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REVIEW
Shiga Toxin-Producing *Escherichia coli* (STEC) Ecology in Cattle and Management Based Options for Reducing Fecal Shedding

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ABSTRACT

Cattle can be naturally colonized with foodborne pathogenic bacteria such as Shiga Toxin-producing *E. coli* (STEC) in their gastrointestinal tract. While these foodborne pathogens are a threat to food safety, they also cause human illnesses via cross contamination of other foods and the water supply, as well as via direct animal contact. In order to further curtail these human illnesses and ensure a safe and wholesome food supply, research into preharvest pathogen reduction controls and interventions has grown in recent years. This review addresses the ecology of STEC in cattle and potential controls and interventions that have been proposed or implemented to reduce STEC in cattle. We focus in this review on the use of management practices and the effects of diet and water management. Implementation of preharvest strategies will not eliminate the need for good sanitation procedures in the processing plant and during food preparation and consumer handling. Instead, live-animal management interventions must be implemented as part of a multiple-hurdle approach that complements the in-plant interventions, so that the reduction in pathogen entry to the food supply can be maximized.

Keywords: *E. coli* O157:H7, EHEC, cattle, management

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INTRODUCTION

One of the largest food safety (and economic) impacts on the cattle industry has been the emergence of Shiga Toxin-producing *Escherichia coli* (STEC) bacteria, which are part of the natural reservoir in ruminant animals such as cattle (Karmali *et al.*, 2010). STEC-caused illnesses cost the American economy more than $1 billion each year in direct and indirect costs from more than 175,000 human illnesses (Scallan *et al.*, 2011; Scharff, 2010). Furthermore, since the emergence of the "poster child" of STEC, *E. coli*

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O157:H7, more than $2 billion dollars have been spent by the cattle industry to combat STEC in processing plants (Kay, 2003).

While post-harvest pathogen-reduction strategies have been largely successful at reducing direct foodborne illness, these processing interventions have not been perfect (Arthur *et al.*, 2007; Barkocy-Gallagher *et al.*, 2003), in large part because avenues of human exposure include indirect routes (LeJeune and Kersting, 2010; Nastasijevic, 2011). In order to further curtail human illnesses and ensure a safe and wholesome food supply, research into preharvest pathogen reduction controls and interventions has grown in recent years (Callaway *et al.*, 2004; LeJeune and Wetzel, 2007; Oliver *et al.*, 2008; Sargeant *et al.*, 2007).

The impact of using pathogen reduction strategies focused on the environmental contamination and exposure routes at the live animal stage are likely to have large impacts on resulting human illnesses (Rotariu *et al.*, 2012; Smith *et al.*, 2012). Reduction of STEC can also yield public health improvements in rural communities (LeJeune and Kersting, 2010) and amongst attendees of agricultural fairs, rodeos and open farms (Keen *et al.*, 2006; Lanier *et al.*, 2011). Thus, the logic underlying focusing on reducing foodborne pathogenic bacteria in live cattle is straightforward: 1) reducing the amount of pathogens entering processing plants will reduce the burden on the plants and render the in-plant interventions more effective; 2) reducing horizontal pathogen spread from infected animals (especially in "supershedders") in transport and lairage; 3) will reduce the pathogenic bacterial burden in the environment and wastewater streams; and 4) will reduce the direct risk to those in direct contact with animals via petting zoos, open farms, rodeos and to animal workers. This review addresses the microbial ecology of STEC colonization of cattle and controls and interventions that have been proposed or implemented to reduce STEC in live cattle in the areas of: 1) Management practices and transport, and 2) Cattle water and feed management.

EHEC, STEC, VTEC AND NON-O157:H7'S: A PRIMER

Although the relatively recent (1982) emergence of *E. coli* O157:H7 into public view makes it seem that this organism is a new arrival in the food chain, data indicates that this organism is far more ancient, having arisen between 400 and 70,000 years ago (Law, 2000; Riley *et al.*, 1983; Wick *et al.*, 2005; Zhou *et al.*, 2010). Although a variety of acronyms have been applied to the "hamburger bug", they belong to a single group that acquired toxin genes from *Shigella* via a gene transfer event (Kaper *et al.*, 2004; Karmali *et al.*, 2010; Wick *et al.*, 2005). Researchers refer to these pathogens often interchangeably as Enterohemorrhagic *E. coli* (EHEC), Shiga toxin-producing *E. coli* (STEC), or Verotoxin-producing *E. coli* (VTEC). While *E. coli* O157:H7 was the first of the STEC's to be recognized as a major food safety threat, recently the other "non O157:H7 STEC" have been increasingly implicated in human illness outbreaks (Bettelheim, 2007; Fremaux *et al.*, 2007). Because of this linkage, the "gang of six" non-O157 serogroups (O26, O45, O103, O111, O121, and O145) have joined O157:H7 as being classified as adulterants in beef (USDA/FSIS, 2012). Since this declaration, focus has shifted on understanding the generalized STEC ecology, rather than simply focusing on *E. coli* O157:H7 (Gill and Gill, 2010).

For years, researchers (including the present authors) assumed that in general, all of the non-O157 STEC would behave similarly to O157:H7 in a physiological and ecological sense. However, recent research has found that in addition to the genetic divergence seen in O157:H7 lineages (Zhang *et al.*, 2007), there appear to be significant physiological differences between and within non-O157 STEC which may play a role in the ecological niche occupied in the ruminant gastrointestinal tract by these non-O157 serotypes (Bergholz and Whittam, 2007; Free *et al.*, 2012; Fremaux *et al.*, 2007). While these and other physiological differences need to be investigated further, and their roles in the gastrointestinal microbial population must be determined, it appears that the O157:H7 serotype is well adapted to survive

in cattle (García *et al.*, 2010; O'Reilly *et al.*, 2010) and that other STEC serotypes can also live in the gastrointestinal tract of cattle (Arthur *et al.*, 2002; Chase-Topping *et al.*, 2012; Joris *et al.*, 2011; Monaghan *et al.*, 2011; Polifroni *et al.*, 2012; Thomas *et al.*, 2012), and be transferred into ground beef (Bosilevac and Koohmaraie, 2011; Fratamico *et al.*, 2011).

While we understand much of how *E. coli* O157:H7 behaves in the gastrointestinal tract and farm environment, we know very little about the ecology of other STEC in those environments (Monaghan *et al.*, 2011; Polifroni *et al.*, 2012). Thus this review focuses on pre-harvest pathogen interventions based upon *E. coli* O157:H7 data. We hypothesize that non-O157:H7 STEC will largely behave in a broadly similar fashion to *E. coli* O157:H7 in the gastrointestinal and farm environment; however this is an educated assumption and that imposed limitation must be understood. Thus readers must be aware that most of the research referred to in this review is based upon *E. coli* O157:H7 specifically, and may or may not apply to all STEC.

ECOLOGY OF STEC AND GASTROINTESTINAL COLONIZATION

Because *E. coli* O157:H7 (and to some degree non-O157 STEC) co-evolved along with its host it is uniquely well-fitted to survive in the gastrointestinal tract of cattle as a commensal type organism (Law, 2000; Wick *et al.*, 2005). While *E. coli* O157:H7 can live in the rumen of cattle (Rasmussen *et al.*, 1999), the site of primary colonization is the terminal rectum (Naylor *et al.*, 2003; Smith *et al.*, 2009a). This organism produces a potent cytotoxin (Shiga toxin) that does not seriously impact its preferred host (cattle) because they lack toxin receptors (Pruimboom-Brees *et al.*, 2000), but this same toxin causes serious illness in humans colonized by *E. coli* O157:H7 (Karmali *et al.*, 2010; O'Brien *et al.*, 1992). Unfortunately, this means that the natural commensal-type relationship between STEC (including O157 and non-O157) and cattle ensures that this organism can be passed on to meat products and consumers of beef (Ferens and Hovde, 2011). This transmission most frequently occurs during summer months, and is linked to a summer increase in the prevalence of *E. coli* O157:H7 in cattle (Edrington *et al.*, 2006a; Lal *et al.*, 2012; Naumova *et al.*, 2007; Ogden *et al.*, 2004; Wells *et al.*, 2009), not just an increase in consumption or a change of cooking habits by consumers (Money *et al.*, 2010; Williams *et al.*, 2010a). It has been suggested that neuroendocrine factors may play a role in *E. coli* O157:H7 (Edrington *et al.*, 2006a; Green *et al.*, 2004), as may signaling between host and intestinal microbial populations or within STEC populations via quorum-sensing (Edrington *et al.*, 2009b; Sperandio, 2010; Sperandio *et al.*, 2001). Further possible interactions within the microbial ecosystem of the rumen are demonstrated in the preferential consumption of *E. coli* O157:H7 by ruminal protozoa (*Epidinium*), and increased populations in the presence of *Dasytricha* (Stanford *et al.*, 2010).

Because of the nature of STEC survival in the ruminant gut, it is no surprise that it persists in fecal deposits (Dargatz *et al.*, 1997; Jiang *et al.*, 2002; Maule, 2000; Yang *et al.*, 2010) and in soils (Bolton *et al.*, 2011; Semenov *et al.*, 2009; Van Overbeek *et al.*, 2010). This allows *E. coli* O157:H7 to cycle within pens and farms in a fecal-oral route (Russell and Jarvis, 2001), recirculating within groups or individual animals (Arthur *et al.*, 2010). The presence of supershedding cattle (Chase-Topping *et al.*, 2008) in the population can further enhance this horizontal transmission within a herd or a pen of cattle (Arthur *et al.*, 2010; Arthur *et al.*, 2009; Cobbold, 2007; LeJeune and Kauffman, 2006). However, the host/dietary/microbial factors underlying the "supershedder" status of cattle remains unknown, as do factors that allow simple gut colonization by *E. coli* O157:H7. Thus it is apparent that the farm/pen/facility environment plays an important role in STEC colonization and recirculation, as well as via direct and indirect transmission to human farm workers/visitors and consumers (Ihekweazu *et al.*, 2012; LeJeune and Kersting, 2010; Smith *et al.*, 2012; Stacey *et al.*, 2007; Strachan *et al.*, 2006).

MANAGEMENT PRACTICES AND TRANSPORTATION

Good management of cattle is critical for efficient animal production, but to date no typical "management" procedures have been shown to affect colonization or shedding of foodborne pathogens (Ellis-Iversen *et al.*, 2008; Ellis-Iversen and Van Winden, 2008; LeJeune and Wetzel, 2007), some practices may reduce horizontal transmission and recirculation of STEC within a herd of cattle (Ellis-Iversen and Watson, 2008). However, the use of management tools like the squeeze chute (crush) to process cattle has been shown to increase the odds of hide contamination with *E. coli* O157(Mather *et al.*, 2007). Yet, in spite of this lack of evidence regarding impacts on food safety, good management practices are critical to ensuring animal health and welfare (Morrow-Tesch, 2001).

Bedding and pen surfaces

E. coli O157:H7 can live for a long period of time in manure, soil and other organic materials (Jiang *et al.*, 2002; Maule, 2000; Winfield and Groisman, 2003) and can be transmitted successively through their environment (Semenov *et al.*, 2010; Semenov *et al.*, 2009). Cattle, especially dairy cows, are bedded on materials that are largely chosen on animal health and welfare grounds. Unfortunately, bedding material can harbor bacteria that are responsible for mastitis, as well as foodborne pathogenic bacteria that can be spread between cattle (Davis *et al.*, 2005; Oliver *et al.*, 2005; Richards *et al.*, 2006; Wetzel and LeJeune, 2006). Researchers have shown that urine increases growth of *E. coli* O157:H7 on bedding, potentially by providing substrate for growth (Davis *et al.*, 2005). Modeling research has shown that an increase in bedding cleaning frequency would increase the death rate of *E. coli* O157:H7 (Vosough Ahmadi *et al.*, 2007). Further studies have demonstrated that the use of very dry bedding reduced *E. coli* O157:H7 prevalence on farms (Ellis-Iversen *et al.*, 2008; Ellis-Iversen and Van Winden, 2008). Researchers have shown that sand bedding reduced transmission of *E. coli* O157:H7 between dairy cows, resulting in lower populations of *E. coli* O157:H7 in cattle bedded on sand compared to sawdust (LeJeune and Kauffman, 2005; Westphal *et al.*, 2011). It is suspected that this difference was due to desiccation or reduced nutrient availability.

Feedlot surfaces were thought to contain manure-like bacterial populations, but recent molecular studies have indicated that the bacterial communities of feedlot surfaces are complex, yet utterly distinct from fecal bacterial populations (Durso *et al.*, 2011). This suggests that traits that favor survival in the gastrointestinal tract (anaerobic, warm, dark) do not favor survival on the feedlot surface (aerobic, cooler, sunlit). Surfaces such as pond ash do not impact survival of *E. coli* O157 (Berry *et al.*, 2010), however studies and anecdotal evidence indicates that a greater number of cattle shed *E. coli* O157:H7 when housed in muddy pen conditions than cattle from pens in normal condition and that the condition of the pen floor may influence the prevalence of cattle shedding the organism and the ability of *E. coli* O157:H7 to survive dry conditions (Berry and Miller, 2005; Smith *et al.*, 2001; Smith *et al.*, 2009b). Studies have recently demonstrated that sunlight can reduce *E. coli* O157:H7 populations on pen surfaces (Berry and Wells, 2012) and in water systems (Jenkins *et al.*, 2011). Overall, bedding or pen cleaning will not eliminate *E. coli* O157:H7 from any farm or feedlot environment, but it may slow spread within a herd or between penmates.

Manure Management

E. coli O157:H7 and other STEC survive in manure and can persist for a lengthy period of time (up to 21 months) (Bolton *et al.*, 2011; Fremaux *et al.*, 2007; Hutchison *et al.*, 2005; Kudva *et al.*, 1998; Varel *et al.*, 2008). Although there are differences amongst STEC strains in their ability to persist in manure, these appear to be related to the oxidative capacity of each strain (Franz *et al.*, 2011). The presence of a native bacterial population in manure reduces *E. coli* O157:H7 survival in soils (Van Overbeek *et al.*, 2010). The amendation of manure in soil can result in

STEC uptake directly by plants, including food crops (Franz and Van Bruggen, 2008; Jiang *et al.*, 2002; Semenov *et al.*, 2010; Semenov *et al.*, 2009). Rainfall events can also wash STEC from cattle feces (stored or in fields) into drinking or irrigation water supplies (Anonymous, 2000; Cook *et al.*, 2011; Ferguson *et al.*, 2007; Oliveira *et al.*, 2012; Pachepsky *et al.*, 2011). As the mean temperature of manure rises during storage the survival of *E. coli* O157:H7 is reduced (Semenov *et al.*, 2007), indicating that composting can enhance manure safety, thus reducing human illnesses (Graham and Nachman, 2010; Kelley *et al.*, 1999).

There have been few studies that have isolated STEC consistently from cattle waste lagoons (Purdy *et al.*, 2010). This is potentially due to the oxidized nature of the lagoon, or the presence of a native microbial population. In waste water lagoons, there are protozoa that preferentially consume *E. coli* O157:H7 (Ravva *et al.*, 2010), possibly explaining at least some of the difference between *E. coli* O157:H7 survival in manure with that of limited survival in dairy lagoons (Ravva *et al.*, 2006). Research has demonstrated that the addition of chemical oxidants to wastewater lagoons can reduce pathogen populations (Luster-Teasley *et al.*, 2011).

Biosecurity

Farm biosecurity is critical for animal health and welfare, especially in regard to animal diseases, but to date there has been little direct impact demonstrated on foodborne pathogenic bacteria such as *E. coli* O157:H7 (Ellis-Iversen and Van Winden, 2008). Research has shown that other animal species, rodents, insects and birds and boars can carry STEC at least transiently (Branham *et al.*, 2005; Cernicchiaro *et al.*, 2012; French *et al.*, 2010; Rice *et al.*, 2003; Sánchez *et al.*, 2010; Wetzel and LeJeune, 2006). Mixing of sheep with cattle has been shown to increase the risk of cattle shedding STEC (Stacey *et al.*, 2007), and a positive correlation between cattle and sheep density was found, at least in the UK (Strachan *et al.*, 2001). Other diverse factors such as the presence of dogs, pigs, or wild geese on the farm have been linked to an increased risk of *E. coli* O157:H7 shedding (Gunn *et al.*, 2007; Synge *et al.*, 2003). Ruminant animals other than cattle do carry *E. coli* O157:H7 (French *et al.*, 2010; Hussein *et al.*, 2000; Sargeant *et al.*, 1999), and this includes sheep and deer that often share the same pasture, feed bunks and water supplies (Bolton *et al.*, 2012; Branham *et al.*, 2005). Other researchers have found that flies and other insects on farms can carry STEC from one location to another (Ahmad *et al.*, 2007; Hancock *et al.*, 1998; Keen *et al.*, 2006; Talley *et al.*, 2009). Furthermore, wild migratory birds such as starlings (Carlson *et al.*, 2011a; Carlson *et al.*, 2011b; Cernicchiaro *et al.*, 2012; Wallace *et al.*, 1997; Wetzel and LeJeune, 2006), cowbirds and egrets (Callaway, unpublished data) can carry STEC (and other foodborne pathogen) between pens, and even between farms long distances apart. While these effects are probably minimal in their direct impact on food safety within a farm, they represent vectors for pathogens to move between "clean" groups of cattle or farms.

Cattle grouping

Many farms are closed to entry by animals from other farms to prevent animal disease transmission. Closed herds prevent spread of *E. coli* O157:H7 (and other pathogens) from one farm to another (Ellis-Iversen *et al.*, 2008; Ellis-Iversen and Van Winden, 2008; Ellis-Iversen and Watson, 2008). However some studies have shown that a closed farm does not impact *E. coli* O157:H7 incidence on farms (Cobbaut *et al.*, 2009). The results of this study suggest that *E. coli* O157:H7 should be considered common to groups of feedlot cattle housed together in pens (Smith *et al.*, 2001), thus keeping groups together throughout their time on a farm, or in a feedlot, without introducing new members to groups appears to reduce horizontal transmission between animals.

A further benefit of grouping cattle involves the use of age as a segregating factor. Young cattle (especially heifers) shed more *E. coli* O157:H7 than do older cattle (Cobbaut *et al.*, 2009; Cray and Moon, 1995; Smith *et al.*, 2001). While it is not possible to segregate calves from cows in the beef industry, there is potential benefit to keeping same-age

groups of calves together as they are transported and enter backgrounding or feedlot operations to prevent horizontal transmission between groups. Off-site rearing of dairy heifers may be an important solution to reducing foodborne pathogens, as has been shown in regard to *Salmonella* (Hegde *et al.*, 2005), and the risk of transmission back to the farm by heifers returning from an off-site facility was found to be low (Edrington *et al.*, 2008).

Animal density may also play a role in the horizontal spread of *E. coli* O157:H7 and other foodborne pathogens (Vidovic and Korber, 2006) as well as the vertical spread to humans (Friesema *et al.*, 2011b). Densely packed animals have a great chance of contamination with fecal spread. However increased animal density reduces the physical footprint and may allow for more efficient and effective waste handling. It has been shown that higher animal density can be linked to an increased risk of carriage of some STEC, including O157:H7 (Frank *et al.*, 2008; Vidovic and Korber, 2006). Other European studies have also found an effect of animal density on human STEC illnesses (Friesema *et al.*, 2011a; Haus-Cheymol *et al.*, 2006), yet Canadian researchers found a variable impact (Pearl *et al.*, 2009). Further studies found that increased stocking density increased shedding of STEC, independent of group size (Stacey *et al.*, 2007; Strachan *et al.*, 2006).

The issue of "supershedders" complicates research into effects of animal density and pathogen shedding (Arthur *et al.*, 2009; Cernicchiaro *et al.*, 2010; Chen *et al.*, 2012; LeJeune and Kauffman, 2006; Stanford *et al.*, 2005). If supershedders do exist long term, rather than simply being a transient phase of infection, then there are interactive effects of animal density and pathogen density in the animal that must be accounted for (Matthews *et al.*, 2006; Matthews *et al.*, 2009). The role of super-shedding animals (even if a transient phenomenon) cannot be discounted in the contamination of hides during transport and lairage, especially in dense conditions (Arthur *et al.*, 2010; Arthur *et al.*, 2009).

Transportation and lairage

Handling and transport to processing plants or feedlots or other farms causes stress (see below) and may spread *E. coli* O157:H7 due to physical contact or fecal contamination, and trailers used may spread pathogens between lots or loads of cattle (Mather *et al.*, 2007). Studies have indicated that transport did not affect STEC populations in cattle, however in these studies *E. coli* O157:H7 populations were very low initially (Barham *et al.*, 2002; Minihan *et al.*, 2003; Reicks *et al.*, 2007; Schuehle Pfeiffer *et al.*, 2009). However, other studies have found that transport caused an increase in fecal shedding of *E. coli* O157:H7 (Bach *et al.*, 2004). Researchers found that transporting cattle more than 100 miles doubled the risk of having positive hides at slaughter compared to cattle shipped a short distance, though the question of time in close-confinement versus being in transit was not examined (Dewell *et al.*, 2008). In another study, longer transport times were correlated with increased levels of fecal shedding of *E. coli* O157:H7 (Bach *et al.*, 2004). It was also demonstrated that a combination of transport and lairage did not lead to an increase in the number or prevalence of *E. coli* O157:H7 from cattle (Fegan *et al.*, 2009).

The presence of a high shedding animal in a trailer has been shown to increase the odds of other animals within the load being hide-positive for *E. coli* O157:H7 (Arthur *et al.*, 2010; Arthur *et al.*, 2009; Fox *et al.*, 2008). However, it should be noted that both low- and high-shedding cattle can be responsible for the spread within and between truckloads (Dodd *et al.*, 2010). Cattle trailers can be important fomites of *E. coli* O157:H7 to uninfected cattle and are frequently positive for *E. coli* O157:H7 when sampled (Barham *et al.*, 2002; Cuesta Alonso *et al.*, 2007; Reicks *et al.*, 2007). It has been shown that the incidence of *E. coli* O157:H7 in transport trailers increases the risk of transmission to farms and feedlots from cattle on these trailers (Cuesta Alonso *et al.*, 2007). To date however, the washing of trailers has only been shown to be effective against *Salmonella* contamination in swine (Rajkowski *et al.*, 1998), yet it is an intuitive, feasible solution to prevent some degree of cross-contamination of cattle during a stressful period.

Lairage and holding facilities are further locations that can impact the prevalence and concentration of *E. coli* O157:H7 on hides of cattle, which is an important route of entry to the food supply (Arthur *et al.*, 2007). Studies have shown that the transfer of *E. coli* O157:H7 to hides that occurs in lairage at processing plants accounted for more of the hide and carcass contamination than did the population of cattle leaving the feedlot (Arthur *et al.*, 2008). Furthermore, the presence of supershedding cattle in these pens can increase the spread of *E. coli* O157:H7 between animals from different farms or feedlots (Arthur *et al.*, 2010; Cernicchiaro *et al.*, 2010). The exact role of lairage and transport/trailers in the spread of *E. coli* O157:H7 (and other pathogens) in cattle is unclear, and is likely time- and animal density-dependent, and may also be affected by stress.

Stress

While we understand stress intuitively, any discussion of "stress" in animals is fraught with anthropomorphism and complexity (Rostagno, 2009; Verbrugghe *et al.*, 2012). Long-term stress may depress immune function in cattle (Carroll and Forsberg, 2007; Kelley, 1980; Salak-Johnson and McGlone, 2007), making them more susceptible to colonization, but the short term effects of stress from weaning, handling or transport on immune status are unknown. Catecholamines rise when animals are under stress, and catecholamines (along with other hormones) have been demonstrated to have an effect on the microbial population, including pathogens (Freestone and Lyte, 2010; Lyte, 2010; Walker and Drouillard, 2012). To date the effect of stress on colonization or shedding of *E. coli* O157:H7 is unclear.

Weaning is stressful to calves, and was shown to increase colonization with STEC (Herriott *et al.*, 1998) and *E. coli* O157:H7 (Chase-Topping *et al.*, 2007). In other studies however, these researchers demonstrated that weaning does not affect the likelihood of shedding (Synge *et al.*, 2003). Interestingly, calving was seen to reduce the likelihood of *E. coli* O157:H7 shedding (Synge *et al.*, 2003). Further studies found that weaning stresses alone did not impact shedding of *E. coli* O157:H7 in dairy calves (Edrington *et al.*, 2011). Other stresses such as movement have been identified as playing a role in *E. coli* O157:H7 shedding in Scottish cattle (Chase-Topping *et al.*, 2007), but this has not been clearly defined in U.S. cattle systems.

When calves were preconditioned to transport stress, they were found to be less susceptible to infection from the environment than were calves not preconditioned to this stressor (Bach *et al.*, 2004). Cattle with excitable temperaments were less likely to shed *E. coli* O157:H7 than were "calm" cattle (Brown-Brandl *et al.*, 2009; Schuehle Pfeiffer *et al.*, 2009). In studies with pigs, it was found that the social stress/excitement of mixing penmates increased fecal shedding of *Salmonella* (Callaway *et al.*, 2006), but this has not been shown to date in cattle, however this implies a potential role of social stresses in cattle during lairage.

Heat stress (and methods to alleviate it) can have effects on animal health and productivity (Brown-Brandl *et al.*, 2003), as well as shedding of *E. coli* O157:H7 and *Salmonella* (Callaway *et al.*, 2006). Water sprinkling to alleviate heat stress in cattle increased measures of animal well-being and decreased *E. coli* O157:H7 populations on the hides of cattle, but did not affect fecal populations (Morrow *et al.*, 2005). In another study with dairy cattle, researchers found that heat stress had no impact on STEC shedding, but *Salmonella* shedding was increased (Edrington *et al.*, 2004). Other researchers have also found that heat stress did not impact *E. coli* O157:H7 shedding in cattle (Brown-Brandl *et al.*, 2009).

CATTLE WATER AND FEED MANAGEMENT

Diet and water supplies can be used to reduce horizontal transmission of STEC between animals on the same farm or in the same feedlot pen. The underlying biology behind these effects has not been elucidated to this point, but it has been suggested that difference could be due to increased fecal pH or

intermediate endproducts of the yeast fermentation (e.g., vitamins, organic acids, L-lactic acid), however these suggestions remain hypothetical (Wells *et al.*, 2009). While the magnitude of the dietary impacts effects is relatively small, it underlines the point that dietary composition can potentially significantly impact *E. coli* O157:H7 populations in the gut of cattle.

Drinking Water treatments

Cattle water troughs can harbor *E. coli* O157:H7 for long periods of time (Hancock *et al.*, 1998; LeJeune *et al.*, 2001a; LeJeune *et al.*, 2001b; Murinda *et al.*, 2004; Polifroni *et al.*, 2012; Wetzel and LeJeune, 2006), and as many as 25% of cattle farm water samples have been shown to contain *E. coli* O157:H7 (Sanderson *et al.*, 2006). These results suggest that these common-use troughs can be vectors for horizontal transmission of *E. coli* O157:H7 within a group of animals. The organic material in the water troughs tends to harbor and protect the STEC, and modeling research has shown that an increase in water trough cleaning frequency would increase the death rate of *E. coli* O157:H7 (Vosough Ahmadi *et al.*, 2007) as well as exposure to sunlight (Jenkins *et al.*, 2011). Chlorination of water supplies has long been used to reduce bacterial populations in municipal water supplies, and this also can be used in cattle water troughs to reduce *E. coli* O157:H7 populations. However, sunlight and organic material in the water reduces the effectiveness of chlorination over time, as has been seen in real world chlorination studies with cattle water troughs (LeJeune *et al.*, 2004). Electrolyzed oxidizing (EO) water has been shown to reduce STEC populations as high as 10^4 CFU/mL (Stevenson *et al.*, 2004), and can be used as an in-plant hide cleaning strategy (Bosilevac *et al.*, 2005). Other treatments such as cinnamaldehyde and sodium caprylate addition to water supplies have been shown to reduce STEC populations, but the effects on palatability are not currently known (Amalaradjou *et al.*, 2006; Charles *et al.*, 2008).

Fasting

Cattle can be fasted for up to 48 h before and during their transport to slaughter, which can affect the prevalence of *E. coli* O157:H7 (Pointon *et al.*, 2012). Ruminal and intestinal VFA concentrations limit the proliferation of *E. coli* because of toxicity of the VFA to the bacteria (Hollowell and Wolin, 1965; Russell and Diez-Gonzalez, 1998; Wolin, 1969). This has created the demand for the use of organic acids/VFA as methods to alter the ruminal fermentation and to reduce pathogen populations in the gut (Ohya *et al.*, 2000; Prohaszka and Baron, 1983; Van Immerseel *et al.*, 2006). However, fasting causes levels of VFA to decline rapidly (Harmon *et al.*, 1999).

Fasting increased *E. coli*, *Enterobacter* and total anaerobic bacterial populations throughout the intestinal tract of cattle (Buchko *et al.*, 2000b; Gregory *et al.*, 2000), and increased *Salmonella* and *E. coli* populations in the rumen (Brownlie and Grau, 1967; Grau *et al.*, 1969). More recent research has demonstrated that fasting can cause "apparently *E. coli* (O157:H7) negative animals to become positive" (Kudva *et al.*, 1995). Fasting made calves more susceptible to colonization by inoculated *E. coli* O157:H7 (Cray *et al.*, 1998). Cattle fasted for 48 h prior to slaughter contained significantly greater *E. coli* populations throughout the gut than cattle fed forage (Gregory *et al.*, 2000). In contrast, it was demonstrated that a fasting period had no effect on *E. coli* O157:H7 shedding (Harmon *et al.*, 1999). When culled dairy cows were reconditioned through feeding high energy diets for 28 d before harvest, the prevalence of *E. coli* O157:H7 declined from 14% to 6% (Maier *et al.*, 2011). In general, studies examining the intestinal environment have repeatedly indicated that low pH and high concentrations of short chain VFA result in lower STEC populations (Bach *et al.*, 2002a; Bach *et al.*, 2005b; Cobbold and Desmarchelier, 2004; Pointon *et al.*, 2012; Shin *et al.*, 2002). Thus the bulk of research supports the concept that fasting increases shedding or population concentrations, or makes cattle more susceptible to colonization due to decreased short chain VFA and increased pH in the gastrointestinal tract. Because feed withdrawal and/or starvation results in decreased VFA concentrations in the gut, it has been

suggested that this shift plays a role in the effects of transport and/or lairage on the shedding of STEC.

Feed types

The first dietary practice shown in early studies to significantly increase the risk of STEC shedding among heifers was feeding corn silage (Herriott *et al.*, 1998). In adult cows, the inclusion of animal by-products in the diet (currently discontinued) was shown to increase STEC shedding (Herriott *et al.*, 1998). Other studies linked feeding whole cottonseed with reduced *E. coli* O157 shedding (Garber *et al.*, 1995; Hancock *et al.*, 1994). Fecal samples from cattle fed dry rolled corn, high-moisture corn and wet corn gluten feed did not contain different populations of generic *E. coli*, or extreme acid-resistant *E. coli* during a limit-feeding period (Scott *et al.*, 2000). However, feces from cattle fed wet corn gluten *ad libitum* contained significantly higher concentrations of extreme acid resistant *E. coli* (resistant to an acid shock simulating passage through the human stomach) than did feces of cattle fed dry-rolled or high moisture corn (Scott *et al.*, 2000).

Barley is often fed to cattle and is ruminally fermented more rapidly than corn by the commensal microbial population. More starch is fermented in the lower gut of corn-fed cattle than in barley-fed cattle, resulting in barley-fed cattle having higher fecal pH and lower VFA concentrations compared with corn-fed animals (Bach *et al.*, 2005a; Berg *et al.*, 2004; Buchko *et al.*, 2000a). Barley feeding was linked (albeit at a low correlation) to increased *E. coli* O157:H7 shedding (Dargatz *et al.*, 1997); and in experimental infection studies barley feeding was again associated with increased shedding of *E. coli* O157:H7 by feedlot cattle (Buchko *et al.*, 2000a). Survival of *E. coli* O157:H7 in manure from corn-and barley fed cattle is approximately equal, therefore simple survival in the feces is not responsible for the increased prevalence of *E. coli* O157:H7 in barley-fed cattle (Bach *et al.*, 2005b).

Distiller's grains

The industrial fermentation of corn to produce ethanol has increased more than 4-fold between 2001 and 2007, and its use doubled by 2010 (Richman, 2007). Thus, an economic incentive to increase the utilization of distillers grains (DG) by-product feeds in the cattle industry has increased dramatically in recent years, especially given DG's role as cost-effective feed supplements for finishing and lactating cattle (Firkins *et al.*, 1985). The inclusion DG into cattle rations has been shown to be an effective replacement for common feedstuffs and has demonstrated an increased daily gain in beef cattle (Al-Suwaiegh *et al.*, 2002) and milk yield and feed efficiency in dairy cows (Kleinschmit *et al.*, 2006). This improvement is likely due to the fact that DG alters the population structure and function of the microbial ecosystem of the rumen and throughout the gastrointestinal tract (Callaway *et al.*, 2010a; Durso *et al.*, 2012; Williams *et al.*, 2010b). Cattle fed 40% corn wet distiller's grains (WDG) were very different than the fecal populations in cattle fed a non DG-containing diet, and populations of generic *E. coli* were higher in their feces (Durso *et al.*, 2012), and in previous studies the survival of *E. coli* O157:H7 in feces was increased by increasing levels of DG supplementation (Varel *et al.*, 2008).

Unfortunately, research has suggested a potential association between DG feeding and an increased prevalence and fecal shedding of the foodborne pathogen *E. coli* O157:H7 in cattle (Jacob *et al.*, 2008a; Jacob *et al.*, 2008b; Yang *et al.*, 2010). Distillers grains were shown to increase the shedding of *E. coli* O157:H7 in cow-calf operations in Scotland (Synge *et al.*, 2003). Other researchers found that feeding a related product (brewers grain) to cattle was also associated with increased *E. coli* O157 shedding, and increased the odds of shedding by more than 6-fold (Dewell *et al.*, 2005). The individual animal prevalence of feedlot cattle shedding *E. coli* O157 on d 122 (but not d 136) was higher in cattle fed 25% wet distiller's grain compared to control diets lacking WDG (Jacob *et al.*, 2008b), but the pen-level shedding was unaffected by WDG feeding. Pen floor fecal sample prevalence of *E. coli* O157 was significantly higher across a 12 week finishing period

in cattle fed 25% DDG and either 15% or 5% corn silage compared with cattle fed 0% DDG and 15% corn silage (Jacob *et al.*, 2008a). However, follow-up studies found no differences in *E. coli* O157:H7 fecal shedding in cattle fed DG (Edrington *et al.*, 2010; Jacob *et al.*, 2009), with no indications of why this difference in results was observed. In a further study utilizing both dry and wet-distillers grains, researchers found that higher levels (40% of the ration) of DG inclusion did increase fecal *E. coli* O157:H7 shedding (Jacob *et al.*, 2010). When cattle were fed 40% wet DG, they had higher populations of *E. coli* O157:H7 as well as higher pH values and lower concentrations of L-lactate (Wells *et al.*, 2009). Further studies found that the DG-associated increase in fecal *E. coli* O157:H7 populations could be mitigated by reducing WDG concentrations to 15% or less for 56 d prior to slaughter (Wells *et al.*, 2011). When corn or wheat DDG were supplemented into cattle on a primarily barley-based diet, there was no difference in impact of DDG supplementation, likely because barley inclusion had already increased the *E. coli* O157:H7 populations through some complementary mechanism (Hallewell *et al.*, 2012). Interestingly, researchers found that the numbers of *E. coli* O157:H7 were greater in fecal *in vitro* incubations that contained corn DG than with wheat DG (Yang *et al.*, 2010).

Grain form

Other scientists have examined the form of corn included in cattle rations can impact *E. coli* O157:H7. In feedlot cattle, steam-flaked grains increased *E. coli* O157 shedding in feces compared to diets composed of dry-rolled grains (Fox *et al.*, 2007). This difference was theorized to be due to dry rolling allowing the passage of more starch to the hindgut where it was fermented to produce VFA thereby killing *E. coli* O157 (Fox *et al.*, 2007). This theory is supported by the fact that post-ruminal starch infusion increased generic *E. coli* populations in the lower gut numerically (Van Kessel *et al.*, 2002). However, to date studies have shown no effect on *E. coli* O157:H7 populations of increasing starch concentrations in the diet (Nagaraja, T. G., personal communication) or by increasing fecal starch concentrations (Depenbusch *et al.*, 2008).

Forage feeding

Escherichia coli can and does thrive in the lower gut of animals fed forage diets (Hussein *et al.*, 2003a; Hussein *et al.*, 2003b; Jacobson *et al.*, 2002). Comparing grain-fed to forage-fed cattle indicates that more *E. coli* (including O157:H7) are present in the feces of cattle fed grain diets. The effects of high grain or high forage diets on the duration or shedding of fecal *E. coli* O157:H7 populations in experimentally inoculated calves have been examined. In these studies the calves that consistently shed the highest concentrations of *E. coli* O157:H7 were fed a high concentrate (grain) diet (Tkalcic *et al.*, 2000). Ruminal fluid collected from steers fed a high-forage diet allowed *E. coli* O157:H7 to proliferate to higher populations *in vitro* than did ruminal fluid from high-grain fed steers (Tkalcic *et al.*, 2000). This was possibly due to differences in VFA concentrations between the ruminal fluids.

Other researchers found that feeding forage actually increased the shedding of *E. coli* O157:H7 in cattle (Van Baale *et al.*, 2004). When cattle were fed forage *E. coli* O157:H7 was shed for 60 d compared to 16 d for cattle on a grain-based diet (Van Baale *et al.*, 2004). Studies examining the effects of forage on survival of *E. coli* O157:H7 in manure found that low quality forages caused a faster rate of death of *E. coli* O157 populations (Franz *et al.*, 2005), indicating a possible role of forage chemical or secondary plant components (such as tannins, see below) in fecal shedding (Min *et al.*, 2007). Feces from cattle fed grain had higher VFA concentrations and lower pH which allowed *E. coli* O157:H7 populations to survive longer than feces from grass-fed cattle (Lowe *et al.*, 2010). Other studies have found that feeding forage rich secondary compounds such as sainfoin, might be a method to manipulate fecal populations of *E. coli* O157:H7 to a limited extent (Aboaba *et al.*, 2006; Berard *et al.*, 2009).

Although *E. coli* O157:H7 populations are generally lower in cattle fed forage diets, it must be

emphasized that STEC are still isolated from cattle solely fed forage, so forage feeding should not be viewed as a magic bullet (Hussein *et al.*, 2003b; Thran *et al.*, 2001). Many outlets have claimed that grass-fed cattle contain fewer pathogens than do cattle fed grain; however this has not been demonstrated scientifically. Researchers have found no difference in food safety parameters of beef from grass-fed cattle versus grain fed cattle (Zhang *et al.*, 2010). Furthermore, research into organic versus conventional rearing systems have demonstrated no difference in the incidence of *E. coli* O157:H7 shedding (Jacob *et al.*, 2008c; Reinstein *et al.*, 2009).

Dietary shifts

A sudden shift from grain to hay appears to cause a severe, widespread disruption in the gut microbial flora population, much like an earthquake in a macrobiological environment (Fernando *et al.*, 2010). Thus the effects of rapid dietary shifts on the microbial population in regards to *E. coli* O157:H7 populations have been examined. Early studies investigating (generic) *E. coli* and dietary effects indicated that a sudden decrease in hay intake by cattle increased fecal *E. coli* populations (Brownlie and Grau, 1967). Other studies using experimentally infected sheep found a sudden switch from an alfalfa pellet diet or a corn/alfalfa ration to a poor-quality forage diet increased *E. coli* O157:H7 shedding (Kudva *et al.*, 1995; 1997).

Cattle fed feedlot-type ration contained (generic) *E. coli* populations that were 1000-fold higher than cattle fed a 100% good-quality hay diet (Diez-Gonzalez *et al.*, 1998). When these cattle were abruptly switched from a 90% grain finishing ration to a 100% hay diet, fecal *E. coli* populations declined 1000-fold within 5 d (Diez-Gonzalez *et al.*, 1998). However, it is important to note that in this study no *E. coli* O157:H7 were detected. Based on these results the authors suggested that feedlot cattle could be switched from high grain diets to hay for 5 days prior to slaughter to reduce *E. coli* contamination entering the abattoir (Diez-Gonzalez *et al.*, 1998). Research indicated that a brief (5 d) period of hay-feeding did not impact carcass characteristics; however, when cattle were fed hay during the final portion of the finishing period, they had lower dry matter intake and lost 2.2 lb/head/d (Stanton and Schutz, 2000). Hay feeding did not significantly impact carcass weight, dressing percentage, carcass grades, or quality parameters, but significantly reduced total coliform counts and (generic) *E. coli* counts (Stanton and Schutz, 2000), but the impact was not as large as that reported by Diez-Gonzalez *et al.* (1998). Cattle fed hay for a 48 h period immediately prior to transport to slaughter did not lose more weight during transport than fasted or pasture fed animals (Gregory *et al.*, 2000). Cattle with a natural *E. coli* O157:H7 infection (53%) were divided into two groups and one was fed grain and the other abruptly switched to hay, 52% of the grain-fed controls remained *E. coli* O157:H7 positive, but only 18% of the hay-fed cattle continued to shed *E. coli* O157:H7; but this switch resulted in a BW decrease of 1.25 lb/hd/d compared to controls (Keen *et al.*, 1999). Other researchers found that cattle fed a high-concentrate diet and switched to a diet containing 50/50% corn silage/alfalfa hay diet had lower *E. coli* counts (0.3 $\log_{10}$) after just 4 days (Jordan and McEwen, 1998). Cattle that were fed an 80% barley ration, fasted for 48 h and then subsequently switched to 100% alfalfa silage did not exhibit any change in *E. coli* O157:H7 shedding (Buchko *et al.*, 2000b). However, when these same animals were again fasted for 48 h and re-fed alfalfa silage, the prevalence of *E. coli* O157:H7 shedding increased significantly (Buchko *et al.*, 2000b). Researchers found that experimentally-infected cattle fed hay shed *E. coli* O157:H7 significantly longer than did grain-fed cattle (42 d vs. 4 d), but *E. coli* O157:H7 populations shed were similar between diets (Hovde *et al.*, 1999). Cattle abruptly switched from a finishing diet that contained wet corn gluten feed to alfalfa hay for 5 d showed an increase in colonic pH and total *E. coli* populations decreased approximately 10-fold (Scott *et al.*, 2000).

Conversely, it was found that when cattle were switched from forage-type diets to a high grain finishing ration, fecal and ruminal generic *E. coli* concentrations increased (Berry *et al.*, 2006). In another

study slightly outside of the "normal" dietary switch structure, switching cattle from pasture to hay for 48 h prior to slaughter significantly reduced the *E. coli* population throughout the gut (Gregory *et al.*, 2000). Gregory *et al.*, found that hay feeding increased intestinal *Enterococci* populations that are capable of inhibiting *E. coli* populations in a fashion similar to that of a competitive exclusion culture. However, the effects of high grain versus forage diets were not examined in this New Zealand-based study (Gregory *et al.*, 2000). Based on their data, the authors concluded, "the most effective way of manipulating gastro-intestinal counts of *E. coli* was to feed hay" (Gregory *et al.*, 2000).

Collectively, these results emphasize that while dietary manipulations such as shifting cattle from a high grain to forage ration could be a powerful method to reduce *E. coli*/STEC populations in cattle prior to harvest, the mechanism remains unknown and the effect is very inconsistent. It appears that a factor in this inconsistency involves forage quality and type, but this remains a hypothesis. It does appear that the presence of endproducts of fermentation (e.g., VFA) and some secondary plant compounds in forages play some role in pathogen population levels. While a dietary switch to forage in feedlots is not advocated due to feasibility, weight loss and other logistical issues, other high fiber feedstuffs (e.g., soy hulls, cottonseed meal) or feedstuffs rich in phenolics or essential oils (see below), may be a more feasible alternative strategy to decrease in *E. coli* O157:H7 populations.

Tannins, phenolics, and essential oils

Plants contain phenolic and polyphenolic compounds, such lignin and tannins, that can affect the microbial ecosystem of the gastrointestinal tract through antimicrobial action (Berard *et al.*, 2009; Cowan, 1999; Hristov *et al.*, 2001; Jacob *et al.*, 2009; Patra and Saxena, 2009). It is theorized that some of these compounds may penetrate biofilms and have an anti-quoroum-sensing effect, which may play a role in STEC colonization (Edrington *et al.*, 2009b; Kociolek, 2009; Sperandio, 2010). Tannins have been demonstrated to significantly inhibit the growth of *E. coli* O157:H7 *in vitro* and generic *E. coli* populations in cattle (Berard *et al.*, 2009; Cueva *et al.*, 2010; Min *et al.*, 2007; Wang *et al.*, 2009). Other researchers have found that the phenolic acids that comprise lignin also demonstrated antimicrobial activity against *E. coli* O157:H7 in fecal slurries, and highly lignified forages showed a reduced period of *E. coli* O157:H7 shedding compared with cattle fed only corn silage (Wells *et al.*, 2005). Phenolic compounds in cranberry extract and sorrel are also effective against *E. coli* O157:H7 growth in vitro (Caillet *et al.*, 2012; Fullerton *et al.*, 2011), also the anthocyanins/proanthocyanidins from lowbush blueberries demonstrated *in vitro* potential to inhibit *E. coli* O157:H7 growth (Lacombe *et al.*, 2012).

Essential oils are most often associated with aromatic compounds in various plants used as spices or extracts (Barbosa *et al.*, 2009). Many of these essential oils exhibit antimicrobial acitivity (Dusan *et al.*, 2006; Fisher and Phillips, 2006; Kim *et al.*, 1995; Pattnaik *et al.*, 1996; Reichling *et al.*, 2009; Turgis *et al.*, 2009), often through the mode of action of dissolving bacterial membranes (Di Pasqua *et al.*, 2007; Turgis *et al.*, 2009). As a result, many plant products have been used for centuries for the preservation and extension of the shelf life of foods (Dabbah *et al.*, 1970). Essential oils have been proposed as potential modifiers of the ruminal fermentation (Benchaar *et al.*, 2008; Benchaar *et al.*, 2007; Boadi *et al.*, 2004; Patra and Saxena, 2009) and to reduce *E. coli* O157:H7 in the live animal via *in vitro* studies (Benchaar *et al.*, 2008; Jacob *et al.*, 2009). Some essential oils have been shown to penetrate biofilms and kill *E. coli* O157:H7 (Pérez-Conesa *et al.*, 2011), which could potentially play a role in reducing colonization in the rumen and/or terminal rectum.

Seaweed (*Tasco*)

Brown seaweed (Tasco-14) is a feed additive that has been included in cattle diets to improve carcass quality characteristics and shelf life, increase antioxidants and to improve ruminal fermentation efficiency (Anderson *et al.*, 2006; Braden *et al.*, 2007;

Leupp *et al.*, 2005). *In vitro* studies have indicated that Tasco-14 can reduce populations of *E. coli* and *Salmonella* (Callaway, unpublished data), and more recent results have linked this antipathogen activity to presence of phlorotannins in the brown seaweed (Wang *et al.*, 2009). The phlorotannin anti- *E. coli* activity was greater than that found in other studies with terrestrial tannin sources (Min *et al.*, 2007; Wang *et al.*, 2009). Studies *in vivo* found that Tasco-14 feeding reduced fecal and hide prevalence of *E. coli* O157 in cattle (Braden *et al.*, 2004). Because Tasco-14 is currently available in the market place, this is a product that can be included in cattle rations; however the extent of anti-pathogen activity in vivo is still not clear, therefore the cost of addition must be weighed carefully by the producer.

Citrus products

Orange peel and citrus pulp have excellent nutritional characteristics for cattle and have been included as low-cost ration ingredients in dairy and beef cattle rations for many years (Arthington *et al.*, 2002). Citrus fruits contain a variety of compounds, including essential oils and phytophenols that exhibit antimicrobial activity against foodborne pathogens (Friedly *et al.*, 2009; Mkaddem *et al.*, 2009; Nannapaneni *et al.*, 2008; Viuda-Martos *et al.*, 2008). Other studies have found that limonids from grapefruit may play a role in inhibiting secretion and intercellular communication by *E. coli* O157:H7 (Vikram *et al.*, 2010).

Research has demonstrated that the addition of > 1% orange peel and pulp reduced populations of *E. coli* O157:H7 and *Salmonella* Typhimurium in mixed ruminal fluid fermentations in the laboratory (Callaway *et al.*, 2008; Nannapaneni *et al.*, 2008). Further studies have demonstrated that feeding orange peel and pulp reduced intestinal populations of diarrheagenic *E. coli* in weaned swine (Collier *et al.*, 2010). In ruminants, researchers demonstrated that feeding of orange peel and citrus pellets (a 50/50 mixture) at levels up to 10% DM reduced artificially inoculated populations of *E. coli* O157:H7 and *Salmonella* Typhimurium in sheep (Callaway *et al.*, 2011a; b). When studies were performed using only dried pelleted orange peel, the reduction in pathogen populations disappeared (Farrow *et al.*, 2012), likely due to the inactivation of essential oils (limonene and terpeneless fraction) during the pelleting process. Continuing studies have demonstrated that orange oils offer a potential method for reducing both STEC and *Salmonella* on beef carcasses as well (Pendleton *et al.*, 2012; Pittman *et al.*, 2011). To date, orange peel feeding has not been examined in large-scale feeding studies, but retains promise as a potential on farm strategy to reduce the burden of pathogens on the farm, reducing environmental contamination and re-infection.

Organic acids

Organic acids have been used in animal nutrition to modify the ruminal fermentation by providing some members of the microbial ecosystem a competitive advantage, and by inhibiting other species (Grilli *et al.*, 2010; Martin and Streeter, 1995; Nisbet and Martin, 1993; Piva *et al.*, 2007). Some organic acids (such as lactate, acetate, propionate, malate) have been shown to have antimicrobial activity against *E. coli* O157:H7 (Harris *et al.*, 2006; Sagong *et al.*, 2011; Vandeplas *et al.*, 2010; Wolin, 1969). These acids have been used on hide and carcass washes to reduce pathogen populations, but only recently has interest in using organic acids to reduce pathogens in live animals received interest (Callaway *et al.*, 2010b; Nisbet *et al.*, 2009). Preliminary results do show some success in inhibiting pathogens in the lower intestinal tract of animals (unpublished data), however, further research needs to be performed to be able to release the appropriate organic acid and concentration in the appropriate intestinal location to reduce populations of *E. coli* O157:H7 in cattle.

Ractopamine

ß-agonists, such as ractopamine, are used in cattle to improve animal performance and carcass leanness. *In vitro*, ractopamine showed no effect on growth parameters of *E. coli* O157:H7 (Edrington *et*

al., 2006c); but when used in sheep, the fecal shedding and cecal populations of *E. coli* O157:H7 were increased (Edrington *et al.*, 2006c). When feedlot cattle were fed ractopamine, the numbers of cattle shedding *E. coli* O157:H7 were decreased (Edrington *et al.*, 2006b). In a follow-up study, researchers demonstrated a negligible effect of ß-agonist (ractopamine and zilpaterol) treatment on fecal shedding of *E. coli* O157:H7 in cattle (Edrington *et al.*, 2009a; Paddock *et al.*, 2011). Taken as a whole, these results indicate that the effects of ß-agonist feeding are minimal or non-existent. Interestingly however, in an *in vitro* swine model norepinephrine was shown to increase *E. coli* O157:H7 adherence (Green *et al.*, 2004), though further research is obviously needed to determine if this applies to cattle colonization.

Ionophores

Ionophores, such as monensin and lasalocid, are antimicrobial compounds included in most feedlot and dairy rations to inhibit gram-positive bacteria, thereby improving feed:gain ratios and production efficiency (Callaway *et al.*, 2003). Because these feed additives affect the gram-positive portion of the microbial population, possibly giving gram-negative bacteria (such as *E. coli*) a competitive advantage, they have been investigated as to their role in the spread of *E. coli* O157:H7 in cattle. Because *E. coli* O157:H7 has a true gram-negative membrane physiology ionophores did not affect the growth of this pathogen *in vitro* when added at concentrations up to 3 fold higher than those normally found in the rumen (Bach *et al.*, 2002b; Van Baale *et al.*, 2004).

Early studies demonstrated a marginal increase of STEC shedding by heifers fed ionophores (Herriott *et al.*, 1998), but other studies found no effect (Dargatz *et al.*, 1997). Further studies examining the effect of ionophoric feed additives (monensin, lasalocid, laidlomycin and bambermycin) on *E. coli* O157:H7 demonstrated no effect of these additives *in vitro* (Edrington *et al.*, 2003b), or on fecal shedding or intestinal populations in experimentally-inoculated lambs in a short-term (12 d) trial (Edrington *et al.*, 2003a). In an *in vivo* study using cattle, it was found that cattle fed a forage ration that included monensin shed *E. coli* O157:H7 for a shorter period of time than forage-fed cattle not supplemented with monensin, but monensin had no effect on shedding when cattle were fed a corn-based ration (Van Baale *et al.*, 2004). In an *in vitro* study, it was found that monensin and the co-approved antibiotic tylosin (tylan) treatment reduced *E. coli* O157:H7 populations up to 2 log10 CFU/mL in ruminal fermentations from cows fed forage, but this did not extend to *E. coli* O157:H7 populations in ruminal fluid from cows fed corn (McAllister *et al.*, 2006). These researchers later found that the inclusion of monensin and tylosin did not alter fecal shedding of experimentally-inoculated *E. coli* O157:H7 when included in barley (grain)-based diet fed to cattle (McAllister *et al.*, 2006). These results suggest there may a potential interaction between diet and ionophore inclusion in the effects on *E. coli* O157:H7 populations. Further studies found that monensin decreased *E. coli* O157:H7 prevalence when fed at 44 mg/kg of feed, compared to the typical 33 mg/kg dosing (Paddock *et al.*, 2011).

CONCLUSIONS

While STEC of many serotypes can be viewed as a commensal organism in the gastrointestinal tract cattle, they represent a significant threat to human consumers and public health. Pre-harvest controls in cattle hold great potential to reduce STEC dissemination on farms, in the environment, and entering the food chain. However, none of the on farm management-based controls discussed herein will completely eliminate STEC from cattle and will certainly not eliminate the need for proper procedures in the processing plant. Instead the live-animal management controls must be installed in a complementary fashion to reduce pathogens in a multiple-hurdle approach (Nastasijevic, 2011) that complements the in-plant interventions as well, so that the reduction in pathogen entry to the food supply can be maximized.

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