Feed development from novel marine resources

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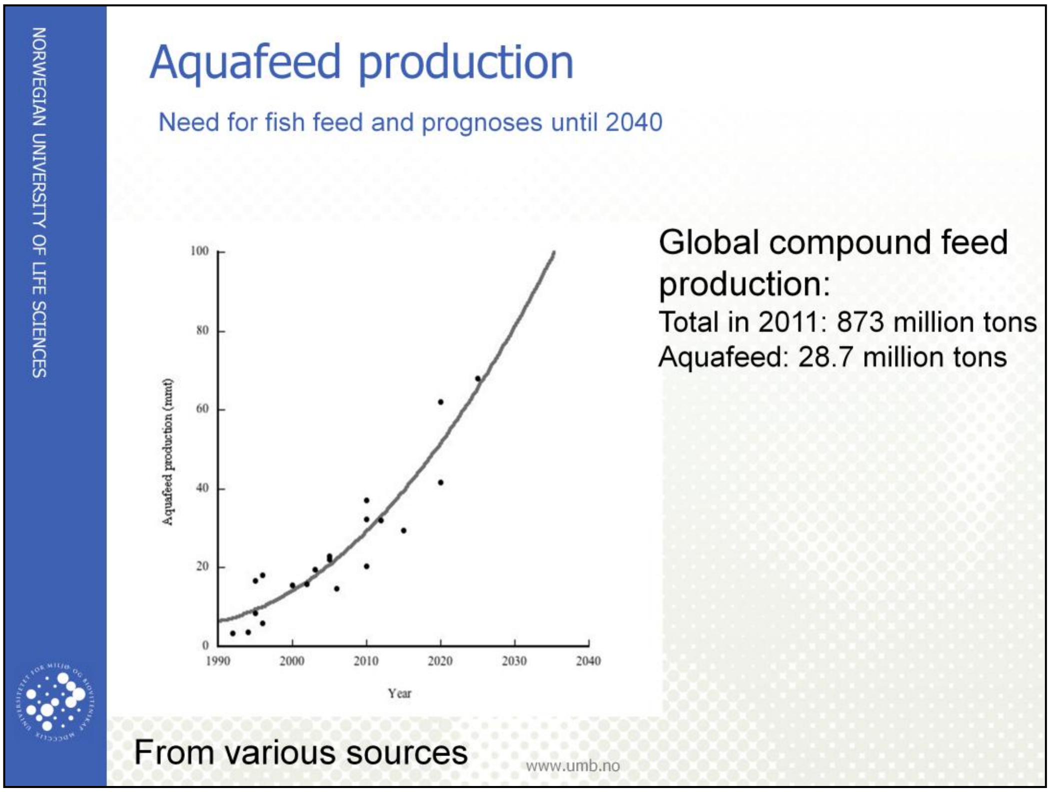


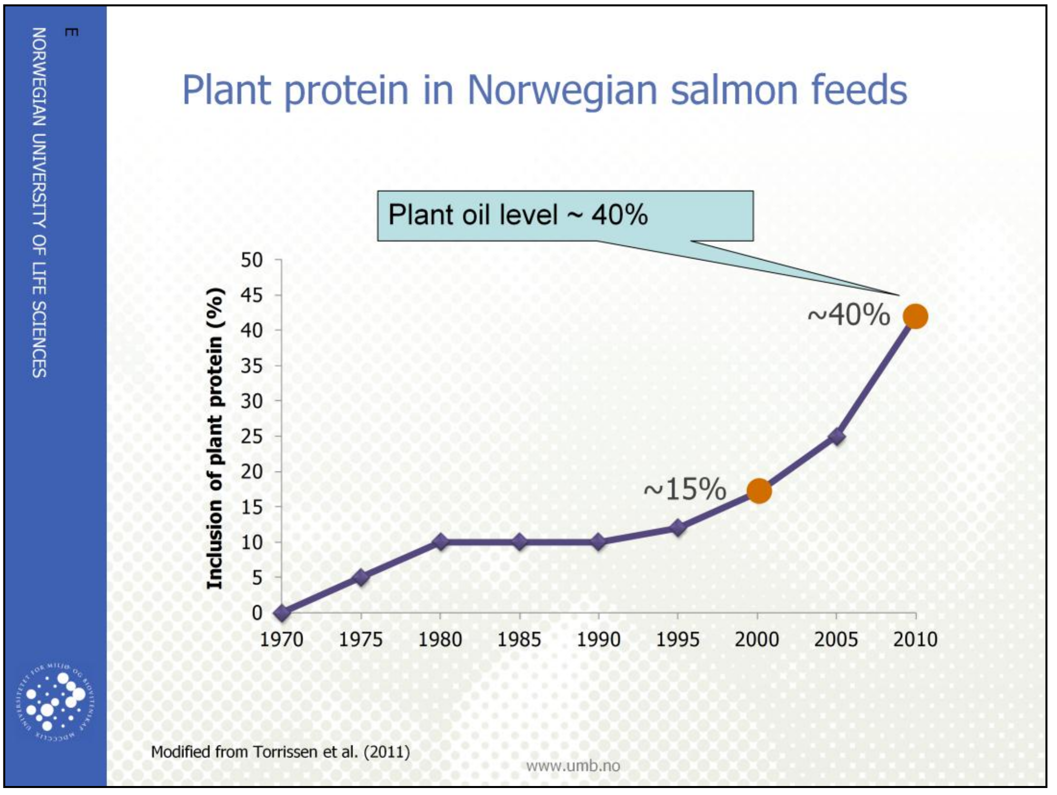
The rapid growth in the aquaculture industry puts large demands on the wild fish stocks we harvest for FM and FO production.

Because these ingredients are limited alternatives are needed.

To day plant ingredients are the most important alternatives.

Currently we are already producing 29 mmt.

By 2030 the need for aquafeed is 80 Mil tonn.

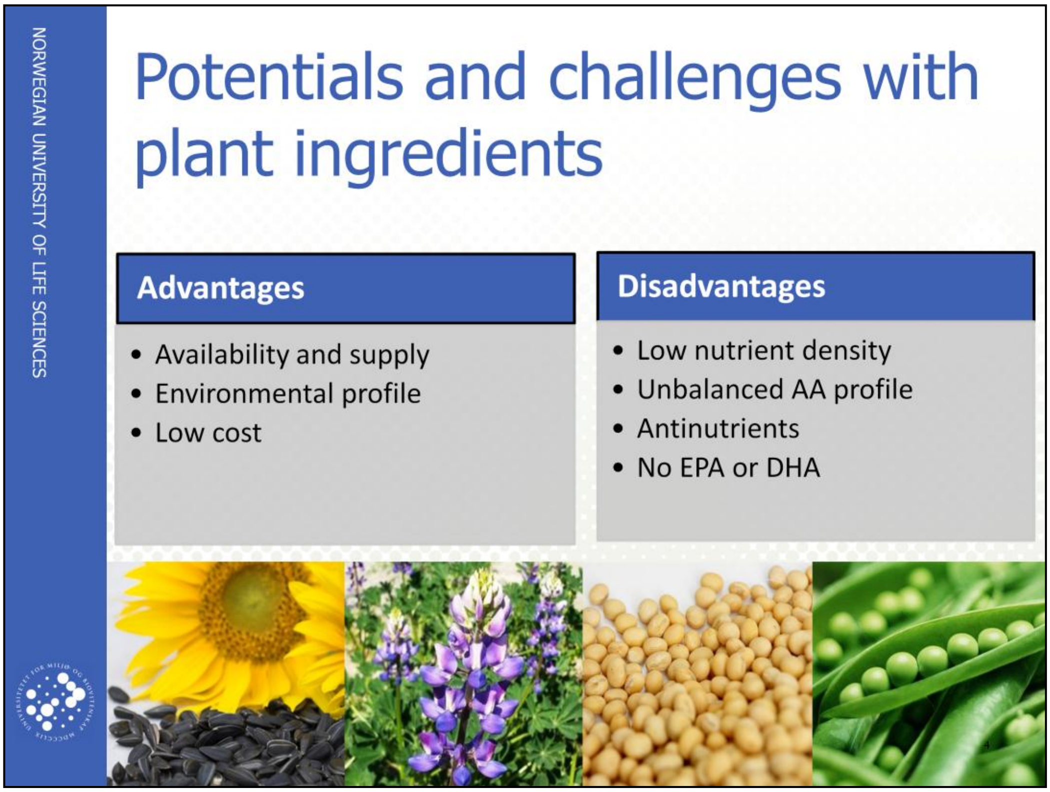


This graph show the inclusion of plant proteins in salmon diets

10 years ago, the level was about 15 %, while in 2010 it is higher than 40 %.

The corresponding inclusion of plant oil is around 40%

The inclusion of plants ingredients continues to increase.

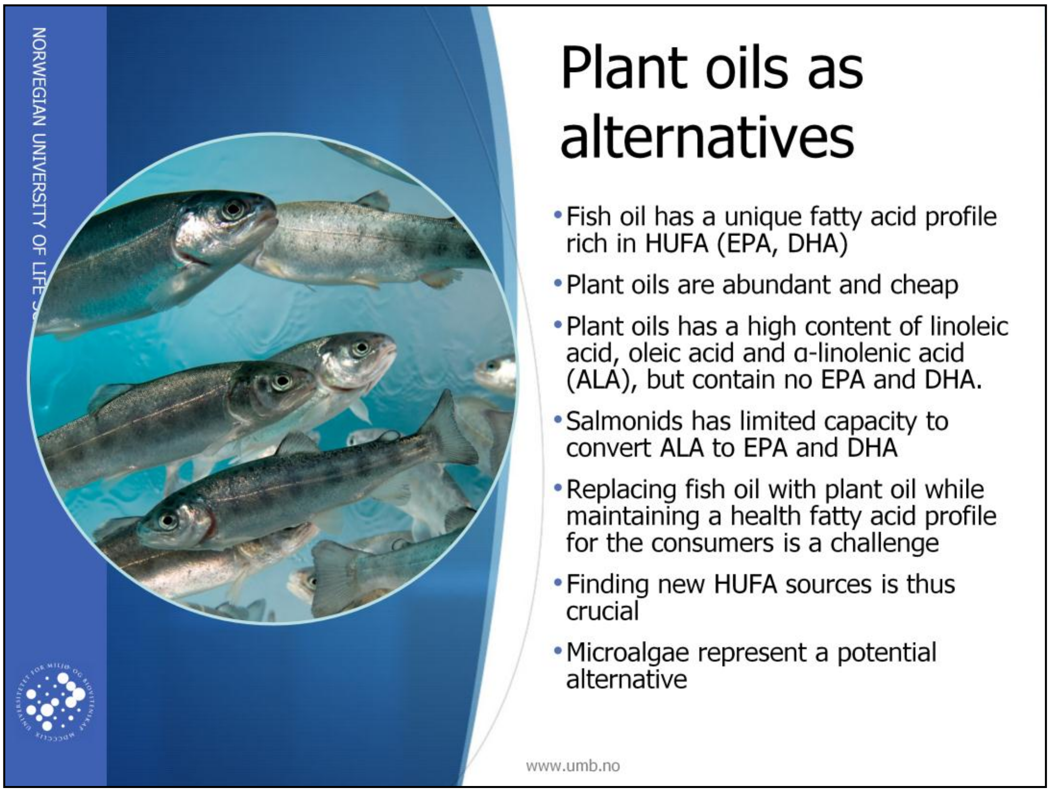


Use of such plant ingredients represent several advantages and disadvantages: They are abundant and cheap, and represent a positive environmental profile.

But plant ingredients for Atlantic salmon do present several challenges such as low nutrient content, low protein quality, bitter taste, and a their content of a wide range of undesirable components.

Some of these challenges can be solved by using optimal combinations of feed ingredients, supplemental amino acids, and by targeted feed processing technology.

Another challenge is than plant oil do not contain HUFA, EPA, DHA



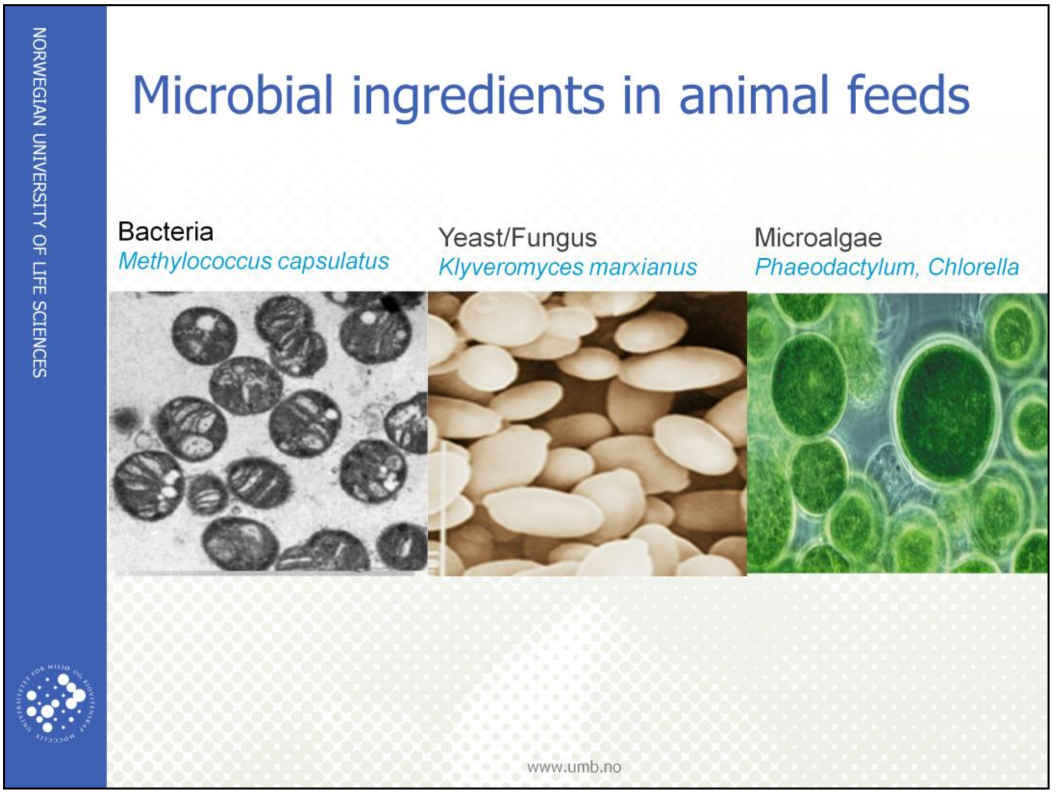
The biggest short tem problem we are phased with currently is to find FO alternatives

While many alternative protein ingredients exits there ar fewer n-3 sources. Ex are krill, micro algae and then you have the GMO story but the market is not yet ready to take this step.

Plant oils have a relatively high levels of linoleic acid, oleic acid and alpha linolenic acid, but they contain no EPA and DHA.

Salmonids are able to convert ALA to EPA, through a series of desaturation and elongation reactions but their ability is limited

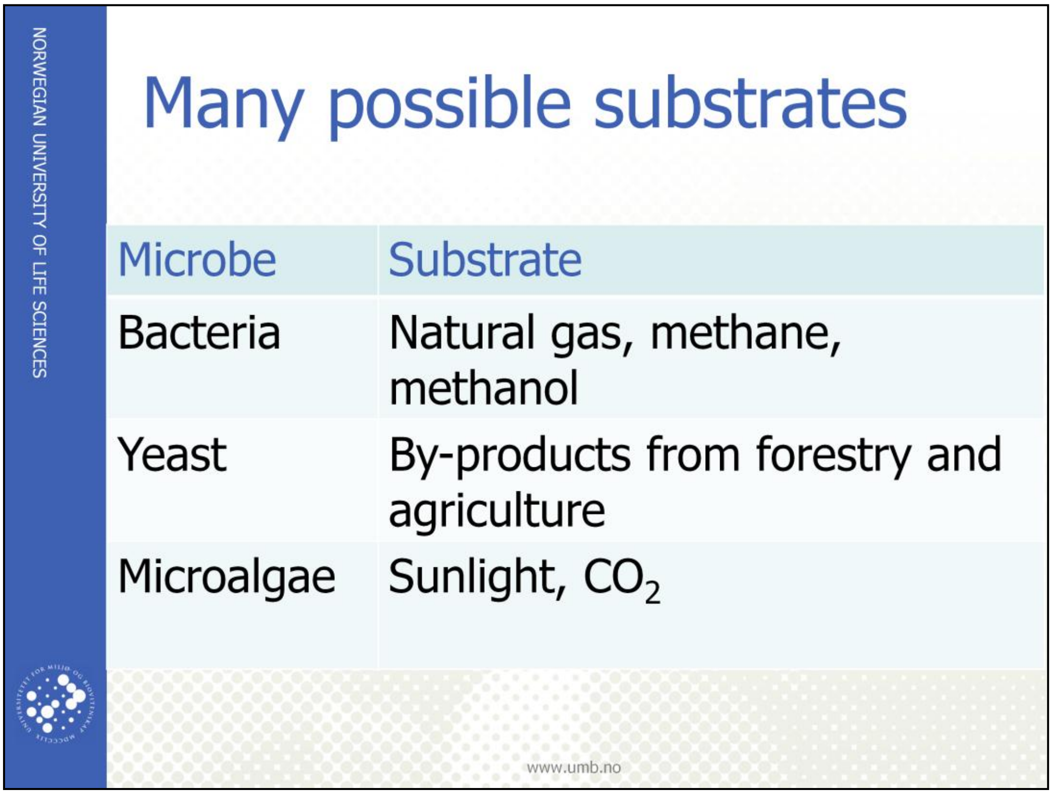
To maintain high levels of EPA and DHA in fish products to ensure a good health profile to meet the demands in the market, replacing fish oil with plant oil is a significant challenge.



Some very promising ingredients I study are microbial products like yeast and bacteria

Because we have more information on yeast and bacteria in aquafeeds, so I will talk about that first because this have given us important experience when microalgae in aquafeeds.

To be successful we must microalgae must have a high nutritional value not only with respect to the lipid content, but also the protein content and content of micronutrient as well as bioaktive components. In addition they must be pallatable for the animlas/fish all this need to be documented when evaluating microalgae.



Microbial ingredients are considered sustainable, they don’t require any agricultural land, they use little fresh water and they can be produced from no-food biomass.

MI can be produced from a wide range of substrates – such as,

Natural gas or methane in the case of metanotroph bacterial

By-products from agriculture and forestry in the case of yeast and fungi

Sunlight and CO2 an the case of micro algae

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Microbial ingredients differ in chemical composition

A relationship exist between nutrient content and growth rate of the microbe:

- Growth rate is reflected in the content of crude protein (DNA/RNA)
- Growth rate: Bacteria > Fungi/Yeast > Fungi (Filamentous) > Alger

Chemical content*	Bacteria	Fungi/Yeast	Fungi Filamentous	Algae
Crude protein,%	60-85	50-70	30-50	45-60
Lipids, %	2-10	2-10	10-25	5-45
Nucleic acids, %	8-20	5-15	5-8	4-6

Chemical content does vary a lot among species within a microbial group and is related to differences in growth conditions.

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There are large variations in chemical composition among the various microbial ingredient sources.

As a rule, chemical composition is dependent on the growth rate of these organisms.

Those with the highest growth rates have a higher content of protein and nucleic acids, while those with slower growth rates have a higher content of lipids and membrane structures, while the content of NA and protein are lower.

Generally, bacteria will grow more rapidly than yeast. Filementarous yeast will grown somewhat slower, while micro algae have the slowest growth rates and thereby the lowest content of CP and NA, while the lipid content in general is higher.

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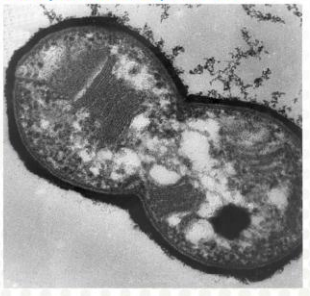
Bacterial meal

Value chain from natural gas to high-value feed resources for the production of human food

Methylococcus capsulatus



Crude protein (including 10% nucleic acids)	70%
Crude lipids (phospholipids)	10%
Carbohydrates	12%
Ash	7%

(Source: Øverland et al., 2011)

Bacterial meal (BM) is produced by aerobe fermentation:

- Methanothroph bacteria and helper bacteria
- Methanol or Methane from natural gas
- Oxygen, ammonia, minerals

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BM is produced from fermentation of natural gas by the methanotroph bacteria Met cap.

In addition, ammonia is added to the process as the N source, as well as minerals salts.

The biomass is harvested and dried.

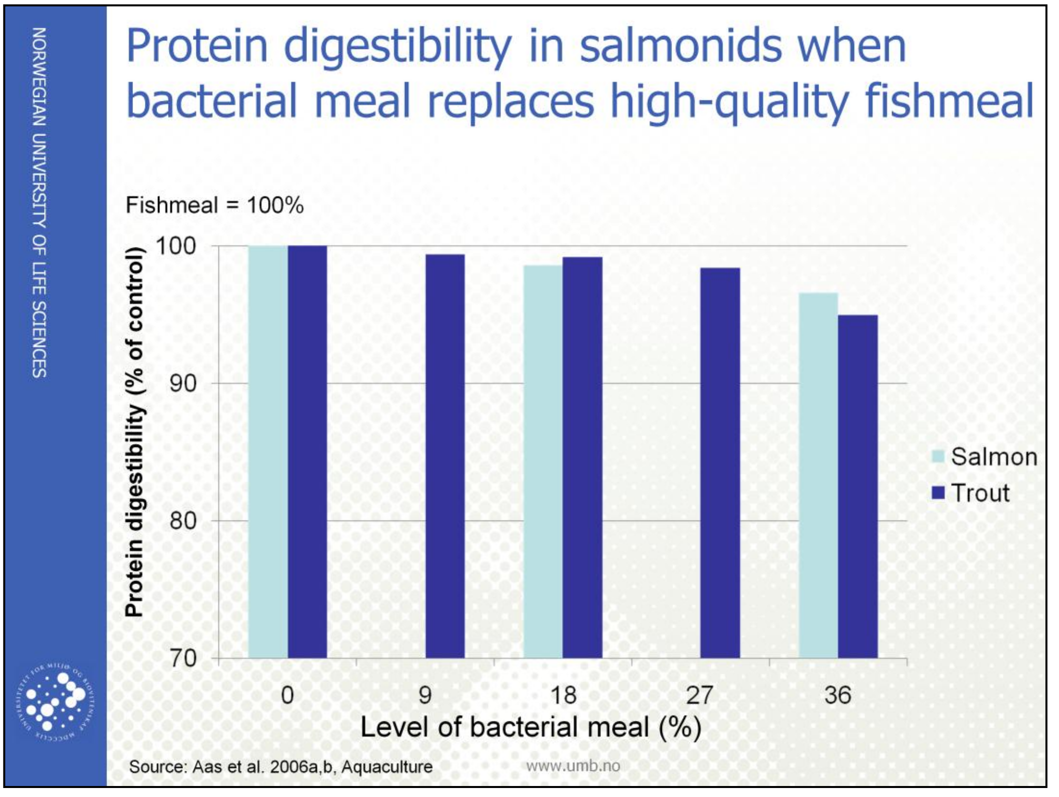
The final biomass is a red-brownish powder

The chemical composition of BM is similar to FM

It contains 70% CP – 10 NA important source of nucleic acids (semi essential)

Crude lipids – Phospho lipids

BM also contain 7% ash - a relative high content of P comparable to fish meal & the P is mostly bound in phospholipid fraction.

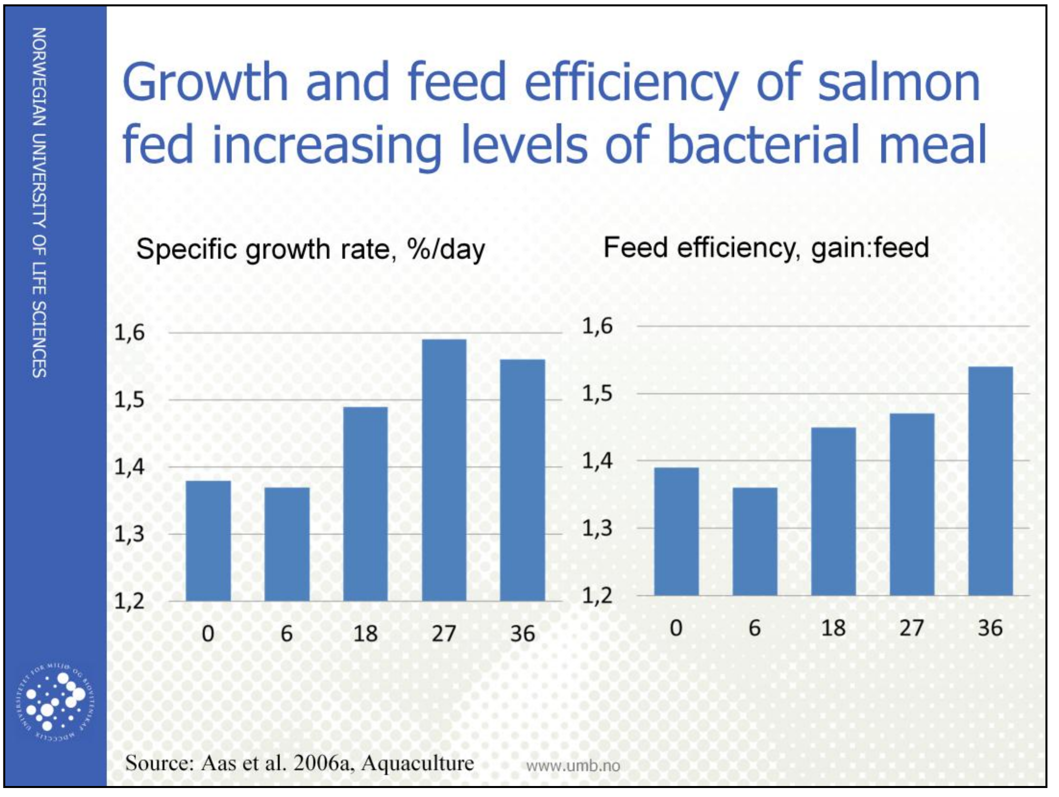


This shows the protein digestibility of diets with increasing levels of CP 0-36% is diets for salmon and Rbt

Values are expressed as a % of the FM control

As you can see, increasing levels of BM in the diet resulted in a slight reduction in the dig of CP. At higher inclusion levels the effect of CP dig was more pronounced.

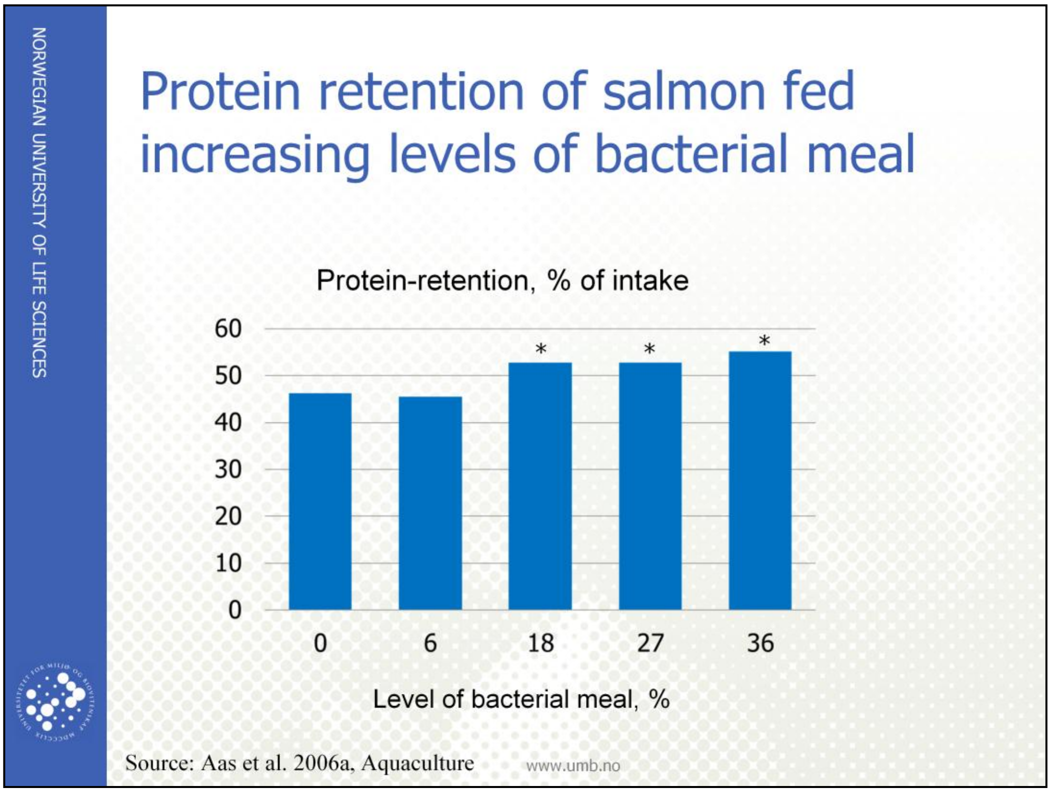
The reduction in digestibility of protein is due to a negative effect of microbial membranes and cell wall components.



This shows results from a trial done with Atlantic salmon where increasing levels of up to 36% BM at the expense of HQ-fish meal.

The results show that increasing levels of BM of up to 36% improved growth rate and feed efficiency of the diets.

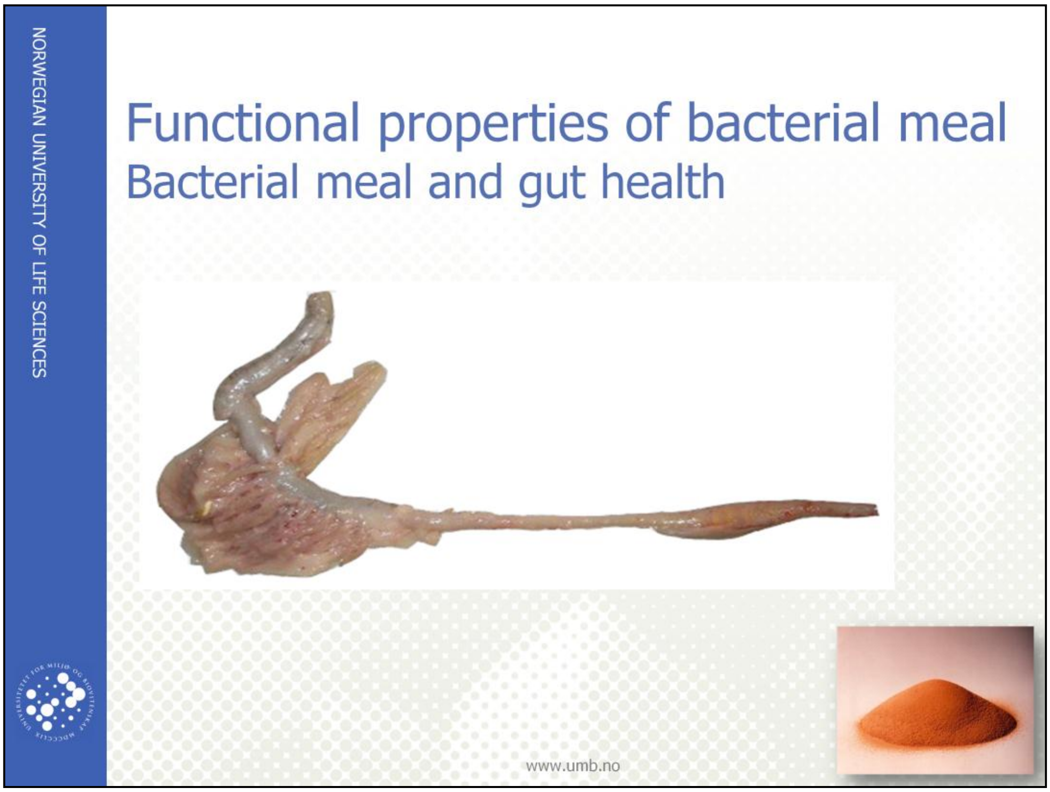
Show that this is a good replacement of FM in salmon diets



This shows the effect of increasign levels of BM on protein retention in the fish

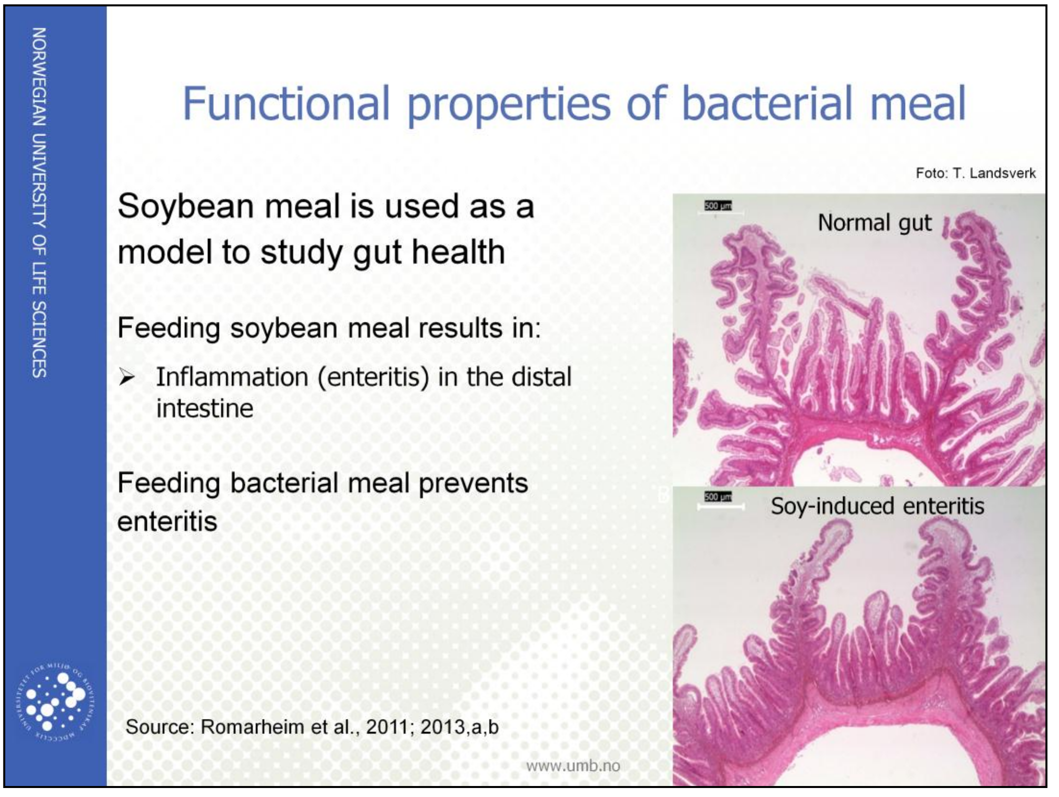
See that proteinretention increases, this occured despite a lower digestibility

Suggest that BM is well utlized could be due to a favorable AA composition and nucleic acids are well utilized.



SC contain a wide range of bioactive components that may have health beneficial effect in the fish.

This include NA - important for regeneration of epitheliacells, components with immunomodulating effects, and antioxidants.



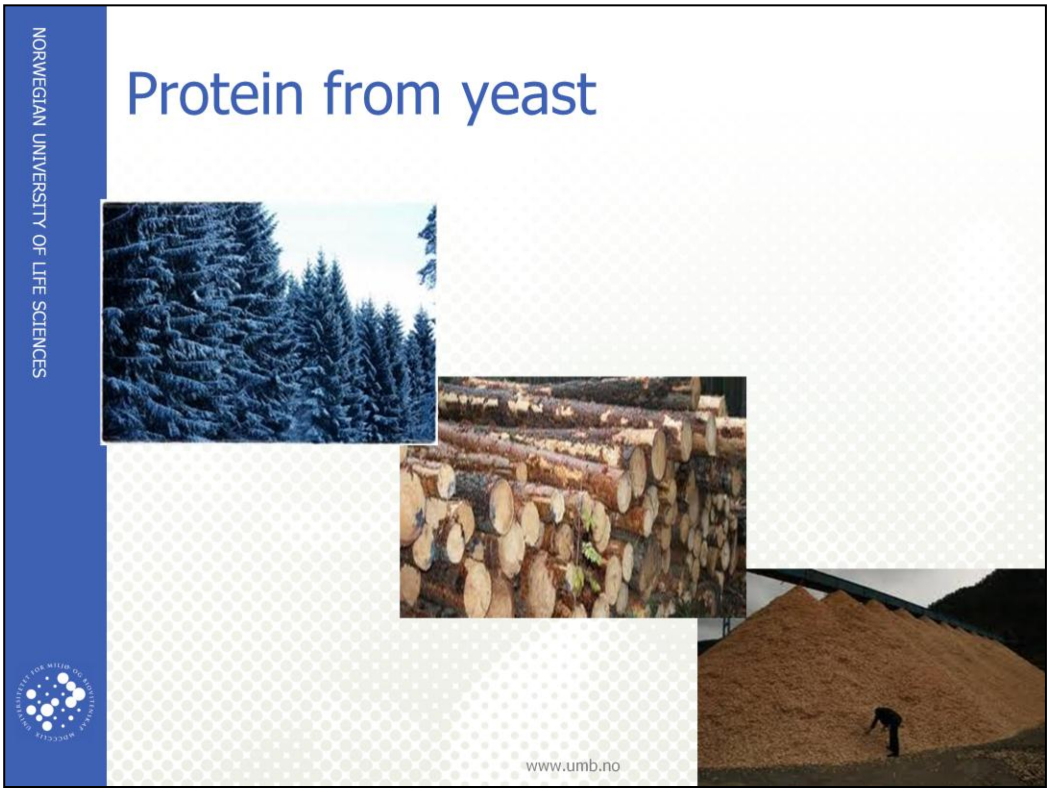
As mention earlier, incrased use of plant ingredients in fish feed may lead to nutritional related disorders like SE and infectious diseases.

Use soy as a model for SE – we know that siy contain a wide range of ANFs that are present in plant ingredients used for fish feed.

Feeding 20% soy gives enteritis in all fish, thus a good model.

BM – prevents this

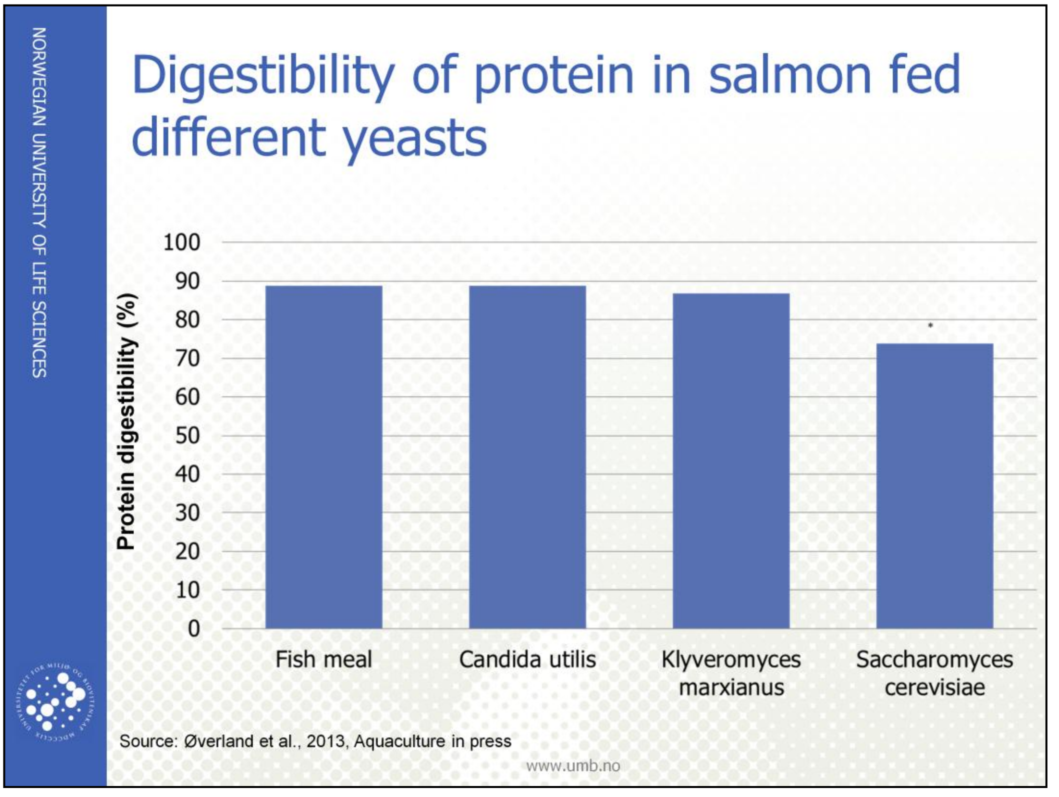
Effect is dose-dependent



We have also evaluated the use of yeast as a source of protein for fish

Yeast have been used in aquafeeds as an aditive with health beneficial effect

But due to advancement in technology, lit is expected that arger quantities will be available, thus we evaluated yeast as a potential protein source



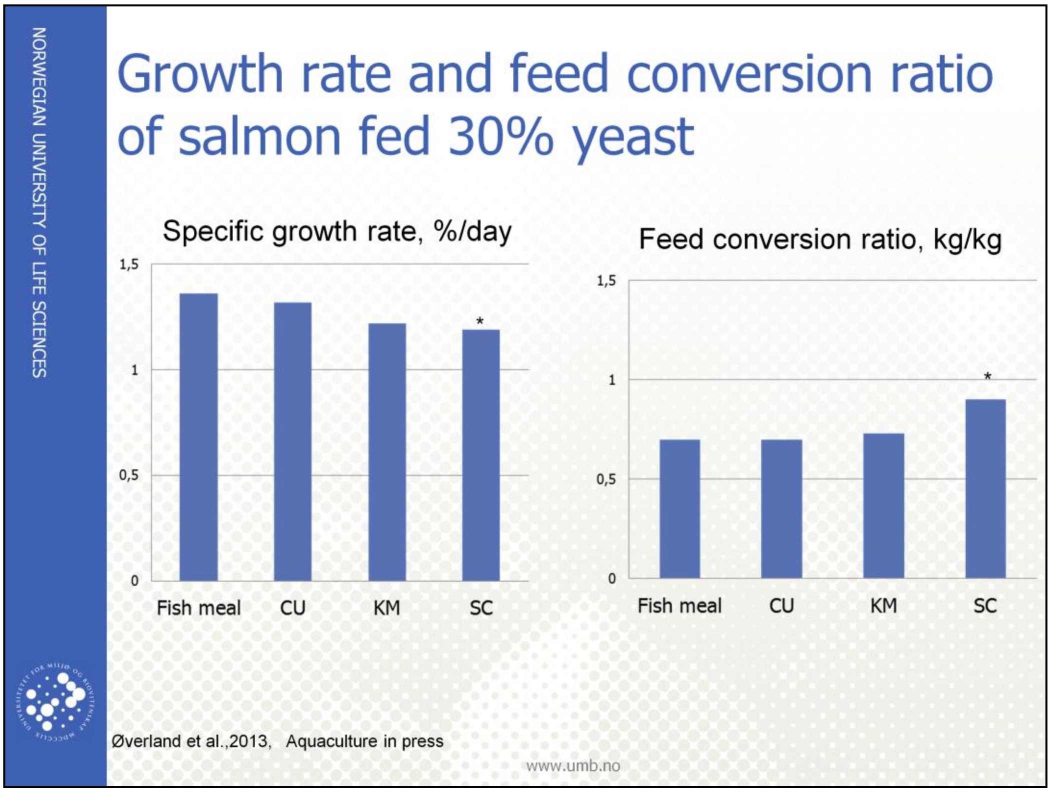
This shows result froman exp with different yeast in diets for atlantic salmon UMB lab

In the exp 30% of the FM was repalced with yeast either from klyveromyces, toula, or sacharomyces

See that the CU gave similaer or better performance while SC gave an inferior results compared to the HQ FM.

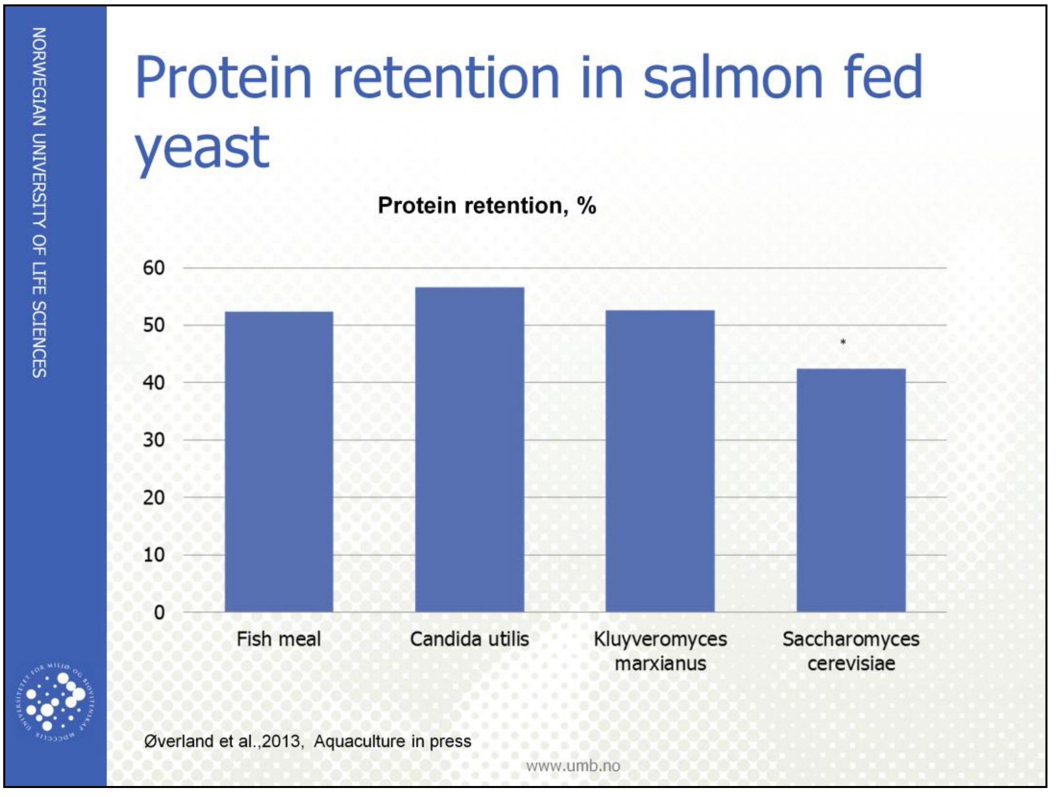
CU, KM use 5 6 6 sugsrs and can thus be produced from lignicellulytic biomass and ag. Waste.

SC uses only hexoses and are thus limited.

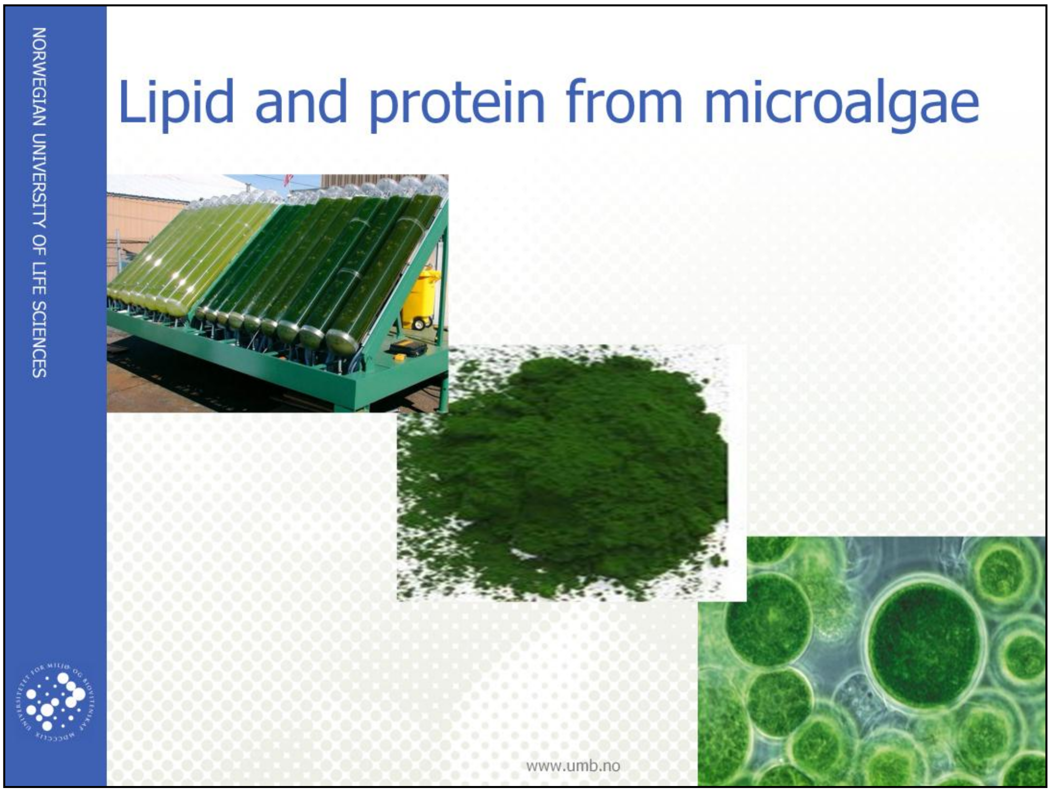


This shows the SGR and F/G of the fish fed HQ-FM & yeast

See that the firsr candiate gave similaer or better performance while the last candiate gave an inferior results compared to the HQ FM.

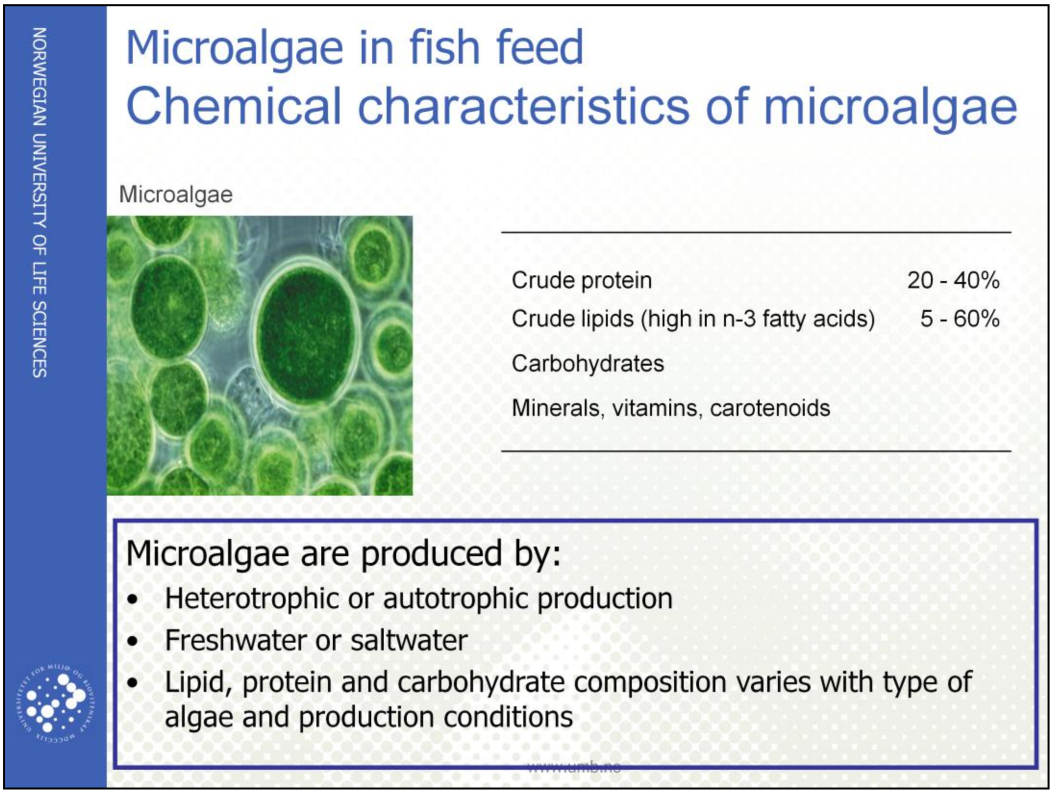


Also see that CP retention increased with CU, similar with KM, reduced with SC



Microalga represents a potential source of both protein and lipids for aqua feeds, but most importantly they serve as a source of the essential omega-3 EPA and DHA for aquafeeds.

To be successful, Microalgae must have a high nutritional value, not only with respect to the lipid content, but also the protein content. In addition the content of micronutrients and bioactive components need to be documented.



Microalga commonly contain 20-40% protein and 5 to 60% lipid but this depend on the species and the growth condition.

In addition they contain a wide range of micronutrients such as minerals, vitamins, chlorophyll and carotenoids that are also important in the search for alternative feed ingredient for farmed fish

Microalgae have been mainly uses as live feed for larvae for many cultured fish species and crustaceans species (directly or for enrichment of rotafers).

To day they are mostly used as additives in animal feed. Their use in aquafeeds is limited mainly due to the challenge to produce these algae in large quantities and for a competitive price.

Microalgae can be cultured photoautotrophically or heterotrophically. With the, photoautotrophic production being the most common method. These are produced from CO_2, inorganic nutrients and light energy, and that they don't require any organic carbon/energy sources which are costly.

Heterotrophic microalgae – (Thraustochytrids) are also interesting as they can contain up to 60% lipids with a DHA content of up to 60%. Ex DHA-gold

Reseach on Microalgae

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1. *Evaluation of nutritional value of :*
   Nannochloropsis oceania, produced at UMB
   Isochrysis galbana, Reed Mariculture, USA
   Phaeodactylum tricornutum, Fitoplankton Marino, Spain
   Collaboration among SINTEF, UMB, and Nofima
2. *Evaluation of functional properties of microalgae, on going*
3. *Chemical profiling of microalga from heterotrophic production, on going*

Production of *Nannochloropsis oceania* at UMB

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We have gain experience with microalgae as a fish feed ingredient from several projects

In the first project we evaluated MA as a source of protein in auqafeeds. This was a part of a lager project in collaboration with x, y, z

Here we tested three marien alga namely …

In the second project which is on going, we are evaluating functional properties of MA in aquafeeds

In the third project we are developing protocols for chemical profiling of MA based on heterotrophic pordiuction for aquafeeds. Lipid stability s a focus.

I will show some results from these projects

NB!Problem with algae is that often small quantities are available for testing thus limted information exist on their nutritional value– especially on marine algae. Need 10 kg! Important criteria are: Nutrrient content, Digestibility, Pallatraility, performance and health.

Chemical composition of microalgae, %				
	Nannochloropsis Oceania	*Isochrysis galbana*	*Phaeodactylum tricornutum*	High-quality fishmeal
Crude protein, %	47.7	20.1	49.0	74.7
Crude fat, %	8.4	16.2	7.4	9.7
EPA, C20:5	2.3	0.08	2.8	1.5-2.0
DHA, C22:6	-	1.6	0.02	0.7-1.3
Amino acids, g/16 g N				
Lysine	4.8	3.1	4.2	6.8
Methionine	1.8	2.5	2.0	2.5
Tryptophan	1.7	2.5	1.3	0.7
Threonine	3.6	4.6	3.7	3.5
Valine	4.6	6.1	4.6	4.0
Isoleucine	3.5	5.1	3.8	3.7
Leucine	6.7	9.2	6.2	6.2
Phenylalanine	3.9	5.7	4.2	3.3
Arginine	4.9	4.1	4.4	5.4

Here you see the chemical composition of the alga tested

CP varied from 20-50%

C Fat varied from 7.4-16.2%,

while the content of EPA was low in Isochrysis, it was 2.8 and 2.3% in Nannocloropsis and Isocrysis, respectively.

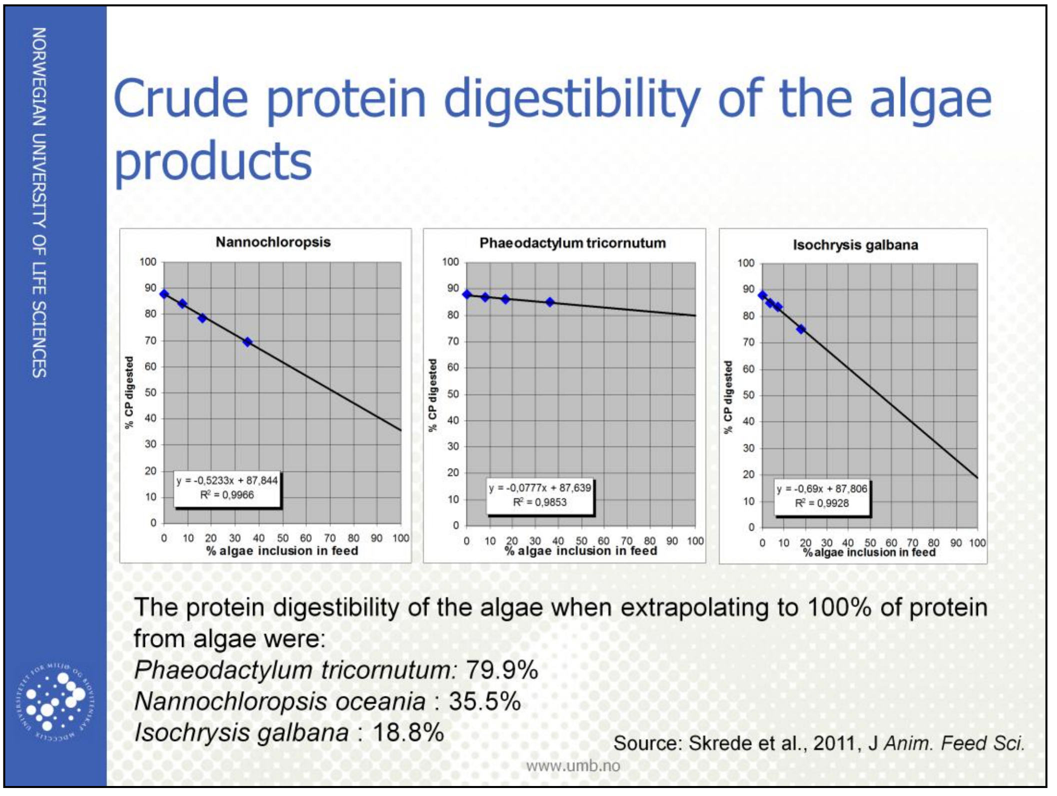
On the other hand, the content of DHA was highest in 1.6% in Isocrysis, but low or non detectable in the other two algae tested

Also the microalgae has a favorable amino acids profile

Here you see AA expressed pr 100 g CP:

The content of lysine is much lower in microalgae vs FM, while the level of trp is relatively high vs FM, while Met is similar

When experess as AA per kg of product, these AA are much lower than FM.



Here you see the digestibility of CP in the diets with increasing levels of algae. The algae was included at 6, 12, 24%, replacing FM in the diet.

See that with increasing levels of algae products the CP digestibiltiy decreases

When extrapolating to 100%, we can calculate the CP dig of the algae product per see, is…

Large variation in CP availability among species

The digestibility of Triconortum is lower compared wth HQ-FM but ut is similar to commonly used plant protein sources such as SBM and rape seed.

Many microalgae species are known to contain carbohydrates, such as chitin and non-starch polysaccharides (NSPs), which are indigestible in salmonids.

Processing methods, such as grinding or enzyme hydrolysis may be required to improve nutrient digestibility,

Usin antioxidat to protect oxidation of fatty acid in microalgae containing high levels of omega-3 fatty acids. Some algae species have also been shown to contain terpene caulerpenyene, a lipid synthesis inhibitor.

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Amino acid digestibility of the algae products

	N. Oceania	*P. Tricornutum*	*I. galbana*	LT fishmeal
Arg	41.2	87.4	56.8	93.6
His	17.2	76.6	37.1	88.9
Ile	30.3	75.9	63.5	92.4
Leu	30.9	81.6	68.6	93.0
Lys	38.1	84.5	12.6	86.8
Met	35.6	83.4	64.8	93.5
Trp	38.3	81.7	69.0	85.6
Phe	31.9	83.2	69.2	90.3
Thr	50.1	83.0	55.0	85.0
Val	31.6	82.2	62.5	91.4

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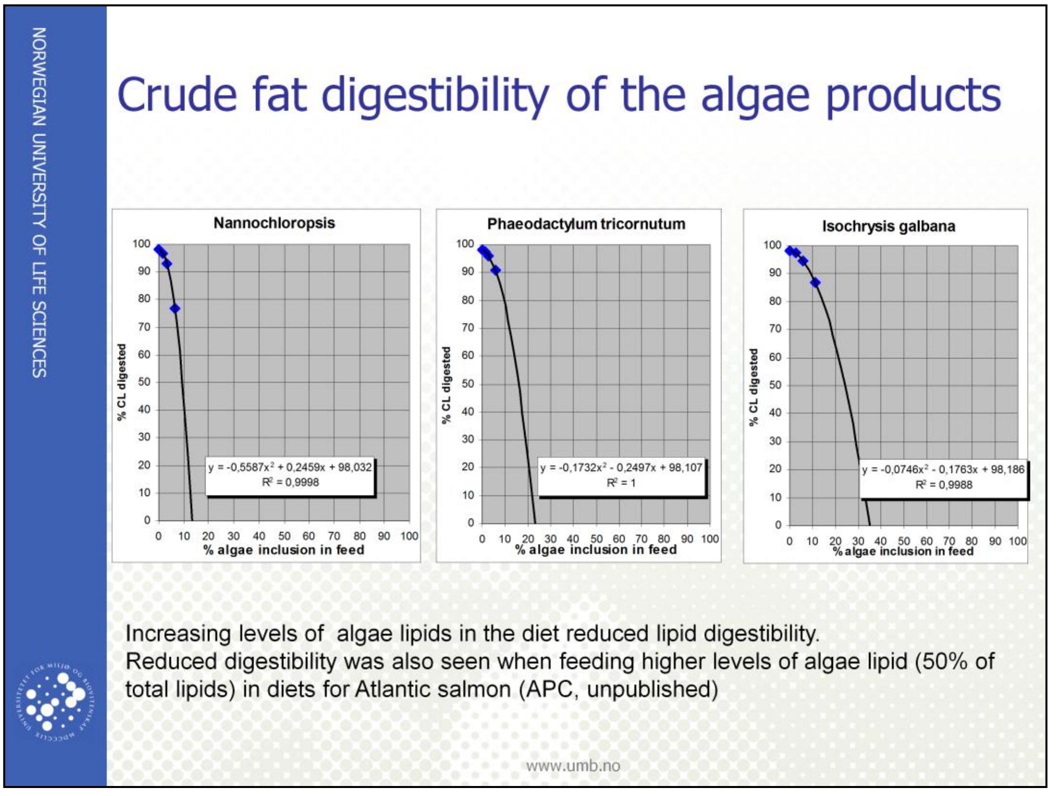
This shows the digestibiltiy of individual amino acids

See that AA dig of N oceania and Isocrysis diet are generally low compared with FM,

but it is interesting to note that the digestibility of the amino acids lys, met, trp in Tricornutum is similar to HQ FM!



Micro algae is an important source of the essential n-3 FAS, EPA and DHA. It is thus it is important to evaluate if these are available for digestion and absorption.

This shows the digestibility of the lipids in the diets see that increasing levels of algae gave a reduction in the lipid digestibility

When extrapolated to 100% inclusion you get an estimate of the digestiblity of the lipid in the algae – see that this is negative

We have recently done a digestibilty trial with higher inclusion levels of algae lipids produced by heterotroph production for both salmon and rainbow trout, where we see a similar patteren in lipid digestiblity 30%

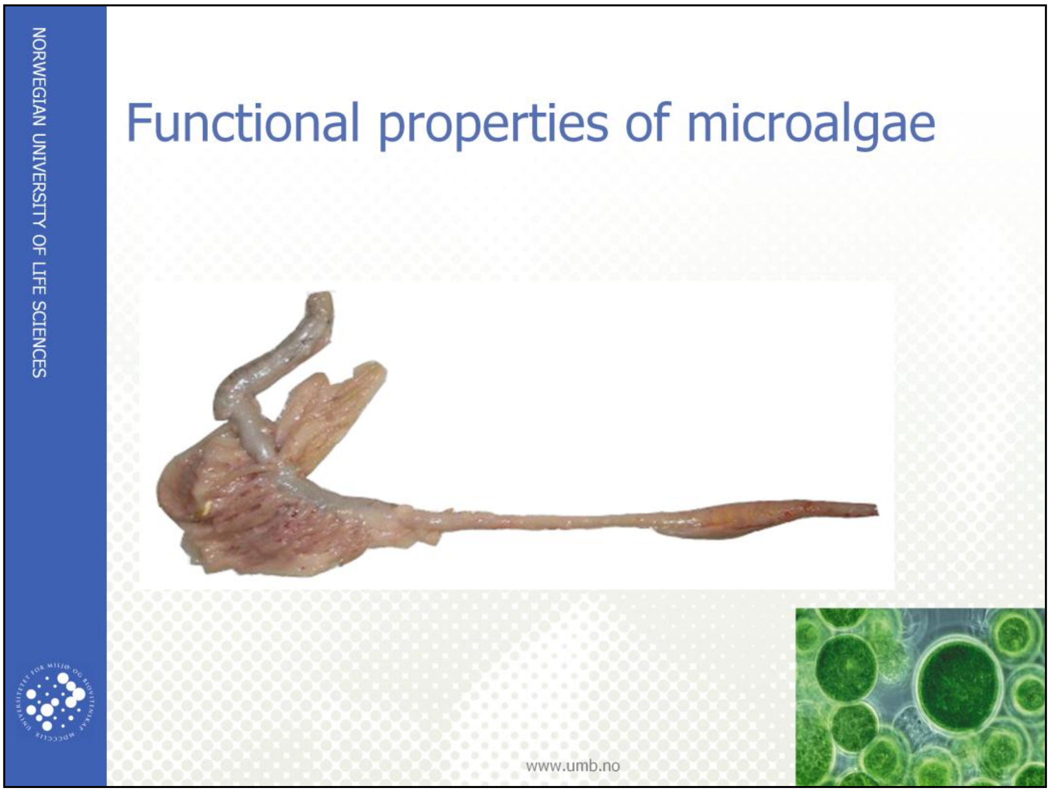
This could be due to the encapsulation of the lipids by the thick cellmembrane or due to a posible content of a lipid inhibitor in the algae.

Especially Isochrysis galbana has a thick cellmembrane and this had the lowest dig of both lipid and CP.

Thus to use these in aquafeeds proper processing to increase their nutritional value is important (there were freeze dried)

Furthermore, these lipids are highly reactive and thus prone to oxidation, thus during downstream processing of the algae biomass it is also important to protect these n-3 fatty acids from oxidation, to aviod rancisity.

Rancid lipids have poor taste and are harmful to the fish. Prevented by the additon os antioxidants.



SC contain a wide range of bioactive components that may have health beneficial effect in the fish.

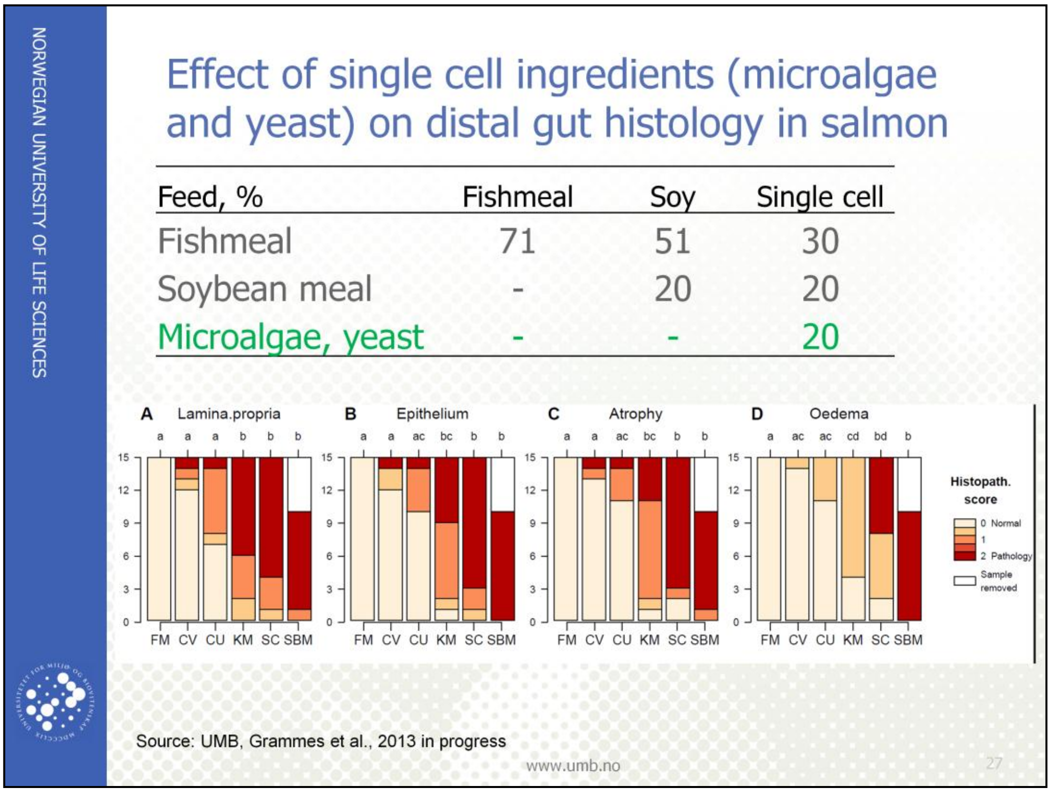
Microalgae also contain a wide range of bioactive components that may have health beneficial effect in the fish.

This include high level of nucleic acids

chlorophyl which has an antinflammatory effect,

beta-carotene which has antioxidants properties.

Chlorella – Fe, B12 content is very high



Evaluated health beneficial effects of MA and yeast using soy-model.

Shows the histology of the DI of the fish fed the different diets

Either a negative FM control or a positive SBM conteol or test diet containing an algae and different types of yeast.

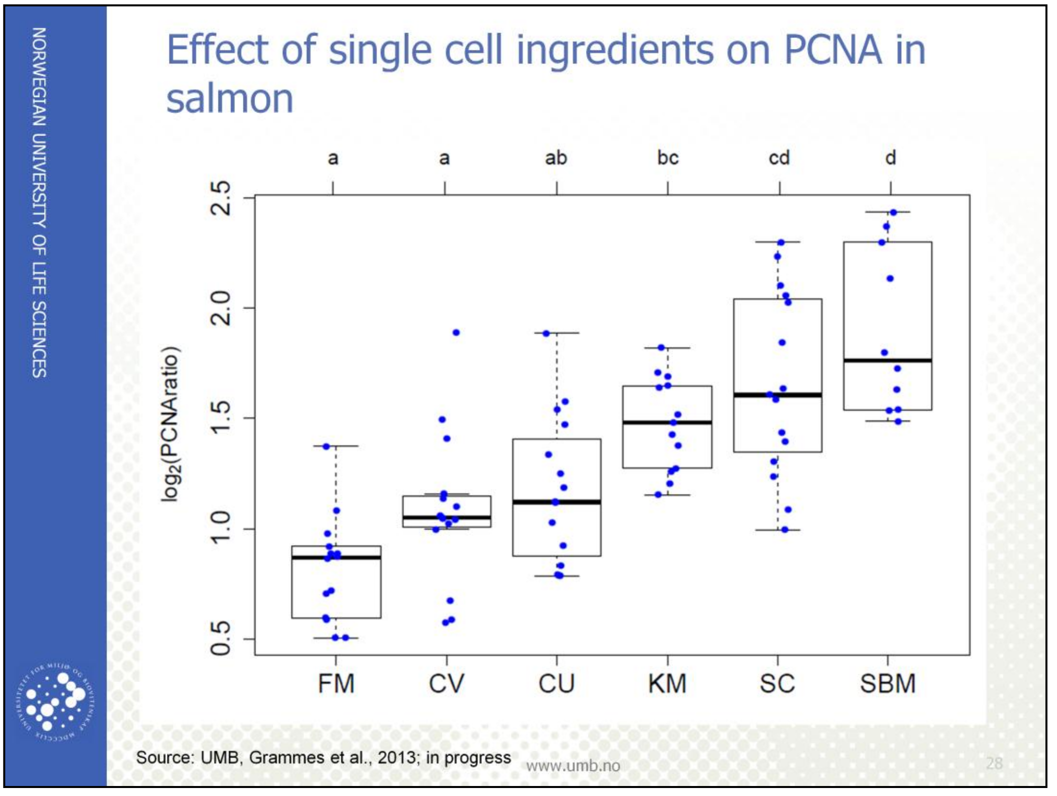
The histology cores are based on a scale from 0 to 2,

with 2 being full enteritis shown in red, while 0 is normal shown in beage.

A score of 1 would indicate slight to moderate enteris

See that FM have a normal gut witout any enteritis, while those fed SBM had full enteritis.

Also see that CV, and the CU yeast gave low histology scores, indicating that these prevented enterritis.

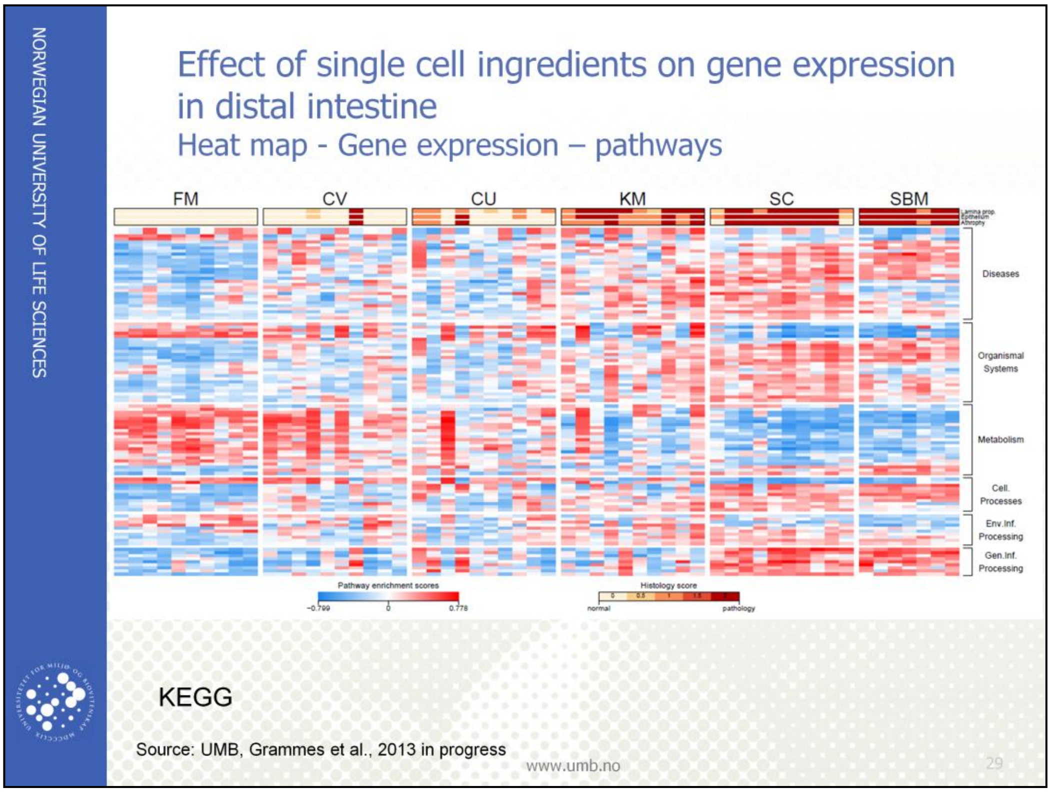


This shown the PCNA staining of the DI,

PCNA is an indication of rapid cell devision, and this suggest that fish fed SBM had increased cell proliferation to compenstae for the loss of epithelial cells due to the enteritis.

Also see that the PCNA scores was low in the groups fed CV & CU – results coinside with the histology

Are length of the PCNA positive region in the crypts of the DI, values are log2 transformed.



We looked at gene expression in the DI of the fish fed the different diets by using microarray.

Shows a heat map of different gene sets, representing different pathways

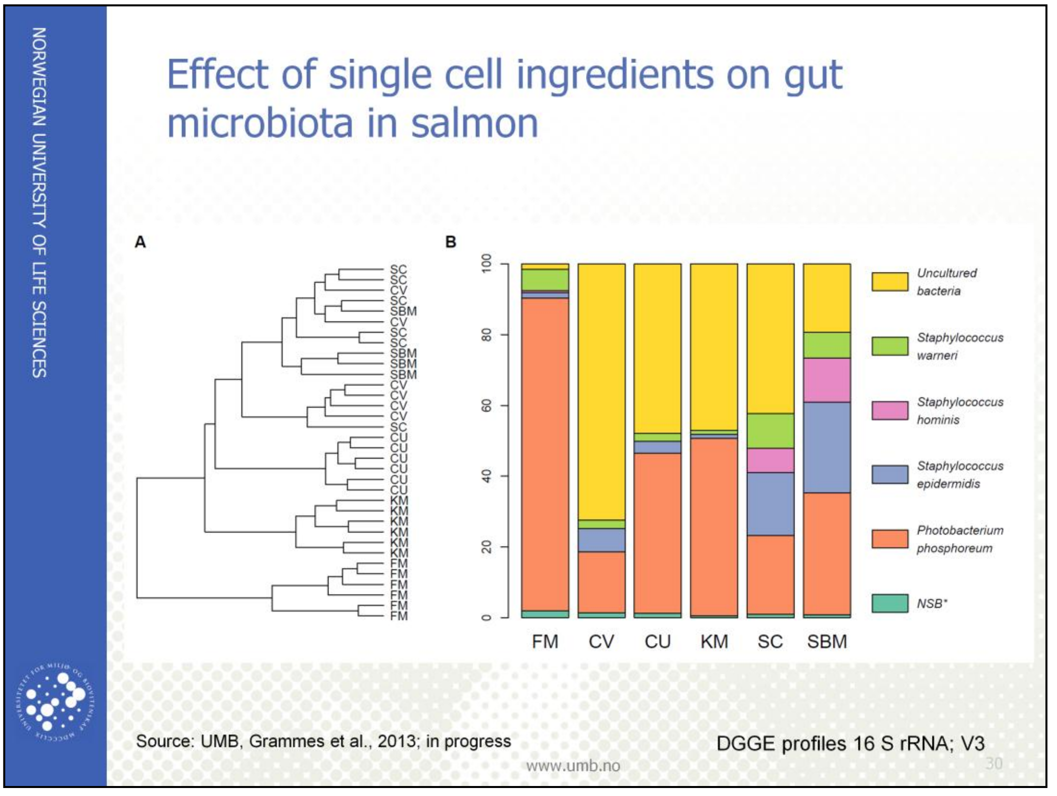
Blue color indicate down regulation, while red indicate up regulation

On the top you can see the different feeding groups, while on the right hand side you can see the different pathways

As you can see, there are changes in gene expression among the groups;

For instance in the metabolism pathway, you can see a clear difference among the group, while the fish fed the FM had very dark blue color, the fish fed the SBM had very dark red color. Also, see dark red colors of the fish fed the CV similar to FM.

This indicate that metabolic patways was affected – this includes lipid metabolism and amino acid metabolism.



Shows profile of the dominant bacteria in the distal intestine by using DNA based methods (relative abundance of mo's)

See that there was a clear differences in the bacteria profile between the FM and the SBM group

Also, the fish fed the CV had a distinct different bacteria profile than the other groups.

In the fish fed the SBM, the staphylococcus which makes up the firmicutes group dominated, while in the fish fed the FM, the protobactera dominated

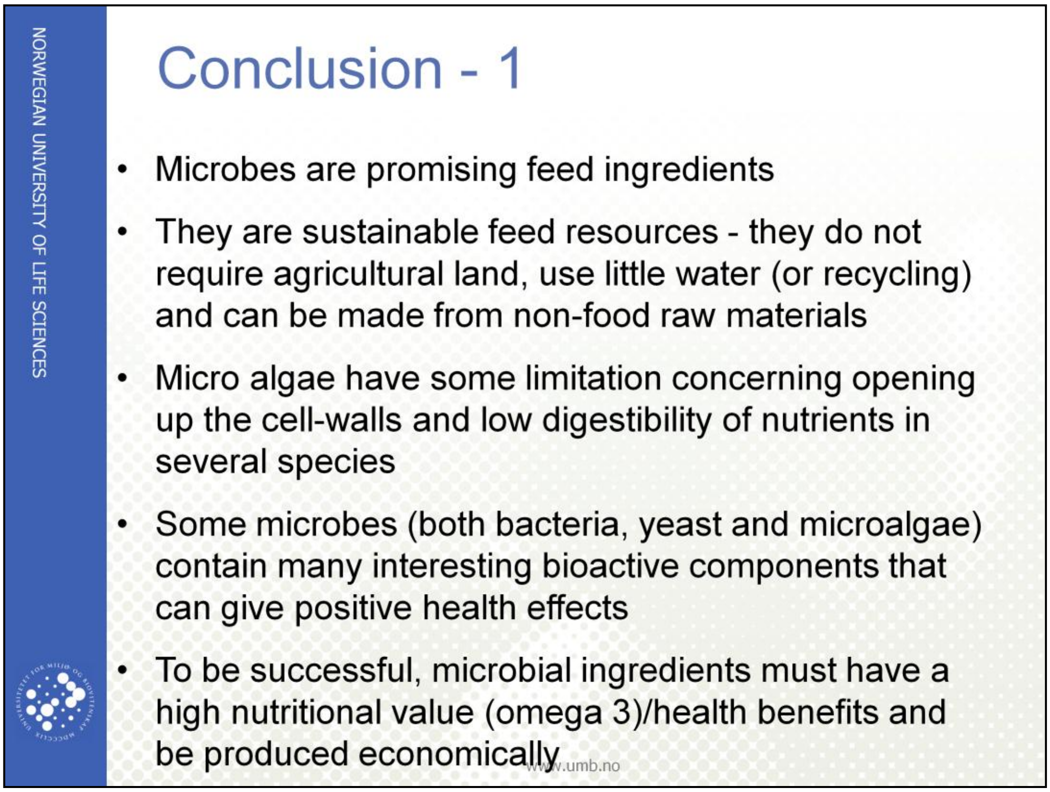
We also saw that this group had a higher diversity of gut bacteria compared with those fed the FM

Fish fed the CV contained a lot of unknown bacteria compared to the other groups.

Suggest that changes in gut Mos may be a causative factor in SE development. Alos, the protection of CV could be assiciated with changes in mo's

We know that SE is associated with increases the gut permeability (disruption of TJ), which may lead to exposure to the gut microbiota. Thus, this indicate that SBM-induced enteritis could be related to changes in gut microbiota. Chlorells has lower CP, beta-carotene, but higher NA and chlorophyl (2x) vs spirulina. Celmembrane in chlorella and bacteria has similarities.

DGGE profiles or pcr-amplified 16S rRNA fragments (V3 region)



The high-energy input for harvesting and processing biomass makes current commercial microalgal production economically unfeasible.

To be successful these ingredients must have a high nutritional value, and beneficial functional properties, and must be produces economically

And finally, the recent changes in the EU regulations allows for an increase use of these ingredient sin the future!