



Immunomodulatory effects of dietary seaweeds in LPS challenged Atlantic salmon *Salmo salar* as determined by deep RNA sequencing of the head kidney transcriptome

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Aim

Seaweeds may represent immunostimulants that could be used as health-promoting fish feed components thereby offering an alternative for the use of antibiotics.

This study was performed to gain insights into the immunomodulatory effects of dietary seaweeds in Atlantic salmon.

Specifically tested were:

- **SW1: 10% inclusion levels of *Laminaria digitata*;**
- **SW2: commercial blend of seaweeds (Oceanfeed®);**
- **against a fishmeal based control diet (FMC).**

Differences between groups were assessed in growth, feed conversion ratio (FCR) and blood parameters hematocrit (Hct) and hemoglobin (Hb). After a LPS challenge in fish representing each of the three groups, RNAseq was performed on head kidneys to determine transcriptomic differences in response to the immune activation, to our knowledge for the first time in fish in this context.



SW1, SW2 and FMC diets were fed for 6 weeks after which fish were either sampled or given a LPS injection (6 mg.kg⁻¹) and sampled after 68-70 h



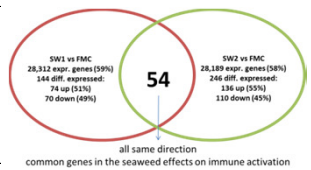
Sampling the head kidney and RNAseq with an Illumina HiSeq2500. Reads were aligned to 48,223 *S. salar* cDNA sequences from NCBI.

Results (RNAseq)

RNAseq resulted in ~154 million single-read 1 x 50 nt reads (~8 Gb data). De novo assembly then resulted in 87,600 cDNA contigs ranging in size from 200 - 14,086 nt. The comparison SW1 vs FMC yielded 28,312 expressed contigs associated with *S. salar* genes: 59% of the NCBI sequences. 144 of these contigs were differentially expressed: 74 up-regulated (51%) and 70 down-regulated (49%). The comparison SW2 vs FMC yielded 28,189 expressed contigs associated with *S. salar* genes: 58% of the NCBI sequences. 246 of these contigs were differentially expressed: 136 up-regulated (55%) and 110 down-regulated (45%). SW1 vs. FMC and SW2 vs. FMC revealed 54 common differentially expressed contigs. Expression of all these contigs was in the same direction, either up- or down-regulated. These contigs can be considered representing the common genes in the immunomodulatory effects of the tested dietary seaweeds. The immune genes were MHC class I and II and HLA class II antigens, and CD209 antigen-like protein E, all involved in antigen processing and presentation.

specific for SW1	fc
MHC class I (UBA) mRNA, UBA*0901 allele	362
T-cell receptor alpha chain V region 2B4 precursor putative mRNA	7.19
MHC class I antigen (UBA) mRNA, UBA*4001 allele	6.61
Macrophage migration inhibitory factor putative mRNA	Inf
MHC class I antigen (Sasa-UBA) mRNA, Sasa-UBA*0902 allele	Inf
BOLA class I histocompatibility antigen, alpha chain BL3-7 precursor putative mRNA	0.154
MHC class I (UBA) mRNA, UBA*0501 allele	0.131
MHC class I mRNA	0.0467
MHC class I (UBA) mRNA, UBA*0701 allele	0.00130

specific for SW2	fc
MHC class I antigen (Sasa-UBA) mRNA, Sasa-UBA*3701 allele	288
H-2 class II histocompatibility antigen gamma chain putative mRNA	137
MHC class II antigen alpha chain (Sasa-DAA) mRNA	39.7
MHC class II alpha mRNA	18.6
Tumor necrosis factor receptor superfamily member 11B precursor putative mRNA	4.14
CD209 antigen-like protein E putative mRNA	3.21
Ig kappa chain V-IV region Len putative mRNA	2.86
partial mRNA for MHC class II antigen beta chain (DAB gene)	Inf
MHC class I antigen (Sasa-UBA) mRNA, Sasa-UBA*1402 allele	Inf
Class I histocompatibility antigen, F10 alpha chain precursor putative mRNA	0.254
MHC class I (UBA) mRNA, UBA*0501 allele	0.131
MHC class I antigen (Sasa-UBA) mRNA, Sasa-UBA*3501 allele	0.125
Proliferating cell nuclear antigen putative mRNA	0.113
MHC class I (UBA) mRNA, UBA*1501 allele	0.00567
Ananorsin putative mRNA	0



common SW1-SW2	SW1(fc)	SW2(fc)
MHC class II antigen beta chain (DAB gene)	309	171
MHC-Sasa class II (clone c144)	54.7	7.76
MHC class I (UBA) mRNA, UBA*0201 allele	36.8	35.0
MHC class I antigen (Sasa-UBA) mRNA	Inf	Inf
MHC class I antigen (Sasa-UBA) mRNA, Sasa-UBA*0202 allele	Inf	Inf
MHC class II antigen alpha chain (Sasa-DAA) mRNA	Inf	Inf
HLA class II histocompatibility antigen, DQW1.1 beta chain precursor putative mRNA	Inf	Inf
CD209 antigen-like protein E putative mRNA	Inf	Inf
MHC class I (UBA) mRNA, UBA*1401 allele	0.350	0.351
MHC-Sasa class II B (clone c22)	0.276	0.00283
MHC class I antigen (Sasa-UBA) mRNA, Sasa-UBA*3901 allele	0.00349	0

The comparisons SW1 vs FMC and SW2 vs FMC revealed 54 common differentially expressed genes all in the same direction. The Table lists the immune genes that were specific for SW1, for SW2 and those in common (fc= fold change; 'inf' indicates expression only in SW diets and not in FMC)

Results (growth performance)

No differences in growth performance were detected between FMC, SW1 and SW2 fish. Interestingly, fish fed with seaweed diets showed much less individual variation in growth performance than the FMC fish. SW fish showed higher FCR than fish fed with seaweed diets. No significant differences existed between groups in Hct and Hb.

Conclusions

- Seaweed fed fish showed higher FCRs but more homogenous growth performance than the control fish.
- Dietary seaweeds caused important immunomodulatory effects changing antigen processing and presentation.

