THE USE OF XYLITOL TO MINIMIZE CONTAMINATION OF BEEF CARCASS SURFACES WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI O157:H7

A Thesis

by

STEVEN THOMAS GREINER

Submitted to the Office of Graduate Studies of Texas A&M University in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE

May 2005

Major Subject: Veterinary Public Health
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May 2005

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ABSTRACT

The Use of Xylitol to Minimize Contamination of Beef Carcass Surfaces with Salmonella Typhimurium and Escherichia coli O157:H7. (May 2005)

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Effects of a 10% xylitol solution (X) on adhesion of Escherichia coli O157:H7 and Salmonella serotype Typhimurium to meat surfaces were examined utilizing three approaches. In Experiment 1, rifampicin-resistant strains of E. coli O157:H7 and S. Typhimurium were dispersed in xylitol or a peptone solution (containing approximately 8.9 mean log per ml of each pathogen) and used to inoculate beef outside round meat surfaces. Samples were then rinsed with water or not rinsed in a 2X2 factorial arrangement. No interaction existed between inoculum type and post-inoculation treatments (P > 0.84). Incubation of pathogens in peptone or xylitol had minimal impact on pathogen adhesion (P > 0.76). Rinsing reduced counts by approximately 0.5 log CFU/cm² (P < 0.01). Experiment 2 meat samples received a pretreatment of a water rinse, xylitol, or no rinse, followed by inoculation with pathogens dispersed in peptone solution (containing approximately 8.6 log mean log per ml of each pathogen). Samples received a post-inoculation treatment of a water rinse, xylitol rinse or no rinse in a 3X3 factorial arrangement. No interactions between pre- and post-inoculation factors were observed for surface pathogen load (P > 0.50). Post-inoculation rinsing reduced counts
by approximately 0.5 log CFU/cm² (P < 0.01) with no difference between water and xylitol (P > 0.64). Experiment 3 carcass surfaces were inoculated with pathogens at an initial level of 5.5 log CFU/cm² and received a hot (35°C) water wash, 2.5% L-lactic acid spray, 10% xylitol spray, lactic acid + xylitol or hot water + xylitol. Pathogen counts were taken at 0 and 24 h post treatment. Lactic acid treatments reduced *Salmonella* by 3.3 log CFU/cm² at 0 h (P < 0.01) and by 2.6 log CFU/cm² after 24 h (P < 0.02). Hot water treatments reduced *Salmonella* by 1.5 log CFU/cm² at 0 h (P < 0.07). Xylitol did not minimize pathogens (P > 0.62) nor did it increase effectiveness of other treatments. These data indicate that xylitol is ineffective at preventing *E. coli* O157:H7 and *S. Typhimurium* adhesion to meat surfaces.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>3</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>5</td>
</tr>
<tr>
<td>Bacterial adhesion</td>
<td>9</td>
</tr>
<tr>
<td>Current interventions in the beef slaughter industry</td>
<td>11</td>
</tr>
<tr>
<td>Xylitol</td>
<td>14</td>
</tr>
<tr>
<td>Hypothesis and research objectives</td>
<td>18</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>24</td>
</tr>
<tr>
<td>Media</td>
<td>24</td>
</tr>
<tr>
<td>Bacterial cultures</td>
<td>24</td>
</tr>
<tr>
<td>Meat samples</td>
<td>25</td>
</tr>
<tr>
<td>Sampling and microbiological analysis</td>
<td>25</td>
</tr>
<tr>
<td>Xylitol preparation</td>
<td>26</td>
</tr>
<tr>
<td>Experiment 1: Pre-treatment of pathogens with 10% xylitol</td>
<td>27</td>
</tr>
<tr>
<td>Experiment 2: Pre-treatment of meat surfaces with 10% xylitol</td>
<td>28</td>
</tr>
<tr>
<td>Experiment 3: Effect of xylitol on meat with pre-existing pathogen contamination</td>
<td>30</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>33</td>
</tr>
<tr>
<td>RESULTS, DISCUSSION AND CONCLUSION</td>
<td>34</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>34</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>38</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>43</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>50</td>
</tr>
<tr>
<td>VITA</td>
<td>61</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mean log reductions in populations of <em>E. coli</em> O157:H7 and <em>Salmonella</em> Typhimurium inoculated onto representative 10-cm$^2$ meat samples after pathogen incubation with either 10% xylitol or 0.1% peptone water</td>
</tr>
<tr>
<td>2</td>
<td>Mean log reductions in populations of <em>E. coli</em> O157:H7 and <em>S.</em> Typhimurium inoculated onto representative 10-cm$^2$ meat samples after sterile water rinse (R) or no treatment (NoR)</td>
</tr>
<tr>
<td>3</td>
<td>Mean populations (log CFU/cm$^2$) of <em>E. coli</em> O157:H7 and <em>S.</em> Typhimurium recovered from pathogen inoculated meat sample rinse water</td>
</tr>
<tr>
<td>4</td>
<td>Mean log reductions in populations of <em>E. coli</em> O157:H7 and <em>S.</em> Typhimurium inoculated onto representative 10-cm$^2$ meat samples after pre-inoculation treatment of no rinse (NoR), sterile water rinse (W) or 10% xylitol rinse (X)</td>
</tr>
<tr>
<td>5</td>
<td>Mean log reductions in populations of <em>E. coli</em> O157:H7 and <em>S.</em> Typhimurium inoculated onto representative 10-cm$^2$ meat samples after pre-inoculation treatment, and followed by a post-inoculation treatment of no rinse (NoR), sterile water rinse (W) or 10% xylitol rinse (X)</td>
</tr>
<tr>
<td>6</td>
<td>Mean populations (log CFU/cm$^2$) of <em>E. coli</em> O157:H7 and <em>S.</em> Typhimurium recovered from sterile water or 10% xylitol used to rinse representative 10-cm$^2$ meat samples after undergoing pre-inoculation treatment of no rinse (NoR), sterile water (W) or 10% xylitol rinse (X) followed by pathogen inoculation and then subjected to a sterile water (W) or 10% xylitol rinse (X)</td>
</tr>
<tr>
<td>7</td>
<td>Mean log reductions in populations of <em>E. coli</em> O157:H7 and <em>S.</em> Typhimurium inoculated onto outside round and brisket surfaces after being subjected to selected treatments and sampled immediately and again after 24 h incubation at 4° C</td>
</tr>
</tbody>
</table>
INTRODUCTION

Today, the average American consumes more than 195 lbs of meat annually. This is an increase of 57 lbs above the annual consumption in the 1950s and through the last half century, beef makes up the majority of the total meat consumed. In 2000, beef made up 33% of the meat consumption followed by poultry with 27% and pork with 25% (7). According to the USDA Agricultural Baseline Projections to 2014, this consumption of approximately 64 lbs of beef per capita is expected to remain rather stable over the next decade (12).

One of the major obstacles to maintaining this level of consumption, and perhaps even increasing it, is the public’s perception of the safety of the beef. Events such as the recent identification of a cow with Mad Cow Disease (bovine spongiform encephalopathy) in the United States (8), and the Jack-in-the-Box Escherichia coli O157:H7 outbreak in the early 1990’s (38) have shaken consumer confidence and potentially reduced beef consumption levels. Foodborne illness remains a major public health issue in the United States and throughout the world (36). Annually, an estimated 76 million illnesses and 5,000 deaths in the United States result from foodborne pathogens. Of these foodborne illnesses, where the etiological agent was identified, it was determined that 30% are caused by bacteria, 3% by parasites and the remaining 67% attributed to viruses, primarily to Norwalk-like viruses (18, 19, 83). However, bacterial pathogens account for 72% of the deaths resulting from foodborne illnesses (74). Two

This thesis follows the style and format of the Journal of Food Protection.
bacterial pathogens, *Salmonella* and *E. coli* O157:H7 are included in the top five pathogens that account for over 90% of the estimated food related deaths (74). It has been shown that these two foodborne pathogens commonly occur in a wide variety of meat and meat products throughout the world (102). Additional studies have established the prevalence of these pathogens in retail meat cuts in the United States (110). Therefore, it can be reasonably concluded that these foodborne pathogens are present in retail meat products and potentially pose a definite risk to the health of the United States population.

Scientific research has the unique ability to investigate, intervene, improve and correct these food safety problems and ultimately reduce the public health risk of a particular food. This thesis research project embraces a potential intervention that, if proven effective, could result in decreasing the public health risk of foodborne disease while increasing the market value and demand for beef through improved consumer confidence and satisfaction.
LITERATURE REVIEW

As previously stated, *Salmonella* and *E. coli* O157:H7 are the two major bacterial foodborne pathogens of concern in beef. In a 1999 Centers for Disease Control and Prevention (CDC) study by Mead et al. (74), non-typhoid *Salmonella* was estimated to account for 1.3 million cases of foodborne illness annually in the United States and was cited as the number one cause of foodborne disease related deaths. While the number of foodborne illness cases and deaths from *E. coli* O157:H7 do not occur with the same magnitude as *Salmonella*, it is still considered one of the greatest challenges to the food industry in the last 80 years. Specific characteristics such as low infectious dose, high infectivity in humans, serious acute illness and long term sequellae, natural occurrence in cattle and global distribution all contribute to making *E. coli* O157:H7 foodborne pathogen of major concern in beef (20). In order to better appreciate the role that these two pathogens play in the beef industry, it is important to comprehend specific information about these pathogens and the diseases that they cause.

*Salmonella.* The genus *Salmonella* belongs to the family *Enterobacteriaceae* and was named after an American veterinarian, Daniel Salmon. It is a rod-shaped, gram-negative, non-sporeforming bacteria that is in most cases motile (6). *Salmonella* can be isolated from the gastrointestinal tracts of a wide rage of domestic and wild animals, including cattle, poultry, swine and even reptiles (35).

The nomenclature system for *Salmonella* is rather complex and one standardized system has still not been agreed upon by all interested parties. The typical one species name per serotype would require over 2,463 species names due to the number of
Salmonella serotypes identified thus far. Perhaps the easiest and most useful system available is the one established by the World Health Organization Collaborating Center (85) and used by the CDC. It classifies Salmonella into Salmonella enterica and Salmonella bongori and further divides Salmonella enterica into six sub-species. The subspecies of primary importance for warm blooded animals is Salmonella enterica subsp. enterica which includes 59% of the known Salmonella serotypes. Salmonella enterica subsp. enterica is further classified by serotype or serovar names which are non-italicized and capitalized. The first citation of Salmonella enterica subsp. enterica serotype Enteritidis is properly written as Salmonella serotype (or ser.) Enteritidis and subsequent references are Salmonella Enteritidis or S. Enteritidis (24).

It is important to distinguish salmonellosis, which is the commonly used term to describe the disease resulting from the zoonotic foodborne infection from a wide variety of Salmonella bacterial serotypes, from typhoid fever, which is the gastrointestinal disease resulting from Salmonella serotype Typhi infection. Typhoid fever is a serious, often fatal gastrointestinal disease in developing third-world countries resulting from poor sanitation practices. The most important distinction of S. Typhi is that it can only infect humans, and no other animal host has been identified. S. Typhi infection is usually acquired from swallowing contaminated water, but foodborne infection may occur if it is washed or irrigated with contaminated water. Ultimately, typhoid fever is generally not considered to be a major problem in the United States (35).

The two most frequently isolated Salmonella serotypes from the United States National Salmonella Surveillance System are S. serotype Typhimurium and S. Enteritidis
In a ten-year analysis of human isolated *Salmonella* serotypes, these two serotypes ranked in the top two, followed by *S.* serotype Heidelberg, *S.* serotype Newport and *S.* Hadar (82). Since most human infections result from the ingestion of animal origin foods, some studies have attempted to determine the prevalence of *Salmonella* in retail food items, while other studies have narrowed the focus to the food animal itself (18, 47, 71, 102, 110, 111).

The typical salmonellosis infection is typified by a 6- to 72-hour incubation period after ingestion of food containing very low numbers of *Salmonella* organisms (75). This is typically followed by acute onset of fever, muscle aches, headache and malaise, which progresses to non-bloody diarrhea, abdominal pain and cramps, fever, nausea and vomiting. In the normal course of illness, clinical recovery follows in two to four days and the recovering carrier may shed for a few weeks to several months in rare situations (1).

**Escherichia coli O157:H7.** *E. coli* belongs to the family *Enterobacteriaceae* that includes enteric or intestinal bacteria such as *Salmonella, Yersinia, Enterobacter* and *Shigella.* *E. coli* is a gram-negative, facultatively anaerobic bacillus that may or may not be motile. It is classified by four different antigens which are the somatic antigen O, the flagellar antigen H, the capsular antigen K, and the fimbrial antigen F. Differentiation between serotypes is based primarily on the O antigen which separates the bacteria into one of more than 170 serogroups, the H antigen then subdivides each serogroup into one of about 56 serotypes (43). An example of such classification is the *E. coli* O157:H7 serotype. The vast majority of *E. coli* bacteria are harmless, normal inhabitants of the
gastrointestinal tract of all warm blooded animals, including man. However, there are six categories of *E. coli* bacteria that cause disease in man. These categories, each differing in virulence, pathogenesis and O:H serotype set, are the enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), the enteroinvasive *E. coli* (EIEC), the enteropathogenic *E. coli* (EPEC), the enteroaggregative *E. coli* (EAggEC) and the diffuse-adherent *E. coli* (DAEC) (1). Although each category causes enteric disease in humans at some level, for the purpose of this thesis, *E. coli* O157:H7 will be the only serotype discussed.

*E. coli* O157:H7 infections in humans can arise directly from animals, from infected humans and from contaminated foods. Cattle are suspected to be the major reservoir of EHEC, providing a continuous replenishing source to maintain the EHEC cycle through fecal contamination of the water and soil (20). Various studies have attempted to determine the worldwide prevalence of EHEC in food animals, carcasses, raw meats and processing plants. According to these studies, the prevalence in samples from the gastrointestinal tract of animals varies widely and may range from zero to 80% (32, 46, 54, 55, 113). Prevalence of EHEC on carcasses was usually less than 10% with a significant stepwise reduction between samples taken pre-evisceration, post-evisceration prior to antimicrobial intervention and post-processing after carcasses enter the cooler (44, 87). Raw ground meats generally show a prevalence of EHEC in less than 5% of the samples and although this prevalence is low, the risk of foodborne illness from EHEC remains (33, 37). The reported incidence in man is relatively low and seems to primarily occur in industrialized countries, such as North America, Europe, Japan and Australia.
In the United States, the reported incidence was 2.8 cases per 100,000 in 1998 (20). EHEC has been associated with alfalfa sprouts, unpasteurized fruit juices, lettuce, dry salami, raw milk and cheese curds (48), but the United States Department of Agriculture – Food Safety and Inspection Service (USDA-FSIS) Risk Assessment of *E. coli* O157:H7 in ground beef (86) estimated based on epidemiological data that anywhere between 16 and 40% of all outbreaks have involved raw or undercooked ground beef. Although EHEC occurs relatively infrequently, the seriousness and potential complications of the illness as well as its classification as an adulterant in ground beef with zero tolerance, indicate that this pathogen warrants special attention (45).

The infectious dose of EHEC is very low and thought to be as few as ten infective bacteria, depending on the nature of the host (57). Children less than 5 years old, the elderly and individuals on antacids or with naturally occurring low stomach acid production are considered to be at a greater risk for EHEC. The incubation period is usually around three to four days but symptoms may appear as early as two days or as late as eight days after ingestion (35). The clinical symptoms may appear as a mild case of diarrhea ranging to severe, copious, hemorrhagic diarrhea, strong abdominal cramps and little to no fever. Typically the diarrhea is watery at the onset, then gradually becoming more hemorrhagic and usually lasts about four days. Vomiting may be seen in half of all observed cases (1). The primary difference between non-pathogenic *E. coli* and EHEC is *E. coli* O157:H7’s ability to produce one or more toxins called Shiga toxins. These toxins (Shiga toxin 1 and Shiga toxin 2) are so named because they resemble the toxin produced by *Shigella dysenteriae*, the agent that causes bacillary
dysentery. These toxins, along with other virulence factors, work in concert to cause various degrees of severity of the disease (73). In most cases the illness is self limiting and resolves in a little more than a week (35).

Hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) are the two major complications associated with EHEC that make it an important foodborne pathogen. HUS occurs in approximately 3 to 7% of sporadic E. coli O157:H7 cases and this rate may increase to nearly 20% in outbreak situations (73). HUS consists of thrombocytopenia, hemolytic anemia and acute renal failure and tends to affect mostly children under five years of age (20, 60). While the exact mechanism is unknown, it is suspected that the Shiga toxins produced by EHEC cross the intestinal barrier and bind to endothelial cell throughout the body, showing preference for the renal tubular endothelial cells. The toxins then shut down the cell mechanisms and cause cellular death. The cellular damage and death is thought to trigger platelet binding causing thrombocytopenia and fibrin deposition which damages passing erythrocytes leading to occlusion of the renal microvasculature and hemolytic anemia (60). Typically, half of HUS patients require dialysis and up to three quarters need blood transfusions. One quarter of all HUS patients develop acute neurological manifestations such as stroke, seizure and coma. Long term complications and sequellae such as pancreatitis, diabetes mellitus, pleural and pericardial effusions, colonic stricture, cholelithiasis and cognitive impairment can occur but are rare. Between 3-5% of HUS patients will develop end stage renal failure and a similar percentage will die (73). HUS is the leading cause of acute renal failure in children of developed countries (20). In older patients,
TTP will typically occur and is most likely the same disorder as HUS, with less renal involvement and more significant neurological involvement (73).

**Bacterial adhesion.** During beef slaughter operations, a new exterior body surface is created as the hide and viscera are removed. However, there are no antimicrobial defense mechanisms associated with this new surface. The exposed surfaces of the carcass provide an environment excellent for bacterial attachment and growth, and this attachment or adhesion is a critical component to the transmission of foodborne disease (81).

The first challenge in the adhesion of bacteria to surfaces of animal cells is being able to overcome an energy barrier based upon electrical charges. Pathogenic bacteria are negatively charged in physiological environments and the surfaces of animal cells and tissues also carry negative charges, thus the repulsion of like charges is the first barrier to adhesion that must be overcome (81). It is theorized that the way bacteria overcome this repulsion is through adhesins and receptors. Adhesins are small, thin, molecules located on fimbria that project from the surface of the bacteria that bind to receptors found projecting from the surface of the animal cells (91). Fimbrial adhesins allow the bacteria to approach, make contact with the surface of the animal cell, and bind before being subjected to the repulsive charge effect. Although lectin–carbohydrate, protein–protein, and hydrophobin–protein are all types of adhesin-receptor interactions involved in bacterial adhesion to mucosal surfaces (81), this thesis will focus on lectin-carbohydrate.
A lectin is defined as a hemagglutinating protein which binds to the branching sugar molecules of glycoproteins and glycolipids on the surface of cells (101). These lectins are classified by their sugar specificity, which can be determined by which simple or complex carbohydrates compete with the binding of the lectin to animal cell receptor sites and block the adhesion (81). One of the body’s major defense mechanisms against infectious diseases is an abundance of decoy carbohydrate molecules in the mucous layer that lines exposed epithelial cells, as well as in tears, saliva, urine, sweat, and breast milk. These decoys bind to the pathogenic organism’s lectins to prevent adhesion and the pathogens are subsequently cleared through the elimination systems associated with the respective tissues involved (112). Therefore, if an innocuous carbohydrate could be found that effectively blocks the adhesion of pathogenic bacteria to animal cells and tissues, then it would potentially serve as a valuable tool to minimize or even prevent bacterial infections. Research has identified a number of carbohydrates that demonstrate this potential and the first successful study dates back to 1979 when Aronson et al. (14) reported the ability of methyl α-D-mannopyranoside to block E. coli colonization of the urinary tract of mice. Carbohydrate-specific adhesion inhibitors have also shown success against a number of bacterial pathogens in monkeys (77), calves (76), rats (58), and rabbits (58). Recent successes have been shown using xylitol as an anti-adhesive molecule in the prevention of human dental caries (99) and acute otitis media (64, 106). It is evident that this new application of carbohydrate molecules could open up a vast new methodology for the treatment of bacterial diseases.
Current interventions in the beef slaughter industry. This section will discuss the currently utilized methods of removing or minimizing the numbers of pathogens on the carcass after the animal is processed. These interventions include chemical disinfecting and dehairing of carcass hides, carcass trimming, water wash, steam pasteurization, organic acid treatment and irradiation.

The two main sources of beef carcass bacterial contamination are intestinal contents and hides. Studies have shown that the majority of contamination actually comes from the hides (16, 17, 27, 44). Therefore, interventions that focus on minimizing the potential pathogen contamination of the hide should logically minimize the subsequent contamination of the carcass itself. Chemical disinfection of hides using a common oral antimicrobial compound called cetylpyridinium chloride (CPC) has shown to be effective in minimizing bacterial populations on the hide surface as well as subsequent reduction of microbial carcass contamination (22, 23). Other approaches have examined the chemical dehairing of carcasses to remove the hair and extraneous matter on the hide with sodium sulfide followed by hydrogen peroxide and water washing. This application was shown to effectively minimize carcass bacterial contamination, yet additional study into the feasibility for commercial application is required (79).

Physical trimming of carcasses stems from the current USDA-FSIS guidelines (9) that state that fecal material shall be “promptly removed from beef carcasses during slaughter/ dressing in a manner satisfactory to the inspector.” It is effective at removing visible fecal contamination of the carcass, when identified, but it generally results in a
significant amount of waste, as well as a concern that contamination may be spread by the trimming knives themselves (52). Fecal contamination must be actively identified and the borders of the contamination subjectively determined by the person conducting the trimming. Since bacterial contamination may not be strictly confined to areas of visible contamination, the effectiveness of this technique poses some significant concerns (52).

A great deal of controversy surrounds the application of water washing in the role of minimizing bacterial contamination on beef carcasses. One study has shown that spray washing minimized bacterial counts and removed surface fecal material without spreading the contamination onto surrounding areas (51). Utilizing hot water (95°C) washes enhanced the reduction in bacterial contamination as well (28-30). Other research concluded that spray washing with cold water was ineffective in removal of microbial contamination and actually caused the contamination to spread (21). In addition, high pressure spraying may result in penetration of the bacteria into the tissue, making many further decontamination interventions ineffective once bacteria reach the interior of the meat (3, 39). In all likelihood, factors such as type of spraying nozzle used, the number, distribution, position, spraying time and pressure, spray angle, water output and operation, along with the carcass size and design of the entire spraying system all play critical roles in determining the ultimate success or failure with regard to water wash to minimize bacterial contamination of carcasses (96).

Steam vacuum was first approved for use in commercial beef slaughter facilities by the USDA-FSIS in April of 1996. The technique serves as a replacement for knife
trimming for removal of carcass fecal contamination and involves the use of applying steam and vacuum or water, steam and vacuum to spot-clean the identified contaminated location on the carcasses (62). Some research has shown this technique to be effective (41, 62), however other research reveals a concern that the surface temperature never gets high enough to achieve an effective kill (30). Regardless, it has been subsequently adapted and grown popular in the United States. The popularity of this intervention most likely stems from its elimination of trim waste, the relatively low cost and ease of application.

One of the most promising advancements in the reduction of carcass bacterial contamination has been the use of organic acid treatments. Even though the antimicrobial mode of action of these organic acids has not yet been satisfactorily explained, (34) acetic, lactic, ascorbic and citric acids have all been utilized in this manner and lactic acid has been the most effective at minimizing bacterial populations. Additionally, the isomer L-lactate has a much greater antimicrobial action than D-lactate or the DL-lactate mixture, depending on the pathogen (72), and residual antimicrobial effects have also been shown during storage of meat after a hot carcass spray (42, 94). Initial research questioned the effectiveness of organic acid sprays on cold meat surfaces (2, 40), however further studies reveal that lactic acid sprays applied post chill are effective at minimizing pathogens (31). Typical application of a hot (55°C) 2% L-lactic acid wash may result in an up to 5.0-log reduction in E. coli O157:H7 and S. Typhimurium numbers on the carcass (28, 52, 84).
**Xylitol.** The rise in antibiotic resistant pathogens has been steadily growing over the years, prompting the drive to find new and innovative ways to fight bacterial pathogens in humans and animals. One promising approach is that of anti-adhesive therapy, which involves the use of certain carbohydrates that prevent the attachment or adhesion of the bacteria to the host cells. Since carbohydrates are typically normal constituents of human and animal cell surfaces and body fluids, they tend to be neither toxic nor immunogenic, and thus are considered to be more mild and safe for the host as well as the environment than traditional antibiotics(92). In addition, since these carbohydrates are not actively involved in killing bacteria, selection of resistant strains is not considered to be a problem (92). As previously discussed in the pathogenesis section, bacterial adhesion is required for colonization, and colonization is required for the ensuing expression of disease symptoms (81). Adhesion is essential in preventing the host natural clearing mechanisms from physically removing the bacteria as in the case of airflow, mucous and cilia in the respiratory tract, or urine flow in the urinary tract. In addition, bacterial adhesion also provides these pathogens access to nutritional sources, permits the delivery of toxic agents into the host tissues, and facilitates bacterial penetration into the host tissues (80). By minimization of the ability of bacterial adherence, it is logical that bacterial removal can be enhanced as well as a reduction of the ability of the pathogens to grow and multiply. This process effectively minimizes the ability of the pathogens to persist and cause disease. Lectins, present on the surface of many pathogenic bacteria, mediate adhesion by binding to complimentary carbohydrate portions of glycoproteins or glycolipids expressed on the surfaces of the host tissues.
Minimized adhesion of bacteria in the presence of soluble carbohydrate moieties may result from a disruption of bacterial metabolism through accumulation of non-metabolizable intermediates \((100, 103)\). Additional studies have shown that specific bacterial lectins on the cell surface fimbria are blocked from binding to animal cells \textit{in vitro} by these soluble carbohydrate moieties and in some cases they can detach bacteria that are already bound to cells via surface lectins \((76, 93, 101)\). Additional studies examined the effects of a specific carbohydrate, xylitol on the adhesive properties of otopathic and cariogenic bacteria, as well as bacteria involved in the development of cystic fibrosis, upper respiratory infections and gastrointestinal infections. These studies found that xylitol was effective at minimizing bacterial adherence in these pathogens \((64, 78, 95, 99, 100, 106, 109)\). These studies have shown that a wide array of pathogenic bacterial genera express lectins that are bound by xylitol. They also demonstrate that adhesion and presumably colonization and infection can be minimized appreciably when the bacteria, the tissue surfaces that they adhere to, or both are treated with xylitol. This premise that xylitol can minimize bacterial adherence to tissue surfaces forms the basis of our interest in the application of xylitol to minimize bacterial pathogen contamination of beef carcasses.

Xylitol \((1,2,3,4,5\) pentahydroxy pentane) has the molecular formula \(C_5H_{12}O_5\) and is a five-carbon, naturally occurring carbohydrate that is classified as a sugar alcohol or a pentitol. Plums, strawberries, raspberries and rowanberries are all natural sources of xylitol \((68)\). The predominant commercial production method is the chemical reduction of D-xylose derived from xylan. Major sources of xylan include fresh cut wood,
especially birch trees, sugarcane byproducts and other plant waste materials such as nut shells. In addition to these plant sources, yeasts, fungi and bacteria have also been shown to produce xylitol (98).

Xylitol has a sweetening property that matches sucrose and is extensively used as a non-glycemic sweetener (glycemic index -5) in diabetic diets. Xylitol produces a perceived coolness sensation in the mouth when it comes into contact with saliva due to its negative heat of solution. This property has made it popular as a non-sugar sweetener for chewing gum, chewable vitamins, tablets, cough syrups, mouthwashes and toothpaste. Xylitol is also used as the primary sweetener in hard candies, mints, sugarless chocolates, gelatin, puddings, jams, baked products and ice creams (97).

Medical uses are also extensive. Xylitol has been used as a metabolic energy source in shock patients (50, 53, 66), in resuscitation for diabetic coma (66), in therapy for the deficiency of glucose-6-phosphate dehydrogenase in red blood cells (107), in reducing the rate of gluconeogenesis (50), in promoting endogenous fat mobilization and oxidation in well nourished patients (50), and in reducing drug induced hemolysis (67). Other research has revealed that xylitol inhibits the growth of nasopharyngeal bacteria (63), prevents acute otitis media in day care center children (104, 105), prevents experimental osteoporosis in rats (70), improves the biomechanical properties of bone (69), prevents ketosis in cattle (89), improves udder health in cattle, and inhibits certain food spoilage microorganisms (67). The most relevant studies that formed the inspiration for the proposed xylitol application to beef carcasses center on the demonstrated reduction of the adhesive properties of Streptococcus mutans and its ensuing effect on dental caries
The inhibition of growth and attachment of both pneumococci and *Haemophilus influenzae* on epithelial and nasopharyngeal cells (64, 100), as well as the inhibition of *Clostridium difficile* adhesion to human intestinal epithelial cells (78). The cited studies indicate that xylitol is effective at minimizing the ability of a variety of bacteria to adhere to cell surfaces thus potentially preventing attachment and infection. Application of xylitol as an intervention step in minimizing bacterial contamination of beef carcasses is an intuitive extension of the anti-adhesive properties demonstrated by these studies.

While xylitol is not specifically listed by the FDA as an item that is “Generally Recognized As Safe” (GRAS), it is classified by the US Code of Federal Regulations under Title 21 Chapter 1, Subchapter B, Part 172 (10) as a food additive permitted for direct addition to food for human consumption. It further denotes that xylitol may be safely used in foods for specific dietary uses, provided the amount used is not greater than that required to produce its intended effect.

The oral and metabolic safety of xylitol has been assessed by various international and national regulatory authorities. The Joint Expert Committee on Food Additives (JECFA) of two United Nations agencies (Food and Agricultural Organization and the World Health Organization) in 1983 allocated an "Acceptable Daily Intake" (ADI) definition of "not specified" for xylitol. An ADI of “not specified” indicates that no consumption limits are required for xylitol. JECFA specifically recommended an unlimited ADI based on the safety of xylitol which reflects the safest category that this committee can place a food additive. The specification is comparable to that of sorbitol which is listed in Title 21 CFR Chapter 1, Part 182.90 as GRAS (11). In addition,
JECFA reported that no additional toxicological studies were recommended (4). In 1986, the Federation of American Societies for Experimental Biology (FASEB) was commissioned by the FDA to investigate the safety of sugar alcohols and lactose (5). Opinions of knowledgeable investigators engaged in work in relevant areas of biology and medicine and comprehensive literature reviews form the basis for these FASEB reports. Their conclusions were that no significant safety concerns would be expected from use of xylitol in humans, and that xylitol appears to have the same safety profile as other sugar alcohols, such as sorbitol and D-mannitol.

**Hypothesis and research objectives.** It is clear that the minimization or elimination of pathogenic bacterial contamination on fresh beef products has major implications for decreasing the public health risk of foodborne diseases. Many current practices have focused primarily on microbial minimization through bactericidal action (steam vacuum, organic acid treatments, and irradiation). These treatments have, in many cases, proven effective in minimizing microbial contamination, but they may be perceived as 'unnatural' by consumers. Studies focusing on minimization of ante mortem microbial concentration of animal carcasses have shown some modest improvements in minimizing carcass pathogen contamination (23, 26, 27, 90). Additional studies have examined changes in diet prior to slaughter in an attempt to alter gastrointestinal microflora to minimize potential pathogenic bacterial numbers (88). One approach to the problem of carcass contamination focuses on the physiological processes that occur when the bacteria make contact with the surface of the carcass. It is generally accepted that attachment involves two main stages. The first is a loose association between the
bacteria and the meat surface, through electrical forces, followed by a time-dependent and irreversible attachment. The irreversible attachment involves the formation of polymers that anchor the organism to the surface making removal next to impossible (56). Treatments with products that minimize microbial adhesion have the potential to reduce pathogen loads by preventing this adherence and subsequent microbial colonization. These types of products may increase the effectiveness of bactericidal treatments by exposing more pathogens to these actions. Finally, if such product is perceived as a 'natural' compound, it could increase consumer acceptance.

Carbohydrate moieties minimize bacterial adhesion through the binding of carbohydrate specific bacterial lectins on cell surface fimbria (25), or through disruption of bacterial metabolism through accumulation of non-metabolizable intermediates (76, 101). One particular pentitol, xylitol has been shown to minimize adhesion of bacteria involved in development of dental caries (99), middle ear infections (106), lung disease such as cystic fibrosis (109), upper respiratory infections (63) and gastrointestinal infections (78, 99). These studies have shown that a wide array of pathogenic bacterial genera express lectins that are bound by xylitol. They also demonstrated that adhesion and presumably colonization and infection were minimized appreciably when the cells, the tissues surfaces that they adhere to, or both were treated with xylitol.

The safety of xylitol has been well established due to its common use in foods and extensive data exists. Minimized microbial binding and subsequent colonization coupled with its status as a food may make xylitol an attractive compound for use in pathogen minimization on the surface of meat products. As a single step process,
application of xylitol may minimize adhesion, and thus minimize colonization and proliferation of pathogenic microbes. Xylitol treatment coupled with a water rinse may also minimize loading, without the use of organic acid treatment of meat. However, a xylitol application followed by a bactericidal rinse (e.g., lactic acid) may increase the bactericidal activity of the acid treatment by exposing more cells to the treatment.

*Salmonella* and *E. coli* O157:H7 are significant foodborne pathogens associated with beef and beef products. Research has previously demonstrated that interventions placed into the beef slaughter process that minimize carcass pathogen contamination levels can potentially minimize the risk of foodborne illness from these pathogens. Finally, evidence has demonstrated xylitol to be effective in minimizing bacterial adhesion in dental caries, middle ear, respiratory and gastrointestinal infections, and it has promise for this use in other applications. Based on these presumptions, it is hypothesized that treatment of carcass surfaces with xylitol alone will minimize bacterial proliferation, but may not result in depletion of bacteria currently present at the time of treatment. Furthermore, treatment with xylitol in combination with a water rinse will suppress proliferation and result in minimized initial bacterial loads. It is also proposed that xylitol treatment followed by treatment with an organic acid will minimize total loads and subsequent proliferation to a greater extent than other known treatments. In addition, any xylitol treatment will minimize the rate of recontamination by preventing bacteria introduced post-treatment from adhering and colonizing carcass surfaces. Finally, if the hypothesis regarding xylitol’s interference with the adhesion of bacteria on the meat surface is supported, application of this process will lead to a safer retail beef
supply for the consumer and subsequently reduce the occurrence of beef associated foodborne illnesses caused by *Salmonella* and *E. coli* O157:H7 pathogens.

In order to evaluate these hypotheses, multiple experimental objectives were established. Primary objectives were to evaluate the potential for xylitol to interact with pathogenic bacteria, specifically *S. Typhimurium* and *E. coli* O157:H7, and subsequently minimize their adherence to meat surfaces. Specifically, the following was to be evaluated:

1) The effect of direct interaction of the pathogens with xylitol on adherence and colonization properties

2) The effect of application of xylitol to meat surfaces as a barrier to adhesions

3) The effect of subsequent rinsing strategies to remove xylitol treated bacteria.

Objective 1 examined the interaction that xylitol has with the pathogenic bacterial lectins. Hypothetically, pathogens exposed to xylitol prior to the inoculation of meat surfaces should result in the binding of the bacterial lectins used to adhere to meat surfaces. With their lectins blocked, adhesion to the meat surfaces should not occur, and a decreased pathogen recovery from the meat surfaces that were inoculated with xylitol treated pathogens over the non-xylitol treated pathogens is expected. Objective 2 examined the effect that xylitol treatment of meat surfaces might have on pathogen adhesion. With xylitol already available to the pathogens on the meat surfaces,
minimized adhesion of these pathogens should occur, since the xylitol blocked the bacterial lectins as the pathogens make contact with the xylitol coated meat surface and prevented adhesion. As such, the xylitol pre-treated meat surfaces would result the in recovery of fewer pathogens over the no-treatment and sterile water pre-treated meat surfaces. Objective 3 examined the effects of rinsing strategies to remove xylitol treated bacteria. Xylitol treated pathogens, whether exposed in the inoculum or on the meat surface, were expected to be unable to adhere to the meat surfaces. Subsequently, rinsing the meat surfaces of xylitol treated pathogens should result in greater recovery of pathogens in the rinse solutions over the non-xylitol treated pathogens. In addition, the rinsing of xylitol treated pathogens with a xylitol solution should also result in a greater recovery of pathogens over the sterile water rinse since it provides even greater exposure of the pathogens to xylitol and further blocks the adhesion of the pathogens to the meat surfaces.

Second, simulated commercial application of xylitol to subprimal cuts alone and in combination with other decontamination strategies, such as an automated hot water wash and a 2.5% L-lactic acid spray as a confirmation of in vitro findings was evaluated. In this application, the ability of xylitol to detach pre-existing pathogen contamination from meat surfaces was examined. It was expected that xylitol treated meat surfaces would result in detachment or blocked adhesion of the pathogens, which would be evident by lower pathogen counts recovered from meat surfaces treated with xylitol over non-xylitol treated cuts. It was also expected to observe a synergistic effect with the hot water and lactic acid treatments in combination with the xylitol with a pathogen count
reduction over and above the individual treatments alone. Satisfaction of these objectives completely addresses both the mechanisms and effectiveness of this intervention, and thus addresses the hypotheses.
MATERIALS AND METHODS

Media. Lactose-sulfite-phenol red-rifampicin agar (LSPR), a selective differential medium developed by Castillo et al. (29) that simultaneously allows enumeration of rifampicin-resistant *E. coli* O157:H7 and *S. Typhimurium* marker organisms, was utilized for all experiments. Per liter, this medium contains 40 g tryptic soy agar (TSA, Difco, Detroit, MI), 3 g yeast extract (Difco), 3 g beef extract (Difco), 5 g lactose (EMI industries, Inc., Gibbstown, NJ), 2.5 g sodium sulfite (MCB Reagents, Cincinnati, OH), 0.3 g ferrous sulfate (MCB Reagents), 25 mg phenol red (Fisher Scientific, Fair Lawn, NJ), 0.1 g cycloheximide (Sigma Chemical, St Louis, MO), and 0.1 g rifampicin (Sigma Chemical). The phenol red was dissolved in 2 ml of 0.1 N sodium hydroxide before adding it to the medium. Prior to adding the rifampicin, the medium was autoclaved at 121°C for 15 min and then cooled to 50°C. The rifampicin was dissolved in 5 ml of methanol and filter-sterilized prior to adding to the sterile medium. The medium was poured into petri dishes and dried at 25°C overnight before use. When grown on this medium the rifampicin-resistant *E. coli* O157:H7 produces yellow colonies and the rifampicin-resistant *S. Typhimurium* produces colonies with a black center surrounded by a pink halo.

Bacterial cultures. Rifampicin-resistant mutants used as inocula in this study were *S. Typhimurium* obtained from *S. Typhimurium* ATCC 13311 and *E. coli* O157:H7 obtained from ground beef implicated in a Washington outbreak in 1993 from P.I. Tarr, Children’s Hospital and Medical Center, Seattle Washington. The growth curves, heat
resistance and acid sensitivity of these mutant strains were determined by previous studies to be identical to the parent strains (52, 59). These rifampicin-resistant cultures were produced by a procedure previously described by Kaspar and Tamplin (61). The rifampicin-resistant organisms were maintained on cryogenic beads (Protect™ Bacterial Preservers; Key Scientific Products, Round Rock, TX) at -80°C and a stock solution was prepared by transfer of these bacteria to tryptic soy broth (TSB) and incubated at 35°C for 12 to 14 h. Confirmation of rifampicin-resistance was confirmed by streaking TSB cultures onto LSPR plates and incubating at 35°C for 24 h. Characteristic colonies were inoculated into TSB and incubated at 35°C for 12 h for use as a stock marker organism inoculum.

**Meat samples.** For Experiments 1 and 2, randomly selected outside rounds were purchased from a local commercial establishment and sliced into approximately 5-mm thick sections, which were tray packed and polyvinyl chloride (PVC) overwrapped.

For Experiment 3, six hot-boned carcass outside rounds and six hot-boned carcass briskets were obtained from a local commercial processing plant and transported from the slaughter floor to the Texas A&M University Food Microbiology Laboratory (College Station, TX), approximately 10 miles away, wrapped in shrouds and placed in insulated containers to maintain temperature. The carcasses were not washed nor decontaminated in any manner before the cuts were obtained for this study.

**Sampling and microbiological analysis.** For Experiments 1 and 2, packages were aseptically opened and slices were individually removed using flame-sterilized scalpel and forceps. Representative samples measuring approximately 10-cm² x 5-mm
were then excised from each slice using a flame-sterilized stainless steel borer, scalpel and forceps, and placed in sterile disposable petri dishes to serve as sample meat surfaces. For Experiment 3, representative meat samples were obtained from the brisket and outside round cuts by using a sterile stainless steel borer, scalpel and forceps to excise sections approximately 10-cm$^2$ x ~2-mm thick. Each meat sample was composited in a stomacher bag to which 99 ml of sterile 0.1% peptone water was added before examination.

Counts of rifampicin-resistant *E. coli* O157:H7 and *S. Typhimurium* were obtained by plating appropriate dilutions of the composited samples onto the selective LSPR agar plates. The LSPR agar plates were incubated for 24 hours at 37°C after which visual counts were performed. The countable range for each pathogen was between 25 to 250 colonies per plate and all counts were transformed into logarithms prior to data analysis for comparability.

**Xylitol preparation.** Xylitol (Sigma) was prepared as 10% solutions in each experiment. Studies examining the effects of xylitol on *S. mutans, H. influenzae, C. difficile, M. catarrhalis* and other pneumococci, utilized a variety of concentrations of xylitol ranging from 0.5 % up to 10% (63, 64, 95, 100, 106). One study examining the inhibition of adhesion of *C. difficile* in the presence of xylitol achieved the greatest reduction in bacterial adherence with 10% xylitol (78). In addition, a 10% solution equated to xylitol’s maximum solubility in water (13). Consequently, a 10% xylitol solution was selected for these experiments in order to provide the maximum concentration of xylitol with respect to the solubility limitations of water as a diluent.
**Experiment 1: Pre-treatment of pathogens with 10% xylitol.** One ml of each stock bacterial pathogen culture was transferred to sterile, freshly prepared TSB and incubated for 24 h at 37°C then 5 ml of each pathogen was combined into a sterile centrifuge tube and designated as the “cocktail.” The inoculum was prepared by dispensing 3 ml of the cocktail into 297 ml of 0.1% peptone water and another 3 ml of the cocktail into 297 ml 10% xylitol solution. The inoculum contained approximately 9.0 mean log per ml of *E. coli* O157:H7 and 8.8 mean log per ml of *S. Typhimurium* throughout the experiment. The preparation of the xylitol inoculum was conducted immediately prior to the experiment allowing the pathogens approximately 5 min of exposure to the 10% xylitol. Six bottles containing 50 ml of sterile water were also prepared to serve as individual rinses for selected samples.

After inoculation of fifteen meat samples, three samples were randomly selected as baseline samples and were transferred into individual sterile stomacher bag containing 99 ml of sterile 0.1% peptone diluent. Each sample was pummeled for 1 min using a Stomacher-400 (Tekmar Company, Cincinnati, OH) after which appropriate dilutions were plated on LSPR agar for enumeration of *E. coli* O157:H7 and *S. Typhimurium* to determine the background load of marker organisms. The remaining meat samples were subjected to one of 4 treatment combinations, with three samples per treatment. Treatment combinations consisted of one of two inoculation types (NoX, 0.1% peptone inoculum, or X, 10% xylitol inoculum) followed by one of two post-inoculation treatments (R, rinsed in sterile water, or NoR, not rinsed). Control (NoX+NoR) samples were immersed in the 0.1% peptone inoculum for 5 s, removed and allowed to drain for
5 s, then allowed a 10-min dwell time, after which samples were stomached, diluted and plated as previously described. Samples in the NoX+R combination were immersed in the 0.1% peptone inoculum for 5 s, removed and allowed to drain for 5 s, then allowed a 10-min dwell time, after which samples were rinsed by immersion for 5 s in individual sterile water bottles, removed, drained for 5 s and then stomached, diluted and plated as previously described. Samples treated with the third combination (X+NoR) were immersed in the 10 % xylitol inoculum for 5 s, removed, drained for 5 s and allowed a 10-min dwell time, after which those samples were stomached, diluted and plated as previously described. The final combination (X+R) samples were immersed in the 10 % xylitol inoculum for 5 s, removed, drained for 5 s and allowed a 10 min dwell time and were rinsed by immersion for 5 s in individual sterile water bottles, removed, drained for 5 s and then stomached, diluted and plated as previously described. Residual solution from the sterile rinse water bottles was also sampled, diluted and plated on the LSPR agar. The entire experimental procedure was repeated on a second day to yield a sample size of n = 6 for each treatment combination.

**Experiment 2: Pre-treatment of meat surfaces with 10% xylitol.** One ml of each stock bacterial pathogen culture was transferred to fresh TSB and incubated for 24 h at 37°C then 5 ml of each pathogen was combined into a sterile centrifuge tube and designated as the “cocktail”. An inoculum solution was prepared by dispensing 3 ml of the cocktail into 297 ml of 0.1% peptone water. Three inoculum solutions as described were prepared for each experiment day. The inoculum solutions contained approximately 8.8 mean log per ml of *E. coli* O157:H7 and 8.4 mean log per ml *S.*
Typhimurium over the two days. One container of 300 ml of sterile water, one container of 300 ml of 10% xylitol, nine bottles containing 50 ml of sterile water and nine bottles containing 50 ml of 10% xylitol were also prepared to serve as collective and individual rinses for selected samples.

As in Experiment 1, three samples were selected as baseline samples for marker organisms and were stomached, diluted and plated on LSPR agar according to the previously described procedure. Treatments consisted of combinations of one of three pre-inoculation strategies and one of three post-inoculation strategies. Pre-inoculation strategies included no pre-inoculation treatment (NoR), pre-inoculation immersion in sterile water (W) or pre-inoculation immersion in a 10% xylitol solution (X). These same strategies were employed post-inoculation, yielding nine treatment combinations (NoR+NoR, NoR+W, NoR+X, etc.) Nine meat samples were randomly selected as the first group and received no pretreatment (NoR) before being immersed into the first 300-ml inoculum container for 5 s, drained for 5 s and allowed a 10 min dwell time. Three of these nine samples received no post-inoculation treatment and were stomached, diluted and plated on LSPR agar in accordance with the previously described procedures (NoR+NoR). Three more samples from the first group were rinsed in individual 50-ml bottles of sterile water for 5 s, drained and then stomached, diluted and plated (NoR+W). The remaining three samples were rinsed in individual 50-ml bottles of 10% xylitol for 5 s, drained and then stomached, diluted and plated (NoR+X). The second group (W) consisted of nine meat samples and these samples were aseptically immersed in a 300-ml container of sterile water for 5 s, removed and allowed to drain for 5 s before being
immersed into a second 300-ml inoculum container for 5 s, drained for 5 s and allowed a 10 min dwell time. Three of these nine samples received no post-inoculation treatment and were stomached, diluted and plated on LSPR agar in accordance with the previously described procedures (W+NoR). Three more samples from the first group were rinsed in individual 50-ml bottles of sterile water for 5 s, drained and then stomached, diluted and plated (W+W). The remaining three samples were rinsed in individual 50-ml bottles of 10% xylitol for 5 s, drained and then stomached, diluted and plated (W+X). The third group (xylitol pre-inoculation; X) consisted of the last nine meat samples and these samples were aseptically immersed in a 300-ml container of 10% xylitol for 5 s, removed and allowed to drain for 5 s before being immersed into the last 300-ml inoculum container for 5 s, drained for 5 s and allowed a 10 min dwell time. Three of these nine samples received no post-inoculation treatment and were stomached, diluted and plated on LSPR agar in accordance with the previously described procedures (X+NoR). Three more samples from the first group were rinsed in individual 50-ml bottles of sterile water for 5 s, drained and then stomached, diluted and plated (X+W). The remaining three samples were rinsed in individual 50-ml bottles of 10% xylitol for 5 s, drained and then stomached, diluted and plated (X+X). The post-inoculation sterile rinse water and 10% xylitol bottles were also sampled, diluted and plated on the LSPR agar. The entire experimental procedure was repeated on a second day to yield a sample size of n = 6 for each treatment.

**Experiment 3: Effect of xylitol on meat with pre-existing pathogen contamination.** Inoculum preparation for Experiment 3 consisted of transferring 1 ml of
each stock pathogen culture into fresh TSB and incubating for 24 h at 37°C. These incubated TSB stock cultures were each diluted by placing 1 ml into 9 ml of 0.1% peptone and vortexing the cultures, then removing 0.1 ml from each and placing them into one vial containing 9.8 ml of 0.1% peptone. Approximately 10 g of fresh bovine feces was obtained from the Animal Science Teaching, Research and Extension Complex (ASTREC) at Texas A&M University and placed into a sterile stomacher bag. The 10-ml pathogen culture previously prepared was added to the feces and hand kneaded for 1 min to disperse the marker pathogens.

Meat samples for this experiment consisted of six outside rounds and six briskets. One brisket (B) and outside round (OR) were selected as negative controls and representative samples were removed by excising three 10-cm² sections approximately 2 mm in depth using a sterile stainless steel borer, scalpel and forceps. These samples were stomached, diluted and plated on LSPR agar as previously described. Representative samples were obtained and sampled throughout the experiment in the same manner as described. All twelve cuts were then inoculated with the marker pathogen containing feces by uniformly spreading a slurry with a sterile spatula over the exposed tissue surface on one side. Positive control samples were then taken from one OR and one B at time zero and again in 24 h after storage at 4°C. The initial pathogen load for positive controls averaged 5.5 log CFU/cm². The remaining ten cuts received a low pressure manual wash to remove gross fecal matter. Each cut was hung and 1.5 liters of water (25°C) was sprayed at 10 psi for 90 s using a hand-held non-corrosive polyethylene compressed air sprayer (10.56 L, Universal-Gerwin, Saranac, MI). The no-treatment
The group (N) consisted of 0-h and 24-h samples (after incubation at 4°C) taken from one OR and one B that received only the hand wash. The hot water treatment (HW) group comprised one OR and one B that received a high pressure wash in an automated spray cabinet (Chad, Co., Lenexa, KS) as described in Hardin et al (52). Five l of potable water at 35°C was sprayed on meat cuts hung in the cabinet for a total of 9 s. The spray started at an initial pressure of 250 psi for 4 s, gradually increasing to 400 psi over 2 s and maintaining this pressure for 3 s to complete the treatment. Representative samples were obtained from these cuts after treatment as well as after 24 h of incubation at 5°C.

The lactic acid treatment (LA) group of one OR and one B received acid decontamination of the carcass surface regions using a 2.5 % v/v L-lactic acid (pH 2.2) which was prepared from 88% L-lactic acid stock (Purac, Inc., Arlington Heights, IL). The acid treatment was applied as a hot (55°C) fine mist and consisted of approximately 200 ml sprayed at 40 psi at a distance of 80 cm from the meat surface for 11 s using a hand-held non-corrosive polyethylene compressed air sprayer as previously described. Representative samples again were obtained from these cuts after treatment as well as after 24 h of incubation at 5°C. The xylitol treatment (X) group consisted of one OR and one B to which a 10% xylitol solution was sprayed as a fine mist (25°C) and applied in the same manner as the lactic acid spray. Approximately 200 ml of 10% xylitol was applied to the meat surfaces. Representative samples were taken immediately after treatment and after a 24-h incubation at 4°C. The next treatment group (HW+X) received a hot water wash followed by the xylitol treatment as previously described for each. Samples were taken after treatment was complete and again after 24 h incubation.
at 4°C. The final treatment group (LA+X) consisted of the last OR and B receiving a
lactic acid treatment followed by a xylitol treatment as described previously.
Appropriate samples were taken in the same manner as the other treatments.

**Statistical analysis.** Data were analyzed by general linear models (GLM)
procedures utilizing SAS version 9.0 (SAS Institute Inc., Cary, NC). Results from
Experiment 1 were evaluated as a 2x2 factorial arrangement of treatments in a
randomized complete block design, with day used as a blocking factor. Experiment 2
was evaluated as a 3x3 factorial treatment arrangement in a randomized complete block
design with day serving as the blocking factor. Experiment 3 was analyzed as a
randomized complete block design with cut serving as the blocking factor. In all
experiments, means were separated by overall F-test protected Fisher’s Least Significant
Difference for pairwise comparisons.
RESULTS, DISCUSSION AND CONCLUSION

**Experiment 1.** Experiment 1 examined the potential effects of pathogen exposure to 10% xylitol prior to inoculation of meat surfaces on the ability of *E. coli* O157:H7 and *S. Typhimurium* to attach to the meat in combination with or without a sterile water rinse. No significant interaction was observed between the type of inoculum and post inoculum rinse (P > 0.84), therefore; only main effects will be presented.

Table 1 depicts the log reductions of each pathogen after treatment. There was no difference in the log reduction of either pathogen between X and the NoX samples (P > 0.76). However, R reduced the pathogen load on the meat surface by approximately 0.4 log CFU/cm$^2$ as shown in Table 2 (P < 0.01).

Table 3 portrays the mean populations (log CFU/cm$^2$) of each pathogen recovered from the rinse water samples after treatment. If bacterial attachment is hindered by pretreatment bacterial exposure to 10% xylitol, more pathogens should be recovered from the rinse water used on X than the NoX samples. However, there was no difference in the mean population of *S. Typhimurium* recovered from the two different rinsing solutions (P = 0.27). Although the population mean difference for *E. coli* O157:H7 between rinse water used on X and NoX treated samples approached statistical significance (P = 0.08), it is doubtful that this separation of no more than 0.2 log cycles is biologically meaningful, as it is less than the enumeration method minimum detection level of 0.5 log/cm$^2$ (29). If it were biologically meaningful, significant differences in population means between X and NoX treated meat samples would
TABLE 1. Mean log reductions in populations of *E. coli* O157:H7 and *Salmonella Typhimurium* inoculated onto representative 10-cm² meat samples after pathogen incubation with either 10% xylitol or 0.1% peptone water

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Microorganism</th>
<th>10% Xylitol (X)</th>
<th>0.1% Peptone (NoX)</th>
<th>S.E.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em> O157:H7</td>
<td>0.2 0.2 ± 0.05</td>
<td>0.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>S. Typhimurium</em></td>
<td>0.2 0.2 ± 0.06</td>
<td>0.76</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( ^{a} \text{n} = 12. \)
TABLE 2. *Mean log reductions in populations of E. coli O157:H7 and S. Typhimurium inoculated onto representative 10-cm² meat samples after sterile water rinse (R) or no treatment (NoR)*

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>None (NoR)</th>
<th>Sterile Water (R)</th>
<th>S.E. (^a)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>0.0 (^b)</td>
<td>0.4 (_B)</td>
<td>± 0.05</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><em>S. Typhimurium</em></td>
<td>0.0 (_A)</td>
<td>0.4 (_B)</td>
<td>± 0.06</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

\(^a\) n = 12.  
\(^b\) Mean log reductions within row with same letter (\(_A\) or \(_B\)) are not significantly different (P > 0.05).
TABLE 3. Mean populations (log CFU/cm²) of E. coli O157:H7 and S. Typhimurium recovered from pathogen inoculated meat sample rinse water

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Inoculum</th>
<th>10% Xylitol (X)</th>
<th>0.1% Peptone (NoX)</th>
<th>S.E. (^a)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli O157:H7</td>
<td></td>
<td>4.6</td>
<td>4.8</td>
<td>± 0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td></td>
<td>4.6</td>
<td>4.7</td>
<td>± 0.07</td>
<td>0.27</td>
</tr>
</tbody>
</table>

\(^a\) n = 6.
have been expected. However, there was no observed difference in the population means between X and NoX, thereby supporting the conclusion that the separation observed for the rinse water samples is not biologically meaningful.

This experiment revealed that pre-treating *E. coli* O157:H7 and *S. Typhimurium* with X had minimal effect on these two pathogen’s ability to attach to meat surfaces, contrary to the hypothesis that by subjecting these pathogens to xylitol, their lectins would become bound and thus preventing or hindering bacterial attachment to meat surfaces. It did however support the preponderance of evidence that rinsing meat surfaces after exposure to pathogenic bacteria will significantly minimize subsequent pathogen attachment (15, 28, 52, 96).

**Experiment 2.** Experiment 2 examined the effects of pre- and post-inoculation treatments of a representative meat sample with 10% xylitol on the ability of *E. coli* O157:H7 and *S. Typhimurium* to attach to the meat. There was no interaction between pre- and post- inoculum treatment (P > 0.50), so again only main effects will be presented.

Pre-treating meat surfaces X had no effect on subsequent load of *E. coli* O157:H7 (P > 0.19) as depicted in Table 4, but revealed a statistically significant smaller mean log reduction in the number of *S. Typhimurium* recovered from X+X treated samples (P < 0.01). While this numerical difference may be statistically significant, again, the biological relevance of a 0.3 log cycle difference is questionable. Table 5 shows that post-inoculation rinsing of samples, regardless of type of pre-inoculation
TABLE 4. *Mean log reductions in populations of E. coli O157:H7 and S. Typhimurium inoculated onto representative 10-cm² meat samples after pre-inoculation treatment of no rinse (NoR), sterile water rinse (W) or 10% xylitol rinse (X)*

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>None (NoR)</th>
<th>Sterile Water Rinse (W)</th>
<th>10% Xylitol Rinse (X)</th>
<th>S.E. (^a)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli O157:H7</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>± 0.06</td>
<td>0.19</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>0.4(^b)</td>
<td>0.4(^A)</td>
<td>0.1(^B)</td>
<td>± 0.07</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(^a\) n = 18.

\(^b\) Mean log reductions within row with same letter (\(A\) or \(B\)) are not significantly different (P > 0.05).
TABLE 5. Mean log reductions in populations of E. coli O157:H7 and S. Typhimurium inoculated onto representative 10-cm² meat samples after pre-inoculation treatment, and followed by a post-inoculation treatment of no rinse (NoR), sterile water rinse (W) or 10% xylitol rinse (X)

<table>
<thead>
<tr>
<th></th>
<th>No Rinse (NoR)</th>
<th>Sterile Water Rinse (W)</th>
<th>10% Xylitol Rinse (X)</th>
<th>S.E. (^a)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microorganism</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli O157:H7</td>
<td>-0.1(^b)</td>
<td>0.2(_B)</td>
<td>0.2(_B)</td>
<td>± 0.06</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>-0.1(_A)</td>
<td>0.5(_B)</td>
<td>0.5(_B)</td>
<td>± 0.07</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

\(^a\) n = 18.  
\(^b\) Mean log reductions within row with same letter (\(_A\) or \(_B\)) are not significantly different (P > 0.05).
treatment solution, minimized the subsequent pathogen load of *E. coli* O157:H7 and *S. Typhimurium* (P < 0.01).

These results are in agreement with Experiment 1’s conclusion that rinsing meat surfaces after exposure to pathogenic bacteria will significantly minimize subsequent pathogen attachment. It also supports the previous findings and conclusion that there is no difference between X and W when they are used as a rinse.

A significant interaction existed between pre- and post-inoculation treatments for both *E. coli* (P = 0.02) and *S. Typhimurium* (P = 0.05) recovery from rinsing media (Table 6). Sampling results of the rinses used as post-inoculation treatments reveals that for *E. coli* O157:H7, X+X treatment led to an increased pathogen recovery from the rinsing agent compared to other treatment combinations. This result is consistent with the hypothesis, but is contradicted by previous results from this experiment. Specifically, no decreases in pathogen numbers on the meat surface were observed. Interestingly enough however, for *S. Typhimurium*, the opposite effect was seen, as between 8–10% more CFU/cm² were recovered from W than X (P < 0.02). If this interaction is real, it means that rinsing in X actually removes fewer pathogens than rinsing with W, unless X had been applied prior to the inoculation; however this is not supported by reductions in pathogen counts on the meat surfaces. Upon closer examination of the *E. coli* O157:H7 data, an outlier was identified, where more than 5.9 log CFU/ cm² were reported to have been recovered. The removal of the outlier, made the difference between the rinsing agent population means for *E. coli* O157:H7 insignificant (P > 0.09). The question as to biological relevance again applies to these results. Even with the outlier included, the
TABLE 6 Mean populations (log CFU/cm²) of E. coli O157:H7 and S. Typhimurium recovered from sterile water or 10% xylitol used to rinse representative 10-cm² meat samples after undergoing pre-inoculation treatment of no rinse (NoR), sterile water (W) or 10% xylitol rinse (X) followed by pathogen inoculation and then subjected to a sterile water (W) or 10% xylitol rinse (X)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Sterile Water (W)</th>
<th>10 % Xylitol (X)</th>
<th>Sterile Water (W)</th>
<th>10 % Xylitol (X)</th>
<th>Sterile Water (W)</th>
<th>10 % Xylitol (X)</th>
<th>S.E.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli O157:H7</td>
<td>4.5&lt;sub&gt;AB&lt;/sub&gt;</td>
<td>4.4&lt;sub&gt;A&lt;/sub&gt;</td>
<td>4.4&lt;sub&gt;A&lt;/sub&gt;</td>
<td>4.6&lt;sub&gt;AB&lt;/sub&gt;</td>
<td>4.3&lt;sub&gt;A&lt;/sub&gt;</td>
<td>5.0&lt;sub&gt;B&lt;/sub&gt;</td>
<td>± 0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>4.2&lt;sub&gt;A&lt;/sub&gt;</td>
<td>3.8&lt;sub&gt;BC&lt;/sub&gt;</td>
<td>4.0&lt;sub&gt;AB&lt;/sub&gt;</td>
<td>3.7&lt;sub&gt;C&lt;/sub&gt;</td>
<td>4.0&lt;sub&gt;AB&lt;/sub&gt;</td>
<td>4.1&lt;sub&gt;A&lt;/sub&gt;</td>
<td>± 0.10</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<sup>a</sup> n = 6

<sup>b</sup> Means within row with same letter (<A, B or C>) are not significantly different (P > 0.05).
separation between rinses is at most 0.7 log cycles making the true significance of this result questionable, especially in the face of the previous experimental results.

**Experiment 3.** Experiment 3 examined the practical application of xylitol as an intervention to minimize existing pathogen contamination on meat surfaces by comparing it to and combining it with the proven interventions of hot water wash and lactic acid sprays. Samples were obtained immediately after the final treatment (0-h) as well as after 24-h incubation at 4°C (24-h). Experiment 3 results are depicted in Table 7. Treatments had minimal impact on population means for the 0-h samples of *E. coli* O157:H7 (P = 0.68), while for *S. Typhimurium*, treatments resulted in differences in pathogen populations (P < 0.01). Treatment with only the X did not reduce pathogen load relative to the N group (P = 0.62). LA or LA+X resulted in significant reductions of *Salmonella* (P < 0.01). HW and HW+X resulted in *Salmonella* log counts intermediate to N and LA. For the 24-h samples, again no difference was detected in the *E. coli* O157:H7 samples (P > 0.08), but for *S. Typhimurium*, LA and LA+X significantly minimized the pathogen load over all other treatments (P < 0.04).

Scrutiny of the results from this experiment shows a relatively high standard error between 24 and 32% of the overall mean for the *E. coli* O157:H7. While the source of this variability is unknown, the high variability observed is understandable due to the small sample size (n = 2) of this study, and most likely accounts for a reduction in the sensitivity of mean comparisons relative to the first two experiments. However,
TABLE 7. Mean log reductions in populations of *E. coli* O157:H7 and *S. Typhimurium* inoculated onto outside round and brisket surfaces after being subjected to selected treatments and sampled immediately and again after 24 h incubation at 4° C

<table>
<thead>
<tr>
<th>Treatments</th>
<th>None (N)</th>
<th>Hot Water (HW)</th>
<th>2.5% Lactic Acid (LA)</th>
<th>10% Xylitol (X)</th>
<th>HW + X</th>
<th>LA + X</th>
<th>S.E.a</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microorganism</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>0.0</td>
<td>1.4</td>
<td>1.3</td>
<td>-0.1</td>
<td>1.5</td>
<td>1.7 ± 1.0</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td><em>S. Typhimurium</em></td>
<td>0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;D&lt;/sup&gt;</td>
<td>-0.4&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;C&lt;/sup&gt;</td>
<td>3.4&lt;sup&gt;D&lt;/sup&gt;</td>
<td>± 0.4</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>24 hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>0.0</td>
<td>0.7</td>
<td>2.1</td>
<td>-0.4</td>
<td>-0.3</td>
<td>2.6 ± 0.7</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td><em>S. Typhimurium</em></td>
<td>0.0&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;B&lt;/sup&gt;</td>
<td>-0.1&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.3&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;B&lt;/sup&gt;</td>
<td>± 0.5</td>
<td>0.04</td>
</tr>
</tbody>
</table>

<sup>a</sup> n = 2.

<sup>b</sup> Mean log reductions within row with same letter (A, B, C, or D) are not significantly different (P > 0.05).
trends in *E. coli* O157:H7 responses follow observations for *S. Typhimurium*, suggesting that increasing the sample size would probably bring these population mean differences into statistical significance.

Overall, the data from Experiment 3 supports that the proven intervention of LA is effective in minimizing pre-existing pathogen loads on meat surfaces, while X is virtually ineffective as a sole intervention. Combining X with HW and LA interventions did not prevent them from being effective at minimizing pathogen loads, but most likely contributed nothing to the effect. The data suggests that HW interventions will make an initial reduction in pathogen numbers, followed by a recovery and subsequent regrowth by 24 h. In addition, it reiterates the fact that LA seems to show a residual effect on pathogen loads and steadily minimize their counts. Castillo et al. (31) demonstrated this residual action in ground beef produced from rounds that had received lactic acid treatment during storage where the data trend lines indicated small reductions in the pathogen counts over 21-day period.

While the combined results from these experiments fail to support the hypothesis that X can be used to effectively minimize adhesion of the bacterial pathogens *E. coli* O157:H7 and *S. Typhimurium* to carcass surfaces, it is plausible that the underlying concepts were and still are correct. A number of factors might well have played a role in the failure to reject the null hypothesis.

The first consideration is that the specific bacterial pathogens used as marker organisms may not express any lectins that are specifically blocked by xylitol. Previous research has shown that *S. Typhimurium* adhesion can be blocked by specific
oligosaccharides in vitro (112) and that a specific glycoprotein lectin expressed by this pathogen has an affinity for mannose (92). Although no specific mention of E. coli O157:H7 is made, E. coli strains have also been shown in vitro as well as in vivo to have its adhesive properties inhibited by a variety of specific oligosaccharides (112). In fact, Mouricout et al. (76) found that administration of an anti-adhesin preparation containing 250 mg of oligosaccharides to calves on average led to a 2.1-log reduction in the number of adherent E. coli K99 challenge bacteria per g of intestinal epithelial tissue over controls. This anti-adhesin activity was consistent with their in vitro results demonstrating the successful employment of an oligosaccharide in preventing adhesion and colonization of the calf intestine by a pathogenic E. coli. While xylitol has been shown to affect the adhesion and colonization of a wide array of pathogenic bacteria (64, 78, 99, 106, 109), it is very possible that it does not affect E. coli O157:H7 and S. Typhimurium in the same manner.

Another consideration might be that these pathogens do express lectins that react with xylitol, but do not block adhesions because of additional binding sites on the lectin. Bacterial lectin-adhesins have been identified that recognize terminal saccharide structures, but many may recognize internal sequences as well (81). What may occur is that the xylitol may bind the terminal receptor of the bacterial lectin, but additional binding sites on the lectin are unaffected by the xylitol and subsequently permit binding to the tissue surfaces. An extension of this possibility is that some tissue receptors have multiple attachments sites for two or more adhesins. While the lectin adhesin may be
itself blocked by the xylitol, it still may permit a protein-protein, or hydrophobin-protein adhesin to bind (81).

It was theorized in the planning of these experiments that a 10% solution of xylitol (~ 600 mM), its maximum solubility at room temperature, should overcome any problems associated with the appropriate concentration of xylitol being available. It has been shown that the affinity of sugars for lectins in usually very low, with the concentration required for 50% inhibition being in the millimolar range. The possibility exists that the xylitol concentration may still have not been high enough to observe the desired blockage of bacterial lectins. However, suitable chemical derivatization can increase this affinity by several orders of magnitude (92). As an example, hydrophobic α-mannosides of methylumbelliferyl or p-nitrochlorophenyl were 500 to 1000 times more inhibitory that the methyl α-mannoside of the adhesion of type 1 fimbriated *E. coli* to yeasts or rabbit ileal epithelial cells (49). Another method to increase affinity of the inhibitors of bacterial lectins includes attaching them to polymeric carriers to form multivalent ligands (65). It might be possible that xylitol’s affinity to the lectins of *E. coli* O157:H7 and *S. Typhimurium* may be too low at the 10% concentration and that chemically altering the xylitol may render a molecule that successfully blocks the lectins and prevents adhesion of the bacteria to tissue receptors.

Our results could have been confounded by how the meat samples were prepared for Experiments 1 & 2. Strong bacterial attachment to meat surfaces has been attributed to exposed collagen surfaces (108). Since the samples used in these first two experiments were cut from outside rounds that were sliced, and then representative 10-
cm² samples excised from the slices, there was not any surface of the sample that truly would be consistent with the typical surface of a carcass. Carcass surfaces contain adipose and connective tissue and, for the most part, the muscle tissues remain intact, limiting exterior exposure to muscle fiber collagen. Our samples maximized exposure to the cut surfaces of the muscle fibers thus maximizing exposure to collagen. The strong attachment interaction with the ample exposed muscle collagen may have been far too extensive for the xylitol to overcome.

In conclusion, this research suggests that 10% xylitol solutions yield no effect on minimizing the attachment of \textit{E. coli} O157:H7 and \textit{S. Typhimurium} to meat surfaces, regardless of whether the pathogens are incubated with the xylitol or if the xylitol is present on the meat surfaces prior to contamination by the pathogens. In addition, 10% xylitol solution utilized as a post-contamination intervention failed to minimize \textit{E. coli} O157:H7 and \textit{S. Typhimurium} load on meat surfaces. However, the effect that 10% xylitol may exert on non-pathogenic spoilage bacteria was not examined by this study, and it is possible that xylitol could prove useful in reducing economic loss from spoilage organisms. While this study may potentially eliminate use of 10% xylitol as an intervention for minimizing \textit{E. coli} O157:H7 and \textit{S. Typhimurium} contamination of beef carcasses, the underlying value of this study is its new approach to dealing with bacterial contamination of beef carcasses and is an important venture into the novel application of anti-adhesion compounds. Traditional intervention methods have relied on bactericidal methods to minimize contamination; whereas, this is one of the first examinations of the
potential use of anti-adhesion compounds to minimize bacterial contamination and pathogen load on beef carcasses.
REFERENCES


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