

EFFECT OF SALT REDUCTION ON GROWTH OF *LISTERIA MONOCYTOGENES* IN
BROTH AND MEAT AND POULTRY SYSTEMS

by

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B.S., Purdue University, 2006
M.S., Kansas State University, 2009

AN ABSTRACT OF A DISSERTATION

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Abstract

Salt is used as a preservative in food. Reducing sodium in food, due to its link to hypertension, and replacing NaCl with other types of salt could have an effect on food safety. The main objective was to determine differences in salts and salt substitutes on growth of *Listeria monocytogenes* in broth and meat and poultry systems. Salts (NaCl, KCl, CaCl₂, MgCl₂, sea salt, and replacement salt) were added (0.5, 1, and 2.5%) to *Listeria monocytogenes* (five-strain cocktail) inoculated *Listeria* enrichment broth at 25 °C and sampled at 0, 24, and 48 h. Results showed that MgCl₂, regardless of concentration, caused *Listeria monocytogenes* populations to grow approximately 0.6 log CFU/mL more ($P < 0.05$) than the other salts. Fresh ground beef, pork, and turkey with NaCl, KCl, CaCl₂, MgCl₂, sea salt, and replacement salt (2.0%) were inoculated with *Listeria monocytogenes* (five-strain cocktail) to determine growth/survival during 5 d at 4 °C to simulate a pre-blend process. *Listeria monocytogenes* populations significantly decreased (0.41 log CFU/g) during the storage time in beef, however no differences ($P > 0.05$) were observed over time in pork or turkey. Salt type did not affect ($P > 0.05$) *Listeria monocytogenes* populations during pre-blend storage. However, salts (MgCl₂ and NaCl) allowed growth ($P < 0.05$) of aerobic populations during storage. Emulsified beef and pork products were processed with NaCl, KCl, sea salt and a NaCl/KCl blend (2%) and post-processed surface inoculated with *Listeria monocytogenes* (five-strain cocktail) to determine growth/survival at 4 °C for 28 d. Pork products showed greater ($P < 0.05$) *Listeria monocytogenes* population growth at all sampling times (0, 7, 14, 21, and 28 d) than beef products; whereas salt type had no effect on *Listeria monocytogenes* populations with sampling times pooled for data analysis. Although salt types were not shown to have an impact on *Listeria monocytogenes* growth/survival in pre-blend and emulsified post-processed surface inoculated meat products, pork and turkey pre-blends and emulsified pork had greater *Listeria monocytogenes* populations compared to beef products. These studies demonstrate that sodium reduction or replacement may not affect safety of pre-blends and emulsified meat and poultry products.

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Approved by:

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Dedication

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Chapter 1 - Introduction

Salt serves several purposes in food; it is a flavor enhancer, extracts myofibrillar proteins in meat, enhances water holding capacity, a preservative, a leavening aid, and it controls fermentation and provides nutritional content (CSU 2011; Miller and Hoseney 2008; Gordon and Klimek 2000; Bauman 1982; Jensen 1954; Marsden 1980). While sodium chloride is the most popular salt used in food, the Food and Drug Administration (FDA) defines salt in 21 CFR 100.115, as any salt intended for human consumption that contains iodide (CFR 2011a).

Salt has been used throughout history as a preservative for food. Theories explaining the preservative effect of salt range from its dehydrating capacity to its ability to interfere with substrate utilization causing cellular function to stop (Csonka 1989; Woods and Wood 1982; Erecinska and Deutsch 1985; Rockwell and Ebertz 1924; Smith and others 1987). Many bacteria are either resistant or tolerant to salt. Two of the most halotolerant foodborne pathogens are *Staphylococcus aureus* and *Listeria monocytogenes* (Jay and others 2005).

Listeria monocytogenes is a Gram positive, non-spore forming, rod shaped bacterium that can grow at a water activity as low as 0.90 and a salt concentration as high as 10% (Jay and others 2005). *Listeria monocytogenes* causes foodborne listeriosis, a foodborne illness characterized by loss of appetite, vomiting, fever, diarrhea, and lethargy (FDA 2009; Jay and others 2005; NIH 2009a). Pregnant women with listeriosis may have stillbirths or miscarriages due to the bacterium crossing the placenta (FDA 2009; Jay and others 2005; NIH 2009a).

Listeria monocytogenes can be found throughout nature in soil, water, animal feces, or vegetation (Jay and others 2005). Raw dairy products are common foods associated with *L. monocytogenes* contamination (CDC 1985; CDC 2001; Jay and others 2005; Marler 2006; Marler 2007; Olsen and others 2004). *Listeria monocytogenes* is also associated with ready-to-eat (RTE) products, such as meat and poultry deli meats as well as hot dogs (CDC 1998; CDC 2011b; CSU 2010; Food Safety News 2011a; Jay and others 2005; Olsen and others 2005). In 1998, over 3,500 samples of RTE products were sampled for Listeriae and 2.5% (90 samples) tested positive for *Listeria monocytogenes* (USDA/FSIS 2000b).

Due to several recalls and outbreaks involving *L. monocytogenes* in RTE meat and poultry products in 1998 and 1999, FSIS placed a zero tolerance on *L. monocytogenes* in these products (USDA/FSIS 2000b). Due to *L. monocytogenes* outbreaks involving post-process

contamination in RTE meat and poultry products, FSIS (USDA/FSIS 2006) established three alternatives that food processors can use to minimize risk of post-process contamination. Alternative 1 states that the establishment must use a post-lethality treatment to reduce or eliminate *L. monocytogenes*, and an antimicrobial agent or process to suppress or limit the growth of the pathogen. Establishments under Alternative 1 are subject to the lowest frequency of testing by FSIS. Under Alternative 2, an establishment must use either a post-lethality treatment or an antimicrobial agent or process. The definition of a post-lethality treatment is a process that must reduce *L. monocytogenes* by 1 log CFU/g of food. Establishments falling under Alternative 2 are subject to more frequent FSIS verification than Alternative 1. Alternative 3 is when an establishment relies on its sanitation program to control *L. monocytogenes* in RTE meat and poultry products. An establishment using Alternative 3 is subject to the most frequent FSIS verification because they do not have a plan in place for reducing post-process contamination (USDA/FSIS 2006).

Many different ingredients and processes have been researched to fit under the category of “post-lethality treatment” or “antimicrobial agents.” These include addition of salt, lowering of pH, and changing of the packaging atmosphere (Lobaton-Sulabo and others 2011; Samelis and others 2001; Samelis and others 2005; Uppal and others 2011).

Sodium is important to overall health in many ways. It is important in early cellular development as well as for active and passive cellular transport (Solomon and Galey 1982). Sodium is also very important to kidney function, which functions to remove waste and water from the blood to form urine (NIH 2010). Sodium is needed in the diet to maintain osmotic balance within cells as well as in the function of nerve impulses and muscle contraction (CSU 2011). However, too much sodium can cause hypertension, or high blood pressure by forcing the body to take in more water than usual to dilute the concentration of sodium in the blood (Cleveland Clinic 2010).

The average American consumes much more sodium than is recommended. The 2010 Dietary Guidelines recommends that 2,300 mg of sodium be consumed per day in a person’s average diet. This number drops to 1,500 mg of sodium if the person is 51 years old or older, African American, or suffers from hypertension, diabetes, or chronic kidney disease (USDA 2010a). However, the average American consumes 3,400 mg of sodium per day, far exceeding the USDA’s recommendations (Mayo Clinic 2011c). Men consume far more sodium on average

than women. A study performed by the Division of Health Examination Statistics of the Centers for Disease Control and Prevention (CDC) surveyed 4,206 men and 4,398 women of all ages about their diet. The study found that men consumed an average of 3,877 mg of sodium per day while women consumed an average of 2,896 mg of sodium per day (Wright and others 2003).

In the past several decades, many organizations have called for a reduction of sodium in food (NYCDOH 2010; WASH 2009; WHO 2006). In the U.S., the Center for Science and the Public Interest has petitioned FDA to reclassify common salt (sodium chloride) as a food additive (FDA 2007). However, the reduction of salt could cause important changes to the safety of food products that should be thoroughly studied (Taormina 2010). According to Linda Kragt of Morton Salt, Inc., the food industry typically uses potassium chloride or a blend of potassium chloride and sodium chloride to reduce sodium levels in food (personal communication, October 3, 2011).

The meat and poultry industry has been researching ways to reduce the amount of sodium in meat and poultry products. In a study by Roenbaugh (2011), a deli-turkey roast was inoculated with *L. monocytogenes* and one treatment contained 1.50% of sodium chloride whereas, the other treatment contained a mixture 0.75% of sodium chloride and 0.75% of potassium chloride. No differences ($P > 0.05$) in *L. monocytogenes* populations were observed between the two treatments after 91 days of storage at 4 °C.

The food industry has been researching ways to reduce the sodium content in various processed food products; however, sodium reduction also affects food safety, and several reviews of the use of salt in the area of food safety have been published in the past (Reddy and Marth 1991; Sofos 1983; Taormina 2010). There is limited research on how different salts (NaCl, KCl, CaCl₂, and MgCl₂) may impact *L. monocytogenes* growth in broth and meat and poultry systems (Sofos 1983). The USDA National Nutrient Database for Standard Reference states that raw ground turkey and beef (15% fat), contains approximately 19 mg and 18 mg of magnesium per 100 grams of food, respectively. Comparatively, raw ground pork (16% fat) contains approximately 16 mg of magnesium per 100 grams of food (USDA 2011). Magnesium sulfate has a greater effect on water activity and dehydration than NaCl does, but it does not have an increased bacteriostatic effect (Rockwell and Ebertz 1924). Conda (Madrid, Spain), a Spanish microbial media producer, states that magnesium is a growth cofactor that aids in enzymatic

reactions, including DNA replication (Conda 2011). Due to these factors, *L. monocytogenes* may be able to grow at a faster rate in poultry products versus pork or beef products.

Therefore, the main objective of this study was to evaluate how salts and salt replacements effects growth of *L. monocytogenes* when added to broth and meat and poultry systems. Secondary objectives were: 1) to determine the effect that different salts have on the growth of *L. monocytogenes* in a broth system during storage at room temperature; 2) to determine the effect that different salts have on the growth of *L. monocytogenes* in ground beef, pork, and turkey during 5 d storage at 4 °C; and 3) to determine differences in *L. monocytogenes* growth and survival (due to post-process contamination) in beef and pork emulsions with different salts during storage at 4 °C for 28 d. Multiple species of meat will be used to determine if there is a species effect for *L. monocytogenes* growth or survival.

Chapter 2 - Review of Literature

Salt

Salt serves several purposes in food; it is a flavor enhancer, extracts myofibrillar proteins in meat, enhances water holding capacity, a preservative, a leavening aid, and it controls fermentation and provides nutritional content (CSU 2011; Miller and Hosenev 2008; Gordon and Klimek 2000; Bauman 1982; Jensen 1954; Marsden 1980). Sodium chloride is the salt that is used most in foods; however calcium chloride is used as a leavening aid in bread systems (FDA 1996). Some of the foods that use salt for various functional purposes include ready-to-eat (RTE) deli meats, hot dogs, and sausages as well as pickles, olives, cheeses, baked pastries, and pies (CSU 2011). One study found that 77% of sodium in the American diet comes from processed and restaurant foods while 12% of sodium was naturally occurring in food (Mattes and Donnelly 1991).

Definition of Salt

The Food and Drug Administration (FDA) defines salt in 21 CFR 100.115 as any salt intended for human consumption that contains iodide. If table salt does not contain iodide, then it must be labeled "This salt does not supply iodide, a necessary nutrient." The FDA further describes salt in 21 CFR 101.4 as any suitable salt used in canning vegetables leaving the definition open for many types of salts. Furthermore, FDA states in 21 CFR 101.22 that "any salt (sodium chloride) used as an ingredient in food shall be declared by its common or usual name 'salt'" (FDA 2011b). Thereby, the definition of salt as defined by FDA is open to include all types of salt from sodium to calcium chloride.

History of Salt

Salt has been very important throughout recorded history. Egyptians used salt to preserve mummies. Ancient Hebrews believed that salt is the eternal nature of God's covenant, which modern Jewish faith still believes. In fact, on Friday nights Jews dip Sabbath bread in salt to symbolize their faith between God and his people. It is also important in Christianity. The Catholic Church dispenses both holy water and holy salt (Salt of Wisdom) because they believe

that salt is associated with truth and wisdom (Kurlansky 2002). In fact, wars have been fought over access to salt since salt was regarded as a form of money (Laszlo 2001).

In 1912, Welsh psychologist Ernest Jones published an essay on the human obsession with salt. Jones, much like his friend Sigmund Freud, thought that the human obsession with salt was subconsciously sexual. Jones' argument was built on the fact that Egyptian priests abstained from salt because it excited sexual desire and Dayak tribesmen were required to abstain from sex and salt after they returned from taking heads (Kurlansky 2002). Jones also stated:

“Homer calls it a divine substance, Plato describes it as especially dear to the Gods, and we shall presently note the importance attached to it in religious ceremonies, covenants, and magical charms (Jung and others, 1995).”

In the early 1920's, Diamond Crystal Salt Company published a booklet entitled “One Hundred and One Uses for Diamond Crystal Salt.” Some of these uses were related to food (keeping colors bright in boiled vegetables, making ice cream freeze, or making water boil at a higher temperature) while others uses not related to food included: removing rust, removing spots from clothes, putting out grease fires, and keeping cut flowers fresh. In modern day, there are more than 14,000 uses for salt which include manufacturing of pharmaceuticals, melting of ice on roads, making soap, fertilizing fields, and softening water (Kurlansky 2002).

Sources of Sodium in Food

Salt is not the only source of sodium found in foods. Sodium is used in connection with many different ingredients that are used in food products. Most of these ingredients use sodium to stabilize or solublize the ingredient into the food product.

Sodium nitrite

One important ingredient used in meat products is sodium nitrite. It is used as a curing agent for meat products and also to control pathogenic bacteria growth, including *Listeria monocytogenes* and *Clostridium botulinum* (AMI 1999). However, the amount of sodium nitrite allowed in food products is strictly restricted. Section 172.175 of Title 21 of the CFR states that sodium nitrite levels cannot exceed 200 ppm in whole muscle products, 156 ppm in comminuted

meat products and bacon levels must be below 120 ppm (CFR 2011b). Nitrite levels are restricted because nitrite poisoning can cause methemoglobinemia which can be fatal by causing abnormal buildups of hemoglobin in the blood (NIH 2011c).

Sodium lactate

Another important ingredient is sodium lactate. Sodium lactate is a lactic acid salt used in fermented products as a flavor enhancer (Igoe and Hui 2001). It is also a humectant that is highly hygroscopic and an antilisterial agent (Igoe and Hui 2001; Farber, Pagotto, and Scherf 2007). Sodium lactate is an important food additive because FDA has given it Generally Recognized as Safe (GRAS) status (FDA 2011b). This means that it can be used at any level in a food product. Samelis and others (2002) found that sodium lactate used alone at 1.8% in frankfurter formulations inhibited the growth of *L. monocytogenes* for 50 days. However, Schlyter and others (1993) found that adding a combination of 2.5% sodium lactate and 0.1% sodium diacetate prevented growth of *L. monocytogenes* at 4 °C for 42 days.

Sodium diacetate

Sodium diacetate is a preservative and flavoring agent used in many types of foods (Igoe and Hui 2001). This ingredient is a white crystalline, hygroscopic powder that acts against molds and bacteria. Sodium diacetate has also been given GRAS status by FDA (FDA 2011b). Schlyter and others (1993) found that sodium lactate works best to inhibit *L. monocytogenes* when combined for a synergistic effect with sodium diacetate.

Sodium erythorbate

Sodium erythorbate is an important antioxidant that is used in food products. It is inert in dry air, but it becomes a powerful antioxidant when it combines with water (Igoe and Hui 2001). It also is a curing accelerator and controller in the meat curing process (Igoe and Hui 2001). It can be used at 550 ppm as a cure accelerator (Epley and others 2011; CFR 2011b).

Monosodium glutamate

Monosodium glutamate, commonly known as MSG, is a very popular flavor enhancer in food. It is a sodium salt of amino acid glutamic acid. It is mostly used in meats, sauces, and soups at a level of 0.1 to 1% (Igoe and Hui 2001). The FDA also has granted monosodium glutamate GRAS status (CFR 2011c). However, a certain population is sensitive to MSG. The

symptoms include headache, sweating, facial pressure or tightness, numbness, tingling or burning in face, neck and other areas, rapid and fluttering heartbeats (heart palpitations), chest pain, nausea, and weakness (Mayo Clinic 2011b).

Salt and Food Safety

There are several theories as to why salt (NaCl) inhibits bacterial growth and destroys bacteria. One hypothesis is that the preservative effect of salt is due to its dehydrating capacity lowering of environmental water activity. However, others have shown that the dehydrating effect of bacteria does not always equate to improved bacteriostatic properties (Rockwell and Ebertz 1924). Other research has shown that NaCl interferes with substrate utilization so that cellular function ceases (Csonka 1989; Woods 1982; Erecinska and Deutsch 1985; Smith and others 1987).

Csonka (1989) reviewed cellular osmotic regulation and stated that in hyperosmotic shock, cells shrank due to cytoplasmic shrinkage, also called plasmolysis. The author further explained that plasmolysis can cause inhibition of macromolecular biosynthesis through inhibition of nutrient uptake and deoxyribonucleic acid (DNA) replication. Woods (1982) also found that with the decrease of glucose utilization from increased concentrations of NaCl, adenosine triphosphate (ATP) levels decreased significantly in *Clostridium sporogenes*. Erecinska and Deutsch (1985) observed cellular respiration decrease in *Paracoccus denitrificans* due to increased NaCl levels. Smith and others (1987) noted that *Staphylococcus aureus* was unable to produce enterotoxin A and that glucose utilization and cellular respiration decreased in *S. aureus* due to NaCl concentration increasing over 10%. In a study by Galinski and Truper (1994) it was shown that environments with increased NaCl levels (2.6 M and higher) changed the composition of lipid membranes which changed gene expression.

Bacteria have been found to have varying levels of resistance to salt concentrations and changed water activity levels. Table 1 summarizes the levels of resistance for some of the more common pathogenic bacteria. This table lists both minimum salt concentrations for inhibition of bacterial growth as well as minimum water activity for growth. *Staphylococcus aureus* is the most halotolerant pathogen and its growth is not inhibited until a water activity of 0.85 or a salt level of 10% (Jay and others 2005). *Listeria monocytogenes* is extremely halotolerant as well

and its growth is not inhibited until a water activity of 0.90 or a salt concentration of 10% (Jay and others 2005).

Many other pathogens, including *Clostridium botulinum*, *Clostridium perfringens*, *Salmonella* spp., *Vibrio parahaemolyticus*, *Vibrio cholera*, and *Vibrio vulnificus*, are inhibited at a water activity of 0.93 (Jay and others 2005). However, some studies have incorporated other interactions such as temperature and pH into the conditions along with NaCl concentration (Thayer and others 1987). Some of the more salt tolerant and salt resistant foodborne pathogens include *Listeria monocytogenes*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus* (Taormina 2010).

Table 1. Inhibitory conditions for bacterial pathogen growth as related to salt concentrations and water activity levels (Jay and others 2005).

Microorganism	Water Activity	Salt Concentration
<i>Clostridium botulinum</i> (toxins A-G)	0.93-0.97	4.5-10%
<i>Clostridium perfringens</i>	0.93-0.95	7-8%
<i>E. coli</i> O157:H7	-	8%
<i>Listeria monocytogenes</i>	0.90-0.97	10%
<i>Salmonella</i> spp.	0.93	Temperature dependant
<i>Shigella</i> spp.	-	3.8-5.2%
<i>Staphylococcus aureus</i>	0.86	10%
<i>Vibrio parahaemolyticus</i>	0.93	8-10%
<i>Vibrio cholera</i>	0.93	8-10%
<i>Vibrio vulnificus</i>	0.93	8-10%
<i>Yersinia enterocolitica</i>	0.95	5-7%

Research is needed to determine what the cause of salt's bactericidal effects are including the reduction of water activity, the effect of the sodium ion, and the effect of the chloride ion. Some studies have shown that chloride is needed for growth in high salt concentrations (Roebler and others 2003). However, another study showed that chloride causes sporulation in spore-forming bacteria (Mah and others 2008).

***Listeria* spp.**

Listeria spp. are Gram positive, non-spore forming, rod shaped bacterium (Bell and Kyriakides 1998; Jay and others 2005; FDA 2009). They are capable of fermenting glucose and

rhamnose and are catalase positive (FDA 2011a; Jay and others 2005). Table 2 shows test results for how to differentiate different *Listeria* spp. based on biochemical reactions

Listeria spp. are broken down into six different species: *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii*, and *L. grayi* (FDA 2011a; Jay and others 2005). However, Bergey's Manual of Systematic Bacteriology only recognizes five distinguishable species, excluding *L. grayi* from the group of recognized species (Seeliger and Jones 1986). While *L. ivanovii* and *L. seeligeri* can cause human listeriosis, *L. monocytogenes* is the species that causes most of these human illnesses. *Listeria ivanovii* and *L. monocytogenes* are both pathogenic to mice. *Listeria monocytogenes* displays tumbling motility due to its peritrichous flagellum and shows incomplete β -hemolysis (*Staphylococcus aureus* positive, *Rhodococcus equi* negative) according to the Christie-Atkins-Munch-Peterson (CAMP) test (FDA 2011a; FDA 2009). Table 3 shows the hemolytic reactions for the *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, and *L. seeligeri*.

Table 2. Differential tests for *Listeria* spp. (FDA 2011a; Jay and others 2005).

Species	β -Hemolysis ^a	Mannitol	Fermentation (acid production)			Xylose	Virulence ^b
			Lactose	Galactose	Rhamnose		
<i>L. monocytogenes</i>	+	-	Variable	Variable	+	-	+
<i>L. ivanovii</i>	+	-	+	+	-	+	+
<i>L. innocua</i>	-	-	+	-	Variable	-	-
<i>L. welshimeri</i>	-	-			Variable	+	-
<i>L. seeligeri</i>	+	-			-	+	-
<i>L. grayi</i>	-	+	+	+	Variable	-	-

^aSheep blood agar

^bMouse test

Table 3. Results of Christie-Atkins-Munch-Peterson (CAMP) test for hemolysis of *Listeria* spp. (FDA 2011a).

Species	Hemolysis enhancement from CAMP test	
	<i>Staphylococcus aureus</i> (S)	<i>Rhodococcus equi</i> (R)
<i>L. monocytogenes</i>	+	- ^a
<i>L. ivanovii</i>	-	+
<i>L. innocua</i>	-	-
<i>L. welshimeri</i>	-	-
<i>L. Seeligeri</i>	+	-

^aSome strains produce S+ and R+ but the R+ reaction is less pronounced than the reaction produced by *L. ivanovii*.

Listeria monocytogenes

Known originally as *Bacterium monocytogenes*, *Listeria monocytogenes* was later named after British surgeon Lord Joseph Lister who is famous for developing antiseptic surgery (Bell and Kyriakides 1998; Murray and others 1926). The species name for this bacterium comes from the fact that *L. monocytogenes* causes increased levels of monocyte production which help the body fight off infection (Bell and Kyriakides 1998; Encyclopædia Britannica 2011). It was first known as an animal pathogen in the mid 1920s, but *L. monocytogenes* has been an important food contaminant in the past several decades (Bell and Kyriakides 1998). The main serotypes of importance to food safety are types 1/2a and 4b (Jay and others 2005; Nightingale and others 2005). While serotypes are not specifically confined to geographic areas, 65-80% of all strains found in the US and Canada are type 4b (Seeliger and Hohne 1979).

Growth requirements for *Listeria* spp. are not different from other Gram positive bacteria. *Listeria* spp. require four B-vitamins for growth: biotin, riboflavin, thiamine, and thioctic acid. They also require the amino acids cysteine, glutamine, leucine, isoleucine, and valine for growth. *Listeriae* possess hydrolase, a bile salt which allows it to grow in the gall bladder. Unlike other Gram positive bacteria, *Listeria* spp. can grow on MacConkey agar. It can grow in the presence of 40% bile, 10% NaCl, 0.025% thallos acetate, 0.04% potassium tellurite and in a pH range of 4.1-9.6. One of the most unique properties of *Listeria monocytogenes* compared to other foodborne pathogens is its ability to grow at refrigeration temperatures as well as above body temperature (1-45 °C). *Listeria monocytogenes* is also able to grow at a water activity of 0.90 by adding glycerol, 0.93 by adding sucrose, and 0.92 by adding NaCl (Jay and others 2005).

Listeriosis

Listeria monocytogenes causes listeriosis in humans. Non-invasive listeriosis is characterized by loss of appetite, vomiting, fever, diarrhea, and lethargy (FDA 2009; NIH 2009a). Severe complications to invasive listeriosis can cause stillbirths or miscarriages in pregnant women due to the bacterium crossing the placenta (FDA 2009; Jay and others 2005; NIH 2009a). Invasive listeriosis can also cause endocarditis, meningitis, pneumonia, and septicemia (Jay and others 2005; NIH 2009a). Most healthy humans are resistant to listeriosis but those who are immuno-compromised are very susceptible (Jay and others 2005). Examples of those in a high risk group for listeriosis include those with neoplasm, Acquired

Immunodeficiency Syndrome (AIDS), or diabetes (Jay and others 2005). People who suffer from alcoholism, cardiovascular disease, or renal issues are also susceptible to listeriosis (Jay and others 2005).

Invasive listeriosis is characterized by intracellular invasion by *L. monocytogenes*, which is a complicated and unique process. When the bacterium is ingested, it then colonizes in the intestinal tract and then moves to tissues (including the placenta) before entering the blood stream where it can be carried to all areas of the body. In non-phagocytic cells, surface proteins InlA and InlB are required for uptake into the cells. InlA (internalin) contains a surface receptor called *E-cadherin* that is required for invasion of epithelial cells. *E-cadherin* is a calcium-dependant cell adhesion molecule. After the bacterium invades the cell, its movement is caused by ActA that controls actin polymerization. Once inside the host cell, listeriolysin O (LLO; a cholesterol-dependant cytolysin) causes the bacterium to be relocated to the cytosol, evading macrophages (Jay and others 2005; Pizarro-Cerda and Cossart 2007).

The infective dose of non-invasive *L. monocytogenes* is very low. Some studies show that as low as 100 bacterial cells can cause foodborne listeriosis (Jay and others 2005). However, the FDA (2009) believes that fewer than 1,000 cells can cause invasion of the gastrointestinal epithelium. One of the major problems with epidemiological studies of *L. monocytogenes* outbreaks is that the bacterium has an incubation period of 3-70 days (CDC 2011b; FDA 2009; NYDOH 2006). This means that it could take up to 70 days after ingestion of the bacterium before the patient shows any clinical signs of listeriosis.

While listeriosis is very rare and affects only 1-9 people per 1,000,000 people each year (0.02% of all foodborne illnesses each year), listeriosis accounts for 28% of all deaths due to foodborne illness (Tompkin 2002). Scallan and others (2011) reported that *L. monocytogenes* had a 94% hospitalization rate which was the highest of all bacterial foodborne pathogens. *Listeria monocytogenes* also had a 15.9% mortality rate which was 3rd of all bacterial pathogens behind *Vibrio vulnificus* (34.8%) and *Clostridium botulinum* (17.3%).

Distribution of Listeria spp.

Listeria spp. can be found throughout nature in soil, water, animal feces, or vegetation (Jay and others 2005). Listeriae also are known to be commonly found in dairy products, especially raw dairy products (Jay and others 2005). One study of milk bulk tanks in England

and Wales in the early 1990's found that over 5% of raw milk contained *Listeria monocytogenes* (O'Donnell 1995). In fact, FDA requires that all milk and milk products that are intended for human consumption be pasteurized at predetermined time and temperature combinations stated in the Pasteurized Milk Ordinance (PMO) (FDA 2010). Twenty-two states have laws or statutes that override this part of the PMO to allow for the sale of raw milk for human consumption (Kennedy 2004).

Listeria spp. also are found in ready-to-eat (RTE) products such as meat and poultry deli meats as well as hot dogs. In 1998, over 3,500 samples of RTE products were sampled for Listeriae and 2.5% (90 samples) tested positive for *Listeria monocytogenes* (USDA/FSIS 2000b). A group of researchers (Levine and others 2001) from the United States Department of Agriculture's Food Safety and Inspection Service (USDA/FSIS) published results from their monitoring of RTE meat and poultry products during the 1990's which showed that overall the prevalence of *L. monocytogenes* decreased during the decade (Table 4). It was observed that sliced ham and luncheon meats consistently had a higher prevalence than all other meat products.

Table 4. Prevalence of *Listeria monocytogenes* in ready-to-eat meat and poultry products during the years of 1990 to 1999 (Levine and others 2001).

Year	Cooked, roast, corned beef	Sliced ham and luncheon meats	Small cooked sausages	Large cooked sausages	Jerky	Cooked poultry products	Salads/ Spreads/ Pates	Fermented sausages
1990	6.38%	7.69%	4.21%	5.32%	0.00%	2.79%	5.48%	NA
1991	4.02%	5.48%	7.24%	4.60%	0.00%	2.62%	3.17%	NA
1992	3.86%	7.89%	6.03%	0.42%	0.00%	2.01%	3.32%	NA
1993	3.04%	8.05%	5.30%	2.13%	0.00%	1.91%	2.19%	NA
1994	2.09%	5.46%	4.81%	1.14%	2.22%	2.37%	2.41%	NA
1995	2.68%	5.00%	4.09%	1.14%	0.00%	2.25%	4.69%	NA
1996	3.35%	7.69%	3.74%	0.95%	0.00%	3.17%	2.17%	NA
1997	2.08%	4.20%	2.74%	1.62%	0.00%	0.95%	2.43%	9.26%
1998	2.15%	4.18%	3.49%	1.19%	1.56%	2.22%	3.11%	2.87%
1999	2.71%	4.58%	1.76%	0.43%	0.00%	1.44%	1.15%	2.09%
Cumulative	3.09%	5.16%	3.56%	1.31%	0.52%	2.12%	3.03%	3.25%

In 2010, USDA published the results of a 2008 risk-based sampling study of *Listeria monocytogenes* (Mamber 2010). This study involved sampling of ready-to-eat (RTE) food products (959 samples), food contact surfaces (3,322 samples), and environmental samples (1,725 samples). In total, five (0.52%) of the 959 food products and 19 (0.57%) of the 3,322 food contact samples contained *L. monocytogenes*. However, 35 (2.03%) of the 1,725 environmental samples contained *L. monocytogenes*. FSIS performed a surveillance study of *L. monocytogenes* and found that when comparing RTE meat and poultry deli products, beef products were the highest in prevalence of *L. monocytogenes* at 1.3% while pork and poultry products contained *L. monocytogenes* in 0.8% and 0.7% of products tested, respectively (USDA/FSIS 2010).

The United States government has a program called Healthy People in which they specifically outline goals for foodborne illness. The target of this program is to have 0.2 cases of listeriosis per 100,000 people per year. The actual incidence was 0.3 cases of listeriosis per 100,000 people per year (CDC 2010b).

***Listeria monocytogenes* Outbreaks**

Listeria monocytogenes has been of great concern in a number of foods. As was stated earlier, *L. monocytogenes* can be readily found in dairy products. Therefore, several *L. monocytogenes* outbreaks have been linked to dairy products. An outbreak of *L. monocytogenes* in Abbott cheeses caused 21 illnesses and two miscarriages in British Columbia (Marler 2006). Another *L. monocytogenes* outbreak occurred in the state of North Carolina in late 2000 to early 2001 involving Mexican cheeses (CDC 2001). All 12 cases were people of Hispanic origin and a majority of people were recent immigrants from Mexico. This outbreak was linked to homemade Mexican-style cheeses made from raw milk. This was not the first outbreak connected to Mexican-style cheeses. In 1985, 85 cases (mostly Hispanic patients) of listeriosis were reported in Los Angeles and Orange Counties in California (CDC 1985). During this outbreak, 29 people died due to contamination of Mexican-style cheeses. Eight of those deaths were neonatal while 13 were stillbirths. The bacterium involved in this outbreak was *L. monocytogenes* serotype 4b.

In 2007, an outbreak involving pasteurized milk caused four people to become sick and two died due to the illness (Marler 2007). The company involved, Whittier Farms, eventually

closed its doors permanently due to this outbreak. This outbreak was extremely rare because it involved pasteurized milk. Olsen and others (2004) reported a list of 12 other outbreaks (dating back to 1966) connected to pasteurized milk. Two of the outbreaks involved *L. monocytogenes* while others involved *Shigella flexneri*, *Salmonella* spp., *Yersinia enterocolitica*, and *Campylobacter jejuni*.

In August, 2010, Louisiana's Department of Agriculture and Forestry called for a recall of 500,000 pounds of cooked sausages and hog head cheese (RTE meat product) because *L. monocytogenes* serotype 1/2a was isolated from the products produced by Vernon Foods (CDC 2011b; Food Safety News 2011a). This outbreak caused 14 illnesses with 7 people being hospitalized and two deaths occurring.

The New York Department of Health and Mental Hygiene reported an outbreak of *L. monocytogenes* that was tied to turkey deli meat (Olsen and others 2005). This outbreak occurred in 2005 and caused 30 people to become ill, ultimately four people died and three women had miscarriages. The serotype responsible for this outbreak also was 1/2a.

In 2011, *Listeria monocytogenes* caused an outbreak in cantaloupe from Jensen Farms that affected 26 states. This outbreak caused 123 people to become sick and 118 of those were hospitalized due to their illness. Twenty-five people ultimately died due to the outbreak and one pregnancy ended in a miscarriage (CDC 2011a). The FDA found *Listeria monocytogenes* on unspecified equipment of the production area (Food Safety News 2011b). The *L. monocytogenes* involved in this outbreak were serotypes 1/2a and 1/2b (CDC 2011a).

One of the most important outbreaks involving *L. monocytogenes* occurred in 1998. Twenty-two states reported listeriosis cases due to a serotype 4b strain (CDC 1998; CSU 2010). Over 100 people were affected by this outbreak and 21 people eventually died. Six of those deaths were miscarriages or stillbirths while the other 15 were adults. Eventually, the outbreak was determined to have originated at Bil Mar Foods due to the demolition of a refrigeration unit. Brand names that were affected included Ball Park, Bil Mar, Bryan Bunsizer, Bryan 3-lb. Club Pack, Grillmaster, Hygrade, Mr. Turkey, Sara Lee Deli Meat, and Sara Lee Home Roast.

Following this outbreak, FSIS established three short-term initiatives: (1) FSIS published a notice requiring plants producing ready-to-eat (RTE) meat and poultry products to reassess their HACCP plans to ensure they are adequately addressing *L. monocytogenes*; (2) FSIS provided guidance to the industry on practices that have been used successfully by other meat

and poultry establishments to prevent the occurrence of *L. monocytogenes* in ready-to-eat products; and (3) FSIS provided educational efforts targeted to those individuals who are at an increased risk of developing listeriosis (USDA 1999). They also established four long term initiatives: 1) FSIS is drafting a protocol to study the post-production growth of *L. monocytogenes* in a wide variety of ready-to-eat products, U.S. Department of Agriculture's Agricultural Research Service will be asked to conduct the study; 2) FSIS will develop an in-depth verification protocol to evaluate whether plants producing ready-to-eat products have reassessed their HACCP plans; 3) FSIS is working with FDA to conduct a risk assessment for *L. monocytogenes*; and 4) FSIS developed performance standards for ready-to-eat products that will address the need to control all pathogens, including *L. monocytogenes*, in these products (USDA/FSIS 2000b). This led to several important emphasis areas in risk management of RTE foods as well as research involving *L. monocytogenes* in these products.

FSIS Guidelines for Listeria monocytogenes

Due to several recalls and outbreaks involving *Listeria monocytogenes* in RTE meat and poultry products in 1998 and 1999, FSIS placed a zero tolerance (no detectable level permitted) on *L. monocytogenes* in these products. FSIS has also had a zero tolerance for *L. monocytogenes* in hot dogs and luncheon meats since 1987 (USDA/FSIS 2000b).

FSIS has also published other regulations to guard against *L. monocytogenes* contamination. Typical thermal processing of RTE meat and poultry products will adequately kill *L. monocytogenes*. Since *L. monocytogenes* is ubiquitous, it is of great concern for post-process contamination during slicing or packaging. A study by Saunders and others (2009) highlighted importance of this when they observed presence of *L. monocytogenes* in various areas of a retail environment. They determined that 12.5% of produce preparation areas tested positive for *L. monocytogenes* while 12.2% of deli sink areas were positive. The study also found that 3.3% of slicer and deli case surfaces tested positive for *L. monocytogenes*.

Quite a few studies have researched the topic of cross contamination of *L. monocytogenes* from slicing equipment to food products. Keskinen and others (2008b) studied the transfer of *L. monocytogenes* from stainless steel knife blades to cut roast turkey breast. Knives were inoculated with 5 and 7 log CFU/cm² of *L. monocytogenes* that had formed a biofilm on the blade. They found that *L. monocytogenes* populations decreased to 2 log CFU per slice of meat

after 16 slices of the knife. They also found that *L. monocytogenes* in a biofilm survived dry environments on the blade after 24 h at 22 °C and 78% relative humidity. Keskinen and others (2008a) also performed a similar study on *L. monocytogenes* that were stressed due to environmental conditions. They tested the ability of the bacterium to transfer after forming either a weak or strong biofilm as well as after being stressed due to cold-shock (4 °C for 2 h) or chlorine-injured (100 ppm for 1 min). They found that stronger biofilm production caused significantly greater transference and cold-shocked cells were transferred at a significantly greater amount than uninjured cells or chlorine-injured cells. Finally, they found that *L. monocytogenes* was transferred at a significantly greater amount to turkey than salami.

The 2010 outbreak involving *L. monocytogenes* in hog head cheese and cooked sausage displays the importance of post-processing contamination. In this outbreak, the products were contaminated from multiple pieces of processing equipment (CDC 2011b). The well-known Bil Mar outbreak was connected to environmental contamination due to the removal of a refrigeration unit that caused *L. monocytogenes* to become a major environmental contaminant at the processing facility (CDC 1998; Marler 1998).

Due to *L. monocytogenes* outbreaks involving post-process contamination in RTE meat and poultry products, FSIS (USDA/FSIS 2006) established three alternatives that food processors can use to minimize risk of post-process contamination. Alternative 1 states that the establishment must use a post-lethality treatment to reduce or eliminate *L. monocytogenes*, and an antimicrobial agent or process to suppress or limit the growth of the pathogen. The establishment must provide validation of the post-lethality treatment for eliminating or reducing *L. monocytogenes*. This validation should specify the log reduction or suppression achieved by the post-lethality treatment and antimicrobial agents. Establishments under Alternative 1 are subject to the lowest frequency of testing by FSIS (USDA/FSIS 2006).

Under Alternative 2, an establishment must use either a post-lethality treatment or an antimicrobial agent or process. Again, the establishment must validate the post-lethality treatment of an antimicrobial agent that is used. The definition of a post-lethality treatment must reduce *L. monocytogenes* by 1 log CFU/g of food. Establishments falling under Alternative 2 are subject to more frequent FSIS verification than Alternative 1 (USDA/FSIS 2006).

Alternative 3 is when an establishment relies on its sanitation program to control *L. monocytogenes* in RTE meat and poultry products. An establishment using Alternative 3 is

subject to the most frequent FSIS verification because they do not have a plan in place for reducing post-process contamination (USDA/FSIS 2006).

Many RTE meat and poultry products use the reduction of water activity as a post-lethality treatment (USDA/FSIS 2006). This can be achieved by adding salt. Other non-lethality treatments may include reducing the product's pH via fermentation or addition of an acidulant (Samelis and others 2001; Samelis and others 2005). Modified atmospheric packaging (MAP) has also been studied and found to be an effective post-lethality treatment for RTE meat and poultry products (Lobaton-Sulabo and others 2011; Uppal and others 2011). Lotaton-Sulabo and others (2011) studied the effects of packaging (vacuum, heat seal without oxygen scavenger, heat seal with oxygen scavenger, and nitrogen flushed) on *L. monocytogenes* populations in beef jerky over a 30 day storage period at 22.5 °C. Results showed that all packaging types caused a 1 log CFU/cm² reduction of *L. monocytogenes* after 48 h of storage and a 3.5 log log CFU/cm² reduction after 30 days of storage. The water activity of the jerky was <0.79 for all samples. Uppal and others (2011) performed a similar study on kippered beef steak and turkey tenders. Samples were stored for 72 h at 25.5 °C. Kippered beef steak had a water activity of 0.81 while turkey tenders had a water activity of 0.77. For kippered beef steak, *L. monocytogenes* was reduced by >1 log CFU/cm² after 24 h of storage and 2.1 log CFU/cm² after 72 h of storage for all packaging treatments. *L. monocytogenes* populations were reduced by >1 log CFU/cm² after 24 h of storage of turkey tenders for all packaging treatment except vacuum packaged (0.9 log CFU/cm²). All packaging treatments caused a reduction of >1 log CFU/cm² after 72 h of storage for turkey tenders.

Sodium lactate and sodium diacetate have also been studied as an antimicrobial against *L. monocytogenes*. Grosulescu and others (2010) studied the effect of sodium lactate and sodium diacetate on starved *L. monocytogenes* on bologna. Five levels of sodium lactate (0, 1.2, 2.4, 3.6, and 4.8%) and sodium diacetate (0, 0.0625, 0.125, 0.1875, and 0.25%) were studied. Results showed that at elevated levels of sodium lactate (4.8%), *L. monocytogenes* was actually protected from heat injury. The D-value was lowest for sodium lactate levels of 1.2% and a temperature of 60 °C. Sodium diacetate had a similar effect at higher concentrations (0.25%). However, the lowest D-value was observed with no sodium diacetate at 59 °C.

In 2000, the USDA/FSIS published a final rule on the use of sodium diacetate, sodium acetate, sodium lactate and potassium lactate in meat and poultry products (USDA/FSIS 2000a).

The rule permits the use of sodium acetate as a flavor enhancer, sodium diacetate as a flavor enhancer and anti-microbial, and sodium lactate and potassium lactate as anti-microbials in meat and poultry products (USDA/FSIS 2000a). Potassium lactate and sodium lactate as an antimicrobial are both permitted in products at a 4.8% level of the total formulation while sodium diacetate can be use at 0.25% by weight of the total formulation (USDA/FSIS 2000a).

Sodium levulinate is a flavoring agent with GRAS status (CFR 2011c). It has also been studied for the use of an antimicrobial against *L. monocytogenes*. Sodium levulinate at 1% concentration was found to inhibit *L. monocytogenes* growth on turkey breast roll for 12 weeks of storage at 4 °C and was effective at 2% to inhibit *L. monocytogenes* growth in bologna after 12 weeks of storage at 4 °C (Thompson and others 2008).

Lauric arginate is another GRAS substance that can be used for its antimicrobial properties (CFR 2011c). Martin and others (2009) performed a study involving the use of lauric arginate, potassium lactate, and sodium diacetate on post-process contaminated frankfurters. No difference ($P > 0.05$) was observed due to concentration when lauric arginate was added at 2, 2.5, and 3 mL of 2.5% lauric arginate solution. All *L. monocytogenes* populations decreased 1 log CFU/cm² after 12 h of storage at 6 °C. Potassium lactate and sodium diacetate used in combination with lauric arginate in packaging suppressed *L. monocytogenes* growth below 2 log CFU/cm² for shelf-life of 156 d refrigerated storage.

Sodium and Health

Sodium (Na⁺) is important to overall health in many ways. During early cellular evolution, one of the important developments was the beginning of ionic gradients (Solomon and Galey 1982). Ionic transport of sodium can be achieved through either active (requiring energy) or passive (does not require energy) transport.

Sodium is very important to kidney function. Kidneys remove waste and water from the blood to form urine. As the kidneys sift through the waste, they determine the amount of sodium, phosphorus, and potassium that is needed to return to the blood. Hypertension can injure small blood vessels in the kidneys. This can lead to kidneys not filtering properly. People with chronic kidney disease may require dialysis. Dialysis uses a filter that mimics kidneys to clean the patient's blood. Patients needing dialysis are treated three times per week with treatments lasting 3-4 h per day (NIH 2010).

Potassium and Health

Potassium (K^+) has many different functions in the body and is extremely important to the function of the human body. Potassium is mainly used in the body for smooth and skeletal muscle contraction (GHS 2009). In fact, potassium and sodium pumps account for 20-40% of resting energy expenditure in an adult (OSU 2010). Potassium also is important to transmission of nerve impulses throughout the body (OSU 2010). It also is a cofactor for the enzyme pyruvate kinase which is involved in glycolysis (OSU 2010).

The Adequate Intake, or the amount adequate for most of the population, for potassium for adults is 4,700 mg per day (USDA 2010b). It is rare for a healthy person to have a potassium deficiency (hypokalemia) because so many foods are good sources of potassium (OSU 2010). However, people who suffer from inflammatory bowel diseases such as ulcerative colitis or Crohn's disease have difficulty absorbing potassium in their intestine (GHS 2010). Overconsumption of potassium is called hyperkalemia and occurs when the kidneys cannot eliminate potassium from blood (OSU 2010). Hypokalemia can also occur from extreme diarrhea, vomiting, or sweating. Symptoms include tingling of the extremities, muscle weakness, and temporary paralysis. Serious complication can cause a cardiac arrhythmia which may lead to cardiac arrest (OSU 2010).

The effect of potassium on blood pressure, coronary heart disease, and risk of stroke has been well documented (Barri and Wingo 1997; OSU 2010). In a study of 17,000 adults in 2001, higher dietary potassium was associated with a significant decrease in blood pressure (Hajjar and others 2001). A Dietary Approaches to Stop Hypertension (DASH) study found similar results (Appel and others 1997). In this study, 459 people were separated into three groups and each group was assigned a different diet. First was a control diet that contained about 1,750 mg/day of potassium while the other two diets (fruits and vegetables diet and a combination diet of fruits, vegetables, and low-fat dairy foods with reduced amounts of saturated fat, total fat, and cholesterol) each contained over 4,000 mg/day of potassium. Results showed that the control diet and fruits and vegetables diet had a higher ($P < 0.05$) percentage of participants who had elevated systolic and diastolic levels.

Calcium and Health

Calcium (Ca^{2+}) is another important cation for the human body. In fact, calcium is the most abundant mineral in the human body (NIH 2011a). Calcium is used for a wide range of functions in the body including vascular contraction and dilation, muscle contraction, nerve transmission, intracellular signaling, and hormonal secretion (NIH 2011a). Up to 99% of calcium is stored in bones and the body uses bone tissue as a source of calcium to maintain constant levels in blood, muscle, and intercellular fluids (NIH 2011a). Serum calcium levels do not fluctuate very often which causes the body to only need around 1% of calcium that it intakes (NIH 2011a). USDA recommends that adults consume 1,000 mg of calcium per day (USDA 2010c). Risk of bone fractures increases with calcium deficiency, which can cause osteoporosis with prolonged deficiency (USDA 2010c).

One meta-analysis pooled results from 11 studies, including 12,000 women and found that women taking calcium supplements (about 1,000 mg/day in most of the studies) had a 27% increased risk of myocardial infarction (Bolland 2010). However, these findings have come under scrutiny because the analysis considered only studies involving calcium supplements and not calcium plus vitamin D, none of the studies were designed primarily to examine cardiovascular disease. Results also showed no significant increase in stroke or death (Dawson-Hughes 2010; Grove and Cook 2010; Heiss and others 2010; Nordin and others 2010). In fact, two studies have shown that increased calcium intake can reduce risk of stroke and lower blood pressure.

In another study, almost 44,000 men were surveyed about their diet. The participants were tracked for 8 years to determine their risk of stroke. The study found that as calcium level increased from 0.5 to 1.4 g/d, risk of stroke significantly decreased (Ascherio and others 1998).

Magnesium and Health

Magnesium (Mg^{2+}) is the fourth most abundant mineral in the human body (NIH 2009b). Half of the magnesium in the human body is found in the bones while the other half is dispersed throughout cells (NIH 2009b). Much like calcium, the body highly regulates serum magnesium levels which causes these levels to remain very constant at all times (NIH 2009b). This mineral is extremely important to the body and is important to biochemical reactions, muscle and nerve function, a healthy immune system, healthy bones, and a healthy heart. It also regulates blood

sugar and blood pressure (NIH 2009b). The Recommended Dietary Allowance (RDA) for magnesium varies due to gender and age (Table 5).

Table 5. Recommended dietary allowances (RDAs) for magnesium for adults and children (USDA 2010c).

Age	Male (mg/day)	Female (mg/day)
1-3	80	80
4-8	130	130
9-13	240	240
14-18	410	360
19-30	400	310
31+	420	320

Hypertension

Hypertension or high blood pressure is a condition where force of the blood against artery walls is high enough that it may eventually cause health problems (Mayo Clinic 2011a). The study of hypertension has been one of great importance in the last several decades. Sodium is needed in the diet to maintain osmotic balance within cells as well as in the function of nerve impulses and muscle contraction (CSU 2011). During heavy exertion, the body will excrete sodium through sweat (CSU 2011). However, over-consumption of sodium can lead to hypertension (CSU 2011; Cleveland Clinic 2010).

The Mayo Clinic defines blood pressure (BP) as “the amount of blood your heart pumps and the amount of resistance to blood flow in your arteries (Mayo Clinic 2011a).” Blood pressure is measured as the systolic (when the heart beats while pumping blood) pressure over the diastolic pressure (when the heart is at rest). Categories for blood pressure can be found in Table 6.

Table 6. Categories of blood pressure levels for adults, ages 18 and over (NIH 2011b).

Category	Systolic (mm Hg)		Diastolic (mm Hg)
Normal	Less than 120	And	Less than 80
Prehypertension	120-139	Or	80-89
Stage 1 High blood pressure	140-159	Or	90-99
Stage 2 High blood pressure	160 or higher	Or	100 or higher

A person can have high blood pressure without showing any symptoms, but if it is left uncontrolled, it can eventually cause heart attack and stroke (Mayo Clinic 2011a). Blood pressure increases as a person gets older (Mayo Clinic 2011a; NIH 2011b). The National Heart, Lung, and Blood Institute states that one out of three Americans suffer from hypertension (NIH 2011b).

Hypertension is caused by many factors. These include amount of water or salt in a person's diet; condition of a person's kidneys, nervous system, or blood vessels; or levels of hormones in a person's body (NIH 2011d). A person is more at risk for hypertension if a person is African American, obese, under continued stress, diabetic, or a smoker (NIH 2011d). Secondary hypertension is caused by another medical condition or medication. Examples include alcohol abuse, atherosclerosis, chronic kidney disease, and endocrine disorders (NIH 2011d). Some medications that can cause secondary hypertension include appetite suppressants, birth control pills, corticosteroids, and migraine medications (NIH 2011d).

Sodium and Hypertension

The effect of sodium intake on hypertension has been thoroughly studied in the last several decades and it is common knowledge that reducing sodium in ones' diet can lower the chances of hypertension (Dickinson and Havas 2007; Karppanen and Mervaala 2006; He and MacGregor 2008). Sodium causes hypertension by forcing the body to take in more water than usual to dilute the concentration of sodium in the blood. This, in turn, causes blood pressure levels to increase (Cleveland Clinic 2010).

In 1972, National Institutes of Health (NIH) started the National High Blood Pressure Education Program as part of the National Heart, Lung, and Blood Institute to educate and research hypertension in the U.S. (NIH 1996; NIH 2011b). One of the primary goals of this program was to determine pharmacological and non-pharmacological treatments that could be used in the public health arena to lower blood pressure (NIH 1996).

Not all research has shown that sodium intake has a strong relationship or correlation with hypertension. A group of Flemish researchers studied nearly 3,700 individuals for eight years to determine the effects of sodium on cardiovascular disease (Stolarz-Skrzypek and others 2011). They found that systolic BP changed with the level of sodium excretion while diastolic pressure did not change at all. However, a 100-mmol increase in sodium excretion was

associated with 1.71 mm Hg increase in systolic blood pressure. They also found that mortality rate from cardiovascular disease was lower in cohorts with medium and high sodium excretion levels. The death rate for low (106 mmol) urinary sodium excretion was 4.1% while rates decreased to 1.9% and 0.8% for medium (165 mmol) and high (250 mmol) urinary excretion levels. The same trend was found when studying all fatal and nonfatal coronary events as well as strokes. However, this study seems to be the only evidence that increasing sodium intake could be beneficial to health.

Sodium Intake in the United States

The average American consumes much more sodium than is recommended. The 2010 Dietary Guidelines recommends that 2,300 mg of sodium be consumed per day in a person's average diet. This number drops to 1,500 mg of sodium if the person is 51 years old or older, African American, or suffers from hypertension, diabetes, or chronic kidney disease (USDA 2010a). However, the average American consumes 3,400 mg of sodium per day, far exceeding the USDA's recommendations (Mayo Clinic 2011c). Men consume far more sodium on average than women do. A study performed by the Division of Health Examination Statistics of the Centers for Disease Control and Prevention (CDC) surveyed 4,206 men and 4,398 women of all ages about their diet (Wright and others 2003). The study found that men consumed an average of 3,877 mg of sodium per day while women consumed an average of 2,896 mg of sodium per day.

Recommendations for Sodium Reduction

Reduction of sodium in the American diet is not a new issue and several reviews of the use of salt have been published in the past (Reddy and Marth 1991; Sofos 1983; Taormina 2010). Salt levels in various food products have been lowered throughout the 20th century for many reasons including discovery of health effects, use of refrigeration, and use of other antimicrobial ingredients (Sofos 1983). However, World Action on Salt and Health (WASH), a consumer advocacy group, has recently stated that salt intake should be lowered to the WHO standards of 5 grams per day all over the world (WASH 2009).

Both the New York City Department of Health and the World Health Organization (WHO) has accepted the many studies that show a correlation between hypertension and sodium

intake and has called for lowering sodium levels in foods worldwide (New York City Department of Health 2010; WHO 2006). In the U.S., the Center for Science and the Public Interest has petitioned FDA to reclassify common salt (sodium chloride) as a food additive (FDA 2007).

The World Health Organization (WHO) has recommended that food processors around the world reformulate and lower the amount of sodium in their products due to health effects linked to over consumption of sodium (WHO 2006). Several studies have cited excess sodium in the diet as a major cause of high blood pressure levels (Dickinson and Havas 2007; Karppanen and Mervaala 2006; He and MacGregor 2008). Other studies have found that reduced sodium levels in the diet can reduce the risk of cardiovascular disease (Cutler and Roccella 2006; Cook and others 2007). However, little research has focused on the food safety implications when salt is reduced in food products (Taormina 2010).

Chapter 3 - Effect of salt and salt replacements on *Listeria monocytogenes* growth in a broth system

Introduction

Salt serves several purposes in food; it is a flavor enhancer, a preservative, a leavening aid, and it controls fermentation and provides nutritional content (Gordon and Klimek 2994; Miller and Hoseney 2008). Salt has been used for centuries as a preservative for food (Jensen, 1954; Marsden 1980). *Listeria monocytogenes*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus* are some of the more salt-tolerant and salt-resistant foodborne pathogens (Taormina 2010).

The mechanism of salt's microbial inhibition effect is still of significant importance in the area of food safety research. One of salt's preservative effects is its ability to decrease water activity levels in food (Csonka 1989). However, research has shown that lowering water activity is not the only way salt controls foodborne microbial growth (Galinski and Truper 1994; Riberiro, Manha, and Brito 2006; Rockwell and Ebertz 1987). Other studies have documented that NaCl interferes with substrate utilization so cellular function ceases (Csonka 1989; Erecinska and Deutsch 1985; Smith and others 1987; Woods and Wood 1982).

Although salt's favorable qualities are known, its unfavorable health effects are also prominent. Salt levels in food have been lowered throughout the 20th century because of refrigeration and the use of other antimicrobial ingredients, but also because of the discovery of health effects (Sofos 1983). The impact of sodium intake on hypertension, for example, has been thoroughly studied over the last several decades, and reducing sodium in one's diet is known to have the potential to lower the chances of hypertension (Dickinson and Havas 2007; He and MacGregor 2008; Karppanen and Mevaala 2006). Both the New York City Department of Health and the World Health Organization (WHO) have accepted these research findings and called for lowering sodium levels in foods worldwide (NYCDOH 2010; WHO 2007). In the United States, the Center for Science and the Public Interest petitioned the Food and Drug Administration (FDA) to reclassify NaCl as a food additive (FDA 2007). The World Action of Salt and Health (WASH) recently stated that salt intake should be lowered to the WHO standards of 5 grams per day (WASH 2009).

The reduction of salt is not a new issue. The food industry has been researching ways to reduce the sodium content in various processed food products. However, sodium reduction also affects food safety, and several reviews of the use of salt in the area of food safety have been published in the past (Reddy and Marth 1991; Sofos 1983; Taormina 2010). The objective of our study was to determine the effect that different salts have on the growth of *L. monocytogenes*.

Materials and Methods

Experimental Design

This study consisted of three phases: 1) determining the ability of *L. monocytogenes* to grow in modified *Listeria* enrichment broth (mLEB) containing the chemical salts NaCl, KCl, and MgCl₂ at different levels, 2) determining the ability of *L. monocytogenes* to grow in mLEB containing CaCl₂, and 3) determining the ability of *L. monocytogenes* to grow in mLEB containing commercial salts (replacement salt and sea salt). Inoculated controls (no salt) were used for each phase of research. A replication consisted of an inoculated control as well as all of the salts at 0.5, 1.0, and 2.5%. Each replication was done in duplicate, with three replications performed for each phase.

Listeria monocytogenes cultures

A five-strain cocktail of *L. monocytogenes* was used for this study. The cultures were ATCC 13932, ATCC 19115, ATCC 19118, ATCC 19912, and ATCC SLR-2249 and were all obtained from American Type Culture Collection (Rockville, Md., U.S.A.). ATCC 13932 (serotype 4b) was isolated from the spinal fluid of a child with meningitis in Germany while ATCC 19112 (serotype 1/2c) was isolated from the spinal fluid of a man in Scotland. ATCC 19115 (serotype 4b) was a human isolate, while ATCC 19118 (serotype 4e) was isolated from a chicken in England. ATCC SLR-2249 is a laboratory strain with the *ActA* gene removed. All cultures were grown for 24 h in tryptic soy broth (Becton Dickinson, Franklin Lakes, N.J., U.S.A.) and incubated at 35 °C. Cultures were removed, aseptically combined into a 5-strain cocktail and diluted 3 (1 mL into 9 mL) times by using peptone Becton Dickinson, Franklin Lakes, N.J., U.S.A.). After the final dilution, 1 mL of the culture was added to the modified *Listeria* enrichment broth.

Listeria enrichment broth salt solutions preparation

Modified *Listeria* enrichment broth (mLEB) (Becton Dickinson, Franklin Lakes, N.J., U.S.A.) was produced without sodium chloride and dipotassium phosphate. The sodium chloride was removed because the research was studying the effect of this salt. The dipotassium phosphate was removed because when CaCl₂ and MgCl₂ were added to LEB, a precipitant of calcium phosphate or magnesium phosphate was formed. Ingredients used (on a per-liter basis) were pancreatic digest of casein (17 g), soytone (3 g), dextrose (2.5 g), yeast extract (6 g), cycloheximide (0.05 g), acriflavine HCl (15.0 mg), and nalidixic acid (0.04 g) (Becton Dickinson, Franklin Lakes, N.J., U.S.A.). Salts were added at ingoing levels of 0.5, 1.0, and 2.5% by adding 2.5, 5.0, and 12.5 mg of salt to a half (0.5) liter of mLEB prior to autoclaving. Salts were all chemical grade salts supplied from Fisher Chemical (Fairlawn, N.J., U.S.A.). Samples were inoculated with *L. monocytogenes* at a level of 3.5 log CFU/mL by serial diluting the stock culture four times prior to inoculation into the mLEB solutions.

Sampling and incubation procedures

All treatments were stored at 22 °C sampled at 0, 24, and 48 h after inoculation for *L. monocytogenes* populations. A sample consisted of 1 mL that was removed from mLEB and serially diluted in a 0.1% peptone solution for enumeration on modified oxford medium (MOX) (Becton Dickinson, Franklin Lakes, N.J., U.S.A.). All serial dilutions were vortexed for 3 to 4 s on high speed before they were transferred to the next dilution. Samples were spread plated on MOX (0.1 mL per plate) agar plates for each dilution, allowing for a detection limit of 2.0 log CFU/mL. Plates were incubated at 35 °C for 48 h prior to counting. Plates were incubated in both aerobic and anaerobic conditions for phase 1 and only in aerobic conditions for phases 2 and 3 (Knabel and others, 1990). No difference ($P > 0.05$) was found between the two incubation methods, so data were pooled.

Mineral analysis for industrial salts

Atomic absorption (AOAC Official Method Number 968.08) was performed on the replacement salt and sea salt from phase 3 to determine the concentrations of sodium, potassium, calcium, and magnesium in each salt.

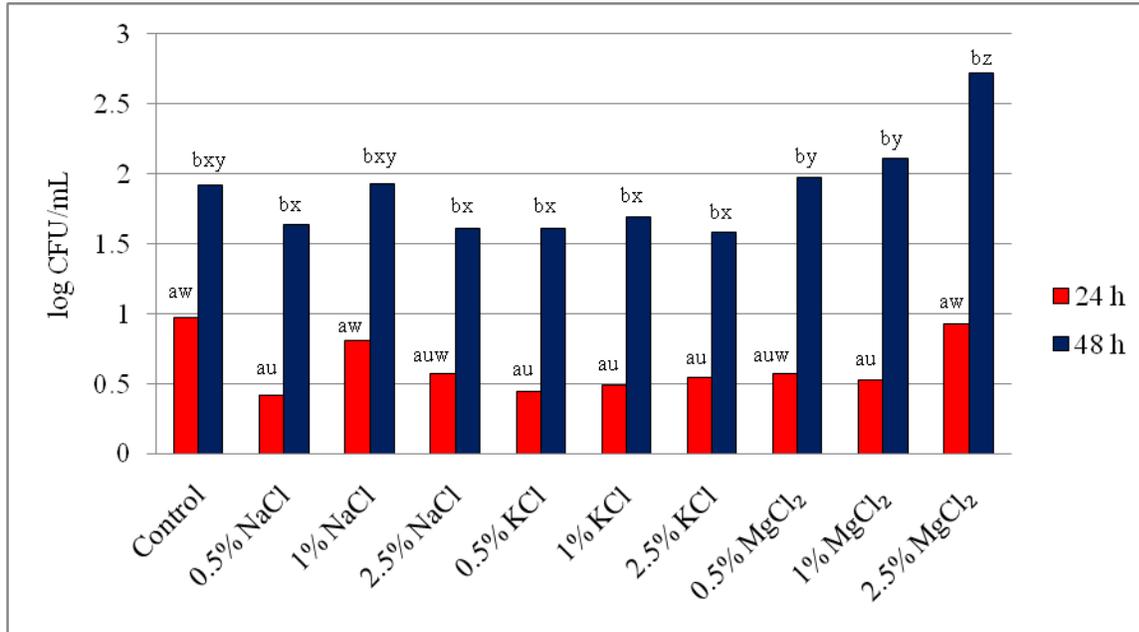
Statistical analysis

Phase 1 of this experiment was a 3 x 3 factorial design with type of salt and concentrations as the two factors. Phase 2 of this study was a completely randomized design and phase 3 was a 2 x 3 factorial design. *Listeria monocytogenes* population data were analyzed using PROC MIXED in SAS version 9.0 (SAS Institute, Cary, N.C., U.S.A.). No differences ($P > 0.05$) were found using Tukey test among storage type (aerobic versus anaerobic) for phase 1, so data were pooled for overall populations within type of salt, concentration, and sampling time. Fixed effects for statistical analysis were salt type, concentration, time, salt type by concentration, salt type by time, concentration by time, and salt type by concentration by time, and the random effect was replication. Least squares means ($P < 0.05$) were used to compare the interactions.

Results

For phase 1, initial *L. monocytogenes* populations (at 0 h) for all treatments averaged 3.88 log CFU/mL. Populations for all treatments increased over time; however, this occurred at different rates for all treatments (Figure 1). Populations differed significantly ($P < 0.05$) due to time, salt type, time x salt type interaction, salt concentration, and salt type x salt concentration interaction. The interactions that involve time were expected because the time would be sufficient for *L. monocytogenes* to grow. A difference between salt concentrations was also expected because as concentration increases, growth should decrease. This occurred with some salt types, but results were mixed with other types. The main goal of this research was to determine the effect of salt type on *L. monocytogenes* growth.

Figure 1. Growth of *Listeria monocytogenes* (log CFU/mL) in modified* *Listeria* enrichment broth with added NaCl, KCl, and MgCl₂ salts (phase 1 research) (n=6).



**Listeria* enrichment broth was modified by removing sodium chloride and dipotassium phosphate;

^{ab} superscripts indicate differences ($P < 0.05$) within treatments for 24- and 48-h populations;
^{uvwxyz} superscripts indicate differences ($P < 0.05$) across all treatments

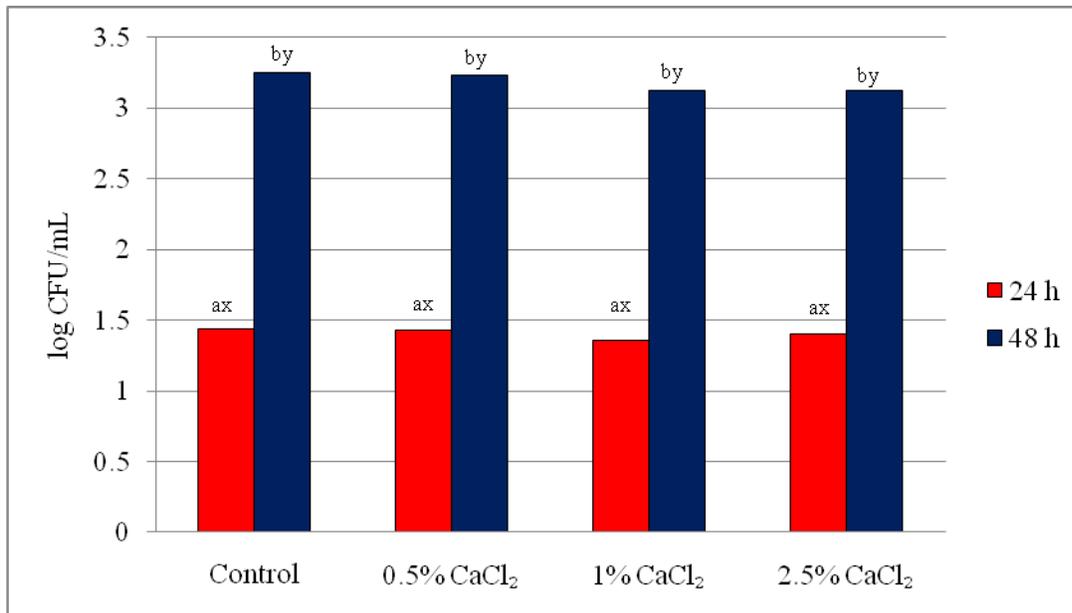
Magnesium chloride treatments had greater ($P < 0.05$) *L. monocytogenes* population at the final sampling time compared with the other salt treatments and the control. Samples increased 1.97, 2.11, and 2.72 log CFU/mL for treatments of 0.5%, 1%, and 2.5% of MgCl₂, respectively, at 48 h after inoculation. Comparatively, NaCl treatment populations increased only 1.64, 1.93, and 1.61 log CFU/mL for 0.5%, 1%, and 2.5%, respectively, whereas KCl treatment populations increased 1.61, 1.68, and 1.58 log CFU/mL for 0.5%, 1%, and 2.5%, respectively, after 48 h. Control samples increased 1.92 log CFU/mL from 0h to 48 h.

Growth also occurred after 24 h for phase 1 (Figure 1), but was minimal due to the lack of time for *L. monocytogenes* to grow. The greatest growth at 24 h was the control with 0.97 log CFU/mL growth. For phase 2, samples grew after 24 h, but all calcium treatments and control averaged around 1.42 log CFU/mL after 24 h. For phase 3, all populations grew after 24 h, but only to a level of 0.60 log CFU/mL.

Initial populations of *Listeria monocytogenes* for the calcium chloride treatments (phase 2) were approximately 4.07 log CFU/mL for 0.5%, 1%, and 2.5% of CaCl₂ (Figure 2).

Populations increased 3.23, 3.12, and 3.12 log CFU/mL for 0.5%, 1%, and 2.5% of CaCl₂, respectively, after 48 h. Inoculated controls increased 3.25 log CFU/mL after 48 h. There was no difference ($P > 0.05$) between the concentrations or between any of the salt treatments and the control.

Figure 2. Growth of *Listeria monocytogenes* in modified* *Listeria* enrichment broth with added CaCl₂ salt (phase 2 research) (n=6).



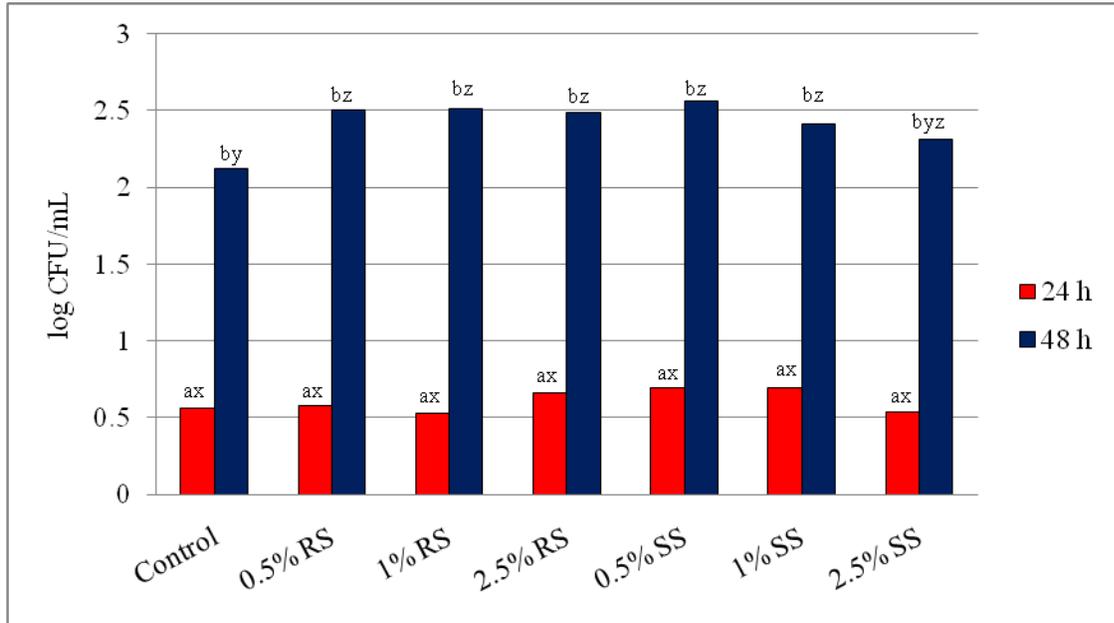
**Listeria* enrichment broth was modified by removing sodium chloride and dipotassium phosphate;

^{ab} superscripts indicate differences ($P < 0.05$) within treatments for 24- and 48-h populations;

^{xy} superscripts indicate differences ($P < 0.05$) across all treatments

For phase 3, average initial population was 3.43 log CFU/mL. A significant ($P < 0.05$) difference was observed due to time, time x type of salt, salt concentration, and time x type of salt x salt concentration. No significant ($P > 0.05$) difference was observed due to type of salt or type of salt x salt concentration. After 48 h, *L. monocytogenes* populations increased approximately 2.50 log CFU/mL for replacement salt at 0.5%, 1%, and 2.5% (Figure 3). The populations grew 2.56, 2.41, and 2.31 log CFU/mL for sea salt at 0.5%, 1%, and 2.5%, respectively, at the same sampling time. Control populations increased 2.12 log CFU/mL at the final sampling time.

Figure 3. Growth of *Listeria monocytogenes* (log CFU/mL) in modified* *Listeria* enrichment broth with added replacement salt (RS) and sea salt (SS) (phase 3 research) (n=6).



**Listeria* enrichment broth was modified by removing sodium chloride and dipotassium phosphate;

^{ab} superscripts indicate differences ($P < 0.05$) within treatments for 24- and 48-h populations;

^{xyz} superscripts indicate differences ($P < 0.05$) across all treatments

Analysis of the industrial salts for phase 3 research was performed to determine the levels of sodium, potassium, calcium, and magnesium (Table 7). Results for the salt replacement showed it was composed of 26.71% sodium and the other cations (K^+ , Ca^{2+} , and Mg^{2+}) ranged from 2.20% to 2.91%. The sea salt had higher levels of sodium (32.40%) and the calcium level was 4.40%, but potassium and magnesium levels remained low at 2.40% and 2.19%, respectively.

Table 7. Analysis (mean \pm standard error) of the industrial salts used in phase 3 research (n=3).

	Sodium	Potassium	Calcium	Magnesium
Sea salt	32.40 \pm 1.05%	2.40 \pm 0.13%	4.40 \pm 0.03%	2.19 \pm 0.01%
Salt replacement	26.71 \pm 0.87%	2.91 \pm 0.15%	2.39 \pm 0.24%	2.20 \pm 0.03%

Discussion

Phase 1 research showed that the magnesium cation caused greater ($P < 0.05$) outgrowth of *Listeria monocytogenes* than sodium and potassium salts. In fact, the treatment with 2.5% of $MgCl_2$ grew almost 1 log CFU/mL more than the control. Phase 2 shows that this phenomenon might be restricted to magnesium because calcium (a divalent cation) did not show an increase ($P > 0.05$) compared with the control. Interestingly, magnesium sulfate has a greater effect on water activity and dehydration than NaCl, but it does not have an increased bacteriostatic effect (Rockwell and Ebertz 1924). Conda (Madrid, Spain), a Spanish microbial media producer, states that magnesium is a growth cofactor that aids enzymatic reactions, including DNA replication (Conda 2011), which could be why the magnesium chloride caused greater outgrowth than the other types of salt, even though it has a greater effect on moisture binding and removal. Magnesium has been shown to be necessary for cellular respiration and glycolysis (Peiss and others 1949). Therefore, our study indicated that $MgCl_2$ facilitated the growth of microorganisms in the meat samples during the 5 d storage time.

The effect of time on *L. monocytogenes* population in all three phases of the research was expected. One of the more important things to note from the data is that even at the highest concentration of 2.5% for the salts, none of the salt treatments actually caused significant inhibition of *L. monocytogenes* during 48 h of storage time at room temperature compared with the control (no salt). This result suggests that salt at these low levels does not inhibit bacterial growth by itself and highlights the importance of hurdles used in food formulations and processing to prevent growth of microorganisms.

Concentration of the various salts also had very little effect during this study. $MgCl_2$ was the only salt that caused a significant increase in *L. monocytogenes* populations as the concentration increased. Because magnesium can be a growth cofactor for microorganisms, it is not surprising that as concentration increased, so did *L. monocytogenes* populations. The fact that the sodium and potassium treatments slowed growth over 48 h of storage time even at 0.5% concentration is surprising considering *L. monocytogenes* is extremely salt-tolerant (Taormina 2010).

Conclusion

This research shows that using industrial salts that contain even low levels of magnesium could cause potential problems in the area of food safety. Even compared with systems with no salt added, magnesium caused significantly greater outgrowth of *Listeria monocytogenes*.

Chapter 4 - Effect of various salts on *Listeria monocytogenes* populations in pre-blended raw ground beef, pork and turkey

Introduction

The reduction of salt is not a new issue and several reviews of the use of salt in the area of food safety have been published in the past (Reddy and Marth 1991; Sofos 1983; Taormina 2010). Salt levels have been lowered throughout the 20th century for many reasons including the discovery of negative health effects, use of refrigeration, and use of other antimicrobial ingredients (Sofos 1983). Recently the World Action of Salt and Health (WASH) stated that salt intake should be lowered to the WHO standards of 5 grams per day (WASH 2009).

The effect of sodium intake on hypertension has been thoroughly studied in the last several decades and it is common knowledge that reducing sodium in one's diet can lower the chances of hypertension (Dickinson and Havas 2007; Karppanen and Mervaala 2006; He and MacGregor 2008). Both the New York City Department of Health and the World Health Organization (WHO) has accepted these research findings and have called for lowering sodium levels in foods worldwide (New York City Department of Health, 2010; WHO 2006). The Center for Science and the Public Interest petitioned the Food and Drug Administration (FDA) to reclassify NaCl as a food additive (FDA 2007).

The food industry has been researching ways to reduce the sodium content in various processed food products. Salt can be added to raw meat materials during pre-blending for sausage formulation. Some of the benefits of pre-blending include allowing more time for fat and water binding, more uniform product, and better production efficiency (Romans and others 2001). Salt is used in meat products as a preservative and to help bind water and fat as well as extract myofibrillar proteins (Miller and Hosney 1996; Gordon and Klimek 2000; Iqoe and Hui 2001 Romans and others 2001).

Previous research (Chapter 3) indicated that using industrial salts that contain even trace levels of magnesium could cause potential problems in the area of food safety. Even compared with systems with no salt added, magnesium caused significantly greater outgrowth of *Listeria monocytogenes*. The USDA National Nutrient Database for Standard Reference states that raw ground turkey and beef (15% fat), contain approximately 19 mg and 18 mg of magnesium per

100 grams of food, respectively. Comparatively, raw ground pork (16% fat) contains approximately 16 mg of magnesium per 100 grams of food (USDA 2011).

Based on our previous research, this could make turkey or beef products more susceptible to *L. monocytogenes* growth because of the greater levels of magnesium. Therefore, the objective of this study was to determine the effect that different salts have on the growth of *L. monocytogenes* in fresh ground beef, pork, and turkey during pre-blend storage.

Materials and Methods

Experimental Design

This study consisted of six different salt treatments (NaCl, KCl, CaCl₂, MgCl₂, replacement salt, and sea salt) and three fresh ground meat and poultry products (ground beef, ground pork, and ground turkey). The NaCl, KCl, CaCl₂, and MgCl₂ were all supplied by Fisher Chemical (Fairlawn, N.J., U.S.A.). The study also consisted of two controls: a positive control that contained no salt but was inoculated with a five-strain cocktail of *Listeria monocytogenes* and a negative control that contained no salt and no *L. monocytogenes*. Each replication consisted of all six salts added at 2% (ingoing) along with the two controls to each of the three ground meat products (ground beef, ground pork, and ground turkey). Three replications were used for this study with meat being purchased on separate days for each replication.

Listeria monocytogenes cultures

A five-strain cocktail of *L. monocytogenes* was used for this study. The cultures were ATCC 13932, ATCC 19115, ATCC 19118, ATCC 19912, and ATCC SLR-2249 and were all obtained from American Type Culture Collection (Rockville, Md., U.S.A.). ATCC 13932 (serotype 4b) was isolated from the spinal fluid of a child with meningitis in Germany while ATCC 19112 (serotype 1/2c) was isolated from the spinal fluid of a man in Scotland. ATCC 19115 (serotype 4b) was a human isolate, while ATCC 19118 (serotype 4e) was isolated from a chicken in England. ATCC SLR-2249 is a laboratory strain with the *ActA* gene removed.

Sample preparation

One lot (8.17 kg) of each meat was purchased from local grocery stores (ground beef and pork from one store and ground turkey from another) in Manhattan, Kan on the same day for

each replication. Duplicate samples were used for each salt and meat combination for each replication. Samples were purchased frozen and allowed to thaw in cold water for 2 h prior to adding salt. Before inoculation with *Listeria monocytogenes*, each salt was added to meat at a 2% level (9.08 g of salt into 454 g of meat). Both negative and positive controls had no salt added to the meat. After salt was added to the meat, samples were hand mixed for 1 min.

Inoculum preparation, sample inoculation, and sample storage

Inoculum was grown in 50 mL of tryptic soy broth (Difco; Franklin Lakes, N.J., U.S.A.) at 35 °C for 24 h prior to inoculation into the meat samples. The five strains of *L. monocytogenes* were aseptically combined (25 mL of each strain) into a sterile beaker and then diluted (10 mL of inoculum into 1 L of peptone). Ten mL of diluted inoculum was added to 454 g of ground meat in a sterile stomacher bag (Stomacher 400 bag, Seward, Bohemia, N.Y., U.S.A.). The negative control was the only sample that was not inoculated with *L. monocytogenes*. After samples were inoculated, bags were twisted and then taped closed and then sampled for enumeration for day 0 before being taped closed again and placed in a refrigerator at 4 °C.

Sampling and enumeration procedures

All treatments were sampled at 0, 3, and 5 d after inoculation for *L. monocytogenes* populations. On each sampling day, a 75 g sample was aseptically removed from the 454 g of meat (in the stomacher bag) by using a sterilized spoon and the sample was then placed in a sterile Filtra-Bag (Fisherbrand, Houston, Tex., U.S.A.). One hundred and fifty mL of 0.1% peptone (Difco; Franklin Lakes, N.J., U.S.A.) was aseptically added to the Filtra-Bag with the meat sample and the bag and its contents were stomached on medium speed for 1 min (Seward Stomacher 400; Bohemia, N.Y., U.S.A.). Samples were then serially diluted in a 0.1% peptone solution for *L. monocytogenes* enumeration on modified oxford medium (MOX) (Becton Dickinson, Franklin Lakes, N.J., U.S.A.) and aerobic count Petrifilm (3M, Minneapolis, Minn., U.S.A.) for aerobic populations. All serial dilutions were vortexed for 3 to 4 s on high speed before they were transferred to the next dilution. Dilutions were spread plated on MOX (0.1 mL per plate) agar plates for each dilution, allowing for a detection limit of 1.3 log CFU/g for 0 and 3 day and <2.3 log CFU/g for 5 d. Plates were incubated at 35 °C for 48 h prior to counting. Dilutions were plated on Petrifilm (1 mL per Petrifilm) and incubated at 35 °C for 24 h prior to

counting. After sampling, all original meat samples in stomacher bags were taped closed and placed back in a refrigerator at 4 °C.

Proximate analysis

Atomic absorption (AOAC Official Method Number 968.08) was performed on replacement salt and sea salt to determine the concentrations of sodium, potassium, calcium, and magnesium in each salt. Three samples were analyzed for each of the salts with duplicate readings for each sample (n=3 for each salt). Moisture and crude fat content of fresh ground beef, pork, and turkey were determined using AOAC Official Method PVM-1:2003. Protein content for the same products was determined using AOAC Official Method 990.03. Results can be found in Table 8. For each replication of research, two samples were analyzed for each lot of meat (n=3 for each meat).

Table 8. Proximate analysis results of ground beef, pork, and turkey (n=3).

	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Calcium (ppm)	Magnesium (ppm)	Sodium (ppm)	Potassium (ppm)
Ground Beef	67.02±3.27	18.55±1.04	13.19±4.02	88.53±26.44	176.88±23.40	1513.63±274.81	1785.46±78.24
Ground Pork	58.77±2.35	15.33±1.18	21.92±2.07	27.87±18.21	151.70±13.27	2738.10±590.08	1837.97±94.29
Ground Turkey	65.40±2.89	13.39±0.05	19.55±2.84	604.11±26.24	132.64±14.30	1714.35±2179	906.42±357.18

Statistical analysis

This study was a 3 (species) by 8 (salt types) factorial design with time as a repeated measure. *Listeria monocytogenes* and aerobic population data were analyzed using PROC MIXED in SAS version 9.0 (SAS Institute, Cary, N.C., U.S.A.). Fixed effects for statistical analysis were salt type, species, sampling time, salt type by species, salt type by time, species by time, and salt type by species by time, and the random effect was replication. Least squares means ($P < 0.05$) were determined using the Tukey test to compare the interactions.

Results

None of the non-inoculated controls (no salt and no *Listeria monocytogenes* added) had any *L. monocytogenes* populations below the detection limit (<1.3 log CFU/g for 0 and 3 day and <2.3 log CFU/g for 5 d). For non-inoculated controls (no salt and no *Listeria monocytogenes* added) for all species, *Listeria monocytogenes* populations for day 0 were below the detection limit of 1.3 log CFU/g and below the detection limit of 2.3 log CFU/g for day 5.

For *L. monocytogenes* populations, no significant differences ($P > 0.05$) were observed for salt type, species, species by salt type, salt by time or species by salt type by time. A two-way interaction ($P < 0.05$) was observed for species by time for *Listeria monocytogenes* populations. *Listeria monocytogenes* populations in beef significantly decreased ($P < 0.05$) from 0 d to 5 d while no significant decrease ($P > 0.05$) was observed for pork and turkey (Table 9). Initial populations for all species were approximately 4.87 log CFU/g on 0 d and decreased throughout sample storage. *Listeria monocytogenes* populations in beef decreased by approximately 0.41 log CFU/g whereas, *L. monocytogenes* populations in beef and turkey decreased by <0.15 log CFU/g.

Table 9. Mean \pm standard error *Listeria monocytogenes* populations (log CFU/g) through the 5 day sampling period for each species with data pooled for salt type (n=36).**

Sampling time*	Beef	Pork	Turkey
0 day	4.87 \pm 0.21 ^a	4.88 \pm 0.21 ^a	4.85 \pm 0.20 ^a
3 day	4.48 \pm 0.21 ^b	4.84 \pm 0.21 ^a	4.74 \pm 0.20 ^a
5 day	4.46 \pm 0.21 ^b	4.74 \pm 0.21 ^a	4.79 \pm 0.20 ^a

*Denotes time sampled after inoculation.

**NaCl, KCl, CaCl₂, MgCl₂, replacement salt, sea salt, control.

^{ab}Indicates significant differences in a column within species.

For aerobic populations, no significance ($P > 0.05$) was observed for species by salt type, species by time, or species by salt type by time interactions. A two-way interaction ($P < 0.05$) was observed for salt type by time for aerobic populations. Salts (NaCl and MgCl₂) showed growth ($P < 0.05$) of aerobic populations during storage (Table 10). However, the differences were 0.32 and 0.47 log CFU/g for NaCl and MgCl₂, respectively.

Table 10. Mean \pm standard error of aerobic populations (log CFU/g) for NaCl, KCl, CaCl₂, MgCl₂, sea salt, salt replacer, control and non-inoculated treatments for sampling days 0, 3, and 5 d pooled on species (n=18).**

Sampling time*	NaCl	KCl	CaCl₂	MgCl₂	Sea Salt	Salt Replacer	Control	Non-Inoculated Control
0 day	5.21 \pm 0.25 ^{ab}	5.12 \pm 0.26 ^a	5.16 \pm 0.25 ^a	5.07 \pm 0.25 ^a	5.04 \pm 0.25 ^a	5.18 \pm 0.25 ^a	4.84 \pm 0.25 ^a	3.86 \pm 0.25 ^a
3 day	5.07 \pm 0.25 ^a	5.29 \pm 0.25 ^a	4.94 \pm 0.25 ^a	5.03 \pm 0.25 ^a	5.10 \pm 0.25 ^a	5.15 \pm 0.25 ^a	5.05 \pm 0.25 ^b	4.43 \pm 0.25 ^b
5 day	5.53 \pm 0.25 ^b	5.56 \pm 0.25 ^a	5.19 \pm 0.25 ^a	5.54 \pm 0.25 ^b	5.29 \pm 0.25 ^a	5.51 \pm 0.25 ^a	5.77 \pm 0.25 ^c	5.22 \pm 0.25 ^c

*Denotes time sampled after inoculation.

**Ground pork, beef, and turkey.

^{ab}Indicates significant differences in a column with salt treatments across sample storage time.

Analysis of the industrial salts was performed to determine levels of sodium, potassium, calcium, and magnesium (Table 11). The salt replacement was composed of 26.71% sodium with the other cations (K^+ , Ca^{2+} , and Mg^{2+}) ranging from 2.20 to 2.91%. Sea salt had higher levels of sodium (32.40%) and calcium (4.40%) than the replacement salt, but potassium and magnesium levels remained low at 2.40 and 2.19%, respectively.

Table 11. Analysis (mean \pm standard error) of the industrial salts used in raw ground beef, pork, and turkey (n=3).

	Sodium	Potassium	Calcium	Magnesium
Sea salt	32.40 \pm 1.05%	2.40 \pm 0.13%	4.40 \pm 0.03%	2.19 \pm 0.01%
Salt replacement	26.71 \pm 0.87%	2.91 \pm 0.15%	2.39 \pm 0.24%	2.20 \pm 0.03%

Discussion

It was expected that the non-inoculated controls would have no *L. monocytogenes* populations below the detection limit (<1.3 CFU/g for 0 and 3 d and <2.3 CFU/g for 5 d) considering they were not inoculated with *L. monocytogenes*. For raw ground beef, United States Department of Agriculture’s Food Safety and Inspection Service (USDA/FSIS) conducted surveillance testing of domestic raw ground beef and found that *L. monocytogenes* was only present in 18% of the samples tested. Therefore, the level of *L. monocytogenes* is relatively low in raw ground beef samples (Farber and others 2007).

A species by time interaction was observed for beef samples since *L. monocytogenes* populations decreased ($P < 0.05$) after 3 and 5 d storage. The pork and turkey sample *L. monocytogenes* populations decreased as well, but not by a significant level ($P > 0.05$). The USDA National Nutrient Database for Standard Reference states that raw ground turkey and beef (15% fat), contain approximately 19 and 18 mg of magnesium per 100 grams of food, respectively. Comparatively, raw ground pork (16% fat) contains approximately 16 mg of magnesium per 100 grams of food (USDA 2011). Although the magnesium level is greater in turkey and beef, results of this study showed that native magnesium in the meat probably had little effect on the growth/survival of *L. monocytogenes* in meat during the 5 d storage time.

It was hypothesized that as sodium was decreased in a formulation and replaced with other salts, *L. monocytogenes* or aerobic populations may have the ability to grow or survive in a food system. In our study, no differences ($P < 0.05$) were found for *L. monocytogenes* populations for any of the salt treatments during the 5 d storage time. However, all aerobic populations grew for all salt treatments, though only the NaCl and MgCl₂ treatments had significant growth during the 5 d storage. One reason that the aerobic populations grew while the *L. monocytogenes* populations decreased could be due to *L. monocytogenes* being outcompeted by the aerobic microflora for nutrients in the food.

Previous research (Chapter 3) has shown that MgCl₂ salt can induce bacterial growth. A Spanish microbial media producer, Conda, states that magnesium is a growth cofactor that aids in enzymatic reactions for bacteria, including DNA replication (Conda 2011). Furthermore, magnesium has been shown to be necessary for cellular respiration and glycolysis (Peiss and others 1949). Therefore, our study indicated that MgCl₂ facilitated the growth of aerobic microorganisms in the meat samples during the 5 d storage time.

Conclusion

There was little effect on *L. monocytogenes* growth/survival by replacing sodium chloride in raw ground beef, pork, and turkey during a 5 d pre-blend storage time at 4 °C. Our data showed that when comparing species during a 5 d storage time at 4 °C, that ground pork and turkey could enable better survival of *L. monocytogenes* when compared to ground beef. More research is needed to determine if these results would differ during an extended storage period or for a fully-cooked product.

Chapter 5 - Effect of various salts on *Listeria monocytogenes* populations on post-contaminated emulsified pork and beef products

Introduction

The reduction of salt is not a new issue and several reviews of the use of salt in the area of food safety have been published in the past (Reddy and Marth 1991; Sofos 1983; Taormina 2010). Salt levels have been lowered throughout the 20th century for many reasons including the discovery of health effects, use of refrigeration, and use of other antimicrobial ingredients (Sofos 1983). However, recently the World Action of Salt and Health (WASH) have recently stated that salt intake should be lowered to the WHO standards of 5 grams per day (WASH 2009). The reduction of salt could cause important changes to the safety of food products that should be thoroughly studied (Taormina 2010)

Listeria monocytogenes has caused outbreaks and recalls linked to RTE meat and poultry products (CDC 1998; CDC 2011b; Food Safety News 2011a; Levine and others 2001; Olsen and others 2005). Due to the nature of these *L. monocytogenes* outbreaks involving post-process contamination in RTE meat and poultry products, FSIS established three alternatives that food processors can use to minimize risk of post-process contamination (USDA/FSIS 2006). Many RTE meat and poultry products use reduction of water activity as a post-lethality treatment (USDA 2006). This can be achieved by adding salt. Other non-lethality treatments may include reducing the product's pH via fermentation or addition of an acidulant (Samelis and others 2001; Samelis and others 2005). Modified atmospheric packaging (MAP) and holding times, combined with salt levels of approximately 4.6% and low water activity (approximately 0.80), has also been studied and found to be effective post-lethality treatments for RTE meat and poultry products (Lobaton-Sulabo and others 2011; Uppal and others 2011). Sodium lactate and sodium diacetate have also been effective as antimicrobial agents against *L. monocytogenes* in bologna and frankfurters (Grosulescu and others 2010; Igeo and Hui 2001).

Post-process contamination of *Listeria monocytogenes* is an important issue in the food industry (CDC 1998; CDC 2011b; CSU 2010; Food Safety News 2011a; Olsen and others 2005). To combat this problem, FSIS required that ready-to-eat meat and poultry processors employ one

of three alternatives to control *L. monocytogenes* due to a post-process contamination (USDA/FSIS 2006). Alternative 1 states that the establishment must use a post-lethality treatment to reduce or eliminate *L. monocytogenes*, and an antimicrobial agent or process to suppress or limit the growth of the pathogen. Under Alternative 2, an establishment must use either a post-lethality treatment or an antimicrobial agent or process. The establishment must provide validation of the post-lethality treatment for eliminating or reducing *L. monocytogenes* for either Alternative 1 or 2. The definition of a post-lethality treatment must reduce *L. monocytogenes* by 1 log CFU/g of food. Alternative 3 is when an establishment relies on its sanitation program to control *L. monocytogenes* in RTE meat and poultry products (USDA/FSIS 2006).

Our previous research (Chapter 3) indicated that using industrial salts that contain even trace levels of magnesium could facilitate the growth of pathogenic and spoilage bacteria in the meat samples. Even compared with systems with no salt added, meat samples with salts containing magnesium caused significantly greater outgrowth of *Listeria monocytogenes*. In a second study (Chapter 4), no difference was observed for the reduction of *L. monocytogenes* for various salts used in pre-blend ground pork, beef, and turkey. However, *Listeria monocytogenes* populations significantly decreased (0.41 log CFU/g) during the storage time in ground beef. No differences ($P > 0.05$) were observed over time in raw ground pork or turkey. Therefore, the objective of our study was to determine the effect of *L. monocytogenes* growth/survival in post-process contaminated emulsified beef and pork products formulated with different types of salts.

Materials and Methods

Experimental Design

This study consisted of four different salt treatments (NaCl, KCl, sea salt, and a blend of 50% NaCl and 50% KCl) and two emulsified meat products made from ground beef and ground pork. The salts were all supplied by Morton Salt (Chicago, Ill., U.S.A.). Each replication consisted of all four salts added separately at 2% (36.3 g of salt into 1.82 kg of meat) to each of the two emulsified meat products (beef and pork). Duplicate samples were used for each salt and meat combination for each replication. Three replications were used for this study with meat being purchased on separate days for each replication.

Listeria monocytogenes cultures

A five-strain cocktail of *L. monocytogenes* was used for this study. The cultures were ATCC 13932, ATCC 19115, ATCC 19118, ATCC 19912, and ATCC SLR-2249 and were all obtained from American Type Culture Collection (Rockville, Md., U.S.A.). ATCC 13932 (serotype 4b) was isolated from the spinal fluid of a child with meningitis in Germany while ATCC 19112 (serotype 1/2c) was isolated from the spinal fluid of a man in Scotland. ATCC 19115 (serotype 4b) was a human isolate, while ATCC 19118 (serotype 4e) was isolated from a chicken in England. ATCC SLR-2249 is a laboratory strain with the *ActA* gene removed.

Sample preparation

One lot (8.2 kg) of each meat (beef and pork) was obtained by the Kansas State University Meat Laboratory. The meat was received frozen and as whole muscle pieces. Samples were trimmed to an appropriate leanness level (80% lean) and then ground in a Hobart Model 4732 grinder (Troy, Oh., U.S.A.) through a 0.953 cm (3/8th inch) plate. Following the first grinding, meat was weighed and separated into 1.82 kg batches. Following this step of the process, equipment was cleaned between each 1.82 kg batch. The salts were then added to the batch and mixed in a Mainca Rm-20 mixer (St. Louis, Mo., U.S.A.) for 2.5 min. Meat was then transferred to the grinder and passed through a 0.476 cm (3/16th inch) plate. Each batch was then run through a Model GL 86 emulsifier (Griffith Design and Equipment Company; Chicago, Ill., U.S.A.), hand stuffed (5 lb capacity; F-Dick, Germany) into pre-hydrated 22 mm cellulose casing (VisKase; Loudon, Tenn., U.S.A.) and then made into 13 cm long links. Meat products were then stored on a smokehouse rack for 24 h (pork) and 48 h (beef) prior to thermal processing in a smokehouse (Maurer; Northbrook, Ill., U.S.A.). The smokehouse cycle consisted of four stages: 1) 30 min at 54.4 °C dry bulb (DB), 2) 30 min at 60 °C DB and 48.9 °C WB (55% relative humidity), 3) 30 min at 71.1 °C DB and 54.4 °C WB (43% relative humidity), 4) 30 min at 82.2 °C DB and 76.7 °C WB (79% relative humidity). Dampers were 50% open and the fan speed was 1,200 rpm for all four stages.

Inoculum preparation, sample inoculation and storage

Inoculum was grown in 50 mL of tryptic soy broth (Difco; Franklin Lakes, N.J., U.S.A.) at 35 °C for 24 h prior to inoculation into the meat samples. The five strains of *L.*

monocytogenes were aseptically combined (20 mL of each strain) into a sterile beaker (Corning; Corning, N.Y., U.S.A.) and then diluted (900 mL of peptone into 100 mL of inoculum cocktail). One hundred mL was removed from the inoculum prior to dipping product to prevent overflow of the inoculum. Each link was dipped in the inoculum for 1 min (2 links at a time) and then aseptically transferred to sterile test tube racks to dry. Links were allowed to dry for 30 min prior to packaging. Links were then individually vacuum packaged at 118 ± 45 mm Hg using a Multivac C100 packaging machine (Multivac Sepp Hagenmüller GmbH and Co.KC; Wolfertschwenden, Germany) in a 5-mil-thick, clear, low-linear-density polyethylene pouch (OPP30/PE15/PET12/EVOH COEX38/LLDP30-125 microns; O₂ transmission rate at 35 °C, 0% relative humidity: 0.33 cc/m², moisture vapor transmission rate at 40 °C, 90% relative humidity: 1.47g/m²) size 19 by 22 cm (Golden-Tech Intl., Inc. Redmond, Wash., U.S.A.). Following packaging, samples were stored at 4 °C in a dark retail refrigeration unit (McCormick Distributing and Service; Urbana, Ill., U.S.A.).

Sampling and enumeration procedures

All treatments were sampled at 0, 7, 14, 21, and 28 d after inoculation for *L. monocytogenes* populations. On each sampling day, a 95 cm² link was aseptically removed from its package and placed in a sterile Filtra-Bag (Fisherbrand, Houston, Tex., U.S.A.). Thirty-five mL of 0.1% peptone (Difco; Franklin Lakes, N.J., U.S.A) was aseptically added to the polyethylene packaging to rinse any bacteria that was in the package and an additional 60 mL was aseptically added directly to the Filtra-Bag and the bag was then stomached on medium speed for 1 min (Seward Stomacher 400; Bohemia, N.Y., U.S.A.). Samples were then serially diluted in a 0.1% peptone solution for *L. monocytogenes* enumeration. All serial dilutions were vortexed for 3 to 4 s on high speed before they were transferred to the next dilution. Dilutions were spread plated on modified oxford medium (MOX) (Becton Dickinson, Franklin Lakes, N.J., U.S.A.) (0.1 mL per plate) agar plates for each dilution, allowing for a detection limit of 2.0 log CFU/cm² on 0 and 7 d and 3.0 log CFU/cm² on 14, 21, and 4.0 log CFU/cm² for 28 d. Plates were incubated at 35 °C for 48 h prior to counting.

Proximate analysis

Moisture and crude fat content of raw ground beef and pork were determined using AOAC Official Method PVM-1:2003. Protein content was determined using AOAC Official

Method 990.03. For pH measurement, 10 g of meat was combined with 90 mL of distilled water and stirred prior to measurement. For each replication of research, two samples were analyzed for each sample of meat (n=6 for each meat) (Table 12).

Table 12. Mean ± standard error for moisture (%), crude protein (%), crude fat (%), and pH of ground beef and pork prior (n=6).

Species	Moisture (%)	Crude Protein (%)	Crude Fat (%)	pH
Beef	68.73±0.89	18.96±0.34	11.09±1.10	5.86±0.05
Pork	69.22±2.13	18.47±0.58	11.10±2.77	6.18±0.05

Mineral analysis for industrial salts

Atomic absorption (AOAC Official Method Number 968.08) was performed on the replacement salt and sea salt from phase 3 to determine the concentrations of sodium, potassium, calcium, and magnesium in each salt. Results for mineral analysis can be found in Table 13.

Table 13. Mean ± standard error of sodium (%), potassium (%), calcium (%), and magnesium (%) analysis of sea salt, KCl, and NaCl (n=3).

	Sodium (%)	Potassium (%)	Calcium (%)	Mg (ppm)
Sea Salt	36.66±0.61	0.24±0.00	0.88±0.03	0.00±0.00
KCl	0.00±0.00	38.17±1.06	0.44±0.01	0.00±0.00
NaCl	38.34±0.16	0.04±0.00	0.00±0.00	0.00±0.00

Statistical analysis

This study was a 2 (species) by 4 (salt types) by 5 (sampling times) factorial design with time as a repeated measure. *Listeria monocytogenes* population data were analyzed using PROC MIXED in SAS version 9.0 (SAS Institute, Cary, N.C., U.S.A.). Fixed effects for statistical analysis were salt type, species, sampling time, salt type by species, salt type by time, species by time, and salt type by species by time, and the random effect was replication. Least squares means ($P < 0.05$) were determined using the Tukey test to compare the interactions.

RESULTS

No differences ($P > 0.05$) were observed for salt type and salt type by time. A three-way interaction ($P < 0.05$) was observed for species by salt by time (Table 14). Within each species, all *Listeria monocytogenes* populations increased ($P < 0.05$) over time for all salt treatments. Within sampling times, only the blended salt (50% NaCl and 50% KCl) caused differences ($P < 0.05$) across species at 7 and 14 d. While *L. monocytogenes* populations finished at similar levels after 28 d of storage, the growth rate was different at 7 and 14 d for the blended salt across species.

Table 14. Mean \pm standard error of *Listeria monocytogenes* populations (log CFU/cm²) for emulsified beef and pork products during the 28 d storage time across salt type (n=6).**

Sampling time*	Beef				Pork			
	NaCl/KCl				NaCl/KCl			
	NaCl	KCl	Sea Salt	Blend	NaCl	KCl	Sea Salt	Blend
0 d	4.12 \pm 0.19 ^{ax}	4.24 \pm 0.48 ^{ax}	4.45 \pm 0.35 ^{ax}	3.94 \pm 0.26 ^{ax}	4.37 \pm 0.33 ^{ax}	3.98 \pm 0.09 ^{ax}	4.18 \pm 0.22 ^{ax}	4.25 \pm 0.20 ^{ax}
7 d	4.96 \pm 0.41 ^{abx}	4.79 \pm 0.49 ^{abx}	4.73 \pm 0.66 ^{abx}	4.18 \pm 0.35 ^{ax}	4.52 \pm 0.56 ^{ax}	5.33 \pm 0.84 ^{bx}	5.58 \pm 0.76 ^{bx}	5.82 \pm 0.68 ^{by}
14 d	5.62 \pm 0.64 ^{bcx}	4.93 \pm 0.36 ^{abx}	4.90 \pm 0.76 ^{abx}	4.89 \pm 0.38 ^{abx}	5.13 \pm 0.23 ^{abx}	5.52 \pm 0.99 ^{bx}	5.92 \pm 0.57 ^{bx}	6.14 \pm 0.99 ^{by}
21 d	6.15 \pm 0.66 ^{bcx}	6.02 \pm 0.27 ^{bcx}	5.85 \pm 0.38 ^{bcx}	6.02 \pm 0.79 ^{bcx}	6.19 \pm 0.60 ^{bcx}	6.26 \pm 0.90 ^{bx}	6.51 \pm 0.73 ^{bx}	6.55 \pm 0.69 ^{bx}
28 d	6.59 \pm 0.40 ^{cx}	6.33 \pm 0.29 ^{bcx}	6.62 \pm 0.15 ^{cx}	6.49 \pm 0.31 ^{bcx}	6.92 \pm 0.37 ^{cx}	6.41 \pm 0.47 ^{bx}	6.58 \pm 0.52 ^{bx}	6.58 \pm 0.46 ^{bx}

*Denotes time sampled after inoculation.

**NaCl, KCl, sea salt, and NaCl/KCl blend.

^{a-c}Indicates differences ($P < 0.05$) in a column within the species and salt type across sampling times.

^{xy}Indicates differences ($P < 0.05$) in a row within the salt type and sampling time across species.

Discussion

The objectives of this study hypothesized that as sodium was decreased in a formulation, replacement salts may facilitate better growth or survival of *L. monocytogenes* in emulsified beef or pork system. In this study, there was no difference ($P < 0.05$) among salt treatments for *L. monocytogenes* populations during a 28 d storage time at 4 °C. The results show that if processors would like to change salt to a low sodium salt, it would not have an impact on *L. monocytogenes* populations during a 28 d storage time at 4 °C.

Salt types were not shown to have an impact on *L. monocytogenes* growth/survival in pre-blend raw beef, pork, and turkey (Chapter 4). However, *L. monocytogenes* populations in ground beef decreased by 0.41 log CFU/g during 5 d storage at 4 °C whereas, ground pork and ground turkey remained the same. In this study, emulsified pork products tended to have greater *L. monocytogenes* populations than emulsified beef products.

FSIS performed a surveillance study of *L. monocytogenes* and found that when comparing RTE meat and poultry deli products, beef products were the highest in prevalence of *L. monocytogenes* at 1.3% while pork and poultry products contained *L. monocytogenes* in 0.8% and 0.7% of products tested, respectively (USDA/FSIS 2010). In our study, all *L. monocytogenes* populations for pork samples were greater ($P < 0.05$) than all *L. monocytogenes* populations for beef samples. When comparing results from this study and FSIS prevalence data, the difference may be caused by the lack of listeristatic compounds (sodium nitrite, sodium lactate, and sodium diacetate) used in RTE beef products compared to RTE pork and poultry products to control growth/survival of *L. monocytogenes*. Roenbaugh (2011) found that addition of sodium nitrite had a listeristatic effect on *L. monocytogenes* populations in RTE sliced turkey meat. Farber and others (2007) also discuss that sodium lactate is an effective antilisterial compound that can be used in RTE meat products. Schlyter and others (1993) found that sodium lactate works best to inhibit *L. monocytogenes* when combined for a synergistic effect with sodium diacetate. This suggests that the compound system of salt mixed with sodium nitrite, sodium lactate, or sodium diacetate may be what causes the antilisterial effect on *L. monocytogenes*.

Time caused a significant ($P < 0.05$) difference in both the *L. monocytogenes* populations. It is expected that populations increase over time because *L. monocytogenes* has the ability to

grow at refrigeration temperatures (Jay and others 2005). However, it is important to realize that hurdles, including packaging and sodium nitrite, are added to food to inhibit or even destroy bacteria in food (AMI 1999; Jay and others 2005; Lobaton-Sulabo and others 2011; Uppal and others 2011).

Some of the interactions had differences of >1.0 log CFU/cm² of *L. monocytogenes* populations. However, many of these were not statistically different ($P > 0.05$). In real-world scenarios, a >1.0 log CFU/cm² difference would be significant to the food industry. In fact, the USDA sets this level as the cut off for approval of a post-lethality treatment for eliminating or reducing *L. monocytogenes* for either Alternative 1 or 2 (USDA/FSIS 2006).

Conclusions

Overall, this study shows that there is little effect of replacing sodium chloride on the safety of emulsified beef and pork products during a 28 d storage time at 4 °C. The results of this study indicate the importance of hurdle effects and adding other antimicrobials to inhibit the growth of *L. monocytogenes*. More research is needed to determine if these results would differ for other types of food products or for longer storage times.

Chapter 6 - Conclusions and Implications

- Overall, replacing sodium salts with potassium, calcium, magnesium, or blended salts will not show differences ($P > 0.05$) between salts relative to the ability of *Listeria monocytogenes* to grow in pre-blended raw ground beef, pork, and turkey or emulsified beef and turkey.
- However, our research on the ability of salts (NaCl, KCl, CaCl₂, MgCl₂, sea salt, and replacement salt) at different concentrations (0.5, 1, and 2.5%) to inhibit *Listeria monocytogenes* in broth systems showed that magnesium may cause *Listeria monocytogenes* to grow to greater levels than other salts. Consequently, salts high in magnesium should be avoided.
- Pork consistently supported greater *Listeria monocytogenes* populations than beef. In pre-blended raw ground meat, *Listeria monocytogenes* populations decreased ($P < 0.05$) in raw ground beef during the 5 d storage at 4 °C while they did not decrease ($P > 0.05$) for raw ground pork in the same conditions. In emulsified meat, *Listeria monocytogenes* populations were again greater ($P < 0.05$) in pork than in beef for all salt treatments.
- The overall conclusion from our studies is that sodium chloride or replacement salts added at 2% do not effectively control *Listeria monocytogenes* in raw ground beef, pork or turkey or emulsified beef and pork products. Thus, other antilisterial compounds added to meat systems appear critical in eliminating or inhibiting growth of *L. monocytogenes*.

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Appendix 1 - SAS code for aerobic populations for raw ground beef, pork, and turkey study

```
options nocenter ls = 120;
```

```
data;
```

```
Input sample salt $ species $ time population
```

```
datalines;
```

1	NC	Beef	0	4.117
1	NC	Beef	3	4.204
1	NC	Beef	5	4.544
2	NC	Beef	0	3.519
2	NC	Beef	3	4.228
2	NC	Beef	5	4.663
3	NC	Beef	0	5.267
3	NC	Beef	3	5.613
3	NC	Beef	5	5.613
4	NC	Beef	0	5.143
4	NC	Beef	3	5.602
4	NC	Beef	5	5.568
5	NC	Beef	0	3.477
5	NC	Beef	3	4.771
5	NC	Beef	5	6.064
6	NC	Beef	0	4.124
6	NC	Beef	3	4.973
6	NC	Beef	5	5.643
1	Cont	Beef	0	4.903
1	Cont	Beef	3	4.279
1	Cont	Beef	5	4.806
2	Cont	Beef	0	5.342
2	Cont	Beef	3	4.544
2	Cont	Beef	5	5.076
3	Cont	Beef	0	4.732
3	Cont	Beef	3	5.519
3	Cont	Beef	5	5.303
4	Cont	Beef	0	4.412
4	Cont	Beef	3	6.452
4	Cont	Beef	5	5.299
5	Cont	Beef	0	4.833

5	Cont	Beef	3	5.090
5	Cont	Beef	5	5.690
6	Cont	Beef	0	4.845
6	Cont	Beef	3	4.869
6	Cont	Beef	5	5.881
1	Na	Beef	0	5.350
1	Na	Beef	3	4.881
1	Na	Beef	5	4.740
2	Na	Beef	0	5.230
2	Na	Beef	3	4.886
2	Na	Beef	5	4.708
3	Na	Beef	0	5.681
3	Na	Beef	3	5.987
3	Na	Beef	5	5.544
4	Na	Beef	0	5.079
4	Na	Beef	3	4.477
4	Na	Beef	5	5.875
5	Na	Beef	0	5.279
5	Na	Beef	3	4.978
5	Na	Beef	5	5.672
6	Na	Beef	0	4.908
6	Na	Beef	3	4.898
6	Na	Beef	5	5.477
1	K	Beef	0	4.964
1	K	Beef	3	4.898
1	K	Beef	5	4.940
2	K	Beef	0	5.301
2	K	Beef	3	4.919
2	K	Beef	5	4.462
3	K	Beef	0	5.919
3	K	Beef	3	5.690
3	K	Beef	5	5.898
4	K	Beef	0	4.785
4	K	Beef	3	5.633
4	K	Beef	5	5.799
5	K	Beef	0	4.417
5	K	Beef	3	5.613
5	K	Beef	5	5.699
6	K	Beef	0	5.196
6	K	Beef	3	5.505

6	K	Beef	5	5.493
1	Ca	Beef	0	5.041
1	Ca	Beef	3	4.748
1	Ca	Beef	5	4.301
2	Ca	Beef	0	5.477
2	Ca	Beef	3	4.021
2	Ca	Beef	5	4.447
3	Ca	Beef	0	5.410
3	Ca	Beef	3	4.386
3	Ca	Beef	5	5.568
4	Ca	Beef	0	5.378
4	Ca	Beef	3	5.716
4	Ca	Beef	5	5.771
5	Ca	Beef	0	4.898
5	Ca	Beef	3	5.093
5	Ca	Beef	5	4.491
6	Ca	Beef	0	5.041
6	Ca	Beef	3	4.833
6	Ca	Beef	5	5.580
1	Mg	Beef	0	5.556
1	Mg	Beef	3	5.072
1	Mg	Beef	5	4.415
2	Mg	Beef	0	4.623
2	Mg	Beef	3	4.653
2	Mg	Beef	5	4.886
3	Mg	Beef	0	5.792
3	Mg	Beef	3	5.633
3	Mg	Beef	5	5.602
4	Mg	Beef	0	5.270
4	Mg	Beef	3	4.663
4	Mg	Beef	5	5.845
5	Mg	Beef	0	4.792
5	Mg	Beef	3	5.049
5	Mg	Beef	5	5.342
6	Mg	Beef	0	5.318
6	Mg	Beef	3	5.114
6	Mg	Beef	5	5.602
1	SS	Beef	0	4.420
1	SS	Beef	3	4.375
1	SS	Beef	5	4.505

2	SS	Beef	0	5.568
2	SS	Beef	3	4.875
2	SS	Beef	5	4.785
3	SS	Beef	0	5.568
3	SS	Beef	3	5.398
3	SS	Beef	5	5.591
4	SS	Beef	0	5.090
4	SS	Beef	3	5.265
4	SS	Beef	5	5.633
5	SS	Beef	0	4.699
5	SS	Beef	3	5.057
5	SS	Beef	5	5.260
6	SS	Beef	0	5.382
6	SS	Beef	3	5.090
6	SS	Beef	5	5.201
1	SR	Beef	0	5.179
1	SR	Beef	3	5.380
1	SR	Beef	5	4.653
2	SR	Beef	0	5.170
2	SR	Beef	3	4.462
2	SR	Beef	5	4.491
3	SR	Beef	0	5.799
3	SR	Beef	3	5.405
3	SR	Beef	5	5.491
4	SR	Beef	0	5.362
4	SR	Beef	3	5.568
4	SR	Beef	5	5.623
5	SR	Beef	0	5.167
5	SR	Beef	3	4.968
5	SR	Beef	5	5.462
6	SR	Beef	0	5.167
6	SR	Beef	3	5.097
6	SR	Beef	5	5.378
1	NC	Pork	0	2.903
1	NC	Pork	3	3.114
1	NC	Pork	5	3.301
2	NC	Pork	0	3.255
2	NC	Pork	3	3.146
2	NC	Pork	5	3.301
3	NC	Pork	0	4.991

3	NC	Pork	3	5.431
3	NC	Pork	5	6.708
4	NC	Pork	0	4.491
4	NC	Pork	3	5.491
4	NC	Pork	5	6.792
5	NC	Pork	0	4.863
5	NC	Pork	3	4.568
5	NC	Pork	5	6.013
6	NC	Pork	0	4.591
6	NC	Pork	3	4.505
6	NC	Pork	5	6.064
1	Cont	Pork	0	5.146
1	Cont	Pork	3	4.477
1	Cont	Pork	5	4.778
2	Cont	Pork	0	5.580
2	Cont	Pork	3	5.207
2	Cont	Pork	5	7.477
3	Cont	Pork	0	5.580
3	Cont	Pork	3	5.207
3	Cont	Pork	5	7.477
4	Cont	Pork	0	3.959
4	Cont	Pork	3	6.330
4	Cont	Pork	5	7.272
5	Cont	Pork	0	5.199
5	Cont	Pork	3	5.386
5	Cont	Pork	5	6.033
6	Cont	Pork	0	5.143
6	Cont	Pork	3	5.107
6	Cont	Pork	5	5.732
1	Na	Pork	0	5.225
1	Na	Pork	3	4.756
1	Na	Pork	5	4.792
2	Na	Pork	0	5.025
2	Na	Pork	3	5.196
2	Na	Pork	5	5.152
3	Na	Pork	0	5.387
3	Na	Pork	3	5.732
3	Na	Pork	5	6.350
4	Na	Pork	0	5.037
4	Na	Pork	3	5.672

4	Na	Pork	5	7.124
5	Na	Pork	0	5.201
5	Na	Pork	3	4.903
5	Na	Pork	5	5.290
6	Na	Pork	0	5.384
6	Na	Pork	3	5.362
6	Na	Pork	5	6.270
1	K	Pork	0	4.623
1	K	Pork	3	4.477
1	K	Pork	5	5.017
2	K	Pork	0	5.164
2	K	Pork	3	5.288
2	K	Pork	5	4.778
3	K	Pork	0	5.286
3	K	Pork	3	6.340
3	K	Pork	5	7.179
4	K	Pork	0	4.914
4	K	Pork	3	5.886
4	K	Pork	5	7.204
5	K	Pork	0	5.215
5	K	Pork	3	5.158
5	K	Pork	5	4.898
6	K	Pork	0	5.215
6	K	Pork	3	5.334
6	K	Pork	5	5.771
1	Ca	Pork	0	6.477
1	Ca	Pork	3	4.602
1	Ca	Pork	5	4.672
2	Ca	Pork	0	4.653
2	Ca	Pork	3	4.740
2	Ca	Pork	5	4.845
3	Ca	Pork	0	4.944
3	Ca	Pork	3	5.959
3	Ca	Pork	5	6.233
4	Ca	Pork	0	4.940
4	Ca	Pork	3	6.318
4	Ca	Pork	5	5.959
5	Ca	Pork	0	5.124
5	Ca	Pork	3	4.826
5	Ca	Pork	5	5.663

6	Ca	Pork	0	5.502
6	Ca	Pork	3	4.826
6	Ca	Pork	5	4.886
1	Mg	Pork	0	4.869
1	Mg	Pork	3	4.556
1	Mg	Pork	5	4.591
2	Mg	Pork	0	5.398
2	Mg	Pork	3	5.114
2	Mg	Pork	5	6.799
3	Mg	Pork	0	5.021
3	Mg	Pork	3	6.281
3	Mg	Pork	5	6.806
4	Mg	Pork	0	5.111
4	Mg	Pork	3	5.851
4	Mg	Pork	5	6.982
5	Mg	Pork	0	5.449
5	Mg	Pork	3	4.633
5	Mg	Pork	5	4.949
6	Mg	Pork	0	5.064
6	Mg	Pork	3	4.716
6	Mg	Pork	5	5.140
1	SS	Pork	0	4.799
1	SS	Pork	3	4.672
1	SS	Pork	5	4.919
2	SS	Pork	0	4.623
2	SS	Pork	3	5.196
2	SS	Pork	5	5.756
3	SS	Pork	0	5.283
3	SS	Pork	3	6.236
3	SS	Pork	5	3.000
4	SS	Pork	0	4.991
4	SS	Pork	3	5.903
4	SS	Pork	5	7.204
5	SS	Pork	0	5.215
5	SS	Pork	3	4.924
5	SS	Pork	5	4.851
6	SS	Pork	0	5.358
6	SS	Pork	3	5.134
6	SS	Pork	5	5.477
1	SR	Pork	0	5.127

1	SR	Pork	3	4.944
1	SR	Pork	5	4.763
2	SR	Pork	0	4.708
2	SR	Pork	3	5.049
2	SR	Pork	5	6.826
3	SR	Pork	0	5.199
3	SR	Pork	3	6.373
3	SR	Pork	5	6.806
4	SR	Pork	0	4.869
4	SR	Pork	3	6.158
4	SR	Pork	5	6.820
5	SR	Pork	0	4.959
5	SR	Pork	3	4.826
5	SR	Pork	5	5.531
6	SR	Pork	0	5.401
6	SR	Pork	3	5.258
6	SR	Pork	5	5.000
1	NC	Turkey	0	1.000
1	NC	Turkey	3	3.491
1	NC	Turkey	5	4.398
2	NC	Turkey	0	1.000
2	NC	Turkey	3	3.602
2	NC	Turkey	5	4.643
3	NC	Turkey	0	3.415
3	NC	Turkey	3	3.881
3	NC	Turkey	5	5.597
4	NC	Turkey	0	3.230
4	NC	Turkey	3	3.886
4	NC	Turkey	5	5.134
5	NC	Turkey	0	4.806
5	NC	Turkey	3	4.255
5	NC	Turkey	5	4.556
6	NC	Turkey	0	5.320
6	NC	Turkey	3	4.908
6	NC	Turkey	5	5.342
1	Cont	Turkey	0	5.342
1	Cont	Turkey	3	4.763
1	Cont	Turkey	5	5.107
2	Cont	Turkey	0	5.491
2	Cont	Turkey	3	4.580

2	Cont	Turkey	5	5.324
3	Cont	Turkey	0	3.041
3	Cont	Turkey	3	4.418
3	Cont	Turkey	5	6.100
4	Cont	Turkey	0	3.041
4	Cont	Turkey	3	4.190
4	Cont	Turkey	5	5.944
5	Cont	Turkey	0	5.045
5	Cont	Turkey	3	5.107
5	Cont	Turkey	5	5.340
6	Cont	Turkey	0	5.447
6	Cont	Turkey	3	5.334
6	Cont	Turkey	5	5.233
1	Na	Turkey	0	6.477
1	Na	Turkey	3	5.241
1	Na	Turkey	5	4.792
2	Na	Turkey	0	4.903
2	Na	Turkey	3	4.778
2	Na	Turkey	5	5.584
3	Na	Turkey	0	4.708
3	Na	Turkey	3	4.716
3	Na	Turkey	5	5.771
4	Na	Turkey	0	4.881
4	Na	Turkey	3	4.748
4	Na	Turkey	5	5.987
5	Na	Turkey	0	4.869
5	Na	Turkey	3	4.987
5	Na	Turkey	5	5.104
6	Na	Turkey	0	5.236
6	Na	Turkey	3	4.982
6	Na	Turkey	5	5.281
1	K	Turkey	0	6.394
1	K	Turkey	3	4.748
1	K	Turkey	5	5.230
2	K	Turkey	0	4.342
2	K	Turkey	3	5.360
2	K	Turkey	5	4.845
3	K	Turkey	0	-
3	K	Turkey	3	5.380
3	K	Turkey	5	6.348

4	K	Turkey	0	4.869
4	K	Turkey	3	4.826
4	K	Turkey	5	6.021
5	K	Turkey	0	5.009
5	K	Turkey	3	5.049
5	K	Turkey	5	5.267
6	K	Turkey	0	5.253
6	K	Turkey	3	5.033
6	K	Turkey	5	5.210
1	Ca	Turkey	0	5.114
1	Ca	Turkey	3	4.785
1	Ca	Turkey	5	5.173
2	Ca	Turkey	0	5.079
2	Ca	Turkey	3	4.362
2	Ca	Turkey	5	4.944
3	Ca	Turkey	0	4.806
3	Ca	Turkey	3	4.964
3	Ca	Turkey	5	5.281
4	Ca	Turkey	0	4.940
4	Ca	Turkey	3	4.869
4	Ca	Turkey	5	5.415
5	Ca	Turkey	0	5.061
5	Ca	Turkey	3	5.013
5	Ca	Turkey	5	5.083
6	Ca	Turkey	0	4.903
6	Ca	Turkey	3	4.886
6	Ca	Turkey	5	5.158
1	Mg	Turkey	0	4.954
1	Mg	Turkey	3	4.663
1	Mg	Turkey	5	4.881
2	Mg	Turkey	0	3.903
2	Mg	Turkey	3	4.591
2	Mg	Turkey	5	5.149
3	Mg	Turkey	0	4.792
3	Mg	Turkey	3	4.833
3	Mg	Turkey	5	5.364
4	Mg	Turkey	0	4.968
4	Mg	Turkey	3	4.996
4	Mg	Turkey	5	6.459
5	Mg	Turkey	0	5.124

5	Mg	Turkey	3	4.908
5	Mg	Turkey	5	5.410
6	Mg	Turkey	0	5.262
6	Mg	Turkey	3	5.185
6	Mg	Turkey	5	5.477
1	SS	Turkey	0	4.813
1	SS	Turkey	3	4.845
1	SS	Turkey	5	4.778
2	SS	Turkey	0	5.294
2	SS	Turkey	3	4.531
2	SS	Turkey	5	5.428
3	SS	Turkey	0	4.380
3	SS	Turkey	3	5.037
3	SS	Turkey	5	6.104
4	SS	Turkey	0	5.064
4	SS	Turkey	3	4.792
4	SS	Turkey	5	6.398
5	SS	Turkey	0	4.799
5	SS	Turkey	3	5.276
5	SS	Turkey	5	5.207
6	SS	Turkey	0	5.332
6	SS	Turkey	3	5.149
6	SS	Turkey	5	5.111
1	SR	Turkey	0	4.934
1	SR	Turkey	3	4.991
1	SR	Turkey	5	4.924
2	SR	Turkey	0	5.471
2	SR	Turkey	3	4.468
2	SR	Turkey	5	4.845
3	SR	Turkey	0	4.869
3	SR	Turkey	3	5.064
3	SR	Turkey	5	5.934
4	SR	Turkey	0	5.164
4	SR	Turkey	3	4.613
4	SR	Turkey	5	5.756
5	SR	Turkey	0	5.294
5	SR	Turkey	3	4.978
5	SR	Turkey	5	5.491
6	SR	Turkey	0	5.312
6	SR	Turkey	3	5.033

```

        6 SR      Turkey      5      5.447
RUN;
options nocenter ls = 120;
data;
input Rep Species $ Moisture Protein Fat Calcium Magnesium Sodium Potassium
datalines;

```

1	Beef	69.29	19.60	10.86	64	182	1806	1781
1	Beef	68.97	18.74	10.34	80	200	1538	1852
2	Beef	69.29	19.60	10.86	64	182	1806	1781
2	Beef	68.97	18.74	10.34	80	200	1538	1852
3	Beef	62.87	17.21	18.47	126	148	1245	1639
3	Beef	62.74	17.38	18.28	117	149	1148	1808
1	Pork	57.12	14.79	21.31	16	147	3330	1874
1	Pork	57.41	14.38	24.47	16	140	2857	1723
2	Pork	57.12	14.79	21.31	16	147	3330	1874
2	Pork	57.41	14.38	24.47	16	140	2857	1723
3	Pork	61.42	16.56	20.25	50	170	2062	1955
3	Pork	62.14	17.08	19.72	53	167	1993	1879
1	Turkey	63.65	13.38	21.48	591	120	283	666
1	Turkey	63.46	13.44	21.28	595	127	334	688
2	Turkey	63.65	13.38	21.48	591	120	283	666
2	Turkey	63.46	13.44	21.28	595	127	334	688
3	Turkey	69.73	13.41	15.63	658	149	4622	1416
3	Turkey	68.44	13.30	16.17	595	152	4430	1315

```

RUN;
/*Arrange population data set*/
data time0; set apc;
drop Population time;
if Time=0 then Time1=Population;
else delete;
data time3; set apc;
drop Population time;
if Time=3 then Time2=Population;
else delete;
data time5; set apc;

```

```

drop Population time;
if Time=5 then Time3=Population;
else delete;
data apc1; merge time0 time3 time5; Sample=_N_;run;
/*Combine Population & Measurement data sets*/
proc sort data=average; by week Species;
data apc2; merge apc1 average; by week Species;
data apc3; set apc2;
DROP time1-time3;
TIME=0; Population=time1; OUTPUT;
TIME=3; Population=time2; OUTPUT;
TIME=5; Population=time3; OUTPUT;
run;
/*Mixed with covariance*/
proc mixed data=apc3 covtest cl;
class Week Species Salt Sample Time;
Model Population= Species|Salt|Time Fat/DDFM=satterth outp=resout;
random Week Week*Salt*Species Time*Week*Salt*Species Sample(week species Salt);
estimate 'Fat slope' fat 1;
/* Compare species of meat against each other */
Lsmeans Species/pdiff adjust=tukey;
ods output lsmeans=lsmeans;
run;
/*Mixed with covariance*/
proc mixed data=apc3 covtest cl;
class Week Species Salt Sample Time;
Model Population= Species|Salt|Time Fat/DDFM=satterth outp=resout;
random Week Week*Salt*Species Time*Week*Salt*Species Sample(week species Salt);
estimate 'Fat slope' fat 1;
/* Compare salt against each other */
Lsmeans Salt/pdiff adjust=tukey;

```

```

ods output lsmeans=lsmeans;
run;
/*Mixed with covariance*/
proc mixed data=apc3 covtest cl;
class Week Species Salt Sample Time;
Model Population= Species|Salt|Time Fat/DDFM=satterth outp=resout;
random Week Week*Salt*Species Time*Week*Salt*Species Sample(week species Salt);
estimate 'Fat slope' fat 1;
/* Compare times */
Lsmeans Time/pdiff adjust=tukey;
ods output lsmeans=lsmeans;
run;
/*Mixed with covariance*/
proc mixed data=apc3 covtest cl;
class Week Species Salt Sample Time;
Model Population= Species|Salt|Time Fat/DDFM=satterth outp=resout;
random Week Week*Salt*Species Time*Week*Salt*Species Sample(week species Salt);
estimate 'Fat slope' fat 1;
/* Salt*Time Interactions */
Lsmeans Salt*time/pdiff Slice=Salt adjust=tukey;
ods output lsmeans=lsmeans;
run;
/*Plot for two-way interaction*/
symbol1 v=star h=2 w =1 i = j c = blue;
symbol2 v=square h=2 w =1 i = j c = red;
symbol3 v=circle h=2 w =1 i = j c = green;
proc gplot data=lsmeans;
where effect= 'Salt*TIME';
plot estimate*Salt=Time;
title 'Salt by Time';
run;

```

```
/*Test residual*/  
proc univariate normal plot data=resout; var resid; run;  
proc gplot data=resout; plot resid*pred /vref=0; run;
```

Appendix 2 - SAS code for *Listeria monocytogenes* populations for raw ground beef, pork, and turkey study

```
options nocenter ls = 120;
```

```
data;
```

```
Input sample salt $ species $ time population
```

```
datalines;
```

1	Cont	Beef	0	5.236
1	Cont	Beef	3	4.265
1	Cont	Beef	5	4.724
2	Cont	Beef	0	5.387
2	Cont	Beef	3	4.201
2	Cont	Beef	5	5.093
3	Cont	Beef	0	2.954
3	Cont	Beef	3	2.903
3	Cont	Beef	5	3.477
4	Cont	Beef	0	4.158
4	Cont	Beef	3	4.000
4	Cont	Beef	5	4.362
5	Cont	Beef	0	4.919
5	Cont	Beef	3	4.833
5	Cont	Beef	5	4.748
6	Cont	Beef	0	4.771
6	Cont	Beef	3	4.778
6	Cont	Beef	5	4.279
1	Na	Beef	0	4.279
1	Na	Beef	3	4.898
1	Na	Beef	5	4.763
2	Na	Beef	0	5.176
2	Na	Beef	3	4.845
2	Na	Beef	5	4.792
3	Na	Beef	0	4.633
3	Na	Beef	3	4.279
3	Na	Beef	5	3.602
4	Na	Beef	0	4.940
4	Na	Beef	3	3.556
4	Na	Beef	5	3.602
5	Na	Beef	0	5.127

5	Na	Beef	3	4.792
5	Na	Beef	5	4.908
6	Na	Beef	0	4.792
6	Na	Beef	3	4.763
6	Na	Beef	5	5.140
1	K	Beef	0	4.924
1	K	Beef	3	4.929
1	K	Beef	5	4.908
2	K	Beef	0	4.881
2	K	Beef	3	4.978
2	K	Beef	5	4.556
3	K	Beef	0	3.929
3	K	Beef	3	3.987
3	K	Beef	5	4.230
4	K	Beef	0	4.708
4	K	Beef	3	4.093
4	K	Beef	5	4.230
5	K	Beef	0	5.344
5	K	Beef	3	5.152
5	K	Beef	5	5.041
6	K	Beef	0	4.290
6	K	Beef	3	5.033
6	K	Beef	5	5.130
1	Ca	Beef	0	4.964
1	Ca	Beef	3	4.699
1	Ca	Beef	5	4.176
2	Ca	Beef	0	4.258
2	Ca	Beef	3	3.255
2	Ca	Beef	5	4.531
3	Ca	Beef	0	4.061
3	Ca	Beef	3	4.164
3	Ca	Beef	5	3.477
4	Ca	Beef	0	4.732
4	Ca	Beef	3	3.079
4	Ca	Beef	5	3.301
5	Ca	Beef	0	4.778
5	Ca	Beef	3	5.114
5	Ca	Beef	5	3.778
6	Ca	Beef	0	5.111
6	Ca	Beef	3	4.672

6	Ca	Beef	5	4.914
1	Mg	Beef	0	5.320
1	Mg	Beef	3	5.176
1	Mg	Beef	5	4.322
2	Mg	Beef	0	4.663
2	Mg	Beef	3	4.525
2	Mg	Beef	5	4.863
3	Mg	Beef	0	4.903
3	Mg	Beef	3	3.362
3	Mg	Beef	5	3.301
4	Mg	Beef	0	4.982
4	Mg	Beef	3	3.000
4	Mg	Beef	5	3.301
5	Mg	Beef	0	4.520
5	Mg	Beef	3	4.771
5	Mg	Beef	5	4.771
6	Mg	Beef	0	5.299
6	Mg	Beef	3	4.944
6	Mg	Beef	5	4.892
1	SS	Beef	0	4.318
1	SS	Beef	3	4.212
1	SS	Beef	5	4.544
2	SS	Beef	0	5.312
2	SS	Beef	3	5.033
2	SS	Beef	5	4.857
3	SS	Beef	0	3.869
3	SS	Beef	3	3.944
3	SS	Beef	5	3.301
4	SS	Beef	0	4.954
4	SS	Beef	3	3.556
4	SS	Beef	5	3.903
5	SS	Beef	0	4.500
5	SS	Beef	3	4.968
5	SS	Beef	5	5.079
6	SS	Beef	0	5.303
6	SS	Beef	3	4.833
6	SS	Beef	5	5.161
1	SR	Beef	0	5.260
1	SR	Beef	3	5.004
1	SR	Beef	5	4.556

2	SR	Beef	0	5.369
2	SR	Beef	3	4.433
2	SR	Beef	5	4.613
3	SR	Beef	0	4.708
3	SR	Beef	3	4.049
3	SR	Beef	5	3.477
4	SR	Beef	0	5.279
4	SR	Beef	3	3.996
4	SR	Beef	5	3.301
5	SR	Beef	0	4.964
5	SR	Beef	3	4.756
5	SR	Beef	5	4.732
6	SR	Beef	0	5.146
6	SR	Beef	3	5.045
6	SR	Beef	5	5.248
1	Cont	Pork	0	5.377
1	Cont	Pork	3	4.456
1	Cont	Pork	5	4.740
2	Cont	Pork	0	5.540
2	Cont	Pork	3	5.362
2	Cont	Pork	5	4.415
3	Cont	Pork	0	4.929
3	Cont	Pork	3	5.301
3	Cont	Pork	5	5.326
4	Cont	Pork	0	4.519
4	Cont	Pork	3	4.924
4	Cont	Pork	5	4.881
5	Cont	Pork	0	5.176
5	Cont	Pork	3	5.207
5	Cont	Pork	5	5.238
6	Cont	Pork	0	5.176
6	Cont	Pork	3	4.886
6	Cont	Pork	5	5.033
1	Na	Pork	0	4.193
1	Na	Pork	3	5.068
1	Na	Pork	5	4.785
2	Na	Pork	0	5.064
2	Na	Pork	3	5.279
2	Na	Pork	5	5.090
3	Na	Pork	0	4.978

3	Na	Pork	3	4.672
3	Na	Pork	5	4.740
4	Na	Pork	0	4.041
4	Na	Pork	3	4.898
4	Na	Pork	5	4.778
5	Na	Pork	0	5.223
5	Na	Pork	3	4.919
5	Na	Pork	5	4.869
6	Na	Pork	0	5.021
6	Na	Pork	3	5.253
6	Na	Pork	5	5.152
1	K	Pork	0	4.763
1	K	Pork	3	4.716
1	K	Pork	5	4.763
2	K	Pork	0	5.262
2	K	Pork	3	5.418
2	K	Pork	5	4.653
3	K	Pork	0	5.013
3	K	Pork	3	4.964
3	K	Pork	5	4.778
4	K	Pork	0	4.954
4	K	Pork	3	5.294
4	K	Pork	5	5.130
5	K	Pork	0	5.111
5	K	Pork	3	5.017
5	K	Pork	5	4.863
6	K	Pork	0	4.964
6	K	Pork	3	5.152
6	K	Pork	5	4.826
1	Ca	Pork	0	5.170
1	Ca	Pork	3	4.857
1	Ca	Pork	5	4.771
2	Ca	Pork	0	5.057
2	Ca	Pork	3	4.973
2	Ca	Pork	5	4.763
3	Ca	Pork	0	4.580
3	Ca	Pork	3	4.303
3	Ca	Pork	5	4.176
4	Ca	Pork	0	4.158
4	Ca	Pork	3	4.929

4	Ca	Pork	5	4.000
5	Ca	Pork	0	4.949
5	Ca	Pork	3	4.447
5	Ca	Pork	5	4.886
6	Ca	Pork	0	5.107
6	Ca	Pork	3	4.663
6	Ca	Pork	5	4.623
1	Mg	Pork	0	5.025
1	Mg	Pork	3	4.699
1	Mg	Pork	5	4.568
2	Mg	Pork	0	5.260
2	Mg	Pork	3	5.236
2	Mg	Pork	5	4.954
3	Mg	Pork	0	4.310
3	Mg	Pork	3	4.260
3	Mg	Pork	5	5.037
4	Mg	Pork	0	5.072
4	Mg	Pork	3	4.412
4	Mg	Pork	5	5.049
5	Mg	Pork	0	5.398
5	Mg	Pork	3	4.580
5	Mg	Pork	5	4.785
6	Mg	Pork	0	4.991
6	Mg	Pork	3	4.643
6	Mg	Pork	5	5.041
1	SS	Pork	0	4.763
1	SS	Pork	3	4.763
1	SS	Pork	5	4.934
2	SS	Pork	0	5.185
2	SS	Pork	3	5.387
2	SS	Pork	5	4.748
3	SS	Pork	0	4.369
3	SS	Pork	3	4.708
3	SS	Pork	5	3.301
4	SS	Pork	0	4.833
4	SS	Pork	3	4.623
4	SS	Pork	5	4.531
5	SS	Pork	0	5.013
5	SS	Pork	3	4.934
5	SS	Pork	5	4.813

6	SS	Pork	0	5.281
6	SS	Pork	3	5.072
6	SS	Pork	5	5.290
1	SR	Pork	0	4.763
1	SR	Pork	3	5.033
1	SR	Pork	5	4.716
2	SR	Pork	0	5.185
2	SR	Pork	3	5.223
2	SR	Pork	5	5.009
3	SR	Pork	0	4.991
3	SR	Pork	3	4.708
3	SR	Pork	5	4.833
4	SR	Pork	0	4.653
4	SR	Pork	3	4.643
4	SR	Pork	5	4.477
5	SR	Pork	0	4.813
5	SR	Pork	3	4.869
5	SR	Pork	5	5.375
6	SR	Pork	0	5.413
6	SR	Pork	3	5.137
6	SR	Pork	5	4.833
1	Cont	Turkey	0	4.949
1	Cont	Turkey	3	4.820
1	Cont	Turkey	5	4.944
2	Cont	Turkey	0	5.260
2	Cont	Turkey	3	4.615
2	Cont	Turkey	5	4.875
3	Cont	Turkey	0	2.301
3	Cont	Turkey	3	2.301
3	Cont	Turkey	5	3.301
4	Cont	Turkey	0	2.301
4	Cont	Turkey	3	2.301
4	Cont	Turkey	5	3.301
5	Cont	Turkey	0	4.903
5	Cont	Turkey	3	5.158
5	Cont	Turkey	5	5.283
6	Cont	Turkey	0	5.498
6	Cont	Turkey	3	5.373
6	Cont	Turkey	5	5.230
1	Na	Turkey	0	4.708

1	Na	Turkey	3	5.491
1	Na	Turkey	5	4.908
2	Na	Turkey	0	4.954
2	Na	Turkey	3	4.940
2	Na	Turkey	5	4.903
3	Na	Turkey	0	4.869
3	Na	Turkey	3	4.724
3	Na	Turkey	5	4.973
4	Na	Turkey	0	5.037
4	Na	Turkey	3	4.041
4	Na	Turkey	5	4.531
5	Na	Turkey	0	4.964
5	Na	Turkey	3	5.029
5	Na	Turkey	5	5.149
6	Na	Turkey	0	5.340
6	Na	Turkey	3	4.954
6	Na	Turkey	5	5.167
1	K	Turkey	0	5.009
1	K	Turkey	3	4.799
1	K	Turkey	5	4.881
2	K	Turkey	0	4.328
2	K	Turkey	3	4.449
2	K	Turkey	5	4.740
3	K	Turkey	0	-
3	K	Turkey	3	5.358
3	K	Turkey	5	4.919
4	K	Turkey	0	4.908
4	K	Turkey	3	4.643
4	K	Turkey	5	5.100
5	K	Turkey	0	4.959
5	K	Turkey	3	5.124
5	K	Turkey	5	5.204
6	K	Turkey	0	5.281
6	K	Turkey	3	5.045
6	K	Turkey	5	4.826
1	Ca	Turkey	0	4.934
1	Ca	Turkey	3	5.076
1	Ca	Turkey	5	4.519
2	Ca	Turkey	0	5.064
2	Ca	Turkey	3	4.243

2	Ca	Turkey	5	4.690
3	Ca	Turkey	0	4.944
3	Ca	Turkey	3	4.934
3	Ca	Turkey	5	4.398
4	Ca	Turkey	0	5.100
4	Ca	Turkey	3	4.623
4	Ca	Turkey	5	4.415
5	Ca	Turkey	0	4.934
5	Ca	Turkey	3	4.771
5	Ca	Turkey	5	4.851
6	Ca	Turkey	0	5.093
6	Ca	Turkey	3	4.954
6	Ca	Turkey	5	4.949
1	Mg	Turkey	0	4.991
1	Mg	Turkey	3	4.813
1	Mg	Turkey	5	4.954
2	Mg	Turkey	0	4.653
2	Mg	Turkey	3	4.699
2	Mg	Turkey	5	4.771
3	Mg	Turkey	0	4.903
3	Mg	Turkey	3	4.892
3	Mg	Turkey	5	4.851
4	Mg	Turkey	0	5.053
4	Mg	Turkey	3	4.987
4	Mg	Turkey	5	4.623
5	Mg	Turkey	0	5.111
5	Mg	Turkey	3	5.004
5	Mg	Turkey	5	4.875
6	Mg	Turkey	0	5.217
6	Mg	Turkey	3	5.155
6	Mg	Turkey	5	4.898
1	SS	Turkey	0	4.708
1	SS	Turkey	3	4.833
1	SS	Turkey	5	4.813
2	SS	Turkey	0	4.934
2	SS	Turkey	3	4.708
2	SS	Turkey	5	4.857
3	SS	Turkey	0	4.544
3	SS	Turkey	3	4.799
3	SS	Turkey	5	5.079

4	SS	Turkey	0	5.086
4	SS	Turkey	3	4.663
4	SS	Turkey	5	5.083
5	SS	Turkey	0	4.940
5	SS	Turkey	3	5.158
5	SS	Turkey	5	5.013
6	SS	Turkey	0	5.369
6	SS	Turkey	3	5.083
6	SS	Turkey	5	5.068
1	SR	Turkey	0	5.037
1	SR	Turkey	3	5.111
1	SR	Turkey	5	4.748
2	SR	Turkey	0	4.863
2	SR	Turkey	3	4.547
2	SR	Turkey	5	4.708
3	SR	Turkey	0	5.057
3	SR	Turkey	3	4.716
3	SR	Turkey	5	4.716
4	SR	Turkey	0	5.155
4	SR	Turkey	3	4.929
4	SR	Turkey	5	4.531
5	SR	Turkey	0	5.230
5	SR	Turkey	3	5.083
5	SR	Turkey	5	5.143
6	SR	Turkey	0	5.324
6	SR	Turkey	3	5.029
6	SR	Turkey	5	5.158

options nocenter ls = 120;

data;

input Rep Species \$ Moisture Protein Fat Calcium Magnesium Sodium Potassium

datalines;

1	Beef	69.29	19.60	10.86	64	182	1806	1781
1	Beef	68.97	18.74	10.34	80	200	1538	1852
2	Beef	69.29	19.60	10.86	64	182	1806	1781
2	Beef	68.97	18.74	10.34	80	200	1538	1852
3	Beef	62.87	17.21	18.47	126	148	1245	1639
3	Beef	62.74	17.38	18.28	117	149	1148	1808
1	Pork	57.12	14.79	21.31	16	147	3330	1874
1	Pork	57.41	14.38	24.47	16	140	2857	1723

2	Pork	57.12	14.79	21.31	16	147	3330	1874
2	Pork	57.41	14.38	24.47	16	140	2857	1723
3	Pork	61.42	16.56	20.25	50	170	2062	1955
3	Pork	62.14	17.08	19.72	53	167	1993	1879
1	Turkey	63.65	13.38	21.48	591	120	283	666
1	Turkey	63.46	13.44	21.28	595	127	334	688
2	Turkey	63.65	13.38	21.48	591	120	283	666
2	Turkey	63.46	13.44	21.28	595	127	334	688
3	Turkey	69.73	13.41	15.63	658	149	4622	1416
3	Turkey	68.44	13.30	16.17	595	152	4430	1315

RUN;

/*Arrange population data set*/

data time0; set mox;

drop Population time;

if Time=0 then Time1=Population;

else delete;

data time3; set mox;

drop Population time;

if Time=3 then Time2=Population;

else delete;

data time5; set mox;

drop Population time;

if Time=5 then Time3=Population;

else delete;

data mox1; merge time0 time3 time5; Sample=_N_;run;

/*Combine Population & Measurement data sets*/

proc sort data=average; by week Species;

data mox2; merge mox1 average; by week Species;

data mox3; set mox2;

DROP time1-time3;

TIME=0; Population=time1; OUTPUT;

TIME=3; Population=time2; OUTPUT;

TIME=5; Population=time3; OUTPUT;

run;

```

/*Mixed with covariance*/
proc mixed data=mox3 covtest cl;
class Week Species Salt Sample Time;
Model Population= Species|Salt|Time Fat/DDFM=satterth outp=resout;
random Week Week*Salt*Species Time*Week*Salt*Species Sample(week species Salt);
estimate 'Fat slope' fat 1;
Lsmeans Species|Salt|Time/cl;
ods output lsmeans=lsmeans;
run;

proc mixed data=mox3 covtest cl;
class Week Species Salt Sample Time;
Model Population= Species|Salt|Time Fat/DDFM=satterth outp=resout;
random Week Week*Salt*Species Time*Week*Salt*Species Sample(week species Salt);
estimate 'Fat slope' fat 1;
/* Compare times */
Lsmeans Time/pdiff adjust=tukey;
ods output lsmeans=lsmeans;
run;

proc mixed data=mox3 covtest cl;
class Week Species Salt Sample Time;
Model Population= Species|Salt|Time Fat/DDFM=satterth outp=resout;
random Week Week*Salt*Species Time*Week*Salt*Species Sample(week species Salt);
estimate 'Fat slope' fat 1;
*Species*time;
Lsmeans Species*Time/pdiff Slice=Species adjust=tukey;
ods output lsmeans=lsmeans;
run;

/*Plot for two-way interaction*/
symbol1 v=star h=2 w =1 i = j c = blue;
symbol2 v=square h=2 w =1 i = j c = red;
symbol3 v=circle h=2 w =1 i = j c = green;

```

```
proc gplot data=lsmeans;
where effect= 'Species*TIME';
plot estimate*species=time;
title 'Species by Time';
run;
/*Test residual*/
proc univariate normal plot data=resout; var resid; run;
proc gplot data=resout; plot resid*pred /vref=0; run;
```

Appendix 3 - SAS code for *Listeria monocytogenes* populations for emulsified beef and pork study

options nocenter ls = 120;

data;

input Rep SampleSpecies \$ Salt \$ Day Pop;

datalines;

1	1	Beef	Na	0	3.9566
1	1	Beef	Na	7	4.9395
1	1	Beef	Na	14	5.9165
1	1	Beef	Na	21	5.6767
1	1	Beef	Na	28	6.6075
1	2	Beef	Na	0	4.3424
1	2	Beef	Na	7	5.1271
1	2	Beef	Na	14	6.0626
1	2	Beef	Na	21	5.9031
1	2	Beef	Na	28	6.7202
1	1	Beef	K	0	3.9058
1	1	Beef	K	7	5.0846
1	1	Beef	K	14	5.4624
1	1	Beef	K	21	6.2553
1	1	Beef	K	28	6.5378
1	2	Beef	K	0	3.8420
1	2	Beef	K	7	4.9138
1	2	Beef	K	14	5.2430
1	2	Beef	K	21	5.8573
1	2	Beef	K	28	6.6580
1	1	Beef	SS	0	4.0107
1	1	Beef	SS	7	4.5119
1	1	Beef	SS	14	5.0663
1	1	Beef	SS	21	5.1367
1	1	Beef	SS	28	6.7243
1	2	Beef	SS	0	4.3617
1	2	Beef	SS	7	4.6021
1	2	Beef	SS	14	6.0607
1	2	Beef	SS	21	5.9934
1	2	Beef	SS	28	6.7364
1	1	Beef	Bl	0	3.7520
1	1	Beef	Bl	7	4.7118

1	1	Beef	Bl	14	5.6484
1	1	Beef	Bl	21	7.1255
1	1	Beef	Bl	28	6.5378
1	2	Beef	Bl	0	3.6385
1	2	Beef	Bl	7	
1	2	Beef	Bl	14	4.7993
1	2	Beef	Bl	21	5.3118
1	2	Beef	Bl	28	6.5315
2	1	Beef	Na	0	4.3617
2	1	Beef	Na	7	5.0569
2	1	Beef	Na	14	4.8062
2	1	Beef	Na	21	6.7672
2	1	Beef	Na	28	5.7889
2	2	Beef	Na	0	4.0682
2	2	Beef	Na	7	5.0149
2	2	Beef	Na	14	6.4472
2	2	Beef	Na	21	5.4698
2	2	Beef	Na	28	6.8096
2	1	Beef	K	0	3.9685
2	1	Beef	K	7	5.2430
2	1	Beef	K	14	4.9165
2	1	Beef	K	21	6.3541
2	1	Beef	K	28	6.2553
2	2	Beef	K	0	4.2041
2	2	Beef	K	7	3.9085
2	2	Beef	K	14	4.8513
2	2	Beef	K	21	5.6767
2	2	Beef	K	28	6.4065
2	1	Beef	SS	0	4.3711
2	1	Beef	SS	7	4.0294
2	1	Beef	SS	14	4.7993
2	1	Beef	SS	21	5.9708
2	1	Beef	SS	28	6.6284
2	2	Beef	SS	0	5.0899
2	2	Beef	SS	7	4.1430
2	2	Beef	SS	14	4.9469
2	2	Beef	SS	21	6.0550
2	2	Beef	SS	28	6.7364
2	1	Beef	Bl	0	4.1206
2	1	Beef	Bl	7	4.0569
2	1	Beef	Bl	14	4.6767
2	1	Beef	Bl	21	6.4843

2	1	Beef	Bl	28	6.7818
2	2	Beef	Bl	0	4.3424
2	2	Beef	Bl	7	3.8633
2	2	Beef	Bl	14	4.6532
2	2	Beef	Bl	21	6.5441
2	2	Beef	Bl	28	5.9420
3	1	Beef	Na	0	4.0864
3	1	Beef	Na	7	5.4265
3	1	Beef	Na	14	4.9868
3	1	Beef	Na	21	7.1477
3	1	Beef	Na	28	6.8482
3	2	Beef	Na	0	3.9112
3	2	Beef	Na	7	4.1973
3	2	Beef	Na	14	5.5250
3	2	Beef	Na	21	5.9420
3	2	Beef	Na	28	6.7889
3	1	Beef	K	0	4.3711
3	1	Beef	K	7	4.5185
3	1	Beef	K	14	4.6435
3	1	Beef	K	21	6.1239
3	1	Beef	K	28	6.3222
3	2	Beef	K	0	5.1335
3	2	Beef	K	7	5.0414
3	2	Beef	K	14	4.4914
3	2	Beef	K	21	5.8261
3	2	Beef	K	28	5.8261
3	1	Beef	SS	0	4.4314
3	1	Beef	SS	7	5.4843
3	1	Beef	SS	14	3.6857
3	1	Beef	SS	21	5.7076
3	1	Beef	SS	28	6.4314
3	2	Beef	SS	0	4.4065
3	2	Beef	SS	7	5.5911
3	2	Beef	SS	14	4.8162
3	2	Beef	SS	21	6.2109
3	2	Beef	SS	28	6.4393
3	1	Beef	Bl	0	3.9494
3	1	Beef	Bl	7	4.3222
3	1	Beef	Bl	14	4.8603
3	1	Beef	Bl	21	5.4232
3	1	Beef	Bl	28	6.7889
3	2	Beef	Bl	0	3.8420

3	2	Beef	Bl	7	3.9395
3	2	Beef	Bl	14	4.7284
3	2	Beef	Bl	21	5.2553
3	2	Beef	Bl	28	6.3711
1	1	Pork	Na	0	4.3010
1	1	Pork	Na	7	5.5966
1	1	Pork	Na	14	5.0334
1	1	Pork	Na	21	6.3522
1	1	Pork	Na	28	7.0043
1	2	Pork	Na	0	4.9661
1	2	Pork	Na	7	4.4150
1	2	Pork	Na	14	4.9269
1	2	Pork	Na	21	6.2292
1	2	Pork	Na	28	7.2162
1	1	Pork	K	0	3.8976
1	1	Pork	K	7	5.8543
1	1	Pork	K	14	6.0792
1	1	Pork	K	21	7.0065
1	1	Pork	K	28	6.0663
1	2	Pork	K	0	3.8976
1	2	Pork	K	7	5.8751
1	2	Pork	K	14	6.4983
1	2	Pork	K	21	5.9566
1	2	Pork	K	28	6.6128
1	1	Pork	SS	0	3.9345
1	1	Pork	SS	7	4.7782
1	1	Pork	SS	14	4.9845
1	1	Pork	SS	21	6.8028
1	1	Pork	SS	28	6.9191
1	2	Pork	SS	0	3.9004
1	2	Pork	SS	7	4.6075
1	2	Pork	SS	14	5.6812
1	2	Pork	SS	21	7.0294
1	2	Pork	SS	28	7.0917
1	1	Pork	Bl	0	4.2923
1	1	Pork	Bl	7	6.2945
1	1	Pork	Bl	14	5.9112
1	1	Pork	Bl	21	5.6812
1	1	Pork	Bl	28	6.9708
1	2	Pork	Bl	0	4.0043
1	2	Pork	Bl	7	6.2355
1	2	Pork	Bl	14	6.7118

1	2	Pork	Bl	21	6.6484
1	2	Pork	Bl	28	6.3711
2	1	Pork	Na	0	4.0952
2	1	Pork	Na	7	4.5315
2	1	Pork	Na	14	4.9058
2	1	Pork	Na	21	6.7889
2	1	Pork	Na	28	7.3617
2	2	Pork	Na	0	4.0453
2	2	Pork	Na	7	4.0107
2	2	Pork	Na	14	5.1106
2	2	Pork	Na	21	5.1303
2	2	Pork	Na	28	6.9685
2	1	Pork	K	0	3.9191
2	1	Pork	K	7	3.9217
2	1	Pork	K	14	4.9868
2	1	Pork	K	21	7.3222
2	1	Pork	K	28	6.5185
2	2	Pork	K	0	4.0719
2	2	Pork	K	7	5.0846
2	2	Pork	K	14	4.1614
2	2	Pork	K	21	6.7672
2	2	Pork	K	28	6.6628
2	1	Pork	SS	0	4.2355
2	1	Pork	SS	7	6.5185
2	1	Pork	SS	14	6.5378
2	1	Pork	SS	21	7.1461
2	1	Pork	SS	28	6.4698
2	2	Pork	SS	0	4.3979
2	2	Pork	SS	7	6.0394
2	2	Pork	SS	14	6.4624
2	2	Pork	SS	21	6.9031
2	2	Pork	SS	28	6.1553
2	1	Pork	Bl	0	4.0022
2	1	Pork	Bl	7	6.2553
2	1	Pork	Bl	14	5.8865
2	1	Pork	Bl	21	7.3522
2	1	Pork	Bl	28	7.0864
2	2	Pork	Bl	0	4.3711
2	2	Pork	Bl	7	4.5798
2	2	Pork	Bl	14	6.9031
2	2	Pork	Bl	21	7.1644
2	2	Pork	Bl	28	5.8293

3	1	Pork	Na	0	4.4314
3	1	Pork	Na	7	4.3617
3	1	Pork	Na	14	5.3010
3	1	Pork	Na	21	5.9566
3	1	Pork	Na	28	6.3802
3	2	Pork	Na	0	4.3617
3	2	Pork	Na	7	4.1917
3	2	Pork	Na	14	5.4914
3	2	Pork	Na	21	6.6990
3	2	Pork	Na	28	6.6180
3	1	Pork	K	0	4.0212
3	1	Pork	K	7	5.0334
3	1	Pork	K	14	4.8663
3	1	Pork	K	21	5.4150
3	1	Pork	K	28	5.6484
3	2	Pork	K	0	4.0952
3	2	Pork	K	7	6.2330
3	2	Pork	K	14	6.5378
3	2	Pork	K	21	5.1055
3	2	Pork	K	28	6.9638
3	1	Pork	SS	0	4.2317
3	1	Pork	SS	7	6.0128
3	1	Pork	SS	14	5.9590
3	1	Pork	SS	21	5.5250
3	1	Pork	SS	28	5.8195
3	2	Pork	SS	0	4.3802
3	2	Pork	SS	7	5.5441
3	2	Pork	SS	14	5.8692
3	2	Pork	SS	21	5.6284
3	2	Pork	SS	28	7.0065
3	1	Pork	Bl	0	4.4314
3	1	Pork	Bl	7	6.0682
3	1	Pork	Bl	14	7.0453
3	1	Pork	Bl	21	5.7782
3	1	Pork	Bl	28	6.7324
3	2	Pork	Bl	0	4.3979
3	2	Pork	Bl	7	5.4624
3	2	Pork	Bl	14	4.3892
3	2	Pork	Bl	21	6.6946
3	2	Pork	Bl	28	6.4771

;

*input Rep SampleSpecies \$ Salt \$ Day Pop;

```
proc mixed;
title 'ANALYSIS 1';
title2 'basic 3 way ANOVA with all 3-way means and with stat tests for slice by day';
class rep sample species salt day;
model pop = species|salt|day/ddfm = satterth;
random rep;
lsmeans species/pdiff adjust=tukey;
lsmeans day/pdiff adjust=tukey;
lsmeans species*salt/pdiff adjust=tukey;
lsmeans species*day/pdiff adjust=tukey;
lsmeans species*salt*day/pdiff adjust=tukey;
lsmeans species|salt|day /slice = day;
run;
```

Appendix 4 – Statistics for *Listeria monocytogenes* and aerobic populations in raw pre-blended ground beef, pork, and turkey and emulsified beef and pork products

Table 15. Probability values for species, salt, species by salt, time, species by time, salt by time, and species by salt by time interactions for *Listeria monocytogenes* and aerobic populations in raw ground beef, pork and turkey.

Effect	<i>L. monocytogenes</i> populations	Aerobic populations
Species	0.4089	0.0072
Salt	0.3726	0.0004
Species*salt	0.7419	0.8498
Time	<0.0001	<0.0001
Species*time	0.0103	0.0924
Salt*time	0.9229	0.0492
Species*salt*time	0.3017	0.9992

Table 16. Probability values for species, salt, species by salt, time, species by time, salt by time, and species by salt by time interactions for *Listeria monocytogenes* populations in emulsified beef and pork products.

Effect	<i>L. monocytogenes</i> populations
Species	<0.0001
Salt	0.6117
Species*salt	<0.0001
Time	<0.0001
Species*time	0.0335
Salt*time	0.8559
Species*salt*time	0.0505