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Modulation of innate and adaptive immunity in Atlantic salmon (*Salmo salar*) fed diets containing commercial-like additives under farming conditions in seawater phase

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ABSTRACT

Diseases and environmental issues (e.g., increased seawater temperature and algal blooms) threaten Chilean aquaculture by causing salmon mortality. Feed additives can help solve these problems by modulating fish physiological responses, but field-level data is still limited. Our study assessed the potential immunomodulation of innate and adaptive biomarkers (e.g., cytokines, effector molecules and cell-surface markers) in farmed post-smolts Atlantic salmon (*Salmo salar*). Fish were fed either a commercial diet (CD) or experimental diets containing additives (PS: 0.25 % Pack Salud; FT: 0.1 % Futerpenol®) for 90 days. Results showed that *ifng*, *il-10*, *inos*, *il-12*, *cath2*, *hepcidin*, *igm* and *igt* were mainly up-regulated in fish fed PS or FT for 34 days (t1). Furthermore, between t2 (90 days of feeding) and t3 (42 days after finishing functional feeding), a natural algal bloom was reported at the facilities. Hence, our study considered the challenge-induced modulation of fish health. Although the accumulated fish mortality throughout the trial did not show significant differences between dietary groups, in the period related to the algal bloom, PS and FT groups reduced dead fish by 10 % and 61 % (respectively, compared to CD). Also, head kidney from FT group (and to a lesser extent in the spleen) had an enhanced innate immune response at t3 with an up-regulation of *inos*, *hepcidin* and *cath2*, which were coordinated by cytokines (*il-1b*, *ifng*, *il-12* and *il-10*) and cell-surface markers (*cd86*, *mhci* and *mhcii*). Thus, since t3 was six weeks after the ending of the functional feeding, FT could induce sustained immunomodulation in Atlantic salmon.

1. Introduction

Substantial economic losses occur in Chilean salmon farming due to infectious diseases and environmental problems [1]. For example, the increasing incidence of algal blooms is a threat to the aquaculture industry in southern Chile, as eutrophication of seawater has been linked to the death of farmed salmonids [2]. Furthermore, *Piscirickettsia salmonis*, an intracellular bacterium that causes Salmonid Rickettsial Septicemia (SRS) accounts for 52.8 % of total fish mortalities at seawater phase [3]. To deal with bacterial pathogens, antibiotics are the most used strategy by Chilean aquaculture [4]. However, their use has raised concerns related to antimicrobial resistance in marine bacteria [5].

During the last decade, nutritional programming and immunomodulatory strategies, which consider aspects like the source and dosage of ingredients and/or additives with bioactive properties, specific feeding periods or regimes, animal development, season or farming stage (among others), have been gaining interest to strengthen overall fish health and welfare [6,7]. For instance, feed additives such as paraprobiotics (e.g., heat-killed bacteria) may reduce the level of Annexin 1 in distal intestine, while also strengthen the systemic humoral response in Atlantic salmon (*Salmo salar*) [8]. Another example is the inclusion of *Spirulina* spp extracts into rainbow trout (*Oncorhynchus mykiss*) feeds during a 7-week trial, which demonstrated that the upregulation of immune-related genes (e.g., *lysozyme*, *c3*, *tnfa*, *igm*) can reduce fish mortal-

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ity against *Yersinia ruckeri* [9]. Additionally, the oral administration of antimicrobial peptides such as bovine lactoferrin also induces long-term modulation of pro-inflammatory genes (*il-1b*, *il-6* and *il-8*) and T helper cells, improving the survival of rainbow trout against *Aeromonas salmonicida achromogenes* [10]. On the other hand, Atlantic salmon fed yeast products from *Debaryomyces hansenii* had an early increase (at 6 h) of pro-inflammatory cytokines in head kidney leukocytes, as well as an improved production of specific immunoglobulins against *Moritella viscosa* [11].

Although there is extensive data on the immunomodulatory properties of additives in functional feeds (FFs) for salmonids, the available information is generally based on lab-scale experiments. Therefore, our main objective was to increase the knowledge about the impact of FFs on biomarkers related to innate and adaptive immunity (e.g., cytokines, effector molecules and cell surface markers) of post-smolts Atlantic salmon under commercial field conditions. For this, fish were fed supplemented diets (using Pack Salud or Futerpenol® additives) for 90 days in seawater phase. Pack Salud contains beta-1,3/1,6-glucans, nucleotides and vitamin C, while Futerpenol® has seaweed's fucoidans and labdane diterpenes from the *Acanthaceae* family [12]. In fish, these compounds have already been reported with immunomodulating properties (e.g., antioxidant capacity, coordinating humoral and cellular immunity and disease resistance) [13–15].

Furthermore, after the feeding period using additives, an algal bloom was reported on the fish farm. Thus, our study focus incorporated the challenge-inflicted modulation of the immune response. The overall results showed that Pack Salud or Futerpenol® induced changes in immune organs such as the head, kidney, gills, and spleen, mostly after 34 days of feeding, whereas after the algal bloom, fish fed FT had a higher innate response, suggesting a potential to strengthen the immune system of Atlantic salmon at long-term.

2. Materials and methods

2.1. Experiment

A fish trial at field scale with post-smolts Atlantic salmon in seawater phase was conducted under the approval of the Bioethics and Biosafety Committee of Pontificia Universidad Católica de Valparaíso (BIOEPUCV-BA-280-2019). All fish were handled by scientists and farm staff certified in working with animals.

Fish (~1.5 kg) in a marine farm (75,000 fish per sea pen) from Salmones Camanchaca S.A. (Contao, Los Lagos region, Chile) were fed in duplicate sea pens during 90 days with one of three diets: Control diet: New Power® – commercial diet from BioMar Chile (CD), Pack salud diet (PS): CD supplemented with 0.25 % Pack salud additive from Salmones Camanchaca S.A. (containing beta-1,3/1,6-glucans, nucleotides and vitamin C), or Futerpenol diet (FT): CD supplemented with 0.1 % Futerpenol® additive from MNL Group that contains seaweed's fucoidans and labdane diterpenes of Acanthaceae family. After this period, fish were fed CD for a further 42 days (Fig. 1). The basal chemical composition of CD is shown in Table 1.

Regarding sampling, 15 fish per sea pen were randomly selected at t0 (before starting with experimental diets), t1 (34 days of feeding with PS or FT), t2 (90 days of feeding with PS or FT) and t3 (42 days after ending use of FFs). In each sampling, fish were euthanized by benzocaine overdose, weighed and measured. Then, samples of gills (G), head kidney (HK) and spleen (S) were removed, preserved in RNAlater® (Merck) and stored at −80 °C until later molecular analysis.

During the experiment, between t2 and t3, an algal bloom was declared in the fish farm. Moreover, throughout the experiment, fish mortality and its cause (e.g., SRS, algal bloom) were recorded.

2.2. Total RNA extraction and cDNA synthesis

Each sample was homogenized using 3 mm metal beads and 1 mL of TRIzol® (Thermo Fisher Scientific) in an automatic homogenizer (FastPrep-24, MP-Bio) through 3 cycles of 35s (5.5 m s^{-1}). The homogenates were centrifuged (10,000 × *g* for 10 min at 4 °C) and the supernatants were transferred to new nuclease-free tubes. Total RNA extraction was performed using the TRIzol® method according to manufacturer's instructions (Thermo Fisher Scientific) and adding a DNA digestion step (DNAse 1-unit μL^{-1}, Thermo Fisher Scientific). RNA quantification was performed in a NanoDrop® Lite equipment (Thermo Fisher Scientific) and the integrity of the samples were visualized in 2 % agarose gels with TAE buffer (Tris-Acetate-EDTA 1 ×). Thereafter, 30 samples (per

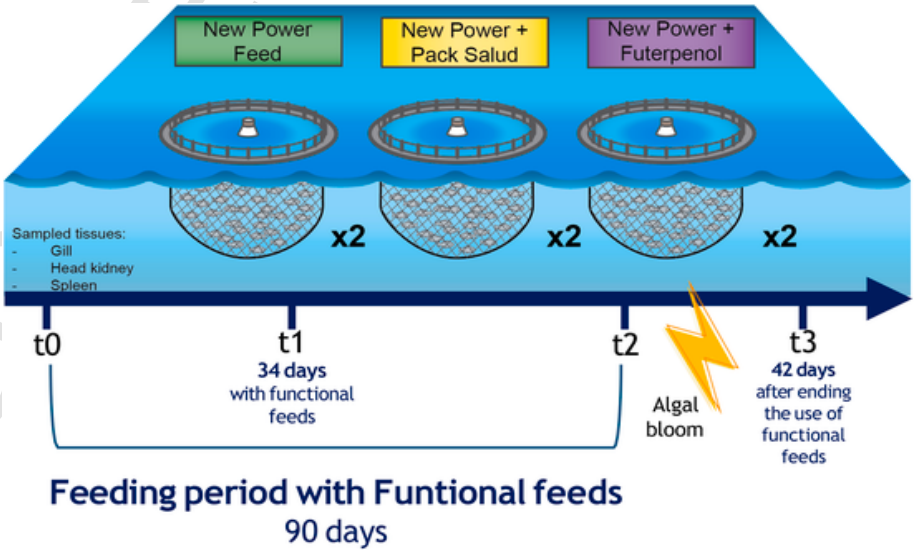

Fig. 1. Experimental design using post-smolts Atlantic salmon at farming conditions. Control group (in green): fish fed New power® from BioMar Chile throughout the experiment. PS group (in yellow): New power® + 0.25 % Pack salud additive from Salmones Camanchaca S.A. FT group (in purple): New power® + 0.1 % Futerpenol® additive from MNL Group. Sampling times: t0 (before functional feeds), t1 (34 days feeding functional feeds), t2 (90 days feeding functional feeds) and t3 (42 days after ending feeding functional feeds). A natural algal bloom was declared in the fish farm between t2 and t3. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Chemical composition of control diet.

Nutritional composition (%)	
Protein content	39.9
Lipids	34.9
Carbohydrates	15.5
Ash	4.7
Total Dry matter (%)	95.0

organ from each dietary group and sampling time) were pooled into 5 samples. This was done by mixing 1 μg of RNA from 6 randomly selected fish. To synthesize cDNA, 1 μg of RNA per pool and the RevertAid First Strand cDNA synthesis Kit (Thermo Fisher Scientific) was used in 20 μL (as a total volume per reaction) according to the supplier.

2.3. Gene expression by reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Immune-related genes (Table 2) were evaluated in head kidney, gills and spleen samples from all experimental conditions. RT-qPCR reactions were performed in triplicate using an AriaMx Real-Time PCR System (Agilent Technologies). Each reaction consisted in a total amount of 15 μL containing Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix 2X (Agilent Technologies), 0.2 μM of each primer (Table 2) and 1 μL of 1:5 diluted cDNA. The RT-qPCR conditions were a denaturation step (3 min at 95 °C) followed by 40 cycles at 94 °C for 5 s and annealing step (60 °C for 35 s). Also, a melting curve was carried out. Primer pair efficiencies (E) were calculated from six serial dilutions of pooled cDNA (for each primer pair) according to the equation: $E = 10^{[-1/slope]}$. Regarding relative expression of each gene, this was calculated using the $2^{-\Delta\Delta Ct}$ method [16] and *ef-1a* was chosen as a reference gene for normalization following Olsvik et al. [17].

2.4. Data analysis

GraphPad v8.0.2 was used to display the results by bar plots and calculate means, SEM and normality test (Shapiro-Wilkins). After this, natural logarithm transformation was performed, and normality was re-evaluated. Based on these results, it was decided to use either parametric statistics (one-way ANOVA and Dunnett's test for comparing PS or FT with CD for each sampling time; or one-way ANOVA and Tukey's test for multiple comparisons among sampling times) or non-parametric statistics (by Krustal Wallis test with Dunn's multiple comparisons test between groups per time or diet). Differences were considered significant when p-value was <0.05. Also, results related to fish mortality and physical parameters such as final weight gain and fish length (as a t4:t0 ratio) were analyzed between CD and experimental diets by Mann-Whitney test. Differences were considered significant when p-value was <0.05.

To identify patterns between the dietary treatments, the corrplot package [18] in R v.4.2.0 [19] was used to calculate correlations based on the data from the immune-related genes (per organ or sampling

Table 2
Specific primers for RT-qPCR. E: primer pair efficiencies. F: forward primer. R: reverse primer. Gene reference: National Center for Biotechnology Information (NCBI).

Gene	Description	Sequence	E	Gene reference
cath2 (Cathelicidin)	Antimicrobial peptide	F: AAGCCAGAAAATGCTCCAGA R: CTCAGGCGACCAATTAAGGA	1.92	NM_001123586.1
cd4 (Cluster of differentiation 4)	Cell-surface marker	F: AGTGGACCTGTGTGGTGACA R: AGGAGGAGGATTGGAAAGGA	1.91	NM_001171848.1
cd8 (Cluster of differentiation 8)	Cell-surface marker	F: CGTCTACAGCTGTGCATCAATCAA R: GGCTGTGGTCATTGGTGTAGTC	1.93	NM_001123583.1
cd83 (Cluster of differentiation 83)	Cell-surface marker	F: AGGATTCGGTTCTGAGATGTAAAG R: GTGACAGCCTCTTCATCAGTAG	1.99	ABC68619.1
cd86 (Cluster of differentiation 83)	Cell-surface marker	F: GATGTTGAGTGGAGCCTGAA R: GACGACAGAGAACAGCATAGAG	2.07	CAQ51440.1
ef-1a (Elongation factor 1 alpha)	housekeeping	F: GCTTACAAAATCGGCGGTAT R: CTTGACGGACACGTTCTTGA	2.02	NM_001141909.1
eomes (Eomesodermin)	Transcriptional factor	F: TATTGGTTCCATGGCTCTCC R: ATCATCTCCGTCTGGAATCG	1.93	NM_001204100.1
hepcidin	Antimicrobial peptide	F: GAAGGCCTTTAGTGTTGCAGTGGT R: GTTGATGTTCCCCAACTGGACTGT	1.93	XM_014170044.2
ifng (Interferon gamma)	Pro-inflammatory cytokine	F: GTGAGCGGAGGGTGTGGATG R: CAGGAAGTAGTGTTTCTGGGTC	1.99	NM_001123558.1
ifng R1 (Interferon gamma receptor 1)	Cytokine receptor	F: TTGGACTGAAGGACCAGGAG R: TTGTCCCCAGGACTCATCTC	2.20	NM_001360942.1
ifng R2 (Interferon gamma receptor 2)	Cytokine receptor	F: TCTTGGTAGTGGGCCTTACG R: CACAGGGACTGGTTGTGATG	2.06	NM_001361122.1
il-1b (Interleukin 1 beta)	Pro-inflammatory cytokine	F: GTCACATTGCCAACCTCATCATCG R: GTTGAGCAGGTCCTTGTCCTTGA	2.00	NM_001123582.1
il-10 (Interleukin 10)	Anti-inflammatory cytokine	F: ATGAGGCTAATGACGAGCTGGAGA R: GGTGTAGAATGCCTTCGTCCAACA	1.94	XM_045705802.1
il-12 (Interleukin 12)	Polarizing cytokine	F: CTTCTCCTGCTCCATTCACAG R: GTAGAACGGTGTAACGGTGTAG	2.06	XM_014198097.2
inos (Inducible nitric oxide synthase)	Production of reactive nitrogen species	F: CCAGCATAAGTGGTTCCAAGACCT R: CCAATCTCAGTGCCCATGTACCAG	2.01	XM_014214975.2
igm (Immunoglobulin M)	Effector molecule	F: TAGAAACCGACAGGGACAGC R: TTTCACCTTGATGGCAGTTG	1.93	S48658.1
igt (Immunoglobulin M)	Effector molecule	F: CAACACTGACTGGAACAACAAGGT R: CGTCAGCGGTTCTGTTTTGGA	1.94	GQ907004.1
mhci (Major Histocompatibility Complex 1)	Presentation of intracellular antigens	F: CAACGCCACAGGCAGTCA R: CGGTACTCATTCTGAGCTGTGTTAC	1.97	XM_045709561.1
mhcii (Major Histocompatibility Complex 2)	Presentation of extracellular antigens	F: AATCAGAGTGACCTGGTTGAG R: GTGGGAGAGGATCTGGTAGTA	2.13	CAD27720.1
tnfa (Tumor Necrosis Factor alpha)	Pro-inflammatory cytokine	F: GCAGCCATCCATTTAGAGGGTGAA R: CTAAACGAAGCCTGGCTGTAAACG	1.84	NM_001123589.1

times). Significant correlations were determined when p-value was <0.05 (degrees of freedom: n-2).

3. Results

3.1. Fish mortality and physical parameters

Accumulated fish mortality throughout the trial (by dietary group) was 1.67 % (CD), 1.58 % (PS) and 1.02 % (FT). This parameter did not show significant differences between groups. However, specifically during the natural algal bloom (between t2 and t3), the mortality in number of dead fish was 1522 in CD, 1366 in PS and 589 in FT (Fig. 2). Thus, PS and FT groups showed a decrease in mortality of 10 % and 61 %, respectively (compared to CD) during this period. In addition, no significant differences in weight gain (CD = 2.82-fold, PS = 2.80-fold and FT = 2.62-fold) or fish length (CD = 1.46-fold, PS = 1.45-fold and FT = 1.44-fold) were detected between dietary groups.

3.2. Gene expression of immune related biomarkers

3.2.1. Comparison of experimental diets containing additives (PS or FT) with CD per sampling time

The expression of *ifng* in PS group (Fig. 3A) was up-regulated at t2 in HK and at t1 in spleen, while FT showed an upregulation of this gene at t1 and t3 in HK and spleen, as well as in gills (t1). On the contrary, the expression of *il-1b* (Fig. 3B) was down-regulated in PS at t3 in HK, but up-regulated at the same time in FT (HK) and at t1 in spleen. Furthermore, *il-10* (Fig. 3C) was down-regulated in PS (HK) at t3, while the upregulation of this cytokine was detected in spleen from FT at t1, along with HK (FT) and gills at t3 (PS and FT). Regarding *il-12* in HK (Fig. 3D), an upregulation was found in PS (t1) and FT (t1 and t3), as well as a downregulation at t3 in PS. On the other hand, *tnfa* (Fig. 3E) showed an upregulation at t3 in HK from FT, whereas in spleen an upregulation was detected in FT at t1.

Cell surface markers such as *cd4* and *cd8* in HK (Fig. 4A and B) showed an upregulation in FT at t2 or t3 (respectively). Moreover, *cd83* (Fig. 4C) was up-regulated in HK and gills from PS at t2, while an upregulation of this gene was detected in gills from FT at t3. In contrast, *cd86* (Fig. 3D) was found down-regulated in HK at t1 (PS) and in gills at t2 (FT), but with an upregulation in HK at t3 (both PS and FT). Regarding *ifng r1* in HK (Fig. 3E), data showed an upregulation at t3 in FT. However, PS (in HK and gills) and FT (in gills and spleen) had a downregulation of *ifng r1* at t3. A different expression pattern was detected for *ifng r2* (Fig. 4F). For instance, this gene showed an upregulation in HK (at t3 from PS) and spleen (at t2 from FT), as well as a downregulation in gills at t3 (PS and FT). Additionally, *mhci* (Fig. 4G) was up-regulated in spleen at t1 (FT), and t3 (PS and FT), while *mhcii* was only up-regulated at t3 in HK from FT (Fig. 4H).

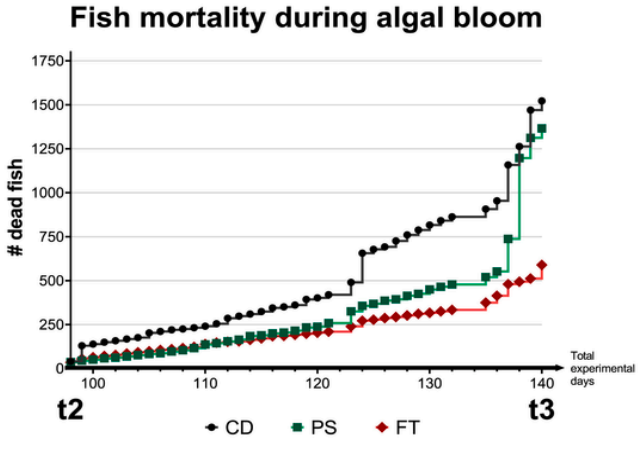

Fig. 2. Accumulated fish mortality between t2 and t3 (during algal bloom). t2: 90 days feeding functional feeds); t3: 42 days after ending with functional feeds. CD (in black): fish fed control diet. PS (in green): fish fed Pack salud additive by 90 days until t2. FT (in red): fish fed Futerpenol® additive by 90 days until t2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Linked to effector molecules, *cath2* in HK (Fig. 5A) was up-regulated at t2 (PS) and t3 (FT), but also down-regulated at t3 (PS). In spleen, this gene was up-regulated at t1 from PS and FT groups. On the other hand, *hepcidin* (Fig. 5C) showed an upregulation at t1 (spleen from PS and FT), t2 (gills from PS) and t3 (HK from FT). Immunoglobulins such as *igm* (Fig. 5D) and *igt* (Fig. 5E) were up-regulated in HK (PS) and gills (from PS and FT) at t1. Also, *igm* was found up-regulated in HK from FT at t1, whereas *igt* had an upregulation in spleen at t1 (FT).

Regarding *inos* (Fig. 5F), its upregulation was detected at t1 (gills from FT), t2 (HK from PS) and t3 (HK from FT and spleen from PS). Furthermore, *eomes* (Fig. 5B) was up-regulated at t2 (spleen from FT) and at t3 (HK and gills from FT).

3.2.2. Intra-dietary comparison across sampling times

In CD group, only *il-1b* (Fig. 3B), *ifng r2* (Fig. 4F) and *hepcidin* (Fig. 5C) showed differences in their expression between sampling times. Specifically, *il-1b* was up-regulated in t3 compared to t0, *ifng r2* was down-regulated in t3 compared to t2 and *hepcidin* was up-regulated in t1 and t3 compared to t0.

In contrast, fish fed PS showed an upregulation of *ifng* (HK) at t3 compared to t0 and t1 (Fig. 3A), while in spleen, this dietary group had a downregulation of *ifng* at t3 (compared to t1). For *il-1b* in HK (Fig. 3B), PS showed an upregulation at t0 (compared to t3), t1 (compared to t0 and t3), and t2 (compared to t0, t1 and t3), whereas *il-10* (Fig. 3C) from PS group was up-regulated at t1 (compared to t3) in HK, up-regulated at t3 (compared to t0) in gills, and up-regulated at t1 (compared to t0) in spleen. Regarding *il-12*, *cd8*, *ifng r1*, *cath2* and *inos* (Figs. 3D, 4B and . 3E, Fig. 5A and F, respectively), PS group showed a downregulation at t3 compared to t0, t1 and t2. Also, PS had an upregulation of *cd83* at t2 compared to t0 (in HK and gills, Fig. 4C), as well as an upregulation of *cd86* (PS) at t3 compared to t1 (Fig. 4D). In gills from PS group, *ifng r1* and *ifng r2* were down-regulated at t3 compared to t1 and t0 (Fig. 4E) and compared t2 (Fig. 4F), respectively. In spleen from fish fed PS, *ifng r2* was down-regulated at t1 compared to t0, t2 and t3 (Fig. 4F). Other comparisons in the PS group showed an upregulation of *cath2* (t1 compared to t0 in spleen, Fig. 5A), *eomes* (t2 compared to t0 in spleen, Fig. 5B), *hepcidin* (t2 compared to t0 and t3 in HK; t1 compared to t3 in HK, Fig. 5C), *igm* (t1 compared to t0 and t3 in HK, Fig. 5D), *igt* (HK: t1 compared t0 and t3 compared t0; gills: t1 compared t0 and t3; spleen: t2 compared t0, Fig. 5E), and *inos* (t1 compared t0 in gills, Fig. 5F).

Regarding fish fed FT, *ifng* was up-regulated in HK at t1 and t3 compared to t0 (Fig. 3A), as well as at t1 compared to t3 in gills. In spleen, *ifng* was up-regulated at t1 (compared to t0, and t2) and t3 (compared to t0, t1 and t2). In contrast, *il-1b* was only upregulated in HK at t3 compared to t0 (Fig. 3B). In addition, both *il-10* (Fig. 3C) and *il-12* (Fig. 3D) were up-regulated at t3 (compared to t0) in HK. Also, *il-12* (HK) was up-regulated at t1 compared to t0, whereas *tnfa* (Fig. 3E), *cd86* (Fig. 4D), *ifng r1* (Fig. 4E), *mhcii* (Fig. 4H) and *inos* (Fig. 5F) from HK had an up-regulation at t3 compared to the other sampling times. However, *tnfa* in gills was down-regulated at t2 compared to t0 (Fig. 3E), similar to *cd8* (down-regulated at t2 in gills compared to t0 and t1, Fig. 4B). In *cd83* (Fig. 4C), t3 had a downregulation (compared to t0 and t1) in HK, while in gills, t0, t1 and t3 were up-regulated (compared to t2). In the spleen this pattern was reversed, with an upregulation of *cd83* at t2 (compared to t0 and t3). Related to *cd86* in gills (Fig. 4D), t2 was down-regulated compared to t0, but in spleen t3 was up-regulated compared to t0. In

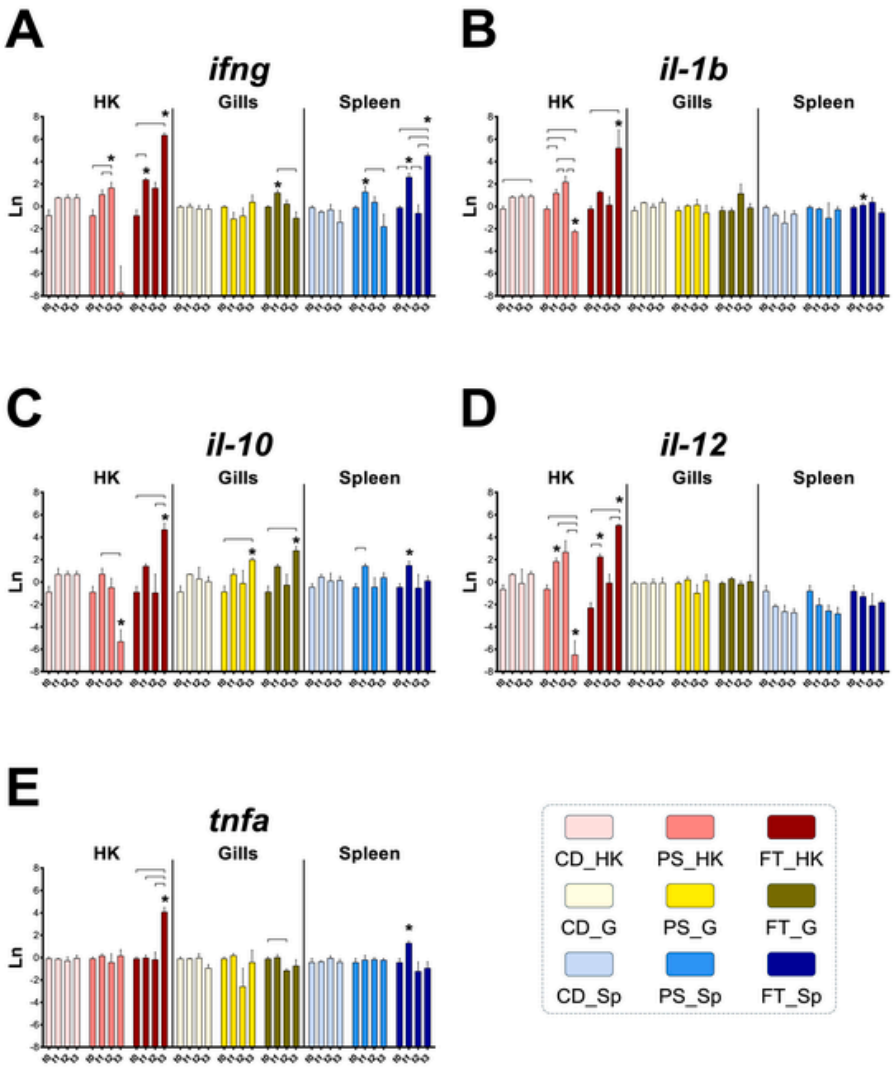

Fig. 3. Relative expression of cytokines (compared to CD at t0) in head kidney (HK), gills (G) and spleen (Sp). The results were displayed in natural logarithm (Ln). Sampling times: t0 (before functional feeds), t1 (34 days feeding functional feeds), t2 (90 days feeding functional feeds) and t3 (42 days after feeding functional feeds). In pink, red and dark red: head kidney, gills and spleen (respectively) from fish fed control diet (CD) throughout the experiment. In light yellow, yellow and dark yellow: head kidney, gills and spleen (respectively) from fish fed Pack salud additive (PS). In light blue, blue and dark blue: head kidney, gills and spleen (respectively) from fish fed Futerpenol® additive (FT). A: Interferon gamma (*ifng*). B: Interleukin 1 beta (*il-1b*). C: Interleukin 10 (*il-10*). D: Interleukin 12 (*il-12*). E: Tumor necrosis factor alpha (*tnfa*). *: Significant difference ($p < 0.05$) among experimental groups (PS or FT) and CD. Also, the line between bars (within each dietary group) shows a significant difference ($p < 0.05$) in the expression of biomarkers between sampling times. From A to E: t0 per organ is an average using 15 pooled samples (baseline) before starting to use functional feeds, while the other bars (t1, t2 and t3) are $n = 5$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

ifng r1 (Fig. 4E), a down-regulation was detected at t3 in gills and spleen (for instance, compared to t2). This was similar to *ifng r2* in gills (compared to t0 and t1), but opposite to the upregulation of *ifng r2* at t2 (compared to t0) in the spleen (Fig. 4F). In *mhci*, t3 and t1 had an upregulation compared to t2 (Fig. 4G).

Furthermore, FT group showed an upregulation of *cath2* (Fig. 5A) in HK (at t2 compared to t0; t3 compared to t0 and t1), as well as in spleen (t1 compared to t0 and t2. In *eomes* (Fig. 5B), both HK and gills had an upregulation at t3 compared to t1, and at t3 compared to t0, whereas in spleen, t1 was up-regulated compared to t0. On the other hand, *hepcidin* (Fig. 5C) showed an upregulation at t3 compared to t0 (HK). In spleen, this gene was up-regulated at t1 and t2 compared to t3. The expression of *igm* (both in HK and gills) was up-regulated at t1 compared to t0 (Fig. 5D), which was similar to *igt* expression in gills (Fig. 5E). However, *igt* also showed upregulation at t1 and t2 (compared to t3) in HK, while *inos* (Fig. 5F) had an upregulation at t1 compared to t0 and t3.

3.3. Correlations

Among dietary groups per organs (Fig. 6A), CD and FT were negatively correlated in HK, which was opposite to the positive correlation between these diets in the gills. Also, PS and FT were positively corre-

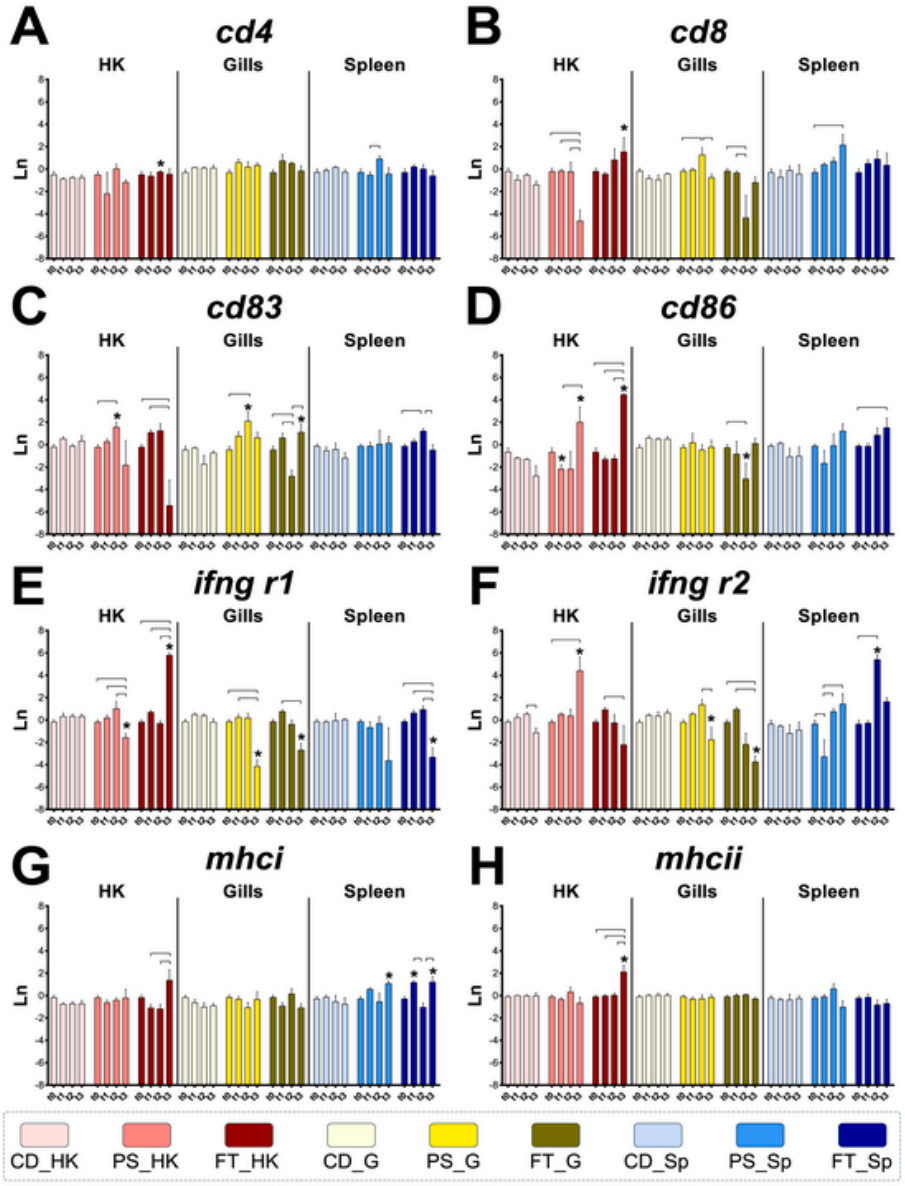

Fig. 4. Relative expression of cell-surface markers (compared to CD at t0) in head kidney (HK), gills (G) and spleen (Sp). The results were displayed in natural logarithm (Ln). Sampling times: t0 (before functional feeds), t1 (34 days feeding functional feeds), t2 (90 days feeding functional feeds) and t3 (42 days after feeding functional feeds). In pink, red and dark red: head kidney, gills and spleen (respectively) from fish fed control diet (CD) throughout the experiment. In light yellow, yellow and dark yellow: head kidney, gills and spleen (respectively) from fish fed Pack salud additive (PS). In light blue, blue and dark blue: head kidney, gills and spleen (respectively) from fish fed Futerpenol® additive (FT). A: Cluster of differentiation 4 (*cd4*). B: Cluster of differentiation 8 (*cd8*). C: Cluster of differentiation 83 (*cd83*). D: Cluster of differentiation 86 (*cd86*). E: Interferon gamma receptor 1 (*ifng r1*). F: Interferon gamma receptor 2 (*ifng r2*). G: Major histocompatibility complex I (*mhci*). H: Major histocompatibility complex II (*mhcii*). *: Significant difference ($p < 0.05$) among experimental groups (PS or FT) and CD. Also, the line between bars (within each dietary group) shows a significant difference ($p < 0.05$) in the expression of biomarkers between sampling times. From A to H: t0 per organ is an average using 15 pooled samples (baseline) before starting to use functional feeds, while the other bars (t1, t2 and t3) are $n = 5$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

lated in gills, whereas in the spleen, PS showed positive correlations with CD and FT.

Regarding comparisons between sampling times and dietary groups (Fig. 6B), CD and PS had a positive correlation at t0, t2, while PS was positive correlated with FT at t2 and negatively correlated to t3. Within the diets, only CD was positively correlated between different sampling times (t1 with t2 and t3, as well as t2 with t3).

4. Discussion

The gut is a key organ for nutrient absorption, but it can also organize local immunity and modulate systemic responses [20–24]. This can be achieved by pro- or anti-inflammatory cytokines and humoral components (e.g., antimicrobial peptides and immunoglobulins), as well as through the coordination of cells such as macrophages, antigen-presenting cells (APCs) and lymphocytes [20,21,25]. Furthermore, to improve overall fish health throughout farming process, and considering salmon mortality due to infectious diseases and environmental

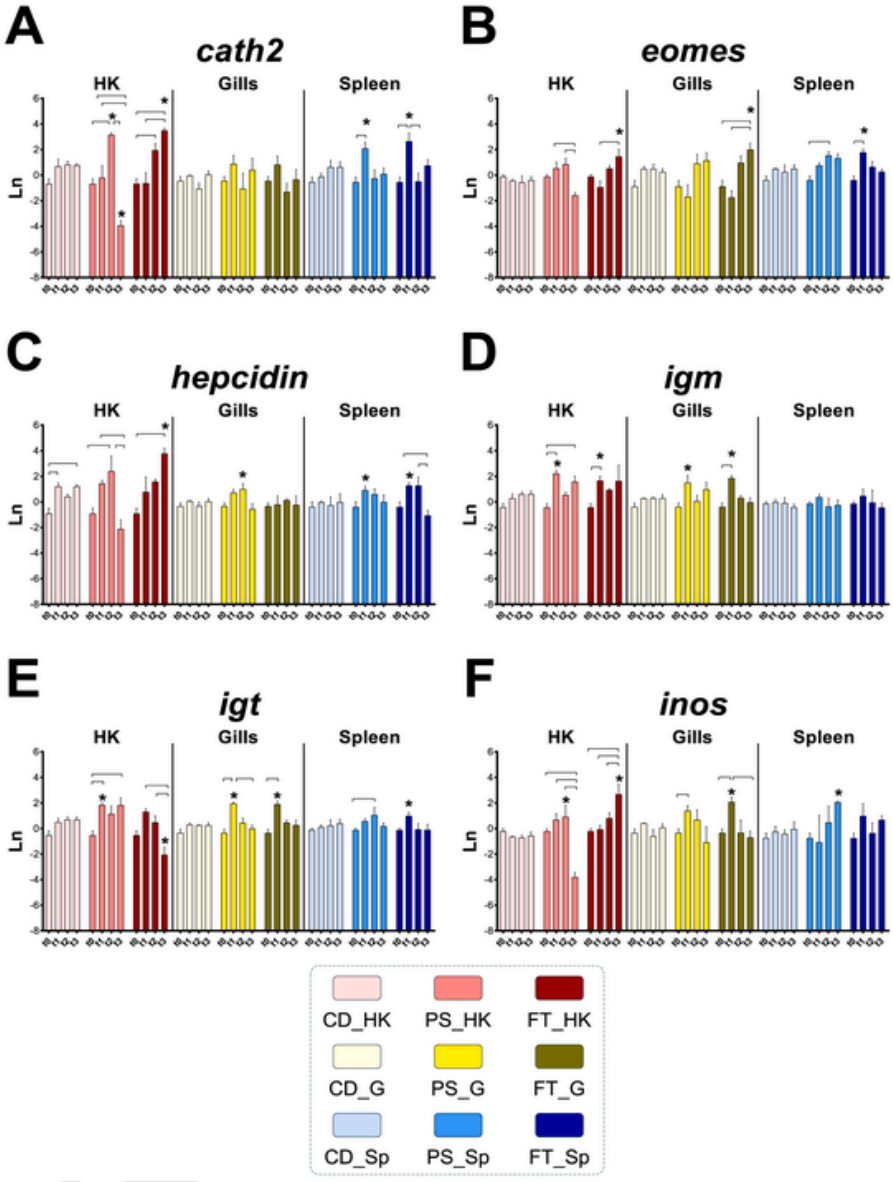

Fig. 5. Relative expression of effector molecules and transcriptional factors (compared to CD at t0) in head kidney (HK), gills (G) and spleen (Sp). The results were displayed in natural logarithm (Ln). Sampling times: t0 (before functional feeds), t1 (34 days feeding functional feeds), t2 (90 days feeding functional feeds) and t3 (42 days after feeding functional feeds). In pink, red and dark red: head kidney, gills and spleen (respectively) from fish fed control diet (CD) throughout the experiment. In light yellow, yellow and dark yellow: head kidney, gills and spleen (respectively) from fish fed Pack salud additive (PS). In light blue, blue and dark blue: head kidney, gills and spleen (respectively) from fish fed Futerpenol® additive (FT). A: Cathelicidin 2 (*cath2*). B: Eomesodermin (*eomes*). C: Hepcidin (*hepcidin*). D: Immunoglobulin M (*igm*). E: Immunoglobulin T (*igt*). F: Inducible nitric oxide synthase (*inos*). *: Significant difference ($p < 0.05$) among experimental group (PS or FT) and CD. Also, the line between bars (within each dietary group) shows a significant difference ($p < 0.05$) in the expression of biomarkers between sampling times. From A to F: t0 per organ is an average using 15 pooled samples (baseline) before starting to use functional feeds, while the other bars (t1, t2 and t3) are $n = 5$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

problems [1,26], the immune functions of the gut during specific feeding regimens are a target for FFs containing bioactive additives (with a focus that goes beyond nutritional aspects) [7,27]. However, the characterization of functional properties on feed additives has been largely performed in small-scale trials or controlled experiments. Therefore, results from fish under commercial aquaculture systems are still necessary to validate the effects of these compounds on fish physiology [27].

In our study with farmed post-smolts Atlantic salmon, the inclusion of PS or FT additives in FFs during seawater phase induced the modulation of cytokines, effector molecules and cell-surface markers linked to innate and adaptive immunity in head kidney, gills and spleen (compared to fish fed a commercial diet). This proposes a potential interaction from the intestine to other organs, which has already been suggested in challenged fish [28]. For instance, in Atlantic salmon exposed to a nutritional challenge (causing Soybean meal-induced enteritis, SBMIE), fish fed diets containing autolyzed *Cyberlindnera jadinii* yeasts showed a regulatory capacity to control the inflammatory response both in distal intestine [29] and spleen [30]. Specifically, in the spleen, data showed that the immunomodulation was related to the antigen presentation process (increase of MHCII and CD4; decrease of ZBTB46) and cytokines (increase of IL-10 and decrease of TNFa). In addition, the physiological response of salmon can also be coordinated by functional

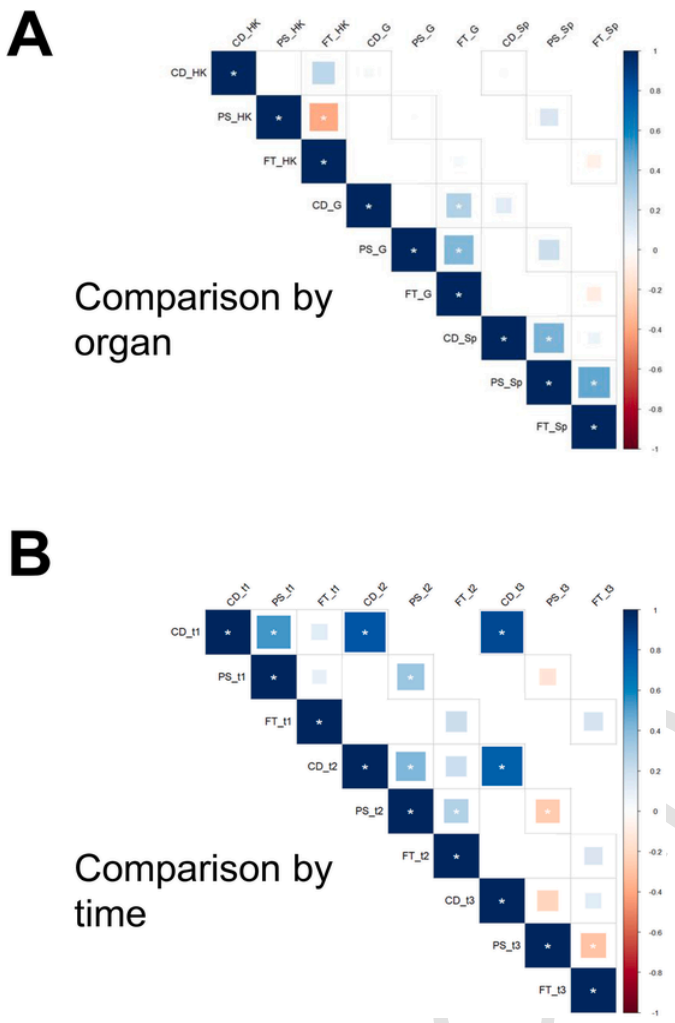

Fig. 6. Correlations. A: Comparison by organs. B: Comparison by sampling times. HK: head kidney. G: Gills. Sp: Spleen. CD: fish fed control diet. PS: fish fed Pack salud additive by 90 days until t2. FT: fish fed Futerpenol® additive by 90 days until t2. In blue: positive correlation. In red: negative correlation. *: Significant difference among data groups ($p < 0.05$; degrees of freedom: n-2). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

feeds in gills since it has already been reported that fish fed *D. hansenii*-based products and subjected to a short-term hypoxia can induce the upregulation of genes related to oxygen transport, hemoglobin complex, and glutathione transferase, as well as reducing plasma cortisol and IL-10 levels [31].

Compared to fish fed CD and/or among sampling times within dietary groups, during the 90-day feeding period with additives (PS or FT), the activation of immune-related genes was primarily at t1 (34 days of feeding). For example, both PS and FT groups had an upregulation of *igm* (gills and HK) and *igt* (gills) at this sampling time, as well as antimicrobial peptides such as *cath2* (in HK and spleen) and *hepcidin* (in spleen) were up-regulated in fish fed additives at t1. These results were linked to the upregulation of *eomes* (transcriptional factor related to NK-like cells) in spleen from FT group, and the increased expression of pro/anti-inflammatory cytokines (e.g., *ifng*, *il-10* and *il-12, tnfa*). Thus, this implies a modulation of innate and adaptive responses [25,32–34] likely by immune cells (e.g., macrophages, lymphocytes and NK-like cells) from myeloid or lymphoid lineages [20] during the first month using feed additives.

Nevertheless, throughout the trial, the main modulation of biomarkers was observed at t3, probably related to the algal bloom at the fish farm (between t2 and t3). These events affect Chilean aquaculture [2,35] by increasing fish mortality due to a lower oxygen content in the water [36,37]. Also, algal blooms can make fish more susceptible to bacterial pathogens (e.g., *P. salmonis* or *Tenacibaculum dicentrarchi*) [3]. Therefore, it is relevant to characterize the immune response of Atlantic salmon during this type of environmental challenges. In our case, although the accumulated mortality from t0 until the end of the experiment did not show differences among groups, specifically during the algal bloom period, fish previously fed with PS or FT additives showed a 10 % and 61 % mortality reduction (respectively, compared to CD). In addition, the comparison of these findings with gene expression at t3 showed that CD group only modulated few innate immune-related biomarkers in HK (upregulation of *il-1b* and *hepcidin*; downregulation of *ifng r2*), whereas FT group had the highest upregulation of several genes. For example, cytokines in HK (*ifng*, *il-1b*, *il-10*, *il-12* and *tnfa*), gills (*il-10*) or spleen (*ifng*), as well as cell-surface markers (*cd8*, *cd86*, *ifng r1*, *mhcii* in HK; *cd83* in gills; *mhci* in spleen), effector molecules in HK (*cath2*, *hepcidin* and *inos*), and eomes (HK and gills) had increased transcriptional levels at t3 in fish previously fed with FT. Particularly in HK, these results imply a role for macrophages during the innate response associated with the inflammatory process (via *ifng*, *il-1b*, *tnfa*, *il-10* and *inos*) and antigen presentation (by *mhcii*, *cd86*, *il-12*, *ifng*, *il-10*) [32], as well as the potential involvement of NK-like cells (or non-specific cytotoxic cells) by the modulation of genes such as *eomes* and *ifng* [33,38–42]. However, further research focused on cellular features and phenotypic mechanisms should deepen these findings.

The assessment of gene expression patterns by time showed that fish fed CD had positive correlations among all samplings. In contrast, FT did not show these patterns and even a negative correlation at t3 was detected (FT and PS comparison). In addition, HK had a negatively correlated gene expression between PS and FT, while in the other organs this comparison had positive correlations (as well as with CD group). These results contribute to proposing that among additives and sampling times, it was the gene expression of HK from FT at t3 which showed the most important differences. It should be noted that t3 was 42 days after ending the feeding period with additives, thus, FT could induce sustained immunomodulation in Atlantic salmon. Hence, FT is an interesting feed additive for continuing research under field conditions. In Chilean salmon farming, the use of FT could be to strengthen the fish immune response for specific periods of time. For instance, when the temperature of seawater increases during the transition from winter to summer. This period has also been associated with natural outbreaks of *P. salmonis* and a higher incidence of SRS, which is the major cause of salmonid mortality in Chile due to infectious diseases [43,44].

5. Conclusions

The inclusion of feed additives such as PS and FT in functional diets did not compromise the growth of post-smolts Atlantic salmon at the farm level in seawater. Also, during the 90-day feeding period with diets containing PS or FT, fish modulated their immune response both compared to CD, as well as within dietary groups over time. This was linked to a differential gene expression of pro- and anti-inflammatory cytokines, effector molecules and cell-surface markers in all three organs tested (head kidney, gills and spleen). However, the effects of the feed additives were mostly at t1 (34 days) in head kidney and spleen. For instance, the upregulation of innate immunity (e.g., *ifng*, *il-10*, *il-12*, *hepcidin and cath2*), and adaptive biomarkers (e.g., *igm*, *igt*) were observed at this sampling time (Fig. 7).

Although no differences in accumulated fish mortality were detected between dietary groups (from t0 to t3), in the specific time period between t2 and t3 (related to the natural algal bloom at the fish

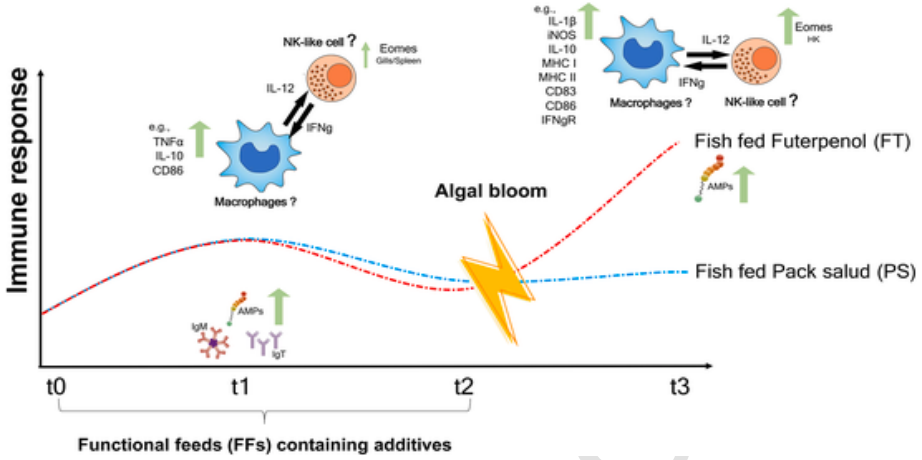

Fig. 7. Conceptual diagram summarizing the potential immunological mechanisms modulated by functional feeds in farmed Atlantic salmon post-smolts. Sampling times: t0 (before functional feeds), t1 (34 days feeding functional feeds), t2 (90 days feeding functional feeds) and t3 (42 days after feeding functional feeds). In red: fish fed Futerpenol® additive (FT) until t2. In blue: fish fed Pack salud additive (PS) until t2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

farm), PS and FT groups showed a decrease of 10 % and 61 %, respectively (compared to CD). FT group also showed an enhanced innate immune response (both cellular and humoral) at t3. This was mainly in the head kidney and to a lesser extent in the spleen (compared to fish fed control diet throughout the trial). For instance, the data showed the upregulation of effector molecules such as *inos*, *hepcidin* and *cath2,* and the coordination of cytokines (e.g., *il-1b*, *ifng*, *il-10 and il-12*) and cell-surface markers related to antigen presentation process (e.g., *cd86*, *mhci* and *mhcii*) at t3 (Fig. 7).

The t3 sampling was 42 days after ending the use of the FFs. Thus, FT could induce immunomodulatory effects that remain in the fish after its use. This is relevant given the multi-stressor conditions that salmonids face throughout the farming process (i.e., different infectious pathogens and/or environmental issues), which threaten fish health and welfare, with adverse economic repercussions for aquaculture.

CRediT authorship contribution statement

Byron Morales-Lange: Methodology, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Felipe Stambuk:** Methodology, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Felipe Ramírez-Cepeda:** Methodology, Formal analysis, Writing – review & editing. **Cristian Valenzuela:** Conceptualization, Methodology, Writing – review & editing. **Jörn Bethke:** Methodology, Formal analysis, Writing – review & editing. **Carlos Soto:** Conceptualization, Resources, Funding acquisition, Writing – review & editing. **Luis Mercado:** Conceptualization, Methodology, Funding acquisition, Writing – review & editing.

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Declaration of interest

During this study, CS was employed by Salmones Camanchaca S.A (Chile).. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that may constitute a potential conflict of interest.

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Data availability

Data will be made available on request.

References

[1] Sernapesca, informe sectorial de pesca y acuicultura, 2023. https://www.subpesca.cl/portal/618/articles-118321_documento.pdf. (Accessed 10 June 2025).
[2] D. Soto, J. León-Muñoz, R. Garreaud, R.A. Quiñones, F. Morey, Scientific warnings could help to reduce farmed salmon mortality due to harmful algal blooms, Mar. Pol. 132 (2021), https://doi.org/10.1016/j.marpol.2021.104705.
[3] sernapesca, informe con antecedentes sanitarios de agua dulce y mar año 1° semestre 2023, 2023.
[4] SERNAPESCA, informe sanitario con información sanitaria de agua dulce Y mar año 2022, 2023.
[5] S. Higuera-Llantén, F. Vásquez-Ponce, B. Barrientos-Espinoza, F.O. Mardones, S.H. Marshall, J. Olivares-Pacheco, Extended antibiotic treatment in salmon farms select multiresistant gut bacteria with a high prevalence of antibiotic resistance genes, PLoS One 13 (2018), https://doi.org/10.1371/journal.pone.0203641.
[6] W. Wang, J. Sun, C. Liu, Z. Xue, Application of immunostimulants in aquaculture: current knowledge and future perspectives, Aquac. Res. 48 (2017) 1–23, https://doi.org/10.1111/are.13161.
[7] S.D.C. Rocha, C.A. Valenzuela, B. Morales-Lange, Immunonutrition-contributing to the future of sustainable aquaculture by supporting animal performance, health and welfare, Animals 14 (2024), https://doi.org/10.3390/ani.
[8] S.D.C. Rocha, P. Lei, B. Morales-Lange, L.T. Mydland, M. Øverland, From a cell model to a fish trial: immunomodulatory effects of heat-killed lactiplantibacillus plantarum as a functional ingredient in aquafeeds for salmonids, Front. Immunol. 14 (2023), https://doi.org/10.3389/fimmu.2023.1125702.
[9] N. Sheikhzadeh, S. Mousavi, G. Hamidian, M. Firouzamandi, A. Khani Oushani, K. Mardani, Role of dietary Spirulina platensis in improving mucosal immune responses and disease resistance of rainbow trout (Oncorhynchus mykiss), Aquaculture 510 (2019) 1–8, https://doi.org/10.1016/j.aquaculture.2019.05.009.
[10] T.D. Khuyen, S.N.M. Mandiki, V. Cornet, J. Douxfils, S. Betoulle, P. Bossier, F.E. Reyes-López, L. Tort, P. Kestemont, Physiological and immune response of juvenile rainbow trout to dietary bovine lactoferrin, Fish Shellfish Immunol. 71 (2017) 359–371, https://doi.org/10.1016/j.fsi.2017.10.027.

[11] B. Morales-Lange, J.Ø. Hansen, B. Djordjevic, L.T. Mydland, M. Castex, L. Mercado, M. Imarai, H. Sørum, M. Øverland, Immunomodulatory effects of hydrolyzed Debaryomyces hansenii in Atlantic salmon (Salmo salar L): from the in vitro model to a natural pathogen challenge after seawater transfer, Aquaculture 578 (2024), https://doi.org/10.1016/j.aquaculture.2023.740035.
[12] A.J. Hernández, A. Romero, R. Gonzalez-Stegmaier, P. Dantagnan, The effects of supplemented diets with a phytopharmaceutical preparation from herbal and macroalgal origin on disease resistance in rainbow trout against Piscirickettsia salmonis, Aquaculture 454 (2016) 109–117, https://doi.org/10.1016/j.aquaculture.2015.12.016.
[13] F. de O. Roberti Filho, J.F.A. Koch, C. Wallace, M.C. Leal, Dietary β-1,3/1,6-glucans improve the effect of a multivalent vaccine in Atlantic salmon infected with Moritella viscosa or infectious salmon anemia virus, Aquac. Int. 27 (2019) 1825–1834, https://doi.org/10.1007/s10499-019-00436-9.
[14] E. Paduraru, E.I. Flocea, C.C. Lazado, I.A. Simionov, M. Nicoara, A. Ciobica, C. Faggio, R. Jijie, Vitamin c mitigates oxidative stress and behavioral impairments induced by deltamethrin and lead toxicity in zebrafish, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms222312714.
[15] P. Li, D.M. Gatlin, Nucleotide nutrition in fish: current knowledge and future applications, Aquaculture 251 (2006) 141–152, https://doi.org/10.1016/j.aquaculture.2005.01.009.
[16] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method, Methods 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.
[17] Pål A. Olsvik, Kai K. Lie, Ann-Elise O. Jordal, Tom O. Nilsen, Ivar Hordvik, Evaluation of potential reference genes in real-time RT-PCR studies of Atlantic salmon, BMC Mol. Biol. 6 (2005) 21, https://doi.org/10.1186/1471-2199-6-21.
[18] Wei Taiyun, Viliam Simko, R package "corrplot": visualization of a correlation matrix, https://github.com/taiyun/corrplot, 2021. (Accessed 10 June 2025).
[19] R Core Team, R A language and environment for statistical, https://www.R-project.org/, 2021. (Accessed 10 June 2025).
[20] M. Stosik, B. Tokarz-Deptuła, W. Deptuła, Haematopoiesis in zebrafish (Danio Rerio), Front. Immunol. 13 (2022), https://doi.org/10.3389/fimmu.2022.902941.
[21] S.A.M. Martin, C.E. Dehler, E. Król, Transcriptomic responses in the fish intestine, Dev. Comp. Immunol. 64 (2016) 103–117, https://doi.org/10.1016/j.dci.2016.03.014.
[22] D. Parra, F.E. Reyes-Lopez, L. Tort, Mucosal immunity and B cells in teleosts: effect of vaccination and stress, Front. Immunol. 6 (2015), https://doi.org/10.3389/fimmu.2015.00354.
[23] I. Salinas, The mucosal immune system of teleost fish, Biology 4 (2015) 525–539, https://doi.org/10.3390/biology4030525.
[24] P.T. Lee, F.Y. Yamamoto, C.F. Low, J.Y. Loh, C.M. Chong, Gut immune system and the implications of oral-administered immunoprophylaxis in finfish aquaculture, Front. Immunol. 12 (2021), https://doi.org/10.3389/fimmu.2021.773193.
[25] Y. Yu, Q. Wang, Z. Huang, L. Ding, Z. Xu, Immunoglobulins, mucosal immunity and vaccination in teleost fish, Front. Immunol. 11 (2020), https://doi.org/10.3389/fimmu.2020.567941.
[26] I. Sommerset, J. Wiik-Nielsen, T. Moldal, V.H.S. Oliveira, J.C. Svendsen, A. Haukaas, E. Brun, Fiskehelserapporten, 2023, 2024 Norway.
[27] A. Zuberi, M. Kamran, N. Younus, M. Abdel-Tawwab, Editorial: functional feed additives: current trends, Front. Aquacult. 3 (2024), https://doi.org/10.3389/faquc.2024.1385508.
[28] D. Gomez, J.O. Sunyer, I. Salinas, The mucosal immune system of fish: the evolution of tolerating commensals while fighting pathogens, Fish Shellfish Immunol. 35 (2013) 1729–1739, https://doi.org/10.1016/j.fsi.2013.09.032.
[29] J.O. Agboola, D.D. Mensah, J. Hansen, D. Lapeña, L.T. Mydland, M. Arntzen, S.J. Horn, O. Øyås, C.M. Press, M. Øverland, Effects of yeast species and processing on intestinal health and transcriptomic profiles of Atlantic salmon (Salmo salar) fed soybean meal-based diets in seawater, Int. J. Mol. Sci. 23 (2022), https://doi.org/10.3390/ijms23031675.
[30] B. Morales-Lange, J.O. Agboola, J.Ø. Hansen, L. Lagos, O. Øyås, L. Mercado, L.T. Mydland, M. Øverland, The spleen as a target to characterize immunomodulatory effects of down-stream processed Cyberlindnera jadinii yeasts in Atlantic salmon exposed to a dietary soybean meal challenge, Front. Immunol. 12 (2021), https://doi.org/10.3389/fimmu.2021.708747.
[31] B. Morales-Lange, B. Djordjevic, A. Gaudhaman, C.M.L. Press, J. Olson, L.T. Mydland, L. Mercado, M. Imarai, M. Castex, M. Øverland, Dietary inclusion of hydrolyzed Debaryomyces hansenii yeasts modulates physiological responses in plasma and immune organs of Atlantic salmon (Salmo salar) parr exposed to acute hypoxia stress, Front. Physiol. 13 (2022), https://doi.org/10.3389/fphys.2022.836810.
[32] J. Zou, C.J. Secombes, The function of fish cytokines, Biology 5 (2016), https://doi.org/10.3390/biology5020023.
[33] D.M. Mokhtar, G. Zaccone, A. Alesci, M. Kuciel, M.T. Hussein, R.K.A. Sayed, Main components of fish immunity: an overview of the fish immune system, Fishes 8 (2023), https://doi.org/10.3390/fishes8020093.
[34] R. Sinha, Macrophage: a key player of teleost immune system, in: Macrophages - Celebrating 140 Years of Discovery, IntechOpen, 2022, https://doi.org/10.5772/intechopen.103804.
[35] R.M. Montes, X. Rojas, P. Artacho, A. Tello, R.A. Quiñones, Quantifying harmful algal bloom thresholds for farmed salmon in southern Chile, Harmful Algae 77 (2018) 55–65, https://doi.org/10.1016/j.hal.2018.05.004.
[36] P.A. Díaz, G. Álvarez, Effects of microalgal blooms on aquaculture and fisheries, Fishes 8 (2023), https://doi.org/10.3390/fishes8090461.
[37] M. Abdel-Tawwab, M.N. Monier, S.H. Hoseinifar, C. Faggio, Fish response to hypoxia stress: growth, physiological, and immunological biomarkers, Fish Physiol. Biochem. 45 (2019) 997–1013, https://doi.org/10.1007/s10695-019-00614-9.
[38] A.S. Büchau, S. Morizane, J. Trowbridge, J. Schauber, P. Kotol, J.D. Bui, R.L. Gallo, The host defense peptide cathelicidin is required for NK cell-mediated suppression of tumor growth, J. Immunol. 184 (2010) 369–378, https://doi.org/10.4049/jimmunol.0902110.
[39] J. Kumari, J. Bøgwald, R.A. Dalmo, Eomesodermin of Atlantic salmon: an important regulator of cytolytic gene and interferon gamma expression in spleen lymphocytes, PLoS One 8 (2013), https://doi.org/10.1371/journal.pone.0055893.
[40] J. Acosta, F. Roa, I. González-Chavarría, A. Astuya, R. Maura, R. Montesino, C. Muñoz, F. Camacho, P. Saavedra, A. Valenzuela, O. Sánchez, J.R. Toledo, In vitro immunomodulatory activities of peptides derived from Salmo Salar NK-lysin and cathelicidin in fish cells, Fish Shellfish Immunol. 88 (2019) 587–594, https://doi.org/10.1016/j.fsi.2019.03.034.
[41] Y. Hu, A. Alnabulsi, A. Alnabulsi, C. Scott, C. Tafalla, C.J. Secombes, T. Wang, Characterisation and analysis of IFN-gamma producing cells in rainbow trout Oncorhynchus mykiss, Fish Shellfish Immunol. 117 (2021) 328–338, https://doi.org/10.1016/j.fsi.2021.07.022.
[42] M. Huang, Z. Zhang, X. Li, J. Feng, Y. Huang, A. Kwaku, Y. Huang, J. Jian, Molecular characteristics and functional analysis of non-specific cytotoxic cell receptor (NCCRP1) in golden pompano (Trachinotus ovatus), Fish Shellfish Immunol. 145 (2024), https://doi.org/10.1016/j.fsi.2023.109344.
[43] F. Bravo, J.P.S. Sidhu, P. Bernal, R.H. Bustamante, Hydrodynamic connectivity, water temperature, and salinity are major drivers of piscirickettsiosis prevalence and transmission among salmonid farms in Chile, Aquacult. Environ. Interact. (2020) 263–279, https://doi.org/10.3354/aei00368.
[44] G. Carril, H.C. Winther-Larsen, M. Løvoll, H. Sørum, Cohabitation of Piscirickettsia salmonis genogroups (LF-89 and EM-90): synergistic effect on growth dynamics, Front. Cell. Infect. Microbiol. 13 (2023), https://doi.org/10.3389/fcimb.2023.1253577.