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

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Pleotropic 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

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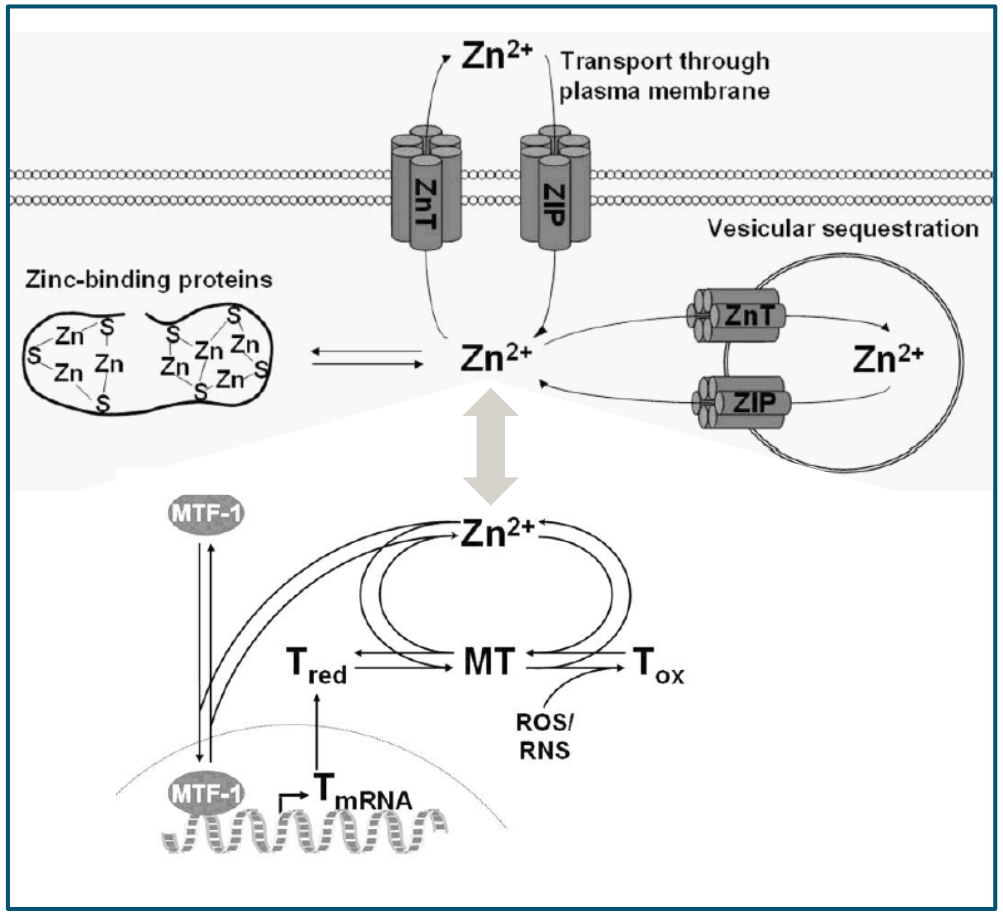
Background – cellular zinc homeostasis



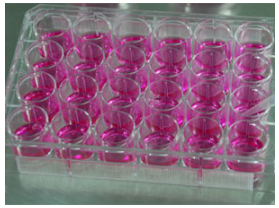
Zn transporters
Zn binding proteins
Metallothionein

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

+ ZnO → 2 hr / 6 hr → RNA → Microarray → 73 / 153 regulated genes

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**

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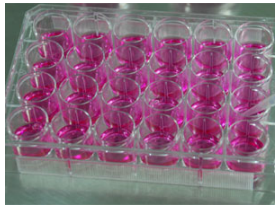
Zinc reduces immune response towards Salmonella



IPEC-J2 cells

+ ZnO
+ Salmonella
→ 2 hr / 6 hr → RNA → Microarray

Regulated immune genes

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

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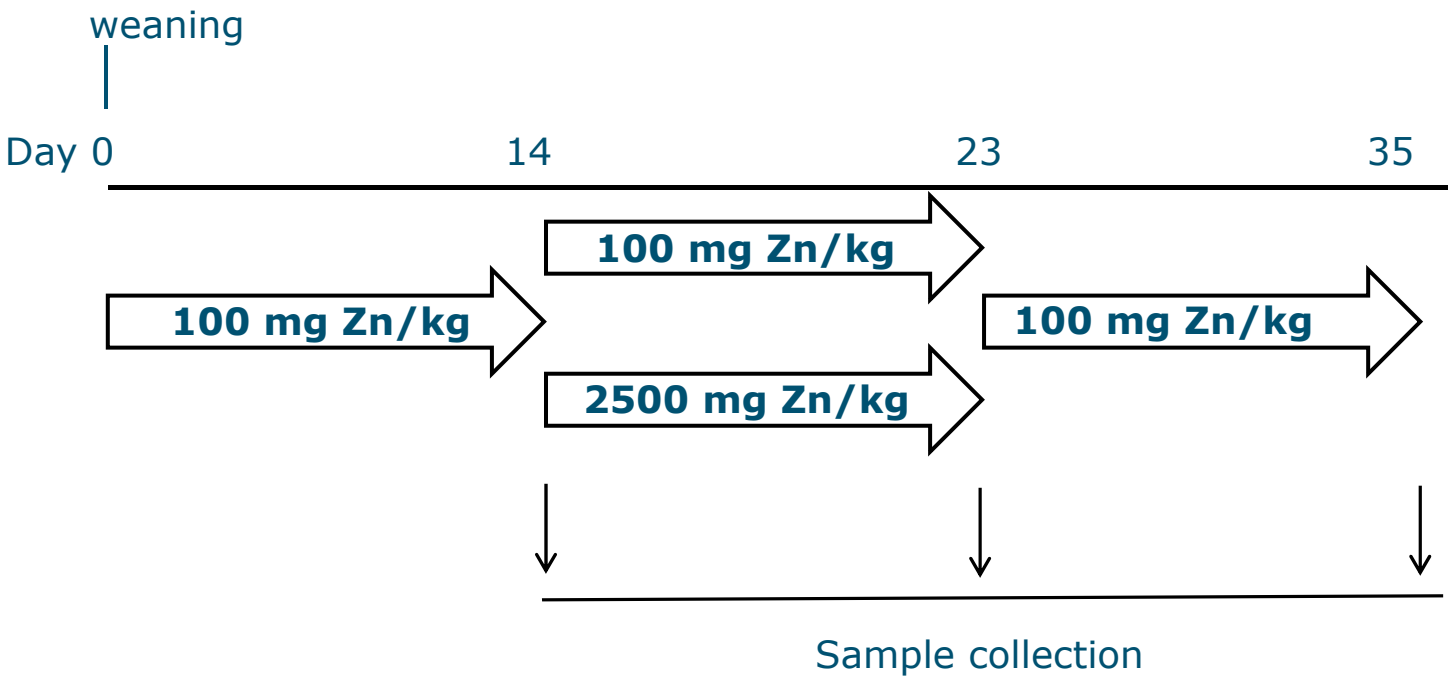
Design *in vivo* study



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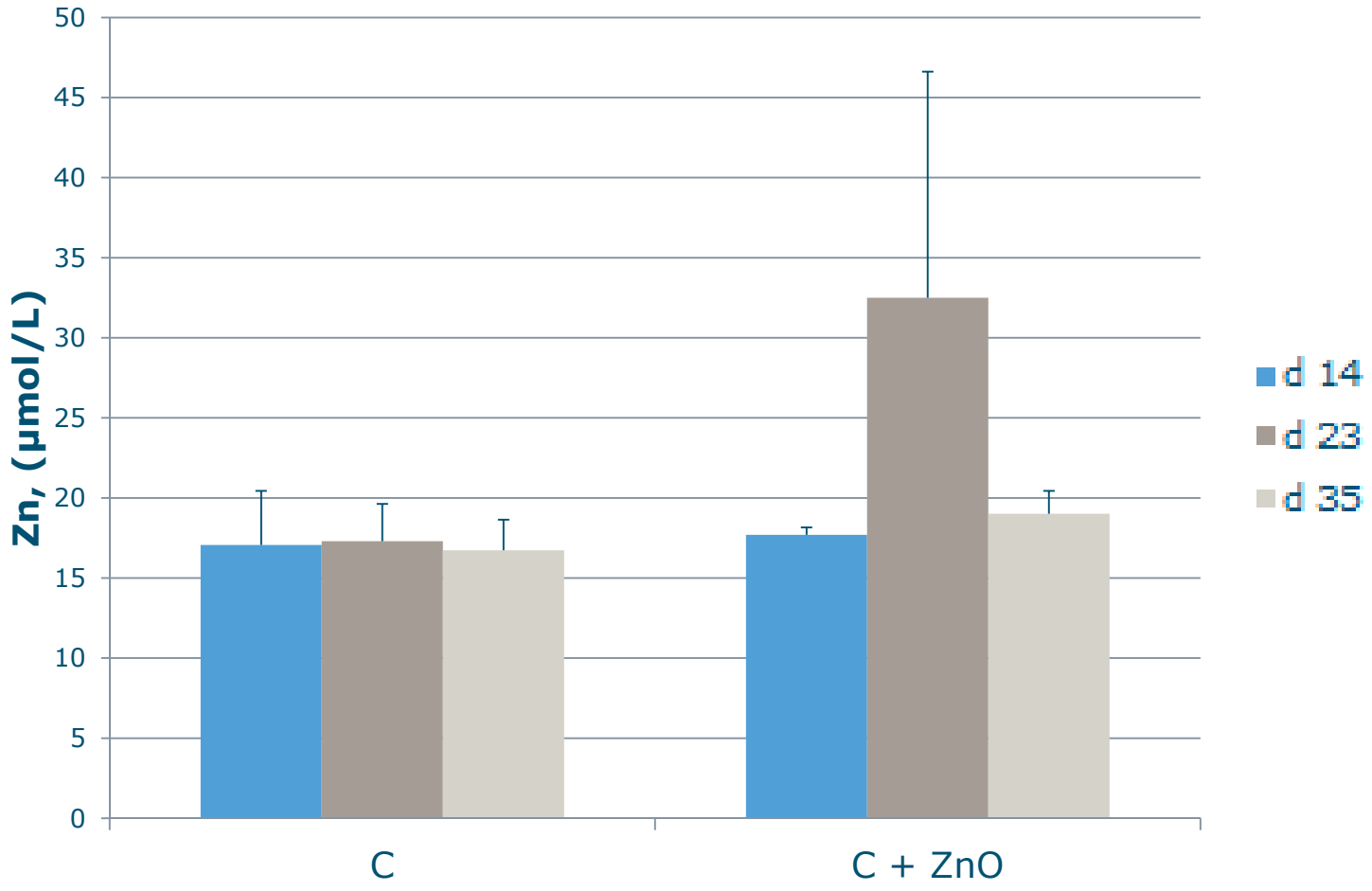
Growth, feed intake, feed conversion

	100 ZnO	2500 ZnO
Body weight gain (g/d/d)		
D0-14	219	226
D14-23	**442**	**485**
D23-35	682	618
D0-35	**395**	**396**
Feed intake (g/d/d)		
D0-14	285	291
D14-23	**635**	**672**
D23-35	1004	923
D0-35	**558**	**556**
Feed Conversion Ratio		
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



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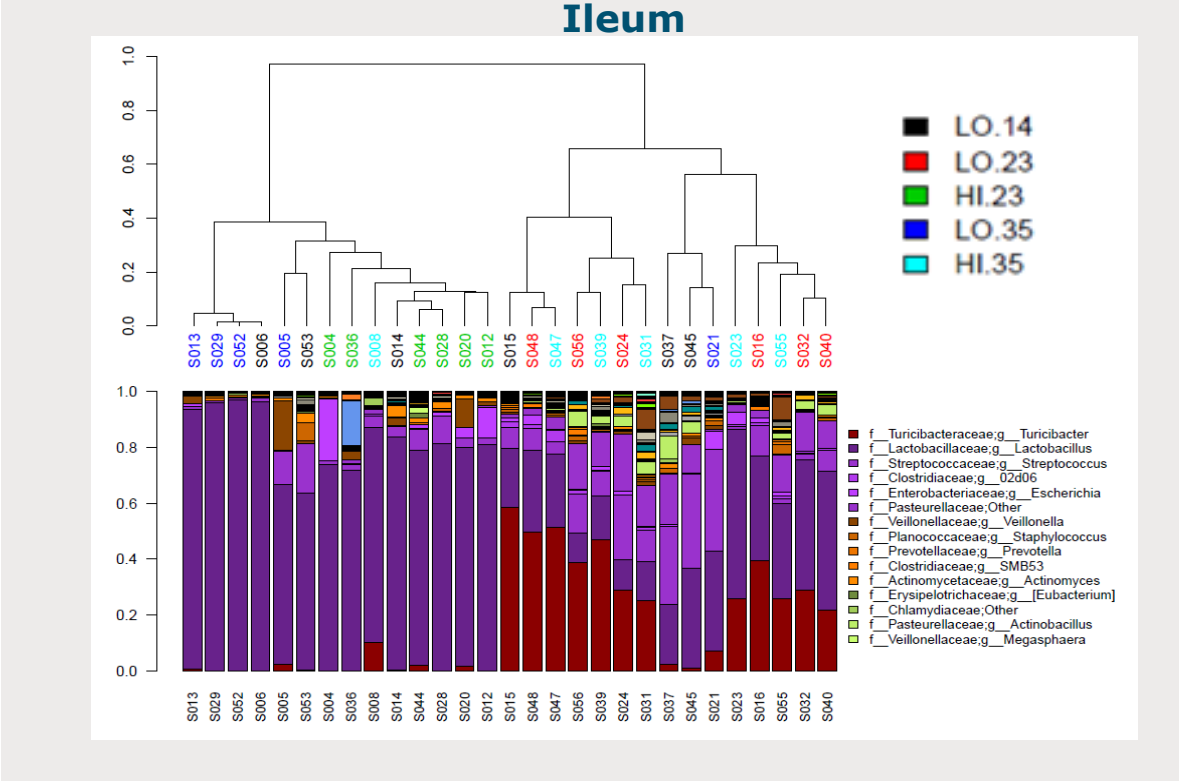
Change in microbiota composition

	Jejunum	Ileum
Day 23	yes	yes
Day 35	no	no



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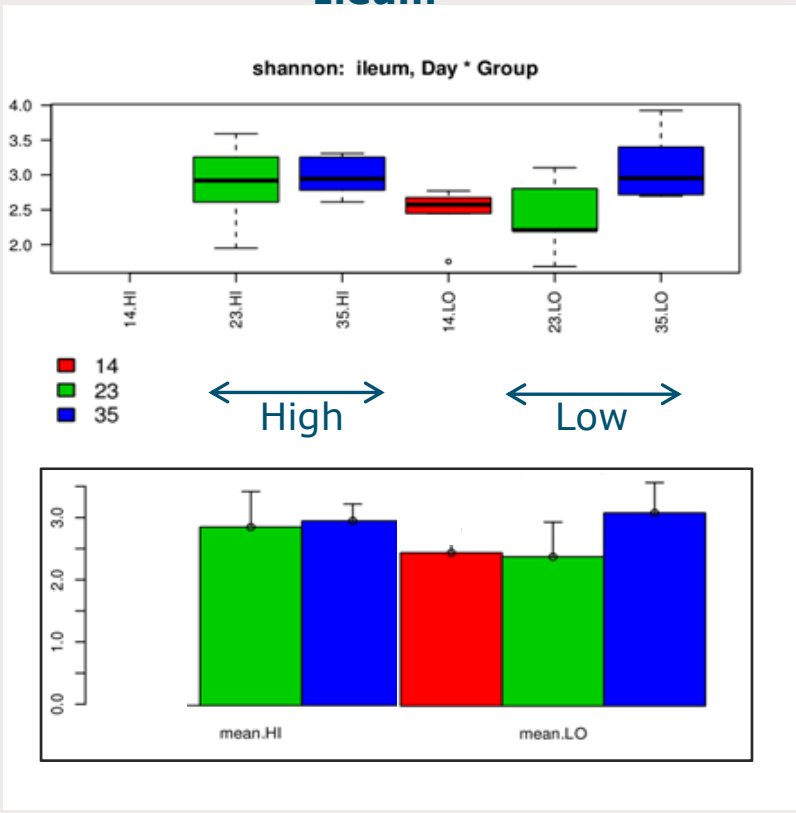
Change in microbiota diversity

	Jejunum	Ileum
Day 23	no	yes
Day 35	no	no

Ileum



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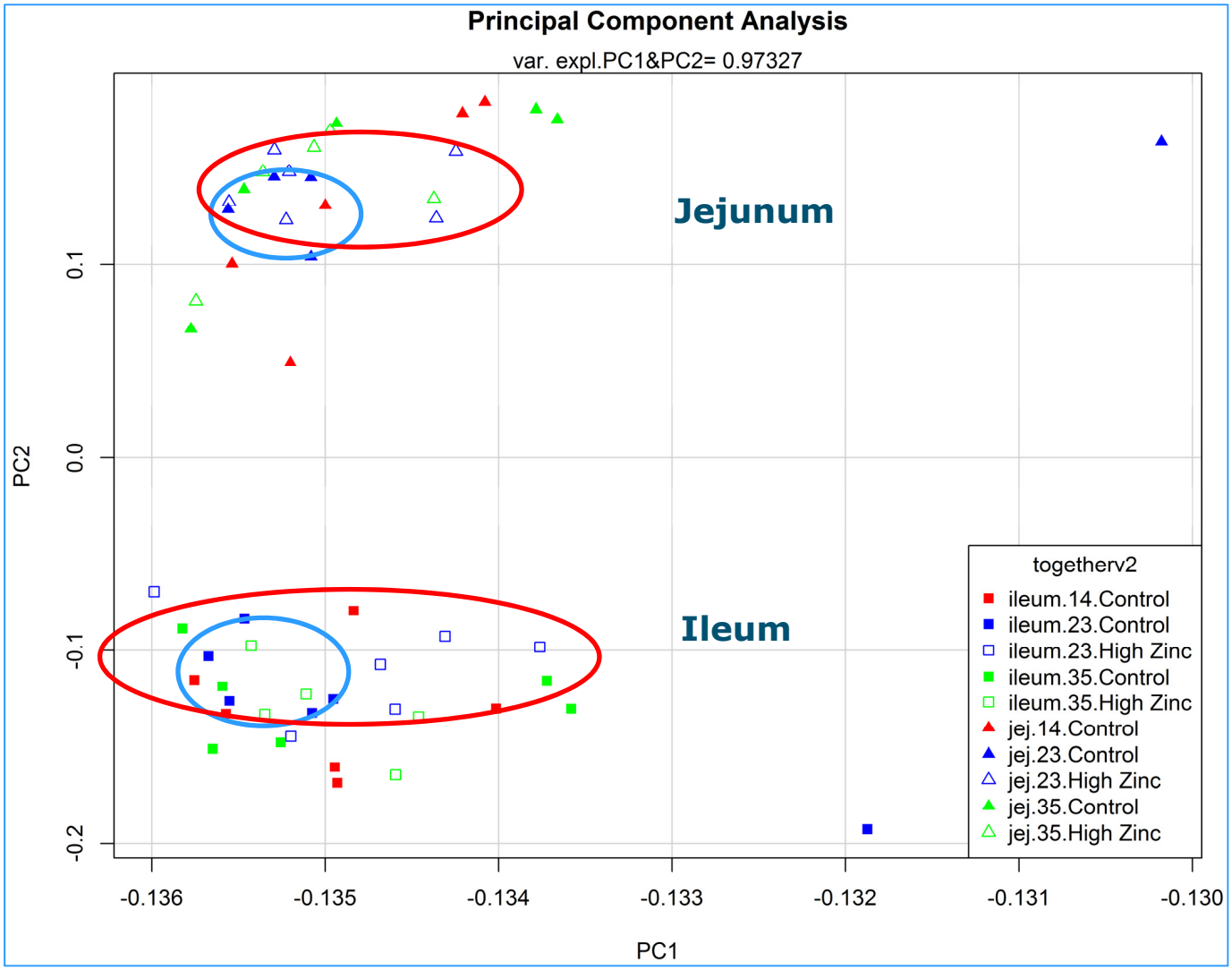
Mucosal gene expression

low

high



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No differences at day 35

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Number of regulated genes[ab] (high vs low zinc)

	Jejunum	Ileum
Day 23	19	15
Day 23 up	8	10
Day 23 down	11	5
Day 35	0	0
Day 35 up	0	0
Day 35 down	0	0

[a] Annotated genes

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

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Summary: functional analysis *in vivo* gene expression

Day 23	Jejunum	Ileum
Metallothonein (MT1A)	+++	+++
Zinc transporters	++	++
Zinc binding	+	+
Metabolic control / rate	+	-
Metabolism	+	+
Cell proliferation /differentiation	++	-
Immune function	++	+

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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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