



A microscopic image showing a large, textured, brownish cell on the right and several smaller, yellowish, rod-shaped bacteria scattered throughout the field of view. The background is a soft, out-of-focus purple and pink hue.

In Vitro and In Vivo Evaluation of Therapeutic Effects of NeutraPath™ Against *Salmonella* Typhimurium

H. Xue¹, D. Wang¹, M. Handtmann¹, B.M. Hargis², & G. Tellez²

¹ Amlan International, Chicago, IL, 60061, USA

² Department of Poultry Science, University of Arkansas, Fayetteville AR 72701, USA

Salmonella Infections

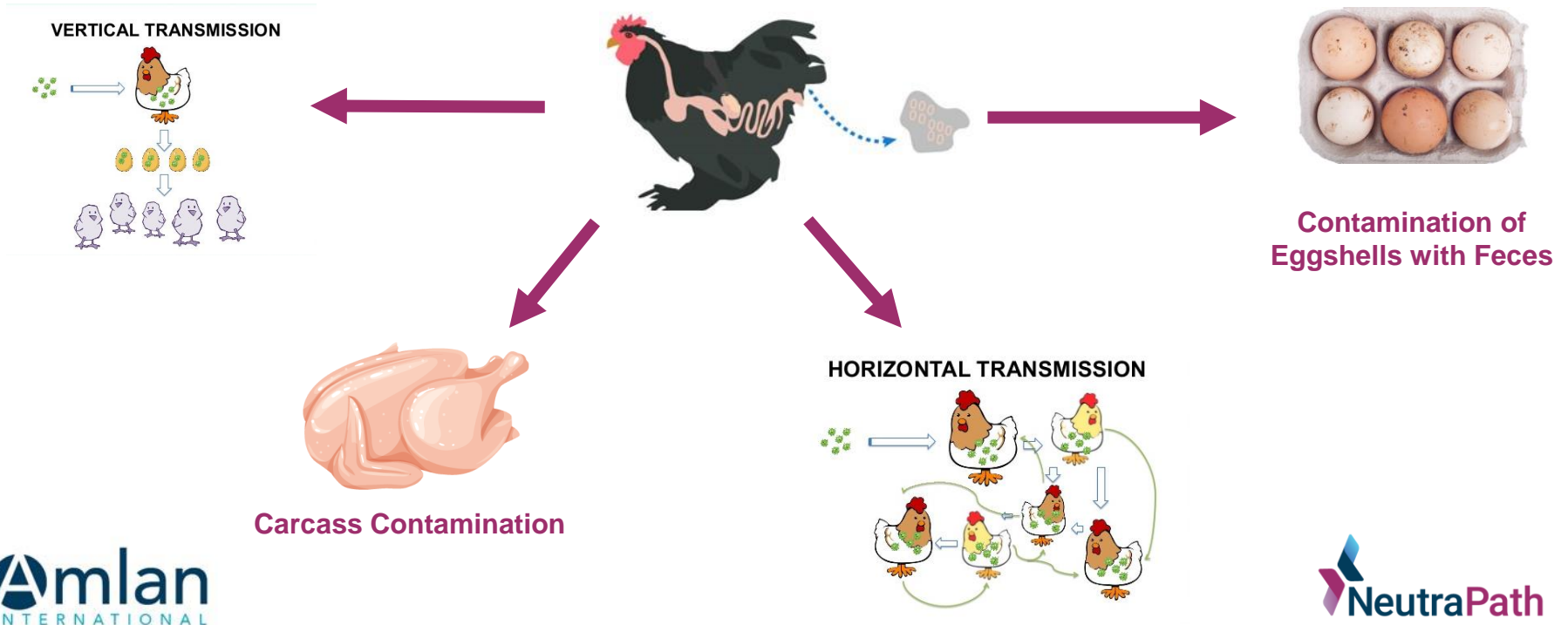
A Significant Public Health Concern

- In the US, 1.4 million cases of human salmonellosis annually; 100,000 cases due to antibiotic-resistant Salmonella
- Poultry serves as the major reservoir of Salmonellas
- Preharvest Salmonella control is of utmost importance;
- Feed additives are a key pre-harvest measure which can help control Salmonella at the farm level



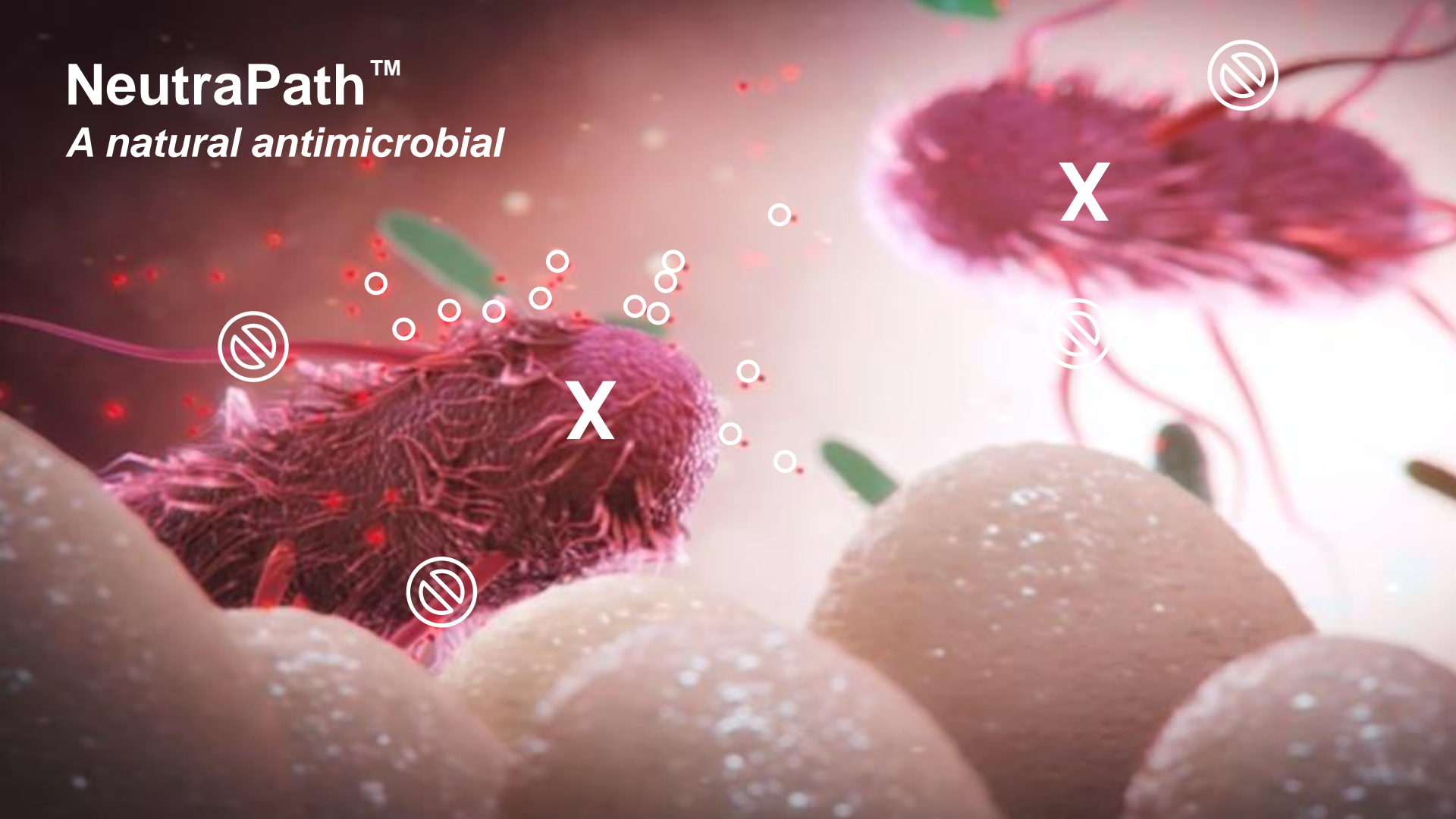
Colonization in chicken intestinal tract

Central to Entry into the Human Food Chain



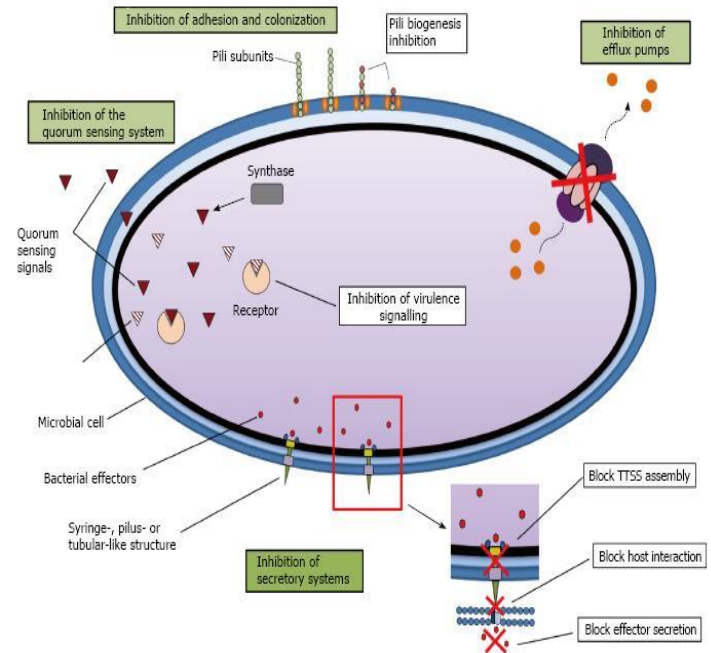
NeutraPath™

A natural antimicrobial



Antivirulence Strategy May Pinpoint a Paradigm Shift for Pathogen Control

- **Toxins**
- Adhesins
- **Biofilm formation**
- **Secretion systems**
(e.g., Type III Secretion systems)
- Cell-to-cell communication
 - **Quorum sensing**
- Siderophores
- Immune evasion



NeutraPath™

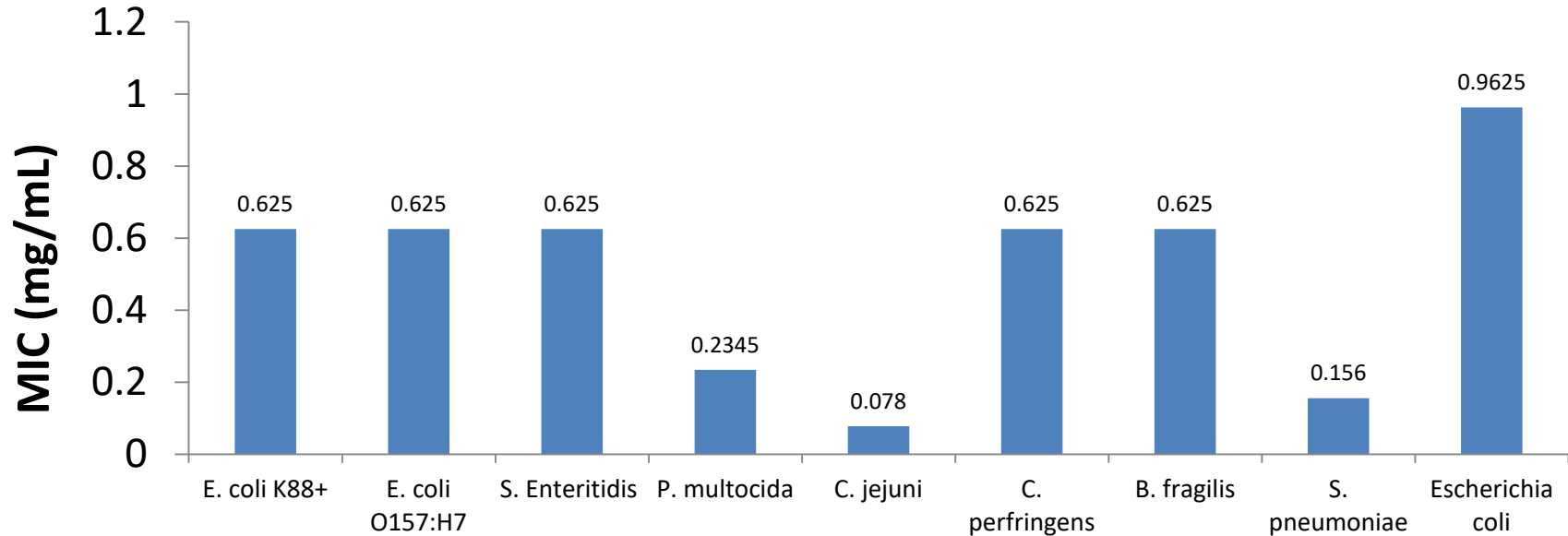
Proven Synergistic Formula



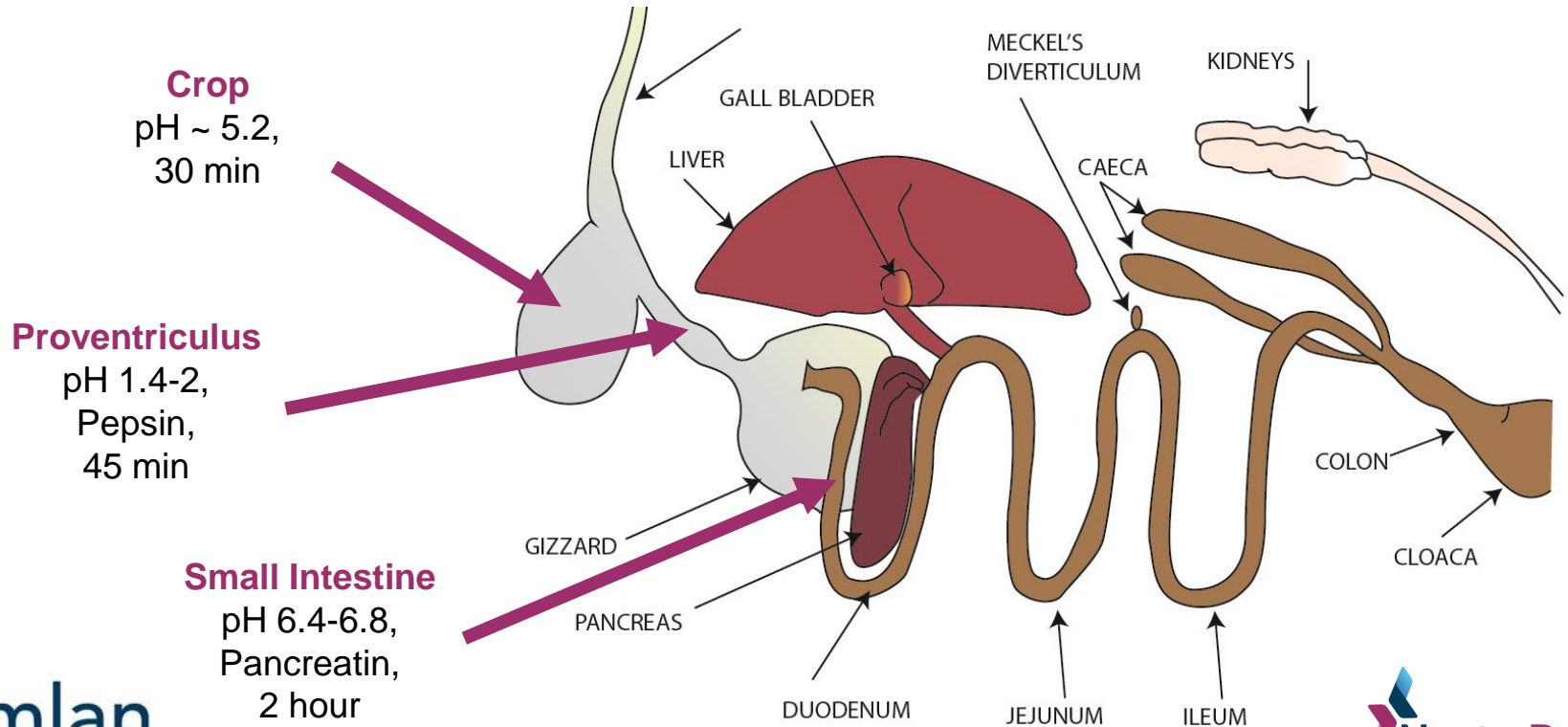
Bacteriocidal and
bacteriostatic effect

Neutralizing virulence factors

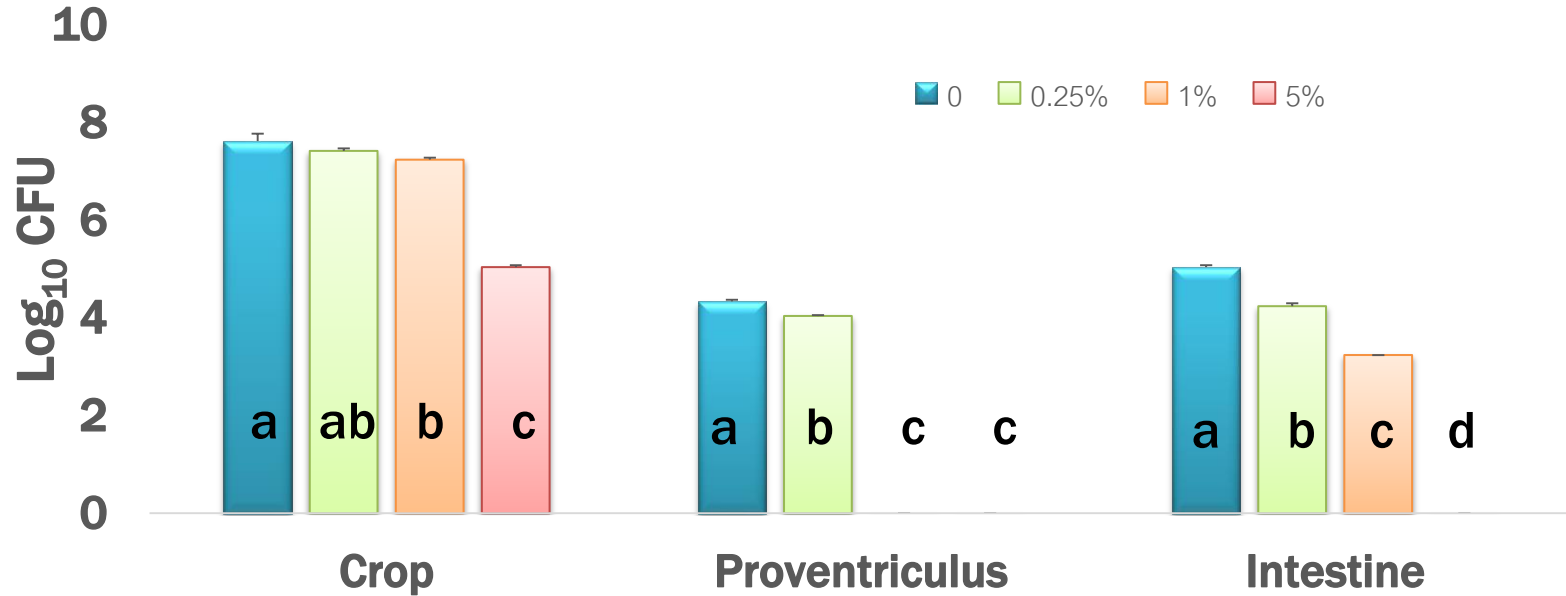
NeutraPath's minimum inhibitory concentration (MIC)



Ability of NeutraPath to Kill *Salmonella* in SIMULATED GI environment *in vitro*



NeutraPath reduced *Salmonella* Typhimurium bacterial load in the *in vitro* digestion system



In Vivo Validation

Objectives

To evaluate in vivo effects of NeutraPath on:

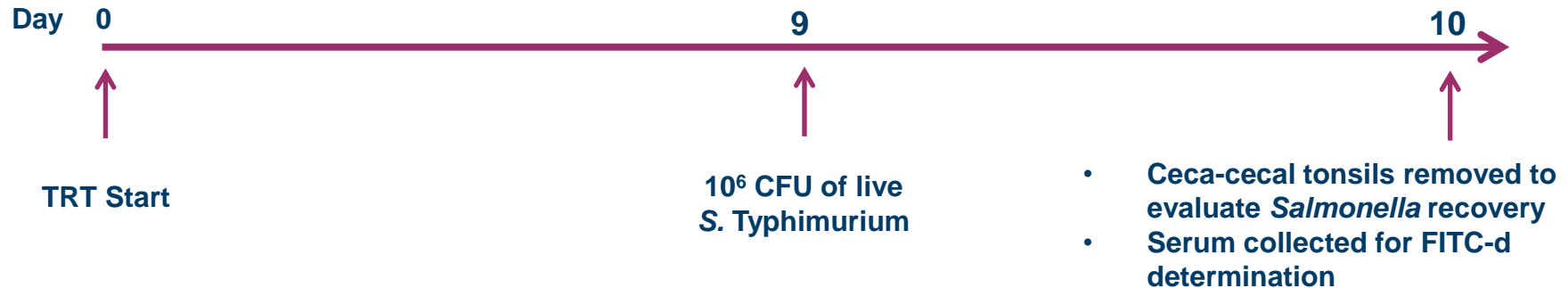
- *Salmonella enterica* sv. Typhimurium cecal colonization in broiler chickens;
- Functional integrity of the host's gut barrier

In Vivo Validation

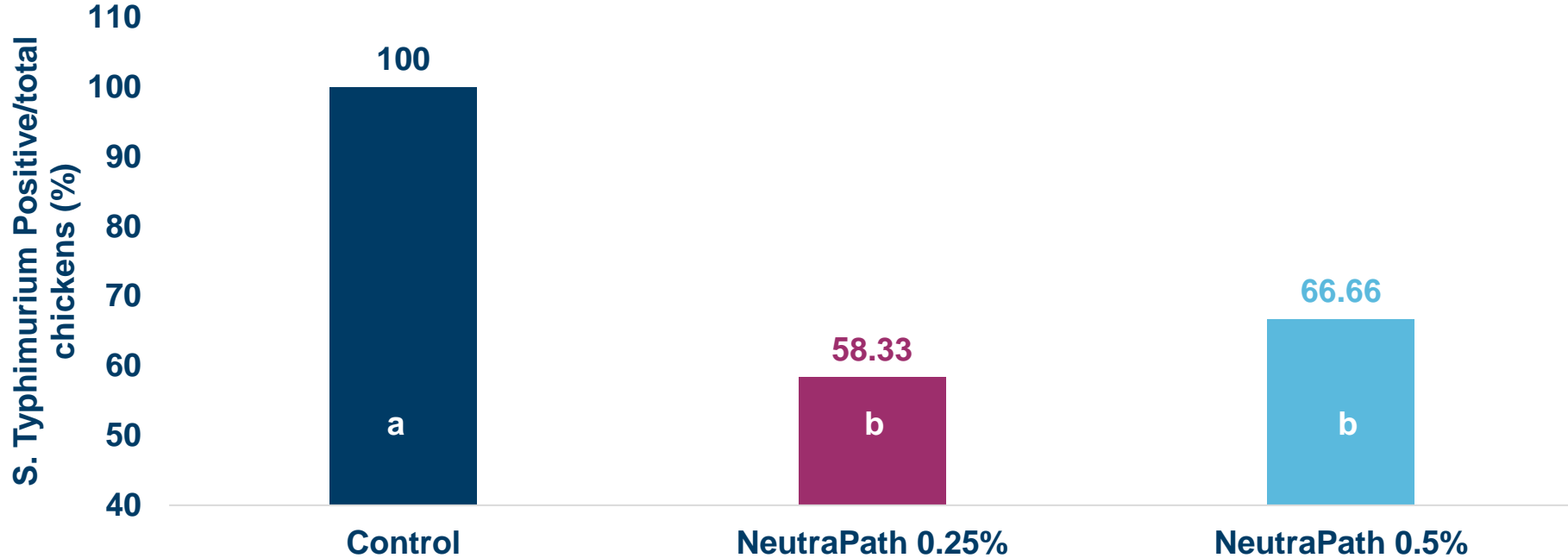
Experimental Design

One-day old male broiler chicks were randomly allocated to one of three groups (n=30 chickens):

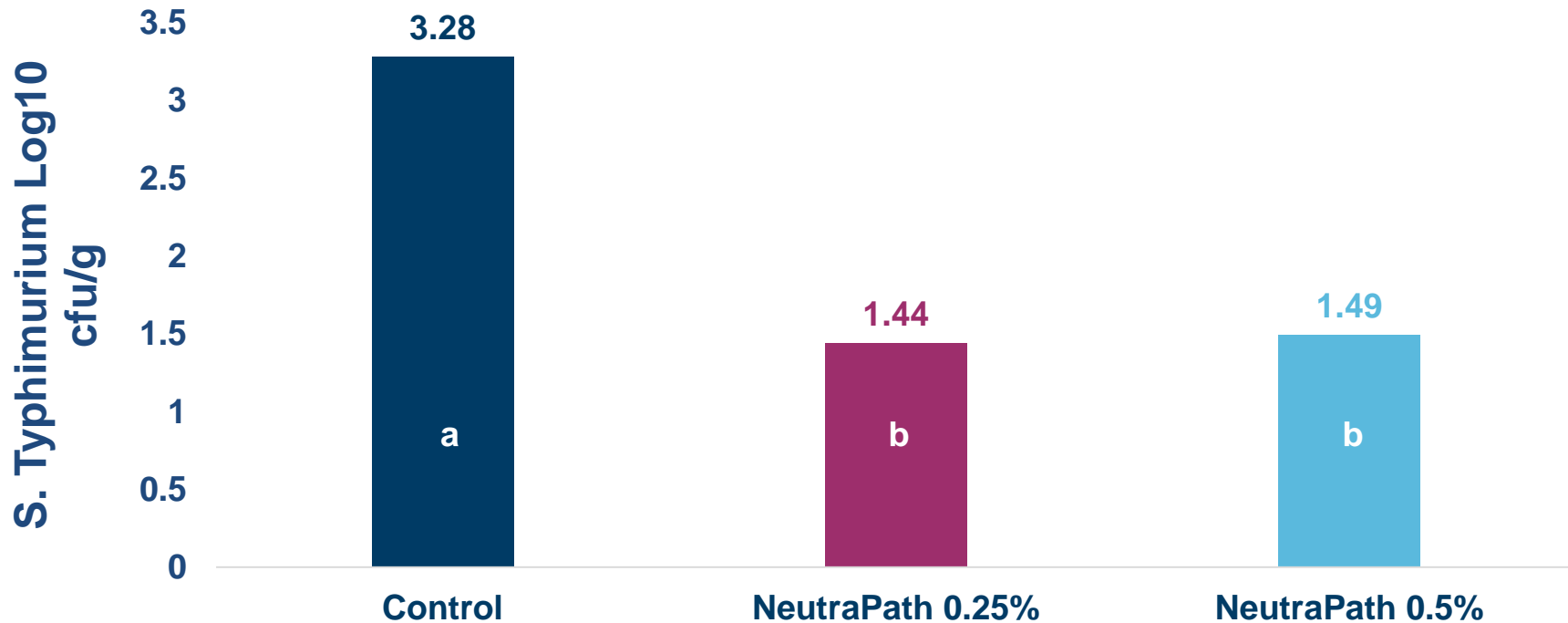
- Challenged control with non-treated feed
- NeutraPath supplemented at 0.25%
- NeutraPath supplemented at 0.5%



NeutraPath Reduced Prevalence of *Salmonella* Typhimurium in Ceca

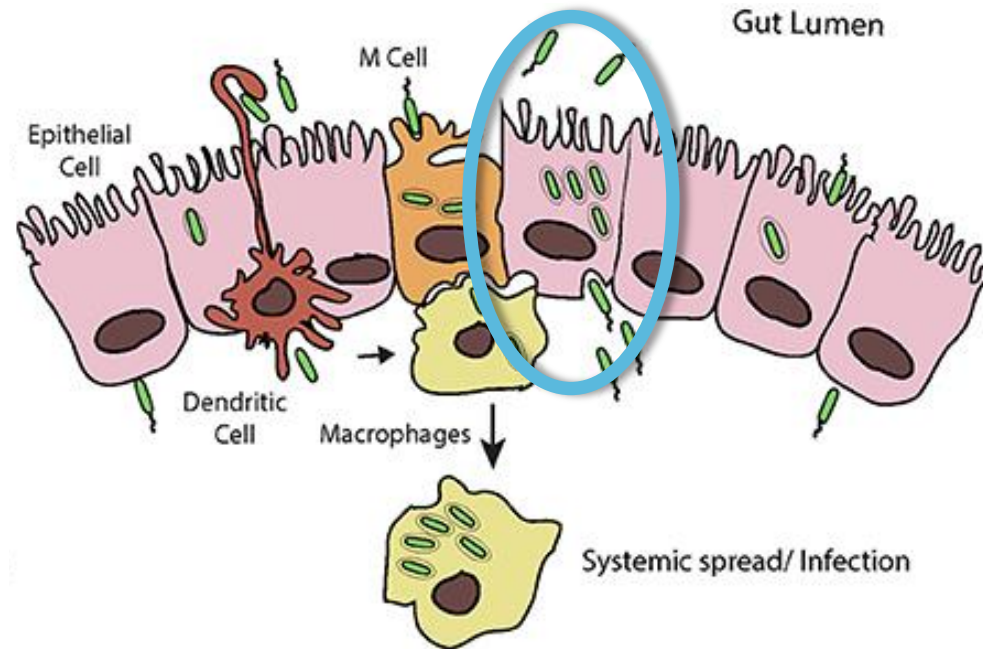


NeutraPath Reduced *Salmonella* Typhimurium Bacterial Load in Ceca

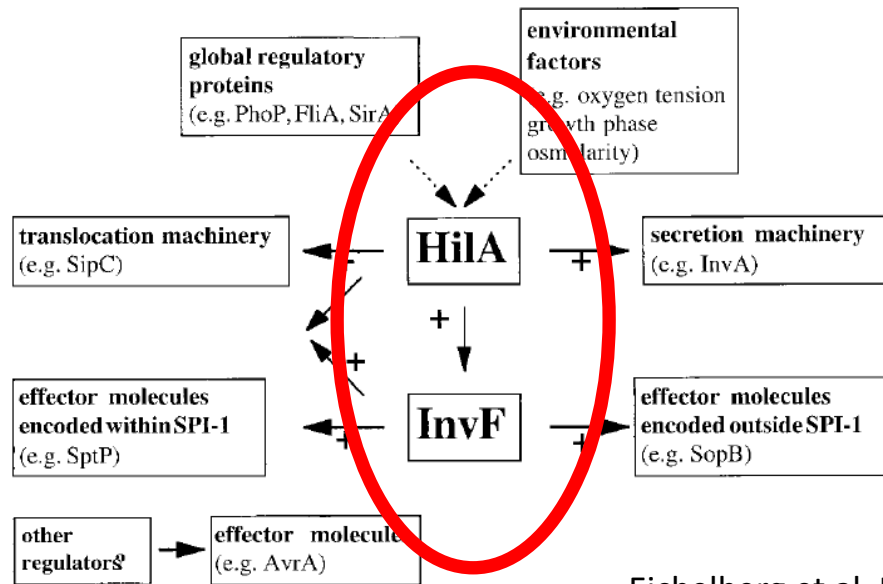
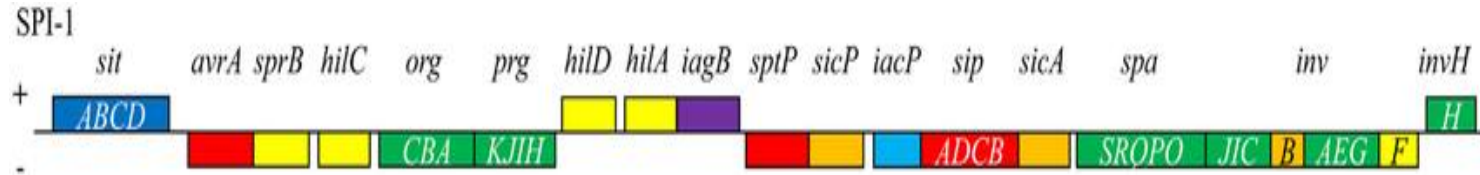


Key Property of *Salmonella*

Ability to invade non-phagocytic epithelial cells



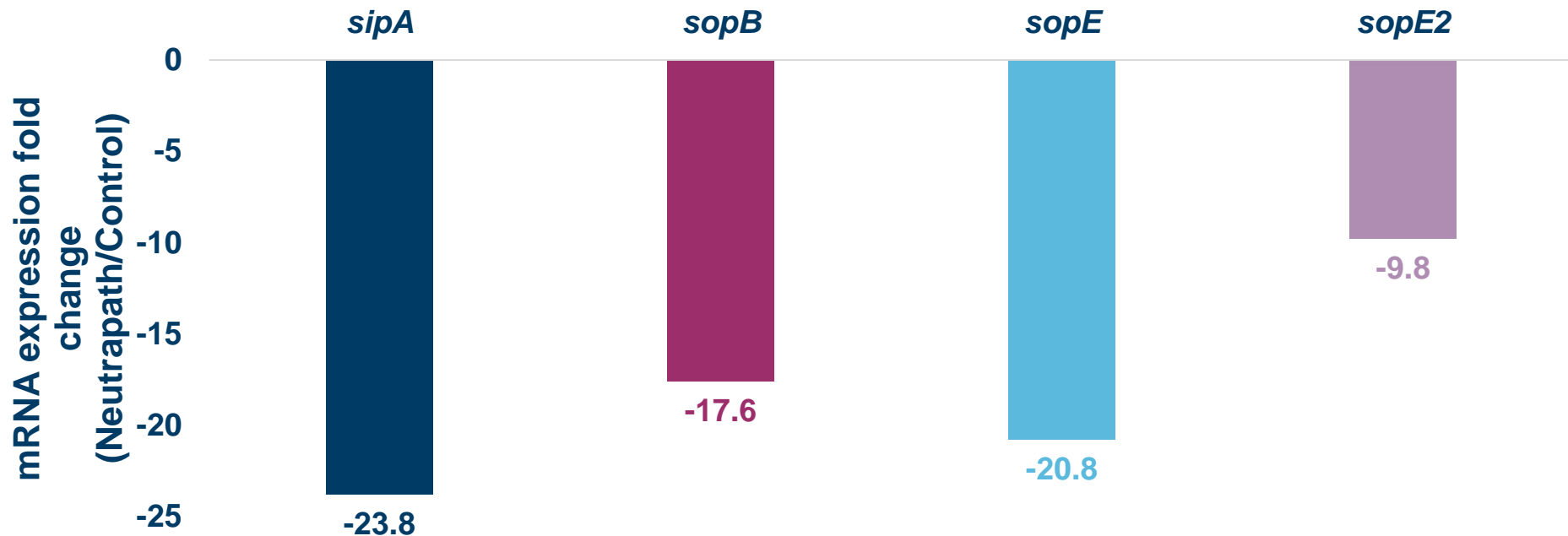
HilA-InvF Axis - Master Regulators For Salmonella Pathogenicity Island 1 (SPI1) TTSS Apparatus Gene Transcription



NeutraPath down-regulated *S. Typhimurium* *hilA* and *invF* mRNA expression at subinhibitory concentration (SIC)

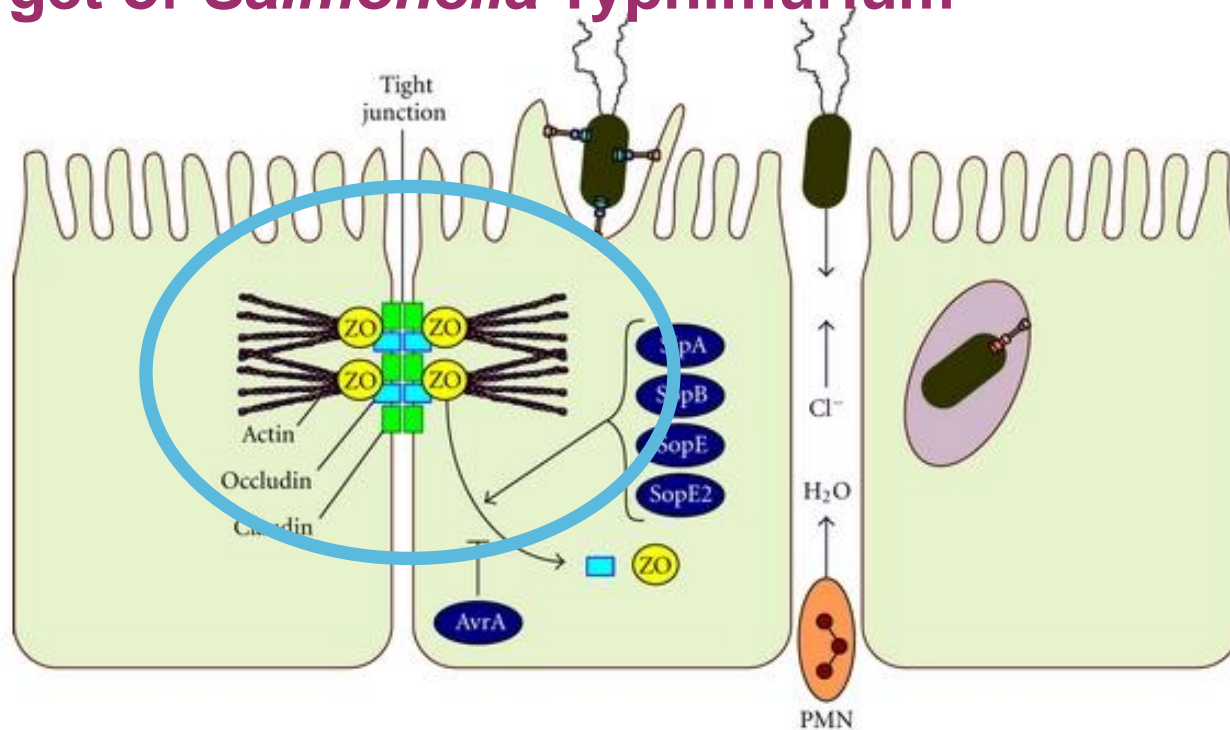


NeutraPath's Suppression of HilA-InvF Axis Further Blocked Expression of Key Downstream Effectors Involved in Invasion

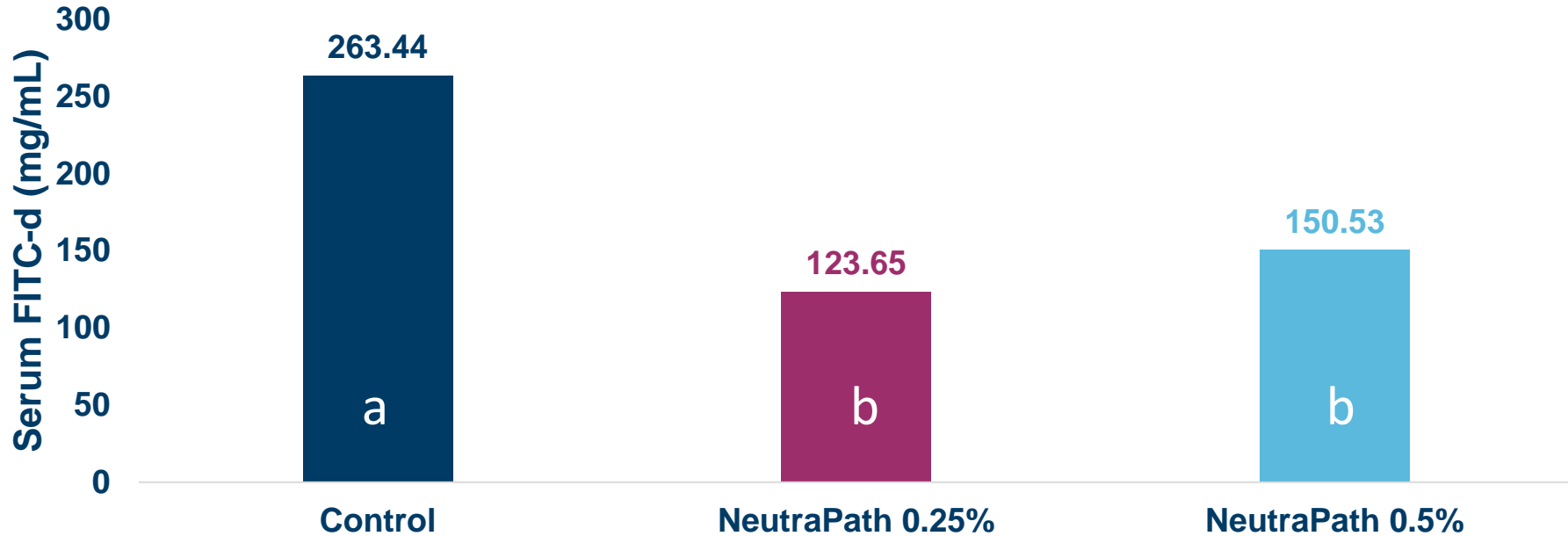


Tight junction:

Key Target of *Salmonella Typhimurium*



NeutraPath Reduced *In Vivo* Gut Permeability after *Salmonella* Typhimurium Challenge



Conclusions

- NeutraPath treatment had the therapeutic potential to reduce *S. Typhimurium* intestinal colonization in broiler chickens;
- Mechanistically, NeutraPath strikingly tuned down SPI-1 TTSS virulence machinery and modified the bacterial 'behaviors' to make them more benign;
- As a result of the blockade of SPI-1 virulence development, NeutraPath further preserved gut barrier integrity during *S. Typhimurium* challenge.

The background of the slide is a microscopic image. It features a large, detailed cell on the right side, showing internal structures like a nucleus and various organelles. Scattered throughout the scene are numerous yellow, rod-shaped bacteria, likely Bacillus or similar species, some of which are in focus while others are blurred in the background. The overall color palette is warm, with yellows, oranges, and purples.

Thank you

Hongyu Xue MD, PhD

Life Sciences Director

Amlan International

hongyu.xue@amlan.com