PRESENT STATUS OF COCCIDIOSIS IN BROILER AND ITS CONTROL MEASURES AT BOGRA DISTRICT

A THESIS

BY

KHAIRUL ALAM

EXAMINATION ROLL NO. 11 VPHY JD 5M
REGISTRATION NO. 0171
SESSION: 2001-2002
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MASTER OF SCIENCE (M.S.)

IN

PHARMACOLOGY

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY
FACULTY OF VETERINARY AND ANIMAL SCIENCE
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Submitted to the
Department of Pharmacology and Toxicology
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Approved as to style and contents by

(Prof. Dr. Md. Anwar Hossain) **Research Supervisor**	**(Prof. Dr. Md. Siddiqul Islam)** **Research Co-Supervisor**

(Prof. Dr. Md. Siddiqul Islam)
Chairman
Examination Committee
and
Chairman
Department of Pharmacology and Toxicology
Faculty of Veterinary and Animal Science
Sylhet Agricultural University
Sylhet-3100

DECEMBER, 2013

DEDICATED

TO MY

BELOVED PARENTS

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The Author

December, 2013

LIST OF ABBREVIATIONS

AST	–	Anticoccidial Sensitivity Test
@	–	At the rate of
c.m	–	Centimetre
dpi	–	Day post infection
DHFR	–	Dihydrofolic reductase
et al.	–	Associates
E	–	Eimeria
^{0}F	–	Degree fahrenheit
g	–	Gravity
i.e.	–	That is
J.	–	Journal
Kg	–	Kilogram
K/cal	–	Kilo calorie
Ltd.	–	Limited
Mg	–	Milligram
Ml	–	Millilitre
µg	–	Microgram
No	–	Number
PABA	–	Para-amino benzoic acid
®	–	Registered
Ppm	–	Parts per million
OPG	–	Oocysts per gram
THFA	–	Tetrahydrofolic acid
Vol.	–	Volume

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PRESENT STATUS OF COCCIDIOSIS IN BROILER AND ITS CONTROL MEASURES AT BOGRA DISTRICT

ABSTRACT

Coccidiosis has negative economic impact on the commercial poultry industry and sulfonamides have a good coccidiostatic effect on control and treatment of broiler coccidiosis. The objective of this study was to assess the prevalence of broiler coccidiosis in Bogra district and comparative investigation of efficacy on four locally used Sulfonamides Esb3®-30%, Coccino®, Navacox®, Coccicure® in broiler coccidiosis experimentally infected by *Eimeria spp* based on the mortality rate, body weight gain, feed consumption, feed conversion ratio, number of oocysts per gram (OPG). To know the prevalence, fifteen farms were selected in different part of the Bogra district and observed every day, during the experimental period out of 15 farms , 12 farmes (80%) were found affected with coccidiosis. For comparative investigation sixty, one day old broiler chicks, Cobb-500 breed were divided equally and randomly into 6 groups (10 chicks /group) and reared in litter up to 28 days of age. Groups were designed as group T_0 as negative control: non-treated and non-infected, group T_i as positive control: non-treated and infected and then group T_1, T_2, T_3 and T_4 were infected and received Esb_3®-30%, Coccino®, Navacox®, Coccicure® respectively. Groups T_i and T_1, T_2, T_3 and T_4 were infected at 12 days old by 100,000 oocystes. Groups T_1, T_2, T_3 and T_4 were treated at 17 days of age. OPG test was done on 11st, 15th, 17th, 21st, 23rd, 24thand 25th days of rearing period. Also weekly mortality, weight gain and feed conversion rate (FCR) were recorded. In the negative control group T_0 and treated group T_1, T_2, T_3 and T_4 didn't show any mortality but infected positive control group T_i had 20% mortality after 28 days of rearing period. The mean live weight in T_2 group was 39.56% increase (1368.7±17.7) on 28 days which was significant dissimilar from positive control group T_i. Positive control group T_i was 25.02% decrease (980.7±20.5) on 28 days from negative control group T_0 which was 1308.0±17.8. The live weight was statistically significant ($p<0.05$) on 21 days and 28 days compared to other treated groups. The feed conversion ratio were 1.45 and 1.61 on 3rd and 4th week respectively in T_2 group which were significantly different ($p<0.05$) compared to other treated groups. Number of less mean OPG shedding was 9300±321, 3500±208 and 1200±115 on after 3 days, 5 days and 6 days treatment respectively in T_2 group which were significantly different ($p<0.05$) compared to other treated groups . Also, they completely inhibit the oocyst shedding in infected groups' one week after treatment. Based on this experiment, the efficacy of four locally used sulfonamides Esb_3®-30%, Coccino®, Navacox®, Coccicure® on treatment of broiler coccidiosis induced by *Eimeria spp* have a significant difference ($p<0.05$). The result provided that the birds of Coccino® treated group showed the best live weight, feed conversion rate and less oocystes shedding as compared to other treated groups.

Key word: Sulfonamides, Esb_3®-30%, Coccino®, Navacox®, Coccicure® Prevalence, Eimeria, broiler chickens, body weight, FCR, OPG.

CHAPTER I

INTRODUCTION

Bangladesh is a sub-tropical country with an agro-based economy. Majority of the people are directly or indirectly associated with agriculture. Two major problems affecting the country are unemployment and malnutrition due to shortage of quality protein in the daily diet. Poultry rearing can create a positive impact on the economy of the country by creating employment opportunity for the unemployed people. Broiler chicks are also a major source of good quality dietary protein. Because of its tenderness and acceptance a healthy food compared with other meats, broiler meat has an increasing demand all over the world.

Because of an almost assured and quick return and relatively small scale capital investment poultry rearing has been considered as the best method of supplying quality protein compared with other sectors in agriculture. The concept of poultry rearing as an industry is relatively new in Bangladesh. But, it is a matter of hope that in the recent years the poultry industry has been a tremendous development in the country. Although the impressive achievement is it possibly represents a small part of what could be done. This is largely because of not addressing the health and safety issues properly in many instances. Despite this tremendous increase in commercial poultry rearing, rural poultry still constitutes around 80% of the total poultry population in Bangladesh (Anon, 1998). More than 2 million rural women are involved in poultry rearing under the poverty alleviation program of different Non-Government Organization (NGO), and the poverty alleviation program of the Department of Livestock Services (DLS).

The village chicken often suffer from a high mortality, due to different diseases, sub optimal management, lack of nutritional feeding and predators (Sonaiya *et al.,* 1992 and Ojok, 1993). Among the different diseases parasitic infection pose a major threat to the chicken. Parasitic infection includes helminth, protozoa and arthropod infection causing anemia, mortality, sub-optimal production, and less body weight gain. Free-range chicken are in permanent contact with soil and insects which act as reservoirs for helminth eggs and larvae (Kabatange and Katule, 1990 and Pandey, 1992).

Among the parasitic disease coccidiosis is one of the major problems for any profitable farm. All age groups of chicken are susceptible, but most outbreaks occur in chicks of 2-4 weeks old. It was third most prevalent (9.4%) diseases of chicken in Bangladesh during 1994-1995 (Bhattacharjee *et al.,* 1996). An obligate intracellular protozoa of the genus *Eimeria* is the causal agent of coccidiosis in broiler. Coccidiosis has been identified as one of the major diseases in chicken in Bangladesh (Karim *et al.,* 1990). Coccidiosis costs the US poultry industry approximately US $ 300 million per year (Danforth, 1986). In Britain only the cost of anticoccidial drugs is estimated to be *5* million Sterling pound (Tress, 1987) and US $ 250 million on a worldwide basis (Long and Jeffers, 1986). There is no exact data on the economic loss due to coccidiosis in Bangladesh, but considering the worldwide losses, it can be assumed that the losses in chicken industry would be enormous. Based on post-mortem examination. Kutubuddin (1973) and Sarker (1976) recorded 14.66% and 12% mortality, respectively, due to coccidiosis in Birds from BAU poultry farm. Later Mondal and Qadir (1978) recorded 54.14% infection with coccidiosis by faeces examination in BAU poultry farm.

Affected birds shed oocysts in their faeces that after being sporulated caused infection to new birds. A single oocyst can give rise to at least over a million progeny oocysts in a single infection cycle. Wet litter and warm weather favour sporulation of oocysts. Signs of coccidiosis vary with the species of *Eimeria.* The most pathogenic species causes bloody diarrhoea and a higher rate of mortality in untreated birds. Subclinical infections also cause serious economic loss by growth retardation.

Levine (1939) first discovered the anticoccidial activity of Sulfonamide that has been in use since 1950s to control the coccidiosis in the poultry industry. But due to continuous uses of sulfonamides *Eimeria spp*. developed a degree of resistance against these drugs. The existence of a sulfonamide resistant strain of *Eimeria tenella* was first reported by Waletzky *et al.* (1954). Some other authors also have reported the resistance of *Eimeria spp.* against sulfonamides and other modern drugs (Warren *et al.* 1966; Hodges *et al.* 1969; Chapman 1976, 1978,182, 1997; Jefferes 1974, 1978; Ryley 1980 and McDougald 1981).

In Bangladesh, few commercial poultry farms use anticoccidial drug in feed during preparation of feed. Besides, those who use ready (Pellet) feed they get the benefit because the manufacturer mixes the anticoccidial drug while mixing the feed. Most of the farms use sulfonamides for treatment of clinical coccidiosis while there is any outbreak. But this can not prevent the damage already occurred due to the clinical disease. On the other hand in feed medication using a good anticoccidial drug causes an improvement in body weight gain and feed conversion ratio (FCR) and a decreased mortality (Ruff *et al.* 1980 and Hayat *et al.* 1996). A number of sulfonamides are available in the market for treatment and prevention of chicken coccidiosis.

Vaccination can be feasible alternative for controlling coccidiosis in layers but not used in broiler because of shortage life cycle of broiler. Two different vaccines viz. Imrnucox®, made with virulent oocyst of different pathogenic *Eimeria* spp. are available in Bangladesh.

This study was designed to fulfill the following objectives:

i. To assess the present status of coccidiosis in broiler chicks in Bogra District.
ii. To evaluate the comparative efficacy on four locally available sulfonamides used to control coccidiosis in broiler chickens.
iii. To determine the effect of selected sulfonamides on experimental infection on broiler.
iv. To evaluate the comparative growth performance of broiler.

CHAPTER II

REVIEW OF LITERATURE

The sulfonamides are one of the oldest groups of antimicrobial compounds still in use today. It was derived from the azo dye prontosil and all other sulfonamides produced since have structurally resembled it. Sulfonamide-diaminopyrimidine combinations have been used to reduce the incidence of sulfonamide resistance and these combination sulfonamide treatment regimens previously used in small animal medicine.

Structure of Sulfonamides

All sulfonamides are derivatives of sulfanilamide, which was, in the 1940s, the first sulfonamide discovered to have antimicrobial activity. Since then many derivatives of sulfonamide with differing pharmacokinetic and antimicrobial spectrums have been used in veterinary medicine to treat microbial infections of the urinary, gastrointestinal, and central nervous systems. Susceptible organisms include many bacteria, coccidia, chlamydia and protozoal organisms, including

Taxoplasma spp. Sulfonamides are white crystalline powders that are weak organic acids, are relatively insoluble in water and tend to undergo crystallization in the urine (acid pH) in vivo, especially in animals that are over dosed, dehydrated, acidosis from disease, or given large doses of a sulfonamide via bolus injection.

Para-amino benzoic acid (PABA), pterdines, glutamic acid and the enzyme dihydropterate synthase interact to form dihydropteroic acid, the immediate precursor to dehydrofolic acid. Dihydropteric acid is enzymatically converted to dihydrofolic acid by dehydrofolate synthase, followed by another enzymatic conversion of dihydrofolic acid to tetrahydrofolic acid (THFA) via dihydrofólic reductase (DHFR). THFA continues in this pathway to permit RNA production and bacterial reproduction. PABA and sulfonamide bear strong enough structural resembles for one to be chemically. Mistaken for the other in the folic acid production pathway. Sulfonamides inhibit the biosynthesis of folic acid by being mistakenly substituted for PABA. Trimethoprim inhibit the biosynthesis of tetrahydrofolic acid from dehydrofolic acid as presented below:

Dihydropteridine + P-aminobenzoic acid (PABA)

↓ ← Block by sulphonamides

Dihydropterioc + glutamic acid

↓

Dihydrofolic acid

↓

Tetrahydrofolic acid

↓

RNA production and bacterial reproduction

Vitamin K is essential for the control of coccidiosis. Vitamin K is originally discovered in 1935 to be an essential factor in the prevention of haemorrhagic symptoms produced in chicks. The discovery was made by a group of Danish Scientist who gave the name ‘Koagulation Factor’ to the vitamin, which become shortened to the K factor and eventually to vitamin K.

A number of chemical components are known to have vitamin K activity. The most important naturally occurring compound is vitamin K, (phylloquinone) which is chemically 2-methyl-3-phytyl-1, 4-napthoquinone. bacteria contain a wide range of molecular forms of the vitamin K_2 (multiprenylmenaquinones) in which is the n designates the number of isoprene units in the side chain.

Vitamin K_3 (menapthone) is synthetic compound which is about 3.3 time as potent, biologically, or the natural occurring vitamin K_2. Vitamin K_3 is more helpful for the blood clotting.

Orton and Hambly (1971) reported that combination of sulfadimethoxine and oretroprin as 0.02% of the ration successfully controlled mortality due to coccidiosis, and caused an increased body weight gain and improved feed conversion rate.

Biely (1973) reported an improved feed efficiency, growth rate and reduced mortality in birds when monensin was used in feed @ 110 gm/ton of feed.

Mitrovic and Schildknecht (1973) found that administration of sulfadimethoxine @ 0.05% was found equally effective compared with other anticoccidial in maintaining weight gain, feed conversion, preventing mortality and reducing gross pathological lesions due to coccidiosis.

Kennett *et al.* (1974) reported that robenidine had broad-spectrum anticoccidial activity and afforded complete protection when given in feed @30 gm/ton. There was no body weight gain and feed conversion at the time of using robenidine.

Yvore *et al*. (1974) reported that stenorol (@ 3ppm.) afforded a complete protection against *E. tenella, E. acervulina, E. maxima* and *E. necatrix* when given in the feed

Bajwa and Gill (1977) found amprolium more effective compared with decoquinate against low-level infection with *Eimeria tenella.* Decoquinate when given 24 hours before or after infection completely inhibited the infection, and the chicks remained susceptible to subsequent infection. The authors concluded that amprolium is more suitable for replacement flocks and decoquinate for broilers.

Greie and Raether (1979) studied the efficacy of halofuginone as coccidiostats and found that the medicated birds produced only a few oocysts in their faeces, and had more body weight gain compared with non medicated birds.

Manuel *et al.* (1997) reported a comparative efficacy of the salinomycin, monensin and clopidol in broiler raised on litter. Birds receiving salinornycin @ 60 ppm had the highest weight gain followed by birds receiving 125 ppm clopidol and then 100 ppm monensin. Feed consumption was more in salinomycin treated birds than the other two anticoccidials. Birds treated with clop idol had the lowest oocysts count followed respectively by monensin and salinomycin. The authors concluded that the three anticoccidials were equally effective against caecal coccidiosis.

Tuller and Modder (1979) reported an increase body weight, feed utilization and feed intake in birds on salinomycin.

Hera *et al.* (1981) studied the efficacy of different anticoccidials in pullets and broilers. Lasalocid @ 75 gm/kg, monensin @ 100 mg/ kg, buqoquinate @ 125 gm/ kg feed, and amprolium and ethopabate @ 500 mg/ kg for *5* days, 250 mg/ kg for 14 days and 125 mg/kg for the rest of the period were used for coccidiosis control. Lasalocid and monensin were found equally effective in pullet. In broiler lasalocid was found to be the best drug compared with all other anticoccidials used.

Haberkornt *et al.* (1986) studied the efficacy of toltrazuril and monensin as coccidiostats. The authors found toltrazuril @ 25 ppm in drinking water for 2 days/week produced the best result. Monensin @ l25ppm feed also gave a good result. However, toltrazuril was found superior in gaining body weight.

Chroustova and Pinca (1987) examined the performances in three large farms used different methods for coccidiosis control, Clinical coccidiosis with approximately 4% mortality was recorded in farms rearing chicks on permanent bedding using 0.05% amproliurn. In farms using lasalocid and monensin only sporadic occurrence of coccidiosis was noticed in chicks reared on wood shavings. In cage rearing birds using lasalocid no clinical coccidiosis was seen.

Kutzer (1987) reported that one of the major problems in coccidiosis control is the development of resistance of *Eimeria* spp. to certain anticoccidial.

Perez *et al.* (1987) compared the efficacy of halofuginone @ 3 ppm and zoalene @ 125 ppm in controlling coccidiosis in broiler chicken strain S47, and found a lower mortality rate (2.49%) in halofuginone treated birds compared with zoalene treated birds in which the mortality rate was 3.6%. Halofuginone was also found superior in overall performances including live weight gain and suppression of coccidiosis.

Kutzer *et al.* (1988) reported that diclazuril @ 0.5, 1 and 2 mg/kg feed were more effective in controlling coccidiosis in broilers compared with narasin @75mg/kg, and maduramicin @ 5 mg/kg feed. The authors did not found any oocyst or any lesion due to coccidiosis 6 days after withdrawal of diclazuril from feed.

Taschirch (1988) observed that the use of maduramicin in feed @ *5* ppm had on adverse effect, and was 12-15 times more effective than other ionophorous compound against *E. tendla, E. acervulina, E. maxima* and *E. mivati.* The author also reported an increase feed intake, feed utilization and body weight gain, and have lowest index of pathological changes and oocysts production.

Bunzoubaa *et al.* (1989) found coccidiosis as one of the major diseases in poultry industry in morocco based on a survey in different poultry farms over a period of 11 years. The authors suggested putting more emphasis on hygiene, good housing and vaccination over drug particularly antibiotics.

Georgiev and Vladimirova (1989) found good efficacy of maduramicin as an anticoccidial agent in broiler chicken when used @ 0.5 kg/ ton of feed.

Lee and Yvore (1989) compared the performance of vaccination against chemoprophylaxis for controlling chicken coccidiosis, and observed a better survival rate and better weight gain in birds vaccinated with Immucox® then birds receiving halofuginone in feed.

Salisch *et al.* (1989) found that the use of diclazuril @ 0.5, 1 and 2 mg/kg were highly effective against *E. tenella* and *E. acervulina* in broilers. The author also noticed a complete suppression of lesion due to coccidiosis and oocyst production after feeding diclazuril mixed feed.

Karim and Trees (1990) reported coccidiosis as a major disease in poultry operation in Bangladesh, and identified *E. tenella, E. acervulina, E. necatrix, E. maxima* and *E. brunetti.* The authors reported coccidiosis outbreak in 7 of 16 farms under investigation, and observed that the much-practiced tactical use of sulphonamides and hygiene fails to control coccidiosis.

Greuel *et al.* (1992) studied the efficacy of diclazuril, maduramicin and halofuginone in coccidiosis control, and observed better result with diclazuril and halofuginone then maduramicin. Birds receiving diclazuril had on intestinal lesion, and had the highest weight gain.

Busch *et al.* (1993) reported that maduramicin @ 5mg/kg teed successfully controlled coccidiosis in turkey and found the drug as the safest one for turkey,

Karim *et al.* (1994) reported that *E. acervulina* was the most common species followed by *E. tenella* in chicken in Bangladesh. The authors also noticed that *E. acervulina* occurs early where as *E. acervulina* never occurs before 2 weeks of age. The authors also described an easy method of lesion scoring of naturally infected chicken.

Karim and Begum (1994) identified *E. praecox* and *E. mitis* in addition to the 5 species identified earlier in chicken in Bangladesh. The authors highlighted the usefulness of oocysts dimensions in conjunction with the prepatent period and site of infection for identification of chicken *Eimeria spp.*

Shukia *et al.* (1995) reported successful control of coccidiosis in pullets by using Duocoxin® (arnprolium and sulphaquinoxaline) in drinking water @ 1 gm/liter without any adverse effect.

Chapman (1997a) found that the use of Coccivac-B® in broiler had a good result against certain Eirneria spp. which are resistant to ionophore antibiotic such as monensin.

Alam *et al.* (2000) compared control of chicken coccidiosis by immunization with stored live oocysts with that of routine chemoprophylaxis. The authors found that the immunized chicks had a significantly higher weight gain compared with control groups, but lower when compared with the groups received chemoprophylaxis. The authors concluded that the apparently lower weight gain in immunized chicks compared with the chemoprophylactic group. was due to the early depression in weight gain caused by the vaccinating dose.

Cernik and Bedrnik (2000) found that continuous or long time treatment with monensin or salinomycin can induce the development of drug resistance against coccidiosis, where as immunization with live attenuated vaccine gave a good result.

Hooge *et al.* (2000) studied the effects of ionophore coccidiostat (monensin or salinomycin), sodium or potassium bicarbonate or both and bactracin methylene disalicylate in broiler chicken. The authors observed an improved body weight gain, feed conversion ratio and reduced mortality if sodium or potassium bicarbonate were added to feed containing monensin or salinomycin.

Hadorn *et al.* (2001) compared the efficacy of Paracox® with dietary coccidiostat on the development and performance of growing pullets and laying hens. The authors observed a minor negative effect on weight gain, feed consumption, weight un-uniformity or mortality. Egg production was slightly decreased in laying hens due to vaccination. The vaccinated birds had a slightly higher feather picking habits. The authors concluded that vaccination with Paracox probably had no additional positive effects on development, performance and mortality rates of growing pullets and laying hens.

Natour *et al.* (2002) performed an experiment entitled "Flock level prevalence of *Eimeria* species among broiler chicks in Northern Jordan." in this experiment six chicks (3-6 weeks of age) were taken randomly, from each of 200broiler farm in Northern Jordan, and submitted for post-mortem and parasitological examination. Seven Eimeria species were identified *Eimeria necatrix, E. acervulina, E. tenella, E. brunetti, E. maxima, E. mivati,* and *E. mitis.* About 5o% of the farm surveyed had all six chicks infected, 23% of the farm were free of the infection. *E. tenella* was most prevalent species (10%).

Rahimi *et al.* (2002) conducted an experiment to determine the effect of betaine on the number of oocysts excreted in the faeces of coccidiosis infected broiler and its effect on feed intake, feed conversion ratio and body weight gain. The chicks were divided into eight groups of fifteen birds is the effected of dietary betaine at levels 0 and 66 ppm were studied. The experiment result showed that betaine increased feed intake and body weight gain significantly ($p<0.05$) in coccidia infected birds. The result indicated that combination of betaine with salinomycin had better effect ($p<0.05$) on broiler performance as compared with betaine or salinomycin alone. It is concluded that betaine increase feed intake and body weight gain coccidiosis infected broilers, but it did not affected the performance of healthy birds.

Fetterer *el al.* (2003) conducted *an* experiment on the effect of dietary betainc on intestine and plasma level of betaine in uninfected and coccidia infected broiler chick. Following experiment, they found that chicks fed betaine supplemented diets and infected with *Eimeria acervulina* and *Eimeria maxima* had markedly higher levels of betaine in the duodenum and mid-gut than unsupplement infected chicks. Uninfected chicks fed betaine exhibited almost twice the levels where lower in *E.* maxima-infected chicks than in *E. acervulina* or *E. tenella* infected chicks. Coccidia infected diet in normal diets exhibited increased in plasma NO_2 and NO_3.

Peek and Landman (2003) worked on resistance to anticoccidial drugs of Dutch *Eimeria* spp. field isolates originating from 1996, 1999 and 2001. Fifteen *Eimeria* spp. field isolate sampled on Dutch broiler farm were subjected to anticoccidial sensitivity test (AST) in a battery cage study. Four isolates from 1996, another four from 1999 and the last seven from 2001. The selected anticoccidial drugs were monensin, narasin, salinomycin, lasalocid, nicarbazin, diclazuril, halofuginone, maduramicin and meticlorpindol. *Elmeria acervulina* present in each of the four 1996 field isolates showed resistance for almost all products tested except maduramicin (1/4) and salinornycin (1/4), which appear to be reduced sensitive. In 1999, the same species presented in a similar resistance pattern for most products. In the year 2000 increased sensitivity to various products was found. The *Eimeria maximum* from 1996 was resistant to all products except naracin (sensitive) and halofuginone reduced sensitive.

Naphade *et al.* (2010) studied the efficacy of homoeopathic medicine (Mercurius Corrosivus) against experimental caecal coccidiosis in broiler chicks.The infection was caused by giving the dose of 50,000 sporulated oocyst of E. tenella. It was observed in transverse section of caeca, that the gross pathological changes observed in an infected untreated control group were paleness of the mucosal membrane, ballooning of the caeca with clotted and non clotted blood. Caecal walls also showed thickening with hemorrhages. However, less severe changes were observed in the groups treated with Amprolium and Mercurius Corrosivus. The studies on the histopathology of intestinal caeca were also undertaken in different groups of birds (treated and untreated). In the infected untreated control group the intestinal mucosa was hypertrophied containing schizonts and gametocytic stages of coccidial pathogen.There was desquamation of intestinal mucosa and denudation of intestinal villi cells, where as the other groups showed less severe histopathological changes.The drug was found to be effective as a curative remedy against experimental caecal coccidiosis.

Hirani *et al*. (2011) studied epidemiological surveillance based on examination of litter material from 318 farms and faecal droppings of 1911 birds which revealed the overall prevalence of coccidian as 20.44 and 17.27 percent, respectively. Prevalence was higher in layers than the broilers and under deep litter system than the cage housing system among layers.

Amare *et al.* (2012) conducted a study to evaluate the prevalence of coccidiosis, to identify the prevalent species of Eimeria and to assess the predisposing factors. Overall prevalence 25.24% (95 % CI: 22.28 -28.20) of clinical coccidiosis was assessed. Prevalence rates of 22.3% (95 % CI: 19.20 -25.65) and 35.3% (95 % CI: 28.47-42.05) was recorded in White leghorn (WLH) grower chickens and in parent stocks of WLH and Rhode Island Red (RIR) breeds, respectively. The prevalence of clinical coccidiosis was significantly higher in adults than the growers (P <

0.05). However, no statistically significant difference was observed between sexes and breeds. *E. tenella, E. brunetti, E. necatrix, E. acervulina* and *E. maxima* were identified. *E. maxima* were found for the first time in the farm. Coccidiosis prevalence has decreased among growers, but is reciprocal in the adults alarming the need for control and prevention measures in the parent stocks.

Awais *et al.* (2012) reported the prevalence of coccidiosis in industrial broiler chickens in Faisalabad, Punjab, Pakistan to determine the occurrence of different species of Eimeriain the area and to assess their correlation with the environmental conditions including temperature, humidity and rainfall. The highest prevalence (27.04%) was recorded of *Eimeria tenella* followed by *Eimeria maxima* (22.42%), *Eimeria acervulina* (19.89%) and *Eimeria necatrix* (4.02%). The prevalence of disease was significantly higher ($P < 0.05$) in autumn (60.02 ± 4.38) followed by summer (47.42 ± 2.92), spring (36.92 ± 2.27) and winter (29.89 ±3.43).

CHAPTER III

MATERIALS AND METHODS

The study on the comparative efficacy of four locally used sulfonamides on controlling of broiler coccidiosis was performed in the Department of Pharmacology and Toxicology, Sylhet Agricultural University, Sylhet. The field survey on the present status of coccidiosis was conducted by asking carefully designed questionnaire to the farmers of 15 selected farms located in different parts of Bogra district. The study period was January to March 2013.

3.1 Study-1: Prevalance of broiler coccidiosis in different farm

3.1.1 Field level survey

Fifteen selected commercial poultry farms were visited in different areas of Bogra district. Several questions were asked to the farmers. The questionnaires used for the survey includes following information:

- (i) Farm with location
- (ii) Owner with address
- (iii) Type of farm
- (iv) Flocks size
- (v) Prevalence of coccidiosis (during the last year)
- (vi) Any recent outbreak of coccidiosis, if any, with mortality
- (vii) Drug used for treatment of clinical coccidiosis (Any report of drug failure)

3.1.2 Selection of experimental farms

The experimental farms were randomly selected. List of the selected farm are given below in the Table 1.

Table 1. Name of the experimental farms with quantity of chicks and type of breed

Sl. No.	Name of farm	No. of chicks	Breed
1.	Mithu poultry farm	600	Cobb-500
2.	Saju poultry farm	500	Cobb-500
3.	Alamin poultry farm	800	Ross -308
4.	Jalal poultry farm	500	Cobb-500
5.	Golam Azam poultry farm	900	Ross-308
6.	Roni poultry farm	1000	Ross -308
7.	Sowrob poultry farm	1200	Ross -308
8.	Saidur poultry farm	1400	Cobb-500
9.	Raja poultry farm	1100	Cobb-500
10..	Zulfikar Ali poultry farm	1000	Ross-308
11.	Chonchol poultry farm	700	Ross-308
12.	Solaiman poultry farm	800	Cobb-500
13..	Liton poultry farm	500	Cobb-500
14.	Sariful poultry farm	1600	Cobb-500
15.	Mokles poultry farm	1200	Ross-308

3.1.3 Management of coccidiosis

During the visit the farmers were also asked about the management practices which they are adopting. These included information on cleaning and disinfection, type and depth of litter, litter management, use of drugs against coccidiosis.

3.2 Study-2: Comparative efficacy of sulfonamide drugs against broiler coccidiosis

3.2.1Experimental chick

Sixty, day old chicks "Cobb-500" were collected from CP Bangladesh Co. Ltd. (Bogra hatchery).

3.2.2 Experimental design

Sixty, day old Cobb-500 broiler chicks were collected from a private commercial hatchery. At the 1st day of life, all the chicks (60) were randomly divided into 6 equal groups T_o, T_i, T_1, T_2, T_3 and T_4 where 10 chicks were in each group. At the 11th day of life dropping were examined to confirm that they were free from oocysts of Eimeria. Chicks of Group T_0 were kept as negative control (10 chicks) while chicks of groups T_i, T_1, T_2, T_3 and T_4 were orally inoculated with 0.5ml containing 100,000 mixed sporulated oocysts/chick as mentioned above at 12 days of age. At the 16th day of age appearance of clinical signs, chicks of Group T_1, T_2, T_3 and T_4 were treated with Esb3®-30%, Coccino®, Navacox® and Coccicure® respectively while birds of Group T_i were kept as infected positive control. All groups were kept under daily observation for signs with collection of dropping for calculations of oocysts per gram (OPG) of feces at 11, 15, 17, 21, 23, 24 and 25 days of age. Body weight and feed intake were recorded for calculation of feed conversion rate (FCR) at 7, 14, 21 and 28 days of age.

3.2.3 Feed

The feed for the experimental chicks were collected from CP Bangladesh Co. Ltd. (Rajshahi feed mill). Feed used for the present experiment is presented in Table 2 with their nutritional composition.

Table 2. Nutritional composition of feed

Name of components	HI-PRO-VITE		
	Starter-510 (01-10days)	Grower-510S (11-21days)	Finisher-511 (22-upto sell)
Moisture	12%	12%	12%
Protein	21.50%	20%	19%
Fate	3.50%	3%	3.50%
Ash	5%	5%	5%
Phosphorus	0.9-1%	0.9-1.05%	0.9-1%
Calcium	0.7-0.9%	0.7-0.9%	0.7-0.9%
Salt	0.3-0.4%	0.3-0.4%	0.35-0.40%
Energy (K/Cal)	3000	3000	3100

Source: CP Bangladesh co. Ltd.

3.2.4 Litter

Rice husk were used as litter in the floor pens at a depth of 3.5 cm.

3.2.5 Brooding

The chicks were provided with temperature (95^0F) during the first week and then gradually decreased at the rate of 5^0F per week up to 4 weeks of age. After one week chick guard was removed. Temperature was maintained by adjusting the height of the heat source, i.e, electric bulb used for heating.

3.2.6 Feeding and drinking

The chicks were given feed thrice a day in the morning, afternoon and evening and water *adlibitum.* During the first week, feed was supplied on fresh news paper and water was supplied in the drinker in each room. At the beginning of second week feed was supplied in feeder. Feeder and drinker were cleaned at every alternate day.

3.2.7 Immunization

All the birds were vaccinated against Infectious Bronchitis & Ranikhet Disease by Avipro ND-IB-HB1(Lohmann animal health) Vaccine following the instruction of vaccine manufacturer. One ampoule of Avipro ND-IB-HB1 vaccine was diluted with 15 ml of supplied solution and the birds were vaccinated at 5th day followed by a booster dose at 21th day with Avipro ND-lasota(Lohmann animal health) by intraoccular method. The birds were also vaccinated against Gumboro disease by Bursine-2(Novartis) following the instructions of the manufacturer. One ampoule of Bursine-2 was diluted with 15 ml of supplied solution and the birds were vaccinated at the age of 11th day-old, followed by a booster dose at 18th day with bursine plus (Novertis) by intra ocular method. Vaccines were collected from local market.

Table 3: Vaccination Schedule.

SI. No.	Name of the vaccine	Manufacturer	Age of the bird	Dose of diluted vaccine	Route of administration
1	Avipro ND-IB-HB1	Lohmann Animal Health	5^{th} day	One drop in one eye	Eye drop
2	Bursine-2	Novartis	11^{th} day	One drop in one eye	Eye drop
3	Bursine plus	Novartis	18^{th} day	One drop in one eye	Eye drop
4	Avipro ND lasota	Lohmann Animal Health	21^{th} day	One drop in one eye	Eye drop

3.2.8 Eimerial oocysts

Sporulated oocysts were isolated from field cases and purified. Collected oocysts were sporulated and passed in susceptible chicks in 3 times. Virulence of the 3^{rd} passage sporulated oocysts was tested according to Walelzky (1970) & McDougald *et al*. (1994). Sporulated oocysts were kept in 2.5% potassium dichromate in screw capped bottles at 4-8^{0}C till used for challenge test.

3.2.9 Experimental infection

Each bird of infected and medicated groups was inoculated orally with 0.5ml solution containing 100,000 mixed species of sporulated oocysts of Eimeria on the 12^{th} days of age.

3.2.10 Oocyst per gram

Oocyst per gram (OPG) of feces was measured at 11, 15, 17, 21, 23, 24 and 25 days of age using McMaster Counting chamber.

3.2.11 Collection of litter sample

Litter samples were collected 11,15,17,21,23,24 and 25day of the experimental period. The samples were collected in properly labeled polythene bags, and were brought to the Bogra district veterinary hospital laboratory.

3.2.12 Preparation of litter sample for oocysts count

Five litter sample collected from each of the six groups was put into their separate bottles filled with water and left at the refrigerator for overnight. Next morning, the bottles were shacked vigorously to dislodge the oocysts from litter cake where applicable.

3.2.13 Use of drugs against coccidiosis

The following drugs were use as recommended by the manufacturer.

- Esb3®-30%- Sulfaclozine sodium monohydrate soluble powder produces by Novertis Animal health. It was given as 2.5gm/liter of the drinking water for 5 successive days.
- Coccino®- Sulfaclozine sodium monohydrate, vitamin K3 enriched soluble powder produce by Eskayef Banglabesh Ltd. Agrovet division. It was given as 2.5gm/liter of the drinking water for 5 successive days.
- Navacox®- Sulfaclozine sodium monohydrate soluble powder produce by Navana Animal health. It was given as 2.5gm/liter of the drinking water for 5 successive days.
- Coccicure®- Sufaclozine sodium monohydrate soluble powder produce by Ranata Animal health. It was given as 2.5gm/liter of the drinking water for 5 successive days.

3.2.13 Counting of litter oocysts

The sample from bottle was sieved by using a 150 µm sieve to remove the large particles. Then the filtrate was centrifuged at 1100rpm for 5 minutes. The supernatant was discarded. The sediment was resuspended in saturated salt solution and made up 50ml in measuring cylinder. The suspension was mixed thoroughly by gently inverting the cylinder 15-20 times. Adequate amount of suspension was taken by plastic Pasteur pipette as quickly as possible and two chambers of the McMaster counting slide were filled with the suspension. The slide was left for 3-5 minutes to allow the oocysts to float. The numbers of oocysts in two chambers were counted by using the x6 eyepiece and xl0 objective of a compound microscope. The number of oocysts per gram of litter was calculated by dividing the number by 0.3 and multiplied by the dilution factor. The number of oocyst per gram of litter (OPG) was calculated by using the following formula:

$$\text{Number in one gram} = \frac{\text{Number of oocyst in two chambers}}{0.3} \times \text{dilution factor}$$

$$* \text{ Dilution factor} = \frac{\text{Total volume of suspension in ml}}{\text{Total volume of feces}}$$

3.3 Statistical analysis

The replicated data for different traits (body weight, feed conversion rate, oocyst per gram of feces) were used to analyze using statistical approaches. Descriptive statistics, analyses of variances (ANOVA), multiple comparisons were determined using statistical software MINITAB version 14.0 (Anon, 1996). Differences between means were considered the significant level at p<0.05.

CHAPTER IV

RESULTS

The present research work was performed to know the present status of broiler coccidiosis in Bogra district and its control measure. To know the prevalence of broiler coccidiosis, fifteen farms were selected and observed every day. During the observation period, most of the farms were found coccidian affected at different age of birds. The severity of the disease was observed and these are sown in the Table 4.

4.1 Clinical signs

During observation, the birds showed signs of reduced feed intake, drowsiness and bloody faces as the common signs.

During the experimental period, out of 15 farms, 12 farms were found affected with coccidiosis. The degree of severity of the disease was low in first day of affection and after one or two days it was more severe than first day of commencement of coccidiosis.

4.2 Prevalence of coccidiosis in the selected farms

The experiment was conducted in fifteen farms. Among these farms coccidial infection was not found in three farms only; that is, coccidiosis was prevalent in the rest of the twelve farms (80%).

Table 4. Severity of coccidiosis in selected farms

Sl. No.	Name of farm	No. of chicks	Time of occurrence (age)	Severity after affected with coccidiosis			
				1st day	2nd day	3rd day	4th day
1.	Mithu poultry farm	600	12th day	+	++	+++	++
2.	Saju poultry farm	500	14th day	+	++	+++	++
3.	Alamin poultry farm	800	16th day	+	+++	++++	++
4.	Jalal poultry farm	500	20th day	+	++	+++	++
5.	Golam Azam poultry farm	900	–	–	–	–	–
6.	Roni poultry farm	1000	15th day	+	++	+++	++
7.	Sowrob poultry farm	1200	18th day	+	++	+++	++
8.	Saidur poultry farm	1400	19th day	+	++	++++	++
9.	Raja poultry farm	1100	–	–	–	–	–
10.	Zulfikar Ali poultry farm	1000	17th day	+	++	+++	++
11.	Chonchol poultry farm	700	15th day	+	++	+++	++
12.	Solaiman poultry farm	800	–	–	–	–	–
13.	Liton poultry farm	500	20th day	+	+++	++++	++
14.	Sariful poultry farm	1600	23th day	+	++	+++	++
15.	Mokles poultry farm	1200	16th day	+	++	+++	++

++++ = Sever infection, +++ = Moderate, ++ = Mild

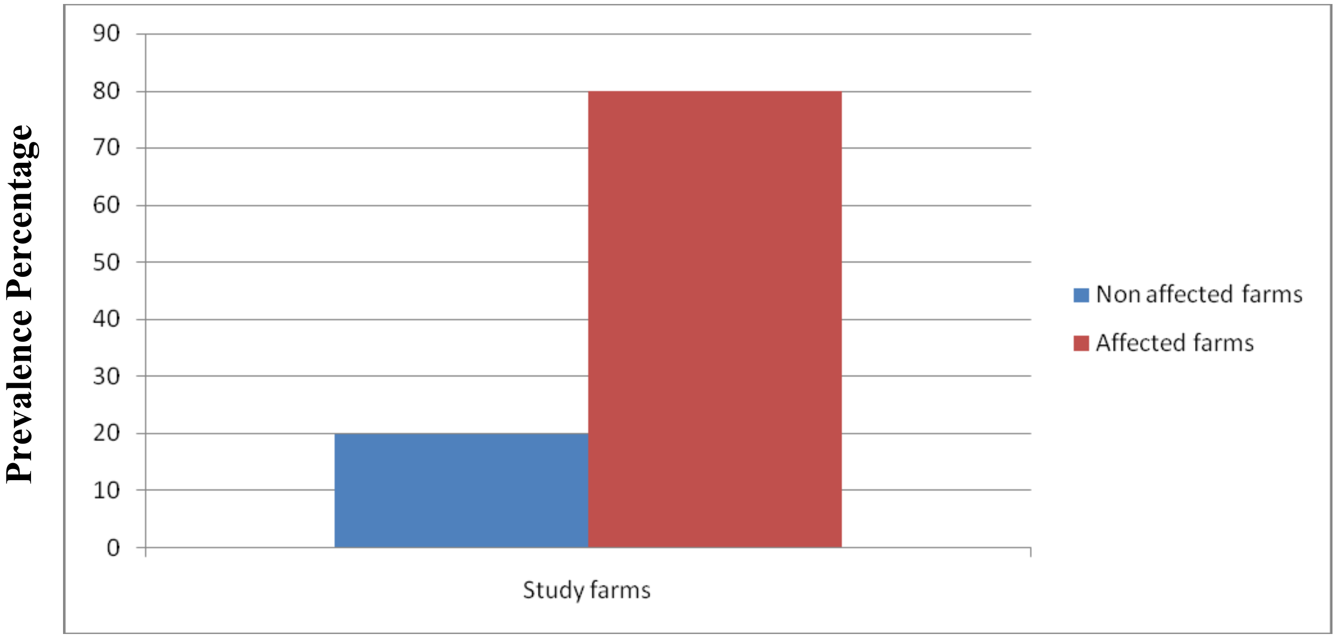

Figure 1: Prevalence of coccidiosis affected farm.

Comparative efficacy of sulfonamides against broiler coccidiosis was conducted to evaluate the comparative growth performance of broiler on the basis of mortality rate, body weight gain, feed conversion ratio and number of OPG shedding.

In this study six group were kept under daily observation. During this observation the infected birds showed general signs of reduced feed intake, drowsiness, ruffled feather, huddling together and bloody faces as the common signs on the 4^{th} dpi.

4.3 Effect on the Mortality:

Mortality rates of treated and controlled group of broiler chicks in case of coccidiosis treated with different sulfonamides were shown in Table 5 and graphical analysis also showed the similar result (Figure 2).

Table 5. Effect of administration of different sulfonamides on mortality% of broiler chickens in experimental coccidiosis

Treatment n=10	**Mortality (%)**			
	After 7 days	After 14 days	After 21 days	After 28 days
T_0 (Negative control)	0	0	0	0
T_i (Positive control)	0	0	10%	20%
T_1 (Esb3®ab-30%)	0	0	0	0
T_2 (Coccino®)	0	0	0	0
T_3 (Navacox®)	0	0	0	0
T_4 (Coccicure®)	0	0	0	0

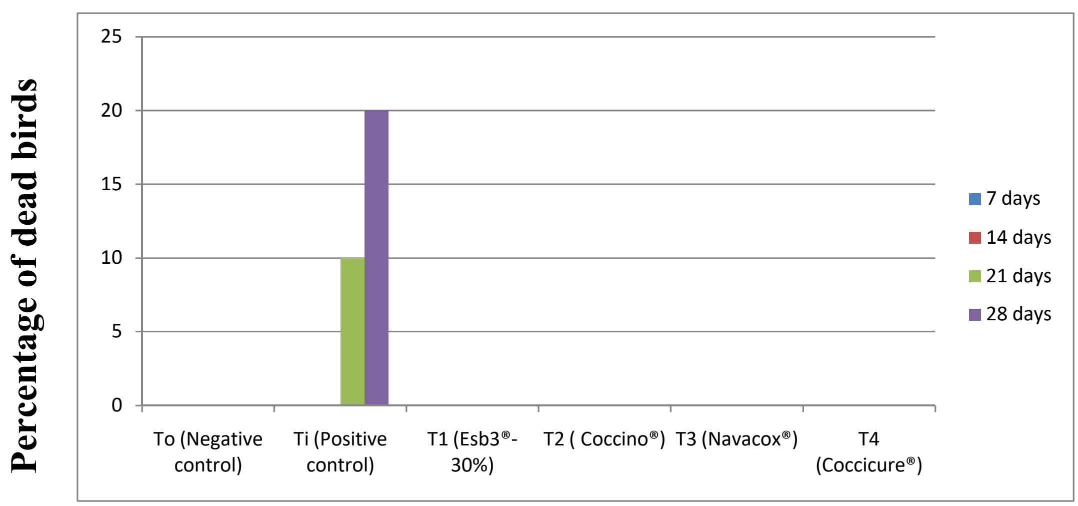

Figure 2: Effect of different sulfonamides on mortality % of broiler chicks

In the negative control group T_0 and treated group $T_{1,}$ $T_{2,}$ T_3 and T_4 didn't show any mortality (zero) during 28 days of rearing period but infected positive control group T_i had 10 % mortality after 21 days that was one week after infection and 20 % mortality after 28 days that was 2 weeks after infection.

4.4 Effect on the Body weight

The body weight of birds in uninfected untreated T_0, infected untreated T_i and treated groups $T_{1,}$ $T_{2,}$ T_3 and T_4 on 0 day, 7 days, 14 days, 21 day and 28 days presented in Table 6.

Weights of six groups were not significant difference before infection (0 days and 7 days). After 2 days experimental infection (14 days in rearing period) body weight were not significantly different.

At the 21 days of experiment it was 9 days after infection and 3 days after treatment the mean body weight was statistically significant at ($p<0.05$) level of probability. After infection, weight of positive control T_i was significantly lower than negative control T_0 and also $T_{1,}$ $T_{2,}$ T_3 and T_4 at $p<0.05$ (Table 6). In negative control group T_0 recorded 794.3±24.7, positive control group Ti was recorded 673.7±29.3 and treated group T_2 was recorded 842.7±20.3. The highest mean body weight was recorded in the treated group T_2 (Table 6). Graphical analysis also showed the similar result (Figure 3).

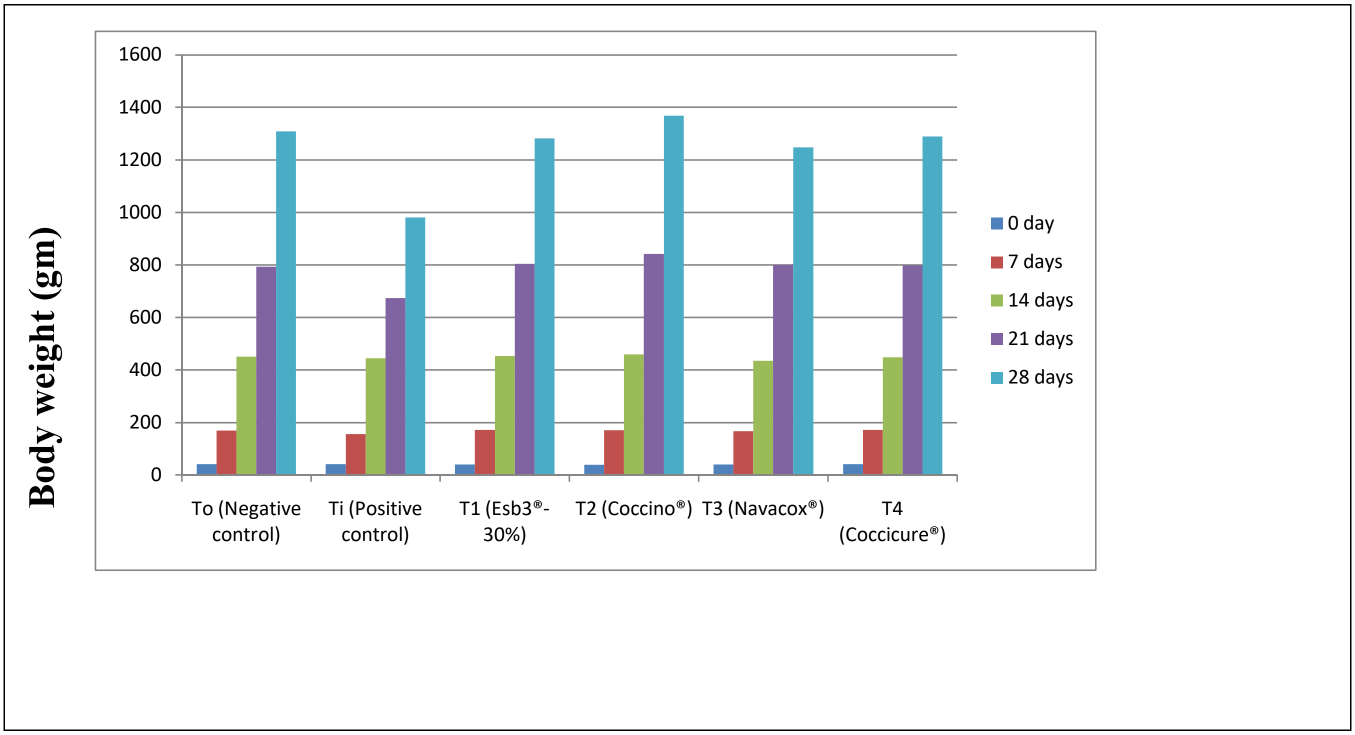

Figure 3: Effect of different sulfonamides on body weight of broiler chickens in experimental coccidiosis.

Finally, 28 days of age of experiment the mean body weight in negative control group T_0 recorded 1308.0±17.8, positive control group T_i was less increases 25.02% (980.7±20.5) compare to T_0 group and compare to T_i group, treated group T_1 was increases 30.62% (1281.0±15.9), treated group T_2 was increases 39.56% (1368.7±17.7), treated group T_3 was increases 27.22% (1247.72±10.5) and treated group T_4 was increases 31.43% (1289.0±23.2). Body weight gradually increased in all sulfonamide treated group and positive control group T_0 recorded lowest body weight but the highest body weight was recorded in the treated groupT_2 (Table 6). The average body weight of all treated group were highly significant ($p<0.05$) than the positive control group.

Data categorized on 0 day, 7 days, 14 days, 21 days and 28 days of age shows that before experimental coccidiosis body weight increased in all group relatively are not statistically significant difference. But after coccidiosis infection occurred and treated with four sulfonamides body weight were increase more in treated group then positive control group. Within four sulfonamides T_2 groups showed significantly higher body weight among the treated groups.

4.5 Effect on the Feed conversion ratio

Feed conversion rate on 7 days, 14 days, 21 days and 28 days of different groups of birds is shown in Table 7. Mean feed conversion ratio at 7 days was significantly difference at ($p<0.05$). Feed conversion ratio at 14 days that was 2 days after infection were significantly difference at ($p<0.05$) level of probability. At 14 days the mean feed conversion ratio recorder 1.24±0.005 in T_0 negative control group, 1.22±0.005 in T_i positive control group, 1.21±0.005 in T_1 treated group, 1.23±0.005 in T_2 treated group, 1.24±0.005 in T_3 treated group and 1.22±0.005 in T_4 treated group.

Table 7. Effect of administration of different sulfonamides on FCR of broiler chickens in experimental coccidiosis.

Treatment	**Feed Conversion Ratio**			
	After 7 days	After 14 days	After 21 days	After 28 days
T_0 (Negative control)	0.96^{d}±0.005	1.24^{a}±0.005	1.47^{c}±0.005	1.63^{d}±0.005
T_i (Positive control)	0.98^{c}±0.005	1.22^{ab}±0.005	1.84^{a}±0.005	1.92^{a}±0.005
T_1 (Esb3®-30%)	0.97^{c}±0.005	1.21^{b}±0.005	1.49^{b}±0.005	1.65^{c}±0.005
T_2 (Coccino®)	0.96^{d} ±0.005	1.2^{a}±0.005	1.45^{c}±0.005	1.61^{e}±0.005
T_3 (Navacox®)	1.02^{a}±0.005	1.24^{a}±0.005	1.51^{b}±0.005	1.68^{b}±0.005
T_4 (Coccicure®)	0.99^{b}±0.008	1.22^{ab}±0.005	1.48^{c}±0.005	1.65^{c}±0.005

Figures with same superscript or without superscript do not differ significantly whereas figures with dissimilar superscript differ significantly. Data were calculated at 5% level of significant ($p<0.05$).

At 21 days of age the mean feed conversion rate was significant difference at ($p<0.05$). The result in positive control group T_i was 1.84±0.005 and the treated group T_2 was 1.45±0.005. The highest mean feed conversion ratio was recorded in positive control group T_i and lowest from the treated group T_2. The best FCR was recorded in T_2 treatment group (Table 7)

Finally at 28 days of experiment the mean feed conversion ratio was significant different at ($p<0.05$). The result in positive control group T_i was recorded 1.92±0.005, T_0 negative control group recorded 1.63±0.005 and in the treated group T_2 was 1.61±0.005. The highest mean feed conversion ratio was recorded in treated group T_i and lowest from the treated group T_2. The best FCR was recorded in T_2 treatment group (Table 7). Graphical analysis also showed the similar result (Figure 4).

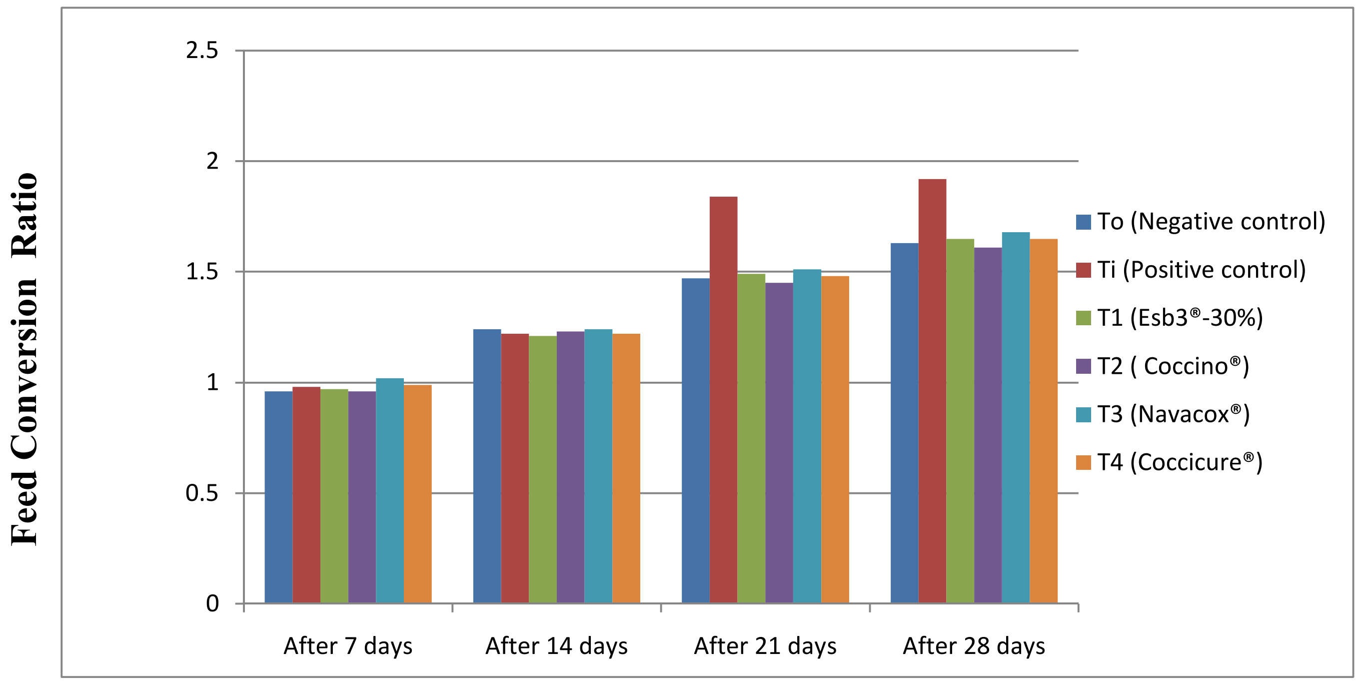

Figure 4: Effect of sulfonamides on FCR of broiler chicks in Experimental coccidiosis

It can be interpreted that because the positive control group had lower body weight and lower feed intake (data wasn't shown) than the other groups. FCR was the best in the treated group T_2.

4.6 Effect on the frequency of OPG shedding:

Frequency of OPG shedding during 1 day before infection and 3 and 5 days after infection and 3, 5, 6 and 7 days after treatment in 6 groups were shown in Table 8.

First day before infection number of mean OPG shedding was zero in all treatment group. OPG in negative control group T_0 remained zero during rearing period.
After 3 days post infection mean OPG shedding was significantly different among the treated groups. In treated group T_i was 51667±841, treated group T_1 was 46333±612, treated group T_2 was 55833±780, treated group T_3 was 52267±769 and treated group T_4 was 46667±953.

After 4dpi clinical sign of coccidiosis appear and after 5dpi mean OPG shedding was significantly different among the treated groups. In treated group T_i was recorded 94500±551, treated group T_1 was 101033±176, treated group T_2 was 92667±899, treated group T_3 was 95500±458 and treated group T_4 was 87167±145.

After 3 days post treatment mean OPG shedding in positive control group T_i was recorded highest 173167±145, treated group T_1 was 1220±208, treated group T_2 was 9300±321, treated group T_3 was 14200±208 and treated group T_4 was recorded 11000±153. Within treated group, T_2 group showing comparatively less OPG shedding at 3 days post treatment. Group T_i have significant difference with all other treated group. Within T_1, T_2 and T_4 groups have no significant different but T_2 group was significantly different at ($p<0.05$) with other treated groups.

After 5 days post treatment mean OPG shedding was significantly different among the treated groups. In positive control group T_i was recorded highest OPG shedding 105100±173, treated group T_1 was 4200±265, treated group T_2 was 3500±208, treated group T_3 was 6300±153 and treated group T_4 was 5500±173. Within treated group, T_2 group showing comparatively less OPG shedding at 5 days post treatment. Graphical analysis also showed the similar result (Figure 5).

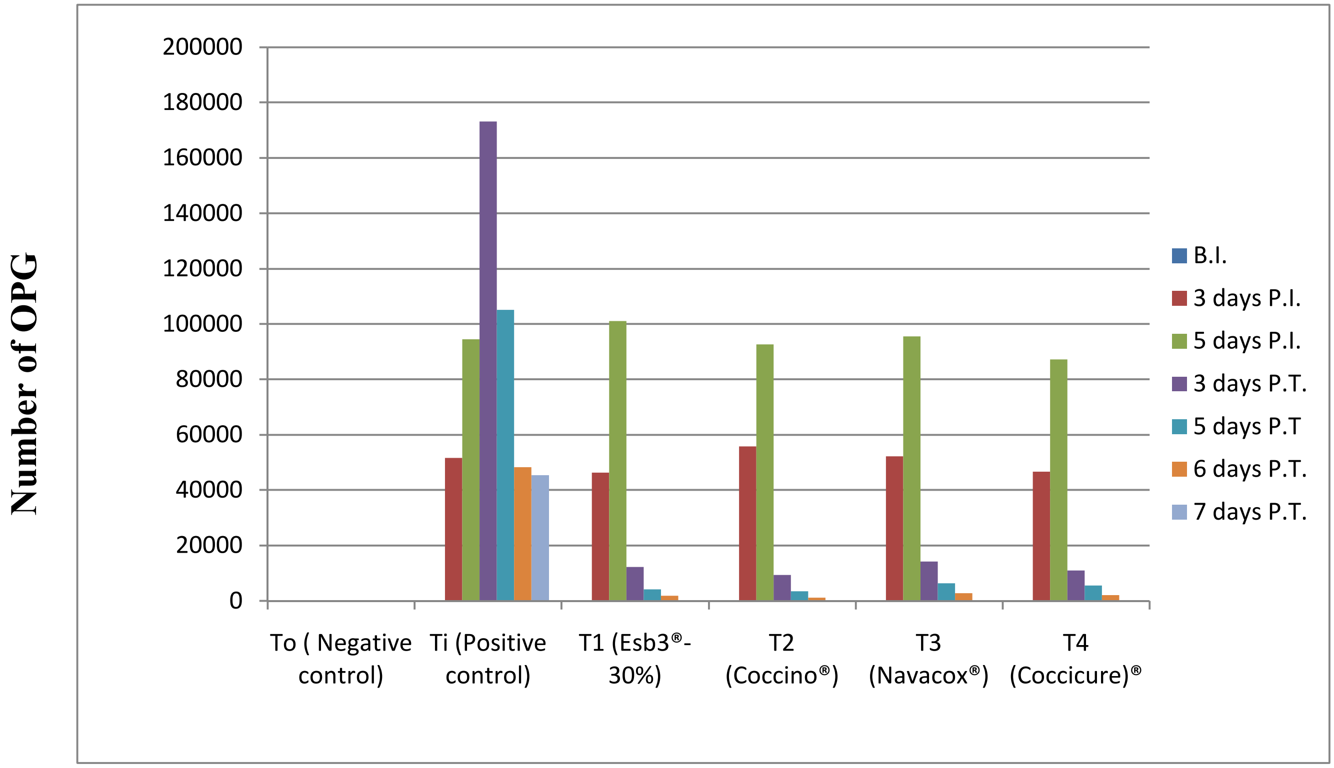

Figure 5: Number of OPG shedding before and after treatment with different sulfonamides in broiler coccidiosis.

After 6 days of post treatment mean OPG shedding was significantly different among the treated groups. In positive control group T_i was recorded highest OPG shedding 48300±115, treated group T_1 was 1800±115, treated group T_2 was 1200±115, treated group T_3 was 2766.7±88.2and treated group T_4 was 2133.3±88.2. Within treated group, T_2 group showing comparatively less OPG shedding at 6 days of post treatment.

After 7 days post treatment mean OPG shedding was significantly different at among the treated group. OPG shedding were decreased in T_i group due to increased body immunity against coccidiosis and OPG shedding was remained zero in all sulfonamides treated groups T_1, T_2, T_3 and T_4. In positive control group T_i was highly significant different with other treated groups and recorded highest OPG shedding 45500±115 (Table 8).

Figure 6. Coccidia affected chicks showing drowsiness, weakness and bloody faeces.

Figure 7. Faeces with blood tint.

Figure 8. Necropsy finding of a coccidia affected bird showing swollen cecum filled with caseous mass.

Figure 9. Necropsy finding of a coccidia affected bird showing severe intestinal hemorrhage.

CHAPTER V

DISCUSSION

The present research work was performed to know the present status of broiler coccidiosis in Bogra district and its control measure. To know the prevalence of broiler coccidiosis, a total of 15 farms were examined during the study period. Of which, 80% farms (12 farms) were found to be affected with coccidiosis. The prevalence was higher than the reports of previous investigations recorded by Sarker (1976), Ahamed (1977), Anjum (1990), Kamal and Hossain, (1992), Moslehuddin *et al.,* (1993), Islam & Samad (2004). They observed 12.0%, 20.0%, 15.0%, 17.0%, 23.0%, 13.5% prevalence of coccidiosis respectively in their study area. The higher prevalence of disease as recorded in the present study in semi-intensive management might be due to differences in management system, level of husbandry including more contamination of feed and water with oocysts and delayed or absence of medication at all.

Coccidiosis caused by an obligatory intracellular protozoa which, are responsible for severe injury to the poultry with an eventual heavy losses to the poultry industry. Use of anticoccidial drugs in feed is a widely practiced method for the prevention and control of chicken coccidiosis in the ever expanding poultry industry (Long, 1983). There are a number of anticoccidial drugs available for use in chicken coccidiosis. Different anticoccidial drugs differ in their mode of action in fighting against the protozoa. Most of these drugs allow a very low level of development of the parasite which however, is desired, for the development of immunity. For this reason birds which receive anticoccidial in feed passes few oocysts through their feces, but not enough to cause any remarkable injury to the birds, This is often referred to as leakage of oocysts, a phenomenon normally seen in case of ionophore (Narsapur *et al.,* 1997).

Different iodine based compounds are normally used as disinfectant in poultry houses. Since the oocysts wall is impermeable to these ordinary chemicals these can not kill the oocysts. However, because of the smaller molecular size ammonia can penetrate the oocysts wall and thus kill the oocysts (Xie *et al.,* 1983). Therefore in this study ammonia was used for fumigation of brooder, feeder and the experimental rooms, which gave a good result.

Despite the use of feed medication reporting of the reasonable percentage of outbreak of coccidiosis in the commercial poultry farms is alarming. In the present study among the selected farms 12 farms (out of 15 farms) were affected by the coccidiosis. It is very dangerous because 80% farms were reported with coccidiosis. It is very high. A number of factors might be contributing to these effects. First of all, the poor management might be the vital predisposing factor for coccidiosis in farms.

Since during the visiting times of study period of the farmers it was noticed that the farmers were quite ignorant of the possible danger of wet litter in particular. Moreover in a number of cases the houses were not properly cleaned and disinfected before introducing day-old chicks. Therefore it is likely that relatively large number of oocysts remained in the floor to initiate a primary infection that after recycling eventually produced a large number of oocysts. This means within a short time a huge number of oocysts become available in the litter to be picked up by the birds at a time when the birds remain totally susceptible. In addition, the intake of large number of oocysts helps to overcome the barrier produced by the anticoccidial drug. It may also be possible that, the anticoccidial drugs were not properly or evenly mixed with the feed, since in most case, they were hand mixed. This means the birds may be receiving a sub optimal dose of the drug.

Comparative efficacy of locally available sulfonamides performed for controlling of broiler coccidiosis. The efficacy was judged on the basis of mortality percentage, body weight, feed conversion ratio and number of oocysts shedding. Coccidiosis is one of the most relevant diseases concerning economic losses on poultry production. The world losses attributable to avian coccidiosis were estimated in 1.5 billion dollar per year (Duquette, 2005). Despite the introduction of live vaccines, in most countries chemotherapy is still the preferred method for the prevention and control of coccidiosis. Significant improvements in the performance of commercially reared poultry have been made during the last half of the twentieth century. It has often been assumed that these improvements would not have been possible without the introduction of a succession of ever more effective anticoccidial agents to control coccidiosis (Chapman, 2005). Without anticoccidial drugs and vaccines, the growth of the broiler industry would have been impossible, as coccidia are ubiquitous and extremely deleterious to the growth and survival of broilers. Fortunately, introduction of the sulfonamide drugs in the 1930 started an era of treating and preventing coccidiosis. Sulfonamides have a broad spectrum of activity against *Eimeria spp.* of the anterior and lower part of the intestine in chickens. Feeding of sulfonamides may prevent clinical signs and reduce oocyst production, decrease mortality there by allowing development of protective immunity.

Based on the results of this study, treatment by sulfonamides could improve body weight, FCR, compared to untreated group (positive control). This result are supported by Orton and Hambly (1971), who reported that combination of sulfadimethoxine and oretropin as 0.02% of the ration successfully controlled mortality due to coccidiosis and caused an increased body weight gain and improved feed conversion rate.

Mortality study indicated that a high variation among treated and non treated group. In the negative control group T_0 and treated group T_1, T_2, T_3 and T_4 didn't show any mortality (zero) during 28 days of rearing period but infected positive control group T_i had 20 % mortality after same rearing period. It indicated that administration of four sulfonamides could inhibit mortality in treated groups. These results are in agreement with Guha *et.* al. (1991), who reported that anticoccidial shows less mortality (3%) and (2%).

In case of mean body weight at 0 day, 7 days and 14 days body weight are not significantly different, at the 21 days of experiment it was 9 days after infection and 3 days after treatment the body weight were significantly different. Positive control group T_i was highly significant different compared to other groups. In negative control group T_0 recorded 794.3±24.7, positive control group Ti was recorded 673.7±29.3, T_1 was recorded 804.3±17.1, T_2 was recorded 842.7±20.3, T_3 was recorded 801.7±12.2 and T_4 was recorded 799.0±17.7. The highest body weight was recorded in the treated group T_2 due to after 3 days treated with Coccino®.

At 28 days of age of experiment the mean body weight in negative control group T_0 recorded 1308.0±17.8, positive control group T_i was decrees 25.02% (980.7±20.5), treated group T_1 was increases 30.62% (1281.0±15.9), treated group T_2 was increases 39.56% (1368.7±17.7), treated group T_3 was increases 27.22% (1247.72±10.5) and treated group T_4 was increases 31.43% (1289.0±23.2). Body weight gradually increased in all sulfonamide treated group and positive control group T_0 recorded lowest body weight but the highest body weight was recorded in the treated groupT_2.The mean body weight of all treated group were significantly different. After 5 days treated with Coccino® the T_2 groups are showing highest body weight. Using four sulfonamides all are containing Salfaclozine sodium monohydrate but Coccino® is enriched with vitamine K_3. Vitamin K is essential for the control of coccidiosis. Vitamin K is originally discovered in 1935 to be an essential factor in the prevention of haemorrhagic symptoms produced in chicks. The discovery was made by a group of Danish Scientist who gave the name 'Koagulation Factor' to the vitamin, which become shortened to the K factor and eventually to vitamin K.

A number of chemical components are known to have vitamin K activity. The most important naturally occurring compound is vitamin K, (phylloquinone) which is chemically 2-methyl-3-phytyl-1, 4-napthoquinone. Bacteria contain a wide range of molecular forms of the vitamin K_2 (multiprenylmenaquinones) in which is the designates the number of isoprene units in the side chain. Vitamin K_3 (menapthone) is synthetic compound which is about 3.3 time as potent, biologically, or the natural occurring vitamin K_2. Vitamin K_3 is more helpful for the blood clotting. Sulfonamides treatment allowed normalization of weight gains due to improvement of the integrity and function of he intestinal mucosa. Significant different in the body weight between the group of healthy, the group of infected and the group of infected with treated chickens were in line with the reported changes that occurs due to coccidiosis (Kettunen *et al.*, 2001; Nodeh *et al.,* 2008).

In case of feed conversion ratio at 7 days, 14 days, 21 days and 28 days mean FCR of different groups were significantly different. All isolates of Eimeria cause significant weight suppression and impaired FCR (Logan *et al*., 1993). The reason for the impairment is that the organism destroys the absorptive mucosal surface, competes for micro nutrients resulting into metabolic imbalance and hence adversely effects nutrient utilization. At 14 days the recorded feed conversion ratio was 1.24±0.005 in T_0 negative control group, 1.22±0.005 in T_i positive control group, 1.21±0.005 in T_1 treated group, 1.23±0.005 in T_2 treated group, 1.24±0.005 in T_3 treated group and 1.22±0.005 in T_4 treated group. At 21 days of age, the result in positive control group T_i was 1.84±0.005 and the treated group T_2 was 1.45±0.005. The highest mean feed conversion ratio was recorded in treated group T_i and lowest from the treated group T_2. The best FCR was recorded in T_2 treatment group. At 28 days of experiment the feed conversion ratio in positive control group T_i was recorded 1.92±0.005, T_0 negative control group recorded 1.63±0.005 and in the treated group T_2 was 1.61±0.005. The positive control group T_i was highly significant difference with other treated group. The highest feed conversion ratio was recorded in treated group T_i and lowest from the treated group T_2. Group T_2 shown the best FCR which were five days treated with Coccino®.

In case of mean OPG shedding at 15, 17, 21, 23, 24 and 25 days were significantly different. On day 11, first day before infection number of OPG shedding were zero in all treatment group. OPG in negative control group (T_0) remained zero during rearing period. On day 15 number of OPG shedding were statistically significant differences in all group, except between $T_{i\,\&}$ T_3 have no statistical significant difference. In treated group T_i was recorded 51667±841, treated group T_1 was 46333±612, treated group T_2 was 55833±780, treated group T_3 was 52267±769 and treated group T_4 was 46667±953. On day 17 OPG shedding treated group T_i was 94500±551, treated group T_1 was 101033±176, treated group T_2 was 92667±899, treated group T_3 was 95500±458 and treated group T_4 was 87167±145. After 3 days post treatment i.e. on day 21, mean OPG shedding was in positive control group T_i was highest 173167±145, treated group T_1 was 1220±208, treated group T_2 was 9300±321, treated group T_3 was 14200±208 and treated group T_4 was 11000±153. Within treated group, T_2 group was showing comparatively less OPG shedding at 3 days post treatment. Group T_i have significant difference with all treated group but $T_{1,}$ T_2 and T_4 have not significant difference but T_2 are significant difference with other groups. On day 23 days in positive control group T_i was recorded highest OPG shedding 105100±173, treated group T_1 was 4200±265, treated group T_2 was 3500±208, treated group T_3 was 6300±153and treated group T_4 was 5500±173. Within treated group, T_2 group showing comparatively less OPG shedding at 5 days post treatment with Coccino®. On day 24 mean OPG shedding was statistically significant and in positive control group T_i was recorded highest OPG shedding 48300±115, treated group T_1 was 1800±115, treated group T_2 was 1200±115, treated group T_3 was 2766.7±88.2and treated group T_4 was 2133.3±88.2. Positive control group T_i was highly significant different with other treated groups. Within treated group, T_2 group was showing comparatively less OPG shedding at 6 days of post treatment with Coccino®. On day 25 that were after 7 days post treatment mean OPG shedding was statistically significant different. Positive control group T_i was highly significant difference with other treated groups. Mean OPG shedding was decreased in T_i group due to increased body immunity against coccidiosis and OPG shedding remained zero in all sulfonamides treated groups T_1, T_2, T_3 and T_4. In positive control group T_i was recorded highest mean OPG shedding 45500±115. This observation supported in many investigation (Laczay *et al*., 1995, Siddiki *et al*., 2008, Ilie *et al*., 2009) the efficacy of the sulfonamide drug against the oocyst at different stage of development was compared with other anticoccidial drugs.

Despite a high rate of infection of coccidiosis by inoculation of 100,000 Eimeria oocysts, positive control group T_i high rate of mortality 10% after first week of infection and 20% after second week of infection. Treatment group T_1, T_2, T_3 and T_4 have no mortality due to treated with sulfonamide drugs Esb_3®-30%, Coccino®, Navacox®, Coccicure® respectively. A high degree of resistance in field isolates of *Eimeria* spp. has been demonstrated earlier (Siddiki *et al.,* 2000). Farmers are trying to overcome the problem of the drug resistance by increasing the dose of the drug, as encouraged by the manufacturer, often knowing or ignoring the possible toxic effect of these sulphonamides on the birds, and also the possible residual effect on human health. This is an important factor to be looked into.

In poultry, in particular treatment of the birds after onset of the disease can not be economically viable. The target should always be the prevention and/or control of the diseases. It is particularly true for coccidiosis, since once the clinical disease develops treatment can only prevent mortality, but it can not overcome the losses already occurred in terms of production and weight gain particularly in broilers.

Therefore it is recommended that in addition to the sanitary practices specific control measures either by using anticoccidial in feed or by vaccination should be adopted for coccidiosis control. It is further emphasized that proper mixing of the anticoccidial with feed must be ensured.

This experiment indicated that the treatment effects of four drugs (Esb_3®-30%, Coccino®, Navacox®, Coccicure®) on broiler coccidiosis induced by *Eimeria spp* have significantly different ($p<0.05$). Comparatively Coccino® treated group have shown the highest body weight gain, feed conversion rate and less oocyst shedding may be the presence of vitamin K_3 with salfaclozine sodium monohydrate.

CHAPTER VI
SUMMARY AND CONCLUSION

6.1 Summary

The experiment was conducted to study prevalence status of broiler coccidiosis and the comparative efficacy of locally available sulfonamides on controlling of broiler coccidiosis, their effects on the body weight gain, FCR, mortality rate and number of OPG shedding. In the experimental prevalence study two breeds (Cobb-500 and Ross-308) were found in randomly selected fifteen farms. Most of the farms were found affected with coccidiosis at different ages of birds. Degree of affection was mild in first exposure but after two or three days it was high. Among fifteen farms, twelve farms (80%) were found affected with coccidiosis. The disease was mostly occurred in between 12-23 days of age.

In the comparative efficacy of sulfonamides "Cobb-500" breed was selected and sixty, day-old chicks were randomly equally distributed in six groups and reared in litter up to 28 days of age. Groups were designed as follows: Group T_0 as negative control: non-treated and non-infected. Group T_i as positive control: non-treated and infected and then Group T_1, T_2, T_3 and T_4 are infected and received Esb3®-30%, Coccino®, Navacox®, Coccicure® respectively. Groups T_i, T_1, T_2, T_3 and T_4 were infected at 12 days old by 100,000 oocystes. Groups T_1, T_2, T_3 and T_4 were treated at 17 days of age. OPG test was done on 11st , 15th , 17th , 21st , 23rd, 24th and 25th days of age. Also weekly mortality, weight gain and feed conversion ratio (FCR) were recorded.

During observation, the mortality rate of positive control group was 10% after one week infection and 20 % after two week infection. But the mortality rate of negative control group and treated group were zero in study period. At the 21, 28 days the highest mean body weight was recorded in the treated group T_2 were 842.7±20.3gm and 1368.7±17.7gm, respectively, and the lowest mean body weight was recorded in the treated group T_i were 673.7±29.3gm and 980.7±20.5gm, respectively. At 21, 28 days of age the highest FCR was recorded in the treated group T_i were 1.84±0.005 and 1.92±0.005, respectively, and the best FCR were recorded in T_2 treatment group were 1.45±0.005 and 1.61±0.005, respectively. After 3, 5, 6 days post treatment OPG shedding in positive control group T_i was highest were 173167±145, 105100±173 and 48300±115, respectively, and T_2 group showing comparatively less mean OPG shedding was 11000±153, 5500±173 and 2133.3±88.2, respectively. After 7 days post treatment mean OPG shedding was decreased in T_i group due to increased body immunity against coccidiosis but highest in T_i group and OPG shedding is remained zero in all sulfonamides treated groups T_1, T_2, T_3 and T_4. Results indicated that Coccino® treated T_2 group showing the best result.

6.2 Conclusion

Coccidiosis is an immunosuppressive disease in poultry that increases the host susceptibility of other diseases. It posses a great challenge to the poultry industry in terms of morbidity, mortality and loss of production due to subclinical and clinical forms of disease. Coccidiosis has been identified as a major disease in chicken in Bangladesh which causes a great economic loss to the poultry industry. So, coccidiosis is a very important disease of broiler. Differences of mortality rate between treated and untreated birds were very high. In treated birds, mortality was zero and it protected a great economic loss. So treatment of coccidiosis is very necessary for the poultry industry. Considering all the facts and findings of the present experiment the use Coccino® might be recommended to the farmer to control broiler coccidiosis for economical benefit. However, the results of this research work will certainly help the future researchers to have a guideline in carrying out further detail study on treating coccidiosis in broiler.

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Table 6. Effect on administration of different sulfonamides on body weight of broiler chickens in experimental coccidiosis

Treatment n=10	Body weight (gm)					Body weight Increase/ less increase
	Initial 0 days	After 7 days	After 14 days	After 21 days	After 28 days	
T_0 (Negative control)	41.333±0.333	170.33±4.37	451.67±8.82	794.3^{b}±24.7	1308.0^{b}±17.8	-
T_i (Positive control)	41.667±0.667	157.33±2.85	445.00±8.33	673.7^{c} ±29.3	980.7^{c}±20.5	25.02% less increase
T_1 (Esb3®-30%)	40.67±1.20	172.33±3.84	453.3±15.6	804.3^{b} ±17.1	1281.0^{b}±15.9	30.62% increase
T_2 (Coccino®)	39.33±1.86	171.67±3.76	459.0±13.9	842.7^{a}±20.3	1368.7^{a}±17.7	39.56% increase
T_3 (Navacox®)	39.67±1.76	167.33±2.06	435.00±8.08	801.7^{b}±12.2	1247.72^{b}±10.5	27.22 % increase
T_4 (Coccicure®)	41.333±0.882	172.33±4.26	449.00±8.02	799.0^{b} ±17.7	1289.0^{b}±23.2	31.43% increase

Figures with same superscript or without superscript do not differ significantly whereas figures with dissimilar superscript differ significantly. Data were calculated at 5% level of significant ($p<0.05$).

Table 8. Number of OPG shedding on infected treated and control groups of broiler chickens

Treatment n=10	**Number of oocysts per gram of feaces**						
	11 days	15 days	17 days	21 days	23 days	24 days	25 days
	B.I	3 days P.I	5 days P.I	3 days P.T	5days P.T	6 days P.T	7 days P.T
T_0 (Negative control)	0	0	0	0	0	0	0
T_i (Positive control)	0	51667^b±841	94500^b±551	173167^a±145	105100^a±173	48300^a±115	45500±115
T_1 (Esb3®-30%)	0	46333^c±612	101033^a±176	12200^c±208	4200^c±265	1800^c±115	0
T_2 (Coccino®)	0	55833^a±780	92667^b±899	9300^c±321	3500^c±208	1200^c±115	0
T_3 (Navacox®)	0	52267^b±769	95500^b±458	14200^b±208	6300^b±153	2766.7^b±88.2	0
T_4 (Coccicure®)	0	46667^c±953	87167^c±145	11000^c±153	5500^b±173	2133.3^c±88.2	0

B.I. = before infection, P.I. = post infection, P.T. = post treatment,
Figures with same superscript or without superscript do not differ significantly whereas figures with dissimilar superscript differ significantly. Data were calculated at 5% level of significant ($p<0.05$).