IMPROVING THE SAFETY OF BEEF – PRE-HARVEST FEEDING AND MANAGEMENT

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I. INTRODUCTION

Beef cattle are one of the most important sources of meat for the American consumer. In recent years, the consumption of ground beef has been linked to numerous outbreaks of food-borne illness caused by enterohemorrhagic *Escherichia coli* O157:H7, a highly virulent bacterium. Because cattle are the natural reservoir of this pathogen, a significant number of illnesses have also been caused by produce and water contaminated with manure. Outbreaks of food-borne illness have seriously diminished the confidence of consumers in their food supply. Frequent recalls of beef after the detection of *E. coli* O157:H7 continue to have serious losses in the beef industry, and in many cases had a major impact on profitability. This public health problem demands the development of sound and effective intervention strategies at different stages of the food supply.

The development of control strategies against *Escherichia coli* O157:H7 is urgently needed to prevent further enterohemorrhagic outbreaks and meat recalls. Post-harvest strategies such as carcass rinses, irradiation and thorough cooking will surely prevent a significant number of infections caused by ground beef, but due to the pervasive nature of *E. coli* O157:H7 in cattle populations, interventions at the farm level are required to control most sporadic cases and outbreaks caused by meat and other foods. Among those pre-harvest solutions, the improvement of cattle management practices, the identification of inhibitory feeds, cattle immunization, antimicrobial compounds, probiotic cultures, and water trough treatments are currently being investigated (Callaway et al., 2004; Callaway et al., 2002; Duncan et al., 2000; Sanchez et al., 2002). The application of pre-harvest interventions will be essential to reduce the presence of *E. coli* O157:H7 in cattle populations and ultimately prevent foodborne diseases. In this review we will concentrate in those interventions that are included in the diet: inhibitory feeds, antimicrobial feed additives, and probiotic cultures.

In the last 10 years, a significant amount of research has been devoted to develop effective pre-harvest interventions against a variety of foodborne pathogens, including *E. coli* O157:H7. Some of those mentioned above have shown promising results, however, their implementation and application have been limited by a variety of factors. Probably the most important factor limiting their adoption is the lack of regulatory incentives for those producers that incorporate interventions into their animal operations. To date the adoption of commercially available technologies has been done on a voluntary basis, but the lack of a perceived benefit discourages a long term acceptance. The future of pre-harvest interventions will depend on the establishment of government and industry incentives.

II. MICROBIAL RISKS ASSOCIATED WITH BEEF PRODUCTION

A variety of foodborne pathogens are natural flora of the gastrointestinal tract of cattle. Pathogenic bacteria can be disseminated to the environment from the ruminant's intestine via manure and can contaminate carcasses at the slaughterhouses. Contaminated meat, water and other foods such as fresh vegetables can ultimately serve as vehicles of food borne infections if consumed untreated. Many foodborne poisoning cases have been associated to foods from animal origin and to fresh fruits and vegetables fertilized with animal manure. The most important pathogens responsible for these illnesses are *Salmonella*, *Campylobacter* and enterohemorrhagic *Escherichia coli* (EHEC) (Sivapalasingam et al., 2004).

The CDC has estimated that *Campylobacter* and *Salmonella* are bacterial foodborne pathogens that cause the largest number of illnesses, as they are responsible for a total of 2 and 1.3 million infections every year, respectively (Mead et al., 1999). The majority of infections with *Campylobacter* and *Salmonella* appear to be due to contaminated eggs and poultry, but red meat is a frequent vehicle for their transmission (CDC, 2005; Jay, 2000). EHEC causes less total infections than those two pathogens (approx. 100,000 in the U. S.), but these bacteria are very often associated to outbreaks of gastroenteritis caused by the contaminated ground beef (Mead et al., 1999; Rangel et al., 2005). Infections due to EHEC are the most important public health concern in relationship to beef products.

Enterohemorrhagic *Escherichia coli*

In the last 20 years, an increased number of entherohemorrhagic diarrhea outbreaks have been caused by highly virulent *E. coli* strains. The gastrointestinal infections caused by EHEC often develop into more serious complications such as hemolytic uremic syndrome in children and vascular disease in elderly people. *E. coli* serotype O157:H7 has been the major etiological agent of outbreaks by EHEC, but other serotypes such as O26 and O111 can produce similar infections. The Centers for Disease Control estimated that *E. coli* O157:H7 causes more than 70,000 infections and 60 deaths, and other EHEC strains are responsible for approximately 36,000 illnesses and 30 deaths, every year in the U. S. (Mead et al., 1999). As a result of the continued occurrence of outbreaks, in 1994 *E. coli* O157:H7 was declared an adulterant in ground beef in the U. S. (USDA/FSIS Directive 10,010.1, 2004). As a result of this regulation, the detection of serotype O157:H7 causes frequent recalls to the meat industry every year with millions of dollars of losses.

Different EHEC serotypes appear to be natural inhabitants of cattle populations, but most of the research to understand their ecology in livestock has been directed towards serotype O157:H7. The preference for this pathogenic strain is based on epidemiological data and its unique phenotypic characteristics that allow a relatively easy identification as compared to other serotypes. Shiga toxin-producing *E. coli* (STEC) are a larger group of potentially pathogenic *E. coli* that includes EHEC and have also been found in ruminant populations at relatively large prevalence. Because the pathogenicity of many STEC serotypes isolated from cattle has not been proven, we will focus our discussion to serotype O157:H7.

III. EFFECT OF DIET ON ENTEROHEMORRHAGIC *E. COLI*

A. Types of cattle feeds

The typical cattle diets include a combination of ingredients to provide the necessary nutrients to maintain a satisfactory weight gain, breeding and milk production (Church, 1991). The feed ingredients that supply most of the energy for weaned and adult animals are grasses and grains (Church, 1991). Energy-supplying feeds can be divided into two main types: concentrates and forages. The first are largely composed of highly digestible carbohydrates such as starch and the latter have relatively high fiber content. The term "fiber" is typically defined as non-starch polysaccharides (NSP) and include a variety of polymers that are not easily digested (Grieshop et al., 2001).

Beef cattle systems that feed high-fiber feeds can be classified depending on whether the animal eats them as living plants as pasture (grazing) and if they are provided as harvested and dried plant material (forage). The rumen is a complex and specialized organ that provides cattle with the needed energy and nutrients from high-fiber feeds using a complex consortium of microorganisms. Cellulose is the predominant type of fiber in grasses and forages, and its conversion into absorbable volatile fatty acids (VFA) is a rather complex microbial process (Dehority, 2003). The hydrolysis of cellulose is catalyzed by cellulases produced by cellulolytic bacteria. The fermentation of cellobiose produces VFA, which are converted in the liver to glucose (Forsberg et al., 1997). The slow cellulose degradation limits the utilization of fiber-rich feeds for high-productivity systems.

High-energy feeds (concentrates) are those that contain a large proportion of readily degradable carbohydrates. The two types of readily degradable carbohydrates in animal feeds are starch and sugars. High-starch feed ingredients include cereals such as corn, barley, sorghum, wheat, and oats and the most frequent high-sugar feed ingredient is molasses (Owens et al., 1997). In the rumen, starch and sugars are rapidly fermented and this fast utilization of carbohydrates to produce VFA allows cattle to grow rapidly.

B. Influence of feeding type on the prevalence of *E. coli* O157:H7

The most common strategy to determine the effect of ruminant feed on the prevalence of pathogenic microorganisms in cattle populations is the collection and analysis of a large number of fecal samples obtained from as many animals as possible and subsequent microbiological analysis. By knowing the percentage of animals that are positive in a herd for EHEC, associations could be established between high or low pathogen incidences and related to management practices.

Some of the first cattle surveys reported that the prevalence of *E. coli* O157:H7 in cattle populations was less than 3% (Garber et al., 1995; Hancock et al., 1997b; Shere et al., 1998), but more recent studies using immunomagnetic separation (IMS) techniques have estimated that approximately 25% of cattle can shed this pathogen in their feces (Chapman et al., 1997; Elder et al., 2000; Sargeant et al., 2003; Smith et al., 2001). The prevalence of *E. coli* O157:H7 in cattle population is markedly affected by seasonality. Several studies have indicated that the prevalence during the colder months is significantly lower than in the summer (Mechie et al., 1997). Cattle are not normally affected by the presence of EHEC in their intestine and the only way to identify a carrier animal is by conducting microbiological tests.

The relationship between the type of cattle feeding practices and the prevalence of *E. coli* O157:H7 has been reported by large samplings of cattle. Garber et al. (1995) initially associated some management factors with EHEC prevalence and reported that grain-fed calves had a greater chance of carrying O157:H7 than forage fed calves (Garber et al., 1995). Barley was also linked to increased O157:H7 prevalence, but a positive association was not found for corn and wheat (Dargatz et al., 1997). Feeding corn silage was reported to increase the probability of finding positive fecal samples (Herriott et al., 1998). From these initial studies it appeared that high-energy feeds increased the prevalence of serotype O157:H7 in cattle populations.

The fecal shedding of serotype O157:H7 in cattle is characterized to be sporadic and transient, and this variability might have a great impact on the results of prevalence studies (Besser et al., 1997; Khaitsa et al., 2003; Sargeant et al., 2000). Several reports from different parts of the world have reported consistently lower prevalence of O157 in pasture-fed animals. In Argentina, no O157-positive cattle was found among grazing herds (Sanz et al., 1998), but the same research group observed that 6.8% of grain-fed animals in feedlots had EHEC in their feces (Padola et al., 2004). In the United States, Lagreid et al. (1999) indicated that approximately 83% of range beef calves had been exposed to *E. coli* O157:H7 before reaching the feedlots, but the fecal prevalence of this pathogen in the 15 herds tested was only 7.4%.

In a study of range cattle, the prevalence of serotype O157:H7 in fecal samples was 1% and this parameter was only 0.6% in animals that were on cow-calf pasture (Renter et al., 2003). New Zealand is a major cattle-producing country that relies mostly on pasture feeding, but it appears that the prevalence of *E. coli* O157:H7 is extremely low (Cook, personal communication). Buncic and Avery (1997) reported only two O157-positive animals in a study that included 531 cattle in 55 dairy farms. In studies conducted by the New Zealand's Ministry of Agriculture and Forestry *E. coli* O157:H7 was never detected from a total of 3,000 bovine and 500 ovine carcasses (Cook, 2001). Based on these reports, the prevalence of EHEC in cattle appears to be lower in animals fed grass, but further work is needed to separate if the effect is due to the pasture feed itself or to the physical separation of the animals.

A number of cattle surveys, however, did not find a close link between the type of diet and prevalence of EHEC fecal shedding. The prevalence was almost the same in drylot and pasture herds (Hancock et al., 1997a). In a longitudinal study of dairy farms, no link was observed between different feeds and pathogen prevalence (Garber et al., 1999). The fecal prevalence was also not different in beef cattle when compared with dairy cattle (1.8 vs. 1.66%) (Hancock et al., 1997b; Herriott et al., 1998). These studies, however, obtained their results using only culture methods that could have affected their final outcome.

In contrast to pasture feeding, the use of cereals has been associated with increased risk of fecal shedding of *E. coli* O157:H7 by at least three separate studies. In a large-scale survey of feedlots in the U. S., feeding barley was identified as a factor that was positively associated with fecal shedding this pathogen (Dargatz et al., 1997). In a report from Denmark, the number of O157-positive dairy cows that were fed cereal was two-fold greater than those that were not fed grains

(Rugbjerg et al., 2003). More recently, barley-fed cattle had a 2.4% O157-fecal prevalence, but corn-fed animals had only 1.3% (Berg et al., 2004).

The inhibition of fecal prevalence by grazing has been investigated by investigators in different parts of the world. In Scotland, Ternent et al. (2001) reported that the O157-prevalence was markedly decreased when cattle were turned out to grass after winter housing. A similar effect was observed in a Swedish study in which switching a group of calves from concentrate feeding to pasture completely eliminated fecal EHEC while the control group that remained indoors receiving grains continued to shed serotype O157:H7 (Jonsson et al., 2001). In two consecutive years in a cow-calf herd in Alberta, the O157:H7 prevalence in cows and calves was decreased from approximately 20% to less than 2% after five weeks on native grass pasture (Gannon et al., 2002).

One of the cattle trials that have produced some of the strongest evidence for the inhibitory effect of roughages on EHEC prevalence was conducted by USDA researchers (Keen et al., 1999). In that study, 200 feedlot cattle that had an EHEC prevalence of 54% were divided into two equal groups after eating a finishing grain diet. One of the groups receiving the same diet and the other group was fed only alfalfa hay. After seven days, the fecal prevalence of the hay-fed cattle was only 14%, but the grain-fed cattle still shed the pathogenic *E. coli* at a 52% rate. This result clearly supported the idea that hay feeding could reduce the *E. coli* O157:H7 fecal prevalence.

Because several studies have failed to confirm the inhibitory effect of forage feeding on *E. coli* O157:H7 fecal shedding the issue has not been completely resolved. In addition to the uncertainty whether forage could have an effect, feeding very high roughage diets for long periods of time could have a significant impact on productivity and on meat quality. Despite the apparent simplicity of the proposed hay feeding period, those limitations preclude any widespread utilization as a pre-harvest strategy.

C. <u>Effect of different forage types on *E. coli* O157:H7 persistence and shedding</u>

Considerable attention from researchers has been devoted to compare the effect of grain and forage feeding on the fecal shedding of *E. coli* O157:H7 since it was suggested that forage could be used to reduce carriage of this pathogen by cattle (Diez-Gonzalez et al., 1998). However, there are very few studies that have compared the potential impact of different types of high-fiber feeds such as alfalfa, clover, and silage. In one of the first reports that examined the association between *E. coli* O157:H7 prevalence and feed practices, Garber et al. (1999) reported that none of the farms in which dairy calves were given clover hay as their first forage had an *E. coli* O157-positive animal. In another longitudinal study among dairy herds, feeding corn silage was correlated with an increased fecal prevalence of *E. coli* O157:H7 in heifers (Herriott et al., 1998). These contrasting results suggest that each forage type could have a different impact on fecal shedding of this particular pathogen and may explain the variability of effects observed by different research groups (Buchko et al., 2000; Hovde et al., 1999; Magnuson et al., 2000).

In the first study that investigated the potential effect of forage type on the fecal shedding of *E. coli* O157:H7, Kudva et al. (1995) inoculated sheep with O157:H7 strains and compared alfalfa pellet feed to grazing on sagebrush-bunchgrass. The authors observed that alfalfa pellet-fed animals shed the pathogenic strains for longer periods of time than pasture-fed rams, and suggested that nutritionally deficient and high fiber diets could increase the bacterial count, but may cause clearing of serotype O157:H7 from the GI tract. The effect of fiber concentration on fecal shedding was also investigated using an inoculated sheep model by Lema et al. (2002). In that report, groups of lambs were fed seven different diets formulated with various amounts of fescue hay and cottonseed hull to contain acid-digestible fiber (ADF) from 5 to 35%. After inoculation of lambs with *E. coli* O157:H7 strains, the population of this bacterium was significantly reduced in all six diets that had 10% or more forage as compared with the diet that had no fescue hay or cottonseed hull.

The mechanism of the potential inhibitory effect of forages on the fecal shedding of serotype O157:H7 has not been elucidated but some hypotheses have been explored. Duncan et al. (1998) have suggested that some plant metabolites can be inhibitory and even lethal for *E. coli* O157:H7. In pure cultures, the growth of this bacterium was inhibited by the coumarins; esculetin, umbelliferone and scopoletin, and in mixed ruminal incubations esculin, a coumarin glycoside was also capable of inactivating this organism. More recently, when inoculated calves were given a daily dose of esculetin, the prevalence of *E. coli* O157:H7 in feces was reduced from 37% to 18% (Duncan et al., 2004). An alternative explanation for the inhibitory forage effect is the lack of readily available carbon sources that *E. coli* O157:H7 could compete for when cellulose and lignin are the most important energy sources. However, this latter hypothesis has no experimental support, yet.

IV. USE OF DIRECT FED MICROBIALS AND PROBIOTIC BACTERIA

The terms probiotic and competitive exclusion are typically referred to beneficial microorganisms that can provide a favorable health effect on the host animal after ingestion. Probiotic bacteria are defined as those bacterial strains that are capable of inhibiting other intestinal microorganisms and improving body functions. The term "direct-fed microbial" (DFM) has been defined by the FDA as those microorganisms generally recognized as safe for feeding to animals. The beneficial effect of probiotics or direct fed microbial preparations is probably due to nutrient competition, production of organic acids, competition for intestinal adhesion sites and production of inhibitory compounds such as bacteriocins and colicins (Fuller, 1999; Nurmi et al., 1992).

Several studies have investigated the utilization of probiotic bacteria and direct fed microbial preparations to reduce O157:H7 carriage in cattle. The two types of bacteria used were lactic acid bacteria (LAB) and other *E. coli* strains. Brashears et al. (2003) used a combination of LAB to treat naturally infected cattle against *E. coli* O157:H7. In this experiment, groups of 60 animals were fed daily doses of *Lactobacillus acidophilus* strains NPC747 or NPC750. The fecal prevalence of this EHEC serotype was reduced by 50% when animals were provided with LAB strain NPC747. Not only the fecal prevalence was reduced, but also the number of O157-positive hides declined by as much as 83% compared with the control group. In an independent study conducted by Moxley and coworkers (Moxley et al., 2003) the same LAB strain reduced the prevalence of *E. coli* O157:H7 prevalence from 21 to 13%, but this difference was not statistically significant.

For more than three years these *Lactobacillus* strains have been commercialized in combination with a *Propionibacterium freudenreichii* strain in a commercial DFM, as a beef performance additive (Bovamine®, National Physiology Corp., Ankeny, IA). It was estimated that more than one million cattle in the U. S. received this DFM. Elam et al. (2003) conducted an additional cattle trial to determine the effective doses of these strains and they observed that strain NP51 (formerly NPC747) required 10^9 CFU per animal to obtain a reduction from 26 to 14% in fecal prevalence of EHEC. In a separate study, a 50% reduction in fecal prevalence was reported when cattle were fed Bovamine and at least 95% reduction when used in combination with a vaccine and neomycin feeding (Ransom et al., 2004). These results indicated that Bovamine could have great potential as a pre-harvest intervention strategy against EHEC in cattle populations, but little is known about the actual effect on fecal counts of *E. coli* O157:H7, its performance compared to other probiotic organisms, and the potential for resistance development.

There have been a few reports that have investigated the effectiveness of probiotic *E. coli* to reduce O157:H7 carriage in cattle. Zhao et al. (1998) were the first to use several *E. coli* strains to inhibit *E. coli* O157:H7 and to feed them to cattle to reduce EHEC fecal shedding in cattle. A total of 18 isolates were obtained from cattle that included seventeen *E. coli* and one *Proteus mirabilis*. These isolates were capable of inhibiting five *E. coli* O157:H7 strains. The ability to colonize cattle by these probiotic strains was tested after recovering the four predominant strains from the gastrointestinal tract of calves after 27 days of administration. Those recovered strains were then used to treat calves inoculated with *E. coli* O157:H7. In a cattle trial, calves were fed cultures of the four probiotic *E. coli* strains and two days later, the treatment and control calf groups were administered a mixture of *E. coli* O157:H7 strains. Feeding the probiotic mixture resulted in a complete lack of detection of serotype O157:H7 in fecal samples after 18 days. After 30 days, all control calves still had EHEC detected in their feces, while only one calf fed probiotic tested positive. Three *E. coli* (strains 271, 786, 797) were selected for inclusion in the first anti-O157:H7 probiotic patent and its commercial utilization was subjected to further assessment (Doyle et al., 1999).

In a follow-up study, Doyle (2001) reported the results of an experiment that used adult cattle fed the patented strains. In this latter experiment, 20 steers received 10^{10} cells of five O157:H7 strains and after 48 and 72 hours, ten of them were treated with oral doses of 10^{10} cells of strains 271, 786 and 797. In those steers that received the probiotic strains, no EHEC was detected in their feces after 12 days, but 9 out of 10 untreated steers had counts greater than 2 logs of O157:H7 by day 30. This probiotic mixture was under commercialization trials by Alpharma, Inc. However, after more than three years, there are no signs of market introduction.

Using the same probiotic strains, Zhao et al. (2003) reported that calves less than a week old inoculated with mixtures of the probiotic *E. coli*, were given a 5-strain mix of either O26, O111, or O157 after 38 h. Treatment of neonatal calves with the probiotic *E. coli* resulted in a marked reduction of the fecal shedding of serotypes O26:H11 and O111:NM as compared to the control animals. However, no difference was found between the control and probiotic administered animals in the level of serotype O157:H7. It was hypothesized that the GI tract of milk-fed calves did not favor the colonization by *E. coli* O157:H7. In the most recent article using the three probiotic *E. coli* strains, a similar experiment was conducted with weaned calves inoculated with three serotypes of EHEC (Tkalcic et al., 2003). The inhibitory ability of the probiotic mixture against serotype O157:H7 was confirmed, but there was no effect on the fecal shedding of *E. coli* O26:H11.

We have recently identified and characterized a group of 14 colicinogenic *E. coli* originally isolated from different animal species that were capable of inhibiting as many as 96 *E. coli* O157:H7 *in vitro* (Schamberger and Diez-Gonzalez, 2002; Schamberger and Diez-Gonzalez, 2004). None of these isolates tested positive for typical *E. coli* virulence factors and none of them were antibiotic resistant. This strain collection was found to produce seven different types of colicins, representing three different modes of action. Colicins B, E1, Ia/Ib, and K are pore formers, colicins E2 and E7 are DNases, and colicin M inhibits peptidoglycan synthesis (Schamberger and Diez-Gonzalez, 2004). These colicinogenic strains were also capable of inhibiting other enterohemorrhagic and enteropathogenic *E. coli* strains.

Among previously characterized colicins, colicin E7 was the only one with that could inhibit all of the *E. coli* O157:H7 strains tested. In addition, eight of the selected colicinogenic strains had E7-homologous genes (Schamberger and Diez-Gonzalez, 2002; Schamberger and Diez-Gonzalez, 2004). Based on these results, a cattle trial was recently conducted to determine the effect of feeding colicin E7-producing *E. coli* strains on the fecal shedding and colonization of *E. coli* O157:H7 in artificially inoculated calves (Schamberger et al., 2004). That experiment used a crossover design in which calves were fed two different doses of a mixture of those eight colicinogenic strains. When the average fecal counts of nalidixic acid-resistant *E. coli* O157:H7 were compared between the control and treatment groups during the same period, the counts of treated calves were consistently lower than the control groups (from 0.2 to 1.5 $\log_{10}$ CFU/g), but these differences were only statistically significant 21 days after inoculation with the pathogenic strains. However, if the comparison was made within the same group of animals that had been first served as control and later been fed 10^8 CFU/g of the colicinogenic mixture, an overall statistical difference of $P= 0.001$ was calculated and a maximum reduction in O157:H7 count of 1.8 $\log_{10}$ CFU/g was observed.

At the end of that experiment, the calves were euthanized and the presence of *E. coli* O157:H7 in tissue samples obtained from the rumen, cecum, ileum, colon and rectum was determined. *E. coli* O157:H7 was isolated from 44% of the tissue samples of animals that were fed the colicinogenic strains before dying and from 64% of samples from control calves (Schamberger et al., 2004). Because this difference was statistically significant it was concluded that the colicin E7-producing strains reduced the colonization of the pathogenic organisms. While that research provided encouraging evidence that colicinogenic *E. coli* could reduce fecal shedding and colonization of serotype O157:H7, the extent of reduction was relatively limited. Supported by in vitro data on the long-term inhibition of multiple colicins on O157:H7 strains, the use of colicinogenic strains that produce multiple colicins will very likely increase the reduction in fecal shedding and colonization of *E. coli* O157:H7 (Schamberger and Diez-Gonzalez, 2005).

V. ANTIMICROBIAL FEED ADDITIVES

A few of compounds have been proposed for feeding to cattle to reduce the colonization of *E. coli* O157:H7. These include sodium chlorate and neomycin sulfate. In 2000, Anderson et al. (2000)

realized that sodium chlorate could have potential against facultative anaerobic bacteria. *E. coli* has a nitrate reductase enzyme when growing anaerobically that can also reduce chlorate to chlorite, but chlorite is toxic for the producing cells. When rumen contents containing *E. coli* O157:H7 were incubated in the presence of 5 mM of sodium chlorate, this pathogen was rapidly inactivated. Feeding of sodium chlorate to experimental sheep and pigs was able to significantly reduce the colonization and fecal shedding of *E. coli* O157:H7 (Callaway et al., 2004). Additional studies have reported that the administration of sodium chlorate in the drinking water decreased the populations throughout the gastrointestinal tract of cattle and sheep dramatically (Callaway et al., 2002). The impact of chlorate feeding has been evaluated and it appeared that it has little effect on other rumen microorganisms and almost no effect on animal performance. More recent data has also indicated that sodium chlorate has little effect on the production of shiga toxins by enterohemorrhagic *E. coli*.

Long-term feeding of sodium chlorate could have serious consequences on livestock, but its utilization as a pre-harvest intervention strategy has been proposed within 24 hours before the animals are sent to slaughter. Because of safety issues, any regulatory approval has not been granted and is still under review by the Food Drug Administration. If this technology is approved it could be a very useful tool to reduce the contamination of carcasses, but it may have very little impact in reducing the prevalence of *E. coli* O157:H7 in cattle populations.

Neomycin is an aminoglycoside antibiotic that is currently approved for cattle use. Elder et al. (2002) reported that the shedding of enterohemorrhagic *E. coli* was drastically reduced when cattle were fed this antibiotic. In a follow up study from researchers at Colorado State University, feeding neomycin was confirmed as the most effective intervention treatment to reduce the fecal count of this pathogenic organism, when compared with vaccination and direct-fed microbial lactobacilli (Ransom et al., 2004). Despite these promising results the long-term application for the reduction of zoonotic pathogens in cattle appears to be very unlikely because of the current trend of reduction in antibiotic usage to prevent development of antibiotic resistance.

VI. TAKE-HOME MESSAGE

The development of pre-harvest antimicrobial strategies is essential to significantly reduce the incidence of foodborne infections transmitted by meat, but their adoption and widespread utilization will depend on novel government and industry incentives. *Escherichia coli* O157:H7 is the most serious concern to meat safety because it is broadly found in cattle populations and frequently contaminates carcasses. Three feeding strategies have been investigated for reducing *E. coli* O157:H7: type of feed, use of beneficial bacteria and use of antimicrobial compounds. The inhibitory effect of forage has not been fully confirmed and it is currently not a viable strategy. Probiotic bacteria such as *Lactobacillus* and antagonistic *E. coli* strains have been effective in reducing pathogenic bacteria, but only direct-fed microbial preparations of *Lactobacillus* are commercially available. Other strategies include the use of sodium chlorate right before slaughter and neomycin sulfate.

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