

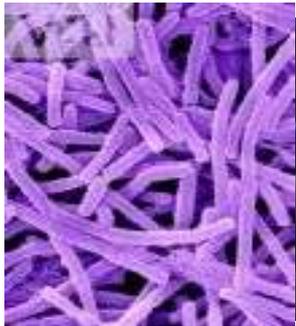
# Strategic application of direct-fed microbials to livestock for growth efficiency and production

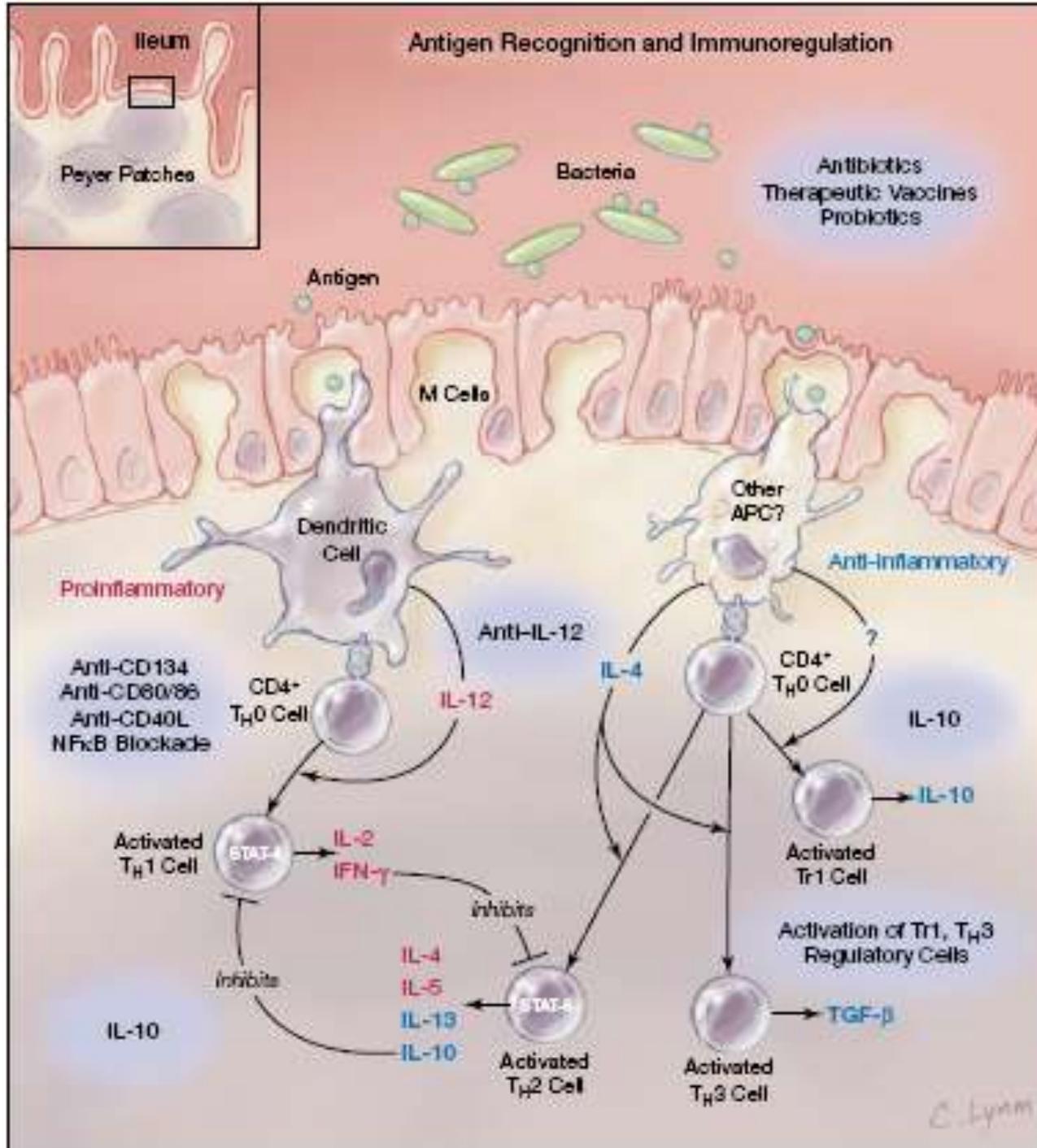
E. Davis and T. Rehberger; Danisco USA; Waukesha, WI, USA

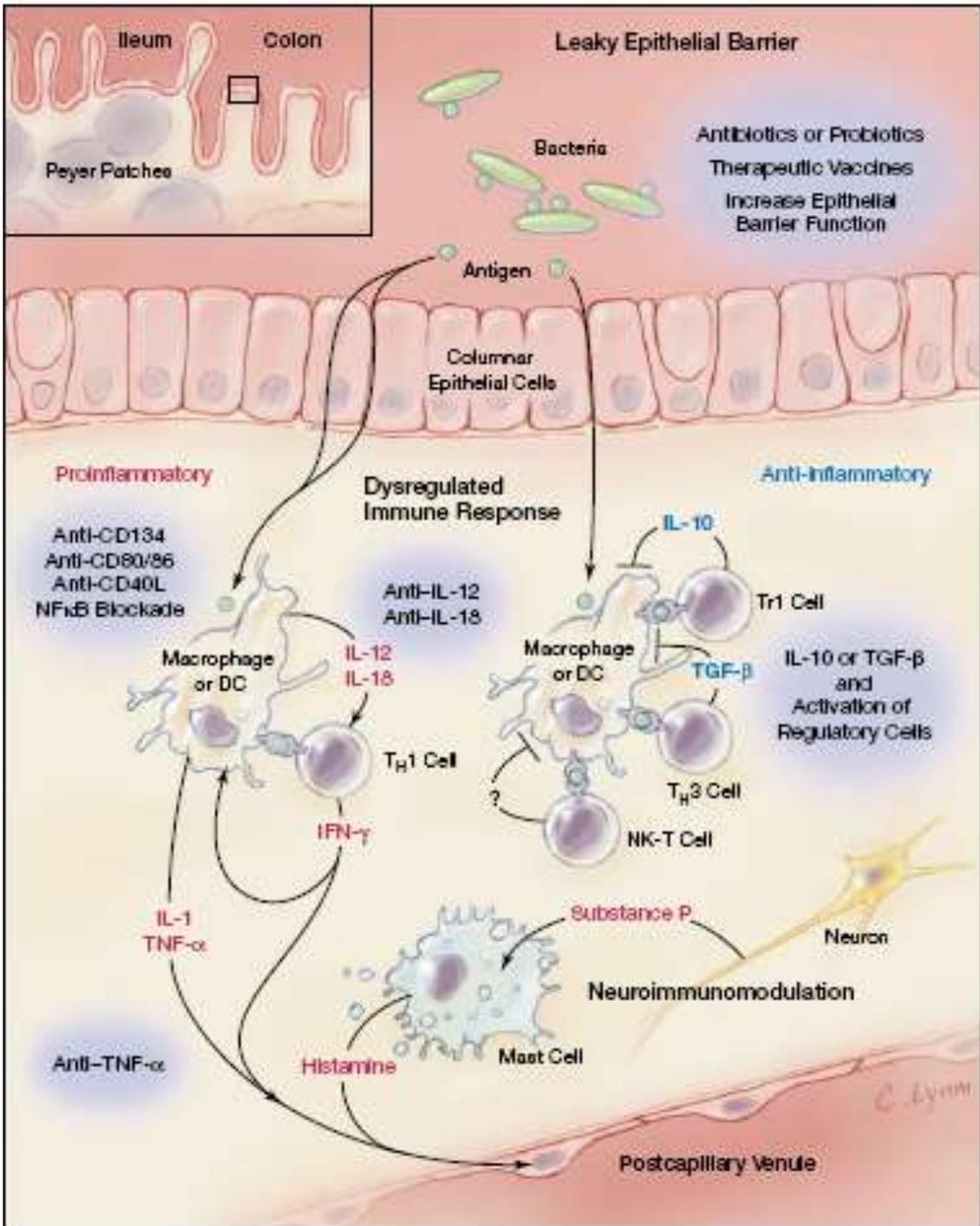


# Presentation Outline

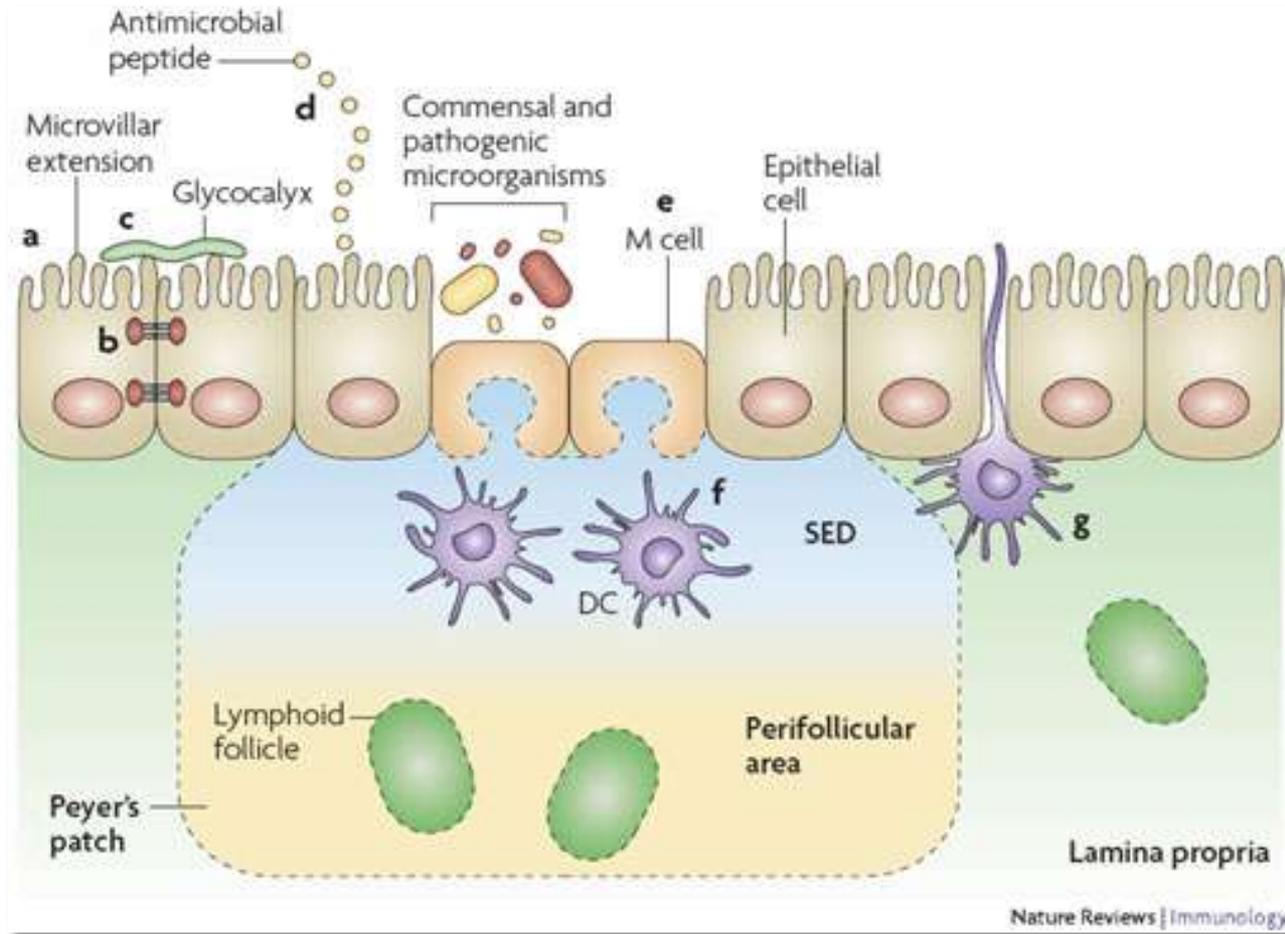
- › Gastrointestinal Microbiota → Immune Development → Performance
- › Direct-fed microbial application and neonatal development
- › Direct-fed microbial application in later production
- › Importance of strategic selection and application of DFMs





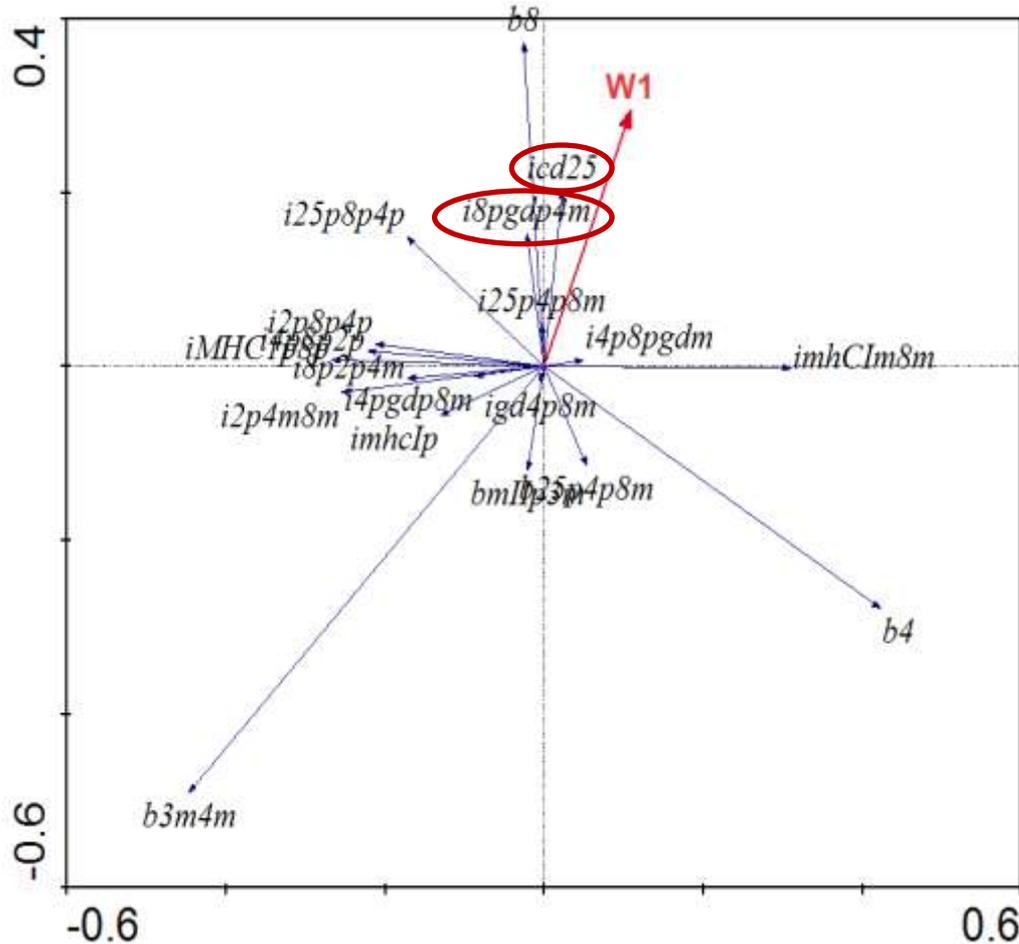


# Immune Homeostasis in the Gastrointestinal Tract

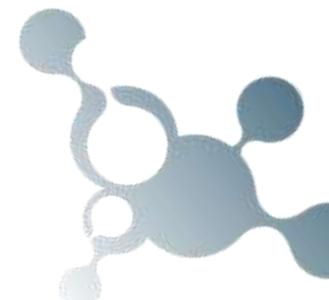


# Growth Performance Association with Intestinal Immune Cell Phenotypes

Jejunal intraepithelial lymphocyte phenotypes positively correlated to pig body weight 11 days post-weaning



Immune cell (IEL) phenotype	P-value
CD4 <sup>+</sup> TCR1 <sup>+</sup> CD8 <sup>-</sup>	0.009
CD8 <sup>+</sup> TCR1 <sup>+</sup> CD4 <sup>-</sup>	0.013
CD4 <sup>+</sup> CD8 <sup>+</sup> CD2 <sup>+</sup>	0.019
CD2 <sup>+</sup> CD8 <sup>+</sup> CD4 <sup>+</sup>	0.023
TCR1 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup>	0.040
CD25 <sup>+</sup>	0.055



Performance measurement

Gain



DNA Isolation

Blood/Tissue Isolation

T-RFLP

Community Analysis

Functional Immunology

Multivariate Correlations: Bacterial Species, Performance, Immune Factors

Use of molecular approaches in combination with “natural” models to identify important microbial contributors

# DFM and Neonatal Development

- › DFM—*Bacillus* strains selected for ability to inhibit clostridia in neonatal pigs
- › 208 mixed parity sows housed at a commercial swine research farm
- › Diets
  1. Control – Standard corn-soybean meal diet
  2. DFM – Control diet supplemented with *Bacillus* combination ( $3.75 \times 10^5$  cfu's feed) fed 6 weeks prior to and throughout lactation
- › Characterization of gastrointestinal microbiota

## **Samples**

- 21 piglets were euthanized from each sow treatment group on day 3 of lactation
- 15 piglets were euthanized from each sow treatment group on day 10 of lactation

## **Dissection – ileum and colon samples**

# Litter performance with DFM supplementation to the sow

	Litter size	Initial litter weight, lbs.	Litter weaning weight, lbs.	Litter ADG lbs.	Weaned litter size	PWM %
DFM	10.99	37.14	136.27	5.01	9.86	10.41
Control	10.95	33.97	128.76	4.74	9.56	12.76
SEM	0.06	1.28	3.15	0.16	0.16	1.54
<i>P</i> value	0.54	0.01	0.02	0.09	0.06	0.12

# Administration of a Bacillus-based DFM shifts intestinal microbiota toward specific Lactobacillus populations

## Day 3 Colon

**Peak P Value Putative Identification**

### DFM

B423	0.009	<i>Lactobacillus gasseri, L. johnsonii</i>
M187	0.07	<i>Lactobacillus gasseri, L. johnsonii, L. sakei</i>
H330	0.08	<i>Lactobacillus gasseri, L. johnsonii, L. crispatus</i>

### Control

M220	0.003	<i>Clostridium sp., Eubacterium sp.</i>
M495	0.003	<i>E. coli and other enterics</i>
M89	0.01	<i>Flexibacter sp., Flavobacterium sp., Bacteriodes sp.</i>
B387	0.02	<i>Clostridium ramosum, Mycoplasma sp., Pseudomonas sp., Brevibacillus sp.</i>
B518	0.03	<i>Bacteroides sp., Flavobacterium sp., Prevotella sp.</i>
B394	0.04	<i>E. coli and other enterics</i>
M96	0.04	<i>Bacteroides sp., Prevotella sp.</i>
H251	0.04	<i>Clostridium sp., Mycoplasma sp.</i>
M214	0.04	<i>Clostridium hungatei</i>
M486	0.07	<i>Eubacterium sp., Flavobacterium sp., Pseudomonas sp.</i>
M92	0.07	<i>Bacteriodes sp., Flavobacterium sp., Flexibacter sp., Prevotella sp.</i>
H240	0.08	<i>Eubacterium sp., Paenibacillus alvei</i>
M539	0.08	<i>Mycoplasma sp.</i>

# Administration of a Bacillus-based DFM shifts intestinal microbiota toward specific Lactobacillus populations

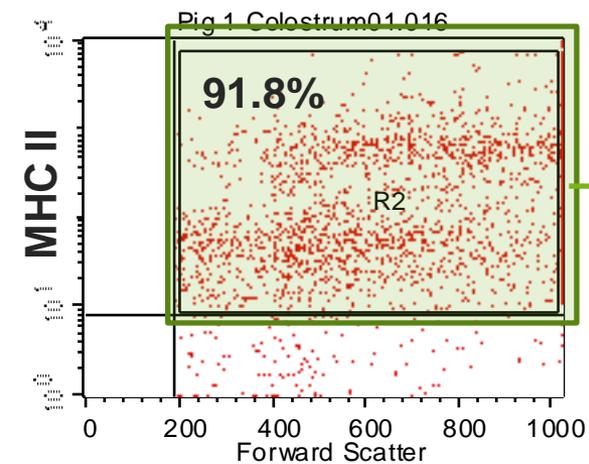
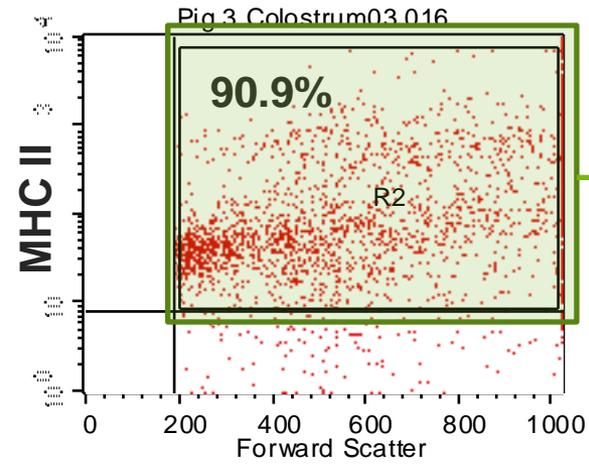
## Day 10 Colon

Peak	P Value	Putative Identification
<b>DFM</b>		
B417	0.003	<i>Lactobacillus alimentarius, L. bifementas</i>
H333	0.01	<i>Lactobacillus sakei, L. hilgardii</i>
M280	0.02	<i>Lactobacillus sp., Bifidobacterium sp., Eubacterium sp., Fusobacterium sp., Microbacterium sp.</i>
B423	0.02	<i>Lactobacillus gasseri, L. johnsonii</i>
H330	0.02	<i>Lactobacillus gasseri, L. johnsonii, L. sakei</i>
B247	0.02	<i>Lactobacillus reuteri</i>
H279	0.05	<i>Lactobacillus brevis, L. delbrueckii, L. salivarius</i>
B262	0.05	<i>Lactobacillus crispatus, L. fructovorans, L. sanfranciscensis, Pediococcus acidilactici</i>
B250	0.07	<i>Lactobacillus acidophilus, L. brevis, L. delbrueckii, L. manihotivorans, L. plantarum, L. salivarius</i>
M569	0.07	<i>Lactobacillus sp.</i>
<b>Control</b>		
M134	0.08	<i>Bacillus sp., Bifidobacterium sp., Mycoplasma sp., Paenibacillus sp.</i>

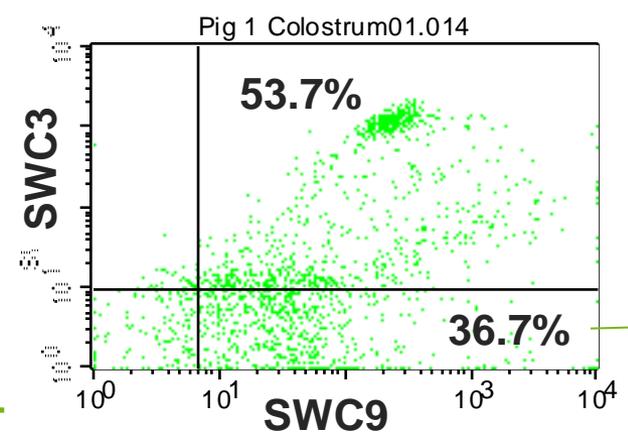
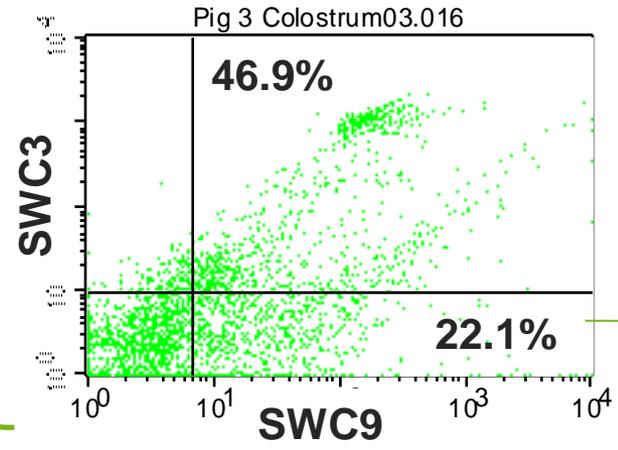


# SWC3- and SWC9-defined myeloid cell subsets within the MHC II<sup>+</sup> immune cell population in colostrum

## CONTROL



## Bacillus DFM

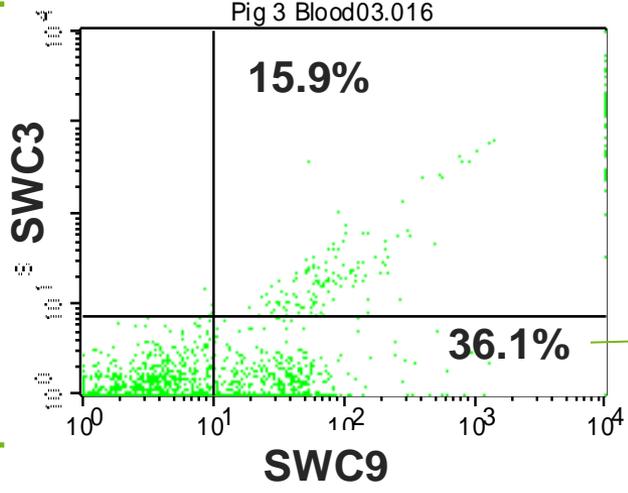
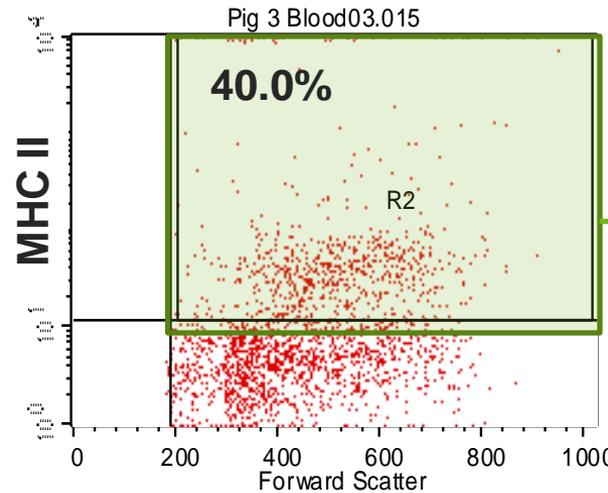
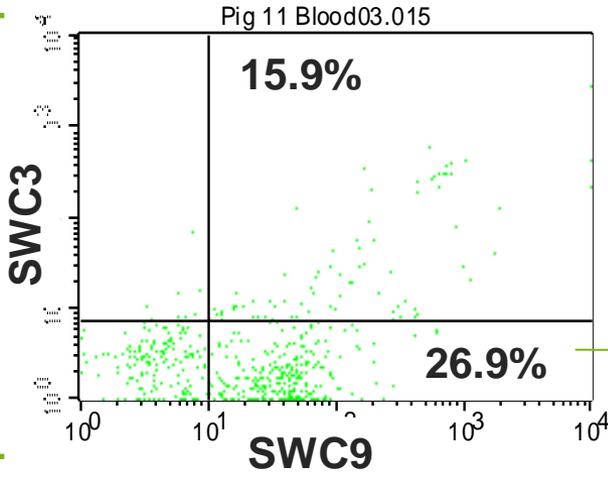
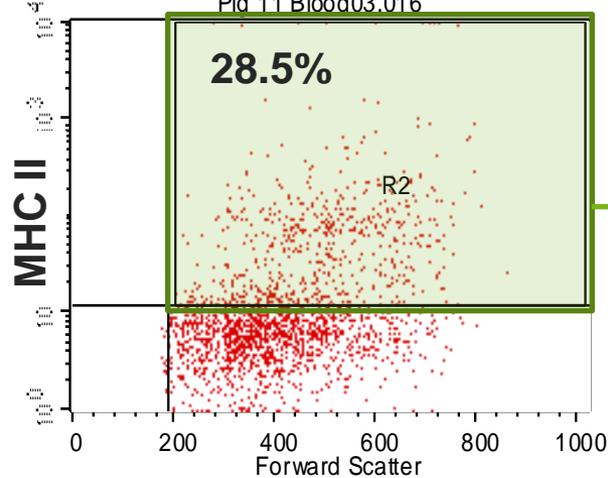


$P = 0.02$



# SWC3- and SWC9-defined myeloid cell subsets within the MHC II<sup>+</sup> population—piglet PBMC at 3 days of age

## CONTROL



**P = 0.09**

**Bacillus DFM**



# Bacillus-based DFM Application



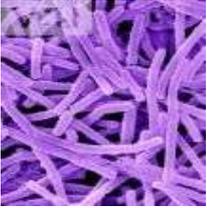
**Sow**



**Piglets**



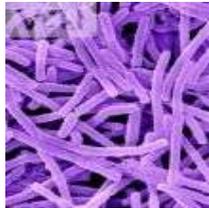
Colostrum



Microbial  
??



Systemic and  
Intestinal  
Development

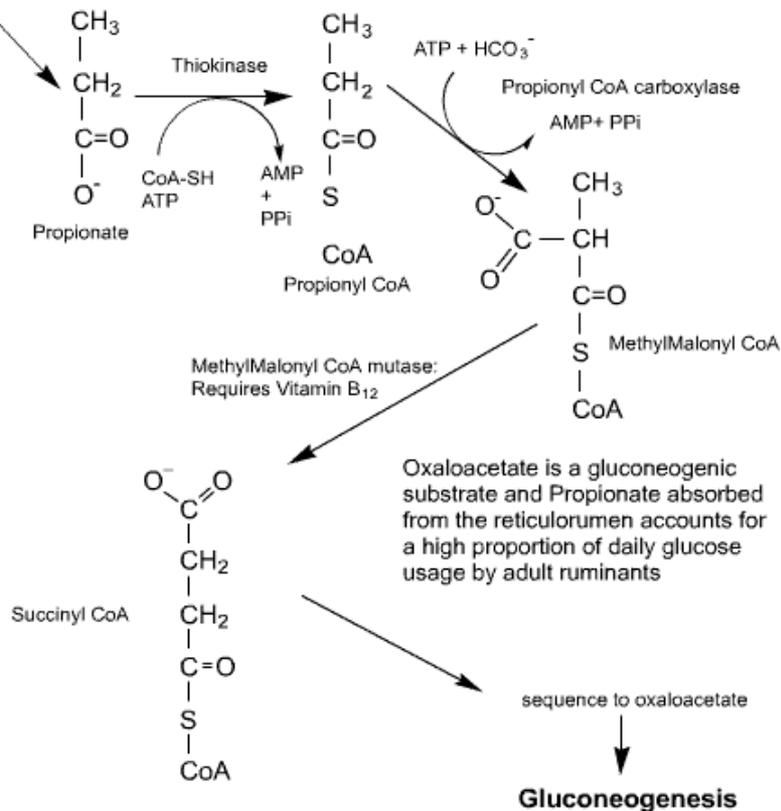


Microbial  
Development

# DFM Application to Dairy Cows for Increased Milk Production

## Metabolic Effects of Propionate

Uptake from the reticulo-rumen via portal blood into the host ruminant's hepatocytes



› Increase in ruminal propionate production results in:

- Increased gluconeogenesis
- Increased plasma insulin
- Increase plasma leptin
- Increased feed efficiency
- Increase milk production
- Increase reproductive efficiency



# *Propionibacterium*

## *Applications for Dairy Cows*

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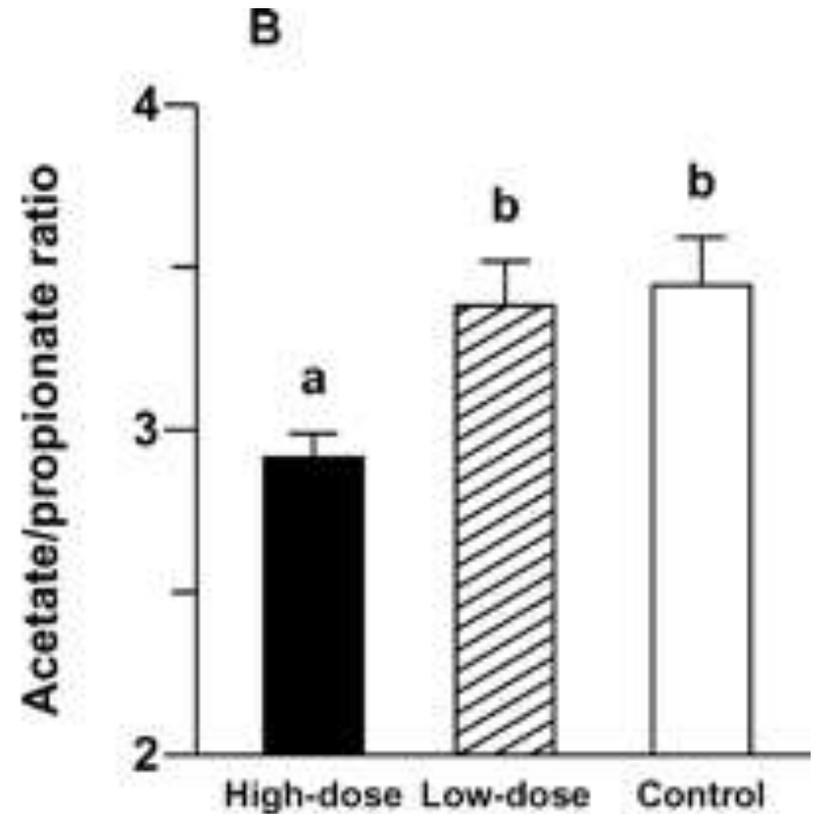
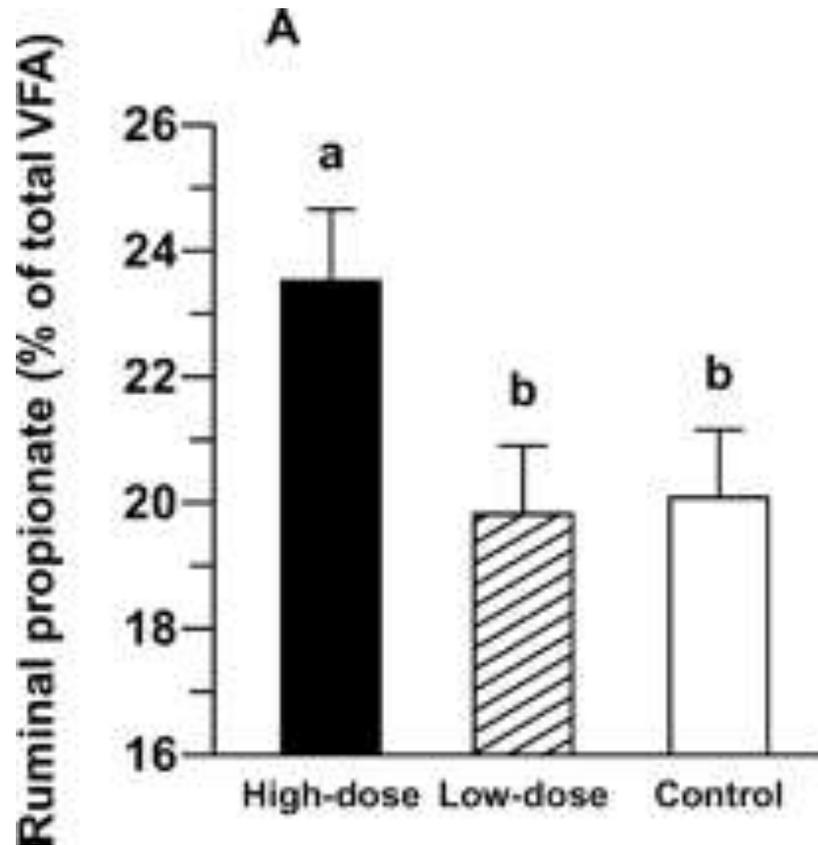
### **Objectives:**

- 1. To isolate and characterize native propionibacteria strains from the rumen of dairy COWS*
- 2. To select propionibacteria strains capable of producing high levels of propionate under typical conditions in the rumen of lactating dairy cows*

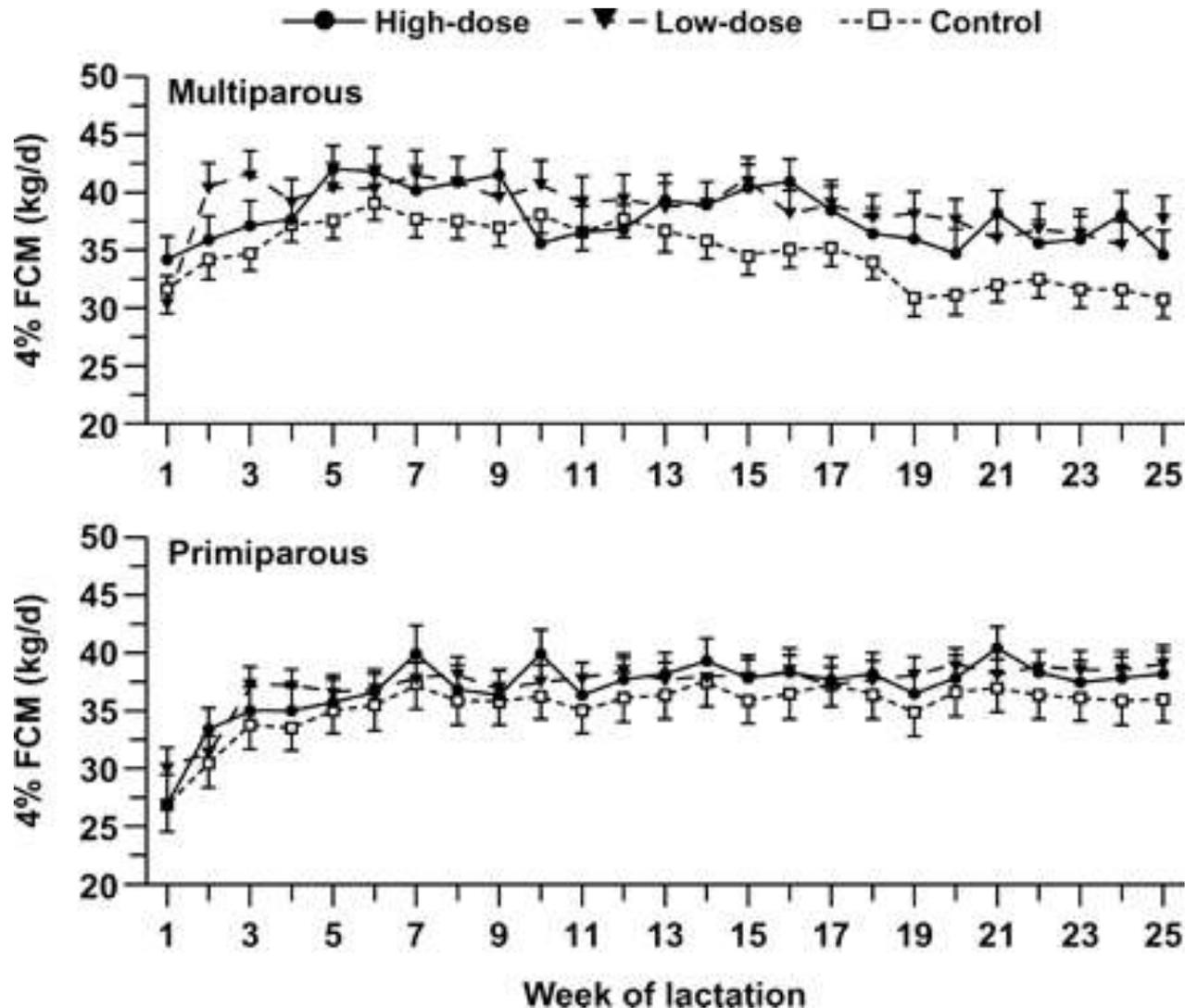
### ***Propionibacterium* Strain P169**



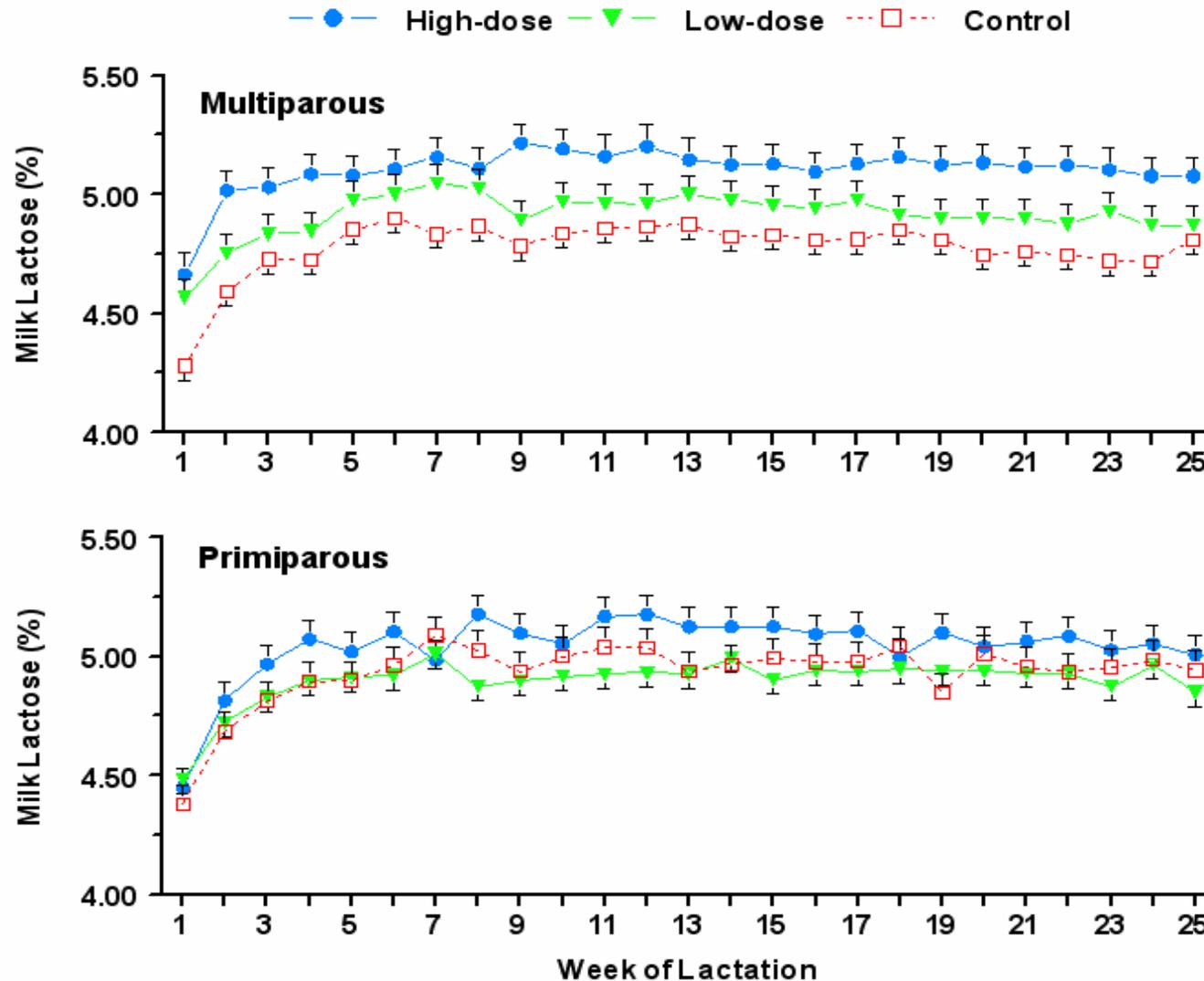
# Effects of feeding *Propionibacterium* strain P169 on Ruminant VFAs



# Effects of feeding *Propionibacterium* strain P169 on Fat Corrected Milk (FCM)

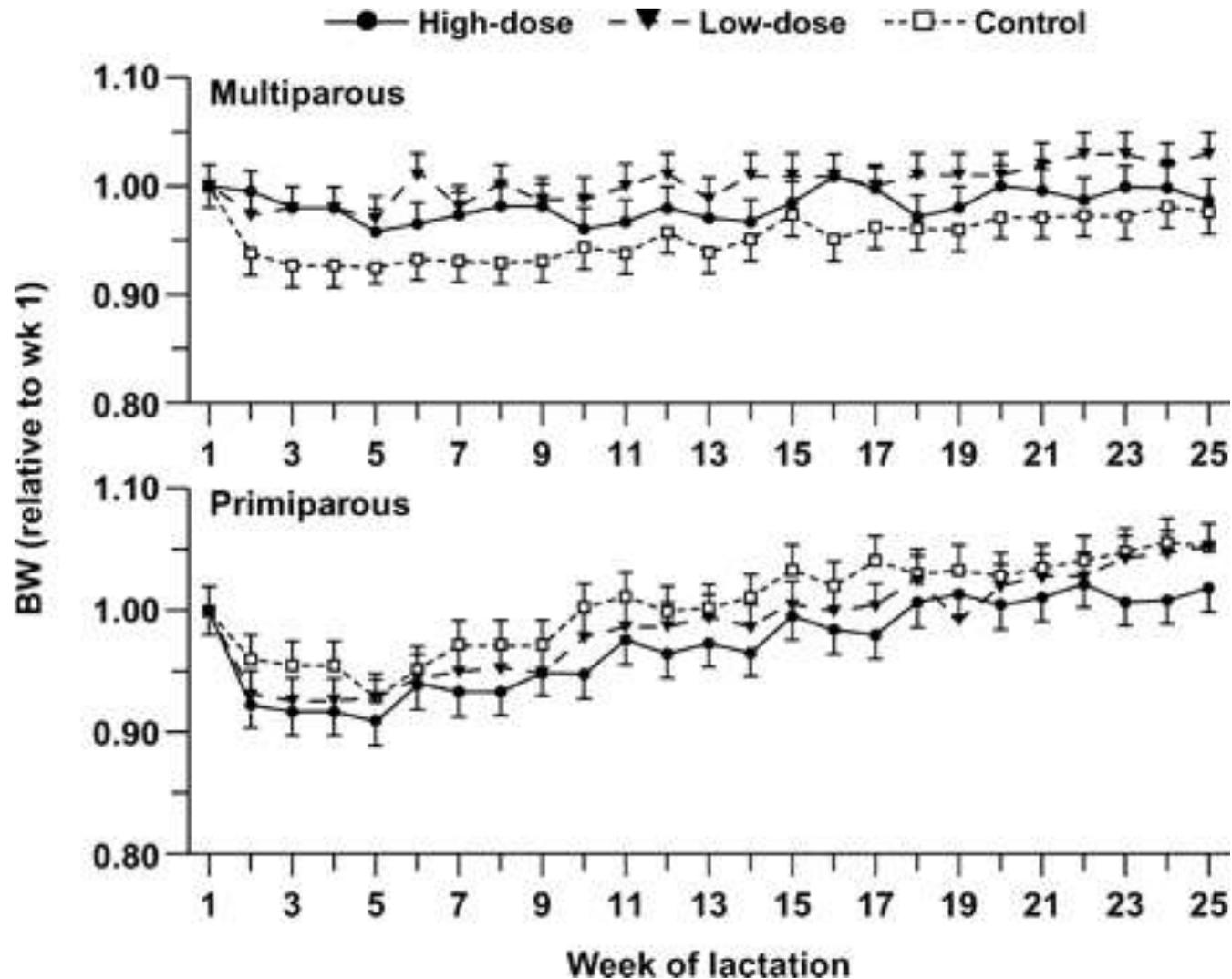


# Effects of feeding Propionibacteria strain P169 on Milk Lactose



Stein et al., 2006 (*J. Dairy Sci.* 89:111-125)

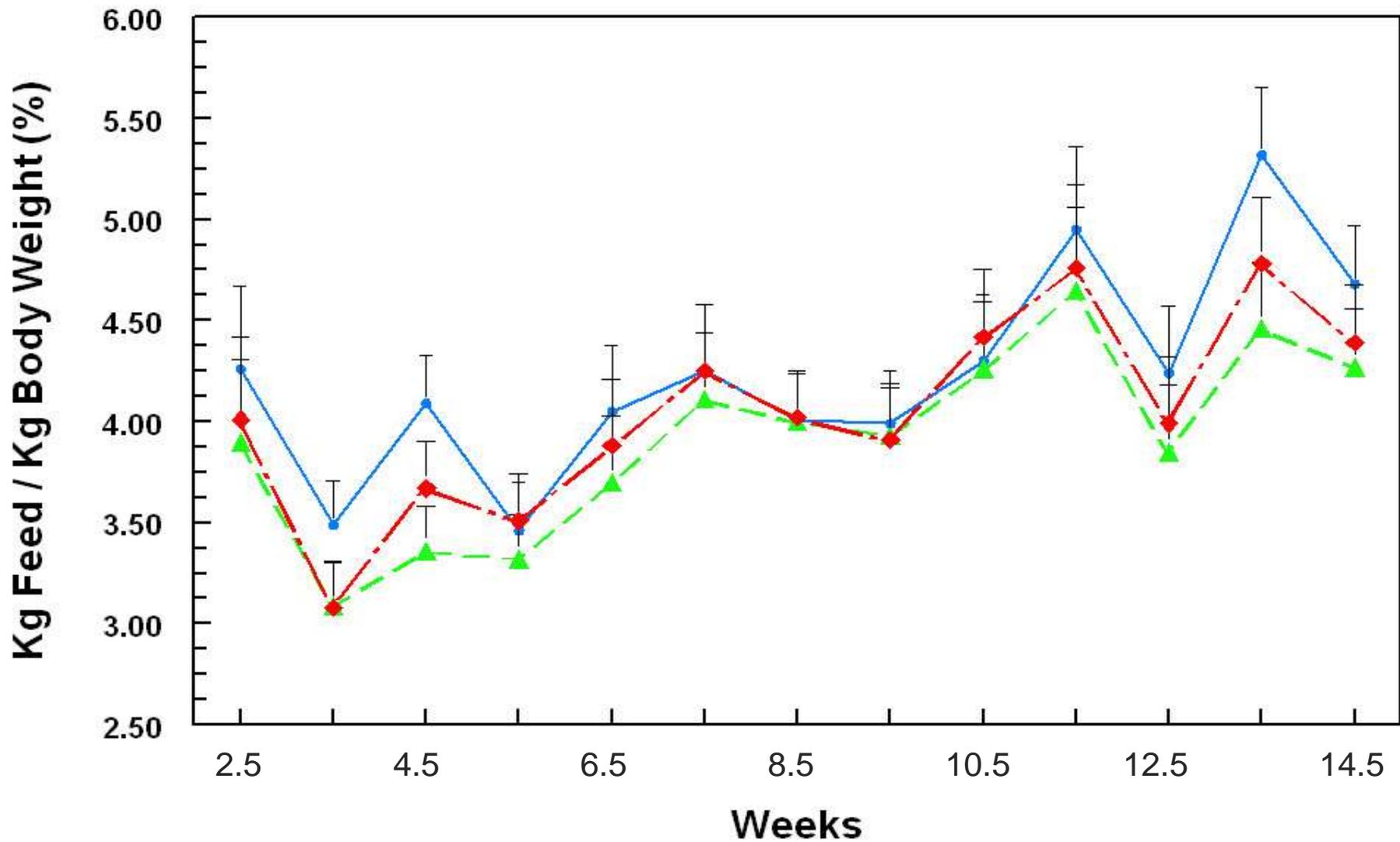
# Effects of feeding Propionibacteria strain P169 on Cow BW during 25 wk of lactation

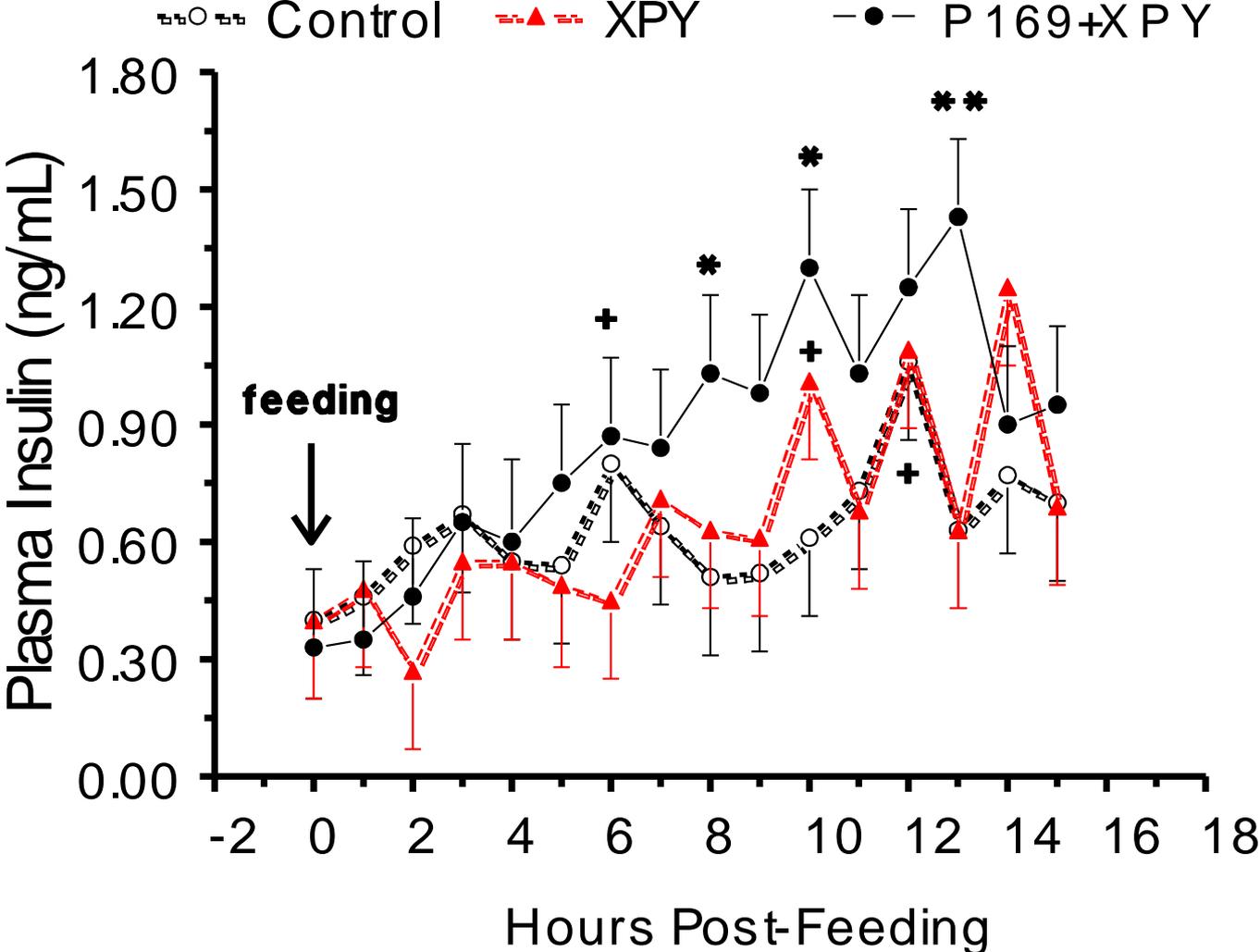


# Feed Consumption

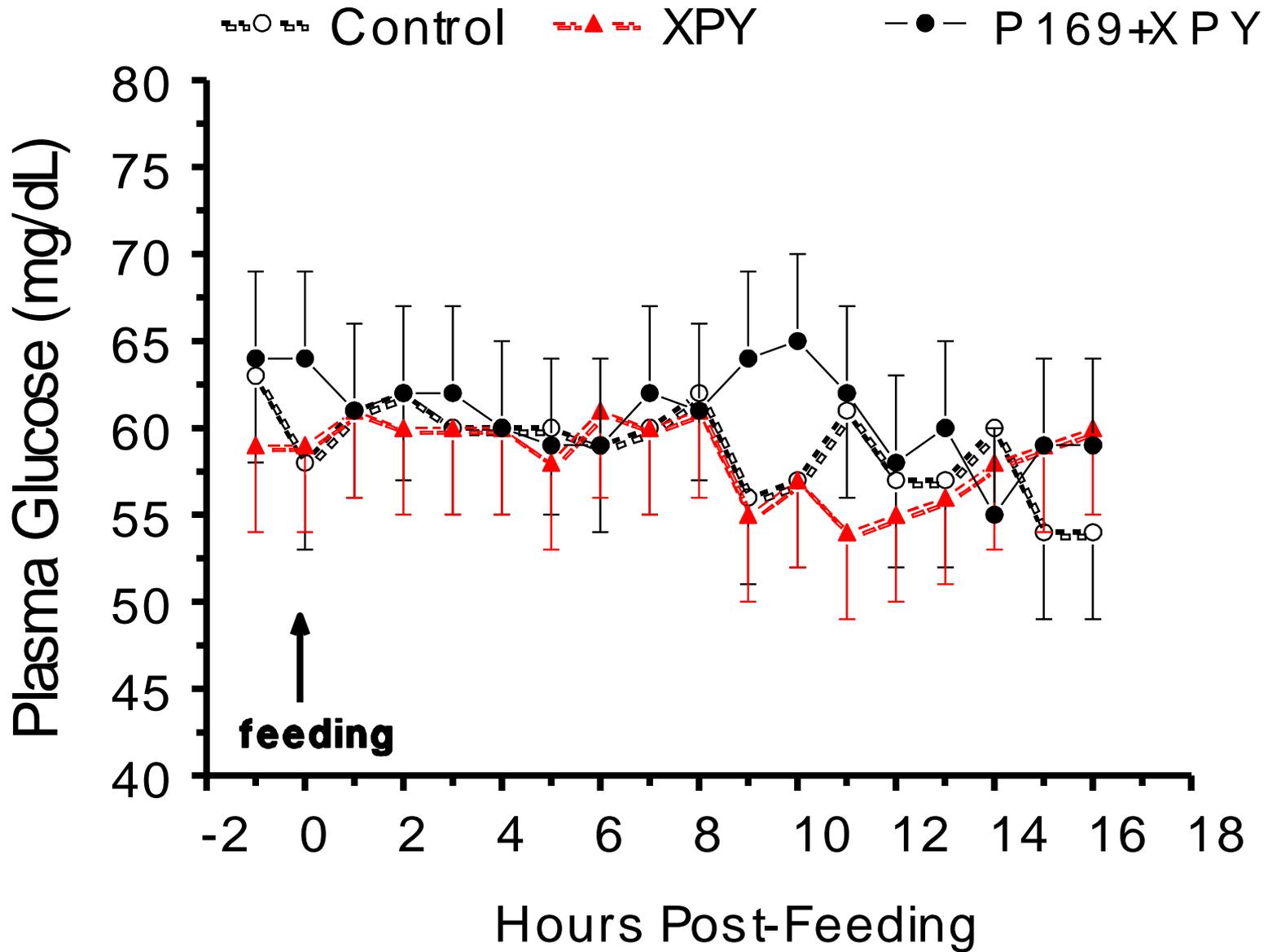
## Treatment x Week

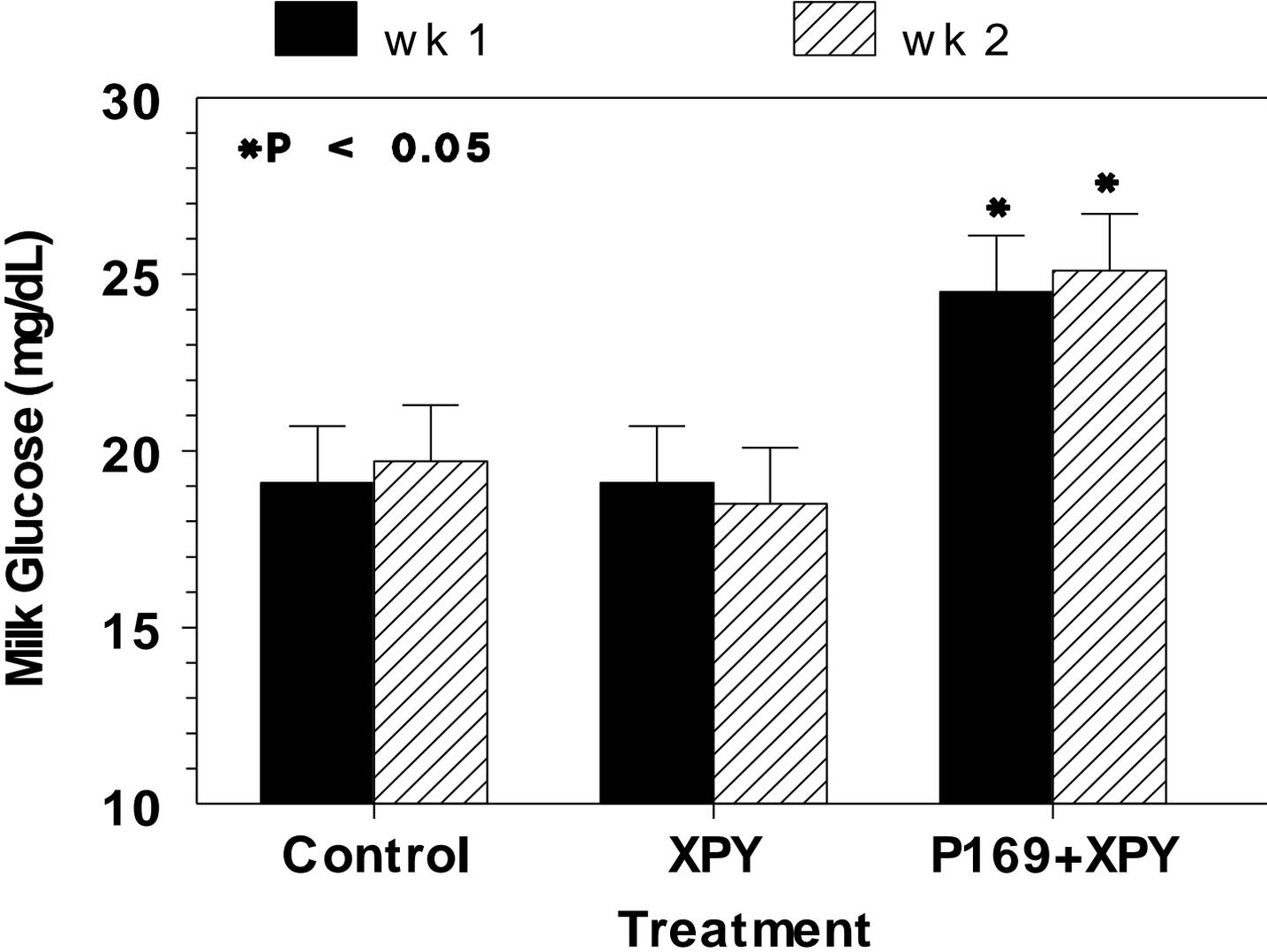
High-dose      Low-dose      Control



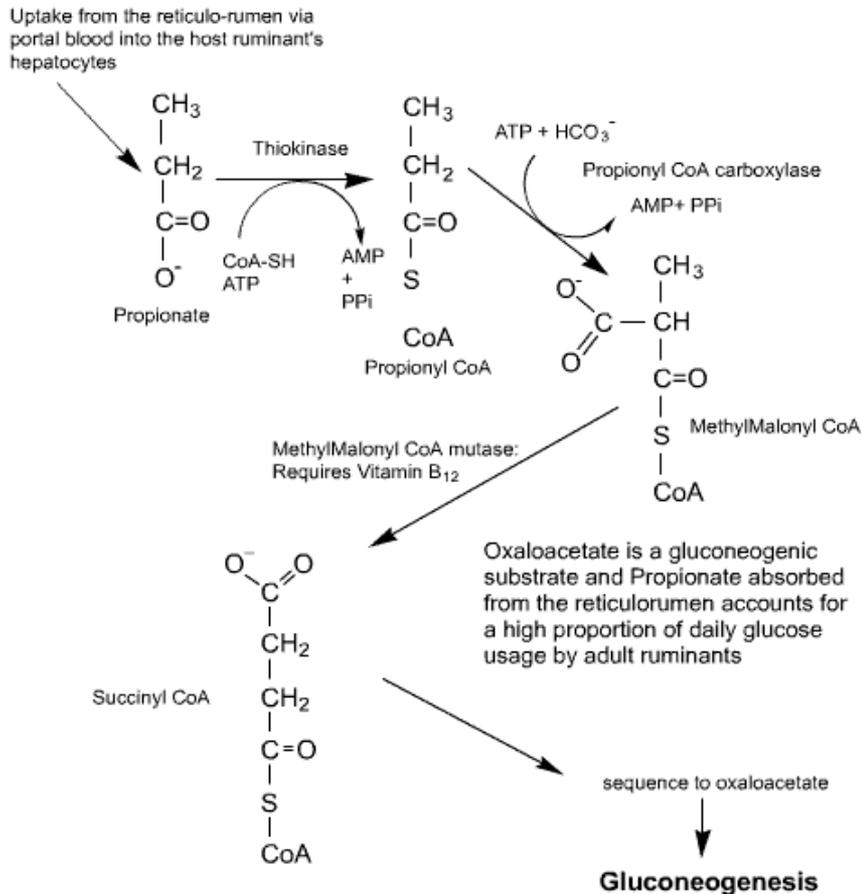


Lehloenya et al., 2006 (unpublished)





# Metabolic Effects of Propionate

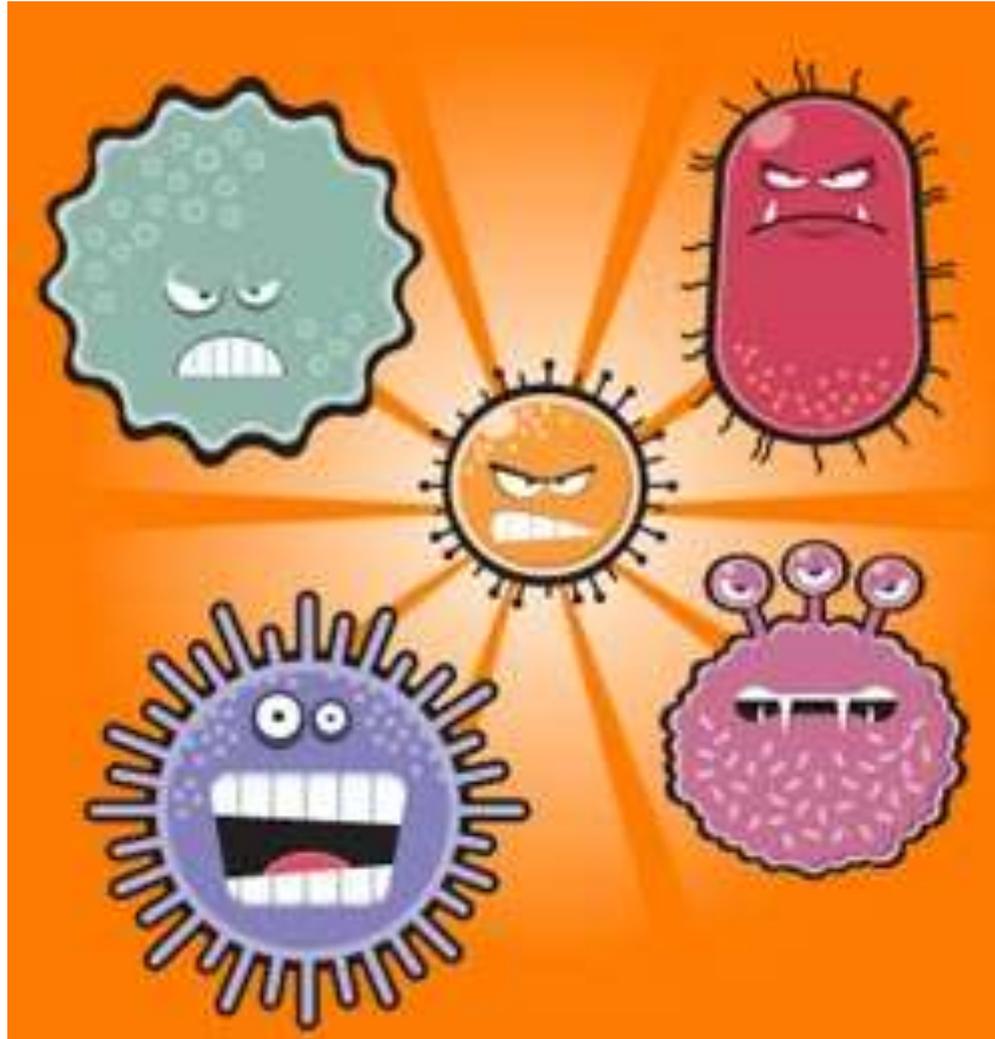


➤ Addition of *Propionibacterium* P169 to dairy cattle diets results in

- Increased ruminal propionate
- Increased insulin
- Increased milk production
- Increased milk lactose and glucose
- Increased feed efficiency

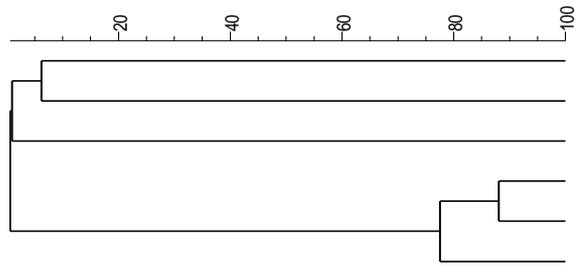


# Our microbial consortia have distinct “personalities”

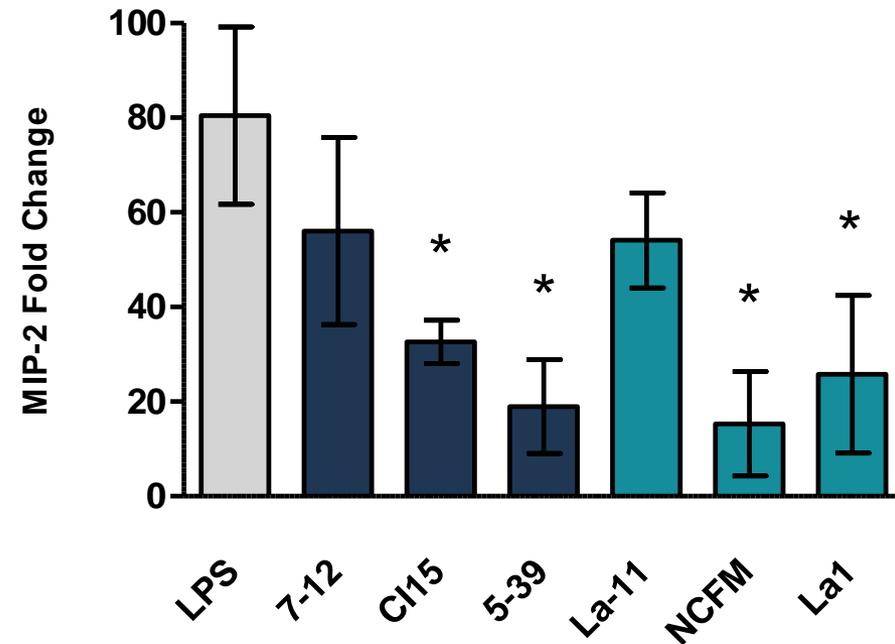
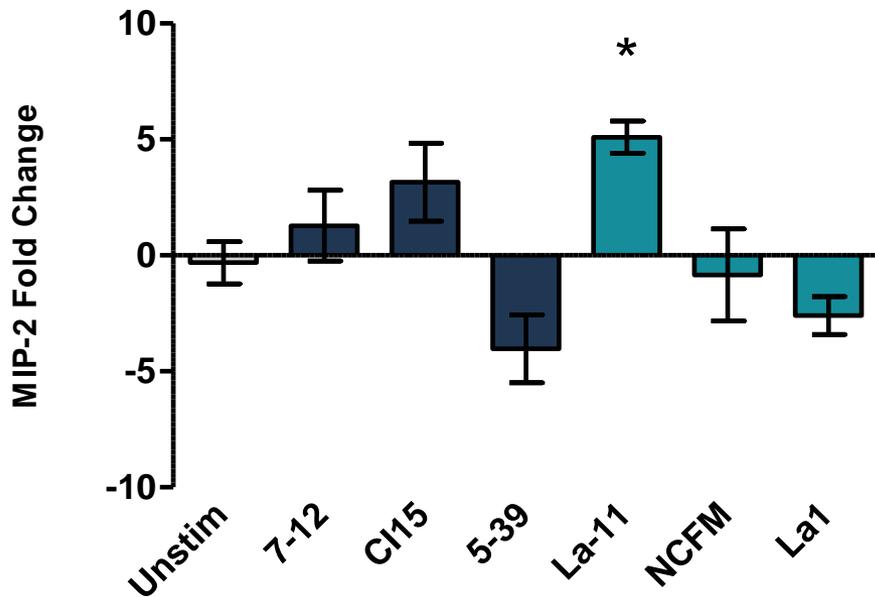


Even within a bacterial species

# Fold change in gene expression of macrophage inflammatory protein-2 (MIP-2) in IEC6 cell line



"5-39	L. plantarum
"7-12	L. plantarum
"CI15	L. plantarum
"La-11	L. acidophilus
"La1	L. acidophilus
"NCFM	L. acidophilus



# Conclusion

- › Strategic application of appropriate DFMs can positively impact neonatal development and production efficiency
- › Importance of strategic selection and application of DFMs
- › Dictate a portion of the microbial consortia to exert strategic outcome

