

# Supplementation of *Bacillus subtilis* Combined with Plant Extracts Effect on Growth Performance and Post-weaning Diarrhea in Pigs

Charlotte Ludorf<sup>1</sup>, Mariah Jansen<sup>1</sup>,  
Jacqueline Connelly<sup>1</sup>, Sophia Reynolds<sup>1</sup>, Hsinhung John Hsu<sup>2</sup>,  
Chung–Nan Chen<sup>2</sup>, Kwangwook Kim<sup>1</sup>

Abstract  
230

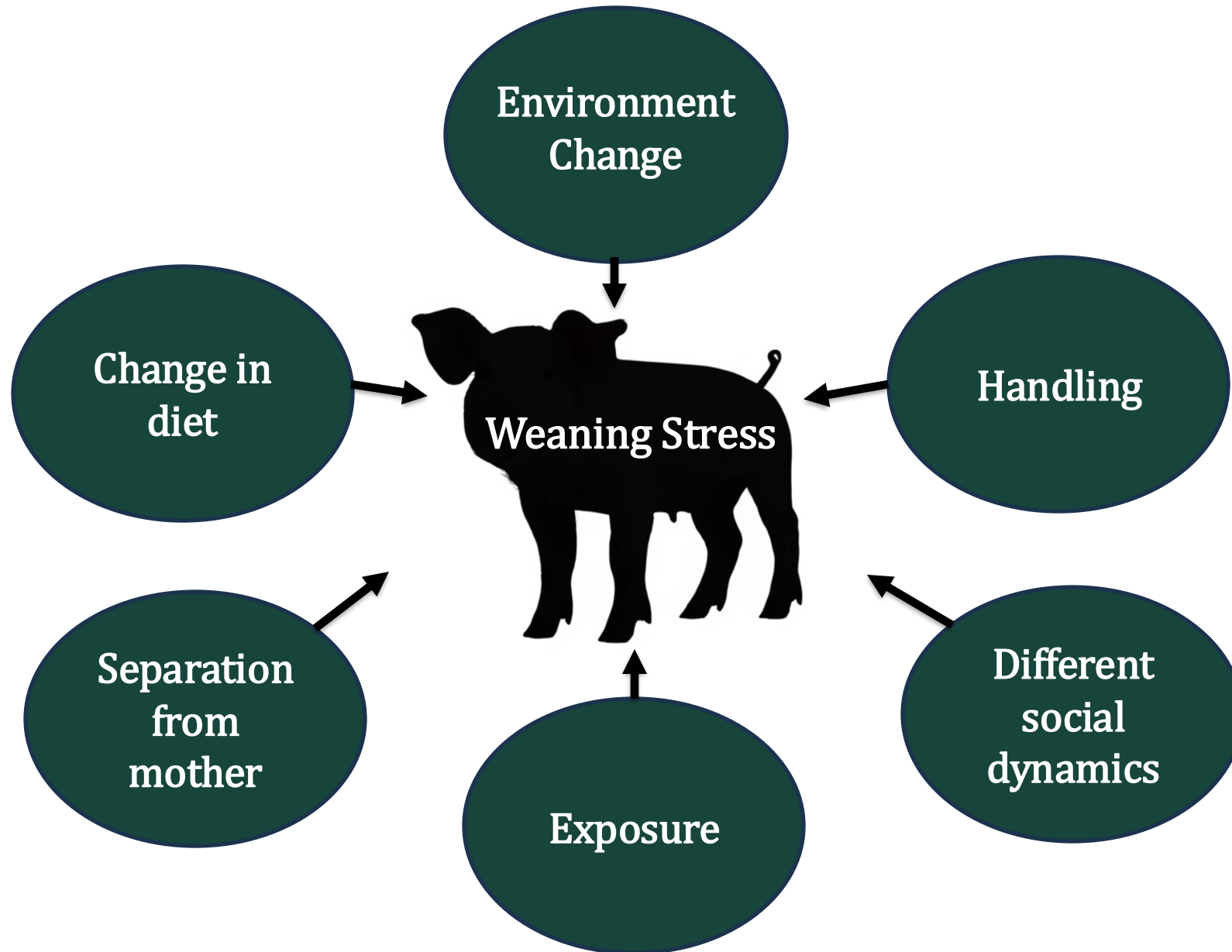
<sup>1</sup>Department of Animal Science, Michigan State University

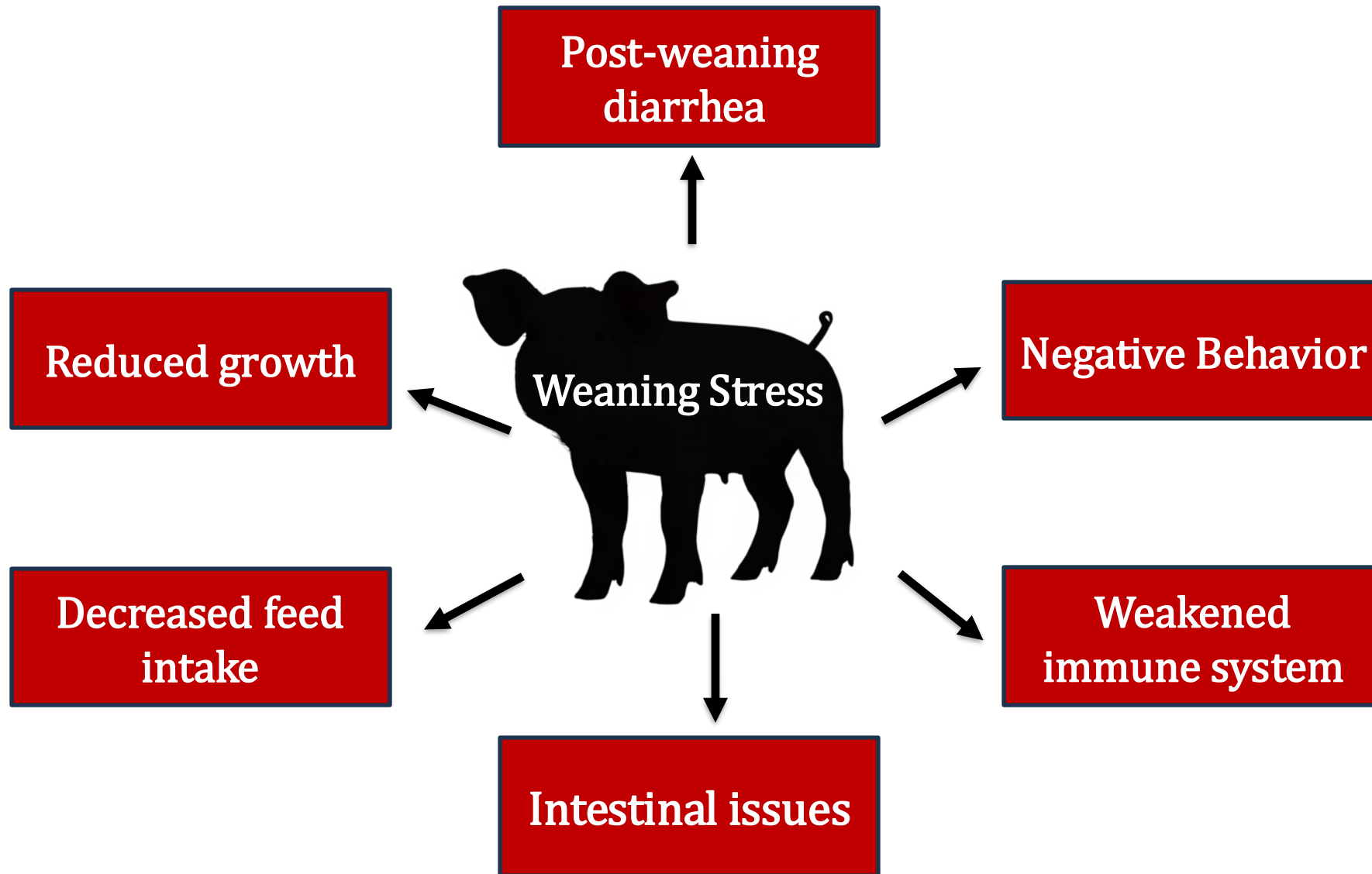
<sup>2</sup>JH Biotech, Inc.



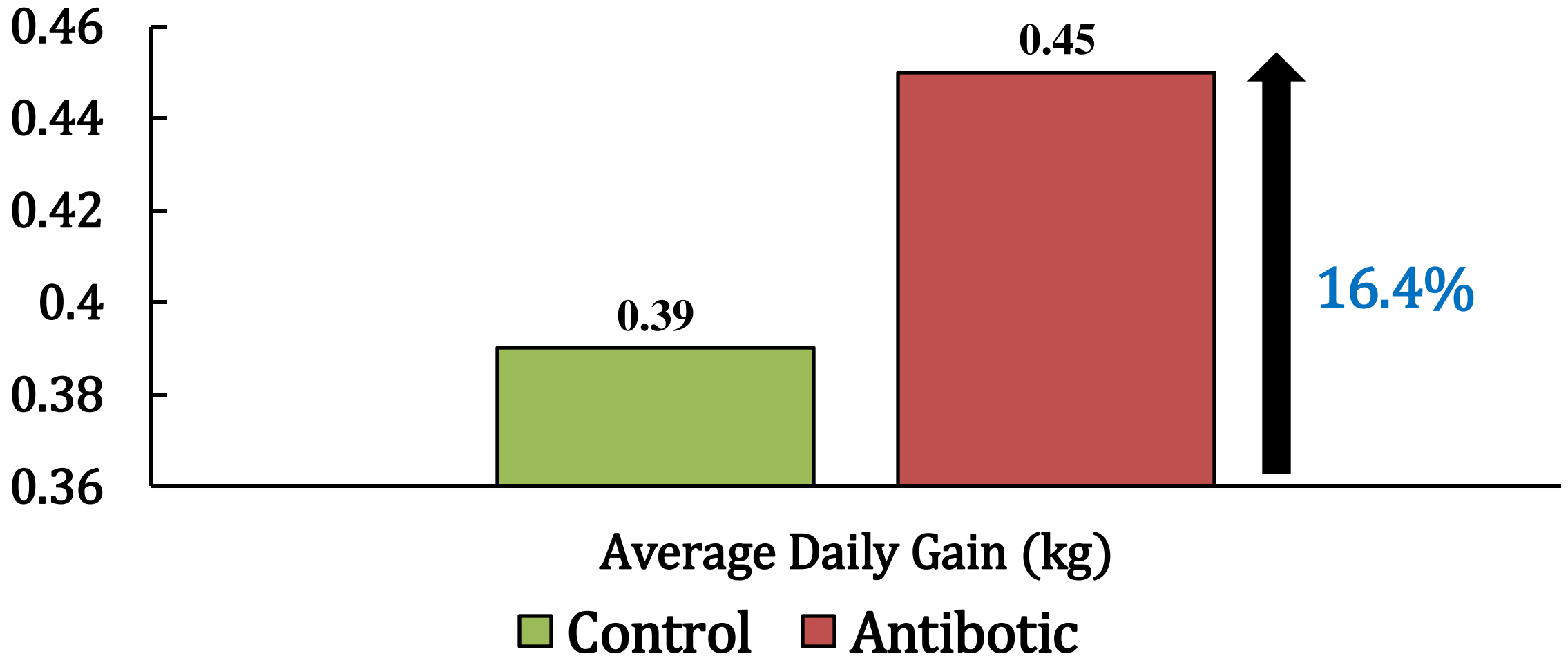
# Outline

- **Introduction**
  - ✓ **Weaning Stress**
  - ✓ **Antibiotics**
  - ✓ **Feed Additives**
- **Objective & Hypothesis**
- **Results & Conclusion**
- **Future Research**



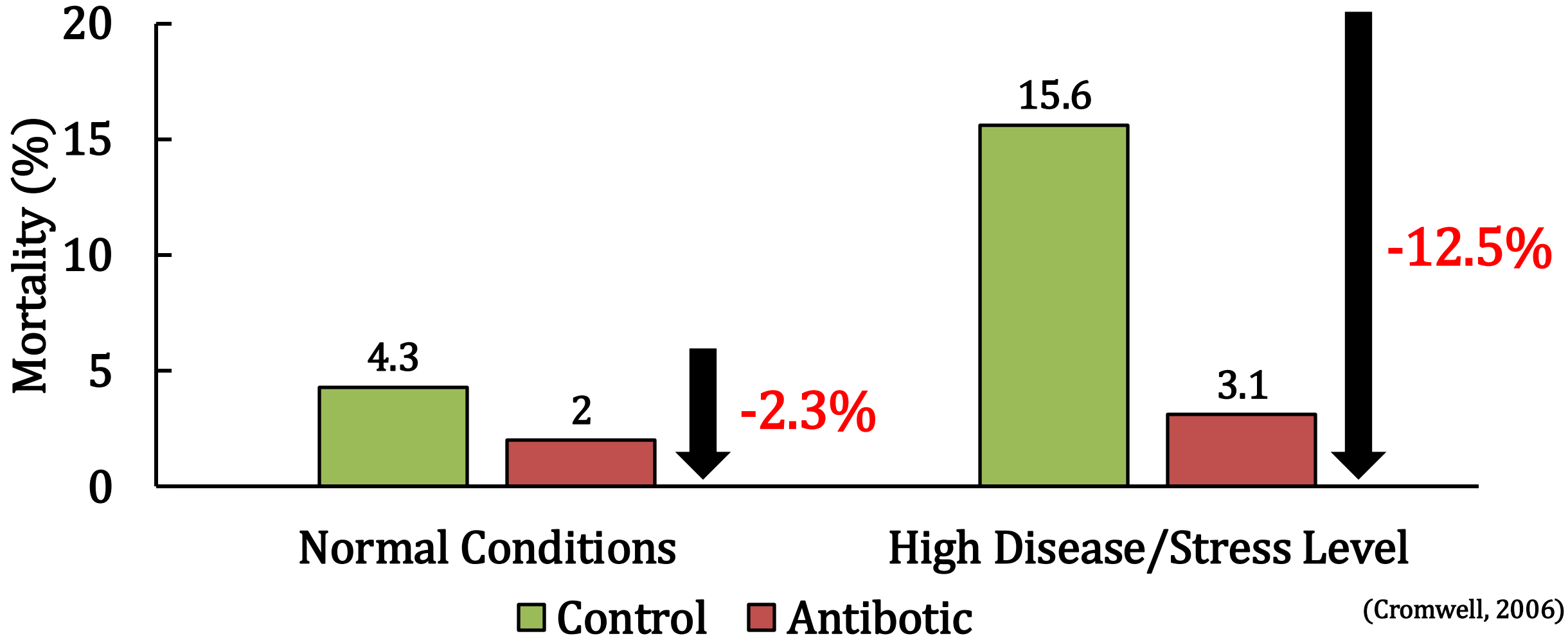


## In-Feed Antibiotics in Starting Phase (7-25 kg)



(Cromwell, 2006)

# Effects of In-Feed Antibiotics on Mortality of Young Pigs



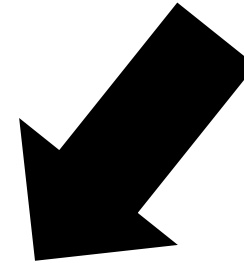
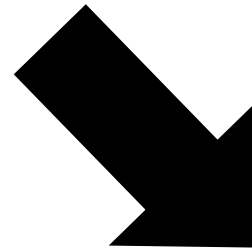
(Cromwell, 2006)

## Concerns on Antibiotic Use

Antimicrobial  
resistance

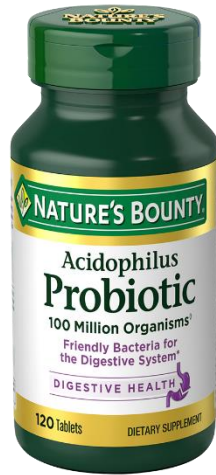


Antibiotic residues  
in products



Industry is turning to  
alternatives to antibiotics

# Potential Alternative to Antibiotics



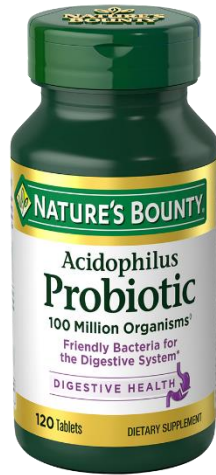
Direct-fed microbials

- Known as probiotics
- Live beneficial bacteria
- Improve microbial balance, digestion, and immune system





# Potential Alternative to Antibiotics



Direct-fed microbials

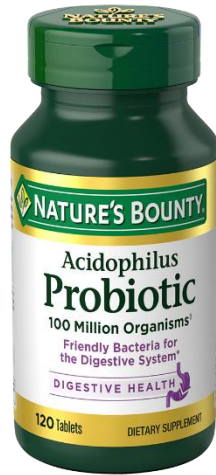
- Known as probiotics
- Live beneficial bacteria
- Improve microbial balance, digestion, and immune system



## Effectiveness

- Stable
- Ability to replicate
- Block growth of harmful bacteria

# Potential Alternative to Antibiotics



Direct-fed microbials

- Known as probiotics
- Live beneficial bacteria
- Improve microbial balance, digestion, and immune system



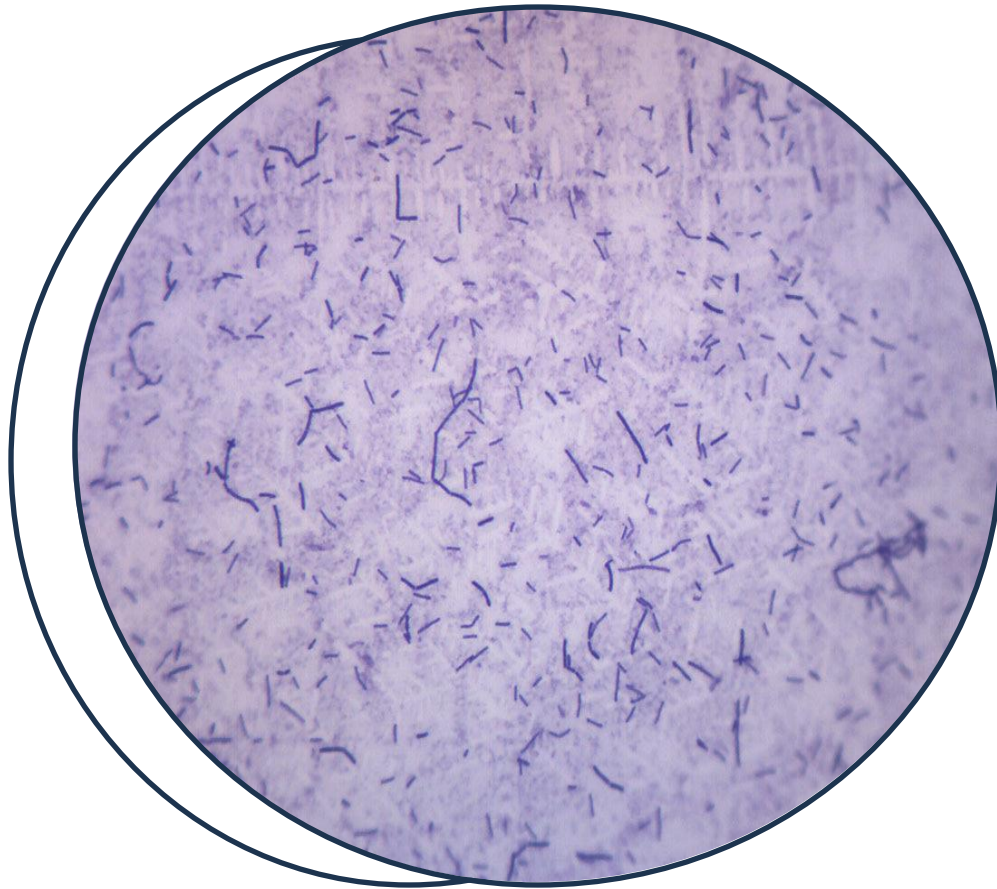
## Effectiveness

- Stable
- Ability to replicate
- Block growth of harmful bacteria

## Common strains

- *Lactobacillus* species
- *Enterococci* species
- *Bacillus* species

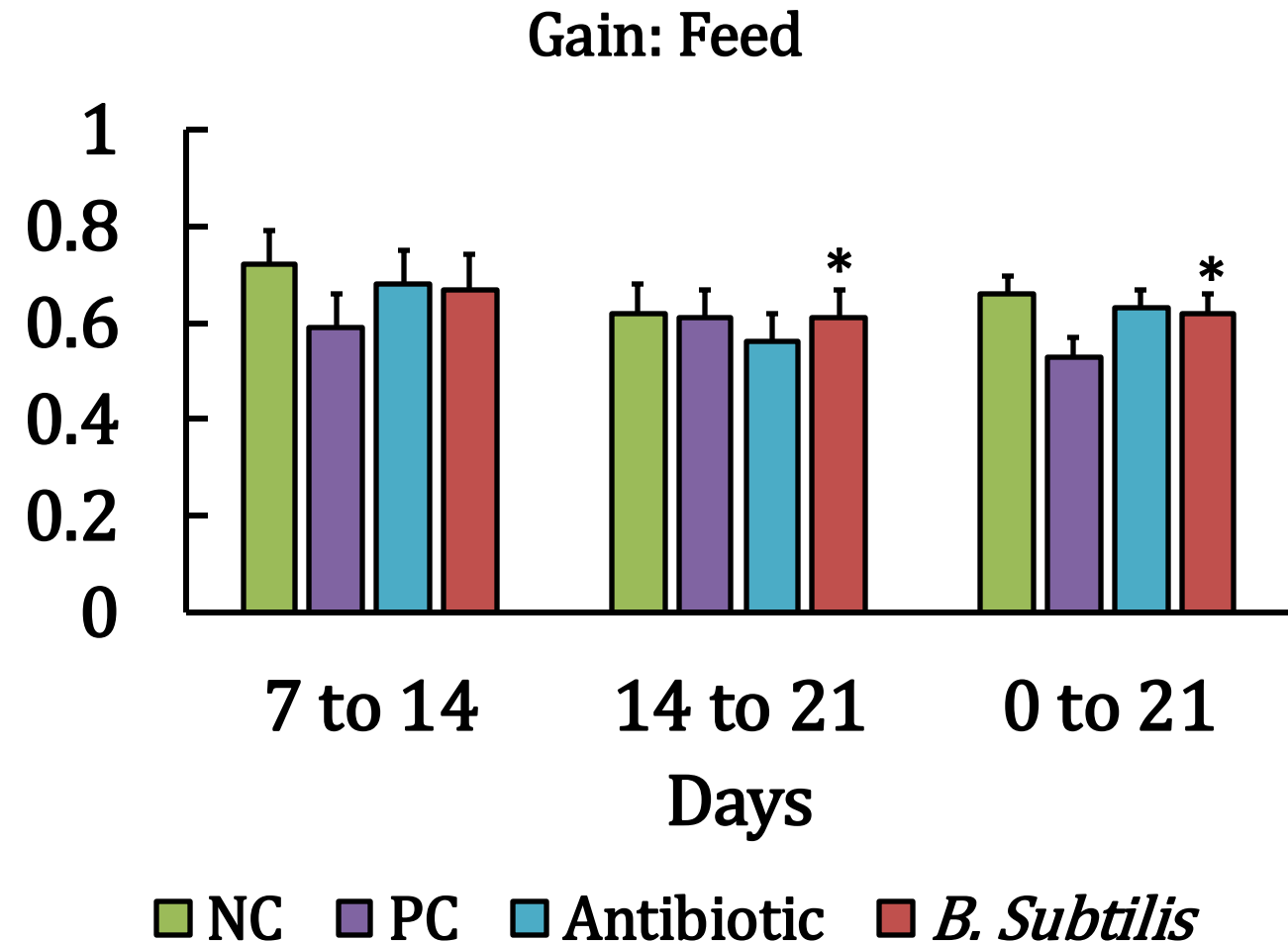
## *Bacillus subtilis*



- Best studied gram-positive organism
- Fast growing
- Stable
- Modifiable
- Easy cultivation
- Improve:
  - Digestion
  - Intestinal health
  - Disease prevention

## Previous Study: *Bacillus subtilis*

- Study on the impact of *Bacillus subtilis* inclusion in ETEC infected pigs
- Improved growth performance and disease resistance
- $2.56 \times 10^9$  CFU/kg of *Bacillus subtilis*



\*  $0.05 < P < 0.10$ : Statistical trend

# Plant Extracts: made from plants using a solvent



## **Cinnamon**

Anti-inflammatory, antimicrobial,  
antioxidant

# Plant Extracts: made from plants using a solvent



## **Thyme**

Anti-inflammatory, antiviral,  
antibacterial

(Halat, et al., 2022)



# Plant Extracts: made from plants using a solvent



**Cinnamon**

Anti-inflammatory, antimicrobial,  
antioxidant

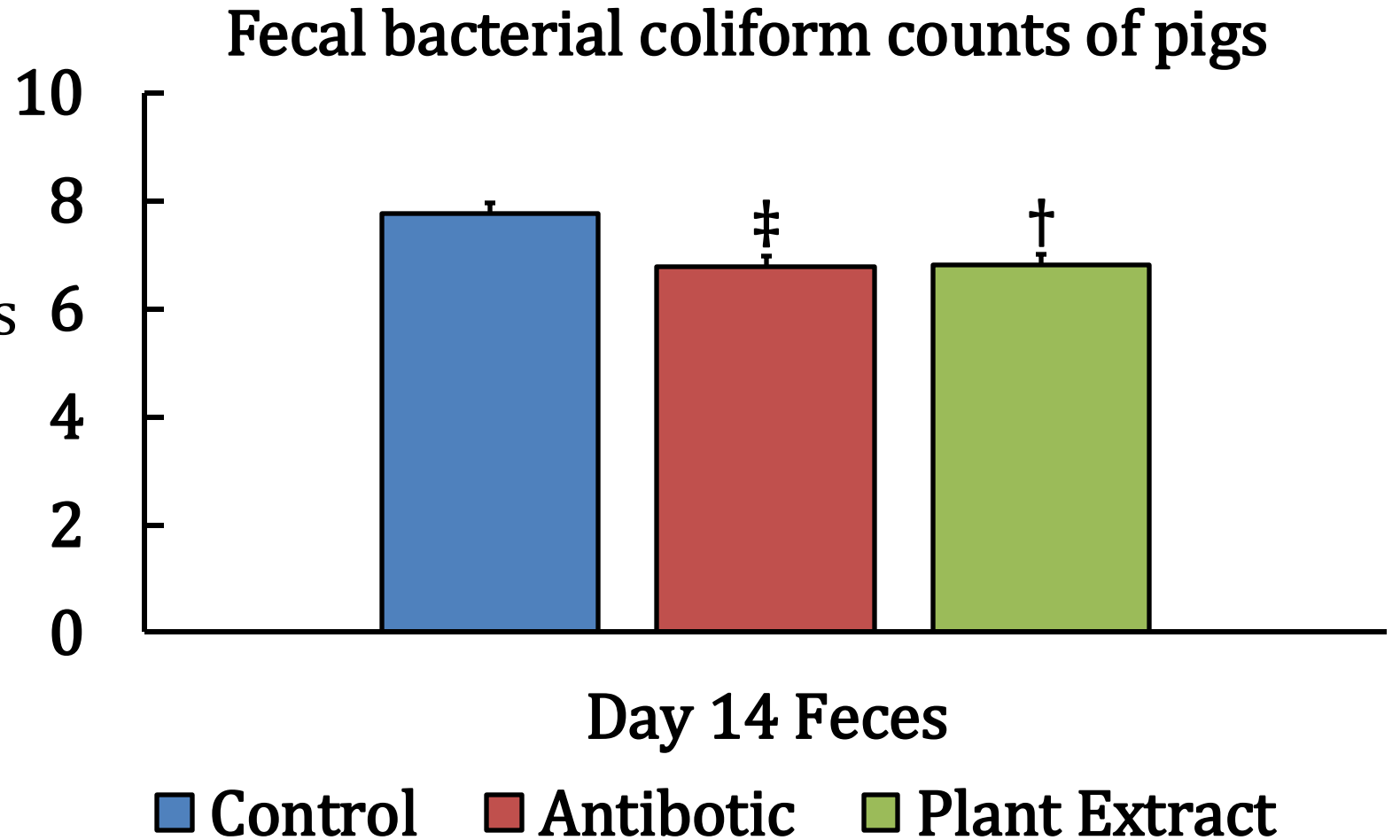


**Thyme**

Anti-inflammatory, antiviral,  
antibacterial

# Previous Study: Plant Extract

- Study on the impact of plant extract inclusion in post weaning piglet's diets
- Plant extract: including cinnamon and thyme



†  $0.05 < P < 0.10$ : Statistical trend

‡  $P < 0.05$ : Significant difference

(Namkung et al., 2004)



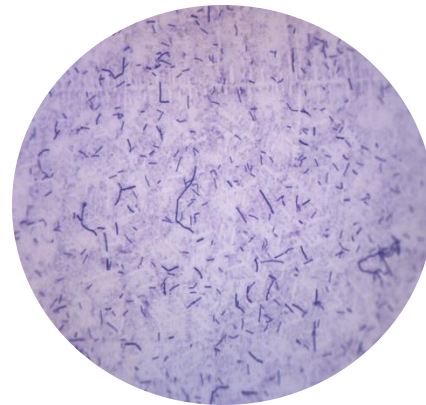
# Objective



To investigate the effects of dietary supplementation with *Bacillus subtilis* and **plant extracts** on growth performance and diarrhea of weanling pigs

# Hypothesis

Supplementation of *Bacillus subtilis* combined with plant extracts will have a **positive impact on growth** and **decrease the incidence of diarrhea**



# Experimental Design

- Randomized Complete Block Design (Blocks: BW x Sex)
- 240 weanling pigs
  - Average Body Weight:  $6.12 \pm 0.53$  kg
  - Average Age:  $19 \pm 1$  d old
- 4 treatment groups:
  - 6 pigs/pen
  - 10 replicates

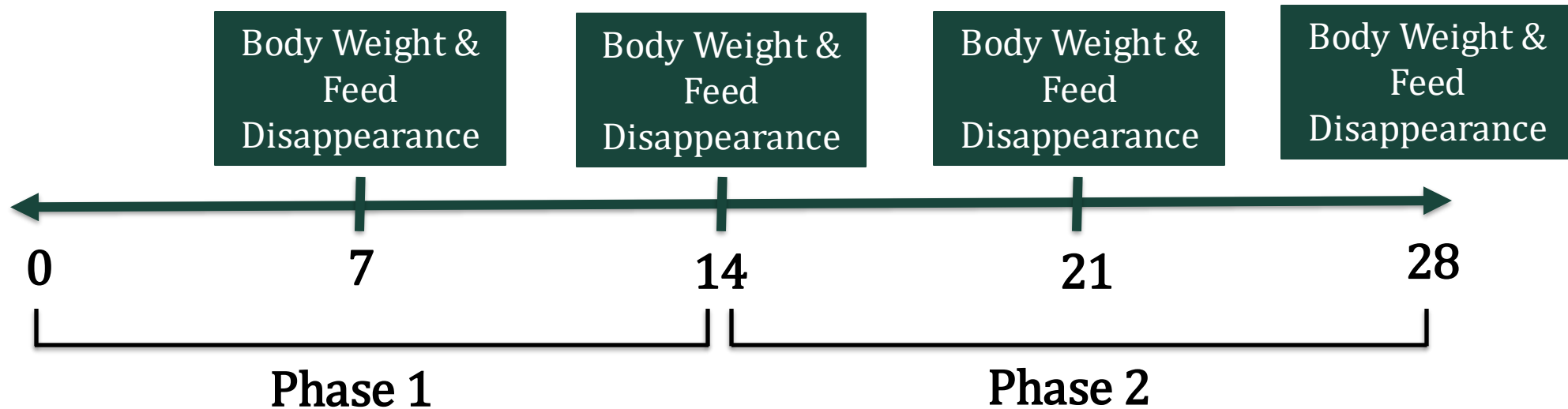
**1. Control (CON)**  
basal nursery diet

**2. *Bacillus subtilis* single dose (BPS)**  
CON + 500 mg/kg of *Bacillus subtilis* and  
plant extract

**3. *Bacillus subtilis* double dose (BPD)**  
CON + 1000 mg/kg of *Bacillus subtilis* and  
plant extracts

**4. Antibiotic (ATB)**  
CON + 50 mg/kg carbadox

# Experimental Design

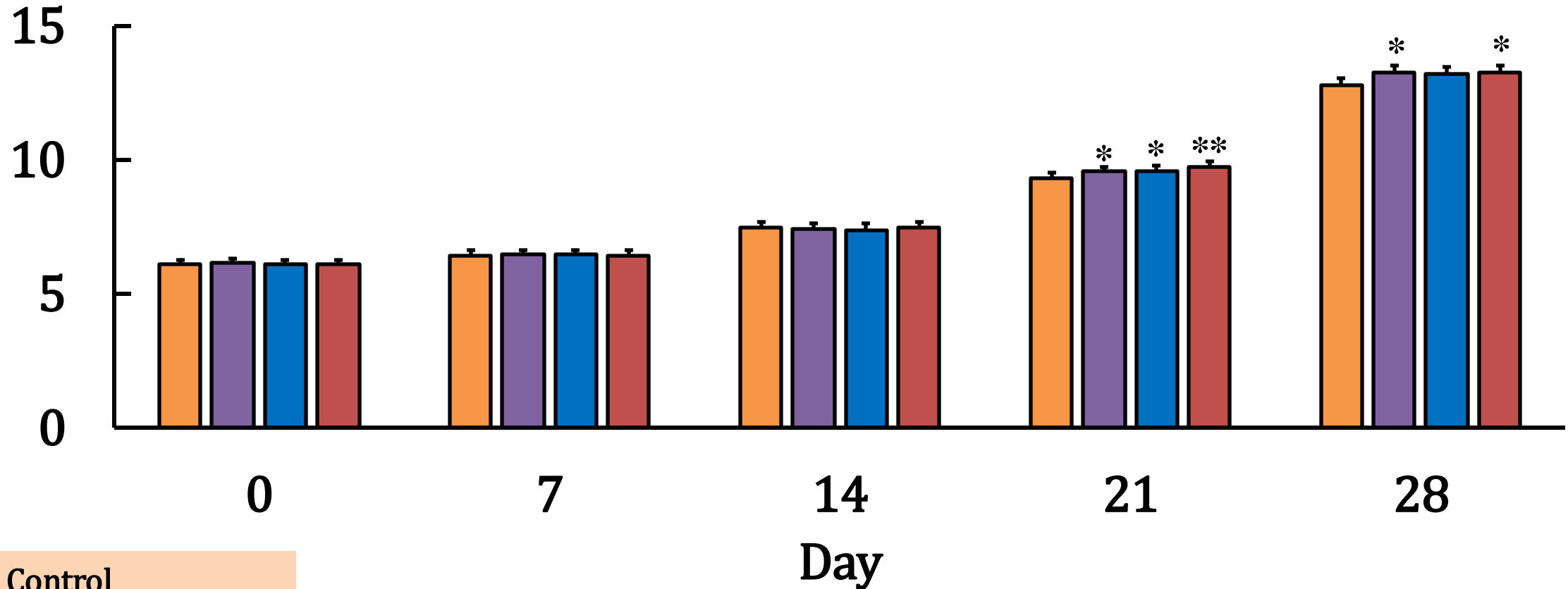


- Calculation of average daily gain, average daily feed intake, and feed efficiency
- Diarrhea scores were recorded twice daily during experiment
  - Scores ranging from 1 to 5 (1 = normal to 5 = watery diarrhea)
  - Frequency of diarrhea:
    - ✓  $3 \geq$  incidence of diarrhea
    - ✓  $4 \geq$  severe diarrhea

# Statistical Analysis

- Statistical Model:
  - Response Variable:  $Y_{ijk} = \mu + D_i + B_{jk} + \epsilon_{ijk}$
  - Experimental Unit: Pen
  - Fixed effect: Diet
  - Random Effect: Block
- Growth Performance:
  - Analyzed by ANOVA using the PROC MIXED of SAS
- Diarrhea Frequency:
  - Analyzed by Chi-square test
- Significance at  $P \leq 0.05$  and tendency at  $0.05 < P \leq 0.10$

# Body Weight, kg

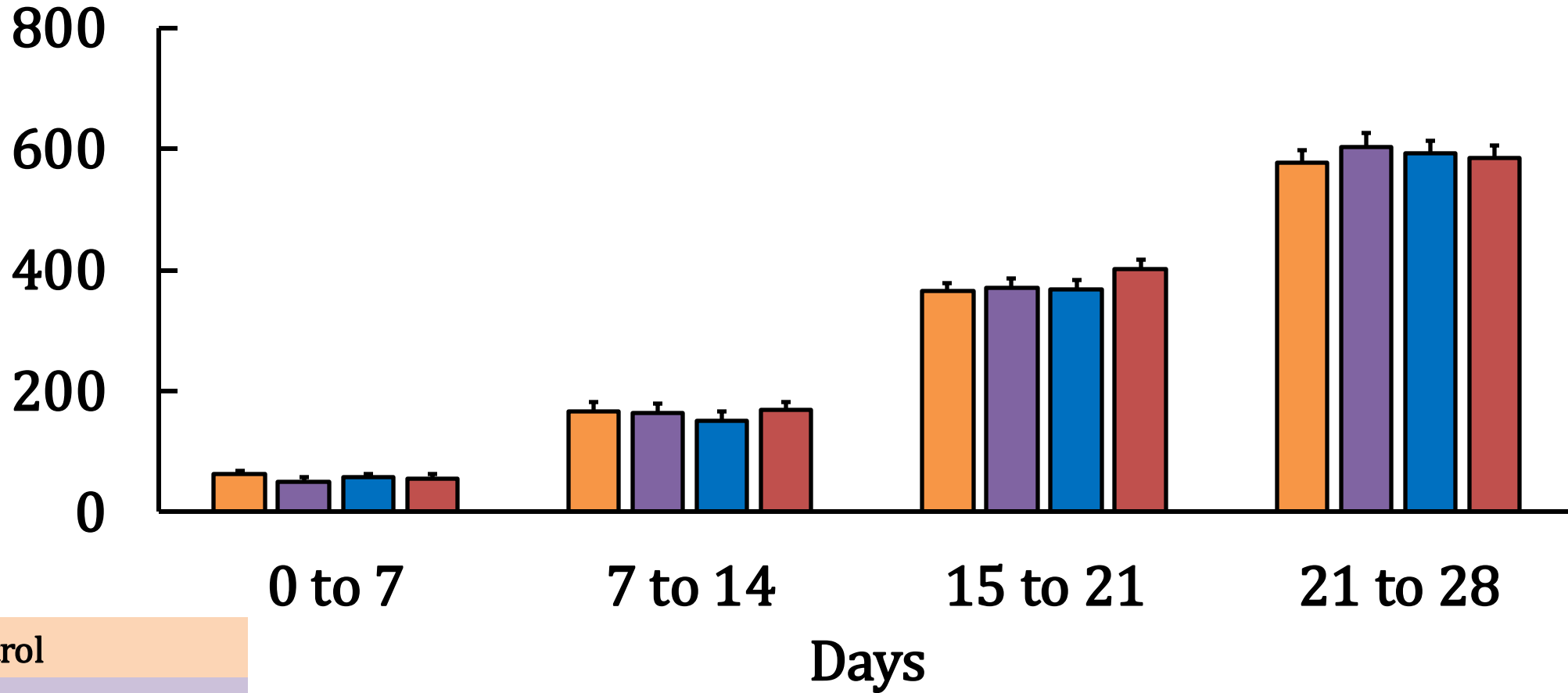


CON: Control  
 BPS: *B. subtilis* single  
 BPD: *B. subtilis* double  
 ATB: Antibiotic

■ CON ■ BPS ■ BPD ■ ATB

\* 0.05 < P < 0.10: Statistical trend  
 \*\* P < 0.05: Significant difference

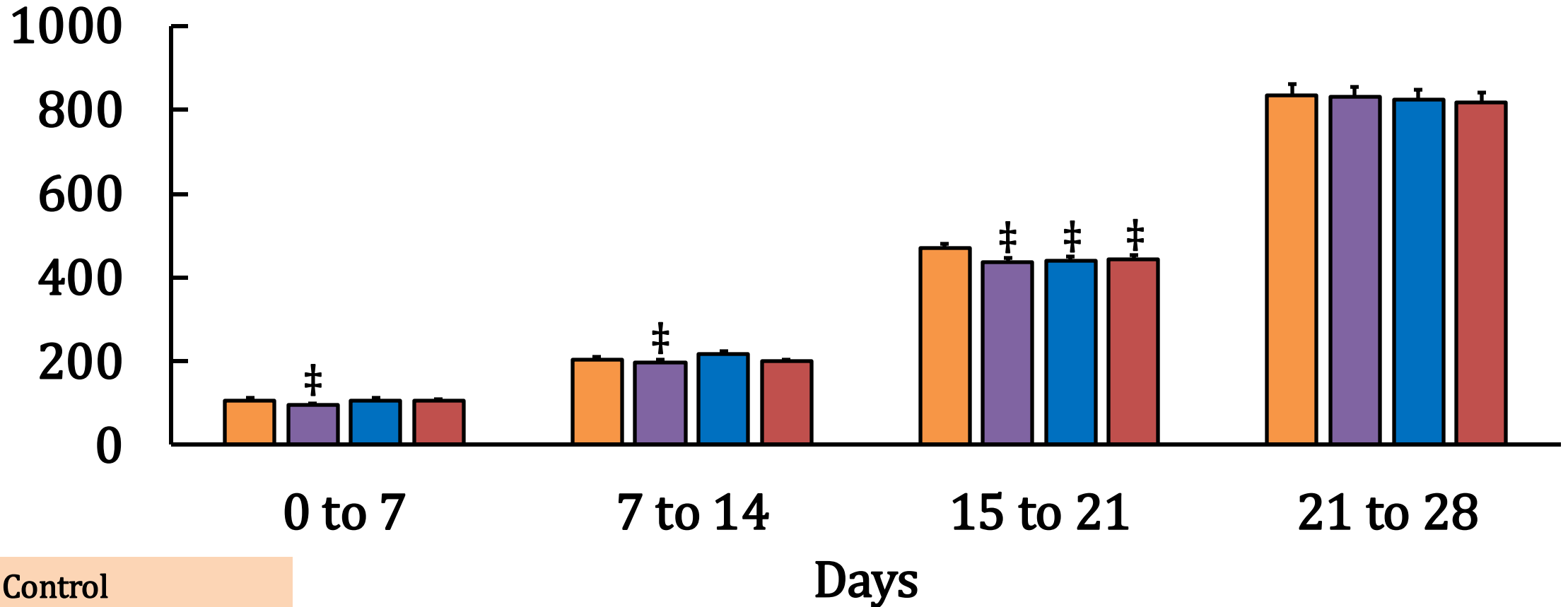
# Average Daily Gain, g/d



CON: Control  
 BPS: *B. subtilis* single  
 BPD: *B. subtilis* double  
 ATB: Antibiotic

CON BPS BPD ATB

# Average Daily Feed Intake, g/d



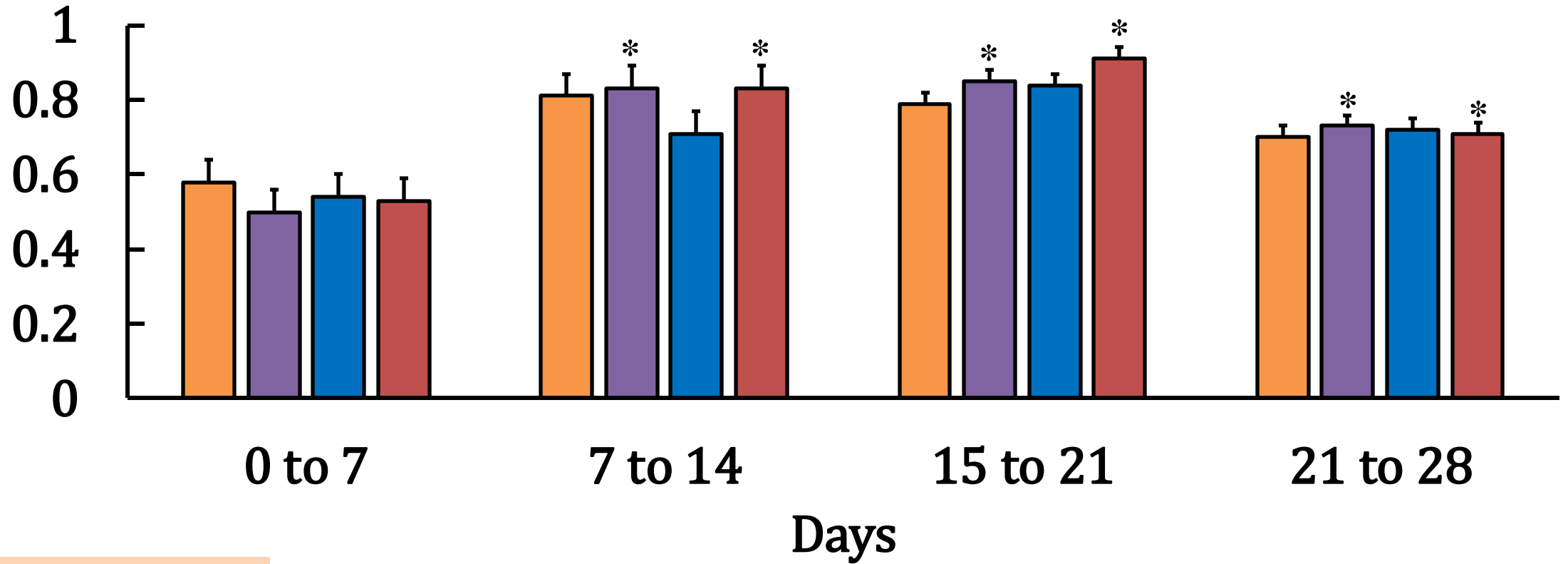
CON: Control  
 BPS: *B. subtilis* single  
 BPD: *B. subtilis* double  
 ATB: Antibiotic

CON BPS BPD ATB

‡  $P < 0.05$ : Significant difference



# Feed efficiency (Gain to Feed Ratio)

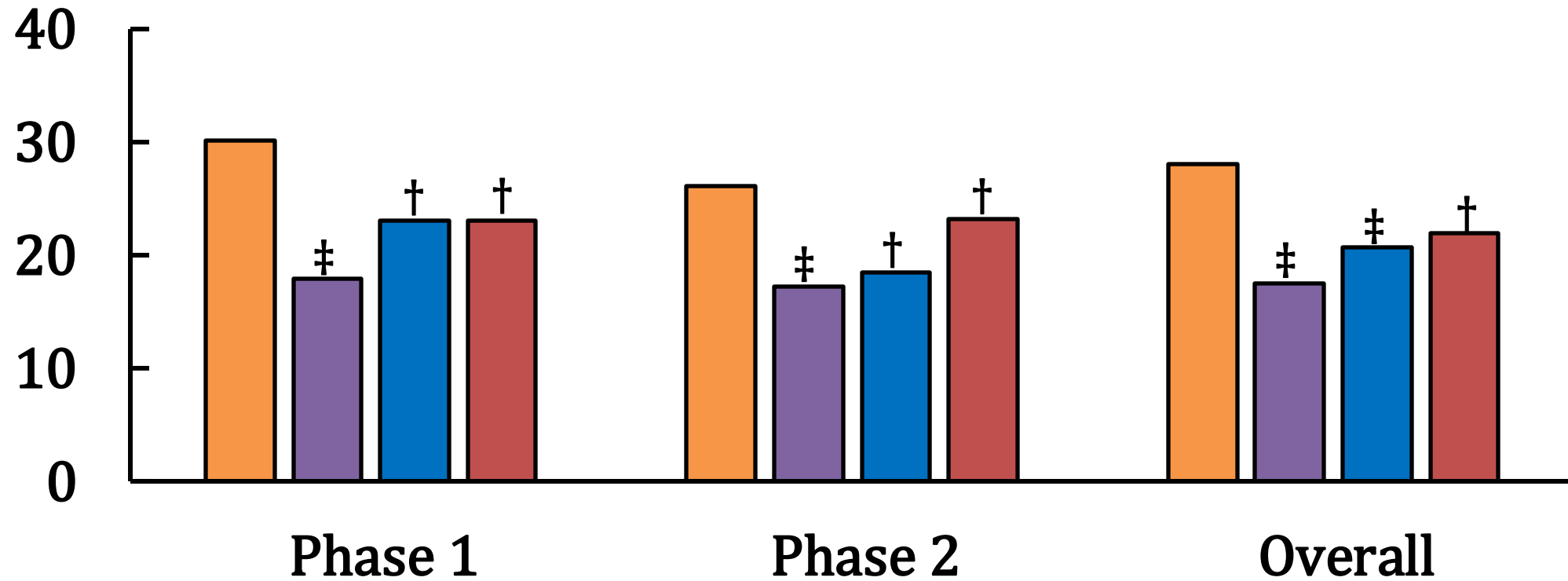


CON: Control  
 BPS: *B. subtilis* single  
 BPD: *B. subtilis* double  
 ATB: Antibiotic

CON BPS BPD ATB

\* 0.05 < P < 0.10: Statistical trend

## Frequency of Incidence of Diarrhea $\geq 3$ ,%



CON: Control

BPS: *B. subtilis* single

BPD: *B. subtilis* double

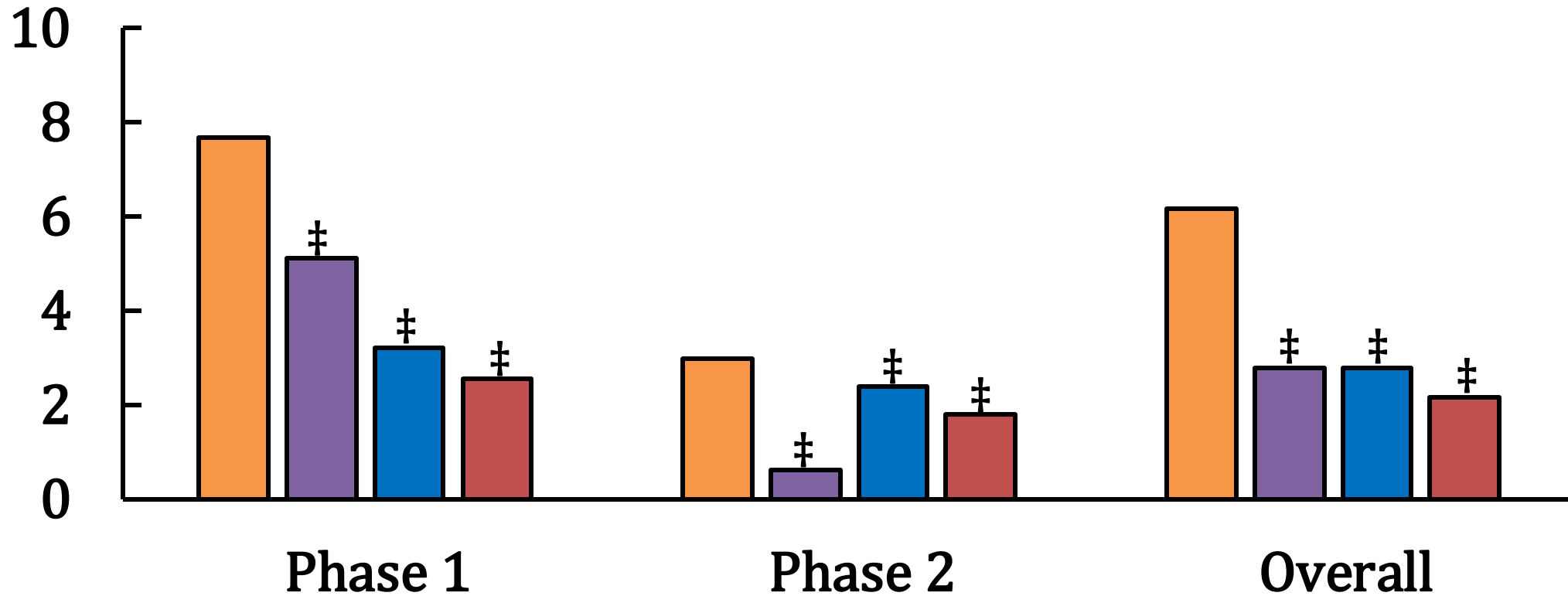
ATB: Antibiotic

■ CON 
 ■ BPS 
 ■ BPD 
 ■ ATB

† 0.05 < P < 0.10: Statistical trend

‡ P < 0.05: Significant difference

## Frequency of Severe Diarrhea $\geq 4$ , %



CON: Control  
 BPS: *B. subtilis* single  
 BPD: *B. subtilis* double  
 ATB: Antibiotic

■ CON ■ BPS ■ BPD ■ ATB

‡  $P < 0.05$ : Significant difference

# Conclusions

Dietary supplementation with *Bacillus subtilis* and plant extracts may help

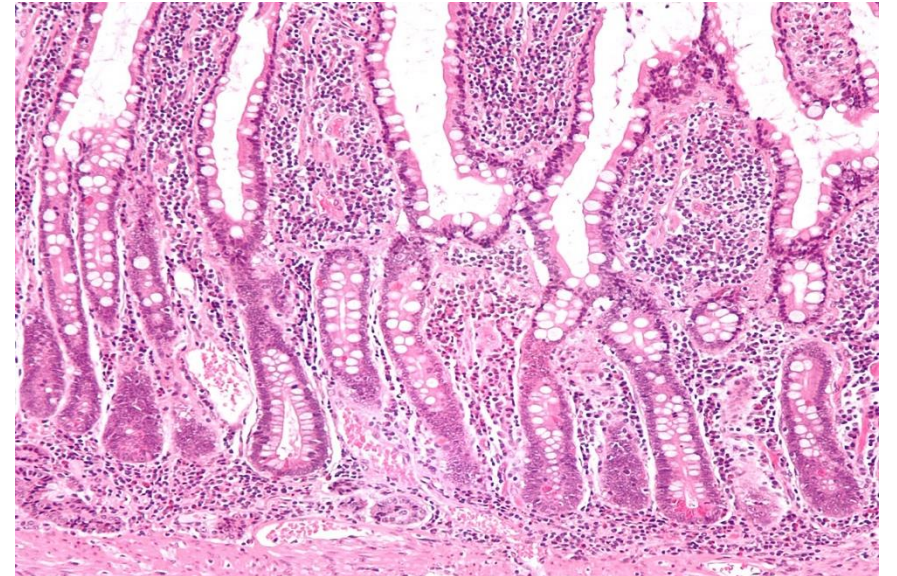
- Improve growth performance during the early weaning stage
- Reduce the incidence and severity of diarrhea in the post-weaning period

# Future Research



## ELISA Assays

- Proinflammatory cytokines
- Stress response biomarkers
  - Acute phase proteins



## Intestinal Development

- Villi height
- Villi width
- Crypt depth

# Acknowledgments



JH Biotech, Inc.



Michigan State University's  
Animal Nutrition, Health,  
and Physiology Lab



Provost's Undergraduate  
Research Grants distributed  
through the College of  
Agriculture and Natural  
Resources at Michigan State  
University

# Thank you for your attention!

