January 15, 2015 MicroBasics Technical Summary

Effects of Surveillance against enteric and food-borne pathogens

Upgrade resistance to infectious organisms as consumers demand the elimination of antibiotics

Introduction: Food-borne illness linked to pathogens in meat and processed foods has led to increased attention to food safety concerns at all stages of production, including the farm. Livestock agriculture is recognized as a potent source of microorganisms, many of which are potential pathogens, and with the increased rearing densities of these intensive production systems, susceptibility to pathogenic challenges is on the rise.

Successful operations implement regular health and herd maintenance programs in an effort to minimize risk of disease, and feed additives including yeast culture and probiotic/prebiotic preparations are often administered to enhance performance. However, with the increased concern surrounding food safety, the focus of feed additive preparations is now shifting towards immune-modulation and pathogen reduction.

May we rely on feed additives to upgrade resistance to infectious organisms? In an effort to evaluate the effectiveness of popular feed additives against different pathogens, two studies were conducted in 2015 by DairyExperts, Inc. (Tulare, CA) in conjunction with Bioscience Laboratories, Inc. (Bozeman, MT).

Methods: Objective I. Evaluate the ability of test products to agglutinate various bacterial strains: In this study, Celmanax SCP, Actigen W.S., Original XPC, Surveillance Dry and Surveillance iL (Surveillance iL evaluated at two concentrations), were evaluated to agglutinate six bacterial strains. Bacterial populations of *Escherichia coli* (0157:H7) (ATCC #51657), *Escherichia coli* (09:K35:K99) (ATCC #31616), *Salmonella enterica enterica* serovar Dublin (ATCC #BAA-1514), *Salmonella enteric enterica* serovar Newport (ATCC #6962), *Salmonella enterica enterica* serovar Typhimurium (ATCC #14028), and *Clostridium perfringens* (ATCC #13124) were used to inoculate separate test tubes containing the test products. The resulting suspensions were allowed to set for 30 minutes, allowing agglutinated cells to settle. Upon elapse of the exposure time, the cells remaining in the supernatant were enumerated. The percent and $\log_{10}$ reductions of the microbial population of each challenge strain were determined by comparison to untreated controls numbers. All testing was performed in quadruplicate and all agar-plating was performed in duplicate.

Methods: Objective II. Evaluate the ability of test products to inhibit attachment of Cryotosporidium to bovine epithelial cells: In this study, Celmanax SCP, Actigen W.S., Original XPC, Surveillance Dry and Surveillance iL (Surveillance evaluated at two concentrations), as well as positive and negative controls, were evaluated to inhibit the attachment of *Cryptosporidium parvum* sporozoites to Madin-Darby Bovine Kidney epithelial cells (MDBK [ATCC #CCL-22]). The positive control substance was 0.1 mg/mL Bovine Submaxillary Mucin (BSM). The negative control was Phosphate Buffered Saline (PBS). The study utilized an immunofluorescent staining procedure using *Cryptosporidium* specific antibodies. Controls for product cytotoxicity and initial population were also performed. The Cytotoxicity Control received no parasite and the initial population received no test product. *Cryptosporidium parvum* oocyst were excysted, mixed with a test product, placed onto monolayers of MDBK cells and incubated at 37°C for 2 hours for attachment. Following incubation, the cells were washed and fixed. Indirect immunofluorescent assay was performed to visualize fluorescently stained sporozoites attached to the cells. Sporozoites from 30 random fields (5 fields from each of 6 replicates) were counted.

Experimental Results & Interpretation: Objective I. Evaluate the ability of test products to agglutinate various bacterial strains: In this study, Celmanax SCP, Actigen W.S., Original XPC and Surveillance Dry reduced microbial populations of all challenge strains, except *Clostridium perfringens*, by less than 0.1 $\log_{10}$ following a 30-minute exposure and settling. *Clostridium perfringens* was reduced by an average $\log_{10}$ of 0.4, 0.1, 1.0, and 1.5 by the different products, respectively. Simply stated, all four test products did not reduce by more than 17% bacteria populations of challenge strains other than *Clostridium perfringens*. *Clostridium perfringens* was reduced by 97% by Surveillance Dry, 92% by Original XPC, 66% by Celmanax SCP, and 31% by Actigen W.S. Surveillance iL when diluted to 2.642 mL product/L (preventive dose), reduced the microbial populations following a 30 minute exposure and settling by an average $\log_{10}$ of 1.2 for *Clostridium perfringens*, 0.44 for *Escherichia coli* 09:K35:K99, 0.32 for *Escherichia coli O157:H7*, 0.28 for *Salmonella enterica enterica* serovar Newport, 0.26 for *Salmonella enterica enterica* serovar Typhimurium, and 0.25 for *Salmonella enterica enterica* serovar Dublin. The preventive dose reduced by 44% or more the previously mentioned bacteria populations, and specifically by 94% for *Clostridium perfringens.* Surveillance iL when diluted to 10.567 mL product/L (treatment dose), reduced the microbial populations following a 30 minute exposure and settling by an average $\log_{10}$ of 5.6 for *Clostridium perfringens*, 5.3 for *Salmonella enterica enterica* serovar Newport and *Salmonella enterica enterica* serovar Typhimurium, more than 4.0 for *Salmonella enterica enterica* serovar Dublin and Escherichia *coli 0157:H7*, and 1.6 for *Escherichia coli* 09:K35:K99. Surveillance iL at treatment dose reduced all of the microbial populations from 98% to 100%. Figure 1.

Experimental Results & Interpretation: Objective II. Evaluate the ability of test products to inhibit attachment of Cryotosporidium to bovine epithelial cells: In this study mean recoveries of Cryptosporidium *parvum* sporozoites following treatment with Celmanax SCP, Actigen W.S., Surveillance Dry and Surveillance iL at preventive and treatment doses were significantly different ($p < 0.05$) from the mean recoveries of the negative control. The largest reduction in sporozoites was experienced with Surveillance iL at treatment dose. There was no significant difference between the negative control and Original XPC. Figure 2.

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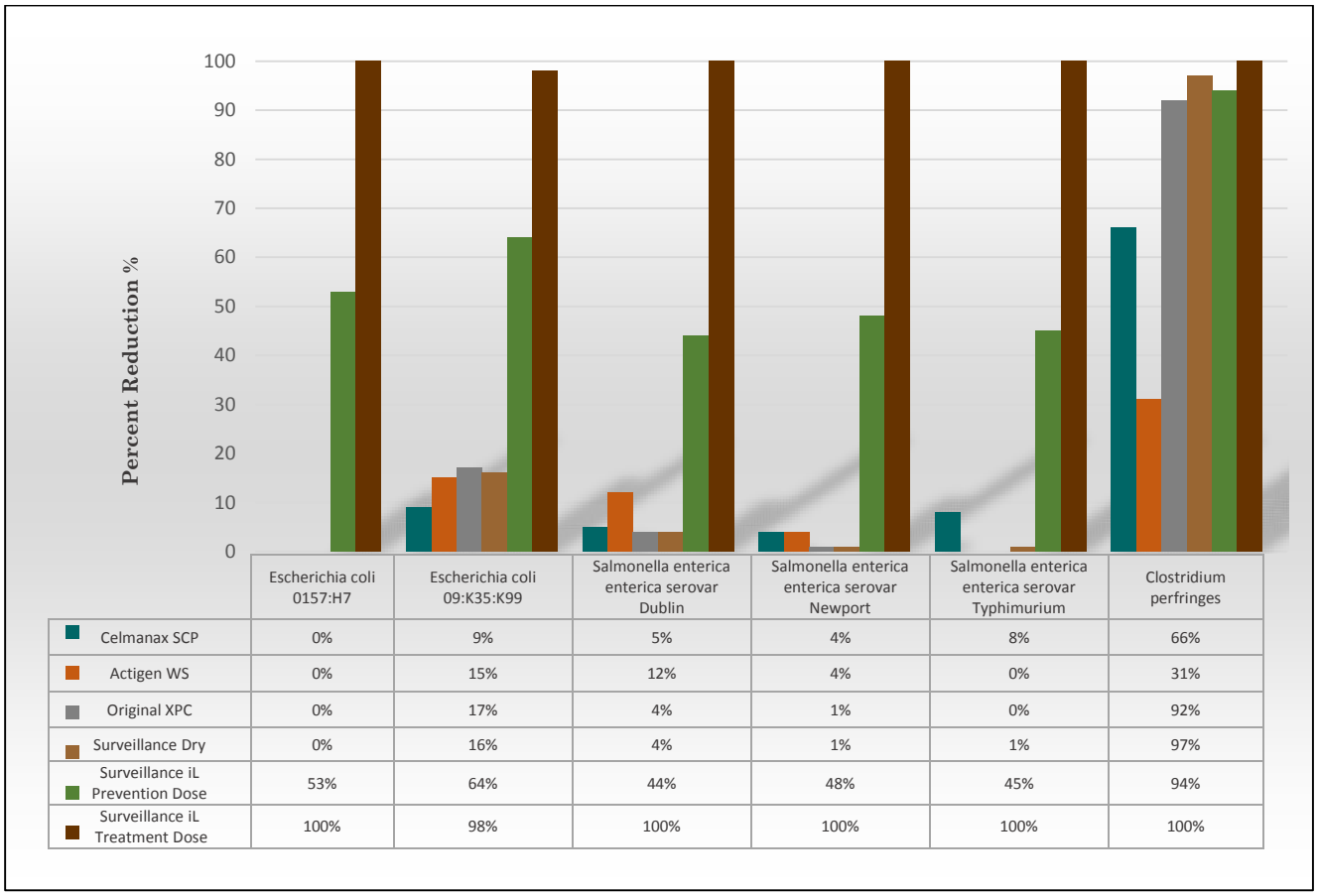
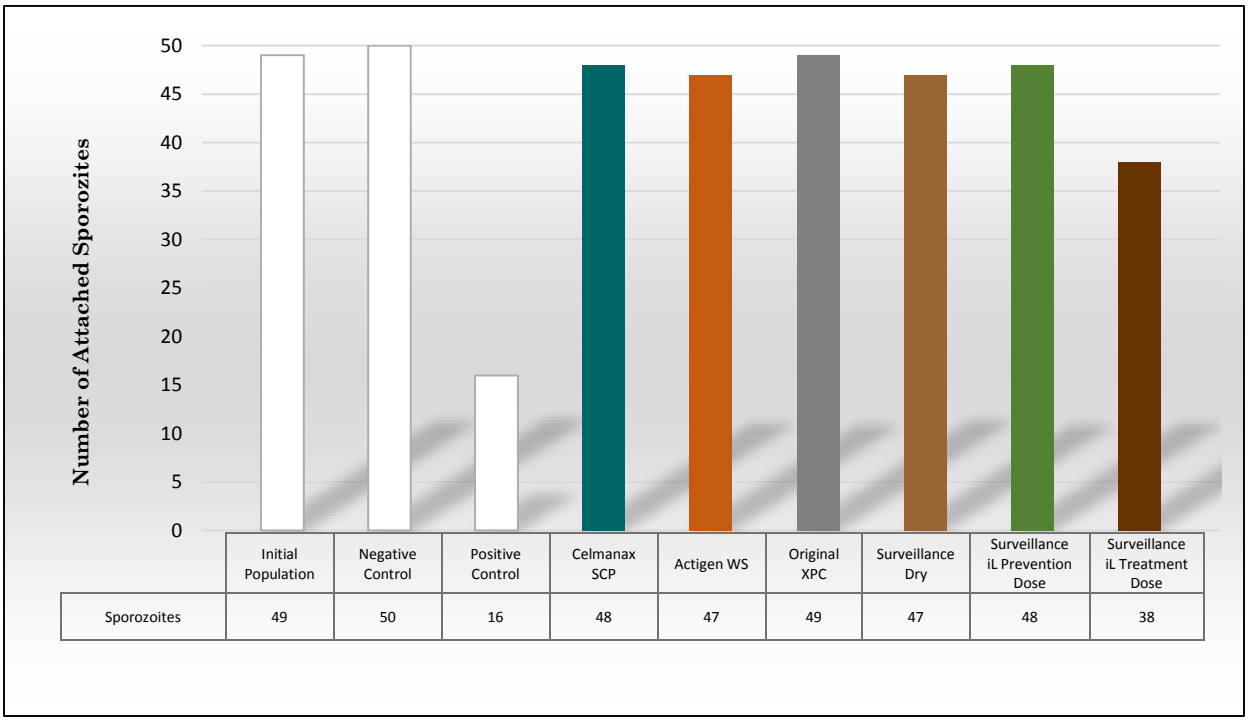
Figure 1. Reduction in bacteria populations for the different products.



	Escherichia coli 0157:H7	Escherichia coli 09:K35:K99	Salmonella enterica enterica serovar Dublin	Salmonella enterica enterica serovar Newport	Salmonella enterica enterica serovar Typhimurium	Clostridium perfringes
Celmanax SCP	0%	9%	5%	4%	8%	66%
Actigen WS	0%	15%	12%	4%	0%	31%
Original XPC	0%	17%	4%	1%	0%	92%
Surveillance Dry	0%	16%	4%	1%	1%	97%
Surveillance iL Prevention Dose	53%	64%	44%	48%	45%	94%
Surveillance iL Treatment Dose	100%	98%	100%	100%	100%	100%

Figure 2. Mean number of *Cryptosporidium parvum* sporozoites bound to MDCK cells per visual field for the different products.



	Initial Population	Negative Control	Positive Control	Celmanax SCP	Actigen WS	Original XPC	Surveillance Dry	Surveillance iL Prevention Dose	Surveillance iL Treatment Dose
Sporozoites	49	50	16	48	47	49	47	48	38