Predicting the Food Safety and Shelf-Life Implications of Less-Than-Truckload (LTL) Temperature Abuse (TA) on Boneless Skinless Chicken Breast Fillets

by

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Abstract

Maintaining food safety and preventing spoilage are paramount during food distribution. However, in the last segment of the cold chain (Last Mile), less-than-truckload (LTL) shipping practices can lead to cyclic temperature abuse (TA). Therefore, two experiments were conducted to develop predictive models of the effects of LTL TA on the safety and shelf-life of a model temperature sensitive food (boneless skinless chicken breast). For both experiments, simulated LTL TA conditions (cyclic 2 h at 4°C, then 2 h 25°C) were used. In experiment 1, inoculated (*Salmonella* Typhimurium) fillets were subjected to TA in a programmable incubator. Using temperature and microbial results, an acceptable tertiary model for the prediction of *Salmonella* growth was created. Experiment 2 was conducted using a commercial pallet of chicken breast fillets and a walk-in cooler. Using Monte Carlo methods, predictions were obtained for risk-of-loss and shelf-life. The research presented in the thesis is an amalgamation of two distinct fields of food microbiology and supply chain to create a broader impact on food safety and security.

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List of Abbreviations

APC Aerobic Plate Count

PSY Psychrotrophs

CFU Colony Forming Unit

BPW Buffered Peptone Water

AF Accuracy Factor

BF Bias Factor

TA Temperature Abuse

LTL Less-Than-Truckload

FTL Full-Truckload

FIFO First-In, First-Out

FEFO First-Expire, First-Out

CHAPTER I.

LITERATURE REVIEW

Introduction to *Salmonella*

Salmonella is the second leading cause of foodborne illness each year in the United States with the Center for Disease Control and Prevention (CDC, 2022a) estimating approximately 420 deaths and over 26,000 hospitalizations are the result of *Salmonella* infections. Also, *Salmonella* infections result in a large economic burden. The economic burden of the leading 15 foodborne pathogens is $17.5 billion with *Salmonella* contributing $4.1 billion (Hoffmann and Ahn, 2021). *Salmonella* cases in poultry alone may account for approximately $2.8 billion (Scharff, 2020). Because of its impact, it is of extreme importance to understand the characteristics and tendencies of *Salmonella* growth. *Salmonella* is a gram-negative, rod-shaped facultative anaerobe (Giannella, 1996). Being a facultative anaerobe, *Salmonella* can grow in environments with and without the presence of oxygen. Regarding the taxonomy of *Salmonella*, there are 2 species, with *Salmonella enterica* having 6 subspecies, and approximately 2600 serovars (Grimont and Weill, 2007). *Salmonella enterica* subspecies *enterica* serovars account for nearly all illnesses in both humans and animals (Jajere, 2019). The disease caused by these serovars in known as nontyphoidal salmonellosis which can be characterized by gastroenteritis and in more severe cases bacteremia (Shimoni et al., 1999). The infectious dose of *Salmonella* can vary depending on the serovar (Kothary and Babu, 2001; Hara-Kudo and Takatori, 2011). Also, individuals from different age groups and immunity levels may be susceptible to more serious cases of salmonellosis. (Shimoni et al., 1999). For example, stomach acid is one of the major barriers *Salmonella* must overcome to infect its host and elderly people have a greater chance of decreased acidity in their stomachs (Smith, 1998). Therefore, the elderly may have an increased risk for infection. To determine the connection with age and susceptibility to *Salmonella* Typhimurium, a study was conducted using inoculated mice (Ren et al., 2009). It was determined that old mice had higher bacterial loads in

the GI tract and liver, lost more weight, and had decreased production of neutrophils than younger mice.

Salmonella and Poultry

According to the Food and Drug Administration (FDA, 2020a), chickens naturally have *Salmonella* thriving in their guts and poultry products are a leading cause of salmonellosis. The carcasses may become contaminated throughout the chicken supply chain particularly during the transport and processing of the birds (Slader et al., 2002; Mainali et al., 2009). If fecal matter comes into contact, directly or indirectly, with the chicken carcasses it is possible for there to be a presence of *Salmonella* (Guerrero et al., 2020). The poultry industry has implemented many practices to mitigate the growth of *Salmonella* on poultry carcasses and reduce food safety risk (Kataria and Morey, 2020). However, *Salmonella* outbreaks continue to be a public health issue (CDC, 2022b). According to a report from the National Antimicrobial Resistance Monitoring System (FDA, 2020b), 4% of retail chicken is contaminated with *Salmonella* in the United States. A study done in Seattle, Washington by Mazengia et al. (2014) studied the prevalence of *Salmonella* on poultry in retail markets and found that 11% of samples were positive with organic production methods resulting in a higher recovery of *Salmonella*. Guran et al. (2017) found that skin-on chicken breasts from retail markets in Atlanta, Georgia may be as high as 44.7%. Internationally, *Salmonella* prevalence was found to be 52.2%, 26.7%, 31.5%, and 45.9% for China, Columbia, Russia, and Vietnam, respectively (Alali et al., 2012). Another study in China found an overall *Salmonella* prevalence of 52.2%, and additional work in Vietnam resulted in 45.9% of samples from markets testing positive for *Salmonella* (Yang et al., 2011; Ta et al., 2012). Lastly, research in Selangor, Malaysia found a prevalence of 20.08% in wet and hypermarkets (Thung et al., 2016). The serovars most responsible for *Salmonella* infection related to poultry can

vary based on region. Many studies have been conducted to determine which *Salmonella* isolates were present on poultry samples. Kumar et al. (2019) and others found the most common poultry related serovars to be *Salmonella* Typhimurium, *Salmonella* Gallinarum, and *Salmonella* Enteritidis in India with *Salmonella* Montevideo, *Salmonella* Newport, *Salmonella* Infantis, and *Salmonella* Pullorum also being detected. In Guatemala, *Salmonella* Paratyphi B, *Salmonella* Heidelberg, and *Salmonella* Derby were determined to be the most prevalent in retail markets (Jarquin, et al., 2015). Also, it has been shown that markets in Greece have higher occurrences of *Salmonella* Hadar, *S*. Enteritidis, and *Salmonella* Blockley (Zdragas et al., 2012). According to the CDC's National Center for Emerging and Zoonotic Infectious Diseases (2016) the 4 most common serovars from human sources are *S*. Enteritidis, *S*. Typhimurium, *S*. Newport, *Salmonella* Javiana.

Salmonella Growth and Temperature on Poultry Meat

Temperature remains a primary factor in preserving the safety and quality of food. Studying the connection between temperature and bacterial growth is not a new concept (Hampil, 1932). Understanding the optimal temperature range as well as the extremes under which a particular microorganism can grow is extremely important to be able to develop a plan to combat its growth at every step along the cold chain. Regarding *Salmonella*, Matches and Liston (1968) investigated the minimal growth temperatures for different serovars including *Salmonella* Typhimurium and *Salmonella* Heidelberg with results of 5.5-6.5°C. Also, it has been shown that *Salmonella* struggles to survive at temperatures above 50°C (Elliot and Heiniger, 1965). Because of its temperature requirements (>5°C and <50°C), *Salmonella* is classified as a mesophile (Wiegel, 1990). When bacteria are heated to greater than their tolerable temperature ranges, proteins denature, enzymes deactivate, and intracellular materials leak out of the cell (Russel, 2003). Also, low temperature can result in damage to cell membranes (El-Kest and Marth, 1992).

The USDA (2020) lists the temperature range of 4°C to 40°C as the "danger zone" because it is the range in which most bacteria that cause foodborne illness grow the fastest. There has been extensive research, on the connection between *Salmonella* growth on poultry products and temperature (Ackbar and Anal, 2015; Borges et al., 2018; Kiel et al., 2018; Biswas et al., 2019; Trinetta et al., 2019). A study in China collected whole chicken carcasses from large, small, and wet markets and found that frozen fillets had a significantly lower prevalence of *Salmonella* (45.7%) than those stored at ambient (56.0%) and chilled temperatures (52.4 %) (Yang et al., 2011). A study by Morey and Singh (2012) compared the growth of both *S.* Typhimurium and *S.* Heidelberg in sterile broth and chicken slurry. The growth mediums were held at 4°C, 7°C, or 10°C, and the results showed a significant difference in the growth of both strains at 4°C compared to other temperatures in both sterile broth and the chicken slurry. Also, *Salmonella* growth was greater in the sterile media because of the lack of competition provided by microflora (Morey and Singh, 2012). Pradhan et al. (2012) investigated the growth of *Salmonella* Typhimurium on chicken breasts at −20°C, −12°C, 0°C, 4°C, and 8°C up to 21 days. The results indicated that *Salmonella* growth on breasts kept at 8°C were significantly different than the other temperatures with a 1.2 log CFU/g increase after 1 week. Another study was conducted looking at the effect of storage temperature, time, and gas environment on the growth of a *Salmonella* and *Listeria* cocktail on cooked chicken patties (Murphy et al., 2001). The patties were inoculated with a *Salmonella* cocktail and processed using a convection oven at high and low humidity levels, and the patties were then stored at 4°C, 8°C, and 15°C under air, vacuum, and CO_2 conditions. The results indicated that lower temperatures, lower storage times, and vacuum packaging reduced growth. In a study done by T.P. Oscar (2009), *Salmonella* Typhimurium growth on chicken skin was found to be optimal at 40°C with a lag time of only 2.5 h and growth rate of 1.1 logs per h. Growth

occurred in the temperature range of 25 °C to 45°C with survival happening at 5°C and 50 °C. Another study was conducted to compare the lag times and specific growth rates of 11 different *Salmonella* serovars on sterile chicken breast burgers at 25°C (Oscar, 2000). The average lag time and specific growth rates were 2.8 h and 0.35 $\log_{10}$ per h, respectively with *Salmonella* Harrdt having statistically different lag time than *Salmonella* Brandenburg and *Salmonella* Agona. Next, *Salmonella* growth in the presence of native microflora was studied at isothermal temperatures ranging from 8°C to 33°C (Jia et al., 2020). Results indicated that *Salmonella* grew at all temperatures above 8°C. A study on ready to eat poultry meat sausages was conducted by inoculating the sausages with *S*. Enteritidis (Akbar and Anal., 2014). The sausages were stored at either 2-4°C or 6-8°C and kept for 35 days. The higher temperature scenario had a climax of approximately 6.5 log CFU/g after 7 days and then decreased back to initial levels after 35 days. In comparison, at lower temperatures the *Salmonella* concentration never rose above inoculation levels. Ferreira, and Lund (1987) studied the connection between pH and temperature on the growth of different *Salmonella* serovars. They found that higher incubation temperatures resulted in the ability to grow at lower pH values. Because of *Salmonella* growths connection to temperature, Akil et al. (2014) collected meteorological and infection data in 3 southeastern states to determine if climate change could influence the amount of salmonellosis cases. Their model concluded that a rise in environmental temperature will likely result in an increase in *Salmonella* infections with an increase of 1°F correlating with 4 additional positive cases.

While large amounts of scientific literature exist for *Salmonella* growth during various isothermal conditions, there is less literature on *Salmonella* growth on poultry during dynamic temperature conditions. An experiment was completed where chicken was inoculated to a concentration of 10^2 CFU/mL and moved between incubators set at 10°C and 30°C (Bovill et al.,

2000). *Salmonella* concentrations reached 9 logs CFU/mL at isothermal 30°C after 30 h. However, 4 fluctuations of 10°C to 30°C resulted in 7-8 logs CFU/mL after 150 h. Bovill et al. (2001) studied the effect of rapid temperature changes on the survival and growth of *S*. Typhimurium. This was accomplished by transferring 200 mL of culture between water baths set at 1°C and 15°C in 3 different time intervals. The scenarios were: 1) Every 6 h, conicals kept at 1°C were moved to 15°C for 20 min, 2) Alternating 2 h at 15°C and 1°C, and 3) Dropping from 15°C to 1°C for 6 h then increasing temperature again to 15°C. Results showed little effect of temperature changes on the growth or survival of *Salmonella*. Another experiment on *Salmonella* growth during fluctuating temperatures was completed in cow manure (Semenov et al., 2007). This study incorporated 4 temperatures (7°C, 16°C, 23°C, and 33°C) and 4 temperature fluctuation ranges (0, ±4°C, and ±7°C), and results indicate the greatest increase of *Salmonella* concentration at 37±7°C. Next, a study was done to investigate the effects of breaks in the cold chain on *S*. Typhimurium growth on goat cheese (Tamagnini et al., 2008). Three treatments were created: 1) A control kept at 5°C for up to 40 days, 2) 8 h at 25°C then 5°C until the experiment finished, 3) 24 h at 25°C then 5°C until the experiment finished. The cheese was inoculated to an initial concentration of approximately 5 log CFU/g. *Salmonella* in the third treatment increased the most dramatically with a maximum increase of 2.4 logs, while treatments 1 and 2 decreased to approximately 4 logs CFU/g.

Temperature and *Salmonella* Biofilms

Salmonella is known to form biofilms and studies have been performed to discover the connection between temperature and biofilm formation (Steenackers et al., 2012). A study on the effects of environmental conditions on *S*. Enteritidis biofilm formation on stainless steel was performed and found 20°C to be the ideal temperature for biofilm development (Giaouris et al., 2005). In addition, after 7 days at 20°C, biofilm formation was no longer connected to the pH

values tested. As similar study was completed on *S*. Typhimurium on stainless still and acrylic at 28°C, 37°C, and 42°C (Nguyen et al., 2014). Results indicated a correlation between higher pH and temperatures and faster biofilm formation. Furthermore, Obe et al. (2022) showed differences in the strength of biofilm formation on plastic and stainless steel at different temperatures with the highest formation rate (40%) being at 25°C on plastic. Additionally, *S*. Kentucky biofilm formation at 5 different temperatures (4°C, 10°C, 25°C, 37°C, and 42°C), 3 surfaces (plastic, rubber, and chicken skin), and 5 pH values has been studied with optimal biofilm conditions found to be at a pH of 7.0 and a temperature of 37°C (Roy et al., 2021). Next, De Oliveira et al. (2014) studied *Salmonella* biofilm formation on 3 different surfaces at 4 different temperatures (16°, 20°, 28°, and 35°C). They found the rdar morphotype at 28°C to be >50% and <5% at 35°C. Also, they found stainless steel to be the easiest material for biofilm formation with growth at the lowest temperature studied. Lastly, a study demonstrated biofilm formation of different *Salmonella* serovars and strains is not uniformly affected by temperature (Borges et al., 2018). Results showed a biofilm formation rate of 71.6%, 63%, 52.3%, and 39.5% at 37°C, 28°C, 12°C, and 3°C, respectively.

Antimicrobial Resistance of *Salmonella*

According to the CDC (2019a), the development of antibiotic resistant strains of *Salmonella* is increasing and can result in more serious illness. These strains are the result of the us of antimicrobials in the animals, and if a person becomes seriously ill the resistance to antibiotics of the bacteria can make it more difficult to treat them (CDC, 2019b). This is a principal reason as to why proper temperature management is so important. Because the continual use of antibiotics in poultry is creating antibiotic resistant strains, understanding proper cold chain management will likely continue to be paramount to prevent outbreaks in the future. A study by

Arslan and Eyi (2010) looked at the antimicrobial resistance of *Salmonella* recovered from retail poultry meat and beef. The results showed a prevalence of 29% in poultry meat with *S.* Typhimurium being the most common serovar. Ampicillin and cephazolin were the 2 most resisted antibiotics, and it was found that 62% of the *Salmonella* isolates recovered were resistant to more than 3 drugs. Another study in Pakistan investigated the prevalence of antibiotic resistant strains of *Salmonella* in 100 retail broiler meat samples (Soomro et al., 2010). From the 38 positive samples, all were resistant to ampicillin, and most were resistant to streptomycin, tetracycline, and nalidixic acid. In China, it was found that 37.5% of poultry meat samples tested were positive for *Salmonella* with *S.* Enteritidis, *Salmonella* Indiana, and *S.* Typhimurium being the most common (Yang et al., 2020). The antibiotic resistance to a minimum of 3 of the used antimicrobials was determined to be 60.1%, and the highest resistance rates were 72.3 % for nalidixic acid, 55.3% for ampicillin, and 48.7% for streptomycin. Abd-Elghany et al. (2015) did a similar experiment in Egypt and found an overall positive for *Salmonella* rate on chicken samples to be 34% (2015). *S.* Typhimurium, *S.* Enteritidis, and *Salmonella* Kentucky were the 3 most common serovars. Regarding the isolates, all were resistant to erythromycin, penicillin, and amoxycillin, and greater than 90% were resistant to nalidixic acid and ampicillin. Another study looked at the pervasiveness and resistance to antibiotics from processed poultry carcasses (Parveen et al., 2007). In this experiment, carcasses were collected prechill and postchill. The results showed a *Salmonella* prevalence of 88.4% on prechilled carcasses and 84.1% on post chill carcasses. *S.* Kentucky and *S.* Typhimurium were most prevalent and greater than half of the isolates were resistant to at least 3 antimicrobial drugs. The drugs the isolates were most resistant to (>50%) were tetracycline, ampicillin, amoxicillin-clavulanic acid, and ceftiofur.

Food Waste

Both food waste and food loss are massive problems globally. According to the United Nations (UN, 2022), 14% of food is lost and 17% of food is wasted globally. The Food and Agriculture Organization of the United Nations (FAO, 2022a) defines food loss as a decrease in the food supply in the supply chain before retail stores, households, or food service establishments, while food waste occurs from the actions of retail stores, households, and food service establishments. In the U.S. alone it is estimated 30-40% of the food supply is lost or wasted (USDA, 2022). The loss or waste of food can have negative impacts on hunger, the economy, and the environment. The FAO (2021) reports that 768 million people could have struggled with hunger in 2020 with nearly 400 and 300 million coming from Asia and Africa, respectively. In the U.S. over 10% off the population may be experiencing some level of food insecurity (USDA, 2021). Globally, food waste costs $2.6 trillion each year (FAO, 2022b). The United States Environmental Protection Agency (2021) states the consequence for food loss and food waste in the U.S. amounts to nearly $220 billion annually. Next, the reduction of food loss and food waste can improve resource efficiency and mitigate the pressure on natural resources (Cattaneo et al., 2021). Read et al. (2010) investigated which points along the supply chain would have the greatest positive impact on the environment. They found that food service, processing, and households have the greatest positive impact on energy use, land use, and water use, respectively. At the retail level, it has been shown that the causes of food waste vary but are likely due to erratic demand and replenishment policies (Teller et al., 2018). In addition, up to 35% of food wasted at retail stores may still be fit for human consumption (Cicatiello et al., 2017). Because of both the food safety and food waste risk associated with improper management at the retail level of the food chain, innovative methods are needed to effectively combat the microorganisms responsible.

Management decisions may be a foundational change that a company can make to improve the food safety and reduce the waste of their operations.

Spoilage Background

Spoilage is a major factor causing food waste in the world today. There are 3 main types of food spoilage: 1) Microbial, 2) Chemical, and 3) Physical (Petruzzi et al., 2017). Chemical spoilage is caused by chemical reactions in or on the food product such as oxidation, while physical spoilage is usually characterized by water entering or leaving a food product such as freezer burn (Blackburn, 2006). However, for the purposes of this review the focus will be on microbial spoilage. Some of the most common microorganisms responsible for the spoilage of poultry meat are: *Carnobacterium* sp., *Psuedomonas* sp., *Yersinia* sp., *Serratia* sp., *Enterobacteriaceae*, *Brochothrix thermosphacta*, and lactic acid bacteria (Höll et al., 2016; Rouger et al., 2017). The composition of bacteria present on meat samples can be affected by temperature and packaging conditions as certain conditions can inhibit or promote growth (Cerveny et al., 2009). All poultry meat naturally has a "background microflora" and will eventually spoil due to the metabolites produced from the metabolic byproducts (Rouger et al., 2017). Microorganisms are placed in different groups based on the temperatures in which they grow (Fung, 2009). Mesophilic bacteria grow best between 15-45°C and have an optimum growth temperature of approximately 37°C (Fung, 2009). Aerobic plate counts (APC) are often used to measure the growth of mesophilic bacteria (Mendonca et al., 2020). In contrast psychrotrophs (PSY) have an optimum growth temperature of approximately 21°C but can still grow at refrigeration temperatures (Fung, 2009). Research has been completed that demonstrates the viability of the use of PSY rather than APC for shelf-life (Pothakos et al., 2012).

Spoilage During the Last Mile

One challenge in controlling spoilage is ensuring that temperature sensitive foods never experience temperature abuse (TA). While maintaining refrigeration temperatures (≤4°C) does not outright prevent spoilage (PSY), it does decrease the rate of spoilage and prolong shelf-life (USDA, 2015). Therefore, preventing TA during the cold chain is essential in preventing a reduction of shelf-life (Global Cold Chain Alliance, 2020). These "breaks" in the cold chain can happen at different segments (Freiboth et al., 2013; Goedhals-Gerber and Khumalo, 2020). This is particularly true for the last segment of the supply chain known as the "last mile" (Balcik et al., 2008; Shu et al., 2015). Two of the primary methods used for shipping freight are "less-than-truckload" (LTL) and "full-truckload" (FTL) (Vega et al., 2021). LTL shipping is used when there is not enough product to fill an entire trailer, and the shipper wishes only to rent the space that their product occupies (FedEx.com, 2022). Therefore, when using LTL, there may be different products from multiple shippers present on the trailer. In contrast, FTL is used when a single company has enough product to fill an entire trailer (Jothi Basu et al., 2015).When products are shipped using LTL methods, multiple stops may be required for unloading (Montecinos et al., 2021). Temperature heterogeneity in refrigerated trucks has been observed with the air around the doors being the highest risk for TA (Moureh and Flick, 2004; Jedermann et al., 2009). In addition, the opening and closing of doors on refer trucks has presented issues with maintaining safe temperatures (Taher et al., 2021). This problem can cause complication later in the food chain when decisions regarding product rotation are made at the retail level (Kozak et al., 2014). The "first-in, first-out" (FIFO) and "first-expire, first-out" methods are two of the most common strategies used (Mendes et al., 2020) In the FIFO model, product rotation is based on the arrival date of a product and assumes all products that arrive at the same time will have the same remaining

shelf-life (Hertog et al., 2014). In contrast, the FEFO model considers the remaining shelf-life of each individual product (Hertog et al., 2014). The FEFO policy has been shown to be better for the environment and reduce waste in comparison to the FIFO policy (La Scalia et al., 2019). However, implementation requires greater communication throughout the supply chain so that managers can have a greater understanding of the remaining shelf-life (Hertog et al., 2014).

Popularity of Poultry Meat

The statistics provided by The National Chicken Council (NCC, 2022a) report that over the last 60 years per capita consumption of chicken has increased by nearly 70 pounds while red meat has decreased from 138 pounds to 111 pounds. In addition, poultry meat has had the largest difference in consumer availability over the last 50 years with per capita availability increasing by 30 pounds in the United States (Bentley, 2019). The shift in consumers choice from red meat to poultry is likely in part due to the reduced price of poultry meat. As of 2021, the wholesale prices (cents per lb.) of beef and chicken are 370.9 and 85.4, respectively (NCC, 2022b). Also, 724.9 and 209.4 are the retail prices of beef and chicken, respectively (NCC, 2022b). Next, poultry meat is proven to be healthier than red meat with 100 g portions of chicken breast and t-bone steak having approximately 32 g and 27 g of protein and 1 g and 4.7 g of saturated fat, respectively (USDA, 2019a; USDA, 2019b). These differences make chicken the more appealing option to lower income consumers looking for a source of healthy protein in their diets. The production of poultry has also increased largely to meet the increase in demand. In 1950, there as nearly 1400 million pounds of broilers produced in the U.S., while in 2022 it is estimated that over 44,700 million pounds of broilers will be produced (NCC, 2022c). Lastly, the market segments for poultry meat have shifted to almost 50% retail 50% foodservice over the last 50 years (NCC, 2022d). The increase in both consumption and production of poultry meat indicates the strict attention that

needs to continue to be given to the food safety and spoilage challenges that plague the poultry industry.

Mathematical Modeling of Bacterial Growth

Mathematical models for the prediction of bacterial growth are helpful tools to maintain food safety. Mathematical modeling consists of primary, secondary, and tertiary models (Whitling, 1995). Primary models describe changes in bacterial concentrations, in specific conditions, over time (Fakruddin et al., 2011). Secondary models describe parameter from primary models (such as growth rate and lag time) at different environmental conditions (Fakruddin et al., 2011). Lastly, tertiary models are a combination of primary and secondary models in a user interface (Stavropoulou and Bezirtzoglou, 2019). Having a method for the reliable prediction of bacterial growth under different environmental conditions is extremely important because it allows users to investigate many scenarios and their impact on food safety (McMeekin et al., 2008). Popular examples of primary models are the Gompertz and Baranyi models (Baranyi and Roberts, 1994). However, the Gompertz model is without biological basis, and the Baranyi model was developed so that it would not share the shortcomings of the older Gompertz model (Baranyi et al., 1993). Also, research has shown that the Baranyi model outperforms the modified Gompertz model (Juneja et al., 2007). Regarding secondary modeling, the Arrhenius relationship and the Ratkowsky square root model are popular models (Ratkowsky et al., 1982). Ratkowsky et al., (1983) later expanded the model to include the entire biokinetic temperature range. Models have been developed to predict both pathogen and spoilage microorganism growth and have wide applicability in keeping food safe and reducing food waste (Bovill et al., 2000; Juneja et al., 2007; Racioppo et al., 2022). Additionally, the Huang model has proven its validity when has been compared with Ratkowsky model (Huang et al., 2011). To test a model's validity, accuracy factors

(AF) factors and bias factors (BF) can be considered (Ross, 1996; Oscar, 2005) The AF shows the overall prediction error with a value of 1 being perfect, and the BF indicates whether a model is overpredicting (>1) or underpredicting (<1) (Ross, 1996). Ross et al. (2000) states that acceptable value for accuracy factors increases by up to 0.15 for each additional variable (pH, temperature, etc.) included in the model. Additionally, Ross et al. (2000) defined the acceptable range for BF to be approximately 0.7-1.15. Modelling the growth of *Salmonella* has been performed in a number of ways previously. Bovill et al. (2000) developed a model for the growth of *Salmonella* and another pathogen in three animal products. Milkievicz et al. (2020) created a model for the growth of *Salmonella* on chicken meat using the Huang primary model and the Ratkowsky and Huang square root secondary models. A tertiary model for the growth of *S*. Typhimurium on chicken skin has been developed (Oscar, 2009). Additionally, Kim et al. (2018) designed a growth model for *Salmonella* on eggs, and Velugoti et al. (2011) designed a *Salmonella* growth model for pork. In many cases, the models in the literature have acceptable BF and AF. However, many models are limited as they can only reliably predict growth under the specific conditions in which they were designed. For example, models have been developed that consider dynamic or isothermal temperatures, sterile growth medium or nonsterile, and individual strains or bacterial cocktails (Zwietering et al., 1994; Oscar, 1999; Oscar, 2009; Fang et al., 2015). There is a need for a model developed specifically for use in the supply chain to address both for food safety and spoilage concerns.

Introduction to Monte Carlo Simulations

A potential tool for risk analysis and predictions are simulation models. There are many simulation models used in agriculture today. One popular example is the Monte Carlo method. According to Johansen (2010), the method was most likely named in honor of the casinos in Monte

Carlo by physicists in the 1940's and involves the use of random numbers to replace physical experiments. Through its use, it is possible for the user to have an estimate of the sampling distribution from the generation of a pseudo-population that is similar to the actual population (Mooney, 1997). A benefit of simulation models is they allow the user to observe potential outcomes of different inputs to answer "what if?" questions (Bonate, 2012). The uses for Monte Carlo methods are diverse, and its implementation has spanned across several fields (Raychaudhuri, 2008). Andreo (1991) states that Monte Carlo methods have been studied in a variety of areas under the umbrella of medial radiation physics. Boda (2014) showed how Monte Carlo simulations can be used in electrolyte solutions. Markov chain Monte Carlo has been used in the field of cognitive psychology (Sanborn et al., 2010). In business, Alrabadi and Aljarayesh (2015) found Monte Carlo simulations to be an accurate method in forecasting stock market returns. Lastly, Monte Carlo has been used in a wide variety of agricultural applications such as modeling light transport in food products (Hu et al., 2020).

Monte Carlo in Agriculture

Monte Carlo methods have been used in a variety of applications within the agricultural industry. There is no singular "Monte Carlo Model" as there are many different versions of the method, and it is often customized on a case-by-case basis (Harrison, 2019). Talwariya et al. (2019) used Monte Carlo simulations to simulate power usage of different types of consumers. They were able to determine that agricultural consumers benefit from the use of renewable energy. Second, Monte Carlo simulations have been used in agricultural sampling in Kenya (Maeda et al., 2010). They were able to estimate the proportion of different crop types in a synthetic crop field before actual physical sampling was carried out. Their method was useful in reducing the labor and funds necessary to perform agricultural sampling with a root mean squared error (RMSE) of less than

1% when over 1,000 samples are used. Gibbons et al. (2006) designed a model to investigate the effects of uncertainty of greenhouse emissions data on cost minimizing solutions. Using Monte Carlo and a farm-level optimization model, they were able to breakdown the implications of greenhouse gas uncertainty on the cheapest methods for combating farm emissions. Next, Hong et al. (2016) used Monte Carlo simulations to test for the lethality of microwave assisted pasteurization with a goal of a 6-log reduction in *Clostridium botulinum* spores. Through Monte Carlo methods, they were able to determine what percentage of processes will result in 5 and 6 log reductions in beef and salmon samples. Further, Monte Carlo has been used to estimate health risk of pesticides on dates in Iran (Eslami et al., 2021). Researchers were able to determine that date consumption does not present a significant health risk due to pesticide residues. Also, Monte Carlo methods have been used to determine the feasibility of alternative farming practices on rice farms in Tanzania (Kadigi et al., 2020). They were able to perform risk analysis so that decision makers can consider the best pathways to take to increase rice production. Lastly, Guarav and Sharma (2020) used the Monte Carlo method to analyze the uncertainty of the parameters for negative health risks (carcinogenic and noncarcinogenic) from heavy metals as a result of the use of wastewater in agriculture.

Monte Carlo in the Poultry Industry

The poultry industry has found use for the Monte Carlo method in a variety of ways. Market risks of the poultry industry in Indonesia was performed using Monte Carlo methods (Purwaningsih et al., 2018). Results showed a market risk of loss of approximately 54% on Indonesian farms. Rico-Contreras et al. (2017) used Monte Carlo to estimate economic risk associated with different moisture levels (40%, 35%, 30%, and 25%) of poultry litter used for energy production, and they found the 40% moisture content scenario to require the least economic

investment. While, the 25% scenario produces more energy, financially it was found to be the worse option due to the effort required to reduce moisture to this level. Risks of negative impacts because of volatile organic compounds in slaughterhouses was investigated (Omidi et al., 2019). Results indicated concentration and frequency of the exposure were the most important variables relating to negative health risks. Also, a study done in the United Kingdom to determine the environmental impact of the 4 major types of egg production systems (Leinonen et al., 2012). They were able to utilize Monte Carlo to quantify the impact uncertainties on the outputs of their Life cycle assessments model. Next, Monte Carlo methods were used to investigate the impact of predetermined parameters on the likelihood of *Salmonella* infection from the consumption of a common chicken dish in South Korea (Jeong et al., 2018). Because of the benefits of the Monte Carlo method, they were able to run 100,000 iterations, and they found that *Salmonella* prevalence at retail and cooking temperature were the 2 most important parameters contributing to the likelihood of *Salmonella* infection. In a different experiment, Coleman and others (2003) utilized Monte Carlo methods to simulate unrestrained bacterial growth and growth inhibited by the "Jameson effect." Results for ground beef and poultry meat at were obtained from the simulations of backroom refrigeration, meat case refrigeration, and home refrigeration conditions. Only 1% of simulations reached temperatures where *Salmonella* could grow, but over 80% had temperatures that allowed the growth of the native microflora on chicken.

Markov Chain Monte Carlo

Markov Chain Monte Carlo (MCMC) is a popular method that takes advantage of the principles of Monte Carlo by combining it with Markov chains (Geyer, 2011). MCMC is predominantly used in Bayesian models where probability is used to measure uncertainty (Jackman, 2000). A Markov chain is a mathematical system that describes the probability of

changing from one state to another; however, the predictions are only dependent on the current state as the model does not remember past states (Geyer, 2011). In stochastic processes, it can be difficult to obtain independent samples. Therefore, dependent sampling via MCMC is a useful tool to increase the efficiency of sampling if gathering independent samples proves to be an issue (Geyer, 1992). MCMC has been used in a variety of applications including agriculture. For example, Parson et al. (2005) compared 3 models, including MCMC, for risk assessment using *Salmonella* and the poultry production chain. They found the MCMC method to be beneficial because it does not require discrete variables and allows the formation of inferences from the results. Also, Huang and Li (2020) used MCMC to compare Bayesian analysis with deterministic methods in the prediction of the growth of *Clostridium perfringens* spores in chicken meat during cooling. They were able to determine through MCMC that Bayesian analysis had more accurate predictions. Next, Ali et al. (2018) created a hybrid model called Markov chain Monte Carlo-copula integrated with genetic programming. In their model, they are able to use climate parameters to estimate the yield of cotton. The ability of MCMC to predict soil moisture profile has been studied (Yan et al., 2015). MCMC was compared with another particle filter soil moisture prediction model, and it was concluded that MCMC was the more accurate scheme. Additionally, Gibson (1997) used MCMC to fit a stochastic model to citrus tristeza virus data in an effort to aid in the control of plant epidemics. In addition, MCMC has been used to study the effects of closing and reopening poultry markets on the infection of humans with the avian influenza virus A (Yu et al., 2014; Lu et al., 2016). The studies concluded that closing markets when there are outbreaks of avian influenza is an effective measure in preventing its transmission. Diagnostic tests for avian influenza have been compared using MCMC (Yamamoto, 2007). Because of MCMC, a more sensitive test was identified and implemented for viral screening. Finally, Wang et al. (2012) were

able to investigate substitution rates of a viral gene as a result of mass vaccinations for avian influenza in chickens. Results showed that mass vaccinations can lead to vaccination resistant viruses thus limiting the effectiveness of future vaccinations.

Monte Carlo Shelf-life Predictions

Monte Carlo methods have been previously used by researchers to estimate the remaining shelf-life of both food and pharmaceutical products (Waterman et al., 2007; Lau et al., 2022). This is an extremely helpful technique because it allows managers to investigate what the effects on spoilage might be under a wide range of possible scenarios. In an experiment on milk, shelf-life was investigated with Monte Carlo being used to construct probability distributions of storage temperature, initial bacterial concentration, and generation times (Schaffner, 2003). Results from this study showed that a decrease in storage temperature of 2.1°C correlated with a 50% and 75% decrease in psychrotrophic and mesophilic spoilage, respectively after 2 weeks. Next, Giannakourou and Taoukis (2020) utilized Monte Carlo method with cold chain distribution and temperature data. They were able to compare the shelf-life predictions of their model with the 180-day shelf-life predicted by the "use-by" date, and they concluded that the uncertainty calculations built into their model resulted in more accurate shelf-life predictions. Additionally, Giannakourou et al. (2001) investigate the applicability of a shelf-life decision system (SLDS). They implemented Monte Carlo methods to simulate the results of the SLDS method, and they determined in local markets 12% and 2% of products were spoiled at the time of consumption for the FIFO system and the SLDS system, respectively. Also, in export markets unacceptability due to quality deterioration was reduced from 38% to 20% when the SLDS method was used. In a study performed by Escobedo-Avellaneda et al. (2012), Monte Carlo was used to monitor the variability of parameters on the predicted shelf-life of vegetables with different moisture levels. They were

able to predict the remaining shelf-life of tomatoes, onion flakes, and sliced green beans. When comparing the Monte Carlo simulations with the deterministic values, they determined that 51.6%, 48.6%, and 53.0% were the probabilities that shelf-life was shorter than the deterministic values for tomato slices, onion flakes, and sliced green beans, respectively

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CHAPTER II.

PREDICTING THE GROWTH OF *SALMONELLA* TYPHIMURIUM INOCULATED ON CHICKEN BREAST FILLETS DURING THE SIMULATED LESS-THAN-TRUCKLOAD CYCLIC TEMPERATURE ABUSE CONDITIONS

Abstract

Current less-than-truckload (LTL) shipping practices, in the last mile of the cold chain, allow for temperature abuse (TA) of perishable goods, resulting in potential food safety issues at the retail level. Commercially produced boneless skinless chicken breast, were inoculated with *Salmonella* Typhimurium (10^4 CFU/mL). Non-inoculated breast fillets were used for evaluation of spoilage. Breast fillets were placed in sterile bags then placed in a programmable incubator set to cycle between 4°C (2 h) and 25°C (2 h) for 24 h time period. Additionally, trials were completed at isothermal 4°C and 25°C for 24 h. Microbial sampling was performed every 6 h (n=3 breast fillets/trial x 3 trials) by plating serial dilutions of breast fillet rinsates on xylose lysine tergitol 4 agar for inoculated fillets and plate count agar or non-inoculated fillets. Starting with an existing model for the growth of *Salmonella* Typhimurium on chicken skin, temperature and microbial data were used to develop a new predictive model. This tertiary model was developed to predict the growth of *Salmonella* Typhimurium on cyclically TA fillets and fillets kept at 25°C. *Salmonella* Typhimurium and aerobic microorganisms increased, 0.93 and 0.84 logs, respectively when experiencing cyclic TA. The bias factors (BF) and accuracy factors (AF) of the model were 0.993 ("fail-safe") and 1.037 for TA fillets and 1.082 ("fail-dangerous") and 1.087 for 25°C. These values are in the acceptable range indicating the model performs well. This study demonstrates that current LTL practices during the last mile of the cold chain does impact the food safety and spoilage of perishable foods. Predictive modeling can be used in food supply chains to predict the growth of *Salmonella* spp. and allow retailers to make decisions regarding shelf-life, while improving food safety and reducing food waste.

Highlights

- Current LTL practices during the "last mile" may lead to temperature abuse.

- Temperature abuse leads to more rapid growth of pathogens such as *Salmonella*.
- Mathematical models can aid retailers in reducing food safety risks.

Maintaining food safety during the food supply chain is one of the biggest challenges faced by the agriculture industries of the world today. Over 600 million (48 million in U.S.) people suffer from foodborne disease each year with over 418,000 deaths (3,000 in U.S.) (WHO, 2015; CDC, 2022a). Bacteria cause 58% of foodborne illnesses with non-typhoidal *Salmonella enterica* contributing nearly 79 million cases alone (WHO, 2015). Of the pathogens responsible for illness, nontyphoidal *Salmonella* ranks second, first, and first in illnesses, hospitalizations, and deaths in the U.S., respectively (Scallan et al., 2011). *Salmonella* infection is characterized with stomach pain, fever, and diarrhea with most cases the result of eating contaminated food (CDC, 2022a). Also, successful treatment of salmonellosis (the disease caused by *Salmonella* infection) is becoming more complicated because of rising antibiotic resistance (Nair et al., 2019). There are a variety of foods in which consumption could lead to infection, but raw animal products present the highest risk (CDC, 2022b). According to the United States Food and Drug Administration (FDA; 2020a), poultry meat is a primary source of *Salmonella* infection with 4% of retail chicken testing positive in 2018. *Salmonella* can live in the gastrointestinal tract of animals such as chickens and food may become contaminated from the pathogen containing feces (FDA, 2020b). Studying the connection between poultry and *Salmonella* has been a focus of food scientists for many years (Morris et al.,1960). Because it presents a major challenge in poultry production, extensive *Salmonella* research has been performed at the live, processing, and storage segments of poultry meat production (Chia et al., 2009; Wales and Davies, 2020; Nychas and Tassou, 1996). There are over 2500 serovars of *Salmonella* (Grimont and Weill, 2007). The most common serovars to cause human illness are *Salmonella* Typhimurium and *Salmonella* Enteritidis, both of which are common

in poultry (European Food Safety Authority and European Centre for Disease Prevention and Control, 2019; Guillén et al., 2020). In addition to the negative health impacts, *Salmonella* infection produces a large economic burden from the federal government to the consumer. These costs may come from hospitalizations, drugs, or outpatient visits (Hoffmann and Ahn, 2021). In 2018, nontyphoidal *Salmonella* cases were found to have the largest associated costs (>$4 billion) in the United States, among the top 15 foodborne pathogens (Hoffmann and Ahn, 2021). *Salmonella* is a gram negative facultative anaerobe that grows best in the mesophilic temperature range (D'Aoust, 1991; Andino and Hanning, 2015). However, it has been demonstrated that *Salmonella* can grow at temperatures as low as 5.5°C in sterile broth and >10°C in the presence of background microflora (Matches and Liston, 1968; Morey and Singh, 2012). Also, *Salmonella* grows at a pH range of approximately 4.1-9.0 (Catalano and Knabel, 1994; Chung and Goepfert, 1970). Chicken breast pH may range from 5.91 to 6.36, and raw meats have water activities >0.95 (Swatland, 2008; Schmidt and Fontana Jr, 2020). In addition to its role as a possible conduit for *Salmonella* infection, chicken requires extra attention from food safety experts because of its continued increase in popularity. The National Chicken Council (NCC) reports a dramatic rise in consumption of chicken meat since the 1960's (2022). Furthermore, the marketing of chicken has trended from predominantly whole carcasses in the 1960's to parts and further processed chicken in more recent years (NCC, 2019a). Consumers are receiving approximately 50% of their poultry meat from retail grocery stores where these individual cuts are sold (NCC, 2019b). It has been shown that retail markets may have the highest incidence of contaminated carcasses (NidaUllah et al., 2016).

For the purposes of this paper, the term "Supply Chain" is defined as all the inputs (supplies, people, technology, etc.) necessary to deliver a product to an end user. The food supply

chain is composed of many segments that make up the journey from raw product to finished product at the end user (National Research Council, 2015). The end user may be a customer purchasing food from a retail store or a restaurant using the food to prepare products for consumers. Since many foods are temperature sensitive (e.g., chicken breast), they must travel along a version of the supply chain referred to as the "cold chain". When cold chain integrity is maintained, the quality, shelf-life, and safety of perishable products is protected (Global Cold Chain Allinace, 2020). When there are breaks in the cold chain, it allows for the opportunity of temperature abuse (TA) (Goedhals-Gerber et al., 2017). Bacteria grow most rapidly on foods between 4°C and 160°C (USDA, 2017). The FSIS refers to this as the "Danger Zone" for bacterial growth. It is advantageous for companies that do not have a sufficient freight to fill an entire trailer and wish only to pay for the space their products are occupying on the trailer to use less-than-truckload (LTL) shipping (FedEx, 2022). Next, the "last mile" of the supply chain is the final segment that comprises the journey of a product to an end user (Lim et al., 2018). During the last mile, delivery vehicles must make multiple stops along the route due to multiple shippers using the same truck/trailer. This may allow for TA to occur at each stop. At the retail level, stores often utilize the "First-In, First-Out" (FIFO) model which operates under the principle that products that arrive first, will expire first (Mendes et al., 2020). In contrast, the "First-Expire, First-Out" (FEFO) model considers the shelf-life dynamic and it utilizes a remaining shelf-life to make decisions regarding perishable products (Hertog et al., 2014).

Modeling of bacterial growth on foods can be a valuable tool in combating food safety and food waste. The advantage of models are they allow the decision maker to have and educated guess on where that product stands without having to perform microbial sampling. There are 3 levels of predictive modelling: 1) Primary models, 2) Secondary Models, and 3) Tertiary models

(Stavropoulou and Bezirtzoglou, 2019). Primary models measure the reaction of bacteria to specific conditions over a set period of time, and secondary models measure the response of primary model parameters during changes in environmental conditions such as temperature (Fakruddin et al., 2011). Tertiary models are user interfaces that utilize secondary and primary models in order to make predictions (Stavropoulou and Bezirtzoglou, 2019). Perhaps the most popular primary model prior to the 1990's was the Gompertz model. (Gompertz, 1825). This model did not originally contain parameters relevant to bacterial growth but was later modified to do so (Zwietering et al., 1990). Additionally, in the early 1990's the Baranyi model was developed with biological basis and introduced a variable for lag time (Baranyi et al., 1993). A popular secondary model uses the Arrhenius Law that has been modified for nonlinear regression (Schoolfield et al., 1981). However, this equation was originally used to determine the effect changes in temperature will have on the rate of a chemical reaction (McKeen, 2017). This equation often struggled to fit growth data because the Arrenhius law predicts a linear relationship between growth rate and temperature; however, the result of plotting the logarithm of the growth rate constant by the reciprocal of the absolute value of temperature (Arrenhius Plot) results in a curve that poorly fits bacterial growth data (Ratkowsky et al., 1982). A new secondary model for bacterial growth below optimum temperature was created where a linear relationship was discovered between the square root of the growth rate constant and temperature (Ratkowsky et al., 1982). This model was improved to include the entire temperature range by adding variables for maximum and minimum temperature (Ratkowksy et al., 1983). Growth models have been designed to accurately predict the proliferation of bacteria on many different foodstuffs (Alavi et al., 1999; Juneja et al., 2009; Sutherland and Braxton, 1995). Several studies modeling the growth of *Salmonella* on raw poultry meat using different isothermal temperatures have been completed (Dominguez and Schaffner,

2008; Juneja et al., 2007; Pradhan et al., 2012). Also, similar studies for *Salmonella* modelling on cooked chicken products have been performed (Oscar, 2002; Oscar, 2009; Li et al., 2017).

There is a need for predictive models that consider TA during the last mile of the supply chain in LTL conditions. In many cases, these conditions more accurately reflect the journey the product will endure. The resulting TA could have a negative effect on the shelf-life and food safety of temperature sensitive food products. Also, the severity of TA may vary according to the number of stops that occur before product reaches its destination. Furthermore, this cyclic heating up and cooling down is not accounted for in the FIFO model. There is a gap in the literature on the modelling of pathogen growth during dynamic temperature conditions like those experienced during LTL conditions. The objectives of this research are to determine the effects of simulated LTL conditions during the last mile on the growth of *Salmonella* Typhimurium and develop a predictive growth model for theses specific conditions.

Materials and Methods

Experimental design. Boneless skinless chicken breast (n=270) was used to study *Salmonella* growth. Three replications were completed for 3 separate storage scenarios (4°C, TA, and 25°C). In each replication, 3 breast fillets were randomly placed on 3 levels of a programmable incubator (MIR-554 Cooled Incubator, Japan) to monitor temperature. For all replications, 30 fillets (15 inoculated and 15 uninoculated) were individually bagged in sterile bags (18 x 30 cm, 1650mL, VWR, Radnor, PA) and placed in separate totes according to inoculation status. The inoculated fillets were used to study *Salmonella* Typhimurium growth, and the uninoculated fillets were used for aerobic plate counts. Sampling was conducted during 5 sampling periods (0, 6, 12, 18, and 24 h).

Temperature monitoring and microbial sampling. Commercially produced boneless skinless chicken breast were procured from a local processor. Breast fillets (n=3) were randomly selected for temperature monitoring and thermocouple (Type K) wires attached to a data logger (TM500: 12-Channel Data Logging Thermometer, Extech Instruments, Nashua, New Hampshire) were centrally inserted. The fillets were placed in bags and situated on the top, middle, and bottom racks of a programmable incubator. The data logger recorded temperature data every 60 seconds for 24 h. After completion, the data was downloaded, and the data was analyzed in Excel (version 16, Microsoft Corpporation, Redmond, WA). A culture of nalidixic acid resistant (35 µg/mL) *Salmonella* Typhimurium (isolated from the Auburn University Poultry Research Farm and selected for resistance to 35µg/mL nalidixic acid; Bauermeister et al. 2008; Kataria et al., 2020) was grown for 18 h (10^8 CFU/mL) in brain heart infusion broth (Neogen Corporation, Lansing, MI) and serially diluted in buffered peptone water (BPW; Neogen Corporation) to prepare inoculum. Fifteen fillets were placed in sterile aluminum trays (Heavy Duty Reusable Eco-Friendly Aluminum Foil Full Size Medium Pan, 20.75" L X 12.75" W X 2.2" D, King Zak Industries, Goshen, NY) and inoculated with 1 ml of *Salmonella* Typhimurium to achieve 10^4 CFU per fillet. These fillets were covered and kept at 4°C for 30 min to allow for bacterial attachment. Also, fifteen additional fillets were placed in sterile bags and kept to determine spoilage. Three randomly selected inoculated and non-inoculated breast fillets were sampled at 0, 6, 12, 18, and 24 h during the 24 h storage period, by aseptically transferring the fillet to a sterile bag and rinsing with 50 mL of BPW (Neogen Corporation) for 1 min. Fillet rinsates were used to prepare serial dilutions in BPW. The dilutions were plated on xylose lysine tergitol 4 agar (Neogen Corporation) containing 25µg/mL of nalidixic acid (Sigma-Aldrich, St. Louis, MO) for the inoculated fillets and

plate count agar (Neogen Corporation) for the uninoculated fillets. All plates were placed in a 37°C incubator (5EG, Precision, Winchester, VA) for 24-48 h before counting.

Developing a tertiary growth model for cyclic temperature abuse. To model the growth of *Salmonella* Typhimurium during simulated supply chain conditions, we began with Oscar's (Oscar, 2009) model for *Salmonella* Typhimurium growth on chicken skin. In Oscar's model, a modified version of the Baranyi model (Baranyi and Roberts, 1994), was used for primary modeling of lag time and specific growth rate at 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50°C. Also, in Oscar's model (Oscar, 2009) samplings were conducted after 0, 2, 4, 6, and 8 h. For the model described in this paper we utilized Oscar's (Oscar, 2009) secondary model for lag time (Equation 1) and the associated values for the parameters. In Oscar's (Oscar, 2009) model, secondary modeling for lag time was completed by fitting a 2-phase exponential model to the lag time data (from primary modelling) that was graphed as a function of temperature.

$$\lambda = \lambda_{\min} + (\lambda_{\max} - \lambda_{\min}) \times \exp[-\lambda_{\text{rate}} \times (T - T_{\min})] \quad (1)$$

In equation 1, λ_{min} and λ_{max} are minimum and maximum lag time in h, λ_{rate} is the rate at which the lag time changes as a function of temperature, and T_{min} is the minimum growth temperature. For secondary modeling of specific growth rate, data from Oscar's (Oscar, 2009) primary modeling was used (Table 1). The square root of growth rate was plotted as a function of temperature and fit to the extended Ratkowsky model (equation 2) (Ratkowsky et al., 1983) using MyCurveFit (MyCurveFit, 2022).

$$\sqrt{r} = b(T - T_{\min}) \times \{1 - \exp[c \times (T - T_{\max})]\} \quad (2)$$

In equation 2, b is the regression coefficient, T_{min} and T_{max} are the minimum and maximum temperatures where growth rate is equal to 0, and c is the parameter that allows the

model to fit when temperatures are greater than the optimum (Ratkowsky et al., 1983). The parameter values from secondary modeling of specific growth rate are as follows: 1) T_{min} (0.669°C), 2) T_{max} (48.197°C), 3) b (0.028), 4) c (0.472). However, the b value was adjusted to 0.20 to achieve a better fit to observed data.

For tertiary modeling the secondary models for lag time (λ) and specific growth rate (μ) were combined using Excel. The inputs for the model are initial bacterial concentration (log CFU/mL), time increment (h), and the temperature profile (°C). In addition, the model allows for the assumption of no lag time and a maximum limit in bacterial concentration. The outputs of the model are a bacterial growth curve (Figures 1 and 2) under the simulated conditions, values for lag time and growth rate, and a remaining time (h) until the "max limit" is reached.

To test the validity of the model during TA and at 25°C, bias factors (BF) and accuracy factors (AF) were calculated (Tables 2 and 3) using the formulas (Equations 3 and 4) developed by Ross (Ross, 1996).

$$bias\ factor = 10^{\left(\frac{\Sigma \log\left(\frac{GT_{predicted}}{GT_{observed}}\right)}{n}\right)} \quad (3)$$

$$accuracy\ factor = 10^{\left(\frac{\Sigma \left|\log\left(\frac{GT_{predicted}}{GT_{observed}}\right)\right|}{n}\right)} \quad (4)$$

For equations 3 and 4, $GT_{predicted}$ and $GT_{observed}$ are the predicted and observed growth time and n represents the number of observations (Ross, 1996). For BF, the predicted values are divided by the observed values (P/O). Next, the $\log_{10}$ of the P/O values was summed and divided

by the total number of observations (n). Lastly, 10 is raised to sum/n value. To determine the AF, the same process is followed with the absolute value of the $\log_{10}$ values.

Results and Discussion

A new tertiary model was developed to predict *Salmonella* growth during the last mile of the cold chain, and model predictions were compared to results from samples collected after simulated LTL conditions. Boneless skinless chicken breast fillets that endured the TA scenario spent a large amount of time above 10°C (Figure 3). Morey and Singh (Morey and Singh, 2012) demonstrated that *Salmonella* growth increased at temperatures above 10°C when background microflora is present, such as in a food matrix. The fillets used for a negative control (4°C) maintained a constant temperature (Figure 3) for the duration of the experiment, while positive control (25°C) fillets reached and maintained 25°C after 6 h of elevated temperature storage (Figure 3).

Salmonella Typhimurium and aerobic bacteria growth during simulated last mile cold chain conditions (negative control [4°C], positive control [25°C], cyclical TA) are shown in Figure 4 and Figure 5, respectively. The initial concentrations of *Salmonella* Typhimurium (Figure 4) and aerobic organisms (Figure 5) were approximately 10^3 CFU/ml of rinsate for all simulated temperature scenarios. Similar to Morey and Singh, (Morey and Singh, 2012) there was no growth in the *Salmonella* Typhimurium for the negative control (4°C; Figure 4). The aerobic organisms (Figure 5) followed the same trend indicating that both *Salmonella* Typhimurium and aerobic organisms remained in the lag phase, for the duration of the experiment, with no increase in growth at 4°C. In contrast, the positive control (25°C,) reached log phase after approximately 6 h for both *Salmonella* Typhimurium (Figure 4) and aerobic organisms (Figure 5) with both approaching 10^8 CFU/ml of rinsate, by the end of the experiment. When exposed to simulated cyclic TA conditions

in the cold chain, the *Salmonella* Typhimurium (Figure 4) increased by 0.93 logs, and the aerobic organisms (Figure 5) increased by 0.84 logs after 24 h. Also, it longer for both bacterial populations (Figures 4 and 5) to reach log phase (~12 h) during the simulated cyclic TA conditions, compared to the positive control (25°C). In addition, it is important to note that under simulated cyclic TA conditions, the log phase of bacterial growth was reached and increasing the growth rate of both *Salmonella* Typhimurium and spoilage (aerobic) microorganisms in comparison to the negative control (4°C). Boneless skinless chicken breast fillets that have been TA during the last mile of the cold chain, is at a higher risk for *Salmonella* growth than breast fillets that have been maintained in a controlled (4°C) environment. This TA data would likely be applicable to other meat and poultry parts that are exposed to TA conditions during the last mile of the cold chain.

If control measures are not taken to protect temperature integrity during the last mile in the cold chain, companies may be allowing the proliferation of pathogens and spoilage organisms on their food products. However, many supply chains are not monitoring this closely to determine if TA is occurring and affecting product shelf-life and safety. Admittedly, the simulated cyclical breaking of the cold chain described in this paper is an extreme scenario, and it would not be likely that there would be the same level of TA at each stop of a delivery route. However, for experimental purposes if the extremes are understood and proven to potentially result in a problem, then companies can individually focus on more realistic scenarios and determine what changes may need to be made to their own cold chains based on the nature of their products, delivery routes, etc. Temperature is the only mechanism protecting the quality and safety of the food once the product has reached the last mile. This means heterogeneity of the temperature must be maintained. The impact of cold chain temperature shifts of the shelf-life of pork and poultry meat has been studied (Bruckner et al., 2012). Results indicated a single 4 h temperature shift to 7°C resulted in

shelf-life reductions of 10.7% and 8.5% for pork and poultry respectively. Also, it has been shown that quality and shelf-life of another perishable food, fish, was negatively impacted by a fluctuation of only 2°C (Tingman and Xiaoshuan, 2010). While these temperatures are much less of a fluctuation than in our experiment, the result is the same, a reduction of shelf-life. To record cold chain temperatures more diligently, advances in technology are needed. Radio-frequency identification (RFID) research has been suggested to help record temperatures in food and pharmaceutical supply chains (Grunow and Piramuthu, 2013; Vivaldi et al., 2020). The technology is rapidly advancing, and it may be a viable option for companies in the future. In addition, standards and regulations would need to be developed for its use. Also, a rise in global temperatures will pose a risk for cold chains. Only 1% of CO_2 production is the result of cold chains (James and James, 2010). However, if temperatures rise it would likely place more strain on cold chain integrity, lead to increased spoilage and food safety risk, and require the use of more energy to maintain temperature homogeneity during the last mile. It is important for companies to maintain and update their cold chains while considering future challenges. Cold chain research has been primarily performed in developed countries with the status of less developed countries being more of a mystery (Ndraha et al., 2018). It could be inferred that the lack of technology and understanding of the importance of cold chain integrity is negatively impacting the populations that are the most food insecure.

Preventing favorable growth conditions for pathogenic and spoilage bacteria are the principal reasons controlling cold chain temperatures in the last mile is paramount. Our microbial data (Figures 4 and 5) demonstrate that the simulated cyclic TA scenario was approaching 1 log of growth of *Salmonella* and spoilage organisms, in contrast to the negative control (4°C) scenario that had no growth. Microbial spoilage in meat has been shown to occur when aerobic bacteria

reach 7 logs CFU (Pothakos et al., 2014; Reid et al., 2017). APCs did not reach spoilage levels during the TA scenario. However, this is likely due to the experiment stopping at 24 h. All spoilage and pathogenic bacteria present challenges if temperature homogeneity is not maintained. For example, a study was completed in Iran, showing dynamic temperature conditions allowed for the proliferation of *Pseudomonas* on chicken (Ghollasi-Mood et al., 2016). Also, when a *Salmonella* spp. and *Listeria* cocktail was used to inoculate chicken patties, experimentation showed that *Listeria* and *Salmonella* spp. growth correlated with higher temperatures (Murphy et al., 2001). Future research on the shelf-life of poultry products in simulated supply chain conditions is needed. This study only looked at the aerobic plate count data which is an indication of mesophilic bacterial growth. Psychrotrophic data would also be valuable information since these are the bacteria that are more likely to grow in cold storage conditions (Ercolini et al., 2009). The bacterial species that are most responsible for poultry spoilage include lactic acid bacteria, *Enterobacteriaceae*, and *Pseudomonas* spp., and the most common pathogens are *Salmonella* spp. and *Campylobacter* (Rouger et al., 2017). However, bacterial diversity on chicken meat is greater when fluctuating temperatures are present rather than isothermal conditions (Zhang et al., 2012). Meat spoilage is subjective; however, a few generally accepted characteristics of spoilage include the formation of slime and meat discoloration (Nychas et al., 2008). Current practices during meat distribution may need to be refined to protect meat and poultry products from TA that leads to the formation of these undesirable characteristics on perishable foods.

Ultimately, the issue with spoilage is the resulting food loss and food waste. Food loss occurs before the product reaches the consumer, while food waste occurs at the retail level and beyond (FAO, 2022). A large portion of food waste takes place in consumer homes, but 26% and 13% occurs at food service and retail, respectively (UN, 2021). Regarding TA during the last mile

of the cold chain in LTL shipping, food waste is a major concern. The “use-by” date that is visible on product packaging is determined before the product enters the last mile. Therefore, any TA abuse occurring during the last mile is not considered in shelf-life determinations for product “use-by” dates. This is the primary issue with the FIFO model for product rotation in retail stores. The temperature and microbial data in this paper and previously published studies indicates that TA is likely having a negative impact on the shelf-life of products. Furthermore, different products within a reefer truck or van may be experiencing different levels of temperature abuse based on location (Jedermann et al., 2009). Detailed temperature modeling of products is needed for the development of a “First-Expire, First-Out” (FEFO) model for retailers. This would give the retailers valuable data to make data based decisions regarding the product rotation of perishable goods in their store. The principle behind such a model would be as follows. First, pallets or boxes of goods would be fit with individual temperature monitoring devices such as RFID technology. Second, as the products travel along the last mile of the cold chain, the TA data would be collected and stored with the RFID. Lastly, when the product arrives to the end user, managers could use the data to determine which products are most likely to expire first and put them on display first. This method is more objective than the current FIFO model and does not operate under the assumption that products that arrive first, expire first. Also, by following the FEFO model food producing companies could limit their food safety risk as the most TA product will likely be the products with higher pathogen counts. The benefits of this change would be felt throughout the supply chain. Companies could better protect their reputations, reduce recalls, reduce food waste, and have larger quantities of sales. Food waste and foodborne illnesses are large and complicated problems, but it is best to address them with data driven methods.

The temperature and microbial growth data collected in this study were used to develop mathematical models (Tables 2 and 3), to describe the growth of different microorganisms under various last mile temperature scenarios (cyclic TA and 25°C). No modeling was completed for the negative control (4°C) because the *Salmonella* Typhimurium counts did not change in this scenario. For *Salmonella* Typhimurium growth during simulated cyclic TA (Table 2), the lowest P/O value was 0.900 and the highest was 1.045, while at 25°C (Table 3) the lowest was 0.863 and the highest was 1.038. A P/O value for the comparison of predicted and observed values equal to 1.0 indicates a perfect match (Ross, 1996). BF of 0.987 and 0.939 were calculated for simulated cyclic TA and 25°C, respectively. While, the AF were 1.032 and 1.073 for cyclic TA (Table 2) and 25°C Table 3), respectively. The BF indicate a prediction model that is on average 1.3% less than our observed data for cyclic temperature abuse (Table 2) and on average 6.1% less than our observed data for 25°C (Table 3). This indicates a "fail-safe" model rather than a "fail-dangerous" model for both TA and 25°C. A fail-dangerous model predicts, on average, longer generation times than are observed, and a fail-safe model predicts, on average, shorter generation times than are observed (Ross, 1996). Regarding the AF, the model's predictions are on average factors of 1.032 (during TA; Table 2) and 1.073 (during 25°C; Table 3) different from the observed values (Ross, 1996). Ross et al. (Ross et al., 2000) recommended limits for BF ranging from 0.7-1.15. There is more forgiveness in a model that under predicts than over predicts bacterial generation times because of the consequences associated with each scenario. For example, an under prediction in generation time leads to the interpretation of higher bacterial numbers, than are actually present. The consequence of this might be discarding product that is still safe/fresh. However, and over prediction of generation time leads to the belief that there are fewer bacterial numbers than are present. Therefore, product may be saved that is spoiled or presenting food safety risk. The BF for

the models described in Tables 2 and 3 fall within the acceptable range. Additionally, Oscar (Oscar, 2005) proposed a more specific method of validating predictive growth models using the BF and AF in tandem. This method of validation is more robust. The recommendation states if a model has a BF less than or equal to 1.0, then the AF should be less than 1.3. On the opposite side, if the BF is greater than 1.0, the AF should be less than 1.5. In both cases, the model described in this paper was an acceptable model using the recommended limits defined by Ross et al. (Ross et al., 2000) and Oscar (Oscar, 2005).

Many other mathematical models have already been developed to describe the growth of microorganisms on meat in isothermal conditions, however, there is a gap in the literature that considers a simulated cold chain LTL TA scenario. Bruckner et al. (Bruckner et al., 2013) developed a predictive model to describe the shelf-life of pork and poultry, as well as *Pseudomonas* growth (Bruckner et al., 2013). Their model used the Gompertz model (primary) and Arrhenius model (secondary) for prediction. They found that their model underestimated the shelf-life of poultry by 11.1%. In comparison, our model underestimated poultry subjected to TA by approximately 1.3% (Table 2). Also, we chose the Ratkowsky model for secondary modeling because of its biological basis (Ratkowsky et al., 1983*)*. Different primary models for the growth of *Salmonella* on chicken have been compared (Juneja et al., 2007). Experimentation found that the Baranyi model performed the best based on R^2 and mean square error values. Another model was developed to describe the shelf-life of packaged chicken in dynamic storage conditions (Yimenu et al., 2019). Like Oscar's model (Oscar, 2009) model, this model utilized the Baranyi model and fluctuating temperatures. The AF and BF for this model were 1.045 and 0.991, respectively. Also, similarly to our model for dynamic temperatures, they had "fail-safe" predictions. In addition, a *Salmonella* prediction model for chicken stored at low temperatures was

developed (Oscar, 2011). For this model, chicken skin was inoculated with *Salmonella* Typhimurium and kept at 4°C to 12°C and predictions were found to be over 84% accurate in all scenarios. The importance of these models is they provide the framework for a FEFO model and show that both pathogenic and spoilage bacteria can be modeled confidently. They provide a necessary tool to battle foodborne illness and food waste and could be extremely useful in managing cold chains. Models can even predict the remaining shelf-life, essentially making the decisions for the retailers. For example, the remaining shelf-life of chicken meat using several primary models and *Pseudomonas* spp. growth data has been developed, with the Baranyi model performing the best (Tarlak and Pérez-Rodríguez, 2021). There have been many different models developed for *Salmonella* and spoilage microorganism growth on chicken. The range of parameters considered in these models has been thorough. They include different temperatures (both isothermal and dynamic), microflora (sterile or with native microflora), and different types of packaging (Oscar, 2005; Oscar, 2008; Wei et al., 2001). With an abundance of models to choose from, companies should implement these models or develop their own to best combat some of the biggest food chain problems in the world today. By combining more diligent temperature recording with these mathematical models, it will be possible in real time to make decisions regarding the food safety and spoilage that are best for business and the consumer.

In conclusion, the current practices in the “Last Mile” of the cold chain are allowing for cyclic TA when trucks are stopping to unload cargo in LTL scenarios. However, this TA is not accounted for when retailers are making decisions on how to rotate their products to the shelves. Both our data and data from others indicate that fluctuating temperatures can result in the increased rate of growth for foodborne pathogens and spoilage microorganisms on temperature sensitive products. Temperature data shows products may spend most of their time above 10°C allowing for

the growth of *Salmonella* therefore increasing food safety risk of chicken. Mathematical models can utilize temperature and microbial data and reliably predict pathogen growth and spoilage of chicken. These models can be used to implement an objective FEFO model rather than the existing FIFO model used by retailers today.

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CHAPTER III.

LAST MILE TEMPERATURE ABUSE IN LESS-THAN-TRUCKLOAD SHIPPING AND ITS IMPLICATIONS ON FOOD LOSSES AT RETAIL

Abstract

Current less-than-truckload (LTL) shipping practices in the last mile lead to temperature abuse (TA) of perishable products causing rapid food spoilage and ultimately contributing to food losses. There is a need to study the implications of TA in LTL on the shelf-life of perishable foods such as raw poultry and design data-based decision models to predict the remaining shelf-life of the products. A pallet of boxes (n = 5 boxes/layer x 4 layers) each containing commercially packaged trays of boneless skinless chicken breast fillets was exposed to cyclic TA (2 h at 4°C followed by 2 h 25°C) for 24 h. Temperature of the chicken fillets in each box on the pallet was recorded via thermocouple data loggers every 5 min for 24 h and infrared images were taken at the beginning of each temperature change (every 2 h). Microbiological sampling (aerobic plate counts (APC) and psychrotrophic plate counts (PSY) was conducted on breast fillets before the beginning of TA (h 0) and at the end of TA (h 24). After the 24 h TA, the tray packs from the most TA box and the control trays (maintained at 4°C without TA) were stored at 4°C sampled (APC and PSY) on alternate days until the bacterial counts reached the spoilage limit of 7 log CFU. Temperature data from each box was analyzed using Monte Carlo simulations (500 iterations) to determine the risk of temperature reaching the food safety and microbiological spoilage "danger zone" (> 4°C) after 0, 6, 12, 18, and 24 h. Additionally, Monte Carlo simulations were used to calculate risk-of-loss (shelf-life of <4 days) and remaining shelf-life based on time spent above 4°C. Temperature data indicates that the 4 layers of boxes on a pallet, as well as individual boxes on each layer, experience TA differently. The top and bottom layers were most affected due to TA compared to the middle layers while the boxes located on the perimeter crossed 4°C the fastest. TA reduced the shelf-life of raw chicken breast fillets to 4.1 days compared to 7.2 days for the control samples. Monte Carlo results indicate top layer boxes have the highest risk (94.96%

average) of reaching 4°C after 24 h of TA with bottom layer boxes having the second highest risk (75.12% average). However, the middle layers demonstrated a decreased risk of TA with averages of 43.2% and 27.2% for layers 2 and 3, respectively. Also, remaining shelf-life was reduced by 42.4% and had a risk-of-loss of 43.8% after 8 h of simulated LTL TA. The study demonstrates heterogeneity of TA during the last mile and emphasizes the need to replace the traditional "First-In, First-Out" model with a "First-Expire, First-Out Model" in the food supply chain.

Introduction

Supply chain management can be difficult to master for any corporation especially those transporting temperature sensitive products such as perishable food commodities. According to the Global Cold Chain Alliance (GCCA, 2020), the cold chain is a version of a supply chain that places focus on maintaining product integrity and quality through temperature management, and it runs from a products origin to the final consumer. If appropriate refrigeration temperatures are not maintained throughout the supply chain, temperature sensitive and high-value products, such as raw poultry, may experience temperature abuse (TA; Ndraha et al., 2018). The refrigerated warehousing industry has large financial implications. The industry generates over $6 billion in revenue and employs over 62,000 people (GCCA, 2020). A product may need to be transformed, packaged, and shipped all while preventing breaks in the cold chain and allowing TA. It is known that 2 popular shipping methods utilized by companies are full truckload (FTL) and less-than-truckload (LTL) shipping (Vega et al., 2021). LTL shipping is utilized when the cargo requires only a portion of the space in a trailer (FedEx, 2022) such that the shipper does not pay the rent of an entire truck but only for the space their cargo occupies (Özkaya et al., 2010). In contrast, FTL is used when a shipper has enough cargo to fill a truck/trailer entirely (FreightQuote, 2019). Because of the rise in e-commerce (United States Department of Commerce, 2020), the popularity

of LTL shipping is expected to continue to rise in popularity with Old Dominion Freight Line (2022) reporting an 18.3% increase in LTL tons per day and a 19.8% increase in LTL shipments per day from February 2021 to February 2022. Also, consumers prefer home delivery (64%) over pickup (23%) for all categories of products except groceries (Pitney Bowes, 2022). The Covid-19 pandemic had a major impact on this trend with online sales increasing by 43% in 2020 (United States Census Bureau, 2022). One of the biggest challenges of LTL is understanding how to approach the "Last Mile" problem (Deutsch and Golany, 2017).The last mile is the last segment of a supply chain where product is transported to the end user (Shu et al., 2015). Addressing how to best approach last mile problems is a key component of the Food and Drug Administration's (FDA) "New Era of Smarter Food Safety" (2021). During the last mile, temperature fluctuations and condensation can occur on perishable food products (Mirzaee and Bishop, 2009). A potential cause of this may be that delivery vehicles must make multiple stops to deliver packages during the last mile in LTL scenarios (Aljohani and Thompson, 2020). It is known that TA occurs in the food supply chain, the effects of Covid-19 has been studied (Skawińska and Zalewski, 2022). Additionally, studies have been completed on warm air infiltration during unloading (Tassou et al., 2009). Rai et al. (2018) studied how the use of curtains can limit warmer air entering reefer trucks during unloading. The effect of different loading patterns on the temperature and airflow inside refrigerated trucks has been studied (So et al., 2022), and de Frias et al. (2020) investigated the temperature implications of opening doors to a refrigerated display case with the most extreme scenarios (opening every 5 min for 60 s) resulting in significant differences in product temperature. Because of the importance of proper temperature management many different technologies have been developed to improve refrigerated trucks and containers (Kehinde et al., 2022). However, limited research has been completed on the effects of last mile TA on shelf-life and food safety.

TA during the last mile of the cold chain may create an environment conducive to the growth of spoilage and pathogenic microorganisms (Labuza and Fu, 1995; Oscar, 2009). According to the United States Department of Agriculture (USDA, 2021a), optimal growth temperature for most bacteria is between 40°F-140°F or 4°C and 60°C. This range is referred to as the "danger zone". Foods are kept below the "danger zone" temperatures to prevent spoilage and mitigate food safety risks (Godwin et al., 2012). For example, poultry meat should be kept at or below 4°C after slaughter to help control the growth of spoilage bacteria and foodborne pathogens (USDA, 2021a). Microbial growth is characterized by lag phase, exponential phase, stationary phase, and death phase (Maier, 2015). The purpose of refrigeration is to extend the lag phase, or the postponement of log phase, of microorganisms thus slowing down the growth (Berk, 2013). Common spoilage microorganisms include bacteria, molds, and yeast (Rawat, 2015). Some common spoilage bacteria in foods are lactic acid bacteria, Pseudomonas spp., Acinetobacter spp., Bacillus spp., and many others (Quintela-Baluja et al., 2014). When investigating the bacteria responsible for spoilage, there are 2 groups that require special interest. These are the mesophilic bacteria (APC) and psychrotrophs (PSY) (Gill and Newton, 1978; Ercolini, et al., 2009). The APC is often used as an overall estimation of the bacterial concentrations in foods (Maturin and Peeler, 2001). It has been demonstrated that TA leads to increases in the total viable counts on many different food products (Oblinger et al., 1982; Rothenberg, 1982; Senter et al., 2000;). Additionally, shelf-life studies have been conducted using APC (Reddy et al., 1994; Rogers et al., 2014; Pfeiffer et al., 2019; Zhou et al., 2022). However, it may be more beneficial to look at the PSY counts because these are the bacteria adapted to survive at storage temperatures of perishable food products such as poultry (Wei et al., 2019). Also, it has been shown that APC can underestimate the bacterial populations present on food products (Pothakos et al., 2012). Many

studies have been completed on PSY spoilage in foods (Russel et al., 1992; Jensen et al., 2001; Zhou et al., 2022). Furthermore, the prevention of spoilage and extension of shell life concerning PSY have been research interests. Tomac et al. (2013) modeled the effect of gamma irradiation of PSY to extend shelf-life of squid. Munsch-Alatossava (2010) determined the use of nitrogen gas can limit PSY growth in Raw milk. Also, legume proteins have proven to control PSY in buffalo milk (Sitohy et al., 2011). For both APC and PSY, 7 logs of colony forming units (CFU)/mL is considered the point spoilage for meat products because this is when the meat has reached an undesirable status (Dainty and Mackey, 1992).

Addressing the food waste problem is important to feed a continuously growing population which could reach 8.5 billion by 2030 (United Nations, 2022a). This increase in population will likely apply pressure on agricultural supply chains of many countries around the globe (Da Silva, 2012; Chapman et al., 2021). The countries without the infrastructure to manage large complicated cold chains may be affected the most (Gligor et al., 2018). According to the UN (2022b), up to 660 million people may be battling hunger in 2030. The Food and Agriculture Organization of the United Nations (FAO, 2021) states the prevalence of moderate and severe food insecurity has grown over the past 5 years reaching approximately 30% in 2020. Resources, money, and labor are all required to produce healthy food, and it is paramount that these inputs are not fruitless (Khan and Hanjra, 2009; Zhichkin et al., 2020; Luckstead et al., 2021). According to the FAO (2019), food loss occurs in the supply chain before the retail segment, and food waste occurs at retail and beyond. Spoilage of foods contributes to the global problem of food waste (USDA, 2022a). Globally food waste has been a focus for researchers. In the United States, food waste could be as high as 40%, and 133 billion pounds may be lost at the levels of retail and consumption (USDA, 2022a). Brancoli et al. (2017) conducted supermarket research in Sweden and found that

meat and bread are the foodstuffs whose associated wastes have the largest negative environmental impact. Cicatiello et al. (2016) quantified the amount of food waste at a supermarket in Italy and determined 23.5 tons of food were able to be recovered over 300 days that would have otherwise been wasted. Another study in Italy revealed that bread, fruits and vegetables were the most wasted foods at the retail level (Cicatiello et al., 2017). In New Zealand, Goodman-Smith et al. (2020) found that vegetables, baked goods, and meat were the 3 most wasted food types. German consumer acceptance of produce that was less than perfect in appearance was found to be a major driver in retail food waste (Hermsdorf et al., 2017). Bilska et al. (2018) found that a single polish supermarket could waste over 3 tons of food in 2 weeks. Additionally, the environmental impact of food waste has been investigated (Hall et al., 2009; Scherhaufer et al., 2018; Dilkes-Hoffmann et al., 2018). It has been demonstrated that 7% of greenhouse gas emissions originate from the retail and distribution steps in Europe (Scherhaufer et al., 2018). Lastly, High value products such as meat and seafood have been shown to be wasted at the retail and distribution levels (Nychas et al., 2008; Love et al., 2015). Karwowska et al. (2021) investigated the loss and waste of meat with results indicating consumption, manufacturing, and distribution account for 96%.

The negative effects of LTL TA on food waste at the retail level may be impacted by managerial decisions regarding product rotation (Hertog et al., 2014). Two of the dominant methods of product rotation at retail stores are “First-In, First-Out” (FIFO) and “First-Expire, First Out” (FEFO) (Mendes et al., 2020). The FIFO model operates under the assumption that products that arrive first should be rotated out first because they will expire first (Pikora et al., 2021). In contrast, the FEFO model takes into consideration the remaining life of a product (Mendes et al., 2020). Issues with cold storage during transportation and retail of temperature sensitive products may lead to spoilage and food waste (Buzby et al., 2014). Additionally, the environment inside

refrigerated trailers and containers is not temperature homozygous; therefore, the level of TA experienced by products inside may be different (Jedermann et al., 2009; Getahun et al., 2017). The heterogeneity of temperature of a TA pallet of berries has been demonstrated previously (do Nascimento Nunes et al., 2014). It is possible that FIFO is leading to the waste of high value temperature sensitive products that could be saved with the FEFO method (do Nascimento Nunes et al., 2014). For example, 50% of the chicken market was projected to be in retail grocery in 2022 (NCC, 2022a). A dynamic shelf-life has been demonstrated to have greater financial gains and reduce food waste when compared to a fixed shelf-life (Buisman et al., 2019). The FEFO model has been demonstrated to outperform the FIFO model in the reduction of food waste in strawberries (Leithner and Fikar, 2018). Additionally, other nonfood products, such as pharmaceuticals, could benefit from the use of FEFO (Sukasih et al., 2020; Rezeki at al., 2022). A primary disadvantage of FEFO is it requires information sharing between different members of the cold chain to work properly (Hertog et al., 2014). However, in industries that have vertical integration, such as poultry (Vukina, 2001), the task of information sharing becomes much easier. In recent years, the development of new technologies, such as RFID, has made the use of a dynamic shelf-life more realistic (Grunow and Piramuthu, 2013; Gaukler et al., 2017).

Because of its continuous growth in popularity and its economic importance, poultry meat was chosen as a model temperature sensitive food product. The National Chicken Council (NCC, 2021) reports that in last 60 years the amount of chicken consumed per person has increased by almost 70 pounds in the United States. However, red meat consumption has decreased by 22 pounds in the same period (NCC, 2021). This shift in consumer choice may be due to the reduced price and health benefits that come with consuming poultry meat as compared to red meat with 100g of boneless skinless chicken breast having less calories and fat than that of a T-bone steak

(USDA, 2019a, 2019b). Additionally, in the year 2021, the retail price of chicken was 209.4 cents per pound while beef was 724.9 cents per pound (NCC, 2022b). This difference in price is likely to be appealing for lower income consumers looking for a cheaper option to have protein in their diets. Also, to have adequate supply for this increase in demand, production of broilers has increased from 1,381 million pounds in 1950 to an estimated 44,933 million pounds in 2022 (NCC, 2022c). Also, the poultry production has a positive effect on the economy because of its export to other countries with approximately 18% (8,000 million pounds) exported in 2021 (USDA, 2021b). This increased in both consumption and production of poultry meat makes it a great example to investigate how decisions in the cold chain can affect temperature sensitive products.

The development of models to aid in FEFO decision making are needed. One such tool for retailers could be through the use of Monte Carlo simulations (La Scalia et al., 2019). Monte Carlo simulations are a vast group of algorithms that solve mathematical problems using random number generation (Johansen, 2010). Essentially, the Monte Carlo method allows the user to approach problem through the generation of random numbers (Joy, 1991). Previously, Monte Carlo simulations have been used in a variety of fields including, finance, engineering, and physics (Raychaudhuri, 2008). Monte Carlo simulations have been used in retail settings before in applications such as scheduling, customer satisfaction, and demand estimation (Blodgett and Li, 2007; Wan et al., 2018; Abello et al., 2020). Additionally, researchers have taken advantage of Monte Carlo methods to combat food loss and food waste (Aiello et al., 2011; Jedermann et al., 2014; La Scalia et al., 2019). However, there is limited research on TA during LTL in the last mile and its effects on shelf-life and food waste. Therefore, we conducted experiments to test the microbiological shelf-life implications of TA in the last mile during LTL shipping on a temperature sensitive product. Additionally, Monte Carlo simulation models are developed using temperature

data to assess the risk of TA and predict remaining shelf-life of different boxes on a TA pallet. The objective of this research is to provide mangers at the retail level to have better tools at their disposal to make better decisions regarding product rotation and storage of temperature sensitive products to maximize profits and reduce food waste.

Methodology

Recording Pallet Temperature

A commercially produced pallet containing boxes with tray packs of fresh, raw, boneless, skinless chicken breast fillets was transported to the Charles C. Miller Jr. Poultry Research Center at Auburn University under refrigeration and stored in a walk-in cooler (4°C for 12-16 h) prior to experiment. The dimensions of the pallet were as follows: 4 layers of boxes x 5 boxes per layer x 24 tray packs per box x 3-6 fillets per tray pack. Boxes were serially marked from 1-20 (layer 1: 1-5; layer 2: 6-10; layer 3: 11-15 and, layer 4: 16-20). Temperature at the center of the breast fillet and the air inside the box were measured every 1 to 5 min interval for 24 h using TM500 12-channel thermocouple dataloggers connected with wire temperature probes (Extech Instruments, Nashua, New Hampshire, USA) with 2 temperature probes per box. The tray located approximately in the center of each box was chosen and the temperature probe was inserted in the center of the middle fillet in the tray and the wire was secured by sous vide tape. At the conclusion of the experiment, temperature data was retrieved from the data loggers using MS Excel (Version 16, Microsoft Corporation, Redmond, WA). This experiment was repeated 5 separate times, and the temperature patterns was analyzed using line graphs generated using Excel.

Simulated Temperature Abuse

TA during LTL was simulated as follows: The pallet was exposed to 2 h at 4°C, simulating a refrigerated truck, and 2 h at 23±2°C, simulating hypothetical TA that occurs when the truck

doors are open. The experiments were conducted by moving the pallet in and out of the walk-in cooler maintained at 4°C for a total of 6 TA cycles. A separate representative box was kept in the walk-in cooler for the duration of the 24 h experiment which acted as the control. The temperature of the control box and the outside room temperature were recorded with a probe thermometer every 2 h.

Thermal Imaging

During the TA trials, thermal images of the pallet were taken using an infrared camera (BCAM 9Hz 120 x 120 Thermal Infrared Camera, Teledyne FLIR, Wilsonville, OR, United States) every 2 h of the experiment. The camera was pointed centrally at the pallet from all 4 sides at a distance of 4 ft when shooting images. The images were saved to a removable memory card in the camera and were retrieved at the conclusion of each trial of the experiment.

Spoilage Study

Microbiological sampling was conducted before (0 h) and immediately at the end of the simulated TA (24 h) to determine if TA impacted the bacterial levels of the raw poultry breast meat. Two predetermined boxes were chosen from each layer to have microbial sampling. The boxes sampled were as follows: layer 1: boxes 1 and 4, layer 2: boxes 7 and 10, layer 3: boxes 12 and 14, and layer 4: boxes 17 and 19. Two tray packs were randomly chosen from each box and 1 fillet per tray was sampled for microbiological analysis. One fillet from each selected tray pack was aseptically placed in a sterile bag (18 x 30 cm, 1650mL, VWR, Radnor, PA, United States) and manually rinsed with 50 mL of buffered peptone water (Neogen Corporation, Lansing, MI) for 1 min, and the rinsate was serial diluted and spread plated in duplicate onto Standard Methods Agar Petri plates (Neogen Corporation). The Petri plates were incubated either at 37°C for 24-48 h to estimate aerobic plate counts (APC) or at 4°C for 10 days to estimate psychrotrophic plate

counts (PSY). After the incubation period, viable colonies on the Petri plates were counted and reported as log CFU/mL of rinsate.

Shelf-life Assessment of Raw Breast Fillets After Temperature Abuse

The effect of TA on the changes in shelf-life of raw chicken breast meat was assessed using 2 boxes from a TA abused pallet. The trays from 2 boxes that crossed 4°C the fastest were pooled together, stored at 4°C and sampled for microbiological analysis every 2 days until the APC counts reached 7 logs. Non-TA chicken trays were stored and sampled in a similar manner to act as a control. During each sampling time, 3 trays from the TA and control samples were sampled in the same manner as in the spoilage study. Plates were counted after 24 h (APC) and 10 days (PSY), and a two-sample equal variance t-test was performed using Excel at each sampling day to determine if bacterial concentrations are significantly different at the chosen sampling times.

Monte Carlo Simulations

Two methods were developed using Monte Carlo simulations. One method predicts the risk of TA based on box number, and the other predicts the remaining shelf-life according to the level of TA experienced. The simulations allow us to take our existing data at specific time points and increase it to a large degree. The data generated through these simulations enabled us to develop predictions based on 500 data points rather than only using the limited amount of data acquired through physical testing. For the first method, Monte Carlo simulations using temperature data from h 0, 6, 12, 18, and 24 were performed. Temperature recordings from 4 or 5 of the trials at these time points was used to calculate an average temperature and standard deviation of temperature for each box at each time point. Next, using the mean and standard deviation, we generated a random number using the Excel formula: NORMINV(rand(),mu,sigma) In this formula, rand() creates a random value between 0 and 1, mu is the sample mean, and sigma is the

sample standard deviation (Winston, 2022). When using this formula, the user is calculating the pth percentile of a normal random variable occurring with the chosen mean and standard deviation (Winston, 2022). Following the initial number generation, 500 iterations were run at each timepoint for each box. From the 500 generated numbers, we obtained an average, standard deviation, maximum, and minimum temperature for each box at each time point. Lastly, we calculated what percentage of the 500 numbers was >4°C at each time point. Formula 1 illustrates how risk of TA was calculated with $n_{>4}$ being the number of simulations >4°C.

$$\%Risk\ of\ TA = n_{>4°C} \times \frac{100}{500} \quad (1)$$

The second method used temperature data and APC data from the control and TA fillets. The remaining shelf-life was calculated for 3 control fillets (0 h >4°C) and 3 TA fillets (8 h >4°C). A graph was constructed with shelf-life graphed vs. time >4°C. Next, a trendline was fit to the data and a linear regression equation was obtained (Formula 2).

$$Y = -0.379x + 7.1597 \quad (2)$$

Estimates of shelf-life were obtained for different levels of TA by substituting values in for "x" (0, 2, 4, 6, and 8 h) and solving for "y." The shelf-life values (y) and a standard deviation (1) were used to run Monte Carlo simulations (500 iterations) in the same manner as previously described using Excel. From the 500 generated numbers, the percentage l<4 days were calculated. This value is referred to as "risk-of-loss" because of the assumption retailers cannot sale all the product before spoilage before 4 days. Formula 3 shows how risk-of-loss was calculated with $n_{<4\ days}$ being the number of simulations being <4 days of shelf-life.

$$\%Risk\ of\ Loss = n_{<4\ days} \times \frac{100}{500} \quad (3)$$

Results

Pallet Temperature Profile

The graphs in Figures 6-9, illustrate the average temperature profiles of each layer of the pallet from 4 replications of the TA experiment. For each layer, the individual temperature profiles for each box are illustrated, and a line has been placed at 4°C to make it easier to see layers and boxes crossed into the "temperature "danger zone". Overall, the TA affected the top and bottom layers of the pallet more severely than the middle 2 layers. Figure 6 shows all the boxes in layer 1 crossing 4°C by approximately 12 h. Next, Figure 9 shows that all but 1 of the boxes (box 19) had crossed 4°C by 24 h. The middle 2 layers of the pallet (Figures 7 and 8) were less impacted as shown by the less dramatic increase in temperature. In these layers, most of the boxes never reached 4°C with only boxes 6 and 13 crossing 4°C for layers 2 and 3, respectively. Box 13 in layer 3 had a much more dramatic increase than any of the other boxes in the middle 2 layers of the pallet. In all layers, the temperature profiles show peaks and valleys. These peaks and valleys of temperature are the more dramatic in the top and bottom layers (Figures 6 and 9) than in the middle layers (Figures 7 and 8).

Figure 10 shows how the boxes were oriented in each layer. Layer 1 has a different orientation than the rest of the layers. The boxes are color coded for each layer according to the order in which they reached 4°C. Regarding the severity of TA, the box order is as follows from hottest to coldest: red, orange, yellow, blue, and purple. Also, each box has the average time (h) required to reach 4°C listed inside it. In layer 1, all boxes crossed 4°C by 12.03 h on average. The fastest box to reach 4°C was box 5 which averaged 9.68 h, and the slowest box was box 4 which averaged 12.03 h. In layers 2 and 3, only 1 box reached 4°C. In layer 2, box 6 required 16.95 h on average to surpass 4°C, and in layer 3 box 13 required 20.02 h on average to reach 4°C. In the

bottom layer (layer 4), 4 boxes reached 4°C by 24 h. Boxes 16, 17, 18, and 20 surpassed the "temperature danger zone" threshold at 20.98, 16.85, 15.87 and 17.73 h, respectively. From a layer-by-layer perspective, layer 1 had 5 boxes, layers 2 and 3 had 1 box, and layer 4 had 4 boxes reach 4°C. The impact of box position is indicated by the colors in each layer. In all layers, the centrally placed box is colored purple indicating it was either the least abused box or it never crossed 4°C. Also, the most abused box was always a perimeter box. In the case of layers 2, 3, and 4, the most TA box were stacked at the same location of the pallet.

Thermal Images

The images in Figures 11 and 12 show the immediate and long term impacts of cyclically removing the pallet of chicken breast from the walk-in cooler. Figure 11's images were taken less than 5 min after the pallet was pulled out of the cooler for the first time. The darker colors show where the pallet is the coldest and the lighter colors show where it is the hottest. The center of the pallet (layers 2 and 3) shows darker purples and blues because they are the coldest areas. The outside edges have already demonstrated lighter colors after only 5 min of TA. In the images from Figure 12, the pallet has experienced 6 TA cycles and is at the conclusion of the experiment. These images show most of the pallet is reddish yellow. Therefore, box temperatures have increased in comparison to original temperatures (Figures 11 and 12). However, even after 24 h of TA, thermal imaging shows heterogeneity in box temperatures based on location. The is exemplified by the darker colors still visible in the central regions.

Microbiological Results

Results were obtained from 2 kinds of spoilage microorganisms. The results are from immediately before TA and immediately after TA or 24 h later. For both APC and PSY there was no growth on the chicken breasts sampled after 24 h. The APC decreased by ~0.2 logs and the

PSY decreased by ~0.1 logs at the conclusion of the experiment. However, the similarity in bacterial concentration at the beginning and end of TA is not conducive of potential growth if fillets are kept for shelf-life.

Figures 13 and 14 show the shelf-life results of the fillets kept after the TA experiment had concluded. The fillets from the 2 hottest boxes from layer 1 was kept for sampling with the control box. The results of both the APC and PSY show a difference in shelf-life of a couple of days between TA and control fillets. Regarding APC, the TA fillets reached spoilage (7 logs) after approximately 4.1 days. While the control fillets required approximately 7.2 days. This is about a 3 day difference in time required for spoilage to occur. The PSY results showed the control fillets took 5.5 days, while the TA fillets required only 3.9 days to reach spoilage. This is a difference of approximately 1.5 days in shelf-life. When comparing the values at each sampling time, it was found that the means were significantly different at day 4 for both the APC and PSY. The values at day 4 for APC were 5.8 ±0.64 and 6.8 ±0.11 log CFU/mL for control and TA, respectively. Further, the values for PSY at day 4 were 6.1 ±0.33 and 7.2 ±0.15 logs for control and TA, respectively. However, no other days were observed to be significantly different with all p-values being greater than 0.05. For APC, variance in bacterial concentrations ranged from 0.29 on day 8 to 0.99 on day 6 for control fillets and 0.11 on day 4 to 1.04 on day 2 for TA fillets. Regarding PSY, variance in bacterial concentrations ranged from 0.03 on day 8 to 0.40 on day 2 for control fillets and 0.15 on day 4 to 1.11 on day 2 for TA fillets.

Monte Carlo Results

Tables 4-7 show the results of the Monte Carlo simulations on the 4 layers of the pallet and have the percent chance of each box within that layer of reaching 4°C at 5 different time points. The results of these simulations are consistent with the temperature profile results in that the

middle layers (Tables 5 and 6) appear to be less affected than the top (Table 4) and bottom layer (Table 7). For layer 1, there is a linear growth in risk of reaching the "temperature danger zone" as time goes on. After only 12 h, all boxes are approximately 50%, and all layer 1 boxes have at least an 86.5% chance of reaching 4°C by the end of the experiment (Table 4). Layer 2's results show a risk of less than 41% after 24 h (Table 5). However, box 6 has a 91% risk level after 24 h (Table 5). Next, layer 3 boxes have the lowest overall risk for reaching 4°C (Table 6). At 12 h, layer 3 boxes were all below 11% with 4 out of 5 boxes being below 4%. Only 1 box in layer 3 (box 13) crossed 50% after 24 h. Lastly, layer 4 results show 2 boxes above 10% after 12 h. Four of the boxes in layer 4 were above 64% with the remaining box being at 47.4% after 24 h. Layer 4 boxes were relatively similar to layers 2 and 3 after 12 h (Table 7). However, after 18 h, the percentages spike and results start approaching the numbers seen for layer 1 (Table 7). This is in contrast to layer 1 where there was a linear increase in risk. Results from the second Monte Carlo method are shown in Table 8.Values for the prediction of shelf-life and risk-of-loss at 5 different TA scenarios (0, 2, 4, 6, and 8 h >4°C) are shown. The risk-of-loss increases dramatically after 6 h >4°C (17.8%) and reaches a maximum of 43.8% after 8 h >4°C. Also, shelf-life reduction increase by approximately 10% every additional 2 h spent >4°C with a maximum value of 42.4% after 8 h. Lastly, the shelf-life decreases by approximately 3 days if 8 h above 4°C is achieved (Table 8).

Discussion

Temperature Profiles

The temperature profiles of the 4 layers seem to follow a trend. The top and bottom layers were most affected by the TA, while the middle 2 layers seem to be less affected. The top layer was the most affected as by the halfway point of the experiment all boxes had reached 4°C (Figure

6) and entered the “danger zone” for bacterial growth. This is the layer that needs to be managed differently as it is in the most danger of spoilage. Next, the bottom layer (Figure 9) is the second most affected. By the end of the experiment all boxes had reached, or were approaching, 4°C. It is likely the reason for this is the top layer of the pallet is more exposed than the other layers, and the sides are in contact with the air surrounding the pallet. This is also evident in how the peaks and valleys of the boxes on layer 1 is more dramatic than that of the other layers. Furthermore, the bottom layer (layer 4) is in direct contact with the wooden pallet which is in direct contact with the floor likely resulting in more dramatic influence by the TA. However, layers 2 and 3 of the pallet (Figures 7 and 8) are shielded by the top and bottom layers of the pallet. Also, the pallet has a plastic wrap that is applied around the perimeter of the box. This wrap may be helping the middle 2 layers stay insulated and less impacted by the TA. TA studies have been conducted using different foodstuffs previously. In South Africa, a study was done to record the temperature profiles of fruits and vegetables being shipped in reefer trucks (Emenike and Hoffmann, 2014). They used temperature loggers to measure the deviations from the set temperature of 2°C at different locations inside the reefer truck. However, like our experiment they found that it is possible to have serious deviations from the set temperate with average temperatures being as high as 12.5°C to 14.9°C. Margeirsson et al. (2012) determined that box position did result in different levels of TA when investigating air and sea transport conditions for cod fillets. While this experiment differs in the mode of transport the principles are similar. They found that TA boxes had shorter shelf-lives when compared to control boxes. A study on home delivery services in Taiwan estimated the impact of TA on frozen shrimp (Ndraha et al., 2019). In this study, they observed that there was temperature greater than the target -18°C in over 50% of the time during transport. Not unlike our study on the pallet, experiments on meal delivery kits or home delivery

services face similar challenges throughout the cold chain. They will need to meet temperature standards as well so that the customers do not receive spoiled or unsafe food.

When looking at the boxes on a more individual level (Figure 10), it appears that box position has an impact on the level of TA experienced. The more centrally located boxes were all purple indicating they experienced the least TA in that layer of the pallet. However, a perimeter box was always the most abused for that layer. This indicates that not all boxes are equal in terms of the level of abuse they experience. If this is the case, it could be inferred that the remaining shelf-life of each box is not equal. However, up to this point all boxes on a pallet would be treated the same because of the same arrival time. In layer 1, after only 9.68 h there is already a box that has crossed into the "temperature danger zone". While in the middle layers (layers 2 and 3) 8 out of 10 boxes never crossed 4°C (Figure 10). The results of the temperature profiles indicate the need for more intense temperature recording because of the diverse levels of abuse experienced by different boxes. In contrast to our results, a study was done by Göransson et al. (2018) where they found that the position on the pallet does not have an impact on shelf-life. However, they calculated thermal time constants and used it to find the most at risk spots on the pallet. From there they estimated the difference in shelf-life. Lastly, our experiment only looks at a single pallet, However, in LTL shipping it is likely there will be multiple pallets of different sizes in the same reefer truck. It has been shown that it is possible for different zones of the refrigerated truck to have different air velocities resulting in higher or lower temperatures (Moureh and Flick, 2004).

Thermal Images

For this experiment, the overall thermal picture of the pallet was of primary interest. The goal was to determine if a noticeable difference in temperature between layers or boxes existed. However, the goal was not to record the actual temperatures since individual thermocouple wires

were already placed in each box. The results of are thermal images are consistent with that of the temperature profile results (Figures 11 and 12). Layers 1 and 4 of the pallet are hotter than layers 2 and 3 (Figure 11 and 12). Also, the interior boxes are cooler than the perimeter boxes (Figures 11 and 12). Thermal images are useful in that they provide a quick and straightforward way to visualize the current state of a food product. Also, thermal imaging is a nondestructive method since the user can stand several feet away when taking the images (Vadivambal and Jayas, 2011). Perhaps loading docks or cold storage facilities could benefit in the investment in thermal imaging cameras because it will aide in the decision making process of rotating their product onto shelves. Thermal cameras allow decision makers to have an idea of which product is in the most danger of spoiling. In agriculture thermal imaging is already being studied in a multitude of ways. For example, Ding et al. (2017) investigated using thermal imaging as a classification tool for grapes at different levels of decay. They were able to categorize the grapes with greater than 90% accuracy. In India, a study was conducted using thermal imaging to aide in the decision making process regarding the storing of grains (Nanje et al., 2013). Additionally,, thermal imaging is helpful in detecting bruises on fruit (Varith et al., 2003). In this experiment, Varith et al. (2003) were able to use the thermal imaging technology to detect differences in temperature between the bruised and healthy tissue of the fruits. Cold chain management could be another area of use tin the future for thermal imaging technologies. However, the results in terms of an actual temperature reading may not be accurate enough if the pallet has certain types of covers (Badia-Melis et al., 2017).

Bacterial Growth During TA and Shelf-life

APC and PSY both had almost no differences in their initial and final concentrations when sampled immediately before and immediately after TA. This is likely because the conditions chose

by us to TA the chicken was not severe enough to cause the bacteria to immediately enter log phase. However, differences are visible when the fillets are kept for shelf-life. Results indicate, TA can result in a difference in shelf-life up to approximately 3 days for boneless skinless chicken breast fillets (Figure 13). Our temperature results indicate that there is a difference in the amount of abuse experienced based on the box location on the pallet (Figures 6-12). Therefore, the boxes chosen to be kept for the shelf-life study may have impacted the remaining shelf-life. In our case, only 2 TA boxes were kept. The shelf-life of boxes that did not cross 4°C (Figure 10) may have been similar to that of the control had they been selected. The shelf-life experiment demonstrates the most TA boxes spoil before the control. If decision makers in the cold chain had this knowledge, new practices could be put in place to sale temperature sensitive products more efficiently. It is possible that a pallet received a day before may have boxes that will keep for longer than boxes on a more recently received pallet. However, under current practices, it is likely that the boxes from a pallet that arrives first will be put on display first. This may allow for food waste to occur due to spoilage. Both food waste and monetary loss are likely outcomes in this situation. In contrast, the product that is more likely to expire first could be displayed first allowing for less waste. This could benefit consumers and poultry meat suppliers at poultry consumption is likely to continue to rise in the coming years (NCC, 2021a).

A study by Senter et al. (2000) found that holding chicken meat samples at a temperature of 13°C for 48 h had similar APC results to their TA scenario (3 days at 4°C then 1 day at 13°C then 1 day at 4°C). They recorded APC values of 8.19 and 9.48 log CFU/mL for the 13°C and TA scenarios, respectively. Like our experiment, this study was attempting to simulate TA during the shipping of temperature sensitive products. However, in our study cyclic TA was used to simulate LTL rather than 1 prolonged TA situation followed by cold storage. Another bacterial growth

study on TA chicken was performed by exposing inoculated chicken breast to different temperatures for 12 h before storage at 5°C (Casanova et al., 2021). The chicken breasts exposed to 20°C and 25°C were found to only be fit for consumption for up to 12 h, while the control was good for 12 days. In Iran, Ghollasi-Mood et al. (2016) exposed chicken to 4°C, 10°C, and 15°C for 8 h each. They found that it took approximately 125 h for total viable counts to reach spoilage levels under ideal storage conditions, while TA (cyclic 8 h at 0°C then 8 h at 10°C then 8 h at 15°C) chicken took approximately 50 h. Russell et al. (1996) exposed whole chicken carcasses to TA of 25°C for 12 h before returning them to ideal storage temperatures. This was done each day for 7 days before all carcasses were sampled after 9 days. They found that the day in which the TA occurred had little impact on the bacterial growth. Also, a study was done on the affects TA has on bacterial diversity growing on the surface of chicken (Zhang et al., 2012). In this work, chicken meat was packaged in air and subjected to isothermal 4°C and 2 fluctuating temperature conditions (0-4°C and 4-10°C). Zhang et al. (2012) found that when the temperature is fluctuating there is a more intricate diversity of microflora present.

Monte Carlo

Our analysis of the risk associated with our TA conditions using the Monte Carlo method was consistent with what we observed in our temperature profiles. The results indicate an increased risk of crossing 4°C for the top (layer 1; Table 4) and bottom layer (layer 4; Table 7) of the pallet. The top layer seems to follow a linear trend in that the risk increases in a relatively consistent manner over the 24 h (Table 4). However, the bottom layer is perhaps more interesting in that up to h 12 all boxes are below 15%, but once 18 h is reached the risk increases more dramatically for all boxes (Table 7). Also, box 6 is an outlier with a higher risk (91.4%) than all other boxes in the middle 2 layers (Tables 5 and 6). The reason for this is unknown, but we do know that box 6 is a

perimeter box and because of this it may have been more impacted than the others. This does not explain why the other perimeter boxes were not influenced in a similar manner. This information demonstrates the complexity associated with the TA that might occur in the supply chain and why more intense temperature monitoring is needed. Perhaps a retailer could have a program already in place at the store and have the temperature history uploaded via other technologies (for example RFID) allowing the retailer to take advantage of the information at their disposal. Once this data is observed, the managers could easily and quickly make the most beneficial decision regarding product spoilage. Having simulations or predictive models at their disposal would allow the end user in the cold chain to make decisions without having to have an in depth understanding of microbiology. There would be no microbiological sampling involved, and it would not be necessary to have a lab to perform tests on the samples to determine the remaining shelf-life.

Shaffner et al. (2003) used the Monte Carlo method to present its usefulness in risk assessment of milk. Using the method, they found that decreasing the temperature of the milk by approximately 2°C resulted in a decrease in greater than 50% of spoilage of milk after 14 days. Next, a predictive model using Monte Carlo was developed to predict the spoilage of milk that has been contaminated after pasteurization (Lau et al., 2012). This model allowed the developers to investigate the effectiveness of different measure in controlling the spoilage of milk. Monte Carlo simulations are not limited to just assessing risk of spoilage microorganisms, and they can be used for food safety implications. For example, risk assessment of illness caused by *Salmonella* Enteritidis in eggs has taken advantage of Monte Carlo simulations (Schroeder et al., 2006). Using this method, they were able to estimate the decrease in illnesses that would occur if the eggs were pasteurized to different levels of bacterial reduction. Zeng et al. (2014) studied pathogen growth in lettuce due to fluctuating temperatures during the last mile. They used Monte Carlo methods to

perform over 8 billion simulations of scenarios that might occur. This level of sampling might prove to be costly and labor intensive. However, because of the simulations they were able to quicky and cheaply get feedback on how best to manage leafy greens to control pathogen growth. Also, fungal spoilage of yogurt has been studied using similar simulations (Gougouli and Koutsoumanis, 2017). They were able to estimate the diameter of fungal growth in the yogurt and develop a model that may be useful in quality decisions regarding the production of yogurt products.

The risk-of-loss predictions via Monte Carlo methods indicate that approximately 44% of chicken breast fillets will have a shelf-life of less than 4 days after 8 h >4°C. This method is assuming a retailer will be unable to sale product fast enough once a minimum acceptable shelf-life is reached. Therefore, the product would have to be discarded leading to food waste. In this experiment, 4 days was chosen, but the method could be altered to predict risk-of-loss at a different value for minimum shelf-life. The significance of a 44% loss becomes evident when observing the monetary impact. If a pallet of chicken breasts weighs 1000 pounds, 440 pounds could potentially be wasted. According to the USDA (2022b), the price of tray packed chicken breasts is $3.06/pound. Therefore, 1 pallet could result in a financial loss of $1,346.40 if 8 h of LTL TA occurs. This highlights the need for a reliable prediction method to mitigate this food waste and associated monetary loss. Monte Carlo methods have been used to predict shelf-life of various perishable products (Hutter et al., 2001; Schaffner et al., 2003; Giannakourou and Taoukis, 2019). These products include different foodstuffs (Ndraha et al., 2019; Lau et al., 2022) and pharmaceuticals (Su et al., 1994; Waterman et al., 2007), but there is limited literature on the use of Monte Carlo simulations for the prediction of poultry meat shelf-life. A study was conducted using Monte Carlo to validate another method (delta method) to predict the shelf-life of several

cultivars of tomatoes (De Ketelaere et al., 2004). In this study, it was determined that the delta method's predictions closely matched the predictions of the Monte Carlo method making their model an acceptable method. Also, Mataragas and Drosinos (2007) used Monte Carlo simulations and time-temperature profiles to create probability distributions of shelf-life and food safety risk associated with of ready to eat sliced ham. Lactic acid bacteria and *Listeria monocytogenes* were used for shelf-life and food safety risk, respectively. However, they determined time-temperature profiles were the better choice for shelf-life because of the overpredictions of the Monte Carlo methods. Additionally, the variability of parameters associated with the deterioration of frozen fruits and vegetables has been successfully implemented in remaining shelf-life models via Monte Carlo methods (GiannKouro and Taoukis, 2019).

Conclusions and Future Work

The last mile presents challenges to cold chain management in LTL scenarios. Therefore, the current FIFO model may be outdated and need to be replaced with the FEFO model. To achieve this a pallet of boneless skinless chicken breast was subjected to simulated supply chain TA conditions. From this experiment, we found that when exposed to cyclic TA, the pallet's layers react differently to the TA. The top and bottom layers (layers 1 and 4) of the pallet increased in temperature at a faster rate than the middle 2 layer likely due to the level of protection experienced by the middle layers (layers 2 and 3). Also, the boxes that were centrally placed in each layer experienced less TA than the boxes positioned on the perimeter of the pallet. Thermal imaging followed a similar trend with the outside edges of the pallet already appearing warmer than the middle of the pallet after minimal exposure to hotter temperatures. Microbial sampling of both APC and PSY showed a difference in shelf-life between TA fillets and control fillets. This indicates that LTL TA has a negative result on the spoilage of palletized chicken breasts. Monte

Carlo simulations demonstrated an increased risk to reach abuse level temperatures in the boxes located on the top and bottom layers (layers 1 and 4) of the pallet with the top layer being the highest risk (>86%) after 24 h. Lastly, shelf-life and risk-of-loss predictions were completed (Table 8). After 8 h of exposure to temperatures >4°C, the risk-of-loss reaches nearly 44%, and shelf-life reduces from 7.2 days to 4.1 days. This study's results show the significance of improper management of the cold chain, especially at the retail level. Simulations and can be useful tools for managers when deciding how to treat their stock of temperature sensitive products, and they can allow them to more accurately judge which products to sell first.

In the future, more research is needed in last mile TA. More scenarios regarding the duration and levels of TA are needed. Also, merging modern technologies into similar experiments will be essential. For example, the use of RFID technology to record temperatures is a more realistic way of collecting TA data in the cold chain. Therefore, the same experiments should be conducted with RFID rather than dataloggers. Also, using the temperature and microbial data, work on calculating actual remaining shelf-life of products based on their level of TA during other durations and temperatures would be beneficial. Next, our experiment was on a single pallet of chicken breast, but we were unable to use an actual reefer truck. Exploring how other temperature sensitive products behave in similar scenarios as well as how the conditions in different positions of a reefer trick trailer would help to provide a clearer picture of what is happening in industry today.

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Tables and Figures

TABLE 1. Primary Modeling results: specific growth rate (μ) and the square root of specific growth rate (√μ) of *Salmonella* Typhimurium on chicken skin[a] and predictions of the square root of specific growth rate and growth rate using the extended Ratkowsky model[b].

Temperature (°c)	μ[c]	√μ[d]	Predicted √μ	Predicted μ
25	0.469	0.685	0.670	0.449
30	0.600	0.775	0.807	0.652
35	0.928	0.963	0.943	0.890
40	1.118	1.057	1.060	1.124
45	0.904	0.951	0.951	0.904

[a](Oscar, 2009)

[b](Ratkowsky et al., 1983)

[c]Specific growth rate

[d]Square root of specific growth rate

TABLE 2. Tertiary model results: Growth of *Salmonella* Typhimurium on inoculated boneless skinless chicken breast fillets during simulated temperature abuse less-than-truckload (LTL) conditions in the last mile of the cold chain.

	Time (h)[a]	Observed	Predicted	P/O[b]	Log_{10}[c]	ABS[d]
Trial 1	0	3.2	3.2	1.000	0.000	0.000
	6	3.2	3.2	1.000	0.000	0.000
	12	3.3	3.2	0.970	-0.013	0.013
	18	3.8	3.4	0.900	-0.046	0.046
	24	4.2	3.9	0.921	-0.036	0.036
Trial 2	0	3.0	3.0	1.000	0.000	0.000
	6	2.9	3.0	1.034	0.015	0.015
	12	3.1	3.0	0.968	-0.014	0.014
	18	3.1	3.2	1.045	0.019	0.019
	24	3.9	3.7	0.938	-0.028	0.028
Trial 3	0	3.1	3.1	1.000	0.000	0.000
	6	3.0	3.1	1.033	0.014	0.014
	12	3.1	3.1	1.000	0.000	0.000
	18	3.5	3.4	0.980	-0.009	0.009
	24	3.8	3.9	1.021	0.009	0.009
					BF[e]	**AF[f]**
				Sum	-0.088	0.202
				Sum/n	-0.006	0.013
					0.987	1.032

[a]Time (h): Time in h.

[b]P/O: Predicted value divided by observed value during TA conditions.

[c]Log_{10}: Log base ten of P/O value.

[d]ABS: Absolute value of Log_{10}.

[e]BF: Bias factor; sum of Log_{10} values divided by the number of observations used.

[f]AF: Accuracy factor; sum of ABS values divided by the number of observations used.

TABLE 3. Tertiary model results: Growth of *Salmonella* Typhimurium on inoculated boneless skinless chicken breast fillets kept at 25°C.

	Time (h)[a]	Observed	Predicted	P/O[b]	Log_{10}[c]	ABS[d]
Trial 1	0	2.6	2.6	1.012	0.005	0.005
	6	3.0	2.6	0.871	-0.060	0.060
	12	4.2	4.0	0.943	-0.025	0.025
	18	6.2	5.4	0.863	-0.064	0.064
	24	7.8	6.8	0.871	-0.060	0.060
Trial 2	0	2.9	2.9	0.998	-0.001	0.001
	6	3.2	2.9	0.916	-0.038	0.038
	12	4.5	4.0	0.890	-0.051	0.051
	18	5.8	5.4	0.922	-0.035	0.035
	24	7.3	6.8	0.927	-0.033	0.033
Trial 3	0	2.7	2.7	1.009	0.004	0.004
	6	2.8	2.7	0.964	-0.016	0.016
	12	3.9	4.1	1.038	0.016	0.016
	18	5.5	5.4	0.987	-0.006	0.006
	24	7.6	6.8	0.897	-0.047	0.047
					BF[e]	**AF[f]**
				Sum	-0.410	0.461
				Sum/n	-0.027	0.031
					0.939	1.073

[a]Time (h): Time in h.

[b]P/O: Predicted value divided by observed value during 25°C conditions.

[c]Log_{10}: Log base ten of P/O value.

[d]ABS: Absolute value of Log_{10}.

[e]BF: Bias factor; sum of Log_{10} values divided by the number of observations used.

[f]AF: Accuracy factor; sum of ABS values divided by the number of observations used.

TABLE 4. Monte Carlo derived risk (% chance) of top layer (layer 1) boxes of palletized chicken breasts reaching 4°C during 24 h of simulated less-than-truckload temperature abuse (2 h at 4°C then 2 h at 25°C).

	Box 1	Box 2	Box 3	Box 4	Box 5
Hour 0	1.6	1.6	0	0	0
Hour 6	12.6	5.6	0.2	18	0.8
Hour 12	46.2	55	75	51.4	81.6
Hour 18	99	98.2	94	66.8	98.4
Hour 24	100	100	88.2	86.6	100

TABLE 5. Monte Carlo derived risk (% chance) of second layer from the top (layer 2) boxes of palletized chicken breasts reaching 4°C during 24 h of simulated less-than-truckload temperature abuse (2 h at 4°C then 2 h at 25°C).

	Box 6	Box 7	Box 8	Box 9	Box 10
Hour 0	0	0	0	0	0
Hour 6	0.2	0	0	0	0
Hour 12	3.8	0	0	2.6	0.2
Hour 18	52.2	6.2	9.6	23.6	0.8
Hour 24	91.4	26.4	40.2	36.8	21.2

TABLE 6. Monte Carlo derived risk (% chance) of third layer from the top (layer 3) boxes of palletized chicken breasts reaching 4°C during 24 h of simulated less-than-truckload temperature abuse (2 h at 4°C then 2 h at 25°C).

	Box 11	Box 12	Box 13	Box 14	Box 15
Hour 0	0	0	0	0	0
Hour 6	0	0	0	0	0
Hour 12	2.6	0	10.6	0	3.8
Hour 18	8	0.4	35.4	3	7.8
Hour 24	27	11.8	54.2	11.6	31.4

TABLE 7. Monte Carlo derived risk (% chance) of bottom layer (layer 4) boxes of palletized chicken breasts reaching 4°C during 24 h of simulated less-than-truckload temperature abuse (2 h at 4°C then 2 h at 25°C).

	Box 16	Box 17	Box 18	Box 19	Box 20
Hour 0	0	0	0	0	0
Hour 6	0	0	0	0	0
Hour 12	0	6.6	13.2	0	19.8
Hour 18	24.4	43.6	66	15.2	44.8
Hour 24	64.4	82.8	94.2	47.4	86.8

TABLE 8. Monte Carlo derived shelf life predictions for cyclically temperature abused (TA; 2 h at 4°C then 2 h at 25°C) chicken breasts fillets determined by time (h) spent above 4°C.

TA >4°C (h)	Risk-of-Loss (%)	Shelf life remaining (days)	Shelf life remaining (%)	Shelf life reduction (%)
0	0	7.2	100	0
2	0.8	6.4	89.4	10.6
4	5.8	5.6	78.8	21.2
6	17.8	4.9	68.2	31.8
8	43.8	4.1	57.6	42.4

FIGURE 1. Predicted and observed growth curves from tertiary modelling of the growth of *Salmonella* Typhimurium on chicken breasts during 24 h of simulated LTL temperature abuse (2 h at 4°C then 2 h at 25°C).

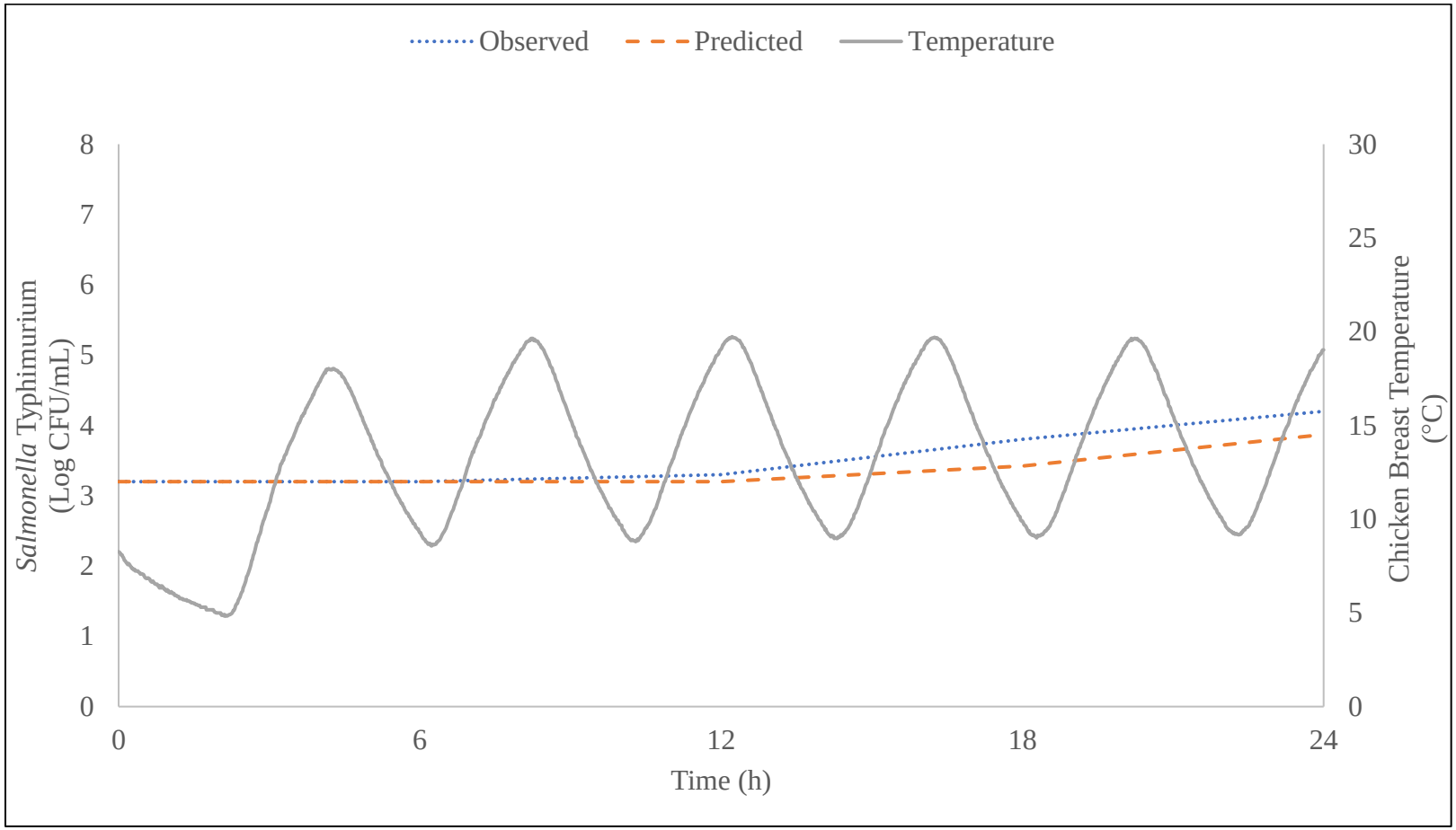

FIGURE 2. Predicted and observed growth curves from tertiary modelling of the growth of *Salmonella* Typhimurium on chicken breasts during 24 h of temperature abuse at 25°C.

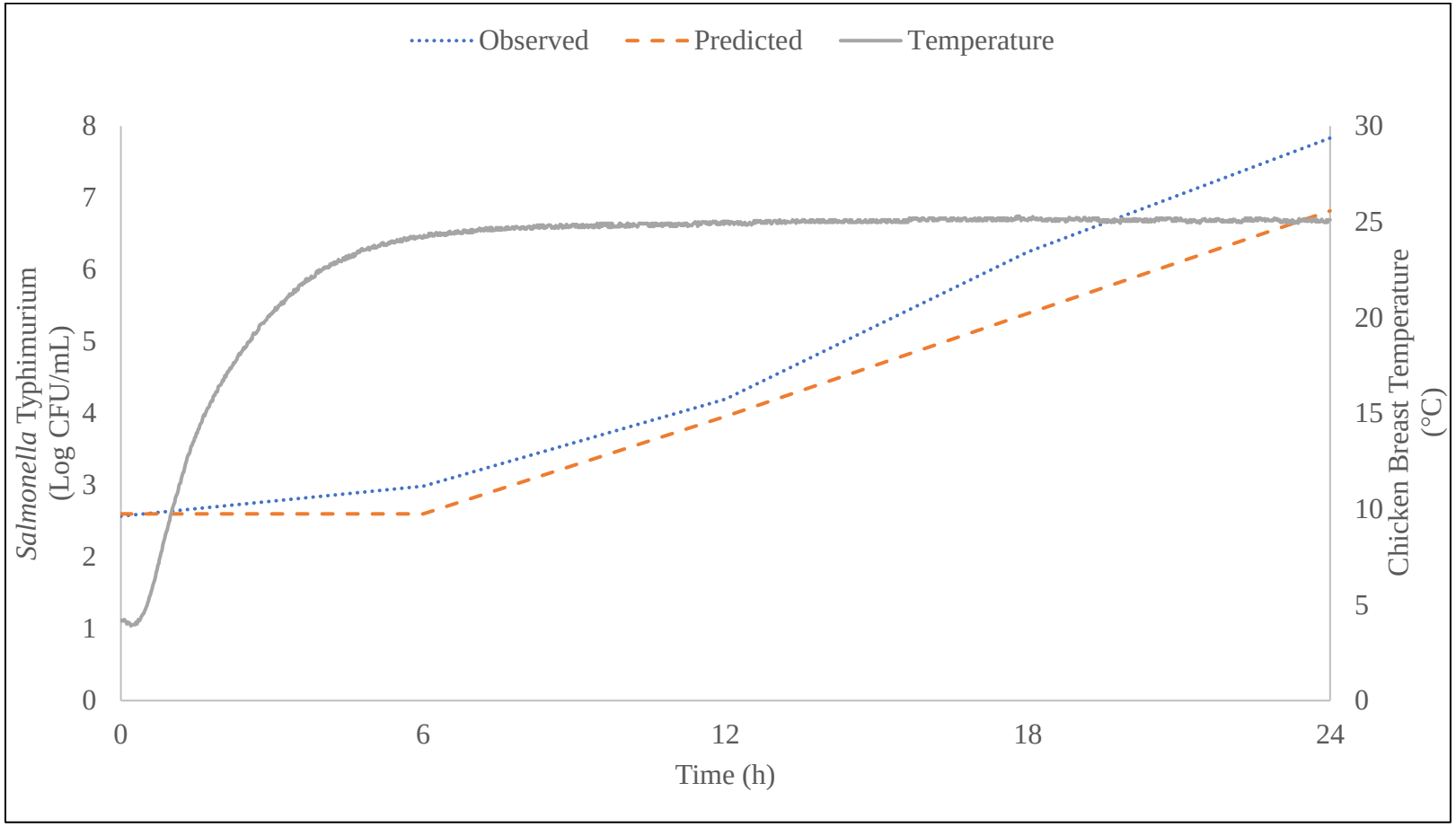

FIGURE 3. Temperature profiles of boneless skinless chicken breast fillets during three simulated supply chain storage scenarios (4°C, 25°C, and cyclic temperature abuse [TA]).

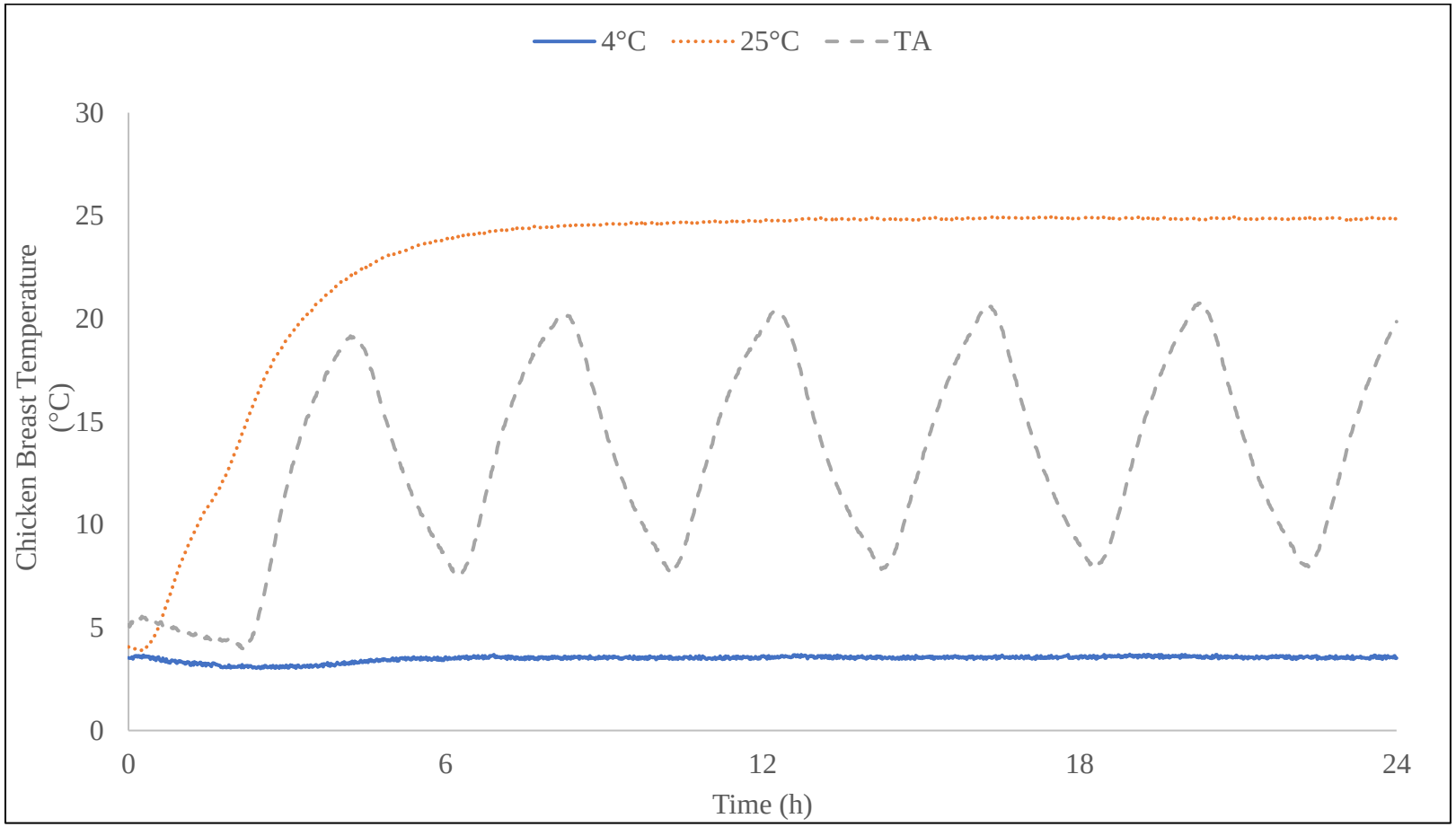

FIGURE 4. *Salmonella* Typhimurium growth during three simulated supply chain transportation temperature scenarios (4°C, 25°C and cyclic temperature abuse [TA]).

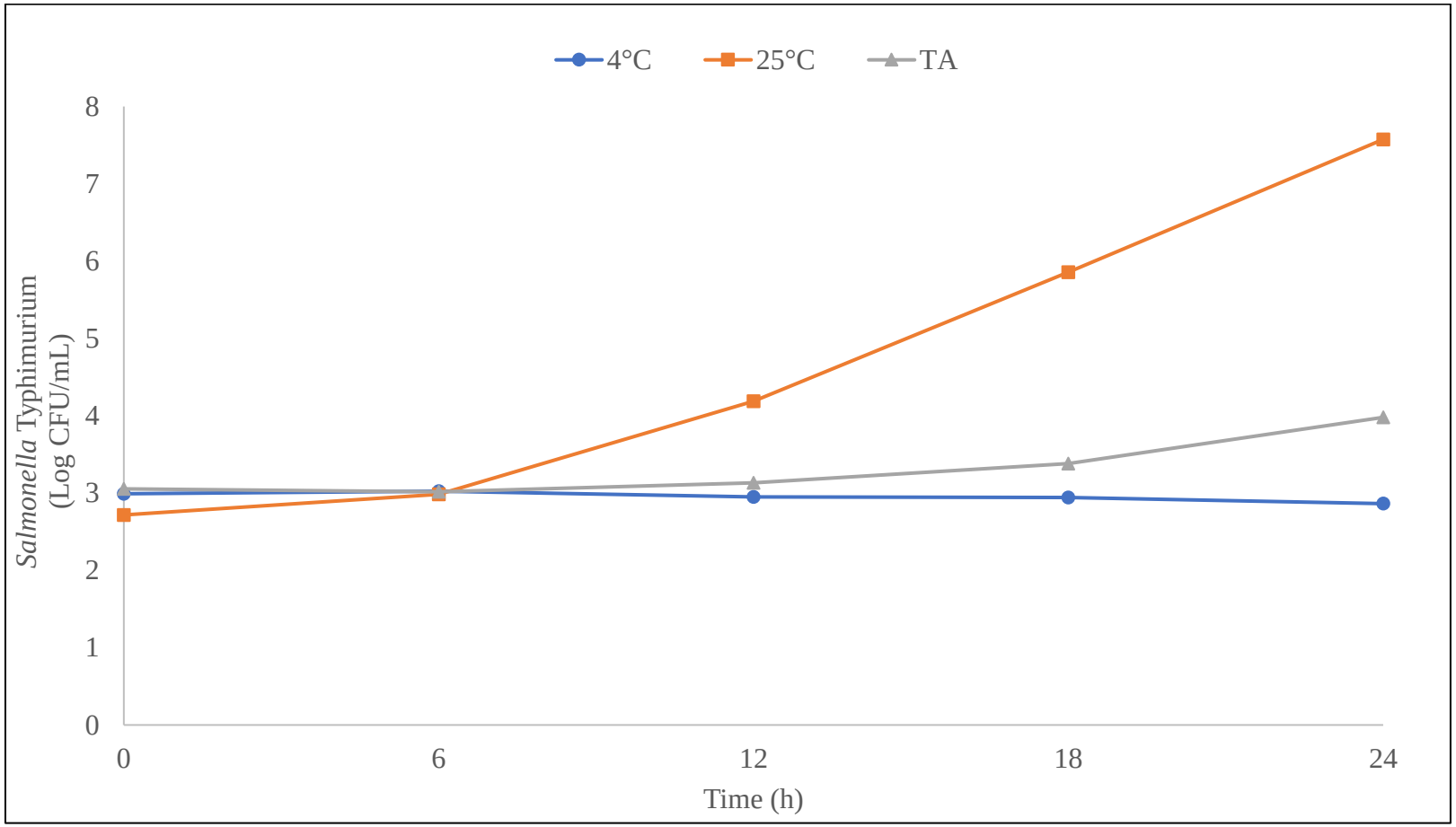

FIGURE 5. Aerobic plate counts during three simulated supply chain transportation temperature scenarios (4°C, 25°C and cyclic temperature abuse [TA]).

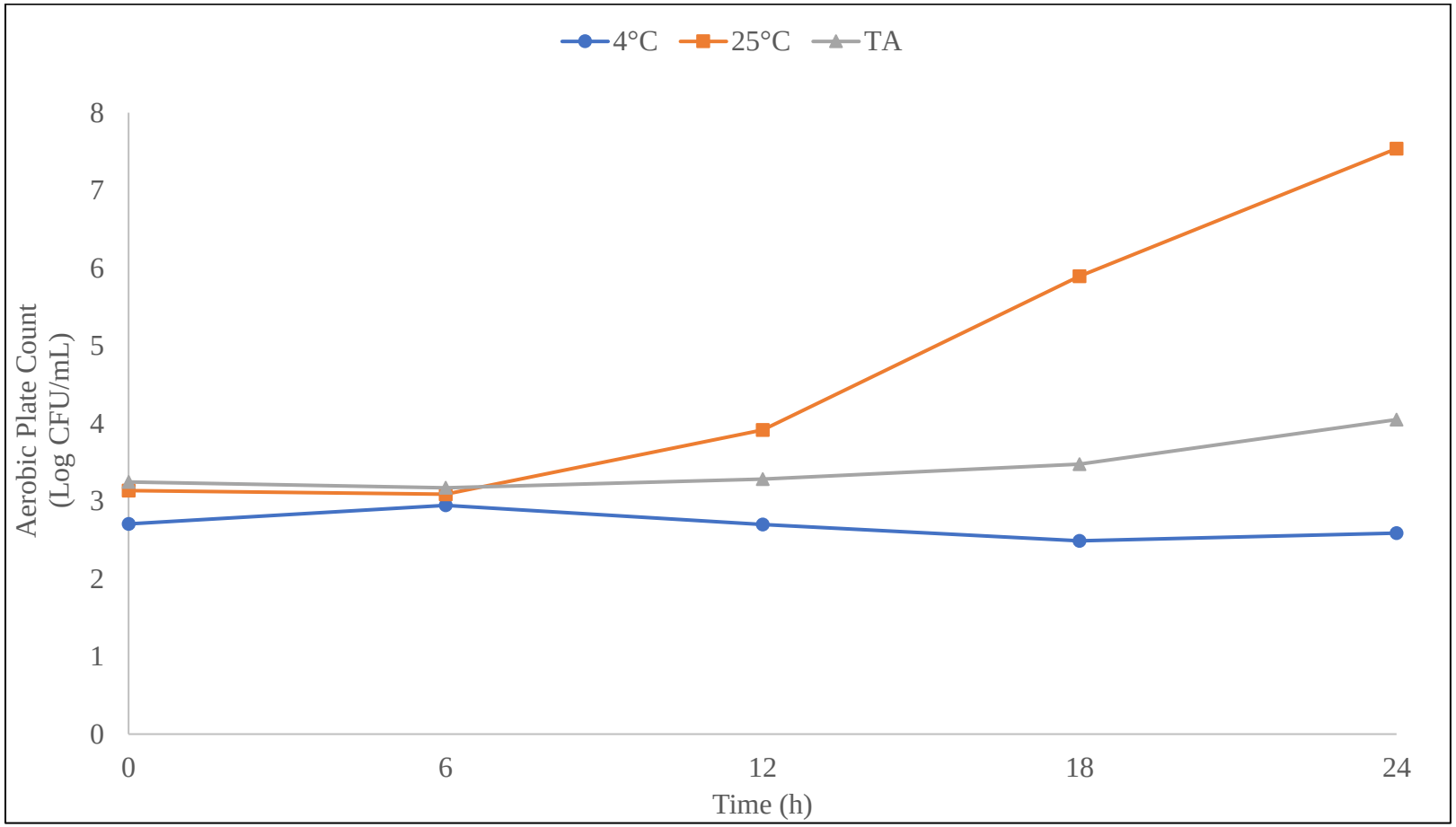

FIGURE 6. Averaged temperature profiles (4 replications) of top layer (layer 1) boxes of palletized chicken breasts while experiencing simulated LTL cyclic TA (2 h at 4°C then 2 h at 25°C).

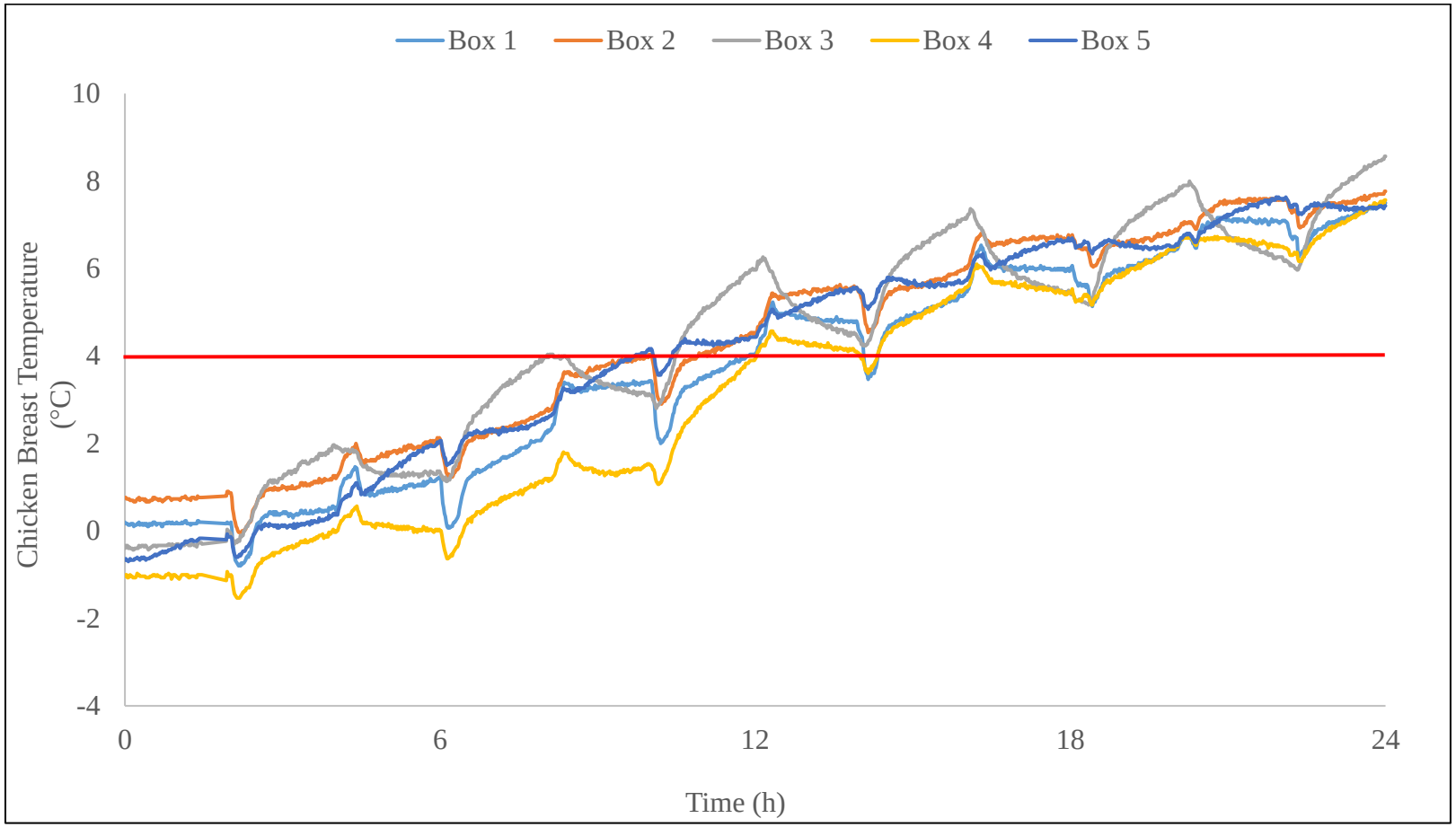

FIGURE 7. Averaged temperature profiles (4 replications) of second layer from the top (layer 2) boxes of palletized chicken breasts while experiencing simulated LTL cyclic TA (2 h at 4°C then 2 h at 25°C).

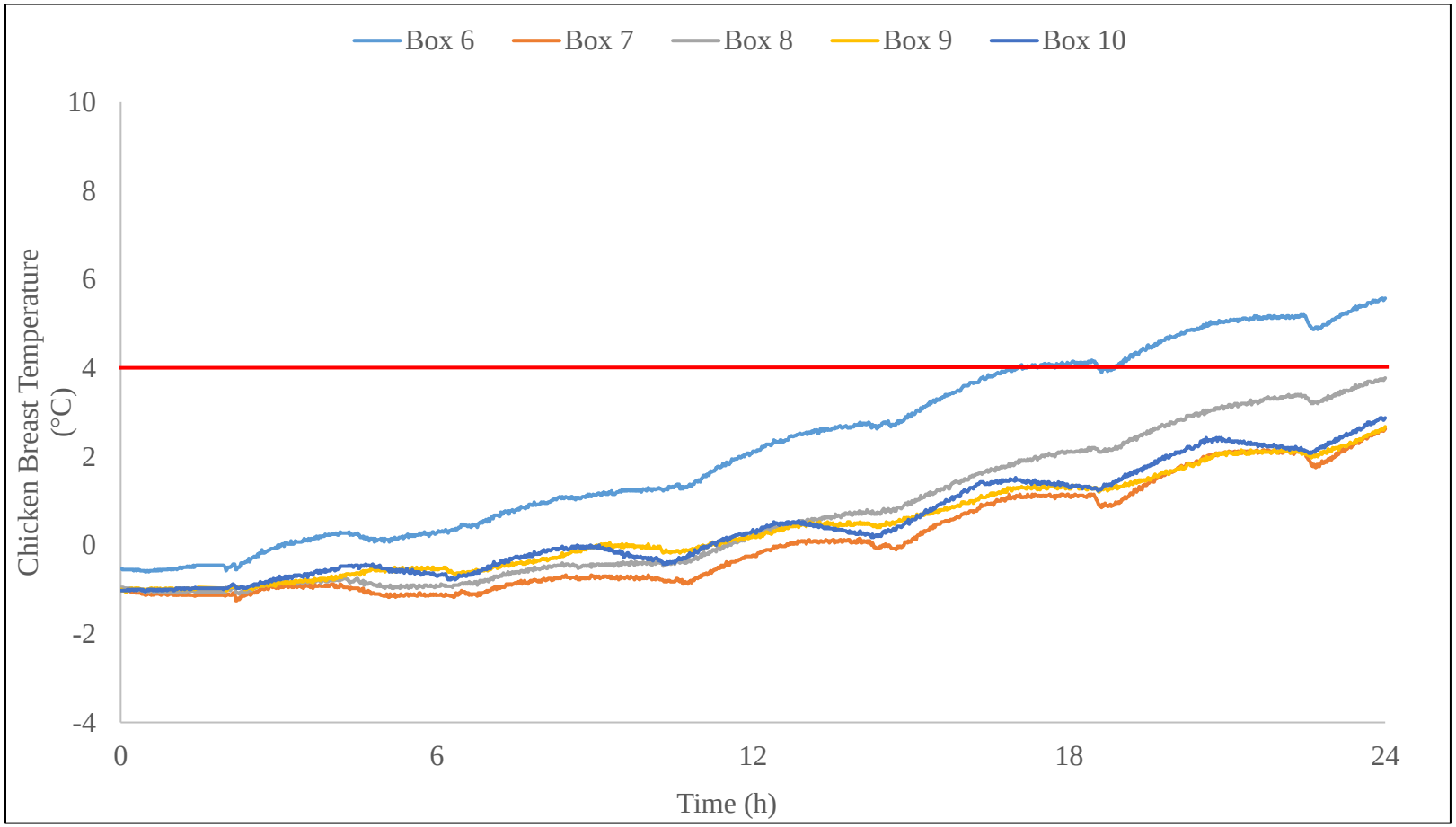

FIGURE 8. Averaged temperature profiles (4 replications) of third layer from the top (layer 3) boxes of palletized chicken breasts while experiencing simulated LTL cyclic TA (2 h at 4°C then 2 h at 25°C).

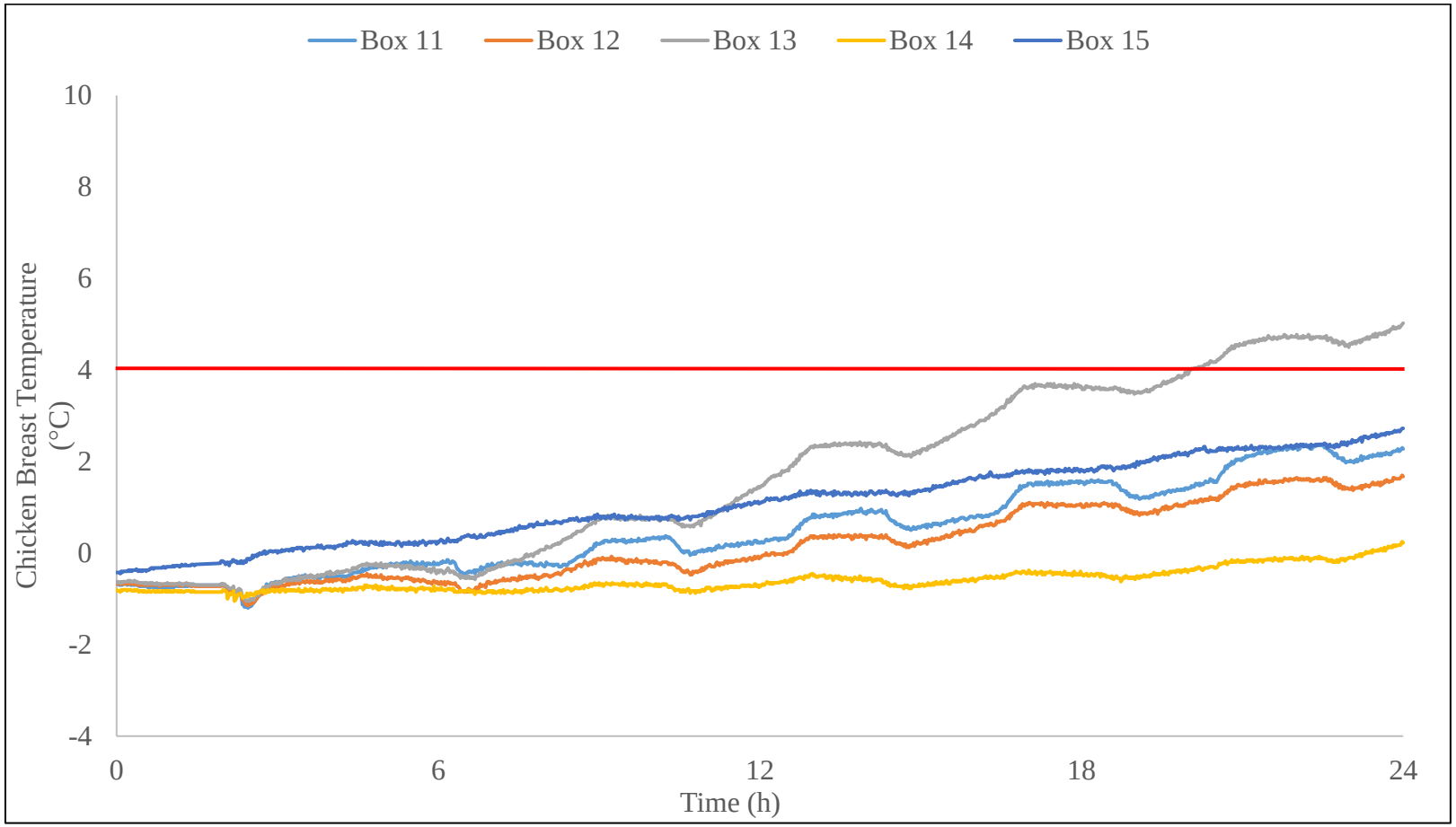

FIGURE 9. Averaged temperature profiles (4 replications) of bottom layer (layer 4) boxes of palletized chicken breasts while experiencing simulated LTL cyclic TA (2 h at 4°C then 2 h at 25°C).

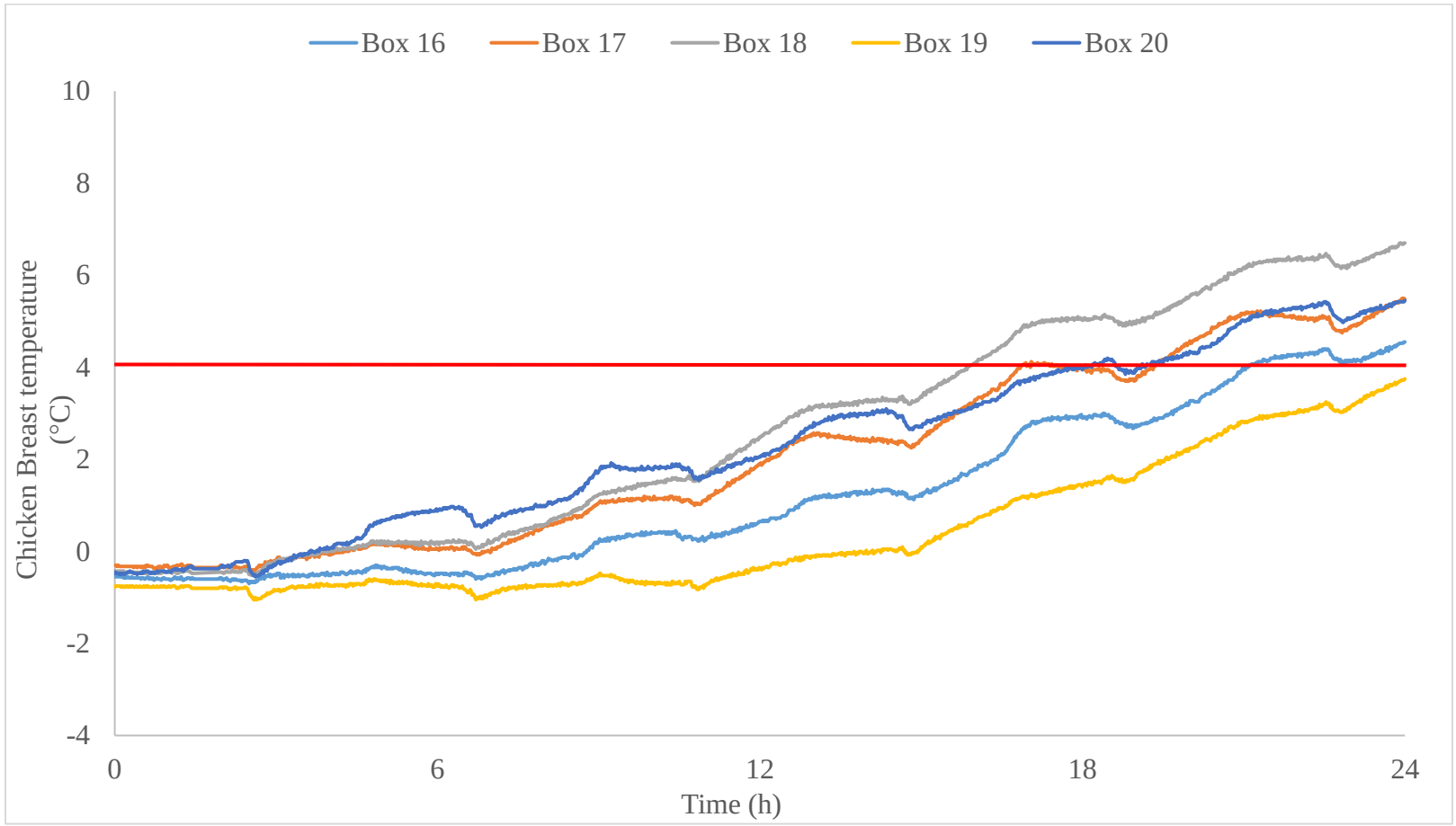

FIGURE 10. Cross sections of 4 layers of palletized boxes of chicken breasts with time required for each box to reach 4°C.

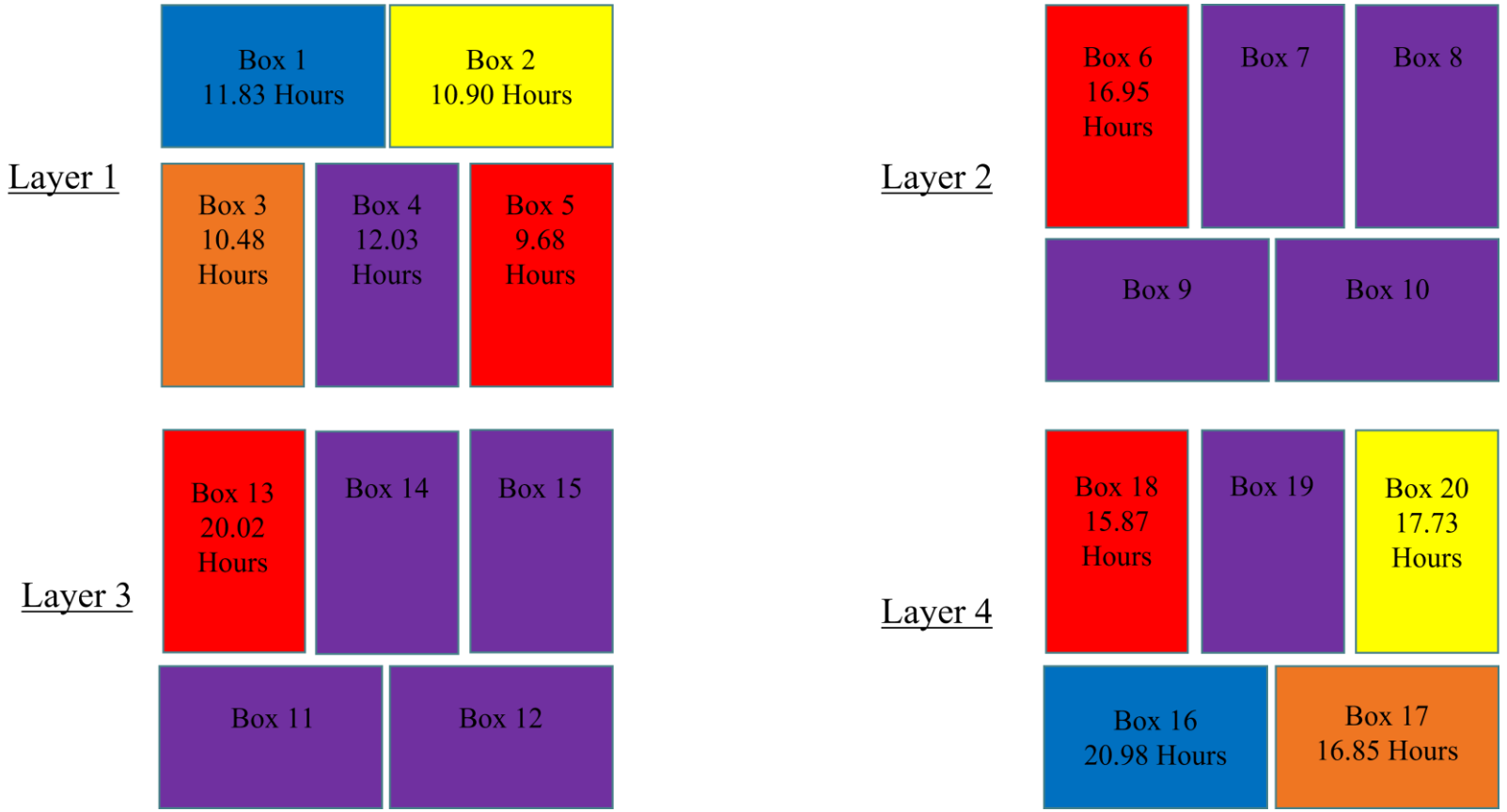

FIGURE 11. Thermal images of all sides of a pallet of chicken breasts immediately after being removed from a walk-in cooler (4°C).

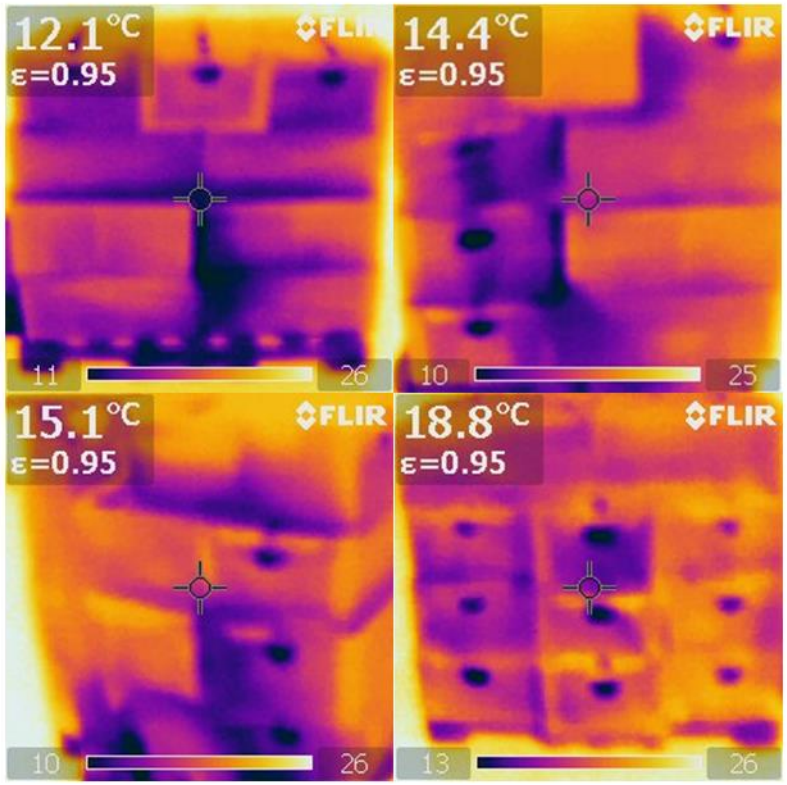

FIGURE 12. Thermal images of all sides of a pallet of chicken breasts after 24 hours of cyclic TA (2 h at 4°C then 2 h at 25°C).

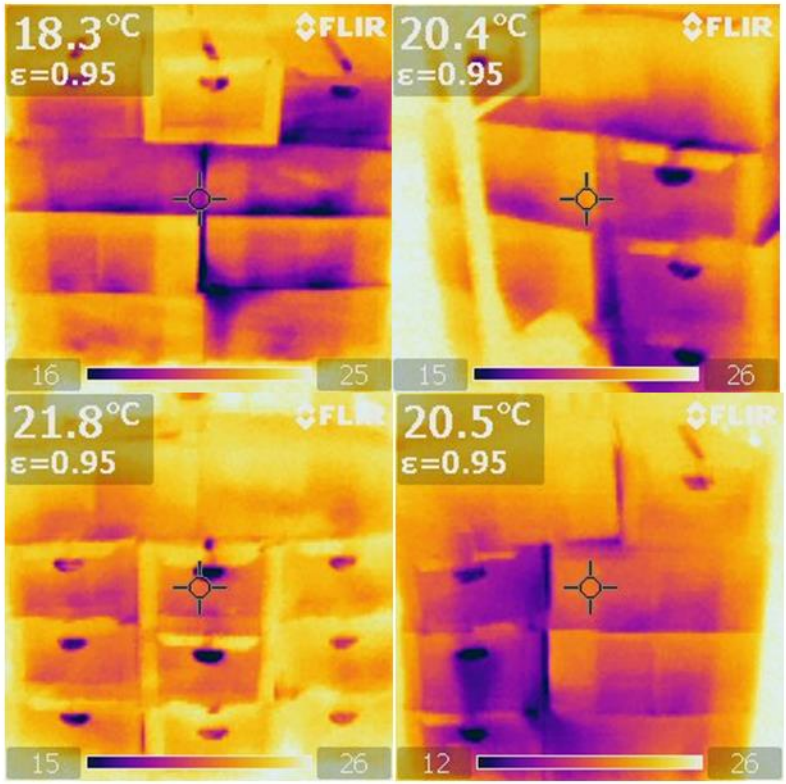

FIGURE 13. APC shelf life of simulated less-than-truckload temperature abused (TA; 2 h at 4°C then 2 h at 25°C) and control chicken breast fillets kept in simulated retail conditions.

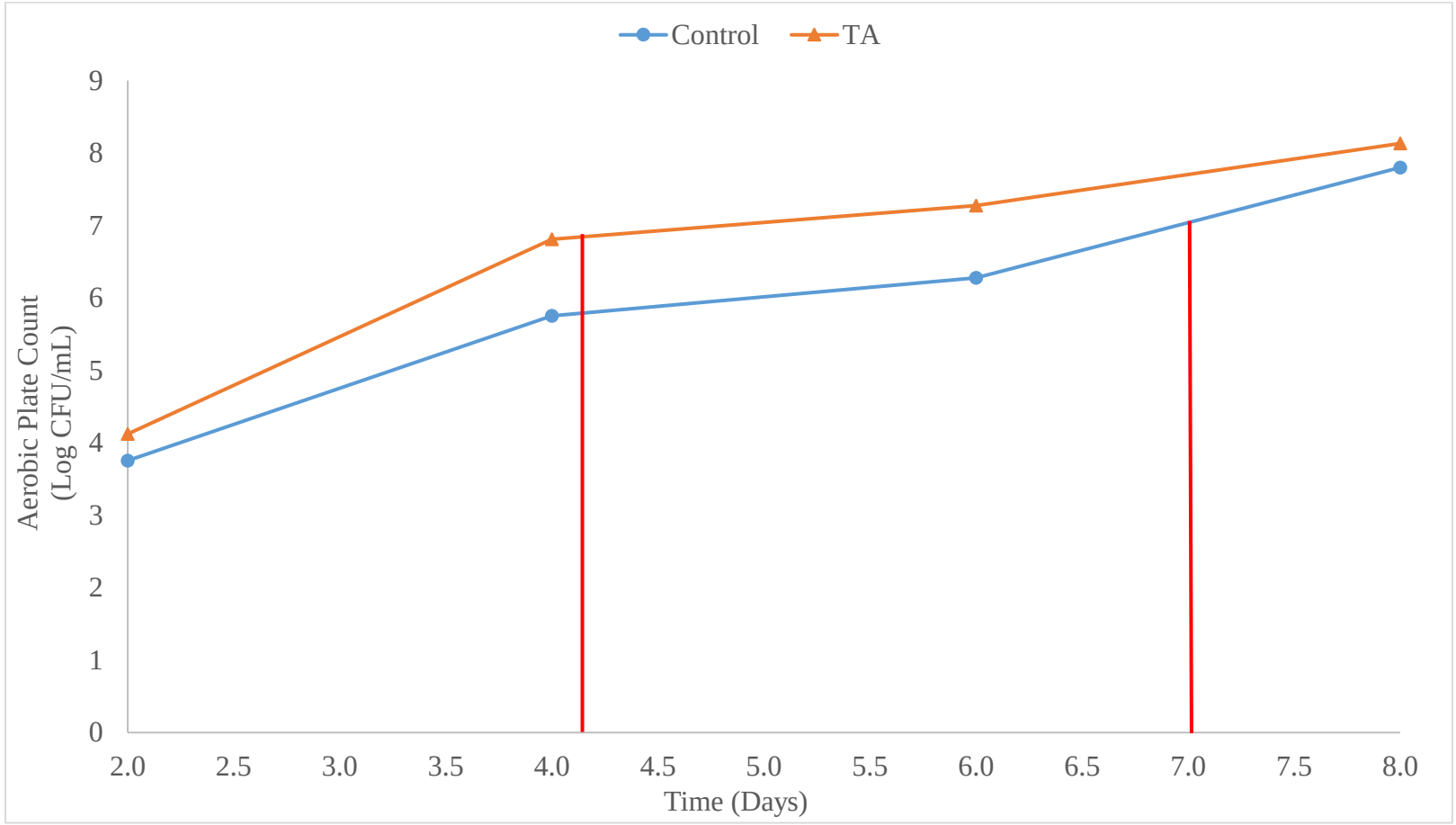

FIGURE 14. Psychrotroph shelf life of simulated less-than-truckload temperature abused (TA; 2 h at 4°C then 2 h at 25°C), and control chicken breast fillets kept in simulated retail conditions.