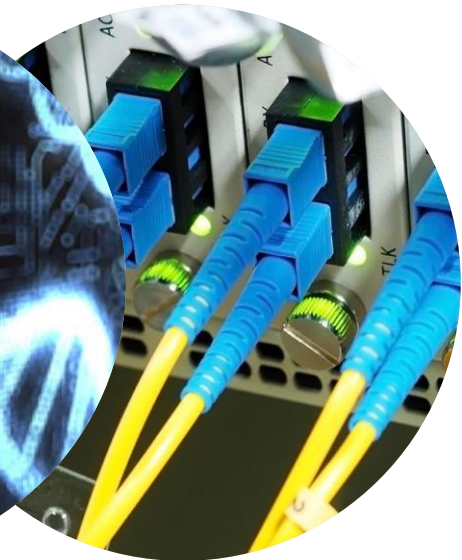


# Data mining of *in vitro* and *in vivo* dietary intervention datasets

Dirkjan Schokker, Ilonka Wijers, Mari Smits, and Marcel Hulst



# Acknowledgements



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**MSD - Animal Health**

**VanDrie Group**

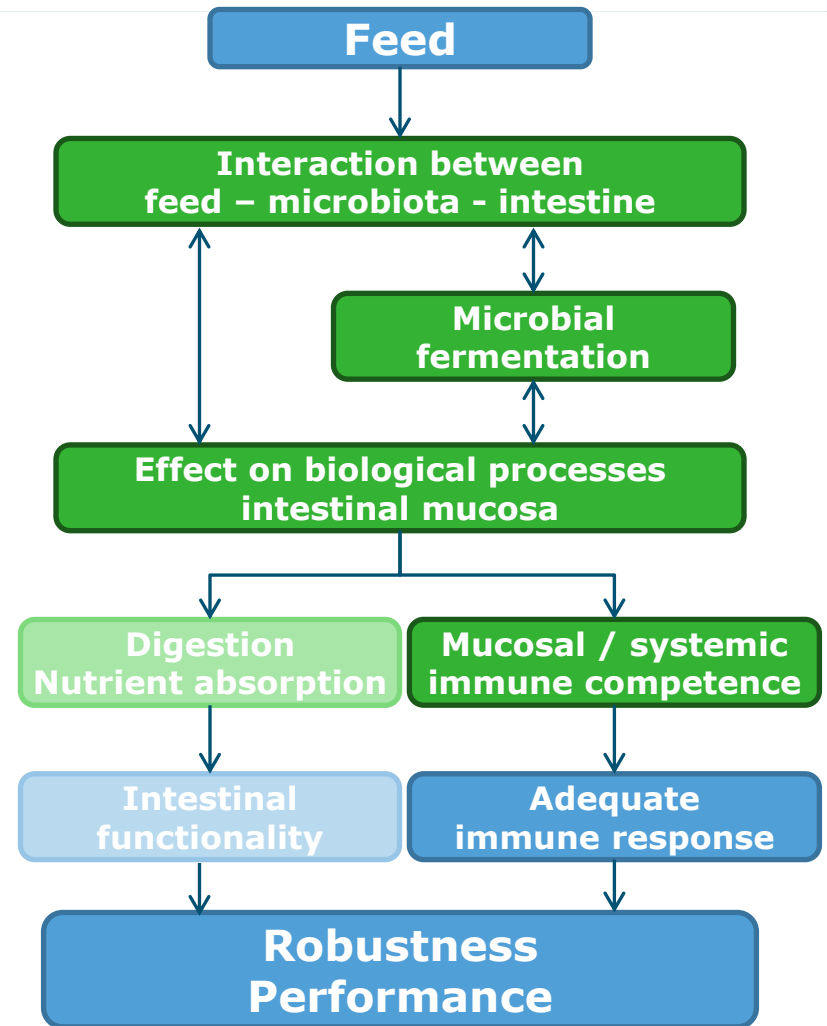
**Denkavit**



**Animal Breeding &  
Genomics Centre**

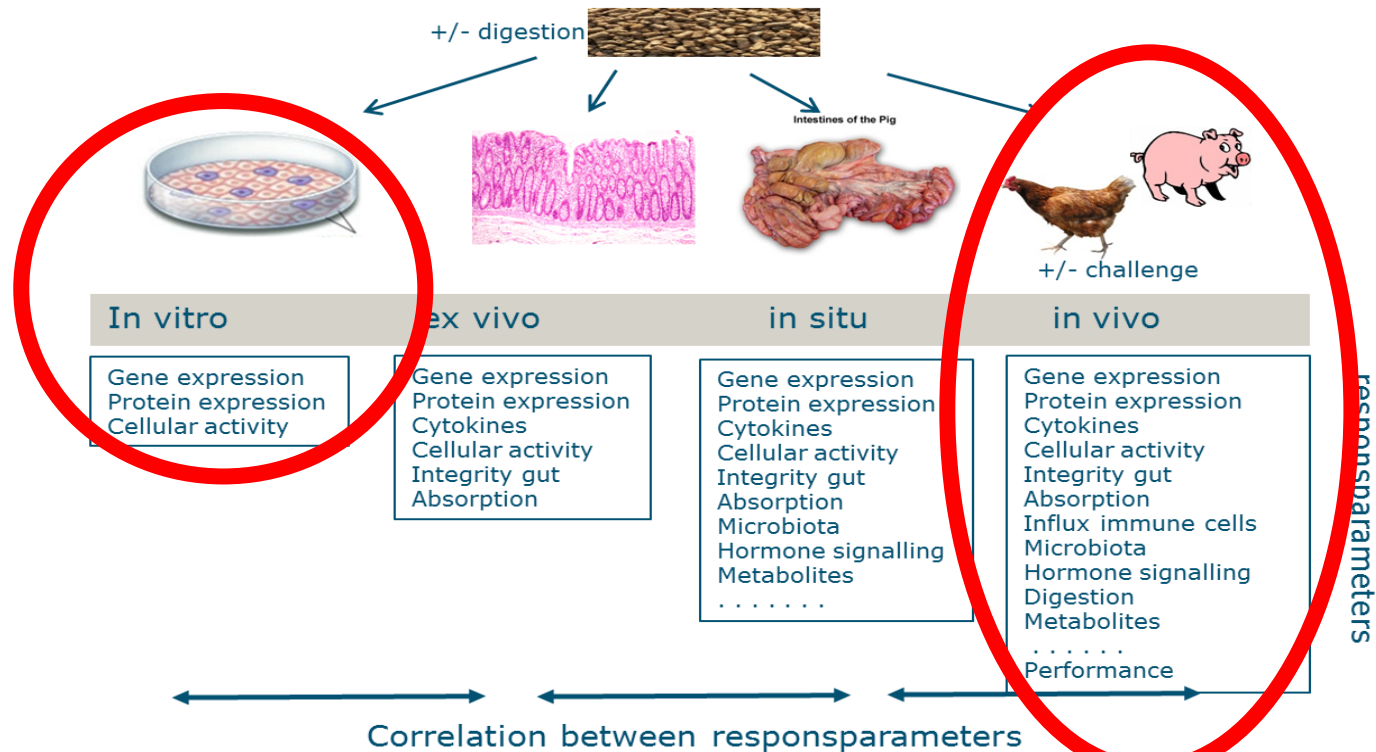
# Background

- **Goal of Feed4Foodure**  
Determine immune competence of livestock after dietary interventions
- **Immune competence**  
potential to adequately respond to stimuli



# Objective

- Identify overlap in the response between *in vitro* and *in vivo* dietary interventions



# *in vitro* → IPEC-J2 cells

Modulation of *Salmonella*-induced cytokine/chemokine response by additives.

gene	hrs	Salm./M	ZnO/M	ZnO-Salm./M	Oct/M	Oct-Salm./M	5% rye-Salm./5% rye
IL8	2	↑↑	↓↓	-	↑	↑	-
CXCL2	2	↑↑	↑	↑	↑	↑	↓
CXCL2	6	↑	↓↓	↑	↓↓	↓↓	-
CSF2	2	↑↑	↑↑	↑	↑↑	↑↑	-
CSF2	6	↑	-	-	-	-	↑↑
IL1A	2	↑↑	↑	-	↑	↑	-
IL1A	6	↑↑	↓↓	-	↓↓	↓↓	-
IL6	2	-	↓	-	↓	↓	↓
IL6	6	-	-	↑	↓	↓	-
CCL20	6	↑	↓	-	-	↓	-
IFNA4	2	-	-	-	-	-	↓↓*
IFNA4	6	↓	-	↑↑	-	-	-
IFNL1	2	-	↑↑	-	-	-	-
IFNL1	6	-	-	-	↑↑	↑↑	-
IL18	6	↓	-	↓	-	↑	-
IL21	6	↑	-	↑↑	-	-	-
IL1B	6	-	-	↑↑	-	-	-
LTA	6	-	-	↑↑	-	-	-

**System is able to express a range of immune parameters**

# Approach - Data mining + resources



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# Results (1) Separate analysis

## *in vitro*

<i>vs.</i>		<i>in vitro</i> 2h	<i>in vitro</i> 6h
<b>ZnO</b>	<b>Mock</b>	<b>178</b>	<b>722</b>
<b>Amox.</b>	<b>Mock</b>	<b>92</b>	<b>707</b>
<b>10%rye</b>	<b>Mock</b>	<b>571</b>	<b>23</b>
<b>5%rye</b>	<b>Mock</b>	<b>208</b>	<b>28</b>
<b>10%rye</b>	<b>5%rye</b>	<b>125</b>	<b>47</b>

Number of differential expressed genes

## *in vivo*

<i>vs.</i>		<i>Time 1</i>	<i>Time 2</i>
<b>ZnO</b>	<b>Control</b>	<b>101</b>	<b>11</b>
<b>ZnO</b>	<b>Control</b>	<b>20</b>	<b>11</b>
<b>Amox.</b>	<b>Control</b>	<b>54</b>	<b>38</b>
<b>10%rye</b>	<b>Control</b>	<b>239</b>	<b>223</b>
<b>5%rye</b>	<b>Control</b>	<b>320</b>	<b>34</b>
<b>10%rye</b>	<b>5%rye</b>	<b>48</b>	<b>192</b>

# Results (2) Overlap

<i>in vivo</i> comparison	Day	Genes		Pathways	
		<i>in vitro</i> 2h	<i>in vitro</i> 6h	<i>in vitro</i> 2h	<i>in vitro</i> 6h
<b>Zinc/Control</b>	23	4_1*	3_3*	7_1*	2_0*
<b>Zinc/Control</b>	35	0_1*	2_1*	2_3*	2_4*
<b>Rye; 10%/Control(0%)</b>	1	11	1	1	1
<b>Rye; 5%/Control(0%)</b>	21	9	1	4	2
<b>Rye;10%/5% day 21</b>	21	0	0	1	0
<b>Rye; 10%/Control(0%)</b>	28	8	0	3	3
<b>Rye; 5%/Control(0%)</b>	28	1	0	0	0
<b>Rye;10%/5%</b>	28	6	0	4	0
<b>Amoxicillin/Control</b>	5	0	1	2	2
<b>Amoxicillin/Control</b>	14	0	2	1	1



# Dietary interventions tested

## ■ Zinc supplementation

- Improve health
- Decrease incidence of post weaning diarrhoea

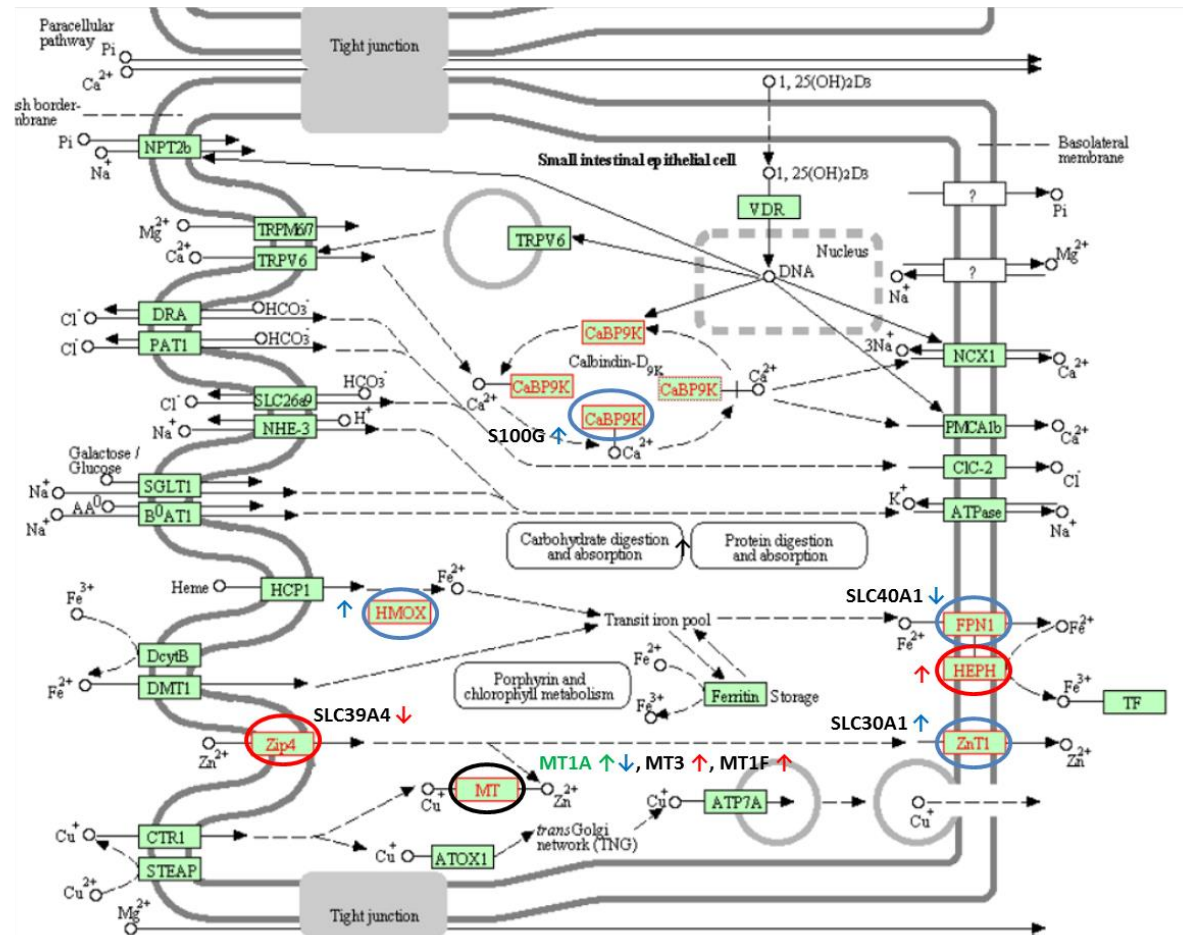
## ■ Increasing rye concentrations

- Higher viscosity
- Anti-nutritive activity
- Positive effect intestinal mucosal immunity

## ■ Addition of amoxicillin

- Perturb microbial community
- Immune development

# Zinc - Mineral absorption pathway

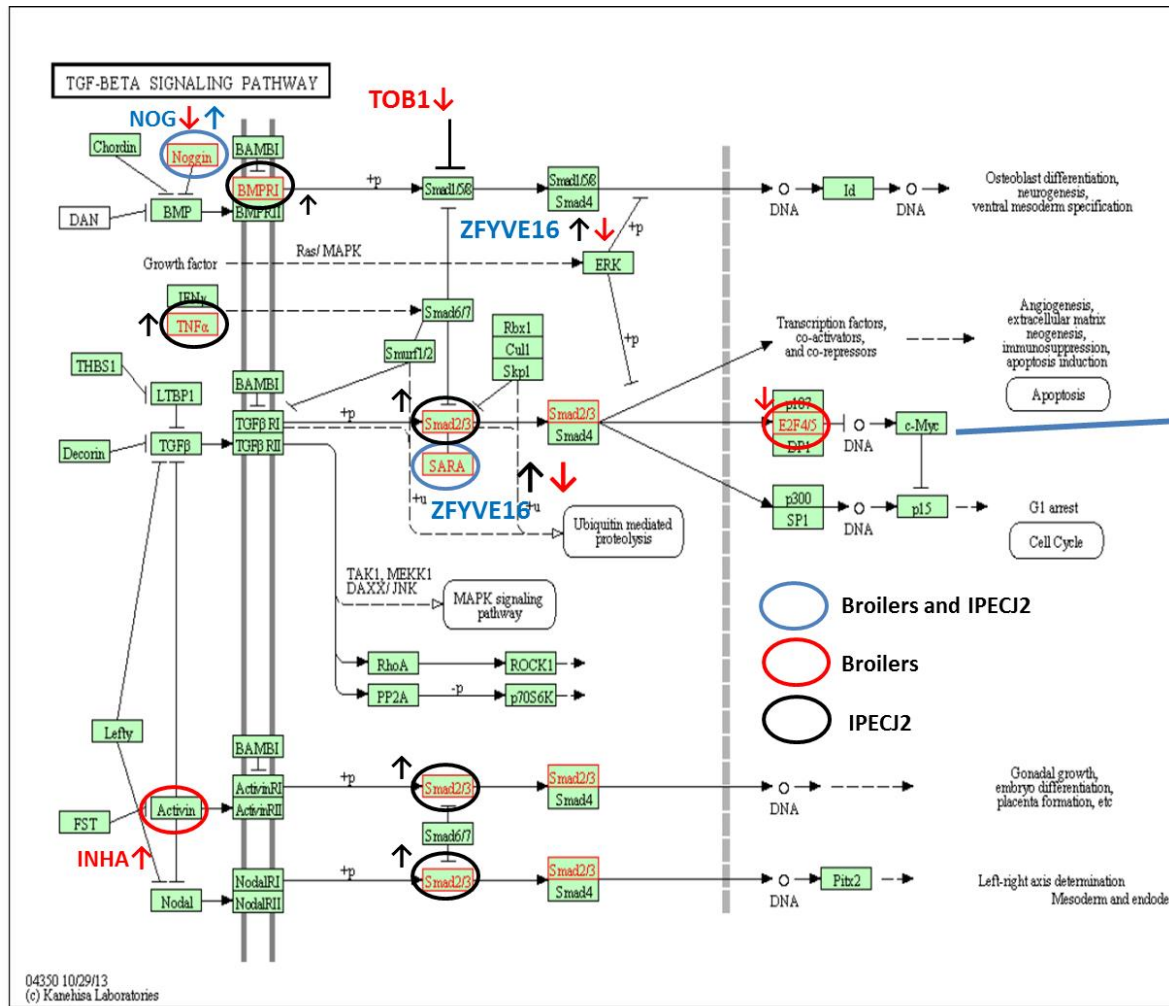


Jejunum 23 days; SLC39A4, MT1A, MT3, MT1F / Ileum 23 days; HEPH, SLC39A4, MT1A, MT3, MT1F / Ileum 35 days; MT1A

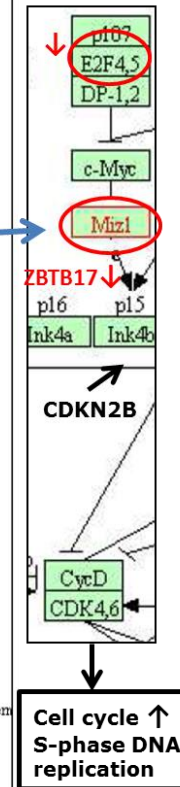
IPEC-J2 2 hrs; MT1A ↑, HMOX1, SLC40A1

IPEC-J2 6 hrs; MT1A ↓, S100G

# Rye - TGF-beta signalling



## Cell-cycle



# Amoxicillin

- Reactive oxygen species (ROS) was found to be the overlapping process, ROS may activate CREB activity which may lead to apoptosis
- Disturbs the microtubule filaments in cells of the intestinal mucosa (maybe even barrier function)
  - Down-regulation of the STMN1 gene, responsible for induction of “MTub catastrophe”

# Amoxicillin

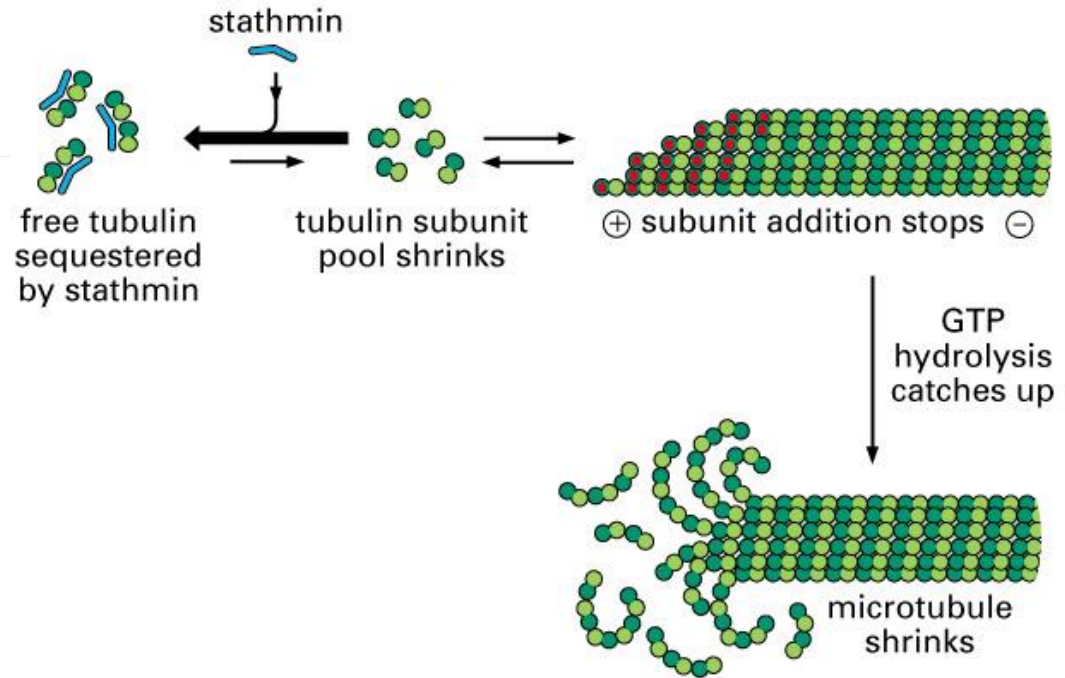


Figure 16–31. Molecular Biology of the Cell, 4th Edition.

- Disturbs the microtubule filaments in cells of the intestinal mucosa (maybe even barrier function)
  - Down-regulation of the STMN1 gene results in less disassembly

# Dietary interventions tested

## ■ Zinc supplementation

- Improve health
- Decrease incidence of post weaning diarrhoea

## ■ Increasing rye concentrations

- Higher viscosity
- Anti-nutritive activity
- Positive effect intestinal mucosal immunity

## ■ Addition of amoxicillin

- Perturb microbial community
- Immune development

## Overlap analysis

**Immune**

**Cell  
Proliferation**

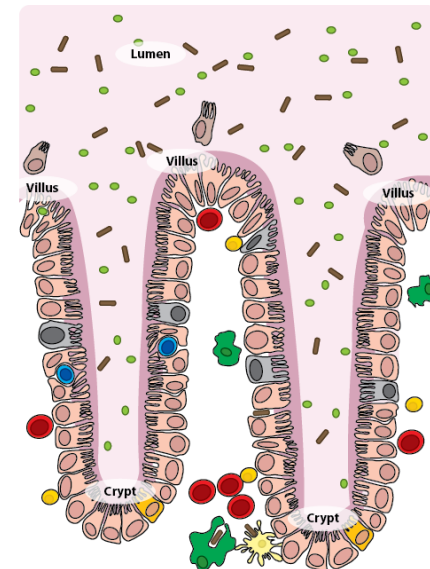
**No direct  
effects**

# Conclusions

- **Minor overlap but meaningful**



VS.



- Insight in direct effect on epithelial cells
  - Almost no direct effect of amoxicillin
- Stress response / ingredient specific?



# Discussion

- *in vitro* vs. *in vivo*  
**predictive value limited in this set up**
- Alternatives to *in vitro* models to better mimic *in vivo*
  - Mixed cell cultures
  - Organoids
  - Small Intestinal Segment Perfusion
- Focus on direct effects



# Thank you for your attention



[dirkjan.schokker@wur.nl](mailto:dirkjan.schokker@wur.nl)



+31 (0)317 480537



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