Effect of Functional Ingredients on Intestinal Health and Growth Performances of Weaned Piglets

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Wageningen Livestock Research, Animal Nutrition

AVANT

Alternatives to Veterinary ANTimicrobials

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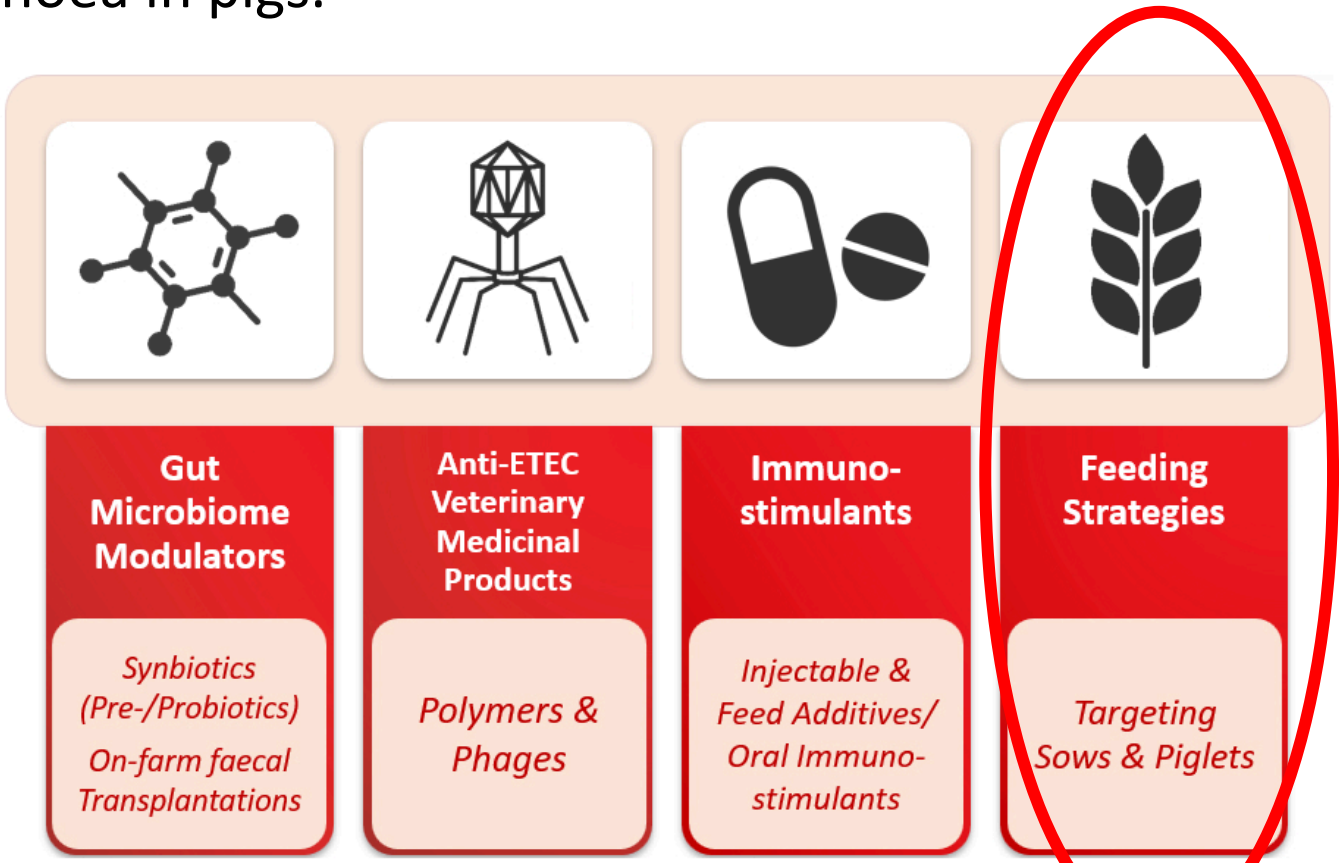
This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No 862829.

The AVANT Project

Develop alternatives to antibiotics and bring them to a TRL 7-8 for treating or preventing post-weaning diarrhoea in pigs.



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PROJECT AIM and DATASETS

Evaluate the impact and get a **deeper understanding of the mode of action** of various functional ingredients on growth, health, and microbiota in post-weaning pigs.

Key Metrics:

- **Growth:** Body weight, feed intake, ADG, FCR
- **Health:** Cytokines, chemokines, C-reactive proteins, faecal consistency, general health status
- **Microbiota:** Composition in ileum and cecum
- **Gene Expression:** In ileum
- **Blood Analysis:** Metabolites, APPs, cytokines

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Dietary interventions

- 4 diets based on “functional ingredients”, interventions lasts **13 days**
- Two reference diets: basal diet and the same diet with supplemented 3000 mg Zn/kg as ZnO

Ingredients	Inclusion (%)	Functionality	Possible Target
Alfalfa meal	5	Low-fermentable, structural fibre	Gut functionality, water binding properties
Sugar beet pulp	5	High fermentable fibre, prebiotic effects	Intestinal microbiome composition/activity
Insect oil from black soldier (BSF) fly larvae	3	Medium chain triglycerides, antimicrobial properties	Intestinal microbiome composition/activity
Chitin rich protein meal from BSF	5	Chitin, functional and antimicrobial proteins and peptides	Local immune system, intestinal microbiome composition and activity

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Reference diet

- Diet based on maize (27%), wheat (27%), barley (19%), soyabean meal (13%), potato protein (3%), molasses (3%) and soya oil (2.2%) as main ingredients.
- Balanced AA profile (11 g SID Lys/kg (100); Met + Cys (61), Thr (65), Trp (22), Val (70))
- No inclusion of organic acids or other additives potentially influencing gut health, but inclusion of phytase
- Cu: 25 mg/kg; Zn: 100 mg/kg

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Experimental trial

- Piglets followed for 35 days post-weaning (12 replicates per treatment, 9 piglets per pen)
- Dietary treatments from weaning until two weeks after (till day 13).
- The reference diet given to all of them is from 3 to 5 weeks p.w. (from day 13 to day 34).
- Diets were nutrient and energy balanced.

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Performances Results, D 0-13

Treatment	CTRL	CTRL_ZnO	Alfalfa	Sugar beet	BSF_oil	BSF_Prot	SEM	P -value
BW at D0 (kg)	8.18	8.17	8.14	8.15	8.16	8.13	0.019	0.48
BW at D13 (kg)	10.48^{a}	11.68^{b}	10.52^{a}	10.61^{a}	10.37^{a}	10.35^{a}	0.106	<0.001
ADG (g/d)	177^{a}	270^{b}	183^{a}	189^{a}	170^{a}	171^{a}	8.1	<0.001
ADFI (kg/d)	0.26^{ab}	0.33^{c}	0.26^{ab}	0.28^{b}	0.25^{a}	0.26^{ab}	0.008	<0.001
FCR	1.46^{b}	1.24^{a}	1.46^{b}	1.47^{b}	1.49^{b}	1.51^{b}	0.028	<0.001

ADG ZnO -> +58%!

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Performances Results (2), D 13-34

Treatment	CTRL	CTRL_ZnO	Alfalfa	Sugar beet	BSF_oil	BSF_Prot	SEM	P -value
BW at D13 (kg)	10.48^{a}	11.68^{b}	10.52^{a}	10.61^{a}	10.37^{a}	10.35^{a}	0.106	<0.001
BW at D34 (kg)	23.55	24.40	23.64	23.96	23.62	23.52	0.303	0.29
No of days	21	21	21	21	21	21		
ADG (g/d)	622	606	624	636	631	627	12.3	0.62
ADFI (kg/d)	0.85	0.85	0.86	0.88	0.86	0.86	0.014	0.86
FCR	1.38^{xy}	1.40^{y}	1.37^{x}	1.38^{xy}	1.36^{x}	1.37^{x}	0.011	0.08

The effects of ZnO on performance parameters are temporary.

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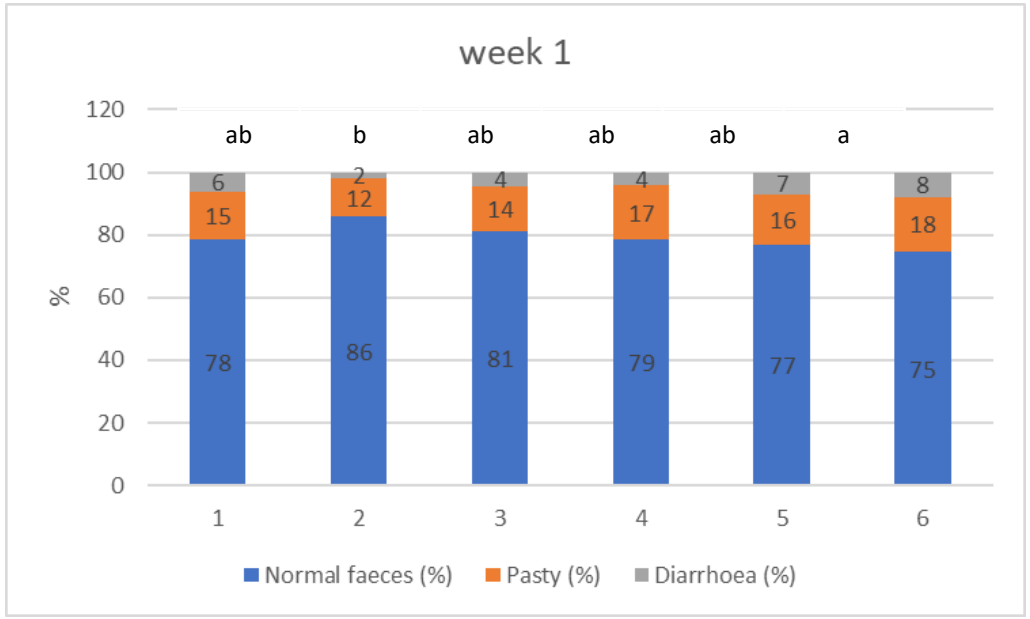
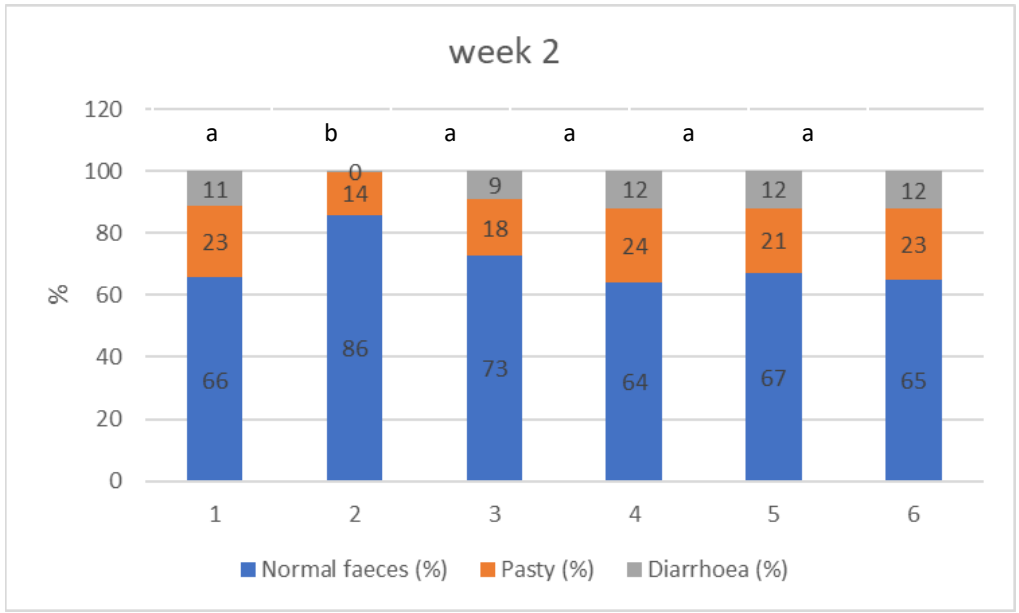
Faecal consistency

P<0.05



P<0.05



Treatments:

T1 = Reference diet (negative control)

T2 = Reference diet + 3000 mg Zn/kg diet as ZnO

T3 = Diet with non-fermentable fibre (5% alfalfa meal)

T4 = Diet with fermentable fibre (5% sugar beet pulp)

T5 = Diet with medium chain triglycerides (3% BSF insect oil)

T6 = Diet with a novel protein source (5% BSF insect chitin rich protein meal)

Ctrl_ZnO shows significantly better faecal consistency than the rest.

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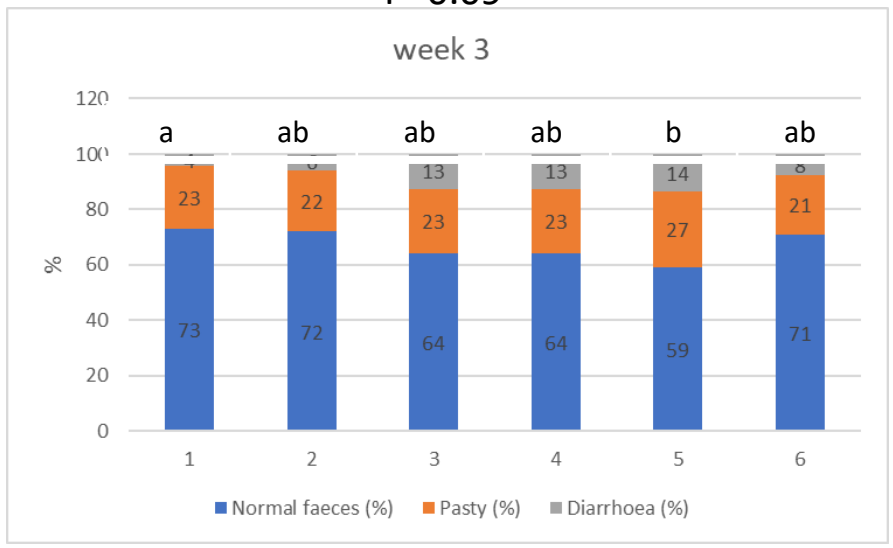
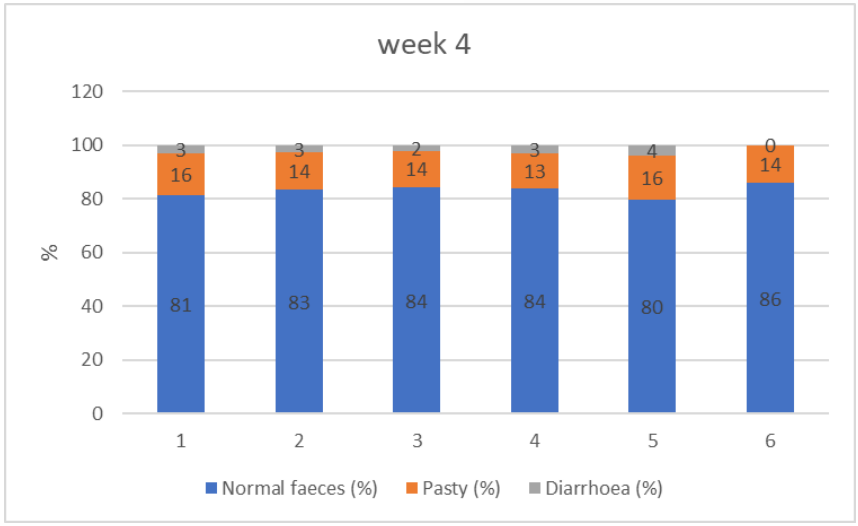
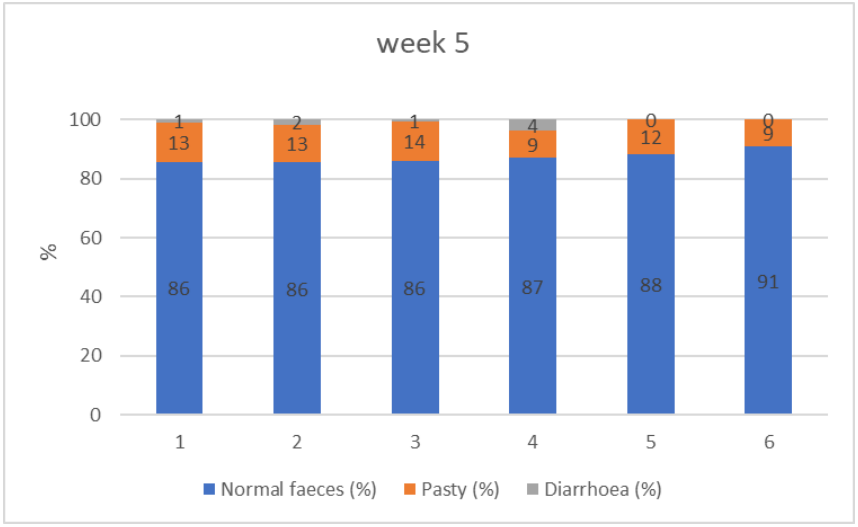
Faecal consistency (2)

P=0.09







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Also in this case the effects are temporary

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ETEC detection via qPCR

- qPCR on the virulence factor **F4 fimbriae** of *E. coli*
- **Colon digesta** samples collected at d6 and d34
- **Undetected** (Ct < 36 cycles) in about **~91%** of the samples
- Among detected samples (~9%), all were from **Day 6** across all treatment groups
- Insufficient data to perform statistical analysis for the treatment effect

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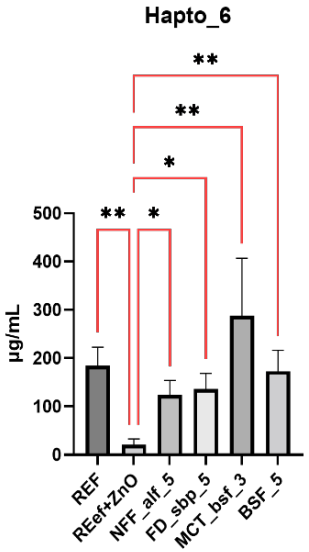
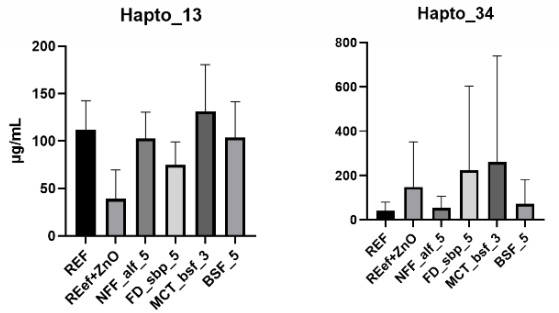
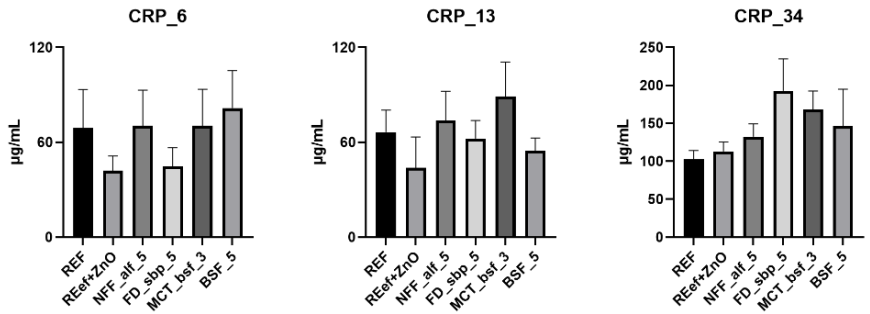
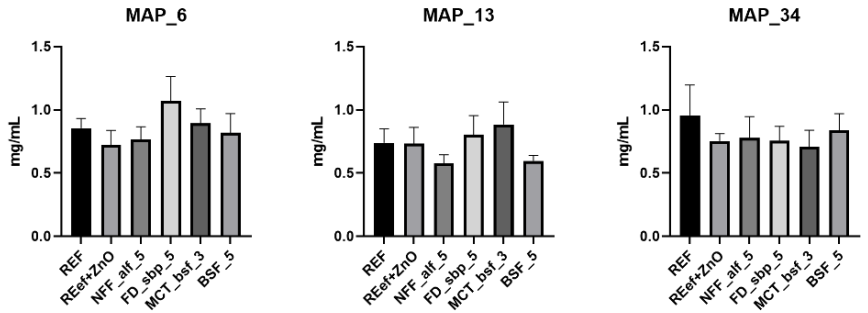
Inflammatory biomarkers

- Systemic inflammation by monitoring key immune inflammatory biomarkers.
- A significantly lower level of **Haptoglobin** was recorded for ZnO supplemented group on d6
- **C-Reactive Protein (CRP)** & **Porcine Major Acute Phase Protein (PigMAP)** recorded no statistically significant differences
- Comparison was made with both REF & Ref+ZnO group
- Small and temporal decrease of inflammation due to ZnO, no difference in the others









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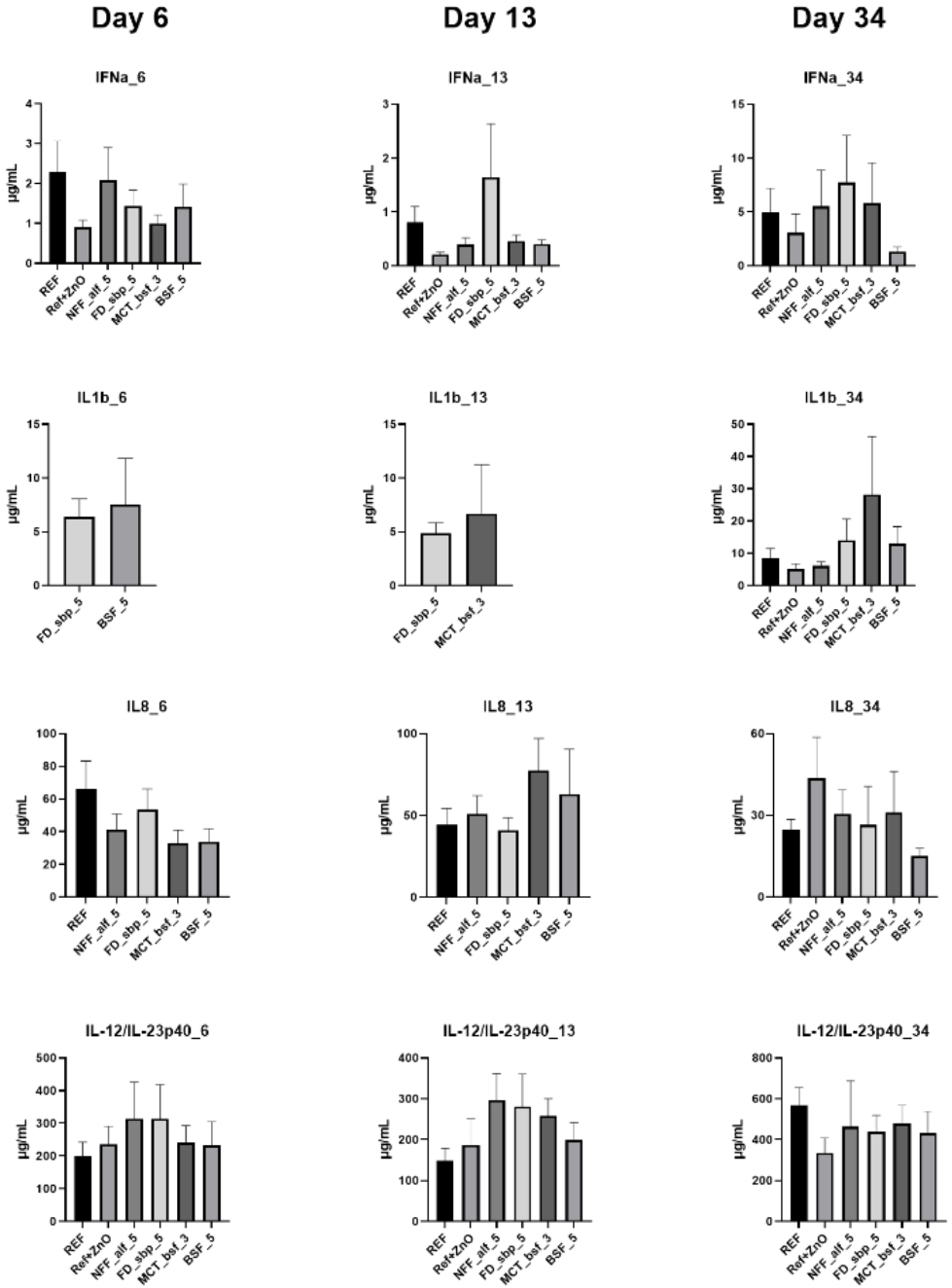
Cytokines & Chemokines

- **IFNγ**, **IL4**, **IL10**, and **TNFα** measures were filtered* out and not considered for further statistical analysis
- **IL1b**: d6, *FD_sbp_5* & *BSF_5*; d13, *FD_sbp_5* & *MCT_bsf_3* passed the filter.
- **IL6**: d6 & d13, all treatments failed to pass the filter; d34 all treatments, except *NFF_alf_5*, *FD_sbp_5* & *BSF_5* , passed the filter
- **IL8**: *Ref+ZnO* failed to pass the filter in all days; *FD_sbp_5* failed to pass the filter in d34
- **No statistically significant differences (comparison was made with both REF & Ref+ZnO group) were observed**

*For statistical analysis, only treatment groups with detectable analyte levels in at least six pig serum samples per group were included.



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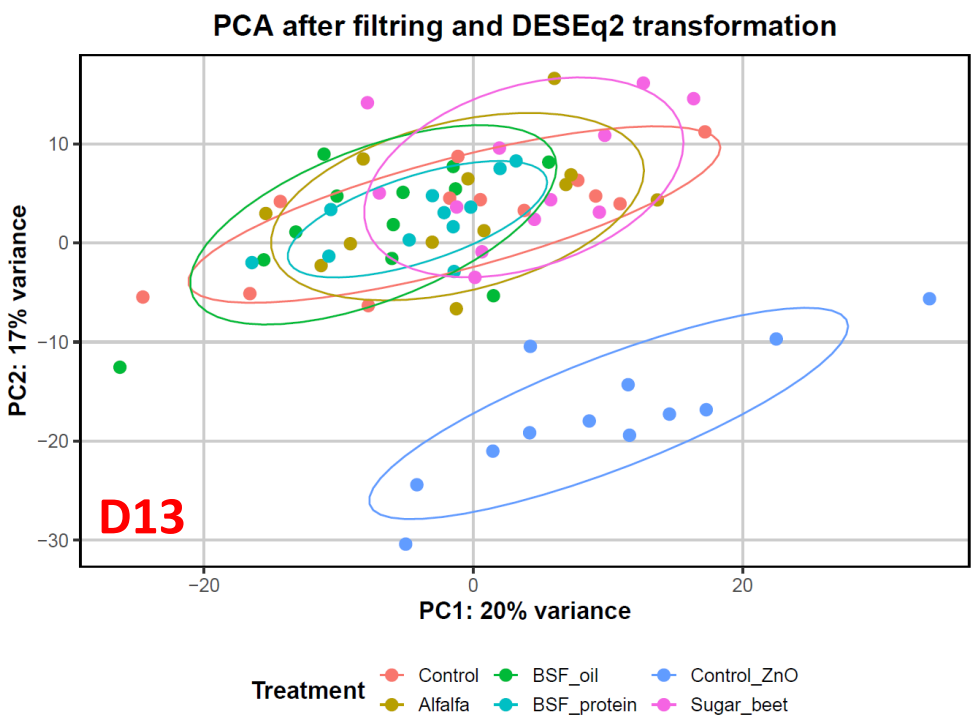
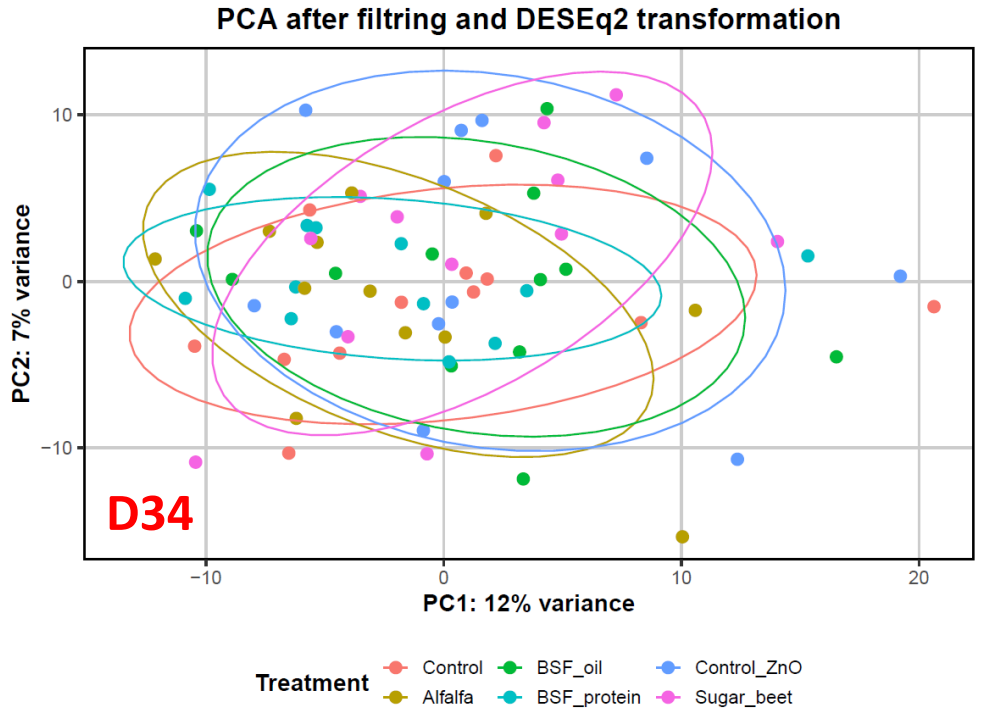
Transcriptomics

- Ileum samples taken at D13 and D34, RNA seq analysis





- Distinct **clustering** in the PCA indicates a **unique expression profile influenced by ZnO treatment**.
- **Temporary effect!**

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Transcriptomics Day 13 (2)

- Zn0 supplementation results in 39 DE genes vs the control diet

Contrast	Genes AdjP & FC	Genes Pvalue & FC	Genes AdjP	Gene Pvalue
Alfalfa_vs_Control	0	2	0	302
BSF_protein_vs_Control	0	1	0	1243
BSF_oil_vs_Control	0	4	0	996
Sugar_beet_vs_Control	0	2	0	1344
Control_ZnO_vs_Control	39	39	128	1458

(adj_)Pvalue < 0.05, |Log2FC| > 1

(adj_)Pvalue < 0.05

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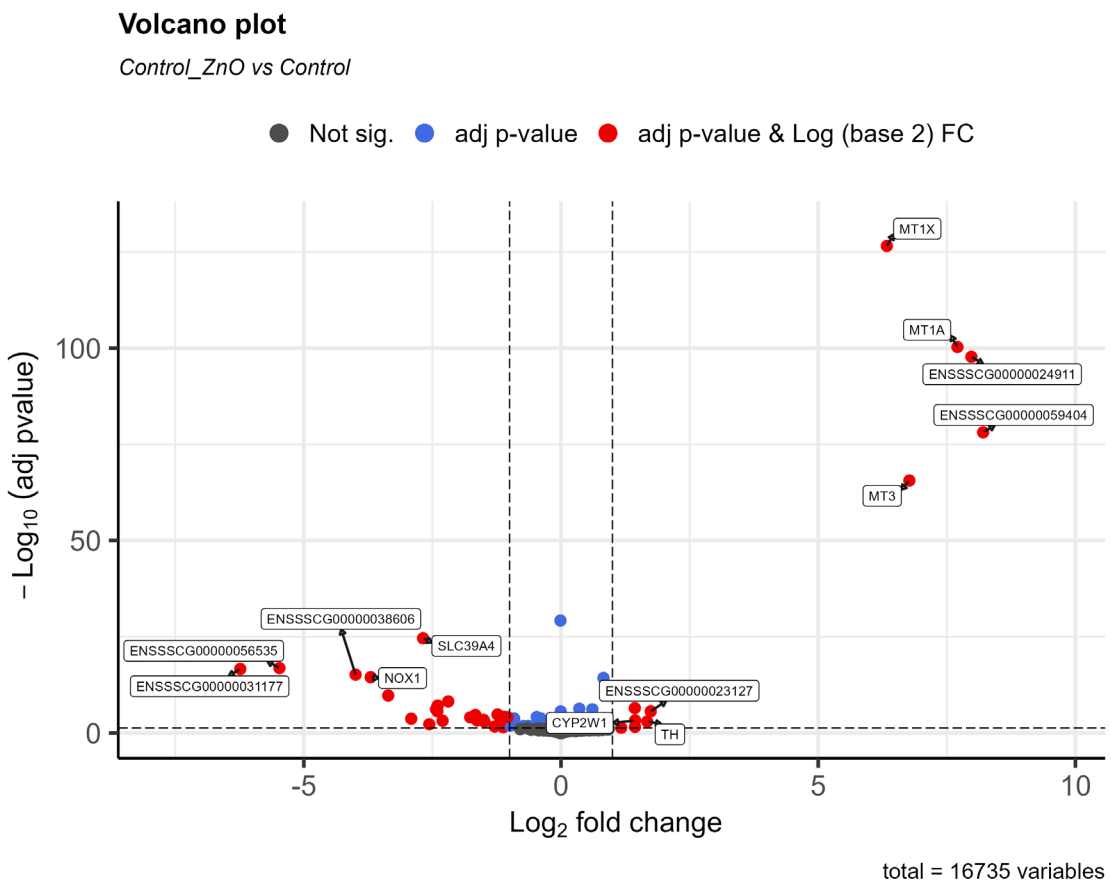
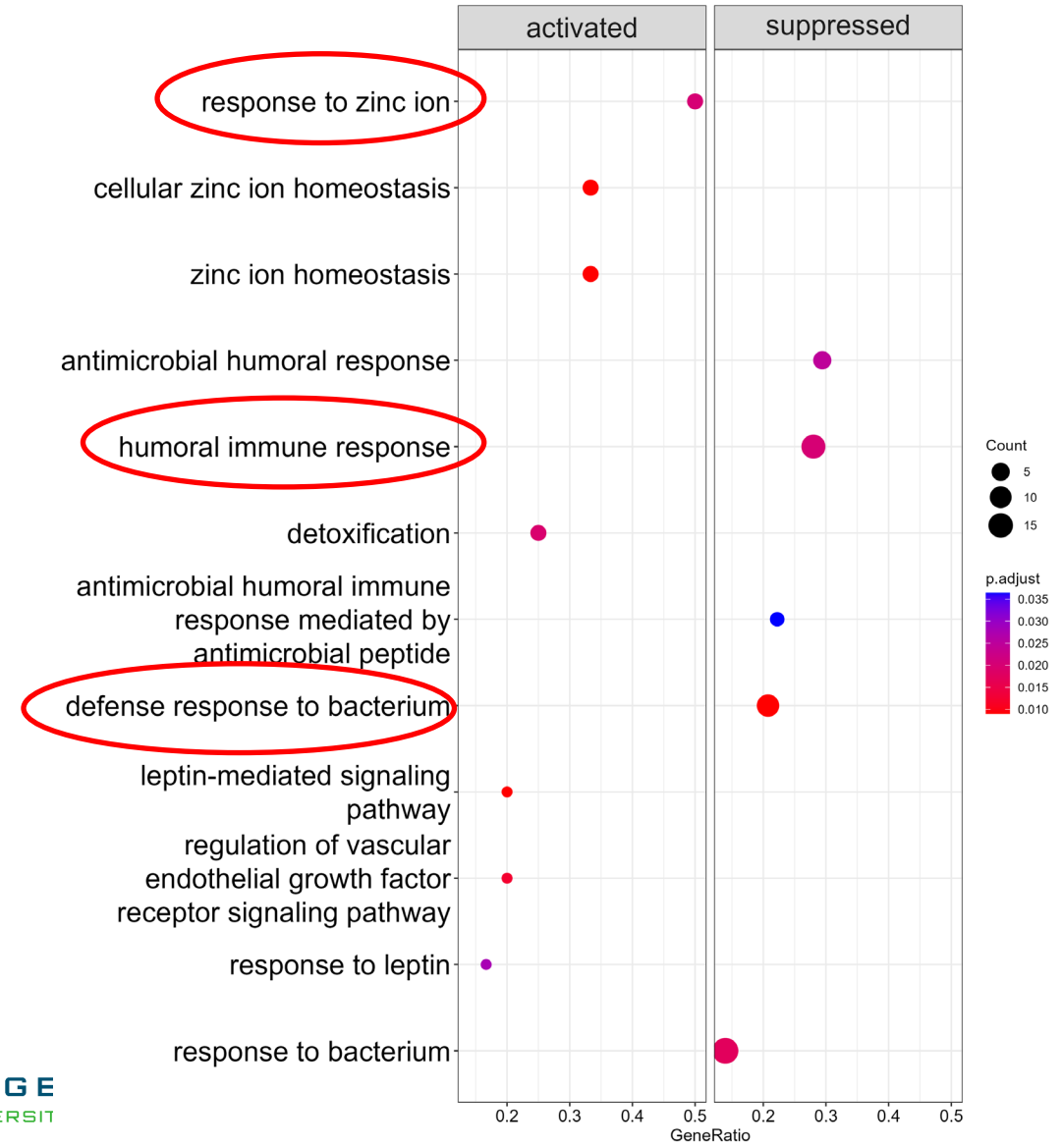
Transcriptomics (3) DEA & GSEA





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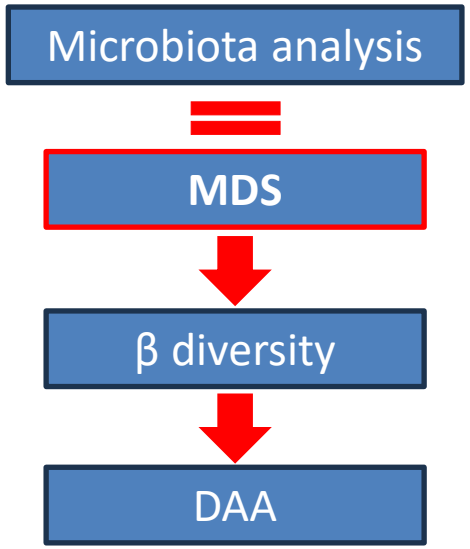
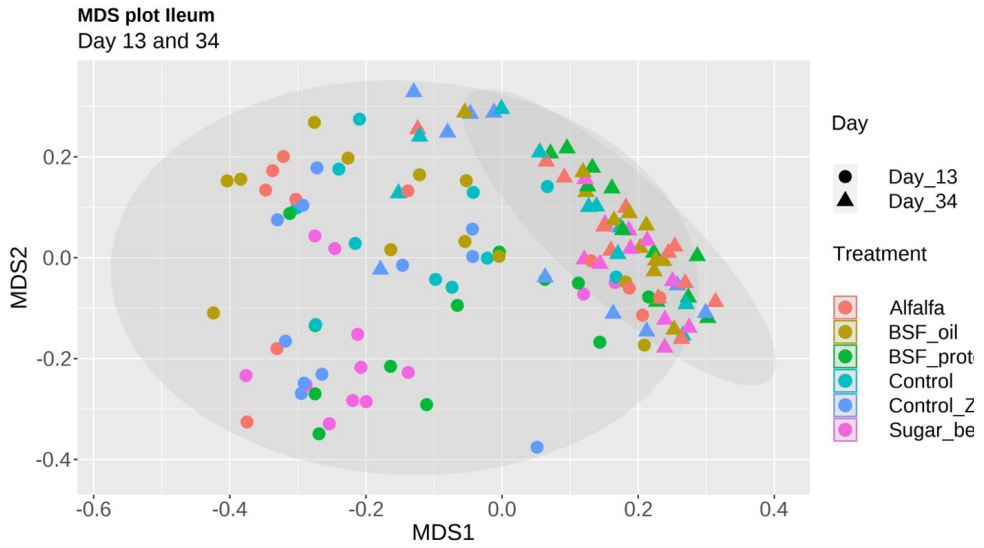
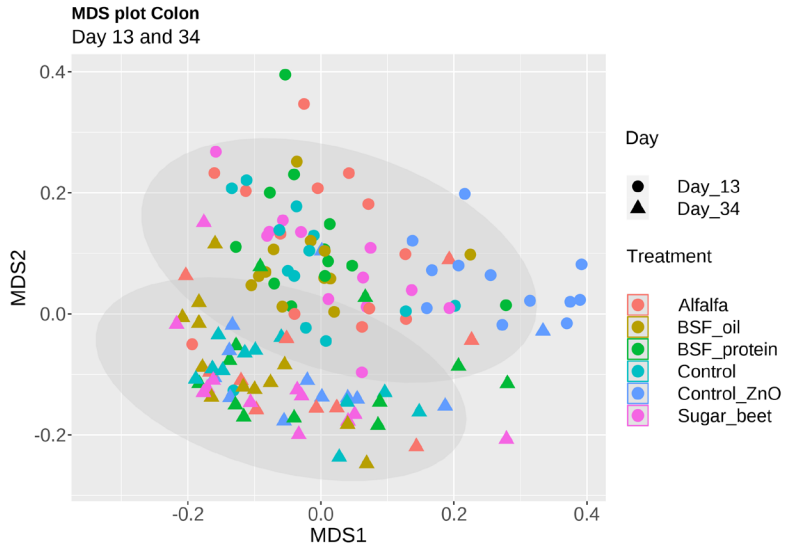
Microbiota

- Ileum and colon samples taken at D13 and D34 and analyzed via 16S analysis







- Distinct **clustering** per Time point
- No difference at day 34

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Microbiota Beta Diversity day 13

Microbiota analysis

=

MDS

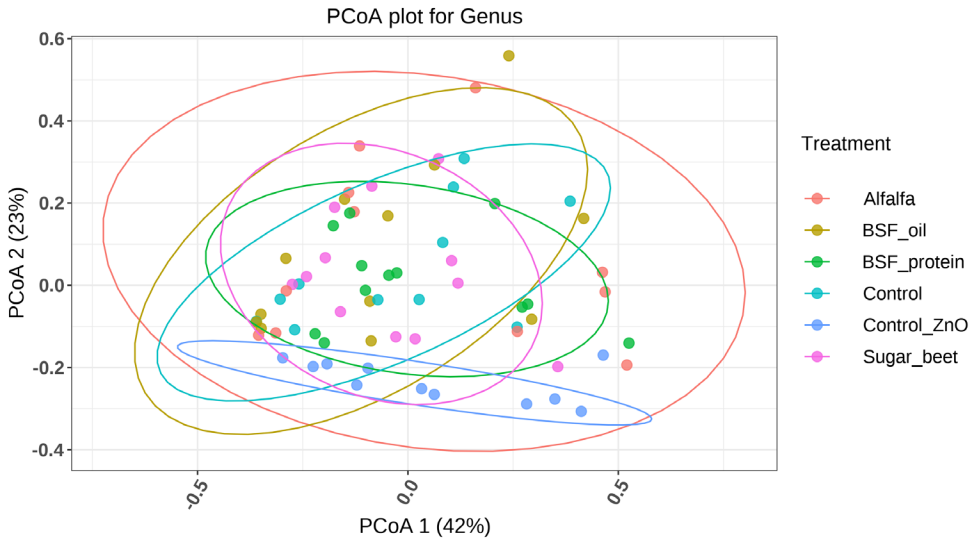
↓

β diversity

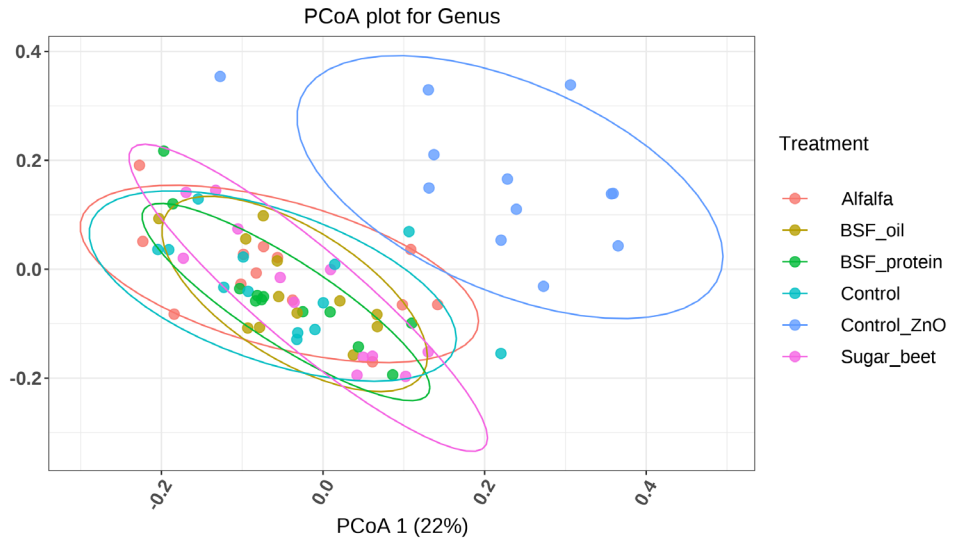
↓

DAA

Ileum



Colon



- The dietary interventions **significantly affect the overall microbial population** in both Ileum and Colon (PEMANOVA p-value 0.003, 0.001)
- The Control_ZnO group, in particular, shows a **distinct microbiome profile**

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Microbiota Differential Abundance Analysis

Microbiota analysis

MDS

β diversity

DAA

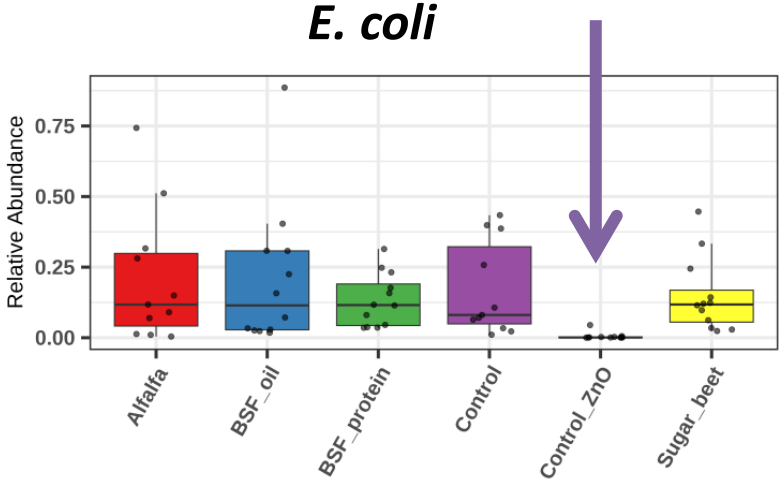
Ileum

Treatment	Increased	Decreased
Control ZnO	14	12
Alfalfa	3	/
Sugar beet	8	5
BSF protein	3	2
BSF oil	3	1

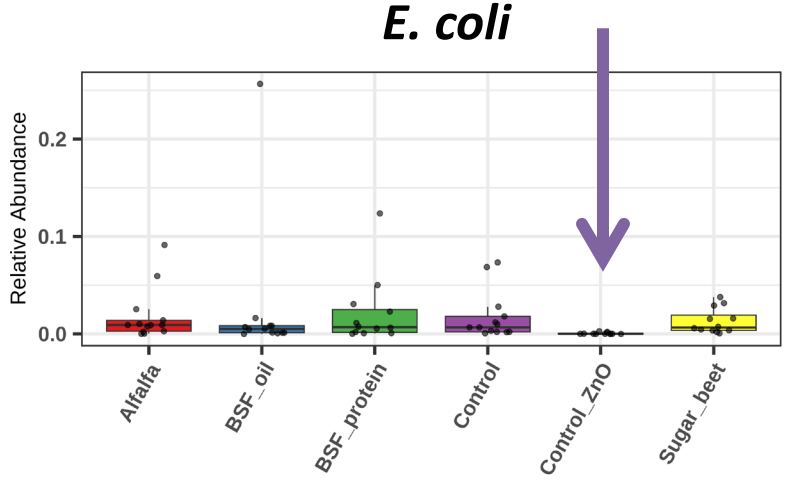
Colon

Treatment	Increased	Decreased
Control ZnO	20	49
Alfalfa	1	/
Sugar beet	2	1
BSF protein	1	1
BSF oil	1	1

E. coli



E. coli



Future perspective

- **Metabolites** analysis in the process (untargeted metabolomics)
- Offers a framework for granular information on dietary interventions
- Apply the **FeedOmics** approach to connect:
  - Transcriptomics
  - Microbiota
  - Metabolomics (untargeted, almost done!)
  - Blood parameters
  - Animal performances
- Presented only the ZnO results, the multi-omics approach will enable us to **deeply explore the underlying biological mechanisms of functional ingredients**, offering a more holistic view of their impact.

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Key Findings

- **No negative nor positive impact of function ingredients**
- **ZnO Supplementation:**
  - Significant improvement in early growth (ADG, ADFI) and feed conversion ratio (FCR)
  - Reduced haptoglobin levels, indicating a potential reduction in inflammation.
  - No long-term performance effects observed after 34 days.
- ZnO is particularly effective against **Gram-negative bacteria**, including *E. coli*
- *Needs to find alternatives*

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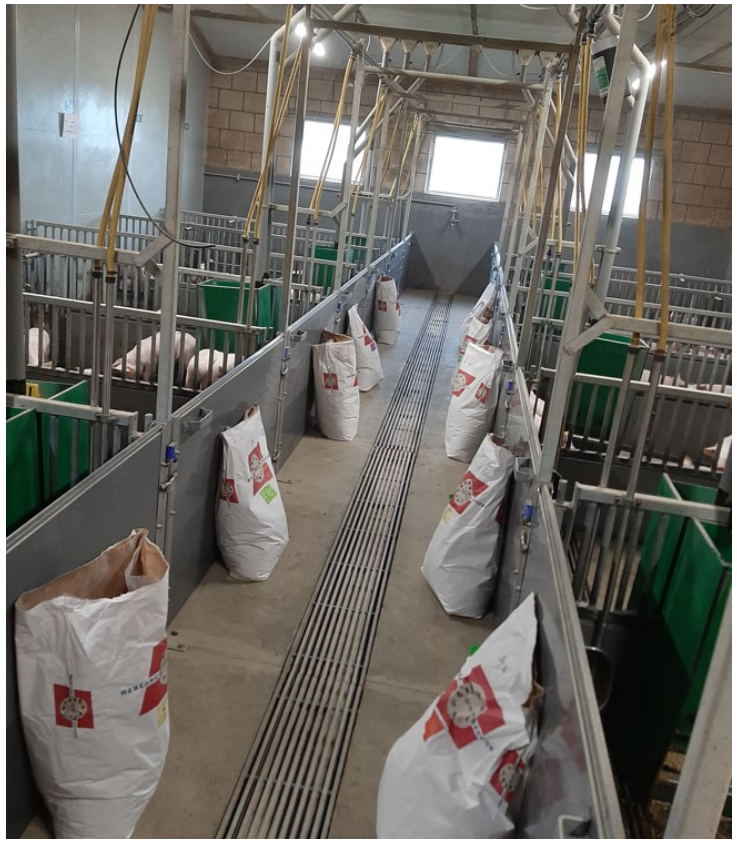
Thank you for your attention



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ACKNOWLEDGEMENTS

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Horizon 2020
European Union Funding
for Research & Innovation

Special thanks to the WLR teams

Soumya (Sam) Kar

Gisabeth Binnendijk

Piet van Wikselaar

Alphons Jansman

And

Mattia Pirolo for the 16S data

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No 862829.

PROJECT CONSORTIUM

Academia
University of Copenhagen (DK)
The Royal Veterinary College (UK)
Wageningen Research (NL)
Complutense University of Madrid (ES)
Swiss Federal Institute of Technology (CH)

Industry
BIOMIN (AT)
SEGES (DK)
Cooperl (FR)
Overjero Laboratories (SP)

SMEs/Associations
Easy-Agricare (DK)
Klifovet (DE)
Schotthorst Feed Research (NL)
Federation of Verterinarians of Europe (BE)
RTDS Group (AT)

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Luzernemeel/-brok-RE < 140 g/kg 5004.610/1/0

Weende analyse en koolhydraten (g/kg)

	DS	RAS	RE	RVET	RVETh	RC	OK	OKh
gem.	918	90	100	18	-	301	410	-
sdc	11	11	27	7	-	26	-	-

	ZETew	ZETam	GOS	SUI	NDF	ADF	ADL	NSP	RNSP
gem.	32	11	-	28	450	333	-	672	222
sdc	-	-	-	-	-	-	-	-	-

Mineralen (g/kg)

	Ca	P	IP	Mg	K	Na	Cl	S-a	S-o
gem.	9.1	2.4	0.1	1.5	18.0	0.9	5.3	2.1	0.6
sdc	2.7	0.4	-	0.3	5.6	0.5	1.3	-	-

Spoorelementen (mg/kg)

	Fe	Mn	Zn	Cu	Mo	J	Co
gem.	678	37	25	9	-	-	2.2
sdc	319	11	5	2	-	-	-

IP/P	5	SUIe/SUI	-	EB (meq/kg)	350
		CF_DI	0.97	KAV (meq/kg)	181

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Sugarbeet pulp, dried-SUG < 100 g/kg 4004.209/1/0

Weende analysis and carbohydrates (g/kg)

	DM	ASH	CP	CFAT	CFATh	CF	NFE	NFEh
mean	893	61	75	9	14	175	573	568
sdc	5	17	6	2	3	8	-	-

	STAew	STAam	GOS	SUG	NDF	ADF	ADL	NSP	RNSP
mean	-	7	-	68	377	200	11	671	299
sdc	-	-	-	11	-	-	-	-	-

Minerals (g/kg)

	Ca	P	IP	Mg	K	Na	Cl	S-i	S-o
mean	9.9	0.8	0.2	2.4	4.0	0.5	0.4	1.4	0.5
sdc	1.9	0.1	-	0.6	1.4	0.3	0.3	-	-

Trace elements (mg/kg)

	Fe	Mn	Zn	Cu	Mo	J	Co
mean	475	57	17	5	0.4	0.1	0.3
sdc	182	10	8	1	0.2	0.1	0.2

IP/P	30	SUGe/SUG	95	EB (meq/kg)	115
		CF_DI	0.97	CAD (meq/kg)	-6

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