
Perturbation of gut homeostasis in pigs by a high dietary concentration of zinc

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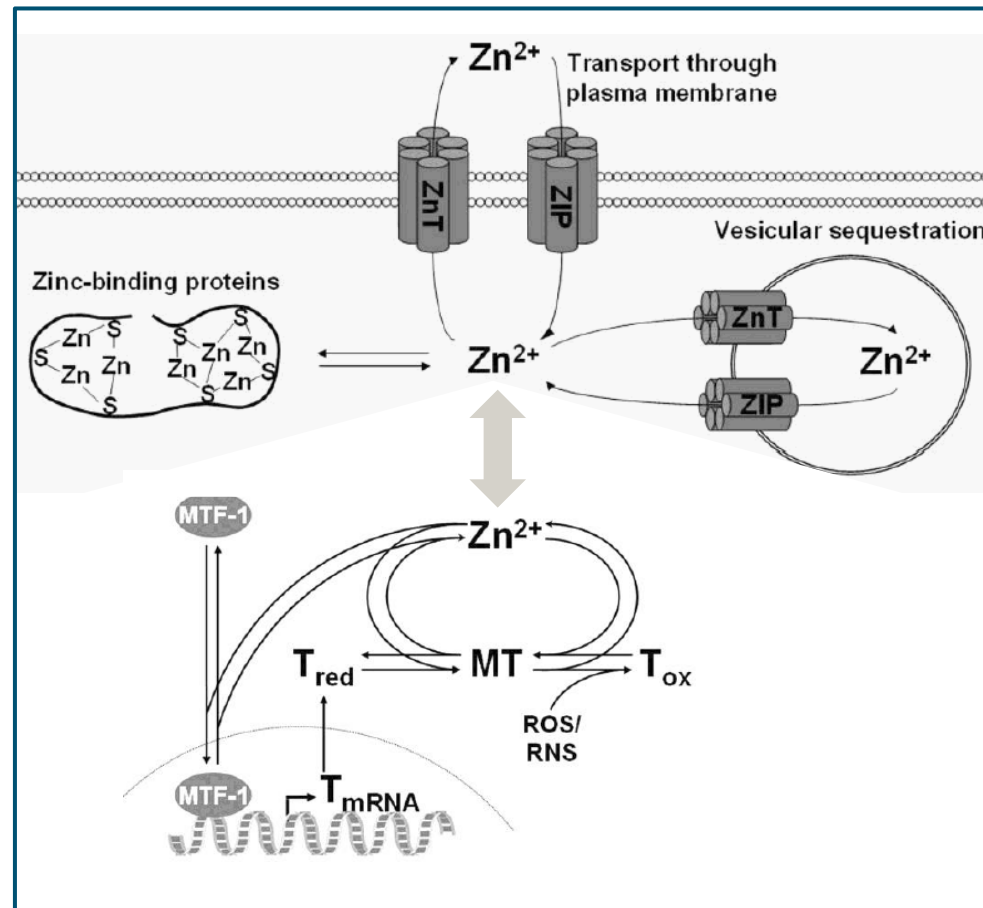


Pleiotropic effects of high dietary zinc concentrations in pigs and mode of action

- Prevents diarrhoea
- Growth promoting effect

- Intestinal microbiota
- Barrier function of the gut
- Immune system
- Digestion / nutrient absorption
- Metabolism

Background – cellular zinc homeostasis



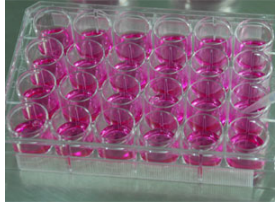
Zn transporters
Zn binding proteins
Metallothionein

Zinc: 300 enzymes, > 300 proteins (for example zinc-finger proteins)

Objective

- **Study effects of exposure to high zinc concentration**
 - **Using *in vitro* cultured cells**
 - **Using genome-wide gene expression analysis**
 - *By *in vivo* study in pigs*
 - *Using genome-wide gene expression analysis*
 - *Using community scale analysis of microbiota*

Exposure of *in vitro* cultured porcine intestinal epithelial cells



IPEC-J2 cells



Zinc homeostasis: Zinc transporters (SLC30A9, SLC39A4)
Metallothionein (MT1A)

Immune function: IL6, TNF, interferon, complement

Cell proliferation: p53, ErbB4 pathways

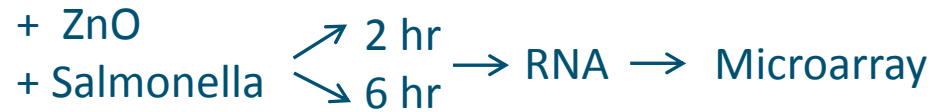
Metabolism: Insulin signalling

Hypoxia: HIF1-network, HMOX1

Zinc reduces immune response towards Salmonella



IPEC-J2 cells



Regulated immune genes

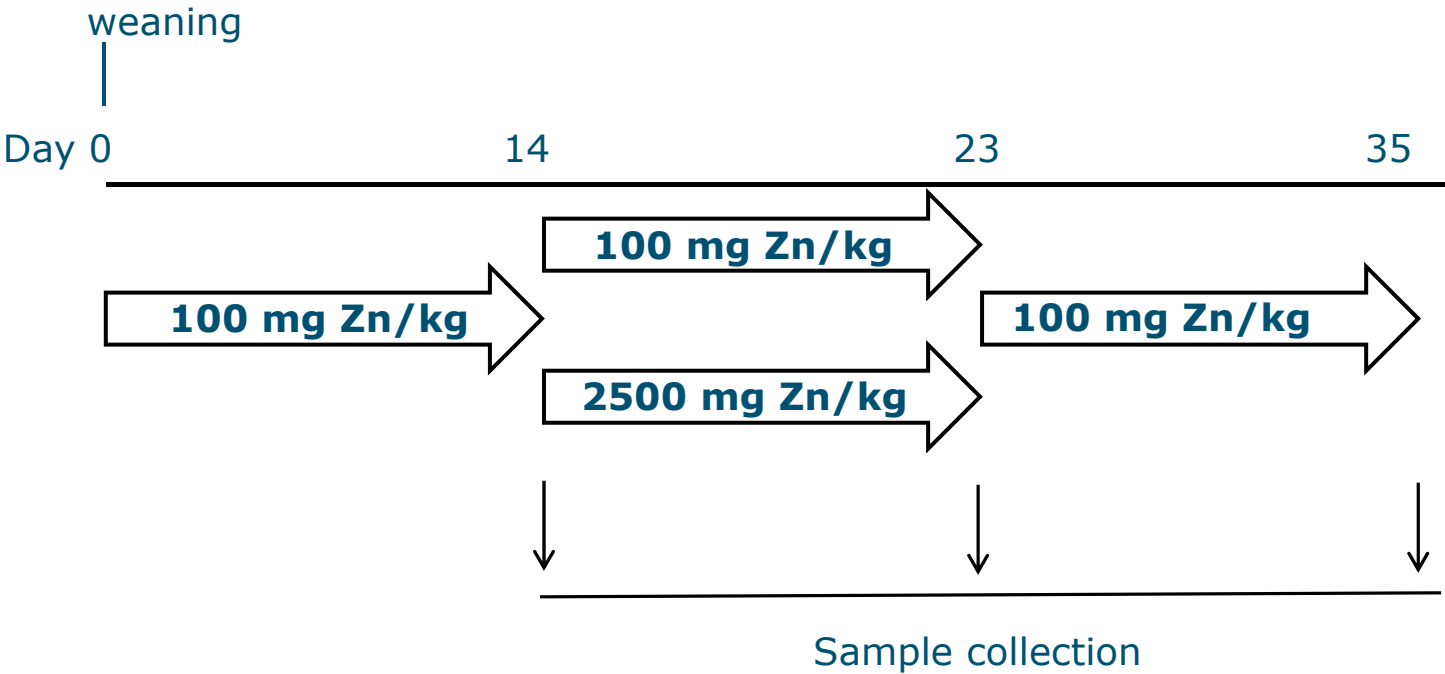
2 h		6 h	
Salmonella	ZnO + Salmonella	Salmonella	ZnO + Salmonella
IL8 IER3 NFKBIE TFAP2C NFKBIA ARHGEF2 FOS CSF2 TNFAIP3 CHAC1 DDIT4	IL8 IER3 NFKBIE TFAP2C NFKBIA ARHGEF2 FOS	CSF2 DDIT4 FOS IL8 MGAT3 TEX14 NFKBIA CTHRC1 TNFAIP3 IL1B NFKBIE CLDN3 CCN2 HSPA1A	CSF2 DDIT4 FOS IL8

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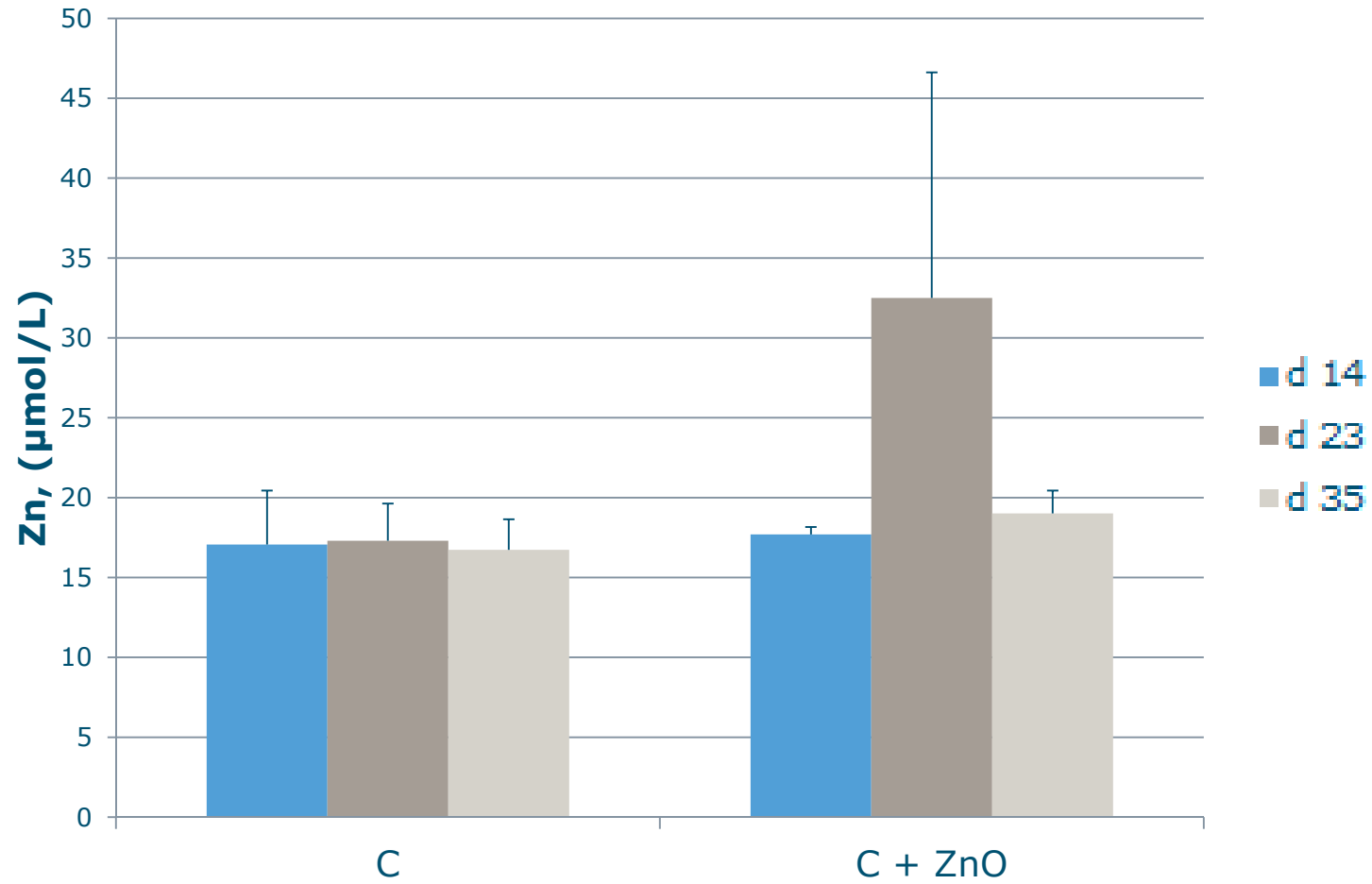
Design *in vivo* study



Growth, feed intake, feed conversion

	100 ZnO	2500 ZnO
<u>Body weight gain (g/d/d)</u>		
D0-14	219	226
D14-23	442	485
D23-35	682	618
D0-35	395	396
<u>Feed intake (g/d/d)</u>		
D0-14	285	291
D14-23	635	672
D23-35	1004	923
D0-35	558	556
<u>Feed Conversion Ratio</u>		
D0-14	1.30	1.29
D14-23	1.44	1.39
D23-35	1.47	1.49
D0-35	1.41	1.40

Zinc in blood



Change in microbiota composition

Jejunum

Ileum

Day 23

yes

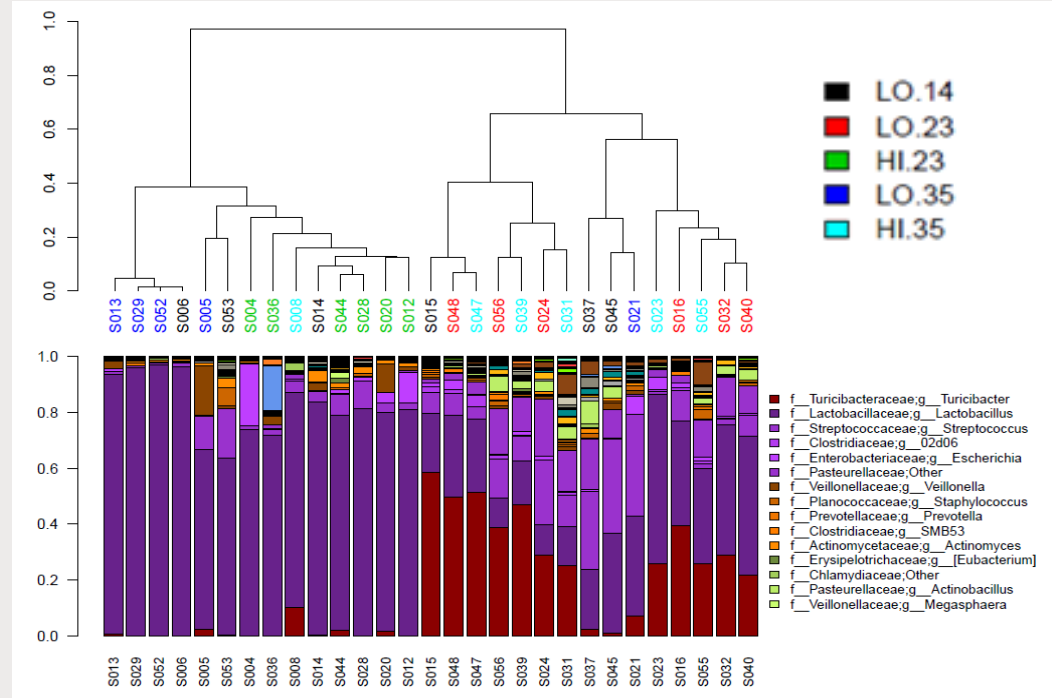
yes

Day 35

no

no

Ileum



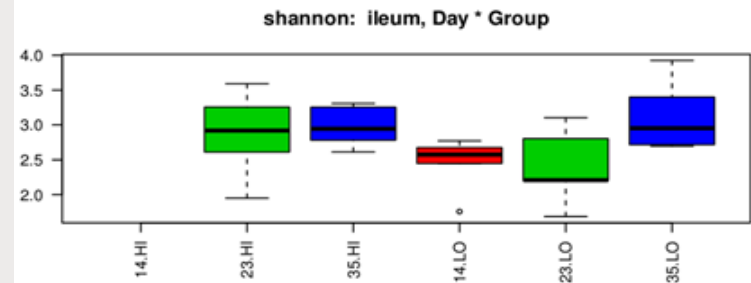
Change in microbiota diversity

Jejunum

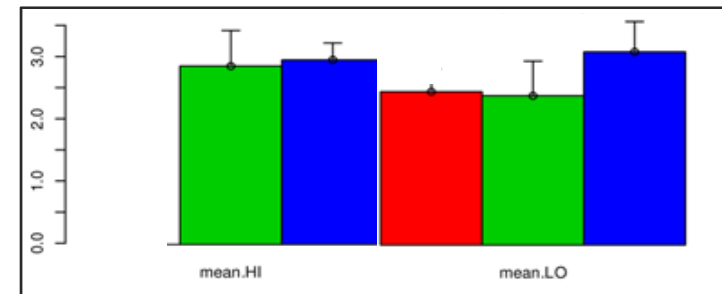
Ileum

Day 23	no	yes
Day 35	no	no

Ileum



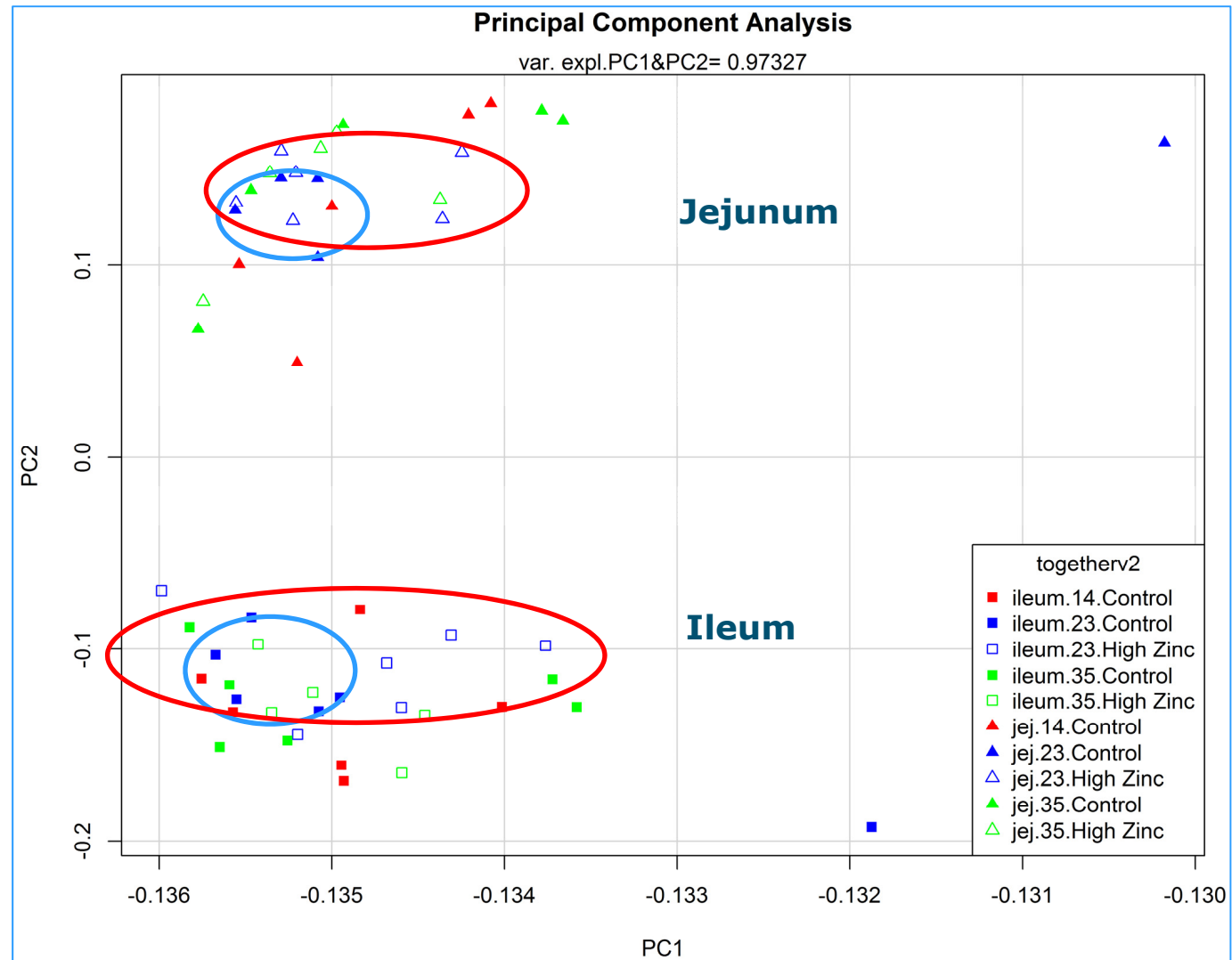
■ 14 ■ 23 ■ 35
 ← High ← Low →



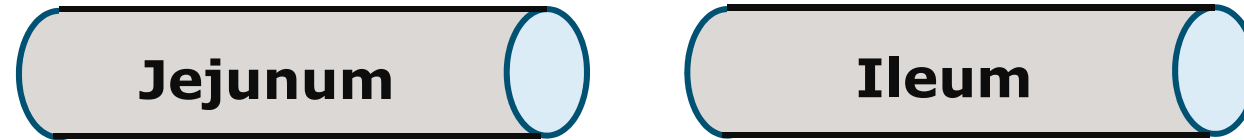
Mucosal gene expression

low

high



Number of regulated genes^{ab} (high vs low zinc)



Day 23	19	15
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Day 23 up	8	10
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Day 23 down	11	5
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Day 35	0	0
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Day 35 up	0	0
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Day 35 down	0	0
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^a Annotated genes

^b Adjusted P value < 0.05 and log Fold Change > |1.5|

Summary: functional analysis *in vivo* gene expression

Day 23

Jejunum

Ileum

Metallothionein (MT1A)	+++	+++
Zinc transporters	++	++
Zinc binding	+	+
Metabolic control / rate	+	-
Metabolism	+	+
Cell proliferation / differentiation	++	-
Immune function	++	+

Conclusions

- Temporary effects of high zinc on intestinal microbiota and gene expression in the intestinal mucosa

- High dietary level of zinc affects microbiota composition (jejunum, ileum) and diversity (ileum)

- High dose of zinc affects host gene expression
- Functional overlap between *in vitro* and *in vivo* systems
 - *In vitro*: stronger response / stress response
- Number of affected processes in jejunum > ileum
- Affected processes: zinc homeostasis, metabolism, cell proliferation / differentiation, immune function

Thank you for your attention



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