ABSTRACT

Title of Thesis: EVALUATING DIFFERENCES IN BODY WEIGHT, GUT MORPHOLOGY, IMMUNE RESPONSE, AND SICKNESS BEHAVIOR IN FAST- AND SLOW- GROWING BROILER CHICKENS WHEN INFECTED WITH *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM

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Fast growth rate in broilers comes with welfare concerns and research is needed to determine if fast- and slow-growing broilers differ in pathogen resistance. The objective of this study was to evaluate differences in fast- (FG) and slow-growing (SG) broilers when challenged with *Salmonella* Typhimurium or broth (control) 14 days post-hatch. Plasma IgA and IgG, jejunum and ileum histomorphology, and behaviors were measured. FG had greater d12 and d24 body weight and d7 jejunum measures, indicating

better absorption, and earlier increases in plasma IgA and IgG, indicating earlier immune development. SG had greater d7 IgG, indicating stronger maternal immunity. Post-challenge, FG gut morphology was more impaired, and SG had greater IgA and reduced sham foraging, indicating a stronger immune response to challenge. The results illustrate fast- and slow-growing broilers differ in *Salmonella* resistance, which can help breeders make selection decisions to prevent *Salmonella* transmission into the human food supply.

EVALUATING DIFFERENCES IN BODY WEIGHT, GUT MORPHOLOGY, IMMUNE RESPONSE, AND SICKNESS BEHAVIOR IN FAST- AND SLOW-GROWING BROILER CHICKENS WHEN INFECTED WITH *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM

by

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AGP - antibiotic growth promoter

AMR - antimicrobial resistance

BSA – bismuth sulfite agar

BSL-2 – biosecurity level 2

CON – TSB control treatment

DPC – Days Post-challenge

FG – Fast Growing breed (Ross 308)

IFN-γ - interferon-gamma

IgA – Immunoglobulin A

IgG – Immunoglobulin G (also referred to as IgY)

IgM – Immunoglobulin M

IL-1 – interleukin 1

IL-1β – interleukin 1 beta

IL-6 – interleukin 6

IL-12 – interleukin 12

IL-18 – interleukin 18

LPS – lipopolysaccharide

NAL – nalidixic acid antibiotic

SG – Slow Growing breed (Redbro)

SPF – Specific-pathogen-free

ST – *Salmonella* Typhimuriu*m* challenge treatment

Th1 – T helper type 1 response

Th2 – T helper type 2 response

TNF-α – tumor necrosis factor alpha

TSB – Tryptic Soy Broth

VFD - veterinary feed directive

CHAPTER 1 Literature Review

1.1 INTRODUCTION

The United States has the largest broiler chicken industry in the world (NCC, 2019), producing 43 billion pounds of chicken in 2019 and 44 billion pounds in 2020 (NCC, 2021a). Production is predicted to continue to increase beyond 2021 (NCC, 2021a). In order to meet high consumer demand, broilers are genetically selected for increased feed efficiency and greater breast yield (Torrey et al., 2021), resulting in birds that reach heavier market weights at incredible growth rates (NCC, 2021b). However, genetic selection for fast growth and other production parameters may inadvertently negatively affect immune function (Iuspa et al., 2020). This is particularly important because unavoidable stressors can occur in modern intensive production systems and result in greater risk of disease (Quinteiro-Filho et al., 2012). It is critical to determine if growth rate affects how chickens respond to infection and colonization by pathogens, such as *Salmonella*.

Salmonella is a frequent cause of human foodborne illness around the world (Knodler and Elfenbein, 2020) and source of an estimated 1.35 million infections and 420 deaths per year in the United States (CDC, 2020). Some of the largest meat recalls in the United States have been caused by *Salmonella* contamination (Bearson et al., 2017) and many of these recalls were linked to chicken meat and eggs. While eliminating *Salmonella* from chicken products reduces human infection risk, it also reduces the economic gains to the poultry industry and bird welfare because it can lead to poor bird performance, increased drug costs, and mortality (Janardhana et al., 2007; Yunis et al., 2000). However, *Salmonella enterica*, the genus often responsible for human infection by *Salmonella* (called Salmonellosis), rarely induces clinical symptoms in poultry (Barrow

et al., 2012). *Salmonella enterica* serovars (subtypes) *S. enterica* ser. Enteritidis and *S. enterica* ser. Typhimurium are considered commensal-like members of the microbiome in poultry (Oakley et al., 2014; Chambers and Gong, 2011; Humphrey, 2006), and chickens serve as persistent, asymptomatic carriers that rarely show behavioral or clinical signs of infection by these *Salmonella* serovars (Shanmugasundaram et al., 2019; Bearson et al., 2017). Not all chicken immune responses successfully clear *Salmonella*, either (Humphrey, 2006). This makes *Salmonella* transmission in flocks and contamination of chicken products difficult to control. It is particularly clear in young chicks with naive immune systems that are more vulnerable to *Salmonella* infection and disease (Bearson et al., 2017). *Salmonella* infection may induce immune stress in chickens, resulting in an inflammatory response in the intestines (Gomes et al., 2014), reduced appetite and growth performance (Liu et al., 2014), depression, lethargy, fever, and diarrhea (Xie et al., 2000) in symptomatic birds. Due to the risk chicken *Salmonella* infection poses to human health, broiler welfare, and broiler performance, it is important to control *Salmonella* in broiler flocks.

Salmonella control measures currently exist, including restricted antibiotic use, vaccination programs, sanitation programs, and more recently dietary changes such as probiotic and prebiotic supplementation. However, some methods of disease control in poultry can be ineffective (Yunis et al., 2000). Even rigorous cleaning and disinfection standards cannot completely eliminate *Salmonella* in broiler flocks (Shanmugasundaram et al., 2019). Control measures to mitigate an outbreak can become expensive due to the sheer number of birds produced and the presence and rapid spread of *Salmonella* among poultry (Lalsiamthara and Lee, 2017). Since *Salmonella* spreads horizontally via the

fecal-oral route, droppings from a single infected bird may be consumed by dozens of other birds, whose droppings may then infect even more birds until an entire house is *Salmonella* positive. Additionally, antibiotic growth promoters (AGP) had previously been widely used to improve growth and manage bacteria in broilers (Brisbin, 2011) but their usage has contributed to the increasingly critical global issue of antimicrobial resistance (AMR) (Montoro-Dasi et al., 2020; Zhou et al., 2012). Some *Salmonella* serovars are already resistant to antimicrobials (Bearson et al., 2017). Global and national concerns also exist over antibiotic use regarding human medical efficacy, human allergic reactions, and drug residues (Brisbin, 2011; Al-Ankari and Homeida, 1996). So much so that in 2017, the Veterinary Food Directive (VFD) made it illegal to include AGPs in broiler feed for production purposes, effectively restricting use to health-related circumstances that require veterinary approval to treat disease (FDA, 2021). Lastly, antibiotics can also reduce gut biodiversity and impair the intestinal microbiota, leading to dysbiosis (Cryan and O'Mahoney, 2011), or inhibit the immune system with prolonged administration (Al-Ankari and Homeida, 1996).

Dietary supplementation of prebiotics and probiotics can alter the gut microbial community and serve as an alternative method of reducing *Salmonella* in poultry. Modulation of gut microbes improves competitive exclusion, reduces pathogen colonization, boosts animal performance, and reduces mortality (Borda-Molina et al., 2018; Clavijo and Florez, 2018; Oakley et al., 2014). Probiotics have additionally been reported to enhance control of *Salmonella* infection in broilers and improve the immune response by introducing beneficial bacteria to the gut (Brisbin, 2011), which compete with *Salmonella* for binding sites and resources. However, knowledge on the application

of probiotics and prebiotics as they pertain to chicken disease resistance and immune response is limited and their usage remains challenged (Brisbin, 2011; Chambers and Gong, 2011). Thus, it is important to validate and utilize alternative, effective methods of reducing *Salmonella* in broilers and preventing its entry into the human food supply. One example could be the development of *Salmonella*- resistant birds through genetic selection of birds with higher tolerance to *Salmonella* (Montoro-Dasi et al., 2020).

An alternative strategy to combat *Salmonella* in poultry is through genetic selection of broilers for disease resistance, which may in turn improve productivity and overall welfare (Schou et al., 2010). There is a need for broiler chickens to be more capable of handling stress and challenges that arise from current production standards, such as overcrowding and heat stress. Genetics have an influence on several health aspects linked to broiler welfare, including development of the immune system and gut microbial community (Schokker et al., 2015). Additionally, selective breeding can achieve differences in early life coping skills and stress adaptation (Schokker et al., 2015; Cheng et al., 2004). Most importantly, differences in pathogen susceptibility between breeds have been observed among poultry and other species, which can be utilized to improve disease resistance (Schokker et al., 2015; Flock et al., 2005). Genetics have an influence on the ability of *Salmonella* to colonize in the chicken gastrointestinal tract (Bearson et al., 2017) and varying degrees of susceptibility to certain bacteria, such as *Salmonella*, can exist across chicken flocks (Li et al., 2018; Cheeseman et al., 2006; Bumstead and Barrow, 1988). Chickens from different breeds can differ in their response to infection. As a result, genetic selection for broilers with greater resistance is an attractive alternative to reduce *Salmonella* in broiler production.

Not only is it important for broilers to have innate *Salmonella* resistance, but it is also important to be capable of identifying subclinical infection in broilers prior to harvest and processing. Chickens do not often show clinical signs of *Salmonella* infection until it is too late to treat either the individual bird or the flock. Thus, it is challenging to detect sick chickens on the farm, resulting in increased risk of spreading *Salmonella*. Sickness behavior provides a possible solution to this problem, as sickness behaviors also arise as an indicator of an immune response (Dantzer, 2004). However, sickness behaviors in chickens are muted except in more severe circumstances. Chickens are stoic; their nature as a prey species is to hide signs of weakness, especially in the presence of a perceived threat such as humans (Tizard, 2008). Domestication has reduced the hiding of sickness in some species, including chickens, to an extent (Tizard, 2008). Additionally, differences in immune function and disease tolerance could influence the severity of an infection, thereby influencing observable sickness behavior. Thus, it is possible that different breeds of chickens may display varying degrees of sickness behavior in response to the same infection due to their genetic background or immune function. However, there is a gap in knowledge to what extent broiler breeds might differ in their sickness behavior, if any, in response to *Salmonella* infection.

1.2 The Genetic Selection of Broilers

Broiler chickens have been selectively bred for greater feed efficiency and performance to yield more meat products and meet consumer demand. However, selection for productivity traits may unintentionally neglect other health and welfare traits, such as stress tolerance (Altan et al., 2003), leg health (Mohammadigheisar et al., 2020), and resistance to disease (Swinkels et al., 2007). So much so that the Global

Animal Partnership (GAP) funded research at the University of Guelph, as part of the Better Chicken Project, to investigate the effects of breeding for enhanced broiler growth rate on a multitude of health and welfare parameters (GAP, 2020). Torrey and colleagues (2021) investigated the growth, efficiency, and mortality of 16 broiler breeds that varied in growth rate, classified as conventional (fastest-growing), fast-slow, moderate-slow, and slow-slow. Conventional birds grew fastest and were most feed efficient compared to the other breeds, as expected (Torrey et al., 2021). Mortality did not significantly differ between breeds, but there was a greater likelihood for the slowest-growing breeds to have fewer culls and more birds found dead (Torrey et al., 2021). Initial reports from the project also noted that the fast-growing breeds were less active, more susceptible to contact dermatitis and muscle damage, and had poor organ development (Torrey et al., 2020). Additionally, differences have been investigated between fast- and slow-growing breeds regarding gastrointestinal, tibia, and plasma metabolites by Mohammadigheisar and colleagues (2020), in which the fast-growing breed had greater concentrations of plasma enzymes, greater body weight and numerically greater villus heights, and less tibia ash content. These findings suggest that the fast-growing breed was more efficient on the same feed and had greater surface area for absorption in the intestine, but that the fast-growing breed may have also had impaired hepatic function and poor skeletal development relative to body weight (Mohammadigheisar et al., 2020). However, the effects of selection for enhanced growth rate on resistance to foodborne pathogens such as *Salmonella* have not been investigated.

Pathogen immunity is a particularly important trait to consider because disease negatively affects both performance and welfare (Xie et al., 2000; Cheng et al., 2004;

Marcq et al., 2011). For example, prior research has shown that laying hens bred for higher group productivity (egg laying) and survivability were less affected by LPS challenge compared to birds from the low group productivity and survivability and commercial breeds (Cheng et al., 2004). This was evidenced by less severe reductions in body weight gain and little to no increase in organ (spleen, liver, heart, and adrenal gland) weight, indicating greater resistance to LPS challenge (Cheng et al., 2004). Multiple studies have explored the link between growth rate or body weight and immune function (Yunis et al., 2000; Leshchinsky and Klasing, 2001; Humphrey and Klasing, 2004; Parmentier et al., 2010; van der Most et al., 2011), often noting an inverse relationship. This may be due to prioritizing the allocation of bodily energy and resources to growth as opposed to immune function, which compromises the immune system (Humphrey and Klasing, 2004). Selecting animals for disease resistance may be a vital tool in restricting the spread of foodborne diseases such as *Salmonella* from the chicken to the table. Thus, it is important to evaluate the effect genetic selection for growth rate and body weight have had on broiler health, particularly regarding *Salmonella* resistance.

1.3 *Salmonella* in Poultry

Gut colonization or infection by *Salmonella enterica* serovars (*S. enterica* ser. Enteritidis and *S. enterica* ser. Typhimurium) in broilers can prompt an immune response, induce morphological changes in the gut, and influence behavior. When injected intravenously, *S.* Typhimurium and *S.* Enteritidis have caused an observed inflammatory response, depression, fever, diarrhea, reduced feed intake, and reduced body weights in broiler chickens (Xie et al., 2000; Quinteiro-Filho et al., 2012). Cobb chicks orally challenged with *S.* Typhimurium at 1 week of age had reduced body

weights and increased mortality rates, incidences of lameness, and diarrhea (Dar et al., 2019). Additionally, intensive broiler production and poor welfare can heighten the risk, prevalence, and severity of *Salmonella* infection. Heat stress, a common and extensively studied issue in broiler production, increases *S.* Enteritidis counts in the spleens of infected broilers (Quinteiro-Filho et al., 2012) and increases broiler intestinal permeability to *S.* Enteritidis and endotoxins through impairment of intestinal structure (Burkholder et al., 2008; Alhenaky et al., 2013). Feed deprivation also increases *S.* Enteritidis attachment in the broiler gut (Burkholder et al., 2008), and overcrowding stress induces immunosuppression, reducing broiler *S.* Enteritidis resistance (Gomes et al., 2014). Furthermore, *Salmonella* infection of broilers can cause reductions in body weight gain and death with severe infection (Fasina et al., 2010). Given these welfare concerns and the effect *Salmonella* infection can have on broilers, further research is needed to understand the impact of selective breeding on broiler pathogen resistance.

1.3.1 Effect of Breed on Pathogen Resistance and *Salmonella* Infection

Susceptibility and resistance to pathogens and the effect of infection on welfare and performance can differ between chicken breeds. When male broilers from two commercial breeds were challenged with coccidial pathogen *Eimeria acervulina*, Iuspa and colleagues (2020) found that birds from one commercial broiler breed had greater fecal oocyst counts and intestinal lesion scores, reduced feed intake and body weight, and a greater increase in feed conversion ratio after challenge compared with the other commercial breed. In a study that evaluated *Salmonella* Pullorum resistance between Rhode Island Red, local Chinese breed Beijing You, and a synthetic layer breed, birds were challenged at 4 d and evaluated for mortality and *Salmonella* carriage (or presence)

in the spleen post-inoculation (Li et al., 2018). Breeds varied significantly in mortality and carriage rates, in which the Beijing You had the lowest mortality rate (8.3%) and *Salmonella* Pullorum carriage rate (0.6%) compared to other breeds (Li et al., 2018). Williams and colleagues (2013) reported no differences in *Campylobacter* carriage between Ross and Hubbard broilers orally infected at 21 days of age, but Ross displayed more incidences of contact dermatitis than Hubbard broilers. Additionally, Han and Smyth (1972) studied the development of Marek's disease in sub-breeds of White Plymouth Rock divergently selected for multiple generations for increased growth rate (Massachusetts High Growth and Virginia High Growth) versus one generation of breeding for faster growth (Massachusetts Low Growth and Virginia Low Growth) following abdominal injection of the virus at 1 day post-hatch. The high growth breeds exhibited greater mortality and lesions in the liver, gonads, and spleen than the slow growing breeds (Han and Smyth, 1972).

Differences in susceptibility and infectivity of *S.* Enteritidis have also been observed across breeds, with greater potential resistance in breeds less heavily selected for productivity traits. Schou and colleagues (2010) reported greater *S.* Enteritidis resistance in the indigenous Vietnamese Ri chickens compared to the commercial Luong Phuong breed, inclusive of a stronger total mixed-antibody response to infection and less severe reductions in body weight gain compared with controls. In another study, newly hatched Rhode Island Red chicks orally inoculated with different strains of *S.* Enteritidis had lower mortality following infection with any of the *S.* Enteritidis strains than a commercial broiler breed, with mortalities between 2% and 30% (pending strain virulence) compared to 20% to 96% mortality rate in the broiler breed (Barrow, 1991).

Lastly, Li and colleagues (2017) evaluated differences in the *TLR4* (toll-like receptor) gene involved in resistance to *S.* Enteritidis in 10 chicken breeds, including specific-pathogen-free (SPF) White Leghorns and Chinese native breeds. The White Leghorns had a strong association between the locus G247A on the *TLR4* gene and increased *S.* Enteritidis resistance when compared with the native breeds (Li et al., 2017).

Genetic selection impacts chicken resistance to pathogens, such as *Salmonella*, but it is unknown if selection specifically for growth rate in broilers affects pathogen susceptibility. Research is needed to identify differences in pathogen resistance between fast- and slow-growing breeds of broilers, which may be elucidated through studying gut morphology, immune response, and sickness behavior in response to infection.

1.4 Gut Integrity

The gut microbiome is linked to gut health, morphology, and physiology. Direct fed microbial (DFM) feed supplements provide beneficial gut microbes and have been found to increase villus height and crypt depth in broiler chicks (Lee et al., 2010). On the other hand, infection by pathogens may induce dysbiosis and impair nutrient absorption and broiler performance. Cobb broiler chicks co-infected with *Eimeria* and *Clostridium perfringens* had reduced intestinal villus heights (Golder et al., 2011), which may correspond to reduced performance (Yamauchi et al., 2010). *Salmonella* colonization also negatively impacts gut integrity. Prior studies have shown that broilers infected with *S.* Typhimurium exhibited intestinal inflammation and damage to the epithelium (Kaiser et al., 2000; Dar et al., 2019). *S.* Enteritidis infection additionally alters gut physiology by reducing intestinal ion permeability, a possible reason for why diarrhea is often not observed in infected broilers (Awad et al., 2012). In contrast, Quinteiro-Filho and

colleagues (2012) reported no effect of *S.* Enteritidis infection on intestinal histology across all segments of the small intestine in broilers despite signs of enteritis. However, enteritis could indicate *Salmonella* invasion of epithelial cells, which is often associated with damage to the intestinal structure (Clark et al., 1998). A link exists between intestinal structure and function (Yamauchi et al., 2010), and as such, breed-related differences may exist that improve intestinal structure strength and resilience to pathogens in broilers selected for increased growth.

1.4.1 Effect of Breed on Gut Integrity

Gut integrity and morphology are important to animal health and nutrition, and as a result, performance and welfare. Thus, decades of selective breeding for increased performance in broilers has resulted in heavier and more efficient birds. Havenstein and colleagues (2003) compared the growth rate of commercial broiler breeds from 2001 (Ross) and 1957 (Arbor Acres), in which the 2001 Ross breed required 3x less time and 3x less feed to reach 1,815 g than the Arbor Acres breed. This change in growth and efficiency may reflect changes in gut morphology (Yamauchi et al., 2010). Broilers with well-developed intestines are more efficient at utilizing energy from feed and perform better than birds with poorly developed or damaged intestines. As a result, it makes sense that broilers genetically selected for fast growth rates, greater feed efficiency, and higher mature body weights might have better developed intestinal morphology for the absorption of nutrients. Yamauchi and Isshiki (1991) studied the intestinal villi of the duodenum, jejunum, and ileum in White Leghorns and broilers between days 1 and 30 of age and reported that broilers had further developed and larger villi than White Leghorns by day 10 post-hatch. These differences suggest that the broiler had more active intestinal

function and greater absorptive capacity due to increased surface area, which in combination support increased growth rate. However, a comparison of a fast-growing broiler breed and 4 slower-growing breeds revealed no significant differences between breeds in jejunal villus height and crypt depth (Mohammadigheisar et al., 2020).

Little is known regarding breed-related differences in intestinal structure resilience to gastrointestinal infection, but research by Gao and colleagues (2013) involving *E. coli* infection in Jinhua and Landrace pigs suggests that the Jinhua breed may typically have more resilient intestinal structure than Landrace. More resilient gut integrity equates to less epithelial damage during infection, resulting in a greater retention of intestinal structure and function (Yamauchi et al., 2010). Breeds that are less resilient may lose productivity due to reductions in absorptive function. Broiler intestinal function is critical to the broiler production industry, and as such it is imperative to ensure broilers have both well-developed and resilient gut integrity to avoid economic losses. It is unclear if selection for enhanced growth rate in broilers has also resulted in improved intestinal structure or intestinal resilience to disease. More research is needed to understand the consequences of growth rate on intestinal structure and resilience, as well as the influence or intervention of the immune response on gut health in fast- versus slow- growing broilers when infected by pathogens such as *Salmonella*.

1.5 Immune Response to *Salmonella*

The broiler chicken immune response against *S. enterica* serovars varies from little or no response to severe disease and comes with potential welfare and performance complications. Severity of infection can be affected by multiple factors such as age (Song et al., 2021), genetics (Bumstead and Barrow, 1988; Schou et al., 2010), environment and

stress (Burkholder et al., 2008; Gomes et al., 2014), microbiome (Chambers and Gong, 2011), and nutrition (He et al., 2020). Infection by *Salmonella* prompts innate and adaptive immune responses in chickens, inclusive of both cytokine and antibody involvement (Barrow et al., 2012).

To combat an intracellular bacterium such as *Salmonella*, the chicken immune response utilizes a combination of T cell and B cell responses. The T cell response confers cellular immunity and consists of two cellular subsets: cytotoxic T cells and helper T cells (Th cells) (Cano and Lopera, 2013). Cytotoxic T cells function to destroy infected and damaged cells, while Th cells aid other immune cells in mounting a response (Cano and Lopera, 2013). Th cells produce both produce cytokines and induce antibody production by B cells (Cano and Lopera, 2013), and Th cell responses can further be divided into Th1 and Th2 subtypes which produce Th1- and Th2-type cytokines, respectively (Berger, 2000). The Th1 response typically produces pro-inflammatory cytokines, such as interferon-gamma (IFN-γ), to support the immune response (Berger, 2000; Wigley and Kaiser, 2003). Pro-inflammatory cytokines contribute to tissue inflammation, lymphocyte recruitment, and induction of proteins involved in the inflammatory response (Berger, 2000; Wigley and Kaiser, 2003). Other pro-inflammatory cytokines include interleukins (IL), such as interleukin-1 (IL-1), interleukin 1-beta (IL-1β), interleukin-6 (IL-6), interleukin-12 (IL-12), interleukin-18 (IL-18), and tumor necrosis factor alpha (TNF-α) (Wigley and Kaiser, 2003). IFN-γ production is stimulated by *Salmonella* lipopolysaccharide (LPS) endotoxin, which is a component of the *Salmonella* outer membrane wall, and as such IFN-γ plays a vital role in both the innate and adaptive immune responses to *Salmonella* infection (Barrow et al., 2012; Penha-

Filho et al., 2012). Brisbin (2011) observed a Th-1 dominant response in commercial broilers infected with *S.* Typhimurium and increased levels of IFN-γ in cecal cells associated with clearance. In ISA Brown chicks infected with *S.* Enteritidis at 1, 4, and 16 days of age, a Th1 response was observed with increased IFN-γ mRNA expression (Crhanova et al., 2011). Dar and colleagues (2019) also reported increased IFN-γ, IL-12, and IL-18 mRNA expression in the liver, spleen, and ceca of Cobb broiler chicks orally challenged with *S.* Typhimurium at 3 days. External administration of IFN- γ has additionally been shown to support the immune response against *Salmonella.* When recombinant IFN-γ was administered to turkeys, it reduced *S.* Enteritidis invasion in the liver and spleen by up to 38.4% (Farnell et al., 2001).

The B cell response is also important in the body's immune response to *Salmonella* infection. B cells confer humoral immunity and are responsible for producing antibodies to help fight disease when antigens are detected (Cano and Lopera, 2013). The antibodies found in birds consist of immunoglobulin M (IgM), immunoglobulin G (IgG; may also be referred to as IgY), and immunoglobulin A (IgA) (Tizard, 2002). IgM is found primarily in the serum and supports the primary immune response soon after first exposure to an antigen occurs (Tizard, 2002). IgG predominates avian blood and, like mammalian IgG, has a role in immunity against pathogens and bacterial toxins and is produced following the IgM response (Tizard, 2002). Lastly, IgA is found primarily in intestinal and respiratory secretions and is responsible for protecting these epithelial surfaces against invasion by pathogens such as *Salmonella* (Tizard, 2002). IgA may also be released into the bloodstream when produced by B cells beneath mucosal surfaces, in

which blood IgA responses could be indicative of a humoral response to infection in the respiratory or gastrointestinal tracts (Tizard, 2002).

Given this information, it is expected to see a humoral immune response following infection by *Salmonella*. Cobb broilers orally challenged at 3 days of age with *S.* Typhimurium had significantly greater levels of IgG and IgM antibodies in the blood serum (Dar et al., 2019). Hassan and colleagues also reported increased blood serum concentrations of IgG, IgM, and IgA in Sussex chickens inoculated with *S.* Typhimurium 4 days post-hatch (Hassan et al., 1991). While increased IgA can be associated with pathogen clearance, it does not necessarily indicate an efficient or effective immune response. Research by Holt and colleagues (1999) reported most White Leghorn chicks challenged with a sublethal dost of *S.* Enteritidis at day 1 did not appear to have a plasma IgA or IgG response. In the White Leghorn chicks that did have an IgA response to challenge, high counts of *Salmonella* in the intestine indicated that the IgA response was insufficient to clear *S.* Enteritidis (Holt et al., 1999). However, both vaccination and direct antibody administration have been shown to reduce or inhibit *S.* Enteritidis. A *Salmonella* chitosan-nanoparticle (CNP) vaccine administered to Cobb chicks at day 1 of age and challenged with *S.* Enteritidis at day 14 resulted in increased IgA levels in bile and serum, increased IgG levels in serum, and reduced *S.* Enteritidis in the ceca (Acevedo-Villanueva et al., 2020). When Lee and colleagues (2002) introduced *Salmonella*-specific IgY (IgG) to *S.* Enteritidis and *S.* Typhimurium in liquid medium in vitro, *Salmonella* growth was successfully inhibited.

It is evident that *Salmonella* infection in broilers elicits an immune response that may involve both T cell and B cell involvement, and as such it is important to understand

how this immune response might differ between different breeds. Broiler breeds selected for different growth rates may have also undergone changes in immune development or function, but the consequences of this selection on the immune system is unclear.

1.5.1 Effect of Breed on Immune Response

Genetics influence immune response, which can be modulated by the gut microbiome resulting in variances in susceptibility and resistance to pathogens (Pan and Yu, 2013; Humphrey, 2006). The gut microbial communities significantly differed between two breeds of chickens divergently selected from the same ancestor for high and low mature body weights and provided the same diets and management, varying by up to 68 of 190 bacterial species (Zhao et al., 2013). Schokker and colleagues (2015) observed significant differences in microbiota composition between two broiler breeds divergently bred for their immune response to pathogenic infection, although the breeds showed no difference in body weight or feed conversion ratio.

Chicken breeds can also differ in their response to stressors, such as heat or immune stress. Layers from four breeds that differed in their humoral immune response and survival rate were challenged to chronic heat stress (23ºC for 23 days) and short-term hygienic stress (LPS challenge) to determine the effect on performance such as egg production, egg quality, feed intake, and body weight (Star et al., 2008). While breeds responded similarly to stressors regarding production measures (greater weight loss, reduced feed intake, and reduced egg weight and shell thickness), they differed in degree of response (Star et al., 2008). Rhode Island Reds had the greatest reductions in body weight, feed intake, and egg production in response to heat stress, whereas the White Leghorn breeds had less severe reductions in the same parameters (Star et al., 2008). The

contrast in response levels may be attributed to the ability of each bird's immune system to adapt to stressors. Additionally, Leghorns bred for high group productivity and survival have shown a greater stronger cell-mediated immune response when administered an LPS challenge compared to those selected for low group productivity and survival. (Cheng et al., 2004).

Prior research has studied differences in immune response between broiler breeds. Swaggerty and colleagues (2003) investigated two breeds of commercial broilers and their crosses for biomarkers indicating *S.* Enteritidis resistance by isolating white blood cells and recording their protective actions against *S.* Enteritidis, including phagocytosis (consumption of the bacteria) and degranulation (release of antimicrobial molecules). The heterophils (white blood cells) of one pure breed and a related cross breed phagocytized more *S.* Enteritidis and were more capable of degranulating when exposed to *S.* Enteritidis, indicating greater immunocompetence than the other pure and cross breeds (Swaggerty et al., 2003). Additionally, broilers with known resistance to extra-intestinal *S.* Enteritidis infection have been reported to have greater mRNA expression of pro-inflammatory cytokines following oral challenge with *S.* Enteritidis at 1-day-old than known susceptible breeds (Ferro et al., 2004). Differences in immune function have also been observed between broiler breeds that differ in growth rate. Swinkels and colleagues (2007) orally inoculated fast-growing (Ross 308) and slow-growing (Hubbard) broiler chicks 7 days post-hatch with *Eimeria acervulina*. The fast-growing breed displayed a rapid increase in cytotoxic T cells in the duodenum paired with a lower *Eimeria acervulina* parasite load, suggesting that the fast-growing breed had a stronger and more successful immune response to challenge (Swinkels et al., 2007). However, research by

Cheema and colleagues (2003) comparing the immune responses of commercial 2001 (Ross) and 1957 (Arbor Acres) broiler breeds reported greater lymphoid organ weight relative to body weight and greater total serum antibody response in the 1957 breed compared to the 2001 breed, suggesting that selection for enhanced growth in broilers may have instead impaired adaptive immune function. Lastly, recent work by Rothschild (2019) compared antibody responses to an Infectious Bronchitis Virus vaccine between one fast-growing broiler breed and 3 slow-growing breeds, recording greater serum antibody levels indicating greater short-term humoral immune responses in the conventional fast-growing breed than in the slow-growing breeds.

Despite these known differences, it is unclear if differences exist between modern fast- and slow-growing broiler breeds regarding immune response to *Salmonella*. More research is needed to evaluate the effect of growth rate on immune system development and function as they pertain to food pathogen resistance. Such differences may also produce variability between fast and slow-growing breeds regarding sickness behavior in response to infection, as sickness behavior is symptomatic of an immune response.

1.6 *Salmonella* and Chicken Sickness Behavior

As a prey species, chickens are stoic, hiding signs of illness that may identify the animal as an easy meal to predators. Birds are adept at hiding sickness until a point at which it is too late to treat, particularly when a potential threat such as a human is present (Tizard, 2008). In this case, it may be beneficial to observe comfort behaviors and other behaviors that can indicate an animal's welfare. Poultry comfort behaviors include walking, stretching, wing flapping, dustbathing, preening, and scratching the ground (Mauldin, 1991). When these behaviors are performed at greater intensities, for longer

durations, and with high frequency, it may indicate better welfare (Costa et al., 2012), and reductions in comfort and exploratory behaviors could precede clinical signs of illness (Abeyesinghe et al., 2021).

Sickness behavior is an observable indicator of an immune response in action. It occurs as a motivational state and adaptive response to a disease, encouraging distinct behavioral patterns that support recovery from the disease, such as lethargy, decreased appetite, and reduced social behaviors (Johnson, 2002; Dantzer, 2004; Tizard, 2008). Behaviors that promote long term health (e.g., play, sexual, and learning behaviors) are reduced, while those that support short term fitness (e.g., resting and anorexia) increase in frequency (Weary et al., 2014). Sickness behavior can be affected directly by pathogens, but the immune system is largely responsible for what animals experience as sickness (Tizard, 2008; Millman, 2006). More specifically, it is a result of the onset of an acute phase immune response due to pro-inflammatory cytokine signaling, including IL-1, IL-6, and TNF-α (Tizard, 2008; Millman, 2006; Dantzer, 2004, Johnson, 2002). Cytokines have been shown to influence specific sickness behaviors. For example, TNF- α can mitigate animal weight loss (Tizard, 2008) and pro-inflammatory IL-1β has been observed to reduce appetite in rats, as evidenced by reduced feed intake (Finck and Johnson, 1997). While the link between pro-inflammatory cytokine response and sickness behavior has been studied (Kelley et al., 2003; Dantzer, 2004; Dantzer and Kelley, 2007), it is unclear if there is any relationship between antibody (immunoglobulin) response and sickness behavior.

Behavioral and physiological signs of sickness in chickens include changes in body temperature (and as a result, changes in thermoregulatory behavior), activity levels,

thirst, feed consumption, weight loss, and social and reproductive behaviors. A study by Cheng and colleagues (2004) reported fever as well as reduced standing, moving, eating, and drinking behaviors and increased time sitting in White Leghorns following LPS injection at 6 weeks of age. In broiler chicks experimentally infected with *E. coli* at 8 days of age, it was determined that birds who failed to respond to a hand clap for attention as an indicator of lethargy also had reduced body temperature, reduced heart rate, reduced respiratory rate, and even dyspnea (Matthijs et al., 2017).

The effect of *S.* Typhimurium on sickness behavior in broilers has been studied previously. Following oral challenge 1-week old Cobb chicks with *S.* Typhimurium, the broilers displayed clinical symptoms of illness including depression, dullness, anorexia, inactivity, weakness, and closing of the eyes (Dar et al., 2019). Xie and colleagues (2000) reported that intravenous LPS injection in 3-week-old broilers caused behavioral changes such as depression and feed avoidance within 48 hours of injection. However, broilers may be even less prone to display behavioral changes from sickness than other birds, due to their selection for faster growth (Berghman, 2016). When a broiler breed and a Brown Nick layer breed were injected with LPS 1-day post-hatch, the broilers had lower mRNA expression of IL-1β and IFN-γ and a reduced febrile response compared to the layer line (Leshchinsky and Klasing, 2001). Reductions in pro-inflammatory cytokine response may reduce the intensity of sickness behaviors in broilers compared to other breeds which might even be observed between broiler breeds that vary in growth rate. More research is needed to evaluate the differences between fast- and slow- growing broiler breeds regarding sickness behavior following infection by a pathogen such as *Salmonella.*

1.6.1 Effect of Breed on Sickness Behavior

Behavioral differences between breeds of chickens can be indicative of their welfare. Notably, there are clear differences between breeds that differ in their production purpose and growth rate. The University of Guelph studied 16 different breeds of broiler chickens varying in growth rate for differences in behavior, performance, mortality, and mobility at the (GAP, 2020). The conventional breed (fastest growth rate) spent the most time sitting (73.6%) compared to the rest, which averaged 63%, and almost half as much time standing (4.2%) and moving (2.3%) as all slower growing breeds (Torrey et al., 2020). Additionally, the breeds with the slowest growth rates spent more time engaging with enrichments than faster growing breeds throughout the study and crossed a physical barrier more times to access feed and water (Torrey et al., 2020). A different study by Dixon (2020) compared the behavior of fast- and slow-growing broilers and reported comparatively greater engagement in active behaviors such as standing, locomoting, foraging, and preening in a slow-growing Hubbard breed than 3 commercial breeds (Ross, Cobb, and Hubbard). Additionally, exploratory behaviors such as foraging activity can vary between genotypes of broilers, in which slow-growing broilers tend to engage in exploratory behaviors more than medium (Almeida et al., 2012) and fast-growing breeds (Yan et al., 2021). In another study, no differences were reported between a fast- and slow-growing hybrid broiler breed regarding foraging behavior (Wallenbeck et al., 2016).

Differences in activity can be key to identifying where welfare or health concerns may arise, for example, reduced mobility may signal an inability of birds to move, and sitting can increase the risk of contact dermatitis and leg health. These behavioral changes, alongside reductions in normal behaviors such as preening and foraging, could

additionally signal disease. While breed-related behavioral differences exist between fast- and slow- growing broiler breeds, it is unknown if they differ in sickness behavior.

Differences in sickness behavior between fast- and slow- growing broilers could signify the severity of infection and resulting presence or strength of an immune response. Increased sickness behavior may indicate a stronger immune response, and thus greater resilience to pathogenic infection by bacteria such as *Salmonella* (Hart, 1988; Johnson, 2002; Cheng et al., 2004). Sickness behavior may additionally serve as an indicator of disease, which can aid producers in identifying illness within flocks and either treat or cull ill birds.

1.7 SUMMARY, OBJECTIVE, AND HYPOTHESIS

Genetic selection for a specific set of traits, such as performance characteristics in broilers, can inadvertently affect other traits of importance, such as pathogen resistance, gut health, immune function, and behavior. The implications of selection for fast growth in broilers is already a hot topic in animal welfare for its impact on broiler health. However, pathogen resistance is highly important as well, particularly as broilers serve as a major reservoir for *Salmonella* transmission into the human supply. *Salmonella enterica* serovars Typhimurium and Enteritidis are frequent causes for human foodborne illness around the globe, and while they are less harmful to poultry, they cause issues for the broiler industry. *Salmonella* infection can cause reduced body weight gain and increased mortalities, resulting in economic losses. However, little is known if differences exist between modern fast- and slow-growing broiler lines relative to *Salmonella* infection, making it increasingly important to understand if selective breeding for a faster-growing, heavier broiler has also resulted in a more (or less) *Salmonella*-resistant broiler.

To evaluate and understand these differences, fast-growing Ross 308 and slow-growing Redbro broiler chicks were orally challenged with either *S.* Typhimurium or Tryptic Soy Broth control at 14 days of age and sampled up until 24 days of age (10 days post-challenge). Differences in body weight, immune function, and gut morphology can elucidate the effect of *Salmonella* challenge, so birds were weighed and blood and intestinal samples were collected throughout the study to evaluate for challenge-induced body weight losses, damage to intestinal structure, and changes in plasma IgA and IgG antibodies indicative of a humoral immune response. Changes in behavior can also reflect sickness as a symptom of an immune response in action, so video recordings were taken of 8 isolators on multiple days to evaluate breed differences and the effect of *S.* Typhimurium challenge on behavior.

The objective of this study was to evaluate differences in body weight, immune response, gut morphology, and sickness behavior between fast- and slow-growing broiler chickens when challenged with *Salmonella* Typhimurium. The first hypothesis of this study was that fast-growing broilers would have greater body weight, greater intestinal morphology measures, lower plasma immunoglobulin concentrations, and reduced behavioral repertoire when compared to the slow-growing breed independent of challenge. The second hypothesis was that post-challenge, the challenged fast-growing broilers would be less negatively impacted than the slow-growing broilers with regard to body weight and gut morphology, but the slow-growing breed would have greater plasma IgA and IgG responses to challenge paired with more significant behavioral signs of sickness.

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CHAPTER 2 Evaluating differences in body weight, gut morphology, and immune response in fast- and slow- growing broiler chickens when infected with *Salmonella enterica* serovar Typhimurium

2.1 ABSTRACT

Fast growth rate in broilers comes with welfare concerns and research is needed to determine if fast- and slow-growing broilers differ in pathogen resistance. The objective of this study was to evaluate differences in fast- (FG) and slow-growing (SG) male broilers when challenged with *Salmonella* Typhimurium (ST) or broth (control; CON) 14 days post-hatch. FG (N=156) and SG (N=156) were raised in the same pen with litter shavings until d7, when they were transferred to 24 isolators. On d7, 13, 17, 21, and 24 body weights, plasma IgA and IgG, and jejunum and ileum histomorphology were measured (N=48 birds/sampling). FG were 70g heavier (P=0.03) on d21 and 140g heavier (P=0.007) on d24 than SG. On d7, FG jejunum villus height and crypt depth were 22 μm and 7 μm greater (P≤0.001) than SG, which can mean better nutrient absorption. SG IgG at d7 was 344 μg/mL higher than FG IgG, which may indicate greater maternal antibody protection. FG plasma IgA was 38 μg/mL higher (P=0.01) than SG at d21, and FG plasma IgG increased with age (P<0.0001) and were higher (P≤0.03) than SG at d21 and d24 by 689 μg/mL and 1,474 μg/mL, respectively, but SG IgG did not increase after d13, which may mean earlier humoral immune development in FG. Day 24 ST ileum villus height was reduced (P=0.009) by 95 μm, but FG-ST were more impaired than SG-ST. Challenge increased (P=0.03) IgG in ST at d21 by 44 μg/mL, but the difference was only significant in SG-ST, indicating a stronger SG-ST IgA response. The results illustrate fast- and slow-growing broilers differ in *Salmonella* resistance, which can help breeders make selection decisions to prevent *Salmonella* transmission into the human food supply.

Keywords: broiler, growth rate, *Salmonella*, immune response, gut morphology

2.2 INTRODUCTION

In order to meet the high consumer demand for chicken, broilers are genetically selected for increased feed efficiency and greater breast yield, resulting in birds that reach heavier market weights at incredible growth rates (NCC, 2021). However, selection for productivity traits may unintentionally neglect other health and welfare traits, such as pathogen resistance. Pathogen immunity is a particularly important trait to consider because disease negatively affects broiler performance, health, and welfare (Xie et al., 2000; Marcq et al., 2011). Despite this, the effect of selection for enhanced growth rate on resistance to foodborne pathogens such as *Salmonella* has not been investigated.

Gut colonization by *Salmonella enterica* serovars in broilers can prompt an immune response and induce morphological changes in the gut. When injected intravenously, *S.* Typhimurium and *S.* Enteritidis can cause an inflammatory response, fever, diarrhea, reduced feed intake, and reduced body weights in broiler chickens (Xie et al., 2000; Quinteiro-Filho et al., 2012). Additionally, Fasina and colleagues (2010) reported reduced body weight and gut morphology in broilers orally challenged with *S.* Typhimurium. Furthermore, infection by *S.* Typhimurium can cause intestinal inflammation or enteritis in broilers (Kaiser et al., 2000; Dar et al., 2019). Little is known regarding broiler breed-related differences in intestinal structure resilience to gastrointestinal infection, but research by Gao and colleagues (2013) involving *E. coli* infection in Jinhua and Landrace pigs suggests that the Jinhua breed may typically have more resilient intestinal structure than Landrace. Resilient gut integrity equates to less epithelial damage during infection, resulting in a greater retention of intestinal structure and function and better nutrient absorption and productivity (Yamauchi et al., 2010).

Salmonella infection prompts innate and adaptive immune responses in chickens inclusive of cytokine and antibody involvement (Barrow et al., 2012). In Cobb broilers orally challenged with *S.* Typhimurium at 3 days, mRNA expression of pro-inflammatory cytokines IFN-γ, IL-12, and IL-18 were increased in the liver, spleen, and ceca (Dar et al., 2019). Increased circulating concentrations of antibodies IgG, IgM, and IgA have also been observed in both Cobb broilers and dual-purpose Sussex chickens challenged with *S.* Typhimurium 3-4 days post-hatch (Hassan et al., 1991; Dar et al, 2019).

Prior research has studied differences in immune response between broiler breeds. Swaggerty and colleagues (2003) investigated two breeds of commercial broilers and their crosses for biomarkers indicating *S.* Enteritidis resistance by isolating white blood cells and recording their protective actions against *S.* Enteritidis, including phagocytosis (consumption of the bacteria) and degranulation (release of antimicrobial molecules). The heterophils (white blood cells) of one pure breed and a related cross breed phagocytized more *S.* Enteritidis and were more capable of degranulating when exposed to *S.* Enteritidis, indicating a greater immunocompetence than the other pure and cross breeds (Swaggerty et al., 2003). Additionally, multiple studies have explored the link between growth rate or body weight and immune function (Yunis et al., 2000; Leshchinsky and Klasing, 2001; Humphrey and Klasing, 2004; Parmentier et al., 2010; van der Most et al., 2011), often noting an inverse relationship in which selection for fast growth reduces immune function. This may be due to the allocation of bodily energy and resources to growth as opposed to immune function, compromising the immune system (Humphrey and Klasing, 2004). However, it is unclear if fast- and slow-growing broilers differ in their immune response to *Salmonella* infection.

It is unknown if differences exist between modern fast- and slow-growing broiler lines relative to *Salmonella* infection, making it increasingly important to understand if selective breeding for a faster-growing, heavier broiler has also resulted in a more or less *Salmonella*-resistant broiler. To evaluate and understand these differences, fast-growing Ross 308 and slow-growing Redbro broiler chicks were orally challenged with either *S.* Typhimurium or Tryptic Soy Broth control at 14 days of age and sampled up until 24 days of age (10 days post-challenge). Birds were weighed and blood and intestinal samples were collected throughout the study to evaluate for challenge-induced body weight losses, damage to intestinal structure, and changes in plasma IgA and IgG antibodies indicative of a humoral immune response following *S.* Typhimurium infection. The objective of this study was to evaluate differences in body weight, immune response, and gut morphology between fast- and slow-growing broiler chickens when challenged with *Salmonella* Typhimurium. The hypotheses were that fast-growing broilers would have greater body weight, greater intestinal morphology measures, and lower plasma immunoglobulin concentrations, and that challenged fast-growing broilers would be less impaired than slow-growing regarding body weight and gut morphology, but the slow-growing breed would have greater plasma IgA and IgG responses to challenge.

2.3 MATERIALS AND METHODS

2.3.1 Animals and Housing

All procedures and protocols were approved by the University of Maryland (UMD) Animal Care and Use Committee (IACUC#: R-NOV-19-55). Three-hundred and twelve male day-of-hatch chicks from two breeds, Ross 308 (Aviagen) (N=156) and Redbro (Hubbard) (N=156), were transported from a local hatchery (Freedom Ranger

Hatchery, Reinholds, PA) to the University of Maryland Animal and Avian Sciences Animal Wing. Chicks were placed together in a single 3 m by 6 m pen with wood shavings litter (d0) (**Figure 2.1**). This encouraged commingling and permitted consumption of pen-mate fecal material to establish a similar gut microbiome. Three brooder lamps were hung above the pen to provide supplemental heat and were removed on d3. Water was provided *ad libitum* through a nipple water line. Chicks were provided Purina Start and Grow Non-Medicated crumbles *ad libitum* in 3 gravity-fed hanging feeders. Temperature was maintained at 32.2°C for d0-1 and gradually reduced by 0.6°C daily until 17.8°C at d13. Ambient temperature, humidity, and photoperiod was maintained according to the Ross Broiler Management Handbook (Aviagen, 2018) throughout the study. Photoperiod was 23h light and 1h dark on d0 and light hours were gradually decreased to 20h light with 4h dark on d7. Ambient temperature was also checked at the floor level inside the pen using an infrared temperature gun (Lasergrip 1080, Etekcity, Anaheim, CA). Chicks were housed in the UMD ANSC Animal Wing from d0-7.

On d7, a total of 264 birds (N=132 per breed) were moved into isolators in BSL-2 rooms at the University of Maryland Avrum Gudelsky Veterinary Medical Center (**Figure 2.2**). Eleven birds from each breed were exclusively placed into 24 isolators (Model 934-1, Federal Designs Inc., Comer, GA) within 4 ABSL-2 rooms (N=6 isolators per room). There were 3 isolators that contained birds from each breed per room. Isolators were 5,195 cm^2 and stocking density did not exceed the minimum space allowance outlined in the Ag Guide (FASS, 2010) throughout the study. Isolator floors consisted of a metal grate with a bin below to collect fecal matter, and isolator lights were

left off for the duration of the study as it was observed to increase aggression (**Figure 2.2**). Each isolator had separate airflow and HEPA filters that were replaced once weekly. Air flow pressure (negative) was monitored twice daily through an attached gauge (Model 25 Manometer, Dwyer Instruments, Inc., Michigan City, IN) to ensure that air pressure fell within an acceptable range (**Figure 2.2**). Chick paper was placed in isolators over the metal grating prior to bird placement to prevent leg injury, then later removed at d10 (**Figure 2.2**). Fecal collection bins were emptied every other day to maintain good air quality. Commercially available feed (Purina Start and Grow Non-Medicated pellets) and water were provided *ad libitum* via a metal gravity-fed trough and a plastic gravity waterer, respectively. Birds were checked for wellness, isolator temperature and humidity were monitored and recorded, and waterers were cleaned and refilled with fresh water twice daily. Individual isolator heaters were on from d7- 10 to maintain temperature. The temperature and humidity of each room was also monitored and recorded once daily. Light hours were 20h light and 4h dark (20:4) at d7 and gradually shifted to 18h light and 6h dark (18:6) until d14, then they were maintained at 18:6 for the remainder of the experiment. Exterior room windows were covered for the duration of the study.

2.3.2 Experimental Design and Procedures

The experimental design was a 2 x 2 split plot design, in which all Ross 308 (FG) and Redbro (SG) birds were raised together in a single pen for the first week (d0-7) and then randomly assigned and split between 24 isolators across 4 rooms by breed and designated challenge treatment group at d7 (**Figure 2.3**). The experimental unit was the isolator (N=24). Each room held 6 isolators total, split into 3 isolators/breed/room, and each room was assigned a challenge treatment (N=2 rooms per treatment) (**Figure 2.3**).

Challenge treatments were given on d14, in which 2 rooms of birds (rooms 3 and 4) received 1 mL of 10^8 CFU/mL *Salmonella* Typhimurium challenge culture (ST) (N=108 birds, 54 per breed) and 2 rooms (rooms 1 and 2) received 1 mL Tryptic Soy Broth control (CON) (N=108 birds, 54 per breed) via oral gavage. Quantitative bacteriology of *Salmonella* spp. presence or absence were collected on d0 and d13 via vent swabs. Sampling was performed on d7, 13, 17, 21, and 24. At each sampling event, 2 birds per isolator were randomly selected and body weight (BW), blood samples, gut histology samples (jejunum and ileum segments), and microbiome samples (ileal and cecal contents) were collected from each bird. **Table 2.1** outlines a brief summary of events.

2.3.3 Culture and Challenge

A lab-cultured nalidixic acid (NAL)-resistant culture of *Salmonella* Typhimurium (Strain #289-1; Cox and Blankenship, 1975) was received one month prior to challenge day. To determine the growth curve of the challenge culture in advance of challenge culture preparation, the culture was transferred to a 10 mL Tryptic Soy Broth (TSB) tube and incubated at 37°C at a shaking speed of 148 rpm for 24 hours. Beginning at 6 hours post-inoculation and every 2 hours thereafter until 14 hours of growth, 2 mL of the culture was removed for serial dilutions (**Figure 2.4a**) and measured at 450 nm for dilutions of 1:1, 1:10, and 1:20 against a blank for optical density (OD). Throughout culture growth, negative and positive controls were used to confirm appropriate microbial growth and verify that there was no contamination during culturing of the NAL-resistant *S.* Typhimurium culture (**Figure 2.4b**). The negative control consisted of TSB and mock inoculation using a sterile pipette, and the positive control consisted of TSB + NAL inoculated with the *S.* Typhimurium culture. The ideal growth time was determined to be

7 hours at 37°C and shaking speed of 148 rpm to achieve a 1.0 x 10^8 colony forming units (CFU)/mL culture of *S.* Typhimurium (**Figure 2.5**).

On d13, the challenge culture was prepared in 20 mL TSB (no NAL) and incubated at 37°C at a shaking speed of 148 rpm in the incubator for 14 hours alongside a negative (TSB) control (**Figure 2.6**). NAL was not included when growing the challenge culture to avoid disruption of the gut microbiome by the antibiotic. On day 14, the challenge culture was diluted six-fold via addition of 100 mL sterile TSB in order to achieve the goal challenge concentration of 1 x 10^8 CFU/mL *S.* Typhimurium, and 2 mL were removed for serial dilution plating and measuring OD using the same procedures described on d13. Challenge culture purity was verified through use of a negative control flask during incubation and plating of growth challenge onto both NAL-resistant Tryptic Soy Agar (TSA) media plates and regular TSA plates.

On d14, half of FG birds (N=6 isolators) and half of SG birds (N=6 isolators) were challenged with 1 mL of 1.3 x 10^8 CFU/mL *S.* Typhimurium (ST) via oral gavage, while the controls received 1 mL sterile TSB (CON).

2.3.4 Qualitative Bacteriology for *Salmonella* spp. Presence/Absence

Qualitative bacteriology was performed on d0 and d13 to ensure the birds were negative for *Salmonella* spp. prior to challenge. On d0, 30 birds (N=15 per breed) were randomly selected and their vents aseptically swabbed. Swabs were individually placed in 15 mL conical tubes with 10 mL TSB diluted with phosphate-buffered saline (PBS) and incubated 24 hours at 37°C. Each swab was plated on a half-plate section of bismuth sulfite agar (BSA) plates (N=2 swabs per plate) with care taken for samples not to touch and then incubated at 37°C for 24 hours. Qualitative bacteriology for *Salmonella* spp.

was repeated on d13 with 48 birds (N=24 per breed) using the same protocol for collection, storage, and incubation of swab samples as on d0. Each swab from d13 was plated on regular BSA as well as BSA + NAL control plates in quarter-plate sections (N=4 swabs per plate, 16 plates per media). BSA + NAL plates were utilized to confirm birds were negative for NAL-resistant *S.* Typhimurium.

2.3.5 Sampling

Birds were aseptically sampled in the University of Maryland ANSC Animal Wing prior to being moved on d7, and on d13, 17, 21, and 24 they were sampled in room 1416M in the 1494 ASBL-B2 Corridor of the UMD Avrum Gudelsky Veterinary Medical Center. On sampling days, birds were weighed and then euthanized via cervical dislocation. Blood samples were collected immediately via cardiac puncture and stored in plasma separation tubes immediately following euthanasia. Tubes were refrigerated and centrifuged within 24 hours of collection for 10 minutes at 2000 x g and 15°C to separate plasma.

Ileal and cecal contents and tissue sections were collected in a biosafety cabinet using aseptic technique. Ileal and cecal contents were expressed into separate tubes, put on ice, and then shipped to collaborators at Purdue University to determine alpha- and beta- diversity of the microbial communities. In challenged birds, one of the two ceca per bird was expressed into a separate tube with 40% glycerol solution, put on ice, and shipped to collaborators at Purdue University to assess *Salmonella* Typhimurium enumeration. A 2 cm segment was removed from the jejunum (2 cm anterior to Meckel's diverticulum) and the ileum (2 cm anterior to the ileocecal junction), carefully cut

longitudinally, and ends stapled to bibulous paper. Jejunum and ileum samples were stored in tubes containing 70% buffered formalin.

2.3.6 Immune Markers

Plasma samples were stored at -80°C until analysis. Commercial ELISA kits were utilized to determine IgA (E33-103 Bethyl Laboratories Inc., Montgomery, TX) and IgG (E33-104, Bethyl Laboratories Inc., Montgomery, TX) concentrations and manufacturer protocol was followed. A practice plate was run for each kit to determine appropriate sample dilution for IgA and IgG concentrations. IgA concentrations were examined at sample dilutions of 1:100, 1:500, 1:1,000, and 1:2,000 using the provided dilution buffer, and the recommended dilution (1:1000) was determined appropriate. IgG concentrations were examined at sample dilutions of 1:100, 1:10,000, 1:100,000, and 1:200,000 using the provided dilution buffer, and the recommended dilution (1:100,000) was determined appropriate.

Briefly, 100 uL of standard or diluted plasma sample were added to wells in duplicate on pre-coated plates, then incubated at 23°C for 1 hour. Plates were then washed 4 times during the wash step using the provided wash buffer, 100 uL of detection antibody was added to each well, and the plates were incubated at 23ºC for 1 hour. Plates were washed 4 times, 100 uL of HRP solution was added to each well, and then plates were incubated at 23°C for 30 minutes. Plates were washed 4 times, then 100 uL of TMB substrate was added to each well, and plates were incubated in the dark at 23°C for 30 minutes. After, 100 uL of stop solution was added to each well and absorbance was measured on a plate reader at 450nm. A standard curve was generated for each plate

using MyCurveFit software (MyAssays Ltd.) and sample IgA and IgG concentrations were calculated using the generated curve and reported in μg/mL.

2.3.7 Gut Morphology

Histological preparation was performed by Histoserv, Inc. (Germantown, MD). Fixed tissues were rinsed using tap water and later dehydrated with graded alcohol. Samples were cleared in xylene and infiltrated with paraffin before being embedded in paraffin. Block samples were sectioned on a microtome at 5 μm thickness per section. Unstained slides were deparaffinized using xylene and hydrated with graded alcohols up to water. Slides were stained with Crazzi's hematoxylin, washed in tap water and 95% ethanol, placed in an eosin-phloxine staining solution, and ran through graded alcohols to xylene. Stained slides were coverslipped with permount as the mounting media.

Slide images were taken at 40x magnification with a camera-mounted microscope and stored on an external hard drive. SVS slide images were loaded into Qupath Quantitative Pathology & Bioimage Analysis software and 5-10 paired villus and crypt measurements per intestinal segment (jejunum and ileum) per bird were recorded electronically for villus height and crypt depth (Burkholder et al., 2008) (**Figure 2.7**). Villi were measured from the tip of the villus to the base at the villi-crypt junction, and crypts were measured from the villi-crypt junction to the crypt base at the basolateral membrane (Golder et al., 2011). Only well-oriented, untorn villi and their paired crypt were measured. Villus-crypt ratio (VCR) was calculated by dividing the villus height by its corresponding crypt depth for each villus-crypt pair measured.

2.3.8 Statistical Analysis

The isolator (N=24) was the experimental unit for all data except d7. Day 7 body weight, histology, and immune marker data were run in JMP using 1-way ANOVA for the fixed effect of breed. Day 13, 17, 21, and 24 body weight, histology, and immune marker data were all run in JMP (SAS Institute, Inc., Cary, NC) using 2-way ANOVA tests for the fixed effects of breed, challenge, and their interaction. The random effect of isolator nested within room was not included for d7 data, but it was included for all other days. Immune marker data was also run using two across age models for the fixed effects of age, breed or challenge, and their interaction with the random effect of isolator nested within room. Additionally, immune marker data was run in an age by breed analysis independent of challenge for the fixed effects of age, breed, and their interaction without the random effect of isolator nested within room. Due to collection issues at sampling, several histology sample villi were torn. As a result, there were major imbalances between breed and challenge treatments for d13, d17, and d21 and several birds were excluded. The data was determined to be unsuitable for analysis and only d7 and 24 histology results were analyzed. Pearson's pairwise correlations were compared for body weight, immune markers, and histology data. Multiple comparisons of means were separated with LSMeans and were considered significant at a $P \leq 0.05$ and a tendency at $P \leq 0.10$.

2.4 RESULTS

Results figures are color coded by treatment to aid in the visualization of treatment differences and changes over time. **Table 2.2** describes each color and represented treatment. Only significant results are presented and discussed.

2.4.1 Mortality

More birds were received (N=339) than ordered and required for the study (N=312) and the birds were raised together until d7. Day 7 mortality was 6 FG and 1 SG and mortality after d7 (N=312) was 0.32% (0.96% adjusted mortality). After d7 there were no SG mortalities, and all mortality was from the FG-CON group (2 birds) between d19 and d24.

2.4.2 Qualitative Bacteriology for *Salmonella* spp. Presence/Absence

On d0, all birds were negative for *Salmonella* spp. growth and 5 (33%) FG birds and 3 (20%) SG birds were positive for bacterial growth on BSA plates. Twenty-two (92%) FG and 22 (92%) SG were positive for bacterial plate growth on d13 on BSA plates. All birds were negative for *Salmonella* spp. growth on BSA as well as NAL-resistant *S.* Typhimurium growth on BSA + NAL plates on d13.

2.4.3 Production

There was no effect of challenge on BW, but breed had an effect as the birds aged. The effect of breed was trending at d13 and d17 and was significant at d21 and d24 (**Table 2.3**). FG birds (297 g) tended to weigh more (P=0.09) than SG birds (273 g) by 24g on d13 (**Table 2.3**). On d17, FG (451 g) tended to weight more (P=0.06) than SG (406 g) by 45g (**Table 2.3**). On d21, FG (695 g) weighed 70 g more (P=0.03) than SG (625 g; **Figure 2.8**). Day 24 FG birds (891 g) weighed 140 g more (P=0.007) than SG birds (751 g; **Figure 2.8**).

2.4.4 Immune Response

Three models were used to analyze the effects of age (d), breed, and challenge on broiler plasma immunoglobulin A and G (IgA and IgG) concentrations: 1) effects of age, breed, and their interaction independent of challenge (**Table 2.4**), 2) effect of age, challenge, and their interaction, within each breed (**Table 2.5**), and 3) across age for the effects of age, breed, and their interaction on plasma immunoglobulin concentrations within each challenge treatment (**Table 2.6**). Additionally, a model was run independent of challenge to evaluate the effects of age, breed, and their interaction across all ages.

IgA

Within age, the effect of challenge on plasma IgA concentration was significant at d13 and d21, and the effect of breed was significant at d24 (**Table 2.4; Figure 2.9a**). At d13, CON bird plasma IgA (72 μg/mL) was 20 μg/mL higher (P=0.009) than ST (52 μg/mL) (**Figure 2.9b**). Plasma IgA was higher (P=0.03) for ST birds at d21 (166 μg/mL) than CON (122 μg/mL) by 44 μg/mL (**Figure 2.9c**). At d24, FG bird plasma IgA (118 μg/mL) was 38 μg/mL higher (P=0.01) than SG (80 μg/mL) (**Figure 2.9d**).

The effect of age was significant (**Tables 2.5 and 2.6**). Independent of breed and challenge, IgA concentrations were similar between d7 (47 μg/mL), d13 (62 μg/mL), and d17 (59 μg/mL), then increased (P<0.0001) to 144 μg/mL at d21 and later decreased to 99 μg/mL at d24 (**Figure 2.10a**). Additionally, the effect of breed was significant. FG plasma IgA was generally greater (P=0.005) than SG at any age by 15 μg/mL (**Figure 2.10b**). The interaction of breed and age was not significant, and FG was only numerically greater than SG from d7 to d21 but statistically greater (P=0.005) at d24 by 38 μg/mL (**Figure 2.10c**).

Within breed, the effect of age was significant for both FG and SG broilers, but there was no effect of challenge (**Table 2.5**). FG plasma IgA concentrations were similar at d13 (67 μg/mL) and d17 (62 μg/mL), increased (P<0.0001) to 153 μg/mL at d21, then decreased to 118 μg/mL at d24 (**Figure 2.11a**). SG followed a similar pattern: IgA concentrations were similar at d13 and d17 (56 μg/mL at both days), increased (P<0.0001) at d21 to 136 μg/mL, then decreased to 80 μg/mL at d24 (**Figure 2.11b**). The effect of age was significant on CON birds. CON plasma IgA concentrations were 72 μg/mL and 56 μg/mL at d13 and d17 respectively, then increased to 122 μg/mL at d21 and decreased to 97 μg/mL at d24 (**Figure 2.12a**). Additionally, there was a breed effect within CON (**Table 2.6**). Independent of age, FG-CON birds had greater (P=0.008) IgA than SG-CON birds by 24 μg/mL (**Figure 2.12b**). The effect of age was also significant on ST birds and followed a similar pattern as CON: plasma IgA concentrations were similar between d13 (52 μg/mL) and d17 (63 μg/mL), increased at d21 (166 μg/mL), and reduced at d24 (102 μg/mL) (**Figure 2.12c**).

IgG

In the within age analysis, the effect of breed on plasma IgG concentrations was significant at d17, d21, and d24, but the main effect of challenge was not significant within any age (**Table 2.4; Figure 2.13a**). At d7, FG birds (2,693 μg/mL) had lower (P=0.05) plasma IgG than SG (3,037 μg/mL) by 344 μg/mL (**Figure 2.13b**). However, on d21 FG birds (1,859 μg/mL) had greater (P=0.03) plasma IgG than SG (1,170 μg/mL) by 688 μg/mL (**Figure 2.13c**). FG birds (2863 μg/mL) also had greater (P=0.0003) plasma IgG at d24 than SG (1,389 μg/mL) by 1,473 μg/mL (**Figure 2.13d**).

The effect of age on IgG concentrations was significant (**Tables 2.5 and 2.6**). Independent of challenge, the effects of breed and the interaction of breed and age were also significant (**Figure 2.14**). From d7-d17, the plasma IgG of both FG and SG birds were similar and decreased (P<0.0001) from 2,865 μg/mL at d7 to 1,719 μg/mL and 1,201 μg/mL at d13 and d17, respectively, after which the FG and SG values diverged (P<0.0001) (**Figure 2.14**). FG plasma IgG increased to 1,859 μg/mL at d21 and 2,863 μg/mL at d24, while SG plasma IgG remained lower (P<0.0001) than FG and remained lower with concentrations of 1,170 μg/mL at d21 and 1,389 μg/mL at d24 (**Figure 2.14**).

Within breed, only the effect of age was significant on FG and SG plasma IgG, and there was no effect of challenge (**Table 2.5**). FG plasma IgG was 1,838 μg/mL at d13, decreased (P<0.0001) to 1,280 μg/mL at d17, then increased to 1,859 μg/mL at d21 and 2,863 μg/mL at d24 (**Figure 2.15a**). SG plasma IgG was greatest at d13 (1,584 μg/mL), then decreased (P=0.04) and remained similar between d17 (1,122 μg/mL) and d21 (1,171 μg/mL) before rising to an intermediate level at d24 (1,390 μg/mL) that was similar to both d13 and d17-d21 (**Figure 2.15b**). Within challenge, the main effects of age, breed, and their interaction on plasma IgG concentrations were significant but only among CON birds. FG-CON plasma IgG decreased (P=0.003) from 1,991 μg/mL at d13 to 1,341 μg/mL at d17 before increasing to 2,120 μg/mL at d21 and 3,051 μg/mL at d24 (**Figure 2.16a**). SG-CON plasma IgG was similar to FG-CON at d13 and d17 (1,589 μg/mL and 1,281 μg/mL, respectively) and remained lower (P=0.0004) than FG-CON and unchanging across age to d21 (985 μg/mL) and d24 (1,199 μg/mL) (**Figure 2.16a**). Among ST birds, only the effect of age was significant, in which IgG decreased

(*P*=0.0001) between d13 (1,630 μg/mL) and d17 (1,090 μg/mL) then increased to 1,477 μg/mL at d21 and 2,127 μg/mL at d24 (**Figure 2.16b**).

2.4.5 Gut Morphology

Due to collection errors at sampling, several sample villi were torn, and many samples did not have enough viable villi (>5 intact villi) to be measured for analysis. As a result, there were major imbalances in the data for d13, 17, and 21 (**Table 2.7**) and data for those days were determined to be unsuitable for analysis. Thus, histology results are only reported for d7 and d24 (**Table 2.8**).

On d7, all histological measures were significant ($P \leq 0.04$) for the main effect of breed (**Table 2.8**). FG jejunum villus (JV) height (508 μm) and jejunum crypt (JC) depth (104 μm) were both greater ($P \leq 0.001$) than SG JV height (486 μm) and JC depth (97 μm) by 22 μm and 7 μm, respectively (**Figures 2.17a-b**). FG ileum villus (IV) height (344 μm) was shorter (*P*=0.008) than SG IV height (358 μm) by 14 μm (**Figure 2.17a**). However, FG ileum crypt (IC) depth (91 μm) was greater (*P*=0.007) than SG IC (87 μm) by 4 μm (**Figure 2.17b**). The jejunum villus-crypt ratio (JVCR) of FG (5.0) was lower (*P*=0.003) than SG JVCR (5.2) by 0.2, and FG ileum villus-crypt ratio (IVCR; 3.9) was lower (*P*<0.0001) than SG IVCR (4.3) by 0.4 (**Figure 2.17c**).

On d24, there was a main effect of challenge on IV height but not JV height (**Table 2.8**). CON IV height (517 μm) was greater (*P*=0.009) than ST IV (422 μm) by 95 μm (**Figure 2.18a**). The effect of challenge was also significant for JC depth among FG birds but not SG on d24 (**Table 2.8**). FG-CON JC depth (119 μm) was greater (*P*=0.05) than FG-ST JC (142 μm) by 23 μm (**Figure 2.18b**). The main effect of challenge on

IVCR was significant on d24 in which CON IVCR (5.2) was greater (P=0.007) than ST IVCR (4.1) by 1.1 (**Figure 2.18c**).

2.5.6 Correlations

Overall, plasma IgA concentrations correlated with several measures, but all correlations were moderate or weak in strength (**Table 2.9**). There were positive correlations between IgA and BW (r=0.43; $P \leq 0.01$), JV (r=0.41; $P \leq 0.01$), JVCR (r=0.35; $P \leq 0.01$), and IV (r=0.32; $P \leq 0.01$). IgG did not correlate with any measure independent of breed. Within FG, IgA was positively correlated with BW (r=0.46; $P \leq 0.01$), JV (r=0.35; $P \leq 0.05$), JVCR (r=0.33; $P \leq 0.05$), IV (r=0.31; $P \leq 0.05$), and IgG (r=0.21; $P \leq 0.05$). Within SG, plasma IgA positively correlated with BW (r=0.34; $P \leq 0.01$), JV (r=0.45: $P \leq 0.01$), and IC (r=0.43; $P \leq 0.05$). SG plasma IgG, correlated negatively with BW (r=-0.38; $P \leq 0.01$), IV (r=-0.54; $P \leq 0.01$), and IVCR (r=-0.42; $P \leq 0.05$).

2.5 DISCUSSION

The objective of this study was to evaluate differences in body weight, immune response, and gut morphology between fast- and slow-growing broiler chickens when challenged with *Salmonella* Typhimurium. Chicks from fast- (Ross 308) and slow-growing (Redbro) breeds of broiler chicken were housed together between day of hatch and day 7, when they were randomly assigned and exclusively placed into BSL-2 isolators. At day 14, half of the birds were orally gavaged with the *S.* Typhimurium challenge and half received Tryptic Soy Broth control. Throughout the 24-day study, birds were weighed and sampled for blood and intestinal segments to evaluate differences in weights, plasma IgA and IgG, and jejunal and ileal gut morphology (villus height, crypt depth, and villus height to crypt depth ratio). The first hypothesis was that fast-

growing broilers would have greater body weight, greater intestinal morphology measures, and lower plasma immunoglobulin concentrations when compared to the slow-growing breed independent of challenge. The second hypothesis was that after challenge, challenged fast-growing broilers would be less negatively impacted than the slow-growing broilers with regard to body weight and gut morphology, but the slow-growing breed would have greater plasma IgA and IgG responses to challenge.

2.6.1 Qualitative Bacteriology for *Salmonella* spp. Presence/Absence

While no *Salmonella* spp. was detected prior to challenge, by day 13 all birds were positive for non-*Salmonella* bacterial growth. BSA agar is a selective and differential media commonly used to identify *Salmonella* spp. growth, but other species of bacteria can grow on BSA despite inhibition, such as coliforms (Rijal, 2021). Based on the low overall mortality and lack of clinical symptoms of disease and sickness behaviors, it is likely that these species of bacteria were commensal or otherwise had no detectable negative impact on broiler health throughout the study.

2.6.2 Mortality

Day 7 mortality was below the industry average expected mortality of 0.7% (Aviagen, 2018), indicating that the birds were in good health. Most mortalities belonged to the fast-growing breed, while only one slow-growing breed mortality occurred, which might indicate breed-related differences in survivability particularly prior to day 7. Such differences in mortality between fast- and slow-growing breeds has been reported previously, in which conventional fast-growing breeds have had greater mortality than slower-growing breeds (Yunis et al., 2000; Fanatico et al., 2008; Abeyesinghe et al., 2021). However, Torrey and colleagues (2021) noted fewer culls but more birds found

dead among slow-growing breeds compared to moderate- and fast- growing broiler breeds. Other research has observed no differences in mortality between fast- and slow-growing broiler breeds (Fanatico et al., 2005; Weimer et al., 2020).

Throughout the rest of the study, only 1 mortality and 1 cull (lameness) occurred, both among the fast-growing control group. No mortalities were associated with the challenge treatment, which is consistent with findings from Marcq and colleagues (2011). However, breed-specific differences in mortality can exist between fast- and slow-growing breeds when challenged with other pathogens. For example, Han and Smyth (1972) recorded 30.8% mortality in a fast-growing breed when infected with Marek's Disease compared to 5.7% in a slow-growing breed.

2.6.3 Body Weight

In this study, birds of both breeds and treatments were below their projected body weights by day 24 of age when compared with other research using the same breeds (Kazemi et al., 2018; Weimer et al., 2020). The fast- and slow-growing breeds in this study were 891 g and 751 g on day 24, respectively, while Weimer and colleagues (2020) reported body weights of approximately 1,200 and 900 g at the same age. When compared to the Aviagen Ross 308 Performance Objectives handbook (2019), the fast-growing Rosses in this study were 334 g lighter than expected at day 24. It is possible these differences occurred as a result of using a commercial feed as opposed to a feed specifically formulated for either of the breeds used in this study. Feed composition can greatly impact body weight gain of broilers (Havenstein et al., 2003). For example, methionine is an amino acid known for its role in increasing broiler body weight (Mirzaaghatabat et al., 2010). The Purina feed used in this study had a methionine ratio of

0.34%, which meets the minimum NRC recommendation for meat chickens (NRC, 1994). Additionally, research by Torrey and colleagues (2021) involving a comparison between 16 broiler breeds varying in growth rate used a feed formulated for a moderate slow-growing breed, which reduced the growth of the fast-growing conventional breeds in the study.

Beginning at day 13, the fast-growing breed was heavier than the slow-growing breed, for which this difference was significant at days 21 and 24. This divergence in body weight is expected, as the breeds used are reported to grow similarly for about two weeks after hatch, after which the fast-growing breed should gain weight at a faster rate than the slow-growing breed (Weimer et al., 2020). At day 24, challenged fast-growing birds had numerically (but not significantly) lower body weight than control fast-growing birds, while slow-growing body weights did not differ between challenge and controls. It is possible that prolonged infection with *S.* Typhimurium was beginning to cause reductions in body weight gain or feed efficiency. Marcq and colleagues (2011) reported a body weight gain reduction of 14.5% in broilers at 42 days when challenged with *S.* Typhimurium at 21 days of age.

Other research involving *S.* Enteritidis or *S.* Typhimurium infection, LPS challenge, or *Eimeria* spp. have also reported reduced body weight gain (Xie et al., 2000; Liu et al., 2014; Sakkas et al., 2018; Iuspa et al., 2020). Infections require a diversion of bodily energy and resources from growth and maintenance functions towards mounting an immune response, which then results in reduced body weight (Greer, 2008). Reduced body weight in challenged compared to control birds could also result from increased levels of proinflammatory cytokines, which induce anorexia or reduced feed consumption

(Finck and Johnson, 1997). Though the difference was merely numerical in the current study, it is possible that beyond day 24 (10 days post-infection), the fast-growing challenged birds may have had greater body weight reductions in compared to controls due to *S.* Typhimurium infection. On the other hand, the body weight of the slow-growing breed did not appear to be impacted by challenge, indicating that the slow-growing breed may be more resistant to prolonged *S.* Typhimurium infection at day 24 (10 days post-challenge) than the fast-growing breed.

2.6.4 Immune Response

IgA

Expected levels of IgA in the plasma of an adult chicken are 600 μg/mL, while IgG (IgY) levels can be between 4,500 and 5,000 μg/mL (Tizard, 2002). The plasma immunoglobulin concentrations recorded in this study at 24 days (IgA 99 μg/mL; IgG 2,127 μg/mL) appear consistent with these findings, given that broilers are not fully matured at 24 days of age. However, across multiple studies there is a wide variance in plasma immunoglobulin levels in broilers, even with use of the same commercial ELISA kit as the present study (Chaudhari et al., 2012; Li et al., 2012; Gomes et al., 2014). Li and colleagues (2012) found 330 μg/mL of IgA and 630 μg/mL of IgG in the plasma of broilers at day 21 days, while in the current study broiler IgA and IgG levels at 21 days were 192 μg/mL and 1,553 μg/mL, respectively. Differences in immunoglobulin levels could have resulted from degradation over time, as samples were stored in either -20ºC (4 months) or-80ºC (10 months) for a total of 14 months in storage prior to, and some were partially or fully thawed once or twice prior to assay use (Abcam). However, it is also

noted that antibodies, including IgG, can remain stable through multiple freeze-thaw cycles (Rastawicki et al., 2012; Castejon et al., 2017; Maelegheer et al., 2018).

Baseline plasma IgA concentrations were elevated for both breeds within the control group at day 13, the day just prior to challenge. This difference could be attributed to randomly sampled individual control birds naturally that had a greater concentration of plasma IgA, but it is also possible that this difference resulted from the time of day that which birds were sampled. Due to biosecurity protocol and consistency across sampling days, all assigned control birds were sampled prior to assigned challenge birds at each sampling. Sampling began in the morning and lasted several hours, resulting in the first sampling of challenge birds occurring between 2-3 hours after the first control bird. As a result, circulating levels of immunoglobulins may have dropped prior to challenge bird sampling. Prior research has reported that levels of white blood cells differed throughout the day in broiler chicken blood (Makeri et al., 2017). However, Cernysiov and colleagues (2009) reported an effect of melatonin on serum IgM and IgG production in mice by using light modulation to influence melatonin production, noting that increased lighting (and therefore, reduced melatonin) increased levels of IgM and IgG in the serum. Melatonin plays a role in regulating the circadian rhythm and is produced in response to darkness and suppressed by the presence of light (Cernysiov et al., 2009). If plasma antibody levels were affected by melatonin in response to lighting, we would have expected the control birds to have lower immunoglobulin levels during sampling than challenge birds, as they were sampled temporally closer to the end of dark hours. Additionally, this pattern of increased plasma IgA was not observed any other day within the present study, nor was it observed in IgG levels on any sampling day.

Plasma IgA levels remained similar from days 7 to 17 in both breeds, increased at day 21 in both challenge and control treatments, then decreased to an intermediate concentration at day 24. These general increases in concentration with age may reflect development of the immune system and immune organs (Fellah et al., 2014). On day 21, plasma IgA concentrations peaked in both challenge and control treatments but were greater in challenge birds than in control birds. This can indicate a potential humoral immune response to the *S.* Typhimurium challenge within the intestines. Avian IgA predominates in intestinal secretions and protects intestinal surfaces from invasion by pathogens (Tizard, 2002). Since IgA is produced by B-cells located under intestinal mucosal surfaces, IgA can also enter the bloodstream from the intestines (Tizard, 2002). In one study, broilers were orally challenged with *S.* Typhimurium at 21 days, resulting in an increase in intestinal IgA antibody titers 8 days following infection which was later associated with apparent clearance by 22 days post-infection (Marcq et al., 2011). Research by Hassan and colleagues (1991) involving dual-purpose chickens supports the finding that *S. enterica* serovars can elicit a strong humoral immune response in serum, inclusive of IgA, when orally challenged. In another study, White Leghorns injected intramuscularly at 1 day post-hatch with *S.* Enteritidis had elevated serum IgA levels (Sheela et al., 2003).

Though challenge birds had greater IgA levels at day 21 (7 days post-challenge), it is important to note that IgA also peaked in control birds. In the present study, control birds were gavaged with TSB, rather than saline, to control for the effects of the challenge culture media. TSB is a medium that supports the growth of a multitude of microbes and its sudden introduction into the chicken gut could have great potential for

causing imbalances in the gut microbiome, otherwise known as dysbiosis (Kogut, 2013). As the predominant antibody secreted in the intestines (Tizard, 2002), IgA has dual roles in protecting the intestinal epithelium from pathogens as well as regulating the gut microbial community (Yang and Palm, 2020). Thus, it is possible that IgA production was increased at day 21 in control birds due to gut microbial disturbance from gavaging TSB at 14 days. Brown and colleagues (2000) reported increased plasma corticosterone levels in rats gavaged with lipids (such as corn oil, soybean oil, and peanut oil) but not in rats gavaged with water, indicating that gavaging with non-saline liquids causes stress. The link between stress and gastrointestinal issues, including microbiome imbalances or dysbiosis, has been thoroughly studied, especially in humans (Hawrelak and Myers, 2004). A negative control (no gavage) or a gavage control of saline might have elucidated the potential effects of TSB on the IgA response in broilers in the present study. More research is needed to clarify the effects of gavage use and liquid type on the gut microbiome and intestinal humoral immune response of chickens.

Holt and colleagues (1991) noted that in White Leghorn chicks infected 1 day post-hatch with *S.* Enteritidis, birds that had an increased IgA response in the intestine were unable to clear *S.* Enteritidis. Though both the fast- and slow-growing breeds in this study exhibited numerical increases in plasma IgA concentration within the challenge groups at day 21, the difference between challenge and control birds was only significant in the fast-growing breed. A greater magnitude of a difference in IgA concentration in response to challenge could be indicative of a stronger humoral immune response to *S.* Typhimurium in the fast-growing breed compared to the slow-growing breed (Kramer et al., 2003). Alternatively, it has been noted by Humphrey and Klasing (2004) that the

adaptive immune response and growth rate are inversely related due to the competition for nutrients between the immune response and metabolism.

Peaks in antibody levels are generally associated with clearance of a pathogen, but a strong humoral immune response may not be effective or indicative of strong immune function (van der Most, 2010; Barrow et al., 2012). By day 24, plasma IgA concentrations in challenged birds of both breeds were similar to controls. However, IgA levels remained numerically higher in slow-growing challenged birds than slow-growing controls, potentially indicating a less effective humoral response to *S.* Typhimurium and reduced likelihood of clearance compared to fast-growing (Holt et al., 1999; Barrow et al., 2012). At day 24, the fast-growing breed had higher plasma IgA concentration than the slow-growing breed, and all treatments had lower plasma IgA at day 24 than day 21. The effect was only significant among the control birds due to the elevated plasma IgA concentration in the slow-growing challenged birds compared to controls.

Natural antibodies increase with age in the chicken (Parmentier et al., 2004). In this study, plasma IgA and IgG generally both increased with age. Exceptions to this trend were the greater levels of IgA at day 21 compared to day 24 in both challenge and control birds (which may signal an intestinal humoral response to both challenge and control treatments), the lack of change in slow-growing IgG levels after day 13 (which may signal delayed immune development), and the high levels of IgG in both breeds at day 7 (which may be due to remnant maternal antibody).

IgG

Plasma IgG levels were highest at day 7 in both breeds compared to all other ages except day 24, which may be remnant maternal antibody (Gharaibeh and Mahmoud,

2013; Fellah et al., 2014). IgG, also termed IgY (or immunoglobulin of the yolk), is transferred to chicks through the egg yolk and lasts up to 10 days post-hatch, providing critical early life immune protection to chicks (Carlander et al., 1999; Gharaibeh and Mahmoud, 2013). Maternal IgG is especially important in broiler health due to their relative short lifespan on-farm (Gharaibeh and Mahmoud, 2013). The slow-growing breed had greater plasma IgG concentrations than the fast-growing breed at day 7, reflecting greater circulating concentrations of maternal antibodies. The secondary immune organs – such as the spleen and cecal tonsils – and the resulting humoral immune response in chicks are not mature enough to mount an immune response until approximately 12 days (Mast and Goddeeris, 1999) and not fully developed until approximately 30 days of age in broilers (Song et al., 2021). Thus, the slow-growing breed in the current study may have been better protected by maternal antibodies than the fast-growing breed prior to immune system maturation.

At days 21 and 24, the fast-growing breed had higher levels of plasma IgG than the slow-growing breed. This magnitude of difference was even greater at day 24. Interestingly, IgG concentrations in both breeds decreased between days 7 and 17, concentrations were at their lowest on day 17, then diverged thereafter. The fast-growing breed plasma IgG increased greatly between days 17 and 24, whereas the slow-growing breed plasma IgG plateaued. These differences could be attributed to differences in overall growth rate and organ development. Nitsan and colleagues (1991) found breed-related differences between a commercial broiler breeder and two Plymouth Rock breeds selected for either high or low body weight. The commercial broiler had the greatest absolute weight for all organs measured at day 15 except the residual yolk, including the

lung, heart, liver, pancreas, crop, proventriculus, gizzard, duodenum, jejunum, and ileum (Nitsan et al., 1991). The broiler breed also had the greatest relative organ weight to body weight in the heart, pancreas, crop, proventriculus, gizzard, and all components of the small intestine (Nitsan et al., 1991). Should lymphoid organ growth also correlate with body weight, it is possible that selection for fast-growing broilers may have inadvertently selected for broilers whose lymphoid organs–and as a result, immune system–develop earlier in life, resulting in higher natural antibody levels at an earlier age. However, research by Cheema and colleagues (2003) compared a 1957 broiler breed (Arbor Acres) and a 2001 broiler breed (Ross 308) and noted that the slower-growing Arbor Acres broiler had greater Bursa of Fabricius, spleen, and cecal tonsil weights at 24 days than the Ross 308 broilers, potentially due to resource allocation towards muscle development rather than organ development. On the other hand, the Ross 308 broilers had a greater thymus weight (Cheema et al., 2003). Alternatively, Rothschild (2019) found no differences in Bursa of Fabricius weight between a conventional fast-growing broiler breed and 3 slower-growing breeds.

There was no effect of challenge on plasma IgG concentration. However, challenged slow-growing birds had numerically greater IgG concentrations than controls at days 21 and 24, indicating a potential muted IgG response to infection. Prior research by Hassan and colleagues (1991) noted a plasma IgG response in response to *S.* Typhimurium infection in Sussex chickens. IgG is the most prominent immunoglobulin in the blood (Tizard, 2002), and due to its high prevalence, any effect of challenge on plasma IgG levels might have been hidden as a result of a low magnitude of an increase in antigen-specific IgG to *S.* Typhimurium compared to the high levels of natural IgG.

The commercial IgG kit used in the present study (Bethyl Laboratories Inc., Montgomery, TX) did not detect *S.* Typhimurium-specific IgG and only measured total plasma IgG concentration. Some researchers developed IgG-specific assay antibodies and have reported differences in serum IgG in response to *S.* Typhimurium infection (Hassan et al., 1991; Dar et al., 2019). Dar and colleagues (2019) reported greater optical densities (492 nm) in Cobb broilers orally challenged with *S.* Typhimurium 4 days post-hatch by approximately 0.025 to 0.05 between 1- and 13-days post-challenge using an assay previously developed by Holt and colleagues (1999). The present study would have benefitted from utilizing a *S.* Typhimurium specific IgG assay to measure the concentrations of circulating IgG antibodies in response to the *S.* Typhimurium challenge.

The lack of a significant response could also have been influenced by feed composition. Arginine and vitamin E in feed have been shown to improve broiler immune response to *S.* Typhimurium challenge, evidenced by increased antibody titers compared to controls (Liu et al., 2014). Broilers need 1.25% arginine and 10 IU/kg of vitamin E to meet their minimal nutritional requirements (NRC, 1994). Muted differences in IgG response could have also been affected by the use of TSB to gavage control birds. TSB may have induced dysbiosis in the gut (Kogut, 2013) and resulted in increased plasma IgG production in addition to increased plasma IgA.

The IgA and IgG results of this study suggest that the fast-growing breed might also mature more quickly with regard to the humoral immune response. Though the slow-growing breed had potentially greater maternal antibody early in the study, supporting its survival early life, the fast-growing breed ultimately had higher plasma antibody

concentrations at days 21 (IgG only) and 24 (IgA and IgA) in addition to a stronger IgA response to challenge at day 21 followed by a return to control levels at day 24. A relationship between growth rate and humoral immunocompetence might be revealed through mortality. Dixon (2020) recorded mortality in a slow-growing Hubbard broiler versus 3 faster-growing commercial breeds and found the faster growing breeds had an overall greater proportion of culls but only 1 fast-growing breed had more birds found dead after 3 weeks of age when compared to the slow-growing breed. Additionally, fewer slow-growing birds were found dead in the first 2 weeks than any of the fast-growing breeds (Dixon, 2020).

Multiple studies have explored the relationship between selection for growth or body weight in relation to immune function (Yunis et al., 2000; Leshchinsky and Klasing, 2001; Humphrey and Klasing, 2004; Parmentier et al., 2010; van der Most et al., 2011). Several have observed an inverse relationship between growth and immune function. Humphrey and Klasing (2004) discussed that an elevated immune response impairs growth due to alterations in nutrient needs by different bodily functions (metabolic versus immune). As a result, it could be that broilers genetically selected for enhanced growth rate may have a weaker immune system. When a broiler breed and a laying breed (Brown Nick) were injected with LPS, a weaker febrile (innate immune) response was observed in the broilers than the layers (Leschinsky and Klasing, 2001). Van der Most and colleagues (2011) also noted that selection for increased growth in broilers greatly impairs immune function in response to infection, agreeing that selection for growth comes with an energy cost that can compromise the immune system. However, it should be noted that differences may exist between the relationship of growth rate and innate

immune response and the relationship of growth rate and adaptive or humoral immune response. The innate immune response, inclusive of fever and inflammation, requires a greater amount of energy than the adaptive immune response (Iseri and Klasing, 2013), meaning that selection for increased growth rate may not negatively impact the humoral immune response as it does the innate immune response. Selection for increased growth rate may simply only increase the rate at which lymphoid organs and immunocompetency are developed independent of immune function or strength and efficacy of the immune response. More research is needed to explore the effect of growth rate on the development of lymphoid organs and immunocompetence in broilers.

2.6.5 Gut Morphology

The birds in this study had shorter villi and crypt measures than broilers of the same or younger age in other studies (Fasina et al., 2010; Lee et al., 2010; Golder et al., 2011). Fasina and Colleagues (2010) reported jejunum villus heights and crypt depths in 14-day-old broiler chicks approximately 392 μm and 58 μm greater, respectively, than those of the 24-day-old broilers in the present study. Additionally, Lee and colleagues (2010) reported ileum villus heights and crypt depths in 21-day-old broilers that were 243 μm and 64 μm greater, respectively, than those of the broilers in the present study.

It is possible that feed composition may have also impacted gut morphology measures as it did body weight, as it was a commercial feed not specifically formulated for either breed used in the present study. In rats, added dietary methionine increased villus heights and villus height to crypt depth ratios throughout all sections of the small intestine (Seyyedin and Nazem, 2017), so it may be expected that reduced methionine may limit intestinal morphological growth in broilers. The methionine ratio of the Purina

feed used in this study (0.34%) met the minimum NRC recommendation for methionine ratio for broilers (NRC, 1994). Additionally, gavaging the control birds with TSB may have impacted gut morphological measures. Since TSB supports the growth of wide variety of microbes, it may be capable of causing imbalances (or dysbiosis) in the gastrointestinal tract (Kogut, 2013). Prior research by Li and Colleagues (2020) observed impaired jejunal and ileal intestinal structure in rhesus macaques following antibiotic-induced dysbiosis. On the other hand, the provision of probiotics and direct-fed microbials can increase intestinal morphological measures in broilers (Kazemi et al., 2018). Thus, it is possible that the use of TSB as the control group liquid in the present study caused dysbiosis, therefore impacting gut morphology in the control birds and possibly muting any differences between breeds or challenge treatments. A negative control (no gavage) or gavaging saline solution instead of TSB might have given clarity as to the effect TSB might have had on gut morphology. As such, more research is needed to understand how TSB influences the gut microbiome and gut morphology in chickens.

At day 7, the fast- and slow-growing breeds differed in all histomorphological measures. The fast-growing breed had greater jejunal villus height and crypt depth than the slow-growing breed, which may suggest greater absorptive capacity in the jejunum of birds bred for faster growth (Yamauchi et al., 2010; Kiela and Ghishan, 2016). The slow-growing breed, on the other hand, had greater ileum villus height but shallower crypts than the fast-growing breed. Growth-related differences in gut morphological measures, specifically villus height, have been documented, noting that selection for growth in broilers has resulted in villi that are larger than those in White Leghorns (Yamauchi and

Isshiki, 1991). Between both intestinal segments, however, the slow-growing breed had greater villus height to crypt depth ratios than the fast-growing breed, which indicates reduced cellular turnover in the jejunum and ileum of the slow-growing breed (Seyyedin and Nazem, 2017). Gut morphology measures at the intermediate days might have provided further insight on the development of intestinal structure as each breed aged.

These aforementioned breed differences disappeared by day 24 (10 days post-infection), but challenge impacted gut morphology, particularly in the fast-growing breed. Within the jejunum, the challenged fast-growing birds had deeper crypts than the fast-growing controls and the slow-growing birds, which may be indicative of increased enterocyte production in the crypts to compensate for enterocyte loss at villi tips due to infection. Fernando and McCraw (1973) found that *Eimeria acervulina* infection in 14-week-old male White Leghorn chicks reduced jejunal villus heights and caused increased crypt depths, which then returned to normal or greater lengths or depths 5-6 days post-infection. However, the effect of challenge had no impact on jejunum villus height in either breed, a finding which is supported by Gomes and colleagues (2014) who challenged 1-day-old male Ross chicks with *Salmonella* Enteritidis and noted no significant differences in any gut morphology measures. On the other hand, Shao and colleagues (2013) noted reductions in jejunum villus height and villus height to crypt depth ratio in broilers challenged with *S.* Typhimurium at 7 days of age. Other research has found differences in jejunal gut morphological measures in response to other challenges, such as necrotic enteritis (Golder et al., 2011; Xue et al., 2018). The lack of difference in jejunum villus height between the fast-growing control and challenged birds, paired with the increased crypt depths in the challenge group, could signal

increased enterocyte turnover and regeneration by the crypts in response to infection and villus damage. At day 24, the jejunum villi of the challenged fast-growing birds might have regenerated enough to be comparable to control birds while the jejunum crypts had yet to return to normal depths. Viable gut morphology measures at days 17 and 21 (3 and 7 days post-challenge) might have provided insight as to if the fast-growing birds in the challenge treatment had reduced jejunum villus height prior to day 24.

Within the ileum, challenged fast-growing birds had reduced villus heights and as a result, was reflected in the reduced villus height to crypt depth ratio, when compared to controls. Reduced villus height and villus height to crypt depth ratio can result from bacterial infection in the small intestine (Fasina et al., 2010). The slow-growing breed displayed similar numerical reductions, but they were not statistically significant. Thus, the magnitude of the difference between control and challenged birds was greater in the fast-growing breed than the slow-growing breed. Combined with the increased jejunum crypt depth, it is possible that the fast-growing breed intestinal structure may be less resilient to infection than the slow-growing breed. Gao and colleagues (2013) found that, in two breeds of pigs (Jinhua and Landrace) challenged with *E. coli*, the Jinhua breed's intestinal villi heights and villus height to crypt depth ratio were less impacted than the Landrace breed, suggesting that Jinhua pigs had stronger intestinal structure. The difference in magnitude between the fast- and slow-growing broiler breeds in the present study may result in reduced absorptive capacity of nutrients and ultimately impact feed efficiency and body weight gain (Yamauchi et al., 2010).

Data from the intermediate sampling days following challenge at day 14 (days 17 and 21) might have provided further insight on the effect of challenge on intestinal

morphology, especially early in infection. The increased crypt depth in the jejunum of challenged fast-growing birds but lack of a difference in jejunum villus height might indicate that the fast-growing breed experienced villus damage in the jejunum but increases in crypt depth allowed for heightened enterocyte production to replace lost cells (Williams et al, 2014**).** As a result, fast-growing breed jejunum villus height may have been restored following challenge, but the jejunum crypts did not yet return to normal (control) depth (Fernando and McCraw, 1973). If the study had been carried out past day 24, we hypothesize that a return to control jejunum crypt depth may have occurred in the challenged fast-growing birds. Although Mohammadigheisar and colleagues (2020) found no difference in jejunum villus height and crypt depth in 48-day-old fast- and slow-growing mixed sex broilers, measures from older ages might have also provided insight to the development of intestinal structure in fast- versus slow-growing broilers.

2.6.6 Correlations

In this study, correlations were only reported for the relationships between plasma IgA and IgG concentrations and all other variables, as the relationship between gut morphologies and body weight are already known (Yamauchi and Isshiki, 1991; Yamauchi et al., 2010). There was a positive correlation between IgA concentration and body weight among both breeds, which was slightly stronger in the fast-growing breed. Additionally, the correlation between IgG and body weight was negative in the slow-growing breed, but a very weak and insignificant positive correlation was detected in the fast-growing breed. IgA and IgG concentrations positively correlated with one another in the fast-growing breed, but not the slow-growing breed. Combined, these support the possibility that selection for increased growth rate in the fast-growing breed may

unintentionally select for earlier development of immunocompetence. Plasma IgG concentrations little to no significant relation to gut morphological measures, except in the slow-growing breed where IgA and ileum villus height (and ileum villus height to crypt depth ratio) negatively correlated. On the other hand, plasma IgA concentration positively correlated with multiple gut morphology measures, such as jejunum villus height. This may reflect a relationship between intestinal morphology and intestinal humoral secretions, as plasma IgA concentration can be representative of intestinal IgA concentration (Tizard, 2002).

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Table 2.1. Summary of events from start (d0) to end (d24) of the study, including a timeline of events and the number of birds involved at each event.

Age (d)	**DPC**	**Event**
0	DPC 0	312 chicks placed in a single pen (N=156/breed).
7	DPC -7	BW, blood, jejunal and ileal segments, and cecal contents collected from 48 birds (N=24/breed). Remaining birds (N=264 birds, 132/breed) moved into isolators after sampling.
12	DPC -2	Video of 8 isolators (N=4/breed, 2/room) recorded for 1 hour.
13	DPC -1	BW, blood, jejunal and ileal segments, and cecal contents collected from 48 birds (N=24/breed, 2/isolator).
14	DPC 0 Challenge	Treatments administered via oral gavage to 216 birds. Challenge: 1 mL 10^8 CFU/mL *S.* Typhimurium given to 108 birds (N=54/breed). Control: 1 mL Tryptic Soy Broth given to 108 birds (N=54/breed).
16	DPC 2	Video of 8 isolators (N=4/breed, 2/room) recorded for 1 hour.
17	DPC 3	BW, blood, jejunal and ileal segments, and cecal contents collected from 48 birds (N=24/breed, 2/isolator).
20	DPC 6	Video of 8 isolators (N=4/breed, 2/room) recorded for 1 hour.
21	DPC 7	BW, blood, jejunal and ileal segments, and cecal contents collected from 48 birds (N=24/breed, 2/isolator).
23	DPC 9	Video of 8 isolators (N=4/breed, 2/room) recorded for 1 hour.
24	DPC 10	BW, blood, jejunal and ileal segments, and cecal contents collected from 48 birds (N=24/breed, 2/isolator).

Table 2.2. Orientation to figure color coding and representative treatments.

Color	Treatment/Effect
Light blue	Slow-Growing Control (SG-CON)
Dark blue[1]	Slow-Growing Challenge (SG-ST) and Slow-Growing (SG)
Light red	Fast-Growing Control (FG-CON)
Dark red	Fast-Growing Challenge (FG-ST) and Fast-Growing (FG)
Gray	All Birds and Treatments
Green	Control (CON)
Purple	Challenge (ST)

[1]Dark blue and dark red are used to represent both their respective breed and challenge interaction as well as breed independent of challenge.

Table 2.3. Effect of breed and challenge on broiler body weight (BW, g) at d7, 13, 17, 21, and 24. Data shown as mean BW (± SEM) of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with S. Typhimurium (ST) or TSB (CON) on d14.

	Treatment					**P-Value**[2]		
Age (d)	**FG-CON**[1]	**FG-ST**	**SG-CON**	**SG-ST**	**SEM**	**Br.**	**Chal.**	**Int.**
7	129	-	127	-	4.0	0.69	-	-
13	279	316	271	276	9.8	0.08	0.12	0.23
17	441	461	399	413	22.7	0.06	0.45	0.91
21	682[a]	709[a]	625[b]	626[b]	31.3	0.04	0.66	0.69
24	906[a]	876[a]	754[b]	747[b]	47.0	0.007	0.70	0.80

[1]FG = fast-growing (Ross), SG = slow-growing (Redbro), ST = 1 mL 1.3x10^8 CFU/mL *S.* Typhimurium challenge, CON = control (TSB).
[2]P-values represent the main effect of breed (Br.; FG or SG), challenge (Chal.; ST or CON), and the interaction of breed and challenge (Int.).
[ab] Rows not sharing the same letters indicate a significant difference at $P \leq 0.05$.

Table 2.4. Effect of breed and challenge on broiler plasma IgA and IgG concentrations (μg/mL) at d7, 13, 17, 21, and 24. Data shown as mean concentration (± SEM) of immunoglobulin in the plasma of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) on d14.

	Treatment					P-Value[2]		
Age (d)	**FG-CON**[1]	**FG-ST**	**SG-CON**	**SG-ST**	**SEM**	**Br.**	**Chal.**	**Int.**
IgA								
7	47	-	47	-	5	0.93	-	-
13	74[a]	59[b]	69[a]	44[b]	7	0.13	0.009	0.47
17	61	64	51	61	7	0.41	0.37	0.64
21	138[b]	168[a]	107[b]	164[a]	19	0.36	0.03	0.49
24	121[a]	115[a]	72[b]	88[b]	14	0.01	0.73	0.4
IgG								
7	2693[b]	-	3037[a]	-	120	0.05	-	-
13[3]	1991	1709	1589	1578	189	0.19	0.44	0.47
17	1341	1218	1281	962	157	0.33	0.18	0.54
21	2120[a]	1597[a]	985[b]	1356[b]	284	0.03	0.79	0.13
24	3051[a]	2675[a]	1199[b]	1580[b]	334	0.0003	0.99	0.27

[1]FG = fast-growing (Ross), SG = slow-growing (Redbro), ST = 1 mL 1.3x10[8] CFU/mL *S.* Typhimurium challenge, CON = control (TSB).

[2]P-values represent the main effect of breed (Br.; FG or SG), challenge (Chal.; ST or CON), and the interaction of breed and challenge (Int.).

[3]d13 FG-ST SEM (IgG) = 205

[ab] Rows not sharing the same letters indicate a significant difference at $P \leq 0.05$.

Table 2.5. Effect of age (d) and challenge on broiler plasma IgA and IgG concentrations (μg/mL) at d7, 13, 17, 21, and 24, within breed. Data shown as mean concentration (± SEM) of immunoglobulin in the plasma of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) on d14.

	d13		d17		d21		d24			P-Value[3]		
Br.[1]	**CON**[2]	**ST**	**CON**	**ST**	**CON**	**ST**	**CON**	**ST**	**SEM**	**Age**	**Chal.**	**Int.**
IgA												
FG[3]	74[c]	60[c]	61[c]	64[c]	138[a]	168[a]	121[b]	115[b]	12	0.0001	0.74	0.24
SG	69[c]	44[c]	51[c]	61[c]	107[a]	164[a]	72[b]	88[b]	12	0.0001	0.17	0.009
IgG												
FG[4]	1991[b]	1686[b]	1341[c]	1218[c]	2120[b]	1597[b]	3051[a]	2675[a]	289	0.0001	0.23	0.89
SG	1589[a]	1281[a]	985[b]	1199[b]	1578[b]	962[b]	1356[ab]	1580[ab]	179	0.04	0.46	0.14

[1]Br. = breed; FG = fast-growing (Ross), SG = slow-growing (Redbro)
[2]CON = control (TSB), ST = 1 mL $1.3x10^8$ CFU/mL *S.* Typhimurium challenge.
[3]P0-values represent the main effect of age (d), challenge (Chal.; ST or CON), and the interaction of age and challenge (Int).
[3]d13 FG-ST SEM (IgA) = 13
[4] d13 FG-ST SEM (IgG) = 312
[ab] Rows not sharing the same letters indicate a significant difference at $P \leq 0.05$.

Table 2.6. Effect of age (d) and breed on broiler plasma IgA and IgG concentrations (μg/mL) at d7, 13, 17, 21, and 24, within challenge. Data shown as mean concentration (± SEM) of immunoglobulin in the plasma of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with S. Typhimurium (ST) or TSB (CON) on d14.

	d13		d17		d21		d24			P-Value[3]		
Chal.[1]	**FG**[2]	**SG**	**FG**	**SG**	**FG**	**SG**	**FG**	**SG**	**SEM**	**Age (d)**	**Br.**	**Int.**
IgA												
CON	74^{b}	69^{b}	61^{b}	51^{b}	138^{a}	107^{a}	121^{a}	72^{b}	11	0.0001	0.008	0.23
ST[3]	60^{c}	44^{c}	64^{c}	61^{c}	16^{a}	164^{a}	115^{b}	88^{b}	13	0.0001	0.31	0.71
IgG												
CON	1991^{b}	1589^{bc}	1341^{c}	1281^{c}	2120^{b}	985^{c}	3051^{a}	1199^{c}	221	0.003	0.0004	0.0004
ST[4]	1682^{b}	1578^{bc}	1218^{c}	962^{c}	1597^{bc}	1356^{bc}	2675^{a}	1580^{a}	258	0.0001	0.13	0.08

[1]Chal. = challenge; CON = control (TSB), ST = 1 mL $1.3x10^{8}$ CFU/mL *S.* Typhimurium challenge.
[2]FG = fast-growing (Ross), SG = slow-growing (Redbro).
[3]P-values represent the main effect of age (d), breed (Br.; FG or SG), and the interaction of age and breed (Int).
[3]d13 FG-ST SEM (IgA) = 14
[4] d13 FG-ST SEM (IgG) = 277
[ab] Rows not sharing the same letters indicate a significant difference at $P \leq 0.05$.

Table 2.7. Counts (N) and mean proportions (%) of individual birds with viable histological samples. Counts and means (μm) of viable intestinal measures of male broilers from fast- (FG) and slow-growing (SG) breeds at d7, 13, 17, 21, and 24 when challenged with S. Typhimurium (ST) or TSB (CON) at d14.

		Birds		Jejunum				Ileum			
Age (d)		**N**[1]	**%**	**N**	**JV**[2]	**JC**	**JVCR**	**N**	**IV**	**IC**	**IVCR**
7	**FG**[3]	23	95.8	228	508	104	5.0	230	344	91	3.9
	SG	20	83.3	197	486	97	5.2	198	358	87	4.3
13	**FG-CON**	9	75.0	79	548	84	6.8	90	351	75	4.9
	FG-ST	0	0.0	0	-	-	-	0	-	-	-
	SG-CON	8	66.7	78	524	88	6.3	79	330	62	5.6
	SG-ST	0	0.0	0	-	-	-	0	-	-	-
17	**FG-CON**	4	33.3	33	688	95	7.5	40	466	80	6.1
	FG-ST	4	33.3	40	684	103	6.9	40	414	90	4.7
	SG-CON	4	33.3	39	683	86	8.4	38	449	75	6.4
	SG-ST	0	0.0	0	-	-	-	0	-	-	-
21	**FG-CON**	1	8.33	10	471	115	4.2	6	331	70	4.8
	FG-ST	5	41.7	45	767	105	7.7	41	404	78	5.5
	SG-CON	2	16.7	20	716	127	5.9	17	485	112	4.6
	SG-ST	3	25.0	20	783	98	8.4	20	543	87	6.7
24	**FG-CON**	10	83.3	99	820	119	7.3	100	519	104	5.2
	FG-ST	8	66.7	79	837	141	6.2	75	406	110	3.9
	SG-CON	8	66.7	77	803	127	6.5	79	523	107	5.1
	SG-ST	6	50.0	56	826	123	6.9	60	442	106	4.3

[1]Counts of birds are those with 5-10 measurable villi. Intestinal measures include jejunum villi height (μm), jejunum crypt depth (μm), jejunum villus-crypt ratio, ileum villi height (μm), ileum crypt depth (μm), and ileum villus-crypt ratio.
[2]JV = jejunum villus height, JC = jejunum crypt depth, JVCR = jejunum villus-crypt ratio, IV = ileum villus height, IC = ileum crypt depth, IVCR = ileum villus crypt ratio.
[3]FG = fast-growing (Ross), SG = slow-growing (Redbro), CON = control (TSB), ST = 1 mL $1.3x10^8$ CFU/mL *S.* Typhimurium challenge.

Table 2.8. Effect of breed and challenge on broiler intestinal histology. Data shown as mean (± SEM) jejunum villi height (μm), jejunum crypt depth (μm), jejunum villus-crypt ratio, ileum villi height (μm), ileum crypt depth (μm), and ileum villus-crypt ratio of male broilers from fast- (FG) and slow-growing (SG) breeds at d7 and 24 when challenged with *Salmonella* typhimurium (ST) or TSB (CON) at d 14.

		Treatment				P-Value[3]		
Age		**FG-CON[2]**	**FG-ST**	**SG-CON**	**SG-ST**	**Br.**	**Chal.**	**Int.**
7	**JV[1]**	508 ± 5[a]	-	486 ± 5[b]	-	0.001	-	-
	JC	104 ± 1[a]	-	97 ± 1[b]	-	0.0001	-	-
	JVCR	5.0 ± 0.1[b]	-	5.2 ± 0.1[a]	-	0.03	-	-
	IV	344 ± 4[b]	-	358 ± 4[a]	-	0.008	-	-
	IC	91 ± 1[a]	-	87 ± 1[b]	-	0.007	-	-
	IVCR	3.9 ± 0.1[b]	-	4.3 ± 0.1[a]	-	0.0001	-	-
24	**JV**	822 ± 42	836 ± 51	806 ± 46	838 ± 47	0.88	0.62	0.85
	JC	119 ± 4[b]	142 ± 5[a]	126 ± 5[b]	123 ± 5[b]	0.24	0.05	0.01
	JVCR	7.2 ± 0.5	6.2 ± 0.5	6.6 ± 0.5	7.0 ± 0.5	0.89	0.54	0.16
	IV	518 ± 29[a]	404 ± 35[b]	515 ± 32[a]	440 ± 32[b]	0.61	0.01	0.55
	IC	102 ± 6	110 ± 7	107 ± 6	107 ± 6	0.87	0.52	0.54
	IVCR	5.3 ± 0.3[a]	3.9 ± 0.4[b]	5.0 ± 0.4[a]	4.3 ± 0.4[b]	0.88	0.008	0.37

[1]JV = jejunum villus height, JC = jejunum crypt depth, JVCR = jejunum villus-crypt ratio, IV = ileum villus height, IC = ileum crypt depth, IVCR = ileum villus crypt ratio.
[2]FG = fast-growing (Ross), SG = slow-growing (Redbro), CON = control (TSB), ST = 1 mL $1.3x10^8$ CFU/mL *S.* Typhimurium challenge.
[3]P-values represent the main effect of breed (Br.; FG or SG), challenge (Chal.; ST or CON), and the interaction of breed and challenge (Int.).
[ab] Rows not sharing the same letters indicate a significant difference at $P \leq 0.05$.

Table 2.9. Correlations (r) between plasma IgA and IgG concentrations (µg/mL) and body weight (BW; g), jejunum villus height (µm), jejunum crypt depth (µm), jejunum villus-crypt ratio, ileum villus height (µm), ileum crypt depth (µm), ileum villus-crypt ratio, and plasma IgA and IgG concentrations of male broilers from fast- (FG) and slow-growing (SG) breeds at d7 and 24 when challenged with *Salmonella* typhimurium (ST) or TSB (CON) at d 14.

	BW[1]	**JV**	**JC**	**JVCR**	**IV**	**IC**	**IVCR**	**IgA**	**IgG**
Overall									
IgA	0.43**	0.41**	0.21	0.35**	0.32**	0.28	0.12	1.00**	0.05
IgG	-0.08	-0.14	-0.02	-0.12	-0.14	0.01	-0.15	0.05	1.00**
FG									
IgA	0.46**	0.35*	0.17	0.33*	0.31*	0.14	0.23	1.00**	0.21*
IgG	0.10	0.06	0.12	0.01	0.10	0.18	-0.01	0.21*	1.00**
SG									
IgA	0.34**	0.45**	0.22	0.33	0.32	0.43*	-0.06	1.00**	-0.17
IgG	-0.38**	-0.34	-0.15	-0.31	-0.54**	-0.17	-0.42*	-0.17	1.00**

[1]BW = body weight, JV = jejunum villus height, JC = jejunum crypt depth, JVCR = jejunum villus-crypt ratio, IV = ileum villus height, IC = ileum crypt depth, IVCR = ileum villus crypt ratio.
*$P \leq 0.05$
**$P \leq 0.01$

Figure 2.1. Ross 308 and Redbro chicks in a single pen.

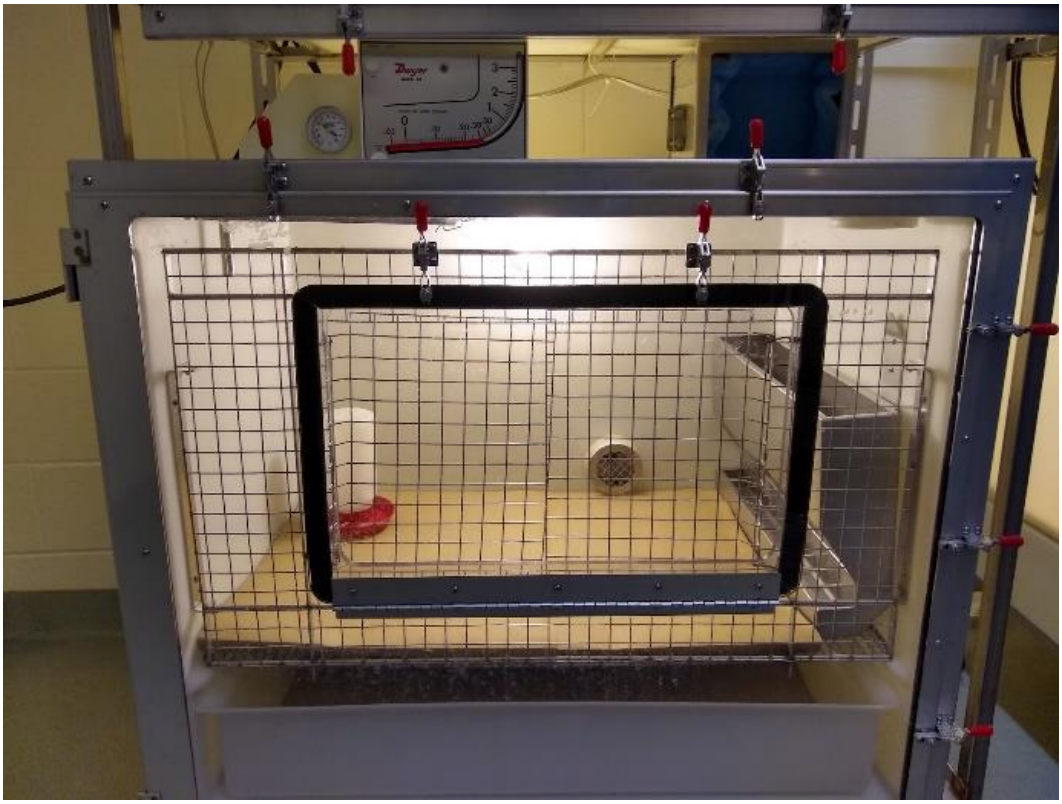


Figure 2.2. A BSL-2 isolator prior to movement of birds on DPC -7, with lights on to show the interior. The isolator is prepared with chick paper and a gravity waterer and equipped with a gravity feed trough. Below the flooring is the feces collection bin. Above the isolator are monitors for temperature and pressure.

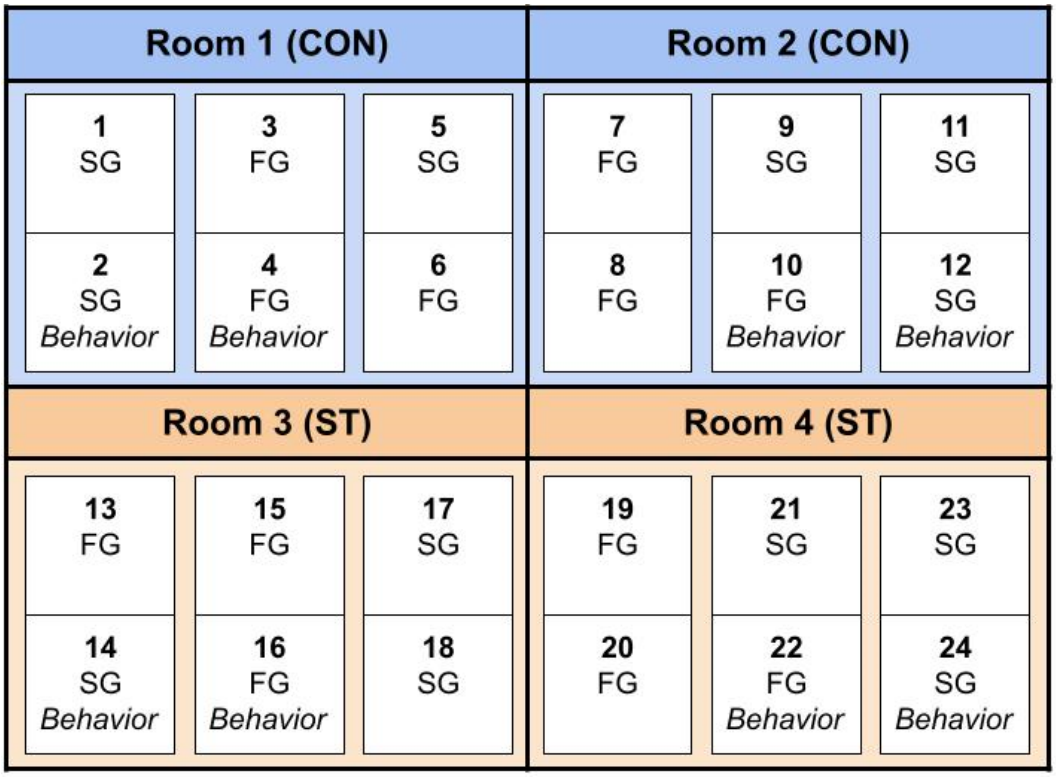


Figure 2.3. Simple depiction of room treatments (CON and ST), isolator set-up, breed assignments (FG and SG), and designated isolators for behavior video recording. Rooms 1 and 2 were assigned the control treatment, while rooms 2 and 4 received the challenge treatment.

a)

b)

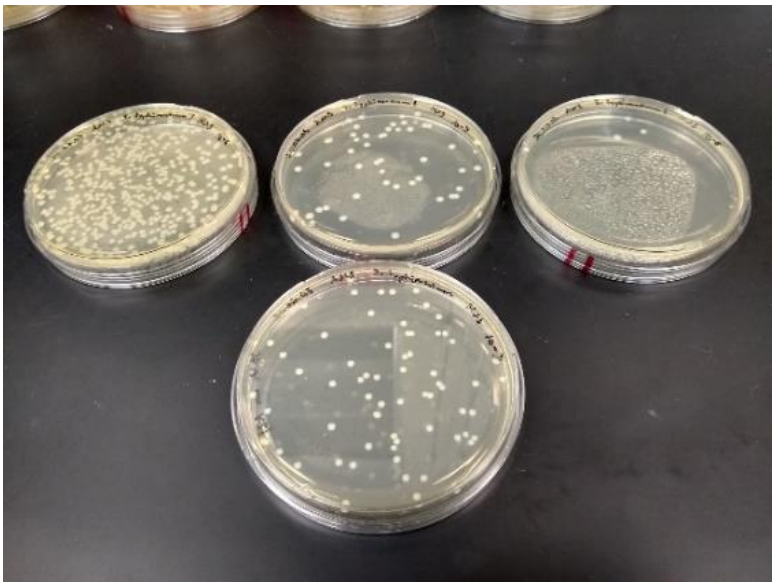
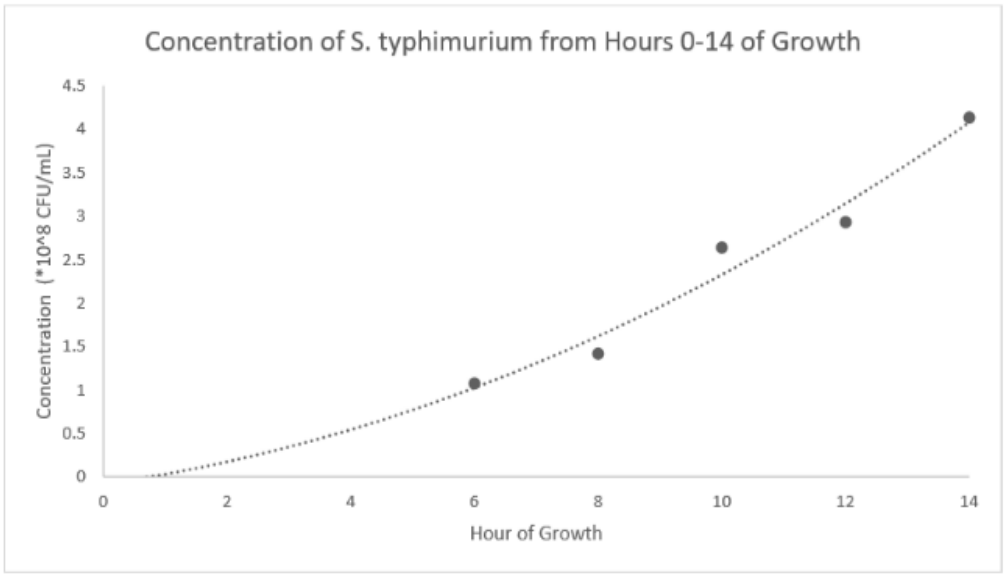
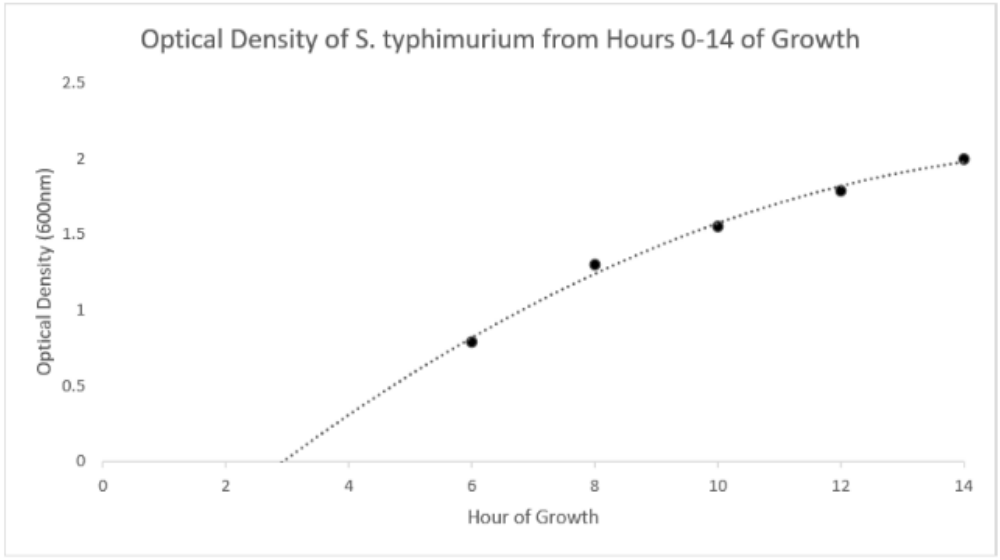


Figure 2.4. Determination of the culture's growth curve. **a)** Negative (left) and positive (right) controls were incubated alongside the *S.* Typhimurium culture (center). **b)** Serial dilutions were used to determine *S.* Typhimurium concentrations every 6 hours.



a)



b)

Figure 2.5. Growth curves of the challenge culture of *S.* Typhimurium from hours 0-14 of growth. **a)** Concentration of *S.* Typhimurium. **b)** Optical density of *S.* Typhimurium.

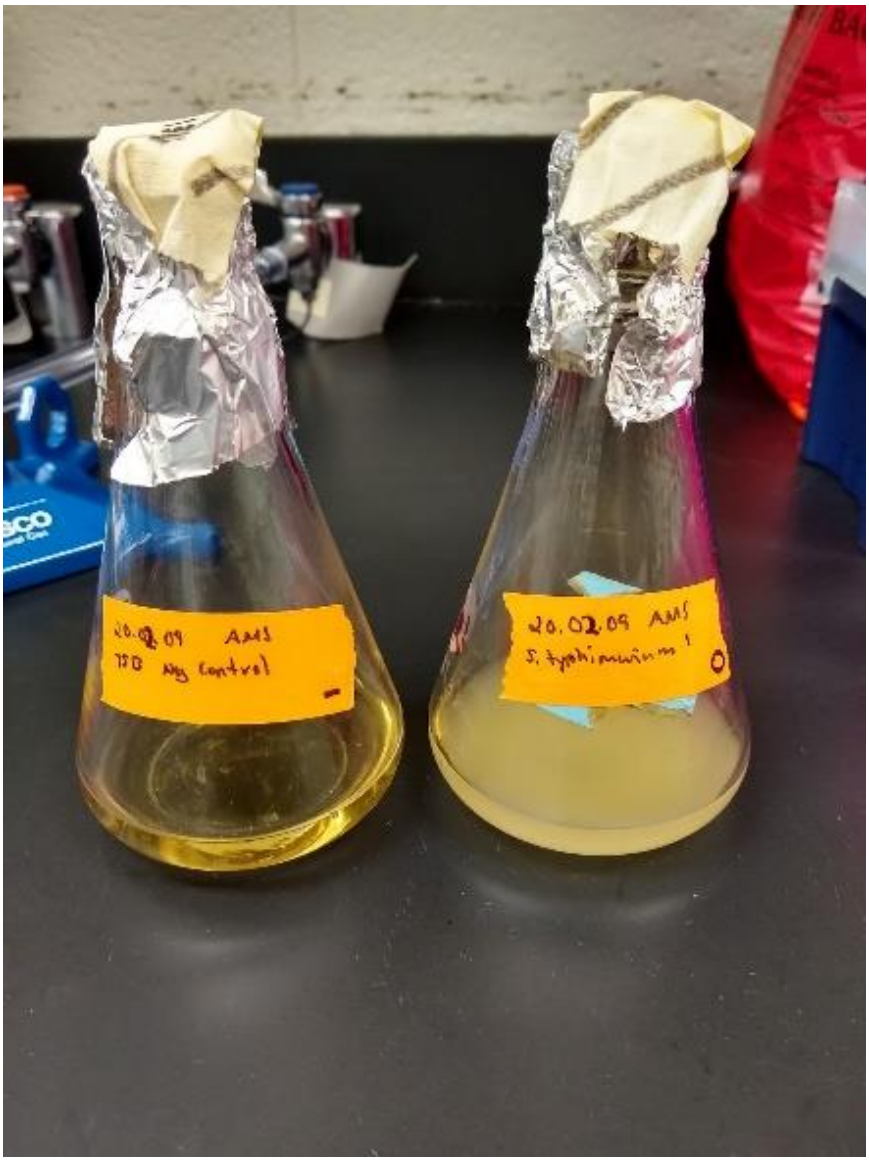
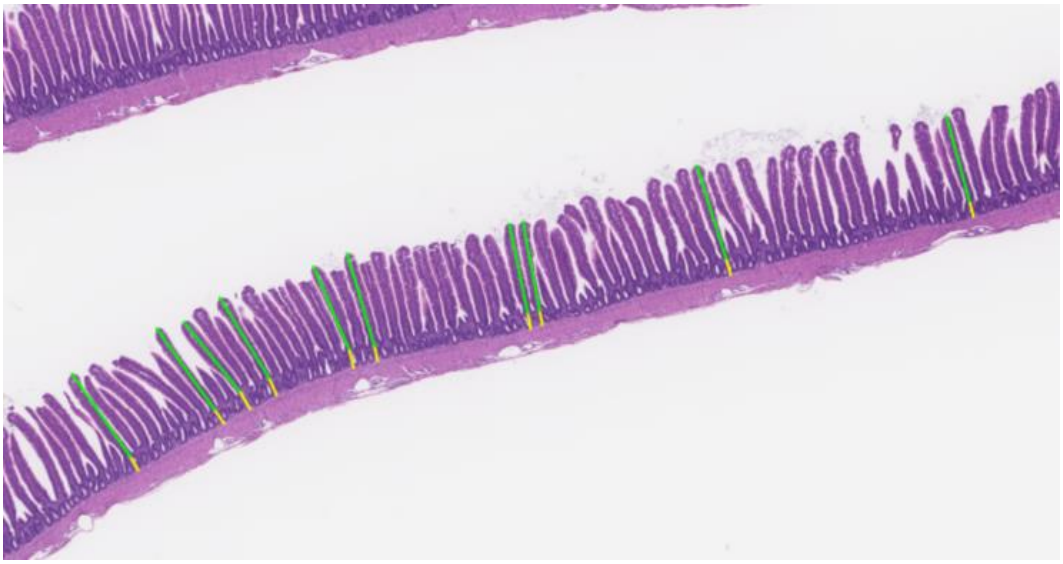


Figure 2.6. Negative control (left) and undiluted challenge *S.* Typhimurium culture (right) after 14 hours of growth.

a)



b)

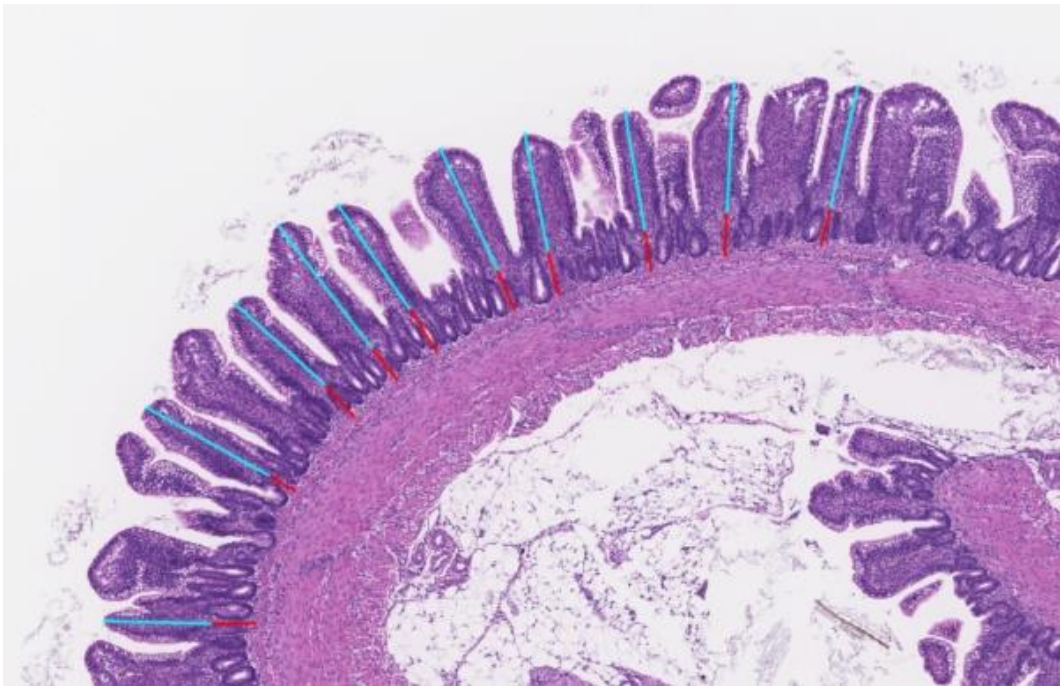


Figure 2.7. Measurement of villi heights and crypt depts. **a)** Measurement of a jejunum sample, with green lines measuring villi heights and yellow lines measuring crypt depths. **b)** Measurement of an ileum sample, with cyan lines measuring villi heights and yellow lines measuring crypt depths.

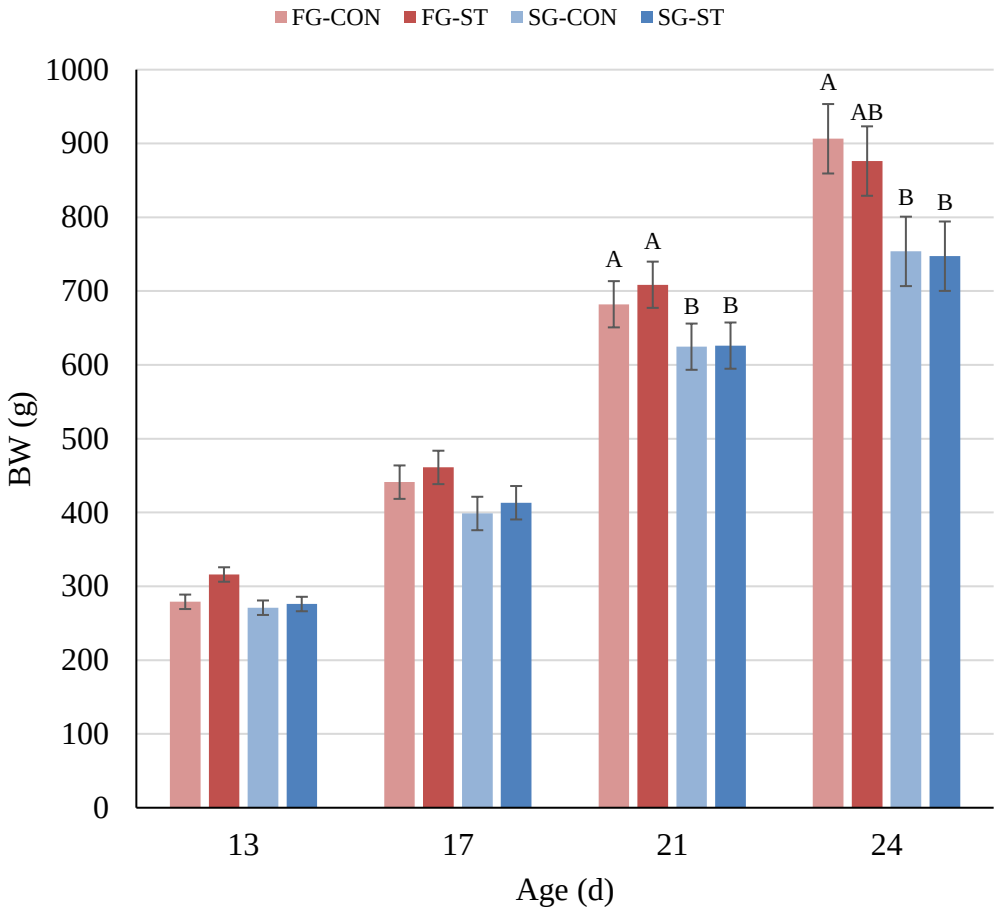


Figure 2.8. Effect of breed and challenge on broiler body weight (BW, g) at d13, 17, 21, and 24. Data shown as mean BW of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) on d14. ^{AB}Columns within each age not sharing the same letters are significantly different.

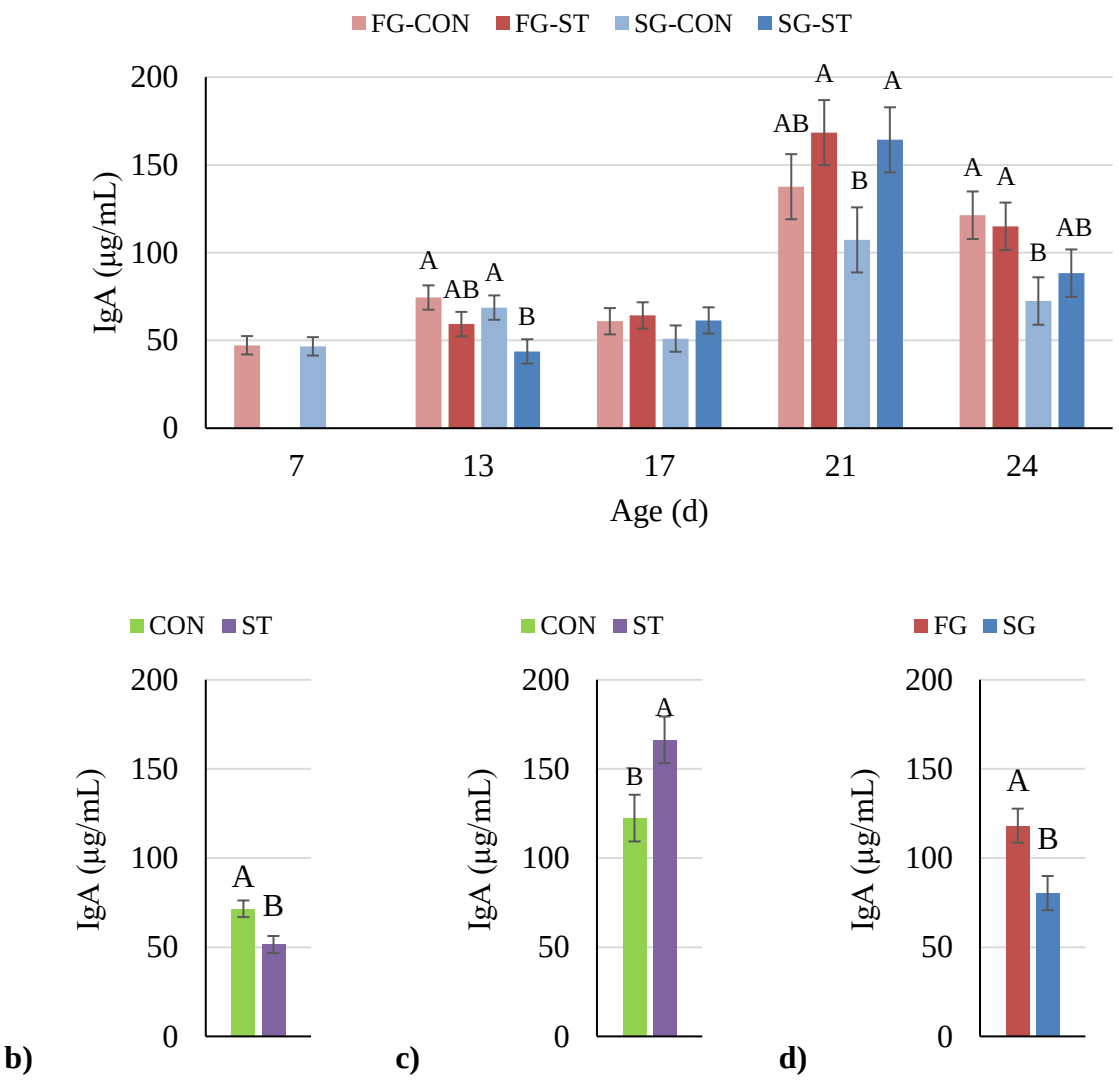


Figure 2.9. Effect of breed and challenge on broiler plasma IgA concentrations (µg/mL) at d7, 13, 17, 21, and 24. Data shown as mean IgA concentration of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) on d14. **a)** The interaction of breed and challenge at each age. **b)** Challenge effect at d13. **c)** Challenge effect at d21. **d)** Breed effect at d24. ^{AB}Columns within each age not sharing the same letters are significantly different.

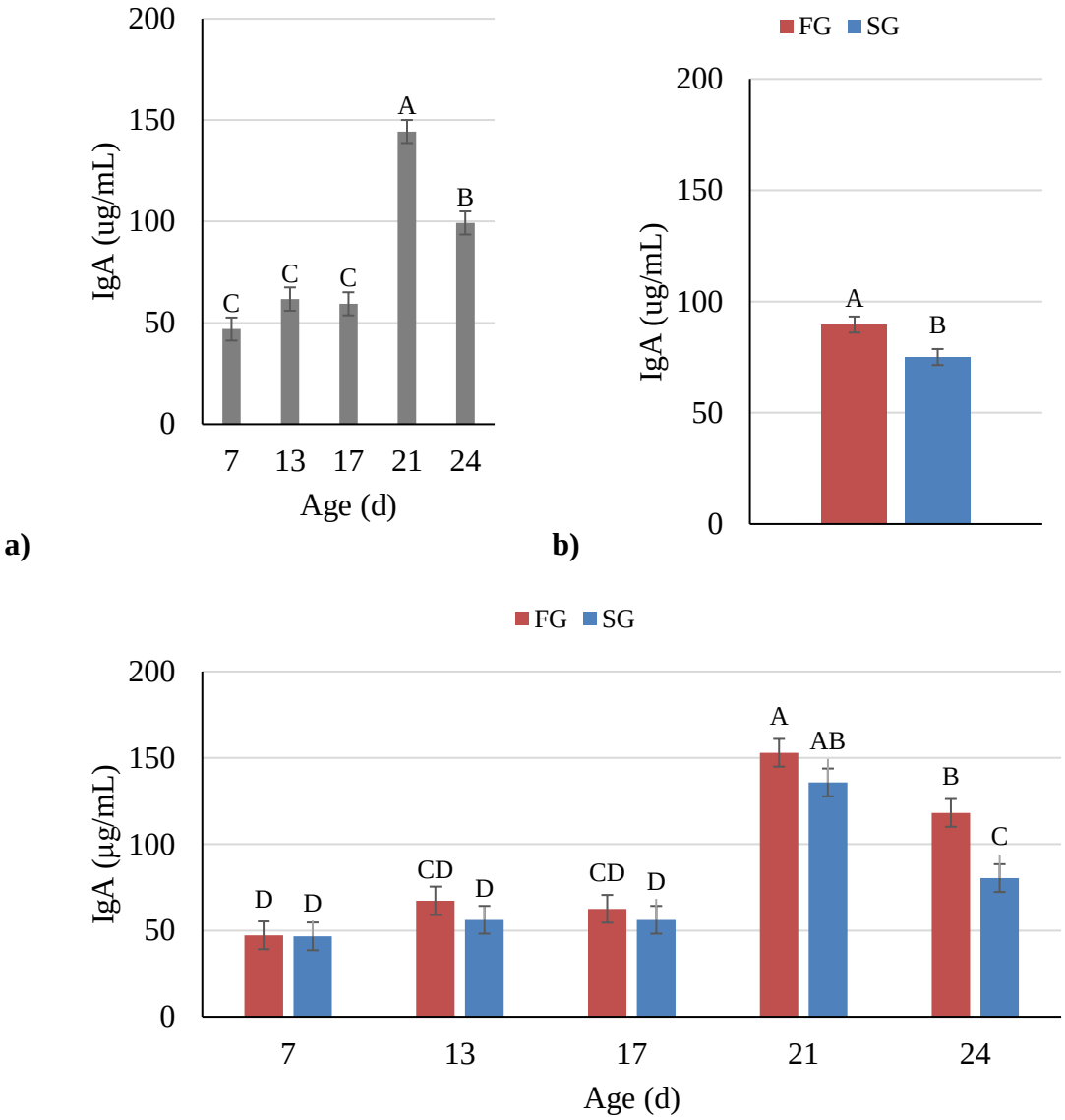
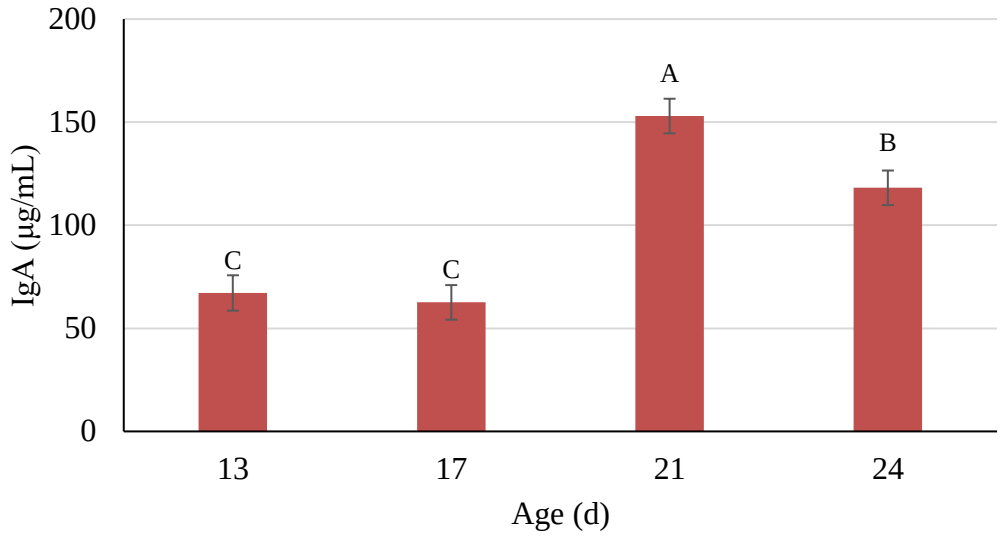


Figure 2.10. Effect of age (d), breed, and their interaction on plasma IgA concentrations (µg/mL) of birds at d7, 13, 17, 21, and 24. Data shown as mean IgA concentration of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14. **a)** Age effect on IgA. **b)** Breed effect on IgA. **c)** Interaction of age and breed effects on IgA. ^{ABCD}Columns not sharing the same letters are significantly different.



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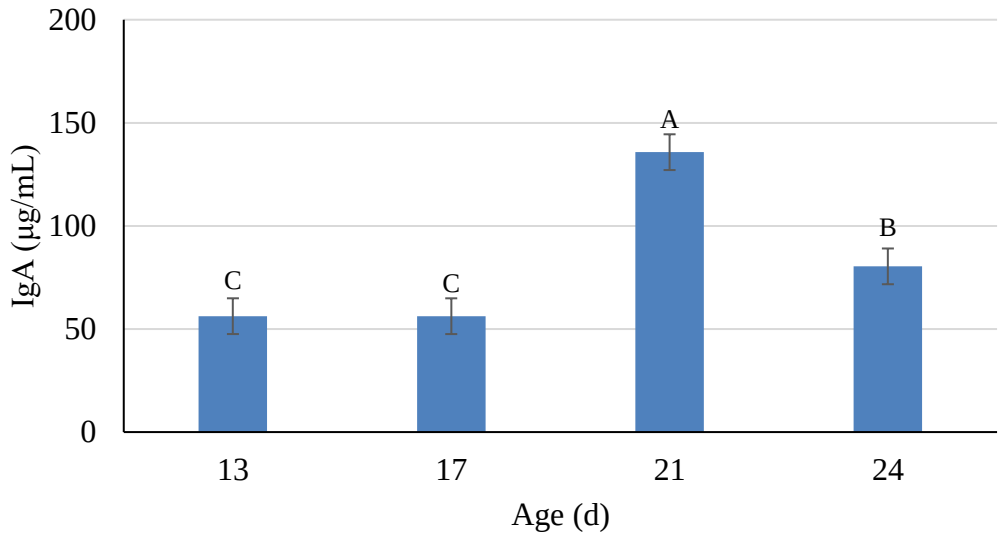


b)

Figure 2.11. Effect of age (d) on plasma IgA concentrations (µg/mL) of birds at d13, 17, 21, and 24. Data shown as mean IgA concentration of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14. **a)** IgA within FG. **b)** IgA within SG. ^{ABC}Columns not sharing the same letters are significantly different.

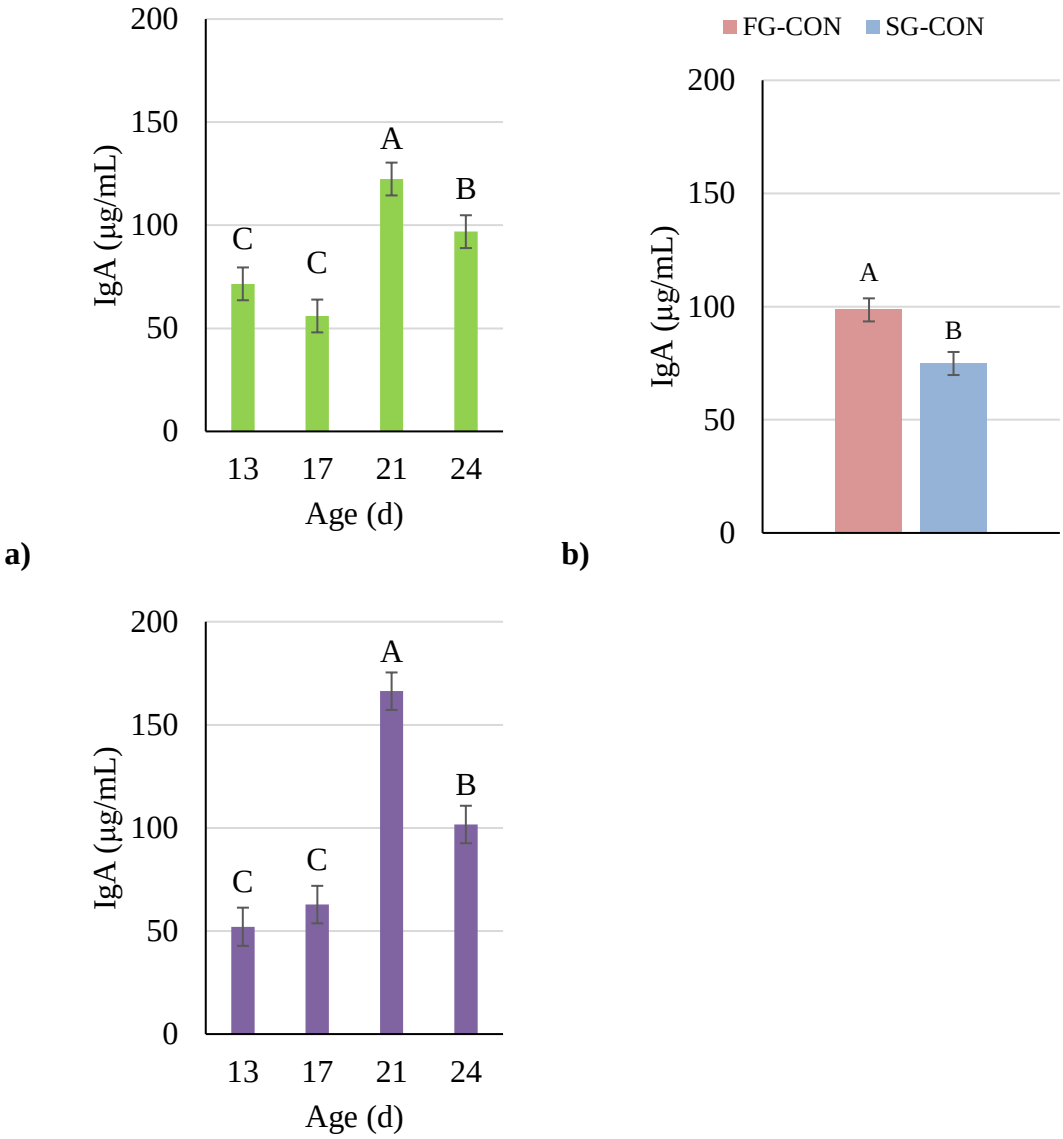


Figure 2.12. Effect of age (d) and breed on plasma IgA concentration (µg/mL) of birds at d13, 17, 21, and 24. Data shown as mean IgA concentration of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with S. Typhimurium (ST) or TSB (CON) at d14. **a)** Age effect within CON. **b)** Breed effect within CON. **c)** Age effect within ST**.** [ABC]Columns not sharing the same letters are significantly different.

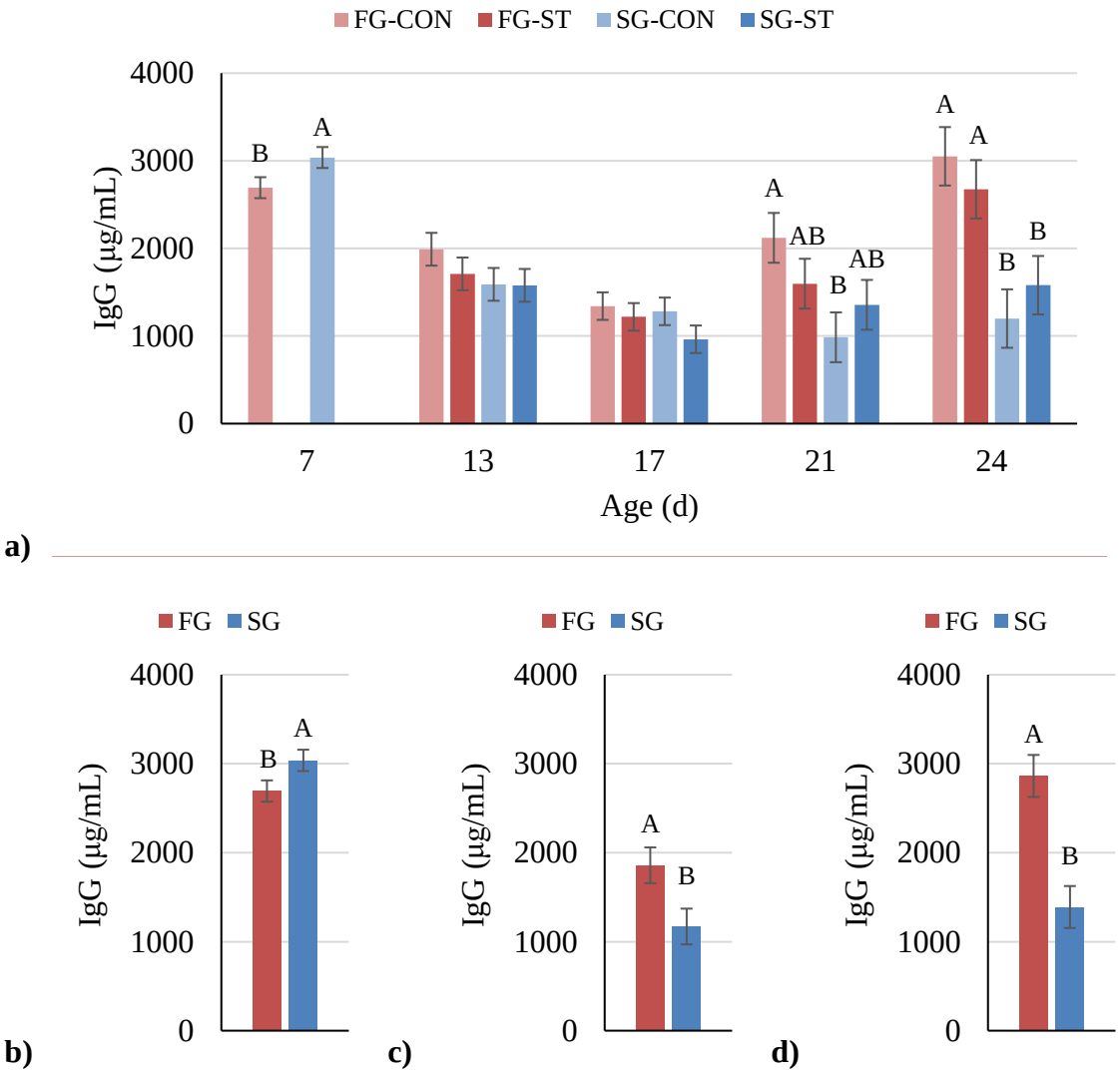


Figure 2.13. Effect of breed and challenge on broiler plasma IgG concentration (µg/mL) at d7, 13, 17, 21, and 24. Data shown as mean IgG concentration of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) on d14. **a)** Within age interaction of breed and challenge at each age. **b)** Breed effect at d7. **c)** Breed effect at d21. **d)** Breed effect at d24. ^{AB}Columns within each age not sharing the same letters are significantly different.

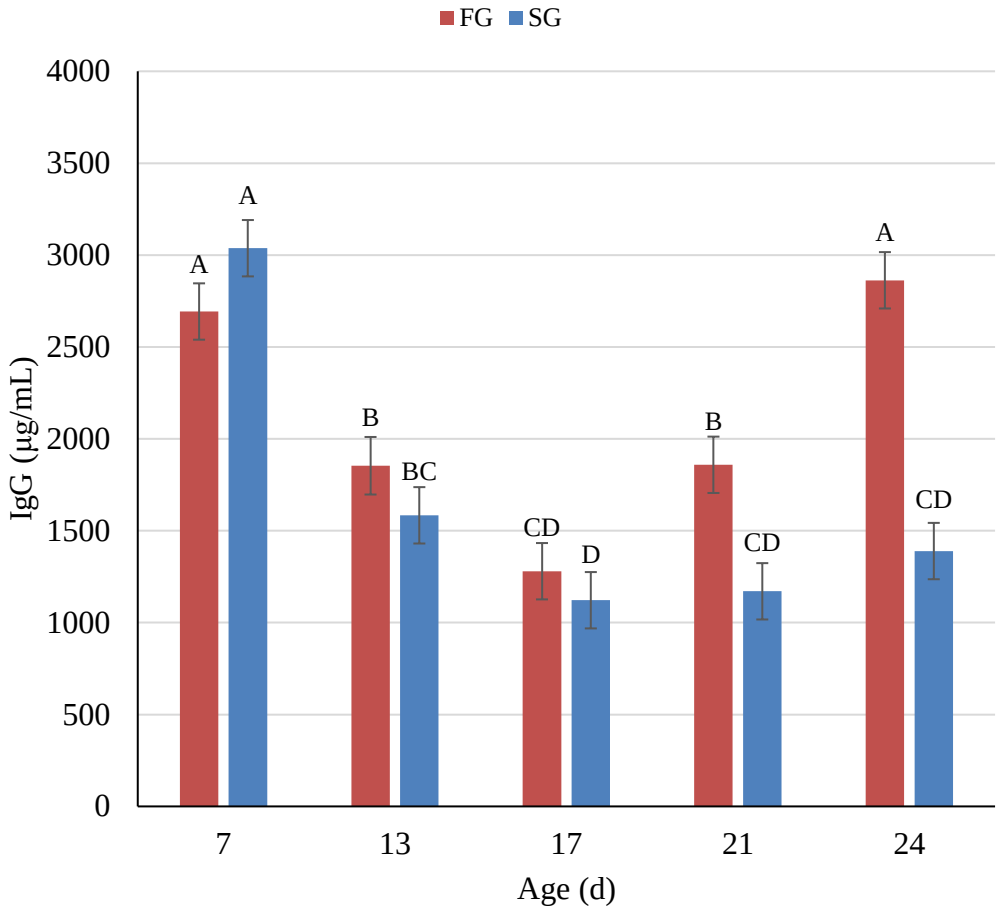
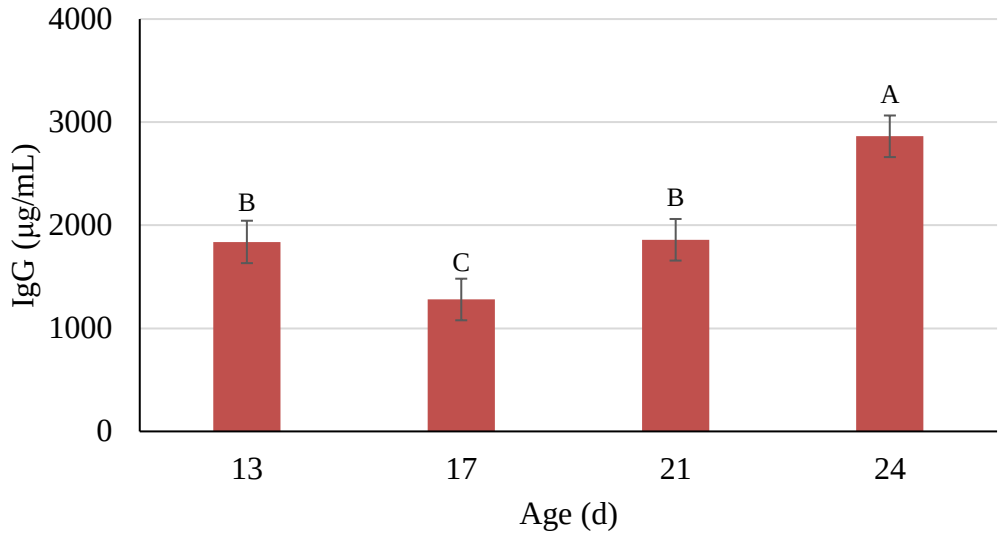


Figure 2.14. Effect of age (d), breed, and their interaction on plasma IgG concentrations (µg/mL) of birds at d7, 13, 17, 21, and 24. Data shown as mean immunoglobulin concentration of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14. ^{ABCD}Columns not sharing the same letters are significantly different.



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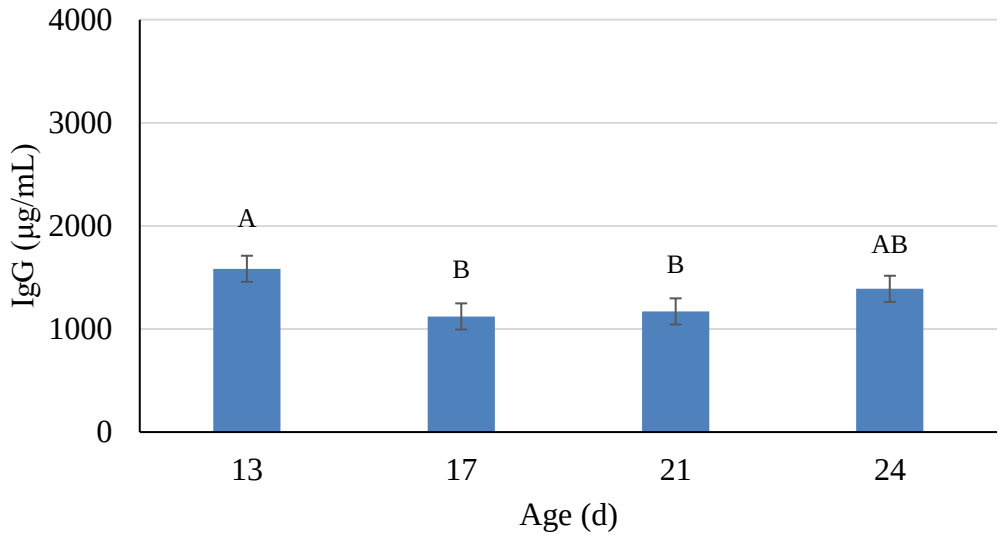
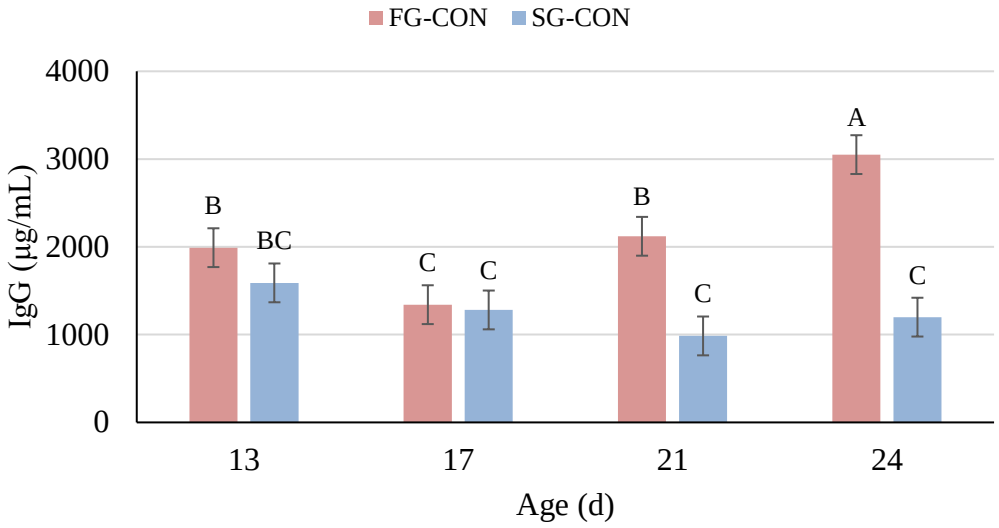


b)

Figure 2.15. Effect of age (d) on plasma IgG concentrations (µg/mL) of birds at d13, 17, 21, and 24. Data shown as mean IgG concentration of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14. **a)** IgG within FG. **b)** IgG within SG. ^{ABC}Columns not sharing the same letters are significantly different.



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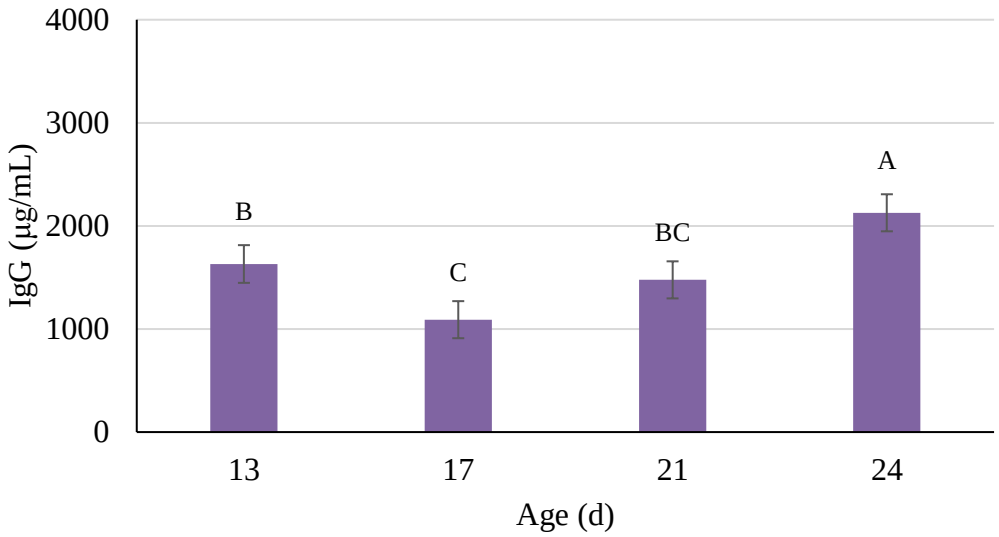

b)

Figure 2.16. Effect of age (d), breed, and their interaction on plasma IgG concentration (µg/mL) of birds at d13, 17, 21, and 24. Data shown as mean IgG concentration of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14. **a)** Interaction of age and breed effects within CON. **b)** Age effect within ST. ^{ABC}Columns not sharing the same letters are significantly different.

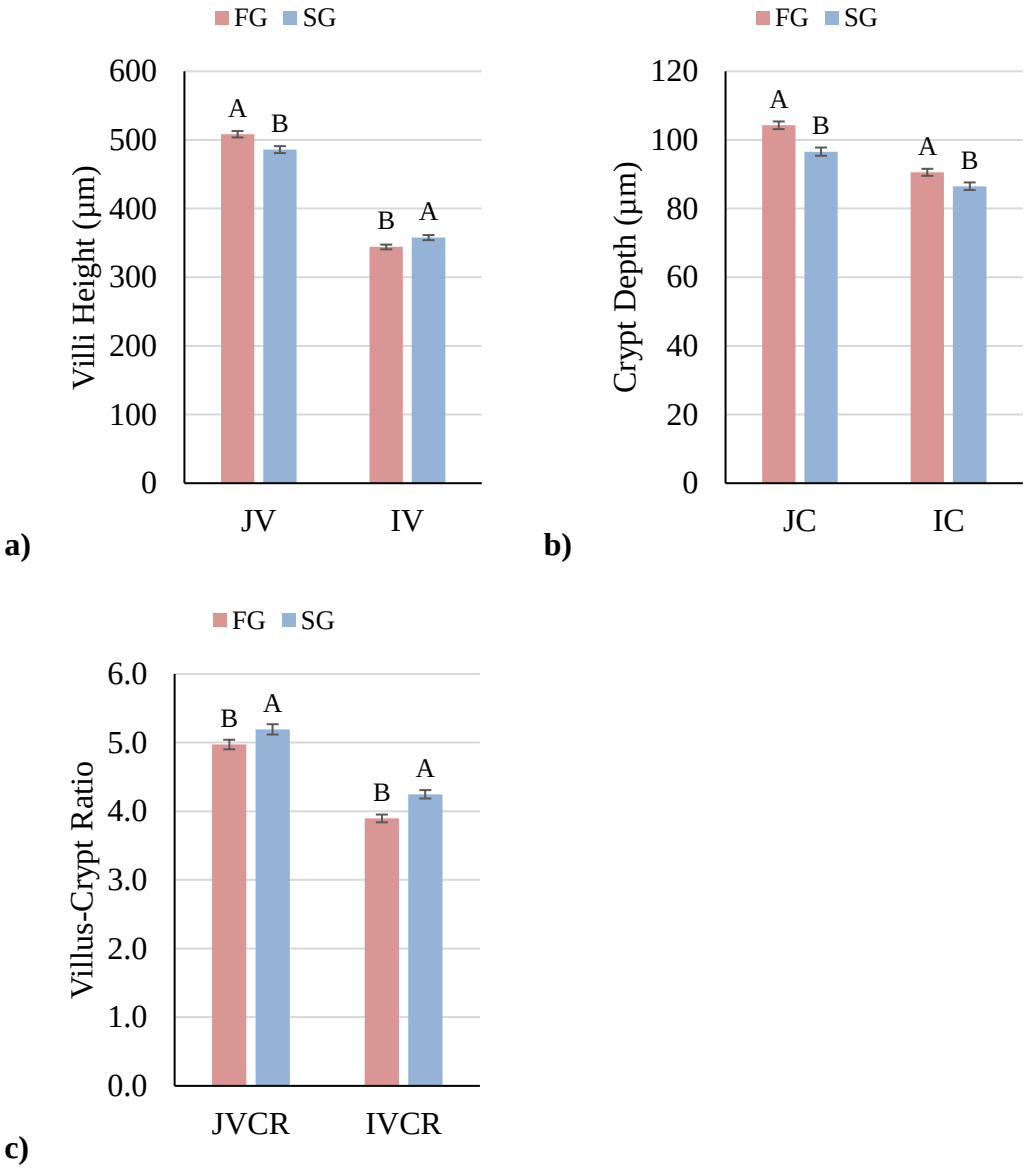

Figure 2.17. Effect of breed on broiler intestinal measures at d7. Data shown as mean jejunum villi height (JV; µm), jejunum crypt depth (JC; µm), jejunum villus-crypt ratio (JVCR), ileum villi height (IV; µm), ileum crypt depth (IC; µm), and ileum villus-crypt ratio (IVCR) of male broilers from fast- (FG) and slow-growing (SG) breeds when challenged with *Salmonella* typhimurium (ST) or TSB (CON) at d 14. **a)** Villi height by breed at d7. **b)** Crypt depth by breed at d7. **c)** VCR by breed at d7. ^{AB}Columns within each morphology measure not sharing the same letters are significantly different.

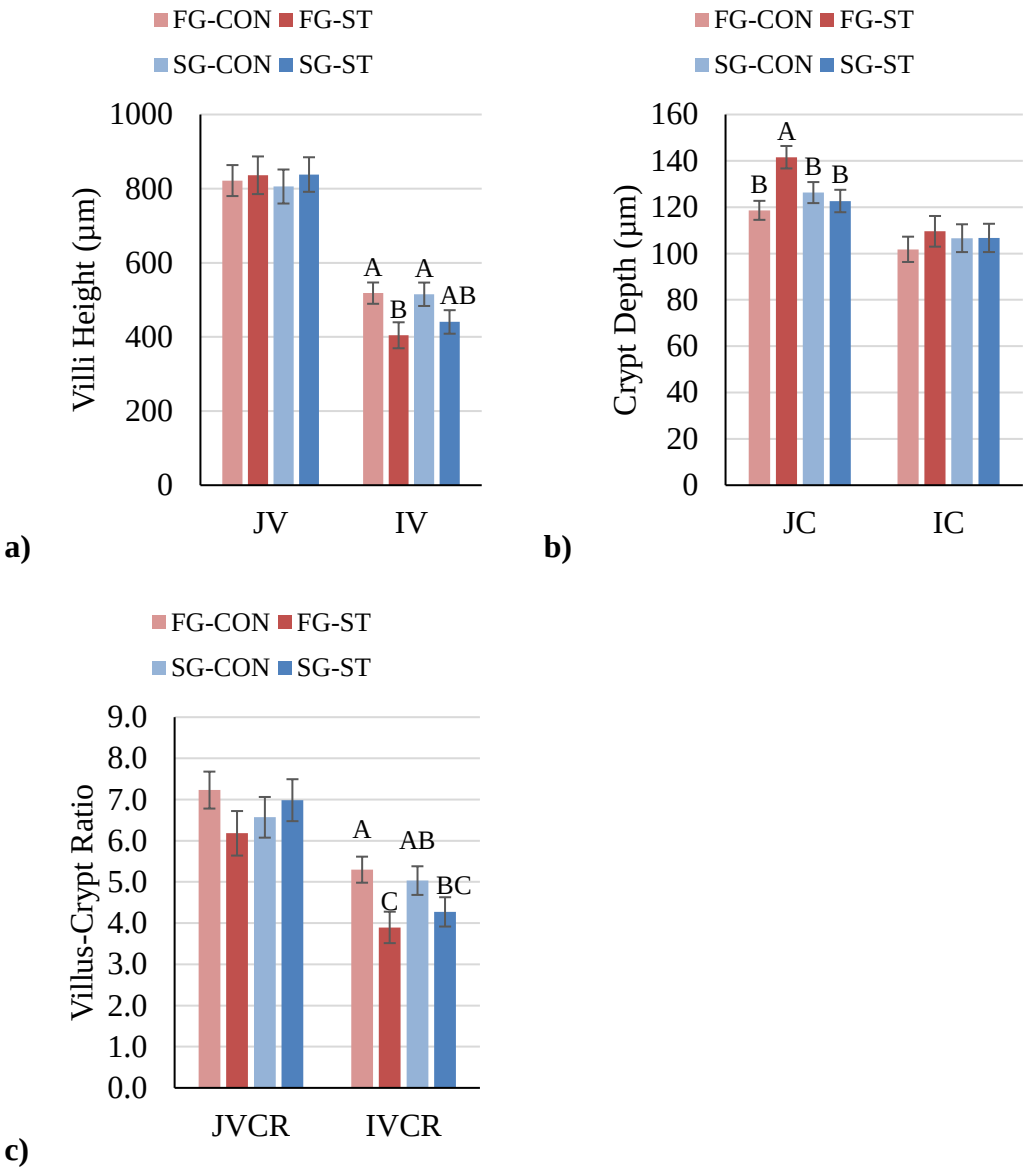

Figure 2.18. Effect of breed and challenge on broiler intestinal measures at d24. Data shown as mean jejunum villi height (JV; µm), jejunum crypt depth (JC; µm), jejunum villus-crypt ratio (JVCR), ileum villi height (IV; µm), ileum crypt depth (IC; µm), and ileum villus-crypt ratio (IVCR) of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *Salmonella* typhimurium (ST) or TSB (CON) at d14. **a)** Villi height by breed at d24. **b)** Crypt depth by breed at d24. **c)** VCR by breed at d24. ^{AB}Columns within each morphology measure not sharing the same letters are significantly different.

CHAPTER 3 Evaluating differences in sickness behavior in fast- and slow- growing broiler chickens when infected with *Salmonella enterica* serovar Typhimurium

3.1 ABSTRACT

Fast growth rate in broilers comes with welfare concerns and research is needed to determine if fast- and slow-growing broilers differ in pathogen resistance and sickness behavior. The objective of this study was to evaluate behavioral differences in fast- (FG) and slow-growing (SG) broilers when challenged with *Salmonella* Typhimurium or broth (control; CON) 14 days post-hatch. FG (N=156) and SG (N=156) were raised in the same pen with litter shavings until d7, when they were transferred to 24 isolators. On d12, 16, 21, and 23 video was recorded for 8 isolators and postures (sitting, standing, or locomoting) and behaviors (eating, drinking, preening, stretching, sham foraging, allopreening, and aggression) were analyzed. Generally, more FG sat (P=0.03) and fewer locomoted than SG with age by 5.2-11.9% and 1.6-2.7%, respectively. More birds sham foraged as they aged (P<0.0001), but on d23 SG-CON sham foraged more (P<0.02) than SG-ST and FG by 2%, indicating both that SG may be more naturally motivated to forage and challenge may have reduced sham foraging in SG-ST. The effect of breed on aggression was trending, in which SG tended to be more aggressive than FG by 0.1%, which could indicate that SG are generally more aggressive than FG. The results show that fast- and slow-growing breeds differ behaviorally as they age, and that slow-growing birds are more active and show greater behavioral signs of sickness but may generally be more aggressive. This information can help breeders make selection decisions to improve broiler welfare and prevent *Salmonella* transmission into the human food supply.

Keywords: broiler, breed, growth rate, *Salmonella*, behavior

3.2 INTRODUCTION

In order to meet high consumer demand, broilers are genetically selected for increased efficiency and greater breast yield, resulting in birds that reach heavier market weights at incredible growth rates (NCC, 2021). However, selection for productivity traits may unintentionally neglect other health and welfare traits, such as pathogen resistance. Multiple studies have explored the link between growth rate or body weight and immune function (Yunis et al., 2000; Leshchinsky and Klasing, 2001; Humphrey and Klasing, 2004; Parmentier et al., 2010; van der Most et al., 2011), often noting an inverse relationship. This may be due to prioritizing the allocation of bodily energy and resources to growth as opposed to immune function, compromising the immune system (Humphrey and Klasing, 2004). Despite this, the effect of selection for enhanced growth rate on resistance to foodborne pathogens such as *Salmonella* and sickness behaviors has not been investigated.

General behavioral differences exist between fast- and slow-growing broilers which may indicate welfare status. The University of Guelph studied 16 different breeds of broiler chickens varying in growth rate for differences in behavior, performance, mortality, and mobility at the (GAP, 2020). The conventional breed (fastest growth rate) spent the most time sitting almost half as much time standing and moving compared with the slower growing breeds (Torrey et al., 2020). Dixon (2020) compared the behavior of fast- and slow-growing broilers and reported less engagement in active behaviors (standing, locomoting, foraging, and preening) in 3 commercial breeds (Ross, Cobb, and Hubbard) than a slow-growing Hubbard breed. Other studies have also reported less engagement in exploratory behaviors among medium (Almeida et al., 2012) and fast-

growing breeds (Yan et al., 2021) than slow-growing breeds. It may additionally be possible to detect differences between fast- and slow-growing breeds by observing comfort behaviors and exploratory behaviors, which can also indicate health and welfare status (Costa et al., 2012). Reductions in these behaviors may even predict illness clinical signs of disease (Abeyesinghe et al., 2021) as a form of sickness behavior.

Sickness is a motivational state and sign of an immune response in action, causing distinct behavioral patterns that support recovery from the disease, such as lethargy, decreased appetite, and reduced social behaviors (Johnson, 2002; Dantzer, 2004; Tizard, 2008). Sickness behaviors can occur as a result of an acute phase immune response involving pro-inflammatory cytokine signaling (Kelley et al., 2003; Tizard, 2008; Millman, 2006; Dantzer, 2004; Dantzer and Kelley, 2007; Johnson, 2002). As such, increased sickness behavior may indicate a stronger immune response, and thus greater resilience to pathogenic infection by bacteria such as *Salmonella* (Hart, 1988; Johnson, 2002; Cheng et al., 2004).

Infection by *Salmonella enterica* serovars in broilers can prompt an immune response and influence behavior. Broilers injected intravenously with *S.* Typhimurium and *S.* Enteritidis have been observed to exhibit an inflammatory response, depression, fever, diarrhea, reduced feed intake, and reduced body weights (Xie et al., 2000; Quinteiro-Filho et al., 2012). Cobb chicks challenged with *S.* Typhimurium at 1 week of age experienced reduced body weights and increased mortality rates, incidences of lameness, and diarrhea, as well as dullness, inappetence, inactivity, weakness, and anorexia (Dar et al., 2019).

Prior research has found differences between chicken breeds regarding pathogen resistance and immune response to infection (Barrow, 1991; Cheng et al., 2004; Schou et al., 2010; Li et al, 2017). Leshchinsky and Klasing (2001) reported that in broilers and Brown Nick Layers injected with LPS 1 day post-hatch, broilers had had lower mRNA expression of IL-1β and IFN-γ and a reduced febrile response. As such, broilers selected for faster growth may be less prone to display sickness behaviors (Berghman, 2016).

Little is known if differences exist between modern fast- and slow-growing broilers relative to *Salmonella* infection, making it increasingly important to understand if selective breeding for a faster-growing, heavier broiler has also resulted in a more (or less) *Salmonella*-resistant broiler. To evaluate and understand these differences, fast-growing Ross 308 and slow-growing Redbro broiler chicks were orally challenged with either *S.* Typhimurium or Tryptic Soy Broth control at 14 days of age and sampled up until 24 days of age (10 days post-challenge). Video was recorded of 8 isolators on multiple days to evaluate breed differences and the effect of *S.* Typhimurium challenge on behavior. The objective of this study was to evaluate differences in sickness behavior between fast- and slow-growing broiler chickens when challenged with *Salmonella* Typhimurium. The hypotheses were that fast-growing broilers would have reduced behavioral repertoire when compared with the slow-growing breed independent of challenge, and that challenged slow-growing broilers would have more significant behavioral signs of sickness than fast-growing broilers, indicating a stronger immune response to the *S.* Typhimurium challenge.

3.3 MATERIALS AND METHODS

3.3.1 Animals and Housing

All procedures and protocols were approved by the University of Maryland (UMD) Animal Care and Use Committee (IACUC#: R-NOV-19-55). Three-hundred and twelve male day-of-hatch chicks from two breeds, Ross 308 (N=156) and Redbro (Hubbard) (N=156), were transported from a local hatchery (Freedom Ranger Hatchery, Reinholds, PA) to the University of Maryland Animal and Avian Sciences Animal Wing. Chicks were placed together in a single 3 m by 6 m pen with wood shavings litter (d0) (**Figure 2.1**). This encouraged commingling and permitted consumption of pen-mate fecal material to establish a similar gut microbiome. Three brooder lamps were hung above the pen to provide supplemental heat and were removed on d3. Water was provided *ad libitum* through a nipple water line. Chicks were provided Purina Start and Grow Non-Medicated crumbles *ad libitum* in 3 gravity-fed hanging feeders. Temperature was maintained at 32.2°C for d0-1 and gradually reduced by 0.6°C daily until 17.8°C at d13. Ambient temperature, humidity, and photoperiod was maintained according to the Ross Broiler Management Handbook (Aviagen, 2018) throughout the study. Photoperiod was 23h light and 1h dark on d0 and light hours were gradually decreased to 20h light with 4h dark on d7. Ambient temperature was also checked at the floor level inside the pen using an infrared temperature gun (Lasergrip 1080, Etekcity, Anaheim, CA). Chicks were housed in the UMD ANSC Animal Wing from d0-7.

On d7, a total of 264 birds (N=132 per breed) were moved into isolators in BSL-2 rooms at the University of Maryland Avrum Gudelsky Veterinary Medical Center (**Figure 2.2**). Eleven birds from each breed were exclusively placed into 24 isolators

(Model 934-1, Federal Designs Inc., Comer, GA) within 4 ABSL-2 rooms (N=6 isolators per room). There were 3 isolators that contained birds from each breed per room. Isolators were 5,195 cm^2 and stocking density did not exceed the minimum space allowance outlined in the Ag Guide (FASS, 2010) throughout the study. Isolator floors consisted of a metal grate with a bin below to collect fecal matter, and isolator lights were left off for the duration of the study as it was observed to increase aggression (**Figure 2.2**). Each isolator had separate airflow and HEPA filters that were replaced once weekly. Air flow pressure (negative) was monitored twice daily through an attached gauge (Model 25 Manometer, Dwyer Instruments, Inc., Michigan City, IN) to ensure that air pressure fell within an acceptable range (**Figure 2.2**). Chick paper was placed in isolators over the metal grating prior to bird placement to prevent leg injury, then later removed at d10 (**Figure 2.2**). Fecal collection bins were emptied every other day to maintain good air quality. Commercially available feed (Purina Start and Grow Non-Medicated pellets) and water were provided *ad libitum* via a metal gravity-fed trough and a plastic gravity waterer, respectively. Birds were checked for wellness, isolator temperature and humidity were monitored and recorded, and waterers were cleaned and refilled with fresh water twice daily. Individual isolator heaters were on from d7- 10 to maintain temperature. The temperature and humidity of each room was also monitored and recorded once daily. Light hours were 20h light and 4h dark (20:4) at d7 and gradually shifted to 18h light and 6h dark (18:6) until d14, then they were maintained at 18:6 for the remainder of the experiment. Exterior room windows were covered for the duration of the study.

3.3.2 Experimental Design and Procedures

The experimental design was a 2 x 2 split plot design, in which all Ross 308 (FG) and Redbro (SG) birds were raised together in a single pen for the first week (d0-7) and then randomly assigned and split between 24 isolators across 4 rooms by breed and designated challenge treatment group at d7 (**Figure 2.3**). Each room held 6 isolators total, split into 3 isolators per breed per room, and each room was assigned a challenge treatment (N=2 rooms per treatment) (**Figure 2.3**). Challenge treatments were given on d14, in which 2 rooms of birds (rooms 3 and 4) received 1 mL of 10^8 CFU/mL *Salmonella* Typhimurium challenge culture (ST) (N=108 birds, 54 per breed) and 2 rooms (rooms 1 and 2) received 1 mL Tryptic Soy Broth (CON) (N=108 birds, 54 per breed) via oral gavage (**Figure 2.3**). *S.* Typhimurium culture was prepared as outlined in Chapter 2.3.3. Videos were recorded for an hour on d12, 16, 20, and 23 for behavior analysis. **Table 2.1** outlines a brief summary of events.

3.3.4 Video Recording

GoPro cameras (GoPro Hero7 Black, GoPro Inc., San Mateo, CA), were mounted to tripods (**Figure 3.1**) and positioned to record 2 isolators per room (one per breed per room; isolators #2, 4, 10, 12, 14, 16, 22, and 24) for 1 hour on d12, d16, d20, and d23 from 14:00-15:00 (**Figure 2.3**). Days selected for recording video preceded each sampling day by 24 hours to avoid the interference of any stressful events such as handling with the accuracy of the behavior data recorded.

3.3.5 Behavior Coding

Video recordings were coded for mobility, production, comfort, exploratory, and social behaviors in the laboratory by 3 students using instantaneous scan sampling every 15 seconds for an hour of recorded time, beginning 5 minutes after the researcher started the camera and left the room (N=241 scans/video) (**Figure 3.2**). During each scan, first the total number of birds performing each posture (sit, stand, locomotion), then each behavior (eat, drink, preen, stretch, sham forage, allopreen, aggression) were recorded using the ethogram (**Table 3.1**) for reference. Thus, all birds were coded for posture but only those exhibiting behaviors were also coded for behavior. If a bird was obstructed from view for any reason and its posture could not be determined, it was coded as not visible and could not be coded for a behavior. Birds could be recorded for both a posture and behavior, a posture and no behavior, or not visible. Counts were later transformed into proportions of birds performing a posture and/or behavior out of the total number of birds in the isolator for statistical analysis. The 3 observers coded between 6 and 15 videos each out of 32 videos total. Interobserver agreement was determined to be over 95% in total for all behaviors.

Postures and behaviors were mutually exclusive within themselves but not to each other (**Table 3.2**). Birds could be coded for one posture and one behavior, just one posture and no behavior, or not visible. All birds were coded for a posture or Not Visible, but not all were coded for a behavior. For example, a bird could be coded for both sitting and preening, while another bird may be coded for standing but not performing any behavior listed in the ethogram.

3.3.6 Statistical Analysis

The isolator (N=8) was the experimental unit for all behavior data. Behavior proportion data was analyzed using the GLIMMIX procedure in SAS (v9.4, SAS Institute, Inc., Cary, NC). Data were tested for normality. The combined total counts of behaviors (eating, drinking, preening, stretching, sham foraging, allopreening, and aggression) were summed and calculated as proportions to create a total behavior category of behaviors for analysis. Behavior data were analyzed using a within-age model including the fixed effects of breed, challenge, and their interaction, as well as using two across-age models including the fixed effects of age, either breed or challenge, and their interaction, organized by either breed or challenge. The random effect of isolator nested within room was included in the analysis of all models. Additionally, behavior data were analyzed using an age by breed model independent of challenge for the fixed effects of age, breed, and their interaction including the random effect of isolator nested within room. Multiple comparisons of means were separated using LSMEANS and differences between measures were detected using PDIFF. Aggression behavior had very low frequency and there was insufficient data for statistical analysis of aggression in the across age models including the main effect of challenge, but it was able to be run in the age by breed model independent of challenge. Aggression is instead reported as raw means in the across age analyses involving challenge and preening at d16 is reported using raw means in the within age analysis. Data were considered significant at a $P \leq 0.05$ and a tendency at $P \leq 0.10$.

3.4 RESULTS

Results figures are color coded by treatment to aid in the visualization of treatment differences and changes over time. **Table 2.2** describes each color and represented treatment. Only significant results are presented and discussed.

First, as a reminder, all birds in each isolator were coded for a posture (sitting, standing, and locomoting [changing location]). Next, only birds displaying a behavior defined in the ethogram were coded for a behavior (eating, drinking, preening, stretching, sham foraging, allopreening, or aggression). A total behavior category was calculated by summing the behaviors at each scan. Thus, postures and behaviors were mutually exclusive within their own respective categories, but not to each other, and birds were either coded once (for a posture only) or twice (for both a posture and a behavior).

Three GLIMMIX models were used to analyze the effects of age (d) breed, and challenge on the proportion of birds performing each posture or behavior, with each model split into two tables for postures and behavior, respectively: 1) across age for the effects of age, breed, and their interaction within challenge on postures (**Table 3.3**) and behaviors (**Table 3.4**), 2) across age for the effects of age, challenge, and their interaction within breed on postures (**Table 3.5**) and behaviors (**Table 3.6**), and 3) within age for the effects of breed, challenge, and their interaction on postures (**Table 3.7**) and behaviors (**Table 3.8**). Additionally, challenge had minimal effects on postures and behaviors, and a model was run independent of challenge to evaluate the effects of age, breed, and their interaction across all ages. There was insufficient data for statistical analysis of preening at d16 in the within age analysis (**Table 3.8**), as well as aggression in the across age analyses inclusive of challenge, and only the raw means are reported (**Tables 3.4 and**

3.6). Aggression was able to be run in the age by breed analysis independent of challenge. There was no effect of challenge independent of breed on any behavior in the across age analyses (**Tables 3.4 and 3.6**).

The effect of age on the proportion of birds sitting, standing, and locomoting was significant for all treatments and tended to increase for sitting and decrease for standing across age except at d16 and d20 (**Tables 3.3 and 3.5**). More (P<0.0001) birds were sitting at d16 (65.9%) and d20 (66.3%) than at d12 (53.9%) and d23 (59.6%) (**Figure 3.3a**). Fewer (P<0.0001) birds were standing at d16 (29.7%) and d20 (28.3%) compared with d12 (39.2%) and d23 (33.7%) (**Figure 3.3b**). Fewer (P<0.0001) birds locomoted at d16 (3.9%) and d20 (4.5%) than d12 and d23 (6.1%) (**Figure 3.3c**).

There was no effect of challenge on proportion of birds sitting over time, but the effect of breed independent of challenge was trending, in which more (P=0.09) FG birds sat than SG by 9.1% and 7.0% at d20 and d23, respectively (**Figure 3.4**). The effect of breed across age was more pronounced when analyzed within challenge in the CON treatment, in which generally more (P=0.03) FG-CON sat than SG-CON by 5.2%-11.9% over time, particularly at d12 and d20 (**Figure 3.5a**). The effect of breed across age was not observed within the ST treatment (**Figure 3.5b**). Within age, there was no effect of breed or challenge on the proportion of birds sitting (**Table 3.7**).

There was no effect of breed or challenge on the proportion of birds standing over time (**Tables 3.3 and 3.5**). Only the effect of age was significant on the proportion of birds standing, in which generally fewer (P<0.0001) birds stood at d23 (39.2%) compared to d12 (33.7%) except in the SG-ST treatment, where a similar proportion of

SG-ST were standing at d12 (36.3%) as at d23 (38.6%) (**Figure 3.6**). Within age, there was no effect of breed or challenge on the proportion of birds standing (**Table 3.7**).

The main effect of age and breed and their interaction on the proportion of birds locomoting was significant (**Tables 3.3 and 3.5**). Fewer (P<0.0001) FG birds locomoted than SG at d12, 20, and 23 by 2.7%, 1.6%, and 2.6%, respectively, but at d16 the proportion of birds locomoting was similar between breeds (**Figure 3.7**). Within age, there was no effect of breed or challenge on the proportion of birds locomoting (**Table 3.7**).

The effect of age was significant on the proportion of birds eating, drinking, preening, and sham foraging, as well as on total behavior (the cumulative proportion of birds coded for eating, drinking, preening, stretching, sham foraging, allopreening, and aggression) (**Tables 3.4 and 3.6**). The proportion of birds eating increased (P<0.0001) with age from 21.2% at d12 to 25.9% at d23 (**Figure 3.8a**). A similar proportion of birds drank at d12 (1.9%), d16 (2.1%), and d20 (1.8%), but increased (P=0.01) to 2.5% at d23 (**Figure 3.8b**). The proportion of birds drinking at d16 was also similar to d23 (**Figure 3.8b**). The proportion of birds preening was similar between d12 (5.8%), d16 (6.1%), and d20 (6.4%) and was the greatest (P<0.0001) at d23 (7.4%) (**Figure 3.8c**). The proportion of birds sham foraging was similar between d12 (1.1%) and d16 (1.2%) but increased (P<0.0001) to 1.8% at d20 and 2.6% at d23 (**Figure 3.8d**). Additionally, the proportion of total behavior was similar between d12 (32.1%) and d16 (32.8%) but increased (P<0.0001) to 37.2% at d20 and 40.1% at d23 (**Figure 3.8e**).

The effects of breed and challenge on the proportion of birds eating across age were not significant (**Tables 3.4 and 3.6**). However, the proportion of birds eating

increased with age within FG and SG-ST but not SG-CON (**Figures 3.9a-b**). Fewer (P<0.0001) SG-CON (5.0%) were eating at d23 than at d12 (**Figure 3.9b**). Additionally, at d12 the effects of breed, challenge, and their interaction were significant (**Table 3.8**). More (P=0.004) SG-CON birds (27.0%) were eating at d12 than any other treatment (19.3%) by 7.7% (**Figure 3.10**).

The effects of breed and challenge on the proportion of birds sham foraging over time were significant, but only within CON and SG, respectively (**Tables 3.4 and 3.6**). There was no effect of challenge on FG birds (**Figure 3.11a**) but more (P=0.01) SG-CON birds (4.1%) sham foraged than SG-ST (2.5%) at d23 (**Figure 3.11b**). Additionally, more (P<0.0001) SG-CON were sham foraging than FG-CON on all days except d16 by 0.7% (d12), 1.0% (d20), and 2.3% (d23) (**Figure 3.11c**). The proportion of ST birds sham foraging did not differ between breeds across age (**Figure 3.11d**). Within d23, the effects of breed, challenge, and their interaction were significant (**Table 3.8**). More (P=0.02) SG-CON sham foraged (4.1%) than any other treatment (2.1%) on d23 (**Figure 3.12**).

There was no effect of breed or challenge on total behavior across age (**Tables 3.4 and 3.6**). Within d12, the effects of breed and challenge were significant, but not their interaction (**Table 3.8**). The proportion of SG-CON total behavior (39.5%) was greater (P≤0.05) than all other treatments (29.7%) by 9.8% (**Figure 3.13**).

There was no effect of challenge on the proportion of aggressive birds across age independent of breed. Independent of challenge, fewer (P=0.09) FG birds tended to exhibit aggression than SG at each age by 0.1% (**Figure 3.14**). Within d12, the effects of breed and challenge were significant but not their interaction (**Table 3.8**). Fewer

(*P*=0.008) FG birds displayed aggression than SG birds by 0.2%, and more (*P*=0.03) CON birds displayed aggression than ST birds by 0.1% (**Figure 3.15**).

3.5 DISCUSSION

The objective of this study was to evaluate differences in sickness behavior between fast- and slow-growing broiler chickens when challenged with *Salmonella* Typhimurium. Chicks from fast- (Ross 308) and slow-growing (Redbro) broiler breeds were housed together between day of hatch and day 7, when they were randomly assigned and exclusively placed into BSL-2 isolators. At day 14, half of the birds were orally gavaged with either the *S.* Typhimurium challenge or a Tryptic Soy Broth control. Throughout the study, 2 isolators per room (1 per breed per room) were recorded and coded for postures (sitting, standing, or locomoting) and behaviors, (eating, drinking, preening, stretching, sham foraging, allopreening, or aggression). Total behavior was also calculated as the sum of all behaviors. The first hypothesis of this study was that fast-growing broilers would have a reduced behavioral repertoire when compared to the slow-growing breed independent of challenge. The second hypothesis was that post-challenge, challenged slow-growing broilers would have greater behavioral signs of sickness.

In this study, there were few differences between breeds in the proportion of birds performing each posture (sitting, standing, or locomoting) and no differences between control and challenged birds. When comparing the first (day 12) and last (day 23) recording days, more birds sat and fewer stood as they aged. This finding is typical and expected among broilers as they grow (Bokkers and Koene, 2003; Sultana et al., 2013; Wallenbeck et al., 2016; Dixon, 2020). However, the proportion of birds locomoting was similar between the first and last day. Generally, locomotion-type behaviors, such as

walking or running, also become less frequent as broilers age (Bokkers and Koene, 2003; Sultana et al., 2013; Dixon, 2020). The birds in this study were housed in BSL-2 isolators with appropriate space allowance and stocking density according to the Ag Guide (FASS, 2010), but isolator space still restricted opportunities for movement. It is possible that due to space limitations and resulting inability or need to locomote greater distances, birds were observed locomoting less often, therefore restricting any ability to capture differences across age. When comparing breeds, generally fewer slow-growing birds sat and more slow-growing birds locomoted than the fast-growing breed. These differences in activity have also been reported in previous research (Bokkers and Koene, 2002; Wallenbeck et al., 2016; Dixon, 2020; Yan et al., 2021).

A unique finding in this study was that at the two recording days following challenge (days 16 and 20), more birds of both breeds and treatments sat and fewer stood and locomoted. There was no effect of challenge on the proportion of birds sitting, standing, or locomoting, so these changes cannot be attributed to challenge. A likely cause for the increase in birds sitting and reduction in birds standing or locomoting is the behavioral stress response following gavage use at day 14, though this pattern in postures was not observed in any other behaviors. Orally gavaging animals is an invasive and stressful event, as evidenced by increases in the plasma corticosterone of rats orally gavaged with corn oil (Brown et al., 2000). Stressful events may cause long-term behavioral consequences, resulting in reduced activity manifested through increased sitting and reduced standing and locomoting.

The inclusion of a negative (no gavage) control group would have been beneficial to this study to determine if the gavage process caused stress. A study by Walker and

colleagues (2012) reported that mice orally gavaged with water had increased fecal corticosterone metabolites paired with increased mean arterial pressure and heart rate up to 5 hours after the gavage when compared to mice provided an oral pill or no pill or gavage (control). However, another study found no differences in fecal corticoid metabolite levels in rats gavaged with water 4 weeks after gavaging compared to rats that received no handling, restraint handling, or dry gavaging (no liquid) (Turner et al., 2012). It is unknown to what extent the gavage causes stress in chickens and research is needed to investigate these effects.

Additionally, stress could be caused by the gavage liquid. Findings by Brown and colleagues (2000) noted that plasma corticosterone increased in rats gavaged with corn oil, but not rats gavaged with water, indicating that stress in response to gavage may be dependent on the liquid being administered. TSB was administered to the control birds in this study as opposed to saline solution to control for the effect the broth in the challenge treatment might have had on physiological, microbial, and behavioral measures. The use of TSB versus saline may have thus caused more stress due to the composition of the liquid, or otherwise altered broiler gut microbiome, causing dysbiosis (Kogut, 2013). The microbiome-gut-brain axis (MGBA) defines the relationship between the gut microbiome and behavior, in which gut microbes transmit signals to the brain that can influence social, feeding, learning, and anxiety behaviors (Kraimi et al., 2019). Alterations of the microbiome can result from dietary changes, such as the provision of TSB in this study, and cause anxiety or stress (Kraimi et al., 2019), which might lead to increased sitting and reduced standing and locomoting. Further research is needed to investigate the

differences between administering saline versus other liquids through oral gavage and their effects on broiler physiological measures and behavior.

While more of the slow-growing birds generally locomoted than fast-growing throughout the study, the proportion of fast- and slow-growing broilers locomoting was similar at day 16. The proportion of slow-growing birds locomoting greatly decreased from day 12 to day 16 but increased thereafter. The reduction in slow-growing broilers locomoting at this age may also have been caused by stress from the gavage. As the reduction was much greater in slow-growing birds than in fast-growing birds, it may indicate greater levels of stress in the slow-growing breed 2 days after gavaging followed by successful coping or recovery.

The proportion of birds eating, drinking, and preening increased, as did total behavior. Increases in eating across age are consistent with findings from Dixon (2020), though other studies report no change in time spent feeding possibly due to a combination of reduced visits (Weeks et al., 2000) or increased consumption (Bokkers and Koene, 2003) paired with increased duration at the feeder. In the present study, there was less distance for broilers to move to reach the feed compared to other research and commercial settings, which may have resulted in a greater likelihood of birds being coded at the feeder as eating. Increases in proportion of birds drinking across age are also consistent with findings from Dixon (2020). Several studies have reported increased preening with age, which is speculated to serve as a displacement behavior as a result of frustration from the inability to move as much at heavier weights (Bokkers and Koene, 2003; Dixon, 2020). Though compliant with the Ag Guide (FASS, 2010), limited space in the isolators could have impacted this finding. Lastly, increased total behavior across

age was observed. Though broilers are less mobile with age their overall activity of other behaviors, such as foraging or preening, increase (Weeks et al., 2000; Dixon, 2020; Almeida et al., 2021).

The proportion of birds eating over time increased in all groups except the slow-growing control group, where the proportion of slow-growing control birds eating decreased between day 12 and day 23. Additionally, more slow-growing control birds were eating on day 12 than any other treatment. This difference might suggest that the slow-growing breed may consume more while remaining less feed efficient, or consume less feed per bite, resulting in increased likelihood of a slow-growing bird eating at the feeder than a fast-growing bird. However, feed consumption was not measured in this study, and actual differences in feed consumption cannot be concluded. Differences in proportion of birds eating could also be related to the proportion of birds sham foraging because both are appetitive behaviors (Weeks et al., 2000).

The proportion of birds sham foraging generally increased with age, but further analysis revealed this to be most significant among slow-growing birds. On the other hand, the proportion of fast-growing birds sham foraging generally remained low with little to no change over time. The slow-growing breed sham foraged more than the fast-growing breed over time except at days 12 and 16, where a similar proportion of each breed sham foraged. Dixon (2020) noted in a comparison between fast- and slow-growing broiler breeds that the breeds differed more greatly in their observed behaviors later in life and the fast-growing breed allocated more time to sitting than to other behaviors. Foraging is observed to have some variance between genotypes of broilers, with a tendency for slower-growing broiler breeds to forage or engage in exploratory

behaviors more than medium (Almeida et al., 2012) and fast-growing breeds (Yan et al., 2021). Breed differences in chicken foraging behavior are especially prominent between broilers and layers. When a Cobb/Ross hybrid broiler breed and Calder Ranger layer breed were provided free access feed and feed mixed in wood shavings, the broilers showed less interest in foraging in the wood shavings and greater inactivity than the layers (Lindqvist et al., 2006). However, Wallenbeck and colleagues reported no differences between fast- and slow-growing broiler breed foraging behavior, as well as decreased foraging behavior over time (Wallenbeck et al., 2016).

Within the slow growing breed, the control birds sham foraged significantly more than the challenge birds, but there was no difference between control and challenge birds among the fast-growing breed. These results suggest that foraging behavior in the slow-growing, but not the fast-growing breed, may have been reduced by *S.* Typhimurium infection. Abeyesinghe and colleagues (2021) observed that engagement in exploratory behaviors such as foraging can indicate better welfare, and that the reduction in these behaviors might precede clinical signs of poor health, as evidenced by negative associations with poor gait and leg abnormalities. The reduction of sham foraging behavior among challenged slow-growing birds could be symptomatic of a stronger immune response to the challenge. Alternatively, in this study the proportion of slow-growing control birds sham foraging increased significantly over time compared to all other groups, but unlike all other groups, the proportion of slow-growing birds eating decreased. It is possible the heightened sham foraging and reduced eating may be related, though correlations between behaviors were not run. Lastly, the proportion of slow-growing birds sham foraging might have been affected by lighting. Kristenson and

colleagues (2007) reported an increase in foraging behavior in broilers housed in dim lighting than in bright lighting. The BSL-2 rooms used in this study had notable differences in rom overhead light intensity, but these differences were not recorded. Additionally, the isolator location in the room relative to the location of the room lights might have altered light exposure. Isolators were stacked 2 high, with an upper and lower isolator per unit, and not all isolators in each room were able to be arranged to face the same direction. Thus, light exposure may have varied between isolator based on stack (upper or lower) or direction faced. It is possible that differences in lighting between isolators and rooms accounted for differences in sham foraging between breeds and challenge treatments. However, random assignment of birds into isolators should have eliminated the possibility of isolator location affecting behavior.

Total behavior increased with age in this study and a similar proportion of birds exhibited total behaviors between days 12 and 16 but increased thereafter. Although broilers may allocate more time to sitting or resting as they age (Weeks et al., 2000; Dixon, 2020), it is also observed that the overall repertoire of behaviors increases with age (Almeida et al., 2012). At day 12, numerically more slow-growing birds were coded for a behavior than the fast-growing breed. The slow-growing breed, as a result, may utilize more diverse behavioral repertoire than the fast-growing breed at this age. In a study by Almeida and colleagues (2012), slow-growing broilers were more active throughout the day than a medium-growth broiler breed.

Across age, more slow-growing birds tended to be aggressive than fast-growing birds. Additionally, the proportion of aggressive birds differed at day 12, in which the slow-growing breed was observably more aggressive than the fast-growing breed, and the

assigned control birds were more aggressive than the assigned challenge birds. Previous research has reported breed-related differences in aggression in laying breeds (Cheng et al., 2001), mice (Sandnabba, 1996), and canines (Duffy et al., 2008). There was insufficient data to analyze aggression across age inclusive of the effect of challenge and aggression occurred with very low frequency (<0.5%). Challenge did not affect challenge across age independent of breed, but evaluation of the raw means revealed numerical differences between challenge treatments in which challenge birds were less aggressive than control birds. Prior research reports that aggression can either increase or decrease in animals in response to a pathogenic infection (Weary et al., 2014). However, the proportion of aggressive challenge assigned birds was similar both before and after challenge. A larger study involving more birds might have resulted in more observations of aggressive behavior and would have provided sufficient data to statistically analyze differences in the occurrence of aggressive behavior. Further research is needed to investigate differences between fast- and slow-growing broiler breeds regarding aggression, as well as to determine the effect of *S.* Typhimurium infection on the frequency of aggressive behaviors.

On the other hand, differences in lighting between rooms and isolators as discussed previously might have affected the frequency of observations of aggressive birds. Prior research shows that increased light intensity can increase aggression in broilers (Prayitno et al., 1997; Mahmood et al., 2014). The potential effect of light intensity on aggression conflicts with the effect on sham foraging, because the slow-growing breed and control treatment had the greatest proportion of birds performing each behavior. If differences in lighting intensity existed, we would expect an inverse

relationship between the proportion of birds engaging in sham foraging and proportion of birds engaging in aggression, as increases in sham foraging are associated with dim lighting (Kristenson et al., 2007) but increases in aggression are associated with bright lighting (Prayitno et al., 1997; Mahmood et al., 2014). As light intensity was not recorded in this study, it is unknown if differences in lighting impacted behavior, particularly aggression or sham foraging, or if there was any interaction of lighting and challenge on behavior.

In this study, breed and age differences were apparent regarding behavior, but there was little to no effect of challenge on broiler behavior. Differences were observed between challenge and control birds in sham foraging behavior (slow-growing only) and the raw means of aggressive behavior, but these differences could be explained by confounding factors sigh as light intensity. Berghman noted that due to their genetic background and selection, broilers are less prone to respond behaviorally to sickness (Berghman, 2016). Thus, it is possible that the *S.* Typhimurium challenge in this study induced no observable behavioral consequences.

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Table 3.1. Ethogram of postures and behaviors.

Type	Behavior	Description
Posture	Sitting	Resting, hocks on the ground, or lying on the side. Head may or may not be visible. Eyes may be open or closed. Not changing location.
Posture	Standing	One or both feet on floor. Immobile, not changing location. If in between movement, feet are together.
Posture	Locomotion	Mobile and changing location. Includes taking steps in any direction at any speed, and jumping, hopping, or lunging. Also includes moving while hocks are resting on the ground.
Not Visible	Not Visible	Obstructed from view. Posture cannot be accurately determined.
Behavior	Eating	Adjacent to the feeder with head over or in trough. May or may not be actively pecking feed.
Behavior	Drinking	Adjacent to the waterer and actively dipping beak in water or raising beak to swallow.
Behavior	Preening	Self-grooming by running beak through feathers, pecking self, or scratching self with feet.
Behavior	Stretching	Extending one leg and/or wing away from the body while standing. Not mobile.
Behavior	Sham Foraging	Physically investigating the environment, but not other birds, by pecking or scratching.
Behavior	Allopreening	Preening directed at conspecifics. Includes light, non-forceful and brief pecking. Contact cannot be defined as aggression.
Behavior	Aggression	Vigorous pecking or kicking at a conspecific with intent of injurious physical contact, or threats (no contact; erect necks, raised neck feathers, intentional movement). Interaction establishes pecking order. Only the aggressor bird is coded for aggression.
Behavior	Total Behavior	The sum of all behaviors (eating, drinking, preening, stretching, sham foraging, allopreening, and aggression).

Ethogram adapted from: Baxter et al., 2019; Bailie et al., 2013; Bokkers and Koene, 2003; Bizeray et al., 2002; Bizeray et al., 2000; Prayitno et al., 1997.

Table 3.2. Table of possible combinations of coded postures and behaviors for a single bird.

	Sitting	Standing	Locomotion	Not Visible
Eating	✔	✔	✔	✘
Drinking	✔	✔	✘	✘
Preening	✔	✔	✘	✘
Stretching	✔	✔	✘	✘
Sham Foraging	✔	✔	✔	✘
Allopreening	✔	✔	✘	✘
Aggression	✔	✔	✔	✘
No Behavior	✔	✔	✔	✔

Table 3.3. Across age (d) analysis of the effect of age, breed, and their interaction on proportion (%) of birds performing a posture (sitting, standing, or locomoting) or not visible, within challenge (CON or ST), at d12, 16, 20, and 23. Data shown as mean proportion (± SEM) of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with S. Typhimurium (ST) or TSB (CON) at d14.

		d12		d16		d20		d23			P-Value[4]		
Beh.[1]	**Chal.**[2]	**FG**[3]	**SG**	**FG**	**SG**	**FG**	**SG**	**FG**	**SG**	**SEM**	**Age**	**Br.**	**Int.**
SIT	CON	57.8^{c}	46.5^{d}	68.2^{a}	62.9^{abc}	70.9^{a}	59.0^{bc}	64.4^{ab}	58.3^{bc}	3.0	0.0001	0.03	0.005
	ST	54.8^{b}	56.3^{b}	65.5^{a}	67.1^{a}	70.9^{a}	64.6^{a}	61.7^{b}	53.8^{b}	4.7	0.0001	0.67	0.0001
STD	CON	37.4^{a}	43.5^{b}	28.0^{b}	33.1^{b}	25.7^{b}	35.5^{b}	29.9^{b}	33.4^{b}	2.9	0.0001	0.11	0.03
	ST	39.5^{a}	36.3^{a}	29.3^{c}	28.4^{c}	24.5^{d}	27.4^{d}	32.9^{b}	38.5^{b}	4.3	0.0001	0.85	0.0001
LOC	CON	4.3^{bcd}	7.8^{a}	3.6^{cd}	3.6^{d}	3.1^{d}	5.2^{bc}	5.3^{b}	7.5^{a}	0.6	0.0001	0.001	0.001
	ST	4.4^{c}	6.3^{ab}	4.4^{c}	4.2^{c}	4.2^{c}	5.4^{bc}	4.4^{c}	7.3^{a}	0.5	0.002	0.0002	0.004
NVS	CON	0.4^{bc}	2.1^{a}	0.2^{c}	0.4^{bc}	0.3^{c}	0.4^{c}	0.5^{bc}	0.7^{b}	0.2	0.0001	0.0002	0.0001
	ST	1.4^{b}	1.1^{b}	0.9^{c}	0.3^{c}	0.5^{a}	2.7^{a}	1.0^{c}	0.4^{c}	0.3	0.0001	0.47	0.0001

[1]Beh. = behavior; SIT = sitting, STD = standing, LOC = locomoting, NVS = not visible.
[2]Chal. = challenge; CON = control (TSB), ST = 1 mL $1.3x10^{8}$ CFU/mL *S.* Typhimurium challenge.
[3]FG = fast-growing (Ross), SG = slow-growing (Redbro).
[4]P-values represent the main effect of age (d), breed (Br.; FG or SG), and the interaction of age and breed (Int.).
[abcd] Rows not sharing the same letters indicate a significant difference at $P \leq 0.05$.

Table 3.4. Across age (d) analysis of the effect of age, breed, and their interaction on proportion (%) of birds performing a behavior (eating, drinking, preening, stretching, sham foraging, allopreening, or aggression) and total coded behavior (total behavior), within challenge, at d12, 16, 20, and 23. Data shown as mean proportion (± SEM) of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14.

		d12		d16		d20		d23			P-Value[4]		
Beh.[1]	**Chal.**[2]	**FG**[3]	**SG**	**FG**	**SG**	**FG**	**SG**	**FG**	**SG**	**SEM**	**Age**	**Br.**	**Int.**
EAT	CON	19.7^{ab}	27.0^{ab}	22.3^{b}	21.2^{b}	26.6^{a}	22.6^{a}	26.8^{a}	21.9^{a}	1.3	0.004	0.67	0.0001
	ST	18.7^{c}	19.4^{c}	21.0^{c}	19.4^{c}	25.2^{b}	25.5^{b}	26.8^{a}	28.0^{a}	2.0	0.0001	0.96	0.27
DRK	CON	1.7^{b}	1.9^{b}	2.8^{a}	2.1^{a}	2.0^{b}	1.4^{b}	2.6^{a}	2.7^{a}	0.3	0.007	0.30	0.32
	ST	1.6	2.5	1.9	1.5	1.8	2.0	2.6	2.0	0.4	0.17	0.94	0.03
PRN	CON	5.5^{b}	6.1^{b}	6.1^{b}	5.6^{b}	5.5^{b}	6.7^{b}	7.2^{a}	7.6^{a}	0.6	0.0005	0.51	0.31
	ST	5.1^{c}	6.6^{c}	6.3^{bc}	6.2^{bc}	7.6^{ab}	6.0^{ab}	8.0^{a}	6.8^{a}	0.8	0.005	0.77	0.003
STR	CON	0.7	0.9	0.7	0.9	0.7	0.7	0.8	0.9	0.2	0.78	0.24	0.93
	ST	0.3^{b}	0.9^{a}	0.9^{b}	0.9^{a}	0.5^{b}	1.3^{a}	0.6^{b}	0.7^{a}	0.2	0.13	0.03	0.01
SHF	CON	1.0^{cd}	1.7^{bc}	1.0^{cd}	0.8^{d}	1.1^{cd}	2.1^{b}	1.8^{b}	4.1^{a}	0.3	0.0001	0.0003	0.0001
	ST	0.8^{c}	0.9^{c}	1.8^{b}	1.1^{b}	1.9^{a}	2.0^{a}	1.8^{a}	2.5^{a}	0.3	0.0001	0.81	0.05
ALP	CON	0.8^{b}	1.6^{a}	1.3^{b}	0.9^{a}	0.6^{b}	2.3^{a}	1.1^{b}	1.5^{a}	0.2	0.25	0.001	0.0001
	ST	1.2^{b}	1.4^{b}	2.4^{a}	1.6^{a}	1.0^{b}	1.2^{b}	1.2^{a}	2.4^{a}	0.5	0.0001	0.75	0.0003
AGR[5]	CON	0.1	0.3	0.1	0.1	0.1	0.3	0.0	0.3	-	-	-	-
	ST	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	-	-	-	-
TBEH	CON	29.5^{bc}	39.5^{bc}	34.3^{c}	31.6^{c}	36.6^{b}	36.2^{b}	40.4^{a}	39.0^{a}	2.1	0.0001	0.61	0.0001
	ST	27.8^{d}	31.8^{d}	34.3^{c}	30.8^{c}	38.0^{b}	38.0^{b}	41.0^{a}	42.5^{a}	2.4	0.0001	0.87	0.0006

[1]Beh. = behavior; EAT = eating, DRK = drinking, PRN = preening, STR = stretching, SHF = sham foraging, ALP = allopreening, AGR = aggression, TBEH = total behavior.
[2]Chal. = challenge; CON = control (TSB), ST = 1 mL $1.3x10^{8}$ CFU/mL *S.* Typhimurium challenge.
[3]FG = fast-growing (Ross), SG = slow-growing (Redbro).
[4]P-values represent the main effect of age (d), breed (Br.; FG or SG), and the interaction of age and breed (Int.).
[5]Only raw means are reported for aggression in the across age analyses due to insufficient data for the statistics software.
[abcd] Rows not sharing the same letters indicate a significant difference at $P \leq 0.05$.

Table 3.5. Across age (d) analysis of the effect of age, challenge, and their interaction on proportion (%) of birds performing a posture (sitting, standing, or locomoting) or not visible, within breed, at d12, 16, 20, and 23. Data shown as mean proportion (± SEM) of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14.

		d12		d16		d20		d23			P-Value[4]		
Beh.[1]	**Br.**[2]	**CON**[3]	**ST**	**CON**	**ST**	**CON**	**ST**	**CON**	**ST**	**SEM**	**Age**	**Chal.**	**Int.**
SIT	FG	57.8^{d}	54.8^{d}	68.2^{b}	65.5^{b}	70.9^{a}	70.9^{a}	64.4^{c}	61.7^{c}	3.1	0.0001	0.61	0.53
	SG	46.5^{d}	56.3^{d}	62.9^{a}	67.1^{a}	59.0^{b}	64.6^{b}	58.3^{c}	53.8^{c}	4.7	0.0001	0.56	0.0001
STD	FG	37.4^{a}	39.5^{a}	28.0^{c}	29.3^{c}	25.7^{d}	24.5^{d}	29.9^{b}	32.9^{b}	3.0	0.0001	0.76	0.19
	SG	43.5^{a}	36.3^{a}	33.1^{c}	28.4^{c}	35.5^{c}	27.4^{c}	33.4^{b}	38.5^{b}	4.2	0.0001	0.52	0.0001
LOC	FG	4.3^{ab}	4.4^{ab}	3.6^{b}	4.4^{b}	3.1^{b}	4.2^{b}	5.3^{a}	4.4^{a}	0.5	0.01	0.58	0.05
	SG	7.8^{a}	6.3^{a}	3.6^{c}	4.2^{c}	5.2^{b}	5.4^{b}	7.5^{a}	7.3^{a}	0.6	0.0001	0.67	0.17
NVS	FG	0.4^{cd}	1.4^{a}	0.2^{d}	0.9^{bc}	0.3^{d}	0.5^{cd}	0.5^{cd}	1.0^{b}	0.2	0.004	0.0002	0.12
	SG	2.1^{a}	1.1^{a}	0.4^{b}	0.3^{b}	0.4^{a}	2.7^{a}	0.7^{b}	0.4^{b}	0.2	0.0001	0.42	0.0001

[1]Beh. = behavior; SIT = sitting, STD = standing, LOC = locomoting, NVS = not visible.
[2]Br. = breed; FG = fast-growing (Ross), SG = slow-growing (Redbro).
[3]CON = control (TSB), ST = 1 mL $1.3x10^{8}$ CFU/mL *S.* Typhimurium challenge.
[4]P-values represent the main effect of age (d), challenge (Chal.; CON or ST), and the interaction of age and challenge (Int.).
^{abcd} Rows not sharing the same letters indicate a significant difference at $P \leq 0.05$.

Table 3.6. Across age (d) analysis of the effect of age, challenge, and their interaction on proportion (%) of birds performing a behavior (eating, drinking, preening, stretching, sham foraging, allopreening, aggression, or aggression) and total coded behavior (total behavior), within breed, at d12, 16, 20, and 23. Data shown as mean proportion (± SEM) of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14.

		d12		d16		d20		d23			P-Value[4]		
Beh.[1]	**Br.**[2]	**CON**[3]	**ST**	**CON**	**ST**	**CON**	**ST**	**CON**	**ST**	**SEM**	**Age**	**Chal.**	**Int.**
EAT	FG	19.7^{c}	18.7^{c}	22.3^{b}	21.1^{b}	26.6^{a}	25.2^{a}	26.8^{a}	26.8^{a}	2.0	0.0001	0.73	0.82
	SG	27.0^{b}	19.4^{b}	21.2^{c}	19.4^{c}	22.6^{ab}	25.5^{ab}	21.9^{a}	28.0^{a}	1.3	0.0001	0.94	0.0001
DRK	FG	1.7^{c}	1.6^{c}	2.8^{ab}	1.9^{ab}	2.0^{bc}	1.8^{bc}	2.6^{a}	2.6^{a}	0.4	0.005	0.50	0.33
	SG	1.9	2.5	2.1	1.5	1.4	2.0	2.7	2.0	0.3	0.11	0.80	0.05
PRN	FG	5.5^{c}	5.1^{c}	6.1^{b}	6.3^{b}	5.5^{b}	7.6^{b}	7.2^{a}	8.0^{a}	0.8	0.0001	0.49	0.04
	SG	6.1^{ab}	6.6^{ab}	5.6^{b}	6.3^{b}	6.7^{b}	6.0^{b}	7.6^{a}	6.8^{a}	0.7	0.03	0.92	0.22
STR	FG	0.7	0.3	0.7	0.9	0.7	0.5	0.8	0.6	0.2	0.16	0.45	0.17
	SG	0.9	0.9	0.9	0.9	0.7	1.3	0.9	0.7	0.2	0.63	0.30	0.11
SHF	FG	1.0^{c}	0.8^{c}	1.0^{b}	1.8^{b}	1.1^{ab}	1.9^{ab}	1.8^{a}	1.8^{a}	0.3	0.0002	0.32	0.03
	SG	1.7^{cd}	0.9^{de}	0.8^{e}	1.1^{de}	2.1^{bc}	2.0^{bc}	4.1^{a}	2.5^{b}	0.3	0.0001	0.02	0.002
ALP	FG	0.8^{bc}	1.2^{bc}	1.3^{a}	2.4^{a}	0.6^{c}	1.0^{c}	1.1^{b}	1.2^{b}	0.5	0.0001	0.45	0.06
	SG	1.6^{ab}	1.4^{ab}	0.9^{b}	1.6^{b}	2.3^{a}	1.2^{a}	1.5^{a}	2.4^{a}	0.2	0.02	0.71	0.0001
AGR[5]	FG	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	-	-	-	-
	SG	0.3	0.1	0.1	0.1	0.3	0.0	0.3	0.0	-	-	-	-
TBEH	FG	29.5^{d}	27.8^{d}	34.3^{c}	34.3^{c}	36.6^{b}	38.0^{b}	40.4^{a}	41.0^{a}	2.6	0.0001	0.98	0.41
	SG	39.5^{b}	31.8^{b}	31.6^{c}	30.8^{c}	36.2^{b}	38.0^{b}	39.0^{a}	42.5^{a}	2.0	0.0001	0.76	0.0001

[1]Beh. = behavior; EAT = eating, DRK = drinking, PRN = preening, STR = stretching, SHF = sham foraging, ALP = allopreening, AGR = aggression, TBEH = total behavior.
[2]Br. = breed; FG = fast-growing (Ross), SG = slow-growing (Redbro).
[3]CON = control (TSB), ST = 1 mL $1.3x10^{8}$ CFU/mL *S.* Typhimurium challenge.
[4]P-values represent the main effect of age (d), challenge (Chal.; CON or ST), and the interaction of age and challenge (Int.).
[5]Only raw means are reported for aggression in the across age analyses due to insufficient data for the statistics software.
[abcd] Rows not sharing the same letters indicate a significant difference at $P \leq 0.05$.

Table 3.7. Within age (d) analysis of the effect of breed, challenge, and their interaction on proportion (%) of birds performing a posture (sitting, standing, locomoting, or not visible) at d12, 16, 20, and 23. Data shown as mean proportion (± SE) of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with S. Typhimurium (ST) or TSB (CON) at d14.

		Treatment				**P-Value**[3]		
Beh.[1]	**Age**	**FG-CON**[2]	**FG-ST**	**SG-CON**	**SG-ST**	**Br.**	**Chal.**	**Int.**
SIT	12	57.8 ± 3.5	54.8 ± 3.5	46.5 ± 3.5	56.3 ± 3.5	0.17	0.34	0.07
	16	68.2 ± 4.3	65.5 ± 4.3	62.9 ± 4.3	67.1 ± 4.3	0.67	0.86	0.43
	20	70.9 ± 5.7	70.9 ± 5.7	59.0 ± 5.7	64.6 ± 5.7	0.11	0.63	0.62
	23	64.4 ± 5.8	61.7 ± 5.8	58.3 ± 5.8	53.8 ± 5.8	0.23	0.54	0.87
STD	12	37.4 ± 2.5	39.5 ± 2.5	43.5 ± 2.5	36.3 ± 2.5	0.55	0.29	0.06
	16	28.0 ± 3.3	29.3 ± 3.3	33.1 ± 3.3	28.4 ± 3.3	0.52	0.61	0.37
	20	25.7 ± 5.9	24.5 ± 5.9	35.5 ± 5.9	27.4 ± 5.9	0.28	0.42	0.56
	23	29.9 ± 3.8	32.9 ± 3.8	33.4 ± 3.8	38.5 ± 3.8	0.23	0.28	0.79
LOC	12	4.3 ± 2.1	4.4 ± 2.1	7.8 ± 2.1	6.3 ± 2.1	0.19	0.73	0.69
	16	3.6 ± 1.0	4.4 ± 1.0	3.6 ± 1.0	4.2 ± 1.0	0.91	0.51	0.94
	20	3.1 ± 0.9	4.2 ± 0.9	5.2 ± 0.9	5.4 ± 0.9	0.08	0.50	0.63
	23	5.3 ± 1.8	4.4 ± 1.8	7.5 ± 1.8	7.3 ± 1.8	0.17	0.76	0.83
NVS	12	0.4 ± 0.9	1.4 ± 0.9	2.1 ± 0.9	1.1 ± 0.9	0.43	0.97	0.27
	16	0.2 ± 0.3	0.9 ± 0.3	0.4 ± 0.3	0.3 ± 0.3	0.58	0.36	0.20
	20	0.3 ± 0.9	0.5 ± 0.9	0.4 ± 0.9	2.7 ± 0.9	0.20	0.15	0.24
	23	0.5 ± 0.5	1.0 ± 0.5	0.7 ± 0.5	0.4 ± 0.5	0.76	0.90	0.37

[1]Beh. = behavior; SIT = sitting, STD = standing, LOC = locomoting, NVS = not visible.
[2]FG = fast-growing (Ross), SG = slow-growing (Redbro), CON = control (TSB), ST = 1 mL $1.3x10^8$ CFU/mL *S.* Typhimurium challenge.
[3]P-values represent the main effect of breed (Br.; FG or SG), challenge (Chal.; CON or ST), and the interaction of breed and challenge (Int.).

Table 3.8. Within age (d) analysis of the effect of breed, challenge, and their interaction on proportion (%) of birds performing a behavior (eating, drinking, preening, stretching, sham foraging, allopreening, or aggression) and total coded behavior (total behavior) at d12, 16, 20, and 23. Data shown as mean proportion (± SEM) of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14.

		Treatment				**P-Value**[3]		
Beh.[1]	**Age**	**FG-CON**[2]	**FG-ST**	**SG-CON**	**SG-ST**	**Br.**	**Chal.**	**Int.**
EAT	12	19.7 ± 1.2[b]	18.7 ± 1.2[b]	27.0 ± 1.2[a]	19.4 ± 1.2[b]	0.0006	0.0003	0.004
	16	22.3 ± 2.6	21.0 ± 2.6	21.2 ± 2.6	19.4 ± 2.6	0.60	0.55	0.92
	20	26.6 ± 1.9	25.2 ± 1.9	22.6 ± 1.9	25.5 ± 1.9	0.36	0.71	0.27
	23	26.8 ± 3.9	26.8 ± 3.9	21.9 ± 3.9	28.0 ± 3.9	0.63	0.44	0.43
DRK	12	1.7 ± 0.4	1.6 ± 0.4	1.9 ± 0.4	2.5 ± 0.4	0.16	0.50	0.44
	16	2.8 ± 0.3[a]	1.9 ± 0.3[b]	2.1 ± 0.3[a]	1.5 ± 0.3[b]	0.05	0.009	0.60
	20	2.0 ± 0.4	1.8 ± 0.4	1.4 ± 0.4	2.0 ± 0.4	0.48	0.59	0.30
	23	2.6 ± 0.4	2.6 ± 0.4	2.7 ± 0.4	2.0 ± 0.4	0.50	0.38	0.31
PRN[4]	12	5.4 ± 0.8	5.1 ± 0.7	6.0 ± 0.8	6.6 ± 0.9	0.20	0.91	0.53
	16	6.1	6.3	5.6	6.2	-	-	-
	20	5.5 ± 0.8	7.6 ± 1.1	6.6 ± 1.0	5.9 ± 0.9	0.83	0.49	0.13
	23	7.2 ± 1.6	7.5 ± 1.6	7.5 ± 1.7	6.8 ± 1.5	0.91	0.90	0.76
STR	12	0.7 ± 0.2[b]	0.3 ± 0.2[b]	0.9 ± 0.2[a]	0.9 ± 0.2[a]	0.02	0.31	0.26
	16	0.7 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.76	0.69	0.63
	20	0.7 ± 0. 2 [b]	0.5 ± 0.2[b]	0.7 ± 0.2[a]	1.3 ± 0.2[a]	0.006	0.18	0.02
	23	0.8 ± 0.3	0.6 ± 0.3	0.9 ± 0.3	0.7 ± 0.3	0.68	0.49	0.83
SHF	12	1.0 ± 0.6	0.8 ± 0.6	1.7 ± 0.6	0.9 ± 0.6	0.48	0.42	0.61
	16	1.0 ± 0.4	1.8 ± 0.4	0.8 ± 0.4	1.1 ± 0.4	0.35	0.19	0.61
	20	1.1 ± 0.3[b]	1.9 ± 0.3[b]	2.1 ± 0.3[a]	2.0 ± 0.3[a]	0.03	0.15	0.09
	23	1.8 ± 0.4[b]	1.8 ± 0.4[b]	4.1 ± 0.4[a]	2.5 ± 0.4[a]	0.0001	0.02	0.02

Table 3.9. cont.

		Treatment				P-Value[3]		
Beh.[1]	**Age**	**FG-CON**[2]	**FG-ST**	**SG-CON**	**SG-ST**	**Br.**	**Chal.**	**Int.**
ALP	12	0.8 ± 0.6	1.2 ± 0.6	1.6 ± 0.6	1.4 ± 0.6	0.41	0.89	0.59
	16	1.3 ± 0.7	2.4 ± 0.7	0.9 ± 0.7	1.6 ± 0.7	0.38	0.18	0.83
	20	0.6 ± 0.3[b]	1.0 ± 0.3[b]	2.3 ± 0.3[a]	1.2 ± 0.3[a]	0.0003	0.11	0.003
	23	1.1 ± 0.6	1.2 ± 0.6	1.5 ± 0.6	2.4 ± 0.6	0.24	0.43	0.51
AGR	12	0.1 ± 0.1[b]	0.0 ± 0.1[b]	0.3 ± 0.1[a]	0.1 ± 0.1[b]	0.008	0.03	0.43
	16	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.43	0.43	0.69
	20	0.1 ± 0.1	0.0 ± 0.1	0.3 ± 0.1	0.0 ± 0.1	0.33	0.07	0.21
	23	0.0 ± 0.2	0.0 ± 0.2	0.3 ± 0.2	0.1 ± 0.2	0.22	0.46	0.62
TBEH	12	29.5 ± 2.4[b]	27.8 ± 2.4[b]	39.5 ± 2.4[a]	31.8 ± 2.4[b]	0.003	0.05	0.21
	16	34.3 ± 3.2	34.3 ± 3.2	31.6 ± 3.2	30.8 ± 3.2	0.33	0.90	0.89
	20	36.6 ± 1.9	38.0 ± 1.9	36.2 ± 1.9	38.0 ± 1.9	0.93	0.40	0.90
	23	40.4 ± 3.2	41.0 ± 3.2	39.0 ± 3.2	42.5 ± 3.2	0.98	0.52	0.66

[1]Beh. = behavior; EAT = eating, DRK = drinking, PRN = preening, STR = stretching, SHF = sham foraging, ALP = allopreening, AGR = aggression, TBEH = total behavior.
[2]FG = fast-growing (Ross), SG = slow-growing (Redbro), CON = control (TSB), ST = 1 mL $1.3x10^8$ CFU/mL *S.* Typhimurium challenge.
[3]P-values represent the main effect of breed (Br.; FG or SG), challenge (Chal.; CON or ST), and the interaction of breed and challenge (Int.).
[4]Only raw means are reported for preening on d16 in the within age analysis due to insufficient data for the statistics software.
[ab] Rows not sharing the same letters indicate a significant difference at $P \leq 0.05$.

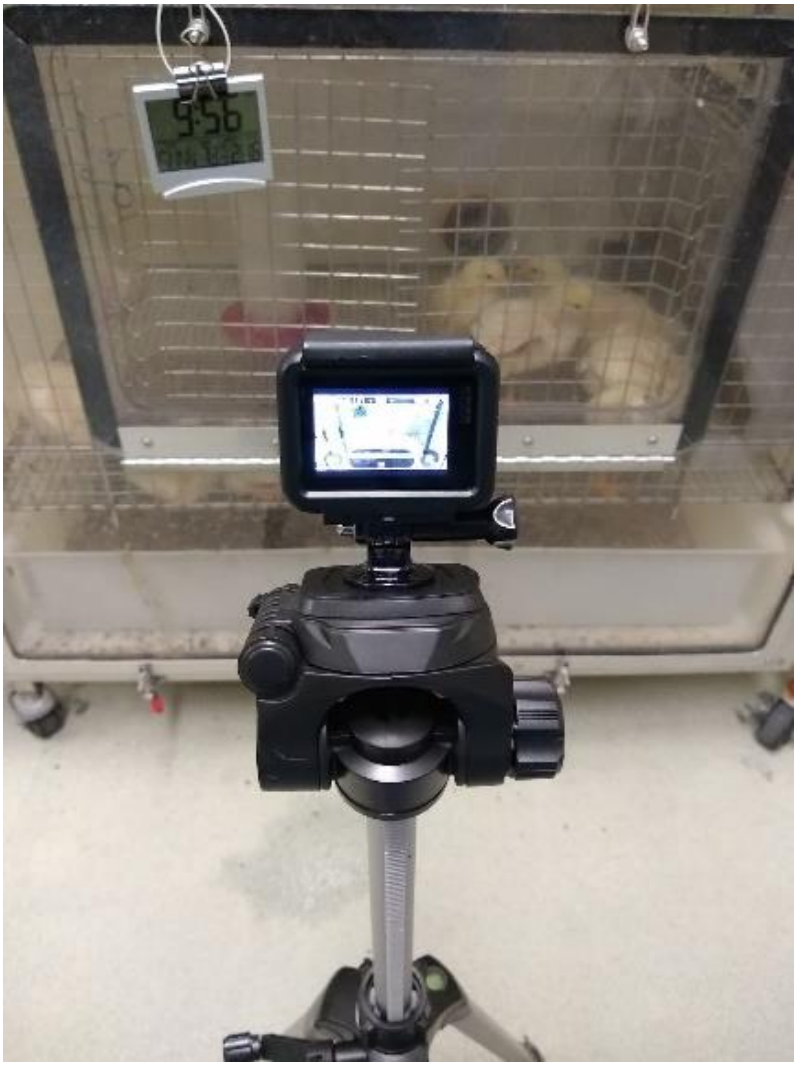


Figure 3.1. GoPro camera set-up facing an isolator. On the isolator door is a hanging clock displaying real time.

Figure 3.2. View of an isolator within a video recording which observers used to code behavior. Observers were able to adjust brightness settings accordingly within each video recording to improve vision of the birds.

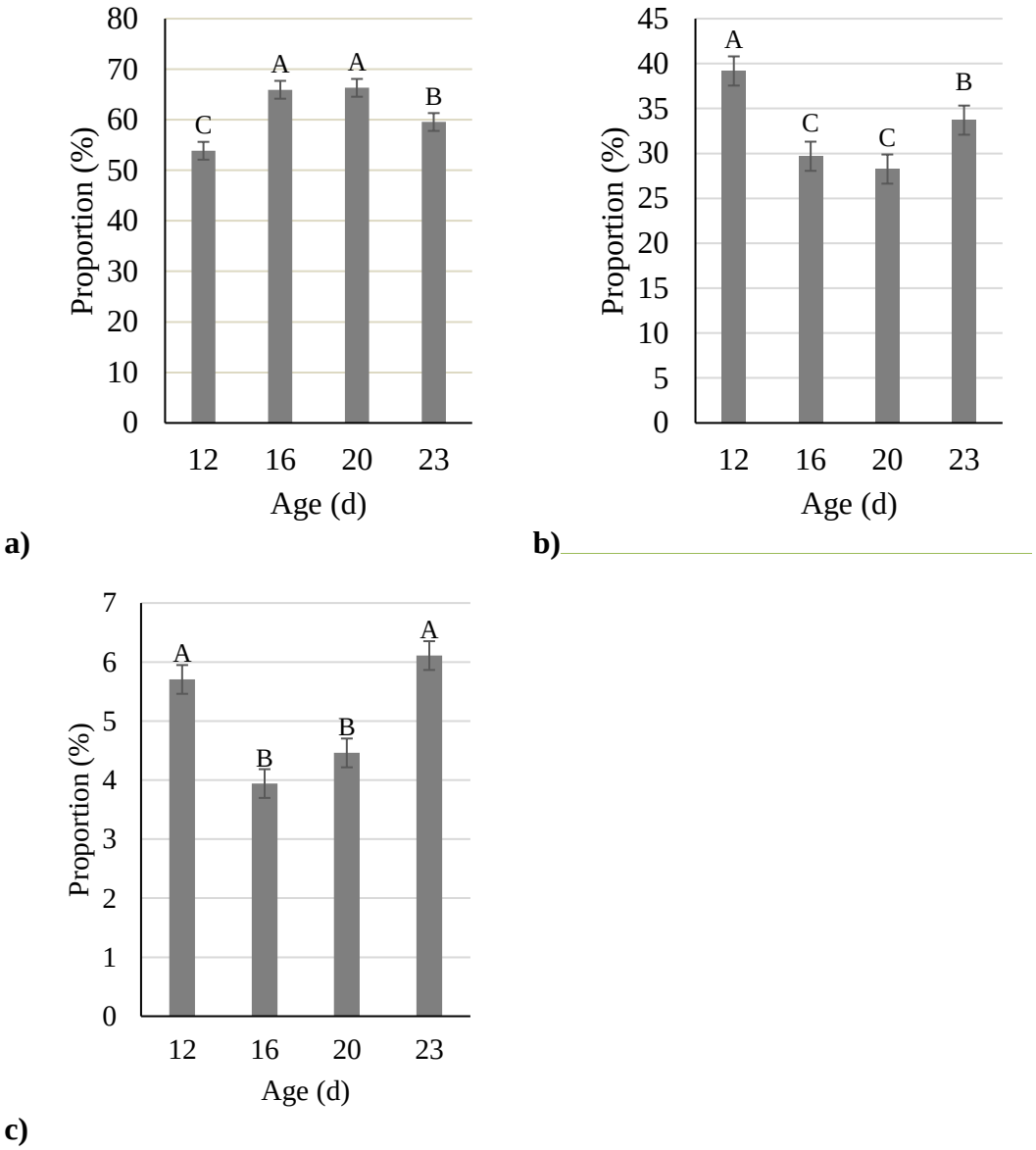


Figure 3.3. Effect of age (d) on mean proportion (%) of birds performing a posture at d12, 16, 20, and 23. Data shown as mean proportion of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14. **a)** Sitting. **b)** Standing. **c)** Locomoting. ^{ABC}Columns not sharing the same letters are significantly different.

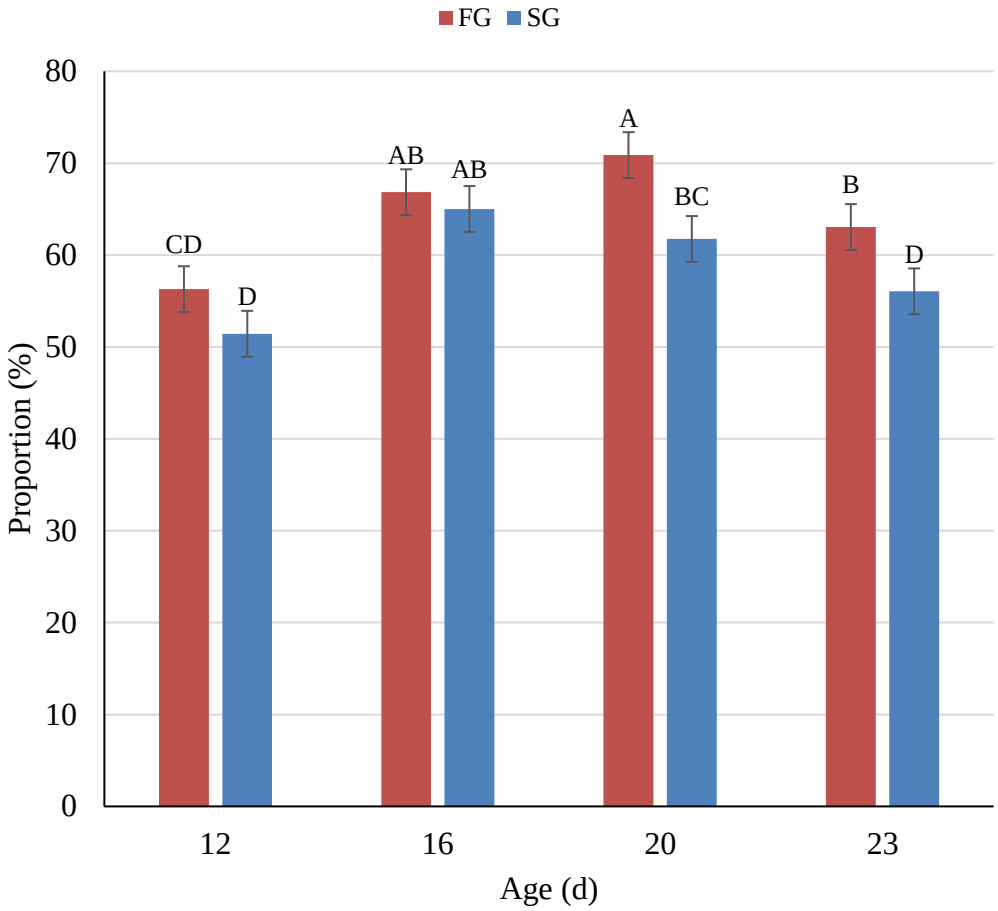
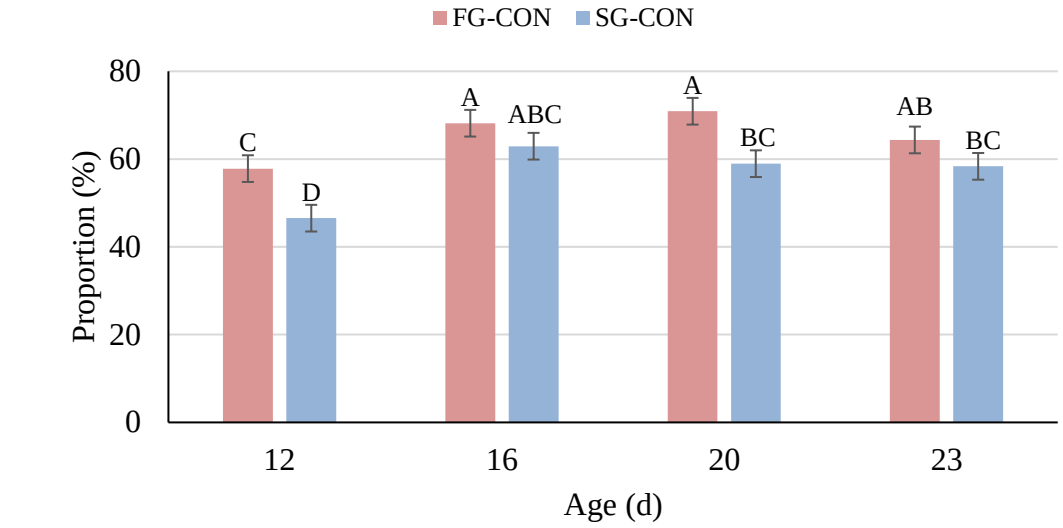
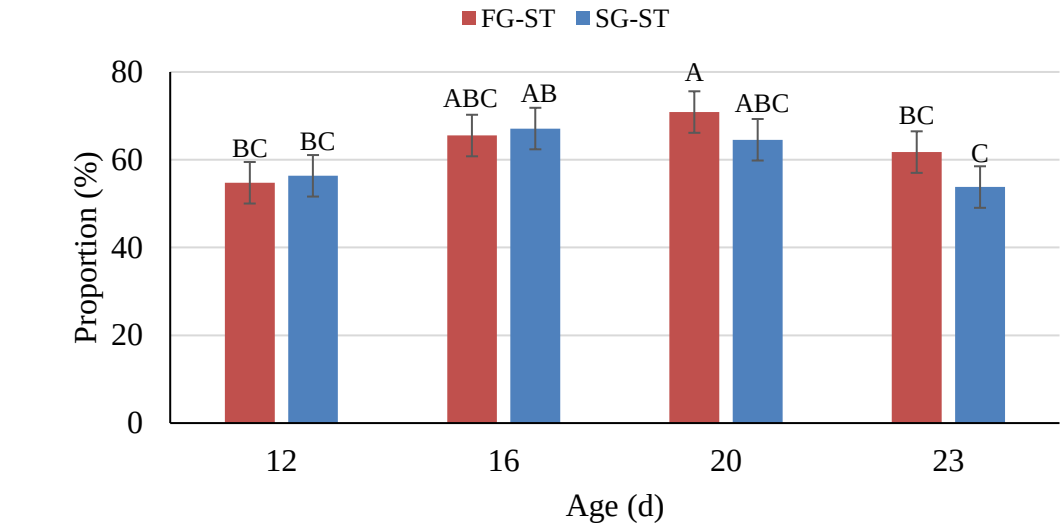


Figure 3.4. Effect of age (d), breed, and their interaction on mean proportion (%) of birds sitting at d12, 16, 20, and 23. Data shown as mean proportion of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14. ^{ABCD}Columns not sharing the same letters are significantly different.



a)



b)

Figure 3.5. Effect of age (d), breed, and their interaction on mean proportion (%) of birds sitting at d12, 16, 20, and 23. Data shown as mean proportion of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14 on **a)** CON birds, **b)** ST birds. ^{ABCD}Columns not sharing the same letters are significantly different.

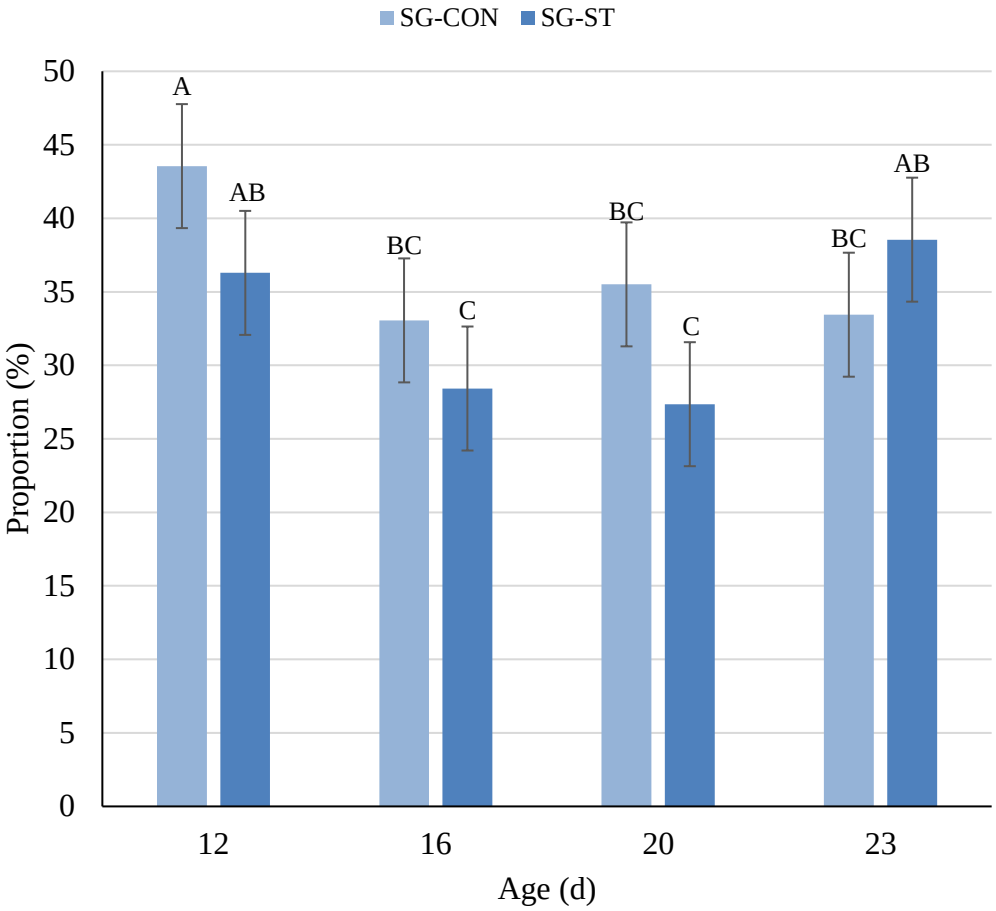


Figure 3.6. Effect of age (d), challenge, and their interaction on mean proportion (%) of birds standing at d12, 16, 20, and 23. Data shown as mean proportion of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14 on SG birds. ^{ABC}Columns not sharing the same letters are significantly different.

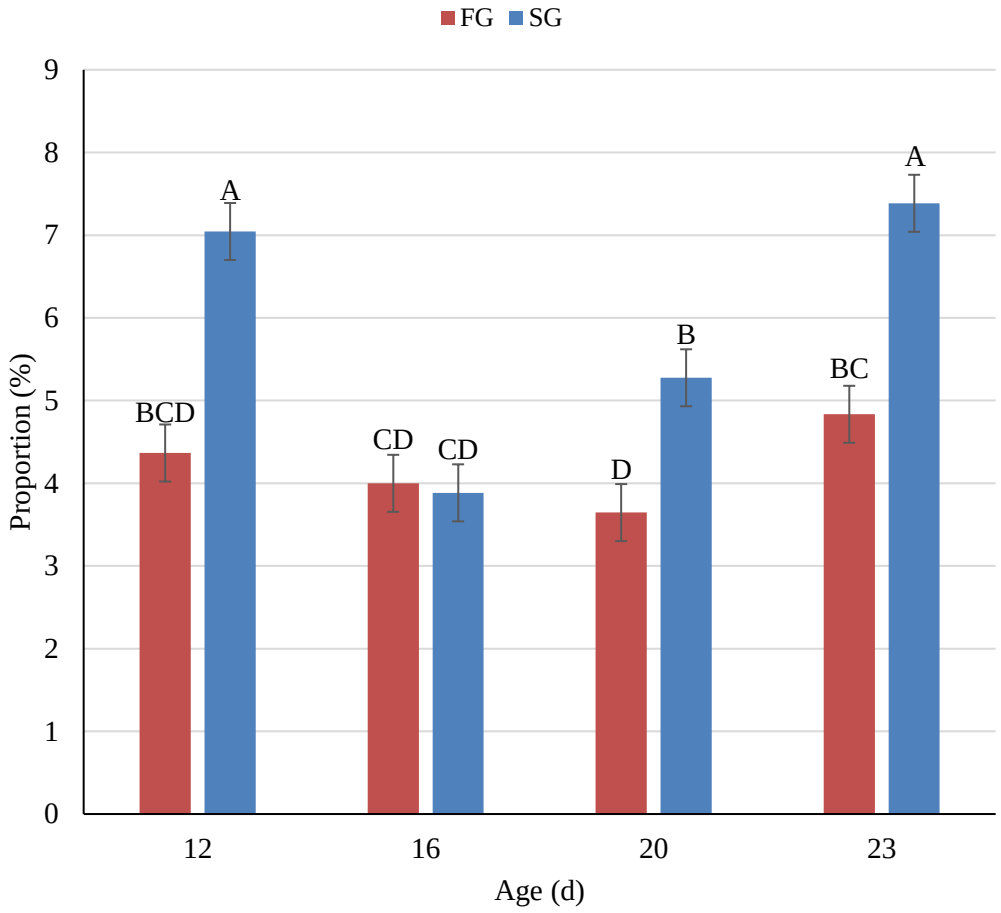


Figure 3.7. Effect of age (d), breed, and their interaction on mean proportion (%) of birds locomoting at d12, 16, 20, and 23. Data shown as mean proportion of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14. [ABCD]Columns not sharing the same letters are significantly different.

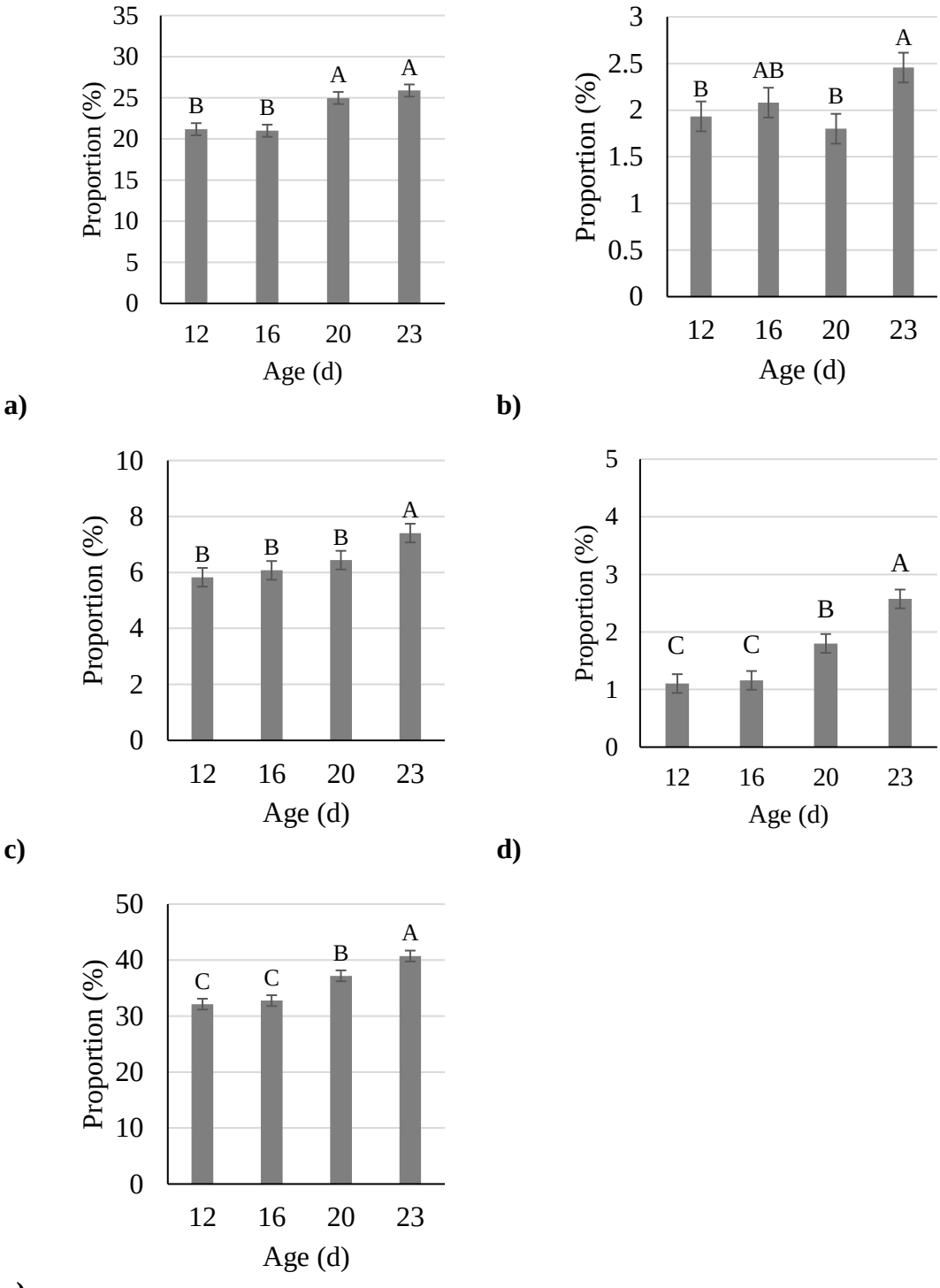
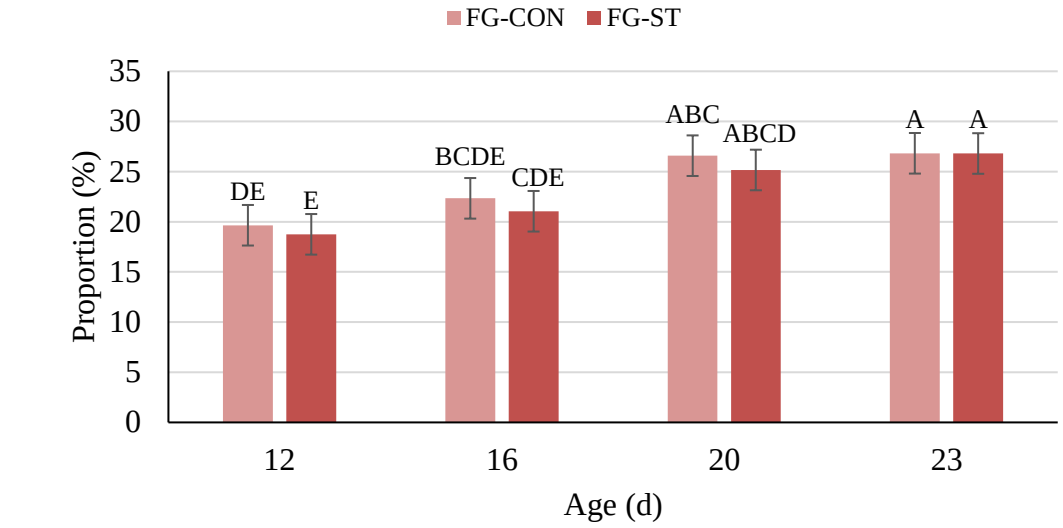
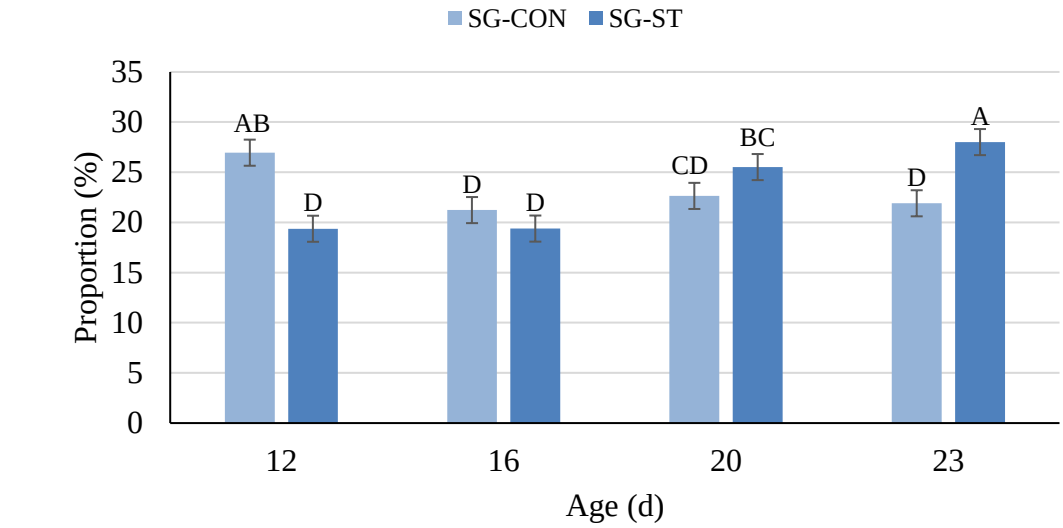


Figure 3.8. Effect of age (d) on mean proportion (%) of birds performing a behavior at d12, 16, 20, and 23. Data shown as mean proportion of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium (ST) or TSB (CON) at d14. **a)** Eating. **b)** Drinking. **c)** Preening. **d)** Sham foraging. **e)** Total Behavior. ^{ABC}Columns not sharing the same letters are significantly different.



a)



b)

Figure 3.9. Effect of age (d), challenge, and their interaction on mean proportion (%) of birds eating at d12, 16, 20, and 23. Data shown as mean proportion of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14 on **a)** FG birds, and **b)** SG birds. ^{ABCDE}Columns not sharing the same letters are significantly different.

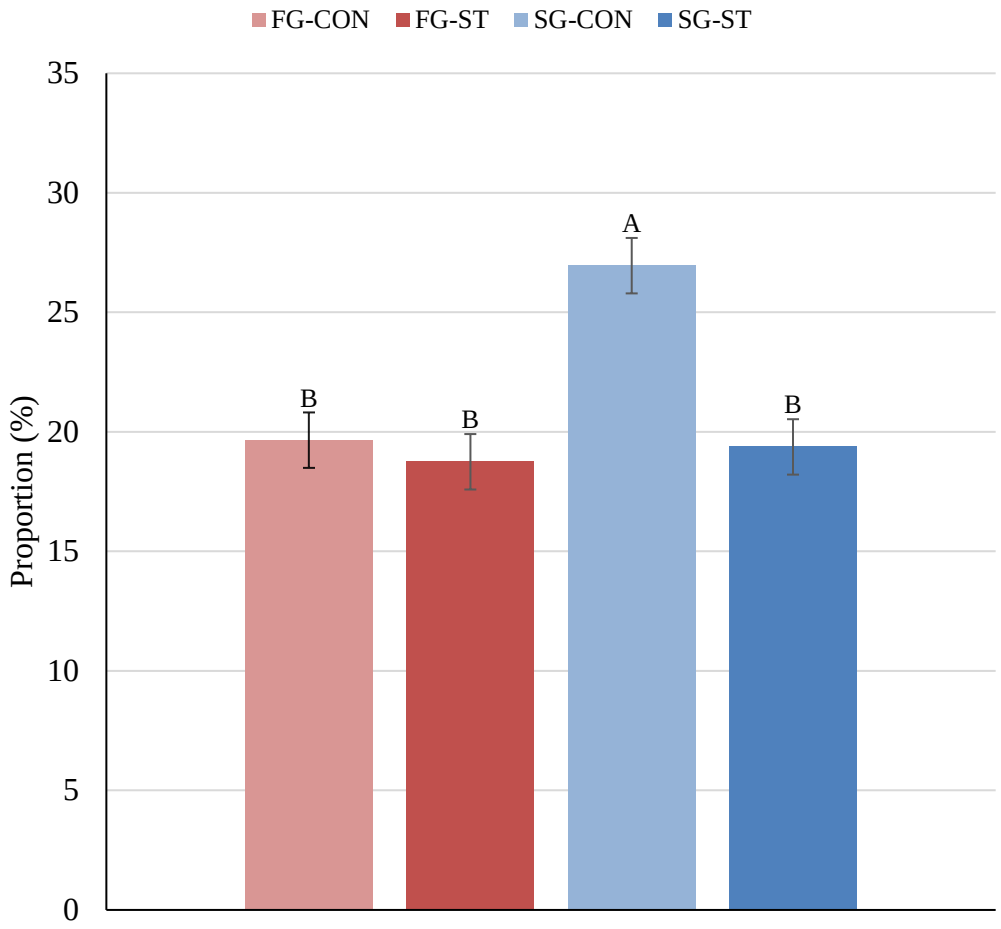


Figure 3.10. Effect of breed, challenge, and their interaction on the mean proportion (%) of birds eating at d12. Data shown as mean proportion of male broilers from fast- (FG) and slow-growing (SG) breeds prior to challenge with *S.* Typhimurium *(*ST) or TSB (CON) at d14. ^{ABC}Columns not sharing the same letters are significantly different.

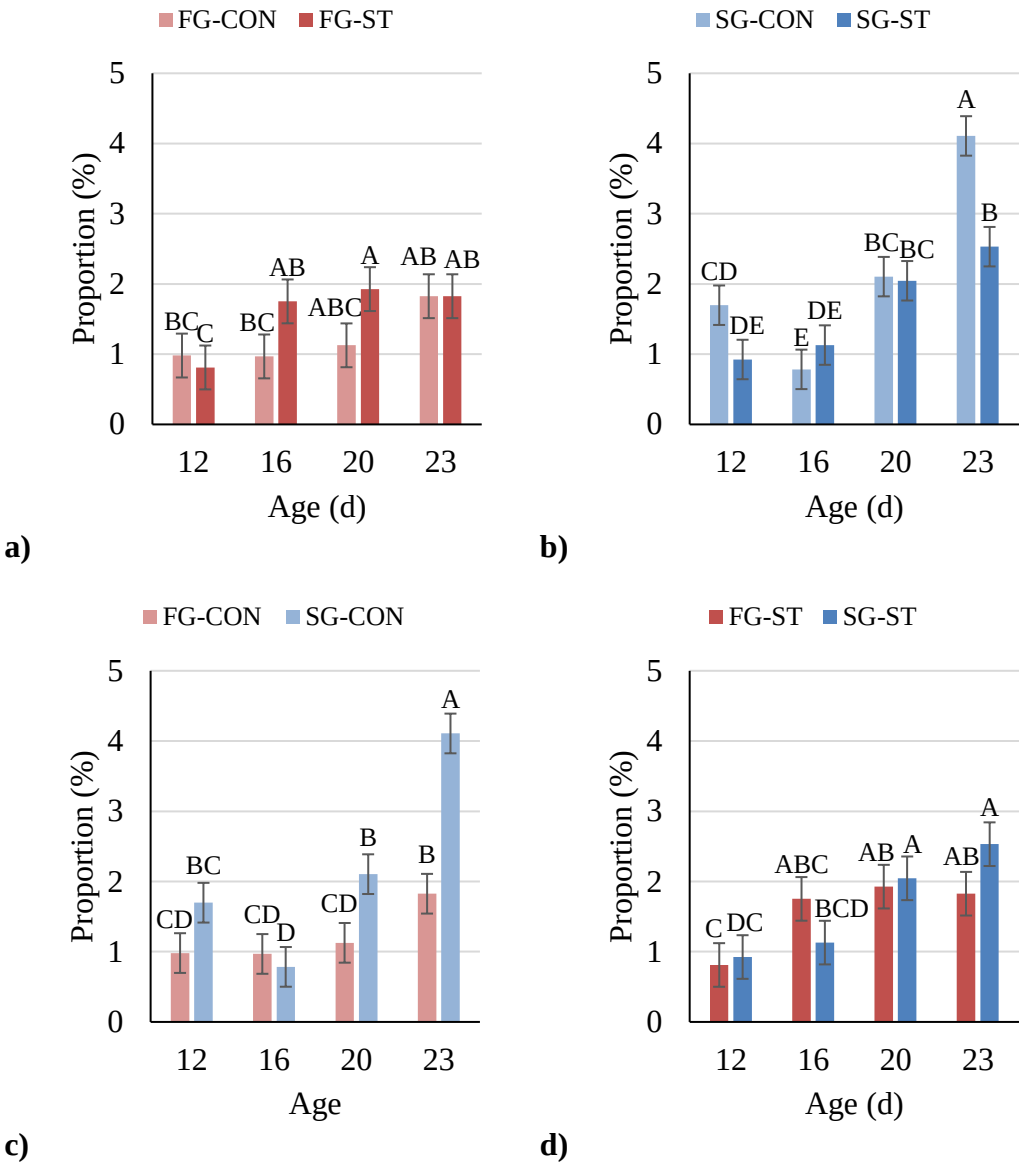


Figure 3.11. Effect of age (d), breed, challenge, and their interaction on mean proportion (%) of birds sham foraging at d12, 16, 20, and 23. Data shown as mean proportion of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14 on **a)** FG birds, **b)** SG birds, **c)** CON birds, and **d)** ST birds. [ABCDE]Columns not sharing the same letters are significantly different.

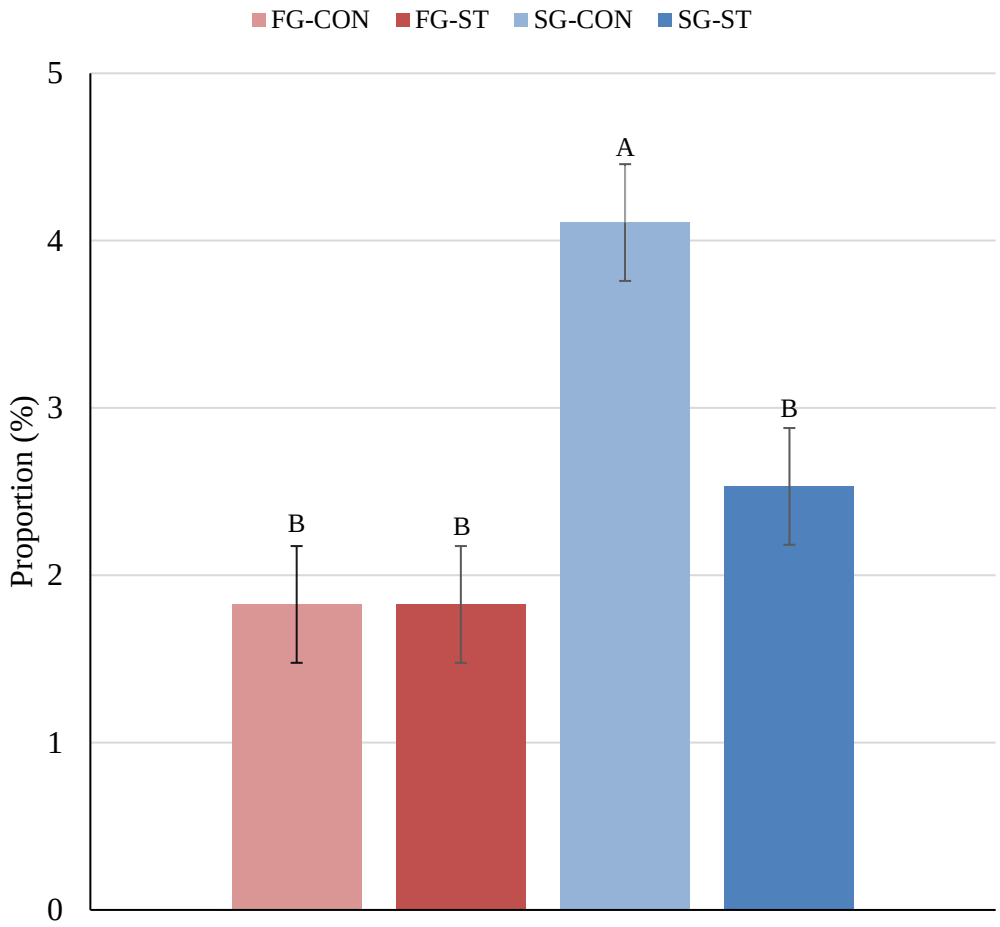


Figure 3.12. Within age (d) analysis of the effect of breed, challenge, and their interaction on the proportion (%) of birds sham foraging at d23. Data shown as mean proportion of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14. ^{AB}Columns not sharing the same letters are significantly different.

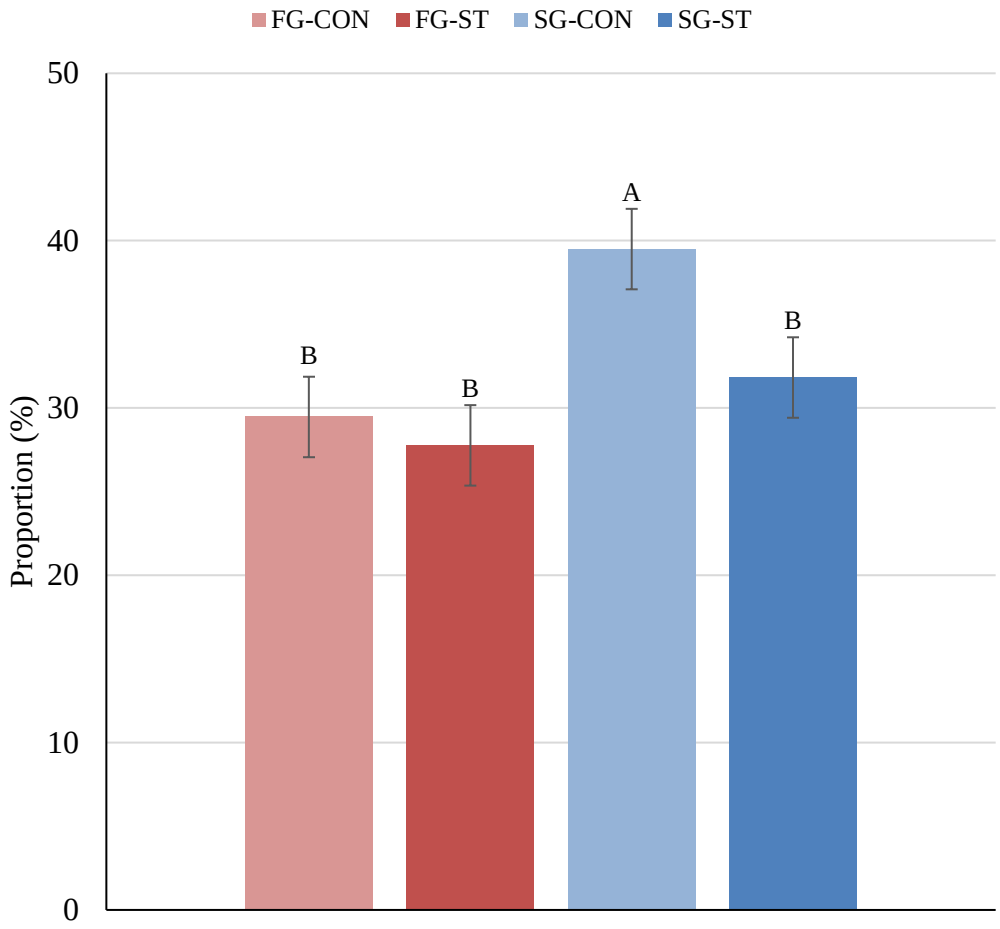


Figure 3.13. Within age (d) analysis of the effect of breed, challenge, and their interaction on the proportion (%) of total behavior at d12. Data shown as mean proportion of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14. ^{AB}Columns not sharing the same letters are significantly different.

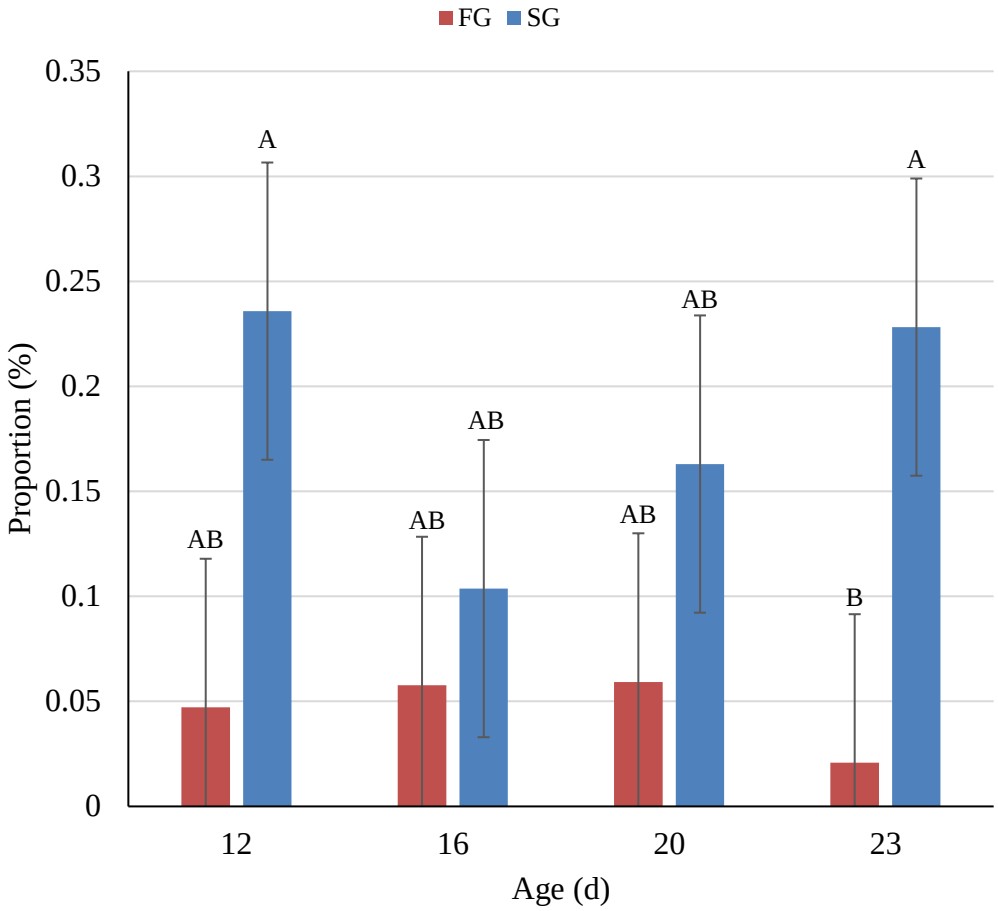


Figure 3.14. Effect of age (d), breed, and their interaction on mean proportion (%) of aggressive birds at d12, 16, 20, and 23. Data shown as mean proportion of male broilers from fast- (FG) and slow-growing (SG) breeds challenged with *S.* Typhimurium *(*ST) or TSB (CON) at d14. [AB]Columns not sharing the same letters are significantly different.

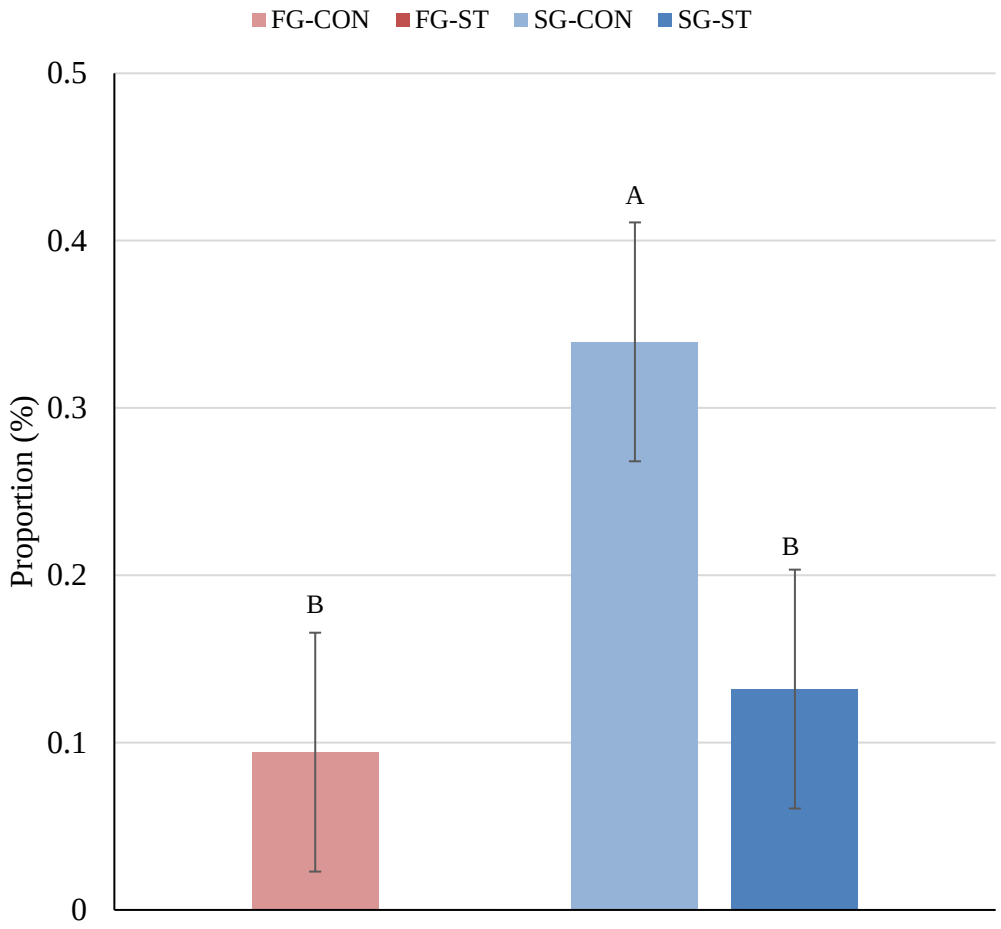


Figure 3.15. Within age (d) analysis of the effects of breed and challenge on proportion (%) of aggressive birds at d12. Data shown as mean proportion of male broilers from fast- (FG) and slow-growing (SG) breeds prior to challenge with *S.* Typhimurium *(*ST) or TSB (CON) at d14. ^{AB}Columns not sharing the same letters are significantly different.

CHAPTER 4 Conclusion

4.1 Summary and Implications

Due to present public concerns regarding the welfare of conventional fast-growing broilers, the increasing use of slower-growing broiler breeds has become a hot topic in animal welfare. Additionally, the ever-present threat of *Salmonella* to the broiler industry and human food supply remains an animal welfare and public health issue that must be faced. The results of this study indicate that meaningful differences exist between fast- and slow-growing broiler breeds regarding body weight, immune response, gut morphology, and behavior when challenged with *S.* Typhimurium and independent of challenge. The objectives of this study were to evaluate differences between fast- and slow-growing broiler breeds when challenged with *S.* Typhimurium and to identify signs of *Salmonella* infection in broilers.

The fast-growing breed was numerically heavier at days 13 and 17 and significantly heavier at days 21 and 24, but challenged birds had numerically lower body weight at day 24 (10 days post-challenge) compared to controls. There is the possibility that challenged fast-growing broilers might have continued to have reductions in body weight compared to controls after day 24. In this case, reduced body weight gain would have been a more pronounced symptom of *S.* Typhimurium infection in the fast-growing breed. On the other hand, slow-growing broiler body weight did not appear to be affected by the challenge, indicating *S.* Typhimurium infection might have caused the fast-growing breed to suffer greater performance losses beyond day 24.

Immune response differed between breeds. Both breeds had an elevated plasma IgA response to challenge 1 week following challenge, but by day 24 the fast-growing breed had higher plasma IgA concentration than the slow-growing breed. While no

plasma IgG response was detected in either breed, fast-growing broiler plasma IgG increased after day 17, while slow-growing broiler IgG did not change. Selection for increased growth may have additionally resulted in faster development of lymphoid organs or earlier maturation of the immune system in the fast-growing breed, though the rate of development or heightened antibody levels do not necessarily indicate a stronger or more efficient immune response (van der Most, 2010; Barrow et al., 2012). Further research is needed to investigate the relationship between growth rate and the development of immunocompetence in broilers. Another unique finding in this study was the slow-growing breed had greater plasma IgG at day 7 which may be remnant maternal antibody conferred from the yolk (Gharaibeh and Mahmoud, 2013). Higher concentrations of maternal antibody improve immune protection early life until immunocompetence is attained (Marcq et al., 2011).

The results suggest that the fast-growing breed had greater jejunum intestinal morphology at day 7, implying greater nutrient absorption. The fast-growing breed's gut morphology was more impaired by challenge than the slow-growing breed in both the jejunum and ileum at day 24 but appeared to recover in the jejunum. The slow-growing breed had a more resilient intestinal morphology in both segments and thus could better maintain normal intestinal function. Less resilient intestinal morphology, as observed in the fast-growing breed, causes reduced nutrient absorption and as a result, reductions in performance.

Lastly, select behaviors differed between breeds. Generally, more fast-growing birds sat and fewer locomoted than the slow-growing breed. The proportion of slow-growing birds sham foraging increased over time, in which fewer challenged slow-

growing broilers sham foraged than the controls. The fast-growing breed, however, showed little to no difference in sham foraging behavior over time nor between challenge and control treatments. Though the frequency of aggression was low, the slow-growing breed was more aggressive than the fast-growing breed.

4.2 Limitations

In the present study, several limitations were encountered. First, we were limited by facility use and animal housing options, which in turn limited the length of the study. Originally, the trial was to last until both breeds reached market weight (42 days for the fast-growing breed, and 62 days for the slow-growing breed) with challenge occurring on day 22. Due to limited availability of BSL-2 rooms and isolators for animal housing, we were only able to house birds in isolators smaller than those planned for the original trial. Thus, to avoid exceeding appropriate stocking density within the available isolators, the study was shortened to 24 days. Shortening of the study reduced our ability to collect samples that might reflect breed differences at greater ages or weights as well as long term effects of *S.* Typhimurium challenge in either breed. Based on the data in the present study, it is quite possible that differences in body weight, gut morphology, immune response, and behavior would have been observed after day 24. Future studies should investigate these differences beyond day 24 or 10 days post-challenge.

Secondly, limitations existed regarding sampling methods and times. In this study, we followed methods that resulted in extreme damage to histological samples collected on days 13, 17, and 21, and they were thus excluded from the study. An attempt was made to analyze the remaining good samples from these sampling days, but imbalances between treatment groups made the data unsuitable for statistical analysis.

Gut morphology data from days 13, 17, and 21 would have been highly beneficial in elucidating breed differences in gut morphology as well as the more immediate effect of *S.* Typhimurium challenge on morphological measures. The gut morphology results from day 7 suggest that further breed differences might have been observed, and the results from day 24 suggest that challenge impacted the gut much earlier. Future studies should take histological samples at these days to better illustrate these differences and changes in the gut morphology of fast- and slow-growing birds when infected with *S.* Typhimurium.

Sampling time and duration, in conjunction with limited availability of BSL-2 trained lab members, may have impacted data. Since few trained individuals were available on some sampling days and the number of birds sampled and samples collected, each sampling day lasted several hours. Additionally, due to biosecurity and facility protocol, control birds had to be sampled prior to challenge birds. Time of day can affect biological functions, particularly circulating concentrations of molecules and white blood cells in the blood, and it is possible this led to the challenge effect observed at day 13. Several immune markers of the innate immune response, such as proinflammatory cytokine IL-6, respond acutely to infection in which the response may not be observed longer than 48 hours post-infection (Xie et al., 2000). Thus, there were few options to study the innate immune response, and of which, fewer commercial kits available. There was an attempt to study plasma IFN-y concentrations using a do-it-yourself ELISA kit, but issues with both the reliability of the kit and stability of IFN-y in the plasma following storage and freeze-thaw cycles limited our ability to do so. Future research should consider having shorter but more frequent collection days, particularly post-challenge, to allow for further investigation and a more accurate understanding of

differences between the breeds in innate and adaptive immune responses to *S.* Typhimurium challenge. This is particularly important as clearance of a gastrointestinal bacterial pathogen such as *S.* Typhimurium can occur without a B-cell or antibody response (Barrow et al., 2012).

Another limitation of the study was the failure to have a negative control group for the effects of the gavage, or to have a saline control for the effects of TSB. TSB may have induced dysbiosis in the present study, therefore causing reductions in gut morphology and increasing the IgA response in control broilers and possibly muting any differences that might have been observed between breeds and challenge treatments. Reductions in gut morphology could have further caused reductions in body weight. Additionally, stress from orally gavaging birds may have also hindered our ability to observe behavior differences due to challenge or breed at days 16 and 20 of behavior recording.

This study was limited in available materials for recording. Only 8 cameras were able to be used to record videos for behavior analysis across merely 4 days, which could have limited the number of observations of each behavior (particularly aggression and preening) and therefore reducing statistical power. In the future, studies should record the behavior of all birds to account for a greater number of recordings for behavioral observations. This would result in a greater number of birds recorded, and as a result, permit successful statistical analysis and more accurate representation of the effects of breed and challenge on behavior.

The broilers in this study had lower body weights and intestinal morphology measures when compared to birds of similar breeds and ages reported in the scientific

literature and management guides. As this study was not directly focused on nutrition, a commercial Purina Start & Grow feed was used throughout the duration of the trial that was presumed to meet the minimum nutritional needs of both breeds. Often, experimentally formulated diets are used to both meet broiler nutritional requirements and supplement their needs beyond baseline requirements when used in research. The disparity between these measures observed in the study and those of other studies might reflect differences in feed formulation. As a result, limitations on these measures could additionally have muted breed and challenge effects that might have otherwise been significant.

Lastly, the COVID-19 pandemic posed as a limitation on the present study. Fortunately, the animal experiment had ended in February 2020, which was prior to the temporary shutdown of university animal research facilities, but lab access was not permitted for several months. Once lab access was restored, orders for several needed lab materials were on back-order due to their demand regarding COVID-19 testing and research, such as pipette tips, which delayed the ability to run ELISAs for months after the study was completed. This reduced the options for plasma immune markers because some degrade with time during storage.

4.3 Impact

Overall, the fast-growing breed had better performance, jejunum gut morphology, and immune development and was observed to be less aggressive and remained relatively unaffected behaviorally by *S.* Typhimurium challenge. The slow-growing breed appeared more resilient to challenge regarding body weight and gut morphology, and had greater plasma IgG concentration in life, indicating greater early life immune protection.

Additionally, *S.* Typhimurium induced a small variety of responses, including impaired intestinal morphology, elevated IgA 1-week post-challenge, reduced exploratory behavior in the slow-growing breed, and an apparent reduction in aggression in both breeds. This information can be used by broiler breeders to make genetic selection decisions that may improve flock welfare, boost *Salmonella* resistance among their birds, reduce the risk of *Salmonella* transmission into the human food supply, and prevent economic losses due to *Salmonella* infection.

4.4 REFERENCES

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