PRE-HARVEST ANIMAL MANAGEMENT CONTROLS AND INTERVENTION OPTIONS FOR REDUCING *ESCHERICHIA COLI* O157:H7 AND OTHER STEC SHEDDING IN CATTLE: an update 2024

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Abstract

Shiga Toxin Producing *E. coli* (STEC; also known as Enterohemorrhagic *E. coli*, EHEC) are virulent foodborne pathogenic bacteria that are frequently isolated from the gastrointestinal tract of beef and dairy cattle. The beef industry is committed to ensuring a safe and wholesome food supply; as a result, the industry has investigated a number of methods to reduce STEC by implementing changes on the farm and in the processing plant. While interventions can reduce STEC entry into the food chain, the introduction of hurdles does not remove the need to ensure animal health and utilize proper animal management and sanitation procedures on the farm and in the processing plant. This review addresses the latest literature investigating potential pre-harvest STEC (both *E. coli* O157:H7 and other non-O157:H7 STEC serotypes) intervention strategies. On-farm interventions to reduce STEC in cattle can be divided up into 3 broad categories: 1) animal management and transport practices, 2) feed and water management, and 3) live animal treatments. Ultimately, pre-harvest intervention strategies are imperfect and may not always be complementary with other pathogen reduction strategies as part of a coordinated multiple-hurdle approach. By reducing the burden of STEC entering processing plants we can increase the effectiveness of in plant interventions and further reduce human illnesses.

Introduction

Shiga Toxin Producing *E. coli* (STEC) are foodborne pathogenic bacteria that can be naturally present throughout the gastrointestinal tract of cattle (Wells et al., 1991; Chapman et al., 1993; Karmali et al., 2010; Ferens and Hovde, 2011; Shridhar et al., 2017; Sapountzis et al., 2020). The most-well characterized member of this group is *E. coli* O157:H7, which frequently colonizes the gastrointestinal tract of its natural reservoir, cattle (Gyles, 2007; Karmali et al., 2010; Ferens and Hovde, 2011; Sapountzis et al., 2020). An estimated more than 175,000 human illnesses in the United States yearly are caused by STEC (Scallan et al., 2011; Hoffmann, 2021), at a cost of more than $1 billion each year in direct and indirect costs (Scharff, 2010) and more than 70% of these total human illnesses are transmitted via food (Scallan et al., 2011).

The cattle industry has invested well in excess of $2 billion dollars to combat STEC in processing plants (Kay, 2003). While post-harvest and during-processing pathogen reduction strategies are effective at reducing STEC populations, multiple interventions are not perfect in preventing human disease outbreaks. The changing pathway of human exposures from cattle underscores the fact that STEC in cattle are still a threat that the beef industry must address on the farm with pre-harvest intervention strategies (Besser et al., 2014; van Overbeek et al., 2014; Browne et al., 2021).

In order to curtail human illnesses and ensure a safe and wholesome food supply, research into pre-harvest *E. coli* O157:H7 and non-O157:H7 STEC reduction controls and interventions has expanded (LeJeune and Wetzel, 2007; Sargeant et al., 2007; Oliver et al., 2008; Smith et al., 2010; Callaway et al., 2013; Duffy et al., 2014). This review provides up-to-date research on pre-harvest animal management interventions that have been proposed or explored to reduce STEC and divided into three broad application-based categories: 1) Animal management and transport practices, 2) Feed and water management, and 3) Live animal treatments.

Animal management practices and transportation

Cattle Pen and Bedding Surfaces: Bacteria can survive and thrive in environments where cattle are raised for a considerable time, and STEC are no exceptions. *E*. *coli* O157:H7 can live for long periods of time (>100 d) in manure, soil and other organic materials (Maule, 2000; Jiang et al., 2002; Winfield and Groisman, 2003) and can be transmitted successively through their environment (Semenov et al., 2009; Semenov et al., 2010). Cattle are bedded on materials that are used to improve animal health and welfare (Davis et al., 2005; Westphal et al., 2011; Eckelkamp, 2014; Rowbotham and Ruegg, 2016; Leso et al., 2020). While this improves animal care and welfare, bedding material can harbor bacteria responsible for mastitis as well as foodborne pathogenic bacteria (Davis et al., 2005; Oliver et al., 2005; Richards et al., 2006; Wetzel and LeJeune, 2006). Bedding contaminated with urine can stimulate the growth of *E*. *coli* O157:H7 and other bacteria either by providing substrate for growth (Davis et al., 2005). Microbial populations in the bedding were linked with reduced *E*. *coli* O157:H7 populations in bedding of all types (Westphal et al., 2011). The use of sand for bedding substrate reduced transmission of *E*. *coli* O157:H7 between dairy cows, resulting in lower *E*. *coli* O157:H7 populations in bedding compared to sawdust (LeJeune and Kauffman, 2005).

Traditionally it was assumed that feedlot surfaces behaved like uncomposted manure, but bacterial communities on the feedlot surface are both complex and distinct from fecal populations (Durso et al., 2011; Berry et al., 2017). Some pen surface conditions can increase the prevalence of STEC. Research found that muddy pen conditions increased STEC prevalence compared to cattle from maintained pens (Smith et al., 2001). Moreover, the type of surface material used may also impact STEC prevalence, but as a study reported pond ash did not impact survival of *E*. *coli* O157 (Berry et al., 2010). Diet components can increase the persistence and survival of *E*. *coli* O157:H7 in manure and on pen surfaces (Berry et al., 2017). In all, Bedding or pen cleaning will not eliminate *E*. *coli* O157:H7 from any farm or feedlot environment, but may slow spread within a herd or between pen mates.

Cattle Grouping: Grouping cattle into age specific groups can minimizes disease spread and prevents exclusion by dominant animals. Younger animals have greater susceptibility to STEC colonization and STEC fecal shedding prevalence is higher in young milk-fed animals as compared to mature adult cattle (Cray and Moon, 1995; Smith et al., 2001; Cobbaut et al., 2009). Differences in colonization are thought to be related to changes in the animal genetic and physiological factors, diet, management, and environmental factors that occur as cattle approach physiologic maturity (Ekong et al., 2015).

Keeping young cattle in the same groups throughout rearing without introducing new animals is recommended to reduce STEC O157:H7 prevalence (Sanderson et al., 2006; Ellis-Iversen and Van Winden, 2008; Ellis-Iversen and Watson, 2008). Young calves have increased risk of excretion and carriage of STEC compared to older calves. To reduce the transmission of STEC, calves that test positive on the hide and carcasses for STEC O157:H7 and non-O157, should be housed individually as opposed to in groups, to reduce animal contact. 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), as 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). If possible, separation of calves from adult cows and replacement dairy heifers and lowering the density of cattle in pens may reduce STEC prevalence by reducing opportunities for animal-to-animal spread (Ekong et al., 2015).

Cattle Density: Animal density may play a role in the horizontal spread and recirculation of *E*. *coli* O157:H7 as well as other foodborne pathogens (Vidovic and Korber, 2006; Gunn et al., 2007). Densely packed animals have an increased chance of co-contamination via fecal spread through fecal-oral pathways (Gunn et al., 2007). This increased animal density can be linked to increased risk of carriage of some STEC, including O157:H7 (Vidovic and Korber, 2006; Frank et al., 2008). Further studies found that increased stocking density increased shedding of STEC, independent of group size (Strachan et al., 2006; Stacey et al., 2007; Spencer et al., 2015). Herd size was positively correlated with an increased shedding of STEC on 126 Swedish farms (Widgren et al., 2015). Even in pasture and range situations where animal density is low, cattle behaviors can create localized higher density areas around shade, feeders, and water sources which increases exposure to STEC in feces (Mitloehner et al., 2002; Wells et al., 2017; Dawson et al., 2018). In addition to increasing prevalence of STEC on farms, increased human STEC illnesses are correlated with greater animal density (Haus-Cheymol et al., 2006; Friesema et al., 2011).

Handling, Transportation, and Lairage*:* Handling and transport causes stress to animals which impacts animal welfare, meat quality, and the microbial population and carriage of foodborne pathogenic bacteria, and increase the spread of *E*. *coli* O157:H7 and other STEC due to physical contact or fecal contamination (Schwartzkopf-Genswein et al., 2012). Measuring the direct impact of transportation on *E. coli* shedding is difficult, and research has reported conflicting results. Multiple studies 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). Alternatively, transport was shown to cause an increase in fecal shedding of *E. coli* O157:H7 (Bach et al., 2004), suggesting further studies are warranted.

The use of squeeze chutes on cattle increased the odds of hide contamination with *E. coli* O157 (Mather et al., 2007). Additionally, trailers used during the transportation and handling process may spread pathogens between lots or loads of cattle due to fecal material on surfaces (Mather et al., 2007). Cattle trailers can be important vectors of *E. coli* O157:H7 and are frequently positive for *E. coli* O157:H7 when sampled (Barham et al., 2002; Cuesta Alonso et al., 2007; Reicks et al., 2007). In fact, the incidence of *E. coli* O157:H7 in transport trailers increased 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).

In addition to conditions in the trailer itself, distance traveled also impacts STEC in cattle. Cattle transported more than 100 miles had a doubled risk of having *E. coli* O157:H7 positive hides at slaughter compared to cattle shipped a shorter 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). Bach *et al.* (2004) evaluated the effects of pre-conditioning and the duration of animal transport from the pasture on fecal excretion of *E. coli* and STEC O157:H7 by beef calves. However, a recent study demonstrated a combination of transport and lairage did not lead to an increase in the number or prevalence of *E. coli* O157:H7 (Fegan et al., 2009).

Lairage and holding facilities can also impact the prevalence and concentration of *E. coli* O157:H7 on hides of cattle (Arthur et al., 2007a). A study found that more hides were contaminated in lairage at processing plants than from direct contact as cattle leave the feedlot (Arthur et al., 2008). Furthermore, the presence of super-shedding cattle in holding pens can increase the cross-transmission of *E. coli* O157:H7 between animals from different farms or feedlots (Arthur et al., 2010b; Cernicchiaro et al., 2010). The exact role of lairage and transport in the spread of *E. coli* O157:H7 in cattle is unclear, and is likely time, age, and animal density-dependent.

Stresses: Livestock stresses should be minimized to promote proper animal husbandry and well-being. It is evident that stresses of transport and mixing impact the gastrointestinal microbial population and foodborne pathogenic bacterial colonization of food animals (Lyte and Freestone, 2009; Rostagno, 2009; Lyte, 2013). It is also known that Long-term stress may depress immune function in cattle (Kelley, 1980; Carroll and Forsberg, 2007; Salak-Johnson and McGlone, 2007), making them more susceptible to colonization with a variety of pathogens on farms (Roth, 1985; Carroll and Forsberg, 2007), but the short term effects of stress from weaning, handling or transport on immune status are unknown.

Weaning is stressful to calves but the impact of weaning stress on STEC is not clear (Edrington et al., 2011). Studies observed increased colonization of STEC (Herriott et al., 1998) and *E. coli* O157:H7 in recently weaned calves (Chase-Topping et al., 2007). In a different study however, researchers demonstrated that weaning does not affect the likelihood of shedding and interestingly in the same study, demonstrated that calving reduced the likelihood of *E. coli* O157:H7 shedding (Synge et al., 2003). Other stresses such as movement and handling 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 demonstrated in U.S. cattle systems. When calves were preconditioned to transport stress, they were less susceptible to infection from the environment than calves not preconditioned to this form of stress (Bach et al., 2004). The impact of social stresses on STEC in cattle have not been observed, however, the potential role of social stress on pathogen transmission in needs to be further explored.

***Environmental Stressors*:** The two most common environmental stressors are heat stress and cold stress, both of which can negatively impact animal health, productivity (Brown-Brandl et al., 2003), and possibly shedding of *E. coli* O157:H7 and *Salmonella* (Barham et al., 2002; Callaway et al., 2006b). Extreme temperature periods have been linked to High Event Periods of STEC shedding (Stanford et al., 2017b). When comparing cattle in a thermoneutral zone to a heat stress inducing environment, immune challenge studies reported significantly different responses to lipopolysaccharide (LPS) derived from *E. coli* O157:H7 (Stanford et al., 2017a). Furthermore, in a two-year heat stress study using lactating dairy cows, *E. coli* O157:H7 shedding increased in the heat stress group in the first year, but did not differ in the second year (Edrington et al., 2004). Studies measuring the impact of heat stress on STEC shedding, however, are conflicting. In a two-year feedlot study, researchers found no correlation between heat stress or cattle temperament and *E. coli* O157:H7 shedding in cattle (Brown-Brandl et al., 2009). 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). One possible component influencing heat stress studies is relative temperature change or change in temperature compared to seasonal norms. A recent study reported an increase in *E. coli* O157:H7 prevalence during the winter months, when weather was 10.5º C warmer than seasonal norms (Stanford et al., 2017a). While the relationship between heat stress and STEC shedding is contradictory, heat stress reduction systems can affect microbial activity as well (Callaway et al., 2006b). Water sprinkling systems alleviated heat stress in cattle and increased measures of animal well-being, decreased *E. coli* O157:H7 populations on the hides of cattle. Providing shade to cattle did not alter shedding of STEC compared to unshaded controls (Wells et al., 2017). The impacts of cold stress on STEC shedding however, needs to be studied further.

Cattle Water and Feed Management

Drinking Water treatments: Both STEC and *E. coli* O157:H7 can be deposited in water troughs and can survive there for long periods of time (Hancock et al., 1998; LeJeune et al., 2001a; LeJeune et al., 2001b; Murinda et al., 2004; Wetzel and LeJeune, 2006). Up to 25% of cattle farm water samples have been shown to contain *E. coli* O157:H7 (Sanderson et al., 2006), which indicates that troughs can be a route that exposes cattle to STEC. Results suggest that common-use troughs can be significant vectors for horizontal transmission of *E. coli* O157:H7 within a group of animals. Modeling research reported an increase in water trough cleaning frequency would significantly increase the death rate of *E. coli* O157:H7 (Vosough Ahmadi et al., 2007). Increasing water-trough levels has shown progress with potentially reducing *E. coli* O157:H7 shedding in cattle feces (Beauvais et al., 2018). Sunlight and residual organic material in livestock water supplies can reduce the effectiveness of chlorination over time, which has been studied in real world chlorination trials with cattle water troughs (LeJeune et al., 2004). Electrolyzed oxidizing (EO) water reduced 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., 2005b). Maintaining water cleanliness and quality is good farming practice but the impact of water treatments specifically on STEC populations is limited.

***Fasting**:* Fasting cattle causes levels of volatile fatty acids (VFA) in the gastrointestinal tract to decline rapidly (Harmon et al., 1999; Wood et al., 2006), which leads to increased *E. coli* throughout the intestinal tract of cattle (Buchko et al., 2000; Gregory et al., 2000), and increased *Salmonella* and *E. coli* populations in the rumen (Brownlie and Grau, 1967; Grau et al., 1969). A similar study reported that fasting can cause “apparently *E. coli* (O157:H7) negative animals to become positive” (Kudva et al., 1995). Moreover, fasted calves were more susceptible to colonization by inoculated *E. coli* O157:H7 (Cray et al., 1998) and 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 (Rasmussen et al., 1993; Harmon et al., 1999). 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; Shin et al., 2002; Cobbold and Desmarchelier, 2004; Bach et al., 2005). Feed withdrawal results in decreased VFA concentrations in the gut, suggesting this shift plays a role in the effects of transport and/or lairage on subsequent STEC shedding, thus the period of time cattle undergo feed withdrawal should be minimized (Pointon et al., 2012).

Distiller’s Grains: The industrial fermentation of corn to produce ethanol increased almost 10-fold between 2001 and 2020 (Richman, 2007; EIA, 2021; Lee et al., 2021). Distiller's grains (DG) are fed as either wet (WDGS) or dry (DDGS) forms, and have been associated with increased fecal excretion of STEC O157:H7 (Jacob et al., 2008a; Jacob et al., 2008b; Yang et al., 2010). 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 (WDG) 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).

Follow-up studies, however, found no differences in *E. coli* O157:H7 fecal shedding in cattle fed DG (Jacob et al., 2009; Edrington et al., 2010), with no indications of why this difference in results was observed. In a further study utilizing both dry and wet-distillers grains, researchers found supplementation of 40% DG (wet or dry) increased fecal *E. coli* O157:H7 shedding (Jacob et al., 2010). Cattle fed a diet containing 40% WDG had higher populations of *E. coli* O157:H7 as well as increased pH values and lower concentrations of L-lactate (Wells et al., 2009). Interestingly, researchers found that the numbers of *E. coli* O157:H7 were greater in fecal in vitro incubations containing corn DG than with wheat DG (Yang et al., 2010). Populations of STEC O157:H7 had greater persistence in manure of cattle fed WDGS at >40% of the ration (Berry et al., 2017). Feeding DDGS at 40% of the ration resulted in cattle shedding STEC O157:H7 at a level similar to that from cattle fed typical barley-based finishing rations (Hallewell et al., 2013). It is suggested that utilizing DG at levels < 15% of the cattle ration could reduce STEC populations compared to feeding at higher levels in the ration (Wells et al., 2011).

Forage versus Grain Feeding: *E. coli* (including STEC) can and does thrive in the lower gut of animals fed forage diets (Jacobson et al., 2002; Hussein et al., 2003a; Hussein et al., 2003b). Comparing grain-fed to forage-fed cattle indicates that in general more *E. coli* are present in the feces of cattle fed grain diets. Research 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 similarly demonstrated no difference in the incidence of *E. coli* O157:H7 shedding (Jacob et al., 2008c; Reinstein et al., 2009). While most data indicates that grain feeding is associated with fecal shedding of *E. coli* O157:H7, some researchers demonstrated that feeding forage 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 days compared to 16 days for cattle on a grain-based diet (Van Baale et al., 2004). Furthermore, steers fed a high-forage diet allowed *E. coli* O157:H7 to proliferate to higher populations in test tube studies than ruminal fluid from high-grain fed steers (Tkalcic et al., 2000), this was possibly due to differences in VFA concentrations between the ruminal fluids. 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 components or secondary plant metabolites in fecal shedding (Min et al., 2007). 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 "silver bullet" (Thran et al., 2001; Hussein et al., 2003b).

Dietary Shifts: A sudden shift from grain to hay appears to cause a severe, widespread disruption in the gut microbial flora population. Cattle fed feedlot-type ration contained *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). 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). Moreover, hay feeding during the final portion of the finishing period did not impact carcass weight, dressing percentage, carcass grades, or quality parameters, but reduced total coliform counts and *E. coli* counts. Additionally, data found that cattle fed hay for a 48 h period immediately prior to transport to slaughter lost no more weight during transport than fasted or pasture fed animals (Gregory et al., 2000). Naturally *E. coli* O157:H7 colonized cattle (53% positive) were divided into two groups, a control group that was fed grain during the entire study and the treatment group which was abruptly switched to hay. At the end of the study, 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; however, forage-fed cattle were 1.25 $\text{lb}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$lighter than controls (Keen et al., 1999). Other research 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., 2000). 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., 2000). 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).

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 probiotic or competitive exclusion culture. The authors concluded that "the most effective way of manipulating gastro-intestinal counts of *E. coli* was to feed hay" (Gregory et al., 2000).

Live animal interventions

Tannins, Phenolics, and Essential Oils: Plants contain phenolic compounds, such as lignin and tannins, that can affect the microbial ecosystem of the gastrointestinal tract through antimicrobial action (Cowan, 1999; Hristov et al., 2001; Berard et al., 2009; Jacob et al., 2009; Patra and Saxena, 2009). Tannin research demonstrated a significant ability to inhibit the growth of *E. coli* O157:H7 *in vitro* and generic *E. coli* populations in cattle (Min et al., 2007; Berard et al., 2009; Wang et al., 2009; Cueva et al., 2010). Other researchers 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).

Many of these essential oils exhibit antimicrobial activity (Kim et al., 1995; Pattnaik et al., 1996; Dusan et al., 2006; Fisher and Phillips, 2006; Reichling et al., 2009; Turgis et al., 2009), often by dissolving bacterial membranes (Di Pasqua et al., 2007; Turgis et al., 2009). Furthermore, one recent study found essential oils associated with the plant species: ajowan, thyme, and cinnamon leaf completely or partially inhibited bovine respiratory bacterial pathogens growth (Amat et al., 2017). Essential oils have been proposed as potential modifiers of ruminal fermentation (Boadi et al., 2004; Benchaar et al., 2007; Benchaar et al., 2008; Patra and Saxena, 2009) and to reduce *E. coli* O157:H7 in the live animal (Helander et al., 1998; Benchaar et al., 2008; Jacob et al., 2009).

Seaweed (Tasco): Brown seaweed is a feed additive that improves carcass quality characteristics and shelf life, increase antioxidants, and improve ruminal fermentation efficiency (Leupp et al., 2005; Anderson et al., 2006; Braden et al., 2007). Supplementation of Tasco in ram diets, altered the ruminal and intestinal microbial populations, and found that Tasco reduced fecal shedding of generic *E. coli* and common STEC by up to 36% (Zhou et al., 2018). While the exact interaction between Tasco-14 and STEC is not understood, research linked the antipathogen activity to the presence of dietary phlorotannins (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). However the extent of Tasco-14 anti-pathogen activity in vivo is still not clear.

Citrus products*:* Citrus fruits contain a variety of compounds, including essential oils and phytophenols that exhibit antimicrobial activity against foodborne pathogens (Nannapaneni et al., 2008; Viuda-Martos et al., 2008; Friedly et al., 2009; Mkaddem et al., 2009). Research 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., 2008a; Nannapaneni et al., 2008). Further studies 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 orange peels 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., 2011b, a). To date, orange peel feeding needs to be examined in large-scale and retains a potential on-farm strategy to reduce pathogens.

Ionophores: Ionophores, such as monensin and lasalocid, are antimicrobial compounds included in most feedlot and dairy rations to inhibit gram-positive bacteria, thereby improving F:G ratios and production efficiencies (Callaway et al., 2003b). *E. coli* O157:H7 has a true gram-negative membrane physiology and ionophores do 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). Further studies examining the effect of ionophores on *E. coli* O157:H7 demonstrated no effect of these additives *in vitro* (Edrington et al., 2003c), on fecal shedding, or intestinal populations in experimentally-inoculated lambs in a short-term 12 d trial (Edrington et al., 2003b). In an *in vivo* study, 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 another *in vivo* study conducted on steers, researchers found a greater percentage of *E.coli O157:H7* shedding in steers fed their monensin supplement versus the control supplement (Hales et al., 2017). In an *in vitro* study, it was found that monensin and the co-approved antibiotic tylosin treatment reduced *E. coli* O157:H7 populations up to 2 $\log_{10}$ 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).

Direct Fed Microbials (DFM) are typically composed of live cultures or fermentation end-products from yeast, fungi, or bacteria. The DFM is fed to animals daily to improve ruminal fermentation and production efficiency (Martin and Nisbet, 1992; Beauchemin et al., 2018). Researchers compared several commercially-available growth enhancement probiotics and yeast products and found that feeding these probiotics provided no effect in regards to pathogen levels in cattle (Keen and Elder., 2000). A probiotic culture comprised of *Streptococcus bovis* and *Lactobacillus gallinarum* from the rumen of cattle reduced *E. coli* O157 shedding when given to experimentally-infected calves(Ohya et al., 2001). There are probiotic products developed to specifically reduce

E. *coli* O157:H7 shedding in cattle. A monoculture of *S. faecium* reduced *E. coli*, however, the greatest reduction was observed in sheep treated with a mixed culture of *S*. *faecium* or a mixture of *S*. *faecium*, *L. acidophilus*, *L*. *casei*, *L. fermentum* and *L*. *plantarum* (Lema et al., 2001). Additionally, studies have also indicated that cultures of *Lactobacillus acidilacti* and *Pediococus* could directly inhibit *E*. *coli* O157:H7, likely through the production of organic acids and low pH (Rodriguez-Palacios et al., 2009). A monoculture DFM comprised of *Bacillus subtilis*, however, did not affect the fecal prevalence or concentration of *E*. *coli* O157:H7 and did not impact average daily gain in feedlot cattle (Arthur et al., 2010a).The addition of *L*. *reuteri* along with glycerol reduced *E*. *coli* O157:H7 populations *in vitro*, but interestingly the addition of glycerol was required to exhibit this anti-STEC effect (Bertin et al., 2017). One monoculture that has demonstrated an STEC reducing effect is a *L. acidophilus* culture derived directly from the rumen of cattle. This DFM reduced *E*. *coli* O157:H7 shedding by more than 50% when fed to feedlot cattle (Brashears and Galyean, 2002; Brashears et al., 2003a; Brashears et al., 2003b). In an independent evaluation, this DFM reduced fecal shedding of *E*. *coli* O157:H7 in cattle from 46% to 13% (Ransom et al., 2003). In a further refinement of this DFM, the *L*. *acidophilus* cultures were combined with *Propionibacterium freudenreichii* and reduced the prevalence of *E*. *coli* O157:H7 in the feces from approximately 27% to 16% and reduced the prevalence on hides from 14% to 4% (Elam et al., 2003; Younts-Dahl et al., 2004). Further work with this DFM has again shown that it reduced *E*. *coli* O157:H7 in feces and on hides (Stephens et al., 2007; Wisener et al., 2015). However recent research utilizing *L. acidophilus* and *L. casei* on a finishing diet in commercial feedlot cattle did not find a significant reduction in *E. coli* O157:H7 fecal shedding (Dewsbury et al., 2021).

Competitive Exclusion (CE) is a specific type of probiotic approach that is used to eliminate *E*. *coli* O157:H7 from cattle gastrointestinal tracts (Brashears and Galyean, 2002; Brashears et al., 2003a; Brashears et al., 2003b; Zhao et al., 2003). Competitive exclusion as a technology, utilizes non-pathogenic bacterial culture of one or more species to the intestinal tract to reduce colonization or decrease populations of pathogenic bacteria (Fuller, 1989; Nurmi et al., 1992). In a study with calves colonized with the *E*. *coli,* CE product shed less *E*. *coli* O111:NM and O26:H111 (Zhao et al., 2003). Depending on the stage of production of the animal (i.e., maturity of the gut), the goal of CE can be the exclusion of pathogens from the naïve gut of a neonatal animal, or the displacement of an already established pathogenic bacterial population (Nurmi et al., 1992).

Colicins and colicin-producing E. coli. Colicins are antimicrobial proteins produced by certain *E*. *coli* strains that kill or inhibit the growth of other *E*. *coli* strains (Konisky, 1982; Smarda and Smajs, 1998; Lakey and Slatin, 2001), including *E*. *coli* O157:H7 (Murinda et al., 1996; Jordi et al., 2001; Schamberger and Diez-Gonzalez, 2002). The concept of using colicins as an intervention strategy to kill food borne recently has been limited by cost to use as a treatment on finished meat products (Abercrombie et al., 2006; Patton et al., 2008; Liu et al., 2011) or vegetables (Nandiwada et al., 2004). Recently however, the costs of production and purification of colicins was lowered by recombined protein expression work where plants can produce colicins in sufficient quantities for commercial use in food animals (Stahl et al., 2004; Callaway and Sheridan, 2015; Schulz et al., 2015). Recently, specific studies have examined the use of specific colicins against *E*. *coli* O157:H7 *in vitro* in gastrointestinal simulations (Callaway et al., 2004c) and against other *E*. *coli in vivo* (Cutler et al., 2007). Approximately 13% of fecal *E*. *coli* isolates from cattle, human, and sheep were colicinigenic, and many of these colicins inhibited growth of STEC O157:H7 and O26 (Mushtaq et al., 2021).

In spite of the seemingly simple addition of a colicin to animal diets to control *E*. *coli* O157:H7, studies have indicated that the sensitivity of *E*. *coli* O157:H7 strains to any single colicin can be highly variable (Murinda et al., 1996; Murinda et al., 1998; Schamberger and Diez-Gonzalez, 2002). Because some *E*. *coli* O157:H7 strains are colicinogenic and produce specific concomitant immunity proteins (Murinda et al., 1998), these strains of *E*. *coli* O157:H7 can be resistant to certain added colicins or even a broad category of colicins (Alonso et al., 2000). As a way of getting colicins into the lower gut of cattle, researchers have proposed a specific form of DFM/CE of feeding colicin-producing *E*. *coli* in cattle rations (Zhao et al., 1998; Schamberger and Diez-Gonzalez, 2002; Schamberger et al., 2004). These strains have been shown to colonize the lower gut of cattle, but the reduction in concentration of *E*. *coli* O157 was approximately 2 $\log_{10}$ CFU/g, not a complete elimination (Nandiwada et al., 2004).

Bacteriophages: All bacteria can be infected by bacteriophages that naturally occur in the environment (Lederberg, 1996; Kutter and Sulakvelidze, 2005), including the intestinal tract of cattle (Callaway et al., 2006a; Kropinski et al., 2012; Niu et al., 2012; Litt and Jaroni, 2017; Bumunang et al., 2019). Phages target specific receptors on the surface of the bacterium (Lederberg, 1996). This specificity allows phages to be an anti-pathogen treatment, targeting the species we wish to eliminate, without perturbing the overall microbial ecosystem (Johnson et al., 2008). One potential drawback to the use of phages is the rapid development of bacterial resistance to a single phage, thus much of the effort has been focused on the development of multi-phage cocktails (Tanji et al., 2005).

Commercial phage-based anti-*E*. *coli* O157:H7 focused on the use of lytic phages in hide wash and surface cleansing products (Liu et al., 2015; Arthur et al., 2017; Tolen et al., 2018). Company-based research indicates a significant reduction in positive trim samples from cattle that were sprayed with this product. However, large scale experimental studies have found little effect of phage spray on STEC prevalence on cattle hides (Arthur et al., 2017). Phages have been used successfully in several *in vivo* research studies examining the effect of phage on diseases that impact animal production efficiency or health (Smith and Huggins, 1982, 1983, 1987; Huff et al., 2002). Bacteriophage treatment reduced enterotoxigenic *E. coli* (ETEC)-induced diarrhea and splenic ETEC colonization in calves (Smith and Huggins, 1983, 1987). Bacteriophages have also been used to control food-borne pathogenic bacteria, especially O157:H7 in cattle gastrointestinal tracts (Kudva et al., 1999; Bach et al., 2003; Callaway et al., 2008b; Niu et al., 2008; Bach et al., 2009; Rozema et al., 2009). Several different phages that lyse O157 and other serotypes of STEC have been isolated from feedlot cattle (Callaway et al., 2006a; Oot et al., 2007; Hallewell et al., 2014; Wang et al., 2015) and other sources (McLaughlin et al., 2006; Lennon et al., 2020) in cattle from around the world (Bumunang et al., 2019). These phages reduced *E. coli* O157:H7 strains in artificially infected animals (Callaway et al., 2008b; Bach et al., 2009; Schaut et al., 2018) and naturally phage-infected ruminants were more resistant to *E. coli* O157:H7 colonization (Raya et al., 2006). Phage treatment impacts in live animals has been mixed, with little change in STEC populations or a cyclic response where STEC and phage numbers exist in an inverse relationship wave throughout the gut (Sabouri et al., 2017). Company sponsored studies for these on-farm products has had mixed results (Stanford et al., 2010), but development of multi-phage cocktails for use in cattle continues.

Cattle Hide Washing: Hide washes can contain antimicrobial compounds, sodium hydroxide, trisodium phosphate [TSP], cetylpyridinium chloride [CPC], and sodium dodecyl sulfate [SDS], which serves to reduce some of the bacterial contamination entering the processing plant on the hide (Bosilevac et al., 2004; Bosilevac et al., 2005a; Bosilevac et al., 2005b; Arthur et al., 2007b; Elramady et al., 2013). The most common hide/carcass rinse additive has been organic acids such as lactic or acetic acid (Berry and Cutter, 2000; Loretz et al., 2011; Zhilyaev et al., 2017). Hide washes significantly reduce the load of *E. coli* O157:H7 entering the plant on the hide, which has been linked to lower final carcass contamination levels (Arthur et al., 2007a; Arthur et al., 2010b), thus improving food safety; but they do not reduce the prevalence of *E. coli* O157:H7 entering the plant within the animal. Interestingly, hide washing at various stages of production will impact the native microbial population of the hide, a lower microbial diversity of the hide population is correlated with higher STEC prevalence (Chopyk et al., 2016).

Sodium Chlorate: Addition of chlorate to *E. coli* cultures kills bacteria because *E. coli* can respire under anaerobic conditions by reducing nitrate to nitrite via the dissimilatory nitrate reductase enzyme (Stouthamer, 1969). Chlorate treatment *in vitro* quickly reduced populations of both *E. coli* O157:H7 and *Salmonella* (Anderson et al., 2000a). Chlorate addition to animal rations reduced experimentally inoculated *E. coli* O157:H7 populations in swine and sheep intestinal tracts (Anderson et al., 2001; Edrington et al., 2003a). Other studies indicated that soluble chlorate administered via drinking water significantly reduced *E. coli* O157:H7 ruminal, cecal and fecal populations in both cattle and sheep (Anderson et al., 2002; Callaway et al., 2002; Callaway et al., 2003a). Hide contamination with *E*. *coli* O157:H7 plays a significant role in carcass/product contamination (Arthur et al., 2009; Arthur et al., 2010a; Arthur et al., 2010b), and chlorate treatment reduced both fecal and hide populations of *E*. *coli* (Anderson et al., 2005). *In vitro* and *in vivo* results have indicated that chlorate treatment does not adversely affect the ruminal or the cecal/colonic fermentation (Anderson et al., 2000b). Additional studies have demonstrated that chlorate alters neither the antibiotic resistance, nor toxin production by *E*. *coli* O157:H7 (Callaway et al., 2004a; Callaway et al., 2004b).

Vaccination: The immune system of animals is a very potent anti-pathogen mechanism against bacteria that are pathogenic to the animal, and vaccination is widely used in the cattle industry to prevent diseases that impact animal health and productivity (Van de Walle et al., 2013; Smith, 2015). Unfortunately, because STEC do not cause disease in cattle, the immunostimulation provided by these foodborne pathogens is not as potent, because it appears that natural exposure to *E*. *coli* O157:H7 does not appear to confer protection to the host against subsequent infection (Gyles, 1998). However, infection with *E. coli* O157 stimulated the innate immune response in calves in a unique fashion (Larzábal et al., 2020).

Various vaccines have been tested for preventing colonisation and/or reducing fecal excretion of STEC in cattle. Additionally, because of the short-term nature of the feedlot studies, examination of the duration of immunity is unknown (Smith, 2015). To date, two vaccine types have been tested and found to be effective at reducing STEC O157:H7 fecal shedding, and have been commercialized: a type III secretion protein (Bioniche), and a Siderophore protein based vaccine (Epitopix) (Snedeker et al., 2012; Varela et al., 2013; Besser et al., 2014).

***Type III Secreted Protein Vaccines**:* A vaccine produced from *E*. *coli* O157:H7 extracts (type III secreted proteins) has been produced as Econiche^{TM}. Preliminary experimental results indicated that this vaccine reduced *E*. *coli* O157:H7 shedding in feedlot cattle from 23% to less than 9% (Moxley et al., 2003; Potter et al., 2004; Van Donkersgoed et al., 2005). In an evaluation study, it was demonstrated that vaccination reduced fecal shedding from 46% to 14% (Ransom et al., 2003). Multiple dosing is effective because it reinforces the immunostimulation, and studies have shown an experimental three dose regimen reduced *E*. *coli* O157:H7 shedding by 65%, but that a second dose system was less effective (Moxley et al., 2009). However, in a follow up study, a two dose regimen was shown to reduce rectal colonization by *E*. *coli* O157:H7 in feedlot cattle (Smith et al., 2009b). Other research demonstrated that the shedding could be reduced from 51% to 21% by a three dose series of vaccinations (Allen et al., 2011). The benefits of vaccinating cattle in reducing cattle hides positive for *E*. *coli* O157:H7 can be lost by comingling with non-vaccinated cattle during transport (Smith et al., 2009a). Overall, it was estimated that this vaccine reduced the risk of new STEC O157:H7 infections occurring by 58% (Vogstad, 2012).

Siderophore Receptor and Porin (SRP) protein vaccines: Siderophores are proteins excreted by bacteria in an effort to obtain iron from its environment, and *E. coli* O157:H7 secretes siderophores in the intestinal tract of cattle. The SRP vaccine targets this protein and disrupts iron transport into the bacterium, resulting in cell death. The Epitopix^{TM} SRP vaccine has been conditionally approved for use in cattle in the U.S. and is undergoing additional safety and efficacy tests. Preliminary research results are promising when the vaccine is utilized in a three dose treatment regimen (Thornton et al., 2009). Other researchers found that vaccination with the SRP reduced fecal concentrations of *E. coli* O157:H7 in cattle by 98%, but the vaccine did not affect cattle performance (Thomson et al., 2009). Vaccination of cattle with this SRP in another study reduced the prevalence of *E. coli* O157:H7 by nearly 50% (Fox et al., 2009). A large scale study of more than 17,000 cattle found that treatment with the SRP vaccine reduced shedding of *E. coli* O157:H7, but they found no impact on fecal prevalence of the six non-O157 serogroups (Cernicchiaro et al., 2014).

Conclusions

Pre-harvest interventions that reduce *E. coli* O157:H7 and other STEC in cattle hold the potential to reduce foodborne pathogen dissemination on farms, in the environment, and from entering the food chain. Yet, development of successful pre-harvest strategies does not eliminate the need for good sanitation and procedures in the processing plant and in the food preparation environment. However, each cattle producer is focused on economic sustainability, but are also committed to wholesome/safe beef production, and pre-harvest food safety techniques could be used during each phase of cattle production. Live-animal interventions must be implemented along the production chain in a complementary fashion to reduce pathogens in a multiple-hurdle approach that complements the in- plant interventions that currently exist, as well as future improvements. Combining the use of pathogen reduction interventions from the farm and in the plant means that beef products leaving the processing facility will have a significant reduction of hazardous foodborne pathogens entering the post-harvest beef supply chain. Increasing interest into the role of the microbiome has sparked new research and theories about how the microbial population of the gut and the host interact and impact STEC populations and shedding (Kim et al., 2017). However, before we attempt to eliminate STEC from the ruminant gastrointestinal microbial ecosystem, we must be aware of potential adverse effects. Because the microbiome of the ruminant gut is so diverse and highly competitive, if a single member of the ruminal microbiome were eliminated, a potentially more dangerous bacteria could emerge and occupy a novel ecological niche, because nature abhors a vacuum (Kim et al., 2017). Therefore, in all our efforts to eliminate STEC from animals prior to slaughter, we must be aware that some other bacteria will always fill a vacancy in any microbial ecosystem.

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