# EFFECTS OF COPPER SULPHATE ON THE GROWTH PERFORMANCE AND REDUCTION OF PATHOGENIC BACTERIAL LOAD IN THE GUT OF COMMERCIAL BROILER

**A THESIS**

**BY**

**MD. SHIPON AFROSE**
Registration No. 1605154
Session: 2016-2017
Semester: January – June, 2018

**MASTER OF SCIENCE (M.S.)**
IN
**POULTRY SCIENCE**

**DEPARTMENT OF DAIRY AND POULTRY SCIENCE**
**HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR - 5200**

**MAY, 2018**

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**A Thesis**

Submitted to the Department of Dairy and Poultry Science
Hajee Mohammad Danesh Science and Technology University, Dinajpur in partial fulfillment of the requirements for the degree of

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**DEPARTMENT OF DAIRY AND POULTRY SCIENCE**
**HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR - 5200**

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*Dedicated to*

*My*

*Beloved Parents*

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*The Author*

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# ABSTRACT

A study was carried out to investigate the effect of copper sulphate on the performance of broilers. A total number of 144 day-07 old chicks were randomly divided into four dietary treatment groups having 3 replications containing 12 birds in each replication among which four dietary group were considered: $T_0$ (Basal diet with no supplemental $CuSO_4$); $T_1$ (Control + $CuSO_4$ @ 100 mg/Kg feed); $T_2$ (Control + $CuSO_4$ @ 175 mg/Kg feed) and $T_3$ (Control + $CuSO_4$ @ 250 mg/Kg feed) respectively. Data on live weight and feed intake were collected and finally three birds from each replication were randomly selected and slaughtered for measuring carcass characteristics at 35 days of experimental periods. By using the SPSS software the collected data were analyzed at 5% level of significance. Total feed intake were non-significant (P>0.05) among the treatment groups but final live weight, live weight gain and FCR were significantly differed among the treatment groups. Significantly (P<0.05) higher body weight gain was found in $T_2$ (2501.25±34.95) which was followed by $T_1$ (2424.74 ±39.97) compared to $T_4$ (2367.11±28.4) and $T_0$ (2355.89±42.7) group respectively. The best and lowest FCR was found in $T_2$ (1.40±0.022) than $T_0$ (1.49± 0.03) while FCR in $T_1$ and $T_4$ group was 1.45±0.062 and 1.48± 0.23 respectively. In the present study, it was found that copper sulphate had significant (P<0.05) effect on dressing %, abdominal fat weight (g) and gizard weight (g) but no significant (P>0.05) effect on heart weight (g) and liver weight (g). The load of *E. coli* and *Salmonella* sp. was counted from the sample of intestine, cloaca and caeca of 35 days aged broiler. The *E. coli* and *Salmonella* sp. load in intestine, cloaca and caeca was significantly decreased in $CuSO_4$ treatment groups compared to control group. Cost effective analysis of the present study revealed that net Profit/ broiler (Taka) was significantly highest in $T_2$ (51.86±3.72) which was followed by $T_1$ (42.62±4.34), $T_3$ (36.23±4.18) and $T_0$ (36.06±4.59) respectively. As comparing all the parameters, $CuSO_4$ @ 175 mg/Kg feed can be added in commercial broiler diet for profitable broiler production.

Key words: Broiler, Copper sulphate, Growth performance, Carcass characteristics, Profit

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| | | |
|---|---|---|
| % | : | Percentage |
| & | : | And |
| < | : | Less than |
| > | : | Greater than |
| °C | : | Degree Celcius |
| ADG | : | Average Daily Gain |
| AGP | : | Antimicrobial Growth Promoter |
| ARC | : | Agricultural Research Council |
| CF | : | Crude Fiber |
| CP | : | Crude Protein |
| Cu | : | Copper |
| $CuSO_4$ | : | Copper Sulphate |
| d | : | Day |
| DM | : | Dry Matter |
| EC | : | European Commission |
| *et al.* | : | Associates with |
| FCR | : | Feed Conversion Ratio |
| FI | : | Feed Intake |
| Fig. | : | Figure |
| g | : | Gram |
| GfE | : | Gesellschaft fur Ernahugsphysiologie |
| HSTU | : | Hajee Mohammad Danesh Science and Technology University |
| Kg | : | Kilogram |
| ME | : | Metabolizable Energy |

| | | |
|---|---|---|
| mg | : | Miligram |
| MUFA | : | Monoenic Fatty Acids |
| NRC | : | National Research Council |
| NS | : | Not Significant |
| ppm | : | Parts Per Million |
| PUFA | : | Polyenic Fatty Acids |
| SEM | : | Standard Error of Mean |
| SFA | : | Saturated Fatty Acids |
| SPSS | : | Statistical Package for Social Science |
| TBCC | : | Tribasic Copper Chloride |

# CHAPTER I

# INTRODUCTION

Bangladesh is a country with over population and growth of population is increasing very fast day by day in comparison to its land size, as a result huge pressure is created on people's basic need. Demand of protein of this booming population is a great threat for us. It is evident that a substantial majority of the population suffers from varying degrees of malnutrition, including protein-energy malnutrition, micro-nutrient deficiencies, iodine deficiency disorder, iron deficiency, anemia, and vitamin deficiencies (Sorwar *et al.*, 2016). There are so many sources of protein but it is not possible to fulfill the demand without broiler. Because the duration of broiler rearing is very short and within 36-42 days it is ready for marketing and suitable for human consumption (Kamal *et al.*, 2015).

The poultry production especially broilers is one of the largest and fastest growing agro-based industries in the World and provides the opportunity of meeting animal protein needs for human. Broilers are produced specifically for meat production, these includes small fryers to large roaster type chicken (Begum *et al.*, 2010).

According to our socio-economic situation, the knowledge of our farmer is very little because most of them are not properly trained for broilers production, but unemployed young generation is coming in this business for short return of value and profit. Pharmaceutical companies take this advantage. They are convincing farmers for using antibiotics as a growth promoter or life savings for chicken. As a result, each and every broiler is a depot of antibiotics. When these broilers are consumed by human this antibiotic residue enters into human body and causing serious human health hazards with drug residues (Kamal *et al.*, 2015). Due to the prohibition of most of antimicrobial growth promoters (AGP), plant extracts and different trace elements have gained interest in animal feed strategies (Charis, 2000).

Copper (Cu) is an essential trace element which is necessary for various enzymatic systems in the body (Lim and Paik, 2006). Copper is required for skin pigmentation, proper functioning of the central nervous system, immune and cardiovascular system (Jegede *et al.*, 2011). The minimum requirement of Cu per day for most avian species was reported as 5-8 mg. kg-1 (Leeson, 2009). Copper is normally added as a feed additive into the poultry ration. However, due to its growth promoting and antimicrobial effects, Cu has been included at rather high concentrations far above pharmacological levels in feed for poultry (Skrivan *et al*., 2002; Adegbenjo *et al*., 2014).

Copper (Cu) has also received great attention because of its antimicrobial properties that improve animal growth performance when fed over the minimum requirement (Kim *et al*., 2011; Lu *et al*., 2010). The last edition of the NRC (1994) recommended 8 mg/kg of Cu as the minimum requirement for broiler diets and it has been reported that its sulphate form ($CuSO_4$) is more effective than other forms (Chiou *et al.,* 1997). However, Cu improves growth and feed efficiency in broilers when it is provided at much higher pharmacological levels (Skrivan *et al.*, 2002).

The deficiency of Cu causes anemia, diarrhoea, bone disorder, change in hair and wool pigmentation, cardio vascular disorder and impaired glucose and lipid metabolism. Despite of its wide physiological role copper has great role to lower the plasma and meat cholesterol (Samanta *et al*., 2011).

Copper compounds were used for medicinal purpose as early as 4000 BC, it was not until the 1920's that copper has first recognized as an essential nutrient for animal. Copper a nutrient that is required for the activity of different metallo enzymes associated with iron metabolism, elastin and collagen formation, melatonin production and the integrity of central nervous system (Samanta *et al*., 2011).Cu is also required for the formation of normal red blood cell by allowing iron absorption from small intestine and release of iron in tissue into the blood plasma (Samanta *et al*., 2011).

However, excess use of Cu in the diet of broiler depresses growth and feed efficiency in broilers (Funk & Baker, 1991), damages both gizzards (Robbins & Baker, 1980) and liver functions (Chen *et al*., 1996, 1997 a,b). Independent of changes in blood lipids, difference in fatty acid composition of cardiac tissue may be gender dependent (Srinivasan *et al*., 1987). Lee *et al*. (1986) demonstrated gender linked effects on the basal activities of lipogenic enzymes in response to starvation and re-feeding in rats. Chiou *et al.* (1999) observed that higher amount of Cu (500 mg kg-1) added in diet have damaged duodenal villi, therefore, have impact on nutrient absorption, depress food intake resulting in poor growth performance of broiler chickens. Inclusion of 500 mg/kg of Cu in the diet did not disturb normal protein metabolism of broiler chicken (Chiou *et al*., 1999). Chen *et al*. (1996) observed an increase in the enzymes level as a result of increase in the level of dietary Cu in country chicken and pullet chickens. No such report is published that deals with the supplement Cu that is higher than 250 ppm and less than 500 ppm to see its effects on the haemato chemicals and hormonal profiles in broiler chicken.

Copper had received considerable attention due to its antimicrobial properties that improve performance in animals when fed over the minimum requirement (Jenkins *et al*., 1970). Studies have shown that supplementation with various Cu sources (e.g., Cu sulfate, Cu citrate, or Cu chloride) increased growth in poultry (Arias and Andkoutsos, 2006; Karimi *et al*., 2011) without inducing adverse effects on birds.

Ruiz *et al*. (2000) reported that copper stimulated poultry growth and increased its performance while Nys (2001) stated that copper promoted the growth of chickens weakly if it was administered at high doses. It was also confirmed by Chiou *et al*. (1999), who did not find any significant effect of copper supplementation in the form of copper sulphate pentahydrate (250 mg/kg).

Therefore, the present study was under taken with the following objectives:

- To know the effect of copper sulphate on the growth performance and carcass yield in broiler
- To observe the effect of copper sulphate on bacterial load in different parts of intestine of broiler

# CHAPTER II

# REVIEW OF LITERATURE

## 2.1 Copper

Copper was an essential trace element for poultry and livestock (Davis & Mertz, 1987). And copper can maintain proper body functions and obtain the optimal growth performance of poultry (Banks *et al.*, 2004). Also, copper is a cofactor for various enzymes such as cytochrome oxidase, lysyl oxidase and Cu-Zn superoxide dismutase (Davis & Mertz, 1987).

Copper (Cu) is an integral part of cytochrome system. The enzyme tyrosinase, ascorbic acid oxidase, feroxidase (ceruloplasmin), superoxide dismutase contain copper and their activity dependent on this element (Swenson and Reece, 1996; Samanta *et al.*, 2011).

Copper (Cu) is an essential nutrient and it has been reported that sulfate form (CuSO4) is more effective than oxide (CuO) (Cromwell *et al.*, 1989). Its level 125 to 250 ppm as CuSO4 improves growth and feed efficiency in broiler (Choi & Paik, 1989; Baker *et al.,* 1991).

## 2.2 Function of Cu

Copper is required for bone formation by promoting structural integrity of bone collagen and for normal elastin formation in the cardiovascular system. Immuno-regulation of body system is a greatly dependent on copper (Samanta *et al*., 2011).

Cu has a positive influence on the activities of some digestive enzymes such as trypsin, chemotrypsin, amylse and lipase (Tang *et al.,* 2013).

Copper additive in feed has a beneficial effect on weight gain, the conversion of feed and modification of the bacterial microflora in the alimentary tract (Ruiz *et al*. 2000, Nys 2001, Makarski 2002). One possible mechanism by which Cu may benefit birds is shifting the gastrointestinal microbiota, thereby reducing the susceptibility of birds to disease, reducing intestinal lymphocyte recruitment and infiltration and thus increasing nutrient absorption (Arias and Koutsos 2006). Some researchers have demonstrated that supplementing broiler diets with high dietary Cu regulates intestinal microbiota through its bactericidal or bacteriostatic functions (Xia *et al*. 2004, Arias and Koutsos 2006).

The mode of action of high Cu might due to its antibacterial function in the lumen (Shurson *et al*., 1990) directly or indirectly through absorption from lumen to liver, secretion to the bile, and then back to the lumen loop. This theory is partially supported by Zhou *et al.* (1994), whose research indicated that intravenous injection of copper histidinate, which bypass the lumen, had similar growth promoting effect as dietary supplementation.

It is involved in different biochemical processes of animal metabolism such as

- enzyme-coenzyme catalytic reactions. It is associated with the function of anumber of enzymes such as oxygenases including cytochrome C oxidase, and copper-zinc superoxide dismutase
- ion transport, for instance with ceruloplasmin (ferroxidase I), a putative copper transport protein required for the incorporation of iron into transferrin for its transport in plasma (EC, 2003).

### 2.3 Requirements and recommendations of Cu

It is advised by the NRC (1994) that copper is added at a rate of 2.5 mg/kg to laying hen diets and at a rate of 8 mg/kg to broiler chicken diets.

Copper requirements of and recommendations for poultry are usually established for the Gallus species (Table 1).

Table 1: Copper requirements (NRC) of and recommendations (GfE) for poultry by scientific bodies

| Animal category | NRC (1994) | GfE (1999) |
|---|---|---|
| | mg/kg diet | mg/kg DM |
| Growing chickens | 4 | 7 |
| Laying hens | 2.5 | 7 |
| Broilers | 8 | 7 |

Copper for other avian species (ducks, guinea fowl) are generally extrapolated from these recommendations.

In the case of turkey, requirements and recommendations from NRC (1994) and GfE (2003a) are generally higher than those for the Gallus species (Table 2).

Table 2. Some copper requirements (NRC) of and recommendations (GfE) for turkeys (mg/ kg) by scientific bodies.

| Animal category | NRC (1994) (90%DM) | GfE (2003a) (DM) |
|---|---|---|
| | mg/kg diet | mg/kg DM |
| Turkey poults 0-4 weeks | 8 | 15 |
| 5-8 weeks | 8 | 6 |
| 9-24 weeks | 8 | 6 |

## 2.4 Absorption and excretion of copper

Copper is absorbed in the upper small intestine. Its absorption as that of all trace elements is difficult to measure and published results are consequently highly variable. Copper absorption is affected by the physiological stage of the animal, dietary level of copper (Jenkins and Hidiroglou, 1989) and interactions with phytate, ascorbic acid, fibre, tannin etc. which appear to complex with copper (Cousins, 1985) and other trace elements. Lonnerdal *et al.* (1985) report that copper absorption is higher in neonates than in adults. Thus, absorption rate hovered 60-70 % of dietary copper in calves compared to less than 5% in adult ruminants (ARC, 1980; Bremner and Dalgarno, 1973).

• Bile has been shown to be the major pathway for copper excretion in many animal species (Aoyagi *et al.*, 1995). Faeces are therefore the main route for excretion of copper, the urinary excretion representing only small losses through kidneys.

## 2.5 Effect on poultry or other animals

Copper additive in the diet, proved that it has a beneficial effect on weight gain, feed conversion ratio and modification of the bacterial microflora in the alimentary tract (Ruiz *et al.* 2000; Nys, 2001).

Cu supplementation (125-250 ppm) in broiler diet was reported to confer improved feed consumption, body weight gain, feed conversion ratio and protein anabolism (Paik, 2001; Karimi *et al.*, 2011).

Samanta *et al.*, 2011 determined the efficiency of copper (Cu) supplementation; a feeding experiment was carried out with 240 day old broiler chicks (vencobb-100). Birds were divided into four dietary treatments: i) C - no additives, ii) $T_1$-75 mg inclusion of Cu/kg diet, iii) $T_2$-150 mg inclusion of Cu/kg diet, iv) $T_3$-250 mg inclusion of Cu/kg diet. The present study was carried out in the Department of Animal Physiology, West Bengal University of Animal and Fishery Sciences, Kolkata, India for a period of 42 days (6 weeks). Growth performance was measured in terms of live weight gain, cumulative feed intake and feed conversion ratio at the end of $21^{st}$and $42^{nd}$day of the experiment and the result was found to be encouraging for commercial enterprises when the chickens were fed at 150 mg Cu/kg ($T_2$) of their diet.

Hashish *et al*. (2010) found significantly higher values on live weight during $4^{th}$ and $5^{th}$ week of age and lowest value for live weight gain for overall period compared to controls in copper sulfate supplement groups. But in $2^{nd}$ week of experiment, there were no significant difference for live weight and live weight gain between the groups fed diets supplemented with copper sulfate and those fed control diet.

Prajapati *et al.* (2010) reported numerical higher live weight of different treatment group compared to the control for the starting two weeks but statistically ($P>0.05$) remained comparable, and at third week of the study significant ($P<0.05$) improvement in the live weight was observed, which indicates that long term effect of feeding 125 mg of copper sulfate per kg is beneficial in respect of live weight gain in meat type colored birds.

Ševčíková *et al*., (2003) conducted an experiment to study the effect of a diet consisting of soybean, wheat and maize with 5% of rapeseed oil and different supplements of copper in the form of $CuSO_4.5H_2O$ or bioplex on cholesterol content and fatty acid composition in breast meat of broiler cockerels. The experiment was conducted on 600 straight-run broiler cockerels randomly divided into 4 groups according to the type of diet: group I – control, no Cu supplement; experimental groups II – 35 mg Cu/kg and III – 175 mg Cu/kg in the form of $CuSO_4.5H_2O$; IV – 175 mg Cu/kg in the form of bioplex. Chickens were sacrificed at 42 days of age. No statistically significant differences were determined in productive parameters except for mortality that was lowest in group IV receiving copper in the Cu-glycine. Cu supplementation decreased ($P< 0.05$) cholesterol content by 24.9% in group IV (493 mg/kg) compared to the control group (656 mg/kg) and significantly decreased the concentration of Polyenic Fatty Acids (PUFA) in groups III and IV (25.90 and 26.02%) in comparison with control group (27.73%) and group II (27.55%). The content of total Saturated Fatty Acids (SFA) increased significantly in group II (27.01%) against the control group (25.41%). The contents of total Monoenic Fatty Acids (MUFA) were significantly higher in groups III and IV (44.01% and 43.14%) than in group II (40.66%).

Arias and Koutsos (2006) studied the effect of dietary copper sulfate (CuSO4) and Tribasic Copper Chloride (TBCC) on intestinal physiology and growth of broiler chickens. In 2 experiments (Experiments 1 and 2), day-old broiler chicks were fed 1 of 4 diets: a basal diet with no supplemental copper (Cu; negative control), a basal diet + 188 mg of Cu/kg of diet from TBCC or CuSO4, or a basal diet + sub therapeutic antibiotics (bacitracin and roxarsone; positive control). In experiment 1 (recycled litter), CuSO4 and TBCC increased carcass weight (d 45 post hatch) compared with the negative control ($P < 0.05$ for each). In experiment 2 (fresh litter), negative control and TBCC increased carcass weight (d 42 post hatch) compared with the positive control ($P < 0.05$ for each). At d 30 to 31 post hatch, intestinal histology was measured. In Experiment 1 (recycled litter), dietary TBCC, CuSO4, and positive control decreased the number of lamina propia lymphocytes or intraepithelial lymphocytes (IEL), or both, compared with the negative control ($P < 0.05$). However, in Experiment 2 (fresh litter), TBCC and positive control increased the number of duodenum IEL compared with the negative control ($P < 0.05$), and negative control and TBCC increased the number of ileum IEL. These data demonstrate that broiler performance and intestinal physiology can be influenced by dietary Cu source and level as well as microbial environment (fresh vs. recycled litter).

Yang *et al.*, (2018) conducted a study to estimate the growth performance, slaughter performance, nutrient content of fecal and liver copper concentrations of growing Goslings from 28 to 70 d of age. Two hundred healthy male Yangzhou geese with similar body weight were randomized to four groups with five replicates per treatment and ten geese per replicate. Average daily feed intake, average daily gain and feed conversion ratio of geese for each pen were measured from 28 to 70 d of age. At 70 d of age, two geese were selected randomly from each pen and slaughtered to evaluate carcass quality. A significant effect of dietary copper was found on body weight, feed conversion ratio, carcass yield, fecal copper concentrations and liver copper concentrations. The result showed that dietary Cu addition can improve growth by increasing the use of the feeding stuff and improving carcass yield in growing Goslings.

Lu *et al.,* (2010) conducted an experiment using a total of 840, 1-day-old, Arbor Acres commercial male broilers to compare copper (Cu) sulfate and tribasic Cu chloride (TBCC, Cu(2)(OH)(3)Cl) as sources of supplemental Cu for broilers fed in floor pens. Chicks were randomly allotted to one of seven treatments for six replicate pens of 20 birds each, and were fed a basal corn-soybean meal diet (10.20 mg/kg Cu) supplemented with 0, 100, 150, or 200 mg/kg Cu from either Cu sulfate or TBCC for 21 days. Chicks fed 200 mg/kg Cu as TBCC had a higher ($P < 0.05$) average daily gain (ADG) than those consuming other diets. Liver Cu contents of broilers fed diets supplemented with TBCC were numerically lower ($P > 0.05$) than those of broilers fed diets supplemented with Cu sulfate. The vitamin E contents and the phytase activities in the feed fortified with TBCC were higher ($P < 0.01$) and numerically higher ($P > 0.05$) compared with those in the feeds fortified with Cu sulfate stored at room temperature, respectively. The vitamin E contents in liver and plasma of broilers fed diets supplemented with TBCC were higher ($P < 0.05$) than those of birds fed diets supplemented with Cu sulfate. This result indicates that TBCC is more effective than Cu sulfate in improving the growth of broilers fed in floor pens, and it is chemically less active than Cu sulfate in promoting the undesirable oxidation of vitamin E in feeds.

Neethu *et al*. (2016) undertook study to evaluate the effect of different concentrations of copper sulphate on the performance and carcass trait of broiler birds. The results revealed that the highest weight gain and lowest FCR was observed in group supplemented with 200 ppm copper sulphate.

Lou *et al*. (2005) conducted an experiment using a total of 420, 1-d-old, Arbor Acres commercial male chicks to compare copper sulfate and tribasic copper chloride (TBCC) as sources of supplemental copper for broilers. Chicks were randomly allotted to 1 of 7 treatments for 6 replicates of 10 birds each and were fed a basal corn-soybean meal diet (11.45 mg/kg copper) supplemented with 0, 150, 300, or 450 mg/kg copper from copper sulfate or TBCC for 21 d. Chicks fed 450 mg/kg copper as copper sulfate had lower ($P < 0.01$) average daily feed intake and average daily gain than those consuming other diets. Feeding supplemental copper increased linearly ($P < 0.0001$) liver copper concentrations regardless of copper source.

Miles *et al*. (1998) conducted three experiments to study Cu sulfate and tribasic Cu chloride (TBCC) as sources of supplemental Cu for poultry. In Experiment 1, 252 chicks were fed the basal corn-soybean meal diet (26 ppm Cu) supplemented with either 0, 150, 300, or 450 ppm Cu from Cu sulfate or TBCC for 21 d. Chicks fed 450 ppm Cu from sulfate had lower ($P < 0.05$) feed intake than those consuming other diets. Feeding supplemental Cu increased ($P < 0.0001$) liver Cu concentration linearly with increasing dietary Cu regardless of Cu source.

Banks *et al*. (2004) reported the broiler supplementation with increasing concentrations of Cu to a diet containing 600 FTU phytase/kg resulted in decreases in 21 d BW, BW gain, feed consumption, feed conversion. In this experiment, Cu supplementation did not reduce the efficacy of phytase (i.e., improvement in apparent P retention with phytase supplementation) but did decrease apparent P retention, BW gain, feed consumption, feed conversion, and tibia ash and toe ash weights.

**2.6 Effect on carcass characteristics in poultry**

Adegbenjo *et al.* (2014) reported that dietary inclusion of CuP showed increased ($P<0.05$) liver, heart and tibia bone Cu concentration when compared with birds fed diet supplemented with $CuSO_4$, Cockerels fed diet supplemented with 100 mg/kg Cu sourced from CuP recorded the highest ($P<0.05$) liver, heart and tibia bone Cu concentration.

Arias and koustos (2006) conducted a study with the supplement 188 mg/kg of copper sulfate in broiler birds and reported an improvement in the carcass weight when used either recycled or fresh litter.

Kumar *et al.* (2013) found significantly ($P<0.05$) higher live weight when the birds were treated with 400 mg/kg copper sulfate on diet compared to control and other two groups treated with copper sulfate at 100 mg/kg and 200 mg/kg throughout the experimental period.

Prajapati *et al.* (2010) observed improvement on dressing weight of treatment group as compared to the control, while the weight of liver get reduced numerically ($P>0.05$) by copper sulfate supplementation.

Xiang-Qi *et al*. (2009) conducted an experiment to compare the effects of dietary Tribasic Copper Chloride (TBCC) and Copper Sulfate ($CuSO_4$) on carcass characteristics, copper deposition and tissular nutrients oxidation in broilers. Results indicated that: when added copper level was 150 mg/kg, half-evisceration yield and breast yield of broilers increased. The evisceration yield of broilers was raised by feeding TBCC and supplementation with 50 mg/kg copper from TBCC got the biggest evisceration yield; when added copper levels increased from 50-350 mg/kg, contents of copper or crude fat in liver or muscles and the level of $V_E$ in heart were increased significantly, while, the levels of $V_E$ or MDA in liver decreased; when added copper levels were less than or equal to 150 mg/kg, the copper levels in liver or muscles were similar between copper sources; while added copper levels were >150 mg/kg, the smaller quantities of copper in liver were gained by using TBCC comparing with feeding $CuSO_4$.

Neethu *et al*. (2016) undertook a study to evaluate the effect of different concentrations of copper sulphate on the performance and carcass trait of broiler birds. The dressing percentage was increasing with the supplementation of 200 to 400 ppm of copper. The best recommended level of copper sulphate in feed was 200 ppm.

Mondal *et al*. (2007) were also unable to found any improvement in carcass weight with the supplementation of copper at 200 and 400 mg/kg and 128 mg/kg on diet as copper sulfate (Sunder *et al*., 2009). Zhang *et al*. (2009) also did not observe any effect on carcass yield when supplied copper as either copper sulfate or tri-basic copper chloride at 50,150,250 and 350 mg/kg on diet.

### 2.7 Cost benefit analysis

Abdallah *et al*. (2009) showed the comparatively better economic efficiency compared to the control when treated with organic copper sulfate in diet. Another study was conducted to evaluate the profitability of broiler production with available growth promoter where it was shown that single growth promoter has the more profitability over the combination with another.

# CHAPTER III

# MATERIALS AND METHODS

## 3.1 Statement of the experiment

The experiment was conducted for a period of 5 weeks from 1st February to 5th March, 2018 to investigate the dietary effect of Copper sulphate in different doses on growth performance of broiler and their microbial load.

## 3.2 Experimental site

The study was conducted in a personal farm at Lalbag, sadar upazila of Dinajpur and the microbial load was analyzed in Microbiological Laboratory of the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU).

## 3.3 Preparation of research shed

The shed was cleaned and washed using fresh water and disinfectant (Iodin, Non Ionic Surfectant, Sulfuric Acid, Phosphoric Acid, FAM 30®, Renata animal health, Bangladesh).Then the shed was kept free for 5 days before placing experimental birds. All necessary equipment were disinfected and set properly to care the broiler chicks successfully.

## 3.4 Management of broilers

One hundred forty four commercial broiler day-old chicks were collected from Kazi Chicks Limited, Thakurgaon. The birds were kept on a floor litter system, each group in separate pens measuring 0.9×1.2 meters. The pens were thoroughly cleaned, white-washed and disinfected before use. Chicks were acclimatized for 7 days in the experimental shed and brooded at 35°C during 1stweek and the temperature was reduced by 3°C every week until the temperature reached room temperature at 25 ± 1°C. All the groups were reared under the similar conditions of temperature, humidity, light, ventilation and floor space throughout the experimental period. Birds were fed commercial feed from Nourish Poultry Feed Limited and water ad libitum under strict biosecurity (Molla *et al.*, 2012).

### 3.5 Biosecurity and sanitation

Proper hygienic and sanitation programs were followed during the experimental period. To prevent the outbreak of disease strict biosecurity was maintained during the experimental period. The following measures were taken to maintain the biosecurity: Visitors were not allowed to enter in the house, all equipment's in the experimental house were kept clean, and dead birds were removed promptly.

### 3.6 Experimental Birds

A total of 144, day old broiler chicks (Cobb 500) were purchased from the dealer of Kazi chicks limited. Then the chicks were properly exposed to heat (brooding) and other management was carefully maintained for up to 7 days. At the age of 7 day, all birds were randomly allocated to four dietary treatment groups as $T_0$, $T_1$, $T_2$ and $T_3$, each group consisted of 3 replications containing 12 birds in each replication. Feed and water were supplied ad libitum throughout the trial. All birds were fed with standard broiler starter and grower (Nourish Poultry & Hatchery Ltd.) ration throughout the experimental period.

### 3.7 Layout of the experiment

The layout of the experiment is shown in table 3.There were three replications in each dietary phase treatment. Thus total number of replicate was 12.

Table 3: Table showing the distribution of broiler chicks to different dietary treatment groups

| **Replication (R)** | **Treatment (T)** | | | |
|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | $T_3$ |
| **$R_1$** | 12 | 12 | 12 | 12 |
| **$R_2$** | 12 | 12 | 12 | 12 |
| **$R_3$** | 12 | 12 | 12 | 12 |

* $T_0$ = Control (Basal diet) with no supplemental Cu

* $T_1$ = (Control + $CuSO_4$ @ 100 mg/Kg feed)

* $T_2$ = (Control + $CuSO_4$ @ 175 mg/Kg feed)

* $T_3$ = (Control + $CuSO_4$ @ 250 mg/Kg feed)

### 3.8 Experimental layout

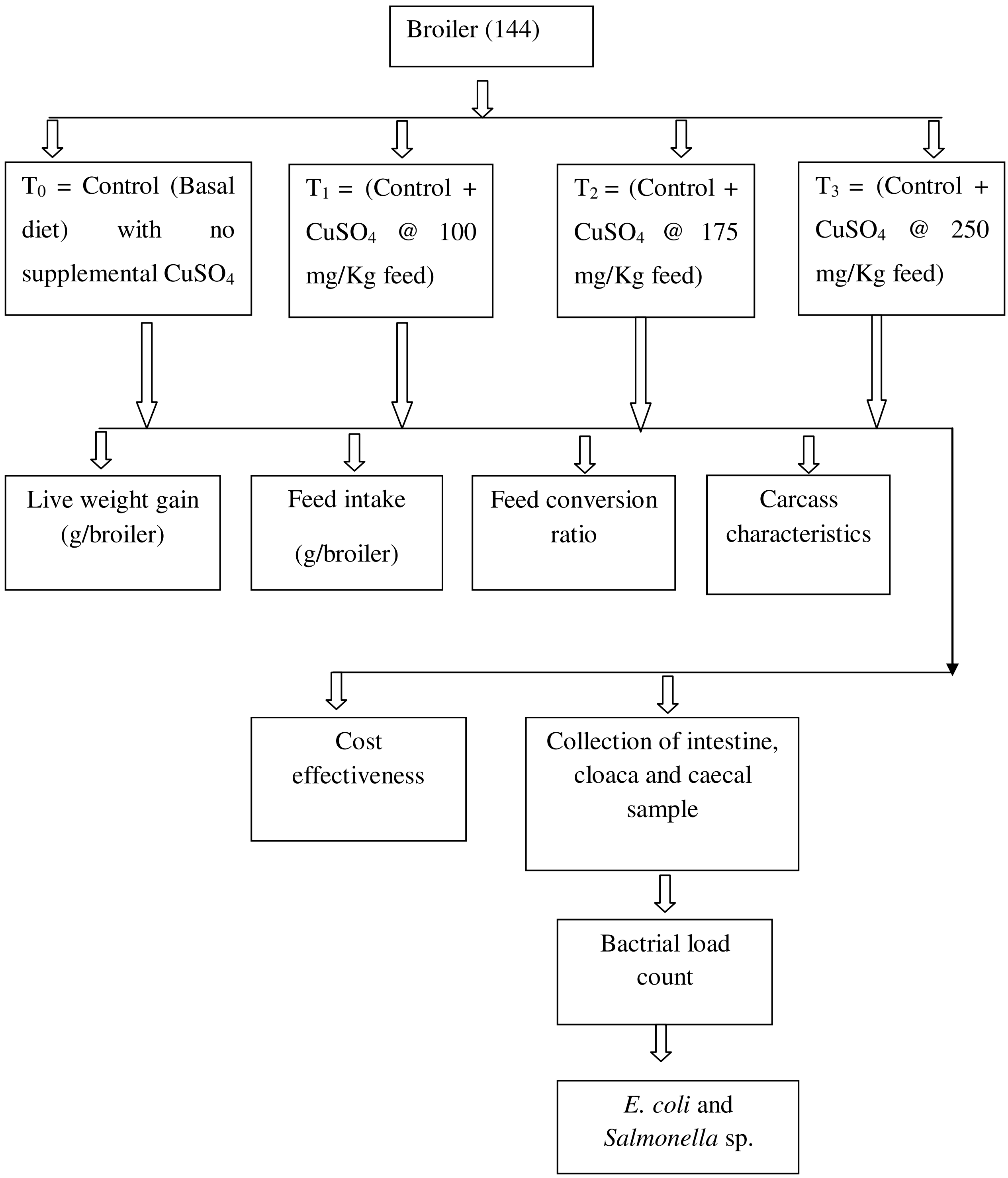


Fig 1: Layout of the Experiment

### 3.9 Collection of commercial feed

Commercial broiler starter and broiler grower feed was purchased and collected from the local market of Dinajpur. This commercial diet was designated as the control diet.

Table 4: Composition of commercial diet

| Ingredients | Broiler starter | Broiler grower |
|---|---|---|
| ME (Kilo Cal/Kg) | 3000 | 3050 |
| CP (%) | 21 | 20 |
| CF (%) | 5 | 6 |
| Fiber (%) | 5 | 5 |
| Ash (%) | 8 | 8 |
| Methionine (%) | 0.5 | 0.45 |
| Lysine (%) | 1.2 | 1.15 |

Source: Kazi Feed Ltd., Bangladesh.

### 3.10 Collection of copper sulphate

Copper sulphate was collected from the Chalk Bazar of Dinajpur.

### 3.11 Experimental Procedure and Data Collection

The growth performance of broiler was evaluated by using change in body weight (growth rate), feed intake and feed conversion ratio.

#### 3.11.1 Live weight

The live weight of each bird was measured with the help of digital balance on $7^{th}$day (initial weight) and $35^{th}$ days (final weight) and recorded.

#### 3.11.2 Live weight gain

The broilers were weighed at start (initial live weight) and then at the end of the experiment (final live weight). Live weight gain/loss was calculated by the difference of initial live weight and final live weight.

Live weight gain = Final live weight - initial live weight

### 3.11.3 Feed intake and feed conversion ratio

Feed intake was determined by weighing the amount of feed offered and feed refusal in each pen (replicate) on a daily basis. Feed intake per pen was obtained by calculating the difference between the total weight of feed given and weight of the refusal feed (left over feed). However, Feed conversion ratio (FCR) was obtained by dividing the total feed intake with total weight gain of all birds in each pen. The feed intake and feed conversion ration were obtained by using following formula:

Feed intake (FI) = Feed offered – Feed refusal

Feed Conversion ratio (FCR) = Feed intake/ Average weight gain

### 3.11.4 Carcass characteristics

A total of 36 broilers were sacrificed and weight of abdominal fat, heart, liver, and gizzard was taken with the help of electric balance of each broiler. Dressing percentage was calculated by the following formula:

$$\text{Dressing \%} = \frac{\text{Dressed weight (g)}}{\text{Live weight (g)}} \times 100$$

## 3.12 Bacterial load count

The samples (intestine, caecum and cloaca) were collected from bird of each replication and carried to Microbiology laboratory, Department of Microbiology for colony count. The identification of bacteria and colony count were performed by following standard microbiological methods.

## 3.13 Statistical analysis

Data were collected from each treatment and entered into the computer data base (excel sheet) ready for statistical analysis.

The data were analyzed by one-way analysis of variance, followed by the Duncan post hoc test to determine significant differences in all the parameters among all groups using the SPSS computer program (Version 20.0; SPSS,). Differences with values of $P<0.05$ were considered to be statistically significant.All data were expressed as Mean ± Standard Error of Mean (SEM).

Fig 2: Experimental groups

Fig 3: Copper sulphate

Fig 4: Mixing of copper sulphate with commercial feed

Fig 5: Weighing of broiler

Fig 6: Weighing of dressed broiler


Fig 7: Weighing of abdominal fat


Fig 8: Weighing of heart weight


Fig 9: Weighing of liver weight


Fig 10: Collection of intestine, caecum and cloaca


Fig 11: Sample inoculation onto media

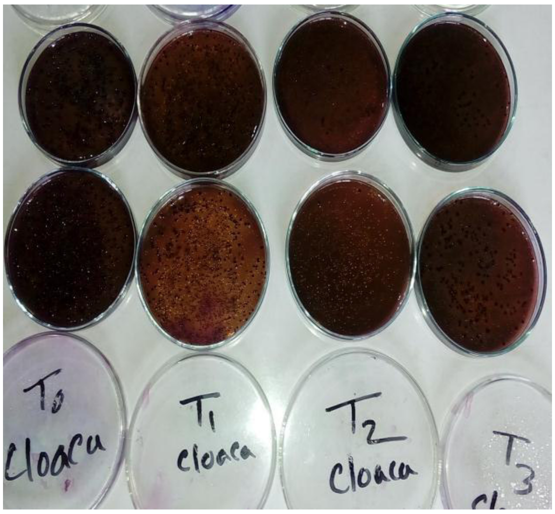


Fig 12: Growth of bacteria (colony) in media

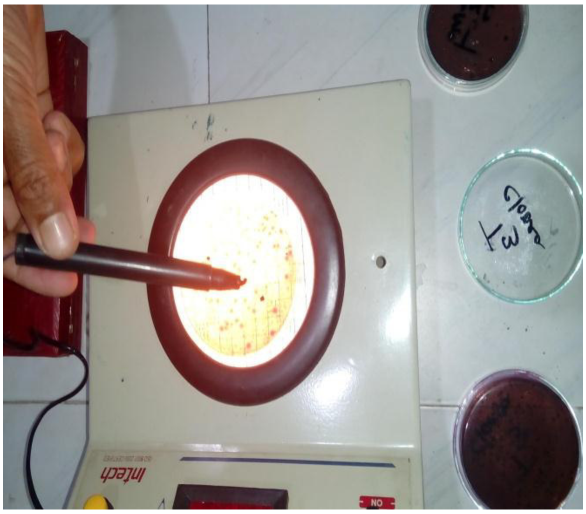

Fig 13: Colony counting in colony counter

# CHAPTER IV

# RESULTS AND DISCUSSION

## 4.1 Effect of $CuSO_4$ on the growth performance of broiler

The effect of feeding copper sulphate on the growth performance of broiler is presented in table 5 and described under following subheading:

Table 5: Effect of copper sulphate on the growth performance of broiler

| Parameter | Treatment | | | | P value |
|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | $T_3$ | |
| Initial live weight (7 days) | 184.44±3.29 | 185.19±3.30 | 189.47±3.02 | 187.61±3.35 | 0.681 (NS) |
| Final live weight (35 days) | $2540.33 \pm 42.33^{b}$ | $2608.74 \pm 40.04^{ab}$ | $2690.72 \pm 34.82^{a}$ | $2554.72 \pm 38.64^{b}$ | 0.029* |
| Weight gain (g/bird) | $2355.89 \pm 42.7^{b}$ | $2423.55 \pm 39.97^{ab}$ | $2501.25 \pm 34.95^{a}$ | $2367.11 \pm 38.4^{b}$ | 0.036* |
| Feed intake (g/bird) | 3483.33±3.98 | 3486.18±4.7 | 3465.00±4.4 | 3474.72±5.17 | 0.164 (NS) |
| FCR | $1.49 \pm 0.03^{a}$ | $1.45 \pm 0.029^{ab}$ | $1.4 \pm 0.022^{b}$ | $1.48 \pm 0.23^{a}$ | 0.047* |

Here, $T_0$ = Control (Basal diet) with no supplemental $CuSO_4$, $T_1$ = (Control + $CuSO_4$ @ 100 mg/Kg feed), $T_2$ = (Control + $CuSO_4$ @ 175 mg/Kg feed) and $T_3$ = (Control + $CuSO_4$ @ 250 mg/Kg feed). All values are presented as Mean ± SEM (Standard Error of Mean). NS means statistically not significant (P>0.05). * means significant at 5% level of significance (P<0.05).$^{a, b, c}$ means bearing different superscripts in the same row differ significantly.

### 4.1.1 Live weight and live weight gain

Table 5 shows the effect of $CuSO_4$ on live weight and live weight gain in broiler. There was no significant (P>0.05) difference among the treatment groups but final live weight significantly (P<0.05) varied at different treatment groups. The initial live weight was 184.44 ± 3.29; 185.19 ± 3.30; 189.47 ± 3.02 and 187.61 ± 3.35 in $T_0$, $T_1$, $T_2$ and $T_3$ group respectively.

The final live weight was significantly ($P<0.05$) higher in $T_2$ (2690.72 ± 34.82) which was followed by $T_1$ (2608.74 ± 40.04), $T_3$ (2554.72 ± 38.64) and $T_0$ (2540.33 ± 42.33) respectively.

The feeding copper sulfate had significant ($P<0.05$) effect on body weight gain in broiler. The significantly ($P<0.05$) highest body weight gain was found in $T_2$ (2501.25 ± 34.95) which was followed by $T_1$ (2423.55 ± 39.97) compared to $T_4$ (2367.11 ± 28.4) and $T_0$ (2355.89 ± 42.7) respectively. The present study is in the line of Hashish *et al.* (2010) who found significantly higher values on live weight during 4th and 5th week of age compared to controls in copper sulfate supplement groups. Similar results are observed by Prajapati *et al.* (2010) reported higher live weight of different treatment group compared to the control for the starting two weeks but statistically ($P>0.05$) remained comparable, and at third week of the study significant ($P<0.05$) improvement in the live weight was observed. Neethu *et al.* (2016) recorded highest weight gain in group supplemented with 200 ppm copper sulphate is closely similar to the present findings. Yang *et al.* (2018) also reported that a significant effect of dietary copper was found on body weight in geese. Banks *et al.* (2004) reported that broiler supplementation with increasing concentrations of Cu to a diet containing 600 FTU phytase/kg resulted in decreases in body weight and body weight gain which is similar to the present study. Lu *et al.* (2010) observed that broiler chicks fed 200 mg/kg Cu as TBCC had a higher ($P < 0.05$) average daily gain (ADG) than those consuming other diets. Kumar *et al.* (2013) found significantly ($P<0.05$) higher live weight when the birds were treated with 400 mg/kg copper sulfate on diet compared to control and other two groups treated with copper sulfate at 100 mg/kg and 200 mg/kg throughout the experimental period. Karimi *et al.* (2011) reported Cu supplementation (125-250 ppm) in broiler diet improved bodyweight gain.

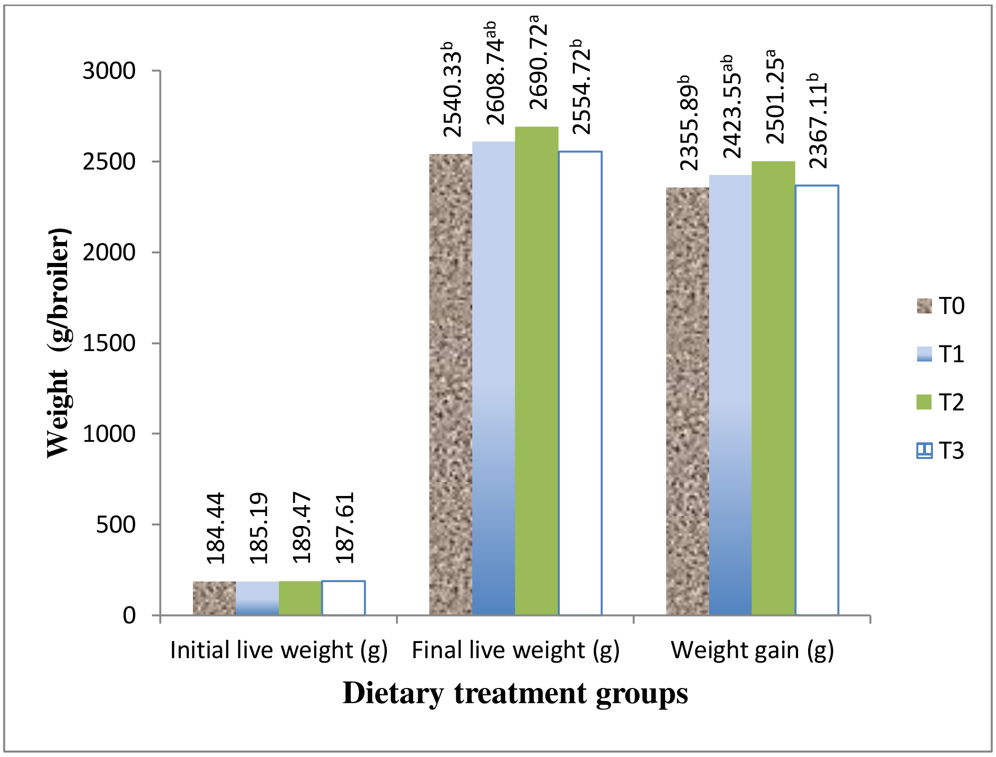


Fig 14: Effect of copper sulphate on the live weight and live weight gain of broiler

Here, $T_0$ = Control (Basal diet) with no supplemental $CuSO_4$, $T_1$ = (Control + $CuSO_4$ @ 100 mg/Kg feed), $T_2$ = (Control + $CuSO_4$ @ 175 mg/Kg feed) and $T_3$ = (Control + $CuSO_4$ @ 250 mg/Kg feed). All values are presented as Mean ± SEM (Standard Error of Mean). [a, b, c] means bearing different superscripts in the same row differ significantly (P<0.05).

### 4.1.2 Feed intake

CuSO4 had no significant effect on the feed intake (g/broiler) in broiler. The feed intake (g/broiler) in $T_0$, $T_1$, $T_2$ and $T_3$ was 3483.33 ± 3.98, 3483.18 ± 4.7, 3465 ± 4.4 and 3474.72 ±5.17 respectively (Table 5). Banks *et al.* (2004) reported that broiler supplementation with increasing concentrations of Cu to a diet containing 600 FTU phytase/kg resulted in decreases feed consumption. Karimi *et al.* (2011) reported Cu supplementation (125-250 ppm) in broiler diet improved feed consumption.

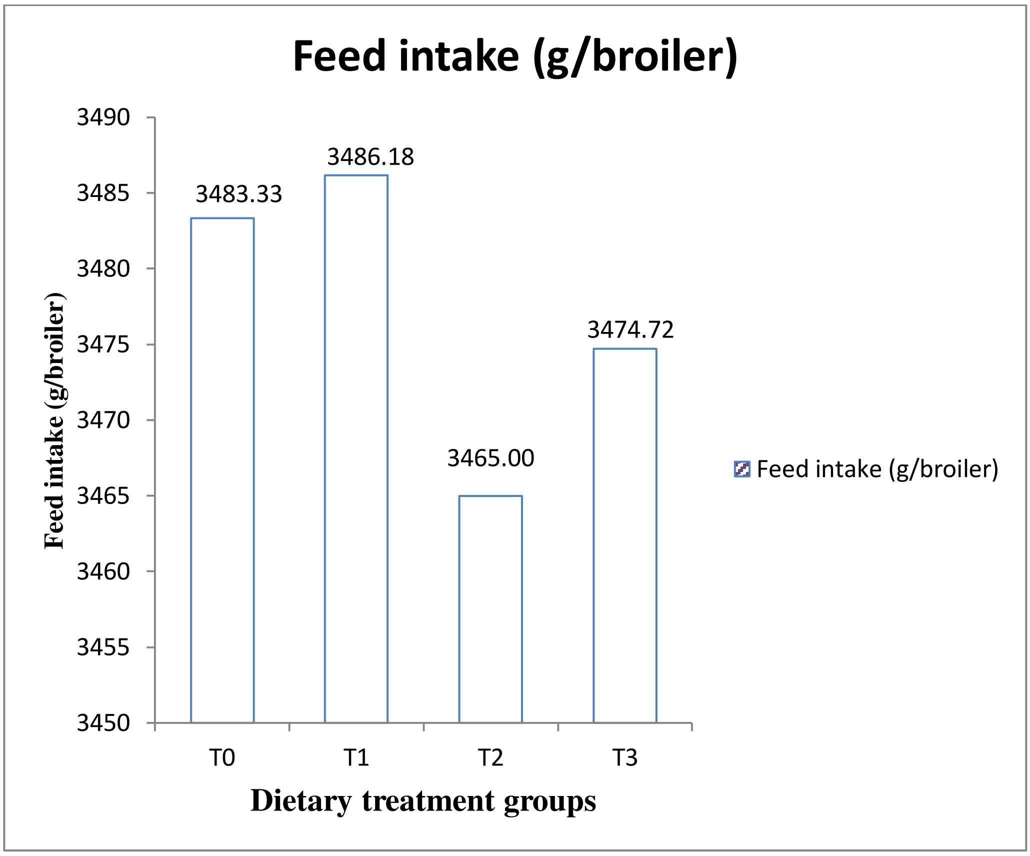


Fig 15: Effect of copper sulphate on feed intake (g/broiler) of broiler

Here, $T_0$ = Control (Basal diet) with no supplemental $CuSO_4$, $T_1$ = (Control + $CuSO_4$ @ 100 mg/Kg feed), $T_2$ = (Control + $CuSO_4$ @ 175 mg/Kg feed) and $T_3$ = (Control + $CuSO_4$ @ 250 mg/Kg feed). All values are presented as Mean ± SEM (Standard Error of Mean).

### 4.1.3 FCR (Feed conversion ratio)

The effect of $CuSO_4$ on FCR in broiler is presented on Table 5. FCR (Feed conversion ratio) was significantly affected by the feeding of CuSO4. The best and lowest FCR was found in $T_2$ (1.40±0.022) than $T_0$ (1.49± 0.03) whereas FCR in $T_1$ and $T_4$group was 1.45±0.062 and 1.48± 0.23 respectively. The present finding is related to the findings of Neethu *et al.* (2016) who recorded lowest FCR in group supplemented with 200 ppm copper sulphate and Karimi *et al.* (2011) who reported Cu supplementation (125-250 ppm) in broiler diet improved feed conversion ratio. Yang *et al.* (2018) also reported that a significant effect of dietary copper was found on feed conversion ratio in geese.

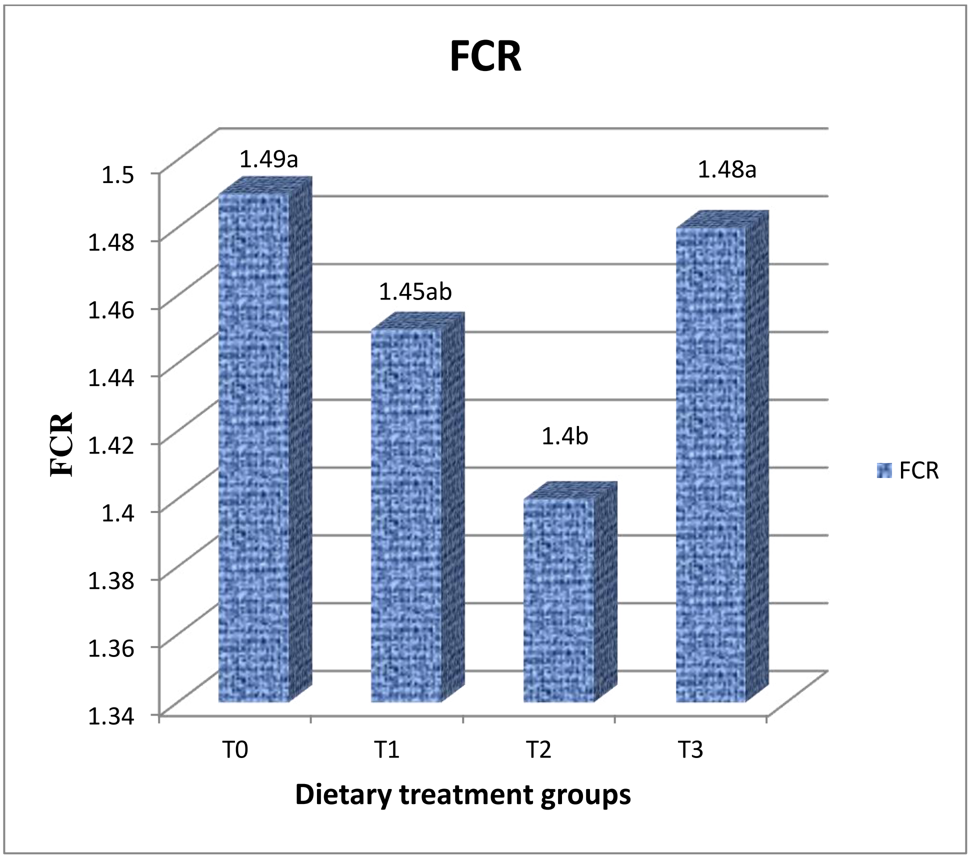


Fig 16: Effect of copper sulphate on FCR of broiler

Here, $T_0$ = Control (Basal diet) with no supplemental $CuSO_4$, $T_1$ = (Control + $CuSO_4$ @ 100 mg/Kg feed), $T_2$ = (Control + $CuSO_4$ @ 175 mg/Kg feed) and $T_3$ = (Control + $CuSO_4$ @ 250 mg/Kg feed). All values are presented as Mean ± SEM (Standard Error of Mean).$^{a, b, c}$ means bearing different superscripts in the same row differ significantly.

### 4.2 Effect of copper sulphate on carcass characteristics

The effect of copper sulphate on the carcass characteristics in broiler is presented in Table 6. In the present study, it was found that copper sulphate had significant ($P<0.05$) effect on dressing %, abdominal fat weight (g) and gizard weight (g) but no significant ($P>0.05$) effect on heart weight (g) and liver weight (g). It was revealed that significantly higher dressing % was found in $T_2$ (61.0±0.58) compared to $T_0$ (58.0 ± 0.58) in where it was 59.33 ± 0.33 and 59.0 ± 0.58 in $T_3$ and $T_1$ group respectively. In the present study, the significantly higher abdominal fat weight (g) was found in $T_0$ (8.67 ± 0.88) followed by $T_1$ (6.67 ± 0.88), $T_2$ (4.00 ± 0.58) and $T_3$ (3.33 ± 0.17) group respectively. The heart weight (g) in $T_0$, $T_1$, $T_2$ and $T_3$ was 13.0 ± 1.15, 12.5 ± 0.29, 11.33 ± 0.88 and 12.00 ± 0.39 respectively. The liver weight (g) was 60.00 ± 1.15, 56.33 ± 1.52, 57.33 ± 0.67 and

59.67 ± 1.2 in $T_0$, $T_1$, $T_2$ and $T_3$ group respectively. The gizard weight (g) was significantly ($P<0.05$) highest in $T_3$ (40.00 ± 0.58) which was followed by $T_2$ (38.00 ± 0.58), $T_1$ (35.83 ± 0.6) and $T_0$ (35.33 ± 0.88) respectively. The findings of the present study are in the agreement with Neethu *et al.* (2016) who observed increased dressing percentage with the supplementation of 200 to 400 ppm of copper and Xiang-Qi *et al.* (2009) who found that half-evisceration yield and breast yield of broilers increased when added copper level was 150 mg/kg. The present study is closely similar to the results of Prajapati *et al.* (2010) who observed improvement on dressing weight of treatment group as compared to the control, while the weight of liver get reduced numerically ($P>0.05$) by copper sulfate supplementation. Adegbenjo *et al.* (2014) who reported that dietary inclusion of CuP showed increased ($P<0.05$) liver, heart weight when compared with birds fed diet supplemented with $CuSO_4$. Arias and koustos (2006) reported an improvement in the carcass weight in broiler supplemented with 188 mg/kg of copper sulfate. Yang *et al.* (2018) also reported that a significant effect of dietary copper was found on carcass yield in geese. The present study is differed with the findings of Mondal *et al.* (2007) who found no improvement in carcass weight with the supplementation of copper at 200 and 400 mg/kg and 128 mg/kg on diet as copper sulfate and Zhang *et al.* (2009) who also did not observe any effect on carcass yield when supplied copper as either copper sulfate or tri-basic copper chloride at 50,150,250 and 350 mg/kg on diet.

Table 6: Effect of copper sulphate on carcass characteristics of broiler

| Parameter | Treatment | | | | P value |
|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | $T_3$ | |
| Dressing% | $58.0\pm0.58^b$ | $59.0\pm0.58^b$ | $61.0\pm0.58^a$ | $59.33\pm0.33^{ab}$ | 0.023* |
| Abdominal fat weight (g) | $8.67\pm0.88^a$ | $6.67\pm0.88^a$ | $4.00\pm0.58^b$ | $3.33\pm0.17^b$ | 0.002** |
| Heart weight (g) | 13.0±1.15 | 12.5±0.29 | 11.33±0.88 | 12.00±0.39 | 0.527(NS) |
| Liver weight (g) | 60.00±1.15 | 56.33±1.52 | 57.33±0.67 | 59.67±1.2 | 0.084(NS) |
| Gizard weight (g) | $35.33\pm0.88^c$ | $35.83\pm0.6^{bc}$ | $38.00\pm0.58^{ab}$ | $40.00\pm0.58^a$ | 0.004** |

Here, $T_0$ = Control (Basal diet) with no supplemental $CuSO_4$, $T_1$ = (Control + $CuSO_4$ @ 100 mg/Kg feed), $T_2$ = (Control + $CuSO_4$ @ 175 mg/Kg feed) and $T_3$ = (Control + $CuSO_4$ @ 250 mg/Kg feed). All values are presented as Mean ± SEM (Standard Error of Mean). NS means statistically not significant (P>0.05). * means significant at 5% level of significance (P<0.05) and ** means significant at 1% level of significance (P<0.01). $^{a, b, c}$ means bearing different superscripts in the same row differ significantly.

**4.3 Effect of copper sulphate on bacterial load count**

Copper sulphate has antibacterial effect. The table 7 shows the effect of copper sulphate on microbial load count in different sample. The load of *E. coli* and *Salmonella* sp. was counted from the sample of intestine, cloaca and caeca of 35 days aged broiler. The *E. coli* and *Salmonella* sp. load in intestine, cloaca and caeca was significantly differed in different treatment groups. In intestine, the *E. coli* load was significantly (P<0.01) higher in $T_0$ (245.00±7.64), followed by $T_1$ (240.00±5.77), $T_2$ (73.33±4.41) and $T_3$ (48.33±4.41) respectively and *Salmonella* sp. load was also significantly (P<0.01) highest in $T_0$ (266.67±8.82) where as it was 256.67±12.02, 110.00±5.77 and 90.00±5.77 in $T_1$, $T_2$ and $T_3$ respectively. Significantly (P<0.01) higher *E. coli* and *Salmonella* sp. count was found in $T_0$ (288.33±4.41 and 270.00±5.77) which was followed by $T_1$ (265.00±2.89 and 246.67±3.33), $T_2$ (83.33±14.53 and 163.33±8.82) and $T_3$ (70.00±15.28 and 70.00±5.77) respectively in cloacal sample. The *E. coli* count was significantly (P<0.01) higher in $T_0$ (296.00±5.77) and lower in $T_3$ (120.00±5.77) in caecal sample. Control group $T_0$ (298.00±11.55) had significantly (P<0.01) highest *Salmonella* sp. count in caecal sample, followed by $T_1$ (293.33±8.82), $T_2$ (283.33±8.82) and $T_3$ (130.00±21.6) respectively.

Copper in feed has a beneficial effect on the modification of the bacterial micro flora in the alimentary tract (Ruiz *et al.* 2000, Nys 2001, Makarski 2002).Some researchers have demonstrated that supplementing broiler diets with high dietary Cu regulates intestinal micro biota through its bactericidal or bacteriostatic functions (Xia *et al.* 2004, Arias and Koutsos 2006).

Table 7: Effect of copper sulphate on bacterial load count in intestinal, cloacal and caecal sample of broiler

| Parameter | | Treatment | | | | P value |
|---|---|---|---|---|---|---|
| | | $T_0$ | $T_1$ | $T_2$ | $T_3$ | |
| Intestine | *E. coli* | 245.00±7.64[a] | 240.00±5.77[a] | 73.33±4.41[b] | 48.33±4.41[c] | 0.000** |
| | *Salmonella* sp. | 266.67±8.82[a] | 256.67±12.02[a] | 110.00±5.77[b] | 90.00±5.77[c] | 0.000** |
| Cloaca | *E. coli* | 288.33±4.41[a] | 265.00±2.89[a] | 83.33±14.53[b] | 70.00±15.28[b] | 0.000** |
| | *Salmonella* sp. | 270.00±5.77[a] | 246.67±3.33[a] | 163.33±8.82[b] | 70.00±5.77[c] | 0.000** |
| Caeca | *E. coli* | 296.00±5.77[a] | 293.33±6.66[a] | 233.33±56.96[a] | 120.00±5.77[b] | 0.006** |
| | *Salmonella* sp. | 298.00±11.55[a] | 293.33±8.82[a] | 283.33±8.82[a] | 130.00±21.6[b] | 0.000** |

Here, $T_0$ = Control (Basal diet) with no supplemental $CuSO_4$, $T_1$ = (Control + $CuSO_4$ @ 100 mg/Kg feed), $T_2$ = (Control + $CuSO_4$ @ 175 mg/Kg feed) and $T_3$ = (Control + $CuSO_4$ @ 250 mg/Kg feed). All values are presented as Mean ± SEM (Standard Error of Mean). NS means statistically not significant (P>0.05). * means significant at 5% level of significance (P<0.05) and ** means significant at 1% level of significance (P<0.01). [a, b, c] means bearing different superscripts in the same row differ significantly.

### 4.4 Cost effective analysis

Cost effective analysis of dietary effect of Copper sulphate on broiler is shown in table 8. The present study revealed that total cost/ broiler (Taka) was 238.30±0.17, 239.12±0.19, 238.74±0.18 and 239.67±0.21 in $T_0$, $T_1$, $T_2$ and $T_3$ respectively. Broiler of $T_2$ (290.60±3.76) group had higher sale price/ broiler (Taka) than $T_0$ (274.36±4.57), $T_3$ (275.91±4.17) where as $T_1$ (281.74±4.32) group was significantly similar to $T_2$.Net Profit/ broiler (Taka) was significantly highest in $T_2$ (51.86±3.72) which was followed by $T_1$ (42.62±4.34), $T_3$ (36.23±4.18) and $T_0$ (36.06±4.59) respectively. The present study is similar to the results of Abdallah *et al.* (2009) who showed the comparatively better economic efficiency compared to the control when treated with organic copper sulfate in diet.

Table 8: Cost effective analysis of dietary effect of Copper sulphate on broiler

| Parameter | Treatment | | | | P value |
|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | $T_3$ | |
| Chick cost/ chick | 50 | 50 | 50 | 50 | |
| Total feed cost/ broiler | 146.30±0.17 | 146.42±0.19 | 145.53±0.18 | 145.93±0.20 | NS |
| Cost of CuSO4 | 0 | 0.70 | 1.21 | 1.74 | |
| Miscellaneous cost | 42 | 42 | 42 | 42 | |
| Total cost (Taka) | 238.30±0.17 | 239.12±0.19 | 238.74±0.18 | 239.67±0.21 | NS |
| Sale price/ Kg live weight (Taka) | 108 | 108 | 108 | 108 | |
| Sale price/ broiler (Taka) | 274.36±4.57$^{b}$ | 281.74±4.32$^{ab}$ | 290.60±3.76$^{a}$ | 275.91±4.17$^{b}$ | 0.029* |
| Net Profit/ broiler (Taka) | 36.06±4.59$^{b}$ | 42.62±4.34$^{ab}$ | 51.86±3.72$^{a}$ | 36.23±4.18$^{b}$ | 0.027* |

Here, $T_0$ = Control (Basal diet) with no supplemental $CuSO_4$, $T_1$ = (Control + $CuSO_4$ @ 100 mg/Kg feed), $T_2$ = (Control + $CuSO_4$ @ 175 mg/Kg feed) and$T_3$ = (Control + $CuSO_4$ @ 250 mg/Kg feed). All values are presented as Mean ± SEM (Standard Error of Mean). NS means statistically not significant (P>0.05). * means significant at 5% level of significance (P<0.05) and ** means significant at 1% level of significance (P<0.01). $^{a, b, c}$ means bearing different superscripts in the same row differ significantly.

# CHAPTER V

# SUMMARY AND CONCLUSIONS

Antimicrobial resistance is now one of the most important health problems in man and animal. In order to decrease this problem, it is necessary to limit the use of antimicrobials in livestock and poultry production and substitute antibiotics growth promoter with alternative compound that has positive effect on growth performance and profitability as soon as possible. All antimicrobial drugs have adverse effects when exposed to human and animals with greater dose or persistent period than suggested. For these reasons, a study was conducted to investigate the dietary effect of copper sulfate as growth promoter alternative to antibiotic in commercial broiler performances. The study was conducted for a period of 5 weeks from 1$^{st}$ March to 5$^{th}$ April, 2018 in a personal farm at Lalbag, sadar upazila of Dinajpur and the microbial load was analyzed in Microbiological Laboratory of the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU) to examine the effect of copper sulphate on the growth performance such as live weight gain, feed intake, feed conversion ratio, carcass characteristics such as dressing percentages, heart, liver and abdominal fat weight of broilers.

In the present study, a total number of 144 day-07 old broiler chicks (Cobb 500) were randomly divided into four dietary treatment groups having 3 replications in each group containing 12 birds in each replication. The four dietary treatment groups were as $T_0$ (Basal diet with no supplemental $CuSO_4$); $T_1$ (Control + $CuSO_4$ @ 100 mg/Kg feed); $T_2$ (Control + $CuSO_4$ @ 175 mg/Kg feed) and $T_3$ (Control + $CuSO_4$ @ 250 mg/Kg feed) respectively.

Data on initial and final live weight and feed intake were collected and finally three birds from each replication were randomly selected and slaughtered for measuring carcass characteristics at 35 days of experimental periods. By using the SPSS software the collected data were analyzed at 5% level of probability.

The present study revealed that supplementation of $CuSO_4$ had significant ($P<0.05$) effect on different parameters. Dietary supplementation of copper sulphate @ 175 mg/Kg feed in broiler shows significantly ($P<0.05$) highest results in live weight gain, dressing % and net profit (Taka) per broiler and lowest value in FCR and abdominal fat weight (g). But the *E. coli* and *Salmonella* sp. load in intestine, cloaca and caeca was significantly lower in copper sulphate @ 250 mg/Kg feed treated group, followed by copper sulphate @ 175 mg/Kg feed treated group than control group.

By analyzing all results and calculating the cost benefit, it might be concluded that copper sulfate @ 175 mg/Kg feed might be profitable to use as a growth promoter in commercial broiler ration.

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