ABSTRACT

IBIWOYE, DEMILADE ISRAEL. Development of Vaccination Strategies against Turkey Coccidiosis: Molecular and Immunological Insights (Under the direction of Dr. Lisa Bielke).

Turkey production faces significant economic and health problem from coccidiosis, which is caused by *Eimeria* spp., and requires novel control methods because of the constraints of conventional anticoccidial medications and vaccinations, including resistance and regulatory restrictions. Using pharmacological, immunological, and nutritional interventions, this dissertation examines different methods for managing mixed *Eimeria* infections in turkey poults.

500 tom poults were divided into five groups for Experiment 1, study 1, which assessed the effects of low and high dosage vaccination and amprolium supplementation on oocysts per gram (OPG): non-vaccinated, non-inoculated (NVNI), low dose (L), LA (lose dose with amprolium), H (high dose), and HA (high dose with amprolium). On d1, 40% of poults in the L & LA group were vaccinated with 100 oocysts/ml of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis,* while 70% of poults in the H & HA group were vaccinated with 500 oocysts/ml of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis*. There were significant differences ($p < 0.05$) in OPG on all days, except days 21 and 27. In Experiment 1, study 2, 345 tom poults were divided into three groups: non-vaccinated, non-inoculated (NV), vaccinated (V), vaccinated and supplemented with amprolium (VA), from d8-12. V and VA group were vaccinated with 100 oocysts/mL of mixed *E. adenoiedes, E. gallopavonis*, and *E. meleagrimitis* on d1 and challenged with 25,000 oocysts/mL of the mixed *Eimeria* species on d21. Results showed no significant differences ($P>0.05$) in BW between treatment groups. Fecal oocyst shedding was significantly reduced in the treatment groups, with the VA group showing the lowest oocyst count ($P < 0.05$) on d10, d14, d18, d26, and d28. On d28, scores for duodenal and ceca lesions revealed less damage in the VA group. The VA group showed the greatest immune regulation, according to

immunological testing, with higher numbers of γδ T-cells ($P<0.05$), $CD4^+$ ($P<0.05$), and $CD8^+$ ($P>0.05$) and enhanced CD40 expression ($P=0.043$) in the cecal tonsils. 280 male Nicholas turkey poults were used in Experiment 2 to evaluate the effects of dietary dried egg products (DEPs) in seven different treatments, including non-challenged (NC), challenged controls (CC), and different concentrations of turkey (DEPt) and chicken-derived (DEPc) DEPs. On d14, all pouts, aside from NC received 16,000 oocysts/mL of mixed *Eimeria*. BWG and FCR were numerically improved ($P>0.05$) by high-dose DEPt (300), and there was no mortality in this group. DEPt downregulated ($P=0.02$) IL-10 and upregulated ($p=0.006$) IL-18 expression in the cecal tonsil and jejunum, and it decreased ($P<0.05$) the output of fecal oocysts on several post-infection days. There was no difference ($P>0.05$) in the subpopulations of T-cells. In Experiment 3, 104 Nicholas poults was randomly allotted to two treatment groups, Control and Challenge with the Challenge group inoculated with 25,000 oocysts/mL of mixed *Eimeria* species on d4 and had their immune gene expression evaluated. The expression of IL-8, IL-10, IL-18, IFN-γ, IL-1β, LITAF, and HSP70 was examined in the tissues of the ceca, cecal tonsils, liver, and spleen from 4dpi to 15dpi. The challenge group had significantly reduced BW ($P<0.001$), higher oocyst shedding ($p=0.03$), and higher mortality ($P=0.04$) compared to controls. Peak infection periods were associated with elevated levels ($P<0.05$) of pro-inflammatory cytokines, IFN-γ and IL-1β, in the ceca and cecal tonsils. The infected poults' cecal tonsil showed an increase ($P>0.05$) in IL-10 at 15 dpi, but their spleen showed a significant drop ($P<0.05$) in IL-8. Various tissue-specific expressions of HSP70 and LITAF indicated systemic and mucosal inflammatory and stress responses. Overall, these studies show that combining DEPs, amprolium, and targeted vaccination can lower parasite load, improve mucosal immunity, and maintain performance, providing long-term options for managing coccidiosis in turkeys.

Development of Vaccination Strategies against Turkey Coccidiosis: Molecular and Immunological Insights

by
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A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Animal Science and Poultry Science

Raleigh, North Carolina
2025

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DEDICATION

To God, my refuge, my strength, and the unfailing light who guided me through every storm. Without Your grace, I would never have made it. To my beloved parents, whose sacrifices, love, and unwavering belief built the foundation of this achievement. To those I lost along the way, you are not here to witness this moment, but your memory lives on in my heart and in every milestone I reach. And to every yearning African child: dream on, your dreams are valid, your voice matters, and no dream is beyond your reach.

BIOGRAPHY

Demilade Israel Ibiwoye was born and raised in Ilorin, Kwara State, Nigeria, with his two other siblings. Demi is a researcher with a deep passion for poultry science and avian health. His curiosity for animal life and agricultural development was sparked early in life and has since shaped a journey of relentless academic pursuit and discovery.

He earned a B. Agric in Animal Production and a Master of Science in Animal Production from the University of Ilorin, Nigeria, graduating with distinction. These formative years cemented his fascination with poultry health and performance, particularly under tropical conditions. He began his PhD in Animal Science at The Ohio State University before transferring to North Carolina State University, where he is currently completing his doctoral work in Poultry Health and Disease.

His dissertation, titled "Development of Vaccination Strategies Against Turkey Coccidiosis: Molecular and Immunological Insights", explores innovative approaches to controlling enteric diseases in turkeys, with broader implications for food security, animal welfare, and sustainable poultry production. Through extensive laboratory and fieldwork, including molecular diagnostics and immunological assays, he aims to contribute to the development of practical, science-based solutions in poultry enteric health.

Throughout his academic and professional journey, he has been inspired by mentors whose dedication to poultry research and mentorship has deeply influenced his path. Like many, his journey has not been without challenges, as he has navigated cross-continental education systems, transferred programs, and balanced research with service and teaching. Yet, these hurdles have refined his resilience and fueled his passion for impactful research.

ACKNOWLEDGMENTS

First and foremost, I express my deepest gratitude to Dr. Lisa Bielke, my advisor, for her unwavering mentorship, encouragement, and belief in me throughout this PhD journey. Your patience, guidance, and excellence have been instrumental in shaping both my academic and personal growth.

To my advisory committee, Dr. Aaron Kiess, Dr. Jesse Grimes, Dr. Yewande Fasina, and Dr. Ravi Kulkarni, thank you for your expertise, insightful feedback, and consistent support throughout this process.

I am thankful to my lab colleagues at The Ohio State University and North Carolina State University, especially Kate McGovern, Thaci Amaral, and Muhammad Hassan. Special appreciation goes to Toni Sullivan and Tracy Omasanuwa for being incredibly reliable and supportive lab managers whose organization and dedication made daily research smoother and more productive. My heartfelt thanks to Philip Yeboah and his family for their unwavering friendship, warmth, and encouragement during the past four years. You have been more than colleagues, you've been family. To the visiting scholars, Majid Ali, Ziaul Islam, and Renata Gomez, your presence has enriched this journey immensely. I also appreciate the TTEU and PDPS teams, particularly Dr. Shelly Nollin, for their behind-the-scenes contributions.

To my cherished friends who became family, Yabaiz Tahir, King Abbey, Papi, Blinks, Bidex, Kero, Lapa, Sir Tommy, and Femi Falade (Evang), thank you for your support across continents. A special thank you to Dr. Ademola Duduyemi, you've been more than a friend; your brotherhood, wisdom, and loyalty have sustained me through some of the toughest moments.

I thank my spiritual mentors, Rev. (Dr.) B.M. Owojaiye, Pastor Ajiboye, both of First ECWA, Ilorin, and Evangelist Femi, for laying the foundation of my faith. I'm also grateful to

Pastor Yomi & Bisi Oshikoya (RCCG Raleigh), Pastor Yisa & Busola Sunmonu (Jesus House Akron), and my fellowship families in Ohio and North Carolina, including Dr. Warren Dick & Carol, Dr. Lapman & Ching Ching Lun, and Dr. Tom Oey, for their spiritual support and kindness.

To my mentors, Dr. Frank Siewerdt, Dr. Kim Livingston, Dr. Steve Clark, Dr. Elizabetta Giuffra, Dr. F.E. Sola-Ojo, Dr. A.A. Toye, Dr. Opeyemi Ayinde, and Dr. Kazeem Adeyemi, thank you for shaping my scientific path.

I also appreciate the families of Dr. Lanre Suleiman, Mr. Funsho Falegan, and my uncles, Mr. Kehinde Alabi, Mr. Segun Ajiboye, Dr. Tayo Abikoye, along with Mrs. Bunmi Lawal and Olumide Lawal, for their enduring support. To my wife's family and the family of Alhaji Wale Anidu, thank you for your love, prayers, and generosity.

To my amazing parents, Mr. and Mrs. T.O. Ibiwoye, thank you for the sacrifices and love that made this possible. To my siblings, Damilola and Damilare, your encouragement kept me grounded. And to my dear wife, Olaitan Comfort Shekoni, your love and belief in me have been my anchor. Thank you for walking this path with unwavering faith and grace.

Finally, I gratefully acknowledge the support of the Prestage Endowment Funds for Turkey Research and Optum Immunity. Your generous funding has been vital to the success of this work. To all whose names may not appear here, but who contributed in one way or another, may God bless you abundantly.

AI tool was used to assist in grammar and sentence structure editing.

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 2

Chapter 3

Chapter 4

Chapter 5

LIST OF TABLES

Chapter 3

Chapter 4

Chapter 5

CHAPTER 1

Dissertation Introduction

Eimeria parasite causes coccidiosis disease, a significant disease affecting the turkey industry, leading to decreased performance, decreased feed efficiency and increases mortality in affected flocks. According to Blake et al. (2020), about $14.5 billion is lost to poultry coccidiosis globally. It leads to decreased performance, decreased feed efficiency and increases mortality in affected flocks. Challenges associated with the control of coccidiosis includes the development of resistant *Eimeria* stains to anticoccidial drugs and this has led to a reduction in effectiveness of control programs (Mathis et al., 2025). Although somewhat successful, live vaccinations can result in minor infections, elicit varying immune responses, and pose management issues, especially in the interim between initial exposure and the development of protective immunity. Due to the aforementioned, there is a need to search for other methods in controlling the disease in turkey poults.

Immune response against *Eimeria* infection include both innate and adaptive response (Jebessa et al., 2025; Lillehoj et al., 2011). The first line of defense is innate immunity, which is mostly mediated by macrophages and γδ T-cells and keeps *Eimeria* pathogen in check early in infection. $CD4^+$ and $CD8^+$ T-cells generate adaptive immunity, which produces pro-inflammatory cytokines like IFN-γ, IL-1β, and IL-18 to aid in the removal of the parasite (Kim et al., 2019; Lee et al., 2022). In addition, regulatory cytokines such as IL-10 and TGF-β, help to control inflammation by preventing excessive tissue damage. Immune responses gets complicated especially in the field settings where birds are challenged with infection from mixed *Eimeria* species that damages the gut, leads to increases in oocysts shedding and increases

disease severity (Walston et al., 2016). Therefore, to characterize immune responses, ceca and cecal tonsil tissues are key players in mucosal immunity in birds, while spleen and liver helps to amplify immune responses (Dalloul & Lillehoj, 2006; as reviewed by Shivaramaiah et al., 2014). Thus, an understanding of how turkey poults respond to *Eimeria* infection is needed to create better control strategies.

Due to these difficulties, current studies have concentrated on improving mucosal immunity and modifying host reactions to *Eimeria* using different strategies. One interesting strategy is nutritional immunomodulation, especially when done with dried egg products (DEPs) as dietary supplements. DEPs have the ability to enhance immune responses and promote intestinal health in poultry since they are abundant in bioactive compounds such as immunoglobulins and cytokine-neutralizing agents (Rasheed et al., 2020; Sand et al., 2016). Although DEPs have shown promising outcomes in broilers, nothing is known about how effective they are in turkeys, especially when faced with mixed *Eimeria* infection.

The immunological reaction to *Eimeria* infection in turkeys, which varies from that of broiler chickens because of differences in their immune systems, is another crucial research topic. Evidence indicates that the time and tissue specificity of cytokine production, including IFN-γ, IL-10, IL-1β, and IL-18, are crucial for disease resolution and recovery in turkeys, who have a complex mucosal immune system (Gadde et al., 2011). Nonetheless, little is known about the temporal and spatial dynamics of immune responses in turkeys, and the majority of research that is now available is on broiler chickens, who have unique immunophysiological characteristics.

Another important strategy for controlling coccidiosis is to enhance vaccination schedules and minimize oocyst cycling. Overcycling can make infections worse by causing

vaccine-derived oocysts to multiply unchecked before protection is completely developed (Gadde et al., 2009). Combining vaccine use and coccidiostats, such as amprolium helps to reduce parasite burden while improving birds' performance and maintaining gut homeostasis.

Therefore, a comprehensive understanding of host-pathogen interactions, assessing alternative feeding methods, and developing vaccination strategies, to ensure management and control of coccidiosis in turkeys is the aim of this research. Integration of these strategies aims to augment immune response against mixed-species *Eimeria* infections, improve growth performance, decrease parasite load, and increase feed conversion efficiency. In the end, these strategies will lessen the need for conventional anticoccidial medications and vaccinations while preserving the production and health of turkeys.

Hypothesis

- Adding dried egg products (DEPs) to diets of turkey poults challenged with mixed *Eimeria* infection will decrease oocyst shedding and alter the immunological response without affecting growth performance. The immunomodulatory effects of higher DEP inclusion levels will be more noticeable, with decreased IL-10 expression and increased IFN-γ and IL-18 activity.
- Turkey poults exposed to a mixed-species *Eimeria* infection will display immune gene expression profiles that are dynamic and tissue specific. The study also postulates that knowledge of host-pathogen interactions would lead to better treatment and vaccine development strategies.
- A short-term amprolium treatment combined with a low-dose live *Eimeria* vaccination will lessen excessive oocyst cycling, minimize intestinal damage, and encourage a more

balanced immunological response in poults. This strategy will improve mucosal immunity while maintaining performance indicators, as opposed to vaccination alone.

Objectives

- Assess how growth performance metrics (BW, BWG, FI, and FCR) in poults challenged by mixed *Eimeria* are affected by varying degrees of DEP supplementation.
- Quantify changes in fecal oocyst shedding after DEP supplementation.
- Measure immune modulation by looking at the expression of important cytokines (IFN-γ, IL-10, IL-18, and IL-1β) in the tissues of the ceca tonsils, jejunum, and spleen, and use flow cytometry to ascertain how DEPs affect T-cell subsets.
- Characterize temporal gene expression profiles of important immune mediators (IL-8, IL-10, IL-18, IFN-γ, IL-1β, LITAF, and HSP70) in liver, spleen, cecal tonsils, and ceca after a mixed *Eimeria* infection.
- Determine how immune gene expression relates to mortality, body weight loss, and oocyst shedding.
- Assess how low-dose vaccination and amprolium administration affect lesion scores, BWG, FI, and FCR in turkeys with mixed *Eimeria* species.
- Monitor the oocyst cycling in vaccinated turkeys, measure oocyst production over time, and identify the best circumstances to reduce over-cycling while immunity is still developing.
- Use flow cytometry to examine immune cell profiles ($CD4^+$, $CD8^+$, $\gamma\delta TCR^+$, and $MHC\text{-}II^+$ cells) and describe immune responses in cecal tonsils and ceca utilizing cytokine gene expression (IFN-γ, IL-10, IL-6, CD40, FOXP1, TGF-β2).

CHAPTER 2

Literature Review

Introduction

Coccidiosis is a major enteric disease affecting poultry, including turkeys (*Meleagris gallopavo*), and has significant economic and production implications. It affects the intestinal tract of birds (reviewed by Abebe & Gugsa, 2018a), leading to a reduction in performance, gut integrity, poor feed conversion, and mortality. It leads to production losses associated with direct losses and cost of preventing or managing the disease.

1.1 Etiology and Clinical Signs

The etiological agent of coccidiosis, *Eimeria*, are potozoan parasites that affect specific regions of the intestines, leading to localized pathology characteristic to each species. *Eimeria* species are known for their host specificity, often infecting only one species and even only very distinct regions of the gastrointestinal tract (GIT; Chapman, 2008). Seven species of *Eimeria* infect turkeys, including *E. meleagridis, E. meleagrimitis, E. dispersa, E. adenoeides, E. gallopavonis, E. innocua,* and *E. subrotunda*. *Eimeria subrotunda* primarily invades the upper portion of the GIT, whereas *E. meleagridis, E. gallopavonis,* and *E. adenoeides* affects the lower intestinal tract (Chapman, 2008). Three species, *E. gallopavonis, E. adenoeides,* and *E. meleagrimitis* are the most prevalent and economically important species to the turkey industry.

Intracellular replication of *Eimeria* leads to tissue damage, manifesting as bleeding, crypt hyperplasia, epithelial erosion, and villous atrophy. Intestinal congestion and extensive necrosis are brought on by lysis of infected cells, while mucosal surface injury disturbs the gut flora and reduces absorption of nutrients (as reviewed by López-Osorio et al., 2020; Weng et al., 2024).

These pathological alterations make infected turkeys more susceptible to secondary infections (Swanson et al., 2023).

Significant intestinal lesions, including development of caseous cecal cores, are known to be caused by *E. adenoeides*, one of the most harmful species in turkeys. Guelph strain of *E. adenoeides* has been especially destructive, causing weight loss and elevated mortality rates in infected flocks, as noted by El-Sherry et al. (2014). Lesions of *E. adenoeides* show up as congestion, edema, petechiae, strands of mucus, blood, and cottage cheese-like material in the ceca, lower small intestine, and rectum, white caseous plugs in caecal pouches, and petechial hemorrhage without a lot of blood loss (El-Sherry et al. 2014). This makes it one of the more important *Eimeria* species in the industry. This strain severely disrupts the absorption of nutrients by mainly damaging the lower gastrointestinal system. Its quick growth in the cecum, which causes significant harm to the intestinal lining, has been connected to pathogenicity of *E. adenoeides* (Chapman, 2008).

Although severity of infections varies according to dose and flock sensitivity, it is one of the most important coccidia species affecting turkey production in terms of economic impact, as millions of dollars are spent on coccidiosis control yearly (El-Sherry et al., 2014). Lesions of *E. gallopavonis* consist of edema, caseous material in the intestinal lumen, sloughing, and white caseous material at the ileo-caecal junction (El-Sherry et al. 2019).

Turkeys are susceptible to *E. meleagrimitis*, another extremely harmful species that primarily affects the lower gastrointestinal tract. In extensively infected flocks, *E. meleagrimitis* causes severe intestinal lesions, especially in the cecal region, which results in significant weight loss and increased mortality, as explained by Chapman (2008). According to Clarkson (1959), lesions of *E. meleagrimitis* consist of an inflated, swollen duodenum with a reddish-brown

necrotic core that occasionally extends into the upper small intestine. Hein (1969) observed that lesions were hemorrhagic enteritis, significant vascular congestion, and a mucosa covered in tissue debris stained with blood. In addition, there is congestion and the buildup of a reddish brown mucoid necrotic core or exudate in the duodenum and jejunum, epithelial sloughing, ulceration, petechiae, and mucoid casts (El-Sherry et al., 2014b). Watery contents are also observed in the ileum, with petechiae, thickening of the walls, and mucus discharges. Its capacity to significantly disturb intestinal epithelial cells and elicit a strong inflammatory response, which impairs nutrient absorption, is frequently linked to its pathogenicity. Much like *E. adenoeides*, *E. meleagrimitis* is known for producing large quantities of oocysts, which aid in the persistence and spread of infection within flocks. Turkey producers must continuously implement control plans against *Eimeria*, particularly for these three most common species, through vaccination, anticoccidial rotational programs, and strict biosecurity procedures to mitigate risk of clinical disease and large production losses.

1.2 History

1.2.1 Discovery and Identification

History of poultry coccidiosis dates as far back as the 1870s, which was prompted by chickens showing severe diarrhea and intestinal lesions. This infection was linked to a possible protozoan infection. By 1910, researchers had concluded that coccidiosis was a parasitic disease caused by a number of species of *Eimeria* (Nawarathne et al., 2021). There are seven pathogenic chicken *Eimeria* species that are known now; six out of these seven species were described by Tyzzer in (1929), namely, *E. acervulina*, *E. tenella*, *E. mitis*, *E. praecox*, *E. maxima*, and *E. necatrix*. Later Levine (1942), described the seventh specie as *E. brunetti*. Among these, *E. praecox* and *E. mitis* were considered the least pathogenic while *E. acervulina, E. maxima,* and

E. tenella are the most common that affect the chicken industries. Occasionally, *E. necatrix* and *E. brunetti* cause disease, especially in older birds, and are included in some vaccines.

In the late 19th century, early research on *Eimeria* was done in Germany, France, and Italy. Rivolta and Silvestrini were the first to identify coccidia and describe the way in which oocyst sporulation occurs. Early testimony from Raillet and Lucet stated that *E. tenella* was formerly referred to as *Coccidium tenellum* and was described in France. In the early part of the 20th century, a more detailed understanding of coccidiosis and *Eimeria* was developed by E.E. Tyzzer when he was at Harvard University (Chapman, 2003). It was during this time period that more thorough research and understanding of the *Eimeria* species happened, most especially in the United States and United Kingdom. Agricultural experiment stations fostered the spread of knowledge about *Eimeria* more effectively. Important *Eimeria* species like *E. tenella E. maxima,* and *E. acervulina* in chickens were identified during this period. This period is also well noted for advancements in microscopy, which ensure the successful identification of the species. A comprehensive account of the lifecycle of *Eimeria* in poultry was later provided by H.B. Fantham (Chapman, 2003).

1.2.2 History of treatment

Gradually, more information about *Eimeria* started to be known by researchers in the field. According to McDonald & Shirley (2009), live *Eimeria* vaccines were developed in the 1950s, showcasing a significant milestone in coccidiosis research and eventual development of recombinant and subunit vaccines in the late 20th century. This helped achieve a more strategic and sustainable method of controlling the disease.

Sulphonamides were introduced later on in the mid-20th century, which served as the first effective chemotherapeutic against coccidiosis. Ionophore antibiotics were also introduced later,

after the introduction of sulphonamides. A major detriment of using these medications is the development of resistant *Eimeria* strains associated with the prolonged use of the medications. Therefore, creating a need for vaccination strategies that can allow for a return to susceptibility by strains that dominate poultry barns is important to ensure the control of coccidiosis (Campbell, 2008). In addition to the use of vaccines, use of rotation programs, which involves the process of alternating the use of different drugs, is effective in alleviating the problems associated with resistance development (as reviewed by Chapman 2008). Also, the use of bioshuttle strategies that involve the use of different chemicals and ionophores at different cycles of production has been proven effective against development of resistance by *Eimeria* strains (Mathis et al. 2025a).

1.2.3 History of cocci in turkeys

Classification of turkey *Eimeria* was established by Tyzzer, (1929). He described three species: *E. meleagridis, E. meleagrimitis,* and *E. dispersa*, which serve as the basis for the foundational taxonomy class of turkey *Eimeria.* Tyzzer also predicted other species of *Eimeria* based on morphology patterns and biology of *Eimeria* oocysts. His findings were consistent with earlier findings by (Johnson 1923) who stated that turkey *Eimeria* are species-specific.

Eimeria gallopavonis was first described by Hawkins (1950). Moore et al. (1954) explained in detail about *E. gallopavonis* and its lifecycle. Generally, *Eimeria* lifecycle was studied by Moore, and he focused on the sporulation and differentiation of *Eimeria* oocysts in the intestinal tract of birds. Understanding the biology of *E. gallopavonis* helps to lessen its effects on poultry production, making it a species that is still significant for research.

E. innocua and *E. subrotunda* are lesser-known species of *Eimeria* in turkeys and are not linked to any outbreaks because of their mild pathogenicity (Chapman, 2008). They have been incorrectly classified as *E. gallopavonis* in previous research (Moore et al., 1954). Even though they cause minimal damage to birds, they are an important addition to *Eimeria*'s taxonomic class. To achieve complete management of coccidiosis, we need an equal understanding of both pathogenic and non-pathogenic strains of *Eimeria*.

A major problem in the research field of coccidiosis is the lack of access to reference isolates from previous studies (as reviewed by Chapman, 2008), which could have facilitated an understanding of the pathogenicity and evolutionary linkages of earlier known strains and newer strains, thereby ensuring a better understanding of *Eimeria* species and possibly a more effective control strategy.

1.3 Turkey Production in the U.S

1.3.1 Economics

The United States produces the most turkeys in the world and exports its turkeys and products. In addition to chicken production, turkey production is a major sector in the U.S. poultry industry (National Turkey Federation 2022; Poultry & Eggs, Economic Research Service 2025). Turkey products like meat, eggs, and its products adds up to make the turkey industry an essential component of the poultry industry. In the year 2024, the U.S. sold about $70.2 billion worth of produce, and out of this, turkey production accounts for 5% of the value, about $3.7 billion. Due to reduced production in the sector, there was a decline in turkey production in the year 2024, with 2023 sales amounting to $6.57 billion (USDA 2024c). Reports from the National Agricultural Statistics Service (USDA, 2024a) stated that the decline in the year 2024 was caused by outbreaks of highly pathogenic avian influenza (HPAI). Other reports stated that, in

addition to the outbreak of HPAI, there was a decline in consumers' preference for turkey products (USDA, 2024b).

The industry has gone through lots of changes in terms of efforts to achieve better efficiency over the past years. Interestingly, the industry now prioritizes bigger birds over increasing their flock size. A combination of management, nutritional, and genetic efforts has helped to increase the weight of turkeys, thereby optimizing the meat yield (USDA, 2014). Minnesota leads the states in the U.S in terms of turkey production, and it accounts for 17% of the total turkey production in the country. Other important states include North Carolina, Arkansas, and Indiana, which all contribute their own quota to making the U.S. the largest producer of turkeys in the world.

Although traditional production techniques continue to rule the U.S. turkey market, the proportion of organic and free-range production has been gradually rising in response to customer demand. Recent reports in 2019 showed that about 1.85 million turkeys were certified organic out of the total production of 229 million turkeys in that year (NASS/USDA 2020), which represents less than 1% production of organic turkeys. In a positive trend in 2021, there was an increase in the amount of organic turkeys produced. 217 million turkeys were produced, and 3 million of those were certified organic, thereby showing an increase in the organic turkey industry and a corresponding increase in their demand. According to USDA (2021), the production of organic turkeys grew by over 500% between 2010 and 2020.

1.3.2 Management

The U.S turkey sector faces many challenges, which include disease outbreaks. Coccidiosis is one of the diseases that threatens turkey production because of potential losses to production. thereby necessitating the need for strategies to manage the disease to ensure a

productive, sustainable, and profitable sector. Several efforts have been directed towards reviving management approaches that challenge the industry. Efforts in water conservation and adoption of renewable energy technologies have grown in importance, directed towards achieving sustainable turkey production (as reviewed by Salihoglu et al., (2018).

Another critical aspect in managing disease in turkey production involves formulating feed to optimize the performance of birds. Formulation of diets takes into comsideration the dietary requirements of birds and the digestibility of ingredients used to provide the necessary nutrients, such as energy and amino acids needed by the birds (as reviewed by Adhikari et al., 2025). Corn and soybean meal are used primarily as sources of energy and protein in poultry diets. The composition, nutritional content, and digestibility of feed have an impact on gut health, which is essential for managing gut integrity against opportunistic or infectious diseases such as *Eimeria*. Also, use of enzyme supplements and other sources of proteins in the diet to improve sustainability and ultimately reduce feed costs have been noted by the industry. The enzymes help in influencing the viscosity and microbial makeup, which helps to maintain a healthy gut by hindering or preventing the formation of lesions in birds challenged by *Eimeria* (as reviewed by Pesti & Choct, 2023).

Despite advancements in nutrition, disease control remains a persistent challenge in turkey production, particularly due to a lack of approved medications for use in an economically small industry such as turkey production (Hafez et al., 2021). Among various health threats facing industry, coccidiosis is particularly concerning (Clark and Chiaia 2024). According to a review by Shivaramaiah et al. (2014), strict biosecurity procedures, vaccination plans, and surveillance systems work together to prevent spread and minimize losses related to coccidiosis. A better understanding of the immunology surrounding vaccination and infection with *Eimeria*

will help researchers and veterinarians develop more effective prevention and treatment options for coccidiosis in turkeys. Presently, much of our understanding of how turkeys react to vaccination or infection is based on chicken knowledge, but turkeys and chickens are separated by millions of years of evolution, and while they are genetically quite similar, they do have some significant differences (Axelsson et al. 2005; Griffin et al. 2008).

In light of the aforementioned, production of an adequate quality and quantity of turkey meat requires a conscious management approach to controlling coccidiosis. To do this, an understanding of the turkey sector and ways to manage the disease is required, which ultimately helps to improve the growth of the turkey industry.

2. Economic Effect of Coccidiosis (Economics, mortality, production loss, and other losses)

2.1 Economics, cost of disease

Coccidiosis costs the poultry industry approximately $14.5 billion yearly (Blake et al., 2020). It is regarded as the second most prevalent enteric disease affecting poultry production in the U.S (Clark and Chiaia 2024)

2.2 Mortality & production loss

The U.S. poultry sector is an important arm of the agricultural sector of the country. Any potential harm or setback in the poultry sector leads to a significant reduction in productivity of agriculture as a whole, making the poultry industry a key component. Losses from coccidiosis include financial losses due to the death of birds, reduction in weights of births, reduction in productivity, which all add up to the estimated cost stated by Blake et al. (2020). In addition, there is an increase in production costs due to mortality, poor weight gain, treatment costs, etc (Suo et al., 2006; Zou et al., 2019). Development of lesions adds to this problem because birds

have reduced performance due to the reduced absorption of nutrients needed for their maintenance and growth (Mesa-Pineda et al., 2021; Taylor et al., 2022).

Drug resistance is a significant problem in the turkey industry, which is caused by prolonged use of specific drugs, and this leads to resistant strains of *Eimeria* (Mathis et al., 2025). This scenario forces producers to search for alternatives to the use of drugs, which are costly and add to the production cost of the birds. In order to avert this, different management strategies such as rotation, bioshuttle, or the administration of vaccines can help increase the sensitivity of these drugs to *Eimeria* strains (Mathis & Broussard, 2006).

Even with the use of the above strategies, coccidiosis can still lead to minimal mortalities, which are more prominent in young birds whose immune system is still developing (Dalloul & Lillehoj, 2006a). Failure to treat the disease might lead to significant damage and secondary infections, such as necrotic enteritis and clostridial dermatitis, which adds to the economic burden. Vaccination is a common way to control coccidiosis, but its use is still being hampered by high production costs, species-specific immune responses, and vaccine overcycling, among many others. In the end, vaccination contributes to more sustainable disease control by lowering drug reliance, enhancing flock performance, and lowering the chance of drug resistance (Mathis & Broussard, 2006a; Mathis & McDougald, 1989). Vaccines help in lowering the cost that is associated with treatments because they help to reduce the incidence of the infection and ultimately help improve production (Augustine, 1988).

2.3 Other losses

Coccidiosis may have an indirect impact on managing poultry health in addition to causing direct financial losses. Use of antibiotics may rise in coccidiosis-affected birds due to their increased susceptibility to secondary diseases. Antibiotic use raises concerns about

antibiotic residues in poultry products and adds to the larger problem of antimicrobial resistance, even if it has no direct impact on food safety (Gómez-Osorio et al., 2024). Consumer satisfaction is reduced when birds are diseased or sick, which results in the quality of the produce being reduced (Freitas et al., 2023; Martins et al., 2022a).

3. Lifecycle of *Eimeria*

The avian mucosal system serves as the defense mechanism against diseases and infections. Therefore, developing effective control or management strategies is dependent on the understanding of this system (Kaiser 2010). The system wholly helps to fight and prevent diseases like *Eimeria* from colonizing the bird's body. Lifecycle in *Eimeria* strain follows transmission through the fecal-oral route, in which uninfected birds pick up the infective *Eimeria* oocysts from feces shed by infected birds. The lifecycle is made up of two phases: exogenous and endogenous phases.

3.1 Exogenous Phase

Here, the *Eimeria* parasite develops outside the host body in the environment (Imai 2018). This phase is also known as sporogony and this phase starts when infected unsporulated oocysts are shed in the feces of infected turkey poults. These oocysts go through the process of meiosis to develop into infectious oocysts, consisting of four sporocysts and eight sporozoites (Price, 2012). This process requires adequate moisture, temperature, and oxygen to ensure the development of oocysts into sporozoites, which is the infective form of the oocysts (Chen et al., 2025). Time for sporulation or development of oocysts is dependent not only on the aforementioned environmental factors but also on the *Eimeria* species. Infection starts in poults when an uninfected poult picks up the infective oocyst (as shown in Figure 2.1).

3.2 Endogenous Phase

The endogenous phase occurs within hosts (Imai, 2018; López-Osorio et al., 2020) and involves both asexual and sexual reproduction, and begins when digestive enzymes facilitate the release of sporozoites from ingested oocysts within the intestine, marking the initial stage of infection (Mesa-Pineda et al., 2021). Asexual reproduction, also known as schizogony, occurs first in the gut. After the sporozoites have invaded the gut, they transform into trophozoites, which undergo multiple divisions to form schizonts. The rupturing of the schizonts leads to the release of merozoites that infect new epithelial cells. Additionally, merozoites go through cycles of reproduction to form a generation of second and third schizonts, thereby increasing the risk of damage done to the gut (Stevens, 1998).

Merozoite differentiation marks the transition to sexual reproduction or gametogony. Here, the merozoites differentiate into microgametes (male) and macrogametes (female) within the gut. Biflagellate microgametes, which are released from microgametocytes, are highly motile and move around host cell environment in search of macrogametes that are close by. The combination of microgametes and microgametes leads to fertilization and formation of a diploid zygote (Mesa-Pineda et al. 2021). Unsporulated oocyst is therefore shed out as feces with the zygote covered with a protective oocyst wall (Burrell et al., 2019).

4.1 Genetic, Maternal Antibodies, and Environmental Factors influence in the development of coccidia immunity in birds

4.1.1 Genetic factors

Genetic background is an important factor to consider in determining the ability of poultry to withstand coccidiosis disease. Genetic factors play an important role in providing

protective immunity to birds against infection or disease. Results from Kim et al. (2010), confirm that specific single-nucleotide polymorphisms (SNPs) are associated with resistance to coccidiosis. In their study, TCR-β SNP and 2 MLF2 SNPs offer protective immunity and resistance to coccidiosis in chickens, and the MLF2 gene could be used as a marker for selecting for resistance in birds. According to Gul et al. (2022), some genes help with resistance to *Eimeria* in birds. Instances of Myeloid leukemia factor 2 and MHC haplotypes are cited as offering protection against *Eimeria.* The action of other genes, such as TGFβ 2-TGFβ 4, IL-2, and prosaposin, has been shown to offer disease resistance protection in poultry. Other reports also stated that the use of lactose compounds and butyrate has helped to improve resistance in birds.

As stated above, genetic factors help in early immune protection against disease, which is critical in helping to reduce the severity of infection. Additionally, toll-like receptors (TLRs) are essential for identifying pathogens and initiating the production of cytokines, whilst T-cell receptors (TCRs) support T-cell activation and cytotoxic reactions against the parasite (Gadde, 2013; Gomez-Osorio et al., 2023; Hong et al., 2006a; Lillehoj, 1998). Some chicken breeds are naturally more resistant to infections than others, offering them adequate protection against diseases. Selective breeding of these resistant lines would lead to more resistant breeds and ultimately, better control of coccidiosis.

4.1.2 Role of Maternal Antibodies

IgY, which is transferred through egg yolk to chicks by hens as maternal antibodies. It is responsible for offering early immunity to the chicks, however, this protection is not long-lived, which gradually reduces from 3 days to three weeks (Kaze et al., 2018). Although IgY is important in the development of the immune system, it can affect the effectiveness of vaccines

used early in the life of birds. Interestingly, there is not enough evidence to support the idea that maternal immunity protection against one species of *Eimeria* may provide some protection against others, perhaps because of conserved proteins or epitopes being recognized by other species. Maternal antibodies produced against an *E. maxima* infection, for instance, have been demonstrated to protect hatchlings from subsequent *E. maxima* infection partially (Smith et al., 1994). Correspondingly, in a study where birds were vaccinated against *E. tenella* and *E. acervulina* by the use of gametocyte antigens from *E. maxima,* the birds had a positive protection in the form of reduced oocyst output (Wallach et al. 1995). Further research is needed to fully understand this protection, which is not well understood because of the cross-specificity of *Eimeria* strains. As stated earlier, maternal immunity does not lead to long-term coverage against coccidiosis (Ahmad et al., 2023b). Therefore, for birds to develop long-term immunity, they need to have access to vaccines or natural exposure to *Eimeria* in their environment (as reviewed by Shivaramaiah et al. 2014).

4.1.3 Environmental Factors

Another important factor in developing immunity in birds are environmental factors. Oocysts survive in areas that are moist and warm. A warm and moist environment helps improve immunity in birds and creates an enabling environment for oocysts to become infective (Gerhold, 2023; Rao et al., 2015). High temperatures between 27°C and 38°C over the course of 24 hours can cause adverse health effects in poultry (as reviewed by Vandana et al., 2021), which can impair immunological function, even while they encourage sporulation. Conversely, low humidity can decrease the chance of infection, impede oocyst production, and interfere with the natural exposure that birds require to build immunity. Therefore, establishing controlled exposure, which is required for immunological priming in poultry, requires controlling these

environmental factors. Extreme temperatures, such as below freezing or over 32°C, can destroy oocysts, limiting transmission, whereas high humidity and warmth promote sporulation within 1-2 days, increasing infection rates (Chanie, Negash, and Tilahun 2009). Seasonal variations affect disease prevalence, with higher incidence rates reported in autumn than in other seasons (Ahad et al., 2015).

Moreover, oocyst sporulation, transmission, and persistence are all increased by poor litter quality, which also creates the perfect conditions for an infection (Chapman & Johnson, 1992; Edgar 1955). Repeated low-level challenges can help build immunity through this increasing exposure, but if it is not adequately controlled, it also increases the risk of clinical disease. Therefore, maintaining dry conditions and routinely removing wet litter are two aspects of effective litter management that assist in reducing oocyst burdens while providing enough exposure for immunological priming.

Thus, in order to ensure proper development of immunity, the risk of coccidiosis must be balanced by proper housing conditions, which include regular cleaning, disinfection, and sufficient ventilation. Although oocysts can withstand many disinfectants, they can be rendered inactive by ammonia or high pH treatments, which lowers environmental contamination (Peek 2010). In addition, high stocking densities increase fecal contamination and the risk of infection among birds (Shirzad et al. 2011). Overcrowding, also raises stress levels, which can impair immune function by raising corticosteroid levels, impairing T cell responses, and making infection more likely to occur (as reviewed by Abebe & Gugsa, 2018; Hadipour et al., 2011). Lastly, environmental stresses that worsen coccidiosis include extremes in temperature, inadequate ventilation, or co-infections with bacterial pathogens such as *Clostridium perfringens*, *Escherichia coli*, and *Enterococcus cecorum* (Williams et al., 2003).

5 Immunity and Immune Responses

5.1 Avian Immune System

The avian mucosal system is made up of innate and adaptive immune systems and they both play an important role in protecting birds against pathogens (Mehrzad et al., 2024; Nochi & Kiyono 2006; Warrington et al., 2011). Humoral and cell-mediated immunity are a major part of the avian immune system, responsible for protecting birds against disease (Figure 2.2). Humoral immunity helps produce antibodies against infections, while cell-mediated immunity involves the action of T cells in preventing or reducing the severity of infections (Mehrzad et al., 2024). In addition, other immune cell component that are important are dendritic cells, NK (Natural Killer) cells, and macrophages and they are essential components of the mucosal immune system and help fight off infections (Nagura, 1992; Tezuka & Ohteki, 2019).

A vital part of the mucosal immune system, mucosa-associated lymphoid tissue (MALT) is essential for shielding mucosal surfaces. MALT is a group of lymphoid tissues that are positioned near the mucosal surfaces of the reproductive, gastrointestinal, and respiratory tracts, where infections are most likely to infiltrate. MALT system acts as the first line of defense against pathogens in mucosal areas, making up for the lack of encapsulated lymph nodes in birds compared to mammals (Nochi et al. 2018; Panda and Colonna 2019; Saez et al. 2021). Part of MALT, the gut-associated lymphoid tissue (GALT) is essential for triggering defenses against *Eimeria* infections in the gut. GALT is the first line of defense against *Eimeria* infections, including Peyer's patches, the bursa of Fabricius, and cecal tonsils, which are host immune cells that contribute to both innate and adaptive immunity (Beller et al., 2020; Mesa-Pineda et al., 2021).

Eimeria infection in birds causes intestinal epithelium damage due to the release of sporozoites from the pathogen. Peyer`s patches, and cecal tonsils are important GALT structures in birds that helps in immune cell activation and response to fight off the infection (Langkamp-Henken et al., 1992; Shira & Friedman, 2018). Early activation of GALT cells helps to regulate the way this pathogen replicates and multiply. On the other hand, the adaptive immune responses support healing of damaged tissues and clearance of infected cells. Although, the progression of infection can alter or change the immune response and activation (Cesta, 2006; Kunisawa et al., 2005). However, as the infection clears off, the GALT immune modulation system enables the control of inflammation, avoids long-term damage and restores regular intestinal function. Moreover, protective immune response to *Eimeria* infection are cell-mediated, but secretory IgA antibody production and B-cells response can also provide mucosal immunity defense (Wallach 2010).

GALT's tissue resident T-cells such as $CD4^+$ and $CD8^+$ can stimulate and coordinate cytokine production and cytotoxicity response. These cells promote the release of cytokine to clear off infected epithelial cell and reduce intestinal damage, thereby limiting parasite replication (Dalloul & Lillehoj, 2006b). Likewise, MALT innate immune cells, such as dendritic and macrophage cells, help to recognize and capture *Eimeria* oocysts, expose T cells to antigens, and initiate adaptive immune response. The intestinal barrier must be protected at all times to preserve nutrient absorption in the gut through the activities of the GALT, thereby lessening the severity of coccidiosis (Gao et al., 2024).

The severity of *Eimeria* infection can be influenced by the modulation of immune response because during the early stage of infection, an acute inflammatory response is triggered to prevent the spread of the disease. Additionally, macrophages and dendritic cells can also

release pro-inflammatory cytokines like TNF-α and IL-6, in order to attract more immune cells to the site of invasion. Although, this inflammatory response can help to control the infection and short-term parasite replication, but if it is not controlled, it can also result in overall severe intestinal damage, tissue necrosis and worsen the disease. (Chen et al., 2023).

The immune system of birds needs to balance this early inflammatory reaction with a resolution phase to avoid causing too much harm. A subpopulation of T cells known as regulatory T cells (Tregs) is essential for preserving immunological homeostasis because they inhibit excessive immune activation and aid in the reduction of inflammation (Barbara et al., 2021; Cheng et al., 2019). Tregs ensure that the immune system targets the pathogen without causing undue damage to host tissues by inhibiting the activation of immune cells that are too aggressive (Choi et al., 1999; Velavan & Ojurongbe, 2011). Preventing chronic inflammation is essential for avoiding long-term intestinal damage, decreased nutrient absorption, and eventually increased financial strain on poultry farms.

Cytokines are secondary messengers that regulate immune responses and help modulate host immunity against coccidiosis (Chen et al., 2022). The network of cytokines and chemokines that regulate inflammation and immunological signaling is another crucial component of the mucosal immune response. In response to infection, the body produces cytokines, including TNF-α, IL-2, IL-4, IL-8, and IL-10 (Ouyang et al., 2011; Wei et al., 2020). The anti-inflammatory cytokine IL-10 reduces excessive inflammation and modifies immunological responses by inhibiting the action of pro-inflammatory cytokines, such as interferon-gamma (Beller et al. 2020; Santhakumar et al., 2017).

Cytokine expression levels during *Eimeria* infection can vary depending on the *Eimeria* species causing the infection and the changes that occur during repeated exposure to the

pathogen (reviewed by Chen et al., 2022; Kim et al., 2019). There are several important cytokines such as IFN-γ, IL-2, IL-12, and IL-10 with distinct functions that help to determine the immune system reaction to *Eimeria*. For instance, IFN-γ which is a cytokine that is produced by TH1 and γδ T cells can stimulate macrophage response which helps to boost cytotoxic T-cell activity to target *Eimeria* infected cells (reviewed by Lillehoj, 1998; Lillehoj & Trout, 1996).

Likewise, IL-2 can help to promote the differentiation and proliferation of T-cell in order to increase the number of effector and memory T cells that is needed for both initial and secondary immune response (as reviewed by Lillehoj, 1998). IL-12 can also help naïve T-cell to differentiate into TH1 cells. This can increase the production of this cytokine to stimulate a strong pro-inflammatory response that helps to clear the parasite (Hong et al., 2006a, 2006b; Kim et al., 2008; Min et al., 2001). In contrast, IL-10 acts as a regulatory cytokine to reduce excessive inflammation and prevent immunopathology during recurrent or chronic infection (reviewed by Kim et al., 2019; Saraiva & O'Garra, 2010). Tregs, macrophages, and epithelial cells also produce TGF-β, which is essential for reducing inflammation and encouraging tissue healing during *Eimeria* infections (Jakowlew, Mathias, and Lillehoj 1997). By suppressing excessive immunological activation, TGF-β helps Tregs differentiate. This ensures that the immune response is correctly controlled when the parasite is eliminated, restoring immune homeostasis.

Furthermore, pro-inflammatory cytokines, like IL-1β, can increase the inflammatory cascade in the intestinal mucosa during *Eimeria* exposure and attract immune cells to the infection site, to promote early innate responses (Laurent et al., 2001). Chemokines such as CXCLi2 (avian IL-8) can also improve local parasite clearance by promoting infiltration of

leukocytes like heterophils and macrophages to the infected cecal tissues (Gadde, 2013). IL-18 and IL-12 often work together to increase T cell IFN-γ expression which then strengthen TH1- T cell response against intracellular parasite (Gadde, 2013; Hong et al., 2006a, 2006b; Lillehoj et al., 2004). In mammalian models of gut inflammation, IL-13 has been shown to regulate barrier defense and tissue remodeling (Mannon & Reinisch 2012). While this has not been directly studied in *Eimeria* infections, the parallels suggest that IL-13 may also contribute to limiting inflammation and supporting repair in avian coccidiosis. Therefore, effective control of *Eimeria* infection depends on maintaining a balance between pro-inflammatory cytokines (e.g., IFN-γ, IL-2, IL-12) and regulatory ones (e.g., IL-10) to clear the parasite without causing severe tissue damage. According to Soutter et al. (2020), cytokine-mediated immune response analysis offers important insights into how B and T-cell responses are modulated against coccidia infections. Gaining insight into cytokine networks and how they govern adaptive immunity may help develop cytokine-adjuvanted vaccines or targeted immunomodulatory treatments, which could enhance vaccination plans and immune-based control techniques against *Eimeria* infections in poultry.

5.1.1 Innate Immunity

The first line of defense against *Eimeria* infection is innate immunity, which helps to fight against *Eimeria* parasites when birds are exposed. However, a key component of adaptive immunity, specifically the T-cell response, also plays a crucial role in the complete eradication of the infection. Innate cells identify and react to infection before the adaptive immune response is fully activated. The innate response helps in early containment of infection and helps prepare the adaptive immune cells for a more targeted response. In addition, innate immunity response can be mediated by the innate cells, such as Natural killer (NK) cells, dendritic cells, and

macrophages. Cells of the innate immunity use phagocytosis as a means of eradicating parasites or infections (Fan et al., 2019; Wang et al., 2024). Immune system reactions to *Eimeria* change as the infection progresses. In the case of *Eimeria* infection, the immune system primarily relies on the adaptive immune system, due to the actions of natural killer cells, dendritic cells, and macrophages. They help in identifying and fighting off the *Eimeria* parasite. These cells present antigens, activate T cells, and rapidly produce cytokines to initiate an adaptive immune response.

5.1.2 Adaptive Immunity (Acquired B-cell & T-cell)

In contrast, adaptive immune system action depends on the activities of T and B cells, which can be triggered by the infection (Lee et al., 2020; Warrington et al., 2011). B cells are essential for the synthesis of antibodies, whereas T cells mediate cellular immunity by activating cytotoxic T-lymphocytes ($CD8^+$) and T-helper ($CD4^+$) cells (Brown et al., 2023; Riera Romo et al., 2016). Therefore, as infection progresses and the parasites multiply, adaptive immune responses become stronger, with T-helper cells and B cells generating antibodies to fight the parasite. IFN-γ and other cytokines stimulate $CD8^+$ T lymphocytes to target infected cells and destroy them, while secretory IgA antibodies aid in neutralizing the parasite (Rami A Dalloul and Lillehoj 2006b).

Furthermore, important immunoglobulins such as IgA, IgM, and IgY are produced by B cells (Boyaka, 2017; Pietrzak et al., 2020; Tezuka & Ohteki, 2019). IgM is the first antibody that is expressed during the early phase of immune development. It can act as a broad-spectrum scout, helping the immune system to detect antigens before undergoing class switching to other antibody types (Boyaka 2017; Li, Jin, and Chen 2020; Pastoret et al. 1998). Also, B cells can change classes in response to antigen exposure in order to produce more specialized antibodies like IgA and IgY. Moreover, mucosal-specific IgA often offers localized protection at the

epithelial surfaces, especially in the respiratory and gastrointestinal systems (Boyaka 2017). The main systemic antibody in birds is IgY. IgY has two functions: it acts as an agent of passive immunity and is also actively generated after antigen exposure to infection. Likewise, maternal IgY antibody can be passed to egg yolk from the hen to the chick to provide early protection before their immune system develops (Hamal et al. 2006; Lamm et al. 1995; Leslie, Stankus, and Martin 1976).

T-cells are essential for immunosuppression, cytotoxic activity, and memory T cell development, which confers immunity against subsequent infections (Russell et al., 2020; Zhang et al., 2017). In the event of an *Eimeria* infection, dendritic cells, macrophages, and NK cells, which are examples of innate immune cells, identify invasive sporozoites and start inflammation when an infection occurs. Additionally, these cells stimulate the adaptive immune system by presenting antigens to T cells, thereby activating the immune response. T-cell responses become more crucial when the infection worsens because the parasite replicates intracellularly within epithelial cells. T-helper cells (Th1) and cytotoxic T lymphocytes (CTLs) are essential for locating and eliminating contaminated cells. This cell-mediated immune response is critical since antibodies alone are less effective against intracellular infections such as *Eimeria* (Lee et al., 2022b). Important components of avian defense against *Eimeria* infection include intraepithelial lymphocytes (IELs), cecal tonsils, and Peyer's patches. These elements support T-cell activation, antibody synthesis, and antigen processing, all essential for protection against *Eimeria* (Lee et al., 2022; Lillehoj & Trout, 1996).

During invasion of epithelial cells by *Eimeria* (as shown in Figure 3), the immune system recognizes the pathogen through Pathogen Recognition Receptors (PRRs), such as Toll-like receptors (TLRs). These TLRs can activate antigen-presenting cells, e.g., macrophages, and also

initiate a T-cell response (Kumar et al., 2009; Smith & Hayday, 2000). This results in the activation of two T-cell populations:

1. $CD4^+$ (T-helper) cells: These cells facilitate immune responses by recruiting neutrophils, enhancing macrophage phagocytosis, and helping B cells to produce antibodies (Horton & Vidarsson, 2013; Owens et al., 1987). $CD4^+$ cells can also recognize Major Histocompatibility Complex Class II (MHC II) molecules and mediate cytokine production, including IFN-γ and IL-10, and they can help to maintain balance in the immune response during *Eimeria* infection (Assenmacher et al., 1994; Horton & Vidarsson, 2013; Lee et al., 2022; Nagata & Nishiyama, 2021).
2. $CD8^+$ (Cytotoxic T-lymphocyte) cells: These cells also play an important role in eliminating intracellular parasites, such as *Eimeria*. The pathogen antigen is recognized by the MHC I molecules and induces apoptosis in infected epithelial cells (Lillehoj & Bacon, 1991; Rodrigues et al., 2003; Rothwell et al., 1995). In addition, tissue resident $CD8^+$ cells can also differentiate into memory T cells, which provide long-term immunity and enhance protection against subsequent *Eimeria* infection (Campos et al., 2023; Cloft et al., 2023; Rothwell et al., 1995).

5.2 Immunity against Coccidiosis (Eimeria Specific Immunity)

For a long time, it has been believed that immunity to coccidiosis is a complex and slow process, and that birds need to be exposed to *Eimeria* more than once before immunity is developed (Allen & Fetterer, 2002; Yun et al., 2000). However, research shows that, even in cages with wire floors that prevent reinfection, broiler chicks acquired immunity following a single exposure. Here, Cobb500 broilers were vaccinated with transgenic *Eimeria* expressing antigens resulted in notable protection against *Eimeria,* indicating that restricted exposure under

regulated circumstances can successfully elicit immunity (Pastor-Fernández et al. 2020a). Complete immunity is achieved in birds after about seven weeks of being exposed to *Eimeria* parasite, which might be through the use of vaccines or direct exposure in their environment (Casteleyn et al., 2010). This process is mediated by the activation of natural killer (NK) cells, dendritic cells, macrophages, and T lymphocytes, which coordinate the immune response against the parasite (Khater, 2020).

Severity of infection and its timing affect the development of immunity against *Eimeria*. A repeat infection may transpire prior to the establishment of complete T-cell immunity, even if a single exposure can elicit immunological responses. The immune system can effectively develop a response and avert clinical disease if the parasite load is low or moderate (Li et al., 2015). The primary immune response is mediated by T cells, particularly $CD4^+$ T helper cells, which coordinate immune signaling through cytokine production, and $CD8^+$ cytotoxic T lymphocytes, which play a direct role in eliminating the infected cells (Kim et al., 2019). A key cytokine involved in this process is interferon-γ (IFN-γ), which regulates immune response and inhibits intracellular *Eimeria* development (Kim et al., 2019; Li et al., 2015).

The role of humoral immunity in the protection against *Eimeria* remains unclear (Kim et al., 2019; Tellez et al., 2014). Although birds produce parasite-specific antibodies in both circulation and across mucosal surfaces following a primary infection (Lillehoj & Trout, 1996), their role in direct protection against *Eimeria* remains unclear. IgA may help hinder the movement of sporozoites and translocation of merozoites in the intestinal epithelium of the bird's gut, which potentially helps to reduce damage (Kim et al., 2019). Maternal antibody, IgA, also offers protection to young birds by contributing to early protection against *Eimeria* infection (Smith et al., 1994). Some studies have suggested that antibodies, particularly IgA, may help

prevent sporozoite and merozoite translocation along the intestinal lumen, potentially reducing the degree of invasion (Kim et al., 2019). Maternal immunity may also play a role in early protection, with passively transferred IgY antibodies contributing to chicken resistance (Smith et al., 1994). However, research indicates that humoral immunity does not play a dominant role in *Eimeria* protection because cell-mediated immunity is the primary driver of resistance (Lillehoj & Trout, 1996).

Although the details of protection offered against *Eimeria* infection by humoral immunity are not well understood, cell-mediated immunity remains the primary force of protection against *Eimeria* infection, and methods to manipulate this immunity should be extensively studied to provide more effective protection for birds.

5.3 Cell-mediated immune responses to Eimeria

T-cells are the most important effector mechanisms in response to primary or complicated *Eimeria* infections (Lee et al., 2022).

5.3.1 Eimeria-Induced Innate Immune Activation

Two main categories of T-cells seen in poultry are $CD8^+$ cytotoxic T-cells (TC) and $CD4^+$ (cluster of differentiation 4^+) helper T-cells (TH). Only a small percentage of T-cells express the γδ version of the T-cell receptor (TCR), whereas the majority express the αβ type. γδ T cells are primarily present in intestinal intraepithelial lymphocytes (IELs); however, they are not widely distributed in the bloodstream. It is thought that γδ T cells are a crucial first line of defense because *Eimeria* infections mainly affect epithelial cells (Smith & Hayday, 2000). Increases in γδ T cell numbers in the duodenum after an initial *E. acervulina* infection have been noted, suggesting their possible involvement in local immunological responses (Lillehoj et al.,

2004; Mathis et al., 2004; Smith & Hayday, 2000). Although TH cells are responsible for starting the immune response, specifically through the production of TH1 cytokines such as IFN-γ, γδ T cells function earlier in the infection to offer quick, local responses against the parasite.

Major histocompatibility complex (MHC) molecules are needed for the activation of T-cells. MHC class I is recognized by $CD8^+$ cells, while class II is recognized by $CD4^+$ cells. According to Lillehoj & Trout, (1996), helper T cells help to activate cytotoxic T-cells that destroy *Eimeria* parasites and aid B-cells in the production of antibodies, which are essential for limiting *Eimeria* infection. Cytotoxic T-cells target and kill only infected cells, not the host, by employing a defense mechanism known as T-cell-mediated cytotoxicity in immune cells. This mechanism is important to ensure the specificity of the actions of $CD8^+$ T-cells.

TH cells help in initiating and coordinating immune response after infection. Research on *E. mivati* infection in chickens shows that when birds are infected, T helper cells are very active and help in reducing *Eimeria* effects by producing IFN-γ to inhibit the *Eimeria* parasite (as reviewed by Lillehoj & Trout, 1996). According to these results, $CD4^+$ TH cells initiate immunological reactions, whereas $CD8^+$ TC cells perform effector tasks necessary for parasite management. Another part of the T-cells is known as IELs, intraepithelial lymphocytes, which are lymphocytes that reside in the intraepithelial portions of the gut. In case of coccidial infections, IELs are highly active, and between 75 and 80 percent of IELs express the $CD8^+$ TC marker, which supports their function in *Eimeria* immunity (Yun et al., 2000).

Host Immune responses in *Eimeria* infections are both species and strain-specific. Previous reports on protective immunity in *Eimeria* observed that immunity can be achieved in birds with access to a small number of oocysts in some species of *Eimeria* at a single timepoint (as reviewed by Shivaramaiah et al., 2014), while in some other species, there may be

a need for more than one exposure, and they may require more oocysts to achieve protective immunity. Although effective protective immunity depends on memory T-cells activation and the initial immune priming that helps in a quick response to secondary *Eimeria* infection.

5.3.2 Eimeria-specific Memory T cells

Memory T-cells are formed after a primary immune response has occurred in birds. Firstly, when birds encounter *Eimeria* parasite, a primary immune response will be initiated, which will be followed by the activation of naive T-cells. These naive T-cells, after responding to and limiting the infection, die, leaving a few remaining as memory cells in the birds. These memory T-cells are initiated when another infection occurs, and they help clear the *Eimeria* parasite. $CD4^+$ memory T-cells belong to the TH1 subgroup, and they help B cells mature into memory cells, which then secrete antibodies into the body. They also contribute to the production of cytokines, such as IL-2 and IFN-γ, which help activate immune cells (Isobe and Lillehoj 1993). $CD8^+$ memory T-cells function to kill the infected cells and prevent the spread of infection. They are also essential in preventing reinfection due to their ability to limit the parasites in epithelial cells. $CD8^+$ T-cells help provide the necessary long-term immunity needed against *Eimeria* infections (Shi et al., 2023). Previous reports from Wattrang et al. (2023), reiterated the importance of $CD4^+$ and $CD8^+$ in immunity against *Eimeria* infection in their in vitro study against *E. tenella*. They evaluated the roles of T-cell subtypes such as $CD4^+CD8^-$, $CD4^+CD8\alpha\alpha^+$ in immunity against *Eimeria* and stated that $CD4^+$ subsets support memory function and provide protection for the immune cells of birds. It is therefore important to have an understanding of how to prime the immune system effectively to get more memory T-cells. This can lead to sustained protection against *Eimeria* infection, which is essential for developing effective vaccines against single or multiple species of *Eimeria.*

6. Factors Influencing Disease Severity

Multiple factors influence the severity of *Eimeria* infection. These factors include the species of *Eimeria* affecting the birds, host immunity, environmental conditions, stocking density, hygiene, and nutrition, among others. These factors determine disease progression and the overall health of the flock, and they can be grouped into two major categories, namely pathogen-related and host-related factors.

6.1 Pathogen-related factors

Severity of *Eimeria* infection is directly impacted by the pathogenicity, which varies significantly according to the parasite species, strain, and oocyst load.

6.1.1 Species of Eimeria

Eimeria species vary in their pathogenicity and are very specific in the part of the gut they affect and colonize. *E. adenoeides, E. meleagrimitis*, and *E. gallopavonis* causes distinct severe intestinal lesions leading to significant production losses (Moore et al. 1954). As opposed to *E. innocua*, which frequently causes subclinical infections with ambiguous pathogenicity, *E. dispersa* is slightly pathogenic and causes few lesions (Dakpogan et al., 2018).

6.1.2 Strain Diversity

Eimeria species have unique strain diversity associated with increased virulence in each individual strain. According to Matsubayashi et al. (2016), some isolates of *Eimeria* expressed higher replication ability and caused more intestinal damage than others, as observed in the case of *E. tenella* isolates in their study. In addition to causing more tissue damage, highly pathogenic strains elicit stronger inflammatory responses (such as increased production of IFN-γ), which might overwhelm immune systems in vulnerable birds and worsen clinical outcomes (Hong et al.

2006b). Understanding these variations and their diversity leads to a more comprehensive control of *Eimeria*.

6.1.3 Oocyst load

Increased oocyst loads raise the risk of serious infections since the quantity of sporulated oocysts consumed is correlated with the degree of intestinal damage and clinical symptoms such as hemorrhagic lesions, malabsorption, and decreased weight gain (El-Shall et al., 2022). In addition, increased oocyst load has been linked to greater disease severity (Jenkins et al., 2017).

6.2 Host-related Factors

Factors such as age, genetic background, previous exposure, drug resistance, and nutritional variables all affect the development of protective immunity, which in turn affects the severity of *Eimeria* infections in poultry.

6.2.1 Age

Young poults ranging from two to six weeks of age are more susceptible to an increased incidence of coccidiosis when exposed to *Eimeria* due to their immature immune system, which matures over time. This is because their immune system lacks enough T-cell responses, and no memory T-cells have formed yet to mount a robust adaptive immune response against *Eimeria* infection (McDougald et al., 2020). Birds that are exposed to *Eimeria* as they become older start to establish immunological memory, primarily through memory T-cell activation. Stronger immunity and fewer signs of disease result from these T-cells' ability to recall prior infections and help the birds react more quickly and efficiently to future exposures (reviewed by Shivaramaiah et al., 2014). Protective immunity is developed through moderate exposure to

coccidia, which is made possible by T-cell-mediated responses, including $CD4^+$ Th1 and $CD8^+$ cytotoxic T cells, which release IL-2 and IFN-γ to inhibit the reproduction of parasites (reviewed by Kim et al., 2019). This exposure can happen in the environment or through vaccinating poults with a small amount of oocysts, which stimulate the production of memory cells and protective antibodies by the immune system (Shivaramaiah et al., 2014).

6.2.2 Genetic background

Genetic variation is crucial; resistant chicken lines show stronger innate and adaptive responses, such as increased macrophage activity and earlier IFN-γ production, than susceptible lines (Bremner et al. 2021). Resistant lines of chickens have a lower amount of oocyst output and reduced lesions in their gut (Williams et al. 2003). In contrast, immature or immunocompromised birds suffer poor formation of T-cells, which can be primed through vaccinations or exposure to oocysts in their environment (Lee et al., 2022b).

6.2.3 Environmental Factors and Biosecurity Measures

Biosecurity is an important component that aids in the reduction of the severity of *Eimeria* infections in the environment. Effective biosecurity involves thorough prevention and sanitation of the poultry environment, as well as the strict authorization of personnel working on the farm. Inadequate biosecurity, which includes poor sanitation and cleaning, raises the possibility of parasite exposure and prevents the development of immunity. An efficient management technique called the "All-In-All-Out" system efficiently cleans and disinfects poultry buildings in between production cycles, hence reducing oocyst buildup. Using this system helps minimize the risk of infection and build-up of *Eimeria* oocysts in litter (Ahmad et al., 2023).

High stocking densities favor more oocysts in the environment, easy spread of *Eimeria*, thereby leading to an overload of the gut with oocysts, which can potentially inhibit immunological responses in birds (reviewed by Salem et al., 2022).

In addition, high humidity levels in the poultry environment resulting from poor ventilation can promote oocyst survival and dissemination. Overcrowding also induces stress, weakens the immune system of birds, and increases their susceptibility to infections. Factors such as poor sanitation and inadequate cleaning favor the accumulation of *Eimeria* oocysts in the environment, aiding in the spread of *Eimeria* oocysts, increasing the severity of the disease (Ahmad et al., 2023). Poor litter conditions and management also favor the growth of *Eimeria* oocysts, thereby increasing the risk of infection. Conversely, extremely dry litter may hinder the establishment of protective immunity in birds vaccinated with live coccidiosis vaccines because the heat in the litter prevents the sporulation of *Eimeria* oocysts (Attree et al., 2021). Adding sick or unquarantined birds can increase the spread of the disease and make it worse (Mesa-Pineda et al., 2021).

6.2.4 Drug Resistance

Drug resistance can be a result of improper use of anticoccidial drugs, timing or rotation, which reduces treatment effectiveness and raises the possibility of severe outbreaks. *Eimeria* can become resistant to routinely used therapies due to drug resistance, which is mainly brought about by poor medication scheduling or rotation (Abbas et al. 2011; Dalloul and Lillehoj 2006). Between 1948, when the first anticoccidial was developed, and 1980, over 80% of anticoccidial drugs were introduced and used extensively for coccidiosis control. Since then, various studies have documented resistance or decreased sensitivity of *Eimeria* species to these drugs, with

significant reductions in their efficacy observed in turkeys (Arabkhazaeli et al., 2013; Lan et al., 2017).

6.2.5 Nutritional Variables

Nutritional variables also have an influence on the severity of *Eimeria* infection in birds. Diets of birds deficient in vitamins A, E, and selenium have an effect on antioxidant defenses in birds, which can predispose birds to damaged gut, severe lesions, and increased oocyst output (Colnago et al., 1984). Antinutritional factors, such as aflatoxins, in diets impair immunological function by inhibiting the generation of cytokines, which increases the severity of coccidiosis (Gómez-Osorio et al., 2024). Some nutritional components, when added to diets, can help alter the gut of *Eimeria*-challenged birds, helping to make a healthy gut and maintain a state of homeostasis. Additives like probiotics and sufficient micronutrients are part of a balanced diet that promotes immunological priming, lessens the severity of disease, and increases flock resilience (Bedford & Apajalahti, 2022).

Development of protective immunity and decrease of the risk of severe clinical coccidiosis depend on efficient control of environmental elements, which can be achieved through improved temperature, humidity, litter conditions, stocking density, and stress reduction (reviewed by Chapman, 2008; El-Shall et al., 2022; Salem et al., 2022). A conscious effort to implement sound nutrition programs and practice biosecurity can help minimize the risk of infection while allowing birds to develop protective immunity.

7. Diagnosis of *Eimeria* Infections in Turkeys

Traditional methods of identifying *Eimeria* species involve the use of a light microscope to visualize *Eimeria.* Interestingly, over the years of constant research, molecular techniques

have evolved to diagnose infections within a short time accurately. These techniques include the use of PCR, LAMP, and NGS to identify *Eimeria* species (Duff et al., 2022; Wang et al., 2014). In addition to the techniques being used for identification, they are also used for quantifying the burden caused by the parasite and characterizing immune gene responses in birds. PCR and qPCR enable the comprehensive monitoring of immune gene dynamics (Blake et al., 2008; Cheng et al., 2022). Apart from diagnosing disease, these molecular techniques can also be used for monitoring the severity of infection as opposed to the conventional methods of diagnosing, such as oocyst per gram (OPG) quantification and lesion scoring. Molecular methods can monitor the replication ability of *Eimeria* parasites within the gut of birds (Haug et al. 2008; Williams 2001). Another accurate and adequate method of diagnosing, detecting, and quantifying intestinal damage caused by *Eimeria* is the use of histopathological assessment (Sun et al., 2024). This can help track *Eimeria* parasite loads that are present in birds. Therefore, to effectively control coccidiosis on farms, integrating the traditional and molecular methods of detection can help in its timely control.

8. Coccidiosis Management, Control, and Challenges in Poultry

Different methods have aided in the control of coccidiosis, including the use of vaccines, anticoccidial medications, management practices, among many more, but the use of drugs is the most widely used (Mesa-Pineda et al., 2021; Peek & Landman, 2011). Prevention of coccidiosis is the most essential approach to controlling the disease, which involves measures such as access to clean water and feed, biosecurity, and sanitation. Biosecurity measures include limiting the personnel, vehicles, and equipment between poultry buildings to prevent the spread of parasites. Also, cleaning and disinfecting poultry facilities helps prevent the spread of oocysts or

potentially limit their amount (Allen & Fetterer, 2002). Timely isolation of sick animals from pens can help reduce the spread and thereby minimize cross-contamination on farms (Ahmad et al., 2023).

8.1 Anticoccidials

One method of controlling coccidiosis is the use of anticoccidial drugs, which have been proven to be effective in preventing infection and, in some cases, to prevent clinical coccidiosis (Chapman, 1997; Grumbles et al., 1948). Anticoccidials such as amprolium, monensin, salinomycin, and lasalocid can be added to feed or water to prevent coccidiosis. According to Noack et al. (2019), administering amprolium in water is more effective and faster in treating birds than supplementing it in the diet.

8.2 Vaccination in Coccidiosis Management

The use of live or attenuated *Eimeria* oocysts as vaccines is one of the most prominent methods for controlling coccidiosis. Regulated exposure of birds to *Eimeria* oocysts helps to develop the immunity of birds by protecting them from future *Eimeria* challenges (Xicheng et al. 2004). Immunity is developed before the birds are exposed to the disease in commercial settings by immunizing day-old chicks in hatcheries or farms. Vaccination helps to enhance flock performance, reduce mortality, and lower losses related to disease control. Therefore, vaccinating birds in a timely manner helps to prevent clinical disease and guarantees protective immunity (Snyder et al., 2021).

It is advantageous to include vaccines as a part of a coccidiosis control strategy. Vaccines reduce treatment and medication expenses by decreasing the need for costly anticoccidial medications and fostering a protective immune response. It also helps to increase *Eimeria'*s

susceptibility to medications by lowering the likelihood of drug resistance, ensuring that medications continue to work. Drug-sensitive *Eimeria* vaccines, for example, have been demonstrated to decrease the selection pressure on drug-resistant strains, increase the efficacy of anticoccidial drugs, and decrease the parasite's mutation rates (Mathis & Broussard, 2006a; Mathis & McDougald, 1989). Additional strategies to manage coccidiosis include the use of rotation, shuttle programs, and anticoccidial sensitivity tests (ASTs). According to Peek and Landman (2011), ASTs are frequently used to assess the effectiveness of medications in regulatory trials and help choose anticoccidial medications that work.

Rotation programs have been demonstrated to delay the development of drug resistance, even though anticoccidial medications and vaccinations continue to be the principal control methods (Blake et al., 2017). In addition to reducing the dangers of medication resistance, integrated control measures seek to improve flock health, maximize disease management, and limit financial losses (Chapman & Rathinam, 2022). To expose *Eimeria* to a variety of modes of action and lessen the chance of resistance developing, rotation schemes alternate between different anticoccidial medication classes (Mathis & Broussard, 2006b). Altering anticoccidial drugs either annually following each production cycle or within a single cycle is how poultry breeders carry out rotation programs (Peek & Landman, 2011).

Bioshuttle programs, sometimes referred to as shuttle programs, combine in-feed anticoccidial medications with immunizations. Prior to the drug withdrawal period, birds are vaccinated against live coccidiosis after receiving anticoccidial drugs at their most sensitive early growth stages (Kimminau & Duong, 2019). Bioshuttle programs improve immunity and lower the risk of serious infections by combining vaccinations with medication (Chapman & Jeffers, 2014).

Dietary manipulations are becoming an important component of an integrated coccidiosis control in addition to vaccination and anticoccidial medications, which is a viable approach for lessening the severity of *Eimeria*-associated lesions and improving performance in turkeys (Rami A. Dalloul and Lillehoj 2006). In addition to the above, the use of phytochemicals in diets has also proven to be effective in controlling coccidiosis (Lee et al., 2022).

8.3 Challenges

8.3.1 Resistance to Anticoccidial Medications

There are still many obstacles to overcome despite improvements in control methods. Drug-resistant *Eimeria* strains have emerged because of the widespread use of anticoccidial medications in poultry farming, making the treatment of coccidiosis more difficult (Abbas et al. 2011). According to Tewari & Maharana (2011), prolonged exposure to these medications can cause genetic alterations in *Eimeria* species, rendering them resistant to widely used anticoccidials. This trend presents a substantial obstacle since it necessitates the ongoing creation of novel drugs or substitute preventative measures, such as vaccinations. Still, the lack of practical molecular methods to track genetic modifications in the parasite makes controlling these resistant strains even more difficult (Blake et al., 2020). This makes monitoring and managing resistance in poultry production a challenging and continuous problem necessitating a timely solution.

8.3.2 Resilience of Oocysts

In addition to the emergence of drug resistance, the environmental resilience of *Eimeria* oocysts is another significant obstacle. It can be difficult to eradicate these oocysts from poultry housing since they can last for long periods in soil and poultry litter under a variety of

temperature and humidity conditions (Tewari & Maharana, 2011). Moreover, *Eimeria*'s short and intricate life cycle enables the parasite to multiply rapidly within hosts, leading to high ambient contamination levels and frequent reinfection within flocks (López-Osorio et al.,2020). Therefore, controlling coccidiosis in turkeys has been compounded by the combination of drug resistance, environmental persistence, and rapid parasite replication which makes eradication of the oocysts in the environment almost impossible.

8.3.3 Challenges of Eimeria Vaccination

Vaccination is a crucial instrument in the fight against the disease, but it also has its challenges, which must be overcome to ensure effective control. Its advantages must be balanced against the difficulties posed by high production costs, complicated logistics, and breed-specific variations in immune responses (Zaheer et al., 2022). Effective vaccines are produced by propagating *Eimeria* strains, which adds to high manufacturing costs and logistical obstacles, such as handling, storage, and administration (reviewed by Soutter et al., 2020) and making vaccines costly. The composition and consistency of vaccines are further complicated by the variation in immune responses among bird breeds and ages. Ineffective vaccine administration can result in uneven immunity within flocks which can lead to overcycling of oocysts and a potential diseased state (reviewed by Shivaramaiah et al., 2014). Despite these obstacles, vaccination remains a practical way to reduce the total cost of coccidiosis. Vaccines provide a more economical option to the ongoing use of anticoccidial medications by improving feed conversion, increasing growth rates, and eventually lowering production losses by averting major disease outbreaks (Chapman, 1996). Most vaccines on the market today are based on live oocysts, which can be challenging to administer and have inconsistent efficacy and performance trade-offs. Therefore, manageable vaccine platforms that can induce long-lasting T-cell memory

without using live organisms for effective coccidiosis control should be created. Therefore, creating more manageable vaccine platforms that can induce long-lasting T-cell memory without using live organisms is crucial for effective coccidiosis control.

8.3.4 Immunity Issues

Incomplete immunity conferred by natural *Eimeria* infection, specifically in the case of single-species infections, can also lead to disease outbreaks on the farm. Following exposure to *Eimeria*, birds develop a certain level of immunity which is species-specific and insufficient for long-term prevention of disease caused by other species (Imai 2018). Consequently, birds remain susceptible to reinfection by the same *Eimeria* species if their immune response weakens due to factors like stress, nutrient deficiencies, or immune system overload from high parasite burdens, and remain vulnerable to infection by different *Eimeria* species (Gadde et al., 2009). Moreover, they continue to be susceptible to infections by different *Eimeria* species (Gadde et al., 2009), underscoring the intricacy of these birds' immunity. The difficulty of sustaining efficient control measures, such as switching up anticoccidial medications and putting vaccination strategies into place, exacerbates this problem (Peek & Landman, 2011).

8.3.5 Farming Systems

Farming systems being practiced can predispose birds to a higher incidence of *Eimeria* infection. One of such systems is intensive and high-density farming systems, which have contributed to the high prevalence of coccidiosis in poultry. Here, birds ingest feces that contain the infective oocyst, become infected, and spread the infection rapidly due to the close contact they have with other birds in the environment (Gao et al., 2024). This coprophagy behavior aids in the spread of the disease because birds are constantly interacting with one another in their

environment. Furthermore, overcrowding has been shown to prompt birds to experience stress, which can manifest as immune suppression and increased susceptibility to disease conditions. Also, stress caused by high stocking densities leads to an elevation in corticosteroid levels and might weaken the immune systems of birds (Ahmad et al., 2023b). Therefore, a conscious effort at controlling coccidiosis outbreaks in such systems needs to be made.

8.3.6 Cost of Management or Control

Another significant concern is the cost of putting complete coccidiosis management measures into place, especially for small-scale or resource-constrained poultry farms (Kadykalo et al., 2018). These expenses include investments in better housing, increased biosecurity, and regular monitoring activities meant to reduce the spread of disease, in addition to vaccines and anticoccidial drugs. Furthermore, due to mild pathogenic effects or the physiological demands of generating an immune response, the administration of live vaccines may result in short-term losses in production performance, such as decreased feed efficiency or weight gain. The financial burden on farmers is exacerbated by economic losses resulting from slower growth rates, inefficient feed conversion, and increased mortality.

Economically driven poor disease control can extend disease outbreaks and increase financial losses by sustaining the *Eimeria* infection cycle (Martins et al., 2022a). Although vaccinations are thought to be cost-effective in the long run, particularly when contrasted with the significant output losses linked to uncontrolled diseases, there are obstacles to overcome in their actual use.

A combination of several methods, including ventilation, humidity control, temperature regulation, appropriate stocking density, and nutritional management, is necessary for effective disease management. These measures all enhance immune function and reduce the spread of

Eimeria (Ahmad et al., 2023). In poultry buildings, keeping the litter dry and clean lowers oocyst survival, while proper ventilation helps regulate humidity, which lowers oocyst survival and infection risk (Mesa-Pineda et al., 2021).

Lesion scoring during necropsies, mortality tracking, and routine fecal examinations to track oocyst shedding enable producers to gauge the severity of the disease and the efficacy of control methods (Snyder et al., 2021).

Coccidiosis management requires a proactive, all-encompassing approach to reduce the disease's impact, safeguard flock health, and keep poultry production economically viable. For complete disease management, a multifaceted strategy combining several control techniques is needed. Reducing the economic effect of coccidiosis requires a comprehensive management strategy that includes vaccination, rotation programs, and bioshuttle tactics (Glorieux et al. 2022). Through reduction of mortality, enhancement of performance, and reduction of the need for medication treatments, these integrated techniques contribute to the economic viability of poultry production.

Reducing the economic effect of coccidiosis requires a comprehensive management strategy that includes vaccination, rotation programs, and bioshuttle tactics (Glorieux et al. 2022). This holistic approach reflects both scientific progress and improved understanding of the interactions between environmental factors, parasite biology, and host immunity (Khater, 2020). Given its persistent economic and production-related impacts, coccidiosis remains a focal point in poultry health research (Attree et al., 2021). Through reduction of mortality, enhancement of performance, and reduction of the need for medication treatments, these integrated techniques contribute to the economic viability of poultry production.

9. Future Directions and Research Gaps in Turkey Coccidiosis Research

There have been several methods of controlling coccidiosis in the poultry industry, which were explained in the previous sections. A combination of rotation programs, vaccinations, and anticoccidial drugs offer a sustainable long-term solution for disease management (Chapman et al., 2002). Proper biosecurity strategies, well-managed litter, and a combination of other control strategies all work to successfully manage coccidiosis. In addition, rotation and shuttle programs, which involve alternating between different anticoccidial medications or incorporating vaccination into drug cycles, help to mitigate the constant menace of drug resistance (Peek & Landman, 2011).

9.1 Vaccination Techniques

The emergence of next-generation vaccines, such as recombinant and DNA-based vaccines, holds promise for providing more precise and long-lasting protection, thereby reducing the need for repeated vaccinations. New generation vaccines consist of viral vector, DNA-based, and recombinant vaccines. The process of creating recombinant vaccines involves introducing *Eimeria* antigens into a different organism, usually a virus or bacterium, which then expresses the antigens. These vaccinations provide more targeted protection than conventional immunizations because they can be made to target *Eimeria* species. Their ability to target several *Eimeria* species at once, including the most harmful to turkeys, *E. adenoiedes, E. gallopavonis*, and *E. meleagrimitis*, can offer broader protection. Immunity against a variety of *Eimeria* species is a problem that is being investigated with recombinant vaccines (Pastor-Fernández et al. 2020a). The use of plasmids containing *Eimeria* antigens is also being used. Plasmids containing *Eimeria* antigens are introduced into host cells as part of DNA vaccines, and the host cells express the antigens to elicit strong humoral and cellular immune responses.

DNA vaccines have shown promise in studies such as Chen et al. (2023), where multiepitope DNA vaccines utilizing conserved antigens from *E. tenella, E. maxima*, and *E. acervulina* improved body weight gain, lesion scores, and oocyst output by 45–63% in chickens against mixed challenges, providing cross-protection. *Eimeria* antigens are introduced into the body by use of modified viruses in viral vector vaccines. These vaccines imitate real infections and use biologically realistic antigen presentation to elicit robust immune responses. For this reason, vectors such as fowl pox viruses are being investigated. Viral vectors, which offer more protection, have also been reportedly used as vaccines against *Eimeria*. Multiple *Eimeria* species may be targeted at once by the use of viral vectors, providing more protection and lowering the possibility of negative consequences (Tewari & Maharana ,2011). Research efforts are currently focused on designing new vaccine formulations that offer broader protection, including against recently identified *Eimeria* species (Pastor-Fernández et al. 2020b).

9.2 Advances in Molecular Diagnostics

Improvements in molecular diagnostics, such as the use of next-generation sequencing and molecular methods, have increased our understanding of the disease in poultry, particularly in turkeys. According to Lee et al. (2022), there have been several strategies that have been explored in understanding host resistance to *Eimeria.* Strategies such as the use of novel vaccine candidates against *Eimeria* were reported. Immune mechanisms governing resistance should be studied to ensure an effective control of coccidiosis. More research on RNA-based vaccines and the modulation of the microbiome can add to the control methods in coccidiosis (Madlala et al., 2021). Knowledge from PCR-based diagnostics helps in early tracking and management of *Eimeria* infections (Caliendo et al., 2013). More molecular advances are expected in a few years that can help in tracking the severity of *Eimeria* infections.

9.3 Selective Breeding

Breeding poultry birds and selecting for resistance to infections can help in the universal control of coccidiosis. By choosing birds with a natural resistance to *Eimeria* infections, the poultry industry can reduce reliance on anticoccidial drugs while aligning with consumer preferences for organic production methods (Quiroz-Castañeda & Dantán-González, 2015).

9.4 Nutritional Strategies

Nutritional strategies such as the use of probiotics, synbiotics, and prebiotics have been used to reduce disease susceptibility. These products help to enhance immune function (Shehata et al., 2022). There has been increasing emphasis on gut health and the role of probiotics in promoting intestinal integrity and immune responses against coccidiosis. Nutritional advancements help to improve vaccine efficacy, address drug resistance issues, and provide a cost-effective management strategy for coccidiosis (Chapman & Jeffers, 2014).

9.5 Conclusion

Coccidiosis is a continuous menace, constantly present in the environment. Oocysts of *Eimeria* are resilient and remain in the environment for a long time in the presence of favorable environmental conditions. Various methods for controlling the disease have been explored in the literature, along with their advantages and challenges. The more we know and understand control strategies in *Eimeria*, the more effective control we can have on the disease. The turkey industry is a small industry that has fewer options available to aid in the control of coccidiosis. The few available drugs that are on the market are becoming resistant to *Eimeria* strain, and vaccines currently available don’t offer cross-protection. Therefore, there is a need to develop more effective strategies to control the *Eimeria* parasite in turkeys. Therefore, this research will aid in

developing more effective strategies for coccidiosis control, which can help address production losses from coccidiosis and prevent the emergence of drug-resistant *Eimeria* strains.

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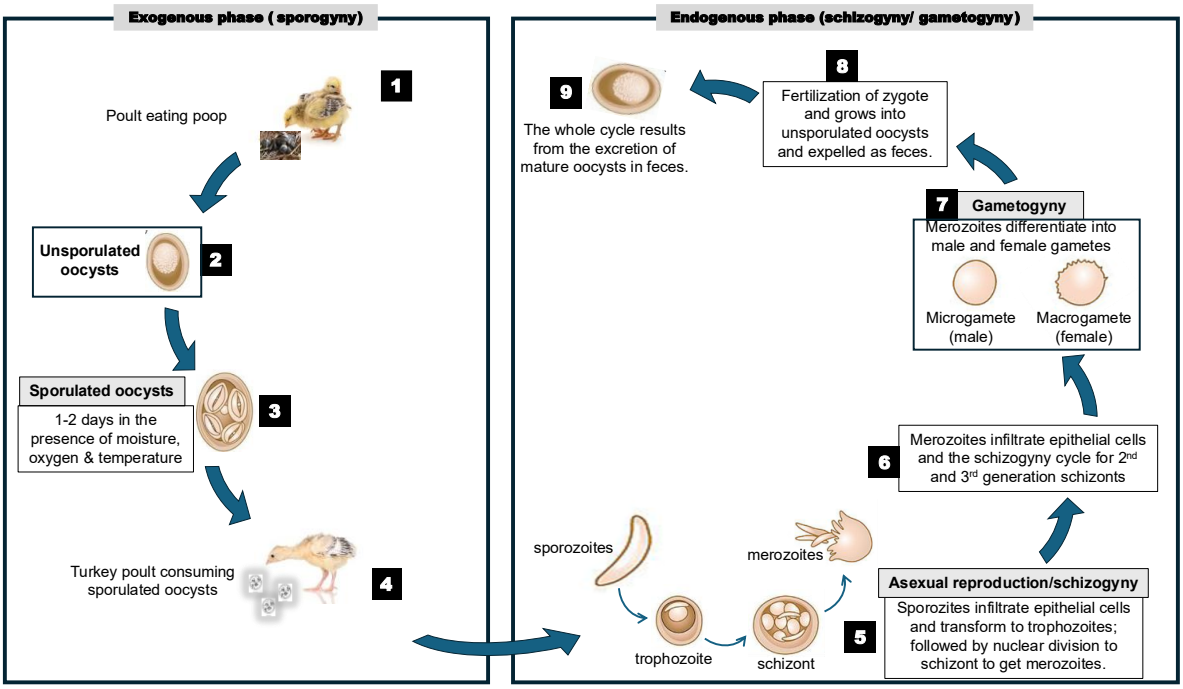

Figure 2. 1 *Eimeria* Lifecycle

The exogenous (sporogony) and endogenous (schizogony/gametogony) phases of the *Eimeria* life cycle alternate. When poults consume sporulated oocysts, sporozoites are released, invading epithelial cells. Merozoites, the result of asexual reproduction (schizogony), differentiate into male and female gametes. The cycle is completed by fertilization, producing zygotes expelled as unsporulated oocysts.

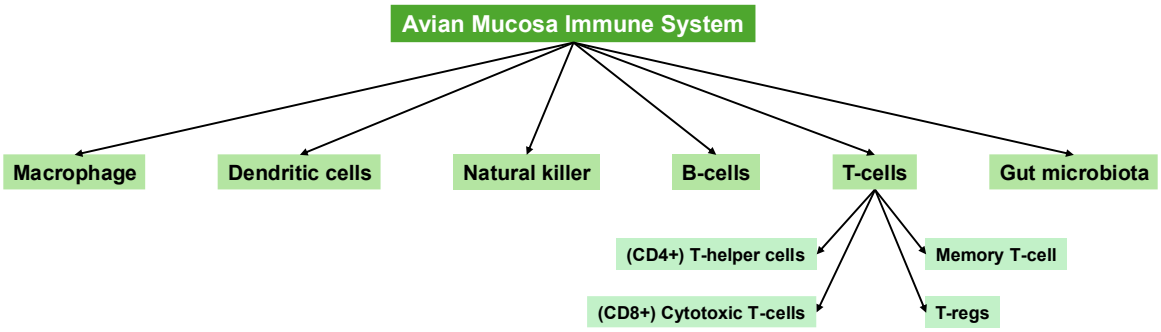

Figure 2. 2 Avian Mucosa Immune System

An illustration showing the various makeup and parts of the Avian Mucosal System, comprising Macrophages, Dendritic cells, Natural killers, B cells, and T cells. T cells further differentiate into helper, cytotoxic, memory, and regulatory subsets. Gut microbiota plays a crucial role in immune modulation, pathogen defense, and maintaining mucosal homeostasis.

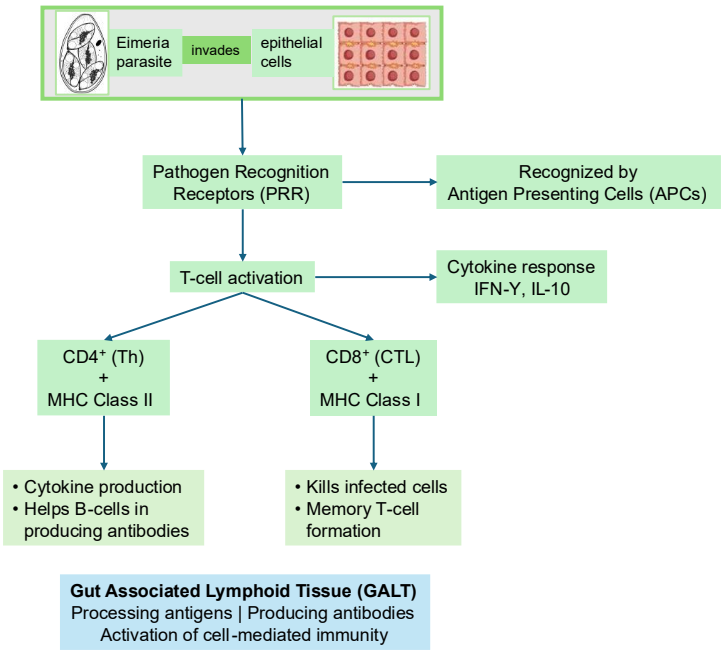

Figure 2. 3 Mechanisms of avian host T-cell responses against *Eimeria* pathogens

Eimeria parasites activate pathogen recognition receptors (PRRs) by invading epithelial cells. The pathogen is recognized by antigen-presenting cells (APCs), which then trigger T-cell activation and cytokine responses (IFN-γ, IL-10).

CHAPTER 3

Evaluating the Role of Vaccination and Oocyst Management in Modulating Immune Responses of *Eimeria*-Challenged Turkey Poults

Abstract

This study aimed to assess the effects of *Eimeria* vaccination and amprolium treatment on growth performance, fecal oocyst shedding, and immune response in tom poults challenged with mixed *Eimeria* species. It was hypothesized that vaccination and amprolium treatment would reduce fecal oocyst shedding, improve growth performance, and modulate the immune response in poults challenged with *Eimeria*. Two experiments were completed; experiment 1 evaluated the effect of vaccine and amprolium supplementation on OPG cycling. A total of 500 tom poults were assigned to five groups in Experiment 1: (a) non-vaccinated, non-inoculated (NVNI), (b) low dose (L), (c) low dose with amprolium (LA), (d) high dose (H), and (e) high dose with amprolium (HA). On d1, 40% of poults in the L & LA group were vaccinated with 100 oocysts/bird of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis,* while 70% of poults in the H & HA group were vaccinated with 500 oocysts/bird of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis*. For experiment 2, a total of 345 tom poults were randomly assigned to three groups: (a) NV, (b) V group vaccinated with 40% of poults receiving 100 oocysts of *E. adenoeides, E. gallopavonis,* and *E. meleagrimitis* on d1, and (c) VA group vaccinated with 30% of poults receiving 100 oocysts of mixed *Eimeria* on d1 and medicated with amprolium from d8 to d12. On d21, V and VA were inoculated with 25,000 oocysts/poult of mixed *Eimeria* species. Performance was measured on d1, d21, and d28, while fecal oocyst shedding was monitored from d6 to d28 at 2-day intervals. Lesions in the ceca and duodenum were scored on d28. Immune responses were assessed by mRNA expression (IFNγ, IL-10, IL1β, CD40, FOXP1, IL-

6, and TGFβ2) analysis in the ceca and cecal tonsils, and Immune cell profiling in the cecal tonsils. Data were analyzed using one-way ANOVA, with significance at $P < 0.05$. There were no significant differences ($P > 0.05$) in weight gain between the treatment groups. Fecal oocyst shedding was significantly reduced in the treatment groups, with the VA group showing the lowest oocyst count ($P < 0.05$) on d10, d14, d18, d26, and d28. Immune analysis revealed that the most significant immune modulation occurred in the VA group. Overall, vaccination combined with amprolium treatment improved performance, reduced oocyst shedding, and mitigated intestinal damage while modulating the immune response, thereby offering an effective strategy for managing coccidiosis in poultry.

Keywords: *Eimeria*, Poults, Vaccination, Amprolium, Mucosal Immunity, Coccidiosis

Introduction

The poultry industry faces significant challenges owing to a variety of severe diseases, including those caused by *Eimeria* species, which are responsible for coccidiosis in turkeys (Attree et al. 2021; Cervantes et al., 2020). *Eimeria* affects and multiplies in different parts of the gut. They vary by species, and in turkeys, several species affect different regions throughout the life cycle, often beginning in early stages of replication in one portion and moving on to another in later stages (Gumina et al. 2021; Lillehoj and Trout 1996). Coccidiosis leads to production losses by impairing growth, increasing mortality, and increasing the cost of controlling disease. The direct and indirect costs of enteric diseases such as coccidiosis represent a significant threat to both poultry productivity and profitability (Blake et al. 2020). It is therefore pertinent to find ways to control coccidiosis with strategies that promote immune modulation without risking clinical coccidiosis that leads to production losses.

Vaccination against *Eimeria* using live oocysts, either with wild type or attenuated strains, is an effective method for the management of coccidiosis by promoting immunity through regulated infection (Xicheng et al. 2004). However, vaccination success is closely tied to the immunological response triggered by the parasite and the dynamics of the oocyst cycle in the environment (as reviewed by Shivaramaiah et al., 2014). *E. meleagrimitis*, *E. gallopavonis*, and *E. adenoiedes* are among the most common *Eimeria* species that infect turkeys and are associated with high morbidity and mortality (Duff et al. 2022; Imai and Barta 2019). Assessment of immune responses to vaccination can be done by using organs of birds associated with infection and also systemic immune response, such as ceca, liver, spleen, and cecal tonsils (as reviewed by Shivaramaiah et al., 2014).

The period during which birds are exposed to live *Eimeria* and the time it takes them to develop protective immunity is essential to prevent clinical coccidiosis, which can be managed by effective control of *Eimeria* oocyst cycling (Snyder et al. 2021). Infection from *Eimeria* might occur when unvaccinated poults are exposed to high amounts of *Eimeria* oocysts from vaccinated poults. Also, high vaccine dose can lead to increased morbidity and mortality while oocysts cycle through a flock before immune protection is completely developed (Yeboah et al., (2025). Consequently, managing oocyst cycling, especially after vaccinating a flock, can help prevent overcycling before protective immunity is achieved by birds (Gadde et al., 2009).

Understanding and studying oocyst cycling patterns after vaccination and developing strategies to prevent overcycling during this critical period before immunity is developed are important. Developing preventive measures against *Eimeria* infection needs a thorough understanding of immune gene responses in infected turkeys. In this study, we used low-dose vaccine containing mixed *Eimeria* species to vaccinate day-old turkey poults, followed by a challenge with the mixed *Eimeria* species and supplementation with amprolium. Oocyst cycling patterns, performance indices, immune gene responses of some tissues, and cellular responses in the cecal tonsils were all monitored.

Materials and Methods

For both experiments described below, poults were reared under age-appropriate temperature and lighting conditions according to Aviagen Turkeys guidelines (Aviagen Turkeys 2024). A nutritionally complete diet and water were provided *ad libitum* throughout the duration of each study. An autogenous mixed *Eimeria* vaccine created in our laboratory from field isolates in 2019 was propagated for use in both experiments. Challenge strains were homologous to the vaccine.

Experiment 1

The animal handling procedures for this experiment adhered to The Ohio State University's Institutional Animal Care and Use Committee guidelines, as documented in protocol number **2019A00000015**.

Experimental Design

A total of 500 tom poults were randomly assigned to five treatments, with five replicates and 20 birds per pen, using a completely randomized design. The treatments were as follows: (i) non-vaccinated, non-inoculated (NVNI), (ii) low dose (L), (iii) low dose with amprolium (LA), (iv) high dose (H), and (v) high dose with amprolium (HA). On d1, 40% of poults in the L & LA group were vaccinated with 100 oocysts/bird of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis,* while 70% of poults in the H & HA group were vaccinated with 500 oocysts/bird of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis*. The litter of birds in all groups except NVNI was lightly sprayed with water daily through d7 to help maintain moisture content needed for sporulation of oocysts. Amprolium at 1.25 mL/L was added to the water of birds in the LA and HA groups from d8-12.

Oocysts per Gram (OPG)

Fecal samples were collected from each pen between d6 and d32 to calculate OPG. The study lasted 32 days.

Experiment 2

The Institutional Animal Care and Use Committee of North Carolina State University (IACUC #: **23-256-01**) approved this study.

Experimental Design

Experiment 2 was built off experiment 1. A total of 345 tom poults (Aviagen Turkeys) were randomly assigned to one of the three treatment groups, with five replicate pens and 23 poults per pen. The three treatment groups were: i) non-vaccinated, non-inoculated (NV), ii) vaccinated (V), and iii) vaccinated with amprolium (VA). VA group was treated with amprolium at 1.25 mL/L in water from d8-12. A commercial turkey management guide (Aviagen Turkeys) was adhered to in terms of temperature and illumination.

On d1, 40% of the poults in the V and VA groups were vaccinated with 100 sporulated oocysts/bird of *E. adenoiedes, E. gallopavonis*, and *E. meleagrimitis.* On d21, poults in the V and VA groups were orally challenged with 25,000 sporulated oocysts/bird of *E. adenoiedes, E. gallopavonis*, and *E. meleagrimitis* to simulate coccidiosis challenge after vaccination.

Body weight (BW) and body weight gain (BWG) of the poults were measured on d1, d21, and d28. Feed intake (FI) and feed conversion ratio (FCR) were calculated concurrently with BW and were corrected for mortality. Mortality was checked and recorded daily.

Oocysts per gram (OPG)

From d6 to d28, feces were collected at 2-day intervals to monitor oocyst shedding for Experiment 2. At each collection, for both Experiment 1 and 2, 6-8 fresh excreta droppings were collected from each pen and stored at 4 °C until further processing. In accordance with Chasser et al., (2020), fecal samples were gathered in bags, weighed, and suspended in a three-fold dilution of 2% PDC (Sigma-Aldrich, Co., 3050 Spruce Street, St. Louis, MO, USA). For OPG, feces were thoroughly mixed, and 1 mL of the mixture was diluted with 9 mL of saturated salt solution and allowed to float for 5 minutes. The number of oocysts shed was evaluated using a McMaster chamber (Jorgensen Laboratories, Loveland, CO, USA).

Lesion Scoring

Following the protocol of Gadde et al. (2020) in Experiment 2, the ceca and duodenum were collected on d28 for lesion scoring (n=5/replicate) on a scale of 0-4. Lesion scores were determined based on intestinal content, intestinal wall thickening, and lesions unique to *Eimeria*.

Tissue Collection for Gene Expression Study

Ten poults per treatment group from Experiment 2 were obtained by randomly selecting two poults from each pen on d28 and euthanized for tissue collection. Cecal tonsil and cecal samples were collected for gene expression analysis. PBS (Sigma–Aldrich, St. Louis, MO, USA) was used to thoroughly rinse the tissues to remove any remaining debris and luminal content, thus ensuring clean samples for molecular analysis.

Gene expression

The ceca and cecal tonsils of turkey poults were preserved in RNA preservation solution (RNAlater, Invitrogen, Carlsbad, CA) and stored at -80 °C until they were processed. To isolate RNA, a 1-cm slice of tissue from each treatment group's poults was removed. Following the labeling of each screw cap homogenization vial, these pieces were placed into a milliliter of TRI Reagent™ Solution. Each sample was homogenized for 20 s using a bead beater. An RNeasy kit (QIAGEN, Hilden, Germany) was used to isolate RNA following the manufacturer's instructions. A NanoDrop2000 (Thermo Fisher Scientific, Waltham, MA) was used to measure RNA levels and ensure correct dilution. The RNA was subsequently transformed into cDNA for the quantitative polymerase chain reaction (qPCR) using a high-capacity synthesis kit (Applied Biosystems Waltham, Massachusetts, USA). Each well received 2.5 ng of sample, 500 nM gene-specific forward and reverse primers (Table 3.1), and 2X Power SYBR Green Master Mix (Applied Biosystems, Waltham, Massachusetts, USA). The volume was equalized to 20 µL by

adding H_2O. Applied Biosystems StepOnePlus real-time PCR system IFNγ, IL-10, CD40, FOXP1, IL-6, and TGFβ2 levels in the cells were evaluated using qPCR. For each reaction, 40 cycles were performed at 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, followed by 10 mins of initial denaturation at 95 °C. The data were then standardized using β-actin expression, a fundamental housekeeping gene. For ease of interpretation, the reciprocal was computed using the formula: 1/ (target gene cycle threshold/beta-actin cycle threshold). Each piece of data is shown as the CT^{-1} reciprocal cycle threshold. ΔΔCT was calculated by averaging each treatment CT^{-1} after setting NVNI as the control. The average NVNI was subtracted from each CT^{-1} to determine ΔCT. The ΔCT readings and ΔΔCT results were the same, since NVNI was used as the control. The $2^{-\Delta\Delta Ct}$ technique was employed to estimate relative gene expression by standardizing the ΔCt values of the target gene against the reference gene β-actin.

Flow Cytometry of Cecal Tonsil Tissue

For samples collected on d28 in Experiment 2, a previously established procedure was used to generate single-cell cecal tonsillocyte preparations from 28-day-old poults for immunophenotyping using flow cytometry (Gaghan et al. 2022). Anti-chicken monoclonal antibodies of mouse origin were acquired from Southern Biotech (Birmingham, AL, USA) and were used in this study. Antibodies against mannose receptor C1-like B (MRC1L-B) (Monocyte/Macrophage lineage, clone KUL01), γδTCR (TCR-1), CD4 (CT-4), CD8 (CT-8 recognizing CD8a chain), CD28 (AV7), and MHC-II (2G11) were used in the first panel. Dead cells were eliminated using live/dead near IR, a cell viability dye (Invitrogen, CA, USA). The cells were stained using a cocktail that included TCR1, 2, or 3 linked to Rphycoerythrin (R-PE, 0.14 mg antibody/10^5 cells), CD4 coupled to fluorescein isothiocyanate (FITC, 1.2 mg antibody/10^5 cells), and CD8 coupled to Cyanine 5 (Cy5, 0.4 mg antibody/10^5 cells). Prior to

data collection on an LSR-II Flow Cytometer (BD Biosciences, Franklin Lakes, NJ, USA), stained cells, fluorescent minus one staining controls, and single-stained compensation were cleaned and fixed with 4% paraformaldehyde (Kulkarni et al. 2017). FlowJO Software (Tree Starr, Ashland, OR, USA) was used to analyze the data. Using the forward scatter (FSC) and side scatter (SSC) height and width coordinates, doublet cells were excluded as part of the gating method. Only living cells that tested positive for CD4 were gated.

Statistical Analysis

The Generalized Linear Model (GLM) process was used to analyze the data using JMP Pro 18.0.2 software (SAS Institute Inc., Cary, NC, USA). OPG data were analyzed as a completely randomized design using the Proc Mixed Procedure of SAS (SAS 6.4 software, SAS Inc., 2016). Tukey's Honestly Significant Difference (HSD) test was used to determine significant differences between the treatment means. Statistical significance was set at $P < 0.05$.

Results

Oocyst counts

There were significant differences ($p < 0.05$) in OPG on all days, except days 21 and 27 in Experiment 1 (Table 3.2). At d15, the L group exhibited very high oocyst shedding (2,388,809 ± 1,430,196 OPG), possibly related to simulated low uptake (40% inoculation) on DOH. The LA group consistently showed low oocyst shedding throughout the experimental period, peaking at only 107,858.8 ± 97,919.97 OPG on d21, and consistently lower than all other groups. Both H (807,192 ± 86,192.34 OPG) and HA (318,081.6 ± 57,697.42 OPG) peaked earliest on d9, and HA exhibited low OPG levels throughout the rest of the experiment after amprolium treatment. The H group oocyst shedding remained high for another week, but the poults likely showed

immune protection after d15 (366,033.6 ± 137,013.4 OPG) when oocyst shedding declined. By d12 NVNI began showing signs of cross-contamination between pens with OPG of 659.6 ± 213.36 that rose to 296,819.8 ± 138,775.5 OPG on d24 before beginning to decline.

Table 3.3 shows the oocyst counts in the second study. Significant differences ($P<0.05$) were observed in OPG on d8, d10, d14, d18, d26, and d28. Consistent with experiment 1, the V group had a spike in oocyst shedding that was notably higher than VA, which peaked on d8 with only 34,845.12 ± 13,253.27 OPG. After disease challenge, both V (90,442.8 ± 37,564.05 OPG) and VA (61,325.28 ± 29,675.7 OPG) exhibited a slight increase in OPG on d28.

Regression Analysis of Total OPG

Regression analysis in Experiment 1 (Table 3.4) revealed that treatment is an important predictor of total OPG ($P = 0.0163$). 44% of the variation in total oocyst counts was explained by the model ($R^2 = 0.44$). Poults in the L group (3,293,260.2 ± 1,414,550.133 OPG) shed considerably more ($P<0.05$) oocysts than those in the NVNI group (396,663.2 ± 133,667.89 OPG). NVNI did not differ significantly from the H, HA, or LA groups in terms of total oocyst shedding ($P>0.05$). According to these results, the L treatment shed more total OPG, while the other treatments had no discernible effect ($P > 0.05$) on shedding levels compared to the NVNI group. Additionally, in regression analysis, the LA (237,522.4 ± 81,147.39 OPG) group had the lowest estimate for overall shedding, although this difference was not statistically significant ($P > 0.05$; Table 3.4).

Regression analysis in Experiment 2 (Table 3.4) revealed that treatment is an important predictor of total OPG ($P = 0.0616$). Approximately 37% of the variation in total oocyst counts was explained by the model ($R^2 = 0.37$). Poults in the V group (1,110,448.44 ± 567,247.65 OPG) shed significantly more oocysts than those in the control group ($P < 0.01$). NV group (0 ± OPG)

had the lowest overall shedding, though no difference (P>0.05) existed between the treatment groups.

Table 3.5 shows the predictive analysis of total OPG after vaccination and total OPG after challenge in Experiment 2. Total OPG after vaccination (345995.88 ± 220670.26 OPG) does not significantly(P>0.05) predict total OPG after challenge (51,690.48 ± 18,101.11 OPG). Less than 1% (0.000011) of the variation in Total OPG after challenge is explained by Total OPG before vaccination.

Growth Performance & Lesion Scores

The results of the effect of the mixed *Eimeria* vaccine and amprolium supplementation on the growth performance of turkey poults in experiment 2 are shown in Table 3.6. No significant differences were observed in any growth parameters measured (P > 0.05).

The effect of vaccination and challenge with *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis* on ceca and duodenum lesion score is also shown in Table 3.6. In all treatment groups, 72% of the poults exhibited a ceca lesion score of 0, whereas 28% had a ceca lesion score of 1. In the NV group, 88% of poults displayed a duodenum lesion score of 0, with the remaining 12% showing a lesion score of 1. In contrast, 84% of poults in the V group had no duodenum lesions (score 0), 12% had a score of 1, and 4% had a score of 2. Lastly, 84% of the poults in the VA group had a duodenum lesion score of 0, and 16% had a score of 1. This reflected low OPG observed on d28.

Gene expression

The effect of vaccination and amprolium supplementation on the gene expression profile of *Eimeria*-infected turkey poults is shown in Table 3.7. The ceca and cecal tonsil tissues were collected on d28, and gene expression was analyzed. Despite the fact that the VA group had

higher numerical values for every parameter, no significant changes ($P > 0.05$) were found for any of the assessed parameters in the cecal tissue. Nonetheless, there was a significant difference ($P < 0.05$) in the cecal tonsils CD40 expression across the treatment groups, with the VA group exhibiting the highest levels.

Flow Cytometry (Immunoprofiling)

The frequencies of macrophages, γδ T-cells, and different T-cell subsets ($CD4^+$, $CD8^+$, and expression of MHC-II and CD28 activation molecules) were measured using flow cytometry to evaluate vaccine-induced cellular responses in the systemic and mucosal lymphoid tissues of poults challenged with *E. adenoeides*, *E. gallopavonis*, and *E. meleagrimitis*. Figure 3.1A shows the gating approach used for precise characterization of these cell types. Figure 3.1B displays the cellular reactions to the challenge and vaccine in the cecal tonsils.

The frequency of gδ T-cells ($g\delta TCR^+$) in the cecal tonsils increased significantly ($P < 0.05$) in the V group compared to the control (NVNI). However, no significant difference ($P>0.05$) was observed between the VA and either V or NV treatment groups. The frequency of $CD4^+$ T cells in the cecal tonsils at d28 was significantly higher ($P < 0.05$) in both VA and NV groups compared to the V group. Additionally, the frequency of live gated $CD4^+CD8^+$ double-positive (DP) cells was significantly higher ($P<0.05$) in both VA and V groups than in the NV group. Additionally, on d28, the V group had a significantly greater ($P < 0.05$) number of activated $CD4^+$ $MHC\text{-}II^+$ T cells in the cecal tonsils than the NV group. However, there was no significant difference ($P>0.05$) between either the NV and VA groups or the V and VA groups. In addition, the VA group had significantly higher ($P < 0.05$) $CD8^+$ $MHC\text{-}II^+$ T-cells than those in both the NV and V groups. However, there were no appreciable differences ($P > 0.05$) in the frequencies of KUL-01+ macrophages and $CD8^+$ T cells among the treatment groups.

Discussion

Strategies to manage oocyst cycling and develop vaccine strategies against *Eimeria* species in challenged turkey poults using either vaccine or in combination with amprolium were evaluated in our study. Presently, the use of vaccines has been increasing without a detailed characterization of the protection that is gained from their use. To effectively evaluate vaccine potential in challenged turkey poults, we used a vaccine containing *Eimeria adenoeides, E. gallopavonis*, and *E. meleagrimitis* in the study.

Fecal oocyst shedding is used to gauge coccidial infection severity, as oocysts are intermittently shed and re-ingested by birds, leading to increased oocyst numbers in each subsequent cycle (Chapman et al. 2013; Duff et al. 2020). The changes in oocyst patterns that were observed in both studies (Table 3.2 and 3.3) revealed an interaction between immune system priming and the suppressive ability of medical intervention. Birds in the LA and HA groups exhibited some shedding patterns characterized by an initial increase, followed by a moderate or low shedding of oocysts, which was similar in both studies. The reduction in fecal oocyst output was significantly greater in poults receiving both the vaccine and amprolium than in those administered the vaccine alone, corroborating the findings reported by Trujillo-Peralta et al. (2023). This suggests that while amprolium helps limit the rate at which *Eimeria* replicates during the immune-naïve period, and ultimately, vaccination helps improve the host immune response, thereby reducing post-challenge oocyst shedding.

There was a sharp increase in oocyst shedding in the L group on d15, which might be due to a delayed but vigorous replication of *Eimeria* strains from the vaccine. This might also be due to suboptimal initial vaccine amount administered to 40% of birds in that treatment group. This was consistent with earlier reports of variable cycling with partial vaccine dose exposure

(Dasgupta and Lee 2000). Conversely, the LA group maintained a consistently lower oocyst output that peaked modestly on d21, indicating that intermittent anticoccidial treatment that interrupts oocyst output may overcome negative side effects of inconsistent or poor vaccine uptake on day of hatch. Ideally, this strategy could also be implemented in the case of vaccines that contain strains at risk of cycling too high before flock immunity develops.

Conversely, the LA group of experiment 1 maintained a consistently low oocyst output that peaked modestly on d21, indicating that intermittent anticoccidial treatment that interrupts oocyst formation may overcome negative side effects of inconsistent or poor vaccine uptake on day of hatch. Ideally, this strategy could also be implemented in the case of vaccines that contain strains at risk of cycling too high before flock immunity develops. These findings support a type of bioshuttle mechanism, wherein limited replication is sufficient to stimulate protective immunity while minimizing mucosal damage (Trujillo-Peralta et al. 2023). The pattern of fecal oocyst shedding observed in this study mirrored the typical reproductive cycle of *Eimeria*, a trend previously documented in poultry infected with mixed *Eimeria* species (Cha et al. 2018). Thereby highlighting the importance of timing associated with the prevention and control of coccidiosis.

In our study, vaccination did not negatively impact growth performance, aligning with our objective to stimulate an immune response without adverse effects. Additionally, we didn't observe a change in growth performance after *Eimeria* challenge, which suggests that the challenge might not have been strong enough to induce a change in performance, which is consistent with the information from lesion scores. Notably, a non-vaccinated, challenge group was not included in the study to confirm vaccine effect on challenge since the ultimate goal was to document shedding and immune response related to vaccination with or without amprolium.

The findings from our study and comparable results from Li et al. (2004) indicate that the effective use of vaccines with other control strategies could help improve the health of birds. Therefore, combining *Eimeria* vaccine with amprolium can lead to a modulation of *Eimeria* lifecycle, enhancing nutrient absorption, reducing inflammation, and maintaining intestinal integrity. Vaccinating after supplementing the diets of birds with anticoccidials helped prevent reduced growth associated with vaccine use and proper development of protective immunity (Dalloul and Lillehoj 2006a).

Lesion scoring is an effective technique for assessing the severity of *Eimeria* infections; however, lesions are less frequently seen in turkeys as opposed to chickens (Johnson and Reid 1970). Our study shows that lesion scores were low for all treatment groups in the ceca and duodenum (Table 3.6). Our results suggest that the *Eimeria* challenge was abated by vaccination, but not proven due to lack of a non-vaccinated challenge group within the experiment, which is consistent with low OPG results. Vaccinations alone and supplementing with amprolium provided partial protection but did not fully prevent duodenal lesions. The findings align with those reported by Behl, (2011); Conway et al. (1999); Hong et al. (2006) who stated that oocyst levels in infected birds do not directly correlate with lesion scores. Therefore, tracking oocyst shedding, weight gain, feed intake, and feed conversion might be more effective in determining the severity of *Eimeria* infection in turkey poults than lesion scores (Conway et al. 1999).

To further characterize the effect of *Eimeria* on turkeys, gene expression profiling in the ceca and ceca tonsils of turkey poults provided valuable insights into vaccine effect and amprolium supplementation on the immune response. No significant differences were observed in the ceca (Table 8). Our results contradict those of Hong, who showed that moderate *Eimeria* replication is sufficient to induce the transcriptional activation of cytokines crucial for mucosal

immunity in chickens, and this suggests that measurable changes in innate immune function may require a higher level of infection, even though challenge results indicated a likely memory immune response to day of hatch vaccination. It could also indicate that immune responses of chickens to *Eimeria* infection are not a good proxy for predicting turkey immune response and more research is needed to fully understand how turkey poults react to infection.

CD40, a costimulatory molecule essential for T cell-APC interactions, helps in the development of adaptive immunity (Elgueta et al. 2009). In our study, CD40 was significantly higher in the VA group. This indicates that vaccinating and supplementing with amprolium in a challenge state with *Eimeria* leads to an enhanced immune response due to the activation of antigen-presenting cells (APCs) in that treatment group. CD40 upregulation is important for eliciting effective immune response against *Eimeria*. It helps in enhancing antigen presenting cells and with the promotion of Th1 needed for removing intracellular parasites such as *Eimeria* (Grewal and Flavell 1998). Although our study did not reveal significant changes in gene expression within cecal tissue, increased expression of CD40 in the cecal tonsils suggests that the bioshuttle strategy benefits immune responses in birds. Further research, likely involving a stronger infection with *Eimeria*, is necessary to further elucidate the dynamics of gene expression in the general defense against *Eimeria* infection. It is also possible that peak innate response timing did not align with sample collection and should be considered when planning further experiments related to immune response of poults to coccidiosis.

Despite low innate immune function changes, flow cytometric analysis on day 28 (Figure 3.1) revealed differences among macrophages, γδ T cells, $CD4^+$ and $CD8^+$ T-cell populations, and their activation states, as marked by MHC-II and CD28 expression. V and VA groups had significantly higher ($p<0.05$) frequencies of γδ T-cells and numerically increased macrophages

(KUL-01^{+}) seven days after *Eimeria* challenge. This shows enhanced innate immune priming, which is consistent with the known role of macrophages and γδ T cells as early responders in mucosal immunity (Bonneville, O'Brien, and Born 2010). These might be due to the γδ T cells contributing to antigen presentation in the cecal tonsil and moderate replication of *Eimeria* without excessive damage in the birds in both groups (Lee, Lu, and Lillehoj 2022).

The data also revealed increased frequencies of $CD4^{+}$ $CD8^{+}$ T (DP) cells in V and VA poults, with the VA group showing a marginally higher proportion than those in the V group. Notably, V and VA groups showed higher ($P<0.05$) DP cell frequencies than NV, suggesting adaptive immune cell priming was induced in the vaccinated groups. These findings were in line with earlier studies demonstrating the role of T-lymphocytes in controlling *Eimeria* infections (Dalloul and Lillehoj 2006b; Lillehoj and Trout 1996). Activation markers of T-cells, such as MHC-II and CD28, were upregulated in the vaccinated groups (V and VA) as observed in the study, which connotes a robust and prolonged immune activation profile. This shows enhanced antigen presenting capacities. In addition to the increased activation markers, CD28, indicating an environment for T-cells that supports the differentiation of memory T cells (Weng, Akbar, and Goronzy 2009).

These features show that the immune system of turkeys in vaccinated groups are capable of eliciting effective immune responses to secondary infection or challenge. Therefore, the use of live oocyst vaccine is capable of inducing mucosal immunity and supports a bioshuttle strategy for controlling turkey coccidiosis, which helps to promote activation of T-cells, reducing intestinal damage, and providing long-term protection to birds, as emphasized by Kim et al. (2019). Immune response changes in the study reveal that mixed *Eimeria* vaccine can elicit immune response in cecal tonsils of turkey poults.

Conclusion

This study shows that supplementing a low-dose mixed *Eimeria* vaccine with amprolium offers the potential for effective immune protection against coccidiosis in turkey poults. The use of amprolium in combination with vaccine helped reduce fecal oocyst shedding without a negative impact on performance, and protecting the mucosal integrity which is important for immunity against coccidiosis. These are supported by enhanced $CD4^+$ and $CD8^+$ T-cells, elevated CD40 expression in the cecal tonsils, and no change in performance indices as compared to non-vaccinated poults. Therefore, using amprolium mid-cycling can help reduce the risk of clinical coccidiosis without any obvious decrease in vaccine efficiency, especially in farms that tend to over-cycle oocysts and have vaccine-related coccidiosis.

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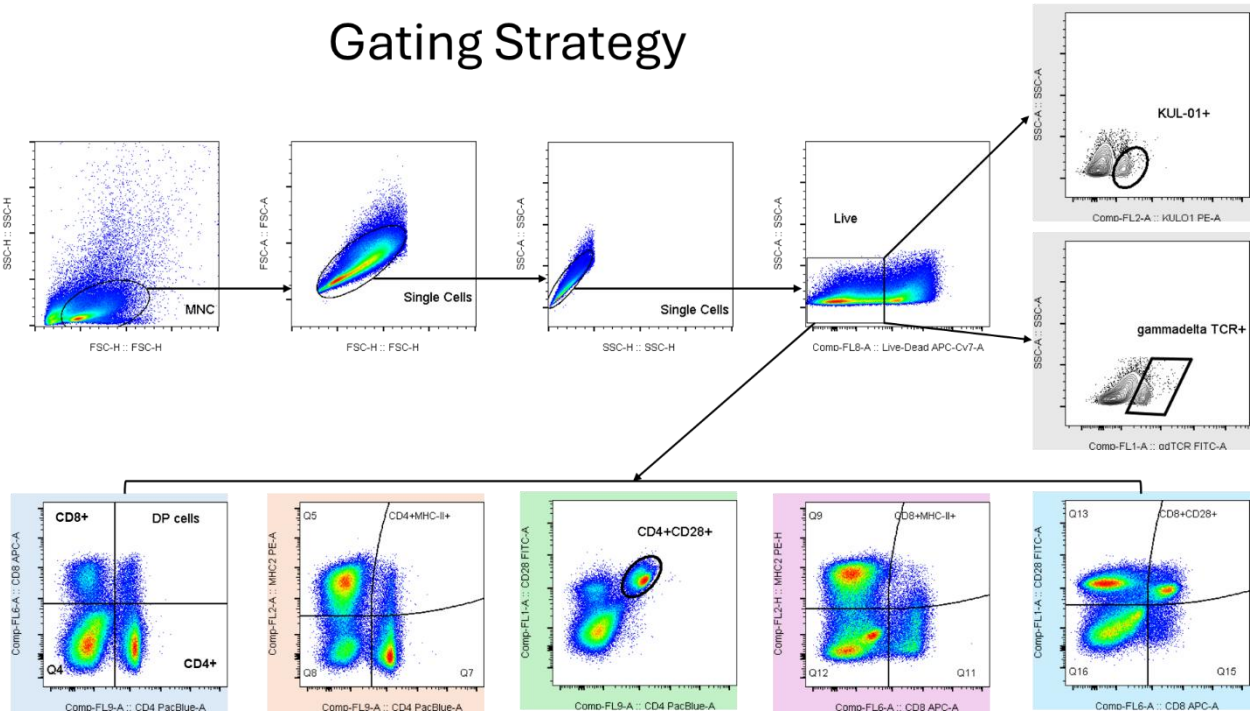
Figure 3. 1 Effect of mixed *Eimeria* vaccine-induced cecal tonsil cellular response in turkey poults. On d1, 40% of poults in the V and VA groups were vaccinated with 100 sporulated oocysts/bird of *E. adenoiedes, E. gallopavonis*, and *E. meleagrimitis.* The poults were challenged with 25×10^3 sporulated oocysts/bird of *E. adenoiedes, E. gallopavonis*, and *E. meleagrimitis* on d21. Cecal tonsillocytes were collected on d28. Two panels were used: panel 1 contained macs (KUL-01^+) and γδTCR^+ cells. All T-cell stains were used in Panel 2 for immunophenotyping analysis using flow cytometry. Part A shows the gating approach used to gate living cells after excluding doublet cells based on their height, width, and forward and side scatter. The % frequencies of gated live cells of macrophages, γδT cells, T-cell subsets ($CD4^+$ and $CD8^+$), and T-cell expression of MHC-II and CD28 activation markers are shown in Part B. Significant differences exist between bars that lack a common letter ($p < 0.05$), as shown in the figure.

A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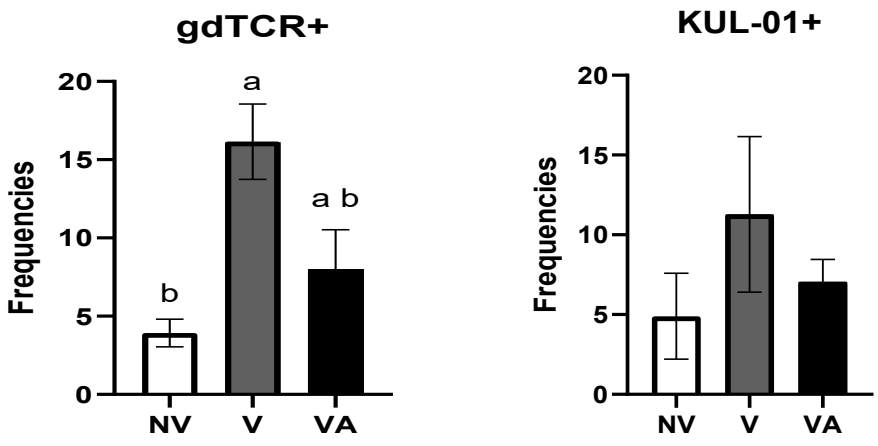
Single Cells
Single Cells
Live
KUL-01+
gammadelta TCR+
CD8+
DP cells
CD4+
Q4
CD4+MHC-II+
Q5
Q8
Q7
CD4+CD28+
CD8+MHC-II+
Q9
Q12
Q11
CD8+CD28+
Q13
Q16
Q15

B.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KUL-01+
Frequencies
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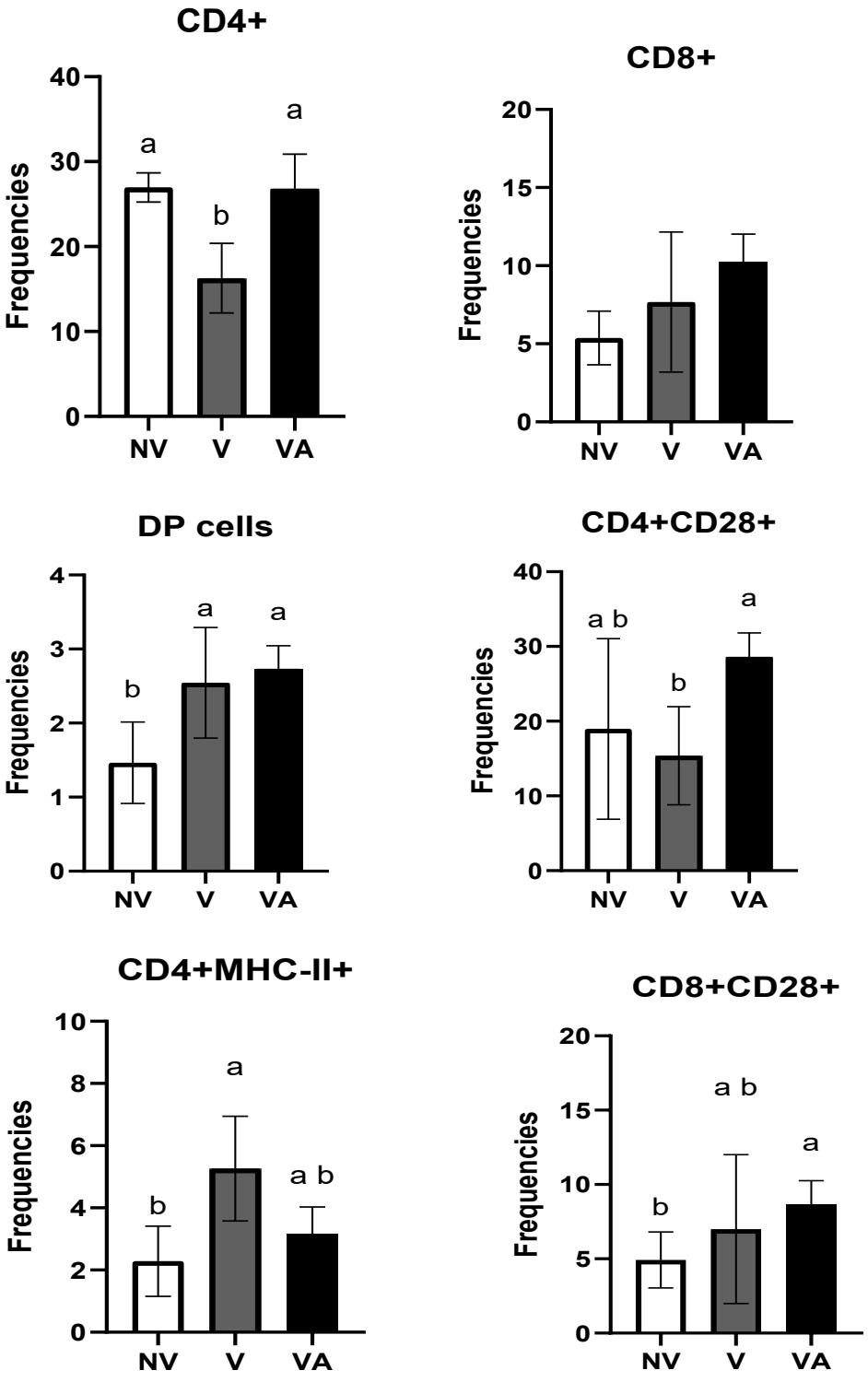
15
10
5
0
a
b
a b
NV
V
VA


CD4+
Frequencies
40
30
20
10
0
a
b
a
NV
V
VA
CD8+
Frequencies
20
15
10
5
0
NV
V
VA
DP cells
Frequencies
4
3
2
1
0
b
a
a
NV
V
VA
CD4+CD28+
Frequencies
40
30
20
10
0
a b
b
a
NV
V
VA
CD4+MHC-II+
Frequencies
10
8
6
4
2
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b
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a b
NV
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CD8+CD28+
Frequencies
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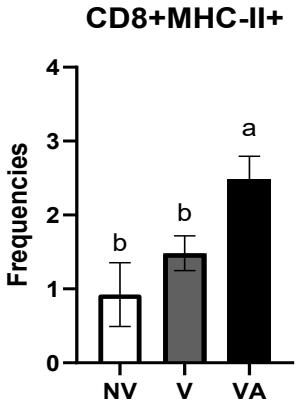
CD8+MHC-II+
Frequencies
4
3
2
1
0
b
b
a
NV
V
VA

Table 3. 1 qPCR Primers, Experiment 2.

Gene	Forward Primer Sequence	Reverse Primer Sequence
β-Actin	GGCTCTATCCTGGCCTCTCT	CATCACAAGGGTGTGGGTGT
IFNγ	TGAACTGAGCCATCACCAAGAA	ACCGTCAGCTACGTCTGAATG
IL-10	CGCTGTCACCACTTCTTCAC	CCCGTTCTCATCCATCTTCTCG
CD40	TTCAGATGTGATCTGTGAGTCGAG	CTCTTGCGCCACGGGCT
FOXP1	AGGCTGGATAGTCAGAAGTGC	AATAGGTCACTCTTCTTACCCCATT
IL-6	CAGAAATCCCTCCTCGCCAA	TCAAATAGCGAACGGCCCTC
TGFβ2	TGTTGCCTGCGTCCACTTTA	TAAATCCTGGGACACGCAGC

Abbreviations: β-Actin, beta actin; IFNγ, interferon gamma; IL-10, interleukin 10; CD40, CD40 molecule; FOXP1, fork head box transcription factor P1; IL-6, interleukin 6; TGFβ2, transforming growth factor beta-2.

Table 3. 2 Total Oocyst Output (d6-32) in turkey poults vaccinated with low and high doses of mixed *Eimeria* vaccine with or without Amprolium supplementation, Experiment 1.

Treatment[1]	NVNI	L	LA	H	HA	P-value
d6	0 ± 0^{c}	146.6 ± 146.6^{bc}	213 ± 132.12^{abc}	$7,059.4 \pm 4,848.28^{ab}$	$6,979.8 \pm 3,571.29^{a}$	0.0163
d9	0 ± 0^{d}	$152,447.4 \pm 38,036.33^{b}$	$21,938.4 \pm 6,964.45^{c}$	$807,192 \pm 86,192.34^{a}$	$318,081.6 \pm 57,697.42^{b}$	0.0002
d12	659.6 ± 213.36^{c}	$9,250.8 \pm 4,217.50^{bc}$	659.6 ± 391.53^{c}	$57,142.8 \pm 14,983^{a}$	$2,757.2 \pm 386.43^{b}$	0.0015
d15	759.2 ± 734.46^{d}	$2,388,809 \pm 1,430,196^{a}$	$3,396.6 \pm 894.67^{c}$	$366,033.6 \pm 137,013.4^{a}$	$15,264.8 \pm 6,230.64^{b}$	0.0003
d18	$20,619.6 \pm 7,474.55^{a}$	$575,424 \pm 224,599.6^{b}$	$79,580.4 \pm 37,229.42^{a}$	$121,078.8 \pm 45,422.54^{ab}$	$64,735.2 \pm 33,234.8^{a}$	0.0125
d21	$51,665.2 \pm 26,659.97$	$16,117.4 \pm 7,858.62$	$107,858.8 \pm 97,919.97$	$18,215.2 \pm 9,876.43$	$39,910.2 \pm 22,318.06$	0.8847
d24	$296,819.8 \pm 138,775.5^{b}$	$52,297.8 \pm 25,833.51^{ab}$	$8,175.2 \pm 3,304.04^{a}$	$7,459.4 \pm 1,666.12^{a}$	$5,811 \pm 2,485.83^{a}$	0.0127
d27	$16,833.4 \pm 12,293.77$	$49,001 \pm 36,227.83$	$3,097.2 \pm 965.28$	$16,550.4 \pm 8,622.42$	$2,381.2 \pm 467.64$	0.0930
d30	$6,143.8 \pm 931.38^{bc}$	$46,087.2 \pm 21,969.32^{a}$	$11,538.6 \pm 10,231.93^{abc}$	$10,696 \pm 2,430.08^{ab}$	$2,847.2 \pm 1,090.45^{c}$	0.0115
d32	$3,163.6 \pm 735.73^{a}$	$3,679.8 \pm 462.92^{a}$	$1,065.4 \pm 431.15b$	$4,995.2 \pm 1,175.20^{a}$	$1,215.6 \pm 169.87^{b}$	0.0022

Abbreviations: OPG, Oocyst per gram; NVNI, non-vaccinated non-inoculated; L, low dose; LA, low dose with amprolium; H, high dose; HA, high dose with amprolium. Samples of feces were collected from every pen on d6-32 at 3-day intervals, and oocyst shedding was quantified as OPG. On d1, 40% of poults in the L and LA groups were vaccinated with 100 sporulated oocysts/bird of *E. adenoiedes, E. gallopavonis*, and *E. meleagrimitis,* whereas 70% of poults in the H and HA groups were vaccinated with 500 sporulated oocysts/bird of *E. adenoiedes, E. gallopavonis*, and *E. meleagrimitis.* LA received amprolium in drinking water on d8-12. Mean values with different superscript letters within a row indicate a significant difference ($P < 0.05$).

Table 3. 3 Oocyst shedding (d6-28) for turkey poults vaccinated with mixed *Eimeria* species and supplemented with amprolium, Experiment 2**.**

Treatment[1]	NV	V	VA	P-value
OPG				
d6	0 ± 0	0 ± 0	0 ± 0	1
d8	0 ± 0[a]	34,845.12 ± 13,253.27[b]	0 ± 0	0.0013
d10	0 ± 0[b]	13,453.2 ± 5,126.51[a]	146.52 ± 95.12[b]	0.003
d12	0 ± 0	39.96 ± 39.96	0 ± 0	0.3679
d14	0 ± 0[b]	10,529.46 ± 5,962.75[a]	139.86 ± 97.88[ab]	0.037
d16	0 ± 0	35,624.34 ± 16,081.41	12,487.5 ± 12,263.96	0.1188
d18	0 ± 0[b]	923,076 ± 601,150.7[a]	7,592.4 ± 7,592.4[b]	0.0024
d20	0 ± 0	0 ± 0	53.28 ± 53.28	0.3679
d22	0 ± 0	0 ± 0	0 ± 0	1
d24	0 ± 0	266.4 ± 250.08	0 ± 0	0.1173
d26	0 ± 0[b]	2,171.16 ± 598.88[a]	865.8 ± 209.55[a]	0.0037
d28	0 ± 0[b]	90,442.8 ± 37,564.05[a]	61,325.28 ± 29,675.71[a]	0.0068

Abbreviations: OPG, Oocyst per gram; NV, non-vaccinated non-inoculated; V, vaccinated; VA, vaccinated and supplemented with amprolium. Samples of feces were collected from every pen on d6-28 at 2-day intervals, and oocyst shedding was quantified as OPG. On d1, 40% of poults in the V and VA groups were vaccinated with 100 sporulated oocysts/bird of *E. adenoiedes, E. gallopavonis*, and *E. meleagrimitis.* Poults in V and VA group were challenged with 25,000 sporulated oocysts/bird of *E. adenoiedes, E. gallopavonis*, and *E. meleagrimitis* on d21. VA received amprolium in drinking water on d8-12. Mean values with different superscript letters within a row indicate a significant difference ($P < 0.05$).

Table 3. 4 Regression Analysis of the Effect of mixed *Eimeria* vaccination and amprolium supplementation on Total OPG, Experiment 1 and 2

	Parameter Estimate	Std Error	t Ratio	Prob>|t|	Mean ± SE
Experiment 1					
Intercept (NVNI)	1,160,770.2	288,276.3	4.03	0.0007*	396,663.2 ± 133,667.89
L	2,132,490	576,552.7	3.70	0.0014*	3,293,260.2 ± 1,414,550.133
LA	-923,247.8	576,552.7	-1.60	0.1250	237,522.4 ± 81,147.39
H	255,652.04	576,552.7	0.44	0.6622	1,416,422.2 ± 221,401.29
HA	-700,787.4	576,552.7	-1.22	0.2383	459,982.8 ± 56,200.22
Experiment 2					
Intercept (NV)	397,686.36	189,636	2.10	0.0578	0 ± 0
V	712,762.08	268,185.8	2.66	0.0209*	1,110,448.44 ± 567,247.65
VA	-315,075.7	268,185.8	-1.17	0.2628	82,610.64 ± 43,431.78

Abbreviations: For Experiment 1, NVNI, non-vaccinated non-inoculated; L, low dose; LA, low dose with amprolium; H, high dose; HA, high dose with amprolium; OPG, oocyst per gram. For Experiment 2: NV, non-vaccinated non-inoculated; V, vaccinated; VA, vaccinated and supplemented with amprolium; SE, standard error. Values represent differences in total oocysts shedding between treatment groups. *Represents groups that are statistically different ($P<0.05$).

Table 3. 5 Predicting Total OPG after Vaccination Vs Total OPG after Challenge

Term	Estimate	Std Error	t Ratio	Prob>|t|	Mean ± SE
Intercept	51,596.146	20,366.91	2.53	0.0250*	345995.88 ± 220670.26
OPG after Vaccination	0.0002726	0.02275	0.01	0.9906	51,690.48 ± 18,101.11

Abbreviations: OPG, oocyst per gram. *Represents groups that are statistically different (P<0.05).

Table 3. 6 Effects of Vaccination with or without Amprolium Supplementation on the Growth Performance and Lesion Score of *Eimeria*-Infected Poults, Experiment 2.

	NV	V	VA	P-value
BW, g				
d1	55.31 ± 0.23[ab]	56.17 ± 0.31[a]	54.97 ± 0.25[b]	0.022
d21	688.87 ± 30.83	673.48 ± 12.32	681.30 ± 11.16	0.867
d28	1,165.02 ± 60.44	1,151.64 ± 28.63	1,185.93 ± 14	0.828
BWG, g				
d1-21	633.56 ± 30.71	617.31 ± 12.21	626.33 ± 11.07	0.851
d22-28	476.15 ± 30.91	478.16 ± 19.13	504.62 ± 6.31	0.579
d1-28	1,109.71 ± 60.33	1,095.48 ± 28.50	1,130.96 ± 14.31	0.817
FI, g				
d1-21	774.19 ± 19.46	765.50 ± 26.03	799.23 ± 23.06	0.58
d22-28	620.87 ± 30.34	629.71 ± 32.95	645.67 ± 16.3	0.82
d1-28	1,394.97 ± 47.27	1,393.15 ± 33.40	1,443.33 ± 27.39	0.57
FCR				
d1-21	1.23 ± 0.05	1.24 ± 0.05	1.29 ± 0.02	0.68
d22-28	1.31 ± 0.03	1.32 ± 0.04	1.30 ± 0.04	0.97
d1-28	1.27 ± 0.04	1.29 ± 0.03	1.28 ± 0.02	0.85
Mortality, %	0.87 (1/5)	1.74 (2/5)	1.74 (2/5)	-
Lesion Score				
Ceca	Score 0, 72% (18/25)	Score 0, 72% (18/25)	Score 0, 72% (18/25)	
	Score 1, 28% (7/25)	Score 1, 28% (7/25)	Score 1, 28% (7/25)	
Duodenum	Score 0, 88% (22/25)	Score 0, 84% (21/25)	Score 0, 84% (21/25)	
	Score 1, 12% (3/25)	Score 1, 12% (3/25)	Score 1, 16% (4/25)	
	-	Score 2, 4% (1/25)	-	

Abbreviations: NV, non-vaccinated non-inoculated; V, vaccinated; VA, vaccinated and supplemented with amprolium; BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio. Mean values with different superscript letters within a row indicate a significant difference ($P < 0.05$). On d1, 40% of poults in V and VA groups were vaccinated with 100 sporulated oocysts/bird of *E. adenoiedes, E. gallopavonis*, and *E. meleagrimitis.* VA received amprolium in drinking water on d8-12. Poults were challenged with 25,000 sporulated oocysts/bird of *E. adenoiedes, E. gallopavonis*, and *E. meleagrimitis* on d21.

Table 3. 7 Effect of low-dose vaccine and amprolium supplementation on Gene expression profile of *Eimeria*-infected turkey poults, Experiment 2.

Treatment[1]	IFNγ	IL-10	IL1β	CD40	FOXP1	IL-6	TGFβ2
Ceca							
NV	0.65	0.6	0.8	0.64	0.67	0.67	0.74
V	0.65	0.6	0.83	0.64	0.65	0.67	0.72
VA	0.67	0.61	0.84	0.65	0.65	0.67	0.74
SEM	0.018	0.006	0.018	0.006	0.01	0.009	0.017
P-value	0.755	0.302	0.293	0.463	0.555	0.876	0.613
Cecal tonsil							
NV	0.73	0.744	1.097	0.81[ab]	0.79	0.88	0.85
V	0.8	0.752	1.1	0.806[b]	0.81	0.86	0.85
VA	0.79	0.75	1.08	0.83[a]	0.77	0.88	0.87
SEM	0.02	0.01	0.03	0.007	0.02	0.01	0.008
P-value	0.115	0.837	0.91	0.043*	0.557	0.617	0.197

Abbreviations: NV, non-vaccinated non-inoculated; V, vaccinated; VA, vaccinated and supplemented with amprolium. [a]Mean values with different superscript letters within a row indicate a significant difference ($P < 0.05$). On d1, 40% of poults in V and VA groups were vaccinated with 100 sporulated oocysts/bird of *E. adenoiedes, E. gallopavonis*, and *E. meleagrimitis.* VA received amprolium in drinking water on d8-12. Poults were challenged with 25,000 sporulated oocysts/bird of *E. adenoiedes, E. gallopavonis*, and *E. meleagrimitis* on d21. On d28, ceca and cecal tonsil tissues were obtained in RNA later solution. Together with the housekeeping gene β-actin, the expression of the genes was measured using real-time PCR ($2^{-\Delta\Delta CT}$).

CHAPTER 4

Performance and Immune Response of *Eimeria*-Infected Turkey Poults with Dried Egg Product Feed Supplementation

Abstract

Coccidiosis, caused by *Eimeria* species, remains a significant health and economic concern in turkey production. Current prophylactic strategies, including anticoccidial drugs and vaccines, face challenges such as resistance and regulatory limitations. Dried egg products (DEPs), which contain bioactive molecules such as immunoglobulins and cytokine-neutralizing agents, have shown promise as nutritional immunomodulators in broilers. However, their efficacy in turkeys under parasitic challenge is not well characterized. This study evaluated the immunomodulatory and performance effects of dietary dried egg products (DEP) in turkey poults experimentally challenged with a mixed-species *Eimeria* infection. A total of 280 male Nicholas turkey poults were randomly allocated to seven dietary treatments, including non-challenged (NC) and challenged controls (CC), and varying inclusion levels of turkey (DEPt) and chicken-derived (DEPc) dried egg products. All groups except the non-challenged control received an oral gavage of 16,000 oocysts/mL containing *E. adenoeides*, *E. gallopavonis*, and *E. meleagrimitis* on d14. Growth performance metrics (BW, FI, and FCR), mortality, fecal oocyst shedding, blood biochemistry, cytokine gene expression (IFN-γ, IL-10, IL-18, IL-1β), and T-cell subsets via flow cytometry were assessed through d28. While growth parameters were not significantly altered by DEP supplementation, high-dose 300 cosabody units DEPt numerically improved BWG and FCR, with zero mortality observed in this group. Importantly, DEPt significantly reduced fecal oocyst output on multiple post-infection days and downregulated IL-10 while upregulating IL-18 expression in the cecal tonsils and jejunum. No significant changes were detected in circulating

T-cell subpopulations. These findings suggest that DEPt, particularly at higher inclusion rates, can reduce parasite burden and modulate cytokine responses in *Eimeria*-challenged turkeys without compromising growth performance. The data underscore the potential of egg-derived bioactive compounds as nutritional immunomodulators in poultry health management.

Keywords: *Eimeria*, turkey poults, dried egg product, gene expression, oocyst shedding, immune response

Introduction

Coccidiosis, caused by protozoa of the genus *Eimeria*, remains a major economic threat to global poultry production. Despite significant advancements in husbandry practices and prophylactic interventions, both clinical and subclinical infections continue to impair growth, nutrient absorption, and intestinal health in poultry flocks (Gautier et al. 2020; Haug et al. 2008; Rochell, Parsons, and Dilger 2016). The risk is heightened in intensive rearing environments, where the spread and persistence of *Eimeria* are more challenging to control. Although anticoccidial drugs and live vaccines are commonly used, their limitations, such as drug resistance, regulatory concerns, and vaccine-induced growth suppression, have prompted interest in alternative nutritional strategies to support host immunity (Gautier et al. 2020; Rasheed et al. 2020b).

Cell-mediated immune responses predominantly mediate protective immunity against *Eimeria*. Key proinflammatory cytokines, including interferon gamma (IFN-γ), IL-1β, and IL-17, are essential for activating T-cells and initiating parasite clearance. However, the anti-inflammatory cytokine interleukin-10 (IL-10), typically upregulated 4–5 days post-infection, can suppress these responses by inhibiting nuclear factor kappa B (NF-κB) signaling, thereby reducing expression of crucial proinflammatory mediators (Dokka et al. 2000; Kim, Chaudhari, and Lillehoj 2019; Wu et al. 2011). Although this mechanism may help limit tissue damage, it can also compromise ability to eliminate the parasite effectively, potentially allowing *Eimeria* to persist (Arendt et al. 2016).

In this context, natural feed-based immunomodulators such as dried egg products (DEPs) have emerged as promising alternatives to help optimize the immune response to *Eimeria*

infection. DEPs are rich in bioactive compounds, including immunoglobulins and anti-inflammatory agents, and have been shown to support gut health and immune function. Notably, DEPs enriched with anti-IL-10 antibodies have demonstrated the ability to neutralize IL-10, potentially restoring proinflammatory responses needed for parasite clearance (Rahman et al. 2013; Shimizu et al. 1993; Wu et al. 2011). Studies in broilers have reported improved performance and immune responsiveness under *Eimeria* challenge following DEP supplementation (Rasheed et al., 2020b; Sand et al., 2016a). However, more recent findings caution that excessive IL-10 suppression during severe infections may trigger overactive inflammation, damaging intestinal tissues, and negating performance benefits (Rasheed et al., 2020b).

While the benefits of DEPs have been explored extensively in broiler chickens, their effects in turkeys, especially under parasitic challenge conditions, remain under-investigated. Turkeys may differ in their immunophysiological responses and susceptibility to coccidia infections, making it necessary to study species-specific dietary interventions. Research has suggested that dried whole egg products can improve feed conversion and support immune organ development in poultry (Lei and Kim 2013) and also serve as an alternative to coccidiostats (Adams 2021). However, the interaction of these products with immune responses under *Eimeria* infection in turkey poults has not been sufficiently characterized. Therefore, the current study evaluated the effects of feeding DEP on performance, oocyst shedding, and immunological response during an infection with mixed species of *Eimeria* in turkey poults.

Materials and methods

The study was performed under the approval of the Institutional Animal Care and Use committee (IACUC) of the North Carolina State University, Raleigh, NC, USA (23-256-01). Ingredient compositions of experimental diets are shown in Table 4.1.

Experimental animal and design

A total of 280 male Nicholas turkey poults were randomly assigned into 7 treatments with 4 replicate pens each, and 10 poults per pen, in a completely randomized design. Treatments included: Non-challenged control (NC), challenged control (CC), 75 cosabody units (cbu) turkey dried egg product (75 DEPt), 150 cbu turkey dried egg product (150 DEPt), 300 cbu turkey dried egg product (300 DEPt), 0.5lbs/ton of chicken dried egg product (1 x DEPc, Optum Immunity, Waterloo WI, USA), and 1 lb./ton of chicken dried egg product (2 x DEPc). DEPc is a commercial product included as a complete product, whereas DEPt was not. Feed treatments were included throughout the entire study. Feed (Table 4.1) and water were provided *ad libitum* while temperature and lighting were maintained at age-appropriate levels.

Preparation of Eimeria and challenge

Purified freshly sporulated *E. adenoeides*, *E. gallopavonis,* and *E. meleagrimitis* oocysts were stored at 4 °C until d14 of age for challenge. Oocysts were floated in a saturated salt solution for enumeration and quantified in a McMaster chamber, and poults in all treatment groups except NC were orally gavaged with 16,000/ml sporulated oocysts of the 3-species cocktail.

Growth performance

Poults were individually weighed in each pen on d1, d14, d19, & d28 while feed weight was recorded on d1, d14, and d19 to calculate BW, body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR).

Mortality

Mortality was checked daily in each pen, and the number of dead birds was recorded daily and checked for signs and cause of death.

Oocysts Per Gram (OPG)

Fecal samples were collected from d18 to d22, and on d28 to evaluate fecal oocyst shedding and determine OPG. Fresh fecal samples were collected from each pen in 50 mL centrifuge tubes and processed in saturated salt solution containing 10 mL of 0.9 % saline at 1:2 (w/v) to form individual samples that were counted via the McMaster method to determine oocyst shedding per cage.

Blood Chemistry

Blood was collected from the brachial vein (n=2 poults/pen; 8 poults per group) on d19, using a 3ml syringe with heparin needles. The groups considered for the blood chemistry were NC, CC, and 300 DEPt group. The blood samples were analyzed immediately using $CG8^{+}$ cartridges of an i-STAT machine (Abbott Point of Care Diagnostics, Princeton, NJ) as recommended by the manufacturer.

Quantitative real-time PCR

Cecal tonsil, spleen, and jejunum were collected in RNA later solution (Invitrogen, Carlsbad, CA) from turkey poults on d19 in three groups: NC, CC, and 300 DEPt (n = 4) and stored at −80 °C until processing. A 1-cm portion of each tissue was removed from the tissue

sample and placed into individually labeled screw cap homogenization vials. Each sample was homogenized using a bead-beater for 20 seconds. The RNA isolation was completed using a RNeasy kit (QIAGEN, Hilden, Germany) that followed the manufacturer's guidelines. A NanoDrop2000 (Thermo Fisher Scientific-Waltham, MA) was used to quantify RNA levels and ensure the correct RNA dilution.

Thereafter, the RNA was transcribed into cDNA for quantitative polymerase chain reaction (qPCR) using a high-capacity synthesis kit (Applied Biosystems Waltham, Massachusetts, USA). All qPCRs were completed on each sample in triplicate. In each of the wells, 2.5 ng of sample, 500 nM of gene-specific forward and reverse primers, and 2X power SYBR green master mix (Applied Biosystems Waltham, Massachusetts, USA) were added, and H_2O was added to finalize the volume to 20 µL. Information on primers of target genes and housekeeping genes is listed in Table 4.2. The Applied Biosystems StepOnePlus real-time PCR system performed qPCR to quantify the cellular expression of IFNγ, IL-10, IL-18, and IL1β. Each reaction involved an initial denaturation of 95℃ for 10 mins, followed by 40 cycles of 95℃ for 30 secs, 60℃ for 30 secs, and 72℃ for 30 secs. The results were then normalized to β-actin expression, a basic housekeeping gene. For ease of interpretation, the reciprocal was then taken using the formula: 1/ (target gene cycle threshold/beta actin cycle threshold). All data presented as reciprocal cycle thresholds of CT^{-1}. ΔΔCT was then calculated by first averaging each treatment CT^{-1}, and NC was set to be the control. The average for NS was then subtracted from each CT^{-1} to obtain the ΔCT. Because NC was used as the control, the ΔΔCT values are the same as the ΔCT values. These values were put into the equation: 2^{-}ΔΔCT (Pfaffl 2001).

Flow cytometry

Cecal tonsils were collected on d19, and single-cell suspensions were prepared for immunophenotyping by flow cytometry. Cecal tonsil tissues from one poult per pen (n=4 poults/group) in three groups: NC, CC, and 300 DEPt were selected for flow cytometry evaluation. Following a cell count, the suspension was adjusted to 1 x 10^7 cells/mL and plated on 96-well round-bottom plates with each well containing 10^6 cells in 100 µL FACS buffer (PBS with 1% BSA). Primary antibodies were added to each well (0.5–1 µg/10^6 cells). Cells were stained in one panel of antibody staining. All antibodies were purchased from Southern Biotech Inc., Birmingham, AL, which were of mouse origin, and their respective stains are given in parentheses below. The panel used antibodies against CD4 (PE-Cy7), CD8 (PB), CD44 (APC), CD28 (FITC), and MHC-II (PE). In the panel, cell viability dye, Live/Dead near IR (Invitrogen, CA) was used to exclude dead cells. Stained cells were washed and fixed with 4% paraformaldehyde before data acquisition using an LSR-II flow cytometer (BD Biosciences). The gating strategy included exclusion of doublet cells through forward and side scatters, height and width, followed by gating on live cells (Figure 1). Live cell gating was furthermore used as a backbone population to obtain $CD4^+$ and $CD8^+$ cells, $CD28^+$ cells, $CD44^+$ cells, and MHC-II^+ cells. Single stains and fluorescence minus one (FMO) control were used for fluorochrome compensation and gating positive cell populations. Data analysis was carried out using the FlowJo software version 10.8.2 (Tree Star, Ashland, OR).

Statistical analysis

All data were analyzed using JMP Statistical software version Pro 18.0.2 (SAS Institute Inc, Cary, NC, USA). OPG data were analyzed by the non-parametric Kruskal-Wallis test followed by Wilcoxon multiple comparison test. All other data were first tested for normal

distribution (Shapiro-Wilk test) followed by one-way ANOVA test analyses. Treatment means were separated using Tukey HSD test. Statistical significance was considered at $P < 0.05$.

Results

Growth performance

Effects of DEP supplementation on growth performance in turkey poults are shown in Table 4.6 (supplementary material). There were no treatment effects on growth performance parameters ($P>0.05$). BW at d1 was similar across all treatments ($P > 0.05$), with values ranging from 59.53 ± 0.87 g (300 DEPt) to 60.74 ± 0.80g (1xDEPc). By d14, BW ranged from 404.72 ± 20.74 g (75 DEPt) to 444.35 ± 7.28 g (NC). On d28, the BW ranged from 1022.53 ± 69.75 g (75 DEPt) to 1159.18 ± 66.19 g (NC), with the highest value still observed in the NC group. BWG at d1-14 ranged from 345.05 ± 20.89 g (75 DEPt) to 384.41 ± 7.27 g (NC) with no significant differences ($P > 0.05$). On d15-19, BWG ranged from 256.07 ± 21.74 g (1 x DEPc) to 298.29 ± 4.33 g (NC), and d20-28 ranged from 547.19 ± 38.46g (1xDEPc) to 622.76 ± 8.11g (NC). FI at d1-14 ranged from 397.79 ± 0.06 g (75 DEPt) to 412.65 ± 0.04 g (CC). FI on d15-19 ranged from 290.69 ± 0.10 g (75 DEPt) to 349.46 ± 0.07 g (NC), with no significant differences in the overall feed intake during the study period ($P > 0.05$). FCR at d1-14 ranged from 1.07 ± 8.79 (CC) to 1.21 ± 6.49 (75 DEPt). From d15-19, the FCR ranged from 1.4 ± 8.15 (NC & 150 DEPt) to 1.65± 17.45 (CC). The overall FCR for the period (d1-19) ranged from 1.19 ± 8.39 (NC) to 1.31 ± 12.35 (75 DEPt).

Mortality in *Eimeria*-infected turkey poults d1-28 was highest in the 150 DEPt group at 36.36%, with two additional deaths compared to other groups (9.09% for NC and CC, 18.18% for 75 DEPt). However, no statistical differences ($P>0.05$) were reported.

Fecal OPG

No oocyst shedding was observed in any of the treatment groups on d18 (Table 4.3). On d19, turkeys receiving 150 DEPt had a mean of 1,098.9 ± 991.01 OPG, while the 300 DEPt group showed a mean of 66.6 ± 38.45 OPG. All other groups had lower mean OPG, with no significant differences ($P > 0.05$) between them. On d20, the 75 DEPt group had the highest mean oocyst shedding of 1,013,985 ± 527,405.67 OPG, followed by the CC group with 578,254.5 ± 206,045.73 OPG, but there were no significant differences ($P>0.05$) among the groups. By d21, turkeys receiving 1× DEPc shed significantly more oocysts ($P < 0.05$) than all other groups, with a mean of 864,135 ± 327,813 OPG, followed by 2× DEPc with 334,665 ± 189,691 OPG. On d22, significant differences persisted ($P < 0.05$), with 75 DEPt showing the highest mean of 199,966.5 ± 92,323.86 OPG, closely followed by 1× DEPc with 161,172 ± 69,920.49 OPG. On d28, oocyst shedding in the 1 x DEPc group remained higher compared to others, with 153,679.5 ± 82,333.15 OPG, while the 2 x DEPc group dropped to 21,145.5 ± 7,839.66 OPG. Supplementation with DEPt (150 and 300) consistently reduced oocyst shedding compared to the other treatment groups throughout the experimental period, while 75 DEPt had the lowest OPG at d28, with a mean of 8,408.25 ± 1924.57 OPG.

Blood Chemistry

Effects of DEPt on Blood Chemistry in *Eimeria*-infected turkey poults are shown in Table 4.4. There was no significant difference ($P > 0.05$) in blood chemistry among the treatment groups. However, DEPt supplementation numerically increased the Glu, and Hb compared to the CC group.

Immune Gene Expression

The effects of DEPt on the relative gene expression of *Eimeria*-infected turkey poults are shown in Table 4.5. There was no significant difference (P=0.0815) in Interferon gamma (IFNγ) mRNA expression in the cecal tonsil among the treatments, but DEPt supplementation showed a significant decrease (P=0.0203) in Interleukin 10 (IL-10) in the cecal tonsils. On the other hand, DEPt significantly increased (P=0.0061) interferon 18 (IL-18) mRNA expression in the jejunum compared to the treatment groups. In addition, although not significant, DEPt numerically increased (P=0.444) IFN-γ and IL-18 (P=0.3862) mRNA expression in the spleen compared with the treatment groups.

Flow Cytometry

As shown in Figure 4.1, no significant (P > 0.05) cellular changes in the $CD4^+$, $CD8^+$, $CD28^+$, CD44+, and MHC-II^+ T-cell frequencies were observed between the treatment groups in cecal tonsils of the poults.

Discussion

This study evaluated the effect of both chicken and turkey-derived DEP during *Eimeria* infection in turkey poults, since DEPc has been proven to help improve performance of *Eimeria* infected birds (Adams 2021; Sand et al. 2016). The study was designed to investigate whether DEPc exhibited cross-efficacy to infected turkeys, and how DEPt performed against *Eimeria* infections in turkey poults. Though oocyst shedding post-inoculation clearly showed infection with *Eimeria*, no changes were observed in growth performance variables, but a few notable observations are discussed below. The findings revealed that body weight (BW) and feed intake (FI) did not differ significantly among the various treatment groups (Table 4.6). Nonetheless, the

150 DEPt group exhibited BW values comparable to the non-infected control (NC) group. This suggests that while DEP supplementation may not robustly enhance growth in early-stage infections, there may be dose-specific benefits under certain conditions. This finding aligns with the literature, which suggests that DEP primarily enhances nutrient utilization efficiency, rather than stimulating feed intake or growth (Sand et al. 2016). Dietary supplementation with egg-derived products has a limited impact on growth performance; more notable benefits emerge with prolonged use or under conditions of mild pathogenic or environmental stress (Fries-Craft and Bobeck 2024). Notably, FCR did not significantly improve in DEP-treated groups compared to the non-infected control (NC) and challenged control (CC) groups, with all DEP-treated groups exhibiting higher FCR values. This suggests that DEP supplementation, at the doses tested, did not enhance feed utilization efficiency under the conditions of this study. The mortality data revealed a dose-dependent but non-linear trend. The 150 DEPt group recorded the highest mortality, whereas the 300 DEPt group had zero mortality, which was comparable to that of the NC group. This suggests that moderate DEPt doses may be insufficient to counteract pathogenic burden or may even disrupt immune homeostasis. In contrast, higher doses might activate more robust protective pathways. This observation emphasizes the importance of dose optimization in nutraceutical interventions. Prior studies have also reported contrasting outcomes at suboptimal dosages, with either neutral or deleterious effects, depending on the disease context and immune maturity of the host (Rasheed et al., 2020a).

Oocyst per gram (OPG) measurements serve as a quantitative proxy for *Eimeria* proliferation and infection burden. In this study, as shown in Table 4.3, DEPt supplementation, particularly at 300 DEPt, numerically reduced oocyst shedding compared to CC. This reduction is of notable relevance, suggesting that DEPt may influence the intestinal milieu in ways that

impair oocyst replication or enhance mucosal immunity. These results align with findings from studies reporting the anticoccidial effects of egg yolk immunoglobulins, which may act by enhancing gut barrier function or modulating local immune responses (Wickramasuriya et al. 2022). However, not all DEP-supplemented groups demonstrated reductions in OPG, indicating that product source (turkey vs. chicken) and dose dependency may be factors. This results aligns with Adams (2021), who reported growth benefits without OPG changes, highlighting species-specific differences and emphasizing the importance of timing, challenge severity, and DEP administration protocols.

The i-STAT analyzer offers a rapid diagnostic option for avian veterinarians, delivering test results within minutes, thereby facilitating timely clinical decisions based on blood chemistry data (Ripplinger et al. 2023). Blood glucose concentrations did not differ significantly among the groups ($P>0.05$), with variability being similar across the treatments (Table 4.4). Minor variability in glucose measurements may reflect natural biological variation or inconsistencies in feed intake, since we did not control feeding time (Khan et al. 2004). Hemoglobin (Hb), the oxygen-carrying molecule in erythrocytes, and hematocrit (Hct), which quantifies red blood cell mass, are commonly used to assess erythrocyte concentration. Together, Hct and Hb measurements provide insights into red blood cell density (Ng et al. 2014). The observed increases in glucose and hemoglobin concentrations in DEPt-treated birds may reflect improved nutrient uptake, erythropoiesis, and systemic oxygen transport. During *Eimeria*-induced inflammation, metabolic derangements are common. Thus, these biochemical trends, although modest, suggest the potential for stabilization of systemic physiology under DEP influence. Although not statistically significant, these results are in concordance with earlier findings that indicate nutritional immunomodulators can buffer against infection-induced

hematological suppression (Oso et al. 2019). The possible differences in blood chemistry caused by an *Eimeria* infection in turkey poults, however, require more investigation.

One of the most notable findings of this study was the significant downregulation of IL-10 and upregulation of IL-18 mRNA in the cecal tonsils and jejunum of DEPt-treated poults (Table 4.5). IL-10 is a pivotal anti-inflammatory cytokine that is often elevated to suppress hyperinflammation. Its induction here suggests that DEPt may help attenuate inflammatory tissue damage during infection. In contrast, IL-18 is a cytokine that promotes inflammation and triggers the production of IFN-γ. Its elevation indicates a dual immune response, containing excessive inflammation while priming the immune system against intracellular pathogens. These outcomes support the hypothesis that DEP functions not merely as a passive nutritional aid but as a dynamic immune modulator, capable of influencing both arms of the immune response. Interestingly, Rasheed et al. (2020) reported no significant modulation of IL-1β, IFN-γ, or IL-10 by DEP. This discrepancy may reflect differences in challenge models, host species, or timing of tissue sampling. Notably, DEP is thought to neutralize IL-10 bioactivity via antibody binding to limit parasite immune evasion (Groux and Powrie 1999; Hong et al. 2006a), a mechanism not directly tested in the current study.

A major pro-inflammatory mediator that is mostly released by activated macrophages, IL-1β is essential for triggering innate immune responses because it encourages immune cell migration to infection sites (Hong et al. 2006b). According to the current study, DEPt supplementation increased cecal tonsil IL-1β expression ($p=0.09$) and indicated an active inflammatory response, which is consistent with its established function in early immune activation during pathogen challenge. Interferon-gamma (IFN-γ), a signature cytokine of cell-mediated immunity, is primarily produced by Th1 lymphocytes, cytotoxic T cells, and natural

killer (NK) cells. As noted by Wigley & Kaiser (2003), these immune cells play a central role in orchestrating cellular immune defenses. It is essential for boosting antigen presentation, stimulating Th1 differentiation pathways, and triggering macrophages to target intracellular pathogens. In this study, IFN-γ expression showed a trend towards upregulation in the jejunum and spleen, indicating enhanced macrophage activation and an intensified inflammatory response. This observation aligns with prior research demonstrating elevated IFN-γ levels in chickens following *Eimeria* infection (Hong et al. 2006a, 2006b; Laurent et al. 2001; Yun, Lillehoj, and Choi 2000). According to Rothwell et al. (2004), IL-10, which is mostly produced by T cells and macrophages, suppresses Th1 responses and controls innate and adaptive immunity. However, it may also increase IFN-γ through NK and cytotoxic T-cells (Abdul-Careem et al. 2008; Shibata et al. 1998). IL-10 expression was consistently lower in the DEPt group in all tissues examined in this study, which is consistent with reports by Shanmugasundaram et al. (2013). However, it was higher in infected poults than in controls. Previous reports of *Eimeria* infection have documented similar elevation of IL-10 (Cornelissen et al., 2009; Hong et al., 2006b). Interleukin-18 (IL-18) is a key regulator of inflammation, primarily by promoting the secretion of interferon-gamma (IFN-γ), which in turn enhances T-helper 1 (Th1) immune responses (Ihim et al. 2022). This immunological activity was particularly evident in the DEPt group, where its jejunum expression suggests a robust early pro-inflammatory response.

Flow cytometry data indicated no significant alterations in major T-cell populations ($CD4^+$, $CD8^+$, $CD28^+$, $CD44^+$, $MHC\text{-}II^+$) within the cecal tonsils (Figure 4.1). This suggests that while DEP modulates cytokine signaling, it may not directly influence lymphocyte recruitment or expansion at the mucosal level. The expansion of intestinal T-cell subsets, including $CD3^+$,

$CD4^+$, and $CD8^+$ populations, plays a crucial role in enhancing immune defense against *Eimeria* infection (Swinkels et al. 2007). The findings from this study agree with prior studies showing that egg-derived products more frequently influence humoral and cytokine-mediated pathways than direct T-cell dynamics (Song et al. 2010). This finding further strengthens the case that DEP's immunological effects are predominantly functional rather than structural.

The results of this study provide compelling evidence that DEPt, particularly at higher doses, can modulate gut immunity, reduce parasite burden, and potentially stabilize performance metrics in *Eimeria*-challenged turkey poults. The data highlights the critical importance of dose selection, as intermediate doses may yield adverse or neutral effects. However, the lack of significant change in cellular immune parameters, coupled with the non-significant trends in BW and FI, underscores the need for combinatory approaches, perhaps integrating DEP with probiotics, prebiotics, or phytogenic additives. Future studies should also assess intestinal histomorphology, oxidative stress markers, and long-term outcomes, including post-infection resilience and shifts in microbiota.

In conclusion, DEP, particularly DEPt, offer a biologically promising avenue for managing *Eimeria* infections in turkeys. This study demonstrates that DEPt, at optimal dosages, can reduce fecal oocyst shedding, modulate immune gene expression, notably IL-10 and IL-18 expression, and supported by FACS (Fluorescence activated cell Sorting) analysis, without adversely affecting growth performance of turkey poults. However, inconsistencies in mortality outcomes and lack of T-cell modulation indicate that DEP efficacy is dose-dependent and context-sensitive, warranting further research to refine administration protocols and uncover underlying mechanisms.

Supplementary Data

Supplementary data can be found at: Table 4.6. Effect of DEPt on growth performance of *Eimeria*-infected turkey poults.

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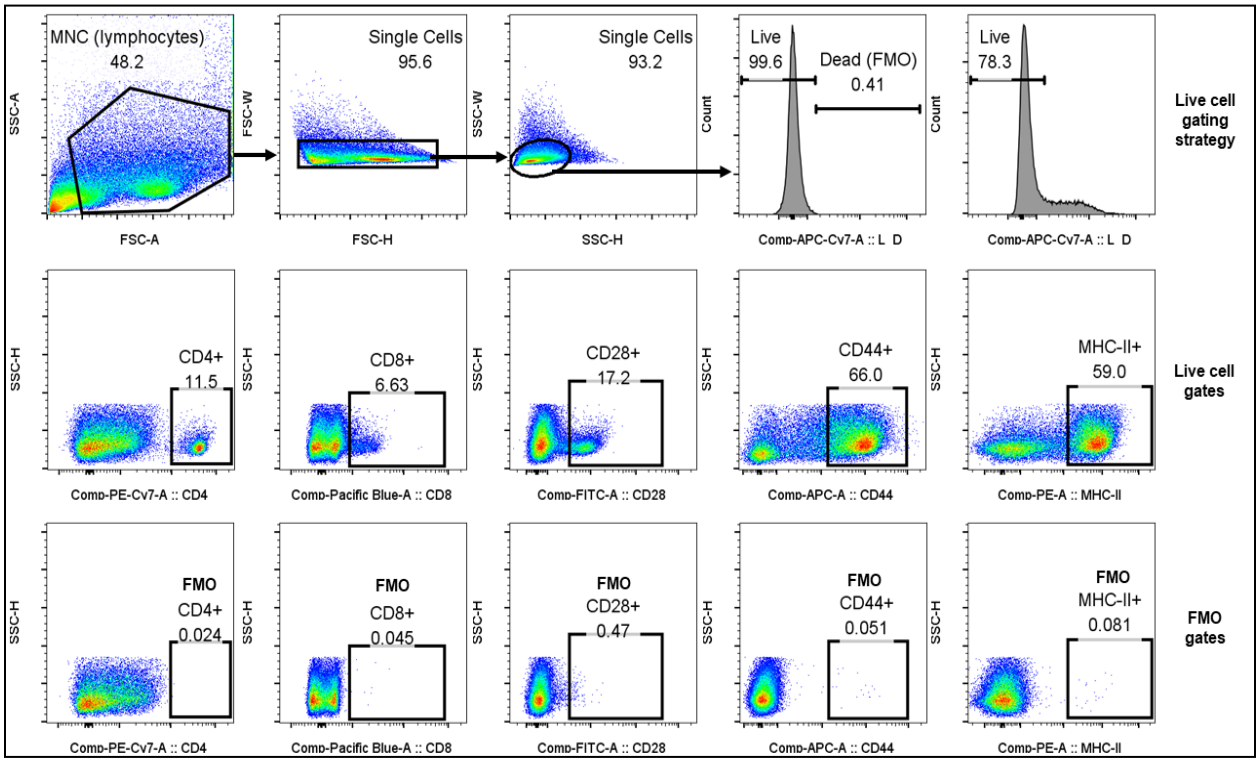
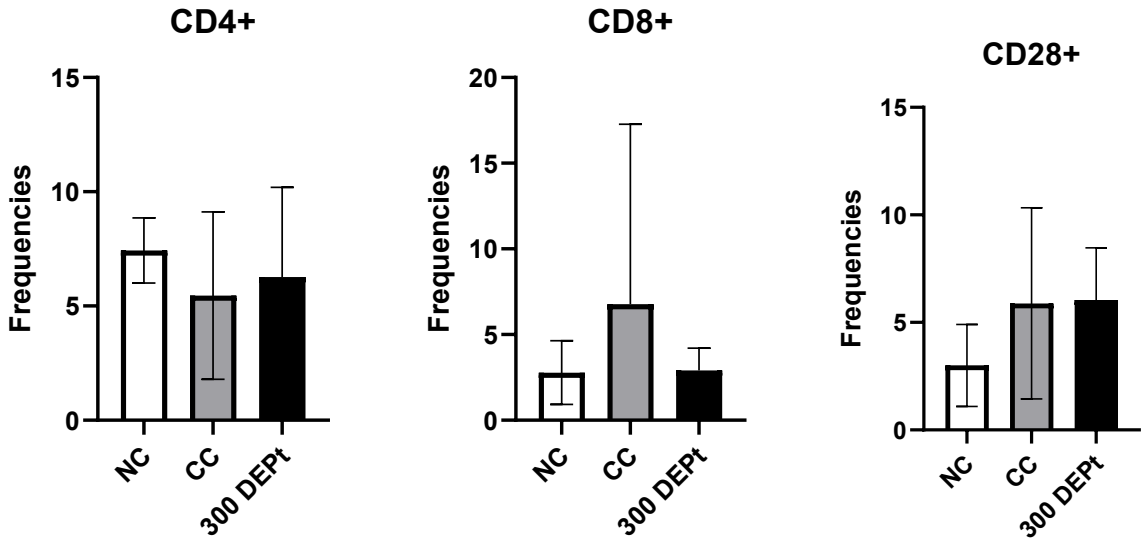
Figure 4.1 Cellular Immune Response in the Cecal Tonsil of *Eimeria*-infected turkey poults. The poults were challenged with 16 x 10^3 oocysts/ml of a 3-species cocktail of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis* by oral gavage at 14 days of age. For flow cytometry analysis, cells were stained with antibodies against CD4, CD8, CD28, CD44, and MHC-II markers. Part A shows the gating strategy used for excluding the doublet cells through forward and side scatters, height, and width, followed by gating on the live cells. Part B shows the frequencies of T cell subsets ($CD4^+$ and $CD8^+$) as well as the T cell expression of CD28, CD44, and MHC-II activation marker.

A


MNC (lymphocytes)
48.2
SSC-A
FSC-A
FSC-W
Single Cells
95.6
FSC-H
SSC-W
Single Cells
93.2
SSC-H
Count
Live
99.6
Dead (FMO)
0.41
Comp-APC-Cv7-A :: L D
Live
78.3
Live cell gating strategy
SSC-H
CD4+
11.5
Comp-PE-Cv7-A :: CD4
CD8+
6.63
Comp-Pacific Blue-A :: CD8
CD28+
17.2
Comp-FITC-A :: CD28
CD44+
66.0
Comp-APC-A :: CD44
MHC-II+
59.0
Comp-PE-A :: MHC-II
Live cell gates
FMO
CD4+
0.024
FMO
CD8+
0.045
FMO
CD28+
0.47
FMO
CD44+
0.051
FMO
MHC-II+
0.081
FMO gates

B


CD4+
CD8+
CD28+
Frequencies
0
5
10
15
20
NC
CC
300 DEPt

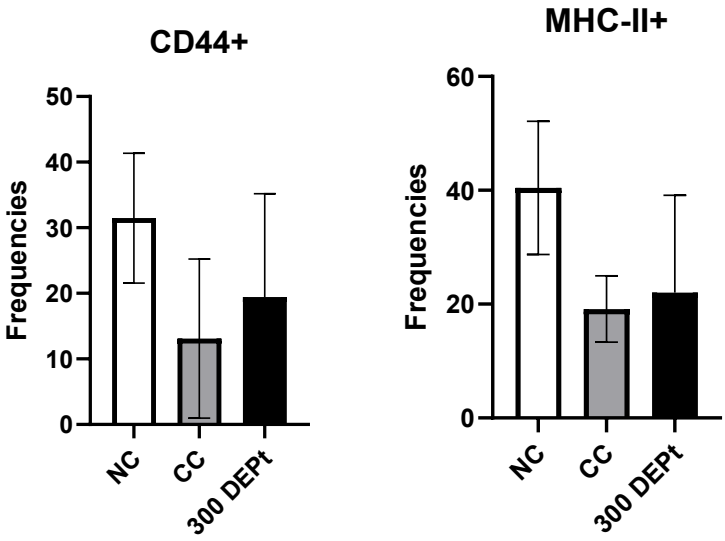

CD44+
Frequencies
50
40
30
20
10
0
NC
CC
300 DEPt
MHC-II+
Frequencies
60
40
20
0
NC
CC
300 DEPt

Table 4.1 Ingredients and composition of experimental diets.

Ingredients	NC	CC	75 DEPt	150 DEPt	300 DEPt	1 x DEPc	2 x DEPc
Corn	18.44	18.44	18.44	18.44	18.44	18.44	18.44
Wheat	19.99	19.99	19.99	19.99	19.99	19.99	19.99
Soybean Meal	36.98	36.98	36.98	36.98	36.98	36.98	36.98
Poultry Meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Limestone	1.87	1.87	1.87	1.87	1.87	1.87	1.87
Mono-dicalcium phosphate	2.35	2.35	2.35	2.35	2.35	2.35	2.35
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral Mix	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin Mix	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Selenium Mix	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline CL	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Methionine	0.48	0.48	0.48	0.48	0.48	0.48	0.48
So. Bicarb	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Threonine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Poultry Fat	8.00	8.00	8.00	8.00	8.00	8.00	8.00
DEPc	-	-	-	-	-	0.05	0.1
DEPt	-	-	0.03	0.06	0.13	-	-

Abbreviations: NC = Non-Challenged Control, CC = Challenged Control, DEPt = Turkey Dried Egg Product, DEPc = Chicken Dried Egg Product

Table 4.2 Genes and their sequences (forward and reverse) utilized for gene expression (qPCR).

Gene	Forward Primer Sequence	Reverse Primer Sequence
β-Actin	GGCTCTATCCTGGCCTCTCT	CATCACAAGGGTGTGGGTGT
IFNγ	TGAACTGAGCCATCACCAAGAA	ACCGTCAGCTACGTCTGAATG
IL-10	CGCTGTCACCACTTCTTCAC	CCCGTTCTCATCCATCTTCTCG
IL-18	GTGTGCAGTACGGCTTAGAGA	AATCTGGACGAACCACAAGCA
IL1β	CCTCCAGCCAGAAAGTGAGG	GAAGTGGGGATGGGATGGG

Abbreviation: β-Actin, beta actin; IFNγ, interferon gamma; IL-10, interleukin 10; IL-18, interleukin 18; IL1β, interleukin 1 beta.

Table 4.3 Fecal OPG of *Eimeria*-infected turkey poults.

Treatment	d18	d19	d20	d21	d22	d28
NC	0 ± 0	0 ± 0	0 ± 0	0 ± 0[b]	0 ± 0[b]	0 ± 0[d]
CC	0 ± 0	183.15 ± 139.64	578,254.5 ± 206,045.73	146,520 ± 30,651.68[a]	98,235 ± 22,151.39[a]	150,016.5 ± 57,712.77[ac]
75 DEPt	0 ± 0	865.8 ± 582.51	1,013,985 ± 527,405.67	215,950.5 ± 137,902.85[a]	199,966.5 ± 92,323.86[a]	8,408.25 ± 1,924.57[b]
150 DEPt	0 ± 0	1,098.9 ± 991.01	371,295 ± 79,566.56	108,225 ± 22,151.38[a]	61,605 ± 18,112.06[a]	47,785.5 ± 18,485.50[abc]
300 DEPt	0 ± 0	66.6 ± 38.45	333,000 ± 82,603.69	104,895 ± 28,435.29[a]	49,950 ± 8,380.32[a]	36,130.5 ± 10,729.89[bc]
1 x DEPc	0 ± 0	233.1 ± 96.12	442,890 ± 208,286.00	864,135 ± 327,813.37[a]	161,172 ± 69,920.49[a]	153,679.5 ± 82,333.15[a]
2 x DEPc	0 ± 0	133.2 ± 54.38	507,825 ± 213,646.17	334,665 ± 189,690.69[a]	55,111.5 ± 11,796.46[a]	21,145.5 ± 7,839.66[bc]
P-value	1	0.4691	0.0783	0.0240	0.0243	0.0022

The poults were challenged with 16 x 10^3 oocysts/ml of a 3-species cocktail of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis* by oral gavage at 14 days of age. There was a significant difference ($p<0.05$) in OPG among the treatments on d21, d22, and d28. [a,b]Mean values with different superscript letters within a column indicate a significant difference ($P<0.05$).

Table 4.3 Effect of DEPt on Blood Chemistry of *Eimeria*-infected turkey poults

Treatment[1]	NC	CC	300 DEPt	P-value
pH	7.47 ± 0.04	7.42 ± 0.04	7.44 ± 0.01	0.484
pCO_2 (mmHg)	32.2 ± 3.67	33.78 ± 3.07	36.52 ± 1.61	0.582
pO2 (mmHg)	42 ± 3.05	41.33 ± 1.53	38.5 ± 1.54	0.494
BE (nmol/L)	-0.17 ± 2.08	-3 ± 1.85	0.33 ± 0.92	0.350
HCO_3 (mmol/L)	23.28 ± 1.89	21.53 ± 1.58	24.52 ± 0.96	0.403
tCO_2 (mmol/L)	24.17 ± 1.87	22.67 ± 1.62	25.67 ± 1.02	0.413
sO_2 (%)	80.33 ± 4.94	75.5 ± 2.96	74.67 ± 1.82	0.481
Na (mmol/L)	144.83 ± 0.79	147.83 ± 1.19	145.67 ± 0.92	0.117
K (mmol/L)	4.88 ± 0.15	5.12 ± 0.17	4.73 ± 0.25	0.400
iCa (mmol/L)	1.38 ± 0.1	1.31 ± 0.08	1.49 ± 0.04	0.298
Glu (mg/dL)	282.17 ± 13.23	258.5 ± 9.36	282.17 ± 7.23	0.203
Hct (%PCV)	23.17 ± 0.61	21.33 ± 1.17	22.5 ± 0.43	0.291
Hb (g/dL)	7.88 ± 0.21	7.25 ± 0.4	7.65 ± 0.15	0.283

[1]NC = Non-Challenged Control, CC = Challenged Control, DEPt = Turkey Dried Egg Product. Abbreviations: pCO_2, carbon dioxide partial pressure; pO_2, oxygen partial pressure; BE, basic excess; HCO_3, bicarbonate; tCO_2, total carbon dioxide; sO_2, saturated oxygen; Na, sodium; K, potassium; iCa, ionized calcium; Glu, glucose; Hct, hematocrit; Hb, hemoglobin. The poults were challenged with 16 x 10^3 oocysts/ml of a 3-species cocktail of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis* by oral gavage at 14 days of age.

Table 4.4 Effect of DEPt on the relative gene expression of *Eimeria*-infected turkey poults

Treatment[1]	IFNγ	IL-10	IL-18	IL1β
Cecal Tonsil				
NC	0.83 ± 0.11	0.698 ± 0.01^{ab}	0.73 ± 0.01	0.81 ± 0.05
CC	0.62 ± 0.07	0.76 ± 0.03^{a}	0.72 ± 0.01	0.79 ± 0.05
300 DEPt	0.83 ± 0.01	0.64 ± 0.01^{b}	0.75 ± 0.01	0.92 ± 0.01
P-value	0.08	0.0203	0.18	0.09
Jejunum				
NC	0.77 ± 0.05	0.72 ± 0.05	0.76 ± 0.02^{b}	0.96 ± 0.12
CC	0.67 ± 0.03	0.84 ± 0.03	0.74 ± 0.01^{a}	0.87 ± 0.05
300 DEPt	0.94 ± 0.11	0.62 ± 0.04	0.88 ± 0.02^{b}	0.94 ± 0.07
P-value	0.050	0.058	0.006	0.756
Spleen				
NC	0.77 ± 0.05	0.7 ± 0.05	0.72 ± 0.03	0.97 ± 0.12
CC	0.70 ± 0.07	0.67 ± 0.03	0.71 ± 0.01	0.81 ± 0.03
300 DEPt	0.79 ± 0.04	0.64 ± 0.04	0.74 ± 0.01	0.84 ± 0.03
P-value	0.44	0.56	0.39	0.33

[1]NC = Non-Challenged Control, CC = Challenged Control, DEPt = Turkey Dried Egg Product. The poults were challenged with 16 x 10^3 oocysts/ml of a 3-species cocktail of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis* by oral gavage at 14 days of age. Significant differences exist between groups that lack a common letter ($p < 0.05$).

Table 4.5 Effect of DEPt on growth performance of *Eimeria*-infected turkey poults

Treatment[1]	NC	CC	75 DEPt	150 DEPt	300 DEPt	1 x DEPc	2 x DEPc	P-value
BW (g)								
d1	59.94 ± 0.63	60.72 ± 0.53	59.67 ± 0.36	59.96 ± 0.64	59.53 ± 0.87	60.74 ± 0.80	60.74 ± 0.46	0.644
d14	444.35 ± 7.28	443.8 ± 10.55	404.72 ± 20.74	441.92 ± 13.68	412.25 ± 27.45	412.6 ± 18.93	432.38 ± 11.18	0.443
d19	682.7 ± 7.77	665.73 ± 16.09	621.1 ± 30.81	672.42 ± 19.59	631.8 ± 40.79	607.93 ± 38.77	655.12 ± 14.89	0.395
d28	1159.18 ± 66.19	1072.61 ± 107.86	1022.53 ± 69.75	1101.24 ± 64.20	1040.81 ± 56.77	1025.15 ± 69.70	1073.53 ± 28.91	0.813
BWG (g)								
d1-14	384.41 ± 7.27	383.08 ± 10.18	345.05 ± 20.89	381.96 ± 13.61	352.72 ± 26.79	351.86 ± 18.52	371.64 ± 10.82	0.436
d15-19	298.29 ± 4.33	282.65 ± 6.55	276.049 ± 10.17	290.46 ± 7.46	279.08 ± 14.88	256.07 ± 21.74	283.48 ± 6.79	0.312
d20-28	622.76 ± 8.11	605.02 ± 15.74	561.43 ± 30.98	612.46 ± 19.62	572.27 ± 40.21	547.19 ± 38.46	594.38 ± 14.51	0.993
d1-19	457.33 ± 43.70	423.76 ± 90.63	401.43 ± 55.69	428.82 ± 45.73	409.01 ± 35.69	417.22 ± 48.88	418.4 ± 15.64	0.386
d1-28	1099.24 ± 66.70	1011.89 ± 107.38	962.86 ± 69.99	1041.28 ± 64.08	981.28 ± 56.32	964.41 ± 69.66	1012.78 ± 28.61	0.812
FI (g)								
d1-14	409.6 ± 0.03	412.65 ± 0.04	397.79 ± 0.06	398.11 ± 0.04	405.28 ± 0.08	398.5 ± 0.04	407.67 ± 0.03	0.54
d15-19	349.46 ± 0.07	328.35 ± 0.19	290.69 ± 0.10	297.4 ± 0.05	311.93 ± 0.02	304.43 ± 0.11	306.35 ± 0.07	0.168
d1-19	758.27 ± 0.03	741 ± 0.07	688.07 ± 0.07	696.1 ± 0.04	717.2 ± 0.06	702.26 ± 0.06	713.66 ± 0.03	0.165
FCR								
d1-14	1.08 ± 4.31	1.07 ± 8.79	1.21 ± 6.49	1.12 ± 9.10	1.17 ± 5.63	1.14 ± 7.12	1.11 ± 1.28	0.454
d15-19	1.4 ± 8.15	1.65 ± 17.45	1.46 ± 13.50	1.4 ± 8.44	1.42 ± 18.36	1.58 ± 26.01	1.46 ± 3.73	0.478
d1-19	1.19 ± 8.39	1.27 ± 26.02	1.31 ± 12.35	1.23 ± 16.58	1.26 ± 21.49	1.29 ± 30.08	1.23 ± 4.24	0.746
Mortality (%)	9.09	9.09	18.18	36.36	0	9.09	18.18	-

[1]NC = Non-Challenged Control, CC = Challenged Control, DEPt = Turkey Dried Egg Product, DEPc = Chicken Dried Egg Product
Abbreviations: BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; SEM, standard error of mean.
The poults were challenged with 16 x 10^3 oocysts/ml of a 3-species cocktail of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis* by oral gavage at 14 days of age.

CHAPTER 5

Temporal Dynamics of Host Immunity in Turkey Poults Following Mixed *Eimeria* Infection

Abstract

Eimeria spp. produce coccidiosis, a serious intestinal illness in turkeys, but little is known about the host immunological response, particularly in cases of mixed-species infections. The purpose of this work was to assess the immunological gene expression profiles in turkey poults across 15 days and in relation to different tissues after they were challenged orally with a mixture of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis*. Random assignment was used to place 104 day-old Nicholas poults into either a control or challenged group. The challenge group was inoculated by oral gavage of a mixed *Eimeria* inoculum on day four, containing 25,000 sporulated oocysts/ml. Gene expression of important immune mediators was measured along with body weight, oocyst output, and mortality. For quantitative real-time PCR examination of IL-8, IL-10, IL-18, IFN-γ, IL-1β, LITAF, and HSP70 gene expression, tissues from the liver, spleen, ceca, and cecal tonsils were obtained on 4, 7, 9, 11, 13, and 15 days post-infection. In comparison to control, the challenge group showed significantly lower body weight, more oocyst shedding, and mortality ($P < 0.05$). Gene expression patterns had distinct temporal and geographical patterns. During peak infection periods, the ceca and cecal tonsils showed increased levels of the pro-inflammatory cytokines IFN-γ and IL-1β. At 15 days post-infection, the spleen of infected poults had a marked decrease in IL-8, but the cecal tonsil of infected poults had an increase in IL-10. Differential expressions of HSP70 and LITAF across tissues suggested inflammatory and stress responses at mucosal and systemic locations. These results demonstrate a coordinated, albeit tissue-specific, immune response in turkeys infected with mixed *Eimeria*. In

addition to systemic modulation in the liver and spleen, these distinct gene expression patterns underscore the significance of local immunity in the ceca and cecal tonsils. This work promotes the creation of focused immunological treatments for the management of coccidiosis and deepens our understanding of host-pathogen dynamics in turkeys.

Keywords: Coccidiosis, *Eimeria* spp, Turkey poults, Immune gene expression, Cytokine profiling

Introduction

The poultry industry is susceptible to a number of extremely severe diseases, including coccidiosis, caused by *Eimeria* species (Attree et al., 2021; Cervantes et al., 2020). In turkeys (*Meleagris gallopavo*), this disease continues to pose critical health and economic challenges, particularly during early developmental stages (Chapman, 2008; Abebe & Gugsa, 2018; Fanatico, 2006). These forms of coccidiosis are primarily caused by *Eimeria adenoeides, E. meleagrimitis*, and *E. gallopavonis*, which are associated with high morbidity and potentially severe mortality in young poults (Gadde et al., 2019). The expenses associated with coccidiosis are among the most significant risks to poultry productivity and profitability (Blake et al., 2020). Although substantial advancements have been made in understanding *Eimeria* infections in chickens, insights into the immune responses of turkeys, especially under natural, mixed-species infection scenarios, remain limited. The pathogenesis of coccidiosis involves intricate interactions between the parasite and host immune system (Lee et al., 2022a). The ceca, a major sites of parasite replication and tissue damage, play a central role in local immune activation. However, systemic organs such as the spleen and liver, along with mucosa-associated lymphoid tissues, including the cecal tonsils, also play essential roles in the immune system regulation and amplification (as reviewed by Shivaramaiah et al., 2014). A complete understanding of host immunity must therefore consider both local and systemic responses, especially during complex infections involving multiple *Eimeria* species. The immune response to *Eimeria* is initiated by innate signaling cascades and reinforced through adaptive pathways. Among the mediators of this response are cytokines and chemokines that govern leukocyte recruitment, activation, and function (Kim et al., 2019). Notably, CXCLi2, commonly referred to as IL-8 in avian systems, serves as a potent chemoattractant for heterophils, orchestrating early inflammation (Jeong et al.,

2012; Taha-abdelaziz et al., 2016). Interleukin-1β (IL-1β) and interferon-gamma (IFN-γ) are central to pro-inflammatory signaling, while IL-10 and IL-13 help regulate immune homeostasis and promote recovery (Abbas et al., 2011; Cyktor & Turner, 2011; Li et al., 1998). IL-18, which enhances IFN-γ secretion, and IL-1 receptor antagonist (IL1RN), a natural modulator of IL-1β signaling, further illustrate the tight regulation of the immune environment during infection (Lee et al., 2022a). In addition to these well-characterized mediators, other molecules such as lipopolysaccharide-induced TNF-α factor (LITAF) and heat shock protein 70 (HSP70) have emerged as key indicators of inflammation and cellular stress (Luo et al., 2008; Wang et al., 2012). LITAF is involved in the transcriptional regulation of pro-inflammatory genes, while HSP70 contributes to protein stabilization and immune signaling during cellular insult.

Despite isolated findings in single-species infection models, a significant gap remains in our understanding of the coordinated expression of these immune markers during mixed *Eimeria* infections in turkeys. Most existing studies fail to capture the complexity of field-relevant infections, and they do not thoroughly assess the spatial dynamics of immune gene expression. To address this gap, the present study investigates the expression profiles of IL-8, IL-10, IL-18, IFN-γ, IL-1β (IL1RN), LITAF, and HSP70 in key immune tissues, specifically the ceca, spleen, liver, and cecal tonsils, following a clinically apparent, mixed *Eimeria* infection in turkey poults. This approach aims to delineate the molecular signatures of host immunity and uncover potential biomarkers of resistance or susceptibility. Ultimately, these findings may inform the development of targeted control strategies, including vaccines and immunotherapeutics, to reduce the effects of coccidiosis in commercial turkey operations.

Materials and Methods

The Study was performed under the approval of the Institutional Animal Care and Use Committee (IACUC) of North Carolina State University, Raleigh, NC, USA **(23-256-01)**.

Animals

A total of 104 day-old Nicholas poults were obtained from Aviagen turkeys (Nicholas Select, Aviagen Turkeys Inc., Lewisburg, WV, USA) and raised under controlled environmental conditions. The poults were randomly assigned to two treatment groups, each with four replicates of 13 poults each. The birds had *ad libitum* access to feed and water for the duration of the 19-day study. Diets were formulated based on the nutrient requirements for turkeys (Aviagen Turkeys, 2015).

Experimental Design

Two treatments were used in the experiment: poults infected with a 3-species mixture of *E. adenoeides, E. gallopavonis,* and *E. meleagrimitis* (Challenge) and an uninfected control (Control). On d4, poults assigned to the Challenge group were inoculated with 25,000 sporulated oocysts of the mixed *Eimeria* mixture. From day of placement to 10 days of age, thick paper was maintained on the wire floor cage. Feces were collected to calculate OPG on 6dpi. Individual body weights (BW) were recorded weekly and calculated as the average weight gain per bird (BWG).

Infection

E. adenoeides, E. gallopavonis, and *E. meleagrimitis* oocysts that had been purified and newly sporulated were kept at 4 °C until the fourth day of the study, when poults in the Challenge group were inoculated. Oocysts were counted and quantified in a McMaster chamber

after floating in a saturated salt solution. The quantity of oocysts per milliliter of solution was used to determine the number of sporulated oocysts.

Tissue Collection

On 4, 7, 9, 11, 13, and 15 days post-infection (dpi), two poults were randomly selected from each pen (8 poults/treatment), weighed, and euthanized to collect cecal and cecal tonsil tissues. Liver and spleen samples were obtained on 4, 7, 9, and 15 dpi. The tissues were collected and rinsed in phosphate-buffered saline (PBS; Sigma Chemical Company, St. Louis, MO) to remove as much material from the lumen as possible. After rinsing, tissues were placed in RNAlater® (Thermo Fisher Scientific, Waltham, MA) and stored at −80 °C until RNA extraction. Due to increased mortality in the Challenge group, only six poults were available for sampling on 13 and 15 dpi in this treatment cohort.

Quantitative real-time PCR

The liver, spleen, cecal tonsils, and ceca of turkey poults were collected in RNA preservation solution (RNAlater, Invitrogen, Carlsbad, CA) and kept at -80 °C until processing. RNA was isolated by cutting and removing a 1-cm section of the tissues from poults in each treatment group. One milliliter of TRI Reagent™ Solution was added after these fragments were put into screw cap homogenization vials with individual labels. Using a bead beater, each sample was homogenized for 20 seconds. Following the manufacturer's instructions, an RNeasy kit (QIAGEN, Hilden, Germany) was used to complete the RNA isolation. In order to measure the RNA levels and guarantee the proper dilution, a NanoDrop2000 (Thermo Fisher Scientific, Waltham, MA) was utilized. A high-capacity synthesis kit (Applied Biosystems Waltham, Massachusetts, USA) was then used to convert the RNA into cDNA for the quantitative

polymerase chain reaction (qPCR). Each qPCR sample was run in triplicate. 500 nM of gene-specific forward and reverse primers, 2.5 ng of samples (Table 5.1), and 2X power SYBR green master mix (Applied Biosystems Waltham, Massachusetts, USA) were added to each well. H_2O was then added to bring the volume to 20 µL. Using qPCR, the Applied Biosystems StepOnePlus real-time PCR system measured the levels of IL-8, IL-10, IL-18, IFNγ, IL1β, LITAF, and HSP 70 in cells. A 10-minute initial denaturation at 95 °C was followed by 40 cycles of 95 °C for 30 seconds, 60 °C for 30 seconds, and 72 °C for 30 seconds for each reaction. A fundamental housekeeping gene, β-actin expression, was then used to standardize the results.

The reciprocal was then calculated using the following formula for convenience of interpretation: 1/ (target gene cycle threshold/beta actin cycle threshold). All information is displayed as CT-1 reciprocal cycle thresholds. After setting NC as the control, ΔΔCT was computed by first averaging each treatment CT^{-1}. The ΔCT was then calculated by subtracting the average for NS from each CT^{-1}. The ΔΔCT results are identical to the ΔCT values because NC was utilized as the control. The equation $2^{-\Delta\Delta CT}$ (Pfaffl, 2001) was used with these values. Information on primers of target genes and housekeeping genes is listed in Table 5.1.

Statistical Analysis

BW and qPCR data were analyzed using JMP software (version 18.0.2, SAS Institute Inc., Cary, NC, USA) using a t-test. All the data were expressed as mean ± SEM for each treatment. OPG data were analyzed as a completely randomized design using the Proc Mixed Procedure of SAS (SAS 6.4 software, SAS Inc., 2016). $P \leq 0.05$ was used to determine statistical significance.

Results

Oocysts count

Poults in the challenge group showed a higher oocyst output, with the control group having zero oocysts at 6dpi after the challenge with 25 x 10^3 oocysts/ml (Table 5.2). The number of total oocysts in the Challenge group, 3,356,640 ± 930002.49OPG, was substantially higher ($p < 0.05$) than that in the Control group, with no oocysts detected.

Performance

The BW at sampling on 7dpi was significantly different ($p < 0.05$) between the control (223.13. ± 12.43 g) and challenge groups (121.88 ± 16.29 g), as illustrated in Table 5.2, consistent with *Eimeria*-induced disease. The Control group consistently had higher BW ($p < 0.05$) than the Challenge group over the whole experimental period. At 7 dpi, the Challenge group's mean BW was 121.88 ± 16.29 g, while the Control group's was 223.13 ± 12.43 g. Similarly, at 13 dpi, Control BW was remained higher (465.63 ± 14.68 g) than Challenge poults (377.50 ± 16.37 g).

Mortality

Table 5.2 shows that the Challenge group had higher mortality ($p < 0.05$) throughout the study than the Control group.

Immune gene expression

The effects of mixed *Eimeria* species on turkey poults' immunological gene expression are displayed in Figures 5.1- 5.7 and are all presented as values relative to β-actin gene. Cecal transcription of IL-8 (Figure 5.1) was elevated ($p < 0.05$) at 7dpi in the Challenge group (0.75 ± 0.02) and the IFNγ gene (Figure 5.4) was also elevated ($p < 0.05$) in the Challenge group on 7dpi (0.83 ± 0.04), 9dpi (0.78 ± 0.02), and 11dpi (0.78 ± 0.02). The challenge group cecal tonsil showed

a significant upregulation of IFNγ (Figure 5.4) on 4dpi (0.71 ± 0.01), IL-1β (Figure 5.5) gene expression at 9dpi (0.86 ± 0.01), and HSP70 (Figure 5.7) at 7dpi (0.79 ± 0.03), respectively .

In comparison to the control group, livers from the challenge group birds showed lower ($p < 0.05$) levels of IL-8 (0.77 ± 0.01) and IL-18 (0.85 ± 0.0009) genes on 7dpi (Figure 5.1 & 5.3 respectively) and HSP 70 (0.89 ± 0.003) on 15dpi (Figure 5.7). In the spleen, on 15dpi (Figure 5.1), the challenge group showed a higher transcription ($p<0.05$) of IL-8 (0.65 ± 0.01) compared to the control birds (0.61 ± 0.01). Finally, splenic transcription of HSP70 was higher ($p<0.05$) for the challenge group on 9dpi (0.71 ± 0.01) and 15dpi (0.66 ± 0.005) dpi (Figure 5.7). In all tissues analyzed in the treatment groups, there were no discernible variations ($p > 0.05$) in the relative gene expression of IL-10 (Figure 5.2) and LITAF (Figure 5.6).

Discussion

The present study assessed the immunological responses in specific tissues of turkey poults following a 3-species *Eimeria* challenge, focusing on ceca, cecal tonsils, liver, and spleen. Understanding tissue-specific immune responses is critical in avian immunology, as different mucosal and systemic sites play distinct roles in pathogen recognition, antigen processing, effector function, and immune memory development. The gene expression results presented here emphasize important cytokines in the ceca, liver and secondary lymphoid organs and offer a thorough description of immunological gene expression in turkey poults after a mixed *Eimeria* infection. Our results show a coordinated elevation of regulatory and pro-inflammatory mediators, which is in line with a complex and dynamic host response meant to minimize tissue damage and manage parasite multiplication. IL-8 enhances the immune response and disease-associated damage in birds, guides the migration of immune cells, and promotes endothelial cell survival and angiogenesis (Li et al., 2003). Recently, Wang et al. (2020), reported that IL-8

expression was significantly higher in the spleen and cecum of *E. tenella*-infected chickens, and other previous reports showed that infection with *E. tenella, E. maxima*, and *E. acervulina* also led to the upregulation of IL-8 (Gadde et al., 2011; Swinkels et al., 2007). Throughout the duration of this study, there was a noticeable variation in the expression of IL-8 in both time and tissue (Figure 5.1), with liver and spleen, which are peripheral to the sites of infection within the gut, expressing intermittently different levels. At 15dpi, a statistically significant elevation of IL-8 was seen in the spleen which is consistent with findings from Wang et al. (2020), who also showed increased IL-8 activity in lymphoid tissues during *Eimeria* infection. In addition, at 7 dpi, IL-8 expression in the ceca increased significantly, indicating an early inflammatory response in the intestines alongside liver tissue. The cecal tonsils of challenged poults had an elevated, but not significant level of IL-8 gene expression at the same time point of cecal samples, indicating that during the first week of infection, inflammatory response is more prominent in the gut, followed by a more systemic inflammation response later in the presented test period.

A key regulatory cytokine, IL-10 exerts its anti-inflammatory effects primarily by inhibiting pro-inflammatory mediators, such as TNF-α, IL-6, and IL-1β and aids in immune system regulation during an *Eimeria* infection by limiting excessive inflammation and IFNγ-driven Th1 responses, both of which are crucial for controlling intracellular parasites (Morita et al., 2001). Increased expression of IL-10 has been associated with an increased risk of coccidiosis (Lee et al., 2022b). Some reports have shown that IL-10 was downregulated in infection-resistant hens but increased in vulnerable ones, perhaps an indication that IL-10 aided immune evasion of *Eimeria* during coccidiosis (Kim et al., 2019; Rothwell et al., 2004). Additionally, IL-10 levels increased when *Eimeria* infection peaked, which may have allowed

the parasite to avoid macrophage-mediated clearance by reducing the inflammatory response (Collier et al., 2008). Greater sensitivity to *Eimeria* infection is indicated by higher oocyst shedding and more serious disease, especially in birds with naturally elevated IL-10 expression (Cupo, 2022). Although variations of IL-10 gene expression in this study were not statistically significant (Figure 2), varying expression of IL-10 in the ceca and cecal tonsils might indicate a temporary alteration of the mucosal immune response to *Eimeria* infection. By inhibiting protective pro-inflammatory responses, even slight elevations in IL-10 at these local sites may contribute to suppression of inflammation and allow uninhibited parasite reproduction.

Macrophages are the primary source of interleukin-18, which, in conjunction with IL-12, activates Th1 cells and triggers the release of IFN-γ, a crucial component of macrophage activation during immunological responses (Göbel et al., 2003). Structurally related cytokines, IL-1β and IL-18, play important roles in early inflammation by stimulating the production of chemokines and the secretion of IFN-γ, respectively (Yamada et al., 2001). According to Cox et al. (2010), the presence of yeast-derived β-glucan in the jejunum of broiler chicks increased the expression of IL-18. In mammals, IL-18 and IL-12 promote Th1-mediated immunity against intracellular infections such as coccidiosis (as reviewed by Biet et al., 2002). In this study, the liver of turkey poults challenged with mixed *Eimeria* species exhibited significantly lower levels of IL-18 expression at 7 dpi (Figure 3). A localized inhibition of systemic inflammatory signals in the early stages of infection could be the cause of this decline. Although IL-18 is generally linked to the stimulation of Th1 responses, its downregulation in the liver may be a sign of *Eimeria*'s immune evasion tactics or a host-driven tactic to prevent excessive inflammation and possible tissue damage. In contrast, Cox et al., (2010) found that broiler chicks exposed to yeast-derived β-glucan had higher levels of IL-18 in their jejunum, demonstrating that IL-18 regulation

in poultry is context-dependent and tissue-specific. A crucial cytokine in coccidiosis, IFNγ is known to prevent the intracellular growth of *Eimeria* (Lee et al., 2022b). This study reported increased IFNγ expression in the ceca of infected poults, which aligns with other findings that connected Th1 cytokine production to *Eimeria* infection. Results by Breed et al. (1997); Dung (2020); Saber et al. (2025) support the role of IFN-γ in host defense against *Eimeria* and lymphocyte activation in poultry. Pro-inflammatory cytokines, including IFN-γ and IL-1β, were significantly upregulated in the ceca at certain times, suggesting that the major location of *Eimeria* replication was experiencing active immune responses. The spleen, cecal tonsils, and, to some extent, the liver all showed increased IFNγ expression in this study, which is consistent with findings from Maes (2011).

Laurent et al. (2001) report that IL-1β, a critical inflammatory cytokine, increases when birds are first infected with *E. acervulina, E. tenella,* or *E. maxima*. According to primary infection investigations and chicken macrophage microarray analysis from Dalloul et al. (2007), their expression is markedly elevated in the duodenum, jejunum, and cecum of *Eimeria*-infected chickens (Hong et al., 2006b). A non-uniform temporal pattern was indicated by the expression of IL-1β in both mucosal and systemic organs, with variations noted in the tissues under investigation. Although there was a statistically significant rise in the cecal tonsils at 9dpi, there was no sustained elevation in the overall expression profile in mucosal tissues (Figure 5.5). The cytokine response to *Eimeria* infection appears to be extremely tissue-specific and dynamically controlled, as seen by the varied IL-1β levels observed in systemic tissues. Instead of a straightforward distinction between mucosal and systemic immunity, these discrepancies may be due to variations in local antigen burden, immune cell composition, and the timing of cytokine signaling cascades. This pattern is partially supported by the findings of Gadde et al. (2011), who

discovered that chickens infected with *E. tenella* or *E. maxima* had greater levels of IL-1β expression. However, they noted that statistical significance was only achieved at 7dpi due to significant heterogeneity. IL-1β response in turkey poults appears to be specific and more time-limited, which may be a result of varying species-specific immunological dynamics and *Eimeria* species composition.

A transcription factor called LITAF influences the production of the TNF-α gene, one of the essential cytokines in the innate immune response. After infection with *Eimeria*, LITAF expression showed varying patterns across several timepoints and tissues (Figure 5.6). In contrast to other tissues that were analyzed, the liver consistently showed non-significant higher levels of LITAF upregulation. This pattern is consistent with the results of Hong et al. (2006), who found that immunological stimulation also increased LITAF expression. During inflammatory stress caused by *Eimeria*, systemic immune activation and gut-liver interaction are reflected in liver-specific LITAF overexpression.

Heat shock proteins (HSPs) help fold proteins and prevent damage, thereby preserving protein stability and shielding cells from external stress. Particularly in intestinal tissues, they facilitate cellular repair and regulate stress responses by rapidly increasing in response to heat, oxidation, and inflammation (Hu et al., 2022; Shaukat et al., 2025). As a defense against outside stressors, HSPs aid in preserving the cell's structural and metabolic integrity (Zhao et al., 2013). Heat shock protein 70 protects produced proteins from further harm by acting as a molecular chaperone that promotes protein-protein interactions (Soleimani et al., 2012). All tissues under investigation showed variations in HSP70 expression after the *Eimeria* challenge. Interestingly, at 7 dpi, a statistically significant upregulation was found in the cecal tonsils (Figure 7); at 15 dpi, in the liver; and at both 9 and 15dpi, in the spleen. These results suggest a pattern of stress

response that varies with tissue and time and is probably influenced by parasite burden dynamics and progressing immune activation from local to systemic activity. The elevated expression of HSP70 and LITAF in several organs suggests a widespread cellular stress response and inflammatory pathway activation, which are likely exacerbated by growing parasite load and related tissue damage. These findings highlight the coordinated participation of systemic and local immune responses to *Eimeria* infection in turkeys.

Conclusion

In conclusion, during *Eimeria* infection, profiling the expression of immune-related genes, such as chemokines and cytokines, can provide vital information for disease management. Notably, when exposed to mixed *Eimeria* infection, turkey poults develop a tissue-specific and temporally dynamic immune response. Considerable localized inflammation at the main sites of parasite replication is indicated by the significant elevation of IL-8, IFN-γ, IL-1β, and HSP70 in the ceca and cecal tonsils. Additionally, systemic organs, including the liver and spleen, displayed more varied expressions, with the liver exhibiting a substantial downregulation of IL-8 and IL-18. These findings highlight the significance of systemic and local immune responses in turkeys, laying the groundwork for the creation of focused immunological treatments to combat coccidiosis.

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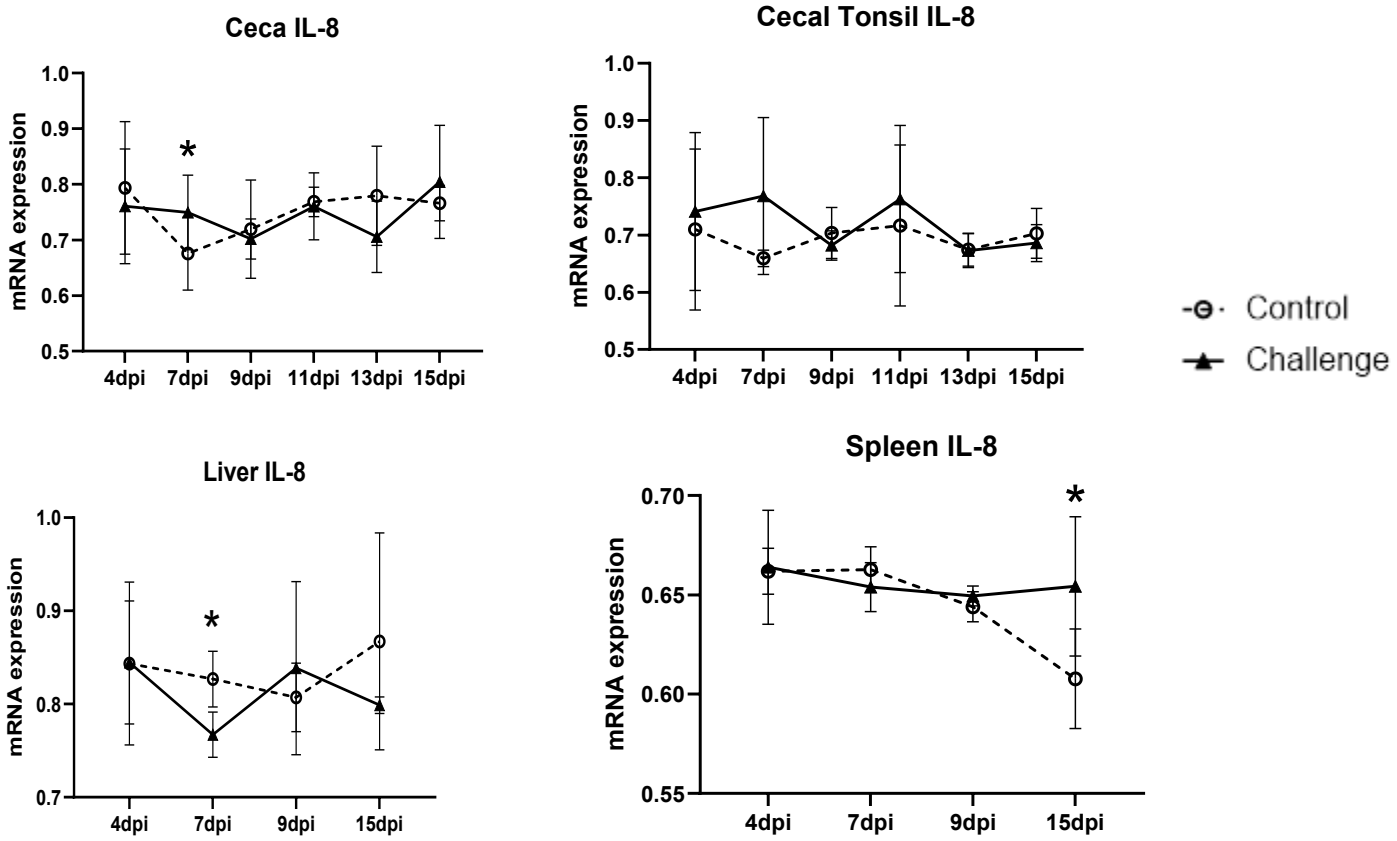

Figure 5. 1 Turkey poults IL-8 immune gene expression in response to mixed *Eimeria* species.

Oral gavage of 25 x 10^3 sporulated oocysts/ml of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis* was used to challenge the poults on d4. At 4, 7, 9, 11, 13, and 15 dpi after inoculation, ceca and cecal tissues were obtained in RNA later solution, while liver and spleen tissues were obtained on 4, 4, 9 and 15 dpi. Together with the housekeeping gene β-actin, the expression of IL-8 was measured using real-time PCR ($2^{-\Delta\Delta CT}$). The levels of expression are displayed in relation to β-actin. A statistical difference ($p < 0.05$) between the treatment groups at each time point is indicated in the figure by an asterisk above the standard error of means lines.

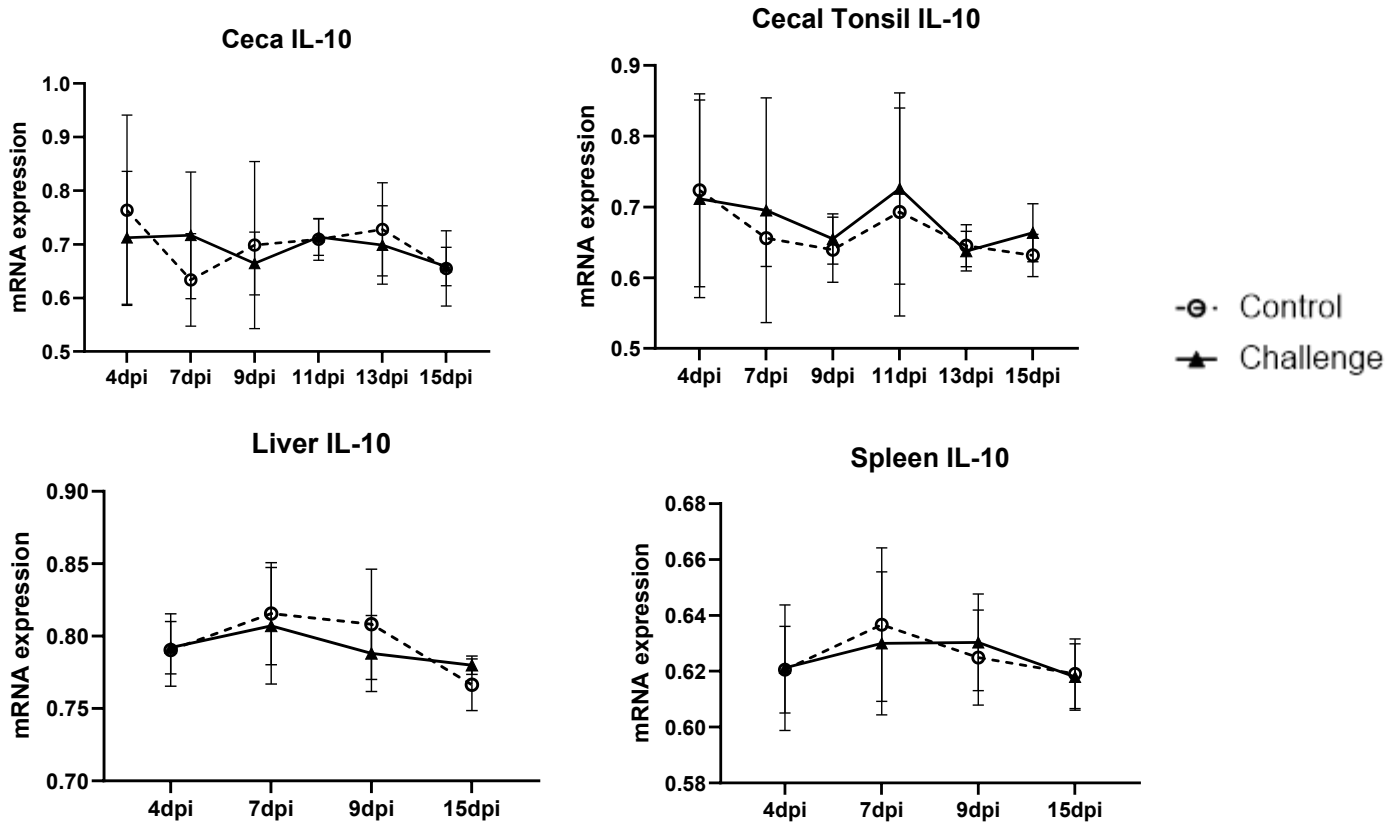

Figure 5. 2 Turkey poults IL-10 immune gene expression in response to mixed *Eimeria* species.

Oral gavage of 25 x 10^3 sporulated oocysts/ml of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis* was used to challenge the poults on d4. At 4, 7, 9, 11, 13, and 15 dpi after inoculation, ceca and cecal tissues were obtained in RNA later solution, while liver and spleen tissues were obtained on 4, 4, 9 and 15 dpi. Together with the housekeeping gene β-actin, the expression of IL-10 was measured using real-time PCR ($2^{-\Delta\Delta CT}$). The levels of expression are displayed in relation to β-actin. A statistical difference ($p < 0.05$) between the treatment groups at each time point is indicated in the figure by an asterisk above the standard error of means lines.

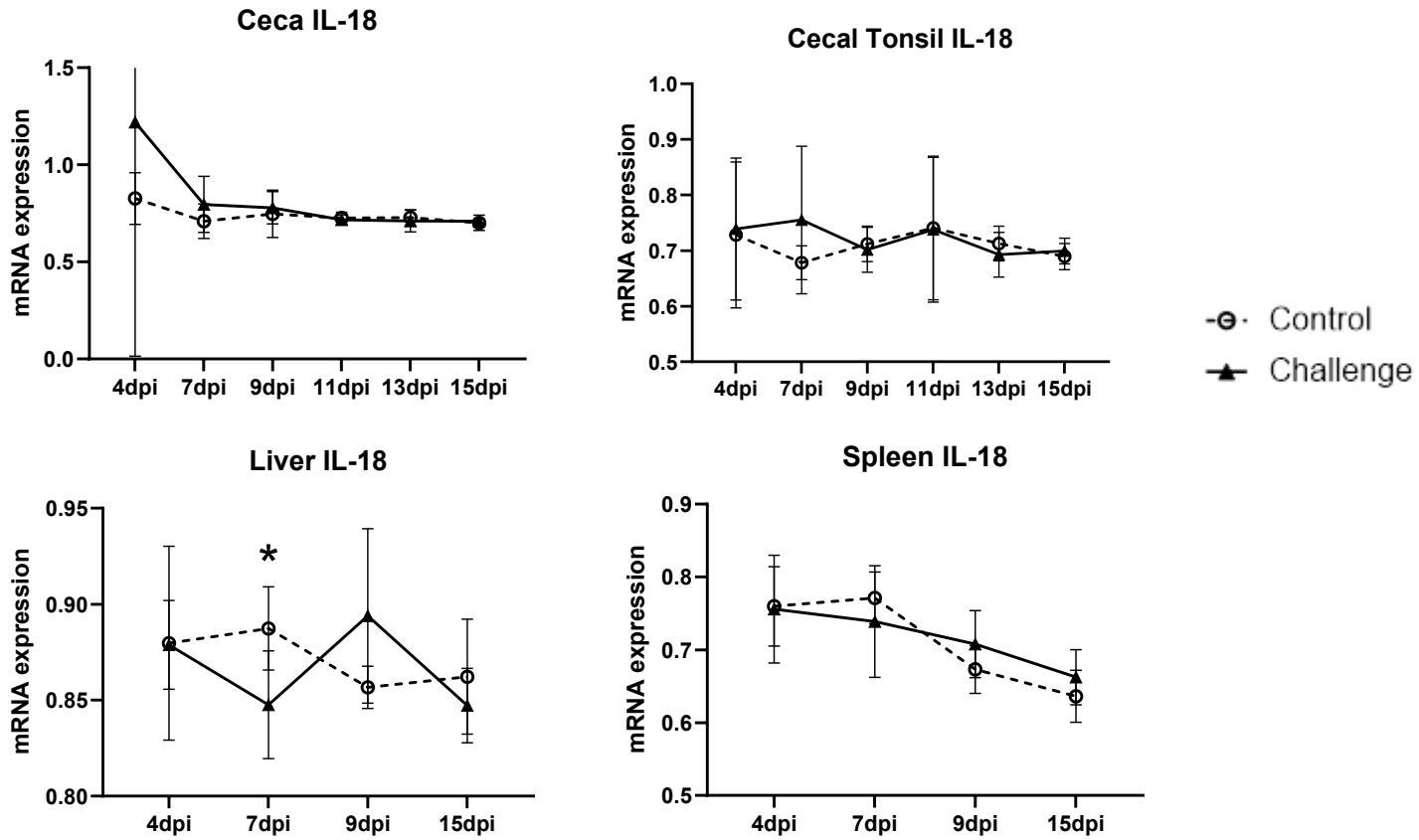

Figure 5. 3 Turkey poults IL-18 immune gene expression in response to mixed *Eimeria* species.

Oral gavage of 25 x 10^3 sporulated oocysts/ml of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis* was used to challenge the poults on d4. At 4, 7, 9, 11, 13, and 15 dpi after inoculation, ceca and cecal tissues were obtained in RNA later solution, while liver and spleen tissues were obtained on 4, 4, 9 and 15 dpi. Together with the housekeeping gene β-actin, the expression of IL-18 was measured using real-time PCR ($2^{-\Delta\Delta CT}$). The levels of expression are displayed in relation to β-actin. A statistical difference ($p < 0.05$) between the treatment groups at each time point is indicated in the figure by an asterisk above the standard error of means lines.

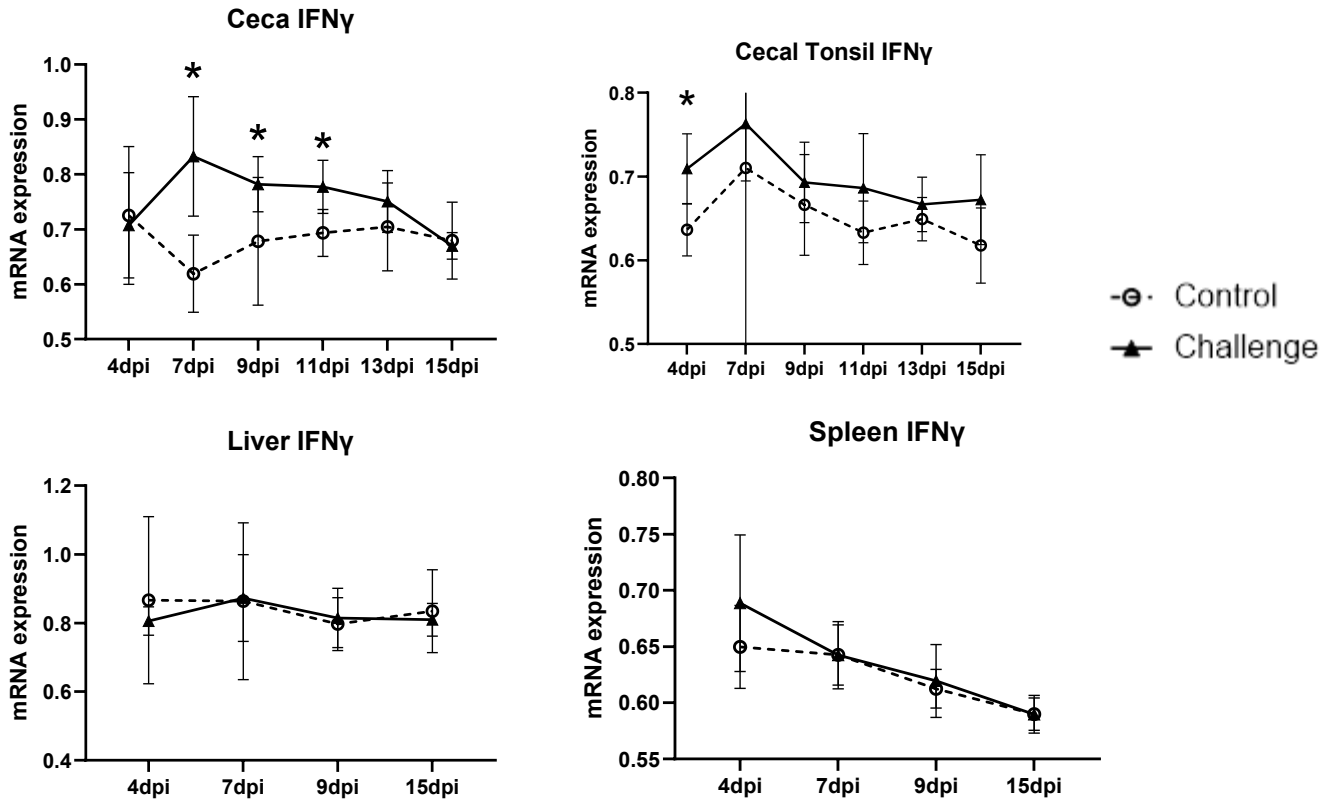

Figure 5. 4 Turkey poults IFNγ immune gene expression in response to mixed *Eimeria* species.

Oral gavage of 25 x 10^3 sporulated oocysts/ml of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis* was used to challenge the poults on d4. At 4, 7, 9, 11, 13, and 15 dpi after inoculation, ceca and cecal tissues were obtained in RNA later solution, while liver and spleen tissues were obtained on 4, 4, 9 and 15 dpi. Together with the housekeeping gene β-actin, the expression of IFNγ was measured using real-time PCR ($2^{-\Delta\Delta CT}$). The levels of expression are displayed in relation to β-actin. A statistical difference ($p < 0.05$) between the treatment groups at each time point is indicated in the figure by an asterisk above the standard error of means lines.

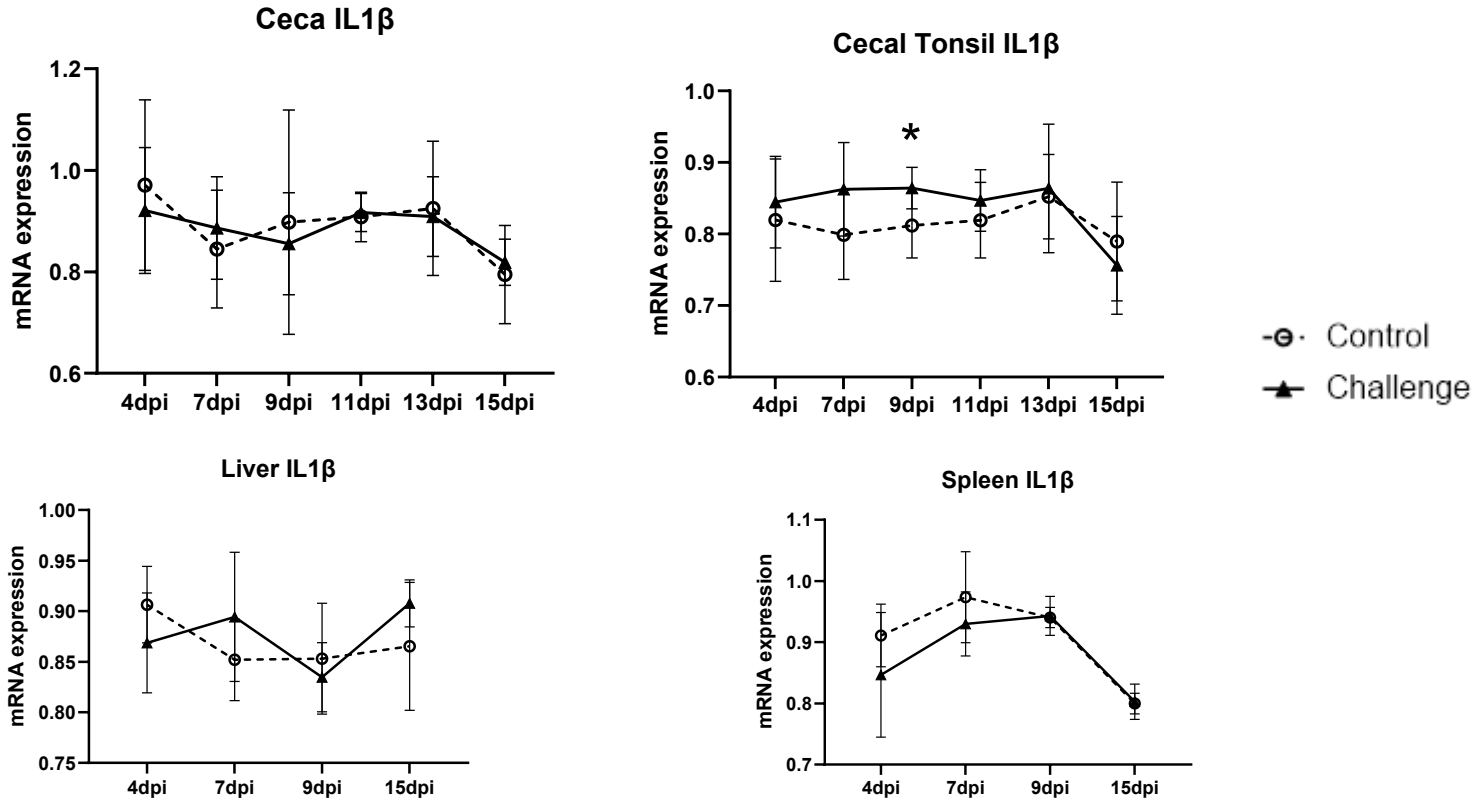

Figure 5. 5 Turkey poults IL1β immune gene expression in response to mixed *Eimeria* species.

Oral gavage of 25 x 10^3 sporulated oocysts/ml of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis* was used to challenge the poults on d4. At 4, 7, 9, 11, 13, and 15 dpi after inoculation, ceca and cecal tissues were obtained in RNA later solution, while liver and spleen tissues were obtained on 4, 4, 9 and 15 dpi. Together with the housekeeping gene β-actin, the expression of IL1β was measured using real-time PCR ($2^{-\Delta\Delta CT}$). The levels of expression are displayed in relation to β-actin. A statistical difference ($p < 0.05$) between the treatment groups at each time point is indicated in the figure by an asterisk above the standard error of means lines.

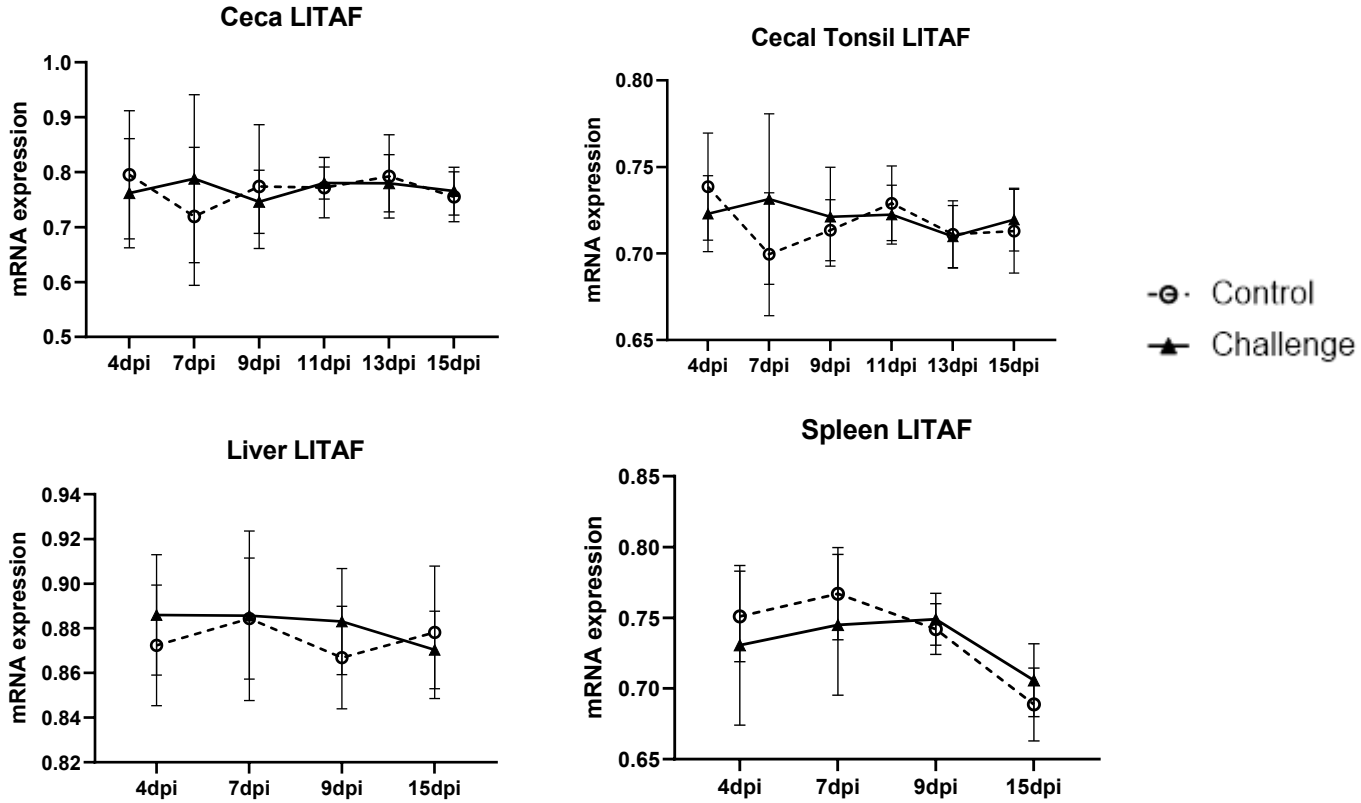

Figure 5. 6 Turkey poults LITAF immune gene expression in response to mixed *Eimeria* species.

Oral gavage of 25 x 10^3 sporulated oocysts/ml of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis* was used to challenge the poults on d4. At 4, 7, 9, 11, 13, and 15 dpi after inoculation, ceca and cecal tissues were obtained in RNA later solution, while liver and spleen tissues were obtained on 4, 4, 9 and 15 dpi. Together with the housekeeping gene β-actin, the expression of LITAF was measured using real-time PCR ($2^{-\Delta\Delta CT}$) . The levels of expression are displayed in relation to β-actin. A statistical difference ($p < 0.05$) between the treatment groups at each time point is indicated in the figure by an asterisk above the standard error of means lines.

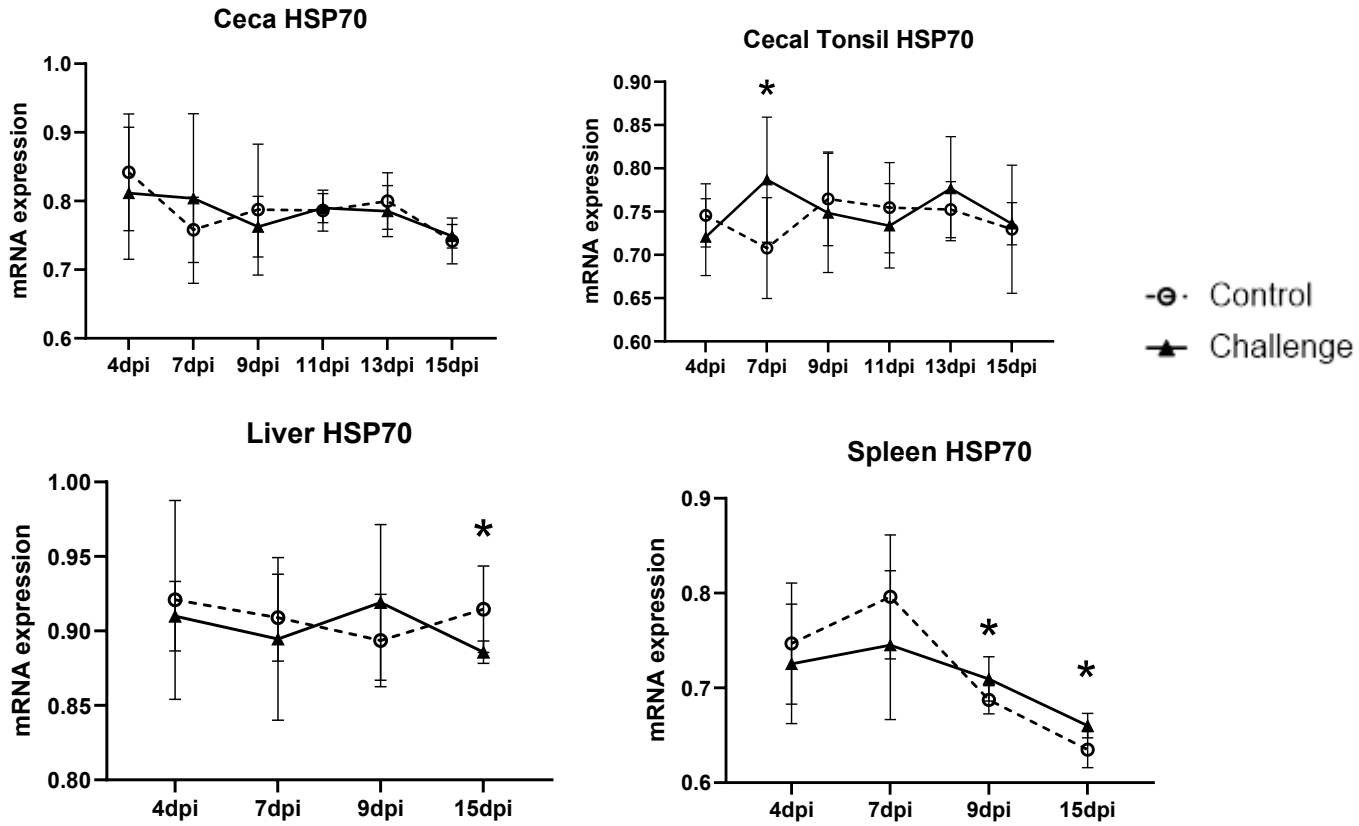

Figure 5. 7 Turkey poults HSP70 immune gene expression in response to mixed *Eimeria* species.

Oral gavage of 25 x 10^3 sporulated oocysts/ml of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis* was used to challenge the poults on d4. At 4, 7, 9, 11, 13, and 15 dpi after inoculation, ceca and cecal tissues were obtained in RNA later solution, while liver and spleen tissues were obtained on 4, 4, 9 and 15 dpi. Together with the housekeeping gene β-actin, the expression of HSP70 was measured using real-time PCR ($2^{-\Delta\Delta CT}$). The levels of expression are displayed in relation to β-actin. A statistical difference ($p < 0.05$) between the treatment groups at each time point is indicated in the figure by an asterisk above the standard error of means lines.

Table 5. 1 The primer sequences utilized in qPCR

Gene	Forward Primer Sequence	Reverse Primer Sequence
β-Actin	GGCTCTATCCTGGCCTCTCT	CATCACAAGGGTGTGGGTGT
IL-8	CCACTGCAAGAACGTTGAAAT	CAGTGGTGCATCAGAATTGAG
IL-10	CGCTGTCACCACTTCTTCAC	CCCGTTCTCATCCATCTTCTCG
IL-18	GTGTGCAGTACGGCTTAGAGA	AATCTGGACGAACCACAAGCA
IFNγ	TGAACTGAGCCATCACCAAGAA	ACCGTCAGCTACGTCTGAATG
IL-1B (IL1RN)	CCTCCAGCCAGAAAGTGAGG	GAAGTGGGGATGGGATGGG
LITAF	ATGACCGCCCAGTTCAGATG	CCAGCTATGCACCCCAGC
HSP 70	TGAATCAACAGCAGTTGCCC	AATTTCCTGCCTCCAAGCGT

Abbreviations: β-Actin, beta actin; IL-8, interleukin 8; IL-10, interleukin 10; IL-18; interleukin 18; IFNγ, interferon gamma; IL-1B, interleukin 1 beta; LITAF, lipopolysaccharide-induced tumor necrosis alpha factor; HSP70, heat shock protein 70.

Table 5. 2 Effect of mixed *Eimeria* species on Total Oocyst Output and Growth Performance of Turkey Poults

Treatment[1]	Control	Challenge	P-value
Total OPG at 6dpi	0 ± 0^{b}	$3{,}356{,}640 \pm 930{,}002.49^{a}$	0.02
BW (g)			
d1	55.77 ± 0.59	55.96 ± 0.55	0.8118
[2]BW (g)			
4dpi	142.5 ± 11.30	128.13 ± 8.13	0.3192
7dpi	223.13 ± 12.43^{a}	121.88 ± 16.29^{b}	0.0002
9dpi	260.63 ± 18.09	210.63 ± 16.89	0.0629
11dpi	338.13 ± 14.30	308.13 ± 6.33	0.0757
13dpi	465.63 ± 14.68^{a}	377.5 ± 16.37^{b}	0.0018
15dpi	583.75 ± 17.26	555.83 ± 34.09	0.4456
Mortality (%)	0.25 ± 0.25^{b}	1.75 ± 0.25^{a}	0.0219

Abbreviations: OPG, oocyst per gram; BW, body weight; [2]BW of sampled birds. The poults in the challenge group were inoculated with 25 x 10^3 sporulated oocysts/ml of *E. adenoeides, E. gallopavonis*, and *E. meleagrimitis* by oral gavage on 4 days of age. Mean values with different superscript letters within a row indicate a significant difference ($P < 0.05$).

CHAPTER 6

Dissertation Conclusions

Characterization of immune response in turkey poults challenged with mixed *Eimeria* infection is one of the main goals of this dissertation. It focused on how turkey poults interact when challenged with *Eimeria* and how effective control strategies, such as addition of additives, use of vaccines can help in controlling the disease. In addition, the dissertation addressed the understanding of tissue-specific cytokine profiling in some tissues in the poults, as a means of creating better control strategies. Important discoveries from the literature highlight the ongoing health and economic problems caused by coccidiosis in turkeys, which is caused by host-specific *Eimeria* species. According to the literature, there is a conscious effort to controlling anticoccidial resistance, a major problem in the use of drugs in the poultry industry.

Chapter 3 evaluated the use of vaccine with amprolium supplementation for managing oocysts cycling which showed improved mucosal immunity, retained intestinal integrity and decreased fecal shedding. Both vaccination and amprolium increased CD40 in cecal tonsils and increased numbers of γδ T cells, $CD4^+$, $CD8^+$, and $CD28^+$ in flow cytometric studies, indicating a balanced innate-adaptive response that reduced disease and increased feed efficiency.

Chapter 4 focused on supplementing the diet of *Eimeria*-challenged turkey poults with dried egg products (DEPs), which have been proven to be effective in chickens to help alleviate the performance losses. Turkey dried product at higher doses helped to reduce oocyst shedding and altered cytokine expression in the poults. Particularly, in the cecal tonsils and jejunum, downregulation of IL-10 and overexpression of IL-18 indicated a mechanism for boosting pro-inflammatory responses that are essential for parasite clearance while preserving growth performance and reducing mortality.

Tissue-specific immune gene dynamics to mixed *Eimeria* challenge in turkey poults was studied in Chapter 5. Results showed cytokines like IL-10 increased in the spleen, indicating systemic resolution, pro-inflammatory mediators like IFN-γ and IL-1β peaked in the ceca and cecal tonsils during active infection stages, reflecting powerful mucosal defense. The result provides a molecular means of tracking the severity of *Eimeria* infection in turkey poults and showed interaction between inflammation, stress, and recovery through increased levels of LITAF and HSP70.

The studies cumulatively confirm the coordinated, dynamic and complex immune response to mixed *Eimeria* species, which is primarily cell-mediated and amenable to strategic control strategies. By adjusting cytokine balance, dietary bioactives such as DEPs provide a non-antibiotic substitute. Bioshuttle strategy that combines the use of low dose vaccines with anticoccidial supplementation helps maximize immune priming maintaining intestinal gut health, meeting industry demands for robust, productive flocks.

Interactions between microbiome and immune response in DEP-supplemented or vaccinated poults should be the focus of future research, possibly employing metagenomics to find probiotics that work in concert with DEP. In addition, the use of next generation vaccines that targets conserved antigens may be informed by comparative genomics of *Eimeria* strains, and scalability would be confirmed by field trials that combine these approaches. To further lessen dependency on treatments, selective breeding may be made possible by investigating genetic markers for resistance in turkeys. The dissertation essentially offers practical insights for managing coccidiosis and advocates for integrated strategies that improve the management and control of coccidiosis in turkey poults. It opens the door for creative, empirically supported

answers to a persistent problem in turkey health by connecting basic with real-world applications.