CLOSTAT®

Active Microbial

CLOSTAT® contains a proprietary strain of *Bacillus subtilis* PB6 — a unique, naturally-occurring probiotic that helps maintain the balance of microflora in the gastrointestinal (GI) tract of livestock and poultry. By strengthening the microflora balance, CLOSTAT has been demonstrated to improve egg production, eggshell weight and eggshell thickness[1].

MODE OF ACTION

The *B. subtilis* PB6 in CLOSTAT has been found to secrete one or more biocidal proteins that are inhibitory towards certain strains of pathogenic bacteria, such as *Clostridium* spp. These proteins disrupt the membrane of bacteria, causing leakage of the cell contents and ultimately killing the pathogenic bacteria without harming the beneficial gut microflora.

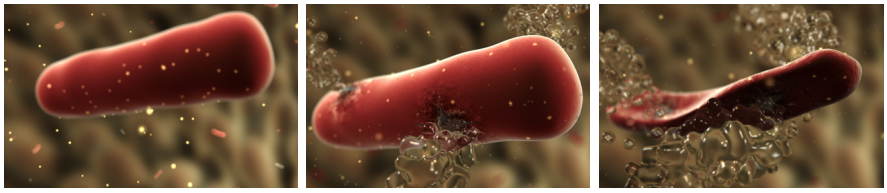

Figure 1: PB6 surfactants impact on *Clostridium perfringens* cell wall structure

WHY IS THIS IMPORTANT?

Pathogenic bacteria like *Clostridium perfringens* create lesions in the small intestine that compromise the integrity of the intestinal lining (increased permeability) — allowing pathogens and toxins to more easily enter the bloodstream. This can result in intestinal inflammation, disease, and an environment in which bacteria can thrive.

By inhibiting the growth of pathogenic bacteria, the PB6 in CLOSTAT helps maintain a healthy microbial balance in the digestive tract and improve nutrient absorption.

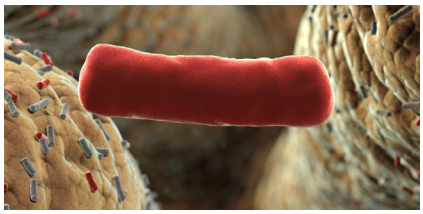

Figure 2: *C. perfringens* in the intestine

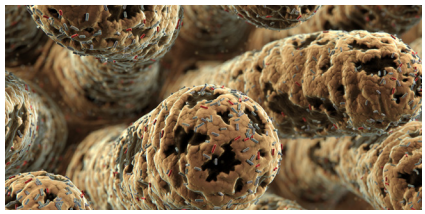

Figure 3: Damaged gastrointestinal villi

CLOSTAT FEATURES AND BENEFITS

- **Contains the probiotic PB6, a unique, proprietary strain of *B. subtilis*[2]**
- **Enhances health status, resulting in disease resistance/reduction and reduced shedding of pathogens**
- **Delivers research-proven efficacy of *B. subtilis* PB6 against a broad range of pathogens *in vitro* and *in vivo***
- **Provides demonstrated safety in livestock and poultry**
- **Heat stable during pelleting, packaging, and storage**

IN VITRO EFFICACY: PROVEN PATHOGEN INHIBITION AGAINST *CLOSTRIDIUM* SPP.

Two common assays are used to evaluate the growth inhibitory activity of direct-fed microbial (DFM) organisms — the first is a cross-streak assay and the second is a zone of inhibition. In a cross-streak assay (Figure 4), the pathogen can be streaked vertically across an agar plate.[3] The DFM is streaked perpendicular to that pathogen. After incubation, any clearing of growth at the intersection of the pathogen and the DFM indicates growth inhibition of the pathogen by the DFM. *B. subtilis* PB6 (top two horizontal streaks on plate) demonstrates clear inhibition, whereas *B. subtilis* ATCC 6633 (bottom horizontal streak on plate) did not inhibit *C. perfringens*.

A zone of inhibition assay (Figure 5) demonstrates the ability of an antimicrobial compound to inhibit the growth of a pathogen.[4] The larger the zone of clearing (reduced growth) around the antimicrobial compound, the more sensitive the pathogen is to that compound. The positive (Enramycin 10 ppm) and negative (sterile water) controls are shown in the bottom two zones of the plate (right and left, respectively), while *B. subtilis* PB6 is shown in the top two zones on the plate. Note, *B. subtilis* PB6 had larger clearing zones than even the positive control — demonstrating inhibition of *C. perfringens.*

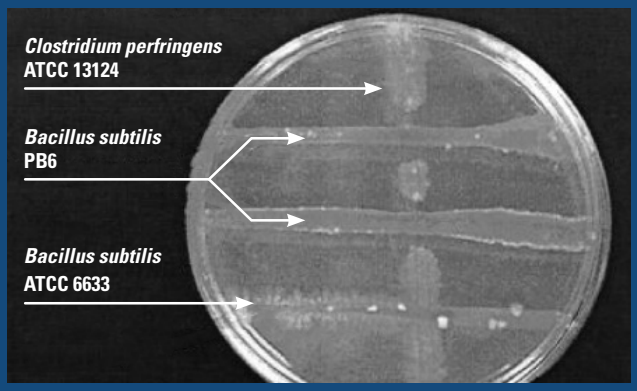

Figure 4: Cross streak assay between *B. subtilis* PB6; *B. subtilis*, ATCC 633; and *C. perfringens*, ATCC 13124. *B. subtilis* PB6 demonstrates clear inhibition, whereas *B. subtilis* ATCC 6633 did not inhibit *C. perfringens.*[2]

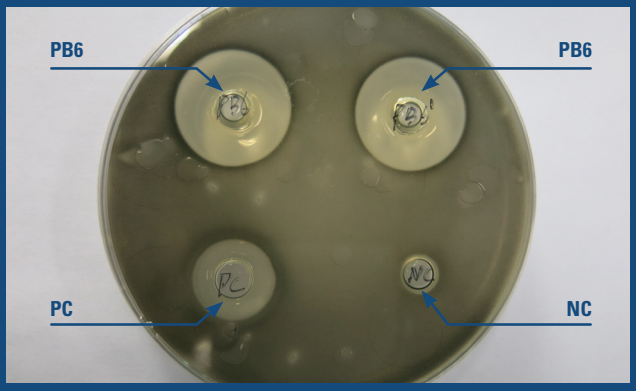

Figure 5: Effect of *B. subtilis* PB6 against *C. perfringens*, ATCC 13124 with positive control (PC, Enramycin 10 ppm) and negative controls (NC, sterile water). *B. subtilis* PB6 had larger clearing zones that demonstrates inhibition of *C. perfringens.*[3]

Table 1: University field isolations of *Clostridium perfringens, Clostridium septicum, Clostridium difficile* and *Clostridium sordelli* demonstrate that *B. subtilis* PB6 has an *in vitro* inhibitory effect in livestock and poultry.[5,6]

C. perfringens strain	*C. septicum* strain
919 (ISU)	ARK124 (UARK)
1537 (UA)	ARK126 (UARK)
1936 (UA)	ARK307 (UARK)
4057 (UA)	40592 (UGA)
ARK07AM (UARK)	51181 (UGA)
TCP3 (UGA)	D07-20687 (UMN)
CP6 (UGA)	D07-21793 (UMN)
CP2 (UGA)	
49824 (UGA)	
008-6130 (UMN)	
008-6429 (UMN)	

ISU — Iowa State University, Veterinary Diagnostic Lab
UA — University of Arizona, Veterinary Diagnostic Lab
UARK — University of Arkansas
UGA — University of Georgia, Poultry Diagnostic Research Center
UMN — University of Minnesota, Veterinary Diagnostic Lab

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References:
1. Abdelqader, A., Irshaid, R. and Al-Fataftah, A. 2013. Effects of dietary probiotic inclusion on performance, eggshell quality, cecal microflora composition, and tibia traits of laying hens in the late phase of production. Trop Anim Health Prod, 45:1017–1024.
2. Lin, A. S. H., A. Y. L. Teo, and T. H. Meng. 2007. Antimicrobial compounds from *Bacillus subtilis* for use against animal and human pathogens. United States Patent 7,247,299.
3. Teo, A. Y. and H. M. Tan. 2005. Inhibition of *Clostridium perfringens* by a novel strain of *Bacillus subtilis* isolated from the gastrointestinal tracts of healthy chickens. Applied and Environmental Microbiology. 71(8):4185-4190.
4. Antibacterial Spectrum of *Bacillus subtilis* PB6 Against Enteric Pathogenic Bacteria in Animals, WP-14-00076.
5. *Bacillus subtilis* PB6 Inhibits a Broad Range of Clostridial Isolates from Poultry, Swine and Ruminants, WP-08-00049.
6. Application of *Bacillus subtilis* PB6 in turkey field trials. WP-09-00105.

PTP-13133
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