EFFECT OF TRACE MINERAL SUPPLEMENTATION AND THE USE OF AN EXPERIMETNAL *ESCHERICHIA COLI* O157:H7 VACCINE ON *ESCHERICHIA COLI* O157:H7 FECAL SHEDDING IN BEEF CALVES

by

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APPROVAL

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November 17, 2005

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ABSTRACT

Two experiments were conducted to evaluate fecal shedding of *E. coli* O157:H7 in newly-weaned calves. In the first experiment, twenty-four heifers were fed a basal diet composed of wheat middlings and corn grain (15% CP and 79% TDN). Twelve heifers were supplemented with trace minerals to provide an additional 399 mg Cu, 1001 mg Zn, and 707 mg Mn/d. The control diet had no supplemental trace minerals added. All heifers were inoculated with an oral dose of 10^{10} CFU of *E. coli* O157:H7. Fecal samples were collected every 18 h for the first three days after dosing and then every three d until d 21 to determine *E. coli* O157:H7 shedding rates. On d 7 venous blood was collected, and on d 21 liver tissue and venous blood were collected. Trace mineral supplementation did not increase IBR titers (P=0.50) but increased (P<0.005) liver Cu concentration. There were no differences in the rate of fecal shedding of *E. coli* O157:H7 between treatments, but the SEM between treatments often were as great as the mean values. *E. coli* O157:H7 decreased in concentration during the first 21 d. Unexpectedly, after d 21, fecal *E. coli* O157:H7 concentration increased to a level measured 18 h post-inoculation. These results suggest that supplemental trace minerals did not influence the rate of *E. coli* O157:H7 shedding. This may be due to a lack of nutritional stress on the animals (no differences in IBR titers), or because the control diet provided adequate trace minerals. In the second experiment, 374 steers were split into two groups to determine if an experimental *E. coli* vaccine would reduce fecal shedding of *E. coli* O157:H7 during the first 56 d after weaning. Calves did not shed *E. coli* O157:H7 during either sampling period (d 0 or d 55). There was no difference in fecal shedding of the bacteria between the control and vaccinated treatments. There was, however, an unexplained difference (P < 0.0001) in ADG, with vaccinated calves gaining 0.11 kg/d more than the control treatment. These data indicate that *E. coli* O157:H7 is not a problem at this ranch in Montana.

INTRODUCTION

It has been reported that there are over 76 million cases of foodborne diseases that occur each year in the United States with over 325,000 people hospitalized and 5,000 of the cases being fatal (Mead et al., 1999). The most prevalent food borne diseases arise from *Campylobacter*, *Salmonella*, Norwalk viruses, and *Escherichia coli* O157:H7. It is estimated that *E. coli* O157:H7 related recalls of hamburger has cost the beef industry as much as $1.6 billion in beef demand (Kay, 2003).

Escherichia coli O157:H7 is a food-born pathogen that can cause significant health risk to consumers and usually causes abdominal pain and bloody or non-bloody diarrhea in humans due to gastroenteritis (Boyce et al., 1995; Phillips et al., 2000). It can also result in hemolytic-uremic syndrome, which can cause acute renal failure.

Cattle are a major reservoir of *E. coli* O157:H7, and if not handled properly beef maybe contaminated during harvesting procedures (Elder et al., 2000; Barkocy-Gallagher et al., 2001; Rivera-Betancourt et al., 2003). Fortunately, current post-harvest methods have proven effective in reducing O157:H7 contamination on carcasses (Elder et al., 2000; Barkocy-Gallagher., 2003; Rivera-Betancourt et al., 2004) through a “multiple-hurdle” intervention system. This system can include live cattle rinses, steam vacuums, organic acid wash cabinets, steam pasteurization wash cabinets, and trimming contaminated areas. It has been proposed that this system needs to be expanded to decrease the amount of *E .coli* 0157:H7 contaminated cattle during the pre-harvest stage.

E. coli O157:H7 is found ubiquitously from the farm to the packing plant (Hancock et al., 1997; Kudva et al., 1997., Rivera-Betancourt et al., 2004). Rice et al.

(2003) and McGee et al. (2004) found that the introduction of one animal which was shedding at high rates infected other cohorts in a pen. Furthermore, Bach et al. (2004) indicated that stress increased susceptibility to O157:H7 shedding.

Preharvest nutrition of cattle has been implicated as a preharvest tool that may decrease *E. coli* O157:H7 shedding (Kudva et al., 1997; Berg et al., 2004). Trace mineral and vitamin supplementation play a critical nutritional role by optimizing the immune status of beef cattle. Trace mineral supplementation has increased humoral and cellular immune response in cattle (Ansotegui et al., 1994; Clark et al., 1995). A functional immune system is necessary for an animal to immunologically respond to foreign antigens (Greene et al., 1998). Furthermore, Greene et al. (1998) stated that "In order to respond immunologically, whether it be to a foreign antigen that has been given, as in a vaccine, or one from the production environment, an animal needs to have an immune system that is responsive and capable of meeting any challenge."

Results from our laboratory (Choat et al., 2005; Standley et al., 2005) showed decreased *E. coli* shedding in Montana feeder cattle compared to cattle from other parts of the U.S. The only common management procedure among different groups of cattle was supplementation with increased levels of trace minerals and vitamins prior to shipment to Midwestern feedlots.

These data concur with data from other researchers who found decreased *E. coli* O157:H7 shedding in Montana cattle. Peterson et al. (2005), in a study that evaluated 1003 Montana calves at four different sampling periods from weaning to harvest, found prevalence rates from 0.0 to 1.24%. These cattle were also supplemented with trace

minerals and vitamins. Additionally, data from Dewell et al. (2005) measured no prevalence of *E. coli* O157:H7 in Montana feedlots, while Colorado feedlots had a prevalence of 21% and Nebraska feedlots had prevalence rates at 45%.

The objective of experiment one was to compare fecal shedding of calves dosed with *E. coli* O157:H7 which were either supplemented with trace minerals and vitamins or not supplemented. The objective of experiment two was to determine the effect an experimental *E. coli* O157:H7 vaccine would have on calves fed similar levels of trace minerals as supplemented calves in experiment one.

LITERATURE REVIEW

Human Infection

Escherichia coli O157:H7 is a food borne pathogen that can pose a significant health risk to the public. *E. coli* O157:H7 commonly causes abdominal pain and bloody or non-bloody diarrhea in humans, due to gastroenteritis (Boyce et al., 1995; Phillips et al., 2000). It can result in hemolytic-uremic syndrome, causing acute renal failure — typically more prevalent in children and the elderly, who may have compromised immune systems.

The resistance of *E. coli* O157:H7 to natural defense mechanisms, as well as the attachment to the intestinal tract and proliferation, is not fully understood. *E. coli* O157:H7 must first elude the natural defense mechanism before it can colonize in the intestinal epithelium. Saliva is the first defense mechanism *E. coli* encounters as it contains mucins, soluble immunoglobin A, and proteins that collect pathogens in a bacteria protein aggregate allowing phagocytic cells to destroy them. Grys et al., (2005) found that StcE (a zinc metalloprotease that is secreted from an etp type II secretion encoded from plasmid pO157), through mucinase activity, reduces the viscosity of saliva allowing the bacteria to move into the stomach. While the stomach is very acidic, *E. coli* O157:H7 is remarkably acid resistant and can move into the colon where it colonizes.

StcE further contributes to the adherence of *E. coli* O157:H7 to host cells of the intestinal epithelium by degrading the protective layer of mucins and glycoproteins on the host cells (Grys et al., 2005). *E. coli* uses long “tether-like” pili to attach onto the host cell membranes effacing micorvilli and cytoplasm. This forms attaching/effacing (A/E)

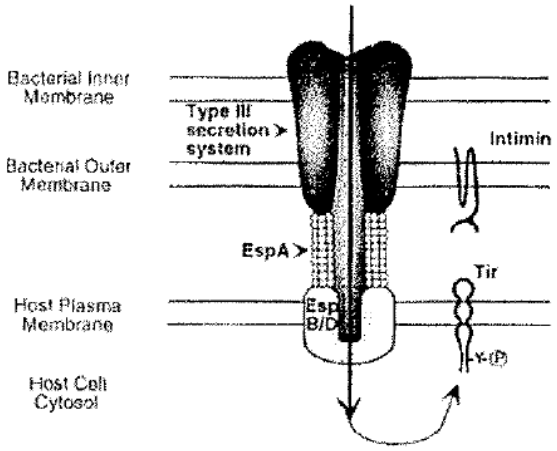
lesions allowing the organism to form a more intimate attachment (Dean Nystrom et al., 1998). The eae gene is necessary for encoding intimin and is associated with forming A/E lesions. This bacterium then uses a specialized injector system known as a type III injection system (Figure 1) that allows it to pump bacterial proteins into the cell allowing infection. This needlelike tube (Esp A) assists the proteins Esp B and Esp D to form an opening in the intestinal membrane, thus allowing the protein Tir into the cell. Tir inserts itself into the membrane with part of it projecting through the cell surface, allowing the protein intimin on the bacterial cell surface to attach intimately to the mucosal cell surface.

The bacterium is locked onto the mucosal cell surface and assists in pedestal formation. Intestinal cytoskeletal proteins bind to the Tir protein imbedded in the cell membrane and form long protein chains called actin. The actin filaments build up under the bacterium, and as they grow they push the cell membrane up forming a pedestal. After many of these pedestals form, and many bacteria have adhered to the intestinal lining, symptoms of infection begin. The locus of enterocyte effacement (LEE) element encodes intimin, Tir, and other type III secretion proteins necessary for pedestal formation (Phillips et al., 2000).

At this stage, it is hypothesized that shiga toxins and other effectors compromise the intestinal epithelial barrier and damage the intestinal endothelium, which results in entry of blood and serum effectors into the intestinal lumen (Grys et al., 2005). Shiga toxin producing *E. coli* (STEC) composes a group of A/E enteric pathogens of animals and humans (Stevens et al., 2002). The release of shiga toxins into the human bowel is

believed to be the central pathogenesis of this disease (Wales et al., 2002). There is a clear difference among *E. coli* O157:H7 strains in their ability to express virulence associated factors (McNally et al., 2001).

Figure 1. The type III secretion system of EPEC used to deliver virulence factors, including Tir, into the host cell cytosol or membrane. Several gram-negative pathogens use this conserved secretion system to deliver diverse effectors into host cells to mediate several different effects within mammalian and even plant cells (Goosney et al., 2000)



Incidence

There are over 76 million cases of foodborne diseases that occur each year in the United States with over 325,000 people hospitalized and 5,000 of the cases ending in fatality (Mead et al., 1999). These data indicate the importance of reducing foodborne pathogens, which means addressing bacteria like *E. coli* O157:H7 and *Salmonella* at the point of contamination, most likely the harvesting phase. Common foodborne diseases arise from Campylobacter, Salmonella, Norwalk viruses, and *E. coli* O157:H7. Steven

Kay (Meat and Poultry, 2003) estimated the cost of *E. coli* O157:H7 in lost beef demand for the last ten years could be as much as $1.6 billion.

MacDonald et al. (1988) reported that 8/100,000 people are infected with *E. coli* O157:H7, and in a more recent report by Mead et al. (1999) indicated that 1.34/100,000 people are infected with *E. coli*. A 1998 *E. coli* outbreak, caused by lettuce grown in Montana, identified 40 residents with *E. coli* O157:H7 infection, with 13 hospitalized (Ackers et al., 1998). However, due to the difficult detection methods and low awareness of this disease to the public, it has been estimated that underreporting of this pathogen in one major city in Canada could vary from 78% to 88% (Michel et al., 2000).

E. coli infection of humans is most prevalent during the month of July (Michel et al., 1999); this coincides with a higher rate of hamburger and beef consumption during the grilling season. In a study conducted by Michel et al. (1999), more cases of *E. coli* were reported in areas with mixed agriculture, typically areas with higher cattle density. It was not determined whether this increase in incidence was attributed to people on the farm who may have come into contact with this organism, or if it was from surrounding suburbanites contracting it from contaminated vegetables, well and/or surface water, or locally grown food.

Wilson et al. (1996) found that of 80 southern Ontario dairy farms sampled, with 335 residents and 1458 cattle tested, 6.3% of the people on 20.8% of the farms and 46% of cattle from 100% of the farms were shedding Vero cytotoxin producing *E. coli* (VTEC) in their feces. Additionally, 12.5% of people had antibodies to *E. coli* O157:H7. These data suggest that continuing or recurrent exposure to VTEC, especially at an early

age, in the farm environment may offer some immunity and protection against infection of *E. coli* O157:H7 and other virulent VTEC serotypes.

Transmission

People are typically infected via three routes: 1) contaminated meat, milk, and produce; 2) a contaminated water supply; and 3) person to person (Boyce et al., 1995; Bach et al., 2002; Lahti et al., 2003). Contaminated beef is a source of human infection typically occuring when raw beef is handled without washing hands and with beef that is not thoroughly cooked (Mead et al., 1999). Ackers et al. (1998) found that the cause of an *E. coli* outbreak in Montana was likely contaminated by either tainted irrigation water or fertilizer, or ground water contaminated by either sheep or cattle feces.

Additionally, the consumption of a contaminated water supply not chlorinated or infection from swimming in a fecally contaminated lake can provide ample opportunity to create infection (Boyce et al., 1995). *E. coli* O157:H7 is not just transmitted through food as previously indicated, and contact with feces is strongly associated with risk of infection (Locking et al., 2001).

A minimal amount of *E. coli* O157:H7 bacteria are needed to cause human infection. An infectious dose of 100 CFU (colony forming units) can easily create infection and colonization of *E. coli* O157:H7 in the colon (Dean-Nystrom et al., 1998; Nataro and Kaper, 1998).

Reservoir

The lower intestines of cattle are suspected of being the major reservoir of *E. coli* O157:H7 (Chapman et al., 1997; Elder et al., 2000; McGee et al., 2004). However, while cattle are typically colonized with, and actively shedding *E. coli* O157:H7, they are asymptomatic (Cray and Moon, 1995; Buckho et al., 2000; Wray et al., 2000). Sheep have been indicated as another important ruminant reservoir for *E. coli* (Chapman et al., 1997; Kudva et al., 1997; Cornick et al., 2000). Sheep also remain healthy with no signs of morbidity while colonized with *E. coli* (Kudva et al., 1995; Wales et al., 2001) and were found to be an appropriate model of *E. coli* etiology in cattle (Cornick et al., 2002).

Additional reservoirs identified as a potential source for human infection are deer (Keene et al., 1997) and goats (LaRagione et al., 2005). *E. coli* has been isolated in other species as well, but they have not been identified as a primary reservoir.

Beef Contamination

In a 1993 study, it was determined that 4% of cattle at slaughter tested positive for *E. coli* O157:H7 in their feces, and 30% of these carcasses were positive while 8% of carcasses from recto-swabbed negative cattle were also positive (Chapman et al., 1993). These data indicate that carcasses became contaminated during the harvest process. Data from Barkocy-Gallagher (2001) indicated that the majority of *E. coli* found on the carcass was a result of pre-evisceration contamination. However, *E. coli* has been found virtually everywhere in the packing plant including door knobs, conveyor belts, floors, locker rooms, and toilet seats (Tutenel et al., 2003; Rivera-Betancourt et al., 2004).

Additionally, it was also reported that hides, fence panels, and holding panels were contaminated with *E. coli*. These data suggest that contamination of carcasses can occur from the environment and personnel anytime during the processing phase.

Fecal and hide prevalence of *E. coli* O157:H7 was significantly correlated with carcass contamination, yet *E. coli* has been detected predominantly on hides suggesting they are a more significant source of contamination than direct contact of feces with the carcass (Barkocy-Gallagher, 2003; Tutenel et al., 2003). This indicates that there was secondary contamination of the hide, and the hides' contamination could either be from feces or the environment. This also suggests that contact between animals after leaving the feedlot can have large affects on *E. coli* contamination among cattle, as well as contamination of cattle can vary significantly from day to day in the packing plant (Tutenel et al., 2003). In a study performed by Barkocy-Gallagher (2001) it was found that *E. coli* detected on the carcasses was primarily a result of transfer within a lot rather than cross contamination among lots.

Tutenel et al. (2003) found that hide from the anal region and shoulder area were found positive every day sampled, and shoulder hide was twice as likely to be contaminated as hide from the anal area. These results were similar to results from Elder et al. (2000), who reported that bacterial loads can differ significantly between animals and even adjacent sites on hides and carcasses. These observations emphasize the importance of reducing hide contact with the carcass.

Antimicrobial interventions and other in plant processing practices substantially reduced *E. coli* prevalence (Elder et al., 2000; Rivera-Betancourt et al., 2004). Bacon et

al. (2000) found that in eight plants, cattle entered with mean *E. coli* counts of 5.5-7.5 (log CFU/100 cm^2) which decreased significantly following hide removal. After multiple hurdle decontamination interventions, including steam vacuuming, pre-evisceration carcass wash, pre-evisceration organic acid rinse, hot water carcass wash, post-evisceration carcass wash, and post-evisceration acid rinse there was a 52.2% reduction in *E. coli* counts. Furthermore, following chilling, *E. coli* counts were decreased by 98.4%. Berry and Koohmaraie (2001) reported that proper sanitation and processing practices prevent and reduce the contamination of carcasses with *E. coli,* regardless of background microflora levels. These data indicate that multiple hurdle technology, with current hazard analysis critical control points (HACCP) requirements for bacterial decontamination purposes, was effective in reducing microbiological contamination of beef carcasses.

Temperature control is critical in the handling and storage of meat to prevent the growth of this pathogen. Berry and Koohmaraie (2001) reported that viable numbers of all microflora remained the same at 4º C. However, at 12ºC *E. coli* grew on beef carcass tissues at all microflora levels.

Cattle Infection

Cattle are infected in the exact same mechanism in which humans are infected. It is important to note however, that cattle lack intestinal receptors for shiga toxins, indicating why cattle are resistant to the enterotoxigenic effects of shiga toxins (Pruimboom-Brees et al., 2000). Infectious doses can be as low as 260 CFU – 10^4 CFU

with both dose and age related effects playing a role in the probability of infection (Besser et al., 2001).

While Cornick et al. (2000) did report that there were no consistent differences in the frequency, magnitude, or colonization among *E. coli* pathotypes, STEC tended to persist longer than other pathotypes and was better adapted to persist in the alimentary tracts of sheep. Shiga toxin negative *E. coli* did not cause neurological disease but colonized and caused A/E lesions in cattle (Dean-Nystrom et al., 2000).

E. coli is predominantly found in the lower gut with the colon as the site of *E. coli* persistence and proliferation in ruminant animals (Harmon et al., 1999; Grauke et al., 2002; LaRagione et al., 2005). Naylor et al. (2003) found that *E. coli* O157:H7 specifically colonized in the recto-anal junction, and intimin was required with the eae gene for colonization, A/E lesion formation, and disease in cattle (Dean-Nystrom et al., 1998; Cornick et al., 2002).

E. coli is not pathogenic in weaned calves or adult cattle (Brown et al., 1997). Calves less than 36 h old inoculated with EHEC O157:H7 developed diarrhea and enterocolitis with A/E lesions in the large and small intestine within 18 h after inoculation. Ingestion of colostrum prior to inoculation with antibodies against shiga toxin 1 and O157:H7 did not prevent disease (Dean-Nystrom et al., 1997). These data and data from Widiasih et al. (2004) suggest that some EHEC strains are pathogenic in neonatal calves possibly due to an undeveloped digestive system.

Cattle that are reinoculated or reintroduced to the same strain or new strain of *E. coli* began shedding the organism again (Cray and Moon, 1995; Besser et al., 1997;

Kudva et al., 1997; Wray et al., 2000). After infection there have been differences in IgG levels of animals. Wray et al. (2000) measured some calves with increased IgG levels (possibly due to colonization in the tonsil or lymphoid tissue) while other calves saw no increase in IgG levels. Other reports also have shown no increase in humoral immunity after infection with *E. coli* O157:H7 (Shere et al., 2002).

Fecal Shedding

Little is known about the exact process of *E. coli* O157:H7 shedding (Cray and Moon, 1995; Brown et al., 1997). There was a wide variation in the amount and duration of *E. coli* O157:H7 fecal shedding (Cray and Moon, 1995; Besser et al., 1997). Cattle appear to shed *E. coli* in their feces sporadically and intermittently (Wray et al., 2000; Shere et al., 2002).

E. coli has been shown to shed at levels from <30 CFU/g to 10^7 CFU/g (Besser et al., 2001) and was detected for as little as a day or as long as two years (Shere et al., 1998). Long term fecal shedding of cattle was probably the result of infection and reinfection.

E. coli shedding is shown to peak in the summer and early fall, declining through the winter in the pre-harvest phase, carrying over to less contamination of carcasses in the harvesting phase (Elder et al., 2000; Barkocy-Gallagher et al., 2003; Rivera-Betancourt et al., 2004). Lahti et al. (2003) did not measure an absence of *E. coli* shedding at the farm level. This may be due to the relatively small amount detected at the farm level. There is a difference in age of animals and the amount and duration of shedding, with older

animals shedding lower counts of *E. coli* in their feces for a shorter period than younger animals (Wray et al., 2000; Van Donkersgoed et al., 2001; Lahti et al., 2003). Naylor et al. (2003) found that *E. coli* bacteria were unevenly distributed in the feces of calves with higher concentrations on the outside of the feces. High shedding rates of *E. coli* O157:H7 resulted from its colonization at the recto-anal junction.

Rice et al. (2003) found that when *E. coli* attached and colonized in the gastrointestinal tract, bacteria were shed for longer durations, but when there was no colonization it was transiently shed for a shorter time. These results were consistent with another study that showed *E. coli* was rapidly eliminated from the rumen environment but still persisted in the feces for up to 67 d (Buckho et al., 2000). LeJeune et al. (2001) also reported that calves appeared to passively shed the organism after drinking contaminated water for a period of four wk before shedding it on a consistent basis.

Detection Methods

Enrichment of samples was utilized with best results of growth measured from tryptic soy broth (TSB) for 2 h at 25º C and then for 6 h at 42º C (Barkocy-Gallagher, 2002; Dodd et al., 2003; Rice et al., 2003). Enrichment of samples has had a large impact on the ability to detect *E. coli* in fecal samples and has more accurately measured prevalence of *E. coli* O157:H7 than direct plating.

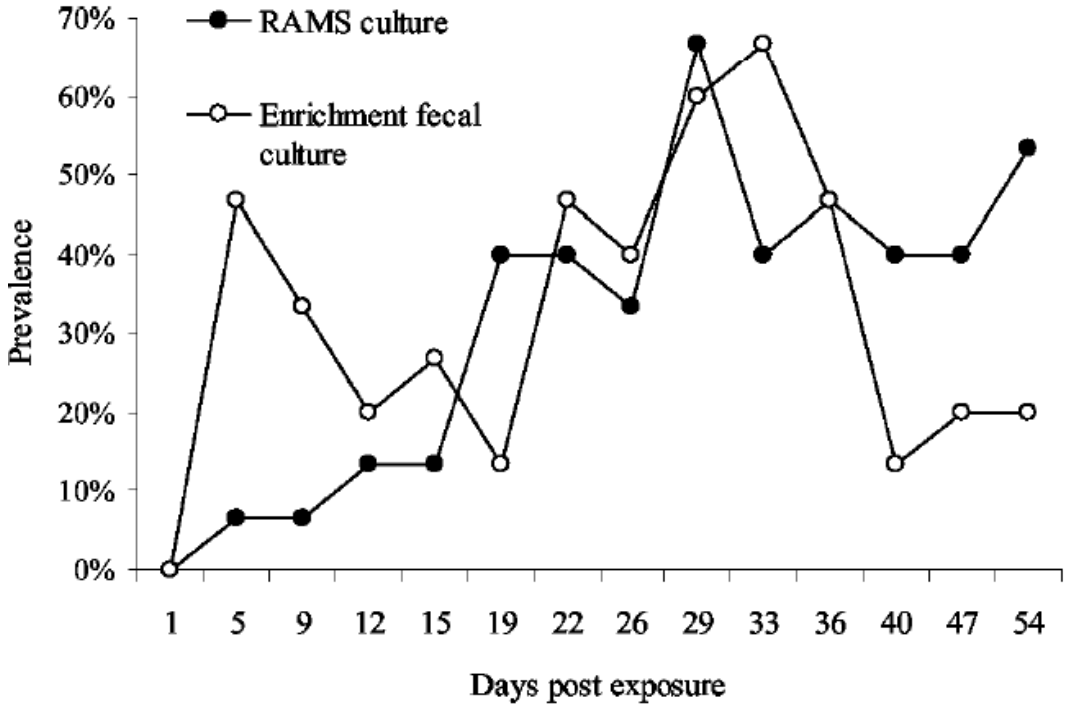
Immuno-magnetic separation (IMS) is an effective tool for the selective concentration of *E. coli* O157:H7 from enrichment concentrations (Barkocy-Gallagher, (2002). This is the preferred method used (MRU method) in determining whether a

sample was positive or negative for *E. coli* O157:H7. More positive samples were found by MRU than any previous methods, primarily because it was developed to recover actual organisms, creating a gold standard of identifying isolates for *E. coli*. This was a more accurate test because it measured damaged pieces of *E. coli* bacteria that previous methods were unable to detect.

Additionally Dynabead isolation detected 74 of 75 samples positive for *E. coli* while direct plating only detected 22 of 75. However, it is important to remember there are innate problems with comparing data from studies in which detection methods with disparate sensitivities were compared.

Rice et al. (2003) reported that recto-anal mucosal swabs (RAMS) more directly measured the relationship between cattle and *E. coli* O157:H7 infection than a fecal culture, especially if the recto-anal junction is the site of colonization (Figure 2). *E. coli* has been detected more from rectal fecal grab samples that have 10g of feces than from samples with 1g of feces (Kudva et al., 1995; Lahti et al., 2003). It was also reported that RAMS cultures could have test results determined in 24 h where fecal cultures require 48 h (Rice et al., 2003).

Figure 2. Detection by recto-anal mucosal swabs (RAMS) culture and fecal culture of *E. coli* O157:H7 from calves experimentally exposed to *E. coli* O157:H7 by penning them with culture positive Trojan calves. Significant differences in sensitivities of detection exist early ($P < 0.01$) and late ($P < 0.05$) in the course of infections (Rice et al., 2003)



Prevalence

Farm and Ranch

Early data from Hancock et al., (1994) reported that *E. coli* fecal prevalence was 0.28% with 8.3% of herds showing one or more positive samples. It also was found that fecal prevalence in beef cattle was 0.71% and was in 16% of herds. Additionally, a study in Georgia found that 2.5% of animals were fecal positive for *E. coli* with herd prevalence at 17.7% (Dunn et al., 2004).

The Federal Register (2002) reported that in five multi-state studies the prevalence in herds containing one or more cattle infected with *E. coli* O157:H7 was 24%, 61%, 75%, 87%, and 100%. The pre-harvest intervention group from the National Cattlemen's Beef Association, (2003) stated that 25% of calves shed *E. coli* O157:H7 within a week of birth with 87% exposed to it prior to weaning.

A study conducted in 14 northwestern herds indicated an overall prevalence of 1.0% with *E. coli* found in 9/14 of the herds (Hancock et al., 1997); however, there were no positive samples in 63% of the visits to the farms. These data indicate that a single sample date could easily over or underestimate *E. coli* O157:H7 prevalence. Therefore, the study done by Laegreid et al. (1999) was accurate when it reported a mean *E. coli* prevalence of 7.4% and standard deviation of 6.2% in beef calves at weaning.

These data indicate that shedding is sporadic and variable. Accuracy of farm *E. coli* prevalence cannot be determined with one sampling date and must be continually monitored. However, generally it can be concluded that individual animal prevalence was typically at a lower rate (<10%) while herd prevalence was at a much larger rate.

Feedlot

Seventy-two percent of 29 Midwestern feedlots sampled had at least one EHEC O157 positive fecal sample, and 38% had at least one positive hide sample (Elder et al., 2000), with overall prevalence in the feces and the hides at 28% and 11% respectively. Other studies indicate that prior to shipping, *E. coli* prevalence was 9.5% to 23% in feces, 18% on hides, and 20% in pens (Smith et al., 2001; Barham et al., 2002; LeJeune et al., 2004). In a tri-state study, it was reported that the prevalence of feedlots containing one

or more cattle infected with *E. coli* O157:H7 was 63%, 100%, and 100% (Federal Register, 2002).

Dewell et al. (2005) found that 86.7% of pens from three states and twelve feedlots had at least one positive *E. coli* fecal sample, and the within pen prevalence varied from 3.3% to 77.8%. In a larger study encompassing 73 feedlots, 711 pens, and 10,622 fecal samples, *E. coli* prevalence was 95.9% in feedlots, 52.0% in pens, and in 10.2% of samples (Sargeant et al., 2004).

Packing Plant

Chapman et al. (1993) reported that *E. coli* was isolated from 4% of cattle at slaughter. In other studies, 5.5 % to 7.5% of cattle tested positive for *E. coli* O157:H7 (Van Donkersgoed et al., 1999; Barham et al., 2002). Sixty-one percent of hides from three Midwestern beef processing plants were positive for *E. coli* O157:H7 (Barkocy Gallagher et al., 2003).

In another study with 30 lots sampled at pre-evisceration, 87% had at least one EHEC O157 positive sample, with 57% of lots positive post-evisceration and 17% positive post processing (Elder et al., 2000). Overall prevalence was 43%, 18%, and 2% at the three respective processing samples.

In a year-long study by Chapman et al. (1997), *E. coli* were isolated from 15.7% of 4800 cattle and 2.2% of 1000 sheep. This study reported that beef prevalence was 13.4% and dairy prevalence was 16.1%. Furthermore, it was found that prevalence varied greatly from month to month with lows at 4.8% and highs at 36.8%.

Mirtsching (2002) stated that based on three years of data (testing hides as they entered the packing plant), if 15-20% of cattle in a pen are contaminated with *E. coli* O157:H7 our multiple hurdle carcass decontamination system prevents occurrence on carcasses. However, if greater than 40% of cattle are contaminated with *E. coli*, our interventions will not prevent occurrence on carcasses. The previous data indicates that with the wide variability of *E. coli* O157:H7 prevalence on cattle entering the packing plant, there needs to be some pre-harvest interventions to decrease prevalence.

Geographic Distribution

Rivera-Betancourt et al. (2004) reported that packing plants in the northern part of the United States had lower prevalence of *E. coli* O157:H7 than southern plants. A tri-state study found that cattle from central Nebraska were nine times as likely to be positive than cattle from eastern Colorado, while no cattle from Montana were positive (Dewell et al., 2005). Results from our laboratory showed decreased *E. coli* shedding in Montana feeder cattle compared to other parts of the U.S. (Choat et al., 2005; Peterson et al., 2005, Standley et al., 2005).

These data contradict earlier reports by Griffin and Tauxe (1991) and Boyce et al. (1995), who suggested that prevalence was greater in northern states and Canada. However, the disparity could be due to more extensive *E. coli* research conducted in the northern states identifying more *E. coli* O157:H7, with less research done in southern states. If these data are correct, there is a disparity between an increased prevalence in

northern states and data that indicates *E. coli* is more prevalent in warmer months and climates.

Cattle Transmission

There were no consistent differences measured in the frequency or magnitude of transmissibility among *E. coli* pathotypes (Cornick et al., 2000). However, in the same study there was evidence of competition between strains that altered colonization and proliferation. Horizontal transmission of *E. coli* O157:H7 has been noted in numerous studies (Kudva et al., 1995, 1997). Animal to animal contact caused *E. coli* infection of calves in adjacent pens with this natural infection lasting 17 to >31 d (Shere et al., 2002). In sheep, shedding as low as 100 CFU to 10,000 CFU, transmitted the organism to other sheep (Cornick et al., 2000). Besser et al. (2001) found that infectious doses could be as low as <260 CFU to 10,000 CFU in calves as well.

Super-shedders, cattle that shed the organism at high rates, have been largely implicated as a main transmission source to other cohorts as well, also recognized as the "Trojan Calf Theory." McGee et al. (2004) found that within two days of the introduction of a super-shedder, 66% of pen cohorts had hide contamination and within two weeks, 50% of the cohorts were shedding the organism as well. Introduction of a super-shedder also causes rapid contamination of the environment.

A study by Collis et al. (2004) investigated transmission of *E. coli* through the market place and packing plant by applying harmless bacterial markers on cattle. Initial prevalence of the marker was 9.1% and increased to 39.4% in the presale pen. With the

same initial values, it increased to 15.1% in the sale ring and to 54.5% in the post sale pen. There was also widespread contamination of the market environment.

The marker was applied at 11.1% prevalence before the animal entered the packing plant and increased to 100% before having the hide removed during processing; after the hide was removed, there was still an 88.8% prevalence on the carcass. Additionally a marker placed on environmental surfaces was detected on 83.3% of hides and 88.8% of carcasses. These data indicate that market auctions and packing plant facilities are also sources of microbial contamination.

Lahti et al. (2003) reported that the finishing unit, and not the introduction of new cattle, seemed to be the source of *E. coli* O157:H7 infection at the farm level. This suggests that the environmental contamination of *E. coli* can play a role in transmitting this organism. *E. coli* has been cultured from mouth swabs from cattle (Buckho et al., 2000). Contamination of animal hides combined with animal grooming can provide a source of *E. coli* O157:H7 infection.

E. coli has been found to persist on barn walls, in feces, feed bunks, water troughs, flies, pigeons, and incoming water supplies (Shere et al., 1998; Buckho et al., 2000; Van Donkersgoed et al., 2001; Lahti et al, 2003). In a study by Sargeant et al. (2004), factors that were associated with *E. coli* prevalence included water tanks, use of mass injectable medication, the use of antibiotics in the water, wetness and pen density, wind velocity, cats, and the height of the feed bunk. Kudva et al. (1998) showed that *E. coli* O157:H7 could persist in feces for 47d to 21 months. The environment has been

shown to be an important source of transmission among cattle (Van Donkersgoed et al., 2001; Shere et al., 2002).

In a study to determine prevalence from the feedlot to the packing plant, it was found that 7.3% of samples from trailers were positive for *E. coli* O157:H7 (Barham et al., 2002)

Van Donkersgoed et al. (2001) found the highest prevalence of *E. coli* O157:H7 in feedlots was in water troughs with water temperature and precipitation affecting its prevalence. Water contamination at low levels of 10^3 CFU was found effective in infecting cattle (Shere et al., 2002). LeJeune et al. (2001) found that *E. coli* O157:H7 survived 245 d in water trough sediments. This *E. coli* was cultured and still able to infect 10 week old calves. *E. coli* also has been shown to persist and survive, at largely reduced numbers, in chlorinated water (Figure 3; LeJeune et al., 2001, 2004). *E. coli* contaminated water has also been shown to disseminate through a cohort of cattle and cause infection (Shere et al., 1998).

Fecal contamination of feeds occurred both in commerce and on farms and could play an important role in transmitting the organism from the feces into the mouth of cattle (Table 1; Lynn et al., 1998). *E. coli* has been reported to be in feed samples at a rate as high as 14.9% (Buckho et al., 2000; Dodd et al., 2003). Other studies have not found *E. coli* O157:H7 in total mixed rations (TMR) and possibly attribute it to pH, organic acids, and feed additives (Van Donkersgoed et al., 2001).

Figure 3. Change in $\log_{10}$ concentrations of *E. coli* O157:H7 in sediments of microcosms simulating cattle water troughs. Chlorine concentration: prior to day 90, 0.15 ppm, 5 to 7 ppm. Bars represent standard errors (LeJeune et al., 2001)

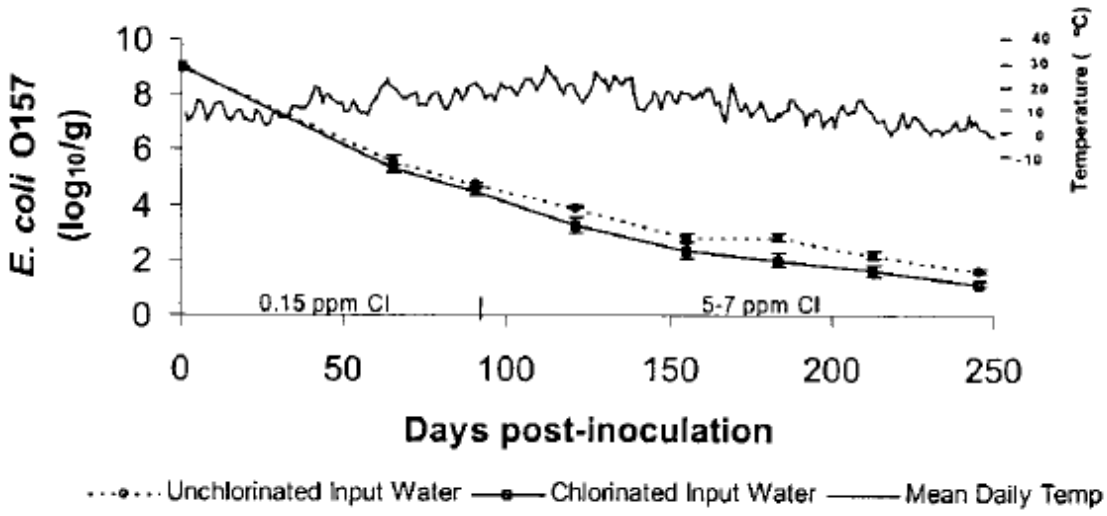


Table 1. Prevalence of *Escherichia coli* in cattle feeds (Lynn et al., 1998)

Feed category	n	Positive culture	
		(no.)	(%)
Fat	3	0	0
Mineral mixes	27	2	7.4
Wet forages	18	2	11.1
Wet by-products	9	4	11.1
Dry by-products	25	8	32.0
Oilseeds	24	8	33.3
Dry grains	37	11	29.7
Dry forages	16	8	50.0
Mixes; unknown	42	18	42.9
Miscellaneous protein	8	5	62.5
Total	209	63	30.1

LeJeune et al. (2004) reported that during the feeding period there appeared to be multiple sources of *E. coli* sporadically entering the population. These and previous data

exemplify the large differences in prevalence and shedding patterns in ruminants as well as indicate the necessity of a pre-harvest multiple hurdle intervention system that decrease the *E. coli* prevalence on animals. This will allow for post-harvest interventions to be most effective as well.

E. coli O157:H7 shedding

Two weeks after cattle are infected, the level of *E. coli* detected in the feces decreased dramatically and was detected intermittently thereafter (Brown et al., 1997 [Figure 4]; Harmon et al., 1999; Buckho et al., 2000; Cornick et al., 2000 [Figure 5]). Sanderson et al. (1999) showed that calves shed *E. coli* in their feces for one month on average. In a study where 56 head of cattle were naturally infected with *E. coli*, 63% of cattle shed for less than a month (Besser et al., 1997).

Pre-treating neonatal calves with probiotic *E. coli* significantly decreased the magnitude of shedding (Zhao et al., 2003). Interestingly, chlorinated water did not cause any differences in *E. coli* prevalence than non-chlorinated pens (LeJeune et al., 2004).

Neomycin sulfate has been shown to decrease fecal shedding of *E. coli* O157:H7 in actively shedding cattle (Elder et al., 2002; Ransom and Belk, 2003). Callaway et al. (2002) also reported that sodium chlorate reduced *E. coli* populations in the rumen by two logs and in the feces by three logs. This was likely because bacteria that aerobically respire on nitrate were exposed to chlorate, and they die due to the intracellular enzyme, nitrate reductase, which converts nitrate to nitrite and reduces chlorate to cytotoxic chlorite.

Figure 4. Time course of fecal shedding of *E. coli* O157:H7 in calves in trials I (A) and II (B). Calves were inoculated with a five strain mixture of *E. coli* O157:H7 on day 0. Fecal samples were collected daily for enumeration of *E. coli* O157:H7 bacteria. Each different symbol represents the CFU (log10) per gram of feces for an individual calf (Brown et al., 1997)

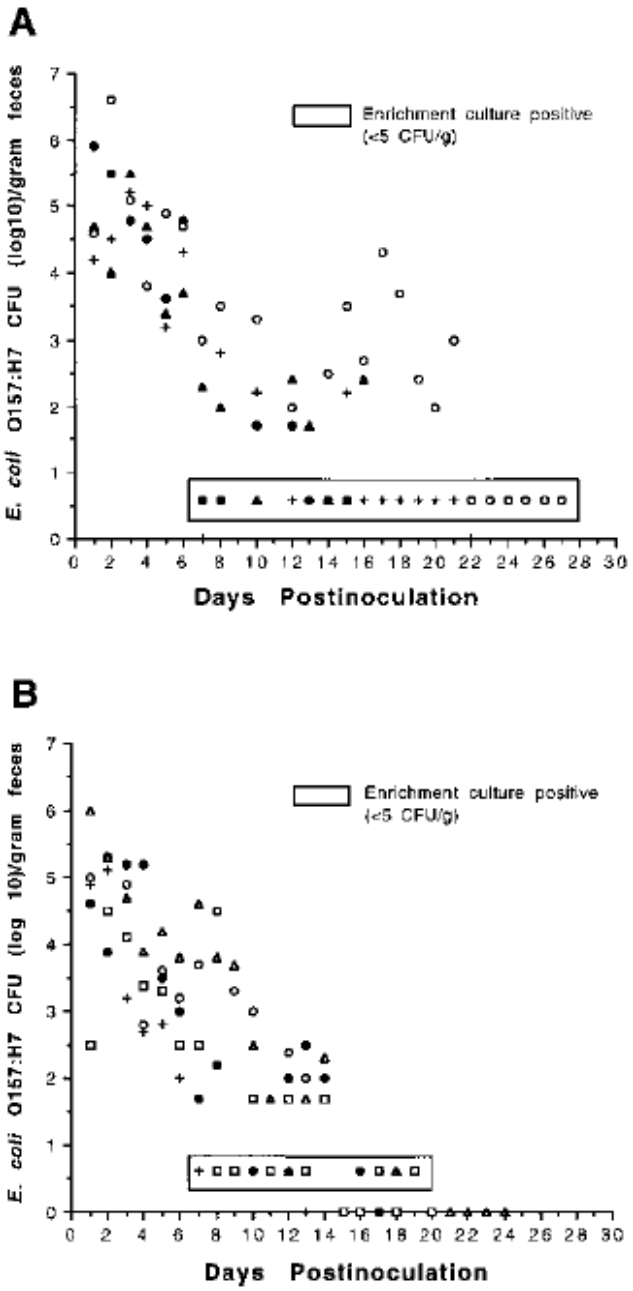
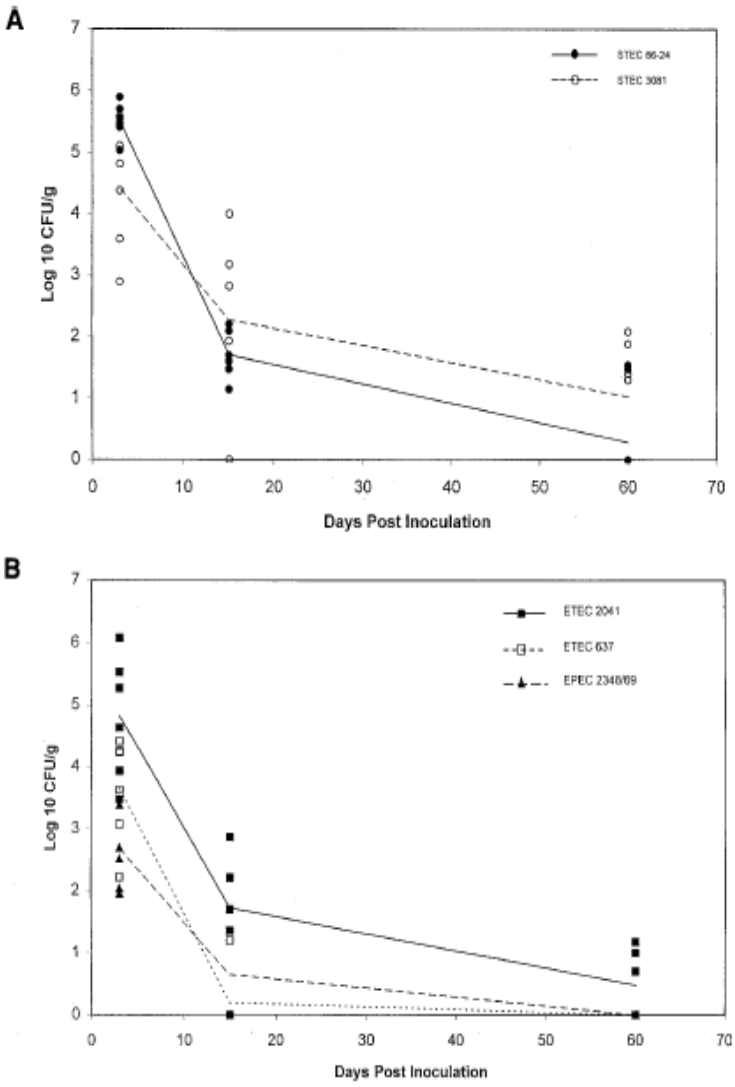


Figure 5. Fecal shedding of *E. coli* strains from six sheep inoculated with a cocktail containing five strains given at a dose of 1010 CFU/strain/animal (treatment 1).Lines represent the means for each strain. (A) Strains STEC86-24; (B) strains ETEC 2041, ETEC 637, and EPEC E2348-69 (Cornick et al., 2000)



Schamberger and Diez Gonzalez (2004) determined that the use of competitive and beneficial bacteria to inhibit or exclude *E. coli* in cattle is very promising in the

control of this organism. The use of coliciogenic *E. coli* to reduce O157:H7 in cattle is a viable method to decrease *E. coli* O157:H7, as well. Colicins are antimicrobial proteins produced by some strains of *E. coli* to inhibit other strains.

E. coli prevalence was greater in barley fed cattle as opposed to corn fed cattle (Buckho et al., 2000; Berg et al., 2004). Jordan and McEwen (1998) found that a high roughage diet fed to cattle for 4 d prior to slaughter reduced *E. coli* concentrations in their feces for the first 24 h but was reversed in the next 24 h. Tkalcic et al. (2000) did not see any difference in prevalence or duration of *E. coli* shedding between cattle fed a high roughage or a high concentrate diet. Dairy herds that were fed corn silage shed at a higher prevalence than those that were not fed silage (Herriott et al., 1998).

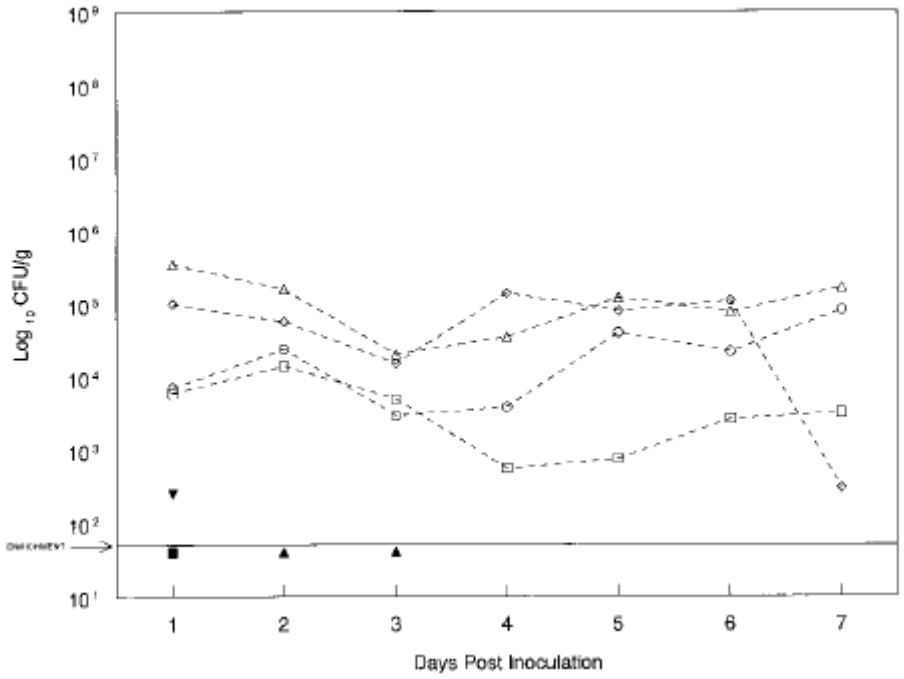
Hovde et al. (1999) found that hay fed animals shed *E. coli* longer than grain fed animals but did not find any difference in acid resistance between treatments. Kudva et al. (1995) found that all sheep shed uniformly for 15 d after turn out on range, even though some were not shedding when they were turned out.

Pre-harvest diets could have potential for reducing the risk of *E. coli* contaminated animals from entering the food chain (Kudva et al., 1995, Berg et al., 2004). Cattle with slower rates of intestinal cell proliferation in the cecum and distal colon were culture-positive longer than cohort cattle with faster cell proliferation (Magnuson et al., 2000).

Fasting caused an increase in *E. coli* fecal shedding of calves shedding at low doses but had little effect on calves already shedding at higher rates but decreased after the resumption of feeding (Brown et al., 1997; Jordan and McEwen, 1998; Magnuson et

al., 2000). Cray et al.(1998) did not observe any differences in rate of fecal shedding of calves that were dietarily stressed and inoculated with 10^{10} CFU. However, calves that were stressed prior to inoculation with 10^{7} CFU were more susceptible to infection and shed significantly more than non-stressed calves (Figure 6). These data indicate that stress could possibly increase shedding of *E. coli* or increase colonization and proliferation of this organism when exposed at lower levels, possibly due to a compromised immune status.

Figure 6. Fecal shedding (d 1 to d 7) of *E. coli* O157:H7 by calves that were well fed (solid symbols) or fasted (open symbols) for 48 h prior to inoculation with 10^{7} CFU of *E. coli* O157:H7 on d 0. *E. coli* O157:H7 organisms were recovered from well-fed calves on d 1 (two of four calves), d 2 (one of four), and d 3 (one of four) (Cray et al., 1998)



Vaccinations to reduce *E. coli* are not yet available to the public, but there are two commercial vaccines that have been used in a series of studies in an attempt to decrease *E. coli* prevalence in the pre-harvest cattle. These vaccines target the attachment mechanism of *E. coli*, specifically the type III secretion system and intimin attachment.

The first vaccine that has under gone extensive trials is likely nearing FDA approval. Potter et al (2004) found in a series of trials that this vaccine increased specific antibody titers to type III secreted proteins, and the vaccinated animals shed *E. coli* in their feces at a significantly lower rates and for shorter duration. In another experiment, this vaccine was used 0-3 times through the feedlot stage; it was found that cattle vaccinated 1-3 times were less likely to shed the organism and the efficacy rate was 59% (Peterson et al., 2005).

The second vaccine has been evaluated in one study by Ransom and Belk (2003) in which feedlot cattle were vaccinated twice in the finishing stage, and there was a 67.9% reduction in fecal shedding of *E. coli*. Another study conducted at Montana State University used passive immunity from cow to calf (Standley et al., 2005). Cows were vaccinated starting 30 d before parturition with a second vaccination 14 d later. Blood serum was collected on both the cow and the calves to determine antibody titers to *E. coli* O157:H7. Titer levels in calves from vaccinated cows had ten times the antibody titers than calves from unvaccinated cows. These data were similar to results reported by Dean-Nystrom (2002), who found that neonatal piglets which suckled from vaccinated dams were protected from EHEC colonization and infection compared to piglets suckling non-vaccinated dams.

The lack of pre-weaning vaccinations and preconditioning, coupled with long haul transport, increased fecal shedding of *E. coli* O157:H7 (Bach et al., 2004). Decreased or poor immune status could contribute to the increase in infection and fecal shedding of the non-preconditioned calves. Herriott et al. (1998) also found that the abrupt weaning of calves caused an increase in fecal shedding of *E. coli*. Prevalence was also found to be the greatest two weeks after calves reached the feedlot (LeJeune et al., 2004). Van Donkersgoed et al. (1999) did not confirm that distance traveled to the plant had any effect on fecal shedding of *E. coli*. However, these cattle were older, and it might not have been noticeable due to the large number of variables evaluated in this study.

In a group of inoculated calves, two calves never shed *E. coli* after four attempted inoculations (Shere et al., 2002). The question raised is what was the immune status of these calves? Further, Wales et al. (2002) saw a large difference in colonization of 6 d old lambs, and one factor that was not considered or evaluated was immune status.

Ransom and Belk (2003) found that vaccination for *E. coli* O157:H7 was effective in two out of three pens of feedlot cattle. The pen in which it was not effective had an unknown nutritional and mineral history. In other studies that showed a marked decrease in *E. coli* O157:H7 prevalence, all had calves that were fed a weaning supplement with increased levels of trace minerals and vitamins (Choat et al., 2005; Peterson et al., 2005; Standley et al., 2005). Cell mediated immunity may be important in controlling STEC as evidenced by rapid and pronounced infiltration of neutrophils into the lamina propria and gut lumen (Stevens et al., 2002).

Immune System

The importance of minerals continues to be an important topic among nutritionists world wide. It has been shown that zinc and copper have a positive impact on immune responsiveness and disease resistance. Mineral and vitamin supplementations play a critical role in optimizing the immune status of beef cattle. Clark et al. (1995) reported that humoral immunity results indicated a positive influence of mineral supplementation. Ansotegui et al. (1994) saw that mineral supplementation for first calf heifers increased neutrophil counts and cellular immune response compared to non-supplemented heifers.

Swenson (1998) showed humoral response was influenced by maternal trace mineral supplementation in newborn calves. Results showed elevated serum *E. coli* (K99) antibody titers, following vaccination with Scour Guard®, in cows fed a complexed mineral 60 d prior to calving compared to cows fed a sulfate-based mineral fed at either 60 d or 30 d prior to calving. Additionally, cows fed a complexed mineral 30 d prior to calving and controls (cows that did not receive trace minerals), had intermediate levels of antibody titers. This indicates that increasing antibody titers enhanced the animal's ability to respond to a pathogenic challenge. Futhermore, the incidence of scours requiring treatment was lower for calves whose mothers had been supplemented with trace minerals compared to controls. Treatment for scours was required for 17 and 16% of the calves in the complexed and sulfate mineral groups, respectively, while 26% of the control calves were treated ($P > 0.10$). Health of calves, born to heifers receiving trace mineral supplementation, was improved as indicated by a 35% decrease in scours.

Greene et al. (1998) reported that stressful conditions at any time during the lifecycle indicate the need for proper mineral supplementation so cattle's immune system has the ability to produce an adequate response. Furthermore, Greene et al. (1998) stated, "In order to respond immunologically, whether it be to a foreign antigen that has been given, as in a vaccine, or one from the production environment, an animal needs to have an immune system that is responsive and capable of meeting any challenge."

E. coli Summary

The previous studies and data indicate a variety of theories and assumptions of *E. coli* O157:H7 shedding and prevalence. It is known that cattle are a main reservoir of *E. coli* and they shed the organism as well as transmit it to other cohorts. It continues to be an issue in food safety to reduce the prevalence of *E. coli* in food sources, public water, and recreation areas. Due to the sporadic and intermittent shedding of the organism by cattle, there needs to be a "common sense" approach to reducing the prevalence of this pathogen. Research to control *E. coli* O157:H7 in the preharvest stage include but are not limited to diet effects, fasting, use of probiotics, vaccination, bacteriophage therapy, and sodium chlorate supplementation. The approach to control these bacteria is most likely a multiple hurdle intervention system at both the pre and post-harvest sections of beef production that will decrease prevalence and prevent carcass contamination with *E. coli* O157:H7.

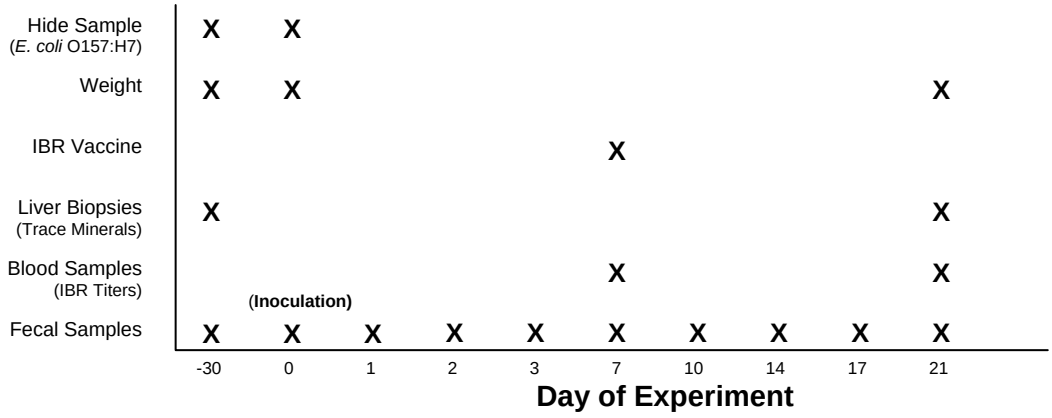
MATERIALS AND METHODS

Experiment 1 - The Effect of Trace Mineral Supplementation on Fecal Shedding of Heifer Calves Experimentally Inoculated with *E. coli* O157:H7

Experimental Design

Twenty-four Angus crossbred heifers (avg. wt. 207 kg) were weaned at approximately 165 d of age. Calves originated from the Montana State University beef herd and had limited access to mineral supplementation prior to weaning. The protocol for the experiment is described in Figure 7. At weaning, heifers were weighed and had fecal, hide, and liver samples collected to determine baseline levels of *E. coli* O157:H7 shedding and liver trace mineral status.

Figure 7. Timeline for experiment in which heifers were supplemented with trace minerals and dosed with *E. coli* O157:H7



Calves were allotted to either a control treatment (no additional trace minerals) or to a supplemented treatment (diet fortified with organic trace minerals) based on initial liver Cu concentrations. Diets were composed of cracked corn grain and a MSU

developed weaning pellet that was either fortified or non-fortified with trace minerals and manufactured by CHS, Inc. in Great Falls, MT. The supplemented diet provided an additional 176mg/d of Cu, 587 mg/d of Zn and 388 mg/d of Mn (Table 2). The control diet did not have additional trace minerals added. Diet samples were analyzed, according to AOAC (2000) procedures for protein and energy and by induction coupled argon plasma methods (Fassell, 1978) for trace minerals.

Heifers were weighed and placed in six pens of four heifers, with three pens per treatment. Heifers were fed to gain 0.68 kg/d (2.27kg corn, 3.18kg weaning pellet) and were fed at 0800 daily using individual feeding gates (American Calan). They were placed on their respective diets 30 d prior to dosing with the *E. coli* O157:H7 cocktail.

Table 2. Ingredient, nutrient composition and calculated trace mineral intakes of diets fed to heifers

Item	Supplemented	Control
Ingredient, % DM basis		
Wheat Middlings	23.21	23.21
Corn Grain	46.12	46.12
Dried Distillers Grain	8.91	8.91
Corn Cob	8.81	8.81
Soybean Hulls	3.71	3.71
Canola Meal (34%)	2.40	2.85
Nutrient analysis		
CP%	15.0	15.0
TDN%	79	79
NE_m, Mcal·kg^{-1}	1.38	1.38
NE_g, Mca·kg^{-1}	0.95	0.95
Trace Mineral Levels		
[a]Cu, mg/day	399	223
[a]Zn, mg/day	1001	414
[a]Mn, mg/day	707	319

[a] NRC requirements = Cu-54.5mg, Mn-109mg, Zn-164mg (NRC, 2000)

Experiment Biosecurity

The barn was hot-pressured washed before calves entered the trial to eliminate any previous pathogens that might be present in the Calan gate facilities. Feed was put in the barn before inoculation to eliminate any contamination of equipment or the barn during the trial period. Separate water troughs were placed in each pen for the heifers' water source, and all tanks were closed off. This was important, as the farm has an overflow watering system in which all water tanks are connected and have the same water source.

After inoculation, the barn was closed to the public, and when entering and exiting the facilities individuals were required to wear plastic boots as well as walk through a disinfectant foot bath containing Virkon S; hand sanitizer (Decon Bacdown, 62% ethyl alcohol) was also used. Coveralls were worn while in the barn and remained in the barn until the next use.

At the conclusion of the trial, all heifers were dosed with Neomycin sulfate via their water source at 6 g/d for 5 d to eliminate any remaining *E. coli* O157:H7 that might be in their intestinal gut. All garbage remained bagged in the barn until the conclusion of the trial, and it was then incinerated. Additionally the barn was hot-pressure washed at the end of the study and was subsequently sprayed down with a Virkon S solution to eliminate any remaining bacteria.

Inoculation

Heifers were inoculated with *E. coli* O157:H7 using the following protocol. Inoculums were prepared by culturing each of five strains of *E. coli* O157:H7 (55AC1, 114AC1, 131AC1, 237AC1, 299AB3) in separate flasks of Luria-Bertani (LB) broth. Inoculum was received from Dr. Terry Arthur at USDA-ARS in Clay Center, Nebraska. The cultures were grown for 18 h at 37º C with agitation until culture densities reached 10^9 CFU of *E. coli* O157:H7/ml. Viable cell counts were estimated by spread plate culture of six serial dilutions on LB agar (Brown et al., 1997; Kudva et al., 1997). The five strains were subsequently mixed, and cells were obtained by centrifugation and resuspended at 10^9 CFU/ml in sterile saline (PBS). Then 10^{10} CFU of inoculum was pipetted onto 0.45 kg of ground corn grain and added to each heifer's diet at 1200 h.

Sample Collection

Hide samples were collected before the heifers ever entered the Calan gate facilities to determine if they had any contact with *E. coli* O157:H7 prior to the trial. Fecal samples were collected at time of dosing and again at 18 h, 32 h, and 50 h post-inoculation. Fecal samples were collected every three to four d thereafter until d 21 to measure presence and concentration of fecal shedding of *E. coli* O157:H7. Heifers were weighed off trial on d 21. The fecal samples were collected via rectal palpation using a new OB glove for each animal and then placing approximately 50 g of feces into a new screw top cup (Fisher Scientific). Cups were labeled with each heifer's individual ID. Fecal samples were shipped in insulated containers containing ice packs for next day

delivery to a commercial laboratory (Food Safety Net, San Antonio, TX) for analysis of prevalence and most probable number of *E. coli* O157:H7.

On d 7 of the experiment, 10mg of liver tissue and 10ml of venous blood samples were collected. Liver biopsy samples were collected in aseptic conditions using a standard procedure (Waterman et al., 1999) and serum samples were obtained via venipuncture.

After sampling, heifers were injected with Pyramid MLV 3 (Fort Dodge Animal Health) for infectious bovine rhinotracheitis (IBR) to determine humoral immune response (Ansotegui et al., 1994) due to trace mineral supplementation. Subsequent blood samples were collected via caudal puncture for IBR titers on d 21 to determine if trace mineral supplementation affected primary and secondary IBR antibody titers

Sample Analyses

Fecal and hide samples were evaluated for prevalence of *E. coli* O157 following enrichment in Trypticase soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) with the use of immunomagnetic separation and selective plating on ctSMAC and ntRainbow agars using the previously described (Barkocy-Gallagher et al., 2002) MARC MRU method. Morphologically typical colonies were tested using the Dryspot *E. coli* O157 latex test (Oxoid, Inc., Ogdensburg, NY). Samples positive for latex agglutination were then subjected to most probable number analysis (MPN) using the previously described method (Barkocy-Gallagher et al., 2003). This method involved a 3X3 MPN setup with 3 rows of 10^{-1}, 10^{-2}, and 10^{-3} dilutions and the inoculation of ImmunoSTAT1 O157:H7 cards to determine final results.

Blood samples were placed on ice and sent to the Montana State University Nutrition Lab where they were centrifuged (2,000 rpm for 20 min) and the serum separated for analysis. The analysis of serum for IBR antibody titers was conducted at Colorado State University Diagnostic Laboratory, Fort Collins, CO. Liver biopsy samples were frozen and shipped on dry ice to Michigan State University Diagnostic Lab for Cu, Zn, Mn, and Co analysis by inductively coupled plasma atomic emission spectroscopy techniques (Braselton et al., 1997).

Statistical Analyses

Data were analyzed using the GLM procedures for SAS (SAS Inst. Inc., 2003. Cary, NC). Changes in *E. coli* O157:H7 CFU/g of feces from d 0 to d 21 were analyzed by repeated-measures in time. Differences were determined using the LSD procedures of SAS (SAS Inst., 2003). Changes in liver Cu concentration and IBR titers were analyzed by a simple ANOVA. Differences were considered significant at the $P < 0.05$.

Experiment 2 – Effect of Vaccination on Fecal Shedding of *E. coli* O157:H7 in Steer Calves during Backgrounding

Experimental Design

Three hundred eighty-two Angus and Angus X Simmental steer calves were used in this experiment to evaluate the effect that backgrounding and an *E. coli* O157:H7 vaccination at weaning had on fecal shedding of *E. coli* O157:H7. Steers primarily born in March were weaned at approximately 204 d of age at an average weight of 242 kg.

Calves originated from one herd (Bair Ranch) in central Montana and were weaned over a 2 wk period with approximately one-third weaned every 5 d. On the day of weaning, each group of steers was removed and hauled from mountain pasture to a back-grounding yard where there were six 150 head capacity pens. Upon arrival, calves were individually processed with all calves receiving an individual electronic identification tag with a corresponding panel tag. Individual weights, fecal samples, and hide swab samples were collected from each steer calf. They were also given an individual dose of 1) Nasalgen IP, Schering Plough; 2) viral respiratory vaccine (Pyramid 4 + Presponse), Fort Dodge Animal Health, administered subcutaneously in the left side of the neck; and 3) antilmenthic (Coopermec), Schering Plough.

Steers were applied to a treatment using a systematic randomization scheme so every third animal through the chute received an experimental *E. coli* O157:H7 vaccination. This vaccine developed by Bioniche was designed to prevent attachment of *E. coli* O157:H7 bacterium to the intestinal wall of cattle. It was administered subcutaneously on the left side of the neck at 2 ml/ hd. As each steer exited the chute, he

was separated into a control group (126hd), a group that would be vaccinated upon arrival to the feedlot (123 hd), and a group that was vaccinated at weaning (125 hd). This left two groups for my study: 249 controls (no vaccine) and 125 treated (*E. coli* O157:H7 vaccine). Each group was fed and transported separately from each other for the rest of the study and during transportation. Treatments remained separate for the rest of the experiment because Peterson et al. (2005) indicated that when a majority of cattle are vaccinated within a pen, vaccinated pen mates confer protection to the non-vaccinated cattle in the same pen.

Calves were backgrounded for a period of 56 ± 8 d. Calves were fed a basal diet of native grass hay and supplemented for four weeks with an MSU-weaning pellet (Table 3). The main ingredients in the pellet were wheat middlings, canola meal, corn, and molasses. However, more importantly, this pellet was designed to provide additional minerals and vitamins to enhance their immune system status and decrease shipping sickness. The ingredients and mineral package are similar to the fortified diet that was used in Experiment 1.

At 56 d, all steers were shipped from central Montana to western Nebraska where they were placed in a feedlot managed by the University of Nebraska. Steers were trucked in their respective separate groups to keep from mixing different treatment groups. Upon arrival, steers were processed and a final weight was collected. Individual fecal samples were again taken to determine the effect that the shipping stress might have on the two treatments.

Response variables measured during the trial were initial weight d 0, weight when received in feedlot d 56, average daily weight gain (d 0 to d 56), individual hide samples (d 0), and individual fecal prevalence of *E. coli* O157:H7 (d 0 and d 56).

Table 3. Nutrient specifications of the MSU weaning pellet

Nutrient	**Recommended amount supplied per day**
Crude Protein	at least 90 grams (g)
Calcium	at least 14 grams (g)
Phosphorus	14 grams (g)
Salt	20 grams (g)
Potassium	18 grams (g)
Vitamin A	40,000 IU
Vitamin D	4,000 IU
Vitamin E	400 IU
Copper (50% inorganic:50% organic)	150 mg
Zinc (50% inorganic:50% organic)	450 mg
Manganese (50% inorganic:50% organic)	400 mg
Selenium	3 mg
Decoquinate (Deccox)	125 mg

Sample Collection

The fecal samples were collected via rectal palpation using a new OB glove on each animal and approximately 50 g of feces were placed into a screw top cup (Fisher Scientific). Cups were labeled with barcodes and were entered in a database with the steer's identification number. Hide swab samples were collected using sponges that were pre-hydrated in 10 ml of buffered peptone water. These sterile sponges were taken out of the bag and were used to swab down the midline of each steer's back with a sterile glove.

After swabbing the hide, each sample was placed back in the bag and re-hydrated with another 10 ml of buffered peptone water. Fecal and hide swab samples were then shipped in insulated containers containing ice packs for next day delivery to a commercial laboratory (Food Safety Net, San Antonio, TX) for analysis of prevalence and most probable number of *E. coli* O157:H7.

Sample Analyses

Fecal samples and hide swab samples were evaluated for prevalence of *E. coli* O157 following enrichment in Trypticase soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) with the use of immunomagnetic separation and selective plating on ctSMAC and ntRainbow agars using the previously described (Barkocy-Gallagher et al., 2002) MARC MRU method.

Morphologically typical colonies were tested using the Dryspot *E. coli* O157 latex test (Oxoid, Inc., Ogdensburg, NY). Samples positive for latex agglutination were then subjected to most probable number analysis (MPN) using the previously described (Barkocy-Gallagher et al., 2003). This method involved a 3X3 MPN setup with 3 rows of 10^{-1}, 10^{-2}, and 10^{-3} dilutions and the inoculation of ImmunoSTAT1 O157:H7 cards to determine final results.

Statistical Analyses

Data were analyzed using the GLM procedures for SAS (SAS Inst. Inc., 2003. Cary, NC). Changes in *E. coli* O157:H7 CFU/g of feces and average daily gain from d 0 to d 56 were analyzed by a simple ANOVA. Differences were determined using the LSD procedures of SAS (SAS Inst., 2003). Differences were considered significant at the $P < 0.05$.

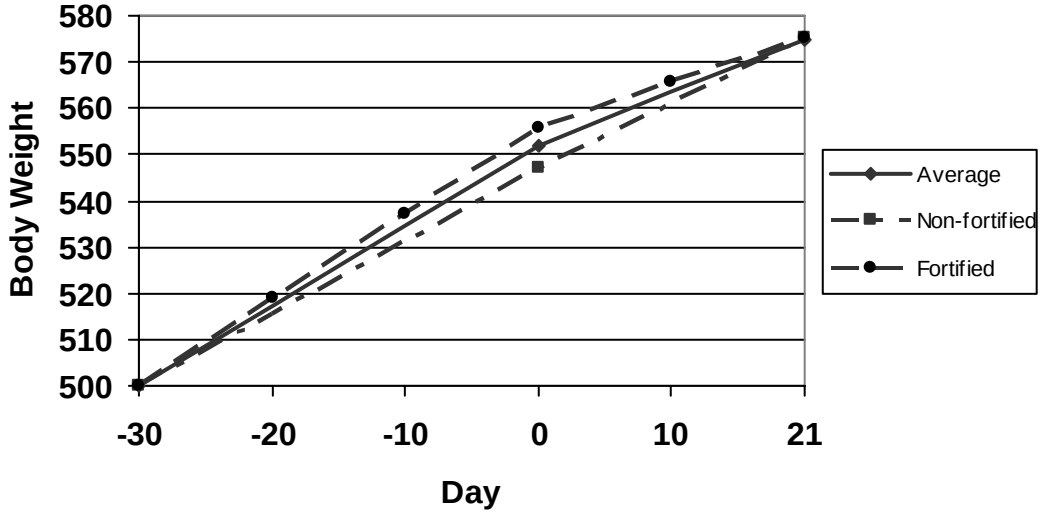
RESULTS AND DISCUSSION

Experiment 1 - The Effect of Trace Mineral Supplementation on Fecal Shedding of Heifer Calves Experimentally Inoculated with *E. coli* O157:H7

Average Daily Gain

There were no differences (P>0.05) in initial body weights measured at the initiation of the experiment. Heifers gained 0.68 kg/d for 51d while on experiment (Figure 8). There was no morbidity measured through the trial. Heifers receiving the fortified ration gained 0.85 kg/d during the first 30 d with heifers receiving the ration which was non-fortified (control) gaining 0.70 kg/d. There were no differences in ADG (P> 0.10) measured between treatments.

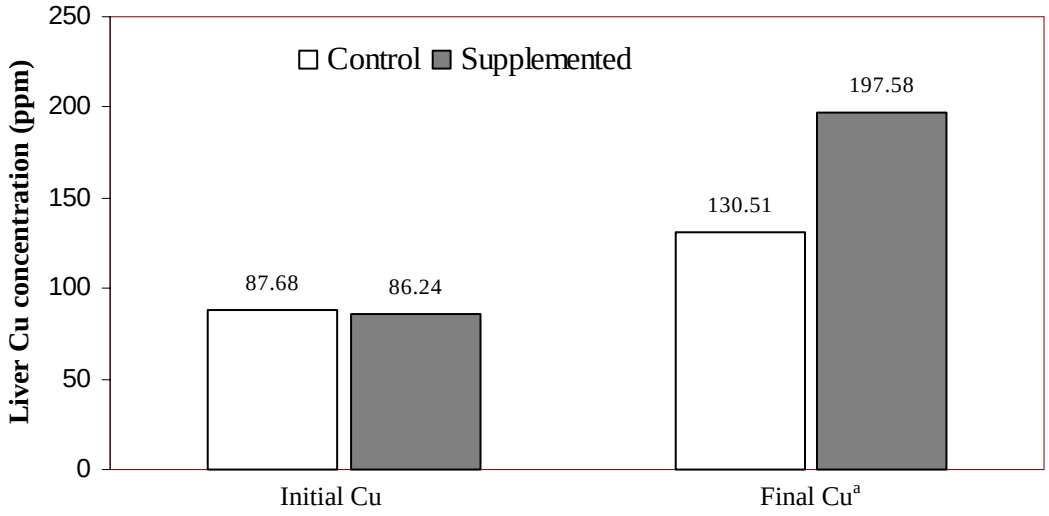
Figure 8. Overall average body weight and treatment average body weight of heifers during 51 d trial period



Copper, Zinc, and Manganese Levels in Liver

There were no differences (P > 0.05) in initial liver Cu concentrations between treatments. Supplementation with Cu significantly increased (P< 0.05) final liver Cu concentration (198 vs. 131 ppm for supplemented and control treatments, respectively (Figure 9). However, Michigan State University results indicate that over 90 ppm Cu in the liver is considered adequate.

Figure 9. Initial and final liver copper concentrations for heifers supplemented with 176 mg/d copper for 50d



[a]Means differ (P<0.005) for final Cu

Unexpectedly, the control heifers consumed 223 mg/d of Cu provided in the non-supplemented diet which was approximately four times their requirement. The source of this mineral contamination was not determined but was likely provided by one of the feedstuffs used to design the pelleted ration. Even though there were high levels of copper detected in the diet, these were probably unavailable to the animal as they were

likely tied up in carbohydrates or could have been less available forms such as copper oxide, which Kegley and Spears (1994) showed had lower bioavailability than either copper lysine or copper sulfate. Suttle (1991) found that molybdenum and sulfur can form insoluble complexes known as thiomolybdates in the rumen that can bind Cu and make it unavailable for absorption.

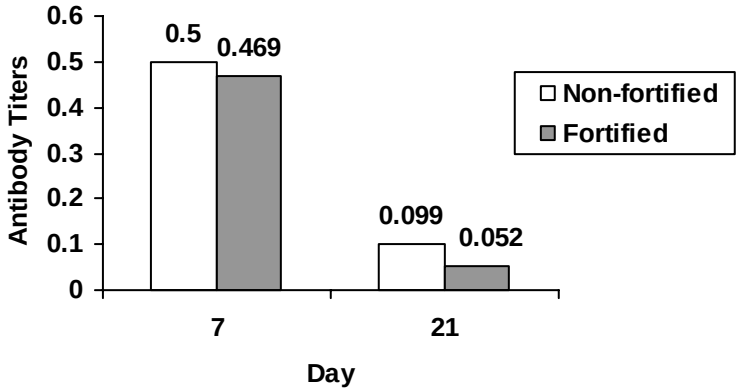
There were no differences in initial levels of zinc concentration in the liver between treatments with fortified and non-fortified means at 129 ppm and 119 ppm respectively. Additionally, there were no differences seen in initial manganese levels in the liver at 7.2 ppm and 7.1 ppm for the fortified and non-fortified treatments respectively. Manganese levels in the liver increased to 8.5 ppm for the fortified diet and 8.5 ppm for the non-fortified diet with no differences between treatments. Interestingly zinc levels decreased in both treatments to 109 ppm and 110 ppm for the fortified and non-fortified diets respectively. This is probably due to the increase that we saw in copper concentrations in the liver, and Greene et al. (1998) reported that these two minerals compete for binding sites on enzymes and metallothioneins.

IBR Antibody Titers

At d 7, there were no differences in IBR antibody titers between treatments with non-fortified heifers at 0.50 and fortified heifers at 0.47. After receiving a modified live IBR vaccine, there were still no differences on d 21 in IBR antibody titers at 0.099 and 0.052 for the non-fortified and the fortified treatments respectively. Supplementation with trace minerals did not increase ($P > 0.50$) IBR antibody titers compared to the control treatment (Figure 10). Lack of a response may be due to the high level of trace

minerals in the control diet which may have prevented nutritional stress/deficiency on the control heifers.

Figure 10. Antibody titers for heifers that were fortified with trace minerals and heifers that were not fortified for d 7 before they received a vaccination, and on d 21



No difference in means at either sampling period; P >0.05.

E. coli O157:H7 Fecal Shedding

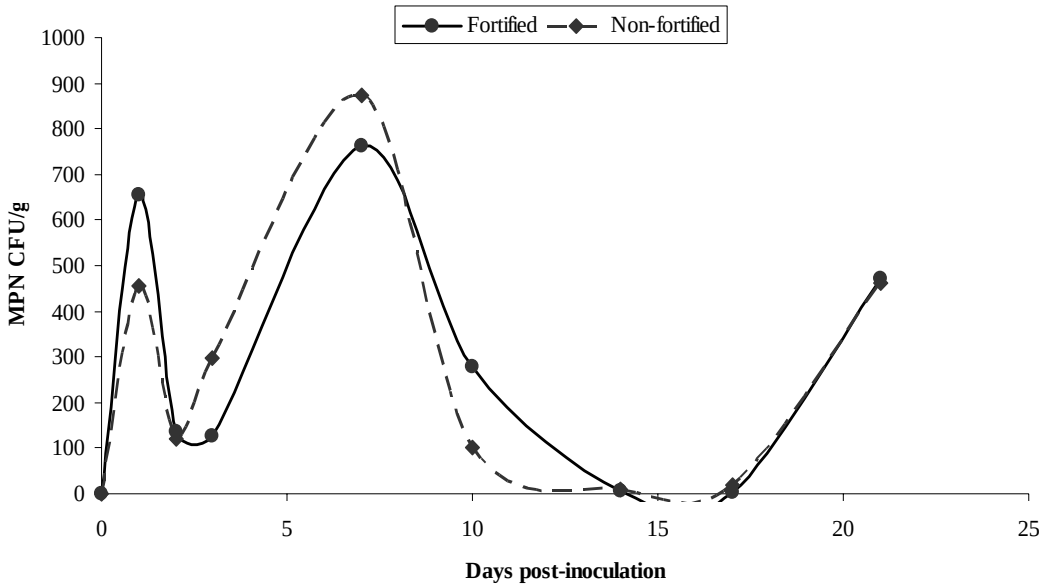
All heifers were tested for *E. coli* O157:H7 shedding and were negative prior to the start of the experiment (d -30 and d 0). Similarly, *E. coli* O157:H7 prevalence was not detected on any of the hide swab samples at d 0.

All heifers were shedding *E. coli* O157:H7 within 18 h of dosing and there was no morbidity observed in any of the heifers post-inoculation (Figure 11). This agrees with other research since cattle are asymptomatic to *E. coli* O157:H7 infection (Cray and Moon 1995; Zhao et al., 2003).

Trace mineral supplementation did not (P=0.71) cause changes in the rate of *E. coli* O157:H7 shedding (Figure 10). The SEM were often larger than the mean values. Additionally this also could be due to treatments having no difference in IBR titers,

therefore no difference in immune system response to dosage with *E. coli* O157:H7 was seen. Since no differences in shedding were measured, values were pooled and are presented in Figure 12 to show the pattern of shedding.

Figure 11. Average *E. coli* O157:H7 fecal excretion patterns between heifers fortified with trace minerals and heifers that were not fortified

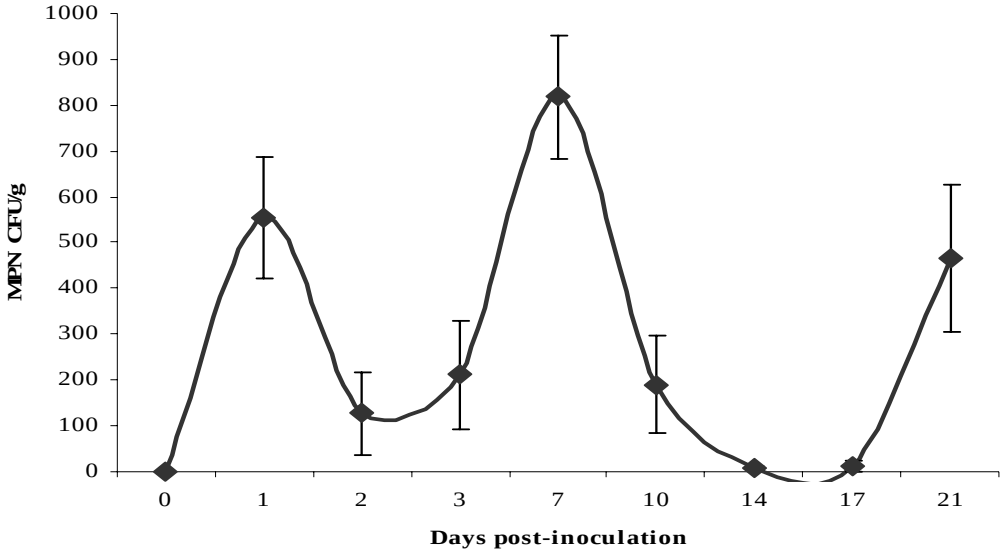


No difference (P > 0.10) between treatments

Following inoculation, there was a peak in *E .coli* O157:H7 concentration by 18 h. This agrees with research of McGee et al. (2004) and Cray and Moon (1995) who also showed a peak concentration of *E. coli* O157:H7 in the calves' feces within 24 h. This would likely be transient shedding of the organism. Although fecal shedding declined by d 2 and 3 post inoculation, there was another unexpected peak in shedding at d 7. Following d 7 there was a reduction in fecal *E. coli* O157:H7 concentration for the next 2 wks. This is consistent with research reported by Brown et al. (1997), who showed a decrease in the fecal concentration of *E. coli* O157:H7 during the first two wks after

inoculation. There was another smaller increase in *E. coli* O157:H7 shedding at d 21 when the experiment was terminated. These periods of high *E. coli* O157:H7 fecal excretion may indicate the colonization of *E. coli* in the lower gut and more specifically at the recto-anal junction agreeing with results from Grauke et al. (2002) and Naylor et al. (2004), who showed that *E. coli* O157:H7 was most prevalent in the lower GIT digesta in the colon and more specifically it was colonized at the recto-anal junction.

Figure 12. Change in average fecal excretion pattern for calves dosed with *E. coli* O157:H7 with standard errors for each sampling date



E. coli O157:H7 Shedding Following
Dosing with Neomycin Sulfate

Following the peak seen on d 21, heifers were fed neomycin sulfate to eliminate any *E. coli* O157:H7 that was still present. Twenty-two of twenty-four heifers ceased fecal shedding of *E. coli* 0157:H7 following doses of neomycin sulfate in their drinking water. However, there were still two heifers that were shedding following dosage. One

heifer was shedding at a rate of less than 3.0 MPN, while the other was shedding at a rate greater than 3.0 MPN. This was not unexpected as Ransom and Belk (2003) indicated that neomycin sulfate was effective in reducing the prevalence of the *E. coli* O157:H7 but not necessarily in eliminating it entirely. Additionally, the heifers that were still shedding may not have consumed enough water to get a full dose of neomycin sulfate to eliminate the bacteria from their hindgut.

Experiment 2 – Effect of Vaccination on Fecal Shedding of *E. coli* O157:H7 in Steer Calves during Backgrounding

E. coli O157:H7 Prevalence

At the time of weaning, no steers were shedding *E. coli* O157:H7 or had O157:H7 on their hides (Table 4). This was expected as calves had been on mountain pasture through the summer. This concurs with Hancock et al. (1994) and Dunn et al. (2004) who found herd O157:H7 prevalence was 0.28% and 2.5% respectively. However, following confinement of cattle, *E. coli* prevalence increases from 9.5% to 28% (Elder et al., 2000; Barham et al., 2002). This is likely due to increased density of cattle and more transmission among animals. Additionally, none of the steers had any prevalence of O157:H7 on their hides. This was more surprising, as from experiences in experiment one there was *E. coli* O157:H7 detected on the hides even when they were not shedding at weaning.

Steers were once again checked for fecal prevalence of *E. coli* O157:H7 when they reached the feedlot. There were no steers shedding *E. coli* O157:H7 in their feces at the end of the backgrounding period. This was unexpected, as previous data has shown that a higher percentage of cattle shed the organism after long-haul transport or during periods following stress (Bach et al., 2004). This could indicate that the organism was not in the environment during the backgrounding phase, preventing steers from being exposed to *E. coli* O157:H7 and therefore, not permitting them to become infected. This concurs with previous studies performed at MSU (Choat et al., 2005; Peterson et al.,

2005; and Standley et al., 2005) who all found minimal levels of *E. coli* in herds in Montana.

This does not indicate that the vaccine was not effective in reducing *E. coli* O157:H7, as it was not detected in any fecal or hide samples from the treatment groups. This agrees with data from Peterson et al. (2005) that found low shedding rates from both 438 steers from the same ranch as experiment two and 588 calves from a ranch in eastern Montana. This study also evaluated the experimental *E. coli* O157:H7 vaccine from Bioniche. Peterson found that the overall pretreatment probability of detecting *E. coli* was 0.40%. These treatment groups were followed through to harvest and sampled three more times with the last sample taken at the packing plant. *E. coli* prevalence was 0.0%, 0.40%, and 1.24% for the second, third, and fourth sampling periods, respectively with no differences measured between treatments. This indicates that the overall prevalence was highest at harvest; however, the probability of detecting *E. coli* at any sampling period during the trial was extremely low. Therefore, similarly to experiment two, it was difficult to measure any response due to vaccination.

Potter et al. (2004) demonstrated that vaccination with the Bioniche experimental vaccine decreased the probability of cattle to shed *E. coli* O157:H7 by 59%. Another study by Peterson et al. (2005) demonstrated that when the probability to detect *E. coli* O157:H7 in the feces is high, the vaccine was shown to be up to 70% effective. Additionally, Potter et al. (2004) and Peterson et al. (2005) showed the probability of detecting *E. coli* O157:H7 was highest at harvest.

Dewell et al. (2005) found that out of 12 feedlots and 15 pens of cattle, there were 0% of cattle shedding in Montana feedlots, 20.7% of cattle shedding in Colorado feedlots, and 45.0% of cattle shedding in Nebraska feedlots. This is similar to data from Choat et al. (2005) that measured only 5.5% of 1835 fecal samples, from 367 steers, were positive for *E. coli.* These steers were followed from the herd of origin through the feedlot to harvest to evaluate the efficacy of an experimental *E. coli* O157:H7 vaccine.

These data, combined with data from experiment two, indicate that cattle are less likely to shed *E. coli* O157:H7 in an extensive management system. In addition, as cattle are confined and later sent to harvest (where they are commingled with other slaughter cattle) they are more likely to become infected and shed *E. coli O157:H7* in their feces.

Table 4. Differences in *E. coli* O157:H7 prevalence, WW, BW, and ADG between vaccinated and non-vaccinated treatments

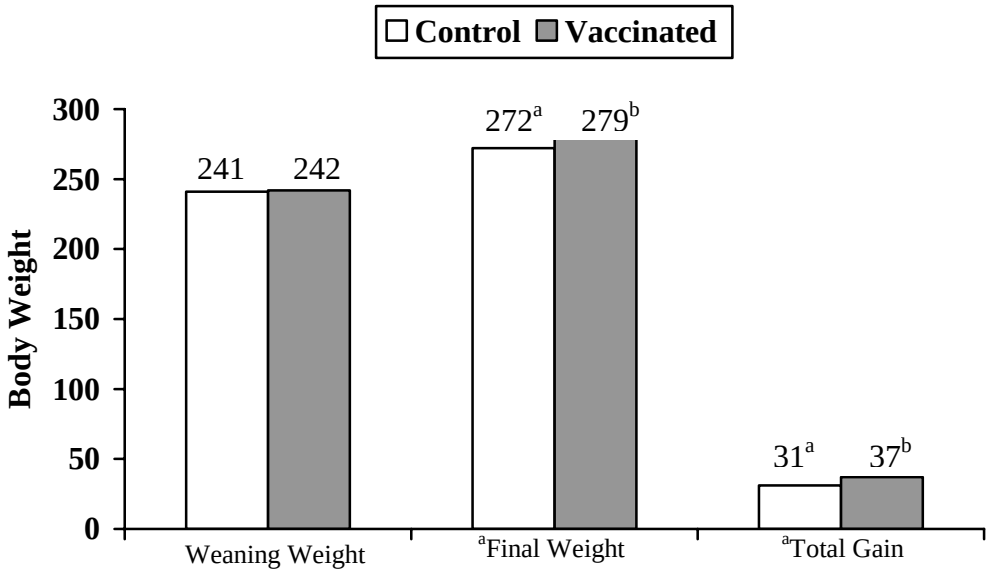
Item:	Vaccinated	Non-vaccinated
No. Animals	125	249
E. coli Hide Prevalence		
d 0	0	0
E. coli Fecal Prevalence		
d 0	0	0
d 56	0	0
WW, kg	242	241
Final BW, kg	279[a]	272[b]
Total gain, kg	37[a]	31[b]
ADG, kg	0.67[c]	0.56[d]

[a,b]Differences significant at $P < 0.05$
[c,d]Differences significant at $P < 0.0001$

Average Daily Gain During
Backgrounding Period

At weaning, there was no difference ($P > 0.05$) in weight between treatments (241 control vs. 242kg). There was a significant difference in starting weights when the steers arrived at the feedlot in Nebraska. Vaccinated steers weighed more than the control steers weighing 279 kg and 272 kg, respectively. This showed a difference of ADG between the two treatments (Figure 13). Average daily gain of control steers was 0.56 kg while vaccinated steers gained 0.67 kg. The ADG between treatments was different at the $P < 0.0001$ level.

Figure 13. Difference in weaning weights, total gain, and final weights between treatments



a,b Differences significant at P < 0.0001

Difference in ADG between treatments was unexpected, as fecal shedding of *E. coli* O157:H7 was not detected in any of the calves. While there was a large amount of variation among the steers at the time of weaning and throughout the backgrounding and

feedlot stages, this was addressed by the randomization scheme separating the observational units into the treatments. This randomization schedule was shown to properly acknowledge this issue by evaluating differences in weaning weights between treatments. There was no difference in weaning weights between treatments at this time (P = 0.74). There is no data that supports or disclaims these differences in ADG, and they can only be explained by speculation.

SUMMARY AND CONCLUSIONS

Experiment 1 - The Effect of Trace Mineral Supplementation on Fecal Shedding of Heifer Calves Experimentally Inoculated with *E. coli* O157:H7

Copper levels in the liver were increased by supplementation with Cu. Zinc levels in the liver were reduced most likely to the competition between copper and zinc for local binding sites. However, in this study trace mineral supplementation did not increase antibody titers for IBR, but controls liver values were more than adequate. Furthermore, there was no difference measured in *E. coli* O157:H7 shedding between heifers receiving additional trace minerals from heifers that were fed diets non-fortified with trace minerals.

The examination of fecal shedding patterns for the two treatments combined showed a clear increase in *E. coli* shedding 18 h following inoculation. This shedding decreased over the next 72 h, after which shedding peaked again, most likely due to *E. coli* O157:H7 colonization in the recto-anal junction. Once again, a decline in the concentration of *E. coli* O157:H7 in the feces was seen until d 21, when another unexpected increase was measured. Further research is needed to determine long term fecal shedding patterns of *E. coli* O157:H7 because a sustained reduction was not measured over the 21 d trial.

Experiment 2 – Effect of Vaccination on Fecal Shedding of *E. coli* O157:H7 in Steer Calves during Backgrounding

There were no differences seen in fecal shedding or prevalence between steers that were vaccinated with an experimental *Escherichia coli* O157:H7 vaccine and steers that were non-vaccinated over the 56 d backgrounding period. This is due to the absence of the organism in the population. This is similar to other research conducted in Montana (Choat et al., 2005; Dewell et al., 2005; Peterson et al., 2005; and Standley et al 2005) and it appears that *E. coli* O157:H7 has less prevalence in Montana. There are probably two main contributing factors to the lower prevalence that was observed in these studies. The first factor is that these cattle have been in a more extensive management system that does not promote the spread or infection of *E. coli* O157:H7. The second factor that could play an important role in reduced prevalence is a colder climate compared to other geographical areas. Just as importantly, strong immune system status cannot be ruled out as an important effect on *E. coli* O157:H7 shedding.

Moreover, there was an unexplained difference ($P < 0.0001$) in the average daily gain of steers between the two treatments with vaccinated steers gaining 0.11 kg/d more than non-vaccinated steers. This measured difference was not expected and could represent some secondary effects of the vaccine on the steers.

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