CHEMOTHERAPEUTIC EFFECTS OF AMPROLIUM TOLTRAZURIL AND *GANODERMA LUCIDUM* IN EXPERIMENTAL COCCIDIAL INFECTION IN WARREN CHICKS

BY

FARUNA PAUL

DEPARTMENT OF VETERINARY SURGERY AND MEDICINE,
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

March, 2008

CHEMOTHERAPEUTIC EFFECTS OF AMPROLIUM TOLTRAZURIL AND *GANODERMA LUCIDUM* IN EXPERIMENTAL COCCIDIAL INFECTION IN WARREN CHICKS

BY

FARUNA PAUL

DVM 2003
MSc/VET-MED/45927/2004-05

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE (M.Sc) IN VETERINARY MEDICINE (AVIAN MEDICINE)

DEPARTMENT OF VETERINARY SURGERY AND MEDICINE,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

March, 2008

DECLARATION

I declare that this work titled "Chemotherapeutic effects of amprolium, toltrazuril and *Ganoderma lucidum* in experimental coccidial infection in Warren chicks" was performed by me in the Department of Veterinary Surgery and Medicine under the supervision of Professor P. A. Abdu, Drs. H. M. Kazeem and M. Y. Fatihu.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Name of student	Signature	Date

CERTIFICATION

This thesis entitled "CHEMOTHERAPEUTIC EFFECTS OF AMPROLIUM, TOLTRAZURIL AND *GANODERMA LUCIDUM* IN EXPERIMENTAL COCCIDIAL INFECTION IN WARREN CHICKS" by Faruna Paul meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

_____ _____

Prof. P. A. Abdu, DVM, MSc, PhD — Date
Chairman, Supervisory Committee

_____ _____

Dr. H. M. Kazeem, DVM, MSc, PhD — Date
Member, Supervisory Committee

_____ _____

Dr. M. Y. Fatihu, DVM, MSc, PhD — Date
Member, Supervisory Committee

_____ _____

Prof. A. B. Ogunkoya, DVM, MSc, Phd — Date
Head of Department,
Department of Veterinary Surgery & Medicine

_____ _____

Prof. S. A. Nkom, BSc, MSc, Phd — Date
Dean, Postgraduate School

DEDICATION

This thesis is dedicated to God Almighty, all members of my family, colleagues and friends.

Acknowledgements

My sincere gratitude goes to Professor P. A. Abdu for all the support, encouragement and understanding throughout the duration of this work. I also wish to thank Dr. H. M. Kazeem and Dr. M. Y. Fatihu for their support and thorough supervision of the research work and their scholarly contribution.

My appreciation goes to the Head of Veterinary Parasitology and Entomology Department, Faculty of Veterinary Medicine, Prof. J. O. Ajanusi and Dr. H. M Kazeem Head of Department, Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine for their cooperation in allowing me to make use of the facilities in their various laboratories. My appreciation goes to Alhaji Sale Usman, Mr. Falaranmi, Mr. Daniel Gimba all of the Veterinary Helminthology Laboratory; Mr. David Leo, Mr. Lawal Musa, both staff of the Veterinary Surgery and Medicine Department, Mr. Edima Obaja and Mr. S. Abdulganiyu both of the Veterinary Teaching Hospital, A. B. U. Zaria. My appreciation also goes to Mr. A. Adaji and Mr. Sunday of the Veterinary Clinical Pathology Laboratory.

The support and encouragement by Drs. Lawal Sa'idu, Mohammed Abdul, Akpabio Uduak, Jatau Isah, Maidawa S. Men, Ahmed Kuburat and Aliyu Wakawa are also appreciated. The assistance from Mr. Atodo Dennis is also well appreciated.

Finally, I wish to register my gratitude to my immediate and extended family members for their support, understanding and patience leading to the successful completion of the work.

Table of contents

Title Page……ii

Declaration……iii

Certification……iv

Dedication……v

Acknowledgements……vi

Table of Contents……viii

List of Tables……xiii

List of Plates……xiv

List of Figures……xvi

List of Appendices……xvii

Abstract……xviii

Chapter One

1.0 Introduction……1

1.1 Background Information……1

1.2 Anticoccidials……2

1.3 Control of Coccidiosis……3

1.3.1 Medication……3

1.3.2 Management and Hygiene……4

1.3.3 Vaccines……5

1.3.4 Genetics……5

1.4 Statement of Research Problem……6

1.5 Justification……7

1.6 Objectives……7

Chapter Two

2.0 Review of the Literature……8

2.1 Definition……8

2.2 Historical Perspective……8

2.3 Epidemiology……9

2.3.1 Distribution……9

2.3.2 Incidence……9

2.3.3 Transmission……9

2.4 Aetiology……10

2.4.1 Classification……10

2.4.2 Species of Eimeria……10

2.4.2.1 *Eimeria tenella*……11

2.4.2.2 Molecular Biology of *Eimeria tenella*……12

2.4.2.3 Life Cycle of *E. tenella*……12

2.4.2.4 Pathogenicity……13

2.4.2.5. Clinical Signs……15

2.4.2.6. Gross Lesions……16

2.4.2.7. Histopathological Lesions……16

2.4.2.8. Diagnosis……17

2.4.3 Public Health significance……19

2.4.4 Prevention and Control……19

2.4.4.1 Management.....20

2.4.4.2 Nutrition.....21

2.4.4.3 Immunization.....21

2.4.4.4 Chemoprophylaxis.....24

2.4.4.4.1 Amprolium (Amprol®, Amprolmix®).....28

2.4.4.4.2 Toltrazuril (Baycox®, Cipcox®, Tolacox®).....29

2.4.4.4.3 *Ganoderma lucidum*.....31

Chapter Three

3.0 Materials and Methods.....34

3.1 Experimental Chicks.....34

3.1.1 Housing for Experimental Chicks.....34

3.1.2 Vaccination.....35

3.2 Source of *Eimeria* Oocysts.....35

3.2.2 Sporulation of oocysts.....38

3.2.3 Preparation of Inoculum.....41

3.3 Anticoccidials used in the Experiment.....42

3.3.1 Amprolium.....42

3.3.2 Toltrazuril.....42

3.3.3 *Ganoderma lucidum*.....42

3.3.3.1 Preparation of *G. lucidum* used for treatment.....44

3.4 Experimental Plan.....44

3.4.1 Experimental Design.....44

3.4.2 Infection with *Eimeria* ……..46

3.5 Samples and Data Collection……..46

3.5.1 Rectal Temperature……46

3.5.2 Body Weight……46

3.5.3 Blood Sample Collection……47

3.5.4 Fecal Sample Collection……..47

3.6 Laboratory Procedures……47

3.6.1 Determination of Packed Cell Volume……47

3.6.2 Determination of Total Plasma Protein……48

3.6.3 Determination of Oocysts Count per gram of Feces……49

3.7 Observation of Inoculated Birds Post Inoculation for Clinical Signs……49

3.8. Postmortem Examination (PM)……50

3.9 Treatment of Infected groups……50

3.10 Statistical Analysis……50

Chapter Four

4.0 Results……52

4.1 Body Weights……52

4.2 Mean Packed Cell Volume (%)……55

4.3 Mean Total Plasma Protein (g/dl)……57

4.4 Clinical Signs……58

4.5 Daily Oocysts Count Post Inoculation……69

4.6 Postmortem Findings……75

Chapter five

5.1 Discussion……..81

5.2 Conclusions……86

5.3 Recommendations……87

References……88

Appendices ……..97

List of Tables

Table Page

3.1 Summary of experimental design……45

4.1 Mean body weight gain (in grams) post inoculation in Warren chicks inoculated with local strain of *Eimeria* at 25 days old……..54

4.2 Mean packed cell volume (%)±SD post inoculation in Warren chicks inoculated with local strain of *Eimeria* at 25 days old……56

List of Plates

Plate Page

I *Ganoderma lucidum*.....32

II Housing for experimental chicks.....36

III Cages made of wire mesh.....37

IV Unsporulated oocysts of *Eimeria* X10.....39

V Unsporulated oocysts of *Eimeria* X40.....40

VI Sporulated oocysts of *Eimeria* X10.....43

VII Uninoculated group showing birds that were alert and well.....59

VIII Day 6 post inoculation showing normal faeces from uninoculated birds (Group A).....60

IX Day 6 post inoculation showing ruffled feathers and somnolence in inoculated birds (Group B).....61

X Day 6 post inoculation showing the presence of frank blood in faeces of inoculated birds (Group B).....62

XI Day 5 post inoculation showing depression, ruffled feathers and somnolence in inoculated birds (Group C).....63

XII Day 5 post inoculation showing presence of blood in faeces of inoculated birds (Group C).....64

XIII Day 6 post inoculation showing hurdling together and somnolence in inoculated birds (Group D).....65

XIV Day 6 post inoculation showing presence of frank blood in faeces of inoculated birds (Group D).....66

XV Day 5 post inoculation showing ruffled feathers in inoculated birds (Group E).....67

XVI Day 5 post inoculation showing presence of blood in faeces of inoculated birds (Group E)……68

XVII Normal caecum at postmortem in uninoculated birds (Group A)……..76

XVIII Caecum containing clotted blood at postmortem in inoculated birds (Group B)……..77

XIX Caecum containing clotted blood at postmortem in inoculated birds (Group C)……78

XX Caecum containing clotted blood at postmortem in inoculated birds (Group D)……79

XXI Ballooned caecum at postmortem in inoculated birds (Group E)……80

List of Figures

Figure Page

1. Mean change in body weight (grams) post inoculation in Warren chicks inoculated with local strain of *Eimeria* at 25 days old……53

2. Graph showing daily oocysts count per gram of faeces in birds inoculated with *Eimeria* and not treated (Group B)……71

3. Graph showing daily oocysts count per gram of faeces in birds inoculated with *Eimeria* and treated with amprolium (Group C)……72

4. Graph showing daily oocysts count per gram of faeces in birds inoculated with *Eimeria* and treated with toltrazuril (Group D)……73

5. Graph showing daily oocysts count per gram of faeces in birds inoculated with *Eimeria* and treated with *Ganoderma lucidum* (Group E)……74

List of Appendices

Appendix Page

1. Mean change in body weight (grams) post inoculation in Warren chicks inoculated with local strain of *Eimeria* at 25 days old……..97

2. Mean total body weight gain (in grams) post inoculation in Warren chicks inoculated with local strain of *Eimeria* at 25 days old ……98

3. Mean total plasma protein (in g/dl) ±SD post inoculation in Warren chicks inoculated with local strain of *Eimeria* at 25 days old……..99

4. Daily oocysts count per gram of feces post inoculation in Warren chicks inoculated with local strain of *Eimeria* at 25 days old……..100

5. Preparation of 2.5% w/v Potassium dichromate solution……..101

6. Preparation of floatation medium (1.3 specific gravity)……..102

7. Preparation of 10% formol saline……..103

Abstract

One hundred and forty five day–old Warren chicks were randomly divided into five groups (A, B, C, D and E). Groups B, C, D, and E were inoculated with about 27,000 sporulated *Eimeria* oocysts per bird at three weeks of age while group A served as the control. Oocyst production and clinical signs were monitored from day zero post inoculation. The values of packed cell volume, total plasma protein, and body weight gain were determined at regular intervals and compared with values from control group A. Three groups (C, D and E) were treated on day seven post inoculation with amprolium, toltrazuril and *Ganoderma lucidum*, respectively after clinical signs and maximum oocyst production were observed. The efficacy of each drug was determined based on the number of oocyst produced per gram of feces until treatment was completed. Bloody droppings, loss of appetite, somnolence and hurddling together were initially observed at day four post inoculation while packed cell volume and body weight gain were significantly ($p<0.05$) reduced in all infected groups. There was no significant difference ($p>0.05$) in the total plasma protein between the inoculated and uninoculated groups. Amprolium, totrazuril and *Ganoderma lucidum* used in the treatment were observed to have anticoccidial effect with toltrazuril having the strongest effect on *Eimeria*, followed by amprolium and *Ganoderma lucidum* based on the number of oocyst produced per gram of feces in each of the groups. In conclusion it could be said that the *Eimeria* used in the experiment was pathogenic for chickens and could be of great economic

importance to the poultry industry as it adversely affected the weight gain of the infected group. It also has an adverse effect on the packed cell volume, with little or no effect on the total plasma protein of inoculated birds. Treatment with amprolium, toltrazuril and *Ganoderma lucidum* showed that they all have anticoccidial effects, but toltrazuril proved to be more efficacious followed by amprolium and lastly *Ganoderma lucidum.*

Chapter One

1.0 Introduction

1.1. Background Information

Fowl coccidiosis is a major disease of chickens caused by protozoan parasites of the genus *Eimeria* (Chapman, 1988). Clinical disease occurs only after ingestion of relatively large number of sporulated oocysts by susceptible birds, and a period of asexual development, in which the parasites multiply in the epithelial cells of the intestine. This is followed by a sexual phase which culminates in the production of new oocysts which is voided out in the feces (Chapman, 1988; Larry and Otto, 2005).

Fowl coccidiosis remains the number one health challenge of birds throughout the world where flock are housed on the floor. It is universally recognized as one of the major disease problems confronting the poultry industry (Evans and Martins, 1991; Adene, 2004). It remains one of the most expensive and common disease of poultry production in spite of advances in chemotherapy, management, nutrition and genetics (McDougald and Reid, 1997).

Coccidiosis outbreaks have been reported in many parts of Nigeria including Shika, Zaria, where the presence of at least two of the most pathogenic species for chickens (*E. tenella* and *E. necatrix*) have been established. However, the

economic losses due to coccidiosis in poultry industry in Nigeria have not been fully estimated (Bishu, 1982; Adene, 2004).

Eimeria tenella infection (caecal coccidiosis) is the best known of the avian coccidiosis, partly because of its widespread importance (McDougald and Reid, 1997). The parasite inhabits the caeca and adjacent intestinal tissues, causing a severe disease characterized by bloody droppings, loss of appetite, huddling together and dehydration, high morbidity, mortality and loss of weight gain and other sings attributed to coccidiosis (McDougald and Reid, 1997; Jordan and Pattison, 1999; Murray and Hanson, 2005).

1.2. Anticoccidials

Anticoccidials are drugs which have activity against coccidia and are used for prevention or treatment of coccidiosis (Chapman, 1988; Jordan and Pattison, 1999; Larry and Otto, 2005). The efficacy of anticoccidials or the sensitivity of the parasite to the drug is strongly related to the intensity with which these products have been in use over the past few years (Suls, 1999).

During the past 25 years, more than 40 chemical products have been offered for sale, while many more have shown anticoccidial activity. The confusion is compounded by differences in efficacy demonstrated by these products when tested against the nine recognized species of chicken coccidia (Reid, 1972). For

many years, drug resistant coccidia have been greatly feared, thus the development of tolerance of drugs by coccidia after exposure to medication is the most serious limitation to effectiveness of products (McDougald and Reid, 1997; Williams, 2006).

Resistance has been reported frequently, involving field isolates and in controlled laboratory conditions following the use of anticoccidial drugs for more or less prolonged periods of time (Hamet *et al.*, 1985). Resistant strains of coccidia may appear suddenly as new mutation, while other strains may have been selected out by long continued use of a product in a given area (Reid, 1972).

1.3. Control of coccidiosis

The methods of control for coccidiosis are medication, management and hygiene, selection for resistance and vaccination (Jordan and Pattison, 1999). Eradication of the parasite is unlikely to be successful, however medication through some form of chemotherapy, remain the main method used in coccidiosis control (Chapman, 1988; Evans and Martins, 1991).

1.3.1. Medication

Anticoccidial drugs are routinely and continuously administered in the feed or drinking water of birds raised in the poultry industry to control coccidiosis (Reck and McQuistion, 1994).

Occasionally when prophylaxis has failed, it is necessary to treat birds showing clinical sings of coccidiosis. The reason for such failure can include accidental omission of a drug from the feed, incorporation of a drug at an inadequate concentration, limited efficacy of a drug against the species of *Eimeria* concerned or the acquisition of resistance to the drug in use (Chapman, 1987a). Unless the disease is recognized and treatment promptly instituted, it may not be possible to limit the effect of infection (Chapman, 1988).

1.3.2. Management and Hygiene

Practical methods of management cannot prevent infection (Larry and Otto, 2005). It is impossible to chemically eliminate coccidia oocyst from a farm environment; good hygiene, however, can substantially reduce the number of oocysts contaminating the environment (Jordan and Pattison, 1999).

Attempt to control coccidiosis through good management procedures on farms are difficult because of the ease of transmission of the organism and its high rate of reproduction (Evans and Martins, 1991). Attention should also be paid to prevention of over flowing waterers and leaking water pipes and roofs (Jordan and Pattison, 1999).

1.3.3. Vaccination

Presently, vaccination programme consists of infecting young poultry with a known dose of live coccidia parasite; this will immunize the birds against the disease (Opitz, 1998).

The use of live or sub–unit vaccines has become the focus of a great deal of research and development although the live vaccines have had the drawback of themselves by causing some degree of pathogenicity and in some cases even leading to clinical disease (Wallach and Waldenstedt, 1999). Commercial vaccines consist of low doses of sporulated oocysts of the various coccidial species and serves only to introduce infection. Chickens are reinfected by the progeny of the vaccine strain on the farm, this constitutes the second dose and this re–exposure is important for good immunity (Opitz, 1998; Larry and Otto, 2005).

1.3.4. Genetics

It had been known for many years that strains of fowl that are less susceptible to coccidiosis can be bred. This approach to control has not been developed partly due to the efficacy and availability of drugs (Jordan and Pattison, 1999).

1.4. Statement of Research Problem

1. Nigeria has an estimated poultry population of about 86 million at a stage in the past, the national poultry stock rose to attain an all time record of 123.9 million in 1979 and about 103 million in 1992 (Akinwumi *et al*., 1979; RIM, 1992). Newcastle disease, Infectious bursal disease and coccidiosis together contributes about 62% of all poultry diseases, while coccidiosis alone tops the list of parasitic diseases affecting poultry (Adene, 2004). Coccidiosis is universally present in poultry–raising operations and chickens of all ages can be infected (Opitz, 1998; Larry and Otto, 2005). *Eimeria tenella* infection (caecal coccidiosis) is characterized by bleeding from the intestines, high morbidity and mortality, loss in weight gain and emaciation (McDougald and Reid, 1997).

2. *Eimeria tenella* develops in the cells of the caeca and is one of the most pathogenic coccidia to infect chickens (Murray and Hanson, 2005). The parasite destroys the integrity of the intestine and interfere with nutrient absorption which results in loss of weight or death (Anonymous, 2002).

3. Many avian diseases including coccidiosis are currently controlled by drug therapy; however, they are increasingly ineffective as drug–resistant coccidia strain rapidly develop (Anonymous, 2002). Today decreased anticoccidial sensitivity and health issues have made it more important than ever to find alternative coccidiosis control measures which include organic anticoccidials (Greg, 2001).

1.5. Justification

The justifications for this research were as follows:

1. Coccidiosis is a disease of great economic importance in the poultry industry.
2. Due to the indiscriminate use of amprolium by poultry farmers in Nigeria over the years, its efficacy has become doubtful.
3. There is the need for research into alternative means of prevention, control and treatment of coccidiosis using organic anticoccidials.

1.6. Objectives

The objectives of this study were therefore to:

1. Re-examine the specific clinical signs and gross pathology associated with infection of chicks with a local isolate of *Eimeria*.

2. Determine the effect of a local isolate of *Eimeria* infection on packed cell volume, total plasma protein and body weight gain.
3. To compare the chemotherapeutic effects of amprolium and toltrazuril.
4. To investigate the potential of *Ganoderma lucidum* as an anticoccidial.

Chapter Two

2.0. Review of the Literature

2.1. Definition

Fowl coccidioisis is an infectious protozoan disease of chickens caused by nine species of *Eimeria* and transmitted by resistant sporulated oocysts and characterized by anemia, anorexia, bloody diarrhoea, severe production losses in the form of morbidity and mortality (Larry and Otto, 2005). The protozoan parasite of the genus *Eimeria* in chickens multiply in the intestinal tract and cause tissue damage, with resulting interruption of feeding and digestive processes or malabsorption leading to dehydration, blood loss and increased susceptibility to bacterial infection (McDougald and Reid, 1997).

Fowl coccidiosis may affect any type of poultry in any type of facility, although the period of high coccidiosis risk is between the 20th and 35th days of a broiler's life (McDougald and Reid, 1997; Suls, 1999).

2.2. Historical perspective

The first comprehensive descriptions of fowl coccidia were made in the 1920s and 1930s (Tyzzer, 1929; Shirley, 2006). Coccidiosis is regarded as the parasitic disease that has the greatest economic impact on poultry production (Duffy and Tucker, 2003).

2.3. Epidemiology

2.3.1. Distribution

Fowl coccidiosis has become increasingly prevalent in the worldwide poultry industry (Anonymous, 2002). Coccidia are distributed worldwide in poultry and wild birds and almost universally present in poultry–raising operations (Larry and Otto, 2005). Survey in North and South America revealed coccidia present in almost all broiler farms (McDougald *et al*., 1986; McDougald *et al*., 1987). Its presence in Nigeria has also been reported (Bishu, 1982; Abdu *et al*., 2006).

2.3.2. Incidence

Fowl coccidiosis occurs year round, but generally occurs more frequently during rainy season (May-September) than dry season (October-April) of the year (Murray and Hanson, 2005). In chickens on litter the disease occurs at 3–6 weeks of age but may occur in older birds (Jordan and Pattison, 1999).

2.3.3 Transmission

Clinical disease occurs only after ingestion of relatively large number of sporulated oocysts by susceptible birds. Both clinically infected and recovered birds shed oocysts in their droppings, which contaminate feed, water, litter and soil (Larry and Otto, 2005). Many different animals, insects, contaminated equipment, wild birds and dust can spread oocysts mechanically. Also, transmission from one farm to another is facilitated by movement of personnel and equipment between farms, viable oocysts have been reported from the dust inside and outside broiler houses, as well as from insects in poultry litter (McDougald and Reid, 1997).

2.4. Aetiology

2.4.1. Classification

Coccidiosis in chickens is caused by species of intracellular protozoan parasites which belong to the phylum *Apicomplexa* and the genus *Eimeria* (Shirley and Tomley, 2006). The host specificity of *Eimeria* in birds is very

strict, so that parasites from different species of birds can be considered different species even though they may have similar-appearing oocysts, sporulated oocyts has four sporocysts each with two sporozoites (McDougald and Reid, 1997).

2.4.2. Species of *Eimeria*

Nine *Eimeria* species have been described in chickens; the most common species are *Eimeria tenella*, which causes the caecal or bloody coccidiosis, *Eimeria necatrix*, which causes bloody coccidiosis and *Eimeria acervulina* and *Eimeria maxima* causes chronic intestinal coccidiosis (Jordan and Pattison, 1999; Murray, 2001). Other species of *Eimeria* which are known to cause coccidiosis in chickens include *Eimeria brunetti, Eimeria mitis, Eimeria mivati, Eimeria praecox* and *Eimeria hagani* (Saville, 1996; Larry and Otto, 2005).

2.4.2.1. *Eimeria tenella*

Eimeria tenella is the cause of caecal coccidiosis especially in young birds, but rarely in less than ten-day old, although chickens of all ages and breed are susceptible to infection (Saville, 1996; McDougald and Reid, 1997).

Microscopically the sporulated oocyst has a characteristic ovoid appearance. It measures between 19.5–26.0 µ (average 22.0 µ) in length and 15.7–19.8 µ (average 19.0 µ) in width. It also has an index length/width of 1.16. *E. tenella* has a characteristic life history of 115 hours minimum prepatent period and a minimum sporulation time of about 18 hours (Jordan and Pattison, 1999; McDougald and Reid, 1997).

The sporulated oocyst is a unique structure in its ability to survive in the external environment for extended periods (Ferguson, 2003). It is protected by a thick oocyst wall and resistant to a fairly wide range of normal environmental conditions and the ability to survive for months or years making control of infection very difficult (Jordan and Pattison, 1990; Ferguson, 2003).

2.4.2.2. Molecular biology of *Eimeria tenella*

The Houghton strain of *E. tenella* has a genome of approximately 60 MB in size, with a Gc content of approximately 53%, there are 14 chromosomes that range in size from 1 Mb to >6 Mb and two major ribosomal DNA clusters, that account for some 2.5% of the genome size, have been mapped as tandem arrays on chromosome 12 (140 copies of an 8kb 18S – 5.8S – 28S unit) and chromosome 10 (500 copies of a 730 bp 5S unit) (Shirley and Tomley, 2006).

2.4.2.3. Life cycle of *E. tenella*

Sporulated oocysts are responsible for infection of new susceptible hosts when ingested (Ferguson, 2003). After ingestion of the infective stage (sporulated oocyst), the parasite also undergoes many developmental stages inside the host cells (Anonymous, 2002). The oocyst wall is crushed in the gizzard (excystation) and the sporozoites enter cells in the mucosa of the caecum and begin the cell cycle leading to reproduction (McDougald and Reid, 1997). Many merozoites are produced and each in turn may enter another epithelial cell of the ceacum, this cycle may be repeated several times thus large numbers of epithelial cells are destroyed (Murray, 2001). The second generation merozoites develop into sexual stages on entering new cells (i.e. macrogametocytes and microgametocytes) which further develop into macro- and microgametes. The macrogametes develop into the oocysts at the time of fertilization by the microgametes which are excreted with the feces (Ferguson, 2003). The oocysts are passed in the feces of infected birds, over 1–2 days, in the presence of moisture, oxygen and a suitable temperature (25–30℃) and four spores develop within the oocyst, each containing two sporozoites (Saville, 1996; Jordan and Pattison, 1999).

2.4.2.4. Pathogenicity

Eimeria tenella is the most pathogenic of the coccidia parasites that infect domestic chickens. It has worldwide distribution with infection becoming noticeable at about three days after infection (Saville, 1996; Ferguson, 2003). *E. tenella* develops in the caeca of chickens under normal circumstances and the severity of infection is dependent on the number of oocysts ingested, age of the bird and nutritional status of the host (Reid, 1968; Murray, 2001).

Eimeria tenella has a very high reproductive potential compared to other *Eimeria* species in chickens and this is governed by the number of schizogonous stages in its life cycle and the number of merozoites in each schizont. In an experimental study aimed at obtaining the maximum achievable oocyst production per bird (one week old) of the *Eimeria* species in chickens, *E. tenella* ranked first with 27,425,190 oocysts/bird followed by *E. acervulina*, while *E. maxima* ranked the least with 2,576,000 oocysts/bird under similar experimental conditions (Williams, 2006).

Schizogony in *E. tenella* occurs in the crypts of Lieberkuhn of the caeca and causes extensive haemorrhage (Larry and Otto, 2005). The most pathogenic stage is the second generation schizonts which mature at 4 days post infection and large groups of the second–generation schizonts

are located within the mucosa of the caecum and cause ulceration of the mucosal surface when the schizonts mature and merozoites are released (Mcdougald and Reid, 1997; Ferguson, 2003).

Coccidial infections are known to be a predisposing factor for other syndrome, in particular necrotic enteritis which is caused by *Clostridium perferingens* (Duffy and Tucker, 2003). Interactions between Reovirus, *E. coli*, and *Salmonella* spp have also been noted (Van der Stroom and Wiebe Vander Sluis, 1999). The onset of mortality in a flock is rapid and mostly occurs between 5 and 6 days post infection. In acute infections, it may follow the first signs of infection by only a few hours, where the exact cause of death is not known, but toxic factors are suspected and in few cases, death may result from gangrenous or ruptured caecal pouches (McDougald and Reid, 1997).

2.4.2.5. Clinical signs

The effects of caecal coccidiosis are due to a number of factors, all of the observed effects are related to disruption of the epithelial cells lining the caeca by the release of parasite stages (Anonymous, 2005a).

Caecal coccidiosis is generally acute in onset and is characterized by depression, ruffled feathers; marked drop in feed consumption, diarrhoea and lethargy (Sainsbury, 1992; Shane, 1997).

Sometimes it is often the loss of performance that gives a hint that there is coccidial infection present, rather than mortality and this loss of performance even in sub–clinical levels of the disease is of economic importance to the farmer (Duffy and Tucker, 2003).

Weight loss and reduced egg production is also observed in layers and infected birds may show pallor of the comb and wattles (Shane 1997; Anonymous, 2006a).

Caecal coccidiosis is characterized by the presence of profuse bloody diarrhoea about five days after infection with high morbidity and mortality. Survivors of severe infection recover in 10–14 days but may never recover lost performance (Saville, 1996). In chronic cases, there are white or pink cores in the caeca, which may be passed in the feces after a long time (Seneviratna, 1969).

2.4.2.6. Gross Lesions

Lesions in *E. tenella* infection are usually observed around the lower intestine and ceaca. Examination of the caeca shows they become swollen and filled with a blood mass; haemorrhagic areas can be seen on the lining and in some cases 'ceacal plugs' form from the accumulation of blood, pus, oocysts and feces, haemorrhagic typhlitis (Saville, 1996; Shane, 1997; Pearson, 2001).

Ceacal wall may appear thickened with much blood and fibrin clots in caecal contents, tissue debris are also found in the caeca of affected birds (Helm, 1999; Kabell *et al*., 2006).

2.4.2.7. Histopatological Lesions

Histopathological studies of the caecum shows necrosis of the mucosa and some necrotic debris close to the mucosal lining in damaged areas. Young oocysts and gametocytes are also observed (Bishu, 1982).

A section through the caecum also shows large number of schizonts protruding through the surface of the mucosa into the lumen (Ferguson, 2003).

Small areas of haemorrhage and necrosis may appear near blood vessels of the inner circular muscles of the muscularis layer, heterophil

infilteration of the submucosa proceeds rapidly as large second–generation schizonts develop in the lamina propria (McDougald and Reid, 1997).

2.4.2.8. Diagnosis

Diagnosis of caecal coccidiosis is based on history, clinical signs, gross lesions, faecal floatation for the presence of coccidia oocysts and microscopic examination of the caeca for coccidia organisms in the tissues (Shane, 1997; Helm, 1999).

Postmortem examination is essential to confirm diagnosis as a result sick birds should be sacrificed for this purpose. Gross lesions may be noticeable from the serosal surface but the mucosal surface should be examined carefully (Jordan and Pattison, 1999). A small amount of mucosal scraping should be diluted with saline on a slide and then covered with a coverslip, oocysts or macrogametes are mostly seen (McDougald and Reid, 1997; Pearson, 2001).

Johnson and Reid in 1970 devised a system for lesion scoring. By this system a score of 0 to 4 is assigned to a bird where 0= normal and 4=most severe case (Johnson and Reid, 1970; McDougald and Reid, 1997).

In laboratory infections, the droppings, score may be used in the same manner as lesion score for a rapid and fairly reliable rating of the infection (Morehouse and Barron, 1970). The extent of abnormal droppings is rated on a scale of 0–4, where 4= maximum diarrhoea, with mucus, fluid and/or blood. Currently, direct microscopical examinations of the fresh intestinal mucosae of culled birds facilitate an immediate, reliable clinical assessment and combines speed and practicality. This is done with the use of a dissecting microscope and the method allow the clinician to distinguish between mucosal damage by coccidiosis or other enteritides and therefore to speedily initiate the most appropriate flock treatment (McDougald and Reid, 1997; Williams, 2005).

Routinely used methods in histopathology are satisfactory for examination of tissues infected with coccidia. Staining of sections with Haemotoxylin & Eosin will demonstrate developing stages of the parasite (McDougald and Reid, 1997).

A capillary electrophoretic approach has been developed for the identification of seven currently recognized species of *Eimeria* infecting chickens. The second internal transcribed spacer (ITS – 2) of ribosomal DNA is Polymerase chain reaction–amplified from any of the seven

species using a single set of oligonucleotide primers. This method is currently employed for fast, specific, cost effective diagnosis of *Eimeria* species present (Morris *et al*., 2005).

2.4.3. Public Health Significance

There is no public health risk from poultry coccidiosis, however, birds which have lost condition as a result of chronic infection may not be suitable for human consumption, also haemorrhagic lesions attracts bacterial contamination from the gut flora (Saville, 1996).

2.4.4. Prevention and Control

The growth of the worldwide poultry industry over the last half–century has been due in part to the use of measures to prevent and control coccidiosis, without adequate control measures, this intestinal disease can cause considerable loss in a poultry facility resulting in poor weight gain and feed utilization in affected chickens (Jenkins, 2005).

The battle against coccidiosis require careful step–by–step planning, thus the possibilities for control of coccidiosis are anticoccidials, management, nutrition, immunization and genetics (Suls, 1999; Jordan and Pattison, 1999).

2.4.4.1. Management

Since coccidial oocysts are ubiquitous and easily disseminated in the poultry house environment, it is very difficult to keep chickens coccidia free, especially under current intensive rearing conditions (Allen and Fetterer, 2002). However, coccidia oocysts can be damaged by bacteria, other organisms and ammonia that are also present and their viability can begin to diminish after 3 weeks (Williams, 1994). Most management control procedures are aimed at providing conditions not suitable for sporulation (Jordan and Pattison, 1999).

Many producers in the United States basically remove caked litter, let the houses air out for 2 to 3 weeks and top dress with fresh litter before placing a new flock. Other management control measures are, to avoid wet litter conditions, especially under drinkers, keep chicks feed and water away from droppings, roost birds over wire netting if brooding arrangement make this possible and avoid overcrowding (Shane, 1997; Helm, 1999 Murray, 2001).

Thus, it should be clear that appropriate management procedures should be in place to regulate environmental conditions so that the appropriate degree of coccidial exposure occurs, and that under – or overexposure to the parasites are avoided (Bafundo, 2006).

2.4.4.2. Nutrition

Basically, antibody and cell mediated responses are protein responses to an antigen. In diets that are limiting in protein and essential amino acids, it will be difficult for the pullets immune system to respond properly (Bafundo, 2006). Addition of vitamin E and Zinc may also be of some value in maintaining a strong immune response. In any case, efforts must be made to fine tune pullet rations so that uniformity of results are obtained (Bafundo, 2006).

2.4.4.3. Immunization

Control of coccidiosis by immunization is feasible since moderate infection with any of the species of *Eimeria* that parasitize the chicken can induce a protective immune response (Rose, 1985). Recently, there has been much interest in the development of "true" vaccines based upon attenuated organisms or non-infective antigens (Chapman, 1988).

Live oocyst vaccine for the control of coccidiosis relies upon the development of acquired immunity to *Eimeria* parasites. The organisms in the vaccine are alive and pathogenic, although some recently introduced vaccines contain attenuated strains of *Eimeria,* these parasites progress through the different stages of their life cycle in the birds intestine, they

serve as the mechanism to stimulate a cell-mediated immune response in the chicken. It is this response that provides the protection as the bird ages, from the adverse effects of the disease (Bafundo, 2006).

A live vaccine has been developed that contains attenuated lines of the seven species of *Eimeria* that infect chickens. The attenuated lines were derived from virulent strains by selection for earlier development in chickens. In 11 field trials, it was observed that the occurrence of coccidial oocysts in the litter and coccidial postmortem lesions was significantly lower in vaccinated birds than in the controls (Shirley *et al.*, 1995).

Gamma-irradiated *Eimeria* parasites have also been used to immunize young chickens against coccidiosis. In preliminary studies it has been shown that one-day-old chickens allowed to feed on irradiated coccidia suspended in an edible gel formulation became completely immune to challenge infection with virulent *Eimeria* species. Another approach is the development of sub-unit vaccines that incorporates DNA from *Eimeria* parasites into live delivery vectors that the poultry industry is using as vaccines (Jenkins, 2005).

The repeated inoculation of oocyts results in a greater degree of immunity than if birds are given a similar number of oocysts on a single occasion, chickens have been effectively immunized by the inclusion of oocyts in the feed over a period of several days (Joyner and Norton, 1973; Norton and Joyner, 1986).

More recently in South Africa a coccidiosis vaccine has come on the scene that uses a very different approach (Anonymous, 2005b). This vaccine, known as CoxAbic is given to broiler breeders so that they can then transfer protective immunity to their chicks. The vaccine is based on Affinity Purified Gametocyte Antigens (APGAs), which are the antigenic part of the coccidial parasite. These APGAs give a strong stimulation to the broiler breeder's immune system so that the mother has adequate immunity for herself and enough to pass on to her progeny chicks. Thus it has been shown that under field condition, vaccinating broiler breeders in order to protect the offspring against coccidiosis is practical and uneventful (Michael *et al.*, 2003; Anonymous, 2005b).

Infectious bursal disease, Marek's disease and chicken infectious anaemia have the ability to impair development and responsiveness to the immune system of the young chicken to coccidiosis. A number of

mycotoxins have also been shown to have negative effect on immune development to coccidiosis (Bafundo, 2006).

2.4.4.4. Chemoprophylaxis

The principal method for the control of coccidiosis involves the administration of drugs continuously in the feed and water almost throughout the life of the bird. Medication via the feed and water has proved to be a convenient, labour–saving, cost–effective method of control and has been a major factor in enabling large number of chickens to be reared in the intensive conditions of the modern poultry industry (Chapman, 1988).

Many products are available for prevention or treatment of coccidiosis in chickens and desirable properties of anticoccidial drugs have been defined (Reid *et al*., 1984; Larry and Otto, 2005). All types of drugs used for coccidiosis control are unique in the mode of action, the way in which parasites are killed or arrested and the effects of the drug on growth and performance of the bird (McDougald and Reid, 1997).

The commonly used anticoccidials are the ionophores (monensin, lasalocid, salinomycin, narasin, maduramycin and semduramycin), folic acid antagonists (sulfonamides, 2, 4-diaminopyrimidine and ethopabate), thiamine analogs (amprolium, dimethalium and beclotiamine) and the quinolones (decoquinate, buquinolate and nequinate). Others include the tetracyclines (oxytetracycline), robenidine, halofuginone, diclazuril, nicarbazin, toltrazuril and clopidol (Reid, 1972; Chapman, 1988; McDougald and Reid, 1997; Suls, 1999; Larry and Otto, 2005).

Some organic and natural products (plant extracts, mineral and omega-3 fatty acids) have been demonstrated to have some useful properties either by controlling *Eimeria* or through improving the animal's ability to resist the disease. One of the first plant extracts to be investigated as a potential source of compounds with anticoccidial activity was *Artemisia annua* (annual wormwood) where tea and extract of this plant have been used to treat human malaria in China for over 2,000 years. The antimalarial activity was found to be associated with artemisinin. Pure artemisinin appears to be effective against coccidia species when used as a feed additive (Duffy and Tucker, 2003).

A liquid powder made from a herbal complex consisting of *Uncariae Ramulus cum uncis, Agrimoniae herba, Sanguisorbae radix, Eclipta*

prostrate herba, Pulsatillae radix, Sophorae flavescentis radix, Rehmanniae radix and Glycyrrhizae radix were studied for anticoccidial activities on *E. tenella* in chickens. The result indicated that the birds administered with the herbal complex showed less bloody feces than those without medication, mild intestinal lesions, significantly higher body weight than unmedicated, thus the herbal complex used in the study was considered effective against *E. tenella* infection in chickens (Du and Hu, 2004).

Various essential oils and spices have been tested for anticoccidial activities, *Tumeric curcuma longa* reduce lesion score and improve weight gain. Recently, a number of Asian herbal extracts were tested for anticoccidial effects against *E. tenella*. Extract from *Sophorae flavscentis* was most effective, betaine (trimethylglycine) extract from sugar beet has also been tested. Zinc has long been known to play a role in epithelial tissue integrity, cell replication, wound healing and in reducing lesions. Also, dietary supplement with selenium has been shown to reduce mortality and increase weight gain in non-immunized chickens infected with *E. tenella* (Duffy and Tucker, 2003).

Research has also been carried out in Zimbabwe and it was found that *Aloe vera* and *Aloe spicata* used by local farmers have anticoccidial activity

even though some of the potentially active constituents are still undefined (Marizvikuru *et al*., 2005). Other herbs used by local farmers in Zimbabwe to treat coccidiosis are *Myrothamnus flabellifoilius*, *Lannea stullmanii*, *Ficus berkei* (wild fig), *Capsicum annum* (pepper), *Parinaria curatellifolia* and *Albizia gumnisera* (Waller *et al*., 1978; Marizvikuru *et al*., 2005).

The development of tolerance to drugs by coccidia after exposure to medication is the most serious limitation to the effectiveness of anticoccidial products. Surveys revealed widespread drug resistance in coccidia in the United States of America, South America and Europe. Even though coccidia develop less resistance to some drugs than to others, long-term exposure to any drug will produce a loss in sensitivity and eventually resistance (Jeffers, 1974; Hamet, 1986; Litjens, 1986; McDougald, 1986; McDougald and Reid, 1997).

Adaptation of *Eimeria* to a drug is first shown by an increase in the number of excreted oocysts, before there is any detectable modification of its efficacy (Hamet, 1978). Under laboratory condition oocyst output is the only criterion that can be used to evaluate *Eimeria* sensitivity to anticoccidials. A strain is said to be resistant to a product when oocyst excretion from infected, treated birds is greater than that obtained from untreated infected birds (Hamet *et al*., 1985).

2.4.4.4.1. Amprolium (Amprol®, Amprolmix®)

Amprolium is a thiamine analoque described in 1960 as an anticoccidial with a high degree of efficacy against seven species of coccidia of chicken and two species of coccidia of turkey. The strongest activity of amprolium was demonstrated for *E. tenella* and *E. necatrix* (Cuckler *et al*., 1960; Rogers *et al*., 1960; Warren and Ball, 1963).

Amprolium is structurally similar to and a competitive antagonist of thiamine (vitamin B1); because rapidly dividing coccidia have a relatively high requirement for thiamine. Amprolium functions on the parasite by a reversible thiamine inhibition mechanism, this mechanism makes possible protection by feeding added thiamine in case of accidental overdosing. Maximum effect of amprolium occurs about day 3 of the life cycle of coccidia (Reid, 1972; Larry and Otto, 2005). It acts primarily upon the first generation schizonts in the cells of the intestinal wall, preventing

differentiation of the merozoites. It may also suppress the sexual stages and sporulation of the oocysts (Mikota and Donald, 2003).

Amprolium is one of the safest anticoccidial drugs to be extensively used in the poultry industry, because it can be fed at several times higher than its recommended dose with no obvious ill effects. It has been used in the treatment of coccidiosis when administered in drinking water (Reid, 1972; McDougald, 1982). Amprolium at 125 mg/kg in the feed of chickens gives acceptable control of *E. tenella* and *E. acervulina*, but less effective against the other species of coccidia which affect chickens. Chickens affected with coccidiosis can be given amprolium at 250 mg/lkg in the feed or drinking water at 250 mg/l for a week (Ryley, 1967; Reece, 1988).

In the absence of coccidiosis, amprolium at 125 mg/kg in the feed of broiler chickens caused a significant increase in the growth rate, but it reduced feed conversion efficiency (Karunajeewa and Reece, 1985). Amprolium also inhibit thiamine utilization (Sharma and Quastel, 1965). Excessive concentration of amprolium (1,000 mg/kg) in the feed given to chickens produced opisthotonus and cerebro-cortical necrosis (Prasad *et al*., 1978). Nervous signs have also been reported in chickens given 125 mg/kg amprolium and 400 mg/kg furazolidone in their feed (Van Stratum, *et al*., 1966). By 1966, drug resistant strains of coccidia became a problem

in the United States. As a result, amprolium was combined with other drugs to extend and strengthen its spectrum of activity (Reck and McQuistion, 1994).

2.4.4.4.2. Toltrazuril (Baycox®, Cipcox®, Tolacox®)

Toltrazuril is a triazinetrione derivative, considered to be the leading drug against coccidiosis; it is widely used in chickens and turkeys for the prevention and treatment of coccidiosis by administration via drinking water (Anonymous, 2006e). It damages all intracellular developmental stages (schizonts, micro- and macrogametes) of *Eimeria* but not the tissue cells of the host animals, as revealed in light and electron microscopic studies. Toltrazuril interferes with the division of the nucleus and with the activity of the mitochondria, which is responsible for the respiratory metabolism of coccidia. Thus it has a coccidiocidal mode of action and its efficacy is independent of the severity of the infection (Melhorn *et al*., 1984; Haberkorn and Stoltefuss, 1987; Anonymous, 2000; Anonymous, 2006e).

It has been proposed that satisfactory control of coccidiosis could be achieved by administering toltrazuril for periods of limited duration, rather than continuously in the feed (Haberkorn, 1984). Experiments have shown that toltrazuril is effective against *E. tenella* when given in

drinking water for periods of 3 days, on three occasions, at weekly intervals. It has also been found that the addition of toltrazuril to the drinking water of broilers infected with *E. tenella* increased their body weight gain and survival rates compared with unmedicated birds (Chapman, 1987b; Ramadan *et al*., 1997).

2.4.4.4.3. *Ganoderma lucidum*

Ganoderma lucidum is a woody mushroom having a nice appearance, a red cap and stipe with white to yellow pores (Plate I). It is unsuitable for direct consumption and it's extracts or tea are extremely bitter. It is sold as tea, capsule or in the form of dried mushrooms and sometimes sold in combination with Ginseng. It is primarily composed of complex carbohydrates called water soluble-polysaccharides, triterpenoids, proteins and amino acids (Oei, 2003). Researchers have identified that water soluble polysaccharides are the most active elements found in *Ganoderma lucidum* that have anti-tumour, immune modulating and blood pressure lowering effects (Oei, 2003; Anonymous, 2006c).

For over 2,000 years *Ganoderma lucidum* (Reishi mushroom) has been recognized by Chinese medical professionals as a valuable remedy. Its Chinese name is Lingzhi, means "spiritual potency". Reishi mushroom are regarded by the Chinese as the "medicine of kings". Dr. Shi-Jean Lee, the famous Chinese medical doctor of the Ming Dynasty, strongly endorsed the effectiveness of Reishi in his famous book, Ban Chao Gang Moo ("Great Pharmacopoeia"). He stated that the "long-term taking of Reishi (Lingzhi) will build a strong, healthy body and ensure a long life (Anonymous, 2006b).



Plate I. *Ganoderma lucidum* (Oei, 2003).

In Japan and China, Reishi (*Ganoderma lucidum*) is ranked as the number one herb, ahead of ginseng because it is non-toxic. It can restore the body to its natural state when taken regularly, enabling organs to function normally and it is an immune modulator as it regulates and fine tunes the immune system. A considerable number of studies in Japan, China, United States of America and the United Kingdom in the past 30 years have shown that the consumption of *Ganoderma lucidum* has been linked to the treatment of a vast range of diseases, common ailments, and conditions. However, most of the scientific research that has been conducted appears to strongly support *Ganodema lucidum's* role as a normalizing substance and nutritional supplement that can yield medical benefits through its normalization and regulation of the body organs and functions (Anonymous, 2006c).

Chapter Three

3.0 Materials and Methods

3.1. Experimental Chicks

Chicks of the Warren breed, of the same age and sex obtained from a commercial hatchery in Ibadan were used for the experiment.

3.1.1. Housing for Experimental Chicks

Two rooms were swept, cleaned and washed with detergent. They were then disinfected with Diskol® (5% Benzalkonium chloride, 7.5% glutaraldehyde, 7.5% formaldehyde, stabilizers and antioxidants q.s) at a recommended rate of 40 ml/10 litres of water. The disinfectant was sprayed on the walls and floors of the rooms. Five cages measuring 60 cm

(length) x 60 cm (width) x 47 cm (height) made of wire mesh at the bottom and raised to a height of 10 cm from the ground to allow fecal dropping to fall unto the floor were constructed (Plate II & III). The cages were also disinfected with Diskol® before the experiment commenced.

To avoid external coccidial contamination throughout the experiment, day-old chicks on arrival were provided with sterilized equipment, feeders and waterers as recommended by Joyner and Davies (1960). The feed used was commercially prepared pelletized standard ration. Chick and grower mash without antibiotics or coccidiostat were provided (Joyner and Davies, 1960). Water was provided from direct tap water, feed and water were supplied *ad libitum*. To avoid contamination due to movement of personnel, foot bath was provided containing Diskol®. To also avoid contamination through the air; the windows were covered with polythene.

3.1.2. Vaccination

One hundred and forty five day-old chicks were purchased from "Chi" hatcheries, Ibadan. They were vaccinated with Newcastle disease virus vaccine B1 strain intraocularly at day old. Glucose and multivitamins were also given in drinking water on arrival. The chicks also received Newcastle disease La Sota vaccine (Pestikal®, manufactured by VETERINA Ltd, Croatia) at three weeks of age. Gumboro vaccine (Gumbokal®,

manufactured by VETERINA Ltd, Croatia) was administered at twelve days old. No antibiotics or anticoccidials were administered.

3.2. Source of *Eimeria oocysts*

An outbreak of caecal coccidiosis involving 200 chickens in Zaria, Nigeria was reported. The caeca from two moribund birds were harvested at postmortem after observing characteristic caecal macroscopic lesions. The caecal content was screened in the Helminthology Laboratory using floatation method to check for the presence of coccidia oocysts (Plate IV & V). The caecal content was then evacuated into sterile Petri dishes



Plate II. Housing showing different cages for experimental chicks.



Plate III. Cages made of wire mesh to allow faecal droppings on the floor.

containing 2.5% potassium dichromate and was allowed to sporulate. This formed the initial pool of oocysts. Fresh sporulated oocysts were made possible by reproducing these initial oocysts in ten coccidial-free chickens which were monitored until bloody diarrhoea and other signs of caecal coccidiosis were observed. Faecal samples were collected daily after signs were observed and postmortem examinations were carried out on moribund birds to examine the caeca in which lesions characteristic of caecal coccidiosis were observed. The caeca were also harvested in order to pool the oocysts together for sporulation and use in the entire experiment.

3.2.1. Sporulation of Oocysts

A saturated sugar solution with specific gravity of 1.3 was prepared and used as a floatation medium. Faeces with fresh saturated sugar solution was mixed thoroughly using a pestle to homogenize the mixture. The faeces and saturated sugar solution was sieved through a tea strainer into a beaker to remove faecal debris. The oocysts were then allowed to float in the saturated sugar solution for about 20 min.

Using a pipette, 1 ml of the filtrate was pipetted from the surface into a test-tube. This was then mixed with 9 ml of distilled water and centrifuged at 251.55 g for 5 min to sediment the oocysts to the bottom of the test-tube and the water was then decanted (Collee *et al*., 1982).

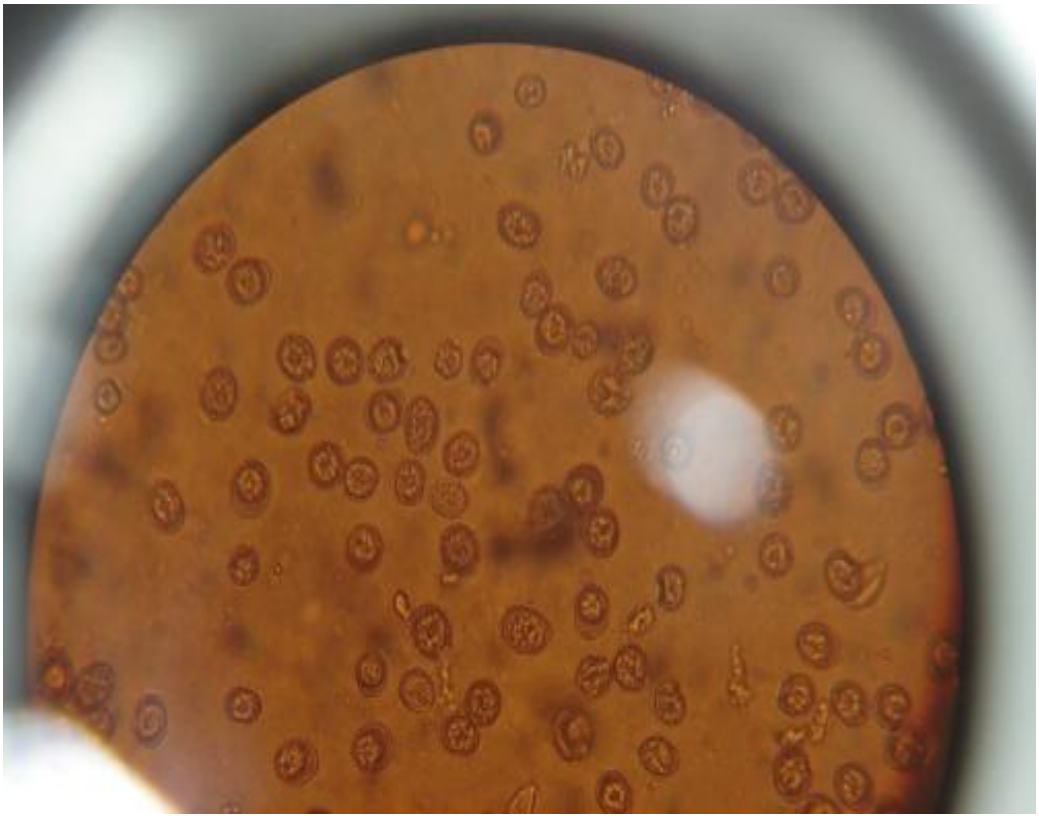


Plate IV. Unsporulated oocysts of *Eimeria* x 10.

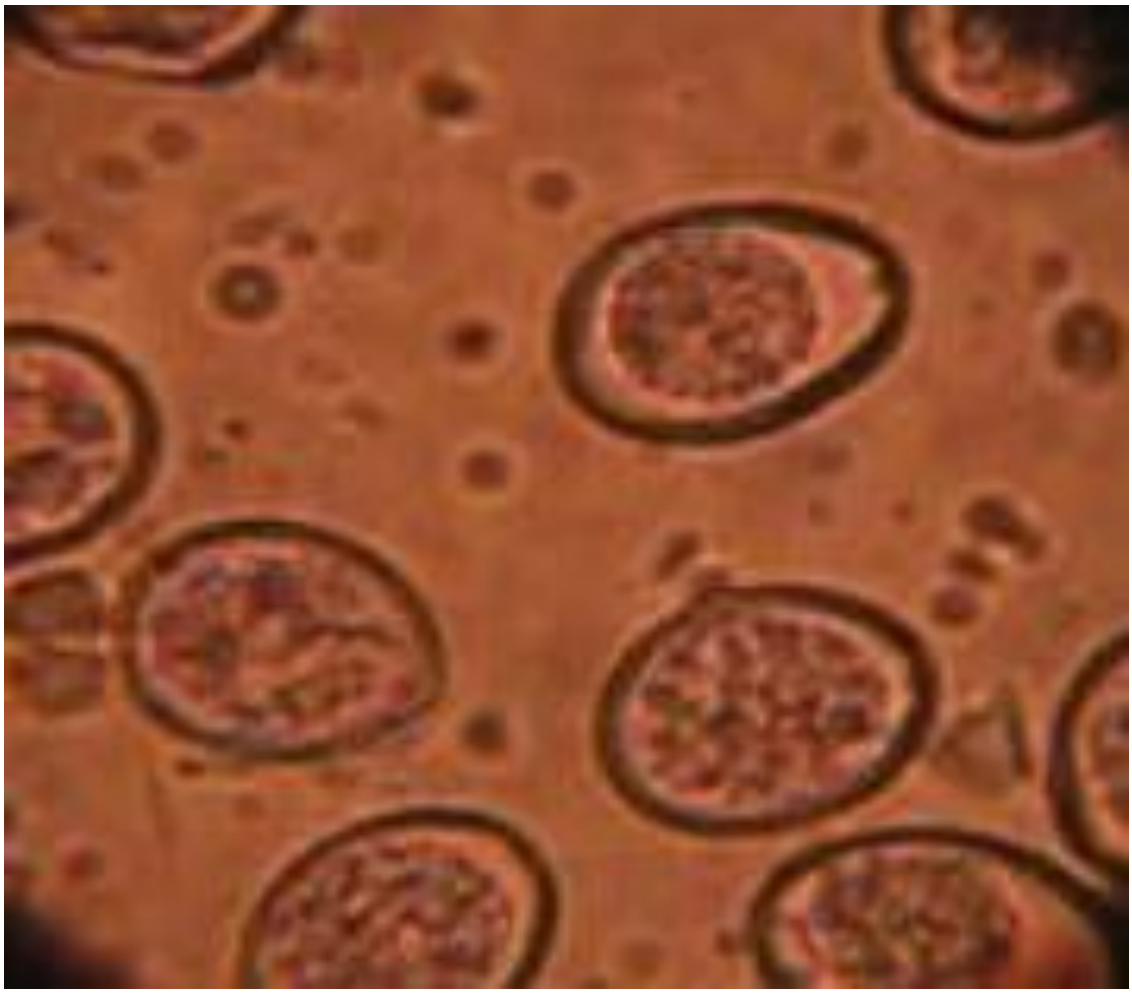


Plate V. Unsporulated oocysts of *Eimeria X* 40.

This was done 3 - 4 times and the sediments containing the oocysts were poured into petri dishes, containing 2.5% potassium dichromate solution and were allowed to sporulate for 1 week at room temperature. Air was blown over the dichromate solution daily to provide oxygen needed for sporulation, evaporated water was also constantly replaced by the addition of dichromate solution.

The oocysts were checked daily by homogenizing the dichromate solution and placing a drop on a glass slide and viewed under a microscope for sporulation (Plate VI). After one week the potassium dichromate solution was washed off by centrifuging at 251.55 g for 5 min in test-tubes to sediment the sporulated oocysts. The solution was then decanted and fresh potassium dichromate was added to the oocysts and stored at 4°C until it was used three weeks later.

3.2.2. Preparation of Inoculum

The number of oocysts per ml of the inoculum was determined using the McMaster technique after washing off the Potassium dichromate solution used to store the sporulated oocysts by centrifugation to sediment the oocysts and the washed sporulated ocysts re-suspended in distilled water; this gave an estimated number of 133,000 oocytst per ml of the inoculum.

3.3. Anticoccidials used in the Experiment

3.3.1. Amprolium

One hundred gram of amprolium hydrochloride (Amprolium-20S®) manufactured by V.M.D.nv/sa-Berendonk 74 – B – 2370 ARENDONK was purchased from a Veterinary Pharmaceutical shop in Kaduna and was used for the treatment of coccidiosis at a dose rate of 0.6 g per litre of drinking water for 7 consecutive days during the experiment.

3.3.2. Toltrazuril

One hundred millilitres of toltrazuril (Kepcox® 2.5% oral solution) manufactured by KEPRO B.V. – Maagdenburgstraat 38, Deventer, Holland was purchased from a Veterinary Pharmaceutical shop in kaduna and was used for the treatment of coccidiosis at a dose rate of 1 ml per litre of drinking water for 2 consecutive days during the experiment.

3.3.3. *Ganoderma lucidum*

About 300 g of *Ganoderma lucidum* was obtained from a local farmer in Jos, Plateau state, Nigeria and was also used for the treatment of coccidiosis at a dose rate of 200 g per litre of drinking water for 5 consecutive days during the experiment.



Plate VI. Sporulated oocyst of *Eimeria* X 10.

3.3.3.1. Preparation of *G. lucidum* used for treatment

Dried *G. lucidum* was ground to reduce the particle size; 1 litre of water was then boiled (100°C), 200 g of *G. lucidum* was then added to the boiled water before it cooled and covered for 12 hr. It was then sieved to remove the water which was used for medication.

3.4. Experimental Plan

3.4.1. Experimental Design

A total of one hundred and forty five chicks were purchased and kept together until three weeks of age before they were randomly assigned into five different groups (A, B, C, D and E) containing 29 birds each (Table 3.1).

Table 3.1: Summary of experimental design

Age (days)	Group A	Group B	Group C	Group D	Group E
[a]0	i/o Newcastle disease (B_1 strain) vaccine				
[a]12	Infectious bursal disease vaccine				
[a]21	Newcastle disease (La Sota) strain vaccine				
[b]25 (Day 0 PI)	Not inoculated with *Eimeria*	Inoculated with *Eimeria*	Inoculated with *Eimeria*	Inoculated with *Eimeria*	Inoculated with *Eimeria*
32 (Day 7 PI)	No treatment	No treatment	Treatment with amprolium	Treatment with toltrazuril	Treatment with *Ganoderm lucidum*

[a]Birds were raised together.
[b]Birds were randomly selected into five different groups before inoculation.

3.4.2. Infection with *Eimeria*

As the period of high coccidiosis risk in chickens is between the 20th and 35th days of age (Suls, 1999), about 27,000 sporulated oocysts/bird of *Eimeria* (approximately 0.2 ml of inoculum) was administered orally at 25 days of age, using an insulin syringe by restraining the birds one after the other and opening the mouth before depositing the inoculum directly into the mouth. The sporulated oocysts suspension in distilled water was agitated before inoculating each bird. Birds in groups B, C, D and E were inoculated with sporulated oocysts while group A served as the control (uninoculated).

3.5. Samples and Data Collection

3.5.1. Rectal Temperature

Daily rectal temperatures in degree centigrade of each bird were taken in all (inoculated and uninoculated) groups using a digital thermometer, manufactured by AGARY, CE 0197, Model – MT301.

3.5.2. Body Weight

The total weight of birds in each group was taken using a weighing balance on days 0, 4, 6, 8, 10, 14, 17, 19 and 21. This was used to calculate the mean body weight of the birds in each group.

3.5.3. Blood Sample Collection

Blood samples used for the determination of packed cell volume and total plasma protein were collected from 12 birds in each group from the heart using 2 ml sterile hypodermic needles and syringes into 5 ml sample bottles containing Ethylene diamino tetra-acetic acid (EDTA). Samples were collected on days 0, 3, 7, 11, 14, 17 and 21 post inoculation. All the blood samples were processed immediately after collection.

3.5.4. Fecal Sample Collection

About 10 g of faeces were collected from each group daily from day zero post inoculation to day 21 post inoculation, for the determination of the number of oocyts/gram of feces. Feces were collected in polythene bags and labeled by group.

3.6. Laboratory Procedures

3.6.1. Determination of Packed Cell Volume

Packed cell volume (PCV) was determined using the microhaematocrit method (Sirois, 1995). Microhaemotocrit capillary tubes were filled with blood by capillary action to about 75% of its length; the outside of the tubes were carefully dried with cotton wool after filling each of the tubes with blood. The opposite ends were then sealed with a flame from a bunsen burner; the sealed tubes were then placed in a haematocrit centrifuge with their sealed ends placed near the outside rim of the centrifuge (to touch the rim). The microhaematocrit centrifuge cover was then replaced and tightened. The centrifuge was then turned on for 5 min. The spun tubes were then placed on a microheamatocrit tube reader one after the other to determine the PCV in percentage.

3.6.2. Determination of Total Plasma Protein

Total plasma protein was determined using the hand refractometric method (Sirois, 1995). After reading the PCV the haematocrit capillary tube was broken by snapping the tube at the point immediately above the buffycoat. The end containing the buffycoat and packed red cells was discarded retaining the end containing the plasma. The plastic flap of the hand refractometer was reflected to expose "glassy" sample surface which was thoroughly wiped with cotton wool. The broken end of the capillary tube was then used to touch the sample surface of the refractometer in order to place plasma sample unto the surface thereafter the capillary

tube was discarded. The plastic flap of the refractometer was then replaced; pressure was then applied on the flap against the plasma sample with the index finger and holding the refractometer against light the concentration of plasma proteins was then read in grams per decilitre (g/dl).

3.6.3. Determination of Oocysts count per gram of Faeces

Oocysts count per gram of faeces was determined using the modified McMaster method (quantitative method). This was done by weighing 4 g of feces into a jar and mixed with 15 ml of saturated sugar solution, the mixture was then poured through a tea strainer into a graduated cylinder, the oocysts were further washed into the cylinder with the floatation medium using a spray bottle through the tea strainer to make it up to 60 ml. The suspension was then mixed by repeatedly blowing small amount of air through a pipette into the suspension, a field of a McMaster slide was then filled with a pipette. The suspension was mixed again and the second field of the slide was filled. The oocysts were allowed to float for at least 15 min and all the oocysts under the two separate grids of the McMaster slide were counted at ×10. This was used to calculate the number of oocysts per gram of faeces.

Oocysts per gram of faeces (o.p.g) = number of oocysts × 50.

3.7. Observation of Inoculated Birds Post Inoculation for clinical signs

Infected birds were monitored daily post inoculation for clinical signs, and any abnormal findings observed were recorded.

3.8. Postmortem Examination

Five birds were sacrificed in each group at day zero, post inoculation to observe for any lesion in the caeca and especially, to ascertain that the birds were free from coccidial infection prior to experimental inoculation on day zero. Subsequently, postmortem examinations were carried out on 5 birds in each group on days 7, 14 and 21 post inoculation and their caeca were observed for lesions. The weights of individual birds were taken before PM examination.

3.9. Treatment of Inoculated groups

Treatment was instituted on day 7 after peak oocysts production and clinical signs were observed. Group A and B were not treated with any anticoccidial. Group C were treated with amprolium at a dose rate of 0.6

g/litre continuously in drinking water for 7 consecutive days. Group D were treated with toltrazuril at a dose rate of 1 ml/litre continuously in drinking water for 2 consecutive days while Group E were treated with *Ganoderma lucidum* at a dose rate of 200 g/litre continuously in drinking water for 5 consecutive days.

3.10. Statistical Analysis

The results obtained were subjected to one way analysis of variance (ANOVA). Statistical tests with P value of 0.05, (all values ≤ 0.05) were considered significant. Test of multiple comparison between the groups were also carried out using statistical package for social sciences (SPSS).

Chapter Four

4.0. Results

4.1. Body Weights

Group A started with an initial average weight of 177.6 g, Group B 160.4 g, Group C 163.8 g, Group D 163.8 g and Group E with 158.6 g on day zero post inoculation. At 14 days post inoculation, Group A had an average weight of 425.0 g, Group B 339.3 g, Group C 346.2 g, Group D 325.0 g and Group E 346.2 g. At 21 days post inoculation Group A had an average body weight of 555.6 g, Group B 450.0 g, Group C 437.5 g, Group D 427.8 g and Group E 437.5 g (Figure 1).

On day zero post inoculation weight gain in all the groups was assumed to be 0.0 g. At day 4 post inoculation, weight gain in Group A was 128.5 g, Group B 90.0 g, Group C 94.6 g, Group D 90.1 g, while in group E the weight gained was 85.1 g (Table 4.1). The total weight gained at the end of the experiment in Group A was 378.0 g, Group B 289.7 g, Group C 273.7 g, Group D 264.0 g, and Group E 278.9 g (Appendix 2).

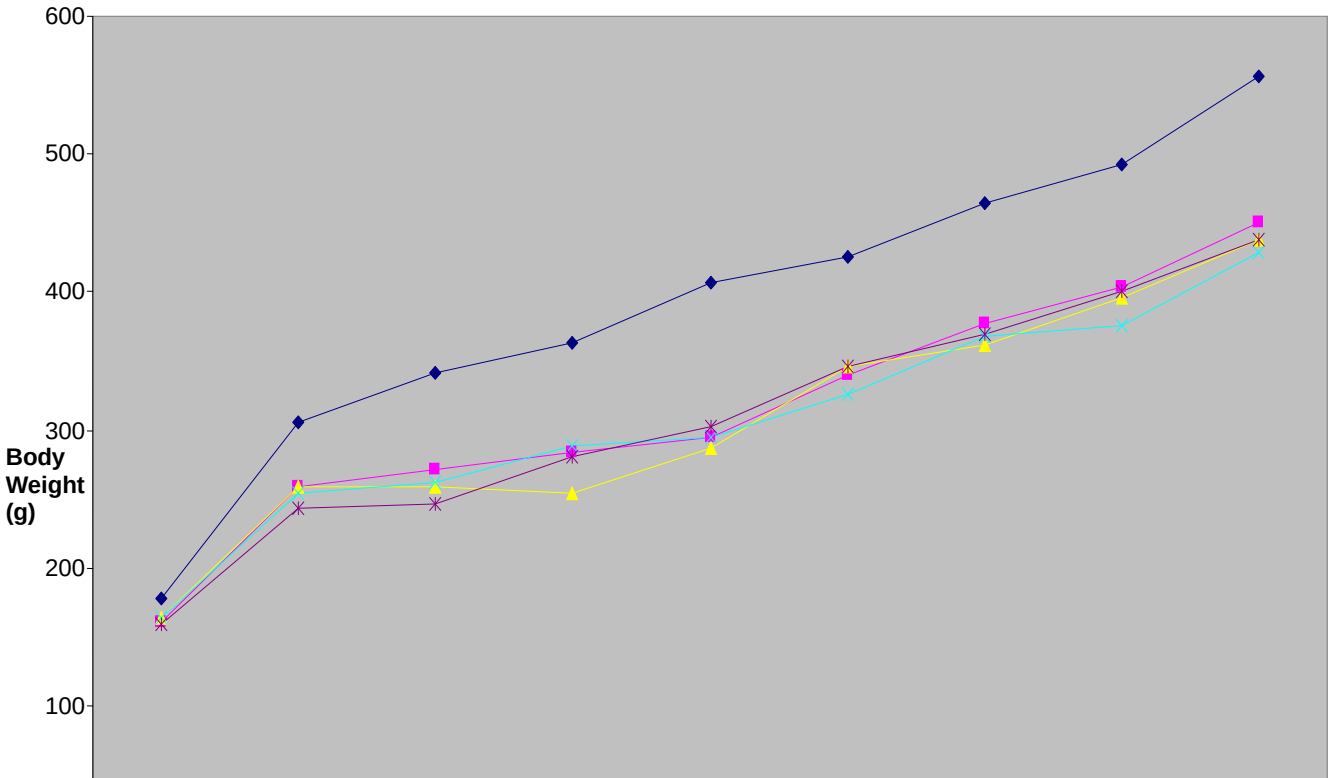


Fig 1: Mean change in body weight (grams) post inoculation in Warren cockerels inoculated with local strain of E. tenella at 25 days old.

Table 4.1: Mean body weight gain (grams) post inoculation in Warren chicks inoculated with local strain of *Eimeria* at 25 days old.

Day(s) post inoculation	Group A	Group B	Group C	Group D	Group E
4	128.5(n=24)	98.0(n=24)	94.6(n=24)	90.0(n=24)	85.1(n=24)
6	35.5(n=24)	12.5(n=24)	0.01(n=24)	8.7(n=24)	2.1(n=24)
8	21.5(n=19)	12.5(n=19)	-4.6(n=18)	26.4(n=19)	34.7(n=18)
10	44.3(n=19)	11.7(n=19)	32.5(n=18)	5.0(n=19)	22.6(n=18)
14	17.5(n=14)	44.3(n=14)	59.9(n=13)	31.1(n=14)	43.0(n=13)

17	39.3(n=14)	37.7(n=13)	15.4(n=13)	42.9(n=14)	23.1(n=13)
19	28.6(n=14)	26.9(n=13)	34.6(n=13)	7.1(n=13)	30.8(n=13)
21	62.7(n=9)	46.2(n=8)	41.3(n=8)	52.8(n=8)	37.5(n=8)

NB: Mean body weight gain is equal to the previous mean body weight minus the current mean body weight on each of the days.

4.2. Mean Packed Cell Volume (%)

Mean packed cell volume (PCV) readings from groups A to E were 30.3±2.1%, 29.4±1.4%, 30.0±2.8%, 31.1±2.5% and 29.6±1.9%, respectively on day zero post inoculation, but at day 7 post infection, the average PCV value for the uninfected group was 30.8±1.5%, while it ranged between 23.6±6.1% and 25.0±2.8% for the inoculated groups by day 11 post inoculation. The average PCV for the uninoculated group was 31.3±2.0 while for the inoculated groups were 33.3±1.8, 30.7±1.8, 31.4±1.9 and 28.9±1.4 for groups B, C, D and E, respectively (Table 4.2).

Some individual PCV in the inoculated groups was found to be as low as 12%.

Table 4.2: Mean packed cell volume (%) ± SD post inoculation in Warren chicks inoculated with local strain of *Eimeria* at 25 days old.

Day (s) post inoculation	Group A	Group B	Group C	Group D	Group E

0	30.3±2.1[a]	29.4±1.4[a]	30.0±2.8[a]	31.1±2.5[a]	29.6±1.9[a]
3	30.3±2.4[a]	30.6±0.9[a]	30.8±1.7[a]	27.7±1.7[b]	29.2±2.5[a]
7	30.8±1.5[a]	23.6±6.1[c]	23.7±6.1[c]	25.7±3.8[b]	25.0±2.8[b]
11	31.3±2.0[a]	33.3±1.8[c]	30.7±1.8[a]	31.4±1.9[a]	28.9±1.4[c]
14	30.0±1.4[a]	29.4±1.7[a]	28.1±2.2[b]	28.1±1.7[b]	27.6±2.0[c]
17	29.2±1.0[a]	29.2±2.2[a]	27.6±1.2[b]	27.8±2.0[b]	27.0±1.4[c]
21	28.1±1.0[a]	29.1±3.0[a]	27.3±2.1[a]	28.2±2.1[a]	27.3±1.9[a]

a b c Different superscripts in a row indicate significant difference between the means, ab: $p<0.05$; ac: $p<0.01$

Normal PCV (%): 24 – 43% (Ritchie *et al.*, 1994; Oladele *et al.*, 2000).

4.3. Mean Total Plasma Protein (g/dl)

The concentration of total plasma proteins were determined for all the groups on day zero post inoculation. The mean plasma protein value was 3.6±0.3 g/dl for the uninoculated group while for the inoculated groups the values were 3.9±0.6 g/dl, 4.0±0.5 g/dl, 3.7±0.3 g/dl and 3.7±0.4 g/dl for groups B, C, D and E respectively. At day 7 post inoculation, the mean total plasma protein values were 3.38±0.3 g/dl for the uninoculated

group, 3.8±0.3 g/dl, 3.7±0.7 g/dl, 3.3±0.4 g/dl, and 3.9±0.4 g/dl for the infected groups B, C, D and E respectively (Appendix 3).

4.4. Clinical Signs

No obvious clinical signs were observed on days 0, 1, 2 and 3 post inoculation, but on day 4 post inoculation blood tinged faeces were observed in the inoculated groups towards the evening. On day 5, loss of appetite, somnolence and ruffled feathers were observed in the inoculated groups (Plates XI, XII, XV and XVI). Diarrhea and presence of frank blood in the faeces of inoculated birds were also observed (Plates IX, X, XIII and XIV) compared to the uninoculated

group (Plates VII & VIII). All the signs observed on day 5 were also present on day 6 post inoculation, but they were more severe in all the infected groups. At day 7, dried caecal cores were observed in the faeces of inoculated birds.



Plate VII. Uninoculated group showing birds that were alert and well (group A).



Plate VIII. Day 6 post inoculation, normal faeces from uninoculated birds (Group A).



Plate IX. Day 6 post inoculation: note the ruffled feathers and somnolence in inoculated group (Group B).



Plate X. Day 6 post inoculation, presence of frank blood in faeces of inoculated birds (Group B).



Plate XI. Day 5 post inoculation, there was loss of appetite, depression, ruffled feathers and somnolence in inoculated birds (Group C).



Plate XII. Day 5 post inoculation, presence of blood in faeces in Inoculated group (Group C).



Plate XIII. Hurdling together, loss of appetite and somnolence in inoculated birds (group D).



Plate XIV. Day 6 post inoculation, presence of frank blood in faeces of inoculated birds (group D).



Plate XV. Ruffled feathers and inappetance in inoculated birds (group E).



Plate XVI. Day 5 post inoculation, presence of blood in faeces of inoculated birds (group E).

4.5. Daily Oocysts Count Post Inoculation

No oocysts were found in the faeces at days zero, 1 and 2 post inoculation in all the inoculated groups. At day three 6,000 oocysts /gram of faeces was recorded for group B, while groups C, D and E had 4,050, 1,400 and 2,250 oocysts/gram of faeces, respectively. Oocysts production peaked in all the groups at day 6 post infection with the highest count in group D (2,347,200 oocysts/gram of faeces) followed by group C (2,158,800 oocysts/gram of faeces), group E (1,904,400 oocysts/gram) and the lowest count was found in group B (1,752,600 oocysts/gram of faeces).

Treatment commenced on day 7 post inoculation. The oocysts count at the beginning of treatment was 184,800 oocysts/gram of faeces for group B, this group received no treatment (Figure 2). Group C which was treated with amprolium had an oocysts count of 691,200 oocysts/gram at the commencement of treatment, also group D which was treated with toltrazuril had an oocysts count of 351,000 oocysts/gram of faeces prior to treatment (Figure 3 and 4) while group E had an oocysts count of 268,200 oocysts/gram of faeces at day 7 before receiving treatment with *Ganoderma lucidum* (Figure 5). At the end of the 7 days treatment with amprolium (day 14 post inoculation) in group C the oocysts count/gram of faeces was 6,600. Group D which received treatment with toltrazuril for

two days had an oocysts count of 2,700/gram of faeces at the end of treatment (day 9 post inoculation) while group E had an oocysts count of 11,700/gram of feces at the end of treatment for 5 days compared to the non treated control group. Oocysts count was observed to increase in groups D and E after withdrawal of treatment.

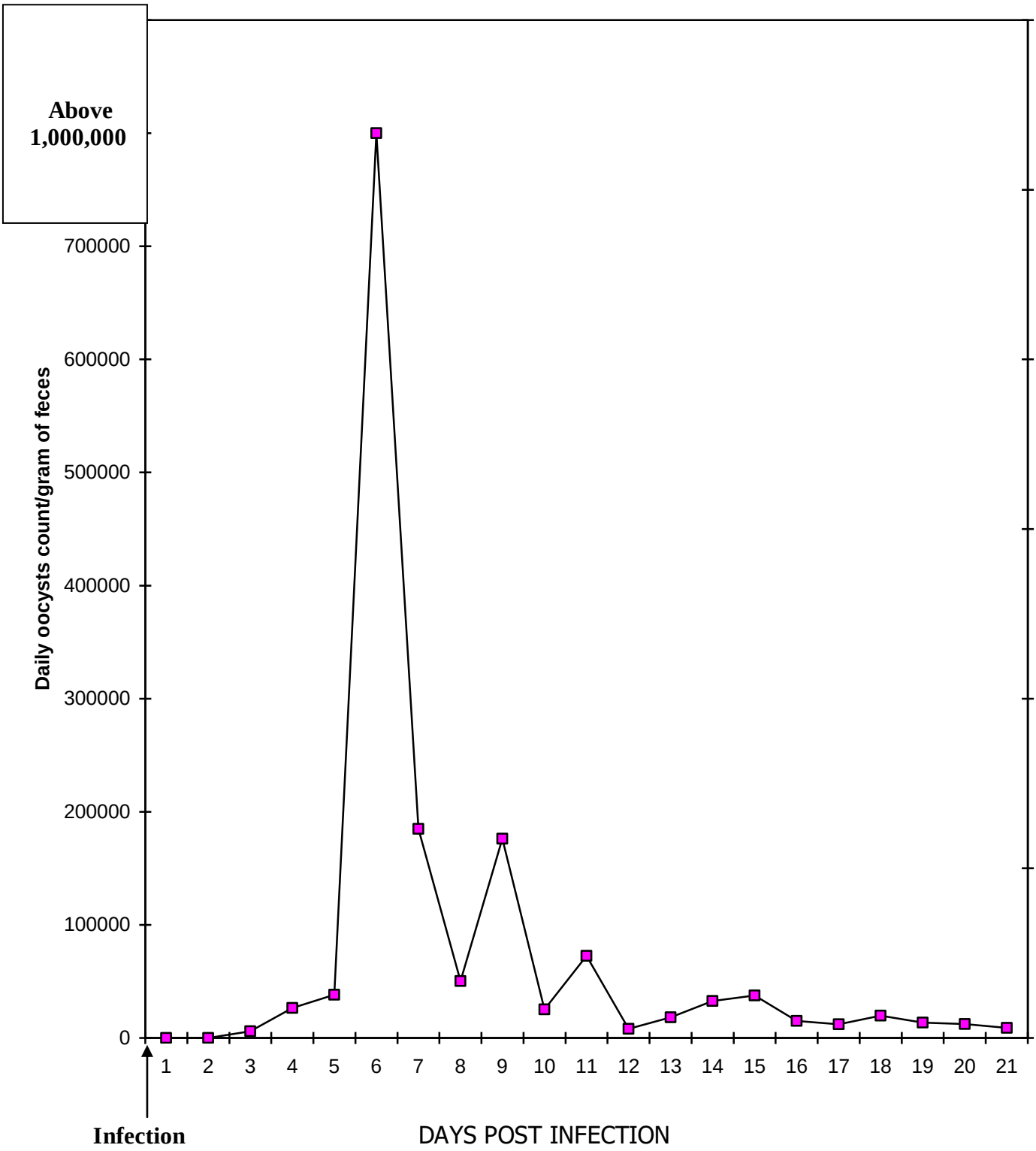

Above 1,000,000
700000
600000
500000
400000
300000
200000
100000
0
Daily oocysts count/gram of feces
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
Infection
DAYS POST INFECTION

Fig.2. graph showing daily oocysts count/gram of faeces in birds inoculated with *Eimeria* and not treated (group B)

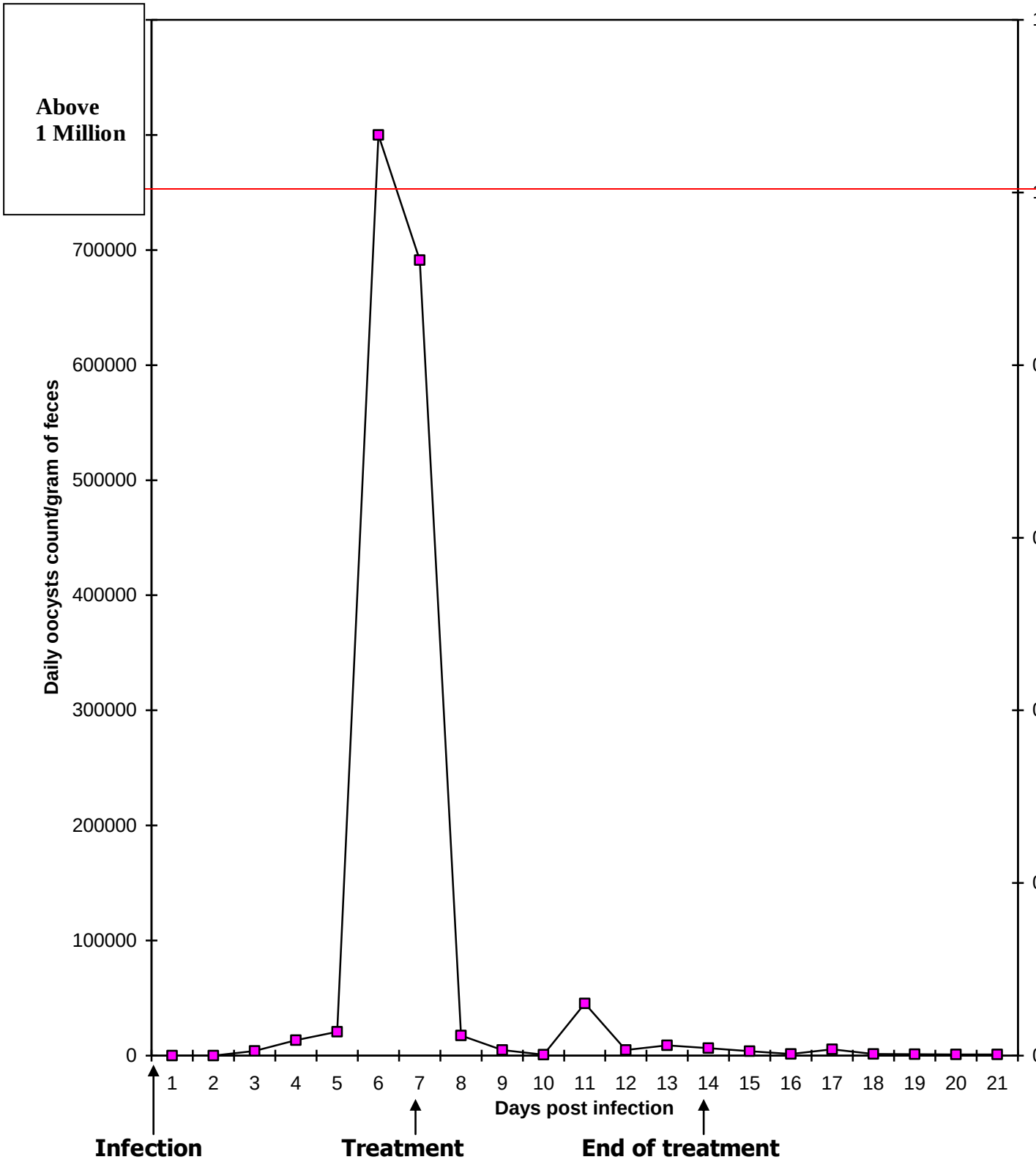


Fig.3. Graph showing daily oocysts count/gram of faeces in birds

inoculated with *Eimeria* and treated with amprolium (group C)

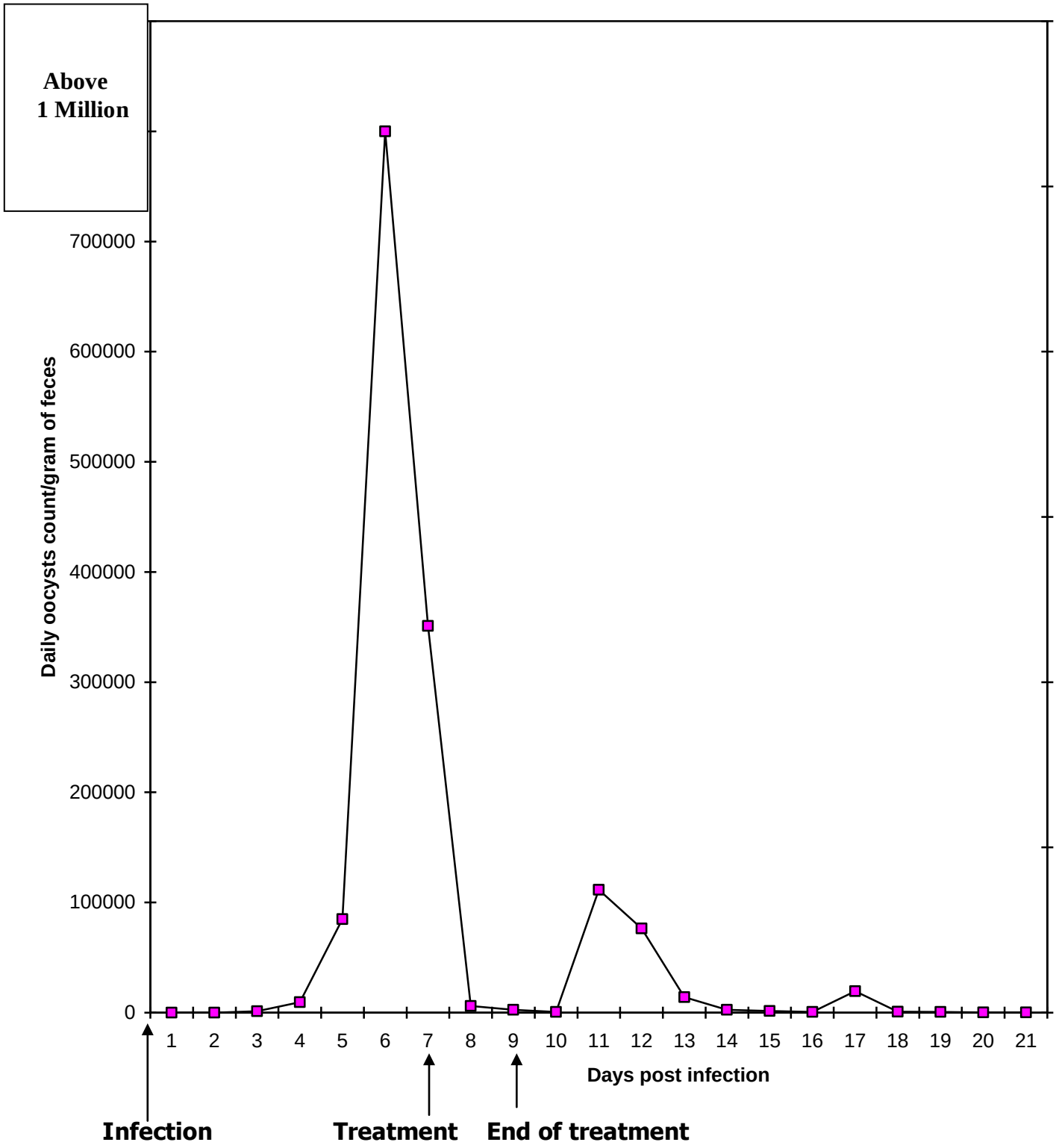


Fig.4. Graph showing daily oocysts count/gram of faeces in birds inoculated with *Eimeria* and treated with toltrazuril (group D)

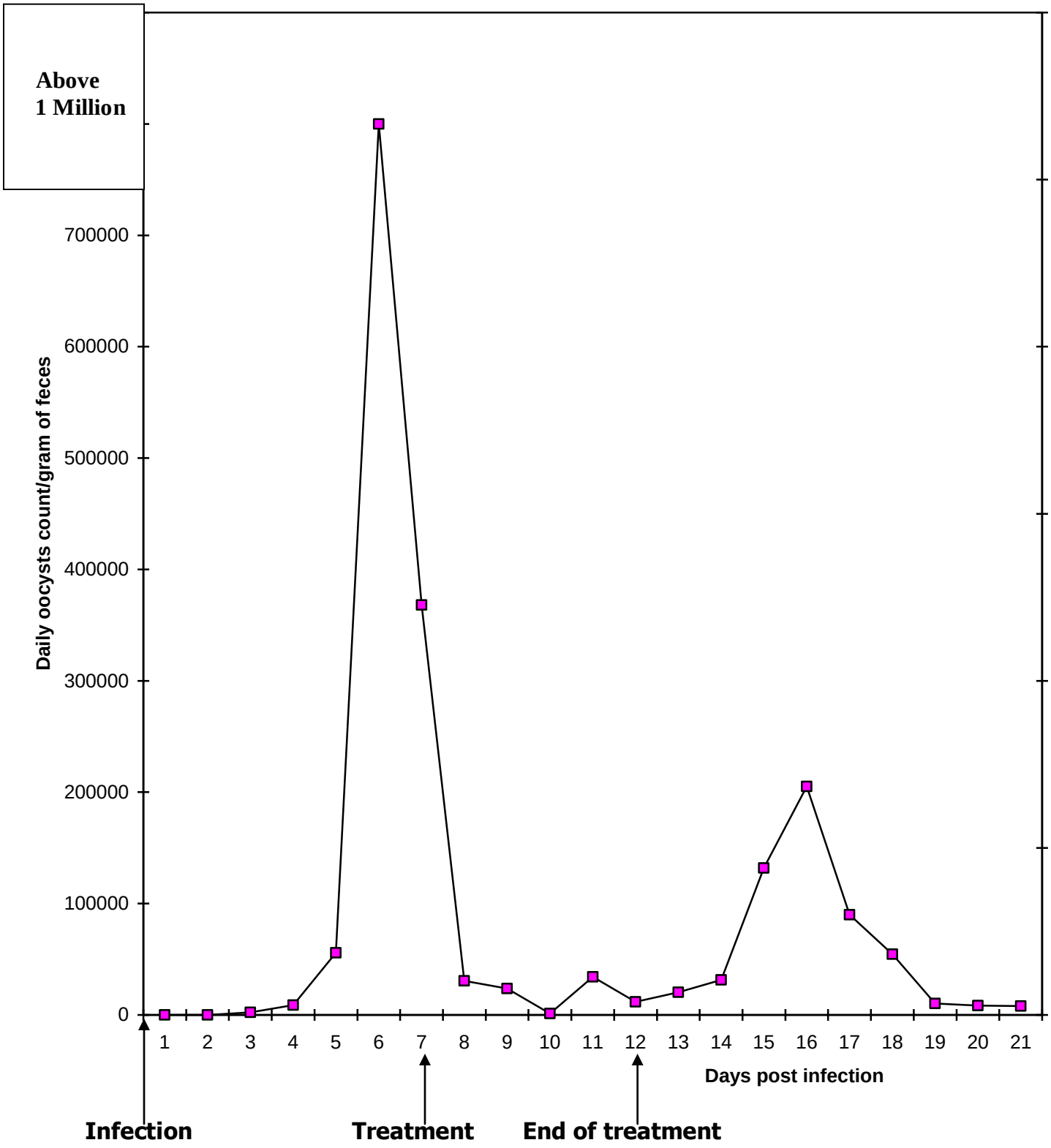


Fig.5. Graph showing daily oocysts count/gram of faeces in birds inoculated with *Eimeria* and treated with *Ganoderma lucidum*

(group E)

4.6. Postmortem Findings

Postmortem examination conducted on five birds from each group at day zero post inoculation showed no visible pathology in the caeca for all the groups and other organs also appeared normal. At day 7 post inoculation, 5 birds were sacrificed from each group; the uninoculated group (group A) showed no visible pathology in the caeca (Plate XVII) and other visceral organs appeared normal. The following lesions were observed in all the inoculated groups at postmortem, pale carcass, enlarged and distended caecal pouches (Plates XVIII, XX and XXI) and presence of clotted blood in caeca (Plate XIX).

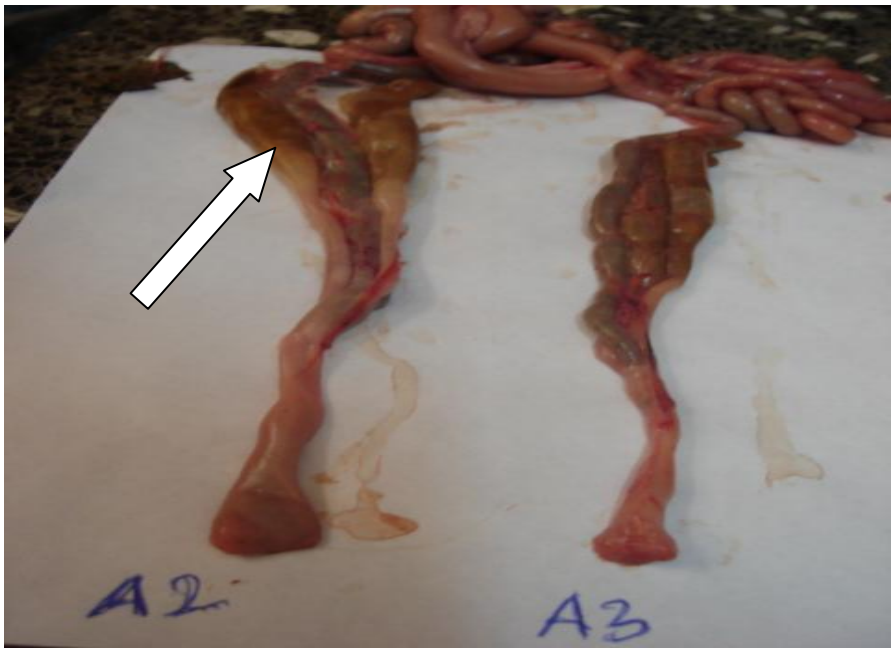


Plate XVII. Arrow showing normal caecum at postmortem for uninoculated birds (group A).

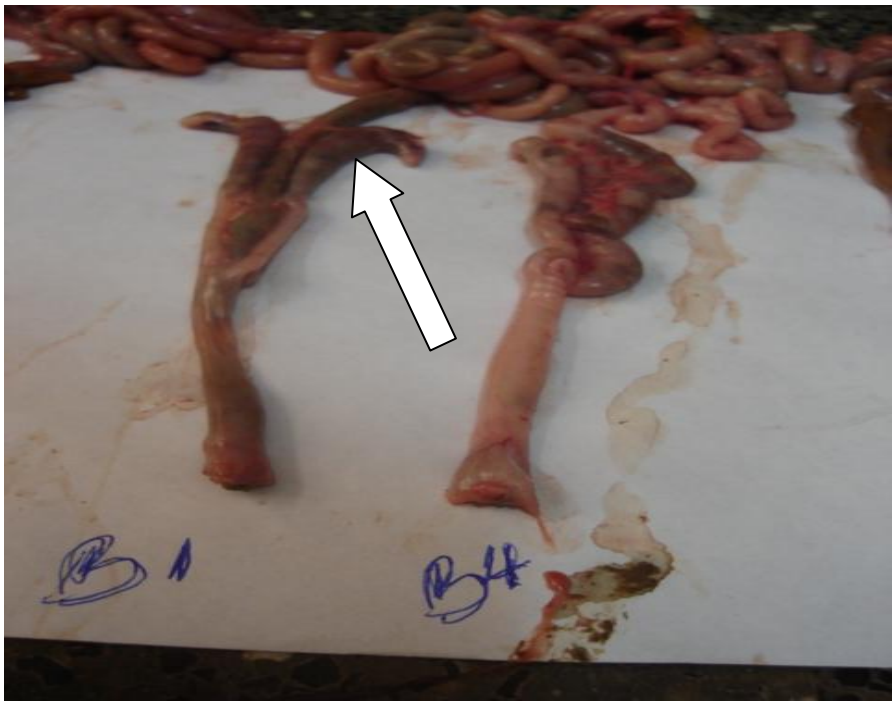


Plate XVIII. Arrow showing caecum containing clotted blood at postmortem in inoculated birds (group B).

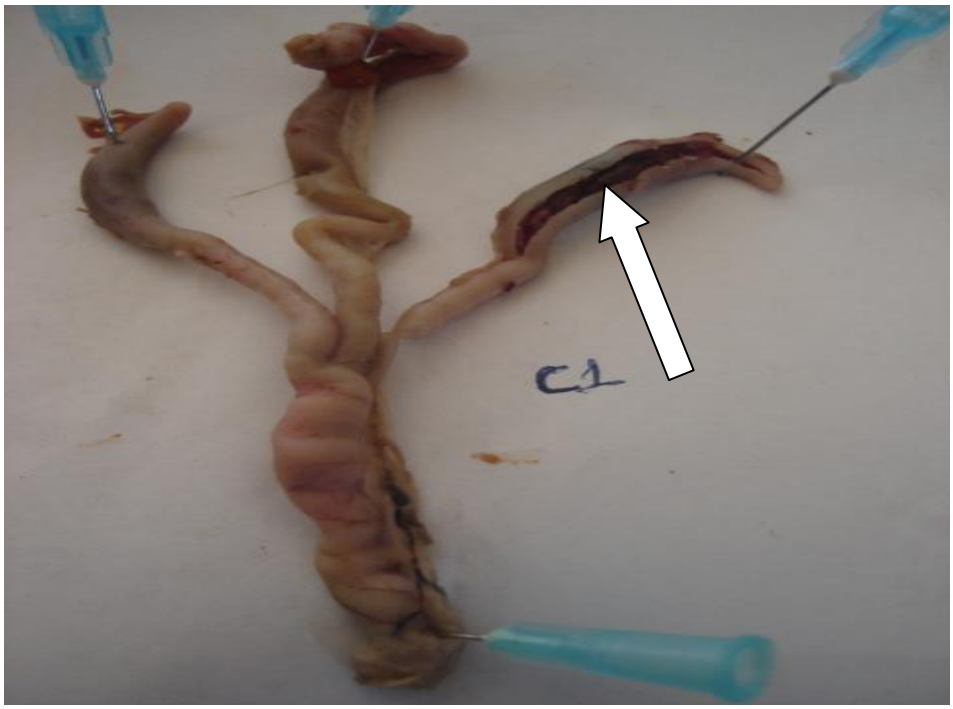


Plate XIX. Arrow showing the presence of clotted blood in caecum at postmortem in inoculated birds (group C).

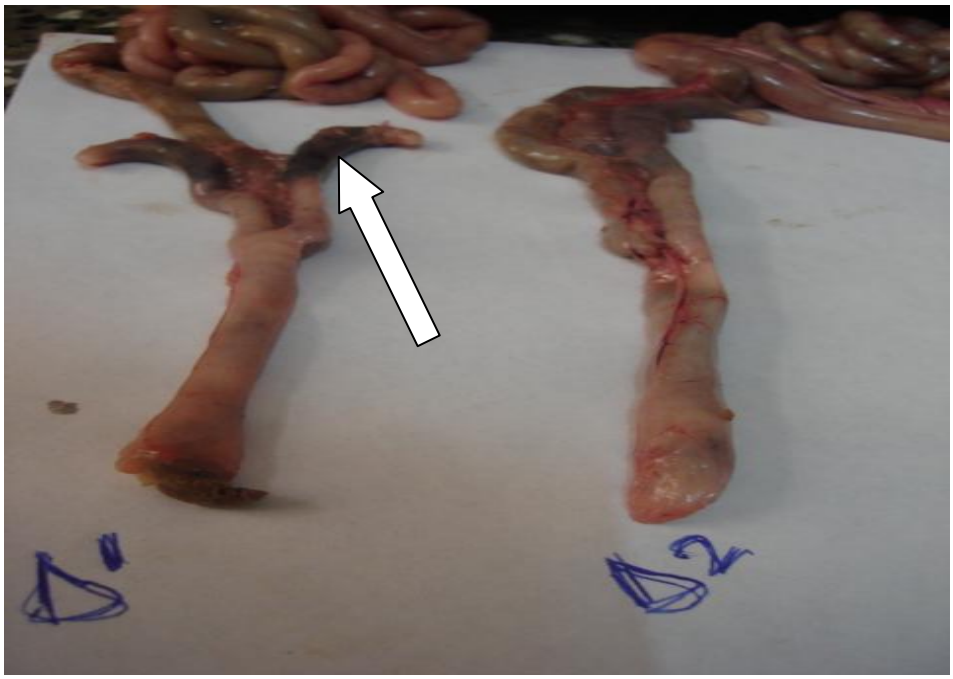


Plate XX. Arrow showing presence of clotted blood in caecum at postmortem in inoculated birds (group D).



Plate XXI. Arrow showing balooned caecum at postmortem in inoculated birds (group E).

Chapter Five

5.1. Discussion

The changes in body weights in the uninfected control group were higher when compared to the infected groups. Mean body weight gain was also reduced throughout the course of the disease in all the infected groups. Highly significant weight loss in the course of caecal coccidiosis has been reported earlier (Reck and McQuistion, 1994; McDougald and Reid, 1997; Jordan and Pattison, 1999). The loss in weight can be attributed to the loss in appetite, impaired feed conversion, diarrhoea and dehydration during the course of the disease (Bishu, 1982; Chapman, 1988.). The weight loss can also be attributed to the activity of the parasite within the intestine, resulting in interruption of absorption of nutrient and consequently weight loss and drop in egg production in layers (Shane, 1997; Larry and Otto, 2005; Anonymous, 2005). The greatest effect of caecal coccidiosis on body weight was noticed between days four and six post inoculation in all infected groups with groups C and E mostly affected. Group C lost weight (-0.042 g) and group E only added a weight of 2.09 g between days four and six, this may be attributed to the high number of oocysts per gram of faeces noticed in the two groups.

Total body weight gain at the end of the experiment (21 days post inoculation) was markedly affected in all the infected groups when compared to the uninoculated control group. Total body weight gain in the control group was 377.97 g, while it ranged between 263.985 g to 289.65 g in the infected groups. This clearly indicates that the disease had an adverse effect on the body weight gain in inoculated birds (Shane, 1997, McDougald and Reid, 1997). Weight loss, especially in broiler production is very important to the poultry farmer since birds will not be able to attain market weight in the course of coccidiosis outbreak and the lost performance may never be recovered (Saville, 1996).

On day 3 post inoculation, there was a decrease in PCV value observed in group D when compared with the uninfected control group A (based on the p value of 0.003 obtained), on the other hand no difference was observed in the inoculated group. On day 7 post inoculation there were significant decreases ($p<0.05$) observed in the PCV in all the inoculated groups (groups B, C, D, and E) when compared to the uninfected control. A decrease of PCV value to as low as 50% of normal value has been reported in caecal coccidiosis (McDougald and Reid, 1997). This can be attributed to the loss of frank blood in feces in the course of the disease which was marked on days 5 and 6 in this study post inoculation. On day 11 post infection there was an increase in PCV values in all the inoculated groups. This increase was highly significant ($p<0.01$) in groups B and E when

compared with the uninoculated control, while on day 21 post infection there was no difference ($p<0.05$) between the inoculated groups and uninoculated control group. This can be attributed to recovery from the effect of caecal coccidiosis in the inoculated groups which is known to occurs within 14 days post inoculation (Saville, 1996).

Studies on the effect of caecal coccidiosis on total plasma protein have not been fully documented. There were no significant differences in the total plasma protein in infected and uninfected groups throughout the course of the experiment except on day 11 post inoculation where a significant increase was noticed in group E (p value =0.004) and in group B (p value =0.016). The effect of caecal coccidiosis on total plasma protein was not obvious, probably because during the course of the disease a combination of processes occurs, some of which contributes directly or indirectly to an increase in total plasma protein while others contributes to its decrease (Sirois, 1995). Disease processes like acute dehydration observed in caecal coccidiosis can result in an increase in plasma proteins while loss of appetite and impaired intestinal absorption can result in decrease plasma protein, thus a highly significant increase or decrease may not be noticed(Sirois, 1995).

In this study it was shown that there was no significant effect of caecal coccidiosis on rectal temperature in infected birds. This can be associated with

the fact that the *E. tenella* is restricted to the gastrointestinal tract in the course of the disease and does not enter into the blood stream at any stage in its life cycle (Saville, 1996; McDougald and Reid, 1997; Jordan and Pattison, 1999; Murray, 2001; Ferguson, 2003).

Blood tinged feces were initially observed on day 4 post inoculation because the average prepatent period of *Eimeria* is between 93 to about 138 hours that is; four days post inoculation (McDougald and Reid, 1997; Jordan and Pattison, 1999). Blood tinged feces observed in all the groups inoculated with *Eimeria* was due to the maturation of the second generation schizonts (Ferguson, 2003). On day five post inoculation, the frank blood observed in the feces in all the inoculated groups was due to ulceration of the mucosal surface of the caeca when the schizonts mature and merozoites are released as reported by other authors (Mcdougald and Reid, 1997; Ferguson, 2003). Other clinical signs observed were ruffled feathers, somnolence, diarrhoea, loss of appetite and weight loss compared to the uninoculated group where in line with the findings of other authors (Jordan and Pattison, 1999). On day 6 post infection, frank blood was still noticed in the faeces of infected birds because of continuous schizogony in *Eimeria* which occurs in the crypts of Lieberkuhn of the caeca and causing extensive haemorrhage (Larry and Otto, 2005). There was no mortality seen in all the infected groups. This may be due to the good nutrition and management maintained during the course of the experiment. The white to pink

cores in faeces noticed on day seven post inoculation were previously reported by Seneviratna (1969) and Jordan and Pattison (1999).

No oocysts were found in the faeces within the first two days post inoculation until the third day post inoculation. The oocysts encountered on the third day post inoculation were most likely extraneous oocysts i.e. inoculated oocysts that pass through the alimentary canal without undergoing excystation (McDougald and Reid, 1997). There was a steady increase in the oocyst count per gram of faeces in all the inoculated groups from day four post inoculation and peaked on day six post inoculation based on the average life cycle of *E. tenella* (Anonymous, 2002).

Treatment was instituted on day seven post inoculation after peak oocysts production was observed on day six and there was a marked decrease in oocysts count per gram of faeces in all the treatment groups when compared to the untreated control group. This is an indication that amprolium and toltrazuril and *Ganoderma lucidum* have anticoccidial effects. The ability of *G. lucidum* to reduce the oocysts count may be due to the fact that it can restore the body to its natural state, enabling organs to function normally and it is an immune modulator as it regulates and fine tunes the immune system (Cuckler *et al*., 1960; Rogers *et al*., 1960; Warren and Ball; 1963, Melhorn *et al*., 1984; Haberkorn and Stoltefuss, 1987; Oei, 2003; Anonymous, 2006c). At the end of the recommended treatment periods in each group, group D which was treated

with toltrazuril for 48 hr (two days) had the lowest oocysts count per gram of faeces followed by group B treated with amprolium for seven days and lastly group E treated with *Ganoderma lucidium* for five days. There was an increase in oocysts count per gram of faeces after withdrawal of treatment in group D which was treated with toltrazuril for two days.

At postmortem on day seven post inoculation, the carcasses of infected groups were observed to be pale. This can be attributed to the dehydration observed and blood loss in the course of the disease. Loss of flesh and discoloration of the shanks as a result of interruption of the absorption of nutrient and pigments through the intestinal mucosa in the course of caecal coccidiosis have been previously observed (McDougald and Reid, 1997; Shane, 1997). Enlarged and distended caeca were observed in all infected groups at postmortem on day seven post inoculation. Blood clots were also seen in the caeca after they were incised. No death was recorded as a result of the infection. This may be attributed to good management and nutrition since coccidiosis is believed to be a self-limiting disease and that most deaths observed in coccidiosis is mostly as a result of concurrent infection (Helm, 1999; Kabell *et al*., 2006).

5.2 Conclusions

The *Eimeria* used in the experiment is pathogenic for chickens and is of great economic importance to the poultry industry as it adversely affected weight gain.

It also has an adverse effect on the packed cell volume with little or no effect on the total plasma protein and rectal temperature of inoculated birds.

Specific clinical sings in *Eimeria* infection can be noticed as early as day four post inoculation. These clinical signs include somnolence, ruffled feathers, marked drop in feed consumption, profuse bloody diarrhoea and the presence of dry caecal cores in faeces on days five and six and seven post inoculation. Gross lesions observed include pale carcass, while other pathology within the intestines was restricted to the caeca which were enlarged, distended and contained clotted blood.

Treatment with amprolium, toltrazuril and *Ganoderma lucidum* shows that they all have anticoccidial effects, but toltrazuril was more efficacious followed by amprolium and *Ganoderma lucidum* respectively.

5.3 Recommendations

1. Considering the deleterious effects of caecal coccidiosis in chickens, it is advisable to place birds on anticoccidials, especially within established period of high coccidiosis risk i.e. between the 21^{st} – 35^{th} days of life.
2. Toltrazuril should be recommended for the treatment of caecal coccidiosis more often than amprolium and *Ganoderma lucidum*.

3. More research into the anticoccidial effect of *G. lucidum* should be conducted to give insight into it's mode of action, toxicity and effective dosage for the treatment of caecal coccidiosis in chickens.

References

Abdu, P. A., Lawal, S. and Wakawa, A. M. (2006). Internal parasitism. In: *Manual of Important Poultry Diseases in Nigeria*. (First Edition) Ahmadu Bello University Press Ltd. Pp. 31-33.

Adene, D. F. (2004). Coccidiosis of poultry: The biology, diagnosis, treatment and control. In: *Poultry Health and Production, Principles and Practices.* Stirling-Horden Publishers (Nig) Ltd. Pp. 129-167.

Akinwumi, J. A., Adegeye, A. J., Ikpi, I. E. and Olayide, S. O. (1979). In: Federal Ministry of Agriculture and Water Resources/ Federal Livestock Department Commissioned Study. 106 pgs.

Allen, P. C. and Fetterer, R. H. (2002). Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. *Clinical Microbiology Reviews*, 15 (1):58-65.

Anonymous (1992). The Nigeria livestock resources. In: *Resource Inventory and Management Report*. Federal Department of Livestock and Pest control services, Federal Ministry of Agriculture, 1992.

Anonymous (2002). Coccidiosis is anything but paltry. *World Poultry*, 18(6): 42-43.

Anonymous (2005a). Coccidiosis. In: *Release by the United States Department of Agriculture, Agricultural Research Service.* www.ars.usda.gov/main/docs.htm?docid=11018 9th January, 2007, 08:12 pm.

Anonymous (2005b). South Africa controls coccidiosis through broiler breeders. *World Poultry*, 21(7). www.Agricworld.nl 10th January, 2007. 04:50 pm.

Anonymous (2006a). Poultry Board Review. www.vetmed.auburn.edu/uploads/images/1567/POULTRY_BOARD_REVIEW.doc, 10th January, 2007, 06:35 pm.

Anonymous (2006b). Reishi: The wonder mushroom that withstood the test of time in China for 4000 years, now re-discovered by the western world. www.naturalpure.com/images/GanodermaLucidum.htm 10th January, 2007, 06:40 pm.

Anonymous (2006c). Information on Reishi; miraculous mushromm in Asia. www.reishi.com 10th January, 2007; 06:55 pm.

Anonymous (2006d). Avian coccidiosis, a parasitic disease that affects chickens and some other poultry: Institute for Animal Health. www.parasitology.information.uni_wuerzburg.de.htm 10th January, 2007. 5:52 pm.

Anonymous (2006e). Toltrazuril – Mode of action. www.baycox.com/17/mode_ofAction.htm 26th February, 2006. 01:47 pm.

Bafundo, K. (2006). The use of live coccidial vaccines in broiler breeder pullets. In: *Understanding coccidiosis, prevention strategies for the breeder pullet.* www.canadianpoultry.ca/understanding%20coccidiosis.htm 10th January, 2007. 06:35 pm.

Bishu, G. (1982). Isolation and identification of two pathogenic *Eimeria* species

in chickens in Nigeria. M.Sc. thesis Ahmadu Bello University, Zaria.

Chapman, H. D. (1987a). Countering drug resistance in *Eimeria* parasites of the fowl. In: Ford, M. G., Holomon, D. W., Khambay, B. P. S. and Sawicki, R. M., *Combating Resistance to Xenobiotics.* Ellis Horwood Ltd. Pp. 54-61.

Chapman, H. D. (1987b). Control of a line of *E. tenella*, partly resistant to monensin, by including toltrazuril discontinuously in the drinking water of chickens. *Journal of Comparative Pathology*, 97:21-27.

Chapman, H. D. (1988). Strategies for the control of coccidiosis in chickens. *World Poultry Science Journal*, 44(3): 187-192.

Collee, J. G., Duguid, J. P., Fraser, A. G. and Marmoin, B. P. (1982). *Practical Medical Microbiology*, Mackie and Mc Mc Cartney Churchhill Longstone. 13th edition, 2 (2):37-39.

Cuckler, A. C., Garzillo, M., Malanga, C. and McManus, E. C. (1960). Amprolium efficacy for coccidia in chickens, *Poultry Science,* 39:1241-1242.

Davies, S. F. M. and Joyner, L. P. (1962). Infection of the fowl by the parenteral inoculation of oocysts of *Eimeria*. *Nature*, 194:996-997.

Du, A. and Hu, S. (2004). Effects of a herbal complex against *E. tenella* infection in chickens, Short Communication. *Journal of Veterinary Medicine*, Series B 51(4): 194, May, 2004. www.blackwel-synergy.com/doi/abs/10 10th January, 2007, 03:15pm.

Duffy, F. C. and Tuker, L. A. (2003). Natural treatments for avian coccidiosis. *World Poultry* – Coccidiosis Special 2003. www.Agricworld.nl pp. 9-10. 11th January, 2007. 04:35 pm.

Evans, T. and Martins, D. (1991). Controlling coccidiosis. *Poultry International*. November Edition, 1991. Pp: 6-7.

Ferguson, J. P. D. (2003). The life cycle of the coccidian parasite, *Eimeria tenella*. In: *World Poultry* – Coccidiosis Special. www.Agriworld.nl Pp: 15-17, 11th January, 2007, 04:12 pm.

Greg, F. M. (2001). New organic anticoccidial can safely be used in feed. *World Poultry* – Elsevier 17(5). www.Agricworld.nl.

Haberkorn, A. (1984). A new coccidial drug and a new concept for prevention of coccidiosis. *Proceedings of the XVII World's Poultry Congress*, Helsinkl, Finland, Pp. 774

Haberkorn, A. and Stoltefuss, J. (1987). Studies on the activity spectrum of toltrazuril, a new anticoccidial agent. *Veterinary Medical Review,* 1:22-32.

Hamet, N. (1978). Mode of action of anticoccidials in prevention: Application, practice, diagnostic and prevention. *Conference Presentation at the Reunion* S.AV-ITAVI, December, 1978.

Hamet, N. (1986). Resistance to anticoccidial drugs in poultry farms in France from 1975 to 1984. *Proceedings Georgia Coccidiosis Conference,* University of Georgia, Athens, Pp. 415-421.

Hamet, N., Josse, J., Robin, B. and Toucas, L. (1985). An epidemiological investigation into coccidiosis and drug resistance in broiler chickens. *World Poultry Science Journal*, 41(3): 210-233.

Helm, D. J. (1999). Coccidiosis in poultry, *Clemson University Livestock Poultry Health Programs.* www.clemson.edu/LPH/coccidia%20in%20Poultrypdf 10th January, 2007. 06:24 pm.

Jeffers, T. K. (1974). *Eimeria tenella*: Incidence, distribution and anticoccidial drug resistance of isolants in major broiler producing areas. *Avian Diseases*, 18:331-342.

Jenkins, M. (2005). Control of avian coccidiosis: Drugs and Vaccines. *Miscellaneous Publishing Information Bulletin.* Feed Information News Service, p.3. www.ars.usda.gov/research/publications.htm 10th January, 2007, 05:58 pm.

Johnson, J. and Reid, W. M. (1970). Anticoccidial drugs: Lesion scoring technique in battery and floor-pen experiments with chickens. *Experimental Parasitology*, 28:30-36.

Jordan, F. T. W. and Pattison, M. (1999). Parasitic Diseases. In: *Diseases of Poultry,* 4th Edition, University Press Cambridge, United Kingdom. Pp. 261-275.

Joyner, P. L. and Davies, S. F. M. (1960). Detection and assessment of sublethal infections of *Eimeria tenella* and *Eimeria necatrix*. *Experimental Parasitology*, 9:243-246.

Joyner, L. P. and Norton, C. C. (1973). The immunity arising from continuous low-level infection with *Eimeria tenella*. *Parasitology,* 67:333-340.

Kabell, S., Handberg, K. and Bisgaard, M. (2006). Impact of coccidial infection on vaccine and vvIBDV in lymphoid tissues of SPF chickens as detected by RT-PCR. *Acta Vet Scad.* 48(1):17-18

Karanajeewa, H. and Reece, R. L. (1985). The effect of coccidiostat and dietry mineral elements on the growth performance of broiler chickens. *Proceedings, 6th Australian Poultry and Stockfeed Convention,* 22-27th September, Melbourne, Pp. 342-349.

Larry, R. M., and Otto, M. R. (2005). Coccidiosis. In: *Mercks Veterinary Manual*, 9th Edition published by MERCK and Co., INC. White House Station, N.J., United States of America, Pp. 2201-2207.

Litjens, J. B. (1986). The relationship between coccidiosis and the use of anticoccidials in broilers in the southern part of the Netherlands. *Proceedings Georgia Cocidiosis Conference*, University of Georgia, Athens, Pp. 442-448.

Marizvikuru, M., Evison, B., Michael, C., Tinyiko, E. H. (2005). Use of herbal plants in poultry health management in the Mushagashe small-scale commercial farming area in Zimbabwe. *International Journal on Applied Research in Veterinary Medicine,* 3(2):163-170.

McDougald, L. R. (1982). Chemotherapy of coccidiosis. In: (Ed) Long, P. L. *The Biology of the Coccidia*, Baltimore, USA, University Park Press, Pp. 373-427.

McDougald, L. R. (1986). Current drugs and programs. *Proceedings Georgia Coccidiosis Conference,* University of Georgia, Athens, Pp. 23238.

McDougald, L. R., Dasilva, J. M. L., Solis, J. and Braga, J. (1987). A survey of sensitivity to anticoccidial drugs in 60 isolates of coccidia from broiler chickens in Brazil and Argentina. *Avian Diseases*, 31:287-292.

McDougald, L. R., Fuller, A. L. and Solis, J. (1986). Drug sensitivity of 99 isolates of coccidia from broiler farms. *Avian Diseases,* 30:690-694.

McDougald, R. L. and Reid, W. (1997). Coccidiosis. In: eds. Calnek, B. W., with John, B. H., Charles, W. B., Larry, R. M. and Saif, Y. M., *Diseases of Poultry*. 10th Edition, Iowa State University Press, Iowa, USA, Pp. 865-878.

Melhorn, H., Orthmann-Falkenstein, G. and Haberkorn, A. (1984). The effect of symetric triazinone on developmental stages of *Eimeria tenella, E. maxima* and *E. acervulina*: A light and electron microscopic study. *Zeitschrift fur Parasitenkunde,* 70:173-182.

Michael, A., Perelman, B., Ashash, U., Kin, E. and Ahahroni, Y. (2003). Update on maternal immunization against coccidiosis. In: *World Poultry* – Coccidiosis Special 2003. www.Agricworld.nl 12th January, 2007, 05:47 pm.

Mikota, K. S. and Donald, C. P. (2003). Amprolium hydrochloride. In: *The Elephant Formulary*. Published by Elephant Care International. www.elephantcare.org/Drugs/amprolium.htm. 10th January, 2007; 07:20 pm.

Morehouse, N. F. and Barron, R. R. (1970). Coccidiosis: Evaluation of coccidiostats by mortality, weight gains, and fecal scores. *Experimental Parasitology,* 28:25-29.

Morris, G., Skinner, R., Fadavi, R., Richards, G. D. and Gasser, R. B. (2005). www.jcu.edu.au/school/bms/avpa/avps_conf_Feb_2005/G%20Morris%20avpa%202005.pdf. 10th January, 2007, 06:25 pm.

Murray, K. (2001). Coccidiosis in chickens: *Alberta Agriculture, Food and Rural Development.* www.agric.gov.ab.ca 9th January, 2007. 05:56 pm.

Murray, J. K. and Hanson, J. A. (2005). Coccidiosis in Chickens: *Alberta Agriculture*.www.millerhatcheries.com/information/diseases/coccidiosis.htm 9th December, 2005. 02:54 pm.

Norton, C. C. and Joyner, L. P. (1986). Avian coccidiosis: The administration of encapsulated oocysts. *Parasitology,* 92: 499-510.

Oei, P. (2003). Mushroom markets and marketing. In: *Mushroom cultivation* (3rd Edition). Backhuy's Publishers Leiden, The Netherlands, P. 58-59.

Oladele, S. B., Ayo, J. O., Esievo, K. A. N. and Ogundipe, S. O. (2000). Effects of seasons and sex on packed cell volume, haemoglobin and total protein of indigenous chickens in Zaria, Nigeria. *Journal of Medical and Allied Sciences*, 3:173-177.

Opitz, H. M. (1998). Biological control of coccidiosis in small poultry flocks. *The University of Maine Cooperative Extension Bulletin* No. 2259.

Pearson, D. (2001). Coccidiosis in broilers: *European Coccidiosis Discussion Group*. Central Veterinary Laboratory, Wey Bridge, UK. www.poultry_health.com/fora/inlhelth/euroc01.htm 10th January, 2007; 06:10 pm.

Prasad, S., Singh, M. M., Jha, G. and Mishra, S. S. (1978). Amprolium poisoning in poultry. *Indian Journal of Poultry Science,* 13:198-202.

Ramadan, A., Abo El-Sooud, K. and El-Bahy, M. M. (1997). Anticoccidial efficacy of toltrazuril and halofuginone against *Eimeria tenella* infection in broiler chickens in Egypt. *Research in Veterinary Science,* 62:175-178.

Reck, M. and McQuistion, E. T. (1994). The anticoccidial effects of amprolium, monensin, and Sodium sulfamethazine in farm – reared chukar partridges (*Alectoris graeca*) in Illinois. *Transactions of the Illinois State Academy of Science*, 87(1&2): 51-59.

Reece, R. L. (1988). Review of adverse effects of chemotherapeutic agents in poultry. *World's Poultry Science Journal*, 44(3):193-216.

Reid, W. M. (1968). A diagnostic chart for nine species of fowl coccidia. *Georgia Agricultural Experimental Station Technique*, Bulletin N. S. 39:1-18.

Reid, W. M. (1972). Anticoccidials used in the poultry industry: Time of action against the coccidial life cycle. Extract from *Folia Veternaria Latina,* 2(3): 641.

Reid, W. M., Long, P. L. and McDougald, L. R. (1984). Coccidiosis. In: eds. Hofstad, M. S., Barnes, H. J., Calnek, B. W. and Reid, W. M. *Diseases of Poultry*. 8th Edition, Iowa State University Press, Ames, Iowa United States of America.

Reyna, P. S., Mathis, G. F. and McDougald, L. R. (1982). A survey of sensitizing anticoccidial drugs to 60 isolates from broiler chickens in Brazil and Argentina. *Avian Diseases*, 31: 287-292.

Ritchie, B. w., Harrison, G. J. and Harrison, L. R. (1994). Galliformes. Hematology of selected gallinaceus birds, differentials. In: *Avian Medicine, Principles and Applications.* Pp. 1340-1341.

Rogers, E. F., Cuckler, A. C., Clark, R. L., McManus, E. C., Garzillo, M., Pessolano, A. A., Malanga, C., Becker, H. J., Otto, W. H., Leanza, W. J., Dickinson, A. M., Sareh, L. H. and Van Iderstine, A. (1960). Antiparasitic drugs. III Thiamine – reversible coccidiostat. *Journal of American Chemistry Society*, 82, 2974.

Rose, M. E. (1985). The *Eimeria*. *Current Topics in Microbiology and Immunology*, 120:7-17.

Ryley, J. F. (1967). Methylbenzoquate, a new wide-spectrum coccidiostat for chickens. *British Veterinary Journal*, 123:513-520.

Sainsbury, D. (1992). The health of poultry. In: *Poultry Health and Management, Chickens, Ducks, Geese, Quail.* Second edition. Professional Books, Oxford London, Edinburgh, 11:129-134.

Sharma, N. N. (1964). Response of the fowl (*Gallus domesticus*) to parenteral administration of seven coccidial species. *Journal of Parasitology*, 50:324-328.

Saville, P. (1996). Coccidiosis in poultry: *Animal Health Advisory Leaflet 3, South Pacific Commission.*
www.spc.int/rahs/publication/leaflets/AHAL%2003Epdf
9th January, 2007. 06:24 pm.

Seneviratna, P. (1969). Parasitic diseases. In: *Diseases of Poultry (Including cage birds)*. Second edition. Bristol: John Wright and Sons Ltd., Pp.80-88.

Shane, S. (1997). Coccidiosis: Enteric diseases. In: *The Poultry Disease Handbook*, www.asasea.com/pd-sect6.htm No.044/11/96 (36). 10th January, 2007, 05:18 pm.

Sharma, S. K. and Quastel, J. H. (1965). Transport and metabolism of thiamine in rat brain cortex in vitro. *Biochemistry Journal*, 94:790-800.

Shirley, M. (2006). Making your coccidiosis control program state of the art: CocciForum Issue No.4, Schering – Plough Animal Health. www.thepoultrysite.com 8th January, 2007. 6:20 pm.

Shirley, M. W., Bushel, A. C., McDonald, V. and Roberts, B. (1995). A live attenuated vaccine for the control of avian coccidiosis: Trials in broiler breeders and replacement layer flocks in the United Kingdom. *The Veterinary Record*, 137(18):453-457.

Shirley, M. and Tomley, (2006). The *E. tenella* genome project. www.sanger.ac.uk/project/E_tenella 9th January, 2007. 08:14 pm.

Sirois, M. (1995). Blood chemistry. In: *Veterinary Clinical Laboratory Procedures*, Mosby's Fundamentals of Veterinary Technology, Pp. 101-105.

Suls, L. (1999). Planning your attack against coccidiosis. *World Poultry* – Elsevier Special, Pp. 10-11.

Tyzzer, E. E. (1929). Coccidiosis in gallinaceous birds. *American Journal of Hygiene,* 10: 269-383.

Van der Stroom, J. H. and Wiebe vander Sluis (1999). The effect of intercurrent diseases on coccidiosis. *World Poultry* – Elsevier Special, pp. 15-16.

Van Stratum, P. G. C., Feron, V. L. and Litjens, J. B. (1966). The effect of furazolidone on broiler chickens fed ration containing amprolium or zoalene. III. Effect of 10 days administration of 400 ppm furazolidone. *Tijdschr Diergeneeskd,* 91:639-647.

Wallach, M. and Waldenstedt, L. (1999). Immunity and the effect of removing coccidiostat from feed. *World Poultry* – Elsevier, 18(6):43-43.

Waller, G. R., Mangiaficos, S. and Ritchey, C. R. (1978). A chemical investigation of *Aloe barbadensis*. *Proceedings of the Oklahoma Academy of Science*, 58:69-76.

Warren, E. W. and Ball, S. J. (1963). The effect of sulfaquinoxaline and amprolium on the life cycle of *Eimeria adenoides.* Moore and Brown, 1951, in turkey poults. *Parasitology,* 53: 653.

Williams, R. B. (1994). Epidemiological studies of coccidiosis in the domestic fowl (*Gallus gallus*). II Physical condition and survival of *Eimeria acervulina* oocysts in poultry house litter. *Applied Parasitology*, 36:90-96.

Williams, R. B. (2005). Direct assessment of physical gut integrity by mucosal examination. *World Poultry*, 21(10) 43-45.

Williams, R. B. (2006). The impact of drug resistant parasites on the epizootiology of coccidiosis. *World Poultry*, 22(1):32.

Appendices

Appendix 1

Mean change in body weight (grams) post inoculation in Warren chicks inoculated with local strain of *Eimeria* at 25 days old.

Day(s) post inoculation	Group A	Group B	Group C	Group D	Group E
0	177.6(n=24)	160.4(n=24)	163.8(n=24)	163.8(n=24)	158.6(n=24)
4	306.1(n=24)	258.3(n=24)	258.4(n=24)	253.8(n=24)	243.8(n=24)
6	341.7(n=24)	270.9(n=24)	258.4(n=24)	262.5(n=24)	245.8(n=24)
8	363.2(n=19)	283.3(n=19)	253.8(n=18)	288.9(n=19)	280.6(n=18)
10	407.5(n=19)	295.0(n=19)	286.3(n=18)	293.9(n=19)	303.1(n=18)
14	425.0(n=14)	339.3(n=14)	346.2(n=13)	325.0(n=14)	346.2(n=13)
17	464.3(n=14)	376.9(n=13)	361.5(n=13)	367.9(n=14)	369.2(n=13)
19	492.9(n=14)	403.8(n=13)	396.2(n=13)	375.0(n=13)	400.0(n=13)
21	555.6(n=9)	450.0(n=8)	437.5(n=8)	427.8(n=8)	437.5(n=8)

Appendix 2

Mean total body weight gain (in grams) post inoculation in Warren chicks inoculated with local strain of *Eimeria* at 25 days old.

Groups	Mean initial Weight	Mean final Weight	Mean weight gain
Group A	177.6 (n=24)	555.6 (n=9)	378.0
Group B	160.4 (n=24)	450.0 (n=8)	289.7
Group C	163.8 (n=24)	437.5 (n=8)	273.7
Group D	163.8 (n=24)	427.8 (n=8)	264.0
Group E	158.6 (n=24)	437.5 (n=8)	278.9

Appendix 3

Mean total plasma protein (in g/dl) ±SD post inoculation in Warren chicks inoculated with local strain of *Eimeria* at 25 days old.

Day (s) post inoculation	Group A	Group B	Group C	Group D	Group E
0	3.6±0.3 [a]	3.9±0.6 [a]	4.0±0.5 [a]	3.7± 0.3 [a]	3.7±0.4 [a]
3	3.6±0.3 [a]	3.6±0.2 [a]	4.0±0.4 [a]	3.4± 0.1 [a]	3.5±0.2 [b]
7	3.4±0.3 [a]	3.8±0.3 [a]	3.7±0.7 [a]	3.3± 0.4 [a]	3.9±0.4 [b]
11	3.1±0.2 [a]	3.6± 0.5 [b]	3.3±0.3 [a]	3.2± 0.7 [a]	3.7±0.3 [c]
14	3.8±0.8 [a]	4.1± 0.4 [a]	3.8±0.5 [a]	3.9± 0.5 [a]	3.6±0.5 [a]
17	3.5±0.5 [a]	3.7± 0.3 [a]	3.9±0.5 [a]	3.9± 0.5 [b]	3.7±0.3 [a]
21	3.4±0.4 [a]	3.1± 0.4 [a]	3.1±0.3 [a]	3.3± 0.5 [a]	3.3±0.5 [a]

a b c Different superscripts in a row indicate significant difference between the means, ab: $p<0.05$; ac: $p<0.01$

Normal total plasma protein: 3.3 – 5.5 g/dl (Ritchie *et al.*, 1994; Oladele *et al.*, 2000)

Appendix 4

Daily oocysts count per gram of feces post inoculation in Warren chicks inoculated with local strain of *Eimeria* at 25 days old.

Day(s) post inoculation	Group A	Group B	Group C	Group D	Group E
1	0	0	0	0	0
2	0	0	0	0	0
3	0	6,000	4,050	1,400	2,250
4	0	26,450	13,450	9,500	8,750
5	0	38,300	20,650	84,950	55,700
6	0	1,752,600	2,158,800	2,347,200	1,904,400
7	0	184,800	691,200	351,000	268,200
8	0	50,400	17,400	6,000	30,600
9	0	176,100	4,800	2,700	23,700
10	0	25,000	900	600	1,200
11	0	72,600	45,300	111,600	34,200
12	0	8,100	4,800	76,500	11,700
13	0	18,100	9,000	14,100	20,400
14	0	32,700	6,600	2,700	31,500
15	0	37,500	3,900	1,500	132,000
16	0	15,000	1,500	600	205,200
17	0	12,000	5,400	19,500	90,000
18	0	19,800	1,500	900	54,600
19	0	13,500	1,200	600	10,200
20	0	12,300	1,100	200	8,300
21	0	9,000	900	200	800

Appendix 5

Preparation of Potassium dichromate solution 2.5% w/v

To prepare this solution 2.5 g of Potassium dichromate was weighed, and was dissolved in 100 ml of distilled water and then stirred gently until all the dichromate was completely dissolved.

Appendix 6

Preparation of floatation medium (1.3 specific gravity)

Seven hundred grams (700 g) of sucrose was weighed and dissolved in 1 litre of warm water, a crater of thymol (about 1 g) was then added and stirred. This was to preserve the medium from fungal growth.

Appendix 7

Preparation of 10% formol saline

One hundred milliliters (100 ml) of 40% formalin was measured using a measuring cylinder, 900 ml of distilled water was also measured, and both were mixed by adding the formalin into the water and then stirred; nine grams of laboratory Sodium chloride was then added into the solution and also stirred. This was then stored until use.