

RAPID INDUCTION OF ENTERIC CYTOKINE CHANGES BY AN EFFECTIVE LACTIC ACID BACTERIA-BASED CULTURE FOR POULTRY



BM Hargis, LR Bielke, G Tellez, SE Higgins*, RE Wolfenden, AD Wolfenden, OB Faulkner, NR Pumford, MJ Morgan, A Menconi, and TE Porter*
University of Arkansas, JKS Poultry Health Laboratory, Fayetteville, AR, USA 72701
*University of Maryland, College Park, MD, USA 20740

Introduction

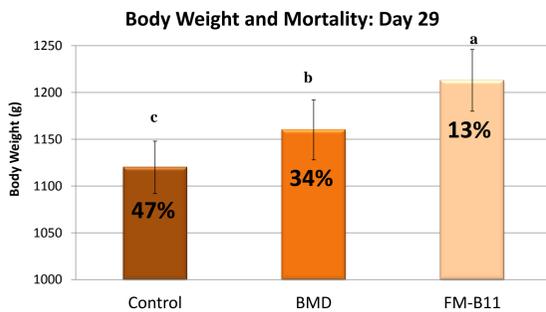
In the United States, a number of performance-enhancing antimicrobials are still permitted for use in broiler and turkey production. Increasing regulatory pressures and consumer preferences have driven a marked increase in poultry meat production when labeled as "Raised Without Antibiotics", a label that includes ionophores for coccidiosis control. While attempting to control human foodborne pathogens, poultry producers are simultaneously challenged to improve production in the face of increasing feed costs while using fewer antibiotics due to increased restriction of antimicrobial usage. Sustainable alternatives to antibiotic growth promoters for animal production include probiotics or direct fed microbials (DFM) consisting of live or dead organisms and spores. Probiotics and DFMs used in animal feed are becoming accepted as potential alternatives to antibiotics for use as growth promoters, and in select cases, for control of specific enteric pathogens. The most common types of probiotics that have been indisputably effective involve some, but not all, lactic acid bacteria (LAB). These bacteria are found normally in the gastrointestinal tract (GIT) of vertebrates and invertebrates, and the use of some LAB cultures are able to restore the natural microflora within the gut (Shahani & Ayebo, 1980). Lactic acid bacteria include the genera *Lactobacillus*, *Pediococcus*, and others that have long been associated with health benefits and which have been used for fermentation of certain foods. While speciation of members of these genera is difficult and inconsistent, these organisms are considered uniformly safe and are not associated with disease in healthy animals or humans (Tellez et al., 2006). A second classification of probiotic cultures are those microorganisms that are not normally found in the GIT (such as allochthonous flora). For example, spore-forming bacteria, normally members of the genus *Bacillus*, are not normally found in high numbers in the GIT, but clearly can enhance performance and disease resistance under some conditions.

Lactic Acid Bacteria-based probiotic

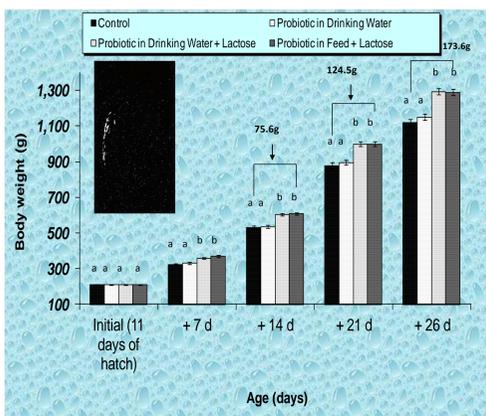
The selection of individual enteric bacteria capable of inhibiting *Salmonella* growth *in vitro* and the ability of selected oxygen-tolerant bacteria to also protect neonatal poult and broilers from *Salmonella* infection following challenge has been a goal of multiple research laboratories (Menconi et al., 2011; Vicente et al., 2008; Bielke et al., 2003; Hollister et al., 1999; Corrier et al., 1998; Hume et al., 1998). Tellez and co-workers (2006) evaluated a simple method to select for individual enteric bacteria capable of inhibiting *Salmonella* growth *in vitro* and the ability of selected oxygen tolerant bacteria, in combination, to protect neonatal poults from *Salmonella* infection following challenge. Concurrently, they also worked toward the isolation, selection, further evaluation and combination of LAB to control additional foodborne pathogens. Extensive laboratory and field research conducted with this defined LAB culture has demonstrated accelerated development of normal microflora in chickens and turkeys, providing increased resistance to *Salmonella* spp. infections (Farnell et al., 2006; J. P. Higgins et al., 2007; J. P. Higgins et al., 2008; J. P. Higgins et al., 2010; S. E. Higgins et al., 2008; Vicente et al., 2008). Published experimental and commercial studies have shown that these selected probiotic organisms are able to reduce idiopathic diarrhea in commercial turkey brooding houses (S. E. Higgins et al., 2005). Large scale commercial trials indicated that appropriate administration of this probiotic mixture to turkeys and chickens increased performance and reduced costs of production (Torres-Rodriguez et al., 2007a; Torres-Rodriguez et al., 2007b; Vicente et al., 2007a; Vicente et al., 2007b; Vicente et al., 2007c).

These data have clearly demonstrated that selection of therapeutically efficacious probiotic cultures with marked performance benefits in poultry is possible, and that defined cultures can sometimes provide an attractive alternative to conventional antimicrobial therapy. Two products developed in our laboratory include Floramax B1[®] (B11) and Sporulin[®], (see <http://www.pacificvetgroup.com> for more information).

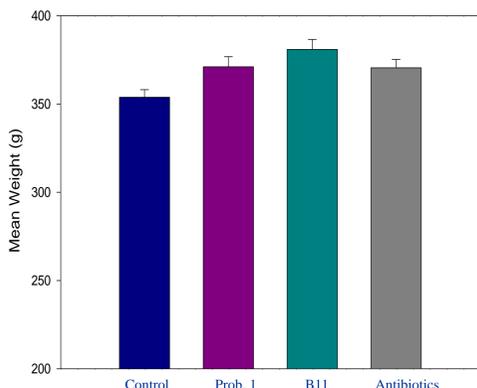
B11 vs BMD for Control of Necrotic Enteritis in a Laboratory Challenge Model



Torres-Rodriguez A. et al. 2007 J. of Applied Poul. Res. 16:635-641
Administration of B11 and dietary lactose at a very low concentration [0.1%] greatly enhanced the growth rates of turkeys under commercial conditions



S. E. Higgins et al., 2005 J. of Applied Poul. Res. 14:345-348
Treatment of idiopathic enteritis in commercial poults with B11 also compared favorably to selected antibiotic therapy



Bacillus Spore-based DFM

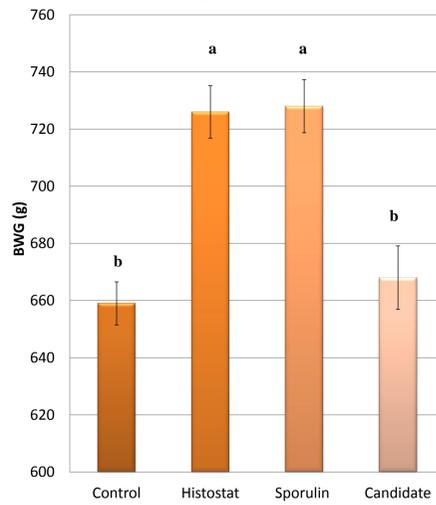
In spite of the success showed by the development of the LAB probiotic for use in commercial poultry as described here, there is still an urgent need for commercial probiotics that are shelf-stable, cost-effective and feed-stable (tolerance to heat pelletization process) to increase compliance and widespread utilization. Among the large number of probiotic products in use today some are bacterial spore formers, mostly of the genus *Bacillus*. Used primarily in their spore form, some (though not all) have been shown to prevent selected gastrointestinal disorders and the diversity of species used and their applications are astonishing. There is scientific evidence suggesting that some but not all isolates of ingested *B. subtilis* spores can, in fact, germinate in the small intestine (Casula & Cutting, 2002; Casula & Cutting, 2002; Duc le & Cutting, 2003; Hoa et al., 2001). Together, these studies not only show that spores are not transient passengers in the gut, but they have an intimate interaction with the host cells or microflora that can enhance their potential probiotic effect. Several commercial spore-forming *Bacillus* cultures have been shown to reduce food borne pathogens (Aureli et al., 2010). However, cost issues associated with achieving necessary concentrations of spores in feed have greatly limited commercial acceptance in the animal industry (Hong et al., 2005).

While the majority of clear-cut research with regard to beneficial probiotic cultures has focused on LAB, as discussed above, a major question in several laboratories is whether or not selected spore-former bacteria (genus *Bacillus* or related) can be as effective as the best known LAB cultures. Recently, one *Bacillus subtilis* spore isolate was as effective as a well-established LAB-based probiotic for *Salmonella* reduction in poultry (Wolfenden R.E. et al., 2010; Shivaramaiah et al., 2011), and was equal to bacitracin for prevention of experimental necrotic enteritis, and was able to markedly reduce necrotic enteritis issues in large scale feed trials (unpublished from the author's laboratory).

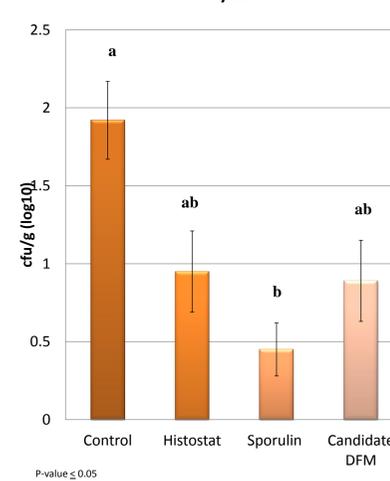
Other isolates or combinations of isolates with increased potency and efficacy may be identified with continued research. Some of these environmental *Bacillus* isolates have been evaluated *in vitro* for antimicrobial activity against selected bacterial pathogens, heat stability, and the ability to grow to high numbers. Unpublished experimental evaluations have confirmed improved body weight gain as well as *Salmonella* sp. or *Clostridium perfringens* reduction in commercial turkey and broiler operations when compared with medicated (nitarsone) or control nonmedicated diets respectively. Indeed, preliminary data suggests that these isolates could be an effective alternative to antibiotic growth promoters for commercial poultry.

Importantly, improved efficiency of amplification and sporulation is absolutely essential to gain widespread industry acceptance of a feed-based probiotic for ante mortem foodborne pathogen intervention, as well as cost effectiveness. Recently, both vegetative growth and sporulation rates have been optimized, which may lead to new efficiencies for commercial amplification and manufacture of a cost-effective product at very high spore counts (Wolfenden R.E. et al., 2010). In order to select even more effective isolates, current research is focused on the mechanistic action of new *Bacillus* candidates. Preliminary studies indicate a potential mechanistic action of these new *Bacillus* candidates at least partially involve rapid activation of innate host immune mechanisms (system or responses) in chickens and turkeys (unpublished data). This data provides an exciting possibility for identification of vastly superior and more potent probiotics in the near future.

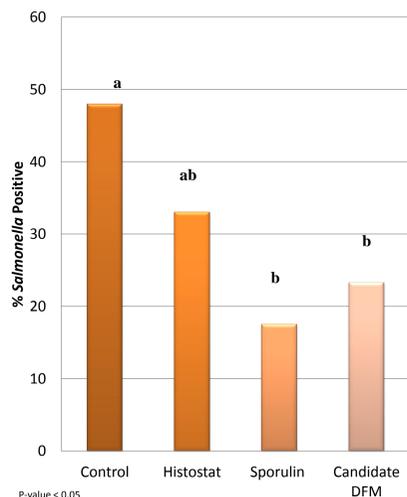
Field Trial Comparison of Turkey Body Weight Gain: Day 23



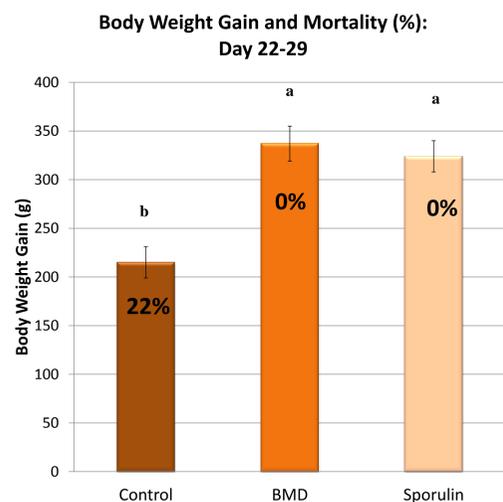
Turkey Field Trial Comparison of Salmonella cfu/g Cecal Content: Day 23



Turkey Field Trial Comparison of Percentage of Poults Positive for Salmonella: Day 23



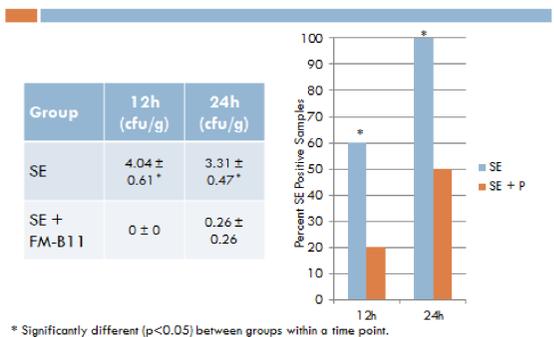
Sporulin vs BMD for Control of Necrotic Enteritis in a Laboratory Challenge Model



Micro-Array Analysis

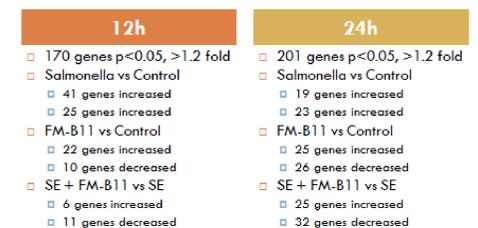
We have recently investigated the effects of B11 with or without *Salmonella* challenge on transcriptional profiling of cecal gene expression in neonatal chicks (Higgins et al., 2011). In this study, day-of-hatch chicks were challenged with *Salmonella enterica* serovar Enteritidis (SE) and treated 1 h later with the poultry-derived, LAB culture (B11, discussed above). Cecae were collected 12 and 24 h post-treatment for *Salmonella* detection and RNA isolation for microarray analysis of gene expression. At both 12 and 24 h, SE was significantly reduced in chicks treated with the probiotic as compared with the birds challenged with only SE ($P < 0.05$). Microarray analysis revealed gene expression differences among all treatment groups. At 12 h, 170 genes were expressed at significantly different levels ($P < 0.05$), with a minimum difference in expression of 1.2-fold. At 24 h, the number of differentially regulated genes with a minimum 1.2-fold change was 201.

Recovery of Salmonella from Cecae

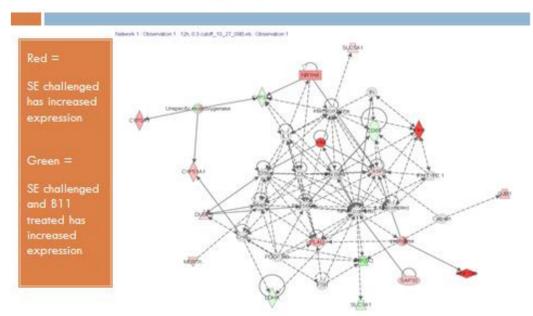


* Significantly different ($p < 0.05$) between groups within a time point.

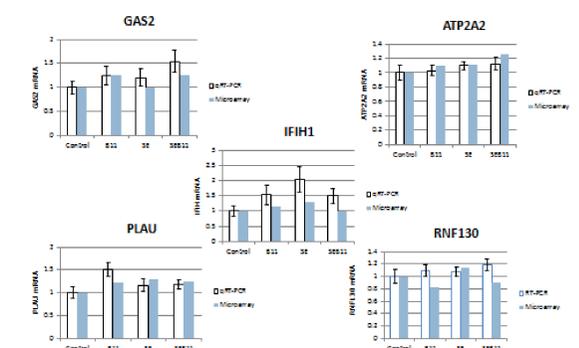
Microarray Analysis



12h IPA Analysis, Network 1



12h Gene Expression



Discussion

Work in our laboratories during the last 12 years has resulted in development and commercialization of two products that are now widely available and used in several countries for reduction of enteric bacterial infections, including those associated with food-borne illness. Importantly, several studies have indicated enhanced performance similar to antibiotics in both field and laboratory evaluations. Remarkably, effects are seen on colonization with *Salmonella* in as little as 12 hr post-administration, and pathway analysis revealed that at both 12 and 14 hr, host genes associated with the nuclear factor kappa B complex, as well as genes involved in apoptosis, were significantly regulated. Based on this analysis, probiotic-induced differential regulation of the genes growth arrest-specific 2 (GAS2) and cysteine-rich, angiogenic inducer, 61 (CYR61) may result in increased apoptosis in the cecae of chicks. Because *Salmonella* is an intracellular pathogen, we suggest that increased apoptosis may be a mechanism by which the probiotic culture reduces *Salmonella* infection. These may be the first evidence indicating that highly effective probiotic/DFM treatments may rapidly alter host gene expression and associated defenses, and may provide clues for selection of even more effective strategies for replacing antibiotics.