Application of Probiotics to Control Foodborne Pathogens from Farm to Fork

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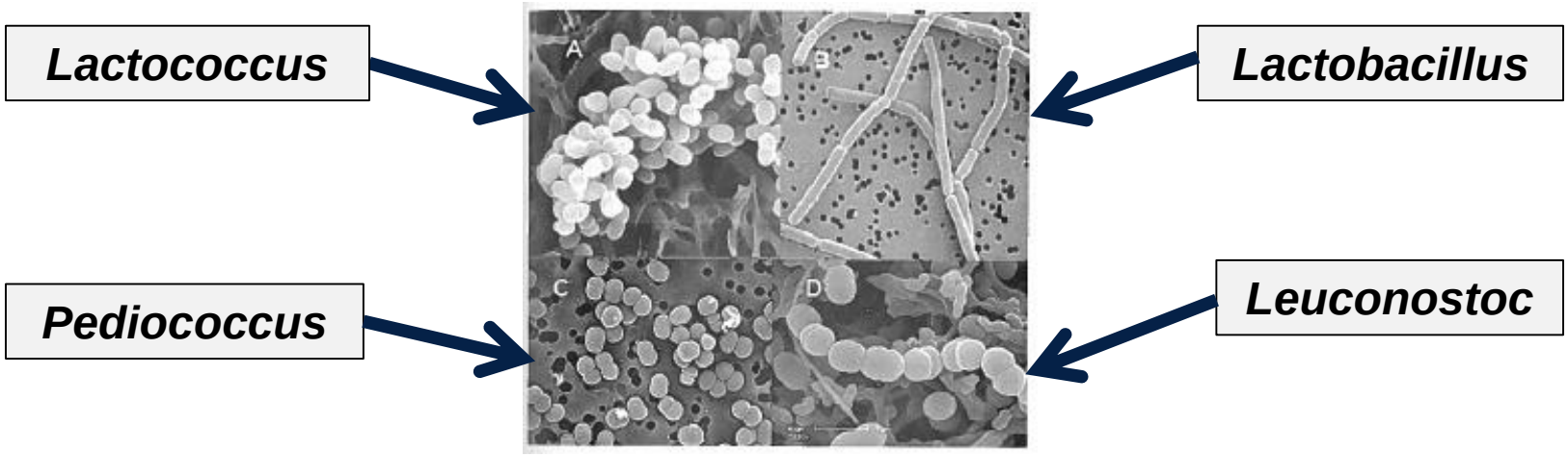
Lactic Acid Bacteria Characteristics

Gram-positive bacteria

Non-sporeforming cocci, coccobacilli, or rod

Usually grow anaerobically, but can also grow in the presence of Oxygen



Lactic Acid Bacteria (LAB)

"Friendly Bacteria"

Lactic Acid Bacteria have a long history of application in the food industry

LAB Benefits (non-exhaustive):

Direct antagonism with enteric pathogens

- Production of antimicrobial compounds (organic acids and bacteriocins)
- Competition for nutrients and minerals
- Occupy adhesion sites in the intestinal tract

Improve intestinal barrier function and activate mucosal immunity

The “ART” of Probiotic Technology

- Microbiological Skill is needed but there is an art to combining strains to meet a specific need
  - Stanley Gilliland
- Some combinations are synergistic, some are antagonistic to each other
- Strains must be selected and screened for the specific purpose and tested in the lab and in real world settings
- There is ALWAYS a dose-response and product must be used by dose

PRE-HARVEST APPLICATIONS

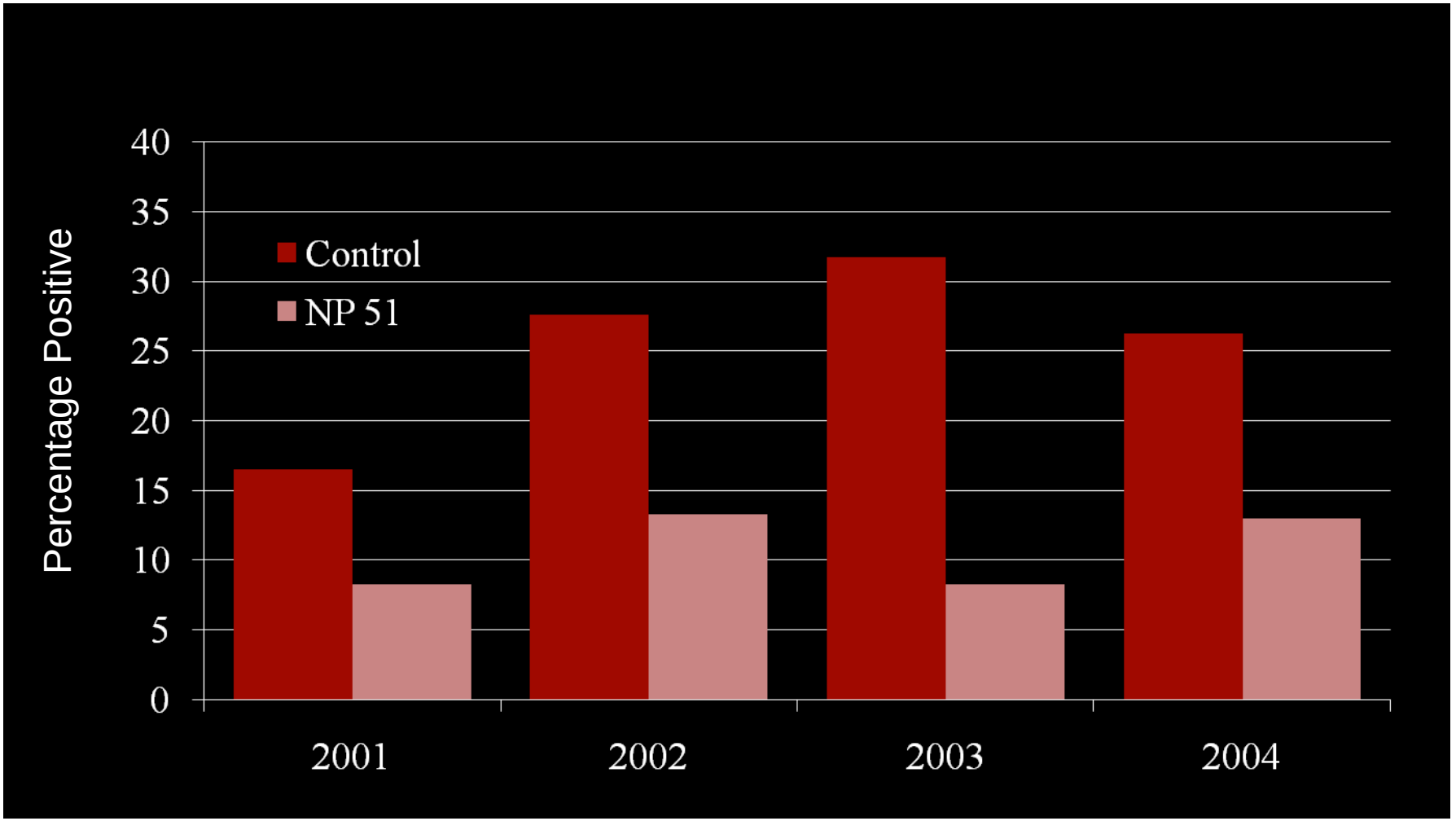
Selection Criteria for NP51

- Began in 1997
- **Sole Purpose**: Identify Strains for Cattle Feeding to Inhibit *E. coli* O157:H7
  - 686 pure cultures isolated and screened
  - 52% showed inhibition ability towards *E. coli* O157:H7
- Several strains inhibitory in manure and rumen fluid
- 4 Strains finally selected for animal studies
    - JFP 66:355

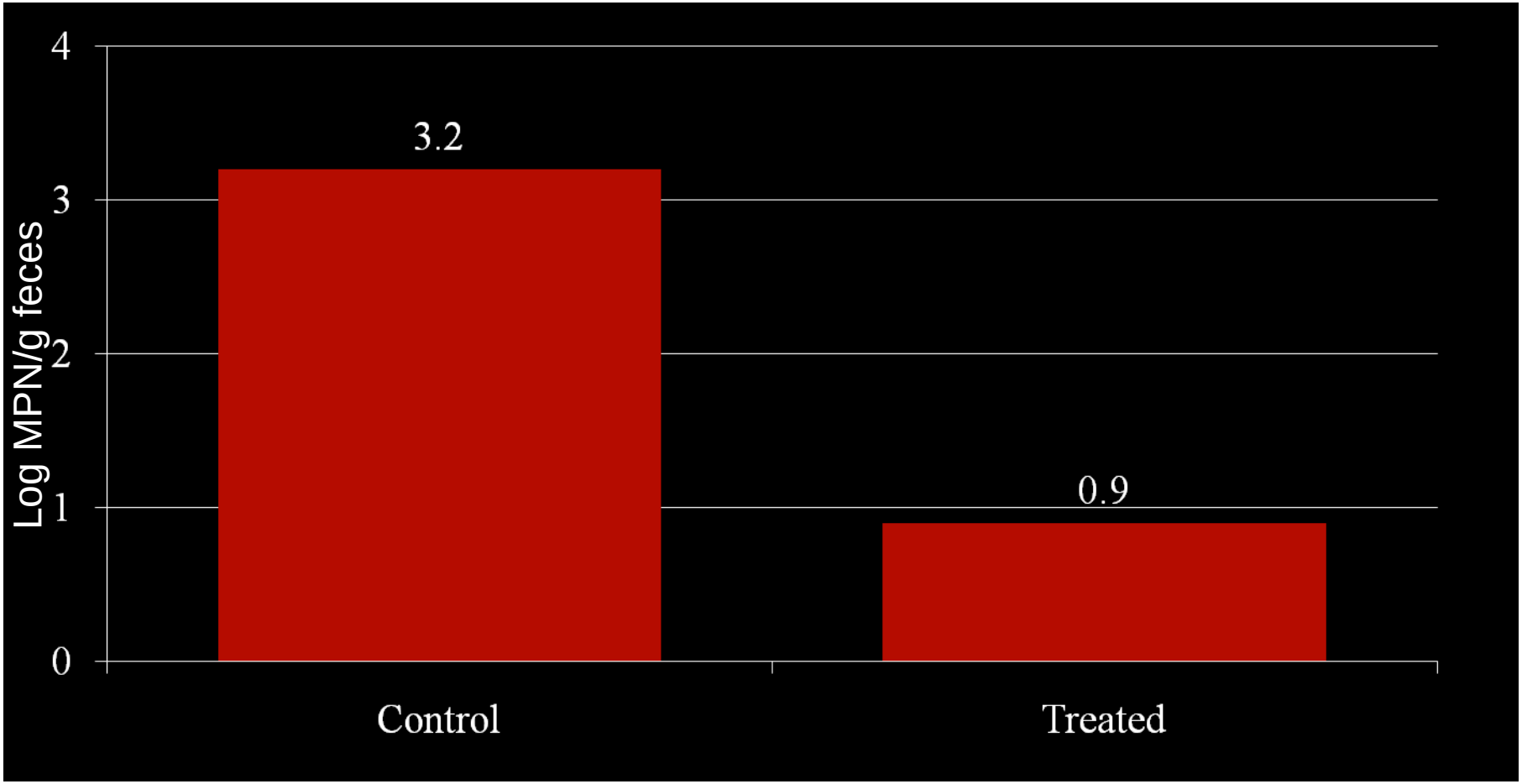
In Vivo Studies with Selected Strains

- 5 Animals Challenged with *E. coli* and Fed Direct-Fed Microbials (DFM)
- 4 of the 5 DFM Combinations Reduced Shedding by 80%
  - Controls – Shed Pathogens for Entire 60 Days of Study
  - DFM Treatments – Animals Shed 3-7 Days
  - 3-5 Log Reduction in Treated Animals that were positive

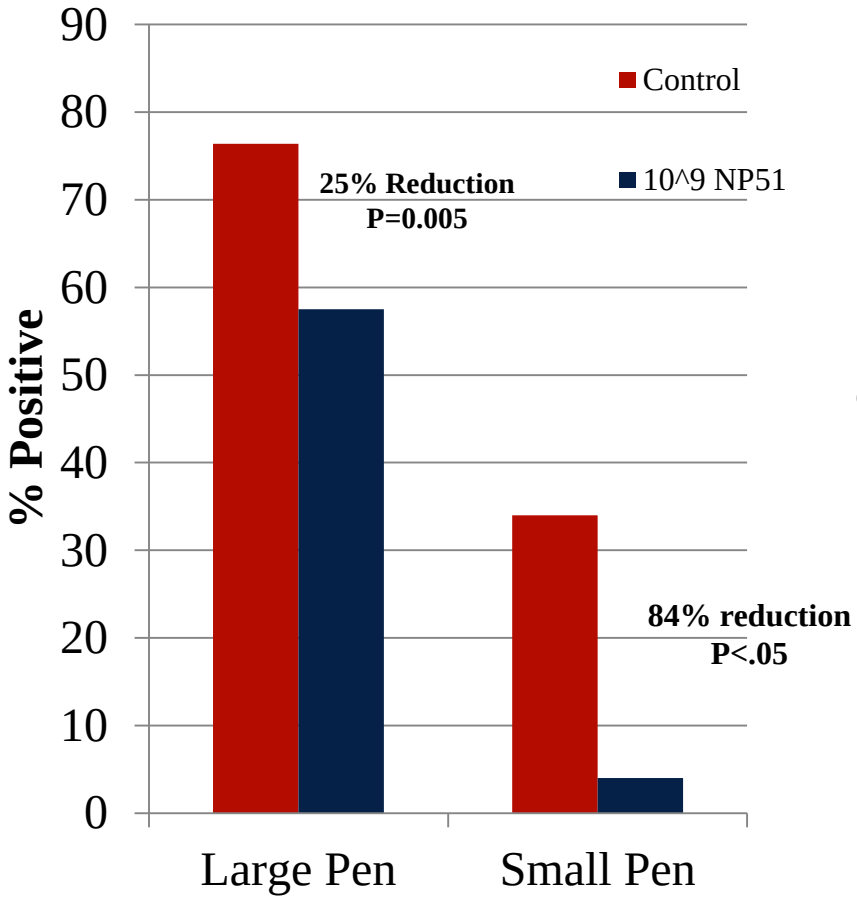
4 Year Cumulative Summary
Reduction of *E. coli* O157 in Beef Feedlot Cattle Using NP 51 (Texas Tech/WTAMU)

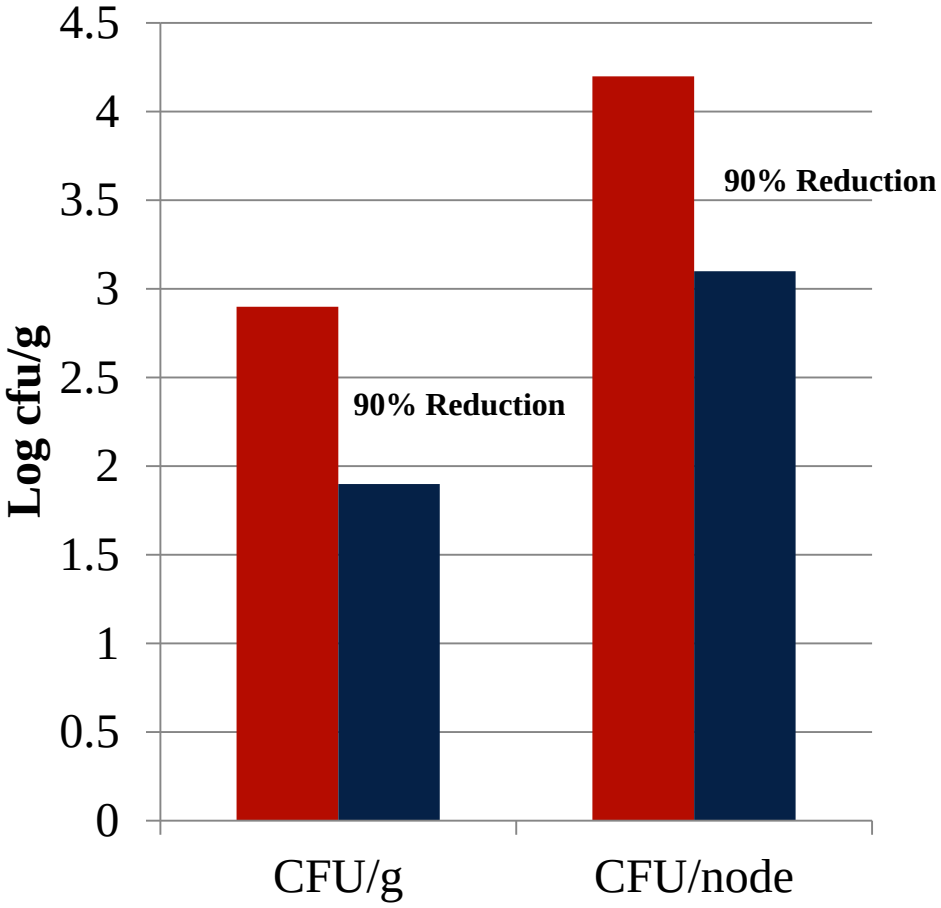


Quantitative Reduction of *E. coli* O157 using a newly developed MPN method in Positive Samples after Treatment with NP 51



2012-Salmonella Reduction in Lymph Nodes using a High Dose of NP51 (10^9/head/day)





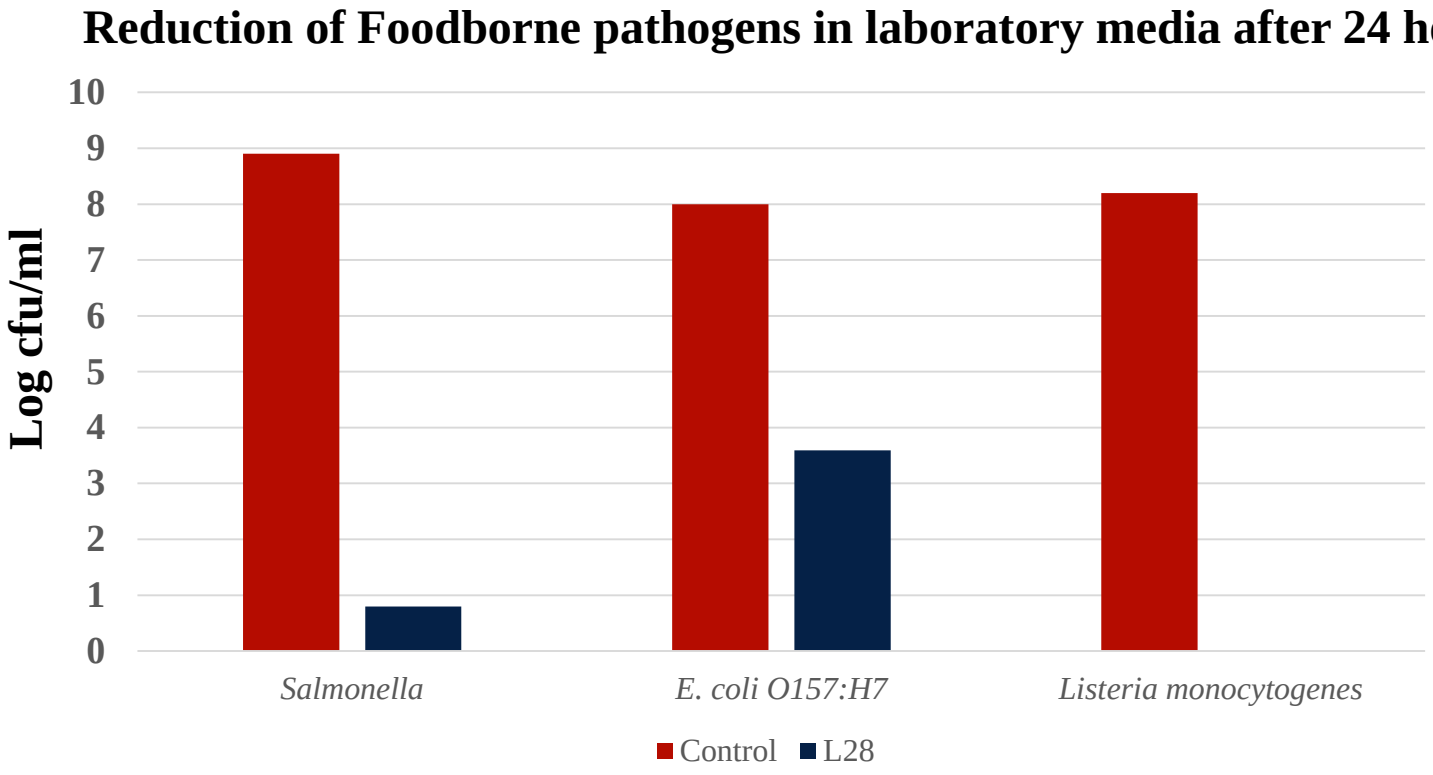
Lactobacillus NP51 Summary

- Supplementing Feed with a 10^9/head/day of *Lactobacillus* NP51 consistently reduces STEC O157 in the feces and on the hide of cattle
- Reductions in prevalence and concentration are observed
- *Salmonella* in lymph nodes is also reduced in prevalence and concentration
- *Salmonella* not reduced in feces/hide
- No detrimental impact on performance and potentially some improvements

“NEXT GENERATION” PRE-HARVEST APPLICATIONS

LAB Isolation Methodology for Next Generation of Probiotics

A systematic method was used to isolate lactic acid bacteria strains for multi-purpose targeted uses



*Experiments were replicated 3 times. A statistical difference was detected between control and treated samples for all pathogens

2016-17 Next Generation Probiotic Study

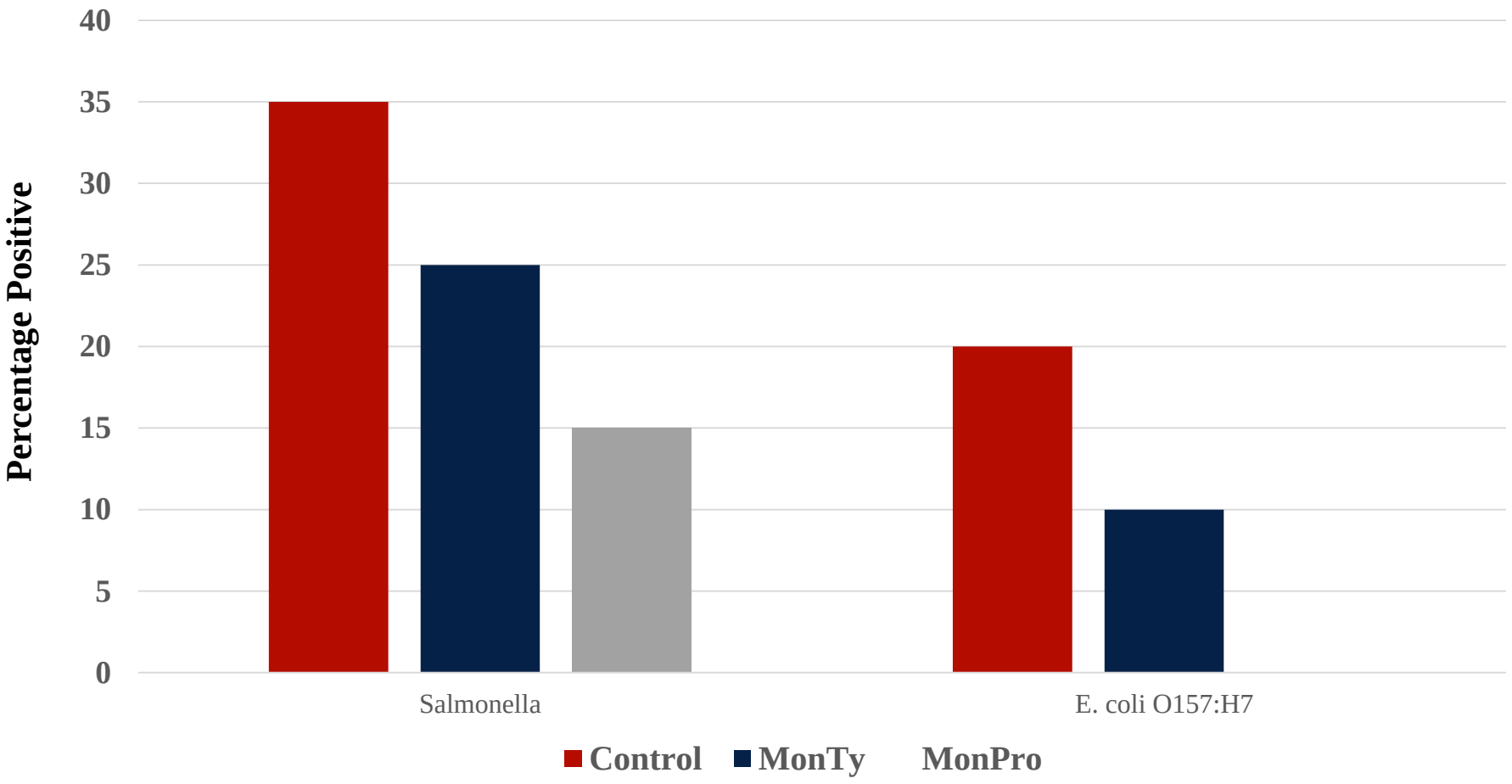
OBJECTIVES

- Determine the pathogen reduction, emergence of antimicrobial resistance patterns of *Enterococcus*, cattle performance, and carcass characteristics of cattle fed diets supplemented with *Lactobacillus salivarious* L28 with and without sub-therapeutic antibiotics.

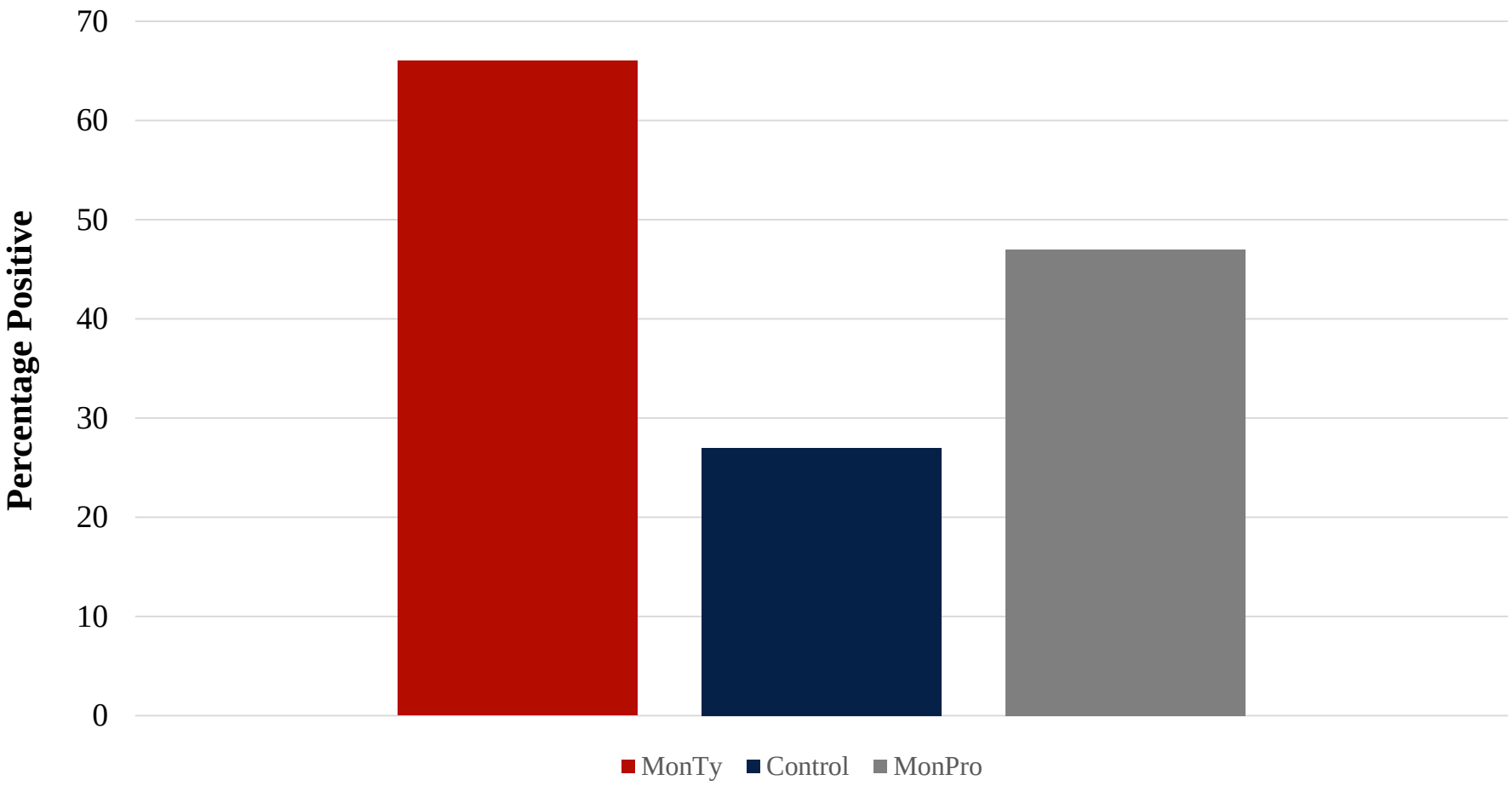
Treatments

- No DFM, no sub-therapeutic antibiotic, and no ionophore (**CON**)
- Monensin (Rumensin 90; Elanco; Greenfield, IN; 33 g/ton DM basis) Tylosin (Tylan 40; Elanco; 11 g/ton DM basis) (**MonTy**),
- Monensin and *L. salivarius* L28 (10^6 CFU hd/d) (**MonPro**).

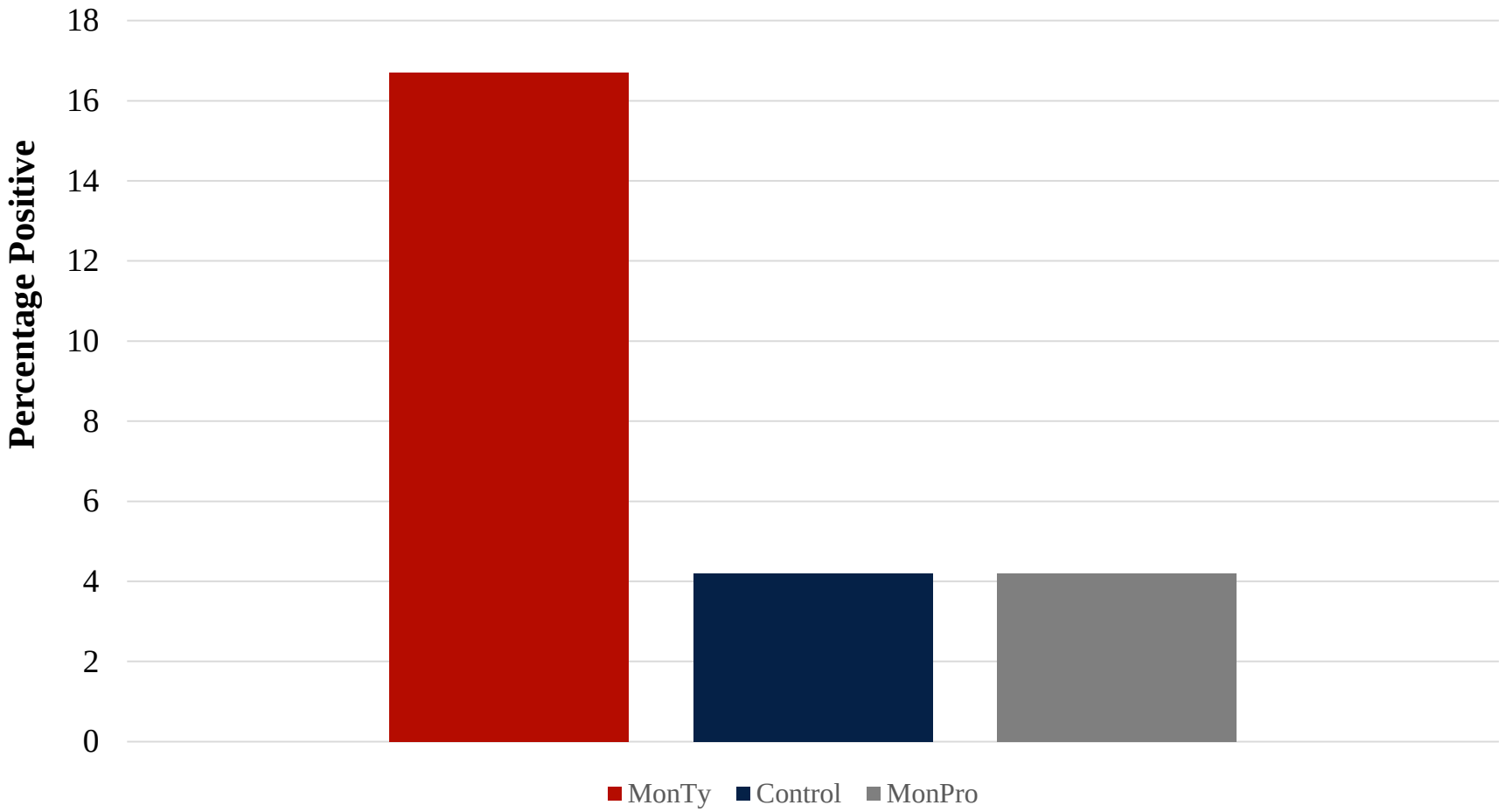
Food Safety Data – Fecal Pathogen Presence



Multi-Drug Resistance of Enterococcus Isolates



Multi-Drug Resistance of Generic *E. coli* Isolates



Performance and Carcass Characteristics

- There were no differences in final BW (P = 0.09) or overall ADG (P = 0.09) across treatments.
- Carcass weight, dressing percent, LM area, and yield grade did not differ (P > 0.23) across treatments.
- All treatments graded USDA Choice or better.

Conclusions

- Supplementation with L28 resulted in reduced pathogen presence of *Salmonella* and *E. coli* O157:H7.
- The presence of L28 along with Monensin resulted in antibiotic resistance patterns similar to the isolates from cattle fed no sub-therapeutic supplementation.
- These results also suggest that *L. salivarius* L28 does not have a negative impact on performance and may have similar performance and carcass responses to beef cattle fed sub-therapeutic levels of antibiotics.

FOOD APPLICATIONS

Determination the reduction of food-borne pathogens in ground beef by a LAB cocktail of 51, 3, 7 and 28

- Lactiguard cocktail (1 x 10^7 cfu/g ground beef):

NP 51 + L7 + D3 + C28

- Pathogens (1 x 10^3 cfu/g ground beef):

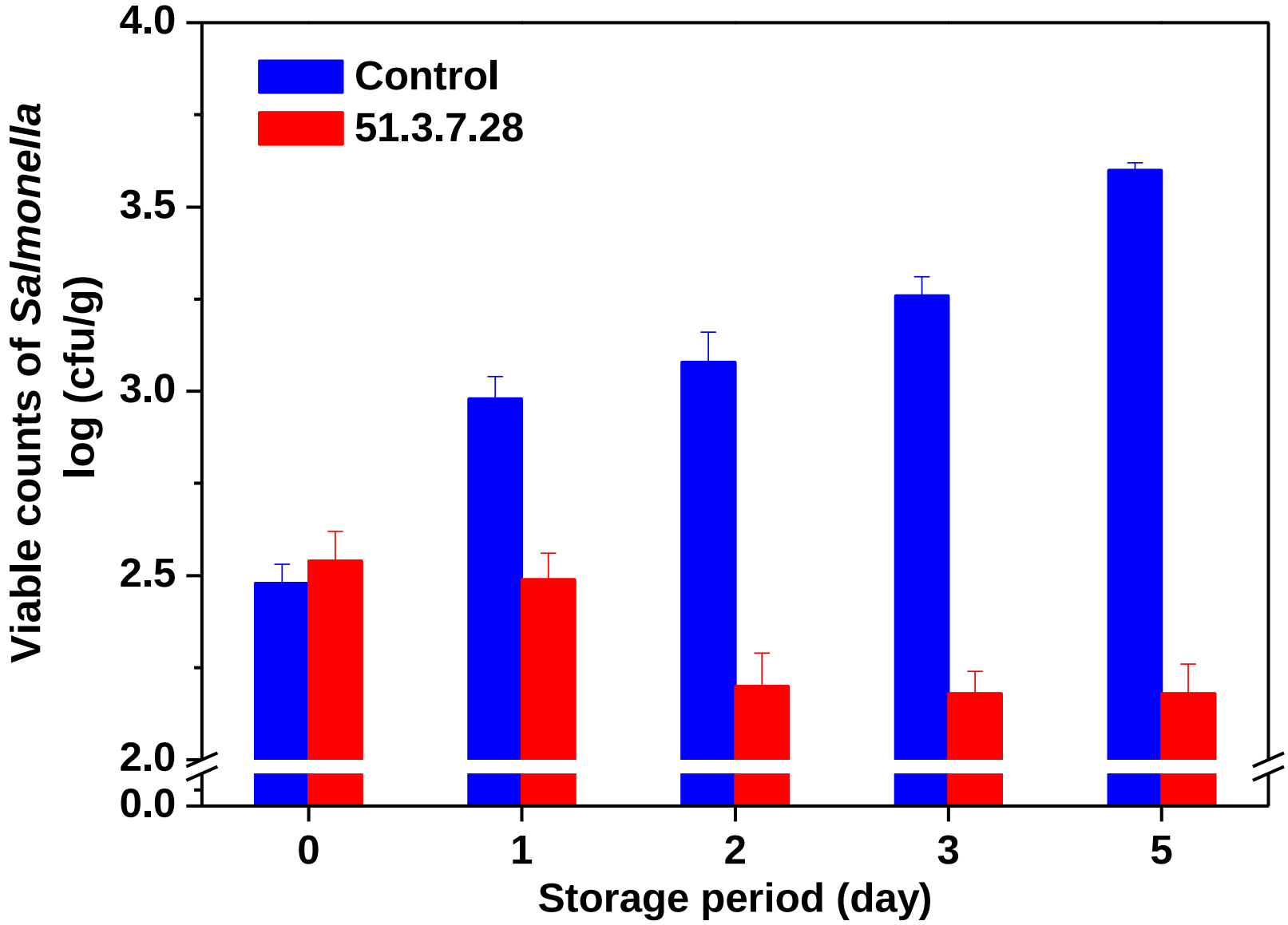
Non O157 STECs EC 026 and EC 0111

Salmonella Typhimurium ATCC 14028, *Salmonella* Heidelburg Sheldon 33471

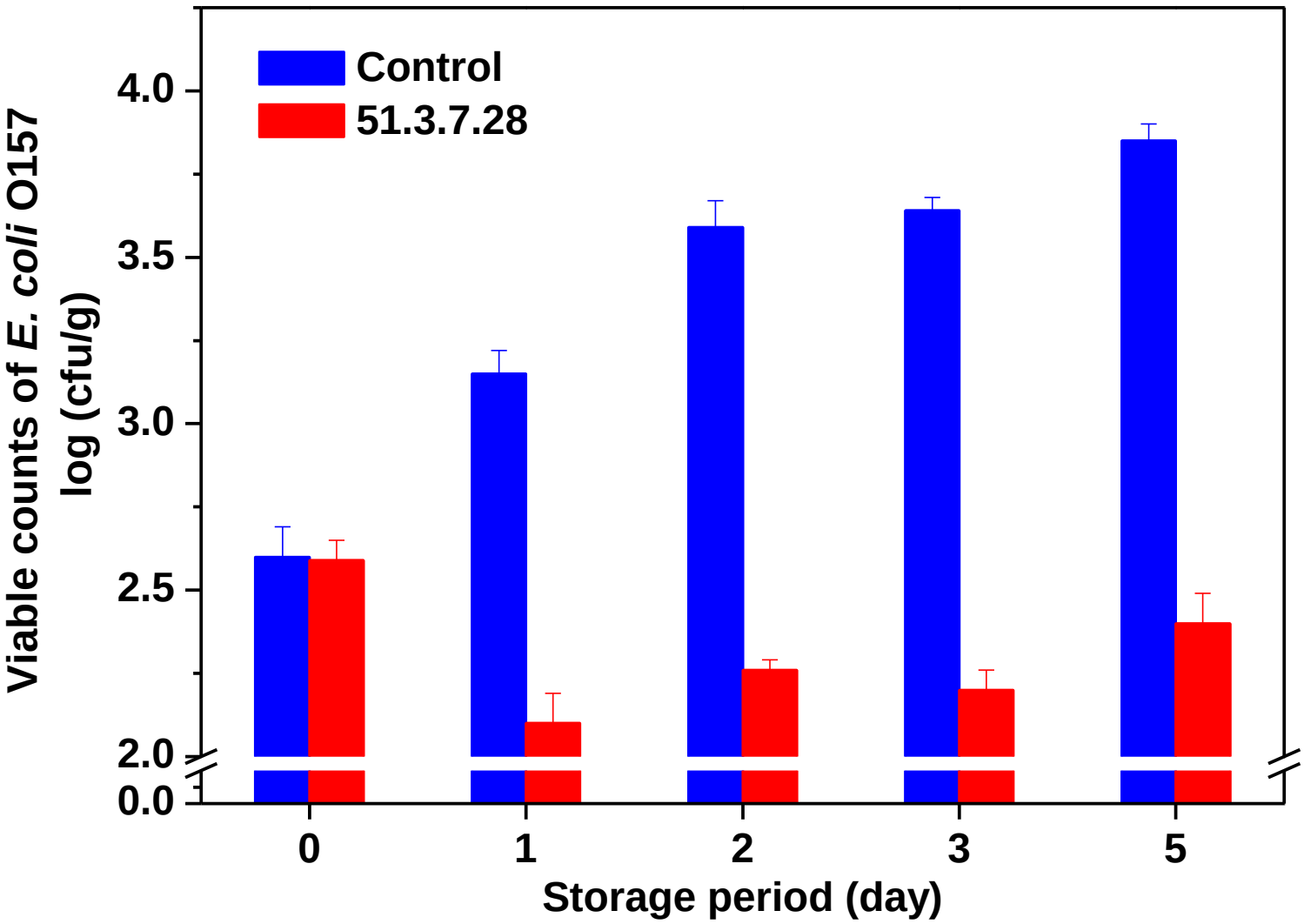
E. *coli* O157: H7 A4 966, E. *coli* O157: H7 A5 528

- Storage conditions: 4 ºC, 5d

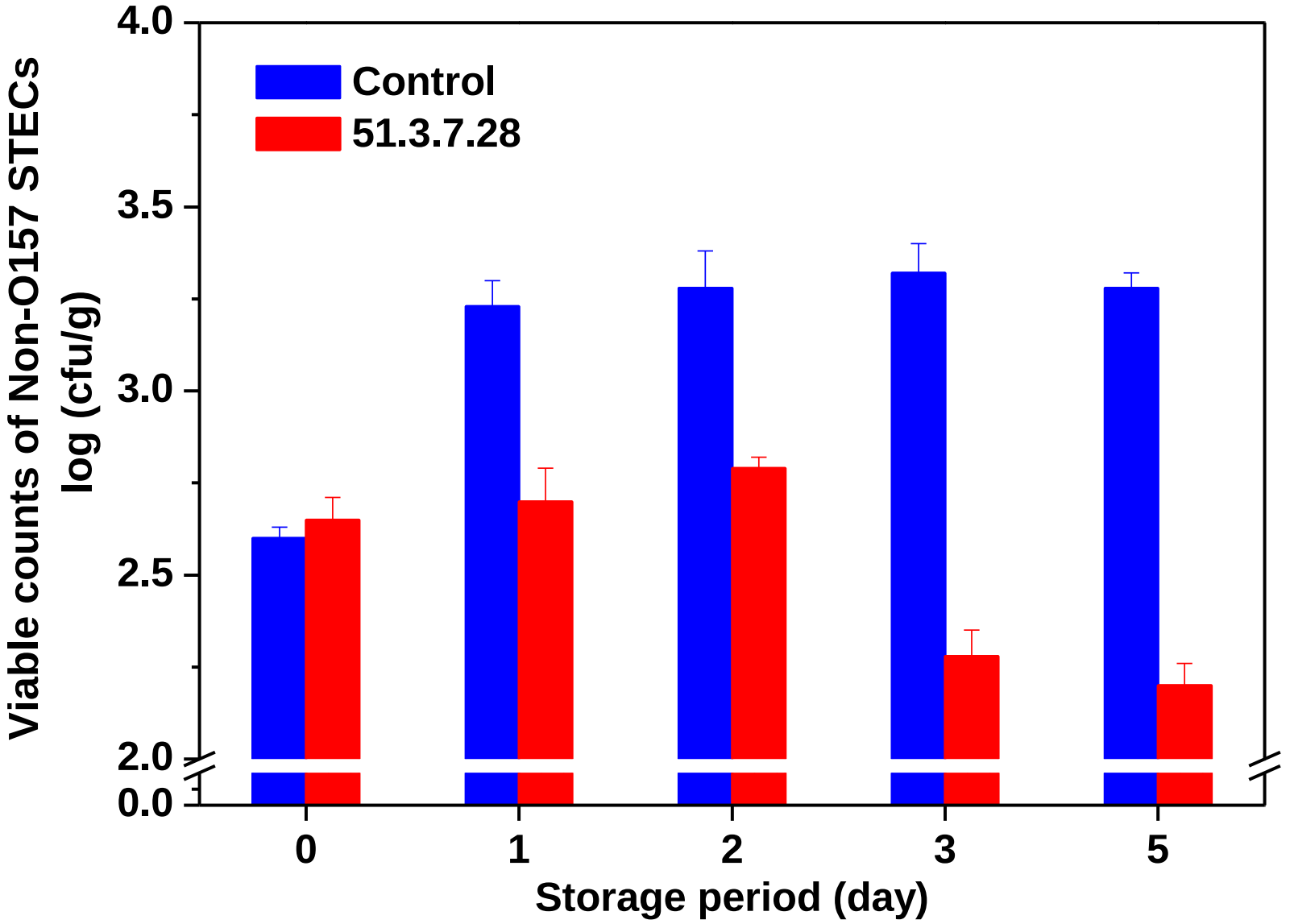
Reductions of *Salmonella* in ground beef after storage with lactic acid bacterial strains, NP51, NP3, NP7, and NP 28



Reductions of *E. coli O157:H7* in ground beef after storage with lactic acid bacterial strains, NP51, NP3, NP7, and NP 28



Reductions of *Non-O157 STECs* in ground beef after storage with lactic acid bacterial strains, NP51, NP3, NP7, and NP 28



Other Applications of L28 (Next Generation)

- Dry Dog Kibble
- Stainless Steel
- Chicken Fat

Application: Pet Kibble, *Salmonella*

In the past year alone, there have been many recalls of pet food attributed to foodborne illness.

Pets that consume contaminated pet kibble can become colonized by *Salmonella* without exhibiting clinical signs and shed the organism in their feces asymptomatically.

- Making the pet a possible source of contamination to people in the household

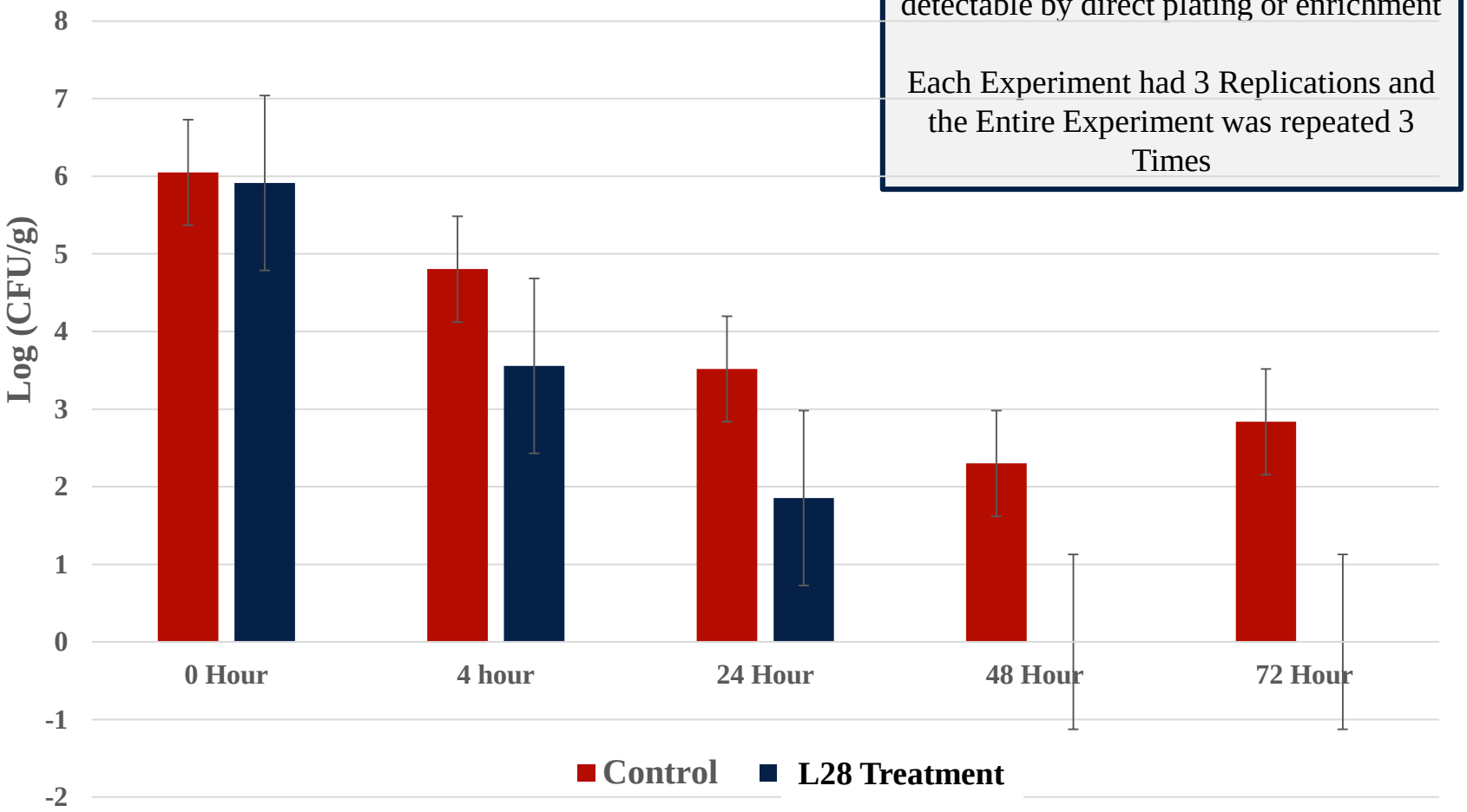
Treatment of Pet Kibble to Reduce *Salmonella*

- Commercially available pet kibble was obtained, inoculated with *Salmonella* and treated with L28 in a chicken fat coating.
- Kibble was bagged and stored at ambient temperature.
- Samples were obtained on hours 0, 24, and 72 to determine pathogen reductions.
- Samples were plated onto XLD with a thin-layer overlay to recover injured cells
- When populations were below detection limits by direct plating, pre-enrichment was conducted to detect survivors.

Pathogen Reduction in Pet Kibble with L28

***After 48 hours of L28 treatment:**
Salmonella was not
detectable by direct plating or enrichment

Each Experiment had 3 Replications and the Entire Experiment was repeated 3 Times



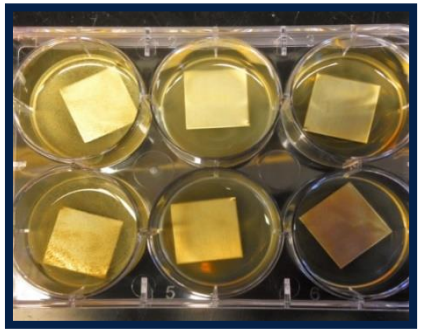
Application: Stainless Steel, *Listeria monocytogenes*

- *L. monocytogenes* is a foodborne pathogen that has caused many recalls in the last couple of decades.
- *L. monocytogenes* is known to have the ability to attach and form a biofilm on many surfaces, including stainless steel.
- Biofilms are not easily removed by common cleaning and chemical sanitizing methods. Therefore, finding innovative ways to control *L. monocytogenes* biofilm formation, growth and subsequent cross-contamination of finished RTE food products is critical.

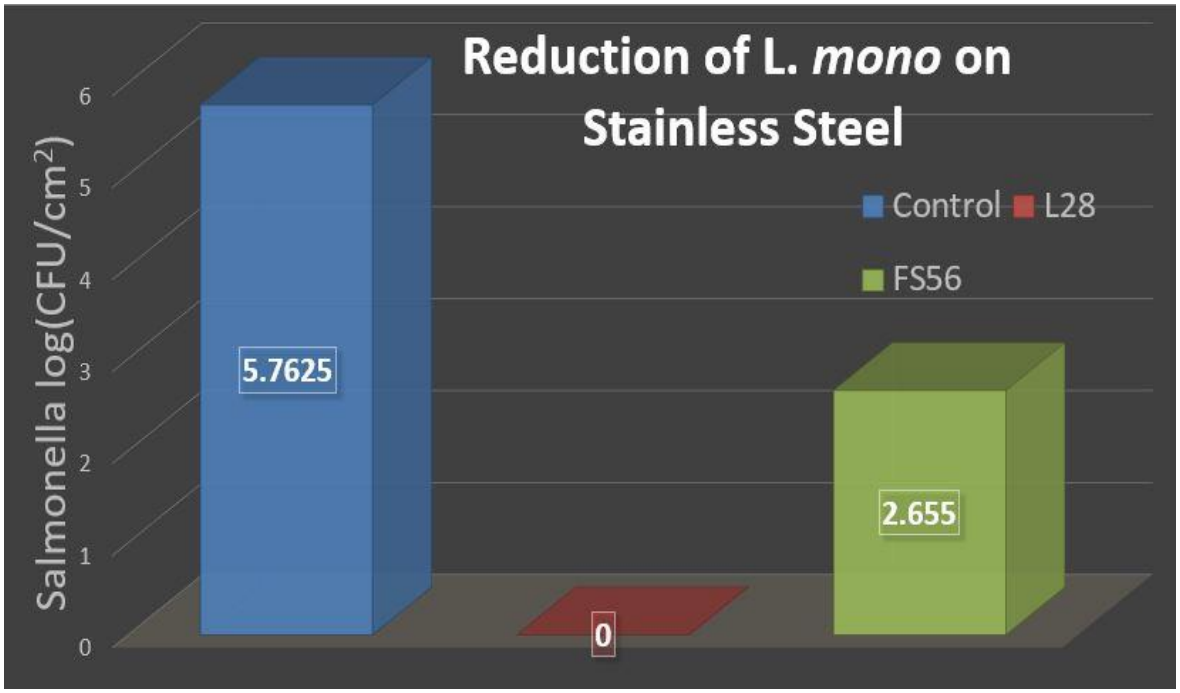
Application: Stainless Steel

Purpose: The purpose of this experiment was to evaluate the ability of L28 and commercially available Lactic Acid Bacteria strain (FS56) to inhibit *L. monocytogenes* (N1-002) on stainless steel coupons.

LAB applied to stainless steel coupons at 7 logs (application concentration)



Pathogen Reduction on Stainless Steel after 24 hours



Listeria monocytogenes was not detectable by means of direct plating or enrichment recovery methods

Experiment replicated 3 times

Application: Chicken Fat, *Salmonella*

- Chicken fat being a rich energy source has many important functions in the canine and feline diet
- It is often used to coat pet food kibble
- However, *Salmonella* is a major pathogen in poultry products and is a frequent vehicle of these bacteria and thus posing a risk to pet food

Chicken Fat Treatments

- Chicken fat was inoculated with 5.0 log cfu/g of *Salmonella.*
- Fat was treated with 7 log cfu/g of L28**.**
- Fat was held at 37°C.
- Resultant populations were enumerated on XLT with a thin-layer overlay to recover inured cells.
- Populations below the detection limit by direct plating were enriched and subjected to molecular screening to detect survivors.

Results: Chicken Fat

After 1 day at room temperature there were statistically significant differences between the control and the treatment samples.

After 3 days *Salmonella* in the control chicken fat had grown to approximately 7.13 log cfu/ml.

On day 3 the **L28** treatment resulted in a **7.13 log cfu/ml reduction** and *Salmonella* was not detectable.

CAUTION!!!!

- NOT ONE PROBIOTIC CAN DO EVERYTHING!!
- “In Plant” studies can be misleading so be sure they are well designed.
  - ONE EXAMPLE – inhibition in the broth instead of in the product/plant
- Some products do not work!!!

Conclusions

- While probiotics are not a “new technology” in concept, the application of the technology is expanding in novel ways.
- Must select specific strains for specific functions.
- Must improve the technology as we learn more about the industry needs
- Applications must be optimized for specific needs

Acknowledgements

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Disclaimer

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QUESTIONS