COMPARATIVE EFFECTS OF PAPAYA LEAVES (*Carica papaya*) SUSPENSION AND TOLTRAZURIL AGAINST COCCIDIOSIS IN SONALI CHICKEN

A THESIS
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MASTER OF SCIENCE (MS)
IN
PHARMACOLOGY

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HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

JUNE, 2019

DEDICATED
TO MY
BELOVED PARENTS

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ABSTRACT

Coccidiosis is recognized as the parasitic disease with the greatest economic impact on poultry industries worldwide. This disease is very common in sonali chicken and other poultry species. Recently, the concerns about possible drug resistance have aroused great caution in the usage of drug in the animal industry. As one of its alternative now a days researchers are concentrating efforts on herbal medicine using different medicinal plants to minimize the adverse effects. Papaya leaves (*Carica Papaya*) having anti-protozoal, anti-inflammatory, anti-microbial and antifungal properties may provide a new therapeutic avenue against coccidiosis. This work was conducted to evaluate the comparative efficacy of papaya leaves suspension (PLS) and toltrazuril on protozoal load, blood constituents, growth performance of sonali chicken infected with *Eimeria spp*. Eighty (80) sonali chicken of seven days old were randomly divided into four groups named T_0, T_1, T_2 and T_3. Each group contained 20 birds. *Eimeria spp.* was administered orally in T_1 T_2 and T_3 groups. T_0 group was the negative control group and after 3 days of infection T_2 group was treated with papaya leaves suspension (0.5%) for 15 days and T_3 group was treated with toltrazuril (coxitril®) solution (1 ml/lit) for 2 days. Results showed that oocyst reduction count in T_2 group was 83.5% and in T_3 group was 100%. Number of oocyst count was increased in T_1 group. Overall, protozoal load significantly ($P<0.01$) decreased in T_2 & T_3 groups whereas significantly ($P<0.01$) increased in T_1 group. In case of haematological parameters total erythrocyte count **(**TEC) and hemoglobin (Hb) in different treatment groups were almost similar and the differences were statistically non-significant ($P>0.05$) except total leukocyte count (TLC) which was statistically significant ($P<0.01$). The highest body weight gain was recorded from papaya leaves suspension treated T_2 group.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations	Full meanings
%	Percentage
° C	Degree celcius
/	Per
&	And
:	Ratio
@	At the rate of
<	Less than
>	Greater than
±	Plus minus
ANOVA	Analysis of Variance
Av.	Average
Contd.	Continued
e.g.	For example
et al.	And others
Fig.	Figure
FAO	Food and Agriculture Organization
DLS	Department of Livestock Service
gm	Gram
HSTU	Hajee Mohammad Danesh Science and Technology University
i.e.	That is
Kg	Kilogram
Max	Maximum

LIST OF ABBREVIATIONS AND SYMBOLS (Contd.)

Mg	Milligram
Mm	Millimeter
Ml	Mililitre
Lit	Litre
No.	Number
NS	Non-significant
OPG	Oocyst per gram
Ppm	Parts per million
T	Treatment
Sq.	Square
SE	Standard Error
E. tenella	*Eimeria tenella*
TEC	Total Erythrocyte Count
TLC	Total Leukocyte Count
Hb	Hemoglobin
mm^3	Cubic millimeter
Ltd.	Limited
B. wt.	Body weight
No.	Number
Contd.	Continued
Conc.	Concentration
PLS	Papaya leaves suspension

CHAPTER I

INTRODUCTION

Bangladesh is an agricultural based densely populated country. About 71% of the population lives in rural areas (BBS, 2010a). The average per capita income is only US $751 (BBS, 2010b). The majority of people are engaged in agricultural operations, particularly crops, fish and livestock, of which both native and exotic poultry are now mainstream. Approximately 20% of the protein consumed in developing countries comes from poultry meat and eggs (Alders and Pym, 2009). Poultry farming in Bangladesh has grown as an emerging and prospective industry and many landless farmers are found to involve with poultry rearing (Huque, 2001). A total of 5 million people are engaged in this sector (Saleque, 2006). At present chicken contributes 51% of total meat production in Bangladesh (Raha, 2007). There are about 110,800 small and large scale poultry farms in this country (Anon, 2006) and per capita annual consumption of meat is 5.99 kg against the universal standard 80 kg per head (Raha, 2007). There are several constraints of poultry industries in Bangladesh including outbreak of infectious diseases causing economic loss and discouraging poultry rearing (Das *et al*., 2005).

Many of these poultry production farms are hampered by internal parasites, such as coccidia. Coccidiosis is recognized as the parasitic disease with the greatest economic impact on poultry industries worldwide (Allen, P. C. and Fetterer R. H. 2002) due to production losses and costs for treatment or prevention. The annual cost of anticoccidial drugs worldwide is estimated at about US $800 million (Williams, 1998). Coccidiosis caused by the genus Eimeria, affecting the poultry industry (Massoud *et al., 2010;* Adnane *et al.,* 2011).

Eimeria may inflict the birds in both clinical and sub-clinical forms. The clinical form of the disease manifests through prominent signs like bloody faeces, diarrhoea, morbidity and mortality and subclinical coccidiosis manifests mainly by poor weight gain and reduced efficiency of feed conversion and gives rise to the highest proportion of the total economic losses (AL-Fifi, 2007). In addition, severe intestinal lesions results in fowl deaths between 5 to 20% owing to Eimeria colonization affect the commercial production.

Chicks are commonly susceptible to species of Eimeria that affect the poultry industry including *Eimeria tenella, Eimeria maxima, and Eimeria acervulina,* of these *E. tenella* causing the caecal coccidiosis is highly pathogenic (Adnane *et al.,* 2011). The incidence of

coccidiosis observed in commercial poultry range from 5 to 70% leading to high rate in morbidity and mortality in the youngest birds (Adhikari *et al*., 2008; Michels *et al*., 2011; Jadhav *et al.,* 2011). The continuing issue for the commercial chicks production is due to high expenses to control coccidiosis.

Chemotherapy has been the main approach for controlling coccidiosis in chickens. *Eimeria spp.* are able to complete their life-cycles without large numbers of infective oocysts building up in the environment. Such subclinical infections result in the development of strong, specific natural immunity without overt disease. However, studies have shown that resistance to anticoccidial drugs develops with time (Usman *et al.,* 2011). Thus, the chemical therapeutic prevention of coccidiosis is either prohibited or not affordable in organic farming. There is therefore a need of treatment that is effective and cheaper to the farmer.

Therefore the control measures can be followed by using available plant resources. The use of medicinal plant extracts in the treatment of both human and animal diseases is gaining popularity because it is affordable by farmers in developing countries (Kubkomawa *et al.,* 2013). According to the World Health Organization (WHO), about 80 to 90% of the world's population still relies on traditional medicine for their health care needs (Ajay *et al.,* 2013). Bangladesh has a rich biodiversity with various plant species, some of which are commonly used in the treatment of several microbial infections and a range of diseases. These plants have been used both for the treatment of human and animal diseases. So, people seek out for the animal products without any chemical drugs for betterment of livestock production with the help of botanical elements as the sustainable alternatives (Brisibe *et al.,* 2008; Giannenas *et al.,* 2012).

Since the ultimate goal of a farmer is to maximize profits, many farmers go for the cheapest and easiest means of fighting diseases such as coccidiosis. *Carica papaya* has been known to be effective against coccidiosis in some countries (Nghonjuyi *et al*., 2015). The leaves of Papaya (*Carica papaya*) as the feed supplement was the most ingested plant due to its less bitterness and the presence of carotene. It can enhance the feed palatability and subsequently increase the chick growth performance. The antihelmintic activity of papaya latex, leaves, seeds and roots attributed to possess the proteolytic enzymes such as papain, chymopapain and lysozyme. The anti-inflammatory property of the Papaya helps in caecal epithelial cell protection and prevents the coccidial reproduction (AL Fifi, 2007).

By realizing all sorts of problem we are planning to treat coccidiosis by using herbal medication like papaya leaves suspension instead of any synthetic agent, to avoid human health hazards as well as economic poultry meat production in Bangladesh.

Papaya leaf *(Carica papaya*) suspension may have anticoccidial effect and effect on blood constituents, growth performance of poultry (sonali chicken) infected with coccidiosis. It may be stated that the papaya leaf (*Carica papaya*) may provide a new therapeutic avenue against coccidiosis because of its availability in our country and effectiveness. This study mainly focuses on the medicinal plant based treatment for the coccidiosis will be the alternative method for prophylaxis and to avoid the increased resistance on the administration of anticoccidial drug for the chicks.

To evaluate the efficacy of papaya leaves suspension against coccidiosis this study was undertaken with the following objectives:

1. To determine the therapeutic efficacy of papaya leaves suspension and toltrazuril against coccidiosis by counting the protozoal oocyst in sonali chicken
2. To evaluate the effects of papaya leaves suspension and toltrazuril on body weight and hematological parameters in sonali chicken

CHAPTER II

REVIEW OF LITERATURE

Many research works have been carried out by numerous researchers to evaluate the efficacy of papaya leaves and toltrazuril against coccidiosis in chickens. Attempts have been made to reflect some of these works, which are directly related with present study.

2.1 Coccidiosis in poultry

A. T. Yohannes *et al.* (2014) studied clinico-pathological study of avian coccidiosis and its economical impact on small scale poultry farming in selected districts of tigray, Ethiopia. The study was designed to provide information on the clinico-pathology and socioeconomic impacts of avian coccidiosis on chickens farmed under small-scale system. Clinico-pathomorphological study, morphometric Oocyte Per Gram (OPG) determination and questionnaire survey were done. Out of 2000 chicken populations, 350 (17.5%) showed clinical coccidiosis clinico-pathomorphological study and 5% mortality rate was reported. The major clinical findings were depression, bloody diarrhea, mucoid droppings and loss of production. Eleven percent of birds examined through necropsy showed mild to moderate thickened, wrinkled and edematous intestinal wall with multiple focal, ecchymotic haemorrahge and congestion and with mucoid content. Seven percent of samples reported to have greatly enlarged and distended caecal pouch with clotted blood and diffused haemorrhagic enteritis. Histopathological examinations of 8.5% of the representative tissue sections showed numerous oocytes invading the mucosal and submucosa layers, loss of enterocells, hemorrhage, necrosis of mucosal layer, infiltration of heterophils and lyphocytes in the submucosal, desqumation and blunting of villi. On morphometric examination, out of 96, 31 and 51 droppings, litter and intestinal content samples, 66 (68.75%), 8 (25.81%) and 39 (76.47%) were found positive for coccidian oocytes, respectively. The questionnaire based survey indicated an estimated 1,375 bird loss per farm per month due to coccidiosis. Risk factors such as poultry farmers/employees with little background on poultry production and poor litter management were found to favour the occurrence of avian coccidiosis. Integrative poultry producer education/extension service and participatory coccidiosis control and prevention approaches need to be in place.

Chapman H. D. (2003) studied origins of coccidiosis in the fowl- the first fifty years. In 1910, H. B. Fantham described the life cycle of a coccidian parasite in birds. Fantham was a

parasitologist at Cambridge University in the United Kingdom working for an enquiry into diseases affecting the red grouse. Despite the growing importance of the poultry industry and the realization that coccidiosis was an important disease of the fowl, little further work was carried out in the United Kingdom until coccidiosis research was initiated at the Veterinary Laboratory, Weybridge almost 30 yr later. Further progress depended upon research carried out at academic and agricultural institutions in the United States. E. E. Tyzzer at Harvard University provided the solid foundation upon which our present knowledge of coccidiosis, and the species of Eimeria involved in the disease, is based. Agricultural experiment stations (AESs) throughout the nation played an important role in communicating advances to the agricultural community. W. T. Johnson at Western Washington and, subsequently, Oregon AES made significant contributions to our understanding of the disease, as did C. A. Herrick and coworkers at Wisconsin AES, J. P. Delaplane and coworkers at Rhode Island AES, and P. P. Levine at Cornell University.

G. Gari *et al.* (2008) studied poultry coccidiosis in tiyo district, arsi zone, Ethiopia. The objective of this study was first to investigate the prevalence of poultry coccidiosis and to identify the coccidial species occurring in the study area on local strain and Rhode Island Red breed chicken. The study involved questionnaire survey, fecal examination, necropsy examination and identification of coccidial species based on their morphology, predilection site in the intestine and sporulation time. More than 75% respondents indicated that poultry production and income generated from poultry production in the rural community is the major income source for females and youth and bloody diarrhea predominantly appeared during wet season than chalky, yellow or green diarrhea. Public and private veterinary service centers have no anti coccidial drugs and other medicaments used for poultry diseases. Frequency detection of oocyst in the fecal samples from Rhode Island Red breed and local strain chicken was 80.65% and 61.25% respectively. This finding indicated that coccidial infection in Rhode Island Red breed was significantly higher than in local strain chicken ($p < 0.05$). The lesion score and mean oocyst output per gram feces was also considerably higher in Rhode Island Red breed than in local strain chicken ($p < 0.05$, $p < 0.001$ respectively), which may be the difference due to management system and breed. Clinical coccidiosis occurrence in Rhode Island Red breed and local strain chicken was 22.58% and 12.25% respectively. There was no statistically significant difference in clinical coccidiosis occurrence between the two genotype chickens and system. Eimeria species identified in descending order of their occurrence were *E. tenella, E. acervulina, E.*

necatrix, E. maxima and *E. mitis*. Mixed infections were the predominant in both production systems. E. mitis was diagnosed for the first time in Ethiopia.

M. M. Amer *et al.* (2010) studied isolation and identification of Eimeria from field coccidiosis in chickens. Oocysts of Eimeria species were collected from 8 Native breed chicken flocks aged 7-8 weeks. These chickens were suffering from bloody dropping, loss of weight, low conversion rate and variable mortalities 3-12% in 6-10 days. Eimeria species' oocysts were sporulated and tested for their infectivity and pathogenicity in male commercial chicks aged 14 days old. The infected chicks showed general signs of ruffled feathers, off food, huddling together with loose dropping and/or bloody dropping with total mortality reached to 90%. The post mortem examination showed hemorrhagic foci in the duodenum, hemorrhagic mucosa in mid intestine and bloody caecal core in two caeci. Eimeria species developmental stages in duodenum, intestine and caecum were histo-pathologically detected at the 6th day post infection. The obtained sporulated oocysts were identified according to morphological features, and the calculated shape index were 1.14, 1.19, 1.25 and 1.23 suggestive to be *E. tenella, E. necatrix, E. acervulina* and *E. praecox*; respectively. Chicks kept individually in a wire cage were inoculated with one sporulated oocyst for obtaining pure isolate from morphologically identified 10 isolates and for detection the site of infection and histopathological features. Egyptian four local isolates in a pure form were obtained. These isolate, including *E. tenella, E. acervulina, E. necatrix,* and *E. praecox*. This isolates were passed in the chicks 14th day old from increasing their number.

2.2 Prevalence of coccidiosis in poultry throughout the world

Jamal Gharekhani *et al.* (2014) studied prevalence of coccidiosis in broiler chicken farms in western Iran. The main goal of current study was to investigate the prevalence of coccidiosis in broiler farms in Hamedan province, western Iran. Chicks and fecal samples were collected in all of the 220 broiler farms in this region. All viscera were examined for gross pathological changes. The mucosa of small intestine and the caeca were examined for the presence and identification of parasitic forms using parasitology methods. The overall rate of coccidiosis was 31.8%; *E. acervulina* (75.7%), *E. tenella* (54.3%), *E. necatrix* (28.6%), and *E. maxima* (20%) were determined. Mixed infections were observed in all of the positive farms. There was a statistical significant difference ($P<0.05$) among infection rate and age groups, dysentery, history of colibacilosis and clostridiosis in farm, and history

of coccidiostats consumption, unlike to breed (*P*> 0.05). This is the first report of coccidiosis rate in broiler farms in this region. Further additional researches and design control strategies for improving management in farms are necessary.

Comfort A. Olanrewaju *et al.* (2014) studied prevalence of coccidiosis among poultry birds slaughtered at Gwagwalada main market, Abuja, FCT, Nigeria. This vibrant sector is seriously affected by coccidiosis, a protozoan parasite disease caused by the genus Eimeria. A survey of the prevalence of Coccidial infections in chickens was assessed by random sampling of bird faecal samples from Gwagwalada market, FCT. A total of 200 faecal samples were examined using concentration by flotation method. 138(69%) of the faecal samples were positive for coccidian oocysts. Eimeria species encountered were *E. tenella*, *E. acervulina* and *E. maxima* (respectively). *E. maxima* and *E. tenella* were found to be most common among young birds examined. Birds with their ages less than 3 months examined were 130 and had a prevalence rate of 70.77%. Birds which were 3 months and above (i.e. adults) examined were 70 and had the prevalence rate of 65%. Prevalence was extremely high (80%) in cockerel birds followed by laying birds (70%), however, local birds and broilers had the lowest, 66% and 60.67% respectively. Two-way ANOVA test shows that there is no significant difference between the types of chicken examined and the rate of infection in this research; meanwhile, there is a significant difference between the different coccideal species infection in chicken examined (p>0.05). There is also no significant difference between the numbers of chicken infected in relation to age of the chicken at 0.05 level of significance. The present findings suggest that this parasite (coccidian) may constitute sources of zoonotic infections for humans especially those who consume the cooked parasitized coccidian chickens and the caretakers. Birds infected with this parasite should be treated and chicken house should be periodically cleaned and disinfected.

Temesigen W Molla *et al.* (2018) studied prevalence of coccidiosis in backyard chicken in around Debere Tabere Town, South Gondar Zone, Amhara Regional State, Ethiopia. A cross sectional study was carried out to estimate the prevalence of chicken coccidial infections and assessment of the associated risk factor in backyard chickens in and around Debre Tabore town, South Gondar Zone, Northwest Ethiopia from October 2015 and May 2016.Floatation techniques were used for qualitative study of coccidial oocyst. Descriptive statistics was used to express prevalence while chi-square test used to assess if there was

statistically significant association between targeted host factors and coccidial infection. The degrees of these associations were quantified using crude and adjusted odds ratio. Statistical significance was assumed if the confidence interval (CI) did not include one among its values or whenever p-value was less than 5%. The overall animal prevalence for coccidial infections was 21.4% (48/224) and the prevalence was significantly associated with breed (p=0.019), age (p=0.028) and sex (p=0.004) of the study animals. In a multivariable logistic regression analysis, female (Adjusted Odds Ratio (AOR)=0.3; 95%CI:0.17-0.68) and exotic breeds (AOR=0.46; 95%CI:0.23-0.93) were found less likely infected with coccidial oocystes as compared to male and local chickens. However, increased risk for coccidial infection was recorded in adult chicken (AOR=2.04; 95% CI:1.02-4.08) as compared to the grower chickens. In conclusion, this study showed coccidial infection could be important in the backyard chickens in the study area even though the overall prevalence was relatively low. Moreover, the prevalence was higher in adults than the growers, which alerts the need to undertake suitable and practically applicable control and prevention measures in the parent stocks.

2.3 Reviews on importance and prevalence of coccidiosis in Bangladesh

S. M. S. H. Belal (2017) studied prevalence of coccidiosis in sonali birds in Sirajgonj district of Bangladesh. The study was conducted to ascertain the prevalence of coccidiosis in Sonali birds in Sirajgonj district of Bangladesh during the year 2015–2016. Diagnosis was made on the basis of history, clinical and post mortem findings. A total of 660 samples (sick and dead birds) were examined where 234 (35.45%) samples were found positive for coccidiosis. The highest prevalence percentage was found in July (53.33%) and the lowest percentage was found in January (16.67%). Birds aged 5 to 6 weeks showed more prevalence percentage (47.50 %), whereas older birds (> 16 weeks) found less susceptible (10%) to coccidiosis. Coccidiosis was slightly more prevalent in female (37.70%) than male (32.22%).

Md. Lipon Talukdar *et al.* (2017) studied prevalence of infectious diseases in sonali chickens at Bogra Sadar Upazila, Bogra, Bangladesh. The study was conducted to determine the prevalence of infectious diseases in Sonali chickens at Bogra Sadar Upazila, Bogra, Bangladesh. A total of 258 sick and dead Sonali chickens were examined for the diagnosis of different infectious diseases based on history, clinical findings and postmortem lesions of dead and sacrificed birds. Infectious Bursal disease (IBD) was recorded in

14.72% (n=38/258) cases. Similarly, Newcastle disease (ND), Coccidiosis, Colibacillosis and Mycoplasmosis were recorded in 11.24% (n=29/258), 13.95% (n=36/258), 14.72% (n=38/258), 12.79% (n=33/258) cases, respectively. Mixed infection of IBD, ND and Coccidiosis found in 16.67% (n=43/258) birds. On the other hand, mixed infection of IBD, ND and colibacillosis was recorded in 15.89% (n=41/258) cases. It is concluded that several infectious diseases are commonly present in Sonali chicken in the study area of Bangladesh. Mixed infections are more prevalent as compared to single infection. Proper hygienic management and appropriate vaccination should be taken in consideration for effective control the diseases. Further microbiological and molecular diagnoses are suggested for detail studies of these diseases and their pathogens.

Kutubuddin (1973) made a study on mortality due to coccidiosis of chicken in BAU poultry farm and recorded 14.66% mortality of the birds due to coccidiosis.

Sarker (1976) collected 300 dead birds from Bangladesh Agricultural University (BAU) Poultry farm and based on post mortem examination about 12% infectivity with coccidiosis was recorded.

Mondal and Qadir (1978) reported subclinical coccidiosis (54.14%) of chicks in (BAU) poultry farms and among the infected birds 23.75% were found to infect with *E. tenella*,the most predominant species in Bangladesh.

Karim *et al.* (1994) recorded higher prevalence of chicken coccidiosis during winter or cold months, and found *E. tenella* (70%), *E. maxima* (40%), *E. brunetti* (30%) and *E.necatrix* (20%) by lesion scoring of birds. The authors reported that *E. acervulina* was the commonest species and found in 80% birds examined.

Bhattacharjee *et al.* (1996) made a study on prevalence of chicken coccidiosis in Bangladesh and reported the prevalence is 9.4% cases.

Islam *et al.* (1996) investigated the prevalence of chicken coccidiosis in dead birds. The authors found that 39.2% of the birds (out of 337) were affected with coccidiosis.

Talha (1999) studied the prevalence of coccidiosis in different poultry farms of Bangladesh. The author reported 45% mortality rate due to chicken coccidiosis in Bangladesh.

Giasuddin *et al.* (2002) did a statistics on mortality due to coccidiosis in Bangladesh and reported 4-5% mortality rate in chicken due to coccidiosis in Bangladesh.

Giasuddin *et al.* (2003) made a study on the prevalence of coccidiosis in chicken in Bangladesh and reported 9.17% prevalence.

Saleque (2003) collected data (1999-2002) from Central Disease Investigation Laboratory and BRAC laboratory, and reported gradual higher prevalence of coccidiosis in poultry farms over the last 3 years.

2.4 Economic impact of coccidiosis in poultry

Adriana Gyorke *et al.* (2016) studied the economic impact of infection with *Eimeria spp*. in broiler farms from Romania. A survey was conducted on chicken broiler farms from Romania in August-November 2010 to evaluate economic losses due to coccidiosis. Data were collected from six broiler farms of different capacity regarding chemoprophylaxis program, weight gain, feed conversion, and mortality, for two previous flocks in two houses of each farm, and finally we evaluated the economic losses. Also, faeces samples were collected and oocysts were classified according to their size, and virulence of each *Eimeria spp.* field isolate was determined by lesion scoring. Correlations between economic performance, oocysts category, and virulence of Eimeria were assessed by multiple linear regression. Total economic losses per 24 flocks of 18,000 chicks each were about €37,948.2, with an average of €3,162.4 per flock, and they were caused by mortality (34.8%) and poor feed conversion (65.2%). Poor body weight gain was associated with AM oocyst category (presumptively *E. acervulina* and/or *E. mitis*), high lesion score in the duodenum, and coccidiostat used for chemoprophylaxis. Feed conversion ratio was linked to the same parameters as body weight gain, minus chemoprophylaxis programme, plus total lesion score. The percentage of mortality was influenced by the lesion score in the caecum and total lesion score. Statistical analysis showed that epidemiological survey of broiler flocks during the grower period can help the farmer to avoid important economic losses due to coccidiosis. As in other countries, the economic losses caused by coccidiosis in Romania are important, and a good prophylaxis programme can reduce the economic impact of coccidiosis.

A. K. Beraa *et al.* (2010) evaluated economic losses due to coccidiosis in poultry industry in India coccidiosis is an old parasitic disease, prevalent all over the country and has a significant impact on poultry production. In this paper, economic loss to poultry industry has been estimated considering the major economic parameters. The estimation has revealed

that commercial broiler industry is a major sufferer due to coccidiosis wherein 95.61 per cent of the total economic loss occurs due to the disease. The commercial layer industry shares 3.53 per cent economic loss, mainly due to cost of chemoprophylaxis and reduced egg production. A comparison across economic traits has revealed that loss is maximum due to reduced body weight gain, followed by increased FCR (23.74%) and chemoprophylaxis (2.83%) in the total loss due to coccidiosis in broiler industry of India. The overall comparison of economic traits for all the types of poultry sector it has shown that reduced body wt gain and increased FCR are the major parameters from which 68.08 percent and 22.70 percent annual loss has occurred in the total loss from coccidiosis in India during the year 2003-04. The total loss due to coccidiosis has been found to be of Rs 1.14 billion (approx) for the year 2003-04. The study has observed that generation of this data across different geographical regions will be helpful to conclude about the global economic loss due to coccidiosis in the poultry industry.

2.5 Efficacy of toltrazuril against coccidiosis

M. M. Amer *et al.* (2010) Studied on the efficacy of some commonly used anticoccidial drugs in controlling of coccidiosis with mixed field isolates in Broiler chickens. One hundred, one day-old Cobb broiler chicks were randomly divided to 5 equal (1-5) groups. First four Chickens groups 1-4 were orally infected with 5x10 mixed sporulated oocysts per chick at 14 day – old. 5th Group (5) were kept as non infected control. After appearance of signs; groups 1, 2 and 3were treated with toltrazuril, amprolium and Sulphaquinoxaline (S.Q); respectively; while group (4) was kept as nontreated control. Results revealed that, group treated with toltrazuril showed great improvement in feed intake average; weight gain and food rate conversion as well as high reduction in the number of oocysts. The results of experimentally infected chicks with mixed field Eimerial oocysts showed some sort of drug resistance to S.Q and amproluim. Our study pointed out that; toltrazuril is still more effective than S.Q or amprolium in elimination of E. oocysts infection in chickens. Area of drug resistance needs more investigation to explore its magnitude, mode and how to overcome.

Ramadan A. *et al.* (1997) studied anticoccidial efficacy of toltrazuril and halofuginone against *E. tenella* infection in broiler chickens in Egypt. The anticoccidial activities of toltrazuril and halofuginone against *E. tenella* were tested in broiler chickens. Comparisons were made between unmedicated infected and uninfected control birds in addition to

infected groups given either toltrazuril at 37·5, 75 and 150 ppm in the drinking water, or halofuginone at 1·5, 3 and 6 ppm in the feed. Both drugs were highly efficacious against *E. tenella*. Treatment improved the bodyweight gain and survival percentage in comparison with the unmedicated, infected group. Intestinal lesions, faecal and oocyst scores and oocyst shedding in droppings were significantly reduced by both drugs. Toltrazuril gave better protection than halofuginone; 75 and 150 ppm toltrazuril in drinking water gave good protection when administered four and five days after inoculation.

M. A. Lovelu *et al.* (2016) evaluated anti-coccidial drug sensitivity against experimental coccidiosis in broiler chicks. This study was undertaken to investigate the effect of sulphaclozine sodium and toltrazuril for the treatment of experimentally induced caecal coccidosis. Day-old chicks (n=96) reared in a coccidia free condition divided into six Groups (A, B, C, D, E and F) each having 4 subgroups of 4 chicks in each. At the age of 23 days the chicks were infected with 104 sporulated oocysts of *E. tenella* except control Group F and treated for 4 consecutive days from 3 days post infection. Then treatment was given in Group A (2 gm/L), B (3 gm/L) with sulphaclozine sodium and in Group C (1 ml/L), D (1.5 ml/L) with toltrazuril. Group E was maintained as infected, but untreated and Group F as negative control. After treatment Group A gained almost similar weight with Group F. A significantly higher ($P<0.05$) weight gain was recorded in Group A and B compared with Group C and D. The infected untreated chicks passed a large number of oocysts from day 5 till the end of the experiment. Chicks receiving sulphaclozine sodium (Groups A and B) had large oocysts count compared with the Groups C and D treated with toltrazuril but lower than the untreated chicks. At the end of experiment the lowest oocysts count was found in Group D (31.32 × 106) and highest in Group E (53.59×106). Hence this study recommends touse sulphaclozine sodium and toltrazuril for chicken coccidiosis.

2.6 Reviews on drug resistance

Jin-Guang Ming *et al.* (2003) selected one hundred and thirty 21-day-old healthy chickens to study the resistance of two *Eimeria tenella* isolates, Nanjing isolate and Fengyang isolate, to diclazuril, maduramicin, clopidol, robenidine and amprolium. The authors reported that two isolates were light resistant to amprolium, sensitive or light resistant to diclazuril, middle resistant to clopidol, middle resistant or complete resistant to maduramicin and robenidine.

Warren *et al.* (1966) examined the response of field strains of coccidia to several coccidiostats during 1964 and 1965. The authors reported strains of all species of Eimeria, and more strains of coccidia had a tendency to be resistant to coccidiostats that were used. The coccidiostats used included 0.0125% amprolium, 0.009% coccidiostat `D' (a mixture of 8 part sulphaquinoxaline and 1 part diaveridine), 0.01% nitrofurazone, 0.0145% coccidiostat `P' (a mixture of 16 percent amprolium, 12 parts sulphaquinoxaline and 1 part ethopabate), 0.0125 percent sulphaquinoxaline and 0.125 percent zoalene.

Joyner (1970) in a study reported resistant response to coccidiostatic drugs in different strains of *Eimeria spp*. The author also reported that the drug resistance may be induced to most coccidiostat in common use and failure of prophylaxis may occur in the presence of resistant strains.

Chapman (1978) studied on the development of resistance in the Houghton strain of *E. tenella* to anticoccidial drugs like amprolium, clopidol and methyl benzoquate. The author reported that amprolium and clopidol developed resistance more rapidly in experiments.

Gill, B. S. and Bajwa (1979) studied on drug resistance of different field isolates of *Eimeria spp.* The authors reported 71.5% resistant to sulphaquinoxaline, 58.8% to bifuran, 34.1% to amprolium, 6.3% to clopidol and 5.6% to nicarbazin against chicken coccidiosis.

Oikawa *et al.* (1975) reported that 97% of the strain of *E. acervullina* was resistant to decoquinate and 93% resistant to clopidol; a considerable percentage of the strain of *E. tenella* and *E. necatrix* were resistant to amprolium, clopidol and decoquinate, and only a small percentage of these strains were resistant to sulfadimethoxime.

Rommel (1987) observing the resistant strains of Eimeria, and stated that it was necessary to seek an alternative to the use of anti-coccidials because of the development of resistant coccidial parasite and need to reduce drug residues in tissues.

2.7 Papaya

It is a tropical fruit tree, also called papaw or pawpaw, which is widely cultivated. It grows rapidly, fruiting within three years and originally from southern Mexico, Central America, and northern South America.

Nutritive value of Papaya:

Nutritional value per 100 g	
Energy	179 kJ (43 kcal)
Carbohydrates	10.82 g
Sugars	7.82 g
Dietary fiber	1.7 g
Fat	0.26 g
Protein	0.47 g
Vitamins	
	(6%)
Vitamin A equiv.	47 µg
beta-Carotene	(3%)
luteinzeaxanthin	274 µg
	89 µg
Thiamine (B1)	(2%) 0.023 mg
Riboflavin (B2)	(2%) 0.027 mg
Niacin (B3)	(2%) 0.357 mg
Pantothenic acid (B5)	(4%) 0.191 mg
Folate (B9)	(10%) 38 µg
Vitamin C	(75%) 62 mg
Vitamin E	(2%) 0.3 mg
Vitamin K	(2%) 2.6 µg

Minerals	
Calcium	(2%) 20 mg
Iron	(2%) 0.25 mg
Magnesium	(6%) 21 mg
Manganese	(2%) 0.04 mg
Phosphorus	(1%) 10 mg
Potassium	(4%) 182 mg
Sodium	(1%) 8 mg
Zinc	(1%) 0.08 mg
Other constituents	
Water	88 g
Lycopene	1828 µg

Source: USDA nutrient database

The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present. Pawpaw (*Carica papaya*) leaves have potential to supply dietary proteins required by the birds. This because they have a high crude protein (CP) content (~30%) with low (~5%) crude fiber levels (Onyimonyi *et al.*, 2009).

2.8 Medicinal use of papaya

Studies at the University of Nigeria have revealed that extracts of ripe and unripe papaya fruits and of the seeds are active against gram-positive bacteria. Strong doses are effective against gram-negative bacteria. The substance has protein-like properties. The fresh crushed seeds yield the aglycone of glucotropaeolin benzyl isothiocyanate (BITC) which is bacteriostatic, bactericidal and fungicidal. A single effective does is 4-5 g seeds (25-30 mg BITC). Papain has been employed to treat ulcers, dissolve membranes in diphtheria, and reduce swelling, fever and adhesions after surgery. With considerable risk, it has been applied on meat impacted in the gullet. Chemopapain is sometimes injected in cases of slipped spinal discs or pinched nerves.

Anibijuwon *et al.* (2009) were investigated for antibacterial activity against some human pathogenic bacteria using the agar diffusion method. The bioactive compound of leaf and root extracts of *Carica papaya* was extracted, using water and organic solvents. The root extracts demonstrated higher activities against all the gram-positive bacteria than the gram-negative bacteria tested, with the highest activity (14 mm zone of inhibition) demonstrated against *Pseudomonas aeruginosa* while the aqueous leaf extract showed pronounced inhibition demonstrating higher activities against the test bacteria than the organic solvents. The extracts demonstrated higher activities against all the gram-positive bacteria than the gram-negative bacteria tested, with the highest activity (4.2 mm zone of inhibition) demonstrated against *Pseudomonas aeruginosa*. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the root extracts ranged between 50-200 mg/ml.

Elisa Friska Romasi *et al.* (November 2011) was reported that the extracts of papaya leaves could inhibit the growth of *Rhizopus stolonifer*. Antibacterial activity of *Carica papaya* leaf extracts on pathogenic bacteria was observed in this study. The objectives of this study were to determine extract ability against pathogenic bacteria, to observe the influence of pH, NaCl, and heat on extracts ability, and to observe extract ability against *B. stearothermophilus* spores. The data showed that ethyl acetate extract could inhibit *B. stearothermophilus, L. monocytogenes, Pseudomonas sp.,* and *E. coli*. From this study, it is found that Papaya leaves are potential natural antibacterial substance, which might be used in certain kinds of food. Atta (1999) found that fresh, green papaya leaf is an antiseptic, whilst the brown, dried papaya leaf is the best as a tonic and blood purifier.

Jyotsna Kiran Peter *et al*. (2014) studied antibacterial activity of seed and leaf extract of *Carica papaya* var. pusa dwarf linn. The study dealt with the antibacterial activity of aqueous, chloroform extract of leaves and aqueous, methanolic extract of seeds of *Carica papaya* var. pusa dwarf through agar well diffusion assay against *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli* and *Salmonella typhi*. It was revealed that the aqueous as well as the methanolic extract of seeds were effective to inhibit the bacterial pathogens while in case of chloroform extract of *Carica papaya* leaves did not show any inhibition against the bacteria and the aqueous leaf extract was potent to inhibit them.

2.9 Efficacy of papaya leaves against coccidiosis

AL Fifi (2007) studied the effect of leaves extract of *Carica papaya,Vernonia amigdalina* and *Azadiratcha indica* on the coccidiosis in free-range chickens. An alternative coccidiosis treatment in response to the side effects of routinely use of conventional anticoccidial drugs was investigated on 600 *E. tenella* experimentally infected thy-old Harco chicks in a factorial design at the Benin Agronomic Faculty Research Station. The effect of *Carica papaya*, *Vemonia amygdalina* and *Azadiratcha indica* dry leaf powder in the conventional feed (15% incolporation) was evaluated. *C. papaya*, the most ingested plant reduced the infected untreated control OPG down to 53% and amygdalina, down to 35%, even though it was less ingested than *C. papaya*. However, this reduction constituted 56 and 37% (*C. papaya* and *V. amygdalina*) ofthe conventional coccidiostatic effectiveness. The OPG reduction might be ascribed on the hand to the bittemess and on the other to the chemical compound, papaine and Vemoside. *A. indica* was less ingested due to it higher bittemess, repellency and toxicity. Consequently it exalted the disease and produce more oocysts than the control group.

Srinivasan Hema *et al.* (2015) studied the invivo anticoccidial effects of *Azadirachta indica* and *Carica papaya* L. with salinomycin drug as a dietary feed supplement in broiler chicks. A total of thirty suspected broiler chicks were screened for coccidiosis, of them 25 chicks were found to be infected with coccidiosis viz. *Eimeria tenella* (15) *Eimeria maxima* (5) *Eimeria necatrix (*6) and *Eimeria mitis* (4). The anticoccidial efficacy of *Azadirachta indica* and *Carica papaya* with Salinomycin as a dietary feed supplement on the representative *E. tenella* (25×103 oocyst) infection challenged in broiler chicks was studied in six groups for the period of six weeks. *A. indica* and *C. papaya* leaves were administered in powder form at the concentration of 0.1% and 0.2% respectively. The Oocysts per gram (OPG) count were observed on 8th, 9th, 10th, 11th day of post inoculation (DPI). The challenged experimental chicks revealed haemorrhage, thickening of mucosa, cores of blood and ballooning of caecum. The experimental group T_5 chicks treated with A. indica were analyzed to possess the maximum weight gain (2.003), better feed conversion ratio (FCR) (2.32), OPG count (5.87), livability percentage (88) and the lesion score (3.33). Chisquare test analysis revealed no significant differences among the treated groups and the performance parameters. Therefore, this study concludes that plant sources used as a remedial curate for coccidiosis is a perforated growth in the commercial broiler industries.

Nghonjuyi N. W. *et al.* (2015) studied the efficacy of ethanolic extract of *Carica papaya* leaves as a substitute of sulphanomide for the control of coccidiosis in kabir chickens in Cameroon. Their work was aimed at evaluating the efficacy of an ethanolic leaf extract of *Carica papaya* with regard to growth, parasitological and haematological parameters in Eimeria-infected KABIR chickens. Fresh leaves were harvested, dried in gentle heat, ground and sieved powder was used to prepare the ethanolic extract. Chickens were infected with 3200 Eimeria oocysts until they began shedding oocysts in their faeces. The ethanolic extract was administered at doses of 0.32g/chicken/day (T_1), 0.80 g/ chicken/day (T_2) and 1.44 g/chicken/day (T_3), directly into the chickens' mouths. Chickens of T4 received a standard anti-cocciadial drug, sulfaquinoxalina-LH, those in T_5 were infected but not treated while T6 was the neutral group. Red blood cell (RBC) and white blood cell (WBC) counts as well as haemoglobin (Hb) values were determined. Among the trial groups, T_3 and T_1 had the highest and lowest feed conversion rates of 1.87% and 1.62% respectively. The highest average growth rate was recorded in T_1 (7.09%) while the least was registered in T_3 (2.74%). Carcass parameters were similar in all groups except for the hearts. In the trial groups chickens in T_3 had the highest oocyst reduction rate (97.4%) while those in T_1 had the lowest (94.5%), but the differences were not statistically significant. Overall, the highest oocyst reduction count was recorded in chickens of T_4 (98.7%) while the lowest was recorded in chickens of T_5. RBC was significantly highest ($P \leq 0.043$) in T_1 and lowest in T_5. WBC was significantly highest ($P \leq 0.031$) in T_4 and lowest in T_5. Hb values were similar in all groups. Ethanolic leaf extract of *C. papaya* could be as effective as standard anti-coccidial drugs if used before the threshold level of parasitic infection.

2.10 Efficacy of papaya leaves on hematological parameters

Salihu. T. *et al.* (2012) were studied in 40 Isa Brown commercial layers infected naturally with nematodes to observe the anthelmintic efficacy of the aqueous and crude extract of *Carica papaya* seeds. The results of this study showed that the powdered and aqueous extract of *C. papaya* after its administration, produced a significant increase ($P < 0.001$) in packed cell volume, red blood cells, haemoglobin concentration, lymphocyte counts and significant decrease in eosinophil counts. In this study, before treatment, the PCV mean values for animals in Groups A (untreated), B (piperazine 322 mg/kg body weight/day), C (powdery *C. papaya*, 300 mg/day/bird) and D (aqueous extracts of *C. papaya*, 1:10 ml water required/day) were 20.00 ± 0.50, 24.00 ± 0.6, 23.00 ± 0.4 and 23.00 ± 0.2. After treatment, this parameter showed a significant increase. Pre-treatment values of hemoglobin

in groups A, B, C and D were 6.30 ± 0.4, 7.5 ± 0.5, 7.4 ± 0.3, and 7.4 ± 0.5, respectively which significantly increased after treatment.

2.11 Efficacy of papaya leaves on body weight

Ahaotu E. O. *et al.* (2018) observed carcass and organ weight indices of feeding different levels of pawpaw leaf meal (*Carica papaya*) on finisher broiler birds. The study was conducted with sixty (60) four weeks old Anak broiler birds. The birds were divided into four treatments with three replicates (containing five birds) per treatment. Broiler finisher rations were formulated in which pawpaw leaf meal was incorporated at levels of 0%, 5%, 10%, and 15% in the control (T_1), T_2, T_3 and T_4 diets respectively. Results showed that the effect of treatments on gizzard weight (g/b/d), liver weight, heart weight and live weight were significant ($p<0.05$). There were improved daily weight gain and daily feed intake as the dietary levels of pawpaw leaf meal increased from T_1 to T_4 in all the parameters evaluated. The incorporation of pawpaw leaf meal into finisher broiler diets had nutritional benefits which led to general improved performance in body weight changes, feed conversion ratio, feed cost/kg gain, carcass and organ weights. It was concluded that 15% pawpaw leaf meal can be included in the diets of broiler finisher birds without any adverse effect on performance.

ABM Mostofa Kamal *et al.* (2015) studied effects of neem, nishyinda and papaya leaves as growth promoters in broiler chicks. The experiment was conducted to evaluate the efficacy of Neem (*Azadirachta indica*), Nishyinda (*Vitex nogundo*) and Papaya (*Carica papaya*) leaves powdered supplementation in drinking water as a growth promoter in broiler chicks. A total of 40 day-old Cobb 500 broiler chicks were purchased from local hatchery (Nourish Poultry & Hatchery Ltd.) and after seven days of acclimatization chicks were randomly divided into two groups, A and B. The group A was kept as a control and not treated. The group B was supplemented with Neem, Nishyinda and Papaya dried leaves powder with feed and water. Weekly observations were recorded for live body weight gain up to 6th weeks and hematological tests were performed at 17th and 35th day's age of broiler to observe hematological changes between control (A) and treatment (B) groups. The initial body weight of groups A and B on 7th day of this experiment were 140±3.56 gm and 140±4.35 gm, respectively and after 35th day of experiment final body weight were 1450±47.35 gm and 1650±58.56 gm, respectively; the net body weight gain were 1310±43.79 gm and 1510±54.25 gm, respectively and economics of production were

analyzed and found that net profit per broiler was Tk. 24.21 and Tk. 34.78, respectively. The body weight was significantly increased ($p<0.01$) in treatment group compared to control group A. The TEC, ESR and PCV value of treatment group showed significant difference ($P<.05$), while Hb estimation did not show significant difference in control group. The results suggest that better growth performance could be achieved in broilers supplemented with Neem, Nishyinda and Papaya extract.

Zulu *et al.* (August 19, 2014) assessed nutrient digestibility and growth performance of Japanese quails (*Cortunix japonica*) fed diets with 0, 2, 4 and 6% pawpaw (*Carica papaya*) leaf meal (PLM) as a protein source. 108 three-weeks-old quails were individually weighed, divided into four groups based on body weights, and groups randomly assigned 0, 2, 4 and 6% PLM-diets. They observed Feed intake, weight gain, body weights, feed conversion ratios (FCR), and digestibility of dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and nitrogen free extract (NFE). Birds fed 6% PLM-diet were superior ($p < 0.05$) in all these parameters, followed by those in 4% PLM group. In this study, optimal results were achieved at 6% PLM replacement level, no significant differences were observed between control & 2% PLM-diet fed birds and partially replacing soybean meal with PLM improved nutrient digestibility.

CHAPTER III

MATERIALS AND METHODS

3.1 Research site

The experiment was conducted at the laboratory & experimental shed under the department of physiology and pharmacology, laboratory of the department of pathology and parasitology, HSTU, Dinajpur.

3.2 Research time

The experiment was performed for a period of 30 days from 3rd January to 2nd February, 2019 to investigate the comparative effects of papaya leaves suspension and toltrazuril against coccidiosis in sonali chicken.

3.3 Preparation of experimental shed

First the room was washed by sweeping with tap water using hose pipe connected with the tap. The room was disinfected with a phenolic disinfectant and allowed to dry leaving the room unused with the electric fan and the bulb switched on overnight. The room was properly ventilated. All the utensils required for the experiment such as feeder, water pot, beakers, pestle and mortar, syringe, needle etc. were collected and the experimental shed was properly designed. During the whole experimental period, all birds were exposed to a 16 hours continuous photoperiod (natural light plus artificial light) in an open sided house. Electrical bulbs were used for additional light at night.

3.4 Collection and acclimatization of experimental birds

Eighty (80) sonali chickens at the age of 7 days were obtained from Bahadur bazar, Dinajpur. Immediately after reaching the destination the birds were shifted to experimental shed. Proper ventilation and lighting was maintained inside the shed. Birds were fed with commercial feed and drinking water *ad-libitum*. Glucose and vitamin C were supplied with drinking water for first three days to overcome the transportation stress. Birds were allowed to acclimatize in their new environment for 3 days before the commencement of the experiment.

3.5 Collection of feed

Sonali mash commercial feed were collected from Griholokkhi Poultry Feed, Kalitola, Dinajpur from a reputed poultry feed exporter. Mash and water were provided *ad-libitum* during the whole experimental period.

3.6 Routine management

Birds were provided to similar care and management in all groups throughout the experimental period. Adequate hygiene and sanitation were maintained properly.

3.7 Collection, preparation and administration of test protozoa

Few coccidiosis suspected (having bloody diarrhoea) birds were collected from different region of Dinajpur district. Faeces from different birds having haemorrhage in caeca were subjected to microscopic examination (using low and high magnification) to confirm the positive cases. Identification of oocyst of *Eimeria spp.* was made on the basis of morphology.

Figure 3.1: Haemorrhage in caeca

Then 5gm faeces from positive case were homogeneously mixed and suspended in 2% potassium dichromate solution overnight. The suspension was filtered through a clean cloth and allowed to sediment for 2 h. The sediment material was then mixed with saturated sodium chloride solution and the suspension was centrifuged at 1500M for 2 min in graduated tubes. The top layer from each tube obtained was immediately pipetted out in a beaker and mixed with 1 L of water in order to dilute sodium chloride solution. The

suspension was allowed to settle down and supernatant fluid was discarded. The sediment containing the oocysts was again suspended in 2% potassium dichromate solution to prevent the development of harmful bacteria. Then the solution was kept overnight for sporulation of oocysts (Ministry of Agriculture, Fisheries and Food, 1986). After that 0.1ml solution was loaded in McMaster counting chamber and the number of oocysts were counted. The total number of oocysts counted was multiplied by 100 to achieve the OPG (AL Fifi, 2007). Then the suspension was shacked properly and the birds of T_1, T_2 & T_3 groups were inoculated orally with 2-3 drops of the suspension.

Figure 3.2: Administration of *Eimeria spp.*

3.8 Collection and processing of plant materials

Papaya leaves were collected from the HSTU, Dinajpur. Young papaya leaves were collected and washed with fresh water. Before chopping it into small pieces, it was soaked with cotton for removing the adhesive water. Then the leaves were chopped into small pieces and mashed with the help of pestle and mortar. Leaves suspension was collected by squeezing mashed leaves. Then it had been produced 0.5% of suspension of grinded papaya leaves with distilled water.

Figure 3.3: Processing of papaya leaves

3.9 Collection, preservation and administration of toltrazuril

Coxitril® liquid (toltrazuril 2.5%) was collected from Square Pharmaceuticals Ltd. It was preserved in a dry place at room temperature and was administered at a dose rate of 1ml/litre of drinking water for 2 consecutive days.

Composition: Each ml contains toltrazuril 25mg.

Figure 3.4: Coxitril® liquid (toltrazuril 2.5%)

3.10 Experimental design

All the 80 birds were randomly divided into 4 groups for assessing the efficacy of papaya leaves suspension and coxitril® liquid (toltrazuril 2.5%) against coccidiosis in sonali chickens.

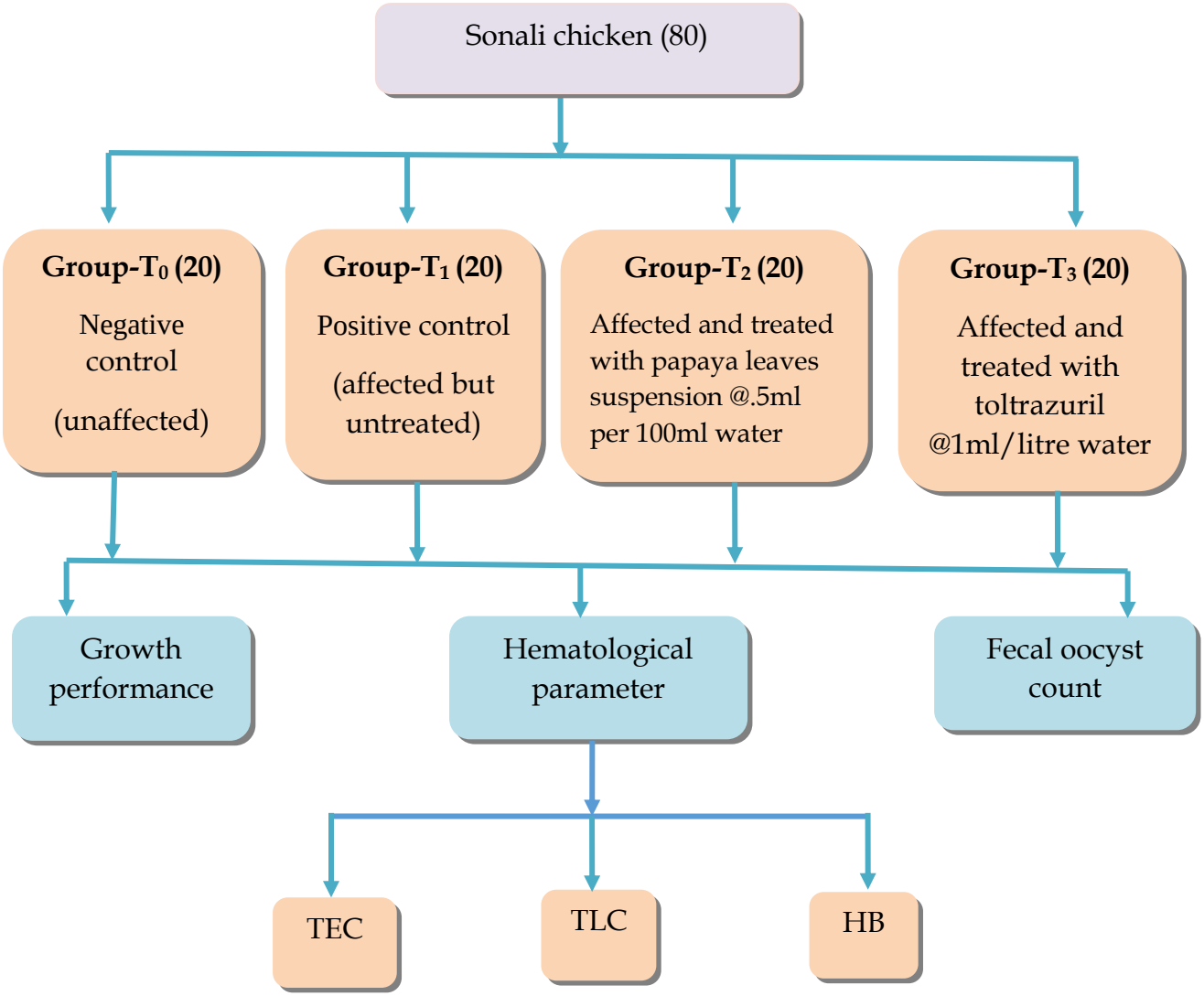

Figure 3.5: Layout of the experiment

3.11 Experimental birds grouping

Eighty (80) sonali chickens were used to evaluate the dietary effect of papaya leaves suspension on *Eimeria spp.* infestation, growth performance and blood parameters. The

birds were assigned into four groups. Birds were randomly distributed in every group. The groups were designated and maintained as follows:

Group T_0: The birds were fed normal diet and given water *ad-libitum* and their body weight was recorded at every 7days interval. Body weights, blood parameters and protozoal load were measured at the times when that of other groups were measured. This group was served as "**Negative control"** group.

Group T_1: The birds were supplied with *Eimeria spp.* protozoa after acclimatization to induce coccidiosis in this group. No anticoccidial treatment was done against *Eimeria spp.* in T_1 group. Adequate feed and drinking water was given. This group served as "**Positive control"** group.

Group T_2: The birds were supplied with *Eimeria spp.* protozoa after acclimatization to induce coccidiosis in this group. This group left as about 3 to 4 days for establishment of coccidiosis. After 4 days, this group was treated with papaya leaves suspension as herbal anticoccidial agent at a dose rate of 0.5ml per 100 ml of drinking water. This group served as **"Papaya leaves suspension"** group.

Group T_3*:* After acclimatization to induce coccidiosis birds of this group were supplied with *Eimeria spp.* protozoa as like as T_1 & T_2 group. This group left as about 3 to 4 days for establishment of coccidiosis. After 4 days, this group was treated with anticoccidial agent (coxitril® liquid @ 1ml/litre water). This group served as **"Toltrazuril"** group to compare the anticoccidial effect with papaya leaves suspension.

3.12 Body weight recording

The body weight was recorded before and during administration of papaya leaves suspension and toltrazuril. Chickens under trial and control groups were weighed with electric weighing machine. The weight of each chicken was taken before feeding in the morning, in noon and afternoon. The average of these three weights was calculated and recorded.

Mean live weight of each group of chicken on 1^{st}, 7^{th}, 14^{th}, 21^{st} and 30^{th} days were recorded with the procedures described above.

Figure 3.6: Recording of body weight

3.13 Protozoal load analysis

3.13.1 Sample collection

The fecal samples were collected early in the morning immediately transferred from the collection site to pathology and parasitology laboratory, HSTU for analysis.

3.13.2 Examination of feces for protozoal oocyst investigation

Feces were examined by two different types of qualitative tests; namely direct smear and flotation techniques to identify the morphological features of eggs, cyst and oocysts (Hendrin C. M. and Robinson N., 2006; Soulsby, 1982).

The direct smear technique was conducted by mixing a drop of water with little bit of feces using an applicator stick on glass slide, covered with cover slip and examined under microscope.

The flotation technique was done by exploiting the density of the parasites, particularly eggs; it allows the parasites to float to the top of a dense solution (specific gravity about 1.20) and can then be skimmed from the top of the tube. For this purpose, 5g of feces were mixed with 5ml of saline. Then the mixture was filtered through sieve and the suspension was centrifuged at 1500 rpm for five minutes. The sediment mixed thoroughly with 5ml of saturated sodium chloride solution and centrifuged for one minute at 2500 rpm. Then the tube placed in a rack in a vertical position and slowly added enough saturated sodium chloride solution with a dropper to fill the tube so that an inverted meniscus forms. Without

shaking the tube, a cover slip placed on top of the tube so that its underside rests on the meniscus and allowed to suspend on top for ten minutes. Then the cover slip was removed, applied to a glass slide and examined under microscope.

3.14 Hematological parameters

Blood samples were collected from the wing vein of chicken of all groups at 15 days interval. The following parameters were observed:

a. Total Erythrocyte Count (TEC)
b. Estimation of Hemoglobin (Hb)
c. Total Leukocyte Count (TLC)

Fig 3.7: Collection of blood from wing vein

Fig 3.8: Blood for haematological test

3.14.1 Total Erythrocyte Count (TEC)

a) The tip of the dry and clean red pipette was placed on the blood sample.
b) The blood was sucked up until it reached the exactly 0.5 mark and carefully wiped the tip of the pipette with a piece of absorbent cotton.
c) Then the tip of the pipette placed immediately in the diluting fluid and filled the pipette exactly upto 101 mark.
d) The number tube around the tip of the pipette was stretched and held with thumb and finger at each end.
e) The contents of the pipette was shaked thoroughly with 8 knot or twisting motion for 1-2 minutes.
f) Then the counting chamber with cover glass was placed under the microscope and made visible the finely rolled area with low power objective.

g) After discarding 2 or 3 drops, a small drop from the pipette was placed to the end of the polished surface of the counting chamber containing the ruling and allowed the space to fill the area under the cover glass.
h) The chamber was allowed to stand for 2 minutes to settle the erythrocytes and counted the cells on the four corner squares and one center square with high power objective (100x).
i) The number of RBC was calculated as follows:
j) Number of RBC = No. of cell counted x 10000
k) The result was expressed in million/ cu.mm.

3.14.2 Determination of Hemoglobin (Hb)

Following procedures were taken for determination of Hemoglobin.

a) N/10 HCl was placed into the perfectly clean and dry diluting tube upto 2 mark.
b) Then the blood sample was drawn into the sahli pipette exactly upto 20 mark and the side of pipette wiped to remove sticking blood to its outside with absorbent cotton.
c) The blood of the pipette was transferred immediately into the diluting tube containing N/10 HCl acid and rinsed the pipette 2 or 3 times by sucking water into the pipette and added these washing to the solution in the tube.
d) Using the dropper, water was added drop by drop, each time mixing the solution with a stirrer until the color of the solution matched the standard.
e) After 5 minutes of first noting time, the result was read in day light from the scale of the measuring tube by observing the graduation mark at the lower edge of the meniscus at the top of the liquid column.
f) The result was expressed in gm%.

3.14.3 Total Leukocyte Count (TLC)

The principles involved in enumeration of TLC were almost same to those of erythrocytes. Here the leukocyte diluting fluid was N/10 HCl solution. Well mixed blood was drawn upto the 0.5 mark of white blood cell pipette. The diluting fluid was filled up to the 11 mark of the pipette and the contents were thoroughly mixed for 2 minutes. 2-3 drops of content were discarded and counting chamber was then filled in the same way as in the red blood cell count. The counting chamber was placed under the microscope and examined under low

power objective (10x). The leukocytes in the 4 large squares (each 1 square mm.) of the counting chamber were counted x 50 and expressed the result in thousand per cu.mm.

3.15 Adverse Effects

After treatment there were no adverse effects on treated chickens.

3.16 Statistical analysis

The data were analyzed statistically between control and treated groups of chicken by IBM SPSS (Version 22.0).

CHAPTER IV

RESULTS AND DISCUSSION

To perform the experiment, eighty (80) sonali chickens were randomly divided into four groups each containing twenty (20) birds. They were fed with Eimeria oocyst for induction of infestation. Group T_0 birds were kept as negative control (unaffected) without giving Eimeria oocyst and any other treatment where as T_1 treated with Eimeria oocyst and next two groups (T_2 & T_3) were treated with 0.5% papaya leaves suspension and coxitril® liquid at a dose of 1 ml/litre water respectively. In this experiment, the efficacy of papaya leaves suspension on protozoal load, blood constituents, growth performance of sonali chicken infected with coccidiosis were investigated. The results of this study are discussed under following headings.

4.1 Anticoccidial effect of papaya leaves suspension and toltrazuril in sonali chicken

Protozoal load in feces of sonali chicken was presented in table 4.1.1. This study showed that, protozoal load in feces was significantly differed among the treated groups. Protozoal load significantly ($P<0.01$) increased in T_1 (positive control) group. Protozoal load significantly ($P<0.01$) decreased in T_2 and T_3 group supplied with 0.5% papaya leaves suspension and toltrazuril. This finding is similar as the result of efficacy of ethanolic extract of *Carica papaya* leaves as a substitute of sulphanomide for the control of coccidiosis in kabir chickens in Cameroon (Nghonjuyi *et al.*, 2015). AL Fifi (2007) showed that papaya leaves have anticoccidial properties.

Table: 4.1.1 Effect of papaya leaves suspension and coxitril® liquid (toltrazuril 2.5%) on number of oocyst per gram (OPG) in feces of sonali chicken

Days	T_0	T_1	T_2	T_3	Significant level
day 1	0 ± 0^{a}	331.6 ± 1.88^{c}	315.2 ± 1.77^{b}	347 ± 1.09^{d}	*
day 15	0 ± 0^{a}	341.8 ± 1.28^{c}	152.4 ± 1.63^{b}	0 ± 0^{a}	*
day 30	0 ± 0^{a}	349.8 ± 1.59^{c}	52.2 ± 1.28^{b}	0 ± 0^{a}	*

This table indicates Mean ± SE (standard error),
a, b, c Means in each row with different superscript are significantly different at (0.05),
*Correlation is significant at the 0.05 level of probability.

4.2 Hematological parameter

Effect of papaya leaves suspension and toltrazuril on blood profile of sonali chicken is given below:

A. Total Erythrocyte Count (TEC):

Total Erythrocyte Count (TEC) is presented in table 4.2.1. The values of TEC in all treated groups and control group were more or less similar and within the normal range at 15^{th} and 30^{th} days of experimental period. These values showed a little fluctuation were not statistically significant ($P>0.05$). This finding is similar as the result of hematological, biochemical and histo-pathological changes caused by coccidiosis in Chickens (Meskerem *et al.*, 2013).

Table: 4.2.1 TEC (million/mm^3) during experimental period

Days	T_0	T_1	T_2	T_3	Significant level
day 15	2.17±0.09	2.45±0.33	2.52±0.30	2.63±0.49	NS
day 30	2.12±0.07	2.71±0.19	2.86±0.17	2.73±0.17	NS

This table indicates Mean ± SE (standard error),
NS= Non significant.

B. Total Leukocyte Count (TLC):

The values of TLC are presented in table 4.2.2. At 15 days interval the values of TLC of all groups are differed followed by different superscripts in the same row. The values are also differed between columns among the treated groups. At the end of experiment the lowest TLC value (6.176±0.11) was recorded from T_3 group and the highest TLC (7.254±0.29) value was recorded from T_2 group. This finding is similar as the result of hematological, biochemical and histo-pathological changes caused by coccidiosis in Chickens (Meskerem *et al.*, 2013).

Table: 4.2.2 TLC (thousand/mm^3) during experimental period

Days	T_0	T_1	T_2	T_3	Significant level
Day 15	6.452 ± 0.45^{a}	6.476 ± 0.27^{b}	6.544 ± 0.48^{d}	6.486 ± 0.52^{c}	*
Day 30	6.588 ± 0.23^{b}	6.722 ± 0.29^{c}	7.254 ± 0.29^{d}	6.176 ± 0.11^{a}	*

This table indicates Mean ± SE (standard error),
a, b, c Means in each row with different superscript are significantly different at (0.05),
*Correlation is significant at the 0.05 level of probability.

C. Estimation of Hemoglobin (Hb):

Hemoglobin (Hb) is presented in table 4.2.3. The values in all treated groups and control group were more or less similar and the values were within normal range. They showed a little fluctuation but were not statistically significant (P>0.05). This indicates that there was no dietary effect of papaya leaves suspension and toltrazuril on Hb. This finding is similar as the result of hematological, biochemical and histo-pathological changes caused by coccidiosis in Chickens (Meskerem *et al.*, 2013).

Table: 4.2.3 Hemoglobin (mg/dl) during experimental period

Days	T_0	T_1	T_2	T_3	Significant level
day 15	8.99±0.41	9.37±0.31	9.37±0.18	9.01±0.65	NS
day 30	8.97±0.43	9.51±0.11	9.61±0.16	9.47±0.13	NS

This table indicates Mean ± SE (standard error),
NS= Non significant.

4.3 Body weight

Data of Table 4.3 indicated that there were a significant (P<0.01) increase in body weight in T_2 (papaya leaves suspension) and T_3 (toltrazuril) group than the T_0 (negative control) and T_1 (positive control). Birds treated with papaya leaves suspension (T_2 group) had the highest body weight gain compared to other groups. This weight gain due to papaya leaf containing papain enzyme. Papain is a protease enzyme that hydrolyzes proteins to short peptides, which is the key factor to increase protein digestibility and fast absorption and helps to

increase growth factors (Battaa *et al*., 2015) also showed significant (P<0.05) body weight gain for Dokki4 laying hens with different levels of papaya latex. These findings were also confirmed upon examination of papaya latex on rabbits (El-Kholy *et al.*, 2008; Zeedan *et al.*, 2009).

Table: 4.3 Effect of papaya leaves suspension and coxitril® liquid (toltrazuril 2.5%) on body weight (gm) of sonali chicken

Days	T_0	T_1	T_2	T_3	Significant level
day 1	182.78±1.14	182.77±1.49	182.42±1.24	184.89±1.44	NS
day 7	$233.56±0.39^{b}$	$214.09±1.63^{a}$	$255.22±0.08^{d}$	$241.52±1.01^{c}$	*
day 14	$290.94±1.19^{c}$	$276.15±0.35^{a}$	$295.89±1.56^{d}$	$289.83±0.77^{b}$	*
day 21	$373.87±1.81^{a}$	$387.98±1.74^{c}$	$429.91±0.61^{d}$	$381.17±1.46^{b}$	*
day 30	$507.11±2.34^{b}$	$498.32±2.67^{a}$	$625.48±2.74^{d}$	$521.96±3.27^{c}$	*

This table indicates Mean ± SE (standard error),
NS= Non significant,
a, b, c Means in each row with different superscript are significantly different at (0.05),
*Correlation is significant at the 0.05 level of probability.

CHAPTER V

CONCLUSION

Herbal medicines are progressively used all over the world. In this study papaya leaves showed 83.5% efficacy and toltrazuril showed 100% efficacy at the end of experiment. So, from the current experimental data, it may be stated that papaya leaves (*Carica papaya*) suspension and toltrazuril have anticoccidial effect. But toltrazuril is more effective than papaya leaves suspension. As toltrazuril shows drug resistance and other side effects, so papaya leaves suspension is more preferable than toltrazuril. Papaya leaves suspension also have significant effect on body weight and total leukocyte count (TLC). No significant effect in total erythrocyte count (TEC) and hemoglobin (Hb). There was no adverse effect found in herbal use of this papaya leaves suspension at the dose rate of 0.5% during this experimental period. In conclusion, this experiment supports the traditional use of papaya leaves (*Carica papaya*) for the control of coccidiosis. It can also be considered as a good growth promoter, leucocyte enhancer in sonali chicken. It may be stated that the papaya leaves (*Carica papaya*) may provide a new therapieutic avenue against coccidiosis because of its availability in our country and effectiveness. To draw a definite conclusion it demands elaborate study specially mechanism of action, contra-indication and histo-pathological effect of this ancient herbal medicine.

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