EVALUATION OF ANTICOCCIDIAL PROPERTIES OF THE METHANOL ROOT-BARK EXTRACT OF *ANNONA SENEGALENSIS* PERS.IN BROILERS EXPERIMENTALLY INFECTED WITH *EIMERIA TENELLA*

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FEBRUARY, 2018

DECLARATION

I declare that the work in this dissertation entitled **Evaluation of anticoccidial properties of methanol root-bark extract of *Annona senegalensis*Pers.in broiler chickens experimentally infected with *Eimeria tenella***has been carried out by me in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The imformation derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Alhaji Bukar MUHAMMA _____ _____

Signature Date

CERTIFICATION

This thesis entitled **EVALUATION OF ANTICOCCIDIAL PROPERTIES OF METHANOL ROOT-BARK EXTRACT OF *ANNONA SENEGALENSIS* PERS. IN BROILERS EXPERIMENTALLY INFECTED WITH *EIMERIA TENELLA*** by Alhaji BukarMUHAMMAD meets the regulation governing the award of the degree of Masters in science in Veterinary protozoology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation

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DEDICATION

This dissertation is dedicated to my caring parents, who have shown me the value of education and also inspired me to pursue it, my brothers and sisters whose support and encouragement were essential to the completion of this study and my success.

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ABSTRACT

The study was conducted to evaluate the effect of methanol root-bark extract of *Anonna senegalensis* against experimental*Eimeria tenella* infection in broiler chickens on the basis of performance (weight gain) and pathogenicity (oocyst production, lesion score and percentage mortality). Phytochemical screening andacute toxicity studies of the extract were carried out using standard methods. Forty-twochicks were randomnly assigned to seven experimental groups of six birds each. The experimental design included positive (infected untreated and negative (uninfected untreated) control groups. Groups 1- 6 were inoculated orally with 2×10^4 sporulated oocysts at the 14th day of age except uninfected untreated group.*In vitro* anticoccidial activity test wasperformed by observing the effect of the extract on oocyst sporulation. Oocysts count was done on 5^{th}, 6^{th} 7^{th} and 8^{th} 9^{th} day after infection.Record of mortality was kept and postmortem of dead birds and lesion score were also performed. The phytochemical analysis of the methanol extract revealed the presence of alkaloids, tannins, saponins, glycosides, flavonoids, cardiac glycosides, steroid and terpenoids. Acute toxicity study of the extract revealed that the LD_{50} methanolic rootbark of the extract of Anonna senegalensis (MREAS) is more than 5000 mg/kg .Inhibition of oocyst sporulation *in vitro* depended on the concentration of the extract, being highest at the maximum concentration of the extract. Chicks medicated with *A. senegalensis* exract attained higher body weight than the non medicated challenged group. The highest mean weight gain after challenge with *E. tenella* at 42 days of age was high in group 5 (1450g). Lesion scores were significantly reduced in groups 4 and 5 (Group treated with 1600 mg/kg and amprolium), while groups 1 and 2 (200mg/kg and 400mg/kg treated groups) showed slight improvement in comparism with the former. Mortality of 5.5%, 2.8%. 2.8% and 16.6% was recorded for groups 1, 2, 3 and 6

respectively. Histopathological findings revealed haemorrhages in the caeca, cellular infiltration, presence of endogenous parasitic stage and desquamation of epithelia. The oocysts output per bird in the treated groups were lower than that in the challenged control group. The reduced oocysts output at 1600 mg/kg dose level suggested that *A. senegalensis* could be beneficial for management and control of coccidiosis in broilers. This anticoccidial effect of the extract was, however, lower than those exhibited by amprolium.

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LIST OF ABBREVIATIONS, DEFINITIONS AND SYMBOLS USED

ABUCAUC: Ahmadu Bello University committee on animal use and care

Ad libitum: Allowed unrestricted access

ALP: Alkaline phosphatase

ALT: Alanine aminotransferase

ANOVA: Analysis of variance

AST:Aspartate aminotransferase

CE: Competetive exclusion

$CD4^+$:T- helper cells

$CD8^+$: C- cytotoxic cells

CGE: Clean green and ethical

DNA: Deoxyribonucleic Acid

dpi:day post infection

E. coli: Eschericial coli

eg: Example

E. tenella: *Eimeria tenella*

et al: And others

etc: et cetera

Etmic2: *Eimeria tenella* microneme recombinant gene

°C: Degree centigrade (degree Celcius)

FAOSTAT:Food and Agriculture Organsation Statistic Division

FOS: Fructoogolisaccaride

G1: Group 1

G2: Group 2

G3: Group 3

G4: Group 4

G5:Group 5

G6:Group 6

G7: Group 7

g: Gram

GIT: Gastrointestinal Tract

H&E: Hematoxyline and eosin

IBD: Infectious Bursal Disease

IgA:Immunoglobulin A

IgG:Immunoglobulin G

IgM:Immunoglobulin M

Kg: Kilogram

ITS: Internal Transcribed Spacer

$LD_{50:}$ Median lethal dose

LPS:Lipopolysaccharide

ml: millimeter

mg:milligram

MOS: Mannanoligossacharide

Nacl: Sodium chloride

NRC: National Research Council

NVRI: National Veterinary Research Institute

OPG: Oocyst per gram

PCR: Polymerase chain reaction

%: per cent

PH: potential Hydrogen ions concentration

rpm: rotation per minute

ROS: Reactive oxygen species

S.D:Standard deviation

Spp:Species

TPGS: D-∝- Tocopheryl Polyethylene Glycol 1000 Succinate

USA: United States of America

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Coccidiosis is a parasitic disease of the intestinal tract of animals caused by single-cell protozoa. The organisms can infect humans, birds, and livestock, as they are usually species-specific. The disease causes enormous financial losses in the poultry industry in Nigeria despite advances in chemotherapy (Chapman, 2000). Avian coccidiosis is caused by any of the species of the genus *Eimeria*, individually or in combination. Outbreaks usually result in enormous economic losses as a result of the associated morbidity and mortality (Oluyemi and Roberts, 2000). The disease leads to the extensive destruction of the intestinal epithelium which results in reduced feedd efficiency and body weight gain, as well as a temporary reduction in egg production (Min *et al*., 2004; Dalloul and Lillehoj, 2005). This has a considerable economic loss at the level of poultry industry.

In broilers the most prevalent species of *Eimeria* that affect poultry are *Eimeria tenella, Eimeria maxima, and Eimeria acervulina* (Shirley, 1995: Gyorke *et al.,* 2013), of which *Eimeria. tenella* is highly pathogenic, causing haemorrhagic diarrhoea and being responsible for great reduction in weight gain and high mortality.*Eimeria* infection is transmitted through hardy, thick walled sporulated oocysts which are able to survive for lengthy periods in the poultry litter and soil particles. Coccidiosis is a rapidly developing intestinal disease presenting with bloody diarrhoea and listlessness. It causes heavy mortality in affected flock. The losses due to coccidiosis are primarily by impaired feed conversion, depressed growth, and downgrading at processing and mortality (McDougald

and Roberts, 1988).The immunosuppressant effect of coccidiosis predisposes birds to other diseases such as viral and bacterial diseases (Fatumbi, 2001). Field experience indicates that a severe *Eimeria tenella* infection, due to contaminated litter is in many cases followed by intestinal *Eschecerial. coli* infection (Stroom and Sluis, 1999).While natural infection with *Eimeria* species induces immunity, vaccination procedures on a commercial scale have shown limited effectiveness, and current disease control remains largely dependent on routine use of anti-coccidial drugs in most countries (Dalloul and Lillehoj, 2005).

Recently several different live vaccines have been commercially developed and these are mostly composed of either virulent or attenuated parasite strains. Major disadvantages of live parasite vaccines are labour intensive in production and high cost due to inclusion of multiple parasite species in the vaccine. Another downside of vaccination is that one vaccine strain may not be efficient in all geographical areas (Chapman, 2000; Abbas, 2012). The lack of efficient vaccines and the increasing incidence of drug resistant strains and escalating public anxiety over chemical residues in meat and eggs mandated the development of alternative control methods. Also, such subunit vaccines would eliminate the danger of emerging resistant strains encountered with live vaccines, but unfortunately until efficient vaccines become commercially available, the poultry industry is forced to rely upon prophylactic chemotherapy to control coccidiosis. Furthermore, the introduction of alternative prevention/treatment measures such as non-chemical feed supplements that effectively enhance productivity and non-specific immunity may help limit the use of anticoccidials.Several Studies has been conducted on the effect of natural products on *Eimeria* infections; and protective immunity against these infections (Giannenas *et al.*,

*2003*and Rondón *et al*.,2006) and to find new substitutes to chemical anticoccidials (El-Khtam *et al*., 2014).Plant extracts have been found to posses antibacterial, antifungal, antiviral, antioxidant, anticarcinogenic, analgesic, insecticidal and growth promoting effects on livestock (Tipu *et al*., 2002; 2006; Ani *et al.,* 2013).Increased interest in the search for alternative treatment of disease (Dalloul and Lillehoj, 2006) led to the development and introduction of a lot of anticoccidial drugs and natural products in the poultry industry all over the world. (Shahbazfar *et al*., 2011; El-Khtam *et al*., 2014).

Anonna senegalenesis Pers (Annonaceae) which grows wild in tropical Africa is commonly known as wild custard apple, it is a sprawling shrub or small tree widely distributed in Africa (Ogbadoyi *et al*., 2007) and particularly in the savannah areas and near streams, and has immense medicinal value (Abubakar *et al*., 2007).The plant has aromatic flowers which are used to flavor food. Its fruit, yellow in colour when ripe, has a pleasant smell with sweet edible jelly. It produces seeds which are ovate in shape, very small in size and open by mechanicalexplosion (Abdullahi *et al.,* 2003). Earlier scientific investigation of the plant has revealed its activity against malaria (Balansard and Timon-David, 1985). The plant has also been shown to be effective as antiulcer/antacid, smooth muscle relaxant (Largasorn *et al*., 1994), antibacterial (Muazu *et a*l., 1994) and in the treatment of sores and colds (Nadkarni, 1988).*Annona* senegalensis have been reported to have antibacterial (Akpa and Oila, 2006), antidiarrheal (Suleiman *et al.,* 2008), anti-trypanocidal activities (Atawodi *et al.,* 2003; Ogbadoyi *et al.,* 2007), antimalarial (Balansard and Timon- David, 1985) and antimollucicidal activities (Sofowara and Adewunmi, 1980) and in cancer treatment (Graham *et al*., 2000).

1.2 Statement of the Research Problem

Coccidiosis is one of the major pandemics in the poultry industry.The disease causes severe economic impact on poultry by reducing performance and decreasing productivity (Zhang *et al.*, 2012) leading to great financial loss in the industry with enormous amount of money being spent towards prevention of the condition. Apart from causing disease and losses, subclinical infections (mild infections without showing symptoms) cause reduced feed conversion. Since feed expenses form some 70% of the cost of producing broiler chickens, the economic impact of coccidiosis is quite enormous (Jordan *et al.*, 2002; Vegad, 2004).

The use of modern anticoccidial drugs added to the feed constitude a good preventive measures against the disease and are convenient for large scale use, however prolonged use of these drugs inevitably leads to the emergence of resistant strains to most anticoccidial drugs (Peek and Landman, 2003). Similarly the use of coccidiostat in poultry feed could lead to the presence of drug residues in poultry products which may be potentially hazardous to consumers (Masiiwa, 1999). Vaccination is also another method for the control of coccidiosis. . Despite the development of good immune response able to protect the birds and its effect on decreasing the resistant to anticoccidials after mixing with the resistant strains, live vaccines induce lesions that may adversely affect the health of the birds and perhaps increase their susceptibility to secondary infection with pathogens, such as *Clostridium perfringes* causing necrotic enteritis (Chapman *et al.*, 2002). Another approach to the control of coccidiosis is by vaccination of birds with live *Eimeria* oocysts.Due to the drawback associated with live vaccines, attenuated vaccines (with

reduced pathogenecity) have been developed, however these vaccines increases production costs in more intensive systems.

1.3 Justification

Poultry is one of the most intensively reared of the domesticated species and one of the most developed and profitable animal production enterprises (Al-Jamaien *et al.,* 2013, Opara *et al.,* 2014). It's importance in improving the nutritional status and income of many small farmers and those with small land holdings has been recognized by various scholars and rural development agencies in the last two decades (Nnadi and Goerge, 2010; Al-Jamaien *et al*., 2013; Letebrhan *et al.,* 2015).

The poultry industry occupies an important position in the provision of animal protein (meat and egg) to man as well as manure for crops and generally plays a vital role in the national economy as a revenue provider and provides employment (Eduvie, 2002; Nnadi and George, 2010). In 1998, the poultry meat represented 28 % of the world total meat consumption compared to 26.5% for beef (Nnadi and George, 2010; Quiroz- Castañeda and Dantán-González, 2015). In Nigeria, the poultry population is estimated to be 160 million; comprising of 72.4 million chickens, 11.8 million ducks, 4.7 million guinea fowls, 15.2 million pigeons, and 0.2 million turkeys, which is estimated at US $250 millions. Backyard poultry constitute about 60% and thus the most important form of poultry production (Akintunde *et al*., 2015; Barde *et al.,* 2015; Omokaro, 2015). The industry contributes up to 15% to the country's gross domestic product (GDP) and accounts for 36% of total protein intake of the country (Akintunde *et al*., 2015

1.4 Aim of the Study

This study is aimed at evaluating the anti-coccidial effects of the methanol root bark extract of *Annona senegalensis* in broiler chickens experimentally infected with *Eimeria tenella.*

1.5 Specific Objectives of the Study

The objectives of the study were to:

- Conduct phytochemical analysis of the methanol rootbark extract of *Annona senegalensis.*
- Establish the safety or acute toxicity of the methanol crude extract of the root-bark of *Anonna. senegalensis*in broiler chickens
- Assess the *In vitro* anticoccidial activity of the methanol extract of the plant
- Determine the effect of the extract on oocyst shedding in broiler chicks infected with *Eimeriatenella* and treated with *Anonna senegalensis* root bark extract
- Determine the effects of the extract on clinical signs andperformance of broiler chicks experimentally infected with *Eimeria tenella* and treated with the extract.
- Determine effect on gross and histopathological changes on caecal lesion score of broiler chicks experimentally infected with *Eimeria tenella* and treated with the extract
- Determinebiochemicalchanges in broiler chickens experimentally infected with *Eimeria tenella* and treated with methanolextract of root-bark of *Anonna senegalensi*

1.6 Research Question

Does the methanol root-bark extract of *Annona senegalensis* have activity against *E tenella*in broiler chickens?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Preamble

Coccidia consist of a wide variety of single celled, parasitic animals in the sub kingdom protozoa of the phylum Apicomplexa (Conway and McKenzie, 2007). This phylum consist of protozoa that posses an apical complex, which by definition is an assembly of organelles located at the anterior end of certain life cycle stages that facilitate attachment and or entry into the host cell (Current *et al*., 1990). Within this phylum are two suborders known as *Adeleorina* and *Eimeriorina*. *Eimeria* which is a pathogen of concern to commercial poultry, belongs to the suborder *Eimeriorina* which consists of 10 famiilies, 37 genera, and around 1500 named species (Levine, 1982).The definition of coccidian genera is based on the morphology of infectious stages - sporulated oocysts, containing a fixed number of sporocysts, each possessing a constant number of sporozoites. However, this rigid definition is often in contradiction to results from molecular phylogeny (Eberhard *et al.* 1999). The genus *Eimeria* is the largest genus within coccidia, with more than 1700 described species. Majority of *Eimeria*species parasitize the gastrointestinal tract of vertebrates. Several species (e.g. *Eimeria intestinalis, E. necatrix, Eimeria stiedai, Eimeria tenella, Eimeria zuernii*) are important parasites of domestic animals that cause serious diseases with high morbidity and mortality. Sporulated oocyst contains 4 sporocysts, each filled with 2 sporozoites (Pellérdy 1974; Levine and Ivens, 1990).All seven species are ubiquitous and can occur on a farm in different combinations (Williams, 1998). *Eimeria acervulina, Eimeria maxima, Eimeria tenella, Eimeria. brunette, Eimeria mitis and Eimeria praecox* were present in litter samples within six weeks after a new flock

placement (Williams, 2005). The ability of the *Eimeria* species to thrive in an environment depends on the characteristics of the species, ubiquity of the species, climate and environmental conditions, sanitary conditions and the susceptibility of the of the host (Williams 1999). The oocysts are able to remain infective outside of the host for long periods, and their protective properties allow them to be resistant to many harsh chemicals and disinfectants (Eschenbacher *et al*., 1996; Jeurissen, 1996; Yun *et al.*, 2000). The *Eimeria* species have an extremely complex life cycle comprising stages both internal and external to the host. Infection occurs by ingesting sporulated coccidial oocysts found in contaminated litter, soil, feed or water. After ingestion, the protozoa go through a series of intracellular, extracellular, asexual, and sexual stages to produce viable oocysts that are excreted in the feces (Rose, 1987). After a brief period outside of the host, the oocysts become infective again through the process of sporulation, and the life cycle is complete.

2.2Avian Coccidiosis and Global Poultry Industry

Avian Coccidiosis is the most frequently reported disease of chickens worldwide and has generated an immense burden on the poultry industry, perhaps as high as US$3 billion globally per year, with a significant cost associated with in-feed anticoccidial medication (McDonald and Shirley, 2009; Dalloul and Lillehoj,2006).Finding a commercialized poultry flock not infected with coccidia is atypical and, in modern commercial setting, subclinical coccidiosis continues to be a persistent problem, whereas clinical coccidiosis is rare (Williams, 1999; Lee *et al*., 2009).

The chicken meat market represents a very dynamically developing area, with a significant increase in production with time. European and American countries dominated the chicken market fifty years ago with 79% of total production. However, by 2012, Asian and American countries contributed approximately 77% of total world production (FAOSTAT,2014).

The poultry industry raises approximately 40 billion chickens annually and coccidiosis is the most frequently reported disease of chickens worldwide (McDonald and Shirley, 2009;Williams, 1999). Lee *et al* (2009) suggested this disease has had the greatest impact on poultry production while Dalloul and Lillehoj (2005) determined that in-feed medication for prevention and treatment of this infection account for the major portion of the economic burden. However, Ruff (1999) estimated that the economic loss annually exceeding $1.5 billion US dollars is from a multitude of factors including decreased weight gain, decreased feed efficiency, decreased egg production in addition to the cost of treatment for the infection. It was determined by Ruff,(1999) that a decrease in feed efficiency where 0.01kg more feed is needed per one kilogram weight gain has the potential to cost the poultry industry $70 million US.

Coccidiosis is a disease that is very difficult to eradicate as the oocyst wall is resistant to environmental factors It has been found that an unsporulated oocyst can survive up to seven months in the caecal tissue and the sporulated oocyst can survive up to two yearsin the exogenous environment (Reid, 1990). Oocysts are resistant to the variety of disinfectants used in commercial poultry houses; thus, these environmentally resilient

oocysts can be transmitted among farms through movement of personnel or equipment (Reid, 1990; .Ruff,1999).

2.3 Causative Agent of Avian Coccidiosis

Coccidiosis is generally a disease of animals but predominantly chickens which are highly susceptible to about 11 different species of this single celled obligate intracellular parasite of almost exclusively the genus *Eimeria* (Phylum: Apicomplexa). *Eimeria* is an intracellular protozoan parasite in the epithelial cell of the intestine (Alberta, 2007; Patrick and Mgbere, 2010; Lai *et al.*, 2011).About 1800 *Eimeria* spp affect the intestinal mucosa of different animals and birds (Nematullahi *et al*., 2008; Muazu *et al*., 2008). In the domestic fowl (*Gallus gallus)*, nine different *Eimeria* species are described (Morgan *et al.*, 2009; Haug *et al*., 2008). Infection with a single species of coccidian are rare in natural conditions, mixed infection being the rule but nevertheless in many outbreaks, the clinical entity can be ascribed principally to one specie, occasionally a combination of two or three (Macmullin, 2001; Patrick and Mgbere, 2010).

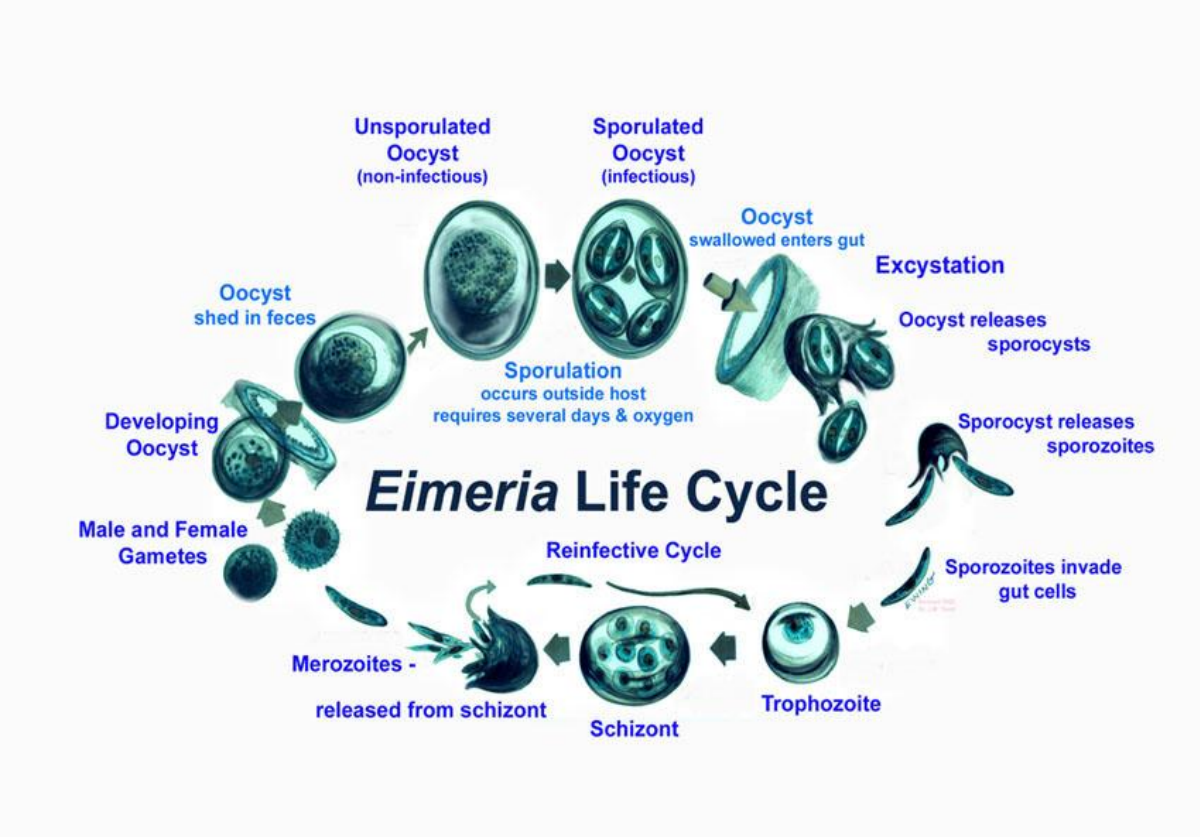
The species of *Eimeria* that are reported as highly pathogenic are *Eimeria brunetti, Eimeria maxima, Eimeria necatrix,* and *Eimeria tenella.* Species reported as mildly pathogenic include *Eimeria acervulina, Eimeria mitis,* and *Eimeria mivati,* whereas *Eimeria praecox* and*Eimeria hagani*are considered to be the least pathogenic (Nematollah*et al*.,2009; Jadhav*et al*., 2011).

2.4Life Cycle of *Eimeria*

The life cycles of *Eimeria* species. are complex, consisting of two developmental stages in the host: an exogenous stage (sporogony) and an endogenous stage (schizogony and gametogony). Some species vary in the number of asexual generations and in the time required for each developmental stage (Blake and Tomley, 2014)

During the exogenous phase, the unsporulated (noninfective) oocyst is excreted from the chicken and undergoes sporulation in the presence of moisture, warmth, and oxygen, thus becoming a sporulated (infective) oocyst.It involves a meiotic division followed by a mitotic division that results in the formation of four sporocysts; a mitotic division then occurs within each sporocyst to form two genetically identical haploid sporozoites. Sporulated oocysts of *Eimeria* contain four sporocysts, each containing two sporozoites. The 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 an unsporulated oocysts (Lal *et al.*, 2009; Norton and Chard. 1983). All subsequent stages in the life cycle after the meiotic division in the oocyst are haploid, thus mitotic division occurring within each schizont results in the formation of haploid merozoites which are all genetically identical and therefore represents a clonal population. Genetic homogeneity is also more probable because there is a real possibility of self-fertilization following gametogony, which will result in clonal offspring (Walker *et al.*, 2013).

Al least two generations of asexual development (sometimes as many as four) give rise to a sexual phase, where small, motile microgametes seek out macrogametes to form the zygote which matures into an oocyst that is released from the intestinal mucosa and is ultimately shed in the feces. Thus, the reproductive potential of a single ingested oocyst is fairly constant (McDoulgald, 2013).



The short life cycle (4–6 days, depending on the species) and the copious production of sporulating oocysts are advantageous for increasing the chances of infecting a large population of chickens.Infection occurs when the host ingests sporulated oocysts. Following ingestion, the microenvironment of the host digestive tract stimulates excystation of the oocyst in the gizzards resulting in the release of sporozoites that invade and destroy cells in the intestinal mucosa and begin the reproductive cell cycle. As a consequence, infected birds display symptoms of disease such as reduced feed intake,

bloody diarrhea, and hampered weight gain (Gilbert *et al.,* 2011; Dalloul and Lillehoj, 2005).

2.5 Immune Response to *Eimeria*Infection

Immune responses of chickens during coccidiosis are complex and specific to each *Eimeria* species, as immunity to one species generally confers little cross protection against others (Rose and Long, 1962). The immune response to coccidia is complex and depends on the *Eimeria* species and site of infection in the intestine. The oocyst lifecycle plays a role in the complexity of the immune response, because at each stage, there are stage specific antigens that are targets for the immune system (Brake *et al*., 1997). Similar to mammals, there are 3 principal antibodies in birds used for host defense: IgM, IgA, and IgY. Shortly after infection, B lymphocytes start to produce all 3 classes of antibodies specific to *Eimeria* antigens (Lillehoj *et al.,* 2004). Macrophages are able to detect surface antigens on sporozoites, together with antibodies they are effective in reducing the sexual reproduction of the oocysts but do not prevent the disease causing effects of sporozoites or merozoites (Lillehoj and Trout, 1996). Role of the bursa of Fabricius in immune response is unknown because bursectomized chickens, which produce a small amount of antibodies, still develop protective immunity from coccidia infection, and if vaccinated with a commercial coccidial vaccine, the bursectomized birds showed little difference from non bursectomized birds (Giambrone *et al*., 1981). Thus, adaptive immunity to *Eimeria i*s primarily cell mediated.Definitive characterization of cell mediated immunity to *Eimeria* infection has proved difficult. This could be due to the fact that cellular immune responses during coccidiosis are impacted by various factors including route, dose, and species of

Eimeria, age and genetics of the host bird, and timing of response measurements following infection (Swinkels *et al*., 2007; Velkers *et al*., 2010). Birds that survive the primary infection develop protective immunity, which allows the bird to be resistant to reinfection for several months (Lillehoj *et al*., 2004). T lymphocytes especially CD+8 T lymphocytes are thought to play the primary role in the immune response of the bird to *Eimeria.* Suppression of T lymphocyte populations increases susceptibility to *Eimeria* infection (Lillehoj and Trout, 1994). Coccidial infection significantly increases the CD+8 subpopulation of T lymphocytes (Swinkels *et al.*, 2007). Resistance to coccidia can be transferred through blood lymphocytes to naïve hosts (Lillehoj and Trout, 1996). T lymphocytes and macrophages work together to secrete cytokines that stop development of *Eimeria* once inside the cells (Dalloul and Lillehoj, 2004).

Though the role of the CD8+ T lymphocytes in controlling *Eimeria* infections is evident, it has been suggested that CD4+ T lymphocytes are important during primary infections while CD8+ cells are essential during secondary infections (Lillehoj, 1998). Chickens treated with anti CD4 antibodies shed more oocysts following a primary infection of *E. tenella* (Trout and Lillehoj, 1996). Single infections of *E. maxima* or *E. tenella* have led to an accession of CD4+ T cells in the duodenum and caeca respectively (Cornelissen *et al*., 2009). Hong *et al*. (2006c) reported a rise in CD4+ and CD8+ cells following an initial exposure to *E. maxima*. These results suggest that the different subsets of T lymphocytes work in concert in order to clear initial infections of *Eimeria* and provide enhanced protection against future encounters.

2.6 Host and Site Specifity of *Eimeria* Species

Eimeria are both host specific, with the denoted species affecting only domestic chickens, and site specific, meaning that certain species parasitize specific regions of the gut and intestinal epithelia. The reason for the exhibition of the host specifity of the parasite is largely unknown, but generally understood to be a combination of genetic, nutritional/biochemical, and immune factors (Yun *et al*., 2000). It should also be noted that while species of *Eimeria* are site specific and vary in the severity of their pathogenicity, interactions between different species as well as the condition of the host can have variable effects on the actual level of pathogenicity exhibited (Fernando, 1982). Investigations of this topic have led to the conclusions that while combined infections of varying concentrations of *Eimeria acervulina, Eimeria brunetti,* and *Eimeria maxima* led to increased weight loss, competition between the species led to reduced oocyst production by individual species except in the instance that the species possessed entirely different infection sites (Hein, 1976). One of the prevalent species of *Eimeria* encountered in commercial poultry is *Eimeria acervulina* which primarily attacks older chickens or replacement hens and possesses a low reproductive potential (Conway and McKenzie, 2007). This species manifests primarily in the mucosal epithelial cells of the duodenal loop, with gross lesions in light infections limited to that specific area. During heavy infections, however, it is possible for lesions to be found lower in the intestinal tract and to result in the destruction of the villus tips (McDougald and Fitz-Coy, 2008). Another species which has a tendency to colonize the upper region of the small intestine is *Eimeria mivati*. This species was originally identified as, and continues to be, incorrectly diagnosed as a strain of *Eimeria acervulina*, but was later named a separate species

(Conway and McKenzie, 2007). While *Eimeria mivati* is considered to primarily infect the upper duodenal region of the intestine, this species is also commonly found throughout the intestinal tract extending even as far as the caeca and cloaca in the most severe infections. *Eimeria mivati*, like *Eimeria acervulina*, affects the mucosal cells of the intestinal villi, but unlike *Eimeria acervulina*, can be commonly found through the entire length of the villi (McDougald and Fitz-Coy, 2008). Although infections of *Eimeria praecox* are not typically known to result in notable gross lesion development, this species is recognized as one which affects the epithelial cells of the sides of the villi inhabiting the duodenal loop (McDougald and Fitz-Coy, 2008).

There are two species of *Eimeria* that are noted for their infection in the mid small intestine. *Eimeria maxima* is considered one of the most prevalent species in chickens while *Eimeria necatrix* is referred to as one of the most virulent species afflicting chickens. Both occur in the midgut, however *Eimeria maxima* is most often found superficially in the epithelial cells of the intestinal mucosa with haemorrhages being found near the tips of the villi. *Eimeria necatrix* is a more invasive species with affected birds showing submucosa and lamina propria containing stages of coccidial development (McDougald and Fitz-Coy, 2008).

2.7Differences Among *Eimeria* Species

The different species of *Eimeria* vary in fecundityand reproductive potential (Parry *et al*., 1992; Al-Badri and Barta, 2012), infection location ranging from the duodenum to the caecum, and pathogenicity – from mild to severe (Reid and Long, 1979). Certain species of

Eimeria (*Eimeria mitis* and *Eimeria praecox*) cause reduce growth rate and feed conversion efficiency, whereas others may cause severe morbidity, with the aforementioned symptoms and bloody diarrhea or mortality (*Eimeria necatrix* and *Eimeria tenella*; (Reid and Long, 1979; Williams, 1999).

Eimeria oocysts can be categorized according to size, with minute variations in length and width, into the following categories small (*Eimeria acervulina*, *Eimeria. mivati* and *E.imeriamitis*), medium (*Eimeria necatrix*, *Eimeria tenella*, *Eimeria praecox*), and large (*Eimeria maxima*) (Reid and Long, 1979). However, species cannot be distinguished by simply using oocyst morphology (Reid and Long, 1979)]. A tentative diagnosis can be made based on the general characteristics of lesions and their location(s) regardless of whether oocysts are being shed (Long and Reid, 1979) Differences between the acquisiton of immunity for each *Eimeria* species have been observed (Rose and Long, 1962; Smith, 2002).

The degree of host specificity may significantly influence intraspecific genetic structure (Johnson *et al.*. 2002b). Many parasites reported as polyxenous form assemblages of morphologically indistinguishable but genetically distinct species/strains (Jousson *et al*., 2000; Demanche *et al*., 2001; Štefka and Hypša, 2008). A model of neutral evolution, depending only on the frequency of new mutations and probability of their fixation, serves as a null hypothesis for assessing genetic variability within a population.

2.8The Coccidian Oocysts

The oocyst is considered a remarkably hard and persistent structure, being resistant to mechanical and chemical damage and to proteolytic degradation (Belli *et al.*, 2006; Mai *et al.,* 2009). In actual case each layer of the oocyst wallresponds very differently to chemical treatments (Belli *et al.,* 2006; Shirley, 1995; Schares *et al.*, 2005). The wall is a structure that is composed of a bilayer, with an outer layer that can be as thick as 500 to 600 nm but that is eventually compacted to 200 nm or less. An inner zone separates the outer and inner layers, with the latter being approximately 40 nm thick (Ferguson *et al.,* 2003). The oocyst is noninfective in its unsporulated state and becomes infective when it sporulates (Lal *et al*., 2009; Norton and Chard, 1983)..

Basically, the composition of the two walls confers the oocyst with an outstanding resistance. The oocyst wall consists mainly of proteins and lipids. In addition, carbohydrates covalently bonded to proteins have also been reported, with varying percentages (1.5–19%). However, the composition of each layer is still unclear (Belli *et al*., 2006; Stottish *et al.,* 1978)..The inner wall is composed of a protein matrix embedded with as well as coated with lipids, while the outer wall is composed of quinone tanned proteins as well as protein-tyrosine crosslinks. Although the presence of cysteines has been reported, no cysteine bridges have been detected (Jolley *et al.,* 1976).

It is likely that the outer layer protects the oocyst from mechanical damage whereas the lipid-rich inner layer insulatesit from chemical attack (Belli *et al*., 2006).Being the result of the fusion between micro and macrogametes and are usually shed in faeces, the oocyst is the defining characteristic of the coccidian. In most cases, the oocyst is unsporulated and is considered as noninfectious when excreted and contains a single, undifferentiated

cytoplasmic mass protected by a wall.Sporulation do occur only under favourable conditions of humidity, temperature and oxygen (Canning and Anwar, 1968).. During this process, the cytoplasm divides into secondary sporoblasts, which develop a resistant wall and are then called sporocysts. Within the sporocysts, the sporozoites are formed and are considered as the infectious stages. The number of sporocysts varies between species of coccidians. For instance, in *Eimeria* there are four sporocysts, each with two sporozoites, while *Isospora* and *Toxoplasma* have two sporocysts with four sporozoites each (Belli *et al.*, 2006; Dubey and Lindsay, 1998)..

2.9Transmission of Avian Coccidiosis

Infection is by oral route, chickens become infected with *Eimeria* species. by ingesting infective oocysts from litter, soil and contaminated feed and water. The infected birds excrete oocysts in their faeces and are a source of infection for other birds as *Eimeria* species. can survive for long periods in infected birds and the environment (Khan *et al*., 2006) The oocysts in faeces become infective through the process of sporulation in about two days (Jeurissen *et al*., 1996)]. Species of *Eimeria* are transmitted via the faecal-oral route (Williams, 1999). The severity of infection is ultimately determined by the number of infective oocysts ingested by the chicken (Johnson *et al*., 1986). Oocysts in the environment may be transported by definitive hosts, asymptomatic avian hosts, rodents, flying insects, other invertebrate pests, contaminated feed, old litter, humans and other equipment used in the rearing facility (Johnson *et al.*, 1986). The ability of the *Eimeria* species to thrive in an environment depends on the characteristics of the species, ubiquity of the species, climate and environmental conditions, sanitary conditions and the

susceptibility of the breed of the birds (Williams, 1999). Oxygen, moisture and warm litter are the ideal environment for excreted oocysts to sporulate (Williams,2002). *Eimeria tenella* sporulation is optimal at 28°C, with a decline in sporulation rates beginning at 20°C and stops at 8°C (Reid *et al*., 1976). A lethal temperature of 55°C has been demonstrated to kill oocysts as well as freezing temperatures; however oocysts survival in the field is extended by the presence of high humidity and above freezing temperatures (Reid *et al*., 1976). Litter containing 31-62% moisture at 25 to 28 °C increased the sporulation rates of *Eimeria acervulina* but this condition became lethal to the oocysts after 5 days (Williams, 1998). All seven species are ubiquitous and can occur on a farm in different combinations (Williams, 1998). *Eimeria acervulina Eimeria maxima, Eimeria tenella, Eimeria brunette, Eimeria mitis* and *Eimeria praecox* were present in litter samples within six weeks after a new flock placement (Williams, 2005). Threat of coccidiosis is less during hot dry weather and greater in cooler damp weather (Saif *et al.,* 2003). Recovered chickens shed oocysts representing a problem in multi-age operations (Sheriff *et al.,* 2008).

2.10 Caecal Coccidiosis

Eimeria tenella infection causes a severe disease characterized by bleeding, hemorrhagic lesion development, high morbidity, mortality and reduced weight gain (Mcdougald and Fitz-coy 2008) Clinical coccidiosis is considered as the most significant parasitic infection affecting the commercial poultry industry. Initial sporadic hemorrhagic lesions occur together with hemorrhages in the cecal lumen and dysentery, which are often accompanied by oocysts formation, caseous cores, and clotted blood (Trees, 2002). The infection is most pathogenic in chickens because schizogony occurs in the lamina propria and crypts of

epithelium of the small intestine and ceca, respectively,, and causes extensive hemorrhage (Kahn, 2005). Thie infection is associated with reducedgrowth rate, poor performance and mortality in broiler chicken.The pathogenic manifestations of *Eimeriatenella* infection are haemorrhages and extensive destruction of caecal tissues caused predominantly by rapid growth ofsecond-generationschizonts within crypt epithelial cells thatmigrate deep into the lamina propria, and the rupture of theseschizont-infected cells to release second-generation merozoites (Allen, 1997)..

2.11 Clinical Signsof Coccidiosis

The disease is manifested clinically by caecal haemorrhage, malabsorption, diarrhoea, characterized by enteritis and bloody diarrhoea. Clinically, bloody faeces, ruffled feathers, anaemia, reduced weight gained are observed(McDougald, and Fitz-Coy. (2008).) *Eimeria tenella* infection causes a severe disease characterized by bleeding, haemorrhagic lesion development, high morbidity, mortality and reduced weight gain (McDougald, and Fitz-Coy. (2008).

2.12Epidemiology of Coccidiosis

Prevalence and Distribution of coccidiosis is influenced by several factors such as high stoking density cramped on a small space, high temperature,humidity, farm/herd structure, quality of feed, as well as all other factors that compromise resistance to the disease and general health status of the birds. Onset of the disease depends on the age of the bird at the time of first infection and number of passages of the infection (for one passage to be completed it is required 10 days), as well as on ability of the bird to develop proper specific immune response (Hofstad, 1984).

It is almost impossible under farming conditions to produce a coccidia free environment (Jordan *et al*., 2002). Birds get oocyst from food or water contaminated by faeces of infected animals. Birds of any age are susceptible to coccidiosis but most birds get infected in the early few weeks of life (Chookyonix*et al*., 2009).Sudden outbreak of coccidiosis occurs following the ingestion of high doses of the sporulated oocysts over a short period of time by non immune young (3-8 week old) birds (Musa *et al*., 2010).Furthermore, clinically infected and recovered adult birds have been shown to shed oocysts in their faeces thereby contaminating feed, water and soil (Trees, 1999; Musa *et al*., 2010). Oocyst sporulated within two days under proper condition can become infective. Chickens pick them up by pecking on the ground or in litter used for bedding in the house (Fanatico, 2006). Coccidial oocysts are normally introduced into new facilities through contaminated equipment or vehicles coming from other poultry operations (Conway and Mckenzie, 2007).

Oocysts are distributed within the poultry building, inside and outside the house by invertebrates and vermin whilst mechanical ventilation systems serve to scatter the oocysts outside the house. Faecal contamination of vehicles and personnel can spread the infection to other farms (Taylor *et al* 2007). People are important vectors of coccidian (Charlton, 2006)in disseminating oocysts, which could be carried over by manure clinging to shoes or by utensils carried about from one pen to the other. Flies ,rodents, pets, cockroaches, beetles, and wild birds have also been incriminated as mechanical vector (Reid, 1978).

2.13 Pathology and Pathogenesis of Caecal Coccidiosis

.After ingestion, infectious oocysts excyst, liberating the infective form: the sporozoite. Sporozoite infect epithelial cells of the intestine. Transfer of the sporozoites up to the locus of the primary lesion is with the help of intraepithelial lymphocytes (Lawn and Rose, 1982; Daszak, 1999). Oocysts infect the cells of intestinal lining, replicate and cause them to burst, change the gut morphology, reducing gut length and truncating the intestinal villi (Joyner and Norton, 1983).As a result, mucosa is sloughed off, which results in nutrient malabsorption (Allen and Fetter, 2002). The intestinal damage leads to the many clinical signs of coccidia infection such as reduced body weight and feed conversion, intestinal lesions, and diarrheoa. Depending on the severity and chronicity of the infection, microscopic lesions include mild to severe caseous and coagulative necrosis with variable numbers of mononuclear and granulocytic inflammatory infiltrates admixed with hemorrhage, fibrin, and/or fibrous connective tissue. Pathology is largely associated with destruction of the epithelial lining of the infected part or intestine which results in reduced ability for the digestion and absorption of nutrient by the bird (Long *et al.*,1980). The severity of the lesions produced by the parasites are a function of numerous factors including the species of coccidia, number of ingested oocysts, the immune status of the host, the age of the host, and nutritional status and concurrent diseases of the host (Long, 1993).

Several factors are associated with the pathogenicity of coccidia including dose of oocysts, viability of oocysts, site of development, age of bird, concurrent disease, bacteria flora, and nutritional status (Fernando, 1982), and strain of the coccidium. *Eimeria necatrix* and *Eimera tenella* are the most pathogenic in chickens because schizogony occurs in the

lamina propria and crypts of epithelium of the small intestine and caeca, respectively, and causes extensive haemorrhage. Most species develop in epithelial cells lining the villi (Kahn, 2005). Gross lesions can vary from mild catarrhal enteritis to multifocal erythematous regions to severe haemorrhage and possible necrotic caseous material (cores) within the intestinal and caecal lumens. The lesions are generally most severe in about 5 dayspost infection that corresponds to the late stages of merogony (Fernando, 1982). The various coccidian life stages may be observed within the affected areas and to a lesser extent unaffected areas.

Coccidiosis goes hand in hand with gut disease, because it damages the gut and allows bacteria to enter and cause secondary infections (Fanatico, 2006).The intestinal epithelial cell damage results in interruption of feeding and digestive processes with subsequent decreases in nutrient absorption, growth, and a decline in the overall health of the host. Dehydration, anemia, and increased susceptibility to other disease agents are common sequelae of coccidiosis (McDougald and Hu, 2001; McDougald, 2008). In severe cases of coccidiosis, mortality can occur. Outbreaks causing 25-50% mortality in the flock are not uncommon (McDougald, 2008).

2.14Histopathology of Caecal Coccidiosis

The caecal wall is composed of four layers namely, mucosa, submucosa, muscularis and serosa[Majeed *et al*., 2009)]. There is a progressive increase in the number of granulocytes, leukocytes and pyroninophillic cells in the submucosa and lamina propria with*Eimeriatenella*(Hammond and Long, 1973). The granulocytes response become most

observed when the first generation merozoites had leftthe glandular tissue and migrate to the tunica propria. Loss of the mucosal epithelial tissue, shortening and atrophy of the villi is associated with coccidian infection (Pout, 1967)This will result in reduction of the surface area available for absorption and also reduction of the number of functional epithelial cells

2.15Diagnosis of Caecal Coccidiosis

The diagnosis of coccidiosis is based on clinical signs, coprology and pathomorphological and pathohistological analysis (Conway and Mckenzie, 2007).Traditionally, the diagnosis of coccidiosis in poultry is performed most accurately during postmortem and histopathology. Macroscopically, this is done by thoroughly examining the location and extents of lesions in the caeca and is complemented by analyzing naive intestinal scrapings with light microscopy in order to morphologically identify schizonts and oocysts. Assessment of coccidiosis lesions is performed standard following the scoring system introduced by Johnson and Reid (1970)

Recently, molecular techniques have been used to compare genetic sequences of different coccidia and create PCR primers for diagnostic testing (Su *et al*., 2003; Schnitzler *et al*, 1998). Studies have demonstrated analysis of the internal transcribed spacer (ITS) regions useful in molecular comparison and distinguishing intraspecific variations in *Eimeria* and other protozoa including *trichomonads* (Felleisen, 1997), Perkinsus marinus (Brown *et al*., 2004), and *Entamoeba* species (Som *et al.,* 2000)..Recently, a PCR-coupled capillary electrophoretic approach using genetic markers of the ITS-2 region of nuclear ribosomal DNA has been developed and investigated for its use for the identification and

epidemiology of seven *Eimeria* species (Gasser *et al*., 2005; Morris and Gasser, 2006). This technique may, in a near future, contribute significantly to the understanding of the epidemiology of each *Eimeria* species This knowledge may be used in formulating and implementing new prevention and control programmes (Morris *et al*., 2007). Generally, the most pathogenic species (*Eimeria tenella, Eimeria necatrix, Eimeria brunetti, Eimeriamaxima*) can be differentiated in the host on the basis of clinical signs, characteristic lesions at particular sites of infection in the chicken intestine, and consideration of the prepatent period, size of oocysts, and morphology of intracellular stages. However, the less pathogenic species such as *Eimeria mitis* and *Eimeria praecox* might be overlooked. A very useful handbook is available (Conway and Mckenzie, 1991) which describes standard diagnostic and testing procedures for *Eimeria* species and includes coloured photographs of gross lesions caused by the most commonly found species

2.16Treatment of Caecal Coccidiosis

Currently, most poultry producers rely on prophylactic measures such as anticoccidial drugsand vaccines to prevent coccidiosis in their flocks. There are two classes of anticoccidials; coccidiostats and coccidiocidals. Coccidiostats are dietary additives fed continuously at low levels, and they act by arresting the growth of intracellular coccidia. Coccidiocidals, on the other hand, act by actively destroying the parasites during their development (McDougald, 2003). These anticoccidials can be classified as chemicals which have specific modes of action against parasite metabolism, such as amprolium, clopidol decoquinate, halofuginone, and polyether ionophores such as monensin lasalocid,

salinomycin, narasin, and maduramycin, which act through general mechanisms of altering ion transport and disrupting osmotoic balance. It is quite clear, however, (Chapman, 1994), that some degree of resistance to all anticoccidial drugs, including ionophores, has developed. To minimize the effects of resistance, poultry producers rotate the use of various anticoccidials with successive flocks, combine chemical and ionophore treatments, or employ shuttle programs during a flock grow out. Application of these treatment programs depends on seasonal conditions and prevalence of various species of coccidia.

2.16.1Anticoccidial drugs

The most commonly used method of controlling *Eimeria* infections in commercial poultry is prophylactic chemotherapy through the use of dietary anticoccidial drugs. The first true anticocidials, known as sulphonamides, were introduced to the poultry industry during the 1940's (Chapman, 1999). Over the past 60 years, anticoccidial drugs have made a major contribution to the growth of the poultry industry due to the effectiveness of the drugs in controlling coccidiosis (Allen and Fetterer, 2002). The two most commonly used types of anticoccidials are synthetic anticoccidials produced by chemical synthesis and ionophorous antibiotics (ionophores) that are produced by fermentation. Synthetic or chemical anticocidials usually have a specific mode of action on parasite metabolism, while ionophores act through a general mechanism of altering membrane ion transport causing a disruption of osmotic balance (Chapman, 1999; Allen and Fetterer, 2002). Examples of chemical or synthetic anticoccidials are amprolium, clopidol decoquinate, nicarbazin, and halofuginone, while some commonly used ionophores include monensin, salinomycin, narasin, and maduramycin. In general most drugs used for control of *Eimeria* are

extremely effective against some species but not very effective against others. As a result, combinations of different drugs may be used to generate better control of the parasite within commercial operations.

A consequence of this extensive drug use has been the development of resistance, which has been documented for all drugs and wherever poultry are reared under intensive conditions (Chapman, 1997; McDougald, 2003). To combat the resistance, shuttle and rotation systems have been employed (McDougald, 2003). In the shuttle programme, different drugs are used during one period of juvenile growth to market size (grow-out); whereas, in the rotation program the type of drug is switched after one or several grow-outs or seasonally (De Gussem, 2007). However, Lee *et al*.,(2009) discovered that careful use of a shuttle or rotation program does not fully prevent drug resistance but does increase the useful longevity of the drug. Some of the new ionophorous antibiotic anticoccidial drugs such as monensin or lasalocid, had been used for some time in the field before, inevitably, drug resistant parasites emerged (Martin *et al*., 1997). Development of new anticoccidial drugs are costly due to stringent testing requirements and few, if any, are being developed for market (Dalloul and Lillehoj, 2005).

Chemical anticoccidial drugs work by acting on specific stages of the *Eimeria* life cycle. Most drugs act upon the asexual stages of the *Eimeria* lifecycle (Chapman, 1993). Ionophores used in coccidiosis control typically target sporozoites, but merozoites can be affected if they come in contact with the drug. Ionophores do not work as well during *in vitro* laboratory tests, but *in vivo* they seem to be the most effective form of control causing

significant damage to the parasite (McDougald, 1990). The ionophorous drug acts on the sporozoites by facilitating the actions of inorganic cations across the cell membrane interrupting important physiological functions such as balance of sodium and potassium (McDougald, 1990). Chemical anticoccidials in general effect the latter stages of the life cycle of *Eimeria* species. One example is the mode of action of amprolium, which disrupts normal function of the parasite by blocking the transport of thymine across the cell membrane (Chapman, 1993). Though not deemed as successful as ionophores, chemical anticoccidial drugs are still used regularly in the industry.

2.16.2 Vaccination

The most feasible alternative to anticoccidal drugs in the prevention of coccidiosis is vaccination (Chapman *et al*., 2002). The three different types of vaccines used in the poultry industry are live unattenuated, live attenuated and recombinant subunit vaccines. The several methods of vaccine administeration includes aerosol application, *in ovo*, intra-nasal and orally. Live vaccines are typically given orally, through the feed or water, or *in ovo* while the birds are still embryos (Tewari and Maharana, 2011). These are given in low doses to facilitate a low level infection to establish immunity to the specific *Eimeria* strain. The risk associated with live vaccines is that it can result in an active infection. Administration through the oral routes of feed and water can result in unequal dosages among individual birds followed by a variable distribution of oocysts in the flock (Williams *et al*., 1999). Most recently, improvements in administration have lessened the problems and improved the oocysts dispersion (Danforth, 1998; Williams, 2002). A unique vaccine that includes a precocious attenuated strain of *Eimeria tenella* and non-attenuated live strains of *Eimeria maxima* and *Eimeria. acervulina* is marketed as Supercox 1 (Osho

Chemical Industries Ltd., Nairobi, Kenya; Tewari and Maharana, 2011). Lightowlers (1994) described live oocyst attenuated vaccines as anavirulent vaccine that demonstrate reduced pathogenicity while maintaining the ability to induce protective immunity.Live oocyst non-attenuated vaccines are defined as vaccinnes which introduce a controlled number of oocysts of one or more wild type *Eimeria* species to induce protective immunity (Innes and vermeulen, 2006).

2.16.2.1 Live vaccines

Live *Eimeria* vaccines consist of sporulated oocysts and are either non-attenuated (wild type strains of *Eimeria* specie) or attenuated. Further differentiation is based on the *Eimeria* species included, their anticoccidial drug sensitivity profile and application. Protective immunity can be achieved if chickens are infected with either a single high dose or multiple low doses (trickle infections) of *Eimeria* (vaccine) parasites (Long *et al*., 1986). It is crucial that anticoccidial drugs are withdrawn from feed in case chicken flocks are vaccinated with drug sensitive live vaccine parasites in order to avoid vaccination failures.

A beneficial side effect of the use of drug sensitive live coccidiosis vaccines is their association with increased sensitivity to anticoccidial drugs (Newman & Danforth, 2000; Mathis & Broussard, 2006; Peek &Landman, 2006).

2.16.2.2Non attenuated (or wild type strains of *Eimeria specie*) vaccines

Non-attenuated vaccines consist of *Eimeria* parasites which have not been modified in any way to change their pathogenicity and originate from laboratory or field strains. Examples of such vaccines are: CocciVac®, Immucox®, VAC M®, Nobilis® COX ATM, Inovocox® and ADVENT®. Many of these vaccines are used worldwide covering the extensive coccidiosis market. Between the year 2001 and 2004 about 2500 million doses CocciVac® and Immucox® were sold (Shirley *et al*., 2005). ADVENT® is a non-attenuated coccidiosis vaccine harbouring *Eimeria. acervulina*, *Eimeria maxima* and *Eimeria tenella*. It is used to immunize broilers against *Eimeria* parasites. ADVENT® enables *in vitro* assessment of parasite viability using ViacystSM (non-viable sporocysts stain with ethidium bromide), an innovative aspect in coccidiosis vaccinology, allowing a better control of the viability of the vaccine dose administrated (Dibner *et al*., 2003).

2.16.2.3 Attenuated vaccines

Attenuated vaccines consist of *Eimeria* spp. strains, which have been manipulated in the laboratory in order to decrease their virulence. Reduced virulence has been performed by serial passages of the parasite in chicken embryos; e.g. *Eimeria tenella* in Livacox® vaccines. Selection for precocity is the second described method for attenuation; e.g. remaining *Eimeria* spp. in Livacox® and all lines in Paracox® vaccines (Shirley *et al*., 1995; Shirley & Miljard, 1986). Precocity is characterized by a shortened endogenous life cycle due to the fact that the number of generations of schizogony is decreased because last generations of schizogony disappear by selecting early oocysts. A consequence is that the number of oocysts produced during infection is reduced; however, the immunizing potential is maintained (Shirley & Miljard, 1986).

2.16.2.4Recombinant vaccine

Recombinant subunit vaccines use antigens from certain stages of the life cycle of *Eimeria* parasites. Early subunit coccidiosis vaccines focused on the asexual part of the life cycle. Microneme proteins are involved in host cell adhesion and penetration (Ryan *et al*., 2000). EtMIC2 is a microneme protein that was shown to stimulate intestinal protective immunity when administered *in ovo* (Ding *et al*., 2005). Since proteins associated with the sexual stages only confer moderate protection, proteins associated with the sexual stages can also be utilized to create a transmission-blocking vaccine (Jenkins, 2001). Coxabic (Phibro Animal Health, Teaneck, NJ) uses sexual stage antigens from *Eimeria. maxima* to inhibit the development of macrogametes into a viable oocyst. It is injected into the breast muscle of broiler breeder hens who produce antibodies which are then passed on to their offspring through the yolk (Sharman *et al*., 2010).

2.16.3 Antioxidants

In most physiological and pathological conditions, the systemic amounts of free radicals and reactive oxygen species are higher than normal (Vladimirov, 2004). Free radical oxidative species are known to be produced during the host's cellular immune response to invasion by *Eimeria* species (Allen *et al*., 1997), which plays important role in defending against parasitic infections. However, their high concentrations may become intolerable to cell causing tissue damage and cytotoxicity and partially contributing to the pathology of infection. In some parasitic infections, changes in the content of some low-molecular antioxidants (Vitamins A, C and E) occur in the host cell (Evans and Halliwell, 2001).

Nutrition/diet also plays a significant role in maintaining health of the animals and preventing them from various diseases. Among all dietary factors, antioxidant rich diets have gained special importance for growth, survival and maintenance, productive and reproductive health of the animals (Surai, 2002). Most of the antioxidants available are found as dietary supplements. One of the most studied antioxidant is vitamin E, known to delay lipid peroxidation in muscles and improve meat quality. Various fruit and herb plants such as plum, cranberries, pomegranate, bearberry, grapeseed extract, pine bark extract, rosemary, oregano, green tea, and other spices function as antioxidants in meat and poultry products (Karek *et al.,* 2013: Dhikl, 2013). Antioxidants are those molecules that inhibit oxidation and reduce oxidative stress caused by increased level of reactive oxygen species (ROS) and free radicals that can start chain reactions in the cell, resulting in death or damage to the cell. Free radicals react with polyunsaturated fatty acids and initiate a process as a chain reaction known as lipid peroxidation. In living systems, lipid peroxidation changes the enzymatic activity and structure of amino acids thus causes damage to DNA and toxic to the cell (Lima and Abdalla, 2001). Normally, the generation and elimination of ROS remains in dynamic equilibrium, but, if the production of ROS becomes elevated beyond the protection capacity of natural antioxidant defense system, the established equilibrium could be disturbed. This impaired equilibrium in support of oxidants is called oxidative stress and it is involved in the pathogenesis of various infections, including parasitic diseases (Costantini and Moller, 2009; Bansal and Bilaspuri, (2011). Therefore, compounds having the antioxidant properties and meeting the demands

of antioxidant defense system or directly interfere with free radicals can restore the balance of oxidants/ antioxidants, leading to improvement of birds in coccidiosis.

Curcumin, present in *Curcuma longa*, could reduce the severity of an infection of the upper and middle part of the small intestine caused by *Eimeria. acervulina* and *Eimeria maxima* (Allen and Danforth, 1998).Artemisinin, an extract isolated from *Artemisia annua*, is effective in reducing oocyst shedding output from *Eimeria acervulina* and *Eimeria tenella* but not *Eimeria maxima* infections when broiler chickens are fed the extract at concentrations of 1 or 2.5 mg/kg. The extract's mechanism of action is thought to involve oxidative stress (Arab *et al*., 2006).

2.17Prevention and Control of Avian Coccidiosis

2.17.1 Poultry house management

Due to the uquibitous nature of coccidian oocysts, they are easily disseminated in the poultry house environment and because they have such a high reproduction potential, it is very difficult to keep chickens coccidia free, especially under intensive rearing conditions. Oocysts sporulate readily in poultry house litter. However, they can be damaged by bacteria, other organisms, and ammonia that are also present, and their viability can begin to diminish after 3 weeks (Williams, 1995).).

Oocysts may remain in buildings from a previous batch of birds, and they may be carried by mechanical means, including equipment, clothing, insects and other animals. Birds introduced to an infected building will quickly become infected. Graat *et al.,* (1998) examined risk factors on 144 broiler farms and found that poor hygiene related to

personnel, feeding and drinking was important, as were the presence of other diseases on the farm and *Eimeria* species found in the previous flock.

Good sanitary measures, avoiding water spillage, overcrowding, and the use of prophylactic anti-coccidia "Shuttle programme" are essential to control the menace of coccidiosis (Musa *et al*., 2010). Litter should always be kept dry and special attention given to litter near fonts or feeding troughs. Good ventilation also reduces the humidity in the house and helps to keep litter dry. The percentage of chicken coccidial sporulation is higher in dry litter than in wet litter, which is most likely due to the buildup of bacteria, ammonia and the poor aeration in wet litter, resulting in loss of oocyst viability (Waldenstedt *et al*., 2001). However, increasing litter humidity is not recommended in broiler production as it causes skin burns, footpad lesions and consequent lameness. In addition, proper ventilation is recommended for better performance of the bird, but it provides a drier litter that favors sporulation. Coccidial oocysts are extremely resistant to environmental conditions and disinfectant agents, so eradication of coccidiosis from chicken houses by litter removal, cleaning and disinfection is not feasible (Carvanates, 2008; Ajao *et al*., 2010). The most proper management practice for reducing oocyst accumulation in the litter and lowering chances of outbreak of clinical coccidiosisseem to be reduction of stocking density(Chapman *et al.*, 2002). Recent advancement has shown that characterisation of the infective *Eimeria* species transcriptome may provide a better understanding of the biology of the parasite and aid in the development of a more effective control for coccidiosis (Amiruddin *et al*., 2012).

2.17.2 Prebiotics

Mannanoligosaccharides (MOS) are derived from the cell wall of the yeast *Saccharomyces cerevisae* and are widely used in animal feed to promote gastrointestinal health and performance. MOS have been described as a prebiotic but the mode of action may be different; they are thought to block the binding of pathogens to mannan receptors on the mucosal surface and stimulate the immune response (Spring *et al*., 2000). Some brand names are: BioMos®, SAF-Mannen®, Y-Mos® and Celmana®.

In poultry MOS enhances the development of *Bifidobacteria* species.and *Lactobacillus* specie in the intestinal tract of young chickens and suppresses the number of enterobacteriacea (Fernandez *et al*., 2002). In an experiment performed with coccidia, dietary MOS (1 g/kg feed) was able to reduce the severity of a single *Eimeria tenella* infection with 3500 or 5000 sporulated oocysts (Elmusharaf *et al*., 2006). In another experiment a dietary supplementation of MOS, at a concentration of 10 g/kg feed, reduced the oocyst excretion and diminished the severity of *Eimeria acervulina* lesions of birds infected with a mixture of *Eimeria acervulina*, *Eimeria. maxima* and *Eimeria tenella* at subclinical doses of 900, 570 and 170 sporulated oocysts, respectively (Elmusharaf *et al*., 2007).

In a study performed by McCann and others (2006) supplementation of MOS (0.5 g/kg feed) or tannin (0.5 g/kg feed) either individually or in combination did not reduce the severity of a mixed coccidiosis infection of *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella* given at clinical doses of 50,000, 15,000 and 15,000 sporulated oocysts,

respectively. These contradictory results are possibly explained by differences in MOS concentrations in feed and the magnitude of *Eimeria* specie inoculation and doses.

2.17.3 Probiotic

Lower intestinal invasion and development of coccidia and lower oocyst production, explained by enhanced local cell-mediated immunity, was achieved with a Lactobacillus-based probiotic supplemented diet in *Eimeria. acervulina* infected broilers (Dalloul *et al*., 2005).

More recently, in a study performed with a Pediococcus-based commercial probiotic (MitoGrow®) given to birds infected with an *Eimeria acervulina* or *Eimeria tenella* infection, increased resistance of birds against coccidiosis and a partial protection against growth retardation was demonstrated (Lee *et al*., 2007a). In another study performed with a Pediococcus- and Saccharomyces-based probiotic (MitoMax®) given to birds challenged with 5000 oocysts of either *Eimeria acervulina* or *Eimeria tenella*, less oocyst shedding and a better antibody response was found in probiotic fed birds compared to non-probiotic controls. These results suggest that MitoMax® may improve the resistance against coccidiosis by enhancing the humoral immune response when included in the diet (Lee *et al*., 2007b).

2.17.4Symbiotic

A way of potentiating the efficacy of probiotic preparations may be the combination of both probiotics and prebiotics as synbiotics (Ali, 2007; Awad*et al*., 2009). Symbiotics is

defined as a mixture of probiotics and prebiotics that beneficially affects the host by activating the metabolism of one or a limited number of health promoting bacteria and/or by selectively stimulating their growth improving the host's welfare (Gibson and Roberfroid, 1995). The main reason for using a synbiotic, is that a true probiotic, without its prebiotic food, does not survive well in the digestive system. Without the necessary food source for the probiotic, it will have a greater intolerance for oxygen, low pH, and temperature (Sekhon and Jairah, 2010)..The combination of a pre- and probiotic in one product has been shown to confer benefits beyond those of either on its own (Gallaher and Khil, 1999). It is characterized by antimicrobial, anticarcinogenic, antiallergic and immune stimulating actions. It also improves the absorption of minerals, protects from diarrhea and optimizes nutrient digestion processes (Gruzauskas *et al*., 2004).

2.17.5Herbal medicine

Since time immemorial, plants and plant parts have been serving as an indispensable source of medicine for indigenous poultry production systems. Medicinal plants and their extracts, herbs and spices are mostly rich in polyphenolic contents, have been used since many years in conventional medicine. In recent times a large number of research studies are more concerned with their antioxidant properties mainly due to their polyphenolic compounds (Robards *et al.,* 1999; Botsoglou *et al*., 2002; Florou-Paneri *et al.,* 2006). Botanical antioxidants are likely to play an increasing role in the control of the disease since they are well accepted by consumers (Brenes and Roura, 2010). The value of plant extracts for controlling *Eimeria* infections has been investigated due to the association of coccidial infection with the lipid peroxidation of the intestinal mucosa (Naidoo *et al*.,

2008) Plants have been reported to be an excellent source of antioxidants (Abbas *et al*., 2012 a and b; Mahmood *et al*., 2012; Atawodi*et al*., 2013). As an alternative of antibiotic growth promoters, medicinal plants are the most popular options. In these plants like neem, garlic, ginger, kalongi, turmeric, cumin, coriander, oregano, rosemary, sage, thyme, clove, mustard, cinnamon, savory, sea-buckthorn and mint etc. have been studied (Durrani *et al*., 2008; Nidallah*et al*., 2010; Ansari *et al*., 2008).}. Which have showed positive effect. In these studies different parts of plants and their extracts viz oil, leaves, bark, seed, roots and other vegetative parts etc. have been experimentally used in poultry species. Growth promotion, antibacterial, anti coccidial, anti-parasitic, anti-fungal, anti- tumor, anti-cancer, pesticide, immune booster and immunogenic properties of these candidate species have been investigated (Subapriya and Nagini, 2005).The anticoccidial effects of dried *Artemisia annua* and pure *artemisinin* were first tested on chickens experimentally challenged with different species of *Eimeria* (Allen *et al.,* 1997). Moreover, Jang *et al*. (2007) reported the anticoccidial effect of green tea against *Eimeria* species in chickens. Other medicinal relief include; *Melia Azadirachta* (NRC, 1993; Biu *et al*., 2006) in Neem leaves which has been reported to produce antiprotozoal, antibacterial and antifungal effects (Schmutterer, 1990). Other aqueous stem back extracts used for the treatment of coccidiosis include; *Khaya senegalensis,* and *Anona senegalensis, Aloe excels, Camellia sinensis, Curcuma longa, Echinacea purpure, Origanum vulgare, Saccharum officinarum, Triticum aestivum, Yucca schidigera* (Abbas *et al.,*2012).Rhois powder increases survival rates and body weight gains of birds, reduces bloody diarrhea symptoms and oocyst excretions from birds infected by *Eimeria tenella* (Youn and Noh, 2001; Zhang *et al*., 2012; Dragan *et al*., 2014). Chandrakesan *et al*., (2009)and Arczewska-Wlosek and

Swiatkiewicz (2012) evaluated the anti-coccidial activity of some herbal extracts blend containing*Aloe vera*.Thus, Herbal mewdicines can be serve as safer alternatives as growth promoter due to their suitability and preference, lower cost of production, reduced mortality, reduced risk of disease, minimum health hazards and environmental friendliness.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1Ethical Approval:

The project proposal to conduct this study was duly approved by the Ahmadu Bello University (ABU) Committee on Animal Use and Care (ABUCAUC). Approval No: ABUCAUC/2016/011

3.2Location of the Experiment

The extraction and phytochemical analysis of the plant material were carried out in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University,(A.B.U.) Zaria, while the anticoccidial test was performed in the Helminthology Research Laboratory, Department of Veterinary Parasitology and Entomology, Ahmadu Bello University (A.B.U.) Zaria.

3.3 Plant Collection and Identification

Fresh root of *A. senegalensis*(Gwandan daji) was collected in the premises of the the Main Campus of Ahmadu Bello University Zaria. Samples of the root and leaveswere sent to the Herbarium, Department of Botany, Faculty of life Sciences, ABU, Zaria, where the plant was taxonomically authenticated and assigned Voucher No. 900167. A voucher specimen number was deposited for future reference.

3.4 Plant Extraction

The root barks were peeled off,cut into pieces, and air dried under shade for two weeks. One 1kg of the dried plant waspulverised and made into a coarse powder. The powdered plant (500g) was extracted with 1.5 litres of methanol using a 2- litre separating funnel.The extraction process was repeated three times using half of the volume of solvent during the second and thirdextraction processes. The liquid extracts were pooled togetherand concentrated *in vacuo* using rotary evaporator to obtainedthe solid extract (Harbone, 1996).

3.5 Phytochemical Screening

Preliminary phytochemical screening of the extract was carried out for possible detection of metabolites present. The method of Trease and Evans (2002) was employed. The Brief description of the technique is as follows:

3.5.1 Test for alkaloid

Few drops of Mayers reagent were added to a portion of the extract, a cream precipitate indicates presence of alkaloids (Trease and Evans 2002).

3.5.2 Testsaponins

Tenml of distilled water was added to a portion of the extract in a test tube and shaken vigorously for about 30 seconds. The tube was allowed to stand in a vertical position and observed for 30 minutes. A honeycomb froth that persists for 10-15 minutes indicates the presence of saponins

3.5.3 Test for tanins:

To a portion of the extract, 3-5 drops of ferric chloride were added. A greenish black precipitate indicates the presence of condensed tannins. Also the appearance of blue or brownish-blue precipitate is indicative of hydrolysable tannins. (Treas and Evans 2002).

3.5.4 Test for carbohydrate

Few drops of Molischs reagent were added to a small portion of the extract,followed by the addition of concentrated sulphuric acid down the side of the test tube to form a lower layer. A reddish coloured ring at the interphase indicates thepresence of carbohydrate (Treas and Evans 2002).

3.5.5 Test for anthraquinones

To a portion of the extract in a dry test tube, 5ml of chloroform was added and shaken for at least 5 minutes. This was filtered and the filtrate shaken with equal volume of 10% ammonia solution, a bright pink colour in the aqueous (upper) layer indicates the presence of free anthraquinones (Treas and Evans 2002).

3.5.6 Test for glycosides

Five milliliters of dilute sulphuric acid was added to a portion of the extract and boiled on water bath for 10-15 minutes. This was then cooled and neutralized with 20% potassium hydroxide. It was then divided into two portions:To the first portion, 5ml of a mixture of Fehlings solution A and B was added and boiled. The presence ofa brick red precipitate

shows the release of reducing sugar as a result of hydrolysis of glycoside (Treas and Evans 2002).

3.5.7 Test for flavonoids

A portion of the extract was dissolved in 1-2ml of 50% methanolandheated then filtered. Also metallic magnesium chips (pieces) is added to the filtrate and followed by few drops of concentrated hydrochloric acid. Appearance of red colour indicates presence of flavonoids (Treas and Evans 2002).

3.5.8 Test for triterpenes

To a porton of the extract, equal volume of acetic acid anhydride was added and mixed gently. One ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Colour changes were observed immediately and over a period of one hour. Blue to blue-green colour in the upper layer and a reddish, pink or purple colour indicate the presence of triterpenes (Treas andEvans, 2002).

3.5.9 Test for sterols

Few drops of concentrated sulphuric acid were added to a small portion of the extract at the side of the test tube. An immediate colour change (cherry red colour)over one hour period usually indicates the presence of unsaturated sterols. (Treas and Evans 2002)

3.5.10 Test for cardiac glycosides

A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of

concentrated was added down the side of the test tube to form a lower layer at the bottom. The interphase was observed carefully for purple-brown ring. A pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides (Treas and Evans, 2002)

3.6 Standard Drug

Amprolium® (250 WSP KEPRO B.V. Magadenburgsteat 17-7421 2AHOLLAND) a commercially available anticoccidial drug for the routine treatment of coccidiosis in Nigeria was used as standard drug.

3.7 Preparation of the Infective Material/Inoculum

3.7.1 Collection of sporulated oocyst

A reference stock of *E. tenella* oocysts propagated in chicken was obtained from the National Veterinary Research Institude(NVRI) Further identification was done by the by the measurement of the oocysts length and width and shape index.

3.7.4 Propagation and storage of of the oocysts

Eimeria tenella oocysts were propagated by orally infecting five two-week-old coccidian-free chicks maintained under strict biosecurity measures at the Parasitology Division, NVRI Jos. Each bird was innoculated per os with one ml suspension containing 1 x 10^4 oocytsusing insulin syringe. The sporulated *Eimeria* oocysts were kept in 2.5% potassium dichromate and refrigerated at 4°C until used for challenge test

3.7.2 Sporulation of oocyst

Samples of unsporulated oocysts were maintained in 2.5% potassium dichromate solution for the purpose of sporulation in clean glass Petri dishes, at the depth of 3-5 mm.The Petri dishes were partially covered and left at room temperature.They were examined under the microscope every 6 hour to monitor the process of sporulation.

3.7.3 Identification of oocyst

Suspension containing sporulated oocyst was drawn from petridish using pasteure pipette. One drop of the suspension was placed on a microscopic slide and covered with cover slip. Five randomly selected oocysts were examined by measuring their length and width with light microscopy equipped with calibrated ocular lens at 40x magnification. Determination of the oocysts shape index(length/width) was also done. Based on the above procedures, the sporulated oocysts were identified as *Eimeria tenella* using the key described by (Soulsby, 1982).

3.8 Acquisition and Management of Experimental Animals (Chicks)

Day old broiler chicks were obtained from a certified commercial hatchery(Yamppy commercial Hatchery, Offa, kwara state) and housed at the poultry pen of the Department of Veterinary Anatomy, Ahmadu Bello University (ABU) Zaria. The birds were brooded for 2 weeks in a cleaned, disinfected and fumigated deep litter system with strict biosecurity measures. The experiment was replicated andone pen was allotted to each replicate.. Each pen was equipped with a feeder and waterer,with 100 watt electric bulbs

and ventilations used to maintain supply of light and temperature respectively.Suitable temperatures wasalso maintained by adjusting the height of the electric bulb. The fumigated pens were sealed for two weeks before introducing the experimental chicks which were vaccinated against Newcastle Disease at day 10 and infectious Bursal disease(Gumboro) at day 14. The birds were fed *ad libitum* on commercial broiler starterbroiler starter marsh from day old to 4 weeks of age, thereafter they were fed broiler finisher from the same source throughout the experiment. The feeds were produced by Rebson Agric Enterprises, Rehoboth feed center located at Samaru Zaria.

3.9 Determination of the Safety Margin of the Plant Extract

3.9.1Acute toxicity studies

The method of Lorke (1983) was used to determine the median lethal dose (LD_{50}) of the extract in the chicks.The study was conducted in two phases. In the first phase, nine chicks were randomly divided into three groups (I, II,III) of three chicks each and treated orally with the extract at doses of 10, 100 and 1000 mg/kg body weight respectively. The chicks were observed for 24 hours for signs of toxicity and possible death. The second phase of the trial was done based on the results obtained during the first trial. In the second phase of the experiment, three chicks were assigned into three groups of one bird each (Lorke, 1983). Chick in each group was given the extract at three different doses of 1600, 2900 and 5000mg/kg respectively depending on the outcome of the first trial. Theywere monitored for signs of toxicity and death within 24 hours as earlier stated and observed till 14 days for any delayed toxic shock signs. The lethal dose was calculated using the formula.

LD_{50}= LD_{50}= $\sqrt{(D_0 \times D_{100})}$

D_0= highest dose that gave no mortality

D_{100}= lowest dose that produced mortality

3.10Assesment of *Invitro* Anticoccidial Activity of the Plant Extract

The assessment of *in-vitro* anticoccidial efficacy was done by observing the effect of the plant extracts on the sporulation of *Eimeria* oocysts. The trial was conducted as follows: Fresh faecal samples were collected from infected birds and the oocyst burden was determined (MAFF, 1977). The oocyst suspension was prepared by serial dilution with 2.5% Potassium dichromate solution to a concentration of 100 oocysts per ml. One ml of this suspension was dispersed in each petri dish labeled appopriately and mixed with the*A. senegalensis*extract of different concentrations as 100, 250, 500, 1000, 1500mg per ml using distilled water as diluents. Simillarvolume of these suspensions was also dispersed each into two petri dishes containing 1.25g/litre amprolium and 2.5% Potassium dichromate solution (Control). The set up was left at ambient temperature and monitored every 6 hours to observe for sporulation of the oocysts over a 48- 72 hour period. The number and percentage of the oocysts sporulation and inhibitionwas determined and recorded. Percentage inhibition was calculated by

$$\text{Percent inhibition} = \frac{\text{number of unsporulated oocyst}}{\text{Total oocyst recovered}} \times 100$$

3.11 Assesment of the *In vivo* Activities of the Plant Extract

3.11.1 Animal Screening and Grouping

Forty-two broiler chicks, 14 days old were randomnly assigned into seven groups of six birds each, namely groups 1-7. Feacal samples from all experimental groups were collected and examined for any contamination by Coccidia parasites prior to the experimental infection. All groups were found negative for coccidial oocysts.

3.11.2 Experimental infection of birds and monitoring

Each bird was infected with 2×10^4 by oral route using 1 ml of normal saline suspension of sporulated oocysts of *imeria. tenella.*Chicks in group 1-6 were infected with *Eimeria tenella,* while group 7 (negative control) were not infected.

The birds were monitored daily for clinical signs of coccidiosis such asdepression, ruffled feathers, bloody diarrhoe, anorexia, inappetance, anaemia and death during the period of treatment and subsequent recovery from the experimental infection.

3.11.3 Administration of the extracts and conventional drug

Immediately after the onset of clinical signs at 4-5 days after infection, chicks in groups 1-4 were treated with the extract using 4 different doses of 200, 400 ,800 and 1600mg/kg for 5 days respectively. Chicks in group 5 were treated with amprolium (1.25g/litre in drinking water), while chickens in group 6 and 7 were given a ml of distill water each andserved as infected non-treated (positive) and uninfected non-treated (negative) controls respectively.

3.11.4 Performance of the birds

Performance index was ascertained by weighing the birds weekly starting from 14 days of age (Day of infection) till 42 days when the experiment was terminated.

3.11.5 Mortality rate

Mortality was recorded in the infected group throughout the period of the experiment and necropsy examination was done. Percentage mortality was calculated by using the formular

$$\frac{\text{No of birds died due to infection}}{\text{No of total birds infected}} \times 100$$

3.11.5Determination of oocyst burden of the infected birds

The number of oocysts per gram of faeces (OPG) was determined using the modified McMaster technique in pooled faecal samples collected randomnly from each replicate at 5^{th}-9^{th} days post infection.Random collection of the samples from each pen was used to obtain an accurate estimate of oocyst excretion.

Briefly the Mcmaster technique is described as follows: Two grams of faeces were soaked in 20 ml of tap water for 24 hours at 4^{o}C.The mixture was homogenized by thorough mixing with a glass rod and the mixture was filtered through a single thickness of muslin. A 10 ml centrifuge tube was filled with filtrate and centrifuged at 1500rpm for five minutes. The supernatant was decantedand the sediment was resuspended in six millilitres of flotation fluid (saturated Nacl solution) and shaken thoroughly to suspend the sediment. It was then centrifuged at 1500rmp (200 x g) for 5 minutes and the supernatant carefully

decanted in a dropper bottle (i.e first oocyst harvest). The sediment was suspended in six ml of floatationfluid,shaken and centrifuged for the second harvest. The supernatant was decanted in the same dropper bottle of the first harvest to make up 12 ml of oocyst suspension. Samples were taken from the solution using a Pasteur pipette and poured on a Mc Masters fill chamber. The oocysts were allowed 3-4 minutes to float to the surface of the counting chamber for easy count. The oocysts was counted under a microscope and the actual number of oocysts was calculated by getting the sum of the count in the 2 chambers and multiplying them by 20 to obtained the OPG.

3.11.5.1Determination of lesion score

Three birds were randomly selected from the infected group were sacrificed onthe 11, 14 and 17 days post- infection, and those that died during the course of the experiment were subjected to necropsy.The caecal mucosa of the birds were examined for gross lesion and the lesion scores were quantitatively graded using the method of Johnson and Reid (1970).

. A score of "0" was given normal caeca with no gross lesions;

+1 Few scattered petechiae in the cecal wall which are reddish or purple in colour.

+2 Numerous petechiae

+3 Extensive haemorrhage

+4 Extensive haemorrhage that gives a dark blue colouration to the caecal intestine.

Dead birds were given lesion score of +4

3.12Histopathological Analysis

Tissue samples of the heart, lungs, kidney, caecum, liverwere collected, fixed in buffered 10% formalin, dehydrated in absolute alcohol, cleared in xylene, and embedded in paraffin for preparation of fine blocks in paraffin wax; sections of 5-μm thickness were cut and subjected to routine hematoxylin and eosin staining as described by Drury and Wallington (1976). The tissue sections were examined under the light microscope for presence of lesions..

3.13Statistical Analysis

Results were expressed as mean standard deviation (S.D). The one way ANOVA and Tukey posthoc test were performed to assess meandifferences among various groups, using Statistica software, version 12 (STAT SOFT inc. 2013; USA). Significance betweencontrol and treated groups was determined by student test. A probability level of ($p<0.05$) was regarded as significant.

CHAPTER FOUR

RESULTS

4.1 Phytochemical Test of the Extract

Phytochemical analysis of the extract revealed the presence of the following constituents alkaloids, glycosides, carbohydrates, terpenoids, flavonoids, steroids, saponins,triterpene, tannins and cardiac glycosides (Table 1).

Table 4.1 : Phytochemical constituents of root bark extract of *Anonna senegalensis*

Constituents	Test	Inferences
Carbohydrates	Molisch	+
Anthraquinones	Bontragens	-
Glycosides	Fehlings	+
Saponins	Froth test	+
Steroids	Liebermann Burchard	+
Triterpenes	Liebermann Burchard	+
Tannins	Ferric chloride	+
Flavonoids	Shinoda	+
Alkaloids	Mayer, Wagners	+
Cardiac glycosides	Keller- Kiliani	+

Key: + present - Absent

4.2 Acute Toxicity

Themedian lethal dose(LD_{50})of the crude extracts of *A. senegalensis* was greater than5000 mg/kg..However the chicks given 5000 mg/kg of the extract exhibited slight depression which resolved after 12 hours of extract administration.

4.3 Morphology and Size of Oocysts

The length, width and shape index ofthe *Eimeria. tenella* oocyst isolated from the infected birds are presented in table 2. The shape index was calculated by dividing the length with the width

Shape index= Length/Width (Soulsby, 1982)

Table4.2: Morphometry of*Eimeria tenella* oocyst

No	Length(µm)	Width (µm)	Shape index
1	20.56	18.64	1.10
2	21.89	17.23	1.27
3	19.95	17.57	1.135
4	21.86	17.86	1.22
5	20,90	17.92	1.17
Average	21.03 ±0.5	17.84 ± 0.3	1.17 ± 3.
Reference value 19.25-26.0 µm		**16.5-21 µm**	

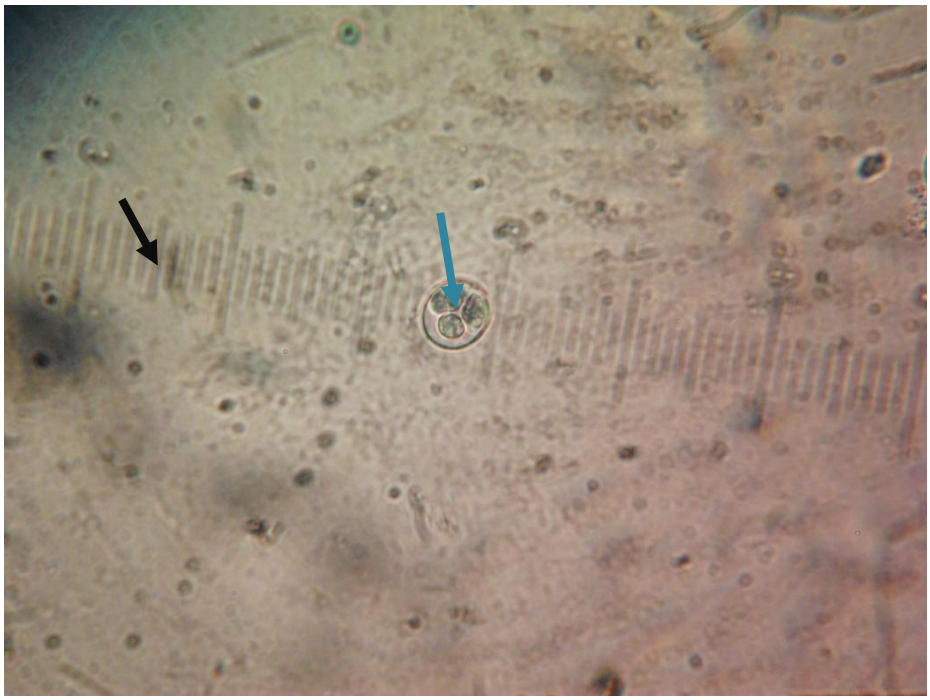


Plate I: Oocyst size measurement using calibrated ocular micrometer



Plate II: Unsporulated oocysts of *E. tenella* (Double arrow)

4.3 *Invitro*AnticoccidialActivity Test

The percentage inhibition of oocyst sporulation produced by the the rootbarkextract of *Anonna senegalensis*at concentrations of between 100 and 1500mg/ml ranged between 78 to 100% (Table 3). It varied according to different concentrations of the extract,being highest at the maximumconcentrations .(100 mg/ml- 78% : 250 mg/ml-85% : 500 mg/ml-90% : 100 mg/mi- 97 : 1500 mg/ml- 100%The percentage inhibition of oocyst sporulation produced by amproliumat the 1.25g/litre concentration was 97% also shown in Table 3

Table 4.3 In vitro anticoccidial effect o A. senegalensis rootbark on sporulation of E. tenella oocyst

Drug/Extract concentrion mg/ml	No of oocysts	No of recovered oocysts	Sporulation (%)	Inhibition (%)
2.5% $K_2Cr_2O_7$	100	110	100	0
A. senegalensis				
100	100	90	22	78
250	100	86	15	85
500	100	88	10	90
1000	100	70	03	97
1500	100	52	0	100
Amprolium g/litre				
0.6	100	115	43	57
1.25	100	112	03	97

4.4 Clinical Manifestation of Caecal coccidiosis byInfected Broiler Chicks

At four to five days post infection, birds experimentally infected with sporulated oocysts of *Eimeria tenella* showed clinical signs of depression, weakness, paralysis of limbsanorexia, ruffled feathers and the easily recongnizable clinical sign of bloody diarrhea. In the treated groups (2-5) the symptoms were milder than other groups (1and 6).Infected non-treated group (G6) showed more severe symptoms.Symptoms started from 5th dpi with maximum strength at 7th and 9th d.p.i. and then regression and disappearance of bloody diarrhea till the end of the experiment.

4.5 Effect of *Eimeria tenella* Infection on Bodyweight Gainof the Infected Chicks

Eimeria tenella infection significantly affected the body weight gain of all infected groups in comparison with negative control chicks (group 7).The decrease in weight was more noticeable in the positive control group (group 6). Mean final bodyweight gain of chicks at 7 weeks of age in groups 1, 2, 3, 4 and 5 was 990.5 g ±19.2, 1011.8 g ±24.6, 1041g ±12.8, 1060 g ± 16.4, 1152 g ±23.9 respectively. The group that received 1.25g/litre of amprolium® (group 5) gain more weight (1041 g ±12.8) than those groups treated with herbal formulation and the positive control (group 6). However, the birds of the negative control (group 7) had better weight gain (1450 g ±28.9) compared to all other groups. Statistically there is no significant difference between the bodyweight gain of group4 and 5. but a significant between group 7 and the rest of the group existed.

Table 4.4: Mean bodyweight gain of broiler chicks infected with *E. tenella* and treated with the extract

Group	Age of birds (days)				
	14	21	28	35	42
	Mean weight gain (g)				
1	390.75 ±13.50[a]	365.75 ± 18.0[f]	503.0 ± 9.4[f]	743.75 ± 44.98[f]	990.5 ± 19.2[d]
2	410.75 ± 8.87[b]	380.0 ± 11.66[d]	573.0 ± 38.3[d]	757.0 ± 36.63[e]	1011.8 ± 24.6[c]
3	396.0 ± 17.74[c]	376.25 ± 2.69[e]	529.0 ± 48.7[e]	781.0 ± 12.64[d]	1041.0 ± 12.8[b]
4	419.75 ± 26.8[a]	397.75 ± 40.5[b]	585.0 ± 48.8[c]	795.5 ± 32.42[c]	1060.0 ± 16.4[a]
5	411.75 ± 35.39	386’25 ± 33.9[c]	605.25 ± 19.4[b]	865.0 ± 30.38[b]	1152.0 ± 23.9[e]
6	372.0 ± 16.31[e]	380.5 ± 9.90[d]	411.75 ± 40.2[g]	410. 50 ±25.2	
7	370.0 ± 14.28f	516.75 ± 24.4[a]	834.0 ± 19.8[a]	1230.0 ± 9.13[a]	1450.0 ± 23.9[e]

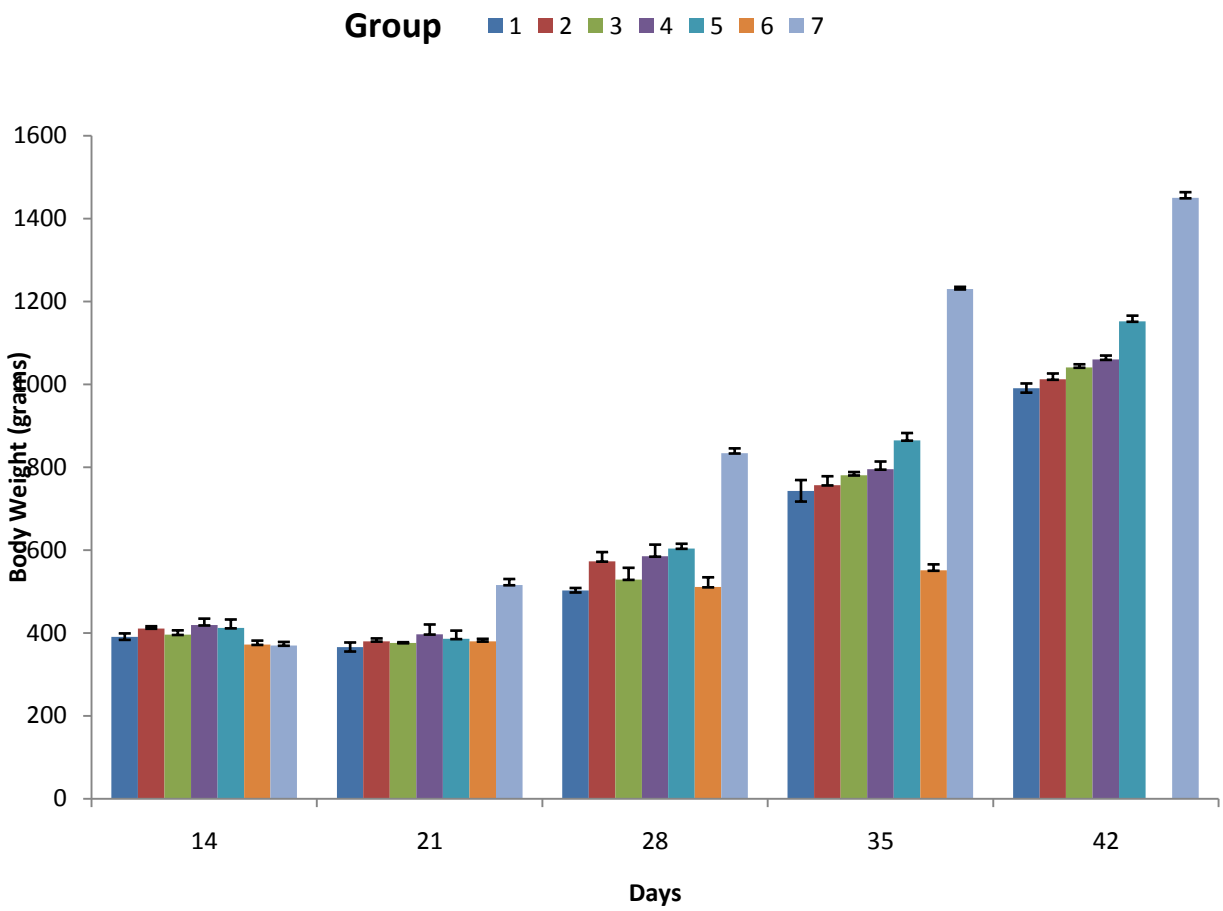


Figure 4.1: Bodyweight gain of chicks infected with *E. tenella*an treated with the graded doses of the extract or amprolium

4.6 Oocysts burden of Broiler chicks Experimentally Infected Birds with *E. tenella* and treated with theMREAS extract or Amprolium

Oocyst shedding by the infected birds peaked at six days post infection, and then declined steadily through the end of the entire measurement period .The highest mean oocysts count in all infected groups (1-6) was recorded at 6th days post infection (dpi), 85250, 122770, 200300, 32240, 52500 and 350350 oocysts per gram respectively. There was significant increase in total oocysts count in positive control (group 6) (352800 oocysts/gram of faeces) compared to other groups. Moreover, there was a significant decrease in total oocysts count in groups 4 (320 oocysts /gram of faeces) and 5(93 oocysts/gram of faeces) compared with other treated groups 1, 2 and 3 and positive control group (6) which were 7500, 31800, 900, 352800 oocysts/gram respectively. Amprolium treated group at 1.25g/liter and *Anonna senegalensis*extractat dose of 1600mg/kginduced thehighest reduction in total oocysts count. (Table 4 and Figure 2)

Table 4. 5: Oocysts per gram (OPG) of chicks infected before and after treatment

	Experimental group						
	1	2	3	4	5	6	
Dpi	Oocyst per gram						
5	45300	31200	30680	35560	51380	40200	0.0
6	85260	122770	200300	32240	52500	350350	0.0
7	35080	40200	40200	21500	20720	198900	0.0

withvarying doses of MREAS and amprolium

8	15480	19400	15900	5310	3200	321700	0.0
9	7500	3180	900	320	93	352800	0.0

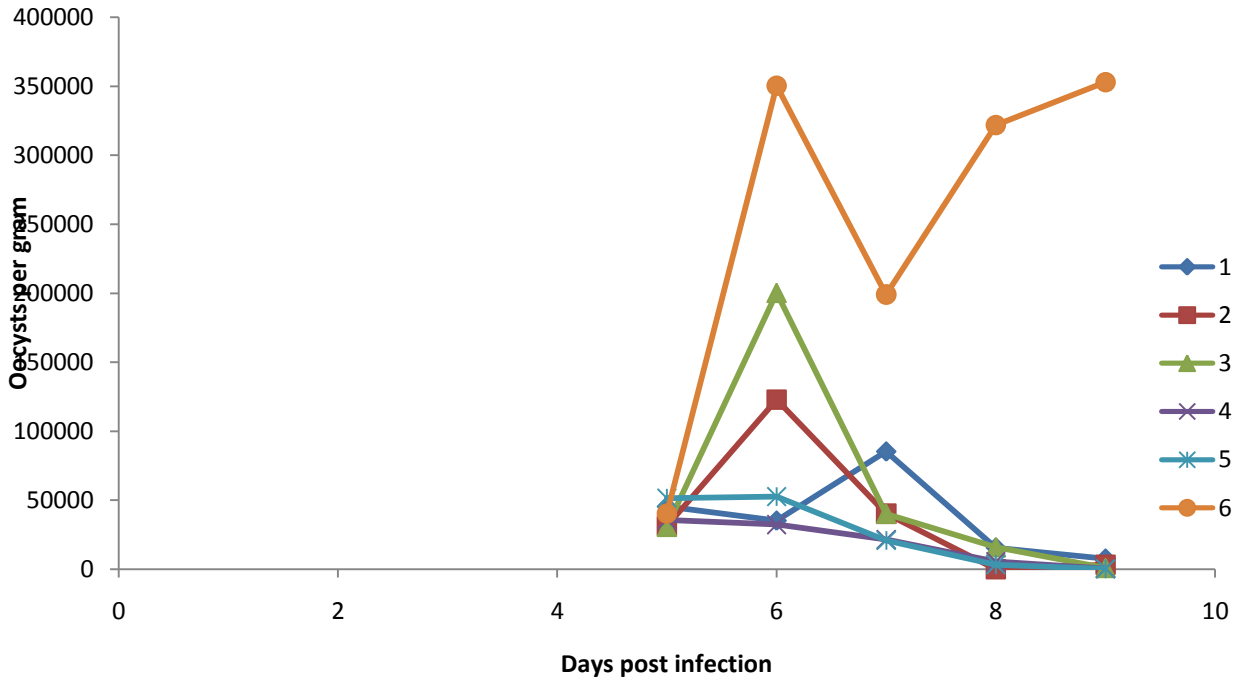


Figure 4.2: Oocyst counts of chicks infected with *E. tenella* and treated with the extract of Anonna senegalensis or amprolium and their control

4.8 Caecal Lesion Score in the Experimental Birds Infected with *E. tenella*

The value of caecal lesion scores of the various groups are: group 1 (2.65)group 2 (2.35)group 3 (2.65) group 4 (1.66) group 5 (1.33) group 6(3.66) and group 7 (0)of the positive control group was the highest (3.6.). challenged birds treated with 1600mg/kg of *A. senegalensis* exhibited significantly (P□ 0.05) lower lesion score (1.6) compared to those observed in the positive control group. Birds treated with amprolium had the lowest lesion score of 1.33. The statistical analysis shows significant difference between the scores of infected treated and group 7 (negative control) (Plate III- XI). Figure 3 and table 4.6

4.7 Mortaity Rate of Experimental *E. tenella* Infection in Broiler Chicks

Mortality was observed in groups treated with 200. 400 and 800 mg/kg doses of the extract i.e 1, 2, 3 and 6 which were 5.5%, 2.8, 2.8% and 16.6% respectively, while the untreated group 6had mortality of 16.6 % . No mortality was recorded in groups 4, 5 and 7. Figure 4

Table 4.6 Mortality and lesion score of chicks infected with *Eimeria tenella* and treated with MREAS

Parameters	Experimental group 1	2	3	4	5	6
Lesion score	2.7^{b} ±0.6	2.3^{bc}±0.6	2.2^{c}±0.6	1.7^{d}±0.6	1.3^{e} ±0.6	3.6^{a} ±0.6
Mortality (%)	5.5	2.8	2.8	0.0	0.0	16.6

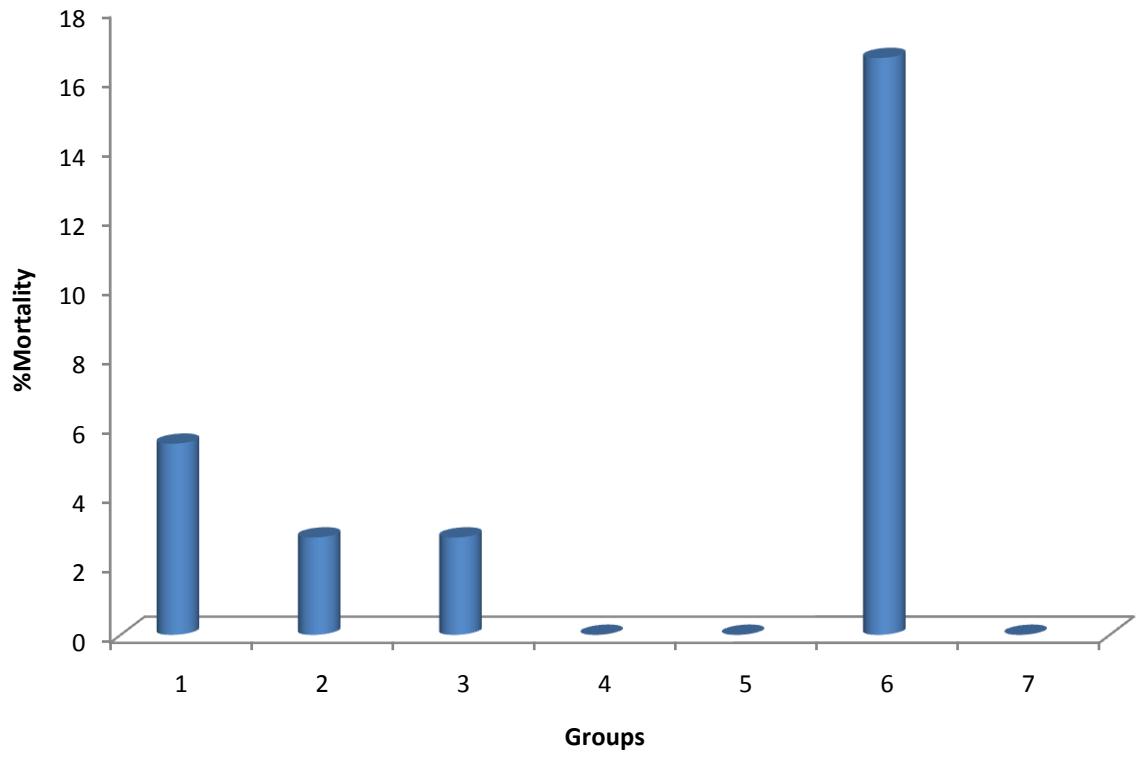


Figure 4.3: Mortality of chicks infected with *E. tenella* and treated with extract

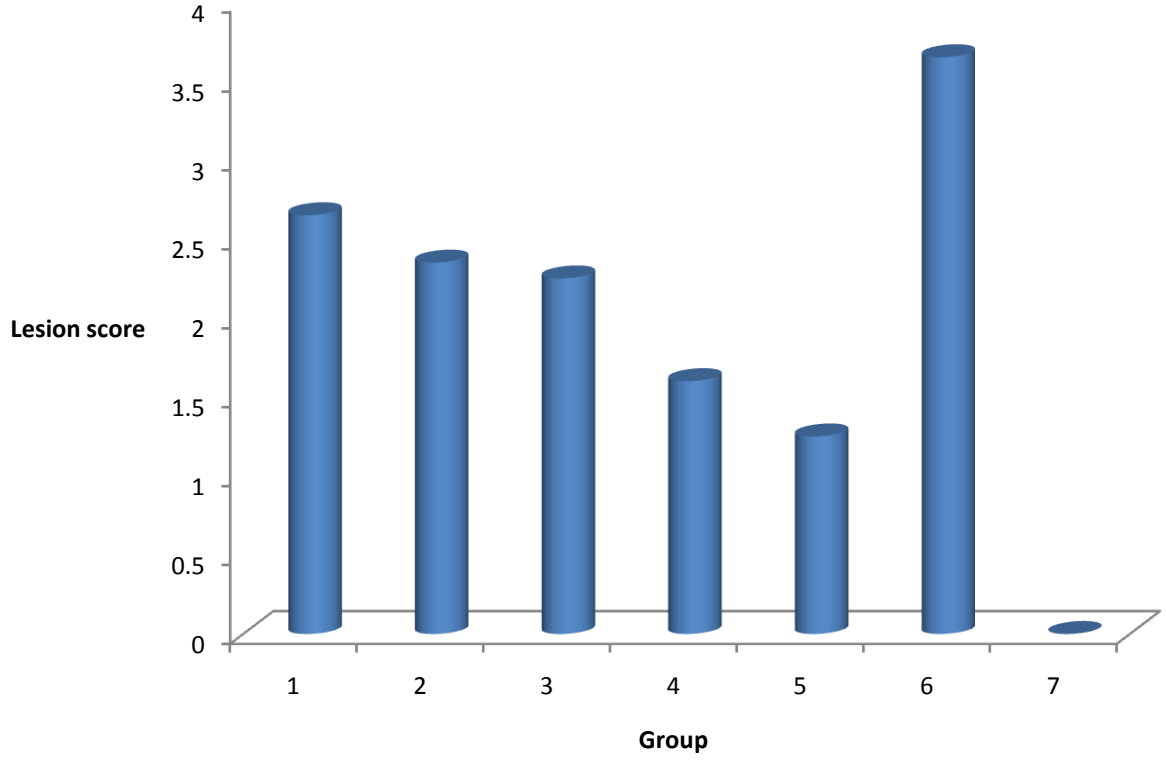


Figure 4.4: Lesion score of chicks infected with *E. tenella* and treated with *A. senegalensis* extract or amprolium and their control

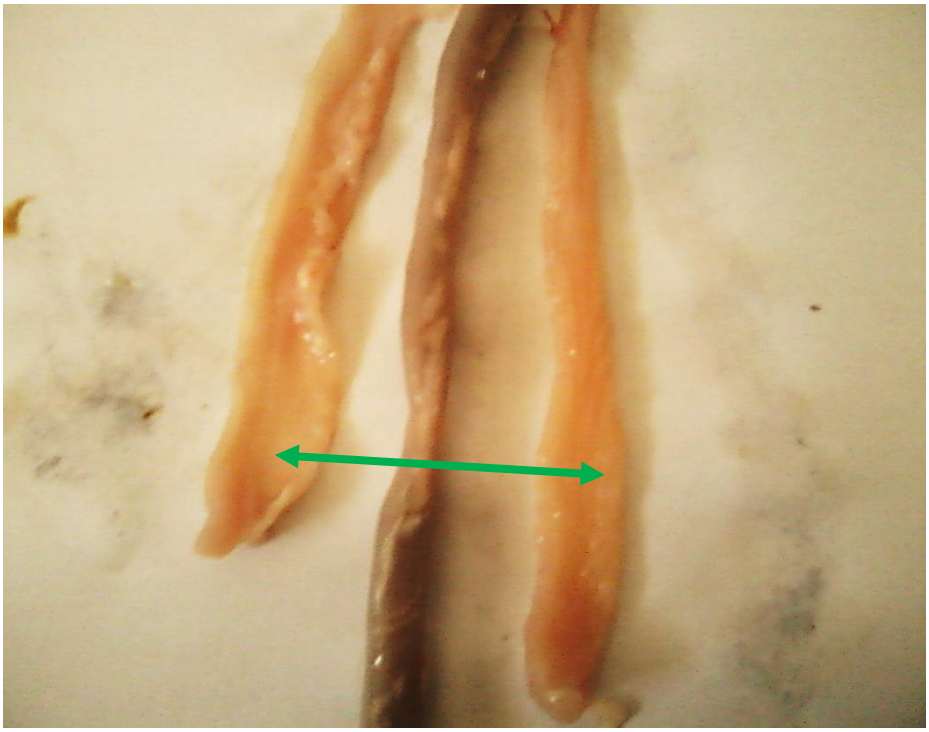


Plate III: Caecal loops of negative control chicks showing normal caeca. Lesion score 0



Plate IV: Few scattered petichiae which are redish or purple on the caecal wall and an unthickened caecal mucosa Lesion score *1



Plate IV: Numerous petechiation and slightly thickened caecal mucosa. Lesion score +2



Plate V: Extensive haemorrhage on the caecal mucosa and greatly thickened caecal mucosa. Lesion score +3



Plate VI: Extensive haemorrhage that gives a dark coloration to the caecal mucosa and greatly thickened mucosa. Lesion score +4

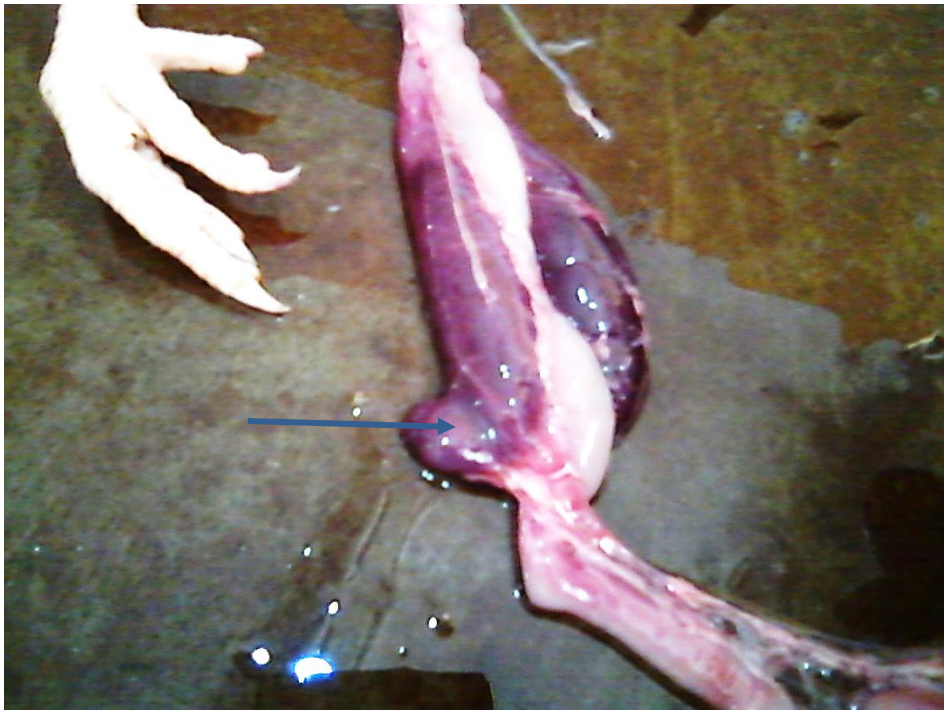


Plate VII: Unopened Caeca (blue arrow) of chickat eight day post infection showing haemorrhage on the caecal wall even before opening the gut. Lesion score +4



Plate VIII:Completely ballooned caecal loops (arrowed)of positive control chick distended with blood. Lesion score +4

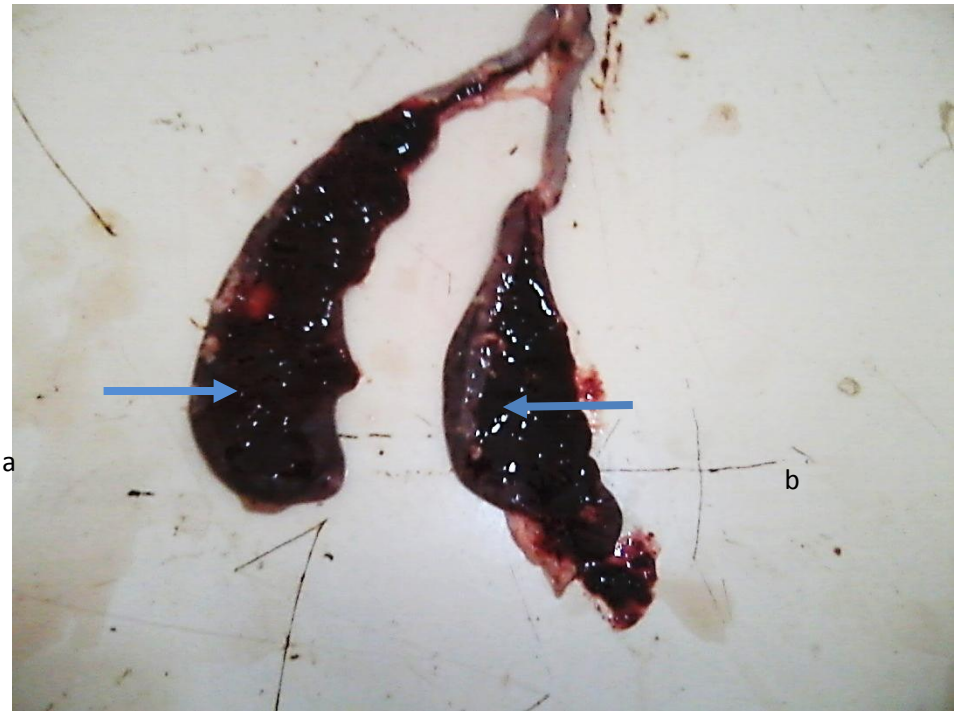


Plate IX: Opened caecal loops (a and b) showing blood mixed with faecal debris and slightly thickened caecal mucosa
Lesion score +4



Plate X: Opened caecal loops showing blood mixed with faecal debris and slightly thickened caecal mucosa. Lesion score +3

4.10 Induced Histopathological Lesions in Broiler Chicks Infected with *E. tenella*

The result histopathological study, showed the presence of high numbers of oocysts, schizonts and desquamation of epithelium in the caeca of group 6 (infected untreated) indicating the severity of infection due to *Eimeria tenella* .Group 7 revealed very normal structure of caecal section as it was used as control group. The group of prophylactic trial (Group 5) also showed some few scattered pinpoint haemorrhages. In the MREAS treated groups, the intensity of caecal lesion was less severe in the 1.6 g/kg (group 4) in comparison to groups 1, 2 and 3 and the positive control group.

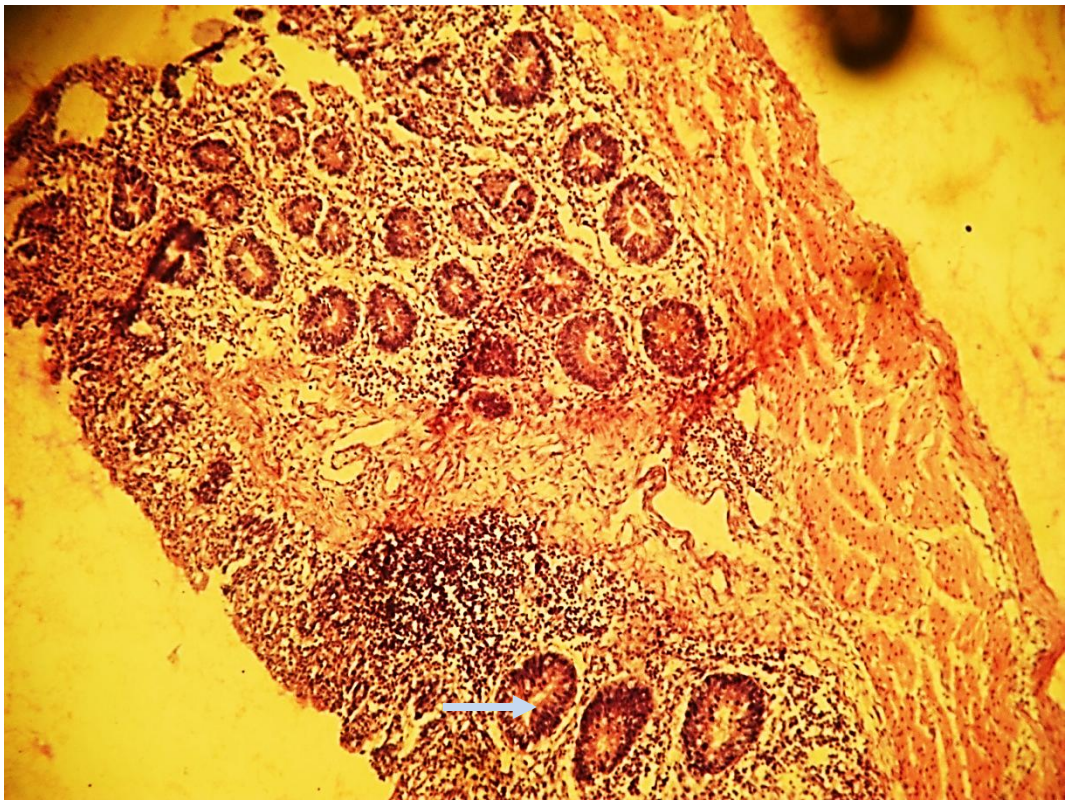


Plate XI: Photomicrograph showing normal glandular tissues of caecum of negative controlbirds. H & E × 500

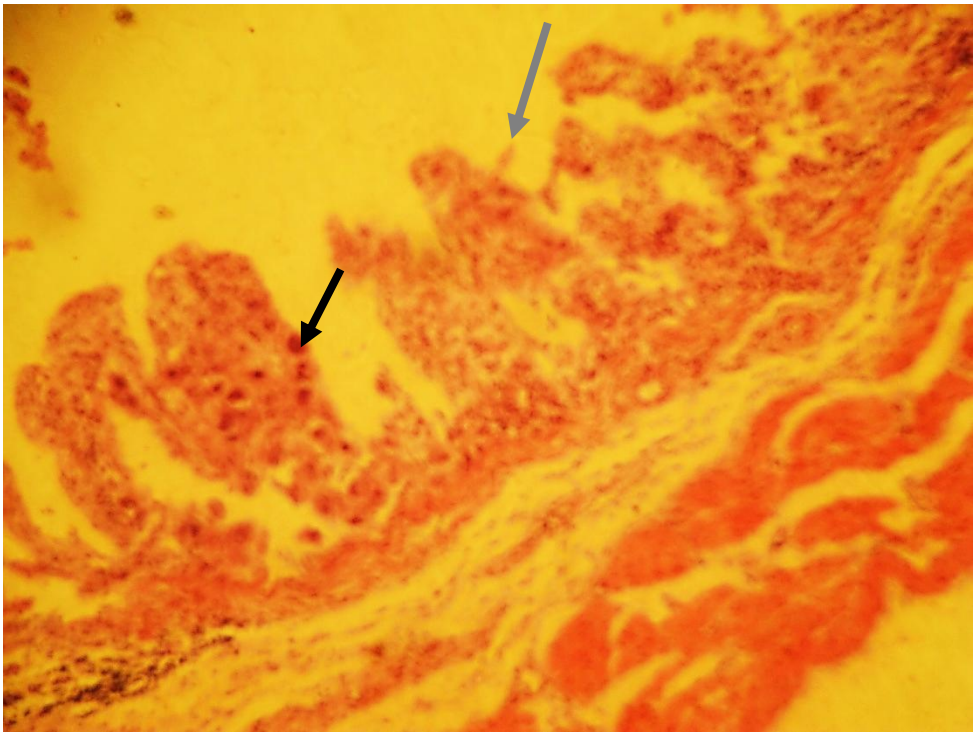


Plate XII:Section of caeca showing desquamation of epithelium (Blue arrow) and developmental stage (schizont) 7 dpi (Black arrow)H & E × 100

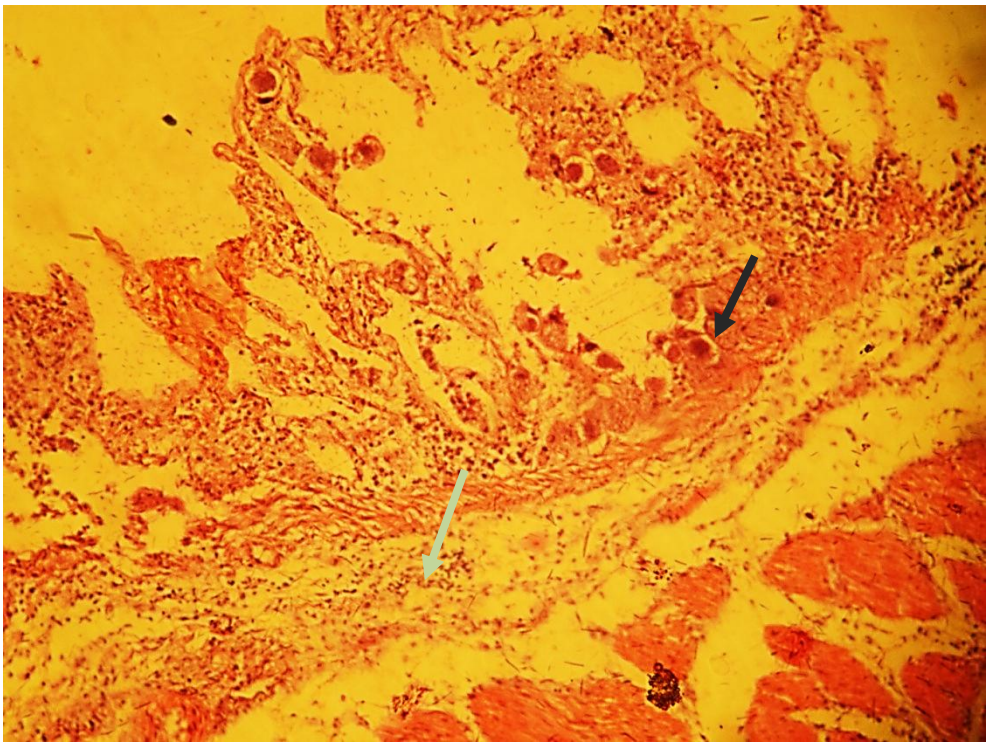


Plate XIII: Section of caeca showing developmental stages of the parasiteat 7 dpi (black arrow). Note the generalised tissue infiltration(green arrow) magnification H & E × 100

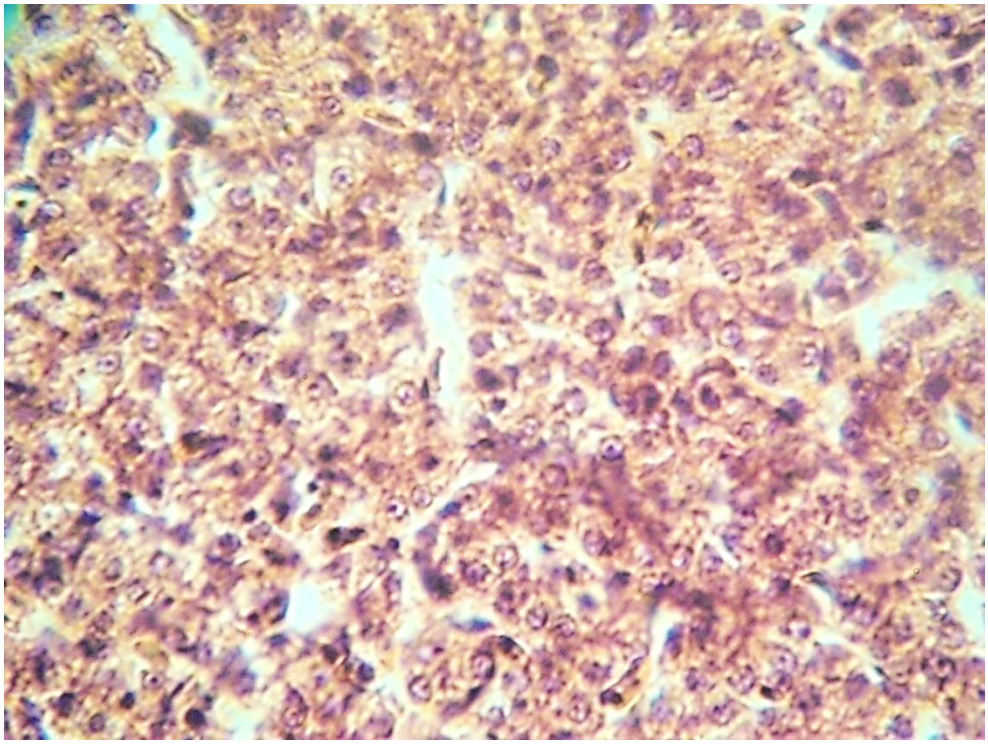


Plate XIV: Photomicrograph showing the histological structure of a liver of negative control H& E × 400

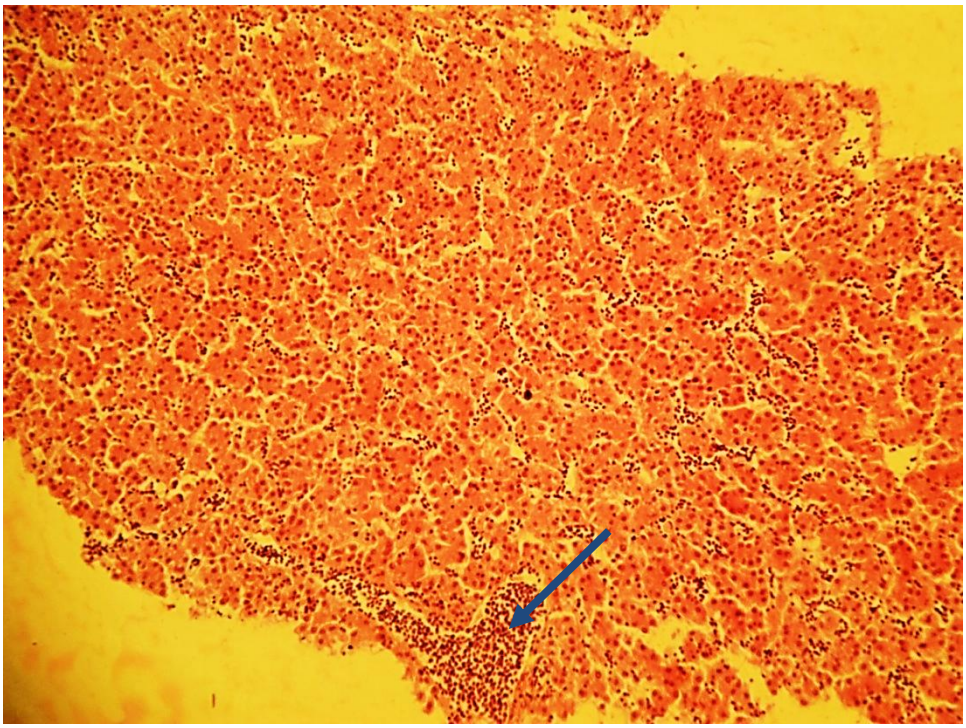


Plate XV:Photomicrograph of liver of bird infected with *E. tenella* showing congestions (Arrow) H & E × 100

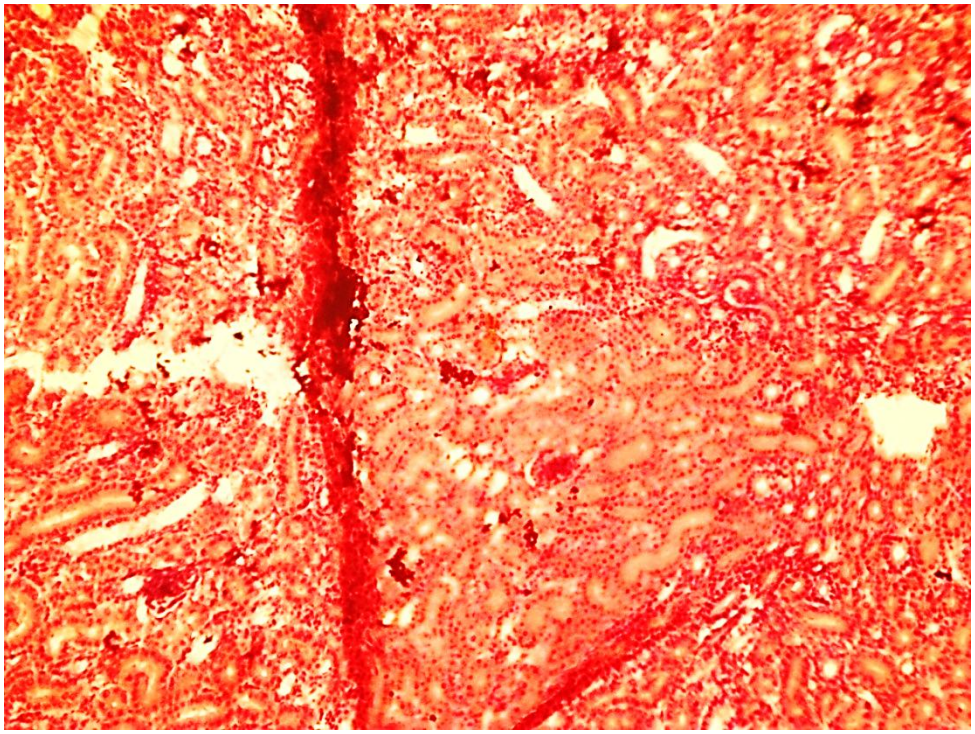


Plate XVI:Photomicrograph showing normal histological structure of kidney of positive control. H & E ×100

CHAPTER FIVE

5.0 DISCUSSION

Plants have been components of phytomedicine since time immemorial, and various assortment of industrial chemicals and bioactive components are extracted from every part of it (Parekh *et al.*, 2006). These phytochemicals exhibit diverse pharmacological and biochemical actions when in animals (Amadi *et al*., 2006). Two separate modes of action can be attributed to the efficacy of plant extracts for treating parasitic infections: their immunomodulatory properties and their antiparasitic effects (Anthony *et al*., 2005). *Annona senegalensis* has been found active on parasites and some opportunistic bacteria like *Pseudomonas, Staphilococcus aureus, Salmonella typhi, Shigella* (Ogbadoyi *et al*.2007). In the current study,phytochemical analysis revealed the presence of phytoconstituents similar to findings of Okoye *et al (*2012) and Adzu *et al* (2005).

In the present study, the sporulation time,the mean values of theoocyst measurments and shape indices are more or less in agreement with the findings of Salim (2001) and Amer *et al* (2010) Saif *et al* (2003), Quraishi *et al* (2009) who reported an oocyst size of 22.2 x17.8 µm, 23 x 19µm and 21.6 x 19µm and sporulation time of 48 hours for the same species respectively.

Acute toxicity test gives an indication on the range of doses that could be toxic to the animal; it could also be used to estimate the therapeutic index (LD_{50}/ED_{50}) of drugs and xenobiotics (Rang *et al*., 2001). In this study, the oral LD_{50} of *Anonna senegalensis*is equal to or more than 5000mg/kg. This result shows that the rootbark extract of*Anonna*

senegalensis can be considered to be acutely non-toxic. According to Hodge and Sterner, (2005), any compound with oral LD_{50} of 5000 mg/kg or more should be considered relatively safe. Simillar results were obtained by Onwusoye *et al* (2014) who reported an LD_{50} of more than 5000 mg/kg of leaf extract of *Anonna senegalensis*. The finding also fall in line with Nwosu *et al* (2011) who worked on the stem extract of the same plant.. It however, disagrees with the findings of Igwe and Nwobodo (2014) and Okoye*et al* (2012) who reported an LD_{50} of 954.99 mg/kg and 3800 mg/kg bodyweight respectively. The result of this findings buttress the fact that local dwellers in different parts of the savanna and rainforest zones have been using parts of *Anonna senegalensis* for medicinal purposes and as edible juicy fruits for many years without complaints of any toxicity (Okoye *et al*., 2012).Thus, any disparity in the toxic effect may be due to differences in geographical location, route of administration, collection time of the plants samples and the extraction procedure.

Inhibition of sporulation is a common criterion to assess anticoccidial properties of new conpounds/ substances(Molan *et al*., 2009). In the present study, the phytochemicals have been shown to exhibit this effect by interfering with oocyst sporulation.*Anonna senegalensis* extract at a concentration of 1500 mg/kg exert the highest percentage inhibition of oocyst sporulation *in vitro,* but had a slightly lower *in vivo* anticoccidial effect. These contrasting features suggest that It is possible that the active components responsible for the high anti-sporulation effect produced *in vitro* were denatured, degraded or inactivated by enzymatic action in the intestine following an *invivo* administration (Nwosu *et al*., 2011).

All the chicks of infected groups developed clinical signs characteristic of acute stages of caecal cocidiosis within a period of 4-5 dayswhich were also supported by post-mortem observations.This is in consistent with the findings ofHaritova*et al.*, (2013) Jatau *et al*(2014) and Tan and Long(2012). In the later stages of the experiment mortality among the infected birds was highest between nineth and fourteenth day post infection owing to the excessive loss of blood. In the present study, the post mortem findings were also in tandem with the findings of (Long,1993) who reported enlarged and thickenedcaecum with clotted blood.

Determination of faecal oocyst output following *Eimeria* infection in animals is used as an indicator of susceptibility, assessment for anticoccidial activity and resistance to *Eimerian* infection (Pouralli *et al*., 2013). The oocyst per gram (OPG) of different groups of chickens revealed a pattern relatively similar to that of lesion scores among the groups. The highest OPG of faeces was recorded in the positive control (group 6) as it was in the untreated group. In the groups administeredwith lower doses of the extract, persistence of infection and high mortality indicated the low doses of the extract was incapable of significantly limiting the progressionof the infection. Conversely, birds treated with 1600mg/kg of the extract and those of group 5 (Amprolium) showed the least evidence of severe coccidiosis as reflected by the low oocyst count, mild clinical signs and absence of mortality.Hence It could be inferred that the effect of *MREA*Sextract on coccidian oocyst output was dose dependent. This in agreement with the findings of Biu *et al*(2006) and El-Khtam *et al*(2014) who reported similar dose related responses in faecal oocyst count and

mortality from coccidian infection in broiler chickens. The results of the present experiment showed that treatment withMREASextract inbroilers reduced oocyst count, severity of *E. tenella* lesions and prevented mortality. Hence., the results also showed that MREAS was effective in the treatment of coccidiosis inbroiler chickens. However amprolium was more potent as shown by lower oocysts count compared to MREAS.The reason for better efficacy of amprolium could be that it is already in the pure state and we can expect a bit low efficacy in the crude extracts of *A. senegalensis.*However neither the extract nor the amprolium could eliminate completely the parasites from the infected birds as they continue to shed oocysts. It is explaned that the continued shedding of oocysts by birds treated with the extract as well as amproliummay be due to the recycling of the oocysts through the faeces which may occur between the chickens and the environment such that the coccidian infection could become chronic Reid (1990) and Price and Barta (2010).

Mortality rate of 2.8-16.6% was recorded in this study, which is in agreement with the mortality range of 4.8- 16.6% reported by Biu *et al*(2006) in chickens respectively. The mortality in this study was mostly that of sudden death, implying that the infection hasa peracute /acute. course.The mortality started 9days post-treatment and persisted till 14 days postinfection. Presumably, the higher mortality was because of the severe pathogenicity of the *Eimeria* species.

Lesion scores in groups of birds have been used for many years to assess quantitatively the severity of infections andeffects of experimental treatments of coccidial infections

(Johnson and Reid, 1970).Lesion scoring was significant as an assessment tool to evaluate the virulence and pathogenicity of field isolates of*Eimeria*species (Conway *et al*., 1990).In this study experimental treatments showed improvement in lesion scores in amprolium treated and MREAS treated groups, but amprolium treated group showed the superior improvement than others. Among the MREAS treated groups,the birds that received 1600mg/kg herbal complex showed the lowest lesion score suggesting that MREAScontain a component that offers protection against coccidiosis, and at a higher dose level, can reduce caecal lesions during an acute primary infection with *Eimeria*. *tenella*. These findings may be attributed to the beneficial effects of herbal extract on intestinal epithelial morphology. Differences among infected groups in curtailing the lesions lasted till the termination of experiment.. However, their scores were at decline as observed at different time spaces.From our results it was concluded that treatment with MREASexhibits anticoccidial activity which was evidenced by lowering of caecal lesion scores.

The abundance of literature on the beneficial effects of herbal agents in the control and management of coccidian infection and performance of broiler chickens (Shaw *et al*., 2011; Tan and Long, 2012; Srinivasan *et al.,* 2015; Quiroz-Castaneda and Dantan-Gonzalez, 2015) indicated that herbal agents are potential present and future remedies to control avian coccidiosis and enhance the in growth and performance.

Evaluation of growth performance revealed that there were significant ($P < 0.05$) differences in bodyweight gain of broilersbetween the treatment groups throughout the experimental period.The significant increase in mean weight gain in treated birds when

compared to the positive control is possibly due to the inhibition of inflammation in the intestinal mucosa which is suggestive of an increased nutrient absorption across the intestinal wall and enhanced feed conversion ratio compared to the non treated positive control (Gotep *et al*., 2016). Nwosu *et al* (2012) and Biu *et al* (2006) reported an increased weight gain and feed conversion ratio in birds treated with*Khaya senegalensis* extracts and *Azadirachta indica*,respectively. The findings of the present study are not in agreement with the findings of Kurkure *et al*(2006), who observed that coccidial challenge had no effect on body weight gain and feed conversion ratio.*Eimeria* cause significant weight suppression and impaired FCR (Logan *et al.,* 1993). The reason for this impairment is that the organism destroys the absorptive mucosal surface, competes for micro nutrients resulting into metabolic imbalances and hence adversely effects nutrient utilization (Mansori and Modirsanei, 2012) However, after treatment with the MREAS, the extract was successful in alleviating growth retardation due to coccidial challenge compared with the non treated posiive control chicks. In clinicalcoccidiosis, normal weights are never fully regained during the growing period because coccidian parasites are difficult to eradicate completely. Despite reduction in the faecal oocyst count and caecal lesions score from the application of the MREAS ,there was no highly significant improvement in the broiler overall growth performance. It is understood that, beyond the anticoccidial mode of action, the magnitude of improvement in growth performance will depend on other factors related to gut ecology (e.g., gut microflora and histomorphology, gut maintenance, mucus production, and host immune response) that consume part of the energy and nutrients the host otherwise uses for production purposes (Mountzouris *et al*., 2011). A number of

similar reports of positive effects of herbal extract on weight gains by Biu *et al* (2006): Nwosu *et al* (2011) and Zulpo *et al* (2007) support the findings of the current study.

The blood biochemical analysis reflected the functional organ impairment (Shibata, 1984), and some enzyme activities are often used as indicators of the site and of the extent of pathological injury (Daniv, 1975).In the present study infection with coccidian parasites showed significant($t < 0.002$) increase in serumAST, ALT and ALP level as compared with the negative control group. The findings agrees with that of Abdel El Maksoud*et al (*2014) who observed elevated levels of ALT, AST and ALP in his study on the effect of coccidian infection on laying hens. Similar findings were reported by Patra *et al*(2010) and Mondal *et al*(2011). The finding also fall in line with those of Koynarsky*et al* (2010) Who reported that elevated ALT and AST activity are indicative of damage to the hepatoytes and mucosal cells which also accompanies coccidiosis.Haematological and biochemical parameters are, however, influenced by factors that include age, gender, feed, drugs, toxic compounds, diseases and parasite infestations (Agbede and Aletor, 2003; Huff *et al.*, 2008; dos Santos Schmidt et *al.,* 2009). In the present study, the elevated levels of AST, ALT andALP were due to infection with *Eimeria. tenella.*However, a reduction in these values was observed on 11 dpi in the treated groups, but normal values were not restored probably due to the short sampling intervals 7^{th}, 9^{th} and 11^{th} dpi.It may be concluded that restoration of these enzymes activities are correlated with the regenerative process in the liver.These indicate that the extract decreased the damaging effects of coccidial infections.

Hypoproteinemia observed in the infected birds is consistent with the findings of Fukata *et al*(1997) in clinical *Eimeria tenella* and *Eimeria acervulina* infections; it may be due to

acute stress that lead to cortisol secretion and catabolism of protein (Kaneko *et al*., 1997). The nutrient malabsorption in the gut is as a result of the damage caused by the parasite.Simillarly acute haemorrhage causes loss of plasma protein followed by rapid movement of interstitial fluids without protein into the plasma compartment to induce acute hypoprrpteinamia (Mondal *et al.,* 2011).In the current syudy,hypoproteinamia is due to stress and excessive loss of blood by the infected birdss. There was a moderate improvement in the serum protein values on 9 and 11 dpi in groups 1-5, but the mean total protein value value remained lower than the negative control (group 7).

In the presnt study a statistically significant increase in albumin level was observed. According to Obikaonu *et al* (2012), the increase in albumin level observed in the current study is suggestive of proper maintenance of the integrity of the liver and other extrahepatic tissues involved in protein synthesis.These findings may be attributed to the improvement effects of *Anonna senegalensis* in the intestinal environment which lead to improvement of the digestion and absorption of the nutrients.In addition, the extract limits the damage caused by the pathogenic parasites.

Elevated urea and uric concentrations can be indicative of high protein diet, congested heart failure, dehydration and increased protein catabolism as a result of stress or renal disease (Patton and Crouch, 1977). Increased concentrations of urea, and uric acid indicating kidney failure, were also reported in previous investigations on infected turkeys by Koynarski (1987) albeit kidney damage are not generally reported. Similar results were obtained by Patra *et al* (2010).In this study, stress of infection, dehydration and dietary

protein could be the cause of the significant elevated urea and uric acid concentrations observed as histopath of the kidney showed no lesions. Hyperuracemia observed in the chickens was not affected by the treatment.

In histopathological study, it was found that Group 7 revealed very normal structure of caecal section as it was used as negative control group. The result of the findings of histopathological study were confirmative and accord with the lesion score. group while the severest lesions were seen in the positive control (group 6) group. Broilers treated with amprolium (group 5) showed the mildest lesions followed by those treated with higher doses of MREAS (group 4)extract.Principal microscopic lesions observed in the caeca showed histologically different stage in the life cycle of the parasite infecting the glandular region as well as the mucosal region. Although these stages were observed in all the groups except the negative control (group 7), intensity was found to be less severe in thetreated group.These findings are also in consonance with those of Rasheda and Bano (1985) and Somro *et al* (2001). Results of the study indicated that the herbal complex at higher doses may have an anticoccidial effect,.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

In conclusion therefore,

i The result of this study showed that the methanol root bark extract of *Anonna senegalensi*s contains many bioactive chemical components.

ii The acute toxicity study showed an LD_{50} of over 5000mg/kg indicating it is relatively non-toxic and safe for use as an anticoccidial agent.

iii Iii Inhibition of sporulation of *Eimeria tenella* oocysts by the extract was achieved and was dose dependent being highest at the highest concentration used. The percentage inhibition produced by the extract at 1000 and 1500mg/kg were similar to that of amprolium at 1.25g/ littre of water.

iv The methanol root bark extract reducuced the oocysts count of chicks infected with 2 $\times 10^4$ sporulated oocysts of *Eimeria tenella*. The group treated with 1600mg/kg ofextract showed the highest reduction in oocysts count followed by 800mg/kg, 400mg/kg,, and 200mg/kg respectively. However, amprolium produced the highest reduction in oocyst count. V

v The result also showed that treatment of chicks with MREASsignificantly improved the bodyweight gain of all infected groups except the positive control (group 6) in comparison

with the negative control group. Average final bodyweight gain of chicks from group 1, 2, 3, 4 and 5 was 990.5 ± 19.2, 1011.8 ± 24.6, 1041 ± 12.8, 1060 ± 16.4, 1152 ±23.9 respectively.

vi The findings of the present study suggest that treatment with herbal extracts of *Anonna senegalensis* at a concentration of 1600mg/kg greatly alleviated the negative impact of *Eimeria* infection by reducing lesion score and improves the performance parameters in broiler chickens but did not suppress coccidial oocysts shading to the level observed in birds treated with amprolium.

vii Basedon the findings of this study, *Anonna senegalensis* has the potential to be used as an alternative to chemotherapeutic drug as it is more affordable as compared to amprolium and specie are ubiquitous throughout Nigeria.

6.2Recommendation

1 Sub acute and chronic toxicity studies should be conducted to observe the effect of long term administration of the extract on chicks

2Further studies are needed to determine the efficacy of root bark extract of *Anonna senegalensis* against other species of *Eimeria* as this would provide imformation on the efficacy of *Anonna senegalensis* against chicken coccidiosis in general.

3.Further studies should be carried out to determine the concentration of*Anonna senegalensis*that is least toxic and more effective in curtailing the disease

4..Use of *Anpnna senegalensis* root extracts in broiler is recommended as inexpensive and efficient herbal remedy for effective coccidiosis controlwithout residual effects.

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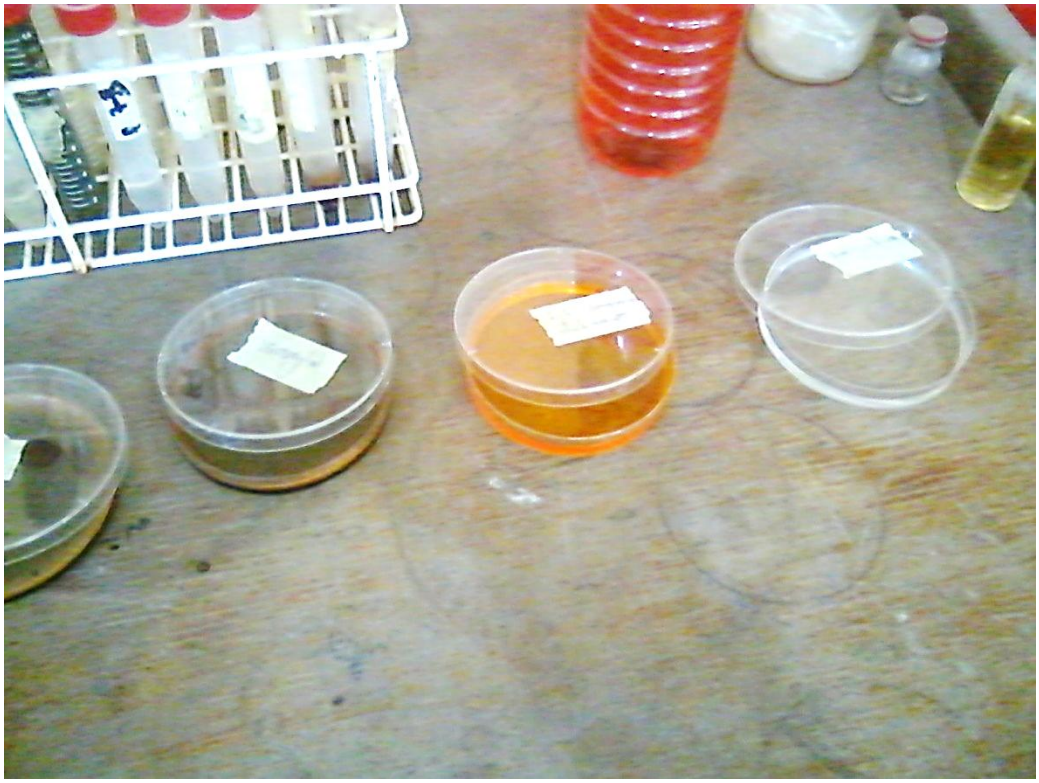
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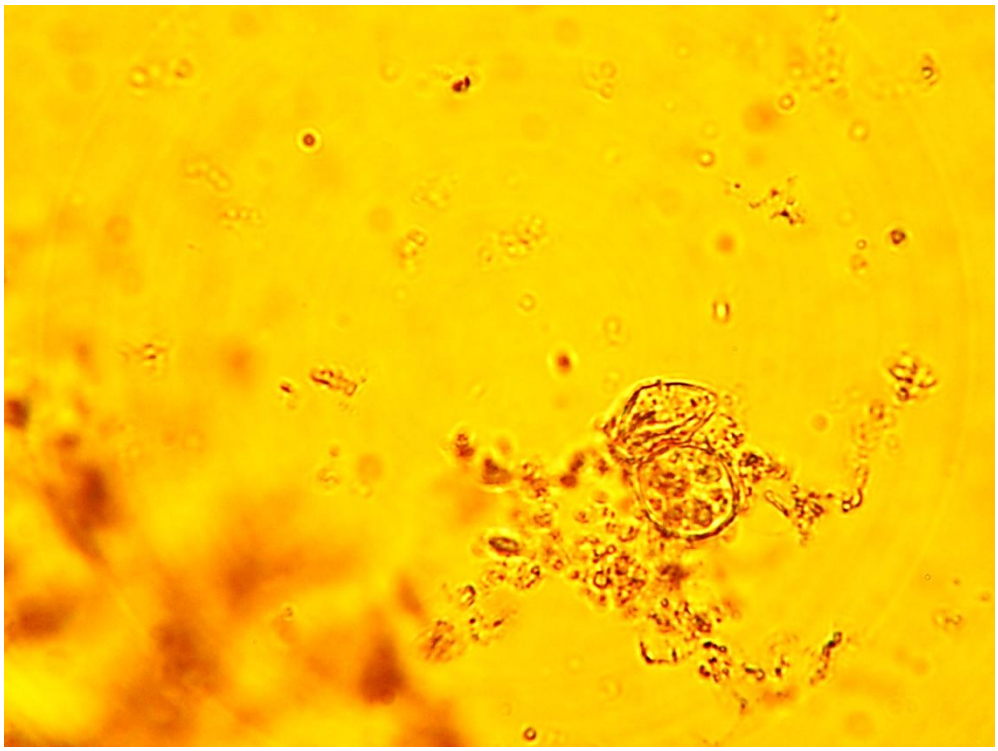
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APPENDICES



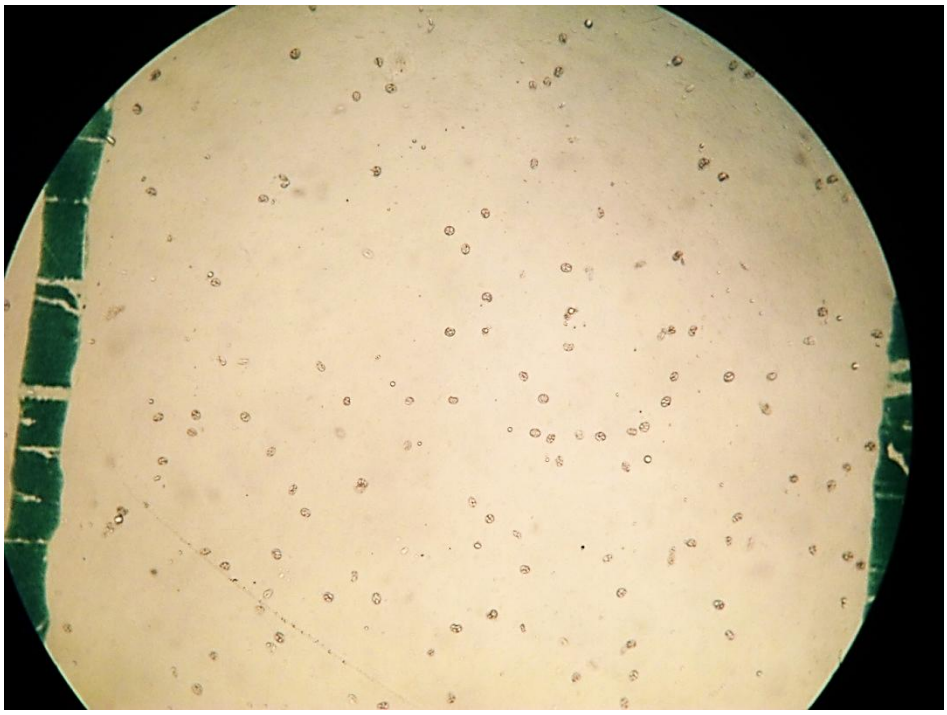
Appendix 1: Invitro inhibition test set up. From left Petri dishes containing oocysts in 1000mg/kg, 1500mg/kg extract, 2.5% potassium dichromate and 1.25g/litre amprolium respectively



Appendix 2: Sporulated oocyst in 100mg/kg of A. senegalensis



Appendix 3: Unsporulated oocyst in petri dish containing 1.25g/ litre amprolium



Appendix 4: Oocyst per gram (OPG) Count using the McMaster counting chamber. Note the green grid lines of the chamber



Appendix 5: Some of the broiler chickens used in the experiment



Appendix 6: Infected chicks (Group 3) showing signs of coccidiosis



Appendix 7 : Measurement of Bodyweightgain using weighing balance