ABSTRACT

CHADWICK, ELLE. The Role of Poultry Parasites in Gut Health and Production. (Under the direction of Dr. Robert Beckstead).

Protozoal infections are becoming more prevalent in the commercial poultry industry with the removal of antibiotics and certain preventative and therapeutic drugs, leading the industry to need a better understanding of these protozoa and their effects on the gut health. *Eimeria* species of coccidia infect all commercial poultry and can cause coccidiosis while *Histomonas meleagris* usually infects longer living poultry and progresses to blackhead disease. Both protozoa cause intestinal damage, resulting in profit losses and animal welfare concerns. In the poultry industry, progression on protozoal disease management and research is dependent on the economic influence of the bird type. The objectives of this dissertation were to identify areas of research needed in relation to protozoal management in commercial poultry production, dependent on bird-type and protozoan, as well as identify potential protozoa outbreak preventative strategies. With broilers, significant economic losses on this highly profitable bird-type has been due to coccidiosis while blackhead disease is rarely observed. Multiple coccidia vaccines are available while the industry is currently testing feed additive gut health modulators to aid the broiler if infection occurs. The use of sodium bisulfate, a natural feed additive, was tested in the diet when broilers were challenged with an increased dosage of a multi-species coccidia vaccine. The sodium bisulfate treatment resulted in infected broilers with greater body weight, improved villi structure and less gut leakage compared to the infected control treatment with sodium bisulfate having no observed effects on the parasite. The use of sodium bisulfate feed additive should be tested in the field to determine its gut health efficacy in coccidia- challenged broilers outside of a research setting.
With longer-living chickens, coccidia vaccines are regularly applied during rearing while infection with *H. meleagridis* during egg production causes animal welfare concerns due to the inflamed cecal tissue. This inflammatory response has a secondary effect of a decrease in performance leading to economic losses. *H. meleagridis* vaccines are currently being examined, but it is not known when to vaccinate the birds to allow for immunity without significant production loss. The conducted broiler breeder trial indicated that *H. meleagridis* infection that led to blackhead disease prior to pullets reaching sexual maturity has limited effects on egg production and quality. This study infers that early introduction of *H. meleagridis* to a chicken laying flock does not lead to production or economic losses and the rearing period should be considered for vaccination purposes.

Turkeys suffering from coccidiosis may have limited to no disease signs, but intestinal health can suffer leading to secondary infections. On the contrary, blackhead disease in turkeys causes high mortality. The sequential infections with *Eimeria* species then *H. meleagridis* exacerbate disease signs in chickens but this has yet to be studied in turkeys. The turkey case report suggests that poor poult quality and improper cocci cycling leads to increased susceptibility to blackhead disease with high mortality and production losses. Controlled, experimental studies still need to be conducted for further explanation of the protozoan interaction. Potential ailments for coccidiosis in turkeys should be examined to decrease secondary infections, like blackhead disease. The data presented herein emphasizes the significance of the health, maturity, and microbe balance of the poultry gut in relation to the entry and establishment of protozoal parasites in poultry production.
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Role of Poultry Parasites in Gut Health and Production

by
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DEDICATION

I would like to dedicate the work of this dissertation to my family. They gave continuous support, love, and interest as I pursued to further my education in a field I am passionate about.
BIOGRAPHY

Elle Victoria Chadwick was born to Drs. Brian and Dianne Chadwick in Hamilton, Ontario Canada. Elle and her three siblings, Monika, Conner, and Rory lived in Canada until 1997 when the family moved to LaGrange, Georgia. Here, Elle grew up in a suburban neighborhood but was able to raise multiple pets. This started her love for animals. From 2010-2014, Elle attended the University of Georgia with hopes of going to veterinary school. While working in a research lab as well as the veterinary teaching hospital, Elle changed her major to avian biology and decided that instead of veterinary medicine, she wanted to pursue a doctorate studying animal diseases. In 2015, Elle started at Auburn University to pursue her Masters degree through the poultry science department. Here, Elle’s projects focused on understanding Salmonella contamination in broilers during grow-out and won numerous awards for the research presentations she gave. She was then offered a doctoral position at North Carolina State University working with protozoal parasites that effect poultry. Her education and training at NC State has broadened her knowledge in nutrition and how this alters disease. She has also been given multiple speaking opportunities as a student and guest-speaker. She currently lives in Raleigh, North Carolina with her dog, Cooper.
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Parasitic relationships occur between a parasite and host, where the parasite gains benefit from the host while the host can suffer from tissue damage, disease or even death. Yet, the biologically ideal parasite might cause morbidity but not mortality for the host in order to establish a continuous relationship (Garrido-Cardenas, et al., 2018). Three classes of animal-host parasites exist: protozoa, helminths and arthropods. Protozoal parasites belong to the Kingdom Protista and are defined as single-celled eukaryotic microorganisms that utilize a host for survival and replication (Taylor, et al., 2015; Mahmud, et al., 2017). Protozoa are found in almost all habitats and their relationship with their host can range from commensal to pathogenic (Yaeger, 1996). In protozoa that use commercial poultry as a host, protozoa can be divided generally into two groups, sporozoite-forming and flagellated (Lillehoj and Lillehoj, 2000; McDougald, 2013). Poultry protozoal parasites that cause animal welfare and economic distress include various *Eimeria* species of coccidia, which form a sporozoite, and *Histomonas meleagris*, which is flagellated (McDougald, 1998; Dolka, et al., 2015). These protozoal parasites are known to induce negative impacts on poultry production due to the pathologies that affect the host’s intestinal health (McDougald, 1998; 2013).

*Eimeria species of Coccidia*

Cocci species fall in the phylum Apicomplexa and are obligate intracellular protozoan parasites that cause intestinal damage to multiple animal species. The genus *Eimeria* is known to have species that infect poultry, leading to economic losses for commercially raised broilers, breeders, turkeys and other poultry production animals (Strout, et al., 1994; Lillehoj and Lillehoj,
Coccidia are distributed globally and can survive environmental extremes due to the oocyst shell acting as a form of natural encapsulation (Blake, et al., 2017). Cocci are host-specific, where each *Eimeria* species can infect select animal species (Chapman, 2008; Taylor, et al., 2015). To date, nine species of *Eimeria* are known to colonize chickens (Strout, et al., 1994; McDougald, 2013) while seven species colonize turkeys (McDougald, 2013; Milbradt, et al., 2014; Rathinam, et al., 2016). Poultry suffering from subclinical coccidiosis can become morbid, leading to poor feed conversion and increased susceptibility to other diseases. Clinical coccidiosis frequently leads to mortality in poultry (Blake, et al., 2017). The negative effects are due to the life cycle of coccidia species, where oocysts invade the enterocytes of various sections of the gastrointestinal tract later to be expelled from the mucosa leaving a damaged epithelial lining (Yun, et al., 2000 Williams, 2005; Milbradt, et al., 2014). This damage leads to inflammation and sloughing of invaded villi, causing malabsorption and enteritis (Blake, et al., 2017). Unsporulated oocysts are then shed into the environment where they can sporulate leading to infection through the fecal-oral route (Yun, et al., 2000). Currently, coccidiosis in commercial poultry is controlled through good husbandry, chemoprophylaxis, vaccination, natural additives or a combination thereof (Blake, et al., 2017).

**Entry and Spread of Chicken and Turkey Eimeria Species in Commercial Poultry Houses**

Coccidia oocysts are considered ubiquitous with poultry rearing, and the *Eimeria* species that infect poultry are found worldwide. These oocysts are brought into production facilities through definitive hosts, paratenic hosts, rodents, flying insects and other pests, contaminated feed and farm equipment, used litter and humans (Reyna, et al., 1983; Williams, 2005). The
entry, transmission, and burden of coccidia in commercial poultry production is best understood through its lifecycle.

One host is utilized for the direct lifecycle of *Eimeria* where three phases of both asexual and sexual reproductive stages occur (Chapman and Jeffers, 2014; Taylor, et al., 2015). First, oocysts in the environment go through their patent period and sporulate to become infective (Tewari and Maharana, 2011). For sporulation, the oocysts need a specific environment of atmospheric oxygen exposure, approximately 40% moisture and warmth of around 29°C (Reyna, McDougald and Mathis, 1983; Williams, 2005; (Price, et al., 2014). The prepatent period starts with fecal- oral recycling of the parasite (Williams, 2005; Soutter, et al., 2020), which is how oocysts enter the gastrointestinal tract of birds (McDougald, 2013). The shell is opened through the mechanical action of the upper digestive tract with help from trypsin, bile, and carbon dioxide. The sporozoites then spread throughout the lumen of the digestive tract. The ingested oocyst will release four sporocysts per oocyst and two sporozoites per sporocyst, totaling eight sporozoites (Taylor, et al., 2015). Host intestinal epithelial cell invasion follows in order for the sporozoites to gain ATP for energy (Roder, 2011). After the sporozoites penetrate the epithelial lining of the intestinal tract, they undergo cell cycling that leads to asexual reproduction within the host epithelial cells and develop as trophozoites (Tewari and Maharana, 2011; McDougald, 2013). Asexual reproduction occurs known as schizogony or merogeny where oocysts are in the intestinal lining and increase in numbers through mitotic division (Chapman and Jeffers, 2014). Merozoites are produced and they penetrate other (healthy) epithelial cells along the host’s intestinal tract (Tewari and Maharana, 2011). Finally, the merozoites penetrate host cells and will differentiate to male (microgamont) or female (macrogamont) (Tewari and Maharana, 2011; Chapman and Jeffers, 2014). Microgamonts will divide and form microgametes (Tewari and
Maharana, 2011). These will fertilize macrogamonts by sexual reproduction leading to zygotes which mature into oocysts (Tewari and Maharana, 2011; Chapman and Jeffers, 2014). Once mature, unsporulated oocysts are released into the environment through the feces and need the specific environmental criteria described above in order to become infective (McDougald, 2013; Taylor, et al., 2015). Oocysts shedding usually occurs at 4-6 days after initial digestion, leading to cycling of the parasite within a flock. Coccidia have a self-limiting life cycle, where the severity of infection is correlated with the number of infective oocysts ingested and epithelial cells available for replication (Williams, 2005). Host protective immunity is developed only after birds cycle coccidia 2-3 times (McDougald, 2013). Management practices and applied treatments will influence both the cycling of this parasite and the host’s immune response to the severity of infection.

**Management Practices that Influence Coccidiosis**

Coccidiosis in poultry production has increased phenomenally as management practices have changed, with more intensive rearing practices and restrictions on certain treatments (Lillehoj and Lillehoj, 2000; Tewari and Maharana, 2011). These protozoa are costly to the industry with interventions and decreased animal productivity leading to billions in revenue losses each year (Tewari and Maharana, 2011; Soutter, et al., 2020). Ideally, coccidiosis is controlled by decreasing oocyst accumulation in the intestinal tract as well as the environment. This will decrease overbearing transmission, reduce clinical signs of disease and improve performance (Tewari and Maharana, 2011; McDougald, 2013; Blake, et al., 2017; Soutter, Werling, Tomley and Blake, 2020). Management practices that can influence oocyst
transmission include the species and age of birds being reared, gut health, diet and litter quality (Blake, et al., 2017).

**Bird species and age** Most published literature and therefore understanding of poultry coccidiosis is through a chicken model, but some mechanisms and treatments can be examined in turkeys. Physical and physiological responses of chickens and turkeys are similar yet a difference between bird hosts is that turkeys lack the severe, gross lesions that are characteristic with some chicken coccidia species (Madden and Ruff, 1979; Milbradt, et al., 2014). Despite differences in the *Eimeria* species host specificity, there are multiple similarities between avian hosts for susceptibility and their immune response to *Eimeria*.

Clinical coccidiosis is seen typically in young animals, although older animals can also be infected following periods of stress (Daugschies and Najdrowski, 2005). In chickens, no age-related differences in innate susceptibilities have been documented (Williams, 2005). Turkeys, at all ages, are susceptible to infection, but resistance to disease has been seen in pouls that are older than 6-8 weeks of age (McDougald and McQuistion, 1978; Hooge, et al., 2000; Chapman, 2008; McDougald, 2013). Age resistance to coccidiosis in turkeys is well-established yet experimental and field research have not verified the mechanisms underlying this phenomenon (Chapman, 2008). Conversely, an oppressive parasite load can lead to disease in older turkeys (McDougald and McQuistion, 1978), but this is rarely observed outside research settings. In congruence with the age of the bird host, the maturity of the bird’s intestinal development and health also play a significant role in infection.

**Gut health** Post-hatch villi development and maintenance has great importance in controlling the response and negative effects of *Eimeria* species. Starvation of chicks and pouls in an experimental setting delays villi development with potentially long-lasting effects on the bird’s
response to microorganisms (Potturi, et al., 2005; Williams, 2005). Coccidia parasites cause impairment to the epithelial lining of the intestinal tract (Cervantes, 2015) by damaging the villi (McDougald, 2013), which compromises the intestinal first line immunological defense mechanisms. Intestinal leakage of plasma proteins and changes in intestinal pH have been observed in chickens suffering from coccidiosis (Williams, 2005). The compromised intestinal health can lead to systemic diseases where changes in microbe populations of the host’s gut lead to secondary or co-infections with viruses, bacteria or other protozoa (Hauck, 2017).

Virus/protozoan interactions have been considered. Disease-causing exposure to viruses, like Marek’s disease, infectious bursal disease and reticuloendothelial virus have been suggested as influencers of the immune system of the host, leading to increased susceptibility to coccidiosis (Williams, 2005). There is little information on the interaction between a cocci vaccine and the other commercial vaccines administered to chickens, but historically, the use of multiple vaccines in chicks indicates that adverse reactions are not an issue if the vaccines are administered in the recommended dosage (Chapman, et al., 2002). These publications indicate that virus load impacts the severity of coccidiosis.

Protozoal populations can also change. In commercial settings, it is common for two or more species of coccidia to infect the bird host’s gut at one time (McDougald, et al., 1986). Since some Eimeria species can overlap colonization sites, multiple species could be competing for the same enterocytes. The interaction between protozoal species of Eimeria and Histomonas meleagridis has been addressed in chickens (McDougald and Hu, 2001) where coccidiosis exacerbates the severity of histomoniasis. This interaction, although common in commercial turkey production, is yet to be addressed in an experimental setting.
The influence *Eimeria* species exert on bacterial populations in the bird gut has been reviewed (Hauck, 2017). A major concern in commercial poultry is necrotic enteritis, where *Eimeria* species cause severe damage to the intestinal mucosa leading to a secondary effect of increased *Clostridium perfringens* populations (Chapman, et al., 2002; Soutter, et al., 2020). Coccidia enterocyte invasion leads to nutrient malabsorption. The excess nutrients, specifically proteins, in the gut provide nutrients for growth and development of *Clostridium perfringens* (Hauck, 2017). Another concern is that some microbes common in the poultry gut, such as *Salmonella* or *Clostridia* species, increase the pathological lesions associated with coccidiosis and/or vice versa. The mucus-associated lymphoid tissue is damaged, during the oocyst invasion potentially leading to changes in the gut microbe populations that can cause secondary infections (Chapman, et al., 2002; Hauck, 2017; Soutter, et al., 2020). Multiple nutritional management strategies have been suggested to alter indirectly the negative effects of coccidiosis by influencing the intestinal health and microbiota of birds (Williams, 2005).

**Feed formulation** A healthy intestine can support the rapid growth of commercial poultry with less nutritional waste (Hooge, et al., 2000). Unfortunately, infection with coccidia alters the passage time of digesta, decreases digesta viscosity, and reduces crude protein, vitamin, and mineral absorption. Protein source and available amino acids also should be considered as a strategy to ensure microbiota balance (Cervantes, 2015; Williams, 2005). Higher crude protein levels increase tryptic activity, which can allow for more efficient excystation of the ingested oocysts. Animal proteins are favored by *Clostridia* species, and higher concentrations of animal proteins can exacerbate the growth of this organism (Williams, 2005). To favor a healthier gut, the addition of exogenous enzymes, like amylases, proteases, phytase or xylanases, can maximize the extraction and digestion of nutrients while maintaining the viscosity of the digesta
(Cervantes, 2015; Walk, et al., 2011). Another option is to maintain a proper electrolyte balance, which will minimize flushing to help birds suffering from a coccidosis-induced diarrhea (Cervantes, 2015). Feed additives is a nonmedicated approach to prevent enteric disease in broilers and turkeys with examples and mechanisms described later.

**Litter quality** The burden of coccidiosis is dependent on the litter management in a commercial poultry house (Chapman, et al., 2002; Price, et al., 2014) due to the parasite lifecycle needing specific environmental conditions for oocyst sporulation (Graat, et al., 1994; Williams, 2005; Price, et al., 2014). The temperature of the house, relative humidity (moisture) and oocyst access to oxygen play major roles in sporulation while bird access to sporulated oocysts will affect cycling of this parasite (Price, et al., 2014). Temperature and humidity alter the speed at which oocysts can sporulate (Edgar, 1955; Reyna, et al., 1983; Graat, Henken, Ploeger, Noordhuizen and Vertommen, 1994). Oocysts remain most viable in various environmental conditions at approximately 4°C while sporulation of the oocysts is optimal around 29°C (Edgar, 1955; Reyna, et al., 1983). Litter condition (dry or damp) effects if sporulation will occur (Graat, et al., 1994) where high moisture levels in the litter lead to bacterial growth, which can promote high ammonia and low oxygen levels (Reyna, et al., 1983; Williams, 2005). After being released in feces and in contact with favorable temperature and humidity, the oocyst will undergo division into the sporocysts if respiration can occur (Young, 1929; Smith and Herrick, 1944). This has been shown experimentally when respiration peaked during the patent period with sporocyst development and maturation then plateaued once the oocysts sporulate (Smith and Herrick, 1944). Another issue is integrity of the oocyst shell that ensures a viable sporocyst. Experimental studies have indicated that if damage to the shell occurs, survivability of the sporocyst is only a few days (Reyna, et al., 1983). Therefore, sporulation of oocysts is negatively affected by
natural degradation and bacterial and ammonia damage in poultry litter (Reyna, et al., 1983; Williams, 2005). These factors in the litter can alter the oocyst’s viability (Tewari and Maharana, 2011).

Bird access to oocysts, whether intended or through natural contamination, will also alter cycling of this parasite. In the first six weeks of rearing, it is common to recover multiple species of *Eimeria* in the litter of chicken and turkey facilities (Long and Millard, 1977; Tewari and Maharana, 2011). The build-up of previous oocyst populations as well as wild-types introduced by other mechanical vectors will increase parasite load in the litter (Reyna, et al., 1983; Chapman, et al., 2002; Chapman and Jeffers, 2014). Improper facility cleaning can lead to unsporulated oocysts which alter the levels of infection if birds are (not) administered a cocci control program. These wild types, due to their fecundity, can resist host immunity developed through attenuated vaccines. With live vaccines, there is the potential for interbreeding of the wild-type and vaccinal populations (Chapman, et al., 2002). Another factor that alters the bird’s access to oocysts is the flock size. The number of birds housed in one facility positively correlates with increased coccidia since more hosts can consume and transmit the parasite (Hooge, et al., 2000). Although the number of produced oocysts is limited by the number of healthy host epithelial cells, oocysts unable to invade will pass through the digestive tract and return to the litter potentially finding a new host (Chapman, et al., 2002). Sporulated oocysts will survive in the litter for approximately three days. Used poultry shavings act a poor reservoir for unsporulated oocysts, however, contaminants in the litter, like invertebrate pests or dust, act as mechanical vectors for oocysts to allow for longer survival of weeks or even months (Reyna, et al., 1983). The fecal-oral route of this parasite greatly influences the flock response and disease
outcome, indicating the importance of litter acting as a vehicle of oocyst transmission as well as a marker of potential disease.

**Diagnostic Tools and Treatment Strategies for Coccidiosis**

Diagnosis of coccidiosis can use a combination of clinical signs and pathology with detection of oocysts in the fecal material or litter. Typically, pathogenic *Eimeria* species can be differentiated in experimental settings based on clinical signs, characteristic tissue lesions, and the size and morphology of the oocysts through microscopic examination when individual or specific species are dosed (Tewari and Maharana, 2011; Blake, Pastor-Fernandez, Nolan and Tomley, 2017).

*Clinical signs* *Eimeria* species cause three levels of disease in poultry: 1. coccidiasis (mild infection), 2. subclinical coccidiosis (reductions in growth and feed efficiency,) and 3. clinical coccidiosis (severe infection). In chickens, coccidiosis can cause diarrhea, morbidity, altered feed efficiency, hemorrhagic lesions of the intestines and potentially death (Williams, 2005). When turkeys are suffering from coccidiosis, they can have clinical signs of reduced feed intake, dehydration, and enteric distress. Growth, feed and water intake, and flock uniformity can suffer (Milbradt, et al., 2014). The clinical signs mentioned above are correlated with multiple enteric disorders in poultry (Hafez, 2011) so coccidiosis diagnosis needs further examination of birds post-mortem using gross pathology and microscopy.

*Gross pathology* *Eimeria* in poultry has general characteristics unique to each species and host (Williams, 2005). In the field and in research settings, chickens can be subjectively scored for severity of intestinal damage due to the *Eimeria* species (Johnson and Reid, 1970) while turkeys do not have a set scoring system. This is because studies using the chicken host and individual
cocci species have led to characteristic and well defined lesions within the intestinal tract while in the turkey, less defined locations are recorded (Chapman, 2008; Milbradt, et al., 2014). The pathology of some species, specifically in turkeys, can change depending on the parasitic (a)sexual reproductive stage. Oocyst load has been suggested to influence the severity of pathological lesions observed post-mortem (McDougald, 2013), but it does not correlate with oocyst output from the bird host (Young, 1929).

The species of *Eimeria* that infect chickens are described below. For this literature review, the species described in greater detail include those that are known to cause losses in commercial chicken production. *E. hagani* has doubtful validity (McDougald, 2013), and therefore, is not described in greater detail. In experimental settings, the nonpathogenic species *E. praecox* and *E. mitis* have resulted in enteritis and reduced feed efficiencies indicating that increased exposure could lead to losses (Tewari and Maharana, 2011), but that level of exposure is rarely observed in the field.

*E. acervulina* is the most frequently encountered coccidia species found in commercial poultry facilities in the Americas (McDougald, et al., 1986; McDougald, et al., 1987; McDougald, et al., 1997). This species will infect the upper portion of the intestinal tract with characteristic white plaque development seen post-mortem in the duodenal loop. *E. acervulina* may have limited effects on body weights but can alter carotenoid absorption, leading to lighter pigmentation. Watery, mucoid feces is observed commonly, potentially leading to litter management issues (Tyzzer, 1929; McDougald, 2013).

*E. brunetti* has been found in about 10-20% of farms surveyed in the Americas (McDougald, et al., 1986; McDougald, et al., 1997). This species infects the lower portion of the digestive tract, below Meckel’s diverticulum to the cloaca. *E. brunetti* can cause reduced weight
gain, poor feed conversion and mortality. Bloody enteritis and ballooning of the lower intestine can be observed post-mortem (Levine, 1942; McDougald, 2013).

*E. maxima* is easily recognized in wet smears due to its characteristic large oocysts. This species infects the mid-intestine from below the duodenal loop through the small intestines. Due to its infection site, this species can influence skin pigmentation in a similar manner to *E. acervulina*. *E. maxima* is moderately to highly pathogenic, where infections can lead to morbidity and mortality. Disease signs from this species can be identified post-mortem from the fluid-filled intestine with yellow or orange mucus and blood in the lumen (Tyzzer, 1929; McDougald, 2013).

If oocysts are not observed microscopically, *E. mivati* is commonly mistaken as *E. acervulina* (Edgar and Seibold, 1964). This is due to this species starting its infection in the upper digestive tract and then moving down to the lower digestive tract and ceca. Infection by this species can reduce weight gain and cause morbidity and mortality. Gross lesions can include red petechiae and round white spots when intestines are observed post-mortem (Edgar and Seibold, 1964; McDougald, 2013).

*E. necatrix* is one of the most recognized species of coccidia for commercial producers due to the macroscopic damage it causes. This species can be found in the mid-intestines, like *E. maxima*. Infections with this species are usually found in older birds, most likely due to its low reproductive capabilities where few oocysts are produced. Infections can cause weight loss, morbidity, and mortality. Droppings can contain blood, fluid, and mucus. *E. necatrix* will cause severe ballooning of the intestines with blood and fluid filling the lumen (Johnson, 1930; McDougald, 2013).
*E. tenella* is one of the best-known species of poultry coccidia due to its defined infection site and high pathogenicity. This species inhabits the ceca and is rarely found in other locations. Infection with this species can be characterized by bleeding, high morbidity, emaciation, feather loss and mortality. During post-mortem examination, it is common to see inflammation and lesions in the ceca. The cecal lumen can be filled with a bloody core (Railliet and Lucet, 1891a, b; Fanthom, 1910; McDougald, 2013;).

Multiple species of coccidia can infect turkeys with seven species of *Eimeria* being described in the United States. Only four species have an economic impact on the turkey industry (McDougald, 2013), and those species’ general characteristics and pathologies are described below.

*E. meleagrimitis* is the most pathogenic of the species to infect the upper digestive tract of turkeys. Morbidity, dehydration, weight loss, and mortality have been observed in experimentally infected poults. Feces can have some blood. Mucus and fluid can be found in the lumen of the duodenal loop and towards Meckel’s diverticulum (Tyzzer, 1929; McDougald, 2013).

*E. adenoides* is known for causing mortality, where up to 100% of infected poults will die. During infection, feces are fluid with some blood. Typically, gross lesions are in the ceca but can extend to the lower intestines, below Meckel’s diverticulum to the cloaca. These lesions can lead to a viscous fluid in the lumen. During severe infections, white or grey cores can develop in the ceca. Lesions quickly heal, making it difficult to diagnose this species (Moore and Brown, 1951; McDougald, 2013).

*E. gallopavonis* can cause varying rates of mortality between 10-100% in poults and usually occurs less than a week after infection. This species infects the lower intestinal tract and
occasional lesions are found in the ceca. A soft, white, caseous necrotic material with oocysts can be shed from the infected birds (Hawkins, 1952; McDougald, 2013).

*E. dispersa* is the only known *Eimeria* species that can infect multiple avian species. *E. dispersa* has low pathogenicity in turkeys, potentially due to its natural host being the bobwhite quail. Mortality is low, but in a high infective dose, this species can cause diarrhea and decreased weight gain in pouls. Oocysts are typically seen in the midgut region and can expand to the cecal necks. The intestinal tract will fill with a cream-colored exudate (Tyzzer, 1929; McDougald, 2013).

**Light microscopy (morphology)** Oocysts can be recovered from fecal material or litter and are generally round in shape (McDougald, 2013). Differentiation between *Eimeria* species is usually based on oocyst size (McDougald, 2013; Taylor, et al., 2015). Because some species overlap in infection sites and have similar macroscopic damage, species that cannot be differentiated by gross lesion examination or oocyst morphometrics can be identified using microscopy (Mattiello, et al., 2000). Currently, there is no *in vitro* model that can be used to mimic all stages that occur *in vivo*. This is due to *Eimeria* species being unable to complete their lifecycle without an animal host (Chapman, 2008; Soutter, et al., 2020).

**Oocyst populations in feces and litter** Oocyst counts in litter and feces is routinely done to understand the oocyst load (Long and Millard, 1977; Chapman, et al., 2002; Conway and McKenzie, 2007). By counting oocysts in fresh feces, researchers can determine the number of oocysts being shed by the birds and can estimate oocyst loads from shedding. This methodology is ideal for studies analyzing vaccination efforts because peak concentrations of oocysts are expected after vaccine administration (Chapman, et al., 2002). Another option is to examine oocysts in the litter. This will give a population estimate of how many oocysts birds could
consume. Oocysts accumulation in the litter is dependent on the management practices used on the flock. Flocks consuming an anticoccidial in their diet will have a single peak of oocysts between 3-8 weeks of age that can be higher than vaccinated birds (Long and Millard, 1977; Hooge, et al., 2000; Chapman, et al., 2002). With an attenuated vaccine, peaks in oocysts occur at 2-4 weeks of age and 4-7 weeks of age with a slightly higher second peak. Unmedicated, unvaccinated flocks have a peak just over a week earlier than medicated, unvaccinated flocks. Either method allows for determination of oocyst cycling, but neither method provides how many oocysts are actually viable (Chapman, et al., 2002).

**Oocyst populations in intestinal villi** Cocci presence and damage on the villi can be observed with hematoxylin and eosin staining (Madden and Ruff, 1979; Milbradt, et al., 2014; El-Sherry, et al., 2019). It is expected that during a coccidiosis challenge, the size and shape of villi suffer due to the damaging effects the parasite has on enterocytes (Milbradt, et al., 2014). The extent of villi damage and oocyst colonization is dependent on the cocci species (Madden and Ruff, 1979) and oocyst load.

With *E. acervulina*, ovoid gametocytes can be found in the mucosal lining of the small intestines, sometimes with tips of the villi sloughing-off, and some epithelial cells may contain multiple parasites. Generally, the villi appear inflamed (Tyzzer, 1929; McDougald, 2013). With *E. brunetti*, damage to the intestinal tract can be so severe that the villi are completely stripped with only the basement membrane remaining intact. *E. brunetti* oocysts will appear ovoid and larger than *E. acervulina* (Levine, 1942; McDougald, 2013). *E. maxima* produce the largest oocyst that invades chickens and causes microscopic hemorrhaging at the tips of the villi and obvious mucosal disruption (Tyzzer, 1929; McDougald, 2013). *E. mivati* are small, round oocysts that look like *E. acervulina*. Unlike *E. acervulina*, this species can be found invading the
tip down to the base of the villi (Edgar and Seibold, 1964; McDougald, 2013). *E. necatrix* has an oblonged ovoid oocyst shape that will form large, crowded clusters in the submucosa and lamina propria (Johnson, 1930; McDougald, 2013). During severe infections with *E. tenella*, heterophil infiltration can be observed while this species colonizes in the lamina propria. The host’s ceca may never recover fully from the severe tissue damage caused by this species, which also has an associated loss of the muscularis mucosa and a densely fibrotic submucosa (Railliet and Lucet, 1891a, b; Fanthom, 1910; McDougald, 2013).

For *E. meleagrimitis*, the small, ovoid oocysts tend to parasitize the tips of turkey intestinal villi with associated high numbers of eosinophils (Tyzzer, 1929; McDougald, 2013). With *E. adenoides*, ellipsoidal oocysts are found at both the tips and deep into the crypt glands of the lower intestines and ceca. The submucosa is congested with heterophils (Moore and Brown, 1951; McDougald, 2013). *E. gallopavonis* also has oocysts with ellipsoidal shape and will invade the lower portion of the intestinal tract. Differentiation is based on *E. gallopavonis* having more rounded oocysts when compared to *E. adenoides* (Hawkins, 1952; McDougald, 2013). For *E. dispersa*, characteristics that can be observed through histology include large and ovoid oocysts that lack the double wall commonly observed in other species (Tyzzer, 1929; McDougald, 2013). Histological examinations offer visual differentiation between *Eimeria* species based on host tissue sampling location, morphology of the oocysts, and location of the parasites within the tissue (McDougald, 2013).

**Disease management** Currently, anticoccidial feed additives (ionophores and/or other chemical compounds) and/or cocci vaccines are used in the poultry industry to control all three levels of disease (Blake, et al., 2017; Chapman, et al., 2002; Chapman, 2008; Tewari and Maharana, 2011; Chapman and Jeffers, 2014; Milbradt, et al., 2014; Agunos, et al., 2019). Anticoccidials have
been and continue to be utilized in some facilities but fear of drug resistance and the movement towards poultry production being “all-natural” decreases their use (Lillehoj and Lillehoj, 2000; McDougald, 2013; Milbradt, et al., 2014). Antimicrobials have been utilized (in conjunction with anticoccidials) for the control of coccidiosis and necrotic enteritis, but current changes in antimicrobial use laws in the poultry industry has decreased this preventative strategy (Agunos, et al., 2019). Cocci mitigation has facilitated development of commercially available vaccines for chickens (Chapman, et al., 2002) and turkeys (Chapman, 2008). In chickens, live and attenuated vaccines are available, but a recombinant vaccine has not yet been developed commercially (Blake, et al., 2017; Soutter, et al., 2020). In turkeys, live vaccines are the only vaccination option (Chapman, 2008). It has become common to use either a shuttle or rotation program, which requires that farms switch among various control methods in order to decrease resistance to specific programs (Chapman, et al., 2002; Chapman and Jeffers, 2014). Chemotherapy and vaccination can be used in combination, whereas drug treatments protect the avian host before protective immunity being initiated (Chapman, et al., 2002; Chapman, 2008; Chapman and Jeffers, 2014). In broiler production, it is common to use a shuttle/dual program, but in turkey production, it is more common to use a continuous program. In either instance, it is rare that only vaccination is used (Agunos, et al., 2019).

Ionophores, produced through fermentation (Chapman and Jeffers, 2014; Blake, et al., 2017), translates literally to “ion bearer” implying that this class of drugs will bind and bring ions across biological membranes. Each ionophore has an affinity towards a specific ion (potassium, sodium, calcium or magnesium) and has a specific ion carrying capacity (Roder, 2011). This affinity aids in coccidiosis control, in which ionophores cause an imbalance of ions within coccidia as this parasite invades the host epithelial cells (Roder, 2011; Chapman and
Jeffers, 2014). This mechanism of action has been shown in chickens and is assumed in turkeys, but experiments to support this claim have not been conducted with a turkey model (Chapman, 2008). A benefit to using ionophores is that they also work against Gram positive bacteria, including *Clostridium perfringens*. Low levels of coccidial parasites have been noted to evade ionophore treatment, allowing for a continuous but minimal exposure which can induce avian immunity against that species. Unfortunately, it can also induce the development of wild-type and resistant coccidia populations in the environment (Blake, et al., 2017). Therefore, ionophores should not be the only cocci control source used for extended periods due to resistance building in wild-type and vaccine populations (Chapman, et al., 2002). Another issue is ionophore toxicity, which has been documented in turkeys (Chapman, 2008). This occurs when the inclusion levels of the ionophore in feed are too great and the turkeys decrease their feed intake, potentially consuming (oocyst filled) litter as an alternative. In a drug surveillance study in Canada from 2013-2017, 86% of broiler production and 82% of turkey production used ionophores during rearing, but overall use dropped by almost 15% by the end of the study (Agunos, et al., 2019). In the United States, ionophores dominate the anticoccidial market but are now classified as an antibiotic so their use has been reduced (Cervantes, 2015; Blake, et al., 2017; Soutter, et al., 2020).

Chemical compounds, also known as synthetic drugs, are produced through chemical synthesis (Chapman and Jeffers, 2014; Blake, et al., 2017). These compounds have a mode of action that differs from ionophores (Chapman, et al., 2002). These synthetic drugs alter oocysts populations by inhibiting parasite microbial respiration, inhibiting the folic acid pathway, and competing with the parasite for thiamine uptake. Some modes of action remain unknown (Chapman and Jeffers, 2014). Many of the commercially available synthetic drugs were
developed for chickens but can be used with turkeys (Chapman, 2008). With these synthetic compounds, an ongoing concern in the poultry industry is the development of parasite resistance (Cervantes, 2015). One way to decrease this resistance is to rotate chemical compounds with vaccinations (Cardenas, et al., 2016).

Coccidial vaccines have been available in the chicken market for seventy years, but the research on and use of various Eimeria species makes vaccine production costly and not always desirable for a grower (Soutter, et al., 2020). Vaccines can be administered in the water, sprayed on the feed, sprayed on the birds as a mist, given in the eye of the bird or sprayed on the birds as an edible gel (Chapman, et al., 2002; Chapman, 2008; Tewari and Maharana, 2011; Chapman and Jeffers, 2014; Soutter, et al., 2020). Vaccine dispensing should be stable, consistent, and easy to administer. Vaccines are sensitive to their environment so prolonged or inadequate temperature storage can decrease their viability (Soutter, et al., 2020). Another issue that can occur is based on automation- it is assumed that even coverage of a vaccine is given to birds at a hatchery. This is not always the case where oocyst coverage can be biased based on the water, feed, or spray consumption. Various oocyst coverage per bird has been noted in literature indicating that administration methodology could influence oocyst cycling (Chapman, et al., 2002). Protective immunity develops after two or three consecutive infections, which occur while birds are being reared over a period of 3 to 4 weeks (Tewari and Maharana, 2011). Currently, live-virulent, live-attenuated and live-ionophore tolerant vaccines are available for commercial chicken production while recombinant vaccines are in development ( Tewari and Maharana, 2011; Blake, et al., 2017).

Live vaccines allow for protection against homologous species, but cross-immunity between parasite or host species is rarely observed. Variation in live vaccine efficiency can be
due to infective species involved in host parasitism, the vaccine dosage, and the host receiving
the vaccine (Soutter, et al., 2020). To maintain the cost of a vaccine, the poultry industry has
targeted the pathogenic strains of *Eimeria* that are known for causing economic burdens on
commercial production. Unfortunately, wild-type and various species populations can
sporadically be found in commercial houses and lead to disease. Certain strains of *Eimeria* also
are undergoing immunological variation which affects the efficacy of the administered vaccines
(Chapman, et al., 2002). Vaccines initiate innate immunity, but low continuous infections are
necessary to develop proper protection and to decrease the potential of inadequate performance
due to this disease (Milbradt, et al., 2014). Another concern is that poor management of the
flock, such as high stocking density, decreased downtime, poor sanitation, improper temperature,
and poor biosecurity (Cervantes, 2015), can trigger severe reactions to live vaccines leading to
disease (Abbas, et al., 2012).

Attenuated vaccines are available for chicken producers but are yet to be available
commercially for turkey producers (Chapman, et al., 2002; Rathinam, et al., 2016). For
attenuation, the oocyst lifecycles are modified or deleted (Chapman, et al., 2002), leading to
“precocious development”. This produces parasites with reduced pathogenicity but similar
immunogenicity to the nonattenuated lines, allowing for immunity of the host to develop
(Rathinam, et al., 2016; Soutter, et al., 2020). Attenuated vaccines have increased safety and
efficacy due to lower levels of replication in the avian host. Unfortunately, attenuated vaccines
require higher vaccine production costs due to the methodology used in the manufacture of
precociously developed parasite lines (Blake, et al., 2017).

Although vaccines provide alternative options, concerns associated with weight gain,
feed conversion and gut health have been raised for vaccinated poultry species, relative
specifically to poor management (Chapman, et al., 2002; Chapman, 2008; Abbas, et al., 2012; Milbradt, et al., 2014; Cervantes, 2015; Rathinam, et al., 2016). The parasite’s mechanism of action of invading, replicating and cycling in the intestines of birds can predispose the host to intestinal health problems (Cervantes, 2015). Therefore, alternative feed additives that focus on maintaining or improving animal performance instead of decreasing parasite interactions are a growing market for coccidiosis control (Cardenas, et al., 2016). Some tested alternatives include probiotics, botanicals, nonspecific immunomodulatory agents, or a combination thereof (Tewari and Maharana, 2011; Abbas, et al., 2012; Cardenas, et al., 2016). Although further work in this area is necessary, some of the proposed mechanisms include: inducing oxidative stress against coccidia acting as immunostimulators or nutrient absorption enhancers (Abbas, et al., 2012). An additive which augments the gut mucosal immune response could act as an immunotherapeutic agent (Tewari and Maharana, 2011). Products that increase the bioavailability of vitamin A, vitamin E and selenium have been considered due to their effects on enhancing the function of the intestinal lining and innate immune system. Maintaining gut integrity has considerable effects on the host’s (immune) reaction to invasive organisms, such as Eimeria species (Williams, 2005).

**Histomonas meleagridis**

*Histomonas meleagridis* (*H. meleagridis*), the causative agent of blackhead disease, is also called infectious enterohepatitis, or histomoniasis during its infectious state (Norton, et al., 1999; Callait-Cardinal, et al., 2007). *H. meleagridis* was first observed before the start of the 20th century in commercial poultry (Cushman, 1893). This protozoan infects avian species that fall in the order Galliformes (Dolka, et al., 2015). Gallinaceous birds can be found all over the world
and include commercially raised chickens and turkeys (Holman, 1964). In conjunction with this
distribution of birds, *H. meleagridis* is worldwide (Liebhart, et al., 2017). Due to its devasting
effects, this protozoan was intensely studied until the mid-1970’s when drug treatment options
became available. Research with this protozoan all but ceased until the removal of the treatments
at the beginning of the 21st century due to public health concerns. *H. meleagridis* is currently
considered a reemerging disease and its effects on poultry production are causing reinforced
interest in the analysis of bird species susceptibility and changes in management practices that
could control the animal’s response to this protozoan. Multiple reviews have been published in
the past decade to aid in a better understanding of *H. meleagridis* with modern poultry genetic
lines, management practices, and research techniques (Hauck and Hafez, 2013; Burgess, et al.,
Liebhart and Hess, 2020). The focus of this section of the current literature review is to highlight
the interactions between *H. meleagridis* and current poultry management, which include
currently applied treatment strategies.

**Entry and Spread of *H. meleagridis* in Commercial Poultry Houses**

The entry of *H. meleagridis* into a commercial poultry flock is dependent on its only
known vector, the cecal worm *Heterakis gallinarum*. A review of this organism and the
interaction with *H. meleagridis* has recently been published and the following summary
highlights its effects on poultry production. *Heterakis gallinarum* is a cecal nematode that is
generally found where poultry are reared. It has limited negative effects on poultry production in
relation to feed conversion and weight gain. Instead, the greatest concern in the poultry industry
about *Heterakis gallinarum* is with its status as the only known carrier of *H. meleagridis*. This
protozoan will disperse to the reproductive tract of *Heterakis gallinarum* and is harbored in the *Heterakis gallinarum* ova as a form of natural encapsulation. *Heterakis gallinarum* eggs can be transferred into poultry facilities by multiple mechanical vectors (flies, darkling beetles, farm equipment, etc.) or by its paratenic host, the earthworm. Once consumed by a host, the embryonated *Heterakis gallinarum* eggs hatch in the intestines and male and female larvae are eventually carried to the ceca of the bird. Sexual maturity of the nematode is reached after approximately 14 days in the ceca and at approximately 25 days, females begin to produce ova.

Fertile but nonembryonated eggs are expelled out of the avian host through fecal and/or cecal droppings and contaminate the surrounding environment. The eggs become embryonated after approximately 2 weeks of aerobic exposure. Therefore, *H. meleagridis* in embryonated *Heterakis gallinarum* eggs can take more than a month to completely cycle (Cupo and Beckstead, 2019).

Due to the lifecycle of *Heterakis gallinarum*, the worm burden in bird species with shorter grow-out periods are rarely observed.

Usually, reproduction of *Heterakis gallinarum* in long-lived commercial chickens cause shedding of contaminated eggs where coprophagy will indirectly pass eggs between flock mates. This mechanism correlates with the heavy worm burden commonly seen in breeding stock (Waters, et al., 1994; Hu, et al., 2006). Because the incidence of blackhead disease is correlated with the worm burden in chicken facilities, a rolling infection with increased morbidity is usually observed with low mortality (McDougald, 2005). For commercial chicken layers and breeders, *H. meleagridis* studies have focused on welfare issues leading to altered production parameters (Gerth, et al., 1985; Esquenet, et al., 2003; McDougald, 2005; Grafl, et al., 2011; Liebhart, et al., 2013).
Consumption of embryonated *Heterakis gallinarum* eggs is the means of *H. meleagrisidis* entry into turkey facilities but consumption of only the cecal nematode eggs does not justify how *H. meleagrisidis* becomes an epidemic outbreak in a short period of time (Hu and McDougald, 2003; Cupo and Beckstead, 2019). Turkeys have been suggested to pass *H. meleagrisidis* within a flock relatively rapidly through horizontal (lateral) transmission via cloacal contact. Muscle movement of the vent brings external material into the ceca of birds. Blackhead disease has resulted by the uptake of *H. meleagrisidis* through this movement in laboratory models therefore the mechanism of rapid spread of this parasite has been suggested in the field (Hu and McDougald, 2003; Hu, et al., 2004; Hess, et al., 2006; Hu, et al., 2006). The cloacal drinking mechanism has been successful in causing direct infection of nonencapsulated *H. meleagrisidis* cells into turkeys and can be directly correlated with horizontal transmission (Hu, et al., 2004). Wet droppings and huddling are more commonly observed with turkeys than chickens, especially if sick. These phenomena have been suggested to increase horizontal transmission of *H. meleagrisidis* (Hu, et al., 2006). A physical barrier between ill and healthy turkeys has been shown effective in decreasing the transmission of *H. meleagrisidis*, indicating the necessity of bird to bird contact in turkeys to pass this pathogen. During a blackhead disease outbreak, it is hypothesized that the general flock population becomes infected by the index case poult(s) who consumed the contaminated *Heterakis gallinarum* eggs (Figure 1.1). Since the course of this disease is approximately 2 weeks before the turkey-host dies, it can also be assumed that the index case became infected approximately 3-4 weeks before the epidemic (Powell, et al., 2009). The number of index cases tend to be low, so it would be easy for a grower to assume that the dead were routine mortality. The rest of the population will likely suffer from a rolling infection
approximately 2 weeks prior to the mortality spiking. To date, it is not known when infected birds are shedding *H. meleagris*.

**Figure 1.1.** Time period illustration of lateral transmission in a turkey flock that consumed *Heterakis gallinarum* to start the blackhead disease outbreak

A typical behavior of gallinaceous birds is ground feeding/pecking (Holman, 1964) and oral consumption of *H. meleagris* without encapsulation has been considered (Norton, et al., 1999; Hu, et al., 2004; Liebhart and Hess, 2009; Armstrong and McDougald, 2011; Liebhart, et al., 2011; Mitra, et al., 2018;). In an experimental setting, oral inoculation of *H. meleagris* cells failed to cause blackhead disease in chickens, with or without a feed stress (Hu, et al., 2006). In turkeys, conflicting data are present in the literature. Liebart and Hess (2009) found that oral inoculation of turkeys on day of hatch resulted in high mortality while Fudge and associates (2019) found that an oral inoculation of *H. meleagris* with turkeys over a week of age undergoing various hours of feed withdrawal did not lead to blackhead disease, but a cloacal inoculation at said hours led to blackhead disease. Some experimental studies have shown that when an oral inoculation of virulent *H. meleagris* has been given to turkeys, the parasite was shed by the bird and caused clinical signs of disease (Liebhart, et al., 2011). Conversely, the
consumption of cecal droppings from an infected turkey did not lead to disease (Armstrong and McDougald, 2011).

Management Practices that Influence Blackhead Disease

Infectious diseases in commercial poultry impact health and therefore economic performance of those animals (Grafl, et al., 2011; Sulejmanovic, et al., 2013; Hess, 2017). The type of poultry farming and hygienic conditions significantly influence the introduction and spread of disease (Callait-Cardinal, et al., 2010; Lotfi, et al., 2012). Since the 1960’s, various rearing stressors given after a *H. meleagridis* inoculation have been considered to influence the outcome of blackhead disease in chickens and turkeys (Welter, 1960). More recently, intensive animal husbandry due to increased animal meat demands and consumer-driven ban of formally licensed medications have led to an upsurge in blackhead disease cases in commercial poultry units (Esquenet, et al., 2003; Dolka, et al., 2015; Hess, 2017).

**Bird species** Multiple *Galliformes* species can have clinical signs associated with blackhead disease (Lund and Chute, 1972) but for commercial poultry production, chickens and turkeys are of primary concern. Chicken varieties are commonly infected while turkey varieties commonly die if they are infected with *H. meleagridis* (Lund, 1967; Waters, et al., 1994; McDougald, 2005; Sulejmanovic, et al., 2013). This difference in mortality is assumed to be due to chickens having a more efficient immune resistance to *H. meleagridis* and recover while turkeys succumb to blackhead disease (Powell, et al., 2009). Because immune status correlates with genetics, the comparison of genetic lines allows for a better understanding of immunological activity against histomoniasis (AbdulRahman and Hafez, 2009). Studies conducted to analyze differences among
genetic lines of chickens and turkeys and their response to blackhead disease are described below.

In relation to chicken responsiveness to *H. meleagridis*, a study in 1967 showed variation in disease status was dependent on chicken breed but more recent studies have shown similar chicken susceptibility, independent of breed (Lund, 1967; Zahoor, et al., 2011). Discrepancies between these two studies could be due to the inclusion of *Heterakis gallinarum* in the 1967 study as well as differences in the host genetic lines and parasite source since these studies were conducted fifty years apart. Currently, it is still debated whether layer or breeder pullets are more susceptible to *H. meleagridis* (Gerth, et al., 1985; McDougald, 2005; Grafl, et al., 2011). Experimental inoculation or natural infection of *H. meleagridis* in broilers has been noted (Hu, et al., 2006). However, the shortened broiler grow-out period for production is known to decrease the frequency of histomoniasis due to less *Heterakis gallinarum* cycling (Waters, et al., 1994; Hess, 2017).

Turkeys, wild and domesticated, fail to have effective immunological activity against *H. meleagridis*, which leads to high mortality rates (Lund, et al., 1975; Waters, et al., 1994; McDougald, 2005; AbdulRahman and Hafez, 2009; Powell, et al., 2009). Like chickens, differences in breed susceptibility to blackhead disease has been considered. A study comparing three breeds of commercial poults found that all are susceptible to *H. meleagridis* infection but differences in the percentage of birds with disease signs and mortality could be based on a genetic component (AbdulRahman and Hafez, 2009). It has also been suggested that mortality is of a greater percentage in turkey breeders compared to commercial meat-type turkeys (30% vs 10%) which is expected to be associated with differences in genetics and/ or rearing conditions.
Utilizing the genetic susceptibility data could aid in breeding a more blackhead disease resistant turkey.

**Age and gender** The influence of age and gender on blackhead disease susceptibility have been investigated (Tyzzer, 1934; Liebhart, et al., 2008). Commercial chickens are highly susceptible to negative effects associated with *H. meleagridis* at 4-6 weeks while turkeys are susceptible at any age (Liebhart, et al., 2008; McDougald, 2013). In a chicken experiment to analyze the interaction between age and disease, Desowitz (1951) found that chickens are susceptible at 6, 13 and 34 days of age but the highest mortality associated with blackhead disease can be found if they are infected around 21 days of age. In commercial production, infections are more commonly seen in pullets during the rearing period, between 1 to 3 months of age (Sigmon, et al., 2019). In field reports, outbreaks in turkey flocks tend to occur at 4-8 weeks of age but have been reported as early as 3 and as late as 17 weeks of age. The age of the flock when infected did not alter the mortality associated with blackhead disease (Callait-Cardinal, et al., 2007). Relative to gender susceptibility to blackhead disease, there is no significant correlation between gender and disease outcome (Callait-Cardinal, et al., 2007; Liebhart, et al., 2008). More information has accumulated on female chicken breeding stock due to *H. meleagridis* effects have on the hens’ performance. With turkeys, unpublished data from the Beckstead lab have shown similar susceptibility to blackhead disease when 1,000 males in one flock and 1,000 females in another were given three cloacal inoculations of *H. meleagridis* over the course of 80 days (Ferrarini and Beckstead, 2019). To date, the age of infection with female chickens can influence production performance, data on male chickens are lacking and there is no evidence that age or gender of turkeys will influence disease outcome.
**Feed restriction** Feed restriction is currently practiced in parent stock houses to maintain weight, ironically in a species of bird that has been genetically selected for increased rates of feed consumption (Jong and Emous, 2017). Short term feed restriction occurs occasionally in commercial turkeys. It has been suggested that feed restriction as part of the *H. meleagridis* challenge could alter chick or poult susceptibility to blackhead disease (Liebhart and Hess, 2009; Liebhart, et al., 2011; Liebhart, et al., 2013; Sulejmanovic, et al., 2013). Welter (1960) and Hu and associates (2006) did not see changes in disease presence with or without feed restriction, indicating that lack of feed intake does not play a role in infection or transmission of *H. meleagridis* for chickens. Instead, feed restriction in a commercial house can change behavior of the birds, where less feed availability increases the likelihood of ground pecking (a stress-related displacement behavior) which can increase the potential for consuming *Heterakis gallinarum* eggs. Feed availability, form and environmental conditions in a rearing facility can lead to litter consumption (Svihus, 2014).

**Housing environment** Whether blackhead disease has a seasonal trend has been debated (Callait-Cardinal, et al., 2007), but it is more likely that the incidence of this disease is based on certain ambient weather effects (Callait-Cardinal, et al., 2010). Rearing temperature has been shown to not influence blackhead disease in chickens or turkeys (Welter, 1960). Increased rain and moisture will stimulate the surfacing and movement of insects and arthropods which could be carrying contaminated *Heterakis gallinarum* eggs (Cupo and Beckstead, 2019). Coincidentally, housing systems that allow access to the outdoors also increase the instance of *H. meleagridis* exposure due to the increased insect and arthropod contact (Callait-Cardinal, et al., 2010; Grafl, et al., 2011).
Within a production house, *H. meleagris* survives best outside a host if it is protected from environmental stressors (Lotfi, et al., 2012; Norton, et al., 1999). Artificially contaminated poultry house materials have been tested for *H. meleagris* survivability over the course of multiple hours. Although *H. meleagris* was viable after various times, the methodology used in this study did not conclude if the infective particles could lead to disease in a bird. This experiment did highlight that litter material, specifically feces, is a potential carrier of this parasite between bird hosts (Lotfi, et al., 2012).

Litter has been suggested as a source for *H. meleagris* contamination, mainly as a carrier of *Heterakis gallinarum* (Waters, et al., 1994) but also as a transmission vector if litter quality is poor and has a high moisture content (Callait-Cardinal, et al., 2010). On conventional deep litter commercial pullet or layer flocks have significantly lower *H. meleagris* antibodies than organic free range pullets or hens but this is likely due to the environmental exposures associated with each rearing husbandry practice (Grafl, et al., 2011). Other studies with layers and *H. meleagris* have utilized deep litter, which yielded disease, indicating that litter does not directly affect pathology (Liebhart, et al., 2013). With turkeys, used litter from a *H. meleagris* positive flock was placed in a pen with susceptible poults and failed to cause blackhead disease, which was likely due to the prolonged time between collecting litter and placing the birds (Norton, et al., 1999). Armstrong and McDougald (2011) found that poults became infected by contact with *H. meleagris* contaminated cages immediately after the infected birds were removed, but at a much lower rate than if they were in contact with an infected poult. *H. meleagris*-contaminated litter can lead to blackhead disease under specific circumstances if contact is made within a short period of time. To date, it is unknown how long *H. meleagris* survives in the litter without *Heterakis gallinarum* while still being infective.
A strong and positive correlation exists between turkeys being placed on used litter from chicken houses and increased blackhead disease outbreaks (Lund, 1967; Waters, et al., 1994). Used chicken litter was previously applied to turkey growing facilities until the recognition that chickens acted as a reservoir for *Heterakis gallinarum*. Litter recovery of *Heterakis gallinarum* varies relative to the commercial chicken varieties that inhabited the litter. Broiler breeders are more likely to be raised on litter throughout their life which allows for continuous consumption of heterakids while conventional layers and broilers are raised in ways which decrease the completion of the nematode lifecycle. In smaller flocks, the rearing of chickens and turkeys together has correlated with increased blackhead disease lesion scores in turkeys and it was hypothesized that this observation is due to a heavier cecal nematode burden causing greater organ damage (Callait-Cardinal, et al., 2010). Because of the carrier contamination associated with chickens, turkeys should be reared separately and on litter that has not come in contact with chickens (Waters, et al., 1994).

**Secondary and co-infections** Coinfections have been suggested to exacerbate blackhead disease (Gerth, et al., 1985; McDougald and Hu, 2001; Dolka, et al., 2015) but this concept has been debated extensively in literature. One study has shown increased susceptibility after pullets were vaccinated against fowl pox (Desowitz, 1951) but another showed no change in disease signs (Welter, 1960). In turkeys, a case report indicated that a flock with clinical signs associated with blackhead disease were associated with the recovery of *H. meleagridis*, *Tetratrichomonas gallinarum* and *Ascaridia dissimilis* (round worm) through tissue sampling and microscopy. Additionally, *Escherichia coli* and *Clostridium* species were cultured in high colony- forming units from sampled tissue. It was not reported if the additional microbes worsened the pathology associated with blackhead disease (Norton, et al., 1999). With recent advancements in
biotechnology, the role of *H. meleagridis* and other microbes in the cecal microbiome can now be better examined (Hauck, 2017).

The interaction between cocci and *H. meleagridis* has been noted in the field and studied in experimental settings (Welter, 1960; McDougald and Hu, 2001; Hu, et al., 2006; Callait-Cardinal, et al., 2010; Hess, 2017). Infection with *Eimeria adenoides* in chickens did not worsen the effects of blackhead disease when broilers were dosed with both protozoa on the same day (Hu, et al., 2006). However, infection of chickens with *E. tenella* led to an increase in liver damage characteristic of blackhead disease, further indicating cocci species specificity might facilitate interaction between host gut damage and these two protozoa (McDougald and Hu, 2001; Hu and McDougald, 2002). Infection with *E. tenella* six days after chickens were fed *H. meleagridis* contaminated heterakid eggs did not alter blackhead disease (Welter, 1960). The interaction among cocci, the microbiome and gut health could (in)directly influence bird susceptibility to blackhead disease (Hu and McDougald, 2002). To date, there is limited information on the interaction between coccidiosis and blackhead disease in turkeys even though the two protozoa are commonly found in the field (McDougald and Hu, 2001; Callait-Cardinal, 2010; Cupo and Beckstead, 2019).

**Diagnostic Tools and Proposed Treatment Strategies for Blackhead Disease**

An enhanced understanding the interaction between these protozoa and the bird host’s intestinal tract is crucial for designing preventative and treatment measures. Multiple diagnostic tools are available for *H. meleagridis*. Infection can be identified through macroscopic lesion damage, microscopic viewing (of cultured media), histological staining as well as other methodologies that are beyond the extent of this literature review (Mattiello, et al., 2000; McDougald, 2013).
**Clinical signs of blackhead disease** Rarely will a literal “black head” be seen with chickens or turkeys infected with *H. meleagris* (Norton, et al., 1999). Chickens suffering from blackhead disease can vary from asymptomatic to severe morbidity while turkeys will suffer from morbidity then mortality (McDougald, 2013). Some chickens will have pathological lesions in the ceca with little to no clinical signs of disease (Zahoor, et al., 2011; Liebhart, et al., 2013). Atypical actions of depression, ruffled feathers, drooping wings and a change in feed and water intake have been noted (McDougald, 2013; Dolka, et al., 2015). In severe cases, chickens can appear emaciated with matted feathers and their cloaca contaminated in sulfur-colored fecal material (Dolka, et al., 2015). Similar to severe cases in chickens, almost all infected turkeys will have sulfur-colored droppings with stained vent feathers (Callait-Cardinal, et al., 2010). They will also suffer from moderate to severe depression and anorexia. Sick turkeys tend to huddle together. Approximately 2 weeks after exposure, chickens can start to recover while turkeys succumb to the disease (Hess, et al., 2006; Zahoor, et al., 2011).

When raising commercial poultry, two of the most important criteria for production performance are 1. target body weight for the age of bird to ensure flock uniformity and 2. efficient feed conversion (Hauck, 2017). In breeder and layer pullets, poor uniformity is associated with high variation in sexual maturation of hens as indicated by altered onset of lay and the quantity and quality of eggs produced (Abbas, et al., 2010). Diseases that infect the ceca of poultry breeding stock have been shown to cause secondary effects, such as decreased egg quantity and quality, which are associated with economic losses for the producer (Smit, et al., 1998; Stephens and Hampson, 2001). The secondary effects of a cecal infection with *H. meleagris* in relation to pedigree production have been addressed (Esquenet, et al., 2003; Grafl, et al., 2011; Liebhart, et al., 2013; Dolka, et al., 2015; Sigmon, et al., 2019). It is estimated that
blackhead disease can cause a decrease in chicken body weight by 10-19% a week after infection (Hu, et al., 2006). This decrease in body weights can continue weeks after inoculation (Lund, 1967; Sigmon, et al., 2019), but this trend was inconsistent in literature (Liebhart, et al., 2013). Lighter body weights of experimentally infected hens did not lead to altered secondary effects, such as egg production and feed consumption, provided that the hens were inoculated before the induction of lay (Sigmon, et al., 2019). However, cecal infection during production has led to issues in performance (Esquenet, et al., 2003; Dolka, et al., 2015). Currently, a controlled experiment focused *H. meleagridis* infection in broiler breeders during the rearing period is not yet available.

In turkey meat production, body weights, uniformity and mortality are correlated with stress and immune function. These production performance parameters also influence the economic return for the producer (Beaulac, et al., 2019). No differences in *H. meleagridis* susceptibility have been reported due turkeys being raised at a lighter or heavier weight. However, mortality due to blackhead disease has been shown to vary from 10-100% in commercial turkey flocks. Higher mortality is associated with increased culling to decrease the potential of an epidemic (Callait-Cardinal, et al., 2007; Callait-Cardinal, et al., 2010). Mortality in the field can occur over a span of 6-20 days, where birds are more morbid but still consuming feed and water all the while infecting flock mates (Lund, 1967; AbdulRahman and Hafez, 2009). The anorexia and loss of muscle mass leads to variation in body weights and therefore, uniformity. The decreased muscle mass and high mortality from this disease will decrease the overall economic yield of the flock.

**Pathogenesis** Oral inoculation through consumption of infected *Heterakis gallinarum* eggs or cloacal inoculation through an infected bird’s freshly-voided liquid feces spreads *H. meleagridis*
to flock mates (Hu and McDougald, 2003; Hu, et al., 2004; McDougald, 2005). Peristaltic and antiperistaltic muscle contractions of the digestive tract bring digesta (Svihus, 2014) and the protozoan (Hu, et al., 2004) to the ceca. It has been suggested that bacterial interaction is necessary for *H. meleagridis*- associated pathogenesis (Norton, et al., 1999; Hauck, 2017; Hess, 2017). With the high bacterial load in the ceca, the microbes can interact with *H. meleagridis* (Hauck, 2017), but the exact mechanisms of the protozoa-bacteria relationships are not well understood (Hauck, 2017; Hess, 2017). Specific bacteria necessary for *H. meleagridis* growth tend to vary depending on the strain of *H. meleagridis*. The bacterial populations in the ceca can be altered based on the inflammatory response of the bird host suffering from blackhead disease (Hauck, 2017). Usually, a fibrinous- hemorrhagic peritonitis will develop with histomoniasis. This inflammation can lead to adhesions of the mesentery and among surrounding organs. In severe cases, a caseous core filled with necrotic material, sometimes described as a “friable plug” will form in the ceca (Esquenet, et al., 2003; Cortes, et al., 2004; Powell, et al., 2009). The macroscopic cecal damage described previously can resemble cecal coccidiosis (Desowitz, 1951). Bilateral cecal *H. meleagridis* infections are not always apparent in birds suffering from blackhead disease (Desowitz, 1951; Windisch and Hess, 2010). Attenuated strains of *H. meleagridis* tend to stay in the lumen of the ceca while virulent strains are more likely to migrate and cause damage to the liver (Sulejmanovic, et al., 2013).

The migration of *H. meleagridis* out of the ceca occurs through the hepatic portal vein to the liver which can lead to a systemic infection (Grabensteiner, et al., 2006). Livers can swell, become discolored and have multiple rounded areas of necrosis. Usually, chickens have minimal, if any, liver damage while severe liver damage is seen in the turkey (Hess, et al., 2006; Powell, et al., 2009; Liebhart, et al., 2011; Liebhart, et al., 2013). Blackhead disease mortality is positively
correlated with the extent of liver damage (Hu and McDougald, 2002; Powell, et al., 2009).

Typical lesion damage for blackhead disease is seen in the ceca and liver (McDougald, 2013). However, other organs have been observed to have lesions (Welter, 1960; Cortes, et al., 2004). Specifically, damage to the reproductive tract has been noted in breeding stock (Liebhart, et al., 2013).

To quantify the extent of the *H. meleagridis* infection based on macroscopic examination, a 0-4 subjective scoring scale has been published. The values are based on visual damage to the ceca and liver. A score of “0” indicates no sign of infection. In the ceca, the scores increase based on subjective identification of tissue thickening then necrosis of the cecal wall and the formation of a caseous core in the lumen. Increasing liver lesion scores are based on the number of necrotic foci, or pinpoint lesions (McDougald and Hu, 2001). If birds are only infected with *H. meleagridis*, chickens will have less severe damage, and therefore lower subjective lesion scores, than turkeys (Hu, et al., 2006). To further determine the extent of the infection and protozoal presence, microscopic analysis can also be used (Liebhart, et al., 2011).

**In vitro growth, recovery, and identification** Because *H. meleagridis* is an anaerobic parasite with a fragile exterior, specific media and growing conditions have been determined. Typically this media consists of Dwyer’s medium which contains medium 199 in Hank’s balanced salt solution, (horse) serum, a starch source (usually rice powder) and a xenic bacterial culture (Dwyer, 1970; Hauck, et al., 2010). Co-cultured bacteria are necessary in the growth medium and are assumed to produce a favorable anaerobic environment while also providing nutrients to the histomonad cells (Gruber, et al., 2017). *H. meleagridis* growth *in vitro* is better supported by mixed bacterial populations as opposed to single isolates (Hauck, 2017). Optimal growth can be seen in cultures grown at a temperature around 40°C and at a neutral pH. This media allows for
ideal growth conditions so that *H. meleagridis* can be observed using light microscopy (Gruber, et al., 2017).

Using light microscopy, *H. meleagridis* appears in a somewhat irregular rounded shape with a clear ectoplasm, a granular endoplasm and range in size from approximately 6-20 µm in diameter (Taylor, et al., 2015; Gruber, et al., 2017). A primary flagellum can be seen *in vitro* or in the cecal lumen. When in the cecal tissue or the liver, the flagellum is lost (Hauck, et al., 2010; Taylor, et al., 2015; Gruber, et al., 2017). A cyst form of *H. meleagridis* has been proposed but this form and its ability to cause disease have yet to be proved (Zaragatzki, et al., 2010). The phenotype of *H. meleagridis* is dependent on isolation location (Taylor, et al., 2015; Gruber, et al., 2017). *Tetratrichomonas gallinarum, Parahistomonas wenrichi* and *Blastocystis* species can have similar morphological characteristics and are commonly found in the host bird’s digestive tract and grow in *H. meleagridis* media. Therefore, *H. meleagridis* in a complex culture cannot always be identified using only light microscopy. Researchers have also used Polymerase Chain Reaction followed by genome sequencing to verify the presence of *H. meleagridis* DNA or used an enzyme-linked immunosorbent assay or antibodies for identification of the parasite’s presence (McDougald, 2005; Stensvold, et al., 2009; Grafl, et al., 2011; Liebhart, et al., 2013; McDougald, 2013; Hess, 2017). Unfortunately, recovering the DNA or detecting antibodies does not guarantee live organisms within the host.

The ability to grow *H. meleagridis* from samples taken during necropsy can vary based the length of time between death of the animal and sampling (Dolka, et al., 2015). This is due to the fragile state of this protozoan, where there is debate on the longevity of its survival outside of a host (Munsch, et al., 2009; Zaragatzki, et al., 2010; Lotfi, et al., 2012). Fortunately, dry media is available to complete epidemiological studies that require prolonged time between media
preparation and sampling (Barrios, et al., 2017). Because the ceca and liver are the predisposing sites for H. meleagridis, sampling for cultures usually occurs using these tissues (Huber, et al., 2006). Cultured fecal and cecal swab samples of chickens and turkeys can recover H. meleagridis as early as 2 days post-inoculation with most birds shedding the protozoan intermittently through the infection (Hess, et al., 2006; Huber, et al., 2006; Liebhart and Hess, 2009; Liebhart, et al., 2010; Grafl, et al., 2011; Liebhart, et al., 2011; Zahoor, et al., 2011; Liebhart, et al., 2013; Sulejmanovic, et al., 2013). The current sampling techniques have aided in recovering live organisms that can be used in epidemiological studies, pathogenicity studies, and transmission of H. meleagridis.

**Histological examination** Differential diagnosis of H. meleagridis can be done through histological staining (Kemp and Reid, 1966). H. meleagridis has been found in various locations within the ceca (contents, mucosa, muscle layer, and serosa) and liver of both chickens and turkeys by histological examination via an in-situ hybridization method (Liebhart, et al., 2006). Acid fast staining has been used for H. meleagridis identification, but this staining produces less vibrant images and therefore is not ideal for identifying H. meleagridis (Cortes, et al., 2004). More commonly, H. meleagridis can be identified via Periodic Acid Schiff (PAS) staining while tissue damage from this protozoan and the host’s immune response can be examined using a standard hematoxylin and eosin staining (Kemp and Reid, 1966; Gerth, et al., 1985; Waters, et al., 1994; Norton, et al., 1999; Esquenet, et al., 2003; Cortes, et al., 2004; Hess, et al., 2015). H. meleagridis is typically identified in a PAS stained sample by its dark purple/magenta color and clear halo surrounding the rounded cell (Kemp and Reid, 1966). Around the cecal villi, high numbers of macrophages and lymphocytes can be seen. The cecal mucosa can be saturated in sloughed epithelial cells and exudate (Esquenet, et al., 2003). Attenuated strains have been
shown to localize in the lumen or crypts of the ceca while virulent strains penetrate the cecal wall (Liebhart, et al., 2011; Sulejmanovic, et al., 2013). Histological examination of *H. meleagridis* is ideal for identification of (live) organism presence and tissue damage in cases with less defined pathological lesions and for diagnostic investigations (Liebhart, et al., 2006).

**Treatment options** No medicated control methods are available for birds suffering from blackhead disease leading to reinforced interest in approved treatment strategies (Sulejmanovic, et al., 2013). For many years blackhead disease prevention and treatment included chemotheurapeutics, specifically nitroimidazoles (metronidazole, dimetridazole, ronidazole), nitrofurans (furazolidone), and arsenical derivatives (e.g., nitarsone, roxarsone, acetarsol) (Dolka, et al., 2015). These products are no longer available due to concerns related to human public health (Hu and McDougald, 2002; Hess, et al., 2006; Liebhart, et al., 2011; McDougald, 2013). Currently, the poultry industry is focused on vaccine development for immunity stimulation as well as additives that decrease the negative production effects of infected birds.

Differences in disease outcome among avian species have been hypothesized as differences in their immune capabilities (Powell, et al., 2009). This has been demonstrated when analyzing vaccine efficacy against *H. meleagridis*. Studies have been conducted on the use of a vaccine with attenuated *H. meleagridis*. Unfortunately, no vaccines are currently available for the commercial chicken or turkey market due to conflicting data in the efficacy, timing of the vaccine and avian species responsiveness (Hess, et al., 2008; Liebhart, et al., 2010; Liebhart, et al., 2011; Liebhart, et al., 2013; McDougald, 2013; Sulejmanovic, et al., 2013; Hess, 2017). Antibodies from infected chickens have been recovered from blood serum and the digestive tract, indicating that an immune response and specifically the gut-associated lymphoid tissue can be stimulated (Windisch and Hess, 2009; Windisch and Hess, 2010; Grafl, et al., 2011). It should be
noted that layers challenged at peak of egg production showed a decrease in egg quantity while layers challenged during rearing had limited to no interruption in production. Although this has not been shown in broiler breeders, utilizing the rearing period for potential *H. meleagrisid* vaccination should be considered due to this time period not having adverse effects on production (Liebhart, et al., 2013; Sigmon, et al., 2019). On the contrary, induction of systemic antibodies against *H. meleagrisid* in turkeys were inefficient in preventing disease. From this information, the stimulation of an antibody response is not an effective control method for blackhead disease in all avian species so alternative products that aid in the mucosa-associated lymphoid tissue should also be considered.

Current control methods are focused on hygienic upkeep of the animal facilities, medications to decrease the cecal worm burden and feed and water additives that lead to variable disease outcomes (AbdulRahman and Hafez, 2009). Deworming practices, specifically against *Heterakis gallinarum*, are still in place to decrease long-lived birds from becoming infected with *H. meleagrisid* due to the widespread cecal worm contamination in these facilities (Hu and McDougald, 2002; Hu, et al., 2006; Dolka, et al., 2015). In commercial turkeys, the worm burden is considered low (Norton, et al., 1999) but the deworming practice is still used to mitigate other diseases associated with other worm species. Acidic products, added to the drinking water, have been attempted in the field but correlates with greater *H. meleagrisid* presence in the ceca. Therefore, acidification of drinking water is not recommended (Callait-Cardinal, et al., 2010). Essential oils have shown promise in *in vitro* experimental settings but have yet to be found consistent in decreasing blackhead disease in the field (Zenner, et al., 2003). Antibiotics and anticoccidials have been proven ineffective in decreasing lesion scores and weight loss associated with *H. meleagrisid* infections (Hu and McDougald, 2002). Control
methods for chickens should decrease the worm burden but control methods for turkeys should
decrease horizontal transmission.

Opportunities for Research with Poultry Protozoal Parasites

In broiler production, with multiple coccidia vaccine and chemotherapeutic options,
producers prioritize improvements in feed conversion efficiency and weight gain (Soutter,
Werling, Tomley and Blake, 2020). Numerous nonmedicated feed additives are available on the
market but need to be tested for their efficacy in aiding in gut health or acting as an
antiprotozoal. Sodium bisulfate, a feed additive that acts as an acidifier, has been hypothesized to
influence gut health and has been shown to improve feed efficiency in broilers (Kassem, et al.,
2012; Ruiz-Feria, et al., 2011). Future work should determine if this feed additive can aid in
enteric health of broilers during a coccidiosis challenge by combining pathological,
parasitological and production analysis.

Currently, only live vaccines are available in the turkey industry and increase risks for
susceptible birds to develop coccidiosis. This risk can increase susceptibility to secondary and/or
coinfections (Rathinam, Gadde and Chapman, 2016). In combination with this concern, poult
quality at placement can vary, altering the initial development of the gut and the microbiome
(Potturi, Patterson and Applegate, 2005). Further research in understanding other enteric diseases
that lead to production issues after coccidiosis in commercial turkeys is necessary.

The majority of blackhead disease research has focused on diagnostics, disease
progression, and vaccination while there is still limited information on certain aspects of general
production. First, there is no information on the effects of *H. meleagris* and broiler breeder
hens in relation to body weights, egg production and egg quality if pullets are infected before lay.
This information could highlight the importance of blackhead disease during pullet rearing and
determine whether broiler breeders will have similar production changes that are seen with layers infected during this time period. Second, the interaction between coccidia species and *H. meleagridis* has been studied with chickens but not turkeys even though both protozoa can be found in turkey facilities. A collection of field data that highlights these two protozoa in commercial production will help determine if experimental studies are necessary.
REFERENCES


Gruber, J., P. Ganas, and M. Hess. 2017. Long-term *in vitro* cultivation of *Histomonas meleagridis* coincides with the dominance of a very distinct phenotype of the parasite exhibiting increased tenacity and improved cell yields. Parasitology. 144:1253-1263. doi 10.1017/S0031182017000646


Hess, M., E. Grabensteiner, and D. Liebhart. 2006. Rapid transmission of the protozoan parasite *Histomonas meleagridis* in turkeys and specific pathogen free chickens following cloacal infection with a mono-eukaryotic culture. Avian Pathol. 35:280-285. doi 10.1080/03079450600815507


Sulejmanovic, T., D. Liebhart, and M. Hess. 2013. *In vitro* attenuated *Histomonas meleagridis* does not revert to virulence, following serial *in vivo* passages in turkeys or chickens. Vaccine. 31:5443-5450. doi 10.1016/j.vaccine.2013.08.098


CHAPTER 2

SODIUM BISULFATE FEED ADDITIVE AIDS BROILERS IN GROWTH AND INTESTINAL HEALTH DURING A COCCIDIOSIS CHALLENGE

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ABSTRACT

Sodium bisulfate (SB) was evaluated on its ability to improve broiler growth and intestinal structure with(out) a coccidia challenge. One thousand two hundred Cobb500 day-old males were randomly assigned within four experimental groups with a 2X2 factorial design, with (out) SB in the diet and with(out) a D0 coccidia challenge using a 10X dose of a commercial vaccine. At D7, oocysts per gram of feces (OPG) was determined. At D: 0, 14, 28 and 41 body weights and feed consumption were measured. At D21, twenty birds per treatment were subjectively scored for coccidia lesions and jejunal histological samples were collected for villi measurements. Twenty additional birds were given FITC-D to determine gut permeability. At D41, ten birds per treatment had histological samples collected. Statistical analysis was conducted in JMP Pro 14 using GLM procedure to compare disease state and diet. Means were separated using Dunnett’s test (P≤0.05) with the nonchallenged- standard diet treatment considered the control. All parameters measured indicated an effect due to the coccidia inoculation. Therefore, effects of diet on (non) challenged treatments were determined using a student t-test (P≤0.05). Limited differences due to diet were seen for the nonchallenged production data. SB had a thinner villi base width (P=0.04) on D21 and greater villi height (P=0.03), smaller base width (P=0.04), thicker muscularis (P=0.03) and lower crypt: height ratio (P=0.01) on D41. Challenged SB had similar gut permeability to the control (p=0.94) on D21. There was no difference in flock uniformity, feed intake, OPG or lesion scores between challenged treatments. Challenged SB had greater body weights on D14 (P<0.0001), 28 (P<0.0001) and 41 (P=0.02). FCR from D0-14 was also lower (P=0.0002). Challenged SB had smaller crypts (P=0.02) and therefore a smaller crypt: height ratio (P=0.03) on D21. Challenged control had a larger apical width (P=0.03) and thicker muscularis (P=0.04) on D41. Overall, the
addition of SB during coccidial enteropathy aided in body weights, FCR and villi health with no observed effects on parasite cycling.

Keywords: broiler, coccidiosis, sodium bisulfate

INTRODUCTION

Sodium bisulfate is an acidic compound that has been conventionally applied to poultry litter and used as a feed additive (Pope and Cherry, 2000; Line, 2002; Ruiz-Feria, et al., 2011; Kassem, et al., 2012; Hunolt, et al., 2015). This compound has shown positive impacts on poultry health and rearing, with binding to ammonia to improve air and litter quality while having various effects on bacteria in the broiler and the broiler’s environment (Pope and Cherry, 2000; Line, 2002; Kassem, et al., 2012; Williams, et al., 2012; Hunolt, et al., 2015). Some studies have indicated a positive correlation with broiler growth if given sodium bisulfate in the diet with or without a disease challenge (Line, 2002; Ruiz-Feria, et al., 2011; Kassem, et al., 2012).

Sodium bisulfate dissociates into its chemical matrix of sodium, hydrogen and sulfate. Sodium and hydrogen are important ions that are regularly transported between the lumen and gut epithelia for acid-base and electrolyte homeostasis. Within the ileum, absorption of sugars and fluids is predominately driven by sodium uptake (Gennari and Weise, 2008). Sulfate is an anion well documented in mammalian plasma that acts as a key component for the maintenance of tissues (Markovich, 2001). It is also essential for sulfonated carbohydrates in mucin, the protective barrier between the lumen and the epithelium of the gastrointestinal tract (Dawson, et al., 2009). Most sulfates come from exogenous sources, either as an inorganic source in the diet or from metabolized sulfur-containing amino acids (Whittamore and Hatch, 2017). Anion substitution of sulfate for chloride has been considered in poultry, mainly during a period of
stress that alters gut homeostasis leading to increased electrolyte losses (Hooge, et al., 1999; Ahmad, et al., 2006; Zdunczyk, et al., 2012). During diarrheal states, fluid losses of electrolytes are common due to improper absorption and sloughing of villi (Gennari and Weise, 2008). Decreased sulphomucins have been identified in humans suffering from gastrointestinal distress (Dawson, et al., 2009). Therefore, increased availability of these compounds during enteropathy could aid the host.

Coccidiosis in poultry is a parasitic disease caused by multiple species in the genus *Eimeria* that compromises the intestinal barrier functionality leading to diarrhea (McDougald, 1998; Yun, et al., 2000; Williams, 2005; Assis, et al., 2010). Coccidia are ingested by the host, invade the mucosa and cause various degrees of damage to the epithelial cells of the intestine, initiating inflammation and macroscopic lesions (Yun, et al., 2000; Assis, et al., 2010). Other clinical signs of coccidiosis include dehydration, nutrient malabsorption and therefore reduced weight gain due to this parasite’s disruptive properties (McDougald, 1998). Intestinal maintenance is of high importance for nutrient absorption and therefore is high energy cost for the bird. Aiding the gut lining and villi during a coccidial infection is essential to maintain performance (Assis, et al., 2010). It is hypothesized that the addition of sodium bisulfate will aid coccidia challenged broilers in growth and intestinal integrity. Therefore, the objective of this study was to determine how the addition of sodium bisulfate in the diet changes broiler production and gut morphology and permeability with or without a multiple coccidia species challenge.

**MATERIALS AND METHODS**

*Animal care and housing* A total of 1200 day-of-hatch broilers from the North Carolina State University Chicken Education Unit (Raleigh, NC) and randomly divided into 4 experimental
groups (10 pens/ treatment) with a 2X2 factorial design, which included combinations of diet and disease (300 birds/ treatment). The treatments included: challenged birds on the SB diet (CSB), challenged birds on the standard diet (standard), nonchallenged birds on the SB diet (SB) and nonchallenged birds on the standard diet (control). The experiment was conducted in 4 rooms, 2 rooms were designated for the coccidia vaccination challenge while 2 rooms were given no vaccination. Each room had diets randomly distributed between 10 pens, with 5 pens on the standard diet and 5 pens on the sodium bisulfate diet. Birds were placed into individual floor rearing pens dressed with new pine shavings. Broilers were reared during the spring in North Carolina, with optimal temperatures, humidity and lighting programs set and maintained in each room based on the Cobb500 management standards. Animal welfare, temperature, humidity and feed and water were checked twice daily over the duration of this study. All experimental procedures for broiler chickens were performed following the guidelines of the North Carolina State University Animal Care and Use Committee.

Diet The starter diets were fed as crumbles, and the grower and finisher diets were fed as pellets. Standard-diet birds (challenged or not) were fed only corn- and soy-based feed. Sodium bisulfate-treated birds (challenged or not) were fed corn- and soy-based feed with 0.5% feed grade sodium bisulfate (Jones- Hamilton Co., Walbridge, OH) which replaced sodium chloride (Table 1). Feed and water were available ad libitum with diets meeting or exceeding the NRC (1994) requirements. No coccidiostats, antibiotics or enzymes were used.

Performance Broilers were individually weighed at hatch, randomly assigned a treatment then placed in the corresponding pen. Individual body weights were also collected on days 14, 28 and 41 to analyze for growth and treatment flock uniformity. Feed added and leftover during each
diet-phase change was recorded to determine feed intake and feed conversion ratio (FCR).

Mortality was recorded twice daily with necropsies performed to determine cause of death.

**Coccidia inoculation** At placement, 20 pens (10 pens per room, 2 rooms per inoculation) were orally inoculated with 10X of a commercial coccidia vaccine (Merck & Co., Inc., Kenilworth, NJ, USA). Each dose contained four *Eimeria* species: *E. mivati, E. tenella, E. acervulina* and two strains of *E. maxima*. For birds not challenged with coccidia, 20 pens (10 pens per room, 2 rooms per inoculation) were sham inoculated with water.

**Oocysts shedding** Fecal material was collected from each treatment on day 7 to determine fecal shedding of coccidial oocysts. Fresh feces from the same treatment group were collected from multiple pens to obtain a pooled sample of approximately 500 grams. Fecal material in each bag was massaged to evenly distribute the collection. Four one-gram replicates per treatment of feces was added to a 50 mL conical and saturated in 2 mL of 2% potassium dichromate following the general guidelines of Haug, et al. (2006). To count, samples were filled with a 58% sugar solution until the sample solution volume reached a total of 30 mL. Each sample was vortexed to ensure even oocysts distribution followed by microscopic counting using a McMaster egg chamber. Each sample had two replicates counted to determine the average number of oocysts per gram of feces (OPG) per treatment.

**Gross lesion scoring** On day 21, twenty birds per treatment (representing the mean treatment population body weight) were euthanized by cervical dislocation and their intestinal tract isolated. Because the treatments included a mixed infection with *Eimeria* species having overlapping colonization sites, one researcher examined the duodenal loop/ upper intestines, mid-intestine and ceca for macroscopic lesions associated with the coccidia species in the vaccination. Birds were subjectively scored based on severity of lesions through the intestinal
tract using the Poultry Coccidiosis Diagnostic and Testing Procedures as a guide (Conway and McKenzie, 2007). Briefly, a 0-4 scale was used to assess macroscopic damage at the duodenal loop/upper intestines to approximately one inch above Meckel’s diverticulum, the mid-intestines approximately one inch above and one inch below Meckel’s diverticulum, and each cecum. A score of “0” represented no visual lesions, “1” was minimal lesions, “2” was moderate lesions, “3” was severe lesions, and “4” was extremely severe. The researcher was blind to which birds correlated with which treatment.

**Histopathology** Twenty birds per treatment on day 21 and ten birds per treatment on day 41 were euthanized and a tissue sample was isolated by cutting at the Meckel’s Diverticulum and approximately 2.5 cm towards the duodenal loop. Intestinal tissue was flushed with phosphate buffered saline (Fisher Scientific, Waltham, Massachusetts) then placed into approximately 10 mL of 10% buffered formalin and stored at room temperature for 3 weeks. Samples were then transferred to 70% ethanol for 3 days then sent to the College of Veterinary Medicine, North Carolina State University for processing and haemotoxylin-eosin (H&E) staining. Samples on slides were microscopically measured using AmScope version 3.7 (Irvine, California). Three villi per bird were examined for oocysts presence and the following villi measurements were collected: villus height, villus apical width, villus base width, crypt depth and muscularis thickness.

**Intestinal permeability** Intestinal macromolecular permeability was carried out using a similar method to Baxter, et al. (2017) with minor modifications. Permeability was determined by giving an oral gavage of 4 milligrams of fluorescein isothiocyanate-dextran (FITC-D) (4 kDa, Sigma-Aldrich Co., St. Louis, MO) dissolved in distilled water per kilogram of broiler body weight. Twenty birds per treatment were randomly selected on days 21, weighed, then dosed with the
appropriate volume of FITC-D solution. Marked birds were placed back into their pens for 1.5-2 hours with free access to feed and water. After, 2 mL of blood per bird was collected from the ulner vein using a heparinized syringe and stored in a capped, heparinized tube. Blood samples were stored in a cooler to decrease light exposure during sampling. Samples were centrifuged (5,000 g for 5 minutes) and the plasma was isolated. Fluorescence in plasma samples was read using a FilterMax F5 microplate reader (Molecular Devices, San Jose, California).

**Statistical analysis** Data was analyzed as a 2X2 factorial in JMP Pro 14 using GLM (SAS Institute Inc., Cary, NC). For the gut permeability data, all four treatments had their means separated with Duncan’s significant difference test (P≤0.05) with the nonchallenged birds fed the standard diet considered the control. Because a treatment effect found with coccidiosis, analysis of diet with treatments having the same disease state were compared using a Student’s T- Test (p≤0.05). This data included growth performance, OPG counts, lesion scoring, histology, and gut permeability. Due to the necessity of separating coccidia- challenged and nonchallenged treatments, each dietary treatment was also compared by room to determine if there was a room effect.

**RESULTS**

**Growth and feed intake** Growth and feed intake were negatively impacted by the coccidiosis challenge (Tables 2, 3 and 4). Body weights were significantly heavier for CSB on days 14 (p<0.0001), 28 (p<0.0001) and 41 (p=0.002). A room effect was observed on day 28 for the body weights of both the challenged treatments. FCR was significantly lower for CSB on days 0-14 (p=0.002). There was no difference in flock uniformity for challenged treatments. Nonchallenged birds had no difference in individual body weights, FCR or flock uniformity after placement. SB treatment was significantly lighter than the control on at placement (p=0.05) but growth and feed
intake was not altered due to this difference based on subsequent measurements. The SB treatment had a room effect on day 28 for body weights.

**Oocysts shedding and lesion scores** None of the nonchallenged treatments had oocysts found in their feces or had lesion scores associated with the coccidia species. Therefore, data presented is based on the challenged treatments (Table 5). There was no significant difference in the average OPG between diets (p=0.72) nor was there a difference in subjective lesion scoring for the duodenal loop (p=0.07), mid-intestine (p=0.25) or ceca (p=1). No room effect was observed for the oocyst shedding or lesion scores. No correlations were seen between the subjective lesion scores and the body weights, gut morphology, or permeability.

**Gut morphology and permeability** Histology measurements were compared between treatments with the same challenge but on varying diets (Table 6). For the challenged treatments, the crypt depth of CSB was significantly smaller (p=0.02) leading to a smaller crypt:height ratio (p=0.03) on day 21. On day 41, CSB had smaller apical width (p=0.03) and a thinner muscularis (p=0.04) compared to the standard. For the nonchallenged treatments, the control treatment had significantly larger base width (p=0.04) compared to SB on day 21. On day 41, control treatment had shorter villus height (p=0.03), thicker base width (p=0.04), thinner muscularis (p=0.03) and a greater crypt:height ratio (p=0.01). Fluorescence levels of blood samples collected on day 21 were compared between the four treatments to determine gut integrity where CSB had the closest fluorescence value to the control (p=0.94) with no statistical differences found between the treatment groups (Figure 1). No room effect was observed for gut morphology or permeability.

**DISCUSSION**

**Performance** General malabsorption of proteins occurs during coccidiosis due to intestinal damage causing challenged broilers to fail to gain weight (Murillo, et al., 1976; Williams, 2005;
Lee, et al., 2011; Chapman, 2014). Specifically, the sulfur-containing amino acids can be broken down for acid-base homeostasis around the intestinal barrier which decreases their potential to be used for growth (Whittamore and Hatch, 2017). Sodium bisulfate has shown to improve feed conversion in broilers (Ruiz-Feria, et al., 2011). Other studies have shown that the addition of sodium bisulfate in litter increases body weights while decreasing the number of broilers with signs of ascites or respiratory distress (Terzich, et al., 1998; Line, 2002). These studies were focused on applying sodium bisulfate to the litter while the current study was focused on applying sodium bisulfate into the feed. In the current study, limited differences in performance were seen with nonchallenged broilers on the varying diets. Hooge, Cummings and McNaughton (1999) showed that the addition of a sodium sulfate, not bisulfate, source in the broiler diet had similar effects on broiler growth and lesion scores compared to other salts if birds were given an acute coccidial challenge. The current study indicates a benefit in growth if sodium bisulfate was supplemented in the feed during a prolonged challenge. Coccidiosis is known for reducing early live performance by increasing FCR while decreasing body weights (Christaki et al., 2004). A lower feed conversion was observed during the starter period for SB challenged birds (p=0.002), the time period at which many broiler producers are concerned about performance when vaccinating meat-producing broilers. A significant increase in body weights for CSB was observed when compared to the standard treatment on days 14, 28 and 41 (Table 2). A room effect was observed for the challenged treatments on day 28. The light timer broke at the beginning of week 4, resulting in no dark cycle for approximately 2 days in one of the challenged rooms. There was no change in the monitored environment to justify the room effect for the SB treatment. It was understood that environmental effects could alter performance results of the study when using multiple rearing facilities. However, due to the inability to control cross-
contamination of oocysts in a pen trial, separation of broilers inoculated with coccidia from the nonchallenged broilers was necessary to ensure that there was no contamination. Even with the room effect, the combination of improved feed efficiency and increased body weight with CSB suggests some alleviation of the negative clinical signs of coccidiosis during early rearing. Growth performance may be altered by factors other than the coccidia challenge, which highlights the necessity to incorporate multiple parameters to measure intervention strategies against coccidiosis within a study (Chasser et al., 2020).

**Disease signs** Macro- and microscopic pathology were used to determine if the addition of SB altered parasite cycling in broilers. Oocyst counts in the feces on day 7 and subjective lesion scoring on day 21 indicated no difference due to diet (Table 5). For this study, a combination vaccine containing *E. acervulina, E. maxima* (2 strains), *E. mivati* and *E. tenella* was used to mimic field conditions, where most cases of coccidiosis have more than one infective species (Reisinger, et al., 2011). Microscopic observations were not used to differentiate species of oocysts in the feces or intestinal tract. The prepatent period of *Eimeria* species used in this study vary from 4-6 days post-inoculation and oocysts can be shed into the litter several days afterward (McDougald, 2013). Day 7 was selected to measure OPG so that a representative population of the *Eimeria* species could be in the fecal samples. Fecal shedding of the oocysts is an indicator of how parasite load is affected by treatments (Chasser, 2020). Based on no significant differences found in OPG between diets, it can be inferred that sodium bisulfate did not alter the shedding of these parasites. Damage of the digestive tract and not damage due to a specific species was analyzed due to the inoculation containing *Eimeria* species that can colonize in similar areas of the digestive tract. Day 21 was selected for measuring macroscopic lesions due to this time corresponding with the greatest intestinal damage due to coccidiosis if *Eimeria* species are
routinely cycling (McDougald, 2013). The recorded intensity of visual lesion damage was low, but this could be due to the subjective nature of the scoring system and the single day observation. Lesion scoring has been suggested to understate the *Eimeria* species impact on gut health indicating that observing only lesion scores may not be indicative of disease (Chasser, et al., 2020). Based on no significant differences detected on the oocysts shedding as well as the lesion scoring, it can be inferred that SB had limited effects on the lifecycle of the *Eimeria* species.

Oocysts alter villi functionality by invading the protective mucosal layer and enterocytes. This leads to innate immune responses and intestinal morphological changes, causing villi sloughing to remove damaged cells, a decrease in nutrient absorption and an inflammatory response (Yun, et al., 2000). In this study, oocysts were identified in both inoculated treatments invading villi on day 21 at the third (and greatest) peak of the cocci cycling. As expected, villi were shortened due to the challenge independent of diet (Table 6). Shortening of the villi and increased crypt depth to compensate for epithelial cell loss has been recorded during a coccidia challenge (Fernando and McCraw, 1973; Al-Sheikhly and Al-Saieg, 1980). This correlates with decreased body weights, potentially due to the increased cell turnover from the crypts, leading to immature and nonspecialized enterocytes and therefore nutrient malabsorption (Fernando and McCraw, 1973). The SB challenged birds had greater body weights with a smaller crypt (p=0.02) and smaller crypt: height ratio (p=0.03) on day 21, supporting the idea that this feed additive favorably altered the intestinal morphology if coccidia were present. On day 41, all measurements were increased for the challenged birds consuming the control diet. This could indicate compensatory hypertrophy, which has been seen in other coccidiosis models (Fernando and McCraw, 1973). A thinner muscularis was observed on day 41 for SB challenged birds
Broilers consuming antibiotic growth promotors tend to have a thinner intestinal wall, potentially due to fewer accumulation of inflammatory cells (Kuttappan, et al., 2015). The changes in muscularis thickness in this study could be due to inflammation. However, further research is warranted before conclusions can be made. Intestinal morphology at 21 and 41 days indicated a favorable response with SB if birds are suffering from coccidiosis.

**Intestinal permeability** During infection, intestinal permeability is altered where plasma proteins leak from the lumen into the bloodstream of challenged broilers due to damage of the mucosa. The intestinal lumen also becomes acidified during a coccidia infection which decreases gut passage time while altering absorption (Williams, 2005; Chapman, 2014). FITC-d is a fluorescent marker that is passively absorbed through tight junctions of the intestinal epithelia and can be detected in blood plasma or serum using a fluorescent reader. Metabolic and immune parameters of the host affect the permeability of FITC-d and it is common to restrict feed in determining intestinal integrity with this marker (Kuttappan, et al., 2015; Baxter, et al., 2017; Woting and Blaut, 2018). This was not done in our model because of the concern with an interaction in feed restriction and disease state as well as feed- to- gain ratio in the broilers. A disease state will cause a change in gut permeability (Kuttappan, et al., 2015). The challenged SB birds had permeability like the nonvaccinated control (p=0.94) (Figure 1). At this point in the coccidia cycling, the greatest intestinal damage is expected. Based on our findings for intestinal permeability using FITC-d, sodium bisulfate consumed during a disease state had intestinal integrity like that of a broiler without a disease challenge.

**Conclusion** Nutritional status of the bird influences it’s response to stressed states, like coccidiosis (Yun, et al., 2000). Sodium bisulfate may indirectly improve growth performance of
coccidia-challenged broilers by supporting intestinal structure and function. No significant differences in parasite shedding or lesion scores between treatments indicates that sodium bisulfate had no direct effect on *Eimeria* oocysts or their replication. This study indicates that the addition of sodium bisulfate is efficacious for broiler performance during a coccidia challenge if birds are reared in ideal environmental conditions.

**ACKNOWLEDGEMENTS**

Thank you to Jones Hamilton Co for financial support for this project.
**Figure 2.1.** Fluorescence of plasma measured on day 21 (mean ± SEM) with treatment groups compared using the Duncan’s test (P≤0.05) with the nonvaccinated control group designated as the control treatment. P-values are indicated above each treatment bar.

CSB= challenged sodium bisulfate treatment group, SB= nonchallenged sodium bisulfate treatment group, FITC-D= fluorescein isothiocyanate-dextran, SEM= standard error of the mean
Table 2.1. Feed ingredients and their inclusion levels for the diets in this study

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Starter Standard / Control</th>
<th>(C)SB</th>
<th>Grower Standard / Control</th>
<th>(C)SB</th>
<th>Finisher Standard / Control</th>
<th>(C)SB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn (yellow, grain)</td>
<td>53.8</td>
<td>53.6</td>
<td>60.4</td>
<td>60.1</td>
<td>63.6</td>
<td>63.4</td>
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<tr>
<td>Poultry fat</td>
<td>4.12</td>
<td>4.12</td>
<td>3.92</td>
<td>3.92</td>
<td>4.03</td>
<td>4.03</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>37</td>
<td>37</td>
<td>31</td>
<td>31</td>
<td>28</td>
<td>28</td>
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<tr>
<td>Calcium carbonate</td>
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<td>1.5</td>
<td>1.2</td>
<td>1.2</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Mono-dicalcium phosphate</td>
<td>1.8</td>
<td>1.8</td>
<td>1.7</td>
<td>1.7</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Salt (plain)</td>
<td>0.4</td>
<td>0.15</td>
<td>0.4</td>
<td>0.15</td>
<td>0.4</td>
<td>0.15</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.21</td>
<td>0.21</td>
<td>0.28</td>
<td>0.28</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.38</td>
<td>0.38</td>
<td>0.36</td>
<td>0.36</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Selenium Premix</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Choline Chloride</td>
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<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Trace Mineral Premix</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Vitamin Premix</td>
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<td>0.2</td>
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<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Sodium Bisulfate</td>
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<td>0.5</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

(C)SB= (challenged) sodium bisulfate treatments
Table 2.2. The mean individual body weights (kilograms) and standard error of the mean (SEM) of each treatment group prior to diet-phase changes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 28*</th>
<th>Day 41</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSB</td>
<td>0.049</td>
<td>0.77</td>
<td>&lt;0.0001</td>
<td>1.81</td>
<td>3.29</td>
</tr>
<tr>
<td>Standard</td>
<td>0.049</td>
<td>0.54</td>
<td>1.74</td>
<td>&lt;0.0001</td>
<td>3.39</td>
</tr>
<tr>
<td>SB</td>
<td>0.048</td>
<td>0.62</td>
<td>1.87</td>
<td>3.36</td>
<td>0.17</td>
</tr>
<tr>
<td>Control</td>
<td>0.049</td>
<td>0.62</td>
<td>1.87</td>
<td>3.39</td>
<td>0.17</td>
</tr>
</tbody>
</table>

CSB= challenged sodium bisulfate treatment group, SB= nonchallenged sodium bisulfate treatment group, SEM= standard error of the mean, differences were considered significant at $P \leq 0.05$

* a room effect was observed
Table 2.3. The average FCR with the standard error of the mean (SEM) of each treatment group prior to diet-phase changes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days 0-14</th>
<th>p-value</th>
<th>Days 14-28</th>
<th>p-value</th>
<th>Days 28-41</th>
<th>p-value</th>
<th>Days 0-41</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSB</td>
<td>1.22</td>
<td></td>
<td>1.72</td>
<td>0.59</td>
<td>1.8</td>
<td>0.34</td>
<td>1.63</td>
<td>0.06</td>
</tr>
<tr>
<td>Standard</td>
<td>1.25</td>
<td>0.002</td>
<td>1.75</td>
<td>0.34</td>
<td>1.85</td>
<td>0.62</td>
<td>1.68</td>
<td>0.51</td>
</tr>
<tr>
<td>SB</td>
<td>1.18</td>
<td>0.47</td>
<td>1.53</td>
<td>0.62</td>
<td>1.77</td>
<td>0.94</td>
<td>1.63</td>
<td>0.51</td>
</tr>
<tr>
<td>Control</td>
<td>1.19</td>
<td></td>
<td>1.57</td>
<td></td>
<td>1.76</td>
<td></td>
<td>1.64</td>
<td></td>
</tr>
</tbody>
</table>

SEM 0.01 0.02 0.04 0.02

CSB= challenged sodium bisulfate treatment group, SB= nonchallenged sodium bisulfate treatment group, FCR= feed conversion ratio, SEM= standard error of the mean, differences were considered significant at P≤0.05
Table 2.4. The coefficient of variation (CV) to calculate treatment flock uniformity at three time periods

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 14</th>
<th>p-value</th>
<th>Day 28</th>
<th>p-value</th>
<th>Day 41</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSB</td>
<td>11</td>
<td>0.75</td>
<td>8</td>
<td>0.26</td>
<td>7</td>
<td>0.64</td>
</tr>
<tr>
<td>Standard</td>
<td>12</td>
<td></td>
<td>9</td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>SB</td>
<td>8</td>
<td>0.33</td>
<td>7</td>
<td>0.38</td>
<td>6</td>
<td>0.08</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td></td>
<td>8</td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.7</td>
<td></td>
<td>0.4</td>
<td></td>
<td>0.5</td>
</tr>
</tbody>
</table>

CSB= challenged sodium bisulfate treatment group, SB= nonchallenged sodium bisulfate treatment group, CV= coefficient of variation, SEM= standard error of the mean, differences were considered significant at P≤0.05
Table 2.5. Oocysts per gram (OPG) counts from day 7 and lesion scoring of the intestine on day 21 for challenged treatments

<table>
<thead>
<tr>
<th></th>
<th>Day 7</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OPG</td>
<td>duodenal loop/ upper intestine</td>
</tr>
<tr>
<td>CSB</td>
<td>46,000</td>
<td>1.3</td>
</tr>
<tr>
<td>Standard</td>
<td>50,000</td>
<td>0</td>
</tr>
<tr>
<td><em>p-value</em></td>
<td>0.72</td>
<td>0.07</td>
</tr>
</tbody>
</table>

CSB= challenged sodium bisulfate treatment group, OPG= oocysts per gram of feces, differences were considered significant at P≤0.05
Table 2.6. Average villi measurements (μm) of the four treatments from days 21 and 41

<table>
<thead>
<tr>
<th></th>
<th>Day 21</th>
<th></th>
<th></th>
<th>p-value</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>p-value</th>
<th></th>
<th></th>
<th>p-value</th>
<th></th>
<th></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSB standard</td>
<td>SB control</td>
<td>p-value</td>
<td>CSB standard</td>
<td>SB control</td>
<td>p-value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villus Height</td>
<td>839</td>
<td>820</td>
<td>0.45</td>
<td>868</td>
<td>891</td>
<td>0.46</td>
<td>1026</td>
<td>1060</td>
<td>0.52</td>
<td>1123</td>
<td>997</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
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</table>

CSB= challenged sodium bisulfate treatment group, SB= sodium bisulfate treatment group, differences were considered significant at P≤0.05


Whittamore, J. M., and M. Hatch. 2017. Loss of the anion exchanger DRA (Slc26a3), or PAT1 (Slc26a6), alters sulfate transport by the distal ileum and overall sulfate homeostasis. Am J Physiol Gastrointest Liver Physiol 313:G166-G179. doi 10.1152/ajpgi.00079.2017


CHAPTER 3

EARLY INFECTION WITH *HISTOMONAS MELEAGRIDIS* HAS LIMITED EFFECTS ON BROILER BREEDER HENS’ GROWTH AND EGG PRODUCTION AND QUALITY

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Manuscript *in press* by The Journal of Poultry Science
A study was conducted to determine differences between *Histomonas meleagridis* infected and control pullets based on disease signs, hen growth and egg production and quality. Ross 708SF females were weighed then placed in pens on day of hatch (92 chicks/pen). At 25 d, 4 pens were infected with *H. meleagridis* in the cloaca while 4 pens were control. At 5, 10 and 20 d post-inoculation, 5 birds per pen (2 birds per pen at 20 d) were subjectively scored for blackhead disease. Birds were feed restricted based on BW and/or egg production. Individual BW were collected at 3, 5, 13, 15, 20 and 64 wk of age. Egg production was recorded at 24-63 wk of age. Egg quality was measured at 30, 34, 39, 42 and 56 wk of age and included: shell and vitelline membrane strength, shell thickness, egg weight, and Haugh units. Hatchability was measured at 27, 37 and 60 and fertility at 27 and 37 wk of age. Treatment effects were determined in JMP Pro 14 using GLM with means separated using the student’s t-test (*P* ≤ 0.05). Cecal lesions were apparent on 5, 10 and 20 d and liver lesions on 10 and 20 d for the infected. The control had no histomoniasis lesions. Flock uniformity differed on wk 13 and 20 (*P* = 0.04; 0.04). Infected birds weighed less at 64 wk (*P* = 0.002). The onset of lay was not delayed. Infected birds produced more eggs during one period (*P* = 0.02). The infected birds produced heavier eggs at 30 wk (*P* = 0.04), eggs with a stronger and thicker shell at 42 wk (*P* = 0.05, 0.03) and a stronger vitelline membrane at 56 wk (*P* = 0.049). Hatchability and fertility did not differ (*P* > 0.05). *H. meleagridis* was observed in the infected birds’ cecal samples at trial termination. This study indicates early infection with *H. meleagridis* has limited effects on pullet egg production and quality.

**Key words:** *Histomonas meleagridis*, blackhead disease, broiler breeder, egg production
INTRODUCTION

*Histomonas meleagridis* is a protozoal parasite that causes histomoniasis or blackhead disease in gallinaceous birds. Histomoniasis is considered a reemerging disease due to the number of outbreaks in intensive chicken and turkey facilities increasing over the past few decades as available treatments decrease (Liebhart *et al.*, 2017). Chickens mount a more effective immune response against *H. meleagridis* compared to the turkey (Powell *et al.*, 2009). Generally, infected chickens suffer from morbidity, specifically lesions in the ceca, but have reduced liver involvement and mortality than turkeys (Liebhart *et al.*, 2011). Negative effects on chicken production performance can include: poor flock uniformity, delayed onset of lay and a decrease in the quality and quantity of eggs produced leading to economic losses (Gerth *et al.*, 1985; Hu and McDougald 2002; Esquenet *et al.*, 2003; Grafl *et al.*, 2011; Dolka *et al.*, 2015).

Conversely, some *H. meleagridis* outbreaks in layer and breeder facilities go unnoticed due to absence of clinical signs or gross pathological lesions (McDougald, 1998; McDougald, 2005; Hu *et al.*, 2006; Sulejmanovic *et al.*, 2013).

Economic losses after a histomoniasis outbreak in commercial chicken facilities are attributed to *H. meleagridis* but do not account for losses due to co-infections or timing of the *H. meleagridis* introduction (Desowitz, 1951; Gerth *et al.*, 1985; Esquenet *et al.*, 2003). Co-infection of *H. meleagridis* with other pathogens has led to an increase in layer mortality as well as a decrease in performance (Gerth *et al.*, 1985; Esquenet *et al.*, 2003). Liebhart *et al.* (2013) found that experimentally inoculating layers with only *H. meleagridis* at the peak of lay caused a decrease in egg production while conversely, Sigmon *et al.* (2019) found that inoculating layers during rearing did not alter their egg production.
Broiler breeders are more likely to cycle *Heterakis gallinarum*, a cecal nematode known for being a vector of *H. meleagrisidis*, than layers due to floor rearing used in commercial breeder facilities (Waters et al., 1994). This indicates an increased potential for the broiler breeder to be exposed to *H. meleagrisidis*. Case reports on the interaction between broiler breeder flocks and *H. meleagrisidis* are documented in the context of a natural infection without other environmental and disease factors controlled (Waters et al., 1994; Dolka et al., 2015). Experimental inoculation has indicated that broiler breeder pullets suffer from inflammation and lesions in the ceca but these studies have only focused on acute disease signs and not production (Hu and McDougald, 2002). The long-term effects of only *H. meleagrisidis* on broiler breeder pullets’ performance has yet to be investigated. This study aimed to determine 1) if an inoculation with a virulent strain of *H. meleagrisidis* would cause morbidity of commercially raised broiler breeder pullets and 2) if histomoniasis during rearing causes alterations to breeder hen egg production and quality.

**MATERIALS AND METHODS**

**Experimental Animals** Seven hundred and thirty-six Ross 708SF female pullets were obtained from a commercial hatchery (Pageland, South Carolina) on day of hatch and individually tagged. The pullets were randomly assigned to 4 pens per treatment (infected or control). Each pen housed 92 pullets. Breeders were vaccinated to control coccidiosis (*Eimeria acervulin*, *E. brunetti*, *E. maxima, E. nectrix* and *E. tenella*) and Marek’s disease at the hatchery. At 2, 5, 12 and 18 wk of age, breeders received vaccinations for Newcastle disease and infectious bronchitis. At 14 wk of age, they received a fowl pox vaccine. Pullets were raised in a blackout rearing facility from 0-21 wk of age then were transferred to a curtain sided laying facility from 21- 64 wk of age. Cockerels were reared in separate pens and were not infected directly with *H. meleagrisidis* during rearing. Eighteen males were housed in each pen during grow out and at 21
wk of age eight males per pen were selected for mating and transferred to the laying house. Birds were monitored twice daily and fed based on the feeding program provided below. Onset of lay and number of eggs produced was recorded daily for the duration of study.

**Housing** Pullet pens during the first 20 wk of age of rearing measured 3.33 m x 4.65 m and had fresh shavings applied prior to bird placement. In the rearing facility, birds were given 23 h of light for the first 2 wk of age, followed by 8 h of light until moving to the laying house. At 21 wk of age, 64 pullets averaging the mean BW of the pen, within one standard deviation of the mean, from each pen were moved to a curtain-sided layer house with each pen measuring 4.65 m x 3.73 m containing 0.51 m high slats, used litter and eight egg boxes. Birds maintained their original treatment status. In the laying facility, birds were given 14 h of light until 22 wk of age, 15 h of light through 24 wk of age, 15.5 h of light on 25-27 wk of age and 16 h of light until trial termination.

**Feeding Program** Diets were formulated to meet or exceed the Aviagen Female Parent Stock Nutrient Specifications as well as the NRC (1994) requirements (Tables 1 and 2). Pullets were provided feed *ad libitum* until 2 wk of age, then were placed on a modified “skip a day” feeding program from 2-22 wk of age. Feed was adjusted for mortality as needed. After 22 wk of age, birds were fed based on hen population and egg production on a per pen basis.

**Inoculation and Detection of Histomonas meleagridis** A culture of *Histomonas meleagridis* was collected from a broiler breeder outbreak in Buford, Georgia then preserved in liquid nitrogen. The isolates were removed from storage and grown at 42 °C in modified Dwyer’s medium consisting of 0.8% (wt / vol) rice powder and 5% horse serum in Medium199 with Hank’s balanced salt solution (Hauck *et al.*, 2010). The number of *Histomonas meleagridis* cells were determined via hemocytometer. At 25 d, pullets in the infected treatment (4 pens) were
inoculated in the cloaca with 100,000 *H. meleagridis* cells per pullet. At 5- and 10- d post inoculation, five pullets per pen per sampling day were euthanized and subjectively scored for histomoniasis based on cecal and liver lesions. On 20 d, two pullets per pen were euthanized and subjectively scored. At 64 wk of age, ten hens per pen were euthanized, cecal samples collected and placed in media then cultures were incubated at 42 °C for 48 h. After incubation, cultures were examined under light microscopy for the presence of *H. meleagridis*.

**Data Collection** Morbidity was assessed by observing disease signs after inoculation, BW and flock uniformity at various points through rearing and at trial termination. Pen weights were collected at placement to ensure initial BW uniformity. At 3, 5, 13, 16, 20, and 63 wk of age, individual BW of the breeder pullets were documented and analyzed for average BW and uniformity.

Monitoring of egg production occurred twice daily from onset of lay until trial termination. To determine egg quality characteristics, twenty-four eggs per treatment representing a sample population were measured for: shell and vitelline membrane strength, shell thickness, egg weight, and Haugh units at 30, 34, 39, 42 and 56 wk of age. Egg weight and Haugh units (Haugh, 1937) were measured using the TSS QCD system (Technical Services and Supplies, Dunnington, York, UK). Shell strength and vitelline membrane strength determinations were conducted using a Texture Analyzer (Texture Technologies, Scarsdale, NY). Shell thickness was recorded with an iGAGING Absolute Origin SpeedMic Micrometer (San Clemente, CA) and the average of two shell thickness measurements are presented. To determine hatchability and fertility, eggs were collected from each pen then stored at 15 °C for 1 wk prior to placement in the incubator. One hundred and eighty eggs were chosen at random from each pen and incubated under standard conditions. Twenty-one d later, hatchability and fertility were
documented based on treatment. Hatchability was determined at 27, 37 and 63 wk of age while fertility was analyzed on wk 27 and 37.

**Statistical Analysis** Feed allocation and egg production were recorded daily and analyzed based on 28-d periods (10 total) during the laying period. The BW, flock uniformity and egg production and quality for the two treatment groups were analyzed in JMP Pro 14 via GLM. Differences between treatments were found using a Student’s *t* test with significance considered if *P* ≤ 0.05.

**Animal Care and Use** This experiment was conducted in agreement with the Institutional Animal Care and Use Committee at North Carolina State University, where husbandry practices and euthanasia were followed keeping animal welfare and wellbeing in mind.

**RESULTS**

**Gross Pathology and Microscopy** Inflammation of the cecal tissue and surrounding mesentery with visually obvious thickening of the cecal wall and changes in the luminal contents were observed in 80% of the infected pullets 5 d post-inoculation, 90% at 10 d and in all infected pullets 20 d (Table 3). Very few white, pinpoint- shaped liver lesions characteristic with *H. meleagris* were found sporadically in the infected pullets at 10 and in all infected pullets at 20 d post-inoculation. None of the control pullets had lesions associated with *H. meleagris*.

Lesions associated with coccidia were apparent in the upper intestinal tract but not the ceca for both treatments during the sampling days. At 64 wk of age, *H. meleagris* was observed via light microscopy in 18 of the 40 cultures from the infected hens sampled and 0 of the 40 control hens. The protozoan *Blastocystis* was apparent in cultures for both treatments.
**Body Weights and Flock Uniformity** Individual BW only differed between treatments at trial termination ($P = 0.002$), where the infected pullets weighed significantly less (Figure 1). Flock uniformity differed between treatments at 13 and 20 wk of age, where the CV was higher for the infected treatment, indicating greater ($P = 0.04$) variation in the pullet size (Table 4).

**Egg Production and Quality** More eggs ($P = 0.03$) were produced in the infected treatment at 48 to 51 wk of age (Figure 2). Egg quality was affected at various sampling times, where at 30 wk of age the infected pullets produced heavier eggs ($P = 0.04$), at 42 wk of age there was a stronger ($P = 0.05$) and thicker shell ($P = 0.03$) and at 56 wk of age there was a stronger ($P = 0.049$) vitelline membrane (Table 5). Hatchability and fertility did not differ between treatments for the time periods analyzed (Table 6).

**DISCUSSION**

**Host-Parasite Interaction** Infection with *H. meleagridis* has varying consequences dependent on the gallinaceous bird host (Lund and Chute, 1972). Chickens have milder clinical signs compared to the turkey, where there is pathological lesions and inflammation in the ceca with minimal, if any, damage to the liver (Lund, 1967; Powell *et al*., 2009; Mitra *et al*., 2018). Cecal lesions and an inflammatory response have been found as early as 3 d post inoculation and can be seen up to 3 wk after (Hauck and Hafez, 2013). At 6 to 8 d post infection, the damaged cecal mucosa recovers and within a month the breeders can gain immunity while remaining positive for the parasite (Dolka *et al*., 2015). Chickens have been shown most vulnerable to *H. meleagridis* at around 4 wk of age, therefore the pullets in this experiment were inoculated at 25 d (Desowitz, 1951). Five d after the inoculation, 80% of the infected treatment pullets sampled had inflammation of the cecal tissue (Table 3). Cecal cores were found in some of the infected...
pullets at 10 d while visually apparent recovery of the ceca via decrease inflammation and core formation was seen at 20 d. Liver damage was minimal and sporadically identified at 10 d while all infected pullets had one to two necrotic pinpoint lesions per liver at 20 d. No mortality due to the \textit{H. meleagridis} inoculation was recorded. The pathogenesis of \textit{H. meleagridis} in the breeders mimics the timepoints observed by Zahoor (2011) who experimentally inoculated various chicken species.

Concurrent infections of \textit{H. meleagridis} with an inoculation of \textit{Eimeria tenella} (10$^4$ oocysts per bird) in broilers at 10 or 14 days of age has been shown to increase pathological lesions in the liver and cause growth stunting (McDougald and Hu, 2001). In this study, the coccidia vaccine given on day of hatch contained \textit{E. tenella} but no macroscopic lesions in the ceca were visually apparent in the control birds and damage from \textit{E. tenella} could not be observed in the infected treatment due to histomoniasis. Additionally, no difference in weight was found at the 5, 10 and 20 d post inoculation necropsies (data not shown). Differences seen in the current study compared to McDougald and Hu (2001) could be explained by the timing and dosage of \textit{Eimeria} administered. Similar to Welter (1960), our findings suggest that proper timing and dosage of vaccines do not exacerbate histomoniasis in breeders. Co-infection disease trials are necessary to conclude if specific pathogens worsen histomoniasis in chickens.

It is hypothesized that \textit{H. meleagridis} inhabits the cecal lumen, becoming part of the microflora for the duration of the chicken’s life (Lund, 1967). At termination, \textit{H. meleagridis} was isolated and grown in culture from the infected birds. The recovery of \textit{H. meleagridis} in almost half of the cecal samples collected from the infected birds supports this claim and indicates that the broiler breeder is an ideal host of this protozoan (van der Heijden and Landman, 2011). It should be noted that \textit{Blastocystis}, a protozoan commonly found in bird’s
intestines, was apparent in the cultures for both treatments (Grabensteiner and Hess, 2006). Generally, *Blastocystis* is an enteric endosymbiont with limited clinical relevance but this statement has been debated (Stensvold *et al*., 2009). It has also been documented to grow well in the media used for *H. meleagridis* (McDougald, 2005). From the current study, it is unknown when the breeders became contaminated with this protozoan. However, the ceca were not inflamed for either treatment during trial termination nor were there signs of gastrointestinal distress due to the protozoan presence. Therefore, the presence of *Blastocystis* most likely did not alter the outcomes from this experiment.

**Flock Growth and Uniformity** Infection with *H. meleagridis* has been reported to cause morbidity, altering BW and flock uniformity in chickens (Lund 1967; Gerth *et al*., 1985; McDougald and Hu, 2001; Esquenet *et al*., 2003). We hypothesized that histomoniasis leads to growth variation due to the inflammation and granuloma development in the ceca. This organ is utilized for immune processes and water and electrolyte absorption so when its functionality is altered by pathogens, bird growth can suffer (Clench and Mathias, 1995; Stephens and Hampson, 2001; Svihus, 2014). Differences in BW were observed only at trial termination, where the infected treatment weighed significantly less than the control (Figure 1). Although BW did not differ during rearing, the infected treatment had significantly poorer uniformity (Table 4). Disease states during rearing that cause poor flock uniformity often correlate with lasting effects on the uniformity of the flock (Abbas *et al*., 2010). This was not seen in the current study, potentially due to the selection process in the transfer of pullets to the laying facility. Greater variation in flock uniformity has been documented with layers suffering from *H. meleagridis* (Gerth *et al*., 1985; Esquenet *et al*., 2003). However, these studies are field reports with birds suffering from concurrent infections unlike the current controlled research experiment. Disease
signs only during rearing and not production could be why there are different growth outcomes between previous *H. meleagris* publications and the current study, however further investigations are necessary.

**Egg Production and Quality** To date, no studies have analyzed the effects of *H. meleagris* on the egg production and quality of broiler breeder pullets. Poor uniformity is associated with variation in the hen sexual maturity, where underweight pullets have a delayed onset of egg production and altered egg quality traits (Abbas *et al.*, 2010). The inoculation during rearing in this study correlated with variation in treatment body weight uniformity but there was no delay in the onset of lay. During one period, the infected produced more eggs than the control (Figure 2). This could be due to the lighter BW of the infected treatment, where heavier hens can have lower egg production (Abbas *et al.*, 2010). The increase in the number of eggs produced per treatment in the current study differ from Gerth (1985) and Liebhart (2013) most likely because of the timing of the infection, where the previous publications had birds suffering from blackhead disease during lay while the current study was during rearing. Like Sigmon (2019), inoculating pullets during rearing did not have a negative effect on egg production. Favorable physical qualities of the eggs were also periodically identified with the infected treatment throughout this experiment (Table 5). To the authors’ knowledge, this is the first study to analyze breeder pullet hatchability and fertility changes due to *H. meleagris* and no effects (*P > 0.05*) were seen (Table 6). Long term benefits of early acute stressors in poultry have correlated with better overall health and performance of the bird (Zulkifili and Siegel, 1995; Smit *et al.*, 1998). In the current study, the *H. meleagris* stressor only during rearing and not production supports this association. Inoculation of only this parasite during lay is necessary to determine there is an effect on older broiler breeders’ egg production and quality.
Based on the recovery of *H. meleagridis* over a year after inoculation, it can be inferred that the broiler breeder is an ideal host for this protozoan and supports the claim that chickens become carriers for life (van de Heijden and Landman, 2011). Breeder pullets have pathological lesions the first few weeks of infection, but mortality was not observed. Negative effects on egg production and quality were not observed when pullets were infected at four weeks of age suggesting that inoculation at this time could offer protection against blackhead outbreaks during the laying period.
Figure 3.1. Average body weight. The average pullet body weight for each treatment for various time points during rearing as well as trial termination presented as the mean body weight ± SEM. An asterisk (*) above the bars indicate statistical differences found between treatments at a specific time period (P ≤ 0.05).
Figure 3.2. Average egg production. The average eggs produced per hen per period within each treatment group ± SEM. An asterisk (*) above a time period indicates statistical differences found between treatments (P ≤ 0.05).
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<th>5% production to 35 wk</th>
<th>35 to 50</th>
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</table>

1 Trace minerals provided per kg of premix: manganese (Mn SO₄), 60 g; zinc (ZnSO₄), 60 g; iron (FeSO₄), 40 g; copper (CuSO₄), 5 g; iodine (Ca(IO₃)₂), 1.25 g.
2 Vitamins provided per kg of premix: vitamin A, 13,227,513 IU; vitamin D3, 3,968,253 IU; vitamin E, 66,137 IU; vitamin B12, 39.6 mg; riboflavin, 13,227 mg; niacin, 110,229 mg; d-pantothenic acid, 22,045 mg; menadione, 3,968 mg; folic acid, 2,204 mg; vitamin B6, 7,936 mg; thiamine, 3,968 mg; biotin, 253.5 mg.
3 Quantum Blue 5G®, 80 g/ton to supply 1.000 FYT (AB Vista) delivering 0.11% of non-phytate phosphorus P, and 0.10% of calcium.
### Table 3.2. Nutrient composition throughout the rearing and laying periods

<table>
<thead>
<tr>
<th>Age fed (wk)</th>
<th>0 to 3</th>
<th>3 to 5</th>
<th>5 to 19</th>
<th>19 to 5% production</th>
<th>5% production to 35 wk</th>
<th>35 to 50</th>
<th>50 to study termination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient</td>
<td>Starter</td>
<td>Grower</td>
<td>Layer Pre-lay</td>
<td>Layer 1</td>
<td>Layer 2</td>
<td>Layer 3</td>
<td>Layer 3</td>
</tr>
<tr>
<td>ME, kcal/kg</td>
<td>2.767</td>
<td>2.653</td>
<td>2.600</td>
<td>2.700</td>
<td>2.800</td>
<td>2.800</td>
<td>2.800</td>
</tr>
<tr>
<td>CP, %</td>
<td>19.43</td>
<td>16.26</td>
<td>14.58</td>
<td>16.00</td>
<td>15.00</td>
<td>14.40</td>
<td>14.30</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.85</td>
<td>0.85</td>
<td>0.80</td>
<td>1.05</td>
<td>2.85</td>
<td>3.05</td>
<td>3.25</td>
</tr>
<tr>
<td>Total P, %</td>
<td>0.79</td>
<td>0.84</td>
<td>0.88</td>
<td>0.73</td>
<td>0.58</td>
<td>0.56</td>
<td>0.54</td>
</tr>
<tr>
<td>NPP, %</td>
<td>0.50</td>
<td>0.45</td>
<td>0.42</td>
<td>0.35</td>
<td>0.35</td>
<td>0.33</td>
<td>0.32</td>
</tr>
<tr>
<td>Total Lys, %</td>
<td>1.08</td>
<td>0.78</td>
<td>0.64</td>
<td>0.76</td>
<td>0.74</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Total Trp, %</td>
<td>0.25</td>
<td>0.22</td>
<td>0.20</td>
<td>0.21</td>
<td>0.18</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>Total Thr, %</td>
<td>0.74</td>
<td>0.59</td>
<td>0.52</td>
<td>0.60</td>
<td>0.60</td>
<td>0.57</td>
<td>0.56</td>
</tr>
<tr>
<td>Total Val, %</td>
<td>0.93</td>
<td>0.78</td>
<td>0.68</td>
<td>0.75</td>
<td>0.71</td>
<td>0.68</td>
<td>0.68</td>
</tr>
<tr>
<td>Total Arg, %</td>
<td>1.30</td>
<td>1.08</td>
<td>0.95</td>
<td>1.05</td>
<td>0.97</td>
<td>0.92</td>
<td>0.92</td>
</tr>
<tr>
<td>Total SAA, %</td>
<td>0.82</td>
<td>0.63</td>
<td>0.59</td>
<td>0.69</td>
<td>0.67</td>
<td>0.64</td>
<td>0.63</td>
</tr>
<tr>
<td>Dig Lys, %</td>
<td>0.96</td>
<td>0.66</td>
<td>0.52</td>
<td>0.64</td>
<td>0.65</td>
<td>0.62</td>
<td>0.62</td>
</tr>
<tr>
<td>Dig Met, %</td>
<td>0.47</td>
<td>0.31</td>
<td>0.28</td>
<td>0.38</td>
<td>0.39</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>Dig Cys, %</td>
<td>0.27</td>
<td>0.24</td>
<td>0.22</td>
<td>0.23</td>
<td>0.22</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Dig SAA, %</td>
<td>0.74</td>
<td>0.54</td>
<td>0.50</td>
<td>0.61</td>
<td>0.60</td>
<td>0.57</td>
<td>0.57</td>
</tr>
<tr>
<td>Dig Thr, %</td>
<td>0.63</td>
<td>0.48</td>
<td>0.41</td>
<td>0.49</td>
<td>0.51</td>
<td>0.48</td>
<td>0.48</td>
</tr>
<tr>
<td>Dig Trp, %</td>
<td>0.22</td>
<td>0.19</td>
<td>0.17</td>
<td>0.18</td>
<td>0.15</td>
<td>0.15</td>
<td>0.14</td>
</tr>
<tr>
<td>Dig Iso, %</td>
<td>0.73</td>
<td>0.55</td>
<td>0.45</td>
<td>0.54</td>
<td>0.55</td>
<td>0.53</td>
<td>0.53</td>
</tr>
<tr>
<td>Dig Leu, %</td>
<td>1.48</td>
<td>1.18</td>
<td>0.99</td>
<td>1.16</td>
<td>1.22</td>
<td>1.18</td>
<td>1.18</td>
</tr>
<tr>
<td>Dig Val, %</td>
<td>0.81</td>
<td>0.66</td>
<td>0.56</td>
<td>0.64</td>
<td>0.62</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>Dig Arg, %</td>
<td>1.20</td>
<td>0.96</td>
<td>0.83</td>
<td>0.93</td>
<td>0.89</td>
<td>0.84</td>
<td>0.84</td>
</tr>
<tr>
<td>Sodium, %</td>
<td>0.20</td>
<td>0.16</td>
<td>0.17</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>Potassium, %</td>
<td>0.81</td>
<td>0.63</td>
<td>0.50</td>
<td>0.60</td>
<td>0.62</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>Chloride, %</td>
<td>0.22</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.24</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>DEB, mEq/100 g</td>
<td>240</td>
<td>182</td>
<td>150</td>
<td>180</td>
<td>170</td>
<td>170</td>
<td>170</td>
</tr>
</tbody>
</table>
Table 3.3. Identification of *H. meleagris* using gross pathology and microscopy

<table>
<thead>
<tr>
<th>Days post-inoculation</th>
<th>Treatment</th>
<th>n</th>
<th>Average Body Weight (kg)</th>
<th><em>Histomonas meleagris</em> Positive$^a$ (%)</th>
<th>Ceca score</th>
<th>Liver score</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>control</td>
<td>80</td>
<td>0.62</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>infected</td>
<td>80</td>
<td>0.59</td>
<td>80</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>control</td>
<td>80</td>
<td>0.68</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>infected</td>
<td>80</td>
<td>0.65</td>
<td>90</td>
<td>3</td>
<td>0.2</td>
</tr>
<tr>
<td>20</td>
<td>control</td>
<td>40</td>
<td>0.84</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>infected</td>
<td>40</td>
<td>0.77</td>
<td>100</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>420*</td>
<td>control</td>
<td>40</td>
<td>4.29</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>infected</td>
<td>40</td>
<td>4.17</td>
<td>45</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

n= number of birds sampled for that treatment during that time period

An asterisk (*) beside days post inoculation indicates statistical differences found between treatments for BW at a specific time period ($P \leq 0.05$)

Alpha ($\alpha$) refers to the two methodologies used to determine if samples were positive for *Histomonas meleagris*. On 5, 10, and 20 d post inoculation, birds were subjectively scored and any bird with *H. meleagris* disease signs was considered positive. On 420 d post inoculation, samples were considered positive if *H. meleagris* cells were observed under light microscopy in culture.
Table 3.4. The flock uniformity presented as the coefficient of variation for multiple time periods during rearing and at trial termination

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>Treatment</th>
<th>Coefficient of variation (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>infected</td>
<td>12.6</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>11.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>infected</td>
<td>12.8</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>infected</td>
<td>12.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>10.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>infected</td>
<td>13.9</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>infected</td>
<td>14.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>11.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>infected</td>
<td>10.9</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>9.8</td>
<td></td>
</tr>
</tbody>
</table>

Values are means of four pens (replicates) per treatment

<sup>a</sup>-<sup>b</sup> Means in a column not sharing a common superscript are significantly different (P<0.05) by Student’s t test
Table 3.5. Treatment effects on physical qualities of the eggs produced throughout lay

<table>
<thead>
<tr>
<th>Age of hens (wk)</th>
<th>Shell strength (g force)</th>
<th>Average shell thickness (mm)</th>
<th>Egg weight (grams)</th>
<th>Haugh Units</th>
<th>Vitelline membrane strength (g force)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>infected 4608.8</td>
<td>0.34</td>
<td>58.78</td>
<td>92.5</td>
<td>2.26</td>
</tr>
<tr>
<td></td>
<td>control 4553</td>
<td>0.338</td>
<td>56.67</td>
<td>94.1</td>
<td>2.36</td>
</tr>
<tr>
<td></td>
<td>SEM 180</td>
<td>0.005</td>
<td>0.69</td>
<td>0.83</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>P-value 0.83</td>
<td>0.81</td>
<td>0.04</td>
<td>0.17</td>
<td>0.45</td>
</tr>
<tr>
<td>34</td>
<td>infected 3800</td>
<td>0.335</td>
<td>60.23</td>
<td>87.2</td>
<td>1.97</td>
</tr>
<tr>
<td></td>
<td>control 4177.9</td>
<td>0.339</td>
<td>59.43</td>
<td>84.5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>SEM 142</td>
<td>0.005</td>
<td>0.83</td>
<td>1.17</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>P-value 0.07</td>
<td>0.65</td>
<td>0.5</td>
<td>0.11</td>
<td>0.81</td>
</tr>
<tr>
<td>39</td>
<td>infected 4297.8</td>
<td>0.341</td>
<td>61.33</td>
<td>87.3</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td>control 3954.8</td>
<td>0.339</td>
<td>60.62</td>
<td>87.6</td>
<td>2.04</td>
</tr>
<tr>
<td></td>
<td>SEM 154</td>
<td>0.005</td>
<td>0.74</td>
<td>1.63</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>P-value 0.12</td>
<td>0.8</td>
<td>0.5</td>
<td>0.92</td>
<td>0.9</td>
</tr>
<tr>
<td>42</td>
<td>infected 4223.7</td>
<td>0.352</td>
<td>63 ± 0.90</td>
<td>83.5</td>
<td>2.29</td>
</tr>
<tr>
<td></td>
<td>control 3842.6</td>
<td>0.334</td>
<td>63 ± 0.92</td>
<td>84.1</td>
<td>2.03</td>
</tr>
<tr>
<td></td>
<td>SEM 135</td>
<td>0.005</td>
<td>1.4</td>
<td>0.1</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>P-value 0.05</td>
<td>0.03</td>
<td>0.99</td>
<td>0.74</td>
<td>0.06</td>
</tr>
<tr>
<td>56</td>
<td>infected 3987.2</td>
<td>0.349</td>
<td>70</td>
<td>83.5 ± 1.4</td>
<td>2.69</td>
</tr>
<tr>
<td></td>
<td>control 4265</td>
<td>0.349</td>
<td>70</td>
<td>80.1 ± 1.2</td>
<td>2.34</td>
</tr>
<tr>
<td></td>
<td>SEM 162</td>
<td>0.006</td>
<td>0.93</td>
<td>0.81</td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td>P-value 0.23</td>
<td>0.99</td>
<td>0.57</td>
<td>0.21</td>
<td>0.049</td>
</tr>
</tbody>
</table>

A significant difference between treatment groups was considered if $P \leq 0.05$

1SEM = standard error of the mean
Table 3.6. Hatchability and fertility of each treatment group and different time periods during lay

<table>
<thead>
<tr>
<th>Wk</th>
<th>Treatment</th>
<th>Hatched eggs (%)</th>
<th>Infertile eggs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>infected</td>
<td>89.65</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>91.93</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.19</td>
<td>0.72</td>
</tr>
<tr>
<td>37</td>
<td>infected</td>
<td>85.05</td>
<td>2.51</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>83.61</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.84</td>
<td>0.99</td>
</tr>
<tr>
<td>63</td>
<td>infected</td>
<td>68.40</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>83.61</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.39</td>
<td>-</td>
</tr>
</tbody>
</table>

Significance was considered if $P \leq 0.05$
REFERENCES


Hess, M., E. Grabensteiner, and D. Liebhart. 2006. Rapid transmission of the protozoan parasite Histomonas meleagridis in turkeys and specific pathogen free chickens following cloacal infection with a mono-eukaryotic culture. Avian Pathol. 35:280-285. doi 10.1080/03079450600815507


CHAPTER 4

TWO BLACKHEAD DISEASE OUTBREAKS IN COMMERCIAL TURKEY FLOCKS WERE POTENTIALLY EXACERBATED BY POOR POULT QUALITY AND COCCIDIOSIS

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Case report accepted by Avian Diseases
SUMMARY

Field visits at two different farms suggest a correlation between commercial turkey flocks having increased mortality from blackhead disease (histomoniasis) if they suffered from poor poult quality at placement and coccidiosis prior to 6 weeks of age. Both cases were all-in/all-out flocks with curtain sided houses and received a coccidiosis vaccine on day of hatch. *Farm I, 2018*: Poult’s from different hatcheries were placed in 2 houses on the same farm (Houses 1 and 2). House 2 had poult’s considered poor quality and suffered from mortality associated with coccidiosis at 2 and 4 wks of age. At 8 wks, blackhead disease was diagnosed in both houses based on necropsies. House 2 had mortality of over 2,000 poult’s and all necropsied had gross lesions characteristic of histomoniasis. Gross lesions associated with blackhead disease were only found in 8 poult’s in House 1, which was populated with good quality poult’s and did not have mortality associated with coccidiosis. *Farm II, 2020*: Poult’s were delivered from the same hatchery onto a 3-house farm (Houses A, B, and C). House C had poult’s that were considered poor quality and had mortality associated with coccidiosis at 3 wks of age. At 8-9 wks, House C had mortality approached 1,000 birds with all necropsied poult’s having clinical signs of blackhead disease. Houses A and B had no mortality with clinical signs of blackhead disease. The similarity of these two cases suggest that poult quality at placement coupled with coccidiosis prior to 6 wks of age can influence the severity of blackhead disease in commercial turkey flocks.

Key words/index terms: Histomoniasis, coccidiosis, *Histomonas meleagridis*, *Eimeria*, turkey
INTRODUCTION

Poor poult quality is a well-known issue in the turkey industry but is currently neglected in research even though it influences overall performance of the poult. Various factors that occur between hatching and placement lead to early poult mortality and other performance issues (Carver, et al., 2002). A stressor that is commonly experienced between hatching and placement is extended transport time and has been mimicked in an experimental study. This stressor caused a delay in the poult’s intestinal development leading to issues in immunity, nutrient absorption and microbiome populations (Potturi et al., 2005). These issues can contribute to pathogen sensitivity as the birds are reared.

Coccidiosis, caused by Eimeria species of coccidia, and Histomoniasis (blackhead disease), caused by Histomonas meleagrisidis, are two protozoal parasitic diseases of economic importance in the poultry industry (McDougald, 1998). Eimeria adenoides, a pathogenic species of coccidia, will parasitize the ceca of their host, overlapping with the site of entry for H. meleagrisidis (Chapman, 2008; McDougald, 2013). It is common to find various coccidia species and vectors of Heterakis gallinarum, the intermediate host for H. meleagrisidis, in commercial poultry production (McDougald and Hu, 2001; Cupo and Beckstead, 2019). Thus, it is likely that infections of both protozoa could occur during rearing. A study of concurrent infections with E. tenella and H. meleagrisidis suggested that clinical effects of histomoniasis in chickens were enhanced when both parasites were given in conjunction (McDougald and Hu, 2001). In turkeys, a similar effect has been speculated but there are no published observations on this interaction.

During field visits to two commercial poultry farms, records indicated a possible connection between poult quality and coccidiosis, leading to increased severity of histomoniasis.
CASE REPORT

Two farms in North Carolina were reared organic and were all-in/ all-out with no transfer of poult from their house once placed. Poults were given a live coccidiosis vaccine containing *E. adenoides* and *E. meleagrimitis* at the hatchery. Litter at both farms had low moisture content and caking before and after the outbreaks of coccidiosis and histomoniasis. During the visits, limited debris and very little plant overgrowth were observed outside of the curtain-sided houses. In both cases, this was the first blackhead disease outbreak on the farms.

**Farm I, February 2018** A blackhead disease outbreak was reported on a farm of two houses (House 1 and House 2) with approximately 5,300 poult placed per house. Poults from each house were from different hatcheries and the farmer noted that poult quality in House 2 was poor. Spikes of mortality at 2 and 4 wks of age in House 2 were diagnosed as coccidiosis, but there were no similar spikes in House 1 (Fig. 1). As a precaution, copper sulfate was added to the drinking water. At 8 wks of age, mortality spiked again in House 2, with a diagnosis of histomoniasis. Extensive culling was done in both houses to remove birds potentially sick with *H. meleagridis*. Of 200 birds removed from House 1, only 8 were found to have lesions associated with blackhead disease at necropsy. In contrast, 2,369 dead or culled poult from House 2 had gross lesions or displayed clinical signs of blackhead disease.

**Farm II, February 2020** Poults from one hatchery were delivered to a farm that had three turkey houses (A, B and C) and approximately 3,700 poult were placed in each house. In relation to poult quality, House C was described by the farmer to have poult of poor or questionable quality while A and B had no such concern. At 3 wks of age, mortality in House C spiked due to coccidiosis while mortality spikes were not observed in Houses A or B (Fig. 2). The farmer remarked that House C was more vocal than Houses A and B through grow-out. At 8-9 wks of
age, House C had a mortality spike and upon necropsy, the flock was diagnosed with histomoniasis with peak mortality reaching 964 poults. Houses A and B did not have an increase in mortality during grow-out and none of the necropsied birds had clinical signs of blackhead disease.

**DISCUSSION**

In these two examples of coccidiosis and blackhead disease outbreaks, both were associated with poor poul quality at placement. Other birds on the same farms, considered normal at placement, did not suffer such outbreaks. Although not well studied, hatchery and transportation risk factors have been correlated with poul morbidity and mortality. These can include, but are not limited to: breeder hen age, season of the year, shipping time, truck temperature, and disease (Carver *et al.*, 2002). Poults delivered from different hatcheries in the 2018 case could have led to the differences observed in poul quality. However, this cannot be assumed for the 2020 case, where poults came from the same hatchery. The farmer in the 2020 case did report increased vocalization with House C during the first 6 wks of rearing, a common behavior of birds suffering from coccidiosis (Chapman, 2008).

Turkey coccidiosis is common in commercial production and it can be controlled by vaccination. Issues with commercially applied live vaccines can include problems with the administration technique or vaccine viability leading to vaccine failure. Poor initial vaccination could result in some birds receiving a high dose, while others receive a low dose or no vaccine. This can result in a ‘rolling vaccine reaction’ with clinical disease. Instability of the vaccine to environmental factors could alter its efficacy due to coccidial oocysts being sensitive to temperature. Improper handling could decrease the vaccine’s effectiveness (Soutter *et al.*, 2020).
In this case, natural coccidiosis outbreaks are possible, through naïve turkeys consuming litter or mechanical vectors contaminated with the prevalent wild types of coccidia found on a farm.

Another organism that can be found in poultry litter or on mechanical vectors is *Heterakis gallinarum*, the only known carrier of *H. meleagridis*. Consumption of *H. meleagridis*-infected ova of *Heterakis gallinarum* introduces blackhead disease to a turkey flock (Cupo and Beckstead, 2019). Once introduced, *H. meleagridis* will spread horizontally among poults and without the need for other carriers or vectors (McDougald, 2013). As in the presented cases herein, the means of *H. meleagridis* introduction is unknown but may involve vectors such as insects or other invertebrates that bring *Heterakis gallinarum* eggs into a poultry house. Precipitation encourages *Heterakis gallinarum* vector migration (Cupo and Beckstead, 2019). The increased precipitation that occurred at a time similar to the potential introduction of *Heterakis gallinarum* suggests the entry of these vectors started the blackhead disease outbreak for both farms in the current case report.

Previous workers suggested that damage caused by coccidiosis could increase susceptibility of the poult to other pathogens (Milbradt et al., 2014). During invasion and replication of coccidial stages, intestinal villi are damaged. Enteritis and sloughing of the epithelium throughout the intestine can occur with *E. meleagridis* while *E. adenoides* causes cecal mucosal surface damage, exposing the basement membrane of the cecal wall (Madden and Ruff, 1979; Chapman, 2008). With cecal tissue irritation and altered gut bacterial populations caused by coccidia, *H. meleagridis* can more easily migrate into the cecal tissue then spread to the liver (McDougald and Hu, 2001). The *H. meleagridis* – related enterohepatitis has been suggested as the key determinant for mortality for poultry suffering from blackhead disease (McDougald, 2013).
In both cases reported, the severity of blackhead disease seems to be exacerbated by poult quality and previous enteritis due to coccidiosis. The lack of the two previously mentioned factors for House 1 in 2018 with only 8 poults suffering with blackhead disease further strengthens the hypothesis that poult quality can influence disease susceptibility and horizontal transmission. The unfortunate timing of high precipitation increased the likelihood of a vector bringing *H. meleagris* into the houses. If a turkey flock with poor health at placement suffers from coccidiosis prior to 6 wks of age, it is suggested to necropsy should be performed on all other mortality the following weeks. This could help identify the index case of blackhead disease and decrease the severity of this disease or prevent an outbreak.
**Figure 4.1.** Mortality over a 9-week period of the 2018 case starting with placement in Houses 1 and 2. The spikes in mortality correlating with bad cocci cycling in House 2 and the potential indicators of initial *H. meleagridis* infection and the blackhead disease outbreaks in both houses are shown.
Figure 4.2. Mortality over a 9-week period of the 2020 case starting with placement of Houses A, B, and C. The spikes in mortality correlating with bad cocci cycling, the potential indicators of initial *H. meleagrisidis* infection and the blackhead disease outbreaks in House C are shown.
REFERENCES


CHAPTER 5
CONCLUSION

The development and efficacy of protozoal management programs in poultry production is based on the economic significance of the type of poultry and clinical significance of the protozoa. For broilers, multiple coccidia vaccines are available commercially due to the economic impact of this type of domesticated poultry. The next step for cocci management in broiler production is determining antibiotic-free feed additives that alleviate the negative effects associated with clinical coccidiosis. The presented broiler research indicates that the use of sodium bisulfate, a feed additive, can be added to the broiler diet to help maintain intestinal health. When broilers were challenged with an increased dosage of a commercial coccidia vaccine, the sodium bisulfate treatment resulted in broilers with greater body weight, improved villi structure and less gut leakage compared to the infected control treatment. Because the broilers were grown in ideal temperature conditions, future work with sodium bisulfate in the field should concentrate on a coccidiosis challenge with an environmental stress to determine effects on broilers in a commercial setting. Future work in the lab should determine sodium bisulfate’s mechanism in aiding intestinal health. With longer-lived poultry, *H. meleagris* is a protozoal parasite of concern where chickens suffer from morbidity while turkeys suffer from mortality. It is unknown when introduction of *H. meleagris* to a chicken flock is appropriate due to the secondary effects the chicken’s immune response can have on the flock’s performance. The broiler breeder trial indicated that the *H. meleagris* introduction should occur prior to the laying period. Pullets infected with *H. meleagris* and suffered from blackhead disease during rearing led to limited effects on egg production and quality, unlike other studies which *H. meleagris* infection during lay caused production losses. With turkeys, the efficacy of
the only available coccidia vaccine needs further research, especially if poult's are of poor quality when delivered to the farm. The turkey case report highlighted that poor poult quality and improper cocci cycling leads to increased susceptibility to blackhead disease. The data presented through this dissertation emphasizes that production and animal welfare issues due to protozoal parasites can be prevented or alleviated based on intestinal health and maturity. This was emphasized using multiple types of domestic poultry with two different protozoa.