EFFICACY OF SOME ANTIMALARIAL DRUGS IN TREATMENT OF *Eimeria tenella* IN CHICKENS

By:

Nada Abdalrahem Ahmed Abdalrhman

(B.V. Medicine, University of Khartoum, 2005)

SUPERVISOR:

Prof. Kamal-Eldeen ElSedig Ibrahim

CO. SUPERVISOR:

Prof. Elgailani Ali Elamin

A thesis submitted to the University of Khartoum in partial fulfillment of the requirements for the degree of Master of Veterinary Medicine

Department of Medicine, Pharmacology and Toxicology

Faculty of Veterinary Medicine

University of Khartoum

June 2009
Dedication

To my parents, brothers and sister I dedicate this work

Nada abd-Elrahim
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>i.</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>ii.</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>v.</td>
</tr>
<tr>
<td>LIST OF FIGURES AND APPENDICES</td>
<td>vi.</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vii.</td>
</tr>
<tr>
<td>Abstract</td>
<td>viii.</td>
</tr>
<tr>
<td>Arabic abstract</td>
<td>x.</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER ONE: LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>1.1 Classification of <em>Eimeria</em> spp.</td>
<td>3</td>
</tr>
<tr>
<td>1.2 Aetiology of poultry coccidiosis</td>
<td>6</td>
</tr>
<tr>
<td>1.3 Geographic distribution</td>
<td>8</td>
</tr>
<tr>
<td>1.4 Coccidian life cycle</td>
<td>9</td>
</tr>
<tr>
<td>1.5 Infection</td>
<td>13</td>
</tr>
<tr>
<td>1.6 Pathogenicity of poultry coccidiosis</td>
<td>15</td>
</tr>
<tr>
<td>1.7 Clinical findings</td>
<td>16</td>
</tr>
<tr>
<td>1.8 Diagnosis</td>
<td>18</td>
</tr>
<tr>
<td>1.9 Histological and physiological changes</td>
<td>19</td>
</tr>
<tr>
<td>1.10.1 Coccidial immunity caused by coccidial infection</td>
<td>21</td>
</tr>
<tr>
<td>1.10.2 Immune responses</td>
<td>25</td>
</tr>
<tr>
<td>1.10.3 Mode of Inheritance</td>
<td>28</td>
</tr>
<tr>
<td>1.11 Isolation of <em>Eimeria</em> spp.</td>
<td>29</td>
</tr>
<tr>
<td>1.12 Control and vaccination</td>
<td>29</td>
</tr>
<tr>
<td>1.13 Coccidiostat and Growth Promoters</td>
<td>33</td>
</tr>
<tr>
<td>1.14 Experimental Medications Mode of Action</td>
<td>36</td>
</tr>
<tr>
<td>1.15.1 Treatment</td>
<td>39</td>
</tr>
<tr>
<td>1.15.2 Anti-malarial Medications used in the Treatment of Coccidiosis</td>
<td>43</td>
</tr>
</tbody>
</table>
CHAPTER TWO: MATERIALS AND METHODS

2.1 Collection of *E. tenella* samples

2.2 Experimental infection

2.3 Experimental treatment and administered doses

2.4 Experimental parameters and data collection

2.5 Laboratory investigation

2.6 Feecal examination

2.6.1 Oocysts count

2.6.2 Oocysts sporulation

2.6.3 Oocyst identification

2.6.3.1 Oocyst morphology

2.6.3.2 Measurements of oocysts

2.6.3.3 Location of infection

2.6.3.4 Sporulation time

2.7 Collection of blood samples

2.8 Haematological methods

2.10 Liver function tests

2.11 Statistical analysis

CHAPTER THREE: RESULTS

3.1 Effects of Anti-malarial and Anti-coccidial drugs on infection symptoums

3.2 Effects of anti-malarial and acoccidial drugs on infection symptoums of the experimental birds

3.3 Effect of control un infected treated primaquine 250 ppm on liver function tests (4 days after treatment)

3.4 Effects of anti-malarial and anti-coccidial medications on haematology at the first period before treatment -4days (MCV, WBCS, PLT, LYM & MON)

3.5 Effects of anti-malarial and anti-coccidial medications on haematology analysis at the first period before treatment - 4days
3.6 Effects of anti-malarial and anti-coccidial medications on haematology after treatment

CHAPTER FOUR: DISCUSSION

CONCLUSION AND RECOMMENDATIONS

REFERENCES

APPENDICES
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Table Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table (1:1):</td>
<td>Classification of <em>Eimeria</em> spp.</td>
<td>6</td>
</tr>
<tr>
<td>Table (3:1):</td>
<td>Means (+SE) of effects of anti-malarial and acoccidial drugs on infection symptoms of the experimental birds</td>
<td>58</td>
</tr>
<tr>
<td>Table (3:2):</td>
<td>Means (+SE) of effects of anti-malarial and acoccidial drugs on infection symptoms of the experimental birds</td>
<td>61</td>
</tr>
<tr>
<td>Table (3:3):</td>
<td>Means (+SE) of effect of control un infected treated primaquine250 ppm on liver function tests (4 days after treatment)</td>
<td>65</td>
</tr>
<tr>
<td>Table (3:4):</td>
<td>Means (+SE) of effects of anti-malarial and anti-coccidial medications on haematology analysis at the first period before treatment -4days.</td>
<td>68</td>
</tr>
<tr>
<td>Table (3:5):</td>
<td>Means (+SE) of effects of anti-malarial and anti-coccidial medications on haematology analysis at the first period before treatment -4days.</td>
<td>72</td>
</tr>
<tr>
<td>Table (3:6):</td>
<td>Means (+SE) of effects of anti-malarial and anti-coccidial medications on haematology analysis after treatment</td>
<td>78</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES AND APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>one</td>
<td>Analysis of Variance for the experimental medications</td>
</tr>
<tr>
<td>one</td>
<td>Oocyst of <em>E. tenella</em></td>
</tr>
<tr>
<td>two</td>
<td>Sporulated oocyst of <em>E. tenella</em></td>
</tr>
<tr>
<td>three</td>
<td>Infected ceacum by <em>E. tenella</em></td>
</tr>
<tr>
<td>four</td>
<td><em>Eimeria tenella</em> life cycle</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

Above all, I have enjoyed tremendous health, motivation, intellect, wisdom, protection, and facilitating circumstances which made it all possible, for which I am forever grateful to (Allah the Greatest) to complete this work which be glory and honor forever and ever.

I am also deeply grateful to Prof. Kamal Eldeen ElSedig, for generously providing me with an enhancing environment that enabled me to concentrate on, and successfully complete my master study.

I also indebted to Prof. Elgailani Ali Elamin who acts as my co-supervisor for unlimited supports and encouragement.

I thank sincerely, Dr. Shawgi M. Hassan for his unlimited help in several aspects of my research including parasitological data collection and suggestions during planning the experiments.

I also thank staff of the departments of veterinary medicine and parasitology for their teaching, availability, and interest in my academic pursuits. The patience of my colleagues encouraged me to go further in my research as well as lab assays during those long days of data collection.

I sincerely appreciate the support of my family (father, mother brothers and sister) who bore the brunt of my frustrations and embarrassing neglect in pursuit of this degree.

At the last not the least I also indebted to whom I were not mentioned here.
ABSTRACT

This research was carried out to study the efficacy of three synthetic anti-malarial drugs (primaquine at level 31.2, 125 and 250 ppm, fancedar (86.625mg) and artesunate (33mg) and one anticoccidial medication (amprolium 60g/100 Liter) as a reference. The drugs were administered in drinking water to treat *Eimeria tenella* in chickens.

A total of 280 3-week old male Hisex strain chicks were divided into 14 even groups, control naive (uninfected unmedicated group), (infected unmedicated group), 6 groups of infected treated by anti-malarial medications (primaquine 31.2, 125 and 250 ppm, fancedar 86.625mg and artesunate 33mg) and anti-coccidial drug (amprolium 60g/100 Liter) and 6 groups of control uninfected medicated, as prophylactic additives and to test their impact on the chicks' behaviour and to test the toxicity. Infection was carried out by inoculation of sporulated oocyst $1 \times 10^4$ in the chicks' crops. Treatment started on the third day of infection.

Results indicated that utilization of primaquine level 125 ppm demonstrated heavier weight gain. All the sick chicks recovered from the disease and none of them died and no oocysts were recovered in the faeces. During treatment of birds with primaquine level 31.2 ppm, feed intake was lower among birds. All the sick chicks recovered from the disease and none of them died and no oocysts were recovered in the faeces. During treatment of chicks with primaquine level 250 ppm, secured the lowest food intake with the highest mortality rate. Among this group the lowest value of mean cell value (MCV) and toxic
symptoms were recorded. The administration of artesunate revealed a higher food intake. All the sick chicks recovered from the disease and none of them died rate and no oocysts were recovered in the faeces and the highest values of MCV were recorded. Administration of fancedar recorded a higher food intake with all the sick chicks recovered from the disease and none of them died, and no oocysts were recovered in the faeces with administration of anticoccidial (amprolium), all of the sick chicks recovered from the disease and none of them died, and exerted the highest values of MCV.

It is concluded that antimalarial drugs (primaquine 31.2, 125 and 250 ppm artesunate 33 mg, fancedar 86.625 mg) for treatment of *Eimeria tenella* was effective compared with anticoccidial drug (amprolium). However, the high level of pimaquine 250 ppm as prophylactic additive was toxic.
المستخلص

لا يظهر النص بشكل بالغ واضحاً. من الواضح أن النص يتحدث عن دراسة بحثية حول ملاءمة أدوية فائقة لدراسة الأداء في مرضى باسستاذ. النص يحتوي على أرقام مثل 125 و 600، وأيضًا ملاحظات على استخدام الأدوية مع بعضها البعض. من الصعب قراءة النص بشكل طبيعي ولذلك لا يمكنني التفاعل بشكل طبيعي معه.
لا يمكنني قراءة النص العربي المكتوب بالخط العربي. يرجى استخدام النص العربي المكتوب باللغة الإنجليزية أو النص العربي المكتوب بالخط الغربي.
INTRODUCTION

Coccidiosis is a common disease that causes great economic losses in poultry industry. Coccidiosis is a self-limiting, infectious disease of the digestive tract caused by host specific intracellular protozoal parasites of the genus *Eimeria*. *Eimeria* species are considered the most pathogenic of the parasitic protozoa of poultry (Kheissin, 1967; Beyer, 1989). The parasite multiplies in the intestinal tract of poultry produces tissue damage, resulting in reduced growth and increased susceptibility to pathogens (McDougald, 2003). *Eimeria* species the rigid host specificity; meaning that species which infects chickens does not infect the other host genus vice versa (Julie and Helm, 1999). Most species of *Eimeria* have characteristic site of invasion (tissue specificity) generally in epithelial cells of endodermal origin and particularly of the intestinal villi. These locations are so characteristic that they are often used as diagnostic features of the individual species (Johnson and Reid, 1970).

Nine species of coccidia occur in poultry. Most of them are pathogenic to varying degrees. *E. tenella* is one of the most common and pathogenic coccidian of poultry. Caecal coccidiosis disease entity high morbidity and high mortality.

Hypothetical constructive and objectives of this study was build on the hypothesis of test efficacy of anti-malarial drugs as a medications of coccidiosis infection by *E. tenella* in accordance with their susceptibility to these compounds, which have similar metabolic function to those of blood cell parasitizing protozoa like plasmodia and prioplasma, which are easily suppressed by this class of compound and have the same phylum.
CHAPTER ONE
LITERATURE REVIEW

1.1: Classification of *Eimeria* spp.:

Coccidiosis is a self-limiting, infectious disease of the digestive tract caused by host specific intracellular protozoal parasites of the genus *Eimeria*. Coccidia are classified under the subkingdom Protozoa of the phylum Apicomplexa (Jeurissen *et al.*, 1996).

Domestic poultry and birds are affected by coccidia called *Eimeria* (scientific name). There are different types or species of *Eimeria* that affect poultry and each are host-specific meaning that a species that infects chickens does not infect turkeys and vice versa (Julie and Helm, 1999).

The complete sequences of the small subunit ribosomal RNA (SSU-rRNA) gene of isolates of seven additional *Eimeria* species (six avian and one mammalian) were submitted to Gene Bank (Barta, *et al.*, 1997). The usage of these sequences, in addition to those previously available for human associated *Cyclospora* and several *Eimeria* species Relman *et al.* (1996) reevaluate the phylogenetic relatedness of *Cyclospora* and *Eimeria*. Both of the previous phylogenetic analyses included sequences for *E. tenella* and *E. mitis* isolaes; thus, two sequences for each of these species we used.

Currently classified as an *Eimeria* species is known to be pathogenic for humans. *Eimeria* is the largest genus of coccidian parasites and reportedly includes more than 1,500 named species
However, the current criteria cited by Sogin (1989) for naming new species of *Eimeria*. This criteria include host specificity, morphologic characteristics of oocysts, duration of prepatent and patent periods, location of the infection in the host, and pathogenicity, which are suboptimal, and the available data for some *Eimeria* species named in the past are incomplete. Thus, some *Eimeria* species may be synonymous, and some organisms thought to belong to the same species may not. Moreover, the possibility even exists that human-associated *Cyclospora* is synonymous with a previously named *Eimeria* species. Although no molecular data are available for the type species of the *Cyclospora* genus or for the *Cyclospora* species that are not known to be human-associated.

Levine, et al. (1980) cited that the sub-kingdom protozoa includes over 65000 named species, of which over half are fossil and approximately 10,000 are parasitic. Among living species, this includes approximately 250 parasitic and 11,300 free-living sarcodines (of which approximately 4,600 are foraminiferids); approximately 1,8000 parasitic and 5,100 free-living flagellates; approximately 5,600 parasitic "Sporozoa" (including Apicomplexa, Microspora, Myxospora, and Ascetospora); and approximately 2,5000 parasitic and 4,700 free-living ciliates. There are undoubtedly thousands more still unnamed. Moreover, they were reported that seven phyla of protozoa are accepted in this classification--sarcomastigophora, labyrinthomorpha, apicomplexa, microspora, ascetospora, myxospora, and ciliophora.

King-Hwa et al. (2007) cited that *Eimeria tenella* is an intracellular protozoan parasite that infects the intestinal tracts of domestic fowl and causes coccidiosis, serious and sometimes lethal enteritis. *Eimeria* falls in
the same phylum (Apicomplexa) as several human and animal parasites such as *Cryptosporidium*, *Toxoplasma*, and the malaria parasite, *Plasmodium*. The authors were reported the sequencing and analysis of the first chromosome of *E. tenella*, a chromosome believed to carry loci associated with drug resistance and known to differ between virulent and attenuated strains of the parasite. The researchers pointed out that the chromosome which appears to be representative of the genome is gene-dense and rich in simple-sequence repeats, many of which appear to give rise to repetitive amino acid tracts in the predicted proteins. Moreover, most striking is the segmentation of the chromosome into repeat-rich regions peppered with transposon-like elements and telomere-like repeats, alternating with repeat-free regions. Moreover, predicted genes differ in character between the two types of segment, and the repeat-rich regions appear to be associated with strain-to-strain variation.

Reclassification, on the basis of phylogenetic analysis, of human associated *Cyclospora* as an *Eimeria* species may stimulate productive research by suggesting possible animal reservoirs of human-associated *Cyclospora* (which may or may not infect other animals). In addition, animal models and cell culture systems that have been developed for *Eimeria* may prove useful for *Cyclospora*. However, it remains to be seen whether the biologic characteristics of *Cyclospora* are similar to those of the *Eimeria* species to which *Cyclospora* is closely related on the basis of phylogenetic criteria (Dispatches, 1997).


1.2: Aetiology of poultry coccidiosis:

Common disease of many species of birds caused by species of genera primarily *Eimeria* and *Isospora* and are quite host specific in chickens: disease of universal importance is *Eimeria tenella* (caecum), *E. acervulina* (upper small int.), *E. maxima* and *E. necatrix* (mid small intestine). In turkeys: common, lesions are less severe than in chickens *E. adenoides* (caecum), *E. meleagrimitis* (mid small intestine). In geese: *E. truncata* and *E. anseris*. Whereas in ducks: renal coccidia due to *E. boschadis* and in quail, partridges, and pheasants causes by various species of *Eimeria* causes enteritis. Moreover, in quail coccidiosis is commonly associated with ulcerative enteritis caused by *Clostridium colinum* (Shivaprasad, 2007).
**Table (1:1):**

**Classification of *Eimeria* spp.**

<table>
<thead>
<tr>
<th>Class</th>
<th>Sub/class</th>
<th>Order</th>
<th>Sub-order</th>
<th>Family</th>
<th>Representative genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perkinsea</td>
<td>Gregarina</td>
<td>Protococcidia</td>
<td>Adelorina</td>
<td>(1) Eimeriidae</td>
<td><em>Eimeria, Isospora, Cryptosporidium</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agamococcidia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sporozoa</td>
<td>Coccidia</td>
<td>Eucoccidia</td>
<td>Eimeriina</td>
<td>(2) Sarcocystidae</td>
<td>Sarcocystis, Toxoplasma Besnoitia, Cystoisospora, Hammondia, Frenkelia</td>
</tr>
<tr>
<td>Piroplasmida</td>
<td>Piroplasmida</td>
<td>Haemosporiina</td>
<td>Plasmodiidae</td>
<td>(1) Babesidae</td>
<td>Plasmodium, Haemoproteus, Leukocytozoon Hepatocystis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2) Theileriidae</td>
<td>Babesia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Theileria</td>
</tr>
</tbody>
</table>

**Phylum: Apicomplexa Levine, 1970**

**Class: Sporozoea leukart, 1879**
Gerald (2007) mentioned that coccidiosis affects several different animal species. In cattle, coccidiosis may produce clinical symptoms in animals from 1 month to 1 year of age but is infective to all age groups. The group of coccidia that are infective to cattle belong to the *Eimeria* genus. Coccidia are very host specific, that is, only cattle coccidia will cause disease in cattle; other species specific coccidia will not cause disease.

Intestinal coccidians from the genus *Isospora* (Protozoa, Apicomplexa) infect a number of songbird species in the wild (Giacomo *et al.* 1997; Duszynski, *et al.* 2000; McGraw and Hill, 2000). Related species (coccidia from the genus *Eimeria*) are common parasites of poultry where they directly inhibit uptake of essential dietary components, including carotenoids in the gastrointestinal tracts of chickens (Allen 1987, 1997; Allen and Fetterer 2002 a, b).

Carmichael (1998) reported that there are many protozoa in the Subclass Coccidia, but emphasized on the family Eimeriina, in particular, the Genus *Eimeria*. Within the genus *Eimeria*, there are hundreds of different species, which cause disease in mammals, birds and reptiles. Each species is identifiable by a particular size and shape, as well as by various other distinguishing characteristics.

### 1.3: Geographic distribution of coccidiosis in poultry:

Jeffers (1974) propagated coccidia from all major broiler-producing regions of the United States. The author obtained *Eimeria tenella* from 308 (29.3%) of the farms which yielded coccidia. This
species being most prevalent in the southeastern and south-central regions.

Poultry coccidiosis in the Sudan was first reported in 1971 then many cases were reported in different regions (Annual Reports of Under Secretary for Animal Resources, 1977-1990).

In Khartoum state incidence the 24% poultry farm which distributed in Khartoum, Khartoum North and Umdourman, whereas the disease showed highly incidence in Khartoum and Khartoum Bahri (Annual Reports under Secretary, Ministry of Agriculture, Animal Resources and Irrigation, 2005).

Mohammed, et al. (1990) reported that five species were identified during an outbreak of coccidiosis in a farm in Khartoum. These species included *Eimeria tenella*, *E. maxima*, *E. mivati*, *E. praecox* and *E. brunette*.

1.4: Coccidian Life Cycle:

Researchers interested in the *Eimeria* species continue to analyze the intricate details of its life cycle in hopes of alleviating the potential hazards (Bruce, 2002).

As *Eimeria* species tends to be very specific in the intestinal region at which they invade, their life cycles are similar with a degree of species specificity (Bruce, 2002). The *Eimeria* complete their life cycle in three distinctive phases including sporogony, merogony, (schizogony) and gametogony; however, the lengths of these phases are unique to the species (Yun et al., 2000).
Oocysts is easily transmissible by mechanical means such as contaminated footwear and equipment, or it can be found in litter, contaminated soil, feed, or water (Conaway and McKenzie, 1991; McDougald and Reid, 1997).

Edgar (1992) mentioned that Coccidiosis, caused by the ingestion of microscopic oocysts. Thus chickens can become infected by coccidia once the oocysts develop into an infective stage outside of the host. Sporogony is the process of a one-celled zygote within the oocyst undergoing a series of divisions to form sporozoites, which are contained within sporocysts. Only oocysts that have undergone this process are able to cause disease.

Bruce (2002) revealed that the sporulated oocysts contain four sporocysts, and each sporocyst contains two sporozoites. Mechanical action of the gizzard and pancreatic enzymes such as trypsin and bile salts cause the destruction of the oocysts’ outer wall, which releases the sporocysts into the digestive tract. Furthermore, the sporocysts are further excysed by trypsin and bile salts that are present in the intestine. The researcher pointed out that sporozoites invade villus epithelial cells along specific locations throughout the digestive tract depending on the species of *Eimeria*. Whereas some species travel within the mucosa, through the lamina propria to the crypt epithelial cells. Once inside villus or crypt cells, the process of merogony takes place.

The sporozoite develops into a rounded body called a trophozoite, and then into an asexually reproductive first-generation schizont (meront). Moreover, the schizont grows and divides rapidly to produce many first-generation merozoites; then, the bodies rupture and release
hundreds of first-generation merozoites, which seek out and invade other epithelial cells. Second-generation trophozoites develop into second-generation schizonts. When the second-generation schizonts mature and rupture, increased quantities of invasive merozoites are released causing widespread infection. The number of asexual stages and time required for each depends on the *Eimeria* species involved; however, most species will have less than four asexual reproductive generations (Rose, 1987; Edgar, 1992).

Merozoites produced by the latter schizont generations develop into sexual forms called gametocytes, some male and some female. This phase of sexual reproduction is termed gametogony. The female gametocyte matures into a macrogamete and the male gametocyte matures and ruptures releasing a large number of motile, biflagellate microgametocytes. The microgametocytes penetrate the mature female macrogamete and fertilization occurs. Following fertilization, a thickened protective wall forms around the zygote. At this stage, the zygote is considered an immature oocyst. When mature, the oocysts rupture the host cell, enter the lumen, and are expelled into the faeces (Jeurissen, *et al.*, 1996; McDougald and Reid, 1997; Yun, 2000).

The sporulated oocyst is the infective stage of the life-cycle. Oocysts can be transmitted mechanically by personnel, contaminated equipment, or in some cases, by wind spreading poultry-house dust and litter over short distances (Simon, 2007). The author mentioned the factors contributing to outbreaks of clinical coccidiosis include:- litter moisture content exceeding 30% due to ingress of rain or leaking waterers, immunosuppression (Marek’s disease, IBD, mycotoxins),
suboptimal inclusion of anticoccidials or incomplete distribution (poor mixing) in feed and environmental and managemental stress such as overstocking, inoperative feeding systems, insufficient ventilation.

David (2007) reported that the transmission stage of the parasite is a microscopic egg shaped cyst (known as the oocyst). Oocysts are shed in the droppings and undergo a process known as sporulation that takes 24-48 hours; after this the oocyst is infective if ingested by a bird. Therefore, an important aspect of the life cycle of these parasites is that the severity of the disease is proportional to the number of sporulated oocysts ingested.

Oocysts excreted from the birds remain in the environment and have the potential to infect other birds. Under favorable environmental conditions (approximately 84°F), sporulation of oocysts is achieved in 24 to 48 hours, and the cycle will continue (Edgar, 1992; Graat et al., 1994; McDougald and Reid, 1997).

Edgar (1992) reported that once the oocyst is sporulated, it can remain infective to birds from several months to one or two years if protected from very hot, dry, or freezing conditions. Coccidial oocysts have rigid characteristics but are not totally indestructible.

According to Lee and Shih (1988) unsporulated oocysts are more susceptible to physical and chemical agents than sporulated oocysts perhaps due to a highly sensitive metabolic state. Moreover, other factors, such as ammonia and anaerobic conditions, are also lethal to oocysts in the environment. Cessation of development occurs when oocysts are introduced to high levels of CO₂ or NH₄, or exposed to
mercury salts, mercuric cyanide, and mercuric chloride due to the ability of these agents to penetrate the oocyst wall (Kheysin, 1972).

The time it takes for oocysts to be seen in the feces after ingestion, is approximately 4 to 7 days for the *Eimeria* species (Henken et al., 1994 a,b; Jeurissen, 1996; McDougald and Reid, 1997).

1.5: Infection:

To initiate infection, sporozoites are released, in the gut, from ingested sporulated oocysts and invade epithelial cells lining the intestine. The entire life cycle occurs in the mucosa and lamina propria, and although light infections are often subclinical, heavy infection can result in severe weight loss, growth depression, and even death (Long, 1973).

It is well established that chickens which survive an initial infection are resistant to re-infection (Rose and Long 1962). Such immunity is species specific (Rose, 1973) and possibly directed against the sporozoite stage (Rose, 1974).

Morishima *et al.* (1984) studied the effect of *Eimeria tenella* infection on *Salmonella typhimurium* infection of chickens was tested using feed experimentally contaminated with *S. typhimurium*. In all research experiments, chickens were necropsied 3 to 14 days after E. *tenella* infection. The number of *S. typhimurium* in the caecal contents was counted and the presence of the organism in the liver and bile was examined. In Experiments 1 and 2, there was no significant difference in
*S. typhimurium* infection between the group infected with *S. typhimurium* alone and the group infected with both *E. tenella* and *S. typhimurium*. In Experiments 3 and 4, *S. typhimurium* counts in the caecal contents of chickens in the concurrently infected group were significantly greater than those of chickens in the *S. typhimurium* alone-infected group.

The specific mechanisms by which each coccidial species causes disease is not clear; however, the establishment of secondary infections due to the altered intestinal mucosa may be responsible for a differential host response (Barker, 1993). This is possible because coccidia are able to interact with other pathogens such as bacteria and viruses, which may amplify the observed effects (Ruff, 1993). As stated above, the ability of the chicken to control the severity of infection and develop protective immunity to coccidial parasites depends on numerous factors. Host responses to the parasite involve a complex series of internal factors that are dependent on developmental stage of the parasite, immune status of the bird, and species and strain of the *Eimeria* parasite.

Al-Natour *et al.* (2002) examined parasitological parameters from chicks were submitted for post-mortem taken randomly from farms in northern Jordan, they were identified seven *Eimeria* spp.: *E. acervulina*, *E. brunetti*, *E. maxima*, *E. necatrix*, *E. mivati*, *E. mitis*, and *E. tenella*. Half (50%) of the farms surveyed had all six chicks infected, 23% of the farms were free of the infection. *E. tenella* was the most prevalent species (39%) followed by *E. necatrix* (12%), *E. brunetti* (12%), and *E. maxima* (10%). The authors concluded that prevalence did not vary by
flock size. Also, neither the use of coccidiostat nor previous coccidiosis clinical outbreaks was associated with the prevalence of coccidiosis.

1.6: Pathogenicity of poultry coccidiosis:

*Eimeria spp.* differ in their pathogenic effect; it was found in caecal coccidiosis due to *E. tenella* occurs most frequently in young birds. On the other hand, Gardiner (1955) pointed out that one to two weeks old chickens were more resistant. Older birds generally immune as a result of previous infection. In general clinical caecal coccidiosis is produced only when heavy infections are acquired over a relatively short period of time, not exceeding 72 hours (Davis and Porter, 1979).

Shuping et al. (1995) investigated the effect of tumor necrosis-like factor (TNLF) on the pathogenesis of coccidiosis. Injection of crude chicken TNLF enhanced the weight loss caused by *Eimeria tenella* infection. Rabbit polyclonal antibody against recombinant human tumor necrosis factor-a (rhTNF) partially restored *E. tenella* induced weight loss in SC chickens, but not in TK chickens. However, injection of chickens with chicken TNLF, rhTNF, and rabbit serum against rhTNF had no significant effect on caecal lesions. Whereas in general, macrophages from SC chickens produced higher levels of TNLF than those from TK chickens. They were observed no significant difference between primary and secondary infection. These results suggest that the excessive TNF production may be involved in weight loss caused by *E. tenella* infection in SC chickens.
In bacteria-free chickens infected with surface-sterilized *Eimeria tenella* oocysts, clinical signs do not develop unlike in chickens with two or more indigenous species of bacteria (Radhhakrishnan, 1971; Johnson and Reid, 1972; Visco and Burns, 1972a; 1972b). Apparently, indigenous bacteria are required for the occurrence of typical caecal coccidiosis in chickens. In the course of development of caecal coccidiosis, the growth of *Clostridium perfringens* and coliforms, especially *Escherichia coli*, is stimulated whereas the growth of *Lactobacillus* spp. is suppressed (Johansson and Sarles, 1948; Radhhakrishnan, 1971). *Lactobacillus* spp have been shown to inhibit the invasion of *Eimeria tenella* in vitro (Tierney et al., 2004).

1.7: Clinical findings:

Reduced weight gain is a major contributor to the production losses that accompany coccidia infections in young chickens. Because inflammatory immune responses divert energy from growth. The clinical signs of coccidiosis are associated with tissue destruction from the release of the merozoites and mature oocysts from the mucosal surface during the last generations of merogony and throughout gametogony. In severe infections, much of the mucosal epithelium is sloughed off and nutrient absorption is compromised (Jeurissen, 1996; McDougald and Reid, 1997; Yun, 2000).

Schwartz (1994) demonstrated that coccidiosis and its infection were one of the most economically important protozoan diseases of poultry is coccidiosis, caused by *Eimeria* spp in intensively reared chickens which are in daily contact with their droppings. The disease is characterized by enteritis and diarrhea.
*Eimeria tenella* is the cause of caecal coccidiosis in young birds, but rarely in those less than ten days old. The disease is characterised by the presence of profuse bloody diarrhoea about five days after infection. On examination the caeca (blind ending sacs) of the large intestine are swollen and filled with a bloody mass; haemorrhagic areas can be seen on the lining. In some cases ‘caecal plugs’ form from the accumulation of blood, pus, oocysts and faeces. Mortality is often high where treatment is not available. Resistance to infection can develop through continued exposure, so outbreaks are less common in older birds (Peter, 1996).

Simon (2007) pointed out that coccidiosis is generally acute in onset and is characterized by depression, ruffled plumage, and diarrhea. Birds infected with *E. tenella* show pallor of the comb and wattles and blood-stained caecal droppings. He mentioned that the Lesions varies in different species in *E. acervulina* and *E. mivati* are 1-2 mm areas of hemorrhage interspersed with white foci visible through the serosa of the distal duodenum and proximal jejunum. Whereas in *E. necatrix* are severe distention of the mid-jejunum with hemorrhages in the mucosa and red-stained fluid in the lumen. Furthermore, in *E. maxima* are distention of the mid-jejunum with hemorrhages in the mucosa. Moreover, in *E. tenella* are hemorrhagic typhlitis. However, in *E. brunette* are hemorrhages of the mucosa of the distal jejunum and colon. Fibrinonecrotic enteritis may occur in chronic cases.

Birds infected with coccidial oocysts do not perform as well as non-infected birds as a result of moderate to severe damage to the intestinal mucosa. Moreover, birds exhibit decreased weight gains, increased feed conversion, and in some cases, birds may appear
asymptomatic, but are limited in their ability to maximize feed efficiency (Edgar, 1992; Henken et al., 1994a; Yun et al., 2000). According to Edgar (1992), it takes only one viable oocyst to establish the presence of coccidia in a poultry house. This is possible because of the parasite’s reproduction index. Ingestion of one Eimeria acervulina oocyst in the infective state has the ability to yield about 72,000 oocysts in one complete life cycle (Henken, 1994).

Eimeria spp. Induce changes in intestinal mucosa, the severity of which is related to parasite density and to the location of parasite within the mucosa; Ali (1989) studied the infectivity of E. mivati in chicken, he cited that the susceptibility to infection increases with age of host and with size of infection dose. In other research revieled that injected sporozoites of E. acervulina, E. maxima and E. mivati in the caeca of checkens produced the infection stages, while E. acervulina and E. maxima failed to produce infection, sporozoites of E. mivati invaded the walls and the life cycle was completed. Moreover, single and mixed infections with E. acervulina, E. maxima and E. mivati indicated that mixed infections produced significantly more severe effects on growth than single infections (Joyner and Norton, 1983).

1.8: Diagnosis of coccidiosis in poulty:

Soulsby (1982) reported that the diagnosis of coccidiosis in chickens is best accomplished by a post mortem examination of a representative number of birds. Moreover, the diagnosis of faecal examination may lead to quite erroneous results. On the other hand in some instances the major pathology is produced before oocysts are shed
in the faeces and, conversely, the presence of large numbers of oocysts may not necessarily indicate a serious pathogenic condition. Thus, with *E. acervulina*, which has a high biotic potential, comparatively larger numbers of oocyst are shed per oocyst given than with *E. necatrix*. Furthermore, the accurate identification of the oocysts of various poultry coccidian is not easy. All of this may be avoided by a post mortem examination. Whereas the location of the major lesion gives a good indication of the species of coccidian concentration. Thus, haemorrhagic lesions in the central part of the small intestine would suggest *E. necatrix*; those in caecum, *E. tenella*; those in rectum, *E. brunette*. The author indicated that it is not sufficient to look for oocysts only, since these may be found with the regularity in the small intestine or caecum of chickens; rather, it is necessary to determine if large number of schizonts are present in the subepithelial tissues for the major pathogens, and in an epithelial position for the other species.

Simon (2007) examined the Lesions of *E. tenella* and *E. brunette* microscopically from intestinal and caecal scrapings reveals oocysts. He cited that to confirm a diagnosis in a commercial operation the following specimens should be submitted to a laboratory: Intestine from a sacrificed, affected bird preserved in 5% potassium dichromate for culture and identification of *Eimeria* spp., intestine showing gross lesions in 10% formalin for histological examination, representative feed samples for anti-coccidial assay and counting oocysts from litter samples.
1.9: Histological and physiological changes:

A closer look at histological and physiological changes that occur during the course of the immune response to coccidial parasites is pertinent to understand the specifics of immunity. Responses to *Eimeria* parasites at the intestinal mucosa may be mediated directly by the parasite or by the host’s immuno-inflammatory response, which may result in changes in the intestinal mucosal morphology (Barker, 1993). Therefore, the author reviewed that physiological changes related to infection have been well documented in experiments with *E. acervulina* (EA) infected chickens. Results indicate that at the level of the intestinal mucosa, local and systemic responses to coccidia are mediated in different ways.

It has been difficult to completely understand these protective mechanisms due to increases in drug-resistant strains and the complexity of the *Eimeria* species life cycle. On the other hand, the past studies have measured the severity of coccidiosis on the basis of weight gains, lesion scores, cessation of total oocyst output, and humoral and cellular responses (Stiff and Bafundo, 1993; Talebi and Mulcahy, 1995). However, a closer look at the histological and physiological changes at the mucosal level is pertinent to understand the specific nature of this immunity.

Fernando and McCraw (1973) measured the intestinal response to a single dose of EA, and infected birds showed a marked increase in total mucosal thickness, decreased villus height, and increased crypt length in the duodenum and to a lesser extent in the anterior jejunum. The mucosal
alterations were the most severe at the height of infection, and a marked increase in the rate of replacement of intestinal epithelial cells was observed. In addition to an increase in duodenal epithelial cell turnover.

Allen (1983) noticed an increase in metabolism of mucosal cells in the lower intestine, which may enhance compensatory growth and help the bird overcome the negative affects of infection on parameters of production. Other morphological changes were evident, such as increased gut length and increased tissue moisture (edema) in the intestine, which were not associated with starvation, but the sole effects of challenge. Moreover, changes in gut environment are also evident from challenge, such as decreased pH of the infected area (Ruff and Wilkins, 1984) and increased feed passage time (Stephens et al., 1974; McKenzie et al., 1987). These changes in gut homeostasis may alter feed intake, which leads to decreased nutrient digestion and absorption, changes in metabolism, and overall decreases in weight gains (Adams et al., 1996).

Interestingly, researchers have contrasting results in experiments where weight gains are measured. Stephens et al. (1974) and Adams et al. (1996) reported that decreased in weight gains associated with EA challenge, while Ogbuokiri and Edgar (1985) and McKenzie et al. (1987) showed no significant weight gain differences when compared to non-challenged controls. This difference in results could be associated with several factors such as genetics of the birds, route of challenge administration, amount of infective oocysts administered for challenge, and strain of EA (Danforth and Augustine, 1989).
Additional studies have shown that initial contact of the sporozoites with the intestinal mucosa produces an inflammatory response including marked cellular infiltration at the site of infection (Rose et al., 1979). This infiltrate consists of multiple leukocyte sub-populations, macrophages, natural killer cells, granulocytes, and lymphocytes that could modulate and enhance immune responses; therefore, altering nutrient absorption and decreasing weight gains (Jeurissen et al., 1996).

1.10.1: Coccidial immunity caused by coccidial infection:

It is well established that B and T-lymphocytes are involved in responses to Eimeria invasion, but there is limited data concerning the possible participation of other effector cells, such as mast cells, which may participate in protective immunity. It is known that mast cell responses contribute to adaptive immunity in mammalian parasitic infections (Rose, 1982; Abraham and Arock, 1998), but their involvement in chickens has been largely overlooked. However, an acute mucosal mastocytophilia response has been reported during secondary Eimeria challenge in chickens (Rose et al., 1980). These responses have been questioned as to if there was an increase in mast cell numbers or if cell migration occurred (Daszak et al., 1993). Mast cells possess distinct attributes that support their role in immune responses, but researchers have been limited in their ability to accurately describe the presence of mast cells during inflammatory responses due to changes in staining properties and morphology associated with tissue location and species.
variation (Yong, 1997). If mast cells are in fact responsible for aiding in protective immunity, it is imperative that they are positively identified. Microscopic differential counting should indicate significantly elevated numbers of mast cells in the small intestine. Until recently, coccidia were classified according to morphological, physiological, and behavioral characteristics such as those described by Brackett and Bliznick (1950).

Today, it is known that different isolates within species of *Eimeria* exist (Chapman, 1982). However, little information is available comparing the immunogenicity and immunovariability of different strains within each *Eimeria* species. Shirley (1985) described new techniques for revealing definitive markers of different strains within the same species of *Eimeria*; however, the differential host response to the different strains was discussed in minute detail. Several authors briefly state events within their experiments in which different strains were noticed (Talebi and Mulcahy, 1995).

It has been shown recently that extracts from the sporozoites of *Eimeria tenella* containing no viable parasites can be used to successfully vaccinate against this infection (Murray, *et al.*, 1986). The effector mechanism(s) of protective immunity induced by live infection or by extract vaccine remains unclear. Although antibodies from various sources demonstrate a variety of anti-parasitic activities, including sporozoite immobilization (Herlich, 1965), sporozoite and merozoite agglutination and lysis (Crane, 1986; Long and Rose, 1972), neutralization (Crane, 1986, Herlich, 1965, Long and Rose, 1972, Rose, 1982), and surface changes (Witlock and Danforth, 1982), the role of
serum antibody in protective immunity is unclear (Joyner and Norton, 1974, Rose, 1982; Rose and Hesketh 1982; Rose and Long, 1971). Recently, emphasis has been directed towards cell-mediated effectors (Joyner and Norton, 1974; Rose, and Hesketh 1979; Rose and Hesketh, 1982) or secretory antibody (immunoglobulin A [IgA]), which has been shown to neutralize sporozoites both in vitro and in vivo (Davis, 1978; Davis and Porter, 1979).

William and William (1968) re-examined acquired immunity to re-infection with *Eimeria tenella*. They were suggested that chickens, immunized by multiple doses of *E. tenella*, develop only a condition of "relative immunity." Immunity, as assessed by clinical signs of the disease, lasted some where between 42 and 63 days after the last immunizing dose. Immunity, where by the host is protected against fatal *E. tenella* infection, moreover, they found to be effective for at least 105 days. Complete immunity, where there is no invasion of host tissue, as assessed by the presence of un sporulated oocysts in the fecal material of challenged immune birds, was not encountered. Although chickens were producing serum lysins 21, 42, 63, 84, and 105 days after the last immunizing dose, they still remained susceptible to challenge inoculations given at that time.

Bumstead and Millard (1987) exposed 3-week old chicks of different breeds and inbred lines of chickens to several *Eimeria* species and measured disease resistance by changes in body weight, mortality and oocyst output. The influence of host strain on the response criteria was clearly demonstrated. Pinard *et al.* (1998) found the Egyptian Fayoumi to be the most resistant of the five outbred lines tested based
upon mortality, lesion scores and growth reduction. Other traits in which variation among populations in resistance to coccidiosis has been expressed include packed cell volume (PCV), and lesion scores (Mathis et al., 1984; Martin et al., 1986; Bumstead and Millard, 1987; Lillehoj and Ruff, 1987). In general, the various measures of response to *Eimeria* did not correlate with one another. Further, host resistance generally depended upon the *Eimeria* species involved (Bumstead and Millard, 1987).

Jeffers (1978) presented data which supported the importance of host strain x coccidial species interactions in the expression of host susceptibility. According to Mathis et al. (1984) PCV was a better measure of disease resistance to *E. tenella* infection than to *E. acervulina* because, compared to the latter, the former causes extensive hemorrhage which substantially depresses PCV.

The relationship between body weight and lesion scores varies among *Eimeria* species. Conway et al. (1990) observed that lesion scores did not fully reflect the degree of disease severity in induced infection. Their results corroborated those of Long et al. (1980), in that weight changes in susceptible broiler birds infected with *E. tenella* did not correspond with those of immunized birds having similar lesion scores. On the other research Long (1973) has suggested that the different results obtained by various investigation concerning the pathogenicity of *Eimeria* might be a result of using experimental animals differing in genetic resistance to coccidiosis.
1.10.2: Immune responses:

*Eimeria* parasites exhibit both extracellular and intracellular stages of life cycles in their infection of the host. The role of the humoral component of the immune system appears to be important during the early phase of the infection and circulating antibodies specific for coccidial parasites are detectable within one week of inoculation with oocysts (Lillehoj, 1991). The level and duration of antibody response depends on host factors such as age and genetics of the host, and the species of *Eimeria* (Lillehoj, 1988). However, bursectomy or transfer of protection by using *Eimeria* immune antibodies show that antibodies are not involved in host resistance to coccidial challenge infection (Rose, 1974; Giambrone *et al*., 1981; Lillehoj, 1987). On the other hand, there is extensive evidence which shows that cell-mediated immune responses are essential in limiting oocyst production in primary and subsequent infections (Wakelin and Rose, 1990; Lillehoj and Trout, 1993; Ovington *et al*., 1995). Generally, antigens of the various *Eimeria* species vary in immunogenecity, with *E. maxima* and *E. brunetti* being the more immunogenic whilst *E. necatrix* and *E. tenella* are the less immunogenic (Ovington *et al*., 1995). The antigenic determinants of the different species are not cross-reactive (Wallach *et al*., 1990). Thus Bumstead and Millard, (1992) noted that chickens from lines which produced the largest numbers of oocysts following infection with *E. tenella* produced the fewest number when infected with *E maxima*, *E. mitis*, or *E. praecox*. Within *Eimeria* species, immunogenecity also varies between stages of development, the asexual stage being more immunogenic than the sexual
stage (Rose and Hesketh, 1976). Even though coccidial merozoite antigens that induced T-cell proliferation were associated with resistance to infection (Jenkins et al., 1998), merozoite fractions that induced lymphocyte proliferation were different from those that induced interferon (IFN-) production, indicating that different antigens favor different cellular responses (Martin et al., 1995). Earlier studies (Prowse and Pallister, 1989) have shown that interferons (IFN) play a role in resistance to primary infection which may be population dependent (Ovington et al., 1995).

*Eimeria* reside outside the host for part of their life cycle but, the majority of it is completed inside the host during asexual and sexual stages of development occurring inside or outside enteric tissues. Once the bird ingests the viable oocyst(s), a cascade of events occurs involving both non-specific and specific defense mechanisms of immunity (Lillehoj and Lillehoj, 2000). It is to be expected that the mechanisms responsible for immunity are complex due to the complexity of the parasite life cycle. Despite all of the research completed on immunity to *Eimeria*, no clear picture has emerged as to how complete resistance is acquired and which mechanisms are sequentially involved in generation of immunity (Rose et al., 1979; Danforth and Augustine, 1989).

In naive chickens, those previously unexposed to *Eimeria*, coccidial infections induce a variety of pathological and immunological responses, which help the host defend against the parasite and acquire protective immunity. However, the level of protection each facet of the immune system provides may vary with the developmental stage of the
parasite (Rose, 1987). Prior to the generation of a specific immune response, the host tries to exclude the *Eimeria* through non-specific immune pathways such as competitive exclusion by normal flora, lysozymes, increased gastric secretions, and peristalsis to quickly flush parasites from the digestive tract (Lillehoj and Lillehoj, 2000; Yun *et al.*, 2000). However, it has been reported that these innate defenses as well as specific immunologically mediated defenses play a role at the intestinal mucosal surface during *Eimeria* invasion (Lillehoj and Trout, 1993). Therefore, the naïve host probably does not eliminate the parasite utilizing only nonspecific pathways, but infection can be controlled to a certain degree prior to the completion of the *Eimeria* life cycle and generation of a specific immune response.

It has been determined that *Eimeria* parasites are vulnerable to the host immune system at three distinct phases of their development: 1) the period between excystation and sporozoite penetration of epithelium, 2) once the sporozoite enters the host epithelium and is exposed to intraepithelial lymphocytes, and 3) during transport of the sporozoite from the surface enterocyte, through the lamina propria and into the crypt epithelium. After these stages in the life cycle, direct interaction between the parasite and cells of the host immune response are unlikely, and the probability of intervention is ceased (Jeurissen *et al.*, 1996).

Stiff and Bafundo (1993) showed the conflicting results in a series of experiments that proved complete immunity can and does exist when birds are continuously challenged on a daily basis with homologous *Eimeria* challenge. Nevertheless, protective immunity hinders fecal
oocyst production, and invasion of the mucosal epithelium is altered. Interestingly, in immune birds, sporozoites penetrate the villus epithelium but are incapable of reaching the crypt epithelium and prevented from further development (Rose et al., 1984; Jeurissen et al., 1996).

1.10.3: Mode of inheritance of coccidiosis in poulty:

Champion (1954) concluded that after a comparison of the survival rate of parental lines (resistant and susceptible), F₁, 1 F₂, and their backcrosses exposed to E. tenella, that sex-2 linkage, maternal effects or cytoplasmic inheritance did not influence susceptibility to E. tenella. His lines which had been selected for only two generations did not show significant evidence of dominance.

In a diallel crossing experiment involving nine lines, heterosis of up to 34% was observed in the survival rate of some individual crosses exposed to E. tenella (Jeffers et al., 1970). Differences in reciprocal crosses were attributed to maternal effects. The importance of maternal effects in resistance to Eimeria spp was confirmed by Buvanendran and Kulasegaram (1972) who tested male progeny of 4 strains crossed reciprocally. According to Smith et al. (1994), maternal antibodies transmitted early immunity against E. maxima which lasted for 2 to 3 weeks.

In two separate experiments in which three different inbred lines were crossed, female cross line chicken had significantly greater survival rates than male chicks (Jeffers et al., 1970) suggesting involvement of the W-chromosome of the dam lines in genetic resistance to E. tenella.
1.11: Isolation of *Eimeria* spp.:

Tomley (1997) worked on the isolation of *E. tenella* from the apical organelles; which contain molecules that are of critical importance for the interaction of all apicomplexan parasites with their target host cells. Thus, there is considerable interest in characterizing and understanding the function of molecules that reside in these organelles. Large numbers of surface-sterilized oocysts of *Eimeria tenella*, an apicomplexan coccidian of the chicken, can be routinely obtained from the bird host, and invasive sporozoites, which contain abundant apical organelles, can be rapidly prepared from these oocysts in the laboratory. Thus, *E. tenella* is proving to be an amenable parasite for sub-cellular fractionation techniques that allow the direct isolation and characterization of apical organelles.

1.12: Control and vaccination:

David (2007) mentioned that management of the coccidiosis disease requires the adoption of sanitary and hygienic procedures to reduce the level of exposure to infection. Sporulation is favored by moisture. Thus, it is important to maintain dry litter, especially around drinkers and feeding areas. Maintaining dry litter will reduce oocyst numbers and the likelihood that birds will be exposed to parasite numbers that will cause clinical coccidiosis.

It has been shown that different strains are capable of having antigenic diversity, so that the immunity conferred by one species strain does not completely protect chickens against further challenge with a different strain of the same species (Talebi and Mulcahy, 1995).
reviews on the use of coccidial vaccines by Danforth and Augustine (1989) they were stated that isolates from different geographical areas do not always show cross-protection following oral immunization. This may be a challenging factor in producing specific vaccines for coccidial control. Additional studies need to be conducted to evaluate the variability in the host’s protective immune response to different strains. This will expand opportunities for the development of alternative control measures; however, it is possible that the coccidia may find new ways to alter their antigenicity.

Historically, coccidial parasites have been controlled through the use of in-feed coccidiostats. However, through the years, drug-resistant strains of *Eimeria* have emerged, which hinder the efficacy of the presently used coccidiostats. Coccidial infections induce a variety of pathological and immunological responses which help the host defend against the parasite and acquire protective immunity. In addition, it has been reported that both immunologically mediated and non-immunological defenses play a role at the intestinal mucosal surface during *Eimeria* invasion (Lillehoj and Trout, 1993).

Watkins et al. (1995) attempted to vaccinate broiler chickens against *Eimeria maxima* by in ovo injection of live oocysts or sporocysts at 17 to 18 days of embryo incubation. Although they found evidence of infection in the newly hatched chicks, these birds showed no protective immunity when challenged at 10 days of age. Furthermore, Provaznikova and Bedrnik (1997) reported successful immunization of broilers with an egg-adapted strain of *E. tenella* by in ovo administration of sporozoites,
although this method was unsuccessful for other species of coccidia tested.

Sousby (1982) cited that the preventive medication of coccidiosis consists of prolonged or continuous use of coccisostatic compounds in the water or feed. These drugs include amprolium, buquinolate, decoquinate, clopidol, momensin, robeniine, zoalene, nicarbazin, furazolidone, nitrofurzone, sulphamethoxazole, methylene blue and salinomycin at the concentrations 0.0125, 0.00055, 0.003, 0.0125, 0.0121, 0.003-0.006, 0.0125, 0.0125, 0.0055, 0.005-0.01, 0.0125, 0.001-0.002, 0.005-0.0075 and 0.006-0.01%, respectively.

David (2007) mentioned that the control of coccidiosis has been achieved by the use of drugs that kill the parasite before it can develop in the chicken. In the 1950’s and occasionally today, drugs were often included in the drinking water to treat sick birds. Unfortunately the onset of coccidiosis is rapid and the signs of disease (such as huddling, ruffled feathers, off feed) are seen with many other poultry diseases. The researcher concluded that the treatment often came too late to prevent serious production losses. A preventative approach is therefore desirable and this is achieved by incorporation of drugs in the starter and grower feeds. The most widely used drugs are known as ionophores. These compounds inhibit the development of the parasite but, do not prevent the acquisition of natural immunity by the bird so that they can be withdrawn from the feed well before the birds are sold.

Williams (2002) studied the establishment and use of live vaccines, either attenuated or non-attenuated, for the control of coccidiosis due to *Eimeria* infections in broiler breeder or layer chickens.
Use in broilers, however, has been slow to gain acceptance. Furthermore, this has been partly for economic reasons, but also because of perceived adverse effects on early chick growth, particularly with non-attenuated vaccines, and concerns about timely onset of protective immunity in such short-lived

David (2007) reported that an entirely different approach to the control of coccidiosis involves the use of vaccines. Which birds are administered small numbers of sporulated oocysts have long been available in the USA. On the other hand in the past vaccines have principally been employed during the rearing phase of egg-laying birds. The author mentioned that the introduction of novel methods of administration (such as with a spray-cabinet in the hatchery) has made vaccination of broilers more feasible. Researchers are actively seeking better means to safely immunize birds against coccidiosis and although there are many technical hurdles to overcome, progress is being made and new vaccines seem likely in the future.

Currently, drugs and live vaccines are the 2 main control measures for the disease; however, due to increasing problems with prolonged drug usage and the high cost of vaccines, alternative strategies are needed for more effective and safer control of coccidiosis in chickens (Dalloul and Lillehoj, 2006; Williams, 2006).

Recent evidence that various dietary and microbial supplements can influence host immunity against enteric diseases prompted us to investigate the role of a commercial probiotic (MitoGrow, Imagilin Technology LLC) on coccidiosis. This probiotic consists of live
Pediococcus acidilactici, which belongs to the homofermentative gram-positive bacteria, able to grow in a wide range of pH, temperatures, and osmotic pressures, and thus able to colonize and inhabit the digestive tract (Klaenhammer, 1993).

1.13: Coccidiostat and growth promoters:

It is controlled by chemotherapy using anticoccidial drugs (synthetic products or antibiotic ionophores) in feed. The appearance of resistance to coccidiostats, consumer demand for fewer feed additives, and European Union regulations (withdrawal of antibiotic feed additives as a precautionary measure) might restrict the use of coccidiostats in the future. If this happens, methods of production, management, and hygiene will be changed. Therefore in the absence of anticoccidial vaccines that are not yet generally available in broiler production or to complement their action, feed composition, or presentation may be used as an alternative to help control coccidiosis. Several dietary supplements such as vitamins, n-3 fatty acids, and plant extracts have been reported to have beneficial effects (Allen et al., 1998; Banfield and Forbes, 1999; Créveu-Gabriel and Naciri, 2001).

Elwinger et al. (1998) mentioned the removal of antibiotic growth promoters is anticipated to result in a significant increase in the incidence of necrotic enteritis due to their direct effect on control of the causative organism, Clostridium perfringens. The disease is most often established after an incidence of coccidiosis. Moreover, the researchers observed Eimeria species most commonly associated with necrotic enteritis seem to be E. acervulina, E. maxima and to some extent E. necatrix. Finn feeds
own necrotic enteritis model simply challenges the birds with E. maxima and necrotic enteritis spontaneously develops.

It is expected that in the near future the coccidiostatics currently used in animal feeds will be banned. Thus there is a need for alternative agent to control coccidiosis in poultry (Iji et al., 2001). Coccidiostats may improve gut health as indicated by increased villi height, uniformity and integrity (Loddi et al., 2002) and they modulate gut and systemic immunity (Ferket et al., 2002).

Riddell and Kong (1992) reported that under normal circumstances, nitrogen is well digested, little enters the caeca and necrotic enteritis is uncommon. However, when viscous diets are fed or coccidiosis increases small intestinal damage, excessive quantities of nitrogen escape digestion and enters the caeca resulting in a more frequent occurrence of necrotic enteritis. Moreover, sloughed cells from damaged villi will provide nitrogen to the caeca in addition to the undigested feed. It is thus evident that E. tenella may not so readily precipitate necrotic enteritis since, although it will provide damaged caecal enterocyte nitrogen to the caeca, it does not affect digestion in the small intestine. Reducing the damage that coccidiosis causes to the small intestine is essential if nitrogen digestibility is to be maintained and necrotic enteritis minimized.

Betaine improves the osmotic condition of the enterocyte (Allen et al. 1998), and when fed with a coccidiostat it appears to reduce the incidence and severity of coccidial lesions thus improving animal performance.

Milan and Janet (2005) revealed that betaine per se had no direct effect on lesion score in isolation. In the presence of a coccidiostat,
however, it is apparent that improving the osmotic status of the gut epithelium through addition of betaine results in further reductions in lesion scores. In other reported studies, betaine plus salinomycin significantly inhibit invasion of both *E. tenella* and *E. acervulina*. However, subsequent development of *E. acervulina* is inhibited more effectively than that of *E. tenella*. (Augustine *et al.*, 1997). Recent work (Waldenstedt *et al.*, 1999) showed no interaction between narasin and betaine in birds which had been inoculated principally with *E. tenella* and *E. acervulina*.

Even before 1907 fundamental discoveries on drug resistance in trypanosomes, it was known that bacteria and protozoa could be adapted to grow and survive in normally toxic concentrations of chemicals by repeatedly exposing the organisms to sub-lethal and increasing concentrations of compounds. Moreover, drug resistant did not become an important practice problem, however, until the development and widespread use of sulfonamide and antibiotics. Furthermore, Sulfaquinoxaline has been in continuous and satisfactory use since 1947 for controlling coccidiosis in a typical New England poultry farm and there was no evidence that drug resistance has developed in the coccidian. Nevertheless, early in 1951, the laboratory studies were began and determine whether resistance could be induced in coccidian by repeated exposure to suboptimal dosages of anticoccidial agents. A short time later, studies were also begun on field strains coccidian. These were obtained from poultry farms where due to intercurrent diseases and other difficulties anticoccidial medication appeared less effective than normally. A strain of caecal coccidian obtained from poultry houses in
which nitrofurazone have been used for three years was fully susceptible to this drug. On the other hand, the caecal coccidia from an area where sulfaquinoxaline had been employed for about for years were fully susceptible to this anti-coccidial agents. It was concluded that there was no evidence of drug fastness in these coccidia (Ashton and Christine, 1955).

Removal of antibiotic growth promoters is likely to increase variability in broiler performance. When unrestrained intestinal microfloral populations are combined with undigested feed substrates, their numbers will increase substantially. The poorer the quality (digestibility) of the feed and the greater the microbial challenge (environment) the greater this problem will be (Waldenstedt et al., 1999).

1.14: Mode of action of experimental medications:

Williams (1997) investigated the anti-coccidial mode of action of quinolones (6-decyloxy-4-hydroxyquinoline-3-carboxylates) against *Eimeria tenella* and *E. acervulina* in chickens. Lower concentrations of quinolones allowed sporozoites to continue their development. Whereas first-stage schizonts were susceptible to a secondary cidal effect, although later schizonts seemed to be rather refractory. Furthermore, the sporulation of oocysts produced by *E. tenella* that completed its life cycle in the presence of suboptimal concentrations of quinolones was inhibited: this probably reflects a drug effect on gametocytes. The researcher reported that quinolones were absorbed rapidly from the chicken intestine, probably in less than 1 hour, whereas the drug withdrawal
experiments showed that quinolones persisted in chicken tissues at active concentrations for up to 48 hour despite their static effect on sporozoites, they may nevertheless be expected to exert a therapeutic effect against drug-sensitive coccidia in interrupted régimes that allow the later cidal effect to come into play. This allows immunity to coccidiosis to develop in the presence of drug.

Artesunate is a semisynthetic derivative of artemisinin, the active principle of *Artemisia annua* L. ART and other artemisinin derivatives are promising novel drugs in the treatment of malaria (Price, 2000). They are recommended by the World Health Organization as salvage treatment options for otherwise unresponsive *Plasmodium falciparum* and *Plasmodium vivax* strains. Large clinical studies with malaria patients have shown that ART is well tolerated, with few and insignificant side effects (*Hien, et al.*, 1992). In addition to the well known antimalarial activity of ART, Efferth, *et al.*, (2001) identified a profound cytotoxic action of ART against cancer cell lines of different tumor types.

There is no consensus regarding the mechanism through which artemisinin derivatives kill the parasites (Kappe, *et al.* 2009). Their site of action within the parasite also remains controversial. At the chemical level, one theory states that when the parasite that causes malaria infects a red blood cell, it consumes hemoglobin within its digestive vacuole, liberating free heme, an iron-porphyrin complex. The iron reduces the peroxide bond in artemisinin generating high-valent iron-oxo species, resulting in a cascade of reactions that produce reactive oxygen radicals which damage the parasite leading to its death (Cumming, *et al.* 1997).
Numerous studies have investigated the type of damage that oxygen radicals may induce. For example, Pandey et al. (1999) observed inhibition of digestive vacuole cysteine protease activity of malarial parasite by artemisinin. These observations were supported by ex vivo experiments showing accumulation of hemoglobin in the parasites treated with artemisinin and inhibition of hemozoin formation by malaria parasites.

Electron microscopic evidence linking artemisinin action to the parasite's digestive vacuole has been obtained showing that the digestive vacuole membrane suffers damage soon after parasites are exposed to artemisinin (Del, et al. 2008). This would also be consistent with data showing that the digestive vacuole is already established by the mid-ring stage of the parasite's blood cycle, a stage that is sensitive to artemisinins but not other antimalarials (Abu-Bakar, et al., 2010).

Li et al. (2005) investigated the mode of action of artemisinin using a yeast model demonstrated that the drug acts on the electron transport chain, generates local reactive oxygen species, and causes the depolarization of the mitochondrial membrane.

Sulfadoxine or sulphadoxine is an ultra-long-lasting sulfonamide often used in combination with pyrimethamine to treat or prevent malaria. It is also used, usually in combination (fansidar is US brand name) with other drugs, to treat or prevent various infections in livestock. Both drugs are antifolates; they inhibit the production of enzymes involved in the synthesis of folic acid within the parasites. Either drug by
itself is only moderately effective in treating malaria, because the parasite Plasmodium falciparum may be able to use exogenous folic acid, i.e. folic acid which is present in the parasite's environment, while in combination, the two substances have a synergistic effect which outbalances that ability (Toby, et al., 2007).

Sulphadoxine and pyrimethamine combination is an antimalarial agent which acts by reciprocal potentiation of its two components, achieved by a sequential blockade of two enzymes involved in the biosynthesis of folinic acid within the parasites. Moreover, Sulphadoxine, like other sulphonamides, is a structural analog of p-aminobenzoic acid (PABA) and competitively inhibits dihydrofolic acid synthesis by inhibiting dihydropteroate synthetase, which is necessary for the conversion of PABA to folic acid. On other hand pyrimethamine is a folic acid antagonist and has a mechanism of action similar to that of trimethoprim. By binding to and reversibly inhibiting dihydrofolate reductase, pyrimethamine inhibits the reduction of dihydrofolic acid to tetrahydrofolic acid (folinic acid). Pyrimethamine interferes with the synthesis of tetrahydrofolic acid in malarial parasites at a point immediately succeeding that where sulphonamides act (Jeffrey, et al., 1984).

1.15.1: Treatment:

Birds medicated with roxarsone and in another experiment with zoalene in the feed produced higher oocyst counts than unmedicated control birds receiving the same oocyst dose of *Eimeria tenella* or a
mixture of six species (*E. tenella, E. necatrix, E. brunetti, E. maxima, E. acervulina, and E. mivati*). These experiments confirm the conclusion that oocyst counts constitute an unsatisfactory and unreliable parameter for judging effectiveness of an anticoccidial even though such increases are a relatively rare occurrence in anticoccidial evaluation experiments (Malcolm, 1975).

A wide variety of drugs is available for the treatment of coccidiosis. Sulphonamides are still the most frequently used drugs, singly or in combination with other drugs such as amprolium and the pyrimidines. Frequent use of drugs has led to widespread drug resistance (Peter, 1996).

Jeffers (1974) found that the incidence of *E. tenella* was high in flocks medicated with Nidrafur or Zoamix, and much lower in flocks medicated with Amprol Plus, Bonaid, Coyden, or Deccox. Tests of 201 of the *E. tenella* isolated showed that their drug sensitivity had been reduced by field exposure to the anti-coccidials. Moreover, reciprocal cross-resistance to Bonaid and Deccox was high among the quinoline-resistant isolants. He concluded that the distribution of multiple drug-resistance phenotypes among the *E. tenella* isolants emphasized a complete drug-resistance profile of strains of coccidia is necessary for determining the anticoccidial drug(s) that would control them most effectively.

Simon (2007) recommended that the administration of amprolium solution or sulfonamides (sulfamethazine or sulfaquinoxaline) in drinking
water. In addition to the administration of water dispersible vitamin A and K supplements may enhance recovery of chicken from coccidiosis.

Du and hu (2004) studied a liquid and a 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 their anticoccidial activities in chickens. They were administered Chickens with herbal liquid, powder, diclazuril or without medication during the study and challenged with oocysts of *Eimeria tenella*. Their results indicated that the birds medicated showed less bloody faeces than those without medication. Moreover, the intestinal lesion was mild in the chicks medicated with herbal liquid without significantly different lesion score when compared with uninfected chicks. The birds with medication had significantly higher body weight gains than birds without medication. Therefore, they were concluded that the herbal complex was effective against *E. tenella* infection in chickens.

Reid (1975) has reviewed that the progress in the control of coccidiosis with anticoccidial drugs. He estimates that new compounds continue to be introduced to the poultry on an average of about one every two years. In general, mortality due to coccidiosis can be eliminated by any of the 25 or more approved anti-coccidial drugs if they used properly. Thus, anti-coccidial drugs are used usually in starter rations for meat-type birds raised under floor-open management. Whereas protection is more important with these fast-growing birds than the egg-production types, where immunity and caging alter the demand for anti-coccidial drugs. However, selection for of an anti-coccidial is based on
the ability of the drug to improve weight and feed conversion and to suppress the development of lesions.

The methods used to avoid the development of drug resistant include switching around 13 classes of drugs and the shuttle program, which is a planned switch of the drug in the middle of the growing period of the birds (Reid, 1975). The speed of emergence of resistant strains of coccidian in the field is given by Reid (1975) which were glycomide - very rapid-; quinolines (buquinolate, deconquinate, nequinate) –rapid; clopidol –less rapid; sulphonamides, nitrofurans, robenidine –moderate; amprolium, zoalene, nitromide –slow; nicarbazin –very slow; monensin – absent or very slow.

Sousby (1982) pointed out that the anti-coccidial drugs dealt with various regulations apply to the pre-market withdrawal of the drug, although some compounds are classed as no-withdrawal which is often substituted for a withdrawal anti-coccidial during the final days before marketing.

Sousby (1982) mentioned that the curative treatment should be instituted immediately after a diagnosis of coccidiosis is made. Furthermore, an interrupted form of treatment is more satisfactory with the sulpha drugs than continuous treatment, which aims to avoid undue concentrationsof the compounds which inhibit the earlier developmental stages of the parasite and thus interfere with the acquisition of immunity. To avoid this, Daivies and kendell (1954 a,b) suggested given sodium sulphadimidine, sodium sulphaquinoxaline and nitrofurazone wih furazolidone at concerations 0.2 m 0.5 and 0.0126%, respectively.
In some cases, birds are given one drug, but two or more drugs (so-called shuttle programs) may be given during the life of a flock. It is convenient to provide a particular compound for a period during which one type of feed is given (Chapman1, 2001).

1.15.2: Anti-malarial Medications used in the Treatment of Coccidiosis:

The well known ant-malarial agents, pamaquine (8-(4-diethylamino-1-methylbutylamino)-6-methoxyquinoline) was synthesized by the modification of the dye methylene blue about 60 years ago and was first synthetic drug applied to the treatment of malaria (Schuhlemann, 1932). Attempts to lessen the hemolytic adverse effect pamaquine result in the synthesis of primaquine, the safer and more potent 8-aminoquinoline compound (8-(4-amino-1-methylbutylamino)-6-methoxyquinoline) (Edgcomb, et al., 1950). This drug had been used until the 1960's when the resistance of the parasite to the drug developed frequently (Thompson and Werbel, 1972).

Pamaquine and primaquine are used in the field of veterinary medicine for treatment of bovine prioplasmosis. Further, primaquine's activity against Sarcocystis muris was found recently (Rommel, et al., 1981).

Toshimi et al. (1991): investigated the activity of anti-malarial drugs pamaquine and primaquine against a laboratory strain of E. tenella, E. necatrix, E. acervulina, E. maxima and E. brunetti revealed that both drugs were effective against E. tenella and E. necatrixbut not against an other three species. Whereas pamaquine suppressed the symptoms of E.
*tenella* at the concentration above 125 ppm in feed. Moreover, primaquine controlled the clinical signs at level above 31.5 ppm. Furthermore, the activity against *E. necatrix* was observed with pamaquine at 250 ppm and with primaquine at level above 125 ppm. On the other hand pamaquine showed tendency apparently to reduce body weight gain at 125-500ppm, whereas primaquine showed the same tendency at 500 ppm.

**1.15.3: Biotechnologies of experimental medications:**

Artesunate is an antimalarial agent. It is a water-soluble hemisuccinate derivative of dihydroartemisinin. Artemisinin is a sesquiterpene lactone isolated from Artemisia annua, a herb that has traditionally been used in China for the treatment of malaria. Artesunate and its active metabolite dihydroartemisinin are potent blood schizonticides, active against the ring stage of the parasite. Artesunate is ideal for the treatment of severe malaria, including cerebral malaria. It is also active against chloroquine and mefloquine resistant strains of *P. falciparum*. It is unstable in neutral solution and is therefore only available for injections as artesunic acid. The injectable formulation must be prepared immediately before use in 5% (w/v) sodium bicarbonate solution to produce the salt sodium artesunate (Taylor *et al.*, 2000).

Artemisinin also known as qinghaosu, and its derivatives are a group of drugs that possess the most rapid action of all current drugs against falciparum malaria (White, 1997). Treatments containing an artemisinin derivative (artemisinin-combination therapies, ACTs) are now standard treatment worldwide for falciparum malaria. The starting compound, artemisinin (a sesquiterpene lactone), is isolated from the
plant Artemisia annua, a herb described in Chinese traditional medicine, though it is usually chemically modified and combined with other medications. Moreover, use of the drug by itself as a monotherapy is explicitly discouraged by the World Health Organization as there have been signs that malarial parasites are developing resistance to the drug. Combination therapies that include artemisinin are the preferred treatment for malaria and are both effective and well tolerated in patients. The drug is also increasingly being used in vivax malaria (Douglas et al., 2010) as well as being a topic of research in cancer treatment (Douglas et al., 2010).

Artemisinin was isolated by Chinese scientists in 1972 from Artemisia annua (sweet wormwood), better known to Chinese herbalists for more than 2000 years as Qinghao. In the early 1970s, initial testing by Chinese scientists of Qinghao extracts in mice infected with malaria showed it to be as effective as chloroquine and quinine in clearing the parasite. Mao Tse Tung’s scientists then began testing in humans and in 1979 published their findings in the Chinese Medical Journal. Artemisinin and other artemether-group drugs are currently the main line of defense against drug resistant malaria in many parts of South-East Asia. To date there have been no reported cases of resistant to artemisinin. Artemisinin is today a very potent and effective antimalarial drug, especially when used in combination with other malaria medicines. Its available in tablets, capsules, injection (Taylor et al., 2000).
Fansidar is an antimalarial agent, each tablet containing 500 mg N1-(5,6-dimethoxy-4-pyrimidinyl) sulfanilamide (sulfadoxine) and 25 mg 2,4-diamino-5-(p-chlorophenyl)-6-ethylpyrimidine (pyrimethamine). Each tablet also contains cornstarch, gelatin, lactose, magnesium stearate and talc. It is only used in certain circumstances because resistance to this treatment is high.

Pyrimethamine or fansidar is used in the treatment of uncomplicated malaria. It is particularly useful in cases of chloroquine-resistant P. falciparum strains when combined with sulfadoxine. It acts by inhibiting dihydrofolate reductase in the parasite thus preventing the biosynthesis of purines and pyrimidines, thereby halting the processes of DNA synthesis, cell division and reproduction. It acts primarily on the schizonts during the erythrocytic phase, and nowadays is only used in concert with a sulfonamide (Baird et al., 2003).

Primaquine (or primaquine phosphate) is a medication used in the treatment of malaria and Pneumocystis pneumonia. It is a member of the 8-aminoquinoline group of drugs that includes tafenoquine and pamaquine (Mihaly et al., 1985). Moreover, Primaquine is mainly used to treat the P. vivax or P. ovale malaria. Once the parasite has been eliminated from the bloodstream, the remaining hypnozoites must be removed from the liver and this is done by administering a 14 day course of primaquine (Baird et al., 2003). This process is called a radical cure. If primaquine is not administered to patients with proven P. vivax or P. ovale infection, there is a very high likelihood of relapse within weeks or
months (sometimes years). When attempting a radical cure, primaquine requires the presence of quinine or chloroquine in order to work (Alving et al., 1955). If primaquine is given alone, the cure rate is only 21%. It is not known if other antimalarials such as mefloquine are likewise able to potentiate the effect of primaquine. Primaquine is not routinely used to prevent malaria in travelers, and is only used as such when no other alternatives are appropriate (Hill et al., 2006).

Primaquine is the recommended prophylactic agent by the Centers for Disease Control and Prevention (CDC) in some instances. Moreover, primaquine is an antimalarial drug. The exact way that primaquine works is unknown. Primaquine is used to treat and prevent malaria (Centers for Disease Control and Prevention, 2005).
CHAPTER TWO

MATERIALS AND METHODS

2.1. Collection of *E. tenella* samples:

A total of 1500 caecal samples were collected on ice from chickens Bovans in Khartoum State.

2.2. Experimental infection:

Three hundred one-day-old male Hisex brought from Koral Farms were reared in Shambat in pen of Faculty of Veterinary Medicine until the age of three weeks, then placed in fourteen different pens which were classified into four groups: infected with treatment (treated group), infected without treatment (infected control), not infected not treated birds (naive) and not infected treated. Whereas, the first two groups was orally challenged with artificial infection *E. tenella* sporulated oocysts doses. The first two groups was have been given \(1 \times 10^4\) sporulated oocysts of *E. tenella*. Therefore each bird was inculated by stomic tube on chickens crops.

2.3. Experimental treatment and administered doses:

The starter feed with non anti-coccidial additives was offer then medications were mixed with water for the four treatments whiched the treatment by primaquine had three levels. The experiment started after the third day of infection i.e. 24 days-old. The birds were thirest a half of
a day then water mixed with medications were offered. The exposure to the sun light was avoided in order to ensure the constituents conservation of the drugs under test and feed ingredients.

The treated experimental birds were separated into seven groups according to the type of medications primaquine, artesunate and fancedar (anti-malarial drugs). Whereas, amprolium acted as anti-coccidial medication, level of the drugs 31.2, 125 and 250 ppm for primaquine group, whereas artesunate 33 mg and fancedar 86.625 mg. The anti-coccidial drug amprolium have got one level 60 grams per 100 liters water which was recommended on database. The subgroup was consisted of twenty birds for each.

2.4. Experimental parameters and data collection:

Parameters studied included body weight gain, the cumulative feed intake, the feed conversion ratio, the survival rate, the lesion score, bloody diarrhoea proportion, diseased and survival chicks, survival time, clinical symptoms, causes of mortality. (No. of died birds divided by No. of total birds all multiplied by 100), and the oocyst per gram (OPG). liver function tests which include serum calcium sodium, potassium, iron, total bilirubin (TBIL), total protein, albumin, alkaline phosphorus, alkaline phosphatase alanine transaminase (ALT) and aspartate transaminase (AST) were investigated of the control uninfected treated with primaquine 250 ppm group after exerted a toxicity symptoms a long the 4 days.

The body weight had been taken twice a week, whereas the ration and dropping weights were collected from steel less trays which was take
three times at the whole of adaptation and examination periods to compare the growth performance between treated and control groups, and within groups. The manure samples were examined the percent of bloody diarrhoea from whole dropping and oocysts count and estimated.

The investigation of the disease causative agents was done by parasites identification. The parasites were identify to levels based on gross lesions in postmortem findings and précised by laboratory examination.

2.5. Laboratory investigation:

The laboratory work was limited to calculate oosyst per gram (OPG) the causes of mortality only and blood parameters and liver functions. The entire manure output of each group that was collected at the first week after artificial infection. Faeces were mixed with an amount of water equivalent to approximately six times the volume of faeces and homogenized using a hand-held blender. A single 1-ml sample of each suspension was mix with 9 ml of 30% (wt/vol) NaNO₃. After being mixed, samples were load into duplicate Mc Masters counting chambers, and the average number of oocysts was determine. The number of oocysts per gram of feces within each pen was estimated. Litter samples had been taken by collecting a handful of litter from each corner and the center of each tray during experiment period. The combined samples were mixed thoroughly and a 10 g sample was mix with 100 ml of tap water and allowed to stand overnight. Then the samples were homogenized in a mechanical blender and the homogenate filtered through cheesecloth. A 15-ml aliquot of the filtrate was
centrifuge and resuspended in 15 ml of 30% (wt/vol) NaNO3. Therefore, the sample was load into duplicate Mc Masters chambers and the oocysts were counted (Herve, 2005).

2.5 1. Faecal examination:

For detection of coccidian oocysts individual fecal samples were floateted in saturated NaCl solution in test tube covered with cover slip for 10 minutes (simple flotation technique) then examined under 10X objective of the microscope.

2.5.1.1. Oocysts count:

Positive samples were used for determination of number of oocysts per gram (opg) using modified McMaster technique (Anon, 1977) as follows: three grams of faeces were mixed with 42 ml of water using a pestle and mortar to make up the suspension, which was strained through 80µ / square sieve to remove debris and the filtrate was collected in clean dry bowl. 15ml of this filtrate were taken into centrifuge tube, centrifuged for 2 minutes at 110x g and the supernatant was then discarded. The sediment was emulsified by gentile agitation and saturated NaCl solution was added until the volume become equal to the initial aliquot of the filtrate. Then the centrifuge tube was inverted several times until the sediment was evenly suspended. The two chambers of Mc master slide were filled using clean pasture pipette. The slide was then left for two minutes to allow the oocysts to float and it was then examined under the low power (10X) of the microscope.
average number of oocysts percent in the two chambers was multiplied by 100 to obtain the oocyst per grams (OPG).

2.5.1.2. Oocysts sporulation:

Either two or three grams of the faecal material was thoroughly mixed with tap water and passed through 100, 80 and 63 mesh screens. The filtrate was transferred into cylinder and allowed to stand overnight. The supernatant fluid was discarded and the sediment was divided into centrifuge tubes and centrifuge at 110 x for two minutes and finally the sediment was suspended in shallow layer of 2.5% potassium dichromate in Petri dishes and was left to sporulation was completed (25-27C) under aeration until sporulation was completed (Osman et al., 1990).

2.5.1.3. Oocyst identification:

The identification of sporulated oocyst was based on oocyst morphology, measurements, location and sporulation time (Goodrich, 1944).

2.5.1.3.1. Oocyst morphology:

Morphological characteristics of the oocysts undertaken included the oocysts shape (ellipsoidal, spherical or ovoidal) and the presence or absence of micropaylar caps (Anon, 1977). In addition to that photographs of oocyst previously documented by (Levine, 1973), were considered as aid in identification of oocyst.
2.5.1.3.2. Measurements of oocysts:

Measurements of *Eimeria* spp. were performed in 50-100 oocyst of each species. Length and width of oocysts were measured using calibrated an eye piece micro-meter using (Olympus, Japan) microscope under objective lens 40X. The measurements were done as described by Levine, 1973 and Anon, 1977.

2.5.1.3.3. Location of infection:

The location of the parasite within the intestine either upper, mid or lower portion was determined.

2.5.1.3.4 Sporulation time:

Every 24 hours the faecal sample in potassium dichromate were examined for detections of sporulated oocyst by placing drop of faecal material in a microscope slide and examined under the low power (10X). The progress was reported until about 90% of the oocysts under the microscopic field were fully sporulated.

2.6. Collection of blood samples:

Blood samples for whole blood and serum were withdrawal from heart before and after infection using vacutainer system (Becton-Dickinson, France).

Wherever blood samples were taken they were divided in two portions; one portion was taken into plain vacutainer tubes and was allowed to clot prior to serum collection while the other one was taken in tubes containing ethylene diamine tetra acetic acid (EDTA)
2.7. Haematological methods:

Red Blood Corpuscles (RBC), White Blood Cells (WBC), Differential WBC Count, Determination of Haemoglobin (HB) Concentration, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) were counted using the digitalized method of haematology systems or coagulation systems (sysmex).

2.8. Liver function tests:

Total serum protein, albumin, phosphorus, calcium, sodium, iron and potassium, Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Total bilirubin (TBIL) were analyzed by sysmex apparatus using the coagulation systems.

2.9. Statistical analysis:

The experimental parameters from each group were subject to analysis of variance using the protected least significant difference (LSD) method to determine the effects of treatments and the variation between means of the same group. A probability of P < 0.05 was considered significant.

The experiment was design accordance to block complete randomize design. Means, standard errored all parameters were computed.

The statistical analysis was carried out using SPSS program version 13.
CHAPTER THREE

RESULTS

Feeding ability exerted two types of birds ability to get feed, the first one was recorded a significantly (p<0.05) higher ability in control infected not treated, treated amprolium, treated primaquine 250, 31.5 and 125ppm, treated artesunate, treated fancedar and not infected not treated (2.3±1.4, 1.8±1.3, 1.7±1.3, 1.5±1.3, 1.7±1.3, 1.7±1.3 and 1.7±1.3), which represented medium ability score (4-0). While the second type secured feed forbidden appeared in not infected treated by amprolium, not infected treated by primaquine 250, 31.5 and 125 ppm, not infected treated by artesunate and not infected treated by fancedar. Moreover, the same pattern was recorded for huddle to keep worm score, where the control infected not treated, treated amprolium, treated primaquine 250, 31.5 and 125ppm, treated artesunate and treated fancedar showed a higher significant (p<0.05) medium values (2.2±1.3, 1.8±1.3, 1.7±1.3, 1.6±1.3, 1.6±1.3, 2.3±1.3 and 1.6±1.3). whereas feed forbidden appeared in not infected treated by amprolium, not infected treated by primaquine 250 31.5 and 125 ppm, not infected treated by artesunate and not infected treated by fancedar recorded the lower score (0).

3.1 Effects of Anti-malarial and Anti-coccidial drugs on infection symptoms:

Number of survival birds during the experiment was exerted in table 3.1, which a significantly (p<0.05) higher survived birds number were
secured in treated amprolium, treated primaquine 31.2ppm, treated artesunate, not infected not treated, not infected treated by amprolium, not infected treated by primaquine 31.2ppm, not infected treated by fancedar and not infected treated by artesunate which get 20 birds, while treated fancedar recorded 19.6 birds. whereas the lower numbers were maintained in control infected not treated, treated primaquine 250 ppm and not infected treated by primaquine 250 ppm, which were get 11.2±1.3, 9.2±1.3 and 11.6±1.3, respectively.

The longest Survival time after treatment were recorded in all group except control infected not treated and not infected treated by primaquine 250 ppm were showed (9±1.334 8.1±1.33, 9±1.12, 9±1.22, 9±0.98, 9±1.45, 9±1.36, 9±1.23, 8.53±1.41, 9±1.52, 8.42±1.11 and 9±0.99 days) significantly (P<0.05) longer than that obtained in control infected not treated and not infected treated by primaquine 250, which were exerted 4±1.334 and 7.22±1.34 days, respectively.

Diseased survival birds number that bird exerted coccidiosis symptoms were secured a significantly (p<0.05) higher number in control infected not treated (8.5±1.5 birds/20 birds in ben), while not infected not treated, not infected treated by amprolium, not infected treated by primaquine 250, 31.2 and 125 ppm, not infected treated by
artesunate and not infected treated by fancedar didn't recorded a survival bird.

Feed intake conception was recorded a higher intake in not infected not treated, not infected treated by amprolium, not infected treated by primaquine 31.2ppm, not infected treated by artesunate and not infected treated by fancedar (1000±0.9, 1000±160, 1000±200, 978±450 and 979±150 grams), compare with treated primaquine 250ppm, which secured the lowest intake (425±91 grams).

Body weight demonstrated a significantly (p<0.05) heavier weight in treated primaquine 125ppm (153±20 grams), compare with control infected not treated, which recorded a lighter weight (78.5±20 grams).

The data in the table 3.1 describes the impact of experimental medications on mortality rate during experiment. The data clearly indicated that mortality rate was decreased by convert of experiment to investigate toxicity. The percentage mortality rate in control infected not treated, treated primaquine 250ppm and not infected treated by primaquine 250 ppm were 2.0±1.1, 2.0±1.3 and 1.7±0.33%, respectively. While the decreased rate rose to 0.1±0.5% in Treated Fancedar and dropped 0% in treated amprolium, treated primaquine 31.5ppm, treated artesunate, not infected not treated, not infected treated by amprolium, not infected treated by primaquine 31.2ppm, not infected treated by artesunate and not infected treated by fancedar.
Table (3:1): Means (± SE) of effects of anti-malarial and acoccidial drugs on infection symptoms of the experimental birds

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Feeding score/ (day)</th>
<th>Huddle to keep worm</th>
<th>survival Bird/day</th>
<th>survival Time/(day)</th>
<th>diseased survival/ (day)</th>
<th>feed intake/gram</th>
<th>Body weight/kg</th>
<th>Mortality(\lambda) (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control infected. not treated</td>
<td>2.3±1.4</td>
<td>2.2±1.3</td>
<td>11.2±1.3</td>
<td>4.9±1.334</td>
<td>8.5±1.5</td>
<td>845±91.</td>
<td>78.5±20</td>
<td>2.0±1.1</td>
</tr>
<tr>
<td>Amprolium 60gm</td>
<td>1.8±1.3</td>
<td>1.8±1.3</td>
<td>20.0±0.0</td>
<td>9.9±1.334</td>
<td>1.7±1.5</td>
<td>730±81.5</td>
<td>86.5±23</td>
<td>0.0</td>
</tr>
<tr>
<td>primaquine 250ppm</td>
<td>1.7±1.3</td>
<td>1.7±1.3</td>
<td>9.2±1.3</td>
<td>8.9±1.33</td>
<td>1.9±1.5</td>
<td>425±91.</td>
<td>94±35</td>
<td>2.0±1.3</td>
</tr>
<tr>
<td>primaquine 31.5ppm</td>
<td>1.5±1.3</td>
<td>1.6±1.3</td>
<td>20.0±0.0</td>
<td>9.9±1.12</td>
<td>1.3±1.5</td>
<td>755±230</td>
<td>87.5±30</td>
<td>0.0</td>
</tr>
<tr>
<td>primaquine 125ppm</td>
<td>1.7±1.3</td>
<td>1.6±1.3</td>
<td>16.2±1.3</td>
<td>9.6±1.22</td>
<td>1.9±1.5</td>
<td>753±430</td>
<td>153±20</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>Artesunate 33mg</td>
<td>1.7±1.3</td>
<td>2.3±1.3</td>
<td>20.0±0.0</td>
<td>9.9±0.98</td>
<td>2.6±1</td>
<td>779±345</td>
<td>110±25</td>
<td>0.0</td>
</tr>
<tr>
<td>Fancedar 86.625mg</td>
<td>1.7±1.3</td>
<td>1.6±1.3</td>
<td>19.6±1.3</td>
<td>9.8±1.45</td>
<td>4.0±1</td>
<td>775±150</td>
<td>101±30</td>
<td>0.1±0.5</td>
</tr>
<tr>
<td>not infected not treated</td>
<td>1.7±1.3</td>
<td>0±0</td>
<td>20.0±0.0</td>
<td>9.9±1.36</td>
<td>0±0</td>
<td>1000±90</td>
<td>117±30</td>
<td>0.0</td>
</tr>
<tr>
<td>not infected treated by Amprolium 60gm</td>
<td>0±0</td>
<td>0±0</td>
<td>20.0±0.0</td>
<td>9.9±1.23</td>
<td>0±0</td>
<td>1000±160</td>
<td>120±15</td>
<td>0.0</td>
</tr>
<tr>
<td>un infected treated by Primaquine 250 ppm</td>
<td>0±0</td>
<td>0±0</td>
<td>11.6±1.3</td>
<td>7.2±1.34</td>
<td>0±0</td>
<td>655±210</td>
<td>102.5±22</td>
<td>1.7±0.33</td>
</tr>
<tr>
<td>un infected treated by Primaquine 31.2ppm</td>
<td>0±0</td>
<td>0±0</td>
<td>20.0±0.0</td>
<td>8.5±1.41</td>
<td>0±0</td>
<td>1000±200</td>
<td>126.5±24</td>
<td>0.0</td>
</tr>
<tr>
<td>un infected treated by Primaquine 125 ppm</td>
<td>0±0</td>
<td>0±0</td>
<td>17.2±1.3</td>
<td>9.9±1.52</td>
<td>0±0</td>
<td>795±230</td>
<td>112.5±23.5</td>
<td>0.5±0.32</td>
</tr>
<tr>
<td>un infected treated by Artesunate 33mg</td>
<td>0±0</td>
<td>0±0</td>
<td>20.0±0.0</td>
<td>8.4±1.11</td>
<td>0±0</td>
<td>978±450</td>
<td>116±25</td>
<td>0.0</td>
</tr>
<tr>
<td>un infected treated by Fancedar 86.625mg</td>
<td>0±0</td>
<td>0±0</td>
<td>20.0±0.0</td>
<td>9.9±0.99</td>
<td>0±0</td>
<td>979±150</td>
<td>108±11</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*Means (± SE) in each column followed by the same letter are not significantly different at 5% level.
3.2. Effects of anti-malarial and acoccidial drugs on infection symptoms of the experimental birds

The data in table 3.2 describing the impact of trail medications on disease symptoms under study.

The significantly (p<0.05) maximum oocyst output value was exerted in control infected not treated (219.38±13.34), while treated amprolium showed (73.0±14.6 oocyst/grams). whereas the minimum value recorded in treated primaquine 250, 31.2 and 125 ppm, treated artesunate, treated fancedar, not infected not treated, not infected treated by amprolium, not infected treated by primaquine 250, 31.2 and 125 ppm, not infected treated by artesunate and not infected treated by fancedar, which were did not showed an oocyst in slides.

Bloody diarrhea score was ranged from 0 to 4. Which was significantly (P<0.05) higher in control infected not treated (2.50±1.64) compare with 1.20±1.11 in treated amprolium, 1.30±1.4 treated primaquine 250 ppm, 1.40 ±1.02 in treated primaquine 31.5 ppm, 1.80±1.3 in treated primaquine 125 ppm, 1.20±1.34 in treated artesunate, 1.60±1.4 in treated fancedar and zero score in not infected not treated, not infected treated by amprolium, not infected treated by primaquine 250, 31.2 and 125 ppm, not infected treated by artesunate and not infected treated by fancedar, respectively.

Dropping score appearance was ranged from 1 to 8, where the significantly (p<0.05) higher scores demonstrated in control infected not treated, treated amprolium, treated primaquine 250, 31.2 and 125 ppm, treated artesunate, treated fancedar and not infected not treated
(5.7±1.334, 4.7±1.334, 3.4 ±1.334, 3.5 ±1.334, 3.2 ±1.334, 3.5 ±1.334, 4.7±1.334 and 4.7±1.334), compare with not infected treated by amprolium, not infected treated by primaquine 250, 31.2 and 125 ppm, not infected treated by artesunate and not infected treated by fancedar, which were get only one grade score.

Lesion scores were ranged from zero to four. control infected not treated exerted was significantly (p<0.05) higher score (2.7±1.4), compare with lower (zero) score recorded in not infected not treated, not infected treated by amprolium, not infected treated by primaquine 250, 31.2 and 125 ppm, not infected treated by artesunate and not infected treated by fancedar, respectively.
Table (3:2):
Means (±SE) of effects of anti-malarial and acoccidial drugs on infection symptoms of the experimental birds

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Oocyst output/gram</th>
<th>drop scores</th>
<th>Bloody diarrhea</th>
<th>Lesion scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control infected not treated</td>
<td>219.38±13.34</td>
<td>5.7±1.334</td>
<td>2.50±1.64</td>
<td>2.70±1.4</td>
</tr>
<tr>
<td>treated by Amprolium 60gm</td>
<td>73.0±14.6</td>
<td>4.7±1.334</td>
<td>1.20bc±1.11</td>
<td>1.60b±0.99</td>
</tr>
<tr>
<td>treated by primaquine 250ppm</td>
<td>0c</td>
<td>3.4±1.334</td>
<td>1.30bc±1.4</td>
<td>1.1bc±0.9</td>
</tr>
<tr>
<td>treated by primaquine 31.5ppm</td>
<td>0c</td>
<td>3.5±1.334</td>
<td>1.40bc±1.02</td>
<td>1.70b±1.4</td>
</tr>
<tr>
<td>treated by primaquine 125ppm</td>
<td>0c</td>
<td>3.2±1.334</td>
<td>1.80b±1.3</td>
<td>1.70b±1.21</td>
</tr>
<tr>
<td>Treated Artesunate 33mg</td>
<td>0c</td>
<td>3.5±1.334</td>
<td>1.20bc±1.34</td>
<td>1.50b±1.3</td>
</tr>
<tr>
<td>Treated Fancedar 86.625mg</td>
<td>0c</td>
<td>4.7±1.334</td>
<td>1.60b±1.4</td>
<td>1.50b±1.3</td>
</tr>
<tr>
<td>not inf not treated</td>
<td>0c</td>
<td>4.7±1.334</td>
<td>0c</td>
<td>0c</td>
</tr>
<tr>
<td>not infected treated by Amprolium 60gm</td>
<td>0c</td>
<td>1.0b±0.0</td>
<td>0c</td>
<td>0c</td>
</tr>
<tr>
<td>not infected treated by Primaquine 250 ppm</td>
<td>0c</td>
<td>1.0b±0.0</td>
<td>0c</td>
<td>0c</td>
</tr>
<tr>
<td>not infected treated by Primaquine 31.2ppm</td>
<td>0c</td>
<td>1.0b±0.0</td>
<td>0c</td>
<td>0c</td>
</tr>
<tr>
<td>not infected treated by Primaquine 125 ppm</td>
<td>0c</td>
<td>1.0b±0.0</td>
<td>0c</td>
<td>0c</td>
</tr>
<tr>
<td>not infected treated by Artesunate 33mg</td>
<td>0c</td>
<td>1.0b±0.0</td>
<td>0c</td>
<td>0c</td>
</tr>
<tr>
<td>not infected treated by Fancedar 86.625mg</td>
<td>0c</td>
<td>1.0b±0.0</td>
<td>0c</td>
<td>0c</td>
</tr>
</tbody>
</table>

*Means (±SE) in each column followed by the same letter are not significantly different at 5% level.
3.3 Effect of control uninfected treated primaquine250 ppm on liver function tests (4 days after treatment):

The data in Table (3.3) summarizes the overall means and standard errors of the investigated traits of liver function of the control uninfected treated with primaquine 250 ppm birds' group, which was exerted a toxic symptoms. The investigation was carried out for four days after treatment. The serum sodium, potassium, calcium, phosphate and ion secured a value of 129.25±23.38, 3.10±2.02, 161.6±24.95, 5.08±0.85 and 79.0±15 respectively. whereas total billirubin., total protein, albumin, alkaline phosphatase, alanine transferase and aspartate transferase, which showed the overall values 0.25±0.2 mlg/deciliter, 1.75±0.72 g/deciliter, 1.35±0.87 g/deciliter, 752.0±52.5 mlg/deciliter, 36.75±9.85 iu/L and 161.0±24.95 iu/L, respectively.

The serum sodium appeared lower value at the first experimental bird's group (125±20.3 µq/L) which differ significantly from the second, third and fourth days, which their values 134±27.8, 130±22.1 and 128.0±23.3 µq/L, respectively (table 3:3).

The effect of experimental treatment on serum potassium of the experimental bird's group secured the highest value on the second group (3.4 ±2 µq/L) where showed a significant difference from the first, third and fourth days of investigation, 4.0±1.8, 3.2 ±0.89 and 3.1±2.02 µq/L, respectively.

The serum calcium of the third group appeared the highest value (13.0±1.38mg/decilitter), whereas the first and second days showed the lowest ones (10.1±1.75 and 11.6±2.0 mg/decilitter).
The highest value of serum phosphate appeared on the third days (5.5±0.7 mg/decilitter), whereas the first day secured the lowest value (4.8±0.9 mg/decilitter).

The serum iron of the second group (91.0±15.3 mg/decilitter) showed a significant difference from the rest days, which the first day value was 73.0±12, the third day secured 66.0±18.1 and the fourth day value was 86.0±14.6 mg/decilitter.

The data on table 3: 3 showing that the total billirubin of the first and fourth days of investigation were reviled nearly similar figures, 0.20±0.06 g/decilitter and 0.20±0.07 mg/decilitter, respectively. Whereas the second and third days secured the similar values (0.30±0.09 and 0.30±0.06 mg/decilitter). On the other hand the total protein values of the second, third and fourth days appeared nearly similar numbers, which they were 1.8±0.4, 1.9±0.9 and 1.9±0.8 g/decilitter, respectively. While the first day showed a significant different number 1.4±0.09 mg/deciliter. On the other hands the blood albumin of the first day represented the lowest value (1.20±0.08 g/decilitter), whereas the fourth day secured the highest value (1.5±0.5 mg/decilitter).

The alkaline phosphatase of the second day showed the highest value (814.0±63.5 iu/litter) which differ significantly from first, third and fourth days of investigation of the liver function test, which secured 790.0±56.8, 699.0±43.7 and 705.0±44.9 iu/litter, respectively. On the other hand the alanine transferase of the third group maintained the lowest value (22.0±9.9 iu/litter) while the second and fourth days showed a consistent pattern of values 31.0 ±7.2 and 30.0±10.0 iu/litter,
respectively, whereas the alanine transferase was witnessed in the first
day (64.0±12.3 iu/litter) and which was significantly (P<0.05) higher
than the cross pounding values. Moreover, the aspartate transferase
exerted nearly similar on the third and fourth days (175.0±31.0 and
163.0±26.5 iu/litter). Which were not differ significantly (P<0.05) higher
than the first and second days (150.0±21.3 and 156.0±21.0 iu/litter) (table
3:3).
Table (3:3):
Means (± SE) of effect of control uninfected treated primaquine 250 ppm on liver function tests (4 days after treatment)

<table>
<thead>
<tr>
<th>parameters</th>
<th>sodium</th>
<th>potassium</th>
<th>Total.Billirubin.</th>
<th>Total.protein</th>
<th>Albumin</th>
<th>Alkaline Phosphatase</th>
<th>Alanine Transferase</th>
<th>Aspartate Transferase</th>
<th>Calcium</th>
<th>Phosphate</th>
<th>Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>125.0±20.3</td>
<td>4.0±1.8</td>
<td>0.20±0.06</td>
<td>1.40±0.09</td>
<td>1.20±0.08</td>
<td>790.0±56.8</td>
<td>64.0±12.3</td>
<td>150.0±21.3</td>
<td>10.1±1.75</td>
<td>4.80±0.9</td>
<td>73.0±12.0</td>
</tr>
<tr>
<td>Second</td>
<td>134.0±27.8</td>
<td>3.4±2.0</td>
<td>0.30±0.09</td>
<td>1.80±0.4</td>
<td>1.40±0.9</td>
<td>814.0±63.5</td>
<td>31.0±7.2</td>
<td>156.0±21.0</td>
<td>11.6±2.0</td>
<td>5.0±1.0</td>
<td>91.0±15.3</td>
</tr>
<tr>
<td>Third</td>
<td>130.0±22.1</td>
<td>3.2±0.89</td>
<td>0.30±0.06</td>
<td>1.90±0.9</td>
<td>1.30±1.0</td>
<td>699.0±43.7</td>
<td>22.0±9.9</td>
<td>175.0±31.0</td>
<td>13.0±1.38</td>
<td>5.50±0.7</td>
<td>66.0±18.1</td>
</tr>
<tr>
<td>Fourth</td>
<td>128.0±23.3</td>
<td>3.8±2.4</td>
<td>0.20±0.07</td>
<td>1.90±0.8</td>
<td>1.50±0.5</td>
<td>705.0±44.9</td>
<td>30.0±10.0</td>
<td>163.0±26.5</td>
<td>11.7±3.23</td>
<td>5.0±0.8</td>
<td>86.0±14.6</td>
</tr>
</tbody>
</table>

*Means (±SE) in each column followed by the same letter are not significantly different at 5% level.
3.4 Effects of anti-malarial and anti-coccidial medications on haematology at the first period before treatment - 4 days (mcv, wbcs, plt, lym, & mon).

The data pertinent to the effect exerted by trail drugs on investigated traits was shown in table 3.4. The effect of anti-coccidial and anti-malarial drugs at first period of experiment (before treatment) on red blood count (RBCs) showed a consistent pattern which were differ insignificantly (p>0.05). The similar values were witnessed in the treated artesunate (2.15±0.4×103/ml) and control inf. not treated, treated amprolium, treated primaquine 250, 31.2 and 125ppm, not infected treated by amprolium and not infected treated by fancedar which showed similar lower values (1.6×103/ml). Whereas haematocrite (HCT) percentages the maximum significantly (p<0.05) figures were recorded in treated artesunate, treated fancedar, not infected treated by primaquine 250, 31.2 and 125ppm and not infected treated by artesunate (30.2±1.5, 29.7±1.4, 29.7±1.1, 29.7±1.34, 29.7±1.5 and 29.7±1.3%), while the minimum values were recorded in control inf. not treated, treated amprolium, treated primaquine 250, 31.2 and 125ppm, control not infected not treated, not infected treated by amprolium and not infected treated by fancedar (24.6±1.4, 24.6±1.2, 24.9±1.5, 24.9±1.3, 24.9±1.1, 24.6±1.7, 24.9±1.6 and 24.6±1.2). Mean concentration haemoglobin cells (mchc) of the experimental birds at the first period before treatment secured three different means which get a significant (P<0.05) variation, the first maximum value was recorded in treated primaquine 31.2ppm (47.3±1.6), while the medium figures secured in control infected not
treated, treated amprolium, treated primaquine, 250 and 125 ppm, control not infected not treated, not infected treated by amprolium and not infected treated by Fancedar (36.2±1.2, 36.2±1.1, 37.3±1.3, 37.3±1.4, 36.2±1.13, 37.3±1.33 and 36.2±1.1), where the minimum values were reviled in treated artesunate, treated fancedar, not infected treated by primaquine 250, 31.2 and 125 ppm and not infected treated by Artesunate (33.1±1.5, 33.7±1.5, 33.7±1.53, 33.7±1.43, 33.8±1.5 and 33.7±1.6). Furthermore, the main plate volume (MPV) get two mean levels of significance the first level maintained in treated primaquine 250, 31.2 and 125 ppm, treated artesunate, treated fancedar, not infected treated by amprolium, not infected treated by primaquine 250, 31.2 and 125 ppm and not infected treated by artemesunate (8.7±0.93, 8.7±0.8, 8.7±0.74, 8.7±0.67, 8.4±0.83, 8.8±0.73, 8.4±0.9, 8.4±0.93, 8.4±0.82 and 8.4±0.57 μm3); while the second level showed a similar value (7.1 μm3) in control infected not treated, treated amprolium, control not infected not treated and not infected treated by fancedar. Moreover, the plate diameter wall (PDW) showed the same pattern as MPV were the first mean level reviled in control infected not treated, treated amprolium treated primaquine 250, 31.2 and 125 ppm, treated artesunate, control not infected not treated, not infected treated by amprolium and not infected treated by fancedar (9.2±1.9, 9.2±1.3, 9.4±1.63, 9.4±1.8, 9.5±1.2, 9.4±1.4, 9.2±1.34, 9.2±1.6 and 9.2±1.4%), while the second level recorded in treated fancedar, not infected treated by primaquine 250, 31.2 and 125 ppm and not infected treated by artesunate, which get the similar percent (8.5%).
Table (3:4)
Means (±SE) of effects of anti-malarial and anti-coccidial medications on haematology at the first period before treatment -4days.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Mean cell volum (MCV)</th>
<th>White blood count (WBCs)</th>
<th>Platlets(PLT)</th>
<th>Lymphocyte(lym)</th>
<th>Granulocyte(GRA)</th>
<th>Monocyte(MON)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control infected not treated</td>
<td>153.8a±5.3</td>
<td>117.4c±5.0</td>
<td>63.0i±3.5</td>
<td>100.8c±4.9</td>
<td>6.1i±1.9</td>
<td>10.4i±3.2</td>
</tr>
<tr>
<td></td>
<td>Treated Amprolium 60gm</td>
<td>153.8i±5.6</td>
<td>117.4i±7.8</td>
<td>63.0i±3.2</td>
<td>100.8i±3.9</td>
<td>6.1i±1.5</td>
<td>10.4i±3.9</td>
</tr>
<tr>
<td></td>
<td>Treated primaquine 250ppm</td>
<td>154.7a±3.7</td>
<td>121.7c±5.8</td>
<td>99.0i±4.6</td>
<td>105.3b±4.8</td>
<td>5.1i±0.9</td>
<td>11.3i±3.0</td>
</tr>
<tr>
<td></td>
<td>Treated primaquine 31.5ppm</td>
<td>154.7a±3.5</td>
<td>121.7c±4.9</td>
<td>99.0i±5.2</td>
<td>105.2b±4.6</td>
<td>5.1i±0.85</td>
<td>11.3i±2.7</td>
</tr>
<tr>
<td></td>
<td>Treated primaquine 125ppm</td>
<td>154.7a±3.9</td>
<td>121.7c±4.7</td>
<td>98.0i±4.8</td>
<td>105.3b±3.5</td>
<td>5.1i±0.73</td>
<td>11.3i±2.4</td>
</tr>
<tr>
<td></td>
<td>Treated Artesunate 33mg</td>
<td>140.8b±3.2</td>
<td>143.3a±5.3</td>
<td>58.0h±5.4</td>
<td>112.6a±3.9</td>
<td>6.3c±1.1</td>
<td>23.4h±3.1</td>
</tr>
<tr>
<td></td>
<td>Treated Fancedar 86.625mg</td>
<td>140.8b±3.2</td>
<td>133.9b±4.9</td>
<td>60.0h±4.0</td>
<td>105.9b±4.0</td>
<td>5.8b±0.9</td>
<td>22.2b±2.8</td>
</tr>
<tr>
<td></td>
<td>Control not infected not treated</td>
<td>153.8a±4.3</td>
<td>117.4b±6.8</td>
<td>63.0i±3.9</td>
<td>100.8b±3.2</td>
<td>6.1i±1.2</td>
<td>10.4i±2.9</td>
</tr>
<tr>
<td></td>
<td>not infected treated by Amprolium 60gm</td>
<td>154.7a±3.8</td>
<td>121.7c±3.5</td>
<td>99.0i±4.7</td>
<td>105.3b±3.5</td>
<td>5.1i±0.9</td>
<td>11.3i±2.84</td>
</tr>
<tr>
<td></td>
<td>not infected Treated by Primaquine 250 ppm</td>
<td>140.8b±4.9</td>
<td>133.9b±3.4</td>
<td>60.0h±4.8</td>
<td>105.9b±4.0</td>
<td>5.8b±0.7</td>
<td>22.2b±3.4</td>
</tr>
<tr>
<td></td>
<td>not infected treated by Primaquine 31.2ppm</td>
<td>140.8b±3.8</td>
<td>133.9b±4.0</td>
<td>60.0h±3.1</td>
<td>105.9b±3.6</td>
<td>5.8b±0.6</td>
<td>22.2b±3.1</td>
</tr>
<tr>
<td></td>
<td>not infected treated by Primaquine 125 ppm</td>
<td>140.8b±2.1</td>
<td>133.9b±5.0</td>
<td>60.0h±3.9</td>
<td>105.9b±3.7</td>
<td>5.8b±0.54</td>
<td>22.2b±2.4</td>
</tr>
<tr>
<td></td>
<td>not infected treated by Artesunate 33mg</td>
<td>140.8b±2.6</td>
<td>133.9b±5.4</td>
<td>99.0i±5.8</td>
<td>105.9b±3.8</td>
<td>5.9ab±0.8</td>
<td>22.2b±2.1</td>
</tr>
<tr>
<td></td>
<td>not infected. Fancedar 86.625mg</td>
<td>153.8a±5.3</td>
<td>117.3c±4.8</td>
<td>98.0a±5.1</td>
<td>100.8c±3.6</td>
<td>6.1a±0.6</td>
<td>10.4i±1.9</td>
</tr>
</tbody>
</table>

*Means (±SE) in each column followed by the same letter are not significantly different at 5% level.
3.5 Effects of anti-malarial and anti-coccidial medications on haematology analysis at the first period before treatment-4days (RBCS,HCT,MCHC,MPV,and PDW%):

Table 3.5 demonstrated the effect of anti-malarial and anti-coccidial drugs on the main cell volume, monocytes, plate count, lymphocytes, granulocytes and white blood count at the first experimental period before treatment.

The main cell volume (MCV), recorded a significantly (p<0.05) higher volume in control infected not treated, treated amprolium, treated primaquine 250, 31.2 and 125 ppm, control not infected not treated, not infected treated by amprolium and not infected treated by fancedar (153.8±5.3, 153.8±5.6, 154.7±3.7, 154.7±3.5, 154.7±3.9, 153.8±4.3, 154.7±3.8 and 153.8±5.3 µm³) compared with treated artesunate, treated fancedar, not infected treated by primaquine 250, 31.2 and 125 ppm and not infected treated by artesunate, which were showed a nearly similar values. Furthermore, white blood count (WBCs) maintained a maximum value recorded in treated artesunate (143.3±5.3×10³/ml), while the minimum values recorded in control infected not treated, treated amprolium, treated primaquine 250, 31.2 and 125 ppm, control not infected not treated, not infected treated by amprolium and not infected treated by fancedar (117.4±5.0, 117.4±7.8, 121.7±5.8, 121.7±4.9, 121.7±4.7, 117.4±6.8, 121.7±3.5 and 117.3±4.8×10³/ml), whereas treated fancedar, not infected treated by primaquine 250, 31.2 and 125 ppm and not infected treated by artesunate showed a similar figure (133.9×10³/ml). Moreover, plate
count (PLT) exerted a significantly \((p<0.05)\) higher figure in treated primaquine 250, 31.2 and 125ppm, not infected treated by artesunate, not infected treated by amprolium and not infected treated by fancedar \((99.0\pm4.6, 99.0\pm5.2, 98.0\pm4.8, 99.0\pm5.8, 99.0\pm4.7\) and \(98.0\pm5.1\)). while control infected not treated, treated amprolium, treated artesunate, treated fancedar, control not infected not treated, not infected treated by primaquine 250, 31.2 and 125 ppm were \(63.0\pm3.5, 63.0\pm3.2, 58.0\pm5.4, 60.0\pm4.0, 63.0\pm3.9, 60.0\pm4.8, 60.0\pm3.1\) and \(60.0\pm3.9\), respectively (Table3:5).

Lymphocytes (LYM) counted at the first period of experiment (before treatment) in treated artesunate, showed a significantly \((p<0.05)\) the highest value \((112.6\pm3.9\times10^3/ml)\), where the treated primaquine 250, 31.2 and 125ppm, treated fancedar, not infected treated by amprolium, not infected treated by primaquine 250, 31.2 and 125ppm and not infected treated by artesunate \((105.3\pm4.8, 105.2\pm4.6, 105.3\pm3.5, 105.9\pm4.0, 105.3\pm3.5, 105.9\pm4.0, 105.9\pm3.6, 105.9\pm3.7\) and \(105.9\pm3.8\times10^3/ml)\). Whereas the lowest value maintained in control infected not treated, treated amprolium, control not infected not treated and not infected treated by fancedar, which were get a similar value \((100.8\times10^3/ml)\). On the other hand the granulocytes (GRA) exerted a significantly \((p<0.05)\) higher values in control infected not treated, treated amprolium, treated artesunate, control not infected not treated and not infected treated by fancedar, which were get similar figure \((6.1\times10^3/ml)\), compare with the lower value secured in treated primaquine 250, 31.2 and 125ppm and not infected treated by
amprolium which were showed also a similar value (5.1×10³/ml). The significantly (p<0.05) maximum value of monocytes (MON) counted in treated artesunate (23.4 ±3.1×10³/ml). While the minimum value showed in control infected not treated, treated amprolium and not infected treated by fancedar, which were recorded same figure (10.4×10³/ml) (table 3.5).
Table (3:5): Means (+SE) of effects of anti-malarial and anti-coccidial medications on haematology at the first period before treatment -4days.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Means±Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Red count(RBCS)</strong></td>
</tr>
<tr>
<td>Control infected not treated</td>
<td></td>
<td>1.6±0.3</td>
</tr>
<tr>
<td>Treated by Amprolium 60gm</td>
<td></td>
<td>1.6±0.4</td>
</tr>
<tr>
<td>Treated by primaquine 250ppm</td>
<td></td>
<td>1.61±0.6</td>
</tr>
<tr>
<td>Treated by primaquine 31.5ppm</td>
<td></td>
<td>1.6±0.5</td>
</tr>
<tr>
<td>Treated by primaquine 125ppm</td>
<td></td>
<td>1.6±0.65</td>
</tr>
<tr>
<td>Treated by Artesunate 33mg</td>
<td></td>
<td>2.15±0.4</td>
</tr>
<tr>
<td>Treated by Fancedar 86.625mg</td>
<td></td>
<td>2.11±0.5</td>
</tr>
<tr>
<td>Control not infected not treated</td>
<td></td>
<td>1.73±0.5</td>
</tr>
<tr>
<td>not infected treated by Amprolium</td>
<td></td>
<td>1.61±0.32</td>
</tr>
<tr>
<td>not infected treated by Primaquine 250 ppm</td>
<td></td>
<td>2.11±0.7</td>
</tr>
<tr>
<td>not infected treated by Primaquine 31.2ppm</td>
<td></td>
<td>2.11±0.83</td>
</tr>
<tr>
<td>not infected treated by Primaquine 125 ppm</td>
<td></td>
<td>2.11±0.63</td>
</tr>
<tr>
<td>not infected treated by Artesunate 33mg</td>
<td></td>
<td>2.11±0.84</td>
</tr>
<tr>
<td>not infected treated by Fancedar 86.625mg</td>
<td></td>
<td>1.6±0.43</td>
</tr>
</tbody>
</table>

*Means (+SE) in each column followed by the same letter are not significantly different at 5% level.
3.6 Effects of anti-malarial and anti-coccidial medications on haematology after treatment

The data in the table 3.6 describes the impact of trail medications on the studied haematological parameters at second period after treatment. The data demonstrated that the white blood count of experimental birds in the second course of the trail (after treatments) showing the following means for the birds treated by amprolium, artesunate and not infected treated by primaquine 31.2ppm to test toxicity were 139.2±24.7, 139.21±14.36×10³/ml and 139.2±17.75×10³/ml, which were significantly (p<0.05) greatest value. While control infected without treatment and not infected treated by primaquine 250 ppm secured similar values were 135.71±23.6×10³/ml, 135.71±16.4×10³/ml, respectively. whereas the lowest similar values appeared on treated primaquine 250ppm, treated fancedar and not infected treated by primaquine 125 ppm (106.5×10³/ml).

The lymphocytes counted at second period after treatment in the first course of experiment (after treatments) for treated primaquine 31.2ppm, treated primaquine 125ppm, control not infected not treated, not infected treated by amprolium and not infected treated by fancedar exerted similar figures which were significant (p<0.05) bigger than other counter parts which were 114.0±10.3, 113.0±13.1, 114.0±12, 113.0±13.3 and 113.0±18.6×10³/ml, respectively. Whereas the lymphocytes was recorded in the treated fancedar, not infected. primaquine 125 ppm and not infected. primaquine 125 ppm and which
was showed similar figures \((101.1 \times 10^3/\text{ml})\) and were represented the lowest values (table 3:6).

The plate count (PLT) at second period after treatment in the control not infected not treated, treated primaquine 31.2 ppm and not infected treated artesunate were had a significantly \((P<0.05)\) higher means \((1063.4 \pm 32.2, 1044.0 \pm 67.9 \text{ and } 1054.0 \pm 31.8 \times 10^3/\text{ml},\) respectively) compare with control infected not treated and not infected treated by primaquine 250 ppm \((846.0 \pm 65.4 \text{ and } 842.0 \pm 36.8 \times 10^3/\text{ml})\) which represented second grade values. Whereas treated amprolium, treated primaquine 125 ppm, treated artesunate, not infected treated by amprolium and not infected treated by fancedar \((406.0 \pm 66.2, 442.0 \pm 68.8, 412.0 \pm 44.3, 441.0 \pm 42.0 \text{ and } 441.0 \pm 21.7 \times 10^3/\text{ml},\) respectively) secured the lowest figure. Moreover, the mean cell volume (MCV) of treated amprolium, treated primaquine 125 ppm, treated artesunate, control not infected not treated, not infected treated by primaquine 31.2 ppm, not infected treated by artesunate, not infected treated by amprolium, treated primaquine 31.2 ppm and not infected treated by fancedar exerted the highest values \((146.0 \pm 20.4, 145.1 \pm 20.6, 146.1 \pm 19.9, 146.8 \pm 20.2, 146.0 \pm 13.8, 146.8 \pm 15.9, 145.1 \pm 15.4, 146.8 \pm 21.5 \text{ and } 145.0 \pm 17.8 \mu m^3)\) compared with control infected not treated and not infected treated by primaquine 250 ppm \((140.0 \pm 19.4 \text{ and } 140.0 \pm 17.6 \mu m^3)\) which maintained the lowest value. Furthermore, the maximum value of mean concentration hemoglobin cells (MCHC) was recorded in control infected not treated and not infected treated by Primaquine 250
ppm, which they were had a similar figure (42.7 g/deciliter), whereas the minimum value was recorded in Treated Amprolium, (33.1±4.6 g/deciliter).

The data in table (3:6) showing the main plate volume (MPV) at second period after treatment as affected by treated primaquine 250, 31.2 and 125ppm, treated artesunate, treated fancedar, not infected treated by amprolium, not infected treated by primaquine 250, 31.2 and 125 ppm and not infected treated by artesunate were 8.8±1.5, 8.6±1.7, 8.7±1.5, 8.6±1.1, 8.6±1.4, 8.8±1.9, 8.4±1.3, 8.4±1.4, 8.4±1.2 and 8.4±1.1 µm³, respectively. Which were demonstrated in table (3.6) higher volume compare with the lower ones in control infected. not treated, control not infected. not treated, treated amprolium and not infected treated by fancedar, which had the similar volume (7.1 µm³).

Plate diameter wall (PDW) at second period after treatment demonstrated in control infected. not treated, treated amprolium, treated primaquine 250, 31.2 and 125ppm, treated artesunate, control not infected. not treated, not infected treated by amprolium, not infected treated by artesunate and not infected treated by fancedar secured a higher percent 9.2±2.4, 9.2±2.2, 9.6±2.5, 9.5±2.1, 9.6±2.2, 9.3±2.4, 9.2±2.1, 9.2±2.7, 9.2±2.5 and 9.2±2.9%, respectively. On the other hand Treated Fancedar, not infected treated by Primaquine 250, 31.2 and 125 ppm reviled the lower percentages 8.4±2.6, 8.5±2.3, 8.5±2.3 and 8.5±2.1%, respectively (Table 3:6).

Monocytes (MON) at second period after treatment secured a higher figure computed in control infected. not treated, treated
amprolium, treated artesunate, not infected treated by primaquine 250 ppm and not infected treated by primaquine 31.2ppm, which were 21.2±6.6, 21.7±5.4, 21.7±5.7, 21.2±5.6 and 21.7±4.3×10³/ml, respectively. While treated primaquine 31.5ppm, Treated primaquine 125ppm, Control not infected, not treated, not infected. Amprolium not infected treated by Artesunate and not infected treated by Fancedar showed the medium values (11.2±3.2, 10.8±3.6, 11.2±2.4, 10.8±3.3, 11.2±3.0 and 10.8±2.2×10³/ml). Moreover, the other rest of experiment medications Treated primaquine 250ppm, Treated Fancedar and not infected treated by Primaquine 125 ppm appeared similar lower value (4.7×10³/ml) (Table 3:6).

The maximum values of granulocytes (GRA) at second period after treatment were recorded in control infected not treated, treated amprolium, treated artesunate, not infected treated by primaquine 250 ppm and not infected treated by primaquine 31.2ppm (5.4±1.3, 5.2±1.8, 5.2±1.2, 5.4±1.1 and 5.2±1.4×10³/ml), whereas the minimum similar values were recorded in treated fancedar and not infected treated by primaquine 125 ppm (0.07×10³/ml).

Red blood count (RBCs) at second period after treatment in Treated Amprolium, Treated Artesunate and not infected treated by Primaquine 31.2ppm were a similar figure (1.91×10³/ml), which were statistically differ significantly (P<0.05) from the lower values counted in Treated Fancedar, not infected treated by Primaquine 125 ppm and not infected treated by Primaquine 250 ppm (1.13±0.84, 1.13±0.7. and 1.13±0.8×10³/ml), while the other rest of experiment had
the medium values $1.77\pm0.34\times10^3$/ml for Control Infected. not treated, $1.58\pm0.7\times10^3$/ml, for treated primaquine 31.2ppm $1.44\pm0.54$ for Treated primaquine 125ppm, $1.58\pm0.1$ for Control not infected not treated, $1.44\pm0.37$ for not infected Amprolium, $1.77\pm0.24$ for not infected treated by Primaquine 250 ppm, $1.58\pm0.6$ for not infected treated by Artesunate and $1.44\pm0.5$ for not infected treated by Fancedar (table3:6).

The maximum percentages of haematocrite (HCT) at second period after treatment exerted in only on the some treated groups, which were not infected treated by Primaquine 31.2ppm, Treated Amprolium and Treated Artesunate ($27.9\pm5.2$ $27.9\pm7.7$ and $27.9\pm8.7$%). Whereas the minimum similar percentages secured in Treated primaquine 125ppm, not infected treated by Amprolium and not infected treated by Fancedar was 20.9% (table 3:6).
Table (3.6): Means (±SE) of effects of anti-malarial and anti-coccidial medications on haematology after treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(WBCS)</th>
<th>(LYM)</th>
<th>(PLT)</th>
<th>(MCV)</th>
<th>(MCHC)</th>
<th>(MPV)</th>
<th>(PDW%)</th>
<th>(MON)</th>
<th>(GRA)</th>
<th>(RBCS)</th>
<th>(HCT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control infected not treated</td>
<td>135.71b±23.6</td>
<td>109.1b±12.2</td>
<td>846.0b±65.4</td>
<td>140.0b±19.4</td>
<td>42.7b±5.9</td>
<td>7.1b±1.3</td>
<td>9.2b±2.4</td>
<td>21.2b±6.6</td>
<td>5.4b±1.3</td>
<td>1.77ab±0.34</td>
<td>24.8b±9.4</td>
</tr>
<tr>
<td>Treated Amprolium 60gm</td>
<td>139.2a±24.7</td>
<td>112.3ab±13.4</td>
<td>406.0c±66.2</td>
<td>146.0a±20.4</td>
<td>33.1d±4.6</td>
<td>7.1b±1.3</td>
<td>9.2b±2.2</td>
<td>21.7b±5.4</td>
<td>5.2a±1.8</td>
<td>1.91ab±0.25</td>
<td>27.9a±7.7</td>
</tr>
<tr>
<td>Treated primaquine 250ppm</td>
<td>106.5d±14.8</td>
<td>101.1ab±11.2</td>
<td>109.0d±71.2</td>
<td>143.2ab±18.7</td>
<td>40.5a±6.4</td>
<td>8.8a±1.5</td>
<td>9.6a±2.5</td>
<td>4.7±1.2</td>
<td>0.8±0.25</td>
<td>1.13c±0.8</td>
<td>16.3d±3.4</td>
</tr>
<tr>
<td>Treated primaquine 31.5ppm</td>
<td>126.91c±13.4</td>
<td>114.0a±10.3</td>
<td>1044.0a±67.9</td>
<td>146.8a±21.5</td>
<td>38.8a±5.3</td>
<td>8.6a±1.7</td>
<td>9.5a±2.1</td>
<td>11.2±3.2</td>
<td>1.8±0.92</td>
<td>1.58c±0.7</td>
<td>23.2b±2.9</td>
</tr>
<tr>
<td>Treated primaquine 125ppm</td>
<td>125.41c±15.35</td>
<td>113.0ab±13.1</td>
<td>442.0c±68.8</td>
<td>145.1ab±20.6</td>
<td>37.8d±3.4</td>
<td>8.7a±1.5</td>
<td>9.6b±2.2</td>
<td>10.8±3.6</td>
<td>1.6±0.94</td>
<td>1.44bc±0.54</td>
<td>20.9±6.8</td>
</tr>
<tr>
<td>Treated Artesunate 33mg</td>
<td>139.2a±14.36</td>
<td>112.3ab±15.6</td>
<td>412.0c±44.3</td>
<td>146.1b±19.9</td>
<td>33.1c±5.2</td>
<td>8.6a±1.1</td>
<td>9.3a±2.4</td>
<td>21.7±5.7</td>
<td>5.2a±1.2</td>
<td>1.91c±0.9</td>
<td>27.9a±8.7</td>
</tr>
<tr>
<td>Treated Fancedar 86.625mg</td>
<td>106.51d±16.3</td>
<td>101.1ab±16</td>
<td>116.0d±23.2</td>
<td>143.2a±17.6</td>
<td>40.5b±4.7</td>
<td>8.6a±1.4</td>
<td>8.4b±2.6</td>
<td>4.7±1.1</td>
<td>0.07c±0.03</td>
<td>1.13c±0.84</td>
<td>16.3d±5.4</td>
</tr>
<tr>
<td>Control not infected not treated</td>
<td>126.91c±6.63</td>
<td>114.0a±12.8</td>
<td>1063.4a±32.2</td>
<td>146.8b±20.2</td>
<td>38.8b±6.2</td>
<td>7.1b±1.6</td>
<td>9.2a±2.1</td>
<td>11.2±2.4</td>
<td>1.8±0.8</td>
<td>1.58±0.1</td>
<td>23.2b±3.4</td>
</tr>
<tr>
<td>not infected treated by Amprolium 60gm</td>
<td>125.42c±7.63</td>
<td>113.0c±13.3</td>
<td>441.0c±42.0</td>
<td>145.1a±15.4</td>
<td>37.8ab±6.4</td>
<td>8.8a±1.9</td>
<td>9.2±2.7</td>
<td>10.8±3.3</td>
<td>1.6±0.5</td>
<td>1.44c±0.37</td>
<td>20.9±3.6</td>
</tr>
<tr>
<td>not infected treated by Primaquine 250 ppm</td>
<td>135.71b±16.4</td>
<td>109.1b±10.8</td>
<td>842.0b±36.8</td>
<td>140.0b±17.6</td>
<td>42.7a±4.8</td>
<td>8.4a±1.3</td>
<td>8.5a±2.3</td>
<td>21.2±5.6</td>
<td>5.4±1.0</td>
<td>1.77ab±0.24</td>
<td>24.8±6.7</td>
</tr>
<tr>
<td>not infected treated by Primaquine 31.2ppm</td>
<td>139.2±17.75</td>
<td>112.3ab±9.3</td>
<td>402.0c±23.6</td>
<td>146.0b±13.8</td>
<td>33.1c±6.5</td>
<td>8.4a±1.4</td>
<td>8.5b±2.3</td>
<td>21.7±4.3</td>
<td>5.2±1.4</td>
<td>1.91a±0.3</td>
<td>27.9a±5.2</td>
</tr>
<tr>
<td>not infected treated by Primaquine 125 ppm</td>
<td>106.51±18.64</td>
<td>101.1ab±8.5</td>
<td>106.0a±27.9</td>
<td>143.2b±19.3</td>
<td>40.5b±4.9</td>
<td>8.4a±1.2</td>
<td>8.5b±2.1</td>
<td>4.7±1.0</td>
<td>0.7±0.24</td>
<td>1.13±0.7</td>
<td>16.3d±3.2</td>
</tr>
<tr>
<td>not infected treated by Artesunate 33mg</td>
<td>126.9±16.9</td>
<td>112.0a±9.9</td>
<td>1054.0c±31.8</td>
<td>146.8±15.9</td>
<td>38.8c±5.5</td>
<td>8.4a±1.1</td>
<td>9.2a±2.5</td>
<td>11.2±3</td>
<td>1.9±0.4</td>
<td>1.58±0.6</td>
<td>23.2b±7.3</td>
</tr>
<tr>
<td>not infected treated by Fancedar 86.625mg</td>
<td>125.42±18.87</td>
<td>113.0±18.6</td>
<td>441.0b±21.7</td>
<td>145.0±17.8</td>
<td>37.8±3.5</td>
<td>7.1b±1.2</td>
<td>9.2±2.9</td>
<td>10.8±2.2</td>
<td>1.6±0.44</td>
<td>1.44bc±0.5</td>
<td>20.9±8.5</td>
</tr>
</tbody>
</table>

*Means (±SE) in each column followed by the same letter are not significantly different at 5% level.
CHAPTER FOUR
DISCUSSION

The effects of aretsunate used in this experiment on whole performance and reduction of coccidiosis symptoms may be attributed to the compound with anti-malarial activity that justified by Allen et al. (1996) due to its potential to elicit oxidative stress which is effective in controlling caecal coccidian and exhibit the disease activity. One of the suppositions of this experiment was primaquine the anti-malarial component of this drug appeared a strong tendency to exhibit anti-coccidial activity.

The protective effects of the medications used in this experiments was suggested by both body weight and lesion scores data (table 4:) although un challenge chicks treated by primaquine 250 ppm exerted a toxicity behavior appeared on the sudden death and liver function tests which was secured in table (4:). Moreover, the effects and toxicity of primaquine at level 250 ppm in control un inoculated group was in agreement with Matsuno et al. (1991) results which they were report primaquine controlled the clinical signs of coccidiosis at level above 31.2 ppm and showed a tendency apparently to reduce body weight at 500 ppm. This toxicity will also be related to Schmidt's theory (Schmidt, 1951) on the extent of substitution of terminal amion-nitrogen, that primaquine with a primary amino terminus.

Whether the differences in anti-coccidial and anti-malarial and their levels observed on the experimental groups perhaps due to the reflection of differences in mode of actions against the parasite and may
be to the differences in their metabolism in chickens. Although the mode of action of the 8-amino-quinolines against blood parasites was not clearly proved, but the most supported one is base on the inhibition of metabolic pathway and protein-synthesis of the protozoa.

In the ensuing years, coccidial lesion scores and weight gain in particular the procedure described by Johnson and Reid (1970) assumed an important role as a measure of anti-coccidial efficacy. Moreover, the medication effects are attributed perhaps due to the efficacy of anti-malarial and anti-coccidial drugs and precision.

Caecal lesion scores obtained in this experiment of medicated groups secured lower lesion scores compare with control infected groups this result was in agreement with the results recorded by Patrica, et al. (1997) in their study on Artemisia annua as supplementary levels on diet, and the authors suggested that A. annua might afford some protection against E. tenella. On the other hand this result was disagreeing with the result obtained by Conway et al. (1999).

A number of workers have shown that significant differences in comparable lesion scores depending on whether they were immunized or un immunized or medicated or un medicated (Long et al., 1980, Augustine et al., 1999, 1993). Hee and Jae (2001) mentioned that lesion scores in the groups treated with umacrocarpa (1.4) or pulsatilla koreena (1.6) which were significantly lower than those of infected control group were their lesion scores was 3. It is possible that tissue damage and changes in the functional integrity of the intestine may allow colonization of the gut by various types of harmful bacteria, leading to
infectedlamination of the intestine, followed by cellular infectedilfiltration (Rose et al., 1979)

Development of typical caecal lesions appeared in infected treated groups was prevented when treatment with artesunate, primaquine, fancedar and amprolium is may be initiated before large of second generation of shizonts appear. Moreover, may be due to the lethal ability against both sexual and asexual stages of E. tenella and properly due to interrupted at a very early stage of oocyst shelling are prevented. Reid (1975) classified that amprolium as a rapid anti-coccidial drug in recovery coccidiosis.

The lowest oocyst out put obtained from treatment groups may be due to the effect of treatment especially the anti-malarial medications that the mode of action of artesunate has been attributed to its potential to induce a state of oxidative stress though the free radical cascade generator by the endoperoxide function (Krung Kraiand Yuthavong, 1987; Levander et al., 1989). and the ability of the free radical alkylate protein (Yang et al., 1994) and the induced oxidative stress can be effective in reducing caecal lesion. Moreover, amprolium competitively inhibits thiamine utilization by the parasite. Prolonged high dosages can cause thiamine deficiency in the host and excessive thiamine in the diet can reduce or reverse the anticoccidial activity of the drug. Amprolium reportedly acts primarily upon the first generation schizont in the cells of the intestinal wall, preventing differentiation of the metrozoites. It may also suppress the sexual stages and sporulation of the oocysts.
Body weight performance in this study was exerted the lowest weight in control infected not treated group compare with infected medicated groups which the highest body weight with in this group recorded in infected treated by primaquine 125 ppm, although the medium weight was recorded in control un infected treated groups, this pattern is similar to that mentioned by Walden Stedt *et al.* (1999) and Hee and Jae (2001). This result may be due to the mechanism of the medications on control un infected treated groups as prophylactic additives and suppression of infection activity, moreover, the result of increasing of body weight in infected treated by primaquine 125 ppm in agreement to the results obtained by Matsuno *et al.* (1991) which showed a tendency apparently to increase body weight compare with control not infected treated by primaquine groups which exerted an observable tendency to reduce body weight, and they were pointed the tendency at 500 ppm. On the other hand the extent of weight gain for all medicated with amprolium groups were susceptible to amprolium. Therefore, Ogbe *et al.* (2008) found that the infected medicated groups exerted a higher body weight compare with infected unmedicated.

The faecal scores were significantly lower (*P*<0.05) in the infected medicated groups compare with infected un medicated groups throughout the experiment.

Death may follow the acute disease either directly or from secondary diseases such as pneumonia. Birds that survive for 5 to 7 days may recover control infected un medicated group, however, permanent damage may occur as lesion scores, oocyst output, bloody diarrhea. Zakia
et al. (1990) mentioned that an outbreak of coccidiosis was reported among 8-week-old white hisex chickens in Khartoum province with 55.5% and 19.5% morbidity and mortality rates, respectively, which they were identified *E. tenella* 52.5%. Moreover, Chapman (1989) recorded that treatment with amprolium prevent mortality in chicken infected with field isolates of *E. tenella*. From above suggested that amprolium was used to control reactions and avoid mortality without interrupting immunity development. The use of this drug, however, in high doses and/or for long time periods can interfere with the development of immunity (cycle uniformity) in birds that were correctly vaccinated. On the other hand while amprolium can interfere with immunity development of all *Eimeria* species, particular care should be taken to avoid interfering with *Eimeria tenella* cycling. The low number of oocysts out put as a consequence, immunity development takes longer. Moreover, the excessive use of amprolium can interfere with the development of immunity against this specie.

Research has indicated that birds may experience reduced food consumption in control un infected un medicated group following clinical infection compare with the infected treated groups. Which may be due to the challenges of infection and in medicated groups may be due to the utilization of these drugs as treatment and control drugs (Apyne, 1977).

Diarrhea may be present in the animal before the oocysts can be found; therefore, a confirmed laboratory diagnosis may not always be
possible. Laboratory findings should be correlated with clinical signs for a diagnosis.

Haematological parameters, particularly haematocrit, are frequently used in assessing condition in birds. Mean corpuscular volume (MCV) overcomes some of the drawbacks associated with measuring haematocrits and therefore should be a better condition index. In this study investigated the relationships between these blood parameters and the breeding performances of challenged and unchallenged birds with *E. tenella*. Moreover, The infection with *E. tenella* resulted in reduction in red blood cell (RBC) counts and haemoglobin content, increase in the mean corpuscular haemoglobin (MCH), the mean corpuscular haemoglobin concentration (MCHC), the mean corpuscular volume (MCV) and the haemoglobin index (HbI), indicating the development of a hyperchromic anaemia. The white blood cell (WBC) counts were elevated as well as the percentages of eosinophils and monocytes following activation of the innate defense mechanisms. Lymphocyte percentages were lower. Both qualitative and quantitative haematological changes observed in chickens, infected with *E. tenella*, were the highest during the period of most intensive invasion.
CONCLUSION AND RECOMMENDATIONS

The present knowledge on the pharmacology and impact of parasitic diseases on productivity performance and health. There is a need to conduct a range of studies in this area in order to fill the gaps in the knowledge base. To date Eimeria spp. and plasmodium spp. are secured similar metabolic function in several recent studies, which build this research hypothesis to utilize the anti-malarial drugs to suppress the infectedection of Eimeria spp. As indicated in Chapters 4 the anti-malarial medications-primaquine, fancedar and artesunate- revealed apparently tendency to prophylactic tendency and recovery the infectedection of Eimeria tenella, in spite of the un infected treated by primaquine secured toxic symptoms at level 250 ppm, causing not only heavy production losses, but also death.

This study recommended that the utilization of anti-malarial medications primaquine, artesunate and fancedar are useful to administrate the Eimeria tenella infectedection especially primaquine inmodrate level 125 ppm which take a short time (5 days) and least cost compare with antimalarial drugs. The other utility of these drugs as control drug and prophylactic additive, which exearted higher performance compare with anti-coccidial drugs represented in amprolium. Moreover, when medicated the coccidiosis with anti-malarial drugs must take care to avoid highly levels and over doses, to avoid the reduction on total performance in chickens and sudden death as symptom of toxicicty.
REFERENCES


Allen, P.C. and Fetterer, R.H. (2002b): 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, 58–65.*


Centers for Disease Control and Prevention (2005): Health Information for Travelers to Argentina, *annual report*.


Conway, D. P., M. E. McKenzie, and A. D. Dayton, (1990): Relationship of coccidial lesion scores and weight gain in infections of Eimeria


Davies, S.F.M., & Kendall, S.B. (1954): The effect of sodium sulphaquinoxaline and sodium sulphamezathine in interrupted schedules of treatment on the development of E. tenella. *Journal of Comparative Pathology & Therapeutics, 64, 87-93.*


Kappe SH, Vaughan AM, Boddey JA, and Cowman AF (2009): "That was then but this is now: malaria research in the time of an eradication agenda". *Science* 328 (5980): 862–6.


Toby Leslie; M. Ismail Mayan; M. Anwar Hasan; M. Hanif Safi; Eveline Klinkenberg; Christopher J. M. Whitty; Mark Rowlan (2007): Sulfadoxine-Pyrimethamine, Chlorproguanil-Dapsone, or Chloroquine for the Treatment of Plasmodium vivax Malaria in Afghanistan and Pakistan. JAMA. 297(20):2201-2209.


## APPENDIX ONE:

Analysis of Variance for the experimental medications

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>not infected not treated</td>
<td>2</td>
<td>4157.267**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>infected not treated</td>
<td>2</td>
<td>29192.494NS</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>not infected not treated</td>
<td>2</td>
<td>76919.530</td>
</tr>
<tr>
<td></td>
<td></td>
<td>infected not treated</td>
<td>2</td>
<td>13194.056</td>
</tr>
<tr>
<td></td>
<td></td>
<td>not infected not treated</td>
<td>2</td>
<td>79425.159</td>
</tr>
<tr>
<td></td>
<td></td>
<td>infected not treated</td>
<td>2</td>
<td>27246.304</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Primaquine</td>
<td>8</td>
<td>6755.9**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fancedar</td>
<td>8</td>
<td>8953.21*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Artisunate</td>
<td>8</td>
<td>10365.907*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amprolium</td>
<td>8</td>
<td>82053.402NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treated not infected</td>
<td>18</td>
<td>28906.785</td>
</tr>
<tr>
<td></td>
<td></td>
<td>not infected not treated</td>
<td>18</td>
<td>76919.530</td>
</tr>
<tr>
<td></td>
<td></td>
<td>infected not treated</td>
<td>18</td>
<td>13194.056</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Primaquine</td>
<td>18</td>
<td>79425.159</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fancedar</td>
<td>18</td>
<td>27246.304</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Artisunate</td>
<td>18</td>
<td>7809.210</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amprolium</td>
<td>18</td>
<td>65840.342</td>
</tr>
</tbody>
</table>

* Significant at 1% level.
* Significant at 5% level.
NS not significant.
FIGURE ONE:
Oocysts of *Eimeria tenella*
FIGURE TWO:
Sporulated oocysts of *Eimeria tenella*
FIGURE Three:
Infected caecum by *Eimeria tenella*
FIGURE Four:

*Eimeria tenella* life cycle