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The Impacts of Administering Metabolites of Saccharomyces cerevisiae on Broiler Performance, Yields and Salmonella Content of Component Portions

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The Impacts of Administering Metabolites of *Saccharomyces cerevisiae* on Broiler Performance, Yields and *Salmonella* Content of Component Portions

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of the requirements for the degree of
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by

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ABSTRACT

The impacts of using health-promoting *Saccharomyces cerevisiae* fermentation metabolites in poultry production and processing can be measured in respect to multiple measures of success. Traditionally this yeast-based compound has been administered to poultry, livestock, poultry, and other species to improve animal performance and production volume output. In addition, *Saccharomyces cerevisiae* fermentation metabolites have also been shown in more recent research to reduce colonization of pathogenic bacteria in the host organism's gastro-intestinal tract. In this dissertation, the impacts of administering a functional ingredient containing *Saccharomyces cerevisiae* metabolites on broiler performance measures and pathogen reduction were measured. One of the studies in this research was conducted in controlled environment research pens, in the absence of disease or stress. In this study, broiler feed conversions and component parts yields were similar between the control and treated broilers. As indicated by studies elsewhere, these results may have been different had the broilers been exposed to a commercial setting, or disease challenges, or other stressors. Also, ceca and parts that were provided by commercial plants that had processed broilers treated with a *Saccharomyces cerevisiae* metabolite product had lower presence and quantities of *Salmonella* in the ceca and component portions compared to samples obtained from non-treated broilers. In addition, the ceca provided by commercial plants which had been exposed to the *Saccharomyces cerevisiae* metabolite treatments had reduced *Salmonella* strength, with increased susceptibility to selected antimicrobials. There are several practical applications of this research. The metabolites of *Saccharomyces cerevisiae* fermentation may be considered in broiler and other meat-animal production systems as one alternative in the multi-hurdle approach to reduce pathogens. This technology may benefit some plants by reducing the need for in-plant

interventions, such as antimicrobial sprays and dips frequently used to achieve regulatory standards. Researchers and integrators that choose to study the administration of the metabolites of *Saccharomyces cerevisiae* fermentation to meat animals can gain valuable knowledge by measuring the impacts on performance, yields, and pathogen presence in settings where the technology has been applied over an extended period of time in a variety of experimental conditions.

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CHAPTER I

INTRODUCTION

INTRODUCTION

Poultry meat producers continually work to improve food safety for the benefits of their consumers and in order to maintain company sustainability. In the meat and poultry production industries, pathogen reduction has been an area of continual emphasis for many years, and there are increasing needs to find alternative methods to reduce bacteria in muscle protein products. While various species typically have a group of pathogenic bacteria that are commonly associated with specific meat types, in poultry two pathogens commensal to the product that are frequently of concern in poultry meat are *Salmonella* and *Campylobacter*. The Food and Agriculture Organisation of the United Nations and the World Health Organisation [sic] has stated that poultry meat has been associated frequently and consistently with the transmission of enteric pathogens, including *Salmonella* and *Campylobacter*, and risk management strategies have been offered (FAO/WHO, 2002, 2003). Furthermore, it has been demonstrated that these pathogens on raw product are frequently transferred to utensils, equipment, and finished product; for example, *Campylobacter* has been shown to transfer from raw chicken parts to cooked chicken slices via cutting boards (Guyard-Nicodème et al., 2013). Therefore, poultry producers and processors are constantly seeking to reduce these pathogens in order to reduce the risk of foodborne illness.

Concurrently to the above challenges to reduce pathogens, poultry producers have the additional challenges from consumers that have expressed a clear shift in opinions toward removing antibiotics in animal agriculture. There have been various degrees of success with this initiative, as some antibiotics have been effectively used under veterinarian prescriptions to prevent and/or treat diseases. However, the public concern for creating antibiotic-resistant pathogens has led many processors to seek alternatives to antibiotics. This has become so

important to some consumers that standards have been developed by the United States Department of Agriculture (USDA) for guidelines such as “Naturally Raised” livestock (USDA, 2009), and auditing has become a growing function within the Agricultural Marketing Service (AMS) certification program in which participating companies have their processes audited for strict antibiotic restriction and controls under a Process Verified Program (USDA AMS, 2018).

Another reason that alternative methods to reduce pathogens are being considered at an increasing pace is that the USDA Food Safety Inspection Service (FSIS) periodically tightens microbial standards to reduce pathogen loads on product. For example, in recent years FSIS has implemented a new pathogen performance standard for various types of poultry products, specifically for the pathogenic organisms *Salmonella* and *Campylobacter* (USDA FSIS, 2015). These pathogen performance standards have been made more stringent over the years, as the government attempts to achieve goals for reducing foodborne illness under its Healthy People initiatives (USDHHS ODPHP, 2018). In order to achieve the goals of improving food safety while meeting the requirements of regulators and consumers, poultry processing managers, researchers, and regulators have focused on manufacturing plant multi-hurdle strategies while using combinations of antimicrobial sprays, dips, and rinses to reduce pathogenic and spoilage bacteria (Berrang and Bailey, 2009; Buncic and Sofos, 2012; Shackelford et al., 1993; USDA FSIS, 2015). However, some consumers prefer to purchase products not treated with antimicrobials to reduce bacteria, due to concerns about chemicals and their impact on consumer health and/or product organoleptic characteristics. Therefore, while antimicrobial interventions have been proven effective at reducing pathogens and bacterial loads, these interventions cannot be used exclusively for all applications. Also, the antimicrobial interventions are most effective when pathogen loads are minimal on incoming animals and raw materials, prior to processing.

For these reasons, research projects to find inventive ways to reduce pathogens in live poultry production are extensive. Research is very limited in determining how novel approaches to pathogen reduction in live poultry production impact reductions of bacteria after product is processed into component parts, packaged, and ready for sale to consumers.

The purpose of this dissertation research is to determine the effectiveness of feeding the dried metabolites of *Saccharomyces cerevisiae* to broilers on bird performance, yields, and the presence and quantity of *Salmonella* in broiler chickens as measured in the ceca and component parts collected at the time of packaging for sale. Bird performance comparisons have been made using a research pen trial setting of control, untreated birds with those fed *Saccharomyces cerevisiae* metabolites, by measuring broiler feed conversion, weight gain, growth rate, and component carcasses parts yields. Also, samples collected in multiple plants representing integrated operations have been studied across multiple growing cycles to fully measure the impact of feeding the dried metabolites of *Saccharomyces cerevisiae* yeast cultures to broiler flocks on the *Salmonella* loads in ceca and parts after processing. A comparison of the impact of *Saccharomyces cerevisiae* on cecal *Salmonella* virulence and susceptibility to selected antibiotics was also conducted. This research dissertation was completed as an independent academic study for the purpose of scientific research.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Common Microorganisms Associated with Poultry Production and Processing

There are numerous microbiological pathogens and spoilage organisms commensal to poultry production. In order for poultry companies to implement effective strategies at reducing bacteria using novel interventions such as *Saccharomyces cerevisiae*, understanding the relationships between microorganisms in poultry and their impact on foodborne illness and/or poultry meat quality and spoilage is relevant. Therefore, below is a brief review of some of the most significant micro-organisms affecting broiler companies and chicken meat products.

Salmonella

Salmonellae are small, Gram-negative, non-sporing rods that are facultatively anaerobic. These organisms are usually motile by peritrichous flagella, although non-motile strains are known to exist. *Salmonellae* colonies are typically 2-4 mm in diameter, and well over 2,500 *Salmonella* serotypes have been isolated (Gast, 2007). The *Salmonella* subspecies names frequently indicate the disease and the animal from which they were isolated, e.g. *typhimurium*, *gallinarium* and *enteritidis*, or the name of the location where the strain was first isolated, e.g. *london* and *arizonae* (Cowan, 1974). More recent classification schemes have been developed based on somatic and flageller antigens (Jay, 1992).

Researchers consistently report that *Salmonella* is one of the most widespread pathogens responsible for foodborne toxic infections in humans (Foley et al., 2008; Jay, 1992). The pathogenesis of the organism has been reported to involve an enterotoxin and a cytotoxin. *Salmonella* enterotoxin acts by elevating intestinal cyclic adenosine monophosphate (cAMP), inducing fluid accumulation, pathogen accumulation, and toxicity (Peterson, 1980). In addition,

intestinal mucosal cells are damaged by a cytotoxin, making these tissues more easily invaded and damaged by the pathogen (Duebbert and Peterson, 1985). Although *Salmonella* organisms generally disappear rapidly from the intestine, up to 5% of infected patients may become carriers upon recovery (Jay, 1992). Cell proliferation in the magnitude of $10^7 – 10^9$ is generally believed necessary to cause salmonellosis, yet outbreaks have been measured at much lower quantities (D'Aoust and Pivnick, 1976). A small number of serotypes are most associated with human and animal diseases, such as, but not limited to, *typhimurium*, *enteritidis*, *newport*, *heidelberg*, and *montevideo* (Foley and Lynne, 2008).

Researchers have described the *Salmonella* infection in poultry species to include interaction of the infection with the immune system in three phases, which include pathogen invasion of the gastrointestinal tract, establishment of a systemic infection to microphages and dendritic cells that replicate in the spleen and liver, and finally a combination immune system reactions such as pathogen clearance, establishment of a carrier state through adaptive response, immunosuppression, or other responses (Chappell et al., 2009).

Campylobacter

The genus *Campylobacter* is a slender, spirally-curved rod possessing at least one polar flagellum (Park et al., 1984), consisting of approximately 14 species, with *Campylobacter jejuni* being one of the primary strains of importance in foods. It requires small amounts of oxygen (3-6%) for growth and carbon dioxide (approximately 10%) for substantive growth, is Gram-negative, oxidase and catalase positive, and will not grow in the presence of 3.5% NaCl or at 25^{o}C. (Jay, 1992). The cells are very sensitive to freezing (Gill and Harris, 1984) and heat (Blankenship and Craven, 1982). The interplay between this organism and other gastrointestinal microbiota in broilers has been analyzed (Kaakoush et al., 2014), and remains an important factor in determining ideal experimental design. Human stool samples taken in hospitals

nationwide have suggested that *Campylobacter jejuni* is a very significant common cause of bacterial diarrhea (Blaser et al., 1982), causing enteritis through production of cytotoxins and enterotoxins, some of which are heat-labile (Ruiz-Palacios et al., 1983), and are very invasive to HeLa cells (Manninen et al., 1982). *Campylobacter jejuni* toxicity has been found to be generally lower and more inconsistent in isolates found in chickens as compared to humans (Gilbert and Slavik, 2004), although intestinal attachment and penetration abilities of the organism have been found to be similar in either host (Gilbert and Slavik, 2005). Improvements in hygiene of poultry processing equipment and water have reduced numbers of *Campylobacter* on packaged poultry carcasses (Mead et al., 1995).

Escherichia coli

The USDA FSIS has deemed *Escherichia coli* as an indicator of choice for measuring process controls for fecal contamination in slaughter facilities for poultry and other animals (USDA FSIS, 1996). The enumeration of this organism has long been used as a process control test important to sanitation and as an indicator of fecal contamination (Mehlman, 1984). The National Research Council also stated that *Escherichia coli* was the best indicator of fecal contamination among the commonly used fecal-indicator organisms (NRC, 1985). This bacteria has strains that cause respiratory diseases, diarrhea, urinary tract infections, and other illnesses (CDC, 2008). Broiler flocks with primary or secondary *Escherichia coli* infections may exhibit airsacculitis (Carter and Chengappa, 1990). Poultry processing interventions such as counter-current scalders, chlorinated rinses, carcasses washes, and reprocessing have been effective at reducing this organism (Waldroup et al., 1992; Waldroup et al., 1993), and growth can be controlled using specified refrigeration time and temperature regimes (Ingham et al., 2004).

Other Microorganisms Associated with Poultry Production and Processing

In poultry production, the processing plant environment has been shown to be a source of *Listeria* contamination in fresh and frozen chicken products (Lawrence and Gilmore, 1994; Lawrence and Gilmore, 1995). Epidemiological studies have demonstrated that *Listeria* can cause gastroenteritis in the absence of invasive disease; however, listeriosis in high-risk individuals, such those that are pregnant, elderly, or immune-compromised, can lead to significant health consequences (Ramaswamy et al., 2007). This organism has significant growth and survival properties, including the ability to adhere to food contact surfaces, making elimination from food processing quite complex (Earnshaw and Lawrence, 1998). *Listeria monocytogenes* isolates have been isolated in poultry processing plants (Lawrence and Gilmore, 1995), and may persist in poultry processing due to development of biofilms and/or plasmid-mediated resistance to commercial disinfectants (Earnshaw and Lawrence, 1998).

Clostridium perfringens is an important pathogen to both broiler producers and processors, since it has a broad distribution, is part of the natural gut flora of warm blooded animals, is capable of forming a spore for survival once exposed to oxygen, and is difficult to eliminate (McCrea and Macklin, 2006). In one study of poultry chill water, *Clostridium perfringens* was found to be a reliable indicator of fecal contamination of the water ecosystem (Voidarou et al., 2007). Typical routes of this organism causing foodborne illness are from food items such as gravies that are not properly cooled and stored after heating (Omaye, 2004).

Pseudomonas is a Gram-negative, aerobic rod (Kreig and Holt, 1984), and one of the primary low-temperature spoilage organisms in poultry and food items (Jay, 1992), especially under aerobic conditions imposed when oxygen permeable packaging materials are used (Kraft, 1992). As a psychrotrophic bacteria, it grows in refrigeration temperatures, but like many other

aerobic bacteria, it is sensitive to carbon dioxide (Montville and Matthews, 2005). Some of the most common off-odor producing bacteria isolated from spoiled broiler chicken carcasses have been *Pseudomonas fluorescens*, *Pseudomonas fragi*, and *Pseudomonas putida* (Russell et al., 1996). Quantification of *Pseudomonas fluorescens* can be used to predict the shelf life of fresh chicken (Russell, 1997).

Shewanella putrefaciens is another spoilage organism associated with fresh poultry. This Gram-negative, facultative anaerobe (Kreig and Holt, 1984) has been described as an an off-odor producing bacteria isolated from spoiled broiler chicken carcasses (Russell et al., 1996). During spoilage biochemical reactions and volitization, it releases methanol, hydrogen sulfides, and trimethlyamines (Jay, 1992). *Shewanella putrefaciens* has been more resistant to chemical sanitizers such as sodium hypochlorite than other spoilage bacteria such as the *Pseudomonas* species (Russell, 1998).

Another common food spoilage organism associated with poultry is *Acinetobacter*. This rod-shaped, Gram-negative aerobe (Kreig and Holt, 1984) has pyschrotrophic characteristics, and is sensitive to carbon dioxide. *Acinetobacter* organisms can be well-managed by controlled atmosphere packaging (Montville and Matthews, 2005). Besides contributing to food spoilage, some species of *Acinetobacter* are also found in soil, water, and human skin, causing pneumonia and blood or wound infections (CDC, 2004).

Even though there are many other bacteria species comprising the poultry microflora, *Salmonella* species will be the emphasis of this dissertation from a microbiological perspective.

Strategies Employed to Remove Pathogens and Spoilage Organisms in Poultry Production and Processing

Poultry integrators and researchers have long recognized that the reduction of bacteria requires a multi-hurdle approach across the wide spectrum of the vertically integrated poultry industry. Thus, any study of one pathogen intervention must be considered with an understanding of the other interventions employed that collectively serve to reduce microorganisms. Therefore, following is a brief review of some common strategies used to remove pathogens and spoilage organisms in poultry production and processing.

Breeding Stock and Hatchery Interventions

The production of commercial poultry originates with pedigree lines, which become the great grandparents of broiler chickens. Unlike production flocks, which are relatively short-lived, elite breeder flocks may have a lifespan in excess of a year, so careful management is necessary to prevent colonization by *Salmonella* and other pathogens (Cox and Pavic, 2010). The control of *Salmonella* colonization is vital and stressed at the apex of production, as this organism can be vertically transmitted from hen to egg (Liljebjelke et al., 2005). Breeder flocks are a primary area of emphasis in reducing pathogens such as *Salmonella* strains, because of the likelihood that a colonized flock may spread the bacterium to a large number of commercial flocks. When designing intervention strategies, the microbial ecology of the chicken is understood in order to avoid unintended negative impacts of such technologies (Callaway et al., 2008). Also, at the elite flock levels, measures such as change-in–change-out of farm or shed-based apparel is commonly practiced, and in some countries shower-in–shower-out practices are employed for using a thorough disinfection strategy prior-to and post-entry to breeder farms (Lewerin et al., 2005).

Eggs received from breeder farms are not necessarily pathogen free, and the external debris or contamination can be transmitted to commercial hatchers and incubators. Enteric pathogens can then spread to other areas of the hatchery based on the design of facility airflow (Bailey et al., 1998; Mitchell et al., 2002). Therefore, many methods are employed in commercial hatcheries to control the spread of pathogens. Methods have included disinfecting eggs with ultraviolet light, ozone, chemicals, electrostatic charging, pulsed light and gas plasma (Coufal et al., 2003; Davies and Breslin, 2003; Dunn, 1996; Mitchell et al., 2002; Rodriguez-Romo and Yousef, 2005). Other egg disinfection methods have included the use of hydrogen peroxide, quaternary ammonium compounds, peroxyacetic acid, and other compounds (Cox et al. 2007). These egg disinfection procedures are often used within the hatchery; however, the benefits of such egg disinfection technologies are frequently also effective if applied to freshly laid eggs on the farm, prior to entering the hatchery environment (Cox and Pavic, 2010).

Broiler Microbial Interventions and Strategies

Researchers and commercial poultry operation managers have gone to extensive efforts to reduce *Salmonella* and *Campylobacter* within vertically integrated broiler production process. Effective cleaning and disinfection of broiler houses has been shown to have an immediate impact of reduction of pathogens present in environmental sample as measured by reduced *Campylobacter jejuni* immediately after cleaning (Burbarelli et al., 2017). Biosecurity measures are used to prevent or reduce the spread of microorganisms in poultry houses through a set of rules and procedures that minimize exposure of bird populations to infectious biological agents (Cox, 2005; Wenzel and Nusbaum, 2007). Reducing the ingress of pathogen carriers including wild birds, rats, insects, and humans is also an effective means of preventing or minimizing enteric pathogens (Arsenault et al., 2007), including maintaining strict hygiene barrier areas for

visitors and farm personnel to properly use designated boots and outer garments (Newell et al., 2011). Broiler stock are typically populated and depopulated on an all-in, all-out basis to minimize cross contamination between flocks that could otherwise occur with multi-stage stocking (Plym-Forshell and Wierup, 2006). Numerous poultry house litter treatments have been used to minimize enteric pathogens, including composting to reduce pathogens (Mohee et al., 2008), or the use of granules that contain compounds such as sulfuric acid (Vicente et al., 2007), sodium bisulfate (Line, 2002), or formic and propionic acid blends mixed with sodium lignosulfate (Garrido, et al., 2004).

Microorganisms in feed may introduce pathogens such as *Salmonella* onto the farm (Davies and Wales, 2010; Davies et al., 2004; Molla et al., 2010). The reduction of *Salmonella* risk may be achieved in feed mills through milling controls such as heat treatment, and the addition of organic acids and their salts and formaldehyde (Berge and Wierup, 2012). Coarse-ground grains and access to growing on litter vs. cages has also been shown to decrease cecal *Salmonella* populations in broilers (Santos et al., 2008). The addition of pathogen interventions to feeds has also been shown to potentially reduce organisms such as *Salmonella* in feed through the inclusion of additives such as organic acids, selected fatty acids, prebiotics, probiotics, competitive exclusion cultures, mannan-based carbohydrates, essential oils, and bacteriophages (Berge and Wierup, 2012).

In poultry production systems, antibiotics have been used in many countries when necessary to prevent, control, or treat bacterial infections, and improve bird health (Seiffert et al., 2013). Some compounds that have reduced pathogens in poultry have included tetracyclines, tylosin, lincomycin, flavomicin, and others. The carboxylic ionophore polyether anticoccidals such as lasolocid, salinomycin, and naracin have also been shown effective at reducing

Clostridium perfringens, necrotic enteritis, and other pathogens (Lanckriet et al., 2010; Martel et al., 2004). However, antibiotic use in animal husbandry has become a topic of public concern due to the potential of developing drug-resistant bacteria; for example, in some Latin-American countries, increased rates of antimicrobial-resistant *Campylobacter* have been reported (Pollett et al., 2012; Sierra-Arguello et al., 2016). This situation is of special relevance in developing countries where the misuse of antibiotics and a lack of controls is a recognized challenge (Reardon, 2014). Research trials with raising antibiotic-free chickens in tropical conditions with conventional practices in the absence of other pathogen interventions was shown to cause extensive hemorrhagic enteritis, typhlitis, *Salmonella* presence, and bird mortality (Ganapathy et al., 2000). Therefore, alternative strategies to reduce pathogens in broilers with the absence of antibiotics has been a focus area for poultry companies.

Processing Plant Interventions

In poultry plants, there are multiple sites where *Salmonella* can originate, be introduced or subsequently spread through cross-contamination (Foley, et al., 2008). Therefore, multi-hurdle strategies have been employed in processing plants, and some of the most common plant interventions are described below.

Initially, a bird brush and washer used prior to the scalder is frequently used to remove incoming dirt and fecal material, to reduce the problem of excessive quantities of pathogens entering the scalding system (USDA FSIS, 2015). Picking machines specialized in the removal of feathers using rotating rubber fingers to rub or beat the feathers off the carcass may cause cross-contamination by enteric pathogens (Dickens and Whittemore, 1997), so picker fingers consisting of certain rubber materials may be used to inhibit microbial contamination (Arnold and Silvers, 2000). Studies using marker organisms and/or pulsed field gel electrophoresis have

demonstrated that bacteria in live poultry can spread on poultry carcasses through the picking process (Mulder et al., 1978; Nde et al., 2007). Therefore, carcass rinses applied during defeathering may be used to decrease microbiological loads. For example, in a study that tested water, acetic acid and hydrogen peroxide as sprays during defeathering, the combination of sprays along with the massaging action of picker fingers on the carcass resulted in antimicrobial effects, as chemicals were forced into the crevices of poultry skin where many bacteria are found (Dickens and Whittemore, 1997).

Carcass washing and/or spraying is a common poultry processing step before, during, and after evisceration. These technologies have shown to be effective in multiple locations in reducing microbial contamination (Shackelford et al., 1993). Inside-outside bird washers have as their primary function the removal of visible fecal material, and have shown to reduce coliforms, *Escherichia coli*, and/or total aerobic bacteria counts on carcasses to various degrees (Northcutt et al., 2003). Carcasses washers have had mixed results on reductions of *Campylobacter* compared to other interventions (Bashor et al., 2004). The use of multiple carcass washing steps for the same carcasses has shown to be more effective than any one step alone. For example, it has been demonstrated that the use of brushes pre-scald in combination with spray washers after feather removal, during evisceration, and prior to chilling have collectively reduced quantities of *Campylobacter* and *Escherichia coli*, and decreased the presence of *Salmonella* (Berrang and Bailey, 2009).

Continuous immersion chilling has been validated as an effective means to rapidly reduce broiler carcass temperatures and microbiological loads (Mulder et al., 1976). Tests of water immersion chilling as compared to air chilling have found aerobic count data to be comparable, although immersion chilling has demonstrated a greater impact on the ability to injure or

inactivate pathogens (Barbut et al., 2009). Immersion chilling has been found to have a negative impact on the growth of *Campylobacter* (Voidarou et al., 2007). Some studies have indicated that air chilling may have additional benefits of further reduced microbiological counts compared to immersion chilling, and lower quantities of spoilage bacteria after held in refrigerated storage (Tuncer and Sirelli, 2008).

Antimicrobial interventions to control *Salmonella* or *Campylobacte*r can be applied in multiple ways, such as spraying or immersion dipping (USDA FSIS, 2015). Immersion is considered generally more effective than spraying because it ensures better coverage and longer contact time (Buncic and Sofos, 2012). For example, acetic acid applied as an immersion dip at 20ppm has been shown to reduce *Salmonella* by 1.4 log colony forming units (CFU's), compared to a reduction of 0.8 log CFU when applied as a spray (Loretz et al., 2010).

The options for various antimicrobial technologies applied as sprays and dips have increased in recent years for poultry processors, as requirements of regulators and consumers have driven the marketplace to use more of these interventions. Chlorine has traditionally been a very commonly used disinfectant in poultry plants (Buncic and Sofos, 2012), and when added to water produces hyphochlorous acid which has lethality to microorganisms (USDA FSIS, 2015). Some other common antimicrobials used in poultry operations which have been compared in research have included acidified sodium chlorite, trisodium phosphate, citric acid, peroxyacetic acid, cetylpyridinium chloride (Chen et al., 2014; Del Rio et al., 2007), acidified lactic acid, and lauric arginate (Moore et al., 2017). The effectiveness of these compounds has frequently been shown to be dependent on contact time, concentration, pH, and application differences in application methods, such as spray verses immersion. Best practices across plants are often compared in research (Wideman et al., 2016).

Plant sanitation has been recognized as critically important in reducing microorganisms that contribute to the growth of pathogens and spoilage organisms. Alkaline chemicals are typically used to remove proteins, fats, oils, and greases (Marriott and Gravani, 2006), and chlorine-based compounds such as chlorine dioxide, chlorine, or chloramines have been used to reduce heterotrophic bacteria (Gagnon et al., 2004). Acidic compounds, such as blends of phosphoric, nitric, sulfuric, and sulfamic acid, are used to remove encrusted surface materials and dissolve mineral scale deposits, including those formed from alkaline cleaners (Marriott and Gravani, 2006). To supplement cleaning compounds, sanitizing agents such as quaternary ammonium, hydrogen peroxide, and other compounds reduce bacteria and prevent biofilms (Dickens and Whittemore, 1997; Russell, 1998, 2000, 2008).

Overview of Alternative Interventions using Probiotics and Prebiotics to Reduce Pathogens in Animals

Probiotics are products that contain viable microorganisms that when administered exhibit health benefits to the host, such as regulation and stabilization of gastrointestinal barrier functions (Gaggia et al., 2010; Salminen et al., 1996), expression of bacteriocins (Gaggia et al., 2010; Mazmanian et al., 2008), immunomodulatory effects (Gaggia et al., 2010; Salzman et al., 2003), and altering the microbiome of the host (Fuller, 1989; Park et al., 2013; Patterson and Burkholder, 2003). Probiotics colonize and are metabolically active in the target site, survive gastric juices and bile, compete with resident microbiota, and produce antimicrobial substances antagonistic towards pathogenic bacteria (Gaggia et al., 2010). Some common genera used for probiotics within the poultry industry are *Lactobacillus*, *Enterococcus*, *Bacillus*, *Saccharomyces*, *Bifidobacterium*, *Streptococcus*, *Aspergillus*, and *Candida* (Gaggia et al., 2010; Kabir, 2009).

Probiotics are derived from beneficial microbial cultures that may be administered to stimulate the local immune system in animals (Gibson and Roberfroid, 1995; Netherwood et al., 1999) and enhance epithelial innate immunity-related gene expression through anti-inflammatory effects and reduced pro-inflammatory cytokine expression (Amit-Romach et al., 2010; Pagnini et al., 2010). With the presence of microorganisms in the gastro-intestinal tract, innate immune system associated pathogen-pattern recognition toll-like receptors may induce expression of pro-inflammatory cytokines and antimicrobial peptides such as defensins, which are direct effector molecules on the innate immune response (Birchler et al., 2001; Ganz, 2003; Kaiser, 2010). These toll-like receptors, also known as pathogen recognition receptors, are part of the innate immune system (Rodríguez-Lecompte et al., 2012), and work by recognizing pathogen-associated molecular patterns, causing a chain reaction that stimulates the immune system to respond (Aderem and Ulevitch, 2000).

One of the benefits of probiotics is the stimulation of the local immune system against colonization by pathogenic microbes (Lan et al., 2005). Changes in mucin profile in response to bacterial colonization, as a function of goblet cell numbers, have been reported to play a role against pathogen invasion of the intestinal mucosa during early development (Forder et al., 2007). The addition of combinations of probiotics and organic acids has been shown to increase goblet cell numbers in the ileum of treated birds (Rodríguez-Lecompte et al., 2012), while increasing the numbers of mucosal-adherent bacteria with a larger mucous layer in the small intestine (Chichlowski et al., 2007).

The use of probiotics has been shown to improve the natural defense of animals against pathogenic bacteria, and can be an alternative and effective approach to antibiotic administration for reduction of bacterial contamination (Ghareeb et al., 2012). *Salmonella* reduction and control

has been widely studied using competitive exclusion, probiotics, and enhancement of intestinal immunity (Revolledo et al., 2006). In vitro experiments have shown that *Enterococcus faecium, Pediococcus acidilactici, Lactobacillus salivarius,* and *Lactobacillus reuteri* isolated from healthy chicken gut inhibit the growth of *Campylobacter jejuni*. Furthermore, broiler chicks challenged with *Campylobacter jejuni* orally and receiving a multispecies probiotic product containing *Enterococci, Pediococci, Lactobacilli,* and *Bifidobacteria* have exhibited significantly reduced *Campylobacter jejuni* colonization in the ceca at both 8 and 15 days post-challenge (Ghareeb et al., 2012).

Prebiotics are non-digestible food ingredients that stimulate the growth or activity of one or a limited number of bacteria (Gaggia et al., 2010; Gibson and Roberfroid, 1995). One prebiotic has been mannon oligosaccharides derived from *Saccharomyces cerevisiae* cell walls, used as an alternative to antibiotic use in pigs (Shen et al., 2009; Rozeboom et al., 2005). Yeast-based derivatives of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have also been used to express *Escherichia coli* phytase in order to affect broiler growth and nutrient utilization (Onyango et al., 2004).

In cattle, use of prebiotics has been a successful alternative in some situations to the ionophore monensin. Historically, monensin has been administered in finishing beef feedlot diets for the prevention of coccidiosis and improved feed utilization. However, with the growing consumer demand for "natural" and/or organically grown beef (Thompson et al, 2007), alternative strategies to monensin have been studied, because the USDA has designated that naturally-raised beef animals are to be grown without the use of growth hormones or antimicrobials (USDA, 2009). An alternative that has been administered is a prebiotic derived from *Saccharomyces cerevisiae* fermentation (Swyers et al., 2014). Some studies have shown

this feed supplement to stimulate the growth of ruminal cellulolytic bacterial species by providing soluble growth factors (Callaway and Martin, 1997), while also improving nutrient digestibility (Wohlt et al., 1991) and increasing mineral retention (Cole et al., 1992). Also, the use of this product has resulted in beef carcass USDA yield and quality grades that were equivalent or better than for untreated control steers, or for those fed monensin (Swyers et al., 2014).

Alternative microbial interventions used in live animals have been studied in the gastrointestinal tract using multiple methods. Although healthy animals have stable microflora in the gastrointestinal tract, molecular methods such as rRNA gene sequence amplification and temperature gradient gel electrophoresis have been successfully used to determine the impact on gut ecology from the use of antibiotics, probiotics, prebiotics, and other food components (Zoetendal et al., 1998).

Saccharomyces cerevisiae and other Yeast-based Feed Supplements and their Modes of Action in Pathogen Reduction

The yeast *Saccharomyces cerevisiae* is an essential component of many important human activities including baking, brewing, distilling, and wine making. In these industries, specialized strains of *Saccharomyces cerevisiae* have been selected for use, and these strains are not readily interchangeable. In addition, this yeast compound is considered a model eukaryotic organism in research activities in hundreds of laboratories worldwide. It has the ability to grow and function both aerobically and anaerobically (fermentative). Wine-making and baking also involve the use of evolving natural strains, in contrast to commercially developed yeast-based products (Mortimer, 2000). Mannoproteins derived from *Saccharomyces cerevisiae* can also protect wines from protein haze and turbidities (Dupin et al., 2000).

Saccharomyces cerevisiae metabolism has been studied extensively as it relates to the fermentation of cultures into alcohols, aldehydes, organic acids, esters, organic sulfides, and carbonyl compounds. Food fermentation by yeast and lactic acid bacteria is accompanied by aliphatic and aromatic alcohols, which at high concentrations can impart off-flavor, but at low concentrations can make an essential contribution to the flavors and aromas of foods and beverages (Hazelwood et al., 2008). The metabolism of yeast cell extracts via the Ehrlich pathway including catabolism of branched-chain amino acids (leucine, valine, and isoleucine), aromatic amino acids (phenyl-alanine, tyrosine, and tryptophan), and a sulfur-amino acid (methionine) in a sequence of transamination, decarboxylation, reduction and export of acids and alcohols has often been studied (Hazelwood et al., 2008). *Saccharomyces cerevisiae* has been an often-used model organism to understand the molecular chemistry of cation homeostasis, an integral characteristic of living organisms that facilitates numerous biochemical reactions by establishing electrochemical gradients across membranes that drive cellular processes such as transport and ATP synthesis (Cyert and Philpott, 2013).

There are differences in yeast-based feed supplements in their compositions and modes of actions. Active dried yeast has been suggested to act mainly as a probiotic. Active dried yeast is defined as yeast that has been dried to preserve its fermenting power and must contain at least 15 x 10^9 live yeast cells per gram. Dried yeast is defined as dried nonfermentive yeast separated from its medium and must contain at least 40% crude protein (AAFCO, 2002; van Heugten et al., 2003). Yeast culture is yeast and the media on which it was grown, dried to preserve its fermenting power (AAFCO, 2002). Researchers have studied the differences in applying active vs. killed dried yeast, with some studies showing effects based on form. For example, irrespective of its viability, yeast supplementation was shown effective in reducing the duration

of subacute ruminal acidosis beef heifers, whereas the proportion of *Ruminococcus flavefaciens*, one of the major anaerobic cellulolytic rumen bacterial species (Brulc et al., 2011), was higher in the solid fraction of digesta in heifers exposed to killed dried yeast (Vyas et al., 2014). Previous studies have shown that autoclaved cells of *Saccharomyces cerevisiae* (Oeztuerk, 2009) and *Saccharomyces boulardii* (Oeztuerk et al., 2005) can stimulate ruminal fermentation by providing nutrients contained within the cells, such as vitamins or other growth factors, to autochthonous microbiota (Opsi et al., 2012).

Yeast-based products may exhibit prebiotic effects in addition to the well-documented probiotic effects (Vyas et al., 2014). Killed dried yeast has demonstrated prebiotic functionality (Oeztuerk et al., 2005; Oeztuerk, 2009) via its stimulatory effects on *Megashaera elsdenii* (Chaucheyras et al., 1995; Chaucheyras et al., 1996) in vitro by providing various growth factors, pro-vitamins, and micronutrients to the autochthonous microbiota (Opsi et al., 2012). The stabilizing effect on ruminal pH in cattle has been ascribed to reduce lactate concentration, due to the nutritional competition between *Saccharomyces cerevisiae* and lactic acid producing bacteria (Chaucheyras et al., 1996) and the stimulation of lactic acid utilizing bacteria such as *Selenomonas ruminantium* (Nisbet and Martin, 1991; Callaway and Martin, 1997) and *Megasphaera elsdenii* (Callaway and Martin, 1997).

Live yeast forms of *Saccharomyces* have been used in a variety of applications as a preventive and therapeutic agent for the treatment of intestinal diseases in humans and animals (Zanello et al., 2009). *Saccharomyces cerevisiae* has been used to suppress the effects of aflatoxicosis in broilers (Stanley et al., 1993). Pathogenic *Escherichia coli* colonization of the digestive tract is mediated by binding of bacterial fimbriae to ligands on gut epithelial cells. The inclusion of yeast components in animal feed is a promising strategy to reduce colibacillosis.

Microscopy has confirmed the ability of enterotoxigenic *Escherichia coli* (ETEC) to adhere to yeast cell walls, indicating the ability of certain yeast cell wall products to contain and/or reduce the intestinal infection from ETEC in young pigs due to the affinity of ETEC to yeast cell walls (Trevisi et al., 2012).

Researchers have repeatedly demonstrated agglutination of pathogenic bacteria onto brewers dried yeast products (Kogan and Kocher, 2007; White et al., 2002). When agglutination of pathogenic bacteria by a yeast extract was tested, one study found 64% of *Escherchia coli* strains that were tested and 67% of *Salmonella* spp. strains that were tested were found to agglutinate to the yeast product, with the pathogen strains *Salmonella Typhimurium* and *Salmonella Enteritidis* having very high agglutination rates of 70% and 86%, respectfully (Kogan and Kocher, 2007).

Anaerobically fermented yeast products are a rich source of nutritional metabolites, mannanoligosaccharides, and β-glucans that may optimize gut health and immunity, which can translate into better growth performance and a reduced risk of foodborne pathogens (Price et al., 2010). Enteric Salmonellosis has been reported to alter microbial ecology, specifically increasing *Clostridia* in the murine gastrointestinal tract prior to diarrhea (Barman et al., 2008). Consumption of *Saccharomyces cerevisiae* fermentation product altered the microbial composition of the gastrointestinal microbial community in pigs, resulting in increased *Bacteriodetes* and *Lactobacillus* after an infection with *Salmonella*. Fecal shedding of *Salmonella* increased from pigs fed a diet with *Saccharomyces cerevisiae* fermentation product, with an increase of beneficial bacteria remaining within the gastrointestinal tract. The addition of *Saccharomyces cerevisiae* fermentation product to the diets of weanling pigs resulted in

healthier intestines, and greater compensatory body weight gains after infection with *Salmonella* than pigs fed conventional nursery diets (Price et al., 2010).

Yeast cells are natural mannose-rich products that can be used as substrates for adhesion of Gram-negative bacteria (Badia et al., 2012). Mannose-rich compounds are believed to mimic the host cell receptor to which the pathogen adheres, as pathogen fimbriae bind to these residues instead of the mannose units of the glycoproteins on the intestinal surface (Gedek, 1999). Mannan ologosaccharides derived from *Saccharomyces cerevisiae* come from yeast cell walls containing approximately 45% mannose residues (Tizard et al., 1989).

Gram-negative bacteria have been shown to be agglutinated by mannan oligosaccharide products by interacting with mannose-specific lectins on the surface of the bacterial organisms (Burkey et al., 2004). Fimbriae of many bacteria bind to the mucosa of the host intestine, facilitating proliferation of the bacteria (Holland, 1990) by acting as surface lectins, which are carbohydrate-binding proteins (Berg et al., 2007). The cell walls of yeasts such as *Saccharomyces cerevisiae* contain mannon components, which can be isolated industrially to produce feed additives known as mannan oligosaccharides (Spring et al., 2000). This product has been shown to bind, *in vitro*, to bacterial cells possessing Type 1 fimbrae, including species of *Escherichia coli* and *Salmonella* (Spring et al., 2000), preventing these pathogens from proliferating at the mucosal surface of the intestine (Rozeboom et al., 2005). This mechanism works in contrast to the infectious activity of *Salmonella* fimbrial adhesins to host mucosa and epithelial cells, which mediates multiple mechanisms during the initial phase of an acute infection (Baumler et al., 1997).

The supplementation of animal diets has been studied with phosphorylated mannans derived from the yeast cell wall of *Saccharomyces cerevisiae*. Mannans have the ability to alter

microbial populations in the intestinal tract. It has been suggested that this modification is accomplished by the ability of mannans to attach to mannose-binding proteins on the cell surface of some strains of bacteria, thereby preventing these bacteria from colonizing the intestinal tract by interfering with the binding carbohydrate residues on the epithelial cell surfaces (Spring et al., 2000). Other studies using *in vitro* colon simulation techniques have suggested that this live yeast probiotic can improve digestion, especially when used at higher doses, due to a shift in bacterial populations (Pinloche et al., 2012).

Glucans with β-1,3 and β-1,6 glycosidic linkages (i.e. β-glucans) are major structural components of yeast and fungal cell walls (Jorgensen and Robertsen, 1995) and have antimicrobial properties by enhancing the immune function (Hetland et al., 2000). Beta-glucans are immunomodulators that can be extracted from the cell walls of yeasts, fungi, and some grains (Eicher et al., 2006). They can enhance the innate immune system by providing protection against bacterial diseases (Onderdonk et al., 1992), protozoan infections (Goldman and Jaffe, 1991), and viral illnesses (Rouhier et al., 1995). Some of the mechanisms of action have been shown to include activation of macrophages, neutrophils, and natural killer cells, along with B- and T- lymphocytes (Eicher et al., 2006). Also, β-glucans have been shown to increase phagocytosis and cytokine production in macrophages *in vivo* (Seljelid et al., 1987) and *in vitro* (Hoffman et al., 1993).

Studies have shown that the yeast survival rate all the way through the gastrointestinal tract of pigs was 1% (Pinloche et al., 2012), which is consistent with other studies using a rat model showing that the major loss of *Saccharomyces cerevisiae* happened in the large intestine rather than in the stomach and small intestine (Garrait et al., 2007). Also, when analyzing the changes in microbiota in the intestine caused by *Saccharomyces cerevisiae*, by using restriction

fragment length polymorphism, a change was observed in solid-associated bacteria; this was believed to be due to high concentrations of *Saccharomyces cerevisiae* (5×10^{11} cfu/kg) in test feeds compared to controls with no supplements (Pinloche et al., 2012). In porcine livers, cytokine responses have been observed in response to feeding various sources of β-glucans derived from beneficial microorgansims such as *Saccharomyces cerevisiae*, *Laminara digitata*, and *Laminara hyperborean* (Ryan et al., 2012b), which is consistent with the view that β-glucans prime the immune system, thereby altering subsequent responses to infection (Volman et al., 2008). It has been reported that stimulation of TLR-4 by lipopolysaccharides in the liver results in production of pro-inflammatory cytokines such as certain interleukins by the Kupffer cells (Seki et al., 2002). A mouse (*Mus musculus*) model of sepsis has also demonstrated that a selected soluble poly-glucan induced significant decreases in IL-6 and IL-10 plasma levels and bacterial colonization of the liver (Newsome et al., 2011). From a therapeutic perspective it is desirable to have the ability to prime host defenses without contributing to a systemic inflammatory response. Differences were evident between the *Laminara digitata* and *Laminara hyperborea* supplementation groups with respect to interleukin expression in the liver, which is consistent with the view that there are differences in how different soluble and insoluble β-glucans are absorbed (Sweeney et al., 2012). The findings of the study indicated that supplementation with β-glucans had systemic effects on the inflammatory response in the liver to a lipopolysaccharide challenge, although it was undetermined whether these effects were attributable directly to the β-glucans following their absorption and uptake within the liver or indirectly due to alterations in the gut microbiota brought about by the β-glucans (Ryan et al., 2012a).

Beneficial changes in bacterial populations induced by *Saccharomyces cerevisiae* intake may be most effective during periods of long sickness, which may be especially relevant in some animals with prolonged exposure to pathogens (Price et al., 2010). Additionally, *Saccharomyces cerevisiae* var. *boulardii* used as a direct-fed microbial in monogastrics has been shown to lead to beneficial microbiome shifts and improved intestinal health (Everard et al., 2014; Feizizadeh et al., 2014). Also, animals fed yeast products tended to have higher serum IgG quantities than controls, enhancing certain other serum immunological traits (White et al., 2007). In addition, experimental phytase enzyme preparations have been developed from genes derived from environmental *Escherichia coli* and cloned into *Saccharomyces cerevisiae* after undergoing gene site saturation mutagenesis to increase thermal stability of the enzyme (Silversides et al., 2004).

***Saccharomyces cerevisiae* Metabolite Compounds Manufacturing and Administration to Poultry and Livestock**

Concentrated β-glucans are extracted from the cell walls of *Saccharomyces cerevisiae* in baker's yeast in various methods. Extraction may be achieved by adding various chemicals such as sodium hydroxide, hydrochloric acid, and hydrogen peroxide to the yeast, in various stages under high heat and pressure, resulting in a highly concentrated β-glucans product (Hunter et al, 2002; Li et al., 2006). *Saccharomyces cerevisiae* strains LL74 and LL83 have been isolated from bakery by-products and found to have probiotic and anti-mycotoxin effects, and were able to tolerate simulated gastrointestinal conditions, while demonstrating a potential to be used in animal feeds, with or without antibiotics, while providing probiotic properties (Poloni et al., 2017).

Saccharomyces cerevisiae has been effectively distributed in various methods to animal feeds. One method studied included adding the organism into silages typically used to feed dairy

cattle in order to study the impact of *Saccharomyces* strains delivered to ruminants. In that study, two strains of *Saccharomyces cerevisiae* and one strain of *Saccharomyces paradoxus* were inoculated individually onto corn forage that was ensiled in mini silos. Fermentation characteristics of silage inoculated with a strain of *Saccharomyces cerevisiae* were similar to control silage. One strain of *Saccharomyces cerevisiae* treatment of inoculation increased ash and decreased organic matter contents of silage, but no differences were observed in nutrient composition or fermentation profiles after 90 days of ensiling. Inoculation with *Saccharomyces* had no detrimental effect on the aerobic stability of silage. In vitro dry matter disappearance, gas production, and microbial protein synthesis were not affected by *Saccharomyces cerevisiae* inoculation. Both *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* populations increased when exposed to aerobic conditions (Duniere et al., 2015).

In another study, *Saccharomyces cerevisiae* supplements were distributed in whole milk to male pigs within three hours of birth and then daily for two weeks until weaning, and distributed in feed for an additional two weeks concurrently with another treatment that was fed the *Saccharomyces cerevisiae* supplement in combination with Vitamin C. An intravenous lipopolysaccharide challenge with *Escherichia coli* 0111:B4 was given at 14 days post-weaning. The *Saccharomyces cerevisiae* treatment, alone and in combination with Vitamin C, increased body weight and average daily gain, and reduced blood cortisol concentrations. After the lipopolysaccharide challenge, within 2 hours of injection differences were observed in the immuno-modulation factors in the combination treatment as compared to the untreated control group, expressed by reduced tumor-necrosis factor - α mRNA in the intestine, spleen, and liver (Eicher et al., 2006). In a separate study, pigs fed *Saccharomyces cerevisiae* cultures within a pelleted and non-pelleted diet displayed no difference in animal performance based on the type

of administration, with both application methods affecting daily intake and gain:feed ratio (Mathew et al., 1998).

In another experiment, Holstein steers received daily treatments of *Saccharomyces cerevisiae* var. *boulardii* with abomasal pulse doses using a veterinary stomach pump over an 18 day period, followed by pulse dose treatments of oligofructose. These doses resulted in stabilization of the intestinal environment during increased carbohydrate fermentation (Gressley, et al., 2016).

Live yeast *Saccharomyces cerevisiae* subspecies *boulardii* were administered to beef heifers via oral paste at the beginning of the experiment (upon arrival to a feedlot), followed by 0.5 grams per animal per day in the diet for 35 days. This combination of paste and dietary administration of *Saccharomyces cerevisiae*, in conjunction with florfenicol treatments upon arrival to a feedlot, was demonstrated to decrease morbidity from bovine respiratory disease, but had little effect on performance as measured by average daily gain and dry matter intake (Keyser et al., 2007).

Saccharomyces cerevisiae has been administered not only as a single probiotic or prebiotic, but also in combination with other supplements and treatments. The combined effects of probiotics and/or prebiotics have been studied repeatedly and the effects appear to be conserved across multiple species in the case of using *Saccharomyces cerevisiae* with other treatments. For example, the combined effects of various combinations of *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Streptococcus faecium*, sorbic acid, and citric acid on intestinal morphology and expression of immune-related genes in young chickens was investigated, as measured by the presence of toll-like receptors and cytokine expression. Birds supplemented with combined probiotics and organic acids showed a beneficial

response, with birds supplemented for 7 days exhibiting similar responses as those supplemented for 14 days, indicating shorter periods of supplementation might be enough to elicit beneficial responses. Some of these beneficial responses included improved villus height in the duodenum, increased number of goblet cells, and greater expression of toll-like receptors and cytokines in the cecum (Rodríguez-Lecompte et al., 2012). In another study, administration of *Saccharomyces cerevisiae* in combination with *Bacillus amyloliquefaciens* improved the intestinal microflora and morphology in broilers (Teng et al., 2017). In a study of weaned piglets, those fed a successive probiotic supplement of *Saccharomyces cerevisiae* ssp. *boulardii* followed by *Pediococcus acidilactici* realized positive effects on feed conversion, intestinal villus length, and crypt depth, with dramatic reductions of fecal *Escherchia coli* counts (Le Bon et al., 2010). A combination of *Bacillus* and active yeast cultures fed to weaned pigs resulted in improved average daily gain, dry matter digestibility, and feed conversion (Min et al., 2004).

Traditionally broiler feed supplements have been combined with vaccines or other compounds to prevent coccidiosis. In one study, four tests groups were compared: a group exposed to a coccidiosis vaccine, a group with the coccidiosis vaccine and a *Saccharomyces cerevisiae* - based feed supplement called Original XPC™ (Diamond V, Cedar Rapids, IA), a group with the coccidiosis vaccine and salinomycin sodium in the grower diet, and a group exposed to all treatments including the coccidiosis vaccine, salinomycin sodium supplement, and *Saccharomyces cerevisiae* XPC product. The inclusion of the *Saccharomyces cerevisiae* - based feed supplement decreased the prevalence of *Salmonella* in the broilers exposed to this treatment. *Salmonella* frequencies in the ceca were reduced the greatest in the group exposed to all three treatments, at all ages sampled including 16 days, 28 days, and 42 days. Similar results occurred

with improvements in growth rate, body weight and feed intake on the birds exposed to XPC, with the greatest benefits occurring from birds exposed to all treatments (Roto et al., 2017).

Research Studies Regarding the Effectiveness and Modes of Action when Administering Saccharomyces cerevisiae Products to Various Species

Saccharomyces cerevisiae derivatives have been successfully used in multiple species for a variety of functions. For example, this microorganism has been used for improvements in intestinal health and strength, improved digestion and nutrient uptake, reduced dependence on antibiotics, and improved immune response. This is in addition to other ancillary benefits such as beneficial microbial action during certain types of food production, wine making, brewing, and other uses. Benefits of this yeast component when administered to various animal species are discussed below.

Humans

Derivatives of *Saccharomyces cerevisiae* have been routinely and extensively used for human foods for many centuries for various purposes, such as in the production of yeast leavened breads and fermented beverages such as beer and wine. Yeast glucans for *Saccharomyces cerevisiae* also occur naturally in other human food products (EFSA, 2011). The most common yeasts, *Saccharomyces cerevisiae* and *Saccharomyces boulardii*, have a 'generally recognized as safe' (GRAS) status and have been used as probiotic biotherapeutic agents against selected human digestive pathologies. In recent decades, *Saccharomyces cerevisiae* has become an attractive host for the production of recombinant proteins and genetic bioconversion because of the relative ease of use and high productivity in genetic engineering (Blanquet et al., 2001). This has led to novel biotechnological research within this yeast compound. For example, mucin-type human glycoproteins have been produced within *Saccharomyces cerevisiae* cells and

may contain many types of glycoprotein forms specific to cancer cells, and thus could serve as tumor-specific antigens used to develop cancer diagnostic and therapeutic agents, such as anti-epitope antibodies (Amano et. al, 2008). In another novel experiment, elemental magnesium administered within a *Saccharomyces cerevisiae* yeast biomass has been shown more effective at penetrating the intestinal cell wall and becoming absorbed than traditional pharmacological supplementation with magnesium salts (Malgorzata et al., 2006).

Probiotics in humans have been postulated to work with multiple modes of action. These include suppression of viable bacterial counts through competition for nutrients or adhesion sites, production of antibacterial compounds, changes in enzyme activity of microorganisms, and stimulation of immunity through increased antibodies and macrophages (Fuller, 1989). Researchers have shown that *Saccharomyces cerevisiae* var. *boulardii* is a preventative and therapeutic agent in humans through various mechanisms including inactivation of bacterial toxins, nutritional effects, quorum sensing, trophic effects, immune-modulatory effects, anti-inflammatory effects, and epithelial cell restitution and maintenance of barrier integrity (Łukaszewicz, 2012). The influence of yeasts was shown to shorten the lag phase duration of bacterial proliferation, while binding pathogenic bacteria to the yeast cell surface, limiting bacterial invasiveness and preventing bacterial adherence and translocation in human intestines (Rajkowska et al., 2012). This has also been demonstrated in other clinical trials, where pooling data from multiple trials and sources of information on acute childhood diarrhea have shown that *Saccharomyces boulardii* significantly reduced the duration of diarrhea by 19.7 hours, stool frequency on day 2 and 3, and the risk for diarrhea on days 3 and 4 after intervention, as compared to untreated control subjects (Feizizadeh et al., 2014). Other studies have shown that *Saccharomyces boulardii* has helped reduce or prevent antibiotic-associated diarrhea, travelers

diarrhea, enteral tube feeding diarrhea, acquired immune deficiency syndrome (AIDS) associated diarrhea, inflammatory bowl syndrome and disease, and recurrent diseases associated with *Clostridium difficile* (Zanello et al., 2009). Prebiotics such as non-digestible oligosaccharides and fructooligosaccharides have been shown to modulate human lipid metabolism via fermentation modes of action, stimulating the growth of endogenous *Bifidobacteria* such that after a short dosage period these become predominant in human feces (Gibson and Roberfroid, 1995). In general, probiotics have been shown to restore the integrity of the ‘‘protective’’ intestinal mucosa-related microbiota that reduce inflammatory barrier diseases of the lower gastrointestinal tract (Kühbacher et al., 2006). Some researchers suggest that probiotic bacteria may stimulate the intestinal innate defense mechanisms through the regulation of induction of antimicrobial peptides such as human beta-defensin-2 (hBD-2), enhancing mucosal barriers to harmful luminal bacteria (Wehkamp et al., 2004). This is supported by the fact that human toll-like receptor 2 meditates induction of the antimicrobial peptide hBD-2 in response to bacterial lipoproteins (Birchler et al., 2001).

Monogastric Animals

The effects of feeding *Saccharomyces cerevisiae* supplements and related compounds to monogastric animals have been studied, for example in horses, dogs, pigs, and fish. Learnings from other species can provide insight to broilers as many physiological characteristics are similarly conserved across species. The impact on these species is discussed below.

Healthy horses fed supplemental *Saccharomyces cerevisiae* metabolites in research did not realize any effect on total nutrient digestibility (Mackenthun et al., 2012). However, supplementation to horses was found to have beneficial effects on the equine fecal bacterial communities and blood stress markers. Stimulation of lactate-utilizing bacteria in the large

intestine was studied and may possibly be explained by the supply of growth factors from yeasts, as fecal concentrations of lactate-utilizing bacteria and cellulolytic bacteria were greater in supplemented horses than in control horses. Transportation for 2 hours disturbed the fecal bacterial ecosystem in horses, increasing the risk of triggering microbial dysbiosis on a longer term in the large intestine, and *Saccharomyces cerevisiae* supplementation may have helped reduce the negative impact of transportation on the equine fecal bacterial ecosystem (Faubladier et al., 2013).

In a study of horses fed a high-fiber or a high-starch diet, with or without *Saccharomyces cerevisiae* supplements, it was determined that the activity of most enzymes, such as polysaccharidase and glycoside hydrolase β-D-xylosidase, increased after the addition of *Saccharomyces cerevisiae* supplement (Jouany et al., 2009). Yeast cultures of *Saccharomyces cerevisiae* added to fecal samples collected from mature horses on a high fiber diet were found to decrease ammonia concentrations when the samples were incubated, and increased acetate production in fecal samples from horses exposed to a diet containing highly concentrated pellets (Lattimer et al., 2007). A *Saccharomyces cerevisiae* supplement had a significant effect on the microbial profile and fermentation pattern in the large intestine of horses fed a high-fiber and high-starch diet, such as modification of gut pH, concentrations of lactic acid and ammonia. When the digestion of starch by equine in the small intestine has been saturated, the effect of adding *Saccharomyces cerevisiae* in the diet has limited the extent of overall undesirable changes in the intestinal ecosystem of the horse (Medina et al., 2002). Another study has shown yeast products with added Selenium can increase the blood concentrations of this element vs. controls (Calamari et al., 2009).

The effects of live yeast *Saccharomyces cerevisiae* have been studied in dogs. When *Saccharomyces cerevisiae* strain CNCM I-4407 was administered to beagle dogs, treated beagles had greater weight gain, higher digestibility of neutral detergent fiber, and lower *Escherichia coli* and enterococci counts in feces (Stercova et al., 2016). This was consistent with other studies using spray-dried *Saccharomyces cerevisiae* in dog diets with similar reductions in *Escherchia coli* counts and increased nutrient digestibility (Middelbos et al., 2006, 2007).

In pigs, *Saccharomyces cerevisiae* cultures have immunologically enhanced of intestinal functions. Yeast products improve pig health by various mechanisms, including inhibition of pathogen adhesion to gastrointestinal epithelial tissues, stimulating immune cells, and adsorption of mycotoxins and inhibiting their actions (Kogan and Kocher, 2007). Pigs experimentally challenged with *Escherichia coli* K88 along with being fed yeast cultures of *Saccharomyces cerevisiae* had reduced quantities of C-reactive proteins in the blood and reduced toll-like receptors in the intestine (Badia et al., 2012). The inclusion of mannan ologosaccharides from yeast in pig diets has contributed to overall animal health and growth through multiple mechanisms, including decreasing pathogenic bacteria (Connolly, 2001). An oral administration of *Saccharomyces cerevisiae* was found to reduce the mortality in pigs associated with immune and cortisol responses to *Escherichia coli*. This may have occurred via suppression of acute responses to pathogenic challenges and, thereby, preventing the diversion of energy away from maintaining innate and adaptive immune responses, as well as liberating it for growth-related purposes. Also, the cumulative *Saccharomyces cerevisiae* induced immune-neuroendocrine response to the lipopolysaccharide (*Escherichia coli*) may have presumably functioned to facilitate short-term clearance of the pathogen, wheras prolonged regulation of the immune

response may have occurred due to heightened cytokine production at the cellular level (Collier et al., 2011).

A feed additive containing multiple components, including processed yeast cell walls as a protein source, improved average daily gain, body weights, feed intake, and innate immunity in pigs at 14 days post-weaning compared to controls (Gerritsen et al, 2012). Other studies showed only a marginal effect of yeast-based β-glucans on the immune response, being less effective than certain antibiotics (Hahn et al, 2006). Physiologically, pigs fed *Saccharomyces cerevisiae* have had a thicker colon wall on day 10 compared to those not fed yeast products, with lower ileal and colonic digesta ammonia concentrations on days 10 and 14, reduced diarrhea, and decreased *Escherichia coli* $K88^+$ attachment to the ileal mucosa (Kiarie et al., 2012).

In pig reproduction operations, the administration of *Saccharomyces cerevisiae boulardii* to sows prior to farrowing was found to modulate development of porcine mucosal immunity, reduce intestinal bacterial translocation to mesenteric lymph nodes after a *Eschericia coli* challenge, and increase IgA concentrations in ileal flushes collected from piglets on days 42 and 52 (Lessard et al., 2009). Dietary supplementation with live yeast *Saccharomyces cerevisiae* to sows and piglets in the late gestation, suckling, and postweaning periods have been useful in the reduction of the duration and severity of postweaning diarrhea (Trckova et al., 2014). This has been shown microscopically to occur due to inhibition of certain *Escherichia coli* from adhering to the brush border region of intestinal villi (Trevisi et al., 2015). In a study of weaning stress followed immediately by transport stress, yeast supplementation suppressed neutrophil and WBC counts that would typically increase after a stressor, wheras differences in *Salmonella* occurrences in the mesenteric lymph nodes and cecum were inconclusive (Weedman et al., 2011). *Saccharomyces cerevisiae* subspecies *boulardii* has favorably influenced the microbiota

of the colon of weaned pigs by enhancing the establishment of *Porphyromonadaceae* and *Ruminococcaceae* bacterial families, suggesting that this feed additive has the potential to modulate bacterial populations associated with gut health *(*Brousseau et al., 2015). Also, fermentation products of *Saccharomyces cerevisiae* have been shown to successfully administer recombinant intracellular epidermal growth factor in young pigs, ameliorating stress and preventing incomplete gastrointestinal development in early-weaned piglets (Wang et at., 2015). Inclusion of yeast cultures in the weanling pig diets has favorably impacted feed intake and performance (Mathew et al., 1998).

The immune systems of various species of fish have been stimulated by the use of selected probiotics such as *Saccharomyces cerevisiae, Vibrio fluviales, Aeromonas hydrophila, Lactobacillus rhamnosus, Bacillus subtilis*, and others (Gómez and Balcázar, 2007). Dietary *Saccharomyces cerevisiae* fermentation products have been reported to improve growth and survival of shrimp *Litopenaeus vannamaei* (Burgent et al., 2004) and rainbow trout *Oncorhynchus mykiss* (Barnes et al., 2006). Also, the yeast product has been shown to improve feed conversion and growth of hybrid tilapia, while also having a favorable impact on non-specific immunity and increased intestinal bacterial count and diversity as compared to the use of florfenicol antibiotics (He et al., 2011). It has also been observed that dietary *Saccharomyces cerevisiae* selectively caused an increase in various bacteria in fish, including uncultured *Mycobacterium*-like species, *Cetobacterium*-like species, and uncultured *Flavobacteria*-like species, while decreasing *Escherichia coli*-like bacteria, uncultured bacilli-like bacteria, and *Pseudomonas fluorescens*-like bacteria (He et al, 2009). Fermented *Saccharomyces cerevisiae* admininstered as a feed supplement to farm-raised rainbow trout resulted in increased feed intake, improved feed conversion, increases in trypsin and amylase activity, and a higher density

of goblet cells per villus in the proximal intestine (Heidarieh et al., 2013). Dietary *Saccharomyces cerevisiae* has boosted the growth and immunity of juvenile Indian Major Carp in aquaculture (Bandyopadhyay et al., 2015).

Ruminants

In dairy cattle, manipulating rumen ecology to promote lactate-uptaking microorganisms, such as *Selenomonas ruminantium* and *Megasphaera elsdenii,* has been long reported to reduce sub-acute ruminal acidosis (Owens et al., 1998). Yeast cultures of *Saccharomyces cerevisiae* have been reported to stimulate lactate utilization by these organisms (Callaway and Martin, 1997), and could contribute to increased rumen pH (Chaucheyras-Durand et al., 2012). *Saccharomyces cerevisiae* supplementation to dairy cattle has also increased milk production (De Ondarza et al., 2010; Desnoyers et al., 2009). *Saccharomyces cerevisiae* live cells have stimulated ruminal microorganism fermentation *in vitro*, with a linear increase of *in vitro* dry matter disappearance, decreased lactate, and a small decrease of methane and hydrogen with hay plus a concentrated feed (Lila et al., 2004). In China, the addition of *Saccharomyces cerevisiae* to rice straw, corn stover, and corn silage was shown to improve rumen fermentation by stimulating the number of fiber-digesting rumen microbes (Mao et al., 2013).

Holstein cattle have been studied extensively in respect to the effects of *Saccharomyces cerevisiae* on digestion. Holstein steers received abomasal pulse doses of *Saccharomyces cerevisiae* var. *boulardii* with a veterinary stomach pump. They were given doses of 0 (control) or 10 grams per day of this live yeast derivative according to a crossover design with 18 day periods. Also, abomasal infusions of 4 pulse doses of 0.25 g/kg of body weight oligofructose were administered every 6 hours on day 16 of each period. During the baseline period prior to the oligofructose challenge, there were no effects of *Saccharomyces cerevisiae* var. *boulardii* on

fecal measures except for an increase in neutral detergent fiber digestibility, suggesting that the yeast product increased intestinal fiber fermentation. During the oligofructose challenge, *Saccharomyces cerevisiae* var. *boulardii* increased the fecal consistency score (1 = watery to 5 = solid: Hulsen, 2006), and tended to reduce fecal short-chain fatty acids. This research suggested that *Saccharomyces cerevisiae* var. *boulardii* abomasal treatments stabilized the intestinal environment during increased carbohydrate fermentation (Gressley et al., 2016).

Holstein heifers fed yeast cultures of *Saccharomyces cerevisiae* were studied to determine the effects of this supplement on rumen microbial fermentation under a dietary challenge with 10:90 forage-to-concentrate ratio to induce digestive upsets. While the addition of yeast cultures during the dietary challenge did not affect the incidence or time to digestive upset, the study found the yeast culture reduced the foam strength in the rumen on the day after a digestive upset, suggesting potential benefits of reducing the risk of developing bloat by using this supplement in highly concentrated diets (Moya et al., 2009). Holstein cows fed diets supplemented with *Saccharomyces cerevisiae* for 23 days prepartum and 56 days postpartum were found to have modest postpartum improvement in body condition scores and milk production (Robinson and Garrett, 1999).

In beef cattle, *Saccharomyces cerevisiae* has been successfully used for many purposes. While the use of *Saccharomyces cerevisiae* in conjunction with *Enterococcus faecium* in feedlot cattle diets has been shown to have little effect on reducing ruminal acidosis or improving nutrient utilization (Beauchemin et al., 2003), feeding this combination to feedlot steers under a high-grain diet had an effect on the animals' immunoregulatory system, as measured by an increase of acute phase proteins in the blood plasma, including an increase in serum amyloid A, lipopolysaccharide-binding protein, and haptoglobin (Emmanuel et al., 2007). Although the

impact on the host animals was not conclusive, this finding was important because it has been reported that during conditions of inflammation, tissue injury, and infection, these acute phase proteins are released in the liver (Suffredini et al., 1999).

Beef cattle heifers raised in Europe with a diet including *Saccharomyces cerevisiae* did not realize any significant impact on growth performance, carcass quality, ruminal fermentation products, and blood metabolites from this supplement (Carrasco et al., 2016). However, other studies have shown that *Saccharomyces cerevisiae* increased feed intake and decreased the gross energy lost as methane in cattle. This phenomenon has significant interest to some countries interested in understanding the potential use of this organism as a tool to decrease the contribution of this methane to greenhouse gasses (McGinn et al., 2004).

The study of biochemical structures in *Saccharomyces cerevisiae* has been used as a model to research the genetic expression of sirtuins in beef cattle, which are involved in regulation of glucose and lipid metabolism (Ghinis-Hozumi et al., 2011). Male crossbred beef cattle fed supplementary amounts of selenium through selenium-enriched *Saccharomyces cerevisiae* yeast were found to have greater activity of glutathione peroxidase in whole blood and longissimus muscle (Juniper et al., 2008b), an important enzyme which catalyzes the reduction of lipid and hydrogen peroxides to less harmful hydroxides through the oxidation-reduction pathway, leading to selenocysteine (Arteel and Sies, 2001).

Yeast derived from a specific strain of *Saccharomyces cerevisiae*, CNCM I-3060, was used to feed selenium enrichments to sheep at 10 times the European Union maximum, or approximately 20 times the United States Food and Drug Administration permitted concentration. No adverse effects were observed in animal health, performance, and voluntary feed intake (Juniper et al., 2008a). Other studies of sheep feed supplements using

Saccharomyces cerevisiae boulardii have demonstrated no improvement in growth performance, possibly due to the fact that the supplement is utilized as a substrate in the rumen and digested as a prebiotic, rather than having an effect as a probiotic (Zerby et al., 2011).

Chickens and Other Avian Species

In this section, the impact of *Saccharomyces cerevisiae* and other yeast-based additives will be reviewed in respect to their impact on broilers, turkeys, and geese. In broilers, yeast culture products have provided multiple benefits, ultimately having a favorable impact on live bird growth performance through improved intestinal morphology, while stimulating increased immunomodulatory functions, such as increased concentration of serum lysozyme that breaks down the polysaccharide walls of many types of bacteria (Gao et al., 2008). The impacts on broilers are summarized below in respect to improved intestinal morphology, reduced pathogenic bacteria, improved broiler performance, and improved meat and egg characteristics.

Improved Intestinal Morphology

In morphometric studies of villus length, an increase has been shown in the ileum for larger-sized birds with diets including *Saccharomyces cerevisiae* (Baurhoo et al., 2007; Chichlowski et al., 2007; Morales-López et al., 2009; Solis de los Santos et al., 2005). Similarly, the efficacy of using a *Saccharomyces cerevisiae* cell wall product derived from the brewery industry had a beneficial influence on broiler performance and intestinal mucosa development, in both litter-floor pen trials as well as field tests (Santin et al., 2001). Researchers have considered that villus development is not a direct effect of yeast-based β-galactomannans on mucosal development, but rather it is an indirect effect exerted by an increase in the population of *Lactobacillus* and *Bifidobacterium* (Baurhoo et al., 2007; Solis de los Santos et al., 2005). In a study comparing the effects of a blend of supplements to chicken diets, which included organic

acids, *Saccharomyces cerevisiae*, and various probiotics, researchers found a difference in the intestinal structure with improved duodenal villus height based on histomorphological assessment (Rodríquez-Lecompte et al., 2012). Birds fed yeast cell wall extract and challenged with necrotic enteritis exhibited increased villus height, decreased crypt depth, and increased villus:crypt ratio when challenged, and did not realize a performance decline from necrotic enteritis (M'Sadeq et al., 2015).

Reduction of Pathogenic Bacteria

In vitro studies have shown that yeast fermentation metabolites reduced *Salmonella Typhimurium* in an anaerobic mixed culture derived from chicken ceca mixed with ground chicken feed (Rubinelli et al., 2016). Birds fed a diet supplemented with *Saccharomyces cerevisiae* and challenged with *Salmonella enteritidis* demonstrated decreased prevalence of the pathogen in the ceca, cloaca, and carcass skin as compared to control diets, while improving feed intake in *Salmonella* challenged birds (Mountzouris et al., 2015).

Studies have shown that chickens fed diets containing β – galactomannans, such as *Saccharomyces cerevisiae*, have realized reduced bacteria through various mechanisms. One mechanism is an increase in the presence of goblet cells, which stimulate the formation of a mucous blanket in the intestine with a large surface area for *Salmonella* adhesion (Baurhoo et al., 2007; Chee et al., 2010; Chichlowski et al., 2007; Leforestier et al., 2009). Another benefit to chickens fed β – galactomannans is the production of a physical protective barrier for the epithelium of the ileum and in the cecal tonsil, causing a reduction of bacteria attached to the epithelium by blocking access to these tissues (Brufau et al., 2015). Scanning images and transmission electron microscopy have revealed a high bacterial load in the mucous blanket, both in the ileum and in the cecal tonsil, and a reduction of bacteria attached to the epithelium in

animals fed β-galactomannans in comparison with animals not fed these treatments (Brufau et al., 2015). This bacterial adherence to the mucus layer has been described as occurring through either lectins or low-affinity bonds (Barnett et al., 2012). *Saccharomyces*-based products in various forms have significantly reduced *Salmonella*, *Campylobacter*, and other pathogens in the broiler cecum or intestinal tract (Fanelli et al., 2015; Guyard-Nicodème et al., 2016; Lensing et al., 2012; Line et al., 1998; Roto et al., 2017). Also, broilers supplemented with a yeast autolysate derived from *Saccharomyces cerevisiae* realized decreased *Escherichia coli* counts in digesta, increased serum antibody titers, and decreased pH of jejunal and ileal digesta compared to birds fed a control diet (Yalçin et al., 2013).

In other studies, *Saccharomyces cerevisiae*-related compounds have been found to reduce pathogens. In a study of competitive exclusion products, a *Saccharomyces cerevisiae* culture, and a *Pediococcus acidilactici* culture, all treatments reduced *Salmonella* percentages in the litter, ceca, and carcass in both summer and winter tests (Al-Zenki et al., 2009). Dietary supplementation with 0.5 g / kg of *Saccharomyces cerevisiae* live yeast in broilers has resulted in alleviation of lipopolysaccharide-induced inflammation, including increased blood serum α-acid glycoprotein, lower serum nitric oxide content, and lower interleukin in the spleen (Wang et al., 2016). Also, poultry exposed to *Saccharomyces cerevisiae*-derived 1,3 *β*-glucan and/or *Escherichia coli*-derived lipopolysaccharide demonstrated immune modulations of airborne pathogen-associated molecular patterns, although they were age and breed dependent (Parmentier et al., 2006). In a trial with broiler chicks in which 3-day-old chicks were orally challenged with 10^4 colony forming units of *Salmonella typhimurium* 29E, birds that received a *Saccharomyces cerevisiae* yeast cell wall preparation had a 1 log reduction of *Salmonella*

typhimurium 29E in the ceca at day 10, and other chicks challenged with *Salmonella dublin* had 34% reduction of that organism in ceca samples at day 10 (Spring et al., 2000).

In a comparison of bacterial interventions in broilers, a monoglyceride mixture, 5 compounds made from various short-chain fatty acids, 2 compounds derived from plant extracts, 3 probiotics, and 1 prebiotic-like compound were studied. The research resulted in the prebiotic-like compound based on *Saccharomyces cerevisiae* yeast to have the highest impact on reduction of *Campylobacter* colonies, at over 3 $\log_{10}$, colonies at 42 days of age (Guyard-Nicodème et al., 2016). This may be an especially effective food safety intervention, because according to some researchers, reducing *Campylobacter* colonization in cecal contents by 2 to 3 $\log_{10}$ would be estimated to reduce human campylobacteriosis cases attributable to broiler meat by 76 to 90% (Romero-Barrios et al., 2013).

Improved Live Bird Performance

Saccharomyces cerevisiae supplements have caused improved breeder, broiler, and layer performance, to various degrees. The impact of this product has effects on a wide number of performance measures, supporting research that has suggested a mature and stable microbiome is an effector of improved growth performance, pathogen control, reduced mortality, and overall health of the host (Patterson, 2012). Inclusion of yeast-based products appears to allow for the entire microbiome of the ceca to reach its stable level at an earlier age in broiler chickens than those in normal diets (Park et al., 2017).

Studies of breeder and layer hen performance have shown positive effects of feeding supplemental *Saccharomyces cerevisiae* products. Breeder hens fed a *Saccharomyces* fermentation product exhibited improvements in hatch-of-fertile eggs, with a reduction in egg contamination from hens at 32 weeks. In addition, the progeny from 39-week-old hens that were

fed *Saccharomyces cerevisiae* fermentation product had improved overall feed conversion and breast meat yield compared to control-fed birds (Kidd et al., 2013). Laying hens fed basal diets supplemented with *Saccharomyces cerevisiae* yeast cultures realized increased body weight, egg weight, and serum uric acid and reduced cholesterol, without affecting other important factors such as hen-day egg production, interior/exterior egg quality, and serum concentrations of protein, triglycerides, and selected alanine amino transferase and aspartate amino transferase enzymes (Yalçin et al., 2008). When challenged with coccidiosis, laying hens and broilers fed a *Saccharomyces cerevisiae* fermentation product had reduced gastrointestinal damage as measured by the occurrence and severity of lesions, increased body weight, and improved feed conversion compared to control birds (Lensing et al., 2012; McIntyre et al., 2013).

In another study, a combination of feed supplements including *Saccharomyces cerevisiae* used concurrently with *Lactobacillus fermentum* at multiple doses were compared to a basal diet and a basal diet with the antibiotic chlortetracycline. In this research, average daily gain and feed efficiency were improved in the broilers fed the probiotic diet during days 1 to 21, exhibiting performance similar to the group fed the antibiotic; concurrently, the probiotic-supplemented diets increased the intestinal mRNA expression of certain toll-like receptors at various ages, suggesting stimulation of the immune system (Bai et al., 2013). A mixture of *Saccharomyces cerevisiae* and *Bacillus subtilis* var. *natto* applied to broiler feed to obtain two-stage fermentation resulted in improved broiler growth performance and feed intake in 21- and 39-day-old chickens (Chen et al., 2009). A combination of *Lactobacillus plantarum*, *Enterococcus faecium*, *Bacilllus subtilis*, and *Saccharomyces cerevisiae* were used to produce a fermented water plaintain (Alisma canaliculatum) compound that exhibited high tolerance to acid, bile, and heat in the broiler gastrointestinal tract, benefitting growth, oxidative stability, and

the composition of broiler fatty acids and meat tissues (Hossain et al., 2012). Also, broilers feed a combination of direct-fed antimicrobials containing *Lactobacillus reuteri, Bacillus subtilis,* and *Saccharomyces cerevisiae* realized improvements in multiple parameters, including body weight gain, feed conversion during ages 0-7 days, greater numbers of white blood cells, and increased plasma IgA, IgG, and IgM concentrations to counteract infections (Salim et al., 2013).

Chickens challenged with aflatoxin have responded to diets containing *Saccharomyces cerevisiae* by displaying greater feed intake, body weight gain, and concentrations of albumin, total protein, and globulin when compared to controls, along with reduced severity of histological lesion scores in the liver and kidney (Bovo et al., 2015). In different studies, breeder hens fed a *Saccharomyces cerevisiae* supplement displayed a significant reduction in toxic effects of aflatoxin on pancreatic lipase and chymotrypsin (Matur et al., 2010), and improvements to the immune system by increasing lymphocytes and phagocytic activity with oxidative bursts of heterophiles (Matur et al., 2011). In addition, broilers treated with *Saccharomyces cerevisiae* during an aflatoxin challenge realized protection against liver lesions and favorable body weight gain (Osweiler et al., 2010). The addition of *Saccharomyces cerevisiae* to broiler drinking water prevented the negative effects of aflatoxin in feed on the relative weight of the liver, histopathology, biochemical parameters, and growth performance, suggesting this yeast derivative may be used as an effective deterrent to mycotoxins (Pizzolitto et al., 2013).

Some *Saccharomyces cerevisiae* fermentation products have improved immune functions and growth performance in the presence of other challenges such as coccidia and Newcastle diseases. Results of Gao et al. (2009) suggested by feeding the yeast metabolites to broilers subjected to *Eimeria tenella* the metabolites could be a replacement for other treatments such as

ionophores used to reduce the impact of coccidiosis in broiler flocks. Some researchers have studied the impact of feeding a *Saccharomyces cerevisiae* yeast extract and prebiotic in the pre-starter phase only, but this demonstrated a minimal effect on the humoral immune response to Newcastle disease virus and infectious bursal disease throughout the production cycle of the broilers (Silva et al., 2009).

Improved Meat and Egg Quality

The impact of *Saccharomyces cerevisiae* on poultry meat and egg characteristics has also been studied. In a comparison on broilers fed a treatment of either whole yeast, *Saccharomyces cerevisiae* yeast extract or *Saccharomyces cerevisiae* yeast cell wall, meat tenderness of raw and boiled samples, as measured by a mechanical shear test, was improved in selected samples from birds fed the whole yeast or yeast extract supplement. Also, the yeast extract and yeast cell wall treatments resulted in lower 2-thiobarbituric acid reactive substance (TBARS) values of breast meat stored for 10 days, suggesting antioxidant effects on the meat derived from broilers raised under those treatments (Zhang et al., 2005). In addition, layers administered this feed supplement have demonstrated decreased blood serum concentrations of triglycerides and cholesterol, resulting in decreased concentrations of egg yolk cholesterol (Yalçin et al., 2010).

Feeding the *Saccharomyces cerevisiae* metabolite product XPC to turkeys has ameliorated the reduction in appetite and growth rate following a live coccidiosis vaccination. Feeding this product at 1.25 g/kg increased body weight of turkey hens, whether or not they were vaccinated (Paiva et al., 2010). Male turkeys fed XPC exhibited increased feed conversion and breast meat yield (Firman et al., 2013). In geese, a commercial fermented liquid feed containing supplementary *Saccharomyces cerevisiae* and *Bacillus subtilis* var. *natto* was found to improve bird growth, feed intake, and the antioxidative status of organs and breast muscle, while

decreasing blood cholesterol. Also, this feed additive mixture improved the indigenous microflora of the geese, due to the fact that the fermented feed contained increased lactic acid and had a low pH, modulating the bacterial ecology of the intestine (Chen, et al., 2013).

Summary: Potential Benefits to Considering Alternative Pathogen Interventions Using Saccharomyces cerevisiae in Poultry Production.

There are many potential benefits to considering the alternative pathogen intervention of using *Saccharomyces cerevisiae* as a prebiotic or probiotic in the production of poultry and livestock. This organism has been shown to reduce many enteric pathogens in the gastro-intestinal tract by various modes, such as by providing mannose binding sites for bacteria agglutination, enhancing the immune function with the presence of β-glucans in the gastro-intestinal tract, and improving the mucin profile in the gut mucosa due to increases in the quantity of goblet cells. This trait of *Saccharomyces cerevisiae* has the benefit of reducing the dependence on antibiotics in poultry and livestock production, thereby reducing the likelihood of antibiotic resistance. Also, these traits of *Saccharomyces cerevisiae* provide the added benefit of strengthening and extending the intestinal villi brush border, leading to improved nutrient uptake and feed efficiency, which can lead to a more economically viable animal production system, especially in the absence of antibiotics. Although the use of *Saccharomyces cerevisiae* alone, or in combination with other compounds, can be an effective strategy to improve broiler health and reduce pathogens, some studies have indicated that the impacts of such interventions on broiler growth and the presence of pathogenic bacteria are not as evident in flocks without disease challenges and other stressors (Hahn-Didde and Purdum, 2016).

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CHAPTER III

The Effects of Feeding Chickens a Supplement with the Dried Metabolites of *Saccharomyces cerevisiae* on Broiler Performance, Carcass Yields, and Component Parts Percentages

The Effects of Feeding Chickens a Supplement with the Dried Metabolites of *Saccharomyces cerevisiae* on Broiler Performance, Carcass Yields, and Component Parts Percentages

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ABSTRACT

Broiler production companies are continuously searching for effective feed ingredient alternatives to optimize bird health, growth performance and carcass characteristics. To achieve those goals, many feed additives have been developed to improve intestinal morphology and gut bacterial profiles to maximize feed utilization and withstand disease challenges that can cause decreased bird performance. In this study, broiler chicks obtained from a commercial hatchery were raised in research pens to study the impact on live bird performance of administering a feed supplement with *Saccharomyces cerevisiae* fermentation metabolites derived from dried yeast. No bacterial challenges were administered to either the control, non-treated flocks, nor to the birds administered the supplement containing *Saccharomyces cerevisiae* metabolites. A comparison of control and treated flocks was conducted based on measurements of live bird growth rate, feed conversion, and carcass yields of component parts including breast meat, tenderloins, wings, and leg meat. The broilers treated with the *Saccharomyces cerevisiae* metabolite exhibited similar live performance metrics and carcass component yields compared to non-treated birds. In this study, the impact of feeding *Saccharomyces cerevisiae* metabolites to broilers on bird performance and carcasses component yields was studied under research pen environmental conditions in the absence of typical broiler disease challenges. In light of other research demonstrating improved growth performance of birds feed *Saccharomyces cerevisiae* metabolites in commercial settings and/or with disease challenges, results of this study suggested that the impact of this supplement on bird performance could be more noticeable in commercial applications than in an isolated and controlled research setting.

Key Words: *Saccharomyces cerevisiae*, feed supplement, broiler

INTRODUCTION

Poultry producers and marketers continuously seek technologies to improve bird performance, maximize carcass yields for economic reasons, and to meet customer expectations. Some of the key measurements of broiler performance for commercial companies are growth rates, feed efficiency, and component yields, which all have a significant impact on production costs that ultimately affect chicken meat prices. In addition, albeit not discussed in this research study, the costs of utilizing feed supplements to maintain bird health and reduce pathogenic bacteria in broilers is an area of emphasis for food safety considerations. Therefore, the impact of feed ingredients and supplements on these metrics is an area of increasing research.

Feed supplements containing *Saccharomyces cerevisiae* fermentation metabolites and related compounds have been studied in multiple applications. Improved feed efficiency, weight gain, and feed consumption at 3 weeks of age has been observed in male broilers fed a dietary supplement of baker's yeast (Shareef and Al-Dabbagh, 2009). Other research has demonstrated the effects of using combinations of feed supplements in conjunction with *Saccharomyces cerevisiae*. For example, in a study comparing the effects of a blend of supplements in chicken diets, which included organic acids, *Saccharomyces cerevisiae,* and various probiotics, researchers found a difference in the intestinal structure with improved duodenal villus height (Rodríguez-Lecompte et al., 2012). A mixture of *Saccharomyces cerevisiae* and *Bacillus subtilis* var. *natto* supplemented in broiler feed to obtain 2-stage fermentation resulted in improved broiler growth performance and feed intake in 21- and 39-day-old chickens (Chen et al., 2009). Also, broilers administered a combination of direct-fed antimicrobials including *Saccharomyces cerevisiae* had improved body weight gain and feed conversion during the first week of age, with greater numbers of white blood cells and increased plasma antibodies and

immunoglobulins to counteract infections (Salim et al., 2013). In turkeys, feeding a *Saccharomyces cerevisiae* fermentation product has ameliorated the typical reduction in appetite and growth rate following a live coccidiosis vaccination (Paiva et al., 2010), as well as increased feed conversion and breast meat yield (Firman et al., 2013).

The use of feed supplements containing yeast-based compounds, such as live yeast, killed dried yeast, and *Saccharomyces cerevisiae* fermentation metabolites, have benefitted the performance of other animals. The metabolites of *Saccharomyces cerevisiae* have been found to increase feed intake and decrease the gross energy lost in beef cattle (McGinn et al., 2004), and increase milk production in dairy cattle (Desnoyers et al., 2009; De Ondarza et al., 2010). Similar feed additives have contributed to overall animal health and growth in pigs (Connolly, 2001). Farm-raised rainbow trout and juvenile carp raised in aquaculture have also realized benefits from *Saccharomyces cerevisiae* metabolites, such as improved feed intake, feed conversion, intestinal villus structure (Heidarieh et al., 2013), and immunity (Bandyopadhyay et al, 2015). Other benefits of using *Saccharomyces cerevisiae* in various species are consistent with the general advantages of administering beneficial bacteria to animals, such as reduced pathogen contamination (Ghareeb et al., 2012), stimulation of the immune system (Gibson and Roberfroid, 1995; Netherwood et al., 1999) and enhanced epithelial innate immunity-related gene expression through reduced inflammation (Amit-Romach et al., 2010; Pagnini et al., 2010).

The purpose of this experiment was to determine the effectiveness of feeding a supplement containing the dried metabolites of *Saccharomyces cerevisiae* to broilers on live bird performance and carcass characteristics. Broiler chickens were raised in a controlled environment in research pens. Control birds were fed a normal diet used in commercial broiler operations, and treated birds were fed the same diet with an added proprietary feed supplement

containing *Saccharomyces cerevisiae* fermentation metabolites, under an FDA-approved usage rate per normal commercial practices. Comparisons were made of growth rate, feed conversion, carcass weights, and component yields. This research was conducted to understand the impact of administering *Saccharomyces cerevisiae* metabolite feed additive on the quantity of marketable chicken carcasses and products, and the live production performance associated with the production of those products.

MATERIALS AND METHODS

Experimental Design and Treatments

A total of 384 one-day-old broiler chicks were randomly allotted to either a control diet (CON) consisting of feed used in commercial operations for broiler production or the same basal diet supplemented with of 1.25 lb / ton (0.62 kg / metric ton) of a proprietary feed supplement containing *Saccharomyces cerevisiae* fermentation metabolites (SCM). Each treatment consisted of 192 broilers, divided into 8 replicate pens of 24 birds each. At one day of age, chicks were evernly divided into each of 16 experimental pens in a controlled research barn setting. The assignment of replicate groups to one of the 16 pens was completed in a completely randomized block design. Environmental conditions for flock density, lighting, and temperature were held constant across the pens, per industry standards consistent with the commercial broiler production industry. Feeds were pelleted prior to administration. Birds were allowed ad libitum access to feed and water throughout the life of the flocks, until feed was withdrawn prior to processing. The birds were then processed in a pilot processing plant using normal industry practices for harvest and processing. A total of 372 birds were captured for processing and yield analysis, including 180 CON and 192 SCM broilers. The research protocols for this experiment were reviewed and approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC).

Measurements of Broiler Performance, Carcass Yields, and Component Parts Percentages

For live production measures of feed conversion and growth, each floor pen of broilers was the experimental unit. At 0, 15, 29, and 47days of age, total broiler weight was measured on a pen-basis to calculate rate of gain, whereas the weights of feed administered and consumed were recorded at each weight age to calculate the feed conversion efficiency. Mortality weights and bird counts were collected throughout the life of the flock to adjust feed conversion by adding mortality weights to the weights of live birds at each stage of data collection.

Individual broilers were also evaluated as individual experimental units for live weights, carcass yields, and component parts percentages. At 48 days of age, birds were collected from the research floor pens, weighed, euthanized, and processed at a pilot processing facility. The processing plant used equipment typical of commercial industry operations, including in-line poultry slaughter equipment, carcass scalding, feather removal, chilling, manual processing and conventional hand deboning of carcasses for component yields. Prior to processing, each individual broiler was weighed on a stationary scale, and then identified with a numbered tag which included a bar code to maintain identity throughout poultry slaughter, chilling, and separation and weighing of component parts. All internal viscera were removed and were not included in the analysis. Data collected after birds were processed included the weights of the carcasses, wings, boneless pectoralis major breast muscles, tenderloins, boneless leg meat portions, pelvic back portions, breast skin tissues, leg meat skin, leg bones, adipose tissue from the abdominal pelvic cavity, and the anterior skeletal frame consisting of the backbone, rib bones, and sternum bone and cartilage material.

Statistical Analysis

Feed conversion for each experimental floor pen unit was determined as the feed consumed ÷ weight of broilers in the pen, adjusted for mortality. For measurements of carcass

yields and component parts percentages, the individual bird and its associated parts percentages were the experimental units. For yield comparisons, the carcass weight from each broiler, and all of the associated component parts, were calculated as a percent of the live weight prior to slaughter. In addition, each component part was calculated as a percent of its associated carcass weight, and evaluated as the component yield for each carcass. The comparative analysis was completed with statistical software (SAS Institute, 2016), with treatments compared using the Oneway Analysis of Variance and Student's t Test, and tests for equal variances were conducted using the Brown-Forsythe analysis.

RESULTS AND DISCUSSION

Broilers and other animals have shown favorable responses in some trials to feed supplements containing *Saccharomyces cerevisiae* fermentation metabolites or other yeast-based organisms. In general, however, many trials have shown that while favorable outcomes have been evident from feeding *Saccharomyces cerevisiae* in reducing intestinal bacteria and improving gut physiology, greater benefits have been demonstrated when challenges, such as diseases, stressors, or abnormalities in feed ingredients, are introduced during the trial. In the present study, no challenges were presented to the broilers, as they were grown in research floor pens and harvested in a controlled environment. Therefore, this may explain why there were few differences noted in performance measures that are considered highly relevant in the commercial chicken industry, such as growth rate, feed conversion, and carcass yields.

The live broiler performance results are reported in Table 1. Overall, the mean growth rates and final bird weights at harvest did not differ ($P = 0.05$) between the CON and SCM birds. Also, feed conversion efficiency was not different between CON and SCM broilers, with overall feed:gain of 1.72 and 1.71 for CON and SCM birds, respectfully. It is important to note that the

environmental conditions were optimal in the research pens, and in a commercial operation the differences in these results may be more apparent.

With the exception that tenderloin weights were greater ($P \leq 0.05$) in CON-fed than SCM-fed birds, carcass component weights did not differ between the CON and SCM broilers (Table 2). Although the tenderloins weights may have been slightly different, favoring the CON broilers, this difference was not considered commercially significant given the small percentage of the total yield attributable to this portion. Moreover, neither carcass yields (expressed as a percent of live weight; Figure 1) nor yields of major portions (Figure 2) and minor portions (Figures 3 and 4) differed ($P>0.05$) between the CON and SCM broilers. It is not surprising that carcass and component yields were not different between CON and SCM birds, especially considering that neither growth rate nor feed:gain differed between the dietary treatments. The fact that all broilers were in a tightly-controlled environment, free of commercial stressors or disease challenges, could have been another major reason for the lack of differences observed in carcass yields and component parts percentages.

While the use of *Saccharomyces cerevisiae* alone, or in combination with other compounds, can be an effective strategy to improve broiler health and reduce pathogens, the results of this research were consistent with other studies which indicated that the impact of some prebiotics and probiotics on broiler growth was not as evident in flocks without disease challenges and other stressors (Hahn-Didde and Purdum, 2016). Furthermore, the use of *Saccharomyces cerevisiae* in conjunction with *Enterococcus faecium* in feedlot cattle diets had little effect on nutrient utilization and growth performance (Beauchemin et al, 2003), wheras feed supplements of *Saccharomyces cerevisiae* var. *boulardii* failed to alter growth performance of sheep (Zerby et al., 2011).

Results of this research differ from other studies which have indicated performance advantages when feeding supplements containing *Saccharomyces cerevisiae* components. Feed supplements including *Saccharomyces cerevisiae* used concurrently with *Lactobacillus fermentum* at multiple doses showed improved average daily gain and feed conversion efficiency in broilers during the first 21 days (Bai et al., 2013). In that research, diets supplemented with *Saccharomyces cerevisiae* and other beneficial bacteria increased the intestinal mRNA expression of certain toll-like receptors at various ages, indicating stimulation of the immune system. Similar yeast culture products used as supplements in broiler diets have shown a favorable impact on live bird growth performance through improved intestinal morphology, stimulation of immunomodulatory functions, and increased concentration of serum lysozyme that breaks down the polysaccharide walls of many bacteria (Gao et al., 2008). Also, broilers fed yeast-based supplements have exhibited increased blood protein and glucose, with corresponding increases in body weight gain at 3 weeks of age (Shareef and Al-Dabbagh, 2009). In a similar analysis, a combination of *Lactobacillus plantarum*, *Enterococcus faecium*, *Bacilllus subtilis*, and *Saccharomyces cerevisiae* produced a fermented water plantain (*Alisma canaliculatum*) compound that exhibited high tolerance to acid, bile, and heat in the broiler gastrointestinal tract, benefitting growth, oxidative stability, and the composition of broiler fatty acids and meat tissues (Hossain et al., 2012).

The benefits of supplementing broiler diets with *Saccharomyces cerevisiae* have been shown to be most evident when birds were challenged than when confined to a controlled environment. For example, dietary *Saccharomyces cerevisiae* and other yeast-based products have been shown repeatedly to suppress the effects of aflatoxicosis (Stanley et al., 1993; Pizzolitto et al., 2013; Osweiler et al., 2010), which is a major challenge in the commercial

chicken feed and production industry. *Saccharomyces cerevisiae* and other compounds derived from yeast have also benefited broilers by alleviating lipopolysaccharide-induced inflammation (Wang et al., 2016). Improved performance in broilers due to *Saccharomyces cerevisiae* has also been realized by stimulation of the immune system (Bai et al., 2013; Yalçin et al., 2013). Feed uptake and intestinal strength against invasive organisms when feeding various compounds derived from *Saccharomyces cerevisiae* may be largely due to improvements in gut morphology (Baurhoo et al., 2007; Morales-López et al., 2009; M'Sadeq et al., 2015; Solis de los Santos et al., 2005). Other research has shown that feeds supplemented with yeast compounds have caused reinforcement of the host's natural defenses by increasing pathogen colonization resistance and stimulating the immune response (Line et al., 1998). Thus, in light of previous research, the results of this experiment suggest that although these *Saccharomyces cerevisiae* metabolites did not appear to provide any significant improvements in performance or yields, the practical benefits of the supplement may not have been observed due to the absence of any significant challenges from environmental conditions, pathogens or other diseases.

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TABLES AND FIGURES

Table 1
Broiler Performance of Birds Fed Control Diets (CON)
vs. CON Diets plus *Saccharomyces cerevisiae* Metabolites (SCM)

Broiler Live Performance Measurements	Treatment Means (kg) CON±SEM	SCM± SEM	*P*-value
Feed Conversion [1]	1.724 ± 0.0214	1.714 ± 0.0106	0.679
Growth Rate per Day [2]			
Day 0 - 15	0.028 ± 0.0005	0.028 ± 0.0005	0.690
Day 0 - 29	0.051 ± 0.0009	0.050 ± 0.0007	0.865
Day 0 - 47	0.065 ± 0.0013	0.066 ± 0.0006	0.730
Live Wt. (kg) in pens Day 47	3.025 ± 0.0591	3.044 ± 0.0352	0.788

[1] feed conversion = feed:gain (kg)

[2] growth rate = gain (kg) / age (d)

Table 2
Carcass and Parts Weights of Broilers Fed Control Diets (CON) vs. CON Diets plus *Saccharomyces cerevisiae* Metabolites (SCM)

Mean Weights (kg)

Component	CON Mean (kg)	CON SEM	SCM Mean (kg)	SCM SEM	*P*-value
Carcass	2.392	0.0247	2.379	0.0238	0.700
Boneless Breast	0.761	0.0094	0.753	0.0089	0.532
Leg Meat	0.464	0.0061	0.468	0.0060	0.580
Wings	0.227	0.0022	0.227	0.0022	0.900
Tenderloins	0.137	0.0013	0.132	0.0014	0.004
Frame	0.263	0.0032	0.263	0.0030	0.984
Back	0.232	0.0034	0.229	0.0031	0.591
Leg Bones	0.135	0.0018	0.131	0.0016	0.138
Breast Skin	0.077	0.0012	0.079	0.0012	0.286
Leg Skin	0.064	0.0009	0.064	0.0011	0.968
Adipose	0.046	0.0009	0.046	0.0010	0.952

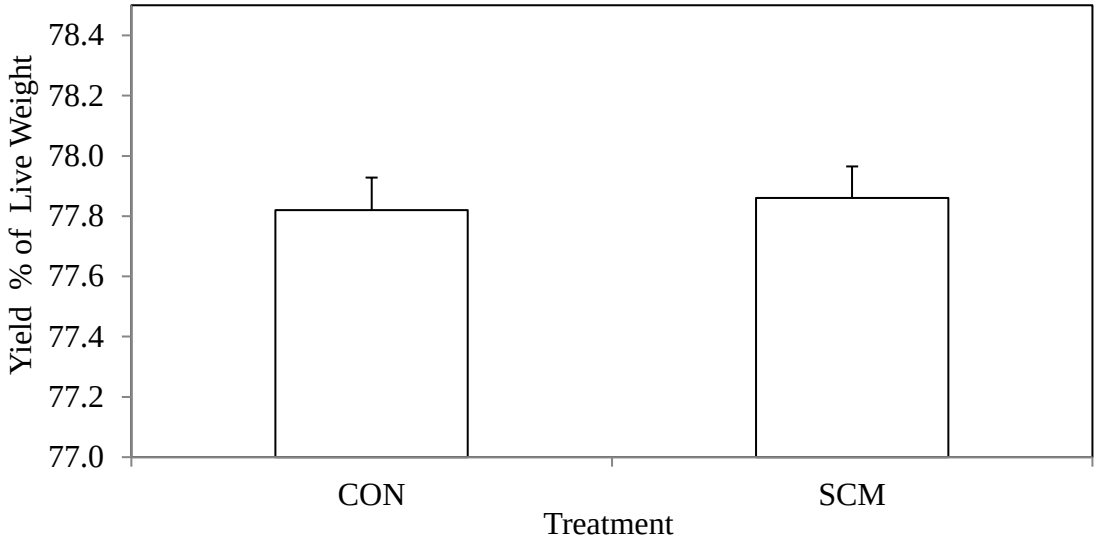

Figure 1. Broiler Carcass Yields as Percent of Live Weight from Birds with Control (CON) Diets vs. Birds Treated with *Saccharomyces Cerevisia* Metabolites (SCM)

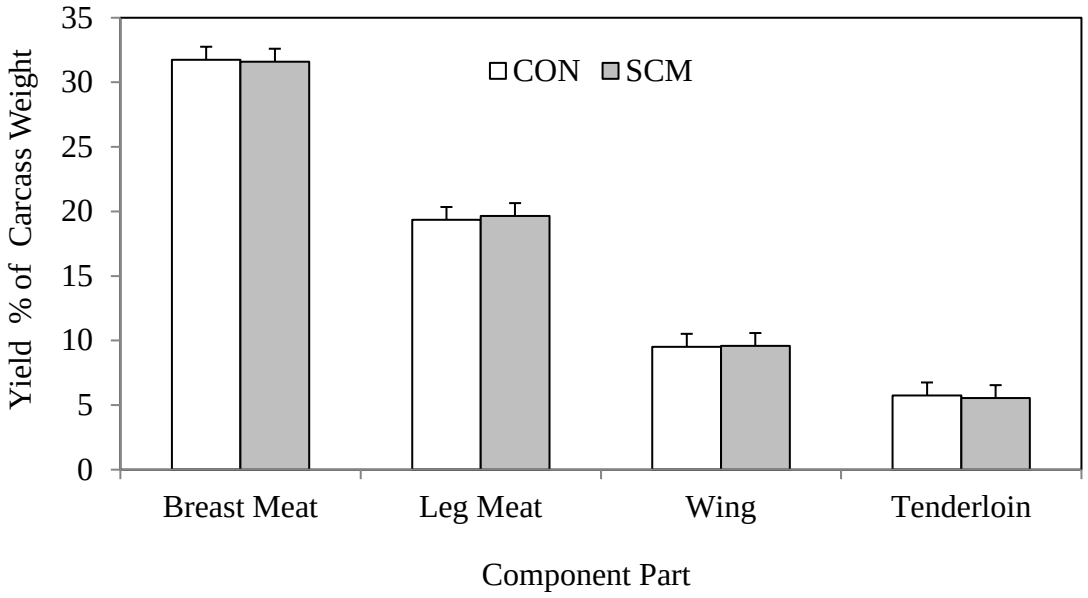

Figure 2. Broiler Yields of Major Component Parts from Birds with Control (CON) Diets vs. Birds Treated with *Saccharomyces Cerevisia* Metabolites (SCM)

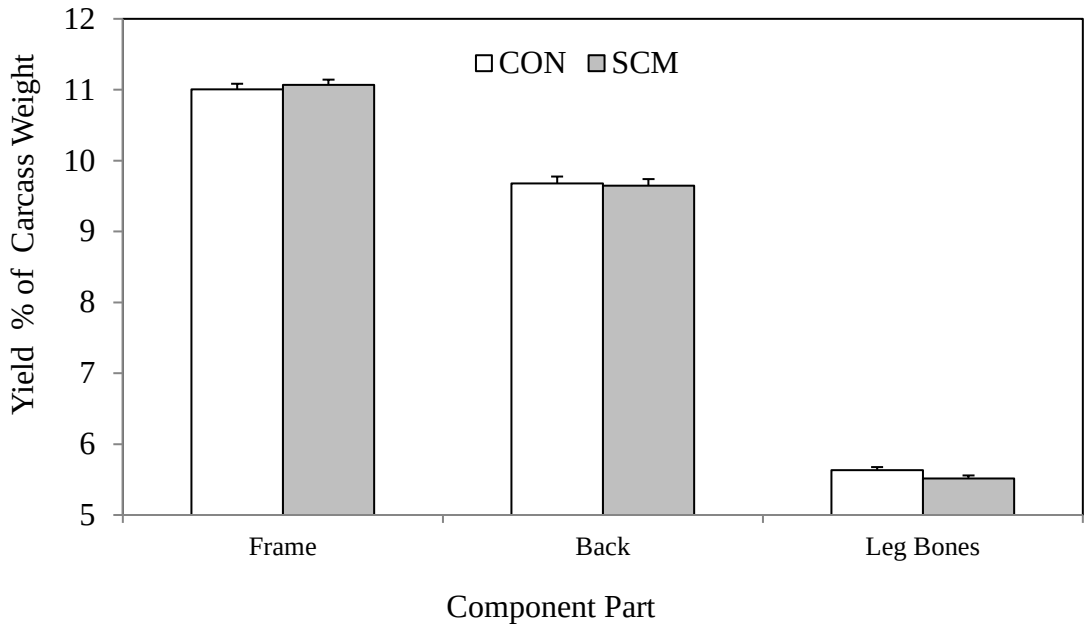

Figure 3. Broiler Yields of Minor Component Frames, Backs, and Leg Bones from Birds with Control (CON) Diets vs. Birds Treated with *Saccharomyces Cerevisia* Metabolites (SCM)

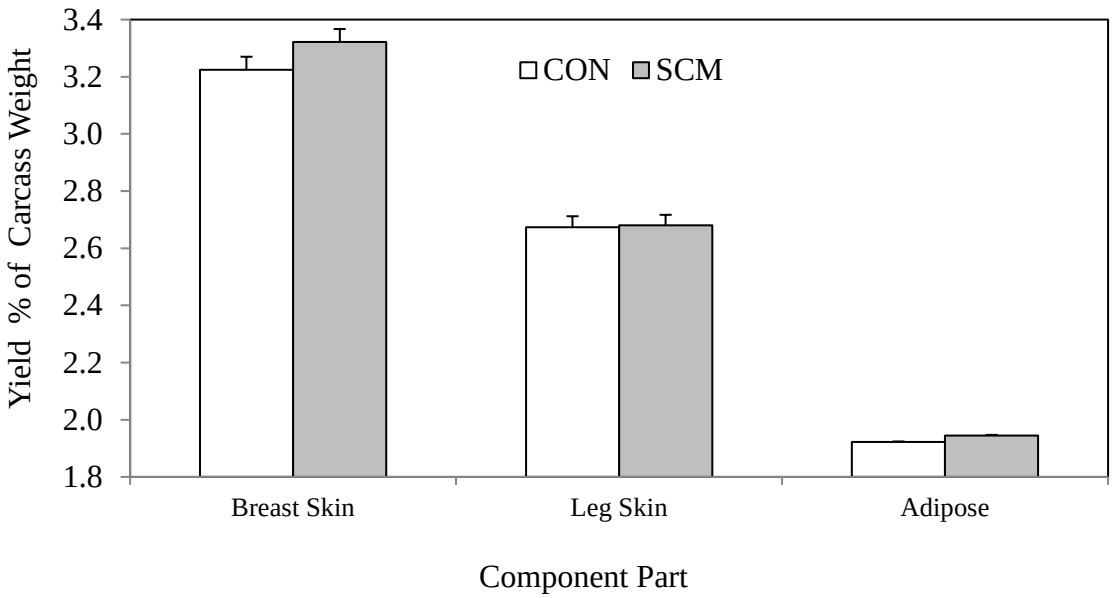

Figure 4. Broiler Yields of Minor Components Skin and Adipose from Birds with Control (CON) Diets vs. Birds Treated with *Saccharomyces Cerevisia* Metabolites (SCM)

CHAPTER IV

The Effects of Feeding Broilers the Metabolites of *Saccharomyces cerevisiae* Fermentation on *Salmonella* Virulence and Antimicrobial Susceptibility in Ceca

The Effects of Feeding Broilers the Metabolites of *Saccharomyces cerevisiae* Fermentation on *Salmonella* Virulence and Antimicrobial Susceptibility in Ceca

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ABSTRACT

The reduction of *Salmonella* and other enteric pathogens in raw poultry products are of interest of poultry producers, processors, and end-users. Increased emphasis is being placed on understanding the impact that interventions have on the *Salmonella* virulence and antibiotic resistance. The influence of microbial interventions in poultry on pathogen virulence and antibiotic resistance is gaining widespread interest, with the sustainability of food safety efforts being a major goal and concern of regulators and consumers. Commercially grown broiler chickens from multiple farms and flocks were administered a prebiotic consisting of *Saccharomyces cerevisiae* fermentation metabolites derived from dried yeast. Broilers exposed to *Saccharomyces cerevisiae* were compared to non-treated chickens, with research metrics including the virulence and antibiotic resistance of the pathogen cultures gathered from cecal samples. The flocks treated with the *Saccharomyces cerevisiae* prebiotic realized reductions in both *Salmonella* virulence as expressed by *hilA*% of controls, and also improved susceptibility of *Salmonella* colonies to selected common antibiotics at the time of harvest. This reduction of *Salmonella* virulence and improved antibiotic susceptibility is a significant component of reduced risk of severe foodborne illness and the ability to effectively treat *Salmonella* infections with selected antimicrobials.

Key Words: *Salmonella*, *Saccharomyces cerevisiae*, prebiotic, broiler

INTRODUCTION

In poultry production, pathogen reduction has been an area of continual emphasis for many years, with increasing needs to find alternative methods to reduce bacteria in a sustainable manner. The Food and Agriculture Organisation [sic] of the United Nations and the World Health Organisation [sic] have stated that poultry meat has been associated frequently and consistently with the transmission of enteric pathogens (FAO/WHO, 2002) While many processing plants utilize a variety of pathogen interventions (Berrang and Bailey, 2009; Shackleford et al., 1993), some consumers prefer to purchase products not treated with antimicrobials to reduce bacteria due to concerns about chemicals and their impact on consumer health and/or product organoleptic characteristics. Therefore, while antimicrobial interventions have been proven effective at reducing pathogens and bacterial loads, these interventions cannot be used exclusively for all applications. Also, the antimicrobial interventions used in processing plants are most effective when pathogen loads are minimized on incoming animals and raw materials prior to processing. For these reasons, research projects to find new, effective ways to reduce pathogens in live poultry production are extensive.

Poultry producers also have the additional challenges from many consumers that have expressed opinions toward removing antibiotics in animal agriculture. Although antibiotics have been effectively used under veterinarian prescriptions to prevent and/or treat diseases, public concern for creating antibiotic-resistant pathogens has led many meat and poultry processors to seek alternatives to antibiotics in animal production operations. Audits for live animal practices regarding antibiotic use have become a growing function of the USDA Agricultural Marketing Service (AMS) certification system for participating companies that have live animal production controls routinely audited under a USDA Process Verified Program (USDA AMS, 2018).

In order to minimize pathogens in raw poultry while reducing the use of antimicrobials and other traditional interventions and antibiotics, many meat and poultry producers continually seek new technologies. *Salmonella* reduction has been widely studied using competitive exclusion, probiotics, and other strategies for enhancement of intestinal immunity (Revolledo et al., 2006). Some probiotics have improved the natural defense of animals against pathogenic bacteria, and can be an alternative approach to antibiotics to effectively reduce bacterial contamination (Ghareeb et al., 2012). Mannon oligosaccharides derived from *Saccharomyces cerevisiae* cell walls have been effective alternatives to antibiotics for some applications in meat animals (Rozeboom et al., 2005; Shen et al., 2009). In poultry, *Saccharomyces cerevisiae* and other yeast based products have been successfully used for a variety of purposes, such as suppressing the effects of aflatoxicosis (Osweiler et al., 2010; Pizzolitto et al., 2013; Stanley et al., 1993), stimulation of the immune system (Bai et al., 2013; Yalçin et al., 2013), and improved gut morphology (Baurhoo et al., 2007; Morales-López et al., 2009).

The purpose of this experiment was to determine the effectiveness of feeding the dried metabolites of *Saccharomyces cerevisiae* to broilers on cecal *Salmonella* virulence and susceptibility to selected antimicrobials. Broiler chicken ceca were collected at commercial processing facilities after normal processing. This study included analyses from poultry ceca collected at the time of harvest from four different processing plants that provided samples representing twelve different integrated broiler houses, by analyzing both control non-treated flocks as well as flocks administered the prebiotic *Saccharomyces cerevisiae* product.

MATERIALS AND METHODS

Experimental design

Broiler plants that provided samples for evaluation were located in multiple regions of the United States. Four plants provided samples from flocks associated with their complexes, representing a total of twelve integrated commercial farms. On each farm, one commercial house of broilers was deemed an experimental flock. The broiler houses were located in regions with extensive poultry production, and were overseen by best management practices typical of the poultry industry, under the oversight of poultry veterinarian services and corporate management. Half of the farms selected were processed at 2 plants that manufactured products for marketing primarily as whole carcasses and bone-in parts, with less production of boneless items. The other half of the farms selected were processed at 2 other plants designed to grow birds for marketing a significant percentage of items as boneless portions, while also marketing some items that included bones and skeletal frame material in addition to the muscle fragment portions.

The experimental units were samples of ceca collected after processing in a commercial plant, to measure *Salmonella* virulence and antibiotic susceptibility at the time of harvest of both control flocks (CON), as well as the same flocks administered a *Saccharomyces cerevisiae* yeast fermentation metabolite product (SCT) with an additional 1.25 lb per ton (0.62 kg / metric ton) of concentrated product added on top of the control formulation. For the SCT samples, the samples were collected as repeated measures over time for a total of 3 treatment repetitions. Prior to harvest, the control flocks were fed a commercial poultry diet formulated by an animal nutritionist to optimize broiler welfare and performance. Flock housing conditions were held constant across the flocks in each complex, per industry standards required by broiler integrators and consistent with the commercial chicken production industry. Feeds were manufactured at a

commercial feed mill and pelleted prior to administration. Birds were allowed ad libitum access to feed and water throughout the life of the flocks, until feed was withdrawn from birds prior to catching for processing. When the broilers reached the market age for slaughter, the birds were transported from the farm in a conventional transport coop, and then birds were harvested at a commercial poultry processing plant under USDA inspection. The birds were processed at one of four selected commercial facilities following published broiler welfare standards (National Chicken Council, 2018a) that were approved by the Professional Animal Auditor Certification Organization (PAACO) (National Chicken Council, 2018b), requiring routine auditing for best management practices in animal wellbeing. The plant and harvesting line were held constant for each flock during the experiment.

The second experimental period consisted of a treatment program for each flock followed immediately after the control period. During the treatment period, flocks were grown under identical conditions as the control period, and at harvest were measured for *Salmonella* presence, quantity, virulence, and antibiotic resistance within each flock. The treatment flocks were fed the identical commercial poultry diet as the control flock, with 1.25 lbs per ton (0.62 kg / metric ton) of *Saccharomyces cerevisiae* yeast metabolite product added on top of the formula. Bird housing was managed in a similar manner as control flocks. Feeds were manufactured at the same commercial feed mill used for controls, pelleted, and presented ad libitum along with water throughout the life of the flocks. Birds were processed at the same plants, using the same procedures as the control flocks. The second experimental period continued for three cycles of broilers on each experimental farm, grown under normal commercial conditions and processed in the same plants and processing lines as the control flocks.

Sampling of Broiler Ceca

Ceca samples were collected after birds were commercially processed and harvested during normal cycles (Table 1). For sampling of ceca, after broilers were harvested by a commercial facility, viscera were collected by commercial procedures and equipment under USDA inspection. Ceca samples were acquired by manually removing the ceca from the viscera by stripping it free of the intestine and pinching at the base at the ceco-colon junction. Aseptic sampling techniques were conducted using sterile gloves and placing samples into sanitary whirl-pack specimen bags. Gloves were changed between each sample collected. A total of 25 ceca were collected after harvest from each flock sampled, and the tissues were individually packed and stored under refrigeration before and during shipment to an offsite laboratory for analysis. During the experiment, subgroups of 25 samples were collected per flock, with a total of 300 samples collected in the baseline group (CON), initial treated flock (SCT1), and subsequent treated flock (SCT2), with 275 samples collected that were provided from the final treatment group (SCT3).

Analysis of Salmonella Presence and Quantity in Broiler Ceca

Ceca samples were shipped to an independent laboratory for analysis. Each of the ceca were weighed and then aseptically placed in 10mL of Lennox L broth (Invitrogen, Carlsbad, CA) and manually massaged to release the cecal contents and dislodge *Salmonella* colonies from adherence sites and vortexed prior to plating. An aliquot of 100µL of each mixture was dispersed onto a Xylose Lysine Deoxycholate (XLD) agar plate (Fisher Scientific, Pittsburg, PA) that was selective for *Salmonella* (Anderson et al., 2015). The agar plates were incubated overnight at 37^{o}C. The following day, white colonies with black centers were enumerated and reported based on a 1:100 dilution factor as CFU/gm of cecal material or CFU/mL of rinsate.

Measurements of Salmonella Virulence in Broiler Ceca

Cecal samples were compared for *Salmonella* virulence using a semi-quantitative RT-PCR analysis to assess the expression of *hilA* (Carlson et al, 2007) in order to measure the regulation of *Salmonella* invasion using this established indicator of virulence (Bajaj et al., 1995). Total RNA was isolated from *Salmonella* colonies (n > 40) using the RNEasy kit (Qiagen) per the manufacturer's protocol. The RNA was subjected to semi-quantitative RT-PCR using primers specific to *hilA*. The number of PCR cycles required to visualize an amplicon under UV light on 2% agarose gel electrophoresis was documented. The comparative expression of *Salmonella* virulence was then reported by comparing the lowest number of cycles required to visualize an amplicon in control samples to the lowest number of cycles required to visualize an amplicon for treated samples, expressed in percent of the number of PCR cycles required for the control samples, using published methodology from previous research (Carlson et al., 2007). Invasion gene expression assays were performed in triplicate for both groups (Control and Test) for each of the flocks analyzed.

Assessment of Salmonella Susceptibility to Selected Antibiotics

The overall susceptibility of *Salmonella* colonies from the aforementioned cecal samples to florfenecol, ceftiofur, and enrofloxacin was measured. Specifically, samples of *Salmonella* cells recovered from the ceca of broiler chickens were obtained by selecting individual black-centered colonies from XLD plates and inoculating them into individual dish wells containing 200µL of LB broth. The bacteria were grown overnight at 37°C to an OD_{600} of approximately 0.3, which corresponds to 3×10^8 CFU/mL. Approximately 3µL of the growth was pin-replicated into fresh dish wells, each containing 32µg/mL, i.e. the breakpoint concentration for each of the selected antibiotics (CLSI, 2011) in 200µL of LB broth. The percent antibiotic resistance (i.e. not susceptible to the antibiotic) of *Salmonella* in cecal colonies was calculated as the percentage of

wells in which *Salmonella* grew in the presence of the selected antibiotic, using published protocols (Feye et al., 2016).

Statistical Analysis

The comparative analysis was completed with statistical software (SAS Institute, 2016). For comparison of percentages, a Contingency Analysis of Proportions was performed using both the Pearson's Chisquare test, with a Likelihood Ratio analysis and Fisher's Exact Test to determine the probability of differences in proportions across treatments. A Kruskal – Wallis test for population differences with unequal variances was also used to compare treatments. For the virulence measurements, each treatment group was measured in relation to the control, and for the analysis of antimicrobial susceptibility the results of the treatment cycles were pooled to increase statistical power in comparison to the control group.

RESULTS AND DISCUSSION

After birds were processed in a commercial facility, ceca samples were recovered and analyzed at a laboratory for *Salmonella* by selecting presumptive colonies from XLD plates and then subjecting them to a tissue culture invasion assay. The method has previously been used (EpiCor®, Embria Health Sciences) to understand *Salmonella* invasion and virulence. In this study, the virulence of the *Salmonella* colonies recovered from SCT flocks was significantly less than the virulence reported from colonies recovered from CON flocks (Figure 1). This reduced virulence in flocks treated with SCT is consistent with previously conducted research (Gantois et al., 2006; Possemiers et al., 2013). This difference may be attributable to the fact that *Saccharomyces cerevisiae* metabolites can increase the presence and concentration of butyrate in the intestine (Possemiers et al., 2013). Similar studies of complex fecal microbial populations exposed to the fermentation metabolites of *Saccharomyces cerevisiae* have shown to effect

volatile fatty acid production *in vitro* (Broomhead et al., 2012), and, more specifically, an overall decrease of growth of specific serotypes, such as *Salmonella Arizonae* and *Salmonella Heidelberg* (Nsereko et al., 2013). An increase in the quantity or concentration of butyrate in the intestine has been shown to decrease *Salmonella* invasion as measured by gene expression *in vitro* (Gantois et al., 2006). The decrease in *Salmonella* invasiveness has also been shown to coincide with a decrease in the expression of *hilA*, which is a major regulator and indicator of *Salmonella* virulence in certain animal species (Baja et al., 1995).

In addition to the study of the invasiveness and virulence of *Salmonella*, colonies of the pathogen recovered from the ceca were subjected to susceptibility to florfenicol, ceftiofur, and enrofloxacin, common drugs used in veterinary medicine that are bactericidal to *Salmonella*. For each type of medication exposed to susceptibility testing, a significant improvement in antibiotic susceptibility was observed for each of the compounds (Figure 2). While this study does not suggest a mechanism to explain how *Saccharomyces cerevisiae* metabolites mediate the improvement in antibiotic susceptibility, studies with other species have reported that this yeast fermentation metabolite can be an important factor in dislodging antibiotic resistance-encoding integrons from the basic *Salmonella* genetic structure (Brewer et al., 2013). For example, a decrease in resistance to selected antibiotics has been observed when the loss of an SGI1 integron occurs. Traditional PCR has been used to identify the presence of the integron within *Salmonella* isolates, followed by agarose gel electrophoresis to measure the sustained presences of the integron when exposed to selected antibiotics such as chloramphenicol (Feye et al., 2016).

Further research is needed to more completely understand how *Salmonella* samples collected differ in antimicrobial resistance at various stages of the poultry processing continuum. In this research, the susceptibility of *Salmonella* to selected antibiotics was analyzed by

collecting samples from cecal material obtained in poultry processing plants after viscera was removed, but prior to final chilling and further processing. There are conflicting results regarding whether commercial chilling has any effect on the persistence of antibiotic resistant *Salmonella* variants (Mohamed et al., 2014). This could be explained by the fact that *Salmonella* invasion depends on multiple host and bacteria factors, one of which is the presence or absence of genes in the bacterium that encode for various virulence factors, and whether these factors are expressed in the bacterium before or during the infection (Olah et al., 2005). While some research has shown that the chilling step may impact the recovery of selected *Salmonella* clonal groups, other data found that the chilling step had no effect on antimicrobial susceptibility, as measured by the presence of class-I integrons, bla_{CMY} genes, and virulence genes including *invA*, *pagC* and *spvC* (Mohamed et al., 2014). By understanding the impact of that *Salmonella* genetic differences have on virulence, targeted interventions can be developed that are effective at reducing the likelihood of severe infections and maintaining the effectiveness of selected antimicrobials. Measures of other factors *in vivo,* such as the production of volatile fatty acids in the presence of specific *Saccharomyces cerevisiae* fermentation metabolites, needs further study to determine the correlation between these measurements and *Salmonella* virulence and susceptibility to antibiotics. The potential benefits to animal and human health from *Saccharomyces cerevisiae* fermentation metabolites modulating *Salmonella* virulence and antimicrobial susceptibility in broiler ceca material have not been clearly quantified, and need further research.

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TABLES AND FIGURES

Table 1
Ceca Sample Collection Information

Sample Cycles	Treatments[1]	Plants
1	CON	A[2]
2	SCT	B[2]
3		C[3]
4		D[3]

[1] Treatments: CON = Commercial broilers fed control diet ; SCT = treatment of commercial birds fed control diet + *Saccharomyces cerevisiae* metabolites

[2] Plants A & B processed products primarily as whole, in-tact, or components

[3] Plants C & D processed boneless products and bone-in component parts

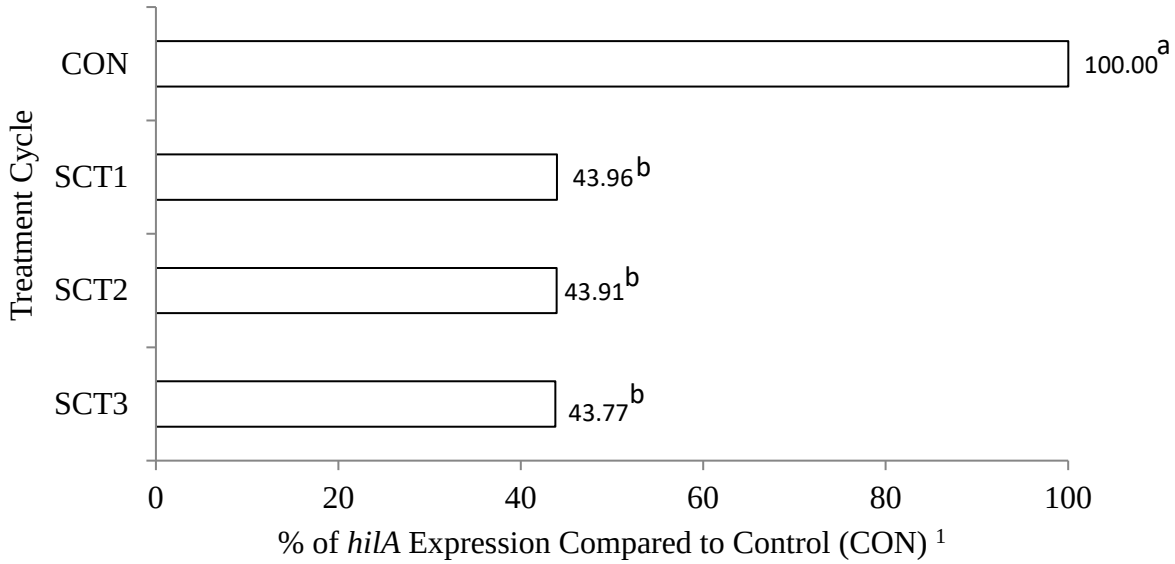

Figure 1. Broiler Ceca *Salmonella* Virulence of Flocks of Control (CON) Birds vs. Flocks Treated with *Saccharomyces Cerevisia* Yeast Product (SCT)

[1] Expression of *hilA* measured at the lowest number of PCR replication cycles required to visualize amplicons under UV light during electrophoresis.
[a - b] percentages are different at $p \leq 0.05$

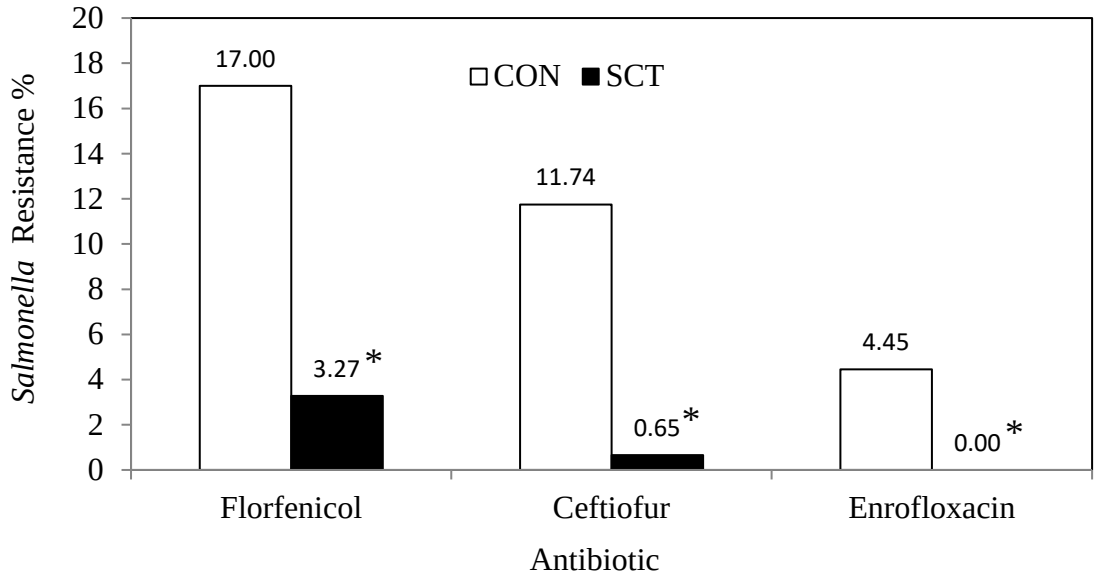

Figure 2. Broiler Ceca *Salmonella* Resistance to Selected Antibiotics Flocks of Control (CON) vs. Treated with *Saccharomyces Cerevisia* Yeast Metabolites (SCT)

* percentages within same antibiotic are different at $p \leq 0.05$

CHAPTER V

The Impact of Feeding Broilers a Supplement with *Saccharomyces cerevisiae* Dried Metabolites on *Salmonella* Presence and Quantity in Broiler Ceca and on Component Portions

The Impact of Feeding Broilers a Supplement with *Saccharomyces cerevisiae* Dried Metabolites on *Salmonella* Presence and Quantity in Broiler Ceca and on Component Portions

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ABSTRACT

Strategies to reduce *Salmonella* and other enteric pathogens in raw poultry products have traditionally involved a multi-hurdle approach by poultry producers, processors, and end-users. Poultry live bird growing operations and processing plants have frequently evaluated the impact of such interventions by evaluating poultry carcasses and/or plant environmental samples. Increased emphasis is being placed on understanding the impact that interventions have on the pathogen loads of specific poultry component parts. The impact of interventions on poultry parts has become more important to regulators and consumers, as these are very common ways to market chicken products compared to whole carcasses. In this study, commercial poultry processing plants provided broiler ceca and carcass portions that had been processed from chickens administered a commercial diet as control, or the same diet with an additional feed supplement which included *Saccharomyces cerevisiae* fermentation metabolites derived from dried yeast. Ceca and parts provided from chickens administered *Saccharomyces cerevisiae* metabolites were compared to the ceca and parts from non-treated chickens, with comparisons made on the presence and quantity of *Salmonella* in the ceca and on the component parts provided after commercial harvest. The ceca and parts obtained from chickens treated with the *Saccharomyces cerevisiae* supplement displayed reduced *Salmonella* presence and quantity compared to ceca and parts from conventional flocks. These results indicated that broilers fed supplemental *Saccharomyces cerevisiae* metabolites may have a reduction in *Salmonella* quantities and presence in ceca, which could potentially alter the overall chicken microflora and lead to reduced *Salmonella* presence and quantity on component parts.

INTRODUCTION

In poultry production, pathogen reduction has been an area of continual emphasis for many years, with increasing needs to find alternative methods to reduce bacteria in a sustainable manner. The United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) has implemented new pathogen performance standards for various types of raw poultry products, specifically for the pathogenic organisms *Salmonella* and *Campylobacter* (USDA FSIS, 2015). These standards have been made more stringent over the years, as the government attempts to achieve goals for reducing foodborne illness under its Healthy People initiatives (USDHHS ODPHP, 2018). In order to achieve the goals of improving food safety while meeting the requirements of regulators and consumers, poultry processing managers, researchers and regulators have focused on multi-hurdle strategies in processing plants by using combinations of antimicrobial sprays, dips, and rinses to reduce pathogenic and spoilage bacteria (Berrang and Bailey, 2009; Buncic and Sofos, 2012; Shackelford et al., 1993; USDA FSIS, 2015). Also, the antimicrobial interventions are most effective when pathogen loads are minimal on incoming animals and raw materials, prior to processing. Therefore, while antimicrobial interventions have been proven effective at reducing pathogens and bacterial loads, these interventions cannot be used exclusively for all applications. Research is very limited in determining how novel approaches to pathogen reduction in live poultry production impact reductions of bacteria after product is processed into component parts, packaged and ready for sale to consumers.

Concurrent to the challenges to reduce pathogens, poultry producers have the additional requirement of meeting increased consumer demand for products with limited exposure to traditional antimicrobial interventions. Some consumers prefer to purchase products not treated with antimicrobials to reduce bacteria, due to concerns about chemicals and their impact on consumer health and/or product organoleptic characteristics. There is also increased demand for

reducing or eliminating antibiotic use in food animals, so veterinarians have worked to minimize prescriptions to prevent and/or treat diseases and are replacing them with pathogen-reduction strategies using novel approaches. The USDA has also increased its oversight of voluntary product claims for products without antibiotics, such as naturally-raised livestock (USDA, 2009), and the USDA Agricultural Marketing Service (AMS) certification systems for companies that have animal production controls routinely audited (USDA AMS, 2018).

Due to the challenges to reduce pathogens in raw poultry while staying cognizant of consumer interests to reduce antimicrobials and other traditional interventions and antibiotics, many meat and poultry producers continually seek new technologies and novel interventions that achieve both requirements. One strategy has been the administration of probiotics and/or prebiotics to animal feeds or at farms during the growing cycle. Beneficial microbial cultures have been shown to stimulate the immune system in animals (Gibson and Roberfried, 1995; Netherwood et al., 1999) and enhance epithelial innate immunity-related gene expression through anti-inflammatory effects and reduced pro-inflammatory cytokine expression (Amit-Romach et al., 2010; Pagnini et al., 2010). Mannon oligosaccharides derived from *Saccharomyces cerevisiae* cell walls have been effective alternatives to antibiotics for some applications in meat animals (Rozeboom et al., 2005; Shen et al., 2009). In poultry, *Saccharomyces cerevisiae* and other yeast based products have been successfully used for a variety of purposes, such as alleviation of lipopolysaccharide-induced inflammation (Wang et al., 2016), and improved strength and structure of gut physiology (M'Sadeq et al., 2015; Solis de los Santos et al., 2005).

The purpose of this experiment was to determine the effectiveness of feeding broilers a commercial supplement with the dried metabolites of *Saccharomyces cerevisiae* on the presence

and quantity of *Salmonella* in broiler chickens in commercial settings, as measured in the ceca and component parts collected at the time of processing. This study included replicate analyses from poultry ceca and products collected at the time of harvest from 4 different processing plants, representing 12 different integrated broiler farms spanning a total of three different states. The ceca and parts from control, non-treated commercial flocks were evaluated as a baseline, so that ceca and parts obtained from flocks administered the *Saccharomyces cerevisiae* metabolites could be compared over multiple repeated analyses.

MATERIALS AND METHODS

Experimental design

Broiler ceca and parts were provided by commercial processing plants, obtained for analysis after production, representing samples from broiler parts processed from twelve integrated commercial farms. The experimental samples were obtained from 4 plants that obtained samples from 3 representative flocks per plant, located in multiple regions of the United States with extensive poultry production. Plants and farms were overseen by best management practices and programs typical in the poultry industry, with oversight by poultry veterinarian services and production management. Each plant and farm was part of an integrated poultry complex, and the farms selected all were designed to grow and harvest birds at two basic types of product mixes for its existing customers. Of the 12 farms, a total of 6 farms were used to process products at 2 plants that manufactured finished products primarily as whole in-tact components, including the skeletal bones, muscles, and outer skin layer. Some examples of markets using these types of component parts included retail groceries, fast food restaurants, and various foodservice entities who utilized chicken component bone-in parts. The other 6 farms were used to provide poultry to 2 other plants that manufactured a significant volume of items marketed as

boneless portions, while still maintaining some sales volume of items that included skeletal bone material.

The control experimental period included collection of control samples of ceca and component parts, obtained after each flock was harvested and processed by a commercial facility. These samples were collected to establish the baseline control for the ceca and portions in respect to *Salmonella* presence and quantities within each flock at the time of harvest. The components collected and analyzed as control were obtained post-harvest from birds that had been administered a conventional diet, typical of the commercial broiler industry, designed for standard poultry performance. Prior to processing, the birds were allowed ad libitum access to feed and water, and feed was withdrawn prior to catching for transport to the commercial processing plant. Flock density, lighting programs, and temperature were managed per industry standards and held constant across the flocks in each complex. When the broilers reached their scheduled harvest dates, the birds were transported from the farm in conventional transport coops, and then birds were harvested at a commercial poultry processing plant under USDA inspection. The plant and processing lines were held constant for each flock during the multiple phases of the experiment. Chickens were processed in one of the 4 commercial plants as previously described. None of the broiler processing facilities were affiliated with the University of Arkansas. The birds were grown and processed under commercial management and were routinely monitored for optimal animal care practices, compliant with the animal welfare requirements of the National Chicken Council, as certified by the Professional Animal Auditor Certification Organization (NCC, 2018).

The second experimental period consisted of obtaining ceca and component parts from commercially-harvested broilers at the same facilities. The samples were from the same plants

as the control samples from the next subsequent time each of the test flocks was harvested. During the treatment period, flocks were grown by the same commercial operators under the same conditions as the control period. At harvest, ceca and component parts were measured for *Salmonella* presence and quantity, in a similar manner as the control period. The treatment flocks were fed the identical commercial poultry diet as the control flock, with 1.25 lb per ton (0.62 kg / metric ton) of *Saccharomyces cerevisiae* yeast fermentation metabolite products added on top of the control formulation. Feeds provided were manufactured at the same commercial feed mills used for controls, pelleted, and presented ad libitum along with water, lighting, and other environmental conditions held constant throughout the life of the flocks. Birds were processed at the same commercial plants, using the same procedures as the control flocks. After birds were processed in the commercial facilities, the ceca and component parts were provided by the commercial integrators for analysis. After the second experimental period, a third and fourth experimental period were also observed, using the same conditions and data collection protocols as the second experimental period.

Sampling of Broiler Ceca and Component Parts

Samples of ceca and component parts were provided after birds were commercially processed and harvested. For sampling of ceca, broilers were initially harvested under management of a commercial facility, who removed viscera from the carcass and produced component broiler parts using industry procedures and equipment under USDA inspection. Ceca samples were acquired by manually removing the ceca from viscera samples by stripping it free of the intestine and pinching at the base at the ceco-colon junction. Aseptic sampling techniques were conducted using sterile gloves and placing samples into sanitary whirl-pack specimen bags. Gloves were changed between each sample collected. A total of 25 ceca were

collected after harvest from each flock, individually packed, and stored under refrigeration before and during shipment to an offsite laboratory for analysis. The total ceca collected included 300 samples, evenly divided between each sample period. The cecal sampling schedule is shown in Table 1.

To collect samples of component parts, the 4 different commercial facilities provided the parts after birds were harvested, carcasses chilled, and component parts manufactured under standard procedures. Parts were selected that were representative of high volume sales from the processing plants. The parts were selected in the normal process flow, but prior to any final parts sprays or dips with antimicrobial chemicals. In this experiment, half of the plants selected had a predominant sales mix of whole component parts that consisted of both edible meat tissue and bones, and the other half of the plants produced a more predominant ratio of boneless products. The parts samples were collected at the point of packaging, with 4 lb collected for each sample. For each 4 lb sample, all parts included were the same unique part type. From the plants that produced products for sale primarily as whole component parts (designated as Parts Group A) , the samples collected from each representative flock were evenly distributed with 2 samples of bone-in split breast quarters, 2 samples of leg quarters, 2 samples of breast tenderloins, 2 samples of wings, and 2 samples of front half frames. For the plants producing products for sales primarily as boneless items (designated as Parts Group B), the items collected were evenly distributed with 2 samples of boneless breast meat, 2 samples of boneless tenderloins, 2 samples of boneless leg meat, 2 samples of wings, and 2 samples of front half frames. Each sample of portions collected was rinsed with media using the ratio of 400mL of buffered peptone water for every 4 lb of portions in the homogenous rinsate solution. The buffered peptone water was deemed an acceptable rinse media as this was the traditional media used in each facility by

regulators and industry personnel, and the samples selected were collected prior to final antimicrobial rinses, thus neutralized media was not required. Each sample of parts with the buffered peptone water solution was placed into a sterile sampling bag, the bag was held closed, and the mixture of parts and buffered peptone water were swirled in a gentle rocking motion for one minute to ensure the surfaces of the cut samples were completely exposed to the sample rinse media. The homogeneous rinsate was transferred to a sterile specimen cup, with 80ml collected, sealed in the container, and stored and shipped under refrigeration to an outside laboratory. A total of 10 samples of parts rinses per flock were collected, for a total of 120 samples during the entire experiment. The parts rinse sampling schedule is shown in Table 1.

Analysis of Salmonella Presence and Quantity in Broiler Ceca and Component Parts.

Samples of ceca and rinsates from parts were shipped to an independent laboratory for analysis. Each of the ceca were weighed and then aseptically placed in 10mL of Lennox L broth (Invitrogen, Carlsbad, CA) and manually massaged to release the cecal contents and dislodge *Salmonella* colonies from adherence sites. Rinsate samples were diluted at a 1:10 dilution in Lennox L broth and were also vortexed prior to plating. An aliquot of 100µL of each mixture was dispersed onto Xylose Lysine Deoxycholate (XLD) agar plates (Fisher Scientific, Pittsburg, PA) selective for *Salmonella* (Anderson et al., 2015). The agar plates were incubated overnight at 37ºC. The following day, white colonies with black centers were enumerated and reported based on a 1:100 dilution factor as CFU/gm of cecal material and CFU/mL of rinsate.

Statistical Analysis

The statistical analysis was completed with SAS JMP® Pro version 13.2.1 (SAS Institute, 2016). For the reporting of colony forming units (CFU's), the enumerated results were converted to log10 values, and compared using the Oneway Analysis of Variance and the Tukey-

Kramer test to determine separation of means. For comparison of percentages, a Contingency Analysis of Proportions was performed using the Pearson's Chi-square test, a Likelihood Ratio analysis, and Fisher's Exact Test for probabilities of differences across treatments. For all variables being compared, significant differences were defined as $P \leq 0.05$. For selected variables that are not different using alpha = 0.05 but are different at $P \leq 0.10$, the differences at this alpha level are noted.

RESULTS AND DISCUSSION

The results of the reduction in *Salmonella* attributable to the *Saccharomyces cerevisiae* metabolite are shown in Figures 1 – 4. The treatment of broilers with the dried metabolites of *Saccharomyces cerevisiae* resulted in significant reductions in the presence and quantity of *Salmonella* in broiler ceca and component parts in the initial and subsequent test cycles after baselines were established from samples collected of untreated broilers.

Figures 1 and 2 show the reduction of enumerated *Salmonella* $\log_{10}$ quantity and overall *Salmonella* percent prevalence in the ceca, respectively. Because the ceca is an established indicator of the impact of live bird interventions on *Salmonella* presence, and is a portion of the broiler anatomy that has been repeatedly studied due to its ability to enhance pathogen durability and growth, the ceca pathogen results in this study may be considered a realiable indicator of the effectiveness of the *Saccharomyces cerevisiae* metabolite on *Salmonella* reduction. The reduction of *Salmonella* $\log_{10}$ counts in the ceca during each period of treatment with *Saccharomyces cerevisiae* supplement was significant ($P < 0.05$) during each treatment cycle as compared to the control period. In the Group A plants producing primarily whole bone-in portions, the overall reduction in *Salmonella* counts was 0.21 $\log_{10}$ during period TRT1, increasing to 0.30 $\log_{10}$ MPN during TRT2, and stabilizing at 0.27 $\log_{10}$ MPN during the TRT3

treatment period. In a similar manner, the Group B plants had an overall reduction of *Salmonella* counts compared to the control of 0.20 $\log_{10}$ during period TRT1, increasing to 0.27 $\log_{10}$ MPN during TRT2, and 0.24 $\log_{10}$ MPN during the TRT3 treatment period. In like manner, the percent prevalence of *Salmonella* was significantly lower in the ceca in all TRT periods as compared to the CON period (Figure 2).

The reduction in the quantities and prevalence of *Salmonella* in the ceca after the implementation of the yeast-based *Saccharomyces cerevisiae* treatment was consistent with findings from other research. Yeast fermentation metabolites reduced *Salmonella typhimurium* in a culture derived from chicken ceca mixed with feed (Rubinelli et al., 2016). Other studies have shown that birds fed a *Saccharomyces cerevisiae* supplement demonstrated had decreased prevalence of the pathogen in the ceca, cloaca, and carcass skin (Mountzouris et al., 2015). The mechanism of decreased quantity and prevalence of *Salmonella* in the ceca may be explained by multiple factors. For example, treatments with *Saccharomyces cerevisiae* have been shown to increase the presence of goblet cells which form a mucous blanket in the intestine, leading to the formation of a large surface area for *Salmonella* adhesion (Baurhoo et al., 2007; Chee et al., 2010; Chichlowski et al., 2007; Leforesteier et al., 2009). This treatment can also form a physical protective barrier for the epithelium in the cecal tonsil, causing a reduction of bacterial attachment to cecal tonsil tissues (Brufau et al., 2015), through biochemical reactions of pathogens with lectins in the mucous blanket and/or through low affinity bonds (Barnett et al., 2012). A reason for the reduction in ceca *Salmonella* may be improved overall gut health, as demonstrated by increased villus length or height in the ileum (Solis de los Santos et al., 2005; Morales-López et al., 2009) and duodenum (Rodriquez-Lecompte et al., 2012). Contributing factors causing the decrease in ceca *Salmonella* may be explained by early reduction of

Salmonella in the gastro-intestinal tract, consistent with findings that broilers inoculated with various *Salmonella serotypes,* such as *Salmonella typhimurium* or *Salmonella Dublin,* at age 3 days had significantly less presence of either *Salmonella* serotype in the ceca at day 10 (Spring et al., 2000).

In addition to the reduction of *Salmonella* in the ceca, the component parts showed a similar reduction in both *Salmonella* MPN counts and prevalence (Figures 3 and 4, respectively). For Parts Group A, this reduction included a consistent reduction in MPN $\log_{10}$ counts of 0.25, 0.26, and 0.25 in the treatment periods TRT1, TRT2, and TRT3, respectively, whereas the reduction of MPN $\log_{10}$ counts in Parts Group B was even greater at 0.51, 0.51, and 0.50 during the TRT1, TRT2, and TRT3 periods, respectively. The average reduction of *Salmonella* incidence rates was 12.4% across all groups and treatment periods. These research results were similar to findings from a comparison of competitive exclusion and probiotic culture products in broilers, including a chicken-origin competitive exclusion product, a *Saccharomyces cerevisiae* probiotic culture, and a probiotic *Pediococcus acidilactici* culture, which all reduced the *Salmonella* percentage in litter, ceca, and carcasses, in both summer and winter tests (Al-Zenki et al., 2009).

The fact that both ceca and component parts showed consistent and predictable reduction of *Salmonella* suggests the *Saccharomyces cerevisiae* metabolite compound had a beneficial effect on the general microbiome of the internal systemic physiology of the chickens, with the overall lower incidence of the pathogen in the gastrointestinal tract contributing to lower presence and quantity on the component parts. Inclusion of products in the diet made from *Saccharomyces cerevisiae* metabolites appear to allow for the entire microbiome in the ceca to stabilize at an earlier age in broilers than those in normal diets (Park et al., 2017). Studies have

revealed that birds fed diets supplemented with *Saccharomyces cerevisiae* maintained decreased *Salmonella* at ages 16, 28, and 42 days (Roto et al., 2017). A mature and stable gut microbiome has been suggested as an effector of pathogen control, reduced mortality, improved growth performance, and overall health of the host (Patterson, 2012). All of these factors support the findings from the present study, suggesting that the use of the *Saccharomyces cerevisiae* metabolite supplement resulted in systemic reduction of *Salmonella* prevalence and quantity, as measured in the ceca and component parts over repeated trial periods.

In summary, the administration of *Saccharomyces cerevisiae* metabolites has been shown to be an effective alternative to reduce the presence and quantity of *Salmonella* in broiler ceca and broiler parts, including whole component bone-in products and a mixture of boneless and bone-in portions. Poultry integrators evaluating options to reduce pathogens in a multi-hurdle approach may choose to use the metabolites of *Saccharomyces cerevisiae* as an intervention option to alleviate some of the other interventions required to achieve regulatory standards while maintaining customer requirements. The effectiveness of *Saccharomyces cerevisiae* metabolites in reducing *Salmonella* in broilers may be the result of multiple physiological mechanisms, such as increased intestinal villus length or height, and the creation of a mucous blanket over parts of the intestine which prevents *Salmonella* adherence to epithelial cells.

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TABLES AND FIGURES

Table 1

Sample Sizes of Broiler Ceca and Component Parts Rinses for Comparison of *Salmonella* Prevalence and Quantification in Control (CON) Broilers to Broilers Treated with *Saccharomyces Cerevisia* Yeast Metabolite (TRT)

Treatment Periods[1]	CON	TRT1	TRT2	TRT3	TOTAL
Ceca[2]	300	300	300	275	1,175
Parts Rinses [3]	120	120	120	120	480
Total	420	420	420	420	1,655

[1] Treatment periods included CON (control, no supplement), TRT1 (first cycle of treated broilers after control period), TRT2 (second cycle of treated broilers), and TRT3 (third cycle of treated broilers).

[2] Broiler ceca samples consisted of 25 per sample group

[3] Broiler parts rinsate samples were conducted using buffered peptone water rinse of 4 lbs. samples, with duplicate rinse samples for each part type. For plants producing primarily whole in-tact parts, rinses were taken of bone-in breasts, bone-in leg quarters, tenderloins, wings, and frames, for a total of 10 parts rinses from each test group. For plants producing primarily boneless meats, rinses were taken of boneless breast meat, boneless leg meat, tenderloins, wings, and frames, for a total of 10 parts rinses for each test group. Total parts rinse samples consisted of 10 rinses per sample group, 12 replicates per treatment.

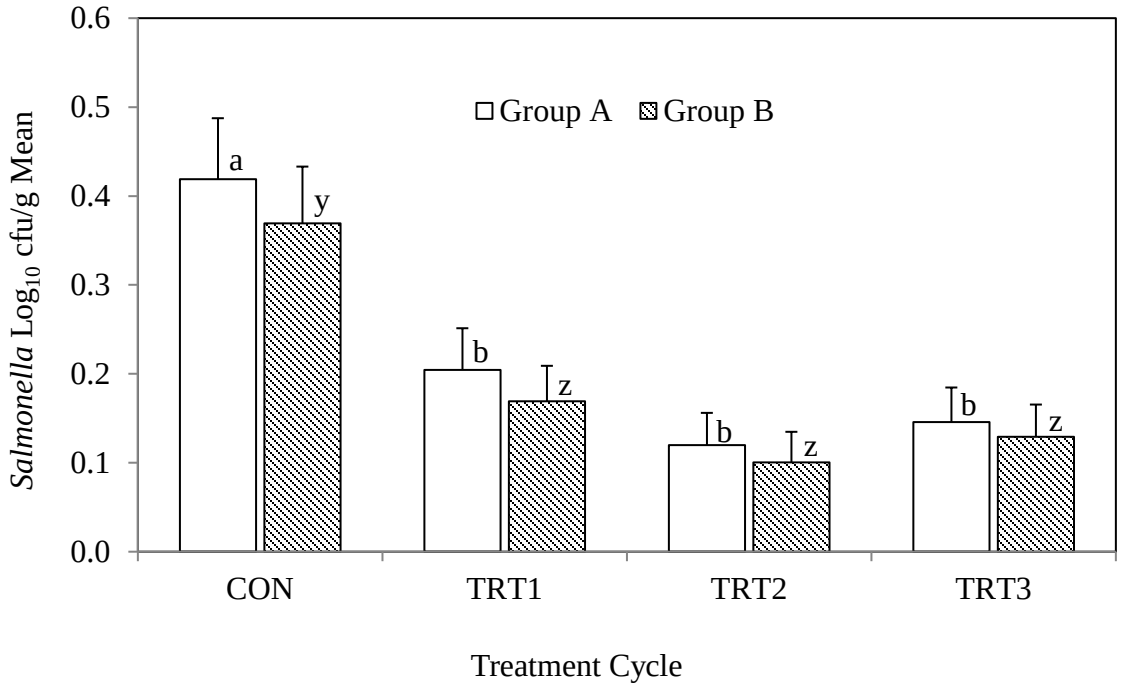

Figure 1. *Salmonella* Log_{10} Counts per Gram in Broiler Ceca from Control Samples (CON) and in Ceca from Broilers Treated with *Saccharomyces Cerevisia* Yeast Metabolite (TRT)[1]

[1] Treatment cycles were analyzed from ceca collected from the same flocks after harvest over sequential treatment periods including non-supplemented control flocks (CON), treated flocks cycle 1 (TRT1), treated flocks cycle 2 (TRT2), and treated flocks cycle 3 (TRT3).

Group A samples were collected from plants that manufactured primarily whole part components and cut-up portions with the bones included in the majority of products sold.

Group B samples were collected from plants that manufactured a significant volume of breast meat and leg meat items, with the bones removed from a significant volume of products sold.

[a – b] Means compared within whole parts products program with different letters are different at $P \leq 0.10$

[y – z] Means compared within boneless meat products program with different letters are different at $P \leq 0.05$

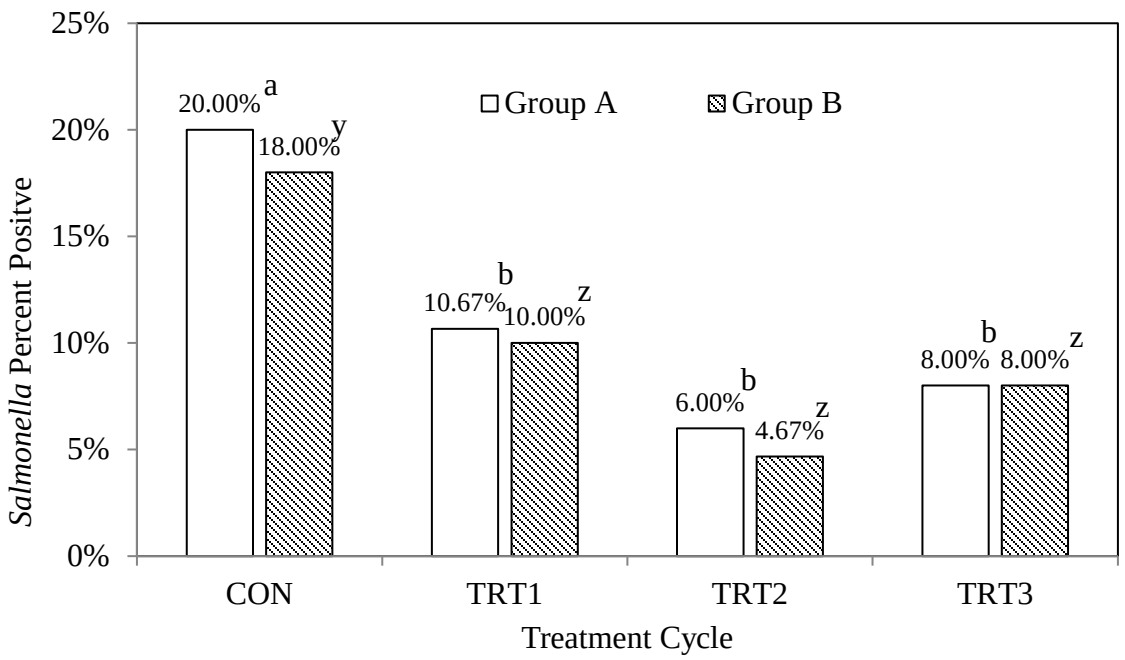

Figure 2. Percent *Salmonella* Prevalence in Broiler Ceca from Control Samples (CON) and in Ceca from Broilers Treated with *Saccharomyces Cerevisia* Yeast Metabolite (TRT)[1]

[1] Treatment cycles were analyzed from ceca collected from the same flocks after harvest over sequential treatment periods including non-supplemented control flocks (CON), treated flocks cycle 1 (TRT1), treated flocks cycle 2 (TRT2), and treated flocks cycle 3 (TRT3).

Group A samples were collected from plants that manufactured primarily whole part components and cut-up portions with the bones included in the majority of products sold.

Group B samples were collected from plants that manufactured a significant volume of breast meat and leg meat items, with the bones removed from a significant volume of products sold.

[a – b] Means compared within whole parts products program with different letters are different at $P \leq 0.05$

[y – z] Means compared within boneless meat products program with different letters are different at $P \leq 0.05$

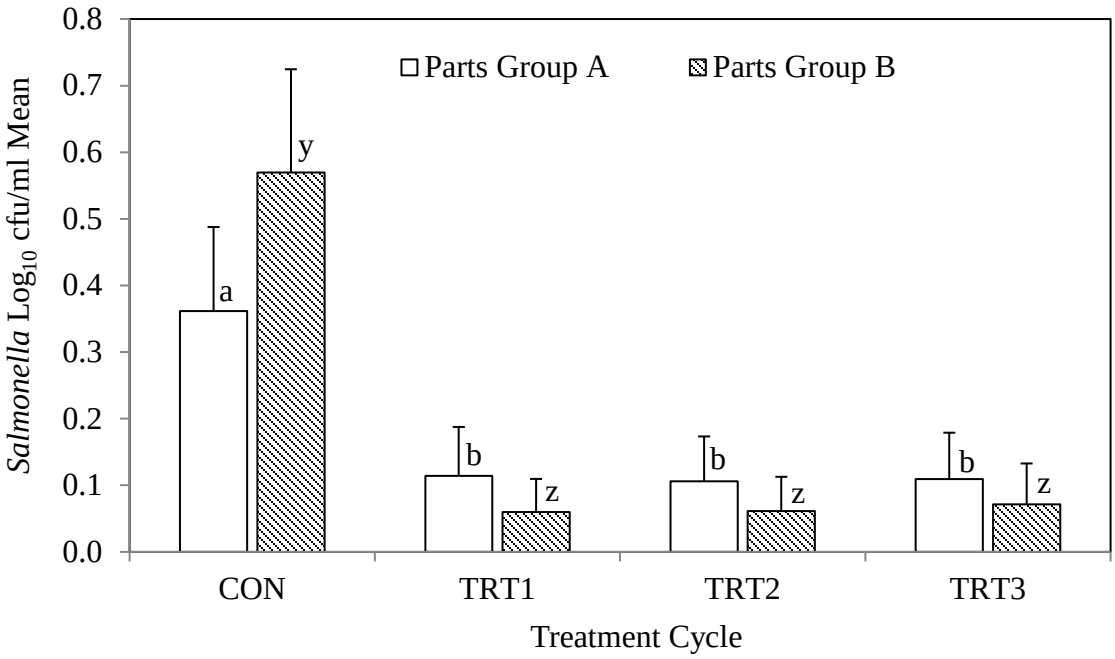

Figure 3. *Salmonella* Log_{10} Counts per ml Rinsate on Broiler Component Parts from Control Samples (CON) and on Parts from Broilers Treated with *Saccharomyces Cerevisia* Yeast Metabolite (TRT)[1]

[1] Treatment cycles were analyzed from portions collected from the same flocks over sequential treatment periods including non-supplemented control flocks (CON), treated flocks cycle 1 (TRT1), treated flocks cycle 2 (TRT2), and treated flocks cycle 3 (TRT3).

Parts Group A consisted of equivalent samples of bone-in split breast quarters, bone-in leg quarters, tenderloins, wings, and front half frames. Samples collected in process prior to final antimicrobial interventions.

Parts Group B consisted of equivalent samples of boneless breast meat, boneless leg meat, tenderloins, wings, and front half frames. Samples collected in process prior to final antimicrobial interventions.

[a – b] Means compared within whole parts products program with different letters are different at $P \leq 0.05$

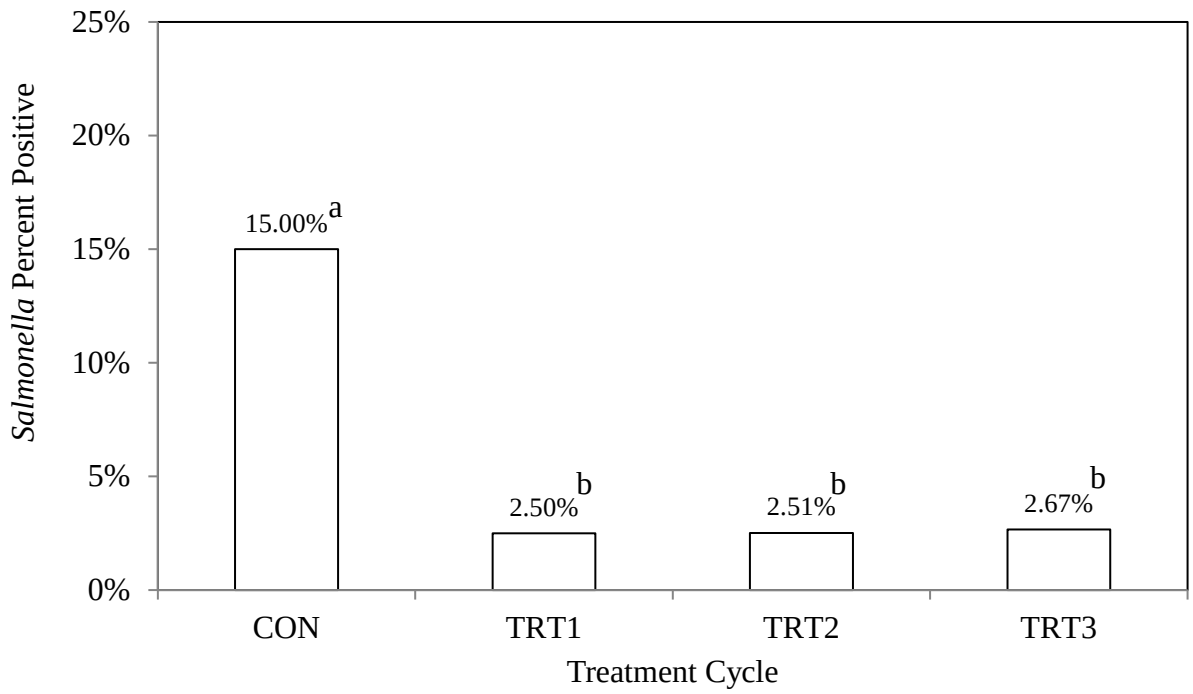

Figure 4. Percent *Salmonella* Prevalence on Broiler Portions from Control Samples (CON) and on Portions from Broilers Treated with *Saccharomyces Cerevisia* Yeast Metabolite (TRT)[1]

[1] Treatment cycles were analyzed from portions collected from the same flocks over sequential treatment periods including non-supplemented control flocks (CON), treated flocks cycle 1 (TRT1), treated flocks cycle 2 (TRT2), and treated flocks cycle 3 (TRT3).

Portions selected were 50% from Group A plants, and 50% from Group B Plants. Portions from Group A plants consisted of equivalent samples of bone-in split breast quarters, bone-in leg quarters, tenderloins, wings, and front half frames. Portions selected from Group B plants consisted of equivalent samples of boneless breast meat, boneless leg meat, tenderloins, wings, and front half frames. Portion samples were collected in process prior to final antimicrobial interventions.

[a – b] Means compared within whole parts products program with different letters are different at $P \leq 0.05$

CHAPTER VI

CONCLUSIONS

CONCLUSIONS

Researchers and integrated poultry companies have a common goal of finding novel new technologies to optimize bird performance while reducing bacteria relevant to food safety and bird health. Food safety, broiler performance, and yield efficiency are inter-connected goals; in this research, those variables were analyzed in respect to a yeast-based supplement that included *Saccharomyces cerevisiae* fermentation metabolites. This research was conducted to determine if this technology could be considered a viable option to consider in the continual search for effective strategies in broiler operations to optimize bird performance and yields, while contributing to pathogen reduction in support of other multi-hurdle food safety interventions.

In this dissertation research, the *Saccharomyces cerevisiae* metabolite intervention was studied to understand its impact on *Salmonella* in the broiler ceca and on component parts. This was a novel research project, as most previous studies of this beneficial micro-organism in broilers have focused on analyzing only internal organs. With growing consumer demand for portioned parts of raw muscle proteins, and tightening regulations regarding pathogens on those parts, the need for understanding the impact of any intervention on edible portions is appropriate. The results from this research indicated that a *Saccharomyces cerevisiae* fermentation metabolite product can have beneficial effects in reducing *Salmonella* in broiler ceca and component portions of the carcass. As previously discussed, the mechanisms of action of some yeast-based products can be the results of several factors. Although not analyzed in this study, such products have been shown to work through improved gut morphology with longer and deeper villi at the brush border (Rodriquez-Lecompte et al., 2012), creation of a protective mucous layer that restricts gut colonization in the intestinal tract (Baurhoo et al., 2007; Chee et al, 2010), and agglutination of pathogenic bacteria to dried yeast (Kogan and Kocher, 2007; White et al., 2002).

One benefit of using the *Saccharomyces cerevisiae* fermentation metabolite functional ingredient technology is that reduced pathogen colonization in the live bird may allow commercial processing plants to streamline or reduce the use of antimicrobials and other interventions during broiler processing. This could serve the dual purpose of offsetting costs of in-plant interventions, while meeting growing consumer interests to purchase chicken that has been exposed to fewer compounds during production that may be required to reduce pathogens and achieve tightening regulatory requirements.

Another part of this research included a study using tightly-controlled pens of broilers not exposed to typical industry challenges. This study resulted in similar bird performance and yields in treated and non-treated birds. Since the birds studied in the research pens were not subject to typical physiological challenges and stressors, the benefits of the *Saccharomyces cerevisiae* metabolite functional ingredient may have been minimized compared to expected impacts in commercial settings, consistent with expectations suggested by other researchers (Hahn-Didde and Purdum, 2016). As noted previously, other researchers have shown improvements in broiler performance using *Saccharomyces cerevisiae* metabolite treatments or other similar compounds, leading to improved broiler production (Bovo et al., 2015; Kidd et al., 2013). Improvements in live animal performance and production have also been observed in other food animal species exposed to products with *Saccharomyces cerevisiae* based compounds or metabolites (Gerritsen et al, 2012; Mao et al., 2013). As previously reviewed, these may have been due to the conserved intestinal structures that were improved across multiple species with similar mechanisms of action realized from various functional ingredients. When used in a commercial setting, companies electing to use a *Saccharomyces cerevisiae* metabolite product

would gain important information by measuring its impacts on pathogen quantities, feed conversion, yields, bird health, and reduction in the use of in-plant antimicrobials.

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APPENDIX

The Institutional Animal Care and Use Committee (IACUC) approval cited in Chapter 3 of this dissertation referred to University of Arkansas IACUC approved protocol #17065: *The Effect of XPC on broiler performance,* April 7, 2017. Data collected in this study was obtained by approved research staff and reported to the dissertation author and committee for analysis. No other IACUC protocols were required for any other chapters, all consisting of data provided by commercial operations.