Diet, *Escherichia coli* O157:H7, and Cattle: A Review After 10 Years

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Abstract
Escherichia coli are commensal bacteria that can account for up to 1% of the bacterial population of the gut. Ruminant animals are reservoirs of the pathogenic bacteria *E. coli* strain O157:H7, and approximately 30% of feedlot cattle shed *E. coli* O157:H7. Feedlot and high-producing dairy cattle are fed high grain rations in order to increase feed efficiency. When cattle are fed high grain rations, some starch escapes ruminal microbial degradation and passes to the hindgut where it undergoes fermentation. Ten years ago researchers demonstrated that populations of total *E. coli* were higher in grain-fed than in forage-fed cattle, and when cattle were abruptly switched from a high grain diet to an all hay diet, total *E. coli* populations declined 1000-fold within 5 days and reduced the ability of the surviving *E. coli* to survive an acid shock mimicking passage through the human gastric stomach. This research provoked many questions about the effects of diet or *E. coli* O157:H7 populations that have not been conclusively answered to date. Subsequent research has shown that diet does affect *E. coli* O157:H7 populations, but the effects have varied in magnitude and impact. Further studies have demonstrated that the effects of forage feeding on *E. coli* O157:H7 populations may be due to concentrations of tannins and phenolic acids in forages. Other ration components such as rapidly ruminally fermented grains (e.g., barley) increase the shedding of *E. coli* O157:H7, and in some situations, feeding distillers grains can increase fecal shedding of *E. coli* O157:H7 due to VFA concentrations. Data from researchers across North America indicate that diet does impact STEC/EHEC populations in cattle prior to slaughter; however the economic, logistic and practical impacts of dietary changes must be examined and accounted for.

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Introduction

Escherichia coli is a facultative anaerobic bacterium commonly found in the mammalian intestinal tract (Drasar and Barrow, 1985). *Escherichia coli* lives a fecal-oral lifestyle and can comprise up to 1% of the gastrointestinal population of mammals and is used as an indicator of environmental fecal contamination of water supplies (Winfield and Groisman, 2003). Most *E. coli* strains are commensal; however, some *E. coli* strains can be pathogenic to humans, and are harbored within food animals (Drasar, 1974, Drasar and Barrow, 1985). Some *E. coli* strains can cause hemorrhagic colitis in humans; but the best known enterohemorrhagic *E. coli* (EHEC) strain remains O157:H7 (Scotland et al., 1990). Each year, more than 60 people die and 73,000 people are made ill by *Escherichia coli* O157:H7 in the U.S. (Mead et al., 1999), and EHEC infections are estimated to cost the economy more than $1 billion per year (USDA-ERS, 2001).

Ground beef is the most frequently implicated source of *E. coli* O157:H7 outbreaks, and bovine-derived products are linked to approximately 75% of *E. coli* O157:H7 outbreaks (USDA-APHIS, 1997, Vugia *et al.*, 2007). Cattle are a major reservoir of *E. coli* O157:H7 and repeated hemorrhagic colitis outbreaks linked to consumption of ground beef, animal contact, manure amendation, or cattle manure-contaminated runoff has firmly established the connection between cattle and *E. coli* O157:H7 epidemiologically, and in public perception (Jay *et al.*, 2007, Keen *et al.*, 2007, Steinmuller *et al.*, 2006). Repeated large-scale recalls of *E. coli* O157:H7 contaminated ground beef, and the well-publicized deaths of children who consumed foods contaminated by exposure to beef products or ruminants have further shaken the confidence of consumers in the wholesomeness and safety of beef (Gage, 2001). In-plant post-harvest sanitation efforts effectively reduce contamination of carcasses with *E. coli* O157:H7 (Arthur *et al.*, 2007a, Bosilevac *et al.*, 2006, Woerner *et al.*, 2006). However, no matter the effectiveness of these strategies, they are not sufficient to ensure human food safety and health.

Studies have shown than up to 30% of all cattle are asymptomatic carriers of *E. coli* O157:H7 (Callaway *et al.*, 2006, Reinstein *et al.*, 2007, Stanford *et al.*, 2005). Manure from cattle production facilities can contain viable *E. coli* O157:H7 and be washed into the water supply and consumed directly in drinking water, or be used as irrigation water on crops, or transmitted by other animal vectors (Hill *et al.*, 2006, LeJeune *et al.*, 2001, Sargeant *et al.*, 2003, Thurston-Enriquez *et al.*, 2005). Therefore, methods that reduce *E. coli* O157:H7 populations in food animals prior to entry to the food chain have great potential to reduce human illnesses (Callaway *et al.*, 2004, Loneragan and Brashears, 2005, Sargeant *et al.*, 2007).

One method proposed to reduce EHEC in cattle is to abruptly change the diet from a high grain to a forage based ration. This suggestion was based on research results first published in 1998 that demonstrated that an abrupt shift from grain to hay-based rations reduced generic *E. coli* populations significantly (Diez-Gonzalez et al., 1998). This study elicited a great deal of subsequent research that has yielded variable results (Hancock *et al.*, 2000, Hovde *et al.*, 1999, Keen *et al.*, 1999). Therefore, as the 10 year anniversary of this hypothesis is reached, this review examines the current state of knowledge about the effects of dietary and other cattle management manipulations on *E. coli* and O157:H7 populations.

Enterohaemorrhagic *E. coli* and their reservoir, ruminant animals

The microbial population of the ruminant is diverse and microbes live throughout the gastrointestinal tract of mammals, and this includes the ubiquitous and adaptable *E. coli* (Drasar and Barrow, 1985, Yokoyama and Johnson, 1988). *Escherichia coli* are rarely cultured in high numbers from the rumen of cattle, typically less than 10^6 cells/ml out of a population of $>10^{10}$ cells/ml (Laven *et al.*, 2003, Min *et al.*, 2007, Wolin, 1969). *Escherichia coli* were not considered "important" ruminal bacteria (Rasmussen *et al.*, 1999, Wolin, 1969) because of the competitive nature of the rumen and the fact that high concentrations of volatile fatty acids (VFA) are bactericidal (Wallace *et al.*, 1989, Wolin, 1969). In the lower intestinal tract, conditions are generally more favorable for *E. coli*, where they can be found at concentrations ranging from 10^2 to 10^7 cells/g feces at slaughter (Davidson and Taylor, 1978, Jordan and McEwen, 1998). In spite of the severity of illness in humans caused by EHEC, they are not predominant members of the *E. coli* population (Laven *et al.*, 2003, Tkalcic *et al.*, 2003, Zhao *et al.*, 1998). In addition to gastrointestinal populations, the oral cavities of cattle also can contain EHEC (Keen and Elder, 2002, Smith *et al.*, 2005), likely due to the process of rumination. However, it must be noted that the genotype of *E. coli* O157:H7 isolates from the oral cavity can differ significantly from fecal isolates (Keen and Elder, 2002).

Researchers have shown that *E. coli* were associated with the digesta rather than with the intestinal wall (Laven et al., 2003). Other studies have demonstrated that the terminal end of the colon was the major site of *E. coli* O157:H7 colonization (Grauke et al., 2002) and the lymphoid tissue located at the recto-anal junction (RAJ) has been demonstrated to be the primary site of colonization of cattle (Naylor et al., 2003). Other researchers have subsequently verified that the RAJ is intimately involved with *E. coli* O157:H7 colonization (Davis, 2006, Greenquist *et al.*, 2005, Lim *et al.*, 2007, Rice *et al.*, 2003) leading to the suggestion that RAJ colonization may be involved in the phenomenon of "super-shedders" (Cobbold, 2007, LeJeune and Kauffman, 2006).

The EHEC strain responsible for most human illnesses and that colonize cattle vary geographically and temporally (Cookson *et al.*, 2007, Fagan *et al.*, 1999, Wang *et al.*, 2000). Studies have found cattle can be concomitantly colonized by up to 26 different serotypes of EHEC (Schurman et al., 2000). In the U.S. most illnesses and subsequent surveys and interest have focused on strain O157:H7, although other EHEC are found in the U.S. in humans and cattle (Hussein *et al.*, 2003b, Midgley *et al.*, 1999). Therefore it is emphasized that although the U.S. focus remains on strain O157:H7, that we not forget the other EHEC (Acheson, 2000), because the natural ecology of EHEC suggests that if strain O157:H7 decreases then another EHEC strain (or another pathogen) would simply fill the vacuum.

Shedding of EHEC in cattle appears to be widespread, but sporadic (Meyer-Broseta et al., 2001). EHEC shedding is highly dependent on season of the year (Gyles, 2007, Sargeant *et al.*, 2007) and can range from as many as 80% of all feedlot cattle in the summer, to as few as 5-10% shedding during the winter (Barkocy-Gallagher *et al.*, 2003, Elder *et al.*, 2000, Naumova *et al.*, 2007). This correlates with an increase in human outbreaks during each summer/early fall, thus highlighting a linkage between the animal (reservoir) populations and consumers via food-borne outbreaks (USDA-APHIS, 1997, Vugia *et al.*, 2007). Seasonality of shedding has long been theorized to be related to temperature or weather (Barkocy-Gallagher *et al.*, 2003, Naumova *et al.*, 2007); however a recent theory has emerged that day length and melatonin or seasonal hormones may play a role in this phenomenon, however much further research is needed to investigate this intriguing hypothesis (Edrington *et al.*, 2007, Edrington *et al.*, 2006a, Schultz *et al.*, 2005).

Hides a reservoir of E. coli O157:H7

Bovine manure can harbor *E. coli* O157:H7 at the typical environmental temperatures for ≥49 d (Wang et al., 1996). Dirt and feces that collects on the hides of cattle can therefore be contaminated with *E. coli* O157:H7 for long periods of time (Arthur *et al.*, 2007b, Barkocy-Gallagher *et al.*, 2004, Keen and Elder, 2002, Reid *et al.*, 2002). Research has indicated that the number of hides positive for *E. coli* O157:H7 is a more accurate predictor for carcass contamination than is fecal prevalence (Barkocy-Gallagher et al., 2003). The incidence of hides contaminated by *E. coli* O157:H7 in studies ranged from 14% to 85% (Elam *et al.*, 2003, Stephens *et al.*, 2007, Woerner *et al.*, 2006, Younts-Dahl *et al.*, 2004). Recently it was demonstrated that when a pen had a >20% fecal incidence rate, then the percentage of hides positive for of *E. coli* O157:H7 rose to 26%; however when the fecal incidence rate in the pens was <20% then the hide contamination level was only 5% (Woerner et al., 2006). Thus a common-sense approach to decreasing the prevalence of *E. coli* O157:H7 on hides has led to the development of methods to remove the hair from hides prior to dehiding/evisceration (Castillo *et al.*, 1998, Nou *et al.*, 2003) or to specialized treatments that reduce the pathogen load on the hide (Bosilevac *et al.*, 2005, Bosilevac *et al.*, 2004). Gregory et al. (2000) found that when cattle arrived at the slaughter plant the hides of cattle fed hay for 48 h prior to transport were as clean as the hides of fasted cattle, and were significantly cleaner than pasture-fed cattle (Gregory et al., 2000).

Do *E. coli* O157:H7 levels in the live animal really matter?

Data indicate that in-plant intervention strategies reduce the spread of *E. coli* O157:H7 on and between carcasses (Arthur *et al.*, 2002, Barkocy-Gallagher *et al.*, 2003, Bosilevac *et al.*,

2006, Woerner *et al.*, 2006). If pre-harvest interventions can be introduced to reduce levels of *E. coli* O157:H7 that enter the abattoir within the live animal, we should be able to further enhance the effectiveness of in-plant intervention strategies (Callaway *et al.*, 2004, Loneragan and Brashears, 2005). Thus reducing the burden of pathogens entering the abattoir should enhance human health (Hynes and Wachsmuth, 2000). However direct infection via food is not the only route of human exposure to foodborne pathogenic bacteria.

The increasing disconnect between the consumer and their agricultural food supply is reflected in a growing number of direct-contact illnesses in humans contracted in farmyards, open farms, petting zoos, and zoological parks (Chapman *et al.*, 2000, Edwards *et al.*, 2008, Keen *et al.*, 2007, Keen *et al.*, 2003, Pritchard *et al.*, 2000). Recent years have seen an increase in human *E. coli* O157:H7 illnesses linked to water contaminated by run-off from feedlots and dairies (Anonymous, 2000, Edwards *et al.*, 2008, Goss and Richards, 2007, Jay *et al.*, 2007). The spread of *E. coli* O157:H7 in runoff from farms has only been recently assessed to understand the movement of pathogens from farms during rainfall events (Berry *et al.*, 2007, Ferguson *et al.*, 2007). Further concerns have been raised about the spread of *E. coli* O157:H7 to humans through crops (e.g., spinach or lettuce) irrigated with water from cattle farms (Gerba and Smith, 2005, Manshadi *et al.*, 2001, Natvig *et al.*, 2002). The recent (2006), widespread *E. coli* O157:H7 outbreaks linked to spinach and lettuce (Jay et al., 2007) contaminated by swine that had foraged on a dairy highlights the ability of EHEC to cause human illnesses through a variety of vectors, further emphasizing the need to reduce foodborne pathogenic bacteria in the live animal before they contact human consumers (Hynes and Wachsmuth, 2000, Loneragan and Brashears, 2005, Sargeant *et al.*, 2007).

Dietary effects on *E. coli* O157:H7 shedding in cattle

Fasting

Before and during transport, cattle can at times be fasted for up to 48 h, which can affect their susceptibility to colonization by *E. coli* O157:H7. Ruminal and intestinal VFA concentrations limit *E. coli* populations because VFA are toxic (Hollowell and Wolin, 1965, Russell and Diez-Gonzalez, 1998, Wolin, 1969). This has allowed VFA and other organic acids to be used to reduce pathogen populations in the gut (Ohya *et al.*, 2000, Prohaszka and Baron, 1983, Van Immerseel *et al.*, 2006). 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 on the shedding of EHEC.

Fasting increased *E. coli*, *Enterobacter* and total anaerobic bacterial populations throughout the intestinal tract (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), furthermore, fasting can cause "apparently *E. coli* (O157:H7) negative animals to become positive" (Kudva et al., 1995). Further studies indicated that fasting made calves more susceptible to colonization by inoculated *E. coli* O157:H7, and demonstrated that fasted inoculated calves shed more *E. coli* O157:H7 than did calves fed normally (Cray et al., 1998). Cattle fasted for 48 h prior to slaughter also were shown to contain significantly greater *E. coli* populations throughout the gut than cattle fed hay or pasture (Gregory et al., 2000). In contrast, it was demonstrated that a fasting period reduced ruminal VFA concentrations, but this did not influence *E. coli* O157:H7 shedding (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 EHEC populations (Bach *et al.*, 2002a, Bach *et al.*, 2005b, Cobbold and Desmarchelier, 2004, Shin *et al.*, 2002). Thus research indicates fasting increases shedding or makes cattle more susceptible to colonization due to decreased short chain VFA and increased pH in the gastrointestinal tract.

Can feed additives and antimicrobials affect E. coli O157:H7?

Ionophores, such as monensin and lasalocid, are 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 can alter the microbial population, possibly giving gram-negative bacteria (such as *E. coli*) a competitive advantage, they were an obvious risk factor to investigate in regard to their role in colonization and shedding of *E. coli* O157:H7. As expected, based upon the gram-negative membrane physiology of *E. coli* O157:H7, the most widely used ionophore (monensin) did not affect the growth or growth rate of this pathogen in vitro when added at concentrations similar to that found in the rumen of cattle fed monensin (Bach et al., 2002b), subsequently *E. coli* O157:H7 has been shown to be resistant to concentrations of monensin as high as 3-fold higher than that normally found in the rumen (Van Baale et al., 2004). Ionophoric feed additives (monensin, lasalocid, laidlomycin and bambermycin) demonstrated no effect on *E. coli* O157:H7 in vitro (Edrington *et al.*, 2003).

Early epidemiological studies demonstrated a marginally significant increase of EHEC shedding by heifers fed ionophores (Herriott et al., 1998), but others could not draw any conclusions (Dargatz et al., 1997). 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 a recent 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 $\log_{10}$ CFU/ml in ruminal fermentations from cows fed forage, but did not affect *E. coli* O157:H7 populations in ruminal fluid from cows fed corn (McAllister et al., 2006). The inclusion of monensin and tylosin did not alter fecal shedding of experimentally-inoculated *E. coli* O157:H7 when either were fed alone or in combination in cattle fed a barley (grain)-based diet (McAllister et al., 2006). These results are intriguing in that they suggest there is a potential interaction between diet type and antimicrobial treatment; however, no definitive proof of this linkage has been demonstrated.

Other feed additives have been examined such as ractopamine and neomycin. Researchers found that the β-agonist ractopamine, which is used to increase animal

growth performance, increased fecal shedding and cecal populations of *E. coli* O157:H7 in sheep (Edrington et al., 2006b). Neomycin is an antibiotic that is used to a limited degree in human medicine and is approved for use in cattle to treat enteric infections. Neomycin was demonstrated to reduce *E. coli* O157:H7 populations in the gut (Elder *et al.*, 2002, Ransom *et al.*, 2003) and on the hides of cattle (Ransom et al., 2003). However, this treatment has not been recommended to reduce *E. coli* O157:H7 in cattle due to antimicrobial resistance concerns.

Probiotics and E. coli O157:H7

In order to enhance ruminant production efficiency, various probiotics (including yeast cultures, competitive exclusion [CE] products, and direct-fed microbials [DFM]) have been widely used in the cattle industry for many years (Dawson *et al.*, 1990, Yoon and Stern, 1996). These probiotic products have been primarily utilized to increase growth rate, milk production, or production efficiency (Callaway *et al.*, 2005b, Fuller, 1989). A commercial *Saccharomyces cerevisiae* DFM culture was found to reduce *E. coli* O157:H7 populations in batch culture but not in a continuous flow culture system that simulated a bovine gut (Bach et al., 2003). 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, and this decrease was attributed to an increase in VFA concentration in the gut (Ohya et al., 2001).

Other researchers have specifically developed probiotic products to reduce *E. coli* O157:H7 shedding in cattle. A probiotic that contained *S. faecium*, or a mixture of *S. faecium*, *L acidophilus*, *L. casei*, *L fermentum* and *L. plantarum* significantly reduced fecal shedding of *E. coli* O157:H7 in sheep from 2-4 $\log_{10}$ CFU/g feces (Lema et al., 2001); however, a probiotic compose solely of *Lactobacillus acidophilus* was ineffective (Lema et al., 2001). A *L. acidophilus* culture that was derived from a cattle rumen 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). This culture reduced fecal shedding of *E. coli* O157:H7 in cattle from 46% to 13% (Ransom et al., 2003). In a further refinement, *L. acidophilus* cultures were combined with *Propionibacterium freudenreichii* (a propionate-producing commensal bacteria) 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 has again shown reductions in *E. coli* O157:H7 and *Salmonella* in feces and on hides (Stephens et al., 2007). This probiotic improves the growth efficiency of cattle, which can economically pay for the cost of its inclusion in cattle rations as a food safety enhancement.

Competitive exclusion (CE) is another strategy to eliminate *E. coli* O157:H7 (as well as *Salmonella*) from cattle intestinal tracts (Brashears and Galyean, 2002, Brashears *et al.*, 2003a, Brashears *et al.*, 2003b, Zhao *et al.*, 2003). A defined population of multiple non-EHEC *E. coli* strains that were isolated from cattle that did not contain *E. coli* O157:H7, and found this generic *E. coli* CE culture could displace an established *E. coli* O157:H7 population from calves (Zhao et al., 1998).

Feedstuffs and *E. coli* O157:H7 populations?

Finishing beef and lactating dairy cattle in the United States are fed high grain rations in order to maximize animal performance and production efficiency (Huntington, 1997). Ruminal bacteria can break down dietary starch, although some starch does reach the colon in an undegraded form where it undergoes microbial fermentation (Huntington, 1997). 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 in cattle rations, and is fermented more quickly than corn by the ruminal microbial population. This means that more starch is fermented in the lower gut of corn-fed cattle than in barley-fed cattle; resulting in barley-fed cattle having a higher fecal pH and lower VFA concentration 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). Feeding of barley-based diets resulted in higher fecal pH compared to corn diets and resulted in higher *E. coli* O157:H7 prevalence and quantity of *E. coli* O157:H7 shedding compared to cattle fed corn-based rations (Berg et al., 2004). 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).

Recent research has demonstrated that steam-flaked grains increased *E. coli* O157 shedding the feces compared to diets composed of dry-rolled grains (Fox et al., 2007). This difference was theorized to be due to dry rolling allowing the passage of more starch to the hindgut where it was fermented to produce VFA thereby killing *E. coli* O157 (Fox et al., 2007). This theory is supported by the fact that post-ruminal starch infusion, increased generic *E. coli* populations in the lower gut numerically (Van Kessel et al., 2002).

Comparing grain-fed to forage-fed cattle still indicates that more *E. coli* (including O157:H7) are present in the feces of cattle fed grain diets. In experimental inoculation 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 for cattle on a grain-based diet (Van Baale et al., 2004). However, it must be emphasized, that although populations *E. coli* O157:H7 are generally lower in cattle fed forage diets, EHEC 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).

One of the most interesting developments involving dietary effects on pathogens in cattle has evolved from the recent surge in the use of corn to produce ethanol. Distillers grains were shown to increase the shedding of *E. coli* O157:H7 in cow-calf operations in Scotland (Synge et al., 2003). Other researchers found that feeding a related product (brewers grain) to cattle was also associated with increased *E. coli* O157 shedding, and increased the odds of shedding by more than 6-fold (Dewell et al., 2005). More recent studies have shown that there is a positive association between distillers grain feeding and an increased prevalence of *E. coli* O157 (Jacob *et al.*, 2008a, Jacob *et al.*, 2008b). 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. In a follow-up study, cattle were fed a steam-flaked corn diet supplemented with 0% or 25% dried distiller's grain (DDG). 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). Further studies found that DDG significantly increased fecal shedding and intestinal populations of inoculated *E. coli* O157:H7 compared to calves fed a steam-flaked corn based ration (Jacob et al., 2008c). It is important to note that the extent of the increase in *E. coli* O157:H7 is variable, and a great deal of variation occurs between sources and even batches of distillers grains. The underlying biology behind this effect has not been elucidated to this point, but it has been suggested that difference could be due to intermediate endproducts of the yeast fermentation (e.g., vitamins, organic acids), however these suggestions remain hypotheses. I*n vitro* studies in our laboratory have detected no effects of DG on *E. coli* O157:H7 populations in mixed ruminal and fecal fluid fermentations (Callaway et al., unpublished). While the magnitude of these DG effects is relatively small and variable, it underlines the point that diet can potentially significantly impact *E. coli* O157:H7 populations in the gut of cattle.

Orange peel and pulp included at 2% of the total volume have been demonstrated to have anti-*E. coli* O157:H7 activity in *in vitro* fermentations (Callaway *et al.*, 2008, Callaway *et al.*, 2005a, Fisher and Phillips, 2006). This effect appears to be a result of the antimicrobial action of essential oils (e.g., limonene) found in the peel (Callaway et al., 2008). Other research has found that feeding an *Ascophyllum nodosum* supplement reduced *E. coli* O157:H7 populations in feces from 35% to 10% and reduced hide sample positives from 85% to less than 50% (Braden et al., 2004). These results underscore that certain feedstuffs and diet can exert potent effects on the microbial population and can be used to control pathogens in certain circumstances and dietary regimens.

Forage to grain dietary shifts: their effects on fecal *E. coli* and *E. coli* O157:H7 populations

Escherichia coli can and does thrive in the lower gut of animals fed high grain diets, as well as those fed forage diets (Hussein *et al.*, 2003a, Hussein *et al.*, 2003b, Jacobson *et al.*, 2002) However, shifting the forage to grain ratio in cattle rations can affect *E. coli* O157:H7 shedding, Early studies investigating *E. coli* and dietary effects indicated that a sudden decrease in hay intake by cattle increased fecal *E. coli* populations (Brownlie and Grau, 1967) and overfeeding of cattle with grain caused an increase in total fecal coliform counts (Allison et al., 1975). Other studies using experimentally infected sheep found a sudden switch from an alfalfa pellet diet to a low quality forage diet increased *E. coli* O157:H7 shedding (Kudva et al., 1995). Sheep shifted from a 50:50 corn/alfalfa ration to poor quality grass hay shed greater populations of *E. coli* O157:H7 than did sheep fed a corn/alfalfa ration (Kudva et al., 1997).

In the study that started a great deal of controversy on this topic, cattle fed a 90% corn/soybean meal ration (feedlot-type ration) contained generic *E. coli* populations that were 1000-fold higher than cattle fed a 100% good-quality hay (Timothy) diet (Diez-Gonzalez et al., 1998). The *E. coli* recovered from the feces of grain-fed cattle in this study were 1000-fold more resistant to an "extreme" acid shock that simulated passage through the human stomach than were *E. coli* from cattle fed only hay (Diez-Gonzalez et al., 1998). When cattle were abruptly switched from a 90% grain finishing ration to a 100% hay diet, fecal *E. coli* populations declined 1000-fold, and the population of *E. coli* resistant to an acid environment similar to that of the human stomach declined more than 100,000-fold within 5 d (Diez-Gonzalez et al., 1998). 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 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).

Although it appears that brief periods of hay feeding can affect *E. coli* populations research indicates that a brief period does not have a significant impact on carcass characteristics but does change final BW (Stanton and Schutz, 2000). When cattle were fed hay during the final portion of the finishing period, they had lower DMI and lost an average of 2.2 lb/head/d and did not significantly impact carcass weight, dressing percentage, carcass grades, or quality parameters, but did significantly reduce total coliform counts as well as generic *E. coli* counts (Stanton and Schutz, 2000), but the impact was not as great as that seen by Diez-Gonzalez et al. (1998). Keen et al., (1999) found that switching cattle from grain to hay caused a decrease in body weight (approximately 1.25 lb/hd/d compared to controls). Over 200 cattle maintained on a grain ration were screened for natural *E. coli* O157:H7 infection and 53% were found to be positive (Keen et al., 1999). When these cattle 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 (Keen et al., 1999).

The proposal of such a dietary switch to reduce *E. coli* O157:H7 shedding provoked a great deal of scientific controversy (Diez-Gonzalez *et al.*, 1998, Hancock *et al.*, 2000) and led to several studies that have subsequently evaluated the effect of radical dietary shifts on *E. coli* populations in cattle, however these studies have also produced conflicting results (Table 1). When cattle were fed a high-concentrate diet and switched to a diet containing 50% corn silage and 50% alfalfa hay, generic *E. coli* counts decreased (Jordan and McEwen, 1998). Cattle fed an 80% barley ration (which as shown previously tends to increase EHEC shedding) were 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 forage-fed animals were again fasted for 48 h and re-fed 100% alfalfa silage, the prevalence of *E. coli* O157:H7 shedding increased significantly (Buchko et al., 2000b). In a study using experimentally infected cattle, Researchers found that 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 dietary regimes and the diet shift did not affect the acid resistance of *E. coli* O157:H7 (Hovde et al., 1999). When cattle were abruptly switched from a finishing diet that contained wet corn gluten feed to alfalfa hay for 5 d, colonic pH increased almost 1 pH unit, total *E. coli* populations decreased approximately 10-fold (Scott et al., 2000). These authors concluded "increased colonic pH was not associated with reduced populations of acid resistant *E. coli*" but "feeding hay for a short duration can reduce acid-resistant *E. coli* populations" (Scott et al., 2000).

In research that approached this question from a different perspective, it was found that when cattle switched from forage-type diets (bromegrass hay or corn silage) to a high grain finishing ration, fecal and ruminal generic *E. coli* concentrations increased (Berry et al., 2006). However, in this study *E. coli* O157:H7 levels were not significantly impacted (Berry et al., 2006). In another study, switching cattle from pasture to hay for 48 h prior to slaughter significantly reduced the *E. coli* population throughout the gut, and found that hay feeding increased intestinal *Enterococci* populations that were capable of inhibiting *E. coli* populations (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 dietary manipulations could be a powerful method to reduce *E. coli*/EHEC populations in cattle prior to harvest.

A theory addressing the effects of forage and dietary shifts on *E. coli* O157:H7 populations

It appears that a dietary shift does cause changes in the microbial populations, including *E. coli* O157:H7, yet studies investigating this phenomenon have often produced contradictory results (Table 1). Shifting the diet abruptly obviously causes a change in the availability and concentrations of substrates available for fermentation in the lower intestinal tract, as well as the members of the microbial intestinal population. Grain-based diets tend to yield higher levels of *E. coli* in the feces due to the higher availability of starch. A change from grain diets to hay shifts the site and extent of digestion toward the rumen, and reduces starch availability in the colon. This change in nutrient availability furthermore causes a rapid shift in the microbial population of the gut, and some organisms selected for by hay feeding (such as *Enterococcus*) can exhibit competitive exclusion type behavior (Gregory et al., 2000), displacing established *E. coli* O157:H7 populations or preventing colonization (Callaway and Martin, 2006, Schneitz, 2005). Increasing the fiber component of the diet also increases the undigestible component of the diet which can physically "scrape" the gut mucosa which has been shown be colonized by *E. coli* O157:H7 (Lim *et al.*, 2007, Low *et al.*, 2005, Naylor *et al.*, 2003), and could physically remove these organisms.

Comparing results from dietary switch studies, it appears that some component of forage quality is involved in some of the differences (e.g, switching to alfalfa hay vs. switching to sagebrush). To date, however it has not been clearly demonstrated which aspect of forage quality is involved because none of these studies have used similar diets, protocols or *E. coli*/EHEC isolation techniques, thereby preventing direct comparison. We theorize that some factors intrinsic to forages may explain some of this effect, as well as some of the inconsistencies found between forages used.

Tannins are anti-nutritional polyphenols that are found in some forages, which have been shown to inhibit the growth of ruminal bacterial (Nelson *et al.*, 1997, Nelson *et al.*, 1998). Tannins inhibit and kill *E. coli* O157:H7 in *in vitro* studies and were found to reduce the shedding of generic *E. coli* over a 15 d period in steers fed tannins (Min et al., 2007). Additionally, as forages mature concentrations of the carbohydrate lignin, which contains carboxylic phenols such as *p*-coumaric acid and vanillin (Heppner, 1968, Martin, 1970). It has been long known that these components of lignin are bactericidal (Martin, 1990), but only recently has research demonstrated that these compounds are capable of killing *E. coli* O157:H7 *in vitro* and in manure (Wells et al., 2005). These data suggest a theory that forage quality and components may play a role in reducing *E. coli* O157:H7 in food animals, and that variation in the concentration of tannins and/or lignin in forages tested in various studies may be responsible for the variance in the dietary shift studies.

In general, the greatest reductions in *E. coli* O157:H7 populations have been observed when cattle were switched to high quality forages; yet high concentrations of tannins and lignin are negatively correlated with forage quality. Therefore, details surrounding this hypothesis obviously need to be examined further. However, given the effects phenolic compounds have in altering the efficiency of the ruminal fermentation (Martin, 1990, Nelson *et al.*, 1997), it is possible that, while tannins and carboxylic phenols do directly kill *E. coli* O157:H7 at physiologically unrealistic concentrations, when phenolics are added to in the intestinal consortium the phenolic compounds could still provide a competitive advantage to organisms that compete against

Table 1. Published reports of effects of diet on fecal *E. coli* populations in cattle					
Authors	Concentrate diet	*E. coli*	Forage diet or alternative feed	*E. coli* CFU	Log_{10} impact
Allison, 1975	Normal diet Over fed grain	8×10^6 CFU coliforms/g 1×10^{10} CFU coliforms/g			
Kudva, et al., 1995	100% Alfalfa pellets	Shed O157:H7 for 4 d	Sagebrush/bunchgrass	Shed O157:H7 for 15 d	
Diez-Gonzalez et al., 1998	90% Concentrate	8×10^7 CFU/g feces	100% Timothy Hay	3×10^4 CFU/g feces	-3.5
Jordan and McEwen, 1998	44% Dry corn 7% Dry Gluten 7% Distiller's Dried Grains	7×10^6 CFU/g feces	50% Corn silage 50% Alfalfa	4×10^6 CFU/g feces	-0.3
Keen et al., 1999	85% Concentrate	52% shedding O157:H7	100% Forage	18% shedding O157:H7	
Hovde et al., 1999	62% Barley/19% corn 90% Corn	7×10^6 CFU/g feces peak (4 d of shedding)	Alfalfa or Grass Hay	7×10^6 CFU/g feces peak (39 or 42 d shedding)	
Stanton and Schutz, 2000	85% Whole Corn	3.2×10^7 CFU/g	30% Millet hay 62% whole corn	1×10^6 CFU/g feces	-1.2
Scott et al., 2000	84% DRC or 41% DRC 45% WCG	3×10^8 CFU/g 5×10^8 CFU/g feces	100% Alfalfa hay	1×10^7 CFU/g 9×10^6 CFU/g feces	-1.2 -1.6
Bucko et al., 2000a	80% Concentrate	5% shedding O157:H7	100% Alfalfa silage (after 48 h fast) Re-fed 100% alfalfa silage (after 48 h fast)	5% shedding O157:H7 42% shedding O157:H7 after 5 d	
Van Baale et al, 2004	80% corn 15% alfalfa hay	16 d of shedding O157:H7	70% prairie hay 15% alfalfa hay	60 d of shedding O157:H7	
Berry et al., 2006	70% corn and 24% corn silage	Approximately 10^6 CFU/g generic *E. coli*	100% bromegrass hay or 87% corn silage	Approximately 10 CFU/g generic *E. coli*	
Fox et al., 2007	Steam flaked corn/wheat and sorghum vs. dry-rolled corn/wheat and sorghum	65% shedding O157 Vs.30% shedding			
Jacob et al., 2008a	85% steam-flaked corn 15% corn silage	3.6% shedding O157	25% of SFC replaced with Dried Distillers Grain	9.8% shedding O157	
Jacob et al., 2008b	84% steam flaked corn	14% shedding O157	25% of grain replaced with Wet Distillers Grain	3% shedding O157	

E. coli O157:H7, helping to reduce its populations in the intestinal ecosystem.

Conclusions

The United States has one of the safest food supplies, yet food-borne pathogenic bacteria are still significant threats to human health, including Enterohaemorrhagic *E. coli*. Post-harvest sanitation strategies have reduced *E. coli* O157:H7 in meat products, but pre-harvest intervention strategies offer methods to reduce pathogen populations in food animals before they enter the food chain. Reductions in *E. coli* O157:H7 shedding on farms can reduce human exposures through water supplies, fruits and vegetables, as well as via direct animal contact. Some feedstuffs do appear to alter shedding levels of *E. coli* O157:H7, but these effects have not always been consistent. Fasting and feeding poor quality forages have been shown to increase shedding of *E. coli* O157:H7 in cattle; however abruptly switching cattle from a high grain ration to a high-quality hay-based diet has been shown to reduce generic *E. coli* and *E. coli* O157:H7 populations. However, switching all feedlot cattle in the U.S. from grain-based diets to hay prior to slaughter is not practical. Further research is needed to elucidate the mechanism (e.g., competitive exclusion, physical removal, forage quality, tannins, lignin, other phenolics) by which forage-feeding impacts the microbial ecology of the bovine intestinal tract, including the ecology of *E. coli* and *E. coli* O157:H7 populations, so that economically viable and practical dietary modifications can be implemented.

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