DIFFERENTIAL RESPONSE OF DIVERGENT GENETIC LINES OF CHICKENS TO MIXED *EIMERIA* INFECTIONS

by

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(Under the Direction of Rami A. Dalloul)

ABSTRACT

Coccidiosis, caused by different species of *Eimeria*, is an enteric disease in poultry that causes significant financial loss to the poultry industry. The objective of this research was to investigate the genetic components conferring resistance or susceptibility to a mixed *Eimeria* infection model in chickens. In this study, pedigreed offspring of White Leghorn chickens were used to evaluate the association of specific haplotypes of the major histocompatibility complex (MHC) B and alloantigen systems A (C4BPM), D (CD99), E (FCAMR), and I (RHCE) with the phenotypic traits being body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), gross and microscopic lesions, and oocyst shedding. The resistance traits such as higher BWG and reduced lesion severity were associated with the MHC B15, CD99-H01, RHCE-H01, and RHCE-H03 haplotypes. Further examination of these haplotype effects on coccidiosis resistance in commercial lines may validate their incorporation in selection programs.

INDEX WORDS: Coccidiosis, *Eimeria*, Major histocompatibility complex, Alloantigens, Chickens, Haplotypes

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DEDICATION

I dedicate this thesis to my family and friends who believed in me when I did not believe in myself. This thesis would not exist today without your love and support.

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Poultry meat and eggs constitute the primary source of animal protein for human consumption globally. The chicken population has significantly increased due to the increasing need for poultry products worldwide. Currently, 74 billion chickens are reared worldwide, and it is estimated that the number will rise to 85 billion by 2032 (Food and Agriculture Organization of the United Nations – with major processing by Our World in Data., 2023). This growth is essential to fulfill the needs of the expanding human population, predicted to be nine billion by 2050. Therefore, sustainable poultry production is necessary to meet the global supply of cheap and safe animal protein sources and safeguard global food security.

Poultry diseases pose significant challenges across the entire industry. One such disease is avian coccidiosis, a critical disease affecting chickens and threatening sustainable poultry production. Coccidiosis is an infection caused by different species of the apicomplexan protozoa *Eimeria*. Coccidiosis manifests in two forms: the clinical form results in high morbidity and mortality, and the subclinical form is characterized by decreased performance parameters like reduced body weight gain (BWG), feed intake (FI), and increased feed conversion ratio (FCR). Coccidiosis also predisposes chickens to secondary bacterial infections such as necrotic enteritis (NE), another significant disease in the poultry industry. Overall, coccidiosis not only impacts the health and productivity of chickens but also poses substantial economic burdens on the poultry industry, estimated to exceed $13 billion annually (Blake et al., 2020).

Coccidiosis has been controlled using drugs such as ionophores (classified as antibiotics) and synthetic anticoccidials. Due to consumer concerns about drug residue in poultry products,

widespread occurrence of drug resistance, and regulatory frameworks, the poultry industry has either banned or reduced the use of these drugs (Peek and Landman, 2011; Owusu-Doubreh et al., 2023). This has urged the poultry industry to focus on alternative strategies for managing coccidiosis in chickens. Therefore, research on vaccines, phytotherapy, and the genetic basis of disease resistance to control coccidiosis has gained more prominence.

In complement with other alternative strategies, researchers could focus on investigating and utilizing genetic markers of coccidial resistance. Genetic selection to resistance to coccidiosis has been investigated since the late 1940's (Palafox et al., 1949; Champion, 1954; Rosenberg et al., 1954). Genetic components, such as the major histocompatibility complex (MHC) and other alloantigen systems A, D, E, and I of chickens, are associated with either susceptibility or resistance to coccidiosis (Miller and Taylor, 2016; Taylor et al., 2016). There is an association of specific haplotypes/alleles of these genetic components with resistance or susceptibility to coccidiosis, highlighting the importance of genetic selection and incorporating valuable markers in the chicken population. However, little research has been done to investigate the genetic markers associated with disease resistance in chickens.

In this thesis, the literature review section discusses coccidiosis: the etiologic agent, the life cycle and transmission, diagnosis, current control strategies, and the genetic basis of chicken resistance to coccidiosis. Previous research conducted to identify the genetic markers for coccidial resistance and such identified markers are also discussed. In our research, we investigated the effects of the genetic components, such as the MHC and other alloantigen systems (A, E, D, and I) on disease outcomes in chickens during mixed *Eimeria* spp. infection. The overall aim was to investigate the association of the haplotypes of these genetic components with the phenotypic traits such as BWG, FI, FCR, gross lesions, microscopic lesions, and oocyst

output. Specifically, we aimed to identify the haplotypes of these genetic components associated with either resistance or susceptibility traits during a mixed *Eimeria* spp. infection.

Coccidiosis presents the poultry industry with complex challenges, warranting a comprehensive understanding of the disease and management strategies. Therefore, this review aims to understand the causative agent, its life cycle and transmission, diagnosis, current control strategies, and the genetic basis of chicken resistance to coccidiosis.

CHAPTER 2

LITERATURE REVIEW

Overview of Coccidiosis

Apicomplexan protozoan parasites of the genus *Eimeria* are the causative agent of coccidiosis in chickens, an important disease. Seven species of *Eimeria* have been shown to cause disease in chickens: *E. acervulina*, *E. necatrix*, *E. tenella*, *E. maxima*, *E. brunetti*, *E. mitis*, and *E. praecox* (Cervantes et al., 2020). Among these, *E. acervulina*, *E. maxima*, and *E. tenella* are the most important in poultry production systems (Chapman, 2014). These species develop in different sections of the gastrointestinal tract and have varying degrees of pathogenicity (Table 1) (Conway and McKenzie, 2007; Cervantes et al., 2020). *Eimeria* is highly host-specific, infecting specific bird species, and site-specific, developing in and infecting certain intestinal sections only.

Eimeria spp. invade and destroy the intestinal epithelium, resulting in poor nutrient absorption, loss of body weight gain, and poor feed conversion efficiency. All seven species have been shown to decrease production in both broilers and layers. Except for *E. mitis* and *E. praecox*, all other *Eimeria* produce pathologic lesions in the intestinal segments they infect (Conway and McKenzie, 2007; Cervantes et al., 2020).

Eimeria life cycle

All the *Eimeria* spp. have similar life cycle and require single host to complete it (direct lifecycle). Yet, this cycle is complex and involves both endogenous (schizogony and gametogony) and exogenous (sporogony) stages (Figure 2.1). The life cycle begins with the ingestion of sporulated oocysts (fecal-oral transmission). These oocysts contain four sporocysts,

each containing two sporozoites. Sporozoites are released from the sporocysts in the gut through mechanical grinding by the gizzard and by the enzymatic actions of different digestive tract enzymes. Depending upon the species involved, sporozoites invade the enterocytes of the various intestinal segments and begin reproduction. The sporozoites transform into trophozoites, start feeding, and perform multiple asexual replication cycles (schizogony), forming a schizont containing merozoites (Cervantes et al., 2020). The schizonts are ruptured, merozoites are released, and they invade the new uninfected cells. The number of schizogony varies between the species (Table 2.1). As sexual reproduction begins, some of these merozoites produce male (microgamete) and female (macrogamete) gametocytes through gametogony. A zygote covered by a double layer of proteins and lipids is formed after microgamete fertilizes with macrogamete, and the sexual cycle is completed. The zygote matures into oocyst, which is non-sporulated inside the bird and excreted into feces. Oocysts become sporulated in about 24 hours when they get favorable environmental conditions (warmth, oxygen, and moisture), known as sporogony (Cervantes et al., 2020). Depending on the species, it takes 4-6 days to complete this entire process.

Transmission

Eimeria has global distribution and is found wherever chickens are reared. Almost all parts of the world have problems with coccidial infection in chickens (Cervantes et al., 2020). The infected birds shed oocysts in their feces, and the only natural way for a chicken to become infected is by ingesting sporulated oocysts. Although the infection caused by *Eimeria* is self-limiting, the outcome of infection is determined by the amount of sporulated oocysts consumed and the health status of chickens. The life cycle of *Eimeria* involves only one host, and no other intermediate hosts have been documented.

Mechanical transmission serves as the primary method of transmission of *Eimeria*. It involves vectors such as poultry farm workers, contaminated equipment such as feeders, waterers, cleaning tools, etc., and insects, wild birds, and dust, facilitating its transmission. Oocysts cannot survive very high temperatures and humidity; once oocysts are shed in feces, their viability is limited in litter due to the practice of composting, which releases heat and ammonia. However, oocysts can remain viable in soil for several weeks. These viable oocysts act as a source of infection to naïve birds.

Diagnosis of coccidiosis

Diagnosis of coccidiosis involves correctly identifying the *Eimeria* species infecting the chickens. Coccidiosis causes damage to the intestinal tract, so post-mortem examination of intestinal segments is the primary method of diagnosis. Upon necropsy, intestinal segments should be scanned thoroughly to identify the characteristic gross lesions in chickens infected with coccidiosis . Additionally, a smear of mucosal scrapping from these intestinal segments can be taken to observe different stages of *Eimeria* under the microscope. The classical method for diagnosing coccidiosis is observing oocysts of different *Eimeria* species in chickens' droppings. Molecular methods such as PCR can be used to make diagnoses more precise. Combining the mentioned techniques will aid substantially in the correct diagnosis of coccidiosis and assessing severity.

Clinical signs

Chickens infected with *Eimeria* have poor gastrointestinal health and show clinical signs like ruffled feathers, depression, huddling, and anorexia. Decreased feed and water consumption, severe diarrhea, and sometimes bloody droppings can be observed. These abnormalities contribute to reduced productivity in chickens with impaired body weight gain and poor feed

conversion efficiency and might lead to mortality in case of severe infections (Greenacre and Morishita, 2021). However, clinical signs should be complemented with other diagnostic approaches mentioned below for accurately diagnosing coccidiosis.

Gross lesion scoring

Intestinal gross lesions are the hallmark of coccidiosis. A standardized lesion-scoring technique is followed to assess the severity of gross lesions (Johnson & Reid, 1970). A scale of 0 to +4 is used to numerically represent the severity of gross lesions caused by different *Eimeria* species. The visible changes observed due to infection by the most prevalent species in the industry- *E. acervulina*, *E. maxima*, and *E. tenella* are summarized in Table 2.2 (Johnson and Reid, 1970; Conway and McKenzie, 2007). Experienced personnel should observe gross lesions in all segments of the intestine, starting from the duodenum.

Microscopic lesion scoring

Microscopic scoring methods can be used to assess the severity of coccidial infections. For some species like *E. maxima*, *E. mitis*, and *E. praecox*, it is hard to visualize gross lesions, which could lead to false negative results (Goodwin et al., 1998). In this case, microscopic scoring can be a valuable method to assess the severity of the infection. This method detects all stages of *Eimeria* life cycle. In conjunction with gross lesion scoring, microscopic scoring can provide a more detailed insight into the impact of infection during experimental and field infections (Idris et al., 1997).

Similar to gross lesion scoring, microscopic scores are also scored on a scale of 0 to +4 (Goodwin et al., 1998). Mucosal scrapings from the intestinal segments are collected, wet mounted on glass slides, and covered with coverslips. Observations are made under a 10X

objective and approximately ten fields of vision per sample. Table 2.3 shows the criteria for the microscopic scoring.

Oocyst count

Oocyst counting in the droppings is a valuable tool for the identification of species as well as for quantifying the infection. It is a quick and non-invasive method to diagnose coccidiosis in chickens. Oocysts are quantified and expressed as oocysts per gram (OPG) translated to infection level in chickens. In this method, fecal samples are collected, soaked in water for 24 hours, floated in supersaturated sugar or salt solutions, loaded in McMaster slide, and counted under a light microscope (Hodgson, 1970). Moreover, recent advances in an automated enumeration of oocysts of different *Eimeria* species have made this method high throughput and more straightforward to conduct (Smith et al., 2023).

Molecular methods

DNA sequence amplification can be used to detect *Eimeria* species. Different materials, such as intestinal tissues, droppings, and litter, can be used as the source of DNA. After DNA extraction, PCR can target genes, such as internal transcribed spacer regions (ITS-1, ITS-2) and ribosomal RNA (Haug et al., 2007; Morris et al., 2007; Cantacessi et al., 2008; Hamidinejat et al., 2010). Similarly, Sequence Characterized Amplified Region (SCAR) primers have been developed and used for the identification of *Eimeria* species (Fernandez et al., 2003; Blake et al., 2008; Ogedengbe et al., 2011; Carvalho et al., 2019). Using specific primers, these genetic regions are amplified for *Eimeria* species detection. This method can detect very low numbers of oocysts, accurately diagnosing *Eimeria* species in chickens with low parasitic load.

Coccidiosis control methods

Coccidiosis had been controlled mainly by chemotherapy in the past. Fermentation products like ionophores (e.g. monensin, narasin, maduramycin, lasalocid, salinomycin) and synthetic (chemical) anticoccidials (e.g. decoquinate, diclazuril, halofuginone, nicarbazin, sulfadimethoxine) have been extensively used in poultry production to control coccidiosis. Ionophore anticoccidials can effectively kill the motile stages of *Eimeria* by causing osmotic imbalance, and synthetic coccidiostats inhibit the biochemical pathways of protozoa and affect parasite metabolism. However, due to the issues of parasite resistance to ionophores and consumer perceptions of chemical anticoccidial residues in poultry products, poultry industry has reduced the use of anticoccidials to control coccidiosis.

Vaccination is one of the methods used to control coccidiosis in the poultry industry (Dalloul and Lillehoj, 2006). Since the 1950s, live vaccines have been used to develop immunity against *Eimeria* at an early age. These vaccines use sporulated oocysts of *Eimeria* spp., which stimulate the immune system of the poultry and reduce the pathogenic effects (Peek & Landman, 2011). Some of the commercially available vaccines that are approved for use in the United States are Coccivac-B52, Coccivac-D, Immucox, Advent, Inovocox, and Hatch-pack Cocci-III . However, issues of vaccine failure might occur if they are not administered correctly or during inappropriate environmental conditions such as low relative humidity in the housing environment (Price et al., 2014; Jeon et al., 2022). Additionally, the high cost associated with vaccines might also be a factor for poultry industry not using them.

Another promising strategy to control coccidiosis involves the use of plant extracts. Many researchers have investigated their protective effects due to their antioxidant, antimicrobial, immunomodulatory, and anti-inflammatory properties (Abbas et al., 2012, 2013;

Saeed and Alkheraije, 2023). Different herbal components, such as polyphenols, essential oils, spices, etc., have been extensively tested during coccidiosis. This research area is still growing and the search for plant extracts with full protection against coccidiosis is yet to be discovered.

Genetic basis of disease resistance

Genetic lines of chickens

The chicken has been a valuable model organism for studying developmental biology, biomedical, and agricultural sciences due to its fecundity, growth rates, and genetic diversity. Chicken lines are the source for immunogenetics research to understand the specific genetic components associated with phenotypic traits. Inbred lines of chickens are produced by selecting for specific genetic components, which is achieved by repeatedly mating siblings or parents and offspring. Due to inbreeding, the genetic pool becomes more and more small with less variation between individuals. This allows for conducting research to investigate the association of specific genetic variation with phenotypic traits. Similarly, congenic lines of chickens have the same background genome and differ only in a specific gene or a small genomic region. Repeated backcrosses with the inbred population and selecting for the specific allele of interest results in the production of congenic lines of chickens. Researchers have developed different congenic lines, which differ only in the B haplotype, which are valuable tools for studying the role of different MHC B haplotypes in traits such as immunity and resistance or susceptibility to diseases (Shen et al., 1984; Abplanalp et al., 1992). Multiple genetic factors influence complex traits such as disease resistance (Demant et al., 1989; Groot et al., 1992). Therefore, the recombinant congenic lines of chickens can be used to identify specific genes or gene combinations influencing disease resistance (Bacon et al., 2000).

Genetic selection

The diploid nature of the chicken genome with much greater genetic diversity at a population level made chickens popular and easier to genetically select for desirable characteristics (Siegel et al., 2006). Natural selection favoring the domestication of chickens has shaped the genetic characteristics of these animals. After domestication, artificial selection in chickens was done by humans to select for food, sport, religious ceremonies, and other objectives. These selections for numerous traits led to the development of diversified breeds with multiple chicken characteristics. Due to various factors such as mutation, genetic recombination, gene flow due to interbreeding, and random mating, modern chickens have more significant variability across different breeds.

Genetic selection has been extensively used to increase the productivity of chickens. Over the last century, breeding programs for increased growth, feed efficiency, and egg production have been extensively used to enhance chicken productivity (Qanbari et al., 2019). Consequently, the dual-purpose chickens produced for eggs and meat have now developed into two separate lines: meat-type broilers and egg-laying birds, playing a significant role in global food security.

Genetic selection for resistance to different diseases could be used to enhance the immune response of chickens, leading to disease resistance. Breeders have been less focused on selection for disease resistance traits than meat and egg production traits. However, with the advancement of technologies for genotyping, it is now cost-effective to identify genetic factors associated with resistance or susceptibility to diseases in chickens. Some genetic markers, such as the MHC and genes for alloantigen systems including A, D, E, and I, have been identified (Silva and Gallardo, 2020; Fulton et al., 2022, 2023, 2024). These markers have a significant role

in the immune response during diseases and could be utilized in breeding programs to enhance disease resistance in chickens (Taylor et al., 2016; Silva and Gallardo, 2020).

These genetic improvements are permanent and help in sustainable agriculture practices. Genetic improvement of disease resistance should be a vital component of disease control programs and combined with other methods, such as housing management, use of plant extracts, probiotics and prebiotics, immunization, biosecurity, and sanitation to control diseases.

Major histocompatibility complex

The chicken MHC is the genomic region located on micro-chromosome 16. Initially classified as B blood alloantigen system (Briles et al., 1950), this locus was associated with the rejection of skin grafts and confirmed as MHC locus (Schierman and Nordskog, 1961). Although chicken MHC is simple and condensed (46 genes covering approximately 240 kb), this region still manages to have all the essential aspects that are present in mammalian MHC (>200 genes covering approximately 4,000 kb) and is considered a minimal essential MHC (Kaufman et al., 1995, 1999). MHC-encoded class I and class II molecules have essential functions in immune response, particularly antigen presentation to T cells. The chicken MHC consists of two regions: MHC B and MHC Y. These regions do not recombine and are genetically independent. The chicken MHC B locus consists of regions BF (class I), BL (class II), and BG (class IV). The BG region is only found in birds. In the MHC Y region, nonclassical MHC class I and class II, and c-type lectin genes are present (Miller and Taylor, 2016). These regions are polygenic (consisting of multiple genes) and highly polymorphic, with multiple alleles across the population.

The high polymorphism in the MHC region could be defined using the sets of genes closely linked and inherited together from one generation to another, known as haplotypes. Numerous haplotypes (more than 78) have already been defined for the MHC B, and further

investigations regarding the MHC Y need to be conducted. The MHC B haplotypes (B haplotypes) and their link to disease resistance have been studied across different studies (Briles et al., 1977; Lamont et al., 1987; Schou et al., 2010; Hunt and Dunn, 2013). Different haplotypes of the MHC B region have been associated to susceptibility or resistance to viral, bacterial, and parasitic diseases.

Rosenberg et al. (1954) first reported on the role of genetic components in resistance and susceptibility to coccidiosis in chickens. Specific B haplotypes are found to be associated with either resistance or susceptibility to coccidiosis (Clare et al., 1989; Caron et al., 1997; Medarova et al., 2003b; Kim et al., 2008). Other studies concluded that resistance or susceptibility arises from the interaction between MHC B and non-MHC genes (Ruff and Bacon, 1989; Briles, 2004) or the background genome (Dunnington et al., 1992). The dissimilarities among the results of different studies suggest that other factors such as environmental influences or interaction with other genetic loci, might be affecting disease resistance. During a challenge with *E. acervulina*, birds with the B2 haplotype showed enhanced resistance to coccidiosis in chickens (Lillehoj and Bacon, 1991). A study on the red jungle fowl population reared in a seminatural conditions in large aviaries showed that the specific B haplotype (CD-c) had increased susceptibility with higher mortality when present in a homozygous condition during coccidiosis (Worley et al., 2010). However, the susceptibility was masked whenever the same haplotype was present in a heterozygous condition, suggesting that the same haplotype can have varying impacts depending on genetic context. Therefore, a better understanding of these haplotypes is necessary for implementing breeding approaches to improve immunity and disease resistance in chickens.

Alloantigen systems

Alloantigens are the antigens present in one or several cell types that are present in some members of the population but are not common to all the members of that population. Inherited polymorphisms of amino acid residues create these alloantigens. They can be detected by antibodies produced by individuals, which have different variants of the molecule. In chickens, 13 alloantigen systems are defined for nucleated erythrocytes, and these systems are designated as letters A, B, C, D, E, H, I, J, K, L, N, P, and R (Briles et al., 1950; Gilmour, 1959; Briles, 1962, 1984). Similarly, B-lymphocytes contain the alloantigen Bu-1, while T-lymphocytes contain Ly-4 and Th-1 (Gilmour et al., 1976, 1979; Fredericksen et al., 1977; Fredericksen and Gilmour, 1981). These alloantigens are highly polymorphic, with multiple alleles in each system across the chicken population. Thus far, the candidate genes for A (CBP4M), D (CD99), E (FCAMR), and I (RHCE) have been identified (Fulton et al., 2022, 2023, 2024).

Resistance to coccidiosis has been linked to genetic components such as alloantigen systems (Crittenden, 1983; Taylor et al., 2016). Different chicken alloantigen systems are significantly associated with resistance and susceptibility to coccidiosis, particularly to cecal coccidiosis with reported association with the closely linked A and E alloantigen systems (Johnson and Edgar, 1984). Specifically, haplotypes like A7E5 and A9E1 were more prevalent in chickens resistant to cecal coccidiosis, while the A9E3 haplotype was more common in birds susceptible to the disease. Similarly, birds with the I4 allele of the I alloantigen system have demonstrated greater resistance to cecal coccidiosis in comparison to those with the I2 allele, suggesting that the I alloantigen system also plays a role in coccidial resistance (Martin et al., 1986). Cecal coccidiosis has also been linked to the L alloantigen system; chickens carrying the

L1L2 or L2L2 genotypes showed reduced lesion scores and higher resistance to coccidiosis in comparison to the L1L1 genotype (Taylor and Briles, 2000).

Methods for haplotype/allele identification

The first attempt to identify haplotypes of the MHC B region in chickens was done by serological typing of the blood involving hemagglutination assays (Briles and Briles, 1982). Although 27 haplotypes were identified, due to the absence of haplotype-specific alloantisera, this method's specificity was unreliable. Similarly, for other alloantigens, serological typing was used to identify alleles of these systems (Briles et al., 1950; Briles, 1962; Schierman and Nordskog, 1965; Medarova et al., 2003a). Immunoprecipitation was another method used to identify variation at the MHC B region. However, a requirement of highly specific antibodies and other processes rendered it impractical to use (Fulton, 2020).

DNA based methods were used to identify haplotypes/alleles of these genomic regions. Methods such as restriction fragment length polymorphisms, single-strand conformation polymorphism, sequencing, and microsatellite markers were used to identify the variability (Miller et al., 1988; Warner et al., 1989; Afanassieff et al., 2001). These approaches were reliable and faster than serological methods in identifying variation across genomic regions. However, multiple factors such as high costs, longer output time, a large amount of input material, and the development of other simpler and faster methods led to the discontinuation of these methods.
A recent advance in genotyping the MHC B region was the development of a single nucleotide polymorphism (SNP) panel (Fulton et al., 2016). SNP panels can be used to identify both serologically defined haplotypes and the new haplotypes associated with these regions. Similarly, SNP panels for alloantigen systems A, D, E, and I have been developed (unpublished data-Dr. Janet Fulton). With the development of SNP panels, detecting variations and subsequently

identifying MHC B haplotypes and alloantigen system alleles would be easier. These advancements in genomics significantly impact studying the association of genetic markers with disease resistance in chickens.

Conclusion

Coccidiosis remains a significant disease in the poultry industry, causing severe economic losses. Different approaches, such as vaccines, use of plant extracts, probiotics, and prebiotics, and chemotherapy, are being evaluated to control this disease. Another promising avenue for effectively controlling coccidiosis is the genetic basis of disease resistance. With the advancements in genomics, the genetic markers associated with resistance or susceptibility to coccidiosis can be easily identified and utilized in selective breeding programs to produce disease-resistant genotypes. Furthermore, the genetic selection for coccidial resistance enhances immunocompetence with improved vaccine efficacy and reduces the need to use chemical drugs, thereby reducing residues. Therefore, the genetic basis of coccidial resistance is a permanent solution and should be augmented with other approaches to coccidiosis management.

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Table 2.1. *Eimeria* species with their site of infections, pathogenicity, immunogenicity, and schizogony number during replication.

***Eimeria* species**	**Site of development**	**Pathogenicity***	**Immunogenicity***	**Schizogony number#**	**References#**
E. acervulina	Duodenum, Jejunum	++	++	4	(Vetterling and Doran, 1966)
E. maxima	Jejunum, Ileum	++	+++	More than 3	(Dubey and Jenkins, 2018)
E. brunetti	Lower Ileum, Rectum	+++	+++	3	(Ryley et al., 1972)
E. tenella	Ceca	+++	+	3	(McDonald and Rose, 1987)
E. necatrix	Jejunum, Ileum	+++	+	3	(McDonald and Rose, 1987)
E. mitis	Ileum	+	++	4	(Novilla et al., 1987)
E. praecox	Duodenum, Jejunum	+	++	More than 3	(Long, 1967; Gore and Long, 1982; Shirley et al., 1984; Salisch, 1990)

* + = Low; ++ = Moderate; +++ = High
Modified from: Mesa-Pineda et al. (2021)

Table 2.2. Gross lesion scoring system

***Eimeria* species**	**Score**	**Description**
E. acervulina	0	Gross lesions not present
	+1	Scattered whitish lesions oriented ladderlike on the mucosal surface, up to five lesions per centimeter square
	+2	Whitish lesions close together on both serosal and mucosal surfaces
	+3	Numerous lesions are beginning to coalesce, some thickening of the duodenal wall, excessive mucous secretion with watery contents
	+4	Coalescing lesion in anterior duodenum, grayish mucosal wall, thickened duodenal wall, creamy exudate in lumen
E. maxima	0	Gross lesions not present
	+1	On the serosal surface, presence of few petechiae, and slightly orange-colored intestinal contents
	+2	More numerous petechiae, more orange-colored intestinal contents
	+3	Ballooning and thickening of the intestinal wall, mucoid and pinpoint blood clots present in intestinal contents
	+4	Ballooned and thickened intestinal wall throughout the small intestine, bloody intestinal contents with more numerous petechiae
E. tenella	0	Gross lesions not present
	+1	Few scattered reddish or purple petechiae on the wall of ceca, normal intestinal contents
	+2	More numerous petechiae on the serosal surface, bleeding on the mucosal surface, cecal contents normal, slight thickening of cecal wall
	+3	More severe bleeding with clots and formation of the cecal core, more thickening of the cecal wall, petechiae on the serosal surface
	+4	Severe bleeding and caseous cecal cores, heavy thickening of the cecal wall, might have ruptured the cecal wall

Adapted from: Johnson & Reid (1970); Conway & McKenzie (2007); Mesa-Pineda et al. (2021).

Table 2.3. Microscopic lesion scoring system

Score	Description
0	No observed stages
+1	1-20 stages
+2	21-50 stages
+3	51-100 stages
+4	More than 100 or considered too numerous to count

Adapted from: Barrios et al. (2017).

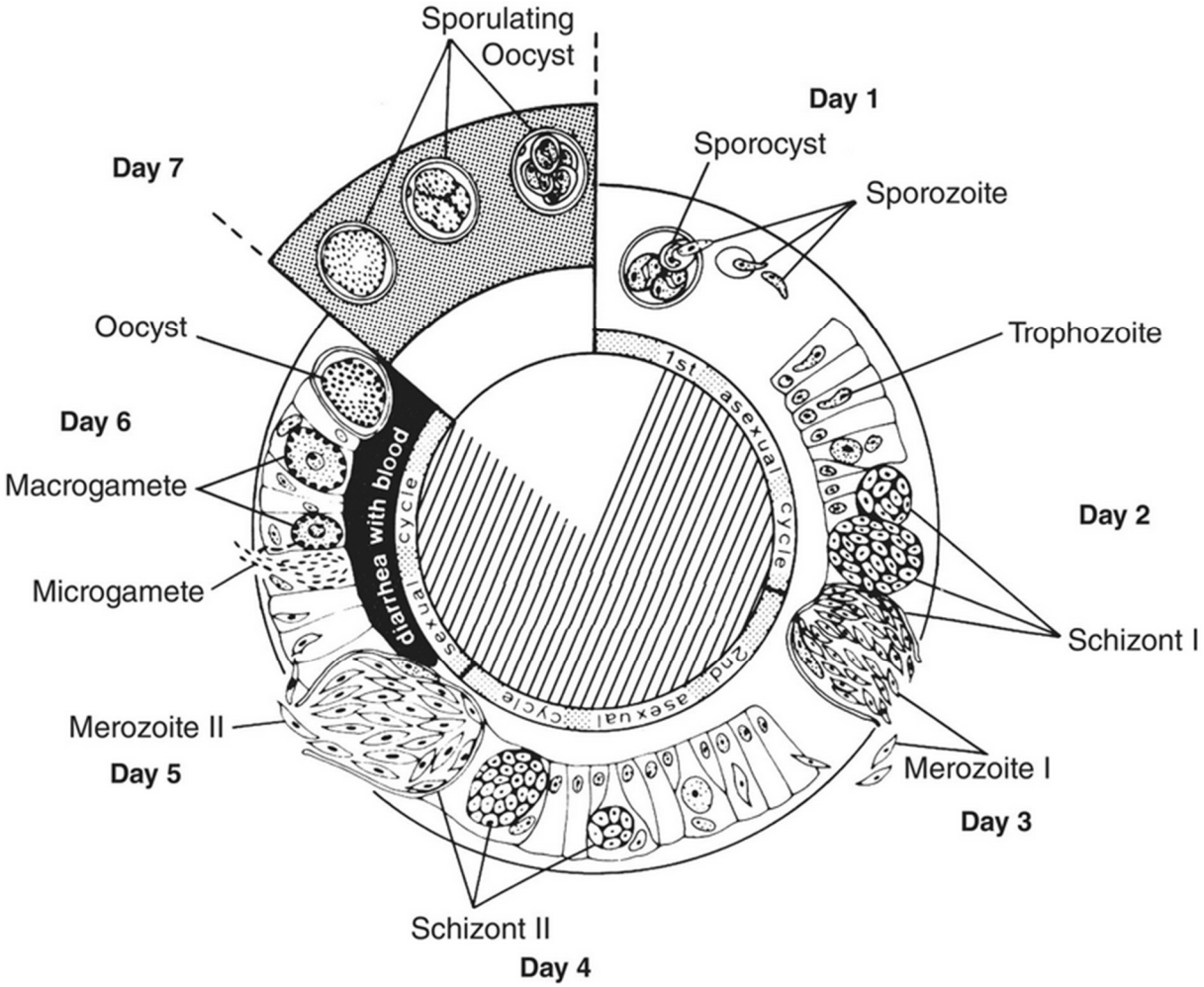

Figure 2.1. Life cycle of *Eimeria tenella* (Cervantes et al., (2020).

CHAPTER 3

CHICKEN MAJOR HISTOCOMPATIBILITY COMPLEX AND ALLOANTIGEN SYSTEMS D, E, AND I INFLUENCE RESISTANCE TO COCCIDIOSIS[1]

[1] Niraula, A., A. Wolc, J. E. Fulton, R. L. Taylor Jr. and R. A. Dalloul. To be submitted to *Journal of Animal Science and Biotechnology*.

Abstract

Major histocompatibility complex (MHC) genes, along with other blood alloantigens, are associated with the level of disease resistance in poultry, including coccidiosis, a major protozoal disease caused by *Eimeria* species. Pedigreed White Leghorn offspring segregating for the MHC B region, plus four additional alloantigen systems A (C4BPM), D (CD99), E (FCAMR), and I (RHCE) were tested for differential resistance to coccidiosis in five 26-day (d) trails (n= 235). For each trial, chicks were hatched, individually wing-tagged, and group housed until challenged. On d 19, all birds were orally inoculated with *E. acervulina* (25,000), *E. maxima* (5,000), and *E. tenella* (10,000) sporulated oocysts and allocated to individual cages. Birds and feed were weighed on d 19 and d 26 to calculate BWG, FI, and FCR. All birds were euthanized on d 26 followed by scoring for gross and microscopic coccidiosis lesions in the duodenum, jejunum, and ceca. Additionally, fecal samples were collected to assess oocyst shedding (oocysts per gram, OPG), and blood samples were obtained for DNA extraction for single nucleotide polymorphism genotyping of the five blood systems to determine the haplotypes present in each chick. Data from the five trials were combined and analyzed using a linear model, generalized linear model, and Kruskal-Wallis tests. Significant differences ($P \leq 0.05$) between genotypes were evaluated by Tukey's HSD/Steel-Dwass tests. A positive and negative association means an increase and decrease in phenotypic trait respectively, with each additional copy (0, 1 or 2) of a given haplotype. The CD99-H01 haplotype association was positive with BWG but negative with FCR. Genotype B21B21 had the highest gross lesion scores (GLS) in the jejunum establishing a positive association between MHC B21 and jejunal GLS. Further, the B12B15 genotype had a lower *E. maxima* OPG compared with the B12B21 genotype. The I system RHCE-H01 haplotype had a negative association with jejunal and cecal GLS. Duodenal GLS

was lower in FCAMR-H02/H02 compared to the FCAMR-H07/H07 genotype of the E system. Specific haplotypes of the MHC and alloantigen systems D, E, and I differed in resistance traits. Further examination of these haplotypes' effects on coccidiosis resistance in commercial lines may validate their incorporation in selection programs.

Key words: Coccidiosis, *Eimeria*, Major histocompatibility complex, Alloantigen systems, Disease resistance

Introduction

Avian coccidiosis is a protozoal disease caused by different species of the genus *Eimeria*. Of the seven species that infect chickens, *E. acervulina*, *E. maxima*, and *E. tenella* are the most prevalent in poultry production systems (Conway and Mckenzie, 2007; Attree et al., 2021). Chickens become infected with *Eimeria* by ingesting the sporulated oocysts that undergo a complex life cycle in the small intestine and ceca. This disease affects the health and productivity of chickens causing economic losses in the poultry industry estimated to exceed $13 billion annually (Blake et al., 2020).

Over the last few decades, coccidiosis has been controlled using different drugs such as ionophores (classified as antibiotics) and synthetic compounds. Due to consumer concerns about drug residues in poultry products, widespread occurrence of drug resistance, and regulatory frameworks, the global poultry industry has reduced the use of these drugs (Peek and Landman, 2011; Owusu-Doubreh et al., 2023). This scenario has shifted the focus toward research on alternative strategies for managing coccidiosis in chickens. Vaccines, plant extracts, and the genetic basis of disease resistance are ongoing research areas to manage coccidiosis. Vaccination with live or attenuated protozoans is used to control coccidiosis outbreaks (Dalloul and Lillehoj, 2006; Soutter et al., 2020). However, issues of vaccine failure leading to vaccine-induced coccidiosis have been observed in the past (Jeon et al., 2022). Another strategy is to use plant extracts in feed and/or water to mitigate the harmful effects of coccidiosis. Many researchers have investigated the protective effects of such extracts due to their antioxidant, antimicrobial, immunomodulatory, and anti-inflammatory properties (Abbas et al., 2012, 2013; Saeed and Alkheraije, 2023). The plant-based compounds have shown promising effects on birds during coccidiosis (Quiroz-Castañeda and Dantán-González, 2015; Yang et al., 2019; Pop et al., 2019).

Another promising avenue for research in controlling coccidiosis is to select for disease resistance. Identifying molecular markers associated with immunocompetence or disease resistance followed by genetic selection is a permanent, cost-effective, and environment-friendly approach to developing coccidiosis-resistant chickens (Lamont, 1998). Different breeds of chickens vary in susceptibility or resistance to coccidiosis which can be attributed to their genetics (Palafox et al., 1949; Champion, 1954; Rosenberg et al., 1954; Long, 1968; Jeffers et al., 1970; Attree et al., 2021; Du et al., 2024). The chicken lines selected for resistance and susceptibility to cecal coccidiosis showed differences in survivability suggesting genetic association to coccidial resistance (Rosenberg, 1948; Champion, 1954; Rosenberg et al., 1954). Genetic components such as the haplotypes of the major histocompatibility complex (MHC) B region and alleles of alloantigen systems including A, E, and I have been associated with resistance or susceptibility traits to coccidiosis (Miller and Taylor, 2016; Taylor et al., 2016). In the congenic lines with differences at the MHC B locus, different B haplotypes showed differences in resistance traits suggesting the genetic association to coccidiosis (Caron et al., 1997). Other studies also have shown the genetic influence of B system during coccidiosis (Clare et al., 1985; Pinard-Van Der Laan et al., 1998; Kim et al., 2008). The allelic frequencies of the closely linked A and E alloantigen systems were different among the divergent lines of chickens selected for resistance and susceptibility to *E. tenella* (Johnson and Edgar, 1984). Similarly, in the high antibody (HA) and low antibody (LA) producing chicken lines divergently selected for the antibody response to sheep red blood cells showed differences in allelic frequencies of A, D, E, and I systems (Dunnington et al., 1984; Martin et al., 1986). The HA and LA lines also have differences in response against *E. tenella* (Dunnington et al., 1992). These studies further reinforced the association of the genetic components to coccidial resistance in chickens.

Furthermore, with the advances in molecular genotyping methods, it is easier to identify the genetic markers of disease resistance. Recently, the genes encoding the A, D, E, and I blood systems in chickens have been identified as C4BPM, CD99, FCAMR, and RHCE, respectively (Fulton et al., 2022, 2023, 2024). The C4BPM gene is located in the regulator of complement activation region and assumed to play a role in complement pathways and modulate the immune response against pathogens. In humans, the FCAMR gene codes for the Fca/mR receptor, which can bind to IgA and IgM antibodies suggesting its role in antibody mediated immune response (Bournazos et al., 2009). During coccidiosis, parasite-specific IgA and IgM are produced by chickens (Yun et al., 2000) and the Fca/mR receptor might help in recognition and internalization of *Eimeria*-coated IgA and IgM complexes by immune cells for their clearance from body. In humans CD99 gene plays important role in peripheral immune responses by involving T cell differentiation and recruiting leukocytes to inflammation sites (Bernard et al., 1995, 1997; Dufour et al., 2008). However, the role of the CD99 gene is not fully understood in chickens. The RHCE gene is supposed to have role in transportation of CO_2 in humans; however, the exact mechanism in chickens is unknown. Therefore, the investigations on the role of the MHC B and A, D, E, and I alloantigen systems in disease resistance against coccidiosis could provide valuable information on genetic basis of resistance to this disease.

Previous studies mostly focused on infection with a single *Eimeria* species at a time (Zhu et al., 2003; Bacciu et al., 2014) as opposed to several *Eimeria* spp. being involved in causing coccidiosis under field conditions. Furthermore, the development of single nucleotide polymorphism (SNP) panels has facilitated the identification of MHC B and (A, D, E, and I) alloantigen systems haplotypes (Fulton et al., 2016, 2022, 2023, 2024). These findings have eased assessment of how these genetic markers influence the resistance traits in chickens.

Therefore, the objective of our study was to examine the association of specific haplotypes of the MHC B and other alloantigen systems A, D, E, and I with the resistance traits during mixed *Eimeria* (*E. acervulina*, *E. maxima*, and *E. tenella*) challenge in White Leghorn (WL) chickens.

Materials and Methods

Breeding scheme

The mating scheme and the genetic information of sires and dams are given in Table 3.1; WL chickens that originated from a commercial source were used. All birds were typed for five alloantigen systems A, B, D, E, and I. Four single male matings using four dams per sire were used. In trials 4 and 5, the mating 4 sire was replaced with a sire having same genetic background except at the MHC B and I locus but the dams remained the same.

Animal husbandry

Five 26-day (d) studies were conducted using the pedigreed WL offspring chicks. For each study, fertile eggs obtained from West Virginia University were incubated for 18 days [37.5°C, 50-55% relative humidity (RH)], then transferred to a hatcher where they were kept for 3 more days (37.0°C and 65-70% RH) to hatch. The hatchability across the five trials ranged from 59.5-89.5 %. Upon hatch, chicks were individually wing-tagged and assigned to cages according to their pedigree. Water and a commercial-based starter diet free of anticoccidial agents in crumbled form were provided ad libitum throughout the trials. All animal handling procedures were performed per the guidelines and approval of the Institutional Animal Care and Use Committee (IACUC) of the University of Georgia.

Eimeria cocktail preparation and inoculation

Sporulated oocysts of *E. acervulina*, *E. tenella* (both field isolates), and *E. maxima* MX8 strain (provided by Dr. Lorraine Fuller, UGA Poultry Science) were combined to produce the required inoculation dose. On d 19, birds were moved to individual cages and all the birds were orally gavaged with 1 mL inoculum/bird containing the sporulated oocysts of *E. acervulina* (25,000), *E. maxima* (5,000), and *E. tenella* (10,000).

Genotyping

On d 26, whole blood samples were collected from the wing veins and approximately 10 µL were transferred to Whatman FTA Elute cards (GE Healthcare Bio-Sciences Corp., Piscataway, NJ). The cards were allowed to dry at room temperature and shipped for genotyping by a SNP panel. The numbers of birds genotyped in each of the five trials are shown in Table 3.2.

Phenotype assessment

All phenotype measurements were conducted in a blinded manner, with genotype information of the individual chicks unknown at the time of assessment, as genotyping was done after completion of experiment.

Growth performance

Birds were monitored and mortality recorded at least twice daily. On d 19 and d 26, birds and feed were weighed to calculate body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR).

Gross lesion scores (GLS)

On d 26, birds were humanely euthanized and coccidiosis GLS from the duodenum, jejunum/ileum, and ceca were assessed to evaluate the pathology caused by *E. acervulina*, *E. maxima*, and *E. tenella*, respectively. Lesion scoring followed the Johnson and Reid (1970)

protocol in which scores from 0 to +4 represent different ranges of gross lesions specific to each species. Typically, a score of 0 indicates no gross lesions present; a score of +1 signifies a few scattered lesions; a score of +2 indicates a larger number of discrete lesions involving a greater area of the affected intestinal zone and significant bleeding in the case of *E. tenella*; a score of +3 denotes extensively developed lesions with some thickening of the intestinal or cecal walls; and a score of +4 represents extensive coalescence of lesions, with marked wall thickening, bloody intestinal contents in the case of *E. maxima*, and large cecal cores in the case of *E. tenella*.

Microscopic lesion scores (MLS)

On d 26, mucosal scrapings were collected from the duodenum, jejunum/ileum, and ceca of all the birds to assess the MLS. Scrapings were wet-mounted onto glass slides, covered with coverslips, and observed under a light microscope as per Goodwin et al. (1998). *E. acervulina*, *E. maxima*, and *E. tenella* developmental stages were included to assess MLS in the duodenum, jejunum/ileum, and ceca, respectively. Briefly, a score of 0 indicated no observed stages; a score of +1 represented 1–20 stages per 10X objective field; a score of +2 denoted 21–50 stages; a score of +3 indicated 51–100 stages; and a score of +4 signified stages that were too numerous to count. Observations were made across approximately 10 fields of vision per sample.

Oocyst shedding

To assess oocyst shedding by individual birds, droppings were collected over a 3-day period (d 23-26) from all the birds. Oocysts per gram (OPG) of fecal droppings, often used to quantify the infection level of *Eimeria* spp., was assessed using the standard McMaster floating technique (Haug et al., 2008). Briefly, fecal samples were suspended overnight in tap water, vortexed, filtered through cheesecloth, centrifuged to discard supernatant, suspended in saturated salt

water, and observed under the 10X objective. McMaster slides were scanned to identify the number of oocysts of *E. acervulina*, *E. maxima*, and *E. tenella*.

Statistical analyses

The data obtained from the individual study were combined and analyzed including both genotypes and trials in the model. For the growth performance and log-transformed OPG data, a linear model and one-way ANOVA were used, and differences across genotypes of each system were evaluated using Tukey's HSD test. Gross lesion and microscopic lesion scores data were analyzed using a generalized linear model with a Poisson distribution and Kruskal-Wallis test. Significant differences across genotypes of each system for GLS and MLS were evaluated using the Steel-Dwass test. The results were considered statistically significant at $P \leq 0.05$.

Results

Genotyping

Birds used for the study segregated for consistent haplotypes within each system across all studies. The haplotypes segregating in progenies were: A) MHC B system: B12, B15, and B21; A system: C4BPM-H02 and C4BPM-H08; D system: CD99-H01 and CD99-H03; E system: FCAMR-H02 and FCAMR-H07; and I system: RHCE-H01, RHCE-H02, and RHCE-H03.

Genotype-phenotype association

Throughout the results and discussion sections, results will be explained with two terms: positive and negative associations. Positive association refers to the quantitative increase in the observed phenotypic traits with each unit increase in the haplotype in the genotype of the birds. A negative association refers to the quantitative decrease in the measured phenotypic trait with each unit increase in the haplotype in the genotype of birds. The effects of the haplotypes of the MHC, A,

D, E and I alloantigen systems on phenotypic traits (a) growth performance & OPG and (b) GLS & MLS are given in Table 3 and Table 4 respectively.

Growth performance

MHC B. During the challenge period (d 19-26), the B21 haplotype of the MHC B system had a significant negative association with FCR. With one unit increase in the B21 in the genotype of birds, FCR decreased by 0.34. However, there was no difference in FCR among different MHC genotypes.

D alloantigen system. The CD99-H01 showed positive and negative associations with BWG and FCR during the challenge period. BWG increased by 4.166 g and FCR decreased by 0.37 when there was a unit increase in the CD99-H01 in the genotype of birds. The H01/H01 genotype had significantly higher BWG than the H01/H03 and H03/H03 genotypes (Figure 1). Similarly, the CD99-H01/H01 genotype had significantly higher FI compared to CD99-H03/H03 genotype. However, FCR was not significantly different across the genotypes of the D system.

I alloantigen system. There was a significant positive association of the RHCE-H01 and a negative association of the RHCE-H02 with FI during the challenge period. A unit increase in RHCE-H01 and RHCE-H02 in the genotype of birds resulted in a 6.44 g increase and a 9.39 g decrease in FI, respectively. However, there were no differences in FI between the genotypes of the I alloantigen system.

Gross lesion scores (GLS)

MHC B. Jejunal and cecal lesion scores were positively associated with the B21 haplotype of the MHC B system ($P \leq 0.05$). One unit increase in B21 in the genotype of chickens, jejunal, and cecal gross lesions increased by 0.39 and 0.23, respectively. The B15B15 genotype had

significantly reduced lesion severity [Lesion Score (L.S.) = 0.34], whereas the B21B21 genotype is linked to increased lesion severity (L.S. = 0.74) in the jejunum (Figure 2). The difference across genotypes was not observed regarding gross lesions in the ceca.

D alloantigen system. The CD99-H01 was positively associated with the gross lesions in the jejunum. With one unit increase in the CD99-H01 in the genotype of the bird, the jejunal GLS increased by 0.32 units. However, the differences across genotypes of the D system were not observed.

E alloantigen system. Although there was no association of specific haplotype to the GLS, the birds with FCAMR-H02/H02 genotype showed significantly lower GLS (L.S.=1.33) compared to FCAMR-H07/H07 genotype (L.S.=1.78) in the duodenum (Figure 3).

I alloantigen system. The RHCE-H01 reduced lesion severity across various sections of the gastrointestinal tract. A unit increase in RHCE-H01 in the genotype of birds led to a decrease of 0.39 and 0.25 units in jejunal and cecal GLS, respectively. In contrast, the RHCE-H03 was associated with increased lesion severity, resulting in a 0.30 unit increase in cecal GLS. In the jejunum, the RHCE-H01/H01 genotype exhibited significantly lower GLS compared to the RHCE-H02/H03 genotype, indicating that the homozygous RHCE-H01 led to a decrease in lesion severity (Figure 4). The RHCE-H01/H01 genotype had the lowest GLS (L.S. = 0.24), whereas the RHCE-H02/H03 had the highest GLS (L.S. = 1.00). The RHCE-H01/H01 genotype showed significantly lower cecal GLS (L.S.= 0.56) than the RHCE-H01/H03 genotype (L.S.=1.17).

Microscopic lesion scores (MLS)

There was no significant association between the haplotypes of alloantigen systems with the MLS in the duodenum, jejunum, and ceca.

Oocyst shedding (OPG)

MHC B. There was a significant difference between genotypes of the MHC B system in terms of E. maxima OPG (Figure 5). The B12B15 genotype had significantly lower OPG (Log OPG= 4.42) compared to the B21B21 genotype (Log OPG=4.78). The B12 haplotype when present with B21 significantly increased oocyst shedding compared to the combination of B12 and B15.

Discussion

Genetic components for disease resistance to coccidiosis have been investigated in the past. Those studies helped to build a foundation for understanding the genetic basis of disease resistance, especially identifying the genomic regions associated with coccidial resistance (Johnson and Edgar, 1984; Clare et al., 1985, 1989). However, these genomic regions need more investigation to pinpoint the exact variants (haplotypes) contributing to the host resistance during chicken coccidiosis. Furthermore, there are no published reports about the effects of the MHC B and other alloantigen systems A, D, E, and I during the mixed *Eimeria* infection in chickens. Therefore, the objective of this study was to assess the association between genetic components and resistance/susceptibility-related phenotypic traits in WL chickens during mixed *Eimeria* spp. infection. The pedigreed offspring of WL were selected for this study because, through genetic selection, these chickens consisted of limited genetic variation at the MHC and the A, D, E, and I alloantigen system locus with the same background genome. This was particularly important in identifying the association of haplotypes of these genomic regions with phenotypic traits such as BWG, FI, FCR, GLS, MLS, and OPG.

Growth performance parameters such as BWG, FI, and FCR are significant indicators during coccidiosis in field conditions. Coccidial infections cause poor gastrointestinal health,

leading to reduced growth in chickens. MHC B haplotype-based genetic association studies have been conducted extensively in chickens (Dunnington et al., 1992; Abplanalp et al., 1992; Schou et al., 2010). In our study, the MHC B21 was associated positively with BWG; however, differences across genotypes were not observed. These results align with a previous study where chickens with different MHC B genotypes challenged with *E. tenella* showed similar relative BWG (Dunnington et al., 1992). Another study showed that chickens with B15B15 genotype had the lowest weight gain during *E. tenella* challenge (Caron et al., 1997). However, this was not the case in our study, which included inoculation with three *Eimeria* spp., which might have influenced the outcome for BWG. According to our study, CD99-H01 showed a positive association with BWG, positively impacting growth performance by increasing BWG. Additionally, the CD99-H01/H01 genotype showed an increased FI compared to CD99-H01/H03 genotype. Thus, the CD99-H01 positively influenced these production traits when present in homozygous condition. However, until now, the effect of the alloantigen D system on the production traits of chickens has yet to be documented.

Gross lesions in intestinal segments caused by *Eimeria* parasites are used to assess disease severity. *Eimeria* spp. are site-specific, each species infecting a particular segment of the intestine. In our study, the MHC B21 haplotype showed a positive association with lesions in the jejunum, infection site of *E. maxima*, with the B21B21 and B15B15 genotypes having the highest and lowest GLS, respectively. Although there were no significant differences between different MHC genotypes in terms of lesions in the duodenum and ceca, the B21B21 genotype exhibited numerically highest cecal lesions (L. S.=0.87). In agreement with these results, a previous study documented that chickens with B21B21 had higher cecal lesions than the B15B21 and B15B15 genotypes (Pinard-Van Der Laan et al., 1998). This suggested that if the birds had at

least one copy of B15 in their genotype, the lesions in the ceca decreased during infection with *E. tenella*. However, cecal lesions were not different across the chickens homozygous for B15 and B21 (Caron et al., 1997). Another study showed that birds with B2 haplotypes had significantly higher lesions in the duodenum and ceca during lighter infections than birds with B15 haplotypes(Ruff and Bacon, 1989). However, the results from this experiment were inconsistent across different *E. acervulina* and *E. tenella* dosages used for infection.

For the D alloantigen system, the CD99-H01 showed a positive association with GLS in jejunum. This association with GLS in jejunum correlates with the increased performance in birds with CD99-H01 haplotype, which might be due to reduced pathology in jejunum supporting the better absorption of nutrients. Similarly, the I alloantigen system also affected the gross lesions across the intestinal segments, with RHCE-H01 and RHCE-H03 showing negative and positive associations with GLS, respectively. Whenever the RHCE-H01 was present in homozygous condition, the gross lesions in the jejunum and ceca were decreased. Surprisingly, this effect of RHCE-H01 was masked during heterozygosity. The combination of RHCE-H02 and RHCE-H03 in the genotype produced the highest lesions across different segments. Previously, Martin et al. (1986) investigated the I alloantigen system during cecal coccidiosis and reported that the birds with the I4 allele in their genotype had a significant reduction in cecal gross lesions, thus showing the association with the I system.

OPG is a quantitative method to assess the protozoal load in the intestine of chickens infected with *Eimeria* spp. In the present study, the B12B15 genotype of the MHC B region showed significantly lower *E. maxima* oocyst shedding compared to the B12B21 genotype. As both groups had B12 in their genotype suggesting that the B21 might be a contributing haplotype to the production of higher number of oocysts resulting in susceptibility to *E. maxima*. This is

also supported by higher jejunal GLS in birds with B21B21 genotype in the present study. Previously, after experimental infection of Fayoumi congenic lines with *E. maxima*, the oocyst output was lower in the M5.1 compared to M15.2 chickens (Kim et al., 2008). The M5.1 and M15.2 lines have B43 and B44 haplotypes respectively. However, in our study only B12, B15 and B21 haplotypes of the MHC region were available, making the comparison difficult. However, another study showed that birds with the same MHC background differed in oocyst output after challenge with *E. tenella* suggesting that non-MHC genes may play a role in resistance to coccidiosis (Lillehoj et al., 1989). Further research would help us better understand the role of these MHC haplotypes in terms of oocyst production after challenge with mixed *Eimeria* species.

In conclusion, haplotype-based association with resistance traits during mixed *Eimeria* infection showed that the MHC and alloantigen systems D, E and I haplotypes have associations with the production (BWG, FI, FCR) and/or intestinal health (GLS, OPG) related traits. The results suggest that the selection for the haplotypes of these genomic region is possible to enhance disease resistance to coccidiosis in chickens. The specific haplotypes associated with the resistance traits could be used in breeding strategies to produce coccidiosis-resistant chickens. However, these haplotypes might have different effects during coccidiosis in commercial chickens, so further association studies should be conducted in commercial lines before employing breeding programs.

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Table 3.1. Genotypes for sires and dams mated to produce pedigree progeny segregating for five alloantigen systems.

Mating	Sire/Dam	Alloantigen System Genotype[3]				
		B (MHC)	**A (C4BPM)**	**E (FCAMR)**	**D (CD99)**	**I (RHCE)**
Mating 1	Sire 1	B12/B15	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H07	CD99-H01/CD99-H03	RHCE-H01/RHCE-H01
	Dam 1	B12/B21	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H02	CD99-H01/CD99-H01	RHCE-H01/RHCE-H01
	Dam 2	B12/B21	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H07	CD99-H01/CD99-H03	RHCE-H01/RHCE-H01
	Dam 3	B15/B21	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H02	CD99-H01/CD99-H01	RHCE-H01/RHCE-H03
	Dam 4	B21/B21	C4BPM-H08/C4BPM-H08	FCAMR-H02/FCAMR-H07	CD99-H01/CD99-H03	RHCE-H01/RHCE-H03
Mating 2	Sire 2	B15/B21	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H02	CD99-H01/CD99-H01	RHCE-H01/RHCE-H02
	Dam 5	B15/B21	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H07	CD99-H01/CD99-H03	RHCE-H01/RHCE-H03
	Dam 6	B15/B21	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H02	CD99-H01/CD99-H01	RHCE-H01/RHCE-H03
	Dam 7	B15/B21	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H02	CD99-H01/CD99-H01	RHCE-H01/RHCE-H01
	Dam 8	B21/B21	C4BPM-H08/C4BPM-H08	FCAMR-H07/FCAMR-H07	CD99-H01/CD99-H01	RHCE-H01/RHCE-H02
Mating 3	Sire 3	B12/B15	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H02	CD99-H01/CD99-H03	RHCE-H01/RHCE-H01
	Dam 9	B12/B15	C4BPM-H02/C4BPM-H02	FCAMR-H02/FCAMR-H02	CD99-H03/CD99-H03	RHCE-H01/RHCE-H02
	Dam 10	B12/B21	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H07	CD99-H03/CD99-H03	RHCE-H01/RHCE-H03
	Dam11	B15/B15	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H02	CD99-H01/CD99-H03	RHCE-H01/RHCE-H01

	Dam 12	B15/B21	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H02	CD99-H01/CD99-H03	RHCE-H01/RHCE-H01
Mating 4	Sire 4[1]	B12/B21	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H02	CD99-H01/CD99-H01	RHCE-H01/RHCE-H03
	Sire 5[2]	B15/B21	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H02	CD99-H01/CD99-H03	RHCE-H01/RHCE-H02
	Dam 13	B12/B15	C4BPM-H02/C4BPM-H02	FCAMR-H02/FCAMR-H02	CD99-H01/CD99-H03	RHCE-H01/RHCE-H01
	Dam 14	B12/B21	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H07	CD99-H01/CD99-H03	RHCE-H01/RHCE-H01
	Dam 15	B15/B21	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H02	CD99-H01/CD99-H01	RHCE-H01/RHCE-H02
	Dam 16	B15/B21	C4BPM-H02/C4BPM-H08	FCAMR-H02/FCAMR-H02	CD99-H01/CD99-H01	RHCE-H01/RHCE-H01

[1] Trials 1-3
[2] Trials 4-5
[3] Genotype across the genomic region of the MHC B, A, E, D, and I alloantigen systems.

Table 3.2. Number of birds genotyped in each of the five trials.

Study #	Number of chicks genotyped
1	61
2	44
3	18
4	56
5	56
Total	**235**

Table 3.3. Effects of haplotypes of the MHC B, A, E, D, and I alloantigen systems on BWG, FI, FCR and OPG during a mixed *Eimeria* infection.

	Estimate table					
Alloantigen haplotype[1]	BWG	FI	FCR	OPG[2]		
				E. acervulina	*E. maxima*	*E. tenella*
B12	0.31	-1.918	0.346	0.017	0.042	0.046
B15	-2.22	-2.531	0.093	-0.013	-0.074	0.015
B21	2.376	4.09	-0.33*	0.004	0.057	-0.049
C4BPM-H02	-0.289	6.935	0.097	0.010	0.040	0.024
C4BPM-H08	0.289	-6.935	-0.097	-0.010	-0.040	-0.024
FCAMR-H02	-0.39	3.4	0.013	0.015	0.023	0.108
FCAMR-H07	0.39	-3.4	-0.013	-0.015	-0.023	-0.108
CD99-H01	4.166**	9.345	-0.387*	0.001	0.0024	-0.019
CD99-H03	-4.166**	-9.345	0.387*	-0.001	-0.0024	-0.019
RHCE-H01	1.395	9.402	-0.020	0.004	-0.035	-0.018
RHCE-H02	-2.3095	-12.784*	-0.041	0.024	-0.053	0.029
RHCE-H03	0.154	-1.176	0.066	-0.025	0.092	-0.001

[1] Haplotypes across different alloantigen systems
[2] Each column shows OPG for *E. acervulina*, *E. maxima*, and *E. tenella*
*Significant at $P \leq 0.05$
**Significant at $P \leq 0.01$

Table 3.4. Effects of haplotypes of the MHC B, A, E, D, and I alloantigen systems on GLS and MLS during a mixed *Eimeria* infection.

	Estimate table					
	GLS[2]			MLS[3]		
Alloantigen haplotype[1]	Duodenum	Jejunum	Ceca	Duodenum	Jejunum	Ceca
B12	0.006	-0.359	-0.057	0.040	-0.040	-0.017
B15	-0.02	-0.179	-0.178	-0.041	0.035	0.014
B21	0.02	0.392**	0.229*	0.020	-0.013	-0.004
C4BPM-H02	-0.05	-0.042	0.102	-0.085	-0.042	-0.006
C4BPM-H08	0.05	0.042	-0.102	0.085	0.042	0.006
FCAMR-H02	-0.11	0.108	-0.143	-0.055	0.005	-0.007
FCAMR-H07	0.11	-0.108	0.143	0.055	-0.005	0.007
CD99-H01	0.018	0.320*	0.019	-0.050	-0.039	-0.00001
CD99-H03	-0.018	-0.320*	-0.019	0.050	0.039	0.00001
RHCE-H01	-0.080	-0.397*	-0.251*	-0.032	-0.015	-0.010
RHCE-H02	-0.022	0.313	0.004	0.064	0.030	0.006
RHCE-H03	0.113	0.200	0.304*	-0.005	-0.004	0.007

[1] Haplotypes across different alloantigen systems
[2] Each column shows GLS in the duodenum, jejunum, and ceca. GLS: Gross lesion scores
[3] Each column shows MLS in the duodenum, jejunum, and ceca. MLS: Microscopic lesion scores
*Significant at $P \leq 0.05$
**Significant at $P \leq 0.01$

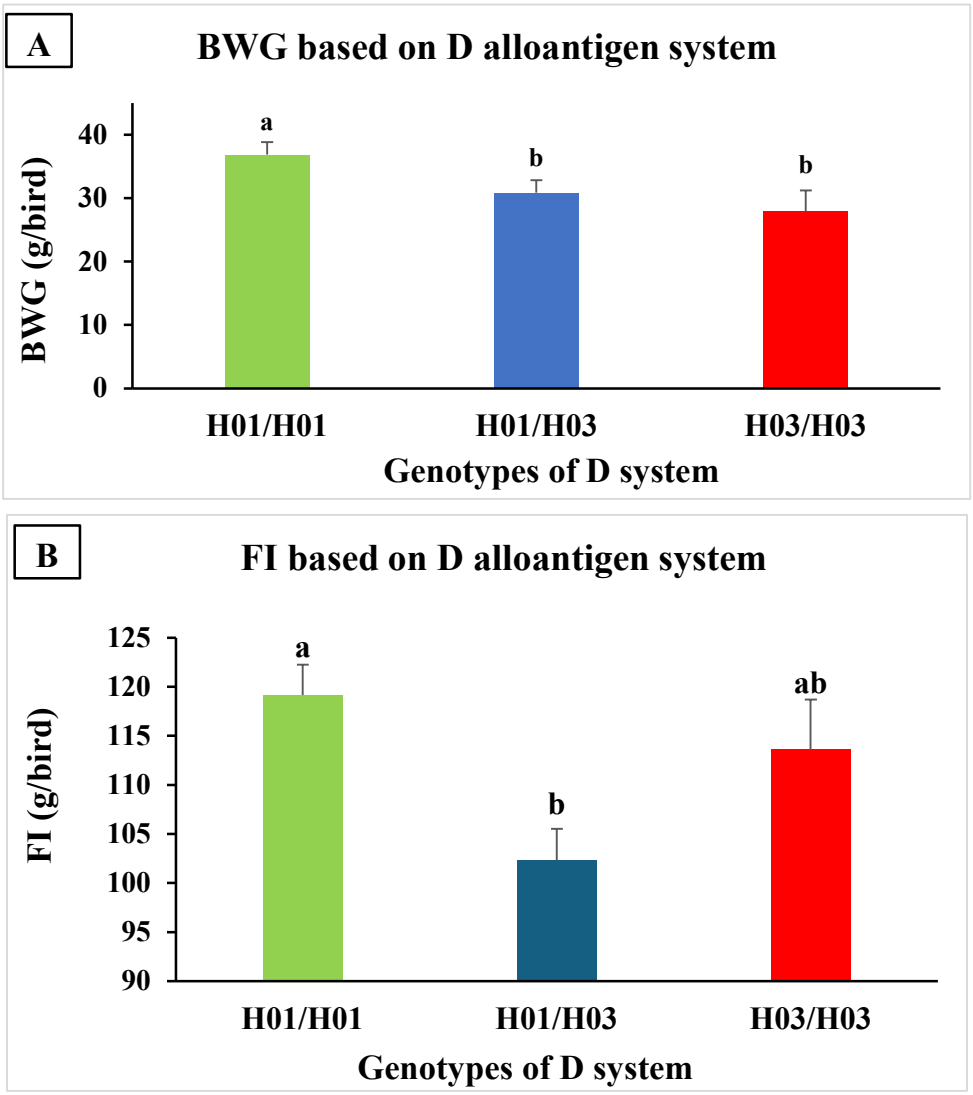

Figure 3.1. Effects of different genotypes of the D alloantigen system on post-challenge BWG and FCR during mixed *Eimeria* infection. Data represent the (A) BWG and (B) FI of the birds with the H01/H01, H01/H03, and H03/H03 genotypes. Each bar represents the mean value of BWG ± SEM and FI ± SEM. Bars without a common letter differ significantly.

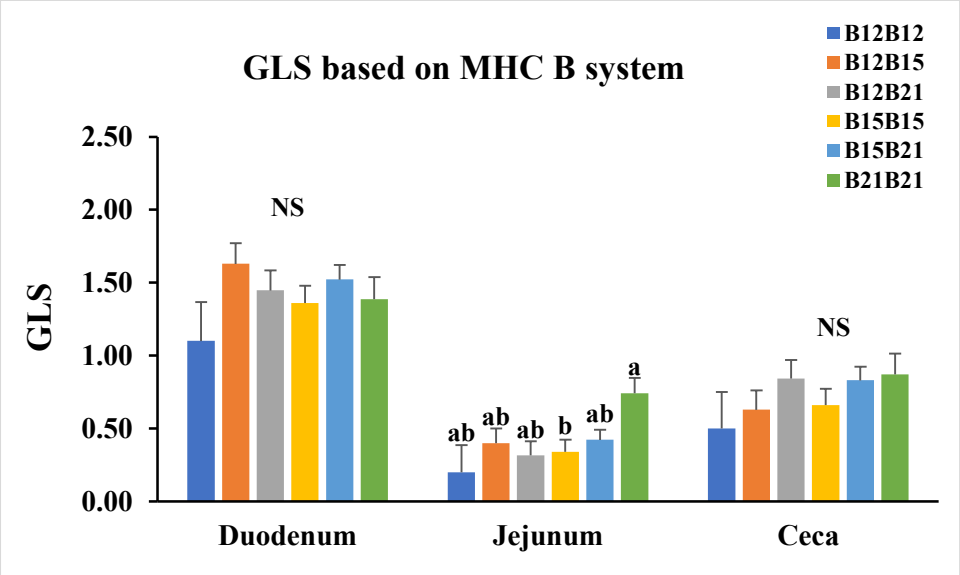

Figure 3.2. Effects of different genotypes of the MHC B system on post-challenge GLS during mixed *Eimeria* infection. Data represent the GLS in the duodenum, jejunum, and ceca of the birds with the B12B12, B12B15, B12B21, B15B15, B15B21, and B21B21 genotypes. Each bar represents the mean value of GLS ± SEM. Bars without a common letter differ significantly. NS=Not significant.

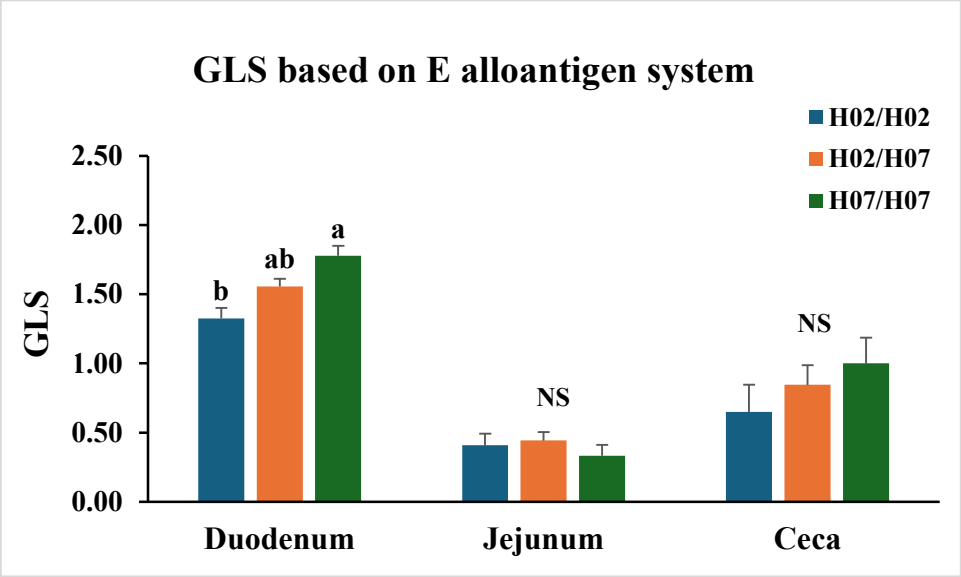

Figure 3.3. Effects of different genotypes of the E alloantigen system on post-challenge GLS during mixed *Eimeria* infection. Data represent the GLS in the duodenum, jejunum, and ceca of the birds with the H02/H02, H02/H07, and H07/H07 genotypes. Each bar represents the mean value of GLS ± SEM. Bars without a common letter differ significantly. NS=Not significant.

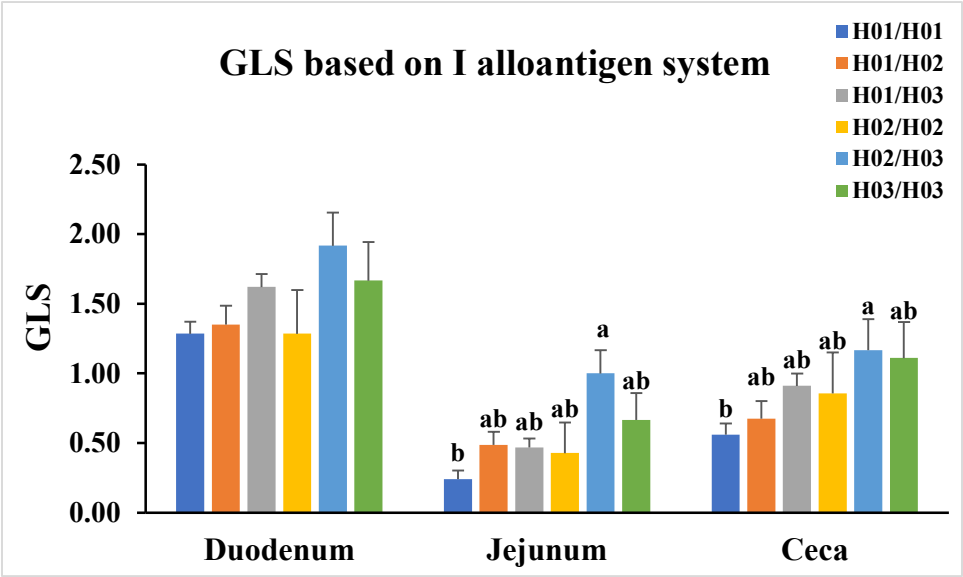

Figure 3.4. Effects of different genotypes of the I alloantigen system on post-challenge GLS during mixed *Eimeria* infection. Data represent the GLS in the duodenum, jejunum, and ceca of the birds with the H01/H01, H01/H02, H01/H03, H02/H02, H02/H03, and H03/H03 genotypes. Each bar represents the mean value of GLS ± SEM. Bars without a common letter differ significantly. NS=Not significant.

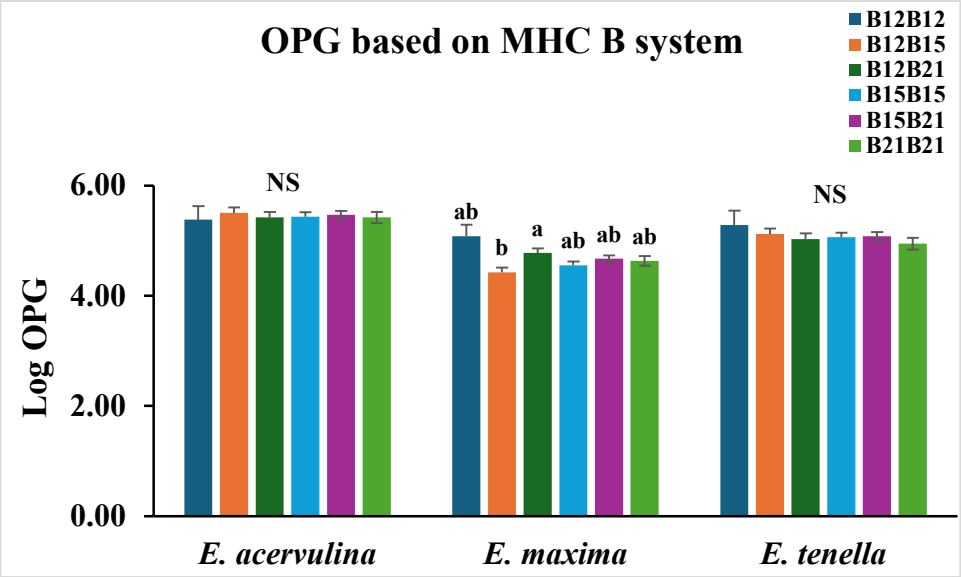

Figure 3.5. Effects of different genotypes of the MHC B system on post-challenge OPG during mixed *Eimeria* infection. Data represent the GLS in the duodenum, jejunum, and ceca of the birds with the B12B12, B12B15, B12B21, B15B15, B15B21, and B21B21 genotypes. Each bar represents the mean value of OPG ± SEM. Bars without a common letter differ significantly. NS=Not significant.

CHAPTER 4

CONCLUSION

Coccidiosis is an enteric disease caused by different species of *Eimeria*, which causes severe economic losses to the poultry industry worldwide. Due to the increasing public concerns regarding using chemical drugs to control coccidiosis, the poultry industry is focusing on research on alternative strategies to manage coccidiosis. The host genetic factors modulate the resistance to coccidiosis in chickens. Therefore, research on genetic factors conferring resistance to coccidiosis is a must for sustainable poultry production.

The research of this thesis provides further insights into the role of specific haplotypes of the MHC B and other alloantigen systems (A, D, E, and I) in chicken resistance to mixed *Eimeria* infection. The pedigreed offspring of White Leghorn chickens, which have the same background genome and only differ at the MHC B and A, D, E, and I alloantigen systems, were utilized to investigate the association of haplotypes of these systems to resistance traits. The traits evaluated include growth performance parameters (BWG, FI, and FCR), intestinal pathology indicators (gross and microscopic scores), and oocysts shed (OPG). The finding suggested that specific haplotypes of these genomic regions have associations with the resistance or susceptibility parameters during mixed *Eimeria* infection. Therefore, the effects of identified haplotypes on coccidiosis resistance should be investigated in commercial lines. Upon further validation of these genetic markers, they could potentially be utilized in selective breeding programs to produce chickens resistant to coccidiosis.