Chapter 1

Prebiotic and Probiotic Approaches to Improving Food Safety on the Farm and Their Implications on Human Health[1]

William B. Smith, Todd R. Callaway, Luis O. Tedeschi, Francis M. Rouquette Jr., Trisha Sheridan and Jennifer Adamski

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/63114

Abstract

Human health is a broad category that encompasses the entirety of the food production system. Livestock production practices have important effects on human health because livestock not only are a primary food source but also can be the source of pathogenic bacteria that may enter the food chain indirectly. As government regulation and public scrutiny restrict the prophylactic use of antibiotic and antimicrobial interventions, other techniques must be used to reduce the burden of animal-borne pathogenic bacteria entering the food system. Prebiotics (isolated compounds that enhance natural microflora and thereby decrease pathogens) and probiotics (live microbes that are administered to livestock to enhance microbial diversity and crowd out pathogens) represent two unique opportunities for alternative measures in pathogen reduction. This review addresses the link between animal production and human health, the agricultural sources of pathogenic organisms, and the probiotic and prebiotic approaches that have been evaluated in an effort to reduce carriage of foodborne pathogenic bacteria by livestock.

Keywords: food safety, livestock, prebiotic, preharvest intervention, probiotic

[1] Proprietary brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product and/or exclusion of others that may be suitable.

1. Introduction: why is farm-based intervention of interest to human health?

This book is dedicated to the understanding and dissemination of knowledge surrounding prebiotic use in human health. Thus, it begs the following questions: When a reader finds this particular manuscript, what is the point? What is the objective of a farm-based perspective when the focus is on human health? While these may be valid questions to the casual observer, a full understanding of potential pathogens and intervention in the subject of human health must by rights include a discussion of the foodstuff at its source. Like all mammals, livestock harbor a diverse collection of bacteria [1]. In fact, the gastrointestinal tract of these animals can harbor in excess of 2000 bacterial species at concentrations of 10^{10} cells/g of digesta [2]. While the majority of these organisms are beneficial to the host and part of the stable native microflora of the gut [3], certain instances or conditions allow pathogenic bacteria to colonize within the animal. Some of these bacteria can make their way from the gut or the hide during processing [4], introducing pathogens into the abattoir (slaughter plant) at harvest that must then be dealt with in final food products. As noted in Reference [1], a great number of these pathogenic bacteria in the realm of human health are also of interest in that of livestock animal health and can commonly be traced back to those very animals. Since these pathogens are a threat to the well-being of both humans and livestock, one must then investigate intervention strategies by which the microbial burden may be reduced at the source so that these pathogenic organisms would never enter the human food chain.

Traditionally, farm-level or feeder/finisher-level control of pathogens has been achieved through prophylactic antibiotic and antimicrobial addition to feeds. The main source of prevention of pathogenic bacterial entry into the food system is through Hazard Analysis and Critical Control Point (HACCP) plans at the abattoir [5]. It should be noted that HACCP control measures are only effective to a certain point (i.e., they are not perfect), but any reduction of pathogen shedding prior to entry into the abattoir will reduce the burden and assist in the efficacy of in-plant HACCP-based controls [6]. In fact, with the subtherapeutic antibiotic use ban in the European Union [7,8] and increased public scrutiny of antibiotic use in livestock in the United States [9], alternative preharvest control strategies must be devised and implemented, especially given the direct correlation between live animals shedding foodborne pathogenic bacteria, such as *Escherichia coli* O157:H7, and the incidence of positive carcasses at the abattoir [5]. Thus, preharvest intervention strategies, such as use of probiotics and prebiotics, need to be viewed as an additional critical control measure that can be included in the food safety continuum.

So how then do preharvest interventions in animals work? Much of the efficacy of products that will be described in the present review can be loosely grouped under an umbrella concept known as a "competitive enhancement" approach to pathogen reduction [1,10–13]. The first facet is based upon the introduction of naturally-occurring microflora isolates from the gastrointestinal tract of an animal of the same species [1], occupying all available ecological niches in the gastrointestinal tract and thereby excluding pathogens [1,14]. When used in neonatal (or newly hatched) animals, this technique is known as "competitive exclusion" (CE),

http://dx.doi.org/10.5772/63114

which reduces pathogen penetration of the naive and essentially sterile neonatal gastrointestinal tract [1,14]. Use of probiotics (also known in the animal industry as direct-fed microbials [DFMs]) is a slightly different approach in which existing gastrointestinal microbial populations can be diversified or modified/attenuated by daily inclusion of a bacterial or fungal population or end-product, and this may have an inhibitory effect on pathogenic bacteria, including foodborne pathogens [1,15]. A further competitive enhancement strategy is the addition of prebiotics, which are limiting nutrients or isolated compounds that are indigestible by the host but give specific innate microbes a competitive advantage that can have a deleterious effect on pathogenic bacteria, to the diet [1]. Furthermore, several of these approaches can be synergistically combined and are termed "synbiotics"; for example, a DFM dependent on the inclusion of prebiotics can be maintained in the gut and given a further competitive advantage to remain in the population to benefit host animal health and production or to improve food safety.

2. Pathogens: what are the sources?

As previously noted, the body , and especially the gut, of most food animals contains many microorganisms [2]. While the vast majority of these are beneficial (commensal) to the host, there are select species and serovars (e.g., *Salmonella*) that exhibit pathogenic or toxigenic effects in both humans and livestock. These pathogens are naturally occurring organisms that, given the opportunity, can colonize the environment of the innate gut microflora and take hold of niches in an otherwise healthy animal. This section provides a discussion of some of the more common pathogenic bacteria in livestock and how these microbes may become a problem in the safety and security of the food chain.

2.1. Campylobacter

Campylobacter has been identified as one of the most common foodborne pathogenic bacteria. Most commonly, *Campylobacter* has been linked to poultry products and linked to human cases of gastroenteritis in most cases as well as the Guillain-Barré syndrome, reactive arthritis, and irritable bowel syndrome or inflammatory bowel disease in the most severe cases [16,17]. *Campylobacter* is a major concern for infection in poultry production [16–18]. One route of contamination, also common to most other pathogens, is through livestock water sources [19]. In an area of intense livestock (dairy) production in England, *Campylobacter jejuni* was found in 14.3% of water sources sampled (predominantly in running water or troughs), *Campylobacter coli* was found in 18.5% (predominantly in stagnant water), and *Campylobacter lari* was identified in 4.2% [20]. In this same study, variables were regressed to show their impact on the prevalence of *Campylobacter* spp. In a multiple regression model, water source and soil type played the most significant role in determining the environmental prevalence of *Campylobacter*, with natural water sources and high clay content both increasing its prevalence [20].

2.2. Enterohemorrhagic *E. coli* (EHEC)

Enterohemorrhagic *E. coli* is a group of highly virulent foodborne pathogenic bacteria that is of great interest to human health. The well-known *E. coli* serotype O157:H7 was first identified in a clinical outbreak of undercooked hamburger patties at a commercial fast food chain in the United States [21]. In fact, this pathogenic serotype has been linked to one of the greatest foodborne pathogen outbreaks in American history [22,23]. In this landmark case, in which over 150 cases were reported and multiple deaths occurred [22], *E. coli* O157:H7 was isolated from ground beef patties and subsequently sourced to the abattoir in which meat was contaminated from pathogenically infected animals [23]. These human infections commonly resulted in postdiarrheal hemolytic uremic syndrome (HUS) and disproportionately affected the young and elderly [22]. While inoculation of the livestock host is generally achieved through fecal-oral contamination or contaminated drinking sources [19,24], this does not account for the transmission of pathogens from the live animal to the meat during processing. Much of the contamination in the plant, especially with regard to *E. coli* O157:H7, can be traced to contamination of the hide and interaction during hide removal and evisceration [25]. In a sampling of over 2500 cattle hides from across North America, researchers discovered that over half of the hides were contaminated with nearly 300 unique isolates of *E. coli* O157:H7 [4]. Additionally, the frequency of the unique isolates obtained from cattle hides was very similar to the prevalence of isolates identified in human clinical cases [4]. In a survey of high-throughput Midwestern United States abattoirs, 11% of all hides, 43% of pre-eviscerated carcasses, and 2% of postprocessed carcasses tested positive for EHEC O157:H7 [5]. This included positive tests for hides in 38% of introduced lots, pre-eviscerated carcasses in 87% of lots, and post-processed carcasses in 17% of lots [5]. While *E. coli* O157:H7 is the best known of the EHEC group, other members (e.g., O26, O111) also pose significant threats to the food supply around the world. Although *E. coli* O157:H7 was quickly categorized by the U.S. Food Safety Inspection Service as an adulterant [26], an additional six serotypes are now included in this important category [27] and thus carry an important public health and economic impact.

2.3. Salmonella

Salmonella is another bacterial pathogen of significant concern both as a foodborne pathogen and as a threat to animal health, having been identified in all vertebrates [28]. More than 2500 separate serotypes comprise *Salmonella enterica* [29], which is the most common species found in food animals. *Salmonella* accounted for 55% of the foodborne illness outbreaks in the United States from 1993 to 1997 [30] and 26% of the outbreaks from 1998 to 2008 [31], with one of the most massive outbreaks being from ice cream hauled in tanker trucks that had improperly handled raw eggs [30]. Although researchers identify *Salmonella* as a ubiquitous microbe, it has been noted that the primary reservoir for such a pathogen is the digestive tract of the animal (indicating fecal-oral transmission or accidental contamination at the abattoir) and conditions under intensive production where animals are in close contact with one another are favored [32]. It should be noted, however, that a common vehicle for *Salmonella* contamination in human food is not livestock per se but instead vine-stalk vegetables [31]. That said, in an evaluation of butcher shop poultry in Portugal, 60% of the products were found to be conta-

minated with *Salmonella* and the pathogen *S. enteritidis* was found to make up 44% of those cases [32].

2.4. Others of interest

While *Campylobacter*, *E. coli*, and *Salmonella* are all identified and targeted as the primary pathogens of interest for reduction in the human food system [19], there are other pathogens of importance that are far less commonly addressed in scientific research. *Clostridium*, like many other pathogens discussed herein, is a Gram-positive, spore-forming pathogenic bacterium [33]. *Clostridium difficile* infection is characterized by severe diarrhea and pseudomembranous colitis [33]. *C. difficile* is a known potential resident of the livestock intestinal tract and has been identified in up to 12% of sampled retail ground beef and ground pork in a Canadian study [34]. *Clostridium perfringens*, the leading cause of necrotic enteritis, can also become a human health issue and has been isolated as a portion of the natural microflora of the jejunum, cecum, and cloaca of poultry [35]. *Listeria monocytogenes* is a pathogen most commonly associated with dairy products [36]. At the time of their review, Skovgaard and Morgen [37] stated that most human cases of listeriosis are of unknown origin, although food was suspected, and recent high-profile outbreaks have definitely confirmed such suspicion [36]. Listeriosis has been linked to central nervous system infections, bacteremia, and endocarditis [37]. Listeria has been isolated from dairy feces as well as feedstuffs and attributed to mastitis in these animals [38]. *Staphylococcus aureus* has been associated with all livestock species [39]. It is an opportunistic pathogen that will colonize both livestock and humans in an infectious nature [40].In dairy cattle, the pathogen is known as one of the leading causes of mastitis, and mastitis is among the leading losses to the dairy industry [41]. In one study, 296 individual isolates of *S. aureus* of animal origin were discovered; while none of the isolates from cattle or swine were found to be common with human infection, a significant number of the poultry isolates were common with those found in the bloodstream of humans [40].

3. Probiotics/direct-fed microbials (DFMs)

A list of probiotics that have been used in food animals to reduce pathogenic bacteria is presented in **Table 1.** Probiotics used in animals are known as DFMs and defined as live, biologically active microbes (bacterial or fungal), or dead cultures that include the end-products of their fermentation, that are administered to an animal in hopes of enhancing the natural gastrointestinal ecosystem and occupying any niches in which pathogenic organisms may thrive [10,42]. Again, this concept is broadly categorized as competitive enhancement in which live, naturally occurring microbes are added to the host animal to enhance the innate population in the gut [10,15]. As noted in Reference [43], the concept of CE specifically originated with the application of mature broiler gastrointestinal contents for the reduction of *Salmonella* [44]. While addition of DFMs to mature animals yields mixed and often negative results, their administration to livestock early in life (as early as the day of hatch in broilers)

has been shown to be effective in reducing pathogenic bacterial loads by kick-starting the natural succession of commensal bacterial colonization of the gastrointestinal microflora [18]. In addition to the direct addition of probiotics to neonatal diets, passive immunity may also be conveyed to the neonate through supplementation of the dam before birth [45].

In addition to the benefits to livestock and human health in terms of a reduction in colonization and shedding of pathogenic microbes, probiotics have also found a niche in the livestock market because of their added benefit of enhanced production performance. Because there are currently no economic incentives to implement food safety interventions in live animals, interventions should be able to "pay for themselves" by improving animal growth or production efficiency. Many studies report the beneficial effects of DFMs on production efficiency in cattle [9,46,47], swine [48], and poultry. The supplementation of feedlot cattle with a combination of *Lactobacillus acidophilus* NP45/NP51 and *Propionibacterium freudenreichii* NP24 resulted in an increase in the graded fat thickness of the animals at slaughter [9], indicative of improved gain and efficiency. The use of *Enterococcus faecium* in feedlot cattle was able to increase the energetic efficiency of the rumen by increasing the proportion of propionate (a glucogenic volatile fatty acid) produced through ruminal fermentation, but all other digestive and production traits were not altered although fecal coliform shedding was increased, potentially due to colonic acidification [46]. Feeding multiparous dairy cows a combination of *Saccharomyces cerevisiae* (Diamond V-XP, Diamond V, Cedar Rapids, IA) and *Propionibacterium* spp. P169 resulted in an increase in fat-corrected milk yield, percent lactose, and weight gain postpartum [47]. When nursery piglets were supplemented with a combination of *Bacillus subtilis* and *Bacillus amyloliquefaciens*, their average daily gain increased and gain-to-feed ratios decreased [48]. However, because the focus of this publication is on human health, the beneficial effects of probiotics on animal production will be disregarded in this review, although it is important to understand that the economic benefits may indeed pay for the inclusion of a food safety enhancement.

Product	Species	Effective against	Reported results	Source
Bacillus spp.	Broilers	*Campylobacter jejuni* *Samonella* Typhimurium[21]	1 to 3 log reduction intracloacally Percentage reduction in the crop and ceca	[17] [55]
B. subtilis	Swine	*Clostridium perfringens* *Escherichia coli*	Increased litter survival, weaning weights and *Lactobacillus* populations	[50]
Biofeed™ (*Bifidobacterium longhum*, *B. thermophylum*, *Lactobacillus acidophilus* and *Streptococcus faecium*)	Swine	- - -	Reduced pathogen load and incidence of diarrhea	[1]
Bovamine™ (*L. acidophilus* and *Propionibacterium freudenreichii*)	Beef cattle	*Escherichia coli*	Reduces populations of O157:H7	[1]
Enterococcus faecium	Swine	Swine influenza A	Up to 4 log reduction in virus titers	[59]

Product	Species	Effective against	Reported results	Source
			Enhancement in nitric oxide production	
Lactic acid bacteria	Cattle	Escherichia coli *Samonella* Typhimurium	High efficacy in reduction by two isolates Moderate efficacy by 12 isolates	[49]
L. acidophilus NPC 747	Cattle	*Escherichia coli*	49% reduction in fecal shedding	[45]
L. crispatus	Cattle (*in vitro*)	*Escherichia coli*	Reduction on agar spot plates, no antibiotic resistance, and survival in manure and rumen fluid	[46]
LiveBac™	Dairy cattle	-	Pathogenic protection agent	[1]
Pedicoccus acidilactici	Cattle (*in vitro*)	*Escherichia coli*	Effective inhibition on agar spots	[46]
Spiromac-C™ (*Bacillus*, *Cellulomonas*, *Lactobacillus*, *Saccharomyces cerevisiae* and *Spirulina*)	Cattle	- - -	Reduced disease incidence	[1]

Table 1. Experimental results reported for selected probiotics for use in control of pathogenic bacteria in livestock species.

3.1. Cattle

In an evaluation of multiple potential candidates as probiotics for use in beef cattle, Brashears et al. [49] found several viable isolates from small and large intestinal and fecal samples *in vitro*, all of the lactic acid bacteria (LAB) family. Twenty-seven of the 86 isolates exhibited greater than 50% survival after 3 hours at pH 3; of these, 8 isolates that could withstand 3 hours in a bile solution with greater than or equal to 60% survival were identified [49]. Finally, LAB S7 and F30 had a high level of efficacy against *E. coli* ATCC 25923 and 80% of the isolates used in bile testing had moderate efficacy in *Salmonella* Typhimurium activity. When feedlot cattle were administered a combination of *L. acidophilus* NP51 and *P. freudenreichii* NP24, fecal shedding of *E. coli* O157 was reduced 1 week prior to and on the day of shipment to the abattoir [9]. However, the trend shifted in terms of hide contamination in which the highest reduction in pathogen incidence was found when a high concentration of *L. acidophilus* NP45 was added to the previously mentioned microbial cocktail [9].

The dietary addition of the DFM *L. acidophilus* NPC 747 reduced shedding of *E. coli* O157:H7 in feedlot cattle [50]. While this trend was observed in the feedlot, fecal shedding was not found to be different at the time of slaughter, mainly due to the overall shedding level to which the animals had been reduced (1.47% of treated animals). A decrease in shedding prevalence in the feedlot, however, was seen as a significant benefit given that the pathogen load at abattoir entry was highly reduced and the subsequent opportunity for contamination by transfer of *E. coli* O157:H7 from hides (1.66% infection) or the environment (in both the feedlot and the abattoir) was therefore not as great [50].

Brashears et al. [51] conducted a systematic review and meta-analysis of studies in which DFMs were used in the suppression of verotoxin-producing or Shiga toxin-producing *E. coli* O157. Their study found that there was an odds ratio of 0.46 (0.46 times as likely to exhibit presence) for the efficacy of DFMs on the suppression of *E. coli* O157 at the conclusion of an experiment, with over 50% of the variability in efficacy coming from the heterogeneity in experiments [51]. When looking at the combination effect of DFMs NP51 and NP24, there was an odds ratio of 0.43, with 58% of the variability due to heterogeneity. This effect somewhat changed, however, when the evaluation was made throughout the individual trial [51]. In this instance, the efficacy of DFMs exhibited an odds ratio of 0.55.

In an effort to isolate and identify LAB for *E. coli* control in cattle, Nurmi et al. [52] were able to identify several microbes with the characteristics necessary for introduction as probiotics. *Pediococcus acidilactici* was identified as having the most control of *E. coli* O157:H7 *in vitro*, exhibiting 129% of the spot plate inhibition of *L. acidophilus* [52]. However, *P. acidilactici* was shown to be resistant to common antibiotics and therefore dropped from the final selection of potential candidate organisms. Based on its lack of antibiotic resistance, effective inhibition of *E. coli*, and survival and efficacy in both manure and rumen fluid, *Lactobacillus crispatus* was recommended for further work as a probiotic for cattle feed inclusion to reduce *E. coli* O157:H7 [52].

3.2. Poultry

Competitive exclusion has its origins in poultry production. Following a severe *Salmonella* outbreak in Finland in 1971, researchers began administering obligate anaerobes to populate the gut of poultry, albeit with little success [44]. However, when natural microflora were taken from adult poultry and administered to newly hatched chicks, the results gave rise to the concept of CE (also known at that time as the Nurmi concept) by early population of intestinal microflora [53]. Stemming from this, most probiotic research studies dealing with CE have taken place in the poultry industry [54], given that poultry production is riddled with concerns surrounding *Salmonella* and *Campylobacter*, the production setting lends itself to immediate inoculation of naive hatchlings, and poultry have a very short growth phase (approximately 42 days from hatch to processing) [1].

The efficacy of probiotics is impacted by the ability of bacteria or isolates to pass through the harsh conditions of the gastric stomach (or proventriculus) to make it to the lower intestine, where conditions are favorable for microbial growth. In an investigation of the administration of *Bacillus* spp. isolated from broiler ceca, oral administration was only able to reduce *C. jejuni* populations in the cecum by 1 log in 1 of 10 instances, whereas intracloacal administration reduced *C. jejuni* populations by 1 to 3 $\log_{10}$ [17]. This was attributed to the inability of the *Bacillus* spp. to survive the conditions of the proventriculus for colonization of the lower gut. It should be noted that this is not a practical route of administration in a commercial setting and thus only demonstrates a need for probiotic survival to demonstrate proof of concept for product efficacy. The results of this trial are supported by the work of Arsi et al. [18], who reported that certain isolates of *Bacillus* spp. and *Lactobacillus* spp. reduced *Campylobacter* colonization *in vitro* by 1 to 2 log. However, when tested *in vivo*, these same isolates were

http://dx.doi.org/10.5772/63114

ineffective in reducing *Campylobacter* populations, demonstrating the inconsistency of probiotic intervention with pathogen colonization of poultry [18]. However, an *in vitro* evaluation of *Bacillus* spp. isolates revealed that three strains (AM 0902, AM1109A, and B2) were able to tolerate pH 2 for up to 4 hours, with an additional two strains (NP122 and RW41) able to tolerate this pH for up to 2 hours, indicating a potential to survive the proventriculus [55]. It was further deduced that NP122 could reduce *Salmonella* Typhimurium concentrations in the crop by 16% and in the ceca by 50%, with AM1109A/B exhibiting a slight reduction in both locations in young broilers [55].

3.3. Swine

While most research studies are directed toward establishing an innate microbial population in neonatal livestock, other work has shown positive results with administration of DFMs to mature animals. *Bacillus* species are Gram-positive bacteria that, in the spore stage, are resistant to acidic conditions (due to the enhanced spore coat that protects the bacteria through the stomach [56]) and have been shown to reduce pathogenic clostridial strains, such as *C. difficile* and *C. perfringens* [45,57]. When *B. subtilis* was administered to mature sows, nursing piglets at 3 days of age were shown to have increased ileal concentrations and piglets at 10 days of age were shown to have increased colonic concentrations of *Lactobacillus gasseri* or *Lactobacillus johnsonii* as well as decreased incidence of *E. coli* and *C. perfringens* [57]. These benefits were linked to a decrease in pathogen shedding in the sows and a more rapid gastrointestinal colonization of commensal bacteria in piglets. A preliminary study demonstrated that when piglets were treated with a porcine-derived bacterial culture at farrowing and weaning, they exhibited decreased *Salmonella* serovar Choleraesuis shedding from 65% to 70% postweaning as well as decreased colonization in both the colon and the cecum [58].

Enterococcus faecium NCIMB 10415 is a recognized probiotic approved by the European Union and has been evaluated for its efficacy in reducing swine influenza virus (SwIV), specifically H1N1 and H3N2 [59]. *E. faecium* was shown to increase cell survivability (40-80%) and reduce viral titers (up to 4 log) of both SwIV strains in two media [59]. In this publication, it was hypothesized and demonstrated that *E. faecium* operates through the adsorption of viral particles as well as the stimulation of nitric oxide production, which in itself has antiviral properties.

The link between livestock production and human health exists not only in their direct relationship through the food chain but also in the coexistence of the species in close proximity to human housing. Puphan et al. [48] reported a reduction in fecal ammonia and hydrogen sulfide, both highly noxious gases, from swine that were supplemented orally with a combination of *B. subtilis* and *B. amyloliquefaciens*. Furthermore, when a combination of *B. subtilis* and *Bacillus licheniformis* was administered to growing pigs, manure from the pens dispersed more quickly, meaning that pens could be cleaned and manure solubilized more quickly for a less noxious waste product [60]. These data indicate that a positive impact on humans that goes far beyond the direct health/non-health dichotomy can be mediated by probiotics.

4. Prebiotics

Prebiotic treatment involves the inclusion of non-host-digestible compounds (often oligosaccharides) in diets to provide a competitive advantage to a segment of the microbial population. Unfortunately, prebiotics have previously not been a common adjunct in livestock production settings, largely due to their cost and the narrow profit margins associated with agricultural production. The use of prebiotics is most often seen coupled with a complementary probiotic (often described as "synbiotics"), and recent research has demonstrated the benefits that may exist with the coordinated use of such a complementary intervention. A list of the prebiotics identified for pathogen reduction in the literature is presented in **Table 2**.

Product	Species	Effective against	Reported results	Source
Avigaurd™ (freeze-dried extract from healthy poultry)	poultry	*Clostridium* *Escherichia coli* *Salmonella*	- - -	[1]
Chitosan	broilers	*Campylobacter jejuni*	1 log reduction with 0.5% *in vitro* and *in vivo*	[16]
FOS	broilers	*Escherichia coli* *Clostridium perfringens*	B cell reduction; increased IgM and IgG titers >Reduced population Reduced population	[7] [8]
Mannan-oligosaccharides (MOS)	broilers	*Escherichia coli* *Clostridium perfringens*	Reduced population Reduced population	[8]
Tasco-14/EX® (brown seaweed; *Ascophyllum nodosum*)	cattle	*Escherichia coli* *Salmonella*	79% reduction in fecal O157:H7 Reduction in shedding	[58] [53]

Table 2. Experimental results reported for selected prebiotics for use in control of pathogenic bacteria in livestock species.

As previously discussed, *Campylobacter* is among the leading foodborne pathogenic bacteria found in livestock and the majority of bacteria are introduced into the human food chain via poultry [16,17]. In an evaluation of *Campylobacter* colonization in hatchling chicks, chitosan (a compound from the chitinous shells of crabs and shrimp) was shown to reduce the population of *C. jejuni* both *in vitro* and *in vivo* when added to the feed [16]. This reduction of colonization was attributed to a down-regulation of *fliA*, *motA*, *motB*, and *CadF* genes, which are all involved in the synthesis and function of the flagella used in cellular function and movement [16].

Fructooligosaccharides (FOSs) and mannan-oligosaccharides (MOSs) have been evaluated for oral administration in broiler chickens in hopes of reducing the colonization of *C. jejuni* [7,8,18]. When used in isolation, neither of these substances was effective in reducing pathogen

http://dx.doi.org/10.5772/63114

colonization in broiler chicks. However, the synergistic combination of *Bacillus* spp., *Lactobacillus* spp., and MOSs reduced *Campylobacter* colonization. As an added benefit, FOSs were demonstrated to induce weight gain in broiler chicks both alone and in combination with probiotics [18]. This is in contrast to the work of Janardhana et al. [7] and Kim et al. [8], with both groups having examined FOSs and MOSs for prebiotic addition to the feed of broiler chicks. The dietary addition of FOSs has been shown to reduce B cells and increase IgM and IgG titers in broiler chicks, both indicators of an enhancement of gastrointestinal immune function [7]. Likewise, FOSs were shown to decrease the incidence of *C. perfringens* and *E. coli* at 0.25% inclusion as well as bolster the population of *Lactobacillus* spp. [8]. This same reduction in *C. perfringens* and *E. coli* was achieved with 0.05% inclusion of MOSs [8].

Essential oils and polyphenolics have also been tested in relation to the reduction of pathogen spread from livestock [61]. Noted essential oil components that have been tested include carvacrol (from savory), curcumin (from turmeric), eugenol (from allspice, betel pepper, and cloves), piperin (from black pepper), and thymol (from thyme) [35,62]. Fecal shedding of *C. perfringens* was reduced up to 30 days following supplementation with two essential oil blends [35]. Intestinal concentrations of *C. perfringens* were reduced for up to 21 days with essential oil administration, but this effect was negated by day 30 [35]. Tedeschi et al. [63] demonstrated that purified coumaric and cinnamic acids, both components of lignin, were able to reduce *E. coli* survival by 10- to 20-fold when mixed with feces, although diets containing forage rich in such compounds had no such effect. Berard et al. [62] also noted that catechol and pyrogallol (hydroxylated phenols) have toxic effects in the presence of microorganisms, mainly through substrate deprivation. Callaway et al. [64] discussed the concept that saponins (natural plant-based detergents) may have an antimicrobial effect by binding cholesterol, thereby disrupting the microbial membrane, in addition to tannins, which may act in substrate deprivation by binding protein and essential cations. Orange peel, a source of essential oils in the citrus family, has been shown to reduce cecal and rectal populations of *E. coli* O157:H7 with 5% and 10% dietary inclusion in sheep 96 hours following inoculation, but fecal shedding was only reduced at 10% inclusion [65]. Inclusion of orange peel at 10% of the diet was also shown to reduce *Salmonella* populations, although diet palatability issues were detected in excess of 10% inclusion [66].

Brown seaweed (*Ascophyllum nodosum*) is another prebiotic additive that has been noted for both its production and antimicrobial characteristics [61,67]. The use of Tasco-14® increased the marbling in carcasses from supplemented animals [67] and reduced fecal shedding of *E. coli* O157:H7 from 34% of the population to 7% of the population with supplementation [68], but there was no effect in *Salmonella*. However, unpublished data from the Callaway laboratory at USDA-ARS in College Station, Texas, demonstrate a small reduction in both *E. coli* O157:H7 and *Salmonella* populations *in vitro*.

5. Conclusions

The gastrointestinal tracts of humans and animals are living ecosystems teeming with diversity, and harnessing that ecology is a vital step toward a full understanding and appre-

ciation of both livestock and human health. As was stated in the beginning, an understanding of the human-animal interface is crucial to the homogeny of food safety protocols and health concerns. While most prebiotic and probiotic innovations in livestock production have sought to increase performance characteristics for maximization of potential, these ventures have often led to the discovery of novel avenues in the improvement of food safety. These new approaches to health and safety come at a crucial time when governmental regulation and public scrutiny necessitate an alteration in current practices in animal health and management. It is through the use of novel and innovative techniques that we will enhance our knowledge of the ecosystem in which we live and will forge new paths in scientific discovery and healthy living.

Author details

William B. Smith[1], Todd R. Callaway[2*], Luis O. Tedeschi[3], Francis M. Rouquette Jr.[1], Trisha Sheridan[4] and Jennifer Adamski[4]

*Address all correspondence to: todd.callaway@ars.usda.gov

1 Department of Soil and Crop Sciences, Texas A&M AgriLife Research, Overton, TX, USA

2 Food and Feed Safety Research Unit, Southern Plains Agricultural Center, Agricultural Research Service, USDA, College Station, TX, USA

3 Department of Animal Science, Texas A&M University, College Station, TX, USA

4 Nell Hodgson Woodruff School of Nursing, Emory University, Atlanta, GA, USA

References

[1] Callaway T.R., Edrington T.S., Anderson R.C., Harvey R.B., Genovese K.J., Kennedy C.N., et al. Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. Animal Health Research and Reviews. 2008;9:217-225.

[2] Hungate R.E. The Rumen and Its Microbes. New York, NY: Academic Press; 1966. 533 pp.

[3] Lu J., Idris U., Harmon B., Hofacre C., Maurer J.J., Lee M.D. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Applied and Environmental Microbiology. 2003;69:6816-6824.

[4] Arthur T.M., Bosilevac J.M., Nou X., Shackelford S.D., Wheeler T.L., Koohmaraie M. Comparison of the molecular genotypes of *Escherichia coli* O157:H7 for the hides of beef

cattle in different regions of North America. Journal of Food Protection. 2007;70(7): 1622-1626.

[5] Elder R.O., Keen J.E., Siragusa G.R., Barkocy-Gallagher G.A., Koohmarie M., Lagreid W.W. Correlation of enterohemorrhagic *Escherichia coli* O157:H7 prevalence in feces, hides, and carcasses of beef cattle during processing. Proceedings of the National Academy of Science (USA). 2000;97(7):2999-3003.

[6] Sargeant J.M., Amezcua M.R., Rajic A., Waddell L. Pre-harvest interventions to reduce the shedding of *E. coli* O157 in the faeces of weaned domestic ruminants: A systematic review. Zoonoses and Public Health. 2007;54:260-277.

[7] Janardhana V., Broadway M.M., Bruce M.P., Lowenthal J.W., Geier M.S., Hughes R.J., et al. Prebiotics modulate immune responses in the gut-associated lymphoid tissue of chickens. Journal of Nutrition. 2009;139:1404-1409.

[8] Kim G.-B., Seo Y.M., Kim C.H., Paik I.K. Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. Poultry Science. 2011;90:75-82.

[9] Elam N.A., Gleghorn J.F., Rivera J.D., Galyean M.L., Defoor P.J., Brashears M.M., et al. Effects of live cultures of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Propionibacterium freudenreichii* on performance, carcass, and intestinal characteristics, and *Escherichia coli* strain O157 shedding of finishing beef steers. Journal of Animal Science. 2003;81:2686-2698.

[10] Callaway T.R., Edrington T.S., Anderson R.C., Byrd J.A., Kogut M.H., Harvey R.B., et al. Using antimicrobial cultures, bacteriocins and bacteriophages to reduce carriage of foodborne pathogens in cattle and swine. In: LaCroix C., editor. Protective Cultures, Antimicrobial Metabolites and Bacteriophages for Food and Beverage Biopreservation. Oxford, UK: Woodhead Publishing; 2011. pp. 204-224.

[11] Callaway T.R., Edrington T.S., Loneragan G.H., Carr M.A., Nisbet D.J. Current and near-market intervention strategies for reducing Shiga-toxin producing *Escherichia coli* (STEC) shedding in cattle. Agriculture, Food and Analytical Bacteriology. 2013;3:103-120.

[12] Callaway T.R., Anderson R.C., Edrington T.S., Genovese K.J., Harvey R.B., Poole T.L., et al. Novel methods for pathogen control in livestock preharvest: An update. In: Sofos J.N., editor. Advances in Microbial Food Safety. Oxford, UK: Woodhead Publishing; 2013. pp. 275-304.

[13] Callaway, T.R., Edrington T.S., Nisbet D.J. Ecological and dietary impactors of food-borne pathogen prevalence and methods to reduce colonization in cattle. Journal of Animal Science. 2014;92:7308-7342.

[14] Steer T., Carpenter H., Tuohy K., Gibson G.R. Perspective on the role of the human gut microbiota and its modulation by pro- and prebiotics. Nutrition Research Reviews. 2000;13(2):229-254. DOI: 10.1079/095442200108729089

[15] Collins D.M., Gibson G.R. Probiotics, prebiotics, and synbiotics: Approaches for modulating the microbial ecology of the gut. American Journal of Clinical Nutrition. 1999;69:1052S-1057S.

[16] Arambel H.R., Donoghue A.M., Arsi K., Upadhyay A., Woo-Ming A., Blore P.J., et al. Chitosan supplementation reduces enteric colonization of *Campylobacter jejuni* in broiler chickens and down-regulates expression of colonization genes. Advances in Food Technology and Nutritional Sciences Open Journal. 2015;1(5):104-111.

[17] Arsi K., Donoghue A.M., Woo-Ming A., Blore P.J., Donoghue D.J. Intercloacal inoculation, an effective screening method for determining the efficacy of probiotic bacterial isolates against *Campylobacter* colonization in broiler chickens. Journal of Food Protection. 2015;78(1):209-213. DOI: 10.4315/0362-028X.JFP-14-326

[18] Arsi K., Donoghue A.M., Woo-Ming A., Blore P.J., Donoghue D.J. The efficacy of selected probiotic and prebiotic combinations in reducing *Campylobacter* colonization in broiler chickens. Journal of Applied Poultry Science. 2005;pfv032. DOI: 10.3382/japr/pfv032

[19] Doyle M.P., Erickson M.C. Reducing the carriage of foodborne pathogens in livestock and poultry. Poultry Science. 2006;85:960-973.

[20] Kemp R., Leatherbarrow A.J.H., Williams N.J., Hart C.A., Clough H.E., Turner J., et al. Prevalence and genetic diversity of *Campylobacter* spp. in environmental water samples from a 100-square-kilometer predominately dairy farming area. Applied and Environmental Microbiology. 2005;71(4):1876-1882. DOI: 10.1128/AEM.71.4.1876-1882.2005

[21] Riley L.W., Remis R.S., Helgerson S.D., McGee H.B., Wells J.G., Davis B.R. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. The New England Journal of Medicine. 1983;308(12):681-685.

[22] Bell B.P., Goldoft M., Griffin P.M., Davis M.A., Gordon D.C., Tarr P.I., et al. A multistate outbreak of *Escherichia coli* O157:H7 — Associated bloody diarrhea and hemolytic uremic syndrome from hamburgers: The Washington experience. Journal of the American Medical Association. 1994;272(17):1349-1353.

[23] Tuttle J., Gomez T., Doyle M.P., Wells J.G., Zhao T., Tauxe R.V., et al. Lessons from a large outbreak of *Escherichia coli* O157:H7 infections: Insights into the infectious dose and method of widespread contamination of hamburger patties. Epidemiology and Infection. 1999;122:185-192.

[24] LeJeune J.T., Besser T.E., Hancock D.D. Cattle water troughs as reservoirs of *Escherichia coli* O157. Applied and Environmental Microbiology. 2001;67(7):3053-3057. DOI: 10.1128/AEM.67.7.3053-3057.2001

[25] Nou, X., Rivera-Betancourt M., Bosilevac J.M., Wheeler T.L., Shackelford S.D., Gwartney B.L., et al. Effect of chemical dehairing on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and Enterobacteriaceae on carcasses in a commercial beef processing plant. Journal of Food Protection. 2003;66(11):2005-2009.

[26] FSIS. FSIS Policy on Non-intact Raw Beef Products Contaminated with E. coli O157:H7 [Internet]. January 1999 [Updated: 17 February 1999]. Available from: http://www.fsis.usda.gov/Oa/background/O157policy.htm [Accessed: 28 April 2016]

[27] USDA/FSIS. Shiga toxin-producing *Escherichia coli* in certain raw beef products. In: FSIS, editor. Federal Register. 2012.

[28] Genovese K.J., Anderson R.C., Harvey R.B., Callaway T.R., Poole D.H., Edrington T.S., et al. Competitive exclusion of *Salmonella* from the gut of neonatal and weaned pigs. Journal of Food Protection. 2003;66(8):1353-1359.

[29] Popoff M.Y., Bockemuhl J., Gheesling L.L. Supplement 2002 (no. 46) to the Kauffman-White scheme. Research in Microbiology. 2004;155:568-570.

[30] Olsen S.J., MacKinon L.C., Goulding J.S., Bean N.H., Slutsker L. Surveillance for foodborne disease outbreak — United States, 1993-1997. Morbidity and Mortality Weekly Report. 2000;49(SS01):1-51.

[31] Gould, L.H., Walsh K.A., Vieira A.R., Herman K., Williams I.T., Hall A.J., et al. Surveillance for foodborne disease outbreaks — United States, 1998-2008. Morbidity and Mortality Weekly Report. 2013;62(SS02):1-34.

[32] Antunes P., Reu C., Sousa J.C., Peixe L., Pestana N. Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. International Journal of Food Microbiology. 2003;82(2):97-103. DOI: 10.1016/S0168-1605(02)00251-9

[33] CDC. Severe *Clostridium difficile*-associated disease in populations previously at low risk — Four states, 2005. Morbidity and Mortality Weekly Report. 2005;54(47): 1201-1205.

[34] Weese J.S., Avery B.P., Rousseau J., Reid-Smith R. Detection and enumeration of *Clostridium difficile* spores in retail beef and pork. Applied and Environmental Microbiology. 2009;75(15):5009-5011. DOI: 10.1128/AEM.00480-09

[35] Mitsch P., Zitterl-Eglseer K., Kohler B., Gabler C., Losa R., Zimpernik I. The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens. Poultry Science. 2004;83(4):669-675. DOI: 10.1093/pa/83.4.669

[36] CDC. Multistate outbreak of listeriosis linked to Blue Bell Creameries products (final update) [Internet]. [Updated: 10 June 2015]. Available from http://www.cdc.gov/listeria/outbreaks/ice-cream-03-15/ [Accessed: 31 January 2016]

[37] Farber J. M., Peterkin P.I. *Listeria monocytogenes*, a food-borne pathogen. Microbiology and Molecular Biology Review. 1991;55(3):476-511.

[38] Skovgaard N., Morgen C.-A. Detection of *Listeria* spp. in faeces from animals, in feeds, and in raw foods of animal origin. International Journal of Food Microbiology. 1988;6(3):229-242. DOI: 10.1046/0168-1605(88)90015-3

[39] Deiters C., Gunnewig V., Friedrich A.W., Mellmann A., Kock R. Are cases of methicillin-resistant *Staphylococcus aureus* clonal complex (CC) 398 among humans still livestock-associated? International Journal of Medical Microbiology. 2015;305:110-113.

[40] Hasman H., Moodley A., Guardabassi L., Stegger M., Skov R.L., Aarestrup F.M. *spa* type distribution in *Staphylococcus aureus* originating from pigs, cattle and poultry. Veterinary Microbiology. 2010;141(3-4):326-331. DOI: 10.1016/j.vetmic.2009.09.025

[41] Barkema H.W., Schukken Y.H., Zadoks R.N. Invited review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. Journal of Dairy Science. 2006;89(6):1877-1895. DOI: 10.3168/jds.S0022-0302(06)72256-1

[42] Fuller R. Probiotics in man and animals. Journal of Applied Bacteriology. 1989;66:365-378.

[43] Bielke L.R., Elwood A.L., Donoghue A.M., Newberry L.A., Neighbor N.K., Hargis B.M. Approach for selection of individual enteric bacteria for competitive exclusion in turkey poults. Poultry Science. 2003;82:1378-1382.

[44] Nurmi E., Rantala M. New aspects of *Salmonella* infection in broiler production. Nature. 1973;241:210-211.

[45] Baker A.A., Davis E., Rehberger T., Rosener D. Prevalence and diversity of toxigenic *Clostridium perfringens* and *Clostridium difficile* among swine herds in the Midwest. Applied and Environmental Microbiology. 2010;76:2961-2967.

[46] Beauchemin K.A., Yang W.Z., Morgavi D.P., Ghorbani G.R., Kautz W., Leedle J.A.Z. Effects of bacterial direct-fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle. Journal of Animal Science. 2003;81:1628-1640.

[47] Lehloenya K.V., Stein D.R., Allen D.T., Selk G.E., Jones D.A., Aleman M.M., et al. Effects of feeding yeast and propionibacteria to dairy cows on milk yield and components, and reproduction. Journal of Animal Physiology and Animal Nutrition. 2007;92:190-202. DOI: 10.1111/j.1439-0396.2007.00726.x

[48] Cai L., Indrakumar S., Kiarie E., Kim I.H. Effects of a multi-strain *Bacillus* species-based direct-fed microbial on growth performance, nutrient digestibility, blood profile, and gut health in nursery pigs fed corn-soybean meal-based diets. Journal of Animal Science. 2015;93:4336-4342. DOI: 10.2527/jas2015-9056

[49] Puphan K., Sornplang P., Uriyapongson S., Navanukraw C. Screening of lactic acid bacteria as potential probiotics in beef cattle. Pakistan Journal of Nutrition. 2015;14(8): 474-479.

[50] Brashears M.M., Galyean M.L., Loneragan G.H., Mann J.E., Killinger-Mann K. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given *Lactobacillus* direct-fed microbials. Journal of Food Protection. 2003;66:748-754.

[51] Wisener L.V., Sargeant J.M., O'Connor A.M., Faires M.C., Glass-Kaastra S.K. The use of direct-fed microbials to reduce shedding of *Escherichia coli* O157 in beef cattle: A systematic review and meta-analysis. Zoonoses and Public Health. 2014;62:75-89. DOI: 10.1111/zph.12112

[52] Brashears M.M., Jaroni D., Trimble J. Isolation, selection, and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of *Escherichia coli* O157:H7 in cattle. Journal of Food Protection. 2003;66(3):355-363.

[53] Nurmi E., Nuotio L., Schneitz C. The competitive exclusion concept: Development and future. International Journal of Food Microbiology. 1992;15(3-4):237-240. DOI: 10.1016/0168-1605(92)90054-7

[54] Nava G.M., Bielke L.R., Callaway T.R., Castaneda M.P. Probiotic alternatives to reduce gastrointestinal infections: The poultry experience. Animal Health Research Reviews. 2005;6(1):105-118. DOI: 10.1079/AHR2005103

[55] Menconi A., Morgan M.J., Pumford N.R., Hargis B.M., Tellez G. Physiological properties and *Salmonella* growth inhibition of probiotic *Bacillus* strains isolated from environmental and poultry sources. International Journal of Bacteriology. 2013;958408:1-8. DOI: http://dx.doi.org/10.1155/2013/958408

[56] Hong H.A., Duc L.H., Cutting S.M. The use of bacterial spore formers as probiotics. FEMS Microbiology Review. 2005;29:813-835.

[57] Baker A.A., Davis E., Spencer J.D., Moser R., Rehberger T. The effect of a *Bacillus*-based direct-fed microbial supplemented to sows on the gastrointestinal microbiota of their neonatal piglets. Journal of Animal Science. 2013;91:3390-3399. DOI: 10.2527/jas2012-5821

[58] Anderson R.C., Stanker L.H., Young C.R., Buckley S.A., Genovese K.J., Harvey R.B., et al. Effect of competitive exclusion treatment on colonization of early-weaned pigs by *Salmonella* serovar Choleraesuis. Swine Health and Production. 1999;7(4):155-160.

[59] Wang Z., Chai W., Burwinkel M., Twardziok S., Wrede P., Palissa C., et al. Inhibitory influence of *Enterococcus faecium* on the propagation of Swine Influenza A virus *in vitro*. PLoS One. 2013;8(1):e54043. DOI: 10.1371/journal.pone.0053043

[60] Davis M.E., Parrott T., Brown D.C., de Rodas B.Z., Johnson Z.B., Maxwell C.V., et al. Effect of a *Bacillus*-based direct-fed microbial feed supplement on growth performance

and pen cleaning characteristics of growing-finishing pigs. Journal of Animal Science. 2008;86:1459-1467. DOI: 10.2527/jas.2007-0603

[61] Crossland W.L., Callaway T.R., Tedeschi L.O. Shiga toxin-producing *E. coli* and ruminant diets: A match made in heaven? In: Ricke S.C., Donaldson J.R., Phillips C.A, editors. Food safety: Emerging issues, technologies and systems. London, UK: Elsevier; 2015. pp. 185-213.

[62] Cowan M.M. Plant products as antimicrobial agents. Clinical Microbiological Reviews. 1999;12(4):564-582. DOI: 0893-8512/99/$04.00+0

[63] Berard N.C., Holley R.A., McAllister T.A., Ominski K.H., Wittenberg K.M., Bouchard K.S., et al. Potential to reduce *Escherichia coli* shedding in cattle feces by using sainfoin (*Onobrychis viciifolia*) forage, tested in vitro and in vivo. Applied and Environmental Microbiology. 2009;75(4):1074-1079. DOI: 10.1128/AEM.00983-08

[64] Tedeschi L.O., Callaway T.R., Muir J.P., Anderson R.C. Potential environmental benefits of feed additives and other strategies for ruminant production. Revista Brasileira de Zootecnia. 2011;40:291-309.

[65] Callaway T.R., Carroll J.A., Arthington J.D., Edrington T.S., Rossman M.L., Carr M.A., et al. *Escherichia coli* O157:H7 populations in ruminants can be reduced by orange peel product feeding. Journal of Food Protection. 2011;74(11):1917-1921.

[66] Callaway T.R., Carroll J.A., Arthington J.D., Edrington T.S., Anderson R.C., Rossman M.L., et al. Orange peel products can reduce *Salmonella* populations in ruminants. Foodborne Pathogens and Disease. 2011;8(10):1071-1075.

[67] Anderson M.J., Blanton Jr. J.R., Gleghorn J., Kim S.W., Johnson J.W. *Ascophyllum nodosum* supplementation strategies that improve overall carcass merit of implanted English crossbred cattle. Asian-Australasian Journal of Animal Science. 2006;19(10): 1514-1518. DOI: 10.5713/ajas.2006.1514

[68] Braden K.W., Blanton Jr. J.R., Allen V.G., Pond K.R., Miller M.F. *Ascophyllum nodosum* supplementation: A preharvest intervention for reducing *Escherichia coli* O157:H7 and *Salmonella* spp. in feedlot steers. Journal of Food Protection. 2004;67(9):1824-1828.