



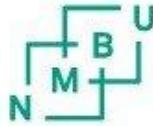
Gut health and microbiota in post-smolt Atlantic salmon fed larvae meal from black soldier fly

Yanxian Li¹, Leonardo Bruni³, Alexander Jaramillo-Torres¹, Trond M. Kortner¹,
Elvis M. Chikwati¹, Ikram Belghit², Erik-Jan Lock², Åshild Krogdahl¹

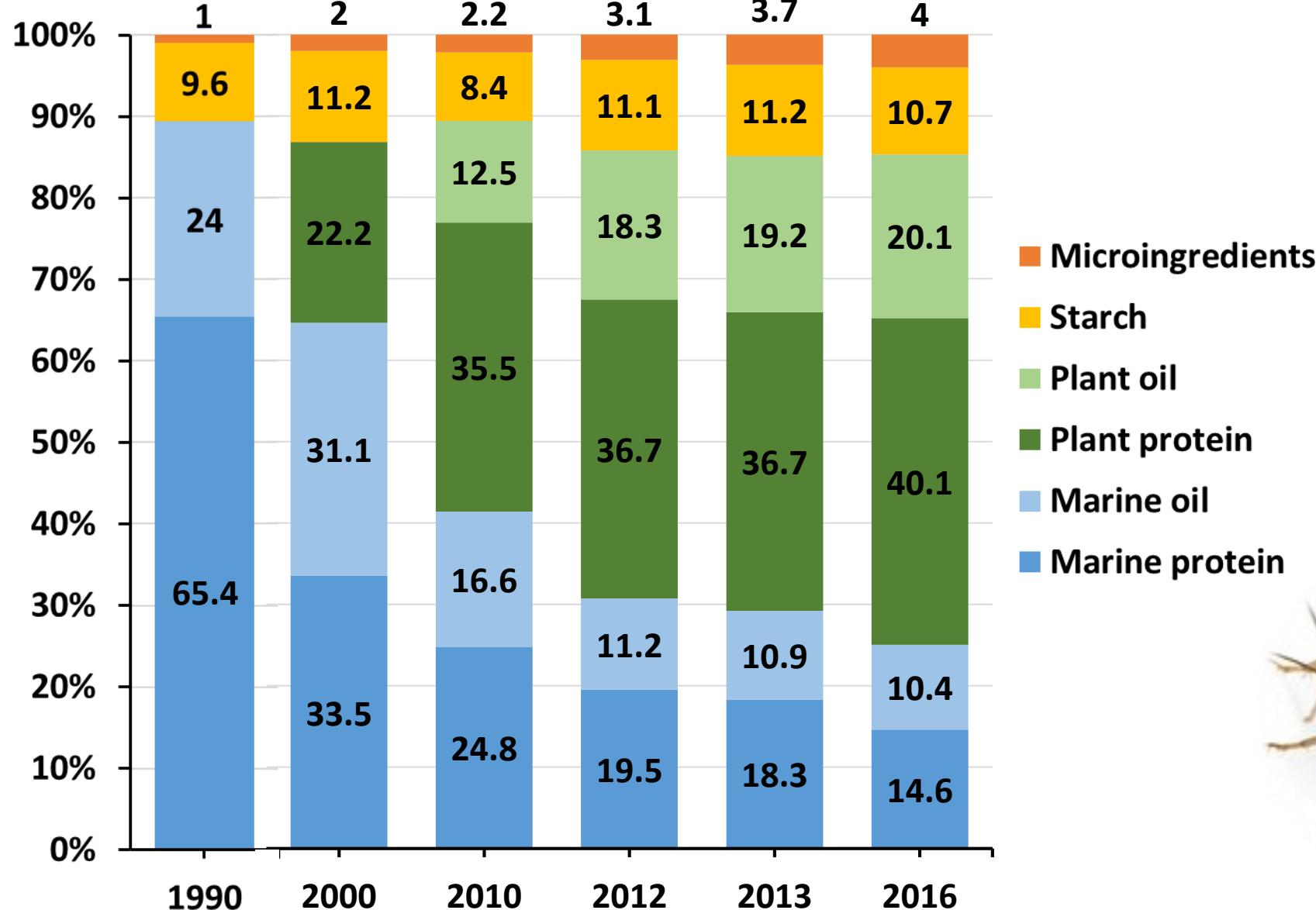
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Norwegian University of Life Sciences (NMBU),
Oslo, Norway

9 Oct. 2019

Norwegian salmon farming in search of alternative feed ingredients



Nutrient sources in Norwegian salmon farming 1990-2016



Insects



Black soldier fly, *Hermetia illucens*



- Proximate composition (DM basis):

- ✓ Protein: 36%
- ✓ Lipids: 18%
- ✓ Ash: 9.3

- Excellent amino acid profile
- Fatty acids: diet dependent

Aquaculture Nutrition



Aquaculture Nutrition 2015

doi: 10.1111/anu.12343

Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (*Salmo salar*) postsmolt

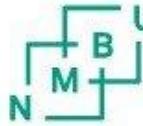
E.R. LOCK¹, T. ARSIWALLA² & R. WAAGBØ¹

¹ National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway; ² Protix Biosystems BV, Amsterdam, The Netherlands

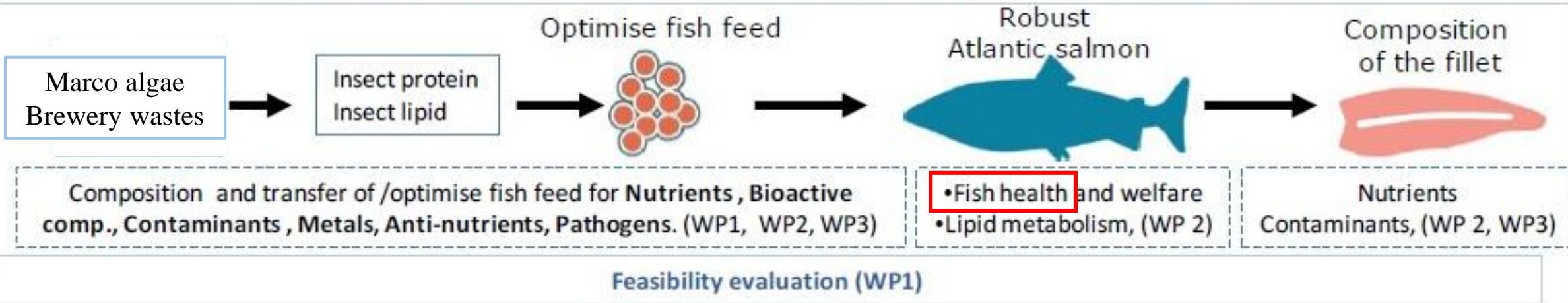
Source:

<http://edge.rit.edu/edge/P17422/public/Photo%20Gallery/Lifecycle%20of%20BSFL.jpg>

Aquafly: Insects as natural feed ingredients for sustainable salmon farming

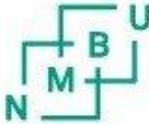


Sustainability evaluation (social, environmental and economic) and ethical considerations (WP4)



Gut health and microbiota

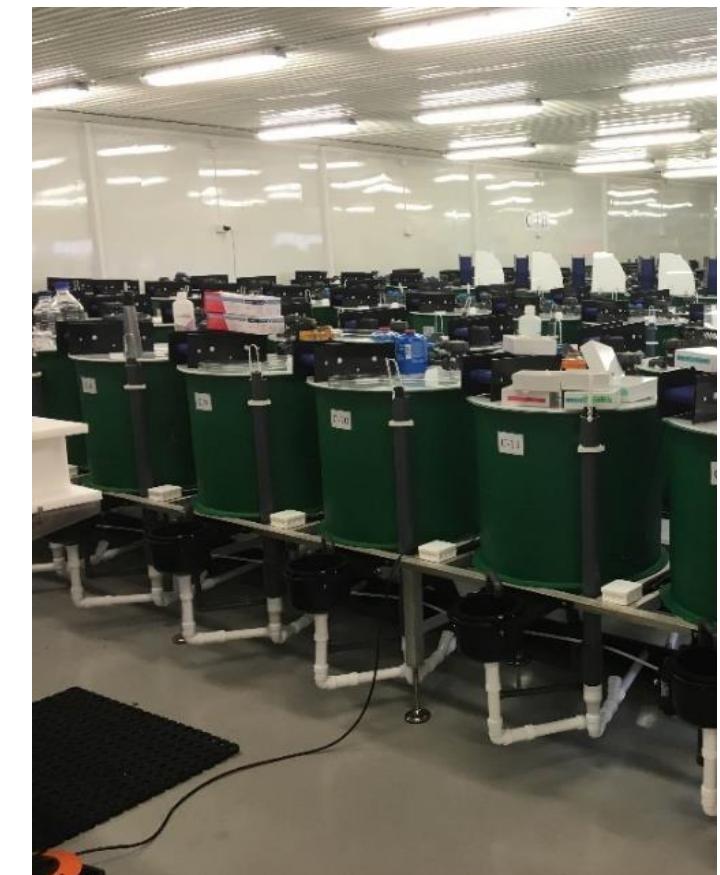
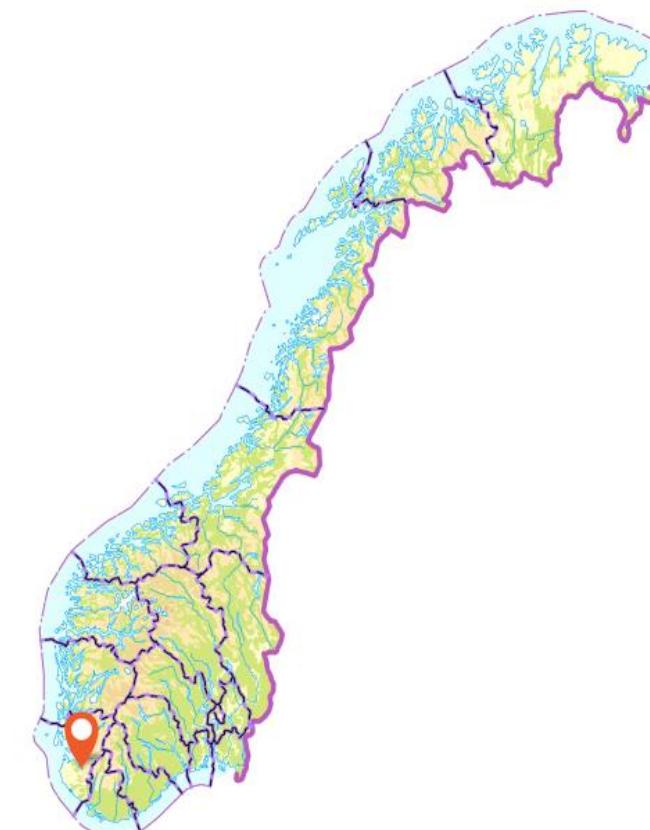
Freshwater feeding trial



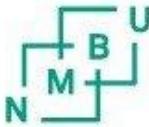
Diet formulation

Ingredients (% dry matter)	REF	IM
Fish meal LT94	35	6
Black soldier fly larvae meal	0	60
Soy protein concentrate	29.6	5
Wheat gluten	14.3	14.4
Fish oil	4.6	6.9
Rapeseed oil	12	4.8
Vitamin & Mineral premix	0.3	0.3
Additives	4.2	2.6
Proximate analysis		
Dry matter	93.6	95.5
Crude lipids	18	22
Crude proteins	47	44
Glycogen	10.5	11.6
Ash	7.9	6.7
TBARS	6.9	16.9

- Initial body weight, 49 ± 1.5 g
- 4 tanks/diet, 100 fish/tank
- Continuous feeding, 24-h illumination
- Dirdal, Rogaland, Norway
- 8 weeks



Seawater feeding trial



Diet formulation:

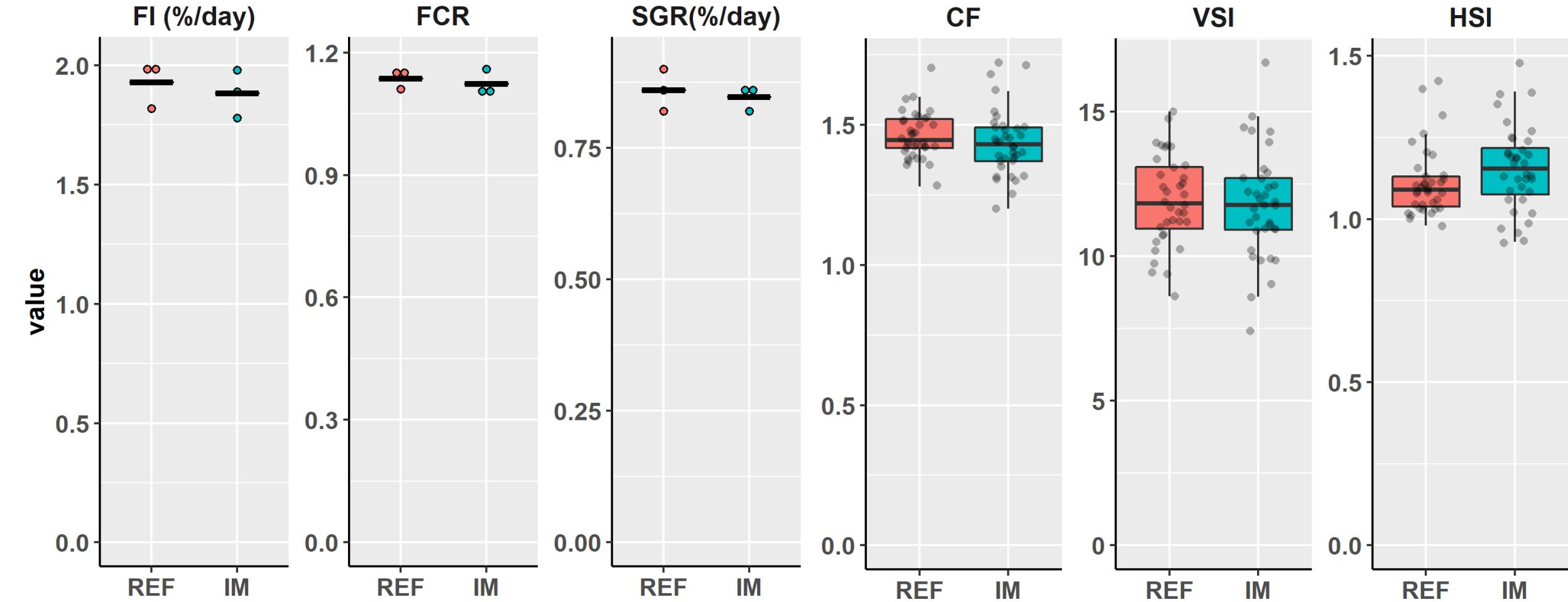
Ingredients (% dry matter)	REF	IM
Fishmeal LT94	10	0
Black soldier fly larva meal	0	14.75
Soy protein concentrate	25	25
Corn gluten meal	7.5	7.5
Wheat gluten meal	3.35	6.88
Pea protein concentrate 55	8.8	2.84
Fish oil	10.18	14.76
Rapeseed oil	20.95	14.73
Binder	12.32	11.24
Additives	1.89	2.29
Yttrium	1.0	1.0
Proximate analysis		
Dry matter (%)	93	95
Crude Protein (%)	38	39
Crude Lipid (%)	29	29
Ash (%)	4.6	4.5
Carbohydrates (%)	11.6	11.4
Gross energy (MJ/kg)	24.6	25.0
TBARS (nmol/g)	3.0	4.9

- Initial body weight, ~1.4 Kg
- 3 net pens/diet, 90 fish/net pen
- Satiation feeding, twice daily
- Inndyr, Nordland, Norway
- 18 weeks

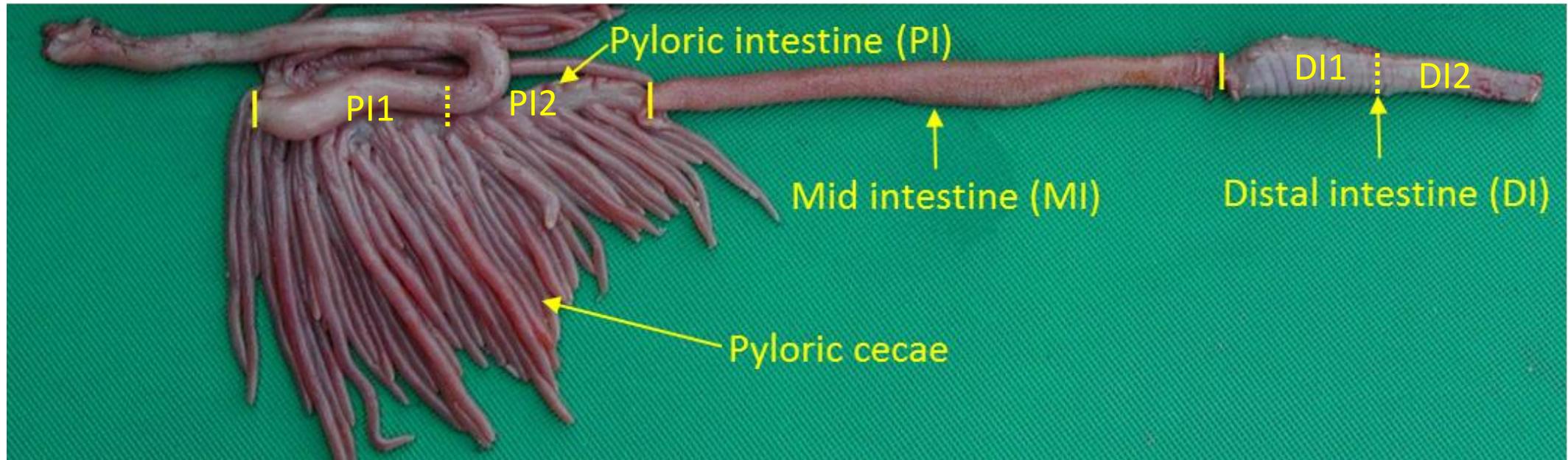
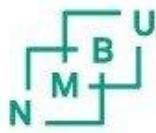


Growth performance and feed utilization

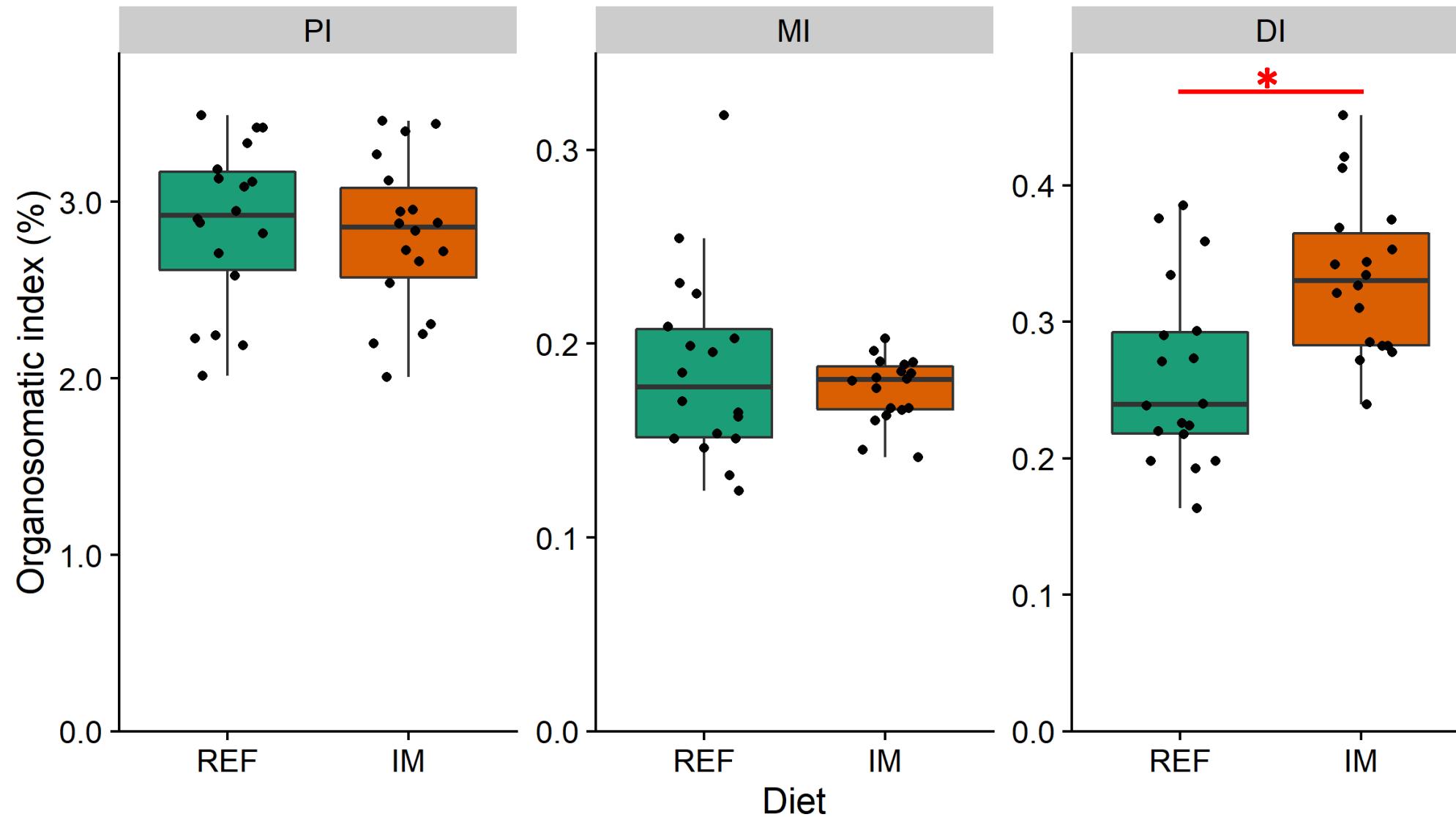
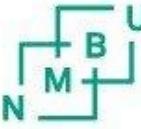
N M B U



Gastrointestinal tract of Atlantic salmon



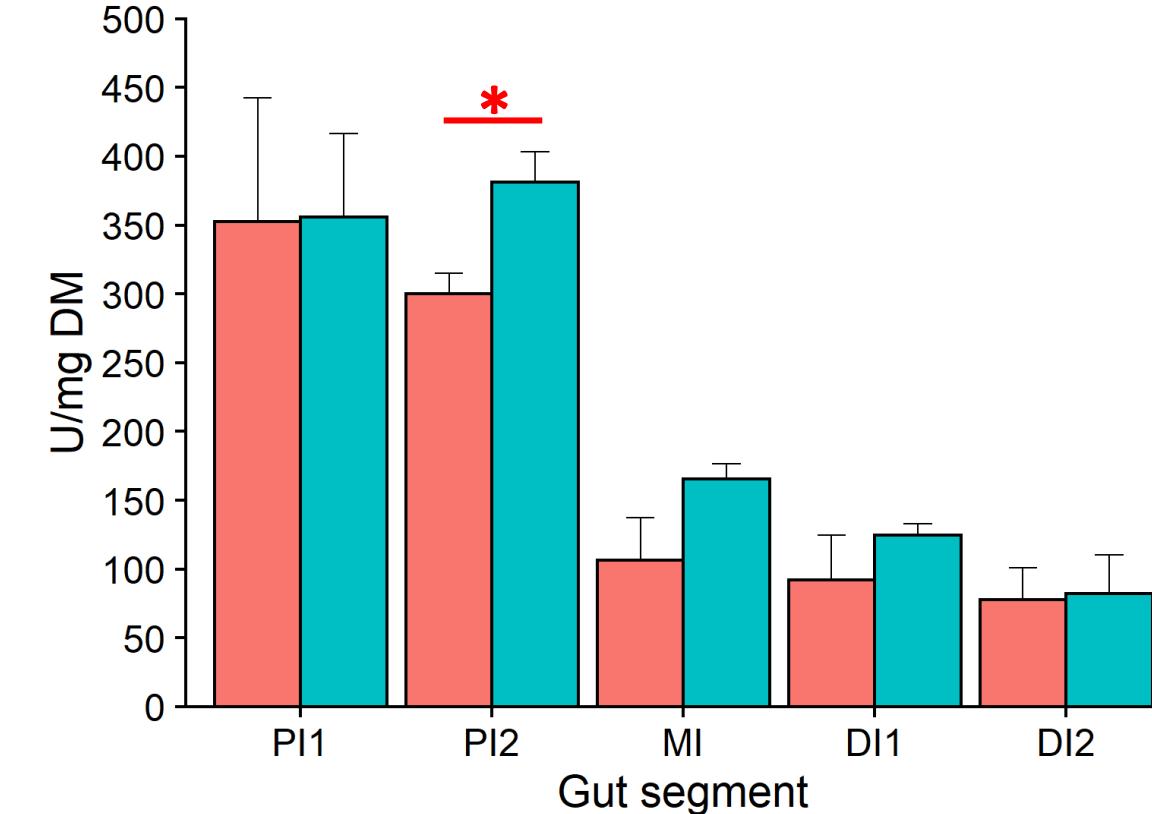
Organosomatic indices



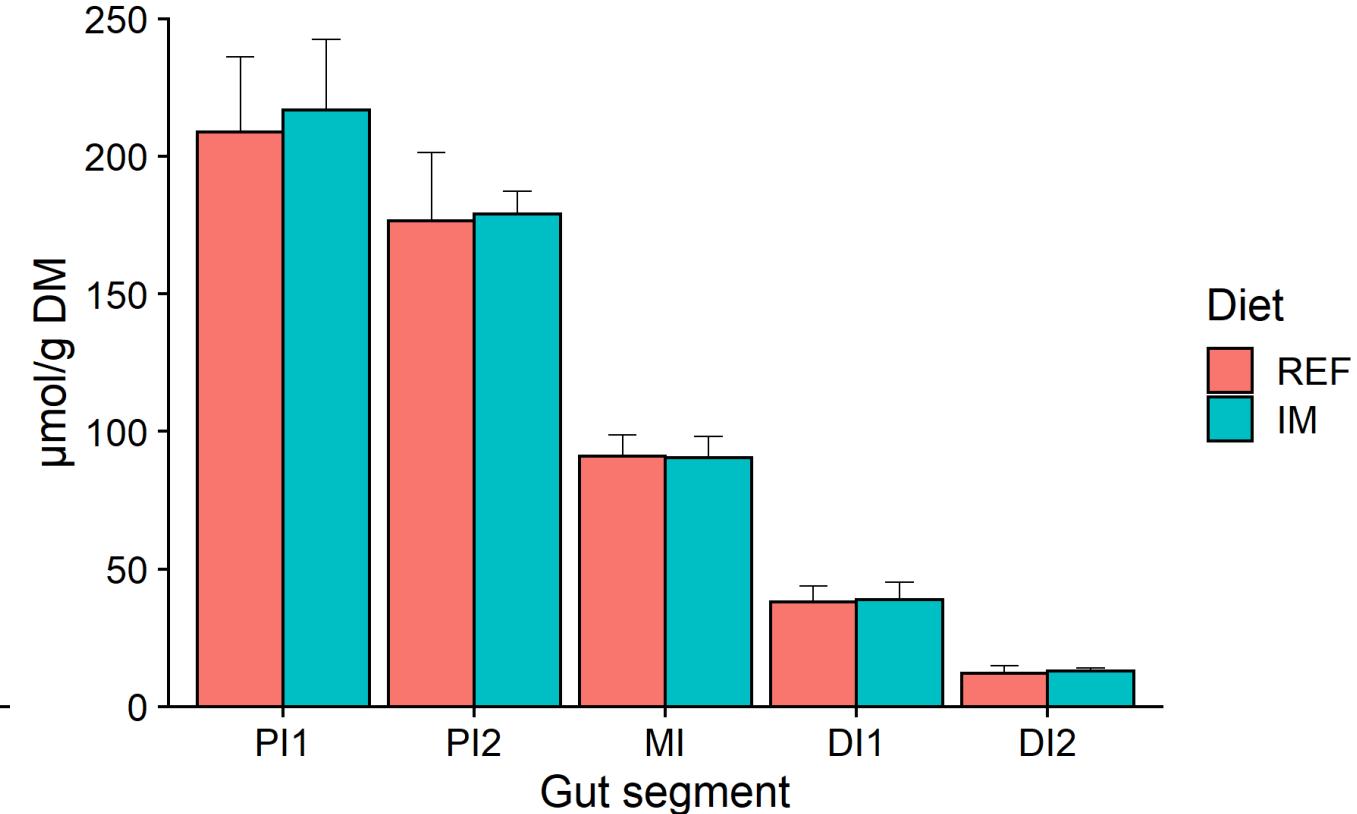
Digestive functions: trypsin activity and bile salts in chyme

B
M
U
N

Trypsin activity

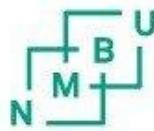


Bile salts concentration

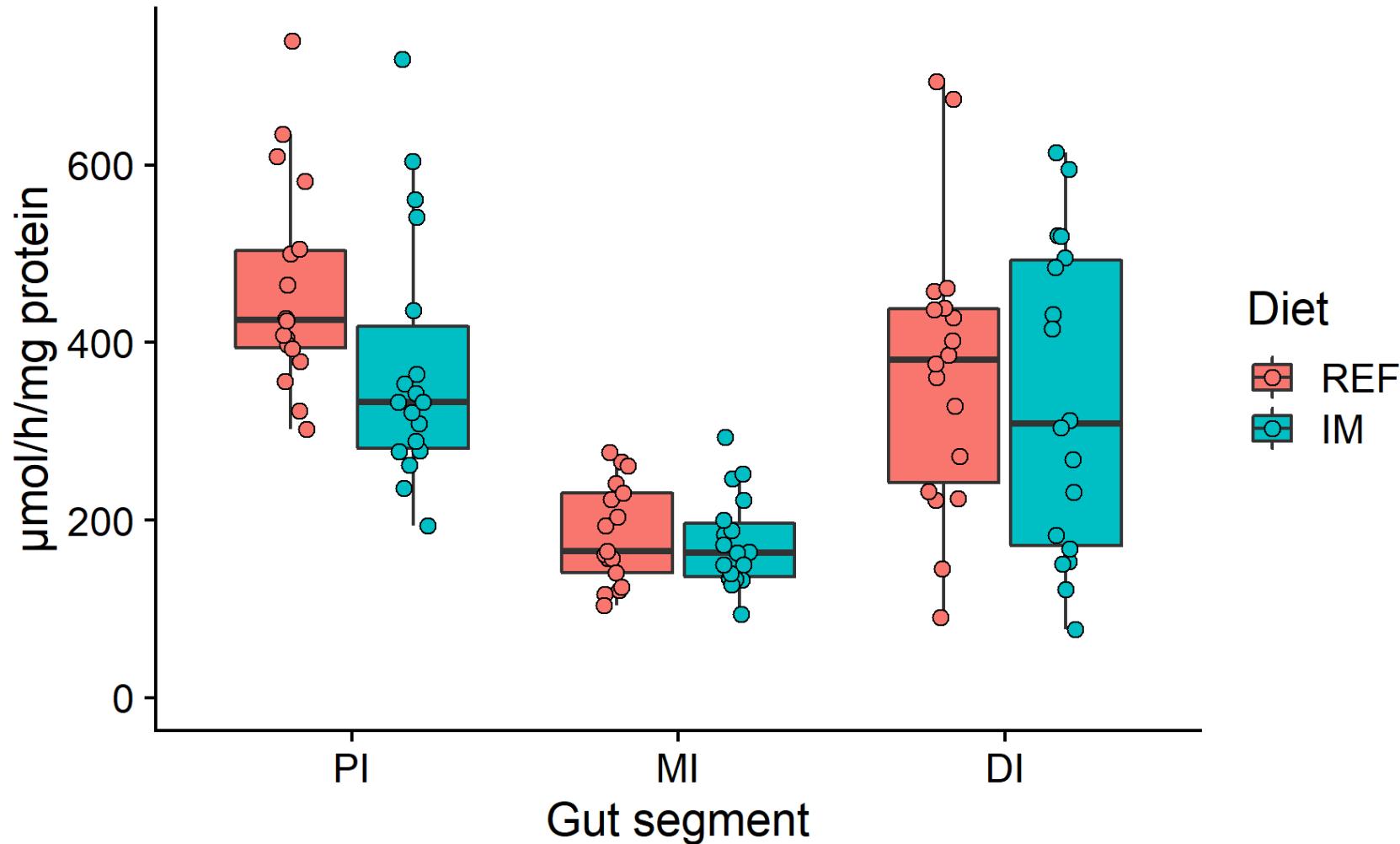


Diet
REF
IM

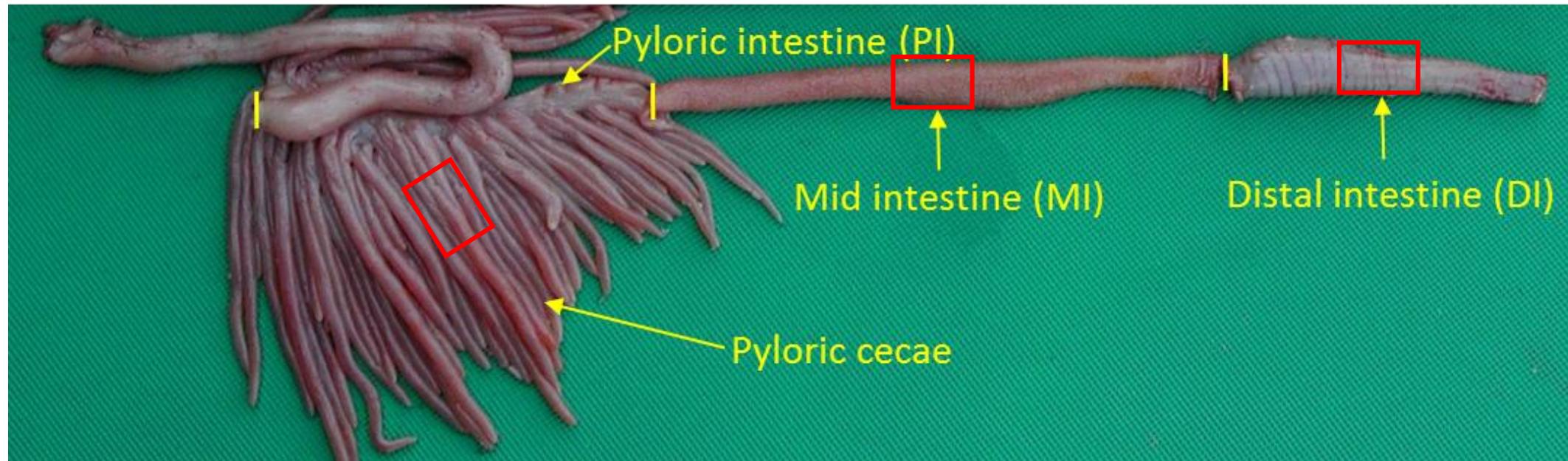
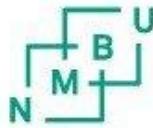
Digestive functions: brush border enzyme activity



Leucine aminopeptidase activity



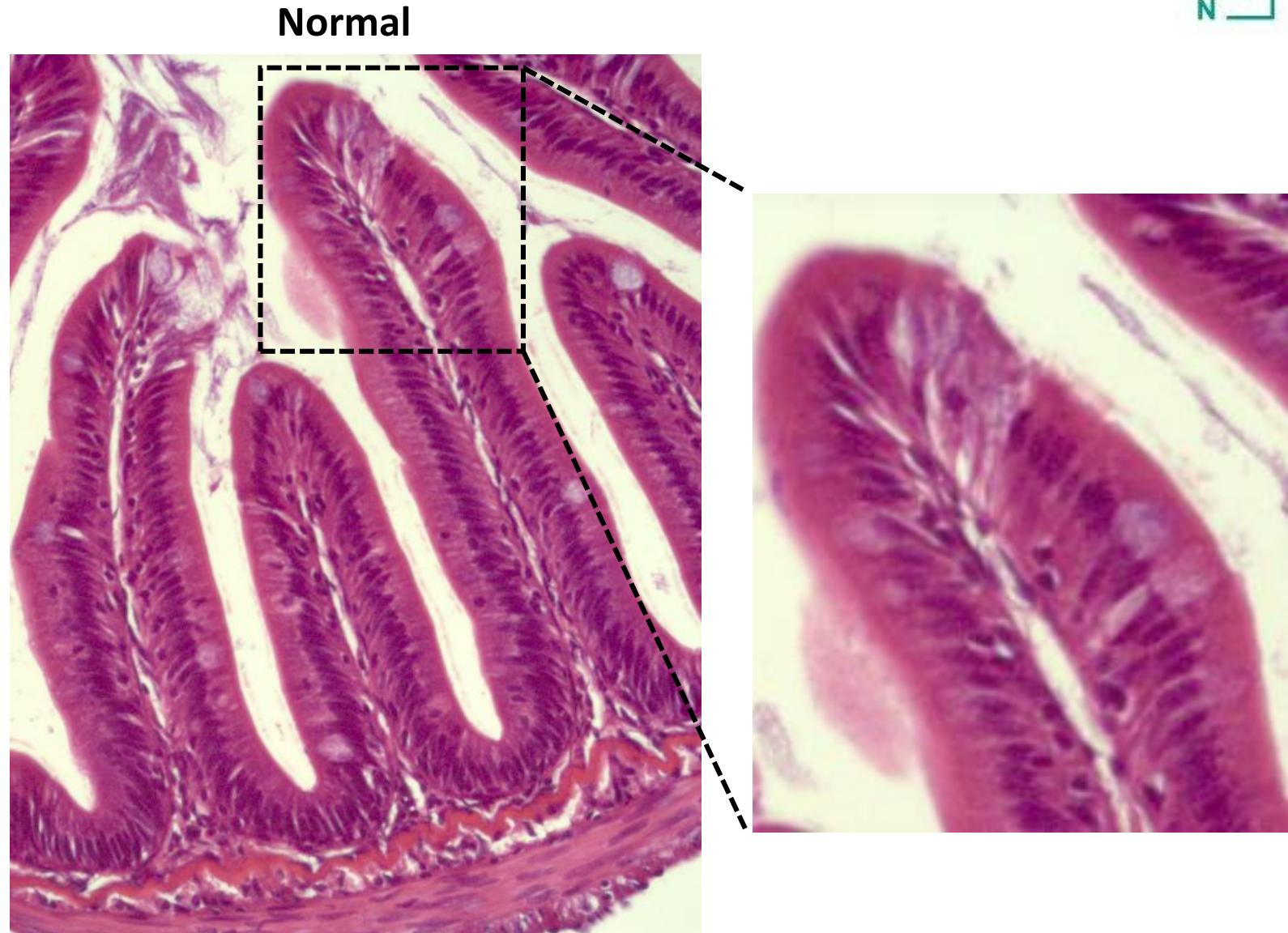
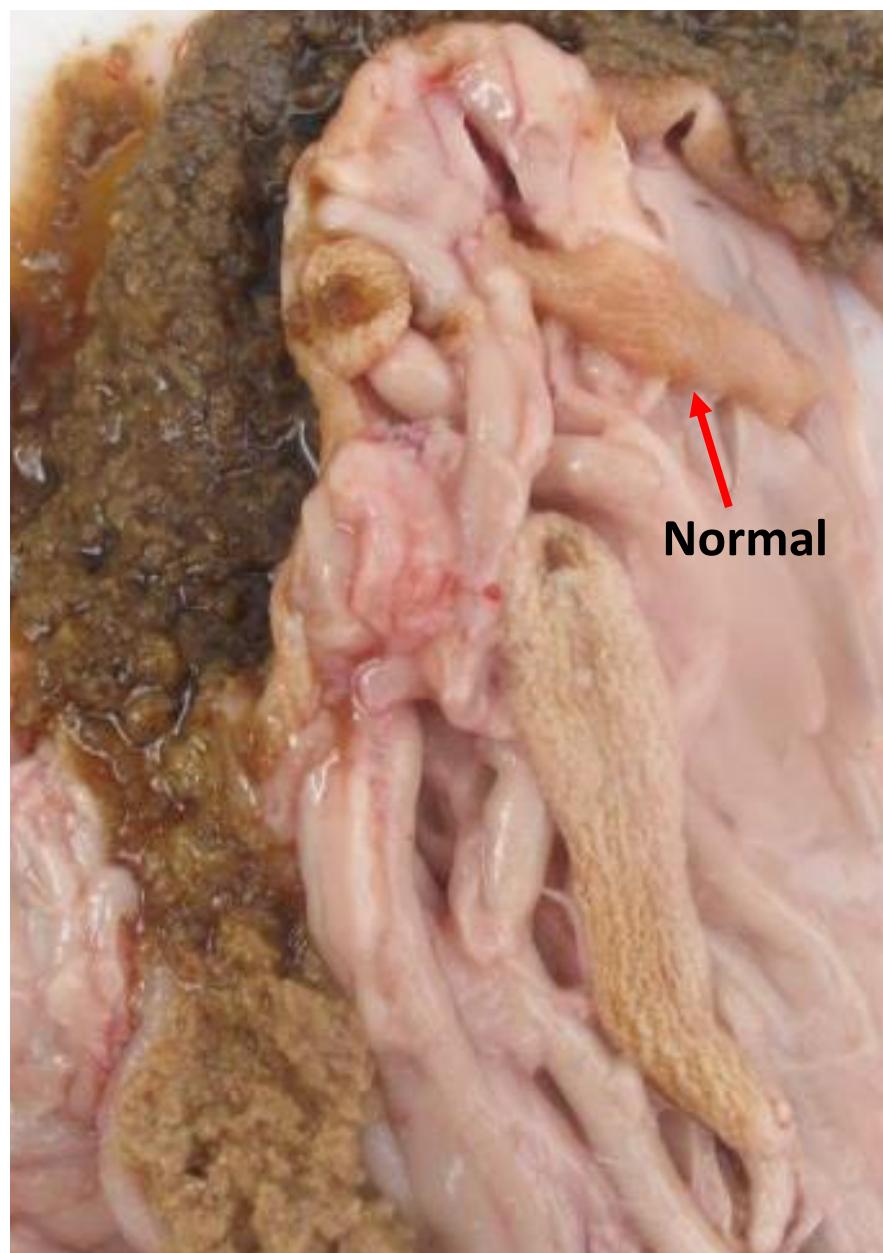
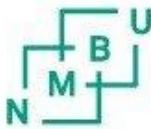
Histological examination



👉 Sub-optimal health conditions:

1. Enterocyte hypervacuolization
2. Inflammation

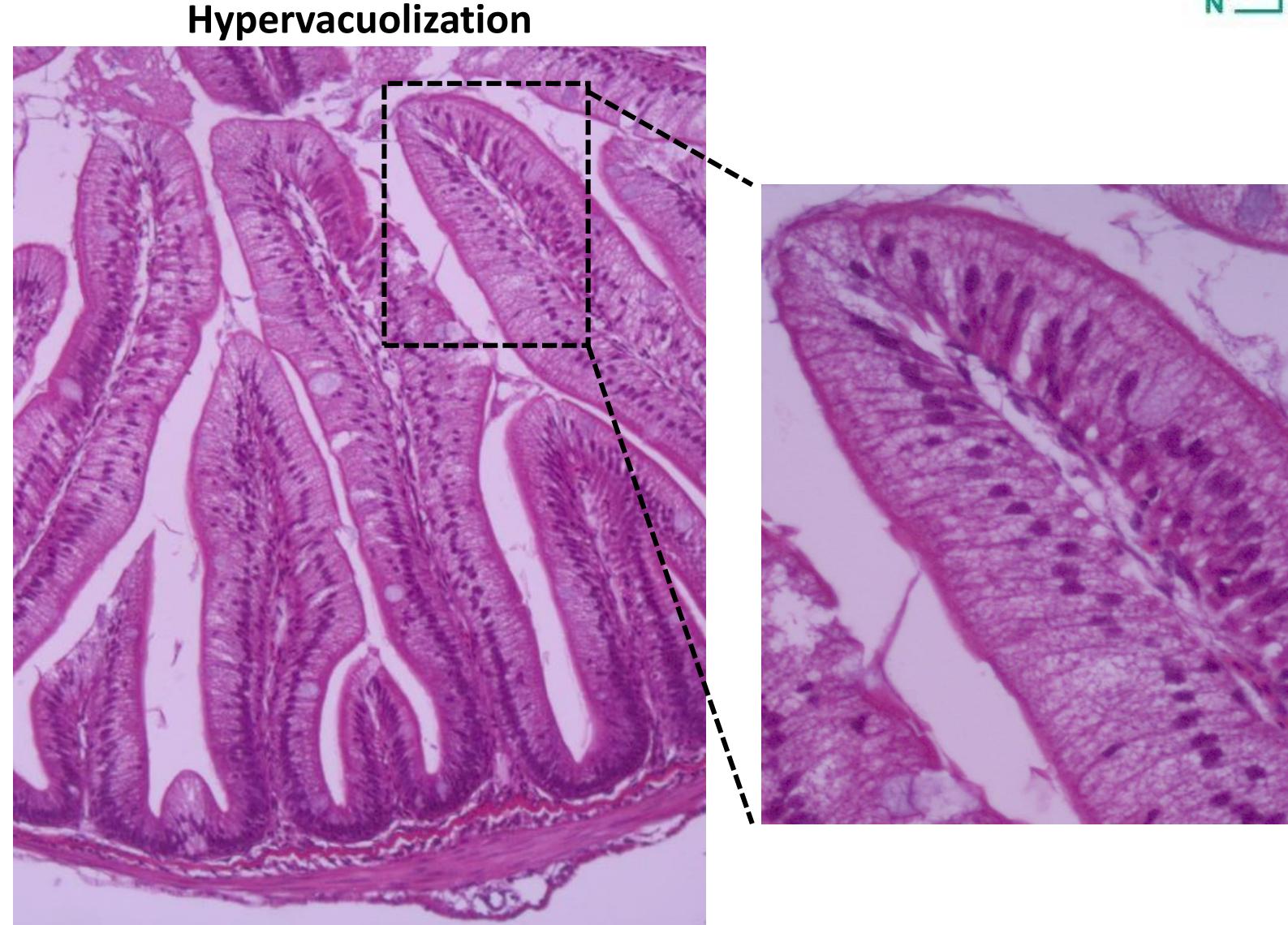
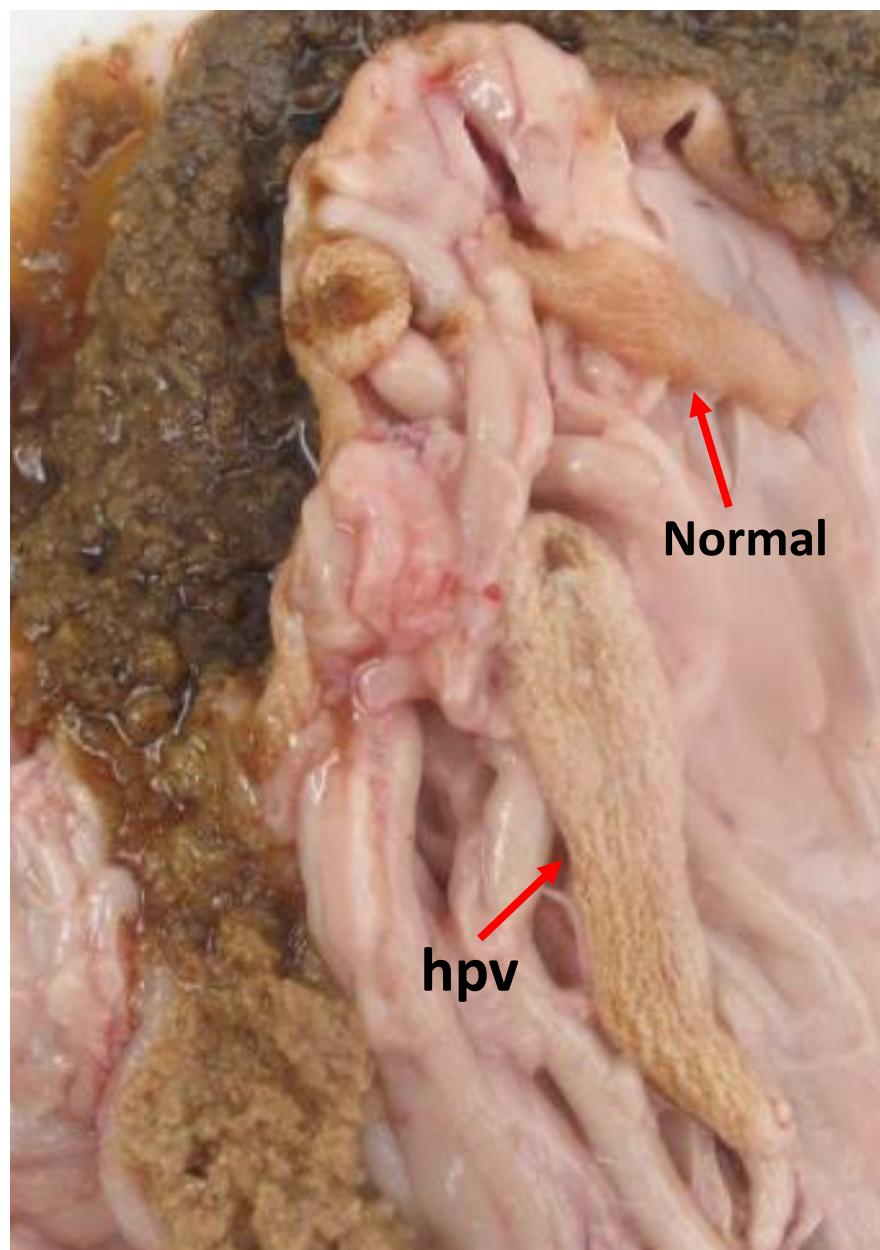
Histological examination: hypervacuolization



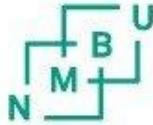
Source: Hanche-Olsen et al., 2013

Histological examination: hypervacuolization

B
M
U
N



Histological examination : hypervacuolization



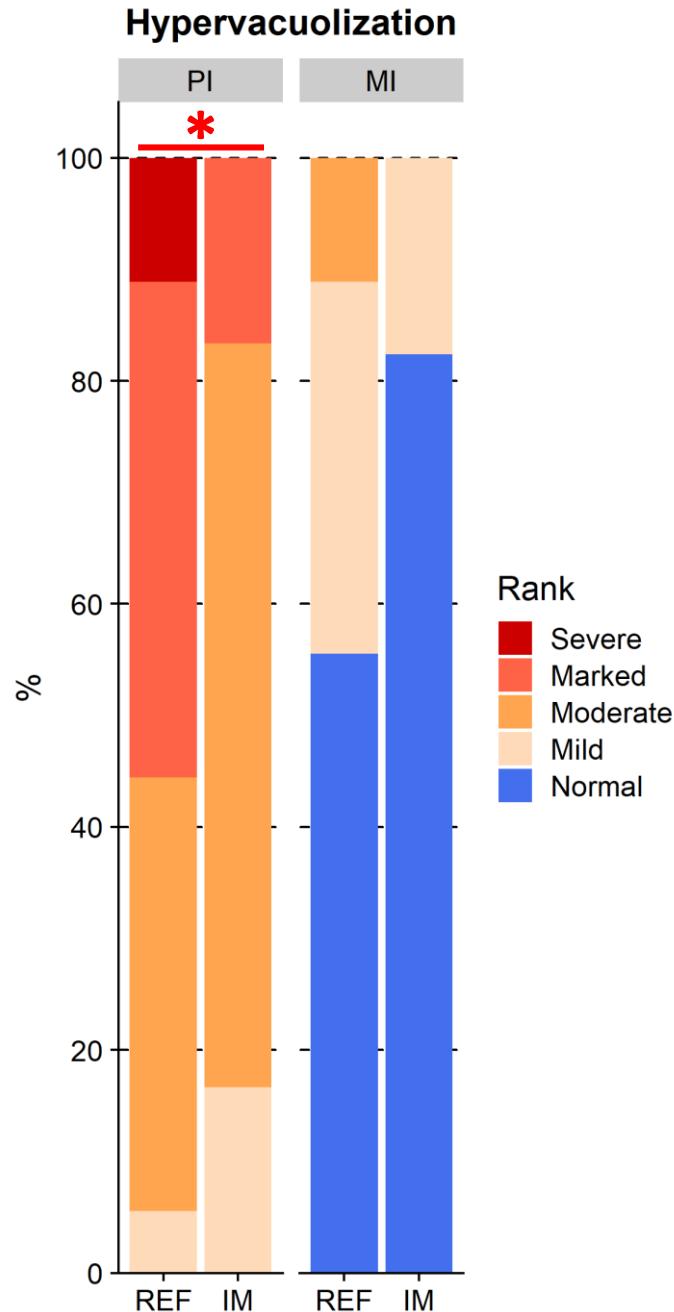
Lipid malabsorption



“Floating feces” problem



Source: Hanche-Olsen et al., 2013



COMPOUNDS IN LIPID TRANSPORT

Time: Thursday, October 10 14.30 - 16.50

Location: Lounge 1-3

Talk 1. Nutrients involved in digestion and transport of lipid across the intestinal mucosa of Atlantic salmon: an overview

Talk 2. Lipid malabsorption in six, Norwegian Atlantic salmon farms

Talk 3. Effects of dietary cholesterol, phospholipids and bile salts on intestinal lipid metabolism

Talk 4. Effects of dietary phosphatidylcholine and choline on intestinal lipid metabolism

Histological examination: inflammation

B
M
U
N

Normal

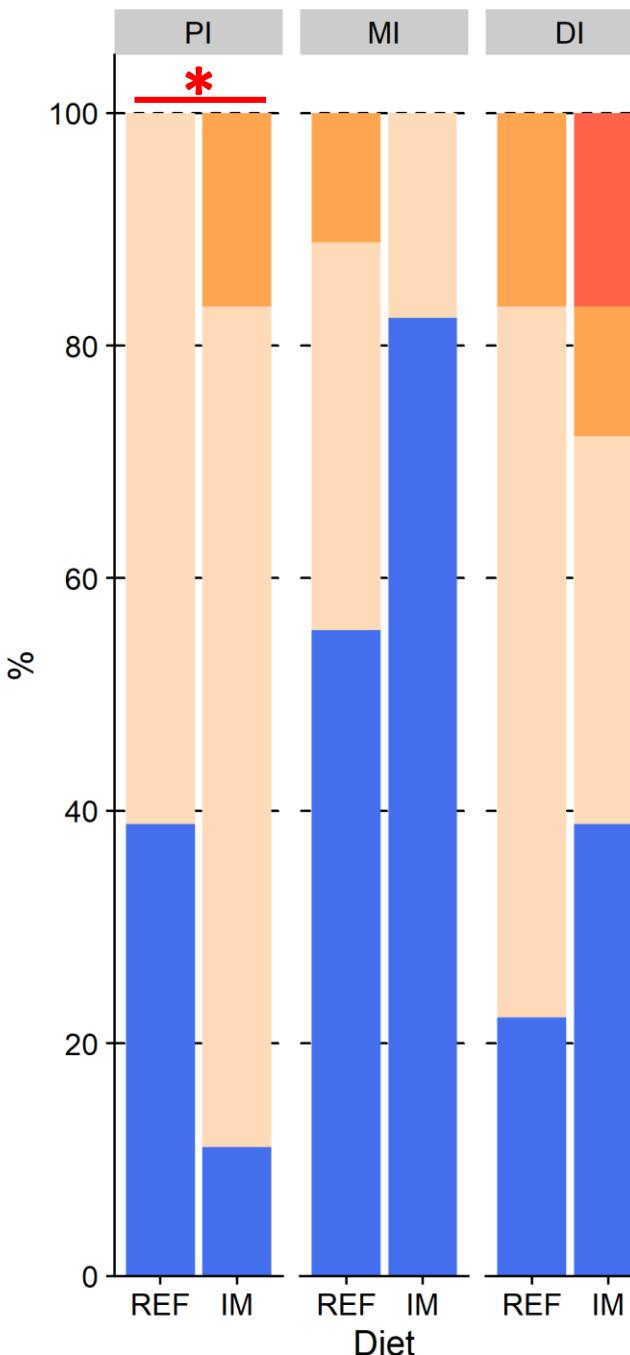


Inflammation



Credits: Elvis M. Chikwati

Submucosal cellularity



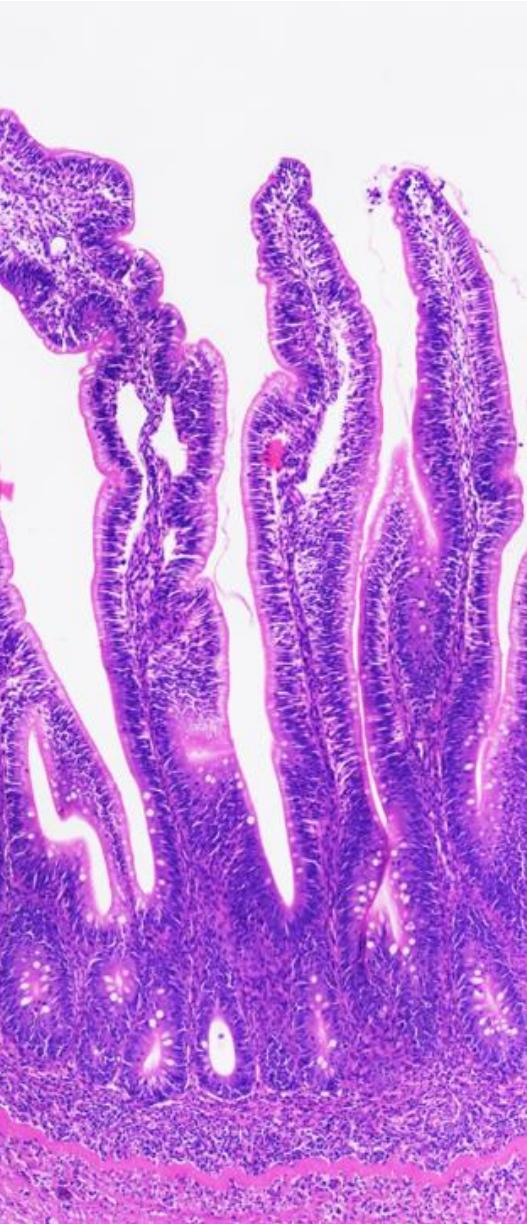
Histological examination: inflammation

B
M
U
N

Normal

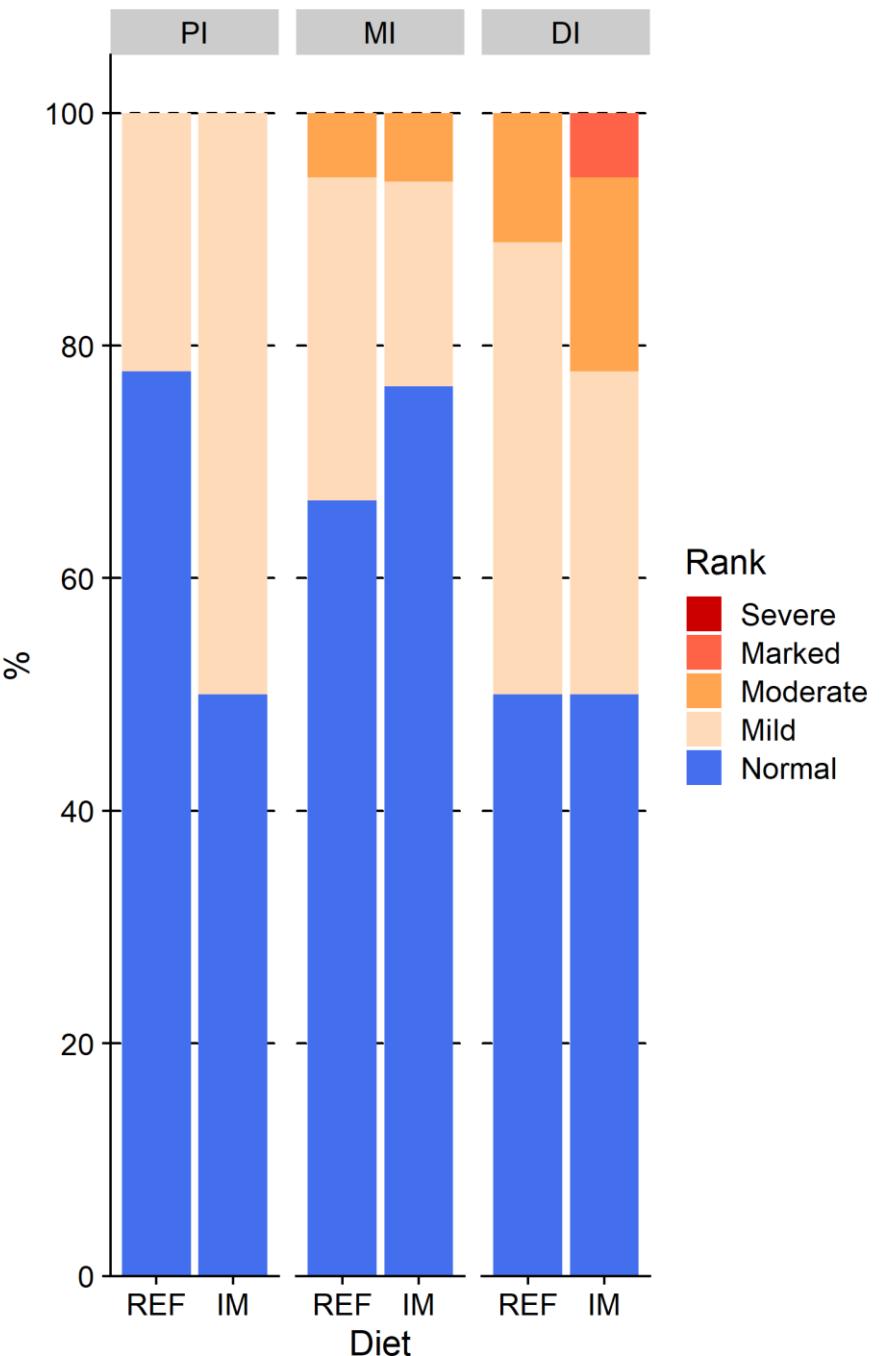


Inflammation



Credits: Elvis M. Chikwati

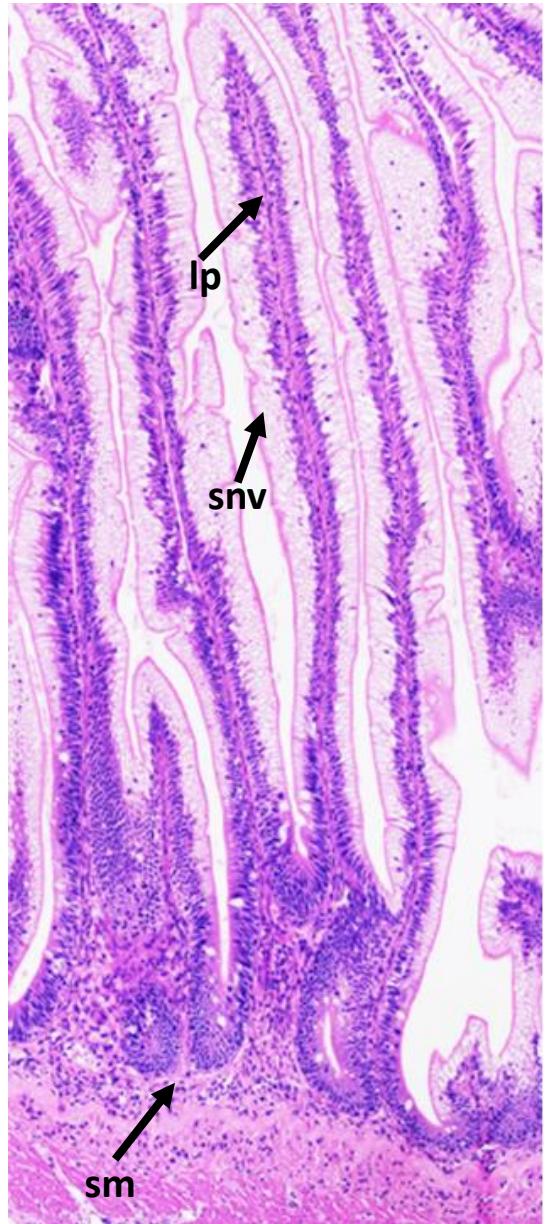
Lamina propria cellularity



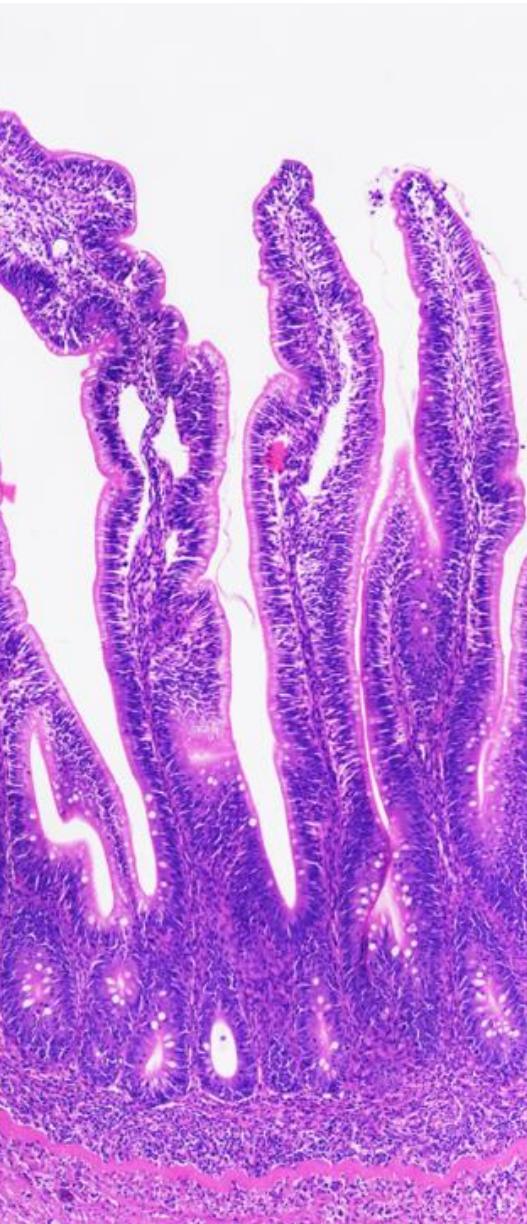
Histological examination: inflammation

B
M
U
N

Normal

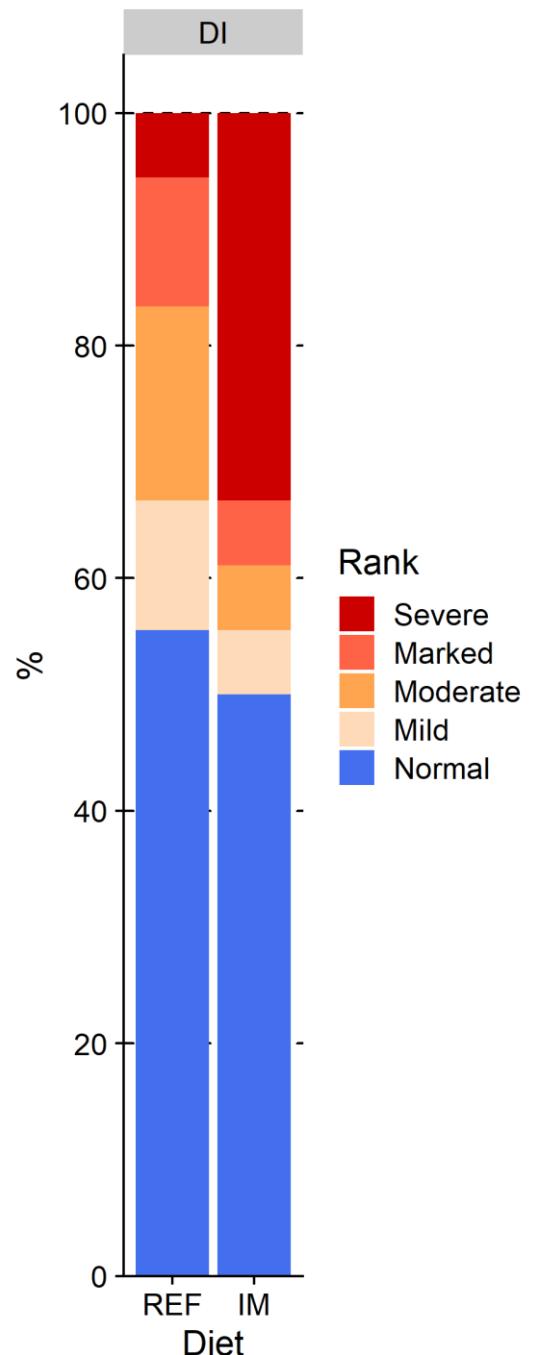


Inflammation

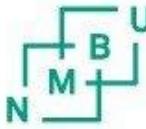


Credits: Elvis M. Chikwati

Supranuclear vacuolization



Gene expression profiling



MyD88 IL1- β IL-8

CD3 $\gamma\delta$ CD8 β MHC-I IFN- γ

IL-17a Foxp 3 IL-10 TGF- β 1

IL4

Immune response

qPCR

Claudin-15
Claudin-25b
ZO-1
E-cadherin
Mucin-2
AQP8ab

Barrier function

Lipid metabolism

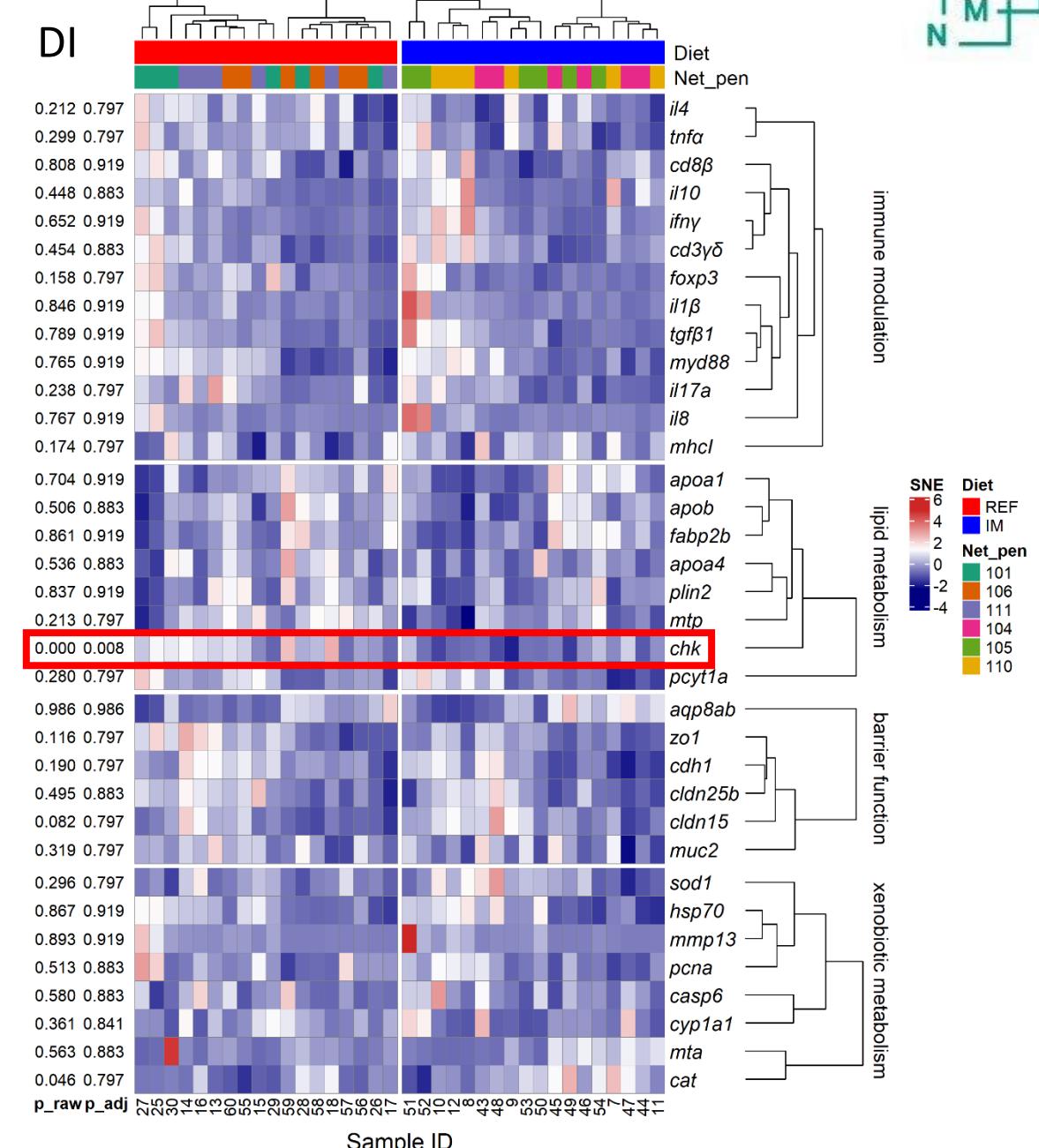
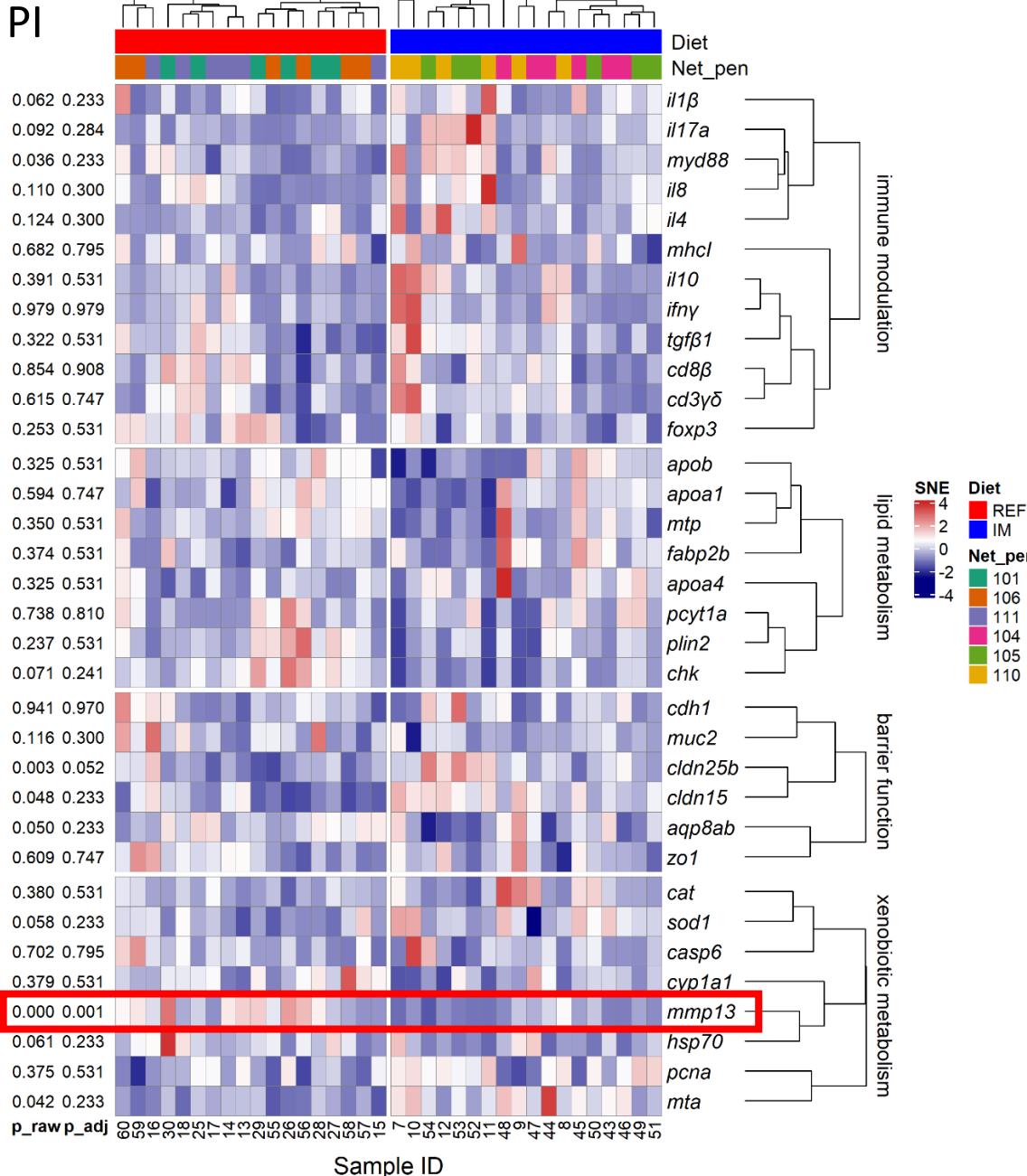
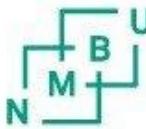
Xenobiotic metabolism

CYP1A1 MT-A HSP70 SOD1

CAT Caspase 6 PCNA MMP13

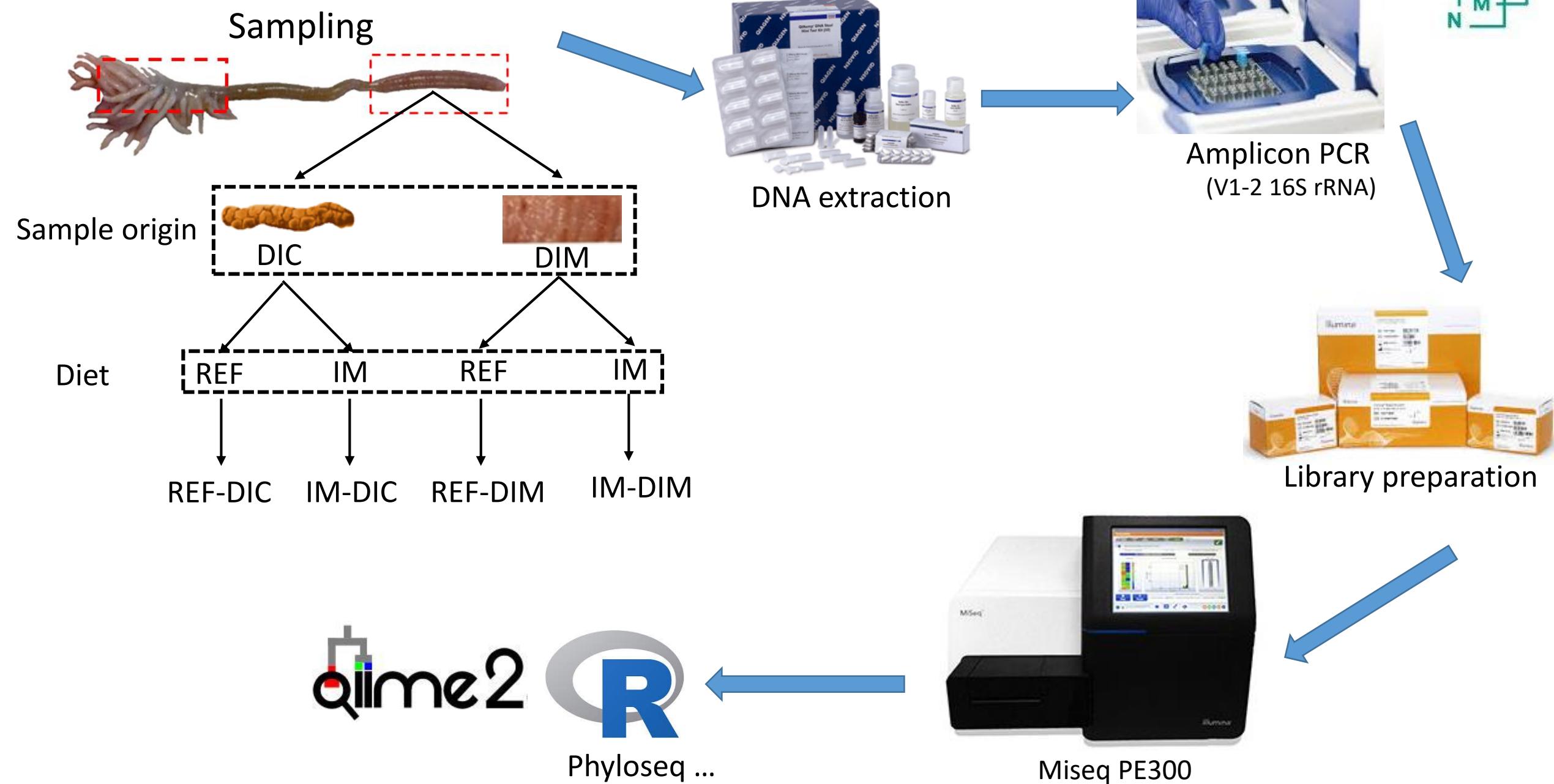
Plin2
MTP
ApoA1
ApoA4
ApoB
CHK
PCYT1A
FABP2b

Gene expression profiling



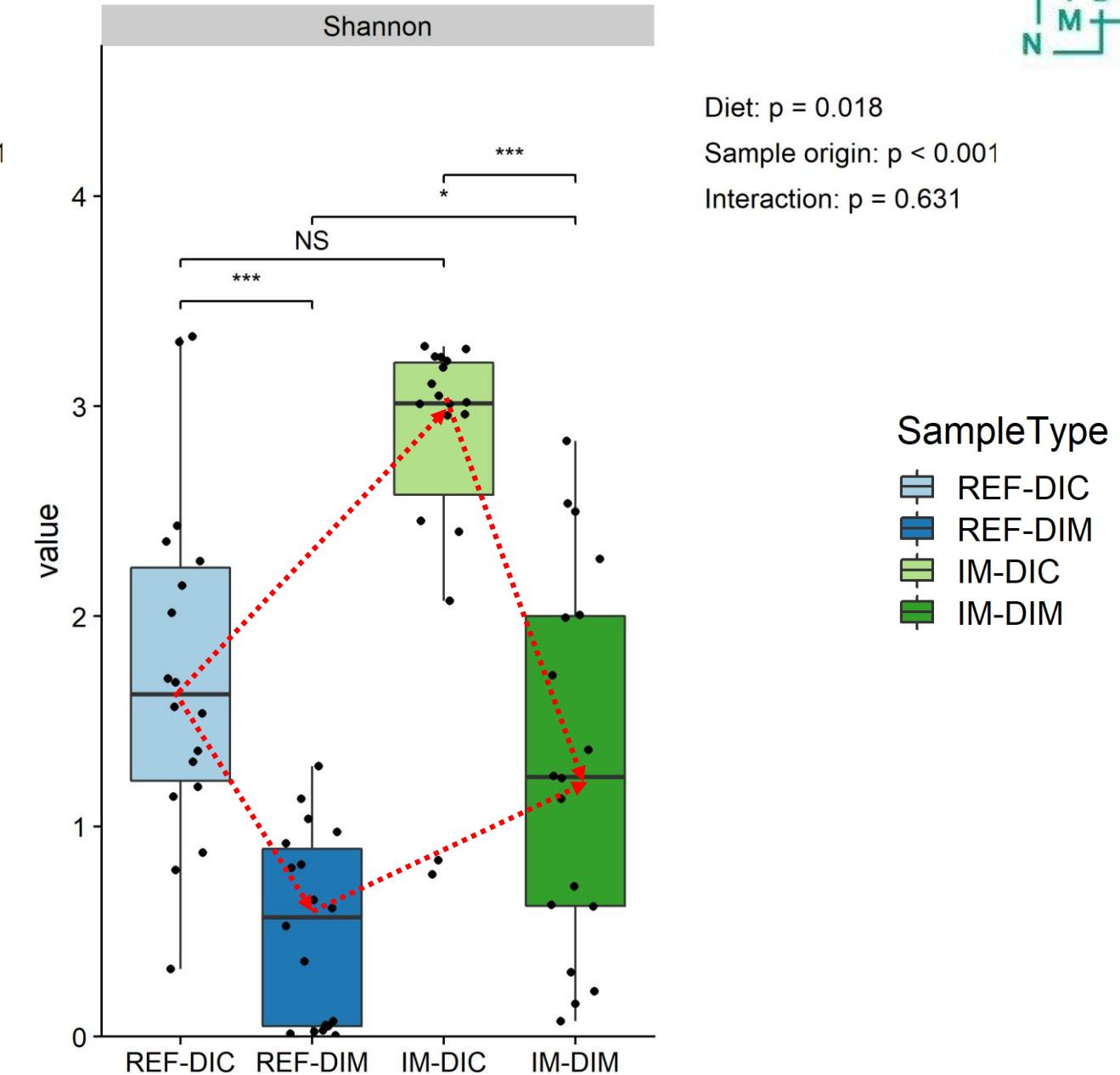
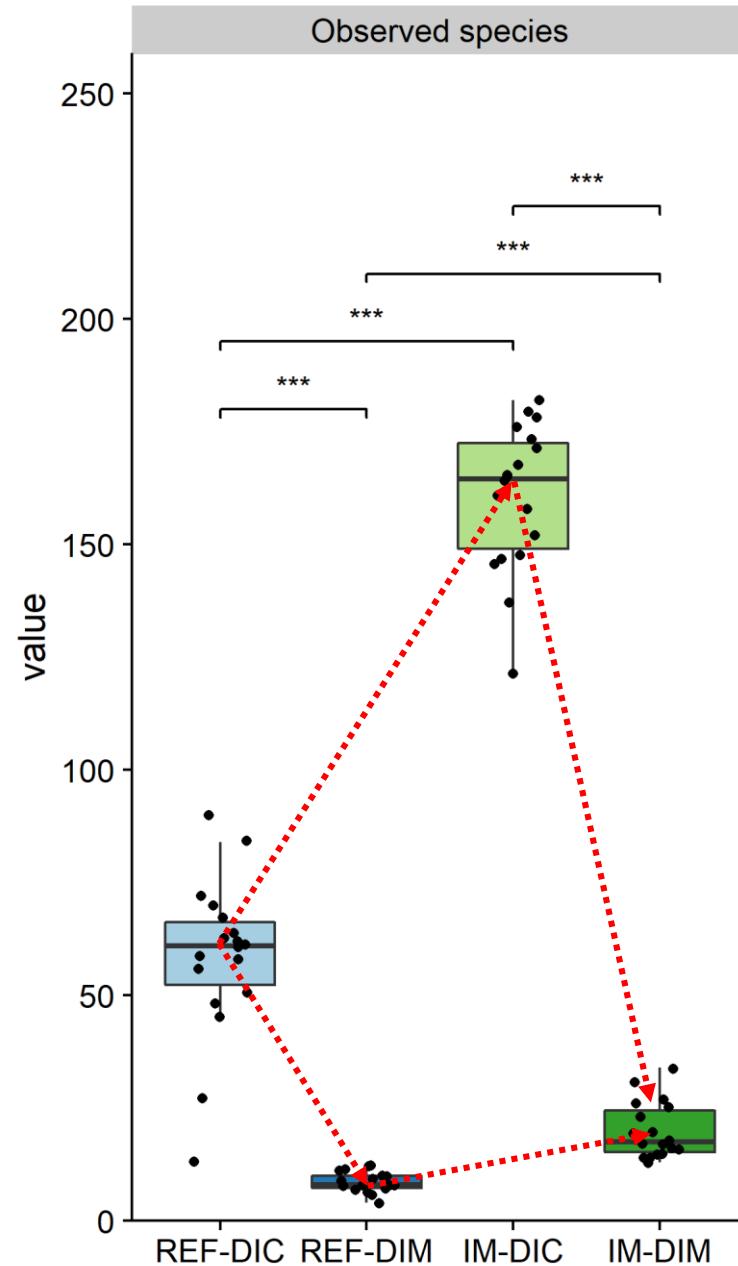
Gut microbiota profiling

N B M U

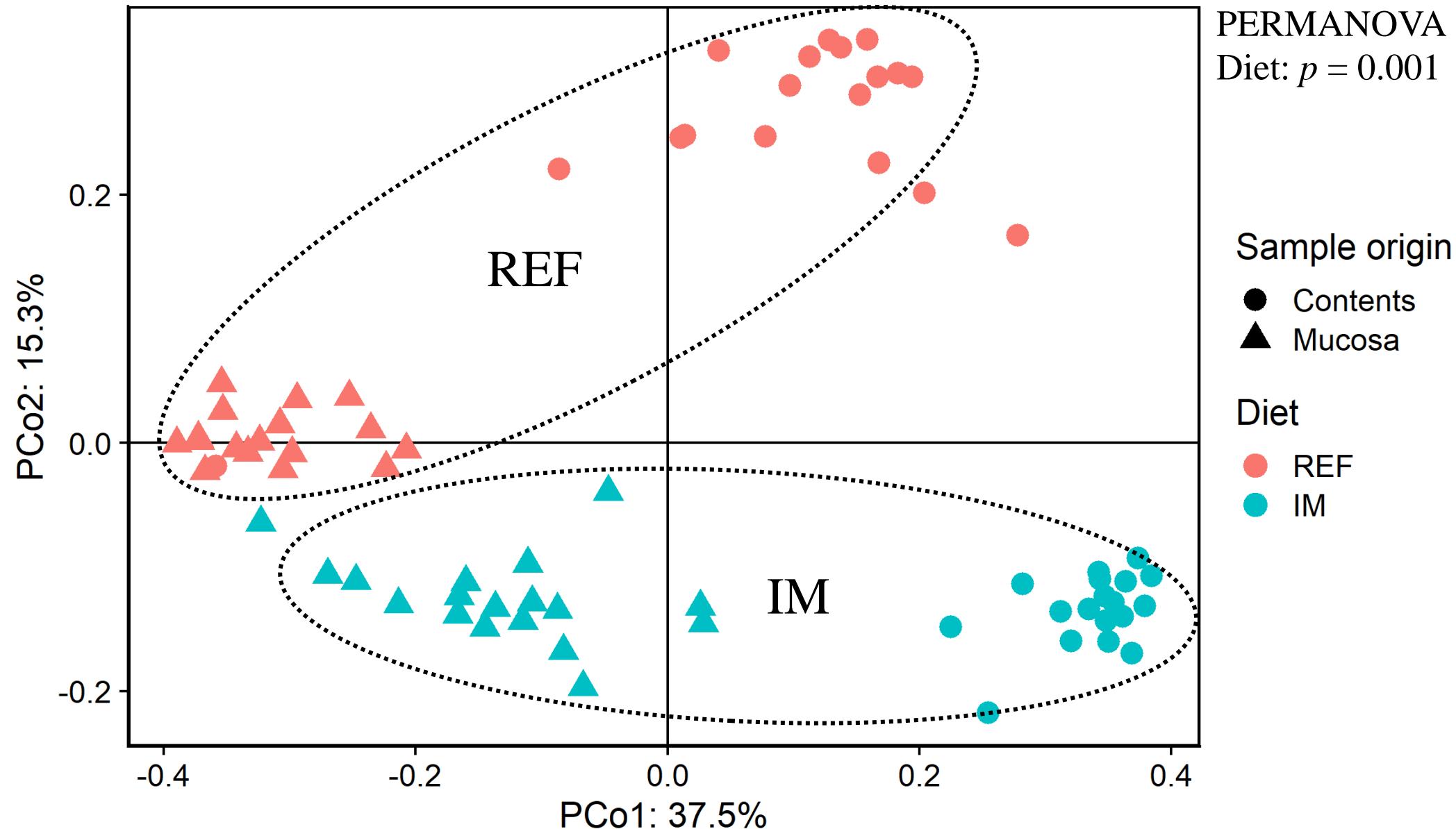


Alpha-diversity

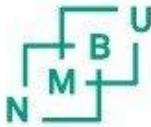
B
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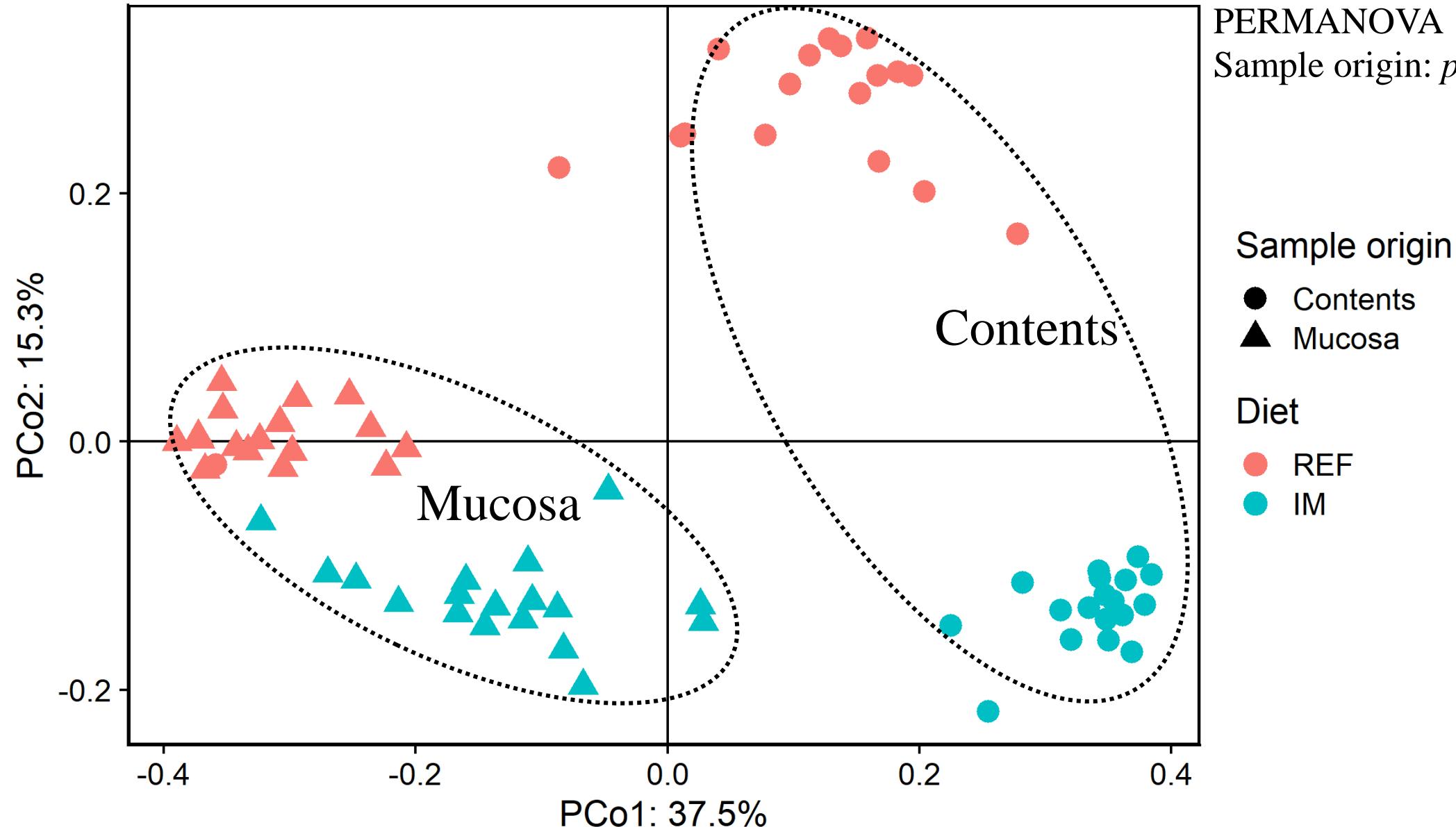
Unweighted-UniFrac distance based PCoA



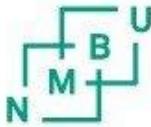
Beta-diversity



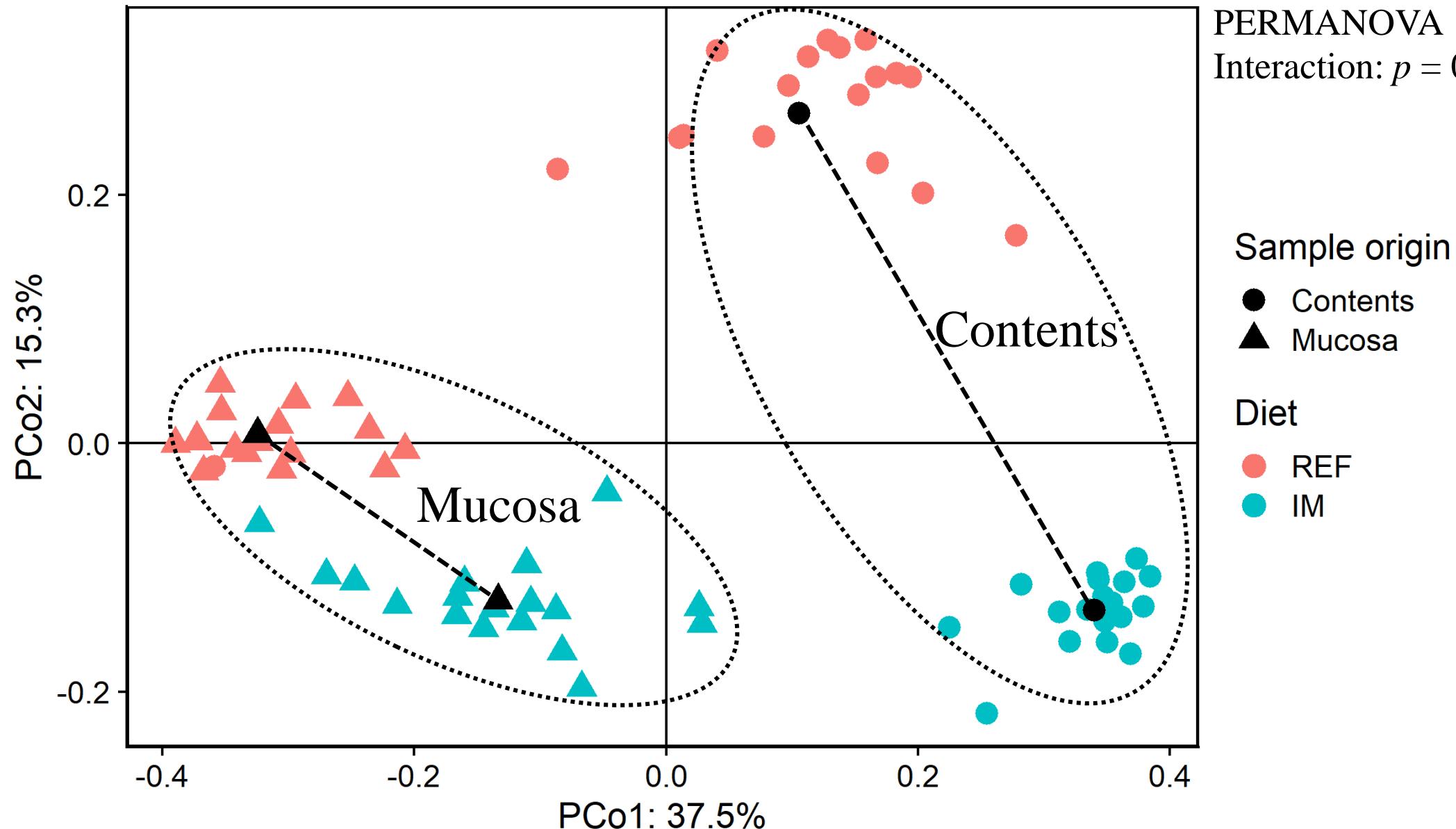
Unweighted-UniFrac distance based PCoA



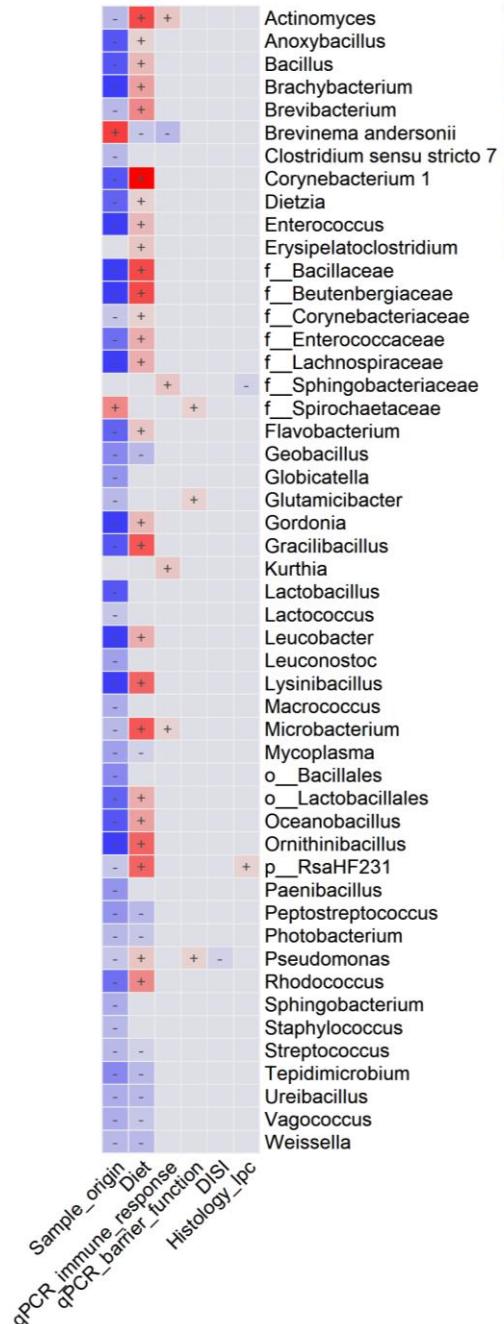
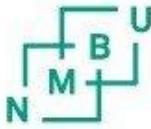
Beta-diversity



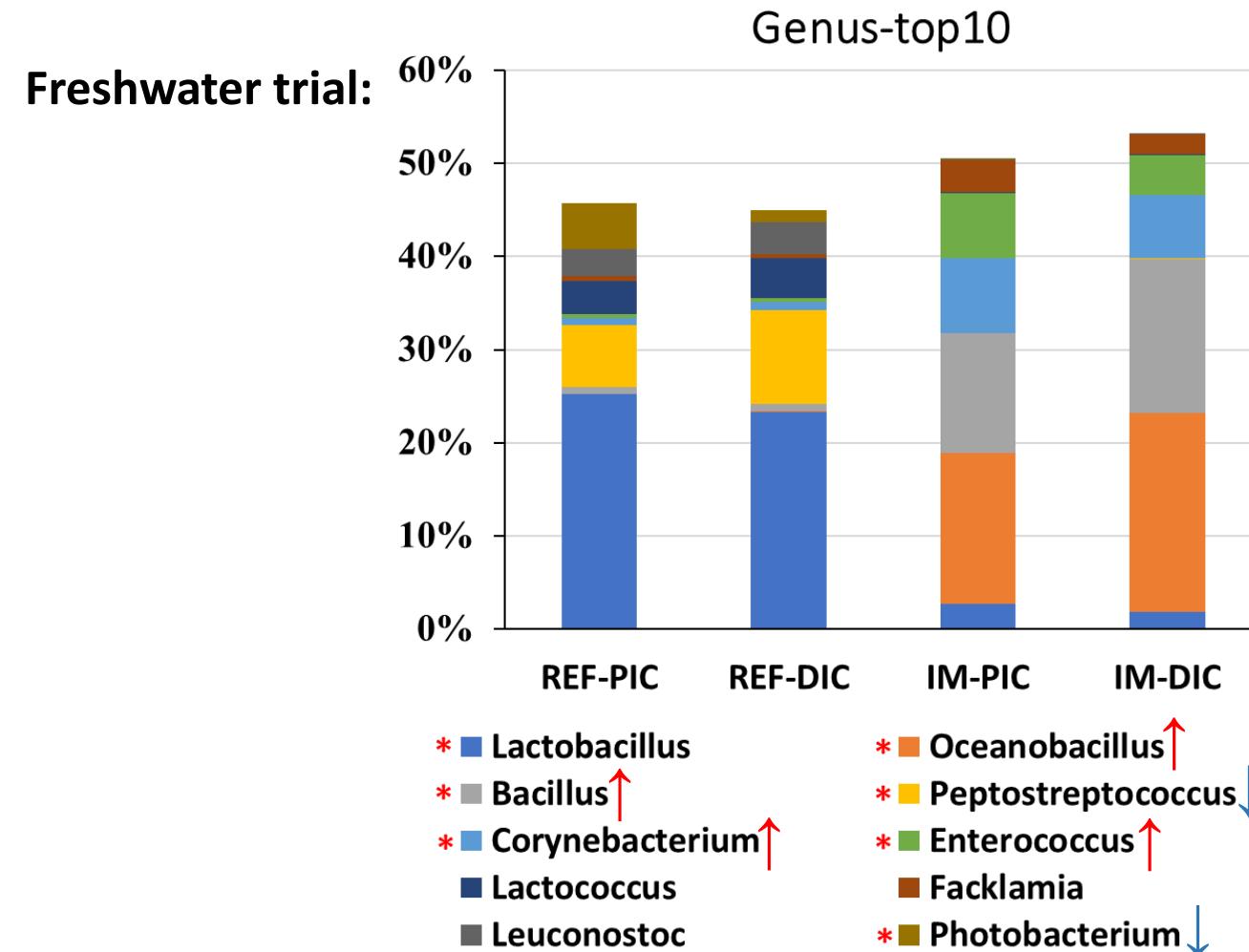
Unweighted-UniFrac distance based PCoA



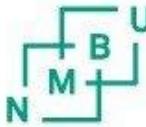
Association testing using MaAsLin2



- Diet effect: 37 differentially abundant taxa
 - ✓ IM: 10 taxa↓, 27 taxa ↑
 - ✓ Reproduced parts of results from the freshwater trial

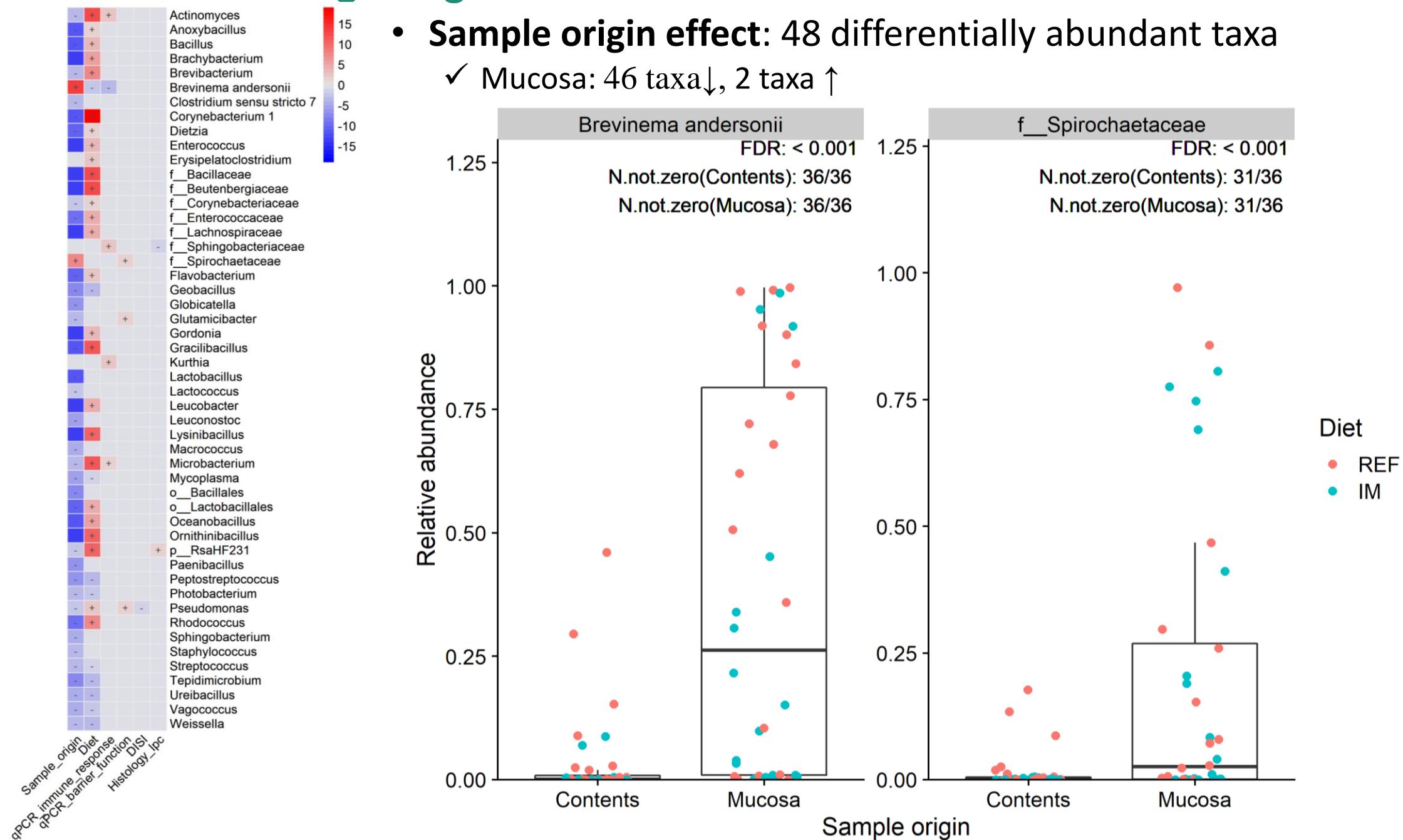


Association testing using MaAsLin2

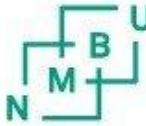


- **Sample origin effect:** 48 differentially abundant taxa

✓ Mucosa: 46 taxa ↓, 2 taxa ↑



Summary



No or minor diet effect on :

- ☛ growth and feed utilization
- ☛ digestive functions
- ☛ expression of selected marker genes

However, IM-based diet,

- ☛ increased submucosal cellularity in proximal intestine
- ☛ increased relative weight of distal intestine
- ☛ reduced enterocyte hypervacuolization in proximal intestine
- ☛ modulated gut microbiota: intestinal contents > mucosa

↑ Bacillus, Oceanobacillus, Corynebacterium, Enterococcus;
↓ Peptostreptococcus, Photobacterium

The Nutrition group, NMBU vet. School



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Assoc. prof,
DVM



Guro Løkka
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Mol biology



Alex Torres
Post doc.
DVM



Ellen Hage
Technician
Lab leader



Kirsti Præsteng
Technician



Elin Valen
Technician



Jintao Liu
Visiting
researcher



Anne K Hansen
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Jie Wang
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Biology



Weiwen Zhou
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**The Research Council
of Norway**

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Bioforsk Christian Brückner



UNIVERSITY OF Andre Gilburn,
STIRLING Isobel Swinscoe



Christoffer Troedsson



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Dorothy Dankel,
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Svenja Heesh



Johan Johansen



Tarique Arsiwalla



Huibert Everdingen

Slides
available at:



figshare

Contact: yanxian.li@nmbu.no