

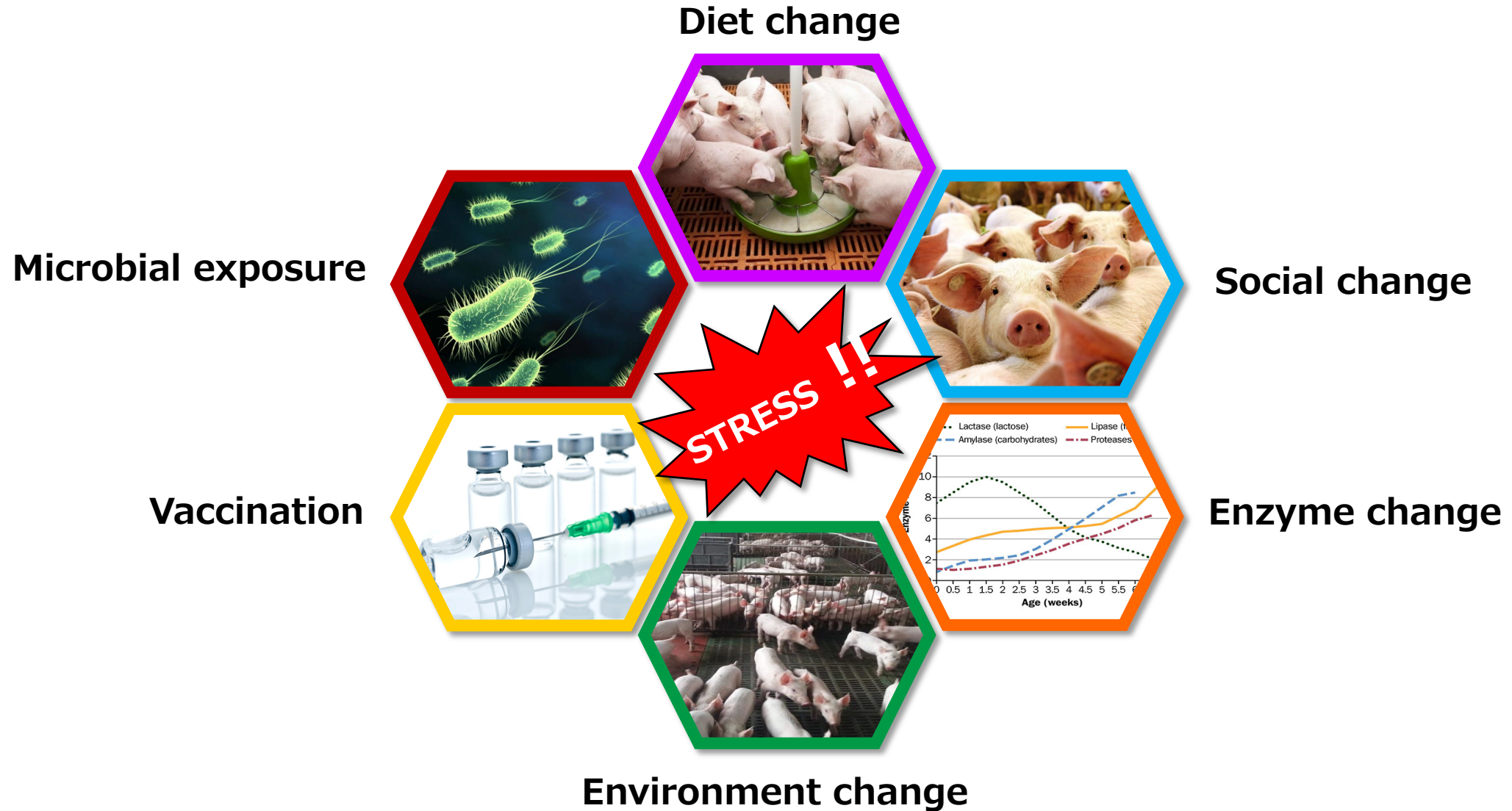
# Effects of L-glutamate and L-aspartate supplementation on intestinal immunity and intestinal barrier integrity of weaned piglets challenged with F18 ETEC

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<sup>1</sup>Department of Animal Science, University of California, Davis, USA;

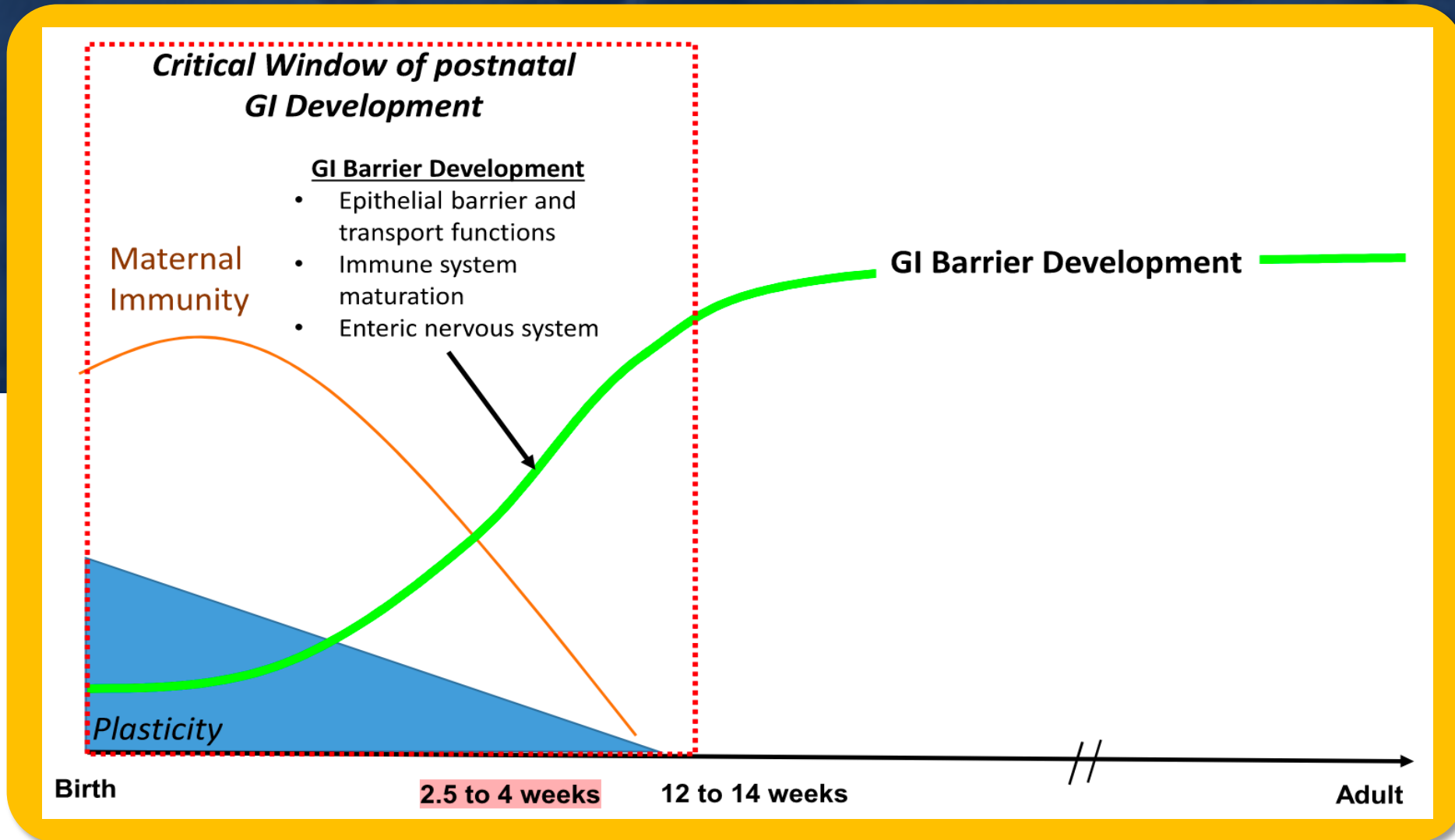
<sup>2</sup>Department of Population Health & Reproduction,  
University of California, Davis, USA

# Why does the weaning phase hold such significance?



# Weaning stress

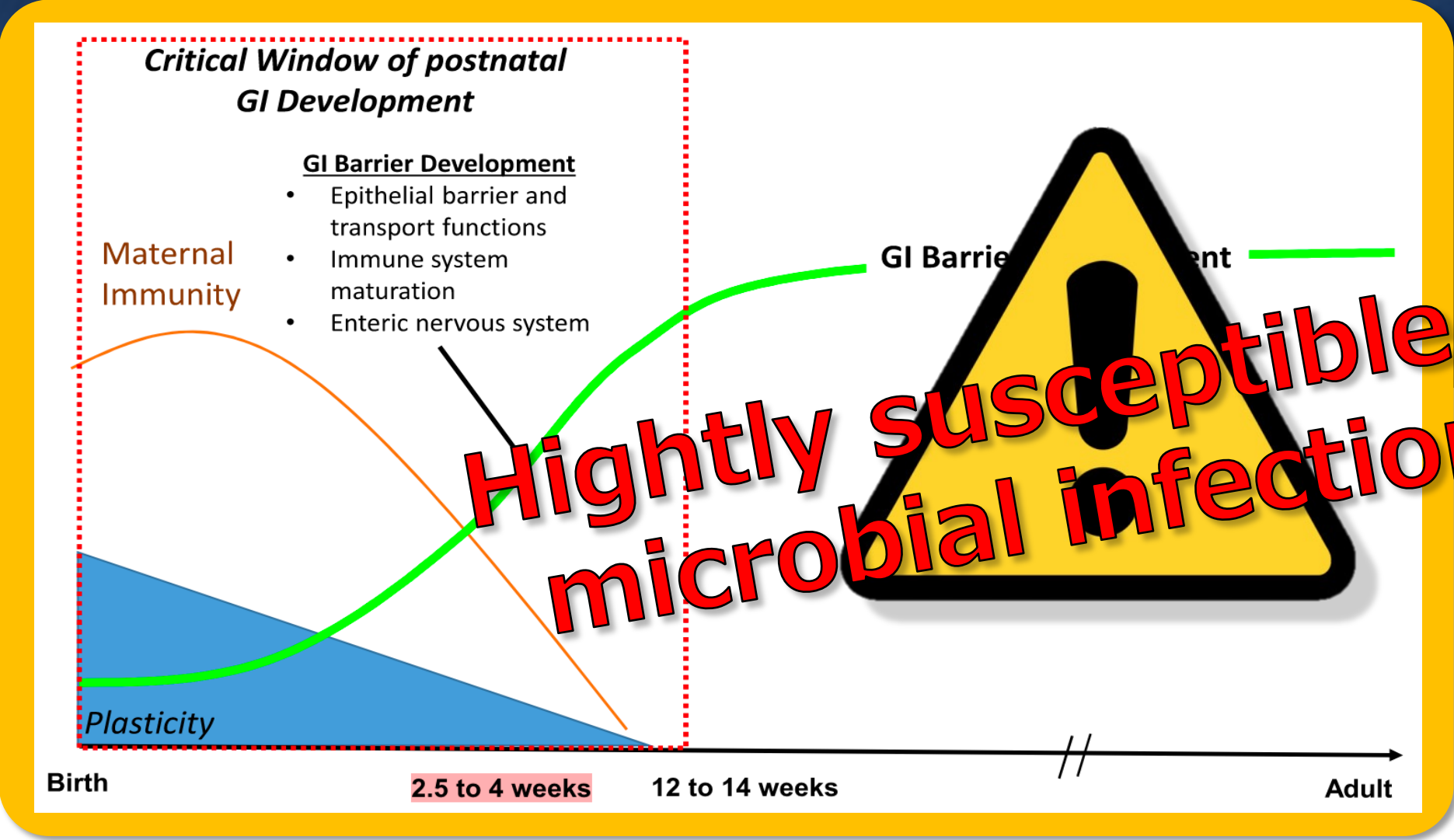
often occur during a critical window of gut development

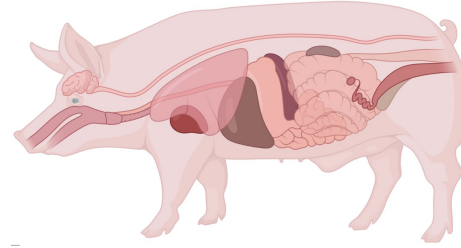
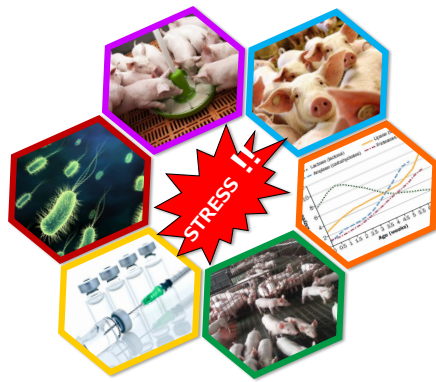




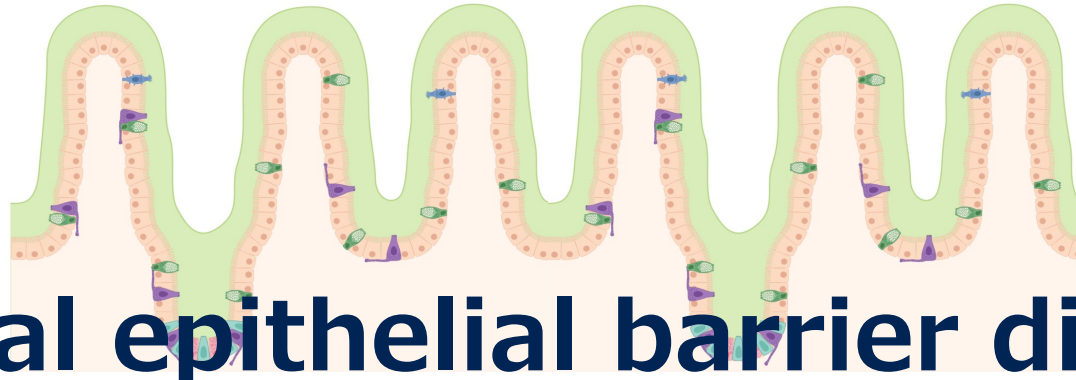
# Weaning stress

often occur during a critical window of gut development





Oxidative stress  
Stress hormones  
Gut infection  
Lower feed intake



# “Intestinal epithelial barrier disruption”



Morphological damage

Goblet cells injury

Disruption of tight junction proteins

Pro-inflammatory cytokines production

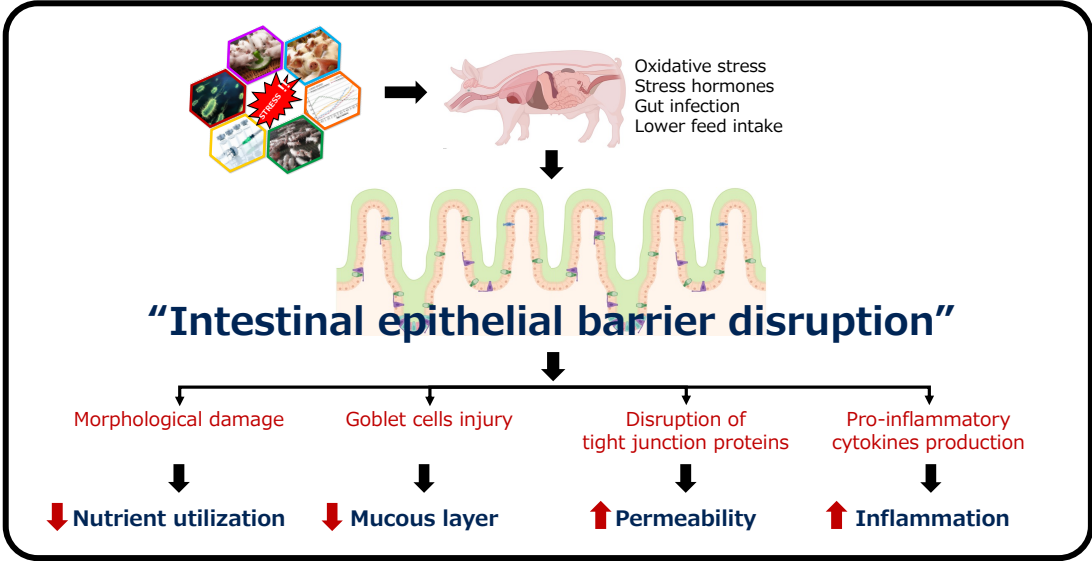


↓ Nutrient utilization

↓ Mucous layer

↑ Permeability

↑ Inflammation

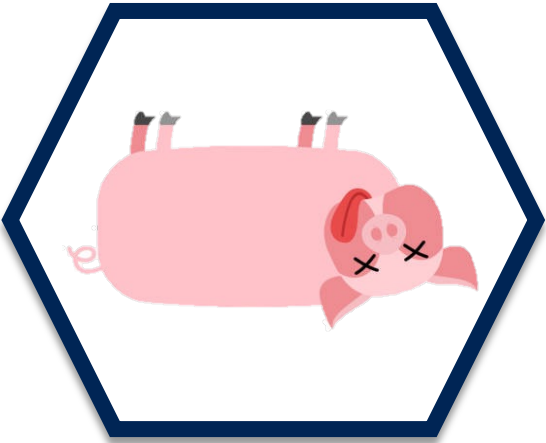


**Diarrhea**

**Growth retardation**

**Death**

**Financial losses**



# What makes **Glu & Asp** so interesting?



*- Dietary non-essential -*

# Glu

Glutamate

## - Functional Amino Acids -

# Asp

Aspartate

Energy source



Energy source

Antioxidant



Antioxidant

Cell proliferation



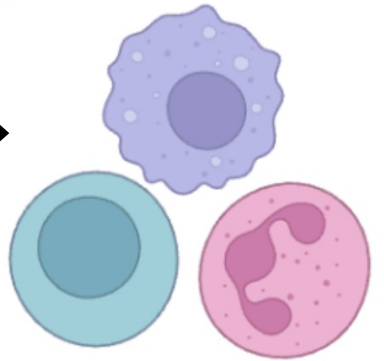
Nucleotide synthesis

Amino acid synthesis



Amino acid synthesis

Immunomodulator

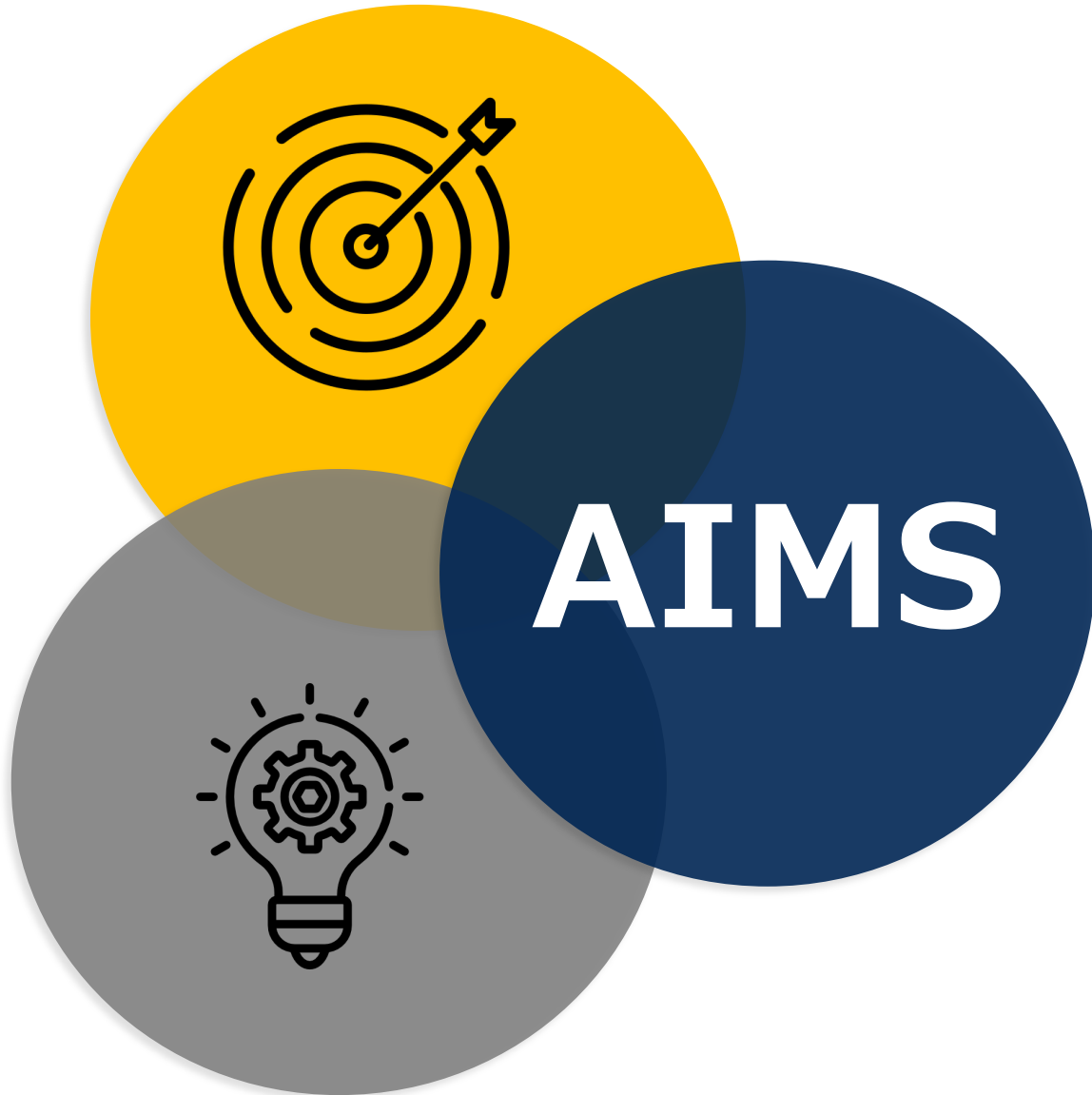


Immune supporter



Can supplementing with  
**Glu & Asp**  
alleviate weaning  
complications ?





To investigate the effects of the dietary supplementation of **Glu or Asp** in weaned pigs challenged with F18 ETEC on

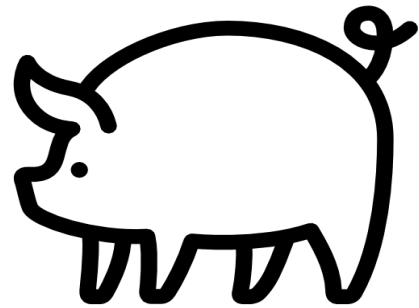


**Intestinal immunity**



**Intestinal integrity**

# Experimental design



weaned piglets

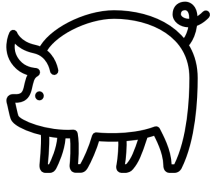


## Dietary treatments

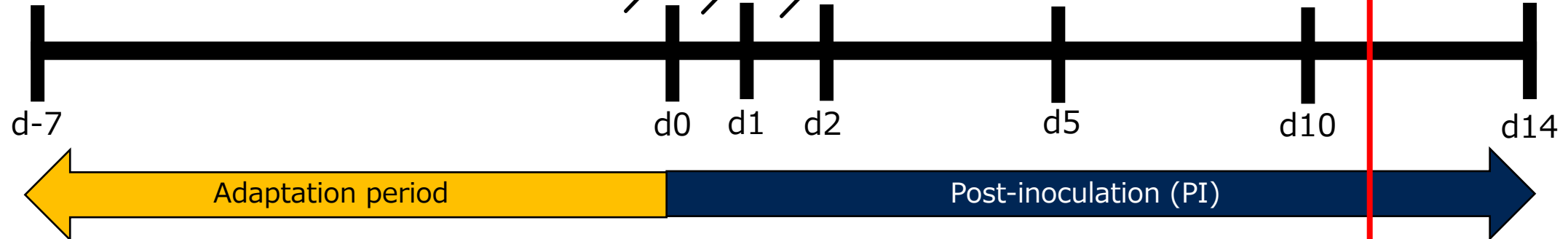
1. Basal diet without ETEC challenge (**NC**)
2. Basal diet with ETEC challenge (**PC**)
3. PC + 1% Glu
4. PC + 2% Glu
5. PC + 1% Asp
6. PC + 2% Asp
7. PC + 50 mg/kg carbadox (**Car**)

# Timeline

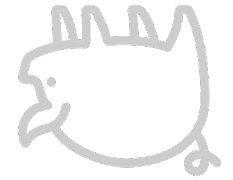
49 weaned piglets  
n = 7 pigs/treatment



Weaning day  
(~24-25 days old)



Intestinal mucosa  
collection

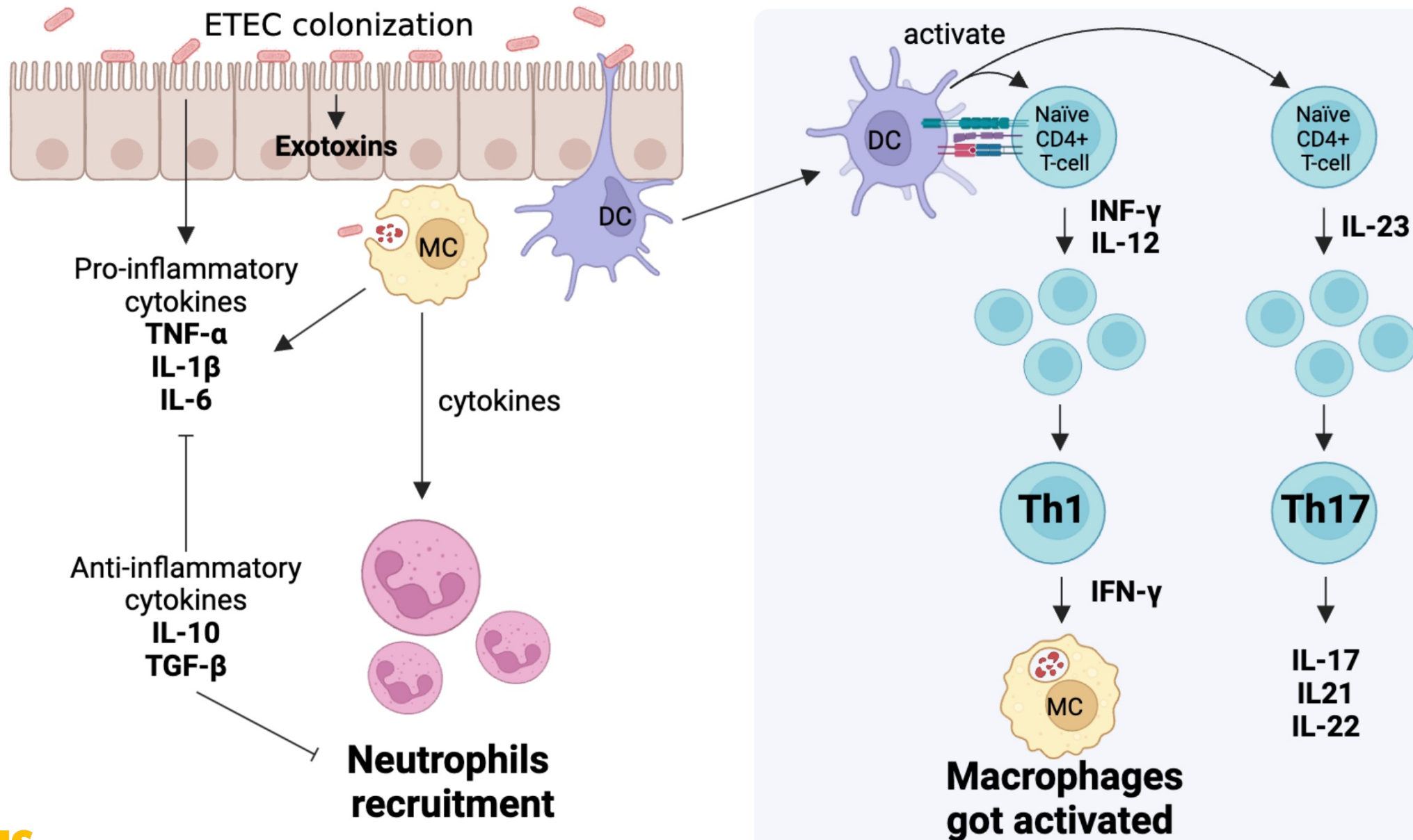


Necropsy  
At recovery

**Analysis:** ANOVA, PROC MIXED of SAS ( $P \leq 0.05$ ), pig as the experimental unit



# ETEC Pathogenesis



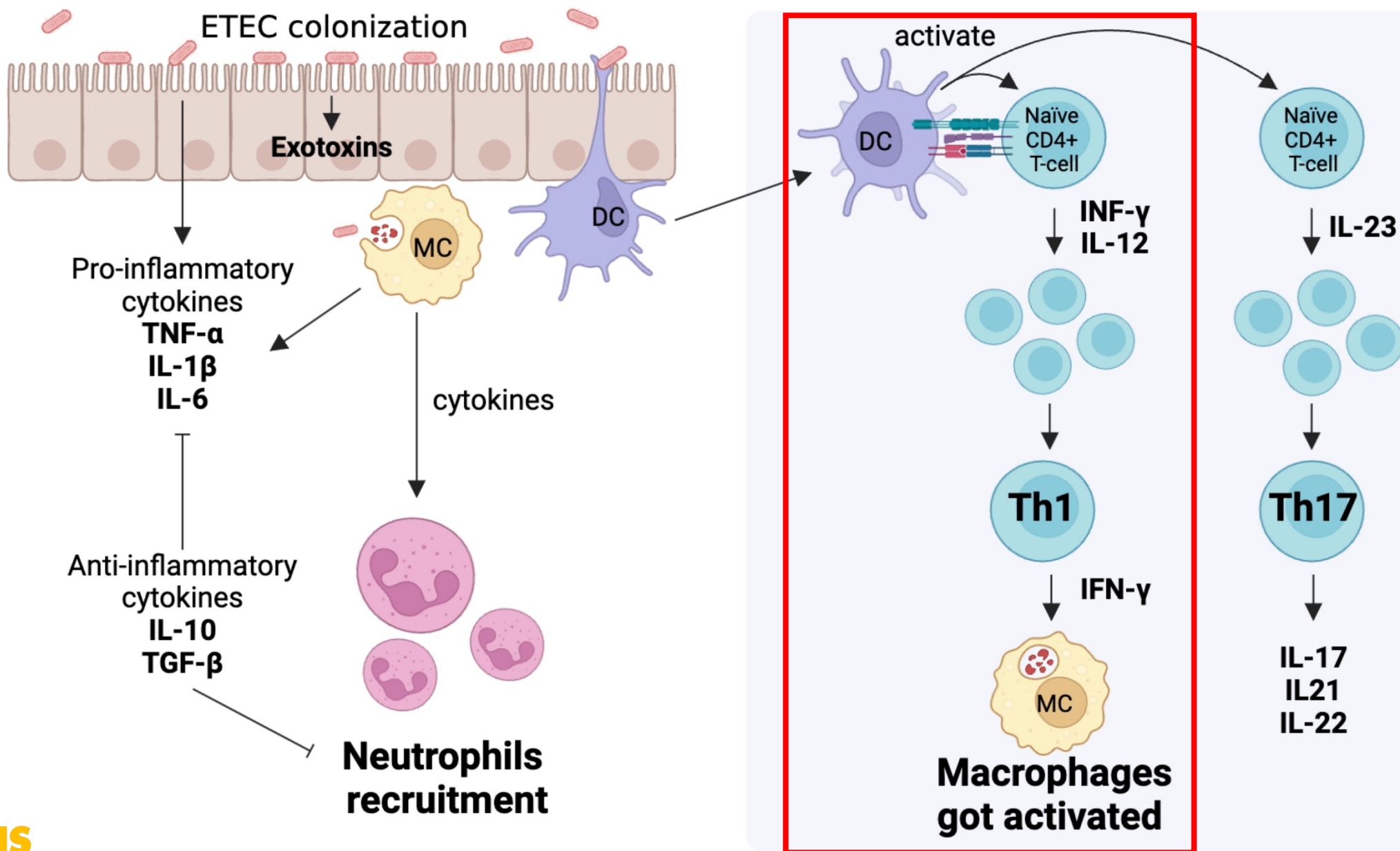
# RESULTS



**Intestinal immune responses**

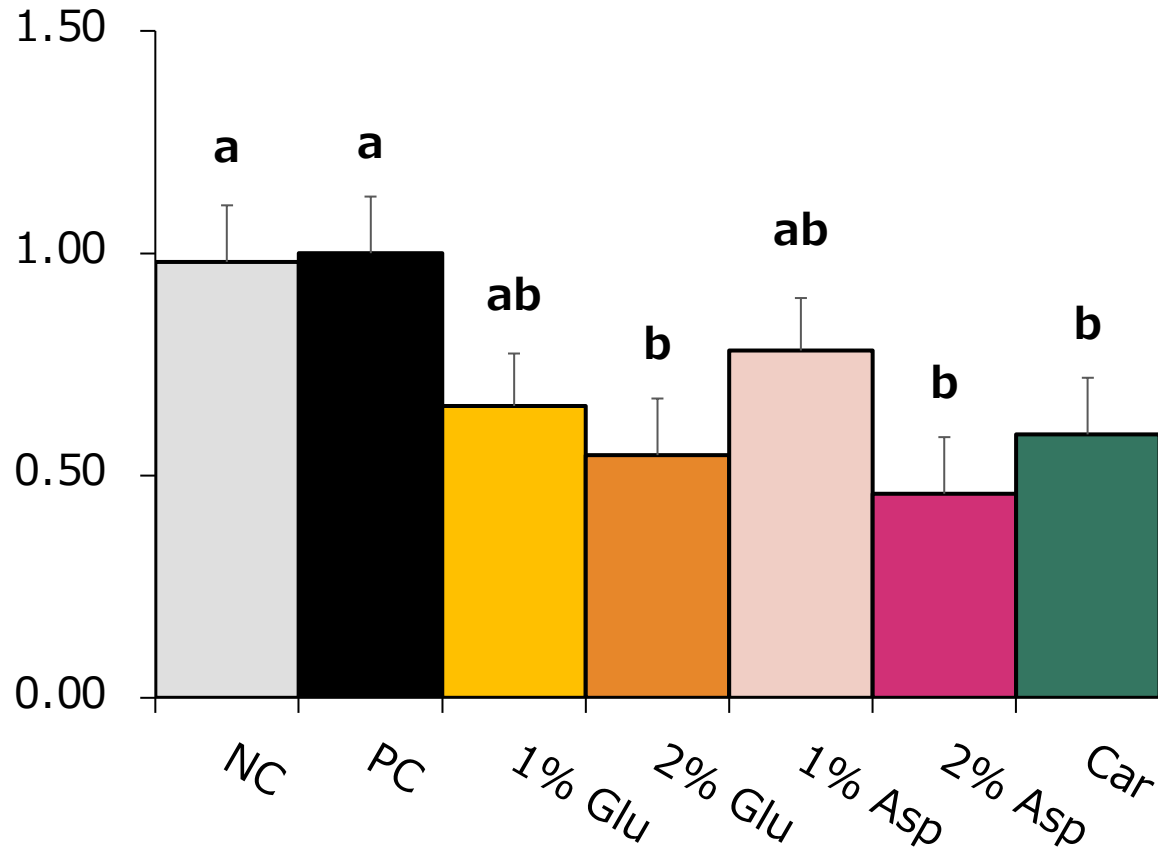
**qPCR**

# Th1-related cytokines

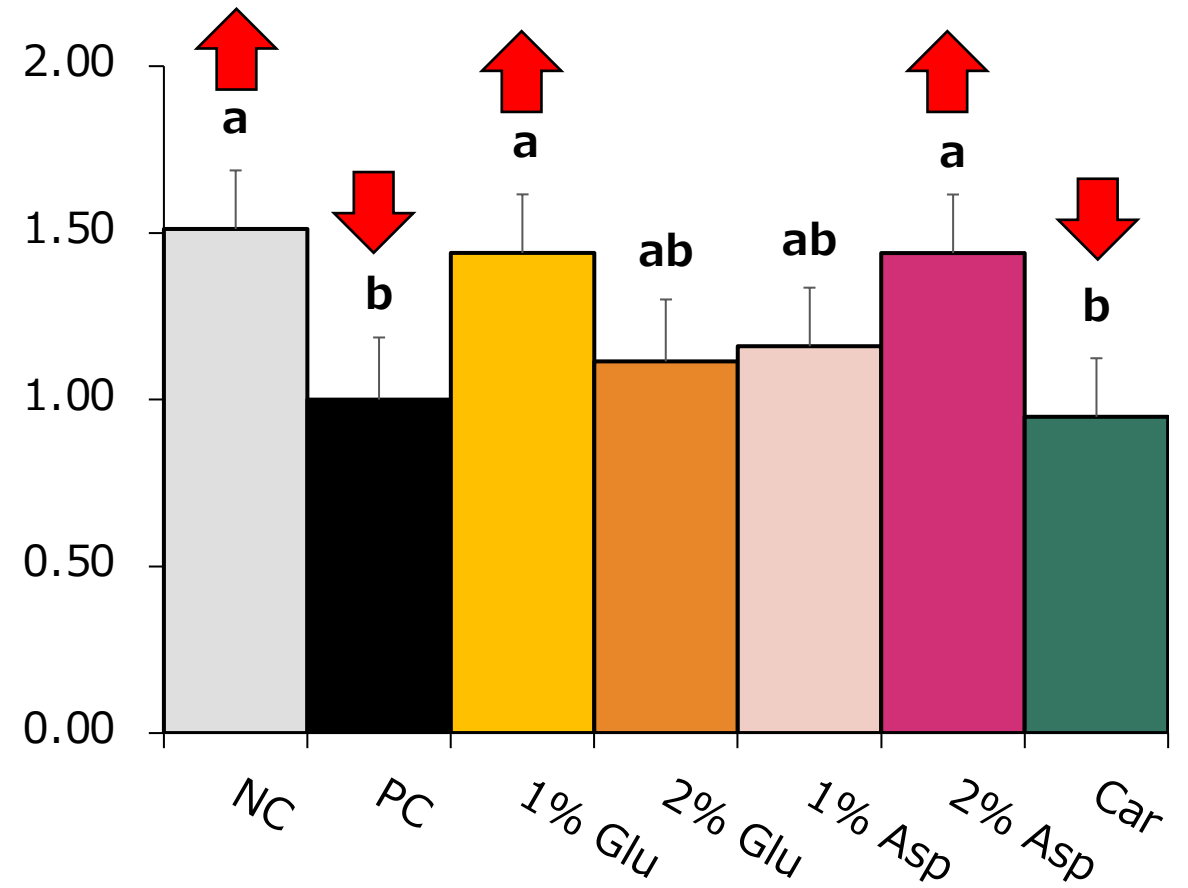


# Relative mRNA abundance: Th1-related cytokines

## Jejunal mucosa, IL-12



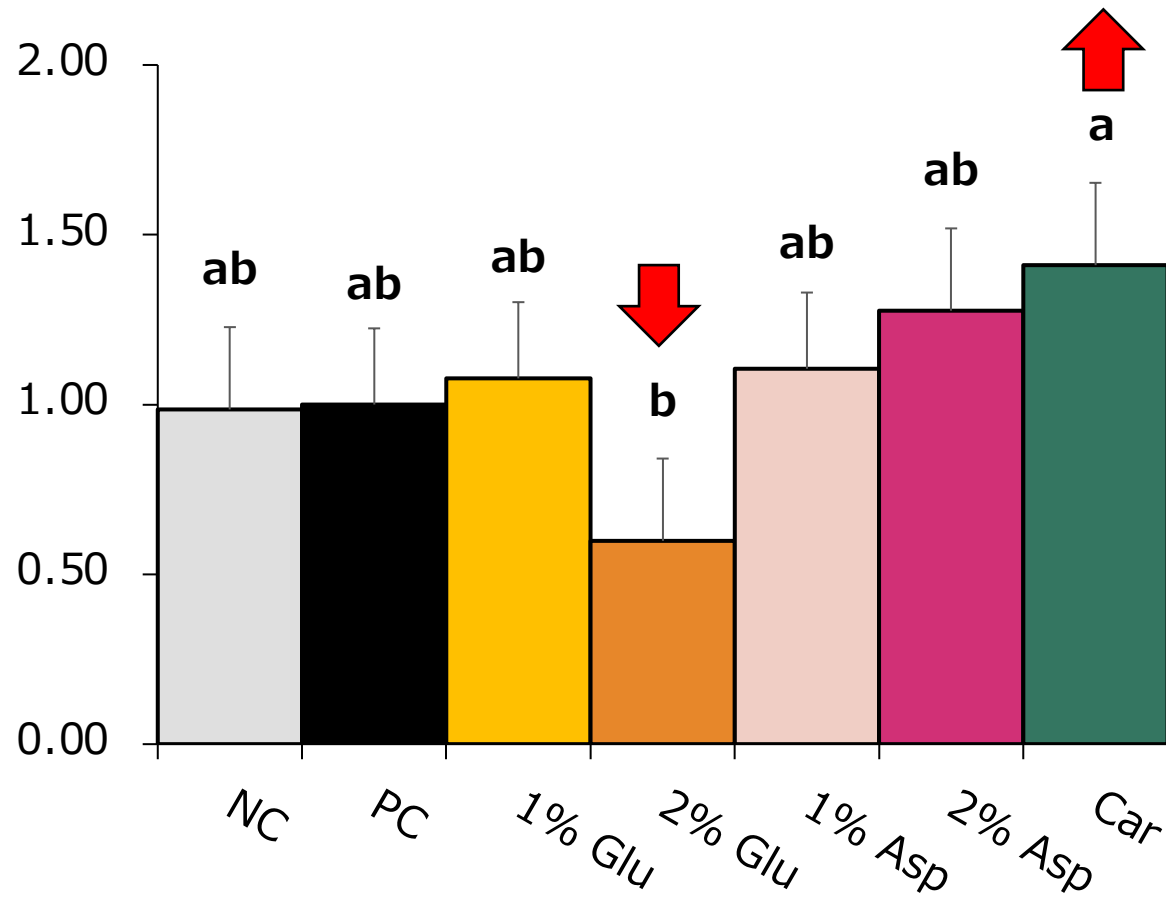
## Ileal mucosa, IL-12



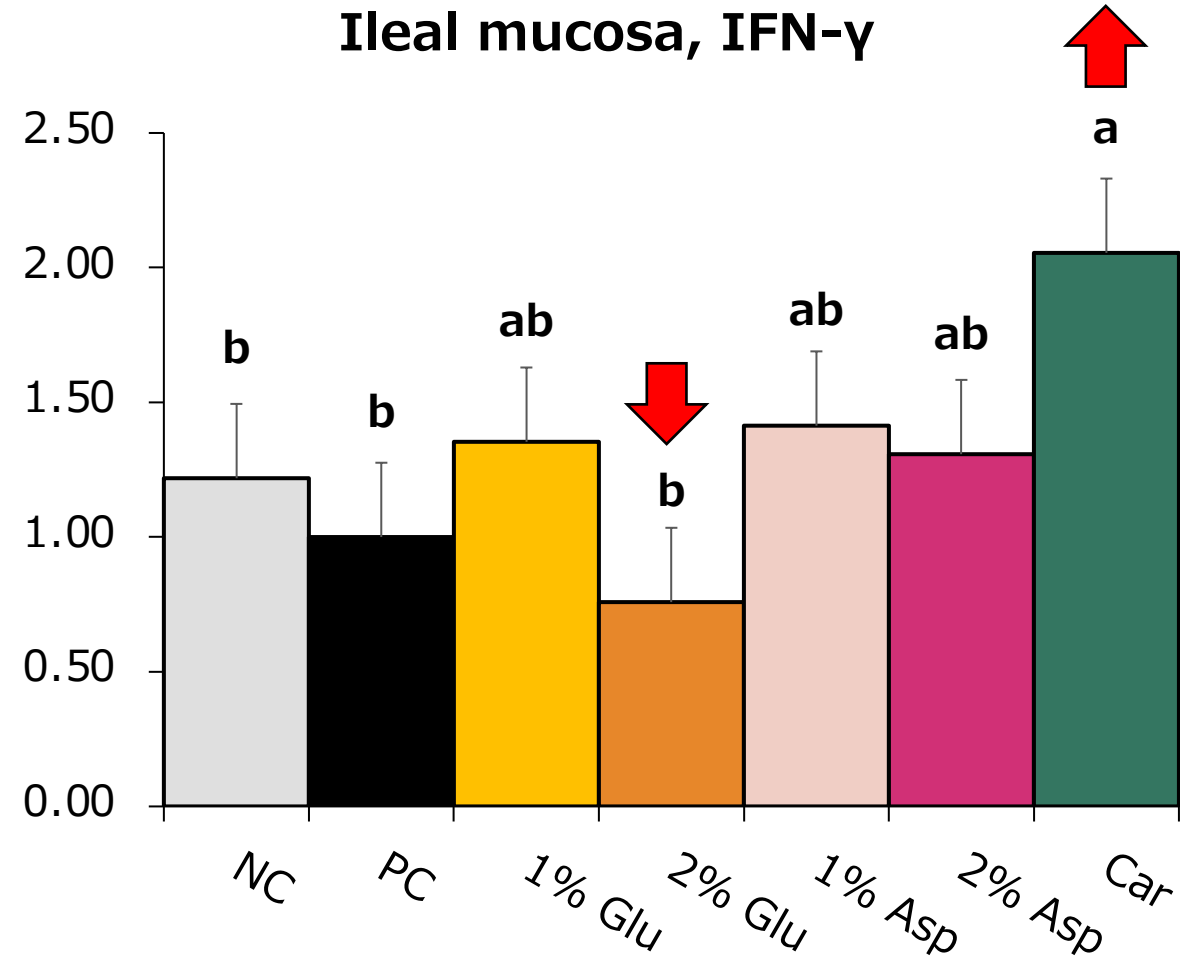


# Relative mRNA abundance: Th1-related cytokines

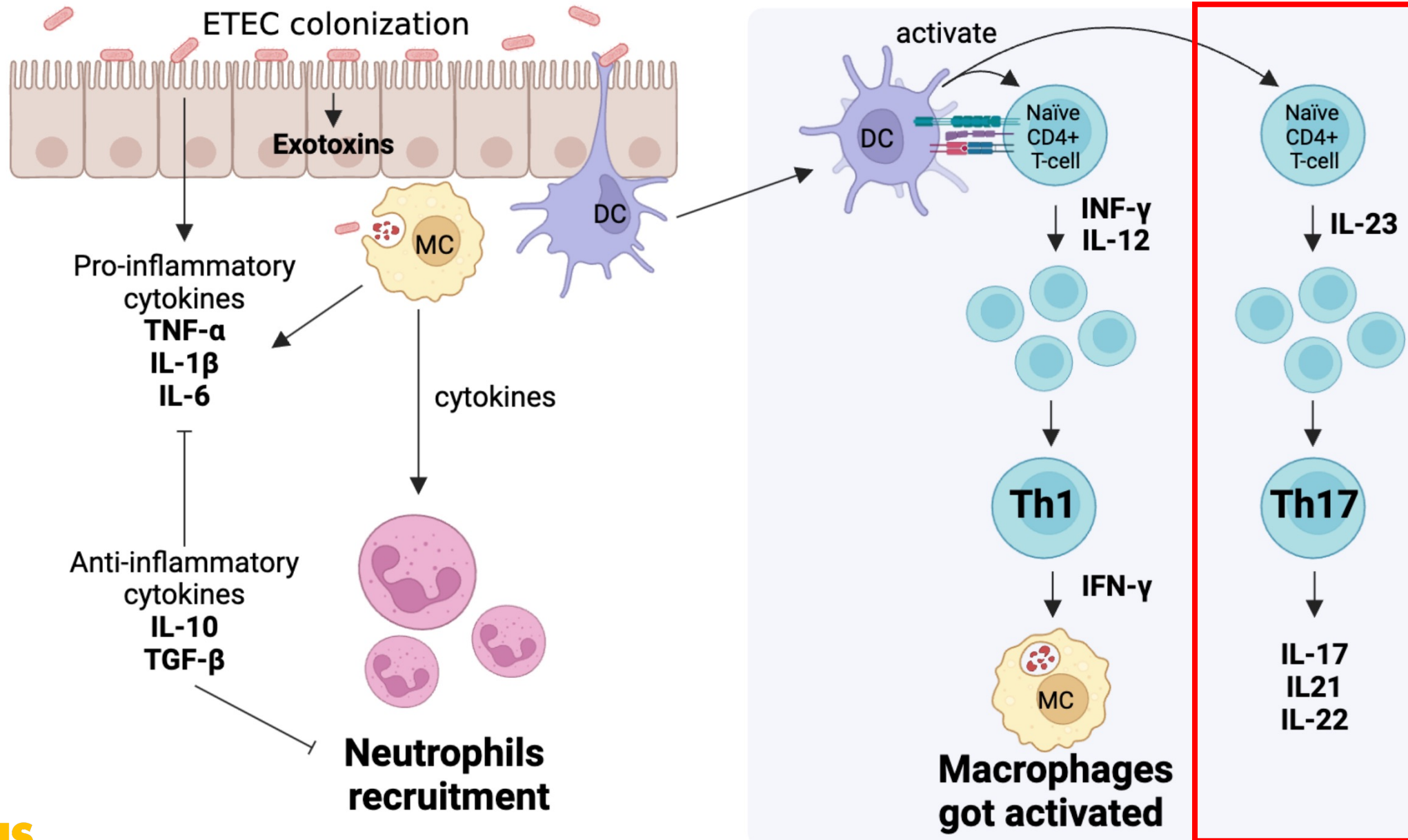
## Jejunal mucosa, IFN- $\gamma$



## Ileal mucosa, IFN- $\gamma$



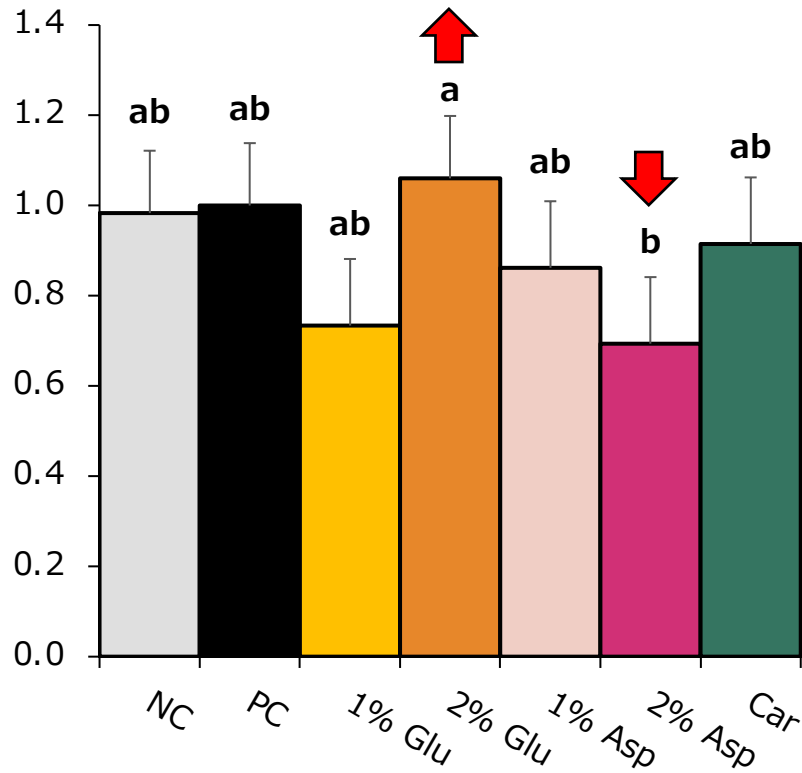
# Th17-related cytokines



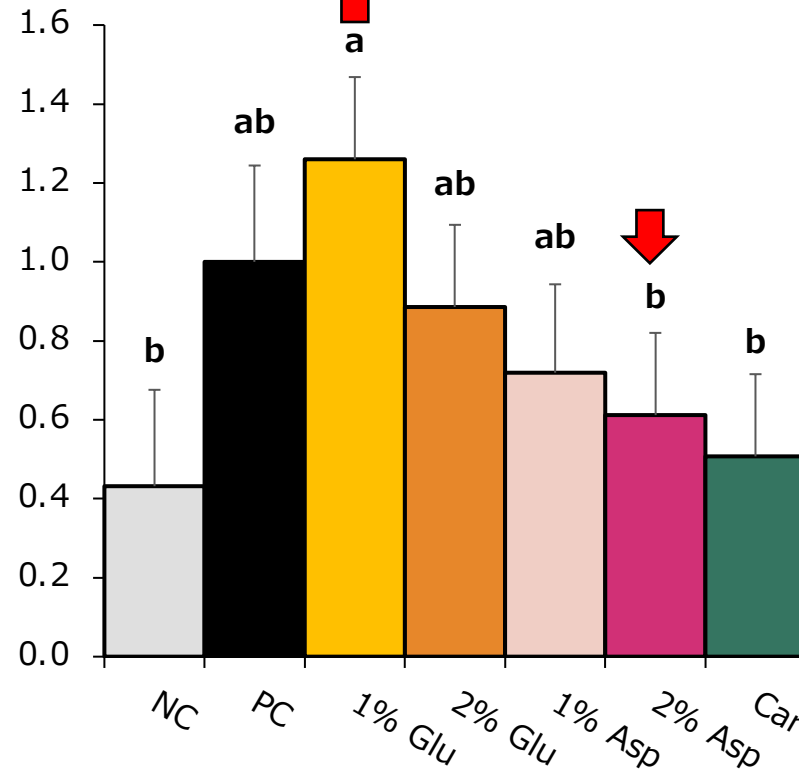
# Relative mRNA abundance: Th17-related cytokines

## Jejunal mucosa

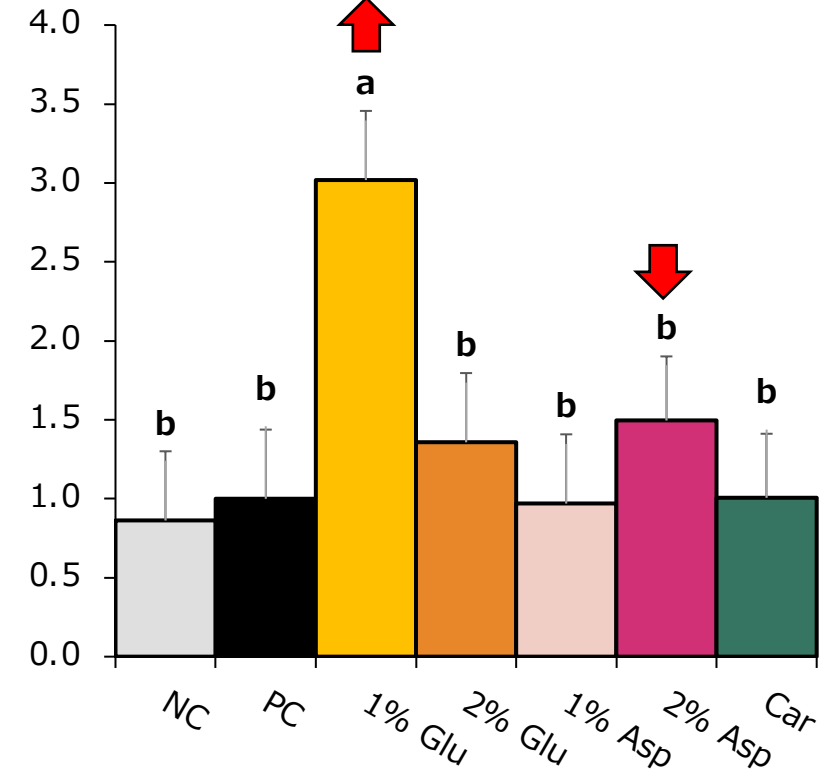
### IL-23



### IL-17A

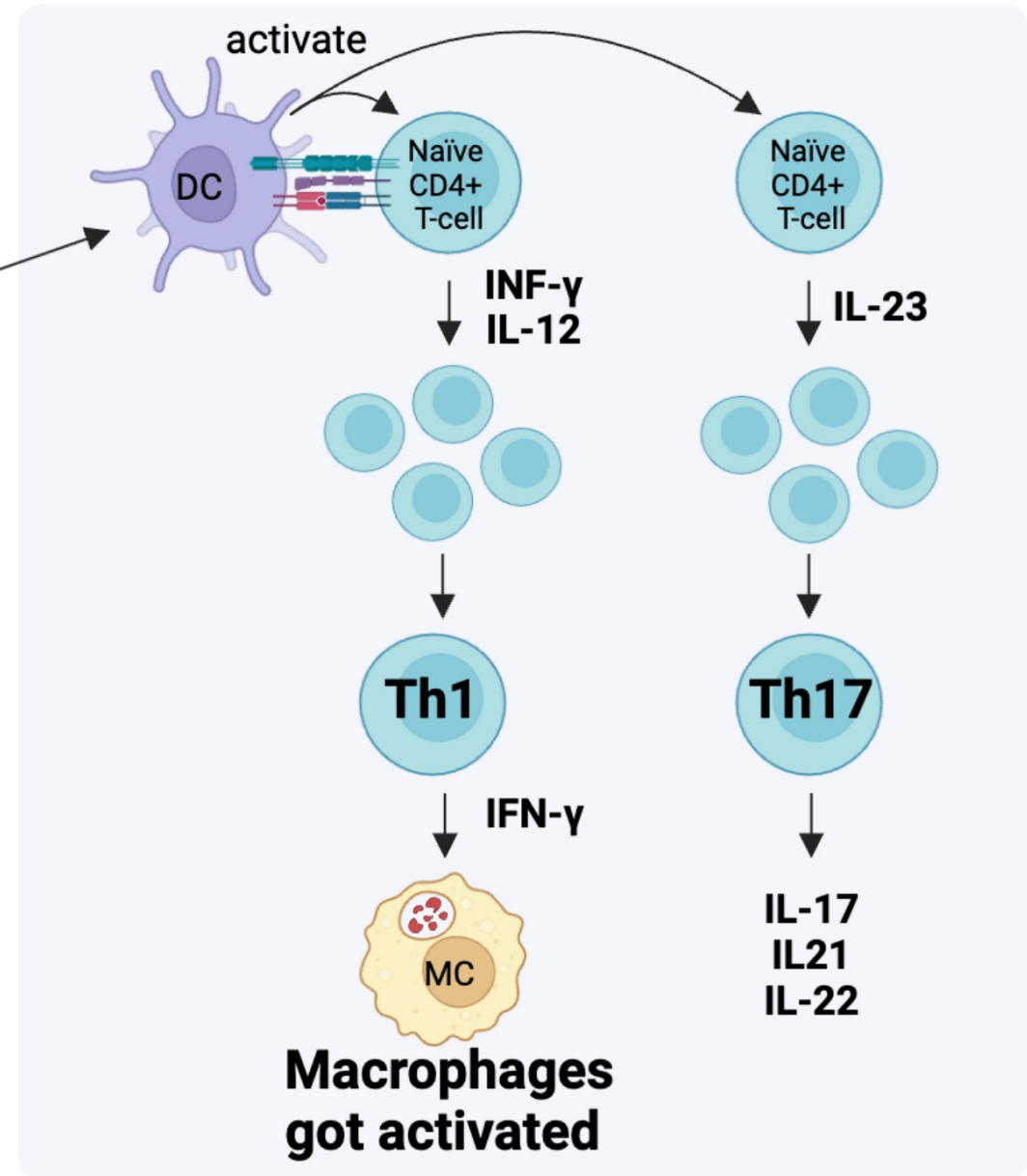
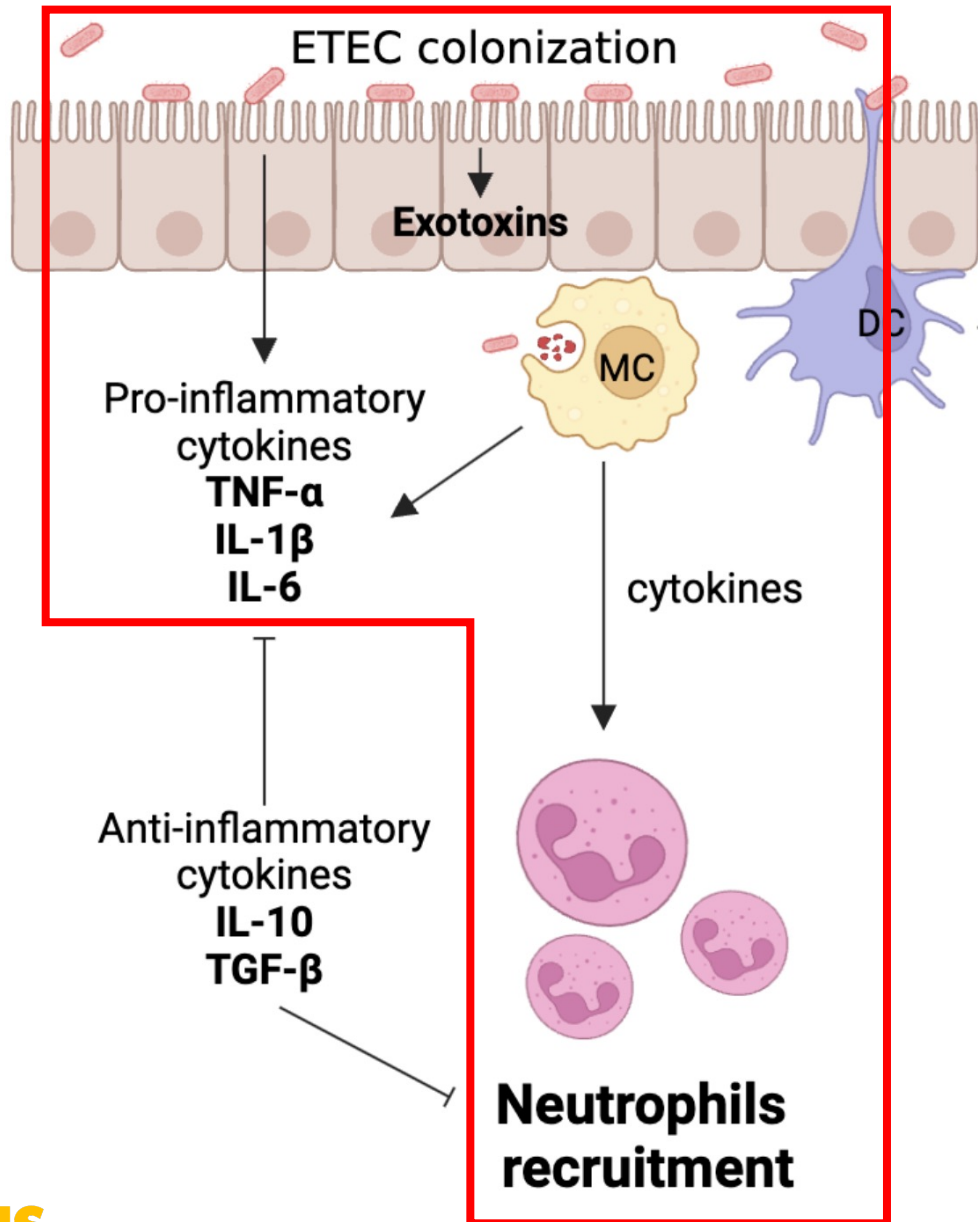


### IL-22



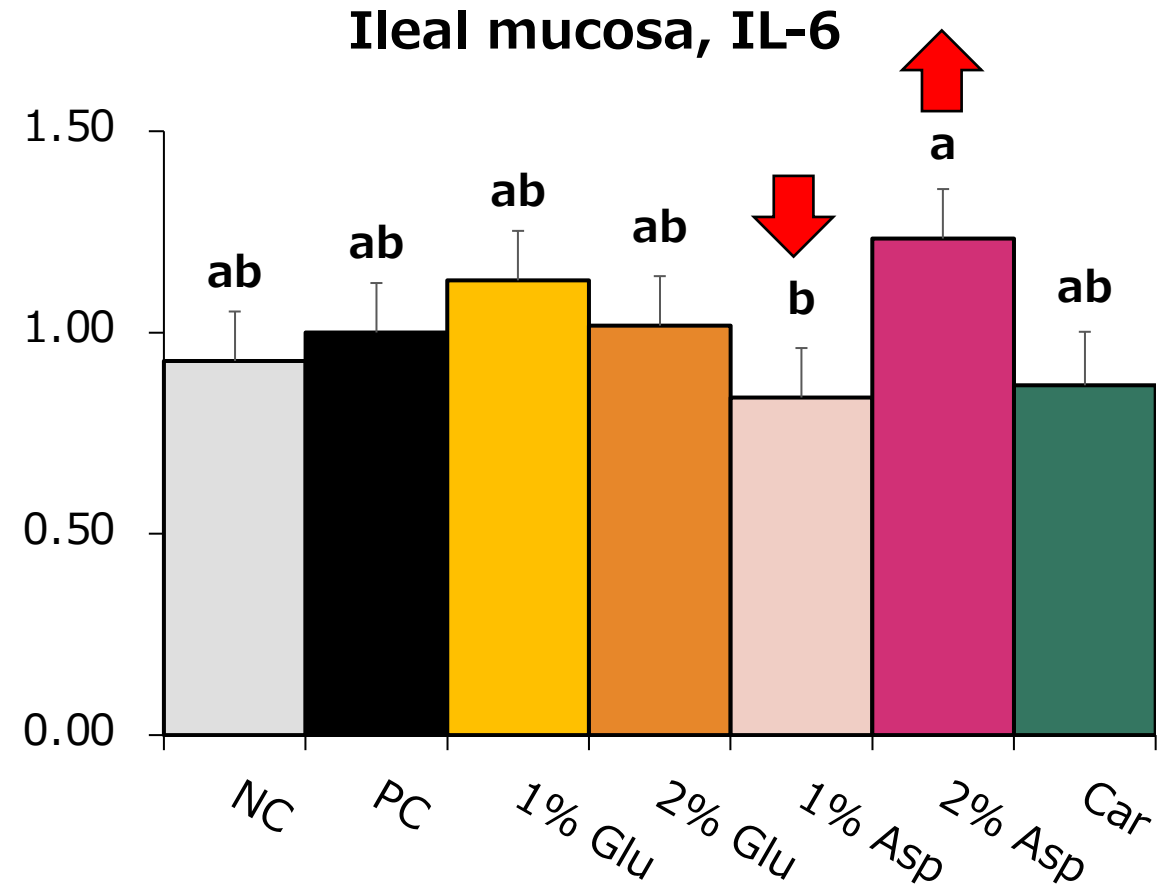
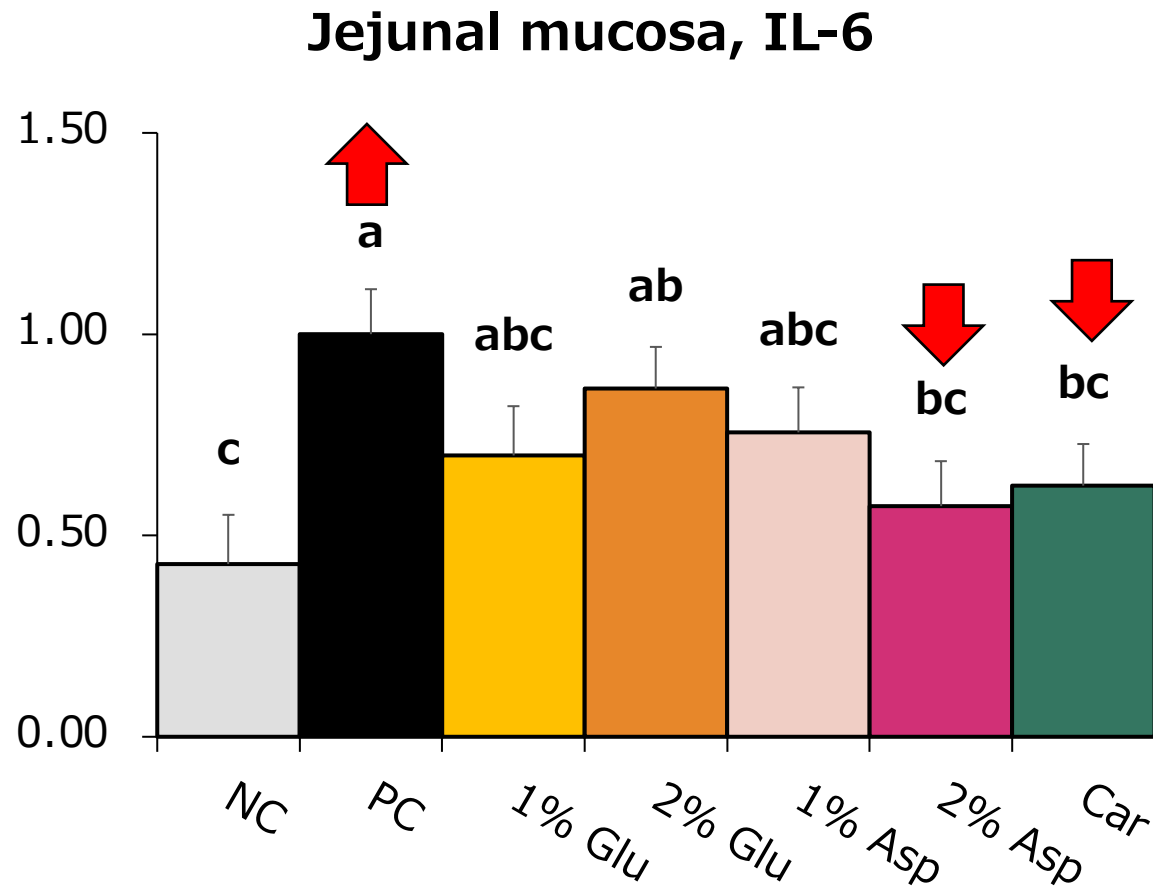
**No differences in mRNA expression of IL-23, IL-17A, and IL-22 in ileal mucosa were observed**

# Pro-inflammatory cytokines



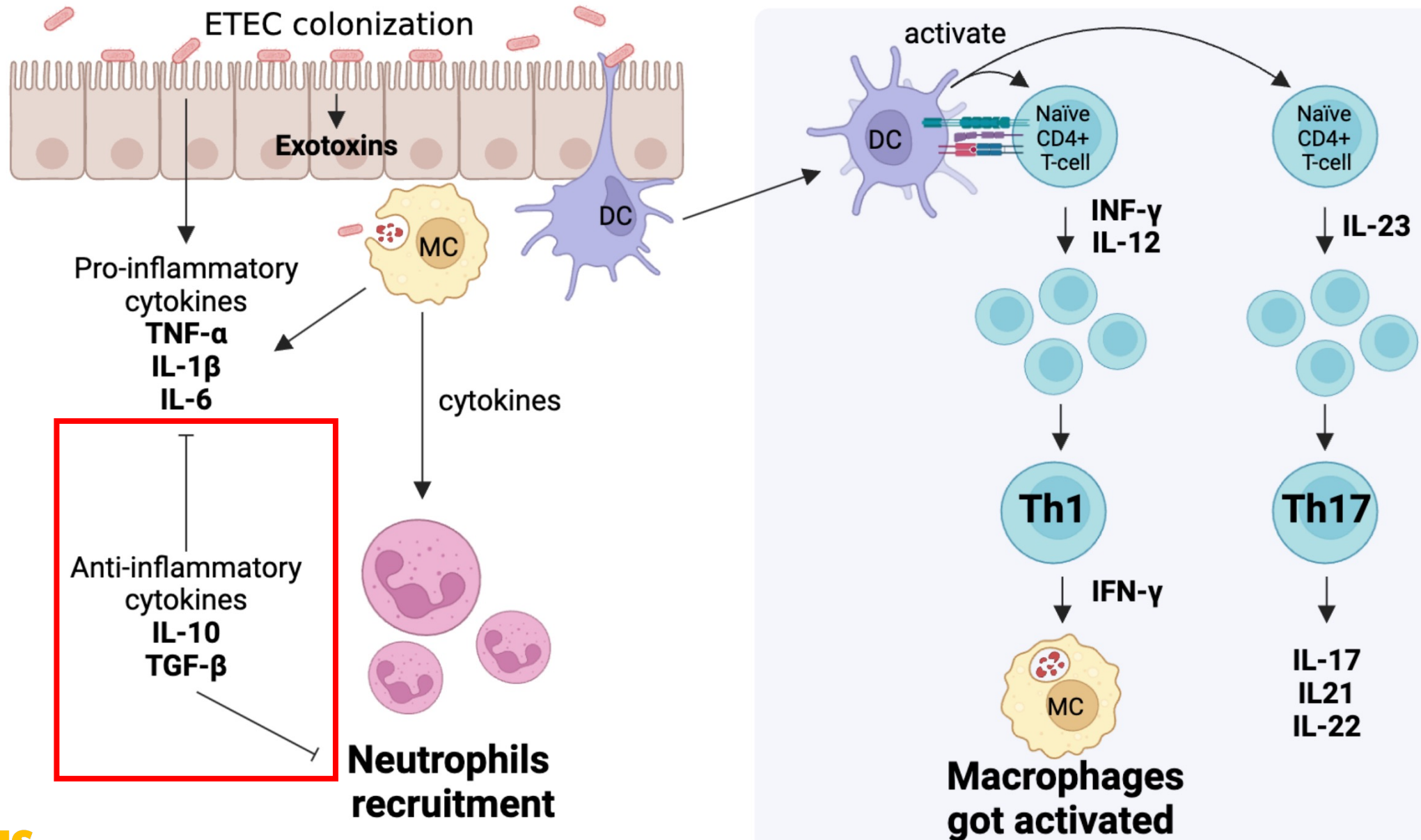


# Relative mRNA abundance: Pro-inflammatory cytokines

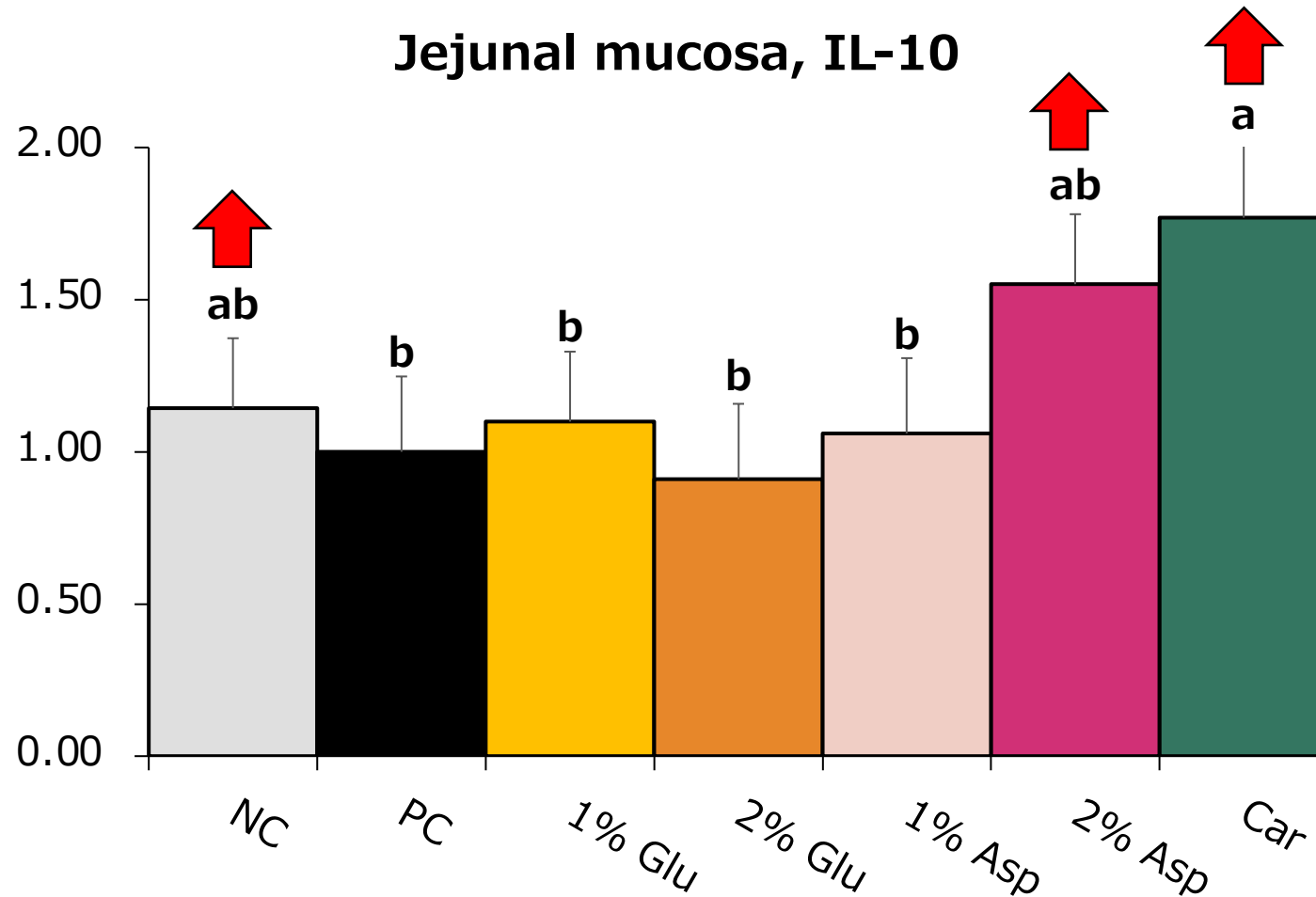


*No differences in mRNA expression of TNF- $\alpha$  and IL-1 $\beta$  were observed*

# Anti-inflammatory cytokines



# Relative mRNA abundance: **Anti-inflammatory cytokines**



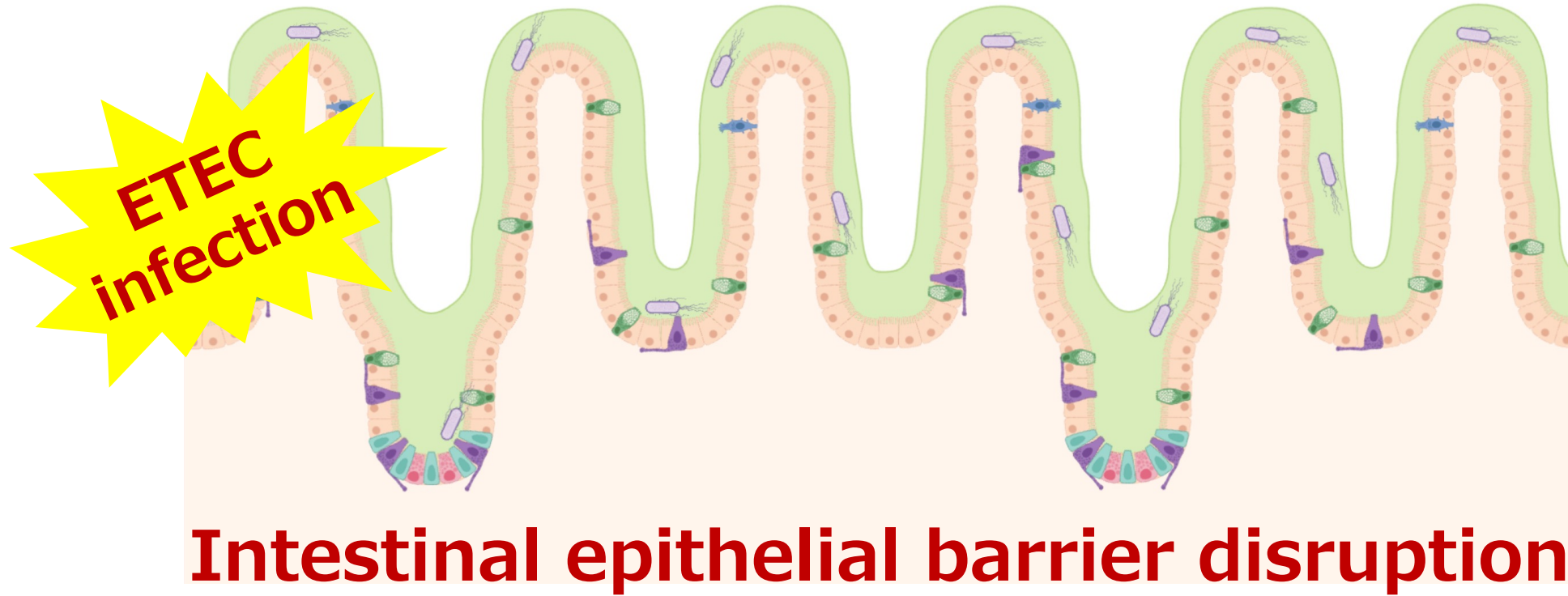
***No differences in mRNA expression of IL-10 in ileal mucosa and TGF- $\beta$ 1 were observed***

# Conclusion: **Intestinal immune responses**

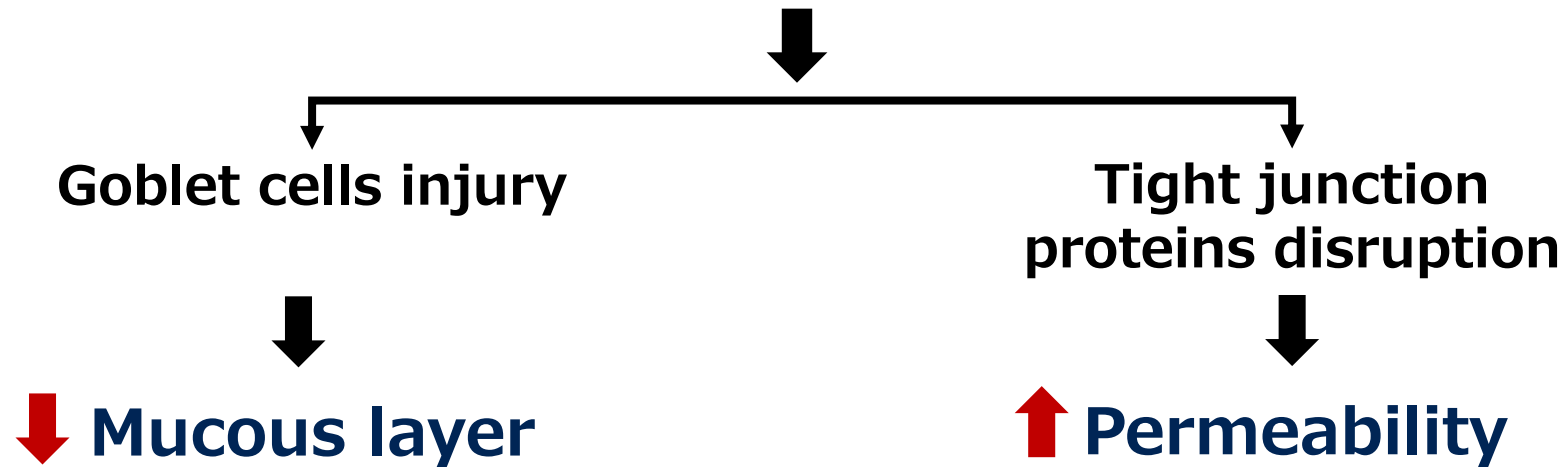
- **1% Glu, 1% Asp, or 2% Asp** modulated mRNA expression of Th1-related cytokines of ETEC infected pigs
- **1% Glu** affected mRNA expression of Th17-related cytokines, especially IL-17A and IL-22 of ETEC infected pigs
- **2% Glu** affected mRNA expression of IFN- $\gamma$
- need to be further investigated







## Intestinal epithelial barrier disruption

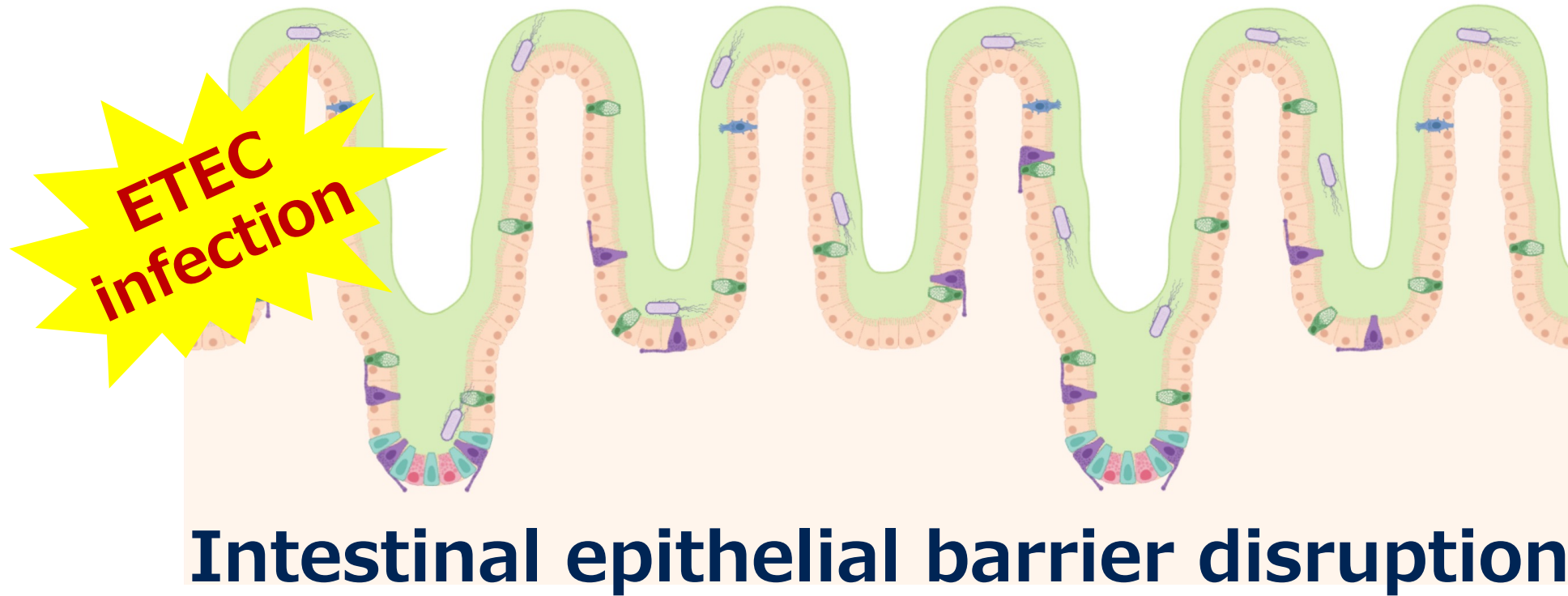


# RESULTS



**Intestinal integrity**

**qPCR**



**MUC2**

Goblet cells injury



↓ Mucous layer

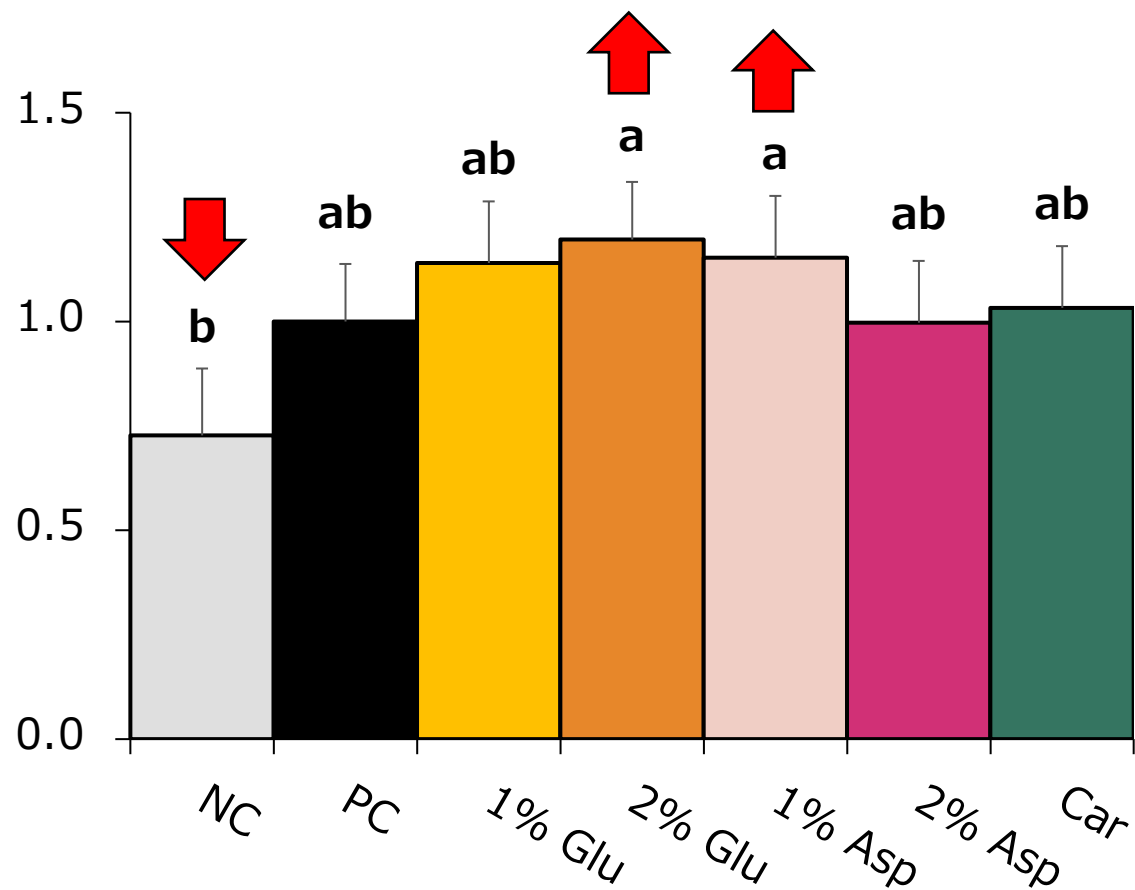
Tight junction proteins disruption



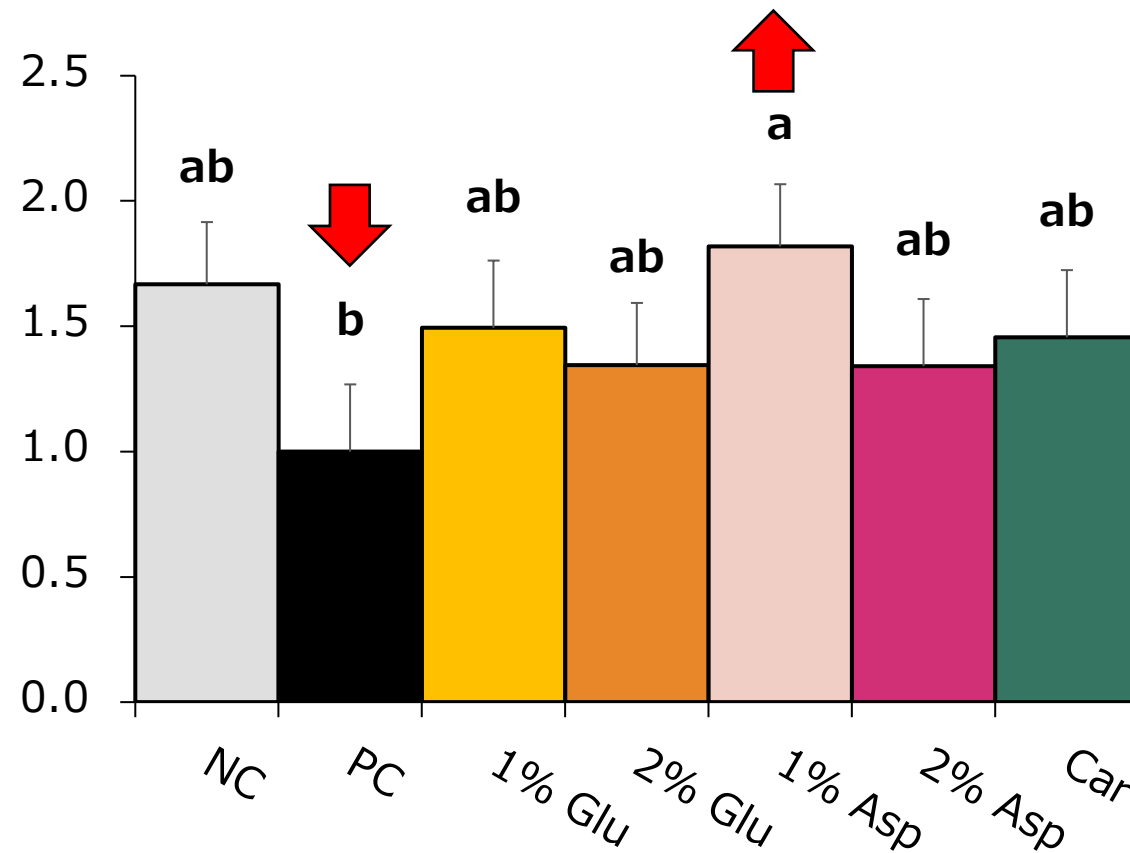
↑ Permeability

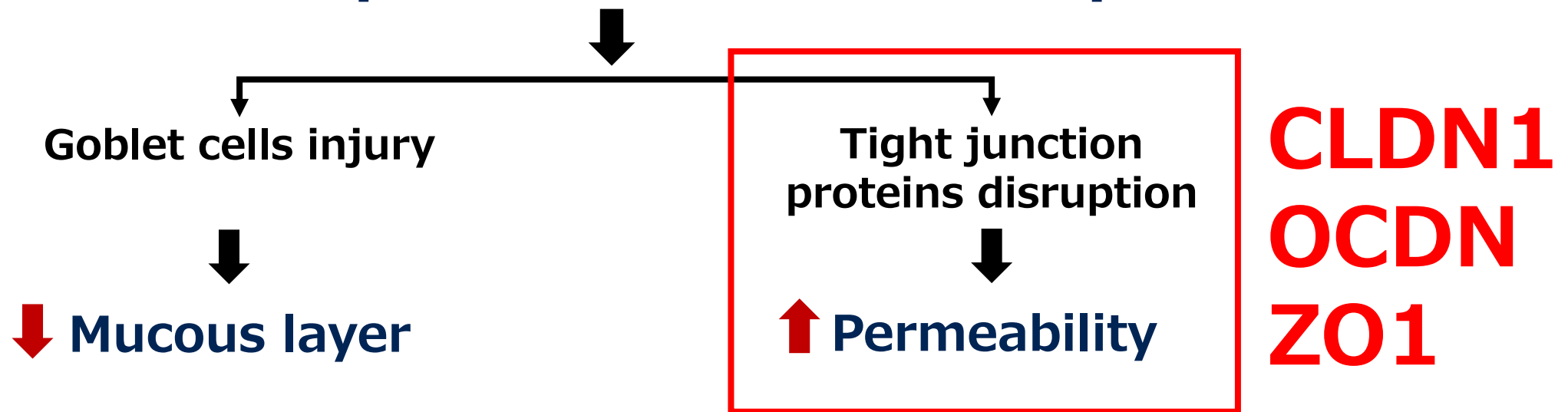
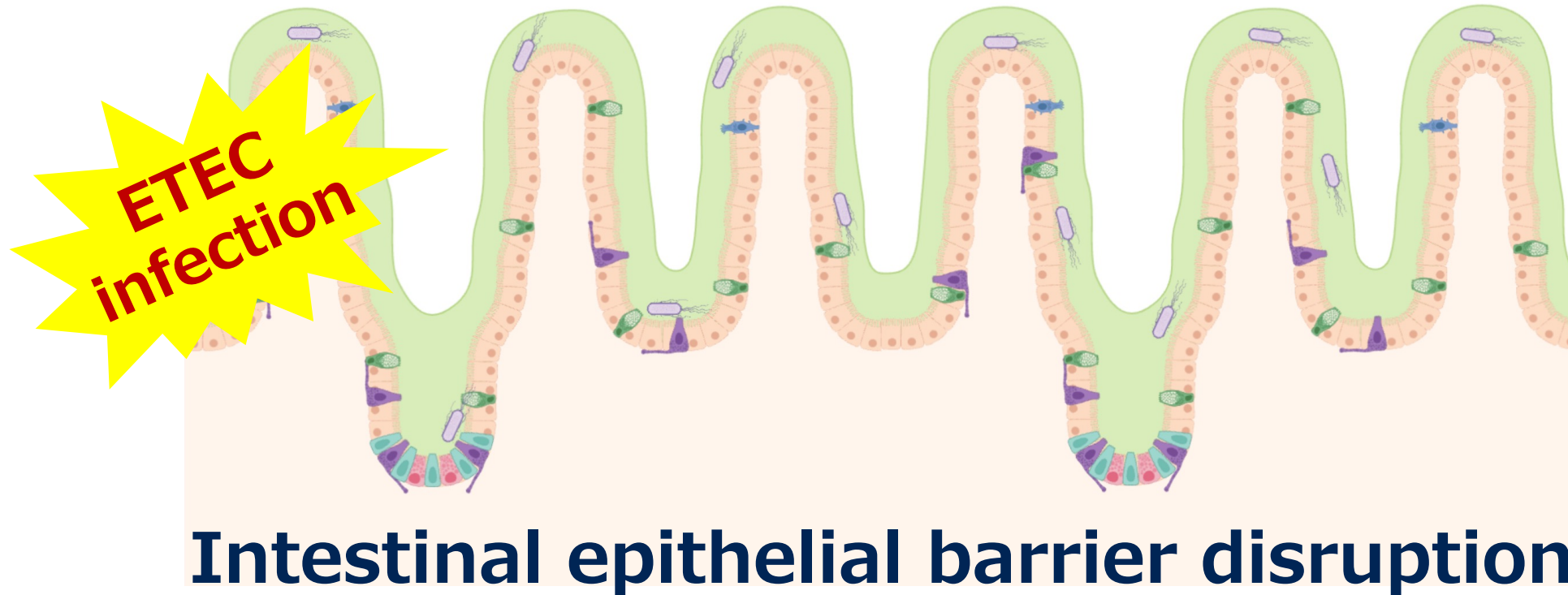
# Relative mRNA abundance: Mucin 2

## Jejunal mucosa, MUC2



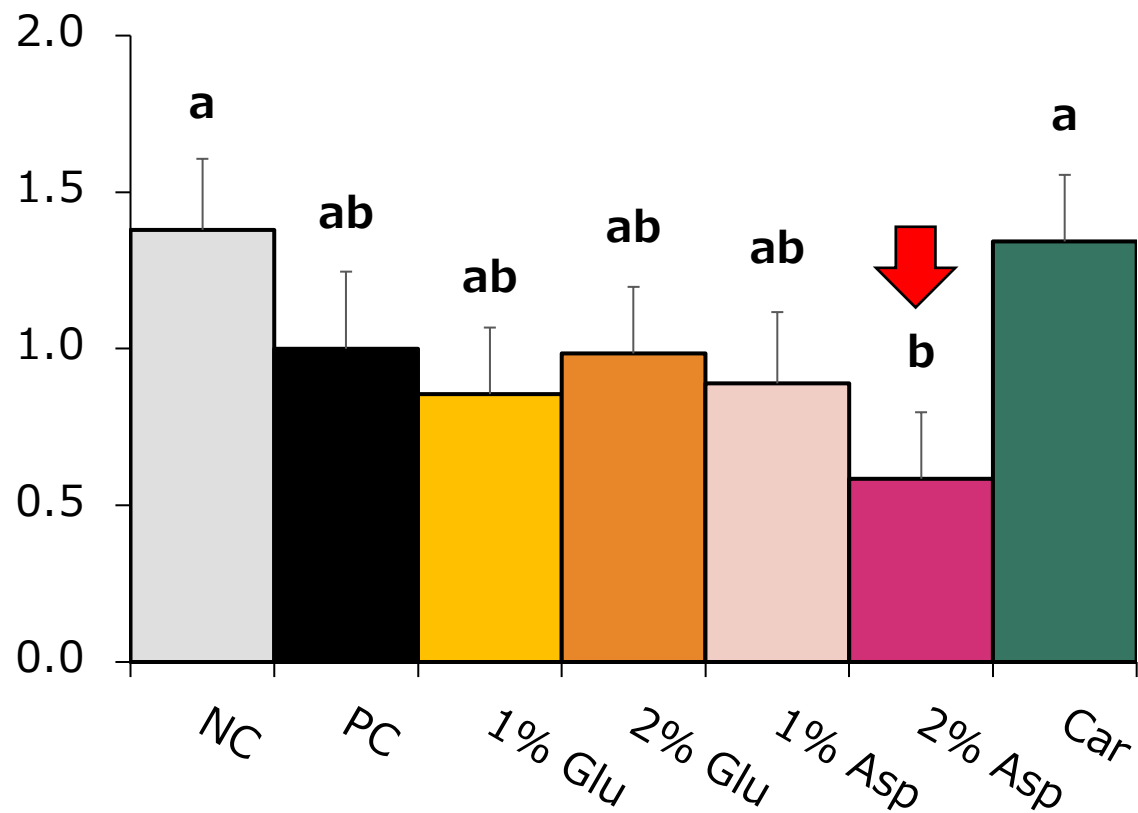
## Ileal mucosa, MUC2



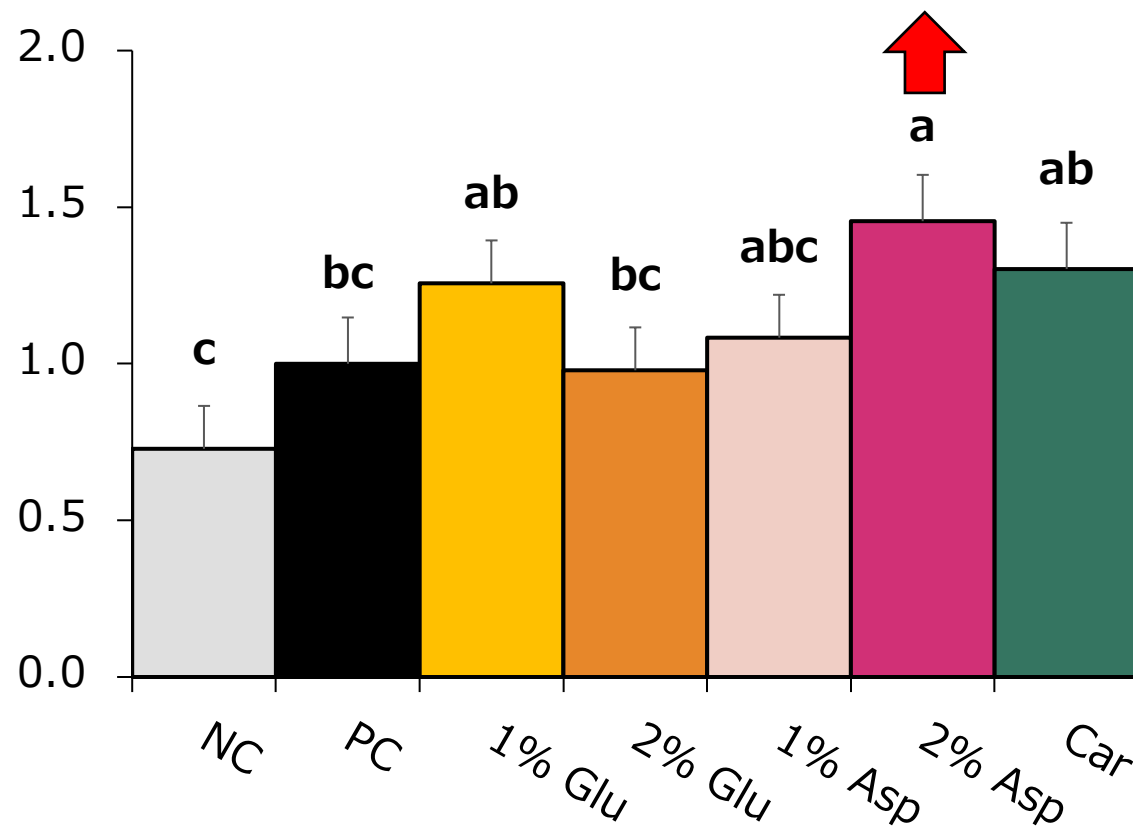


# Relative mRNA abundance: Claudin 1

## Jejunum mucosa, CLDN1

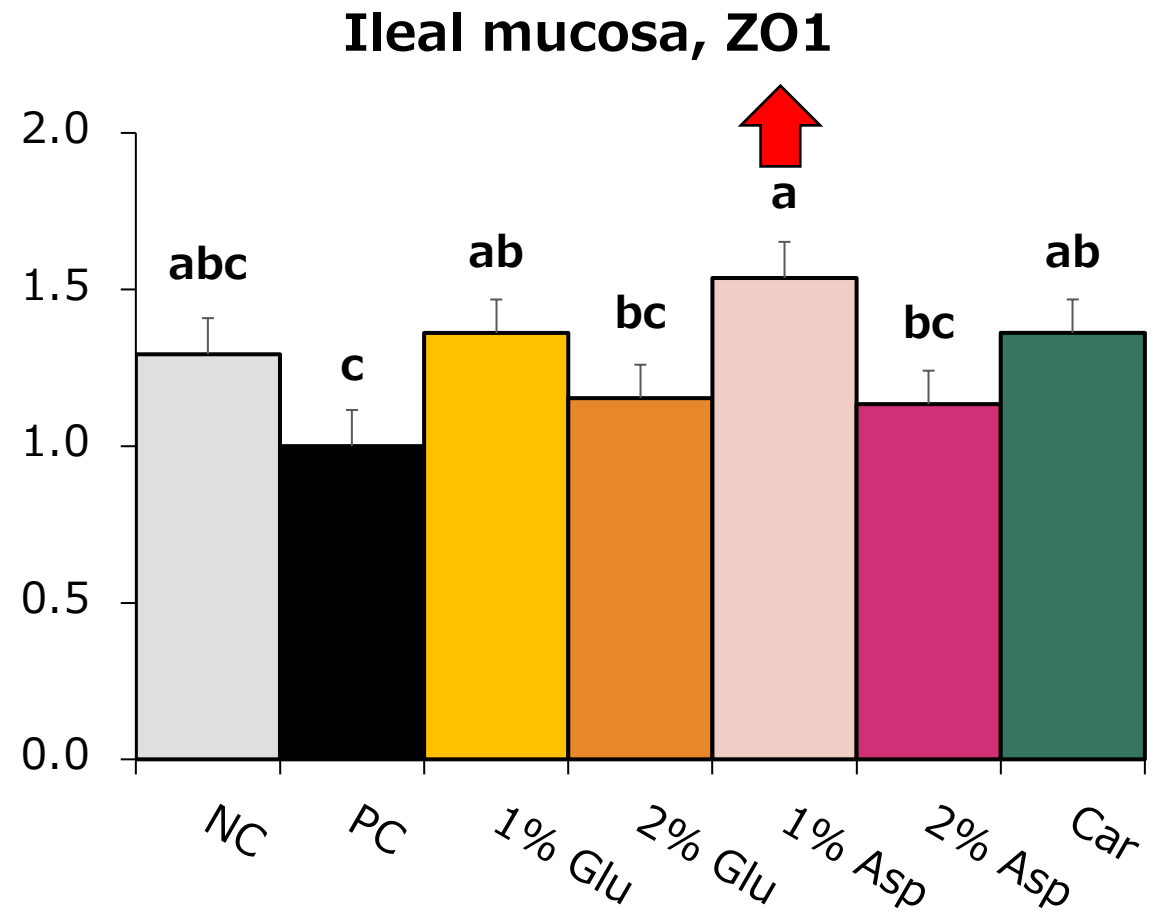
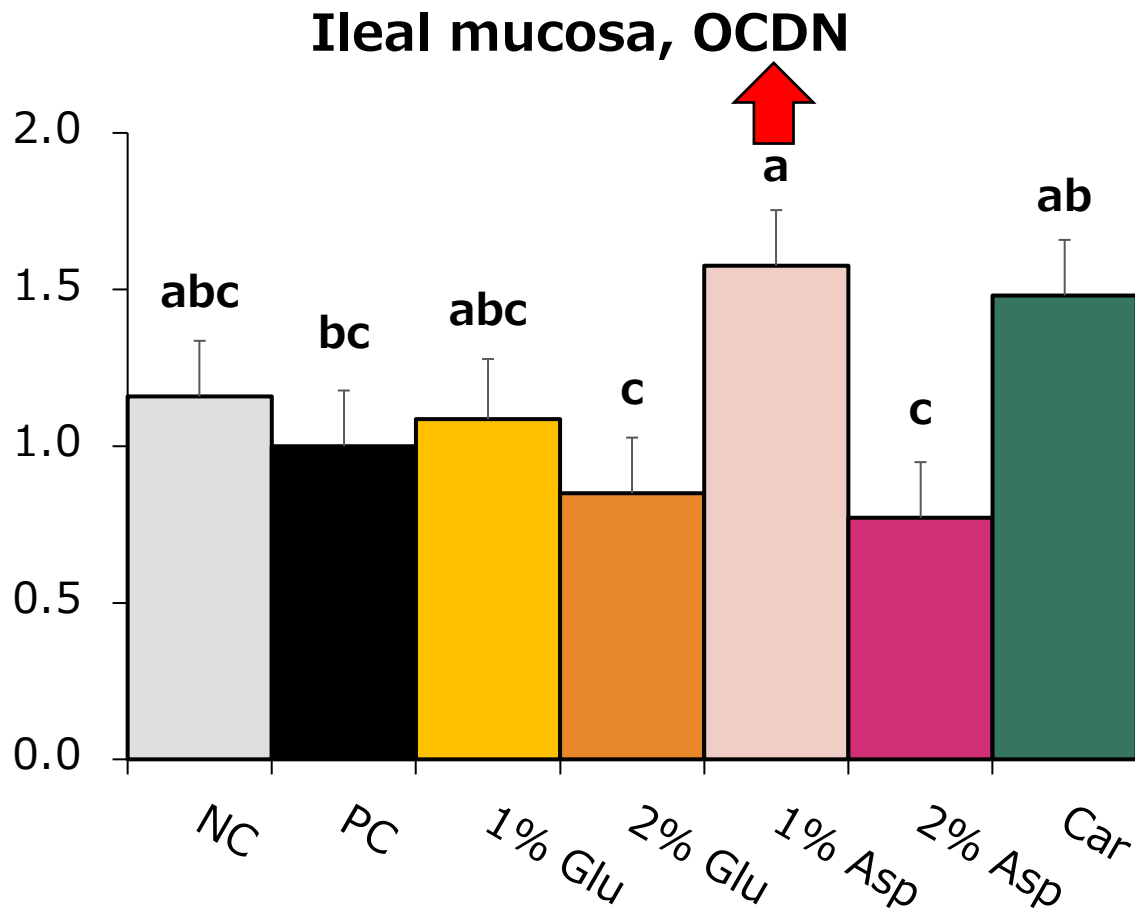


## Ileal mucosa, CLDN1





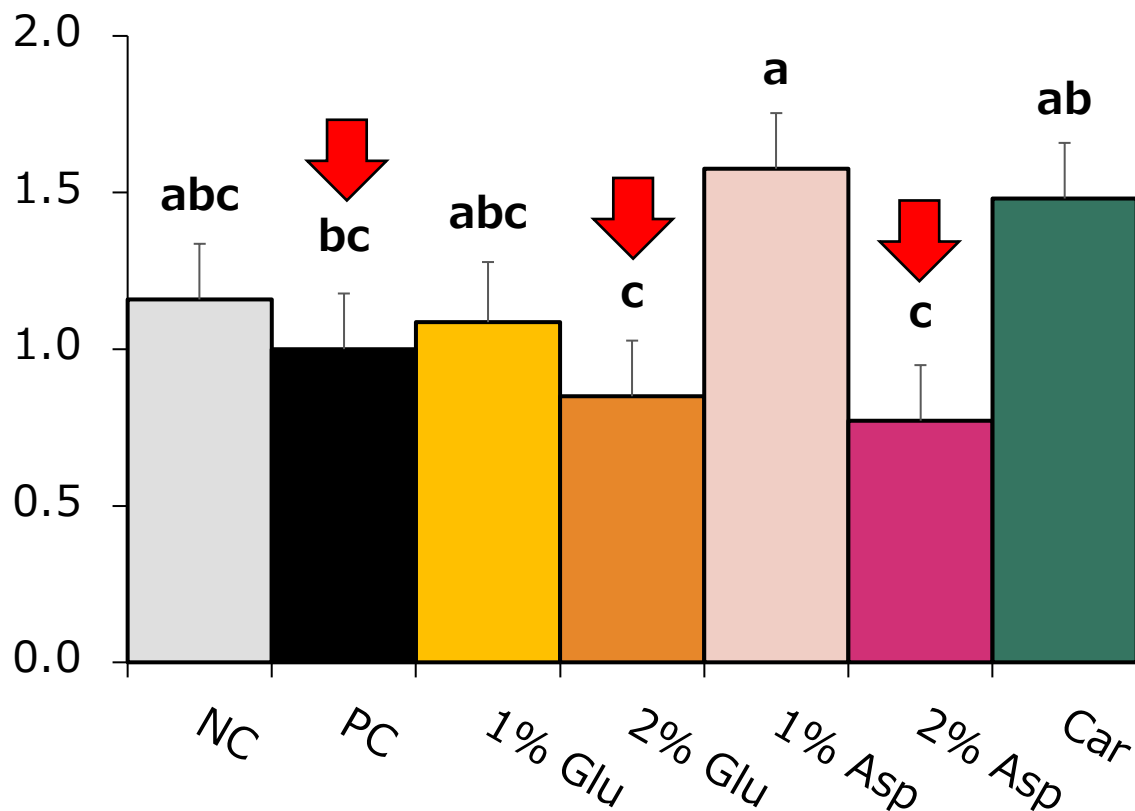
# Relative mRNA abundance: Occludin and Zonular occludens 1



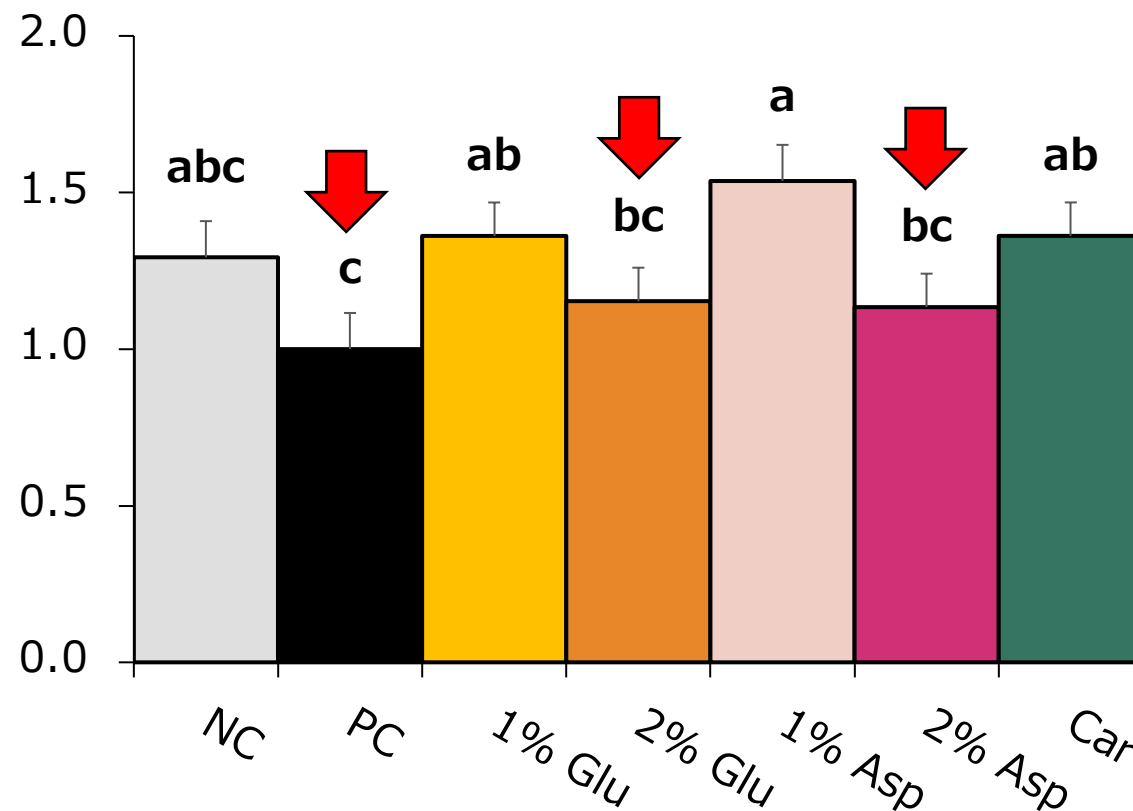
***No differences in mRNA expression of  
OCDN and ZO1 in jejunal mucosa were observed***

# Relative mRNA abundance: Occludin and Zonular occludens 1

## Ileal mucosa, OCDN



## Ileal mucosa, ZO1



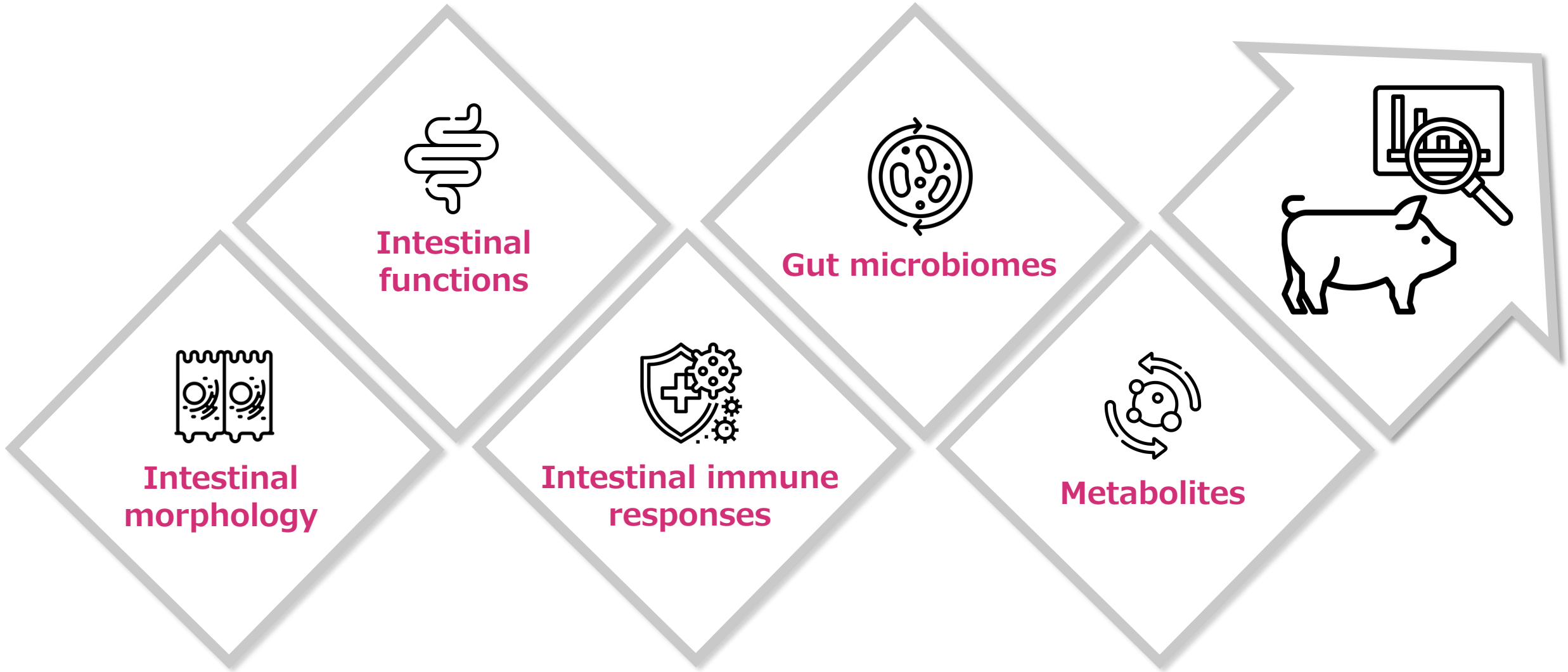
***No differences in mRNA expression of  
OCDN and ZO1 in jejunal mucosa were observed***

# Conclusion: **Intestinal integrity**

- **Glu or Asp** affected mRNA expression of mucin 2 with similar trends as the carbadox group
- **1% Glu or 1% Asp** affected the gene expression of tight junction proteins with similar trends as the carbadox group



# Further *analysis*





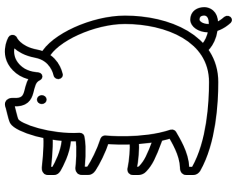
# Take-home message



**Ileal and jejunal mucosa** exhibited different responses in cytokines and tight junction proteins mRNA expression



**Glu and Asp** may promote gut health, reduce diarrhea, and enhance growth performance through different mechanisms

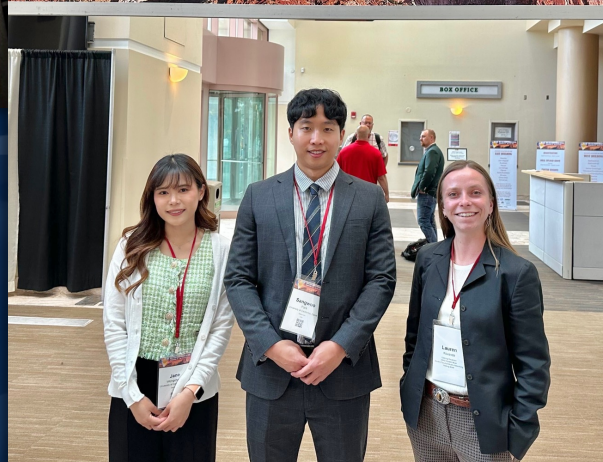


# THANK YOU

## For your attention



ASAS-CSAS-WSASAS Annual Meeting  
Albuquerque, NM - July 16-20, 2023



## Acknowledgments

- Members of Dr. Yanhong Liu, Dr. Peng Ji, and Dr. Maria Marco's lab for assisting in the execution of *in vivo* study.
- Shelby Sopocy, UC Davis Swine Facility manager for assisting in providing piglets.
- My undergraduate interns for helping me conducting the animal experiment and collecting samples.
- Novo Nordisk Foundation (NNF) for funding support.
- PIG-PARADIGM



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