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Evaluating the Microbial Quality and Use of Antimicrobials in Raw Pet Foods

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EVALUATING THE MICROBIAL QUALITY AND USE OF ANTIMICROBIALS IN RAW PET FOODS

by

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Raw meat-based diets (RMBDs) are emerging pet foods that pose food safety risks because of the potential presence of pathogens that could cause illness to humans. In this research, the microbial quality of select RMBD products sold by pet food companies online and the use of chemical antimicrobials to reduce the microbial load in chicken liver, a common RMBD ingredient, were evaluated.

Ground meat blends and livers from four animal species (beef, pork, chicken, turkey) were purchased from four online companies that delivers directly to consumers through parcel businesses. Products were procured at three different times during one year and were assessed for their microbial quality, specifically aerobic plate count (APC), lactic acid bacteria (LAB), yeast and molds (Y&M), *Enterobacteriaceae* (EB), *Salmonella* spp., *Escherichia coli*, and *Listeria* spp. Overall, the microbial quality of the products were poor with some having high levels of indicator microorganisms that exceed acceptable levels of hygienic food criteria, e.g., APC (3.1 %; 2 out of 65) and EB (21.5 %; 14 out of 65). Presumptive *Salmonella*, generic *E. coli* and *Listeria* colonies were also detected in 33.8, 96.9, and 98.5 % of the samples, respectively. All four B2C companies missed at least one required information on their product labels, as well as safe food handling and storage instructions.

The effect of immersing and agitating chicken livers in peracetic acid (PAA, 450 ppm), cultured dextrose fermentate (CDF, 1.5 % w/v) and buffered vinegar (BV, 1 % w/v) on the reduction of *Salmonella* spp. and aerobic bacteria and meat color was investigated. All treatments [including distilled water (control)] resulted in significant reductions in *Salmonella* counts ($p < 0.05$). PAA resulted in the highest numerical *Salmonella* reduction from Day 0 (0.65 ± 0.12 log) to Day 14 (1.31 ± 0.12 log), although there were no significant differences in log reductions compared to control, signaling that immersion and agitation alone can reduce *Salmonella*. BV was the most promising in inhibiting the growth of aerobic bacteria – BV inhibited growth to Day 7, while PAA and CDF inhibited growth until Day 3 only.

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Chapter 1. Introduction

The search for more natural and healthier ways to nourish humans extends to developing and manufacturing foods for companion animals. There is a growing trend of using raw meat–based diets (RMBDs), either in frozen, fresh, or freeze-dried forms, to mitigate chances of cats and dogs from having infectious and degenerative diseases (Billinghurst, 2001; Nüesch-Inderbinen et al., 2019). RMBD generally refers to cat or dog diets based largely on raw meats obtained from different animal sources (Freeman et al., 2013). RMBDs available in the market are prepared differently depending on the manufacturer. Some manufacturers use "human-grade" meat cuts while others use byproducts of meat processing. Regardless of the type of raw meats used, the ingredients are highly perishable and may contain pathogenic and spoilage microorganisms that could proliferate during manufacturing, transportation, retail, and long-term storage. To minimize the risk of transferring pathogens from a contaminated RMBD to human handlers of pet food, manufacturers may treat their products with antimicrobial agents or include a pasteurization step in their process to inactivate and mitigate the growth of pathogens.

Most of the pathogens we associate with RMBD are *Salmonella* spp., Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes* (US FDA, 2021). All of these may be transferred from raw pet food to human handlers through many ways, such as meal preparation, pet shedding, or direct ingestion, which is a common incident with young children (Freeman et al., 2013; Lambertini et al., 2016; Hellgren et al., 2019). In the United States, *Salmonella* causes about 1.35 million infections, 26,500

hospitalizations, and 420 deaths every year (CDC, 2022). Pet food manufacturing is regulated by the United States Food and Drug Administration (U.S. FDA), which has a zero tolerance for *Salmonella* in all pet foods (dry, wet or refrigerated/frozen) and especially those produced with no further heating or "kill" step that destroys the pathogen (U.S. FDA, 2013). There is a high risk for *Salmonella* in raw pet foods as this pathogen is often linked to contaminated raw meat, poultry, and eggs (Montville et al., 2012). Despite this risk, the number of RMBD consumers continue to grow, as evidenced by the fact that raw pet foods and treats are the fastest growing segment of the pet food industry (Semple, 2020).

Growth in U.S. pet food sales through e-commerce has been increasing steadily since the late 2010s and was projected to be 24 % of the market by 2025 (Donaldson, 2021). The COVID-19 pandemic accelerated and blew past this projection, as growth in online pet food sales rose to 30.1 %, compared to store-based retailing which is only 3.0 % (Semple, 2020). Current estimates show that by 2025, pet food e-commerce will account for 53 % of total U.S. pet food sales (Packaged Facts, 2021). While not all pet foods sold online are RMBD, some raw pet food manufacturers are digitally native (i.e., started the business fully online) and sell their products directly to consumers through e-commerce. Because the products they sell are raw or uncooked, they are considered time/temperature control for safety (TCS) food items, which require proper time and temperature measures to minimize the potential for pathogen growth or toxin formation in the product from the point of manufacture, during transportation and storage, to the point of consumption or use. Proper temperature control starts with the temperature of the product at the end of manufacturing (e.g., frozen, chilled or room temperature) and with

how the manufacturer packages the products for shipping, e.g., using insulated shipping containers with adequate dry ice or cold packs (Hallman et al., 2015). Time control depends on the distance and type of courier service used, e.g., overnight, two to three-day delivery, ground. The courier may experience delays in transportation or handle the package in the same way as non-perishable packages that exposes the package containing raw pet foods to temperature abuse conditions, depending on the season and route of the delivery trucks (Hallman et al., 2015).

The U.S. FDA as well as professional veterinary organizations such as the American Animal Hospital Association (AAHA), American Veterinary Medical Association (AVMA) and the Canadian Veterinary Medical Association (CVMA) have issued position statements and warnings that discourage consumers from feeding raw pet foods to their companion animals, especially those that are not subjected to processes that inactivate pathogens (AAHA, 2021; AVMA, 2021; CVMA, 2018). However, as the demand for RMBD products increase, they will continue to be manufactured and available to consumers.

This thesis research focused on evaluating the microbial quality of select RMBD products sold by online retailers and the use of chemical antimicrobials to reduce the microbial load in an RMBD ingredient. The specific objectives of the study were to:

1. determine the microbial quality of ground meat blends and livers sold online by pet food manufacturers or businesses directly to consumers (B2C); and
2. evaluate the efficacy of antimicrobial agents, specifically peracetic acid (PAA), cultured dextrose fermentate (CDF), and buffered vinegar (BV), on *Salmonella* and APC of chicken livers, an organ meat typically used in RMBD products.

For the first objective of the study, microbial quality of the RMBD products was defined by the enumeration of aerobic plate counts (APC), lactic acid bacteria (LAB), yeasts and molds (Y&M), *Enterobacteriaceae* (EB), *Salmonella* spp., generic *Escherichia coli*, and *Listeria* spp. APC, LAB, and Y&M populations greater than 1,000,000 colony forming units per gram (10^6 CFU/g) in any of categories would typically indicate that a raw meat product was spoiled. *EB* counts above 5000 bacteria/g (3.7 log CFU/g) and generic *E. coli* counts exceeding the limit of 500 CFU/g (2.7 log CFU/g) would indicate unsatisfactory hygiene quality per EU Regulation No. 142/2011 and (EC) 2073/2005, respectively. *Listeria* spp. are used often as an index for presence of *Listeria monocytogenes* and their presence by direct plating signifies serious environmental sanitation problems in the facility (Vanderzant & Splittstoesser, 1992; Williams et al., 2011). Moreover, a pet food product is considered adulterated if *Salmonella* is found in the product (U.S. FDA, 2013). It was hypothesized that higher microbial counts will be present in the ground meat blends than in the whole livers because the blends are a mixture of muscle and organ meats – some of which are edible and others inedible – that are inherently high in microbial loads, and the process of grinding increases the surface area of the raw meat product to which microorganisms can adhere and easily spread.

In the second objective, the antimicrobials tested were PAA, CDF and BV, which are either approved for meats and poultry by the U.S. Department of Agriculture Food Safety and Inspection Service (USDA FSIS) or for pet foods by the Association of American Feed Control Officials (AAFCO). PAA is a commonly used antimicrobial in the poultry industry and does not require labeling if its use does not exceed 2000 ppm of

peroxyacids and 1435 ppm of hydrogen peroxide (USDA FSIS, 2021). BV and CDF typically appear as “vinegar” and “cultured dextrose”, respectively”, in product labels. Of these, PAA has been demonstrated as an effective intervention to reduce microbial loads in poultry (Cano et al., 2021). Therefore, PAA was hypothesized to yield the highest reductions in both *Salmonella* and APC when compared to BV and CDF.

This thesis contains five chapters with Chapter 1 discussing the background, rationale, and objectives of the study. Chapter 2 is a review of the literature on RMBDs, their associated food safety risks, supply chain and possible interventions. Chapters 3 and 4 provide technical details of the experiments and results obtained for the first and second objectives, respectively. Lastly, Chapter 5 summarizes the conclusions of this study, as well as future work recommendations.

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Chapter 2. Literature Review

2.1 Food Safety Risks Associated with Raw Meat Based Diets

Raw meat-based diets (RMBDs) for pets, typically comprised of uncooked meat trims, parts, organs, and bones, are a public health risk due to the potential presence of pathogens such as *Salmonella* and *Listeria monocytogenes* that could be transmitted to humans and cause illnesses especially to immunocompromised individuals (Freeman et al., 2013). RMBDs, as all pet food products, fall under the jurisdiction of U.S. Food and Drug Administration (US FDA). Currently, regulations pertaining to pet food states zero tolerance for *Salmonella* (U.S. FDA, 2013). *Salmonella*-contaminated pet food is a well-established risk factor for human salmonellosis and that this may occur if a pet owner unintentionally ingests the bacteria by touching their mouth with their hands while handling the pet food (Davies et al., 2019) or handling unwashed pet food bowls (Luisana et al., 2022). There are already cases linking human infections from dogs due to contamination of dry pet foods with *S.* Schwarzengrund (Behravesh et al., 2010), *S.* Infantis (Imanishi et al., 2014) and multidrug-resistant *Salmonella* (Schnirring, 2018). While these cases involved dry pet food, the Minnesota Department of Health reported recently what are likely the first cases of *Salmonella* infection in the U.S. that are linked to raw pet foods. Specifically, two children living in the same household where pets were fed contaminated raw turkey pet food fell ill and the raw pet food products tested for *S.* Reading (Hassan et al., 2019).

The concern about the presence of pathogens in raw pet food is also reflected in recent product recalls issued by the U.S. FDA (2021a). From 2018-2021, there were 33

product recall announcements associated with frozen raw pet foods. Most of the causes (31 out of 33) for the recall were biological hazards, such as presence of *Salmonella, L. monocytogenes,* Shiga-toxin producing *Escherichia coli,* or a combination of these microorganisms (Figure 2.1). A closer look at the ingredients of some of these recalled products showed they are blends of muscle meat, bones, entrails, and other internal organs. Other recalled raw pet food products had raw eggs in their formulation, which could be a source of *Salmonella*. Besides product recalls, a two-year surveillance study conducted by the U.S. FDA Center for Veterinary Medicine in 2010 revealed that of the 196 raw pet food products purchased from the internet, 15 tested positive for *Salmonella* and 32 for *L. monocytogenes* (Nemser et al., 2014; US FDA, 2018a).

Another factor that elevates the food safety risk of raw pet foods is the increased demand and use of e-commerce. Although U.S. pet food sales from brick-and-mortar retail stores are still greater compared to e-commerce in 2019, sales growth in the latter category (30.1 %) far exceeds that of the former category (3.0 %) (Semple, 2020). Additionally, estimates of pet food sales are expected to grow and account for 53 % of total U.S. pet food sales by 2025 (Packaged Facts, 2021). One concern with U.S. pet food sales via e-commerce is that there are raw pet food manufacturers or businesses that sell their own products directly to consumers (B2C) who may exist strictly online (i.e., digitally native) and not be registered with state regulatory agencies, U.S. FDA or the Association of American Feed Control Officials (AAFCO) (U.S. FDA, 2021b). By not registering, these B2Cs may not be inspected for compliance with the Food Safety Modernization Act (FSMA) of 2011 regularly.

These food safety risks need to be addressed especially since raw pet food products are time/temperature control for safety (TCS) foods. When these products are sold online and directly to consumers, there is a risk for the cold chain from production to storage, transportation, and consumer use to be interrupted. Courier services, such as the United Parcel Service (UPS), FedEx or DHL, may not have a protective service for the transportation of perishable commodities (UPS, 2021). While TCS foods will be accepted for transportation, the food safety risks shall be solely the responsibility of the shipper (manufacturer or retailer of pet food) for any damage (UPS, 2021). In a guidance document on Direct-to-Consumer (DTC) and Third-Party Delivery Service Food Delivery developed by Council III of the Conference for Food Protection, if a food manufacturer will be subscribing to a certain courier, they should verify initially the promised level of service of the courier before trusting it or making any changes in their established temperature-control requirements, including packaging and cooling (Conference for Food Protection, 2020).

2.2 Raw Pet Food Supply Chain

For adequate control measures to be designed and implemented, it is important to understand how raw pet foods are produced, how they are distributed to consumers (pet owners), and what are the applicable regulations. Generally, the supply chain is made up of six key actors who bear some responsibility in promoting the microbial food safety of raw pet foods: (1) the farm, (2) slaughterhouse and byproducts processors, (3) pet food manufacturing plant, (4) transporters or distributors, (5) market and (6) consumers (Figure 2.2). While there are various ingredients used in making raw pet foods, discussion in this section will focus on meat and poultry-based RMBDs.

2.2.1 Farm

Initially, meat and poultry products come from live animals and birds grown in farms of varying scales. The U.S. Department of Agriculture Food Safety and Inspection Service (USDA FSIS) is the agency in charge of inspecting the animals and processed meats bound for human consumption. They inspect raw meats and poultry for their microbial safety and wholesomeness (USDA FSIS, 2013a). Hence, prior to entering slaughter establishments, an appointed inspector from the USDA FSIS shall examine all livestock and poultry and these animals should be healthy and free from diseases (9 CFR § 309.1; 9 CFR § 381.71). Animals showing any sign of disease or illness are removed from the supply chain and are brought for slaughter or dressing in a separate area, where they may be discarded following the procedures for disposal of condemned livestock (9 CFR § 309.13) and poultry (9 CFR § 381.95) products. These requirements are under the Federal Meat and Inspection Act (FMIA) of 1906 and the Poultry Products Inspection Act (PPIA) of 1957.

2.2.2 Slaughterhouse and Byproducts Processors

Healthy animals that passed the initial inspection are brought inside the slaughtering facility and are examined continuously by the designated USDA FSIS inspector. Animal carcasses are contaminated easily in the environment during slaughtering, chilling, and cutting processes, which may aid the proliferation of spoilage microorganisms and pathogens if not handled properly (Koutsoumanis & Sofos, 2004). Hence, these facilities maintain and adhere to their written Sanitation Standard Operating Procedures (SSOP) and Hazard Analysis and Critical Control Point (HACCP) plan (USDA FSIS, 2015). The inspectors are required to verify if the facilities comply with the

requirements pertaining to the humane method of slaughter (9 CFR § 313), post-mortem examination (9 CFR § 310; 9 CFR § 381.76-381.94) and labeling of meat and poultry (9 CFR § 317; 9 CFR § 381.115 – 381.144).

At the end of the slaughter process, meat trims and other byproducts are further inspected and either passed for human consumption or are deemed not intended for human, which are then diverted towards rendering, pharmaceutical use, or pet food manufacturing. Human-grade products are carcasses and parts that can be sold commercially and were inspected and verified by USDA FSIS to be safe, wholesome, and properly labeled (FMIA, 1906). Apart from lean meat and premium cuts sold in the market, byproducts such as hearts, livers and, in poultry carcasses, gizzards, are also sold for human consumption and typically marketed separately from the meat (USDA FSIS, 2013b). However, parts like beef lungs are not recommended for human consumption in the U.S., so they are sold either for animal or pharmaceutical use (9 CFR § 310).

While slaughter management practices are employed in these facilities and are routinely inspected by USDA FSIS, pathogens such as *Salmonella* may still be present in the raw meats and their prevalence are documented (Table 2.1). Depending on the formulations used by pet food manufacturers, raw meat trims and byproducts are delivered and transported to their plants from the slaughterhouse following guidelines for transport (9 CFR § 325; 9 CFR § 381.189-194).

Official terms used for pet food ingredients coming from animal products include meat, poultry, meat byproducts, poultry byproducts, meat, and bone meal (MBM), meat meal, blood meal, poultry byproduct meal, poultry meal, among others (AAFCO, 2021). Meat and meat byproducts are fresh, while ingredients that are defined as “meal” are

rendered parts. Rendering is a process where animal byproducts are converted into usable materials by applying heat to extract moisture and separate fats (Meeker, 2006). Typically, raw pet food manufacturers do not use rendered product but, instead, procures fresh or frozen byproducts directly from meat processors or from a separate processing facility that aggregates and freezes byproducts into huge blocks. It is unknown how often microbial testing of these frozen byproducts are conducted, but most of these facilities are not considered human-grade. Some byproduct processors conduct tests for aerobic plate counts (APC) and biogenic amines, which are important indicators of food safety and meat quality for pet foods, and often label their frozen byproduct blocks to indicate they must be re-processed to control for potential pathogens (Pond, 2021). Hence, the duty to apply a pasteurization or "kill step" to inactivate pathogens in fresh or frozen meat byproduct ingredients is not assumed by slaughterhouse facilities or byproducts processors, but falls on the raw pet food manufacturer.

2.2.3 Pet Food Manufacturing

At the pet food manufacturing facility, the ingredients, processes and finished products are under the regulatory authority of U.S. FDA. The U.S. FDA regulates all food for animals, like human foods, ensuring that they are safe to eat, produced under sanitary conditions, contain no harmful substances, and be labeled truthfully (U.S. FDA, 2021c). In collaboration with the U.S. FDA, a state's Department of Agriculture and AAFCO assist in the regulation of pet food. AAFCO is a private non-profit organization that has no statutory authority to regulate animal food, but it establishes the nutritional standards for complete and balanced pet foods. AAFCO defines ingredients and develops uniform language that state feed control officials may adopt or reference in law (AAFCO,

2019). State feed control officials regulate pet food to ensure that the laws and rules established for the protection of companion animals and their custodians are complied with so that only unadulterated, correctly, and uniformly labeled pet food products are distributed in the marketplace.

Raw pet food manufacturers are required to conduct a hazard analysis, develop a food safety plan, and establish preventive controls to mitigate biological, chemical and physical hazards in their processes and in accordance with the FSMA's Preventive Controls for Animal Food (U.S. FDA, 2021d). One of the main hazards associated with raw meats are the potential presence of pathogens. Most raw pet food manufacturers try to retain the "raw-like" attributes of fresh meats, so they avoid heat treatments for pasteurization. They resort to nonthermal technologies, chemical interventions, and combinations thereof to inactivate or reduce microbial populations in their finished products.

Carcasses can be decontaminated using nonthermal processes, such as irradiation and high pressure processing (HPP). Irradiation is a decontamination strategy used for meats as it inactivates not just the foodborne pathogens, but also the food's indigenous microflora, thereby extending product shelf life (Cummins & Lyng, 2017). Furthermore, key benefits of irradiation are its nonthermal mode of processing, thereby preserving the integrity of meat, and it can be applied after the products are in their final packaging, thereby reducing the risk for cross contamination (Farkas, 2006). However, irradiation is negatively perceived by consumers who associate the technology with carcinogenicity, compromised food quality, risks to production workers, and environmental complications during production (Frewer et al., 2011). Irradiation also produces a certain aroma during

processing which affects the meat's flavor, color and increased oxidative changes (Ahn et al., 2013). To reduce the adverse effect on meat flavor and to increase consumer acceptance, other methods such as vacuum packaging and flushing the headspace with nitrogen or other inert gases may be applied to frozen meats, post-irradiation (Ahn et al., 2013; Brewer, 2009; Farkas, 1998). Generally, pathogens such as *Campylobacter*, *Yersinia* and *Vibrio* have low resistance to irradiation compared to *L. monocytogenes* and *Salmonella* serotypes, which have overlapping radiation resistances (Farkas, 1998). Hence, irradiation doses that can inactivate *Salmonella* in raw meats used for pet foods would also kill any non-sporeforming pathogen present. Currently, irradiation has been applied to pasteurize a wide variety of meat products (e.g., hamburger patties, ground beef, oysters, and shellfish), exotic products (frog legs) and pet treats (Ehlermann, 2016).

HPP is another nonthermal processing technology that can be effective at reducing microorganisms in meats, vegetables, seafoods, fish and other food products (Campus, 2010). Its applicability is not only limited to raw meats, but also as a post-lethality step for ready-to-eat cooked meat products that are contaminated potentially due to slicing and packaging after their processing "kill step" (Jackowska-Tracz & Tracz, 2015). In raw poultry, HPP has been demonstrated to reduce *Campylobacter jejuni* and *Salmonella* populations by at least 5 log (Argyri et al., 2018; Jackowska-Tracz & Tracz, 2015; Sheen et al., 2015). Similar to irradiation, HPP can denature proteins in raw meats and lead to undesirable changes in color, appearance and texture (Campus, 2010; Jimenez-Colmenero & Borderias, 2003). Thus, there are differing opinions towards the adoption of HPP for raw pet foods. Although there are many raw pet food manufacturers using HPP to pasteurize their products (Mehlenbacher et al., 2012), there are also

consumers discouraging its application because of potential changes to the raw pet food product's nutritional and natural components, as well as the high cost it entails (My Pet Carnivore, 2022).

Most consumers prefer to use freeze-dried raw pet food products instead of fresh or frozen forms, because of the ease to mix with dry kibble and long product shelf-life (Dziki, 2020). However, since freeze drying is also one of the preferred methods in preparing stocks of microorganisms, this process only provides a mild effect on microbial reductions (Bourdoux et al., 2018) and should not be considered a pasteurization process. For example, freeze drying did not significantly reduce the mesophilic spores in coriander, but had reduced aerobic plate counts (APC), *Enterobacteriaceae* (EB), and yeasts and molds (Y&M) by 1.23, 0.87 and 0.97 log CFU/g, respectively (Bourdoux et al., 2018). There are no published reports on using freeze drying to reduce the microbial loads in raw meats. Hence, most freeze-dried raw pet foods and treats are manufactured by applying HPP first to inactivate pathogens and reduce spoilage microorganisms in the fresh pet food products, followed by freeze drying.

It is possible to apply chemical treatments to raw meats to reduce their microbial loads. Typical treatments include chlorine, inorganic phosphates, peracetic acid (PAA) and organic acids. Chlorine (hypochlorite) is used in some countries to control microbial growth, contamination, and cross-contamination (Bolder, 1997). The application of 200 mg/L chlorine can significantly reduce bacteria on poultry, pork, and beef except for carcasses with low initial counts wherein changes in bacterial levels was nil. Muhandiramlage et al. (2020) also reported the effectiveness of chlorine in reducing *Campylobacter jejuni* contamination in chicken meat and how it induces physiological

and morphological changes in the said microorganism such as shape change, degeneration of cells and shriveled bacterial cells. When chlorine was used with meat and poultry (carcasses, part, trims, and organs) as a spray, wash, rinse, dip, chiller water, or scalding water, free chlorine should not exceed 50 ppm and should meet the 1-120 seconds dwell time (USDA FSIS, 2021). However, even though it is widely used in meat processing plants, one drawback of using chlorine is that it poses a negative perception to the consumers because of occupational health and safety concerns (Chousalkar et al., 2019). Furthermore, use of chlorine-based treatments in meat is not approved in the European Union (European Parliament, 2004).

Another common chemical treatment of meats is based on phosphates, specifically trisodium phosphate (TSP). This chemical generates superior antimicrobial effect and has been utilized as a surface treatment agent to decrease populations of pathogens and extend product shelf life (Sallam & Samejima, 2004). TSP is approved by USDA FSIS as an antimicrobial agent in raw, chilled poultry carcasses provided that the amount is 8 - 12 % maintained at 7.2 – 12.8 °C (45– 55 °F) and applied by spraying or dipping carcasses for up to 15 s (9 CFR § 424.21). Dinçer and Baysal (2004) mentioned that one of the main mechanisms of TSP in reducing microbial counts is through detachment of bacterial cells from the surface of the poultry skin. Because of its alkaline nature, Gram-negative bacteria are more sensitive to TSP treatment (Dickson et al., 1994). TSP was reported to be effective in reducing populations of *Salmonella, Campylobacter, Escherichia coli* O157:H7, *Listeria, Staphylococcus aureus* and spoilage bacteria such as *Pseudomonas* and *Lactobacillus* on poultry (Capita et al., 2002). TSP was also found to be effective against *Campylobacter* and *Salmonella* in duck carcasses

than in chicken (Sarjit & Dykes, 2015). The protein and lipid content of chicken skin, coupled with the presence of crevices on the surface, tended to diminish the efficacy of TSP on foodborne pathogens (Thormar et al., 2011).

PAA is a commonly used antimicrobial in poultry processing. It is comprised of peroxyacetic acid, octanoic acid, acetic acid, hydrogen peroxide, peroxyoctanoic acid, and 1-hydroxyethylidene-1,1-diphosphonic acid (USDA FSIS, 2021). This chemical agent does not require labeling if it is used at doses below 2000 ppm of peroxyacids and 1435 ppm of hydrogen peroxide when treating carcasses and can be considered a processing aid, following U.S. FDA's definition of the term. The European Food Safety Authority (EFSA) reported that there were no toxicity concerns found on using PAA solution during poultry and meat processing (EFSA, 2014). Its mechanism for controlling growth of microorganisms is through interference of its cell membrane and obstruction of enzymatic and transport process (King et al., 2005). While the efficacy of PAA in decontamination has been observed to be effective in poultry products (Cano et al., 2021), this in contrast with the studies on beef carcasses where it was deemed less effective (King et al., 2005; Gill & Badoni, 2004). Currently, there is an increase popularity of using PAA in the poultry industry compared to use of chlorine, TSP, and other chemical agents (Cano et al., 2021).

Weak organic acids can be used to control for microbial growth and are generally recognized as safe (GRAS) ingredients for meat products by U.S. FDA (Mani-López et al., 2012). As such, weak organic acids are also used as "clean label" ingredients. Even though the term "clean label" has not been defined officially by U.S. FDA, the term characteristically refers to products that are free from additives, artificial colors, and

flavors. Consumers perceive “clean label” products as not being heavily processed or only contain familiar, non-chemical, easy-to-pronounce ingredients (Grant & Parveen, 2017). Organic acids, such as lactic, acetic, and citric acid, have been utilized and studied to reduce bacterial populations in a wide range of meat products (Castillo et al., 2000; Grajales-Lagunes et al, 2012; Reyes Carranza et al., 2013; Hussain et al., 2015). They have been reported to have residual inhibition of foodborne microorganisms after two days of washing, which is important given that continued pathogen growth can occur even after decontamination (Reyes-Carranza et al., 2013). Use of organic acids in decontamination washes coupled with modifications in other intrinsic and extrinsic factors generally reduces the growth of foodborne pathogens. In a study by Christiansen et al. (2009), an additive effect was observed when raw pork jowls were decontaminated with hot water followed by the application of lactic acid at 80 °C for 15 s compared to using hot water for decontamination only. Gonzalez-Fandos et al. (2020) observed a combined effect of lactic acid decontamination and modified atmospheres packaging on the counts of *Campylobacter jejuni* on raw chicken legs. Another study using acetic acid coupled with modifications in spray pressure resulted to reductions in microbial counts spray pressures increased and spray times decreased (Reyes Carranza et al., 2013). Overall, the lethal effects of organic acids will depend on a variety of factors, such as pH of the food, mode of application, and the concentration of the acid used.

Despite their promising antimicrobial effects, organic acids can negatively impact meat texture and its water retention. Hence, organic acid derivatives were developed to address some of these undesirable changes (Totosaus et al., 2002). For instance, buffered vinegar is basically acetic acid that has been buffered using a sodium- or potassium-based

alkali to increase its pH and reduce its impact on functional properties of processed meat and poultry (Badvela et al., 2016). Its mode of action is similar with those other organic acids wherein it disrupts cellular process resulting to reduction of the growth rate of the microorganisms. Some studies have shown buffered vinegar's effectiveness at controlling pathogens like *L. monocytogenes* and *E. coli* O157:H7 as a stand-alone treatment or in combination with other antimicrobials on meat and poultry products (Badvela et al., 2016; Ponrajan et al., 2011). Commercially, there are several manufacturers selling buffered vinegar either in powdered or liquid form.

Weak organic acids that are byproducts of microbial fermentation may also have antimicrobial effects. Their efficacy is coupled with other metabolites and could be modulated by the type of substrate used in the fermentation. One example of these fermentates is MicroGARD® (International Flavors and Fragrances, Inc., New Century, KS), a patented antimicrobial comprised of fermentation metabolites from milk, dextrose, or wheat with propionic bacteria or specific *Lactococci* (Staszewski and Jagus, 2008). These metabolites include diacetyl, lactic, propionic, and acetic acid, and other undefined low-molecular-mass inhibitors around 700 Da (Al-Zoreky et al., 1991). Inhibitory activities of different MicroGARD® products on some spoilage microorganisms were reported previously on yogurt, cheese, dressings, and vegetables (Yang et al., 2021; Serna-Jiménez et al., 2020; Samapundo et al., 2017; Staszewski and Jagus, 2008), yet there is limited information on its ability to control microorganisms in raw meat and poultry.

2.2.4 Transporters or Distributors

After processing, finished raw pet food products are either distributed to retail stores, sold directly to consumers through the manufacturer's website or other retailers' websites, or, in many cases, distributed through both platforms. Key stakeholders in transporting food in the U.S. via motor or rail vehicle such as shippers, receivers, loaders, and carriers should abide by the FSMA rule on Sanitary Transportation of Human and Animal Food (U.S. FDA, 2018b). This guidance document was developed to avoid practices during transportation of foods that will lead to a public health risk (e.g., using unsanitary vehicle, no temperature control for TCS foods). Consequently, since raw pet foods are TCS foods, a written procedure should be developed and implemented by the manufacturer to ensure that food is transported to retail stores and/or cold chain facilities under adequate temperature control (U.S. FDA, 2018b). Often, refrigerated trailers are used to transport TCS foods from manufacturing facilities or cold storage facilities to brick-and-mortar retail stores.

However, when raw pet food products are purchased online, either from a retailer or directly from a B2C, the products are shipped to consumers using parcel package businesses or couriers, such as UPS and FedEx. These courier services are not covered by the stated FSMA rule and most of their fleet handle packaged non-perishable items. Hence, there is a higher food safety risk for this mode of delivery as packaged TCS products may experience temperature abuse in non-refrigerated trucks or trailers and treated by personnel as other non-perishable items. These couriers have stated explicitly on their websites that it is the shipper's responsibility to ensure that the products sent are

declared to be perishable and are packed with sufficient refrigerants and dunnage until they reach the consumers safely (Hallman et al., 2015).

2.2.5 Market

While the raw pet food segment is only a small portion of the total U.S. pet food industry, sales of refrigerated and frozen pet foods are increasing tremendously, making this segment the fastest growing in the industry (Semple, 2020) and is projected to continue to grow. Recent data published by the American Pet Products Association (APPA) showed U.S. households spent 109.6B USD for their pets and 40.2 % of that expenditure was for pet food and treats (APPA, 2022). As the market continues to grow, so too are positive testimonials and demand for RMBDs. However, professional veterinary organizations, such as the American Animal Hospital Association (AAHA), American Veterinary Medical Association (AVMA), and Canadian Veterinary Medical Association (CVMA) have issued their positions particularly discouraging pet owners to introduce raw meats in their pets' diet (AAHA, 2021; AVMA, 2021; CVMA, 2018). As initially mentioned in this chapter, U.S. FDA have also declared caution regarding the danger of raw pet food to pets and their owners (U.S. FDA, 2018a).

There are applicable regulations pertaining to the microbial quality of raw pet food products once they enter the market (Table 2.2). Pet foods sold in the U.S. should not contain *Salmonella* as this shall be considered adulterated as per CPG Sec. 690.800 (U.S. FDA, 2013). In Canada, the regulation of pet food by the Canadian Food Inspection Agency (CFIA) focuses only on imports to prevent diseases from being introduced into their country (Government of Canada, 2021). Requirements pertaining to absence or presence of pathogen like *Salmonella* is not explicitly stated. The European Union (E.U.),

on the other hand, has a more stringent guideline pertaining to the use of meat and animal byproducts for pet food, specifically Annex XIII, “Pet food and certain other derived products” of Commission Regulation (EU) No. 142/2011. The policy states that EB count may not exceed 5000 bacteria per gram (3.7 log CFU/g) and a maximum of two out of five samples may exceed the limit of 10 bacteria per gram (1 log CFU/g). Hellgren et al. (2019) also mentioned that in Sweden, they have recommended guidelines for certain microbial parameters set by the Swedish Board of Agriculture, in addition to E.U. regulations.

While it is only Sweden that have added recommended guideline values, microbial indicators such as APC, EB and generic *E. coli* are already used to evaluate if process hygienic criteria are met by foods intended for human consumption according to the Commission Regulation (EC) No. 2073/2005 in the E.U. Anaerobic bacteria and coliforms are also analyzed and used as indicators for fecal contamination (Wheater et al., 1980; Gerba, 2009). Although the recommended values do not directly indicate that the pet food is not fit for consumption by pets, there is a probability that the product may be unsafe as indicator microorganisms have also been used to indicate presence of pathogens (Borrego, 1978).

2.2.6 Consumers

Finally, the last segment of the supply chain are the consumers. Their role in maintaining the microbial food safety of pet foods is vital because even if the food procured was safe, without proper handling and cold storage in their homes, the food could spoil quickly, causing illness and other food safety-related issues for their pets and

household members, especially children, elderly, pregnant women, or those with compromised immune systems (Langiano et al., 2011; Hołda & Głogowski, 2016).

To help consumers, it is important that food safety information is available readily at the retail store, on product labels, and on websites from which the raw pet food products were procured. However, this is not the case in some parts of the U.S. as observed in Mehlenbacher et al. (2012) study involving commercially available raw pet foods in the Minneapolis/St. Paul area. Mehlenbacher et al. (2012) mentioned that all the stores included in their study advertised raw meat diets, but there were no precautionary statements or other types of communication about possible foodborne illnesses that can be acquired due to mishandling during preparation and storage. This information is essential as such scenario could pave way for pathogens to be transferred from a contaminated pet food to pet owners (Davies et al., 2019; Freeman et al., 2013). With regards to product labeling, there was a lack of foodborne illness warnings or storage instructions on the primary packaging (Mehlenbacher et al., 2012). Currently, U.S. FDA and AAFCO do not require manufacturers or retailers to provide safe handling instructions on the product labels of pet foods. In contrast, the USDA requires safe handling (e.g., storage temperatures, thawing procedures, etc.) and cooking instructions be printed on product labels of ready-to-cook raw meats and poultry, as stated by the Federal Meat Inspection Act (FMIA) (9 CFR § 317). More importance should be given on handling raw pet foods since improper thawing exposes the food surface of the meat to the "temperature danger zone," making it favorable for microorganisms to proliferate (USDA FSIS, 2013c). Another important information that needs to be available in the packaging for the consumers is the product shelf life (Carter et al., 2014). Currently,

AAFCO's Model Regulations for Pet Food and Specialty Pet Food (2021) only listed the following information that should be provided by manufacturers on the product label: product name and brand name, statement specifying the species name of pet or specialty pet for which the food is intended, quantity statement, guaranteed analysis, ingredient list, statement of nutritional adequacy or purpose, feeding directions, calorie content and name and address of manufacturer. Lot numbers or manufacturing dates are not listed on the required information to be printed on the package labels, though they are useful during product recalls and market withdrawals.

Given these gaps in making product and food safety information available information to consumers, there is a risk for pet owners to get sick due to improper storage and mishandling of raw pet foods. The U.S. FDA relies on their monitoring and surveillance activities to detect when a food product distributed in the market presents a risk of illness or injury or gross consumer deception. At which time, U.S. FDA requests the manufacturer to initiate a product recall (21 CFR § 7.45). Manufacturers and distributors could also initiate a recall voluntarily and at any time since they carry out the responsibility to protect the public health and well-being from products that present a risk of injury (21 CFR § 7.40).

However, in the event that a person becomes ill or there is an outbreak of foodborne illness, the Centers for Disease Control and Prevention (CDC) becomes involved to gather evidence, identify potential food source(s), and communicate findings to consumers and retailers to prevent additional illnesses and contain the outbreak (CDC, 2020). State agencies, such as the State Department of Public Health, may help during the

investigation of a foodborne illness or a product recall (U.S. Department of Health & Human Services, 2019).

2.3 Summary

Raw pet foods may be a small segment of the pet food industry, but they are the fastest growing segment of the industry and pose the highest food safety risks to consumers and their companion animals. These products are comprised mainly of raw meat byproducts that have inherently high microbial loads, are often handled in non-food-grade processing environments, and are meant to be ready-to-eat products. Several actors – the farm, slaughterhouse and byproducts processor, pet food manufacturer, market, transporter or distributor, and consumer – along the supply chain of raw pet foods bear some responsibility in promoting or maintaining the microbial food safety of raw pet foods, though most of the responsibility lies with the raw pet food manufacturer. During manufacturing, microbial loads may be reduced using nonthermal processing techniques or chemical interventions.

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2.5 Tables

Table 2.1. *Salmonella* prevalence in raw livers, ground meat and poultry.

Product	Species	*Salmonella* Prevalence		Reference
Ground (comminuted)	Chicken	59.9 %		USDA FSIS (2014)
	Turkey	22.4 %		
	Beef	1.6 %		
	Pork	1.39 %		Broadway et al. (2013)
Liver	Chicken	59.4 %	(148 of 249)	Jung et al. (2019)[a]
	Turkey	25.0 %	(2 of 8)	
	Beef	15.8 %	(9 or 57)	
	Pork	55.6 %	(5 of 9)	

[a] Prevalence values were obtained in retail samples in Delaware, New Jersey, and Pennsylvania.

Table 2.2. Regulatory thresholds for microbial indicators in pet food and animal feed in the USA, Sweden, and European Union.

Microbial indicators	Maximum thresholds (log CFU/g)		
	USA[a]	Sweden[b]	European Union[c]
Aerobic plate counts (APC)	–	6.7	–
Coliforms grown at 37°C	–	4.7	–
Anaerobic bacteria	–	3.7	–
Enterobacteriaceae (EB)	–	3.7	3.7
Salmonella spp.	Absence	0.0	Absence

[a] CPG Sec. 690.800 (U.S. FDA, 2013)

[b] Recommended by the Swedish Board of Agriculture, with thresholds for EB and *Salmonella* based on EU requirements.

[c] E.U. Commission Regulation No. 142/2011, Annex XIII.

2.6 Figures

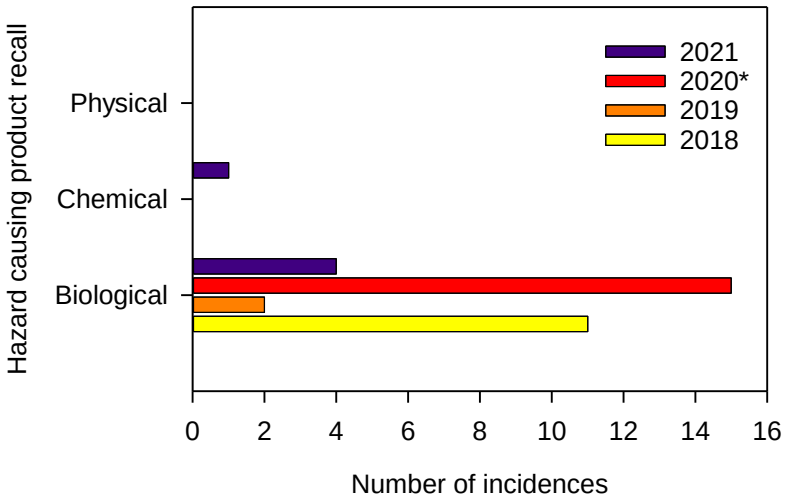

Figure 2.1. Summary of recalled frozen raw pet food products from 2018-2021, as listed in the U.S. FDA Recalls, Market Withdrawals, & Safety Alerts webpage (https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts). *An incident in 2020 was due to risk of *Clostridium botulinum* in freeze dried sardines larger than 12.7 cm (5 in).

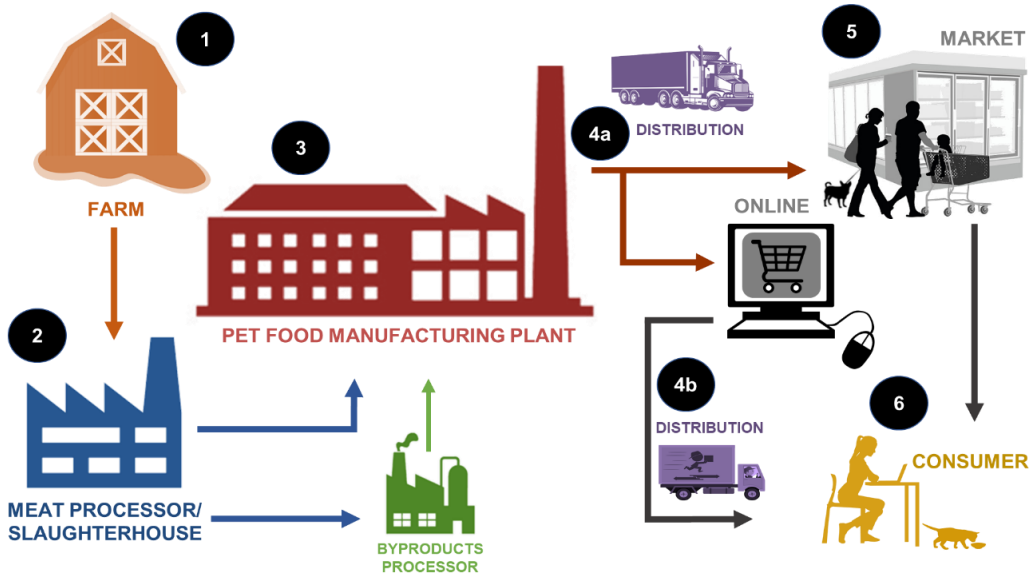

Figure 2.2. Overview of the supply chain of meat and poultry-based raw pet food products.

Chapter 3. A Survey of the Microbial Quality and Food Safety Information on Product Labels of Raw Pet Food Meat Blends and Livers Sold via Business-to-Consumer (B2C) e-Commerce

3.1 Abstract

Raw meat–based diets (RMBDs) are gaining popularity because of their perceived health benefits to companion animals. However, there is a concern as these raw pet food products may not undergo processes or chemical treatments that reduce or eliminate pathogens and spoilage microorganisms. Raw pet food products sold directly by manufacturers or businesses to consumers (B2C) online may also suffer from temperature abuse during transportation if they were not packaged and handled properly. In this study, frozen ground meat blends and whole livers of four species (beef, chicken, pork and turkey) were purchased online from four B2C companies three times in one calendar year. These products were analyzed for the following microbial quality parameters: aerobic plate count (APC), lactic acid bacteria (LAB), yeast and molds (Y&M), *Enterobacteriaceae* (EB), *Salmonella* spp., generic *Escherichia coli*, and *Listeria* spp. Results showed there was an interaction between species and meat type on all microbial parameters ($p < 0.05$), except for *Salmonella*. Sampling time had no effect on any of microbial counts, but the manufacturer or retailer had an effect on LAB, Y&M and *Listeria* counts. Overall, the microbial quality of the raw pet food products was poor as high levels of microorganisms were observed (APC, 4.13- 5.99 log CFU/g; LAB, 3.40-4.98 log CFU/g; Y&M, 1.21- 3.23 log CFU/g; and EB, 1.71-4.07 log CFU/g). Presumptive *Salmonella*, generic *E. coli* and *Listeria* spp. were detected in 33.8, 96.9, and

98.5 % of the 65 pet food products, respectively. Most of the products also lacked some of the required information on the labels based on AAFCO Model Regulations for Pet Food and Specialty food (e.g., calorie content, intended species, guaranteed analysis, etc.), as well as safe food handling and storage instructions that consumers typically see in raw meat products. Additional guidelines for industry should be set to reduce the risk of consumers receiving unsafe raw pet foods via e-commerce.

3.2 Introduction

Pet ownership has steadily risen since 1988 when only 56 % of U.S. households had pets (APPA, 2022). Today, 70 % of U.S. households, which equates to 90.5 million homes, own at least one pet and spend money for their basic needs. In 2021 alone, 109.6B USD were spent for pets with 40.24 % of the total expenditures on pet food and treats (APPA, 2022). Evidently, pet owners put much importance on the food eaten by their dogs and cats and expect the pet foods to provide adequate nourishment and improved resistance to diseases (Clemens, 2014). When it comes to the type of pet food purchased, Semple (2020) reported that a big portion of the sales constitutes dry pet food or kibble. While there are a variety of popular products offered in the market, there are emerging products that pose potential health and food safety risks.

For instance, raw meat-based diets (RMBD) or raw pet foods are gaining popularity to pet owners because they are perceived as both a healthy option and an “all natural” diet, with some manufacturers claiming health benefits for pets (e.g., longer lifespans, improved immune systems, reduced chances for developing illnesses, etc.) (Billinghurst, 2001; Nüesch-Inderbinen et al., 2019). Currently, raw pet food makes up only 2.34 % of the U.S. pet food industry, but it is the fastest growing segment at 396.7M

USD sales in 2020 (Semple, 2020). The term RMBD refers to uncooked or raw meats or meat byproducts obtained from fish, livestock, or poultry which are used as pet food. (Freeman et al., 2013). Specific ingredients may include skeletal muscles, internal organs (offal) and edible or meaty bones (Freeman et al., 2013; Hellgren et al., 2019). Most RMBDs are formulated in a way that minimizes additives and supplements, such as preservatives, stabilizers, coagulating agents, sweeteners, flavors or vitamins and minerals (Nüesch-Inderbinen et al., 2019). However, because RMBDs are raw, the U.S. Food and Drug Administration and professional veterinary organizations have voiced their concerns regarding the microbial loads and minimal processing of RMBDs. Studies have shown poor microbial quality of some of the raw pet food products available in Canada and Europe, where high counts of indicator organisms such as aerobic bacteria, generic *E. coli* and fecal coliforms were found (Weese et al., 2005; Morelli et al., 2019). The presence of pathogens such as *Salmonella* and *Listeria monocytogenes* were equally observed in some raw pet food samples (Nüesch-Inderbinen et al. 2019; Van Bree et al., 2018; Nemser et al., 2014). In the U.S., raw pet foods have been implicated in U.S. FDA's product recalls and market withdrawals in recent years (2021). The majority of the reasons for the product recall or market withdrawal has been the presence of pathogens such as *Salmonella, L. monocytogenes* and Shiga-toxin producing *Escherichia coli.*

Raw meats sold for human consumption may contain low levels of pathogens and other microorganisms, but the consumer is provided safe food handling and cooking instructions so the raw meats are stored, thawed, prepared and cooked properly for safe consumption. RMBDs, on the other hand, are mostly made of raw meat ingredients but the human custodians are not expected to heat or cook the raw pet food products when

preparing and feeding it to their companion animals. As such, RMBDs can be described as "ready-to-eat" foods. Microorganisms present in raw pet foods can be transferred readily to humans during meal preparation, manual handling of pet waste, or by direct ingestion of contaminated raw pet food by children in the household (Freeman et al., 2013; Lambertini et al., 2016; Hellgren et al., 2019). In a report by the Minnesota Department of Health, case investigations revealed that the *Salmonella* infection of two children was linked to a raw turkey pet food product contaminated with *S.* Reading found within their household (Hassan et al., 2019). Animal feeding studies also showed that pathogens could be transferred from the raw pet food to the pet feces (Joffe and Schlesinger, 2002; Finley, 2004). Specifically, *S.* Heidelberg isolated from the raw pet food used in a feeding trial was found in the feces shed by 5 out of the 7 dogs included in the study (Finley, 2007).

Current guidelines of the U.S. FDA (2013) consider a pet food to be adulterated if it is contaminated with *Salmonella* and has no further processing steps to destroy the pathogen. The U.S. FDA (2020a) has also released a notice about raw pet foods stating:

> *"FDA does not believe raw meat foods for animals are consistent with the goal of protecting the public from significant health risks, particularly when such products are brought into the home and/or used to feed domestic pets; however, we understand that some people prefer to feed these types of diets to their pets."*

Professional organizations such as the American Animal Hospital Association (AAHA) and the American Veterinary Medical Association (AVMA) have also issued similar position statements as the U.S. FDA pertaining to RMBDs. The AAHA

expressed, they *"no longer support or advocate the feeding of raw protein diets to pets"* and the AVMA discourages feeding of any animal-source protein not subjected to a process to kill pathogenic microorganisms *"because of the risk of illness to cats and dogs, as well as humans"* (AAHA, 2021; AVMA, 2021).

Despite the precautionary notes from U.S. FDA and professional veterinary societies, specialized online shops of raw pet foods continue to sell frozen mixtures of ground raw meats, customized meal plans, and animal byproducts such as bones, internal organs, and cartilage as pet treats (Morelli et al., 2019). When used as ingredients, the wide array of human-grade (edible) and non-food-grade (inedible) raw meats and byproducts contributes to the variety of natural microflora that can be found in raw pet food products. Furthermore, purchasing raw pet foods from B2C companies online poses a significant food safety risk especially when the B2C processing facilities may not be registered or routinely inspected by state and federal agencies. Some B2C companies also exist strictly online (i.e., digitally native companies) since entering the digital space or selling through social media sites (e.g., Facebook Live) is now very accessible. Hence, there is a concern as to how smaller or entrepreneurial digitally native companies are equipped at getting the required help and training on food safety while the regulatory structure for e-commerce is in the works (Schaffner, 2021).

Bulk distribution of TCS foods typically relies on a network of cold storage warehouses and fleets of refrigerated trucks, trailers, and railcars. However, most B2C companies selling raw pet food products use third party delivery or courier services (e.g., FedEx, UPS, DHL, USPS) that are more accustomed to handling and delivering non-perishable items. These services can handle, transport and deliver perishable items (e.g.,

home meal kits) properly so long as the sender declares the items are perishable and assumes responsibility for proper packaging of TCS foods (i.e., using an insulated container with enough dunnage and dry ice or cold packs). Nevertheless, even if TCS foods are packaged properly, factors such as seasonal temperatures, inclement weather events, and road construction or obstructions could delay timely delivery of TCS foods, making them susceptible to temperature abuse (Hallman et al., 2015).

In this study, the microbial quality of frozen ground meat blends and whole livers of four species (beef, chicken, pork and turkey) were purchased online from four B2C companies at three sampling times in one calendar year and were analyzed for the following microbial quality parameters: aerobic plate count (APC), lactic acid bacteria (LAB), yeast and molds (Y&M), *Enterobacteriaceae* (EB), *Salmonella* spp., generic *Escherichia coli*, and *Listeria* spp. while looking at the interaction between meat type and species and the overall main effect of B2C companies and sampling time (season). Product and food safety information on the raw pet food product labels were also characterized.

3.3 Materials and Methods

3.3.1 Raw pet food samples

Sixty-five frozen raw pet food products (41 ground meat blends and 24 whole livers) were purchased from four online B2C companies (Company A, B, C and D) every 3-4 mos. during one calendar year. These three sampling timepoints represent winter (January – March), summer (May – August), and fall (September – December) seasonal temperatures encountered during shipping or transportation. The ground meat blends and livers were from four animal species, specifically, cattle, chicken, pig, and turkey and

were purchased when available or in stock. The following criteria were set when choosing the four B2C companies:

(a) the company is based in the USA;

(b) the company manufactures and sells their own raw pet food products that strictly follow the prey model diet, i.e., only raw meat ingredients;

(c) the company sells their products online; and

(d) the company is willing to ship their frozen products directly to consumers within the contiguous 48 states.

At the time of purchase, the least expensive option for shipping to the University of Nebraska-Lincoln was selected. Upon receipt, surface temperatures of all products were taken using an infrared thermometer with a stated accuracy of ±2°C (Model No. 800, Etekcity, China). The primary packaging of each product was also checked for adherence to labeling requirements stated in the Model Regulations for Pet Food and Specialty Pet Food by the Association of American Feed Control Officials (AAFCO) (2021), provision of safe food handling instructions (e.g., storage temperature, thawing), and availability of lot codes and manufacturing and expiration dates. All products received were stored at 4 °C for 24 h to thaw fully for adequate sampling prior to microbial enumeration.

3.3.2 Microbial Enumeration

For every product purchased, three 25-g subsamples were taken and were each placed into a sterile 1.627 L (55 oz.) stomacher bag (Whirl-Pak®, Thomas Scientific LLC, Swedesboro, NJ, USA) and diluted in 225 ml of 0.1% Butterfield's Phosphate Buffer (BPB) using an autodilutor (Smart Diluter, Neutec Group, Inc., Farmingdale, NY,

USA). Samples were homogenized for 90 s at 200 rpm in a triple mix paddle blender (Model No. 11-452-120, Fisher Scientific Co. LLC, Waltham, MA, USA).

Aerobic plate count (APC), yeasts and molds (Y&M) and *Enterobacteriaceae* (EB) were enumerated using 3M Petrifilm™ following AOAC Official Methods 990.12, 997.02, and 2003.01, respectively. Samples were also plated using selective media, namely, Oxford Listeria (OX) agar for *Listeria*, MacConkey (MAC) agar for *Escherichia coli*, xylose lysine deoxycholate (XLD) agar for *Salmonella* and De Man, Rogosa and Sharpe (MRS) agar with L-cysteine for lactic acid bacteria (LAB). Inoculated OX agar plates were incubated at 32 °C for 48 ± 2 h, while inoculated MAC and XLD agar plates were incubated at 36 °C for 24 ± 2 h. Lastly, LAB were counted after incubating MRS agar plates at 36 °C for 72 ± 2 h under anaerobic conditions using gas packs (BD GasPak™ EZ Anaerobe container system with indicator 260001, Becton, Dickinson and Company, Sparks, MD, USA). After enumerating the colony forming units (CFU) from each plate or 3M Petrifilm™, counts of subsamples were averaged and reported as log CFU/g.

3.3.3 Statistical Analysis

Estimated means of the different microbial parameters were obtained using 2 x 4 factorial two-way analysis of variance (ANOVA) with sampling time (season) and retailer as fixed block effects. Data were analyzed for the overall main effect of B2C companies and sampling time and the interaction between product type and animal species. This is a nested design since turkey liver was not available or in stock during all three sampling periods. Comparisons across meat type and species were also conducted using Tukey-Kramer's test.

For *Salmonella* species, results for the level of contamination were obtained only on products where presumptive *Salmonella* colonies was found in three or more samples. Additionally, the odds of detecting presumptive *Salmonella* colonies in a 25 g raw pet food based on the EB count was analyzed using logistic regression. All statistical analyses were run using SAS software (Version 9.4, SAS Institute, Cary, NC, USA).

3.4 Results and Discussion

3.4.1 Primary Packaging and Information on Product Labels

AAFCO's Model Regulations for Pet Food and Specialty Pet Food (2021) listed nine components that needs to be included in the product label: product name and brand name, statement specifying the species name of pet or specialty pet for which the food is intended, quantity statement, guaranteed analysis, ingredient list, statement of nutritional adequacy or purpose, feeding directions, calorie content, and name and address of manufacturer. All B2C companies missed at least one piece of required information on their product labels (Figure 3.1a, Figure 3.1b, and Table 3.1), which was similar to the observations made by Mehlenbacher et al. (2012) in their study characterizing raw pet foods purchased from brick-and-mortar retail stores in the Minneapolis-St. Paul area in Minnesota. In this study, the common requirement missing was calorie content, which should be measured in terms of metabolizable energy (AAFCO, 2021). Calorie content is the latest requirement by AAFCO and was added in 2014 (AAFCO, 2012). Calorie content was the only information missing on Company A's labels for ground meat blends but was available on their website as of April 2022. Company B, on the other hand, missed five items on the product labels – calorie content, species the product is intended for, guaranteed analysis, address of the manufacturer, and list of ingredients – but were

available on their website though statement specifying the species is not directly stated. Company C did not include the species the food is intended for, but the information was available on their website. Company D failed to include guaranteed analysis and nutritional adequacy statement on their product labels but provided on their website.

For the liver samples, however, all B2C companies did not provide the guaranteed analysis information and calorie content on their product labels and on their websites. Companies C and D also failed to provide a statement of nutritional adequacy and feeding directions, but these information were available on their websites.

While AAFCO does not have a congressional authority to regulate pet foods, many states have adopted and enforce the AAFCO model regulations. Per U.S. FDA food labeling guidelines, proper identification of product, net quantity statement, manufacturer's name and address, and proper listing of ingredients need to be included in the product label (U.S. Food and Drug Administration, 2020b). All B2C companies complied with U.S. FDA's requirements except for Company B, which failed to mention both the address of manufacturer and list of ingredients. This is concerning given consumers should be able to contact the manufacturer in case there is an issue with the pet food product and the consumers should know all of the ingredients of the food products entering their households and what they are feeding their companion animals.

All B2C companies provided some level of food safety handling information, even though it is not explicitly required by AAFCO or U.S. FDA for raw pet food products. In contrast, USDA FSIS requires food safety handling information for all meat and meat products of cattle, swine, sheep, goat, horse, other equine that have not undergone adequate processing steps that would render them ready-to-eat for humans

(Federal Meat Inspection Act, 1970). Because the USDA does not have jurisdiction once raw meat products are deemed and processed for animal food, such info is not required on pet food labels. Of four B2C companies, Company B and Company C provided food safety handling information on their product labels, while Company A and Company D provided similar information on pamphlets shipped with their products. Specifically, both Company B and Company C stated “products must be kept frozen until feeding time” with Company B providing the suggested number of days at refrigerated and frozen storage conditions in the label. Company D provided specific instructions on handling, storage, and proper thawing of their products on their pamphlet. However, Company A advised on their pamphlet that “thawing the raw product at room temperature before feeding is ideal.” This advice was concerning as it contradicted USDA’s safe defrosting methods of raw meats and perishable goods. Specifically, thawing perishable goods at room temperature is not advisable as this practice exposes the surface or the outer layer of the food in the "temperature danger zone" – between 4-60 °C (40-140 °F) – which are favorable conditions for bacteria to grow (USDA FSIS, 2013). While the four B2C companies attempted to provide safe food handling instructions, it is crucial that they and other raw pet food manufacturers provide appropriate information since in a quantitative assessment on dry pet foods, handling and preparation of pet foods was used as baseline and mentioned to be the most direct exposure route for transmission of pathogens (Lambertini et al., 2016). While the Federal Meat Inspection Act (FMIA) 9 CFR § 317.2 is only applicable to human foods, the addition of requiring that safe handling information be provided in animal food should be considered because there have been

instances wherein a pet owner got sick due to contaminated dry (Behravesh et al., 2010; Imanishi et al., 2014; Schnirring, 2018) and raw (Hassan et al., 2019) pet foods.

3.4.2 Interactions and Effects of Variables

Results showed that there was an interaction between species and meat type on the microbial counts across the variety of products tested (APC, $p < 0.0001$; LAB, $p = 0.0008$; Y&M, $p < 0.0001$; EB, $p < 0.0001$; *E. coli*, $p < 0.0001$; *Listeria* spp., $p = 0.0371$), except for *Salmonella*. This meant there was sufficient evidence that the microbial quality of a raw pet food product was dependent on the species and meat type. Results also showed that the microbial counts in ground meat blends were generally higher compared to those of liver (Figure 3.2). The declared ingredients in the labels of the ground meat blends showed a mixture of meat, organ parts and some bones (Table 3.2). Spread of microorganisms occur in the carcass cutting process and the degree contamination is dependent usually on the extent of exposure in food-contact surfaces, workers, and equipment (Jensen et al., 2004). The physical act of grinding likely promoted the spread of microorganisms throughout ground meat blend samples, so their microbial quality were expected to be inferior to those of whole and sliced liver samples. Moreover, ground beef blend was found to have the highest counts in all microbial parameters.

Previous studies have reported that the season when animals are harvested could influence the prevalence of microorganisms such as *L. monocytogenes* (Pérez-Rodríguez et al., 2010), *Campylobacter* (Smith et al., 2019), and *Salmonella* (Williams et al., 2014) in fresh meats. Since most raw pet food products are sold and kept frozen until use, it was difficult to ascertain the season or time of year when the animals were harvested. While Company A and Company B each provided lot codes or manufacturing dates, Company

C did not provide this information on the label or anywhere on the package. Company D also did not provide lot codes or manufacturing dates, but they provided a “best by” date. In this study, the effect of season was meant to be the environmental or weather conditions at the time of year (sampling time) when the raw pet food products were purchased, transported and delivered. All products were shipped frozen and transported from all four B2C companies to the University within 2-3 days, mostly using 2-3 day air shipping services or 3-day ground transport services. All products arrived in insulated boxes packed with dry ice. Three B2C companies (A, C and D) used some form of dunnage, such as packing peanuts and paper. As a result, all but three out of 65 products arrived at the University with mean surface temperatures below 4 °C. Food safety information were also provided on the secondary packaging, with some companies emphasizing proper storage conditions such as “Keep Frozen” and “Requires immediate attention and cold storage.”

The B2C company (A, B, C or D) influenced the following microbial quality parameters: LAB, Y&M and *Listeria* spp. Products from Company C had the highest counts of LAB and *Listeria* followed by Company D. However, differences in LAB (p = 0.9219) and *Listeria* (p = 0.6176) counts for the two companies were not significant. As for Y&M, counts for Company B products were the highest and were significantly different from those of Company A products (p = 0.0279). LAB and *Listeria* counts are important parameters to monitor in any raw meat product since high LAB counts typically indicate spoilage of the meat (Kreyenschmidt et al., 2010). High *Listeria* counts, on the other hand, indicate the hygienic nature of the processing environment and are used often to measure the effectiveness of a facility’s sanitation protocols. However,

given some of the ingredients used in RMBDs are meat trims and organ meats, it is possible that how these byproducts of meat processing are handled at the slaughter facility and during aggregation prior to freezing and delivery to a raw pet food processing facility could account for their high *Listeria* populations. Overall, LAB and *Listeria* spp. results suggested Company C and Company D may have utilized poorer quality raw meat ingredients and processed them under the least sanitary conditions compared to the other B2C companies.

3.4.3 Aerobic Bacteria

APC ranged from 4.13 log CFU/g (beef liver) to 5.99 log CFU/g (beef blend) (Table 3.3). Bottari et al. (2020) in Italy showed comparable APC in various raw pet foods (ground meat blends and variety meats) which ranged from 4.63 to 6.58 log CFU/g. APC counts of the ground meat blends were comparable to those involving minced and/or blended meat and animal by-products in other RMBD studies, specifically 6.77 log CFU/g (Morelli et al, 2019) and 5.36 log CFU/g (van Bree et al., 2017). While the USA and EU have not enforced limits on APC in raw pet foods, 3.1 % of the products tested (2 out of 65, both ground beef blends) exceeded 5 x 10^6 CFU/g (~6.70 log CFU/g), the maximum limit set by the Commission Regulation (EC) 2073/2005 for human foods. Moreover, APC of 22 % of the samples (14 out of 65 products, all of which are ground meat blends and pork livers) were within 5 x 10^5 CFU/g (~5.70 log CFU/g) to 5 x 10^6 CFU/g (~6.70 log CFU/g). According to the EU Commission Regulation (EC) 2073/2005, if 2 out of 5 samples fell within the said range, this meant that improvements must be made in production hygiene and selection of raw materials in the facility.

Additionally, there was a significant difference in APC between ground meat blends and livers for both beef and chicken (Figure 3.2a). No difference was found for pork blend and pork liver.

3.4.4 Lactic Acid Bacteria

LAB counts ranged from 3.40 log CFU/g (beef liver) to 4.98 log CFU/g (beef blend). Similar to APC, only ground beef and chicken blends were significantly higher than their liver counterparts (Figure 3.2b). While previous studies conducted on level of contamination of RMBDs in the USA, Canada and Europe did not include LAB, Kreyenschmidt et al. (2010) mentioned that the shelf life of raw meat products ends when LAB counts reach 7 log CFU/g. At this point, raw meat products typically no longer have acceptable organoleptic properties, often emitting rancid off-odors and forming slime.

3.4.5 Yeasts and Molds

Y&M counts ranged from 1.21 log CFU/g (chicken liver) to 3.23 log CFU/g (beef blend) (Figure 3.2c). Mean Y&M counts of all products tested, except chicken livers, were higher than those reported for raw beef-based burgers (1.61 ± 1.02 log CFU/g) in retailed stores in Pennsylvania, USA (Luchansky et al., 2020). It is difficult to ascertain if the obtained counts indicated a risk to human health because there is no regulation pertaining to presence of yeasts and molds in fresh/frozen pet foods.

3.4.6 Enterobacteriaceae

EB counts ranged from 1.71 log CFU/g (chicken liver) to 4.07 log CFU/g (beef blend). Generally, EB counts were lower in livers than ground meat blends (Figure 3.2d). While there are no EB limits in raw pet foods in the USA, 21.5 % of the products tested (14 out of 65) had EB counts greater the 3.70 log CFU/g limit set by the EU for raw pet

foods (EU regulation No. 142/2011). Furthermore, 92 % of the products tested (60 out of 65) exceeded 10 bacteria/g, which is a secondary stipulation by the EU regulation. Although EB colonies were not further analyzed beyond enumeration in this study, Hellgren et al. (2019) identified EB found in raw pet foods they tested were mostly coliforms. Monitoring EB in raw pet foods is essential since this family of Gram-negative bacteria also includes well-known pathogens such as *Salmonella, E. coli, Klebsiella* and *Shigella.*

3.4.7 Salmonella spp.

Presumptive *Salmonella* colonies were detected in 33.8 % of the products (22 out of 65, mostly ground meat blends). This result was much higher than reported prevalence of *Salmonella* in raw pet foods (2.6 to 20 %) tested in other studies (Weese et al., 2005; Nemser et al., 2014; van Bree et al., 2017; Hellgren et al., 2019; Nüesch-Inderbinen et al., 2019). Because the U.S. FDA has zero tolerance on any *Salmonella* serotype found in pet foods (raw or cooked) and considers the pathogen an adulterant, its prevalence was expected to be low. In fact, previous studies involving raw pet foods used enrichment steps to detect *Salmonella* (Weese et al., 2005; Nemser et al., 2014; van Bree et al., 2017; Hellgren et al., 2019; Morelli et al., 2019; Nüesch-Inderbinen et al., 2019) as it was expected that levels would fall below culture methods for enumeration, but only Morelli et al. (2019) reported zero presence of *Salmonella* in their pet food samples.

For those products with presumptive *Salmonella* colonies, ground turkey (7 out of 11), chicken (5 out of 12), and beef (5 out of 12) blends had the highest percentage (Table 3.4) which was not surprising as ground meats for human consumption from these species have been recalled in recent years due to *Salmonella* (USDA FSIS, 2022). The

Center for Science in the Public Interest (2013) reported that a major portion of outbreaks associated to *Salmonella* are poultry products.

To date, several pet food products have been linked to human Salmonellosis (Nemser et al., 2014; Hassan et al., 2019). Apart from humans, there are also cases wherein RMBD causes sickness like gastroenteritis in pets and other animals (van Bree et al., 2018). There is also a concern on this growing segment of the pet food industry particularly on possible implications in public health because raw pet foods could be a source of unique *Salmonella* serotypes with unknown pathogenicity (Finley et al., 2006).

Ray (2005) suggested that EB counts can be used as a potential indicator for the presence of *Salmonella* in foods, while others have noted that good correlations of EB to *Salmonella* counts do not always lead to conclusive estimates of *Salmonella* in meats (Ghafir et al., 2008; Corbellini et al., 2016). Nevertheless, logistics regression results of EB to *Salmonella* counts above 1 log CFU/g in the raw pet food products tested in this study showed significant correlation ($p = 0.0363$). The resulting odds estimate for one unit difference was 0.90, implying that the odds of finding *Salmonella* above 1 log CFU/g when EB counts in raw pet food is 4 log CFU/g is about 0.90 times greater than when EB count is 3 log CFU/g. These results, however, should be used with caution as there were only a limited number of products from a handful of B2C companies used in this analysis.

3.4.8 Escherichia coli

One of the ubiquitous coliforms in meat is *E. coli* which are often used as an indicator of fecal contamination (Vanderzant & Splittstoesser, 1992). In this study, generic *E. coli* colonies were observed in 96.9 % of the samples, which was higher than

Weese et al. (2005) in which only 64 % of the 25 commercial raw food diets tested positive for *E. coli*. Morelli et al. (2019) observed similar *E. coli* counts (in RMBDs made of minced meat and byproducts coming from one or two animal species), on average 4.04 log CFU/g, which are similar to what was found in chicken and beef blends (4.07 and 4.75 log CFU/g, respectively) in this study. Liver samples had lower *E. coli* counts than ground meat blends, in general. Ground beef blends had the highest *E. coli* counts, which was significantly different than counts for ground pork and turkey blends (Figure 3.2e). Of the 63 products positive for generic *E. coli*, 69.8 % had counts above 2.7 log CFU/g, which is the limit for process hygiene criteria foodstuffs in the EU (EC 2073/2005).

3.4.9 Listeria species

Listeria spp. were observed in 98.5 % of the samples with mean estimates between 2.00 log CFU/g (chicken liver) to 3.09 log CFU/g (beef blend) (Figure 3.2f). High percentage was noted in this study compared to the assessment done by Nemser et al. (2014) and van Bree et al. (2018), who reported finding *Listeria* in 11.5 and 42.9 %, respectively, of raw pet food products they tested. Even though no further evaluation was conducted to test for the presence of *L. monocytogenes* in the raw pet food products in this study, it is likely some of the colonies observed were pathogenic. Nemser et al. (2014) reported that 48.5 % of the total samples that tested positive for *Listeria* in their study were confirmed to be *L. monocytogenes*. Van Bree et al. (2018) and Morelli et al. (2019) also reported presence of *L. monocytogenes* in more than half of the raw pet food samples they analyzed. Recent recalls or market withdrawals of raw pet foods in the U.S. were due to presence of *Salmonella* and *L. monocytogenes* (US FDA, 2021). While

primary concern is the risk of human pet food handlers acquiring listeriosis, this disease can also affect animals (Dhama et al., 2015). The presence of *Listeria* spp. in pet foods indicates unhygienic processing environments and that sanitation measures were inadequate (Vanderzant & Splittstoesser, 1992; Williams et al., 2011). Performing microbiological test in foods and environment, either by in-house testing or through third party service providers, is vital prior to releasing food products in the market to protect both the manufacturer and consumers. Tompkin et al. (2002) encouraged food processing plants deciding to only test presence *Listeria spp.* to treat all products testing positive as if they were confirmed to be positive for *L. monocytogenes* to avoid potential sanitation failure and consequences.

3.5 Conclusions

The high microbial loads found in raw pet food products sold online by B2C companies showed these products had poor microbial quality and posed a food safety risk to human custodians and their companion animals. Better microbial control measures should be implemented by the B2C companies surveyed in this study. Improving the microbial quality of RMBDs starts at using quality meat ingredients or treating meat byproducts and organ meats destined for raw pet food production as food-grade as economically possible, good manufacturing practices to control for cross-contaminations, and sound sanitation procedures. Raw pet foods should always be treated as TCS foods and proper temperature/time controls should be followed during shipping. Product labels should provide adequate information on ingredients and proper storage, handling and use of the product to mitigate food safety risks.

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3.7 Tables

Table 3.1. Summary of lacking information on the primary packaging labels of raw pet food samples.

Meat type	Company A	Company B	Company C	Company D
Ground meat blends	1. Calorie content	1. Calorie Content 2. Species the product is intended for 3. Guaranteed analysis 4. Address of the Manufacturer 5. List of ingredients	1. Calorie Content 2. Species the product is intended for	1. Calorie Content 2. Guaranteed analysis 3. Statement of nutritional adequacy
Liver	1. Calorie content 2. Guaranteed analysis	1. Calorie Content 2. Guaranteed analysis 3. Species the product is intended for 4. Address of the Manufacturer	1. Calorie content 2. Guaranteed analysis 3. Species the product is intended for 4. Statement of nutritional adequacy 5. Feeding directions	1. Calorie content 2. Guaranteed analysis 3. Statement of nutritional adequacy 4. Feeding directions

Table 3.2. Species and ingredients of ground meat blends purchased in this study.

Species	List of ingredients on product labels or on company website			
	Company A	Company B[a]	Company C	Company D
Beef	Ground beef, beef bones, beef heart, beef liver, beef kidneys, beef lungs	Ground up beef: meat, bone, heart, liver, kidney	Meat including heart & lung, bone, liver, kidney/spleen/ pancreas	Beef round, beef meat, beef heart, beef tongue, beef bone, beef liver, beef kidney, beef spleen, beef suet
Chicken	Whole chicken (includes head, feet, and giblets)	Ground up chicken: meat, bone, heart, liver, kidney	Whole chicken, heart, bone, lung, feet, innards	Chicken breast, chicken leg quarters, chicken heart, chicken backs, chicken liver, chicken gizzard
Pork	Pork meat, pork bones, pork heart, pork liver, pork kidneys, pork lungs	Ground up pork: meat, bone, heart, liver, kidney	Ground pork	NA[b]
Turkey	Whole turkey (including feet and giblets)	Ground up whole turkeys with organs and added 10% turkey liver	Turkey, heart, bone, liver, lung, spleen, innards	Turkey meat, turkey breast, turkey frames, turkey heart, turkey liver, turkey gizzards, turkey fries

[a] Lists of ingredients for Company B products were obtained from their website. Otherwise, lists of ingredients were printed on the product labels.

[b] Not available. Company D does not sell ground pork blend.

Table 3.3. Microbial quality parameters of raw pet food products purchased in this study.

Raw pet food		Microbial counts (Mean ± SE)[a]					
		Aerobic plate count (APC)	*Enterobacteriaceae* (EB)	Lactic acid bacteria (LAB)	Yeasts and molds (Y&M)	*E. coli* (generic)	*Listeria* spp.
Beef	ground blend	5.99 ± 0.26	4.07 ± 0.26	4.98 ± 0.30	3.23 ± 0.23	4.75 ± 0.28	3.09 ± 0.26
	liver	4.13 ± 0.31	2.24 ± 0.31	3.40 ± 0.35	1.96 ± 0.27	2.49 ± 0.33	2.45 ± 0.30
Chicken	ground blend	5.43 ± 0.26	3.26 ± 0.26	4.71 ± 0.30	3.00 ± 0.23	4.07 ± 0.28	3.02 ± 0.26
	liver	4.20 ± 0.31	1.71 ± 0.31	3.47 ± 0.35	1.21 ± 0.28	2.50 ± 0.34	2.00 ± 0.31
Pork	ground blend	5.01 ± 0.38	3.11 ± 0.39	4.34 ± 0.44	2.92 ± 0.34	3.24 ± 0.41	2.14 ± 0.38
	liver	5.06 ± 0.39	2.30 ± 0.39	4.60 ± 0.44	2.02 ± 0.35	2.62 ± 0.42	2.01 ± 0.38
Turkey	ground blend	4.99 ± 0.28	3.07 ± 0.28	4.56 ± 0.31	3.10 ± 0.25	3.48 ± 0.30	2.79 ± 0.27

[a] Mean ± one standard error.

Table 3.4. Levels and percentages of raw pet food products testing presumptive positive for *Salmonella* spp. in this study.

Raw pet food		*Salmonella* level (log CFU/g)[a]	Percent of Products (%)
Beef	ground blend	1.67 ± 0.37	41.67
	liver	2.13 ± 1.03 [b]	11.10
Chicken	ground blend	< 1	41.67
	liver	< 1 [b]	11.10
Pork	ground blend	< 1 [b]	16.70
	liver	< 1 [b]	33.33
Turkey	ground blend	1.17 ± 0.31	63.67

[a] Mean ± one standard error. The limit of quantification was 1 log CFU/g.

[b] Presence of presumptive *Salmonella* colonies was detected in less than three samples.

3.8 Figures

Figure 3.1(a). Primary packaging and label information on meat blends purchased from Company A and Company B.

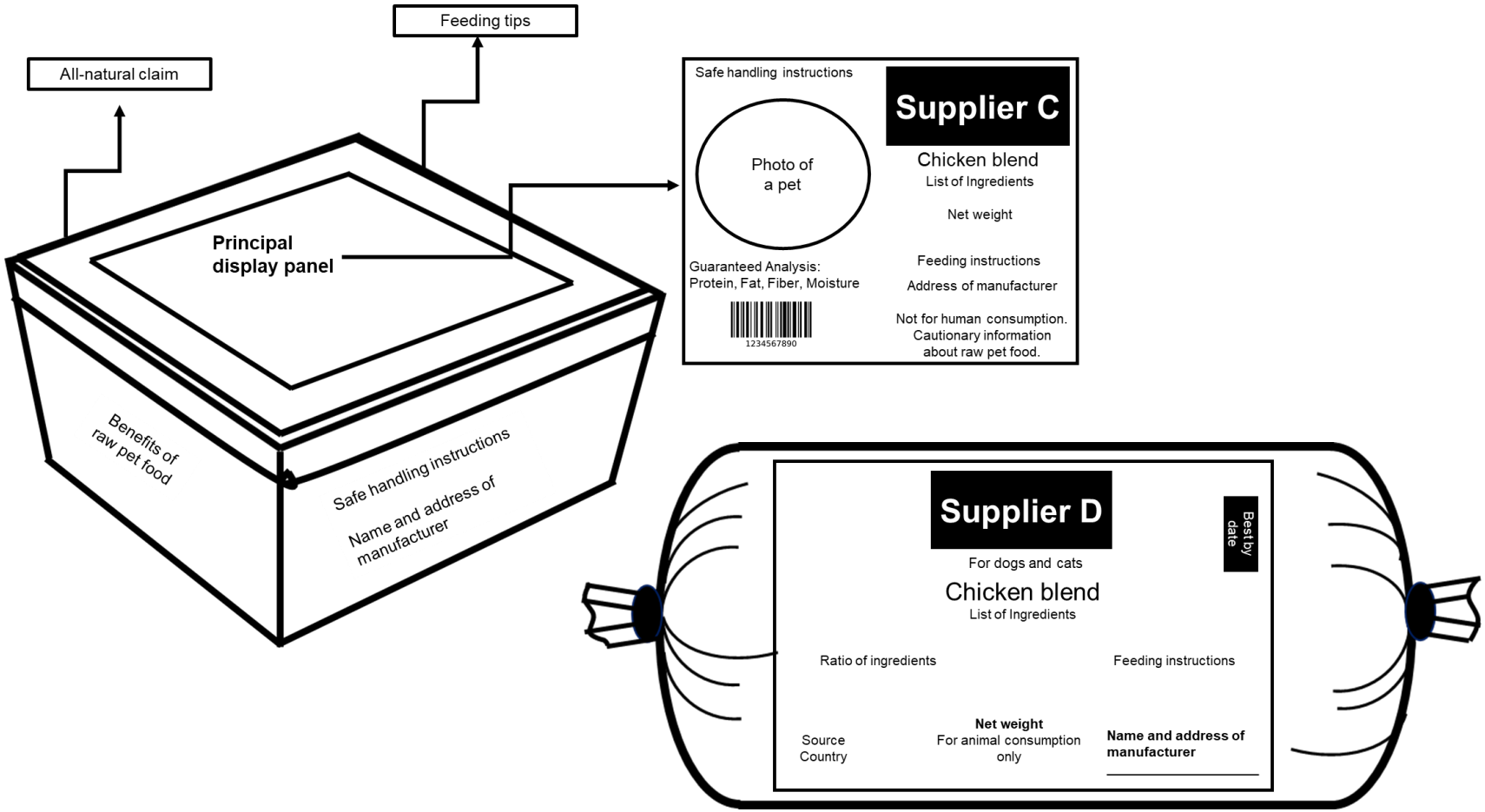

Figure 3.1 (b). Primary packaging and label information on meat blends purchased from Company C and Company D.

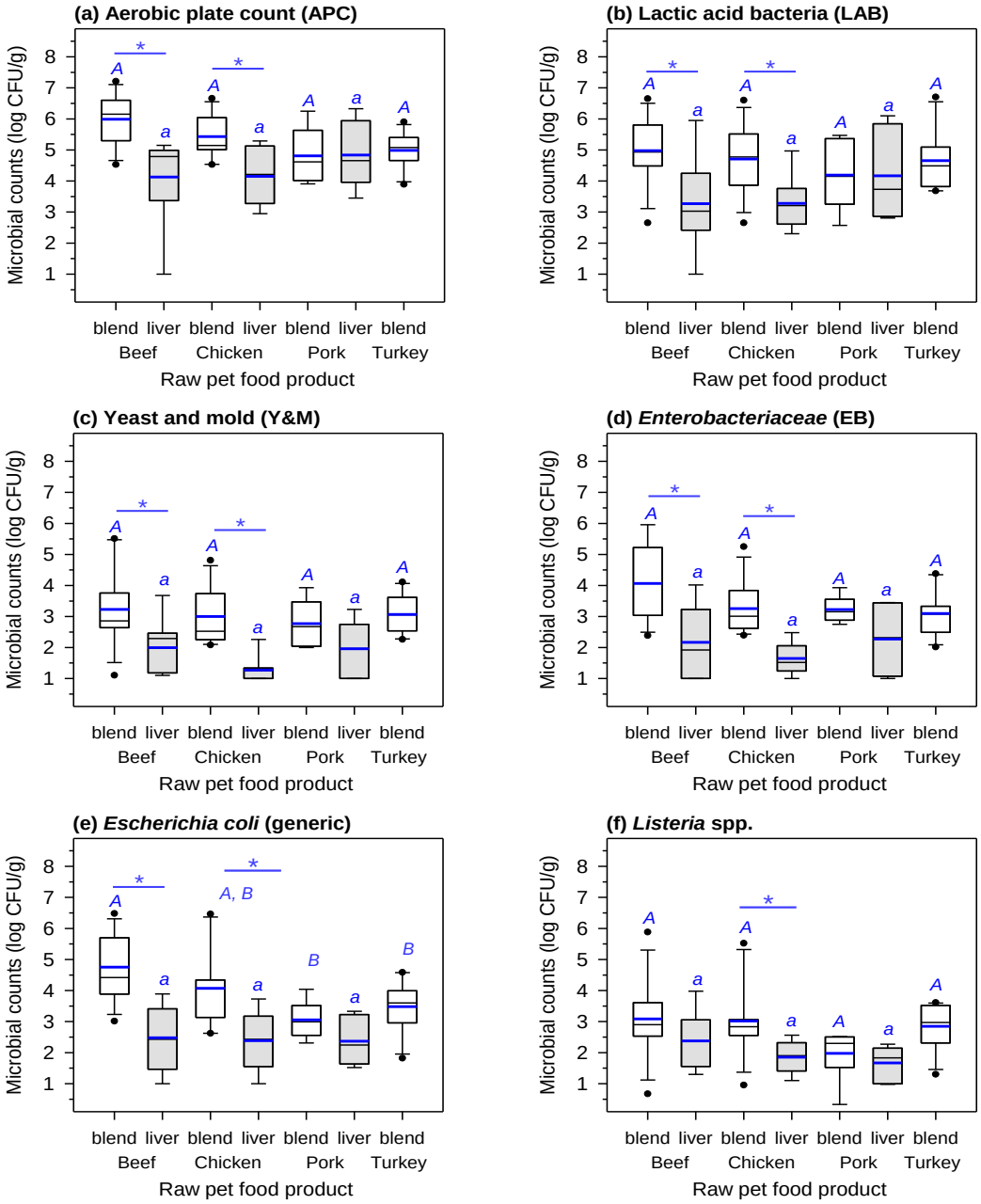

Figure 3.2. Variations in microbial quality parameters of raw pet food products purchased online from business to consumer (B2C) companies. Boxplots represent 25th, 50th (median) and 75% percentiles, while whiskers represent 10th and 90th percentiles. Solid circles below and above the whiskers represent data beyond the 10th and 90th percentiles, respectively. Means are represented by dark blue lines. Asterisks (*) indicate significant difference ($p < 0.05$) between the mean values for ground meat blends and livers within the same species. Uppercase and lowercase letters were used to compare means across species for blends and livers, respectively. Means with no common letters differed significantly ($p < 0.05$).

Chapter 4. Effect of Peracetic Acid, Cultured Dextrose Fermentate, and Buffered Vinegar on *Salmonella* and Aerobic Bacteria in Raw Chicken Livers

4.1 Abstract

Chicken liver is one of the most common offal used as ingredient in raw pet foods. While the demand for raw pet food diets is increasing, presence of pathogens like *Salmonella* remains to be a public health concern. This study aimed on evaluating the use of peracetic acid (PAA), cultured dextrose fermentate (CDF), and buffered vinegar (BV) to reduce *Salmonella* on raw chicken livers. Samples were inoculated with a five-strain cocktail of poultry-borne *Salmonella* to obtain 10^6 CFU/g. Samples were immersed for 90 s with agitation in one of the following treatments: distilled water (control), 450 ppm PAA, 1.5 % (w/v) CDF and 2.0 % (w/v) BV, prior to storing at 4°C. *Salmonella* was enumerated on XLD agar and monitored for 14 days. Data were analyzed using analysis of covariance (ANCOVA). After immersion, there was a significant *Salmonella* reduction ($p < 0.05$) observed in all treatments, including control. PAA resulted in the greatest numerical reduction at 0.65±0.12 log; yet, there were no significant differences in the reductions among all other treatments ($p > 0.05$). After 14 days, higher numerical reductions were still observed for PAA, but the difference was only seen when compared to CDF and not to BV nor the control. Although similar reductions ($p > 0.05$) were noted after 14 days except for CDF, *Salmonella* population was lowest in all timepoints when PAA was used. Effects on aerobic bacteria and liver color were also studied using uninoculated chicken livers. PAA and CDF were able inhibit the growth of aerobic

bacteria until day 3 while BV have inhibited the growth up to 7 days. This indicated that BV was the most effective among treatments in retarding the growth of aerobic microorganisms in chicken liver. Color measurement showed that chicken livers immersed in PAA became lighter, but difference was no longer observed on day 1 and the succeeding days. No differences were also observed in redness and yellowness values across all treatments.

4.2 Introduction

Salmonella is one of the major foodborne pathogens with high public health risk (Jung et al., 2019). In 2019 alone, this pathogen accounted for 8,956 infections, 2,492 hospitalizations and 54 deaths in the United States in (CDC, 2019a). People infected by *Salmonella* may experience diarrhea, fever, stomach cramps, nausea, vomiting and/or headaches (CDC, 2019b). While this pathogen is often associated with consumption of contaminated raw or undercooked chicken, eggs, and beef (Montville, 2012), recent reports documented that *Salmonella* can also come from a wider spectrum of animal and human foods (CDC, 2022).

A well-known health risk factor for human salmonellosis is handling *Salmonella*-contaminated pet food (Davies et al., 2019). Cases of *Salmonella* infection in humans due to contaminated pet food (e.g., kibble) have been previously reported (e.g., Behravesh et al., 2010; Imanishi et al., 2014; Schnirring, 2018). However, newer pet food products, specifically raw meat–based diets (RMBDs), pose a much higher risk as they are made from raw ingredients such as fish, livestock, or poultry (Freeman et al., 2013). Although RMBDs are gaining acceptance from pet owners due to perceived health benefits, no peer-reviewed studies have supported these claims (Nüesch-Inderbinen et al., 2019; van Bree et al., 2019). Instead, disadvantages such as the presence of pathogens in raw pet foods were observed. Nemser et al. (2014) recovered *Salmonella* from 15 of 576 raw pet food, exotic feed and jerky-type treats surveyed from 2011 to 2012. Additionally, reports linking *Salmonella* in raw pet foods to human salmonellosis have been reported (Hassan et al., 2019).

There are different kinds of muscle meat, bones, and variety meats (offal) used in preparing RMBDs. For this diet preparation, liver is the most common variety meat (Morelli et al, 2019) because it is a good source of essential nutrients (Seong et al., 2015). Livers are currently sold by raw pet food manufacturers and specialty pet food shops in packs for pet owners who choose to prepare homemade RMBDs. Most raw pet food manufacturers grind and mix the liver with other ingredients such as meat trims, fruits and vegetables to produce raw pet food diet. Chicken liver has been reported for pathogen outbreaks, one of which is the 2011 *Salmonella* Heidelberg outbreak that sickened 39 individuals (CDC, 2012). Jung and colleagues (2019) also observed a high prevalence of *Salmonella* in chicken livers, recovering *Salmonella* in 59.4 % (148 of 249) of the purchased chicken livers from retail stores in three U.S. states.

Some studies have focused on reducing pathogens in variety meat like livers, giblets, and gizzards but the published data are limited. This is an important area of research as this could have profound implications for the raw pet food safety. The U.S. Food and Drug Administration (U.S. FDA) has stringent guidelines with regards to *Salmonella* in animal foods; pet food is considered adulterated if it is contaminated with *Salmonella* and no subsequent heat step or pasteurization process to kill it (U.S. FDA, 2013).

In determining appropriate interventions, the processes and nature of ingredients should be considered. For instance, when chicken liver is used in ground meat blends, interventions should precede mixing and grinding as these processes allows for surface pathogens to be spread in the end-product (Stelzleni et al., 2013). Furthermore, because RMBDs try to mimic natural diet in the wild and apply it to pets, utilization of non-

thermal processes and use of antimicrobial agents, specifically those that have clean-label designations, generally regarded as safe (GRAS) or are known to be safe and suitable for use in the production of meat and poultry (USDA-FSIS, 2021), should be explored.

At present, U.S. FDA does not have a regulatory definition for “clean label.” However, Grant and Parveen (2017) described clean label products as those that are free from additives, artificial colors, and flavors. One example of clean label products commonly used as antimicrobial in the market is buffered vinegar (BV). BV is an acetic acid combined with a buffer, either sodium or potassium-based alkali, to reduce the impact on the functional properties of the product (Badvela et al., 2016). There are studies reporting its effectiveness in controlling microbial load and pathogens especially when it is used with other interventions (i.e., antimicrobials, carbon dioxide) to package meat and poultry products (Badvela et al., 2016; Desai et al., 2014; Ponrajan et al., 2011). Another widely used antimicrobial approved by FDA in the U.S. is Microgard® fermentates (Al-Zoreky et al., 1991). This patented antimicrobial is comprised of metabolites from milk, dextrose, or wheat with propionic bacteria or specific *Lactococci* (Von Staszewski and Jagus, 2008). Inhibitory activities of the fermentate on dairy products, dressings, and some vegetables have been observed and reported (Yang et al., 2021; Serna-Jiménez et al., 2020; Samapundo et al., 2017; Von Staszewski and Jagus, 2008). BV and fermentates can be listed on the product labels as “vinegar” and “cultured milk/or cultured dextrose”, respectively.

Peracetic acid (PAA), on the other hand, is an antimicrobial agent which is increasingly popular in poultry decontamination (Cano et al., 2021). The U.S. Department of Agriculture does not require labeling for PAA if its use does not exceed

2000 ppm of peroxyacids and 1435 ppm of hydrogen peroxide (USDA FSIS, 2021). This is considered safe as risk assessments by Joint FAO/WHO Expert Committee on Food Additives (2005, 2006) and European Food Safety Authority (2014) showed that there is no potential health concern in using PAA if they were prepared within the conditions they have been evaluated. These include PAA treatment preparations for pre-chill (spray washing or short-duration dip treatment), chill (chiller baths) and post-chill (short-duration dip treatment) steps in poultry processing. Concentrations used were 400-700 ppm for spray washes, up to 230 ppm in the long duration chiller baths and concentrations not exceeding 2000 ppm in the short-term baths (EFSA, 2014).

With the current guidelines and trends in raw pet food manufacturing, the main objective of this study was to evaluate the efficacy of PAA, cultured dextrose fermentate (CDF), and BV on *Salmonella* in raw chicken livers. Because PAA is an effective intervention in microbial reduction for poultry, PAA was hypothesized to yield the highest *Salmonella* reduction as compared to buffered vinegar and cultured dextrose fermentate. Moreover, effect of PAA, CDF, and BV on chicken liver's aerobic bacteria population and color were determined.

4.3 Materials and Methods

4.3.1 *Salmonella* Preparation and Inoculation

Five poultry-borne strains of *Salmonella enterica* subsp. *Enterica* were incubated individually at 35 °C for 24 h in 9 ml of tryptic soy broth (TSB). These strains were the following: *Salmonella* Hadar (JE 322 2013 MI), *Salmonella* Enteritidis (IV/NVSL 94-13062), *Salmonella* Branderup (NVSL 96 - 12528), *Salmonella* Typhimurium (ATCC 14028) and *Salmonella* Heidelberg (2247-1). For each strain, 0.1 ml was transferred in

200 ml TSB using the same incubation time and temperature. Subsequently, cell cultures were pooled together to make a bacterial cocktail (1000 ml of poultry-borne *Salmonella*) with a final concentration of 10^8 CFU/ml.

Chicken livers were procured from Tyson Foods and brought to the University of Nebraska-Lincoln Food Processing Center (UNL FPC). Chicken livers were stored frozen at –20 °C until further use. Approximately 24 h prior to inoculation, chicken livers were thawed at 4 °C. Background aerobic bacteria and *Salmonella* were determined by direct plating. The APC obtained was 2.57 ± 0.25 log CFU/g while *Salmonella* was not observed in the samples (limit of quantification: 10 CFU/g).

Chicken livers were dipped in the bacterial cocktail for 30 s. Samples were then drained on a grill grid and air-dried for 20 minutes. The inoculated chicken livers were placed in a cooler at 4 °C for 24 h to allow for further microbial attachment. Prior to applying the antimicrobial treatments, three subsamples of inoculated livers were obtained in every batch for determining the initial *Salmonella* count which were targeted at 10^6 CFU/g. Mean *Salmonella* counts obtained was 6.79 ± 0.09 log CFU/g.

4.3.2 Preparation and Application of Antimicrobial Treatments

One-liter solutions of 450 ppm peracetic acid (PAA) (Birkoside MP-2, Birko Corp., Henderson, CO, USA), 1.5 % w/v cultured dextrose fermentate (CDF), and 2.0 % w/v powdered buffered vinegar (BV) were prepared by diluting the concentrated solution (for PAA) and dissolving powder (for CDF and BV) in cold (4 °C) sterile distilled water. PAA concentration was tested using a PAA test kit (Peracetic Acid VACUettes kit K-7904B, CHEMetrics, Inc., Midland, VA, USA). The CDF was provided by International Flavors & Fragrances Inc. (MicroGARD® 200, New Century, KS, USA) while the BV

was supplied by Corbion (Verdad® Powder N6 Vinegar, Lenaxa, KS, USA). Distilled water was used as control in this study to demonstrate the amount of reduction due to immersion and mechanical agitation of the chicken liver in the solution.

Chicken livers inoculated with *Salmonella* spp. were then immersed in 4 °C solutions of distilled water (control), PAA, CDF, or BV for 90 sec with agitation at 40 rpm (SHKE6000-7, Thermo Scientific, Marietta, OH, USA). After immersion of samples, extra liquid was allowed to drip for 3 min prior to vacuum packing (Multivac C200, Multivac Inc., Kansas City, MO, USA). The treated samples were individually packaged, stored at 4 °C and were used subsequently for microbial analysis.

4.3.3 Microbial Analysis

Chicken livers were aseptically removed from their packaging material on Days 0, 3, 7, and 14 post-treatment. Two subsamples were analyzed for each treatment and day. Samples were weighed and placed into a sterile stomacher bag (Whirl-Pak®, Thomas Scientific LLC, Swedesboro, NJ, USA) then mixed with the corresponding amount of 0.1% buffered peptone water to prepare a 1:10 dilution. Samples were then stomached for 90 sec at 200 rpm (Stomacher® 400 Circulator, Seward Ltd., Bohemia, NY, USA). Serial dilutions were conducted followed by duplicate plating on xylose lysine deoxycholate (XLD) agar. Plates were then incubated at 37 °C for 24 ± 2 h. After enumeration, *Salmonella* counts were reported as log CFU/g and reductions computed using the initial *Salmonella* count (pre-treatment) and the average count of the subsamples on a specific sampling timepoint.

4.3.4 Aerobic bacteria counts

Non-inoculated chicken livers were treated with antimicrobials using the same procedures in Section 4.3.2. APC were enumerated on Days 0, 3, 7 and 14 post-treatment. Two subsamples from each treatment were plated on Petrifilm™ (3M Microbiology Products, St. Paul, MN, USA) in duplicates and incubated at 35° ± 1 °C for 48 ± 3 h. Microbial counts were reported as log CFU/g.

4.3.5 Liver Color Evaluation

The same liver samples used for APC were tested for color prior to plating. Color measurements were conducted using a handheld portable colorimeter (Model BC-10, Minolta Camera Co Ltd., Osaka, Japan) and expressed as L* (lightness), a* (redness), and b* (yellowness). Calibration was initially performed by placing a standard white Minolta calibration plate inside the same packaging bag used for the chicken liver. This is to nullify the color and light reflectance properties of the packaging material (Petracci and Fletchert, 2002). Color measurements were taken at three different spots on the chicken liver surface that were free from noticeable defects (e.g., uneven surface, bruises) and were averaged. Meat color ($L^*a^*b^*$) measurements were recorded on Day 0, 1, 3, 7 and 14 post-treatment.

4.3.6 Statistical Analysis

Three independent replications were performed for each set of treatments using freshly prepared solutions of antimicrobial treatments and bacterial cocktails. Data were analyzed using four by four (4 x 4) factorial two-way analysis of variance with covariate (ANCOVA) wherein treatment and time (in days) were the independent variables, replications as block and weight as covariate. For color, data were analyzed using four

by five (4 x 5) factorial two-way analysis of variance (ANOVA) with treatment and time (in days) as independent variables and replications as block. When there was no interaction among variables, the main effects were analyzed. When there was significant difference ($p < 0.05$), Tukey-Kramer's post-hoc test was applied to separate means between treatments. All statistical analysis were conducted using SAS software (Version 9.4, SAS Institute, Cary, NC, USA).

4.4 Results and Discussion

4.4.1 *Salmonella*

Statistical analysis showed there was a significant interaction between treatment and day ($F = 2.40$; df = 9,29; $p = 0.04$) but there was no association between liver weight and log reduction achieved ($F = 1.82$; df = 1,29; $p = 0.19$). Hence, *Salmonella* reduction were estimated using the mean weight (31.37 g) of the chicken livers analyzed.

Immediately after treatment, results showed that there was a significant *Salmonella* reduction when using PAA ($p < 0.0001$), CDF ($p = 0.0016$), and BV ($p = 0.0021$), including control ($p = 0.0012$). However, there were no difference in the reduction of *Salmonella* among the treatments and the control (Table 4.1) indicating that immersing chicken livers in antimicrobials were just as effective as immersing or washing in distilled water. Still, higher but non-significant log reductions were observed using PAA than in CDF ($p = 0.2894$), BV ($p = 0.2536$) and control ($p = 0.3505$). While the difference was not significant, PAA was expected to achieve higher reductions as review on its efficacy showed that it was more effective in poultry decontamination in comparison with other antimicrobials like chlorine compounds and cetylpyridinium chloride (CPC) (Cano et al., 2021). PAA controls growth of microorganisms in the food

matrix by denaturing proteins, disrupting the cell membrane and obstruction of enzymatic and transport process (King et al., 2005; Block, 2011). Nagel et al. (2013) observed reductions of 2.02 and 2.14 log CFU/ml rinsate in broiler carcasses when dipping for 20 s in 4 ± 2 °C post-chill immersion tank using 400 ppm and 1000 ppm PAA concentrations, respectively. Chen et al. (2014) also reported greater than a one log reduction in *Salmonella* population on ground chicken parts, specifically 1.5 and 1.3 log CFU/g. Higher concentrations (700 and 1000 ppm) were used in a continuous online pathogen elimination tank with an immersion time of 23 s and a water temperature ranging from 10-15 °C (4°C potable water was used to bring the treatments in their required concentration). Although longer contact time was used in this study, the higher reductions observed in other studies could also be attributed to the design of their decontamination tank wherein rotation is employed to add more mechanical force to the immersion treatment as compared to using an incubation shaker.

For BV and CDF, zero to low *Salmonella* reductions have been observed in other raw poultry and meat matrices. Stelzleni et al. (2013) studied the effects of two types of BV coupled with sodium dodecyl sulfate and levulinic acid against *S.* Typhimurium on ground beef patties and obtained reductions ranging from 0.36 to 0.70 log CFU/g after seven days. The difference on the reductions between the BVs used and control (no intervention) were minimal, ranging from 0.17 to 0.36 logs. In the case of fermentates, a cultured milk fermentate (Microgard® 100) used in an acidified chicken model showed no significant effect on *Escherichia coli* and *Brochothrix thermosphacta* when compared to the control (Lemay et al., 2002). However, these results contradict those observed by

Ponrajan et al. (2011) where beef injected with brine and 2% BV resulted in a 1.0 log CFU/g reduction on *E. coli* O157:H7.

Overall, over the 14-day period after antimicrobial treatment, *Salmonella* populations decreased for the control, PAA and BV, but not for CDF (Figure 4.1). Counts immediately after treatment (Day 0) were significantly higher ($p < 0.05$) compared to counts obtained on subsequent sampling timepoints for control and BV. For PAA, *Salmonella* count on Day 0 became significantly different after Day 7 ($p < 0.05$). For CDF, difference was only observed between Day 0 and 7 ($p = 0.001$).

Although the decrease in *Salmonella* population at Day 0 may be attributed to the different treatments, the decreasing trend on counts could also be attributed to storage temperature. Chicken livers were kept at 4 °C which generally allow *Salmonella* to survive but inhibit their growth. Pradhan et al. (2012) evaluated the effect of refrigerated and freezing temperature on the growth and survival of *S.* Typhimurium in chicken breast and observed similar trend with this study but the change in *Salmonella* populations did not vary significantly until Day 7. Comparable observations were reported by Osaili et al. (2020) in ground camel meat wherein *S.* Typhimurium counts from the initial population had declined slightly after seven days.

Figure 4.1 shows that *Salmonella* counts were almost identical between control and BV until the 14th day of storage further indicating that it is not effective in controlling *Salmonella* in chicken liver for prolonged refrigerated storage. BV used does not have bactericidal effect, but it is marketed to extend the lag phase of microbial growth (i.e., a bacteriostatic effect) of bacteria present thereby extending product shelf life (Corbion, 2022). While the bacteriostatic effect of BV in *Salmonella* was not evident in this study,

this might be because a lower concentration and a shorter immersion time compared to previous studies were used. Heir et al. (2021) experimented different concentrations (2.5 – 18 %) and immersion times (300 s) of the same BV used in this study on raw salmon and have reported complete inhibition of *Listeria monocytogenes* for 12 days. As for CDF-treated chicken liver, *Salmonella* population also decreased similarly with chicken livers treated with water until the 7th day of storage. Although the *Salmonella* counts increased by Day 14, this was not significantly higher than the counts obtained on Day 7 (p= 0.10).

With PAA, even though differences were not significant compared to the control, *Salmonella* counts were numerically lower in PAA regardless of storage time. Additionally, *Salmonella* populations in PAA-treated samples demonstrated similar trends with other studies wherein *Salmonella* did not continue to grow exponentially under refrigerated conditions. In a study by Park et al. (2017) comparing 1200 ppm PAA and 50 ppm of chlorine, results also showed that PAA was the most effective treatment. However, their observed reduction using PAA was significantly higher compared to water-treated ground chicken. In terms of effect in *Salmonella* population after nine days of storage, observed values over time did not change.

For an intervention to be considered practical in the meat and poultry industries, the accepted criterion is at least one-log reduction of the pathogen of interest (Brashears & Chaves, 2017). The mean estimates of *Salmonella* reduction after 14 days of storage were greater than 1 log CFU/g for PAA, BV and the control. But only samples treated with PAA demonstrated reduction that will likely be greater than one log (95% CI = 1.06, 1.56 log CFU/g). While this technically meets the one-log reduction criteria, the

recommended duration for storage at 4 °C of chicken livers for animal consumption are typically four to seven days. By the time PAA-treated chicken livers reach 1 log reduction when stored at 4 °C, the livers may already be beyond their intended shelf life.

4.4.2 Aerobic Plate Count (APC)

Similar to the *Salmonella* challenge study, there was a significant treatment and day interaction ($F = 7.41$; df = 9,29; $p < 0.0001$) but no interaction between liver weight and achieved microbial counts ($F = 0.03$; df = 9,29; $p = 0.86$). Hence, simple effects of treatment and day were further assessed.

Table 4.2 and Figure 4.2 show the APC counts using different antimicrobial interventions. Immediately after treatment (Day 0), no differences in the APC were observed. However, on Day 3, the difference was now seen as APC of PAA-treated samples was significantly lower compared to CDF ($p = 0.0234$) and the control ($p = 0.0024$). Additionally, APC in chicken livers treated with BV is significantly different when compared to control ($p = 0.0146$).

On Day 7, BV continued to show lower microbial counts compared to PAA ($p < 0.0004$), CDF ($p < 0.0001$) and control ($p < 0.0001$). While there was already a difference between chicken livers treated with BV and PAA, the latter was still lower than the control ($p < 0.05$). According to ICMSF (1986), 5.70 log CFU/g APC value is considered an upper microbiological limit for a quality fresh poultry. The data showed that chicken livers treated with water and CDF are already nearing spoilage levels by Day 7 while PAA and BV continued to maintain lower levels of aerobic bacteria. By Day 14, however, counts for all treatments were greater than 5.70 log CFU/g with BV still having significantly lower APC levels than the other treatments.

With regards to comparing the APC as storage time increased, counts on chicken livers treated with distilled water continued to increase significantly from Day 0 to Day 3 ($p = 0.0071$), Day 7 ($p < 0.0001$) and Day 14 ($p < 0.0001$). This was in contrast with chicken livers treated with antimicrobial treatments wherein growth was much slower. No differences were observed in the APC between the day of treatment and the 3rd day of storage ($p > 0.05$), but counts were increasing as the storage time reached the 7th and 14th day. From the three antimicrobials, CDF was the least effective in inhibiting bacterial growth as a marginal difference between counts of Day 0 and Day 3 ($p = 0.18$) was observed.

The most effective treatment was BV as growth of aerobic bacteria was inhibited until Day 7 and counts were not approaching spoilage level until the 14th day of storage. These results agreed with previous observations in chicken retail cuts treated with 1.0 % BV, where product shelf life was extended from approximately 12 to 20 days (Desai et al. 2014). Organic acids such as acetic acid or vinegar are effective at reducing aerobic bacteria in meat and poultry through disruption of the normal cellular process in microorganisms, thus slowing growth (Badvela et al., 2016). Apart from aerobic bacteria, other researchers have measured spoilage by evaluating psychrotrophic microorganisms and the results were similar to those observed in this study. Harris and Williams (2008) observed that 1.0-3.0 % BV retarded the growth of psychrotrophs for 7 days in ground chicken breast meat while Ponrajan et al. (2011) reported delayed growth for 21 days in beef top rounds and top sirloin steak using 2.0% BV.

4.4.3 Meat Color

There was no treatment by day interaction observed for lightness (L*), redness (a*) and yellowness (b*) values. The type of antimicrobial treatment had an effect on the lightness ($p = 0.005$) but not on redness ($p = 0.7381$) or yellowness ($p = 0.2536$) of the chicken liver. Refrigerated storage time influenced all color parameters ($p < 0.05$).

Although there were no interaction effects for any color values, simple effects (effect of days in storage per treatment) were further investigated for lightness (L^*). Use of CDF and BV showed no distinct differences when compared to control. However, chicken livers treated with PAA was significantly lighter than those treated with BV significantly at Day 0 ($p = 0.0229$) although the difference between treatments became marginal by Day 1 ($p = 0.0778$). Prior to packing, the difference in lightness was visibly noticeable between PAA-treated chicken livers and the other treatments (Figure 4.3). This could be due to presence of hydrogen peroxide in the antimicrobial agent which have been reported to cause a bleached appearance (Lillard & Thomson, 1983). However, on Days 3 to 14, there were no differences observed among all treatments showing the initial lightening effect by PAA was temporary (Figure 4. 4 and 4.5). Bauermeister et al. (2008) also reported lighter appearance of poultry carcasses treated with 100 ppm and 150 ppm PAA, but differences were no longer observed by Day 7 compared to the control. Although there were changes observed in some of the treatments as the days in storage increases, generally, chicken livers in this study became lighter which was also observed by Petracci and Fletchert (2002) in broiler skin and meat. For redness (a*) and yellowness (b*), the main effects of prolonged storage also showed increasing values of these two parameters (Figure 4.5).

4.5 Conclusions/Recommendations

Salmonella reductions in inoculated raw chicken livers after immersion in PAA, CDF, or BV were not different when compared to chicken livers immersed in distilled water. No difference in reductions among treatments was also observed on the 3rd and 7th day of storage. However, on the 14th day, a higher reduction was observed for PAA, BV, and control but not for CDF. Additionally, the trend showed a decrease in *Salmonella* population throughout storage of chicken livers at 4 ºC. Nevertheless, *Salmonella* counts in PAA-treated samples was numerically lower from Day 0 to Day 14 compared to other treatments indicating its potential to achieve moderate *Salmonella* reductions in raw chicken livers after treatment and prolonged storage at refrigerated conditions. Moreover, it was seen that all the antimicrobial treatments could be used to inhibit growth of aerobic bacteria as PAA, and CDF were able to demonstrate control until the 3rd day of storage and BV inhibited growth until the 7th day of storage. Overall, no significant differences in *L*a*b** values were observed in extended storage of chicken livers at 4 ºC.

It is recommended to explore use of PAA concentrations higher than 450 ppm to check if it will have a more distinct difference when compared to untreated chicken livers. Furthermore, seeing BV as the most effective treatment in delaying the growth of aerobic bacteria, it may be worthwhile to investigate possible synergistic effect of PAA and BV in controlling pathogens and background microflora of chicken livers.

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4.8 Tables

Table 4.1. Reduction of *Salmonella* (log CFU/g) in chicken livers treated with different antimicrobials after 14 days of storage at 4°C.

Storage time (days)	Log Reduction in *Salmonella* spp. (Mean ± SE)*							
	Distilled water (Control)		PAA		CDF		BV	
0	0.44 ± 0.12	[a,x]	0.65 ± 0.12	[a,x]	0.43 ± 0.12	[a,x]	0.41 ± 0.12	[a,x]
3	0.81 ± 0.12	[a,y]	1.00 ± 0.12	[a,x,y]	0.72 ± 0.12	[a,x,y]	0.83 ± 0.12	[a,y]
7	0.85 ± 0.12	[a,y]	1.08 ± 0.12	[a,y]	0.95 ± 0.12	[a,y]	0.87 ± 0.12	[a,y,z]
14	1.22 ± 0.12	[a,z]	1.31 ± 0.12	[a,y]	0.65 ± 0.12	[b,x,y]	1.20 ± 0.12	[a,z]

[ab]Least squares means within a row without common superscripts are different $p < 0.05$.
[xyz]Least squares means within a column without common superscripts are different $p < 0.05$.
*Abbreviations: standard error (SE); PAA= 450 ppm peracetic acid; CDF= 1.5% cultured dextrose fermentate (Microgard® 200); BV= 2.0% powdered buffered vinegar (Verdad® Powder N6 Vinegar).

Table 4.2. Aerobic plate count (APC) (log CFU/g) in chicken livers treated with different antimicrobials after 14 days of storage at 4°C.

Storage time (days)	Aerobic plate counts (log CFU/g) (Mean ± SE)*							
	Distilled water (Control)		PAA		CDF		BV	
0	2.80 ± 0.25	a,w	2.36 ± 0.25	a,x	2.95 ± 0.25	a,x	2.77 ± 0.25	a,x
3	3.79 ± 0.25	c,x	2.68 ± 0.25	a,x	3.53 ± 0.25	b,x	2.87 ± 0.25	a,b,x,y
7	5.61 ± 0.26	c,y	4.72 ± 0.26	b,y	5.40 ± 0.25	b,c,y	3.61 ± 0.26	a,y,z
14	8.32 ± 0.25	b,z	7.84 ± 0.25	b,z	8.09 ± 0.26	b,z	5.89 ± 0.25	a,z

[abc]Least squares means within a row with different superscripts are different $p < 0.05$.
[wxyz]Least squares means within a column with different superscripts are different $p < 0.05$.
*Abbreviations: standard error (SE); PAA= 450 ppm peracetic acid; CDF= 1.5% cultured dextrose fermentate (Microgard® 200); BV= 2.0% powdered buffered vinegar (Verdad® Powder N6 Vinegar).

4.9 Figures

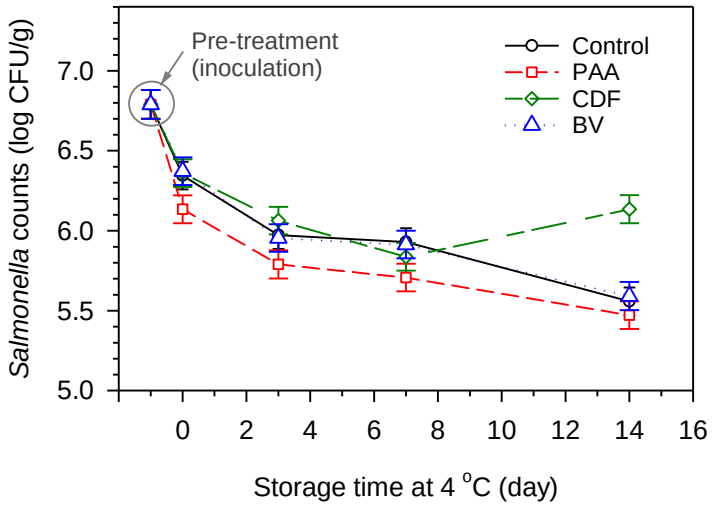

Figure 4.1. Effects of various antimicrobial treatments on chicken livers inoculated with *Salmonella* spp. (error bars represent standard error).

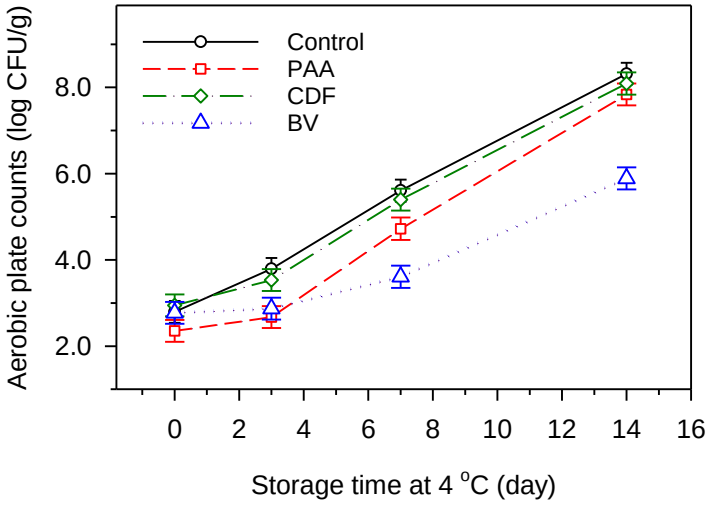

Figure 4.2. Aerobic bacteria in chicken livers treated with various antimicrobials during storage (error bars represent standard error).

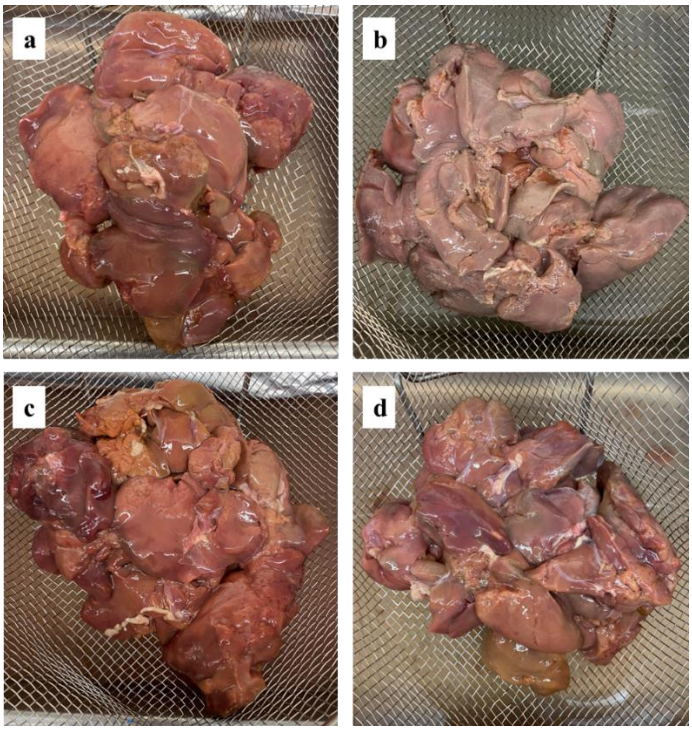

Figure 4.3 Chicken liver after dipping in a. water, b. 450 ppm PAA, c. 1.5% CDF and d. 2.0% BV.

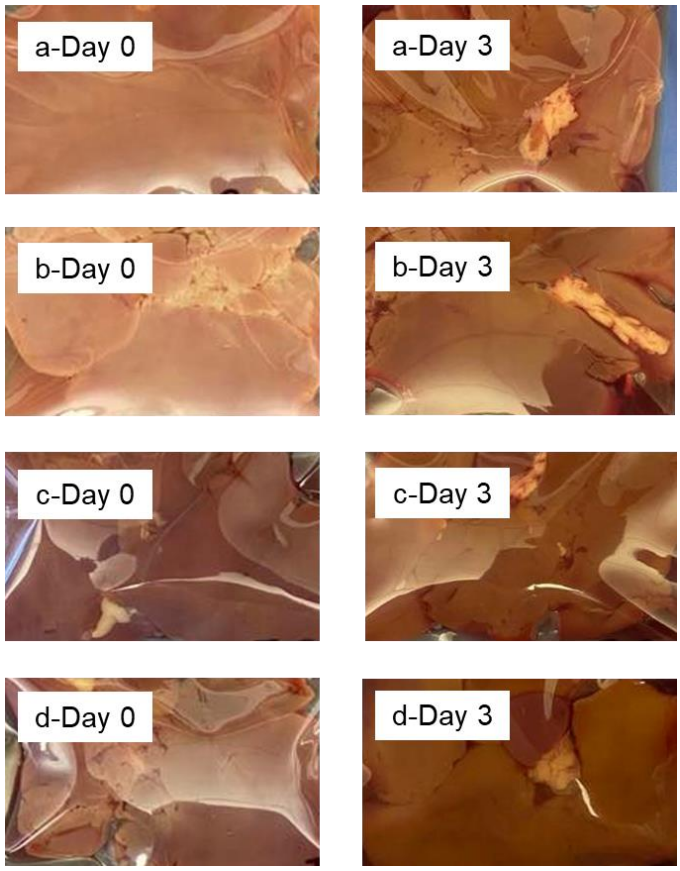

Figure 4.4 Chicken liver inside the packaging material after dipping in a. water, b. 450 ppm PAA, c. 1.5% CDF and d. 2.0% BV at Day 0 and Day 3.

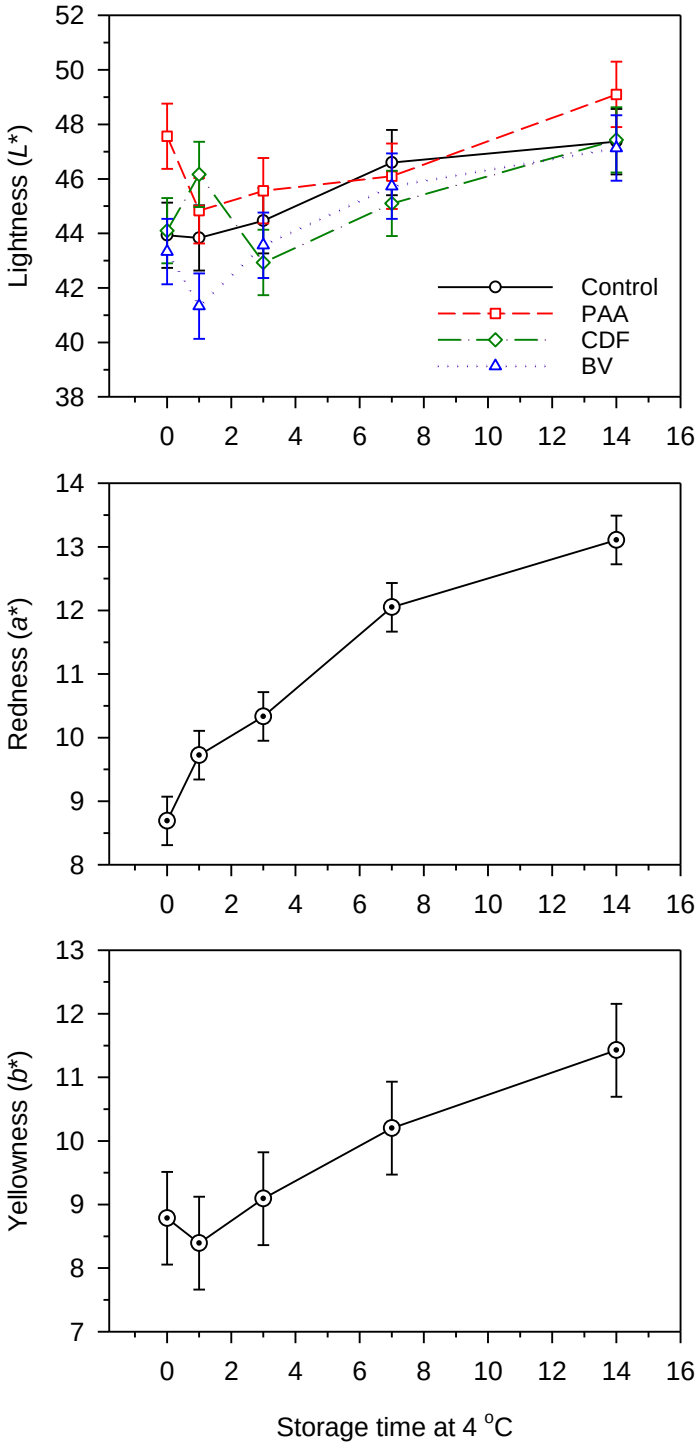

Figure 4.5. Color ($L^*a^*b^*$) measurements of raw chicken livers treated with different antimicrobials and stored for 14 days at 4 °C. Lightness (L^*) values are presented for each antimicrobial treatment to show simple effects of treatment per day, while redness (a^*) and yellowness (b^*) values have been averaged for all treatments due to lack of statistical differences across treatments. Error bars represent standard error (SE).

Chapter 5. Conclusions and Future Work

5.1 Conclusions

The first objective aimed on evaluating the microbial quality of raw pet food products purchased online and the food safety information provided by pet food manufacturers or businesses who sell directly to consumers (B2C) companies. Overall, there was an interaction between meat type (ground meat blends and livers) and species (beef, chicken, pork and turkey) for all microbial parameters except for *Salmonella.* Specifically, aerobic plate counts (APC), lactic acid bacteria (LAB), yeasts and molds (Y&M), *Enterobacteriaceae* (EB), and generic *Escherichia coli* counts in ground meat blends and livers differed for beef and chicken, but not for pork. *Listeria* spp. counts in ground meat blends and livers differed only for chicken. It is crucial that these online companies implement good manufacturing practices (GMP), sanitation standard operating procedures (SSOP), and preventive controls to ensure that raw meat based diet (RMBD) products arriving to the consumers are of good quality and safe. Sampling time (season of transport) did not contribute to the microbial counts of the raw pet food products tested. The packing and shipping procedures implemented by the four B2C companies were sufficient at maintaining low product temperatures to control microbial growth. With regards to product labeling, all B2C companies failed to provide at least one product labeling information required by AAFCO's Model Regulations for Pet Food and Specialty Pet Food (2021). While safe handling instructions are not required for raw pet foods, all four B2C companies provided this information either on the product label or in a separate pamphlet included in the shipment. This practice should be encouraged considering that RMBDs are time/temperature control for safety (TCS) foods.

The second objective focused on evaluating the effects of peracetic acid (PAA), cultured dextrose fermentate (CDF), and buffered vinegar (BV) on *Salmonella*, aerobic plate counts (APC) and meat color of raw chicken livers. *Salmonella*-inoculated raw chicken livers were immersed with agitation in different antimicrobial treatments. Observed reductions in *Salmonella* by the three antimicrobial treatments showed no difference compared to control (distilled water). However, at Day 14, higher *Salmonella* counts were observed for CDF differing from PAA, BV and control. Overall, even though difference is insignificant among treatments and control, this showed significant *Salmonella* reduction in chicken livers. A separate set of uninoculated raw chicken livers were used to evaluate aerobic bacteria and color. All antimicrobial agents inhibited growth of aerobic bacteria until the 3rd day of storage. From the three, BV proved to be the most effective in inhibiting the growth of aerobic bacteria in raw chicken livers. As for color, there was a difference observed in lightness (L*) for PAA compared to BV but this was only reflected on the day of treatment. No difference in *L**, *a**, and *b** were noted from Day 3 to Day 14 of storage.

5.2 Suggestions for Future Work

Since B2C companies (suppliers) influence the microbial level and quality of RMBDs, a quantitative microbial risk assessment (QMRA) could be conducted to develop a model to estimate human exposure to pathogens like *Salmonella* and *L. monocytogenes* through RMBD feeding. Moreover, it is also noteworthy to explore the impact of employing preventive controls (e.g., supplier controls, process controls like high pressure processing, and stringent sanitation procedures) in reducing and increasing human exposure to pathogens.

Furthermore, because there is a growing demand of RMBDs in the USA characterizing labeling information of RMBDs sold online or in retail stores could be documented as well as consumers' knowledge, attitudes, and perception (KAP). At present, EU countries have KAP reports readily available (Morelli et al., 2019, 2021; Bulochova & Evans, 2021).

As PAA and BV showed some reduction in *Salmonella* and aerobic bacteria population, respectively, synergistic effects of these two antimicrobial agents could be explored. Additionally, effect of other chemical interventions which are approved for use in meat and poultry could be investigated on other variety meats such as gizzard, heart, lungs, and green tripes. Non-thermal processes, such as HPP and irradiation, and their effects on pathogens and product shelf-life could be done. Hurdle technology concept, particularly on the combination of chemical and physical treatments, could be studied if it will yield higher microbial reduction compared to employing just one intervention.

5.3 References

Bulochova, V., & Evans, E. W. (2021). Raw Meat–Based Pet Feeding and Food Safety: Netnography Study of Pet Owner Comments and Review of Manufacturers' Information Provision. *Journal of Food Protection, 84*(12), 2099-2108. https://doi.org/10.4315/JFP-21-158

Morelli, G., Bastianello, S., Catellani, P., & Ricci, R. (2019). Raw meat-based diets for dogs: Survey of owners' motivations, attitudes, and practices. *BMC Veterinary Research*, 15, 74. https://doi.org/10.1186/s12917-019-1824-x

Morelli, G., Stefanutti, D., & Ricci, R. (2021). A survey among dog and cat owners on pet food storage and preservation in the households. *Animals*, *11*(2), 273. https://doi.org/10.3390/ani11020273

Appendix A. Levels of Psychrotrophic Bacteria in Raw Pet Foods

During the summer sampling season (May-August), levels of psychrotrophic bacteria in raw pet food products were assessed together with other microbial parameters. Similar procedures discussed in Section 3.3.2 were used and psychrotrophs were enumerated using 3M Petrifilm™ for aerobic plate counts (APC), which were incubated at 7 °C for 10 days. Counts obtained for each product type were averaged and reported as log CFU/g.

Results showed that mean APC counts were comparable to psychrotrophic bacteria counts. Differences in count ranged only from 0.02 to 0.94 log CFU/g.

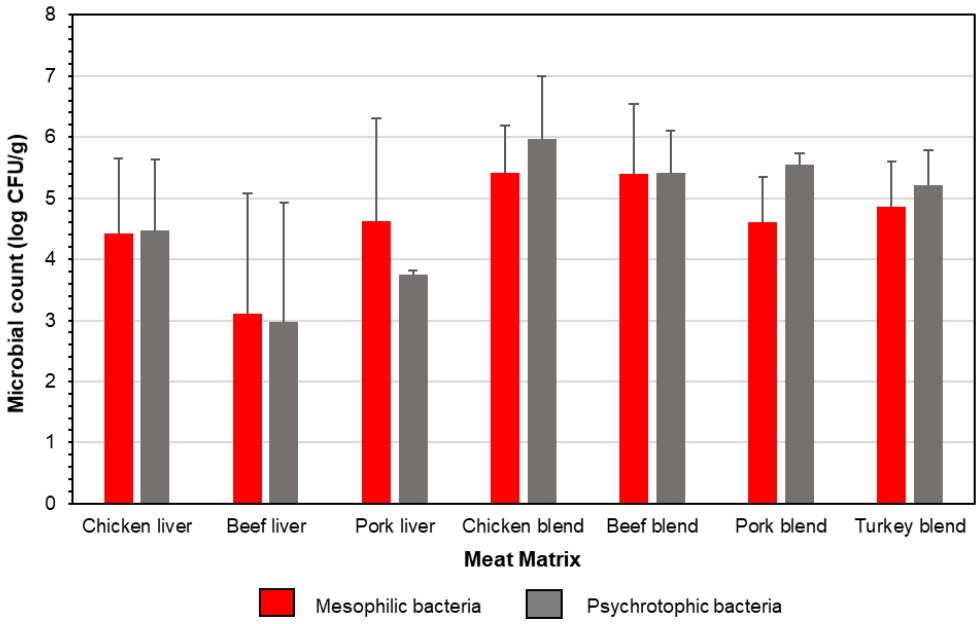

Figure A.1 Level of mesophilic and psychrotrophic bacteria found in raw pet foods purchased in May-August 2021 (error bars represent standard error).

Appendix B. Raw Data and Statistical Analyses

B.1 Microbial Quality of Meat Blends and Livers (Objective 1)

B.1.1 Analysis of Variance (ANOVA)

The code below was used to estimate means of microbial parameters using 2 x 4 factorial two-way ANOVA. Analysis was done to check interaction between meat type and species with sampling time (season) and supplier [a.k.a., business to consumer (B2C) company] as fixed block effects. Additionally, Tukey-Kramer's test was used to compare means. However, this is a nested design since turkey livers were not available at all sampling timepoints. Details about the code are highlighted in green color.

```
*CLEARS SAS LOG AND RESULTS FOR CLEANER WORKING ENVIRONMENT;
dm "log; clear; odsresults; clear;";

* ---------------------------------------------------;
* SAVE OUTPUT TO PDF;
* ---------------------------------------------------;
ODS PDF FILE = '..\results\pet-food-sas-output ';

* ---------------------------------------------------;
* IMPORT EXCEL;
* ---------------------------------------------------;

* Change ..\data\ to your computer directory where this data set is
stored';

PROC IMPORT
      DATAFILE = '..\data\Data for Analysis-Raw Pet Food.xlsx'
      OUT      = data
      DBMS     = xlsx
      REPLACE;
      SHEET    = "ALL";
      GETNAMES = YES;
RUN;

* This is to make sure the data was read in to SAS;
PROC PRINT DATA = data (OBS = 10) NOOBS;
RUN;

* ---------------------------------------------------;
* MODEL CODE;
```

```
* ------------------------------------------------;

* Sort that data by organism so that the model can be run separately by
organism;

PROC SORT DATA = data;
      BY Organism;
RUN;

* This runs the model with the nested design and Season & Supplier as
fixed block effects;

PROC GLIMMIX DATA = data PLOTS = studentpanel;

* Runs a model individually for each organism;

      BY          Organism;

* Tells SAS which variables are categorical (i.e. groups, not numbers);

      CLASS Season Supplier MeatType Species Organism;

* y = Species + Species(MeatType) + Season + Supplier;

      MODEL       Mean = Season Supplier Species Species(MeatType);

* Provides estimates for each Species x MeatType combination;
* Plots the Species x MeatType (MEANPLOT);
* Provides tables for comparing MeatType within Species and Species
within MeatType (SLICEDIFF) with Tukey Adjustment to control Type I
error rates;

      LSMEANS     Species(MeatType) / SLICEDIFF = (Species MeatType)
      PLOT  = MEANPLOT(SLICEBY = MeatType CL JOIN) CL ADJUST = TUKEY;

* Provides estimates for each Season and Supplier overall;
* Plots the Season and Supplier estimates (MEANPLOT);
* Compares between Seasons and between Suppliers (DIFFS);
      LSMEANS     Season Supplier / PLOT  = MEANPLOT(CL) BYLEVEL DIFFS
      CL ADJUST = TUKEY;

      ODS SELECT  ModelInfo ClassLevels CovParms Tests3 LSMeans
      MeanPlot Diffs SliceDiffs StudentPanel;
      ODS OUTPUT  LSMeans = lsmeans;
RUN;

* ------------------------------------------------;
* EXPORT DATA;

PROC EXPORT DATA = lsmeans
      OUTFILE = '..\results\lsmeans.csv'
      DBMS    = csv
      REPLACE;
RUN;
```

```
ODS PDF CLOSE; * move this to the bottom of the page;
```

Table B.1. Mean microbial counts (log CFU/g) collected for evaluating microbial quality of ground meat blends and livers sold online as raw pet food products.

Rep	Season	Supplier	MeatType	Species	Organism	Mean	Above LOD
1	Fall	Supplier A	Ground	Beef	Total Aerobic Plate count	4.97	
1	Fall	Supplier A	Ground	Beef	Enterobacteriaceae	4.01	
1	Fall	Supplier A	Ground	Beef	Total yeast and mold	2.94	
1	Fall	Supplier A	Ground	Beef	*Salmonella* spp	0.00	0
1	Fall	Supplier A	Ground	Beef	Lactic acid bacteria	4.48	
1	Fall	Supplier A	Ground	Beef	*Listeria* spp	2.75	1
1	Fall	Supplier A	Ground	Beef	*E.coli*	3.84	1
1	Fall	Supplier A	Liver	Pork	Total Aerobic Plate count	5.03	
1	Fall	Supplier A	Ground	Pork	Total Aerobic Plate count	3.91	
1	Fall	Supplier A	Liver	Pork	Enterobacteriaceae	3.08	
1	Fall	Supplier A	Ground	Pork	Enterobacteriaceae	2.75	
1	Fall	Supplier A	Liver	Pork	Total yeast and mold	1.89	
1	Fall	Supplier A	Ground	Pork	Total yeast and mold	2.55	
1	Fall	Supplier A	Liver	Pork	*Salmonella* spp	0.00	0
1	Fall	Supplier A	Ground	Pork	*Salmonella* spp	0.00	0
1	Fall	Supplier A	Liver	Pork	Lactic acid bacteria	4.48	
1	Fall	Supplier A	Ground	Pork	Lactic acid bacteria	3.48	
1	Fall	Supplier A	Liver	Pork	*Listeria* spp	0.98	1
1	Fall	Supplier A	Ground	Pork	*Listeria* spp	2.30	1
1	Fall	Supplier A	Liver	Pork	*E.coli*	2.28	1
1	Fall	Supplier A	Ground	Pork	*E.coli*	2.65	1
1	Fall	Supplier A	Liver	Chicken	Total Aerobic Plate count	2.95	
1	Fall	Supplier A	Ground	Chicken	Total Aerobic Plate count	5.10	
1	Fall	Supplier A	Liver	Chicken	Enterobacteriaceae	1.52	
1	Fall	Supplier A	Ground	Chicken	Enterobacteriaceae	3.27	
1	Fall	Supplier A	Liver	Chicken	Total yeast and mold	1.00	
1	Fall	Supplier A	Ground	Chicken	Total yeast and mold	2.41	
1	Fall	Supplier A	Liver	Chicken	*Salmonella* spp	1.48	1
1	Fall	Supplier A	Ground	Chicken	*Salmonella* spp	1.99	1
1	Fall	Supplier A	Liver	Chicken	Lactic acid bacteria	2.30	
1	Fall	Supplier A	Ground	Chicken	Lactic acid bacteria	4.48	
1	Fall	Supplier A	Liver	Chicken	*Listeria* spp	1.23	1
1	Fall	Supplier A	Ground	Chicken	*Listeria* spp	2.83	1

Rep	Season	Supplier	MeatType	Species	Organism	Mean	Above LOD
1	Fall	Supplier A	Liver	Chicken	*E.coli*	1.38	1
1	Fall	Supplier A	Ground	Chicken	*E.coli*	3.20	1
1	Fall	Supplier A	Ground	Turkey	Total Aerobic Plate count	4.90	
1	Fall	Supplier A	Ground	Turkey	Enterobacteriaceae	3.09	
1	Fall	Supplier A	Ground	Turkey	Total yeast and mold	2.26	
1	Fall	Supplier A	Ground	Turkey	*Salmonella* spp	2.31	1
1	Fall	Supplier A	Ground	Turkey	Lactic acid bacteria	4.45	
1	Fall	Supplier A	Ground	Turkey	*Listeria* spp	2.97	1
1	Fall	Supplier A	Ground	Turkey	*E.coli*	2.96	1
1	Fall	Supplier B	Liver	Beef	Total Aerobic Plate count	3.62	
1	Fall	Supplier B	Ground	Beef	Total Aerobic Plate count	6.17	
1	Fall	Supplier B	Liver	Beef	Enterobacteriaceae	2.58	
1	Fall	Supplier B	Ground	Beef	Enterobacteriaceae	4.14	
1	Fall	Supplier B	Liver	Beef	Total yeast and mold	2.33	
1	Fall	Supplier B	Ground	Beef	Total yeast and mold	3.75	
1	Fall	Supplier B	Liver	Beef	*Salmonella* spp	0.00	0
1	Fall	Supplier B	Ground	Beef	*Salmonella* spp	0.00	0
1	Fall	Supplier B	Liver	Beef	Lactic acid bacteria	2.49	
1	Fall	Supplier B	Ground	Beef	Lactic acid bacteria	5.48	
1	Fall	Supplier B	Liver	Beef	*Listeria* spp	2.95	1
1	Fall	Supplier B	Ground	Beef	*Listeria* spp	0.67	1
1	Fall	Supplier B	Liver	Beef	*E.coli*	2.92	1
1	Fall	Supplier B	Ground	Beef	*E.coli*	4.32	1
1	Fall	Supplier B	Liver	Pork	Total Aerobic Plate count	4.28	
1	Fall	Supplier B	Ground	Pork	Total Aerobic Plate count	6.25	
1	Fall	Supplier B	Liver	Pork	Enterobacteriaceae	1.55	
1	Fall	Supplier B	Ground	Pork	Enterobacteriaceae	3.93	
1	Fall	Supplier B	Liver	Pork	Total yeast and mold	2.04	
1	Fall	Supplier B	Ground	Pork	Total yeast and mold	3.32	
1	Fall	Supplier B	Liver	Pork	*Salmonella* spp	0.52	1
1	Fall	Supplier B	Ground	Pork	*Salmonella* spp	0.00	0
1	Fall	Supplier B	Liver	Pork	Lactic acid bacteria	2.87	
1	Fall	Supplier B	Ground	Pork	Lactic acid bacteria	5.33	
1	Fall	Supplier B	Liver	Pork	*Listeria* spp	1.00	0
1	Fall	Supplier B	Ground	Pork	*Listeria* spp	0.33	1
1	Fall	Supplier B	Liver	Pork	*E.coli*	1.67	1
1	Fall	Supplier B	Ground	Pork	*E.coli*	4.04	1
1	Fall	Supplier B	Liver	Chicken	Total Aerobic Plate count	3.53	
1	Fall	Supplier B	Ground	Chicken	Total Aerobic Plate count	5.19	
1	Fall	Supplier B	Liver	Chicken	Enterobacteriaceae	1.40	
1	Fall	Supplier B	Ground	Chicken	Enterobacteriaceae	3.93	

Rep	Season	Supplier	MeatType	Species	Organism	Mean	Above LOD
1	Fall	Supplier B	Liver	Chicken	Total yeast and mold	1.32	
1	Fall	Supplier B	Ground	Chicken	Total yeast and mold	3.68	
1	Fall	Supplier B	Liver	Chicken	*Salmonella* spp	0.00	0
1	Fall	Supplier B	Ground	Chicken	*Salmonella* spp	0.52	1
1	Fall	Supplier B	Liver	Chicken	Lactic acid bacteria	3.44	
1	Fall	Supplier B	Ground	Chicken	Lactic acid bacteria	3.85	
1	Fall	Supplier B	Liver	Chicken	*Listeria* spp	1.65	1
1	Fall	Supplier B	Ground	Chicken	*Listeria* spp	0.95	1
1	Fall	Supplier B	Liver	Chicken	*E.coli*	1.72	1
1	Fall	Supplier B	Ground	Chicken	*E.coli*	4.05	1
1	Fall	Supplier B	Ground	Turkey	Total Aerobic Plate count	5.34	
1	Fall	Supplier B	Ground	Turkey	Enterobacteriaceae	3.14	
1	Fall	Supplier B	Ground	Turkey	Total yeast and mold	3.62	
1	Fall	Supplier B	Ground	Turkey	*Salmonella* spp	1.12	1
1	Fall	Supplier B	Ground	Turkey	Lactic acid bacteria	3.68	
1	Fall	Supplier B	Ground	Turkey	*Listeria* spp	1.30	1
1	Fall	Supplier B	Ground	Turkey	*E.coli*	3.99	1
1	Fall	Supplier C	Ground	Beef	Total Aerobic Plate count	6.48	
1	Fall	Supplier C	Ground	Beef	Enterobacteriaceae	5.48	
1	Fall	Supplier C	Ground	Beef	Total yeast and mold	2.93	
1	Fall	Supplier C	Ground	Beef	*Salmonella* spp	0.82	1
1	Fall	Supplier C	Ground	Beef	Lactic acid bacteria	5.91	
1	Fall	Supplier C	Ground	Beef	*Listeria* spp	3.63	1
1	Fall	Supplier C	Ground	Beef	*E.coli*	5.70	1
1	Fall	Supplier C	Ground	Pork	Total Aerobic Plate count	4.05	
1	Fall	Supplier C	Ground	Pork	Enterobacteriaceae	3.17	
1	Fall	Supplier C	Ground	Pork	Total yeast and mold	2.05	
1	Fall	Supplier C	Ground	Pork	*Salmonella* spp	0.00	0
1	Fall	Supplier C	Ground	Pork	Lactic acid bacteria	2.57	
1	Fall	Supplier C	Ground	Pork	*Listeria* spp	1.92	1
1	Fall	Supplier C	Ground	Pork	*E.coli*	2.64	1
1	Fall	Supplier C	Ground	Chicken	Total Aerobic Plate count	6.07	
1	Fall	Supplier C	Ground	Chicken	Enterobacteriaceae	4.14	
1	Fall	Supplier C	Ground	Chicken	Total yeast and mold	2.57	
1	Fall	Supplier C	Ground	Chicken	*Salmonella* spp	0.00	0
1	Fall	Supplier C	Ground	Chicken	Lactic acid bacteria	5.82	
1	Fall	Supplier C	Ground	Chicken	*Listeria* spp	3.04	1
1	Fall	Supplier C	Ground	Chicken	*E.coli*	4.20	1
1	Fall	Supplier C	Ground	Turkey	Total Aerobic Plate count	5.90	
1	Fall	Supplier C	Ground	Turkey	Enterobacteriaceae	4.38	
1	Fall	Supplier C	Ground	Turkey	Total yeast and mold	3.31	

Rep	Season	Supplier	MeatType	Species	Organism	Mean	Above LOD
1	Fall	Supplier C	Ground	Turkey	*Salmonella* spp	0.82	1
1	Fall	Supplier C	Ground	Turkey	Lactic acid bacteria	5.97	
1	Fall	Supplier C	Ground	Turkey	*Listeria* spp	3.61	1
1	Fall	Supplier C	Ground	Turkey	*E.coli*	4.58	1
1	Fall	Supplier D	Liver	Beef	Total Aerobic Plate count	4.79	
1	Fall	Supplier D	Ground	Beef	Total Aerobic Plate count	7.21	
1	Fall	Supplier D	Liver	Beef	Enterobacteriaceae	1	
1	Fall	Supplier D	Ground	Beef	Enterobacteriaceae	5.96	
1	Fall	Supplier D	Liver	Beef	Total yeast and mold	2.29	
1	Fall	Supplier D	Ground	Beef	Total yeast and mold	5.51	
1	Fall	Supplier D	Liver	Beef	*Salmonella* spp	0	0
1	Fall	Supplier D	Ground	Beef	*Salmonella* spp	3.44	1
1	Fall	Supplier D	Liver	Beef	Lactic acid bacteria	5.95	
1	Fall	Supplier D	Ground	Beef	Lactic acid bacteria	6.17	
1	Fall	Supplier D	Liver	Beef	*Listeria* spp	2.92	1
1	Fall	Supplier D	Ground	Beef	*Listeria* spp	5.88	1
1	Fall	Supplier D	Liver	Beef	*E.coli*	2.32	1
1	Fall	Supplier D	Ground	Beef	*E.coli*	6.48	1
1	Fall	Supplier D	Liver	Chicken	Total Aerobic Plate count	3.57	
1	Fall	Supplier D	Ground	Chicken	Total Aerobic Plate count	6.66	
1	Fall	Supplier D	Liver	Chicken	Enterobacteriaceae	1.38	
1	Fall	Supplier D	Ground	Chicken	Enterobacteriaceae	5.25	
1	Fall	Supplier D	Liver	Chicken	Total yeast and mold	1.00	
1	Fall	Supplier D	Ground	Chicken	Total yeast and mold	4.81	
1	Fall	Supplier D	Liver	Chicken	*Salmonella* spp	0.00	0
1	Fall	Supplier D	Ground	Chicken	*Salmonella* spp	0.52	1
1	Fall	Supplier D	Liver	Chicken	Lactic acid bacteria	2.68	
1	Fall	Supplier D	Ground	Chicken	Lactic acid bacteria	5.52	
1	Fall	Supplier D	Liver	Chicken	*Listeria* spp	2.53	1
1	Fall	Supplier D	Ground	Chicken	*Listeria* spp	4.85	1
1	Fall	Supplier D	Liver	Chicken	*E.coli*	1.93	1
1	Fall	Supplier D	Ground	Chicken	*E.coli*	6.16	1
1	Fall	Supplier D	Ground	Turkey	Total Aerobic Plate count	5.23	
1	Fall	Supplier D	Ground	Turkey	Enterobacteriaceae	3.33	
1	Fall	Supplier D	Ground	Turkey	Total yeast and mold	3.06	
1	Fall	Supplier D	Ground	Turkey	*Salmonella* spp	0	0
1	Fall	Supplier D	Ground	Turkey	Lactic acid bacteria	5.09	
1	Fall	Supplier D	Ground	Turkey	*Listeria* spp	3.53	1
1	Fall	Supplier D	Ground	Turkey	*E.coli*	3.79	1
2	Winter	Supplier A	Liver	Beef	Total Aerobic Plate count	3.11	
2	Winter	Supplier A	Ground	Beef	Total Aerobic Plate count	6.13	

Rep	Season	Supplier	MeatType	Species	Organism	Mean	Above LOD
2	Winter	Supplier A	Liver	Beef	Enterobacteriaceae	1.53	
2	Winter	Supplier A	Ground	Beef	Enterobacteriaceae	4.46	
2	Winter	Supplier A	Liver	Beef	Total yeast and mold	1.16	
2	Winter	Supplier A	Ground	Beef	Total yeast and mold	1.1	
2	Winter	Supplier A	Liver	Beef	*Salmonella* spp	0.00	0
2	Winter	Supplier A	Ground	Beef	*Salmonella* spp	0.00	0
2	Winter	Supplier A	Liver	Beef	Lactic acid bacteria	2.44	
2	Winter	Supplier A	Ground	Beef	Lactic acid bacteria	4.9	
2	Winter	Supplier A	Liver	Beef	*Listeria* spp	1.3	1
2	Winter	Supplier A	Ground	Beef	*Listeria* spp	3.54	1
2	Winter	Supplier A	Liver	Beef	*E.coli*	1.73	1
2	Winter	Supplier A	Ground	Beef	*E.coli*	5.7	1
2	Winter	Supplier A	Liver	Pork	Total Aerobic Plate count	6.33	
2	Winter	Supplier A	Ground	Pork	Total Aerobic Plate count	5.43	
2	Winter	Supplier A	Liver	Pork	Enterobacteriaceae	3.45	
2	Winter	Supplier A	Ground	Pork	Enterobacteriaceae	2.93	
2	Winter	Supplier A	Liver	Pork	Total yeast and mold	1	
2	Winter	Supplier A	Ground	Pork	Total yeast and mold	3.93	
2	Winter	Supplier A	Liver	Pork	*Salmonella* spp	0.52	1
2	Winter	Supplier A	Ground	Pork	*Salmonella* spp	0.00	0
2	Winter	Supplier A	Liver	Pork	Lactic acid bacteria	6.1	
2	Winter	Supplier A	Ground	Pork	Lactic acid bacteria	3.86	
2	Winter	Supplier A	Liver	Pork	*Listeria* spp	2.1	1
2	Winter	Supplier A	Ground	Pork	*Listeria* spp	2.5	1
2	Winter	Supplier A	Liver	Pork	*E.coli*	3.33	1
2	Winter	Supplier A	Ground	Pork	*E.coli*	3.34	1
2	Winter	Supplier A	Liver	Chicken	Total Aerobic Plate count	4.21	
2	Winter	Supplier A	Ground	Chicken	Total Aerobic Plate count	4.55	
2	Winter	Supplier A	Liver	Chicken	Enterobacteriaceae	2.19	
2	Winter	Supplier A	Ground	Chicken	Enterobacteriaceae	2.6	
2	Winter	Supplier A	Liver	Chicken	Total yeast and mold	1.36	
2	Winter	Supplier A	Ground	Chicken	Total yeast and mold	2.08	
2	Winter	Supplier A	Liver	Chicken	*Salmonella* spp	0.00	0
2	Winter	Supplier A	Ground	Chicken	*Salmonella* spp	0.00	0
2	Winter	Supplier A	Liver	Chicken	Lactic acid bacteria	3.26	
2	Winter	Supplier A	Ground	Chicken	Lactic acid bacteria	4.72	
2	Winter	Supplier A	Liver	Chicken	*Listeria* spp	1.90	1
2	Winter	Supplier A	Ground	Chicken	*Listeria* spp	3.07	1
2	Winter	Supplier A	Liver	Chicken	*E.coli*	3.73	1
2	Winter	Supplier A	Ground	Chicken	*E.coli*	2.63	1
2	Winter	Supplier A	Ground	Turkey	Total Aerobic Plate count	4.31	

Rep	Season	Supplier	MeatType	Species	Organism	Mean	Above LOD
2	Winter	Supplier A	Ground	Turkey	Enterobacteriaceae	3.03	
2	Winter	Supplier A	Ground	Turkey	Total yeast and mold	4.11	
2	Winter	Supplier A	Ground	Turkey	*Salmonella* spp	1.30	1
2	Winter	Supplier A	Ground	Turkey	Lactic acid bacteria	4.54	
2	Winter	Supplier A	Ground	Turkey	*Listeria* spp	3.52	1
2	Winter	Supplier A	Ground	Turkey	*E.coli*	1.82	1
2	Winter	Supplier B	Liver	Beef	Total Aerobic Plate count	5.15	
2	Winter	Supplier B	Ground	Beef	Total Aerobic Plate count	5.67	
2	Winter	Supplier B	Liver	Beef	Enterobacteriaceae	4.02	
2	Winter	Supplier B	Ground	Beef	Enterobacteriaceae	2.38	
2	Winter	Supplier B	Liver	Beef	Total yeast and mold	2.35	
2	Winter	Supplier B	Ground	Beef	Total yeast and mold	2.48	
2	Winter	Supplier B	Liver	Beef	*Salmonella* spp	1.70	1
2	Winter	Supplier B	Ground	Beef	*Salmonella* spp	0.00	0
2	Winter	Supplier B	Liver	Beef	Lactic acid bacteria	2.39	
2	Winter	Supplier B	Ground	Beef	Lactic acid bacteria	4.19	
2	Winter	Supplier B	Liver	Beef	*Listeria* spp	3.98	1
2	Winter	Supplier B	Ground	Beef	*Listeria* spp	2.51	1
2	Winter	Supplier B	Liver	Beef	*E.coli*	3.89	1
2	Winter	Supplier B	Ground	Beef	*E.coli*	4.52	1
2	Winter	Supplier B	Liver	Pork	Total Aerobic Plate count	4.13	
2	Winter	Supplier B	Liver	Pork	Enterobacteriaceae	1	
2	Winter	Supplier B	Liver	Pork	Total yeast and mold	2.59	
2	Winter	Supplier B	Liver	Pork	*Salmonella* spp	0.00	0
2	Winter	Supplier B	Liver	Pork	Lactic acid bacteria	2.98	
2	Winter	Supplier B	Liver	Pork	*Listeria* spp	1.86	1
2	Winter	Supplier B	Liver	Pork	*E.coli*	2.21	1
2	Winter	Supplier B	Liver	Chicken	Total Aerobic Plate count	4.52	
2	Winter	Supplier B	Ground	Chicken	Total Aerobic Plate count	5.01	
2	Winter	Supplier B	Liver	Chicken	Enterobacteriaceae	2.48	
2	Winter	Supplier B	Ground	Chicken	Enterobacteriaceae	2.73	
2	Winter	Supplier B	Liver	Chicken	Total yeast and mold	2.26	
2	Winter	Supplier B	Ground	Chicken	Total yeast and mold	3.22	
2	Winter	Supplier B	Liver	Chicken	*Salmonella* spp	0.00	0
2	Winter	Supplier B	Ground	Chicken	*Salmonella* spp	0.00	0
2	Winter	Supplier B	Liver	Chicken	Lactic acid bacteria	3.21	
2	Winter	Supplier B	Ground	Chicken	Lactic acid bacteria	3.77	
2	Winter	Supplier B	Liver	Chicken	*Listeria* spp	2.01	1
2	Winter	Supplier B	Ground	Chicken	*Listeria* spp	2.54	1
2	Winter	Supplier B	Liver	Chicken	*E.coli*	3.05	1
2	Winter	Supplier B	Ground	Chicken	*E.coli*	4.07	1

Rep	Season	Supplier	MeatType	Species	Organism	Mean	Above LOD
2	Winter	Supplier C	Liver	Beef	Total Aerobic Plate count	4.61	
2	Winter	Supplier C	Ground	Beef	Total Aerobic Plate count	6.61	
2	Winter	Supplier C	Liver	Beef	Enterobacteriaceae	3.05	
2	Winter	Supplier C	Ground	Beef	Enterobacteriaceae	3.06	
2	Winter	Supplier C	Liver	Beef	Total yeast and mold	2.57	
2	Winter	Supplier C	Ground	Beef	Total yeast and mold	2.77	
2	Winter	Supplier C	Liver	Beef	*Salmonella* spp	0.00	0
2	Winter	Supplier C	Ground	Beef	*Salmonella* spp	0.00	0
2	Winter	Supplier C	Liver	Beef	Lactic acid bacteria	4.37	
2	Winter	Supplier C	Ground	Beef	Lactic acid bacteria	4.8	
2	Winter	Supplier C	Liver	Beef	*Listeria* spp	1.63	1
2	Winter	Supplier C	Ground	Beef	*Listeria* spp	3.53	1
2	Winter	Supplier C	Liver	Beef	*E.coli*	3.40	1
2	Winter	Supplier C	Ground	Beef	*E.coli*	5.53	1
2	Winter	Supplier C	Ground	Chicken	Total Aerobic Plate count	5.95	
2	Winter	Supplier C	Ground	Chicken	Enterobacteriaceae	2.99	
2	Winter	Supplier C	Ground	Chicken	Total yeast and mold	2.2	
2	Winter	Supplier C	Ground	Chicken	*Salmonella* spp	0.82	1
2	Winter	Supplier C	Ground	Chicken	Lactic acid bacteria	4.83	
2	Winter	Supplier C	Ground	Chicken	*Listeria* spp	2.84	1
2	Winter	Supplier C	Ground	Chicken	*E.coli*	4.37	1
2	Winter	Supplier C	Ground	Turkey	Total Aerobic Plate count	5.08	
2	Winter	Supplier C	Ground	Turkey	Enterobacteriaceae	2.9	
2	Winter	Supplier C	Ground	Turkey	Total yeast and mold	3.15	
2	Winter	Supplier C	Ground	Turkey	*Salmonella* spp	0.52	1
2	Winter	Supplier C	Ground	Turkey	Lactic acid bacteria	4.49	
2	Winter	Supplier C	Ground	Turkey	*Listeria* spp	2.53	1
2	Winter	Supplier C	Ground	Turkey	*E.coli*	3.46	1
2	Winter	Supplier D	Liver	Beef	Total Aerobic Plate count	5.01	
2	Winter	Supplier D	Ground	Beef	Total Aerobic Plate count	6.57	
2	Winter	Supplier D	Liver	Beef	Enterobacteriaceae	1	
2	Winter	Supplier D	Ground	Beef	Enterobacteriaceae	5.96	
2	Winter	Supplier D	Liver	Beef	Total yeast and mold	3.68	
2	Winter	Supplier D	Ground	Beef	Total yeast and mold	5.39	
2	Winter	Supplier D	Liver	Beef	*Salmonella* spp	0.00	0
2	Winter	Supplier D	Ground	Beef	*Salmonella* spp	0.00	0
2	Winter	Supplier D	Liver	Beef	Lactic acid bacteria	1	
2	Winter	Supplier D	Ground	Beef	Lactic acid bacteria	6.65	
2	Winter	Supplier D	Liver	Beef	*Listeria* spp	1.52	1
2	Winter	Supplier D	Ground	Beef	*Listeria* spp	2.17	1
2	Winter	Supplier D	Liver	Beef	*E.coli*	1.00	0

Rep	Season	Supplier	MeatType	Species	Organism	Mean	Above LOD
2	Winter	Supplier D	Ground	Beef	*E.coli*	5.91	1
2	Winter	Supplier D	Liver	Chicken	Total Aerobic Plate count	5.28	
2	Winter	Supplier D	Ground	Chicken	Total Aerobic Plate count	5.01	
2	Winter	Supplier D	Liver	Chicken	Enterobacteriaceae	1.00	
2	Winter	Supplier D	Ground	Chicken	Enterobacteriaceae	2.39	
2	Winter	Supplier D	Liver	Chicken	Total yeast and mold	1.00	
2	Winter	Supplier D	Ground	Chicken	Total yeast and mold	2.48	
2	Winter	Supplier D	Liver	Chicken	*Salmonella* spp	0.00	0
2	Winter	Supplier D	Ground	Chicken	*Salmonella* spp	0.00	0
2	Winter	Supplier D	Liver	Chicken	Lactic acid bacteria	4.08	
2	Winter	Supplier D	Ground	Chicken	Lactic acid bacteria	3.9	
2	Winter	Supplier D	Liver	Chicken	*Listeria* spp	2.11	1
2	Winter	Supplier D	Ground	Chicken	*Listeria* spp	2.35	1
2	Winter	Supplier D	Liver	Chicken	*E.coli*	3.30	1
2	Winter	Supplier D	Ground	Chicken	*E.coli*	2.62	1
2	Winter	Supplier D	Ground	Turkey	Total Aerobic Plate count	4.67	
2	Winter	Supplier D	Ground	Turkey	Enterobacteriaceae	2.49	
2	Winter	Supplier D	Ground	Turkey	Total yeast and mold	2.54	
2	Winter	Supplier D	Ground	Turkey	*Salmonella* spp	0.00	0
2	Winter	Supplier D	Ground	Turkey	Lactic acid bacteria	3.82	
2	Winter	Supplier D	Ground	Turkey	*Listeria* spp	2.52	1
2	Winter	Supplier D	Ground	Turkey	*E.coli*	2.49	1
3	Summer	Supplier A	Liver	Beef	Total Aerobic Plate count	4.88	
3	Summer	Supplier A	Ground	Beef	Total Aerobic Plate count	5.28	
3	Summer	Supplier A	Liver	Beef	Enterobacteriaceae	3.41	
3	Summer	Supplier A	Ground	Beef	Enterobacteriaceae	3.32	
3	Summer	Supplier A	Liver	Beef	Total yeast and mold	1.1	
3	Summer	Supplier A	Ground	Beef	Total yeast and mold	2.79	
3	Summer	Supplier A	Liver	Beef	*Salmonella* spp	0	0
3	Summer	Supplier A	Ground	Beef	*Salmonella* spp	1.70	1
3	Summer	Supplier A	Liver	Beef	Lactic acid bacteria	4.12	
3	Summer	Supplier A	Ground	Beef	Lactic acid bacteria	4.50	
3	Summer	Supplier A	Liver	Beef	*Listeria* spp	2.36	1
3	Summer	Supplier A	Ground	Beef	*Listeria* spp	2.88	1
3	Summer	Supplier A	Liver	Beef	*E.coli*	3.42	1
3	Summer	Supplier A	Ground	Beef	*E.coli*	3.01	1
3	Summer	Supplier A	Liver	Pork	Total Aerobic Plate count	5.81	
3	Summer	Supplier A	Ground	Pork	Total Aerobic Plate count	4.10	
3	Summer	Supplier A	Liver	Pork	Enterobacteriaceae	3.44	
3	Summer	Supplier A	Ground	Pork	Enterobacteriaceae	3.43	
3	Summer	Supplier A	Liver	Pork	Total yeast and mold	1.00	

Rep	Season	Supplier	MeatType	Species	Organism	Mean	Above LOD
3	Summer	Supplier A	Ground	Pork	Total yeast and mold	2.80	
3	Summer	Supplier A	Liver	Pork	*Salmonella* spp	0.00	0
3	Summer	Supplier A	Ground	Pork	*Salmonella* spp	0.00	0
3	Summer	Supplier A	Liver	Pork	Lactic acid bacteria	5.76	
3	Summer	Supplier A	Ground	Pork	Lactic acid bacteria	5.47	
3	Summer	Supplier A	Liver	Pork	*Listeria* spp	2.27	1
3	Summer	Supplier A	Ground	Pork	*Listeria* spp	2.30	1
3	Summer	Supplier A	Liver	Pork	*E.coli*	3.19	1
3	Summer	Supplier A	Ground	Pork	*E.coli*	2.31	1
3	Summer	Supplier A	Liver	Chicken	Total Aerobic Plate count	3.02	
3	Summer	Supplier A	Ground	Chicken	Total Aerobic Plate count	5.04	
3	Summer	Supplier A	Liver	Chicken	Enterobacteriaceae	1.10	
3	Summer	Supplier A	Ground	Chicken	Enterobacteriaceae	2.52	
3	Summer	Supplier A	Liver	Chicken	Total yeast and mold	1.00	
3	Summer	Supplier A	Ground	Chicken	Total yeast and mold	2.15	
3	Summer	Supplier A	Liver	Chicken	*Salmonella* spp	0.00	0
3	Summer	Supplier A	Ground	Chicken	*Salmonella* spp	0.00	0
3	Summer	Supplier A	Liver	Chicken	Lactic acid bacteria	2.54	
3	Summer	Supplier A	Ground	Chicken	Lactic acid bacteria	4.89	
3	Summer	Supplier A	Liver	Chicken	*Listeria* spp	1.10	1
3	Summer	Supplier A	Ground	Chicken	*Listeria* spp	2.71	1
3	Summer	Supplier A	Liver	Chicken	*E.coli*	1.00	0
3	Summer	Supplier A	Ground	Chicken	*E.coli*	3.11	1
3	Summer	Supplier A	Ground	Turkey	Total Aerobic Plate count	5.40	
3	Summer	Supplier A	Ground	Turkey	Enterobacteriaceae	4.23	
3	Summer	Supplier A	Ground	Turkey	Total yeast and mold	2.72	
3	Summer	Supplier A	Ground	Turkey	*Salmonella* spp	1.12	1
3	Summer	Supplier A	Ground	Turkey	Lactic acid bacteria	4.90	
3	Summer	Supplier A	Ground	Turkey	*Listeria* spp	3.48	1
3	Summer	Supplier A	Ground	Turkey	*E.coli*	3.97	1
3	Summer	Supplier B	Liver	Beef	Total Aerobic Plate count	4.97	
3	Summer	Supplier B	Ground	Beef	Total Aerobic Plate count	4.53	
3	Summer	Supplier B	Liver	Beef	Enterobacteriaceae	1.92	
3	Summer	Supplier B	Ground	Beef	Enterobacteriaceae	3.03	
3	Summer	Supplier B	Liver	Beef	Total yeast and mold	1.26	
3	Summer	Supplier B	Ground	Beef	Total yeast and mold	3.76	
3	Summer	Supplier B	Liver	Beef	*Salmonella* spp	0.00	0
3	Summer	Supplier B	Ground	Beef	*Salmonella* spp	0.00	0
3	Summer	Supplier B	Liver	Beef	Lactic acid bacteria	3.03	
3	Summer	Supplier B	Ground	Beef	Lactic acid bacteria	2.65	
3	Summer	Supplier B	Liver	Beef	*Listeria* spp	1.59	1

Rep	Season	Supplier	MeatType	Species	Organism	Mean	Above LOD
3	Summer	Supplier B	Ground	Beef	*Listeria* spp	2.60	1
3	Summer	Supplier B	Liver	Beef	*E.coli*	2.43	1
3	Summer	Supplier B	Ground	Beef	*E.coli*	3.74	1
3	Summer	Supplier B	Liver	Pork	Total Aerobic Plate count	3.45	
3	Summer	Supplier B	Ground	Pork	Total Aerobic Plate count	5.13	
3	Summer	Supplier B	Liver	Pork	Enterobacteriaceae	1.10	
3	Summer	Supplier B	Ground	Pork	Enterobacteriaceae	3.14	
3	Summer	Supplier B	Liver	Pork	Total yeast and mold	3.23	
3	Summer	Supplier B	Ground	Pork	Total yeast and mold	2	
3	Summer	Supplier B	Liver	Pork	*Salmonella* spp	0.00	0
3	Summer	Supplier B	Ground	Pork	*Salmonella* spp	0.52	1
3	Summer	Supplier B	Liver	Pork	Lactic acid bacteria	2.81	
3	Summer	Supplier B	Ground	Pork	Lactic acid bacteria	4.44	
3	Summer	Supplier B	Liver	Pork	*Listeria* spp	1.80	1
3	Summer	Supplier B	Ground	Pork	*Listeria* spp	2.52	1
3	Summer	Supplier B	Liver	Pork	*E.coli*	1.52	1
3	Summer	Supplier B	Ground	Pork	*E.coli*	3.34	1
3	Summer	Supplier B	Liver	Chicken	Total Aerobic Plate count	4.97	
3	Summer	Supplier B	Ground	Chicken	Total Aerobic Plate count	4.53	
3	Summer	Supplier B	Liver	Chicken	Enterobacteriaceae	1.92	
3	Summer	Supplier B	Ground	Chicken	Enterobacteriaceae	3.03	
3	Summer	Supplier B	Liver	Chicken	Total yeast and mold	1.26	
3	Summer	Supplier B	Ground	Chicken	Total yeast and mold	3.76	
3	Summer	Supplier B	Liver	Chicken	*Salmonella* spp	0.00	0
3	Summer	Supplier B	Ground	Chicken	*Salmonella* spp	0.00	0
3	Summer	Supplier B	Liver	Chicken	Lactic acid bacteria	3.03	
3	Summer	Supplier B	Ground	Chicken	Lactic acid bacteria	2.65	
3	Summer	Supplier B	Liver	Chicken	*Listeria* spp	1.59	1
3	Summer	Supplier B	Ground	Chicken	*Listeria* spp	2.60	1
3	Summer	Supplier B	Liver	Chicken	*E.coli*	2.43	1
3	Summer	Supplier B	Ground	Chicken	*E.coli*	3.74	1
3	Summer	Supplier B	Ground	Turkey	Total Aerobic Plate count	4.66	
3	Summer	Supplier B	Ground	Turkey	Enterobacteriaceae	2.40	
3	Summer	Supplier B	Ground	Turkey	Total yeast and mold	3.9	
3	Summer	Supplier B	Ground	Turkey	*Salmonella* spp	1.00	1
3	Summer	Supplier B	Ground	Turkey	Lactic acid bacteria	3.87	
3	Summer	Supplier B	Ground	Turkey	*Listeria* spp	2.31	1
3	Summer	Supplier B	Ground	Turkey	*E.coli*	3.08	1
3	Summer	Supplier C	Liver	Beef	Total Aerobic Plate count	1.00	
3	Summer	Supplier C	Ground	Beef	Total Aerobic Plate count	6.87	
3	Summer	Supplier C	Liver	Beef	Enterobacteriaceae	1.00	

Rep	Season	Supplier	MeatType	Species	Organism	Mean	Above LOD
3	Summer	Supplier C	Ground	Beef	Enterobacteriaceae	4.23	
3	Summer	Supplier C	Liver	Beef	Total yeast and mold	1.20	
3	Summer	Supplier C	Ground	Beef	Total yeast and mold	2.71	
3	Summer	Supplier C	Liver	Beef	*Salmonella* spp	0.00	0
3	Summer	Supplier C	Ground	Beef	*Salmonella* spp	1.85	1
3	Summer	Supplier C	Liver	Beef	Lactic acid bacteria	3.65	
3	Summer	Supplier C	Ground	Beef	Lactic acid bacteria	4.97	
3	Summer	Supplier C	Liver	Beef	*Listeria* spp	3.16	1
3	Summer	Supplier C	Ground	Beef	*Listeria* spp	3.95	1
3	Summer	Supplier C	Liver	Beef	*E.coli*	1.20	1
3	Summer	Supplier C	Ground	Beef	*E.coli*	4.02	1
3	Summer	Supplier C	Ground	Chicken	Total Aerobic Plate count	6.30	
3	Summer	Supplier C	Ground	Chicken	Enterobacteriaceae	3.58	
3	Summer	Supplier C	Ground	Chicken	Total yeast and mold	4.24	
3	Summer	Supplier C	Ground	Chicken	*Salmonella* spp	1.12	1
3	Summer	Supplier C	Ground	Chicken	Lactic acid bacteria	6.60	
3	Summer	Supplier C	Ground	Chicken	*Listeria* spp	5.52	1
3	Summer	Supplier C	Ground	Chicken	*E.coli*	6.46	1
3	Summer	Supplier C	Ground	Turkey	Total Aerobic Plate count	5.49	
3	Summer	Supplier C	Ground	Turkey	Enterobacteriaceae	3.08	
3	Summer	Supplier C	Ground	Turkey	Total yeast and mold	2.76	
3	Summer	Supplier C	Ground	Turkey	*Salmonella* spp	0.00	0
3	Summer	Supplier C	Ground	Turkey	Lactic acid bacteria	6.70	
3	Summer	Supplier C	Ground	Turkey	*Listeria* spp	3.50	1
3	Summer	Supplier C	Ground	Turkey	*E.coli*	3.60	1
3	Summer	Supplier D	Ground	Beef	Total Aerobic Plate count	5.36	
3	Summer	Supplier D	Ground	Beef	Enterobacteriaceae	2.77	
3	Summer	Supplier D	Ground	Beef	Total yeast and mold	2.62	
3	Summer	Supplier D	Ground	Beef	*Salmonella* spp	0.52	1
3	Summer	Supplier D	Ground	Beef	Lactic acid bacteria	5.05	
3	Summer	Supplier D	Ground	Beef	*Listeria* spp	2.92	1
3	Summer	Supplier D	Ground	Beef	*E.coli*	4.26	1
3	Summer	Supplier D	Liver	Chicken	Total Aerobic Plate count	5.29	
3	Summer	Supplier D	Ground	Chicken	Total Aerobic Plate count	5.77	
3	Summer	Supplier D	Liver	Chicken	Enterobacteriaceae	1.84	
3	Summer	Supplier D	Ground	Chicken	Enterobacteriaceae	2.67	
3	Summer	Supplier D	Liver	Chicken	Total yeast and mold	1.26	
3	Summer	Supplier D	Ground	Chicken	Total yeast and mold	2.4	
3	Summer	Supplier D	Liver	Chicken	*Salmonella* spp	0.00	0
3	Summer	Supplier D	Ground	Chicken	*Salmonella* spp	0	0
3	Summer	Supplier D	Liver	Chicken	Lactic acid bacteria	4.97	

Rep	Season	Supplier	MeatType	Species	Organism	Mean	Above LOD
3	Summer	Supplier D	Ground	Chicken	Lactic acid bacteria	5.5	
3	Summer	Supplier D	Liver	Chicken	*Listeria* spp	2.56	1
3	Summer	Supplier D	Ground	Chicken	*Listeria* spp	2.93	1
3	Summer	Supplier D	Liver	Chicken	*E.coli*	2.95	1
3	Summer	Supplier D	Ground	Chicken	*E.coli*	4.24	1
3	Summer	Supplier D	Ground	Turkey	Total Aerobic Plate count	3.89	
3	Summer	Supplier D	Ground	Turkey	Enterobacteriaceae	2.01	
3	Summer	Supplier D	Ground	Turkey	Total yeast and mold	2.33	
3	Summer	Supplier D	Ground	Turkey	*Salmonella* spp	0	0
3	Summer	Supplier D	Ground	Turkey	Lactic acid bacteria	3.7	
3	Summer	Supplier D	Ground	Turkey	*Listeria* spp	2.08	1
3	Summer	Supplier D	Ground	Turkey	*E.coli*	4.57	1

B.1.2 *Salmonella* levels of Meat Blends and Livers

This code was run to analyze the level of presumptive *Salmonella* colonies in products where presence was found in three or more samples. Meat blends were referred as "ground" in the SAS code below.

```
* When there is enough replication (aka Beef blend, Chicken blend, and
Turkey blend) had >= 3 samples Above limit of detection (LOD), what is
the level of contamination? ;

* ------------------------------------------------;

* Summary of Salmonella Above LOD;

PROC MEANS DATA = data;
      WHERE Organism= "Salmonella spp" & AboveLOD= 1 & MeatType =
      "Ground" & Species ne "Pork";
      CLASS MeatType Species;
      VAR   Mean;
RUN;

* This runs the model with the nested design and Season & Supplier as
fixed block effects;

PROC GLIMMIX DATA = data PLOTS = studentpanel;

* Runs a model individually for each organism;

      WHERE Organism= "Salmonella spp" & AboveLOD= 1 & MeatType =
      "Ground" & Species ne "Pork";

* Tells SAS which variables are categorical (i.e. groups, not numbers);
      CLASS Season Supplier MeatType Species Organism;

* y = Species + Season + Supplier;

      MODEL       Mean = Season Supplier Species;

* Provides estimates for each Species, Season and Supplier overall;
* Plots the Season and Supplier estimates (MEANPLOT);
* Compares between Species and between Seasons and between Suppliers
(DIFFS);

      LSMEANS Species Season Supplier / PLOT  = MEANPLOT(CL) BYLEVEL
      DIFFS CL ADJUST = TUKEY;

      ODS SELECT  ModelInfo ClassLevels CovParms Tests3 LSMeans
      MeanPlot Diffs SliceDiffs StudentPanel;
RUN;

ODS PDF CLOSE; * move this to the bottom of the page;
```

Moreover, logistics regression was also conducted to determine the odds of detecting presumptive *Salmonella* colonies in a 25 g raw pet food based on the obtained *Enterobacteriaceae* count.

```
* IMPORT EXCEL;
* ------------------------------------------------;

* Change ..\data\ to your computer directory where this data set is
stored';

PROC IMPORT
      DATAFILE = '..\data\Data for Analysis-Salmonella and EB.xlsx'
      OUT = correlation data
      REPLACE;
      GUESSINGROWS = 50;
RUN;

* This is to make sure the data was read in to SAS;

TITLE "Petfood Correlation Data";
PROC PRINT DATA = correlation_data (OBS = 10) NOOBS;
RUN;

* This is to run the correlation between Enterobacteriaceae and the
presence or absence of Salmonella in a pet food;

DATA micro_data;
      SET correlation_data;
      IF Salmonella_spp = . THEN    SLM_aboveLOD = .;
      ELSE IF Salmonella_spp = 0 THEN SLM_aboveLOD = 0;
      ELSE SLM_aboveLOD = 1;
      Observation = _n_;
      KEEP Observation Rep Season Supplier MeatType Species
      Enterobactericeae Salmonella_spp SLM_aboveLOD;

RUN;

PROC PRINT DATA = micro_data;
RUN;

* This is to create frequency tables for meat type and species
interaction, season and supplier;

TITLE 'Summary with Meat Type x Species';
PROC MEANS DATA = micro_data;
      CLASS       MeatType Species;
      VAR         SLM_aboveLOD;
RUN;

TITLE 'Summary for Season';
PROC MEANS DATA = micro_data;
      CLASS       Season;
```

```
      VAR         SLM_aboveLOD;
RUN;

TITLE 'Summary for Supplier';
PROC MEANS DATA = micro_data;
      CLASS       Supplier;
      VAR         SLM_aboveLOD;
RUN;

* This will run the parameter and odds ratio estimates;

TITLE 'EB vs SLM';
proc glimmix data=micro_data;
      CLASS MeatType Species Supplier Season;
      MODEL SLM_aboveLOD (event='1') = Enterobactericeae Species
      Species(MeatType) Supplier Season / htype = 3 dist=binary s or
      chisq;
      ESTIMATE 'odds EB (1 unit difference)' Enterobactericeae 1,
               'odds EB (2 unit difference)' Enterobactericeae 2 / EXP
               CL;
RUN;
```

B.2 Evaluation of Antimicrobial Interventions (Objective 2)

This code runs a four by four (4 x 4) factorial two-way analysis of variance with covariate (ANCOVA). Treatment (Trt) and time (Day) were the independent variables, replications as block and weight as covariate. Tukey-Kramer's test was also applied compare means among treatments. The same code was run for APC.

```
* IMPORT EXCEL;
* ------------------------------------------------;

* Change ..\data\ to your computer directory where this data set is
stored';

PROC IMPORT
      DATAFILE = '..\data\Data for Clean Label study.xlsx'
      OUT      = data
      DBMS     = xlsx
      REPLACE;
      SHEET    = "Summary";
      GETNAMES = YES;
RUN;

* This is to make sure the data was read in to SAS;

PROC PRINT DATA = data (OBS = 10) NOOBS;
RUN;

* This is to check summary of weight;
PROC MEANS DATA = data mean min Q1 median Q3 max;
      VAR   Weight;
RUN;

* This runs the model with replicates as random block effects;

PROC GLIMMIX DATA = data PLOTS = studentpanel;

* Tells SAS which variables are categorical (i.e. groups, not numbers);

      CLASS Trt Day Rep;

* y = Trt + Day + Days(Trt) + Random Prep Rep;
* y used for both Log (Microbial count) and Log reduction;

      MODEL       Logreduction = Weight Trt Day Trt*Day;
      RANDOM      Rep;

* Provides estimates for each Days x Trt combination;
* Plots the Days x Trt (MEANPLOT);
* with Tukey Adjustment to control Type I error rates;
```

```
* the AT Weight = tells it to estimate them at that given weight (by
default it selects the mean weight);

      LSMEANS     Trt*Day / AT Weight = 31.3670833 PLOT  =
      MEANPLOT(SLICEBY = Trt CL JOIN) CL;

* This is to see differences in log reduction by treatment;
      SLICE Trt*Day / AT Weight = 31.3670833 SLICEBY=Day ADJUST= TUKEY;
      SLICE Trt*Day / AT Weight = 31.3670833 SLICEBY=Trt ADJUST= TUKEY;


      ODS SELECT  ModelInfo ClassLevels CovParms Tests1 Tests3 LSMeans
      MeanPlot Diffs SliceLines SliceDiffs StudentPanel;
      ODS OUTPUT  LSMeans = lsmeans;
RUN;

* EXPORT DATA;
-------------------------------------------------;

PROC EXPORT DATA = lsmeans
      OUTFILE = '..\results\lsmeans.csv'
      DBMS    = csv
      REPLACE;
RUN;


ODS PDF CLOSE; * move this to the bottom of the page;
```

Table B.2. Data collected in evaluating the efficacy of peracetic acid (PAA), cultured dextrose fermentate (CDF) and buffered vinegar (BV) in reducing *Salmonella* in raw chicken livers.

Rep	Day	Trt	Log	Logreduction	Weight
1	0	Control	6.43	0.48	24.8
2	0	Control	6.2	0.41	31.78
3	0	Control	6.43	0.41	33.27
1	0	PAA	6.03	0.88	42.57
2	0	PAA	6.08	0.53	29.92
3	0	PAA	6.23	0.61	32.02
1	0	CDF	6.46	0.45	32.03
2	0	CDF	6.28	0.33	40.92
3	0	CDF	6.35	0.49	19.49
1	0	BV	6.54	0.37	33.07
2	0	BV	6.24	0.37	24.29
3	0	BV	6.38	0.46	29.55
1	3	Control	5.89	1.02	32.71
2	3	Control	6.05	0.56	26.14
3	3	Control	6.02	0.82	28.13
1	3	PAA	5.98	0.93	24.87

Rep	Day	Trt	Log	Logreduction	Weight
2	3	PAA	5.69	0.92	28.26
3	3	PAA	5.78	1.06	27.56
1	3	CDF	5.93	0.98	34.18
2	3	CDF	6.11	0.5	28.34
3	3	CDF	6.13	0.71	34.92
1	3	BV	6	0.91	24.82
2	3	BV	5.88	0.73	37.23
3	3	BV	6.02	0.82	26.08
1	7	Control	5.64	1.27	36.98
2	7	Control	6.11	0.5	36.57
3	7	Control	6.05	0.79	18.68
1	7	PAA	5.56	1.35	38.93
2	7	PAA	5.88	0.73	27.74
3	7	PAA	5.64	1.2	34.53
1	7	CDF	5.76	1.15	28.1
2	7	CDF	5.86	0.75	30.24
3	7	CDF	5.93	0.91	29.27
1	7	BV	5.98	0.93	36.3
2	7	BV	5.81	0.8	30.41
3	7	BV	5.9	0.94	35.95
1	14	Control	5.67	1.24	19.31
2	14	Control	5.61	1	35.18
3	14	Control	5.43	1.41	33.68
1	14	PAA	5.49	1.42	21.13
2	14	PAA	5.7	0.91	34.17
3	14	PAA	5.23	1.61	38.24
1	14	CDF	6.15	0.76	35.3
2	14	CDF	5.84	0.77	37.42
3	14	CDF	6.34	0.5	33.94
1	14	BV	5.46	1.45	27.9
2	14	BV	5.52	1.09	35.4
3	14	BV	5.72	1.12	43.3