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ADDIS ABABA UNIVERSITY, COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE, DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

EXPERIMENTAL STUDY ON PATHOLOGICAL CHANGES AND PERFORMANCE VARIATIONS IN CHICKENS CHALLENGED BY *EIMERIA TENELLA* UNDER DIFFERENT GENOTYPE, AGE AND NUTRITIONAL STATUS

BY:

MISGANA TEFERA ABOMA

MVSC PROGRAM IN VETERINARY PATHOLOGY

JUNE, 2022

BISHOFTU, ETHIOPIA

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Signature and Approval Sheet

Addis Ababa University

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As a member of the Board of Examiners of the MSc. Thesis open defense, we certify that we have read and evaluated the Thesis prepared by Misgana Tefera Aboma entitled "**Experimental study on pathological changes and performance variations in Chickens challenged by *Eimeria tenella* under different genotype, age and nutrition status**'' and examined the candidate. We recommend that the Thesis be accepted as fulfilling the Thesis requirements for the degree of Master of Science in Veterinary Pathology.

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By my signature below, I declare and affirm that the thesis is my own work and all material used for this Thesis has been given acknowledged through citation. I solemnly declare that it has not been previously submitted to any other University or institution for the award of any academic degree, diploma, or certificate. This thesis is submitted in partial fulfillment of the requirements for a Master of Science degree (MSc.) at the Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library.

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LIST OF ACRONYMS/ABBREVIATIONS

ASL	Above sea level
ANOVA	Analysis of variance
AWG	Average weight gain
CSA	Central statistics authority
DNA	Deoxyribonucleic acid
EPG	Eggs per gram
ELISA	Enzyme-linked immunosorbent assay
FCR	Feed conversion ratio
FAO	Food and agriculture organization
GIT	Gastrointestinal tract
IACUC	Institutional animal care and use committee
ILRI	International livestock research institute
LST	Lesion scoring technique
µm	Micrometer
NAHDIC	National animal health diagnostic and investigation center
PCR	Polymerase chain reaction
PI	Post-infection
ITS	ribosomal DNA internal transcribed spacer
rRNA	Ribosomal ribo nueclic acid

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ABSTRACT

Eimeria tenella is a Protozoa which is a causative agent of avian coccidiosis. The disease cause bleeding in the cecum, diarrhea, high morbidity and mortality in chickens. The experimental study was conducted from November 2020 to January 2022 at the ILRI poultry research facility in Addis Ababa. First experiment was aimed to investigate the effect of *Eimeria* infection at different feeding regimes on chicken pathological changes and productivity diversity in adult Sasso T451A and Bovans brown breeds. Chickens were infected with 1 x 10^4 oocysts of *Eimeria tenella*. Three factors; feeding regimes, challenges level and breeds of chicken were seen to evaluate the effects of parasite. The results showed that Bovans brow feeds deficient infected group had significantly higher ($P < 0.05$) gut lesions as compared to the control group. Production performance of feed restricted groups was significantly low as compared to optimum feeding but there was no significantly difference ($P < 0.05$) in production performance between infected and control groups. The second experimental study investigated oocysts output, lesion scores, PCV, pathological changes and production performance following artificial intubation of 3 x 10^4 of *Eimeria tenella* oocysts. The results show that lesion scores in Sasso was significantly higher ($P < 0.05$) than Bovans brown on day 4 post-infection. PCV of infected chicken was significantly ($P < 0.05$) decreased at day 8 post-infection in both breeds, similarly oocyst count and lesion score in the infected group of chicken was higher at day 8 than day 4 post-infection, this indicate that the infection was more severe at day 8 post-infection. Histopathological examination result of infected chicken by *E. tenella* was observed with infiltration of inflammatory cell in the cecum tissue. Therefore, the current result showed that the effect of *E. tenella* on infected chicken intestine had developed pathological lesions than non-infected groups of chickens.

Key Words: Coccidia, *E. tenella*, Bovans brow breed. Sasso T451A breed, young, optimum feeding, deficient feeding, infection, lesion score, oocyst count

1. INTRODUCTION

In Ethiopia, poultry production is an important activity for immediate cash generation and source of quality protein (Gebeyeh and Yizengaw, 2017). Broadly three poultry production systems prevail in the country rearing millions of flocks of chicken namely, traditional, small scale and large-scale commercial farms (Tadelle *et al.,* 2003). The Traditional types of farming are grounded on indigenous chicken types, and scavenging management systems whereas the small and large-scale production systems are defined by more intensively managed exotic chicken breeds (Duguma *et al.,* 2005; Hailu, 2012). Across all the production systems the level of productivity does not commensurate with the number of chickens and the demand for poultry products. Sub-optimal productivity prevails in the country due a number of technical and non-technical problems (Gebeyeh and Yizengaw, 2017).

Infectious diseases such as coccidiosis have been known to contribute to sub-optimal production (Gebeyeh and Yizengaw, 2017). It was reported in various continents and countries of the world including China (Sun *et al*., 2009), Ethiopia (Gari *et al*., 2008), Europe (Williams *et al*., 1996), Netherlands (Graat *et al*., 1988), North and South America (Mattiello *et al*., 2000), Indian (Bera *et al.*, 2010) and Turkey (Akcay *et al*.,2011). It is caused by a protozoan parasite belonging to the genus Eimeria. It is the most commonly encountered and economically important disease adversely affecting poultry production and causing great economic losses worldwide (Abdisa *et al*., 2019). The economic losses amounting to over 3billion US$ has been documented in the poultry industry. The major part of the financial loss was accountable to in-feed and medication for prevention and treatment. In addition, financial losses due to mortality, mal absorption, inefficient feed utilization and impaired growth rate in broilers, as well as a temporary reduction of eggs production in layers has been published (Dalloul and Lillehoj, 2006). The financial impact of prophylaxis and estimates of production losses due to disease put the costs of coccidiosis to the poultry industry in Great Britain to at least £38 million per annum (Williams, 1999). Although studies on the economic losses are lacking in Ethiopia, previous field investigations showed that coccidiosis was identified as a cause of direct and indirect losses in all Ethiopian farms.

Average losses due to culling, mortality and coccidiostat cost was estimated that the economic losses identifying costs of 0.55 and 0.53 Birr per chicken in small and large scale farms, respectively, in Ethiopia (Kinunghi *et al*., 2004). Losses occurred in the form of mortalities, coccidiostats cost, reduced weight gains, the reduced market value of affected birds, culling, delayed egg laying and reduce egg production. Proportional mortality rates due to coccidiosis were 14.5% and 13.3% in small-scale and large-scale poultry farms, receptively (Habtamu and Gebre, 2019)

Apicomplexan parasites of the genus *Eimeria* are organisms that invade the intestinal tract, causing coccidiosis, an enteric disease of major economic importance worldwide. The disease causes high morbidity ranging from acute, bloody enteritis with high mortality, to subclinical disease. However, the presence of intestinal lesions depends on the *Eimeria* species. *Eimeria* species causing diseases to poultry are: *E. tenella, E. necatrix, E.acervulina, E. maxima, E. brunetti, E. mitis*, and *E. praecox*. The differences between these *Eimeria* species include the invasion of specific sites of the intestine, pathogenicity and the type of lesion produced (López-Osorio *et al*., 2020). The most common and pathogenic species that affect the poultry is Emeria tenella, resulting in 100% morbidity and high mortality. In Ethiopia, *E. acervulina, E. necatrix, E.maxima,* and *E. tenella* are endemic in all parts of the country and affect mainly young growing birds (Wondimu *et al*., 2019). The severity and occurrence of clinical coccidiosis are related to several factors like host, agents, management and environment interaction. Detection of an oocyst in feces and postmortem examination of intestinal lesions are the most important procedures for the diagnosis of coccidiosis in chickens (Wondimu *et al*., 2019).Coccidiosis is largely a disease of young birds because immunity is low and it quickly develops after exposure and gives protection against later disease outbreaks. However, birds can be infected at any age if not exposed earlier. Chickens get the infection by eating oocysts once these are sporulated after being shed in the droppings of infected birds. They pick them up by pecking on the ground or in litter used for bedding in the house. Oocysts can also be spread by insects, dust, wild birds and humans through shoes and equipment. Outbreaks of coccidiosis usually occur when birds are between 3 and 8 weeks of age (Gupta, 2009).

A vaccine composed of parasite antigens and antigen encoding genes targeted to elicit specific immunity is eminently preferable but not available for field use. Therefore, introduction of alternative prevention and treatment methods such as feed supplements to simultaneously increase productivity and stimulated non-specific immunity is taught to reduce dependence on coccidiostats (Dalloul *et al*., 2005). Such approaches require the understanding of the pathological changes induced by the *Eimeria* species. Since the biological features of the *Eimeria* parasite are highly specific, the prepatent period, development within the intestine, type of lesion and the morphological appearance of various endogenous stages in the mucosa of the intestine and the lesion scores vary depending on the strain and the resistance of the host. The application of the lesion scoring technique (LST) gained momentum for confirmation and accurate diagnosis of chicken coccidiosis and monitoring of the pathogenicity. Assessment of the virulence and pathogenicity of *Eimeria* species are vital factors for formulating effective control strategies (Raman *et al*., 2011). Therefore, it was hypothesized that plane of nutrition, age and breed affects the production and pathological responses of chicken to coccidian infection.

Objective:

The main objective of this experimental study was to investigate the body weight, egg laying, hematological and pathological responses of chicken following exposure to experimental *Eimeria tenella* infection.

The specific objectives were:

- Assess the effect of chicken breed and age on their responses to *Eimeria tenella* infection
- Investigate the role of plane of nutrition on production performance and pathological response of chicken to *Eimeria tenella* infection

2. LITERATURE REVIEW

2.1 General Description on Avian Coccidiosis

The term 'coccidiosis' can be used to describe clinical manifestations of disease arising from infection with any of the coccidia species (Clark and Blake, 2012). Poultry Coccidiosis is recognized as the major parasitic disease of chickens caused by protozoan parasites of the genus *Eimeria* and it is one of the major diseases of commercial poultry production and causes huge financial losses to the poultry industry every year (Meteab *et al*., 2021). It seriously impairs the growth and feeds utilization of infected birds resulting in loss of productivity (Engidaw and Getachew, 2018). *Eimeria* may lead to massive epithelial destruction. Consequently, the host may suffer with diarrhea, mal-absorption and poor weight gain (Habtamu and Gebre, 2019).

Avian Coccidiosis causes massive destruction of the epithelial cells and characterized by diarrhea, enteritis, drooping wings, paleness of the comb, poor growth and occasional appearance of blood in droppings. The death rate can be quite high in young chickens. Poor management practices, like wet litter and excessive stocking density, can exaggerate the symptoms (Engidaw and Getachew, 2018). The tissue damage can also expose the bird to bacterial infections, like Clostridium and Salmonella. Diseases that suppress the bird's immune system may help with coccidiosis to produce a more severe problem. For example, Marek's Disease and Infectious Bursal Disease may interfere with the development of coccidiosis immunity and intensify a coccidia infection (Vermeulen *et al*., 2001).

2.2 Etiology

Avian coccidiosis is caused by the intracellular protozoan parasite of *Eimeria* species in the kingdom Protozoa, phylum Apicomplexa, class Coccidia, order Eucoccidiorida, family Eimeridae and genus *Eimeria* (Engidaw and Getachew, 2018).

The coccidia of the genus *Eimeria* are predominately host-specific; i.e., each species occurs in a single host species or a group of closely related hosts (Conway and Mckenzie, 2007).

Nine *Eimeria* species are identified as causative agents of poultry coccidiosis but only seven of them have been recognized to infect chickens. The seven species of *Eimeria* that infect chicken are *Eimeria tenella, Eimeria necatrix, Eimeria brunetti, Eimeria maxima Eimeria acervulina* and *Eimeria mivati* (Gupta, 2009), all of them can causes disease, but the clinical signs vary according to the species, and the pathogenicity range from mild to severe intestinal or cecal infection (Williams, 1999). *Eimeria mitis, Eimeria praecox* and *Eimeria hagani* are relatively non-pathogenic species or do not cause significant lesions (Engidaw and Getachew, 2018), whereas *E. necatrix* and *E. tenella* are highly pathogenic and may cause heavy mortality (Williams, 1999). Of this species *E. acervulina*, *E. necatrix, E. maxima* and *E. tenella* which affect mainly young growing birds are considered to be endemic in all parts of Ethiopia (Habtamu and Gebre, 2019). Eimeria species that infect chickens and the site of development in the intestine of chickens are illustrated in (Table 1).

2.3 Morphology of *Eimeria* Oocyst

Oocyst of *Eimeria* spp. is a robust wall resistant to mechanical, chemical and proteolytic degradation. Its wall has been associated with a bilayer structure composed of glycoprotein and lipid bilayer. The proteins provide the oocyst with great structural resistance against extreme heat or cold while the lipid layer supplies protection against chemical damage (Yu and Heo, 2021). The layer of oocytes can be as thick as 500 to 600 nm but that is eventually compacted to200 nm or less. An inner zone of approximately 40 nm separates the outer and inner layers (Quiroz- and Dantán, 2015).

Based on the infectivity, there are two types of oocysts: Non-infective (Unsporulated) oocyst and Infective (Sporulated) oocyst (Nawarathne *et al.*, 2021). Sporulated *Eimeria* oocyst contains four sporocysts and each sporocyst contains two convex/banana-shaped sporozoites (Jeanne *et al.*, 2015). The morphology of sporulated *Eimeria* oocysts are ellipsoidal or circular shape with a thick cell wall. The Majority of *Eimeria* oocysts have an ovoid shape (Engidaw and Getachew, 2018). E. maxima (30.5 x 20.7μm) is the largest while E. mivati (15.6 x13.4μm) and E. mitis (15.6 x 14.2μm) are the smallest as compared to other species of Eimeria. *E. maxima, E. acervulina, E. hagani* and *E. burnetti* are ovoid while *E. necatrix*

is oblong (Habtamu and Gebre, 2019), *E. tenella* (25 × 19 µm) oocysts are ovoid, smooth, colorless, without a micropyle or residuum but with a polar granule (Taylor *et al.*, 2007).

Size, shape and color are helpful in the identification of *Eimeria* species (Mohamoud *et al.*, 2020). Other characteristics that are valuable in species identifications includes: the nature of macroscopic lesions, sporulation time, prepatent period and location of the parasite within the epithelial cells (Abdisa *et al.*, 2019)

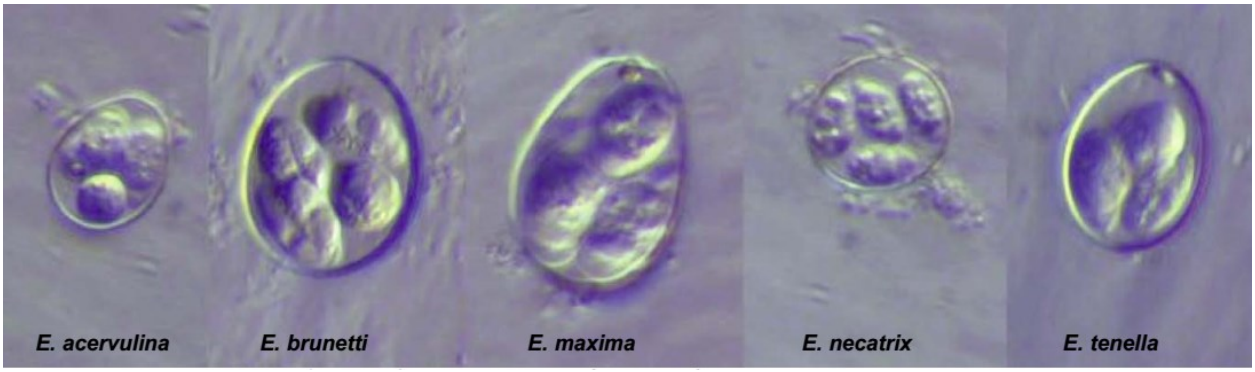


Figure 1: Comparative sizes and morphology of oocysts of 5 *Eimeria* species pathogenic in chicken

Source (Jeanne *et al.*, 2015).

2.4 Life Cycle of a Typical *Eimeria* spp

Eimeria species. follow a typical coccidian lifecycle, characterized by tissue and host specificity (Mesa-Pineda *et al.*, 2021), they are homoxenous with sexual and asexual multiplication taking place in a single host and transmission is exclusively via the fecal-oral route(Sharman, 2013; Haile, 2018). It follows a complex life cycle; consisting of three main developmental stages, one occurs on litter (exogenous phase) under the ideal conditions of humidity, temperature, and oxygen supply, unsporulated (non-infective) oocyst is excreted from the chicken and undergoes sporulation, thus becoming a sporulated (infective) oocyst. This process is known as sporulation (Quiroz-Castañeda, 2018), and two main stages of endogenous phase occurs in the intestine of the host and involves several rounds of asexual reproduction (schizogony) followed by sexual differentiation (gametogony), fertilization, and the shedding of unsporulated oocysts. Some *Eimeria* species vary in the number of asexual generations and in the time required for each developmental stage (Quiroz-

Castañeda and Dantán-González, 2015). All replicative stages occur within infected host cells, with the exception of the oocyst in which formation of sporozoites occurs within the oocyst. Infections are initiated when sporozoites are released from the sporocyst and penetrate host cells (Barta, 2001). During the endogenous phase of the life cycle, each successive developmental stage of the parasite expresses a wide spectrum of antigens that are presented to the immune system of the host (Baker *et al.*, 2005).

Seven distinct phases of the *Eimeria* life cycle have been identified (Jeanne, *et al.*, 2015). Those are; (1) Sporogony: mature non-infective (unsporulated) oocysts are eliminated with fecal materials and can remain in the litter for long periods of time (Quiroz-Castañeda, 2018). Under suitable conditions of temperature(24–28°C), humidity (40–80%) and oxygen supply within 24–48 hours, unsporulated oocyst changed to a sporulated oocyst, At this stage, the sporulated oocysts are ready to infect a new host upon ingestion (Jeanne, *et al.*, 2015). (2) Excystation: Following ingestion, mechanical action of the gizzard breaks the oocyst wall and releases the sporocysts from oocyst. The sporozoites are activated by bile or trypsin when the sporocysts reach the small intestine, and they escape from the sporocysts (Price and Barta, 2010). (3) Cell invasion: upon sporozoites released in the digestive tract, they enter the epithelial cells of the intestine or ceca either directly or following ingestion by a macrophage. Inside the cells, the sporozoites will transform into trophozoites (Taylor *et al.*, 2007);(4) Merogony or schizogony: during this phase, the parasite (schizont) will multiply by a process of asexual multiple division called merogony and each schizont will release several thousand merozoites upon infected cell rupture. Depending on the species of Eimeria, this process will be repeated from 2 to 4 times with the invasion of new epithelial cells (Jeanne, *et al.*, 2015); (5) Gametogony: at a certain time, the merozoites invading the host cells become gametocytes. The male gametocytes multiply by a process of asexual multiple division and release microgametes (mobile cells considered male gametes) into the lumen of the gut. Conversely, the female gametocytes do not multiply any further and mature into macrogametes (immobile cells considered the female gamete) inside the host cells. Microgametes give rise to the release of numerous minute biflagellate microgametes that seek, and fertilize the macrogamete and then form a zygote (Yu and Heo, 2021). (6) Fecundation: A zygote is formed when the macrogamete is fertilized by a microgamete

which then develops a double-layered oocyst wall. The fertilized zygote is now known as an oocyst (Quiroz-Castañeda, 2018).; (7) Transmission: oocysts are released by the rupture of the epithelial and non-sporulated oocysts are passed via the feces into the litter to begin another cycle (Haile, 2018).

The short life cycle (4–6 days, depending on the species) and the numerous productions of sporulating oocysts are advantageous for increasing the chances of infecting a large population of chickens (Quiroz-Castañeda and Dantán-González, 2015). Overall, the ingestion of a sporulated oocyst can potentially lead to the production of about 2-3 million new oocysts in 5 to 7 days (Haile, 2018). Coccidian parasites are transmitted from host to host by accidental ingestion of oocysts contaminate food or water (Mai *et al.*, 2009).

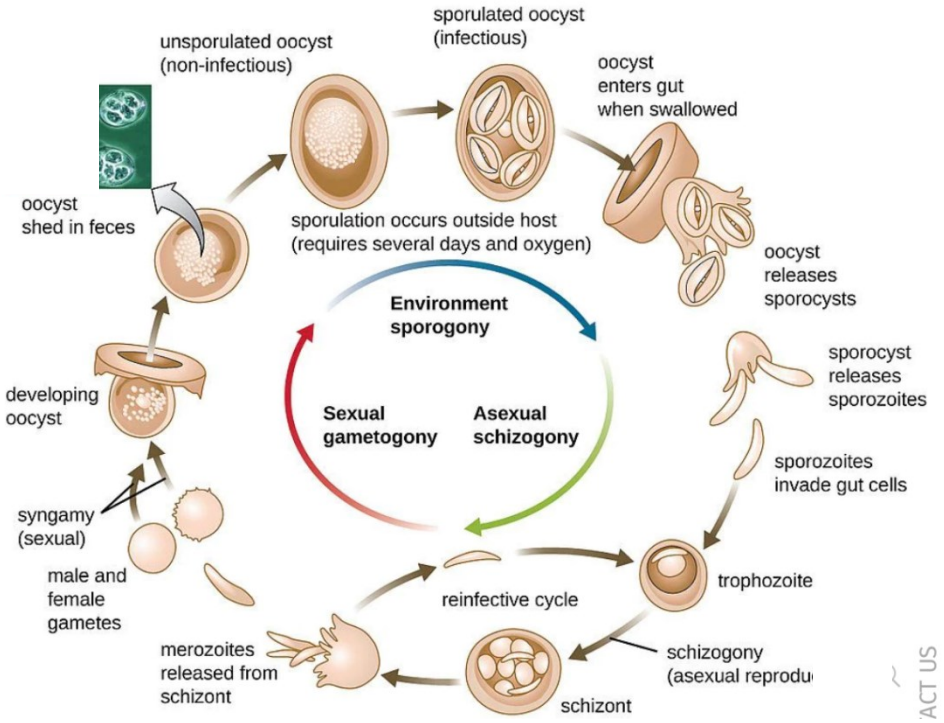


Figure 2 : lifecycle of *Eimeria*

2.5 Pathology and Pathophysiology of Coccidiosis

Depending on the species, magnitude and site of infection, coccidiosis causes enteritis which is consequential for fluid loss and malabsorption of nutrients (*E. acervulina and E. mitis*), inflammation of the intestinal wall with pinpoint hemorrhages and sloughing of epithelial (*E. brunetti and E. maxima*), or complete villar destruction resulting in extensive hemorrhage and death (*E. necatrix and E. tenella*) (Chapman, 2014). There is also catarrhal enteritis and thickening of the intestinal wall and extensive coagulative necrosis and sloughing of the mucosa throughout the entire intestine (*E. brunetti*) (Habtamu and Gebre, 2019).

Eimeria tenella is a highly pathogenic *Eimeria* species, that causes marked typhlitis with occasional involvement of the adjacent areas of the intestine. Blood is often apparent in the ceca and feces in the early stages of the infections; later, cheesy cecal cores may be found. Large clusters of schizonts may be seen in microscopic scrapings of the ceca (boulton,

2018). In longer-standing infections, the caecal contents become caseous and adherent to the mucosa. As the regeneration of the mucosa occurs these caecal plugs are detached and caseous material is shed in the faeces (Taylor *et al.*, 2007). *E. tenella* can cause high morbidity, mortality and reduced weight gain in commercial broilers or layer pullets (Boulianne, 2013).

Table 1:- location and pathological lesion of *Eimeria* species

Species	**Location**	**Lesion**
E. tenella	Caeca	Severe hemorrhage with white red spots in wall of intestine
E. necatrix	Middle intestine	Severe hemorrhage with mucoid discharge whitish and red spot in wall of intestine
E. brunette	Lower half of intestine	thin walled intestine, mucoid on necrotic discharge, distension of intestine
E. maxima	Middle intestine	Distended intestine with hemorrhage spots, mucoid discharge
E. acervulina	Upper intestine	Whitish spots on wall on serous surface hemorrhage streak and whitish lesions on intestinal surface, mucoid enteritis
E. praecox	Duodenum	No lesion but slightly hemorrhagic appearance on intestinal surface of duodenum slight mucoid discharge.

Source (Solomon, 2006).

Eimeria spp. multiplies in the intestinal tract and causes tissue damage (Willams, 2005). This damage can interfere with the food digestion and nutrient absorption and cause dehydration, blood loss (anemia), poor weight gain, poor feed conversion efficiency, reduced feed and water intake, intestinal malabsorption, reduced nutrient digestion, villous atrophy, increased intestinal passage time, intestinal leakage of plasma proteins and increased intestinal acidity (Geetha and Palanivel, 2018).

2.6. Factors Affecting the Coccidia Infection

2.6.1 Chickens Genetic background

Coccidial pathogenicity is influenced by the breed of chicken. All chicken breeds are not equally susceptible to Coccidiosis; some breeds are more resistant than others., subsequently, differences in innate immunity vary a chicken's capacity to resist coccidiosis (Geetha and Palanivel, 2018). Chickens genetic factors influence immunity to Coccidiosis (Smith *et al.*, 2002). Some birds show resistance to *Eimeria* as their immune systems have an improved ability to fight infection agent. This is known for both outbred and inbred chicken lines (Lillehoj *et al.*, 2000). For example, Lee *et al.* (2016) have compared the resistance of congenic Fayoumi lines to *E. tenella* infection and they have evaluated the genetic differences in response to *Eimeria tenella* infection. The Fayoumi M5.1 line had higher BW gain, lower oocyst shedding, and a higher ratio of B and CD4 (+)/CD8 (+) T cells than the M15.2 chickens. These results was suggested that the M5.1 line is least susceptible to *E. tenella* infection than the M15.2 line

The chickens' genetic background influences the development of the parasites and the severity of lesions and is potentially correlated with immunity to infection (Clare *et al.*, 1985). Different lines of chickens displayed a different susceptibility to the same *Eimeria* infections. (El-Shall *et al.*, 2022). The magnitude of clinical signs resulting from *Eimeria* infection is influenced by host genetic factors (Dalloul and Lillehoj, 2006). The proliferation of the parasites, severity of lesions and effects on weight gain are very much determined by the genetic background of the host (Vermeulen *et al.*, 2001).

Many researchers have reported that some breeds are more susceptible to coccidiosis compare to others, for example, in Ethiopia a research finding by Gari *et al.* (2008) report that the Rhode Island Red breed is more susceptible than local chicken in Ethiopia, native Egyptian breeds such as the Fayoumi chicken have also appear more resistant compared with White Leghorns (Pinard-Van Der Laan *et al.*, 1998).

2.6.2 Age

Resistance to the *Eimeria* infection usually increases with the age of the birds (Mohammed and Sunday, 2015). Most of the *Eimeria* spp. affects birds between 3 and 18 weeks of age group (Geetha and Palanivel, 2018; El-Shall *et al.*, 2022). Younger birds have not fully developed their immune system to resist coccidiosis infection, whereas older chickens are relatively resistant to infection (Dalloul and Lillehoj, 2006). Chickens, which faced the pathogens for the first time, are more susceptible than those who have previously encountered the infections or used anticoccidial drugs (Yun *et al.*, 2000).

Avian Coccidiosis outbreaks are more common at 3–6 weeks of age.Chicks at 2 weeks old are more resistant than at any other time during the first 6 weeks, which may be due to maternal antibody received from parent (El-Shall *et al*., 2022). Clinical Coccidiosis rarely occurs in layers and breeders during the laying cycle because of prior exposure to Coccidia and resulting optimum immunity level. If a flock is not exposed to a particular species early in life or if immunity is depressed because of other diseases, outbreaks may occur after layers are moved to production houses (Mcdougald *et al*., 2020).

2.6.3 Number of oocyst

The severity and occurrence of coccidia infection is directly related to the parasite species, the particular isolate or strain involved, and the number of oocysts ingested. Lack of cross immunity between species of *Eimeria* predisposes birds to infection and disease outbreaks caused by different species (Yun *et al.*, 2000). The number of oocysts is an important factor affecting the outcome of infection. Infection with a small number of oocysts may not result in any clinical signs. Rather, it may contribute to the development of acquired immunity to resist the disease in a similar process to vaccination (El-Shall *et al*., 2022).

2.6.4 Concurrent infections

Besides the genetic factors playing a role in the final outcome of the infection, interference of concurrent infection with different *Eimeria* species and interactions with other pathogens such as viruses and enteric bacteria can determine the severity of the disease. Coccidia can occur as outbreak in association with respiratory infections that can intensify the severity of coccidiosis by interfering with immunity and drug intake capacity of chickens. Ill-health due to other disease can be incriminated in outbreaks of coccidiosis in poultry farm as a result of letdown in immunity or reduced capacity of food intake (Habtamu and Gebre, 2019).

Diseases that cause suppression of the immune system can also increase the risk of having clinical disease caused by coccidiosis. Specifically, Marek's disease virus and infectious bursal disease virus will increase a bird's susceptibility to *Eimeria*. These birds may not be able to sustain good production as well as defend themselves from pathogens. This may aggravate coccidiosis, placing a heavier burden on anticoccidial drugs (Mcdougald *et al*., 2020). In chickens, aflatoxins in the diet also act as a main stress factor which increasing the severity of disease or increase susceptibility of chickens to coccidiosis by impairing of cellular and humoral immune responses to infectious (El-Shall *et al.*, 2022).

2.6.5 Environment and management related stress

Deep litter under poor sanitation could provide optimal temperature and relative humidity for sporulation of oocysts (El-Shall *et al.*, 2022). The prevalence rate of coccidiosis is higher during the rainy season, because, rainy season is providing favorable conditions for the growth and development of the infective oocysts (Mohammed and Sunday, 2015).

The key factors for coccidiosis outbreak are the presence of sporulated oocysts in the environment for long period, bad ventilation, absence of proper waste disposal, wetting of litter from leaking pipes, absence of all-in all-out system and the presence of stress-inducing problems in the flock (Chanie *et al.*, 2009). Birds managed on deep litter and high stocking density show a higher incidence of coccidiosis due perhaps to their close contact with the

infective oocysts in the bedding materials (Mohammed and Sunday, 2015). The possible risk factors associated with the outbreak of coccidiosis in Ethiopia were reported as the absence of proper disposal of litters, wetting of litters, absence of all-in all-out system, the presence of stressors (such as a change in diets and concurrent infections) and extensive use of coccidiostats (Habtamu and Gebre, 2019).

2.7. Diagnosis

2.7.1. Detection of Oocyst

Coccidiosis in the Chickens may be diagnosed by using standard faecal flotation techniques. Oocysts can be identified in feces by salt or sugar flotation methods, a small sample of faeces is mixed with a flotation medium (commonly saturated aqueous sodium chloride, sodium nitrate or sugar) and examined for the presence of oocysts (Barta, 2001). In some laboratories today simple flotation and light microscopy is still used as the method of detecting *Eimeria* oocysts. *Eimeria* oocysts isolation depends on the measurements of oocysts by using a calibrated ocular micrometer at 400x magnification (Conway and Mckenzie, 2007).

The number of oocysts present in feces is influenced by the genetically determined reproductive potential of the *Eimeria* species, the number of infective oocysts ingested, stage of the infection, age and immune status of the birds, previous exposure, consistency of the fecal sample and method of examination (Conway and McKenzie, 2007). Large numbers of oocysts may be present in mucosal scrapings from affected birds. Fresh mount wet smears and/or histologic examination of the intestine can confirm the presence of developing stages of coccidia such as sporozoites, merozoites and schizonts (Brash *et al.*, 2013).

Diagnosis by faecal examination may lead to quite inaccurate results. In some instances, the major pathology is produced before oocysts are shaded in the faeces (*E. tenella*) (Habtamu and Gebre, 2019). Thus, diagnosis by fecal examination may lead to quite low sensitivity,

because diarrhea may precede the heavy output of oocysts by 1–2 days and may continue after the oocyst discharge has returned to low levels. Variation in oocyst counts also makes it difficult to link oocyst counts to the severity of the disease. Therefore it is not always possible to find oocysts in a single fecal sample; multiple fecal examinations of one animal or single fecal examinations of animals housed in the same environment may be required (Haile, 2018). Therefore, the results of fecal examinations must be related to clinical signs and intestinal lesions (gross and microscopic) (Conway and McKenzie, 2007).

2.7.2. Gross Pathological

Diagnosis of coccidiosis in poultry flocks depends on necropsy and the examination of birds for intestinal lesions in different areas of the gut (Chapman *et al.*, 2013). *Eimeria* species are identified easily by their gross lesions and the location on which *Eimeria* species is causing the disease, *Eimeria* species like *E.tenella* damage is being limited to the caeca (Haile, 2018). Less pathogenic *E. mitis* and *E. acervulina* result in mild enteritis whereas highly pathogenic *E. tenella* and *E. necatrix* cause the destruction of intestinal villi leading to hemorrhage and death (Chapman, 2014). Macroscopically the infection can be recognized by small white spots usually intermixed with rounded, bright or dull red spots of various sizes (*E. necatrix*), numerous array of whitish transverse patches in the upper half of the small intestine (*E. acervulina* and *E. mivati*) and there is catarrhal enteritis and thickening of the intestinal wall with extensive coagulative necrosis and sloughing of the mucosa throughout the entire intestine (*E. brunette*) (Geetha and Palanivel, 2018). Inflammation of the intestinal wall with pinpoint hemorrhages and sloughing of epithelial (*E. brunetti* and *E. maxima*) (Chapman, 2014).

Eimeria tenella is highly pathogenic, causes the ceca to be dilated, thickened and often filled with unclotted or partly clotted blood and marked typhlitis with occasional involvement of the adjacent areas of the intestine (Brash *et al.*, 2013), finally complete villa destruction resulting in extensive hemorrhage and death (Chapman, 2014). If the birds survived *E. tenella*, the lesions will slowly disappear but the cecal content will typically remain caseous (Jeanne, *et al.*, 2015). These characteristics and others have been utilized in the widely

accepted visual diagnosis and scoring of the severity of lesions in different regions of the gut (Chapman, 2014).

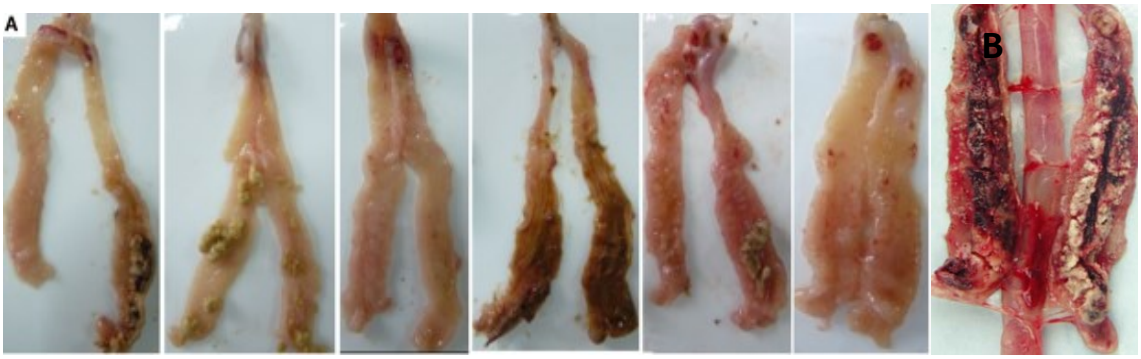


Figure 3*:* Gross lesions in the ceca of *Eimeria tenella*-infected chicks

*(A) = Chickens infected with *E. tenella* (B) = Typical white caseous content in the ceca will be observed after the lesions are resorbed (Jeanne *et al.*, 2015; Cui *et al.,* 2017).

2.7.3. Histopathological changes

Ordinary methods in histopathology are satisfactory for routine examination of tissues infected with coccidia. Staining of sections with H&E or other common histologic stains will demonstrate developing stages of *Eimeria* in the intestinal epithelial cell (Mcdougald, 2020).

The main histopathological lesions of coccidiosis are hyperplastic or proliferative enteritis, the pathological changes vary in detail according to the species of Eimeria. In most cases, loss of surface epithelial cells and villous atrophy is associated with first-generation schizonts, while crypt destruction or hyperplasia is associated with gamonts (Haile, 2018). Histopathological change in the case of *Eimeria tenella* form revealed loss of epithelial tissue, congestion of blood vessels followed by marked hemorrhage, severe muscular oedema, necrosis of sub mucosa, loss of villi, disruption and necrosis of caecal mucosa, a cluster of oocysts and, lymphoid cells showing hyperplasia (Geetha and Palanivel, 2018).

There is also retention of an excessive amount of blood in the tissue (hyperemia) and infiltration of blood around infection (Solomon, 2006). Intestinal lesions were found in the form of complete detachment of the mucosal layer from the sub-mucosal layer accompanied by the accumulation of cell debris in the intestinal lumen, and the lymphoid cells showed hyperplasia. Mononuclear cell infiltration was noted in the mucosal layer (Haile, 2018).

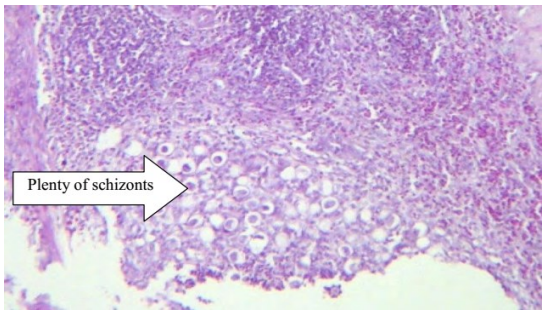


Figure 4: Cecum section infected with *Eimeria tenella* (100x).

Source (Solomon, 2006)

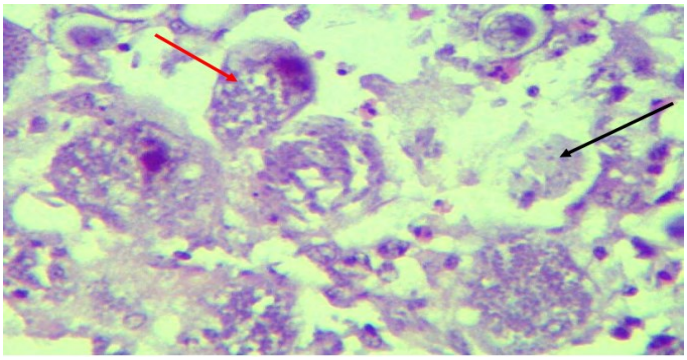


Figure 5: Stained schizointes and schizonts of *E. Tenella*,

Red arrow indicates mature schizointes and black arrow indicate immature schizonts .source (Solomon, 2006)

2.7.4. Molecular diagnosis

Traditional methods of species identification are very important for diagnosis, although they should be complemented by molecular methods that involve PCR diagnostic assays based on DNA amplification (Chapman, 2014). PCR method identification to determine the

incidence and occurrence of the parasite species and its proper documentation will help in future control regimes of coccidiosis (Shameer, 2016).

Polymerase chain reaction has been used for the detection of coccidial infections and species identification (Geetha and Palanivel, 2018). The molecular method uses PCR assay by amplification of specific genes in the DNA sequences of the *Eimeria* parasite. Ribosomal DNA internal transcribed spacer1 (ITS-1) and ribosomal DNA internal transcribed spacer2 (ITS-2 are sequences that are excised from rDNA precursors through post transcription. ITS regions are less conserved than the rRNA genes, showing variations in DNA sequence and length makes the design of primers straightforward and reduces the risk of cross-reactions among different species (Schnitzler *et al.*, 1998). They have been widely used in the identification of all seven species of *Eimeria* in chickens using specific primers. Targeting primers at conserved ribosomal DNA sequences (5.8S and 28S) have also been used in *Eimeria* species identification with a high level of genetic variants (Joseph and Adekunle, 2018).

In addition to the use of ITS-1 and ITS-2, Random Amplified Polymorphic DNA (RAPD) has been used to develop Sequence Characterized Amplified Region (SCAR) primers for the identification of each *Eimeria* species (Fernandez *et al.* 2003). These primers have been tested for the amplification of *Eimeria* species. Fernandez *et al.* (2003) have combined the SCAR primers for the seven species of *Eimeria* for the development of a multiplex PCR assay which is used to simultaneous discrimination of all species of *Eimeria* infecting chickens in a single tube reaction. The limitation of the SCAR method is that it may be less sensitive as compared to ITS-1 and ITS-2 which are present in the multiple copies of *Eimeria* genome (Joseph Adekunle, 2018).

Multiplex PCR techniques have been described that combine all primers for each *Eimeria* species in a single tube. Newer technology includes real-time (quantitative) PCR and loop-mediated isothermal amplification (LAMP) as an alternative to gel electrophoresis (Mcdougald *et al*., 2020). Early assays using ITS1, ITS2 or SCAR markers relied on qualitative assays in which identification of amplification products has been obtained by

agarose or polyacrylamide gel electrophoresis (Chapman *et al.*, 2013). Nevertheless, the application of real-time PCR to both detect and quantify species would be particularly useful for species complexes like *Eimeria* in chickens (Abdisa *et al.*, 2019).

2.7.5 Serological diagnosis

Enzyme-linked immunosorbent assay (ELISA) detects antibodies in blood (sera) samples and can be designed so that they detect species-specific antibodies or antibodies that recognize a range of species. Since they detect antibodies rather than the parasites themselves, ELISAs have the advantage that they are able to identify the species to which chickens have developed an immune response even when the parasites are no longer present (Solomo, 2006). They also provide the ability to assess immune responses following vaccination. ELISAs are cheap assays that would allow rapid screening of large numbers of birds. In addition to screening for the presence of infection in the flock, ELISA was suggested to be a useful tool to estimate the immune state of chickens as a result of vaccination with a live vaccine (Onaga *et al.*, 2005).

2.8. Prevention and Control Strategies

Control of the avian coccidia is essential to the poultry industry because, for the most part, large flocks of chickens are kept on the floor at high stocking densities in warm environments. These conditions are highly favorable for the transmission, replication and accumulation of *Eimeria* spp. in very large numbers (Baker *et al.*, 2005).). Due to the extreme effect of coccidiosis on the poultry, different treatment and control methods have been deployed. Thorough biosecurity coupled with the use of prophylactics were the first strategies deployed in the control of this disease (Joseph and Adekunle 2018). Coccidiosis is by far more easily prevented than treated (Vegad, 2004).

2.8.1. Immunization by vaccination (Immunoprophylaxis)

Live vaccines consist of formulations of sporulated oocysts of several or all of the avian species of *Eimeria*. Most commercially available vaccines contain live oocysts and vary according to the number of species of *Eimeria* included, the numbers of oocysts present, and whether or not they are attenuated (Baker *et al.*, 2005). The number of oocysts of each

Eimeria species provided in the vaccine is critical to initiating immunity without causing clinical disease. Some vaccines contain drug-sensitive strains of *Eimeria*, facilitating the establishment of drug-sensitive populations and extending the usefulness of anticoccidials (Brash *et al.*, 2013).

Vaccines containing all species that infect the chicken are used mainly to immunize egg-laying stock whereas vaccines containing fewer species (usually *E. acervulina, E. maxima* and *E. tenella*) are used in broilers. The first vaccines comprised populations of wild-type oocysts that were potentially pathogenic, but more recently, vaccines containing attenuated parasites which have reduced pathogenicity but retain immunogenicity, have been introduced (Chapman *et al.*, 2013).

The developed vaccines for coccidiosis were mostly live, divided into two categories namely, nonattenuated and attenuated vaccines. Other minor trends in the development of coccidial vaccines include subunit, recombinant, and DNA-based vaccines. Protective immunity against coccidiosis in chicken can be in the form of an active or passive immunity response, which confers resistance in case of exposure to *Eimeria* in the field (Barbour *et al.*, 2015). Planned vaccination of young chicks or poultry to small numbers of oocysts by coarse spray at the hatchery or in feed, water or gel blocks or in ovo at 18 to 19 days incubation has been used successfully (Brash *et al.*, 2013). In some cases, vaccination failure may occur due to the birds are overwhelmed with exposure to wild-type virulent oocysts before they have had time to develop an immune response. It is obviously important that vaccination is undertaken carefully because any chicks that are not exposed to vaccinal oocysts may be vulnerable to potentially high numbers of virulent oocysts when placed on litter (Chapman *et al.*, 2013).

2.8.2 Natural Exposure to Eimeria oocysts

It is known that when chickens are infected with a low number of *Eimeria* parasites, protective immunity is induced after two or three consecutive infections (Joyner and Norton, 1973). If chickens are exposed to curtain numbers of oocysts in their environment, they develop immunity to the species of coccidia represented. Exposure must be moderate or clinical signs will appear. Exposure can be limited if dry litter conditions are maintained. Wet litter (including wet areas around waterers) is particularly to be avoided. This practice is rarely used in large commercial poultry farm operations (Brash *et al.*, 2013).

Prevention of coccidiosis during the initial period of rearing has always been comparatively easy, through the use of anticoccidial drugs, a greater difficulty has been to ensure that hens develop immunity to each of the major species of *Eimeria* by the time they come into lay and anticoccidial drugs are removed. Thus for many years, poultry producers used strategies of husbandry during the initial rearing period that were intended to expose flocks to limited infections with *Eimeria* spp. so that immunity could develop and protect the birds against parasite challenges during the anticoccidial drug-free or laying period (Baker *et al.*, 2005).

2.8.3. Treatment (Chemotherapy)

The chemoprophylaxis control method is by using anticoccidial products or coccidiostat drug in the feed ration is by far the most popular: it is estimated that 95% of the broilers producer have used anticoccidials drugs for the treatment and control of coccidiosis (Gussem, 2006). The effective use of coccidiostat as feed additives over the past 50 years has played a major role in the growth of the poultry industry. These anticoccidials can be classified as (i) chemicals which have specific modes of action against parasite metabolism widely used for the treatment of coccidian including amprolium, sulfadimethoxine, sulfaquinoxaline and sulfamethazine, but Sulfas should not be used in layers. Essential withdrawal times are usually required prior to marketing (Brash *et al.*, 2013), (ii) poly-ether ionophores such as monensin lasalocid, salinomycin, narasin, and maduramycin, which act through general mechanisms of altering ion transport and disrupting osmotic balance of Eimeria. These latter compounds are now the backbone of coccidiosis control (Allen and

Fetterer, 2002). Any treatment will be effective only if given very early (Jeanne, *et al.*, 2015).

The prophylactic drugs used for the prevention of coccidiosis are coccidiostats. An effective coccidiostat should inhibit the schizogonic stage and allow immunity to develop. Almost all broiler chickens reared throughout the world are given anticoccidial drugs for prevention and control of coccidiosis from the time of hatch until a few days before slaughter (Baker *et al.*, 2005). In the egg-production sector, the situation is quite different. Prophylactic anticoccidial drugs must be withdrawn once hens approach the point of lay to prevent any carryover of drugs into eggs destined for consumers (Baker *et al.*, 2005). However, the constraint of this strategy in the control of coccidiosis disease is the emergence of drug-resistant isolates of the *Eimeria* parasite (Joseph *et al.*, 2018).

An early insight was that use of low concentrations of certain drugs in the feed did not necessarily prevent the acquisition of immunity. It is now known that most drugs are effective in the field because they only partially suppress parasite development, allowing birds to acquire natural immunity as a consequence of exposure to parasites that escape drug action (Chapman *et al.*, 2013).

2.8.4. Management and biosecurity

Management and biosecurity measures for the control of coccidiosis should focus on the prevention of the introduction of the parasite into the premises, and control of its multiplication and spread in case flocks have been infected (Haile, 2018). Biosecurity measures aiming to prevent the introduction of *Eimeria* parasites to the farm are similar to those applied for the prevention of other infectious poultry diseases and should focus on isolation, traffic control and sanitation including disinfection of materials, people and equipment entering the farm and poultry house (Peek and Landman, 2011). Vaccination combined with good management practices will provide a feasible and sustainable strategy to control coccidiosis and improve the overall health of poultry (Tellez *et al.*, 2014)

Management of poultry houses plays a significant role in the spread of coccidiosis because coccidial oocysts are omnipresent and are easily spread in the poultry house environment. Good sanitary measures, maintaining good litter quality by use of efficient drinkers to prevent wet litter, cleaning feeders and drinkers, strict hygiene, maximizing downtime between flocks, regular monitoring of bird health, using enough feeders and waterers to prevent overcrowding, timely removal of dead birds if any from the poultry house and keeping the litter dry to reduce sporulation of oocysts are essential to control the risk of coccidiosis (Usman *et al.*, 2011)..

2.8.5 Feeding and feed Natural products as Feed supplement

Nutrition plays a primordial role in chicken health. The immune response is not only improved by vaccination and/or chemoprophylaxis against infectious disease and hygiene but also by an intake of adequate nutritional constituents (Habtamu and Gebre, 2019).

Many different herbal compounds have been investigated for their potential use as a dietary supplement to control coccidiosis(Peek and Landman, 2011). Natural products such as probiotics, plant extracts and fungal extracts as feed supplements are some of the alternative measures discovered in the control and prevention of coccidiosis. A consistent report on the use of probiotics either alone or together with other supplements has been confirmed to reduce microscopic lesions of coccidiosis in birds (Joseph *et al.*, 2018).

A number of natural products or feedstuffs have been tested as anticoccidial dietary additives including mushroom and herb extracts incorporation which resulted in enhancement of both cellular and humoral immune responses in E. tenella infected chickens (Guo *et al.*, 2004). Dietary plum has been used to promote protective immunity against coccidiosis as assessed by reduced body weight loss and decreased oocyst shedding (Lee *et al.*, 2008). Additionally, antimalarial artemisinin, a product extracted from the herb Artemisia annua, was shown to have a deleterious effect upon macrogametocytes of E. tenella by affecting the expression of an enzyme sarcoplasmic endoplasmic reticulum calcium ATPase (Del Cacho *et al.*, 2010). Improved resistance to E. acervulina was

observed when the diet of chickens was supplemented with garlic metabolites (Kim *et al.*, 2013).

2.9. Status of Avian Coccidiosis in Ethiopia

Avian coccidiosis is endemic in Ethiopia and causes great economic losses in all production systems, particularly in young birds (FAO/ILRI, 1995). Comparative mortality rates due to coccidiosis were 14.5% and 13.3% in small-scale and large-scale poultry farms, receptively (Safari *et al.,* 2004). The species of coccidia identified in Ethiopia are *E. tenella*, *E. necatrix*, *E. maxima* and *E. acervulina,* E. *mivati* and E. *brunetti* (Ashenafi, 2000; Methusela, 2001; Lobago *et al.*, 2005). The study conducted by Lobago *et al.*, (2005) reported the occurrence of coccidiosis and distribution of *Eimeria* species in dead chickens from 1- 60 days of age, at Kombolcha Poultry Multiplication and Research Centre indicated that out of 965 dead birds, 370 (38.34%) were found to have clinical coccidiosis. The *Eimeria* species identified with their prevalence were *E. brunette (*45.3%), *E. tenella* (40.8%)*, E. acervulina (*9.7%) and *E. necatrix (*4.1%). In central Ethiopia *Eimeria acervulina* was the most prevalent coccidial species as reported by different Authors (Hagos, 2000; Safari, 2004; Lobago,*et al*, 2005), whereas a study conducted in Kombolcha disclosed that *Eimeria burneti* was the most prevalent coccidian species. The previous and recent studies in different areas of Ethiopia indicated that poultry coccidiosis is highly prevalent and widely distributed (Table 3)

Table 2: Prevalence of coccidiosis in different areas of Ethiopia.

Site of study	Prevalence	Reference
Adama	56.30%	Ermias and Mekonnen (2015)
Adiss Ababa	23.10%	Alemayehu *et al*, (2012)
Ambo	20.57%	Oljira *et al.* (2012)
Arsi Tiyo District	64.40%	Gari *et al*. (2008)
Bahir Dar	40.60%	Abebe & Mekonnen (2016)
Central Ethiopia	25.80%	Ashenafi *et al*.(2004)
Debre zeit	71.70%	Dinka and Yakob, (2012)
Dire Dawa	42.70%	Migbaru & Abdi (2015)
Gonder	53.60%	Belaynew *et al.* (2016)
Hawassa	65.10%	Muluken & Liuel (2017)
Komblcha	25.24%	Abadi *et al*. (2012)
Mekele	20.30%	Brhane & Nibret (2016)
Nekemte	19.50%	Gerbi, *et al.* (2015)
Yabello	19.30%	Addis & Endale (2016)
Debre tabor	21.40%	Temesgen, *et al.* (2018)
Jimma	32.40%	Tadesse and Teshome (2018)
Alage	19.5%	Eshetu and Nigussu, (2019)

3. MATERIALS AND METHODS

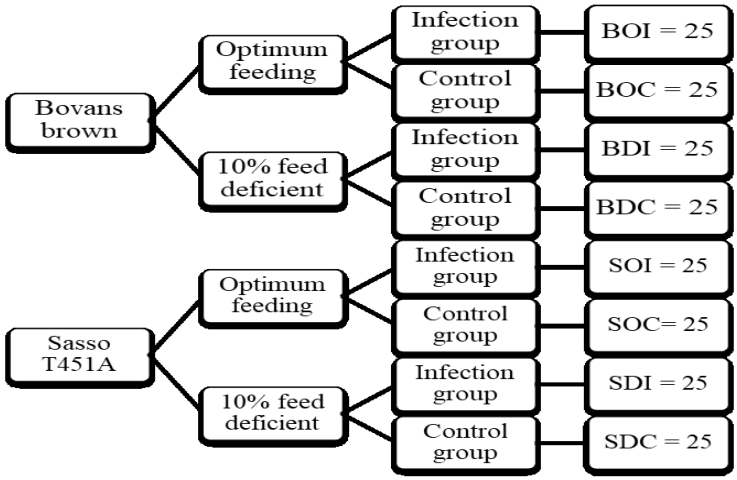
3. 1 Study Area

The experiment was conducted from November 2020 to April 2022 at the International livestock research institute (ILRI) poultry research facility in Addis Ababa, Ethiopia.

3.2 Experimental Animals

The experiment was conducted over two rounds. Three hundred female day-old chicks (DOC) of two commercial breeds (150 Bovans brown and 150 Sasso T451A) for the first round experiment and 100 DOC (50 Bovans brown and 50 Sasso T451A) for the second experiment were bought from Ethio-chicken breeding company. The birds were originated from the same flocks whose parents have subjected to similar husbandry.

In Experiment 1, five chickens were housed in a pen of dimensions 120 cm in height, 62 cm in width and 150 cm in length. Each pen had a drinker and feeder. Experimental grouping was done as described in the flow chart below (Figure 6)



BDI= Bovans 10% feed deficient infected
BDC = Bovans 10% feed deficient Control
BOC = Bovans Optimum feeding Control
BOI = Bovans Optimum feeding infected,
SDI = Sasso 10% feed deficient infected
SDC = Sasso 10% feed deficient Control
SOC = Sasso Optimum feeding Control
SOI= Sasso Optimum feeding infected

Figure 6: Experimental group distribution

In experiment 2, for both breeds, 20 pens were arranged in 2 rooms for infection and control groups. Control groups of both breeds were housed together in one room and infection groups were also housed in another room.

3.3 Housing and Chicken Management

Both experiments were conducted in accordance with the principles and guideline for the care and use of Laboratory Animals and approved by the Institutional animal care and use committee of Addis Ababa University, College of veterinary medicine and Agriculture. For experiment 1, in the first 8 weeks of age, all birds were supplied with feed according to the amount of feed recommended by breed feeding guidelines. From week 9, feed restricted groups in both genotypes were fed 10 % less from optimum feeding regimen and optimum feeding groups were fed to the optimum level as recommended by Bovans brown (Appendix IV) and Sasso T451A (Appendix V) breed feeding guideline. Experimental infection was done on the 31st week of age. In experiment 2, since there was no feeding experiment chicken were provided with optimum feeding regimen until the end of the experiment. However, chicken were kept for only 29 days. Experimental infection was done at the 21st days of age.

Figure 7: Experimental Chicken housing in the pen

All chickens had ad libitum access to water and a coccidiostats-free diet. Experimental birds were housed in pens with proper lighting, heat and other technical management of chicken were applied as recommended in the breed management guideline manuals (bovans brown management guideline, 2020) and (Sasso Chicken Management guideline, 2017). Strict biosecurity measures were taken to avoid any extraneous contamination by poultry pathogens. The Chicken's health status was monitored twice a day for any signs of illness and/or welfare impairment. Disinfected Teff straw was used as bedding material. Gloves, boots and masks were worn at all times. Footbath was used always before and after contacting the experimental chicken. Chickens were vaccinated according to the breeder vaccination schedule.

3. 4. Study Design

A randomized control trial was done for the administration of infection and a completely randomized study design was used to allocate animals into suboptimal and optimal feeding regimens.

Experiment 1: Underweight and weak chicks were excluded before randomly distributing chickens into their experimental groups. From week 9, out of chicken supplied from the breeder company, 200 (100 bovans brown and 100 Sasso T451A) were selected according to their weight uniformity and physical condition. Both breed and feeding regime was randomly distributed into infection and control groups. To avoid cross-contamination the challenge factor was applied at the room level while the other two factors, breeds and feeding regime, were randomized at the pen level. At the beginning four experimental groups were established in a 2 × 2 factorial arrangement before challenging with *Eimeria tenella*, this involved 2 breeds (Sasso T451A and Bovans brown) and 2 feed regimes (optimum feeding group and 10% feed Deficient groups). Then from the points of challenges, an experimental design added a challenge level as another third factor and was established in a 3 x 2 factorial arrangement, which included 2 breeds (Sasso T451A and Bovans brown) x 2 feed regimes (optimum feeding group and 10% feed Deficient groups) x 2 levels of challenge (without and with *Eimeria tenella* challenge). Therefore, the 2 x 2 x 2 factorial arrangement was totally containing eight experimental groups (figure 6).

Experiment 2: A day-old chicks were categorized into different experimental groups by random allocation into four experimental groups. All chickens were reared for 21 days under the same feeding and management conditions. The experiment was arranged into two factors each at two levels of factorial design; which included 2 breeds (Bovans brown and Sasso T451A), x 2 levels of challenge (with *Eimeria tenella* and without *Eimeria tenella)*. Each experimental group contained 5 pens as replicates and five birds per pen. All birds were tagged individually with tags carrying sequential identification numbers. To avoid cross-contamination the challenge factor was applied at the room level while the breed factors were randomised at the pen level.

3.5. Experimental Infection and Observation Schedule

Experiment 1: On week 31, chickens in the *Eimeria* challenged groups were inoculated (gavaged) by 1 x 10^4 of the Houghton laboratory strain of *Eimeria tenella* oocysts in 1 ml of distilled water whereas control groups were inoculated with 1 ml of sterile distilled water.

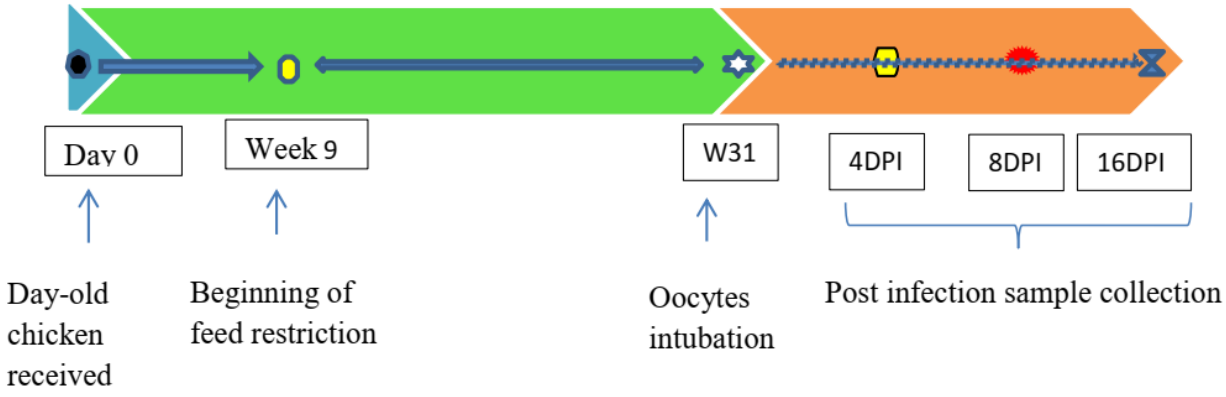


Figure 8: Experimental process of first experiment

Experiment 2: Fecal samples from all experimental groups were collected and examined prior to the experimental infection and all groups examined were found negative for coccidial oocysts. Three weeks after the start of the experiment (at 21 days of the study), chickens in the infection groups were gavaged with 3 x 10^4 of the Houghton laboratory strain of *Eimeria tenella* oocysts in 1 ml of distilled water, whereas chickens in the control

groups (without *Eimeria* challenge) were gavaged 1 ml of sterile distilled water. The experiment was terminated at 29 days of age (figure 9).

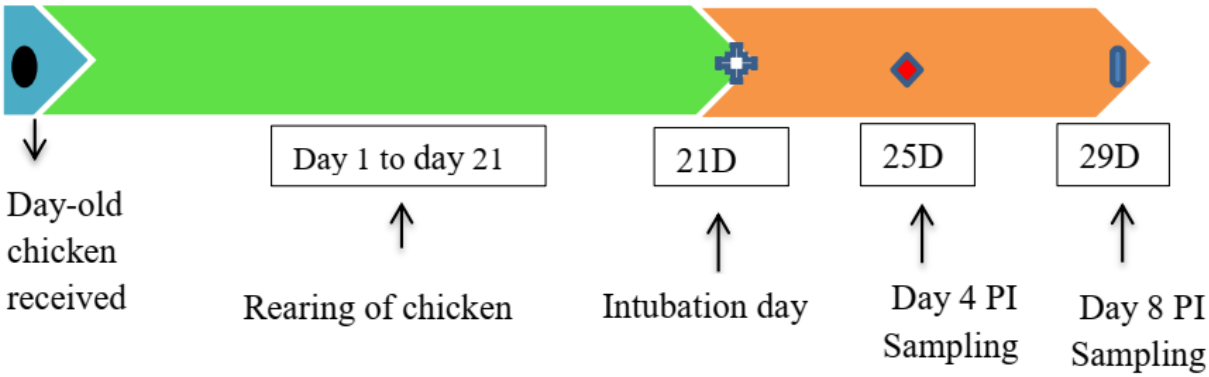


Figure 9: Experimental process of second experiment

3.6. Data Collection

3. 6.1 Faecal oocyst count

To obtain accurate information with regard to the severity of infection, oocyst counting methods were carried out to determine the number of oocytes per gram (OPG) of faeces. In both experiments, faecal sample collection was started on day 4 post-infection. Three fresh droppings were pooled and collected once every 24 hours from each pen and continuing until day 12 PI and day 8 PI for the first and second experiment respectively. Oocysts shading per gram per pen was determined by using sodium chloride solution for the flotation technique and quantified by McMaster's counting technique according to the technique used by Bowman and Georgis (2003) and Ahad *et al*. (2016). To determine oocyst per gram (OPG) of feces, three gram of fecal materials was mixed thoroughly with 42ml of saturates sodium chloride solution (Yu *et al*., 2021) and filtered by sieve. The suspension was equally poured into both right and left McMaster slide by Pasteur pipette. The counting was carried out under 10x objective lenses magnification of the light microscope. The numbers of oocyts in two chambers were multiplied by dilution factors (x50). Oocyst per gram faces (OPG) was calculated based on the technique described by (MAFF. 1979; Gari G. *et al*., 2008).

3.6.2 Hematological Evaluation (PCV)

At day 4, day 8 and day 16 one chicken per pen in the first experiment and at day 4 and day 8 post infection 1 and 2 chicken per pen was selected respectively for sampling. Blood samples were collected from the brachial vein of selected chickens with body weight near to the group's average body weight by using a 3ml sterile syringe and a 23-gauge needle. Each blood sample was transferred immediately into a sterile vacutainer tube containing the anticoagulant, ethylene diamine tetra acetic acid (EDTA). The blood sample was mixed gently by repeated inversion and allowed to flow into a capillary tube by capillary action up to 3/4 of its length, and then sealed with sealant wax. The sealed end outward and the open end was towards the center. The capillary tube was then centrifuged at 10,000 revolutions per minute (rpm) for 5 min using the haematocrit centrifuge. The PCV value was read using the haematocrit reader and recorded as the percentage of whole blood (Audu . *et al*., 2017).

3.6.3. Postmortem examination and lesion scoring

In experiment 1, one chicken/pen was humanely euthanized at day 4, day 8 and day 16 post-challenge with *Eimeria* by cervical dislocation followed by necropsy procedures. Their intestines were removed for post-mortem examination and the cecum was removed for gross or macroscopic examination of cecal lesion associated with *Eimeria tenella* infection.

In Experiment 2, on day 4 and day 8 of post-challenge, 1 and 2 chicks per replicate respectively were killed and cecum was removed for lesion scoring as described in experiment 1. Cecal lesion score was graded according to the guidelines described by Conway and McKenzie, (1990). Johnson and Reid (1970) lesion scoring technique was used as a baseline for assessing the gut lesions from caecal loops of chickens infected with *Eimeria tenella*. Cecal Lesions were scored from 0 to 4: 0 (no lesions), 1 (mild lesions), 2 (moderate lesions), 3 (severe lesions) and 4 (very severe lesions and fatal cases) (Conway and McKenzie, 2007).

3.6.4. Histopathological examination

At day 4, day 8 and day 16 one hen/pen was euthanized in the first experiment and at day 4 and day 8post infection 1 and 2 chicken/pen was euthanized respectively. After killing selected chickens by cervical dislocation, gross pathological changes were observed and lesion scored, about 1-3 cm classical cecum lesions were taken for histopathological slide preparation from the infected groups and a cecum tissue sample was also collected from control groups for histopathological examination. The collected sample was immediately fixed in 10% buffered formalin and submitted to Animal Health Institute (AHI), Sebeta, Ethiopia. The tissue specimens were trimmed and then processed in an automatic tissue processor to dehydrate in ascending order of alcohol concentration, cleared in xylene and impregnated and embedded in paraffin wax for the preparation of fine blocks. Embedded tissue sample was sectioned with a semi-automated microtome at 5µm thickness and fixed on slides. Afterward, sectioned tissue was deparaffinised by heat and xylene. Haematoxylin and eosin staining was used to demonstrate the developmental stages of *Eimeria tenella* in the cecal tissue (Annex II). Finally, the stained tissue was dehydrated in alcohol, cleared in xylene, stained with H $ E, mounted and examined by a light microscope for histopathological changes (Debbou-Louknane *et al*., 2018).

3.6.5 Production performance

Experiment 1: Performance variables evaluated were: laying percentage (%), egg weight and body weight. Individual body weights of all groups were measured weekly. Eggs weight and egg production per pen were recorded on a daily basis and average weekly egg production was calculated by dividing the number of eggs collected by the number of hen days and finally analyzed for any variations among the group. The effect of feed deficiency and *Eimeria* challenge on the chicken's performance was compared with control groups. Body weight was again collected at day 4, day 8 and day 16 post-infection.

Experiment 2: Individual body weight of chickens was collected weekly for three consecutive weeks. On day 21, infection groups were challenged with *Eimeria tenella*. At day 4 and day 8 of post-challenge individual body weights of chickens were again collected to evaluate the effect of challenges on the body weight.

3.7. Data Analysis

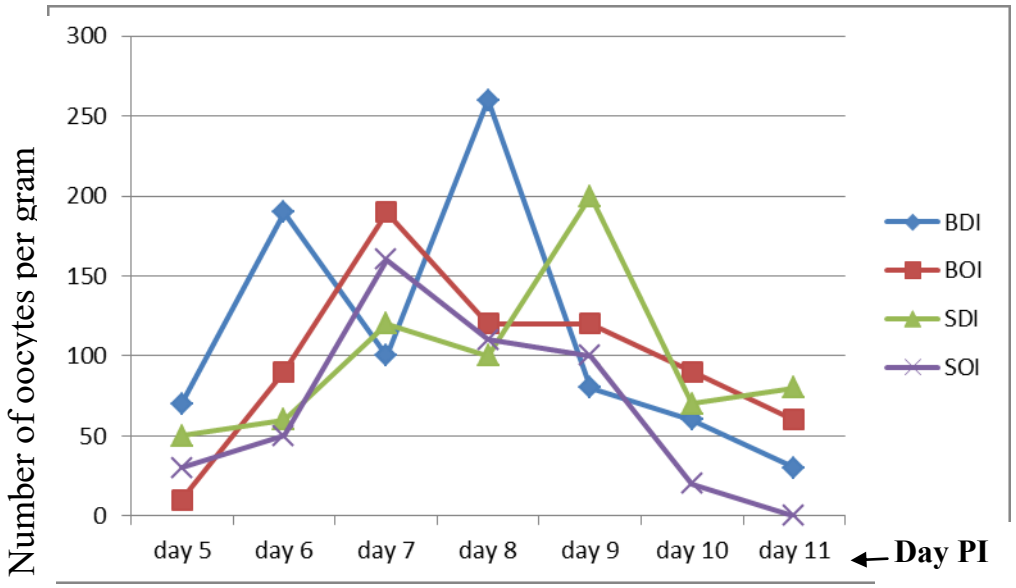
All data were recorded on the experimental log book and the data obtained were stored in Microsoft excel-2010 and analyzed by using the R statistical package. Descriptive statistics and graphs were produced using data analysis tools in Microsoft Excel-2010. The mean and standard deviation were calculated to describe the hematological parameters, body weight gain, oocytes count, and egg production recorded during the study period. Continuous response variables listed above were analyzed using the Statistical tool ANOVA to analyze the variation among the experimental groups.

Linear modeling analysis was used for the comparisons of the association between experimental groups and to determine the association between groups in response to challenge and/or feed restriction was analyzed. The significance level for all statistical tests was determined at 95% confidence limits ($P < 0.05$).

4. RESULTS

4.1. Oocyst Count

In experiment 1: overall, very few oocysts were detected in both breeds of chicken starting from day 5 through day 11 post-infection (32 weeks of age) in infected groups whereas the non-infected control groups in both breeds showed no Ccccidia oocyst in their droppings throughout the experimental period. None of the infected birds has shown signs of clinical coccidiosis. There was no significant difference ($P > 0.05$) in oocyst per gram between the two feeding regimes and the two breeds of infection groups (figure 11)



*BDI = Bovans brown feed deficient infected; BOI= Bovans brown optimum feeding infection, SDI= Sasso feed deficient infected; DOI= Sasso optimum infected, PI = post infection

Figure 10:- Number of oocytes counted in infection groups

In experiment 2, Oocysts were first detected in the feces of the infected group on the 5th day post-infection and continued to day 8 of the observation period (chicken age 29 days) in both breeds. In both breeds, there was a gradual increase in OPG count. All control groups of both breeds showed no oocyst production in their droppings throughout the experimental

period (figure 12). There was no significant difference ($p > 0.05$).in between both breeds of challenged groups in total oocyst production (Table 4). However, there was significantly higher OPG count in challenged groups than in control groups ($p < 0.05$).

Table 3 :- Oocyst per gram count in young chicken

	Average Oocyst per gram			
Breed	5DPI	6DPI	7DPI	8DPI
Sasso infected	110	2,920	152,670	161,620
Bovans infected	30	2,100	107,510	114,360
P-values				
Breed	0.182	0.420	0.907	0.898

*DPI= Day post infection

As described in (Table 4), however the variation among both breed is not statistically significant in oocyst shedding, the oocyst count of *E. tenella* infection showed slightly higher in Sasso breed infection group than Bovans brown infection group.

4.2. Packed Cell Volume

Packed cell volume is an important characteristic measurement to express the resistance to coccidiosis among the bird population.

In experiment 1: The effect of 1 x 10^4 (10,000) Houghton strain *Eimeria tenella* infection on the adult layer of Sasso T451A and Bovans brown breeds did not significantly ($P > 0.05$) affect the PCV value of infection groups as compared to the control groups. PCV at day 4, day 8, and day16 post-infection was not statistically different ($P > 0.05$) between breeds, and between the two feeding regimes (Table 5).

Table 4 : Packed cell volume of adult layer

Experimental groups	PCV value at different sampling points (%)		
	Day 4 PI	Day 8 PI	Day 16 PI
Bovans brown DC	33	33.4	33.4
Bovans brown DI	32.4	32.8	33
Bovans brown OC	33.4	32.8	33
Bovans brown OI	33	33.2	33.2
Sasso DC	32.8	33.5	33.75
Sasso DI	33.8	33.2	33.2
Sasso OC	33.8	33.6	33.6
Sasso OI	33.4	33.6	33.8
P-values			
• **Breed**	0.286	0.331	0.408
• **Infection**	0.667	0.652	0.869
• **Feed**	0.392	0.824	0.866
• **Breed x Infection x feed**	0.830	0.770	0.643

*DC= deficient control; DI=deficient infection; OC= optimum control; OI = optimum infection; PI= post infection; PCV= Packed cell volume

Experiment 2: The effect of coccidiosis on PCV values of young-age chickens of the two breeds was analyzed by comparing the infection groups with control groups. The differences in mean PCV value between the infected and control groups of Sasso and Bovans brown breeds showed statistically significant ($p<0.05$) at day 8 post challenge (Table 6). The packed cell volume level between the breed was not statistically significant at both sampling points.

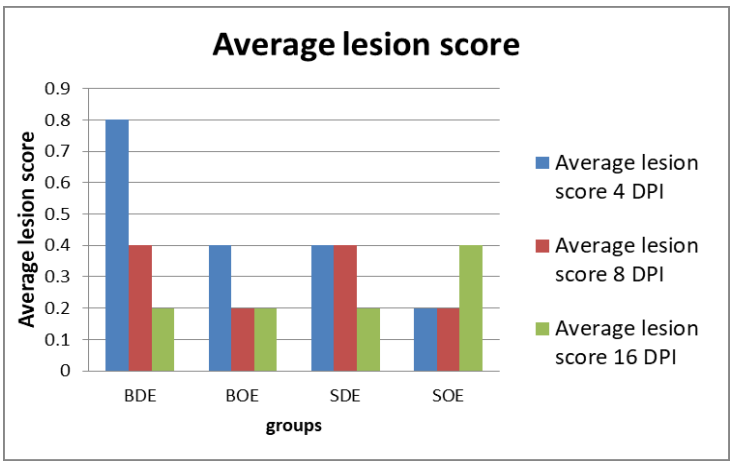
Table 5 : Packed cell volume data young chickens

Breed	Challenge	Days of post infection	
		Day 4 PI	Day 8 PI
Sasso	Control	34.90	34.60
Sasso	Infected	34.30	32.30
Bovans	Control	34.80	35.20
Bovans	Infected	33.60	32.60
P-values			
Breed		0.543	0.466
Challenge		0.176	<.001
Breed x challenge		0.648	0.808

4.3. Pathological Changes

4.3.1. Gross lesion score

Experiment: 1 As described in (Figure 12) the 10% feed deficient infected Bovans brown group showed significantly higher ($P < 0.05$) lesion score as compared to the other groups whereas there was no significant difference among other groups. All chickens in the non-infected control groups showed no detectable cecal lesions. Cecal lesion score due to *Eimeria tenella* infection in the adult layer was less than grade 2.



** BDE= Bovans 10% feed deficient infected with *Eimeria tenella*, BOE = Bovans Optimum feeding infected with *Eimeria tenella*, SDE = Sasso 10% feed deficient infected with *Eimeria tenella* , SOE= Sasso Optimum feeding infected with *Eimeria tenella*

Figure 11: Average lesion score of adult chicken

Table 6 : Adult and young chickens lesion score data

Significance level of lesion score at different sampling point						
		First experiment			Second experiment	
	P-values	4 DPI	8 DPI	16 DPI	4 DPI	8 DPI
➢	**Breed**	0.09	0.95	0.18	0.027	0.318
➢	**Feed**	0.69	0.34	0.17	-	-

*DPI= Day post infection,

In experiment 2: The mean lesion score in the challenged groups was significantly higher than the control groups ($p = 0.005$) at day 4 PI and ($p \leq 0.001$) at day 8 post-challenge. Sasso breeds infection groups were significantly higher($p \leq 0.05$) at day 4 post-infection in lesion score as compared to Bovans brown infection group However, there was no difference in cecal lesion score in both breeds at day 8 post-challenge and no lesion was observed in the control groups (Table 7)

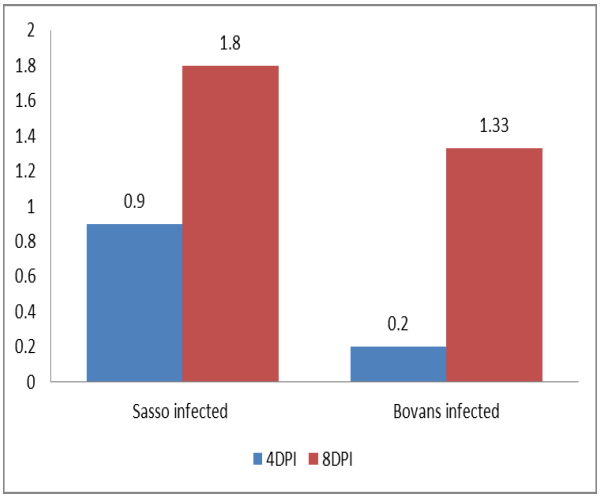
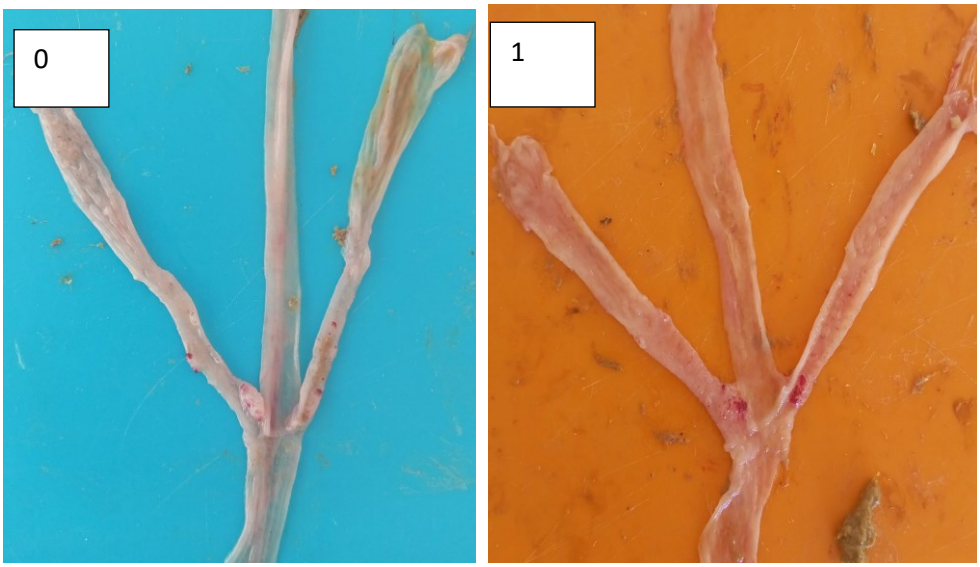
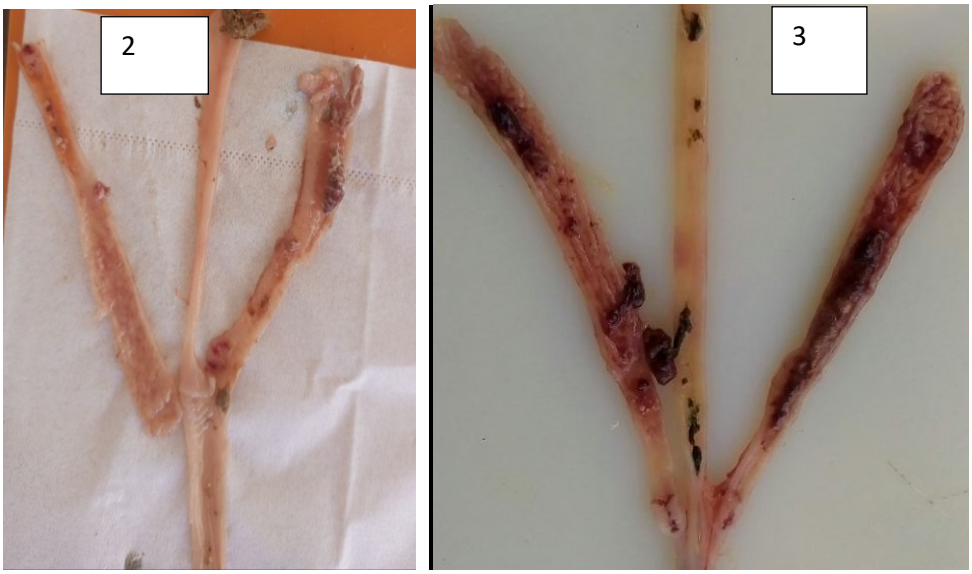


Figure 12: Average lesion score of chicken infected young chicken

Birds with lesion scores of 4 were absent in all infected groups. The Sasso infection groups were presented with a slightly higher macroscopic lesion score in the cecum as compared to Bovans brown infection group. No macroscopic lesion linked to *Eimeria tenella* was observed in the unchallenged groups. Lesion scores observed were $\leq$ 3 in the young chicken experiment (second experiment).



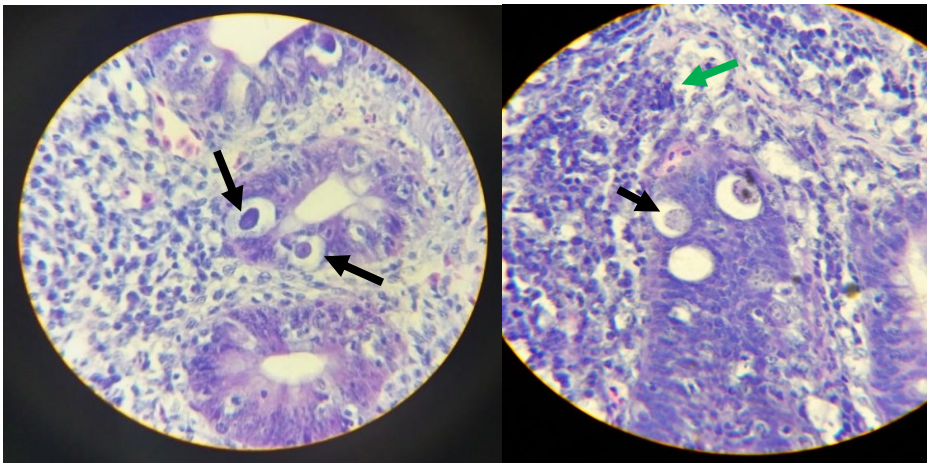


* (0) = normal; (1) = grade 1 lesion score; (2) = grade 2 lesion score; (3) grade 3 lesion score

Figure 13: Gross pathological lesion from control and infected chicken

4.3.2. Histopathological lesions

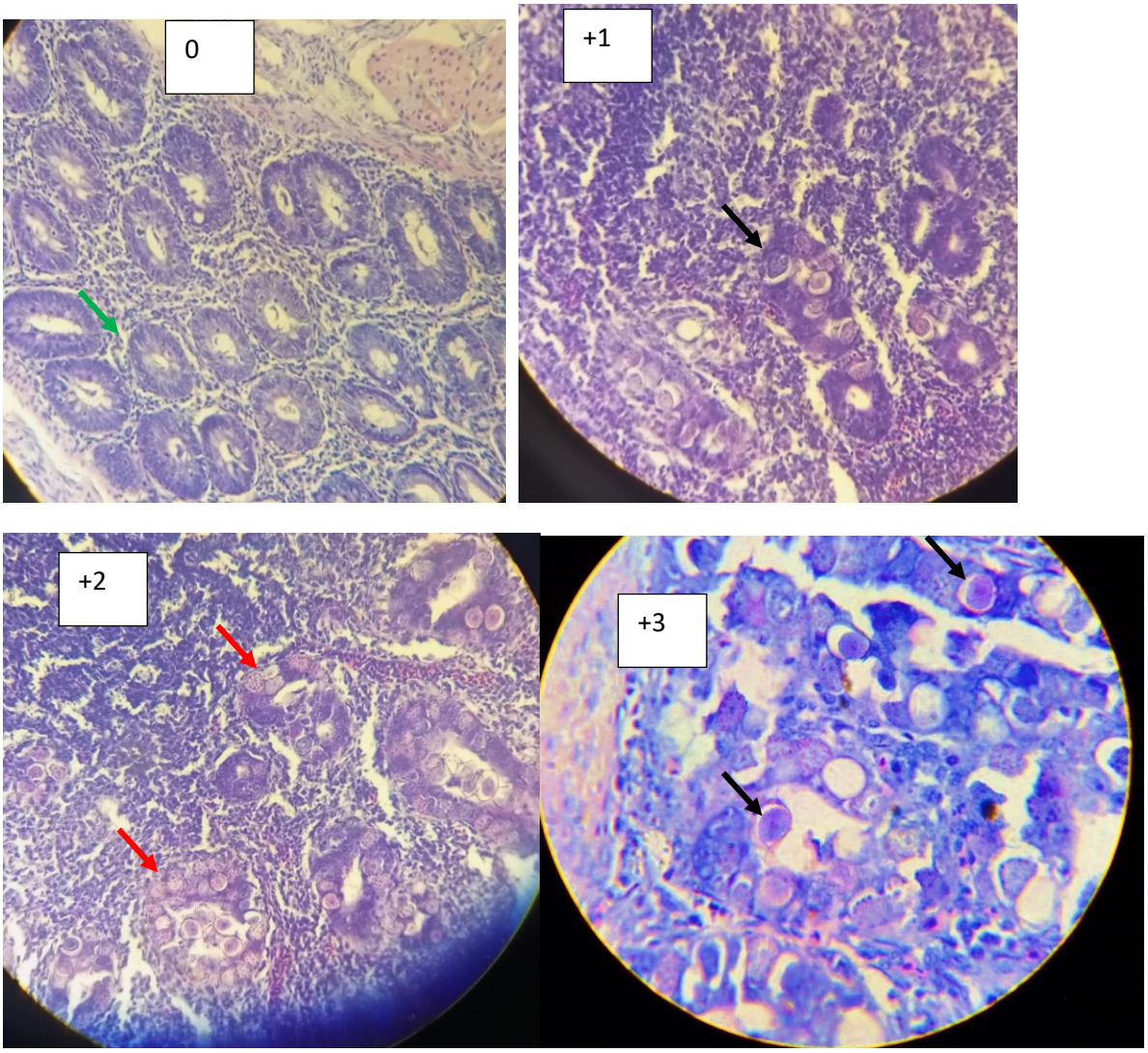
In experiment 1: Histopathology lesions observed in the cecum section showed the presence of few developing oocysts and infiltration of a few inflammatory cells with mildly hypertrophied caecal glands (Figure 15)



*Black arrow showed few developing schizonts in the sectioned cecum green arrow indicate infiltration of inflammatory cell

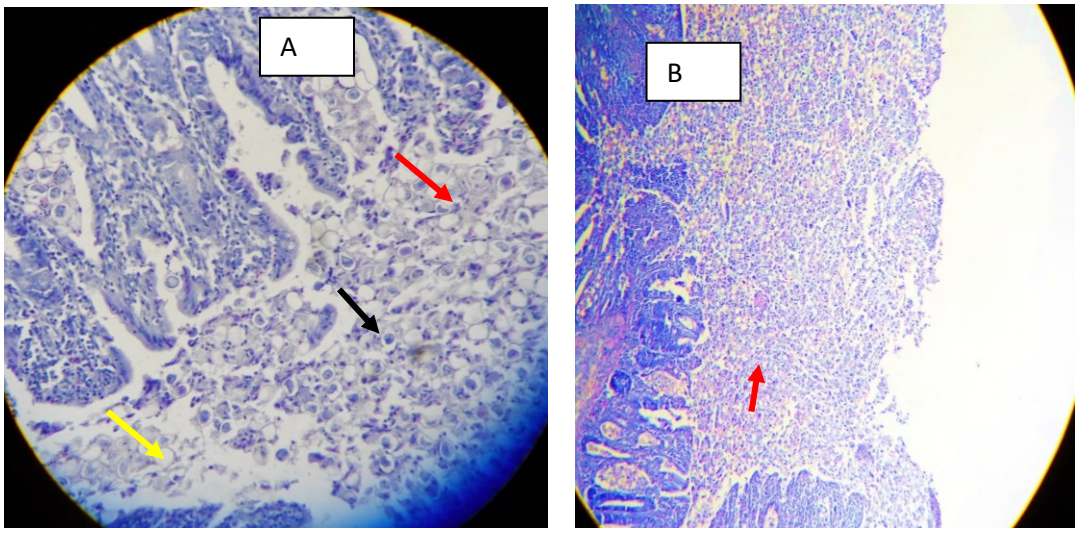
Figure 14: Adult layer Histopathology slide (40x)

In experiment 2, Pathological lesions observed in the cecum section showed necrotized epithelial cells, severe hemorrhage in the lamina propria, thickened muscular layers, the crypt cells were highly invaded with the developmental stages of *E. tenella* that their morphology is almost disappeared and accumulations of the inflammatory cell (mononuclear cells and eosinophils) were observed in the intestinal epithelium. Microscopic examinations of the affected caeca with lesion score grade three showed severe tissue damage and plenty of schizonts and oocysts in the epithelium (Figure 16). Histological slide prepared from cecum of control groups showed no any developmental stage of oocyst and normal structure of cecum with normal size and shape of caecal gland



*(0) = the histological slide prepared from cecum of control groups; (+1) = histological slide prepared from lesion score 1; (+2) = histpathological slide of lesion score grade 2; and (+3) = histpathological slide of lesion score grade 3; Green arrow indicate the normal cecal gland stracture Black arrow indicate developing schizonts in cecal glands, and red arrow shows the hypertrophied cecal gland

Figure 15: second experiment Histopathology slide under microscope (40X and 100X)



Black arrow indicate developing schizonts, red arrow erosion of epithelial cell and yellow arrow points at accumulation tissue debris; * (A)= by 40X objective lenses magnification; (B)= by 10X objective lenses magnification

Figure 16: Photomicrograph of chicken intestine showing sloughing of the villi and *Eimeria* oocyst

4.4. Chicken Production Performance

4.4.1 Body weight gain

In the first experiment, Quantitative feed restriction (QFR) (10%) data of both Bovans brown and SassoT451A commercial breeds showed that there was a statistically significant difference ($p < 0.05$) in body weight gain (BWG). The effect of the feed restriction regime has played a significant role in the reduction of body weight as compared to optimum

feeding at post challenge, but the *Eimeria* challenge was not associated with in body weight gain at day 4, day 8 and day 16 post-challenge period (Table 8).

Table 7: Factorial body weight gain data analysis of during and post challenge (in gram)

Factors	Week 30 (Day 0 of infection)	Day 4PI	Day 8 PI	Day 16 PI
Bovans brown				
• **BDC**	1480.7	1524.1	1555.6	1501.3
• **BDI**	1471	1522	1539.3	1538.7
• **BOC**	1708.5	1756.6	1767.7	1805.8
• **BOI**	1704.2	1743.4	1781.5	1794.1
P- value				
Feed	< 0.05	< 0.05	< 0.05	< 0.05
Challenge	0.765	0.718	0.982	0.829
Feed * challenge	0.914	0.80	0.575	0.656
Sasso T451A P-value				
• **SDC**	2536.4	2566.9	2597	2592.9
• **SDI**	2533.8	2559.2	2588.7	2584.7
• **SOC**	2893.7	2978	3026.4	3085
• **SOI**	3022.1	3090.2	3142.1	3140
P- value				
Feed	< 0.05	< 0.05	< 0.05	< 0.05
Challenge	0.143	0.214	0.637	0.959
Feed * challenge	0.120	0.183	0.283	0.682

*PI = post infection, BOC= Bovans brown optimum feeding control group; BOI= Bovans brown optimum feeding infection group BDC= Bovans brown 10% feed deficient control group; BDI= Bovans brown 10% feed deficient infection SOC= Sasso optimum feeding control group; SDI= Sasso 10% feed deficient infection group

In the second experiment: post-challenge individual body weight was collected at 4th DPI and 8th DPI. The chicks were challenged on day 21. No significant differences were observed in mean body weight gain of both breeds at day 4 and day 8 post challenges between challenged and control groups (Table 9).

Table 8: Growth performance young chicken in gram

Breed	Challenge	BW 1week	BW 4 DPI	BW 8 DPI
Sasso	Control	89	361	444
Sasso	Infected	86	351	436
Bovans	Control	63	212	252
Bovans	Infected	64	200	235
P-values				
Breed		<.001	<.001	<.001
Challenge		0.600	0.088	0.128
Breed x challenge		0.066	0.917	0.567

*BW= Body weight; DPI= days post infection

4.4.2. Eggs production

In the first experiment, Eggs production rate and eggs weight of 10% quantitative feed restricted Bovans brown and Sasso T451A groups were significantly(p <0.05) less than the optimum groups (Tabel 10). As visualized in (figure 18), as chickens have continued with10% qualitative feed deficiency, variation in eggs production between optimum feeding and 10% feed deficient groups of Bovans Brown and Sasso T451A breed remain significant. But, there was no significant variation among the control and challenged groups of similar feeding regimes.

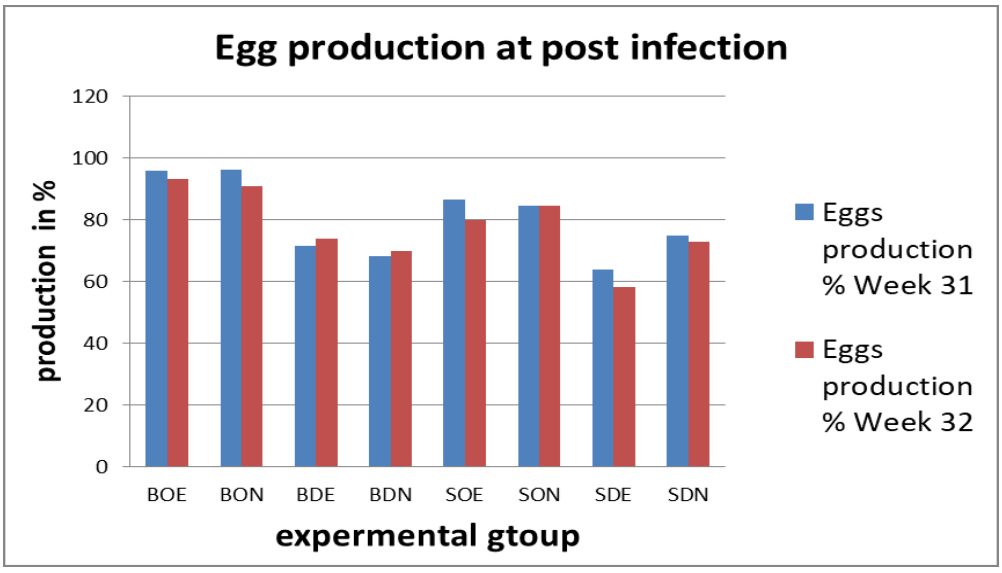


Figure 17: Eggs production in percent %

Table 9 :- Bovans brown and Sasso breed eggs production and eggs weight data analysis

Variable	**Mean**	**95% CL**		**p. value**
		Lower	upper	
BO Eggs production	84.20±20.6	79.43	88.98	< .001
BD Eggs production	65.71± 30.1	58.74	72.67	
SO Eggs production	49.88 ±29.6	42.78	56.98	
SD Eggs production	36.68 ± 33.6	28.62	44.75	0.0155
BO eggs weight	55.8±4.36	54.79	56.81	
BD eggs weight	53.14±4.03	52.2	54.07	< .001
SD eggs weight	39.94±18.63	43.66	52.61	
SO eggs weight	48.14 ± 25.1	33.91	45.96	0.0310

*CL= Confidence interval; BO= Bovans brown optimum feeding group; BD= Bovans brown 10% feed deficient group; SO= Sasso optimum feeding group; SD= Sasso 10% feed deficient group

5. DISCUSSION

Coccidiosis is known to be the most prevalent and most important disease of poultry production worldwide. Its prevalence and economic significance have been reviewed by different authors in the different poultry production systems. In developing countries, poultry production has a principal role in poverty reduction and food security. Thus, this study is important to know the factors increasing the severity of clinical coccidiosis in genetically selected breeds for improved poultry production at commercial poultry farms and the smallholder farmer's level.

The purpose of the study was to investigate the effects of experimental infection of 1 x 10^4 for the first experiment (adult layer) and 3 x 10^4 for the second experiment (young chicken) *Eimeria tenella* sporulated oocyst on chicken's performance, oocyst shedding, hematological change, gross pathological lesion and histopathological change in Sasso T451A and Bovans brown breeds.

Experiment 1

In the first experiment, oocyst count was very low in all infected groups, and consequently, cecal lesion scores were also low. It is assumed that Birds were infected at a relatively older age. Adult chickens are said to be resistant to coccidian infections (Geetha and Palanivel, (2018); El-Shall *et al*., (2022); Dalloul and Lillehoj, 2006). It is also possible that the dose administered to establish infection might have been inadequate to produce the desired change.

Restricted feeding produced relatively higher cecal lesion scores in adult Bovans brown chickens as compared to the other infected groups suggesting that chicken of this breed will be more susceptible to coccidian infection if they are not provided with adequate feeding. A similar finding by McNaughton *et al.* (1990) reported that greater coccidiosis lesion scores were observed in feed restricted broilers compared to that fed *ad libitum*. In contrast, Zulkifli *et al*. (1993) have reported that chickens kept under a feed restriction regime for three weeks before *E. tenella* inoculation had lower lesion scores as compared to *ad libitum*

birds. Such difference could be due to the difference in the duration of restricted feeding; the duration of suboptimal feeding went on for 23 weeks.

Comparative PCV results of the data obtained from the adult layer control group and challenged groups of both optimum feeding and feed deficient groups have shown that oral dosing of *E. tenella* in adult layer chicken did not significantly affect PCV of infected chickens as compared to control groups. Similar finding was reported by Jataul *et al*. (2014) who have reported that the differences in mean PCV between the infected and control groups of both Cobb and Marshal broiler breeds were not statistically significant ($P > 0.05$) in response to the low infection of *Eimeria tenella* sporulated oocysts

In the first experiment where adult layer chickens were used, the first oocysts was detected at day 5 post-infection. The number of oocyst counted was very low as compared to the second experiment in which much younger animals were used suggesting the contribution of the age factor in the development of resistance to coccidian infection. Similar fiding have reported by different authors as young chickens are more susceptible than the adult layers. For example Mohammed and Sunday, (2015); Dalloul and Lillehoj, (2006) have described that resistance to the *Eimeria* infection usually increases with the age of the birds and older chickens are relatively resistant infection. Most of the *Eimeria* spp. affects birds between 3 and 18 weeks of age group (Geetha and Palanivel, 2018; El-Shall *et al*., 2022).

In the first experiment, there was no significant difference between control and infection group in the body weight of both breed. However, the birds assigned to the 10 % quantitative feed restriction groups were significantly lower ($p<0.05$) in body weight gain during feed restriction periods than those assigned to the optimum feeding group. This experimental finding was agreed with the finding of Jang, *et al*. (2009), who find weight gain of broiler birds assigned to the feed restricted groups was significantly lower ($p<0.05$) in body weight gain during the feed restriction period (8 to14 d) than those assigned to the control group. According to the report of (Victoria *et al*., 2005; Pinchasov *et al*., 1993; and Tolkamp *et al.* 2005), the variation in body weight gain was highly affected due to the effect quantitative feed restriction

The optimum feeding groups had significantly higher egg production and egg weight as compared to the feed deficient group. The findings by Robinson *et al.* (2007) reflected that even small degrees of over or underfeeding might negatively affect egg and chick production. Egg weight is better in hens that were given *ad libitum* access to feed than feed restricted chicken. Simeneh. (2019) have also reported that feeding level contributed substantially to egg size. Though, there was no significant difference between control and infected groups of layer on eggs production performance.

Experiment 2

Following infection of young chicken, all infected birds remained negative for oocysts until day 5 at which the parasite starts to appear in feces. On the other hand, all non-infected control groups remained negative throughout the experimental period. However oocysts output was increased from 5 to 8 dpi in the infection groups of young chickens. This finding was similar to the finding of Tippayaporn and Thanyakorn, (2021) who found the first oocyst in the feceas of infected chicken was at day 5 PI. However, this result does not agree with Wageha *et al*. (2018) who found that oocyst shedding started at 6 dpi. There were differences in oocyst shedding because of different reproductive rates, differences in the number of schizont stages and the rate at which the stages develop (Cha *et al*. 2018; Tippayaporn and Thanyakorn, 2021). Oocyst shedding has been shown to be a useful way to determine the level of *Eimeria* infection (Du *et al*., 2005; Jordan *et al*., 2011; Lee *et al*., 2009). These results indicate that the number of oocysts recovered from fecal material is an accurate indication of the infection level and correlated to the grade of average lesion scores (Jordan *et al*., 2011).

The infected young birds have shown clinical signs of weakness, reduced appetite, and bloody diarrhea, and oocysts were present in their faeces from day 5 post-challenge. Similar to the finding of Ogbe (2009) and Tippayaporn and Thanyakorn (2021). Clinical Coccidiosis was highly observed in young chickens challenged at 3 weeks, at this time the maternal antibody of young chickens was considered to be declined and the natural protection level of a chicken was not enough to be capable of the infection. A similar finding was observed by Lobago et al., (2005) they have described that Coccidial infection has significantly affected chickens at age of 3 to 4 weeks as compared to older chickens. Infections in adult chickens

were very mild because adult chickens are relatively more resistant than young chickens. The resistance level of chickens to Coccidiosis increase as the age of the chicken increase (Mohammed and Sunday, 2015; McDougland and Reid, 1997), which has reduced the occurrence of clinical Coccidiosis in the adult layer of both Sasso T451A and Bovans brown commercial breed.

Eimeria tenella were previously reported from different places in Ethiopia by several investigators; for instance, Getachew (2004), Lobago *et al*. (2005) and Chanie *et al.* (2009) have reported *E. tenella* in the different study areas. The severity of coccidial infection may vary with the isolated oocyst, number of oocysts ingested and the immune state of the bird. In this research, gross and microscopic pathological changes were used to demonstrate the severity of the infection in chickens experimentally infected with *E. tenella*. Similarly, Adamu *et al*.,(2013) have used the gross and microscopic pathology change to describe the severity of disease in chickens naturally infected with *E. tenella* and *E.brunette*. Additionally, PCV and OPG parameters have been used to get information about the incidence of infection and severity of coccidiosis. Similar reports have been discussed by other authors as PCV reduction is directly related to the reduction of blood constituents due to hemorrhage caused by the disease Wakenell (2010) and Adamu *et al*. (2013).

Lesion scoring is a common method used for determining the level of *Eimeria* infection (Jordan *et al*., 2011). Several studies have observed and scored the effect of *Eimeria* infection on the gut lesions (Johnson and Reid, 1970; Vermeulen *et al*., 2001; Williams 2005; Conway and Mckenzie 2007; Haug *et al*. 2008; Amer *et al*. 2010). In agreement with these previous studies, our study shows that *Eimeria* infection has a remarkable impact on gut lesions of infected young birds from both breeds (Sasso T451A and Bovans brown) having higher lesions scores as compared to non-infected birds on day 25 (4 days post-infection) and on day 29 (8 days post-infection). Destruction of the host's cecal tissue was caused by the parasite development and multiplication in the epithelial cells of cecal tissues and results in reducing feed intake and nutrient absorption, dehydration, blood loss, and increased susceptibility to other diseases (McDougald, 2003). In the young chickens experiment gross pathological lesion score up to grade 3 was observed in the day 8 post-challenge sampling. Though the high average lesion score was observed on day 8 post

challenges, at day 4 post challenge remarkable gross lesion scores up to grade 2 was observed in the challenged group. Sasso infected breed had a significantly higher lesion score as compared to Bovans brown at day 4 post-infection.

In the present study, observed gross pathological lesions were comparable with the findings of Ashenafi *et al*. (2004). and Adamu *et al*.,(2013), in which the most common post-mortem lesions of E. tenella infection were manifested by petechial to severe hemorrhage in the cecal wall, ballooning and thickened caecal loops of mucosa and enlarged caecum with clotted blood. This characteristic feature of E. tenella infection also agreed with the report of Melkamu, (2018) and Chapman (2014) who identified that caecal coccidiosis cause enlarged and distended caeca filled with blood and petechial hemorrhages were observed in *E. tenella* infected chickens.

In the current study, pathological lesions scores were more extensive in young chickens on 8 DPI than on day 4 PI. A similar finding was reported by Melkamu (2018) average lesion score on day 8 was higher. In this finding, lesion score has a strong relationship with the amount of total oocyst production. High oocyst production during the peak multiplication time of the protozoa in the intestine may result in extensive epithelial cell damage in the cecum. That is why the lesion score on day 8 is more severe than on day 4 and the OPG count on day 8 was a peak oocyst production in both genotypes of chicken.

The parasite is pathogenic and affects the caeca of chickens due to invasion of the mucosal lining of the caeca by different developmental stages of the parasite, loss of villi, disruption of caecal mucosa, cluster of schizonts in epithelial cell of the cecum, necrosis of caecal mucosa considerable numbers of oocyst in lamina propria of the caecum, presence of, lymphocytic and mononuclear cells infiltration in cecal mucosa and sloughing of epithelial lining was observed. Similar findings were reported by Soomro *et al*. (2001), and Adamu *et al*. (2013). The damage that occurs with the changes in the functional integrity of the intestine may lead to secondary bacterial infection and cellular infiltration as described by (Ogbe *et al*., 2010) and Lillehoj *et al*., (2000).

The presence of high numbers of oocysts, schizonts and severe tissue damage in the caeca of the young chicken experiment indicated the severity of infection due to *E. tenella*. similar to

the findings of Adamu *et al*. (2013), and Ashenafi *et al*., (2004), who described the most severe stage caused by *E. tenella* as the second generation schizont, which caused excessive tissue damage, bleeding, disruption of the caecal glands and destruction of the mucosa and muscularis layer.

Eimeria tenella infection is characterized by heavy loss of blood from the cecum and causes reduction in packed cell volume value. Thus, the change in PCV was considered in this study as a parameter to compare the natural resistance to coccidiosis. PCV is a sensitive variable to *E. tenella* infection due to excessive bleeding induced in the cecum. The reduction in PCV value in the infection groups of both young Bovans brown and Sasso T451A breed was significantly reduced (<.001) as compared to the control groups at day 8 PI. This result showed that both genotype chickens have been subjected to high blood loss due to blood vessels were disrupted when the schizonts mature and the merozoites were released. These results are similar with those obtained by Wakenell (2010), Melkamu *et al*., (2021), Dial (2010) and Tippayaporn and Thanyakorn, (2021) who reported lower value of PCV in chickens infected with *E. tenella* when they were compared to the uninfected controls. Ogbe *et al.* (2010) also reported a slight drop in the PCV, Hb and RBC counts in *E. tenella* infected broilers. PCV value at day 4 PI was not significantly reduced in the challenged group as compared to the control group. This finding indicates the presence of variation between day 4 and day 8 post-challenge PCV reduction in both breeds indicate that the severity of infection at day 8 post-infection is higher than day 4 PI which may be a subject for further investigation. A similar finding was carried out by (Adenaike *et al*., 2018) chicken PCV was found to be an important variable in distinguishing between infected and uninfected chickens with clinical coccidiosis.

No difference in body weight was observed between challenged and negative control groups in both breeds of chicken infected at a young age. The time between infection and end of the experiment was only eight days which might have contributed to the lack of difference. Pathophysiological changes may require time to be translated into body weight changes. Zulkifli *et al*. (1993) also reported a similar finding. They used 30,000 *Eimeria tenella* oocysts on 50 days old White Plymouth Rocks dual-purpose chicks and no difference in body weight was found from *E. tenella* inoculation between the control and infected groups.

On the other hand, an increasing levels of *E. tenella* infection have previously been associated with reduced body weight gain over a comparable timeframe in fast-growing broiler chickens (Boulton 2018) and (Conway *et al*., 1990), but this was not expected to be the case in the slower-growing layer lines used in the present study (Soutter *et al*., 2021).

6. CONCLUSION AND RECOMMENDATION

Very low oocyst count in adult chicken has made a significant number of oocyst and the counted number of oocysts was not strong enough to cause a high lesion score. Corresponding to the low oocyst count lesion score and PCV changes were not significant in infected adult chicken. This result indicated that the adult chicken might have better tolerance to cope up the effects of the clinical coccidian infection. Breed comparison shows only the feed restricted Bovans brown looks more susceptible among the adult infected groups. This finding indicate as however adult chickens are resistant to 10,000 *Eimeria tenella* oocyst infection but also 10% feed restricted Bovans brown breeds are prone to coccidian infection.

At a younger age, the body weight gain was significantly high in the Sasso breed and at adult age, egg production was significantly high in Bovans brown inversely at younger age Sasso infected groups was found more affected (more gut lesion) than Bovans brown and in adult layer (second experiment) feed restricted Bovans brown infection groups were significantly scored high lesion score. This result indicates that the Production performance of a chicken is negatively related to resistance capacity of chickens to cecal coccidiosis.

Young Experimental Chicks were highly challenged with *E. tenella* as determined by collected data. This study shows that coccidial challenge using 30,000 oocytes per chicken had a developed prominent cecal lesion, reduced packed cell volume value, and higher oocytes counts per gram of feces as compared to non-infected. Therefore, the following recommendations are forwarded:

- Keep older birds away from young chickens since old birds are relatively resistant and they can act as carriers to young susceptible chicks.
- Young chickens are susceptible to coccidian infection, therefore it is necessary to maintain all coccidial control measurements like the use of effective coccidiostat, good hygiene and sanitation on the farm in order to protect them from infection
- As far as coccidiosis is concerned, the adult Sasso breed might have relatively better tolerance to the disease plus feed deficiency and they could be recommended for sustainable poultry development at the smallholder level.

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Appendix I: Method for estimation of oocyst per gram feces (OPG) (MAFF, 1979).

1. Take 3 grams of fecal sample out of 24 hours pooled dropping and mix thoroughly with 42ml of tap water in a 120ml wide mouthed glass.
2. Pour fecal suspension through a wire mesh screen, collecting the filtrate in a clean bowl.
3. Mix the filtrate thoroughly to ensure that there is a uniform suspension of fecal material and transfer an aliquot to a centrifuge tube.
4. Centrifuge for 2 minutes at 1500 rpm.
5. Discard the supernatant fluid and emulsify the packed sediment by saturated salt solution (NaCl) until the volume equals that of the initial aliquot of filtrate.
6. Invert the tube several times until the sediment is evenly suspended and fill two chambers of a McMaster slide using a clean Pasteur pipette.
7. Count all the oocysts within the ruled area of the chambers using 10mm objective and 10x eyepiece and the average count of the two chambers were recorded. Then the mean value is calculated: (Conway and McKenzie, 1990).

$$OPG = \frac{X}{0.15} * 45 * \frac{1}{3}$$

Where X= number of oocysts counted

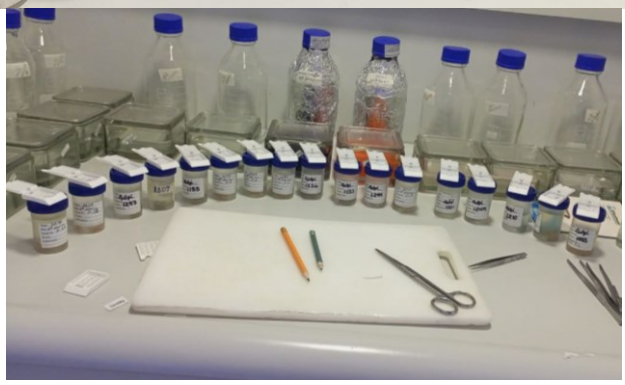
Vol. = 45 ml water that the feces is soaked

0.15= volume of the McMaster counting chamber

1/3 = correction factor

Appendix II Histopathological procedures (Takulder, 2007)

1. **Fixation** of tissue by 10% buffered formaldehyde
2. **Trimming** part of the tissue in a way that the lesion we require be included to fit standard histological processing and put in to plastic tissue cassettes



3. **Tissue Processing**: second fixation, dehydration, clearing and impregnating using an automatic tissue processer
   - Second tissue fixation by 10% buffered formaldehyde(
     - formalin- I for 2 hours and
     - formalin- II for 1 hour)
   - Dehydrating tissue by increasing alcohols concentration
     - 70% alcohol 1hour
     - 95% alcohol 1hour
     - 100% alcohol 1hour
     - 100% alcohol 2hours
     - 100% alcohol 2hours
   - clearing of tissue by xylene
     - xylene –I 1.30hour
     - xylene –II 1.30hours
     - xylene –III 1.30hours
   - impregnation of tissue by paraffin wax
     - Paraffin wax –I 2 hours
     - Paraffin wax –II 3 hours

4. **Embedding or blocking of processed tissue:** Impregnated tissues are placed in a mould with their labels and then fresh melted wax (54-60^{0}C) is poured and allowed to settle and solidify.

5. **Sectioning:** Sectioning of tissue in 5 micron meter thickness using sami-automatic microtome machine, tissue ribbons were spread on warm water bath and tissues were attached to albumenized glass slides. put on water bath to straighten the ribbon, and then adhere on the surface of frost ended and clear slide. Later label and put in incubator overnight to avoid paraffin wax.


6. **Staining:** manualy staining with Haematoxylin and Eosin to give color for sectioned tissue as the following staining procedure

   Hematoxyline eosine stain procedure (Talukder.S, 2007)

   - ❖ Deparaffinize slides in 3 changes of xylene for 5 minutes each.


- ❖ Rehydrate slides in 100% alcohol (3times for 3 minute each) and transfer to 95% alcohol for 3 minute and 70% alcohol for 3 minute
- ❖ rinse in running water
- ❖ Place in Hematoxylin for 10 - 15 minutes.
- ❖ Rinse in tap water.
- ❖ Stain in Eosin (Counter stain) (3dips).
- ❖ Rinse in tap water
- ❖ Dehydrate in ascending alcohol concentration (70 % 3 dips, 95 % 3 dips, 100% I for 3 minutes, 100% for 3 minutes & 100% III for 3 minutes).
- ❖ Cleared it in xylene (Xylene-I for 5 minutes , Xylene-II for 5 minutes & Xylene-III for 5 minutes
- ❖ Mount side with cover glass with DPX or Canada balsam
- ❖ Finally examined

7. **Microscopic examination:** stained slide is examined under microscope starting from the lower to higher magnification (4x, 10x, 40x and 100x) for the presence and identification of the microscopic lesions.

Appendix III: Method of lesion scoring for E. tenella. (Jeanne, 2015)

- The euthanized birds were necropsied to pull out the intestine. Paired ceca was separated from the intestine and transferred to a clean white bottomed metal dish along with their respective wing band.
- Ceca was then cut open to visualize the pathological change after the challenge infection.
- Gross lesion score scores will be recorded following Johnson & Reid (1970).
- The standard lesion scoring system for *Eimeria tenella* is as follows.
  - **0** = Ceca with no gross lesions.
  - **+1** = Very few scattered petechiae on the cecal wall; no thickening of the cecal walls and normal cecal content present
  - **+2** = Lesions more numerous with noticeable blood in the cecal contents; cecal wall is somewhat thickened; normal cecal contents present.
  - **+3** = Large amounts of blood or cecal cores present; cecal walls greatly thickened; little, if any, fecal contents in the ceca.
  - **+4** = cecal wall greatly distended with blood or large casseous cores; fecal debris lacking or included in cores.
- Birds dying of coccidiosis are scored as 4, regardless of the nature and magnitude of lesions that may be present.

Appendix IV: Bovans brown feeding guideline

REARING TABLE

Weeks	Age in days	Feed intake per bird per day (g)		Feed intake per bird cum. (g)		Body weight (g)	
		minimum	maximum	minimum	maximum	minimum	maximum
1	0-7	10	12	70	84	64	67
2	8-14	16	18	182	210	114	122
3	15-21	24	26	350	392	186	197
4	22-28	31	33	567	623	268	283
5	29-35	36	38	819	889	360	380
6	36-42	41	43	1106	1190	459	483
7	43-49	45	47	1421	1519	564	591
8	50-56	49	51	1764	1876	671	702
9	57-63	53	55	2135	2261	776	811
10	64-70	57	59	2534	2674	876	913
11	71-77	60	62	2954	3108	969	1009
12	78-84	63	65	3395	3563	1054	1099
13	85-91	66	68	3857	4039	1136	1186
14	92-98	69	71	4340	4536	1210	1265
15	99-105	72	74	4844	5054	1277	1338
16	106-112	75	77	5369	5593	1344	1411
17	113-119	83	85	5950	6188	1402	1477
18	120-126	84	86	6538	6790	1455	1545

Appendix V: Sasso T451A livebody weight and feed/day of breed standard

T451A FEMALE PERFORMANCE

Growing period:

Week	Age in day	Live body weight	FEED g /day
1	1-7	102	AD LIBITUM
2	8-14	248	33
3	15-21	393	44
4	22-28	539	50
5	29-35	685	55
6	36-42	810	58
7	43-49	910	63
8	50-56	1010	66
9	57-63	1110	69
10	64-70	1210	72
11	71-77	1310	75
12	78-84	1410	79
13	85-91	1510	83
14	92-98	1610	87
15	99-105	1710	91
16	106-112	1810	94
17	113-119	1910	98
18	120-126	2010	102
19	127-133	2130	106
20	134-140	2250	108
21	141-147	2370	110
22	148-154	2470	112

Appendix VI Pathological change data collection format

No	Date	Chickens ID No.	Gross Pathological lesion scoring format				remark
			Types Hemorrhagic	cecal wall thickness	cecal content	grade (0-4)	