

# Supplementation of *Bacillus subtilis* modified fecal microbiota of weaning pigs experimentally infected with pathogenic *E. coli*

**Abstract  
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# Post-weaning Diarrhea

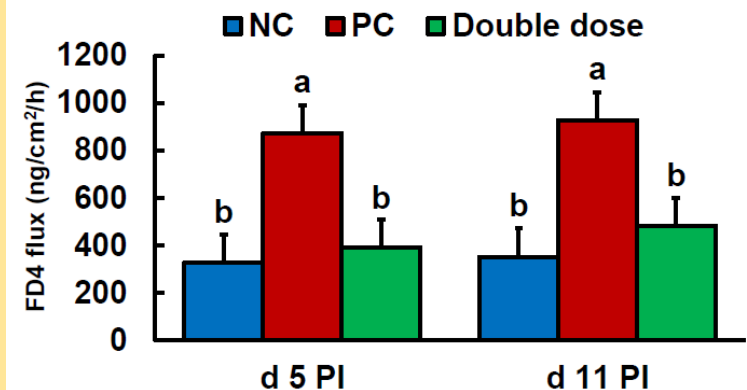
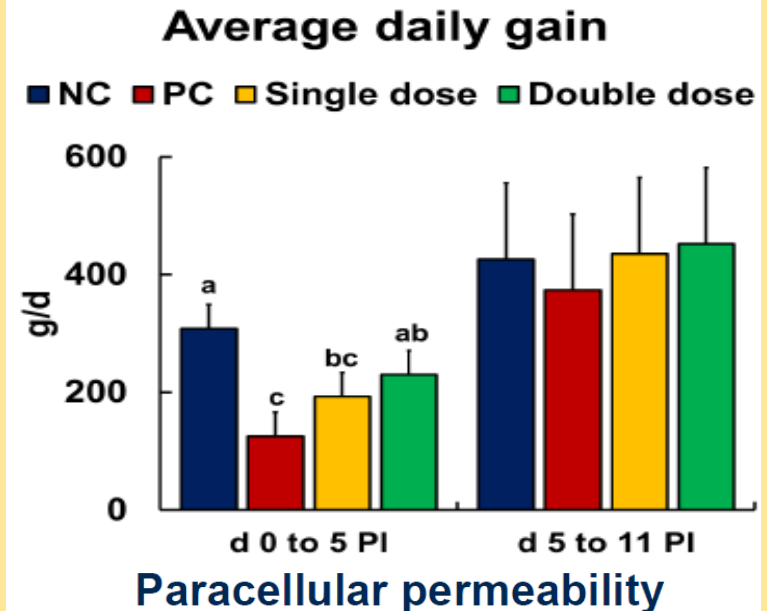
- Can be induced by
  - Stress
  - Sudden change of diet
  - Increased exposure to enterotoxigenic pathogens
    - **F18 *E. coli***
- Often leads to weight loss and possibly death
- Economical loss

# Probiotics

- Live microorganisms confer a health benefit on the host when administered in adequate amounts (UNFAO/WHO 2001)
- Potential alternatives to antibiotics
- *Bacillus subtilis*
  - Gram positive, aerobic bacteria
  - Stable in ambient temperature
  - Spores are resistance to harsh environment

# Previous Studies (Kim et al., 2019)

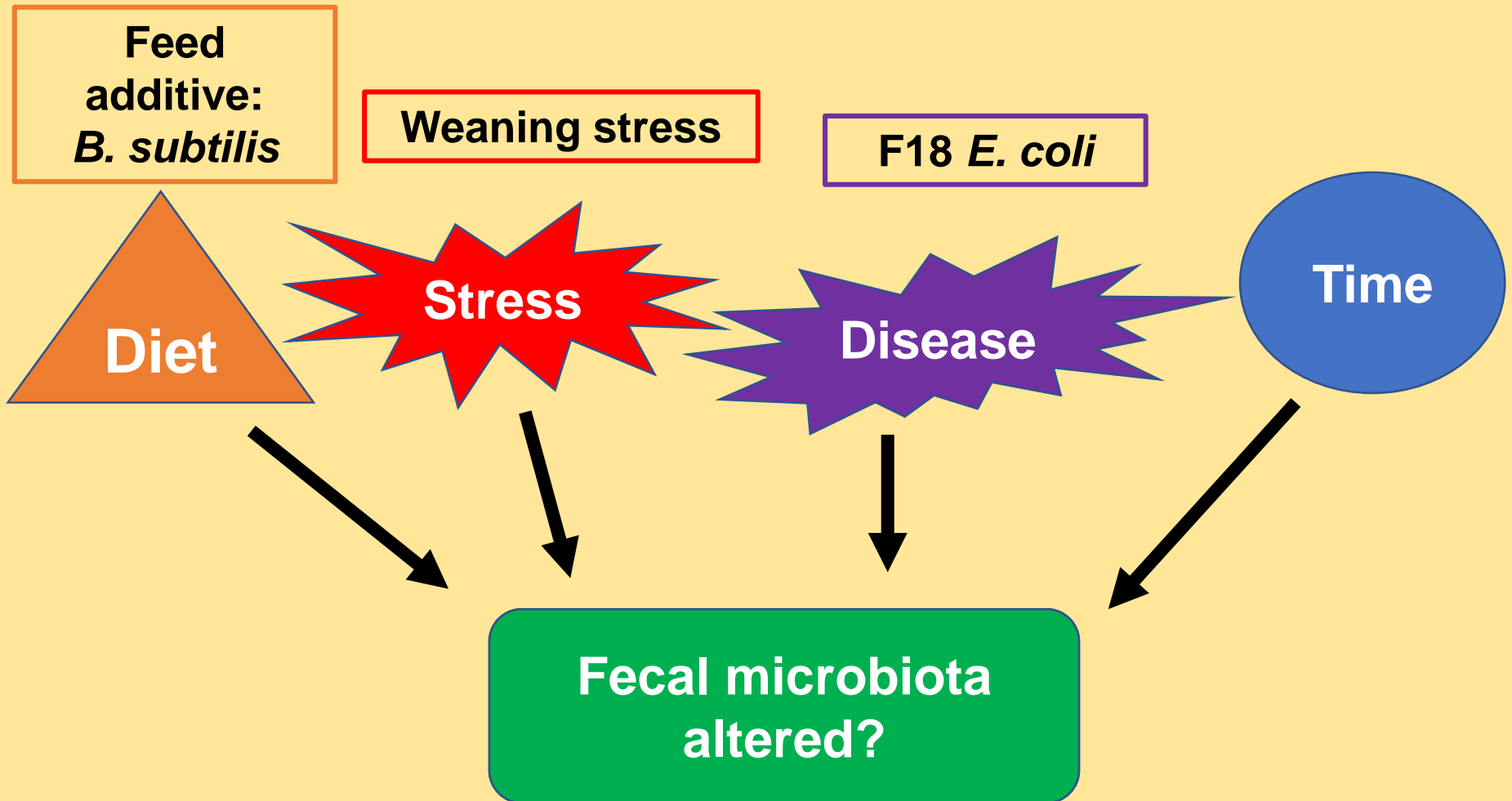
- **Supplementation of *B. subtilis* to weanling pigs challenged with F18 *E. coli*:**
  - Improved growth rate
  - Reduced leaky gut
  - Enhanced gut barrier functions



# Gut Microbiota

- **Gut microbiota** = collection of microbe living inside the gut
- **Gut microbiome** = totality of microbes, their genetic elements, and environmental interactions in the gut
- Microbes in the intestines play a role in keeping the host healthy
  - Nutrition: production of VFAs
  - Physiology: maintenance of gut structure and barrier function
  - Immune system: sIgA regulation

# Gut Microbiota: Objective

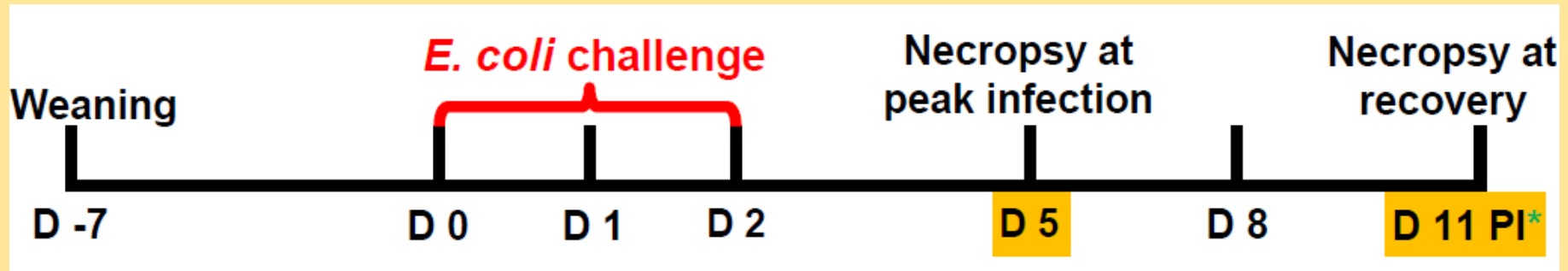


# Experimental Design

- 48 weanling pigs
    - $6.73 \pm 0.77$  kg BW, 21 d old
  - RCBD (Blocks: BW x Sex)
  - 4 treatments (12 pigs/treatment)
    1. Negative control ← *E. coli* non-challenged
    2. Positive control
    3. Single dose *Bacillus subtilis*
      - $1.28 \times 10^9$  CFU/kg
    4. Double dose *Bacillus subtilis*
      - $2.56 \times 10^9$  CFU/kg
- } *E. coli* challenged



# Experimental Design

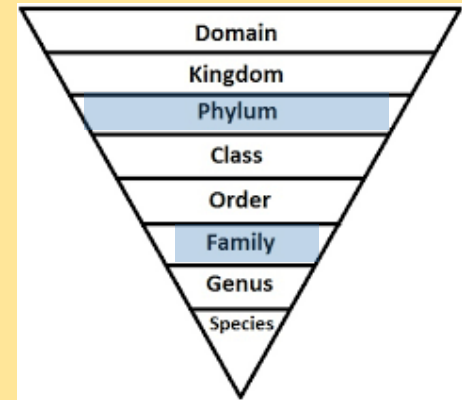


- Oral inoculation of pathogenic F18 *E. coli* challenge with  $10^{10}$  CFU/dose were given to pigs 3 times
- Fecal samples were collected on d 5 and d 11 post inoculation

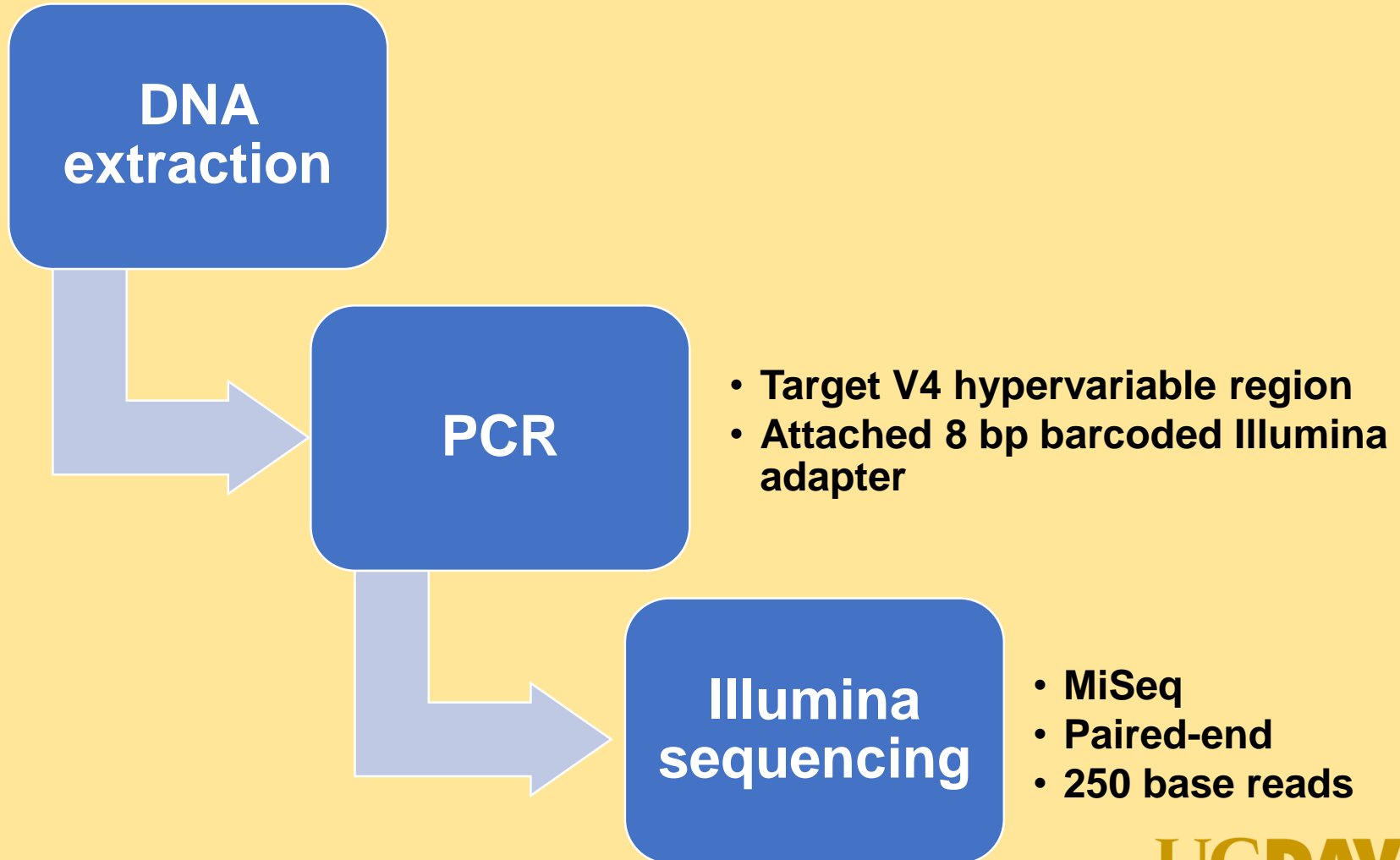


# 16S rRNA Sequencing: Measurements

- **Alpha diversity**
  - Observed: measures richness
  - Shannon: measures richness and evenness
- **Beta diversity**
  - Bray Curtis Dissimilarity
    - Principal Coordinates Analysis (PCoA) plot
- **Relative abundance**
  - Phylum
  - Family: *Firmicutes & Proteobacteria*



# 16S rRNA Sequencing: Library Preparation



# 16S rRNA Sequencing: Bioinformatics

**Fastqc +  
Trimmomatic**

- Raw reads evaluated for quality control and trimmed

**QIIME2 (2018.6)**

- dada2: quality control and feature table construction
- Greengenes: database used to generate taxonomy file

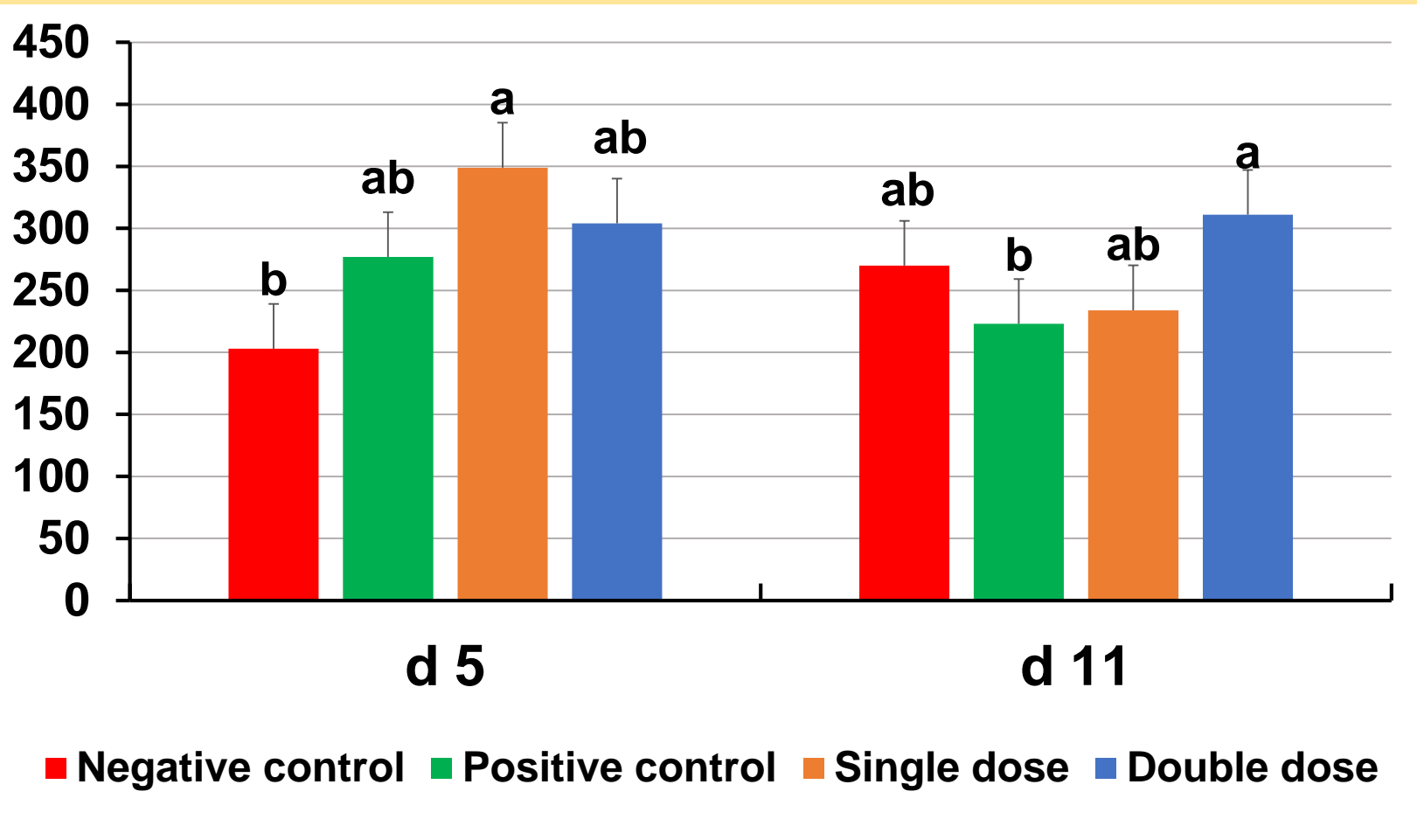
**R program**

- Phyloseq & ggplot2: calculate and visualize **beta diversity** and **relative abundance**; calculate alpha diversity indices

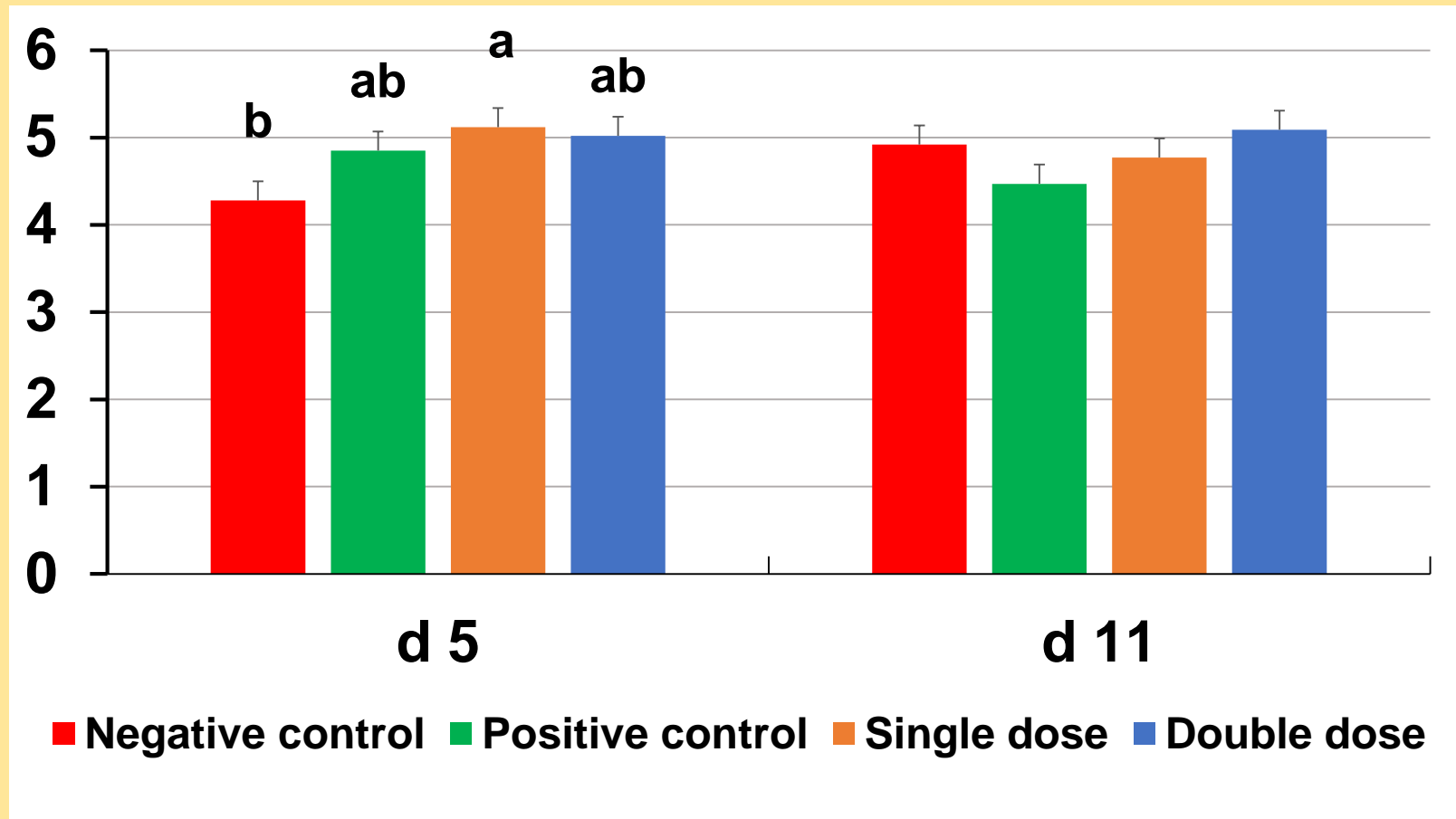
**SAS**

- ANOVA using PROC MIXED of SAS for **alpha diversity** and **relative abundance**; significant difference at  $P < 0.05$

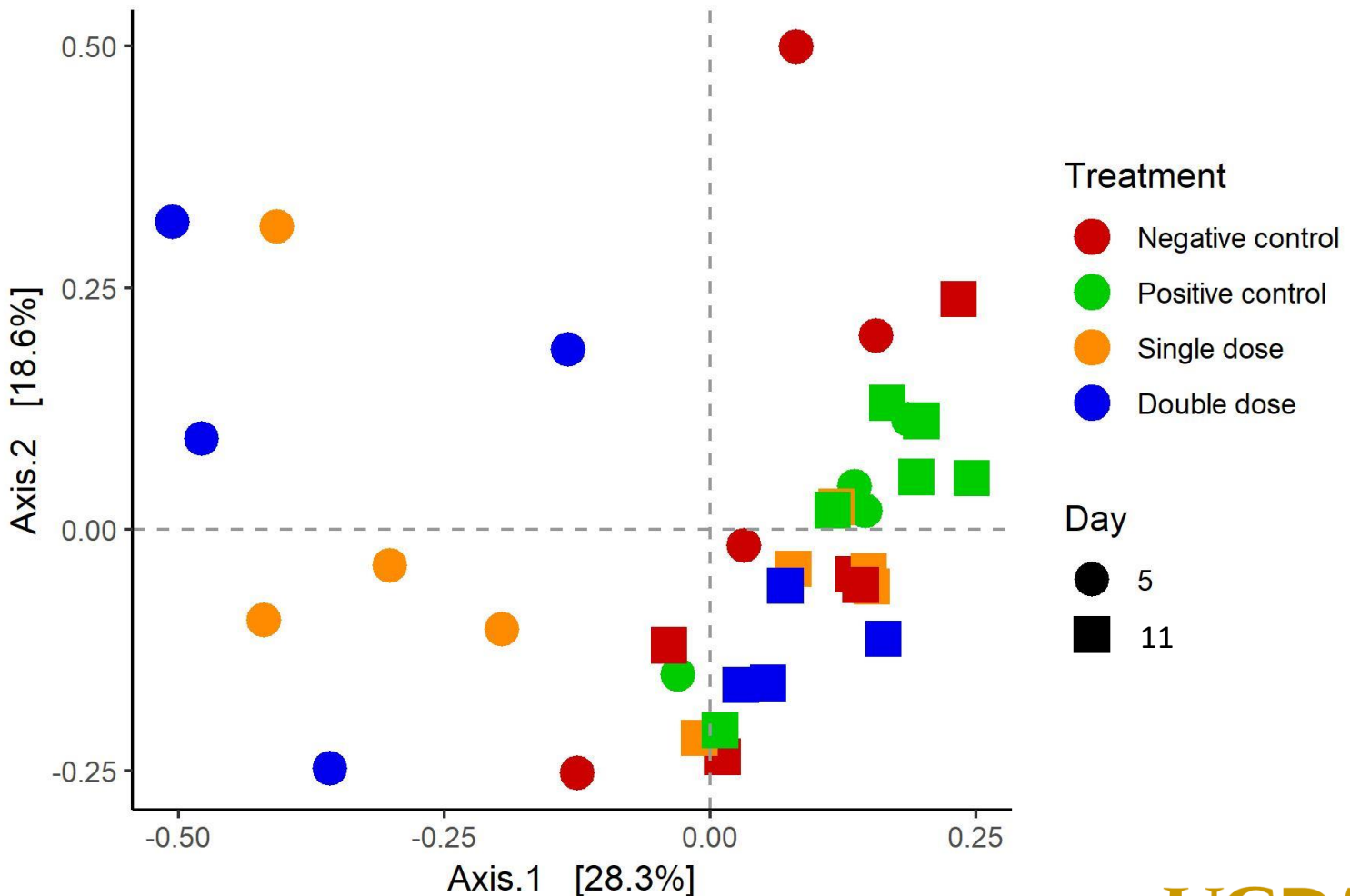
# Alpha Diversity: Observed



# Alpha Diversity: Shannon

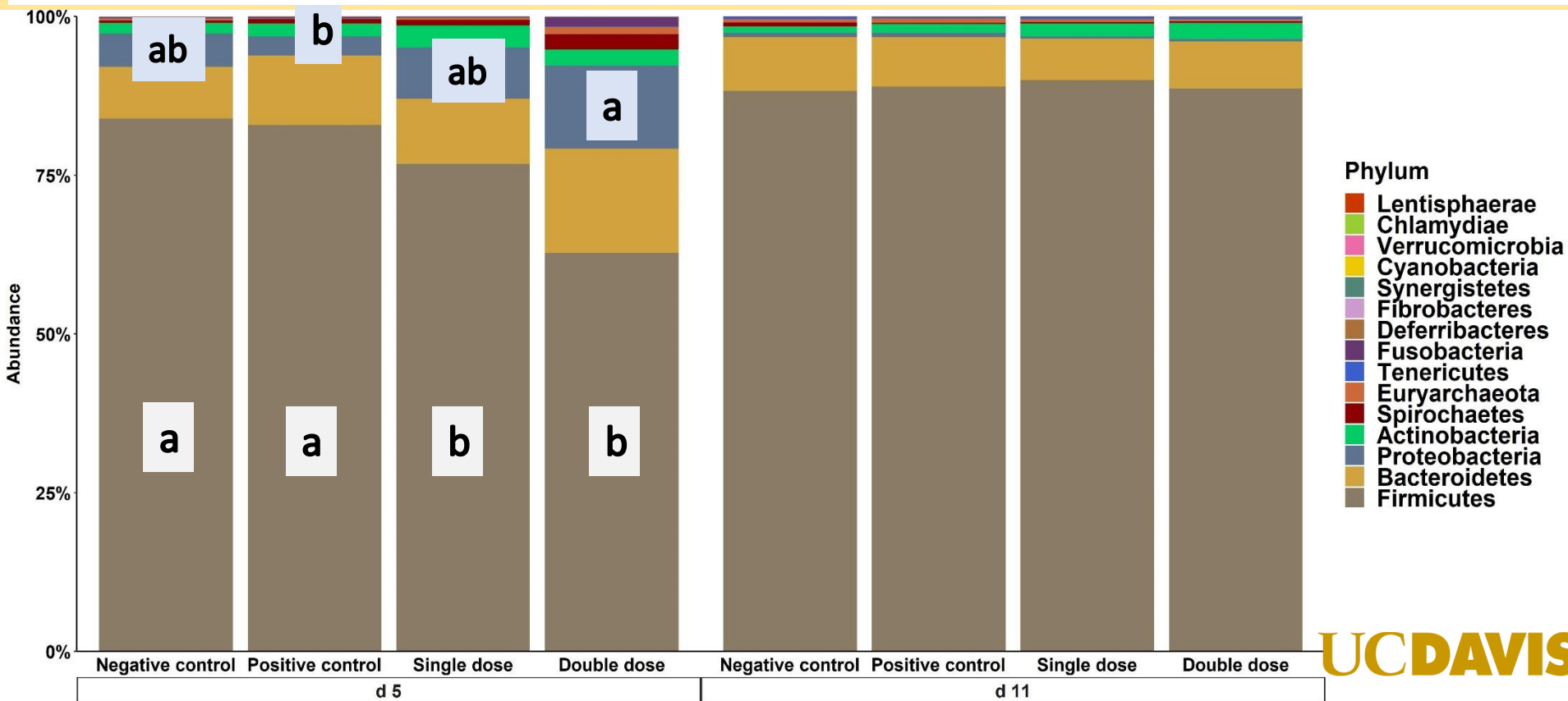


# Beta Diversity: PCoA



# Relative Abundance: Phylum

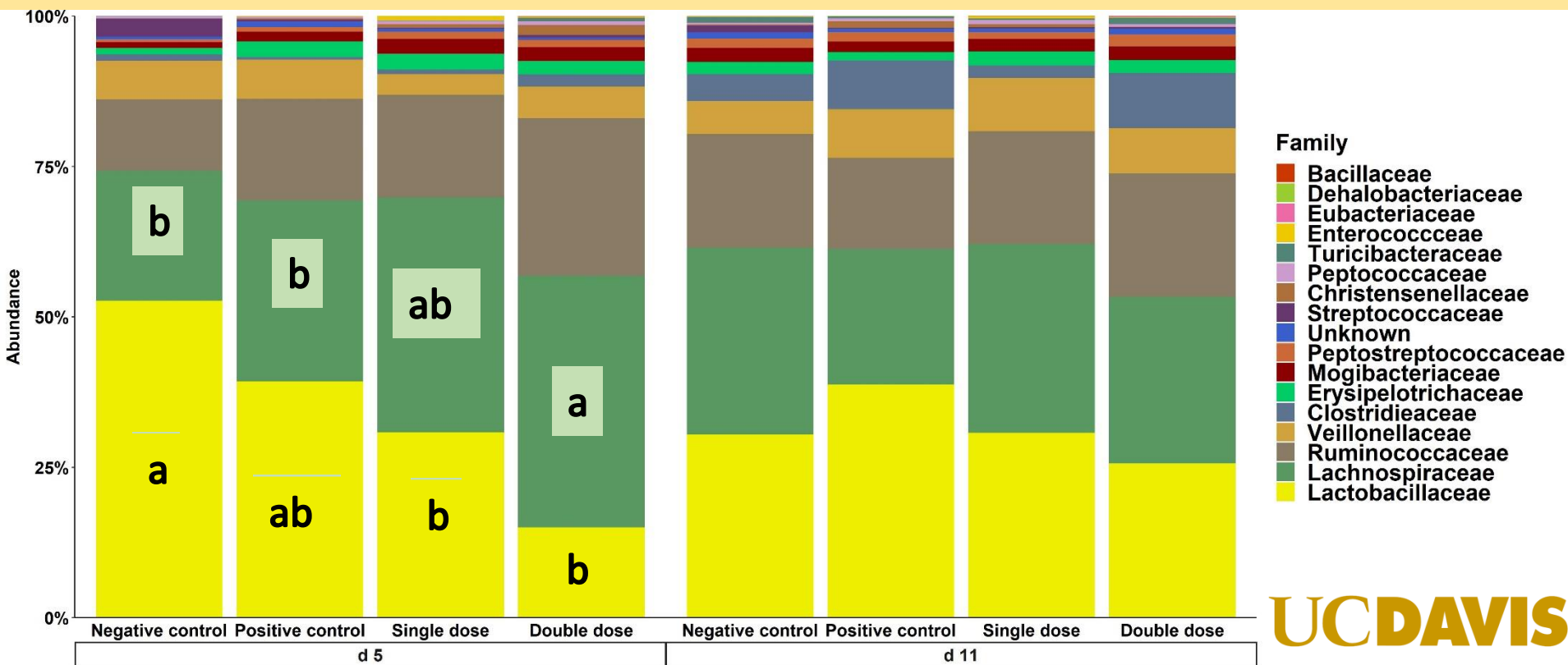
- d 5:
  - Lowest relative abundance of *Proteobacteria* in Positive control
  - Lowest relative abundance of *Firmicutes* in Single & Double doses





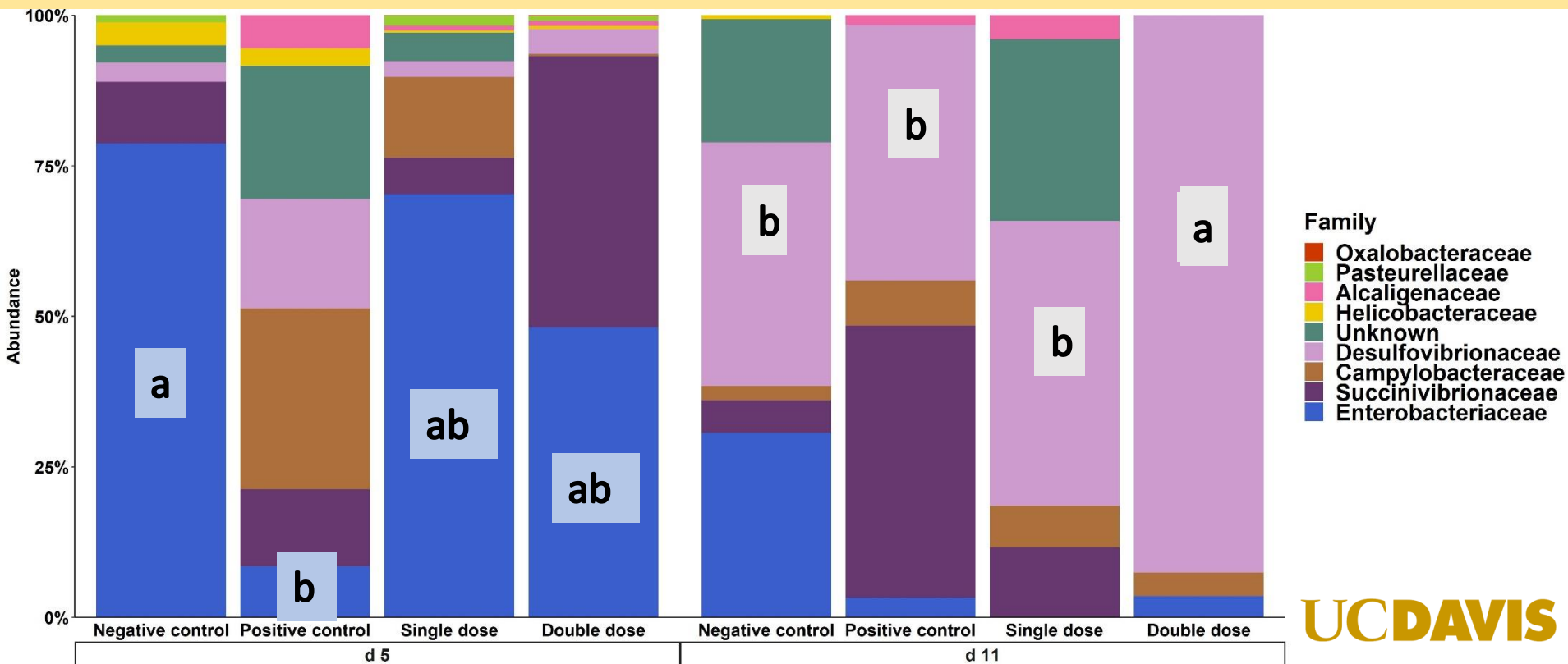
# Relative Abundance: *Firmicutes* Family

- d 5
  - Highest relative abundance of *Lachnospiraceae* in Double dose
  - Low relative abundance of *Lactobacillaceae* Single & Double doses



# Relative Abundance: *Proteobacteria* Family

- d 5: Lowest relative abundance of *Enterobacteriaceae* in Positive control
- d 11: Highest relative abundance of *Desulfovibrionaceae* in Double dose





# Conclusions

- **Supplementation of *Bacillus subtilis* could modify gut microbiota of weaned pigs challenged with F18 *E. coli***
- **Further analysis**
  - **Quantify *Bacillus subtilis* and F18 *E. coli* in fecal microbiota**
  - **Evaluate metagenomics of fecal microbiota**

# Thank you!

