ASSESSMENT OF EFFICIENCY OF DIFFERENT COMBINED COCCIDIOSTATS AGAINST DIFFERENT EMERIA SPECIES INFECTION IN BROILERS IN BANGLADESH

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A Thesis

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CERTIFICATE

*This is to certify that the thesis entitled "***ASSESSMENT OF EFFICIENCY OF DIFFERENT COMBINED COCCIDIOSTATS AGAINST DIFFERENT EIMERIA SPECIES INFECTION IN BROILERS BANGLADESH***" submitted to the Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of Master of Science in Microbiology and Parasitology, embodies the result of a piece of bona fide research work carried out by Mohammad Arifur Rahman, Registration No. 19-10074 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

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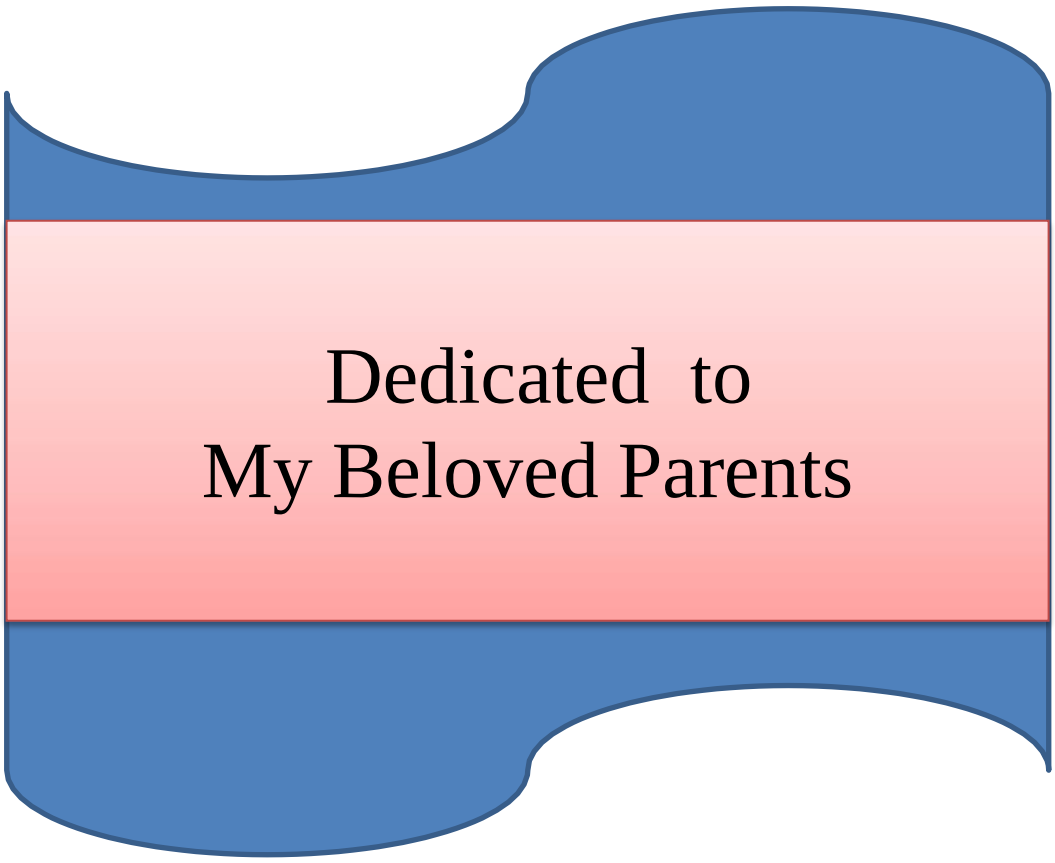
Dedicated to
My Beloved Parents

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The Author

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ACRONYMS AND ABBREVIATIONS

ABBREVIATION		FULL MEANING
G	=	Gram
Kg	=	Killogram
FCR	=	Feed Conversion Ratio
CONT'D	=	Continued
Dr.	=	Doctor
e.g.	=	For example
etc.	=	Et cetera
et al.	=	And others/Associates
i.e.	=	That is
M.S.	=	Master of Science
NO.	=	Number
sp.	=	Single species
spp.	=	Plural species
MT	=	Metric ton
GDP	=	Gross Domestic Product
DOC	=	Day old chick

ASSESSMENT OF EFFICIENCY OF DIFFERENT COMBINED COCCIDIOSTATS AGAINST DIFFERENT EIMERIA SPECIES INFECTION IN BROILERS IN BANGLADESH

ABSTRACT

Coccidiosis is considered as the major parasitic disease of poultry which seriously impairs the growth and productivity. This study was aimed to assess the efficacy of combined coccidiostats in broilers. A total of 1200 broiler chicks (Cobb 500) were reared in different feeding group such as Treatment- 1 (T1), Treatment- 2 (T2), Treatment- 3 (T3), Treatment- 4 (T4), Treatment- 5 (T5) which were treated with anticoccidial combined agents namely without coccidiostat, Maduramicin + Nicarbazin, Narasin + Nicarbazin, Semduramicin + Nicarbazin, Monensin + Nicarbazin respectively. It was found that T3 group consumed lower ration (2.797 kg/bird) followed by T5 group (2.825kg/bird), T2 group (2.835kg/bird), without anticoccidial T1 group (2.880kg/bird) and T4 group (2.888kg/bird) throughout life time. At the culling week, the highest body weight gain (1915g) was recorded in the T5, followed by T3 group (1883g), T4 group (1832g), T2 group (1808g) and T1 group (1805g). The lowest FCR (1.500) was observed in the T5 group, followed by T3 group (1.504), T4 group (1.547), T2 group (1.571), and T1 group (1.576). Highest lesion score recorded in controlled group which was not treated with anticoccidial drugs. On the other hand, lowest lesion score was observed in broilers of T5 followed by T3, T4, T2 and T1. *E. tenella* showed highest lesion score in broilers in comparison to *E. maxima* and *E. acervulina*. Monensin + Nicarbazin combination showed the best efficiency among coccidiostats used in broilers. The findings of the study might be helpful for the prevention and control of coccidiosis in broilers using proper anticoccidial drugs for both prophylaxis and treatment.

Keywords: Efficiency, coccidiostats, Eimeria, Lesion score, floor pen, Bangladesh

CHAPTER 1

INTRODUCTION

Poultry meat obtains a distinguished position as an important source of protein around the world, with a total production of approximately 120 billion tons annually, which represents more than one-third of the human's protein food and is expected to be double by 2050 (Alexandratos and Bruinsma, 2012). Poultry sector is also one of the most vibrant segments of agriculture sector in Bangladesh. It generates employment directly (20%) and partially (50%) for millions of people. Livestock also share GDP (13.44%) in agricultural (Livestock Economy, 2019-2020). Poultry meat provides about 78% of the total meat supply in Bangladesh, and is one of the best available sources for the production of high biological value animal protein (Rony *et al*., 2021). Commercial poultry farming in Bangladesh is expanding day by day. However, this sector is still confronted with many health problems like coccidiosis which is hindering its progress.

Although coccidiosis is a disease known for many years, it is still considered as the most economically important parasitic condition affecting poultry production worldwide. The major threats for commercial broiler farms in Bangladesh are mainly due to coccidiosis which causes a great economic loss to the poultry industry due to mortality, reduced body weight and increased expenses related to preventive and therapeutic control (Farooq *et al*., 1999). Globally, 3 billion dollar per year economic loss is attributed due to coccidiosis that impacts commercial poultry production (Dalloul and Lillehoj, 2006; Williams, 1999). Almost 70% of the estimated cost in poultry production is due to subclinical coccidiosis through the impact on weight gain and feed conversion rate. One of the reasons for these remarkable findings is probably the difficult diagnosis of subclinical coccidiosis, which prevents the industry to evaluate the best possible strategies for control of coccidiosis. The poultry industry spends a significant amount of money in the prevention and treatment of several diseases for making farm profitable worldwide.

Coccidiosis is a disease that is caused by protozoan parasites of the genus *Eimeria,* developing within the intestine of most domestic and wild animals and birds. Seven species of *Eimeria* are recognized to infect chickens, including *E. acervuline, E. maxima, E. mitis, E. tenella, E. necartrix, E. brunetti, E. praecox* (Shirley *et al*., 1986). Among these, *E. acervulina, E. tenella* and *E. necatrix* are the most prevalent species in the field (Alam *et al*., 2020) where each species infecting a specific region of the avian intestine. Based on epidemiological studies from the latest years, in broiler chickens, *E. acervulina*, *E. tenella*, *E. maxima,* are frequently found. *Eimeria* spp. mainly intracellular parasites of the intestinal epithelium having direct life cycle (single host). Both asexual and sexual reproduction take place in same host. Schizogony and Gametogony occur within the host, and sporulation and maturation of the fertilized zygote usually takes place outside the host. It is distributed worldwide in commercial poultry and wild birds. Coccidiosis is usually a disease of young birds, but birds can be infected at any time if never been exposed before. Coccidiosis goes hand-in-hand with other gut diseases, because it damages the gut mucosa and allows bacteria to enter causing secondary infections. Avian coccidiosis is a highly infectious disease which can be transmitted easily through insects, litters, equipment, drinking water, and contaminated diets. Coccidiosis is characterized by dysentery, enteritis, emaciation, drooping wings, poor growth and low production.

An average prevalence of coccidiosis (22.8%) in poultry industry which results huge economic damage in commercial broiler production in Bangladesh (Rahman *et al*., 2019). Coccidiosis is now the biggest challenge for broiler production because it is a constant challenge for the poultry industry, as biosecurity and management of most of the farms in Bangladesh is very poor. Most of the farmers do not maintain rules and regulation of biosecurity and biosafety. As Bangladesh is tropical country, there is a huge seasonal variation in respect to temperature and humidity. Control of the disease by the use of drugs is indispensable to achieve sustainable poultry production. In addition to control programs based upon chemotherapy or vaccination, satisfactory control of coccidiosis in poultry requires strict attention on hygiene and

sanitation, and biosecurity measures that limit human access to poultry facilities (Chapman, 2018).

The use of several drugs (Coccidiostats), alone or in combination, has proven to be an effective alternative in the struggle against avian coccidiosis. Coccidiostats are of two groups like Ionophore and Chemicals. Especially, ionophores in broiler chicken feeds have been the principal choice to control coccidiosis because resistance against ionophore develops slowly and they do not completely suppress parasite development, thus allowing the development of immunity in the host after first exposure (Noack *et al*., 2019). Therefore, these coccidiostats are supplied through feed in every meal to fight against coccidiosis in regular basis. However, the emergence of drug resistant strains, especially after a prolonged use of a drugs in feed and water has created serious drugs resistance problem.

Therefore, this study was designed in broilers in floor pen system to assess different combined products (Narasin + Nicarbazin, Monensin + Nicarbazin, Maduramicin + Nicarbazin, Semduramicin + Nicarbazin) in different trail groups along with control. We followed the intestinal lesion scoring technique described by (Johonson and Reid, 1970), to measure the efficacy of different combined coccidiostat products. The species that are observed *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella* as they are mainly found in broilers in Bangladesh. The ultimate outcome of this research was to find out which drug combination was more effective against which *Eimeria* spp. After all, this outcome may open a gateway for broiler farmers to control coccidiosis in farms for making sustainable farming. So, the objectives of this research were to

1. Find out the effective combination of coccidiostats against *Eimeria* spp.
2. To observe which coccidiostat combination yield the best growth performance of the broiler in floor pen system

CHAPTER 2

RIVIEW OF LITERATURE

Coccidiosis is an infectious disease of the intestinal tract of wild and domestic animals caused by different protozoa. Those parasites are widespread, especially where intensive production systems are used to raise livestock. They cause mortality, poor growth, and impaired performance. The concept of coccidiosis prevention in chickens by inclusion of drugs in the feed (prophylaxis) was first described in 1948 and involved the use of sulfaquinoxaline, the first feed additive for poultry (Grumbles *et al*. 1948). In the chicken, at least seven *Eimeria* species are recognized that parasitize different regions of the intestine (Shirley *et al*. 1986); many species are recognized in ruminants. The parasites are transmitted from one host individual to others via the feces which shed the transmission stage of the life cycle into the environment. Infection occurs from ingestion of sporulated oocysts in the litter of the shed.

Rony *et al*. (2021) reported that overall prevalence of coccidiosis in broiler was 34.48% in Gazipur, Bangladesh. Despite of routine anticoccidial treatment, coccidiosis was documented in all surveyed farms. At the age of 15 to 18 days of broiler, 68.07% of farmers used chemoprophylaxix. Among all coccidiosis usage, amprolium was the highest (74.78%) followed by toltrazuril (55.46), sulphaquinoxaline (23.52%), sulphaclozine (28.57%), sulphadimidine (24.36%) and sulphadimethoxime (24.36%). During coccidiosis outbreak, traditional herbal medicines like extracts of bollygum (*Litsea glutinosa*), sal (*Shorea robusta*) and arjuna (*Terminalia arjuna*) barks and leaves were also used by 4.20% farmers. Majority of the small-scale farms were operated with good quality DOCs, all- in-all-out system, daily cleaning of feeder and drinker, good flow of drinking water, mixture of old and new litter and routine anticoccidial treatment.

Shirley, 1986 found that, in gastrointestinal parasitism, coccidian infection plays a vital role as most important constrain for growth of poultry, various species reported from the chicken are *Eimeria acervulina, E. brunette, E. necartris, E. tenella,*

E. maxima, E. mitis, E. mivati and E. hagani etc. These species have predilection site in the different part of gastrointestinal tract. *E. acervulina* occurs in the epithelial cells of the anterior portionof the small intestine mainly in duodenum. *E. brunette* occurs in the mucosa of the lower portion of the small intestine, caecum, rectum and cloaca. *E. tenella* is present in caecum. *E. necatrix* occurs in the jejunum, mid gut, caecum and other parts of the large intestine. They produce severe damage to the site of their predilection in various mammals including human being.

Finlay, 1993 mentioned that coccidiosis is an intestinal infection that is caused by various species of intracellular protozoan parasites belonging to the genus *Eimeria*. In general, coccidiosis results in intestinal lesions, enteritis, and diarrhea and extensive damage to the digestive tract may lead to death (Cook, 1988). There are different species of avian coccidia, such as *Eimeria tenella, Eimeria necatrix, Eimeria brunetti, Eimeria praecox, Eimeria acervulina, Eimeria mitis,* and *Eimeria maxima*. Each *Eimeria* species has a particular predilection site in the chicken digestive tract; for example, *E. tenella* attacks the cecum.

Ayaz, 2003 found that the most common and pathogenic species that affects the poultry industry in Pakistan is *E. tenella,* which results in high mortality.

Gharekhani *et al*. (2014) showed that in western Iran, 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 from 220 broiler farms in this region. Mixed infections were observed in all of the positive farms. This is the first report of coccidiosis rate in broiler farms in this region.

Looker, 1986 noted that most of the anticoccidials show their greatest efficacy against the 1st and 2nd asexual cycle, some inhibits sexual stages of the life cycle. Only few anticoccidials disturb the chemical metabolic pathway by which the drug block the specific stage of the parasite Selection of an anticoccidial is based on the ability of the drugto improve weight and feed conversion and to suppress the development of lesions (Reid, 1975).

Agtarap *et al.* (1967) showed that monensic acid is a fermentation product of *Streptomyces cinnamonensis,* was first described and have a broad-spectrum effect

against *Eimeria*. It forms lipid-soluble complexes with sodium and potassium cations, leading to increased permeability of the membrane for these ions. Monensin is able to transport sodium ions through membranes in both electrogenic and electroneutral manner (Mollenhauer *et al*. 1990). Horses are particularly susceptible to monensin poisoning (Matsuoka *et al.* 1996). Reid, (1975) reported that Monensin increases the weight gain, feed conversion ratio and in some cases causes suppression of necrotic enteritis. It is more efficient than amprolium, clopidol and zoalene controlling of coccidiosis.

Jeffers, 1988 mentioned that Narasin is a polyether antibiotic obtained from *Streptomyces aureofaciens* It is a derivative of salinomycin having an additional methyl group, therefore alternatively called (4S)-4-methyl salinomycin. When combining different ionophores with nicarbazin, Challey and Jeffers (1973) found that combinations of nicarbazin and narasin had synergistic activity. A combination product containing both active pharmaceutical ingredients (API) in 1:1 ratio was developed (Maxiban®). Very high levels of narasin caused death in sows, leg muscle weakness in turkeys, and cardiopulmonary clinical signs in 15% of the rabbits from Brazilian rabbit farms (Oehme and Pickrell, 1999).

Oehme and Pickrell, (1999) recorded that the ionophores maduramicin was first isolated from the bacterium *Actinomadura yumaensis* (Liu *et al.*, 1983). It is a large heterocyclic compound is widely used for commercial broiler production. Maduramicin is the most toxic of all the ionophores for non-target animals.

Semduramicin can be isolated from *Actinomadura roserufa* (Tynan *et al*., 1992). It is a highly effective drug against *Eimeria* and is well tolerated by chickens (Ricketts *et al*., 1992; Logan 1993).

The speed of emergence of resistant strains of coccidian in the field is given by Reid (1975) as follows Glycomide- very rapid; Quinolones- rapid; Clopidol- less rapid, Sulphonamides, nitrofurons, robenidine- moderate; Amprolium- slow; Nicarbazine- very slow and Monensin- absent or very slow. Resistance is more likely to develop in birds reared under intensive condition than in farm animals (Einstein *et al*., 1994).

Amer *et al*., 2010 reported that 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 Eimeria oocysts infection in chickens. Area of drug resistance needs more investigation to explore its magnitude, mode and how to overcome.

Stallbaumer and Daisy, (1988) studied that the sensitivity was established in vivo of 47 isolates of coccidia obtained from commercial broiler sites, to monensin, narasin, salinomycin and nicarbazin. Seventeen samples were obtained from the UK, 15 from the Netherlands, six from West Germany, six from Spain, and one each from France, Italy and Israel. Fourteen (30%) samples were predominantly Eimeria acervulina, 18 (38%) predominantly *E. tenella*. The remaining 15 (32%) were mixed species, predominantly E. acervulina and E. tenella with some samples showing lesions characteristic of E. maxima and E. brunetti. All isolates were ionophore-tolerant, as judged by lesions, weight gain, feed conversion and mortality. Most isolates were sensitive to nicarbazin. Cross-resistance was evident between all ionophores but not between ionophores and nicarabazin.

Farran *et al*., 2020 conducted a study to assesses the efficacy of two different potentiated ionophores (monensin and narasin) and a chemical (nicarbazin, a shuttle) in programme with narasin to control coccidiosis in challenged male broilers through the evaluation of performance and macroscopic lesions of internal organs. A total of 4400 broiler chicks were housed in 44 flor pens of 100 birds each; birds of the first group were fed a narasin/nicarbazin (NN) containing diet from 0 to 27 day of age followed by narasin diet until market age, and those of group 2 were fed a narasin/nicarbazin (NN) containing diet from 0 to 27 day of age followed by narasin

ration for the same trail periods. The coccidia challenge was performed at day 1 by the inclusion of a proven *Eimeria*- infected litter in the bedding for all floor pens followed by an oral challenge by using a commercial cocci vaccine at 7^{th} day of age with a concentration of 11 times from the normal dose. Although results showed that narasin/nicarbazin and monensin/nicarbazin treatments both demonstrated efficacy at reducing cocci lesions, narasin/nicarbazin significantly increase the body weight and reduced feed conversion ratio ($P < 0.05$) at 27 day (1405 gm vs. 1284 gm and 1.458 vs. 1.566, respectively) and at 33 day of age (2178 gm vs. 2026 gm and 1.549 vs. 1.462, respectively). Weight and weight percentage of the ready to cook carcass and carcass cutup parts were significantly improved by narasin/nicarbazin. Narasin/nicarbazin inclusion in the diet for 0 to 27 days followed by narasin until market weight was a successful strategy, because in addition to preventing and controlling of coccidiosis, it improved performance of broiler chicken.

CHAPTER 3

MATERIALS AND METHODS

3.1. Sampling area

The present study was conducted to find out the efficiency of different combined coccidiostats against the infecting of different species of *Emeria* and overall performance in broiler production in Bangladesh. The trial was conducted in the trial farm of Nourish Poultry and Hatchery Ltd. which is located in Shreepur, Gazipur during the time period from April, 2020 to May, 2020.

Figure 1: The map of Sreepur upzila, Gazipur district indicates geographical location of Nourish Poultry and Hatchery Ltd.

3.2. Experimental birds, feed and management

A total of 1200 broiler chicks (Day old, Cobb–500) were collected from Nourish Hatchery. The chicks were reared under standard nutrition and management practices from day 1 to 33 in a floor pen system. Feeding schedule was pre-starter ration up to 10 days of age, starter ration 11–21 day and finally finished with grower ration. The feed and water were provided ad libitum. The temperature was maintained at 30ºC -

34ºC during the first week of age and then gradually reduced room temperature on 14th day. Lighting was provided for 24 hours throughout the experimental period. All the birds were vaccinated for New Castle disease on 5th day and 25th day, for Infectious Bursal disease on 11th day and 18th day of age. No supplement and antibiotics were used throughout the rearing period except chlorination of water. No group of birds were given challenge for *Eimeria* spp. and no preventive measures were taken for controlling the coccidiosis rather just use of coccidiostats in feed.

Figure 2: Feeding and Management of birds

3.3. Coccidiostats

Four commercially found combined coccidiostats were used in this trail. These are:

1. **Treatment 2** (Maduramicin 0.75 ppm + Nicarbazin 8 ppm)
2. **Treatment 3** (Narasin 8 ppm + Nicarbazin 8 ppm)
3. **Treatment 4** (Semduramicin 3 ppm + Nicarbazin 8 ppm)
4. **Treatment 5** (Monensin 8 ppm + Nicarbazin 8 ppm)

3.4. Trial design

The total shed was divided into 40 pens, each of which was surrounded by iron net. The 30 chicks were placed in each pen. As we were dealt with five treatment group so each treatment group had 8 pens. The pens of each treatment group were placed randomly throughout the shed. Table 1 shows the detail of each group.

Table 1: Bird groups with treatment dosage.

Groups	**Coccidiostats**	**Doses**	**Duration of feeding**
Treatment-1(T1)	Control (Without coccidiostat)		
Treatment-2(T2)	Maduramicin 0.75 ppm + Nicarbazin 8 ppm	500 g/MT	33 days
Treatment-3(T3)	Narasin 8 ppm + Nicarbazin 8 ppm	500 g/MT	33 days
Treatment-4(T4)	Semduramicin 3 ppm + Nicarbazin 8 ppm	500 g/MT	33 days
Treatment-5(T5)	Monensin 8 ppm + Nicarbazin 8 ppm	500 g/MT	33 days

3.5. Data collection

The data for several parameters like mortality, body weight, feed consumption, FCR and intestinal lesion score for coccidiosis were secured for performance analysis of different treatment groups that fed with different coccidiostats. The data were collected on day 7^{th}, 14^{th}, 21^{th}, 28^{th} and 33^{th}.

3.5.1. Body weight gain:

Standard weight machine was used to take the weight in weekly basis and 10% birds from each pen were weighed and make average to represent each group. Only at 33^{th} day, all birds of each pen were weighed.

3.5.2. For feed consumption:

The feed consumption of each pen was calculated once in a week and made average for each group.

3.5.3. Feed conversion ratio (FCR):

Feed conversion ratio was calculated as the kg. of feed consumed to produce one kg of live weight. To calculate the FCR following formula was used-

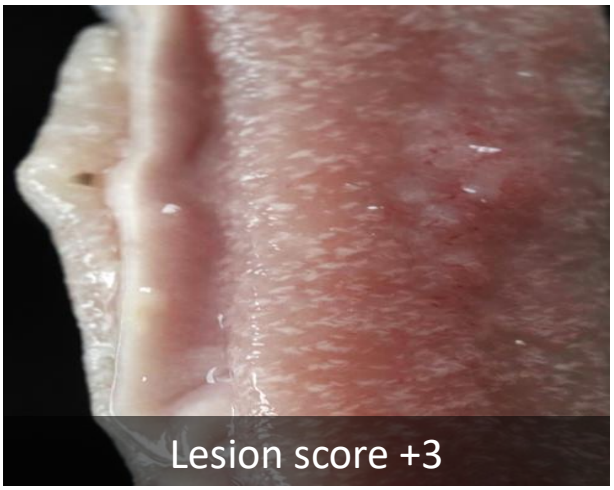
$$FCR = \frac{\text{Feed consumed by birds}}{\text{Live Weight of Birds}}$$

3.5.4. Lesion Scoring:

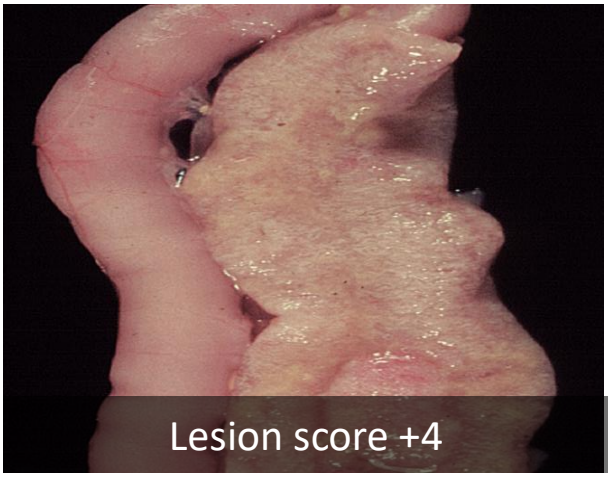
At 28th day, one bird was randomly selected per pen (8 birds per treatment) and then were sacrificed for necropsy analysis. Each *Eimeria* has specific site for their multiplication in the intestinal tract of host. Like, *E. acevulina* multiply in the Duodenum (mostly), *E. Maxima* multiply in the jejunum and ileum and *E. tenella* multiply in the caecum. So, we checked lesions in the intestine and the lesions were scored by the lesion scoring technique described by Johonson and Reid (1970).

Scattered white plaque like lesions, broken rice appearance, look like rungs of ladder. Maximum of 5 lesions per cm² in the duodenum.

Lesions are much closer but not coalescent. No thickening of intestinal wall. Digestive tract contents are normal



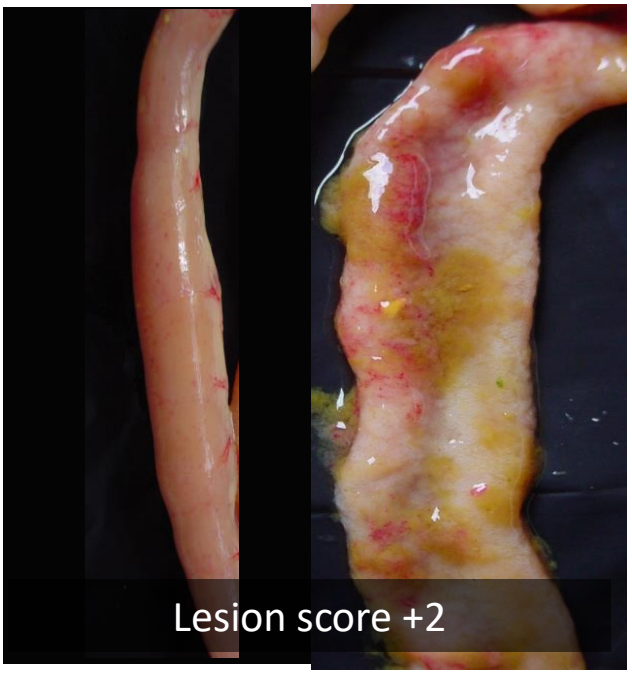
Lesions are numerous enough to cause coalescence and giving the intestine a coated appearance. Thickening of intestinal wall. Digesta becomes watery



Mucosal wall is greyish with individual lesions completely coalescent and indistinguishable. The intestinal wall is very much thickened, creamy exudates in the digesta.

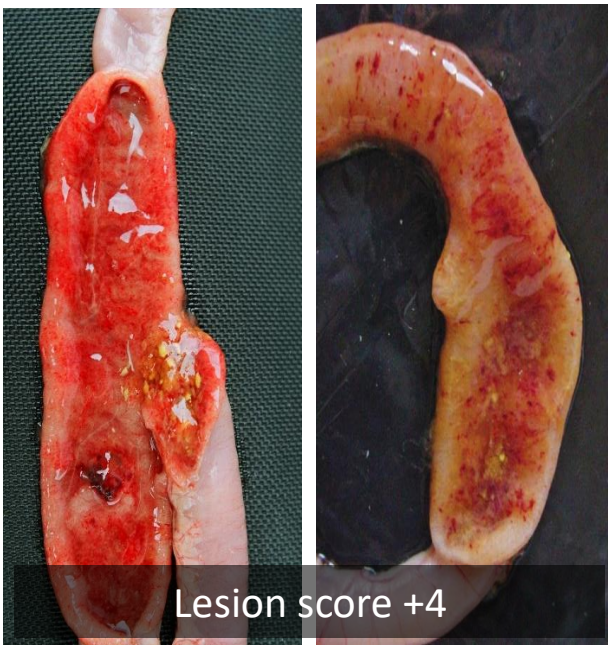
Figure 3: ***Eimeria acervulina***

The serosal and mucosal surface of Jejunum and ileum may be speckled with numerous red petechiae. There is little or no ballooning of the intestine.



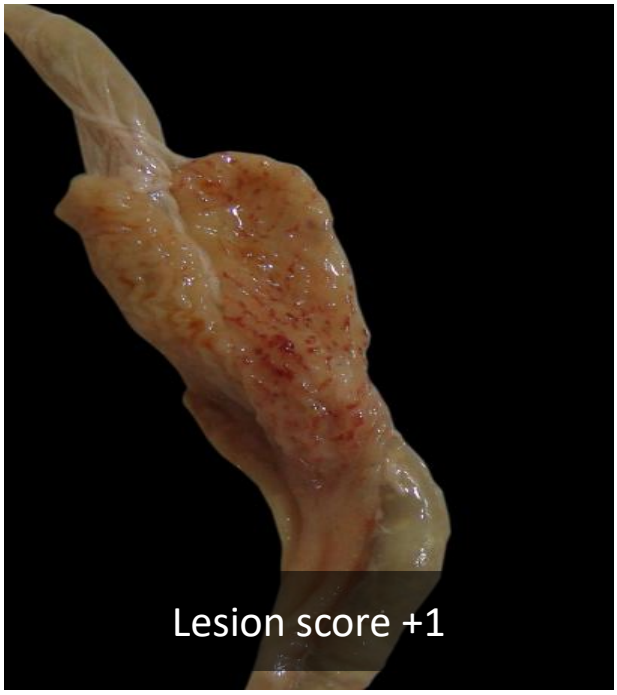
The serosal surface may be speckled with more numerous red petechiae and intestine might be filled with orange mucous. Little ballooning of the intestine. Slightly thickening of the intestinal wall.

Numerous red petechial hemorrhages on serosal surface. The intestinal contents are filled with pinpoint blood clots and mucus. The intestinal wall is thickened and ballooned.

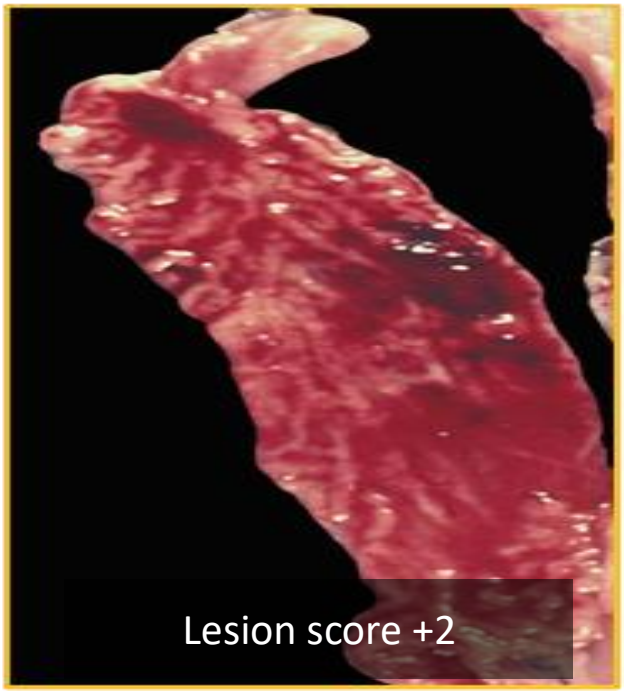


Contains numerous blood clots and digested red blood cells giving a characteristic color and putrid odor. The intestinal wall is greatly thickened and ballooned.

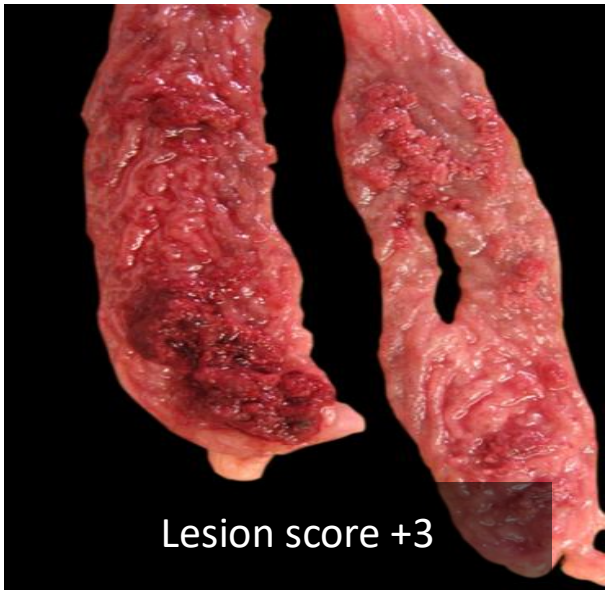
Figure 4: ***Eimeria maxima***



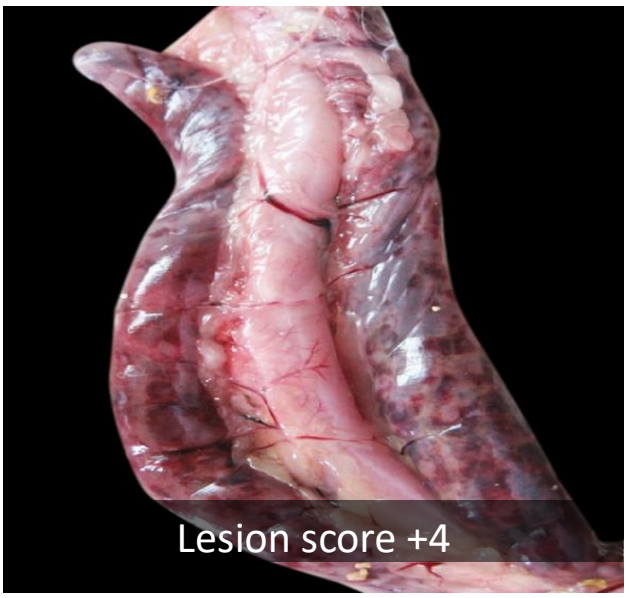
Very few scattered petechiae on the caecal wall. No thickening of the caecal wall. Normal caecal content are present.



Lesions more numerous, with noticeable blood in the caecal contents. Slightly thickening of the caecal wall. Normal caecal content are present.



Large amounts of blood or caecal cores are present. Caecal wall are greatly thickened. Little fecal contents are present in the caeca.



Cecal wall greatly distended with blood or large caseous cores. Fecal debris lacking or included in the cores. Bloody diarrhea (non digested blood) and mortality.

Figure 5: ***Eimeria tenella***

CHAPTER 4

RESULTS

4.1. Weekly feed consumption per bird

A total of 1200 broiler chicks (Cobb500) were reared with different Treatment group such as Treatment-1 (T1), Treatment-2 (T2), Treatment-3 (T3), Treatment-4 (T4), Treatment-5 (T5) which was treated with anticoccidial combined agents. The average feed consumption per bird at the culling week was 2.880kg, 2.835kg, 2.797kg, 2.888kg, 2.825kg for T1, T2, T3, T4, T5 respectively. It was found that T3 treated group consumed lower ration (2.797 kg/bird) followed by T5 treated group (2.825kg/bird), T2 treated group (2.835kg/bird), without anticoccidial T1 treated group (2.880kg/bird) and T4 treated group (2.888kg/bird) throughout life time.

Table 2: Feeding performance based on weekly feed consumption per bird (Cobb 500)

Treatment Group	Total flock	1st week (kg)	11th day (kg)	2nd week (kg)	3rd week (kg)	4th week (kg)	Culling week (kg)
T1 (without Coccidiostat)	Cobb (30*8)	0.149	0.351	0.565	1.193	2.110	2.880
T2 (Md + Nb)	Cobb (30*8)	0.154	0.357	0.574	1.156	2.075	2.835
T3 (Nr + Nb)	Cobb (30*8)	0.151	0.351	0.562	1.132	2.079	2.797
T4 (Sd +Nb)	Cobb (30*8)	0.150	0.352	0.572	1.220	2.131	2.888
T5 (Mn + Nb)	Cobb (30*8)	0.149	0.352	0.572	1.144	2.066	2.825

4.2. Body weight gain

The average body weight of broiler chicks (Cobb500) at the culling week in the dietary group T1, T2, T3, T4, T5 were 1805g, 1808g, 1883g, 1832g, 1915g, respectively. At the culling week, the highest body weight gain (1915g) was recorded in the T5 treated dietary group, followed by T3 treated dietary group (1883g), T4 treated dietary group (1832g), T2 treated dietary group (1808g). The lowest weight gain was observed in T1 treated dietary group (1805g).

Table 3: Body weight (gm) gain ranking weekly in Cobb-500 broiler flock

Treatment group	Total flock	1st week (gm)	11th day (gm)	2nd week (gm)	3rd week (gm)	4th week (gm)	Culling week (gm)
T1(without Coccidiostat)	Cobb (30*8)	195	372	525	823	1412	1805
T2 (Md + Nb)	Cobb (30*8)	197	375	534	850	1435	1808
T3 (Nr + Nb)	Cobb (30*8)	201	376	540	864	1468	1883
T4 (Sd +Nb)	Cobb (30*8)	199	380	535	862	1467	1832
T5 (Mn + Nb)	Cobb (30*8)	201	379	541	913	1517	1915

4.3. Food conversion ratio (FCR)

The average FCR of broiler chicks (Cobb500) at the culling week in the dietary feed group T1, T2, T3, T4, T5 were 1.576, 1.571, 1.504, 1.547, 1.500, respectively. The lowest FCR (1.500) at the culling week was observed in the T5 treated dietary group, followed by T3 treated dietary group (1.504), T4 treated dietary group (1.547), T2 treated dietary group (1.571), and T1 treated dietary feed group (1.576). But only in case of 11th day, the flock nourished with T4 documented the lowest FCR than the other anticoccidial treated feed group. The flock were reared through feed without any anticoccidial drugs showed highest feed conversion ratio (FCR) than the flock were reared through feed with anticoccidial drugs.

Table 4: Feeding performance based on weekly FCR in Cobb-500 broiler flock

Treatment Group	Total flock	1st week FCR	11th day FCR	2nd week FCR	3rd week FCR	4th week FCR	Culling week FCR
T1 (without Coccidiostat)	Cobb (30*8)	0.789	0.960	1.095	1.436	1.485	1.576
T2 (Md + Nb)	Cobb (30*8)	0.757	0.935	1.070	1.405	1.470	1.571
T3 (Nr + Nb)	Cobb (30*8)	0.751	0.933	1.052	1.311	1.408	1.504
T4 (Sd +Nb)	Cobb (30*8)	0.756	0.926	1.060	1.327	1.417	1.547
T5 (Mn + Nb)	Cobb (30*8)	0.740	0.930	1.045	1.306	1.391	1.500

4.4. Lesion score

Cumulative scores of lesions are showed here for each treatment group for each species. In T5, lesion score of *E. acervulina* showed 1 where *E. tenella* showed 3 followed by *E. maxima* showed 4. In T3, *E. acervulina* showed 1 where *E. tenella* showed 7 followed by *E. maxima* showed 5 lesion score. In case of T4, *E. acervulina* showed 2 where *E. tenella* showed 6 followed by *E. maxima* showed 9. On the other hand, in T2, E. *acervulina* showed 2 where *E. tenella* showed 11 followed by *E. maxima* showed 13. But, in T1, *E. acervulina* showed 4 where *E. tenella* showed 15 followed by *E. maxima* showed 13 lesion score. Highest lesion score was recorded in controlled group which not treated with anticoccidial drugs. On the other hand, lowest lesion score was observed in broilers that treated with T5 followed by T3, T4 and T2. *E. tenella* showed highest lesion score in broilers if compared with *E. maxima* and *E. acervulina*.

Table 5: Lesion score of different *Eimeria spp.* treated by different anticoccidial drugs

Treatment Eimeria	Treatment -1	Treatment -2	Treatment -3	Treatment -4	Treatment -5
E. acervulina	4	2	1	2	1
E. maxima	13	13	5	9	4
E. tenella	15	11	7	6	3

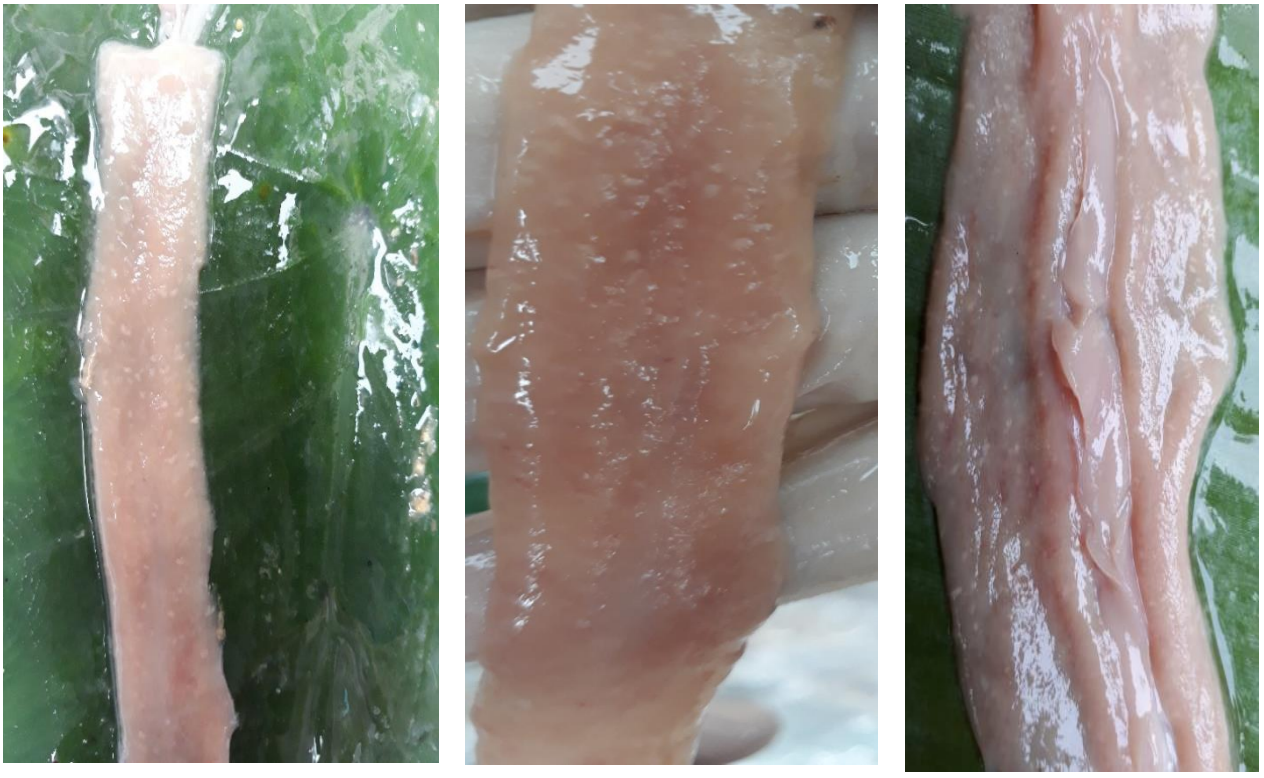


Figure 6: Post mortem lesion for *Eimeria acervuline* in Duodenum.

Figure 7: Post mortem lesion for *Eimeria maxima* in Jejunum.

Figure 8: Post mortem lesion for *Eimeria tenella* in Caecum.

CHAPTER 5

DISCUSSION

Eimeria species are responsible for serious economic losses in the broiler farms in Bangladesh. Several predisposing factors like High stocking density, high temperature and humidity, wet litter, poor ventilation, low immune status of the host, high oocyst challenge, enteritis from any other causes and lack of efficacy of coccidiostats are responsible for higher prevalence of coccidiosis. Wet litter favors the growth/sporulation of oocyst of coccidia causing disease in broiler. On the other hand, proper management of litter reduces the chance of getting litter wet, thus minimizing the problem of coccidiosis. For proper management of coccidiosis, we have to deal with all the predisposing factor carefully. Vaccination is another possible measure which help to reduce infection. Low doses of live sporulated oocyst of various *Eimeria* species (as vaccine) is given on day old chicks either on hatchery or farm. Vaccine serves only to introduce the infection; chickens are reinfected by the progeny of vaccine strain on the farm. Species- specific immunity usually develops after natural infection, primarily a T cell response. The degree of immunity depends on the extent of infection and the number of reinfections.

Proper mixing of anticoccidial drugs in feed is also used for prevention of outbreak of disease (Kabell *et al*., 2006). Anticoccidial drugs are used not only as preventive measures but also prophylactic treatment. Anticoccidial drugs belong to two classes: ionophores and synthetic chemicals. Ionophores (such as monensin, narasin, salinomycin, semduramicin, maduramicin and lasalosid) are chemical compounds that reversibly bind and transport ions through biological membranes in the absence of protein pore. The ionophores can disrupt the membrane potential and thus exhibits cytotoxic properties. So, the ionophores have the ability to disrupt the ion gradients across the parasitecell membrane. Chemical coccidiostat like Nicarbazin enters the sporozoite cell and paralyze the intracellular enzymes that supply ATP. As a result, there is cessation of Na-K pump.

The present study gives an overview on efficacy of different combined coccidiostat drugs with different species of *Eimeria* in broiler in floor pen system in Bangladesh. In this study, we used 4 coccidiostat drugs namely, T2 (Maduramicin 0.75 ppm + Nicarbazin 8 ppm), T3 (Narasin 8ppm + Nicarbazin 8ppm), T4 (Semduramicin 3 ppm + Nicarbazin 8 ppm), and T5 (Monensin 8 ppm + Nicarbazin 8 ppm), in feed of broiler.

During the prophylaxis treatment of broiler, the flock was treated with T3 showed lowest feed intake per bird (2.297 kg) followed by T5 (2.825 kg), T2 (2.835 kg), T1 (2.880 kg) and T4 (2.888 kg). At the culling week, the highest body weight gain(1915gm) was recorded in the T5, followed by T3 (1883g), T4 (1832g), T2 (1808g), and T1 (1805g). These findings are in agreement with Reid (1975) who reported that Monensin increased the weight gain and feed conversion ratio.

The lowest FCR (1.500) was observed in T5, followed by T3 (1.504), T4 (1.547), T2 (1.571), and T1 (1.576) at the culling week which were also in accordance with Reid (1975).

This study documented that weight gain and feed conversion ratio (FCR) were significantly better in broilers reared through coccidiostats compared with without any coccidiostat. Because Coccidiosis does an extensive histological damage of intestinal epithelia. Enterocytes are ruptured by the releasing of first and second generation of merozoites. As a consequence, we found intestinal inflammation, increase mucous production, reduce the intestinal integrity and reduce intestinal barrier function (Destruction of tight junction). Due to this, we found shortening of intestinal villi, that limits the area of absorption which impaired the absorption of nutrients. Also, lot of energy is wasted from the birds to counteract this infection by its immune system which can be used for growth. Coccidiosis also altered the electrolyte balance in gut. An amount of ATP is also used to maintain the balance which can be used for growth. As a result, we found reduced daily weight gain and increased feed conversion ratio. Specific Chemoprophylaxis (use of coccidiostat in feed) significantly reduce the *Eimeria* infection in birds and its detrimental effects on intestinal tract. Thus,

coccidiostat helps to achieve expected weight gain and FCR. This report is similar to Stallbaumer and Daisy, (1988) from Europe in broilers, Morsy *et al*., from Egypt in quails.

The findings of the post-mortem examination were based on naked eyes observation on the intestine of the birds. The findings of this experiment showed that the extreme lesion score was observed in birds reared without Coccidiostat. But chicken treated with (Monensin +Nicarbazin) showed lowest lesion score followed by those treated with (Narasin +Nicarbazin), (Semduramicin +Nicarbazin) and (Maduramicin +Nicarbazin). *Eimeria tenella* was recorded to cause severe lesion score than *Eimeria maxima* and *Eimeria acervuline.* These observations were similar to those of Amer *et al*. (2010). Alam *et al*. (2020) recorded that *E. tenella*, *E. acervulina*, *E. maxima*, *E. brunetti* and *E. necatrix* were mostly found in broiler chicken in Bangladesh and in most of the cases, the broiler had mixed infection with *E. tenella* and *E. acervulina*. These observations are in agreement with the findings of this study. As coccidiosis is an omnipresent disease, in Bangladesh the mixed infection rate is high. It is due to lack of proper knowledge to maintain the farm condition, to develop the best prevention programme and to recognise the factors that influence the incidence of the disease. Bad farm management includes wet litter that encourages the oocyst sporulation, contaminated drinkers and feeders, improper ventilation, high stocking density and less gap between two batch.

Coccidiostats are used usually in all rations for meat type birds like broiler raised under floor pen management. Most of the anticoccidials are decrease susceptibility in broiler production due to their overuse, misuse and abuse; sometimes long-term use of anticoccidial as feed supplements. Resistance is more likely to develop in birds reared under intensive condition than in farm animals (Einstein *et al*., 1994). Reid (1975) documented that the drugs becoming resistant against coccidian oocyst are Glycomide- very rapid; Quinolones- rapid; Clopidol- less rapid; Sulphonamides, Nitrofurons, Robenidine- moderate; Amprolium- slow; Nicarbazin- very slow and Monensin- very slow. So, the efficacy of Monensin Nicarbazin combination recorded as most effective in broiler production.

In our study, birds were reared with different anticoccidial drugs until slaughtering. But some birds showed lesion score of *Eimeria* spp. which was agreement with Nematollahi *et al*. (2009) who reported the failure of chemoprophylaxis to control the disease. This might be due to misuse of coccidiostats or through the development of resistance by the local strain of Eimeria to variable compounds (Hadipour *et al*., 2011). Chapman H.D *et al*., 1997 reported that drug resistance or reduce susceptibility is the ability of a parasitic strain to multiply or to survive in the presence of concentrations of a drug that normally destroy parasites of the same species or prevent their multiplication. Resistance development is a natural selection process. After a period of use, the efficacy of the product (the sensitivity of *Eimeria* towards it) decline. Drug resistance is responsible for subclinical coccidiosis, and subsequently, for impaired economic performance as well as body weight gain and feed conversion ratio (Gyorke *et al*., 2013).

Chapman *et al*., 1997 also reported that there is a relation between oocyst leakage and resistance development. Zero leakage of oocyst initiate rapid resistance development, on the other hand, allowing leakage of oocyst initiate slow resistance development. Chemical coccidiostats show rapid resistance development whereas opposite for ionophores. Nicarbazin is the only chemical which shows slow resistance development. As Chemicals and ionophores have different mode of action, so combination with Nicarbazin and ionophores products develop resistance slower than each constituent independently. The best possible recommendation is gone through rotation programme (Changing the anticoccidial tools to one of another class after a few cycles) in order to prevent drug resistance which was in agreement with Gyorke *et al*., 2013.

CHAPTER 6

SUMMARY AND CONCLUSION

Coccidiosis is a parasitic disease with great economic significance. A holistic approach is needed to manage coccidiosis at farms which includes proper management (cleaning & disinfection, maintain dry litter, stocking density, reduction of stress), good nutrition (balanced diet with low CP), vaccination and specific chemoprophylaxis (Coccidiostats). Coccidiostats are using successfully for decades to manage coccidiosis around the world. However, long-term use of each anticoccidial drugs has led to development of resistance. In order to minimize the occurrence of resistance,various rotation or shuttle programs is practiced. Use of combination drugs are even more successful than single products.

In was concluded from the present investigation that supplementation of coccidiostat in feed affected the body weight and FCR positively along with reduction of intestinal lesion score caused by *Eimeria* spp. Although we didn't provide any challenges artificially, but we found intestinal lesion of *Eimeria* infection. It indicates the presence of natural challenge of *Eimeria* oocyst through out the farm, which again proves its omnipresence. Monensin Nicarbazin combination are found to be most effective among others in terms of growth performance and reduction of intestinal lesions. Hence combination coccidiostat and their proper way of application may be explored as an effective tool for successful control of coccidiosis.

CHAPTER 7

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