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

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*
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 by 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 gastro-intestinal 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 (Ferrens 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 amending 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., 1997; Wetzel and LeJeune, 2006). Research has demonstrated that the addition of chemical oxidants to wastewater (Ravva et al., 2006), cowbirds and egrets (Callaway, unpublished data) can carry STEC (and other foodborne pathogens) 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).

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 (Syne 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 DDG (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 populations of increasing starch concentrations in the 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 microbiological 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-quorum-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 activity (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 limonoids 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 (Herrriott et al., 1998), but other studies found no effect (Dargatz et al., 1997). Further studies examining the effect of ionophoric feed additives (monensin, lasalocid, laid-lomycin 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.
REFERENCES


control to reduce *Salmonella enterica* contamination in a concentrated animal feeding operation in the Texas panhandle. BMC Vet. Res. 7:9-17.


Edrington, T. S., T. R. Callaway, D. J. Smith, K. J. Genovese, R. C. Anderson and D. J. Nisbet. 2006c. Effects of ractopamine HCl on Escherichia coli O157:H7 and Salmonella in vitro and on intestinal...


Water Health. 8:646-670.
Harris, K., M. F. Miller, G. H. Loneragan and M. M. Brashears. 2006. Validation of the use of organic acids and acidified sodium chloride to reduce Escherichia coli O157 and Salmonella Typhimurium in beef trim and ground beef in a simulated processing environment. J. Food Prot. 69:1802-1807.


Lyte, M. 2010. The microbial organ in the gut as a


Piva, A., E. Grilli, L. Fabbri, V. Pizzamiglio and I. Campani. 2007. Free versus microencapsulated organic acids in medicated or not medicated diet for


