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CALANUS *C. finmarchicus* HYDROLYSATE AND SILAGE IMPROVES FEED INTAKE, GROWTH AND HEALTH OF ATLANTIC SALMON *Salmo salar* IN THE FIRST PERIOD AFTER SEAWATER TRANSFER

Calanus (*Calanus finmarchicus*) is a small copepodite with large lipid storage that periodically can constitute as much as 90% of the standing stock of zooplankton in the Norwegian Sea and the Barents Sea. The PUFA rich oil has been commercially utilized for many years and has been explored as a promising source of marine n-3 fatty acids in feed for Atlantic salmon. High production losses of A. salmon have been reported in the first period after seawater transfer of fish. Fish protein hydrolysates have demonstrated effective feed attractant properties after transfer of salmon and shown to improve the growth of fish. In this study, calanus hydrolysate (FPH) and calanus silage (FPC) were evaluated as feed intake, growth and health promoters as additives in post-transfer feed for A. salmon.

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Calanus (Calanus finmarchicus) as potential feed attractant, growth and health stimulant in feed for A. salmon (Salmo salar)

Diets and fish feeding trial

Six experimental diets were produced where the control diet was a standard formulated salmon diet. The calanus ingredients were added at two levels by top coating (FPH 2.5 %, FPH 5.0 %) on the extruded pellets. In addition, a commercial calanus FPH (Zooca) produced by Calanus AS was also added by top-coating (2.5 %). The calanus protein mainly exchanged the dietary fishmeal protein. A 12-weeks feeding trial was carried out with salmon (84 g), starting out from the first day of feeding after seawater transfer. The dietary impacts on daily feed intake, growth, plasma and liver health markers (lipid profile, total tocopherols, stress markers, other's), and on the skin quality (transcriptional profiling) were measured.

Feed intake, growth and protein digestibility

All calanus ingredients (FPH, FPC) significantly increased feed intake and growth of salmon as compared to fish fed the control diet in the 6 first weeks of feeding ($P < 0.05$), while no differences with respect to the dietary inclusion levels were found ($P < 0.05$), Figure 1. A linear correlation (corr. = 0.95, $P < 0.05$) between feed intake and growth was found, indicative of efficient nutrient utilization, while at the same time improved protein digestibility was also found in fish fed calanus FPH 5 % and FPC 5 % compared to control fish ($P < 0.05$), Figure 2. Accordingly, the digestibility of amino acids (AA) and essential AA (EAA) were significantly higher in all groups fed calanus ingredients compared to control fish ($P < 0.05$). Fish fed calanus ingredients gained 84 - 88 % of their initial weight and showed efficient growth rates; mean SGR = 1.50 ± 0.02 % in the first 6 weeks of feeding post-transfer, significantly higher than in fish fed the control diet; mean SGR = 1.20 ± 0.04 % ($P < 0.05$). Compensatory growth in fish fed the control diet in the following 6 weeks of feeding resulted in no significant differences in total growth (Week 0–12).

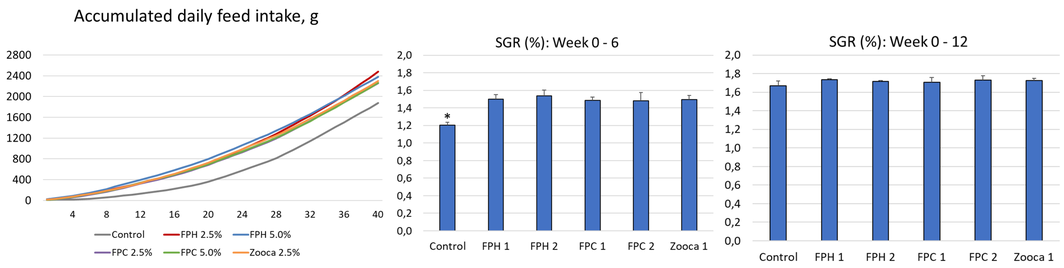

*Figure 1 Accumulated feed intake (g) and daily growth (SGR, %) in A. salmon after seawater transfer of fish fed control diet, pilot produced calanus hydrolysate (FPH) and concentrate (FPC), or a commercial hydrolysate (Zooca) at dietary levels 1 (2.5 %) or 2 (5.0 %). Mean values + STD(x) are given. * Control fish show significantly lower growth compared to all other dietary groups (Week 0 - 6).*

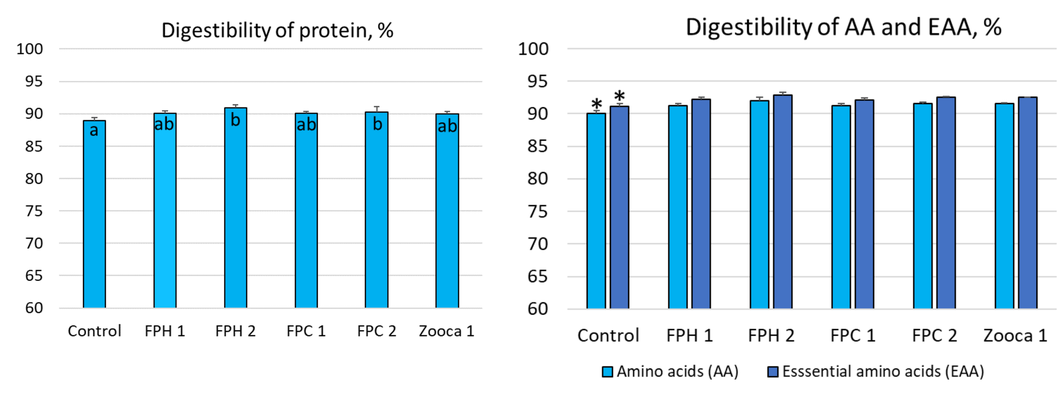

*Figure 2 Protein, amino acid (AA) and essential AA (EAA) digestibility in A. salmon after 12 weeks of feeding. Salmon were fed control diet, pilot produced calanus hydrolysate (FPH) and concentrate (FPC), or a commercial hydrolysate (Zooca) at dietary levels 1 (2.5 %) or 2 (5.0 %). Mean values + STD(x) are given. * Control fish show significantly lower AA and EAA digestibility compared to all other dietary groups.*

Liver and plasma health markers

No dietary differences in liver weight (g), liver somatic index (HSI, %) and lipid contents (%) was found in A. salmon after 12 weeks of feeding (ns). Accordingly, no significant differences in plasma lipid class composition (TG, Total chol, HDL-Chol, PL) or plasma markers for oxidative stress (FRAP, TAC) were found (ns). Liver MDA was significantly reduced in fish fed calanus FPC 2 and reduced in fish fed FPH 2, suggesting reduced oxidative stress (Figure 3), although no dietary effects on liver tocopherols or on reduced (GSH) and oxidized (GSSG) glutathione were detected.

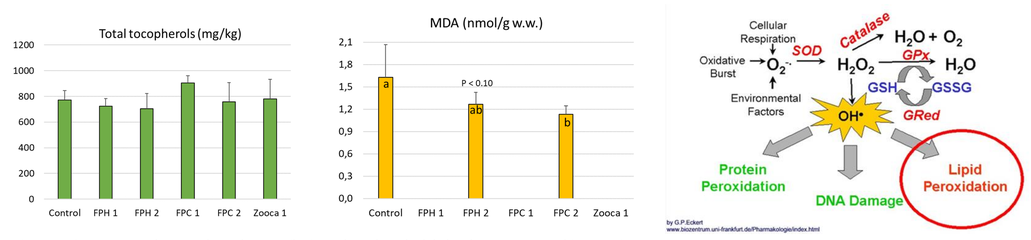

Figure 3 Liver total tocopherols (mg/kg) and malondialdehyd (MDA, nmol/g w.w.) in A. salmon fed control diet, pilot produced calanus hydrolysate (FPH) and concentrate (FPC) and a commercial hydrolysate (Zooca) at dietary levels 1 (2.5 %) or 2 (5.0 %). Mean values + STD(x) are given.

Transcriptional profiling of skin

In the skin, the genetic responses to FPH was much stronger than to FPC, while the correlation was relatively high (r = 0.69). There was not much in common between the pilot produced calanus FPH and FPC versus the commercial produced FPH (Zooca), which only means that the commercial calanus product had other bioactive properties than the pilot produced calanus ingredients.

The expression differences in the downregulated genes in fish fed calanus FPH were greater by scale than the up-regulated genes (Table 1), and many of the downregulated genes encode structural proteins and regulators of tissue differentiation consistent with previous studies of skin wounds healing.

Table 1 Genetic profiling in fish fed (FPH 5%)

Function	Gene	Symbol	FPH
Collagen	Collagen Type XI Alpha2		3.2
Collagen	Collagen 2a1a protein		3.2
Collagen	Collagen_type I_ alpha 2		3.0
Immune effector	TNFa-induced protein 3	TNFAIP3	4.2
B cell	Fc receptor-like protein 2	LOC106583577	3.2
T cell	TCR	LOC106569105	3.0
Ion channel	Chloride intracellular channel 4	clic5	6.6
Protease inhibitor	Calpain-1 catalytic subunit	capn1b	7.2
Protease	Cystatin-B-like	LOC106603058	3.1
Differentiation	Metastasis associated family 1-2	LOC106589409	3.3
Cytoskeleton	Dystonin	LOC106612695	-13.0
Ubiquitin	E3 ubiquitin-protein ligase MARCH4	S34425223	-13.8
Antigen presentation	MHC class I antigen	LOC106588401	-4.4
Chem-okine	Chem-okine CCL-C11b	ccl-c11b	-3.7
Lymphocyte	Growth factor independent protein 1B, Gfi-1b	LOC106565457	-13.4
T cell	Drebrin-like a	LOC106567986	-6.2
TNF-related	TNF receptor superfamily member 1A	tnr1a	-3.2
Lipid metabolism	Aldehyde dehydrogenase family 3-A2a	LOC106602847	-17.8
Cell adhesion	Occludin	LOC106567754	-9.7
Cell adhesion	Claudin-18	cldn18	-9.9
Cell adhesion	Nephronectin	npnt	-10.9
Differentiation	R-spondin-3	rspo3	-5.3
Differentiation	Transmembrane protein 88 b	LOC106563386	-9.4
Differentiation	Glucocorticoid receptor	S22216917	-11.9
Differentiation	Tripartite motif-containing protein 55	trim55	-15.9
Differentiation	Myogenic factor 5	myf5	-4.5
Mucus	GMP Giant mucus protein	LOC106592404	-10.4
Endothelium	Cysteinyl leukotriene receptor 2-like	LOC106603409	-5.4
Endothelium	EGF-like domain-containing protein 7	egfl7	-11.1
Glycan	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase-like protein 1	b3gntl1	-10.2
Neural	Synaptosome-a-associated protein 25a	LOC100194580	-17.1

Summary and conclusion

- Calanus FPH and FPC significantly increased feed intake and growth of A. salmon in the first 6 weeks of feeding after seawater transfer, while no significant differences in growth was found after 12 weeks of feeding due to compensatory growth in fish fed the control diet.
- The protein and amino acid digestibility was significantly improved in all fish groups fed calanus ingredients (FPH, FPC), probably explained by higher levels of water-soluble proteins in the diets.
- Reduced levels of liver MDA suggests antioxidant properties of calanus FPH and FPC, consistent with previous results *in vitro*.
- Transcriptional responses to calanus FPH were relatively strong in the skin and many of the downregulated genes showed similar profiles as in previous skin wound healing studies, suggesting impacts on the transcription or regulation of the cellular composition of the skin.

Conclusion: Calanus FPH and FPC stimulated feed intake, growth and protein digestibility in A. salmon, and reduced markers of oxidative stress in liver, while calanus FPH appeared to improve the skin quality. The functional roles of calanus ingredients on health and robustness of salmon following seawater transfer needs to be further confirmed.