Poultry Science

Controlling *Salmonella*: strategies for feed, the farm, and the processing plant

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ABSTRACT Controlling *Salmonella* in poultry is an ongoing food safety measure and while significant progress has been made, there is a need to continue to evaluate different strategies that include understanding *Salmonella*-poultry interaction, *Salmonella*-microbiota interactions, *Salmonella* genetics and response to adverse conditions, and preharvest and postharvest parameters that enable persistence. The purpose of this symposium is to discuss different strategies to consider from feed milling to the farm to the processing environment. This Poultry Science Association symposium paper is divided into 5 different sections that covers 1) immunological aspects of *Salmonella* control, 2) application of *Salmonella* genetics for targeted control strategies in poultry production, 3) improving poultry feed hygienics: utilizing feed manufacture techniques and equipment to improve feed hygienics, 4) practical on farm interventions for controlling *Salmonella*—what works and what may not work, and 5) monitoring and mitigating *Salmonella* in poultry. These topics elucidate the critical need to establish control strategies that will improve poultry gut health and limit conditions that exposes *Salmonella* to stress causing alterations to virulence and pathogenicity both at preharvest and postharvest poultry production. This information is relevant to the poultry industry's continued efforts to ensure food safety poultry production.

Key words: *Salmonella*, immunology, genetics, feed, postharvest

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INTRODUCTION

There has been a significant growth in poultry production in the United States over the past decade. Broiler production increased by over 23% from 2012 to 2022 with a value of $31.5 billion in 2021 from over 9 billion broilers produced (USDA-NASS United States Department of Agriculture-, 2022). Similar growth was reported in broiler exports (over 7 billion pounds) and per capita consumption (98.9 pounds) in 2022 (NCC, 2022). Despite this increase, the poultry industry has been severely challenged by food safety concerns. According to the Centers for Disease Control and Prevention (**CDC**), contaminated chicken contributes significantly to foodborne illnesses reported annually in the United States; and *Salmonella* and *Campylobacter* are often the culprits (Tack et al., 2020). *Salmonella* is a leading cause of bacterial foodborne infection in the United States, thus a top food safety concern.

This paper is obtained from a food safety symposium held at the 2023 Poultry Science Association Annual Meeting in Philadelphia, Pennsylvania, organized by the Industry Committee for Poultry Science to broadly address different aspects of *Salmonella* control from the bacteria-host interaction to preharvest and postharvest measures. Here, we cover 3 distinct themes that address: First, *Salmonella* control from immunological perspective where *Salmonella*'s evolution and chicken's immune response against *Salmonella* were elucidated. Second, *Salmonella* genetics for control, where we discussed advances in our understanding of *Salmonella* genetics and its use for developing targeted control strategies. Lastly, considerations for preharvest and postharvest control measures, here we shared advances in *Salmonella* interventions from the feed mill to the breeders and broilers and the processing stages of poultry production.

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IMMUNOLOGICAL ASPECTS OF *SALMONELLA* CONTROL

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Immunometabolism

Almost 10 yr ago, we began introducing the concept of immunometabolism into the poultry health and disease lexicon (Arsenault et al., 2013), particularly as part of the overall "gut health" discussion. At that time, the field concentrated on the changes in intracellular metabolic pathways in immune cells, especially macrophages and T cells, during different environmental stimuli that altered their function (activation, proliferation) and regulated inflammation (Michalek et al., 2011; O'Neill and Hardie, 2013; O'Neill et al., 2016). However, this cellular-based characterization of immunometabolism did not include the physiological and metabolic changes that occur in at the tissue level that will also contribute to the outcome of interaction between the host and infectious and noninfectious stimuli (Arsenault et al., 2013; Kogut and Arsenault, 2017). Today, the discussion of immunometabolism has expanded to include the overall physiological and metabolic health of the host organisms (Troha and Ayres, 2020; Lee et al., 2022). In fact, Troha and Ayres (2020) provide a compelling argument that the metabolic interactions between the host-microbiota-pathogen in the intestine directly influence both the virulence of the pathogen and the host defenses. First, the immunometabolic connections between host and microbiota direct not only host defenses, but also the overall physiology of the host against an infection. Second, these metabolic modifications during infection modulate immune function, promote tissue protection, and stimulate antivirulence mechanisms (Ayres, 2016; Rao et al., 2017; Troha and Ayres, 2020, 2022).

Enteric Immunometabolic System

The intestine is a physical, biochemical, and microbiological barrier system that forms the gut immune function.

Microbiota and Microbial Metabolite Production The avian commensal microbiota has a fundamental symbiotic functional association with the host; and thus, are involved in regulating bird health (Oakley et al., 2014; Stanley et al., 2014). Further, the microbiome directs host intestinal metabolism and immunity and drives a metabolome that affects energy balance and body weight in the avian host (Carrasco et al., 2019). Lastly, the residential microbes in the gut play a major role in inhibiting pathogens from colonizing by a process called colonization resistance (Shealy et al., 2021). The microbiota, using a number of biochemical pathways, metabolize diet- and host-derived metabolites that can have a direct impact on the intestinal immune system and inhibit colonization of the intestine by competitor bacteria. Additionally, bacterial metabolites including short-chain fatty acids (**SCFA**) serve as an energy source to the epithelial cells that line the intestine, but these SCFA may also be antimicrobial and limit virulence factor expression on pathogenic bacteria (Zhou et al., 2014; Zou et al., 2019; Gupta et al., 2020). Other examples include the degradation of dietary tryptophan to promote epithelial cell barrier function, the breakdown of dietary arginine which inhibits proinflammatory cytokine production (Fouad et al., 2021)

Intestinal Epithelium The epithelium physical firewall is a single layer of epithelial cells that separates the densely colonized, and environmentally exposed, intestinal lumen from the largely sterile subepithelial tissue. The intestinal epithelial cell layer displays a number of distinctive functions including production of antimicrobial peptides (defensins, cathelicidins, C-type lectins) and the secretion of mucus which are a key defense against luminal microbes. Besides being the primary barrier preventing a microbial breach of the intestine, the epithelial cells should also be considered part of the cellular component of the innate immune response possessing PRRs for sensing microbial-associated molecular patterns (**MAMPs**), but also capable of producing cytokines and chemokines to drive an inflammatory response against pathogen infection.

Cellular Immune System Below the epithelial layer is the final component of the intestinal barrier: the immunological barrier where the professional immune cells (macrophages, dendritic cells (**DCs**), and lymphocytes) reside in the lamina propria (Smith et al., 2021). This intestinal immune barrier has 2 distinct functions: the ability to respond to opportunistic pathogens, invasive pathogens, and microbial products while also maintaining a state of tolerance to the diverse and beneficial commensal intestinal microbes (Broom and Kogut, 2018). Both systems working together through innate immune sensing using PRRs on epithelial cells and professional immune cells in the lamina propria (DCs and macrophages), trigger immune pathways resulting in microbial killing and the activation of various acquired immune effector T cells (Th1, Th2, Th17, and Treg) all while keeping the resident microbiota in check without generating an overt inflammatory response. IgA-producing plasma cells, intraepithelial lymphocytes, and $\gamma\delta$T cell receptor-expressing T cells are lymphocytes that are uniquely present in the mucosa.

Enteric Neuroendocrine System The gut is more than a large complex immune organ, it is also thought to be the largest neuroendocrine organ in the body because of the large numbers of neurons, gut hormones, and secondary messengers involved in regulating an array of physiological functions in the host (Neuman et al., 2015; Cari and Knauf, 2016). The neuroendocrine system (**NES**) of the gut consists of 2 parts: the gut endocrine cells, located in the gut mucosa, and the enteric nervous system (**ENS**) in the gut submucosa. This system regulates several functions of the GI tract, such as motility,

secretion, absorption, microcirculation in the gut, local immune defense, and cell proliferation. The ENS comprises a large variety of neurotransmitters and associated receptors.

The gut contains a large number of enterochromaffin cells (endocrine cells that produce serotonin) that are dispersed among the epithelial cells of the gut mucosa in the intestine of the chicken (Rawdon, 1984; Hiramatsu, 2020). The gut endocrine cells secrete peptides signaling substances into the lamina propria of the gut lining, where they have regulatory activity on the ENS and the afferent and efferent nerve fibers of the central nervous system (**CNS**), in particular the autonomic nervous system (reviewed by Hiramatsu, 2020). These cells regulate several functions of the gastrointestinal tract, including sensation, motility, secretion, absorption, local immune defense, and even food intake (by affecting the appetite). Further, neurochemicals play a recognized role in determining bacterial colonization and interaction with the gut epithelium (Lyte et al., 2021).

Enteric Host Defenses Against Salmonella *Infection*

Preventing, resisting, and repairing infectious damage are integral parts of host defense (Ayres, 2016; Kogut and Arsenault, 2017). Historically, the chicken's immune strategies against a paratyphoid *Salmonella* infection involved a short-lived inflammation mediated by the increased expression of proinflammatory cytokine and chemokine genes in the intestinal tissue (Withanage et al., 2005; Setta et al., 2012; Matulova et al., 2013; Rychlik, 2020; Mon et al., 2021). The activation of the innate immune response induces an influx of heterophils (granulocytes) to the intestine that limits bacterial invasion (Kogut et al., 1994, 2012) but does not lead to a pathological inflammation that is seen in mammals (Patel and McCormick, 2014). New terminology has defined these mechanisms as disease resistance or antagonistic defenses strategies (Ayres, 2016; Troha and Ayres, 2020, 2022).

However, *Salmonella* have evolved the capacity to survive this initial immune response and persist in the gut lumen for weeks without causing clinical disease in birds (Van Immerseel et al., 2004). This persistent colonization of the intestinal tract is an important aspect of a *Salmonella* infection because it results in the silent propagation of bacteria in poultry stocks due to the impossibility to isolate contaminated animals.

This persistence also suggests that a second defense mechanism has evolved in chicken-*Salmonella* infection biology that functions to foster host health similar to that described by Ayres (2016). This alternate defense strategy, known as physiological or cooperative defenses, incorporate disease tolerance (tissue protective systems) and antivirulence mechanisms that inhibit pathogen-induced disease pathogenesis (Sanchez et al., 2018; Troha and Ayres, 2022) and promote asymptomatic carriage. This response in the chicken occurs around 48 to 96 h after initial paratyphoid *Salmonella* infection. These physiological defenses are characterized by an immunometabolic reprogramming of the cecum phenotype involving the host-microbiota-pathogen interactome that drives the pathogen toward a commensal relationship of the chicken host. First, there is a significant alteration in the cecal microbiota composition and metabolome (Lee et al., 2020; Mon et al., 2020). This is accompanied by a profound increase in T regulatory cells in the cecum which increases the expression of the immune regulatory cytokine IL-10 and redirection of the immune response to the bacterial pathogen to an anti-inflammatory, Th2-mediated response (Shanmugasundaram et al., 2015, 2021). *Salmonella* infection then induces a dramatic immunological reprogramming in the cecum that alters the host defenses to disease tolerance. Using kinome array analysis, functional T cell analysis, and mRNA transcriptional analysis of the *Salmonella*-infected cecal tissue, both genotypic and phenotypic alterations led to a tolerogenic local environment that resulted in the establishment of persistent, asymptomatic cecal colonization (Kogut et al., 2016; Kogut and Arsenault, 2017; Mon et al., 2021). In parallel with these alterations in the local immune responses, there is a dramatic metabolic phenotype alteration in the *Salmonella*-infected cecum. The immunometabolic profile of the cecum changes from an mTOR-mediated proinflammatory state to an anti-inflammatory state driven by adenosine monophosphate-activated protein kinase (**AMPK**)-directed oxidative phosphorylation (Kogut et al., 2016; Mon et al., 2020). Lastly, we also found that *Salmonella* intestinal colonization inhibits the release of neurochemicals that regulate the enteric neuroimmunological responses to infection (Redweik et al., 2021) and inhibit enteric neuron functionality, thus blocking the gut-brain axis control of the host response to the pathogen.

Because of the increase in antimicrobial-resistant microbes, the use of antibiotics as growth promoters have either been banned by government regulations or removed by producers due to the consumer demand for "no antibiotics ever" or "raised without antibiotics" poultry products. Thus, there is an ongoing demand for the development and use of alternatives to antibiotics for growth promotion and disease prevention (Kalia et al., 2022). To better understand the mechanisms of *Salmonella* persistence in the chicken gut, we have used a system biology approach to interrogate all the components of the infection biology of the chicken-*Salmonella* interaction. Immunometabolic reprogramming of the cecal phenotype has emerged as a critical mechanism of the establishment of a persistent, asymptomatic *Salmonella* infection in the chicken. The data suggest that these protective immunometabolic connections between the host and its microbiota might be manipulated as a therapeutic strategy and targeting of the regulators of this immunometabolism signify a promising translational approach.

APPLICATION OF *SALMONELLA* GENETICS FOR TARGETED CONTROL STRATEGIES IN POULTRY PRODUCTION

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General Overview

Foodborne *Salmonella* is a public health concern, and the emphasis continues to be directed toward poultry production, particularly raw poultry and eggs (Gast et al., 2022; O'Bryan et al., 2022). *Salmonella* remains challenging in part due to the large number of distinct serovars that comprise the group. In practice, detecting different serovars has evolved considerably with the introduction of molecular methods such as polymerase chain reaction (**PCR**) assays (Ricke et al., 2018). However, not all serovars behave equally regarding physiology, stress resistance, and pathogenesis (Andino and Hanning, 2015). Therefore, a comprehensive functional understanding at the genomic level is critical to delineate individual serovar responses and assign the appropriate risk associated with their presence. In recent years, considerable progress has been made in *Salmonella*'s whole genome sequencing (**WGS**). This progress has led to extensive databases that provide a better understanding of the genetics of *Salmonella*. In-depth genomic databases have provided an opportunity for developing specific strategies to study *Salmonella* and identify critical factors that influence *Salmonella* dissemination and survival. As a result, genomic-based methods can be applied to track-specific serovars and identify environmental factors that influence *Salmonella* virulence response during poultry production. This offers the opportunity to optimize more targeted interventions in both preharvest and postharvest production. Examples of these applications are discussed in the following sections.

Salmonella *and Stress—Genetic Responses*

Salmonella spp. possess numerous physiological systems to overcome encounters with environmental systems considered stressful and, depending on the circumstances, lead to increased virulence (Foster and Spector, 1995; Spector and Kenyon, 2012; Horn and Bhunia, 2018). In poultry production, some of the more likely stressors would be an application of acids to create a hostile lower pH. Acidic pH can occur in the gastrointestinal tract (**GIT**) via the generation of SCFA from the fermentative indigenous microbiota or the application of organic acids in poultry feeds and at the processing plant (Ricke, 2003; Dittoe et al., 2018). However, *Salmonella* can adapt to more inhibitory lethal pH levels below 4 if first exposed to more moderate pH levels (Foster, 1995, 1999). Under such conditions, *Salmonella* expresses acid tolerance by expressing an array of acid-shock inducible proteins that enable them to overcome the lethality of acid shock (Foster, 1995, 1999). This phenomenon also occurs in the presence of SCFA at neutral pH in concentrations comparable to GIT fermentation levels (Kwon and Ricke, 1998). It may be because *Salmonella* can produce SCFA under anaerobic conditions (Dunkley et al., 2009). Intuitively, *Salmonella* might be expected to have some tolerance to their own fermentation end products when inhabiting the poultry GIT.

Combatting Salmonella*: Genetic Identification of Targets*

In the poultry processing plant, acids, such as peroxyacetic acids, comprise some of the more popular antimicrobials applied to control pathogens during processing (Cano et al., 2021). A concern with any emphasis on a particular antimicrobial is the potential for tolerance and/or resistance developing in *Salmonella*. As discussed earlier, this can occur with acids and *Salmonella* and is a concern in meat and poultry products (Mani-López et al., 2012). An additional concern is a cross-resistance development, where global genetic networks of stress response genes allow for resistance responses to multiple environmental stressors (Rangel, 2011). In foods such as vegetables, where minimal processing occurs, combining several interventions, known as a multiple hurdle approach, has been viewed as a means to use lesser amounts of individual interventions assuming the combination will be synergistic (Mogren et al., 2018). While multiple hurdle approaches would appear practical, the potential for a pathogen such as *Salmonella* to express cross-protection to several antimicrobials confounds this strategy to some extent. However, once the genome of *Salmonella* spp. was sequenced, an opportunity to identify unrelated stress responses and avoid cross-protection in poultry production offered a means to design more optimal combinations of antimicrobials using transcriptomic responses such as microarrays to screen candidates (Ricke et al., 2013). As an illustration of this application, Milillo et al. (2011) used microarrays to demonstrate that heated organic acids were synergistic and disrupted the membranes of *S.* Typhimurium, causing intracellular leakage. When the transcriptomic microarray responses were analyzed, an impact was also noted at the genomic level, with both impairments of heat shock protein synthesis occurring due to membrane damage and repression of virulence gene expression. Transcriptomics have advanced since the introduction of microarrays, but the application of this type of screening to identify optimal targets for multiple hurdle combinations remains constant.

Tracking Salmonella *in Poultry Production*

Given the extensive range of serotypes and differences in physiology and pathogenesis, tracking individual isolates is critical to understanding *Salmonella*'s dissemination dynamics in poultry production. Historically,

marker strains of *Salmonella* that could be used in complex ecosystems, such as bird infection studies or inoculation on poultry carcasses, involved the generation of antibiotic-resistant strains. These were created by either isolation of naturally resistant isolates or selection for spontaneous mutants by growing the strain with the candidate antibiotic (Park et al., 2008). These antibiotically selected marker strains could easily be recovered in selective media containing the antibiotic and preventing the appearance of wildtype nonantibiotic-resistant *Salmonella*, which would otherwise have confounded the interpretation of the experiment (Park et al., 2008). Green fluorescent protein (**GFP**) genes have also been inserted in *Salmonella* to create marker strains, but these have been proven to negatively impact *Salmonella* growth kinetics (Oscar, 2003).

Advances in *Salmonella* genetics have led to more precise abilities to track multiple strains of *Salmonella* simultaneously while avoiding detrimental impacts on growth physiology. One outcome is the ability to create DNA-barcoded strains that can be detected. Ideally, a *Salmonella* isolate of interest should be minimally impacted by creating the respective marker strain counterpart. In addition, antibiotic resistance and GFP-based marker strains are limited to single isolates as there is no way to differentiate multiple strains in combinations. Conceptually, a unique sequence can be inserted anywhere on the chromosome of a microorganism such as *Salmonella*, as Yang et al. (2019) described. The key is that the sequence is unique to that particular isolate and is inserted in a region of the chromosome that does not compromise the functional fitness of the recipient. Once inserted, this sequence can be differentiated for detecting and tracking individual serovars or strains within a serovar. Yang et al. (2017, 2018) were able to track transmission routes for *S.* Enteritidis in broilers and demonstrate competitive exclusion in broilers among different barcoded strains of *Salmonella*. This approach should lead to greater precision in identifying specific transmission routes in poultry live bird production and the processing plant. The ability to barcode different *Salmonella* serovars would allow for identifying distribution patterns and frequency of occurrence and provide insight into *Salmonella* ecology in poultry production.

IMPROVING POULTRY FEED HYGIENICS: UTLIZING FEED MANUFACTURE TECHNIQUES AND EQUIPMENT TO IMPROVE FEED HYGIENE

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Feed is thought to be a major vector for pathogens in poultry flocks, with *Salmonella* being identified as one of the most common biological hazards associated with all classes of animal feed (McIlroy, 1996; Jones, 2011). Salmonellosis affects thousands of individuals each year, leading to more efforts to control *Salmonella* pre- and postharvest. Recent concerns regarding food safety have led to more focus being placed on preharvest interventions being implemented to control pathogens, such as *Salmonella*, in feed. Currently, there are a few practices ranging in complexity and management, which can be employed by feed mills to reduce *Salmonella* entering the feed mill and contaminating finished feed.

Efforts to prevent *Salmonella* from entering the feed mill should start before raw ingredients arrive. One attribute that *Salmonella* possesses is the ability to persist in various feed ingredients and survive in low moisture environments and low water activity foods, such as all classes of feed (Jones, 2011; Netto et al., 2019; Boltz et al., 2021). Purchasing ingredients from known suppliers that regularly sample and test their products can greatly reduce the likelihood of bringing pathogens into the facility (Muckey, 2016). Personnel at the feed mill should also visually inspect each load for contamination, such as mold or foreign materials, and reject any loads that appear contaminated (Jones, 2008, 2011). Establishing "clean" and "dirty" zones in the feed mill and limiting access between them can aid in preventing any cross-contamination of *Salmonella* and other pathogens from raw ingredients to finished feed (Morita et al., 2006). These zones can be color-coded to allow personnel to easily distinguish them from one another or the use of walls can physically separate the 2 zones (Muckey, 2016). Having separate cleaning equipment, such as brooms and shovels between the 2 zones will also decrease the likelihood of cross-contamination. If workers must go from "dirty" to "clean," the use of antibacterial foot baths or personal protective equipment (**PPE**) should be utilized to reduce the possible spread of pathogens.

Keeping the feed mill environment well-kept, such as limiting the amount of dust build-up and pests in the facility can minimize sources of introduction and creating a suitable environment for *Salmonella* to reproduce and thrive. Dust has been identified as a major source of *Salmonella* contamination in feed mills, so control for it is vital to keeping a feed mill pathogen-free (Nape, 1968; Butcher and Miles, 1995; Jones, 2011). All raw ingredients should be unloaded in the dirty zone and treated as if they are contaminated and handled with caution while being unloaded (Jones, 2011). Maintaining the dust handling equipment throughout the feed mill will decrease the amount of dust build-up and *Salmonella* spread. Raw ingredient receiving areas are one area of focus since raw ingredients produce the most dust compared to other points in the feed mill (Jones, 2011). Large amounts of dust can also be produced from grinding equipment, such as hammer mills, if air quality equipment is improperly inspected and maintained (McCarty, 2005; McDaniel, 2005). Air vents and flow for a feed mill should also be carefully thought out to reduce the risk of dust cross-contamination. The vents on the outside of the feed mill should be separated from any air intakes since this can decrease the risk of potentially contaminated dust from being pulled back into the

feed mill (Jones, 2008). A good practice is to have air intakes equipped with a filter that is changed regularly to keep any contaminated dust or other possible vectors outside the mill (Jones, 2008).

Along with controlling dust in the feed mill, regular cleaning will improve the feed mill's overall cleanliness. Cleaning up spilled oil and fat and excess accumulation in the feed mill will decrease areas that can harbor *Salmonella* and cross-contamination with finished feed (D'Aoust, 1997; Nayak, 2000; Jones, 2011). Cleaning up spilled ingredients and feed regularly will decrease food sources for pests, such as rodents and wild birds, improving the cleanliness of the mill and reducing possible vectors for *Salmonella* (Morita et al., 2006; Benskin et al., 2009). Limiting any excess moisture in the feed mill will inhibit *Salmonella*'s ability to grow and reproduce (Jones, 2011). Past work has demonstrated that most feed contamination occurs in the system, especially inside the cooler (Israelsen et al., 1996). *Salmonella* has been known to create biofilms, a layer of microorganisms, that form in niche areas that can be difficult to eliminate (Shi and Zhu, 2009). This is especially true in locations with continual water access, such as the cooler deck. As every feed mill has its own unique design, workers must identify any areas in that feed mill that could harbor biofilms, including *Salmonella* (Shi and Zhu, 2009).

During the pelleting process, mash feed is subjected to heat for varying lengths of time before being extruded through a pellet die. Manipulation of steam to generate higher conditioning temperatures and adjustment of equipment settings to allow for extended conditioning times are common and effective ways to control pathogens during pelleting. Standard conditioning temperatures and times can vary greatly depending on the geographical location, diet formulations, production rate, and management. Temperatures over 80°C are commonly used for conditioning due to temperature manipulation being easier to accommodate in feed mills (Perera et al., 2021). Conversely, steam conditioning times can be more difficult to manipulate compared to steam conditioning temperatures due to the complexity of altering the steam conditioning system (Boney et al., 2018). Previous work has shown that conditioning temperature and time is an effective way to reduce the pathogen load of feed as well as increase other important feed quality metrics such as pellet quality (Behnke, 1994; Amerah et al., 2011; Abdollahi et al., 2013; Boney and Moritz, 2017; Boney et al., 2018; Boltz et al., 2019, 2020; Rueda et al., 2022). Boney et al. (2018) demonstrated a 3-log reduction in *Enterococcus faecium* (*E. faecium*; a nonpathogenic surrogate for *Salmonella*) when feed was steam conditioned for 30 s and saw a greater improvement of 4-log reduction when feed was steam conditioned for 60 s. Later work by Boltz et al. (2019) demonstrated a 3- and 4-log reduction in the same *E. faecium* when feed was pelleted using 2 different techniques: standard pelleting at 70°C for 15 s with no additional hygeinizer use and more thermally aggressive pelleting at 80°C for 30 s with an additional 45 s retention time in the hygienizer. A potential downfall of utilizing increased conditioning temperatures and times is that heat-liable nutrients, such as amino acids and exogenous enzymes, can be degraded and result in decreased growth (Cutlip et al., 2008; Boroojeni et al., 2014; Loar et al., 2014; Boltz et al., 2020). Lynch et al. (2023) recently showed that a 10% increase in lysine was needed when feed is pelleted using a hygienic technique (88°C for 60 s and 6 min retention in a hygienizer) to be comparable to a standard pelleting technique (77°C for 30 s with no additional hygienizer retention). Nutritionists could overformulate specific amino acids and enzymes into diets to account for the losses during pelleting, but these may not be feasible due to the availability of some of these ingredients and the cost associated with these synthetic amino acids.

Thermal processing is a valuable tool to control pathogens but leaves the finished feed susceptible to cross-contamination during cooling, bagging/loadout, and transport to the farm. Chemical feed additives can be used to control pathogens during the pelleting process, as well as provide protection after thermal processing is completed. Some of the most used chemicals presently are formaldehyde-based products, organic acids, and essential oils (Ricke, 2003; Muckey, 2016). These products do come with some concerns, mainly the cost associated with specialized application equipment, and the potential of being a hazard to worker health due to prolonged exposure (Sheldon and Brake, 1991; Muckey, 2016). Formaldehyde has been considered one of the most effective antimicrobial treatments for animal feed but has limited use due to labeling concerns and special permits (Gosling et al., 2021). Chemicals, such as formaldehyde and alcohols, have been shown to be viable options to sanitize various surfaces and equipment in the feed mill (Carrique-Mas et al., 2007; Møretrø et al., 2009; Cochrane et al., 2016). However, this may be difficult to accomplish due to needing to physically remove all organic material adhering to the surfaces, and most of the equipment in these facilities was not designed to be clean-in-place.

Salmonella is a ubiquitous pathogen that requires constant diligence to try and prevent it from entering and spreading throughout a feed mill. Receiving ingredients from trusted sources, cleaning up major spills, maintaining dust handling systems, and controlling pests can all be taken to reduce the likelihood of *Salmonella* entering the feed mill. Thermal processing that occurs during pelleting process is another step that can be used to reduce any *Salmonella* that may have been in the raw ingredients. The use of chemicals in the feed and for the sanitation of equipment can aid in preventing contamination of finished feed, but care needs to be taken regarding labeling of feed and for worker safety. All these steps together can help to prevent the spread of *Salmonella* to flocks and ultimately to consumers.

PRACTICAL ON FARM INTERVENTIONS FOR CONTROLLING *SALMONELLA*—WHAT WORKS AND WHAT MIGHT WORK

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Due to the ubiquitous nature of *Salmonella* with poultry, initial contact with the bacterium if it is present in birds or in the environment is almost inevitable. As a result of this, control measures are put in place protect birds, and thus consumers, from colonization. Traditionally, control of these organisms in food occurred through safeguarding the microbial integrity of food or decontamination before consumption, if or when it occurs (Gast, 2007). Antimicrobials and other bacterial reducing agents utilized during processing to decontaminate carcasses cannot always compensate for insufficient control measures during the grow-out period. Thus, highlighting the need for preharvest control measures. Preharvest control measures can range from least specific to most: management and sanitation, gastrointestinal colonization control (**GCC**), and vaccination.

Management and Sanitation

Proper biosecurity and general hygiene are some of the most important and useful management tools necessary to effectively reduce *Salmonella* presence during growout. Without these tools, other methods such as GCC and vaccination implemented to reduce and eliminate *Salmonella* presence are practically useless (van Immerseel et al., 2009). Effective management and sanitation that encompasses all aspects of the poultry production continuum including *Salmonella*-free breeding chicks, hatchery management, proper cleaning and disinfection, effective insect, and rodent management programs, strictly enforced biosecurity programs, and decontamination programs for litter, feed, and water (Hopp et al., 1999).

Salmonella is known to colonize reproductive organs of hens leading to the deposition of the bacteria into the eggs, possibly producing a *Salmonella*-positive chick (Berchieri et al., 2001; Gast et al., 2004). Therefore, sourcing of hatching eggs from *Salmonella*-free breeder flocks is ideal (Gast, 2007). The egg may be exposed to pathogenic organisms from fecal material on the surface of the egg or the air may be contaminated. Sanitization methods that remove the egg cuticle are not preferred because removal can expose egg pores allowing an entry point through eggshell penetration and can impact hatchability (Wang and Slavik, 1998). In its place, methods such as ultraviolet irradiation of hatching eggs can be utilized without affecting hatchability (Coufal et al., 2003).

Since *Salmonella* can also be introduced to the grow-out facility by shared equipment and personnel, enforcement of biosecurity measures including, but not limited to, monitoring of movement onto the farm, use of protective clothing and designated footwear between house, and proper cleaning and disinfection of shared equipment prior to movement around the farm is necessary (van Immerseel et al., 2009). Cleaning and disinfection programs are geared toward reducing the microbial load in the grow-out house. Four basic principles should be followed to ensure the best possible outcome: 1) dry cleaning followed by wet cleaning to remove dirt and organic matter that could impair disinfectant use, 2) appropriate use of disinfectants to kill microorganisms, 3) rinsing to clear residue, and 4) fumigation (Morgan-Jones, 1987). Common disinfectants used include aldehydes, peroxides, quaternary ammonium compounds, and phenolic substances. The efficacy of these programs can vary due to the procedure and the proper use of products (Davies and Breslin, 2003).

Rodents and insects may act as mechanical and biological vectors involved in the introduction of *Salmonella* within the house, but also spreading the organism around the farm. Methods to control these pests can involve physical control methods such as preventing access to the building, traps and bait stations, clearing of vegetation around the house, or rotational chemical control with insecticides and rodenticides (van Immerseel et al., 2009).

Decontamination of water, feed, and litter are important as these could be potential sources of *Salmonella* introduction. Chlorine can be used to sanitize the water lines, with varying efficacy, but does not necessarily result in reduced cecal colonization (Poppe, 2000). Chemical poultry litter amendments such organic acid, formalin, sodium bisulfate, sodium sulfate, and sulfuric acid are used primarily to reduce ammonia emission by pH modification of the litter; however, control of *Salmonella* and other pathogens may be a secondary effect of this pH modification (Vicente et al., 2007).

Gastrointestinal Colonization Control

GCC programs broadly refer to methods involved in reducing pathogen colonization or the numbers of the organism found within the gastrointestinal tract (Gast, 2007). GCC programs can include a wide variety of methods such as dietary modification, antibiotics, organic acids, prebiotics, probiotics, synbiotics (a combination of prebiotics and probiotics), competitive exclusion (**CE**), and others (i.e., antimicrobial peptides, essential oils, and bacteriophages) (van Immerseel et al., 2009; Vandeplas et al., 2010).

With the poultry industry moving toward reducing/eliminating antibiotic use due to concerns about developing resistant bacteria in human health has brought about more "natural" methods (Vandeplas et al., 2010). One such example is organic acids in the form of SCFA and medium-chain fatty acids (van Immerseel et al., 2009). As little as 1.25 minimolar (**mM**) of the medium fatty acid monocaprin in an emulsion resulted in a bactericidal effect (6−7 log decrease) against *Salmonella* Enteritidis (Thormar et al., 2006).

Prebiotics, probiotics, and synbiotics also offer potential in reducing colonization of *Salmonella* in live birds

(Schrezenmeir and de Vrese, 2001; Vandeplas et al., 2010). A common prebiotic are mannooligosaccharides, and its inclusion at 4,000 parts per million led to a 34% decrease in cecal colonization with *Salmonella* Dublin in 10-day-old birds (Spring et al., 2000). Bacterial species typically associated with probiotics are *Lactobacillus, Enterococcus, Pediococcus*, and *Bacillus* spp. (Vandeplas et al., 2010). In vitro, 12 *Lactobacillus* strains used were effective against *Salmonella* attachment to ileal epithelial cells by blocking attachment sites making them unavailable to *Salmonella*, but also through the production of lactic acid as an inhibitory substance against *Salmonella* (Jin et al., 1996). Studies evaluating the use of synbiotics against *Salmonella* in poultry have been conducted through competitive exclusion cultures. One such example is a significant decrease in the recovery of *Salmonella* Enteritidis in birds administered 0.1% fructooligosaccharide and 0.1% fructooligosaccharide plus competitive exclusion culture compared with the control and birds administered CE 1- and 7-days postinoculation (Fukata et al., 1999).

Competitive exclusion treatments utilize a defined or undefined bacteria culture, typically sourced from mature birds, to minimize chick susceptibility to *Salmonella* colonization before the establishment of their own resident microflora (Gast, 2007). Similar to their inclusion as a probiotic, bacterial species most often included are: *Lactobacillus, Bifidobacterium*, and *Bacillus* spp. and can be administered in many ways through installation into the crop, mist, drinking water, or used as a feed additive (Gast, 2007). Protection of the chick by *Salmonella* colonization through competitive exclusion results from interference with *Salmonella* attachment and inhibition of growth through VFA production (Schneitz, 2005).

Vaccination

Use of live or inactivated *Salmonella* vaccines only reduces the susceptibility to *Salmonella* and cannot create an impermeable barrier against infection (Gast, 2007). For broilers, the use of vaccines in production is challenging, but vaccination of broiler breeders is worthy of investigation. In this case, vaccination can reduce the prevalence of *Salmonella* in breeding hens, thus their progeny, but also increases passive immunity in broilers (Dórea et al., 2010). This has been demonstrated with decreased prevalence of *Salmonella* on breeder carcasses compared to unvaccinated breeders, but also in the broiler progeny with differences in *Salmonella* prevalence in chick box liners, litter swabs, dust, and on the carcasses compared to the progeny of the unvaccinated breeders.

MONITORING AND MITIGATING *SALMONELLA* IN POULTRY

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Salmonella—*A Food Safety Concern in Poultry*

Salmonella continues to be problematic in poultry, despite mitigation efforts. The Interagency Food Safety Analytics Collaboration (**IFSAC**) reported an estimated 17.3% cases of foodborne *Salmonella* was attributed to chicken, while the CDC evaluation of incidences and trends of foodborne illnesses for *Salmonella* during 2019 was 17.1 incidences per 100,000 people (Tack et al., 2020; IFSAC, 2022). The most common serovars identified in foodborne outbreak cases include Enteritidis, Newport, Typhimurium, Javiana, I 4,[5],12:i:-, Oranienburg, and Infantis (Collins et al., 2022). *Salmonella* has continued to evolve, and studies have demonstrated the challenges to effectively mitigate *Salmonella* might be attributed to the diversity of *Salmonella* serotypes (Cox et al., 2019; Obe et al., 2021a,b). There are more than 2,500 serotypes of *Salmonella*, and they can be different in their expression both at the genetic and phenotypic level (Grimont and Weill, 2007; Andino and Hanning, 2015). In fact, some studies have suggested that these serovars can coexist, compete, and express different characteristics like host adaptation, pathogenicity, and defense mechanism for survival (Uzzau et al., 2000; Cheng et al., 2019; Obe et al., 2020; Larsen et al., 2021). While differences in serotype dynamics have been shown (Andino and Hanning, 2015; Cox et al., 2019; Obe et al., 2021b), in-depth understanding of whether this diversity extends to strain-strain variation are still limited and perhaps being explored. Notably, the most common *Salmonella* serotypes found in poultry have continued to change, while more pathogenic serotypes like Heidelberg was previously very persistent in poultry, others like Kentucky have emerged and occupied the ecological niche. In fact, in recent times there has been emergence of serovar Infantis in samples from chicken (Foley et al., 2011; McMillan et al., 2020). This shows that as one serotype is being controlled, another is emerging, thereby making mitigation efforts more challenging. Moreover, *Salmonella* tolerance to antimicrobial treatment has not been comparable among all serotypes, as data from many studies are contradictory. This might be because different studies used different serotypes and most likely different strains of the serotype. Differences in experimental conditions might have also contributed to the variation in results. Given these differences, strain-to-strain variation in *Salmonella* survival under different experimental conditions might be inferred, and this variation must be investigated as part of the continued effort to establish effective mitigation practices.

Salmonella *Mitigation—Where Should We Begin?*

Beside vaccination and management practices during preharvest production, *Salmonella* interventions have been focused on the postharvest poultry production, that is, at processing establishments. A possible

explanation is that the processing plant is often the last stage of the poultry production continuum for raw chicken and where the last intervention is applied. In the past 5 yr there have been a few studies that evaluated *Salmonella* in different poultry processing establishments and showed significant progress being made by the industry (De Villena et al., 2022; Rasamsetti et al., 2022; Thames et al., 2022; Rasamsetti and Shariat, 2023). However, there is not a lot of research on *Salmonella* control during live production. Given the recent framework proposed by the Food Safety and Inspection Service (**FSIS**) to reduce cases of salmonellosis linked to poultry, poultry establishments should consider evaluating all stages of poultry production where rigorous mitigation strategies could have an impact on *Salmonella*. It is important for individual establishments to assess current mitigation practices to understand the persistence of *Salmonella* and modify strategies as needed. These efforts should include live production and processing for effective *Salmonella* control.

Surveillance and Mitigation Practices Across Poultry Production

At processing, many improvements in *Salmonella* control have occurred, partly due to the establishment of the Hazard Analysis and Critical Control Point (**HACCP**) final rule by FSIS in 1996 with the goal to reduce foodborne pathogens on meat and products, thereby reducing foodborne illness caused by the consumption of contaminated products. Upon the implementation of HACCP, poultry establishments were expected to meet performance standards that entail FSIS collecting samples of chicken carcasses in each establishment over a 51-moving window (USDA-FSIS, 1996, 2006; Williams and Ebel, 2012; NCC, 2019). The agency publishes the report of each poultry establishment based on 3 categories by targeting a *Salmonella* positive acceptable limit of 9.8% (5 of 51) on carcasses and 15.4% (8 of 52) on chicken parts. For broiler carcass, a processing plant that desired category 1 must achieve 50% or less of the maximum acceptable limit allowed (i.e., ≤4.9%) during the most recent sampling moving window, whereas category 2 signifies that a plant meet the maximum acceptable limit allowed but with results over 50% of the allowed acceptable limit (i.e., >4.9 and ≤9.8%) during the most recent sampling window. Category 3 is less desired because it means that a plant has exceeded the maximum acceptable limit allowed (>9.8%) in the performance standard during the most recent sampling window (USDA-FSIS, 2021). While the implementation of HACCP program has increased *Salmonella* monitoring and influenced control programs, especially as more plants are performing internal pathogen testing, there have been inconsistencies in overall reduction of *Salmonella* occurrence in meat products. Williams et al. (2020) reviewed the changes in *Salmonella* occurrence in meat products since the implementation of the HACCP rule and showed there was an initial reduction in the 1990s that was lost over time. The authors suggested these occurrences are partly due to changes in sampling methods and several other potential factors. In addition, a recent review of FSIS surveillance plant sampling data showed a slight increase of 0.66% *Salmonella* positives from poultry carcasses from 2016 to 2020 and a substantial 46% decrease in parts during the same time (Siceloff et al., 2022). A few other recent studies have biomapped *Salmonella* occurrence throughout processing stages and have shown advances in *Salmonella* surveillance and mitigation specific to the plants studied (De Villena et al., 2022; Rasamsetti et al., 2022). De Villena et al. (2022) utilized microbial biomapping approach and quantified pathogen load in a processing facility that uses high and low levels of antimicrobials like peracetic acid and sodium hypochlorite and concluded that quantification of microbial foodborne pathogens and indicator organisms at different processing stages can help understand where specific interventions and antimicrobial levels can be targeted for effective *Salmonella* control. Moreover, each establishment needs to conduct a biomapping baseline as part of process control to make informed food safety decisions. Similarly, the work of Rasamsetti and Shariat (2023) used biomapping to reveal changes in *Salmonella* serotype populations at different stages of processing. The study showed changes in *Salmonella* serotype dynamic between prechill (before antimicrobial intervention) and postchill (after antimicrobial intervention) as some serotypes are more effectively mitigated with antimicrobials at each processing plant studied. Interestingly, these studies are also vital to understanding changes in virulence patterns of *Salmonella* serotypes and the emergence of antimicrobial tolerant serotypes like Infantis that are quickly becoming problematic in poultry production.

At preharvest, there is no standard regulation for *Salmonella* control, but several poultry integrators perform internal monitoring as part of a company-specific control strategy. Using the data from such monitoring, integrators are able modify their vaccination program and biosecurity measures. While these efforts can be effective, *Salmonella* continues to evolve past common strategies and require a more exhaustive multihurdle approach that includes the farm and the processing plant. Some studies on prevalence, quantity, and serovar population complexities have shed some light into *Salmonella* persistence at preharvest, particularly on some pathogenic serotypes that are seen in processing samples since *Salmonella* isolated at postharvest must have originated from birds at preharvest (Rothrock et al., 2021; Siceloff et al., 2022; Wang et al., 2023). Serotype Kentucky is the most isolated in live production samples, but not as common in processing samples suggesting the effectiveness of processing control measures on attenuating the serovar (Rothrock et al., 2021; Rasamsetti and Shariat, 2023). However, this is not true for other more pathogenic serotypes like Enteritidis, Infantis, and Typhimurium that might be more tolerant to antimicrobial interventions (Siceloff et al., 2022). Furthermore, a recent surveillance of 80 broiler flocks from different

poultry complexes suggest that *Salmonella* occurrence and load from flocks within a farm can differ and further investigation at live production into serotype populations can help poultry establishments amend their control measures before flocks are transported to the processing facilities (Obe et al., 2023). There is a need for more research to understand the mechanism underlying serovar-specific differences in virulence and persistence of *Salmonella*, especially under different environmental conditions both at the farm and processing plant. For mitigation at preharvest, vaccination has commonly been used in breeders and broilers to reduce *Salmonella* colonization and prevalence and some studies have evaluated the use of feed additives like probiotics for competitive exclusion (Micciche et al., 2018; Kimminau et al., 2021; Fulnechek, 2022; Juricova et al., 2022). However, many feed additives have not shown similar efficacy against *Salmonella*. There exists a need for more research to identify effective feed additives. *Salmonella* control at preharvest must follow a multihurdle approach where other interventions are supplemented with vaccination to reduce load and pathogenic serotypes.

The FSIS proposed *Salmonella* framework will require testing incoming flocks for *Salmonella* before slaughter and establishments will have to devise a processing strategy. This will put pressure on poultry companies to adopt a more conscientious strategy beyond vaccination and biosecurity in their fight against *Salmonella*. *Salmonella* mitigation will require routine monitoring across the production chain, including quantification and identifying harborage sites at the farm and plant to understand persistent serotypes and develop effective control measures.

INDUSTRY PERSPECTIVE

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Food safety is the top priority for companies that produce and process chicken products in the United States, and the industry prides itself on delivering safe, affordable, and wholesome food both domestically and abroad. The chicken industry continues to meet food safety challenges head-on and has done an outstanding job of improving the microbiological profile of raw products. The FSIS is the public health agency in United States Department of Agriculture (**USDA**) that is responsible for inspection at broiler chicken processing facilities (those facilities that process chickens for meat). The U. S. meat and poultry inspection system complements industry efforts to ensure that the nation's commercial supply of meat and poultry products is safe, wholesome, and correctly labeled and packaged.

Food safety standards are applied to all chicken products produced in the United States and countries that import chicken products must also meet these federal standards. One of the pathogens monitored by FSIS is *Salmonella*, whose prevalence is determined on a routine basis. In the most recently published report by FSIS that includes data through March 2023, an average of 3.4% of chicken carcasses at processing plants nationwide tested positive for detectable levels of *Salmonella*—well below the USDA performance standard of 9.8% for *Salmonella* on raw chicken carcasses. The same holds true for chicken parts. Since FSIS implemented the parts performance standard in 2016, there has been a 65% reduction in *Salmonella* on chicken parts. In fact, the most recent data out of FSIS indicated that less than 7% of chicken parts tested positive for detectable levels of *Salmonella*—also below the USDA *Salmonella* performance standard which is set at 15.4%.

Modernizations, ongoing research, innovation, and technology have helped the industry better address the food safety challenges of today and tomorrow.

DISCLOSURES

The authors declare that they have no conflict of interests.

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