In Plant Assessment of Lactic Acid Intervention in Variety Meats Through the Beef Processing Line

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Abstract. Beef industries continuously seeks interventions that may reduce bacterial loads in their products to increase shelf life and ensure beef product wholesomeness. This in-plant validation study was conducted to evaluate the effect of spray lactic acid interventions in beef variety meats. Lactic acid solution (2 to 5%) was applied to variety meats by spray application ≥ 103.42 kPa for 5 s. Samples were collected in plant during the processing line by swabbing 500 cm^2 surface area of meat before and after lactic acid applications. A total of 108 samples were collected (54 before and 54 after) for beef liver, cheek, and heart. All samples were shipped to Texas Tech University´s food Microbiology laboratory for microbiological analysis. Composites of three samples were made by adding 3 mL of each sample to a sterile test tube until reaching 9 mL. All composites were serially diluted and plated to determine total aerobic plate counts (APC), coliform counts and generic *E. coli* counts using 3M APC Petrifilm, 3M Coliform/E. coli Petrifilm, respectively. Duplicates were made of each Petrifilm and were incubated at 37 °C. All samples were converted to CFU per cm^2 before statistical analysis. Treatment with lactic acid (2-5%) spray was lower ($P < 0.005$) in APC, generic *E. coli* and total coliforms microbial loads after the intervention on beef cheek, heart, and liver. Therefore, lactic acid (2-5%) spray intervention reduces Aerobic Plate Count, Total Coliforms and generic *E. coli* in beef cheeks, heart, and liver in the processing line.

Key words: Aerobic count**,** cheeks, coliforms, *E. coli,* heart, liver.

Resumen. La industria cárnica busca intervenciones que reduzcan los microorganismos en sus productos para aumentar la vida útil y garantizar la inocuidad. Este estudio se realizó para evaluar el efecto de la intervención con ácido láctico en cachete, hígado y corazón de res. Se aplicó una solución de ácido láctico (2 a 5%) mediante aspersión ≥ 103.42 kPa durante 5 s. Las muestras se recolectaron durante la línea de procesamiento, frotando 500 cm^2 de la superficie antes y después de la intervención. Se recolectaron un total de 108 muestras (54 antes y 54 después) para hígado, mejilla y corazón de res. Todas las muestras se enviaron al laboratorio de microbiología de alimentos de la Universidad de Texas Tech para su análisis. Se realizaron compuestos de tres muestras agregando 3 mL de cada muestra a un tubo de ensayo hasta alcanzar 9 mL. Los compuestos se diluyeron en serie y se sembraron en placas para determinar los aerobios totales, coliformes y *E. coli* genérico utilizando un 3M Petrifilm para aerobias totales, coliformes y *E. coli*. Se hicieron duplicados de cada placa y se incubaron a 37 °C. Todas las muestras se convirtieron a UFC por cm^2 antes del análisis estadístico. La aplicación de ácido láctico al 2 - 5% mostró diferencias estadísticas ($P < 0.05$) en la carga microbiana de APC, *E. coli* genérico y coliformes totales después de la intervención. La intervención en aspersión de ácido láctico (2-5%) redujo el recuento de aerobios totales, coliformes totales y *E. coli* genérica en mejillas de res, corazón e hígado en la línea de procesamiento.

Palabras clave: Aerobios totales, cachete, coliformes, corazón, *E. coli*., hígado.

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1. INTRODUCTION

All meats are sterile if it comes from healthy animals; however, this can be contaminated along the processing line inside the plant, due to the harvest process and the plant conditions (Harris et al. 2006). *Escherichia coli* O157:H7 and *Salmonella* are pathogenic microorganisms that cause concern in the beef industry, since those microorganisms appears frequently on the carcass due to the ability to grow in the digestive tract of ruminants animals (Manyi-Loh et al. 2016). Polygastric animals, such as bovine animals, are asymptomatic to diseases produced by *Escherichia coli* and Salmonella spp. (Pruimboom-Brees et al. 2000). On the other hand, humans can present symptoms if they ingest food contaminated with one of those bacteria, causing damage on their health. It is important that the beef industry continuously search for alternatives process to control and reduce the incidence of spoilage of those microorganisms in food.

During the slaughter process, the plant´s operators must practice good hygienic techniques to prevent further contamination by increasing the bacterial load on the beef carcass. During the slaughter process is where carcasses get contaminated due to lack of hygienic techniques of tools, operators, or the area. Since 1993, the Center of Disease Control and Prevention (CDC) started an intense effort to reduce the incidence of *Escherichia coli* O157:H7 in the red meat supply (Wheeler et al. 2014). This decision was along due to an outbreak in 1992 caused by *Escherichia coli* O157:H7 by the consumption of hamburgers from one restaurant chain (CDC 1993).

The Food Safety and Inspection Service (FSIS) within the United States Department of Agriculture (USDA) supported by the U.S. laws, force the beef industry to design a Hazard and Critical Control Points (HACCP) plan and make sure those are verified and validated continuously (Arthur et al. 2004). The main purpose of a HACCP plan is to identify critical control points during the processing line and introduce corrective and preventive actions to reduce bacterial spoilage risk in food (Kang I-B et al. 2018). In 2014, the Beef Industry Food Safety Council (BIFSCO) was form to unite every beef company and food safety researchers to find solutions to common problems (Anonymous 2014). With the effort of both, beef industries and researchers, the microbiological quality of raw beef have been improved (Koohmaraie et al. 2005). One of the most significant findings was the contamination of the carcass by the beef hide as primary source of contamination (Small et al. 2005).

During the beef processing line contamination in meat happens in multiple ways. Due to that beef industries introduce intervention in different stations in their processing line to reduce the risk of contamination in beef product. Stations such as evisceration or hide removal are one of the most common areas where contamination occur in beef carcasses. Every preventive and corrective action must be validated with scientific data in order ensure the efficacy of it.

In 2012, FSIS declared the implementation of a routine verification of the HACCP plan to control the risk of beef products contaminated by STEC in the beef processing line. Shiga toxin producing *E. coli* is of great concern in meat products due to their morbidity and mortality in many recent large outbreaks related to gastrointestinal disease in the United States. *Escherichia coli* O157:H7 is one of the most controlled pathogens in food, however; recently it had been found other *E. coli* serotypes such as O26, O103, O45, O111, O121 and O145. These other serotypes can produce shiga toxin causing diseases to human by food contaminated with these bacteria (Etcheverría and Padola 2013). In 2012, 265,000 cases of STEC infection were reported in which 34% were from STEC O157, and 64% were from other STECS (CNN 2020). Since American people consume beef products cooked with temperature lower than 160°F, the low infection dose (< 10 cells) and the severity of the disease, the USDA consider as adulterant all shiga toxin-producing *Escherichia coli* (STEC) (Liao et al. 2014). Likewise, *Salmonella spp.* is another pathogen of main concern in beef, due to their presence in the digestive tract in the animal. *Salmonella* is the microorganism that causes the most outbreaks of food illness in the United States (Wolf et al. 2012).

Due to its prevalence along the processing line, the industry uses indicator bacteria to determine the microbiology quality in food such as, generic *Escherichia coli*, aerobic bacteria and total coliforms. In addition, those indicator bacteria determine the presence of *Salmonella* and STEC in food due to the similarity of their multiplication and growth rate (Ghafir et al. 2008; Jiménez Edeza et al. 2012).

To determine the presence of pathogenic microorganisms in carcasses, beef industries continuously make microbiological analysis along the beef processing line. The most used method to determine STEC presence in the carcass is by molecular detection, which main objective is to search for specific virulence genes from specific microorganisms (USDA 2019). Beef industries uses polymerase chain reaction (PCR) to detect presence of the genes *eae* y *stx* which are characteristic for STEC (Gao A et al. 2018). If the results from the PCR are a presumptive positive for STEC, the whole batch is considered as adulterated unless the plant makes an extra procedure to eradicate the microorganism from the carcass. When the PCR stated a presumptive positive sample from one batch, only a confirmatory test (culture) can determine the presence of STEC on the carcass (USDA 2017). Every batch with positive results must be introduced to a different process. Those carcasses must be exposed to a cooking, irradiation, or high-pressure process to achieve the removal of STEC in the product. The presence of STEC on the carcasses causes loss on the plant´s profit because these carcasses go through an extra process, which increases production costs, and the product are sold in a lower price.

Bacterial contamination is inevitable in the transformation process from muscle to meat; therefore, industries should implement intervention where they can assure the elimination or reduction of those bacteria to acceptable levels. Studies had shown that intervention along the process reduces the probability of contamination in the carcasses, which contribute to the shelf-life of the product and reduces the risk of pathogens (Sofos 2005; Wheeler et al. 2014). Chemical rinses and washing are one of the most used intervention due to their effectiveness against microorganisms. However, beef industries use other interventions, such as sanitizing using chlorine solutions, oxidizers, and thermal interventions.

Variety meats are a potential by-product in the beef industry. The demand for these products has been increasing over the years. In 2016, a net export of 280,000 metric tons of beef viscera was recorded in the United States, obtaining a value of USD 754 million as income (U.S. Meat Export Federation 2013). In third world countries, this type of meat is considered as cheap meat, due to its low price for commercialization. Some types of beef viscera are consumed in several countries due to cultural traditions. In addition, variety meats have a potential use as ingredient in processed meat due to its low cost and their water retaining capacity. Since this meat are separate from the carcass in the slaughter process it does not receive the intervention treatment that cold carcass receives along the processing line. There have been many outbreaks on the United States from contamination of pathogens in variety meats. One recent recall was in October 2019, the Canadian Food Safety Agency, recalled various raw beef and veal product which includes beef liver and beef heart of possible spoiled product by *Escherichia coli* O157:H7 (FSN 2019). In addition, beef cheeks also had recalled in the past, on March 2015, a Chicago beef plant establishment recalled possible contamination of *Escherichia coli* O157:H7 in beef cheeks (Illinois Department of Agriculture [updated 07/17/2020 16:36:07]). It is important for the plant to apply intervention to it, to reduce the risk of pathogens.

The main purpose of the research was to evaluate lactic acids interventions on variety meats by counting indicator bacteria. The data obtained were only applicable to the beef plants where the samples were taken. For this research, the following objectives were determined:

- Elaborate a literature review of beef variety meats and antimicrobial interventions in the beef industry.

- Determine total coliform, generic *E. coli* and aerobic count from beef heart, cheek, and liver in the processing line.

- Validate the effect of lactic acid intervention by spray in beef heart, cheek and liver on aerobic count, total coliforms, and generic *E. coli* count on a beef plant facility.

2. MATERIALS AND METHODS

Research location
The research was carried out in a beef processing plant in the state of Nebraska, United States. All the samples were shipped during the night, inside a cooler with ice bags to Texas Tech University located in Lubbock, Texas. The microbiological analysis was conducted in the microbiology laboratory, located in the Experimental Science Building (ESB. Statistical analysis was carried out at Zamorano University.

Research design
The experimental design used was a Completely Randomized Design. During the research, the lactic acid concentration was between 2-5%, and it was applied to cheek, liver, and heart, in a complete uniform spray form with 103.42 kPa for 5 seconds. In the treatment, lactic acid temperature was between 35-45 °C. The samples were taken randomly before and after the lactic acid application along the beef line.

Plant sampling and microbiological analysis

Plant intervention Samples were taken from liver, heart, and cheek inside the beef plant facilities while the plant was operating. For the intervention, all samples were sampled before and after the lactic acid treatment. All viscera pass through a chad cabinet were lactic acid was applied for 5 seconds with a 103.42 kPa spray surrounding the viscera. After the evisceration, pelvic fat was removed from the liver, and the heart had a cut in it. The carcass head was hung on the rail and pass through the chad cabinet, were samples were taken above the cheek over the head.

Variety meats. The collection of samples was carried out randomly in the process line of the plant of the day. Samples were taken from the cheeks, liver, and heart of the beef before and after exposure of lactic acid via spray to these cuts. The swabbing method was performed with a sterile sponge with 25 mL of phosphate buffer on each variety meats. An area of 500 cm^2 was rubbed into the samples, collecting 162 samples (81 before and 81 after). The sponges were immediately cool and transported overnight, inside a cooler to Texas Tech University.

Samples preparation Each bag with its sponge was placed inside a Stomacher (model 400 circulator, Seward, West Sussex, United Kingdom) and these homogenized at 250 rpm for one minute. Consolidation of three samples was performed adding 3 mL of each bag in a sterile test tube until reaching 9 mL of samples, this being the dilution 10^0. Subsequently, a sterile tube with 9 mL of phosphate buffer was taken, and the neck was flamed of the tube for two seconds, with the use of a pipette, 1 mL of the consolidate was transferred and placed in a sterile tube with 9 mL of phosphate buffer, this being the 10^{-1} dilution. Two more dilutions were made, following the same procedure, until the 10^{-3} dilution is reached for the samples taken before the application of lactic acid. For the samples obtained after the application, two dilutions were made, until obtaining the 10^{-2} dilution in a test tube.

Silvering samples Once the 54 samples were diluted, all will be plated. For total Aerobic bacteria 3M ™ Petrifilm™ Aerobic Count Plate was used. The top cover of the test tube was lifted, and 1mL of the test tube was transferred. Once the solution was placed inside, the Petrifilm top cap was released with the objective to fall on the sample. A plate spreader was placed on the drop, which created a circle inside the Petrifilm. The same procedure was performed for the remaining samples and a duplicate of each sample were made.

3M ™ Petrifilm ™ *E. coli* /Coliform Count plates was used to determine the presence of Coliforms and *Escherichia coli*. The top cap of the Petrifilm was lifted and with the use of a pipette, 1 mL of the test tube was placed in the center of the Petrifilm. The upper lid of the Petrifilm was lowered, without letting it fall, avoiding the creation of bubbles inside the plate. The sample spread on its own inside the Petrifilm creating a circle on it. The same process was carried out for the remaining samples and a duplicate of each sample is made. The same Petrifilm was used for the count of Coliforms and Escherichia coli.

The Petrifilm will be collected creating columns of 20 samples and placed on a sterile tray. Subsequently, the trays were placed in an incubator, to proceed with the incubation process of the samples.

Samples incubation and count The trays were placed in an incubator at 37 ºC for a period of 24 hours ± 2 hours to perform the coliform count following the NMKL 147.1993 method). For the total aerobic count and *Escherichia coli,* the samples were incubated at the same temperature for a period of 48 hours ± 2 hours (Jay 2002). After 24 hours, the total Coliforms were manually counted in the Petrifilm, considering the red colonies that produced gas. The data were documented in the assigned logbook and the Petrifilm will be placed back in the incubator to count Escherichia coli after 24 hours. Once the 48 hours were over, the count of total Aerobes and *Escherichia coli* was obtained. The *Escherichia coli* count were done manually, considering only the blue colonies with gas production. Total Aerobic count were done using the 3M ™ Petrifilm ™ Plate Reader kit that counts the red colonies on the plate. Subsequently, the *Escherichia coli* count was added to the Coliform data and the results of total Coliforms, Escherichia coli and aerobic plate counts will be documented in the log.

Statistical analysis for microbiological analysis

With the data obtained, paired T tests were carried out to detect differences before and after the intervention, using a significance level of 0.05. Comparisons were made within each type of microorganism at the same station where the intervention was applied. In addition, comparisons were made in between each type of variety meats before and after the intervention by an analysis of variance. In addition, comparison was made between each type of variety meats from the total reduction of each microorganisms. All comparisons were made in statistical package SAS (9.4).

3. RESULTS AND DISCUSSION

Beef cheeks can be commercialized by whole piece or by processing it obtaining other type of products. This cut is widely used as meat raw material in various meat products such as: hamburger patties, ground meat, manufactured cuts, and canned meat. Due to their low cost, cheeks are used more frequently in the mixture of these processed meat products. In addition, they have a medium cohesion ability; therefore, it can retain water obtaining a juicier texture in the product. When beef cheeks are used as a raw material for the products described above, they must comply with certain regulations described by the United States' Code of Federal Regulation (CFR). Which details that these products must contain no more than 25% of all the mixture used to manufacture these types of food. If in any case, this 25% of the formulation is exceeded, it is the company's obligation to declare it on the label, in the ingredients section or next to the name of the product (The Code of Federal Regulation 1992). Despite its market demand, this meat trimmer has a high probability of contamination by pathogenic bacteria during processing. Studies have shown high levels of bacteria on beef cheeks compared to other muscles, contamination by *Escherichia coli* O157: H7 had also been found on beef cheeks (Carney et al. 2006). It is possible that the carcass is contaminated in the harvest room since the lower part of this, when hanging, is the head and when applying interventions or carcass wash, all the residual water pass through to the carcass head. Carney *et al.* (2006), showed that the prevalence of *Escherichia coli* O157: H7 on the cheeks of cattle was 3% in their study. Therefore, beef cheeks are considered a risk when using this cut in the formulation for ground meat or hamburger patties.

The heart is an involuntary muscle of the animal. It is the main organ in the circulatory system, whose function is to propel blood through the arteries so that it spreads throughout the body. This muscle has been of great importance in the meat industries since it is demanded by the market and can be used in formulations for other meat products. Beef heart is by-product with high demand in China, Hong Kong, Mexico, Japan, and other Asian countries' market. This is because the cultures from these countries have gastronomy adapted to these types of food. Likewise, companies that process sausages and other meat by-products, used beef hearts to introduce them into the formulation. Like beef cheeks, heart is used as ingredients for water retention in the formulation as it has medium cohesion activity. However, this raw material has to comply with certain regulations for its use in meat by-product formulations. For Chili, FSIS only accepts a maximum of 25% of the formulation as beef heart, if it is exceeded it must be declared on the label. In addition, the beef heart can be used for formulations of Salisbury Steak and Mettwurst sausages; however, these do not have a limit of use in those products. In products such as ground meat and hamburger patties the use of beef heart is allowed without any limitation (FSIS 2005), the amount will depend on the company. However, beef´s heart can be infected during the evisceration process or by cross contact of the employees with the product. Like beef cheeks, the heart has a high probability of bacterial contamination during processing. The heart presents a wide microflora, where studies determined the presence of Micrococcus, Streptococcus, *Staphylococcus*, Moraxella-Acinetobacter, *Escherichia coli*, Coryneform, Pseudomonas and Flavobacterium (Hanna et al. 1982). It is important to maintain good controls in plant to avoid contamination of this product. In 2003, an outbreak caused by the consumption of beef heart by the spoilage of Clostridium perfringens was reported in Munich, Germany (Schalch et al. 2003). Hannah et al. (1982) determined that the initial concentration of total aerobes in the heart of the cattle are less than four log CFU/g. It is very important to control the concentration of these products as this determines the shelf life of the food. *Teania saginata*, a parasite of the Cestoda class, has been found in the heart of cattle (Nesbakken 2005). In 1980, beef heart was examined in meat packing plants and as results was determined the presence of *Yersinia enterocolitica* (Stern and Oblinger 1980).

Beef liver is a by-product from beef carcass processing. The function of this organ in the body is detoxification of the blood, storage of glycogen and synthesis of plasma proteins. Like beef hearts and beef cheeks, those organs are used for the formulation of different meat by-products; this product is also consumed as beef cuts in different cultures. The largest importers of liver worldwide are Russia and Egypt followed by Latin American countries (U.S. Meat Export Federation 2013). Beef liver is a very popular food in the Asian and Latin American markets. Both cultures demonstrate frequent consumption of liver in their diets. However, this food can also be used as raw material in formulations of different meat by-products. Liver is an organ with a high probability of contamination by microorganisms at the time of carcass harvest. This organ is found together with the other organs, and a bad procedure during the evisceration of the carcass can contaminate this product by multiple bacteria found in the intestine. Various studies have been carried out to determine the concentration of bacteria in the liver. Hannah *et al.* (1982), determined the presence of Micrococcus, Streptococcus, Staphylococcus, Coryneform, Pseudomonas, Moraxella-Acinetobacter, Flavobacteria, fungi and yeast in liver stored at 2 ºC for five days(Hanna et al. 1982)(Hanna et al. 1982)(Hanna *et al.* 1982)(Hanna *et al.* 1982)(Hanna *et al.* 1982)(Hanna *et al.* 1982). In another study, Im *et al.* (2016) determined that in several meat processing plants in Korea there is no prevalence of pathogenic microorganisms such as: *Escherichia coli* O157: H7, Salmonella, Staphylococcus aureus and *Clostridium perfring*ens in liver (Im et al. 2016). However, the prevalence or not of these microorganisms in the products coming from the beef carcass will depend on the hygiene and safety measures that the plant implements during processing. Still, outbreaks of parasitic toxoplasmosis have been found in South Korea from liver consumption (Choi et al. 1997). In 2011, an outbreak of *Escherichia coli* O157: H7 was reported from consumption of beef liver in Canada (FSN 2011).

Bacterial contamination is inevitable in the transformation process from muscle to meat; therefore, industries should implement intervention where they can assure the elimination or reduction of those bacteria to acceptable levels. Beside contamination, another problem is bacterial growth in food due to acceptable condition for its multiplication. If the product is not properly handled, process, or cooked it can result as threat for public health. In the production of meat, antimicrobials interventions used in various steps in the process may present a significant reduction on the risk of contamination. The beef industry should always assume that a level of contamination would occur; however, use of multiple interventions along the line might reduce the contamination on the carcasses. Studies had shown that intervention along the process reduces the probability of contamination in the carcasses, which contribute to the shelf-life of the product and reduces the risk of pathogens (Sofos 2005; Wheeler et al. 2014). The antimicrobial agents used in those intervention had been studied for their capacity for inactivating or inhibit bacterial growth in foods (Huffman 2002). The transformation from muscle to meat, antimicrobial interventions are applied after the slaughter process. This is due since many researches had shown that beef carcass contamination occurs during the slaughter process (Barkocy-Gallagher et al. 2003). During the hide removal, the beef carcass can be contaminated. In addition, the meat surface of the carcass can be contaminated during the evisceration process, by stomach fluids or by esophagus fluids. After this process, multiple interventions have been used to reduce the microorganism contamination. Chemical rinses and washing are one of the most used intervention due to their effectiveness against microorganisms. However, beef industries use other interventions, such as sanitizing using chlorine solutions, oxidizers, and thermal interventions.

Oxidizer antimicrobials have multiple targets in cells as well in almost every biomolecule. This intervention inhibits enzymes, disrupts membrane layers, and oxidizes oxygen scavengers and thiol groups, disrupts protein synthesis and it ends with cell death (Dean et al. 1997). Peracetic acid are commonly use in beef interventions since it is approved by the FSIS for beef carcasses. Many studies had reported the effectiveness of peracetic acid. Other antimicrobial oxidizer widely used in the beef industry is electrolyzed oxidized water (EO). This antimicrobial reduces *Escherichia coli* O157:H7 in no more than 0.5 log CFU/cm^2 in beef head surface and cheek (Kalchayanand et al. 2008).

Thermal intervention main objective is to destroy with heat treatments cells, yeasts, and molds from the carcass. Steam-vacuuming is approved by the USDA-FSIS as a substitute for knife trimming (Wheeler et al. 2014). Hot water interventions mode of action is by inactivating enzymes for bacterial life, also to cause DNA breakage and RNA degradation (Ray and Bhunia 2014). Steam vacuuming is other common thermal interventions used in the beef industry. The thermal intervention it is only profitable to certain areas of the carcass, the ones who are heavily contaminated since it is not feasible to steam vacuum the entire carcass.

Since thermal intervention is considered one of the best strategies to reduce bacterial load, this can present changes in the physical and chemical characteristics in food. Due to that, non-thermal intervention are other alternatives in which uses low heat to reduce contamination without damaging certain beef characteristics. Electro-beam is one of the non-thermal intervention not commonly used in the beef industry. This intervention consists on applying high-energy electrons on the product. (Arthur et al. 2005) demonstrated that one kGy dose of electron beam reduces *E. coli* O157:H7 up to 4 logs in chilled beef primal.

All intervention can be applied manually or automatized using cabinets. Cabinets or best known as “Chad Cabinet” are automatized system where the carcass pass through. Inside the cabinet the carcass pass through a homogenized spray system applying organic acids, hot water or steam around the carcass. Making interventions along the processing line an efficient, fast and homogenized process.

In chemical rinse intervention, the most common used chemical are organic acids. In 1996, the USDA-FSIS first approved the use of organic acids such as lactic, acetic, and citric acids in a concentration of 1.5-5% in beef process. Organic acids are effective because they affect the microorganism’s cytoplasm by acidifying it. This is due because to the accumulation of undissociated weak acids in the cytoplasm of the cell (Booth 1985). These undissociated molecules cause interference with transmembrane proton gradient of the cell (Brown and Booth 1991). In 1980, Bair Parker stated, that undissociated molecule is the one responsible for the antimicrobial properties of the organic acids, however, this depends on the concentration, pH, pKa and concentration of the undissociated molecule. Which benefits the elimination of bacteria because this interference blocks the nutrient transport and energy generation of the cells, affecting the microbial growth. The effect of organic acids depends on the bacteria concentration, which this depends on the length of the time it has contact with the meat surface. Also, the efficacy of organic acids depends if the bacteria are covered by surfaces like fat, small cuts or if the carcass surface is not even, making impossible for the rinse of organic acid to reach those places. Typically, organic acids are applied by rinsing the carcass or the specific cut; however, new methods have been used such as complete immersion, but this process are applied to small batches. In addition, the temperature of the carcass, presence of moisture and fat solidification, affect the effectiveness of organic acid rinses. Research had shown that organic acid application had better results after hide removal process, when the carcass is still warm (Huffman 2002). When organic acids are applied right after the hide removal, it could reduce the concentration of total aerobic plate counts onto 1.5 log (Snijders et al. 1985). However, some beef industries washes and trims the beef carcass to reduce microbial contamination, but, some research reported that beef carcass with acid spray 2% had a greater reduction in comparison with washing and trimming the carcasses (Hardin et al. 1995). Antimicrobials used in the beef industry are chlorine, sodium hypochlorite, acetic, lactic, peroxyacetic and short chains organic acids, ozone, nisin, lactoferrin and trisodium phosphate (Gill and Badoni 2004). The most used organic acids as intervention in beef plants are lactic acid, acetic acid and citric acid, these organic acids are Generally Recognized as Safe (GRAS). Which means that the Code of Federal Regulations accept the use of these organic acids with food and there is no negative effect for the intake of it. These antimicrobials had shown effective alternatives to reduce microbiological contaminations in beef carcasses. However, beef plant preferred the used of peroxyacetic, lactic and acetic acid for carcass decontamination in the U.S. and Canada meat industries. Most of the U.S. beef industries used those acids into their intervention process; many large packaging plants used lactic acid in 2% concentration by spray to skinned carcasses after their washing with steam process (Gill and Badoni 2004). The FAO in 1973, declared that there is no limitation for the daily intake for acetic, acetate, citric, citrate, lactic and lactate, however, it limited the consumption por D1-lactate to 0-100 mg/kg body weight of this product (Manyi-Loh et al. 2016).

Acetic acid is a monocarboxylic acid. This organic acid has a peculiar characteristic, which it has a bad odor and taste, which limits it use in food. However, acetic acid it had been used in the food industry for interventions for some sausages and pigs' feet (Mani-López et al. 2012). Acetic acid and it derivate as considered by GRAS for miscellaneous and general usages (21 CFR 184.1005). Much research had approved the efficiency of acetic acid as a decontamination intervention in the meat industry.

Citric acid is a hydroxy tricarboxylic acid produced by some natural plants. This acid is soluble in water, and it is considered as GRAS for the use in fresh and processed meat (21CFR184.1033). Various research had proved the effectiveness of citric acid in indicator microorganisms and pathogens. Citric acid is an effective bactericidal organic acid also; this does not affect the organoleptic characteristics of food. Study proved that citric acid in 3% concentration does not produced odors or color change in poultry skin (González-Fandos et al. 2009).

Lactic acid is the most used in the meat industry due to their effectiveness in the decontamination of microorganisms and low costs (Wheeler et al. 2014). The FDA approved the use of lactic acids as an interventions technique, pre and post chilling (< 5% acid solution), sub-primal cuts and trimmings (2-3% solution, < 55 ºC), and to beef heads and tongues (2.0-2.8% in washing systems) (USDA 2010). Many studies had compared the effectiveness of organic acids.

Ransom et al. 2003 reported that lactic acid 2% reduce *Escherichia coli* O157:H7 by 3.3 logs and acetic acids 2% reduced it by 1.6 logs, both on beef carcasses.

Table 1 shows interventions used by the beef industry to gain bacterial reduction load in their process. Lactic acid and steam vacuum are the interventions that reduces the most bacteria in beef. However, lactic acid has lower cost and uses less water in comparison than steam vacuum, in addition, this steam vacuum affects the organoleptic characteristic of meat becoming a negative result.

Table 1. Antimicrobial interventions used in the beef industry.

Intervention	**Microorganism**	**Reduction (Log CFU/cm^{2})**	**References**
Peracetic acid	*E. coli* O157:H7	0.5 - 3.0	(Ransom et al. 2003; King et al. 2005)
Electrolyzed oxidize water	*E. coli* O157:H7,	0.5 - 1.0	(Kalchayanand et al. 2008; Jadeja et al. 2013)
Lactic acid	*E. coli* O157:H7,	1.0 - 5.0	(Delmore et al. 2000; Castillo et al. 2001; Stivarius et al. 2002; Loretz et al. 2011)
Citric acid	*E. coli* O157:H7,	1.5 - 2.3	(Ransom et al. 2003; Midgley and Small 2006; Laury AM. et al. 2009)
Steam Vacuum	*E. coli* O157:H7, APC; total coliforms	2.0 - 5.0	(Dorsa et al. 1996; Kochevar et al. 1997)
Hot water	Total coliforms, *S. Typhimurium,* and *E. coli* O157:H7	3.0 - 4.0	(Huffman 2002; Kalchayanand et al. 2012)
Electro Beam	*E. coli* O157:H7	4.0	

Lactic acid had become of great interest because of their effectiveness against STEC strains inoculated on meat surface, including *Escherichia coli* O157:H7 and non-*Escherichia coli* O157:H7 (Kalchayanand et al. 2012). Used of lactic acid during the pre-evisceration process showed reduction between 1 to 1.6 logs of aerobic bacteria and *Enterobacteriacceae* (Bosilevac et al. 2006). Application of lactic acids and the end of the slaughter process in warm carcasses reduces the concentration of *Escherichia coli* between 0.5 to 1.8 log CFU/cm^2 on beef carcasses (Barboza de Martinez et al. 2002). However, under laboratory condition it has been report that lactic acids reduces inoculated *Escherichia coli, Escherichia coli* O157:H7, *Salmonella Newport and*

Typhimurium by 1.0 to more than 4.8 log CFU/cm^2 (Loretz et al. 2011). In addition, other studies had reported the non-effectiveness of lactic acids application. Intervention of lactic acid (2%) concentration determined no significant difference in the reduction of bacteria in cooled carcasses, however, substantial reduction were obtained in concentration of 4% (Castillo et al. 2001). The effect of lactic acid against microorganisms depends on the time of exposure, and their bactericidal action increases with the time after their application (Dorsa et al. 1998; Gill and Badoni 2004). Other research reported reduction of *Escherichia coli,* Coliforms, Aerobic Plate Count of 0.66, 0.70 and 0.64 log CFU/g, however, this research shown not significant reduction of *Salmonella Typhimurium* (Stivarius et al. 2002). Intervention with lactic acid by spray and immersion reduces APC, total Coliforms and *Escherichia coli* in beef variety meats (cheek, intestine, lips, liver, oxtail and tongue). Immersion of variety meats in lactic acid reduces APC by 0.7 log CFU/g or more on six variety meats. For total coliforms it reduces 0.7 log CFU/g in 5 variety meats being the cheek the lowest with 0.5 log CFU/g reduction and for *Escherichia coli* it reduces 0.7 log CFU/g in four variety meats being cheek, tongue the lowest with reduction between 0.1-0.3 log CFU/g (Delmore et al. 2000). However intervention by spray shows APC reduction of 0.7 log CFU/g in five variety meats in exception for large intestines, total coliforms reduces the same amount in four variety meats except cheek and liver, and for *Escherichia coli* it reduces 0.7 log UFC/g or more in four except for cheek and tongue (Delmore et al. 2000).

Lactic acids in small concentration do not affect the organoleptic characteristics in meat. Research had proven that lactic acids had no effect in the color of ground beef during 3 days of storage after their acid intervention (Harris et al. 2012). (Ellebracht et al. 1999) reported no change of color in lactic acid (2%) intervention or hot water with temperature of 95 ºC in trims prior to grinding. However, some studies reported color change in ground beef exposed to lactic acid intervention (Stivarius et al. 2002) reported that treated beef with lactic acid (5%) presented lighter color and less content of oxymyoglobin compared with the control and hot water treatment. In addition, the same authors showed that beef treated with lactic acids presented “non-beef like odor” by day 3 of the lactic acid intervention in comparison with hot water interventions and the control

As indicated, variety meats are a potential by-product in the beef industry due to their uses in the formulation of process meat; for this reason, , this by-product had also been studied to find an effective intervention to reduce the risk of contamination of pathogens in the product. Table 2 shows antimicrobial interventions used in the processing line of beef cheek, heart, and liver. It is observed that spray lactic acid intervention reduces the most indicator microorganisms on beef cheek, liver, and heart.

Table 2. Antimicrobial interventions in beef cheek, heart, and liver.

Intervention	**Variety Meats**	**Microorganism**	**Reduction (Log CFU/cm^2)**	**References**
Spray Lactic acid (2%)	Cheek, liver, and heart.	APC, coliform count, and generic *E. coli*.	0.0 – 1.96	(Delmore et al. 2000; Kalchayanand et al. 2008; Pokharel et al. 2016)
Immersion Lactic acid (2%)	Cheek and liver	APC, coliform count, and generic *E. coli*.	0.5 – 1.6	(Delmore et al. 2000; Schmidt et al. 2014)
Hot water immersion	Cheek and liver	APC, coliform count, and generic *E. coli*.	0.0 – 1.1	(Delmore et al. 2000)
Hot water spray	Cheek and liver	APC, coliform count, and generic *E. coli*.	0.0 – 1.1	(Delmore et al. 2000)
Steam Vacuum	Cheek and liver	APC, coliform count, and generic *E. coli*.	0.0 – 1.1	(Delmore et al. 2000)

CFU, colony forming units.

Table 3 indicates the results obtained by an Analysis of Variance (ANOVA) and means separation. It shows the effect of lactic acid intervention against coliforms load, APC and *Escherichia coli* in beef liver, heart, and cheek. Samples were taken randomly before and after the intervention from each batch.

Table 3. Aerobic Plate Count (APC), *E. coli*, and Coliforms load (Log CFU/cm^2) in beef cheek, heart and liver sampled before and after 2-5% lactic acid intervention.

Variety Meats	**APC**		***E. coli***		**Coliforms**	
	Before	After	Before	After	Before	After
Cheek	3.35^a	1.63^a	0.93^a	0.00*^{a}	0.98^a	0.00*^{a}
Heart	1.66^b	0.49^b	0.87^a	0.02^{ab}	0.89^a	0.06^{ab}
Liver	1.79^b	0.61^b	1.05^a	0.10^b	1.16^a	0.15^b
CV%	25.88	52.18	53.01	52.47	52.82	55.66
Pr > |F|	<0.0001	<0.0001	0.59	0.04	0.31	0.08

CFU, colony forming units.
*Lower than the detection limit (< 0.05 CFU/cm^2).
^{ab} Means with different small case letters in each row within the same microorganism indicate statistical difference (P < 0.05).

Initial microbial count of Aerobic Plate Count (APC) showed statistical difference between each cheek, heart, and liver. The initial microbial load on cheeks is statically different in comparison with the initial count of heart and liver in which this two remains with no difference. Beef cheeks usually presented a higher number of microorganisms in the microbial load. This happens because the animal is lifted upside down, situating the head in the lower region near the floor and while the carcass is washed the contaminated water make contact with the head, contaminating the area of the cheek. (Carney et al. 2006) demonstrated that strains of *Escherichia coli* O157:H7 have a 3% prevalence in beef cheek after wash interventions. This can be of great risk for human consumption because this type of variety meats is used to make beef patties. In addition, APC counts have a higher microbial load in comparison with *Escherichia coli* and total coliforms because this indicator bacterium when inoculated, counts all the aerobic mesophilic bacteria and it is not a selective media for bacteria. *Escherichia coli* and total coliforms counts does not present statistical difference in the initial count before the intervention.

In addition, there is statistical difference in the microbial count after acid lactic interventions between each variety meats. APC counts after lactic acid have a greater load on cheek than heart and liver after the intervention. However, the bacteria reduction relay in the initial load of the cut and the time of exposure, and as we can see the initial load in cheek double the amount of the initial load of liver and heart. Bacteria reduction depends on the initial microbial load of each microorganism in the variety meats. High values, such as APC in beef cheeks, which shows a mean of 3.35 log CFU/ cm^2 doubling the mean for APC in beef heart and liver, will have a higher count after the intervention. It exists a small difference between cheek and the other two variety meats in *Escherichia coli* and total coliforms counts after the intervention. Cheek is a small cut which area of expose is less than heart and liver. Therefore, the intervention has greater effect in the reduction of microorganisms in small areas. Table 4 shows the microbial count before and after the intervention in beef cheeks. Intervention of 2-5% lactic acid reduces significantly ($P < 0.005$) in aerobic plate count, generic *E. coli* and total coliforms by 1.72, 0.94 and 0.94 CFU/cm^2. These results are close to other research, which stated that 2-5% lactic acid intervention reduces aerobic count and generic *E. coli* by 1.96 and 0.91 Log CFU/cm^2 in beef cheeks (Pokharel et al. 2016). Other researches demonstrated that lactic acid intervention will reduced by aerobic count up to 0.76 Log CFU/cm^2 on beef carcasses (Buege and Ingham 2001). In addition, immersion of beef cheeks in 2.5-5% lactic acid intervention reduces *E. coli* O157:H7, non O157:H7 STEC and *Salmonella enterica* on ranged of 0.6 to 1.4 Log CFU/cm^2 (Schmidt et al. 2014).

Table 4. Microbial count of Aerobic Plate Count (APC), generic *E. coli*, and Coliforms load (Log CFU/cm^2) before and after the 2-5% of lactic acid intervention in beef cheek.

	Log CFU/cm^2 ± SD			
	Before	**After**	**Total Reduction**	**Pr > |t|**
Aerobic count	3.35 ± 0.37	1.63 ± 0.36	1.72 ± 0.37	<0.0001**
E. coli	0.94 ± 0.45	0.00* ± 0.00	0.94 ± 0.32	<0.0001**
Coliforms	0.94 ± 0.57	0.00* ± 0.00	0.94 ± 0.40	<0.0001**

CFU, colony forming units.
*Lower than the detection limit (< 0.05 CFU/cm^2).
**Statistical difference ($P < 0.05$).

Table 5 shows the microbial load before and after the 2-5% lactic acid intervention in beef heart. This intervention in beef heart showed significant reduction ($P < 0.005$) on generic *E. coli*, total coliforms, and aerobic count. Spray lactic acid interventions on beef heart reduces aerobic plate count, generic *E. coli* and total coliforms by 1.19, 0.85 and 0.88 Log CFU/cm^2. (Pokharel et al. 2016) demonstrated that lactic acid intervention reduces generic *E. coli* load up to 0.41 in beef heart, similar as the results presented in Table 6.

Table 5. Microbial count of Aerobic Plate Count (APC), generic *E. coli*, and Coliforms load (Log CFU/cm2) before and after the 2-5% of lactic acid intervention in beef heart.

	Log CFU/cm^2 ± SD			
	Before	**After**	**Total Reduction**	**Pr > |t|**
Aerobic count	1.67 ± 0.76	0.52 ± 0.51	1.19 ± 0.65	<0.0001*
E. coli	0.88 ± 0.50	0.03 ± 0.07	0.85 ± 0.35	<0.0001*
Coliforms	0.95 ± 0.49	0.07 ± 0.19	0.88 ± 0.37	<0.0001*

CFU, colony forming units.
*Statistical difference ($P < 0.05$).

Likewise, lactic acid intervention in beef liver reduces microbial load. Table 6 shows bacterial reduction before and after lactic acid intervention which presented significant reduction ($P < 0.005$) in aerobic count, generic *E. coli* and total coliform. This lactic acid intervention on beef liver reduces aerobic plate count, generic *E. coli* and total coliforms by 1.18, 0.95, 1.02 Logs CFU/cm^2. These results are alike to the one presented by (Delmore et al. 2000) in which was stated that 2% lactic acid intervention reduces aerobic count, generic *E. coli* and total coliform by 0.7, 0.6 and 0.7 log CFU/cm^2 in beef liver. Other research stated that lactic acid rinsed intervention reduces coliform load up to 0.83 Log CFU/cm^2 in beef carcasses (Dormedy et al. 2000).

Table 6. Microbial count of Aerobic Plate Count (APC), generic *E. coli* and Coliforms load (Log CFU/cm2) before and after the 2-5% of lactic acid intervention in beef liver.

	Log CFU/cm^2 ± SD			
	Before	**After**	**Total Reduction**	**Pr > |t|**
Aerobic count	1.79 ± 0.45	0.62 ± 0.43	1.18 ± 0.44	<0.0001*
E. coli	1.06 ± 0.56	0.10 ± 0.18	0.95 ± 0.41	<0.0001*
Coliform	1.17 ± 0.54	0.15 ± 0.26	1.02 ± 0.43	<0.0001*

CFU, colony forming units.
*Statistical difference ($P < 0.05$).

Lactic acid intervention it is a very common process in the United States beef plants. This antimicrobial solution presented effective results against bacteria. As expected, spray lactic acid intervention has statistical difference in the reduction in the microbial load of APC, *Escherichia coli* and total coliforms on beef cheek, heart, and liver ($P < 0.005$). Intervention with 5% lactic acid concentration in laboratory conditions reduces *Escherichia coli* O157:H7 strains up to 2.6 log

CFU/cm^2 (Cutter and Siragusa 1994). Researches had indicated that spray lactic acid intervention is an effective way to reduce bacterial loads. (Castillo et al. 2001) determine that 4% concentration of spray lactic acid intervention reduces more than 4.8 log CFU/cm^2 of *Escherichia coli* lowering the microbial load into undetectable numbers.

Table 7 shows the microbial reduction of APC, *E. coli* and total coliforms after 2 - 5% lactic acid intervention in beef cheek, heart, and liver. There is no statistical difference (P < 0.005) in the reduction of aerobic count, generic *E. coli* and coliform in between each variety meats sampled (cheek, heart, and liver).

Table 7. Microbial reduction of Aerobic Plate Count (APC), generic *E. coli* and Coliforms load (Log CFU/cm2) by 2-5% of spray lactic acid intervention in beef cheek, heart, and liver.

	Log CFU/cm^2		
Variety Meats	**APC**	***E. coli***	**Coliforms**
Cheek	1.72[a]	0.94[a]	0.94[a]
Heart	1.19[a]	0.85[a]	0.88[a]
Liver	1.18[a]	0.95[a]	1.02[a]
CV%	56.80	53.56	58.99
Pr > |F|	0.09	0.81	0.70

CFU, colony forming units.

[ab] Means with different letters in each row within the same microorganism indicates statistical difference (P < 0.05).

4. CONCLUSIONS

- Beef variety meats are a potential by-product from the beef industry, however, there is very little information from different interventions applied to these by-products since it is not the main product from the beef carcass.

- Beef cheeks present a higher Aerobic count microbial load; however, beef liver presented greater microbial load of generic *E. coli* and total coliforms.

- Lactic acid is an effective antimicrobial intervention since the spray of lactic acid (2-5%) intervention reduces Aerobic Plate Count, Total Coliforms and generic *E. coli* in beef cheeks, heart, and liver.

5. RECOMMENDATIONS

- Determine bacterial load in other seasons to analyze if there are differences between the microbial loads.
- Validate the effect of lactic acid in primal, sub-primal and trims from the same beef facility.

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7. APPENDIX

Appendix 1. Slaughter process for beef carcasses.

Before slaughter treatment. The main objective of applying this treatment is to guarantee meat quality and safety to consumers. First, food deprivation in animals is necessary to empty the digestive system and prevent further contamination. Avoid any kind of stress into the animal such as rough handling, long transportation, and fights between other steers. In addition, it is important to reduce long stress periods because this can affect the meat quality, as it depletes body sugar content, which it is useful for lactic acid production. Meat quality relies on the amount of lactic acid produced by the organism. When the organism produced a low amount of lactic acid, this can induce into a dark cutter meat, which it is a negative aspect for meat quality. Lactic acid production influenced the pH of the meat, so a low lactic acid production will not acidify the meat enough making it suitable for microbial growth.

Stunning and bleeding. Many countries have regulations requiring human methods for killing the animal (FAO 2009); however, there are some exceptions for killing the animal due to multiple religions. For beef, the most common stunning method is with a captive-bolt pistol, which the animal it is hit in the forehead making them unconscious after the impact while the other employee exsanguinates the cattle. For this process, the employee cut the carotid artery and the jugular vein while the animal is hanging up in the rail.

Skinning the Cattle. During this process, to prevent cross-contamination, it is important that the outer side of the hide do not touch the skin surface of the carcass. In addition, the employee must not touch the outer side and the skin surface.

Evisceration. The operator must tide the rectum and the esophagus of the cattle during the hide removal. During the evisceration process the operator, make a slight cut in the middle of the abdominal cavity. Since carcass is hanged up, which facilitates the viscera removal because this falls under their own weight. Is important that the operator avoid cuts on the organs to prevent cross-contamination between the intestine and the paunch.

Splitting and washing the carcass. The employee split the carcass in two sides by cutting the backbone from the pelvis to the neck. It is important to sterilize the saw in hot water (82 ºC) between carcasses. Washing the carcass with water removes bloodstains and soil to improve appearance. It is crucial to make sure that the water is clean. However, this practice is not a substitute for good hygienic practices during the slaughter process.