

FECAL pH AND STARCH CONCENTRATIONS IN RELATION TO PREVALENCE
OF *ESCHERICHIA COLI* O157 IN FEEDLOT CATTLE

by

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Abstract

Escherichia coli O157, a food-borne human pathogen, causes hemorrhagic colitis and hemolytic uremic syndrome. Cattle are a major reservoir and the organism resides in the hindgut and is shed in the feces. Cattle feces are a major source of food and water contamination. Houseflies feed on cattle manure and are a source of *E. coli* O157 transmission. We have observed that houseflies have an affinity for a steam-flaked corn product (SFC-36) made from tempered whole corn that is more ruminally digestible than the traditional SFC (SFC-18). Therefore, we investigated whether SFC-36 diets contained and resulted in higher *E. coli* concentrations in the feces of cattle compared to SFC-18 diets. Concentrations of *E. coli* were not different between the two SFC diet samples, but resulted in higher coliforms in diets containing the SFC-36 after exposure to the environment. However, *E. coli* concentrations in feces from cattle fed the two diets were similar. In fact, cattle fed the diet containing SFC-18 flakes actually shed higher concentrations of coliforms. This led us to speculate that starch digestion may have an effect on the growth of *E. coli* O157 in the hindgut. We determined whether fecal *E. coli* O157 was related to fecal starch concentration. Steers (n=263) were sampled for *E. coli* O157 and fecal starch concentration determinations. Steers positive for *E. coli* O157 contained 21% more ($P < 0.05$) fecal starch than steers that were negative for *E. coli* O157. We attempted to alter the concentration of starch escaping rumen fermentation by feeding diets based on SFC and dry-rolled corn (DRC) to 30 heifers prescreened for being culture positive for fecal *E. coli* O157. Heifers were sampled for feces and by rectoanal mucosal swab (RAMS) weekly to monitor fecal pH and fecal starch

concentration, and prevalence of *E. coli* O157. Based on RAMS, prevalence of *E. coli* O157 tended to be higher ($P = 0.08$) for heifers fed SFC than DRC diet. Fecal starch and pH were similar ($P > 0.05$) between positive- or negative-*E. coli* O157 heifers. Apparently, fecal *E. coli* O157 was not related to fecal pH or starch concentration in cattle.

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**CHAPTER 1 - *Escherichia coli* O157 and starch digestion
in finishing feedlot cattle: A review of the literature.**

B. E Depenbusch and J. S. Drouillard

Literature review of *Escherichia coli* O157

Escherichia coli (*E. coli*) a gram-negative and facultative anaerobic bacteria was first described by Dr. Theodor Escherich in 1885. Many different strains of *E. coli* are commonly found inhabiting the mammalian digestive tract. *E. coli* can account for as much as 1% of the colonic bacteria according to Diez-Gonzalez et al. (1998). These microflora are beneficial to the mammal in that they synthesize vitamin K and B-complex vitamins. Generic *E. coli* are harmless commensal organisms; however they can be toxic if they penetrate through the gut wall and into the portal blood system (Russell et al., 2000a). Once in the portal blood system, these bacteria can release an endotoxin (i.e. lipopolysaccharide complex) from their cell wall when they lyse, which can cause fever and even death (Nataro and Kaper, 1998). Isolates of *E. coli* are serologically differentiated on the basis of three major surface antigens O (somatic), H (flagella), and K (capsule). *E. coli* strains that cause diarrheal illness are categorized based on virulence properties, mechanisms of pathogenicity, clinical syndromes, and distinct O:H serogroups. These categories include enteropathogenic *E. coli* strains (EPEC), enterotoxigenic *E. coli* strains (ETEC), enteroinvasive *E. coli* strains (EIEC), diffuse-adhering *E. coli* strains (DAEC), enteroaggregative *E. coli* (EAaggEC), and enterohemorrhagic *E. coli* strain (EHEC) (Doyle et al. 1997; Nataro and Kaper, 1998). The EHEC *E. coli* strains include O157:H7, O26:H11, O103, O104, O11, and O128. *E. coli* O157:H7 is the primary EHEC strain associated with human illness in the U.S. The majorities of *E. coli* O157 is motile and have the H7 flagella. However approximately 10-20% of the *E. coli* O157 are non-motile and are commonly referred to as *E. coli* O157:NM.

Research on *E. coli* O157:H7 started with the finding of *Shigella dysenteriae* as the causative agent of epidemic bacterial dysentery by Kioshi Shiga in 1898 (Park et al., 2001). Hemolytic uremic syndrome (HUS) was first described in 1955 as a triad of clinical features, including acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia (Park et al., 2001). In 1972, Keusch et al. discovered that Shiga toxins can contribute to bloody diarrhea. The first confirmed isolation of *E. coli* O157:H7 in the

United States was in 1975 from a California woman with bloody diarrhea (Doyle et al. 1997). Shortly thereafter, Konowalchuk et al. (1977) found that certain diarrheagenic *E. coli* strains produced a substance that was toxic (cytotoxin) to Vero (African green monkey kidney) tissue culture cells. *E. coli* O157:H7 was first identified as pathogenic to humans in 1982, after it was associated with two outbreaks of hemorrhagic colitis (HC; i.e. bloody diarrhea; Karmali et al., 1983 and Riley et al., 1983). Not all EHEC infections produce overt blood in stools, but *E. coli* O157:H7 infections do have a higher rate of bloody stools (Buchanan & Doyle 1997). *E. coli* O157:H7 commonly produces shiga toxins (STXs), formally called Shiga-like toxins (SLTs) or verotoxins (VTs), all of which resemble the cholera toxin (Natro and Kaper, 1998). These toxins are able to cross the intestinal wall much easier than the bacteria itself and can cause acute diarrhea even if the bacteria never cross the intestinal wall. (Natro and Kaper, 1998) STXs play a key role in the pathogenesis of renal failure by inducing endothelial damage in the glomeruli and arterioles of the kidney (Park et al., 2001). This localized damage is associated with higher expression of Gb3 receptors (receptor for STXs) on the renal endothelial cells as compared to other endothelial cells (Park et al., 2001). Interestingly, children have many more Gb3 receptors than adults and this can explain in part why children are more susceptible to renal failure than adults (Van Setten et al., 1997). Two main classes of STXs are Stx1 and Stx2. EHEC can express either one or both of them together. Most commonly, the *E. coli* O157:H7 isolates from humans express both Stx1 and Stx2 or they will only express Stx2 (Rasmussen & Casey 2001). The ability to produce the STXs is believed to have originated with a bacteriophage, presumably directly or indirectly from *Shigella* (Buchanan & Doyle 1997).

Besides Stx1 and Stx2, there are other suspected virulence factors in *E. coli* O157:H7. Virulence genes contain a variety of specific characteristics. Intimin (eae), translocated intimin receptor (tir), lipopolysaccharide (LPS), and other virulence factors which are located on the so-called pathogenicity islands (Pais). These Pais were found by researchers to be located on discrete segments of DNA on the chromosome (Knapp et al., 1986). Intimin has been shown to mediate histopathological attaching and effacing (A/E) lesion in neonatal calves and in tissue culture cells (Dean-Nystrom et al., 1998). This finding is supported by other work with a gnotobiotic pig model, where *E. coli*

O157:H7 attached intimately to the intestinal epithelial cells located primarily in the large intestine and effaced the microvilli at the site of attachment (Tzipori et al., 1986). The precise mode of action responsible for bacterial adhesion is unclear (Park et al., 2001). Tir is believed to bind with eae, which triggers additional host-signaling events and actin nucleation for the formation of the A/E lesion (Park et al., 2001). The LPS complex that is common to Gram-negative bacteria is known as the 'O' or somatic antigen. Lipopolysaccharide is composed of two polysaccharides; A and O. (Park et al., 2001). Bilge et al. (1996) suggested that the O polysaccharide may interfere with the adherence of *E. coli* O157:H7 to host epithelial cells. They proposed that the O polysaccharide may physically prevent the adherence between the other virulence factors such as intimin and host epithelium cells (Bilge et al., 1996).

Human implications

E. coli O157:H7 and other non-O157 STEC can cause Hemorrhagic Colitis (HC) or Hemolytic Uremic Syndrome (HUS) in humans. Hemorrhagic Colitis (i.e. bloody diarrhea) is the most common and less severe of the two. Hemolytic Uremic Syndrome can potentially be fatal. The young (<5 years old) and the elderly (>65 years old) are at greatest risk of HUS (Griffin and Tauxe, 1991). Hemorrhagic Colitis and HUS have been diagnosed with greater frequency in the last two decades. One thought is that these diseases have always existed, but until recently, we had limited ability to link the symptoms to causative factors. Another thought is that in modern societies we “dine out” more often and consume more processed foods (Buchanan & Doyle, 1997) and thus are more frequently exposed to sources of pathogens. The preliminary symptoms of HUS are severe abdominal cramps, HC, nausea, vomiting, and low-grade fever. Onset of these symptoms occurs 2 to 4 days after infection with a minimum infectious dose of 5 to 10 cells of viable O157:H7 (www.medicinenet.com). On average, these symptoms will persist for 5 to 10 days in mild conditions. The more severe symptoms of HUS will begin about one week after the onset of gastro-intestinal symptoms. Severe symptoms include pallor, intravascular destruction of red blood cells (microangiopathic hemolytic anemia), depressed platelet counts (thrombocytopenia), lack of urine formation (oligo-anurica), swelling (edema), and acute renal failure (Buchanan & Doyle, 1997). Other

complications may include seizures, coma, stroke, colonic perforation, pancreatitis, and hypertension (Buchanan & Doyle, 1997). One half of HUS patients require dialysis, and 15% of the HUS cases led to early development of chronic kidney failure (Buchanan & Doyle, 1997). Hemolytic Uremic Syndrome also can even be fatal with mortality rates ranging from 3 to 5% in humans (Buchanan & Doyle, 1997). Due to its low infectious dose and the severity of the diseases it causes *E. coli* O157:H7 is a legitimate concern for the human population.

Potential vectors of E. coli

Generic *E. coli* is ubiquitous and has been detected in dogs, birds, flies, sheep, humans, deer, cattle, ferrets, and other small mammals (Bach et al., 2002). The large intestine of cattle is a major reservoir for Shiga toxin producing *E. coli* (STEC; Armstrong et al., 1996). Little is known about the mode of transmission of *E. coli* among animals within a herd and between different herds of cattle. Limited work has been done to identify potential transmission vectors such as food, water, insects, birds, flies, deer, and other small rodents. Prevalence of *E. coli* O157:H7 appears to follow a seasonal pattern, with elevated levels in the summer and fall followed by a decline during the winter months. It is conceivable that the seasonality of certain vectors may play a role in this seasonal variation in shedding patterns.

The preponderance of research on potential vectors has isolated water as a likely means of transmission between cattle within a pen and between cattle sharing adjacent pens (LeJeune et al., 2001; LeJuene et al., 2004; Van Donkersgoed et al., 2001). Cattle that are positive for *E. coli* O157 can contaminate water with their mouth or with fecal material. LeJuene et al. (2004) isolated *E. coli* O157:H7 from 37 of 172 (21.5%) water troughs during the months of April and September. Van Donkersgoed et al. (2001) observed similar results with a prevalence of 20% between the months of March and May. Water supply into the water trough also is a possible source of *E. coli* O157 infection (LeJeune et al., 2001 and Van Donkersgoed et al., 2001). LeJeune et al. (2001) noted the feed bunk and the prevalence of generic *E. coli* in the trough. Shorter distances between water trough and feed bunks led to higher prevalence of generic *E. coli* in the trough. This may be due to a greater amount of unconsumed feed entering the water

trough, resulting in more nutrient rich substrate available for the proliferation *E. coli* O157:H7 already present in the water (Lejeune et al., 2001). Climatic temperatures and the weekly precipitation also are thought to be factors affecting *E. coli* prevalence in the water trough (Donkersgoed et al., 2001). Dry and warm conditions in the week prior to sampling yielded a higher prevalence of *E. coli* O157:H7 (Donkersgoed et al., 2001). According to Lejeune et al. (2001), both *E. coli* O157 and Salmonella were more likely to be found in the water troughs that were not cleaned as compared to troughs that were recently cleaned. Lynn et al. (1998) proposed that O157:H7 can proliferate in the sediment and survive for up to 4 months. Competition and predation by other microorganisms, such as protozoa, in the water trough may play an important role in the number of generic *E. coli* in the water trough (Lejeune et al., 2001). All these findings suggest water is an important source of pathogen transmission between cattle and that routine cleaning of the water troughs may be a logical approach to reduce transfer of *E. coli* O157:H7 among cattle.

Occasionally, *E. coli* O157 is found in the total mixed rations fed to cattle (Lynn et al., 1998). Dodd et al. (2003) sampled feed from 54 Midwestern feedlots and found that 8 to 50% of bunks sampled were positive for *E. coli* O157. Research by Van Donkersgoed et al. (2001) showed that the fresh total mixed ration contained undetectable levels of *E. coli* O157:H7 while the prevalence in the feed bunk was 1.7% over the entire feeding period. However, during the next 48 days, the prevalence of *E. coli* O157:H7 in the feed bunk was 10% (Donkersgoed et al., 2001). One possibility is that *E. coli* O157 can replicate in feeds under warm conditions on cattle farms (Lynn et al., 1998). However, you can not rule out the possibility that the cattle themselves contaminated the feed. Thus cattle feed may be a means of horizontal transmission between animals in a pen. Therefore, Lynn et al. (1998) suggested that using propionic acid in cattle feeds may limit bacterial growth and hence reduce the exposure of pathogenic organisms to cattle.

A common subtype of *E. coli* O157 was found in two separate feed yards located approximately 100 km apart from each other (Van Donkersgoed et al., 2001). No connection between the two feed yards was ever made; leading Van Donkersgoed et al. (2001) to believe that birds may have been the common link between the two feed yards.

Birds of all types have tested positive for generic *E. coli* in the past, and 68% of the birds at the Emperor Valley Zoo tested positive for generic *E. coli* (Gopee et al., 2000).

The common housefly also has been identified as being a potential vector (Kobayashi et al., 1999; Moriya et al., 1999). The house fly is commonly found on animal farms and the fly larva commonly develops in animal feces. The labellum, alimentary canal, and the crop of the fly harbored several hundred *E. coli* O157 for up to three days after being artificially infected, according to Kobayashi et al. (1999). Kobayashi et al. (1999) believes that the housefly can transmit *E. coli* O157 via their feces or by simply landing on and making contact with their labellum (mouth). Alam & Zurek (2004) found that the average prevalence of *E. coli* O157:H7 in house flies collected at the feed bunk of a cattle farm was 2.9%. Prevalence during the months of June and July were highest at 4.6 and 6.1%, respectively. Alam & Zurek (2004) also showed that each fly during the months of June and October were harboring 3.0×10^1 to 4.8×10^4 colony forming units of *E. coli* O157:H7. The high concentration of *E. coli* O157:H7 in house flies makes them a suitable candidate for transmission of *E. coli* O157:H7 between animals. Due to the fly's attraction to human food and drinks, house flies can transmit the food borne pathogen directly to humans from animal farms. Another important consideration is that house flies can travel at long distances as long as 10 to 20 miles and typically range from 0.5 to 2 miles (Broce, 1993).

Small mammals such as skunks, raccoons, and rats have access to feed and water supply of feedlot cattle. Work by Gopee et al. (2000) has already identified the mammalian families of these animals to be carriers of *E. coli*. Currently, no work has been done to look at the possibility of these animals as a means of transmission for *E. coli* O157:H7 in current animal production systems. New management practices may be implemented to reduce pathogen transmission between herd mates and between different herds once we have a better understanding of these and other potential vectors commonly found on animal production farms.

Sources of human infection include consumption of contaminated fruits, vegetables, water, and processed meats, with ground beef being identified as one of the more common sources (Armstrong et al., 1996). Bovine feces can serve as a prolonged contamination source, because *E. coli* O157:H7 can survive in feces for up to 70 days

under a wide range of environmental conditions (Wang et al., 1996). Using manure as fertilizer can contaminate fruits and vegetables intended for human consumption (Rasmussen & Cassey 2001). Drinking water and recreational water can be contaminated directly by cattle feces or indirectly via run-off water from farms contaminated with *E. coli* O157:H7 (Callaway et al., 2004). Contamination of beef carcass can occur at the abattoir during the removal of the hide or due to inadvertent contact between the carcass and digestive contents. Previous research has suggested that there is a high correlation between presence of O157:H7 on the hide and O157:H7 contamination of the carcass (Elder et al., 2000). Commercial abattoirs have implemented Hazard Analysis and Critical Control Points (HACCP) programs in an effort to identify and control sources of food-borne pathogens. The HACCP programs, though effective, may become overwhelmed when the pathogen load into the abattoir is excessive. Post-harvest pathogen control strategies include general sanitizing approaches, such as organic acid spraying, hot water spray washing, and steam vacuuming of beef carcasses (Park et al., 2001). Reduction of *E. coli* O157:H7 from mechanically deboned chicken meat and ground hamburger with gamma radiation has proved to be effective (Bitzan, et al., 1993). Research has shown that irradiation is a viable alternative to chemicals and preservatives in reducing food borne pathogens; however, the U.S. population has not been convinced of the safety of irradiated meats (Buchanan, et al., 1998; Clavero et al., 1994).

Prevalence and shedding patterns in cattle

E. coli O157:H7 is principally isolated from the digestive tract and not from the major internal organs of cattle. Necropsy studies by Cray & Moon (1995) showed no evidence of *E. coli* in the liver, spleen, or kidneys. Grauke et al. (2002) and Buchko et al. (2000) further defined the main location of *E. coli* O157:H7 as the cecum and distal colon portion of the large intestine. Recent research by Naylor et al. (2003) suggested that *E. coli* O157:H7 has an affinity for the lymphoid follicle-dense region located 3-5 cm proximally from the recto-anal junction. Rice et al. (2003) and Greenquist et al. (2005) suggested that swabbing this recto-anal junction appears to be more sensitive than traditional fecal grab samples for determining prevalence of *E. coli* O157:H7.

Early experimental model studies in Canada and the U.S. indicated that only 0.3 to 2.2% of cattle were positive for O157:H7 (Cray & Moon 1995). New enumeration techniques, such as immunomagnetic bead separation, have revealed that 30% or more cattle may be positive for *E. coli* O157:H7 (Chapman et al., 1997, Mechie et al., 1997, Hutchinson et al., 2005). More recent reports suggest O157:H7 prevalence may be even higher than previous estimates which reflect improvements in detection methods (Gansheroff and O'Brien, 2000). Elder et al. (2000) and Oot et al. (2007) indicated that 27 to 28% of tested feedlot cattle were shedding O157, and that 70 to 72% of the tested feedlots had at least one animal positive for O157. Results just prior to slaughter showed 43% of the cattle were shedding O157:H7 and 87% of the supplying feedlots had at least one infected animal (Elder et al., 2000). The prevalence of *E. coli* O157:H7 does not appear to be isolated in one geographical region or another. Findings by Hancock et al. (1997b) show that O157:H7 is widely distributed throughout the United States. Hutchinson et al. (2005) found similar prevalence levels and distributions of *E. coli* O157 in the United Kingdom.

Typical shedding patterns are believed to have one period of elevated shedding interspersed with longer periods of no shedding (Hancock et al., 1997a; Hancock et al., 1997c). Studies by Magnussan et al. (2000) and Sanderson et al. (1999) both suggested an average shedding period of 30 days. Actual shedding periods are quite variable and ranged from a few days to one year (Magnussan et al., 2000). Cray & Moon (1995) indicated that shedding peaked about one week after inoculation and decreased continually for 48 to 189 days thereafter for calves (Cray & Moon 1995). Adult cattle shed for a shorter length of time (i.e. 14 to 98 days after being inoculated) as compared to younger calves (Cray & Moon 1995). Shedding appears to be transient and animals can be inoculated multiple times with the same strain (Cray & Moon 1995). However, calves did shed more and for a longer period of time with the first inoculation as compared to the second inoculation (Cray & Moon 1995). In this study, inoculated calves shed more *E. coli* O157:H7 (4.0×10^5 to 1.6×10^9 CFU/g of feces) than the adult cattle (1.2×10^5 to 1.0×10^7 CFU/g of feces; Cray & Moon 1995). Similar results were found by Dargatz et al. (1997) in that lighter cattle (less than 700 lbs) also were observed to have higher prevalence of *E. coli* O157:H7 than heavier cattle (Dargatz et al., 1997). Naylor et al.

(2003) proposed that in any given population of *E. coli* O157:H7 positive animals, a subset of these animals will be shedding high numbers of *E. coli* O157:H7. He coined these as “supershedders”, and identified them as potentially important sources of horizontal transmission between herd mates.

A study by Donkersgoed et al. (2001) indicated that newly arrived cattle in a feedlot had higher prevalence of *E. coli* than cattle fed for more days. Cattle in feedlot for less than 20 days were nearly 4 times more likely to yield a positive sample than the cattle that were fed longer (Dargatz et al., 1997). LeJeune et al. (2004) found similar results with higher prevalence in the early feeding period and then declined below entry levels after 42 days on feed. The reason for these findings is unclear. Perhaps younger and lighter cattle may be more naïve and more susceptible to stress and bacterial infections. Hancock et al. (1997b) suggested that dietary stress, related to transportation of new cattle, may result in replication of *E. coli* O157:H7 in the rumen fluid. Another factor associated with *E. coli* prevalence is the epithelial cell proliferation in the lower G.I. tract (Magnuson et al., 2000). Slower proliferation rates of the cecum and colon resulted in a longer shedding period (Magnuson et al., 2000). However, growing diets of grain or forage did not have an effect on gastrointestinal tract proliferation or on the duration of *E. coli* O157:H7 shedding (Magnuson et al., 2000).

Extreme acid resistance

The gastric stomach is commonly viewed as “the first line of defense against food borne pathogens” (Waterman & Small 1998). Gastric pH typically is around 2.0 in humans (Texter et al., 1968). This acidic environment kills the vast majority of food borne pathogens, including *E. coli* O157:H7. Typically, *E. coli* does not proliferate very well in environments where pH is less than 5.5. Lin et al. (1996) has illustrated that *E. coli* can survive at a pH as low as 2.0 when acid resistant genes have been induced. The *E. coli* that survives an acid shock equivalent to that of the gastric stomach is said to have “Extreme acid resistance”. These extreme acid resistant *E. coli* are believed to be the reasons for the very low infectious dose in humans. *E. coli* that are acid sensitive are more likely to be destroyed by this acid shock than the extreme acid resistant *E. coli*. Acid sensitive pathogens need to be in very high numbers in order to increase their

chances for surviving the acid shock, whereas the extreme acid resistant pathogens may be infectious in low numbers. Previous studies suggested that pH was the driving force behind extreme acid resistance of *E. coli* O157:H7 (Goodson and Rowbury, 1989). It has also been noted that the acid tolerance of O157:H7 is highly dependent on the growth phase of the pathogen (Gorden & Small 1993). Studies by Diez-Gonzalez and Russell (1999) contradicted the idea that pH was the instigator of extreme acid resistance. They theorized that intracellular pH per se does not appear to regulate the extreme acid resistance of O157:H7. They speculated that undissociated acids, particularly acetate molecules, are more influential than the intracellular pH on inducing extreme acid resistance. They concluded that undissociated acids are needed to induce the acid resistant genes. *E. coli* cultures that were grown aerobically needed more volatile fatty acids (VFA) to induce extreme acid resistance than did the cultures grown anaerobically (Diez-Gonzalez & Russell 1999). This study suggests that redox potential may initiate extreme acid resistance. To further support their hypotheses, the addition of cysteine, a reducing agent, to the anaerobic cultures further increased the amount of volatile fatty acids needed to stimulate acid resistance (Diez-Gonzalez & Russell 1999). It appeared that VFA could only induce extreme acid resistance of O157:H7 if they were added during the exponential growth stage according to Diez-Gonzalez and Russell (1999).

Effect of diet on E. coli O157

Manipulation of diets fed to finishing feedlot cattle has been proposed as a means for reducing shedding of *E. coli* O157, though results have been mixed (Tkalcic et al., 2000, Berg et al., 2004, Van Baale et al., 2004). In a recent published longitudinal study by Berg et al. (2004), the authors found that cattle fed corn-based diets shed fewer *E. coli* O157 than cattle fed barley-based finishing diets. The researchers suggested that differences in the site of digestion may have impacted the differences in shedding. The starch portion of the corn grain is not as ruminally digested as the starch in barley grain likely resulting in more starch flow to the hind gut (Orskov 1986) with the corn-based diet. Increasing starch content in the lower gastrointestinal tract will result in a secondary fermentation in the large intestine. As fermentation increases, so will the production of short chain volatile fatty acids (i.e. acetate, propionate, and butyrate), hence reducing pH

in the large intestine. Russell et al. (2000) has speculated that lower a lower pH in the large intestine is a favorable environment for both survival and growth of *E. coli* O157. Buchko et al. (2000) contradict this hypothesis when they suggested lower fecal pH and the associated volatile fatty acids inhibited proliferation of *E. coli* O157 in the large intestine. Some studies have demonstrated an inhibitory effect of volatile fatty acids (particularly propionic acid) on *E. coli* O157 (Horii et al., 1998 and Shin et al., 2002). Results by Berg et al. (2004) are in agreement with this hypothesis. In addition, a cattle study by Hovde et al. (1999) and a sheep experiment by Kudva et al. (1997) demonstrated similar results when comparing hay diets to cereal grain diets. Compared to forage-based diets containing large amounts of cereal grains are more likely to provide readily available starch for secondary fermentation in the large intestine. Again, shifting the site of digestion for a portion of the dietary starch will result in elevated levels of volatile fatty acids, potentially inhibiting *E. coli* O157. In addition, recent work has demonstrated differences in survivability of *E. coli* O157 in the feces of cattle fed forage diets rather than cereal grain-based diets (Bach et al., 2005). Researchers showed that *E. coli* O157 persisted longer in the feces from cattle fed barley when compared to the feces of cattle fed corn diets. This may be in part due to the lower pH and perhaps higher dry matter content of corn fecal samples as compared to barley fecal samples (Bach & McAllister, 2003). These findings are in stark contrast with research by Russell et al. (2000), who suggested that *E. coli* O157 growth was enhanced in the presence of volatile fatty acids and at a lower pH due to starch fermentation in the large intestine. Diez-Gonzalez et al. (1998) showed that generic and acid-resistant *E. coli* in the large intestine were significantly increased when cereal grains were increased in the diet, thereby increasing secondary fermentation in the hind gut. This is in agreement with Berg et al. (2004), who observed a significant increase in fecal generic *E. coli* when corn-based diets were fed compared to barley-based diets. Results from Berg et al. (2004) would suggest that volatile fatty acid production and colonic pH have different effects on generic *E. coli* and *E. coli* O157. Berg et al. (2004) stated that generic *E. coli* in the hind-gut may not be considered a reasonable predictor of *E. coli* O157 in the hind-gut.

E. coli O157 is a human pathogen commonly associated with animal production systems, especially cattle. Contamination of beef carcasses during the slaughter process

is a common route of transmission to humans. Post-harvest strategies during the slaughter process significantly reduce contamination of finished beef products. However, these systems can become overwhelmed when the pathogen load entering the abattoir via contaminated cattle hides and digestive contents is increased. Pre-harvest intervention strategies that reduce the incoming pathogen load into the abattoir will likely increase the effectiveness of post-harvest intervention strategies. One such pre-harvest intervention strategy may be the manipulation of diets fed to finishing cattle. A better understanding of effects of diet on site of digestion of starch and the subsequent effects on fermentation in the large intestine may be useful in identifying successful intervention strategies. Intervention strategies to 1) reduce fecal shedding of *E. coli* O157 and 2) decrease the likelihood that *E. coli* O157 will survive in the feces outside of the animal would be equally beneficial to the beef industry.

Literature review of starch digestion in cattle.

Commercial feedlot operations utilize a variety of different feed ingredients including several different cereal grains. Corn, sorghum, wheat, barley, and oats are among the cereals commonly fed to feedlot cattle, with corn being the most widely used (Vasconcelos and Galyean, 2007). A review by Huntington (1997) summarized starch contents of various grains used in research trials. Wheat contained the highest starch content (77%) followed closely by corn and sorghum with 72%. Barley and oats contained the least starch with 57 and 58%, respectively. Rooney and Pflugfelder (1986) described several factors affecting ruminal digestion of starch including composition and physical form of the starch, protein-starch interactions, and physical form of the grain.

Amylose and amylopectin are the two primary molecules in the endosperm layer of cereal grains. Differences in the endosperm layers results in structural classifications such as vitreous, flinty, waxy, nonwaxy, and opaque. These different attributes have been suggested as the basis for differences, indigestibility of grains (Huntington 1997). Genotypes which contain only amylopectin are classified as waxy and are generally associated with faster rates of digestion (Huntington 1997). Surrounding the highly concentrated starch granules is the floury endosperm, which is a protein-rich matrix

embedded with starch granules called the peripheral and corneous endosperm (Huntington 1997). This protein matrix in wheat and barley is easily penetrable and rapidly fermented in the rumen (McAllister et al., 1994). In corn and sorghum, the peripheral endosperm is extremely resistant to attachment and hence penetration by ruminal bacteria and protozoa, leaving only the ruminal fungi to penetrate this layer (McAllister et al., 1994). Grain processing which disrupts and breaks this peripheral endosperm allows for faster attachment and digestion of the floury endosperm by bacteria and protozoa.

Bacteria that are loosely or tightly bound to the grain will contribute to three-fourths of fiber, protein, and starch digestion in the rumen (McAllister et al., 1994). Kotarski et al. (1992) identified 15 strains of amylolytic bacteria and eight amylolytic enzymes in ruminal samples. Not all amylolytic bacteria are equipped with the complete array of enzymes needed for starch digestion, so a mixture of bacterial species is needed for maximal digestion in the rumen. Protozoa also are present in rumens of cattle fed high grain diets; though their numbers are far less than those of bacteria. Protozoa are slow growing and can engulf large starch molecules and bacteria when feeding. The process of engulfing large starch molecules modulates ruminal fermentation, as protozoa digest starch more slowly than bacteria (Mendoza et al., 1993). With protozoa, the release of organic acids from metabolism of starch is extended over a longer period of time, thereby reducing the accumulation of organic acids and preventing sharp declines in ruminal pH (Nagaraja et al., 1992). As mentioned earlier, the role of rumen fungi in starch digestion is less than that of bacteria and protozoa, but may be important in diets with whole grains due to its ability to attach and penetrate through the peripheral endosperm (McAllister et al., 1994).

Ruminal digestion of starch can be greatly affected by grain processing. Steam-flaking and dry-rolling whole grains are the primary processing methods employed by commercial feedlots (Vasconcelos and Galyean, 2007). Steam-flaking involves steaming of whole corn at atmospheric pressure for 20 to 60 minutes and then passing it through a roller mill. Soaking or steeping the whole corn prior to flaking is also a common practice (Zinn et al., 2002). The combination of soaking and steaming can increase the moisture content of the grain by 20 to 25%. Degree of processing can be altered by changing the

gap between the two rolls (Zinn, 1990). Whereas decreasing the roll gap will increase the degree of processing (Zinn et al., 2002). Steam-flaking typically increases starch digestibility by 19% for sorghum and 13% for corn when compared to dry-rolling (Huntington, 1997). In respect to ruminal starch digestion, corn and sorghum typically respond better to grain processing, such as steam-flaking, compared to barley and wheat (Owens et al., 1997). These differences are attributed to the physical structure of the protein and starch-matrix (Rooney and Pflugfelder, 1986).

A review paper by Huntington (1997) shows that ruminal digestion of starch was 10% higher for finishing diets based on steam-flaked corn compared to dry-rolled corn. A lower ruminal pH and a lower ruminal acetate:propionate ratio have been reported (Zinn et al., 1995; Barajas and Zinn 1998; and Corona et al., 2006) for steers fed finishing diets based on SFC rather than DRC, which suggest a greater ruminal fermentation of starch. As a result, more starch is available for digestion in the small and large intestine of cattle fed finishing diets based on dry-rolled corn. Theurer (1986) estimated that large amounts (up to 600 g/kg) of starch can escape ruminal fermentation and be presented for digestion in the small intestine. However, starch hydrolysis by pancreatic α -amylase in the ruminant animal is limited (Harmon and McLeod, 2001). In addition, research by Walker and Harmon (1995) and Swanson et al. (2004) indicates that secretion of pancreatic α -amylase is decreased by in response to starch. However, other research by Richards et al. (2003) observed that secretion of pancreatic α -amylase is increased if protein and starch is infused postruminally. In any case, increasing the amount of starch entering the small intestine above that which can be digested and absorbed will result in a secondary fermentation in the large intestine (Siciliano-Jones and Murphy, 1989; Harmon and McLeod, 2001).

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**CHAPTER 2 - High moisture tempering of whole shelled
corn before flaking: Effects on fecal shedding of *E. coli*
coliforms in finishing cattle.**

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Abstract

Hydrothermal processing (steam-flaking) of grains is a common method used to improve ruminal starch availability by increasing gelatinization of the starch. Grains typically are conditioned with water, effectively adding 8% moisture to the grain, and then steamed for 20 to 40 min prior to rolling. Increasing moisture levels of the grain above that which is obtained during the normal steaming process may be required for maximal starch gelatinization. However, from a food safety perspective, increasing moisture levels of steam-flaked corn may provide ideal conditions for the proliferation of human pathogens such as *E. coli* O157. The objectives of this study were to quantify differences in generic *E. coli* populations due to environmental contamination of SFC containing 18% or 36% moisture, and to quantify fecal shedding of *E. coli* by feedlot heifers fed these grains. Steam-flaked corn samples exposed to environmental conditions for 21 h contained more ($P < 0.05$) coliforms and fastidious and non-fastidious microorganisms. Increasing moisture content of the flaked corn resulted in elevated levels ($P \leq 0.09$) of coliforms and total plate counts in steam-flaked corn samples exposed to the elements. Coliforms and total plate counts in the total mixed rations were not different ($P > 0.05$) between fresh and samples exposed to the environment. However, increasing moisture content of the flaked corn resulted in elevated levels ($P \leq 0.07$) of coliforms and total plate counts in total mixed rations. Fecal shedding of non-*E. coli* coliforms and total coliforms were greater ($P \leq 0.09$) for heifers fed total mixed rations containing the low moisture steam-flaked corn. Conditions within the steam-flaked corn samples or contamination from environmental sources allowed for proliferation of coliforms and total plate counts. However, no microbial growth was detected in the total mixed rations exposed to the same environmental conditions. Interestingly, fecal shedding of acid-resistant coliforms was higher in heifers fed total mixed rations containing the low moisture steam-flaked corn. It is plausible that something other than microbial populations in the feed is responsible for these differences.

Introduction

Cattle digestive tracts are believed to be important reservoirs for *Escherichia coli* (*E. coli*) O157, a human pathogen and causative agent of hemorrhagic colitis and hemolytic uremic syndrome. Research suggests that shedding of *E. coli* O157 by cattle persists for an average of 30 d, but may vary from a few days to a year (Magnusson et al., 2000). Wang et al. (1996) observed that *E. coli* O157 can survive in feces for up to 70 d under a wide range of environmental conditions. Houseflies (*Musca domestica*) commonly feed on bovine feces, and can themselves carry hundreds of *E. coli* O157 in their alimentary canals (Kobayashi et al., 1999). Houseflies can serve as a vector for transmission of *E. coli* O157 between feces and cattle feed (Lynn et al., 1998).

Hydrothermal processing (steam-flaking) of grains is a method commonly employed to improve ruminal starch availability through gelatinization (Theurer, 1986; Zinn 1987, 1990). Grains typically are conditioned with water and then steamed for 20 to 40 min prior to rolling, effectively adding 8% or more moisture to the grain. Increasing moisture levels of the grain above that which normally is obtained during the conditioning process may be required for maximal starch gelatinization. However, from a food safety perspective, increasing moisture levels of steam-flaked corn (SFC) may provide conditions that are ideally suited to proliferation of human pathogens, including *E. coli* O157. The objectives of this study were to 1) quantify differences in generic *E. coli* populations within grains containing 18 or 36% moisture following a period of environmental exposure, and 2) determine fecal shedding of *E. coli* by feedlot heifers fed diets containing flaked grains with different moisture levels.

Materials and methods

Grain processing

Whole-shelled corn was tempered daily by combining 454 kg of corn (90% DM) and 129 kg of water into a stationary 1.2 m³, water-tight paddle mixer. Corn and water were mixed periodically and tempered overnight to allow ample time for moisture assimilation by the corn. Following the conditioning process, the tempered corn (SFC-36; 36% moisture after flaking) was transferred to a 2.7 m³ stainless steel steam chest and

steam conditioned at atmospheric pressure and temperature of $\sim 100^{\circ}\text{C}$ for 45 min. Ten min prior to flaking the 46×61 cm Ferrel-Ross roller mill was started, and the corrugated rolls were preheated using steam. Non-tempered corn (SFC-18; 18% moisture after flaking) was processed in a similar manner except that corn (90% DM) was added directly to the steam chest for conditioning. Products were flaked to a common bulk density of 335 g/L and was checked periodically by sampling the flaked corn underneath the rolls using a Winchester cup (Seedburo Equipment, Chicago, IL). Each corn type was processed and analyzed daily for susceptibility to hydrolysis by amyloglucosidase to estimate starch availability (Sindt et al. 2006).

Sampling steam-flaked corn

E. coli/coliform and aerobic bacterial counts of SFC-36 and SFC-18 were determined by taking a 250-g aliquot of each grain immediately underneath the rolls of the Ferrel-Ross flaker. Samples were collected using sterile (i.e., autoclaved for 20 min; model 2021 gravity; AMSCO; Erie, PA) 10×15 cm aluminum pans and contents subsequently were placed into a Nasco Whirl-pak plastic bag, and frozen. A second sample (32 kg) was taken directly from the discharge of the 0.5×9 m drag conveyor. Samples were collected into 151-L plastic containers lined with a plastic trash bag and then sealed. Once both SFC-36 and SFC-18 were flaked and collected, samples were then aseptically emptied out onto clean plastic bags which were spread out on the ground. Steam-flaked corn samples were left exposed to the environment for approximately 21 h. After 21 h of exposure, SFC samples were mixed by hand using sterile gloves for three min and a 250-g sub sample was taken and refrigerated for bacterial analysis. Procedures were replicated on three consecutive days.

Total mixed ration sampling

Total mixed rations (TMR) were sampled directly from the discharge of the truck-mounted mixer. A 250-g sample was aseptically collected into a Nasco Whirl-pak bag and refrigerated. In addition, a second 2 kg sample was collected from the discharge into a 10 cm deep \times 30 cm wide \times 46 cm long sterile aluminum pan. TMR samples were covered with aluminum foil until both TMRs containing SFC-36 and SFC-18 were sampled. Immediately following collections, aluminum pans were placed outside in

direct sunlight where they were exposed to flies and environmental conditions for 21 h. All SFC and TMR samples were then taken to Kansas State University Food Safety Laboratory for bacterial enumeration. Procedures were replicated on three consecutive days.

E. coli and coliform enumeration procedures

Upon arrival at the lab, individual samples were thoroughly mixed by shaking the bag vigorously for 30 s. A 50 g sub sample was placed into a plastic bag containing 200 mL of peptone diluent. Samples were then homogenized by hand massaging the bag for 2 min. Homogenized mixtures were subsampled and serially diluted with 0.1 % peptone diluent, vortexed, and plated onto duplicate *E. coli*/coliform Petrifilm™ (3M; St. Paul, MN). Petrifilm™ plates were incubated at 35°C for 24 h and enumerated for typical *E. coli* and total coliforms according to the AOAC method outlined in the *E. coli*/coliform Petrifilm™ package insert. *E. coli* numbers were expressed as log₁₀ colony forming units/g of feed (CFU/g).

Total aerobic plate counts

Upon arrival at the lab, sample contents were thoroughly mixed by shaking the bag vigorously for 30 s. A 50 g sub sample was placed into a sterile stomacher bag containing 200 mL 0.1 % peptone diluent. Samples were then hand massaged for 1 min before serial dilutions were prepared using 0.1 % peptone diluent. The original, second, and fourth serial dilutions were spiral plated using a Whitley automatic spiral plater (model WA02CD, Don Whitley Scientific, West Yorkshire, UK) onto pre-poured tryptic soy agar (TSA) petri plates. The petri plates were allowed to air dry before being inverted, and then were incubated at 35°C for 48-h. After incubation, plates were enumerated using a spiral plate counter grid, and total plate count was calculated.

Cattle diets and experimental design

All experimental procedures involving the use of animals were in accordance with the rules and regulations set forth by Kansas State University Institutional Animal Care and Use Committee. Ninety-six crossbred heifers (initial BW = 374 ± 6 kg) were stratified by BW to 12 pens (6 pens per treatments and 8 heifers per pen). On d 1 heifers

were allocated to pens and treatments were assigned randomly to pens. Pens were concrete surfaced and provided 9.2 m² of surface area and 62 cm of linear bunk space per heifer. A common water fountain was shared between adjacent pens. Fountains were cleaned thoroughly prior to and throughout the duration of the study. Pens were constructed of pipe fences, and therefore did not prevent contact between cattle in adjacent pens. Heifers were fed once daily their respective diet consisting of 73% (DM basis) of either SFC-18 (18 % moisture) or SFC-36 (36 % moisture; Table 1). Heifers were offered ad libitum access to both water and experimental diets for the duration of the study. After 56 d on respective diets, cattle were removed from their pens and restrained in a hydraulic working chute and fecal sampled using rectal palpation. Cattle that produced no fecal sample (< 15 g) at initial sampling were resampled after 5 to 10 min. Fecal samples from all animals within each pen were composited (equal volume) and thoroughly mixed by hand massage, sealed in Whirl-Pak bags (Nasco, Ft. Atkinson, WI), and cooled on ice before being transported to the Kansas State University Food Safety Laboratory.

Acid resistance and enumeration

Upon arrival at the lab, 10 g of feces were added to 20 mL of 0.1% peptone diluent (Difco Laboratories, Detroit, MI) and homogenized for 2 min in a stomacher lab blender 400 (Tekmar, Cincinnati, OH). Two 1-mL aliquots of the homogenized solution were combined each with 10 mL of McIlvaine's citrate buffer and adjusted to pH 2 or 7 with 85% lactic acid or 1 M NaOH. The pH 7 samples were vortexed immediately and pH was recorded. Samples were serially diluted with 0.1 % peptone diluent, vortexed, and plated in duplicate onto *E. coli*/coliform Petrifilm™. The pH 2 samples were “acid challenged” for 15-min at room temperature before being neutralized to pH 7 with 1 M NaOH. Sample pH was then recorded, serially diluted in 0.1 % peptone diluent, and plated onto duplicate *E. coli*/coliform Petrifilm™ plates. Petrifilm plates were incubated at 35°C for 24 h and enumerated for *E. coli* and total coliforms according to the AOAC method outlined in the *E. coli*/coliform Petrifilm package insert. *E. coli* and coliform numbers were expressed as log₁₀ colony forming units/g of feces (CFU/g).

Statistical analysis

E. coli coliforms, non-*E. coli* coliforms, total coliforms, and total plate counts of the SFC and TMR samples were log transformed and analyzed as a randomized complete-block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included effects of block and treatment. Fecal coliforms were analyzed as a randomized complete-block design, with pen of heifers as the experimental unit. For statistical analysis, a value of one half of the detection limit was entered for samples that yielded no detectable colony forming units. Because feed and fecal samples were diluted differently, the detection limit varied with sample type.

Results

Tempering whole shelled corn prior to steam-flaking increased moisture content of both corn flakes (36% and 18% moisture for SFC-36 and SFC-18, respectively) and TMR (65% moisture and 77% moisture for TMR samples containing either SFC-36 and SFC-18, respectively; Table 1). Coliform levels were near or below the detection limit (0.40 Log₁₀ colony forming units/g) for SFC. Generic *E. coli*, non-*E. coli*, and total coliform counts were not different ($P > 0.05$) for SFC-36 and SFC-18 (Table 2). SFC-36 did, however, have more ($P < 0.05$) fastidious and non-fastidious microorganisms when compared to the non-tempered SFC-18 flakes. All SFC samples regardless of moisture content increased ($P < 0.05$) in microbial populations during the 21-h exposure period. SFC-36 samples exposed to environmental conditions were higher ($P < 0.05$) in total aerobic plate counts when compared to environmentally exposed SFC-18 samples.

TMR samples contained more ($P < 0.05$; Table 3) generic *E. coli*, non-*E. coli*, and total coliforms than their respective SFC samples. With the exception of generic *E. coli*, coliform levels were not different ($P > 0.05$) between TMR samples containing SFC-36 and SFC-18. Unlike the SFC samples, environmental exposure did not increase ($P > 0.05$) microbial populations over the 21-h exposure period. Non-*E. coli* coliforms, *E. coli* coliforms, and total plate counts were higher ($P < 0.05$) for environmentally exposed TMR samples containing SFC-36 compared to environmentally exposed TMR samples containing SFC-18.

Numbers of total coliforms and acid resistant *E. coli* shed by yearling heifers (n = 96; 48 heifers per treatment) were not different ($P > 0.05$; Table 4) for cattle fed diets containing SFC-36 and SFC-18. Acid resistant non-*E. coli* coliforms tended ($P = 0.09$) to be higher in fecal material collected from heifers fed SFC-18 based finishing diets. Similarly, acid resistant total coliforms were greater in number ($P < 0.05$) for fecal material derived from cattle fed SFC-18 based finishing diets.

Discussion

Hydrothermal processing (steam-flaking) of grains is a common method used by commercial feedlot producers. Steam flaking increases gelatinization of starch and thereby improves ruminal and total tract starch digestion (Theurer, 1986; Zinn, 1987; Zinn 1990). Temperatures generated in the steam chest are typically around 100°C (depending on elevation) and exposure times range from 20 to 40 min. These combinations of temperature and time are sufficient to kill most bacteria. However, we detected both coliforms and aerobes in our initial samples taken just after the steam-flaking process. The process used in our study to temper whole grain with water overnight likely increased overall microbial growth in the mixer. Regardless of microbial load, it is unlikely that any of the microorganisms that were introduced to the steam chest prior to flaking actually survived the intense heating process. Therefore, flaked corn samples taken just after the flaking process were most likely contaminated due to contact with conveying equipment and ambient air. Total aerobic plate counts were higher for SFC-36 compared to the SFC-18. The SFC-36 contained more moisture which likely fostered an environment for bacterial proliferation.

Lynn et al. (1998) sampled fresh TMR from 16 farms. Seventy-five percent of the samples contained generic *E. coli*, with an average concentration of 2.1 Log₁₀ CFU/g. As with the study described by Lynn et al. (1998), samples in the present study were exposed to a variety of sources of contamination. First, grains were exposed to surfaces of environment and storage areas, as well as ambient air. Another opportunity for contamination occurred in the mixer and mixer discharge. The coliform counts observed in this study (3.3 Log₁₀ CFU/g) were similar to those reported by Lynn et al. (1998). The increase in *E. coli* coliforms observed in the TMR containing SFC-36 may be due to

greater overall moisture content, thus creating an environment that was more ideally suited to bacterial growth. However, one would expect that total plate counts and total coliforms would follow a similar trend in bacterial numbers.

Bacterial levels of SFC, regardless of moisture content, increased over the 21-h exposure period. The observed increase in bacterial contamination over time could have been due to the replication of native microbes in the samples, or due to surface contamination from other sources. Houseflies have been identified as potential vectors of *E. coli* contamination (Kobayashi et al., 1999 and Moriya et al., 1999). Houseflies transmit *E. coli* from bovine feces to other surfaces with which they make contact; including cattle feed (Kobayashi et al., 1999). Alam and Zurek (2004) sampled houseflies at this research facility during the same timeline as the current study, and found that houseflies were harboring between 3.0×10^1 to 4.8×10^4 colony forming units of *E. coli* O157 per fly. During the course of our study, houseflies were commonly found feeding on flaked corn and TMR samples. Based solely on visual observations, houseflies appeared to have a preference for the SFC-36 flakes when compared to the SFC-18. Sindt et al. (2006), when discussing the animal growth performance of the cattle used in this study, suggested that the SFC-36 flakes were over-processed because the added weight in water increased density, thus giving the appearance of an less rigorously processed grain when evaluated by conventional methods (i.e., bulk density). The more extensive processing of SFC-36 increased degree of starch gelatinization compared to SFC-18 resulting in an attractive, maltose-like aroma, which may have attracted flies. Total plate counts were higher in SFC-36 versus SFC-18 after environmental exposure. This difference could be explained by differences in growth conditions in the SFC samples in addition to increased contamination from outside sources such as houseflies. Lynn et al. (1998) suggested that wet grain mixtures and some silage-based mixtures supported a higher growth of *E. coli* O157 (Lynn et al., 1998).

The TMR containing SFC-36 also was more likely to contain greater numbers of bacteria than TMR containing SFC-18 flakes after 21 h of exposure. Again, these differences were likely due to differences in moisture content and attractiveness of SFC-36 by flies. Exposing TMR samples to environmental conditions for 21 h did not result

in increased levels of bacteria. Lynn et al. (1998) found similar results when they compared microbial counts of fresh feed to the same feed after 24 h in feed trough.

Feed delivered to the heifers in our study was adjusted daily so that only trace amounts of unconsumed feed was remaining in the bunk prior to the next days feeding. However, small portions of feed would remain in the bunk for periods of time similar to the exposure time used in Experiment 1. Results from Experiment 1 shows that heifers fed TMR samples containing the SFC-36 consumed more coliforms and fastidious and non-fastidious microorganisms. One might speculate that ingestion of large numbers of a particular microorganism would lead to fecal shedding of the respective microorganism, but this was not the case for *E. coli*, non-*E. coli*, or total coliforms. Our results suggest that moisture levels due to tempering corn prior to flaking did not increase fecal shedding. Interestingly, heifers fed the TMR containing SFC-18 actually shed more acid resistant non-*E. coli* and total coliforms than heifers fed TMR containing SFC-36. This difference in acid-resistance is possibly an indication of different populations of *E. coli*. It appears from our data that the microbial populations in the TMR consumed by the heifers was not responsible for the differences in fecal shedding. A plausible explanation for this apparent difference in *E. coli* populations may be due to the diet and its digestibility.

Manipulation of diets fed to finishing feedlot cattle has been proposed as a means for reducing fecal shedding of *E. coli* O157 (Hovde et al., 1999, Berg et al., 2004, Van Baale et al., 2004). In a recent published longitudinal study by Berg et al. (2004), the authors found that cattle fed corn-based diets shed more *E. coli* coliforms than cattle fed barley-based finishing diets. The researchers suggested that differences in the site of digestion may have impacted differences in shedding. The starch portion of the corn grain is not digested as extensively as that of barley, thus resulting in more starch flow to the hind gut (Orskov 1986). Increasing starch content in the lower gastrointestinal tract will result in a secondary fermentation in the large intestine. As intestinal fermentation increases so will the production of short chain volatile fatty acids (i.e. acetate, propionate, and butyrate), reducing pH in the large intestine. Russell et al. (2000) speculated that a lower pH in the large intestine is favorable for growth and survival of *E. coli* O157. Similarly, Diez-Gonzalez et al. (1998) observed that *E. coli* coliforms and acid-resistant

E. coli in the large intestine were significantly increased when cereal grains were added to the diet, thereby increasing secondary fermentation in the hind gut. On a dry matter basis, SFC-36 corn flakes may have been processed to a lighter flake density than the SFC-18 corn flakes. Sindt et al. (2006) when summarizing the growth performance of heifers used in this study speculated that over processing of SFC-36 may have resulted in the observed reductions in feed intake. Decreasing flake density of corn also increases ruminal digestion of starch and thereby decreasing starch flowing to the large intestine (Swingle et al., 1999; Plascencia and Zinn, 1996). Results from Berg et al. (2004) and Buchko et al. (2000) demonstrated that lower fecal pH and the associated volatile fatty acids inhibited proliferation of *E. coli* O157 in the large intestine. Conflicting results from Berg et al. (2004) would suggest that volatile fatty acid production and colonic pH have different effects on generic *E. coli* and *E. coli* O157 in the bovine large intestine. Berg et al. (2004) stated that generic *E. coli* in the hind-gut may not be considered a reasonable predictor of *E. coli* O157 in the hind-gut.

The significant increase in bacterial counts of the steam-flaked corn samples after 21 h exposure period may be due to contamination by houseflies. However, other factors such as airborne bacteria and proliferation of native bacteria in the steam-flaked corn can not be ruled out. Likewise the increased bacterial numbers in the total mixed rations containing the SFC-36 after the 21 h exposure period cannot be attributed to the houseflies. Whether this difference is due to optimal moisture levels for bacterial growth or truly a difference in affinity for houseflies is not well understood. Regardless, feeding cattle diets with higher levels of coliforms and total plate counts did not increase fecal shedding of these organisms. Fecal shedding of acid resistant coliforms was increased for heifers receiving the TMR containing non-tempered low moisture SFC-18 corn flakes. Differences in site and extent of digestion between the two flaked corns may have resulted in differences observed for acid resistant fecal coliforms. However, we must be hesitant to suggest that differences in starch entering the large intestine would be have similar results on *E. coli* O157 based on this study. Further research quantifying actual fecal starch and *E. coli* O157 in the large intestine would shed light on this relevant question.

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Table 1. Composition of experimental diets containing either 18% moisture or 36% moisture steam-flaked corn.

Item	SFC-18	SFC-36
Ingredient		
SFC-18 ^a	73.3	-
SFC-36 ^b	-	72.9
Wet corn gluten feed	9.8	9.8
Alfalfa Hay	5.9	6.0
Tallow	3.1	3.1
Premix ^c	2.4	2.6
Soybean meal	2.1	2.1
Limestone	1.7	1.7
Potassium chloride	0.4	0.4
Sodium chloride	0.3	0.3
Urea	1.0	1.0
Vitamin/mineral premix ^d	0.1	0.1
Nutrient %, analyzed		
Dry matter	77.2	65.4
Crude protein	13.2	13.2
Calcium	0.70	0.70
Phosphorus	0.36	0.36
Starch availability of flaked corn, %	56.6	58.8

^aLow moisture steam-flaked corn (18% moisture).

^bHigh moisture steam-flaked corn (36% moisture).

^cTo provide per kg of diet DM: 300 mg monensin, 90 mg tylosin, and 0.5 mg melengesterol acetate per heifer daily.

^dTo provide per kg of diet DM: 2,200 IU vitamin A, 0.1 mg cobalt, 0.6 mg I, 60 mg Mn, 0.3 mg Se, 60 mg Zn, and 10 mg Cu.

Table 2. Bacterial counts in steam-flaked corn samples before (initial) and after (final) environmental exposure.

Item	Initial	Final	SEM
Non- <i>E. coli</i> coliforms	-----Log ₁₀ colony forming units/g-----		
SFC-18 ^a	0.25 ^d	2.79 [*]	0.84
SFC-36 ^b	0.75	3.90 [*]	0.84
Generic <i>E. coli</i> coliforms			
SFC-18	0.25 ^d	2.41 [*]	0.75
SFC-36	1.40	4.71 ^{*†}	0.75
Total coliforms			
SFC-18	0.25 ^d	2.95 [*]	0.82
SFC-36	1.42	4.91 ^{*‡}	0.82
Total plate count ^c			
SFC-18	4.82	6.59 [*]	0.63
SFC-36	9.12 [†]	12.14 ^{*†}	0.63

^aSteam-flaked corn samples containing 18% moisture.

^bSteam-flaked corn samples containing 36% moisture.

^cTotal aerobic fastidious and non-fastidious microorganisms.

^dNo colonies were detected therefore one half of the detection limit (0.40) was entered for the value.

^{*}Value after 21 h exposure (Final) is significantly greater than value for corresponding initial value ($P < 0.05$).

[†]Value for SFC-36 is significantly greater than value for SFC-18 ($P < 0.05$).

[‡]Value for SFC-36 is greater than value for SFC-18 ($P < 0.10$).

Table 3. Bacterial counts in total mixed ration before (initial) and after (final) environmental exposure.

Item	Initial	Final	SEM
Non- <i>E. coli</i> coliforms	-----Log ₁₀ colony forming units/g-----		
SFC-18 ^a	4.26	3.86	0.84
SFC-36 ^b	6.28 [‡]	6.90 [†]	0.84
Generic <i>E. coli</i> coliforms			
SFC-18	2.49	2.75	0.75
SFC-36	5.33 [†]	5.96 [†]	0.75
Total coliforms			
SFC-18	4.31	4.01	0.82
SFC-36	6.45 [‡]	7.04 [†]	0.82
Total plate count ^a			
SFC-18	9.30	8.20	0.63
SFC-36	11.75 [†]	12.66 [†]	0.63

^aTotal mixed ration containing low moisture (18%) steam-flaked corn.

^bTotal mixed ration containing high moisture (36%) steam-flaked corn.

^cTotal aerobic fastidious and non-fastidious microorganisms.

[†]Value for SFC-36 is significantly greater than value for SFC-18 ($P < 0.05$).

[‡]Value for SFC-36 is greater than value for SFC-18 ($P < 0.10$).

Table 4. Fecal coliform levels of heifers within a pen receiving total mixed ration containing either low moisture steam-flaked corn (SFC-18; 18% moisture) or high moisture steam-flaked corn (SFC-36; 36% moisture).

Fecal Coliforms	SFC-18	SFC-36	SEM	<i>P</i> -value ^a
Total coliforms (pH 7)	-----Log ₁₀ colony forming units/g-----			
<i>E. coli</i>	6.29	6.18	0.19	0.44
Non- <i>E. coli</i>	5.60	5.57	0.19	0.94
Total	6.45	6.33	0.19	0.30
Acid-resistant coliforms (pH 2)				
<i>E. coli</i>	1.33	0.85 ^b	0.19	0.22
Non- <i>E. coli</i>	2.29	0.85 ^b	0.19	0.09
Total	2.49	0.85 ^b	0.19	0.05

^aProbability that differences of the magnitude observed were due to random chance.

^bNo colonies were detected therefore one half of the detection limit (1.70) was entered for the value.

**CHAPTER 3 - Influence of processed grains on fecal pH,
starch concentration, and shedding of *Escherichia coli*
O157 in feedlot cattle.**

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Abstract

Manipulation of cattle diets has been proposed as a possible pre-harvest control measure for *Escherichia coli* O157. Altering hindgut fermentation through diet changes may be a means to reduce fecal shedding of *E. coli* O157. In Exp. 1, the objective was to determine whether fecal shedding of *E. coli* O157 was related to fecal starch concentration. Starting on d 20, and every week thereafter until d 61, steers in 54 pens (6 to 7 steers per pen) were sampled (n = 122) using feces and by rectoanal mucosal swabs (RAMS) for *E. coli* O157 and fecal starch concentration determinations. *Escherichia coli* O157 prevalence was 3.3% in fecal samples, 4.1% as measured by RAMS, and 4.9% by fecal or RAMS samples. Steers positive for *E. coli* O157 contained 21% more ($P < 0.05$) fecal starch than steers that were negative for *E. coli* O157. In Exp. 2, we attempted to alter the concentration of starch escaping rumen fermentation by feeding finishing diets based on steam-flaked corn (SFC) and dry-rolled corn (DRC) to 30 heifers prescreened for being culture positive for fecal *E. coli* O157. Starting on d 13, heifers were sampled (feces and RAMS) weekly to monitor fecal pH and fecal starch concentration, and prevalence of *E. coli* O157. Prevalence of *E. coli* O157 remained above 30% for the first 13 d and then declined ($P < 0.05$) over weeks. Based on RAMS prevalence of *E. coli* O157 tended to be higher ($P = 0.08$) for heifers fed SFC than DRC diet. After d 20, heifers fed DRC had higher ($P < 0.05$) fecal starch and lower ($P < 0.05$) fecal pH than heifers fed SFC. Fecal pH was negatively correlated ($r = -0.34$; $P < 0.05$; $n = 143$) with fecal starch concentration. Fecal starch concentration and pH were not different ($P > 0.05$) for heifers positive or negative for *E. coli* O157. Our data suggest that fecal shedding of *E. coli* O157 was not related to fecal pH or starch concentration in cattle fed grain-based diets.

Introduction

The serotype O157:H7 one of nearly 250 Shiga toxin producing Escherichia (*E. coli*) coli implicated worldwide (Johnson et al. 2006) is considered the most pathogenic serotype in North America, Japan, and the United Kingdom (Bielaszewska and Karch, 2000). Cattle digestive tracts, particularly the hind gut (Grauke et al., 2003; Naylor et al., 2003; Van Baale et al., 2004), are believed to be the primary reservoir for *E. coli* O157:H7, a human food-borne pathogen that causes hemorrhagic colitis and hemolytic uremic syndrome. Manipulation of diets fed to feedlot cattle has been proposed as a means for reducing fecal shedding of *E. coli* O157:H7, but results have been inconsistent (Buchko et al., 2000, Berg et al., 2004, Van Baale et al., 2004). Berg et al. (2004) found that cattle fed corn-based diets shed more generic *E. coli* than did cattle fed barley-based finishing diets. Berg et al. (2004) suggested that differences in the site of digestion (rumen vs hind gut) may have impacted fecal shedding of *E. coli* O157. In the rumen, the starch fraction of corn grain is not digested as extensively as starch from barley, thus resulting in higher starch concentrations entering the hind gut (Orskov 1986; Huntington, 1997). Increasing starch concentration in the lower gastrointestinal tract will result in a secondary fermentation and increased production of VFA, hence reduced pH in the large intestine. The more extensively cereal grains are processed, the more starch is digested in the rumen and the less that enters the lower digestive tract (Huntington, 1997). Buchko et al. (2000) and Berg et al. (2004) suggested that low pH and the associated VFA inhibited proliferation of *E. coli* O157 in the large intestine. Therefore, it was of interest to determine the relationship between hindgut fermentation to fecal prevalence of *E. coli* O157. Our objective was to evaluate fecal starch concentration and pH in relation to shedding of *E. coli* O157 in feedlot cattle.

Materials and methods

Experiment 1

Care and handling of animals used in this study were conducted under the approval of the Kansas State University Institutional Animal Care and Use Committee protocol number 2315. Three hundred sixty-eight crossbred-yearling steers (BW = 334 ±

17 kg) were obtained from a common source, and offered ad libitum access to chopped alfalfa hay and fresh water upon arrival. Steers were allowed ad libitum access to four step-up diets leading to the final finishing diet that contained 78% dry-rolled corn and 8% alfalfa hay (Table 1). Steers were housed in 54 concrete-surfaced pens (6 to 7 steers per pen) and each pen (36 m²) included an overhead shade (18 m²) covering half of the pen and feed bunk. Each pen contained an automatic water fountain and 3.2-m of a fence-line feed bunk. Neither total feed samples nor individual feed ingredients were analyzed for *E. coli* O157.

Samples and sampling schedule

Starting on d 20, and every week thereafter until d 61 (August through September), steers from 18 out of the 54 pens were individually restrained in a hydraulic working chute and sampled for *E. coli* O157. For our sampling scheme, we sampled the first 18 pens in the first week, second 18 pens in the second week, and third 18 pens in the third week and back to the first 18 pens in the fourth week and so on until the sixth week. To determine the prevalence of *E. coli* O157 in steers, rectoanal mucosal swab (RAMS) samples (Rice et al., 2003; Greenquist et al., 2005) and fecal grab samples via rectal palpation were obtained from all animals within the sampled pen. The RAMS samples were obtained according to the procedure described by Rice et al. (2003), by using a sterile foam-tipped applicator (VWR International, Buffalo Grove, Ill, Catalog #10812-022) inserted approximately 2 to 5 cm into the anus of each steer, and the epithelium surface was sampled using a rapid in-and-out motion. The RAMS samples were then placed into culture tubes containing 3 mL of Gram Negative (GN) broth (Becton Dickinson, Franklin Lakes, N. J.) with cefixime (0.05 mg/liter; Catalog #740.01, Invitrogen Corporation; Carlsbad, CA), cefsulodin (10 mg/liter; Catalog #C8145; Sigma-Aldrich; St. Louis, MO), and vancomycin (8mg/liter; V2002; Sigma-Aldrich) (GN-CCV; Van Baale et al., 2005). Immediately following the RAMS sampling, a fecal grab sample was acquired via rectal palpation. Cattle not producing an adequate fecal sample (> 5 g) were sorted off and re-run through the restraining chute about 10 min after the original sampling time. Fecal samples were sealed in Whirl-Pak® bags (14 × 20 cm, Nasco, Ft. Atkinson, WI), and both fecal samples and RAMS tubes were kept on ice and transported

to the Pre-harvest Food Safety Laboratory in the College of Veterinary Medicine at Kansas State University for *E. coli* O157 isolation.

Isolation of E. coli O157

Whirl-Pak® bags containing the fecal samples were kneaded by hand for 20 to 30 s, and approximately 1 g sub-sample was placed into a culture tube containing 9 mL of GN-CCV broth by using a sterile transfer stick. Culture tubes containing both RAMS and fecal samples were vortexed for 1 min, incubated at 37°C for 6 h, subjected to immunomagnetic separation (IMS; Dynal, Inc. New Hyde Park, NY), and spread plated onto sorbitol MacConkey agar containing cefixime (50 ng/mL) and potassium tellurite (2.5 µg/mL; Sigma-Aldich) (CT-SMAC). Plates were then incubated overnight (16 to 18 h), and up to six sorbitol-negative colonies were streaked onto blood agar plates (Remel, Lenexa, KS) and incubated for 12 to 18 h at 37°C. Growth on blood agar plates was tested for indole production, for O157 antigen by latex agglutination (Oxoid Limited, Basingstoke, Hampshire, England) and species was confirmed by API 20E identification test (Biomérieux, Inc., Hazelwood, Mo; Van Baale et al., 2004).

Fecal starch analysis

After bacteriological sampling, the balance of the fecal material was frozen for subsequent determination of fecal starch concentration. Fecal samples from steers positive for *E. coli* O157 by either sampling method (fecal or RAMS; n = 41) and 239 randomly selected samples from *E. coli* O157-negative steers were analyzed for starch concentration. Before analysis, samples were thawed, dried in a 55°C forced-air convection oven, and then ground through a 1-mm diameter screen by using a Thomas-Wiley laboratory mill (Model 4 Thomas Scientific™, Swedesboro, NJ). Dry matter of each sample was determined by drying sample for 16 h at 105°C. Starch concentration in feces was determined according to procedures described by Herrera-Saldana and Huber (1989).

Statistical Analysis

Fecal starch concentrations were analyzed using the Proc Mixed procedure of SAS (SAS Version 9.1; Cary, NC). The model statement included the effect of presence

or absence of *E. coli* O157 for each of the sampling techniques. Individual animal numbers were used as a random effect.

Experiment 2

Pre-trial phase

Care and handling of cattle used in this study were conducted under the approval of the Kansas State University Institutional Animal Care and Use Committee protocol number 2315. Ninety-two crossbred yearling heifers (BW = 400 ± 5 kg) were started on a common receiving diet and transitioned (step up diets 1 to 6) to a finishing diet (step up diet 6; Table 2) containing predominantly dry-rolled corn (DRC). Dry-rolled corn was processed to a mean geometric particle size of 4,072 µm (n = 23; Baker and Herrman, 2002) by using a single stack roller mill. Heifers were offered ad libitum amounts of water and feed, and were fed once daily at 0800. Neither total feed samples nor individual feed ingredients were analyzed for *E. coli* O157. After 14 d on the DRC diet, heifers were restrained in a hydraulic working chute, and a RAMS and fecal sample were obtained from each heifer as described in Exp. 1. On the day before sampling, heifer diets were switched from step 4 to step 5 (Table 2). Heifers that failed to yield adequate amounts of feces (> 5 g) were separated and re-sampled approximately 10 min after the original sampling time. Fecal samples sealed in Whirl-Pak® bags (Nasco), and RAMS tubes were kept cool on ice, and transported to the Pre-Harvest Food Safety Laboratory for *E. coli* O157 isolation by using procedures described for Exp. 1.

Trial phase

Of the 92 heifers, 30 (33%) were identified as being positive for *E. coli* O157 based on the initial sampling of feces or RAMS. The heifers (n = 30) positive for *E. coli* O157 were used in a randomized complete-block design experiment to compare the impact of grain processing methods on *E. coli* O157 prevalence (Fox et al., 2007). One week after sampling, heifers were stratified by weight and randomly assigned, within strata, to a finishing diet based on either steam-flaked corn (SFC; n = 15) or DRC (n = 15). Heifers assigned to the DRC diet remained on the same diet throughout the duration of the experiment. Heifers assigned to the SFC diet were transitioned over a 10-d period

from the DRC diet to the SFC diet by using three transition diets in which SFC gradually replaced DRC as the grain source (75:25, 50:50, 25:75, DRC:SFC, respectively). Diets were formulated to contain 14% CP, 0.7% Ca, 0.35% P, 0.7% K, 30 mg/kg monensin, and 9 mg/kg tylosin (Elanco Animal Health, Greenfield, IN). The SFC was processed to a flake density of 360 g/L and had a mean geometric particle size of 5,724 μm ($n = 159$; Baker and Herrman, 2002). Cattle were housed in individual pens (1.5 m x 7 m) with a fence-line feed bunk (1.5 m). Half of the pen and the feed bunk were covered by an overhead roof. Dividers between pens consisted of steel pipe, and thus did not prevent contact between animals in adjacent pens. In addition, water fountains were located such that each fountain served two adjacent pens. Fecal material buildup was removed from the concrete-pen surfaces via scraping every 2 to 4 d. Cattle were fed once daily at 0800, and were offered ad libitum amounts of their respective diets. Neither total feed samples nor individual feed ingredients were analyzed for *E. coli* O157.

The RAMS and fecal samples were obtained from each heifer on d 20 and weekly thereafter for 4 weeks as described in Exp. 1. A 10 \times 140 mm non-sterile wooden stick (Catalog #14 410, Fisher Scientific, Pittsburgh, PA) was used to add approximately 1 g of feces to test tubes containing 15 mL of deionized water, and vortexed (Vortex-Genie® 2, Vortexer Scientific Industries, Bohemia, NY). Fecal pH was then determined with a calibrated pH meter (Thermo Orion model 230Aplus, Orion Research Inc., Beverly MA). Cattle not producing an adequate volume of fecal material after the first and second time through the restraining chute were monitored in their respective pens until a visually fresh fecal pat could be collected for pH and starch analysis. The balance of the fecal material after bacteriological sampling was frozen for starch analysis as described in Exp. 1.

Statistical analysis

Escherichia coli O157 prevalence (positive or negative), fecal pH, and fecal starch concentration data were analyzed using the repeated measures analysis of the Proc Mixed procedure of SAS (SAS Version 9.1; Cary, NC). The model statement included the effects grain processing method (dry rolling vs steam-flaking) and sampling day. Weight block served as the random variable.

Results and discussion

Experiment 1

Prevalence data for *E. coli* O157 are shown in Figure 1. Prevalence of *E. coli* O157 was 4.1% (36 of 872) as measured from RAMS samples and 3.3% (29 of 872) from fecal samples. *Escherichia coli* O157 was detected in 44 of 872 (5.0%) steers by either sampling technique or only 11 of 872 (1.3%) steers tested positive by both sampling techniques (Figure 1). Naylor et al. (2003) suggested that *E. coli* O157 specifically colonizes the lymphoid, follicle-dense mucosal epithelium at the terminal rectum. Rice et al. (2003) and Greenquist et al. (2005) concluded that sampling the terminal rectum, approximately 2 to 5 cm proximal to the rectoanal junction, was more sensitive than a fecal culture.

Fecal DM and starch concentrations are summarized in Table 3. Fecal starch averaged 23% across all steers ranging from a minimum of 1.2% up to a maximum of 59.6%, with a standard deviation of 11% or greater. Zinn et al. (2007) compiled fecal starch concentration data from 32 metabolism studies and found a mean value of 5.9% with a wide range (0 to 44%) in fecal starch. Fecal DM was not correlated ($P > 0.05$) with *E. coli* O157 prevalence. Barajas and Zinn (1998) reported similar values for fecal starch concentrations in yearling heifers fed DRC-based diets (25% and 19% fecal starch when fed diets containing either 11.3% or 15.0% CP, respectively). Steers positive for *E. coli* O157 as determined by prevalence in fecal samples had a higher ($P < 0.05$) fecal starch concentration than did steers negative for *E. coli* O157. Likewise, fecal starch was higher ($P < 0.05$) for steers that tested positive by either method. But, fecal starch concentration was not different when prevalence was determined by RAMS method ($P > 0.05$).

Experiment 2

Pre-trial Phase. Prevalence of *E. coli* O157 was 16.3% (15 of 92) using RAMS samples and 23.9% (22 of 92) for fecal samples. Presence of *E. coli* O157 was detected by either in 30 of 92 (32.6%) steers, whereas only 7 of 92 (7.6%) steers tested positive by both sampling techniques. Interestingly, fecal samples were more sensitive than RAMS samples in detecting *E. coli* O157, which is in contrast to other published studies (Rice et

al., 2003; Greenquist et al., 2004). However, Rice et al. (2003) observed that fecal samples were more sensitive than RAMS samples for the first 2 weeks after experimental exposure to *E. coli* O157. Greenquist et al. (2004) suggested that recently exposed cattle could have less colonization of the rectoanal junction thereby leading to lower sensitivity of the RAMS method. Prevalence data from our study suggest that fewer animals were colonized compared the number of animals which were shedding *E. coli* O157.

Trial Phase. Prevalence data for SFC and DRC over the 50-d sampling period are summarized in Figure 2. During the 50-d sampling period, RAMS method was more sensitive than the fecal samples for 6 out of the 7 sampling periods (data not shown). Prevalence of *E. coli* O157 in heifers as measured by fecal samples ($P = 0.66$, data not shown), RAMS ($P = 0.08$, data not shown), or either method ($P = 0.10$, Figure 2) remained greater than 30% for the first 14 d, and then declined ($P < 0.05$) over time. No treatment \times day interactions ($P > 0.05$) were observed, regardless of the sampling method.

Fox et al. (2007) found that *E. coli* O157 prevalence for heifers fed finishing diets based on steam-flaked grains were higher than that observed in heifers fed dry-rolled grains, and that it remained above 30% for the first 30 d, which are in agreement with our data. In addition to dietary treatments, normal shedding patterns of *E. coli* O157 could have affected our prevalence data over time. Fecal shedding period can be quite variable and may range from a few d to one year (Magnusson et al. 2000). Sanderson et al. (1999) and Magnusson et al. (2000) have suggested an average shedding period of 30 d. Cray and Moon (1995) indicated that fecal shedding peaked about one week after inoculation of calves and decreased continually for 48-189 d thereafter. The use of the prescreening model to select positive animals for *E. coli* O157 does not take into account temporal shedding pattern of an animal. Therefore, some animals may be at the end and others at the start of their shedding patterns. However, in order to detect significant differences when analyzing binomial data such as, absence or presence of *E. coli* O157, a high prevalence of *E. coli* O157 is needed. The prescreening model will likely yield prevalence levels near 50% making this model useful in testing pre-harvest intervention strategies such as grain processing (Fox et al., 2007). Naylor et al. (2003) proposed that in any given population of *E. coli* O157 positive animals, a subset of these animals will

be shedding high numbers of *E. coli* O157 called “supershedders”, thereby enhancing horizontal transmission between herd mates sharing the common water fountain was possible.

Type and level of cereal grain fed, as well as degree of grain processing, can affect the site and extent of starch digestion (Huntington, 1997). Ruminal starch fermentation is greater for finishing diets based on SFC when compared to DRC (Huntington, 1997; Barajas and Zinn, 1998). A lower ruminal pH and a lower ruminal acetate:propionate ratio have been reported (Zinn et al., 1995; Barajas and Zinn 1998; and Corona et al., 2006) for steers fed finishing diets based on SFC rather than DRC, which suggest a greater ruminal fermentation of starch. Fecal starch concentration was similar ($P > 0.05$) for SFC and DRC on d 0. After d 20, heifers fed DRC had higher ($P < 0.05$) fecal starch and lower ($P < 0.05$) fecal pH than did heifers fed SFC (Figure 3). The correlation between fecal starch and pH was -0.34 ($P < 0.05$, $n = 143$; Figure 4) and was lower than that previously described (Russell et al., 1980; Ledoux et al., 1985; Barajas and Zinn, 1998; Xiong et al., 1991). Possibly, a better predictor of fecal pH may be the measurement of starch concentration exiting the small intestine rather than the large intestine. Regardless of the correlation, feeding DRC increased starch entering the lower gastrointestinal tract and, thereby, increased starch in the feces, compared with feeding SFC. Figure 5 illustrates fecal starch concentration and fecal pH over time for *E. coli* O157-positive and -negative samples. Regardless of the sampling method used, fecal starch and pH were not different ($P > 0.05$) for *E. coli* O157 positive and negative samples.

Theurer (1986) estimated that large amounts (up to 600 g/kg) of starch can escape ruminal fermentation and be presented for digestion in the small intestine. Increasing the amount of starch entering the small intestine above that which can be digested and absorbed will result in a secondary fermentation in the large intestine (Siciliano-Jones and Murphy, 1989; Harmon and McLeod, 2001). As hindgut fermentation increases, so will the production of VFA, hence reducing pH in the large intestine. Van Kessel et al. (2002) demonstrated a reduction in cecal and fecal pH with abomasal infusion of starch and glucose. Russell et al. (2000) speculated that a lower pH (< 5.5) in the large intestine is a more favorable environment for both survival and growth of acid-resistant *E. coli*.

However, Buchko et al. (2000) suggested that a lower fecal pH (< 6.3) and the associated VFA inhibited proliferation of *E. coli* O157:H7 in the large intestine. Other studies have demonstrated an inhibitory effect of VFA, in particular propionic acid, on *E. coli* O157:H7 (Rasmussen et al. 1993; Horii et al., 1998). Results from Berg et al. (2004) showed that fecal pH and prevalence of *E. coli* O157 were lower for cattle fed corn compared to cattle fed barley based diets. Corn is less digestible in the rumen than barley (Huntington, 1997); thereby presenting more undigested starch to the large intestine which would favor increased hindgut fermentation (Orskov, 1986; Duncan et al., 1991; Berg et al., 2004). Studies with cattle (Hovde et al. 1999) and sheep (Kudva et al., 1997) found that a hay-based diet resulted in greater colonization of *E. coli* O157:H7 compared to a grain-based diet. Berg et al. (2004) suggested that their results agreed with those of Hovde et al. (1999) and Kudva et al. (1997) in that the forage fed animals, which had the highest colonization of *E. coli* O157:H7, would likely have a higher colonic pH. In addition, Bach et al. (2005) showed that *E. coli* O157:H7 persisted longer in the feces from cattle fed barley than in the feces of cattle fed corn diets. The authors speculated that this may be partly due to the lower fecal pH from cattle fed corn, compared with those fed barley. In our study (Figure 2), we observed a lower prevalence ($P \leq 0.10$) of *E. coli* O157 for cattle fed DRC compared SFC. A review by Huntington (1997) showed that starch from SFC is 11% more ruminally digested than the starch from DRC. Therefore, cattle fed DRC based diets would have more starch presented to the large intestine for increased fermentation and VFA production. Our results seem to be in agreement with Berg et al. (2004), Buchko et al. (2000), and Horii et al., (1998).

Van Kessel et al. (2002) abomasally infused either 778 g/d of starch hydrolysate or 888 g/d of glucose and found lower ($P < 0.01$) fecal pH, compared with that of steers abomasally infused with water. Total aerobic bacterial concentrations in the feces were also higher ($P < 0.01$) with starch hydrolysate and glucose infusions, but total coliforms, *E. coli*, and acid-resistant *E. coli* concentrations were not different ($P > 0.05$) with carbohydrate infusions. They concluded that the amount of starch entering the large intestine affects cecal fermentation and microbial populations, but did not affect acid-resistant *E. coli*. Results from this study suggest that fecal shedding of *E. coli* O157 is not correlated to fecal starch concentration or fecal pH.

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Table 5. Dry-rolled corn based finishing diet fed to yearling steers in Exp. 1.

Item	DM, %
Ingredient	
Dry-rolled corn	81.0
Ground alfalfa hay	7.9
Corn steep liquor	6.1
Supplement ¹	5.0
Nutrient %, formulated	
CP	13.0
Ether extract	3.8
Calcium	0.70
Phosphorus	0.38

Table 6. Ingredient composition and formulated nutrient values of diets fed to yearling heifers during pre-trial phase of Exp. 2.

Ingredient	DRC ¹ step up diets, % of DM					DRC ³	(DRC:SFC ²) blends		SFC ³
	1	2	3	4	5		50:50 ⁴	75:25 ⁵	
Steam-flaked corn	-	-	-	-	-	-	39.3	60.0	80.3
Dry-rolled corn	37.4	45.7	54.3	62.6	71.1	79.5	40.1	19.8	-
Ground alfalfa hay	50.3	41.9	33.4	25.0	16.5	8.1	8.2	8.0	7.7
Corn steep liquor	5.9	5.9	5.9	6.0	6.0	6.0	6.0	5.9	5.7
Supplement	6.4	6.5	6.4	6.4	6.4	6.4	6.4	6.3	6.3
Nutrient, % formulated									
CP	16.5	16.0	15.6	15.0	14.5	14.0	14.0	14.0	14.0
Ether extract	2.8	3.0	3.1	3.3	3.5	3.7	3.7	3.7	3.7
Ca	1.2	1.1	1.0	0.9	0.8	0.7	0.70	0.70	0.70
P	0.35	0.36	0.36	0.37	0.37	0.38	0.38	0.38	0.38

Figure 1. Prevalence of *Escherichia coli* O157 by rectoanal mucosal swab (RAMS) and fecal samples in yearling steers (Exp. 1).

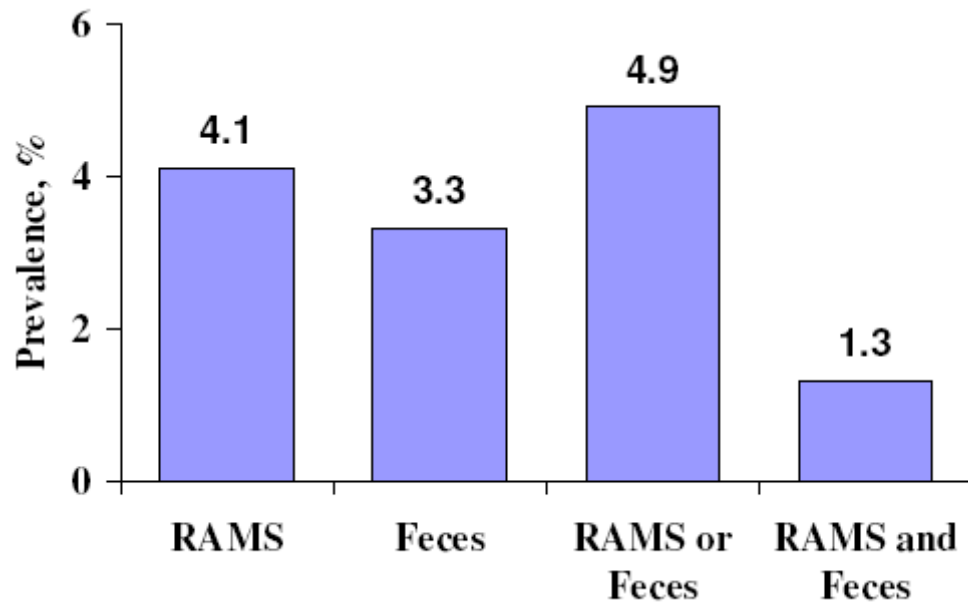


Table 7. Fecal DM and starch concentrations (% of DM) in yearling steers sampled for *Escherichia coli* O157 by rectoanal mucosal swab (RAMS) and fecal samples (Exp. 1).

Item	No. of samples	Fecal DM¹, %	Average	Minimum	Maximum	SE
Feces						
Positive for <i>E. coli</i> O157	26	19.4	26.4 ^a	4.7	57.0	0.8
Negative for <i>E. coli</i> O157	251	18.5	20.8 ^b	1.2	59.6	2.3
RAMS						
Positive for <i>E. coli</i> O157	17	18.6	24.1	4.2	40.0	3.0
Negative for <i>E. coli</i> O157	263	18.6	21.2	1.2	59.6	0.8
Feces or RAMS						
Positive for <i>E. coli</i> O157	41	18.8	25.1 ^a	4.2	57.0	1.9
Negative for <i>E. coli</i> O157	239	18.5	20.8 ^b	1.2	59.6	0.8

^{ab}Means within a column without common superscripts are different ($P < 0.05$).

Figure 2. Prevalence of *Escherichia coli* O157 in yearling heifers fed finishing diets based on steam-flaked corn (□) or dry-rolled corn (■) over a 7-week period (Exp. 2)

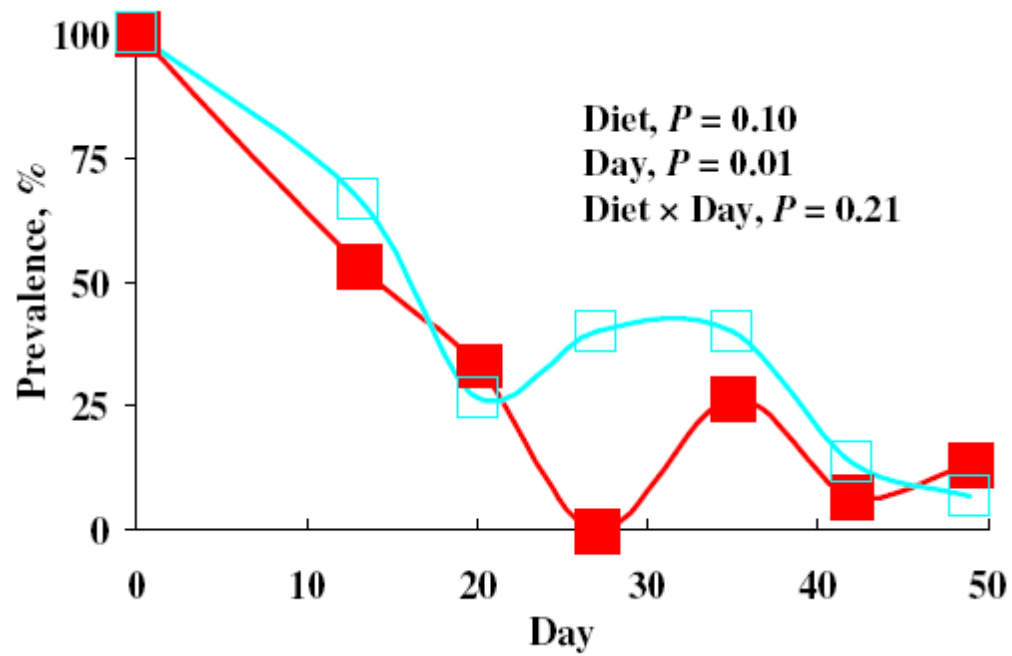


Figure 3. Effect of steam-flaked (\square) or dry-rolled corn (\blacksquare) on fecal starch concentrations (A) and fecal pH (B; Exp. 2).

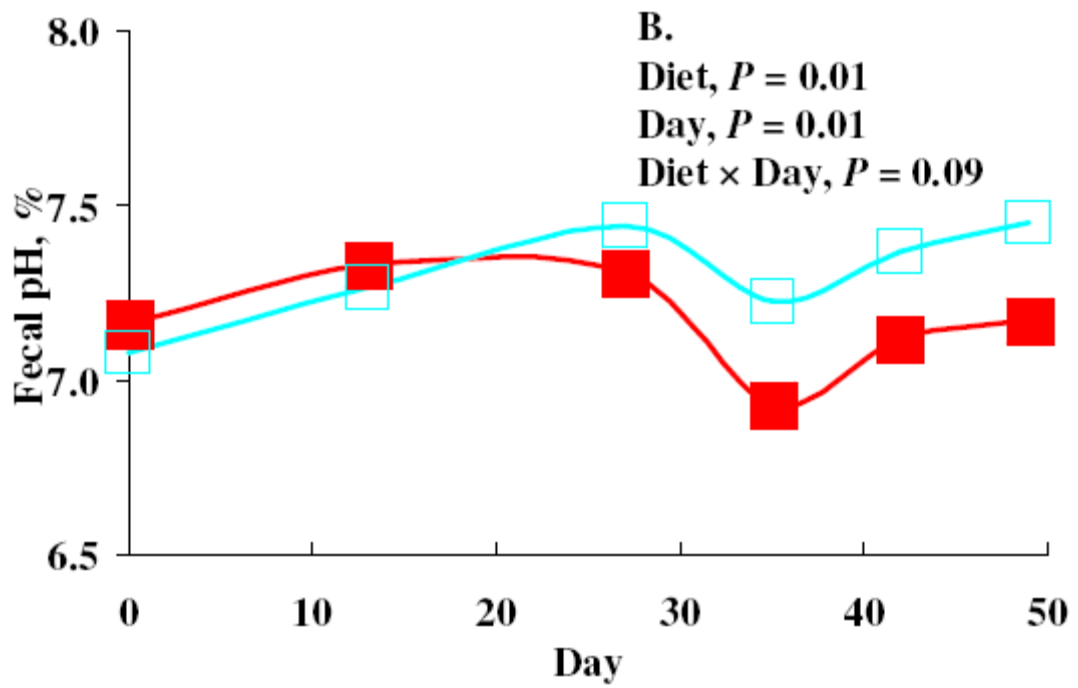
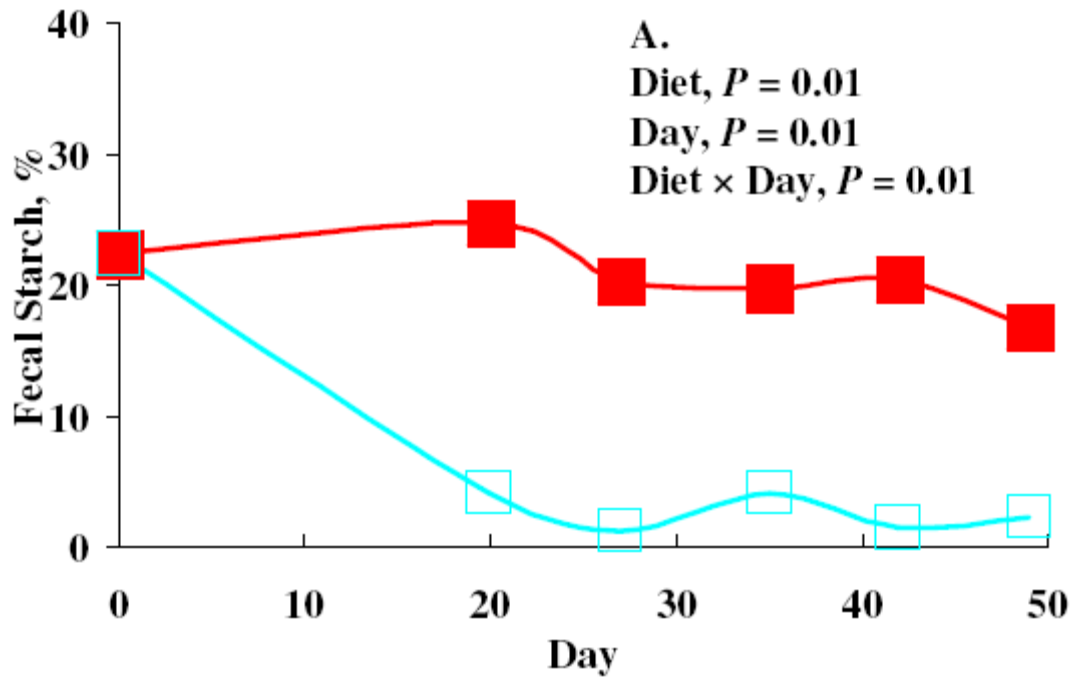


Figure 4. Relationship of fecal pH and fecal starch concentration (Exp. 2).

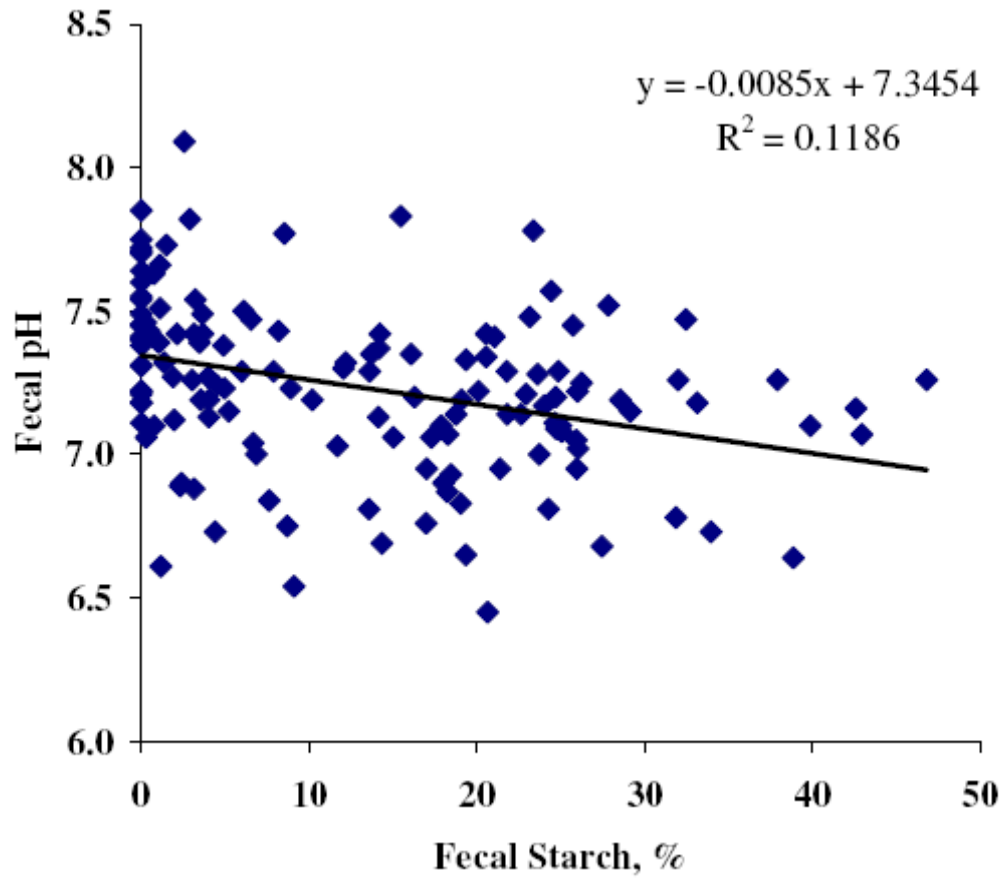


Figure 5. Fecal starch concentration (A) and fecal pH (B) of yearling heifers positive (■) and negative (□) for *Escherichia coli* O157 (Exp. 2).

