FERMENTATIONEXPERTS

Novel mussel-based feed ingredients
for animal health and wellbeing

By Jens Legarth
CEO
Fermentationexperts

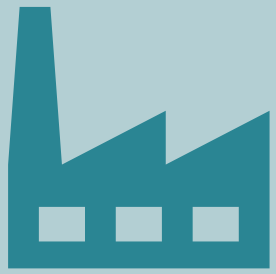
Program

- What is lactic acid fermentation?
- The health influence of the microbiome
- How to implement mussels in a fermentation proces!
- What is the business case?
- Where is the market?

You can have the worlds best idea! But you have to ask yourself 2 questions;
What is the business case and where is the money?

FERMENTATIONEXPERTS AS

- EUROPEAN PROTEIN UKRAINE LLC — 49 %
  - Partner: Agrolife GmbH
- FERMENTATION INC .USA — 50 %
  - Partner: Land O´Lake
- EUROPEAN PROTEIN AS — 100 %
- VÅDFODEREKSPERTEN — 100 %



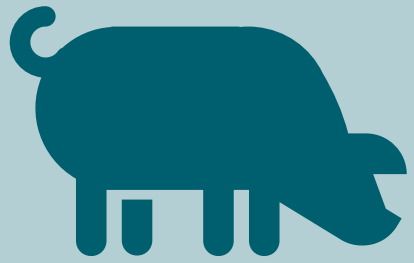
FERMBIOTICS HOLDING

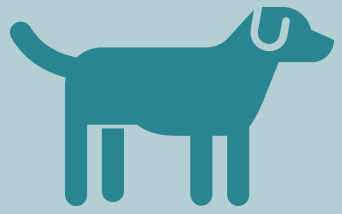
- FERMBIOTICS

FERMENTATION · PROTEIN · HEALTH · SUSTAINABILITY

SEGMENTS

PIGS · POULTRY · AQUA · PETS · RUMINANTS · HUMANS





FermBiotics
Supporting gut health

WHAT **WE DO**

We specialize in fermented feed and dietary supplements

BENEFITS

- ✓ Improved feed efficiency
- ✓ Affordable way to health promoting effects
- ✓ Lower drug consumption

EP100i is fermented rape protein concentrate and wheat containing lactic acid and live lactic acid bacteria and their metabolites. Represses coli and salmonella and modulates the intestine.

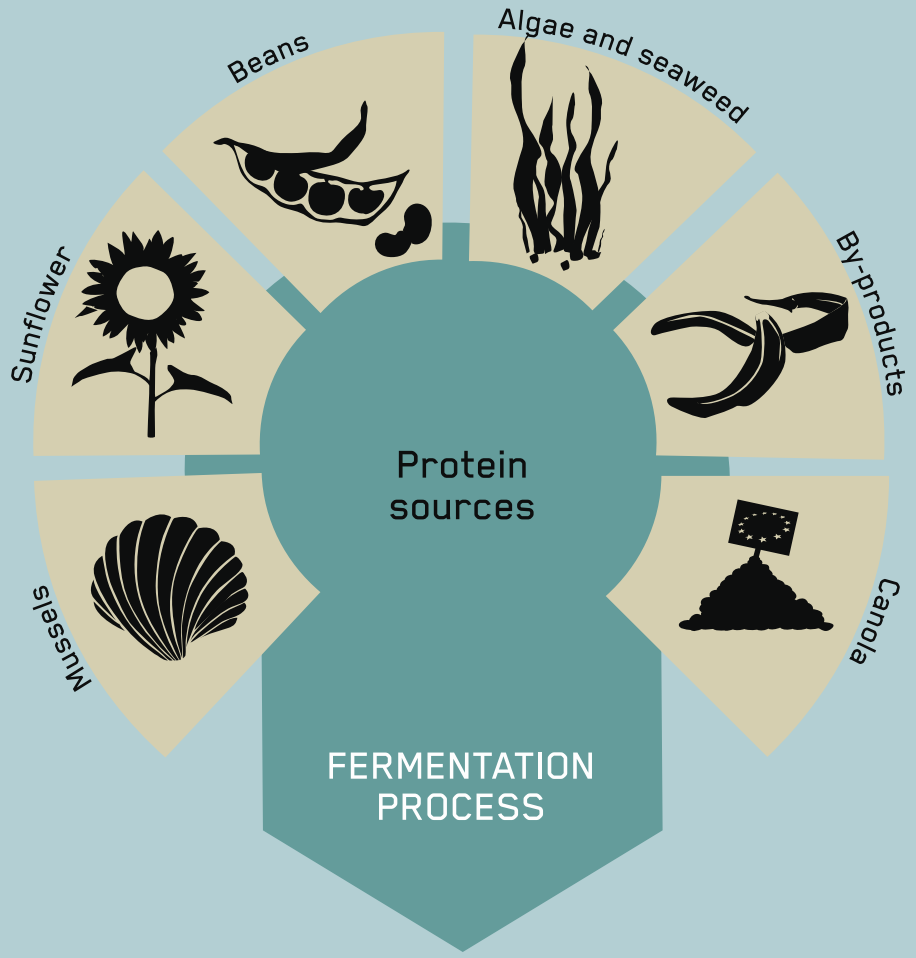
EP200 is fermented soy protein concentrate containing lactic acid and live lactic acid bacteria and their metabolites. Complements EP100 in the feed

EP199 is fermented rape and seaweed.
Reduces Inflammation and optimizes the immune system

DESIGNER **PROTEIN**

Extend the package of possibilities!

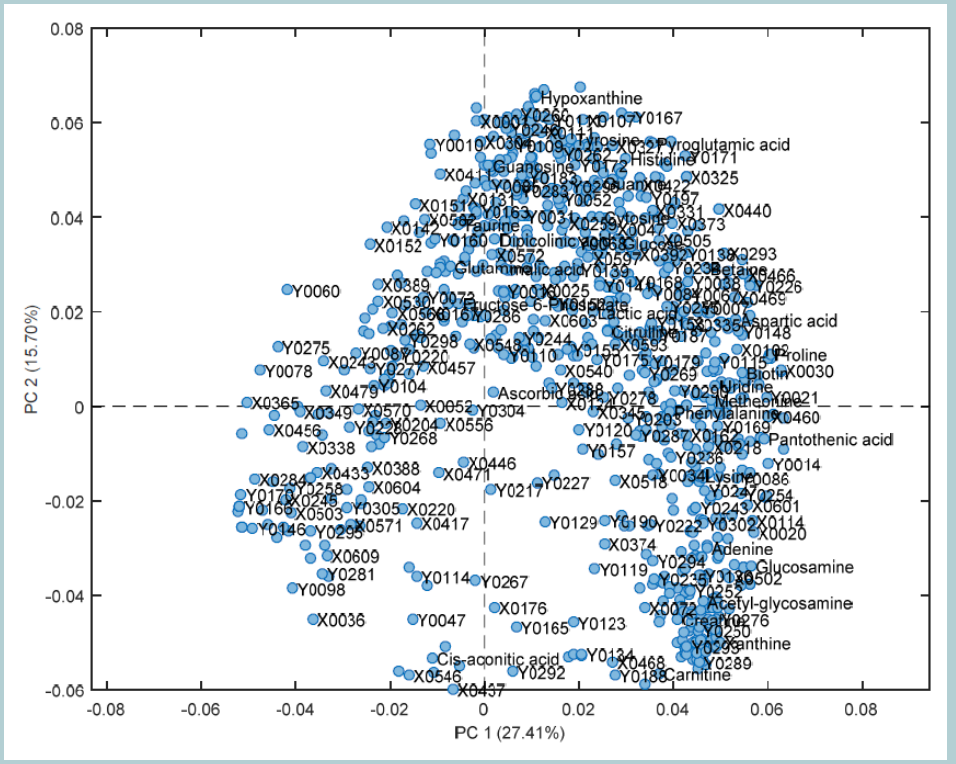
- We can ferment any protein source
- We can convert indigestible sugar into lactic acid – 5-10%
- Not only one but a package of advantages will pay for the drying cost
- Higher protein, phosphorous, fibre and energy digestibility.
- Our dry products contain live bacteria and enzymes



METABOLOMIC STUDY OF FERMENTED MATERIAL

Compounds of interest present in fermented plant and seaweed products

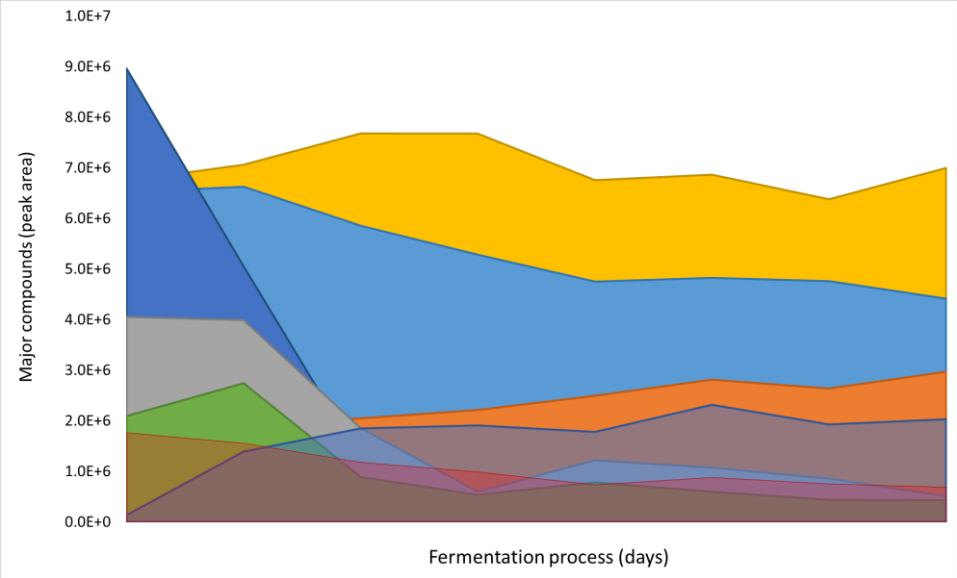
1. **Essential amino acids**
2. **Polyunsaturated fatty acids**
3. **Bioactive phenols**
   a) Anti-oxidants
   b) Anti-microbial
   c) Anti-inflammatory
4. **Vitamins from B-complex**
5. **Phytate solubilization**
   a) Myo-inositol production
6. **Fiber solubilization**
   a) Sugars
   b) Prebiotic oligosaccharides
7. **Lactic acid**
8. **Bacterial biomarkers**

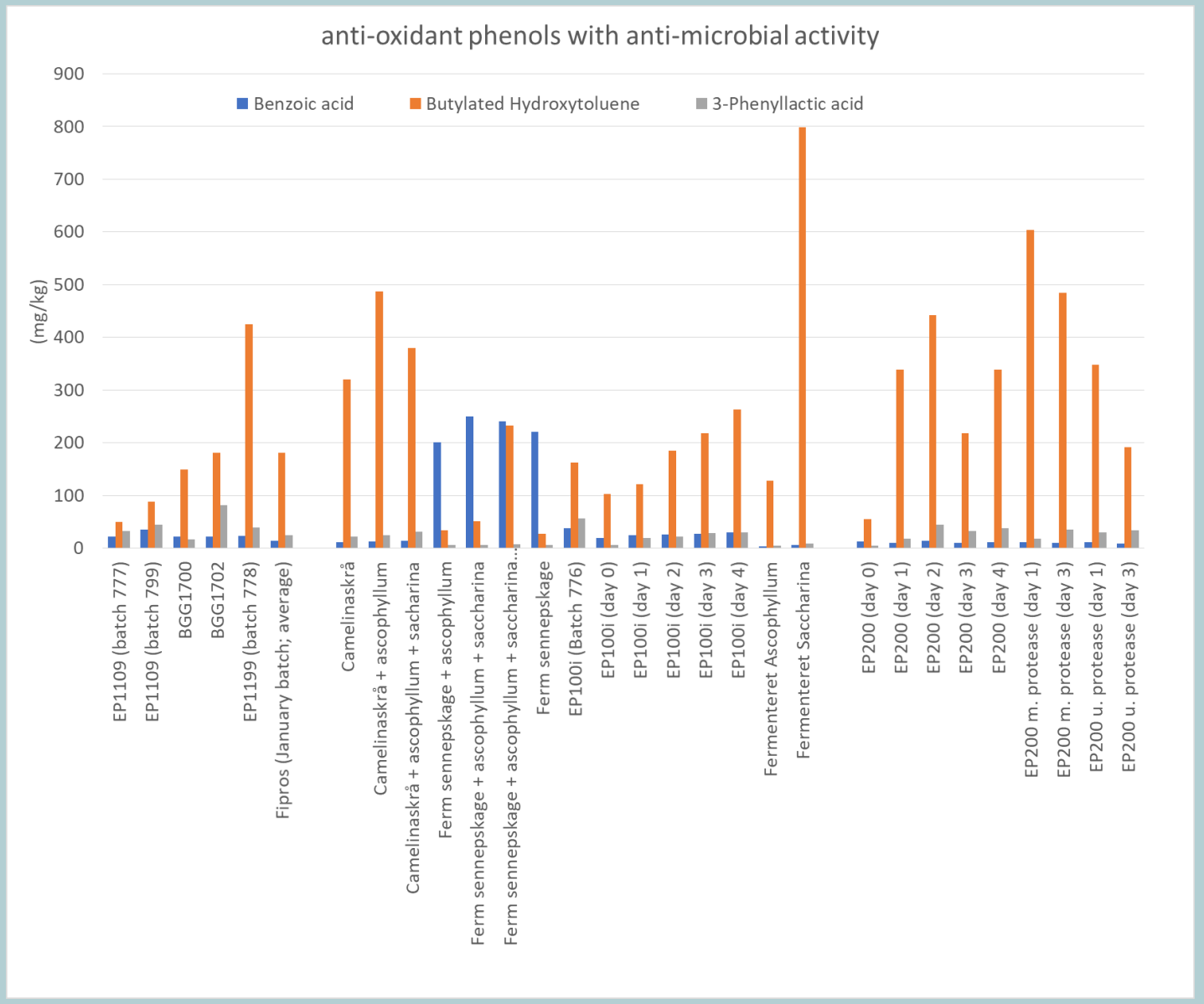


Loading plot from PCA model calculated on the concentrations of identified and unidentified compounds using LC-MS and GC methods

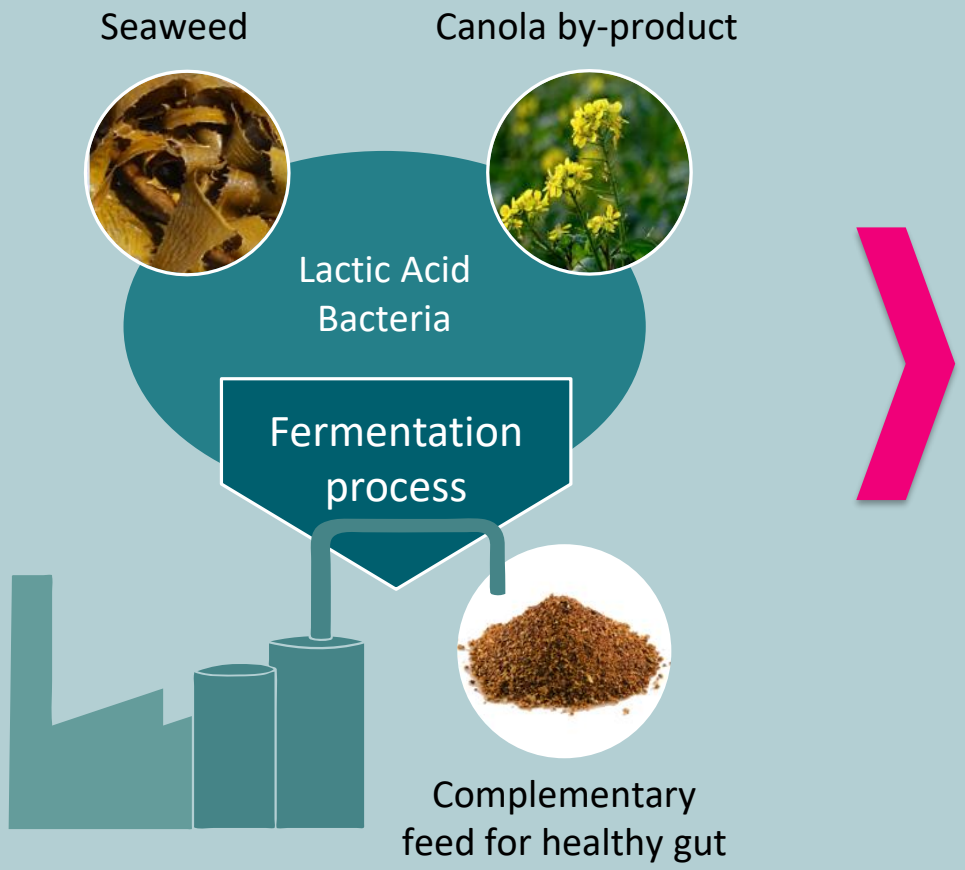
FOREFRONT ANALYTICAL TOOLS FOR **UNDERSTANDING PARAMETERS INFLUENCING FERMENTATION**

Lacto-fermentation of rapeseed meal




anti-oxidant phenols with anti-microbial activity
Benzoic acid
Butylated Hydroxytoluene
3-Phenyllactic acid
(mg/kg)
900
800
700
600
500
400
300
200
100
0
EP1109 (batch 777)
EP1109 (batch 799)
BGG1700
BGG1702
EP1199 (batch 778)
Fipros (January batch; average)
Camelinaskrå
Camelinaskrå + ascophyllum
Camelinaskrå + ascophyllum + sacharina
Ferm sennepskage + ascophyllum
Ferm sennepskage + ascophyllum + saccharina
Ferm sennepskage + ascophyllum + saccharina...
Ferm sennepskage
EP100i (Batch 776)
EP100i (day 0)
EP100i (day 1)
EP100i (day 2)
EP100i (day 3)
EP100i (day 4)
Fermenteret Ascophyllum
Fermenteret Saccharina
EP200 (day 0)
EP200 (day 1)
EP200 (day 2)
EP200 (day 3)
EP200 (day 4)
EP200 m. protease (day 1)
EP200 m. protease (day 3)
EP200 u. protease (day 1)
EP200 u. protease (day 3)

FERMENTATION & **DRYING** PROCESS



Bioactives

Prebiotics
Probiotics
Metabolites

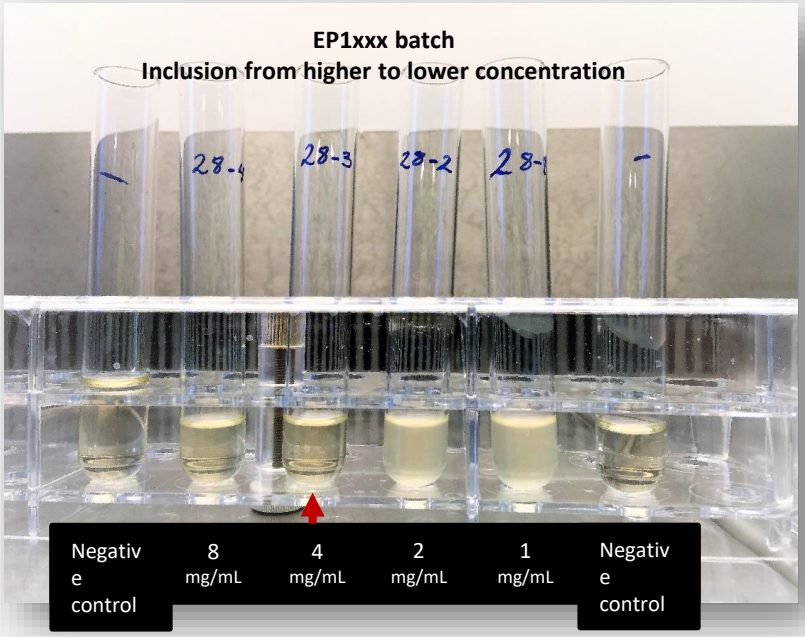
COLI

CLOSTRIDIA PERFRINGENS
IS THE BACTERIA RESPONSIBLE

- Necrotic enteritis in broilers
- Hemorrhagic enteritis in new-born calves
- Enterotoxaemia in sheep
- Food poisoning in humans

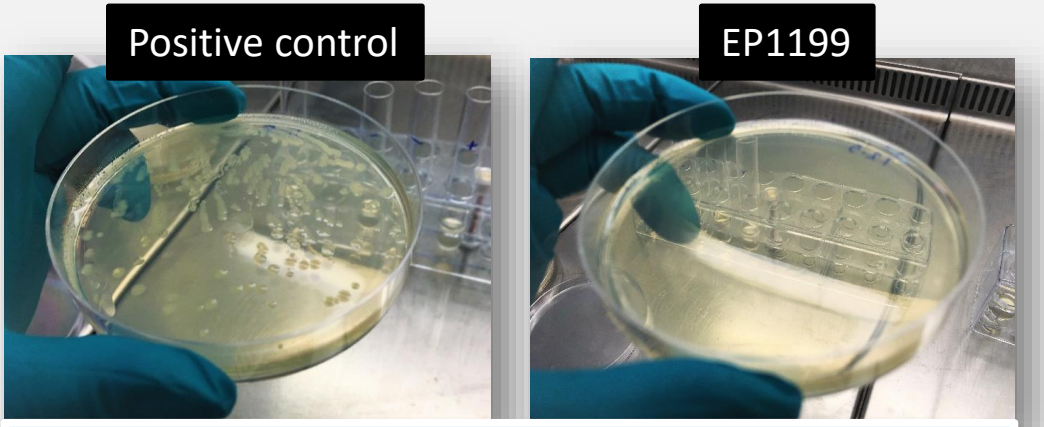
SEVERAL BATCHES CONTAINING FERMENTED SEAWEED WERE USED TO TEST THE **MINIMUM INHIBITORY CONCENTRATION (MIC)**

- The test evaluates the smallest concentration of an antimicrobial agent that inhibits the growth of bacteria.



4 mg/mL of EP1199 is the concentration inhibiting growth of *C. perfringens*.

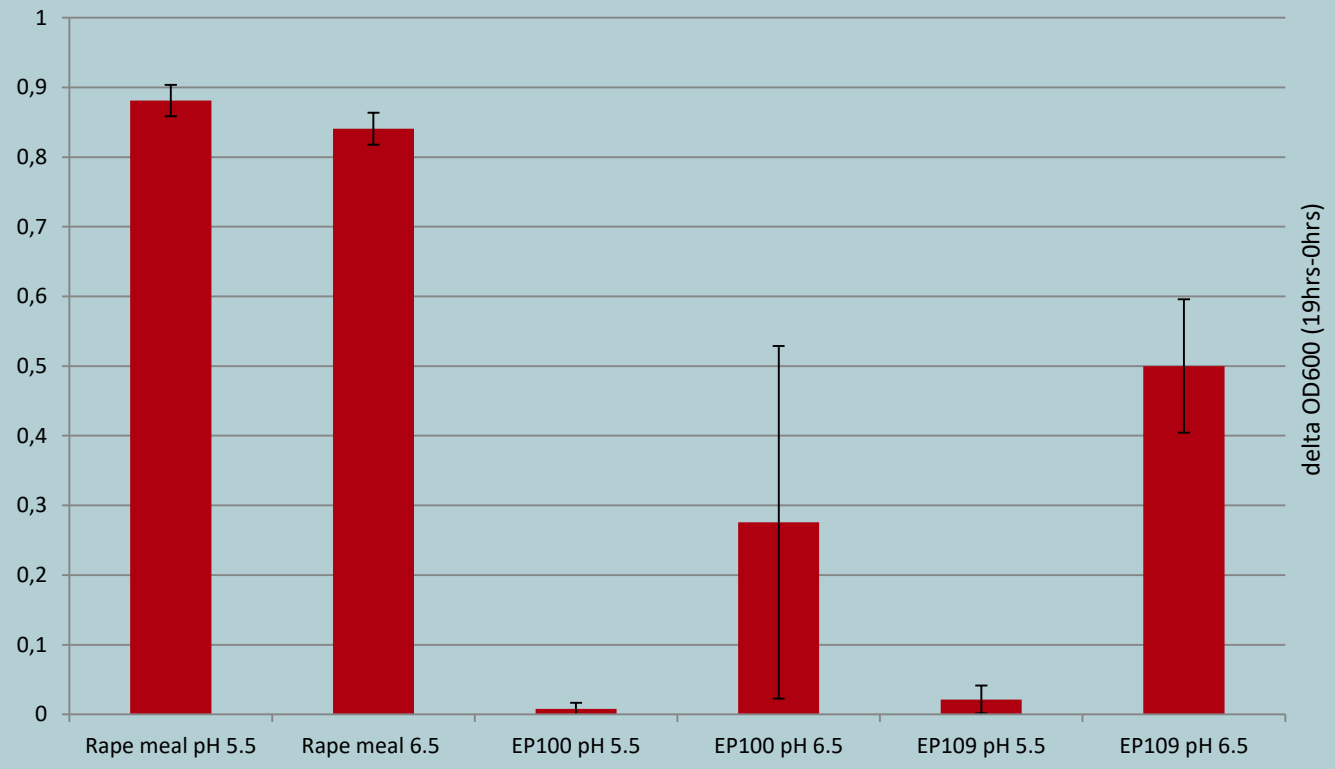
1 g of EP1199 has 150 mg/mL of *C. perfringens* inhibitory compounds!



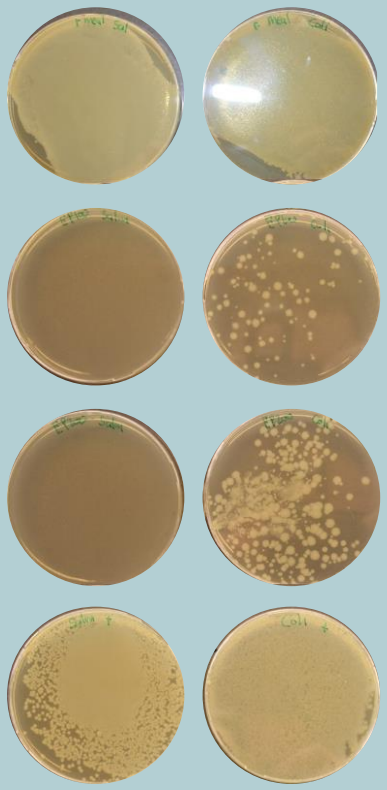
Clostridium perfringens grown on medium Nutrient agar (20 h at 37 °C) without and with EP1xxx

COLI OD600 OF E. COLI ATCC 11229

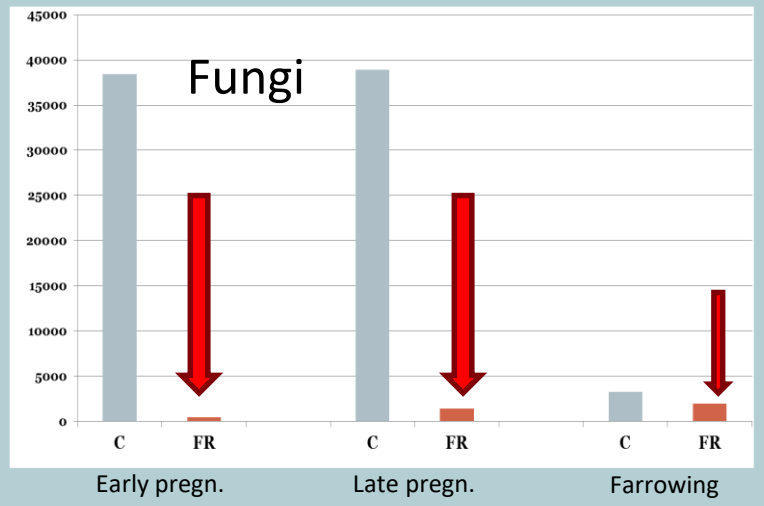
COLI GROWTH
AFTER 19 HOURS

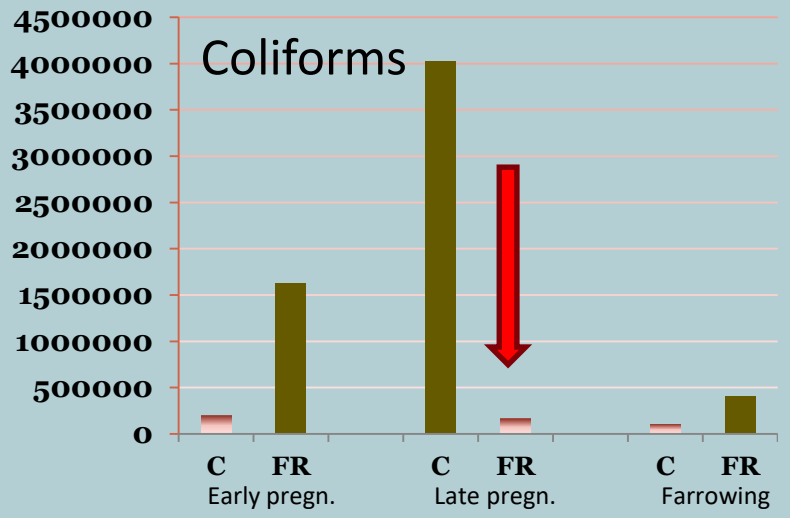


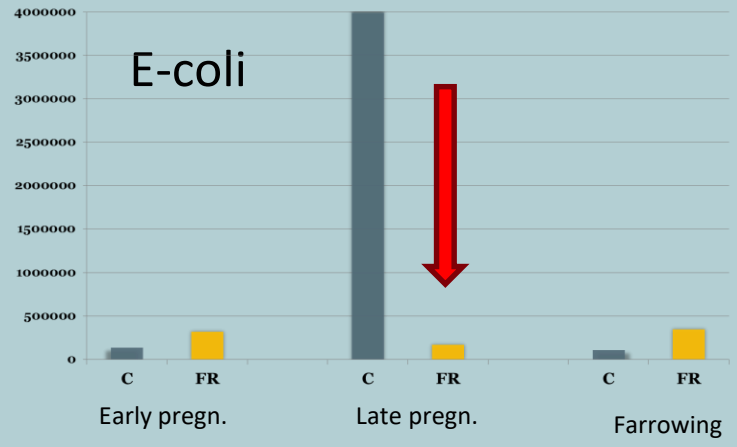
SALMONELLA GROWTH
AFTER 7 DAYS

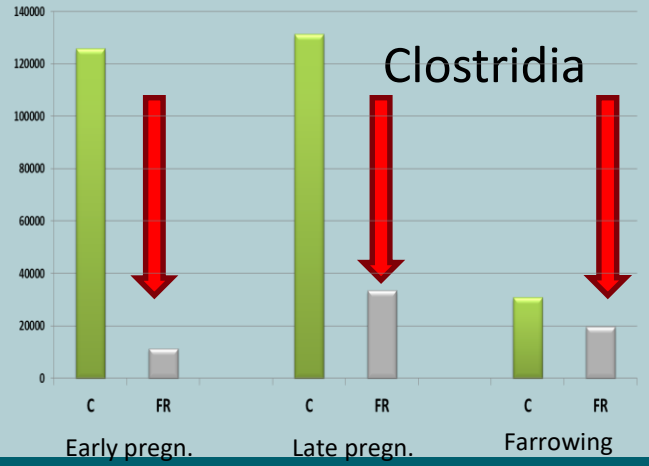


MICRO ORGANISMS IN THE HIND GUT









Anti-microbial activity of fermented rapeseed-seaweed product.

Strain: *Methicillin-resistant Staphylococcus aureus (MRSA USA 300)*

- 3 batches of fermented rapeseed-seaweed product were tested.
- Polar-non polar compounds were extracted with MeOH:DCM.
- Concentrations of the deposited extract on the plate are indicated in the table.

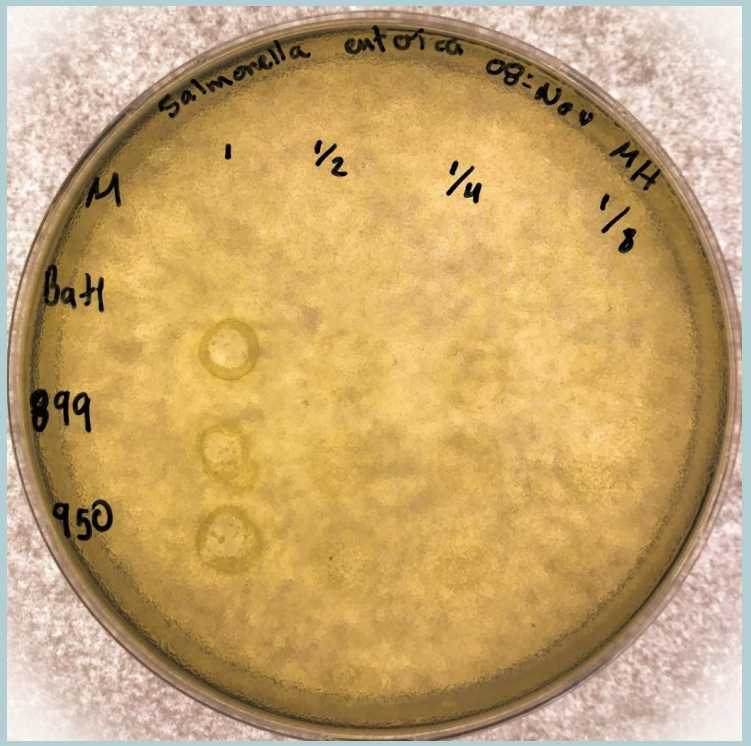
Treatment	Code on plate	1	1/2	1/4	1/8
		mg/ml			
Methicilin	M	0.050	0.025	0.012	0.006
EP1199	Batch 1	100	50	25	12.5
EP1199	899	100	50	25	12.5
EP1199	950	100	50	25	12.5

Spot-on-lawn assay on Methillicin-resistant Staphylococcus aureus (MRSA USA 300) *grown on MH medium overnight at 37 °C. Halos are the areas of inhibition growth of MRSA, were the fermented extracts were deposited into the medium. Negative control is DMSO at 9% (low center, not visible)*

Anti-microbial activity of fermented rapeseed-seaweed product. Strain: *Salmonella enterica* ATCC 43971

- 3 batches of fermented rapeseed-seaweed product were tested.
- Polar-non polar compounds were extracted with MeOH:DCM.
- Concentrations of the deposited extract on the plate are indicated in the table.

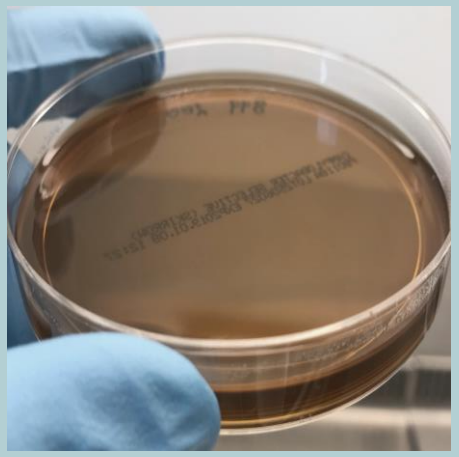
Treatment	Code on plate	1	1/2	1/4	1/8
		mg/ml			
Methicilin	M	0.050	0.025	0.012	0.006
EP1199	Batch 1	100	50	25	12.5
EP1199	899	100	50	25	12.5
EP1199	950	100	50	25	12.5



Spot-on-lawn assay on Salmonella enterica (ATCC 43971) *grown on MH medium overnight at 37 °C. Halos are the areas of inhibition growth of MRSA, were the fermented extracts were deposited into the medium. Negative control is DMSO at 9% (low center, not visible)*

Minimum inhibitory concentration (MIC) assay with fermented rapeseed-seaweed
Strain: *Campylobacter jejuni (CCUG 48890)*

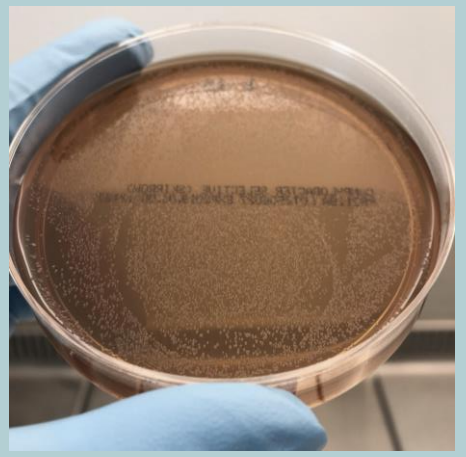
9 mg/mL



5 mg/mL

2 mg/mL

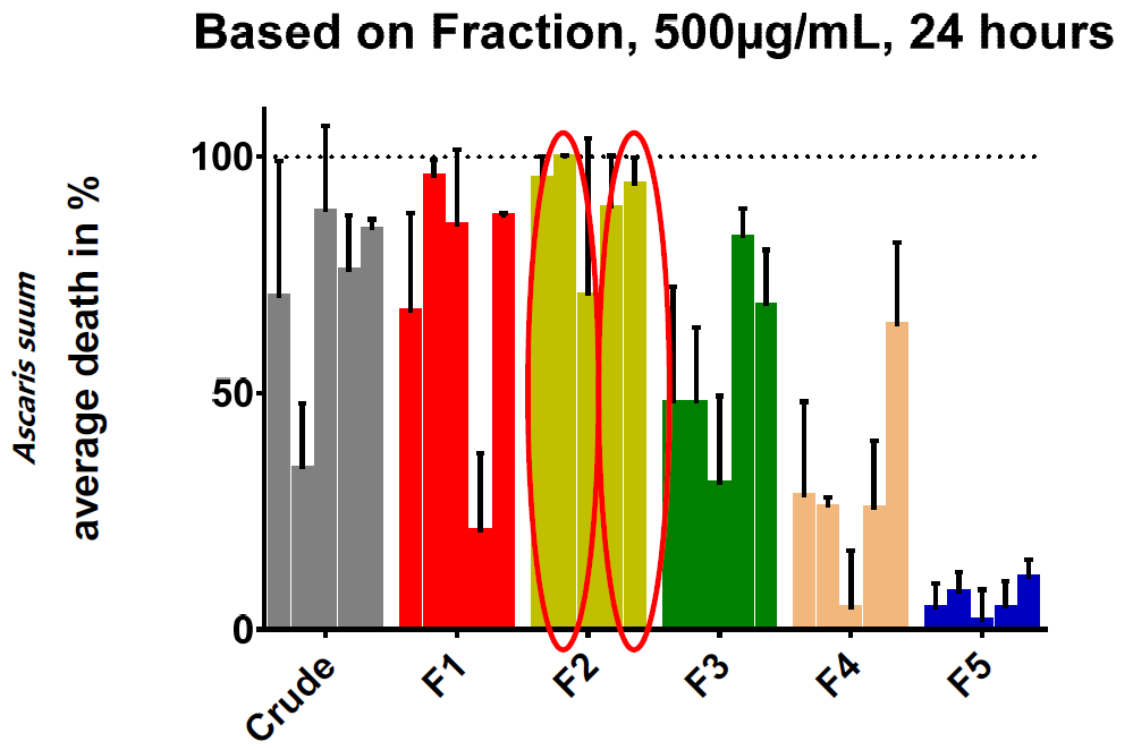
1 mg/mL



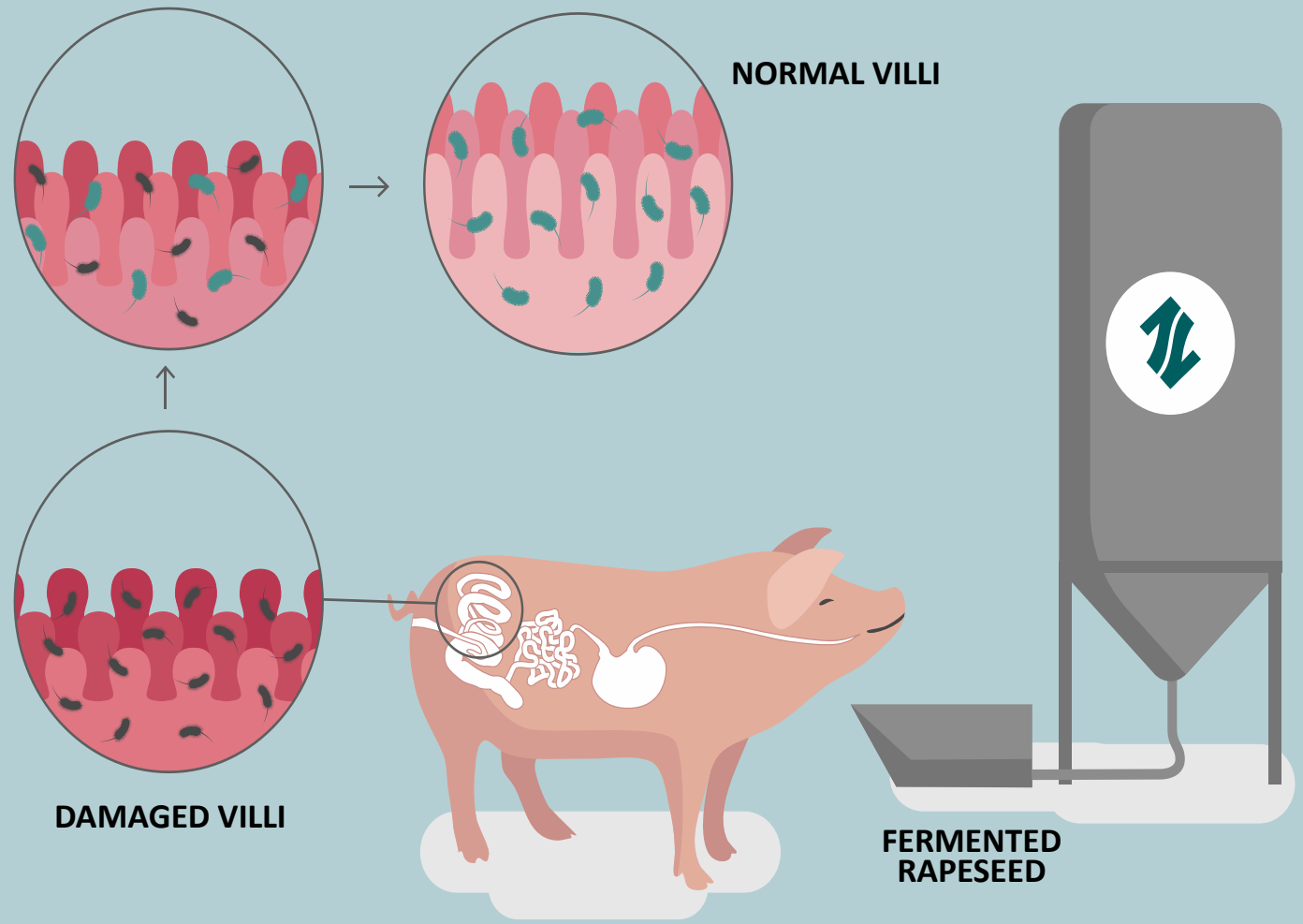
Dilution plates from MIC assay with Campylobacter jejuni (CCUG 48890)
grown on Skirow medium overnight at 37 ^{o}C under reduced oxygen conditions. Plates were diluted

PARASITES IN EP1199

c. *In vitro* studies on parasites (CBS)

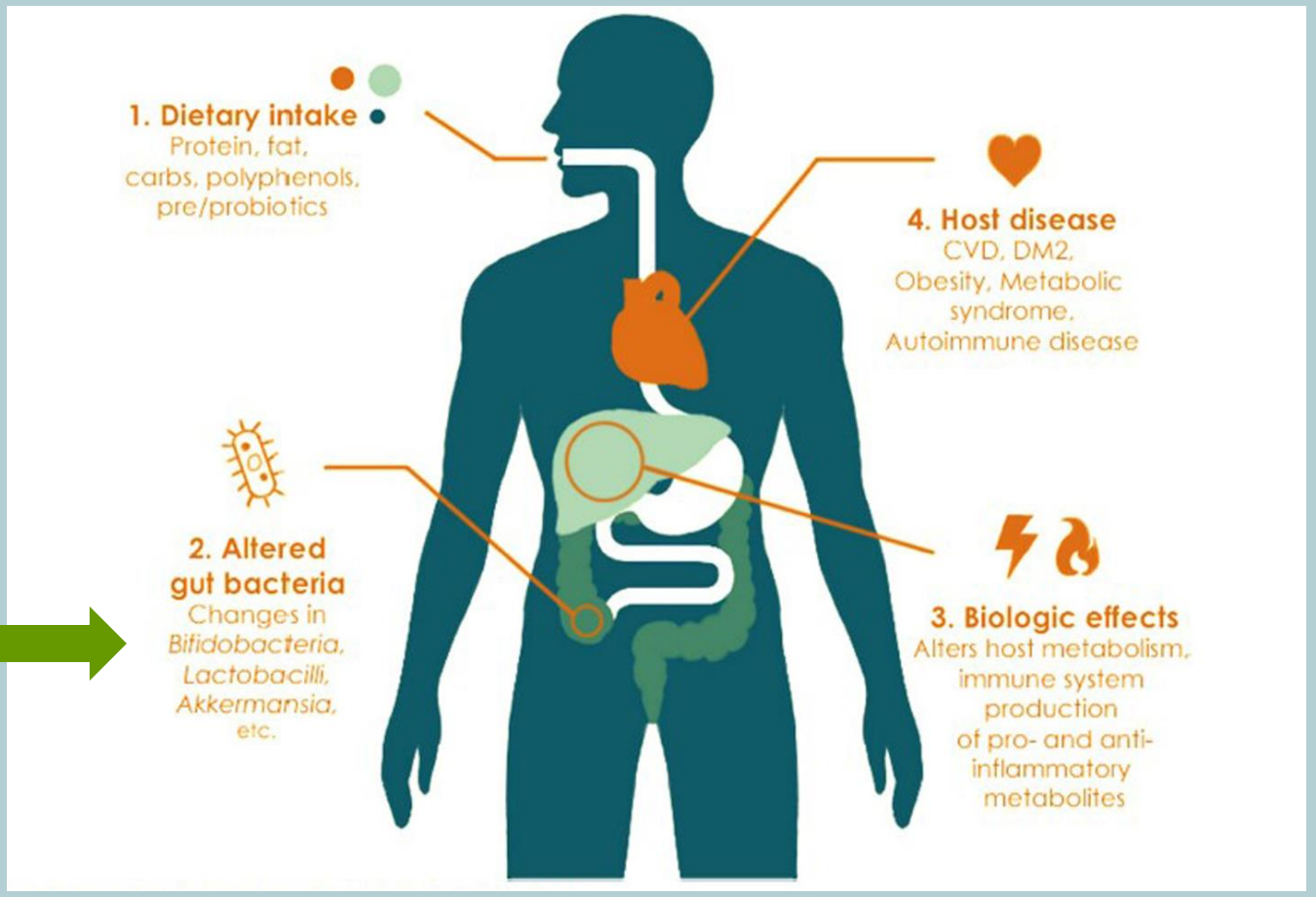


EP100 & **EP199** HAS POSITIVE
INFLUENCE ON ALL ORGANS!



IMPACT OF DIET ON THE GUT MICROBIOTA AND HUMAN HEALTH

Microbiome modulation by fermented canola and seaweed



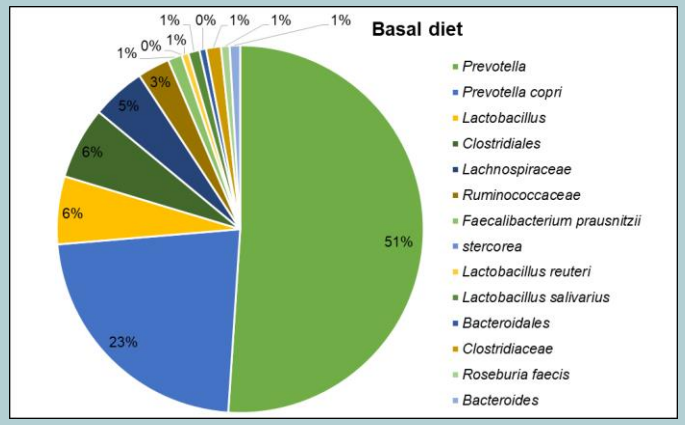
Credit: J Transl Med. 2017; 15(1):73. doi: 10.1186/s12967-017-1175-y

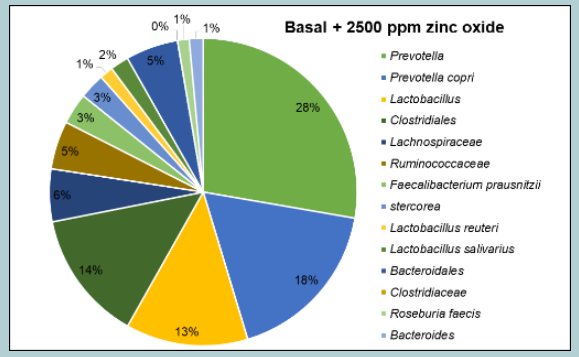
PIG **TRIAL**

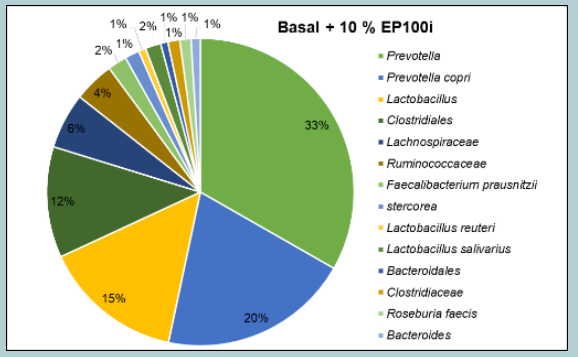
Gut microbiota characterisation (N=2x5)

Increase of *Lactobacillus* in 2.5% fermented product group compared to basal diet

- 2.5% fermented products to diet are enriched in good gut bacteria
- *Lactobacillus* increase 6% -> 19%
  - *Lactobacillus reuteri*
  - *Lactobacillus salivarius*
  - *Faecalibacterium prausnitzii*
- Positive modulation towards anti-low-grade-inflammatory gut bacteria



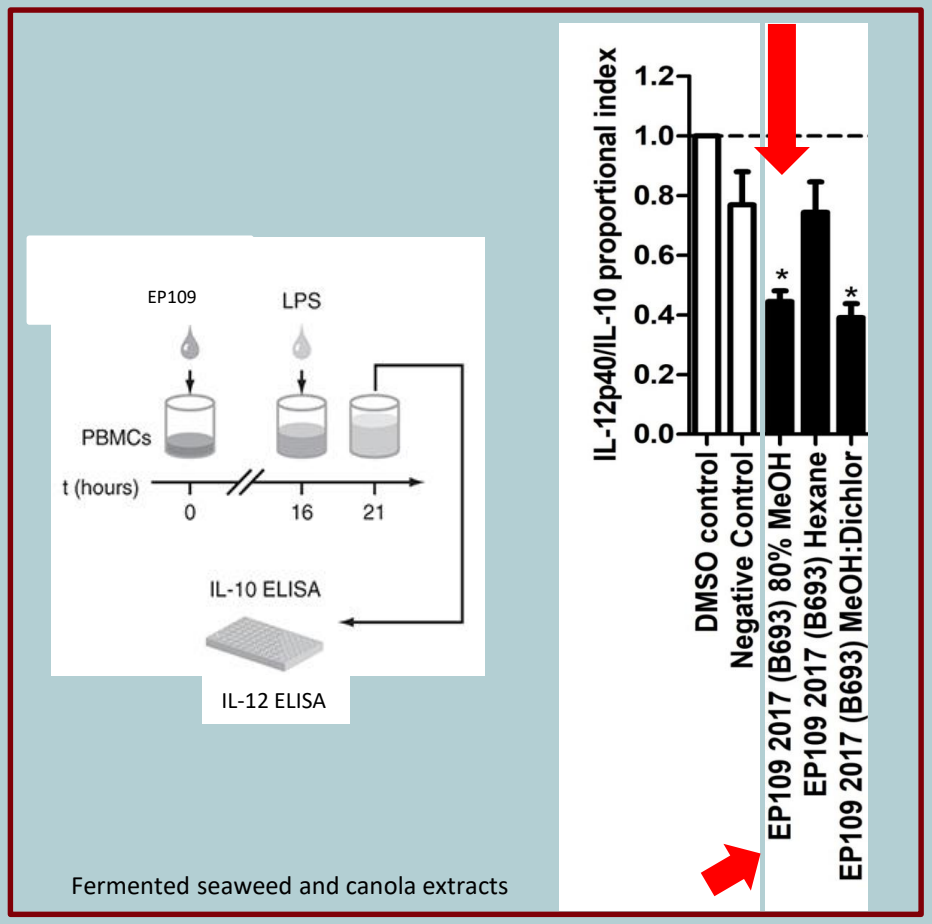




IN VITRO ANTI-INFLAMMATORY EFFECT

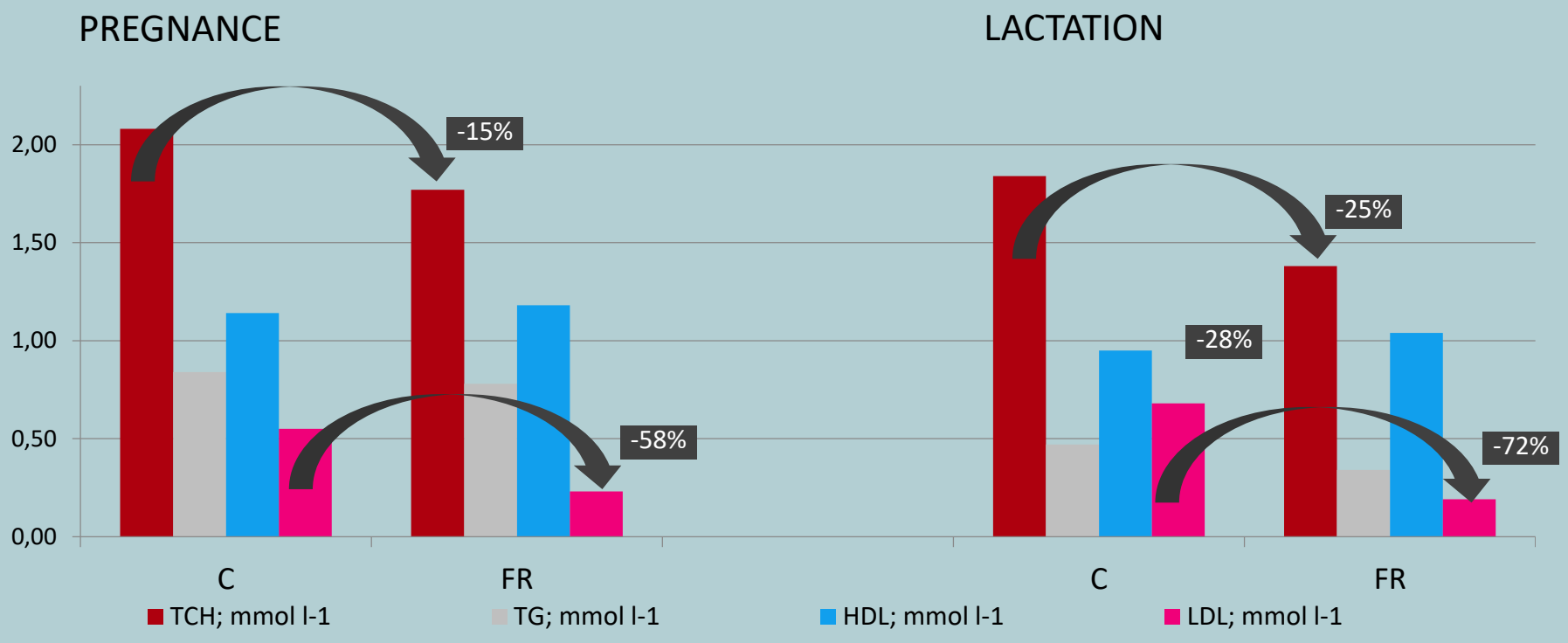
Fermented seaweed and canola extracts

- Test of different fermented seaweed and canola extracts in peripheral blood mononuclear cells (PBMC) assay using 3 different human donors.
- Lipopolysaccharides (LPS) or endotoxins found of the outer membrane of Gram-negative bacteria was used to stimulate an inflammation in matured human PBMCs.
- Response was measured by inhibition of **IL12** proinflammatory cytokine and induction of anti-inflammatory cytokine **IL10** by extracts.



LDL REDUCED IN PIG STUDY

4% GG1715 of diet, n = 10 (C) +30 (GG1715)



RESULTS
FEED CONVERSION RATE (KG FEED/KG BODY WEIGHT GAIN)

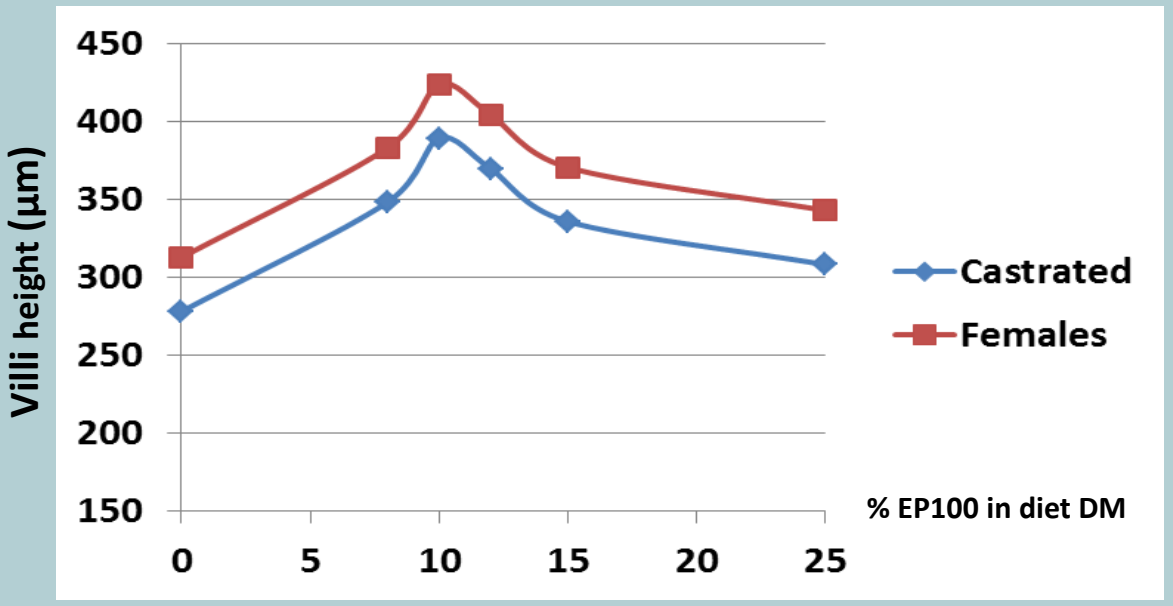
Parameter:	0Zn	2500Zn	8%EP	10%EP	12%EP	15%EP	25%EP	10%EP	+0.6%BA	+1.0%BA
Average daily weight gain after weaning (from average piglet weights at period start and end)**:**										
D15-30	226	230	316	329	293	291	294	329	281	298
D31-64	625	633	594	651	558	647	612	651	540	599
Feed conversion rate after weaning (kg feed/kg liveweight gain):										
D1-14	2.10	2.30	1.55	1.80	1.55	1.77	1.72	1.80	1.60	1.73
D15-37	1.67	1.62	1.62	1.54	1.57	1.54	1.52	1.54	1.60	1.59
D38-65	1.55	1.45	1.64	1.56	1.56	1.53	1.56	1.56	1.61	1.58
Whole weaner unit period (D1-64):										
Exit D64 post-weaning	33.6	33.8	34.7	35.4	32.4	35.0	34.5	35.4	31.7	34.1
Daily weight gain	408	414	425	450	400	437	422	450	382	418
FCR	1.60	1.54	1.63	1.56	1.56	1.54	1.56	1.56	1.61	1.59

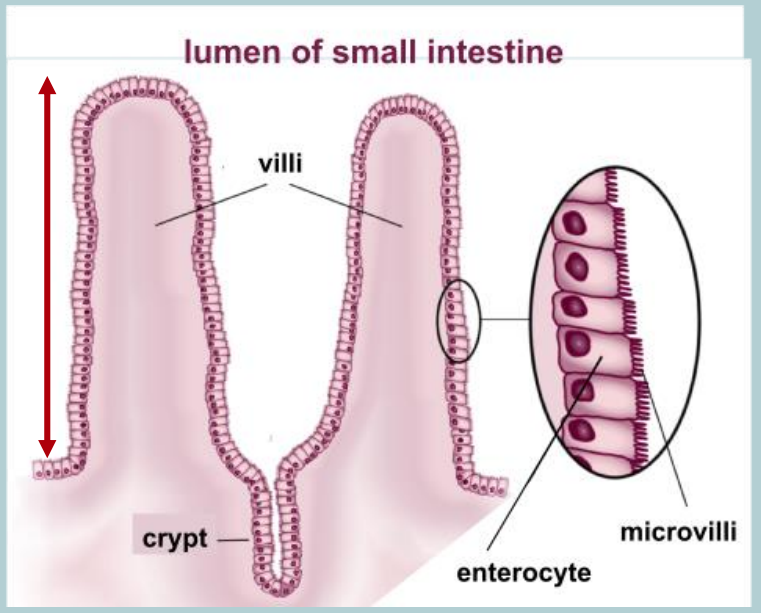
Better than 2500Zn

As good as 2500Zn

RESULTS

SMALL INTESTINAL VILLI HEIGHT (6 SLAUGHTERED PIGLETS D11 AFTER WEANING PER TG)





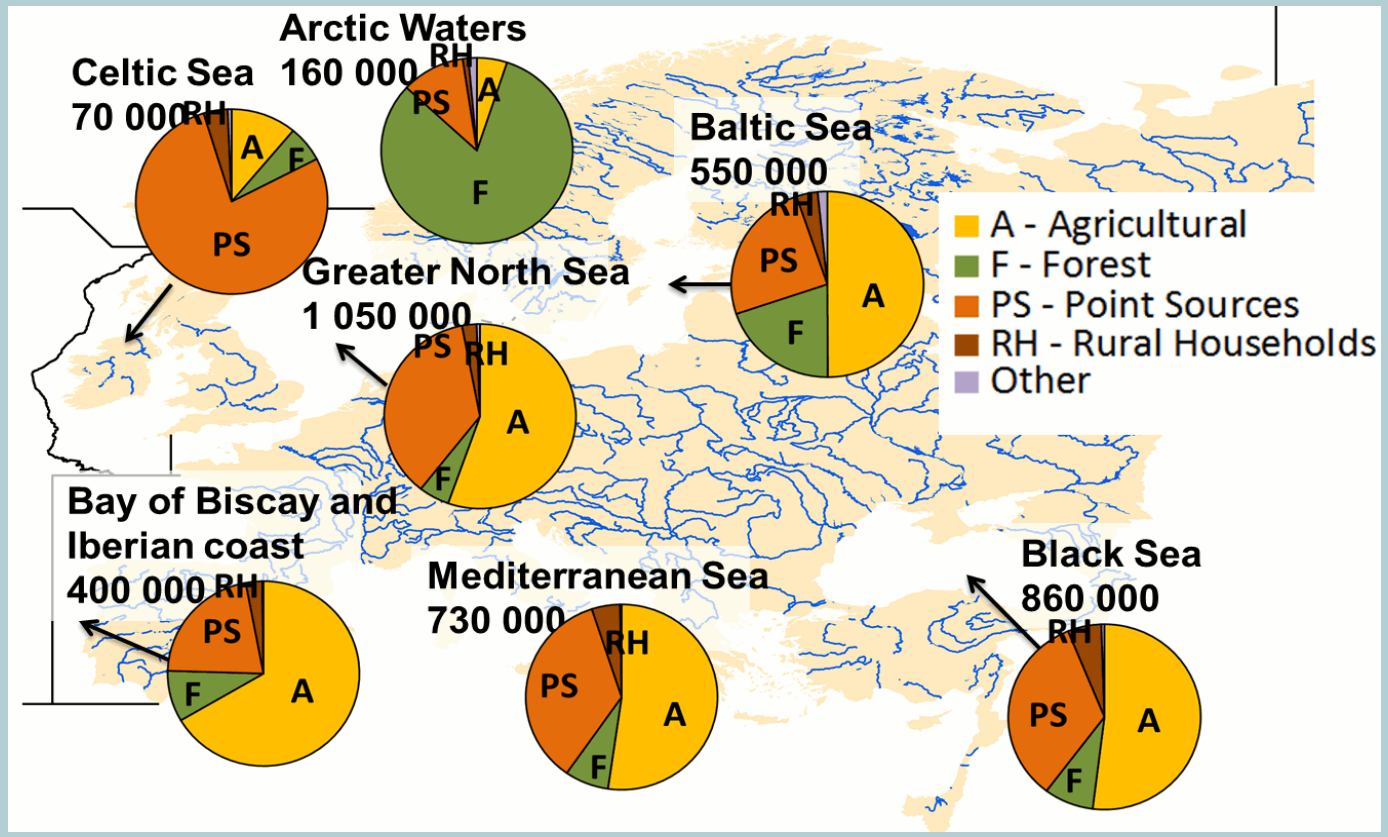
EP100:

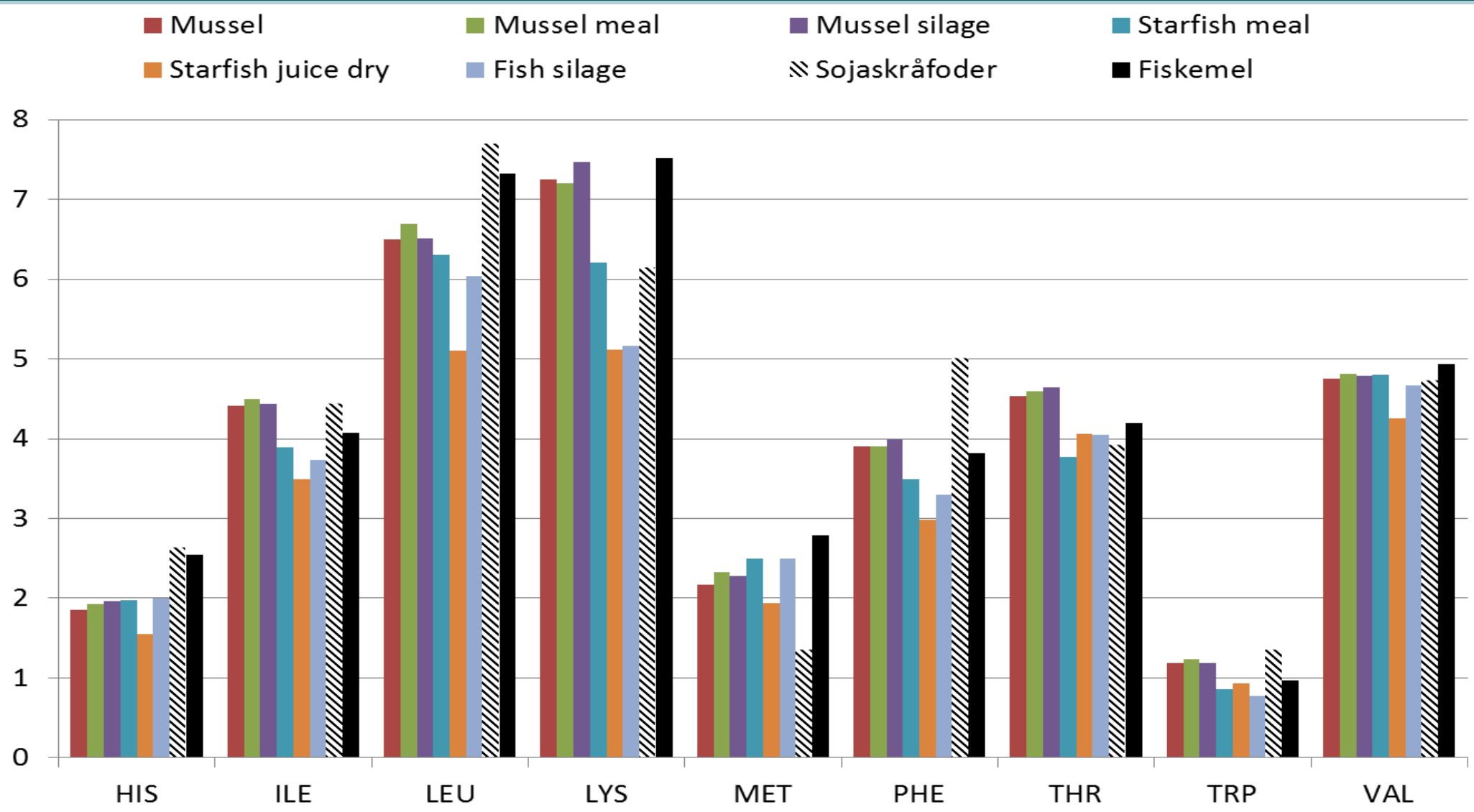
- Increased small intestinal villi height <35% (maximal effect: 10% EP100i in diet)
- Stimulated extensive folding of villi => ↑↑↑ increase in surface area
- Immune cell infiltration and enterocyte morphological changes absent
  - Also in colon

Impact of EP products on weaner piglet performance

	TG1 Basal diet	TG2 2500 ppm zinc	TG4 10 % EP100i	
No. repetitions per TG	2	6	5	
Average pig/Pen	48	46	47	
Total no. pigs per TG	93	277	229	
Average weight at weaning (kg)	6,07	5,86	6,16	
ADWG (g)	272	285	304	TG1: P<0.05
AWG (kg)	17,1	18,0	19,2	
FI/pig (kg)	31,5	30,1	32,5	
FCR (kg feed:kg weight gain)	1,84	1,68	1,69	NS
Average body weight at D63 (kg)	23,0	23,9	25,5	
Average no. pig/pen at start of experiment	46,5	46,2	45,8	
Mortality (%)	12,0	5,2	4,1	NS

We can make 100 million tons of protein from mussels in Europe without discharging any more nutrients to the sea





Kilde: Marine proteinkilder v. Jan Værum Nørgård og Flemming Gertz

How can we make business out of mussels?

Grow the mussels and dry them?

What are we going to do?

Any suggestions?

Could we look outside the traditional box?

Market Competiveness

We have to look at the process innovation and competitiveness now and not in 10 years time!

If you find an alternative protein source now and start scaling it will take 10 - 20 years before it is an industry.

Bacterial resistance, laws and market trends will change the market slowly!

What are the threats?

Our business case!

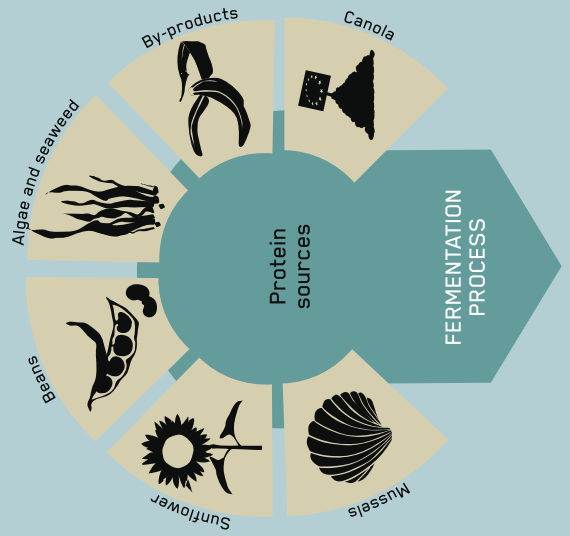
- We will grow the mussels for less than 1 year
- About 9 ton of raw mussel will give us 1 ton of mussel meal
- We will cook them and seperate shells from meat
- We will end up with a cooked mussel soup with 40% drymatter
- We will grind the mussels and use the heated mussel for the fermentation process. It will be as an innoculent
- As result we will use the water to enrich health, vitamins and minerals from other plant materials
- Now we have a basket of benefits to pay for our drying process

Marine protein prices

Price is approximately 85% of fishmeal. 60% protein compared with 70% protein in fish meal

- Current fish meal selling price is 2,000€/ton. Dried mussel meal should cost 1,700€/ton
- We obtain 11% mussel meat. 9 ton of raw mussel is needed for 1 ton mussel meal. Growing cost is 120€/ton x 9 ton = 1080 €/ton meal
- Separation from shells and pasteurisation cost 350€/ton. Then we have a pumpable meat with 40% dry matter
- Logistic, handling and storage matters 200 €/ton
- In total cost 1630 €
- Income from shells (50 – 250€/ton ?)
- Other income?

Where is the market now ?



Fish and Poultry producers
800 kg soya + 450 kg mussel soup = 1 ton of fermented mix

German speaking population = hate soya!
Pig Producers= rapeseed and seaweed + mussel meal

SUSTAINABLE FARMING WITH FERMENTATION

- Fermentation is considered the best means to make Europe self-sufficient in protein. All ANFs degrade in the fermentation process, allowing the use of locally produced proteins such as rapeseed, sunflower, lupines and horse beans.
- Lactic acid fermentation can alleviate ecology with 100 % organic feed. Fully fermented feed from e.g. algae can improve organic protein and replace expensive protein feed.

- Economically beneficial for farmers due to increased weight gain, less use of medication and more affordable raw materials.
- Fermentation releases all minerals. Phosphorus is also released and thereby digestible for the animals, so that no extra phosphorus should be added. Pollution is reduced by up to 75 %. The earth's phosphorus mines are empty within the next century, but still an important element.

- Feeding with fully fermented feed reduces the risk of bacterial outbreaks in animals, including salmonella, campylobacter, coli, and more. In fermented rape seed there is a probiotic effect which can break down pathogenic bacteria, including the resistant bacteria. There is a reduced bacterial pressure in the barn, including resistant bacteria.
- CO2 is reduced as a result of lower soybean imports due to self-sufficiency.

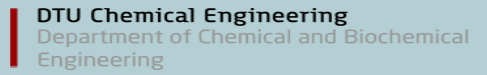
- World population increases each day by 240,000 people. Alternative protein sources are needed. It is only possible at sea and algae is a sustainable way.
- Algae absorbs high amounts of P, N and other aquatic systems in the sea. Algae is the future protein and energy source. It is possible to produce up to 20 times more protein/acre than on land. It is a future food and energy resource.

- Fermentation of protein crops and feed increases feed value by 5 to 15 %.
- Fishmeal and soybean meal can be replaced by cheaper fermented European Protein products with the same effect. Production of fishmeal drains the ocean of a large number of fish. In order to produce 1 ton fishmeal, requires approx. 3-4 tons of fish.

PROJECTS

- Currently attending 10 projects (national/EU-level).
- Total budget of €36,0 million.

SOME OF OUR PROJECT PARTNERS:



Tomorrow's solutions ...today