Susceptibility of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*, Inoculated onto Beef Tissues, Steaks and RTE Products, to Lactic Acid, Lactoferrin and Activated Lactoferrin

Project Summary

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Background

Highly publicized outbreaks of food-borne illness since 1993, primarily caused by bacteria such as *E. coli* O157:H7, *Salmonella spp*. and *Listeria monocytogenes*, elicited intense consumer concern about meat safety. In response, regulatory authorities, researchers and the beef industry initiated efforts to implement food safety management systems that would improve microbiological quality. The USDA Food Safety and Inspection Service (FSIS) began initiating new regulatory requirements during the mid-1990s. Packers were required to knife-trim carcasses to remove all visible contaminants, comply with written sanitation standard operating procedures (SSOP), implement Hazard Analysis Critical Control Point (HACCP) systems, and meet microbiological performance criteria and standards for *E. coli* and *Salmonella* as a means to verify HACCP effectiveness and pathogen reduction.

Researchers and beef packers/processors have addressed consumer food safety concerns by developing a variety of methods that are now implemented, or are being further developed, to reduce numbers of bacteria on beef and beef products and improve microbiological safety. These microbiological decontamination technologies include:

- Animal cleaning;
- Chemical dehairing at slaughter;
- Spot-cleaning of carcasses by knife-trimming or steam/hot water vacuuming; and
- Spraying/washing/rinsing of carcasses before evisceration and/or before chilling, with water, chemical solutions and/or steam or hot water.

The most commonly used decontamination strategies involve the use of water and steam at various temperatures and spray pressures. Other decontamination strategies involve the use of FDA-approved chemicals applied through water-based sprays. The main objective of this study was to determine the effectiveness of lactic acid, lactoferrin and activated lactoferrin in inhibiting growth of *E. coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* inoculated onto beef tissues, steaks and ready-to-eat (RTE) products.

Methodology

This project consisted of four separate studies as follows: (1) RTE, which used bologna prepared in the laboratory as representative of a beef RTE product; (2) Boneless Beef Short Plates, purchased from a local meat processor; (3) Lean Tissue Pieces, purchased from a local meat processor; and (4) Adipose Tissue Pieces, collected from the brisket of carcasses as they exited the thermal decontamination spray cabinet at a large commercial packing plant.

The first three studies (RTE, Boneless Beef Short Plates and Beef Lean Tissue Pieces) received the same inoculations and treatments. All samples received treatment either before (pre) or after (post) inoculation. The samples were then refrigerated at 50-54^{o} Fahrenheit (10-12 ^{o}C) and microbiological counts measured on the days enumerated in Table 1. The RTE and Boneless Beef Short Plate samples were vacuum packaged, whereas the Lean Tissue and Adipose Tissue samples were packaged in Styrofoam trays covered with air-permeable film.

The last study (Adipose Tissue Pieces) received inoculation only before (pre) treatment application and was subjected to two additional sequential treatments (Activated Lactoferrin followed by 2% Lactic Acid and 2% Lactic Acid followed by Activated Lactoferrin). The samples were then refrigerated and microbiological counts measured on days 0, 1 and 2, as shown in Table 1.

TABLE 1

Study	Inoculation	Treatment (Pre and Post)	Days
RTE (Bologna)	*E. coli* O157:H7	No Treatment (Control)	1
	Salmonella Typhimurium	Water	8
	Listeria monocytogenes	Activated Lactoferrin	19
		Non-Activated Lactoferrin	33
		2% Lactic Acid	
Boneless Beef Short Plates	Same as RTE	Same as RTE	0
			4
			8
			15
			29
Beef Lean Tissue Pieces	Same as RTE	Same as RTE	0
			1
			3
			5
			7
Adipose Tissue Pieces	Same as RTE	**Treatment (Pre Only)**	
		No Treatment (Control)	0
		Water	1
		Activated Lactoferrin (ALF)	2
		Non-Activated Lactoferrin	
		2% Lactic Acid (LA)	
		ALF + 2% LA	
		2% LA + ALF	

Findings

The results of this study documented the effectiveness of "activated lactoferrin" (a naturally occurring protein derived from whey and skim milk) for reducing growth of *E.*

coli O157:H7 and *Listeria monocytogenes* in slightly temperature-abused RTE products (bologna) and vacuum-packaged beef cuts.

- Treatment of bologna slices with "activated lactoferrin" (ALF) after inoculation with pathogens allowed less increase (compared to untreated Control) in *E. coli* O157:H7 counts, by 1.4 to 2.4 log (log CFU/cm^2), and in *Listeria monocytogenes* counts, by 6.3 to 6.4 log, at 33 days of vacuum-packaged storage at 10°C while treatment before inoculation allowed less increase in *E. coli* O157:H7 (by 0.7 to 1.3 log) and *Listeria monocytogenes* (by 6.2 log).
- Treatment of beef cuts with ALF after inoculation with pathogens allowed less increase (compared to untreated Control) in *E. coli* O157:H7 counts (by 4.6 log) and *Listeria monocytogenes* counts (by 4.6 log) at 29 days of vacuum-packaged storage at 12°C while treatment before inoculation allowed less increase in *E. coli* O157:H7 and *Listeria monocytogenes* counts by 3.3 and 4.7 log, respectively.
- Results of this research study also revealed that use of a combination of ALF followed by 2% lactic acid (LA) was the most effective treatment used in this study for decontaminating inoculated hot beef adipose tissue, with advantages of 1.3, 1.1 and 0.8 log less increase in counts for *E. coli* O157:H7, *S.* Typhimurium and *Listeria monocytogenes,* respectively, at 2 days storage at 12°C, than the second best decontamination treatment (LA).
- Results of the study support use of LA (prior to, or following, inoculation with pathogens) especially for reducing growth of *Salmonella* Typhimurium on vacuum-packaged bologna, vacuum-packaged beef cuts and retail packaged beef cuts as well as for decontaminating freshly harvested beef carcasses.

Implications

The Centers for Disease Control (CDC) estimates that there are 76 million cases of food-borne illness in the United States annually, with 14 million cases attributed to known pathogens. *E. coli* O157:H7 alone is estimated to account for 76,000 cases of food-borne illness and 76 deaths annually. Multiple intervention strategies to inhibit or eliminate *E. coli* O157:H7 in the beef production process are extremely important to the industry. The results of this study suggest that activated lactoferrin is efficacious in inhibiting the growth of *E. coli* O157:H7, Salmonella Typhimurium and *Listeria monocytogenes* on vacuum-packaged bologna, vacuum packaged beef cuts and (with the addition of a lactic acid treatment) freshly harvested beef carcasses. The results further suggest the bactericidal efficacy of 2% lactic acid for reducing the growth of *Salmonella* Typhimurium on vacuum-packaged bologna and beef cuts, retail-packaged beef cuts and for decontaminating freshly harvested beef carcasses.