperature spanned a range between 3.2 °C (February) and 15.7 °C (September) and a natural photoperiod was observed.

2.2 Feeds

Two series of feeds each comprising four iterations (8 feeds in total) were formulated by Mowi Feed as summarised in Table 1. The first series of feeds (SC) incorporated a seasonally changing package of functional ingredients (FIs). The second series (GSS) included a fixed package of functional ingredients where the FIs chosen were those understood to be of higher benefit in terms gill and skin health. The SC series was manufactured at the Mowi Feed factory at Valsneset in Norway and reflected Mowi Feed's functional ingredient strategy. The GSS series was produced at the Nofima Feed Technology Centre in Bergen (Norway) and represented the alternative FI strategy. Pellet size and formulation were adapted to meet the changing needs of the growing salmon.

Table 1. Formulation and estimated composition of the experimental feeds indicating the relative abundance of the different functional ingredients.

Headlines	Unit	SC	GSS	SC	GSS	SC	GSS	SC	GSS
Crude protein	%	44,1		41,2		37,9		34,5	
Crude fat	%	28,2		32,1		34,2		37,6	
Pelet diameter	mm	5		7		9		9	
Feeding from	Date	10.09.2020		07.11.2020		10.01.2021		17.02.2021	
Feeding to	Date	14.10.2020		14.12.2020		17.02.2021		14.07.2021	
Fish size at start	gram	380		780		1350		1530	
Fish size at end	gram	750		1320		1530		3240	
Functional package									
Vitamin C		+	++++	+	++++	+++	++++	++++	++++
Vitamin E		++	++++	++	++++	++	++++	+++	++++
Mineral premix		+	++	+	++	++	++	++	++
Yeast product 1		+++	+++	+++	+++	+++	+++	+++	+++
Yeast product 2			+		+		+		+
Yeast product 3			+		+		+		+

2.3 Sampling and testing

At each sample point all fish were individually weighed and counted with further post-mortem assessments being carried out on 5 salmon / pen (15 fish / feed type). Each fish was subjected to the following: mucus quantity measurements, visual health assessment of the gills, gill swabbing for qPCR and tissue sampling (two pieces of skin and second gill arch on the right side) for histopathological evaluation.

Table 2. Overview of sample plan

Sample point	Date	Performance	Gill health	Skin health
0 (zero)	02.09.20		AGD Score qPCR (*P.perurans*) Histology	Mucous quantity Histology
1	14.10.20	Growth	AGD Score qPCR (*P.perurans*) Histology	Mucous quantity Histology
2	14.12.20	Growth	AGD Score qPCR (*P.perurans*) Histology	Mucous quantity Histology
3	17.02.21	Growth	AGD Score qPCR (*P.perurans*) Histology	Mucous quantity Histology Welfare Score
4 (Final)	14.07.21	Growth	AGD Score PGD Score qPCR (*P.perurans*) Histology	Mucous quantity Histology

2.2.1 Gill Score

Assessments of gill damage / integrity (gill Score) were carried out on all the left-side gill arches of the fish. All pens were scored blind, and worst gill arch determined the score. The clinical development of AGD (grey/white raised mucoid patches on the gills) was assessed according to Taylor et al., 2008 (modified) at all 5 checkpoints. The clinical signs symptomatic for AGD is the foundation for the scoring system ranging from 0-5, were score 0 is no clinical signs and score 5 illustrates extensive "lesions" covering most of the gill surface. PGD assessment was carried out using a Mowi in-house protocol only at the end of the trial.

2.2.2 Gill swabs measurements followed by real-time quantitative PCR

Quantitative polymerase chain reaction (qPCR) was used to confirm the presence of *Paramoeba perurans* reported as prevalence (a proportion of the fish on which the parasite was found) and to quantify the severity of the infestation by evaluation the cycle threshold (Ct) values as a measurement of the amoeba expression. Samples were collected and analysed according to a commercial method with a standardised protocol supplied by PatoGen AS.

2.2.3 Mucous quantity

Epidermal mucous collection was carried out according to an internal protocol developed by Mowi ASA. In short, mucous quantity was determined according to the change in weight of an absorbent sponge strip which was held in contact with the fish above the lateral line for 30 seconds.

2.2.4 Histology

Gill (second gill arch, right side) and skin samples (head and dorsum) were taken from 5 fish / pen (15 fish / feed) at each check point. Formalin fixed, haematoxylin and eosin stained [?] samples were prepared by PatoGen AS, Norway. An examination of the gill and skin sections was carried out by VeHiCe (Veterinary Histopathology Centre) in Norway. Gill health was rated on a scale of 0-24 according to Mitchell et al (2012) where higher scores indicate an increasing level of damage. For the skin samples, both mucous cell density and epidermal layer thickness were measured.

2.2.5 Welfare Score

An assessment of welfare score was performed in February 2021 due to the likelihood of ulcer development caused by environmental factors (development of typical winter sores). A Mowi internal protocol "Mekanisk avlusning SCoringsveilder, versjon 170522) was used to assess-scale loss, red belly and ulcer/wound development.

2.4 Data analyses

Differences in fish performance and health outcomes between salmon fed the two feeds were assessed using unpaired Student's t-test. A significance level of 0.05 was used for all analyses. Data are presented as mean ± SD. All statistical analyses were performed in GraphPad Prism 8 and IBM SPSS Statistics V25.

3 Results

3.1 Performance

Fish fed the GSS feed had a significantly higher final weight and weight gain than fish fed diet the SC feed at the last sample checkpoint (p<0.001, Table 3). No significant difference in FCR was observed among fish fed diet SC and GSS.

Table 3. Summary of the performance indicators measured from start (02.09.20) to end of the trial (14.07.21)

Performance	Diet SC	Diet GSS	SEM	*P-value*
Initial weight, g	399	400	3,13	0,842
Final weight, g	3153	3368	42,90	0,001
Weight gain, g	2754	2968	42,90	0,001
bFCR (%)	1,16	1,08	0,06	0,206
Mortality (%)	0	0,83	0,22	0,374

3.2 Gill score AGD

The severity of AGD-related gill damage (gill score) generally decreased with time (Figure 1) and whilst the AGD scores for salmon fed the GSS feed were noticeably lower than those of salmon fed the SC feed in December 2020 and February 2021, there were no significant differences in score at any point (Figure 1 & Table 3).

Table 4. Mean AGD gill Score for each sample point.

Mean AGD gill Score Sample point	Date	Diet SC	Diet GSS	SEM	*P-value*
1	14.10.20	1,13	1,07	0,28	0,8147
2	14.12.20	1,20	1,00	0,34	0,5621
3	17.02.20	0,73	0,47	0,26	0,3216
4/Final	14.07.20	0	0	Na	Na

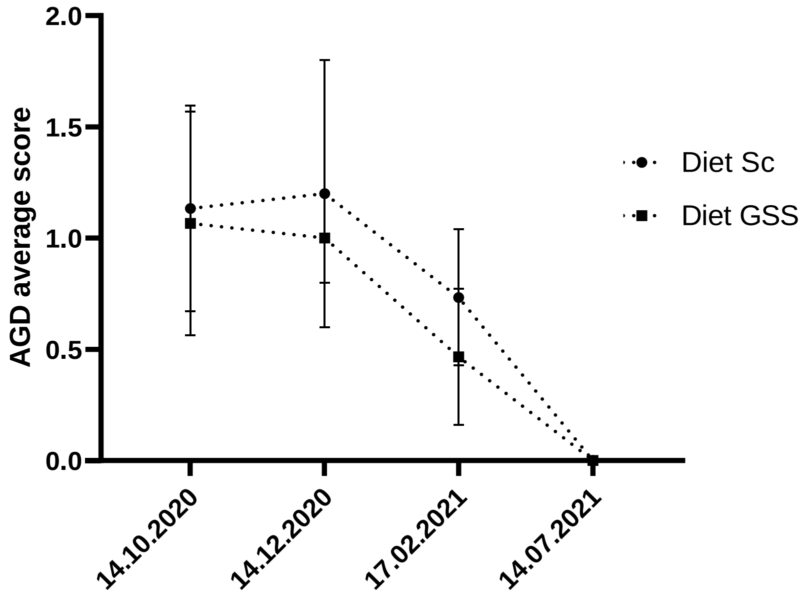


Figure 1. AGD gill score development throughout the trial period. Each point represents mean of 15 fish / feed type at each time point

3.3 Real-time quantitative PCR

At the start of the trial, 15% of the salmon were positive for *P.peruranse* (data not shown) and that proportion increased to 100% prevalence in October and December 2020. By February 2021, *P.peruranse* was absent from the population but, remerged at a very low level (6% of fish infected) in the salmon fed the SC feed in July 2021. At no time point were there significant difference in the prevalence or load (indicated by Ct's) of *P.peruranse*.

Table 5. Data presented as mean Ct values from qPCR against *P.peruranse* and prevalence in Atlantic salmon throughout the trial period.

Mean Ct value and prevalence (%) of *P.perurans*					
Sample point	**Date**	**Diet SC**	**Diet GSS**	**SEM**	***P-value***
1	14.10.20	18.2 (100%)	17.8 (100%)	0,86	0,6851
2	14.12.20	18.9 (100%)	18.8 (100%)	0,66	0,9122
3	17.02.20	0 (0%)	0 (0%)		
4/Final	14.07.20	28.5 (6%)	0 (0%)		0,4561

Note: The cycle threshold (Ct) value of a reaction is defined as the cycle number when the fluorescence of a PCR product can be detected above the background signal. Where the sample contains a large amount of the target species DNA, the number of amplification cycles required to achieve detection will be low. A cut off was set at around ~36, with a mild parasitic load >30, mild (30-25), moderate (25-15), severe <15. High Ct = low amoeba expression, Low Ct = High amoeba expression.

3.4 Mucous quantity

Based on the gravimetric / absorbent strip test, there were no significant differences in epidermal mucous production between salmon fed either of the two feeds at any of the checkpoints.

Table 6. Data presented as mean mucous weight (grams) absorbed by standardised foam strip from the skin of Atlantic salmon at each checkpoint.

Mean mucous weight					
Sample point	**Date**	**Diet SC**	**Diet GSS**	**SEM**	***P-value***
1	14.10.20	0,03842	0,04615	0,008	0,1325
2	14.12.20	0,054389	0,059513	0,007	0,4807
3	17.02.20	0,038667	0,04314	0,008	0,5804
4/Finale	14.07.20	0,059107	0,055293	0,007	0,6126

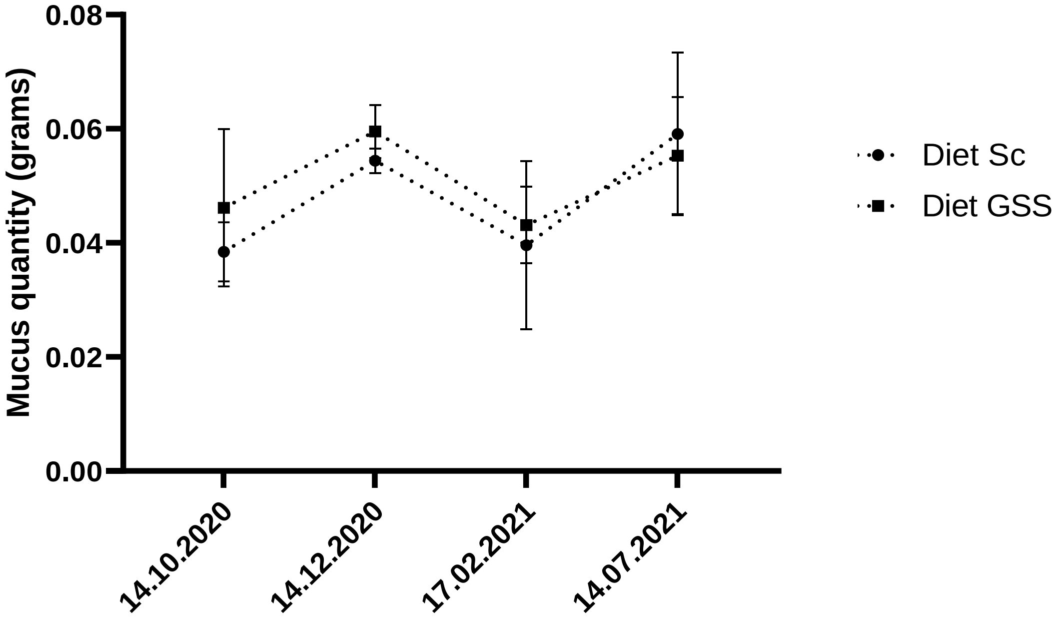


Figure 2. Weight of mucous absorbed by standardized foam strip after placement on salmon. Each point represents average of 15 salmon / feed at each time point.

3.5 Histology

3.5.1 Gills

Salmon fed both feeds exhibited moderate gill pathology (scores 7-8 on a scale of 0-24) at all sample points. Although no significant differences between salmon fed the two feeds were observed, the gill pathology of the salmon fed the SC feed was less severe than for those fed the GSS feed.

Table 7. Histopathology scores for gills from Atlantic salmon.

Histology, gill pathology					
Sample point	**Date**	**Diet SC**	**Diet GSS**	**SEM**	***P-value***
1	14.10.20	8,10	8,50	0,55	0,472
2	14.12.20	7,40	8,10	0,60	0,231
3	17.02.20	8,10	8,10	0,44	0,999
4/Final	14.07.20	7,3	7,5	0,32	0,533

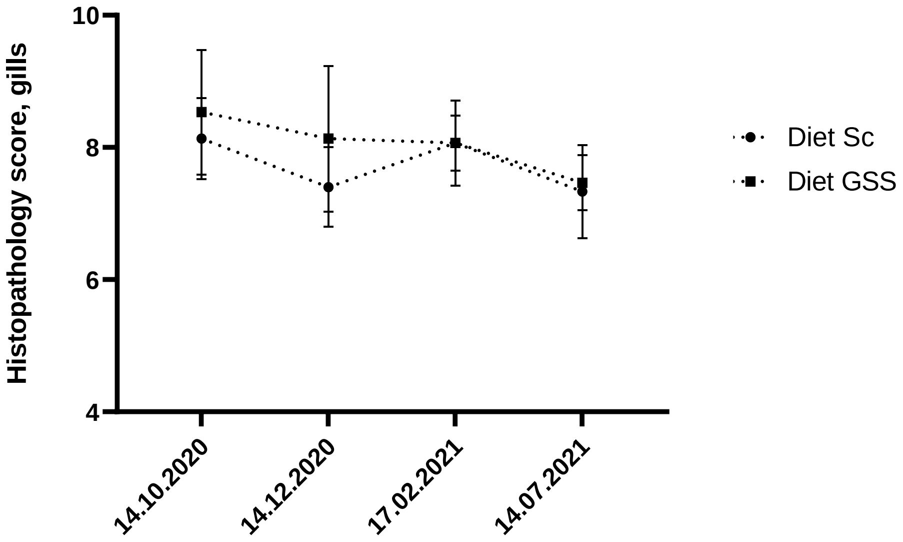


Figure 3. Time series for gill health score (histological examination) according to VeHiCe following a modified version of the Mitchell et al. (2012) schedule. n=15 fish surveyed / feed type / time period.

3.5.2 Mucous cells in Epidermis

The mucous cell density of salmon fed feed SC tended to be higher than that of salmon fed the GSS feed in October and December (P-values 0.05 – 0.10) and was significantly higher (P = 0.0311) than for fish fed GSS in February. However, by July 2021, there were no significant differences in mucous cell density.

Table 8. Data presented as mean cell numbers of mucous cells from Atlantic salmon.

Histology, mucous cell number/ 1500 µm epidermis					
Sample point	**Date**	**Diet SC**	**Diet GSS**	**SEM**	***P-value***
1	14.10.20	92	75	8,8	0,0658
2	14.12.20	119	104	8,01	0,0793
3	17.02.20	118	100	7,81	0,0311
4/Final	14.07.20	109	125	11,01	0,1601

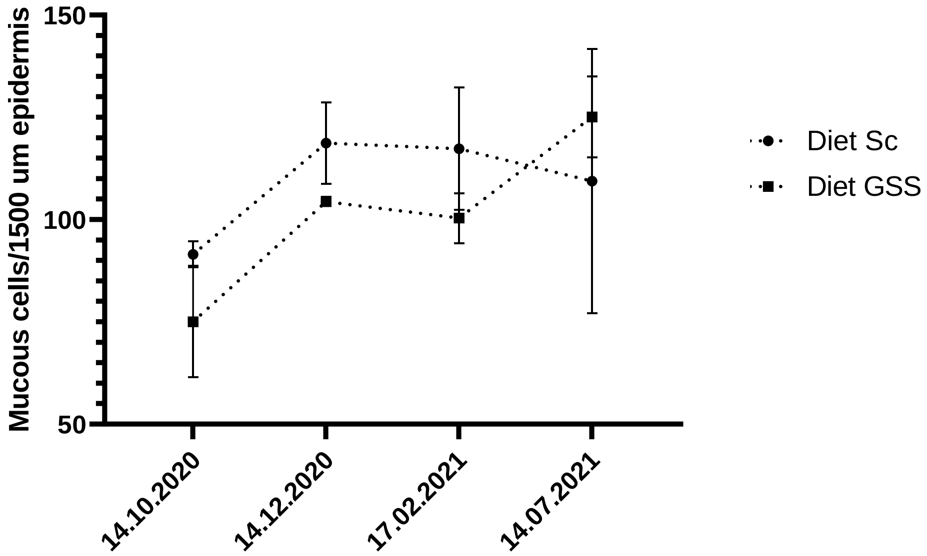


Figure 4. Time series for mucous cell density (histological examination) according to VeHiCe, n=15 fish surveyed / feed type / time period.

3.5.3 Epidermal layer thickness

In October there was a tendency (P = 0.0734) for epidermal thickness to be thicker in salmon fed SC than GSS. Epidermal layer thickness then increased between October and December but, there were no significant differences in thickness between salmon fed either of the feeds thereafter.

Table 9. Epidermal layer thickness in Atlantic salmon fed SC and GSS feeds.

Histology, epidermal layer thickness (µm)					
Sample point	**Date**	**Diet SC**	**Diet GSS**	**SEM**	***P-value***
1	14.10.20	92	79	6,87	0.0734
2	14.12.20	112	119	8,96	0,4291
3	17.02.20	133	118	9,3	0,1204
4/Final	14.07.20	108	115	10,53	0,5300

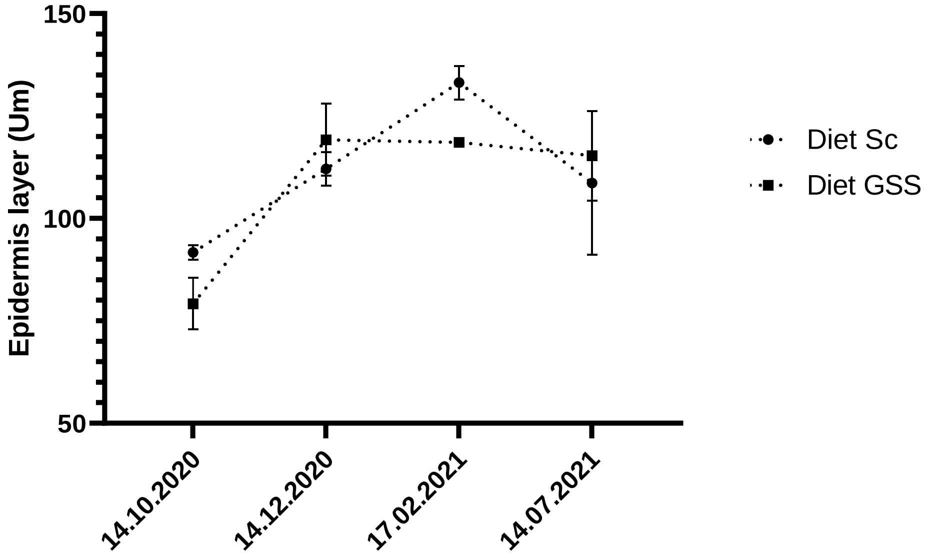


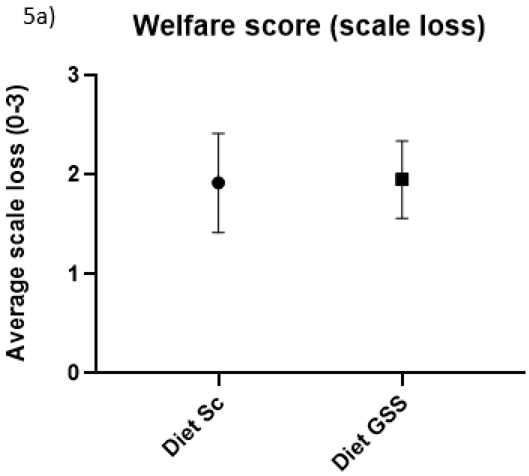
Figure 4. Time series for epidermal thickness (histological examination) according to VeHiCe, n=15 fish surveyed / feed type / time period.

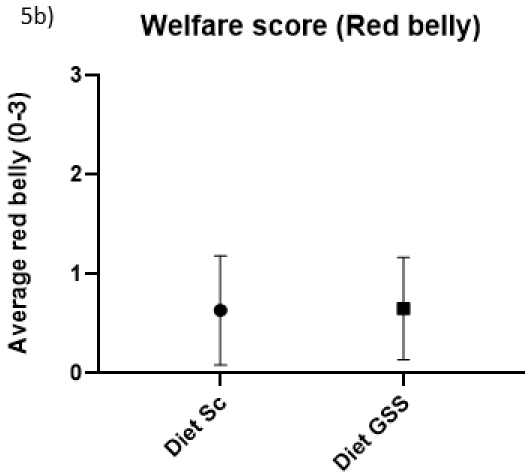
3.6 Welfare Score

An evaluation of welfare score was performed in February 2021. Scale loss, red belly and ulcers / wounds were considered to be advanced, at a low level and at a low level respectively for all fish. There were no significant differences observed amongst salmon fed the two feed types.

Table 10. Welfare score in Atlantic salmon in February 2021. Where scores are from 0 "damage absent" to 3 "damage severe"

Welfare score, sample point 3, 17.02.21				
	Diet SC	**Diet GSS**	**SEM**	***P-value***
Scale loss	1,9	1,95	0,081	0,683
Red belly	0,63	0,65	0,098	0,864
Ulcer/wounds	0,3	0,35	0,133	0,707





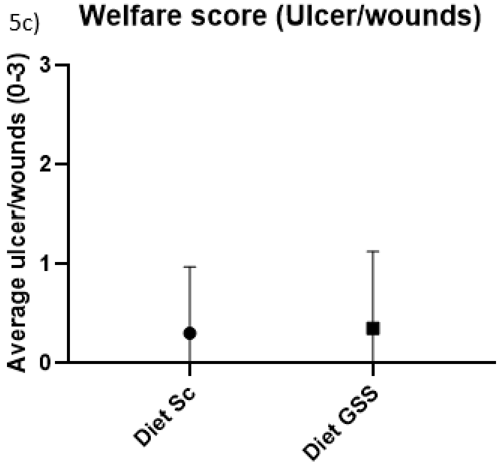


Figure 5a, 5b, 5c. Welfare evaluation in February 2021, 5a) Scale loss, 5b) Red belly, 5c) Ulcer/wounds. n=60 / feed type for each plot.

4. Discussion

Overall, growth and FCR were very good with the salmon fed the alternative (GSS) feed gaining significantly more weight (approximately 7.8%) than those fed the default (SC) feed. This was coupled to a feed conversion ratio that was also 6.9% lower for GSS-fed salmon. It would be tempting to claim this as a victory for the GSS feed but, it must be pointed out that whilst formulated on identical principles for nutrients outside the FI (functional ingredient) scope, the two series of feeds were manufactured using different feed material lots and at different facilities (commercial scale vs pilot scale production). On that basis, we cannot make any specific claims with regards fish performance.

Mortality rates were negligible / non-existent despite the naturally occurring outbreak of amoebic gill disease (AGD) between October and February (with visible gill pathology still apparent in February) and moderate gill damage (scores 7-8 on a 0-24 scale) observable at the cellular level throughout the feeding period. As would be expected for a natural AGD outbreak, when the high salinity / high temperature conditions that encouraged parasite infestation were exchanged for typical winter conditions (lower salinity / low temperature) the infestation ended. Once again, we should not claim any credit for the role played by the functional feeds in alleviating the AGD symptoms. Furthermore, although the AGD pathology scores were lower in the GSS-fed salmon than in the SC-fed salmon in December and February, at no point were there any significant differences (nor tendences towards a difference) in gill health at either the macroscopic or microscopic level.

At the first three checkpoints, epidermal mucous cell density either tended to be higher (October and December) or was indeed significantly higher (February) in SC-fed salmon than in GSS-fed salmon. Additionally, the epidermis tended towards a higher thickness in SC-fed fish than in GSS-fed salmon in August. However, despite an apparent overall increase in thickness for the latter 3 checkpoints, there were no further feed-related difference in epidermal thickness thereafter. Furthermore, this observation at the microscopic level was not accompanied by any differences at the macro level in terms of mucous production or at least, in our ability to collect mucous from the skin. Whilst scale loss was moderate (likely to have been caused by netting to catch the fish), red belly and winter wounds were not remarkable in February and no significant differences were recorded between fish fed the two feed types. This leads to the assumption that consumption of the GSS or SC feed resulted in similar outcomes for skin health.

The present study typifies the challenges associated with attempting to evaluate functional ingredients as both part of a package of measures and also, under real world conditions. The variable environment and changing seasons bring with them differences in the health and welfare challenges encountered by the salmon and potentially reduce our ability to visualize any potential benefits which may or may not be visible under stable conditions. Additionally, on the basis that two functional feeds were compared, there is no means by which we can determine the "absolute" benefits associated with consumption of either or both functional feeds *per se*.

5. Conclusion and recommendation

The objective of this study was to compare two functional feeds as part of the toolkit for the non-medicinal management of naturally occurring challenges to gill and skin health in farmed Atlantic salmon. The default strategy (SC) was based on the year-round application of a mid-market yeast cell wall preparation which overlayed a seasonally changing pattern of vitamin and mineral super-supplementation. The alternative strategy (GSS) was based on continuously high levels of vitamin and mineral supplementation in addition to both the mid-market yeast-cell wall preparation and two further premium yeast supplements. The GSS feed therefore represented a high-cost solution.

We can say that development of severe AGD, PGD and skin damage was avoided by salmon fed both feeds. In the absence of data for a feed without functional ingredients / without super supplementation of vitamins E, C and trace minerals, what we cannot say is whether both feeds made a material difference to that outcome. It is however fair to propose that the gill and skin health focused package (GSS) did not make a material difference to the health of the salmon relative to the default, seasonally adapted FI strategy (SC). On that basis, we would not recommend the proposed new strategy as a replacement or support for the current seasonal package used in today's grower feed.

6. References

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