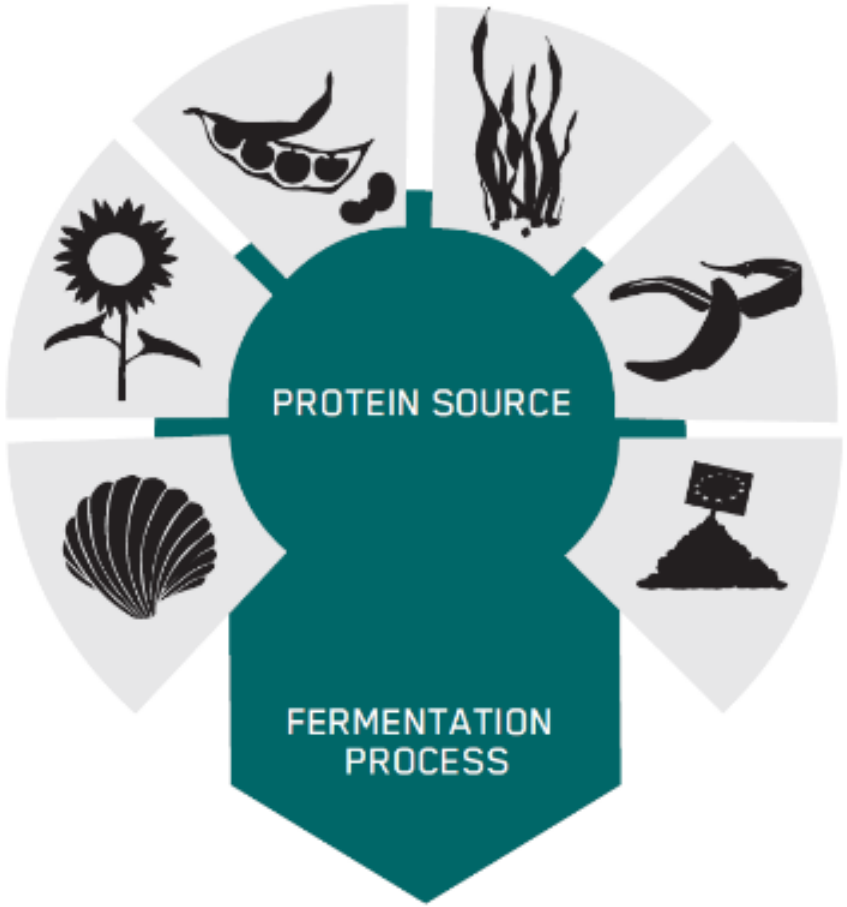
EUROPEAN PROTEIN

FERMENTATIONEXPERTS & EUROPEAN PROTEIN

ABOUT FERMENTATION

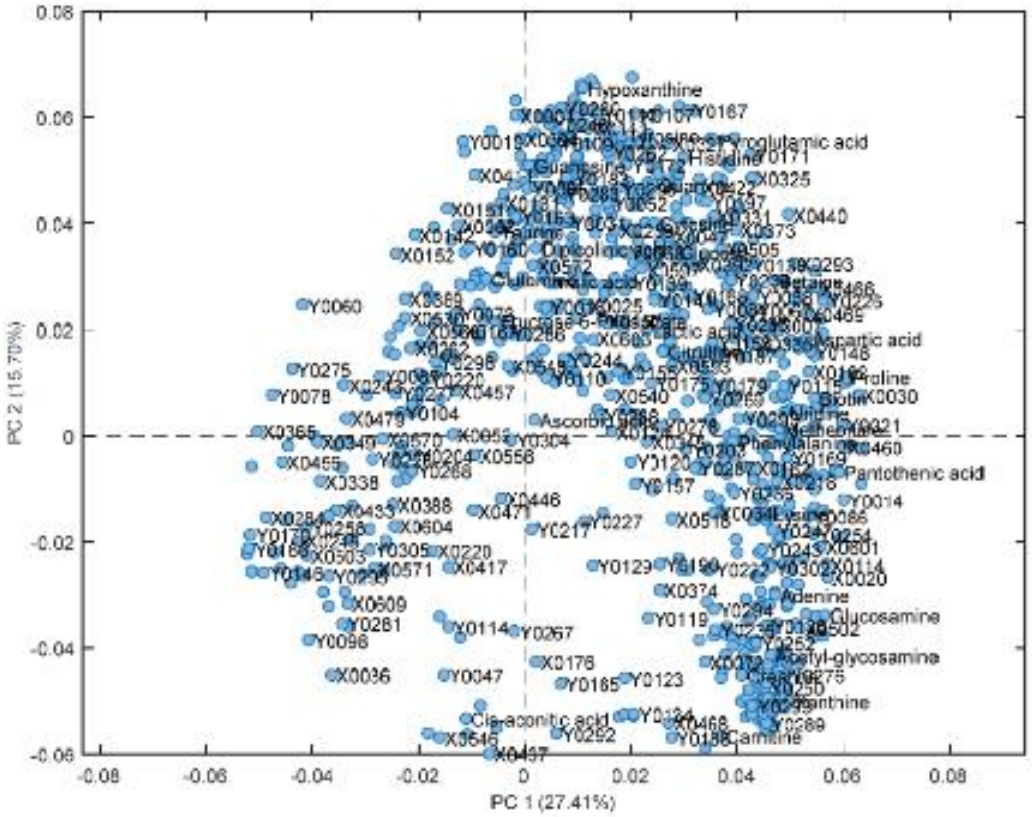


Designer protein

- We can ferment most protein sources
- We can convert indigestible sugar into lactic acid (5-10%)
- Several advantages will pay for the drying cost
- Higher protein, phosphorous, fibre and energy digestibility
- Our dry products contain live bacteria and enzymes

ABOUT FERMENTATION

Compounds in fermented EP products

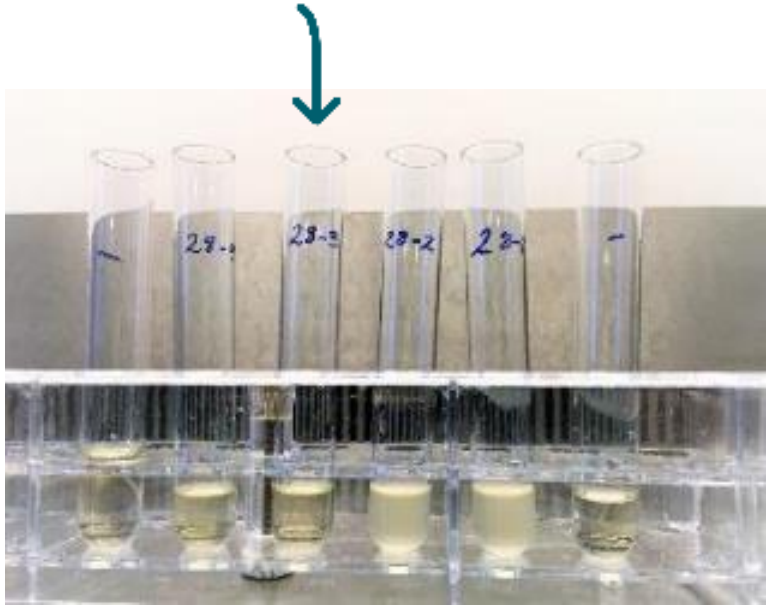


- **Essential amino acids**
- **Polyunsaturated fatty acids**
- **Bioactive phenols**
  - Anti-oxidants
  - Anti-microbial
  - Anti-inflammatory
- **Vitamins from B-complex**
- **Phytate solubilization**
  - Myo-inositol production
- **Fiber solubilization**
  - Sugars
  - Prebiotic oligosaccharides
- **Lactic acid**
- **Bacterial biomarkers**

Source: European Protein laboratory test

BACTERIA*

IN VITRO



Negative control
8 mg/mL
4 mg/mL
2 mg/mL
1 mg/mL
Negative control

Bacteria inhibition:

Clostridium perfringens

EP-products inhibit growth of *Clostridium perfringens*

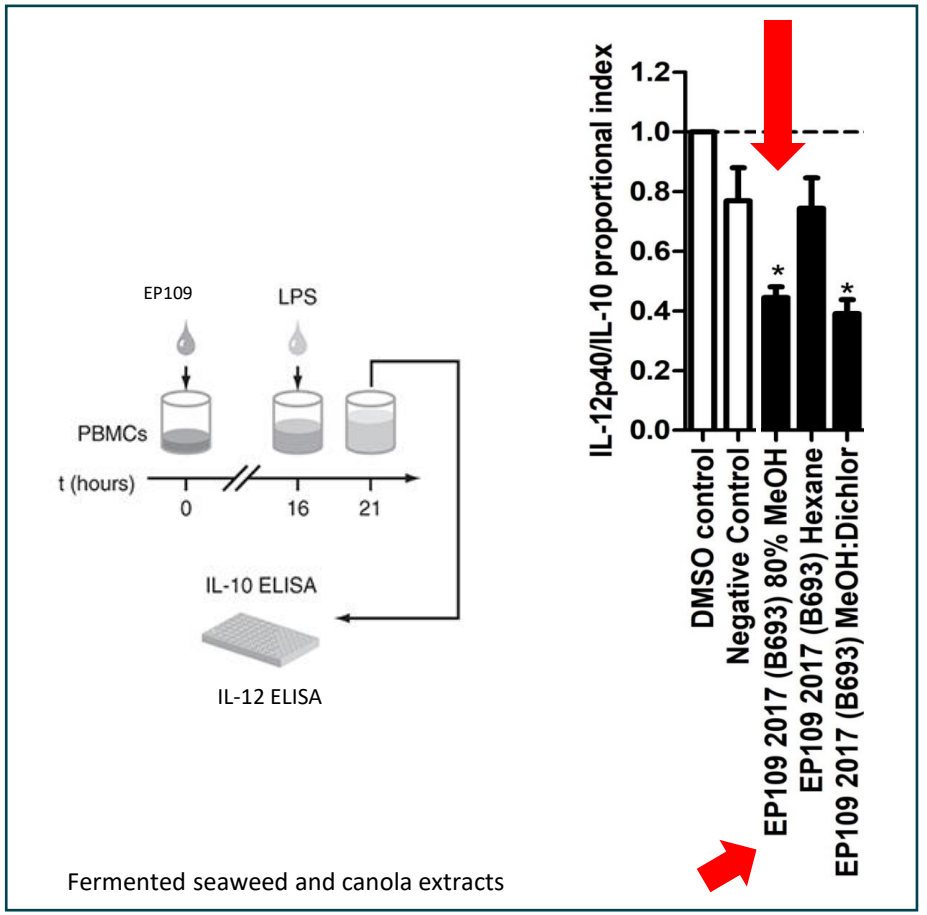
1 g of EP-product has 150 mg/mL of *C. perfringens* inhibitory compounds!

Photo 1: European Protein laboratory test on several batches of an EP-product. The test evaluates the lowest concentration of an antimicrobial agent that inhibits the growth of bacteria (MIC).
Photo 2 and 3: *Clostridium perfringens* grown on medium Nutrient agar (20 h at 37 ^{o}C) without and with EP-products.

IN VITRO ANTI-INFLAMMATORY EFFECT

Fermented seaweed and canola extracts

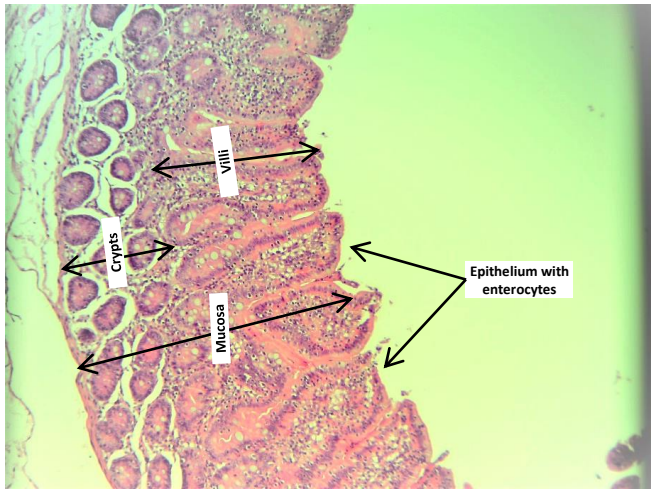
- Test of different fermented seaweed and canola extracts in peripheral blood mononuclear cells (PBMC) assay using 3 different human donors.
- Lipopolysaccharides (LPS) or endotoxins found of the outer membrane of Gram-negative bacteria was used to stimulate an inflammation in matured human PBMCs.
-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



Small intestine - Jejunum

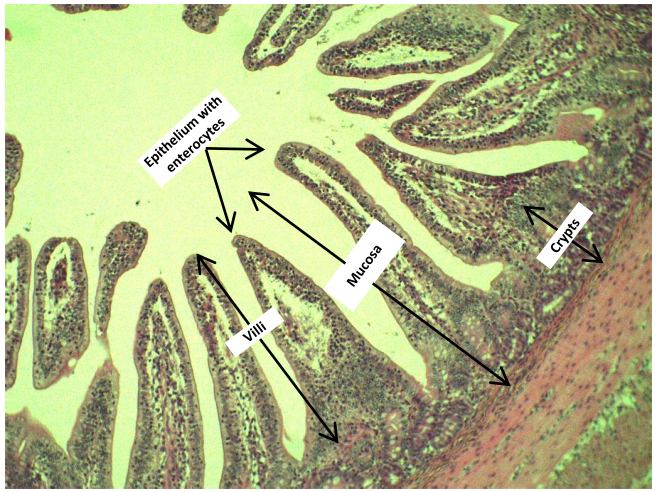
Magnification 10X – slide 1. ***Mucosa including villi and crypts was analyzed under the microscope***

Basal group



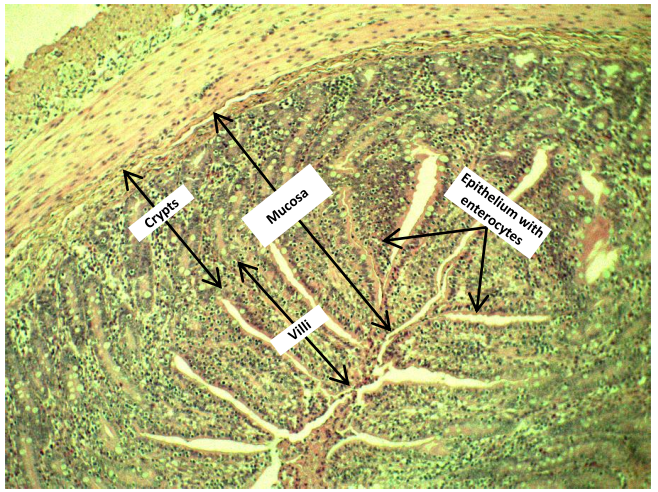
Thinner mucosa in jejunum in comparison with zinc and EP100i groups.
It is a sign of under developed gut

Zinc group



Lose mucosa in jejunum in comparison with basal and EP100i groups.
It is a sign of physiological stress, indicating a gut that is vulnerable to pathogen invasions and inflammation

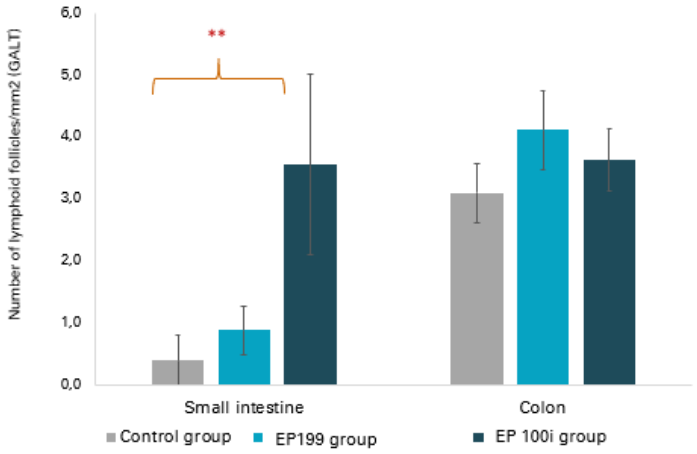
EP100i group



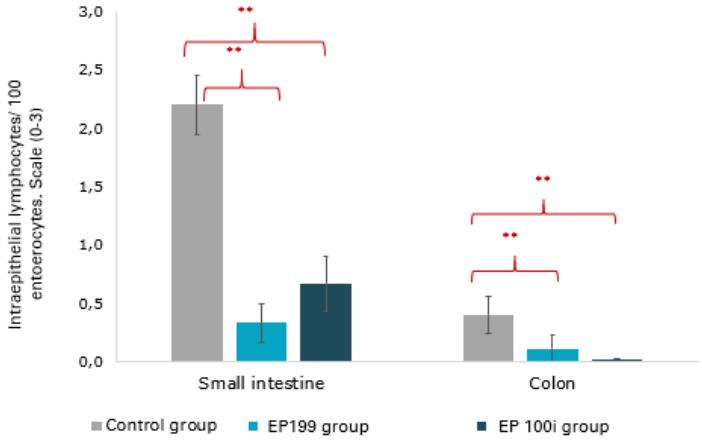
Thigh and packed mucosa in jejunum in comparison with basal and zinc groups.
It is a sign of a well developed gut.

GUT MODULATION

Development of GALT structures



Gut inflammation



Inflammation

0: normal

(no deviation from the norm)

1: low

(slight infiltration, but no damage to the stroma or epithelium)

2: moderate

(signs of weak inflammation with some disruption to epithelial continuity and intestinal blood-barrier)

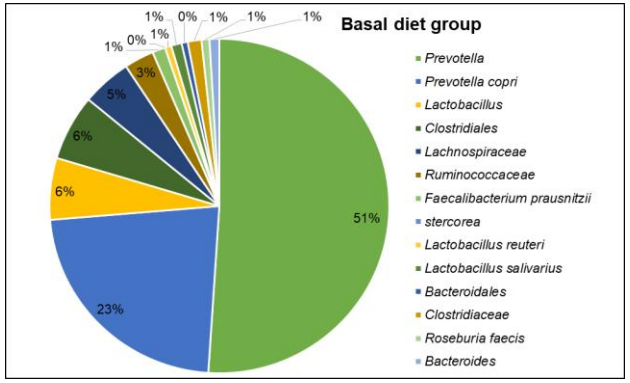
3: severe

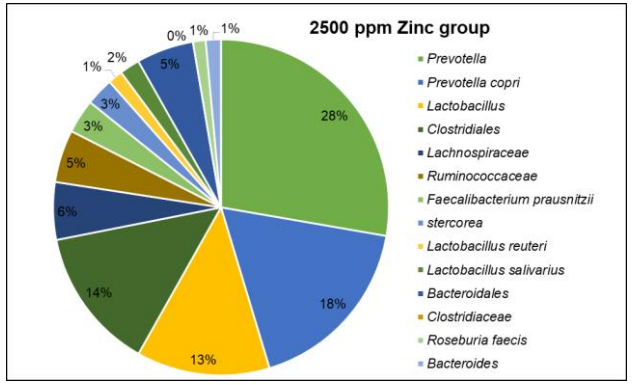
(moderate inflammation with damage to the epithelium and intestinal blood-barrier).

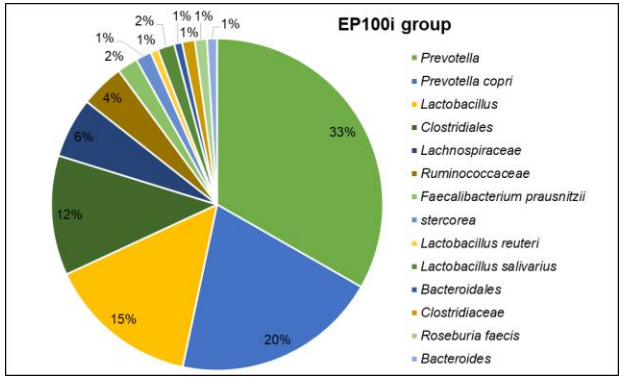
Figures are data from an *in vivo* trial in nursery pigs. Piglets were fed with a commercial basal diet used as positive control and with the addition of fermented seaweed and rapeseed into the basal diet. Blood, gut content and gut tissues were sampled from N=10 piglets after 4 weeks of the dietary regimes. Differences between groups (T-test; $p < 0.05$).

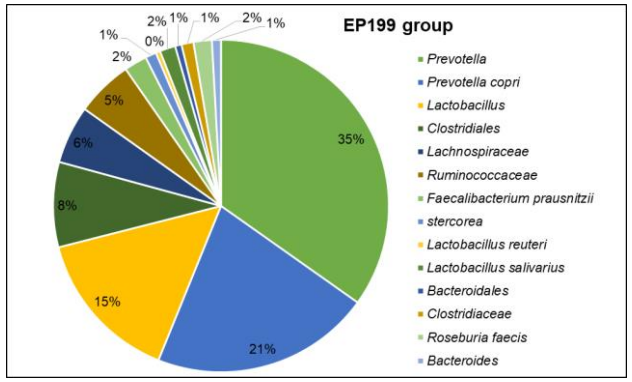
GUT MICROBIOME MODULATION

INCREASED BACTERIAL EVENNESS









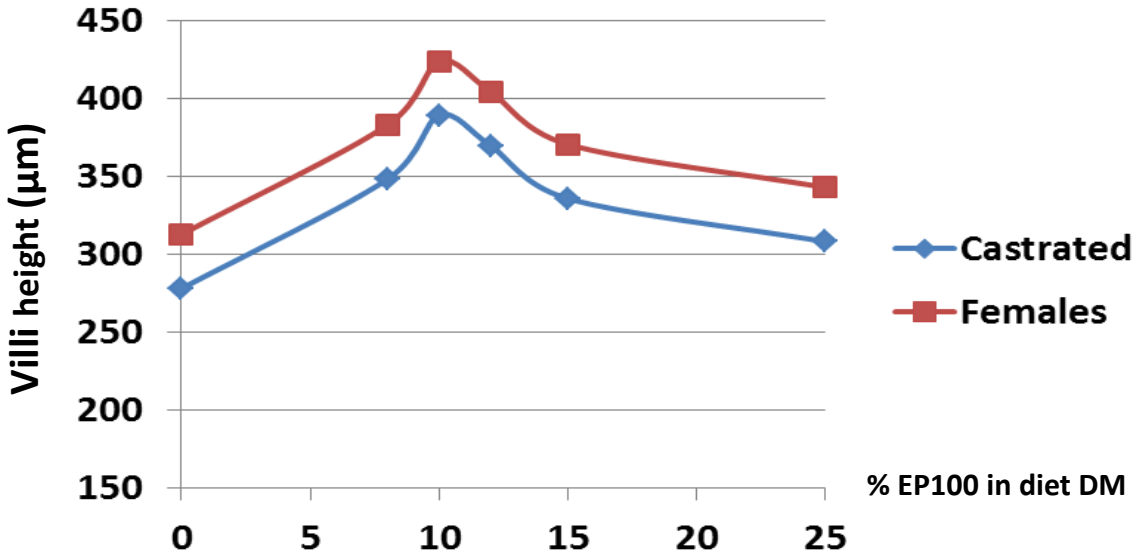
Increased number of recognized beneficial gut bacteria

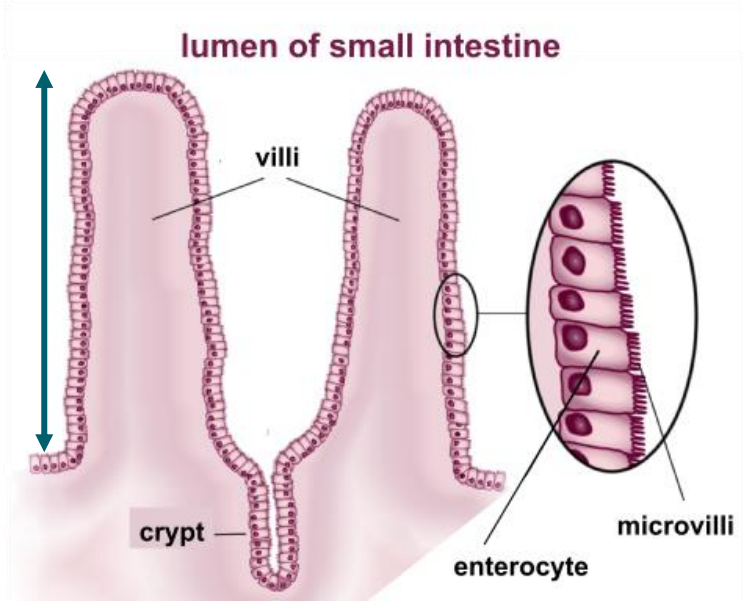
	Basal diet	10 % EP199	10 % EP100i
Lactobacillus spp.	5.9	**14.8**	**14.5**
Lachnospiraceae spp.	4.7	**5.6**	**5.7**
Ruminococcaceae spp.	2.8	**5.4**	**4.2**
Faecalibacterium prausnitzii	1.2	**2.2**	**2.0**
Lactobacillus salivarius	1.0	**1.5**	**1.6**

Even dominance of several bacterial groups corresponds to improvement in animal health and performance as they improve gut-homeostasis

RESULTS

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



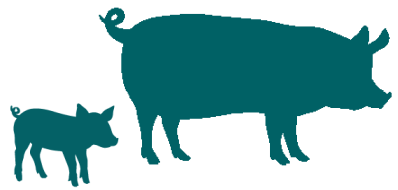


EP100:

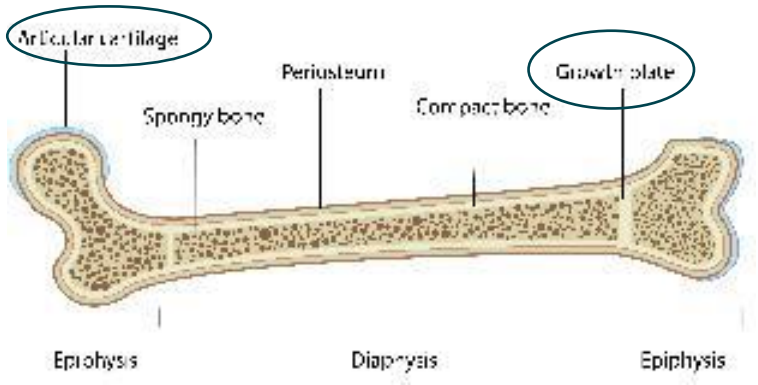
- Increased small intestinal villi height <35% (maximal effect: 10% EP100i in diet)
- Stimulated extensive folding of villi => ↑↑↑ increase in surface area
- Immune cell infiltration and enterocyte morphological changes absent
  - Also in colon

MATERNAL FEEDING OF EP199

Improved bone structure and mechanical properties of offspring[1]



IN VIVO



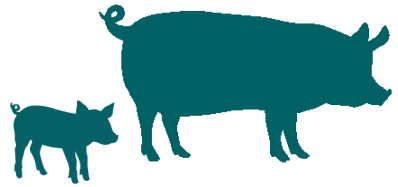
The fermentation process of EP-products makes nutrients more available. Through a better absorption of minerals like calcium and phosphorous during gestation, the piglets development of bone density, growth plate and articular cartilage are improved.

Table 4: Bone properties of samples from knees of 77 days old piglet.

Basal geometric properties	Unit	Group control	DFRSM	P-value
Bone length	mm	143	156	0.006
Bone weight	g	149	156	0.036
Growth plate cartilage	Unit	Group control	DFRSM	P-value
Total cartilage thickness	µm	1369	2100	< 0.001
Articular cartilage	Unit	Group control	DFRSM	P-value
Total cartilage thickness	µm	2067	3640	< 0.001
Structural properties	Unit	Group control	DFRSM	P-value
Yield load	kN	1.64	1.88	0.012
Ultimate load	kN	1.91	2.26	0.038
Stiffness	N/mm	830	1067	0.021
Elastic energy	J	1.74	1.81	0.022
Work to fracture	J	2.87	3.6	0.032
Bending moment	Nm	23.4	29.9	0.002

Source: 2019, Trial: ”A fermented rapeseed meal additive: Effects on Production Performance, nutrient digestibility, colostrum immunoglobulin content and microbial flora in sows. https://www.ncbi.nlm.nih.gov/pubmed/31890914

MATERNAL FEEDING OF EP199



IN VIVO

Table 5: Mineral content of blood in sows and piglets.

		Gilts			Sows		
Minerals	Units	Control	FRS	P	Control	FRM	P
LATE PREGNANCY n=6							
Phosphorus	mmol l^-1	1.42	1.70	P≤0.05	1.38	1.88	P≤0.01
Copper	µmol l^-1	26.19	32.47	P≤0.05	23.37	28.09	P≤0.01
Iron	µmol l^-1	15.38	21.06	P≤0.05	13.88	19.18	P≤0.01
LATE LACTATION n=6							
Phosphorus	mmol l^-1	1.87	2.31	P≤0.05	1.76	2.24	P≤0.01
Magnesium	mmol l^-1	1.04	1.27	P≤0.05	1.03	1.28	P≤0.01
Copper	µmol l^-1	12.66	16.15	P≤0.05	14.65	18.31	P≤0.01
Iron	µmol l^-1	18.78	25.43	P≤0.05	23.54	29.17	P≤0.01
Zinc	µmol l^-1				14.18	17.78	P≤0.01
OFFSPRING n=12							
Phosphorus	mmol l^-1	1.97	2.42	P≤0.05			
Calcium	µmol l^-1	2.58	3.12	P≤0.05	2.53	3.04	P≤0.01
Copper	µmol l^-1	20.66	26.91	P≤0.05			
Zinc	µmol l^-1	7.87	10.29	P≤0.05			
Iron	µmol l^-1	27.81	35.33	P≤0.05	28.02	37.51	P≤0.01

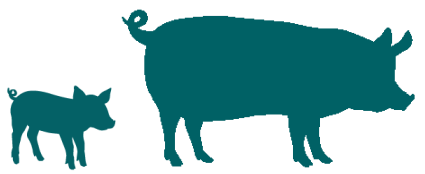
Blank spaces mean, that there is no statistical siginificant difference

Sows: For both gilts and sows the content of phosphorous, copper and iron is significantly higher than in the control group during pregnancy and lactation.

Piglets: Offspring from primiparous sows have a higher content of all minerals in their blood. For piglets from multiparous sows calcium and iron content is improved.

Source: 2019, Trial: ”A fermented rapeseed meal additive: Effects on Production Performance, nutrient digestibility, colostrum immunoglobulin content and microbial flora in sows. https://www.ncbi.nlm.nih.gov/pubmed/31890914

MATERNAL FEEDING OF EP199



IN VIVO

Table 6: Blood analysis in sows and piglets.

		Gilts		
Blood	Units	Control	FRS	P
LATE PREGNANCY n=6				
Haematocrit	%	38.77	41.12	P≤0.05
Haemoglobin	mmol l^-1	7.01	7.88	P≤0.05
Red blood cells	10^12	6.91	8.01	P≤0.05
LATE LACTATION n=6				
Haematocrit	%	36.23	39.17	P≤0.05
Haemoglobin	mmol l^-1	4.56	5.98	P≤0.05
Red blood cells	10^12	6.23	6.96	P≤0.05
OFFSPRING				
Haematocrit	mmol l-	36.24	39.96	P≤0.05
Haemoglobin	µmol l-1	6.92	7.89	P≤0.05
Red blood cells	µmol l-1	6.03	6.76	P≤0.05

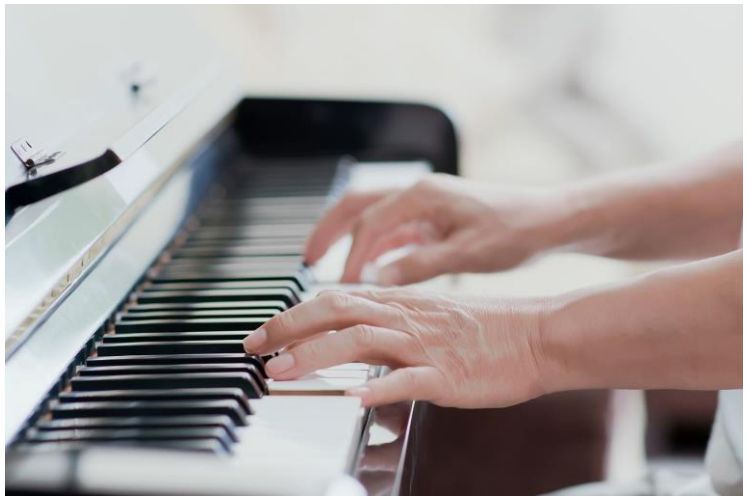
Sows: Red blood cell parameters are increased for both primiparous sows and their piglets meaning more oxygen can be carried to tissues.

Source: 2019, Trial: "A fermented rapeseed meal additive: Effects on Production Performance, nutrient digestibility, colostrum immunoglobulin content and microbial flora in sows. https://www.ncbi.nlm.nih.gov/pubmed/31890914

HOW EP-PRODUCTS MODULATE THE GUT AND ADJUST THE BODY

Try to improve overall health through a single key

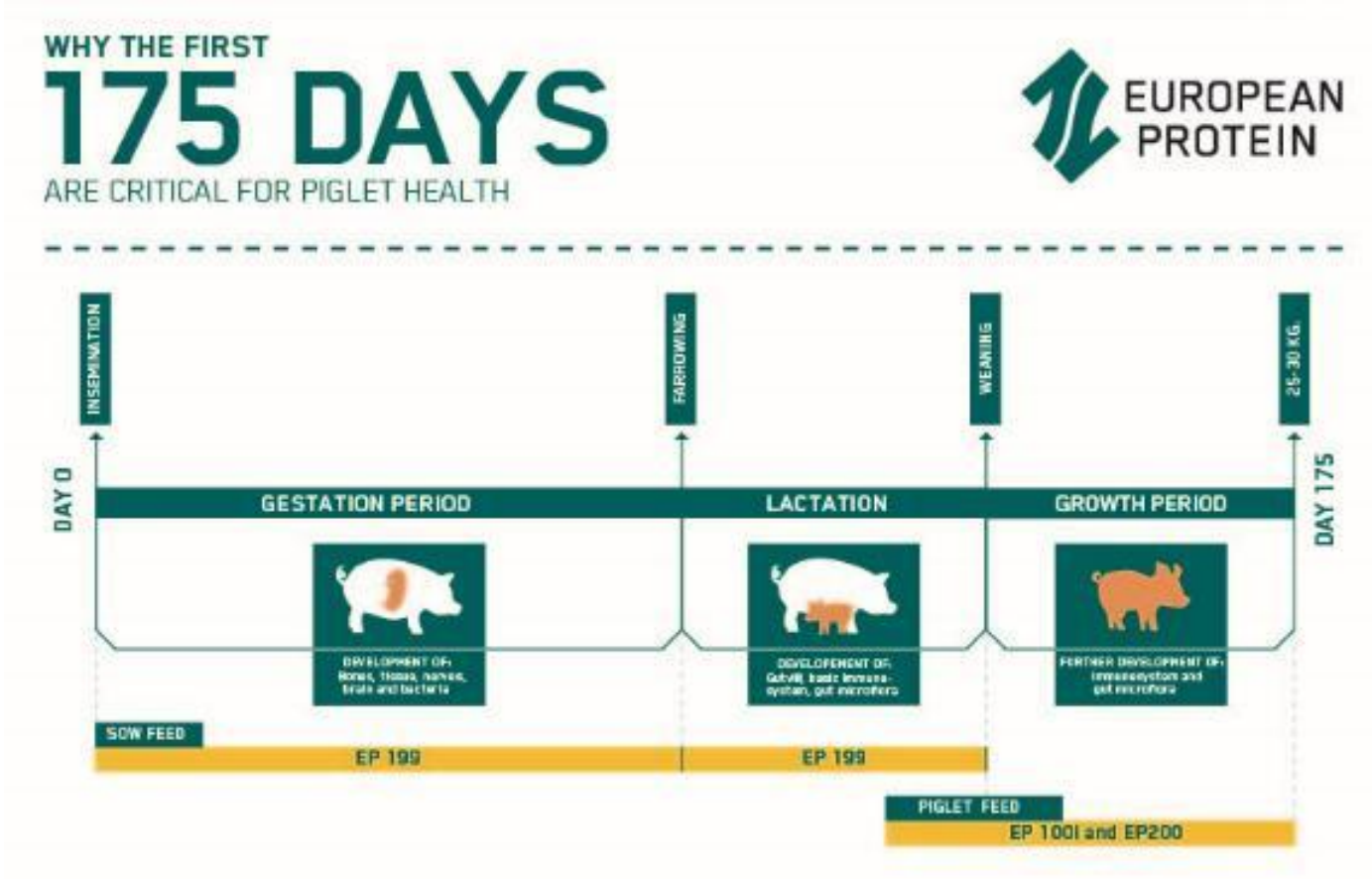
VS



Improve health by using chords to create symbiotic effects and harmony

FEEDING CONCEPT

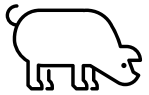
Direct and indirect feeding to improve offspring



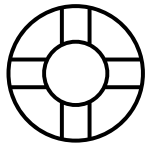
Research shows that up to 80 % of the piglet's DNA is founded during gestation and lactation.

FARM RESULTS – WHAT CHANGES?

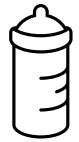
Through maternal feeding, the production parameters change



1-3 piglets more weaned per sow.



Lower mortality in both sows and piglets.



Increased milking ability means more kilos produced per litter.

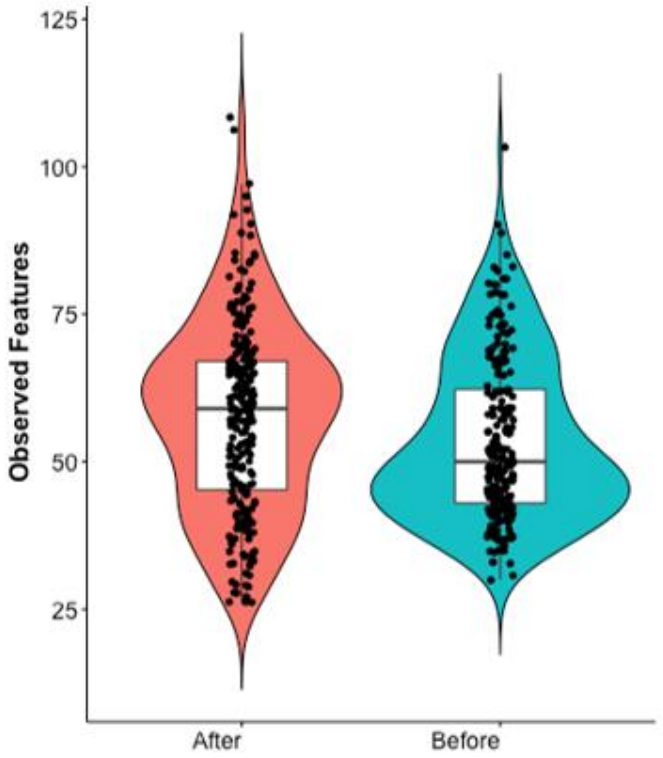
Reduction in the sow feed by 10% with same or better performance.

Reduction of **pathogens** means less handling and treatment of the animals.

Healthy animals **save time** and manual labour.

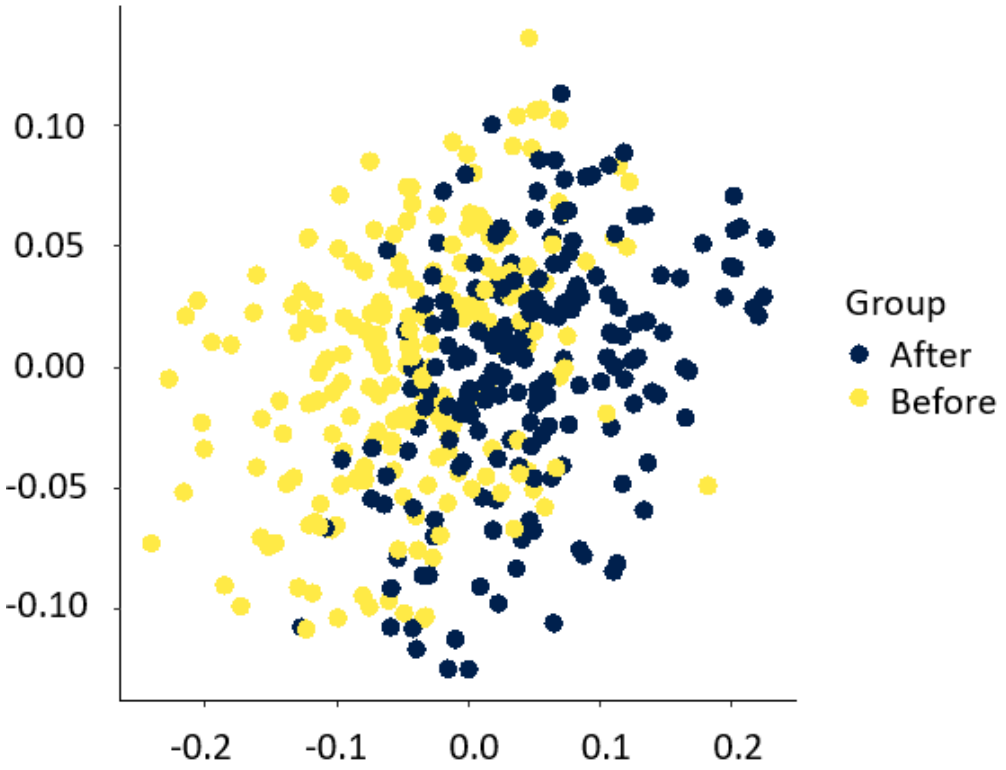
With a healthy herd, you can phase out medicinal **zinc (ZnO) and lower the use of antibiotics**

comparison of 19 farms before/after (n=608 samples)



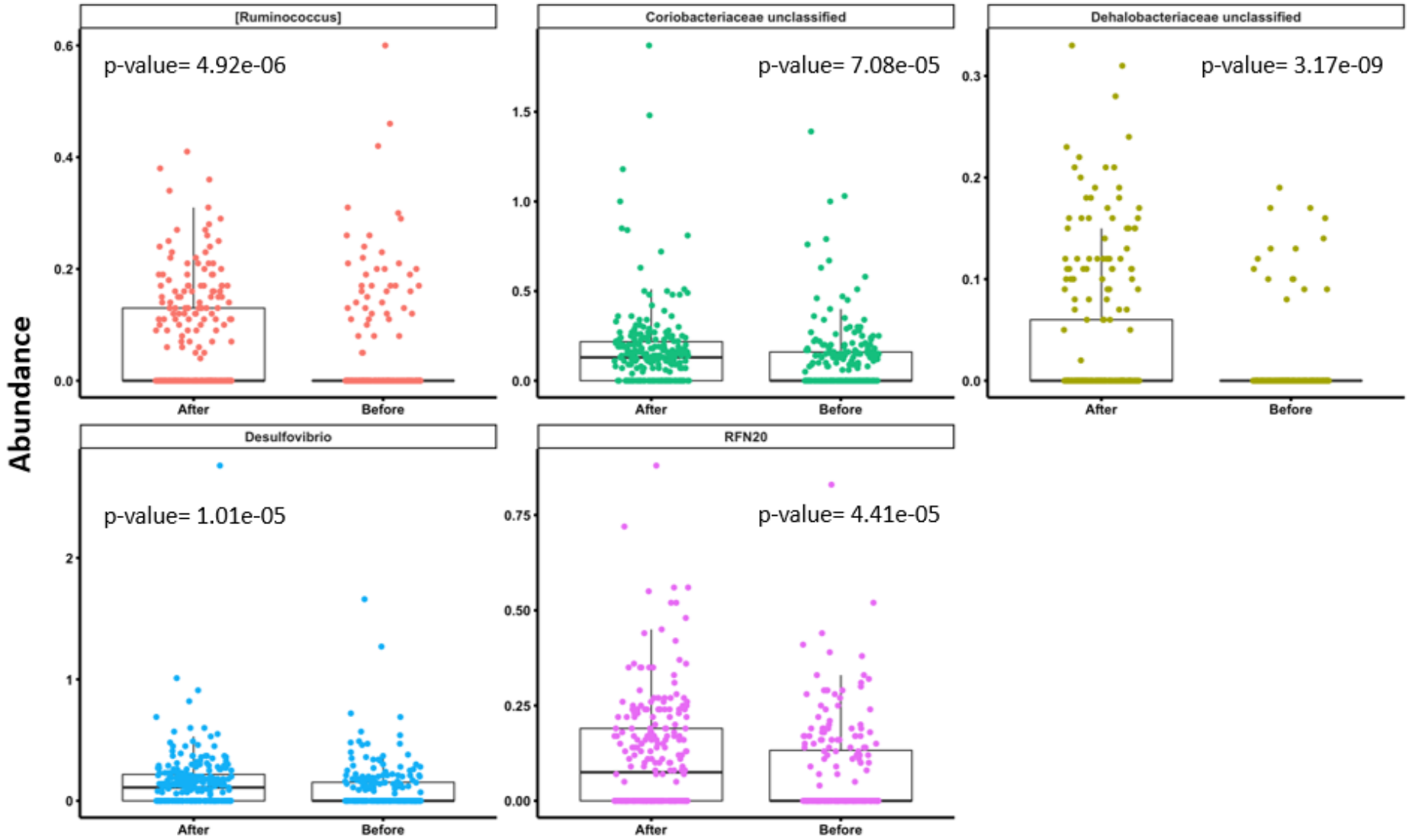
Pairwise Wilcoxon Rank Sum Test

p-value: 0.0037



p-value: 0.001

5 bacteria families (before/after)



OUR CHALLENGES

- Market acceptance of novel functional protein sources
- Market entry - local proof of concept
- Veterinarians and advisors
- Education: What is the microbiome and why is it so important?

Tomorrow's solutions ...today

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https://www.macrocascade.eu/

EUROPEAN PROTEIN

Tomorrow's solutions... today