Extraction of bioactive components in cultivated seaweed

SIG Seaweed workshop IV 4th April 2017, Trondheim.

Liv Torunn Mydland, Dept. of Animal & Aquacultural Sciences, NMBU.



Overview

Photo: FMC

- Brief introduction on bioactive components in seaweed
- Results from a recent experiment:
  - –Extraction of fucoidan & laminarin
  - –Chemical characterization
  - –*In vivo* feeding experiment with Atlantic salmon
- Conclusions and further work

Bioactive components in cultivated seaweed

BROWN SEAWEED (~1800 species)

Nutrients:

+ protein, lipid, vitamins, minerals

– low levels of most nutrients, often too high levels of e.g. arsenic and iodine.

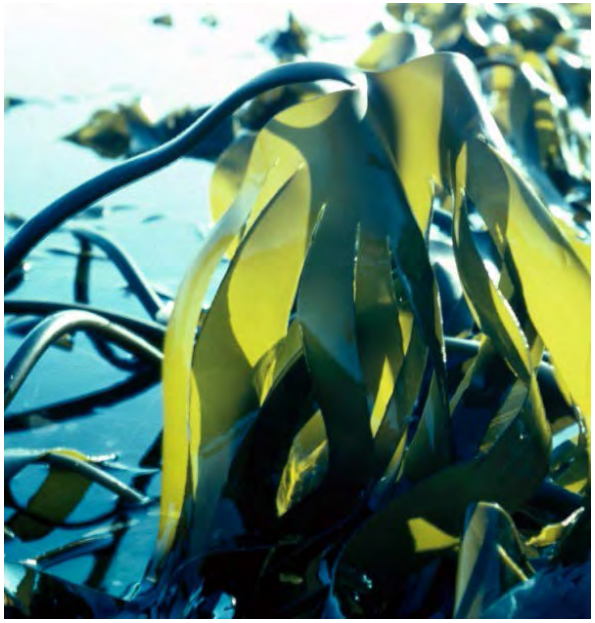
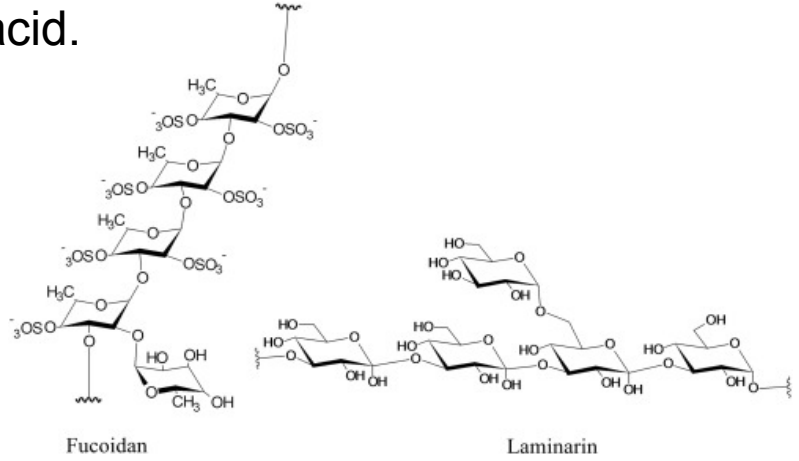


Photo: Seaweed Energy Solutions

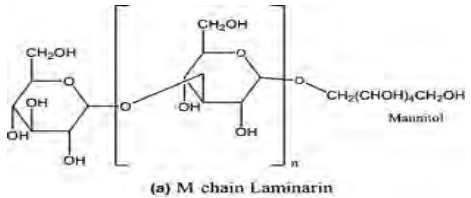
- *Polysaccharides:* fucoidan, laminarin, alginate, cellulose….carrageenan)
- *Sterols:* in brown SW: fucosterol
- Pigments: Chlorophylls / Carotenoids (fucoxanthin, β-carotene)
- Phenols / phlorotannins

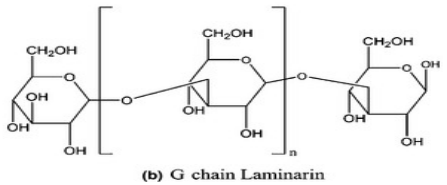
Polysaccharides

- Laminarin: main storage polysaccharide in brown macroalgae, consisting mostly of linear b-1,3-linked glucose units with small amounts of b-1,6-linkages.
- Fucoidan: a heterogeneous polysaccharide in brown macroalgae, consisting primarily of 1,2-linked a-L-fucose-4-sulfate units with very small amounts of D-xylose, D-galactose, D-mannose, and uronic acid.



- **Alginate**: a linear polymer consisting of 1,4-linked b-D-mannuronic acid (M) and a-L-guluronic acid (G) in varying sequences.
-
- **Cellulose** - structural component of both plants and many forms of algae (chain of several hundred to more than 10 000 b-1,4-linked D-glucose units).





Reported bioactivity/medical properties

- Fucoidan:
  - Immunemodulating
  - Antithrombotic
  - Anticoagulant
  - Antiviral (anti-infectious)
  - Antibacterial / probiotic
  - Antitumor
  - Antioxidant
  - Antiinflammatory
  - Antilipidemic
  - Wound healing
- Laminarin:
  - Immunemodulating
  - Antithrombotic
  - Anticoagulant
  - Antiviral (anti-infectious)
  - Antibacterial / probiotic
  - Antitumor
  - Antioxidant
  - Antiinflammatory

Many of the observed effects: possibly indirectly by modulation of gut microbes.

Conflicting results – due to:

- Many challenges when doing the extractions!
- Structural variation of the different polysaccharides with:
  - Different macroalgae species
  - Different harvest seasons & locations
  - Maturity of the samples
- Different extraction protocols – different purities of extract
- Difficult to analyze purity & structure of the components
  - Colourimetric tests
  - Chemical composition/sugar composition
  - Size exclusion chromatography

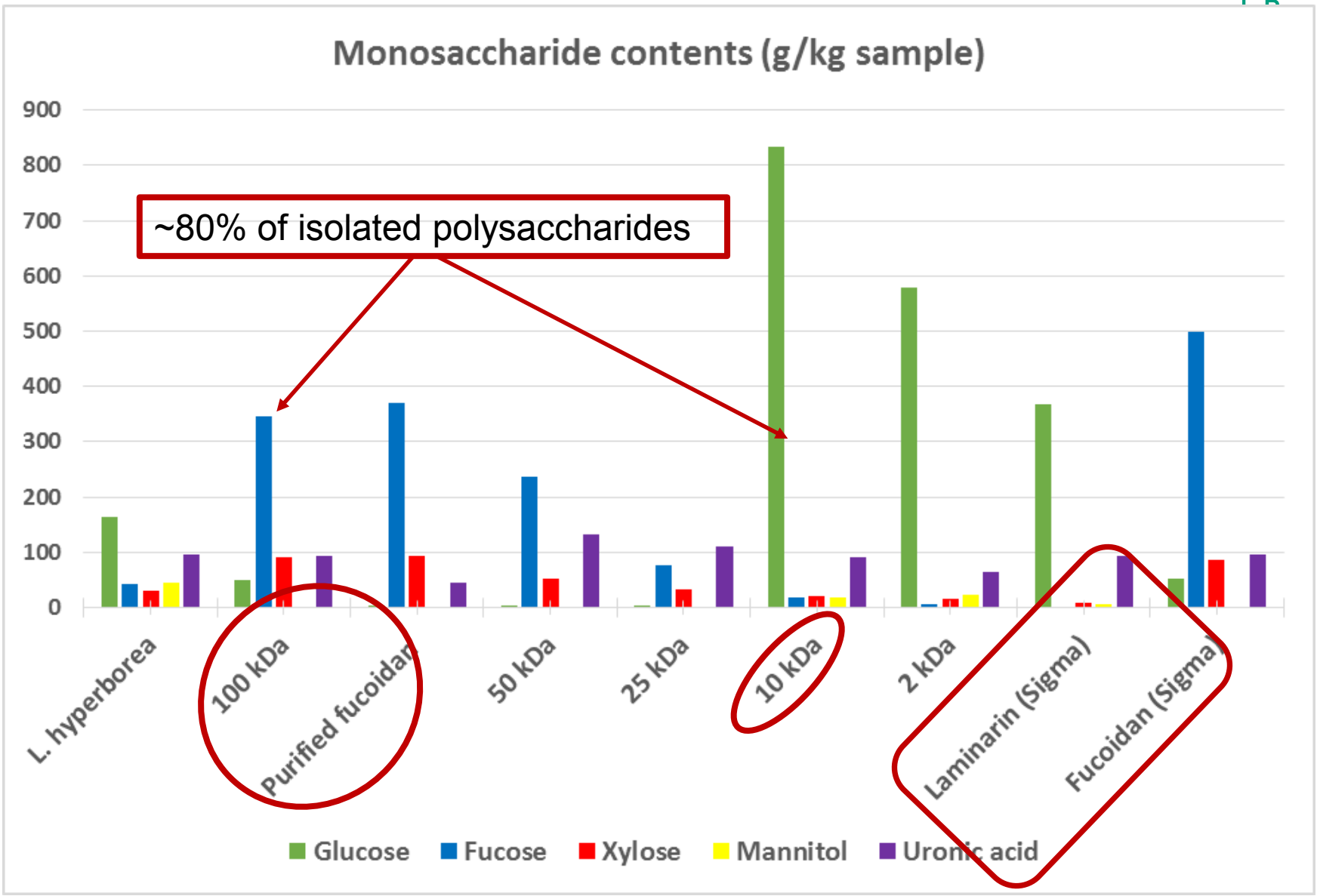
In vivo experiments

- “Currently, there is a need for reproducible, well characterized fucoidan fractions to ensure significant progress” (Chollet et al. 2016).
- Many in vivo experiments with promising results!
- BUT: many of the experiments do not contain details for extraction protocol and chemical analyses/purity

Ion-exchange; size-exclusion; affinity chromatography; Membrane filtration

Extraction procedure:

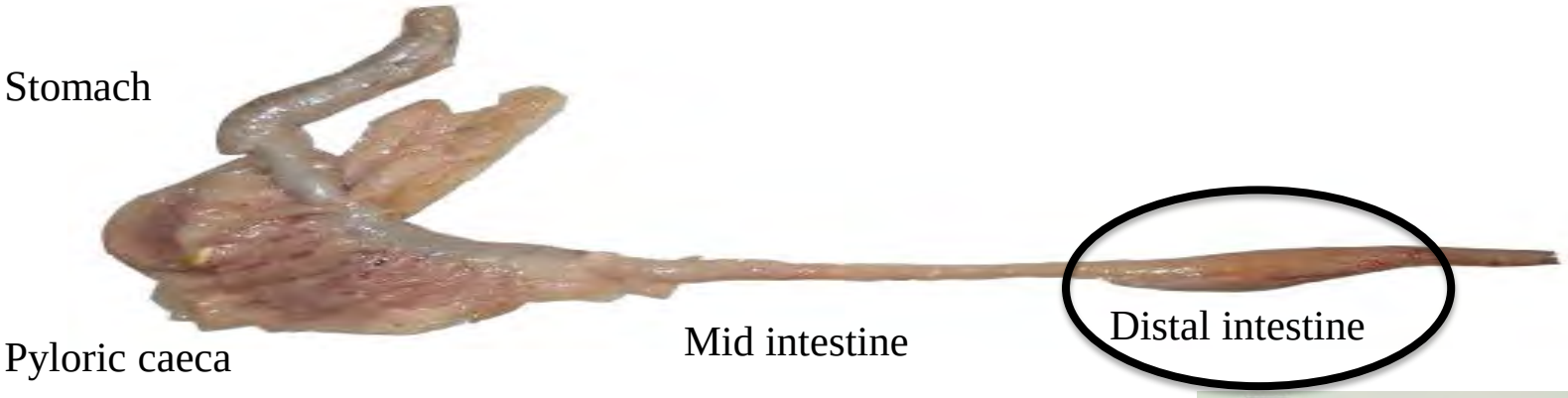
- Starting material: dried winter harvested *L. hyperborea* (stortare/kelp)
- Ground to pass through a 1mm sieve (LH).
- Milled LH was mixed with 0.03M HCl in ratio of 1kg:20L and incubated for 1h at 70^0C.
- Large particles were removed by filtration (nylon filter).
- 1M $CaCl_2$ at a ratio of 1:1 was added to filtrate - stored at 4ºC ON (resulting in precipitation of alginate – filtered & centrifuged to remove precipitate).
- The filtrate was then fractionalized in GEA membrane filtration pilot – sequential filtration through spiral membranes with MWCO of 100, 50, 25, 10 and 2 kDa.
- Retentate from each membrane was collected and freeze dried for further analysis.
- (100kDa fraction was also further purified: re-dissolved and EtOH precipitation of alginate).


Monosaccharide contents (g/kg sample)
900
800
700
600
500
400
300
200
100
0
~80% of isolated polysaccharides
L. hyperborea
100 kDa
Purified fucoidan
50 kDa
25 kDa
10 kDa
2 kDa
Laminarin (Sigma)
Fucoidan (Sigma)
Glucose
Fucose
Xylose
Mannitol
Uronic acid

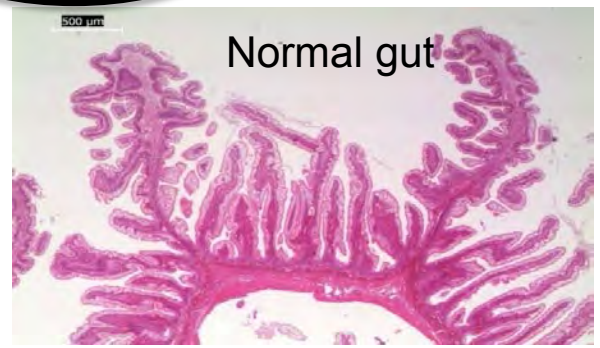
Chemical Composition

Elements (g/kg)	Carbon	Hydrogen	Nitrogen	Sulphur	C:N ratio	C:S ratio
L. hyperborea	303	50	17	16	18	19
100 kDa	237	56	4	88	59	2.7
50 kDa	178	48	3	78	60	2.3
25 kDa	109	30	5	71	23	1.5
10 kDa	368	65	1	8	442	48
2 kDa	373	65	1	7	361	52
Purified Fucoidan	224	50	5	90	48	2.5
Fucoidan Sigma	252	46	0	67	-	3.8
Laminarin Sigma	220	41	1.5	34	150	6.5

Plant ingredients increase risk of gut health disorders in fish



Soybean meal in feed for Atlantic salmon induces a dose-dependent inflammation (enteritis) in the distal intestine



Diets

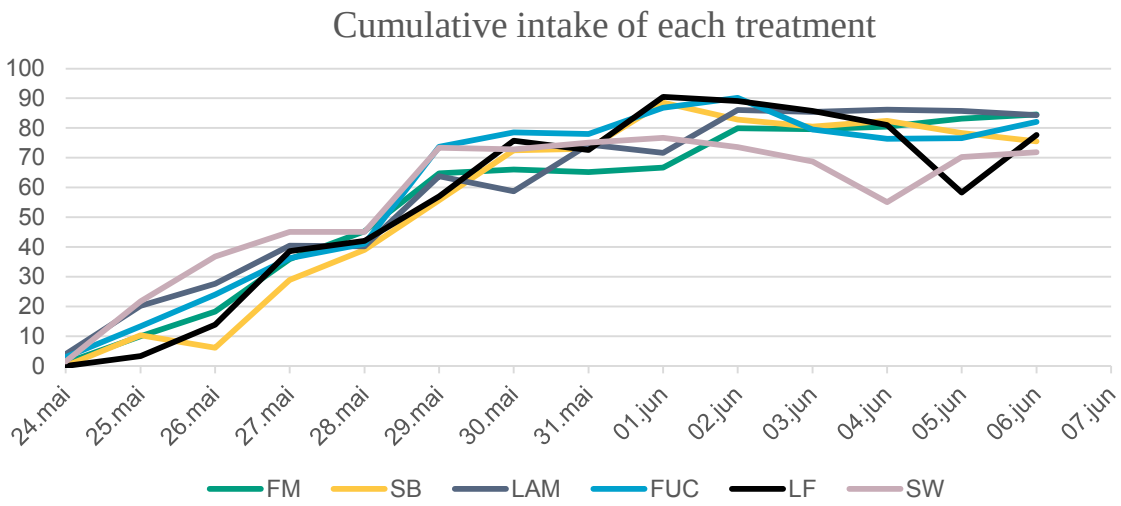
Ingredient (g/kg diet)	FM	SBM	LH	FUC	LAM	LAM+FUC
Soybean meal		200	200	200	200	200
Fish meal	425	193	193	193	193	193
Corn gluten meal		60	35	60	60	60
Wheat flower	169	181	132	181	181	181
Wheat gluten	151	150	170	150	150	150
Fish oil	240	186	191	186	186	186
P, choline, AA's, premiks (min/vit)	15	30	29	30	30	30
Fucoidan (crude extract)	-	-	-	0.75	-	-
Laminarin (crude extract)	-	-	-	-	0.75	-
Laminarin+Fucoidan	-	-	-	-	-	0.75+0.75
Laminaria hyperborea	-	-	50	-	-	-

Chemical comp. (g/kg)	FM	SBM	LH	FUC	LAM	LAM+FUC
Dry matter	963	941	940	932	936	940
Crude protein	445	409	414	407	397	414
Gross energy MJ/kg	24	22.4	22.7	22	23	22.4
Total lipid	253	182	182	181	182	168
Starch	113	144	115	162	155	164
Total ash	90	63	70	64	62	63

Salmon experiment

- Salmon in salt water (NIVA, Solbergstrand)
- Initial BW: 250 g
- Triplicate tanks
- 12 fish / tank
- 28 days duration

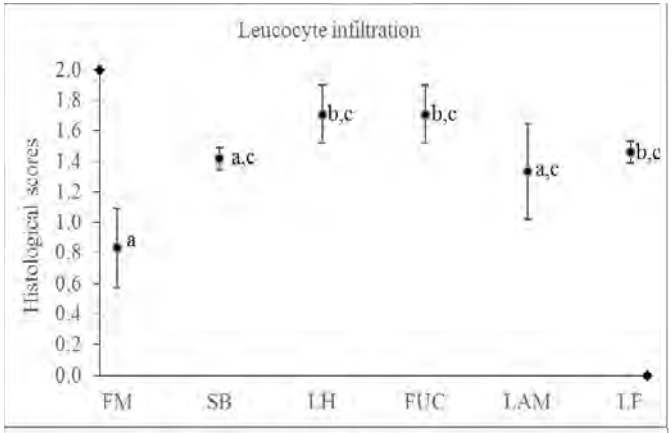
Disease problems.
Several dead fish.

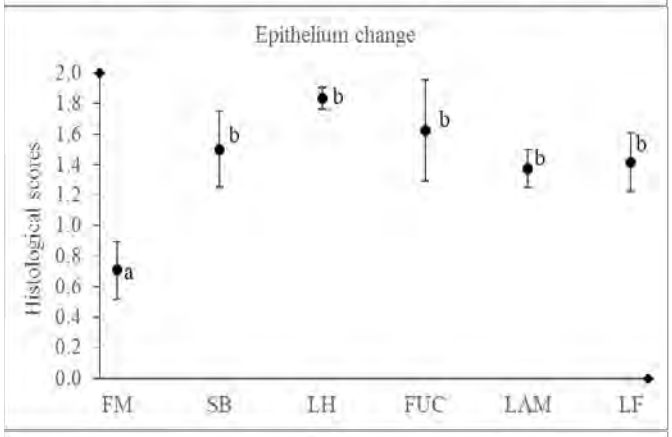


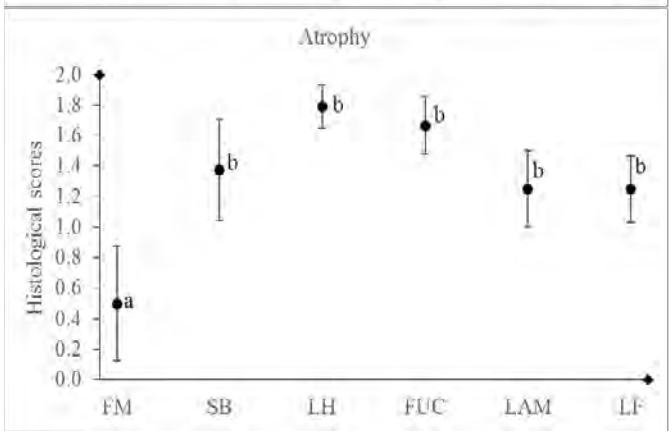
Diets	FM	SBM	LH	FUC[2]	LAM	LF[1]	SEM[3]	P value
Performances								
Initial weight (g/fish)	261	257	261	261	265	251	2.65	0.09
Final weight (g/fish)	333	315	308	324	319	298	7.47	0.12
Feed intake (g DM/ kg initial weight)	245	250	269	298	278	286	17.03	0.37
FCR[4]	0.94	1.12	1.41	1.18	1.29	1.41	0.09	0.04[4]
SGR %	0.98	0.81	0.66	0.86	0.74	0.68	0.08	0.16

[1]Values are least square mean
[2]N=2; One tank excluded
[3]Pooled standard error of mean
[4]Only the difference between FM and LH is significant

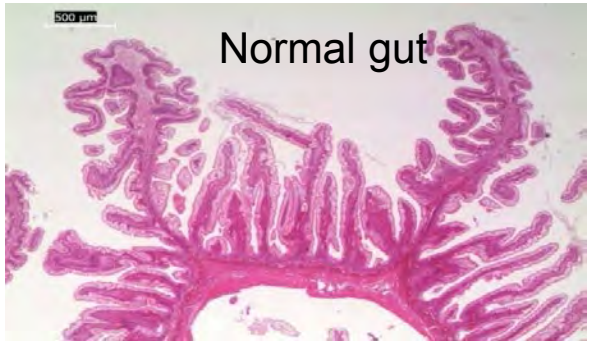
Histology







- (1) Accumulation of leucocytes in the lamina propria.
- (2) Changes in the epithelium – reduced supranuclear vacuolization, reduced cellular height and increased cytoplasmic basophiles.
- (3) Atrophy – reduced height of the intestinal folds.



0 = normal; 0.5 = slight change; 1 = moderate change; 1.5 = distinct change and 2 = severe changes in the DI-sections.

Conclusions:

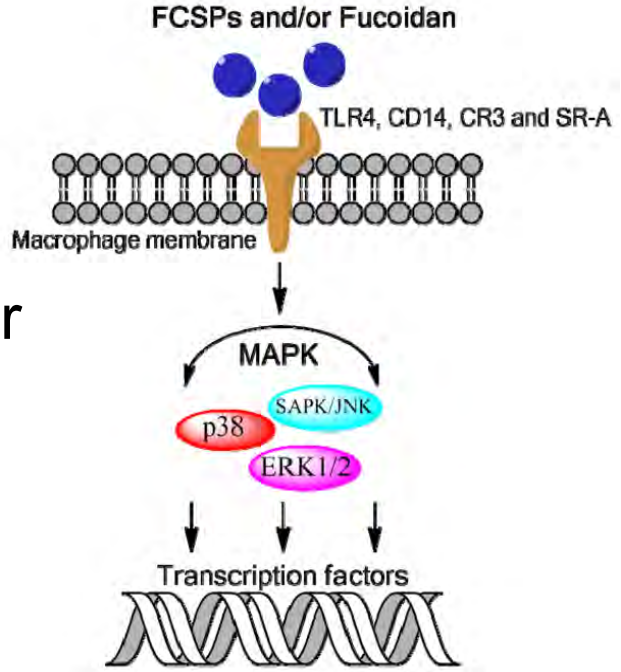
Succesful extraction of laminarin & fucoidan

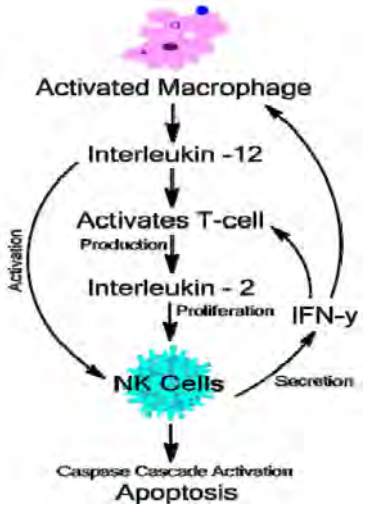
No difference in growth

No positive effect on SBM enteritis

Further work

- Optimization on extraction/isolation.
- Isolate bioactive components from other macroalgae species.
- Work on isolation of other components.
- Effect of up-stream & down-stream processes.
- Chemical analyses & molecular characterizations.
- Mode of action of the bioactive molecules – gene expression, gut microbiota
- *In vitro* assays for bioactivity
- *In vivo* feeding experiments





FOODS OF NORWAY

More information can be found at our web site:
www.foodsofnorway.net

Senter for forskningsdrevet innovasjon
Norges forskningsråd

Key researchers in this experiment:
MSc thesis - Rouzbeh Keihani
Extraction/experimental design: Dr. Liv T. Mydland & Prof. Margareth Øverland
Diets & in vivo experiment: Dr. Felipe Reveco; Dr. Jon Øvrum Hansen
Polysaccharide analyses: PhD-student Sandeep Sharma
Gut health analyses: Prof. Charles Press
Thanks also to Jon Funderud (Seaweed Energy Solutions) for providing large amounts of kelp (Stortare; *L. hyperborea*)