VALIDATION OF THERMAL LETHALITY AGAINST *SALMONELLA ENTERICA* SEROTYPES IN FEATHER MEAL WITH BLOOD, AND COOKIE MEAL

by

WENYI HUANG

(Under the Direction of Todd Callaway)

ABSTRACT

The objective of this study was to validate the lethality of heat processing to *S*. enterica in feather meal with blood (FMB) and Cookie meal (CM). FMB and CM Samples of inoculated *S*. Typhimurium (ST), *S*. Newport (SN), and *S*. Enteritidis (SE) were submerged into water bath tempered to 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, or 95°C for 0, 0.5, 1, 3, 5, and 10 minutes, respectively. *D*- and *z*-values were generated from best-fit line. In FMB, a 7.0-$\log_{10}$ Colony forming Unit/g (CFU/g) inactivation of three *Salmonella* serovars was obtained during the heating period and subsequent rendering of FMB at all temperatures after 10 minutes. However, in CM, the results showed that a 7.0- $\log_{10}$ CFU/g inactivation of three *Salmonella* serovars required a temperature during rendering of CM above 90°C. Results from this study provide short- and long- term benefits to reducing the foodborne pathogens in the rendering industry.

INDEX WORDS: *Salmonella enterica*, foodborne illness, pre-harvest food safety, rendering

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CHAPTER 1

INTRODUCTION

Foodborne Illness

Foodborne illnesses in humans are caused by many types of pathogens (e.g., bacteria, protozoa, or virus). Centers for Disease Control and Prevention (CDC) estimates that each year, approximately 1 in 6 (48 million) Americans suffers from a foodborne illness, 128,000 are hospitalized, and 3,000 die from foodborne diseases (CDC, 2018). About 9.4 million cases of foodborne illness are caused by 31 major pathogens in the United States annually and around 3.6 million of these cases is caused by bacteria (Scallan et al., 2013; USDA-ERS, 2019). According to the U.S. Department of Agriculture (USDA), foodborne illnesses in the United States cost more than 17.6 billion each year (USDA, 2021). This drain on human health and the national economy is caused by a variety pathogenic bacterium and is a major public health concern and imposes a significant cost to the U.S. economy (Scharff, 2012; Marder et al., 2017).

Salmonella Overall

Salmonella is a gram-negative, rod-shaped bacteria that can adapt to various environments (Wray and Wray, 2000). The environmental adaptability makes *Salmonella* a problematic foodborne pathogen because it is widespread and nearly ubiquitous in some food animal species. *Salmonella* thrives in the gastrointestinal tract of animals, where it can establish a symbiotic/commensal relationship with its host, resulting in little or no clinical disease expression. However, the pathogen can be shed in feces, which can contaminate soil, water, and surfaces, potentially infecting other animals (Purdy et al., 2010). This fecal *Salmonella* shedding

can also lead to hide and wastewater contamination during the slaughtering process (Galland, 1997).

Salmonella can grow under both aerobic and anaerobic conditions, depending on the environment. Although its ideal growth conditions are a temperature of 37°C (98.6°F) and a pH of 6.5 to 7.5, different strains of *Salmonella* can grow in a wide range of conditions, such as temperatures ranging from 2°C (36°F) to 54°C (129°F) and pH values between 4.0 and 9.5 (Li et al., 2012a). Salmonella is also capable of surviving in extreme conditions, including being able to persist in frozen meat for over a year (Müller et al., 2012).

Eggs and poultry are commonly associated with transmitting *Salmonella* infections to humans; however, all types of animal-derived food can carry bacteria, including beef, pork, lamb, fish, dairy products, vegetables, fruits, nuts, and others (International Commission on Microbiological Specifications for, 1996). In the United States, approximately one-third of reported *Salmonella*-related diseases are linked to poultry, beef, pork, or lamb consumption (Hsi et al., 2015). The likelihood of contracting *Salmonella*-related illness from consuming poultry is 1 in 40 for the average American over their lifetime, while the lifetime risk from eating beef or pork is 1 in 100 (Hsi et al., 2015). Thus, *Salmonella* poses a significant risk to consumers that must be addressed at many points in the food production continuum.

***Salmonella* as a Normal Member of the Food Animal Environment**

The global food safety challenge is caused by the diverse range of foods that contribute to the burden of *Salmonella*-related illness. Although significant efforts have been made to reduce *Salmonella* contamination throughout the food industry, it remains a challenge because many food animals are natural hosts to *Salmonella*. Therefore, it is crucial to focus efforts on controlling *Salmonella* in food-producing animals.

Food-producing animals have various ways of acquiring *Salmonella*, such as exposure to infected animals or their excrement, unsanitary living conditions, wild birds, rodents, biting insects, use of improperly rendered animal by-products in their feed, and tainted water (European Food Safety, 2009). In the poultry industry, the vertical transfer of *Salmonella* from parents to their offspring or eggs for human consumption through contamination within or outside the eggs is a significant concern (European Food Safety, 2009).

Salmonella can be carried by a variety of animals, including cattle, horses, pigs, cats, dogs, rodents, domestic and wild birds, reptiles, amphibians, and fish (Hoelzer et al., 2011). These animals often carry pathogenic bacteria without showing any symptoms but can occasionally transmit the organism to humans or food intended for human consumption. For example, *Salmonella enterica* can cause systemic disease in newborn poultry chicks, resulting in symptoms such as diarrhea and dehydration. These serotypes can persist and colonize older birds without causing symptoms, leading to horizontal transmission to other birds in the flock or eggs. Persistence and asymptomatic colonization of *Salmonella* within a poultry flock can create a potential pathway for transmission to humans through contaminated eggs or poultry meat.

Importance of *Salmonella* Prevention

One of the most common foodborne illnesses in humans is caused by *Salmonella* infections which results in nearly $4.1 billion costs annually and $88 million cost for loss of productivity in the United States every year (USDA, 2022). Among the bacterial pathogens associated with foodborne illness, non-typhoidal *Salmonella* is one of the leading causes of bacterial foodborne illnesses (Scallan et al., 2011; Marder et al., 2017). Part of the challenge in preventing human infections, is that infectious dose of *Salmonella enterica* can range from 15 to 10^5 cells, depending on the serotype involved, and there are more than 2,500 different serotypes

known, many of which cause human illnesses (Mead et al., 1999; Foley et al., 2008). Livestock animals are a major reservoir for foodborne pathogens and acquire *Salmonella* by ingestion via a fecal-oral route (Russell and Jarvis, 2001; Wilkerson et al., 2020). Contamination of animal feeds during the transportation to and while on the farm contributes to the infection of *Salmonella* into food animals and colonization of those animals (Crump et al., 2002b). *Salmonella* infection in animals may be asymptomatic or cause clinical illness of differing severity (Rukambile et al., 2019). Age of the animal and environmental/production stress can be an important factor influencing the duration of fecal shedding of *Salmonella* and the severity of clinical illness of the food animal (Hoelzer et al., 2011). On the farm, *Salmonella* can survive on surfaces, and in water and soil for long periods of time, and can be recirculated amongst herd members (Rukambile et al., 2019). *Salmonella* then can contaminate animal carcasses during the slaughter process from manure on the hide, or gut leakage during processing and can cross-contaminate food products during further processing or preparation, ultimately leading to human illnesses (Callaway et al., 2014; World Health et al., 2016; Young et al., 2016). Multiple studies have confirmed that *Salmonella* can also be isolated from the lymph nodes of cattle which can be subsequently incorporated into ground beef (Brichta-Harhay et al., 2012; Gragg et al., 2013; Vipham et al., 2015; Edrington et al., 2016; Belk et al., 2018). Therefore, it is apparent that animal feed becomes the first step in a food chain that has direct impact upon food safety and human health.

Pre-harvest Food Safety as a Concept

Preventing the transmission of pathogens to consumers can be a challenging task for many food products, as it may be difficult to control contamination once the food has left the farm. Post-harvest decontamination measures, if available, may not always be effective or

contamination may occur subsequent to the decontamination step. Moreover, raw food items, such as fruits, vegetables, nuts, and some seafood, cannot be cooked before reaching the consumer to eliminate any pathogens. Another complication can arise during home preparation, where raw food items like meat can contaminate other food items in the preparation environment before cooking. The process of food production involves multiple steps, and each stage presents an opportunity for contamination, which can ultimately result in increasing (or decreasing) the risk of foodborne illness reaching a human consumer.

Pre-harvest food safety is essential to ensuring the protection of our food supply (Sofos and Smith, 1998). The addition of safe animal by-products to livestock and poultry feeds plays an integral part in ensuring the safety of our food products. The objective of food safety assurance is to ensure that food products are free from pathogenic microorganisms, disease, and parasites, as well as potentially harmful residues and physical hazards.

About one-third to one-half of each animal produced for meat, milk, and eggs is not actually consumed by humans (Meeker and Hamilton, 2006). Animal by-products provide a large amount of protein feeds used in animal rations which often contain manufactured feed products, such as dogs and cats. After slaughter of livestock animals, the inedible products like hair, feathers, hooves, horns, bones, blood, fat tissues are essential raw materials for the rendering industry (Meeker and Hamilton, 2006). These raw materials are subjected to rendering processes to make them into many useful products, such as feather meal, blood meal, bone meal, and fish meal that are used in animal feeding to provide limiting nutrients. Because food animals can contain foodborne pathogens, the process of by product production can lead to contamination of the final product, and in turn pathogens can be carried forward to the feed provided to animals (Gabis, 1991; Nesse et al., 2003). Providing nutritious products for animal feed and recycling

nutrients, the rendering industry must ensure the microbiological safety of its products. The present research was designed to validate the thermal lethality to three serotypes of *Salmonella enterica* (e.g., Typhimurium, Enteritidis, and Newport) that are associated with food animals and human illnesses in feather meal with blood and cookie meal during the rendering process.

CHAPTER 2

LITERATURE REVIEW

Rendering Industry

The rendering industry is closely related to production of food animals, agriculture, food, and meat industry. Approximately 120 million hogs, 33 million cattle and 8 billion chickens are slaughtered in the United States annually (USDA, 2022). Almost one-third to one-half of every animal raised for food, dairy, eggs, or fiber is not consumed by humans, but enters the food chain in another route, via rendered products (Meeker and Hamilton, 2006). The rendering industry converts raw proteinaceous products, such as dead animals, slaughterhouse by-products, recalled products, and restaurant waste oil into useful products, including protein meal and fat. Rendering is truly a sustainable form of recycling of protein sources. Finished rendered products used in the animal feeding industry include blood meal, feather meal with blood, poultry fat, bone meal, poultry meal, tallow, and lard. The most valuable and important purpose of these products is feeding livestock, poultry, aquaculture, and companion animals (Meeker, 2009).

Throughout history, animal products have been subjected to rendering to convert or recycle both edible and inedible raw animal tissues like heart, tongue, liver, cheeks, feathers, hides, blood, bones, shells, and fat into valuable products for human and animal food industries(Meeker, 2009). To ensure the microbiological safety and quality of rendered products, the duration and temperature of the cooking process during rendering are critical to the lethality step to reduce pathogen transmission to final products. Consequently, all rendering system technologies involve the gathering and hygienic transport of raw material to a facility where it is

ground to a consistent particle size and then conveyed to a cooking vessel, which can either be in a continuous-flow or batch configuration (Meeker and Hamilton, 2006). The majority of the tissues processed originate from slaughterhouses, although they may also include trimmings from butcher shops and restaurant grease (Meeker and Hamilton, 2006). During rendering, animal by-products such as internal organs are crushed, heated to drive off the water, which can account for as much as 65% of the weight, and then separated into tallow, or fat, and greaves, or solids. The crushing of raw materials can release aerosols that contain harmful microbes and spread throughout a rendering facility, even contaminating areas where finished products are handled (Barnes, 1983). Therefore, it is important to have barriers and other protective measures in place. During the rendering cooking process, which typically takes 40 to 90 minutes at temperatures ranging from 240 to 290°F (115.6 to 143.3°C), computers are used to monitor the time/temperature process to ensure appropriate moisture loss (Meeker and Hamilton, 2006). However, the precise relationship between time and temperature required to destroy specific microorganisms in rendering matrices is not yet known. It has been shown that bacterial spores can survive in high-fat, low-water environments, making them more difficult to eradicate through thermal inactivation (Lowry et al., 1979). As a result, certain processing conditions that fall within (or outside) the margins could lead to finished products that contain bacterial spores or other heat-resistant biological materials.

In North America, most of the rendering facilities utilize continuous-flow, dry rendering units(Meeker and Hamilton, 2006). Dry rendering can be carried out using either batch or continuous processing methods. An example of a batch system involves subjecting the raw material to high pressure and temperature, typically at a temperature of at least 80°C and a pressure of 12 atm for a minimum of 40 minutes. This batch cooker serves the purpose of

cooking, drying, and hydrolyzing the raw material. While the batch system used to be commonly used for particle reduction, modern technologies have largely eliminated the need for it. Pressure cooking is essential to break down the keratin proteins present in feathers, enhancing their digestibility and product quality for consumption by animals (El Boushy et al., 1990).

A common continuous processing system for rendering starts by feeding raw materials into a grinder where they are ground into smaller particles. The ground particles are then moved into a continuous cooker, which is designed to handle specific aspects of the rendering process in sequence. In the cooker, the particles are heated to temperatures between 115°C and 145°C for a duration of 40 to 90 minutes, while being subjected to a pressure of 43.5 psi (Meeker and Hamilton, 2006). After the material has been cooked, a drainer conveyor is used to separate the liquid fat from the non-fat solid material. The solid material is then processed through a screw press to reduce its fat content from around 25% to 10-12%, and then combined to produce the final meal products (Ockerman and Hansen, 1988). Animal feeds are primarily designed to meet the nutritional needs of animals for maintenance, activity, production, and reproduction. However, since farmed livestock are raised for human consumption, the quality and safety of animal feed is essential to ensure the safety of human consumers. Therefore, animal feed is considered to be a part of the human food chain, and any assessment of feed safety must consider the potential hazards for both animals that consume it and the human consumers of animal products (Fink-Gremmels, 2012).

The safety of animal feed and pet food in the United States is under the jurisdiction of the Food and Drug Administration (FDA). The FDA is responsible for identifying and addressing any potential hazards in the manufacturing processes of pet foods. To improve the current regulatory system, the FDA created the Animal Feed Safety System (AFSS) to analyze the safety

of animal feed and pet food. Additionally, in 2011, the FDA Food Safety Modernization Act (FSMA) was passed as law, introducing new regulations for feed safety, such as the application of food safety preventive controls (FSPCs). The goal of these controls is to prevent, eliminate, or reduce the presence of food safety hazards, including pathogenic microorganisms such as *Salmonella spp.*

Finished Rendered Products

Each year, the rendering industry in the United States produces approximately 11.2 billion pounds of protein and 10.9 billion pounds of fats. A significant portion, about 85%, of these products are used in the production of animal feed ingredients. The National Research Council (NRC) or the Association of American Feed Control Officials (AAFCO) is responsible for defining the composition of these feed ingredients. The AAFCO manual is updated annually and provides guidelines for feed ingredients (Spragg and Aird, 2003). As of 2006, the AAFCO manual referenced around 125 individual animal byproducts produced in the United States (Meeker and Hamilton, 2006).

Feathers that are not used for poultry meal can instead be used to produce feather meal. The primary market for unused feathers is in meal production, and feather meal must contain a minimum of 75% digestible crude protein for pepsin (Ockerman and Hansen, 1988). The undecomposed feathers are pressure-cooked to break down keratin protein bonds, resulting in a free-flowing and easily digestible feather meal suitable for all types of livestock (Meeker and Hamilton, 2006). Typically, the digestibility of finished feather meal exceeds the guidelines set by AAFCO, but the addition of urea can further increase digestibility in ruminant animals (Ockerman and Hansen, 1988). Feather meal is an excellent source of sulfur-containing amino acids, particularly cystine (Meeker and Hamilton, 2006), but is lacking in lysine, methionine,

histidine, and tryptophan (Ockerman and Hansen, 1988). In addition to feathers, clean blood from slaughtered animals can also be used to add to feather meal to produce feather meal with blood. To begin the process, blood is first treated by either mechanically removing a significant amount of moisture or by coagulating it with steam to create a semi-solid state. The semi-solid masses are then flash-dried to quickly eliminate any remaining moisture content and further provide lysine to feather meals.

Cookie meal is a common bakery waste product containing high amounts of carbohydrates (Lee et al., 2010). The utilization of bakery waste as animal feed is a common practice that helps to reduce the amount of waste that needs to be disposed of, thereby mitigating environmental problems (Westendorf et al., 1996). Bakery wastes refer to products that are derived from the recycling of the bakery and grocery store, including items like croissants, sweet breads, cakes, dough, tarts, and pies, whether they are raw or baked (Simpson, 2012). The primary component of bakery waste is typically unsold bread that is removed from store shelves after just 24 hours(Macgregor, 2000). However, it may also include other edible ingredients like dough, flour, sugar, icing, and even burnt or damaged products (Macgregor, 2000; Crawshaw, 2003). Bakery waste may also contain ground-up plastic bags, which are introduced during the mechanical unwrapping process (Macgregor, 2000).

Microbiological Safety of the Rendering Process

Apart from generating a profitable final product, the rendering industry also plays a significant role in eliminating pathogenic microorganisms from the food supply chain. Some studies have documented the successful elimination of pathogenic microorganisms in the incoming animal by-products through effective treatment (Troutt et al., 2001). The raw material that is received can serve as a reservoir for numerous pathogenic bacteria. Studies have shown

that cattle have a 23% contamination rate of *Escherichia. coli* O157:H7 (Smith et al., 2001), a 50% contamination rate of *Salmonella* (Troutt et al., 2001), and a 39% contamination rate of *Cryptosporidium parvum* (Huetink et al., 2001). Poultry has been reported with a contamination rate of up to 100% for *Salmonella* (Foegeding et al., 1994). Swine have been found to be contaminated with *Salmonella* at a rate of 46% and *Yersina enterocolitica* at a rate of 49% (Foegeding et al., 1994; Swanenburg et al., 2001).

Salmonella is a major type of microbial pathogen contamination found in finished feed, which can be eliminated during the rendering process. However, the rendering industry is facing challenges due to cross-contamination from incoming raw materials, resulting in *Salmonella* contamination in the finished feed given to livestock. This continuous contamination of *Salmonella* in animal feed poses a risk to the food chain, and several studies have linked cases of *Salmonella* infection in humans to contaminated animal feed. For instance, Boyer et al. (1958) found a correlation between the *Salmonella* serotypes present in humans and animals to feed ingredients and animal feed, while Watkins et al. (1959) detected 28 different serotypes of *Salmonella* in 18.5% of animal by-product samples. Pomeroy et al. (1961) built upon Watkins' research by collecting samples from 22 states throughout the US. They tested a total of 980 samples, and *Salmonella* was found in 170 of them, with 43 different serotypes being identified.

The severity of the *Salmonella* issue as a feedborne contaminant was highlighted by an outbreak of *Salmonella* Agona that occurred between 1969 and 1970 in the United States, United Kingdom, Netherlands, and Israel. The initial detection of *S.* Agona in fish meal in each country was followed by the pathogen being found in domestic animals and humans (Clark et al., 1973). By 1972, *S.* Agona was the 8th most commonly identified serotype in the US. Between March and May of that year, a *S.* Agona outbreak took place in Paragould, Arkansas, affecting

seventeen individuals. The source was traced back to a local restaurant that had received poultry products from a Mississippi farm which had used fish meal contaminated with *S. Agona* (Franco, 2006).

Between 1965 and 1970, the animal health division of the United States Department of Agriculture (USDA) and the FDA collaborated to test animal by-products and finished meals to determine baseline levels of *Salmonella* contamination. Commissioner Goddard, in response to some of the early results, decided to include food intended for animals in Section 201 (f) of the Federal Food, Drug and Cosmetic Act in 1967. This inclusion meant that Salmonella contamination in animal feed was now considered as an adulterant under the Act (Franco, 1999), but *Salmonella* remained a pathogen of interest to USDA(FSIS) and FDA and has been continuously monitored in the food supply since..

Although *Salmonella* can be detected in finished animal by-products, the amount of contamination decreases quickly after processing. Sutton et al. (1992) demonstrated that in a sample of meat and bone meal stored at 28.8°C, *Salmonella* levels decreased from 30 CFU/g to below the detection limit within two days of storage. Additionally, even when *Salmonella* is present, animal by-products are still less likely to be contaminated compared to meals made of vegetable proteins. Hamilton (2002) found that the incidence rate of *Salmonella* in animal by-product meals was higher in the Netherlands, Canada, and the U.S., while levels were higher in vegetable proteins in Germany and the UK. Although animal proteins had a higher range of incident rates, the risk factor associated with vegetable proteins was higher because they make up a larger percentage of the finished feed. The animal proteins had a risk factor of 0.9-1.68 for *Salmonella*, while vegetable proteins had a risk factor of 1.743-8.964 (Hamilton, 2002).

The FDA's inclusion of animal feed in the definition of food and the establishment of links between human disease and contaminated animal by-products led to the rendering industry placing a greater emphasis on the biological safety of their products. As a result, the Animal Protein Producers Industry (APPI) was founded in 1984 to regulate biosecurity within the industry, such as screening for *Salmonella* and implementing hazard analysis and critical control plans (HACCP) to ensure product safety. Additionally, the APPI provides continuous education to the industry on new issues that may affect their products (Franco, 2006).

Since the formation of APPI, significant improvements have been made to the rendering process, and studies have shown that finished meals are free of pathogenic bacteria such as *Salmonella* as they leave the cooker. Troutt et al. (2001) tested raw products used in production at 17 rendering facilities in the mid-western United States and found high levels of *Salmonella spp., Listeria monocytogenes, Campylobacter jejuni,* and *Clostridium perfringens* contamination. However, when the processed meals were tested, none of these pathogens were detected. The rendering process is also effective in eliminating viruses. The time and temperature used by the rendering industry are sufficient to kill viruses within the by-products. Pirtle (1997) demonstrated this by using pseudorabies virus (PRV), a common viral disease in pigs, as a model microorganism. Raw materials with varying degrees of viral contamination were processed, and the resulting meat and bone meal (MBM) was analyzed for the presence of the virus. The results showed that the virus was unable to survive the rendering process (Pirtle, 1997).

In 1986, two years after APPI was established, bovine spongiform encephalopathy (BSE) was confirmed in the United Kingdom, which was thought to have originated from sheep by-products (Kimberlin, 1990). The USDA conducted an 8-year study to determine whether the rendering process kills the prions responsible for BSE and whether they can be transmitted orally

to cattle via finished meat and bone meal (MBM). During the study, neonatal calves were fed raw brain or finished MBM from sheep infected with scrapie, and they were monitored for signs of disease, lesions, or prion protein deposits associated with scrapie or BSE (Cutlip et al., 2001). The results showed that the experimental calves fed the MBM diet did not show any clinical signs or develop lesions associated with BSE, and spinal cord and brain samples did not indicate the presence of any prions (Cutlip et al., 2001). However, a decade after the discovery of BSE, Creutzfeldt-Jakob disease, a human disease linked to the consumption of cattle suffering from BSE, was identified, leading to the FDA's decision to ban the use of feed produced from ruminants as a feed ingredient for ruminant animals (Franco, 2006).

Despite the risks associated with finished animal by-products, the rendering process is still considered the best means for disposing of raw by-products and mortalities due to several advantages, including infrastructure, volume reduction, controlled processing, established regulations, and timely processing (Hamilton et al., 2006). To support this claim, a study was conducted by the United Kingdom Department of Health (2001) to evaluate the risks involved with different methods of disposal for animal by-products. The study found that rendering, incineration, and funeral pyres all effectively eliminated biological hazards, but incineration and pyres created chemical hazards associated with burning. The rendering process was the only method of disposal that successfully eliminated both biological and chemical hazards, except for BSE, which was considered a negligible risk to humans if the solid material was incinerated. Overall, while there are some risks associated with finished animal by-products, the rendering process provides several advantages and is the most effective means of disposal for both biological and chemical hazards (Hamilton et al., 2006).

In addition to potential biological and chemical hazards, other methods of disposal for animal by-products are not able to handle the large volume of raw materials. For instance, when by-products are disposed of in landfills, sawdust is added to reduce moisture content. However, the addition of sawdust can increase the volume of material by up to 25%, resulting in the consumption of 25% of total landfill space in the US if all animal by-products and mortalities generated in one year were to be disposed of this way (Sparks Companies, 2001). Composting is another option, but it is limited to small-scale production due to the need for a specific balance of carbon and nitrogen ratio and moisture content in the raw by-products and mortalities (Hamilton et al., 2006). For example, the pork industry alone would require around one trillion cubic feet of space to compost the amount of material rendered each year. Incineration is effective at eliminating biological hazards but is also limited by the volume of raw materials generated each year. Large amounts of fossil fuel would be required, making it cost-prohibitive, and the resulting ash from the raw materials would also need to be disposed of. Overall, these alternative methods of disposal are not practical for large-scale animal by-products, making rendering the most viable and efficient option for their disposal (Hamilton et al., 2006).

Salmonella

Salmonella spp. are a type of bacteria that are shaped like rods, have a negative gram-staining characteristic, and are capable of surviving without oxygen. They belong to the Enterobacteriaceae family and are capable of movement. Typically, *Salmonella* can be identified by the production of gas and acid during glucose fermentation on a medium called triple sugar iron (TSI). These bacteria cannot utilize sucrose or lactose in differential media (D'Aoust and Purvis, 1998). However, Le Minor et al. (1974; 1973) have shown that plasmids can sometimes mediate sucrose and lactose fermentation in certain cases of *Salmonella*.

Throughout the 20th century, the nomenclature of *Salmonella* has been altered multiple times due to changes in biochemical and serological characteristics, as well as advancements in DNA homology. The discovery of somatic (O) and flagellar (H) antigens allowed for the division of the genus into various "groups" (LeMinor, 1979), as proposed by White in 1926. Kauffman built upon White's system of classification in 1941, resulting in the modern Kauffman-White classification system, which currently comprises more than 2,541 distinct serovars of Salmonella (Popoff et al., 2004).

Salmonella Significance as Human Pathogens in Animal Feed Components

According to estimates from the CDC, *Salmonella* cause approximately 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths each year in the United States (CDC, 2018). In a study conducted on breeder/multiplier and broiler production houses, 60% of meat and bone meal were found to contain *Salmonella*, and it was concluded that feed was the source of *Salmonella* due to the nature of the pathogen in poultry breeder/multiplier houses (Jones et al., 1991). It was observed that *Salmonella* contamination in U.S. broiler production remained relatively stable between 1969 and 1989 (Jones et al., 1991).

Salmonella contamination of rendered products is most likely to occur due to failures in sanitation during post-rendering handling. Additionally, recontamination with *Salmonella* may occur through aerosols that flow through processing areas (Magwood et al., 1965; Orthoefer et al., 1968; Davies et al., 1997). A study that involved taking samples from the raw materials area of a rendering processing plant using swabs found that the contamination rate with *Salmonella* was higher, up to 95%, compared to the finished product area, which had a contamination rate of 15.2%(Davies et al., 1997).

Three primary serovars of *Salmonella* that are commonly found in animal feeds are *S.* Senftenberg, *S.* Montevideo, and *S.* Cerro (Jay et al., 2008). Li et al. (2012) shared data gathered by the U.S. FDA's Feed Contaminants Program (2002-2009) and *Salmonella* Assignment (2007-2009) that tracks *Salmonella* contamination trends in animal feeds. During this period, 2,058 samples from animal feeds, feed ingredients, pet foods, pet treats, and supplements for pets were collected and tested for *Salmonella* presence and identification. Among these samples, 257 (12.5%) were found to be positive for *Salmonella*. Out of the 45 *Salmonella* serotypes detected, *S.* Senftenberg and *S.* Montevideo were the most commonly identified. These findings provide useful information for the animal feed industry to address *Salmonella* contamination issues. Several studies have investigated the levels of *Salmonella* contamination and the most commonly found serovars in animal feed and feed ingredient samples taken from animal feed facilities and rendering plants. Some of these studies have also examined surveillance data from previous years to identify trends in *Salmonella* contamination (Davies et al., 1997; Papadopoulou et al., 2009; Li et al., 2012b; Ge et al., 2013). According to Gong and Jiang (2017), the overall prevalence of *Salmonella* in animal feeds in the United States ranged from 12.5% to 22.9% at low contamination levels (<10 MPN/g). However, some feed ingredients such as animal bone meals and blood meals had higher contamination rates, up to 34.4%. Gong and Jiang (2017) discovered that *Salmonella* serovars Typhimurium, Infantis, and Senftenberg were present in both the raw materials receiving area and the finished meal loading-out area of a rendering processing environment. This suggests that cross-contamination may occur between these separate areas, and this is a critical control point that can be targeted to interrupt the transmission of pathogens in the feed supply.

Under section 402(a)(1) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 342(a)(1)), FDA considers non-direct human contact animal feeds or feed ingredients to be adulterated by *Salmonella* if they contain one or more of the following serotypes known to be pathogenic to the intended animal consumers: *S*. Pullorum, Gallinarum, or Enteritidis in poultry feed, *S*. Cholerasuis in swine feed, *S*. Abortuseque in horse feed, *S*. Abortusovis in sheep feed, and *S*. Newport and Dublin in dairy and beef feeds (FDA, 2010). However, any detection of *Salmonella* serotypes in pet food or pet treats classifies the product as adulterated, as these are considered direct-human-contact animal feed and are not subjected to a commercial heat treatment or other commercial processes that could eliminate the pathogen (FDA, 2010). The FDA FSMA has introduced new regulations on current Good Manufacturing Practices (cGMPs) that include preventive controls for *Salmonella* contamination in animal feed and pet food. These regulations aim to assist the rendering industry that produces and uses pet food, animal feed, and raw materials in preventing *Salmonella* contamination (FDA, 2013).

Salmonella contamination has been a global concern, not just limited to the United States. An Expert Meeting on Animal Feed Impact on Food Safety, jointly organized by the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO), concluded that ensuring safe feed is crucial for reducing and preventing microbiological hazards (FAO-WHO, 2008). Consequently, the European Food Safety Authority (EFSA) conducted a risk assessment on feedstuffs for food-producing animals and acknowledged the possibility of introducing *Salmonella* into the animal production system through feed consumption (Fink-Gremmels, 2012).

Pre-harvest Food Safety

Preharvest food safety research and activities have improved over time as more understanding has been gained about the pathogenesis, virulence, and transmission of foodborne pathogens and contaminants(Isaacson et al., 2004). This has led to the expansion of research and policies in recognition of the importance and complexity of the preharvest phase of food production(Blaha, 1999; World Health, 2004). Food safety continues to be a significant concern for public health and agriculture on a global scale. Since 1998, several funding programs and national initiatives have been established and put into action in the United States and other countries. The focus of these initiatives has varied over time, depending on factors such as the emergence of new pathogens or outbreaks, advancements in technology and methodology, and funding structures. There has even been debate over terminology, with different terms such as farm-to-fork, plate-to-table, and preharvest-to-postharvest being used. Recent advancements in methods and technologies have led to increased efforts in developing faster, more accurate, and more specific ways to detect or screen for foodborne pathogens during the preharvest phase of food production.

Furthermore, epidemiological research has gained significant momentum with the help of funded research programs, allowing for the conducting of longitudinal cohort studies, large case-control studies, and ecological studies (Torrence, 2003). The results of these studies continue to be published, providing crucial data for understanding preharvest food safety and implementing potential intervention, control, or prevention strategies (LeJeune et al., 2001; Berghaus et al., 2013). The USDA's Food Safety and Inspection Service (FSIS) has also begun to establish more guidelines at the preharvest level for enterohemorrhagic *Escherichia coli* and *Salmonella*. Internationally, the European Union has set performance standards for Salmonella in poultry at

the preharvest level. Additionally, the World Health Organization and Agriculture Organization (WHO/FAO) of the United Nations, as well as Codex Alimentarius (the international food rule-making body), have organized expert consultations, reports, and established standards programs for preharvest action on specific foodborne pathogens in food animals (WHO, 2004).

The primary objective of preharvest food safety is to develop interventions, controls, prevention, and mitigation strategies that can help reduce foodborne pathogens and contamination early in the food production chain (Torrence, 2018). By doing so, it is possible to minimize the risks to public health downstream by synergistically reducing pathogen burden entering the food chain, allowing processing plant level interventions to have increased efficacy. Over the past 15 years, preharvest research has focused on developing various strategies for mitigating, controlling, and preventing the major pathogens in food animals. These strategies are based on the understanding that the preharvest production system is complex and involves not only the food animal and its microbial ecology but also the animal's interaction with its environment and surrounding microbial ecology. The introduction of potential foodborne pathogens or contaminants to food animals, as well as the environment, is influenced by both production practices and the environment itself (including feed, water, soil, wildlife, and birds). The movement of these contaminants has the potential to spread from preharvest to postharvest and ultimately into food products. Unlike the processing environment, which is more contained, preharvest food production involves the surrounding environment and the movement of various factors such as people, animals, insects, birds, and equipment. This can aid in the transmission and movement of foodborne pathogens and contaminants. Therefore, it is important for researchers and decision-makers to continue to approach preharvest food safety systematically.

Spread of Pathogens from Feed to Animals

Foodborne pathogens such as *Campylobacter* species, non-Typhi serotypes of *Salmonella* enterica, Shiga toxin-producing strains of *Escherichia coli* (STEC), and *Listeria monocytogenes* are commonly found in food-producing animals like cattle, chickens, pigs, and turkeys (Heredia and García, 2018). These animals act as major reservoirs for these pathogens. These pathogens have zoonotic potential, meaning they can be transmitted from animals to humans, and can cause diseases or even death(Heredia and García, 2018). Given the seriousness of this situation, it is important to recognize the potential danger posed by these pathogens and take appropriate measures to prevent their spread.

There is considerable evidence that animal feed is frequently contaminated with foodborne bacterial pathogens. Studies by Hacking et al. (1978) and Loken et al. (1968) have reported that *Salmonella* is a common contaminant in rendered animal products, including meat (81%) and feather meal (40%). In addition, the FDA conducted two studies in 1993 and 1994 to test the presence of Salmonella in rendered products, and found positive rates of 56% and 25%, respectively (McChesney et al., 1995; Crump et al., 2002a). These findings highlight the potential for Salmonella contamination in rendered animal products, which can be a significant food safety concern if these products are used as animal feed or other products that may come into contact with human food.

Thermal Processing Principle

When food or rendering materials are exposed to high temperatures, the reduction of microbial populations typically occurs in a logarithmic manner over time at a constant elevated temperature. Various parameters are used to quantify the impact of high temperatures on microbial populations. Thermal death time (TDT) or F value is a measure of time, temperature,

material matrix, and organism, and it represents the time required to kill or reduce a given number of organisms at a specific temperature (Heldman and Hartel, 1997). TDT can be used to enhance product safety by reducing microbial populations, thereby decreasing spoilage microbes and increasing shelf-life(Teixeira, 2006; Jay et al., 2008). Decimal reduction time or D value represents the time required for a one $\log_{10}$ Colony Forming Unit (CFU)/g reduction of a particular organism at a specific temperature. A large D value at a given temperature indicates increased thermal resistance of a microbial population in a product. The 12-D concept is used as a lethality time in the canning industry and refers to the time required for destroying 12 $\log_{10}$ CFU/g of *Clostridium botulinum* spores (Teixeira, 2006; Jay et al., 2008). The thermal resistance constant or *z* value is the parameter that indicates the temperature increase required to cause a one $\log_{10}$ reduction, as shown by the slope on the thermal destruction curve. A large *z* value typically indicates the presence of heat-resistant vegetative cells or microbial spores in a microbial culture(Heldman and Hartel, 1997).

The food industry has conducted multiple research studies to explore the impact of various factors on the thermal resistance of pathogens, including cooking methods, food composition, packaging type, and product type. According to Jay (2008), *Salmonella* can be effectively destroyed at milk pasteurization temperatures. Ng et al. (1969) conducted a study on the heat resistance of *S.* Senftenberg 775W and found that this strain was more sensitive to heat during the log phase of growth than during the stationary phase. They also found that cells grown at 44°C were more heat resistant than those grown at either 15°C or 35°C. Although *S.* Senftenberg 775W is reportedly 30 times more heat resistant than *S.* Typhimurium, Goepfert (1968) discovered that the latter organism was more resistant to dry heat than the former when testing dry heat resistance in milk chocolate. Murphy et al. (2000, 2004) demonstrated that

various *Salmonella* serovars (*S*. Senftenberg, *S*. Typhimurium, *S*. Heidelberg, *S*. Mission, *S*. Montevideo, and *S*. California) and *L*. monocytogenes exhibited significantly different D- and *z*-values across different commercial products, such as chicken breast meat, chicken patties, chicken tenders, franks, beef patties, and blended beef and turkey patties. For *Salmonella*, kinetic rate constants (approximately 2.303/D) of 0.076 to 9.68 min^{-1} were obtained at a temperature range of 55 to 70°C.

Kinley et al. (2010) conducted a study to assess the level of bacterial contamination in rendered animal products and analyzed *Salmonella* from the samples. The study found that the total bacterial counts varied from 1.7 to 6.7 log_{10} CFU/g, with the highest counts detected in blood meal and the lowest counts in meat meal. The *Salmonella* isolates showed D-values of 9.27-9.99, 2.07-2.28, and 0.35-0.40 min at 55, 60, and 65°C, respectively. Rachon et al. (2016) investigated the suitable storage times of inoculated foods that could be utilized in heat resistance studies or process validations with similar cell viability and heat resistance characteristics. The study utilized the Weibull model and the first-order kinetic (D-value) methods to express inactivation data and calculate the heating time required to achieve 5.0 log_{10} CFU/g reductions at temperatures ranging from 70°C to 140°C. The study found that at higher temperatures (>100°C), the calculated heating times based on D-values to achieve 5.0-log_{10} CFU/g reductions were significantly lower than the times calculated using the Weibull model. This was due to the initial heat shoulder until microbial inactivation being observed to begin, which was not considered, and the product had not yet reached the target temperature. This suggests that the application of first-order kinetics is inadequate when the product temperature is increasing and when holding times at target temperatures cannot be reliably controlled, such as in food processes like extrusion and continuous heat treatments without moisture evaporation.

Channaiah et al. (2017) conducted a study on the thermal lethality of a mixture of three strains of *S.* enterica (*S.* Typhimurium, *S.* Newport, and *S.* Senftenberg 775W) in a commercial muffin baking process. They used an oven temperature of 190.6°C for 21 minutes and were able to achieve a reduction of ≥5.0 $\log_{10}$ CFU/g in *Salmonella* populations after 17 minutes of baking, and a reduction of 6.1 $\log_{10}$ CFU/g after 21 minutes of baking. The study also included a D-value experiment, which showed that the D-values of the *Salmonella* cocktail in muffin batter were 62.2 ± 3.0, 40.1 ± 0.9, and 16.5 ± 1.7 minutes at 55, 58, and 61°C, respectively, with a *z*-value of 10.4 ± 0.6°C. Although the study focused on the baking industry, it serves as an example of a validation standard for a baking or heat treatment process.

Jones-Ibarra et al. (2017) investigated the thermal inactivation of a mixture of *Salmonella* serovars in raw poultry offal at different temperatures and found that a 7-$\log_{10}$ CFU/g reduction in *Salmonella* could be achieved during the heating come-up period. Mean D-values for the *Salmonella* cocktail at 150, 155, and 160°F were 0.254±0.045, 0.172±0.012, and 0.086±0.004 min, respectively, and the z-value was 21.95±3.87°C. On the other hand, Hayes (2013) examined the thermal death of four pathogenic *Salmonella* strains in animal feed materials and found that further research was needed to ensure the complete destruction of *Salmonella* at 240°F (115.6°C) for longer time intervals. Some unidentified microorganisms were observed to reappear at later treatment times, indicating the need for further investigation to identify the impact of particles on thermal conductivity through the rendering matrices.

Conclusion

The Food Safety Modernization Act (FSMA) is a critical piece of legislation aimed to ensure the safety of the US food supply. The law is broad in scope and provides the FDA with enhanced powers to regulate all aspects of food production, including the rendering industry. The

act was signed into law in 2011, and since then, the FDA has been working in partnership with the Food Safety Inspection Service of the USDA to develop and implement regulations that promote food safety.

One of the key challenges facing the rendering industry is the risk of contamination of pet and animal feed products with pathogenic bacteria. Contaminated feed products can introduce harmful pathogens into the food chain, with potentially serious consequences for both animals and humans. To address this risk, the FDA has developed guidelines and regulations that require the validation of thermal lethality of rendering processes to ensure the destruction of bacterial pathogens in animal feed products.

The consequences of a disease outbreak in the animal livestock industry could be significant and profound. Such an outbreak could have a severe impact on the rendering industry, the entire food animal chain, consumers of animal products, pets and their owners, as well as consumer confidence in the safety of the food supply. Therefore, it is essential that the rendering industry takes steps to ensure the safety of its products and that the FDA continues to work to promote food safety through the implementation of effective regulations and guidelines.

The objective of the present study was to validate the thermal lethality against three serotypes of *Salmonella enterica* (Typhimurium, Enteritidis, and Newport) in feather meal with blood and cookie meal during the rendering process.

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CHAPTER 3

VALIDATION OF THERMAL DESTRUCTION OF *SALMONELLA* SPP. IN FEATHER MEAL WITH BLOOD[1]

[1] Huang, W., T.R. Callaway, and R. A. Fernandez. To be submitted *Journal of Animal Science*

Abstract

About one-third to one-half of each animal produced for meat, milk, and eggs is not consumed by humans. This unused animal tissue is often converted into animal co-products through the rendering process which creates valuable protein-rich products used primarily in animal feedstuffs. Unfortunately, many of the animal tissues that enter the rendering chain can contain foodborne pathogenic bacteria, such as *Salmonella*. To ensure that foodborne pathogens are successfully eliminated from the finished animal feed products, the rendering industry must have validation data on the thermal lethality of the rendering thermal process. The objective of this study was to validate the lethality levels of high heat processing to S. enterica in Feather meal with blood (FMB). *Salmonella* (*S*. Typhimurium [ST], *S*. Newport [SN], and *S*. Enteritidis [SE]) inoculated FMB were loaded into glass test tubes with cap and submerged into water bath tempered to 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, or 95°C for 0, 0.5, 1, 3, 5, and 10 minutes, respectively. *D*-values and *z*-values were generated from best-fit model parameters. The results indicate that a 7.0-$\log_{10}$ CFU/g inactivation of three *Salmonella* serovars may be obtained during the heating period and subsequent rendering of FMB at temperature not less than 60°C. D-values of other temperatures are calculated to help achieve 7.0- $\log_{10}$ CFU/g reduction. *D*-values of ST, SN and SE are ranged from 0.82 to 0.54min, 0.60 to 0.50min, and 0.74 to 0.50 min, respectively. Results from this study provide short- and long- term benefits and guidance to the rendering industry to the efficacy of foodborne pathogen reduction steps used during the rendering process, to produce safer feeds for use by pets and in food animal production.

Introduction

The processing of waste materials from harvesting animals used for food, such as livestock and poultry, provides raw materials for different products used in U.S. agriculture and

processing industries (Meeker and Hamilton, 2006). These products include components for livestock and pet food, as well as industrial products like cooking oils, tallows, and soaps(Meeker and Hamilton, 2006). Rendering is a process that enables the entire animal to be used, preventing excessive disposal of animal carcass tissues in landfills and improving sustainability of animal and food production (Meeker, 2009). However, despite the intensity of the rendering process in terms of heat treatment, there may still be microbiological risks associated with rendered products if the treatment does not reach a lethality threshold. Therefore, further research is necessary to develop and validate rendering procedures that can eliminate microbial pathogens from a wide variety of feedstuffs that are produced from the rendering industry.

Hofacre et al. (2001) found *Salmonella* serovars in bovine meat and bone meal and reported a 5% prevalence of *Salmonella* in blended meals. The *Salmonella* serovars were resistant to at least one therapeutic antimicrobial. Similarly, Kinley et al. (2010) discovered *Enterococcus* spp. in various rendered meals at approximately 81% prevalence and *Salmonella* in 8.7% of total sampled rendered material. Poultry meal samples had a higher prevalence of *Salmonella* at 13.7%, indicating a greater potential for cross-contamination in rendered materials sourced from poultry.

Microbial pathogens may be present in rendered animal products due to either the survival of pathogens during processing or post-processing cross-contamination (Kinley et al., 2010; Gong et al., 2014; Jiang, 2016). The rendering industry typically employs a high-heat continuous-type process, where high-moisture raw materials are heated indirectly to temperatures between 250 and 280°F (121.1 and 137.8°C) for periods ranging from 20 to 90 minutes (Jones-Ibarra et al., 2017). After thermal processing, a mechanical press separates excess

fat, producing nonfat solids and extracted fat fractions that can be processed further for animal consumption or use (Meeker and Hamilton, 2006). The increased number of recalls of raw, dry, and processed pet foods since August 2013, and the passing of the U.S. Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA), highlights the need for validated process control and food safety preventive controls to prevent cross-contamination of final products (FDA, 2023). Between 2013 and 2015, 30 recalls of various pet foods were conducted due to concerns over pathogen cross-contamination, which has been reported to contribute to human disease outbreaks following exposure to pet foods. Although the source of contaminating pathogens was not identified in all recalls, product cross-contamination was identified as a contributing factor (CDC, 2014, FDA, 2023).

Establishing thermal process lethality parameters is crucial in ensuring the safe completion of animal rendering. The decimal reduction time (D-value) refers to the constant time required to inactivate 90% of a microbe's population (1.0 log) at a specific temperature (Buchanan, 1993). On the other hand, the z-value indicates the temperature change needed to achieve a tenfold change in the *D*-value (Buchanan, 1993). According to Kinley et al. (2010), the *D*-values of various *Salmonella* serovars in buffered saline decreased as process temperature increased, with z-values approximating 7°C for *Salmonella*. However, the D-values in saline has limited value in predicting the resistance of *Salmonella* to heating in rendering processes. The *D*-values of multiple *Salmonella* serovars in a blend of poultry crax and fat ranged from 0.67 to 0.70 min at 115.6°C (240°F) (Hayes 2013). These results indicate that achieving minimum rendering temperatures can effectively inactivate even large populations of *Salmonella* present on surfaces of raw poultry offal (Hayes 2013).

Previous studies have not considered the impact of the cumulative lethality of rendering processes towards microbial pathogens like *Salmonella*. Therefore, further research is necessary to establish the minimal processing conditions required to achieve a cumulative process lethality of 7.0-log10-CFU/g in *Salmonella* during rendering. This lethality criterion is based on the performance standards set by the U.S. Department of Agriculture (USDA) for fully cooked poultry products. The objective of this study was to perform experiments on the thermal lethality of *Salmonella* enterica serovars (Typhimurium, Enteritidis, and Newport) associated with human or animal disease in feather meal with blood (FMB) at various processing temperatures. The collected data was then used to determine a process z-value to forecast the required lethality needed to attain the performance objective of inactivating *Salmonella* by 7.0 log10-CFU/g cycles.

Materials and Methods

Preparation of *Salmonella* serovars for experimental use

Four pathogenic *Salmonella* serotypes recognized by FDA as hazardous for animal feeds were obtained for this study. Isolates of *Salmonella* serovars Enteritidis were obtained from the culture collection of the Center for Food Safety (University of Georgia, Griffin), Serotypes Typhimurium and Newport were obtained from the gut microbiology laboratory culture collection in the Department of Animal & Dairy Science (University of Georgia, Athens) for use in *D*-value study completion.

All *Salmonella* cultures were stored in 9ml of tryptic soy broth (TSB; BD Difco™, Franklin Lakes, NJ) in glass test tubes and following overnight growth at 39°C. In addition, each culture was prepared on slants of brilliant green agar (BGA; BD Difco™, Franklin Lakes, NJ) from overnight cultures of isolates in TSB. Inoculated BGA broth cultures were incubated for 24

h at 39°C, removed from incubation, and placed at room temperature (25°C) until required for use. New broth cultures were used for each experimental replication, and cultures were not retained for more than 1 week. Cultures from slants were activated by aseptically collecting culture using sterile inoculating loops into TSB, followed by overnight (24 h) incubation at 39°C.

FMB preparation

Hydrolyzed feather meal with blood was obtained from commercial poultry by-products rendering facility from Darling Ingredients (Irving, TX). The obtained product was immediately returned under ambient temperature to the Department of Animal & Dairy Science on the University of Georgia campus and was stored under refrigeration (4°C) until ready for use.

Procedure for *Salmonella* inoculation into FMB

Salmonella cultures (10^8 CFU/mL) were prepared by first growing and reviving *Salmonella* Typhimurium (ST), *Salmonella* Newport (SN), and *Salmonella* Enteritidis (SE) separately at 39°C in 9 mL of TSB filled in 20ml glass test tubes for 18 to 24 h.

A preliminary study was conducted to test the optimal moisture condition of FMB and *Salmonella* culture mixtures mimicking the rendering environment. 1 to 5 ml of ST culture was added to 5g of FMB. 3ml of ST culture in 5g of FMB was chosen for the experiment.

Each *Salmonella* serotype culture, prepared as above, was inoculated into FMB at 3ml cocktail per 5 g sample in a plastic container. The samples were mixed with a sterile plastic rod to homogenize the inoculum throughout the sample and covered with sterile aluminum foil. 1 g sample was transferred to a 20 ml sterilized glass test tube with a plastic cap for thermal validation. Each experiment was conducted in triplicate (n=3)

Thermal Resistance Trials

The 20 ml glass test tubes with plastic caps were autoclaved before the experiment. FMB samples with *Salmonella* cocktail were aseptically transferred into these test tubes. Inoculated samples were placed into a water bath with a scientific thermometer set to 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, or 95°C (140°F, 149°F, 158°F, 167°F, 176°F, 185°F, 194°F, or 203°F). A timer was started immediately upon the sample being placed into the water bath. Samples were removed at 0, 0.5, 1, 3, 5, and 10 minutes of heating for each of the eight experimental temperatures. At each time point, samples were removed and placed immediately into an ice-cold water bath to chill samples and halt heating-induced *Salmonella* inactivation/killing. Samples were held in the cold water bath for at least 1 min, and no longer than 5 min, prior to being removed for enumeration of surviving *Salmonella*. 9 ml of 0.9% buffer saline was added to each tube to avoid *Salmonella* reduction. Samples were serially diluted in 0.9% buffer saline and transferred to brilliant green sulfa agar (BGA) Following the transfer, plates were aerobically incubated for 24 to 48 h at 39°C prior to colony enumeration. Colonies counted were red to pink-white, with red zones surrounding each colony. Plate counts were then log-transformed for statistical analyses and calculation of *D*- and *z*-values.

Salmonella D-value and process z-value determination

The D-values of *Salmonella* cocktails were determined for each process temperature (60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, and 95°C) across three replicates. Linear regression analysis was used to analyze the survivor curves, and the D-values at each temperature were calculated from the negative inverse of the slope of the best-fit line. The process z-value was determined by the log-transformed D-values for the *Salmonella* cocktail plotting against the processing temperature for each individual replication. The negative inverse of the slope of the best-fit line was used to calculate the replicate-specific z-value, which was determined for each

of the three replicates. The process z-value was then obtained by averaging the values of the three replicates.

Experimental design and statistically analysis

The thermal inactivation trials were designed and analyzed using factorial analysis, and each trial was performed in the same way three times (n=3). The mean bacterial counts of the *Salmonella* cocktail obtained from the culture controls and heat-treated samples were converted to log10 cfu/g values ± standard error using Microsoft Excel® (Microsoft® Redmond, WA 2010). The D-values obtained from these trials were averaged and analyzed statistically to determine any significant differences in the heat resistance of *Salmonella* at different heating temperatures. The data was analyzed using two-way analysis of variance (ANOVA) and significantly differing means were separated by use of Tukey's multiple comparisons test at $P < 0.05$ using R-studio (2009-2022 RStudio, PBC).

Results and Discussion

Thermal inactivation of *Salmonella* in FMB

The survival populations of ST, SN, and SE in FMB from 60°C to 95°C were shown in Figure 1. Following 10 minutes of exposure at 80°C to 95°C, each ST population was reduced to <1 $\log_{10}$-CFU/ml and 7- $\log_{10}$-CFU/g reduction was achieved in FMB. Following 10 minutes exposure of heat, an average 8.6 log CFU/mL of SN population was reduced to <2 log CFU/mL at 60°C to 70°C and <1 log CFU/mL at 75°C to 95°C. A 7- $\log_{10}$-CFU/g reduction of SE was achieved after 10 minutes of exposure at 65°C to 95°C. Following 10 minutes of exposure at 60°C, 6.7- $\log_{10}$-CFU/g reduction was observed. Data were analyzed by two-way analysis of

variance (ANOVA). The results showed that time ($p<0.05$), temperature ($p<0.05$), and time x temperature interaction ($p<0.05$) had main effects on ST, SN and SE inactivation.

All ST survivor curves except curve of 60°C demonstrated a shouldered inactivation curve: initially shallow, followed by a steeper rate of pathogen decline and a shallow "tailing" phase with little additional inactivation. Similarly, SN and SE numbers declined while heated and the rate of reduction accelerated after 0.5 min of exposure. A tailing effect was observed after 3 minutes for ST at 75°C to 95°C, after 3 minutes for SN at 70°C to 95°C, and after 3 minutes for SE at 70°C to 95°C. Thermal inactivation of *S. enterica* was previously studied in whole chicken blood(Wong de la Rosa et al., 2020). The survival curve of *S. enterica* possessed shoulder and/or tail components. Therefore, the observed values in the present study are in agreement within previous studies.

_D_-values and _z_-values for Salmonella Inactivation in FMB

Average *D*-value for the three *Salmonella* serotypes at 60°C to 95°C in FMB (Table 1) were 0.82 to 0.54 min, 0.60 to 0.50 min, and 0.74 to 0.50 min for ST, SN, and SE, respectively. Data were analyzed by ANOVA. The ANOVA output indicated temperature did not exert a significant effect on *D*-value ($p=0.07$) of ST. But temperature had a significant effect on *D*-value of SN ($p<0.05$) and SE ($p<0.05$). Jones-Ibarra (2017), whose study *D*-values in raw poultry offal for the *Salmonella* cocktail (*S.* Senftenberg, *S.* Enteritidis, and *S.* Gallinarum) at temperatures of 150, 155, or 160°F were 0.254±0.045, 0.172±0.012, and 0.086±0.004 min, respectively. As the temperature increases, *D*-value decreases, indicating a decreased thermal resistance of microorganisms. (60°C versus 95°C for ST, SN and SE). Overall, shorter *D*-value for the higher thermal treatment, 95°C, were expected and consistently observed during this study when compared to lower thermal treatment process at 60°C.

Following completion of *D*-value calculations from across replications, a *z*-value was the negative inverse of the slope of the best-fit line connecting the *D*-values from the eight process temperatures for each replicate. *Z*-values of three *Salmonella* serovars were shown in Figure 2. The *z*-value for ST, SN and SE were 128.21, 303.03, and 175.44°C, respectively. This unusually high *z*-value was observed for three *Salmonella* serotypes is likely related to the tailing effects seen with these data set (Figure 1).

Conclusion

The USDA Food Safety Inspection Service has previously established criteria for controlling Salmonella in fully cooked poultry products, which require a processing method that reduces the number of microorganisms by 7.0- $\log_{10}$-CFU/g. The objective of this study was to validate the thermal lethality against *Salmonella enterica* in FMB. From in vitro trials and survivor curves, three *Salmonella* serovars perform different resistance of heat. Following 10 minutes of exposure of heat at 60°C, 7- $\log_{10}$-CFU/g reduction was failed to achieve in all *Salmonella* population. *D*-values were calculated from survivor curves to help achieve the requirement of 7- $\log_{10}$-CFU/g reduction. In this study, *D*-values of three Salmonella serovars in FMB at 60°C to 95°C are shown in Table 1. Application of *D*-values to achieve a predicted 7- $\log_{10}$-CFU/g inactivation at 60°C utilized would produce minimum required holding times of 2.61, 2.73, and 2.87 min for ST, SN and SE, respectively. In practical measure, *z*-value is a measure of how susceptible a bacteria population is to changes in temperature. Therefore, it provided more information on heat resistance of bacteria to rendering industry. With the combination with the *D*-value obtained at 95°C in FMB and inactivation of three *Salmonella* to nondetectable numbers within 0.82 mins, indicates that rendering of FMB to 95°C should satisfy renderer's needs to produce safe poultry by-product meal for further processing.

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CHAPTER 4

VALIDATION OF THERMAL DESTRUCTION OF *SALMONELLA* SPP. IN COOKIE MEAL[2]

[2] Huang, W., T.R. Callaway, and R. A. Fernandez. To be submitted *Journal of Animal Science*

Abstract

Cookie meal (CM) is a collective term that refers to bakery wastes such as cookie dough, bread, chips, and cereals. CM is usually utilized as by-product feed that is included in pet food as well as livestock feed. During the collection and transport of CM, there is often opportunity for contamination of this product with foodborne pathogenic bacteria which could then be transmitted to animals and eventually to humans through pet contact or consumption of food animal derived protein. To ensure that foodborne pathogens, such as *Salmonella*, are eliminated from the finished feeds, the rendering industry must have validation data on the thermal lethality of the rendering thermal process. The cross contamination of bakery feed has significant adverse effects on both the rendering industry and the entire food animal chain, ultimately affecting consumers. The objective of this study is to validate the thermal lethality against S. enterica in Cookie meal (CM). Samples of *Salmonella* (*S.* Typhimurium [ST], *S.* Newport [SN], and *S.* Enteritidis [SE]) inoculated CM were loaded into glass test tubes with cap and submerged into water bath tempered to 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, or 95°C for 0, 0.5, 1, 3, 5, and 10 minutes, respectively. *D*-values and *z*-values were generated from best-fit model parameters. The results indicate that a 7.0- $\log_{10}$ CFU/g inactivation of only SE may be obtained during the heating come-up period and subsequent rendering of CM at 80°C to 95°C. A 7.0- $\log_{10}$ CFU/g reduction of ST and SN was failed to achieve following 10 minutes exposure at all treatment temperature. D-values of ST and SN are calculated to help achieve a predicted 7.0- $\log_{10}$ CFU/g reduction in CM. *D*- values of ST, SN and SE are ranged from 1.49 to 0.67min, 1.94 to 0.63min, and 2.22 to 0.54min, respectively. Results from this study provide short- and long-term benefits and guidance to the rendering industry in methods of reducing populations of

foodborne pathogens in cookie meal and other bakery waste products that are used as feeds for pets and food animals.

Introduction

Cookie meal (CM) is a term used for food leftovers like bread, pasta, cereals, snacks, biscuits, and chocolate bars (Simpson, 2012). Cookie meal, and other bakery wastes, are commonly utilized in the production of pet food and livestock feed, especially for monogastric animals, due to their high levels of energy from fats and sugars, as well as the cooked starch's high digestibility (Cornevin, 1892). These products must be rendered to obtain a consistent product, and the cooking process increases digestibility, and is also used as a thermal lethality step to reduce foodborne pathogenic bacterial contamination. The validation of rendering processes' thermal lethality is essential to the safety of the pet food and livestock industries and to regulatory authorities, to ensure the destruction of bacterial pathogens in products that people eat. The cross-contamination of bakery waste with foodborne pathogenic bacteria during the production chain could have severe and profoundly negative consequences for the rendering industry, the pet food industry, along with the entire food animal chain, including consumers.

Rendering facilities use thermal processing that typically lasts between 40 to 90 minutes at temperatures ranging from 240 to 290ºF (115.6 to 143.3ºC) to ensure the microbiological safety of their rendering products (Meeker and Hamilton, 2006). Inadequate processing conditions can potentially lead to the survival of microbes (Crump et al., 2002). The thermal death time (TDT) is a function of time, temperature, material matrix, and organism, while the decimal reduction time (D value) indicates the time required for a one $\log_{10}$-CFU/g reduction of a specific organism at a particular temperature (Heldman and Hartel, 1998). Although the TDT of *Salmonella* has been studied in food products (Murphy et al., 2000; D'Aoust, 2001; Murphy et

al., 2004; Bucher et al., 2007), few studies have been conducted on rendered CM, thus the lethality of these processes remains unknown. Therefore, the objective of this study is to validate the thermal lethality against *Salmonella* enterica serovars (Typhimurium, Enteritidis, and Newport) associated with human or animal disease in CM at various processing temperatures. The collected data was then used to determine a process z-value to forecast the required lethality needed to attain the performance objective of inactivating *Salmonella* by 7.0 $\log_{10}$-CFU/g.

Materials and Methods

Preparation of *Salmonella* serovars for experimental use

Four pathogenic *Salmonella* serotypes recognized by FDA as hazardous for animal feeds were obtained for this study. Isolates of *Salmonella* serovars Typhimurium and Newport were obtained from the microbiology laboratory (Department of Animal & Dairy Science, University of Georgia, Athens), and Enteritidis were donated from the microbiology laboratory (Department of microbiology, University of Georgia, Athens), for use in *D*-value study completion.
All *Salmonella* cultures were stored in 9ml of tryptic soy broth (TSB; BD Difco™, Franklin Lakes, NJ) in glass test tubes and following overnight growth at 39°C. In addition, each culture was prepared on slants of brilliant green agar (BGA; BD Difco™, Franklin Lakes, NJ) from overnight cultures of isolates in TSB. Inoculated BGA slants were incubated for 24 h at 39°C, removed from incubation, and placed at room temperature (25°C) until required for use. New fresh cultures were used for each experimental replication, cultures were routinely transferred by aseptically collecting culture using sterile inoculating loops into TSB, followed by overnight (24 h) incubation at 39°C.

CM preparation

CM was obtained from commercial bakery waste rendering facility from Darling Ingredients in Albertville. The obtained product was immediately returned under ambient temperature to the Department of Animal & Dairy Science on the University of Georgia campus and was stored under refrigeration (4°C) until ready for use.

Procedure for *Salmonella* inoculation into CM

Salmonella cultures (10^8 CFU/mL) were prepared by first reviving *Salmonella* Typhimurium (ST), *Salmonella* Newport (SN), and *Salmonella* Enteritidis (SE) separately at 39°C in 9 mL of TSB filled in 20ml glass test tubes for 18 to 24 h.

A preliminary study was conducted to test the optimal moisture condition of CM and *Salmonella* cocktail mixtures mimicking the rendering environment. 1 to 5 ml of ST cocktail was added to 5g of FMB. 4ml of ST cocktail in 5g of FMB was chosen for the experiment.

Each *Salmonella* cocktail, prepared as above, was inoculated into CM at 4ml cocktail per 5 g sample in a plastic container. The samples were mixed with a sterile plastic rod to homogenize the inoculum throughout the sample and covered with sterile aluminum foil. 1 g sample was transferred to a 20 ml sterilized glass test tube with a plastic cap for thermal validation. Each experiment was conducted in triplicate (n=3)

Thermal Resistance Trials

The 20 ml glass test tubes with plastic caps were autoclaved before the experiment. CM samples with *Salmonella* cocktail were aseptically transferred into these test tubes. Inoculated samples were placed into a water bath with a scientific thermometer set to 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, or 95°C (140°F, 149°F, 158°F, 167°F, 176°F, 185°F, 194°F, or 203°F). A timer was started immediately upon the sample being placed into the water bath. Samples were

removed at 0, 0.5, 1, 3, 5, and 10 minutes of heating for each of the eight experimental temperatures. At each time point, samples were removed and placed immediately into an ice-cold water bath to chill samples and halt heating-induced *Salmonella* inactivation. Samples were held in the cold-water bath for at least 1 min, and no longer than 5 min, prior to being removed for enumeration of surviving *Salmonella*. 9 ml of 0.9% buffer saline was added to each tube to avoid *Salmonella* reduction. Samples were then serially diluted in 0.9% buffer saline and transferred to brilliant green sulfa agar (BGA) Following the transfer, plates were aerobically incubated for 24 to 48 h at 39°C prior to colony enumeration. Colonies counted were red to pink-white, with red zones surrounding each colony. Plate counts were then log-transformed for statistical analyses and calculation of *D*- and *z*-values.

Salmonella D-value and process z-value determination

The D-values of *Salmonella* cocktails were determined for each process temperature (60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, and 95°C) across three replicates. Linear regression analysis was used to analyze the survivor curves, and the D-values at each temperature were calculated from the negative inverse of the slope of the best-fit line. The process z-value was determined by the log-transformed D-values for the *Salmonella* cocktail plotting against the processing temperature for each individual replication. The negative inverse of the slope of the best-fit line was used to calculate the replicate-specific z-value, which was determined for each of the three replicates. The process z-value was then obtained by averaging the values of the three replicates.

Experimental design and statistically analysis

The thermal inactivation trials were designed and analyzed using factorial analysis, and each trial was performed in triplicate (n=3). The mean bacterial counts of the *Salmonella* cocktail

obtained from the culture controls and heat-treated samples were converted to $\log_{10}$-CFU/g values ± standard error using Microsoft Excel® (Microsoft® , 2010). The D-values obtained from these trials were averaged and analyzed statistically to determine any significant differences in the heat resistance of *Salmonella* at different heating temperatures. The data was analyzed using two-way analysis of variance (ANOVA) and significantly differing means were separated by use of Tukey's multiple comparisons test at $P < 0.05$ in R-studio (2009-2022 RStudio, PBC).

Results and Discussion

Thermal inactivation of *Salmonella* in CM

The survival populations of ST, SN, and SE in CM from 60°C to 95°C were shown in Figure 3. Following 10 minutes of exposure at 60°C to 95°C, ST and SN population were not able to reduce to <1 log CFU/mL and failed to achieve 7-log reduction in CM. Average 6.0-log and 6.7-log reduction were achieved in ST and SN in CM, respectively. Following 10 minutes exposure of heat, 7-log of SE population was inactivated at 80°C, 90°C and 95°C. *D*-values in next section were then applied to achieve predicted 7-log-cycle inactivation. Data were analyzed by two-way analysis of variance (ANOVA). The results showed that time ($p<0.05$), temperature ($p<0.05$), and time x temperature interaction ($p<0.05$) had main effects on ST, SN and SE inactivation.

All ST survivor curves except curves of 60°C and 65°C demonstrated a shouldered inactivation curve: initially shallow, followed by a steeper rate of pathogen decline and a shallow "tailing" phase with little additional inactivation. Similarly, SN and SE numbers declined while heated and the rate of reduction accelerated after 1 min and 0.5 min of exposure, respectively. Tailing effect was observed after 3 minutes for ST at 75°C to 95°C, after 3 minutes for SN at 70°C to 95°C, and after 3 minutes for SE at 70°C to 95°C.

D-values and *z*-values for Salmonella Inactivation in CM

Average *D*-value for the three Salmonella serotypes at 60°C to 95°C in CM (Table 2) were 1.49 to 0.67 min, 1.94 to 0.63 min, and 2.22 to 0.54 min for ST, SN, and SE, respectively. Data were analyzed by ANOVA. The ANOVA output indicated temperature had a significant effect on *D*-value of ST ($p<0.05$), SN ($p<0.05$) and SE ($p<0.05$). As the temperature increases, *D*-value decreases, indicating a decreased thermal resistance of microorganisms. (60°C versus 95°C for ST, SN and SE). Overall, shorter *D*-value for the higher thermal treatment, 95°C, were expected and consistently observed during this study when compared to lower thermal treatment process at 60°C.

Following completion of *D*-value calculations from across replications, a *z*-value was the negative inverse of the slope of the best-fit line connecting the *D*-values from the eight process temperatures for each replicate. *z*-values of ST, SN, and SE were 45.66, 31.65 and 25.91°C, respectively (Figure 2).

Conclusion

The thermal lethality against *Salmonella enterica* in CM is largely unknown. Therefore, the objective of this study was to validate the thermal destruction of *Salmonella enterica* in CM. From in vitro trials and survivor curves, three *Salmonella* serovars perform different resistance of heat. Due to the ingredients of CM, the high absorption of water reaches a better growth condition for *Salmonella* and increases the difficulty of achieving 7- $\log_{10}$-CFU/g inactivation. Expected inactivation was not achieved under most temperatures for three *Salmonella* serotypes at the end of heat process in CM. *D*-values were calculated from survivor curves to help achieve the requirement of 7- $\log_{10}$-CFU/g reduction. In this study, *D*-values of three Salmonella serovars in FMB at 60°C to 95°C are shown in Table 1. Application of *D*-values to achieve a predicted 7-

$\log_{10}$-CFU/g inactivation at 60°C utilized would produce minimum required holding times of 1.75, 4.04, and 5.74 min for ST, SN and SE, respectively. In practical measure, *z*-value is a measure of how susceptible a bacteria population is to changes in temperature. Therefore, it provided more information on heat resistance of bacteria to rendering industry. With the combination with the *D*-value obtained at 95°C in CM and inactivation of three Salmonella to nondetectable numbers within 2.22 mins, indicates that rendering of FMB to 95°C should satisfy renderer's needs to produce safe cookie meal for further processing.

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CHAPTER 5

CONCLUSION

As an agenda for future work on the heat inactivation of the *Salmonella enterica* in rendered animal feedstuff, we now summarize the issues we left open in these two studies. The results have been shown that temperature versus time affects the bacteria growth. It would be interesting to develop a more comprehensive method to obtain the bacteria survivor. In this study, two feedstuffs were inoculated with *Salmonella enterica* culture which increase the water activity of samples. The bacterial reduction of *Salmonella enterica* in two feedstuffs might be affected by the higher water activity. Three parameters need to be specified for the detection and control: bacteria identification, samples' surface area and the water activity of the samples.

Finally, a complete investigation of the detection of *Salmonella enterica* and heat inactivation methods presented in this study requires a more thorough performance evaluation. First, the methods should be compared experimentally with those previously proposed in the literature. Second, the method should be tested for a large number and more diverse rendering conditions.

Table 1. *D*-values for S. Typhimurium (ST), S. Newport (SN), S. Enteritidis (SE) in Feather Meal with blood (FMB) at thermal processing temperatures of 60 to 95°C.

Temp (°C)	D-value (min)[a]		
	S. Typhimurium	*S.* Newport	*S.* Enteritidis
60	0.82 ± 0.083	0.60 ± 0.034^{A}	0.74 ± 0.019^{D}
65	0.71 ± 0.088	0.60 ± 0.016^{A}	0.64 ± 0.0077^{E}
70	0.67 ± 0.049	0.60 ± 0.026^{AB}	0.57 ± 0.022^{F}
75	0.67 ± 0.097	0.50 ± 0.020^{C}	0.52 ± 0.0015^{FG}
80	0.56 ± 0.014	0.50 ± 0.011^{C}	0.52 ± 0.0075^{FG}
85	0.55 ± 0.0054	0.52 ± 0.024^{ABC}	0.51 ± 0.0021^{FG}
90	0.54 ± 0.0093	0.51 ± 0.0043^{BC}	0.52 ± 0.0042^{FG}
95	0.54 ± 0.017	0.50 ± 0.0032^{C}	0.50 ± 0.0024^{G}

[a] Values are the means from three replications ± standard errors. Means not sharing a capitalized letter (A, B, C) and (D, E, F, G) differ by Tukey's multiple comparison test at p=0.05.

Table 2. *D*-values for *S*. Typhimurium (ST), *S*. Newport (SN), *S*. Enteritidis (SE) in Cookie meal (CM) at thermal processing temperatures of 60 to 95°C.

Temp (°C)	D-value (min)[a]		
	S. Typhimurium	*S*. Newport	*S*. Enteritidis
60	1.49 ± 0.12^{A}	1.94 ± 0.095^{D}	2.22 ± 0.25^{G}
65	1.12 ± 0.0071^{B}	1.46 ± 0.21^{E}	1.25 ± 0.10^{H}
70	0.99 ± 0.12^{B}	0.88 ± 0.037^{F}	0.85 ± 0.084^{HI}
75	0.92 ± 0.048^{B}	0.80 ± 0.047^{F}	0.73 ± 0.018^{I}
80	0.69 ± 0.015^{C}	0.76 ± 0.032^{F}	0.60 ± 0.030^{I}
85	0.69 ± 0.031^{C}	0.75 ± 0.031^{F}	0.60 ± 0.024^{I}
90	0.67 ± 0.020^{C}	0.72 ± 0.065^{F}	0.54 ± 0.0061^{I}
95	0.67 ± 0.048^{C}	0.63 ± 0.031^{F}	0.54 ± 0.0097^{I}

[a] Values are the means from three replications ± standard errors. Means not sharing a capitalized letter (A, B, C), (D, E, F), and (G, H, I) differ by Tukey's multiple comparison test at p=0.05.

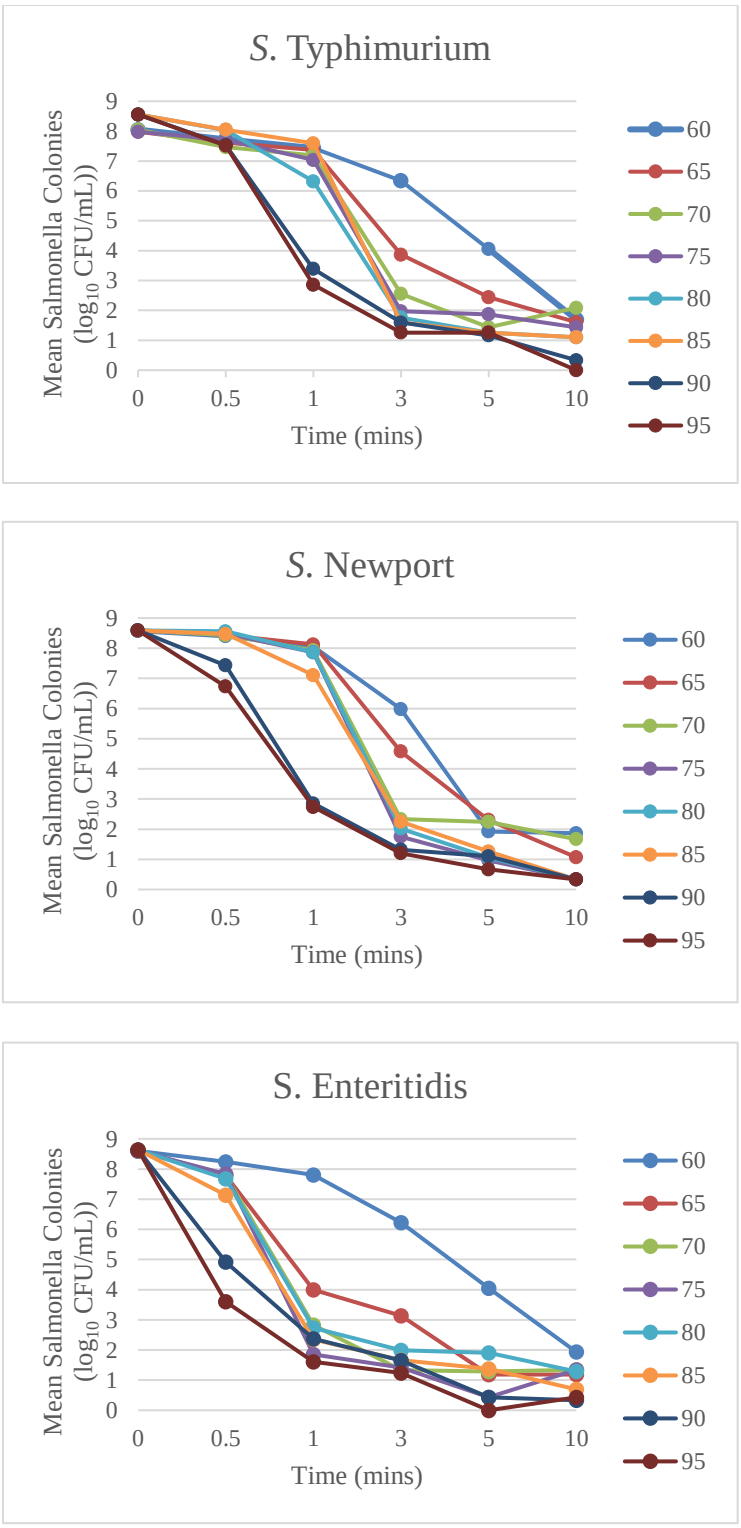
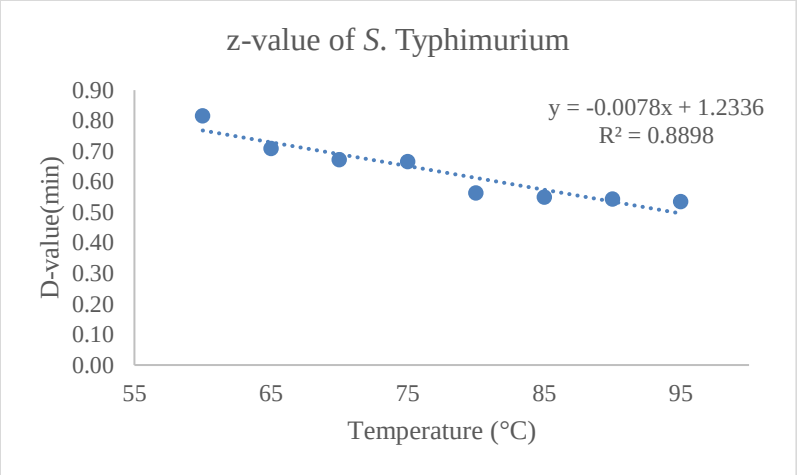
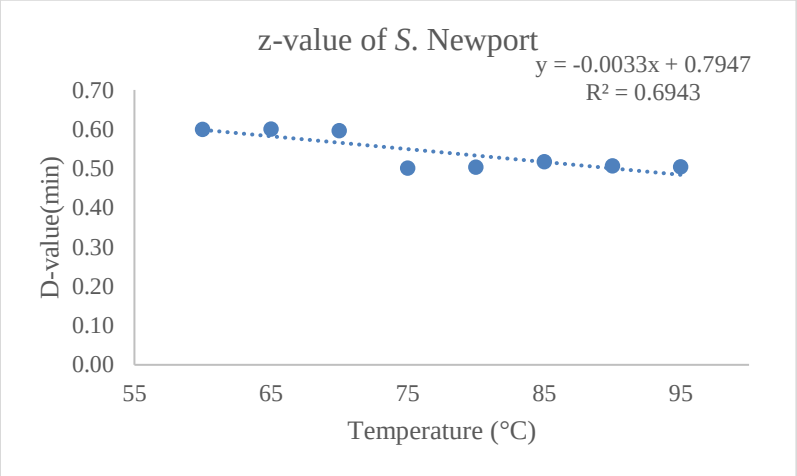


Figure 1. Thermal inactivation of *S.* Typhimurium (ST), *S.* Newport (SN), and *S.* Enteritidis (SE) during heating in Feather meal with blood (FMB) at heat processing of 60 to 95 °C.





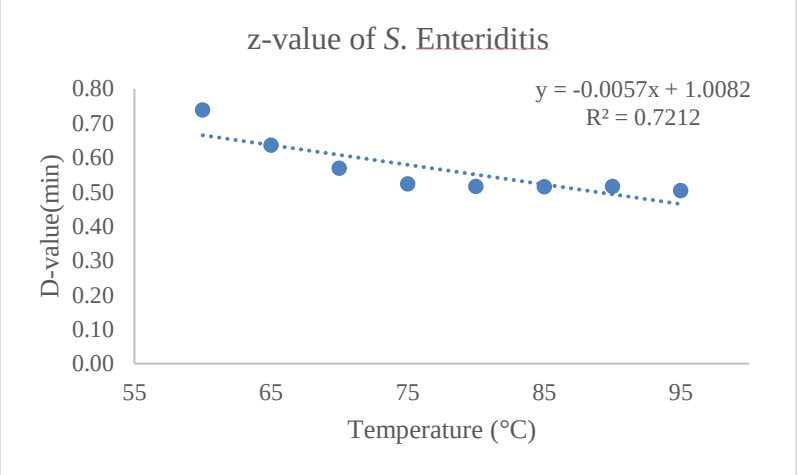
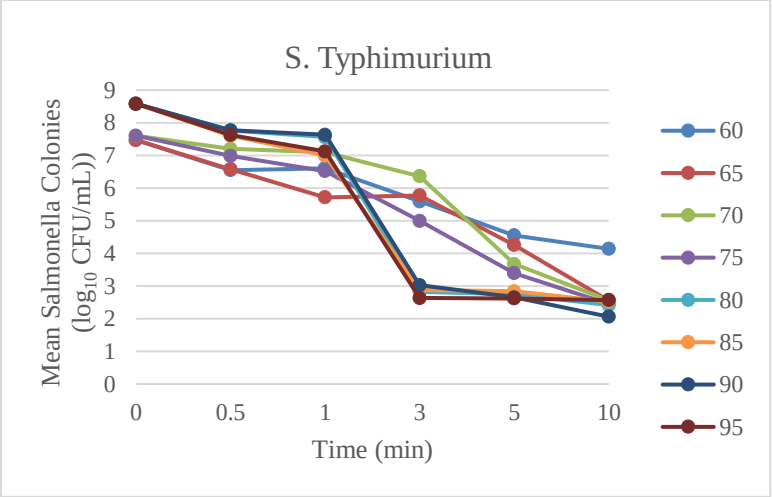
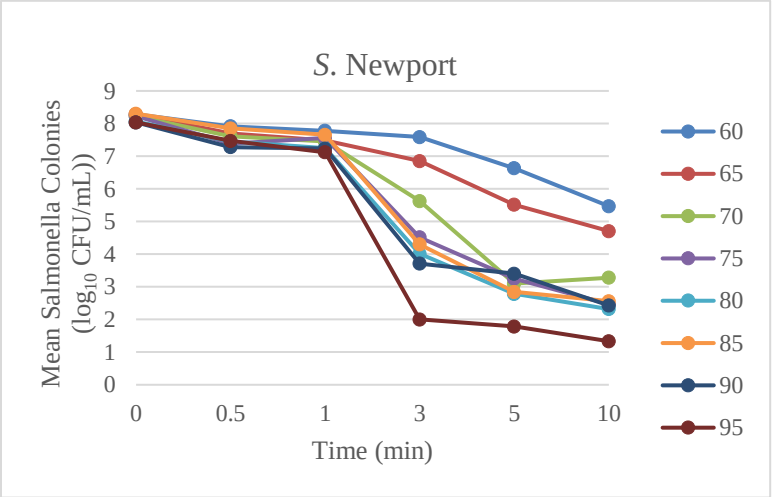


Figure 2. *z*- values of S. Typhimurium (ST), S. Newport (SN), and S. Enteritidis (SE) in Feather meal with blood (FMB) at 60 to 95 °C





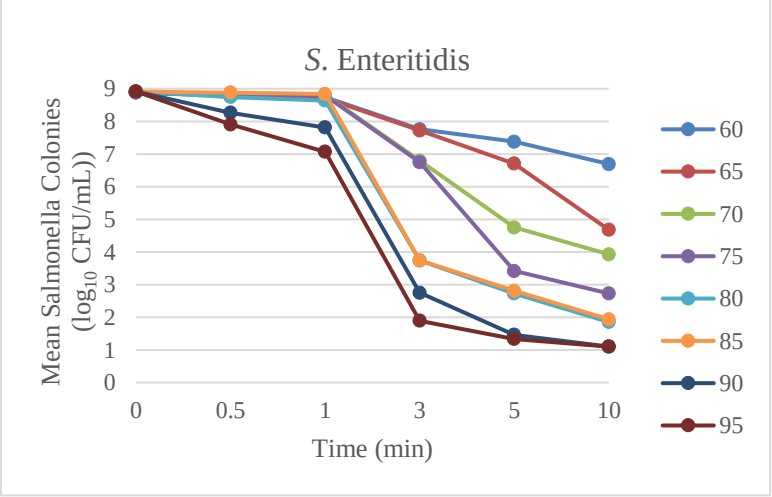


Figure 3. Thermal inactivation of *S.* Typhimurium (ST), *S.* Newport (SN), and *S.* Enteritidis (SE) during heating in Cookie meal (CM) at heat processing of 60 to 95 °C

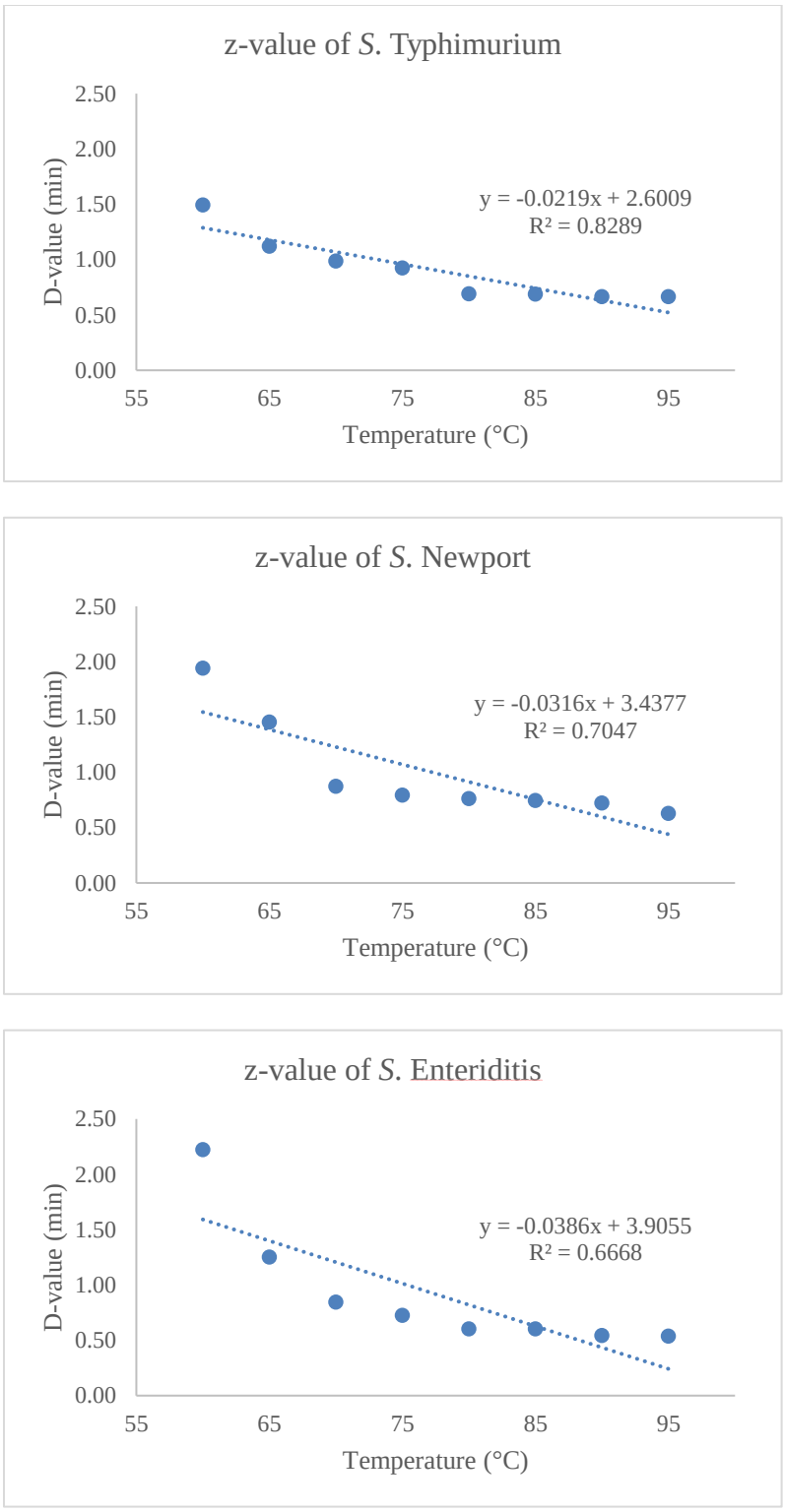


Figure 4. *z*- values of *S.* Typhimurium (ST), *S.* Newport (SN), and *S.* Enteritidis (SE) in Cookie meal (CM) at 60 to 95 °C