CLOSTAT®

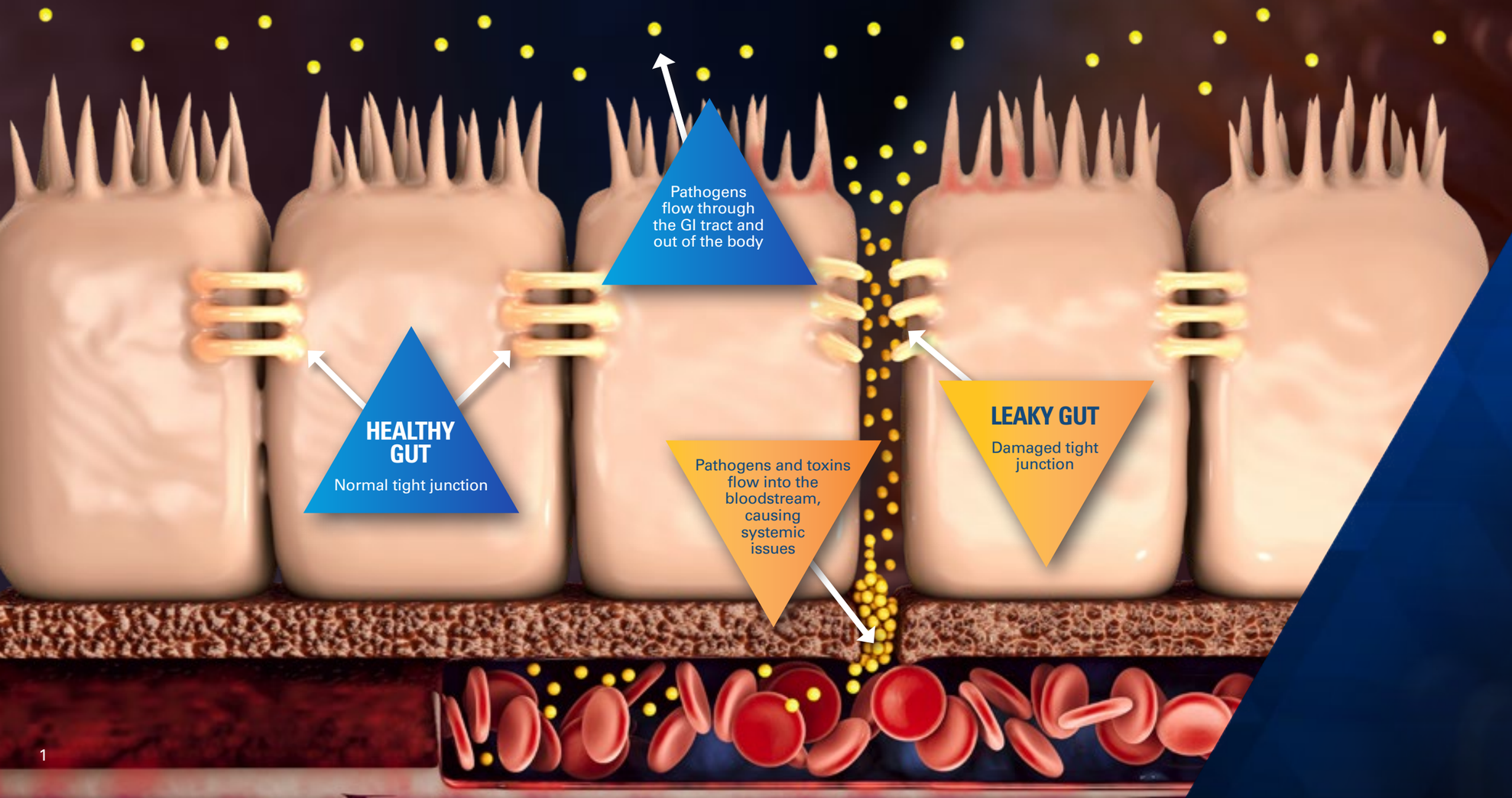
BACILLUS SUBTILIS PB6 ACTIVE MICROBIAL

kemin.com/clostat-cattle

KEMIN 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

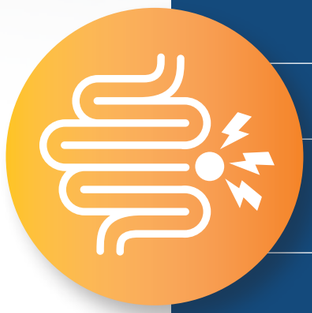
Decreased intestinal integrity of the gut, allowing harmful pathogens and toxins to enter into the host, which can lead to both intestinal and systemic inflammation

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.[1] 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.**[2]

The intestinal barrier is regularly exposed to up to ***10 trillion microorganisms.***[3]



EVERYDAY STRESSORS

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

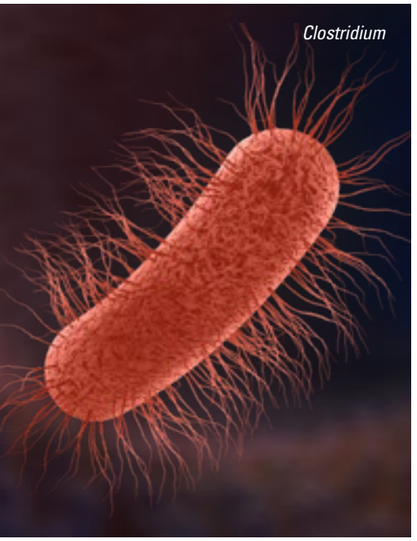


Figure 2: *Clostridium*, *Salmonella* and *E. coli*

SELECTION CRITERIA FOR MICROBIALS

Not all active microbials 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.[4,5] 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.[4,5]

NOT ALL *B. SUBTILIS* ARE CREATED EQUAL

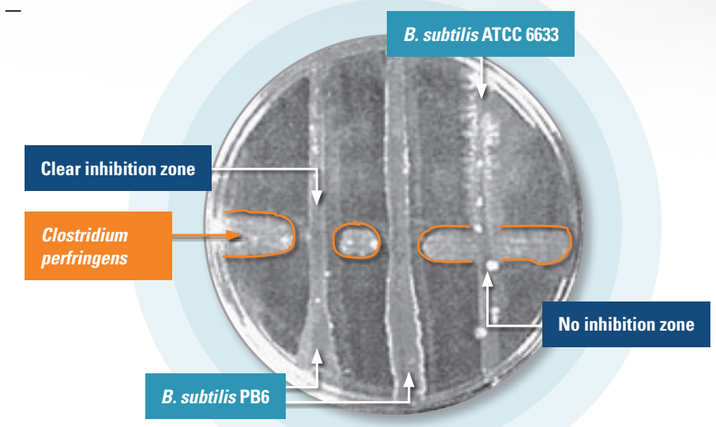


Figure 3: *In vitro* comparison of CLOSTAT (*B. subtilis* PB6) and *B. subtilis* ATCC 6633[4]

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_2.

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.

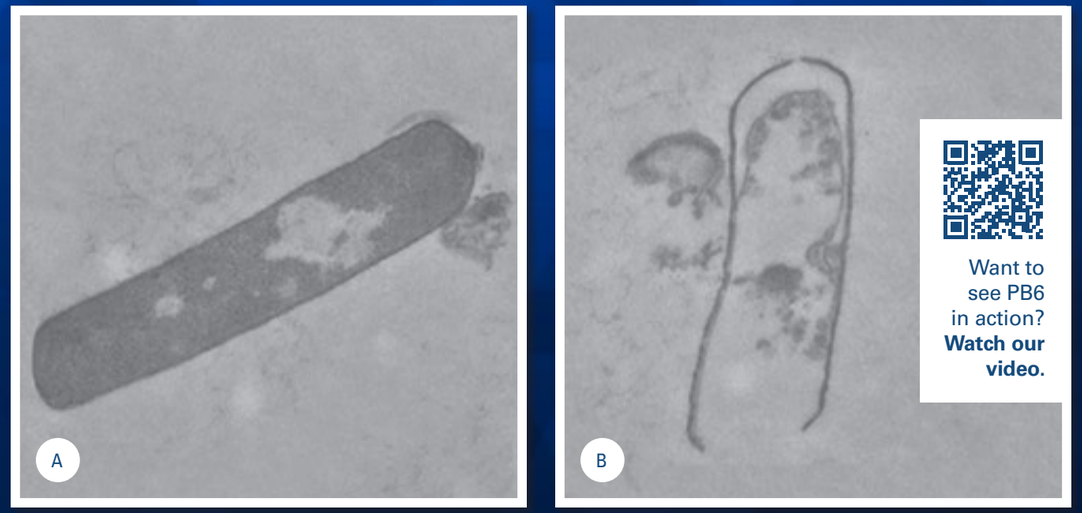
Dive deeper into the CLOSTAT modes of action on pages 6-8

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.[4,5] 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).[6] These proteins disrupt the bacteria membrane, causing leakage of the cell contents and ultimately killing the pathogenic bacteria without harming the beneficial gut microflora.



Want to see PB6 in action? **Watch our video.**

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)[6]

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.[7] This inflammatory process and immune system activation consumes significant amounts of nutrients and pulls nutrients and energy away from other key performance functions.[7]

B. subtilis PB6 has been found to secrete cyclic lipopeptide surfactins through normal metabolism, which inhibit phospholipase A_2 (PLA_2), a rate-limiting enzyme involved in the arachidonic acid (AA)-associated inflammatory pathway.[8] Inhibition of synthesis of proinflammatory cytokines and elevation in the level of anti-inflammatory cytokines re-establish cytokine balance.[7,9]

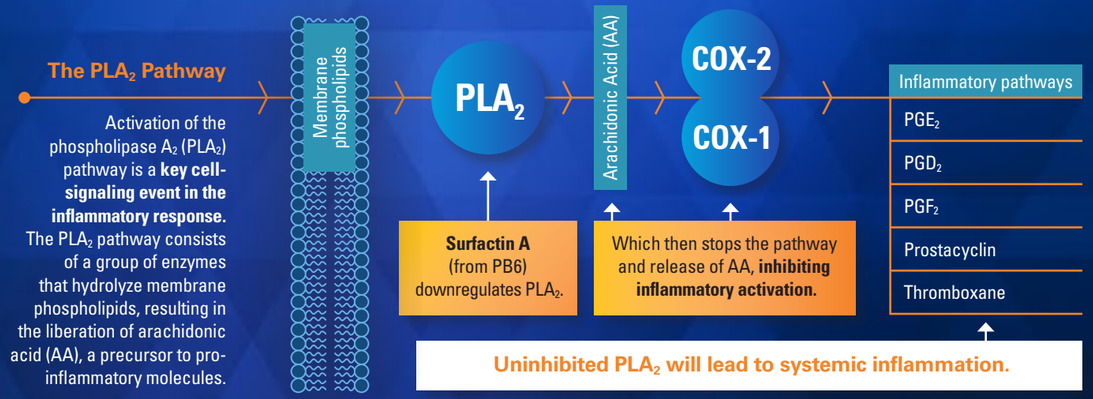


Figure 5: PLA_2 pathway inhibition

MODE OF ACTION 3: QUORUM QUENCHING

What is quorum sensing (QS)?[10-13]

- 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)?[14]

- 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.**[10-13]

***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.[6]

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).[15-17] 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).[18]

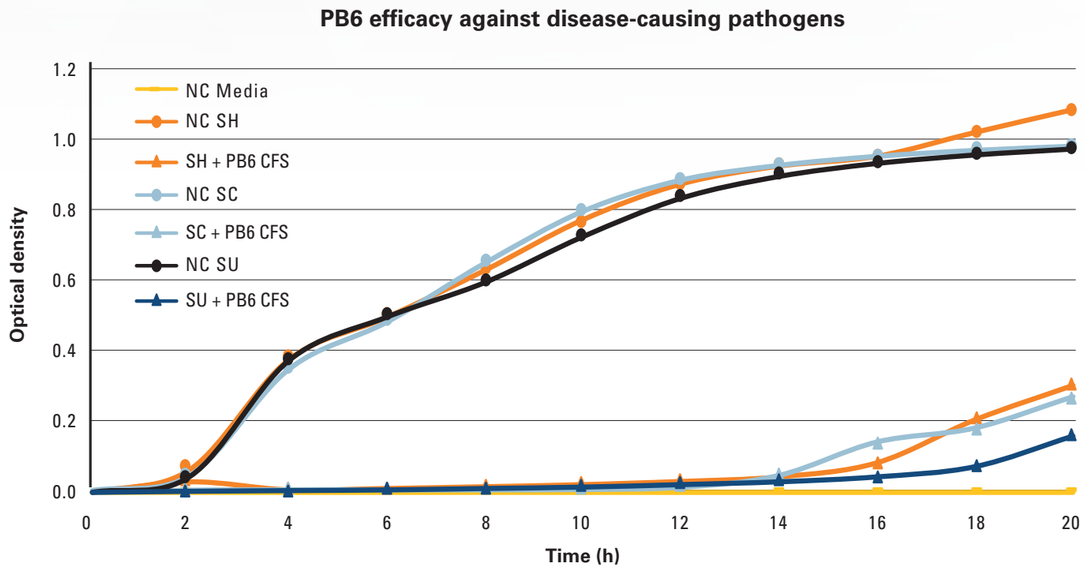


Figure 6: Effect of *B. subtilis* PB6 (PB6) metabolites on the growth of *Salmonella* ser. Heidelberg (SH), Cerro (SC) and Uganda (SU) in a kinetic read assay (CFS = cell free supernatant)[18]

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. KANA6344-16. 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*.[9] 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 x 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.[19] Mediating this change in body temperature would potentially spare glucose, allowing energy to be put towards other productive functions.

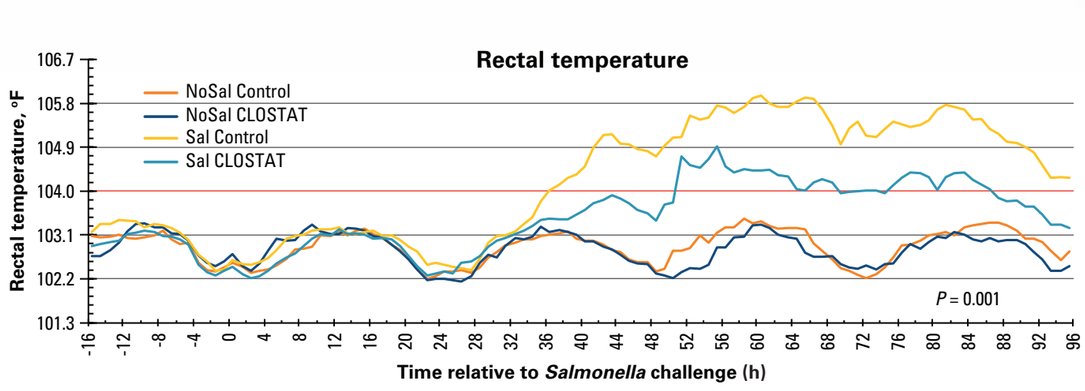


Figure 7: Effect of CLOSTAT on rectal temperatures of weaned Holstein steers challenged with *S. typhimurium*

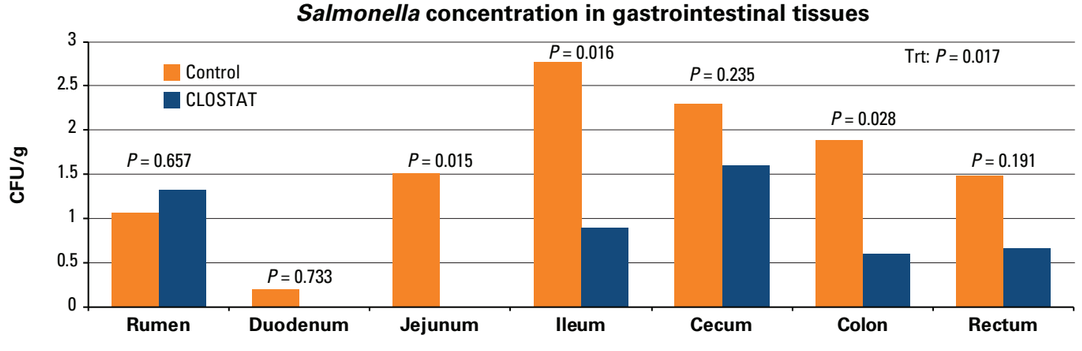


Figure 8: *Salmonella* concentrations in tissues from calves receiving CLOSTAT or not (Control) 48 hours after *Salmonella* inoculation (1.6 x 10^6 CFU). SEM = 0.09.

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[20]

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.[20]

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

	Treatment[1]					*P*-value		
Item	Control	CLOSTAT®	KemTRACE® Chromium	CLOSTAT® + KemTRACE® Chromium	SEM[5]	CLOSTAT®	KemTRACE® Chromium	CLOSTAT® x KemTRACE® Chromium
BRD1[2], %	43.9	34.6		21.3	-	0.02	0.03	0.59
BRD2[3], %	23.4	11.9	16.3	12.4	-	0.10	0.47	0.40
BRD3[4], %	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								
1st treatment	13.4	14.3	9.2	11	2.2	0.44	0.04	0.81
2nd 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
2nd treatment	103.3	102.7	103.0	104.0	0.56	0.68	0.28	0.09
Antimicrobial cost, $/hd[6]	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 (Excede®, 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[21]

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)

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

[a-b] 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

	Treatment[1]					*P*-value		
Outcome	Control	CLOSTAT® 500	KemTRACE® Chromium	CLOSTAT® 500 + KemTRACE® Chromium	Covariate	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[2]	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*10^9) 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[22]

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 β-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

	Treatment[1]			*P*-value		
Parameter	Control	CLOSTAT®	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[2], 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[3] 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
Milk composition						
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

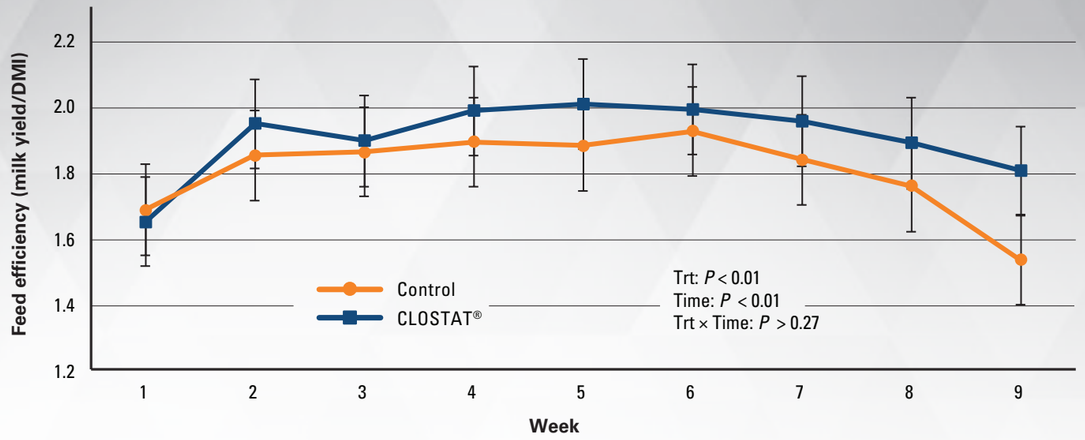
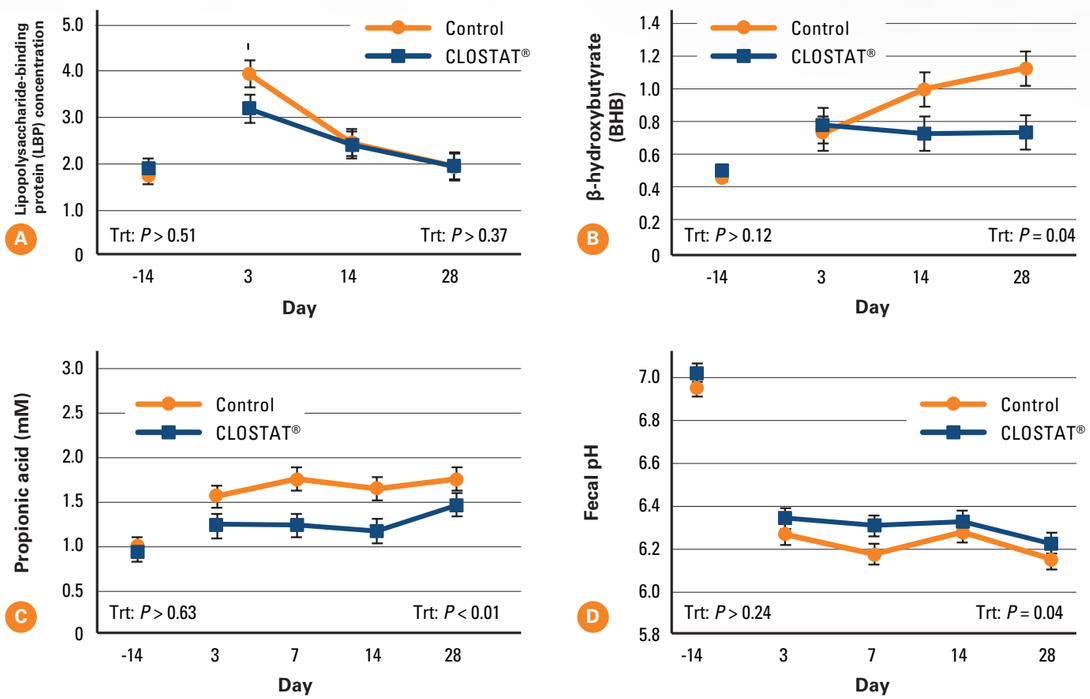


Figure 9: Effect of CLOSTAT (*B. subtilis* PB6) on postpartum feed efficiency (FE = MY/DMI)



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 β-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[23]

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

Footnotes: Table 5, 6

1. 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.
2. 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.
3. 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[1]	CLOSTAT®[1]	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
Carcass				
Carcasses[2], n	972	997	0.56	0.05
Hot carcass weight, lb	957	960	0.93	0.37
Hot carcass weight gain[3], DRI, lb	475	500	3.28	0.03

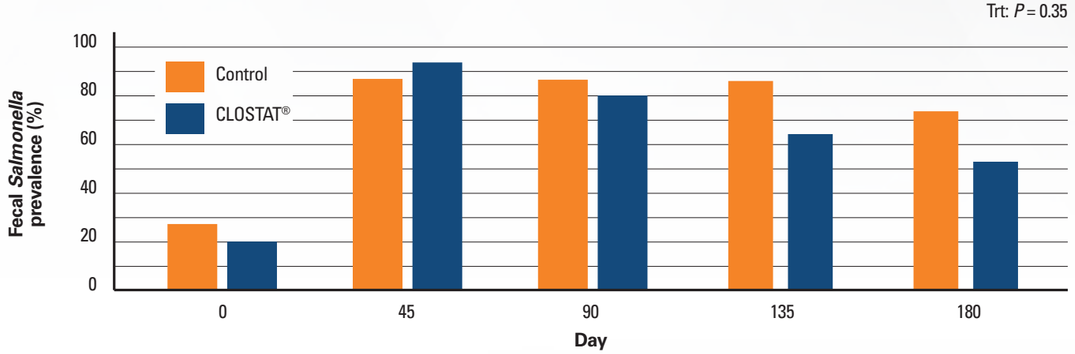


Figure 11: Fecal *Salmonella* prevalence of feedlot cattle sampled throughout the feeding period supplemented with 0.5 g/hd per day. CLOSTAT 500 (*B. subtilis* PB6), CLOSTAT or not (Control). Treatment: *P* = 0.35, Day: *P* < 0.01, Treatment x Day: *P* = 0.76; SEM = 5.4.

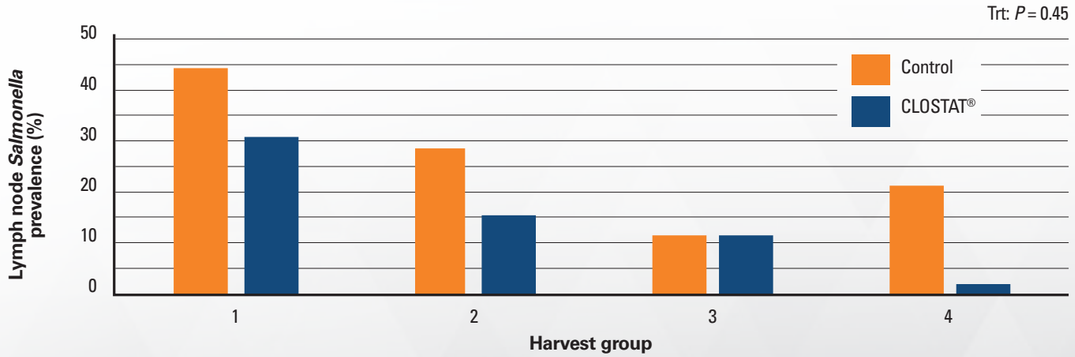


Figure 12: Lymph node *Salmonella* prevalence from feedlot cattle sampled across four harvest dates that were supplemented with 0.5 g/hd/d CLOSTAT 500 (*B. subtilis* PB6), CLOSTAT or not (Control).

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®
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 x 10^8 CFU/g

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

CLOSTAT DRY

Delivers 2.2 x 10^8 CFU/g

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

THE CLOSTAT SUITE OF PRODUCTS[24,25]

- ✔ 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.

kemin.com/cattle | **#TheScienceBehind**

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

KEMIN® INTESTINAL HEALTH | CLOSTAT®

For more information about CLOSTAT, give us a call or visit us at kemin.com/clostat-cattle.

1-800-752-2864

REFERENCES

1. Williams, J. M., et al. (2015, May). Epithelial cell shedding and barrier function: A matter of life and death at the small intestinal villus tip. Veterinary Pathology. 52(3):445-455.
2. Chakaroun, R. M., L. Massier and P. Kovacs. (2020, April). Gut microbiome, intestinal permeability, and tissue bacteria in metabolic disease: Perpetrators or bystanders? Nutrients. 12(4):1,082.
3. Vighi, G., Marcucci, F., Sensi, L., Cara, G. D., & Frati, F. (2008). Allergy and the gastrointestinal system. Clinical & Experimental Immunology, 153, 3-6. doi:10.1111/j.1365-2249.2008.03713.x
4. 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):4,185-4,190.
5. Lin, A. S .H., et al. (2007). Antimicrobial compounds from *Bacillus subtilis* for use against animal and human pathogens. United States Patent 7,247,299.
6. *Bacillus subtilis* strain PB6 — other antimicrobial secondary metabolites next to surfactins. WP-08-00007.
7. Horst, E. A., et al. (2019, Oct.). Causes and metabolic consequences of leaky gut. (conference paper). 2019 Cornell Nutrition Conference.
8. Selvam, R., et al. (2009). Effect of *Bacillus subtilis* PB6, a natural probiotic on colon mucosal inflammation and plasma cytokines levels in inflammatory bowel disease. Indian Journal of Biochemistry Biophysics. 46(1):79-85.
9. Broadway, P. R., et al. (2020, Aug.). *Bacillus subtilis* PB6 supplementation in weaned Holstein steers during an experimental *Salmonella* challenge. Foodborne Pathogens and Disease. 17(8):521-528.
10. LaSarre, B. and M. J. Federle. (2013). Exploiting quorum sensing to confuse bacterial pathogens. Microbiology and Molecular Biology Reviews. 77(1):73-111
11. Yu, Q. et al. (2017).The Agr-like quorum sensing system is required for pathogenesis of necrotic enteritis caused by *Clostridium perfringens* in poultry. Infection and Immunity Journal. 85(6):e00975-16.
12. Vidal, J. E., et al. (2012). Evidence that the Agr-like quorum sensing system regulates the toxin production, cytotoxicity and pathogenicity of *Clostridium perfringens* type C isolate CN3685. Molecular Microbiology. 83:179-194.
13. Ohtani, K., et al. (2009). Virulence gene regulation by the Agr system in *Clostridium perfringens*. Journal of Bacteriology. 191(12):3,919-3,927.
14. Tan, G., T. J. Koh and H. M. Tan. (2021). Quorum quenching by *Bacillus subtilis* PB6 — Abolishing clostridial infections and disease progression in livestock through the disruption of cell-to-cell communication thereby improving broiler performance. efeedlink.com/ebook/1b1cc4e8-a892-488f-b488-8e9e2e432257.html
15. Patchanee, P., et al. (2008). Characterization of multidrug-resistant *Salmonella enterica* serovar Heidelberg isolated from humans and animals. Foodborne Pathogens and Disease. 5(6):839-851.
16. Gieraltowski, L., et al. (2016). National outbreak of multidrug resistant *Salmonella* Heidelberg infections linked to a single poultry company. PLOS ONE. doi.org/10.1371/journal.pone.0162369.
17. Cummings, K. J., et al. (2010). *Salmonella enterica* serotype Cerro among daily cattle in New York: An emerging pathogen. Foodborne Pathogens and Disease. 7(6):659-665.
18. The inhibitory effect of *Bacillus subtilis* PB6 on the growth of *Salmonella* Heidelberg, Cerro and Uganda. WP-17-00016.
19. Kluger, M. J. and B. A. Rothenburg. (1979, Jan.). Fever and reduced iron: Their interaction as a host defense response to bacterial infection. Science. 203(4,378):374-376.
20. Smock, T. M., et al. (2020, July). Effects of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on serum chemistry, complete blood count, and fecal *Salmonella* spp. count in high-risk cattle during the feedlot receiving and finishing periods. Translational Animal Science. 4(3)txaa164.
21. Lago, A., et al. (2021, July). Effect of supplementing dairy cows with *Bacillus subtilis* PB6 and/or chromium propionate on health, culling, reproductive performance and metabolic parameters. (Abstract P413). American Dairy Science Association Annual Meeting. Cincinnati, Ohio.
22. Effects of CLOSTAT® (*Bacillus subtilis* PB6) supplementation on gastrointestinal tract permeability, metabolism, inflammation and production parameters in transition dairy cows, TD-22-7726.
23. The effect of supplementing CLOSTAT® 500 (*Bacillus subtilis* PB6) to yearling steers in a commercial feedyard on health, *Salmonella* spp. prevalence, feedlot growth performance and carcass characteristics, TD-22-7809.
24. Compatibility of *Bacillus subtilis* PB6 with organic and inorganic acids, antibiotics and coccidiostats. INF-19-17802.
25. The role of commercial pelleting on the viability of *Bacillus subtilis* strain PB6, TL-19-17863.

21.01154
PTP-8430
August 2022