Effects of dietary valerate glycerides on diarrhea, inflammatory status, and intestinal barrier integrity in weanling piglets infected with Enterotoxigenic *E. coli*

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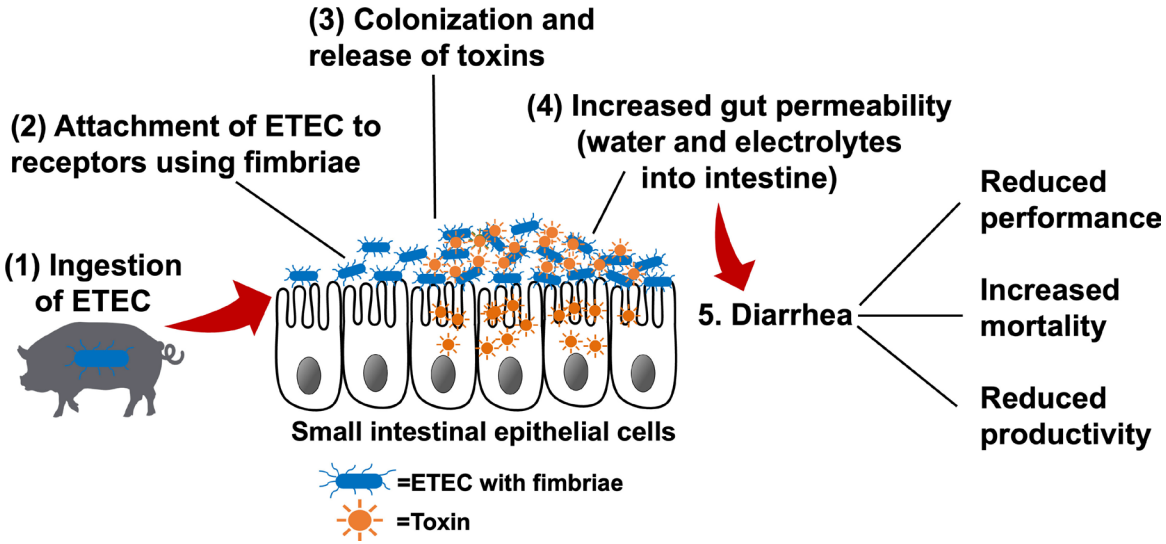
Enterotoxigenic *Escherichia* (*E.*) *coli* (**ETEC**)

- **F4** and **F18** strains in weaned piglets
- **Adherence** to intestinal lining
- **Toxin** production
  - Local and systemic inflammation
  - Impairment of intestinal barrier functions
  - Ion secretion and imbalance



Kim et al., 2022

Diarrhea

Dietary intervention

- ✓ Anti-inflammatory
- ✓ Promotion of barrier function
- ✓ Interaction with ion secretion pathways

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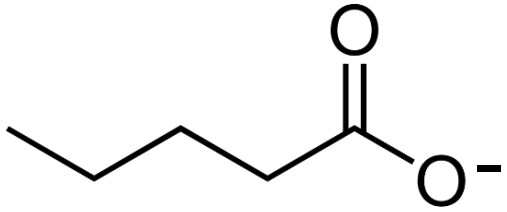
Valerate



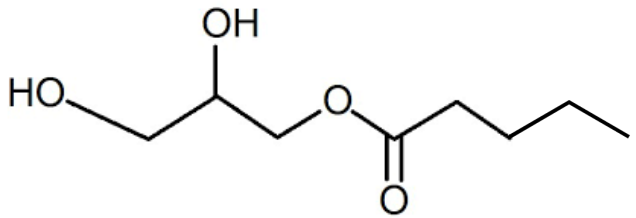
- C5 carboxylic acid SCFA
- **GPR agonism** (Poul et al., 2003)
- **Antimicrobial** (Kovanda et al., 2019)

✓**Improved intestinal barrier function *in vitro*** (Gao et al., 2022)

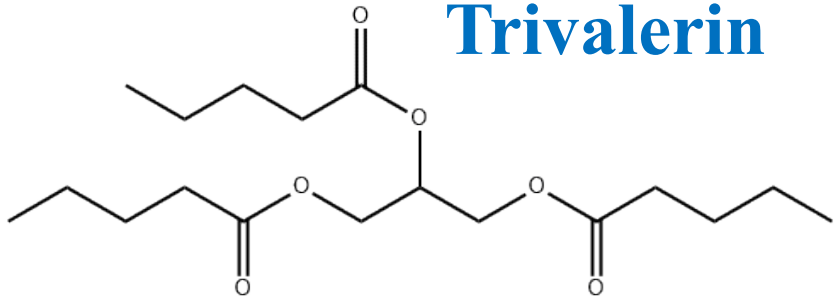
✓**Improved disease resistance** (Onrust et al., 2018; Hofacre et al., 2020)

Valerian plant

Monovalerin



Trivalerin



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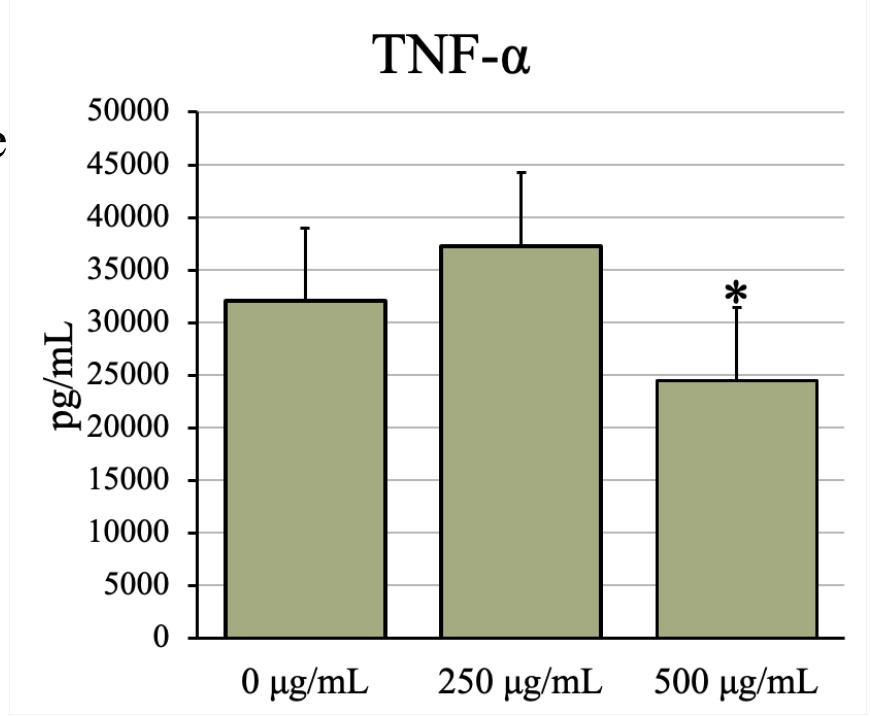
Valerate

In vitro

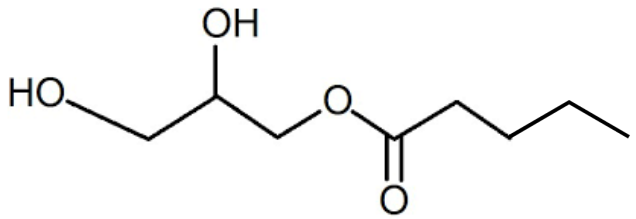
- Reduced ($p < 0.05$) inflammatory cytokine secretion in porcine alveolar macrophages challenged with 1 µg/mL LPS

- Tendency ($p = 0.055$) for maintained initial TEER in IPEC-J2 monolayers challenged with 10 µg/mL LPS



Valerian plant

Monovalerin



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Experiment 1: Pilot

Objectives:

1. Establish a coinfection model utilizing F4 and F18 ETEC
2. Evaluate the effects of dietary **butyrate** and **valerate** glycerides on diarrhea, systemic inflammation, and growth performance of weanling piglets coinfected with two strains of pathogenic ETEC

Experimental design

- **20 weaned piglets** (21-24 d of age), individually housed
- **RCBD**: Blocked by bodyweight within sex
- Treatments: *ad libitum*
  - **0.1% monobutyrin (7 pigs)**
  - **0.1% monovalerin (7 pigs)**
  - **Control (6 pigs)**
- All piglets coinfected with F4+ and F18+ ETEC after 7-day adaptation period
- ETEC coinfection inoculum administered on 3 consecutive days
  - **Dose: 0.5× 10^9 CFU/1.5 mL sterile PBS each strain**

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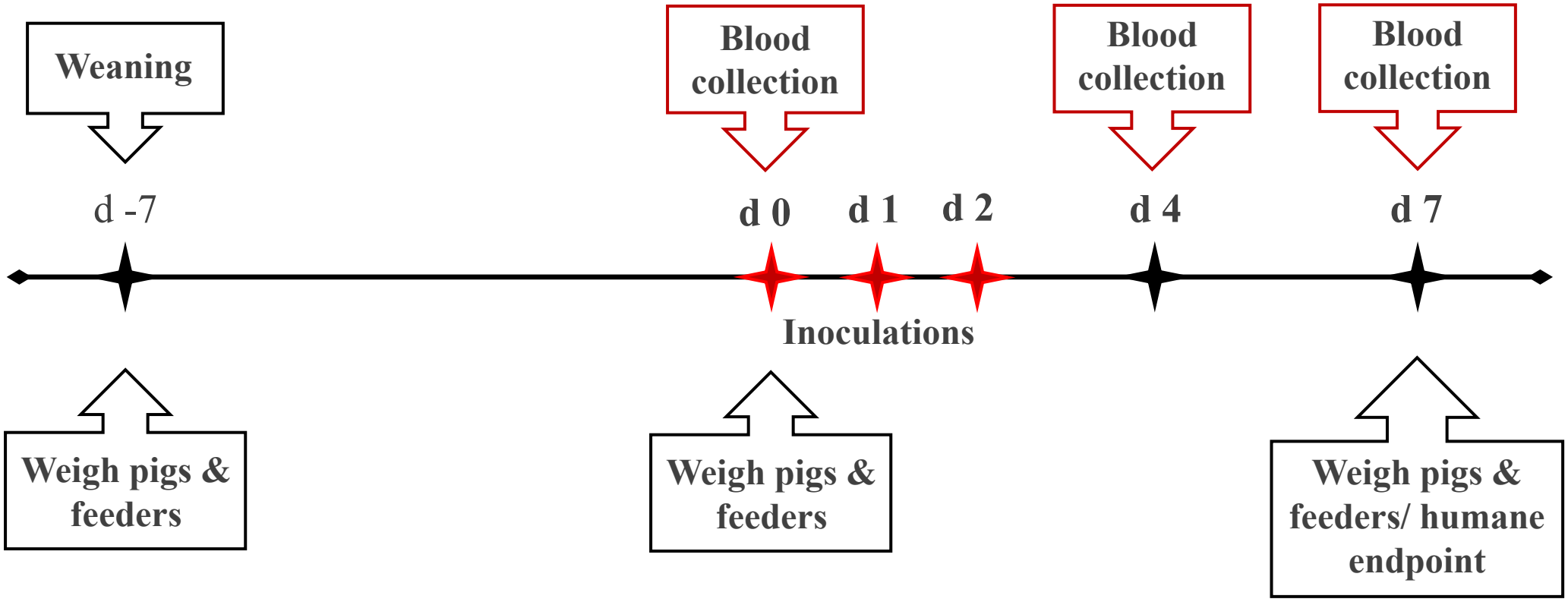
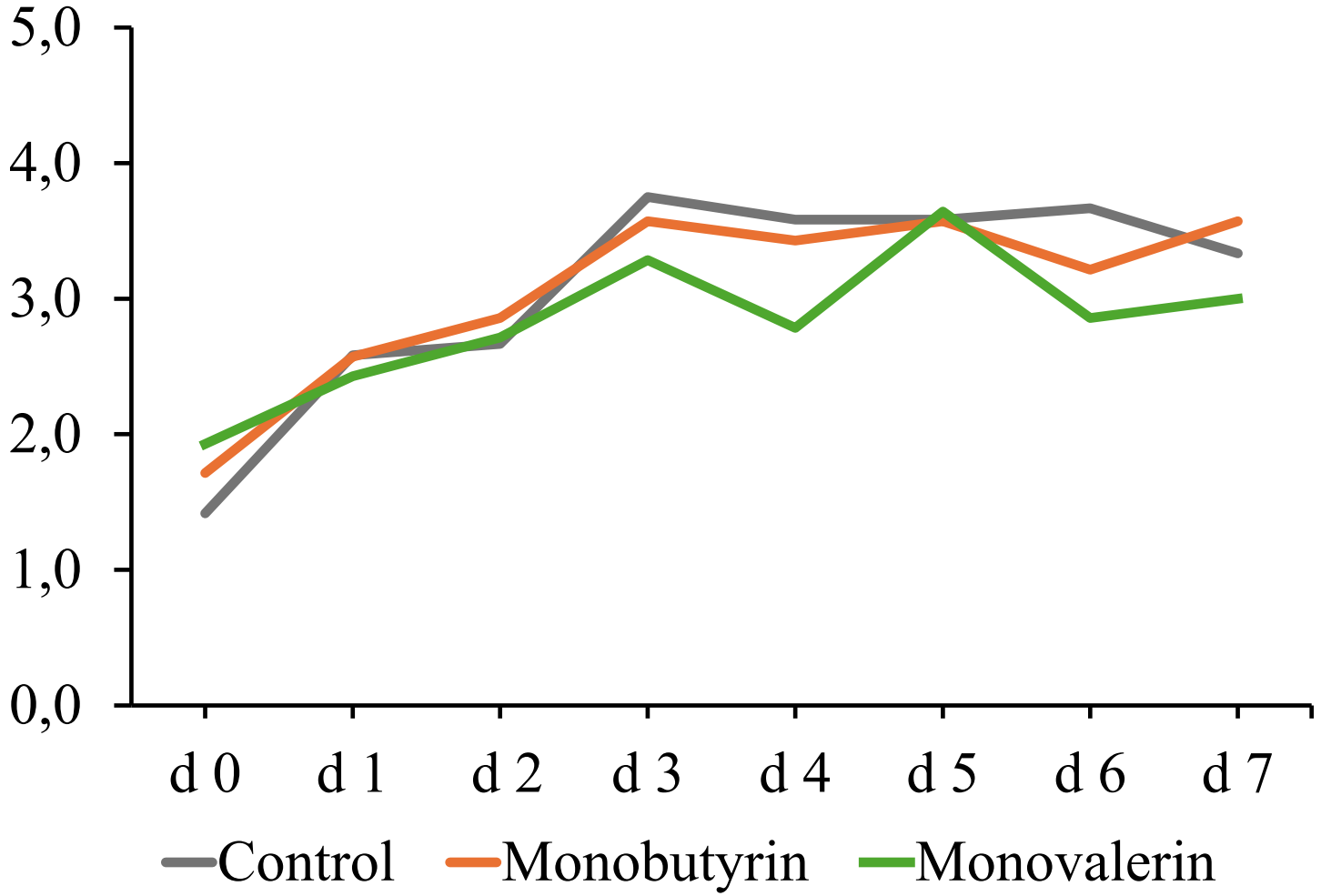
Experimental timeline



Diarrhea scores: d 0 to d 7 PI



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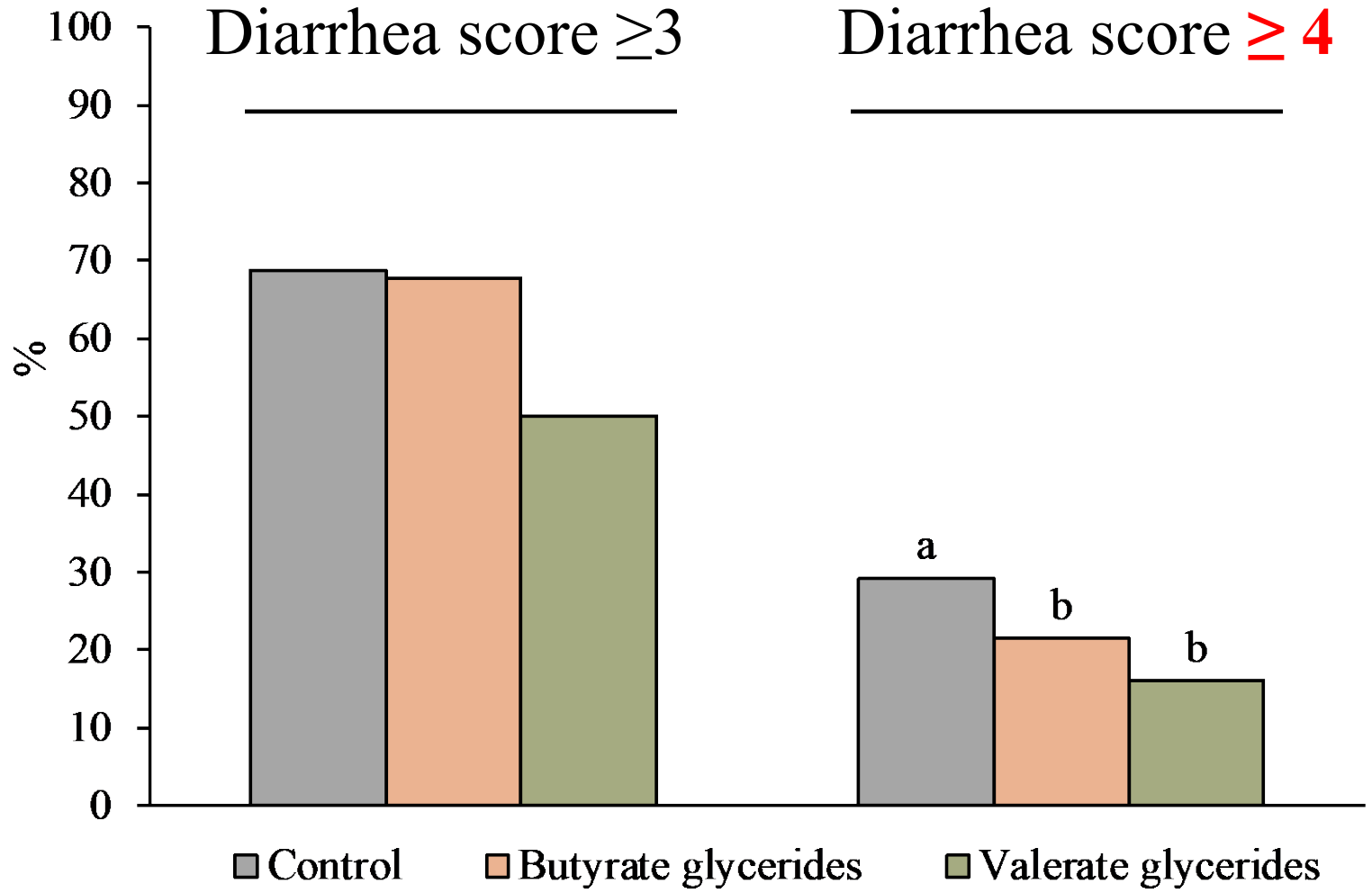
Frequency of diarrhea: d 0 to d 7 PI



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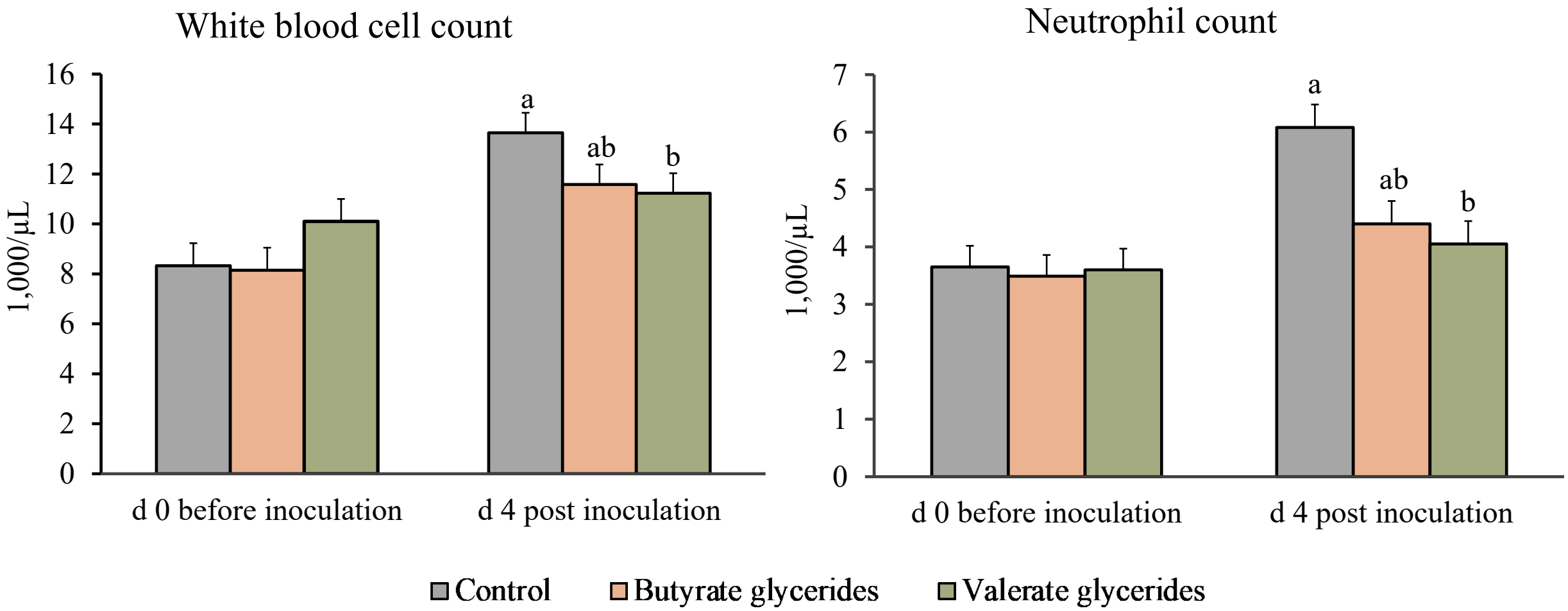
Whole blood hematology



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Blood serum TNF-α

	Control	Butyrate glycerides	Valerate glycerides	SEM	*P*-value		
					Treatment	Day	Interaction
d 0	302	175	199	65.42	<0.05	0.530	0.99
d 4 PI	251	147	121	55.48			
d 7 PI	291	159	194	69.45			

Conclusions after pilot experiment

- ✓Experimental **coinfection** with F4+ and F18+ ETEC **elicited diarrhea**
- ✓**Valerate glyceride** supplementation **reduced**:
  - ✓ **Inflammatory markers**
  - ✓**Frequency of severe diarrhea**

Experiment 2

Objective:

Determine effects of dietary **monoglycerides** and **triglycerides** of **valerate** on intestinal health and immunity in weanling pigs infected with ETEC

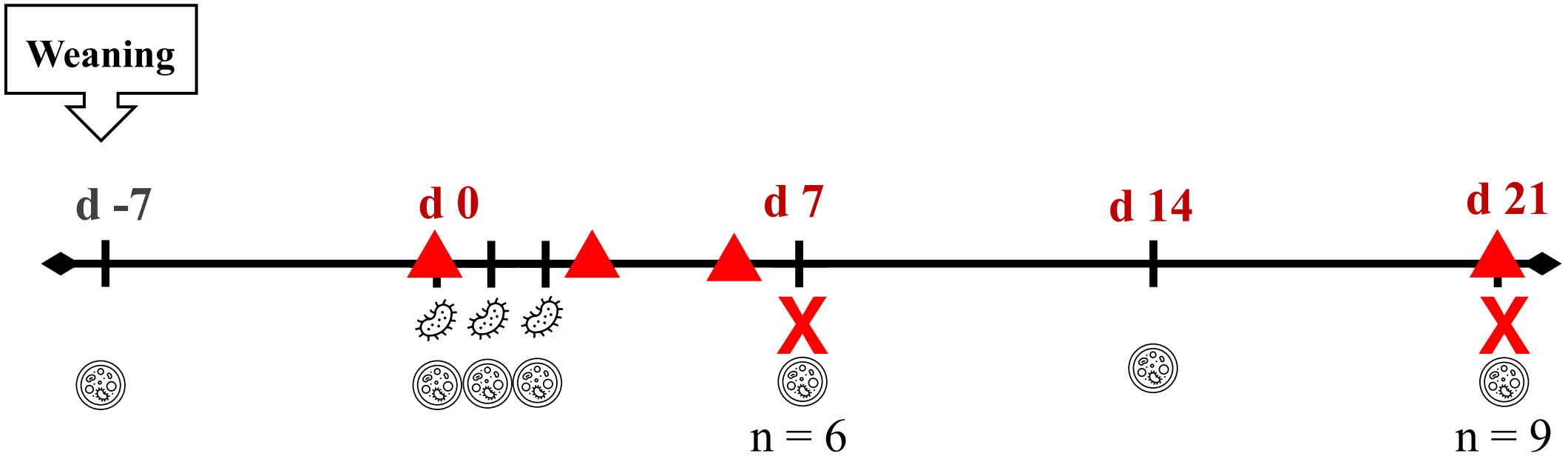
Materials and methods: **Experimental design**

- **60 weaned piglets** (21-24 d of age), individually housed
- **RCBD**: By bodyweight within sex

Control	Low monovalerin	High monovalerin	Trivalerin
Basal diet	**Basal diet +** **0.075%** Monovalerin	**Basal diet +** **0.1%** Monovalerin	**Basal diet +** **0.1%** Trivalerin

- All data analyzed using PROC MIXED in SAS

Materials and methods: **Timeline**



ETEC inoculation (10^{10} CFU/3 mL)

Blood collection

X Tissue sampling

Fecal culture

Materials and methods: **Mucosal intestinal mRNA expression**

- **Cytokines**
  - Tumor necrosis factor alpha (***TNFA***)
  - Interleukin 10 (***IL10***)
- **Tight junction proteins**
  - Zona-occludens-1 (***ZO1***)
  - Occludin (***OCLN***)
- **G-protein coupled receptors**
  - G-protein coupled receptor 43 (***GPR43***)
  - G-protein coupled receptor 41 (***GPR41***)
- **Enteroendocrine cell marker**
  - Cholecystokinin (***CCK***)
- **Ion secretion**
  - Cystic fibrosis transmembrane regulator (***CFTR***)

Materials and methods: **Serum analysis**

ELISA

- Tumor necrosis factor alpha (**TNF-α**)
- C-reactive protein (**CRP**)
- Haptoglobin (**Hp**)

Frequency of severe diarrhea d -7 to d 20 PI

Frequency of hemolytic coliforms d 7 PI

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mRNA expression d 7 PI: **TNF-α**

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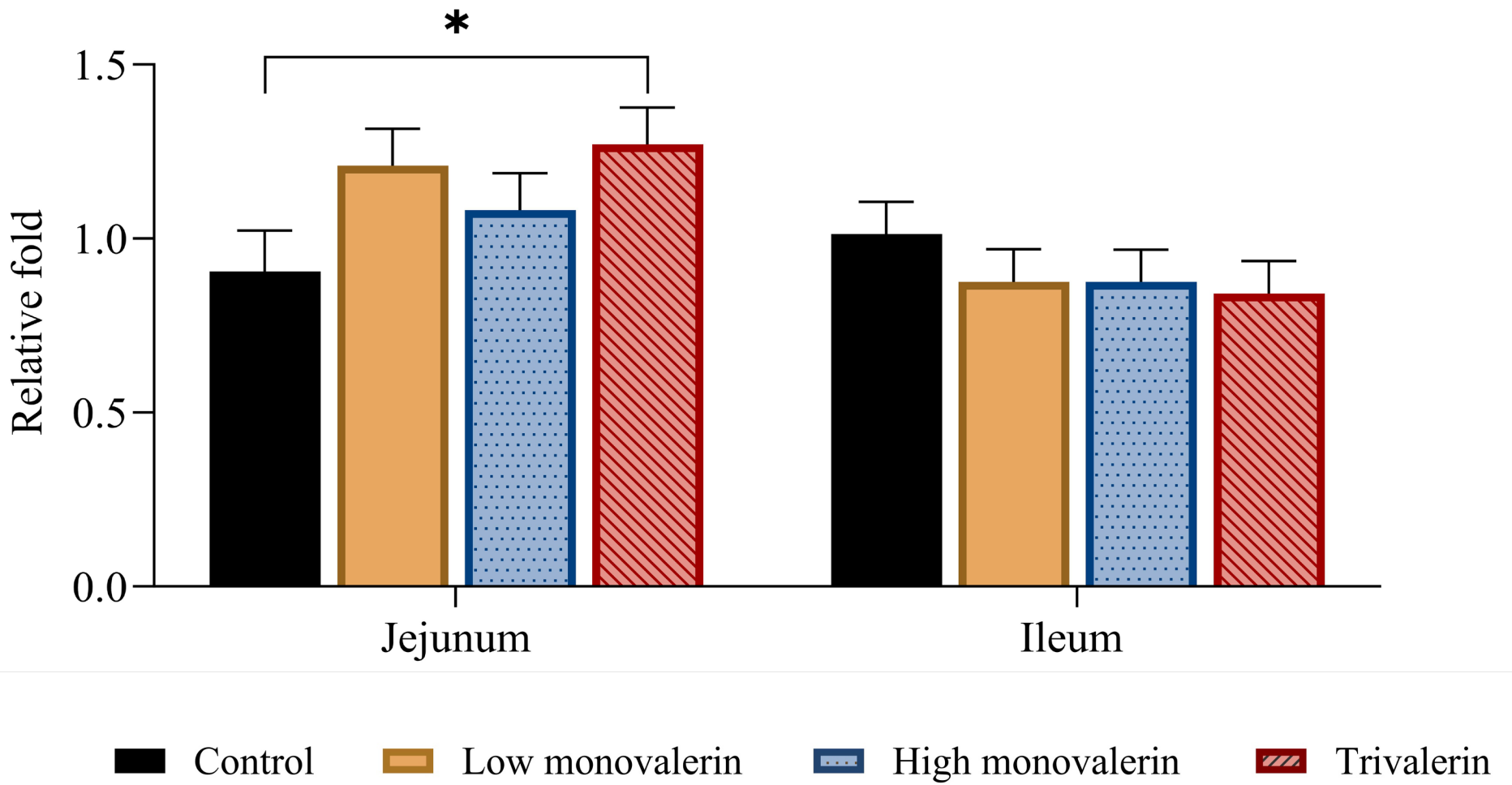
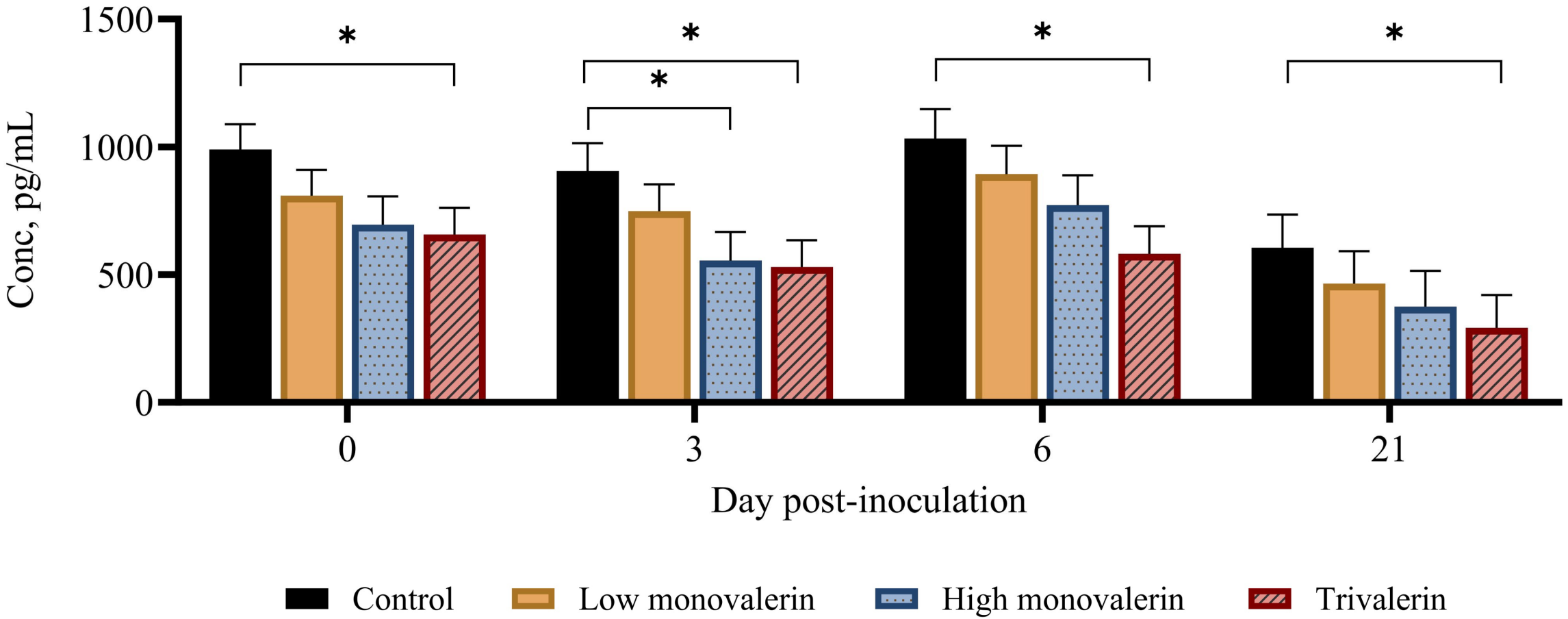
mRNA expression d 7 PI: **Zona-occludens 1**



Serum **TNF-α**



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Conclusions

✓Trivalerin

- **Reduced frequency of hemolytic coliforms**
- **Reduced frequency of severe diarrhea**
- **Increased intestinal gene expression of *ZO1* and decreased gene expression of *TNF-α***

✓0.1% monovalerin ***or*** trivalerin

- **Reduced serum TNF-α**

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Overall conclusions

Feeding valerate glycerides to weaned pigs infected with ETEC

✓**Reduced fecal shedding of hemolytic coliforms**

✓**Reduced frequency of severe diarrhea**

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