COMPARATIVE EFFICACY OF COCCIDIOSTATS ON EXPERIMENTALLY INDUCED *EIMERIA TENELLA* INFECTION ALONG WITH EFFECTS ON GROWTH HAEMATO-BIOCHEMISTRY AND PATHOLOGY IN BROILERS

A
THESIS
SUBMITTED TO THE
ANAND AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE

OF
DOCTOR OF PHILOSOPHY

IN
VETERINARY PARASITOLOGY

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ABSTRACT

COMPARATIVE EFFICACY OF COCCIDIOSTATS ON EXPERIMENTALLY INDUCED *EIMERIA TENELLA* INFECTION ALONG WITH EFFECTS ON GROWTH, HAEMATO-BIOCHEMISTRY AND PATHOLOGY IN BROILERS

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ABSTRACT

The efficacy of three commonly used feed coccidiostats named Diclazuril (T1) Salinomycin (T2), Diclazuril + Salinomycin (T3) in shuttle programme and Maduramicin (T4) on experimentally induced *Eimeria tenella* coccidial infection and their effects on growth, haematology, biochemical and histopathological changes were undertaken in three hundred Cobb400 strain of broiler at University Poultry Complex, Anand Agricultural University, Anand during year 2012. Birds were given feed containing Diclazuril (T1), Salinomycin (T2), and Maduramicin (T4) coccidiostats at dose rate of 1 ppm, 60 ppm and 5 ppm upto 42 days. Weekly body weight and feed consumption were recorded. Various parameters considered for comparative efficacy were studied. Experimental infection of 50,000 oocysts of *E.tenella* was given on 22nd day of age. Blood was collected before experimental infection at 3 weeks and after experimental infection at 4 weeks of age for haemato-biochemical study.

The results of faecal score, oocyst per gram (OPG), lesion score, oocyst index value and mortality indicated better efficacy of coccidiostats as compared to non medicated birds in experimental infection with better efficacy of Maduramicin and Salinomycin as compare to Diclazuril and Diclazuril + Salinomycin shuttle treatment .

Coccidiostats proved to have growth promoting action in broiler chickens during the experimental infection. Birds fed with Maduramicin medicated (5 ppm) performed well in terms of live weight gain and feed conversion ratio and it was followed by salinomycin (60 ppm) for weight gain and Diclazuril (1ppm) for feed efficiency in broiler birds.

Result of sensitivity against *E. tenella* indicated good efficacy of Maduramicin (82%), whereas limited efficacy of Salinomycin (76%), Diclazuril (74%) and Diclazuril + Salinomycin Shuttle group (71%) on the basis of Global index value (GI_{NNC} %)

Haematological studies revealed that haemoglobin concentration, packed cell volume and total erythrocytes counts were significantly ($P < 0.05$) reduced, while total leukocytes counts were significantly increased on account of coccidial infection in all coccidiostat treatment and infected non treated groups. Different Leukocytes Count (DLC) value revealed significant increase in heterophills, lymphocytes and eosinophills and significant decrease in monocytes and basophills on account of coccidial infection. Results on haematological studies indicated comparatively less pathological damage by Salinomycin.

. Studies on biochemical profile revealed significantly ($P < 0.05$) lower serum glucose and serum total protein, while significant increase in serum total cholesterol,

Serum Glutamic Oxalo-acetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and Alkaline Phosphatase (AKP) activities was observed due to coccidial infection as compared to pre infection levels in birds. Results of biochemical studies indicated comparative less pathological damage by coccidiostats treatment as compared to infected non treated group, but there was no consistent trend for drug choice.

From histopathological study it was clear that the Maduramicin and Salinomycin treated group showed very less mechanical damage to tissue hence it could be used as a curative remedy against the caecal coccidiosis. The presence of clusters of large schizonts in the caecum was pathognonomic for *E. tenella.* The magnitude of infection type and dose of coccidiostat and stage of development of the disease could be established by histopathological observation.

ACKNOWLEDGEMENTS

Acknowledgements

I take this opportunity to extend my deep sense of gratitude and words of appreciation toward those, who helped me during the pursuit of my present study.

It gives me a great privilege and immense pleasure in expressing my sense of gratitude to my Major Guide Dr. J. J. Hasnani, Ph.D., Professor & Head , Department of Parasitology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand for his prolific and introspective guidance, encouragement with constructive suggestions, judicious and deliberative discussions, critical appraisal of manuscript, active persuasion and continuous supervision throughout the course of my study.

I wish to express my sincere gratitude and thanks to my Minor Guide Dr. D.J. Ghodasara, Associate Professor, Department of Pathology for his valuable and timely constructive suggestions and guidance. I am also thankful to the members of my advisory committee, Dr. P.V. Patel, Professor, Department of Parasitology, Dr. M. N. Brahmbhatt, Professor & Head, Department of Veterinary Public Health and Director of Student Welfare, Anand Agricultural University, Anand, Dr. R.S. Joshi, Associate Professor, Department of Animal Genetics and Breeding for their timely help and valuable suggestions.

I am extremely thankful to F.P. Savaliya, Principal Scientist & Head, Poultry Complex for providing all necessary facility for smooth conduction of experiment in the farm. I am also thankful to Dr. K.S. Prajapati, Professor & Head, Department of Pathology for help in sample collection as well as for extending facilities in haematological and histopathological work. I am also thankful to all the staff members of Pathology Department, namely Dr. B.P. Joshi, Dr. C.J. Dave, Dr. Purnima Jani, Shri Amitbhai Patel and Ku.Himani Patel, for the assistance rendered by them during the tenure of my work.

I am thankful to P.G. Students namely Dr.Suchit, Dr. Vandeep and entire staff namely Shri Manojbhai, Shri Hemubhai and shri Jasubhai from Department of Parasitology for providing all the facilities and assistance as and when required.

I am very much thankful to Dr. A.B. Patel for his assistance and help for experiment and blood sample collection.

I am also thankful to Dr. D. J. Parmar, Associate professor, Dr. J. S. Patel Professor & Head and Mr. Mayur, Ph.D. Scholar, Department of agricultural Statistics for statistical analysis of experimental data.

I am also thankful to Dr. J.S. Arya, Retd. Professor and Head, Department of Physiology, Dr A.M. Pande, Professor and Head Biochemistry, Dr.Sanjay Patel Research Fellow and Shri Bhattbhai, Retd. Lab Technician for providing necessary facilities in biochemical and haematological analysis of blood and sera samples.

Encouragement and support given by Dr. L.G. Kathiriya, Retired Professor, Dept. of Parasitology, Dr. A. J. Dhami, Professor & Head, Dept. of Gynaecology & Obstetrics and Dr. G.C. Mandali, Associate Professor, Dept. of Medicine, Veterinary College, Anand is heartily acknowledged.

I am extremely thankful to Dr. R.K. Mishra, Dr. N. J. Bhagora and Smt. Josephineben, Shree Ketanbhai and all the staff members and farm workers of Poultry Complex for assisting and helping me in my research work.

I want to express my thanks to staff of College Library for their necessary support.

I further extend my thanks to the Anand Agricultural University authorities namely Dr. A M. Shekh, Former Hon. Vice Chancellor, Dr. K.B.Kathiriya, I/C Hon. Vice Chancellor and Director of Research & Dean P.G. Studies, Dr. M.M.Pathak, Associate Director of Research, Dr. D. M. Korat, Associate Director of Research,

Dr. P.R. Vaishnav, I/c Registrar, for according permission and necessary help for my P.G. studies.

I am also thankful to Dr. J. V. Solanki Retired Principal and Dr. A.M. Thaker, Principal & Dean, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand for providing facilities and support for my P.G. studies.

The love, affection and encouragement given to me by my beloved wife, daughter, son, brother, sister and friends during my study tenure is heartily acknowledged.

Lastly and everly, I would like to pray my respects to parents and Almighty God, who blessed me to achieve this success.

Place: Anand

Date: 16/07/2014
26/07/2014

(N.D. Hirani)

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ABBREVIATIONS

A.A.U	Anand Agricultural University
ACP	Anti Coccidial Products
AKP/ALP	Alkaline Phosphatase
ALT	Alanine Amino Transferase
AST	Aspartate Amino Transferase
a. m.	ante meridian
ANOVA	Analysis of variance
BIS	Bureau of Indian Standards
BW	Body Weight
BW_0	Body weight at day old age
BW_1	Body weight at 1st week
BW_2	Body weight at 2nd week
BW_3	Body weight at 3rd week
BW_4	Body weight at 4th week
BW_5	Body weight at 5th week
BW_6	Body weight at 6th week
BWG	Body weight gain
BWG_{0-1}	Body weight gain from 0-1 week
BWG_{0-4}	Body weight gain from 0 to 4 week
BWG_{0-6}	Body weight gain from 0 to 6 week
BWG_{1-2}	Body weight gain from 1-2 week
BWG_{2-3}	Body weight gain from 2-3 week
BWG_{3-4}	Body weight gain from 3-4 week
BWG_{4-5}	Body weight gain from 4-5 week
BWG_{4-6}	Body weight gain from 4 to 5 week

BWG_{5-6}	Body weight gain from 5-6 week
CD	Critical Difference
Cm	Centimeter
cmm	Cubic millimeter
CHOD	Cholesterol Oxidase
CP	Crude Protein
DCP	Digestible Crude Protein/Di Calcium Phosphate
Df	Degree of freedam
DOT	Dinitro o Toluanimide
DL	Dextro livo
DLC	Differentiate Leukocytes Count
DPI	Day Post Infection
EOB	Energy Oxygen Balance/Ecological Oxidative Balance
et al.	*et alli* (and associates)
FC_1	Feed consumption at 1^{st} week
FC_2	Feed consumption at 2^{nd} week
FC_3	Feed consumption at 3^{rd} week
FC_4	Feed consumption at 4^{th} week
FC_5	Feed consumption at 5^{th} week
FC_6	Feed consumption at 6^{th} week
FCR	Feed Conversion Ratio
FCR_{0-4}	Feed conversion ratio from 0 to 4 week
FCR_{0-6}	Feed conversion ratio from 0 to 6 week
FCR_1	Feed conversion ratio at 1^{st} week
FCR_2	Feed conversion ratio at 2^{nd} week

FCR_3	Feed conversion ratio at 3rd week
FCR_4	Feed conversion ratio at 4th week
FCR_5	Feed conversion ratio at 5th week
FCR_{5-6}	Feed conversion ratio from 5 to 6 week
FCR_6	Feed conversion ratio at 6th week
FI	Feed intake
F_{Ic}	Feed Conversion Ratio Infected Control
F_{IM}	Feed Conversion Ratio Infected Medicated
Fig.	Figure
g/gm	Gram
G I	Global Index
GDP	Gross Domestic Product
GOD	Glucose Oxidase
Hb	Haemoglobin
H & E	Haematoxylene and Eosine
i.e.	That is
IM	Infected medicated control
INC	Infected Non medicated control
INR	Indian Rupees
IU	International Unit
Kcal	Kilo calorie
Kg	Kilogram
LDH	Lactic De Hydrogenase
LS	Lesion Score
ME	Metabolizable energy

µ	Micron
µl	Micro litter
mg	Milligram
ml	milliliter
NNC	Non infected Non medicated Control
n	Number of birds/ samples
No.	Number
OI	Oocyst Index
OPG	OocystPer Gram
POD	Peroxidase
PCV	Packed Cell Volume
P^H	Logarithm of Negative Ions
p.m	past meridian
PI	Post Infection
ppm	parts per millon
P<0.05	Significance at 5% level
P<0.01	Significance at 1% level
Q.S.	Quantum sufficient
RBC	Red Blood Cell
Rs.	Rupees
SCE	Sugar Cane Extract
T_1	Treatment one (with Diclazuril as Coccidiostat)
T_2	Treatment two (with Salinomycin as Coccidiostat)
T_3	Treatment three (with Diclazuril + Salinomycin as Coccidiostat)
T_4	Treatment four (with Maduramicin as Coccidiostat)

T_5	Treatment five (Infected Non Medicated Control =without Coccidiostat called Positive Control)
T_6	Treatment six (Non Infected Non Medicated Control =without Coccidiostat called Negative Control)
TFC	Total Feed Consumption
$TFC_{(0-4)}$	Total feed consumption from 0 to 4 weeks of age
$TFC_{(0-6)}$	Total feed consumption from 0 to 6 weeks of age
$TFC_{(4-6)}$	Total feed consumption from 4 to 6 weeks of age
SGOT	Serum Glutamic Oxaloacetate Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
STC	Serum Total Cholesterol
STP	Serum Total Protein
Spp.	Species
TEC	Total Erythrocyte Count
TLC	Total Leukocytes Count
US	United State
USA	United State of America
viz.	Namely
Wt.	Weight
Symbols	
@	at the rate of
<	Is less than
>	Is greater than
%	Percentage
±	Plus or minus
=	Equal to

INTRODUCTION

CHAPTER – I

INTRODUCTION

Agriculture and livestock contribute greatly to the world gross domestic product, especially in the developing countries where they retain a fundamental role for the economic sustenance of millions of people. In India, poultry industry is booming and emerging as the world's second largest market with a growth of 12-15% year on year. Chicken meat is an important source of animal protein both in rural and urban areas and owing to their relatively low fat and cholesterol contents than other meat, chicken meat is considered as a healthy animal food (Umaya, 2014). Poultry is one of the fastest growing segments among the component of agricultural sector in India. The total poultry population which was only 73.5 million in 1951 made tremendous growth during the past fifty years and has reached 648.83 million during 2007. Today India occupied third position in egg production (which was 5th in 2000) and fifth position in poultry meat production (which was 13th in 2000) in the world. An average growth rate of poultry industry is about 5% in layer sector and 10% in broiler sector over last decade (Singh, 2012). The contribution of livestock sector to the country's GDP is Rs. 2366.5 billion in which contribution from poultry sector is Rs. 411.5 billion during 2010-2011. About 66.7 per cent of the total output from poultry is realized from the poultry meat sector and only 33.3 per cent from egg production. The organised sector of the poultry is contributing nearly 70 per cent of the total output and the rest 30 per cent is by the unorganised sector.

Broiler production is one of the best ways of supplying good quality animal protein for human consumption. Broilers are the quickest, most economic and the most efficient

converter of plant material into food of high biological value (Bootwalla, 2005). Poultry meat constitutes around 20% of total meat production in the country (Singh, 2012). The per capita availability of poultry meat is 2.15 kg/annum which is very less as against the recommendation of 11 kg meat/annum given by National Institute of Nutrition (Prabhakaran, 2012). Hence, there is tremendous scope for further development in poultry meat sector.

In intensive poultry farming, birds are exposed to several stress factors which enhance their susceptibility to various diseases that are already existing or emerging. Intensification of poultry industry and continuous genetic improvement for rapid growth and feed efficiency inevitably increases the prevalence of diseases and losses possibly due to immune suppression, caused by various infectious and non infectious agents (Selvam *et al.*, 2003). Challenges from bacteria, viruses and parasites placed a limit on production potential and high mortality was often noticed at the farms. Poultry sector is still confronted with many enteric diseases like coccidiosis which are hindering its progress (Saima *et al.*, 2010). Over the period we have developed health management systems and with the aid of effective vaccines, antibiotics and coccidiostats, it is now possible to restrict the economic losses from the infectious agents.

Among the diseases, coccidiosis is a widely known, greatly studied and yet incompletely understood protozoan disease of poultry. It is a disease which accounts for 5-10 per cent mortality rate of chickens and an unknown loss due to reduced weight gain and feed efficiency, damage to the digestive tract, decreased egg production and lowered resistance of birds to other poultry diseases. There is no cross immunity between different *Eimeria spp.* and later outbreaks may occur due to different species. The principal effect of coccidiosis is reduced growth rate and poor feed conversion but this is not easy to measure in the field since data from uninfected flocks is unavailable. Coccidiosis may cost the US

chicken industry about $127 million annually (Chapman, 2009). Coccidiosis remains one of the most expensive and common diseases of poultry production in spite of advances in chemotherapy, management, nutrition and genetics (Mc Dougald and Reid, 1991).

This disease is one of the most prevalent significant problems in poultry throughout the tropical countries (Chakrabarti, 1989) and the annual worldwide loss due to coccidiosis was estimated about $ 800 million (Williams, 1998). Bera *et al.* (2010) estimated the total loss of Rs 1.14 billion due to coccidiosis in Indian poultry industries during the year 2003-04. Like many parasitic diseases, coccidiosis is largely a disease of young birds because immunity quickly develops after exposure and it gives protection against later disease outbreaks. The short direct life cycle and higher reproductive potential of coccidia in poultry intensify the potential of severe outbreaks of disease in the modern poultry houses. Moreover, coccidiosis may strike any type of poultry under any type of management. Vaccines against coccidiosis have met with limited success and have been mostly used in breeder pullets (Mc Dougald and Reid, 1991). Thus coccidiosis is probably the most expensive disease that afflicts intensively raised poultry. Almost all chickens are reared with an anticoccidial drug in the feed, at considerable expense, testimony that coccidiosis remains an important problem.

Coccidiosis is a major parasitic disease of poultry and is caused by the Apicomplexan protozoan of the Genus *Eimeria*. The underlying mechanisms of the host specificity are not well understood but most likely include genetic, nutritional, biochemical and immune factors. In addition to host specificity, a given *Eimeria* parasite only infects particular cell types or tissues in a given host (Lillehoj and Okamura, 2003). Coccidiosis causes mortality, malabsorption, inefficient feed utilization, impaired growth rate in broilers and reduced egg production in layers (McDougald, 2003; Lillehoj *et al.*, 2004). Without the administration of anticoccidials in feed or in drinking water, economic broiler production is inconceivable. In

spite of the advances in immunological, biotechnological and genetical methods, control of coccidiosis chiefly depends upon prophylactic chemotherapy with anticoccidial drugs. However, the emergence of drug resistance in coccidia is a great problem with most of the drugs, which, in due course, limit their use (Abbas *et al.*, 2012).

Becker (1959) reviewed eight species of the genus *Eimeria* occurring in chickens. These were *E. tenella* (Railliet and Lucet, 1891), *E. mitis, E. acervulina, E. maxima* (Tyzzer, 1929), *E. necatrix, E. praecox, E. hagani* (Levine, 1938) and *E. brunetti* (Levine, 1942). Edgar and Seibold (1964) described *E. mivati* for the first time in USA and subsequently Reid and co-workers (1965) reported it from poultry in Europe. Ray (1945) described *Wenyonella gallinae* from chicken in India, which is a solitary member of the Genus *Wenyonella.*

Levine (1982) described the tenth species of *Eimeria*, as *E. sporadica* in addition to the above nine species of *Eimeria* in chicken but validity of *E. sporadica* was questioned. Soulsby (1982) listed all the nine species of *Eimeria*, one species of *Wenyonella* and one species of *Crytosporidium,* viz. *C. tyzzeri* in chicken.

E. tenella is the ubiquitous and most pathogenic parasite responsible for caecal coccidiosis with high rate of mortality in poultry. The lesions caused by the parasite disturb nutrient absorption, triggering several changes in carbohydrates, lipid, protein and mineral metabolism (Patra *et al.*, 2010). This protozoa inhabits the caeca and adjacent intestinal tissues causing a severe disease characterized by bleeding, high morbidity and mortality, low weight gain, emaciation and other signs attributed to coccidiosis.

There are basically two means of prevention of coccidiosis: chemoprophylaxis and vaccination. Chemoprophylaxis using so-called Anti Coccidial Products (ACP) or

coccidiotats in the ration is the most popular. It is estimated that 95% of the broilers produced receive anticoccidials (Chapman, 2009).

Generally two groups of anticoccidials are considered, ionophorous antibiotics or 'ionophores' and synthetically produced drugs, also denominated as 'chemicals'. Chemicals were the first type of drugs being used in treatment and later on in prevention of coccidiosis (Abbas *et al.*, 2008). According to McDougald and Reid (1991), the shuttle program is intended to improve control of coccidiosis. Repeated exposure to the same anticoccidial drug can result in the selection of drug-resistant strains of *Eimeria* (Chapman, 2001), which might be expected to result in reduced efficacy of anticoccidial drugs.

In broiler production, numerous anticoccidial drugs are used for prevention and control of coccidiosis. However, development of tolerance to these drugs have lead to search for newer molecules and different classes of anticoccidials have been discovered and used from time to time for prevention and control of coccidiosis.

The use of drugs for coccidiosis control commenced in the early 1940s and many scientists from government, university, and private institutions were involved in this work. A pioneering publication from the Rhode Island Agricultural Experiment Station was the first to show that coccidiosis could be prevented by incorporating a drug in the feed and this was to revolutionize control of the disease (Chapman, 2009). Subsequently many novel compounds were introduced with varying success. Generally, the occidiostats need to be fed for the life of the animal (in the case of broilers) in order to protect against re-infection from the ever-present oocyst stage of the disease. The availability and the continuous preventive use of coccidiostats has significantly contributed to the development and growth of the poultry production as well as health and welfare. A major boost in coccidiosis control occurred in the 1970's with the introduction of monensin as the first ionophore coccidiostat. This allowed

broiler production to develop to its present scope. Prior to this, only chemical coccidiostats were available with their somewhat erratic efficacy due to rapid build up of resistance by the parasite. Coccidiosis outbreaks were common and difficult to treat or prevent.

Ionophore drugs are mono carboxylic polyesters antibiotics, which are the production of fermentation of *Streptomycis* types and are used in a large extent in poultry production industry to control and prevent coccidiosis disease. At present ionophore drugs include salinomycin, lasalocid, narasin, Maduramicin and semduramycin. Ionophoric antibiotics combine with a number of mono and divalent cat ions and in the form of bi-complexes make it possible to transfer ions through lipid hydrophobic membrane, and when they are added to diet, they change bioavailability, gut uptake and absorption and reserves of nutrient in tissues (Elsasser, 1984).

The effective use of anticoccidial feed additives has played a major role in growth of poultry industry. "Continuous feeding low concentration of sulphaquinoxaline for the control of coccidiosis in poultry" by Grumbles and co-workers in 1948, was the first paper to demonstrate that it was possible to control coccidiosis by the continuous inclusion of low level of drug in the feed of chicken. Bedrnik (1983) established that reduced efficacy of an anticoccidial has an adverse effect on weight gain and feed conversion efficiency of chicken. Conway *et al.* (1993) stated that the weight gain is the most sensitive and informative measure of anti coccidial efficacy. The continuous use and misuse of anticoccidials led to the emergence of drug resistant strains. Besides, drug residues in poultry products cause potential hazard to the consumers. This necessitates the exploration of a safe, economic and effective alternative for the control of avian coccidiosis (Shameem, *et al.* 2010). The broiler industries has found that the use of anticoccidials drug is generally cost effective as insurance against losses. However recurring expenditure for feed medication in large scale broiler production

reminds producers that control of this disease will be a continuing expense unless some cheaper method of control is discovered. Today almost all broiler flocks receive preventive medication and therapy used as a last resort (Mc Dougald and Reid, 1991). The poultry industries might be considered fortunate because coccidia resistant to the ionophores are sensitive to some of the other chemical compound. There are about 13 compounds available for the control of coccidiosis in broilers. The worldwide market of anticoccidial drug is to be estimated at about 300 million dollar per year. Despite the availability of many anticoccidial drug, infection caused by the *Eimeria spp.* continued to be a source of significant economic loss to the poultry industry (Chapman 2009).

Coccidiosis causes not only economically important changes such as impaired growth and poor feed utilization, but also produce more subtle changes in metabolism (Allen, 1988). The parasite multiplies in the intestinal tract and causes tissue damage, resulting in diminished feed intake and nutrient absorption, reduced body-weight gain, dehydration, blood loss, and increased susceptibility to other diseases (Davies *et al.,* 1963; Turk, 1978; McDougald, 2003). The induced tissue damage and change in intestinal function may allow colonization by various harmful bacteria, such as *Clostridium perfringens*, leading to necrotic enteritis (Helmboldt and Bryant, 1971, Maxey and Page, 1977). Caecal coccidiosis caused by *Eimeria tenella* may contribute to an increased severity of blackhead disease in chickens. The studies have shown that coccidiosis can affect the ability of poultry to use nutrients and thus produce a variety of nutritional disorders (Ruff *et al.*, 1986). The effect of coccidiosis on poultry health and down- grading poultry carcass should be considered and specific prophylactic measures should be implemented (Trentin *et al.*, 2000).

It produces deviation in the various haematological and biochemical components of the body (Turk, 1985.; McDougald and Reid, 1991; Panda *et al.*, 1997.; Deger *et al.*, 2002.;

Patra *et al.*, 2010). In the organism, enzyme action is influenced by metabolic activities and pathologic condition. To combat clinical and subclinical forms of poultry diseases, accurate and differential diagnosis of the diseases at early stages of infections is necessary (Talebi *et al.*, 2005).

Amer *et al.* (2010) pointed that *E. tenella* showed more lesion score and histopathological changes in caecum as compared to other *Eimeria spp.* Basu *et al.* (1990) reported that *E.tenella* as a predominant cause of heavy economic loss in poultry. In the ensuing years, coccidial lesion scores, in particular the procedure described by Johnson and Reid (1970), assumed an important role as a measure of anticoccidial efficacy.

Coccidiosis continues to be one of most important disease causing significant economic losses to the poultry industry despite the availability of latest anticoccidial drugs. Many anticoccidial drugs were introduced but sooner or later resistance to all these products was reported in *Eimeria* species (Chapman, 1993). The problem of drug resistance to different anticoccidials under field condition has left broiler producers in a dilemma regarding choice of drug for control of disease at their farm. Gujarat state has the broiler population around 33.30 lakhs out of total 1.33 crore poultry as per the Livestock census 2007 (Summary report on 18th Livestock census 2007, Gujarat State, 2009). As per the 29th survey report on estimates of major livestock products for the year 2011-2012 the total poultry population is estimated to be 3.91 crore (Directorate of Animal Husbandry, Gujarat State, 2011-12). Systemic studies on coccidiostats are meagre in Gujarat.

There is a need of systemic studies of coccidiostats in broilers in middle and southern Gujarat being the major pocket of broiler production. The knowledge of comparative efficacy of coccidiostats along with clinico- pathological study in broilers might be helpful for the proper selection of coccidiostat for prevention of most pathogenic caecal

coccidiosis and better improvement in broiler production. The knowledge will also help in diagnosis and control of coccidiosis in this area. So the comparative study of coccidiostats in broiler by giving experimental infection of *E. tenella* is planned with following objectives.

1. To study comparative efficacy of coccidiostats on faecal score and lesion score in broilers.
2. To find comparative efficacy of coccidiostats on
    i) Growth performance and Feed Conversion Ratio in broilers.
    ii) Alteration in haemato - biochemical parameters.
    iii) Changes in histopathological lesions.

REVIEW

OF

LITERATURE

CHAPTER – II

REVIEW OF LITERATURE

The present investigation was undertaken in broiler birds in Anand district of Gujarat. The main objective is to ascertain the efficacy of coccidiostats in caecal coccidiosis with its effect on haematology, biochemical and enzymatic profile and caecal histopathology of different broiler groups managed under cage system of housing. Lot of work has been done on chicken coccidiosis in India and abroad since its first recognition. Anand district being a major pocket of poultry production in Gujarat State, special attention on coccidiosis, an economically devastating poultry disease, becomes mandatory. The literature pertaining to the investigation has been reviewed below in following sub topics:

1. **Efficacy of Anticoccidials**
2. **Body weight and feed efficiency**
3. **Mortality, Faecal and Lesion scoring**
4. **Haemato-Biochemical and Enzymatic Changes**
5. **Histopathology**

2.1 Efficacy of Anticoccidials

Mc Loughlin and Gardinar (1962) conducted a trial with three week old chickens, which were infected with different levels of inoculums varying from 1,000, 5,000, 25,000, 50,000, 1, 00,000 and 2, 00,000 of *Eimeria tenella* oocysts to study the efficacy of amprolium.

Caecal coccidiosis resulted in significant reduction in body weight as a result of stress, feed intake and poor absorption of nutrients from the intestine. Changes in body weight provide a more sensitive indirect measurement of the severity of infection (Mukkur and Bradley, 1969).

Clinical trials with amprolium compounds in caecal coccidiosis in chickens were studied by Johnachan *et al.* (1972). A limited study with White Leghorn chicks was conducted to determine the comparative coccidiostatic efficacy of 0.024 per cent amprolium hydrochloride in drinking water, and 0.043 per cent sulphaquinoxaline in drinking water, amprolium showed better coccidiostatic properties in comparison with sulphaquinoxaline on analysis of data.

Kinashi *et al.* (1973) were the first to isolate salinomycin, a monocarboxylic acid polyether ionophore produced by a strain of *Streptomyces albus* in Japan.

Miyazaki *et al.* (1975) found that the salinomycin drug was effective in reducing the mortality and increasing the average weight of chickens experimentally infected with *E. tenella.*

Bajwa and Gill (1977) infected the birds with different magnitudes of 1000, 5000, 25,000, 50,000 and 100,000 oocysts of *Eimeria tenella* to study the efficacy of amprolium.

Danforth *et al.* (1977[a]) reported that the salinomycin compound had significant anticocoidial activity at 60 to 100 ppm levels when titrated against single or mixed infections of six species of *Eimeria* viz. *E. acervulina, E. mivati, E. maxima, E. tenella, E. necartix* and *E. bruneti.* The drug at 100 ppm treatment level was statistically as effective as 121ppm monensin in controlling coccidiosis.

Danforth *et al.* (1977[b]) compared the efficacy of salinomycin at various treatment levels (60 to 100 ppm) in two experiments against un medicated and either 100 or 121 ppm monensin medicated groups. Salinomycin at all treatment levels showed significant anticoccidial activity for all parameters studied viz. mortality, weight gain, feed conversion, dropping scores and lesion scores. They found no significant differences between the activity of any level of salinomycin and monensin based on mortality, weight gain and feed conversion.

Morrison *et al.* (1979) studied the efficacies of salinomycin, monensin and halofuginone and found that all the three drugs were equivalent in controlling coccidial infections.

Raether (1980) reported that salinomycin at 60 ppm was effective against severe experimental infection of *Eimeria spp.* and multiresistant field isolates of *E. tenella* and *E. acervulina.*

Wornick *et al.* (1980) reported that salinomycin at the rate of 60 ppm was effective in controlling chicken coccidiosis based on weight gain, feed conversion, reduced mortality and clinical observations. They opined that salinomycin was superior over monensin or other anticoccidial.

Chang *et al.* (1982) observed that salinomycin at 60 mg/kg level was superior to monensin (100mg/kg) or clopidol with decoquinate (100 and 8.35 mg/kg) in floor pen trial in broilers in sub tropical Taiwan. In six field trials the drug was comparable or superior to other anticoccidials tested (Clopidol, halofuginone, lasalocid, monensin and nicarbazin with ethopabate) on the basis of weight gain, feed conversion, reduced mortality and clinical observations.

Raether and Bauer (1984) found that salinomycin sodium at a concentration of 50-70 mg/kg of feed controlled experimental infection of *E. acervulina*, *E. tenella*, *E. tenella* with *E. bruneti* and the anticoccidial action was better than that of several other compounds tested in comparative trials.

Gomez *et al.* (1985) inoculated 22 day old hygienically raised White Leghorn chicks with $5x10^4$ sporulated oocysts of *Eimeria tenella* strains to know the prophylactic activity of amprolium on performance of birds.

Hyun *et al.* (1986) to determine the total oocyst production experimentally inoculated the strains of *E. tenella*, *E. maxima* and *E. acervulina* developed from single oocyst isolation. They inoculated to the birds by oral route at a dose rate of $1X10^6$ sporulated oocysts.

Drug sensitivity of 99 isolates of coccidia from broiler farms was studied by Larry *et al.* (1986). They isolated the coccidian from broiler farms and tested for sensitivity to contemporary anticoccidial drugs. The isolates usually comprised of *E. acervulina, E. maxima, E. tenella and E. brunetti.* They concluded that isolates were resistant to monensin, salinomycin, nicarbazin and amprolium + ethopabate, based on intestinal lesion score reduction.

Mc Dougald *et al.* (1986) isolated coccidia from broiler farms in 12 broiler producing states and tested them for sensitivity to contemporary anticoccidial drugs. The isolates usually comprised of 2 or more species including *Eimeria acervulina*, *E. maxima, E. tenella* and *E. bruneti.* Based on intestinal-lesion-score reduction isolates were resistant to monensin (110 ppm), salinomycin (60 ppm), nicarbazin (125 ppm), and amprolium + ethopabate (125 + 4 ppm).

Yun *et al.* (1986) tested salinomycin at 50, 70, 90 ppm in feed against mixed infection of *E. tenella*, *E. acervulina* and *E. maxima* and recommended 70 ppm as the optimal dosage for routine anticoccidial treatment. However in mild or severe infections 50 or 90 ppm dosages were suggested to be effective.

The effect of lasalocid sodium (Avatec) on feed and water consumption and maturity in chicks by experimentally induced caecal coccidiosis was studied by Kaul and Verma (1987). Lasalocid sodium when given at a rate of 150 ppm fairly reduced the feed conversion ratio and was found to be superior to other groups. Further, water consumption by medicated chicks was slightly more than the unmedicated chicks. The maturity of chicks did not vary in treated groups, but it was delayed by over 3 weeks in birds that suffered and recovered without treatment.

Jo and Jang (1987) reported that salinomycin at 50 to 100 ppm was effective against *Eimeria spp.* infections and at 60 ppm level was comparable to monensin at 100 ppm or maduramicin at 5 ppm. They did not observe any evidence of drug resistance to salinomycin.

Muzurkiewez *et al.* (1987) studied the effect of ionophore coccidiostats against *E. tenella* and *E. acervulina* infections and noted that the best anticoccidial index was obtained for maduramicin followed by lasalocid, narasin, salinomycin and monensin.

Srivastava *et al.* (1987) evaluated anticoccidial activity of salinomycin against drug resistant strains of *E. tenella* in broiler chicks. Salinomycin at 60 ppm recorded high significant weight gain over infected non treated control and resulted in significant reduction in lesion scores, whereas Salinomycin treated group recorded an improvement of 23.4 percent in feed efficacy over infected non treated group. Mortality was also checked by addition of

salinomycin to the feed. In overall assessment, the experiment proved that salinomycin had a significant anticoccidial effect in broilers.

Weber *et al.* (1987) compared the efficacy of lasalocid with other ionophorous coccidials against *E. acervulina* and *E. tenella* infection in chicken. They observed that both lasalocid and salinomycin were equally effective in cases of high infection intensity while monensin and narasin proved less effective.

Folz *et al.* (1988) made comparative anticoccidial evaluation of halofuginon, lasalocid, maduramicin, monensin and salinomycin at 3, 125, 5, 120 and 66 ppm respectively and reported that all the five drugs demonstrated significant activity against *E. tenella*, *E. maxima*, *E. necatrix*, *E. brunetti* and *E. acervulina*.

Stallbaumer and Daisy (1988) established the *in-vivo* sensitivity of 47 isolates of coccidia to monensin (100 mg/kg), narasin (70 mg/kg), salinomycin (100 mg/kg) and nicarbazin (125 mg/kg).

Varga *et al.* (1988) studied the anticoccidial efficacy of maduramicin ammonium at 5 mg/kg dietary level in the floor pen trial against *E. tenella* and *E. acervulina* infection and the drug proved to be as good as or superior to other ionophorous anticoccidials ie. monensin, narasin, salinomycin and lasalocid.

Chapman (1989) in a trial obtained field isolates of *Eimeria tenella* from four local broiler farms and infected a dose of 1 x 10^5 sporulated oocysts per bird to know the efficacy of toltrazuril, sulphaquinoxaline / pyremethamine and amprolium / ethopabate.

Salisch and Shakshouk (1989) reported that diclazuril treated chickens performed best followed by uninfected group, maduramicin, salinomycin, narasin and monensin treated groups in terms of feed conversion efficiency, growth rate, and pathogenic effects.

Basu *et al.* (1990) reported that coccidiosis was an acute problem in the Kalyani Kanchrapara areas of West Bengal and *Eimeria tenella* was the common species found. They studied the efficacy of Coyden-25 (Clopidol) and Coxidot (3-5 dinitro- ortho- toludamide) and concluded that both the drugs were found beneficial for weight gain of the broiler birds, and prevent *E. tenella* infection.

Yvore *et al.* (1990) found that salinomycin was highly efficacious at 60 ppm based upon improved performance, lesion score, haematocrit and serum optical density in the control of coccidiosis in chicks and they observed no significant differences between salinomycin, monensin or halofuginone

Muangyai *et al.* (1991) inoculated the birds with 10^4 oocysts via stomach tube in battery trials to study the efficacy of toltrazuril as prophylactic chemotherapeutic agent.

Conway *et al.* (1993) compared the efficacy of a new ionophore semduramicin with salinomycin against different stages of *E. tenella and E. acervulina* in a series of *in-ovo* and *in-vivo* trials. When the two drugs were given at 93 h post inoculation both drugs exerted same effect against late schizogonous stages of *E. tenella*. They studied the stage of action of the anticoccidials and noted that in *E. tenella* infected chickens both drugs exerted their maximum effect on weight gain and feed gain ratio through the first 72 h PI. Both drug acted similarly on early life cycles stages of *E. acervulina* and no improvement in performance was recorded when medicated feed was given for longer than 72 h and indicated that both drugs

were more active against sporozoites and early schizogonous stages of *E . acervulina* than those of *E. tenella.*

Dash *et al.* (1993) harvested the infective sporulated oocysts of *E. tenella* by in vitro culture. Broiler chickens were infected experimentally with sporulated oocysts to know the anticoccidial efficacy of monensin and halofuginone.

Rahman *et al.* (1993) in a mixed infection of *Eimeria tenella* and *E. acervulina* infection in a farm successfully treated broiler chicken with 30 per cent Sulfaclozine sodium salt monohydrate (ESb3).

Sensitivity of field isolates of *Eimeria* to monensin following the use of a coccidiosis vaccine in broiler chickens was studied by Chapman, H.D. (1994) and it was found that monensin was more effective against isolates from farms following the use of a coccidiosis vaccine and was not effective where ionophores had been used.

Mukerjee *et al.* (1994) infected 14 day old experimental birds by dropping suspension containing 50,000 sporulated oocysts of *Eimeria tenella* directly into the pharynx to study the therapeutic efficacy of Supercox, Duocoxin and Zycox (herbal preparation).

Conway *et al.* (1995) studied the anticoccidial efficacy of semduramicin and salinomycin against field isolates of *E. maxima* in broiler chickens in two different treatment groups infected with 10^3 and 10^4 sporulated ooccysts per bird. It was demonstrated that the efficacy of each anticoccidial drug was equal to or greater than 90 per cent in controlling these *Eimeria* isolates. It was also found that 25ppm of semduramicin was more efficacious than 66pmm of salinomycin on improvement in weight gain, feed conversion, plasma carotenoid concentration and lesion scores.

Danforth *et al.* (1997) studied the anticoccidial activity of salinomycin at a dose rate of 60-100ppm in the feed and definite anticoccidial activity resulting in improved weight gain, FCR, lesion score and decreased mortality compared to unmedicated control.

Environmental selection pressure in different geographical location as well as the history of drug used may differ with each other therefore strain resistant in one area may be sensitive to another area (Martin *et al*, 1997).

Pathak *et al.* (1998) obtained oocysts from slaughtered birds and those presented for post- mortem examination from different poultry farms in and around Bikaner. These oocysts were sporulated and identified as *E. tenella* and were experimentally inoculated by oral route at a dose rate of 5×10^4 sporulated oocysts per bird to study the efficacy of amprolium and toltrazuril.

Basith *et al.* (2000) studied the efficacy of salinomycin at 60 ppm in feed given 24 h before experimental infection with 3×10^4 oocysts of *E. necatrix* till the end of the experiment. Three birds from each group were killed at various intervals from 12 to 240 h post inoculation. Histopathological examination of the intestine showed that salinomycin exerted a potent action against early developmental stages of *E. necatrix* (sporozoites, tropozoites and first generation schizonts).

Asim Mahmood *et al.* (2001) studied the chemotherapeutic efficacy of lasalocid sodium, monensin and salinomycin against coccidiosis in day broilers. The birds were experimentally inoculated with mixed field isolates of *Eimeria* spp. (50,000 sporulated oocysts / bird). Lasalocid sodium (180 ppm) medicated birds had better feed conversion and consumption ratio, more weight gain and lower faecal oocyst counts compared to salinomycin (66ppm) and monensin (130ppm) medicated birds.

Use of diclazuril in shuttle programme was highly effective in mixed *Eimeria spp.* infections in comparison with nicarbazine, narasin + nicarbazin and zoaline in starter diet, while salinomycin, monensin and lasalocid in grower diet (Conway *et al.* 2001).

Kiaei *et al.* (2001) conducted an experiment to know the effect of Artemisia sieberi and chemical anticoccidial drug on control of coccidiosis and broiler performance. Birds were infected with *E. tenella*, *E. maxima*, *E. acervulina* and *E. necatrix* at the end of the third week of age. The flock was monitored for signs of disease and the faecal sample was collected to determine Oocyst Per Gram (OPG), body weight gain, feed intake, and feed conversion ratio. The performance of diclazuril treated group was found to be good.

The anticoccidial efficacy of traditional Chinese medicine combined with diclazuril or maduramicin against *Eimeria tenella* was studied by Li-PeiGuo *et al.* (2005). Birds were inoculated with 1.5×10^5 sporulated oocysts of *Eimeria tenella*. They observed the faecal oocyst count and live weight gain. The feeding of diclazuril and traditional Chinese medicine prevented chicken coccidiosis effectively and also promoted the growth of birds, when compared with maduramicin and traditional Chinese medicine.

Control of coccidiosis in chickens has relied upon managerial practices and the prophylactic use of coccidiostatic drugs. The emergence of *Eimeria* strains that are resistant to commercially available drugs and vaccines had increased (Vermeulen *et al.* 2001).

George and Sabu (2002) studied the efficacy of amprolium hydrochloride, monensin and salinomycin at the rate of 125, 100 and 66 ppm respectively, as coccidiostats in broiler chicken (n=100) based on feed conversion ratio, performance index survival (%) ratio, mean gain (%) and faecal score (%). Salinomycin was found to be the best coccidiostat closely

followed by monensin. Amprolium hydrochloride was not able to control coccidiosis based on the above parameters.

Ashraf *et al.* (2002) conducted a study to compare the efficacy of different feed additive anticoccidials and coccidiosis vaccines in 240 broiler chicken. They were given commercial feed with 6 per cent Salinomycin sodium (Coxistac), 12 per cent Salinomycin sodium (Sacox), monensin (Elancoban) and Lasalocid sodium (Avatec) or unmedicated feed and were vaccinated at days 3 and 10. Birds were challenged on days 22 and 35 and the oocyst count, feed consumption and weight gain were recorded. Mortality was recorded and post-mortem examination of dead birds was also performed. It was found that 12 per cent Salinomycin sodium (Sacox) as anticoccidial in the feed was significantly better ($P<0.05$) than all the treatments in terms of live weight gain, feed, FCR, oocyst count and reduced mortality. The results of other anticoccidials were not satisfactory, while both the vaccinated groups performed well in terms of oocyst count, in morbidity, mortality rate. However, their live weight gain and feed efficacy were not good.

Suo-Xun *et al.* (2002) studied the anticoccidial efficacy of decoquinate and maduramicin in broiler chicken experimentally infected with *E. tenella* at a dose of 5×10^4 sporulated oocysts by dribbling. Based on number of oocysts in the caecum of dead birds and number of oocysts passed in droppings, they concluded that toltrazuril was more effective when compared with maduramicin treated birds.

Tipu *et al.* (2002) reported the anticoccidial efficacy of herbal compound neem fruit (*Azadirachta indica*) and compared it with an ionophorous anticoccidial salinomycin. (Kokcisan) against coccidiosis in broilers (n=240). The result revealed that non-infected non-medicated birds had better ($p<0.05$) weight gain as compared to medicated groups. The infected birds of salinomycin group had better weight gain and feed efficacy when compared

with other treated groups but the difference was not significant ($p>0.05$). The neem fruit compound at 150 g/ kg feed gave excellent performance in terms of oocyst count and lower mortality as compared to other treated groups.

Mpoame *et al.* (2003) studied the efficacy of aqueous extract of papaya seeds for treatment of *Eimeria tenella* in broiler chicken based on mortality, oocysts production and average weight gain. They reported that aqueous extract of papaya seeds seemed to be efficient in the *Eimeria tenella* in broiler chickens.

Zhang *et al.* (2003) using *Eimeria tenella* Houghton strain a sensitive reference, studied the resistance of two Hebei and Shandong isolates of *Eimeria tenella* to maduramicin. Birds were inoculated at a dose of 5×10^4 sporulated oocysts and observed the parameters viz. weight gain, lesion score and faecal oocyst count. The result showed that both the Hebei and Shandong isolates of *Eimeria tenella* had high resistance to maduramicin.

Peek and Landman (2003) reported that fifteen field isolates of *Eimeria spp.* were sampled on Dutch broiler farms and were subjected to an anticoccidial sensitivity test (AST) in a battery cage study. The selected anticoccidials included monensin, narasin, salinomycin lasalocid, nicarbazin, diclazuril, halofuginone, maduramicin and meticlorpindol / methylbenzoquate. *Eimeria tenella* showed reduced sensitivity for nicarbazin and was sensitive to narasin, maduramicin, and halofuginone where as all other products showed resistance.

Koinarski (2003) studied the anticoccidial efficacy of semduramicin in broiler chickens infected with *E. tenella* and compared it with monensin, salinomycin, and maduramicin. The experiment was performed on broiler chickens. They recorded clinical signs, feed conversion ratio, the change in blood haemoglobin content and haematocrit

values. Their experiment showed that semduramicin exhibited high anticoccidial activity when compared to monensin and maduramicin treated birds.

Gautam and Gupta (2004) did not find resistant against Salinomysin 60 ppm and Maduramysin 5 ppm in *E tenella* infected broilers at 3 wk of age. All coccidiostats are showing varying degree of efficacy in Haryana. Salinomycin shown 86.8% and maduramicin shown 80.50% efficacy against Kernel isolate of *E. tenella.*

Ebrahimnezhed and Pourreza (2005) studied the effect of ionophore drugs (salinomycin, lasalocid and their combination) on the performance of broiler chicks. Increasing drug level significantly ($p<0.05$) reduced body weight gain and feed intake and the feed conversion was increased at 21 to 42 and 0 to 56 days of the experiment.

Ranade and Desai (2005) conducted an experiment to know the efficacy of Cocciban, a herbal poultry feed supplement from selected herbal plants, diclazuril and salinomycin in broilers. Feed intake, weekly body weight gain, FCR and mortality were recorded. The incidence of coccidiosis was nil in all groups. They concluded that Cocciban can be used to successfully prevent coccidiosis without any adverse effects in broilers.

Additives such as salinomycin, betain and neem seed kernel were used to assess the efficacy of dietary supplements against coccidiosis in broilers. Lesion scores and occyst per gram of faecal sample of broilers revealed a significant reduction in salinomycin supplemented birds when compared to the negative control groups (Susila *et al.,* 2005).

Badran and Lukesova (2006) reported that poultry producer have to rotate the use of various anticoccidial with successive flock, combine chemical and ionophore treatment or comply shuttle programme to minimize the effect of resistant.

Abbas *et al.* (2008) stated that maduramicin has particular mode of action among of

ionophores, which does not allow an easy emergence of resistance against *Eimeria tenella* .

Abbas *et al.* (2009) suggested to use Diclazuril in shuttle programs is highly efficacious against different *Eimeria* spp. in comparison with other chemical anticoccidials and ionophores.

Bray *et al.* (2009) stated that polyether ionophorus compounds have been used extensively in broiler as coccidiostat. Today the efficacy of ionophorous is considered largely due to their ability to control the coccidiosis but permit the acquisition of immunity.

Peek and Landman, (2011) reported use of live anticoccidial vaccine in combination with anticoccidial shuttle programme for prevention of anticoccidial resistance.

Ahaotu *et al.* (2013) stated that control of coccidiosis is largely limited to good husbandry and prophylactic chemotherapy using range of drugs.

Arabkhazaeli *et al.* (2013) stated that mild or subclinical infections are also important because minor intestinal lesion can interfere with growth, feed efficiency and economy. They reported that rotation of the anticoccidials will give better efficacy and prevent economic losses in coccidiosis.

Chapman (2014) suggested that now a day shuttle and rotation programme are widely used in broiler industry because of drug resistant problems.

2.2 Body weight and feed efficiency

Mayhew (1932) opined that the problem of coccidiosis was not of mortality alone but greater importance was the effect of the disease on future weight gains, feed consumption and egg production.

Chicks infected with 50,000, 1, 00,000, and 2, 00,000 sporulated oocysts of *E. tenella* at four weeks of age and above showed severe depression in growth (Gardiner, 1954).

Reid and Pitosis (1965) studied the influence of coccidiosis on feed and water intake in chicken. They observed reduction in feed and water intake by the fifth day following inoculation with *E. tenella*. Moderate inoculation with *E. tenella* resulted in decreased feed consumption up to 50 per cent of the normal level, while water intake was only slightly decreased.

Miyazaki *et al.* (1975) found that the salinomysin drug was effectively in reducing the mortality and increasing the average weight of chickens experimentally infected with *E. tenella*.

Migaki and Babcock (1979) conducted safety evaluation of salinomycin in broiler chickens at 50, 60, 80, 100 and 160 ppm fed continuously from 1 to 56 days of age and observed that 60 ppm salinomycin had an acceptable safery profile for commercial development based on weight gain and feed conversion.

Kos *et al.* (1981) made laboratory trials to evaluate the anticoccidial activity of ionophore antibiotics (monensin, lasalocid, salinomycin) against *E. tenella*, *E. brunetti* or *E. maxima* and found that all the drugs were protective as shown by weight gain, feed conversion and mortality rate.

Witlock (1983) reported a significant reduction in weight of chicks on five day post infection (dpi) when infected with 1, 00,000 sporulated oocysts of *E. tenella*.

Lohner and Wilson (1985) compared efficacy of salinomycin 60 ppm with that of monensin 100 ppm in feed and observed that salinomycin treated birds showed highly significant better feed conversion and production index than monensin treated birds.

Shukla *et al.* (1987) observed that salinomycin at 60 ppm in feed was effective against mixed infection of *E. acervulina, E. necatrix, E. maxima* and *E. tenella* on the basis of weight gain and feed conversions.

Perez *et al.* (1988) reported better weight gain in halofuginone treated group as compare to Dinirto Ortho Toluamide (DOT) in broiler chicks.

Salisch and Shakshouk (1989) reported that diclazuril treated chickens performed best followed by uninfected group, maduramicin, salinomycin, narasin and monensin treated groups in terms of feed conversion efficiency, growth rate, and pathogenic effects.

Srivastava and Sinha (1987) in their study on the efficacy on salinomycin at 60 ppm level against drug resistive strains of *E. tenella* in broiler chickens found significant increase in weight gain.

Thyagarajan *et al.* (1989) observed that caecal coccidiosis resulted in significant reduction in body weight as a result of stress, feed intake and poor absorption of nutrients from the intestine. Irrespective of medication to coccidial infection, it resulted in depressed growth rate and feed efficiency.

There was higher body weight gain in Maduramicin given bird as compared to other coccidiosis in broiler chickens. No change in the sensitivity of *E tenella* parasite to 24 successive experiments with Maduramicin (Felfeldi, 1991).

Majumdar *et al.* (1993) reported better results of body weight, feed consuption, faecal score, and lesion score in coccidiostat given birds as compared to non medicated birds.

Badiola *et al.* (1994) reported that Maduramicin is better coccidiostat for reduction of body weight gain, feed consumption and mortality.

Coccidiostat named lasalocid, maduramicin, monencin, narasin and halofuginone were resulted in improved production performance in broiler chicks but the effect between the groups varied. In several aspects effect of narasin was best (Elwinger, 1994).

Jithendran (2001) found coccidiosis to be the single most important disease in the form of outbreaks in all the poultry farms in his study based on poultry carcasses submitted for routine disease investigation work in private and government farms in Kangra, Una, Solan and Mandi districts of Himachal Pradesh. Caecal coccidiosis due to *Eimeria tenella* and other species affecting the intestinal tract viz. *Eimeria acervulina, Eimeria necatrix, Eimeria brunetti, Eimeria maxima* were found to be predominant.

Rahimi *et al.* (2003) conducted a study to determine the effect of betaine on the number of oocysts excreted in the faeces of *Eimeria tenella* infected broiler chicken and its effect on feed intake, feed conversion ratio and body weight gain. It was concluded that betaine increases feed intake and body weight gain in coccidiosis infected birds, but did not affect the performance of healthy birds and it had no effect on the number of oocysts in droppings of the birds.

Ebrahimnezhad and Pourezza (2005) showed that salinomycin was better than lasalocid in term of body weight gain and feed conversion ratio in broiler chickens.

Singh *et al.* (2006) found lower weight gain and Feed Conversion Ratio (FCR) in mixed *Eimeria spp.* infection in broiler chicks.

El -Ghany *et al.* (2007) stated that toltrazuril was the most effective anti-coccidial in chicken by observing better body weight and body weight gain compared to Amprolium.

Allam *et al.* (2008) found better body weight gain halofuginone treated group as compared to DOT and controlled group in experimental caecal coccidiosis in broilers.

Angel *et al.* (1998) reported that Diclazuril and Maduramicin are good coccidiostat as compare to Salinomycin in broilers at 28 day of age.

Bozorgmehri Fard and Rajat (2008) reported that Diclazuril has highest feed conversion ratio as compare to salinomysin at 42 days of age in broilers.

Abbas *et al.* (2009) suggested for use of coccidiostat diclazuril in rotation and shuttle programmes to avoid resistant to *Eimeria tenella.*

Amer *et al.* (2010) found greater improvement in weight gain and body weight in toltrazuril treatment as compared to amprolium in broiler chicken.

Azizi *et al.* (2010) suggested that maduramicin could decrease the OPG significantly and improve the production performance as compare to salinomycin in experimental produced coccidiosis in broilers.

Anosa *et al.* (2011) found better result in feed conversion ratio and weight gain in amprolium, toltrazuril and embazine treatment against coccidiosis in broilers.

Usman *et al.* (2011) stated the future need of effective herbal experimental investigations for the control of coccidiosis to prevent harm and drug resistance.

Chauke and Siebrits (2012) found significantly lower weight gain in coccidiostat treatment group (219 vs. 560.5 g) as compared to the control group.

2.3 Mortality, Faecal and Lesion scoring

Herrick *et al.* (1942) conducted lesion scoring to assess the minimal protective dose of organic sulphur compound against caecal coccidiosis. The score was based on intensity of lesion as few, slight, many and severe.

Gardiner (1954) reported that four week old chicks suffered the highest mortality among the different age groups, when infected with 50,000, 1,00,000 and 2,00,000 sporulated oocysts of *E. tenella.*

Johnson and Reid (1970) standardized the lesion scoring technique and described a scoring scale. In battery trial using pure species, the six species *Eimeria tenella, E. necatrix E.mivati, E. maxima, and E.brunetti* were scored on a 0 to +4 scales with description of gross pathologic changes for each score. The gross pathologic picture was difficult to correlate with weight gain or other indications of pathology. Birds floor pen trials infected with more than one species were also graded on a 0 to +4 scales. Four sections of intestine (upper, middle, lower and caeca) were scored separately.

Rahman and Anantaraman (1970) reported *Eimeria tenella, E. necatrix, E. maxima, E.mitis* and *E. acervulina* from domestic fowls occurring in the city of Madras, identified based on morphology of oocysts, sporulation time and pathology.

Rahman *et al.* (1971) reported 45.3% incidence of coccidiosis based necropsy studies. Five species of coccidia. viz, *Eimeria tenella, Eimeria acervulina. Eimeria necatrix, Eimeria brunetti* were identified and reported from Mysore for the first time. The main criterion for differentiation of species was the morphology of oocysts, sporulation time and pathology.

Visco and Burn (1972) also reported that complex relationship between host, intestinal bacteria and *E.tenella* in producing the clinical symptoms of the caecal cocidiosis. Host and Parasite strain or isolates may be responsible for result difference.

Karlsson and Reid (1977) found *Eimeria acervulina, E. tenella, E. necatrix* and *Eimeria brunetti* to be widely distributed in north-east Georgia. Using immunity challenge techniques, 17 flocks from 12 to 72 weeks of age showed significantly lower lesion scores for

the first three species compared with susceptible control flocks similarly challenged. Only one flock was found to be susceptible to *E. tenella*. No mortality occurred in the field flocks after challenge while 20 to 60 per cent mortality occurred in the susceptible controls. Partial immunity occurred more frequently in field flocks aged 28 weeks of age or less (62 per cent) than in older flocks (30 per cent). Lesion scores ranked from 0-1 to 2-2 with the former and 0-1 to 0-6 with the later group. A modification for the immunity challenge method was developed by using lesion scores as major criteria for determining immunity. The method permits testing four species infecting different parts of the digestive tracts in one group of birds.

Lee and Onderka (1978) reported clinical coccidiosis from six regional laboratories of the Ontario veterinary Service Branch from years 1973 to 1977. Among broilers, infections occurred in birds as young as one week of age but 80 per cent (112/140 consignments) were found in birds three to seven weeks of age with majority (58 per cent) concentrated in the four to five week old birds. Although the higher incidence of clinical coccidiosis among layers and other chickens was in the younger birds (4 to 12 weeks), infections were recorded in birds of all ages including two flocks of two year old birds. The possible use of drug rotation for better control of coccidiosis in chickens was discussed.

Chappel and Babcock (1979) made comparative studies of salinomycin, monensin and lasalocid in the control of coccidiosis in broilers and found that salinomycin and monensin were equivalent in their potency in lesion control and was statistically found superior ($P<0.05$) to lasalocid. Neither coccidiosis related mortality nor any differences in feed conversions were recorded among medicated groups.

McDouglad (1981) studied anticoccidial drug resistance in the South-eastern United States and found that salinomycin provided the best overall control (64 per cent), followed by lasalocid (56 per cent) and monensin (52 per cent) based on lesion score reduction.

McDouglad *et al.* (1981) recorded significant reduction in lesion score and improved body weight and feed conversion with salinomycin treatment.

Paulillo *et al.* (1986) compared the efficacy of monensin (100 ppm), halofuginone (5 ppm) and salinomycin (60 ppm) against mixed infection of *E. tenella*, *E. maxima* and *E. acervulina* and concluded that salinomycin treated group had fewer coccidial lesions than other groups.

Belot *et al.* (1987) studied the use of salinomycin under farm conditions at 50 mg/kg feed and found that the drug gave good control of coccidiosis based of faecal oocyst counts and intestinal lesion scores.

The role of levamisole in the induction of cell mediated immunity was studied in experimental caecal coccidiosis by Abdul Rahman *et al.* (1989). Levamisole was found to improve the development of resistance to coccidial infection compared to levamisole untreated birds. They concluded that levamisole enhancing effect on cell mediated immune response had special value in the prevention of coccidiosis.

Srivastava and Sinha (1987) in their study on the efficacy on salinomycin at 60 ppm level against drug resistive strains of *E. tenella* in broiler chickens found significant reduction in lesion score and mortality.

The efficacy of duocoxin in clinical cases of coccidiosis in broiler chicks was studied by Guha and Misra (1990). Duocoxin (MSD) administered in drinking water was found

highly efficacious in controlling a field outbreak of of coccidiosis (mixed infection of caecal and intestinal forms caused by *Eimeria tenella* and *E. necatrix,* respectively in broiler chicks.

Karim and Trees (1990) identified five species of *Eimeria*, namely; *Eimeria acervulina*, *E. tenella*, *E. necatrix* and *Eimeria brunetti* and *Eimeria maxima* in chickens in Bangladesh on the basis of lesion seen on post-mortem examination of naturally infected birds, and on the dimension of oocyst and the lesions seen in chicks experimentally infected with single oocyst derived strains. The use of filter tap, polycarbonate cages permitted the isolation of strains without sophisticated animal isolators and appropriate for use in laboratories throughout the developing world. Responses to a questionnaire sent to all known intensive poultry farms suggested that coccidiosis was a major disease. For control, producers relied mostly on management procedures and the tactical use of sulphonamides; but in feed chemoprophylaxis was not widely used. These control measures were not satisfactory and coccidiosis outbreaks were reported from seven of 16 farms. There was evidence of a seasonal incidence in clinical coccidiosis.

Shad-Del and Farahani (1995) found significant positive difference in term of pathological effect in Dinitoluamide (DOT) treated chickens as compare to control.

Panda *et al.* (1997) studied the mortality pattern of coccidiosis in broiler birds in Orissa. The study revealed the presence of six species in droppings, viz, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria brunetti*, *Eimeria mitis*, *Eimeria maxima*, with an mortality rate of 65.83, 37.96, 48.89, 15, 25.46, and 3.05 per cent respectively. Highest number of positive cases was recorded from birds at 3-6 weeks of age. Death due to caecal coccidiosis was higher than that of intestinal coccidiosis.

Conway *et al.* (1999) studied the comparative testing of anticoccidials in broiler chickens. The relationship between oocyst dose and lesion score was evaluated in trials involving 5 fields isolates each of *E. acervulina*, *E. maxima* and *E. tenella*. The relationship between oocyst does and lesion score was examined for different coccidial species using a linear model. In nonmedicated birds, low oocyst doses caused mean lesion scores up to 2.0 but the number required to cause higher mean scores were many times greater. The estimated oocyst dose in salinomycin medicated birds for any given mean lesion score was substantially more than the corresponding estimates for non medicated birds.

The effect of aflatoxin and *Eimeria tenella* infection on mortality and lesion score in broiler chickens was studied by Prabakaran and George (1999). They undertook the study to evaluate the effects of aflatoxin on *E. tenella* infection. Mortality was recorded in each group and expressed as per cent mortality. Lesion scores were made at 5-10 days post infection according to the severity of the caecal lesions. They concluded that the presence of dietary aflatoxin increases the mortality due to *E. tenella* infection in broiler chickens. The lesion score also increased in broilers affected with aflatoxicosis and coccidiosis concurrently.

Asim-Mahmood *et al.* (2001) conducted and experiment to assess the pathology of coccidiosis and its response to lasalocid sodium, monensin and salinomycin treatment using lesion scoring in experimentally infected broiler chickens with field isolates of mixed *Eimeria* species. Their findings indicated that there was decline in lesion score over time. The statistical analysis of lesion scoring revealed noticeable difference between each ionophore antibiotic treated and infected untreated groups. A marked decrease in lesion score was observed within each ionophore antibiotic treated group with the passage of time. This decrease in lesion was more pronounced among the lasalocid treated groups as compared to other ionophore treated groups.

The comparative efficacy of the anticoccidials was evaluated by Rana and Tikaram (2002) on basis of performance index considering weight gain, survival and faecal score. They have found moderate efficacy of Amprolium against caecal coccidiosis . The most succeptible age group found for coccidiosis in broiler was 15-28 day at Jind district in Haryana.

Mathis *et al.* (2003) studied control of coccidiosis with toltrazuril in conjugation with salinomycin and nicarbazin or non medicated feed in broiler chickens infected with *E. tenella*, *E. maxima* and *E. acervulina*. The performance data, lesion score and oocyst counts showed that toltrazuril successfully controlled coccidiosis with no relapse of infection.

Shojadoost *et al.* (2003) also reported that there was low lesion score in broiler birds infected with *Eimeria tenella* (25,000 oocysts) and treated with Salinomycin (66 ppm).

Ashuma *et al.* (2005) found reduced infectivity of sporulated oocyst on basis of lower lesion score and better survivability and reduced oocyst production in *E. tenella* infected chicken given herbal coccidiostat

Ancuceanu *et al.* (2006) studied the efficacy of lasalocid sodium in prevention of mortality due coccidiosis in intensive reared chicken. The result revealed that the efficacy of lasalocid sodium in mortality prevention was 99.3 per cent and the recorded global mortality rate for coccidiosis was 4.66 per cent. The highest mortality rate was observed in Leghorn (5.97) per cent) followed by Rhode Island (4.32 per cent) and Sussex (3.92 per cent) breeds.

Anuradha *et al.* (2007) studied the prevalence of chicken coccidiosis in broiler and broiler breeder farms in Bangalore district. The preliminary study involved copromicroscopy, necropsy and lesion score and it was reported that caecal coccidiosis was found to be the predominant form compared to intestinal coccidiosis. The number of cases was found to be

highest in the age group of 6-8 weeks (47.66 per cent) followed by 4-6 weeks (29.9 per cent). The identification based on morphology and micrometry revealed five different species, viz. *Eimeria tenella*, *Eimeria maxima*, *Eimeria necatrix*, *Eimeria acervulina* and *Eimeria mitis*.

Abbas *et al.* (2008) also reported that maduramyci given @5 ppm at 12 days of age in broilers shown reduction in faecal score and oocyst index as compared to control birds. Reduction in OPG was also recorded after 7 day PI in *E. tenella* oocyst infection @ 75000.

Georgieva *et al.* (2010) reported that Maduramicin at 5 ppm dose rate found to reduce oocyst index and lesion score at 7 day PI given mixed 80000 *Eimeria spp.* oocysts. They found reduced activities of super Oxide Dismutase in infected group compare to healthy group. They have found beneficial effects of maduramicin on lipid peroxidation reducing oxidative stress.

Shammem *et al.* (2010) reported better result in Maduramysin treatment by reduction of OPG, faecal score and lesion score at 5ppm dose rate in 2 week old broiler birds given 32000 oocysts of *E. tenella*.

Anosa *et al.* (2011) found reduction in lesion score in amprolium, toltrazuril and embazine treatment against coccidiosis in broilers.

Chauke and Siebrits (2012) found significantly lower weight gain in coccidiostat treatment group (219 vs. 560.5 g), but had the less lesion score and oocysts output in the faecal samples compared to the control group.

Haritova *et al.* (2013) reported decreased motility of the gastro-intestinal tract during the clinical caecal coccidiosis.

2.4 Haemato-Biochemical and Enzymatic Changes

Pratt (1940) observed a rise in blood sugar level in caecal coccidiosis during fourth and fifth day of infection.

Waxler (1941) demonstrated a significant decrease in blood haemoglobin (Hb) in caecal coccidiosis during fourth and fifth day of infection.

Natt and Herrick (1955) studied the use of haematocrit value as an index of the severity of the haemorrhage resulting from caecal coccidiosis. The haematocrit value was found to decrease approximately by fifty per cent on the fifth and sixth day of infection during the haemorrhagic phase of the disease and by the fourteenth day the value returned to normal. They concluded that the use of the haematocrit was a quick and easy method for determining the severity of the haemorrhage resulting from the coccidial infection.

They (Natt and Herrick, 1956) also studied the changes in the blood volume during the course of caecal coccidiosis and noted that the decrease in the blood volume on the fifth and sixth day of infection was due to the result of the erythrocyte loss. They observed a decrease in the corpuscular volume (haematocrit value) by thirty-eight per cent on the fifth and sixth day of the infection and it required nine days for the haematocrit value to return to normal.

Joyner and Davies (1960) reported that sub-lethal infections of *E. tenella* and *E. necatrix* could regularly be detected by haemogram. They found that the haemoglobin concentration and packed cell volume (PCV) were the most sensitive parameters which

showed steady decline during post-infection with a distinct drop in packed cell volume values between 4^{th} and 7^{th} day in *E. tenella* infection.

Schlueter (1963) reported reduction in total serum protein and albumin with *E. tenella* infection. He also recorded a marked reduction in globulin on 5^{th} day post-infection with a gradual rise to normal after 11^{th} day.

Perk and Hort (1964) noted that the albumin fraction decline and the beta-2 globulin fractin increased in experimental *E. tenella* infection.

Mukkur and Bradley (1969) observed a significant drop in packed cell volume and Hb ($P < 0.01$) on 6^{th} day in *E. tenella* infection and the values returned to normal on day 13 post-infection. They have also reported significant reduction ($P < 0.01$) in total protein at 4^{th} and 7^{th} day, alpha-1 globulin at 7^{th} day, beta and gamma globulin at 9^{th} day after *E. tenella* infection.

Freeman (1970) noted reduced haematocrit value from 4 to 6 days post-infection with *E. tenella* infection. He did not find significant change in the plasma glucose during the 6 days observation in 3-week old chickens infected with *E. tenella.*

Oikawa *et al.* (1971) recorded changes in blood components during *E. tenella* or *E. acervulina* infection. The erythrocyte count, haematocrit value and Hb concentration were observed to decrease extremely on the 5th and 6th days by the 10th day the changes returned to normal range.

Joshi *et al.* (1974) studied the variation in blood constituents during caecal coccidiosis in 2-3 months old birds. The Hb percentage was reduced to 6.5 ± 0.35 and packed cell volume decreased to 20.281 ± 0.89 in infected birds, while blood glucose level was increased

to 275.75 ± 14.70 mg per cent in caecal coccidiosis as compared to 160.00 ± 7.15 mg per cent in normal birds.

Saad *et al.* (1974) reported that 10 days old chickens when infected with *E. tenella* showed decrease in total erythrocyte counts from day 5 post-infection, in packed cell volume from day 6 post-infection and in Hb value from day 4 post-infection. During this period total leukocytes count was increased.

Stephens *et al.* (1974) conducted trials to study the physiological changes due to *E. acervulina, E. brunetti* and *E. mivati* infections in 4-week old chickens and observed significant differences between the ESR of infected and control chickens with an increased buffy blood coat value.

Kumar and Rawat (1975) studied the effects of mixed infection of *E. necatrix* and *E. acervulina* in 3 - 4 months old cockerels. They noted that the blood glucose level increased significantly ($P < 0.01$), but not the total, free or esterified cholesterol in infected birds as compare to control. Affected bird showed an increased serum acid phosphatase and decreased alkaline phosphatase and serum glutamic pyruvic transaminase activity without significant change in total serum protein.

Ruff *et al.* (1975) reported that coccidiosis alone induced slight proteinemia and increased plasma glucose level with decrease in PCV and Hb level in experimental infection of *E. tenella* in male broiler chicks.

Washburn (1975) found significant decrease in packed cell volume in chickens infected with a dose of 1, 00,000 *E. tenella* oocysts.

Constantinescu (1976) found that in chickens with mixed coccidiosis, there was a hypoglycaemia and dysproteinemia, but serum cholesterol level remained normal and alkaline phosphatase increased compared to control birds.

Singh *et al.* (1976) recorded an increase in serum cholesterol (160.00 mg per cent), serum glutamic pyruvic transaminase (15-16 units/l) and pyruvic acid (4-11 mg per cent) on 27^{th} day post-infection in intestinal coccidiosis produced by *E. acervulina, E. maxima, E. mitis* and *E. necatrix* at 1,00,000 oocysts dose level, while at 16,970 oocysts level the values were 128.66 mg per cent, 9.83 units/ml and 2.43 mg per cent, respectively on 24^{th} day of infection.

Turk *et al.* (1977) stated that coccidial infection markedly alters intestinal physiology resulting in changes in morphology, nutrient absorption and micro flora. These changes were most severe in infected area and may be mild or absent in other area. Intestinal pH is markedly decreased during the acute phase of infection in the affected area. Gut motility is decreased during the acute phase but restored during recovery. Digestion and absorption of protein is impaired in the infected mid intestine. Absorption of carbohydrates particularly glucose is not consistently affected, but lipids, fat-soluble vitamins and carotenes are also poorly utilized during infection.

Turk (1978) observed that the circulating Hb and haematocrit values decreased on days 5 and 6 of *E. acervulina* infections, days 5 to 10 of *E. brunetti* infections and days 6 to 10 in *E. tenella* infections.

Witlock *et al.* (1981) conducted trials to study the physiological basis of *E. tenella* induced mortality in 3-week-old chickens. No significant difference was noted in plasma glucose level between the control and infected not dying group of chickens, while decrease in

plasma protein level was observed in the infected not dying group compared to control group. They also recorded decline in PCV and Hb Values in *E. tenella* infection.

Ruff and Augustine (1983) studied the effects of coccidiosis on the electrophoretic patterns of serum proteins in chickens infected with *E. acervulina*, *E. tenella* and *E. maxima* and found decreased total serum protein on days 5 and 7 PI. They further noted that albumin levels were lowest at 5 days PI in *E. acervulina* infection and 7 days PI in *E. tenella* and *E. maxima* infections and the return of values to normal level subsequently indicating a rapid replacement ob albumin.

Witlock (1983) reported that *E. tenella* infection induced haemorrhage resulting in significant decrease in packed cell volume from day 5 to day 7 post-infections.

Chadwick *et al.* (1985) reported that at day 5 post-infection, haematocrit was reduced after inoculation of 50,000 oocysts of *E. tenella* in 3 weeks old cockerels.

Turk (1985) conducted trials to study the macro elements in *E. acervulina, E.necartix*, *E.brunetti* and *E. tenella* infection in 4-week-old chickens. He observed that haemoglobin, erythrocyte concentrations and haematocrit values were decreased during the acute phase of the infections and returned to normal during recovery. In *E. necatrix* infection Hb concentrations were decreased from the 6th through 14th days of infection and the haematocrit values fall from the 5th through 10th day. He also analysed biochemical parameters in experimental *E. acervulina*, *E.necartix*, *E.brunetti* and *E. tenella* infection in 4-week-old chickens. He recorded decreased plasma calcium, magnesium and sodium concentrations and variable potassium concentrations.

Nayak and Rai (1986) studied serum protein profiles in 6-week-old chickens infected with mixed culture of *E. tenella*, *E.necartix* and *E. acervulina*. Total serum proteins were

2.94±0.052 grams per cent in uninfected birds. There was marked fall in relative percentage of albumin and alpha-globulin in infected chickens while beta and gamma globulin increased.

Padmavathi and Muralidharan (1986[a]) studied alteration in haematological parameters in *E. tenella* infection and found significant reduction in RBC count from 1.1 to 0.7 x 10^6/cmm, Hb from 3 to 1.2 gm per cent and packed cell volume from 8 to 2 per cent from 4^{th} to 13^{th} day post-infection. A significant increase in WBC count was observed from 20.8 to 23 x 10^6/cmm from 4^{th} to 13^{th} day post-infection.

Padmavathi and Muralidharan (1986[b]) estimated the levels of serum metabolites during *E. tenella* infection. There was decrease in serum levels of glucose (from 90 to 60 mg/100 ml) from day 4 to 23 post-infection, total protein (2.1 to 0.3 gm/100 ml) from day 4 to 14 post-infection, albumin (from 1.0 to 0.1 gm/100 ml) from day 4 to 14 post-infection and in carotenoids (from 30 to 25 per cent) transmission from day 4 to 8 post-infection. They recorded a drop in serum levels of sodium, inorganic phosphorus and iron and an increase in serum levels of uric acid, potassium and total cholesterol.

Conway *et al.* (1993) estimated the effects of different levels of oocyst inoculums of *E. acervulina*, *E. tenella* and *E. maxima* and noted that PCV value was depressed by *E. acervulina* and *E. tenella* infections starting at 10^4 oocysts per bird. He also reported that carotenoids, lipids and plasma protein values were significantly depressed in *E. Acervulina, E. tenella* and *E. maxima* infections with different dose levels.

Kogut and Powel (1993) stated that AKP activity may be the sensitive marker of the pathogenesis in coccidial infection of the caecum.It is directly proportionate to WG and indirectly proportionate to Oocyst index and LS.

Kalra *et al.* (1996) found significant decrease in serum cholesterol and a significant increase in alkaline phosphatase in 21 days old chicks infected with coccidiosis as compared to control birds. However, no significant changes in SGOT and SGPT were noticed.

Fukata *et al.* (1997) observed that the total erythrocyte count and packed cell volume in chickens infected with *E. tenella* were significant lower than those of uninfected controls.

Panda *et al.* (1997) found significant reduction in Hb, packed cell volume and total erythrocytes count, with significant increase in total leukocytes count on 5^{th} and 7^{th} day post-infection of *E. tenella.*

Kumar and Padmavathi (2000) found significant increase in total leukocytes count and a decrease in total erythrocytes count and packed cell volume, while in biochemical study blood glucose increased between the range of 194 to 214 mg/100 ml in *E. tenella* infected as compared to 185 to 186 mg/100 ml in healthy control 0 to 11days old broiler chicks.

Deger *et al.* (2002) reported higher SGOT (300.8 ± 19.2 units) and SGPT (40.2 ± 5.4 units) values in coccidia infected chickens as compared to normal SGOT (30.0 – 170.0 units) and SGPT (4.0 - 37.5 units) values. He stated that measurement of enzymatic activities is useful in determining the pathological condition in the tissue. Enzymatic activity of SGOT, SGPT, LDH and GCT were found high initially and thereafter decreased noticeably. A parallel decrease in the number of oocyst in the faeces of treated chickens was observed.

Stove *et al.* (2002) found significant increase in serum total protein and serum total glucose in *E. tenella* infected 2-weeks old Plymouth Rock chicks.

Jaipurkar *et al.* (2004) found reduction in Hb, PCV and TEC values in White Leghorn chicks inoculated with *E. tenella* oocytes at 22 days of age as compared to healthy control group.

Hussein and Rahman (2005) observed that Salinomysin causing marked leucopenia, neutropenia and Lymphositosis given Salinomysin without infection.

Talebi *et al.* (2005) reported that with increase in age, haematological parameters like Hb, RBC, PCV and DLC (except Heterophills) significantly increases. Normal haematological value and Leukogram of genetically improved broiler strain are lower than the indigenous chicken That is why this broilers are more succeptible as compare to native birds.

Pangasa *et al.* (2007) reported significant low glucose, protein and AKP value in infected as compared to control given 50,000 oocyst of E. *tenella* during acute phase of disease. Reduction in glucose might be due to defect in absorption of glucose, leakage of glucose in plasma and increase demand of glucose by developing stage of parasite. They also observed reduction in Total Protein.

Rizvi *et al* .(2008) reported higher AST and ALT value in salinomysin 60-120 ppm given coccidiostat feed at 12 week of age in layer type birds.

Patra *et al.* (2009) reported hypoglycaemia with increase alanine amino transferase and change in total protein and total cholesterol level in serum of broiler birds suffered from *E. necatrix* infection in Aizawl District of Mizoram.

Georgieva *et al.* (2010) suggested that *Eimeria tenella* would be reflected as disruption of the ecological oxidative balance (EOB) in infected broiler chickens. They have found beneficial effects of maduramicin on lipid peroxidation reducing oxidative stress.

Anosa *et al.* (2011) found improvement in haematological values in amprolium, toltrazuril and embazine treatment against coccidiosis in broilers.

Mondal *et al.* (2011) reported increase in plasma glucose, cholesterol, AST(SGOT), while decrease in Protein, ALT (SGPT) values in broilers given 20000-25000 doses of *E.tenella* oocysts infection. They also stated that Protein decrease is due to rapid movement of interstitial fluid without protein in to the plasma and also due to acute stress responsible for cortisol secretion and disturbances in protein catabolism.Increase in cholesterol might be due to decrease billiary excretion of cholesterol in anorexia. Incerease in SGOT might be due to significant damage of cell lining of the caecal wall along with inflammation and severe blood loss causing tissue loss from the body.

Adamu *et al.* (2013) found monocytosis, lymphocytosis, heterophilia and eosinophilia in coccidiosis caused by *E. tenella* and *E.brunetti*. . Serum biochemical analysis showed decreases in alanine amino transferase/glutamic pyruvic transaminase (ALT/GPT) and aspartate amino transferase/glutamic oxalacetic transaminases (AST/GOT), and a marked increase in alkaline phosphatase (ALP) activities.

2.5 Histopathlogy

The influence of age of host on infection with *Eimeria tenella* was studied in broiler chickens by Elaine Rose (1967). The excystation of *E. tenella* sporozoites was more rapid in chicks aged 4, 5 and 6 weeks than in those of 0,1,2 and 3 weeks. The greater proportion of un excysted sporulated oocyst was found in the faeces of birds aged 0 and 1 weeks than any other age groups studied, indicating less successful excystation in these birds. They concluded that older birds tended to be more resistant to second and third infection than the younger birds.

Caecal wall thickening was observed because of oedema and cellular infiltration with the formation of scar tissue by Misra and Gautham (1970).

Chappel *et al.* (1974) studied the site of action of aryltriazin CP25, 415 and found that at the concentration of 15 ppm it was effective against single and mixed *Eimeria spp.* infections in chickens. Based on histological observation all stages of *E. tenella*, *E. acervulina* and *E. maxima* were affected by the drug except *E. maxima* developing macrogamounts and the drug affected primarily first generation schizogony of *E. acervulina* and *E. tenella*.

Babu *et al.* (1976) reported extensive vacuolation in the glandular epithelium cells suggestive of increased globlet cell activity in caecal coccidiosis. They found inflammatory cells predominantly pseudoeosinophils, macrophages and lymphocytes around the glands with damaged epithelial cells and merozoites in *E. tenella* infection concluding that the magnitude of infection in stage of development of disease produced should be established by histopathological examination.

Chappel (1979) gave a detailed account of histopathological examination of chickens infected with *E. acervulina*, *E. maxima* or *E. tenella* with restricted and unrestricted medication of salinomycin. He observed that most of the sporozoites failed to transform into trophozoites and impairment in the development of schizonts and complete arrest of asexual states with marked reduction in oocyst shedding.

Smith *et al.* (1981) while studying the effects of different ionophores viz. monensin, lasalocid, narasin and salinomycin on parasite inoculated chicken kidney cells observed marked inhibition of asexual development accompanied by irregular and gross swelling of sporozoites. They found that most of the sporozoites are unable to transform into trophozoites and impairment in the development of schizonts was observed which affects asexual stages of *Eimeria tenella* due to the effect of ionophores treatment

Lawn and Rose (1982) found developing trophozoites and schizonts within enterocytes in the crypts, the host cell became enormously distended but retained connections to neighbouring cells by terminal buds at the lumen surface of caecum in *E. tenella* infection.

Long and Jeffers (1982) in their *in-vitro* studies on different anticoccidial drugs viz. monensin, salinomycin, lasalocid and arprinocid against sporozoietes of *E. tenella* observed that these drugs reduced the viability of sporozoites based on mortality, haemorrhage and specific lesions in embryo chorio allantois scores and concluded that the drugs were effective primarily against the invasive stages.

Maes *et al.* (1984) studied the effect of 1 ppm Diclazuril on *E. tenella* infected birds and reported that 1 ppm Diclazuril was lethal against asexual and sexual stages of parasites. Diclazuril was able to prevent oocyst shedding.

Samal and sinha (1989) reported denudation of epithelium and development of first and second generation Schizont with cellular infiltration in the lamina propria. They observed destruction of muscularis mucosa with few macrophages at the area of damage. They also observed degeneration and vacuolation in treatment group in histopathological evaluation of anticoccidial activity of lasalocid and amprolium against *Eimeria tenella* infection in chicken.

Shukla *et al.* (1990) recorded changes due to mixed infection of *E. acervulina, E. maxima, E. necatrix* and *E. tenella* in chickens. They observed that the small intestine and caecal pouches were distended with crimson appearance and severe hemorrhagic enteritis. The contents were noted as reddish brown in colour and mixed with clots of blood and fibrin

shreds. Caeca were thickened, congested and necrosed. The lesions were more severe on 6th and 7th day post-infection

Mc Dougald and Reid (1991) described the first generation schizonts maturing at two to three days of post infection, heterophil infiltration in the sub mucosa, second generation schizonts in the lamina propria, appearance of oocysts, macro and micro gametocytes on 7th and 9th dpi.

Mandal and Samal (1991) made histopatholical study on the anticoccidial efficiacy of a herbal product IHP-250C against *E. tenella* infection. They observed that the drug at various dose levels showed blunting and shortening of villi with hydropic vacuolar degeneration by the invasion of parasites, as well as arrested development of all the endogenous stages from trophozoites to gametocytes. The efficacy of the drug was directly proportional with the increase of dose levels.

Chaudhri (2000) reported desquamation of intestinal mucosa and denudation of intestinal villi cells in histopathological changes in coccidian infected birds.

Soomro *et al.* (2001) studied post-mortem findings in coccidiosis affected farms and reported lesions in the intestine and caecum. In the intestinal form, extremely ballooned intestines, along with petechial haemorrhages were observed grossly, without having to open the gut. Enlargement of the caecum and the appearance of clotted blood in the area along with haemorrhagic or whitish spots on the caecal wall, inflammation, necrotic patches, dilation of the caecum with consolidation of the caecal contents, were observed in the almost all cases of caecal coccidiosis. It was further observed that the caecal form of infection (96.0 per cent) occurred more frequently on almost all the farms compared with intestinal form (4.0 per cent). The histopathological lesions of the caecal coccidiosis involved loss of

epithelial tissues, congestion of blood vessels, leakage of blood, oedema, necrosis of the submucosa or caecal mucosa and loss of villi. In the cases of intestinal form, lesions were in the form of complete detachment of the mucosal layer from the submucosa with the accumulation of the cell debris in the intestinal lumen. Both the forms exhibited hyperplasia of the lymphoid cells.

The efficacy of toltrazuril and amprolium was evaluated by Lakkundi *et al.* (2002) in experimentally induced caecal coccidiosis in broiler chickens. They found that toltrazuril prevented the establishment of caecal coccidiosis by degeneration and disintegration of first generation schizonts. However, in the amprolium treated birds, few intact first and second generation schizonts and also micro and macro gametocytes were noticed on microscopic examination. They concluded that toltrazuril was coccidiocidal in nature based on histopathological studies.

El- Abasy *et al.* (2003) studied the effect of oral administration of Sugar cane extract (SCE) on *Eimeria tenella* infection. They reported that SCE treated birds showed lower number of schizonts, gametocytes and oocysts when compared to infected untreated birds. Their result suggests that SCE had immune stimulating and protective effects against *Eimeria tenella* infection in broiler chickens

Jaipurkar *et al.* (2004) stated ballooning of caeca with clotted and unclotted blood, hypertrophied intestinal mocosa with various parasitic stages. The mucosa and submucosa were found to be heavily infiltrated with lymphocytes.

Chandrakesan *et al.* (2009) studied the efficacy coccidiostats against caecal coccidiosis in broiler chickens. They found histopathological changes like cellular infiltration, inflammatory changes, hyperplastic changes, necrosis, presence of endogenous

developmental stages of coccidia and sloughing off epithelial layers in all the treatment groups.In addition to this, heavy infiltration of mononuclear cells with marked alteration of cellular integrity was observed in the control group. Although endogenous developmental stages of coccidia were observed in all the groups, intensity was found to be less in the Salinomycin treated group.

McDougald and Fitz-Coy (2008) observed that the most pathogenic stage caused by *E. tenella* by the second generation schizont, which caused excessive tissue damage, bleeding, disruption of the caecal glands and destruction of the mucosa and muscularis layer. Micro gametes and macro gametes of schizonts are seen in the tissue on days 6 and 7 after infection and matured oocysts are released.

Siddiki *et al.* (2008) observed marked degeneration, desquamation of superficial epithelium, leukocyte infiltration in submucosa, loss of epithelial tissue and villi, severe muscular oedema, disruption of caecal mucosa, cluster of oocysts, marked proliferation of epithelial cells, different stages of parasites in caecal tissue. The major histopathological changes observed in caeca of infected chicks revealed denudation of villi and haemorrhages. The tissue contained various stages of schizonts in anticoccidial treatment in Bangladesh.

Patra *et al.* (2009) observed petechial haemorrhage, necrosis and disintegration of glandular epithelial cells and schizont in coccidial infection of *E. necatrix*

Milad (2011) found that there was early recovery in herbal anticoccidial agent treated group indicated by the presence of degenerating schizonts and oocysts on the 7th day of post infection compared to coccidia alone, where in these changes were evident on 14th day of post infection in non treated control broilers

Histopathological examinations of *E. tenella* infected broilers showed excessive tissue damage, haemorrhage, the presence of clusters of large schizonts and merozoites in the caecal tissue, and coccidian oocysts in the lumen as reported by Adamu *et al.* (2013).

MATERIALS AND METHODS

CHAPTER - III

MATERIALS AND METHODS

The present study was undertaken to know the comparative efficacy of commonly used coccidiostats in Anand District of Gujarat. In a study, broiler birds were given experimental infection of *E. tenella* at three week of age to see the haematological, biochemical and histopathological changes in different treatment group along with growth and feed parameters. The study was conducted on Cobb-400 strain of broilers reared on battery cage system with routine standard protocols.

3.1 Collection of Materials

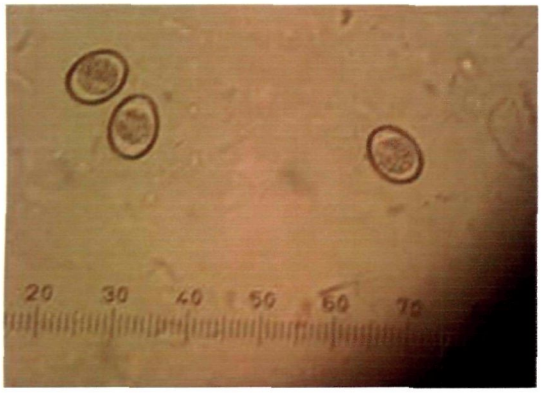
Faecal and tissue samples were collected from commercial broilers brought to the Department of Pathology, Veterinary College, Anand, for routine ante and post-mortem examinations. Faecal, blood and tissue samples were collected from experimental broilers reared at Poultry Complex Farm, Veterinary College, Anand.

3.1.1 Separation of oocysts and sporulation

Oocysts were collected from caecal droppings from gut of birds died due caecal coccidiosis (Plate-9) and were processed and separated by salt floatation technique (Long *et al.*, 1976). The oocysts were then allowed to sporulate in 2 per cent potassium dichromate solution as a shallow layer of 5 mm depth in a petridish at room temperature. The petridish was covered to minimize the evaporation of dichromate solution (Plate- 10). However, sufficient quantities of two percent potassium dichromate solution were added occasionally to make up the loss due to evaporation. The emulsion was frequently disturbed by blowing air

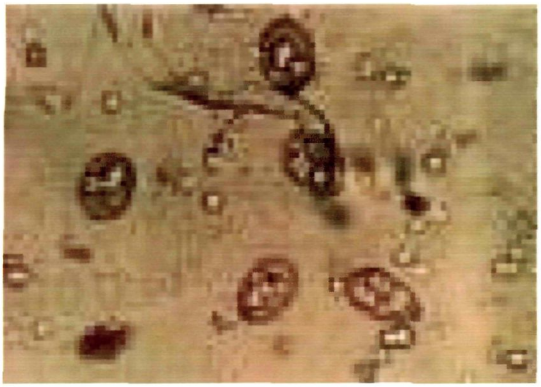
P-7

P-8

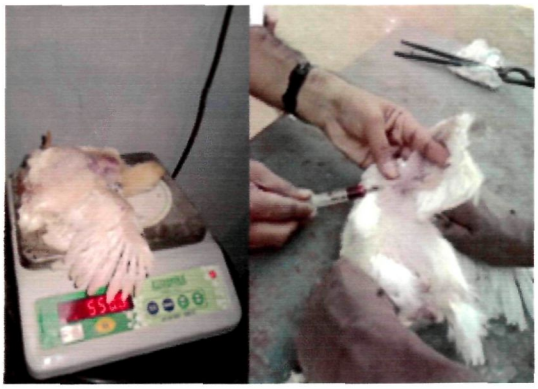


P-9

P-10



P-11



P-12

into it through a Pasteur pipette to facilitate sporulation (Singh and Gill, 1975). Successive samples from the suspension of oocysts in potassium dichromate solution were examined at regular intervals and the progress of sporulation was recorded (Plate- 11). The sporulation process was judged complete when the sporozoites within the sporocysts were fully formed. The time required for fifty per cent of the oocysts to complete the sporulation process was considered to be the sporulation time for comparative purposes (Norton and Chard, 1983).

3.1.2 Measurement of oocysts

Morphology of the oocysts was studied under a high power objective lens. The length and width of the oocysts were measured with an eyepiece scale calibrated by means of a stage micrometer. A total of hundred oocysts were measured and average arrived.

3.1.3 Species identification by morphology and micrometry of sporulated oocysts.

Morphology of oocysts was studied under a low power (10x) objective lens. The identification of oocysts was done as per Levine, 1985; Soulsby, 1982; and McDougald and Reid, 1991. Identification of species of *Eimeria tenella* was done on the basis of the following characteristic features.

a) Location of the lesions in the intestine.
b) Appearance of the gross lesions and the caecal contents.
c) Oocyst morphology viz. size, shape, shape index, colour, appearance of the wall, presence or absence of micropyle.
d) Minimum time for sporulation

3.1.4 Experimental & Treatment Studies

3.1.4.1 Experimental design

Total of three hundred cobb broiler chicks of either sex obtained from Venky India Limited, Mogar, District- Anand, Gujarat were used for the study. They were reared under coccidia-free conditions. Fifty chicks of T1, T2, T3 and T4 group were given (Plate 3 to 6) Diclazuril (0.1%), Salinomycin (12%), Diclazuril (0.1%) + Salinomycin (12%) in shuttle programme (Diclazuril was given for initial three week followed by Salinomycin in last three week) and Maduramicin(1 %) at a dose rate of 100 gm, 50 gm, 100 + 50 gm and 50 gm. per 100 Kg. broiler feed as coccidiostat, respectively. One group of 50 chicks (Plate-7) was kept as infected control (T5) and another group of 50 chicks (Plate-8) was kept as uninfected control (T6) without coccidiostat in feed. All birds were given starter feed up to 4 weeks of age and finisher feed for 5 to 6 weeks of age as per given feed formulation (Table-1 and Plate 1 to 2). Wing banding was done to keep the accurate record of each chick/ bird. They were vaccinated against Marek' Disease, Ranikhet Disease and Gumboro Disease as per the schedule on first, seventh and fourteenth day, respectively.

3.1.4.2 Housing management

Up to first 3 weeks of age 10 birds per cage were housed then after the space was expanded with 5 birds per cage as per the Table-2 showing treatment and agewise broiler rearing in cages. All the managemental practices required for optimum brooding and rearing of birds were followed.

P-1

P-2

P-3

P-4

P-5

P-6

Table-1. Feed formulation for starter and finisher ration with different ingredients

Sr.No.	Ingredients	Broiler starter (0-4 week)	Broiler finisher (5-6 week)
1	Maize	53.900	54.075
2	Deoiled rice bran	1.200	9.200
3	Soyabean deoiled cake	39.755	30.500
4	Trace Minerals[1]	0.100	0.000
5	Shell Grit	2.200	1.700
6	D.C.P.	2.000	1.390
7	Salt	0.300	0.400
8	Furazolidone[2]	0.025	0.050
9	Metabolic activator[3]	0.100	0.100
10	Toxin Binder[4]	0.100	0.100
11	Lysine	0.100	0.100
12	D.L Mithionine	0.135	0.090
13	Natural Performance enhancer	0.025	0.025
14	Vitamin (B_{12})[5]	0.010	0.020
15	Groundnut oil	0.000	2.000
16	Vitamin and Mineral supplement[6]	0.000	0.200
17	CP (%)	23.048	20.07
18	M.E (kcal / kg feed)	2797.77	2907.00
Trace Minerals[1]= Each kg contains: Copper (15 g), Iodine (1 g), Iron (60g), Manganese (80 g), Selenium (0.3 g), Zinc (80 g), Inorganic nutritive care (Q.S.)			
Furazolidone[2] = Each kg contains furazolidone (200 g) and inorganic nutritive carrier (Q.S.)			
Metabolic activator[3] = 1 kg per tonne of feed having Lecithin extract treated with co enzyme			
Toxin Binder[4] = Selected silicates, surfactants, organic acids and salts of organic Acids			
Vitamin (B_{12})[5]= Each kg contains 100 mg Vit. B_{12}			
Vita. and Min. supplement[6] = Each 2 kg contains Vit. A (50 lakh IU), Vit. B_2 (2g), Vit. B_6 (400 mg), Vit. B_{12} (5600 mcg), Vit. D_3 (6.25 lakh IU), Vit. E (800 IU), Choline chloride (10 g), Calcium pantothenate (4 g), Copper (2 g), Manganese (27.5 g), Iron (7.5 g), Zinc (15 g), Iodine (1 gm), Calcium (27.25 %), Phosphorus (7.45 %)			

Table-2. Coccidiostat treatment groups and age wise no. of birds with no. of replicate

Age (wk)	T1 Diclazuril	T2 Salino--mycin	T3 Diclazuril +Salino--mycin	T4 Madura--micin	T5 Control Infected Non Medicated	T6 Control Non Infected Non Medicated
0-3	50 (10x5R)	50 (10x5R)	50 (10x5R)	50 (10x5R)	50 (10x5R)	50 (10x5R)
4-6	50 (5x10R)	50 (5x10R)	50 (5x10R)	50 (5x10R)	50 (5x10R)	50 (5x10R)

3.1.4.3 Feed and water management of birds

Ground maize was given for first day in plastic feed dishes to broiler chicks. From second day, the experimental feed was offered. After first week of age, the feed was offered in the linear cage feeders adjusted outside of the cages up to 6 weeks of experimental period. Weighed quantity of feed was offered thrice a day i.e. at 9:00 a.m., 2:30 p.m. and 10:00 p.m. during entire experimental period. All the measures were taken to minimize the wastage of feed. Stirring and mixing of feeds in the feeder was done 4-5 times per day. Clean, fresh, wholesome drinking water was made available to all experimental birds throughout the experimental period.

3.1.4.4 Counting, Collection and inoculation of oocysts

The oocysts in the droppings were counted by using McMaster slide as described by Joyner (1958). The number of oocysts present on stock suspension was estimated by employing Fuschs-Rosenthal haemocytometer (Joyner and Davies, 1960). The values obtained by this method were compared by counting the total number of oocysts present in 10 µl of uniformly mixed suspension. *Eimeria tenella* oocysts were isolated and utilized for the

experiment. Preparation and administration of the inoculums was carried out in the manner described by Hein (1968).

Fresh suspensions of oocysts were prepared for each inoculation. The age of the oocysts calculated from the day of recovery was 10 days. The oocysts were washed in normal saline before inoculation to remove traces of potassium dichromate if any, to avoid toxicity to chickens. The sporulated oocyst suspension was washed four times in distilled water in order to remove potassium dichromate from the culture. The number of oocysts per ml of distilled water was estimated by using haemocytometer as described by Long *et al.* (1976). The desired dose of 50,000 oocysts for *Eimeria tenella* were adjusted to 1.0 ml of suspension and it was inoculated by intra-crop route to experimental birds in T1 to T5 group at 22 days age.

3.1.4.5 Clinical manifestations, mortality and necropsy records

Clinical signs were recorded during the acute phase of the infection. Droppings were examined daily for the presence of oocysts from all the treatment groups. The pre patent period was also recorded. A daily record of mortality of each group was maintained with bird identification. Reason for death and the weight of dead bird was taken before necropsy. Necropsy lesions of the birds were recorded.

3.1.4.6 Faecal score

Approximately 5-10 g of ten fresh faecal sample were collected from each group in polythene bags and were transported fresh to the laboratory for further processing daily. Faecal score was made upon the qualitative observation of the appearance of the dropping from normal as given in Table-3. Faecal score was observed from day 2 post infection to day 12 post infections regularly up to 11 days (Shameem *et al.*, 2010).

Table-3. Faecal score number with score wise description of faecal dropping consistency

Sr. No.	Faecal Score No	Description of faecal dropping consistency
1	0	normal dropping
2	1	slightly abnormal dropping
3	2	loose motion
4	3	dropping mixed with blood
5	4	fresh bloody diarrhoea

3.1.4.7 Oocyst Per Gram (OPG)

It was done to know the number of oocysts passed in the faeces of each group of caged birds to observe on the ability of a drug to suppress oocyst production. Oocyst per gram was calculated from day 4 post infection to day 12 post infection regularly upto 9 days. The procedure followed for OPG was McMaster chamber method as described by Long and Rowell (1958). Ten gram of faeces was soaked in 100ml of tap water for 24 hr at 4°C in 200 ml beaker, tightly covered with lid. The beaker was shaken vigorously and suspension was filtered through a single thickness of muslin cloth and filtrate adjusted to 100ml. A 15 ml centrifuge tube was filled with filtrate up to 1 cm from the top and centrifuged for 5 min at 800 rpm. The supernatant was discarded. The pellet was then re suspended in a few millilitres of saturated sodium chloride solution with a vortex mixer, or by tapping the tube. More salt solution was added to the original 15 ml volume and tube was inverted several times. Samples were removed with Pasteur pipette and McMaster counting chamber was filled.

3.1.4.8 Lesion score

Lesion score described by Johnson and Reid (1970) according to severity of the intestinal/ caecal changes at the time of necropsy of birds infected with *Eimeria tenella* was

followed. The lesions score were studied on third, fifth, seventh and ninth day post inoculation in battery trials as follows.

Table-4. Lesion score number with score wise description of lesions

Sr. No.	Score No.	Description of Lesions
1	0	No gross lesions
2	+1	Very few scattered petechiae on the caecal walls; no thickening of caecal wall; normal caecal contents present.
3	+2	Lesions more numerous with noticeable blood in the caecal contents; caecal wall was somewhat thickemed; normal caecal contents present
4	+3	Large amount of blood or caecal cores present; caecal walls greatly thickened; little faecal contents in the caeca.
5	+4	Caecal wall greatly distended with blood or large caseous cores; faecal debris lacking or included in cores. Dead birds scored as +4

3.1.4.9 Oocyst index

Oocyst index was determined by microscopic examination of mucosal scrapings from the caeca on day 7 post infection as per the method of Hilbrich (1978) with some modification. The caecal mucosa was scrapped onto the cover slip and the cover slip was then pressed on a microscopic slide in such a way that the mucosal material got spread underneath the whole surface area of the cover slip. Five fields of cover slip were viewed for each scraping i.e. four corners and central field and the oocysts were counted in each field. The oocyst index was graded according to the number of oocysts per field as follows:

Table-5. Oocyst Index number with microscopic field wise number of oocyst

Sr. No.	Oocyst Index No	Field wise number of oocyst
1	0	<1 oocyst/ field
2	1	1-10 oocysts/ field
3	2	11-20 oocysts/ field
4	3	21-50 oocysts/ field
5	4	51-100 oocysts/ field
6	5	>100 oocysts/ field

3.1.4.10 Global index

Stephan *et al.* (1997) suggested a formula for detection of resistance to anticoccidials by finding out global index based on five parameters, viz., per cent weight gain, feed conversion ratio, lesion score, oocyst index and per cent mortality. In this formula weightage to different parameters was given in view of their importance. The following formula was used in the present study.

$$GI=\%WG_{NN}-[(F_{IM}-F_{NNC})\times 10]-[OI_{IM}-OI_{INC}]-[LS_{IM}-LS_{INC})\times 2]-[\%Mortality/2]$$

where, GI = Global index, OI = Oocyst Index, WG = Weight Gain, F = Feed conversion ratio, LS = Lesion Score, NNC = Non infected Non medicated Control, IM = infected medicated control, INC = Infected Non medicated control. In this formula weight gain and feed conversion ratio at the end of experiment was considered. The Global Index of Non infected non medicated Control in percentage (GI_{NNC} %) was calculated from above Global Index values. The global index of Non infected Non medicated Control in percentage (GI_{NNC} %) of >90% indicates very good efficacy, >80% represents good efficacy, >70%

represents limited efficacy, >50% represents resistance and <50% represents complete resistance to the drug.

3.1.4.11 Growth and feed parameters

The most important criterion for evaluating effects of anticoccidial drugs is body weight and body weight gain. The birds were weighed at the end of the every week in the cool morning hours before feeding and the weekly feed consumption was recorded. Feed conversion ratio (FCR) was calculated by using the following formula. Important traits required for study of present experiment are described as follows:

3.1.4.11.1 Weekly Body Weight (BW)

Body Weight (g) at day old (BW_0) and thereafter at weekly interval i.e. at 1^{st} (BW_1), 2^{nd} (BW_2), 3^{rd} (BW_3), 4^{th} (BW_4), 5^{th} (BW_5) and 6^{th} (BW_6) weeks of age were recorded in the cool morning hours before feeding.

3.1.4.11.2 Weekly Body Weight Gain (BWG)

Body weight gain (g) was calculated at weekly interval. Broiler birds were weighed individually at weekly interval up to six weeks of age and weekly body weight gain was calculated by subtracting the average body weight (g) of previous week from that of average weekly body weight (g) of current week and were designated as $BWG_{(0-1)}$, $BWG_{(1-2)}$, $BWG_{(2-3)}$, $BWG_{(3-4)}$, $BWG_{(4-5)}$ and $BWG_{(5-6)}$. $BWG_{(0-4)}$, $BWG_{(4-6)}$ and $BWG_{(0-6)}$ were also calculated. Weight gains of each group were compared in between different treatment group and with non-medicated non- infected control group.

$$\% \text{ Weight gain} = \frac{\text{Mean weight gain of medicated infected group} \times 100}{\text{Mean weight gain of non- medicated non-infected group}}$$

3.1.4.11.3 Feed Consumption (FC)

The weighed quantity of feed was offered daily to birds of each replication in each group. At the end of every week, the left over feed was weighed and recorded in each replicate. Feed consumption was calculated by subtracting the left over feed from total feed offered in each week. Feed consumption during 1^{st}, 2^{nd} , 3^{rd} , 4^{th} , 5^{th} and 6^{th} weeks were calculated and designated as FC_1, FC_2, FC_3, FC_4, FC_5, and FC_6, respectively. Feed consumption up to 4^{th} week, 5 to 6 week and up to 6^{th} week of age were calculated and designated as $FC_{(0-4)}$, $FC_{(5-6)}$, and $FC_{(0-6)}$, respectively.

3.1.4.11.4 Feed Conversion Ratio (FCR)

FCR was calculated during each week (FCR_1, FCR_2, FCR_3, FCR_4, FCR_5, and FCR_6), 0-4 weeks (FCR_{0-4}), 5-6 weeks (FCR_{5-6}) and 0-6 weeks (FCR_{0-6}) and it is derived by the following formula.

$$FCR = \frac{\text{Average Feed Consumption(g)}}{\text{Average body weight gain (g)}}$$

3.2 Collection of blood

Approximately 5 ml of blood was collected from wing vein for haematology and for separation of serum. Blood samples were taken randomly from 10 birds in each group before experimental infection of *E. tenella* at 3 week and at 4 weeks of age after experimental infection of *E. tenella* from same group birds (Plate-12 &13). For haematology, 1.0 ml blood was transferred to a citrated vial and remaining blood was allowed to clot and after retraction of clot, serum was separated and centrifuged. Serum samples were stored at -20°C in deep-freeze until analyzed for the biochemical and enzymatic attributes. In all, 60 blood samples before experimental infection and 60 blood samples after experimental infection of six groups

of broilers were used for haemato-biochemical alterations at Dept. of Animal Physiology and at Dept. of Animal Biochemistry & Biotechnology.

3.2.1 Haematology

Haemoglobin (Hb) content of blood was estimated by Sahli's acid haematin method (Coles, 1986). The values were expressed as gram per cent of blood. Packed cell volume (PCV) was estimated by microhaematocrit method (Coles, 1986). The values of PCV were expressed as percentage. Total Erythrocytes Count (TEC) and Total Leukocytes Count (TLC) and Differential Leukocyte Count (DLC) was estimated as per the methods described by Jain (1986). The values were expressed as 10^6/µl, 10^3/µl and 10^3/µl for TEC, TLC and DLC, respectively.

3.2.2 *Biochemical* and Enzymatic Assay

3.2.2.1 Serum Glucose

Estimation of serum glucose was carried out as per the Glucose Oxidase and Peroxidase GOD/ POD standard method using kit of Crest Biosystems India Ltd., Goa. The values were expressed as milligram per deci litre of serum.

3.2.2.2 Serum Total Protein

Serum total protein content was estimated by Biuret Method using kit of Crest Biosystems India Ltd., Goa, and expressed as gm/ 100 ml of serum.

3.2.2.3 Serum Total Cholesterol

Estimation of serum total cholesterol was done by standard Cholesterol Oxidase (CHOD) method using kit of Crest Biosystems India Ltd., Goa. The values were expressed as milligram per 100 ml of serum.

3.2.2.4 Serum Glutamic Oxalo-acetic Transaminase (SGOT)

SGOT was also estimated by modified International Federation of Clinical Chemistry (IFCC) method (1986) using kit of Crest Biosystems India Ltd., Goa, and the values were expressed as U/L.

3.2.2.5 Serum Glutamic Pyruvic Transaminase (SGPT)

SGPT was estimated by modified International Federation of Clinical Chemistry (IFCC) method (1986) using standard kit of Crest Biosystems India Ltd., Goa. The values were expressed as U/L.

3.2.2.6 Serum Alkaline Phosphatase (AKP)

Serum alkaline phosphatase activity was estimated by P- Nitro Phenyl phosphate (PNPN) method (1954) using kit of Crest Biosystems India Ltd., Goa, and the values were expressed as U/L.

3.3 Collection of tissue

Tissue samples from broilers died of coccidiosis and from two random sacrificed broiler birds in each group at 2, 3, 4, 5, 6 weeks of age were examined for faecal score, lesion score and oocyst study. Caeca were collected in 10 per cent neutral formalin for histopathological study at Dept of Veterinary Pathology.

3.3.1 Histopathological Examinations

Tissue pieces of caeca preserved in 10 per cent neutral buffered formalin were processed by paraffin wax embedding method. Sections were cut at 4-5 micron thickness with the help of microtome and stained with Ehrlich's Haematoxylene and Eosin (H & E)

method for examinations as described by Luna (1960). Typical lesions were photographed at different magnifications.

3.4 Statistical Analysis

Data so generated during the present study were statistically analyzed as per the method of Snedecor and Cochran (1980) by using completely randomized design. Statistical Analysis System (SAS, 2000) was also used for the description statistics of the data.

RESULTS

AND

DISCUSSION

CHAPTER - IV

RESULTS AND DISCUSSION

Coccidiosis continues to be one of the most important disease causing significant economic losses to the poultry industry despite the availability of many anticoccidial drugs. To most of the anticoccidial drugs resistance has been reported in *Eimeria* species (Chapman, 1993). In this scenario, the determination of a relationship between the anticoccidial being used and level of efficacy against it in a given area assumes paramount significance.

In broiler production, numerous anticoccidial drugs are used for prevention and control of coccidiosis. It is achieved mainly based on proper management conditions, which includes the addition of coccidiostats in the feed as well as vaccination. However, development of tolerance to these drugs have lead to search for newer molecules continuously and different classes of anticoccidials have been discovered and used from time to time for prevention and control of coccidiosis.

Coccidiostats are classified based on the mode of synthesis in to two types *i.e.* ionophores and chemicals. The ionophorous compounds produced by fermentation of *Streptomyces spp.* Monensin was the first polyether ionophore marketed, (Shumard and Callnder, 1968) and subsequently activity was found in many other fermentation was marketed. Chemical coccidiostats are synthetically manufactured and further classified by the chemical composition and mode of action. Diclazuril, halofuginone and amprolium are commonly used chemical coccidiostats. Diclazuril is a nucleotide analogue and lethal against endogenous developmental stages of parasites. Arabkhazaeli *et al.* (2013) stated that mild or subclinical infections are also important because minor intestinal lesion can interfere with growth, feed efficiency and economy. Now a day shuttle and rotation programme is widely

used in broiler industry because of drug resistant problems (Chapman, 2014). Diclazuril in shuttle programme is highly efficacious against *Eimeria spp.* in comparision with other anticoccidials and ionophores (Convey *et al.,* 2001; Abbas *et al.* 2009).

The first resistant field strain of *Eimeria tenella* was isolated from USA by Waletzky *et al.* (1954). This strain failed to respond to treatment with sulphaquinoxaline. Today the position is such that resistance has been reported against almost all the anticoccidial drugs that have been introduced (Chapman, 1999). It was opined by Schnitzer and Grunberg (1957) that "Drug resistance has followed the development of chemotherapy like a faithful shadow and history of chemotherapy of drug resistance, aptly characterize this problem". In this circumstance, the determination of a relationship between the anticoccidial being used and level of efficacy against it in a given area needs focused attention.

The efficacy of three commonly used feed coccidiostats named Diclazuril (T1) @ 1 ppm, Salinomycin (T2) @ 60 ppm, Diclazuril + Salinomycin (T3) in shuttle programme and Maduramicin (T4) @ 5 ppm on experimentally induced *Eimeria tenella* coccidial infection and their effects on growth, haematology, biochemical and histopathological changes were undertaken in Three hundred Cobb400 strain of broiler chicken at University Poultry Complex during the year 2012. The findings are presented in various tables/graphs/plates and discussed suitably here in this chapter.

4.1 Comparative efficacy of cocidiostats on faecal score, oocyst output and lesion score in different treatment group after experimental *Eimeria tenella* infection

Faecal score, oocyst output and lesion score values are helpful for knowing the intensity of infection and degree of pathological damage in the intestine.

4.1.1 Comparative efficacy of cocidiostats on faecal score

Qualitative faecal score was made upon the observation of the appearance of the dropping from normal values and their mean value are given in Table-6 and graphically depicted in Figure-1. It was graded on a scale from 0 to 4. In T1 group mean value ranged from 0 to 2.4 between two to twelve days post infection. Highest mean value 2.4 ± 0.25 was observed in T1 and T3 group on 4^{th} and 5^{th} days PI among coccidiostats treatment groups, while on 3^{rd} day PI less faecal score (1.2 ± 0.2) was observed in T3 group as compare to T1 group (2.2 ± 0.2). In T2 and T4 group similar mean value 1.4 ±0.3 and 1.6 ±0.3 were observed on 3^{rd} and 4^{th} days PI, while non significant lower mean value were observed in T4 group as compare to T2 group on 5 to7 days PI. All faecal score mean value of treatment group was significantly lower as compare to infected control T5 group. Similar finding were reported by Majumdar *et al.* (1993). Over all lowest faecal score mean was observed in T4 group followed by T2 group. Similar findings were observed by the Raju *et al.* (2012) in Maduramicin and Salinomycin Treatment infected with 25000 sporulated oocyst of *E. tenella* in broilers . Abbas *et al.* (2008) also reported that maduramycin given @ 5 ppm at 12 days of age in broilers shown reduction in faecal score in 75000 *E. tenella* oocyst infections. Similar results of faecal score reduction by different coccidiostat were described by Rana and Tikaram (2002), Shameem *et al.* (2010) from India and by Danforth *et al.*(1977) from abroad in broiler birds suffering from natural or artificially produced caecal coccidiosis. Reduction in the faecal score values is due to the suppression of schizogony process by the coccidiostats resulting in less intestinal damage as compare to infected non treated control.

4.1.2 Comparative efficacy of cocidiostats on Oocysts Per Gram (OPG)

The efficacy of coccidiostats was evaluated based on their ability to suppress the oocyst production. It was studied from the forth to twelfth day post infection in T1 to T5 group. The mean oocyst per gram of faeces excreted in different five groups experimentally

infected with *E.tenella* on different days of infection are presented in Table-7 and graphically depicted in Figure-2.

4.1.2.1 Forth day post infection

On the fourth day post infection it was observed that there was statistically significant lower OPG counts in all four coccidistat treatment group as compared to control groups. Among treatment group significant lowest OPG (8727.80 ± 34.68) was found in Salinomycin treated group with slight non-significant rise in Maduramicin group (8790.80 ± 134.91) as compared to Diclazuril group (10690.50 ± 312.06) and Diclazulril + Salinomycin group (11069.00 ± 211.78).

4.1.2.2 Fifth day post infection

On the 5th day post infection lowest OPG was observed in Maduramicin treated group (10909.10 ± 167.62) followed by Salinomycin group (10943.00 ± 221.97), Diclazuril + Salinomycin shuttle group (12848.00 ± 164.26) and Diclazuril group (13509.40 ± 245.35). The value of OPG in all coccidiostat given group was significantly lower than the control group (20318.40 ± 97.42).

4.1.2.3 Sixth day post infection

On the sixth day post infection significant lower oocyst production was noted in old treatment group as compared to control group (21970.90 ± 249.91). The OPG value were lowest in Maduramicin group (12725.40 ± 188.39) following Salinomycin group (13912.30 ± 214.74), Diclazuril group (17014.10 ± 371.3) and Diclazuril + Salinomycin group (18103.60 ± 220.57).

4.1.2.4 Seventh day post infection

On seventh day post infection only Maduramicin group have significant lowest OPG (10922.00±168.99), while remaining groups have non-significant lower OPG value as compared to control group (22539.50 ± 171.69).

Table-6. Faecal score value (Mean + S.E.) in different treatment group from 2 to 12 day post infection (n=5)

Day PI	T_1	T_2	T_3	T_4	T5	Period Mean
2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00^{g}
3	2.20 ± 0.20	1.40 ± 0.24	1.20 ± 0.20	1.40 ± 0.24	3.40 ± 0.24	1.92^{b}
4	2.40 ± 0.24	1.60 ± 0.24	2.40 ± 0.24	1.60 ± 0.24	4.00 ± 0.00	2.40^{a}
5	2.40 ± 0.24	1.40 ± 0.24	2.40 ± 0.24	1.20 ± 0.20	4.00 ± 0.00	2.28^{a}
6	2.20 ± 0.20	1.60 ± 0.24	1.80 ± 0.20	1.00 ± 0.00	3.40 ± 0.24	2.00^{b}
7	1.20 ± 0.20	0.80 ± 0.20	1.20 ± 0.20	0.40 ± 0.24	3.40 ± 0.24	1.40^{c}
8	0.40 ± 0.24	0.60 ± 0.24	0.60 ± 0.24	0.60 ± 0.24	3.20 ± 0.20	1.08^{d}
9	0.40 ± 0.24	0.40 ± 0.24	0.60 ± 0.24	0.80 ± 0.20	2.60 ± 0.24	0.96^{de}
10	0.40 ± 0.24	0.60 ± 0.24	0.40 ± 0.24	0.20 ± 0.20	2.40 ± 0.24	0.80^{e}
11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.80 ± 0.20	0.36^{f}
12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.20 ± 0.20	0.24^{fg}
Treatment mean	1.06^{b}	0.77^{c}	0.96^{b}	0.66^{c}	2.67^{a}	
	T		P		T×P	
	S Em	C.D	S Em	C.D	S Em	C.D
	0.06	NS	0.09	0.24	0.19	0.54

The means bearing different superscript within same row differ significantly from each other (P<0.05)

Fig.-1. Faecal score value Mean in different treatment group from 2 to 12 day post infection

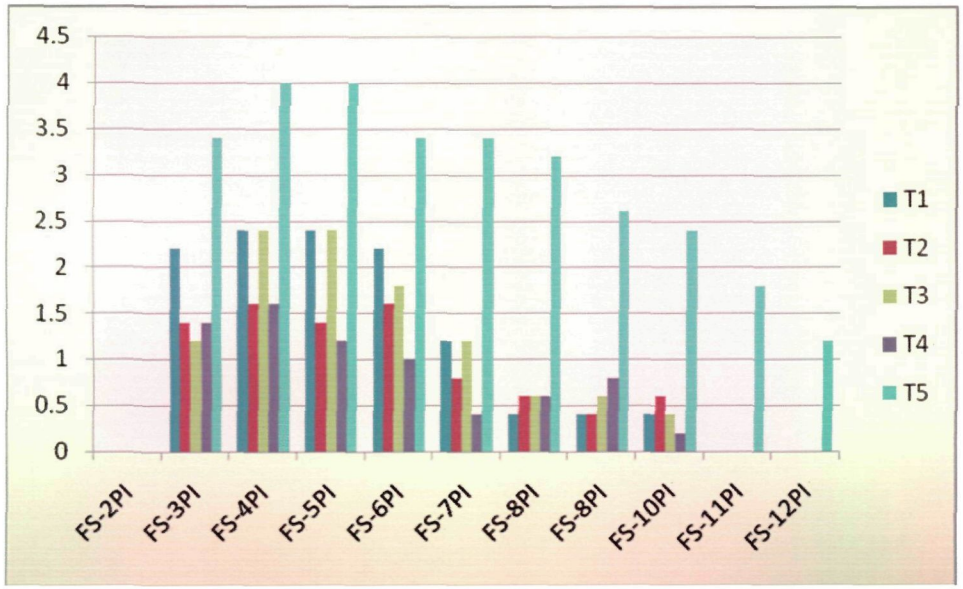


Fig.-2. Oocyst per gram value Mean in different treatment group from 4 to 12 day post infection

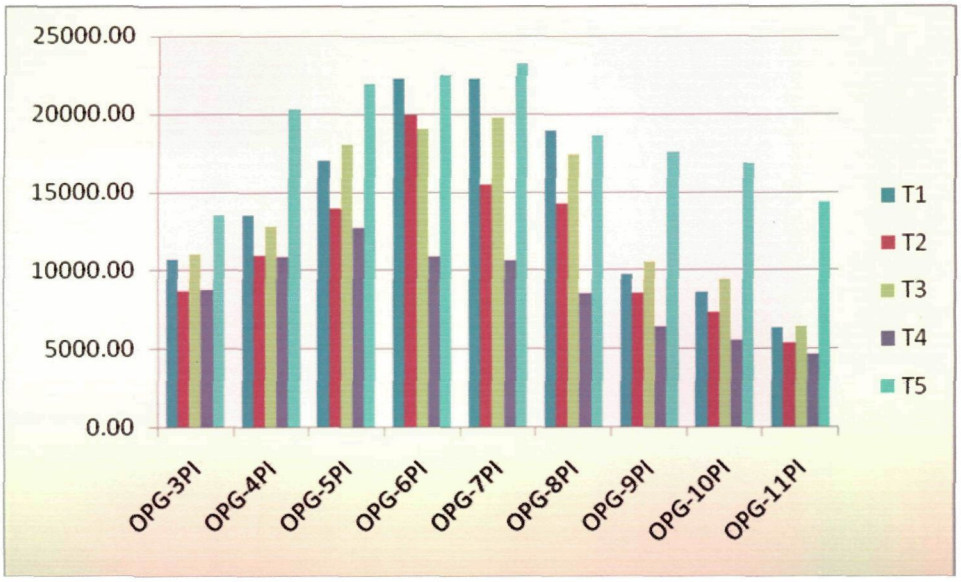


Table-7. Oocyst per gram value (Mean + S.E.) in different treatment group from 4 to 12 day post infection (n=10)

Day PI	T_1	T_2	T_3	T_4	T5	Period Mean
4	$10690.50^{c} \pm 312.06$	$8728.80^{d} \pm 134.68$	$11069.00^{b} \pm 211.78$	$8790.80^{d} \pm 134.91$	$13565.68^{a} \pm 265.23$	10568.94^{f}
5	$13509.40^{b} \pm 245.35$	$10943.00^{d} \pm 221.97$	$12848.00^{c} \pm 164.26$	$10909.10^{d} \pm 167.62$	$20318.40^{a} \pm 97.42$	13705.58^{e}
6	$17014.10^{c} \pm 371.93$	$13912.30^{d} \pm 214.74$	$18103.60^{b} \pm 220.57$	$12725.40^{e} \pm 188.39$	$21970.90^{a} \pm 249.91$	16745.26^{c}
7	$22227.70^{b} \pm 204.65$	$19927.90^{c} \pm 182.69$	$19056.50^{d} \pm 605.72$	$10922.00^{e} \pm 168.99$	$22539.50^{a} \pm 171.69$	18934.72^{a}
8	$22227.90^{b} \pm 259.77$	$15445.60^{d} \pm 154.51$	$19788.90^{c} \pm 137.71$	$10679.90^{e} \pm 125.37$	$23247.40^{a} \pm 212.16$	18277.94^{b}
9	$18951.40^{a} \pm 546.74$	$14237.80^{d} \pm 124.48$	$17425.40^{c} \pm 244.15$	$8568.90^{e} \pm 71.03$	$18595.70^{b} \pm 34.14$	15555.84^{d}
10	$9768.80^{c} \pm 50.04$	$8575.00^{d} \pm 70.24$	$10541.40^{b} \pm 124.21$	$6474.40^{e} \pm 123.59$	$17595.10^{a} \pm 91.54$	10591.54^{f}
11	$8599.70^{c} \pm 56.92$	$7324.00^{d} \pm 131.39$	$9415.30^{a} \pm 177.94$	$5537.30^{e} \pm 90.51$	$16861.80^{a} \pm 256.62$	9548.02^{g}
12	$6393.30^{b} \pm 78.38$	$5403.50^{c} \pm 76.14$	$6441.00^{b} \pm 121.38$	$4675.20^{d} \pm 111.02$	$14358.60^{a} \pm 81.69$	7454.32^{h}
Treatment mean	14375.87^{b}	11611.10^{d}	13854.35^{c}	8809.23^{e}	18784.00^{a}	
	T		**P**		**T×P**	
	S Em	**C.D**	**S Em**	**C.D**	**S Em**	**C.D**
	2.40	70.69	1.96	94.84	1.47	212.07

The means bearing different superscript within same row differ significantly from each other (P<0.05)

4.1.2.5 Eighth day post infection

On eighth day post infection significant reduction in OPG value were observed in Maduramicin group (10679.00 ± 125.37) and Salinomycin group (15445.60 ± 154.51) as compared to control (23247.40 ± 212.16).

4.1.2.6 Ninth day post infection

On ninth day post infection OPG value were highest in Diclazuril group (18951.40 ± 546.74) as compared to control group (18595.70 ± 34.14), while significant lowest values were observed in Maduramicin group (8568.90 ± 71.03) as compared to control as well as other treatment group.

4.1.2.7 Tenth, eleventh and twelfth day post infection

There was significant reduction in OPG count in all treated group as compared to control group. Among treatment groups, trend of reduction was similar with lowest OPG in Maduramicin group followed by Salinomycin group, Diclazuril group and Diclazuril + Salinomycin shuttle group during last three day post infection.

The overall treatment mean results indicates best efficacy of Maduramicin and Salinomycin in OPG reduction followed by Diclazuril and Diclazuril+Salinomycin. The overall trend of period mean showing increase in OPG count was observed upto seven days PI and there after decreasing trend was observed up to 12 days post infection.

In the present study Maduramicin and Salinomycin treated birds also showed lowered oocysts production which was more effective than Diclazuril and Diclazuril+Salinomycin groups. Our findings are in agreement with the following findings.

Salisch and Shakshouk (1990) also reported reduced oocyst output in Maduramicin treatment group when compared with narasin and monensin infected with *Eimeria tenella* (2.5×10^4 oocysts per bird) in broiler chickens. Abbas *et al.* (2008) also reported that maduramicin given @ 5 ppm at 12 days of age in broilers shown reduction in OPG as

compared to control birds after 7 day PI in *E. tenella* oocyst infection@ 75000. Azizi *et al.* (2010) suggested that maduramicin could decrease the OPG significantly and improve the production performance as compare to salinomycin in experimental produced coccidiosis in broilers.

Shojadoost *et al.* (2003) observed that there was reduced oocyst per gram of faeces in broiler birds infected with *Eimeria tenella* (25,000 oocysts) and treated with Salinomycin sodium (66 ppm) as found in the present study with double dose of oocysts. Muangyai *et al.* (1991) evaluated the efficacy of toltrazuril at 8 ppm and Maduramicin 5 ppm in feed, in commercial broilers infected with *E. acervulina, E. maxima* and *E. tenella*. Oocysts per gram of faeces value were slightly higher in the Maduramicin treated group when compared with the toltrazuril group. Difference in the result might be due to species, drug and dose variation.

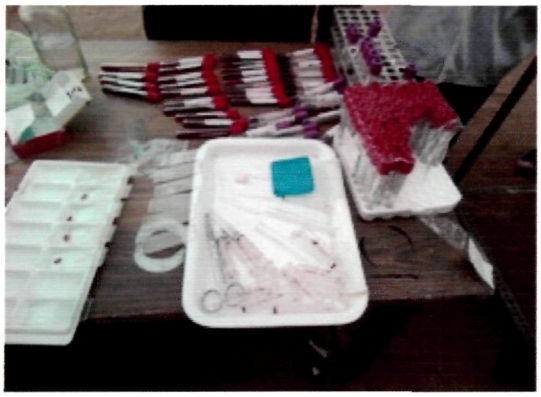
4.1.3 Comparative efficacy of cocidiostats on lesion score

Lesion score was done as per the procedure given by Johnson and Reid (1970). It was carried out on third, fifth, seventh and ninth day post infection and the result is given in Table-8 and graphically depicted in Figure-3. Photograph of different lesion score categories are given in Plate no. 15-18.

4.1.3.1 Third day post infection

Lesion score was +2 in Diclazuril, Diclazuril + Salinomycin and Positive control group with blood in the caecal contents and thickened caecal wall. One bird of positive control group was showing caseous cores at some places.

Maduramicin group (1.20 ± 0.20) and Salinomycin group (1.60 ± 0.24) showed lower lesion score as compare to other treatment and control group.



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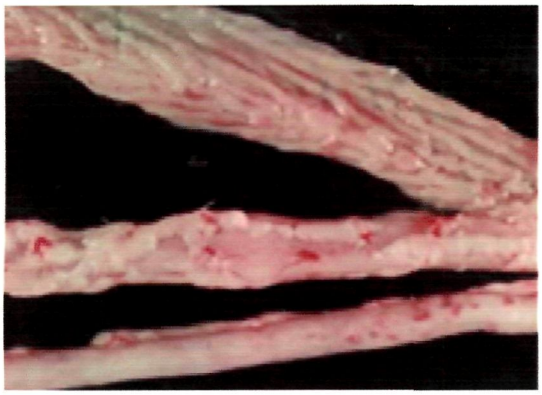
P-14



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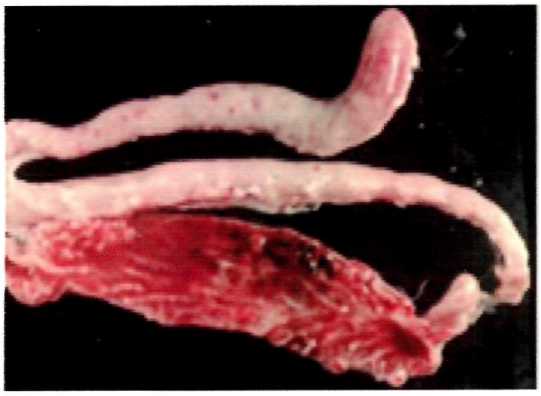

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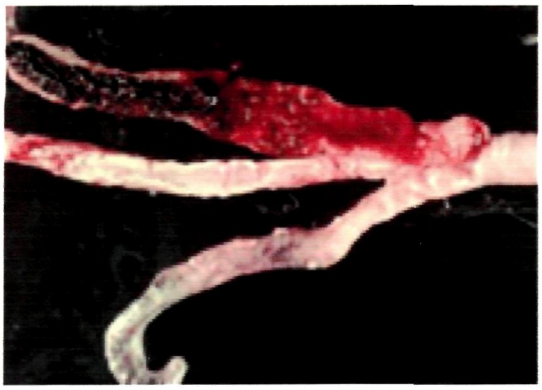

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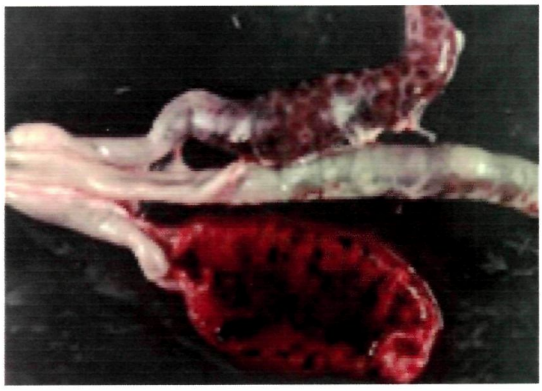


P-18

Table-8. Lesion score value (Mean + S.E.) in different treatment group from 3, 5, 7 and 9 day post infection (n=5)

Day PI	T_1	T_2	T_3	T_4	T5	Period Mean
3	2.40^{a} ± 0.24	1.60^{a} ± 0.24	2.20^{a} ± 0.20	1.20^{a} ± 0.20	2.40^{a} ± 0.24	1.96^{a}
5	1.80^{a} ± 0.20	1.60^{a} ± 0.24	1.80^{a} ± 0.20	1.80^{a} ± 0.20	2.40^{a} ± 0.24	1.88^{a}
7	1.40^{a} ± 0.24	1.20^{a} ± 0.20	1.40^{a} ± 0.24	1.00^{a} ± 0.00	2.40^{a} ± 0.24	1.48^{b}
9	1.20^{a} ± 0.20	0.60^{a} ± 0.24	0.80^{a} ± 0.20	0.40^{a} ± 0.24	1.80^{a} ± 0.20	0.96^{c}
Treatment mean	**1.70^{b}**	**1.25^{cd}**	**1.55^{bc}**	**1.10^{d}**	**2.25^{a}**	
	T		**P**		**T×P**	
	S Em	**C.D**	**S Em**	**C.D**	**S Em**	**C.D**
	0.11	0.31	0.10	0.28	0.22	NS

The means bearing different superscript within same row differ significantly from each other ($P<0.05$).

Fig.-3. Lesion score value Mean in different treatment group from 3,5,7 and 9 day post infection

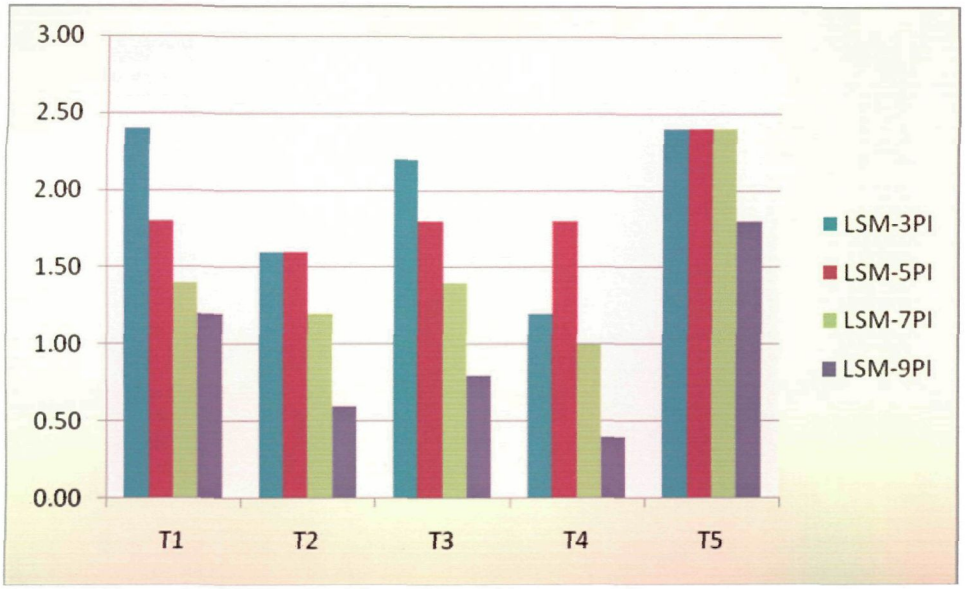
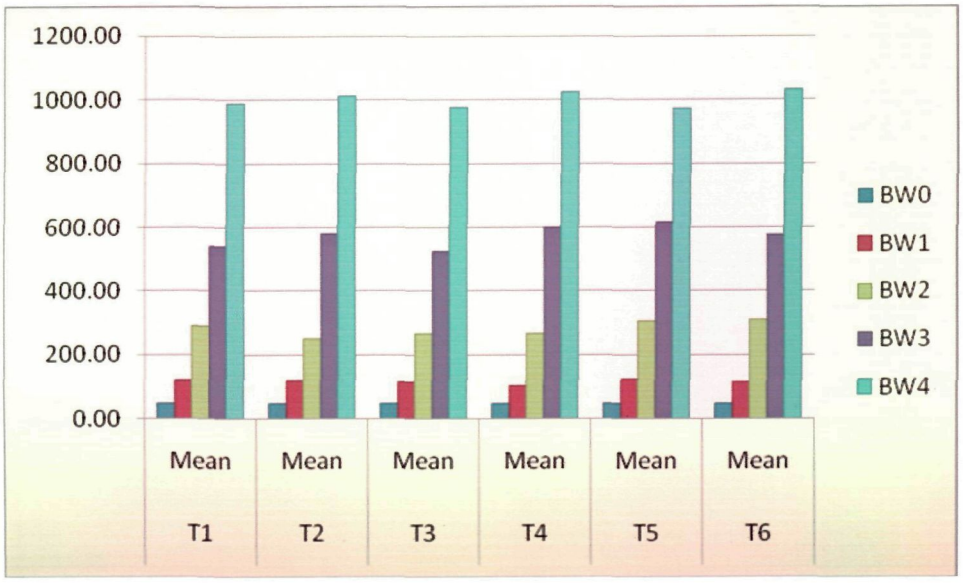


Fig.-4. Weekly Mean body weight in gram in different treatment group at starter stage



4.1.3.2 Fifth day post infection

All treatment groups have lower lesion score as compared to control group. Non medicated infected birds showing large amount of blood with thickened caecal wall, while coccidiostats given groups have less severity as compare to control one.

Statistically lesion score of Dicazuril group, Diclazuril + Salinomycin group and Maduramicin group was similar i.e. 1.80 ± 0.20 where Salinomycin group showing 1.60 ± 0.24 lesion score value.

4.1.3.3 Seventh day post infection

The severity of the lesion was reduced in all treatment groups as compared to control group (2.40 ± 0.24). The lowest lesion score was observed on Maduramicin group (1.00 ± 0.00) followed by Salinomycin group (1.20 ± 0.20) and remaining two group with similar 1.40 ± 0.24 lesion score indicating between efficacy of Maduramicin and Salinomycin causing least caecal damage.

4.1.3.4 Ninth day post infection

Maduramicin group, Salinomycin and Diclazuril + Salinomycin group birds were showing very few petechiae on the caecal wall without caecal wall thickening as compared to Diclazuril and control group.

In the present study the mean lesion score value of *Eimeria tenella* infected and coccidiostat given group was in range of 1.10 to 1.70, which was significantly low as compared to 2.25 in infected non medicated birds. Statistically all treatment groups were showing significant lower lesion score as compare to infected control. Majumdar *et al.* (1993) reported similar better results for lesion score in coccidiostat given birds as compared to non medicated birds.

In the present study also *Eimeria tenella* infected and Maduramicin treated birds showed lower lesion score when compared to infected non medicated birds. The lower

treatment mean lesion score values (1.10) in Maduramicin group followed by Salinomycin group (1.25) indicating better efficacy of Maduramicin and Salinomycin.

Similar findings were observed by Anish *et al.* (2007). They evaluated the effect of currently used ionophores, Maduramicin (5 ppm) and Salinomycin (60 ppm) in battery trials by infecting birds with field isolate *of Eimeria tenella* and observed similar trend of lesion score in both the treatments. The findings are also in accordance with Yvore *et al.* (1990), Varga *et al.* (1988), Salisch and Shakshouk (1990), Shojadoost *et al.* (2003) infected with 25,000 oocysts doses, Shammem *et al.* (2010) infected with 32000 oocysts doses, McDougald *et al.* (1981) under floor-pen conditions and by Raju *et al.* (2012) infected with 25000 oocysts doses of *Eimeria tenella* for Maduramicin and Salinomycin treatment in broiler birds.

Conway *et al.* (1990) observed higher coccidial lesion scores in male broiler chicks experimentally infected with different field isolates of *Eimeria acervulina, E. maxima,* and *E. tenella* given Salinomycin at 60 ppm which might be due to the difference in isolates, age and immune status of the birds.

4. 1. 4 Comparative efficacy of cocidiostats on oocyst index

The Oocyst index was studied on seventh day post infection and the values were given in table-9. It was 0, 1, 2, 2, 3 in Maduramicin group, 0, 3, 3, 2, 1 in Salinomycin group, 2, 0, 3, 4, 4 in Diclazuril group, 2, 2, 3, 3, 4 in Shuttle group and 3, 4, 4, 4, 5 in positive control group. Average oocyst index was highest in positive control (4.0). Maduramicin and Salinomycin groups have lower oocyst index as compare to Diclazuril and Diclazuril + Salinomycin Shuttle group. Result indicates better efficacy of Maduramicin and Salinomycin.

Muzurkiewez *et al.* (1987) studied the effect of ionophore coccidiostats against *E. tenella* and *E. acervulina* infections and noted that the best oocyst index was obtained for

Maduramicin followed by lasalocid, narasin, Salinomycin and monensin. Abbas *et al.* (2008) also reported that maduramicin given @5 ppm at 12 days of age in broilers shown reduction in oocyst index as compared to control birds in *E. tenella* infection of 75000 dose oocysts. Georgieva *et al.* (2010) reported that Maduramicin at 5 ppm dose rate found to reduce oocyst index and lesion score at 7 day PI given mixed 80000 *Eimeria spp.* oocysts. These results are in agreement with our study in which Maduramicin fed group shown lowest oocyst index, which might be due to beneficial effects of maduramicin on lipid peroxidation reducing oxidative stress as reported by Georgieva *et al.* (2010).

Raju *et al.* (2012) found higher oocyst index in Maduramicin and Salinomycin treatment group as compare to our result which might be due to location and oocysts dose difference.

4.1.5 Comparative efficacy of coccidiostats on clinical signs and mortality

The birds infected with *Eimeria tenella* and non-medicated control T5 showed clinical signs of depression, reduced feed and water intake on third and fourth day and bloody diarrhoea on fifth day post infection, where as infected and coccidiostat given groups showed a lower degree of clinical symptoms. Maduramicin(T4) given group birds were moderately active with poor clinical signs observed with increased severity trend in Salinomycin (T2), Diclazuril + Salinomycin (T3) and Diclazuril (T1), respectively after three week in experimentally infected birds. The percent mortality of the birds is given in Table-10.

The rate of mortality in Maduramicin group, Salinomycin group, Diclazuril group, Diclazuril + Salinomycin group and Infected non treated group were 4%, 6%, 10%, 12%, and 40%, respectively. Significant low mortality (4-12%) was found in medicated group as compared to non medicated group. Overall treatment mean mortality was found lowest in maduramicin group (0.67) followed by Salinomycin (1.0), Diclazuril (1.67) and Diclazuril + Salinomycin Shuttle group (2.0). Results indicates better efficacy of Maduramicin and

Table-9. Oocyst index number in different treatment group at 7 day post infection

Sr. No.	Treatment group	Index Value	0	1	2	3	4	5	Average main
1	T1	2,0,3,4,4	1	-	1	1	2	-	2.6
2	T2	0,3,3,2,1	1	1	1	2	-	-	1.8
3	T3	2,2,3,3,4	-	-	2	2	1	-	2.8
4	T4	0,1,2,2,3	1	1	2	1	-	-	1.6
5	T5	3,4,4,4,5	-	-	-	1	3	1	4.0

Table-10. Weekly mortality percent mean in different treatment group

Week	T_1	T_2	T_3	T_4	T5	T6	S.Em	C.D.
I	0	0	0	0	0	0	1.03	2.98
II	0	0	0	0	0	2		
III	0	0	0	0	0	0		
IV	8	5	10	4	32	0		
V	2	1	2	0	8			
VI	0	0	0	0	0	0		
Treatment Mean %	**1.67^a**	**1.00^a**	**2.00^a**	**0.67^a**	**6.67^b**	**0.33^a**		

The means bearing different superscript within same row differ significantly from each other ($P<0.05$)

salinomycin in our study. Badiola *et al.* (1994) also stated that Maduramicin is better coccidiostat having less mortality.

Wornick *et al.* (1980) reported that Salinomycin at the rate of 60 ppm was effective in controlling chicken coccidiosis based on reduced mortality and clinical observations. They opined that Salinomycin was superior over monensin or other anticoccidial. Similarly Wheelhouse *et al.* (1985) observed low mortality in broiler chicken infected with *Eimeria tenella* and treated with Salinomycin sodium (60 ppm) when compared with the lincomycin (2.2 ppm) treated group. Manuel *et al.* (1979) evaluated the efficacy of Salinomycin (66 ppm) treated group performed well when compared with monensin (100 ppm) and clopidol (30 ppm) treated birds. These findings are in agreement with present study where Maduramicin and Salinomycin treated birds' revealed reduced mortality.

In the present study, *Eimeria tenella* infected and non medicated birds had 40.00 per cent mortality and it was less than the reports of Migaki and Babcock (1983) who found average mortality rate of 49.3 per cent in non medicated birds. Raju *et al.* (2012) reported higher (23.8 %) mortality as compare to our study in Maduramicin fed group, which might be due to drug resistant in that particular area.

4.2 Comparative efficacy of cocidiostats on body weight and body weight gain

Body weight and weight gain are the most sensitive and informative parameters of anti coccidial efficacy. The mean body weight (g) at different ages has been presented in Table-11 & Table-12 and graphically depicted in Figure-4 & Figure-5, respectively.

The mean weekly body weight gain (g), weight gain during starter phase i.e. $BWG_{(0-4)}$, finisher phase i.e. $BWG_{(4-6)}$ and overall experimental period i.e. $BWG_{(0-6)}$ has been presented in Table-13 & Table-14 and graphically depicted in Figure-6 & Figure-7, respectively.

Table-11. Weekly body weight in gram (Mean + S.E.) in different treatment group at starter stage

Week	T_1	T_2	T_3	T_4	T_5	T_6
BW_0	46.46 ± 0.52	47.06 ± 0.16	46.80 ± 0.14	47.08 ± 0.42	47.32 ± 0.11	46.84 ± 0.26
BW_1	120.20^{a} ± 1.43	117.96^{a} ± 1.48	116.86^{a} ± 1.47	105.08^{b} ± 0.29	122.46^{a} ± 0.84	116.66^{ab} ± 0.82
BW_2	289.10^{b} ± 4.70	251.00^{d} ± 3.00	265.32^{c} ± 4.51	266.66^{c} ± 3.29	307.34^{a} ± 0.33	310.00^{a} ± 3.30
BW_3	541.9^{d} ± 5.24	581.94^{c} ± 4.12	525.10^{e} ± 5.21	600.10^{b} ± 5.05	617.80^{a} ± 7.49	575.68^{c} ± 2.01
BW_4	989.04^{c} ± 5.57	1013.98^{b} ± 5.15	979.30^{cd} ± 11.04	1023.86^{ab} ± 6.92	974.84^{d} ± 2.85	1031.60^{a} ± 11.14
Treatment Mean	397.35^{c}	402.39^{c}	386.68^{d}	408.56^{b}	413.95^{ab}	416.16^{a}
	T		**P**		**T×P**	
	S Em	**C.D**	**S Em**	**C.D**	**S Em**	**C.D**
	2.00	5.59	1.82	5.11	4.47	12.51

The means bearing different superscript within same row differ significantly from each other ($P<0.05$)

Table-12. Weekly body weight in gram (Mean + S.E.) in different treatment group at finisher stage

Week	T_1	T_2	T_3	T_4	T_5	T_6
BW_5	1413.14^{b} ± 7.96	1416.04^{b} ± 3.85	1339.04^{ab} ± 10.01	1416.54^{b} ± 12.69	1284.18^{d} ±13.84	1533.44^{a} ±8.20
BW_6	1767.14^{c} ± 8.26	1780.56^{c} ± 10.58	1743.44^{d} ± 1.42	1871.72^{b} ± 7.70	1520.12^{e} ± 2.72	1965.10^{a} ±12.93
Treatment Mean	1590.14^{c}	1598.30^{c}	1541.24^{d}	1644.13^{b}	1402.15^{e}	1749.27^{a}
	T		**P**		**T×P**	
	S Em	**C.D**	**S Em**	**C.D**	**S Em**	**C.D**
	4.66	12.92	3.30	9.14	8.08	22.39

The means bearing different superscript within same row differ significantly from each other ($P<0.05$)

Table-13. Weekly and overall body weight gain in gram (Mean + S.E.) in different treatment group at starter stage

Week	T_1	T_2	T_3	T_4	T_5	T_6
$BWG_{(0-1)}$	73.74^{a} ± 1.50	70.90^{a} ± 1.47	70.06^{a} ± 8.52	58.00^{b} ± 0.46	75.14^{a} ± 0.90	69.82^{ab} ± 0.66
$BWG_{(1-2)}$	168.60^{b} ± 5.30	133.04^{d} ± 4.04	148.46^{cd} ± 3.77	161.58^{bc} ± 3.12	184.88^{ab} ± 1.01	193.34^{a} ± 3.77
$BWG_{(2-3)}$	252.84^{c} ± 6.62	330.94^{a} ± 4.95	259.78^{c} ± 2.47	333.44^{a} ± 5.12	310.46^{b} ± 7.19	265.68^{c} ± 1.45
$BWG_{(3-4)}$	447.10^{a} ± 6.27	432.04^{b} ± 6.19	454.20^{a} ± 15.73	423.76^{b} ± 7.16	357.04^{c} ± 10.06	455.92^{a} ± 12.43
$BWG_{(0-4)}$	512.58^{b} ± 5.50	966.92^{a} ± 5.06	932.50^{b} ± 10.96	976.78^{a} ± 17.10	927.52^{b} ± 12.94	984.76^{a} ± 11.07
Treatment Mean	235.64^{bc}	241.73^{ab}	233.12^{c}	244.19^{a}	231.88^{c}	246.19^{a}
	T		**P**		**T×P**	
	S Em	**C.D**	**S Em**	**C.D**	**S Em**	**C.D**
	3.02	8.50	2.47	6.93	6.05	16.98

The means bearing different superscript within same row differ significantly from each other ($P<0.05$)

Table-14. Weekly and overall body weight gain in gram (Mean + S.E.) at finisher stage in different treatment group

Week	T_1	T_2	T_3	T_4	T_5	T_6
$BWG_{(4-5)}$	424.10^{b} ± 8.97	402.06^{bc} ± 4.14	359.74^{d} ± 8.40	392.68^{c} ± 10.35	309.34^{e} ± 14.13	501.84^{a} ± 10.65
$BWG_{(5-6)}$	354.00^{d} ± 8.37	364.52^{d} ± 11.34	404.40^{c} ± 9.67	455.18^{a} ± 10.96	235.94^{e} ± 15.21	431.66^{b} ± 10.51
Treatment Mean	389.05^{c}	383.29^{c}	382.07^{c}	423.93^{b}	272.064^{d}	466.75^{a}
$BWG_{(4-6)}$	778.10^{c} ± 8.96	766.58^{c} ± 11.47	764.14^{c} ± 7.01	847.86^{b} ± 6.03	545.28^{d} ± 4.01	933.50^{a} ± 13.24
$BWG_{(0-6)}$	1720.68^{c} ± 7.49	1733.50^{c} ± 7.45	1696.04^{d} ± 1.32	1824.64^{b} ±1126	1472.80^{e} ± 4.96	1918.26^{a} ± 9.86
	T		**P**		**T×P**	
	S Em	**C.D**	**S Em**	**C.D**	**S Em**	**C.D**
	4.66	12.92	3.30	9.14	8.08	22.39

The means bearing different superscript within same row differ significantly from each other ($P<0.05$)

Fig.-5. Weekly Mean body weight in gram in different treatment group at finisher stage

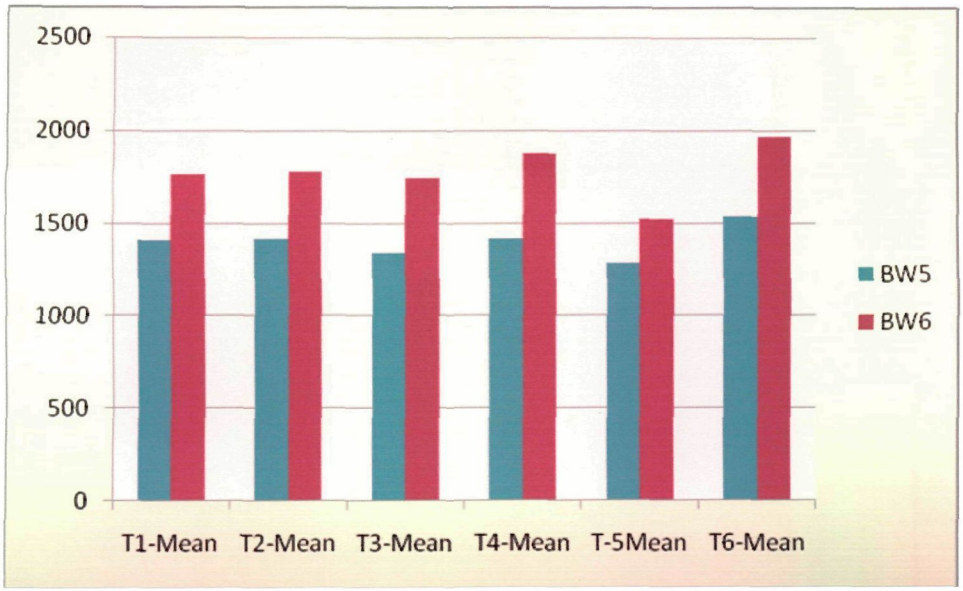


Fig.-6. Weekly Mean body weight gain in gram in different treatment group at starter stage

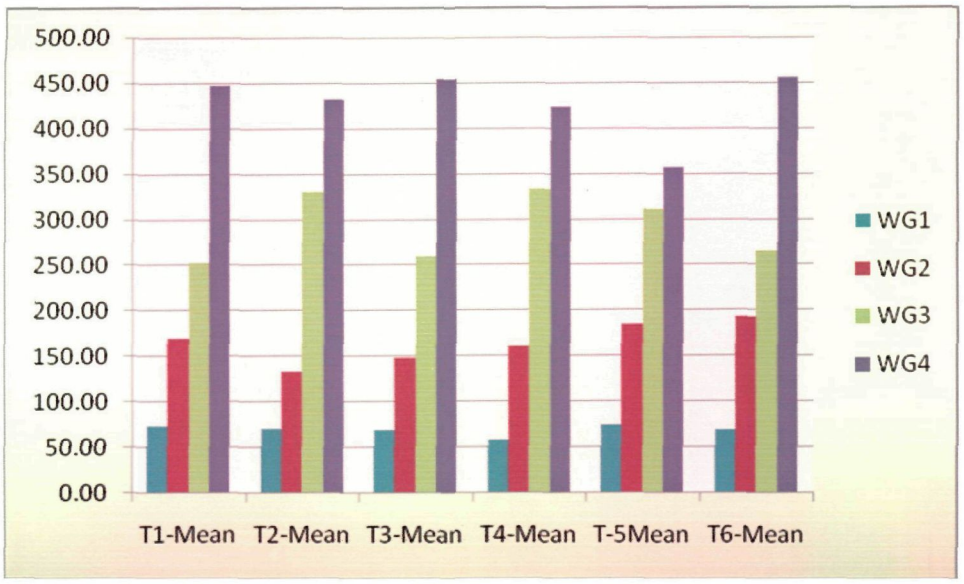


Fig.-7. Weekly Mean body weight gain in gram in different treatment group at finisher stage

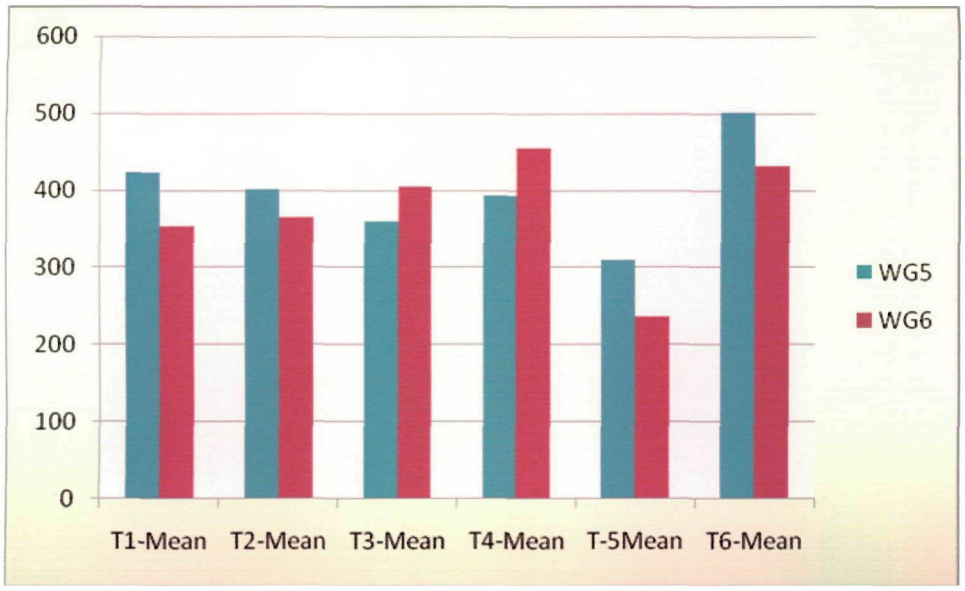
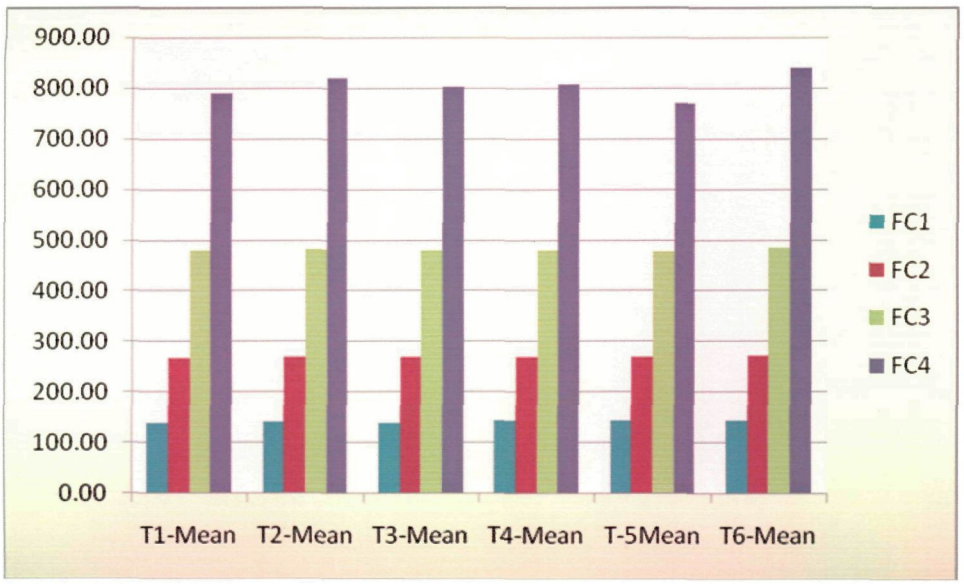


Fig.-8. Weekly Mean feed consumption in gram in different treatment group at starter stage



4.2.1 Comparative efficacy of cocidiostats on body weight

Before experimental infection of *E.tenella* in broiler birds body weight was significantly highest in T5 group (617.80 ± 7.49) followed by T4 (600.10 ± 5.05), T2 (581.94 ± 4.12), T6 (575.68 ± 2.01), T1 (541.94 ± 5.24) and T3 (525.10 ± 5.21), respectively at 3 weeks of age. Results indicating better efficacy of Maduramicin followed by Salinomycin, Diclazuril and Diclazuril + Salinomycin coccidiostat among coccidiostat given treatment group at three week of age. At two week of age, Diclazuril has given highest body weight among four treatment group.

After experimental infection of *E.tenella* on 22nd day of age, highest body weight (1023.86 ± 6.92) was observed in T4 Maduramicin group followed by T2 Salinomycin group (1013.98 ± 5.15), T1 Diclazuril group (989.04 ± 5.57) and T3 Diclazuril + Salinomycin group (979.30 ± 11.04) at 4th week of age. T6 Negative control group birds having highest body weight (1031.60 ± 11.14) among all six groups, while T5 Positive controls birds showing lowest (974.84 ± 2.85) body weight at this age.

Similar trend of body weight was observed up to six week of age among four treatment group. Highest body weight i.e. 1031.60 ± 11.14, 1533.44 ± 8.20 and 1965.10 ± 12.93 was observed in T6 negative control bird, while lowest body weight i.e. 974.84 ± 2.85, 1284.18 ± 13..84 and 1520.12 ± 2.72 was observed in T5 positive control group at 4, 5 and 6 week of age, respectively among all six groups.

Highest body weight (1871.72 ± 7.70) was observed in Maduramicin given group followed by Salinomycin (1780.56 ± 10.58), Diclazuril (1767.14 ± 8.26) and Diclazuril + Salinomycin group (1743.44 ± 1.42) at 6 week of age in coccidiostat treatment groups. Among all groups, negative control group birds shown highest body weight (1965.10 ± 12.93) and positive control group birds shown lowest body weight (1520.12 ± 2.72) at 6 week of age. Results indicate best result of body weight with non infected non medicated

group as compare to four coccidiostat treatment groups. Similar results were observed by Majumdar *et al.* (1993) and Thyagarajan *et al.* (1989) for non infected non medicated group and by Anosa *et al.* (2011) for infected but non medicated group. Maduramicin shown highest body weight followed by Salinomycin, Diclazuril and Diclazuril + Salinomycin Shuttle group among coccidiostat treatment groups.

Similar results were observed by Azizi *et al.* (2010) in mixed infection of *Eimeria spp* for Maduramicin coccidiostat. Felfeldi (1991) stated that sensitivity of *E tenella* did not change after 24 successive trials to maduramicin with higher BW as compare to other coccidiostat. Miyazaki *et al.* (1975) found that the salinomysin drug was effectively in reducing the mortality and increasing the average weight of chickens experimentally infected with *E. tenella.*

4.2.2 Comparative efficacy of cocidiostats on body weight gain

Body weight gain was highest in Maduramicin given group (333.44 ± 5.12) followed by Salinomycin given group (330.94 ± 4.95), Diclazuril + Salinomycin group (259.78 ± 2.47) and Diclazuril group (252.84 ± 6.62) at 3 week of age before experimental infection of *E.tenella.* Controlled birds showing highest weight gain compare to treatment group at 2 week of age while Maduramicin given birds have highest weight gain (333.44 ± 5.12) at 3 week of age as compare to control group as well as other treatment groups. More or less similar body weight gain was observed between 3 – 4 weeks of age but highest weight gain (424.10 ± 8.97) was observed in T1 Diclazuril group between 4-5 weeks of age among four treatment groups. Highest body weight gain (455.18 ± 10.96) was observed in T4 Maduramicin given group followed by T3 Diclazuril + Salinomycin group (404.40 ± 9.67), T2 Salinomycin group (364.52 ± 11.34) and T1 Diclazuril group (354.00 ± 8.37), among four coccidiostats given group at 5-6 weeks of age. Positive control birds showing decreasing

trend of body weight gain after experimental infection to six week of age, while negative control bird showing increasing trend of body weight gain up to 5 week of age.

Over all highest body weight gain was observed in T4 (1824.64 g) group followed by T2 group (1733.50 g), T1 group (1720.68 g) and T3 group (1696.64 g) at the end of six week period in coccidiostats given group. All four values are differing significantly among coccidiostat group as well as from control group. Negative control T6 birds shown significant highest BWG (1918.26 ± 9.86) and positive control T5 birds showing significant lowest BWG (1472.80 ± 4.96) at the end of six week period.

Overall result of body weight and body weight gain indicating better efficacy of Maduramycin among treatment group. Salisch and Shakshouk (1990) reported that broiler chickens infected with *Eimeria tenella* (2.5 × 104 oocysts per bird) given Maduramicin at 5 ppm showed increased weight gain and feed conversion, when compared with narasin and monensin. The efficacy of Maduramicin against *Eimeria tenella, E. maxima, E. necatrix, E. brunetti* and *E. acervulina* in Hubbard - cross were studied by Folz *et al.* (1988) who reported that birds treated with Maduramicin had significantly higher weight gain than birds medicated with lasalocid, monensin, Salinomycin treated group and lower feed conversion ratio when compared to lasalocid treated group. These results are in agreement with our findings.

Raju *et al.* (2012) observed that *Eimeria tenella* infected and Salinomycin treated group had better weight gain when compared to Maduramicin and Lasalocid groups. The efficacy of neem fruit product when compared with Salinomycin sodium against coccidiosis in broilers by Tipu *et al.*, (2002) revealed that the birds medicated with Salinomycin sodium had better ($P>0.05$) weight gain and feed efficacy as compared to other treated groups. These observations are also in accordance with Chappel and Babcock (1979) who found higher relative weight gain and lower lesion score in the Salinomycin than the lasalocid treated

group. These results indicates better efficacy of Salinomycin as compare to Maduramicin which might be due to some resistant against Maduramicin compare to Salinomycin in that area.

4.3 Comparative efficacy of coccidiostats on feed consumption and feed conversion ratio.

In present experiment, weekly feed consumption (g/bird) was recorded up to 6 weeks of age, starter phase (0-4 week), finisher phase (5-6 week) and overall experimental duration (0-6 week). The feed consumption values are presented in Table-15 & Table-16 and graphically depicted in Figure-8 & Figure-9, respectively. Feed conversion ratio (FCR) in terms of feed consumption (kg) per body weight gain (kg) is an important tool to measure the feed conversion efficiency of bird. The average weekly feed conversion ratio recorded at each week (1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th}), starter phase (0-4 weeks), finisher phase (5-6 weeks) and overall (0-6 weeks). The feed conversion ratio values are presented in Table-17 & Table-18 and graphically depicted in Figure-10 & Figure-11, respectively.

4.3.1 Comparative efficacy of coccidiostats on feed consumption

Before experimental infection of *E.tenella*, there was no consistent trend regarding feed consumption in all six groups up to 3 week of age. After experimental infection, feed consumption was highest in T2 group (820.20 ± 0.80, 920.78 ± 0.46, 1030.28 ± 0.50) followed by T4 group (808.50 ± 0.50, 915.20 ± 0.36, 1020.40 ± 0.59), T3 group (802.80 ± 0.53, 910.10 ± 0.37, 1010.00 ± 0.39) and T1 group (790.16 ±0.76, 900.50 ± 23.33, 1000.88 ± 1.23) at 4, 5 and 6 week of age, respectively. There was significant decrease feed consumption observed in positive control T5 group, while significant increase feed consumption was observed in negative control T6 group as compare at coccidiostat treatment group at starter stage.

Table-15. Weekly and overall feed consumption in gram (Mean + S.E.) in different treatment group at starter stage

Week	T_1	T_2	T_3	T_4	T_5	T_6
FC_1	140.20^{c} ± 0.65	142.40^{bc} ± 0.60	141.05^{c} ± 1.60	144.40^{ab} ± 0.26	145.22^{a} ± 0.58	145.25^{a} ± 0.29
FC_2	268.00^{b} ± 0.65	270.22^{ab} ± 0.61	269.44^{b} ± 1.17	271.16^{a} ± 0.80	271.12^{a} ± 0.33	272.24^{a} ± 0.46
FC_3	480.32^{bc} ± 0.62	482.22^{b} ± 0.61	481.16^{b} ± 1.08	480.56^{b} ± 0.69	478.52^{bc} ± 0.64	485.18^{a} ± 0.90
FC_4	790.16^{e} ± 0.76	820.20^{b} ± 0.80	802.80^{d} ± 0.53	808.50^{c} ± 0.50	770.42^{f} ± 0.61	840.66^{a} ± 1.54
TFC_{0-4}	1678.68^{e} ± 2.10	1715.04^{b} ± 1.07	1694.45^{d} ± 1.61	1704.62^{c} ± 1.48	1665.28^{f} ± 0.84	1743.33^{a} ± 2.28
Treatment Mean	419.67^{e}	428.76^{b}	423.61^{d}	426.15^{c}	416.32^{f}	435.83^{a}
	T		**P**		**T×P**	
	S Em	**C.D**	**S Em**	**C.D**	**S Em**	**C.D**
	0.40	1.12	2.70	0.32	0.80	2.23

The means bearing different superscript within same row differ significantly from each other ($P<0.05$)

Table-16. Weekly and overall feed consumption in gram (Mean + S.E.) in different treatment group at finisher stage

Week	T_1	T_2	T_3	T_4	T_5	T_6
FC_5	900.50 ± 23.33	920.78 ± 0.46	910.10 ± 0.37	915.20 ± 0.36	898.62 ± 0.59	960.86 ± 0.62
FC_6	1000.88 ± 1.23	1030.28 ± 0.50	1010.00 ± 0.39	1020.40 ± 0.59	996.64 ± 0.52	1070.58 ± 0.49
Treatment Mean	950.69^{d}	975.53^{b}	960.05^{cd}	967.80^{bc}	947.63^{d}	1015.72^{a}
$TFC_{(5-6)}$	1901.38^{d} ± 23.60	1951.06^{b} ± 0.59	1920.10^{cd} ± 0.67	1935.60^{bc} ± 0.74	1898.26^{d} ± 0.62	2031.44^{a} ± 0.77
$TFC_{(0-6)}$	3579.98^{e}	3666.10^{b}	3614.55^{d}	3640.22^{c}	3560.54^{f}	3774.77^{a}

	T		P		T×P	
	S Em	**C.D**	**S Em**	**C.D**	**S Em**	**C.D**
	4.78	13.40	2.76	7.73	6.76	NS

The means bearing different superscript within same row differ significantly from each other ($P<0.05$)

Table-17. Weekly and overall feed conversion ratio (Mean + S.E.) in different treatment group at starter stage

Week	T_1	T_2	T_3	T_4	T_5	T_6
FCR_1	1.90^{c} ±0.04	2.01^{bc} ±0.04	2.01^{bc} ±0.06	2.49^{a} ±0.02	1.93^{c} ±0.02	2.08^{b} ±0.02
FCR_2	1.59^{c} ±0.05	2.03^{a} ±0.06	1.81^{b} ±0.04	1.68^{c} ±0.03	1.47^{d} ±0.01	1.41^{d} ±0.03
FCR_3	1.90^{a} ±1.05	1.46^{b} ±0.02	1.85^{a} ±0.02	1.44^{b} ±0.02	1.54^{b} ±0.04	1.83^{a} ±0.01
FCR_4	1.77^{c} ±0.03	1.90^{b} ±0.03	1.77^{c} ±0.06	1.91^{b} ±0.03	2.16^{a} ±0.06	1.84^{b} ±0.05
$FCR_{(0-4)}$	1.78^{abe} ± 0.01	1.77^{abe} ± 0.01	1.82^{a} ± 0.02	1.75^{c} ± 0.01	1.80^{ab} ± 0.01	1.77^{bc} ± 0.2
Treatment Mean	1.79^{b}	1.85^{ab}	1.87^{a}	1.88^{a}	1.78^{b}	1.79^{b}
	T		**P**		**T×P**	
	S Em	**C.D**	**S Em**	**C.D**	**S Em**	**C.D**
	0.02	0.06	0.02	0.05	0.04	0.11

The means bearing different superscript within same row differ significantly from each other ($P<0.05$)

Fig.-9. Weekly Mean feed consumption in gram in different treatment group at finisher stage

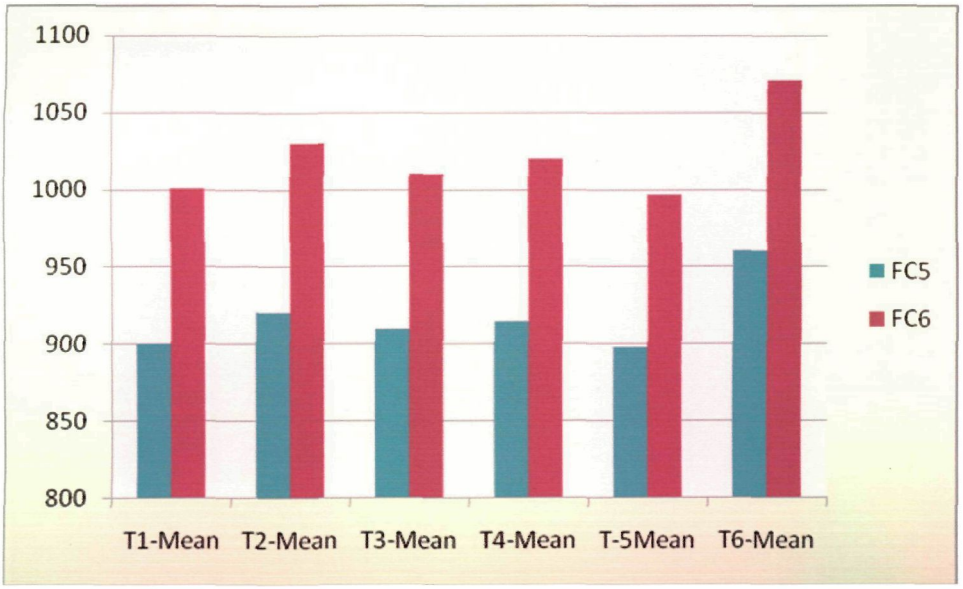


Fig.-10. Weekly Mean feed conversion ratio in different treatment group at starter stage

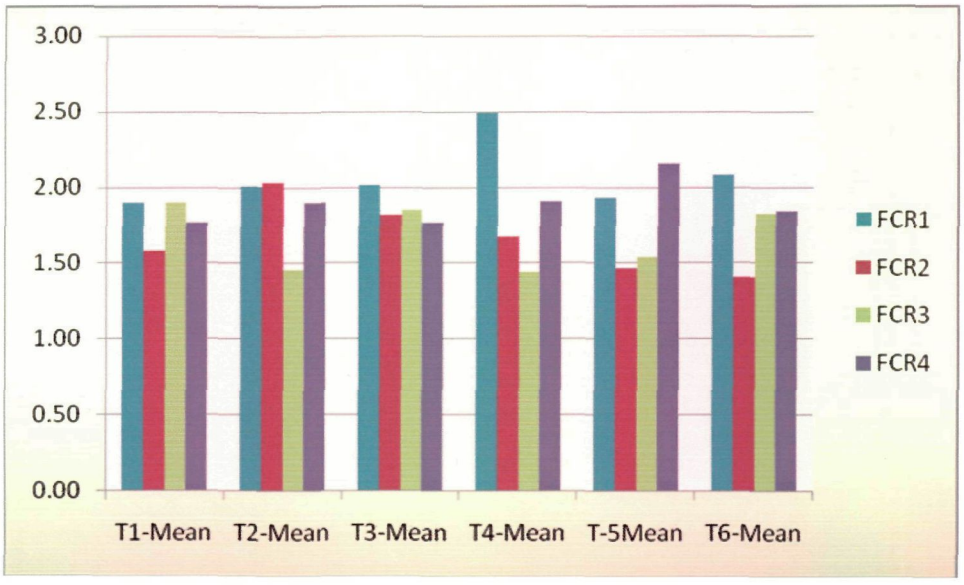


Fig.-11. Weekly Mean feed conversion ratio in different treatment group at finisher stage

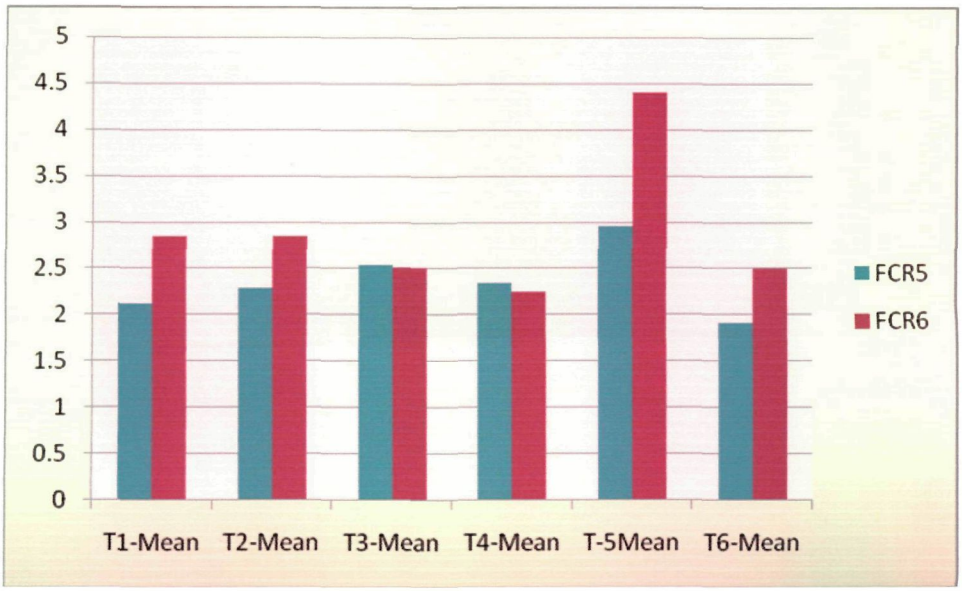


Fig.-12. Heamatological value Mean in different treatment group before and after experimental infection of *E.tenella*

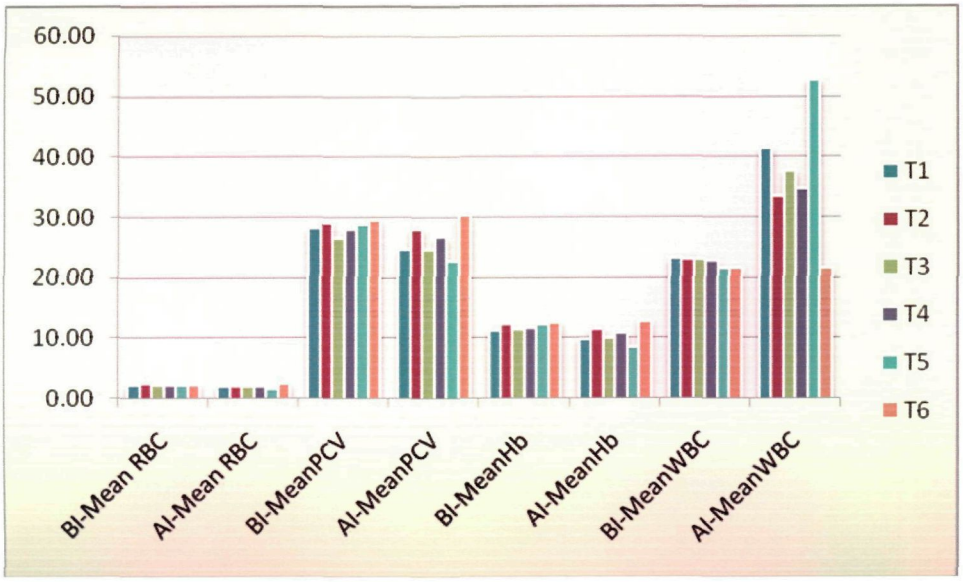


Table-18. Weekly and overall feed conversion ratio (Mean + S.E.) in different treatment group at finisher stage

Week	T_1	T_2	T_3	T_4	T_5	T_6
FCR_5	2.13^{bc} ± 0.06	2.29^{b} ± 0.02	2.54^{b} ± 0.06	2.35^{b} ± 0.06	2.96^{a} ± 0.04	1.92^{c} ± 0.04
FCR_6	2.84^{b} ± 0.07	2.85^{b} ± 0.09	2.51^{c} ± 0.06	2.25^{c} ± 0.05	4.40^{a} ± 0.31	2.49^{c} ± 0.6
Treatment Mean	2.49^{bc}	2.57^{b}	2.53^{b}	2.30^{cd}	3.68^{a}	2.21^{d}
$FCR_{(5-6)}$	2.45^{c} ± 0.03	2.55^{a} ± 0.04	2.51^{bc} ± 0.02	2.28^{d} ± 0.02	3.48^{a} ± 0.03	2.18^{e} ± 0.03
$FCR_{(0-6)}$	2.08^{b} ± 0.01	2.12^{c} ± 0.01	2.13^{c} ± 0.02	2.00^{a} ± 0.01	2.42^{d} ± 0.01	1.47^{a} ± 0.01
	T		**P**		**T×P**	
	S Em	**C.D**	**S Em**	**C.D**	**S Em**	**C.D**
	0.08	0.23	0.05	0.13	0.11	0.32

The means bearing different superscript within same row differ significantly from each other ($P<0.05$)

Among four coccidiostat group, Salinomycin given group have highest feed consumption (1715.00 ± 1.07, 1951.06 ± 0.59) followed by Maduramicin group (1704.62 ± 1.48, 1935.60 ± 0.74) in starter and finisher phase, respectively among coccidiostats groups. Significant increase feed consumption (1743.33 ± 2.28, 2031.44 ± 0.77) in negative control, while significant decrease feed consumption (1665.28 ± 0.84, 1898.28 ± 0.62) in positive control among all groups was observed during starter and finisher phase respectively. At the end of six week significantly highest feed consumption was observed in T2 group (3666.10g) followed by T4 group (3640.22g), T3 group (3614.55g) and T1 group (3579.98g) among four coccidiostat group. All values are differing significantly among coccidiostat treatment group. In positive control group significant lowest feed consumption (3560.54g), while in negative control group significant highest feed consumption 3774.77g was observed at above age among all groups.

4.3.2 Comparative efficacy of coccidiostats on Feed Conversion Ratio (FCR)

Before experimental infection of *E.tenella* lowest feed conversion ratio was observed in T4 group (1.44 ± 0.02) followed by T2 group (1.46 ± 0.02), T3 group (1.85 ± 0.02) and T1 group (1.90 ± 1.05) at 3 week of age. Above result indicate better efficacy of Maduramicin followed by Salinomycin for lowering feed conversion ratio at 3 week of age.

At the age of six week after experimental infection of *E.tenella*, Maduramicin given group have lowest FCR (2.25 ± 0.05) followed by shuttle program of Diclazuril + Salinomycin (2.51 ± 0.06), while Salinomycin given and Diclazuril given groups have 2.85 ± 0.09 and 2.84 ± 0.07 FCR value, respectively.

Over all FCR value were found lowest in T4 group followed by T1, T2, and T3 among treatment group. Significant highest FCR (2.42 ± 0.01) was observed in T5 group and lowest FCR (1.97 ± 0.51) was observed in T6 group among all groups.

An overall result of feed efficiency and feed conversion ratio indicates better result by Maduramicin and Dicalzuril among treatment groups at the age of six week.

Azizi *et al.* (2010) observed that Maduramicin given better BW and FCR as compare to the Salinomysin in mixed isolates of *Eimeria spp.* given at 26 day of age in broilers.

Salisch (1987) evaluated the efficacy of Maduramicin (5 ppm) under experimental and field conditions based on live weight gain and feed conversion. Their result showed that Maduramicin treated birds had significantly improved live weight gain and low feed conversion ratio, when compared to narasin at 70 ppm treated birds. Georgiva *et al.* (2010) reported better results with Maduramicin in *E tenella* infected broilers by improving WG and FCR. Safety of Maduramicin (Cygro) was studied in 600 Tetra-82 broilers by Laczay *et al.* (1989). They fed Maduramicin at the dose rate of 2.5, 5.0, 7.5 and 10.0 ppm in feed during the starter and grower phases between the 1st and 42nd days. It was reported that Maduramicin at 5 ppm did not influence body weight gain, feed consumption, feed conversion, or death rate In the present study, weight gain due to Maduramicin (5 ppm) premix in feed was highest followed by Salinomycin (60 ppm) and Diclazuril (5 ppm). This difference might be due to higher dose of mixed inoculums of *Eimeria spp.* given at later age. These findings are in agreement with our findings of Maduramicin.

Salisch and Shakshouk (1989) reported that Diclazuril treated chickens performed best followed by uninfected group, maduramicin, salinomycin, narasin and monensin treated groups in terms of feed conversion efficiency, growth rate, and pathogenic effects. Bozorgmehri Fard and Rajat (2008) stated that Diclazuril has highest FCR as compared to salinomycin at 42 days of age in broilers. Angel *et al.* (1998) found that Diclazuril and Maduramicin are good coccidiostat as compare to Salinomycin in broilers at 28 day of age.

These findings are in agreement with the findings in the present study in which Diclazuril performed better after Maduramicin.

In the present study, Salinomycin at 60 ppm showed less feed efficiency, while Ashraf *et al.* (2002) used Sacox (12 per cent Salinomycin sodium) as anticoccidial in the feed and reported that it was significantly better (P>0.05) in terms of live weight gain and feed efficacy. Ebrahimnezad and Pourreza (2005) studied the effect of ionophorous anticoccidial drugs, Salinomycin and lasalocid on performance of broiler chicks and results showed that Salinomycin was better than lasalocid sodium. Badstue and Johansen (1986) evaluated the efficacy of Maduramicin ammonium at 5 ppm (Cygro) and Salinomycin 66 ppm (Sacox, coccistac) in broiler chickens. The Salinomycin group had a lower feed consumption/kg live weight than the Maduramicin group in their study. This difference might be due to variation in the dose of infection and age of birds.

Based on the finding of this study, it is concluded that coccidiostats are proved to have growth promoting action in broiler chickens during the experimental infection of 50,000 dose of *E. tenella*. Birds fed with Maduramicin medicated (5 ppm) performed well in terms of live weight gain and feed conversion ratio and it was followed by salinomycin (60 ppm) for weight gain and Diclazuril for feed efficiency in broiler birds. Maduramicin at 5 ppm and Salinomycin at 60 ppm can also be used for prevention and control of coccidiosis with less alteration in body weight and feed efficiency in broiler birds based on the result of mortality lesion sore and OPG values.

4.4 Comparative efficacy of coccidiostats on Global Index with sensitivity

Stephan *et al.* (1997) suggested a formula for detection of resistance to anticoccidials by finding out global index based on five parameters, viz., per cent weight gain, feed conversion ratio, lesion score, oocyst index and per cent mortality. In this formula weightage

to different parameters was given in view of their importance. The following formula was used in the present study.

$$\mathbf{GI=\%WG_{NN}-[(F_{IM}-F_{NNC})\times 10]-[OI_{IM}-OI_{INC}]-[LS_{IM}-LS_{INC})\times 2]-[\%Mortality/2]}$$

where, GI = Global index, OI = Oocyst Index, WG = Weight Gain, F = Feed conversion ratio, LS = Lesion Score, NNC = Non infected Non medicated Control, IM = infected medicated control, INC = Infected Non medicated control. In this formula weight gain and feed conversion ratio at the end of experiment was considered. Global Index with above formula is 88, 91, 85, 98, 56 and 120 for T1, T2, T3, T4, T5 and T6 treatment, respectively. The Global Index of Non infected Non medicated Control in percentage (GI_{NNC} %) was calculated from above Global Index values which was 74, 76, 71, 82, 47 and 100 for T1, T2, T3, T4, T5 and T6 treatment, respectively. Result indicates good efficacy of Maduramicin, where as limited efficacy of Salinomycin, Diclazuril and Diclazuril + Salinomycin Shuttle group.

Similar findings were observed by Fan-Sheng Chao *et al.* (2005). They evaluated the efficacy of Maduramicin (5 ppm) against *Eimeria tenella* (50,000 sporulated oocysts) in broiler chicken of Shanghai, China based on lesion score and live weight gain, Maduramicin had good anticoccidial efficacy. Muzurkiewez *et al.* (1987) studied the effect of ionophore coccidiostats against *E. tenella* and observed best anticoccidial activity in maduramicin followed by lasalocid, narasin, salinomycin and monensin.

In the study of Munoz *et al.* (1993) *E. acervulina* and *E. tenella* were studied in Hungary for their sensitivity to Maduramicin, monensin, Salinomycin, narasin and lasalocid, in battery trials. Maduramicin showed highest anticoccidial Index (AI) activity at the 3 dose rates tested (5, 6 and 7 ppm respectively). Varga *et al.* (1988) studied the anticoccidial efficacy of Maduramicin (5 ppm) in battery and floor pen trials in Germany. The birds were infected with *E. tenella* and *E. acervulina* oocysts at the age of one week. The efficacy of

Maduramicin at the dose level used, proved to be as good as or superior to monensin at 100 ppm, narasin at 70 ppm, Salinomycin at 60 ppm and lasalocid at 75 ppm.

Peek and Landman (2003) reported that fifteen field isolates of *Eimeria spp.* were sampled on Dutch broiler farms and were subjected to an Anticoccidial Sensitivity Test (AST) in a battery cage study. The selected anticoccidials included monensin, narasin, salinomycin lasalocid, nicarbazin, diclazuril, halofuginone, maduramicin and meticlorpindol / methylbenzoquate. *Eimeria tenella* showed reduced sensitivity for nicarbazin and was sensitive to narasin, maduramicin, and halofuginone where as all other products showed resistance.

Better efficacy of Maduramicin observed in present study and reported by many authours might be due to particular mode of action of Maduramicin among ionophores, which does not allow an easy emergence of resistance against *Eimeria tenella* as reported by Abbas *et al.* (2008).

Yadav (2000) in his study found various field isolates of *E. tenella* from Gurgaon district of Haryana, susceptible to Maduramicin at 5ppm though with limited efficacy. The efficacy of Maduramicin and Decoquinante against *Eimeria tenella* in broiler chickens was evaluated by Suo *et al.* (2002) in Beijing, China. They infected birds with 50,000 sporulated oocysts and based on the number of caecal oocysts and anticoccidial index, they observed that *Eimeria tenella* was resistant to Maduramicin at the dose rate of 5ppm.Raju *et al.* (2012) also found good efficacy with Salinomycin treatment (66ppm) in broiler chickens infected with 25,000 sporulated oocysts of *E. tenella*, They observed relatively high percent weight gain, low mortality percent, feed conversion ratio and lesion score, whereas Maduramicin (5ppm) and Laslocid sodium (75ppm) showed reduced efficacy with regard to lower percent weight gain, higher mortality percent, feed conversion ratio and lesion score. These

observations might be due to overuse or repeated use of this compound in that particular area leading to a probable tendency towards development of resistance.

George *et al.* (2002) reported that Salinomycin (66 ppm) showed good efficacy closely followed by monensin (100 ppm) and amprolium hydrochloride (125 ppm) in *Eimeria tenella* infection (50,000 sporulated oocysts) at the higher dose of infection when compared to other coccidiostats and coccidiocidal drugs. The anticoccidial efficacy of Salinomycin (60 ppm) against drug resistant strains of *E. tenella* in broiler chicks was evaluated by Srivastava and Sinha (1987) and Salinomycin was found to have 80 percent anticoccidial efficacy in broiler chickens. In present study, Salinomycin showed limited efficacy (76 per cent) against *E. tenella.* Migaki *et al.* (1978) evaluated the efficacy of Salinomycin at 60 ppm, monensin at 100 ppm and lasalocid at 75 ppm against *Eimeria tenella* infection in broiler chickens and found that Salinomycin was highly efficacious when compared to monensin and lasalocid treated birds.

Gautam and Gupta (2004) did not find resistant against Salinomysin 60 ppm and Maduramysin 5 ppm in *E tenella* infected broilers at 3 wk of age. All coccidiostats are showing varying degree of efficacy in Haryana. Salinomycin shown 86.8% and maduramicin shown 80.50% efficacy against Kernel isolate of *E. tenella.*

Meireles *et al.* (2003) evaluated the efficacy of nicarbazin, robenidine, monensin, narasin, Salinomycin, Maduramicin, Diclazuril, semduramicin and lasalocid sodium against field isolate of *Eimeria tenella* and reported that the isolate was resistant to all the coccidiostats except Diclazuril, while in our study Diclazuril showed limited efficacy.

The difference in the efficacy of various coccidiostats used by the different workers might be due to difference in isolates of *E.tenella* or species of *Eimeria spp.*, variation in dose of infection or coccidiostat, locational changes, change in age and immune status of the birds. Environmental selection pressure in different geographical location as well as the history of

drug used may differ with each other therefore strain resistant in one area may be sensitive to another area (Martin *et al*, 1997).

The reduced efficacy of Diclazuril and Salinomycin in the present study might be due to their use in this area since 10-15 years which resulted in decreased sensitivity to caecal coccidia. Another possible reason may be the lower dose of drugs used by the poultry farmers.

The reduced efficacy of Diclazuril and Salinomycin in the present study indicates that their use should be restricted because there are chances of development of resistant in this area. Results are suggestive for switching over to other anticoccidial to overcome the problems of resistance.

4.5 Comparative efficacy of coccidiostats on haematological value.

Haematological values are the indicators of the pathological damage caused by the chemicals or infection. Values are compared with positive and negative control at different age. Comparative efficacy of coccidiostat on haematological value is given in Table-19 & Table-20 and graphically depicted in Figure-12, respectively.

Literature related to the haemato-biochemical changes in different coccidiostat treatment in the broiler for comparative study is very meager, even though we have tried to compare with available literature and with the literature of coccidial infection without treatment. Talebi *et al* (2005) reported that generally with increase in age, haematological parameters like Hb, RBC, PCV and DLC (except heterophills) significantly increases. Normal haematological value and Leukogram of genetically improved broiler strain are lower than the indigenous chicken that is why these broilers are more succeptible as compare to native birds.

Table-19. Haematological value (Mean + S.E.) in different treatment group before and after experimental infection of *E.tenella* (n=10)

Parameters	(BI or AI)	T_1	T_2	T_3	T_4	T_5	T_6	Period Mean	P		T×P	
									S Em	C.D.	S Em	C.D.
TEC (10^6/µl)	BI	2.33 ±0.03	2.36 ±0.03	2.29 ±0.02	2.30 ±0.03	2.31 ±0.01	2.32 ±0.01	2.32^a	0.03	0.08	0.07	0.20
	AI	2.02 ±0.12	2.13 ±0.12	2.05 ±0.12	2.11 ±0.12	1.69 ±0.01	2.42 ±0.01	2.07^b				
PCV(%)	BI	28.28 ±0.55	29.11 ±0.18	26.47 ±0.75	27.90 ±0.72	28.80 ±0.15	29.51 ±0.14	28.35^a	0.23	0.65	0.57	1.59
	AI	24.56 ±0.26	28.05 ±1.10	24.56 ±0.26	26.72 ±1.00	22.77 ±0.15	30.24 ±0.12	26.15^b				
Hb(g%)	BI	11.16 ±0.23	12.22 ±0.14	11.43 ±0.27	11.63 ±0.32	12.35 ±0.06	12.42 ±0.11	11.87^a	0.08	0.22	0.19	0.54
	AI	9.84 ±0.17	11.51 ±0.29	9.96 ±0.16	10.76 ±0.19	8.54 ±0.07	12.75 ±0.05	10.56^b				
TLC (10^3/µl)	BI	23.14 ±0.21	22.91 ±0.13	23.04 ±0.12	22.70 ±0.20	21.51 ±0.06	21.51 ±0.06	22.47^a	0.15	0.42	0.37	1.04
	AI	41.36 ±0.46	33.36 ±0.39	37.56 ±0.99	34.66 ±0.13	52.71 ±0.38	21.47 ±0.05	36.86^b				

The means bearing different superscript within same column differ significantly from each other ($P<0.05$)
BI = Before Infection AI = After Infection

Table-20. Haematological value (Mean + S.E.) showing treatment mean in different treatment group (n=10)

Parameters	T_1	T_2	T_3	T_4	T_5	T_6	T		T×P	
							S Em	C.D.	S Em	C.D.
TEC (10^6/µl)	2.18^{b}	2.25^{ab}	2.17^{b}	2.21^{b}	2.00^{c}	2.37^{a}	0.05	0.14	0.07	0.20
PCV (%)	26.42^{cd}	28.58^{b}	25.52^{d}	27.31^{c}	25.79^{d}	29.88^{a}	0.40	1.12	0.57	1.59
Hb (g/dl)	10.50^{d}	11.87^{b}	10.70^{d}	11.20^{c}	10.45^{d}	12.59^{a}	0.14	0.38	0.19	0.54
TLC (10^3/µl)	32.25^{b}	28.13^{d}	30.30^{c}	28.68^{d}	37.11^{a}	21.49^{e}	0.26	0.73	0.37	1.04

The means bearing different superscript within same row differ significantly from each other ($P<0.05$)

4.5.1 Effect on Total Erythrocytes Counts (TEC)

Before experiment infection at 3 week of age highest TEC mean counts (2.36 ± 0.03 × 10^6/µl) was observed in T2 group followed by T1 group (2.33 ± 0.02 × 10^6/µl), T4 group (2.30 ± 0.03 × 10^6/µl) and T3 group (2.29 ± 0.02 × 10^6/µl) among treatment group. Positive control group T5 (2.31 ± 0.01 10^6/µl) and negative control group T6 (2.32 ± 0.01× 10^6/µl) have more or less similar values. There was no significant difference in TEC counts between control and treatment group.

After experimental infection, There were significant reductions in all treatment groups with highest reduction in T1 (2.02 ± 0.12× 10^6/µl) followed by similar reduction trend in T3 group (2.05 ± 0.12 × 10^6/µl), T2 (2.13 ± 0.12 × 10^6/µl) and lowest reduction in T4 group (2.11 ± 0.12 × 10^6/µl) as compare T6 group. Result indicates better efficacy of Maduramicin in term of less R.B.Cs damage among four treatments. In comparison with positive control groups, all coccidiostat treatment groups showing significant beneficially affect on TEC value showing less R.B.Cs damage.

Significant reduction in TEC was also recorded by Natt and Herrick (1956), Joyner and Davies (1960), Stephens *et al.* (1967), Padmavathi and Muralidharan (1986[a]), McDoungald and Reid (1991), Fukata *et al.* (1997), Panda *et al* . (1997), Kumar and Padmavathi (2000) and Jaipurkar *et al.* (2004), Ogbe *et al.* (2010) and Adamu *et al.* (2013) in birds affected with *E. tenella* infection. However, Moynihan (1950) and Dakshinkar and Dharmadhikari (1985) did not find any change in total erythrocytes count with *E. acervulina* infection. While Stephens *et al.* (1967) found significant increase in erythrocyte count at 6th, 10th and 14th day of inoculation. The reduction observed in TEC during the acute phase of infection is due to haemorrhage.

4.5.2 Effects on Packed Cell Volume (PCV)

At the age of 3 weeks of age before experimental infection PCV values was lowest in shuttle group T3 (26.47 ± 0.75%) and highest in Salinomycin T2 group (29.11 ± 0.18%) compare to four coccidiostat group indicating least damage with Salinomycin. Both control groups having no significant higher value compare to treatment group.

After experimental infection, the highest reduction was observed in T1 and T3 group (24.56 ± 0.26%) followed by T4 group (26.72 ± 1.00%) and T2 group (28.05 ± 1.10%) compare to pre infection treatment group. Result indicated less damage by Salinomycin for PCV reduction value among for coccidiostat treatment group.

There was significant reduction in positive control group (22.77 ± 0.15%) after infection as compare to pre infection (28.80 ± 0.15%) indicating heavy damage by *E.tenella* protozoa at 4^{th} weeks of age. There was also significant reduction of PCV in positive control post infection group as compare to all treatment post infection group indicating supportive efficacy of coccidiostat in birds for less reduction of PCV value.

. Like present findings, significant reduction in packed cell volume was also recorded by Joyner and Davies (1960) and Stephens (1965). Similarly, Hein (1971) observed significant reduction in PCV on day 5 post-infection in 2 weeks old chickens, while Turk (1985) recorded fall in haematocrit value from the 5^{th} to 10^{th} day with *E. necatrix* infection. The present results were also comparable to those reported previously by number of authors for *E. tenella* infection (Natt and Herrick, 1956; Mukkur and Bradley, 1969; Oikawa *et al .,* 1971; Joshi *et al .,* 1974; Witlock *et al .,* 1981; Witlock, 1983; Turk, 1985; Padmavathi and Muralidharan, 1986[a]; Conway *et al .,* 1993; Panda *et al .,* 1997; Kumar and Padmavathi, 2000; Jaipurkar *et al .,* 2004; Ogbe *et al.,* (2010) and Adamu *et al.* (2013).

The reduction observed in packed cell colume during the acute phase of infection might be due to severe blood loss and the subsequent elevation of packed cell volume values

towards normal level suggested active erythropoiesis following the acute phase. Therefore, interpretation must be made in the light of hydration status of the bird. In fluid loss, the PCV value may be falling in the normal range. So, differential diagnosis must be done by estimating total protein content of serum or plasma (Kaneko and Cornelius, 1971).

4.5.3 Effects on Haemaglobin (Hb)

All treatment group having lower Hb value at 3 weeks of age before experimental infection except Salinomycin group having nearly normal Hb value (12.22 ± 0.14 g/dl) similar to control group, which indicate better efficacy of Salinomycin.

After experimental infection the Hb value were significantly decreased. The trends of decrease was highest in T1 group (9.84 ± 0.17 g/dl) followed by shuttle T3 group (9.96 ± 0.16 g/dl). T4 group (10.76 ± 0.19 g/dl) and T2 group (11.51 ± 0.29 g/dl) among four treatment group. Positive control T5 groups shown significantly lowest Hb value (8.54 ± 0.07) as compare to all treatment groups. Result indicated better efficacy of Salinomycin as compare to other coccidiostat.

The results showed marked reduction in values in the infected group during acute phase of infection and the values returned to normal during recovery. There was no appreciable reduction in values in the infected medicated group when compared to control group. The present findings are in confirmity with the observations made by Waxler (1941), Joyner and Davies (1960), Mukkur and Bradely (1969), Oikawa *et al.*, (1971), Joshi *et al.*, (1974), Turk (1978), Witlock *et al.*,(1981, Turk (1985) and Padmavathy and Muralidharam (1986b) , Ogbe *et al.* (2010) and Adamu *et al.* (2013) for *E.tenella* infection.

The reduction in the value of haemoglobin observed in the infected group of birds might be attributed to haemorrahges in the caeca followed by development of caecal lesions. There may be injury to tissue and liberation of large quantities of histamine, which increase

the local blood flow and also increase the permeability of capillaries and venules allowing large quantities of fluid to come out (Padmavathi and Muralidharan, 1986[a]).

4.5.4 Effects on Total Leucocytes Counts (TLC)

At the age of 3 weeks before experimental infection TLC count was in the range of 22.70 to 23.14 $\times 10^3$/ µl among all four treatment groups, while the TLC count was (21.51 ± 0.06 $\times 10^3$/ µl) in both the control group. Significant higher TLC count was observed among treatment group as compare to control group.

After experimental infection TLC counts were significantly higher in four treatment groups then the pre infection group. Highest counts were observed in T1 group (41.36 ± 0.46 $\times 10^3$/ µl) followed by T3 group (37.56 ± 0.99 $\times 10^3$/ µl), T4 group (34.66 ± 0.13 $\times 10^3$/ µl) and T2 group (33.36 ± 0.39 $\times 10^3$/ µl). Post infection TLC count of positive control group were (52.71 ± 0.38 $\times 10^3$/ µl) significant higher than the pre infection value of same group (21.51 ± 0.06 $\times 10^3$/ µl). The result indicates better efficacy of Salinomycin followed by Maduramicin in *E.tenella* infection.

Like our findings, significant increase in TLC has also been reported by Saad *et al* . (1974), Padmavathi and Muralidharan (1986[a]), McDougald and Reid (1991), Panda *et al* . (1997), Kumar and Padmavathi (2000), Jaipurkar *et al.* (2004) and Ogbe *et al.* (2010). Adamu *et al.* (2013) also reported similar findings of higher TLC with increased numbers of lymphocytes, eosinophils and heterophils in *E.tenella* and *E. brunetti* infected broilers.

The increased total leucocytes count in coccidian affected birds might be due to body immune system to suppress the infection (Stephen, 1965). This increase was suggestive of increase leucopoiesis as a first step of defense mechanism to infection (Padmavathi and Muralidharan, 1986[a]).

4.5.5 Effects on Differential Leucocyte Counts (DLC)

Comparative efficacy of coccidiostat on DLC value is given in Table-21 & Table-22 and graphically depicted in Figure-13, respectively.

4.5.5.1 Effects on Heterophills

All treatment group have 1 to 1.5 $\times 10^3$ / µl higher value as compare to both control group at 3 weeks of age. After experimental infection heterophills counts were significantly increase in all treatments group with highest increase in T1 group ($18.07 \pm 0.07 \times 10^3$ /µl) followed by T3 group ($16.52 \pm 0.56 \times 10^3$/µl), T4 group ($15.34 \pm 0.26 \times 10^3$ / µl) and T2 group ($14.92 \pm 0.27 \times 10^3$ / µl) indicating better efficacy Salinomycin and Maduramicin. The increase in the heterophils was observed because heterophils also contain a variety of granules that contribute to the first line host defense against bacteria, fungi, protozoa and some viruses. Acute or chronic inflammatory disease is the predominant cause of monocytosis or heterophilia in pet birds (Irizaary – Rovira, 2004) because monocytes, macrophages and dendritic cells are important hematopoietic cells that play critical roles in defense and in maintaining homeostasis.

4.5.5.2 Effects on Lymphocytes

Slightly higher lymphocytic values were observed in all treatment group as compare to control group at the age of 3 weeks of age, but significant increase in lymphocytes were observed after experimental infection among all treatment groups with highest increase in T1 group ($19.14 \pm 0.47 \times 10^3$ / µl) followed by T3 group ($16.66 \pm 0.62 \times 10^3$ / µl), T4 group ($15.06 \pm 0.29 \times 10^3$ / µl) and T2 group ($13.90 \pm 0.57 \times 10^3$ / µl). Positive control group after post infection showing significant increase in lymphocytes count ($30.11 \pm 0.29\ 10^3$ / µl) as compare to preinfection ($9.28 \pm 0.12 \times 10^3$ / µl). Result indicates better efficacy of Salinomycin followed by Maduramicin in infected birds compare to Diclazuril in term the pathological damage.

Table-21. Different Leukocyte Count (DLC) value (Mean + S.E.) in different treatment group before and after experimental infection of *E.tenella* (n=10)

Parameters	(BI or AI)	T_1	T_2	T_3	T_4	T_5	T_6	Period Mean	P		T×P	
									S Em	C.D.	S Em	C.D.
Heterophills (10^3 / µl)	BI	9.84 ±0.09	9.74 ±0.06	9.90 ±0.08	9.56 ±0.16	8.40 ±0.09	8.29 ±0.03	9.29^a	0.09	0.24	0.21	0.59
	AI	18.07 ±0.07	14.92 ±0.27	16.52 ±0.56	15.34 ±0.26	17.49 ±0.14	7.44 ±0.02	14.96^b				
lymphocytes (10^3 / µl)	BI	9.53 ±0.16	9.34 ±0.08	9.42 ±0.09	9.38 ±0.08	9.28 ±0.12	9.32 ±0.07	9.38^a	0.13	0.36	0.31	0.87
	AI	19.14 ±0.47	13.90 ±0.57	16.66 ±0.62	15.06 ±0.29	30.11 ±0.29	9.92 ±0.01	17.47^b				
Monocytes (10^3 / µl)	BI	0.72 ±0.02	0.68 ±0.02	0.73 ±0.02	0.69 ±0.02	0.75 ±0.01	0.75 ±0.01	0.72^a	0.02	0.06	0.05	0.15
	AI	0.49 ±0.06	0.61 ±0.08	0.65 ±0.10	0.61 ±0.03	0.67 ±0.11	0.90 ±0.02	0.65^b				
Eosinophills (10^3 / µl)	BI	0.77 ±0.02	0.82 ±0.01	0.75 ±0.03	0.80 ±0.01	0.84 ±0.01	0.81 ±0.01	0.80^a	0.01	0.05	0.05	0.13
	AI	1.84 ±0.07	1.68 ±0.08	1.80 ±0.08	1.46 ±0.07	2.37 ±0.04	0.93 ±0.01	1.68^b				
Basophills (10^3 / µl)	BI	2.28 ±0.02	2.34 ±0.03	2.25 ±0.02	2.28 ±0.03	2.26 ±0.02	2.33 ±0.03	2.29^a	0.02	0.05	0.04	0.12
	AI	1.82 ±0.05	2.25 ±0.04	1.94 ±0.08	2.18 ±0.03	2.06 ±0.07	2.28 ±0.05	2.09^b				

The means bearing different superscript within same Column differ significantly from each other ($P<0.05$)
BI = Before Infection AI = After Infection

Table-22. Different Leukocytes Count (DLC) value (Mean + S.E.) showing treatment mean in different treatment group (n=10)

Parameters	T_1	T_2	T_3	T_4	T_5	T_6	T		T×P	
							S Em	C.D.	S Em	C.D.
Heterophills (10^3 / µl)	13.95^a	12.33^c	13.21^b	12.45^c	12.95^b	7.87^d	0.15	0.42	0.21	0.59
lymphocytes (10^3 / µl)	14.34^b	11.62^d	13.04^c	12.22^d	19.70^a	9.62^e	0.22	0.62	0.31	0.87
Monocytes (10^3 / µl)	0.60^b	0.64^b	0.69^b	0.65^b	0.71^b	0.83^a	0.04	0.10	0.05	0.15
Eosinophills (10^3 / µl)	1.31^b	1.25^b	1.28^b	1.13^c	1.59^a	0.87^d	0.03	0.09	0.05	0.13
Basophills (10^3 / µl)	2.05^d	2.30^a	2.09^{cd}	2.23^{ab}	2.16^{bc}	2.31^a	0.03	0.08	0.04	0.12

The means bearing different superscript within same row differ significantly from each other ($P<0.05$)

Fig.-13. Different leucocyt Count (DLC) value Mean in different treatment group before and after experimental infection of *E.tenella*

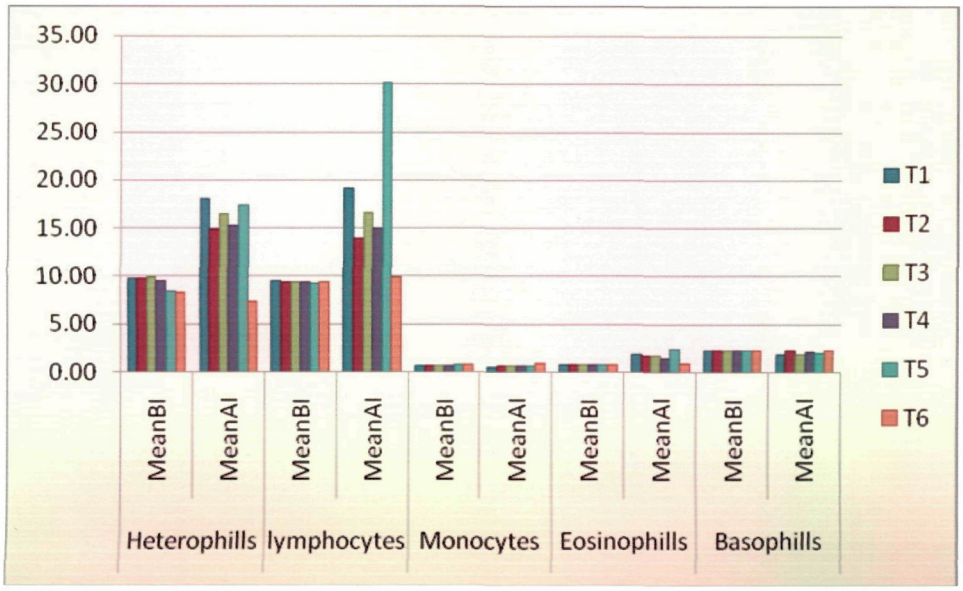
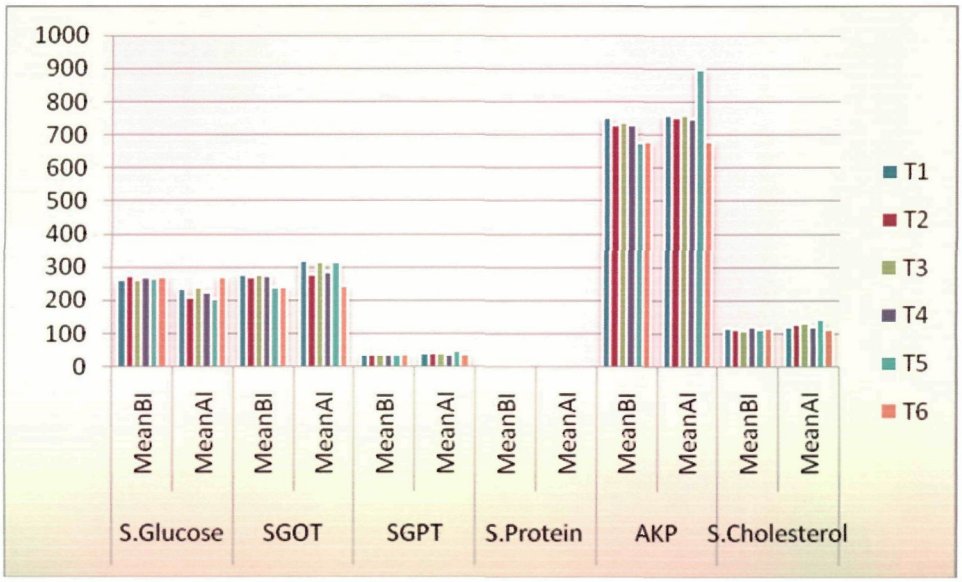


Fig.-14. Biochemichal value Mean in different treatment group before and after experimental infection of *E.tenella*



The increase in the lymphocyte count may be attributed to the effect of the inflammation of the caeca and intestine. Chronic antigenic stimulation may result in a greatly expanded circulating lymphocyte pool because the primary functions of the lymphocytes are immunological response, humoral antibody formation and cell mediated immunity (Irizaary-Rovira, 2004). Hussein and Rahman (2005) also observed that Salinomycin with 60 ppm and higher doses causing neutropenia and lymphocytosis given Salinomycin without coccidial infection.

4.5.5.3 Effects on Monocytes

Coccidiostats used in the experiment have no more effects on monocytes count at 3 weeks of age, but count was decreased after infection with lowest decrease in Salinomycin treatment showing better efficacy. Infection having significant effects on Monocytes count as there was decrease in post infection value in positive control i.e. 0.08×10^3 / µl. Padmavathi and Muralidharan (1986[a]) have also reported similar decrease in the monocytes count at 7 day PI given experimental infection of 50,000 oocysts.

4.5.5.4 Effects on Eosinophils

At 3 weeks of age there were no significant difference among four coccidiostat treatment used as compare to control, but infection causing significant increase in eosinophilic count with highest value of T1 group ($1.84 \pm 0.07 \times 10^3$ / µl) followed by shuttle group T3 (1.80 ± 0.08 10^3 / µl), T2 group ($1.68 \pm 0.08 \times 10^3$ / µl) and T4 group ($1.46 \pm 0.07 \times 10^3$ / µl). Significant increase of eosinophilic count in T5 positive control ($2.37 \pm 0.04 \times 10^3$ / µl) as compare to treatment group were observed indicating more damage by infection which can be reduced by coccidiostat treatment. Similar increase of eosinophils was reported by Adamu *et al.* (2013).

Eosinophilia in birds rarely occurs but may be associated with parasitism (mites, intestinal parasites, parasites with tissue migration) and are known to interact with

homocytotropic antibodies (IgE and IgG), mast cells and basophils. The antibody and T lymphocytes provide specificity to the reaction and the IgE on mast cells attracts eosinophils to modulate the inflammatory reaction (Irizaary-Rovira, 2004).

4.5.5.5 Effects on Basophils

Basophils count were more or less similar in both control as well as all four trearment group at 3 weeks of age, but more reduction was observed in T1(1.82 ± 0.05 $\times 10^3$ / µl) and T3 (1.94 ± 0.08 $\times 10^3$ / µl) group as compare to T2(2.25 ± 0.04 $\times 10^3$ / µl) and T4(2.18 ± 0.03 $\times 10^3$ / µl) after experimental infection indicating better efficacy of Salinomycin and Maduramicin. Significant reduction was observed post infection in control group (2.06 ± 0.07 $\times 10^3$ / µl) as compare to pre infection (2.26 ± 0.02 $\times 10^3$ / µl). Padmavathi and Muralidharan (1986[a]) did not find basophills after experimental infection of 50,000 oocysts of *E.tenella* on 7th day PI.

4.6 Comparative efficacy of coccidiostat on bio-chemical values

Coccidia can produce subtle change in metabolism. In disease condition, enzyme action is also influenced by metabolic activities (Allen, 1988). The result of serum biochemical and enzymatic profile in different coccidiostat treatment before and after experimental infection are presented in Table-23 & Table-24 and graphically depicted in Figure-14, respectively. Turk *et al.* (1977) stated that coccidial infection markedly alters intestinal physiology resulting in changes in morphology, nutrient absorption and microflora. These changes were most severe in infected area and may be mild or absent in other area. Intestinal pH is markedly decreased during the acute phase of infection in the affected area. Gut motility is decreased during the acute phase but restored during recovery. Digestion and absorption of protein is impaired in the infected mid intestine. Absorption of carbohydrates particularly glucose is not consistently affected, but lipids, fat-soluble vitamins and carotenes are also poorly utilized during infection.

Table-23. Biochemical value (Mean + S.E.) in different treatment group before and after experimental infection of *E.tenella*

Parameter	(BI or AI)	T_1	T_2	T_3	T_4	T_5	T_6	Period Mean	P		T×P	
									S Em	C.D.	S Em	C.D.
Serum Glucose (mg %)	BI	262.50 ±3.44	273.90 ±2.73	263.30 ±60.26	268.70 ±3.11	267.90 ±2.58	270.00 ±2.70	267.72	1.18	3.32	2.90	8.13
	AI	237.40 ±1.58	210.40 ±1.63	238.40 ±1.36	224.20 ±5.05	203.60 ±2.73	270.00 ±2.70	230.67				
Serum Total Protein (gm %)	BI	4.50 ±0.11	4.26 ±0.09	4.28 ±0.15	4.52 ±0.10	4.62 ±0.06	4.43 ±0.10	4.44	0.04	0.10	0.09	0.25
	AI	3.58 ±0.08	3.75 ±0.03	3.62 ±0.07	3.46 ±0.08	3.28 ±0.06	4.49 ±0.07	3.70				
Serum Total Cholesterol (mg %)	BI	115.60 ±2.93	114.60 ±2.78	109.40 ±2.50	119.40 ±1.19	112.80 ±2.37	116.60 ±2.48	114.73	0.94	2.64	2.31	6.46
	AI	121.60 ±0.65	130.50 ±3.58	131.90 ±3.12	122.00 ±0.60	142.50 ±1.44	115.20 ±1.61	127.28				
SGOT (U/L)	BI	276.60 ±2.44	271.80 ±2.14	276.20 ±2.88	274.80 ±2.27	240.60 ±2.94	239.80 ±2.33	263.30	1.64	4.59	4.01	11.25
	AI	320.20 ±3.87	278.40 ±5.92	314.60 ±5.24	286.00 ±7.13	314.80 ±4.52	242.10 ±2.52	292.68				
SGPT (U/L)	BI	37.50 ±0.83	36.60 ±0.95	38.80 ±0.68	36.30 ±1.01	36.00 ±1.13	35.60 ±0.76	36.80	0.51	1.42	1.24	3.48
	AI	42.00 ±1.33	41.20 ±1.55	40.80 ±1.84	38.80 ±2.15	46.60 ±085	36.60 ±0.78	41.00				
AKP (U/L)	BI	752.60 ±4.47	728.80 ±5.71	734.80 ±6.70	729.80 ±5.01	676.60 ±4.42	680.24 ±4.37	717.14	2.91	8.17	7.14	20.01
	AI	759.80 ±2.48	751.00 ±1.87	760.80 ±2.69	750.00 ±1.98	896.35 ±20.29	679.20 ±4.19	766.19				

The means bearing different superscript within same row differ significantly from each other ($P<0.05$)

BI = Before Infection AI = After Infection

Table-24. Biochemical value (Mean + S.E.) showing treatment mean in different treatment group

Parameter	T_1	T_2	T_3	T_4	T_5	T_6	T		T×P	
							S Em	C.D.	S Em	C.D.
Serum Glucose (mg %)	249.95^{b}	242.15^{c}	250.85^{b}	246.45^{bc}	235.75^{d}	270.00^{a}	2.05	5.75	2.90	8.13
Serum Total Protein (gm %)	4.04^{b}	4.01^{b}	3.95^{b}	3.99^{b}	3.95^{b}	4.46^{a}	0.06	0.18	0.09	0.25
Serum Total Cholesterol (mg%)	118.60^{bc}	122.55^{b}	120.65^{b}	120.70^{b}	127.65^{a}	115.90^{c}	1.63	4.57	2.31	6.46
SGOT (U/L)	298.40^{a}	275.10^{b}	295.40^{a}	280.40^{b}	277.70^{b}	240.95^{c}	2.84	7.96	4.01	11.25
SGPT (U/L)	39.75^{ab}	38.90^{ab}	39.80^{ab}	37.55^{bc}	41.30^{a}	36.10^{c}	0.88	2.46	1.24	3.48
AKP (U/L)	756.20^{b}	739.90^{c}	747.80^{bc}	739.90^{c}	786.48^{a}	679.72^{d}	5.05	14.15	7.14	20.01

The means bearing different superscript within same row differ significantly from each other ($P<0.05$)

4.6.1 Effects on Serum Glucose

Before experimental infection at 3 weeks of age, serum glucose values were more or less similar in treatment as well as in control group.

After experimental infection there was significant reduction in glucose value. The highest reduction was observed in T2 group (210.40 ± 1.63 mg %) followed by T4 group (224.20 ± 5.05 mg %), T1 group (237.40 ± 1.58 mg %) and T3 group (238.40 ± 1.36 mg%). T5 positive control group had shown significant lower post infection glucose value (203.60 ± 2.73 mg %) as compare to pre infection control value (267.90 ± 2.58 mg %). Results indicates better efficacy of Diclazuril + Salinomycin group as compare to Salinomycin and Maduramicin in relation to effect on glucose value.

Pangasa *et al.* (2007) reported significant low glucose in infected and non medicated broilers as compared to control and Salinomycin coccidiostat fed broilers given 50,000 oocyst of *E. tenella* during acute phase of disease. Salinomycin had given better result with less glucose reduction similar to our findings. Reduction in glucose might be due to defect in absorption of glucose, leakage of glucose in plasma and increase demand of glucose by developing stage of parasite as reported by Mondal *et al.* (2011).

The findings are in agreement with Padmavathi and Muralidharam (1986a) who recorded hypoglycaemia with 50,000 *E.tenella* oocysts infection at 7 day PI and Constantinescu (1976) found hypoglycaemia in mixed infection, and Freeman (1970) and Basith *et al.* (1998) failed to see any change in plasma glucose. In contrast to the above findings significant increase in glucose level of whole blood in cases of coccidiosis was reported by Pratt (1940), Waxler (1941), Joshi *et al.* (1974), Kumar and Rawat (1975[a]) and Mondal *et al.* (2011).

4.6.2 Effect on Serum Total Protein

Serum Total Protein Value are more or less similar in treatment group at 3 week of age, but it was significantly reduced after experimental infection. Highest decrease in serum protein value observe in T4 group (3.46 ± 0.08 g %) followed by T1 group (3.58 ± 0.08 g %), T3 group (3.62 ± 0.07 g %) and T2 group (3.75 ± 0.03 g %). Post infection value of positive control was significant lower (3.28 ± 0.06 g %) as compared to pre infection. (4.62 ± 0.06 g %). Result indicate better efficacy of Salinomycin among four treatments. The present study revealed that there was significant reduction in levels of serum protein among the infected birds. Infected birds had hypoproteinaemia on 7th Day PI.

Pangasa *et al.* (2007) reported similar findings of significant low protein value in infected and non medicated broilers as compared to control and Salinomycin coccidiostat fed broilers given 50,000 oocyst of *E. tenella* during acute phase of disease.

Mondal *et al.* (2011) reported decrease in Protein in broilers given 20000-25000 doses of *E.tenella* oocysts infection. They also stated that protein decrease is due to rapid movement of interstitial fluid without protein into the plasma and also due to acute stress responsible for cortisol secretion and disturbances in protein catabolism.

The findings are also in accordance with Schlueter (1963), Mukkur and Bradley (1969), Oikawa *et al.* (1971), Witlock *et al.* (1981), Ruff and Augustine (1982), Padmavathi and Muralidharan (1986^{b}) and Conway *et al.* (1993) who all recorded lowered total serum protein values in *E. tenella* infected birds. Further, the findings coincided with significant reduction observed by Kouwenhoven and Van Der Horst (1970) and Wiswe (1986) with *E. acervulina*; Conway *et al.* (1993) with *E. maxima* infection and of Nayak and Rai (1986) with mixed infection of *E. tenella*, *E. necatrix* and *E. acervulina* infection. On the contrary, Kumar and Rawat (1975^{b}) did not observe any significant difference in total serum protein

between the control and *E. necatrix* and *E. acervulina* infected birds, while Stoev *et al.* (2002) found significant increase in serum total protein due to *E. tenella* infection.

The significant reduction in serum total protein observed in affected birds might be due to reduced feed intake and/or haemorrahges through the gut leads reduced absorption of amino acids and formation of inflammatory exudates rich in blood proteins (Basith *et al.,* 2000). It might also be due to decreased retention and change in the protein metabolism (Conway *et al.,* 1993), increased vascular permeability in the intestinal tract for formation of inflammatory oedema (Preston-Mafhan and Sykes, 1967; Champan *et al.,* 1982) and due to lowered intestinal pH and subsequent leakage of serum protein into the intestine (Kouwenhoven and Van Der Horst, 1970).

4.6.3 Effect on Serum Total Cholesterol

Before experimental infection at 3 week of age, serum total cholesterol values were more or less similar except Maduramicin group. This T4 group showing significantly higher serum total cholesterol values (119.40 ± 1.19 mg %).

After experimental infection of *E.tenella* serum total cholesterol value were significant increases in control as were as treatment group. Higher Serum cholesterol values were observed in T3 shuttle group (131.90 ± 3.12 mg %) followed by T2 group (130.50 ± 3.58 mg %), T1 group (121.60 ± 0.65 mg %) and T4 group (122.00 ± 0.60 mg %) in post infection treatment group. Positive control T5 group shown significant high post infection value (142.50 ± 1.44 mg %), as compare to pre infection value (112.80 ± 2.37 mg %). Result indicate better efficacy of Maduramicin in all four treatment group at four week of age.

Mondal *et al.* (2011) reported increase in cholesterol value in broilers given 20000-25000 doses of *E.tenella* oocysts infection. Increase in cholesterol might be due to decrease billiary excretion of cholesterol in anorexia.

Our results are in agreement with the observations of Singh *et al.* (1976), Padmavathi and Muralidharan (1986[b]) and Basith *et al.* (2000), while Kumar and Rawat (1975[a]) did not record significant increase in serum total cholesterol, and Kalra *et al.* (1996) reported significant decrease in serum cholesterol in birds given *Eimeria* inoculation at 4[th] day post-infection.

The hypercholesteremia observed in present study among the infected birds might be due to disturbed fat metabolism and loss of fluid resulting in apparent increase (Padmavathi and Muralitharan, 1986[b]) or due to impaired liver function leads to disturbed fat metabolism consequent to injury to intestinal epithelium in coccidiosis (Basith *et al.*, 2000).

4.6.4 Effect on Serum Glutamic Oxalo-acetic Transaminase (SGOT)

At 3 week of age SGOT value were about 30-35 I.U higher in treatment group as compare to control birds.

The post infection values were significantly higher in T1 group (320.20 ± 3.87 U/L) and T3 group (314.60 ± 5.24 U/L) among four treatment groups. Post infection value in control group was significantly higher (314.80 ± 4.52 U/L) as compare with pre infection control (240.60 ± 2.94 U/L). The result show better efficacy of Salinomycin followed by Maduramicin as compared to Diclazuril and Diclazuril + Salinomycin group.

Enzymes like SGOT, SGPT are present in large amount in metabolically hyperactive tissues. Therefore, tissue damage results in the elevation of the levels of these enzymes in serum (Kramer, 1980; Martin *et al.*, 1983).

Serological profiles of these enzymes are altered significantly in cell membrane degeneration, inflammatory and diffuse tissue degeneration and loss. The cause for the decrease in serum enzymatic activity in birds with coccidiosis following treatment can be attributed to the complete or partial healing of the diffuse intestinal tissue damage caused by the parasite (Deger *et al.*, 2002).

Rizvi *et al* .(2008) reported higher AST and ALT value in salinomysin 60-120 ppm given coccidiostat feed at 12 week of age in layer type birds similar to our findings.

Mondal *et al.* (2011) reported increase in AST (SGOT) in broilers given 20000-25000 doses of *E.tenella* oocysts infection. Incerease in SGOT might be due to significant damage of cell lining of the caecal wall along with inflammation and severe blood loss causing tissue loss from the body.

Comparable findings were reported by Singh *et al.* (1976) and by Deger *et al.* (2002) have also shown higher value of SGOT in infected bird before treatment with coccidiostats, while Kumar and Rawat (1975[a]) found no significant difference, but values were slightly less in infected group as compared to normal group. Kalra *et al.* (1996) found no change in SGOT activities.

Significant increase in SGOT activity observed in coccidia affected birds under study might be due to damage to liver and intestine. Coles, (1986) and Montogomery *et al.* (1990) also reported SGOT increase due to cellular membrane and tissue degeneration.

The increase in the SGOT could also be attributed to its leakage from the cystosol and mitochondria due to hepatic and kidney damage, increased cell membrane permeability and decreased overall tissue strength (Tung *et al* .1971).

Gessler (1965) reported that serum levels of transaminases are affected by reduced feed intake. In this study also the birds lost appetite during the peak of infection and this might have affected the serum transaminase levels.

4.6.5 Effect on Serum Glutamic Pyruvic Transaminase (SGPT)

At 3 week of age, SGPT values were found in range of 36 to 39 U/L between control and treatment groups, which were not differing significantly. After experimental infection significant higher value were observed in T1 and T2 group as compare to pre infection. After infection, positive control bird showing significant higher value (46.60 ± 0.85 U/L) as

compared to pre infection value (36.00 ± 1.13 U/L). Result indicated the effect of infection on SGPT value. Among coccidiostat treatment used in experiments, Diclazuril and Salinomycin causing less pathogenic damage as compare to Maduramicin.

Biu *et al.* (2006) also reported similar findings of increased ALT level in mixed coccidia infected chickens.

Singh *et al.* (1976) have shown significant increase in SGPT values on the 12th day post-infection. Deger *et al.* (2002) also found comparable higher SGPT values in coccidia-affected birds before treatment of coccidiostats. On the contrary, Kumar and Rawat (1975^{a}) have shown significant decrease in SGPT activity, while Kalra *et al.,* (1996) found no change in SGPT activity.

Here also this increase in SGPT activities might be due to extensive damage to liver and intestine by coccidial parasites, since the serological profiles of liver enzymes are altered significantly in cell membrane degeneration, inflammation and diffuse tissue degeneration and loss (Coles, 1986; Montgomery *et al.,* 1990). As a result of cellular damage several enzymes like ALT, AST and AP beach out into serum and hence their levels are increased depending upon the type and extent of damage (Hussain and Rahman, 2005).

4.6.6 Effect on Serum Alkaline Phosphatase (AKP)

At 3 week of age AKP value were significant higher in all four treatment group as compare to control group. The highest value was observed in T1 group (752.60 ± 4.47 U/L) followed by T3 group (734.80 ± 6.70 U/L), T4 group (729.80 ±5.01 U/L) and T2 group (728.80 ± 5.71 U/L) indicating better efficacy of Salinomycin and Maduramicin as compared with Diclazuril and Diclazuril + Salinomycin group.

Post experimental infection of *E.tenella* causing less damage in birds given Diclazuril and Salinomycin as compare with other group. Significantly higher post infection AKP value

(896.35 ± 20.29 U/L) was observed as compared with pre infection T5 value (676.60 ± 4.42 U/L).

Kogut and Powel (1993) stated that AKP activity may be the sensitive marker of the pathogenesis in coccidial infection of the caecum. It is directly proportionate to WG and indirectly proportionate to Oocyst index and LS.

Constantinescu (1976) reported similar comparable results in mixed coccidia infected birds, while Kalra *et al.* (1996) reported significant increase in serum AKP activity among infected birds. On the contrary, Wiswe (1986) found decreases activity of AKP in broiler chicks infected with *E. acervulina*, while Kumar and Rawat (1975[a]) reported significant decrease in serum AKP of *E. necatrix* and *E. acervulina* infected 3-4 months old cockerels. The present increase in serum AKP activity might be due to damage to liver, intestine and kidneys, which liberate the enzymes into the circulation (Kalra *et al.,* 1996).

The noticeably increased serum activities of AKP found in the present study might be associated with the metabolic alteration and damage of the bone marrow as compensation for the blood losses; the bone marrow might be forced to produce excessive blood cellular components as reported by Adamu *et al.* (2013).

4.7 Comparative efficacy of coccidiostats on histopathological changes

Histopathology is very much important to differentiate the abnormalities between normal and healthy structure at microscopic level. Histopathology is concerned with the demonstration of minute alterations in tissue structures in diseased conditions (Culling, 1963). Gross and microscopic pathology were specifically used to demonstrate the severity of the disease in chickens infected with *E. tenella*. The presence of high numbers of oocysts, schizonts and severe tissue damage in the caeca indicated the severity of infection due to *E. tenella*. Efficacy of coccidiostats can be judged by observing the histopathological changes at different period of time.

4.7.1 Gross Lesions

The gross lesions observed in the caecum of the birds infected with 50, 000 coccidial oocysts of *E.tenella* in T1 to T5 groups shown enlargement, distention with partially clotted or unclotted blood with reddish brown contents and exudate containing tissue debris on five day of post infection (Plate-14), it was gradually reduced after seven day post infection and was very mild on nine dpi onwards. Caecal wall was greatly thickened because of oedema and cellular infiltration with the formation of scar tissue. These findings are in accordance with those reported in caecal coccidiosis by Misra and Gautham (1970), Shukla *et al.* (1990) and Mc Dougald and Reid (1991). However, these lesions were mild in anticoccidial group T1 to T4 with variation in the intensity of gross pathological changes. Necropsy lesion are of less intensity were observed in T2 and T4 group. Soomro *et al.* (2001) observed enlargement of the caecum and the appearance of clotted blood in the area along with haemorrhagic or whitish spots on the caecal wall, inflammation, necrotic patches, and dilatation of the caecum with consolidation of caecal contents in almost all cases of caecal coccidiosis as seen in our study for infected and non medicated broilers at different post infection days.

4.7.2 Microscopic lesions

The histological examination of normal caeca did not show any changes and have the normal histological structure. The submucosa is only a thin layer except where blood vessels and nerves occur. The muscularis mucosa is very poorly developed and consists of only a few bundles of circular muscle fibres. The mucosa makes up about two thirds of the total thickness of the wall, the villi being well developed both in length and breadth as well as short and blunt but the crypts of Liberkuhn being very short. Within the lamina propria many lymphoid cells and some ymphoid nodules are found, both at the base and within the villi. Large numbers of mucous goblet cells occur in the epithelium but they do not outnumber the chief cells. (Plate-19, 25, 31,37).

Plate-19: Normal caecal section showing mucosa makes up about two thirds of the total thickness of the wall containing well developed villi and lymphoid cells in the lamina propria at 3 day PI in T_6 group bird (100x)

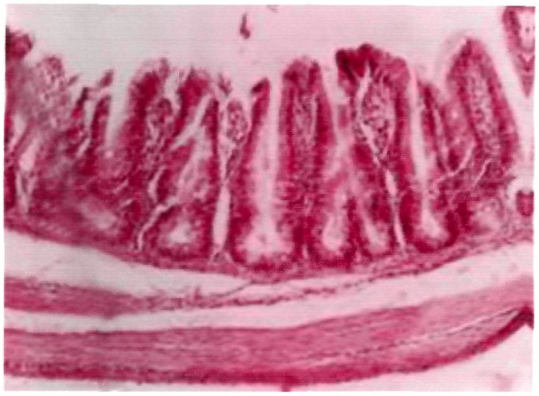
Plate-20: Caecal section showing cellular infiltration in lamina propria and desquamation of the epithelial lining of the vill containing schizonts at 3 day PI in T_5 group bird (100x)

Plate-21: Caecal section showing distortions of villi, edema and congestion of submucosa and muscular layer with leukocytes and development of schizonts at 3 day PI in T_1 group bird (40x)

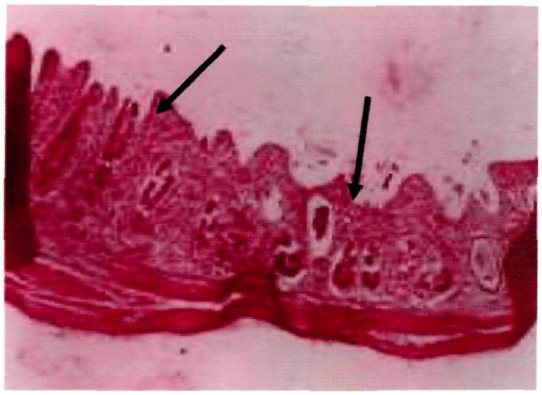
Plate-22: Caecal section showing edema of submucosa and muscular layers and thickening of mucosa with Sloughing of epithelial cells of mucosa at 3 day PI in T_2 group bird (100x)

Plate-23: Caecal section showing denudation and desquamation of the epithelium with distortion of villi and edema of submucosa at 3 day PI in T_3 group bird (100 xs)

Plate-24: Caecal section showing desquamation of the epithelium with edema and cellular infiltration of lymphocytes in the mucous and submucous layers at 3 day PI in T_4 group bird(100x)



P-19



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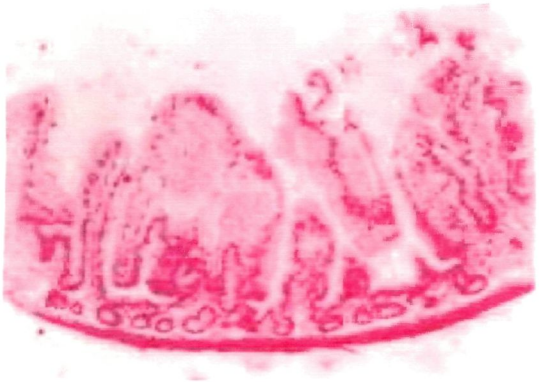

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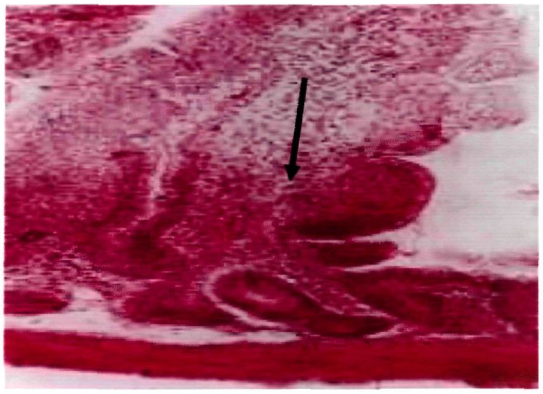

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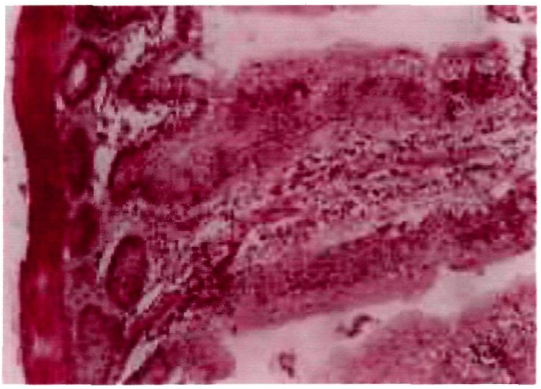
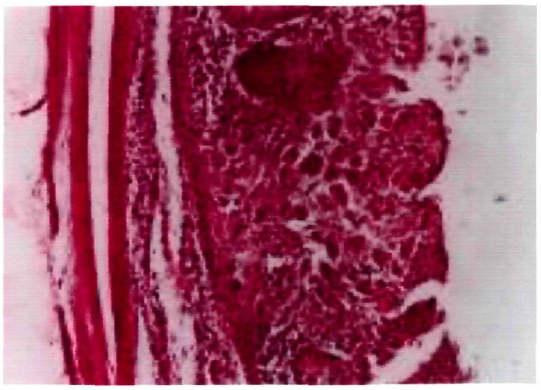


P-23



P-24

On 3rd day of post-infection caeca of group T5 shows cellular infiltration in lamina propria and desquamation of the epithelial lining of the villi. The oedema of submucosa and muscular layers of caecum with more number of Schizonts in the epithelial cells of villi were observed. Due to developing schizonts, the epithelial cells ruptured, resulted in congestion and haemorrhages, which were seen in the mucosa, submucosa and muscular layers (Plate-20). The caeca of group T1 on 3rd day of post-infection shows distortions of villi, oedema and congestion of submucosa and muscular layer. Damage to mucosa, low villous height to total mucosal thickness and the ratio is lowest which is due to maximum cell damage. The number of leukocytes were increased with the schizonts development (Plate-21). In group T2, oedema of submucosa and muscular layers and thickening of mucosa was seen. Sloughing of epithelial cells of mucosa was also seen with changes in epithelial cells (Plate-22). The group T3 shows denudation and desquamation of the epithelium. Distortion of villi and oedema of submucosa and muscular layers and thickening of mucosa was seen. The low ratio of villous height to total mucosal thickness showing the mechanical damage and alteration to mucosa (Plate-23), while in group T4, the denudation and desquamation of the epithelium, oedema of submucosa and muscular layers of the caecum, cellular infiltration of lymphocytes in the mucous and submucous layers and slight distortion of villi were noticed (Plate-24).

At 3rd day post infection Smith *et al.* (1981) and Long and Jeffers (1982) noted that the ionophores cause marked inhibition of asexual development by reducing viability and infectivity of sporozoites. They stated that the ionophores are found to be active against sporozoites and early schizogenous stages.

On 5th day of post-infection caeca of group T5 shows number of second stage schizont with numerous inflammatory cells especially lymphocytes were seen in the disrupted mucosal smear. This stage causing maximum damage during the rupture of the schizont (Plate-26). Microscopic examinations of the group T1 shows showed severe tissue

damage and plenty of schizonts containing merozoites (line) and oocysts (arrow) in the mucosa and tissue (Plate-27). In T2 group, there was development of large second stage schizont in haemorrhagic mucosal smear with presence of blood cells (Plate-28). In T3 group there was cluster of large schizont with severe tissue damage with lymphocytic infiltration (Plate-29), while in T4 group severe haemorrhage was observed in submucosal layer with mild destruction of caecal epithelium (Plate-30).

At seven dpi the caecum revealed a large number of second generation schizonts packed with merozoites and liberating merozoites along with the presence of developing oocysts were observed in T5 group. In addition, congestion of vessels, multifocal areas of haemorrhages and leukocyte infiltration predominately heterophils with degeneration and desquamation of crypt epithelium were also noticed (Plate-32). In T1 group, destruction and desquamation of epithelial cells was observed. Most of the epithelial cells of mucosa contained oocysts. Infilteration of lymphocytes were seen in mucosal, submucosal and muscular layers. Glandular hyperplasia, development of different stages of parasite was observed (Plate-33). The T2 group showed similar histopathological lesions with reduced intensity, along with few developing schizonts and merozoites in the crypt epithelium. However, hyperplastic changes in crypt epithelium characterized by regenerating epithelium with lymphoblast and numerous mitotic bodies were noticed on 7th dpi (Plate-34). In T3 group there was cluster of large developed oocyst in the caecal crypts with number of inflammatory cells (Plate-35). While group T4 shows infilteration of lymphocytes and fibroblasts, hyperplasia of mucosal glands. Numbers of glandular cells were observed in some places, with slight destruction of epithelial cells (Plate-36).

Similarly Chappel (1979) also reported that salinomycin cause arrested development of *E. tenella* schizonts, while Mc Dougald and Reid (1991) revealed a large number of second generation schizonts packed with merozoites along with the presence of developing

Plate-25: Normal caecal section showing well developed villi both in length and breadth with some lymphoid nodules at the base of villi and Large numbers of mucous goblet cells in the epithelium at 5 day PI in T_6 group bird (400x)

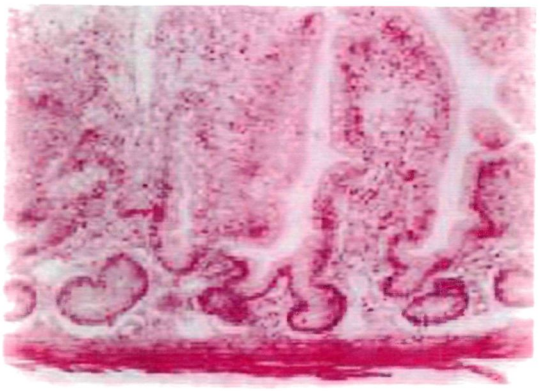
Plate-26: Caecal section showing number of second stage schizont with numerous inflammatory cells especially lymphocytes were seen in the disrupted mucosal smearat 5 day PI in T_5 group bird (40x)

Plate-27: Caecal section showing severe tissue damage with plenty of schizonts containing merozoites (line) and oocysts (arrow) in the mucosa and tissue at 5 day PI in T_1 group bird (40x)

Plate-28: Caecal section showing development of large second stage schzont in haemorrhagic mucosal smear with presence of blood cells at 5 day PI in T_2 group bird (100x)

Plate-29: Caecal section showing cluster of large schizont with severe tissue damage and lymphocytic infiltration at 5 day PI in T_3 group bird (40x)

Plate-30: Caecal section showing severe haemorrhage in submucosal layer with mild destruction of caecal epithelium at 5 day PI in T_4 group bird (10x)



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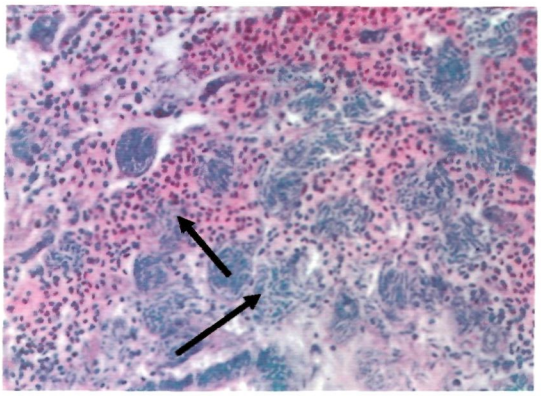

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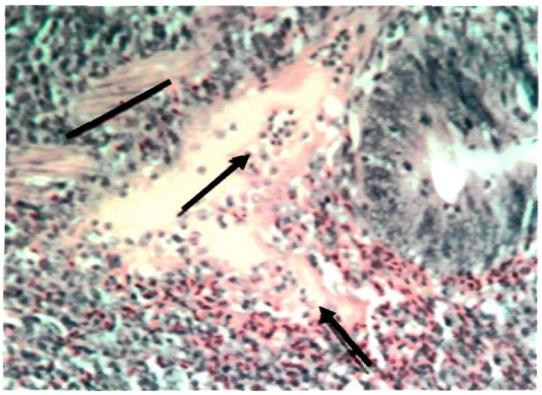

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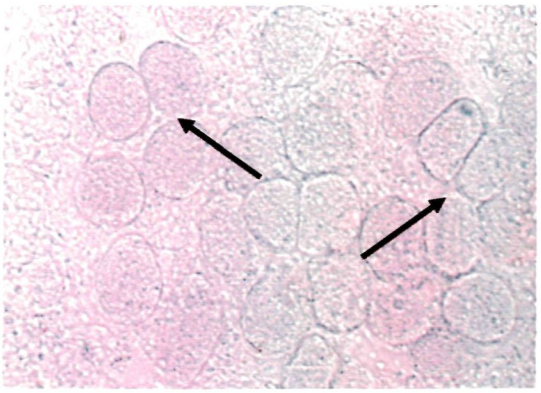

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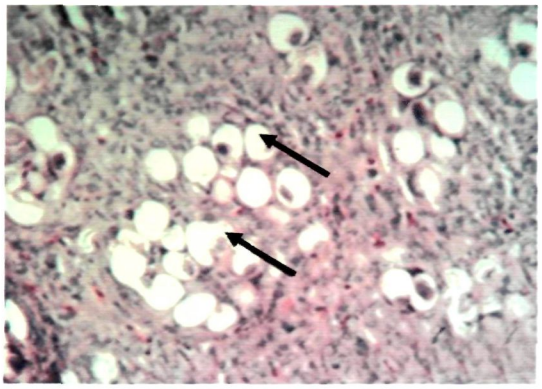

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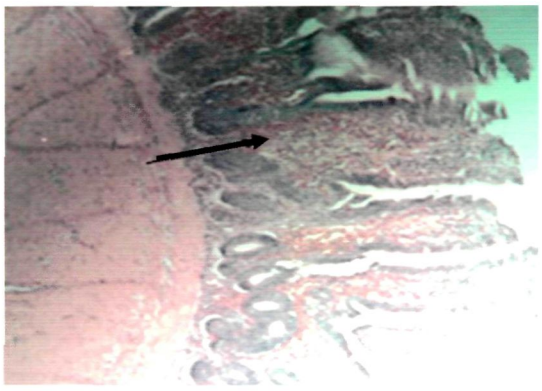

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Plate-31: Normal caecal section showing mucosa makes up about two thirds of the Stotal thickness of the wall containing well developed villi being and lymphoid cells in the lamina propria at 3 day PI in T_6 group bird (100 x)

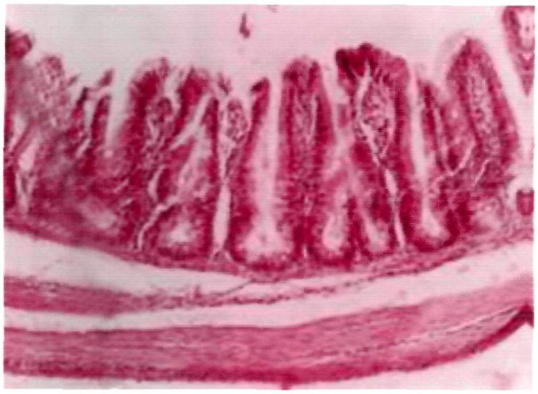
Plate-32: Caecal section showing large number of second generation schizonts packed with merozoites and presence of developing oocysts at 7day PI in T_5 group bird (200x)

Plate-33: Caecal section showing destruction and desquamation of epithelial cells with infilteration of lymphocytes and oocysts in epithelial cells of mucosa at 7 day PI in T_1 group bird (100x)

Plate-34: Caecal section showing few developing schizonts and merozoites in the crypt epithelium with hyperplastic changes in crypt epithelium characterized by regenerating epithelium at 7 day PI in T_2 group bird (200x)

Plate-35: Caecal section showing cluster of large developed oocyst in the caecal crypts with number of inflammatory cells at 7 day PI in T_3 group bird (200x)

Plate-36: Caecal section showing infilteration of lymphocytes and fibroblasts, hyperplasia of mucosal glands with slight destruction of epithelial cells at 7 day PI in T_4 group bird (40x)



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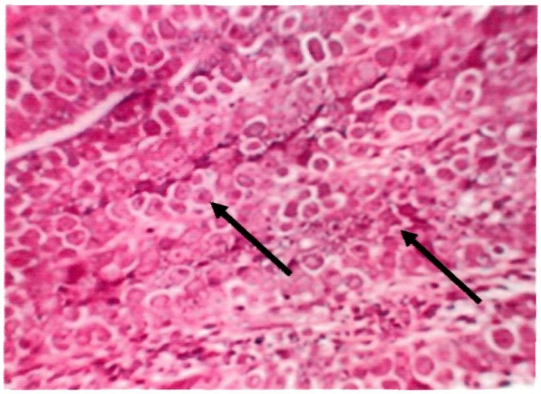

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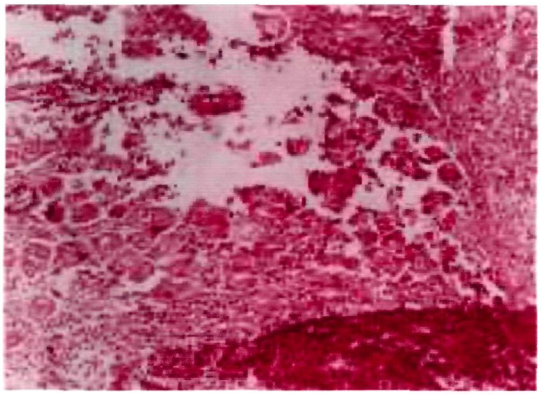

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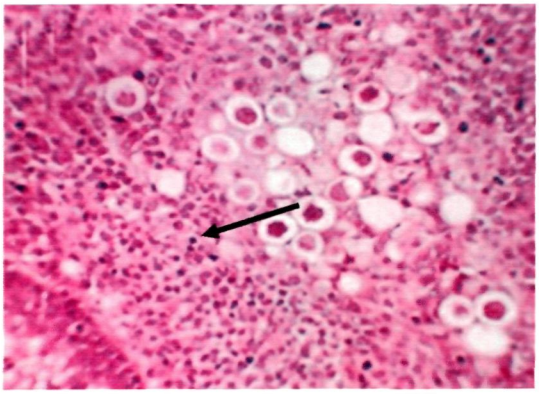

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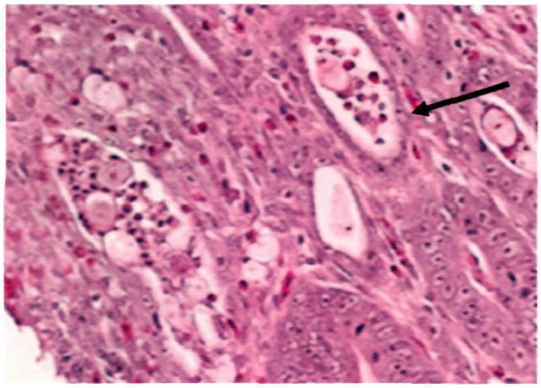

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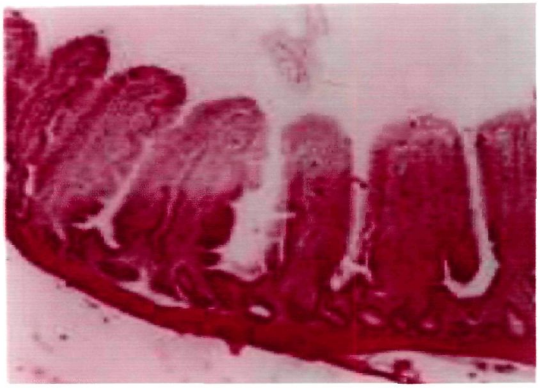


P-36

oocysts at seven dpi in caecal coccidiosis without medication. In addition, congestion of vessels, multifocal areas of haemorrhages and leukocyte infiltration predominately heterophils with degeneration and desquamation of crypt epithelium were also noticed.

At 5-7 day PI Sasmal and sinha (1989) reported denudation of epithelium and development of first and second generation schizont with cellular infiltration along with development of lymphoid follicle in the lamina propria. They observed destruction of muscularis mucosa with few macrophages at the area of damage. They also observed degeneration and vacuolation in treatment group.

The group which treated with anticoccidial agents showed similar histopathological lesions on 5th dpi but with reduced intensity along with few developing schizonts and merozoites in the crypt epithelium on 5th dpi. However, hyperplastic changes in crypt epithelium characterized by regenerating epithelium with lymphoblast and numerous mitotic bodies were noticed on 7th dpi, but similar changes were noticed in coccidian alone infected group on 14dpi (Milad, 2011).

Jaipurkar *et al.* (2004) stated that there was ballooning of caeca with clotted and unclotted blood, hypertrophied caecal mucosa with various parasitic stages at 5-7 days PI were observed in broilers given experimental infection of 50000 oocysts on 22nd day of age. The mucosa and submucosa were found to be heavily infiltrated with lymphocytes. In anticoccidial treatment group less pathological damage were observed. There was desdquamation of intestinal mucosa and denudation of intestinal villi cells. Similar observations on histopathological changes were reported by Chaudhry, (2000). Maes *et al.* (1984) demonstrated that 1 ppm Diclazuril was lethal against asexual and sexual stages of *E.tenella*. It was also preventing oocyst shedding.

On 10th day of post infection caeca of group T5 shows development of numerous oocysts in the distended caecal crypts surrounded by leukocytic infiltration (Plate-38) .T1

group showed connective tissue proliferation in submucosa and muscular layer and cellular infilteration were also observed. Hyperplasia of lymphoid follicles and the tissue starts regenerating (Plate-39). The group T2 shows regeneration of caecal mucosa, hyperplasia of lymphoid follicles with Oocyst (Plate 40), while group T3 showed degeneration of gametocytes with destructed oocyst structures and cellular infiltration in the lamina propria. (Plate 41), while in group T4 shows regeneration of caecal mucosa. No oocysts could be detected in the epithelial cells (Plate-42).

Histopathological examination of the affected caeca showed similar findings with those reported by McDougald and Fitz-Coy (2008). They described the most pathogenic stage caused by *E. tenella* as the second generation schizont, which caused excessive tissue damage, bleeding, disruption of the caecal glands and destruction of the mucosa and muscularis layer. Microgametes and macrogametes of schizonts are seen in the tissue on days 6 and 7 after infection and matured oocysts are released into the lumen in huge numbers. These finding however, correspondingly related to those recorded by Babu *et al* (1976), Soomro *et al.* (2001), Lakkundi *et al.* (2002), Siddiki, *et al.* (2008), and Chandrakesan, *et al.* (2009) in different drugs and infection. They were also observed loss of epithelial tissue and villi, severe muscular oedema, disruption of caecal mucosa, cluster of oocysts, lymphoid cells showing hyperplasia, lymphocytic infiltration, sloughing of epithelial lining, coccidian gametes, schizonts, desquamated epithelial cells, marked proliferation of epithelial cells, different stages of parasites in caecal tissue. The major histopathological changes observed in caeca of infected chicks revealed marked degeneration, desquamation of superficial epithelium, leukocyte infiltration in submucosa, denudation of villi and haemorrhages. The tissue contained various stages of schizonts. The significant reduction in intensity of caecal lesions was observed in treated groups T2 and T4 in present study.

Plate-37: Normal Caecal section showing well developed villi both in length and breadth with some lymphoid nodules at the base of villi and large numbers of mucous goblet cells in the epithelium at 5 day PI in T_6 group bird (400x)

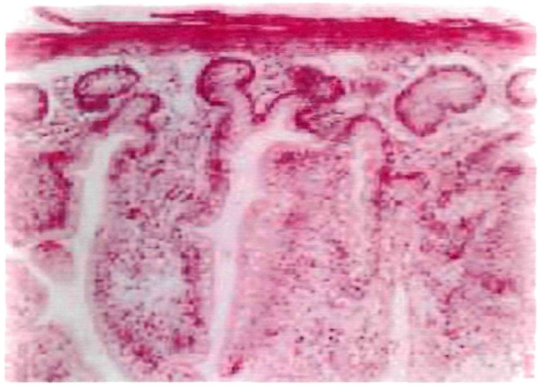
Plate-38: Caecal section showing development of numerous oocysts in the distended caecal crypts surrounded by leukocytic infiltration at 10 day PI in T_5 group bird (100x)

Plate-39: Caecal section showing regenerating changes of connective tissue proliferation in submucosa and muscular layer with cellular infilteration and hyperplasia of lymphoid follicles at 10 day PI in T_1 group bird (400 x)

Plate-40: Caecal section showing regeneration of caecal mucosa, hyperplasia of lymphoid follicles with Oocyst at 10 day PI in T_2 group bird (100x)

Plate-41: Caecal section showing degeneration of gametocytes with destructed oocyst structures and cellular infiltration in the lamina propria at 10 day PI in T_3 group bird (400x)

Plate-42: Caecal section showing regeneration of caecal mucosa without oocysts in the epithelial cells at 10 day PI in T_4 group bird (100x)



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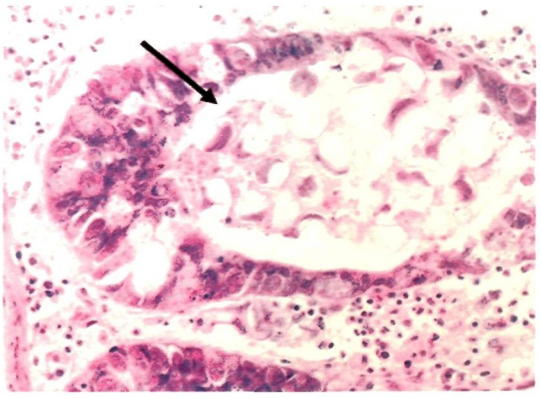

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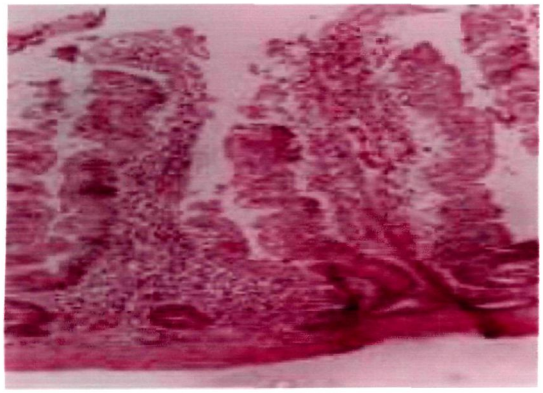

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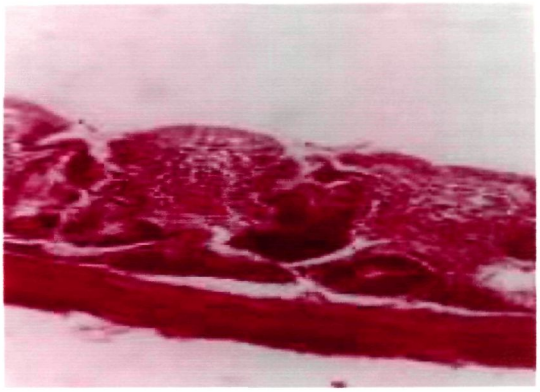

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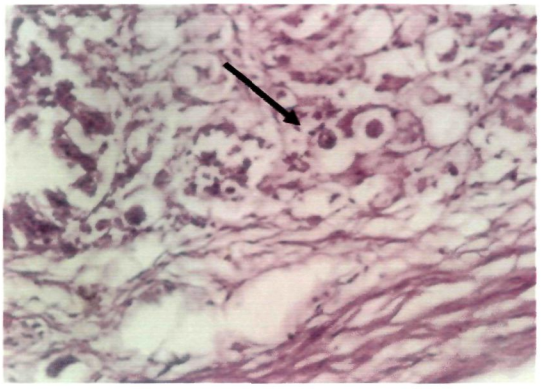

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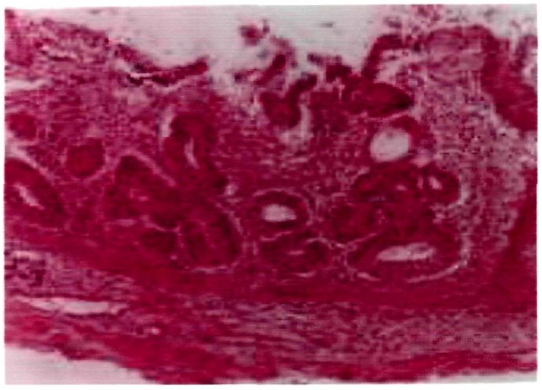

P-42

The severity of coccidial infection may vary with the isolate, number of oocysts ingested and the immune state of the bird. Some species are identified easily by the location and appearance of gross lesions in concert with the size of oocysts or schizonts. The presence of clusters of large schizonts in the caecum is pathognomic for *E. tenella* as reported by Adamu *et al.* (2013).

In the present study, early recovery was also indicated by the presence of regenerative changes on the 10^{th} day of post infection in anticoccidial treated group compared to coccidia alone. Hence it can be concluded that the anticoccidial is partially effective in controlling coccidiosis. However, further studies are required to acertain the effectiveness and economical impact of the anticoccidial usage in the poultry operation

The histopathological changes were observed in the group T5 are due to infection of *E.tenella* pathogen. But there was reduction in these symptoms in infected but treated birds in remaining groups. In group T2 and T4 the regeneration of caecal mucosa is observed, while in group T1 and T3 most of the part of caecal mucosa is seen affected due to parasite. There were no oocysts of *E. tenella* in the epithelial cells in T4 and T2 groups and recovery of caecal tissue was observed during experimentation. From this study it is clear that, the Maduramicin and Salinomycin treated group shows very less mechanical damage to tissue hence it can be used as a curative remedy against the caecal coccidiosis.

The observations of the present study did not show any infective stages in which parasites were infiltrating either macroghage. However, the present observations on caecal coccidiosis have enabled to draw conclusion that the magnitude of infection, type and dose of coccidiostat and stage of development of the disease could be established by histopathological observations.

SUMMARY

AND

CONCLUSION

CHAPTER - V

SUMMARY AND CONCLUSIONS

The efficacy of three commonly used feed coccidiostats named Diclazuril (T1) Salinomycin (T2), Diclazuril + Salinomycin (T3) in shuttle programme and Maduramicin (T4) on experimentally induced *Eimeria tenella* coccidial infection and their effects on growth, haematology, biochemical and histopathological changes were undertaken in Three hundred Cobb400 strain of broiler. Birds were given feed containing Diclazuril (T1), Salinomycin (T2), and Maduramicin (T4) coccidiostats at dose rate of 1 ppm, 60 ppm and 5 ppm. Weekly body weight and feed consumption were recorded. Experimental infection of 50,000 oocysts of *E.tenella* was given on 22nd day of age. Blood was collected before experimental infection at 3 week and after experimental infection at 4 week of age for haemato-biochemical study.

5.1 Comparative efficacy of cocidiostats on faecal score, oocyst output and lesion score in different treatment group after experimental *Eimeria tenella* infection

All faecal score mean value of treatment group was significantly lower as compared to infected control T5 group with lowest faecal score mean being observed in T4 group followed by T2 group indicating better efficacy of Maduramicin and Salinomycin

The overall treatment mean results indicated best efficacy of Maduramicin and Salinomycin in OPG reduction followed by Diclazuril and Diclazuril + Salinomycin. The overall trend of period mean showing increase in OPG count was observed upto seven days PI and there after decreasing trend was observed up to 12 days post infection.

Statistically all treatment groups were showing significant lower lesion score as compared to infected control. The lowest treatment mean value 1.10 was in Maduramicin

group followed by Salinomycin group (1.25) indicating better efficacy of Maduramicin and Salinomycin

Average oocyst index was highest in positive control (4.0). Maduramicin and Salinomycin groups had lower oocyst index as compared to Diclazuril and Diclazuril + Salinomycin Shuttle group. Result indicated better efficacy of maduramicin and Salinomycin on basis of oocyst Index value

Significant low mortality (4-12%) was found in medicated group as compared to non medicated group. Overall treatment mean mortality was found lowest in Maduramicin group (0.67) followed by Salinomycin (1.0) indicating better efficacy of Maduramycin and Salinomycin.

5.2 Comparative efficacy of cocidiostats on body weight and body weight gain

Results indicate best result of body weight with non infected non medicated group as compared to four coccidiostat treatment groups. Maduramicin showed highest body weight followed by Salinomycin, Diclazuril and D+S Shuttle group among coccidiostat treatment groups.

Over all highest body weight gain was observed in T4 (1824.064 g) group followed by T2 group (1733.50 g), T1 group (1720.68 g) and T3 group (1696.64 g) at the end of six week period. T3 and T4 values are differing significantly from T1 and T2 values among coccidiostat group as well as from control group. Negative control T6 birds showing significant highest BWG (1918.26 ± 9.86) and positive control T5 birds showing significant lowest BWG (1472.80 ± 4.96) at the end of six week period.

5.3 Comparative efficacy of coccidiostat on feed consumption and feed conversion ratio.

At the end of six week significantly highest feed consumption was observed in T2 group (3666.10g) followed by T4 group (3640.22g), T3 group (3614.55g) and T1 group (3579.98g) among four coccidiostat group. In positive control group significant lowest feed consumption (3560.54g), while in negative control group significant highest feed consumption 3774.77g was observed at above age among all groups.

Over all FCR value were found lowest in T4 group followed by T1, T2, and T3 among treatment group. Significantly highest FCR (2.42 ± 0.01) was observed in T5 group and lowest FCR (1.97 ± 0.51) was observed in T6 group among all groups indicating better efficacy of Maduramicin followed by Diclazuril.

5.4 Comparative efficacy of coccidiostat on Global Index with sensitivity

The Global Index of Non infected Non medicated Control in percentage (GI_{NNC} %) was calculated from above Global Index values which was 74, 76, 71, 82, 47 and 100 for T1, T2, T3, T4, T5 and T6 treatment, respectively. Result indicated good efficacy of Maduramicin and limited efficacy of Salinomycin, Diclazuril and Diclazuril + Salinomycin Shuttle group.

The difference in the efficacy of various coccidiostats used by the different workers might be due to difference in isolates of *E.tenella* or species of *Eimeria spp.*, variation in dose of infection or coccidiostat, locational changes, change in age and immune status of the birds. Environmental selection pressure in different geographical location as well as the history of drug used may differ with each other therefore strain resistant in one area may be sensitive to another area.

The reduced efficacy of Diclazuril and Salinomycin in the present study might be due to their use in this area since 10-15 years which resulted in decreased sensitivity to caecal coccidia. Another possible reason may be the lower dose of drugs used by the poultry farmers.

The reduced efficacy of Diclazuril and Salinomycin in the present study indicates that their use should be restricted. Results are suggestive for switching over to other anticoccidial to overcome the problems of resistance.

5.5 Comparative efficacy of coccidiostat on haematological value

Result indicated better efficacy of Maduramicin in term of less R.B.Cs damage among four treatments. In comparison with positive control groups, all coccidiostat treatment groups showed significant beneficial effect on TEC value showing less R.B.Cs damage.

Result indicated less damage by Salinomycin for PCV reduction value among for coccidiostat treatment group. There were also significant reduction in positive control post infection group compared to all treatment post infection group indicating supportive efficacy of coccidiostat in birds for less reduction of PCV value

Positive control T5 group had significantly lower Hb value (8.54 ± 0.07) as compared to all treatment groups. Results indicated better efficacy of Salinomycin as compared to other coccidiostat.

Post infection TLC count of positive control group were (52.71 ± 0.38 $\times 10^3$/ µl) significantly higher than the pre infection value of same group (21.51 ± 0.06 $\times 10^3$/ µl). The result indicated better efficacy of Salinomycin followed by Maduramicin in *E.tenella* infection.

After experimental infection heterophills counts significantly increased in all treatments group with highest increase in T1 group (18.07 ± 0.07 × 10^3 /µl) followed by T3

group (16.52 ± 0.56 $\times 10^3/\mu l$), T4 group (15.34 ± 0.26 × 10^3 / µl) and T2 group (14.92 ± 0.27 × 10^3 / µl) indicating better efficacy of Salinomycin and Maduramicin.

Positive control group after post infection had significant increase in lymphocytes count (30.11 ± 0.29 10^3 / µl) as compared to pre infection (9.28 ± 0.12 × 10^3 / µl). Results indicated better efficacy of Salinomycin followed by Maduramicin in infected birds compared to Diclazuril in term the pathological damage.

Salinomycin treatment shown better efficacy with less fluctuation in monocytes counts. Infection having significant effects on Monocytes count as there was decrease in post infection value in positive control birds.

Significant increase of eosinophilic count in T5 positive control (2.37 ± 0.04 $\times 10^3$ / µl) as compare to treatment group were observed which indicating more damage by infection which can be reduced by coccidiostat treatment.

Basophills count indicating better efficacy of Salinomycin and Maduramicin. Significant reduction was observed post infection in control group (2.06 ± 0.07 $\times 10^3$ / µl) as compared to pre infection (2.26 ± 0.02 $\times 10^3$ / µl).

5.6 Comparative efficacy of Coccidiostat on bio-chemical values

Results indicated better efficacy of D+S group as compared to Salinomycin and Maduramicin in relation to effect on glucose value.

Results of Serum Total Protein Result indicated better efficacy of Salinomycin among four treatments, while Serum Total Cholesterol result indicated better efficacy of Maduramicin and Diclazuril in all four treatment group.

The result of Serum Glutamic Oxalo-acetic Transaminase (SGOT) showed better efficacy of Salinomycin followed by Maduramicin as compared to Diclazuril and Diclazuril

+ Salinomycin group, while results of Serum Glutamic Pyruvic Transaminase (SGPT) showed better efficacy of Diclazuril and Salinomycin.

The result of Serum Alkaline Phosphatase (AKP) indicated better efficacy of Salinomycin and Maduramicin as compared with Diclazuril and Diclazuril + Salinomycin group.

5.7 Comparative efficacy of coccidiostat on Histopathological changes

The gross lesions observed in the caecum of the birds infected with 50, 000 coccidial oocysts of *E.tenella* in T1 to T5 groups showed enlargement, distention with partially clotted or unclotted blood with reddish brown contents and exudate containing tissue debris on five day of post infection, which gradually reduced after seven dpi and were very mild on nine dpi onwards.

At 3 day PI ionophores cause marked inhibition of asexual development by reducing viability and infectivity of sporozoites affecting early schizogenous stages.

At 5-7 day PI there was denudation of epithelium and development of first and second generation Schizont with cellular infiltration along with development of lymphoid follicle in the lamina propria. There was degeneration and vacuolation in treatment group.

Widespread damage to absorptive epithelium and destruction of villi were evident on histopathological examination. The mucosa as well as sub mucosa was heavily infiltrated with lymphocytes. Desquamation of superficial mucosal epithelium along with infiltration of mononuclear cells and the presence of schizonts were seen.

The groups which were treated with anticoccidial agent showed similar histopathological lesions on 5th dpi but with reduced intensity, along with few developing schizonts and merozoites in the crypt epithelium on 5th dpi.

On 10th day of post infection caeca of group T5 showed development of numerous oocysts in the distended caecal crypts surrounded by leukocytic infiltration. T1 group showed connective tissue proliferation in submucosa and muscular layer and cellular infiltration.

Based on the above findings following conclusions were drawn.

1. The results of faecal score, oocyst per gram (OPG), lesion score, oocyst Index value and mortality indicated better efficacy of coccidiostats as compared to non medicated birds in experimental infection with better efficacy of Maduramicin and Salinomycin as compared to Diclazuril and Diclazuril + Salinomycin shuttle treatment
2. Coccidiostats proved to have growth promoting action in broiler chickens during the experimental infection. Birds fed with Maduramicin medicated (5 ppm) performed well in terms of live weight gain and feed conversion ratio and it was followed by salinomycin (60 ppm) for weight gain and Diclazuril for feed efficiency in broiler birds.
3. Maduramicin at 5 ppm and Salinomycin at 60 ppm could also be used for prevention and control of coccidiosis with less alteration in body weight and feed efficiency in broiler birds based on the result of mortality lesion score and OPG values.
4. Result of sensitivity against *E. tenella* indicated good efficacy of Maduramicin, and limited efficacy of Salinomycin, Diclazuril and Diclazuril + Salinomycin Shuttle group on the basis of Global index value (GI_{NNC} %).
5. The reduced efficacy of Diclazuril and Salinomycin was found in the present study. Results suggested for switching over to other anticoccidial to overcome the problems of resistance.
6. Haematological studies revealed that haemoglobin concentration, packed cell volume and total erythrocytes counts were significantly ($P < 0.05$) reduced, while total leukocytes counts were significantly increased on account of coccidial infection in all coccidiostat

treatment and infected non treated group. Different Leukocytes Count (DLC) value revealed significant increase in heterophills, lymphocytes and eosinophills, while significant decrease in monocytes and basophills on account of coccidial infection. Results on haematological studies indicated comparativly less pathological damage by salinomycin.

7. Studies on biochemical profile revealed significantly ($P < 0.05$) lower serum glucose and serum total protein, while significant increase in serum total cholesterol, SGOT, SGPT and alkaline phosphatase activities due to coccidial infection as compared to healthy birds. However, were non-significantly higher in infected birds. Results of biochemical studies indicated comparative less pathological damage by coccidiostats treatment as compared to infected non treated group, but there was no consistent trend for drug choice.

8. The severity of coccidial infection might vary with the isolate, number of oocysts ingested and the immune state of the bird. The presence of clusters of large schizonts in the caecum was pathognononic for *E. tenella.*

9. From the histopathological results it was clear that, the Maduramicin and Salinomycin treated group showed very less mechanical damage to tissue hence it could be used as a curative remedy against the caecal coccidiosis. The magnitude of infection, type and dose of coccidiostat and stage of development of the disease could be established by histopathological observations.

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