

# CLOSTAT®

*BACILLUS SUBTILIS* PB6  
ACTIVE MICROBIAL



[kemin.com/clostat-cattle](http://kemin.com/clostat-cattle)

**KEMIN**<sup>®</sup>  
INTESTINAL   
HEALTH

# WHAT IS INTESTINAL HEALTH AND WHY DOES IT MATTER?

The function of the gastrointestinal (GI) tract is to **digest and absorb nutrients, maintain a balanced microbiome, prevent harmful compounds from entering the host and defend against harmful pathogens.**

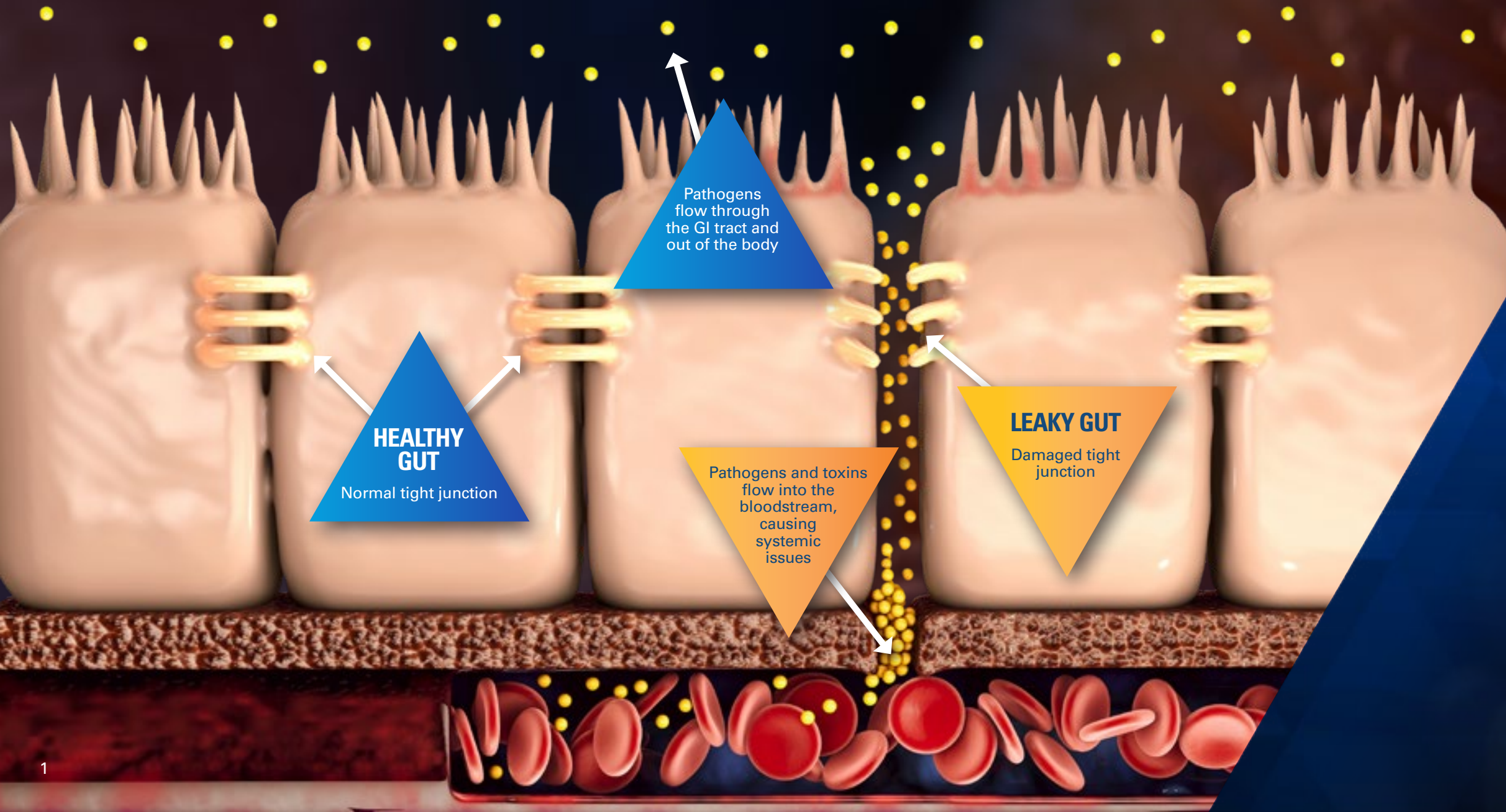
## A HEALTHY GUT:

Breaks down nutrients for optimal absorption

Promotes and maintains immune system health

Maintains structural intestinal integrity

Preserves the balance of microflora



## A DAMAGED GI TRACT, OR LEAKY GUT, COULD RESULT IN:

An unbalanced microbiome, leading to a higher prevalence of enteric pathogens

Reduced digestive and absorptive capacity

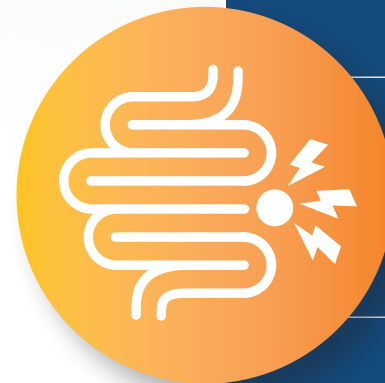
Decreased intestinal integrity of the gut, allowing harmful pathogens and toxins to enter into the host, which can lead to both intestinal and systemic inflammation

A compromised immune system

# STRESSORS IMPACTING THE INTESTINE

Just as exposure to pathogenic bacteria in the environment is inevitable, so are other everyday stress events. Under stress events, both the mucosal layer and the tight junctions are negatively impacted, often leading to inflammation and reduced integrity of the intestinal barrier.<sup>1</sup> Reduced intestinal integrity indicates there is a breakdown in the tight junctions between the epithelial cell membranes, allowing for intestinal permeability. Without these tight junctions, pathogenic organisms like *Clostridium*, *Salmonella* and *Escherichia coli* (*E. coli*) can cross the intestinal barrier, resulting in an immune response that makes cattle more susceptible to diseases and reduces their performance and profitability.<sup>2</sup>

*The intestinal barrier is regularly exposed to up to 10 trillion microorganisms.*<sup>3</sup>



- EVERYDAY STRESSORS**
- Pathogens (Figure 2)
  - Heat, cold or mud stress
  - Diet changes
  - Weaning
  - Handling and transportation
  - Metabolic and production demands
  - Mold and mycotoxins
  - Overcrowding



*Clostridium*



*Salmonella*



*E. coli*

Figure 2: *Clostridium*, *Salmonella* and *E. coli*

# SELECTION CRITERIA FOR MICROBIALS

Not all active microbes are the same. When evaluating active microbial solutions to fight against intestinal-compromising pathogenic bacteria, the following criteria must be considered:



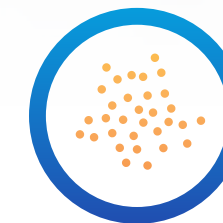
Proven mode(s) of action



Backed by repeatable research



Efficacy against a broad spectrum of disease-causing pathogens



Stable in multiple manufacturing processes, including pelleting



Intestine viability

## WHY DOES THE MODE OF ACTION MATTER?

By understanding the mode(s) of action of an active microbial, one can better predict how the host will respond to the product once it is fed. This helps create confidence in the product, as it has gone through the rigorous testing and research to prove the mode(s) of action.



# WHAT IS CLOSTAT®?

CLOSTAT® contains a proprietary, patented strain of *Bacillus subtilis* PB6 — a gram-positive, spore-forming *Bacillus* that was isolated from a healthy chicken intestine. *B. subtilis* PB6 grows faster than other known *Bacillus* spp.<sup>4,5</sup> and is resistant to low (acidic) pH and high temperatures, enabling it to survive the GI tract and the pelleting process.

In addition, a peer-reviewed article describing the modes of action has been published in the *Journal of Applied and Environmental Microbiology*.<sup>4,5</sup>

## NOT ALL *B. SUBTILIS* ARE CREATED EQUAL

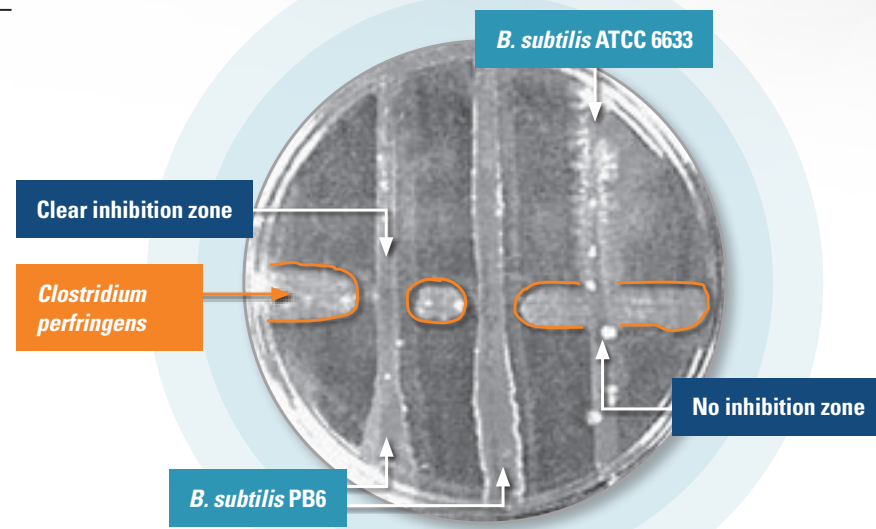


Figure 3: *In vitro* comparison of CLOSTAT (*B. subtilis* PB6) and *B. subtilis* ATCC 6633<sup>4</sup>

## *B. SUBTILIS* PB6 MODES OF ACTION AT A GLANCE

*Bacillus subtilis* PB6

### 1 PATHOGEN INHIBITION

*B. subtilis* PB6 has been found to secrete multiple biocidal proteins that are inhibitory towards certain strains of pathogenic bacteria.

### 2 REDUCED GUT INFLAMMATION

*B. subtilis* PB6 has been found to secrete cyclic lipopeptides surfactins through normal metabolism, which inhibit PLA<sub>2</sub>.

### 3 QUORUM QUENCHING

*B. subtilis* PB6 has been found to prevent the initiation of infection, colonization and disease progression by producing a lipopeptide called fengycin.

Dive deeper into the CLOSTAT modes of action on pages 6-8

## *B. SUBTILIS* PB6 MODES OF ACTION

### MODE OF ACTION 1: PATHOGEN INHIBITION

*B. subtilis* PB6 secretes multiple biocidal proteins that are inhibitory towards certain strains of pathogenic bacteria.<sup>4,5</sup> Using LC/MS and Q-TOF analyses, the active metabolites have been found to possess a cyclic structure comprised of seven amino acids, collectively known as surfactins.

The mode of inhibition action is due to the ability of the surfactin molecules to form pores on the cell walls of the bacteria as shown using electron microscopy (Figure 4).<sup>6</sup> These proteins disrupt the bacteria membrane, causing leakage of the cell contents and ultimately killing the pathogenic bacteria without harming the beneficial gut microflora.

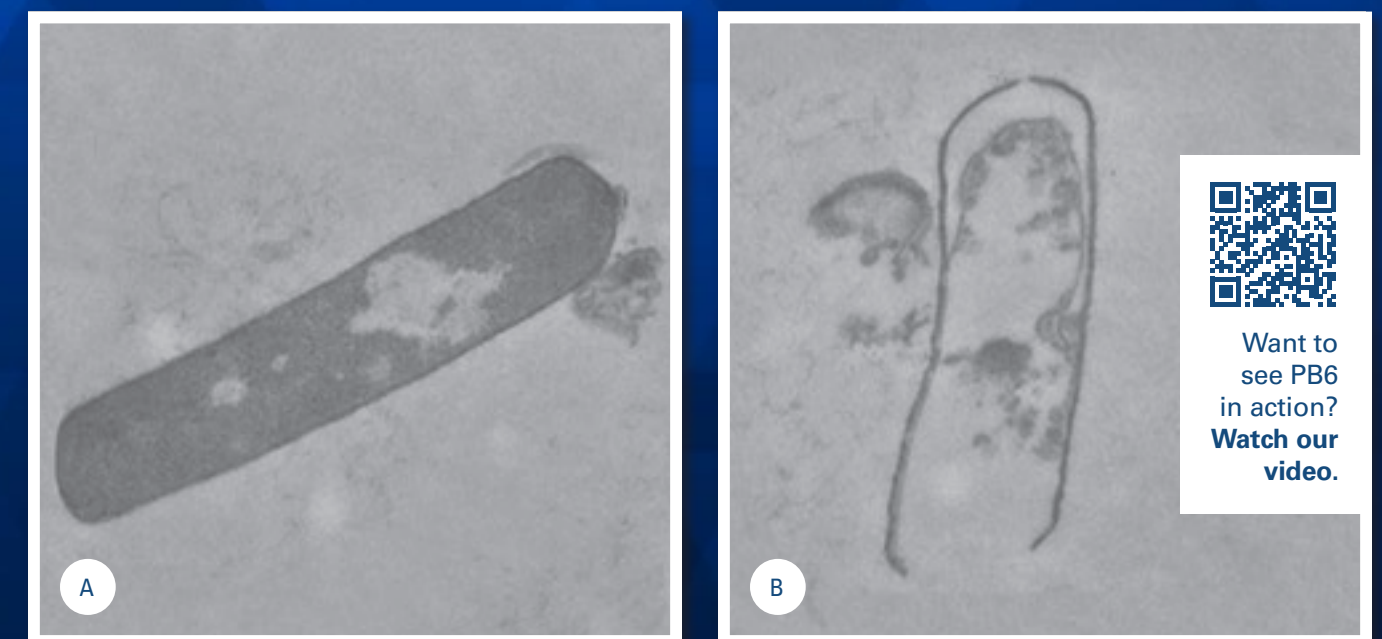


Figure 4: The effect of *B. subtilis* PB6 on *Clostridium perfringens* at 37° C. (a) Disruption of the cell wall and loss of cytoplasmic contents into the exterior after one hour. (b) Rupture and death of cell after 4 hours. (Transmission electron micrograph magnification: 29,000X)<sup>6</sup>

# B. SUBTILIS PB6 MODES OF ACTION

## MODE OF ACTION 2: REDUCED INTESTINAL INFLAMMATION

Intestinal inflammation can occur if the intestinal lining becomes damaged, allowing molecules such as bacteria, pathogens and their toxins to pass between epithelial cells, also known as Leaky Gut Syndrome (Figure 5). Once that occurs, the immune system recognizes these invasive organisms and triggers an immune response to destroy and remove the invaders.<sup>7</sup> This inflammatory process and immune system activation consumes significant amounts of nutrients and pulls nutrients and energy away from other key performance functions.<sup>7</sup>

*B. subtilis* PB6 has been found to secrete cyclic lipopeptide surfactins through normal metabolism, which inhibit phospholipase A<sub>2</sub> (PLA<sub>2</sub>), a rate-limiting enzyme involved in the arachidonic acid (AA)-associated inflammatory pathway.<sup>8</sup> Inhibition of synthesis of proinflammatory cytokines and elevation in the level of anti-inflammatory cytokines re-establish cytokine balance.<sup>7,9</sup>

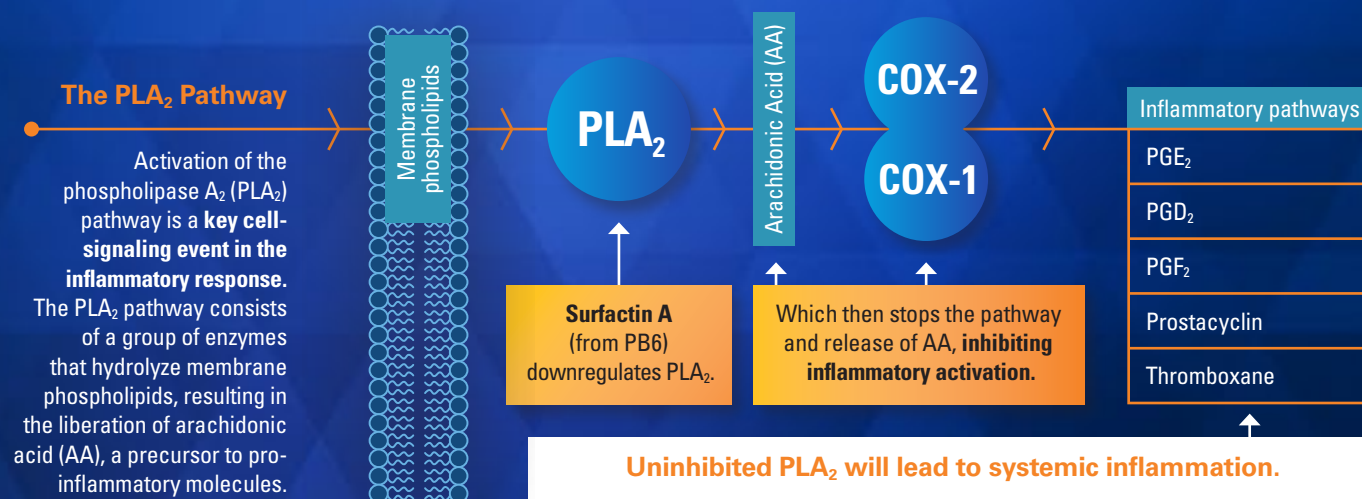


Figure 5: PLA<sub>2</sub> pathway inhibition

## MODE OF ACTION 3: QUORUM QUENCHING

**What is quorum sensing (QS)?**<sup>10-13</sup>

- Initiation of infection, colonization and disease progression
- Production, secretion and detection of diffusible signaling molecules
- Triggering of a cellular response, including the production of toxins and other virulence factors
- Allowance of host colonization and invasion through a variety of mechanisms

**What is quorum quenching (QQ)?**<sup>14</sup>

- Disruption of the QS system
- Abolishment of the expression of virulence factors
- Reduction of pathogenicity and disease severity
- Rendering of pathogens less invasive

Quorum quenching can occur via several routes, including the **degradation of QS signaling molecules, the inhibition of signal biosynthesis and the inhibition of signal detection.**<sup>10-13</sup>

*B. subtilis* PB6 can prevent QS of *C. perfringens* through the production of fengycin, a quorum quenching molecule, that is naturally produced during growth. QQ also aids in the prevention of toxin production and formation of biofilm.<sup>6</sup>

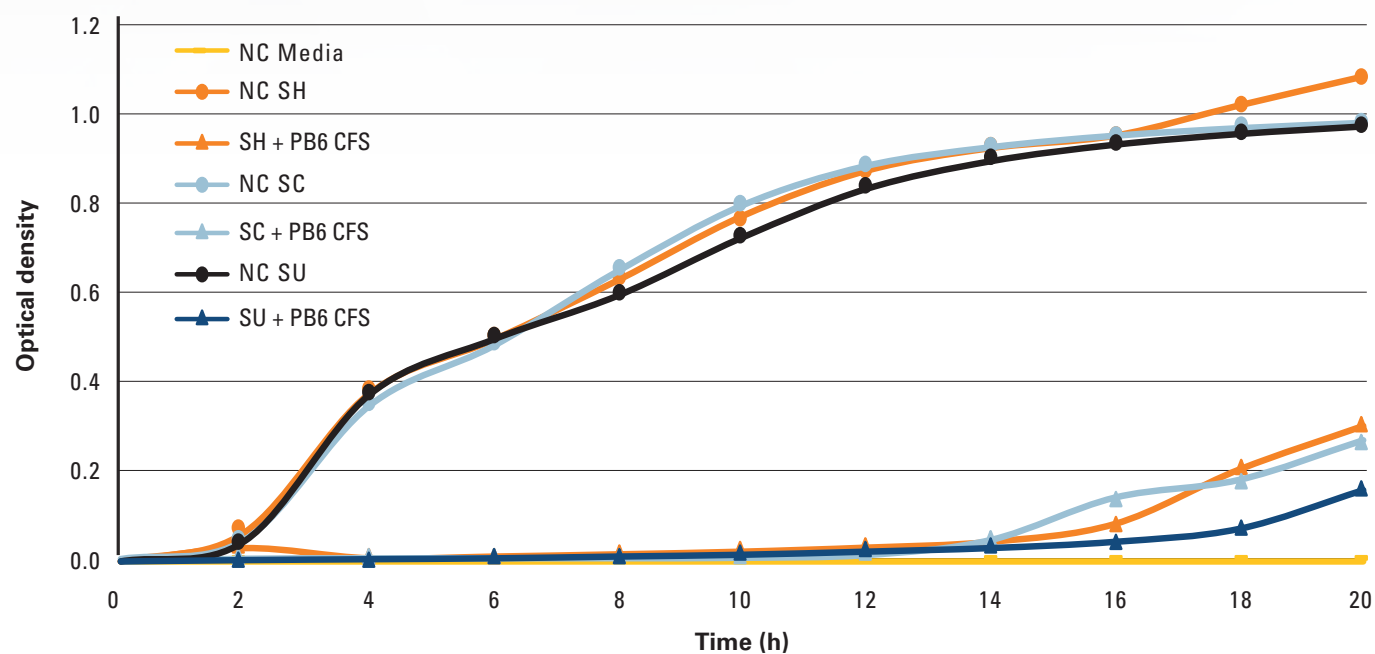


# EFFICACY AGAINST DISEASE-CAUSING PATHOGENS

Understanding and identifying disease-causing pathogens is critical in developing mitigation and treatment strategies. For example, *Salmonella enterica* serovars Heidelberg, Cerro and Uganda are associated with disease and mortality in cattle with each shown to be resistant to many antibiotics (Figure 6).<sup>15-17</sup> While *in vitro* assays cannot mimic *in vivo* conditions, the work reported here demonstrated that the metabolites secreted by *B. subtilis* PB6, a direct-fed microbe, have the ability to inhibit the growth of these pathogens and may be a viable alternative to antibiotic use.

*Salmonella enterica* ser. Heidelberg, Cerro and Uganda were provided by the Wisconsin Veterinary Diagnostic Laboratory (WVDL).<sup>18</sup>

**PB6 efficacy against disease-causing pathogens**



**Figure 6:** Effect of *B. subtilis* PB6 (PB6) metabolites on the growth of *Salmonella* ser. Heidelberg (SH), Cerro (SC) and Uganda (SU) in a kinetic read assay (CFS = cell free supernatant)<sup>18</sup>

**THE BOTTOM LINE:** *B. subtilis* PB6 completely inhibited the growth of multiple strains of *Salmonella* through 14 hours of incubation.

This material is based upon work supported by the U.S. Department of Agriculture, Bioscience Research Lab, Agricultural Research Service, Lubbock, TX, under agreement No. KANAG344-16. Any opinions, findings, conclusion or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

# RESEARCH AND RESULTS

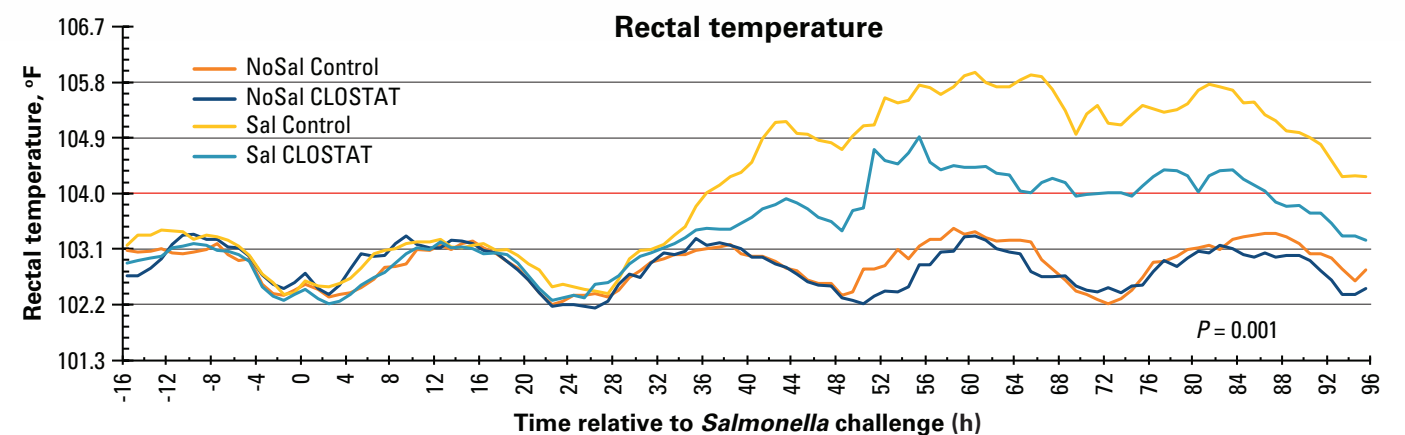
## REDUCING THE NEGATIVE IMPACT OF PATHOGENIC *SALMONELLA TYPHIMURIUM* IN WEANED HOLSTEIN STEERS

USDA-ARS, Livestock Issues Research Unit in Lubbock, Texas

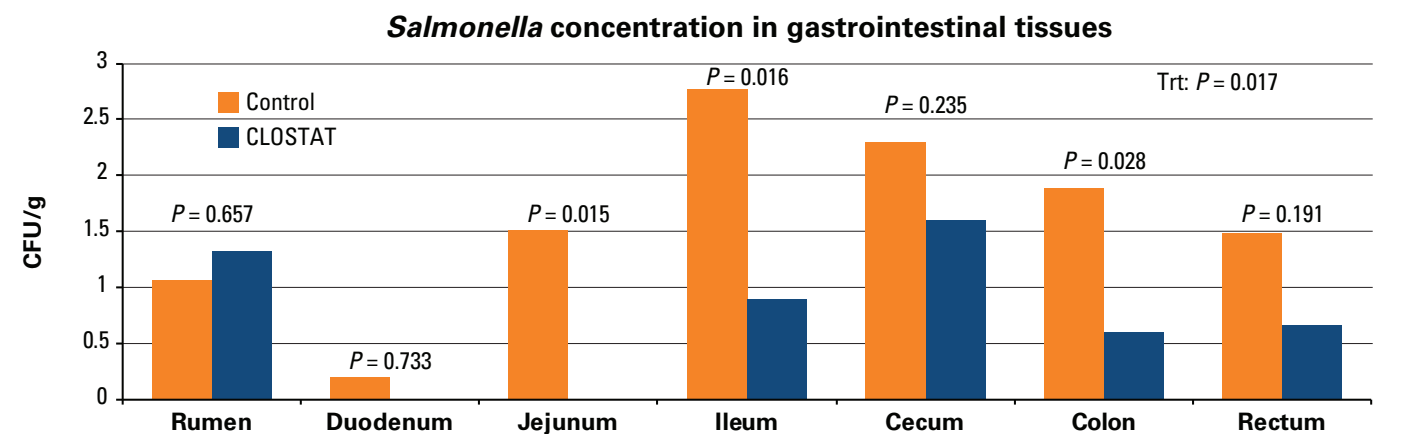
**Study objective:** Research evaluated the potential for CLOSTAT to reduce the severity of salmonellosis in weaned Holstein steers challenged with *S. typhimurium*.<sup>9</sup> Calves were fed either control diets (no CLOSTAT) or 13 g/hd/d of CLOSTAT in a starter ration for 35 days. Calves were then assigned to one of four treatments, consisting of CLOSTAT or no CLOSTAT and *Salmonella* ( $1.6 \times 10^6$  *S. typhimurium*) or no *Salmonella*.

**Results:** The CLOSTAT-fed calves displayed decreased rectal temperatures ( $P < 0.001$ ) after the study, compared to the control calves challenged with *Salmonella* (Figure 7).

Mounting an immune response to a pathogen challenge requires a significant amount of energy. It has been estimated that an increase in core body temperature by 1.8° F (1° C) requires an increase of 10-13% in an animal's metabolic rate.<sup>19</sup> Mediating this change in body temperature would potentially spare glucose, allowing energy to be put towards other productive functions.



**Figure 7:** Effect of CLOSTAT on rectal temperatures of weaned Holstein steers challenged with *S. typhimurium*



**Figure 8:** *Salmonella* concentrations in tissues from calves receiving CLOSTAT or not (Control) 48 hours after *Salmonella* inoculation ( $1.6 \times 10^6$  CFU). SEM = 0.09.

# RESEARCH AND RESULTS

## SUMMARY OF EFFECTS OF *B. SUBTILIS* PB6 AND/OR CHROMIUM PROPIONATE SUPPLEMENTATION ON HEALTH AND PERFORMANCE OF HIGH-RISK BEEF CATTLE DURING THE RECEIVING PERIOD<sup>20</sup>

West Texas A&M University

**Study objective:** Determine the effect of supplementation with CLOSTAT (*B. subtilis* PB6) and/or KemTRACE® Chromium (chromium propionate) on feedlot growth performance, clinical health and subsequent carcass traits of high-risk cattle with the hypothesis that *B. subtilis* PB6 and chromium propionate will improve feedlot health and performance, and the combination of these will result in additive improvement.<sup>20</sup>

**Results:** Study data validated the health and performance benefits for CLOSTAT during the receiving period (Table 1). Supplementation of both CLOSTAT and KemTRACE Chromium reduced the incidence of bovine respiratory disease (BRD) morbidity. These data suggest feeding CLOSTAT during the feedlot receiving period may be a promising alternative to antimicrobials for improvement of health and performance outcomes in high-risk cattle.

**Table 1:** Effect of *B. subtilis* PB6 and/or chromium supplementation on health outcomes of beef cattle during the feedlot receiving period

Item	Treatment <sup>1</sup>				SEM <sup>5</sup>	P-value		
	Control	CLOSTAT®	KemTRACE® Chromium	CLOSTAT® + KemTRACE® Chromium		CLOSTAT®	KemTRACE® Chromium	CLOSTAT® x KemTRACE® Chromium
BRD1 <sup>2</sup> , %	43.9	34.6		21.3	-	0.02	0.03	0.59
BRD2 <sup>3</sup> , %	23.4	11.9	16.3	12.4	-	0.10	0.47	0.40
BRD3 <sup>4</sup> , %	12.7	5.4	8.8	6.7	-	0.19	0.71	0.46
Respiratory mortality, %	4.2	1.1	3.3	2.2	-	0.34	0.95	0.65
Days to								
1 <sup>st</sup> treatment	13.4	14.3	9.2	11	2.2	0.44	0.04	0.81
2 <sup>nd</sup> treatment	21.1	17.4	21.6	17.5	3.2	0.13	0.91	0.93
Rectal temperature, °F								
1st treatment	103.2	103.5	103.5	104.0	0.37	0.16	0.23	0.83
2 <sup>nd</sup> treatment	103.3	102.7	103.0	104.0	0.56	0.68	0.28	0.09
Antimicrobial cost, \$/hd <sup>6</sup>	12.45	8.50	9.62	6.15	1.66	0.03	0.13	0.87

1. Placebo control; CLOSTAT = 13 g/hd/d DM inclusion of *B. subtilis* PB6; KemTRACE Chromium = 450 ppb DM chromium; CLOSTAT + KemTRACE Chromium = 13 g/hd/d DM inclusion of *B. subtilis* PB6 + 450 ppb DM chromium  
 2. Percentage of steers treated for BRD at least once  
 3. Percentage of steers treated for BRD at least twice  
 4. Percentage of steers treated for BRD three times  
 5. Pooled standard error of the mean  
 6. Antimicrobial cost assumes the following: \$0.59/mL for florfenicol (Nuflor®, Merck Animal Health), \$0.47/mL for enrofloxacin (Baytril®, Bayer Animal Health), \$2.00/mL for ceftiofur crystalline free acid (Exceld®; Zoetis)

**THE BOTTOM LINE:** Supplementation of CLOSTAT decreased bovine respiratory disease (BRD) incidence by 25% alone and 50% in combination with KemTRACE Chromium.

## EFFECT OF SUPPLEMENTING DAIRY COWS WITH *B. SUBTILIS* PB6 AND/OR CHROMIUM PROPIONATE ON HEALTH, CULLING, REPRODUCTIVE PERFORMANCE AND METABOLIC PARAMETERS<sup>21</sup>

University of California, Davis

**Study objective:** Evaluate the effect of supplementing dairy cows with CLOSTAT 500 (*B. subtilis* PB6) and/or KemTRACE Chromium 0.4% (chromium propionate) on health, culling, reproductive performance, metabolic parameters, milk yield and components. A total of 680 Holstein cows from a California Central Valley Dairy were individually orally dosed with a syringe once a day from prepartum to 110 days in milk (DIM) with **a)** CLOSTAT 500 0.5 g, **b)** KemTRACE Chromium 2.0 g, **c)** Both CLOSTAT 0.5 g and KemTRACE Chromium 2.0 g, CLOSTAT + KemTRACE Chromium or **d)** placebo.

**Results:** One of the important findings from this study was a 50% reduction in the rate of culling cows assigned CLOSTAT versus placebo (Table 2). Treatments influenced meaningful metabolic parameters that have major long-term implications of lactation success and will help in understanding the interactions of gut health and transition success.

**Table 2:** Culling rate, estimates and 95% confidence interval (CI) by treatment (n = 680)

Outcome	Treatment				Covariate	Trt Effect P-value
	Control	CLOSTAT® 500	KemTRACE® Chromium	CLOSTAT® 500 + KemTRACE® Chromium		
Culling rate, %	18.3 <sup>b</sup> (13.3, 24.7)	9.2 <sup>a</sup> (5.7, 14.6)	14.0 <sup>ab</sup> (9.4, 20.4)	11.2 <sup>ab</sup> (7.2, 16.9)	-	0.072
Sold, %	84.8	81.3	94.7	77.3	-	-
Died, %	15.2	18.7	5.3	22.7	-	-

<sup>a,b</sup> Differing superscripts indicate significant difference  $P < 0.05$

**Table 3:** Effect of CLOSTAT 500 and/or KemTRACE Chromium on milk yield and composition of lactating Holstein cows

Outcome	Treatment <sup>1</sup>				Covariate	P-value		
	Control	CLOSTAT® 500	KemTRACE® Chromium	CLOSTAT® 500 + KemTRACE® Chromium		Trt	Time	Trt x time
n	180	173	170	157	--	--	--	--
Milk yield, lb	104.3	105.2	104.3	106.9	Parity	0.461	<0.0001	0.599
Energy corrected milk, lb	104.2	105.3	105.2	106.8	Parity	0.552	<0.0001	0.879
Fat, %	3.65	3.65	3.71	3.65	--	0.494	<0.0001	0.568
Protein, %	2.81	2.82	2.83	2.82	Parity	0.726	<0.0001	0.880
LSCC <sup>2</sup>	2.22	2.03	2.28	2.44	Parity	0.114	0.017	0.697

1. Control = no supplemental CLOSTAT 500 or KemTRACE Chromium, CLOSTAT = supplemented 0.5 g/hd/d (3.3\*10<sup>9</sup>) CLOSTAT 500, Chromium = supplemented 500ppb lb/DM KemTRACE Chromium, CLOSTAT 500 + KemTRACE Chromium = supplemented 0.5 g/hd/d CLOSTAT 500 and 500ppb lb/DM KemTRACE Chromium  
 2. LSCC = low somatic cell count

**THE BOTTOM LINE:** Feeding CLOSTAT reduced the cow culling rate by 50%.

# RESEARCH AND RESULTS

## THE EFFECTS OF CLOSTAT (*B. SUBTILIS* PB6) SUPPLEMENTATION ON PRODUCTION PARAMETERS IN TRANSITION DAIRY COWS<sup>22</sup>

Iowa State University

**Study objective:** Evaluate the effects of CLOSTAT (*B. subtilis* PB6) supplementation on the inflammation, fecal metrics, energetic metabolism and production parameters in transition dairy cows. A total of 48 multiparous Holstein cows were stratified by previous 305 mature equivalent milk (ME) and parity and assigned to one of two top-dressed dietary treatments. Twenty-one (21) days before expected calving each group of cows were either fed a control diet (no CLOSTAT) or a diet including 13 g/hd/day of CLOSTAT. Cows remained on their respective postpartum dietary treatment for the remainder of the study (i.e., 63 DIM).

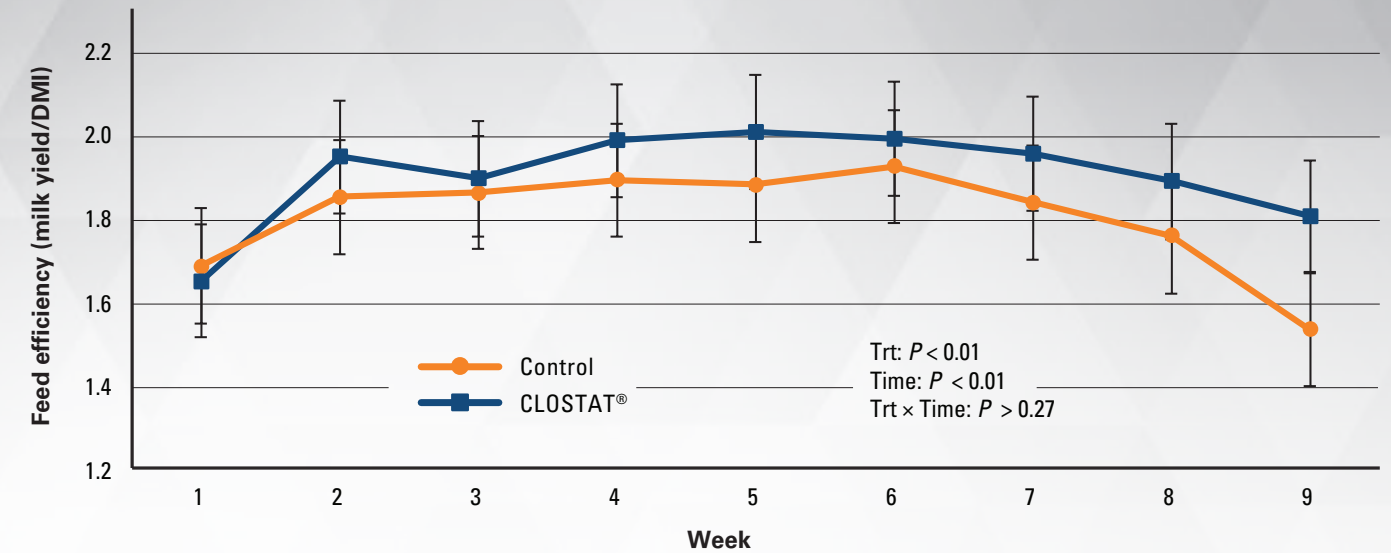
**Results:** Supplementing *B. subtilis* PB6 had the following positive effects on transition dairy cows:

- Increased milk yield even with decreased postpartum dry matter intake (DMI), resulting in improved feed efficiency
- Decreased milk lactose and milk urea nitrogen (MUN) concentrations
- Increased milk protein and lactose yield
- Decreased circulating postpartum  $\beta$ -hydroxybutyrate (BHB)
- Decreased lipopolysaccharide-binding protein (LBP) concentrations on day three
- Increased fecal pH
- Decreased postpartum propionic acid by 24%

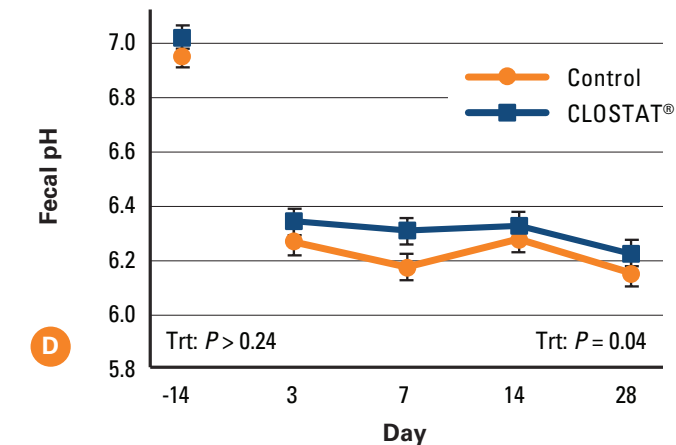
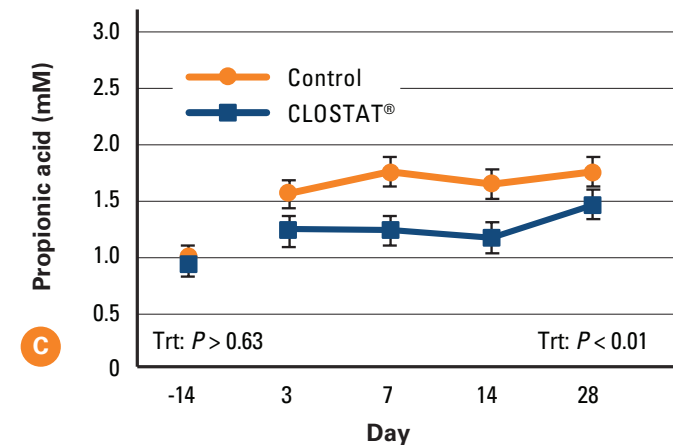
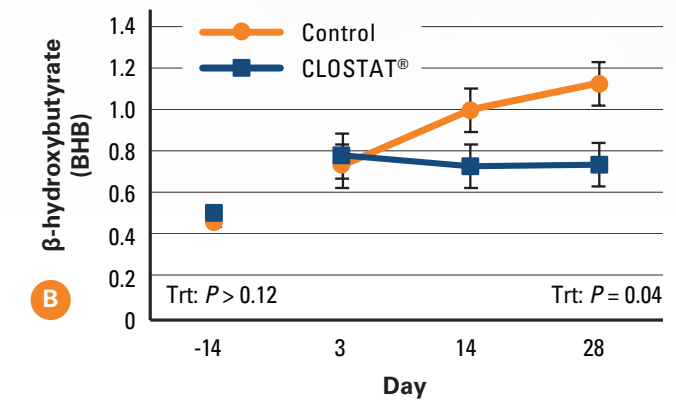
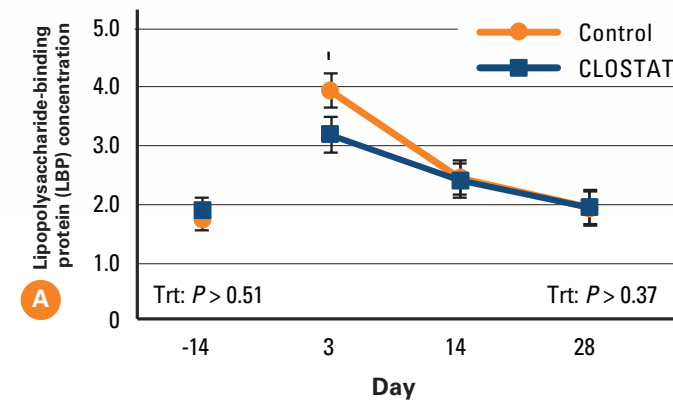
**Table 4:** Effect of CLOSTAT (*B. subtilis* PB6) on production parameters and milk composition in transition dairy cows

Parameter	Treatment <sup>1</sup>			P-value		
	Control	CLOSTAT <sup>®</sup>	SEM	Trt	Time	Trt x Time
DMI, lb/d	58.43	57.00	0.55	0.05	<0.01	0.62
Milk yield, lb/d	103.70	107.27	0.68	<0.01	<0.01	0.10
ECM <sup>2</sup> , lb/d	122.08	126.11	0.97	<0.01	<0.01	0.50
Milk yield/DMI	1.77	1.88	0.02	<0.01	<0.01	0.27
ECM/DMI	2.09	2.21	0.03	<0.01	<0.01	0.31
BCS <sup>3</sup> prepartum	3.68	3.68	0.09	0.99	0.16	0.34
BCS postpartum	3.39	3.41	0.08	0.86	<0.01	0.99
<b>Milk composition</b>						
Fat, lb	5.03	5.16	0.06	0.14	<0.01	0.75
Protein, lb	3.20	3.37	0.04	<0.01	<0.01	0.20
Lactose, lb	4.94	5.07	0.02	0.01	<0.01	0.11
MUN, mg/dL	10.86	10.36	0.15	0.01	<0.01	0.51

1. 13 g/hd/d calcium carbonate (n=24); CLOSTAT = 13 g/hd/d CLOSTAT (*B. subtilis* PB6; n=24)  
 2. Energy corrected milk  
 3. Body condition score



**Figure 9:** Effect of CLOSTAT (*B. subtilis* PB6) on postpartum feed efficiency (FE = MY/DMI)



**Figures 10A-10D:** Effect of CLOSTAT (*B. subtilis* PB6) or not (Control) on Serum LBP (A), Serum BHB (B), fecal propionic acid (C) and fecal pH (D) in transition dairy cows

**THE BOTTOM LINE:** Supplementing CLOSTAT significantly improved feed efficiency (milk yield/DMI + ECM/DMI) and reduced  $\beta$ -hydroxybutyrate (BHB).

# RESEARCH AND RESULTS

## THE EFFECT OF SUPPLEMENTING CLOSTAT 500 (*B. SUBTILIS* PB6) ON YEARLING STEERS IN A COMMERCIAL FEEDYARD ON HEALTH, *SALMONELLA* SPP. PREVALENCE, FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS<sup>23</sup>

Commercial feedyard research

**Study objective:** Evaluate the effects of CLOSTAT 500 (*B. subtilis* PB6) supplementation on health, prevalence of *Salmonella* spp., growth, performance and carcass characteristics of yearling steers in a commercial feedyard. A total of 2,100 beef steers, weighing 689.5 lb (± 83.69 lb), were blocked by arrival date and assigned randomly to pen within block. The pens were randomly assigned to one of two dietary treatments within a block (15 pen/trt; 70 hd/pen).

Half of the pens received control diets (no CLOSTAT), while the other half received a diet that was supplemented with 0.5 g/hd/d of CLOSTAT 500 (*B. subtilis* PB6).

**Results:** This research study showed supplementing CLOSTAT reduced the overall incidence of morbidity and decreased the percentage of cattle treated at least once for bovine respiratory disease (BRD). Overall, CLOSTAT showed a tendency to reduce fecal *Salmonella* counts and had a 46% reduction in the overall mean prevalence of lymph node *Salmonella* (15.48% vs. 28.66%). With dead and removed steers included, CLOSTAT improved final weight, average daily gain and feed efficiency.

**Table 5:** Effect of CLOSTAT (*B. subtilis* PB6) on animal accountability, morbidity and mortality of steers

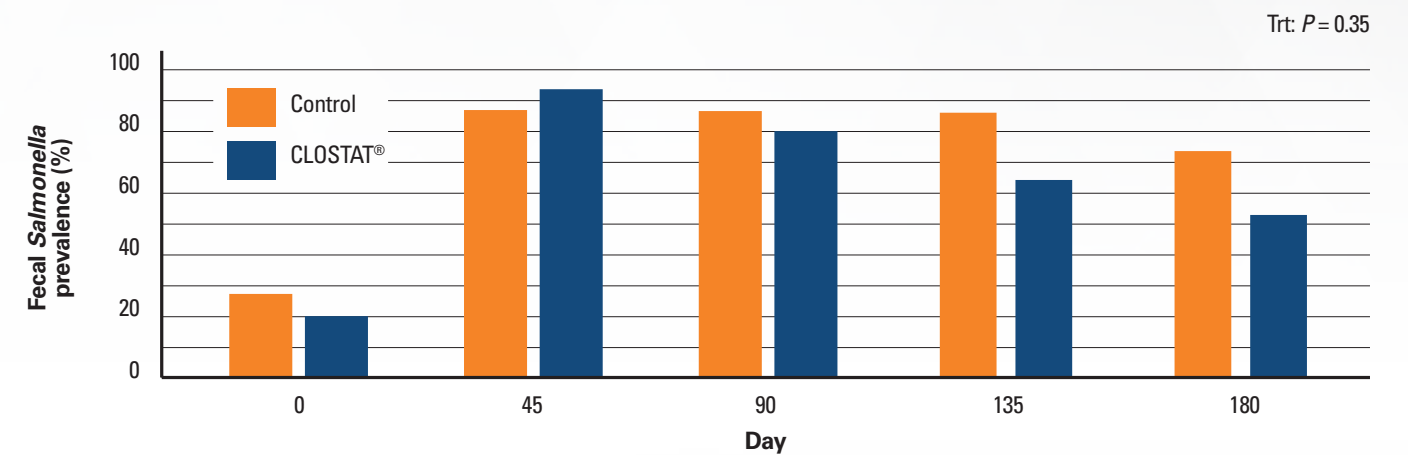
Item	Control <sup>1</sup>	CLOSTAT <sup>®1</sup>	P-value
Day 0 steers, n	1,050	1,050	--
<b>Morbidity</b>			
Total first pull, n (%)	141 (13.43)	109 (10.38)	0.03
<b>Respiratory morbidity</b>			
1st treat, n (%)	134 (12.76)	96 (9.14)	<0.01
2nd treat, n (%)	35 (3.33)	22 (2.10)	0.03
<b>Mortality, n (%)</b>	33 (3.14)	24 (2.29)	0.23
<b>Removed, n (%)</b>	40 (3.18)	29 (2.76)	0.18
<b>Total outs, n (%)</b>	73 (6.95)	53 (5.05)	0.06
<b>Shipped, n</b>	977	997	--

**Footnotes: Table 5, 6**

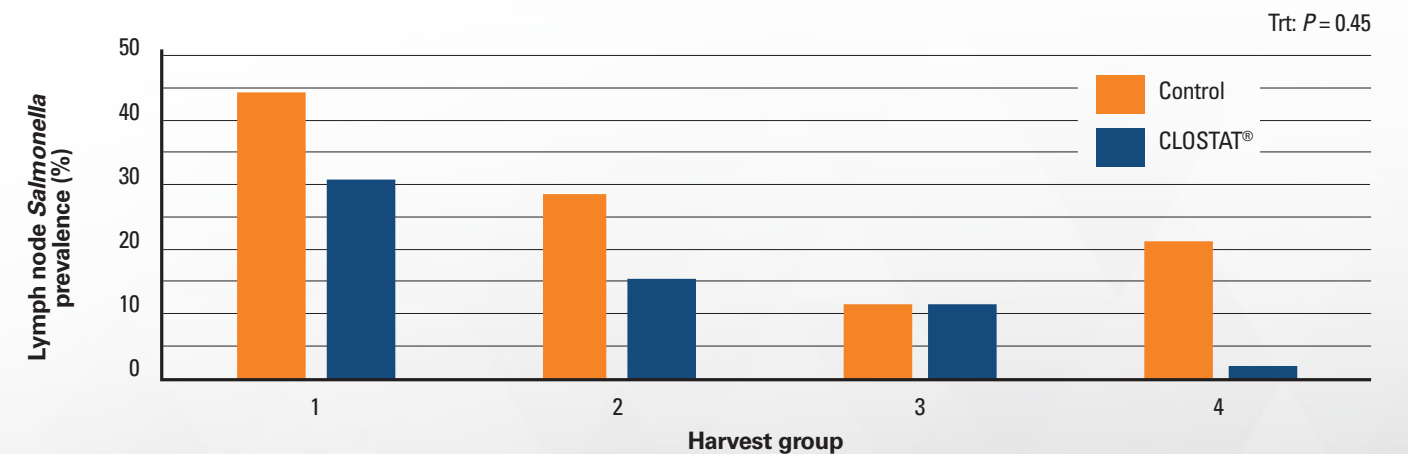
- Control = standard finishing diet; CLOSTAT = fed control diet supplemented with 0.5 g/hd/d CLOSTAT 500 (*B. subtilis* PB6), administered through the micro-ingredient machine.
- Number of carcasses analyzed after reconciliation between carcass data collection service and packing plant data. Carcasses from which lot number could not be confirmed, USDA condemned, or carcasses reported as "could not be tracked through harvest floor", were not analyzed.
- Hot carcass weight gain with dead and removals included (DRI), calculated from the assumption of 58% initial dressing percentage.

**Table 6:** Live growth performance of steers fed 0.5 g/hd/d of CLOSTAT 500 (*B. subtilis* PB6) compared to those that were not.

Item	Control <sup>1</sup>	CLOSTAT <sup>®1</sup>	SEM	P-value
Day 0 body weight, lb	679	680	1.82	0.71
DMI, lb	22.16	22.36	0.092	0.14
Final body weight, lb	1,412	1,442	9.86	0.052
Total gain, lb	733	762	9.25	0.046
Days on feed	196	198	0.975	0.09
Average daily gain (ADG), lb/d	3.74	3.84	0.037	0.08
DMI:ADG	6.01	5.83	0.061	0.06
<b>Carcass</b>				
Carcasses <sup>2</sup> , n	972	997	0.56	0.05
Hot carcass weight, lb	957	960	0.93	0.37
Hot carcass weight gain <sup>3</sup> , DRI, lb	475	500	3.28	0.03



**Figure 11:** Fecal *Salmonella* prevalence of feedlot cattle sampled throughout the feeding period supplemented with 0.5 g/hd per day. CLOSTAT 500 (*B. subtilis* PB6), CLOSTAT or not (Control). Treatment:  $P = 0.35$ , Day:  $P < 0.01$ , Treatment x Day:  $P = 0.76$ ; SEM = 5.4.



**Figure 12:** Lymph node *Salmonella* prevalence from feedlot cattle sampled across four harvest dates that were supplemented with 0.5 g/hd/d CLOSTAT 500 (*B. subtilis* PB6), CLOSTAT or not (Control).

**THE BOTTOM LINE:** Supplementation of CLOSTAT significantly reduced overall morbidities and bovine respiratory disease (BRD) morbidities and showed a tendency to reduce total outs. Supplementation also reduced the prevalence of *Salmonella* in the lymph nodes by 46%.

# CLOSTAT<sup>®</sup> FEATURES AND BENEFITS

CLOSTAT contains a proprietary, patented strain of *B. subtilis* PB6. Kemin selected *B. subtilis* PB6 — a unique, naturally occurring and spore-forming probiotic — because it helps maintain the balance of microflora in the GI tract in an array of animals, including dairy and beef animals. The *B. subtilis* PB6 in CLOSTAT has been shown to have multiple modes of action, including secreting surfactins, reducing gut inflammation and disrupting pathogen quorum sensing abilities.

## CLOSTAT 500

Delivers  $6.6 \times 10^8$  CFU/g

Application rate for dairy/beef/calves:  
0.5 g/hd/d

## CLOSTAT DRY

Delivers  $2.2 \times 10^8$  CFU/g

Application rate for dairy/beef:  
13 g/hd/d

## THE CLOSTAT SUITE OF PRODUCTS<sup>24,25</sup>

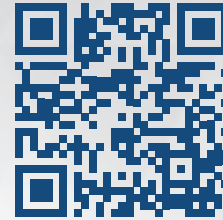
- ✓ Confirmed product stability in feed:
  - Mineral premix concentrate (oxides, sulfates) for three months
  - Long-term temperatures up to 149° F (65° C) for one year
- ✓ Stable in pelleting process at 194° F (90° C) for 10 minutes
- ✓ Stable in lick tub and block production at 180° F (82° C) for 16 hours
- ✓ Resistant to acid conditions of pH 2.0 for 90 minutes
- ✓ Compatible with antibiotics and organic acids

To optimize animal health  
and performance, you must  
**OPTIMIZE  
INTESTINAL HEALTH.**

# KEMIN IS THE SCIENCE BEHIND HEALTHIER CATTLE.

Since 1961, Kemin has used advanced molecular science to develop industry-leading solutions for optimal gut health, ruminant nutrition and feed quality. It's just one of the ways we sustainably transform the quality of life every day for 80% of the world with our 500+ ingredients, which are all backed by technical, lab and product support teams. This ensures you have a partnership where you get the most out of every product.

See more of #TheScienceBehind CLOSTAT. Scan to watch video.



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For more information about CLOSTAT, give us a call or visit us at [kemin.com/clostat-cattle](https://kemin.com/clostat-cattle).

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