EFFECTS OF DIETARY SUPPLEMENTATION OF COPPER AND ZINC ON PRODUCTIVE PERFORMANCE AND HEMATOLOGICAL PARAMETERS OF COMMERCIAL BROILER

A THESIS

BY

MD. TAOHID WASIM SHAON

Registration No. 1405106

Session: 2014-2015

Semester: January-June, 2016

MASTER OF SCIENCE (M S)
IN
ANIMAL SCIENCE

DEPARTMENT OF GENERAL ANIMAL SCIENCE AND NUTRITION

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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[Submitted to the Department of General Animal Science and Nutrition, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur for partial fulfillment of the requirement of the degree]

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Approved as to style and content by

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DEPARTMENT OF GENERAL ANIMAL SCIENCE AND NUTRITION

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

DECEMBER, 2016

Dedicated to my parents for their undefined encouragements

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ABSTRACT

The effects of feeding copper sulfate pentahydrate, zinc sulfate and their combination were investigated in commercial broilers. Total 80 Cobb-500 of 07 days old chicks were randomly divided into 04 dietary groups having 04 replications in each groups. Four diets were considered: control (T_0), copper sulfate pentahydrate ($CuSO_4.5H_2O$) @ 150 mg/kg of commercial diet (T_1); zinc sulfate ($ZnSO_4$) @100 mg/kg on diet (T_2); and combination of $CuSO_4.5H_2O$ + $ZnSO_4$ @ 150 mg/kg + 100 mg/kg of diet (T_3), respectively. Initial live weight, live weight gain and feed intake were recorded. Two birds from each replication were randomly selected and sacrificed at 35 days of ages and the carcass characteristics, blood parameters such as total erythrocytes count (TEC), total leucocytes count (TLC), hemoglobin content (Hb), packed cell volume (PCV) and cost effectiveness of different treatment used, finally the recorded data were analyzed by using SPSS software at 5% level of probability. The results showed that total feed intake were non-significant (P>0.05) among the experimental broiler groups. The final live weight was significantly (P<0.05) differed among the experimental groups and highest live weight was recorded in T_2-2440 g/bird compared to other three experimental groups. Broilers in T_2 group (1.67) shows the highest feed efficiency and significantly (P<0.05) differ compared with rest of the groups. Daily live weight gain was differed significantly (P<0.05) among the experimental groups where highest value at 3rd and 4th weeks of experiment was found in birds of $T_{2.}$ There were no significant (P>0.05) differences observed among the dietary treatment groups in terms of de-feathering percentages, liver, heart and abdominal fat weight. On the other hand, significant (P<0.05) difference were observed in carcass weight, where highest value was recorded in T_3 compared to T_0. Thigh and breast weight was also differed significantly (P<0.05) in T3 group compared to control and other group. Among the hematological parameters, TEC and TLC were found non-significant (P>0.05) compared to control. Birds in T_1 group shows the highest value of Hb content and PCV (8.07 g/dl; 29.50 %), respectively. During the comparison of cost effective ratio among the experimental groups, satisfactory result was revealed in T_1. Benefit over control group was also highest in this group (44.84 Tk.), this was due to lowest cost and availability of $CuSO_4.5H_2O$ compared to $ZnSO_4$. Although the total live weight gain was higher in $ZnSO_4$ supplied group. As comparing the price of both compounds, it might be profitable to provide $CuSO_4.5H_2O$ in commercial broiler diet.

Key words: Copper sulfate pentahydrate, zinc sulfate, broiler performance, blood parameters.

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ABBREVIATION AND ACRONYMS

AGP	Antibiotic growth promoter
BCRDV	Baby chicks ranikhet disease vaccine
CFI	Cumulative feed intake
cmm	Cubic millimeter
Cu	Copper
$CuSO_4$	Copper sulfate
$CuSO_4.5H_2O$	Copper sulfate pentahydrate
DCP	Di calcium phosphate
EDTA	Ethylenediaminetetraacetate
F:G	Feed intake: Live weight gain
FE	Feed efficiency
FI	Feed intake
FLW	Final live weight
g	Gram
Hb	Hemoglobin
HCl	Hydrochloric acid
HSTU	Hajee Mohammad Danesh Science and Technology University
ILW	Initial live weight
kg	Kilogram
LRI	Livestock research institute
LWG	Live weight gain
mg	Milligram
mm	Millimeter
NaCl	Sodium chloride
NS	Non-significant
PCV	Packed cell volume
rpm	Round per minute
SEM	Standard error mean
SPSS	Statistical Package for the Social Sciences
TEC	Total erythrocytes count
TLC	Total leucocytes count
wk	Week
Zn	Zinc
$ZnCO_3$	Zinc carbonate
ZnO	Zinc oxide
$ZnSO_4$	Zinc sulfate

CHAPTER I

INTRODUCTION

Agricultural farming system now a day's largely depends on poultry sectors in Bangladesh and broiler is one of the major harvest of poultry farming. Broiler production plays a vital role to the national economy in case of promoting employment opportunity; improving financial status for households as well as the nutritional level of the people. According to (MoF, 2012) approximately 2.58 percent of national income came from animal production during the 2010/11 financial year. In addition, meat of broiler possesses a tremendous source of protein and nutrients which are necessary for health and growth of the human body (Rana *et al.,* 2012).

Broiler growth are depends on various types of trace minerals such as copper, iron, manganese, and zinc and are involved in many digestive, physiological, and biosynthetic processes within the live. These minerals act as catalysts in enzyme systems within cells or as parts of enzymes and are considered as constituents of hundreds of proteins involved in intermediary metabolism, hormone secretion pathways, and immune defense systems (Dieck *et al.,* 2003) and such trace elements are required in small amounts, usually less than 100 mg/kg dry matter (Bao *et al.,* 2009). Limited use of antibody growth promoter (AGP) in food animal have been established worldwide (Turnidge, 2004) and for this reason copper might be useful as growth promoter because it has important role in immune stimulation, reducing stress and lowering disease challenges. So that there was a practice to supplement an addition of copper sulfate in poultry feed (Cohen, 2002). Similar information as copper improves the growth and feed efficiency in broilers when it is provided at much higher pharmacological levels (Choi and Paik, 1989; Baker *et al.,* 1991) (Fuller *et al.,* 1960; Bunch *et al.,* 1961; Burnell *et al.,* 1988). It was also found in some experiments that feeding copper sulfate ($CuSO_4$) in white leghorn hens has a positive response (Chiou *et al.,* 1997).

Many of the authors support copper sulfate pentahydrate due to cost effectiveness and easy availability, copper sulfate pentahydrate is the main source of copper, however, an excess in the diet may depresses growth and feed efficiency in broilers (Funk and Baker, 1991; Choi and Paik, 1989; Baker *et al.,* 1991).

So as the other studies proves that sulfate form ($CuSO_4$) is more effective than other forms (Cromwell *et al.*, 1989; Baker *et al.*, 1991; Paik, 2001; Choi and Paik, 1989). On the other hand, Pesti and Bakalli (1996) reported copper sulfate pentahydrate and citrate copper supplementation reduced cholesterol levels of serum and breast muscle.

As copper has antimicrobial properties that improve animal growth performance when fed over the minimum requirement so that this mineral received great attention (Kim *et al.,* 2011; Lu *et al.*, 2010; Fuller *et al.,* 1960; Bunch *et al.,* 1961; Burnell *et al.,* 1988; Samanta *et al.*, 2011).

Copper is routinely used as a micronutrient and is necessary for the development of connective tissue, nerve coverings, and bone (Neethu *et al.,* 2015). Copper plays an important role in antioxidant system as has been described in several studies (Ajuwon *et al.,* 2011; Karimi *et al.*, 2011).

A wide variety of physiological processes are largely depending on zinc, both for broiler health and growth. This trace mineral plays vital responsibilities in enzyme systems and acts as promoters in many enzymatic and hormone regulation process (Suttle, 2010; Batal *et al.,* 2001). It is a fundamental part of more than 300 enzyme systems that are involved in metabolism of energy, protein, and nucleic acids (Tabatabaie *et al.,* 2007) and in numerous biological processes (Vallee and Auld, 1990). A continuous dietary intake of zinc is essential for the body's optimum physiological functions as it is not stored in the body (Zalewski *et al.*, 2005). In the chicken, zinc is necessary for optimum growth, feathering, bone development, skin quality and immunity (Roberson and Schaible, 1958; Stahl *et al.,* 1989; O'Dell, 2000).

There are two preferred source of zinc and that is zinc oxide (ZnO) and zinc sulfate ($ZnSO_4.H_2O$) which are mostly used as feed additives in broiler chicken diet (Wedekind *et al.,* 1992). On the other hand (Edwards, 1959; Sandoval and Kawchumnong, 1972; Sandoval *et al.,* 1999; Puchala *et al.,* 1999) had evaluated the bioavailability of zinc among different inorganic sources and reported that, zinc sulfate ($ZnSO_4$), zinc carbonate ($ZnCO_3$) and zinc oxide (ZnO) had 100%, 93%, and 67% respectively in broiler. The most commonly used zinc for supplementation in animal diet is inorganic zinc in the form of zinc sulfate due to cost and commercial availability (Vladimír *et al.,* 2011). Rossi *et al.* (2007) also reported that addition of organic zinc could minimize skin tearing and improve carcass appearance of broilers while another author found that zinc supplementation (as forms of $ZnSO_4$ or zinc picolinate) could improve carcass weight of Japanese quail (Sahin *et al.,* 2005). Burrell *et al.* (2004) reported improved performance when broilers consumed diets formulated to contain 110 mg of zinc per kg of feed.

In poultry, deficiency of zinc is responsible for reduction in weight gain, skeletal abnormalities, disturbance in bone mineralization, and immunological abnormalities (Kidd *et al.,* 1996; Blamberg *et al.,* 1960; Young *et al.,* 1958; Scott *et al.,* 1969). Therefore, zinc is often supplemented in practical poultry diets to elicit a positive response in broiler chickens, particularly during early age. Tissue uptake of zinc in chicks is linearly related to zinc levels in the diet (Sandoval *et al.,* 1997; Bartlett *et al.,* 2003).

Several studies shows some disagreements between copper and zinc and that is high zinc intake inhibit intestinal absorption and hepatic accumulation of copper and induce clinical signs of copper deficiency (Ogiso *et al.,* 1974; Fischer *et al.,* 1981; Bremner and Beattie, 1995; Gonzalez *et al.,* 2005). Some studies also showed that high zinc intake induces a high level of metallothionein in the intestinal mucosa that has high binding affinity for copper (Hall *et al.,* 1979; Fischer *et al.,* 1983; Santon *et* ***al.,* 200**2). However, there is a very limited studies was conducted to observe the dietary effects of optimum and safety levels of copper and zinc or their combination on productive performance of broiler. Therefore, the present study was designed with the following objectives:

i) To investigate the effects of growth performance, feed intake, feed efficiency and carcass characteristics in commercial broiler.
ii) To study the hematological parameters like hemoglobin (Hb), total erythrocytes count (TEC), total leucocytes count (TLC), packed cell volume (PCV) etc.
iii) To evaluate the cost effectiveness of broiler production using dietary copper and zinc.

CHAPTER II

REVIEW OF LITERATURE

Pertinent literature on “effects of dietary supplementation of copper and zinc on productive performance and hematological parameters of commercial broiler” were reviewed in this chapter. The main objective of this chapter was to provide up-to-date information concerning the research work which is addressed here. Important information related to the present study was represented below under the following headings:

2.1 Performance of broiler-

2.1.1 Live weight and live weight gain

2.1.2 Feed intake

2.1.3 Feed efficiency

2.2 Carcass characteristics

2.3 Effect on hematological performance

2.4 Cost effectiveness of production

2.1.1 Live weight and live weight gain

Hall *et al.* (1979) and **Fischer *et al.* (1983)** showed that high zinc intake induced high metallothionein in the intestinal mucosa that has high binding affinity for copper.

Fox *et al.* (1987) treated group of broilers at the rate of 500 mg/kg level of copper sulfate and found no effect on weight gain and feed conversion. Whereas **Wang *et al.* (1987**) observed depression in growth and feed conversion affected minimal at the same dietary level. But **Burnell *et al.* (1988)** reported antimicrobial activity along with the growth promoting action of copper sulfate in their research. Besides these, **Choi and Paik (1989)** and **Baker *et al.* (1991)** reported improve growth in broiler when copper sulfate is supplied at the levels of 125 to 250 mg/kg of diet.

Chen *et al.* (1996, 1997) also reported that significantly reduction of live weight gain when treated with the supplementation of copper sulfate at the rate of 500 mg/kg in the diet of country chicken and layer pullets. Similar effect was found by **Miles *et al.* (1998)** who observed that growth performance was reduced with the dietary supplementation of copper sulfate from 200 to 600 mg/kg of diet.

Ewing *et al.* (1998) reported average live weight increased at 35 days onwards in copper sulfate supplemented groups compared to control. The authors also suggest that in case of evaluation of effectiveness of copper sulfate supplementation in diet under commercial condition average live weight gain (BWG) is a better indicator than feed efficiency (FE) because calculation of adjusted FE is not routinely practiced in the industry.

Lauridsen *et al.* (1999) conduct a study with the addition of 175 mg/kg copper sulfate on diet resultant improved growth rate and feed intake for a short period but did not affect feed utilization. But **Paik *et al.* (1999)** demonstrated that higher level of copper as copper sulfate gave different response in different species on the basis of performances.

Mohanna and Nys (1999) reported that up to 25 mg/kg of zinc sulfate supplementation on diet has the effect to increase the live weight and feed intake. But another author reported that diet with supplementation of copper sulfate at the rate of 350 mg/kg leads to significant decrease in live weight gain as well as reduced the production performance of broiler. The authors stated that due to nutrient absorption decreased in gastro-intestinal tracts, the performance was reduced (**Zia-Ur-Rahman and Akhtar, 2001**).

Skrivan *et al.* (2002) reported significantly reduced weight gain with the supplementation of higher concentration of copper sulfate in chicken. Broilers receiving the diet with 126 mg/kg total copper sulfate between 1 and 14 days of age reported feed consumption to weight gain ratios were significantly higher and 35 mg/kg during days 15 to 41. The authors also reported decrease mortality with the supplementation of copper sulfate found statistically insignificant.

Faundez *et al.* (2004) demonstrated that copper sulfate has antimicrobial activity against highly prevalent entero-pathogenic organisms, *Salmonella spp*. and *Campylobacter spp.*

Xia *et al.* (2004) reported that copper sulfate pentahydrate has ability to improve the activity of digestive enzymes such as protease, amylase and lipase, which ensured better digestion and utilization of the feed. Thus the authors found a positive effect on live weight gain in broiler chicks with the supplementation of $CuSO_4$. $5H_2O$ at 400 mg/kg feed, which might be total reduction of pathogenic organism in the gut that are interfere with the growth of broilers.

Burrell *et al.* (2004) conduct a study in broiler chickens with a maize-soybean meal basal diet along with the supplementation of 110mg/kg zinc sulfate and reported optimum live weight achievement.

Gonzalez *et al.* (2005) showed reduced hepatic copper sulfate concentration when dietary zinc provided in rat diet. The authors hypothesized that it could be due to the sequestration of dietary copper sulfate by intestinal metallothionein induced by high dietary zinc sulfate content.

Ao *et al.* (2007) reported that copper sulfate of birds' intestinal mucosa, fed with organic zinc was significantly higher than that from the birds fed the control diet but did not differ significantly from those fed the inorganic form. This is possibly due to more zinc being absorbed, more metallothionein being produced, and more copper sulfate being trapped in the mucosa of the small intestine when organic zinc was included in the control 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 periods compared to controls in copper sulfate supplemented groups. But in 2^{nd} week of experiment, there were no significant differences 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 numerically 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.

Liu *et al.* (2011) reported increase average daily weight gain (P<0.04) with the addition of 60, 120, or 180 mg/kg of zinc sulfate in diet. On the other hand, **Anil *et al.* (2012)** found no significant (P>0.05) difference on live weight gain in different treatment treated with zinc sulfate at different dosage up to 6 weeks of birds age. But at the end of experiment the authors found slightly increase of live weight gain when compared to control.

Ezzati *et al.* (2013) reported improve performance include increased live weight gain and decreased feed intake without changing the feed conversion ratio at the level of 100 mg/kg zinc sulfate supplementation on diet.

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

Neethu *et al.* (2015) reported statistically significant different among the treatment group from the control as copper sulfate supplementation reduced the live weight gain in all the groups compared to control group of birds.

Neethu *et al.* (2015) reported that increased concentration of copper sulfate in the diet at the level of 600 mg/ kg and 800 mg/ kg showed a significant difference ($P<0.05$) from other groups' i.e. control and 200mg/kg. The authors also reported that increased copper sulfate supplementation, the growth performance of the birds was found to be reducing. Optimal weight gain was observed with addition of 200 mg/kg copper sulfate.

Abhishek *et al.* (2016) reported the lowest live weight of birds in control group then the other 4 treatment groups which are treated with different form of zinc sulfate prove that zinc sulfate is essential for growth. The authors reported with an irrespective source of zinc sulfate has a significant effect on increase in live weight in groups fed with 80 mg/ kg from 21-45 days of age.

2.1.2 Feed intake

Shivanandappa *et al.* (1983) found feed intake reduction due to the gastrointestinal disturbance caused by copper sulfate as hemorrhagic enteritis.

Mohanna and Nys (1999) reported that up to 25 mg/kg of zinc sulfate supplementation on diet has the effect to increase feed intake.

Zia-Ur-Rahman and Akhtar (2001) found significantly depressed in feed intake and poor feed efficiency in broilers when fed at the rate of 350 mg/kg in diet. Therefore, birds in treated with copper sulfate showed significant decrease in growth performance as compared to bird fed normal diet or in control group.

Salabi *et al.* (2011) found improves feed intake when they added zinc sulfate at the level of 90 mg/kg of diet. The authors also suggest that the requirement for early chick growth is satisfied when chicks are fed diets containing 40 mg of zinc sulfate per kg as recommended by the (NRC, 1994).

Liu *et al.* (2011) reported increase average daily feed intake (P<0.07) with the addition of 60, 120, or 180 mg/kg of zinc sulfate in diet.

Anil *et al.* (2012) concluded as the supplementation of zinc in the form of inorganic and organic at 20, 40, 60, 80 mg/kg of diet did not influence much with regard to the feed intake.

Ezzati *et al.* (2013) reported that daily feed intake significantly affected (P<0.01) by dietary zinc sulfate but not the feed conversion ratio. They explained feed intake was improved with the supplementation of 100 mg/kg zinc sulfate on diet, but in those groups which were treated between 100 mg/kg and 125 mg/kg found no significant differences between feed intakes.

2.1.3 Feed efficiency

Paik *et al.* (1999) & **(2000)**; **Paik (2001)** showed improved feed efficiency in broilers when copper sulfate was supplemented where weight gain was the determining factor.

Xia *et al.* (2004) found lowest feed efficiency when the bird was treated with the supplementation of 200 mg/kg. The authors discussed the possible cause of positive effect of copper sulfate at the level of 200 mg/kg on diet could be attributed to the reduction of pathogenic microorganisms of digestive tract.

Chowdhury *et al.* (2004) reported an improvement in feed efficiency at the higher level 200 mg/kg but cumulative feed intake (CFI) was reduced of birds supplemented copper sulfate on feed during 1-6 week resulted in no differences in feed efficiency (FE).

Bao *et al.* (2009) suggested that zinc sulfate level is the first limiting element among zinc, manganese, copper and iron because supplemental organic zinc alone or combined with other elements, manganese, copper and iron, significantly improved feed conversion ratio.

Hashish *et al.* (2010) found the effect of feeding copper sulfate at highest level i. e. 200 mg/kg and 300 mg/kg which consumed more feed in grower period compared to control and the group treated with 100 mg/kg of diet. But the groups which supplied at the rate of 100 mg/kg and 300 mg/kg gave the best values of feed efficiency compared with the control and group treated with 200 mg/kg of diet.

Kumar *et al.* (2013) found significantly ($P<0.05$) higher live weight when the birds were treated with 400 mg/kg on diet compared to control and other two groups treated with copper sulfate at 100 mg/kg and 200mg/kg throughout the experimental period. The authors also reported reduced feed conversion ratio after day 42 of the experiment when a group treated with copper sulfate at 200mg/kg in diet.

Ezzati *et al.* (2013) compared among control and experimental groups (i. e. 100 mg/kg and 125 mg/kg treated groups) and found no significant difference ($P>0.05$) in feed efficiency.

Midilli *et al.* (2014) found an increasing feed efficiency while they supplied inorganic and organic form of zinc alone or combination with microbial phytase.

Dukic *et al.* (2015) treated broilers with inorganic and organic form of zinc and found no significant difference in finisher and feed efficiency in whole 6 period of experiment.

2.2 Carcass characteristics

Barber *et al.* (1960) showed increased dressing percentage might be a cause of reduction in the liver and gut weight when the broiler groups were treated with copper sulfate in diet. Whereas, **Waldroup *et al.* (2003)** tested with the supplementation of 55 and 250 mg/kg copper as copper sulfate and did not found any effect on the dressing percentage and edible carcass yield.

Arias and Koustos (2006) conducted a study with the supplemented 188 mg/kg of copper sulfate in broiler birds and reported an improvement in the carcass weight when used either recycled or fresh litter. But the authors did not observe any effect on weight of spleen and liver with the supplementation copper sulfate. Addition of 128 mg/kg of dietary copper sulfate supplementation did not show any effect on liver weight (**Sunder *et al.*, 2009**).

Ao *et al.* (2006) reported corn soy based diet with 20.1 mg/kg supplemental level of zinc sulfate for optimal growth rate of chicks. Supplementation of zinc sulfate and copper sulfate provided by sulfate forms of these 2 minerals, but not by organic forms, resulted in significantly lower gain: intake ratio compared with supplementation of only inorganic zinc.

Ao *et al.* (2007) reported increased (P < 0.05) live weight gain: feed intake ratio with dietary supplementation of zinc sulfate. The authors also reported a significant interaction between zinc source and copper source on live weight gain: feed intake of chicks. The live weight gain: feed intake of chicks was improved (P<0.01) by supplementation of zinc sulfate compared with chicks fed the basal diet. Chicks fed both zinc sulfate and copper sulfate had lower (P<0.01) live weight gain: feed intake compared with chicks fed the diet supplemented with inorganic zinc only. The authors also found antagonistic behavior between copper and zinc in inorganic forms but not in organic form and they observed this based on the feed efficiency data.

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.

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.

Neethu *et al.* (2015) reported that increased concentration of copper sulfate in the diet at the level of 600 mg/ kg and 800 mg/ kg showed a significant difference (P<0.05) from other groups' i. e. control and 200mg/kg. The authors also reported that relative dressed weights also increase within the different group when treated with different level of copper sulfate pentahydrate ($CuSO_4.5H_2O$) compared to control group.

Liu *et al.* (2011) conduct a study by zinc source or the interaction between zinc source and found carcass traits were not affected (P>0.10). The authors also reported unaffected

($P>0.10$) dressing percentage, percentage of breast muscle or thigh muscle, or percentage of abdominal fat with the supplementation of dietary zinc, but did affect ($P<0.07$) percentage of eviscerated yield. Higher ($P<0.07$) percentage of eviscerated yield in broiler supplemented with 60, 120, or 180 mg of zinc per kg than birds fed the control diets. The percentage of breast muscle in broilers fed diets with supplemental zinc was numerically higher ($P=0.17$) than that in broilers fed the control diets.

2.3 Effect on hematological performance

Chiou *et al.* (1999) found in their study that copper plays a major role as cofactor in hematogenesis when supplemented in commercial broiler rations. **Mpofu *et al.* (1999)** also explain the importance of copper sulfate as this the most compulsory trace elements in livestock because it is necessary for hemoglobin formation, iron absorption from gastrointestinal tract and iron mobilization from tissue stores.

Zia-Ur-Rahman and Akhtar (2001) found the possible responsible for decrease in the plasma glucose concentration in copper sulfate supplemented birds due to increased packed cell volume and hemoglobin.

2.4 Cost benefit analysis of production

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. **Roy *et al.* (2013)** who reported that feeding probiotic to broiler was either similar or more profitable than combination of probiotic and antibiotic growth promoter while better than antibiotic growth promoter alone.

CHAPTER III

MATERIALS AND METHODS

The birds were reared in a personal farm at Kornai, adjacent to the HSTU campus, Basher Hat, Dinajpur. The relevant laboratory works were performed with proper authentication at Physiology Laboratory, Faculty of Veterinary and Animal Science, HSTU, Dinajpur. The following procedures were followed for covering the experiment.

3.1 Preparation of the research shed

The shed was cleaned and washed using fresh water, soapy water and disinfectant (GPC 8®). Then it was kept open for 5 days before placing the experimental birds. All necessary equipment was set properly to care the broiler chicks successfully.

3.2 Experimental birds

A total of 80, day old broiler chicks of "Cobb 500" strain were purchased from the dealer of Nourish Poultry and Hatchery Limited®. Then the chicks were properly exposed to heat (Brooding) and other management was carefully maintained as the company manual for up to 7 days. Finally the 7 days old birds were carefully transferred to the experimental shed, in which proper lighting, ventilation and heating arrangement were insured. One group was consisting of four replication i.e. four cage and 5 birds are occupied in each cage. Each of the cages contains a feeder and a waterer for each of the five birds. Birds were housed in proper atmosphere and hygienic condition. The birds were fed with standard broiler starter and broiler finisher ration throughout the experimental period.

3.3 Research layout

Total of 80 of seven (07) days old "Cobb 500" broilers were randomly divided into four groups (T_0, T_1, T_2 and T_3) having 4 replications in each. T_0 was considered as control and fed with only commercial ration, and T_1, T_2 and T_3were denoted the broiler groups fed the diet supplementation with 150 mg of $CuSO_4.5H_2O$ (Copper Sulfate Pentahydrate),100 mg $ZnSO_4$ (Zinc Sulfate) per kg of feed and their combination(150 mg $CuSO_4.5H_2O$ and 100 mg $ZnSO_4$), respectively. Initial live weight of each bird was recorded (at day 07) just prior to the dietary grouping and kept them into separated bamboo made cage, the birds were reared on slatted floor (Macha). Live weight gain and weekly feed intake were recorded at 7 days interval up to the end of the 28 days of experimental period(i.e. 35 days of bird age) and total 16 birds were sacrificed for taking visceral organ weight and collect blood sample for hematological study (TEC, Hb and PCV).

Photo 1. Separations of bird in each replication by bamboo cage

Layout of Research work

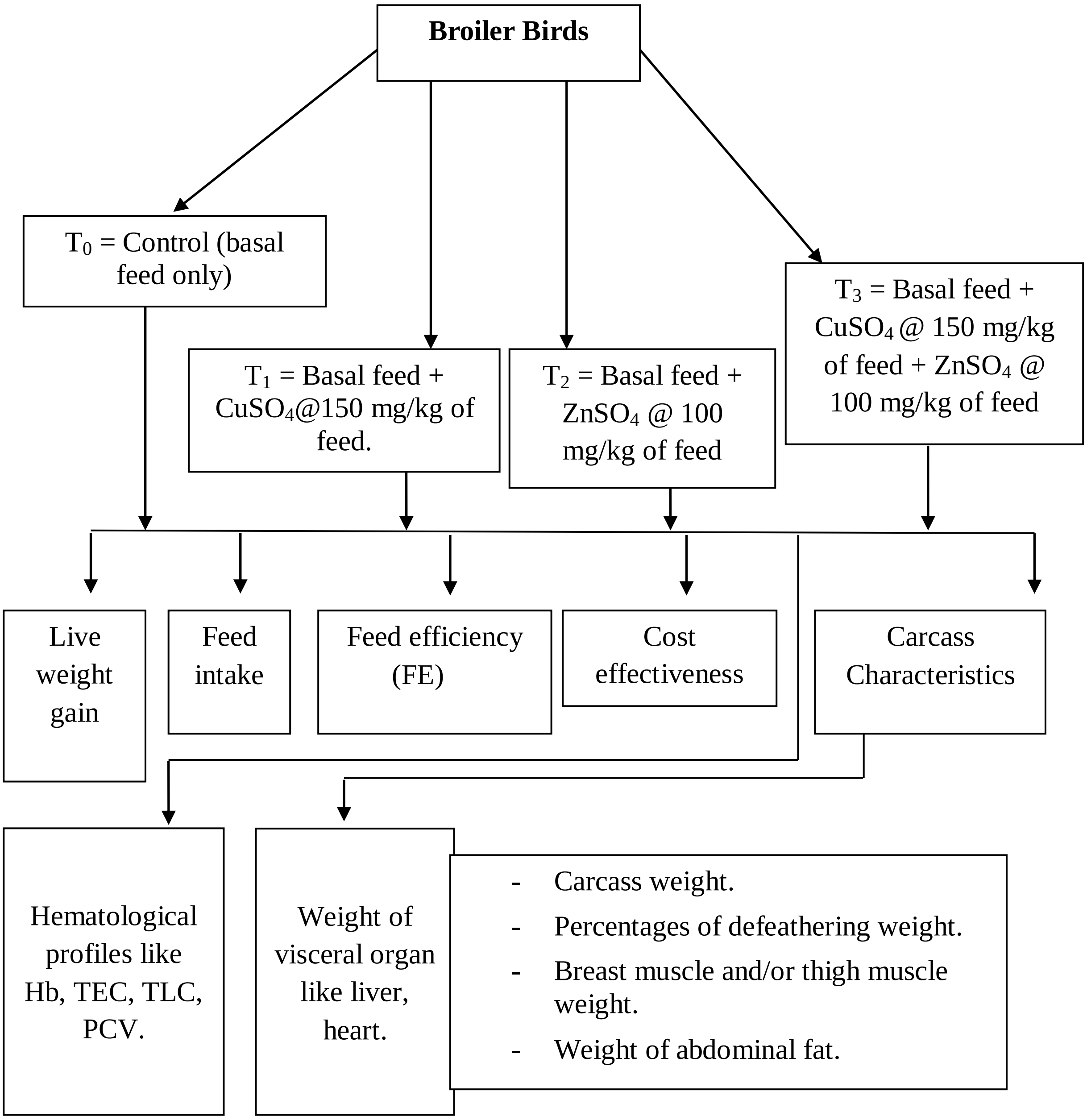

Figure 2. Layout of research work.

3.4 Research diets

T_0 = Basal feed only + fresh drinking water.

T_1 = Basal feed + $CuSO_4.5H_2O$ @150 mg/kg of feed + fresh drinking water.

T_2 = Basal feed + $ZnSO_4$@ 100 mg/kg of feed + fresh drinking water.

T_3 = Basal feed + $CuSO_4.5H_2O$@ 150 mg/kg of feed + $ZnSO_4$@ 100 mg/kg of feed + fresh drinking water.

Composition of the commercial diet (kg/100kg)

Table 1. Formulation of commercial ration

Ingredients	Broiler starter	Broiler finisher
Maize	43.00 kg	43.64 kg
Wheat	10.00 kg	10.00 kg
Rice polish	4.00 kg	10.00 kg
Soybean	26.00 kg	22.50 kg
Meat and Bone meal	9.00 kg	8.00 kg
Oyster shell	1.00 kg	1.00 kg
Salt	300 g	250 g
Methionine	200 g	180 g
Lysine	30 g	30 g
Vitamin Premix (broiler)	250 g	250 g
Feed zyme	-	50 g
Soybean oil	6.5 kg	4.00 kg
DCP	2.50 g	-
Choline chloride	100 g	100 g
Total	100.00 kg	100.00 kg

Source**:** Nourish Poultry and Hatchery Ltd. ®, Bangladesh.

3.5 General management practices

Fresh, clean and cool drinking water was made available for all times. Each bamboo cage was 2.5 ft × 2 ft and was allotted for 5 birds. Fresh and dry rice husk was used as litter at a depth of about 5 cm from day 1 to day 10 and after 10 days when the birds are free from the risk of trapped, the litter materials were removed. In order to maintain required temperature and humidity inside the cage all the windows of the farm were kept open during day and at night electric bulbs were provided as a source of heat and light. The birds were exposed to a continuous lighting of 12 hours; a day. The experimental birds were vaccinated against Newcastle (Ranikhet) disease and Infectious bursal disease (Gumboro) as per Table 2.

Table 2. The vaccination schedule of commercial broiler.

Age of birds (day)	Name and type of vaccine	Preparation of dilution	Dose and route of administration
5	BCRDV freeze dried live vaccine	1 ampoule was diluted with 6 ml of distilled water	One drop in each eye
11	Nobilis Gumboro D 78 freeze dried live vaccine	1 ampoule was diluted with 36 ml of distilled water	One drop in each eye
18	BCRDV (Booster dose) freeze dried live vaccine	As used in day 5	One drop in each eye
21	Nobilis Gumboro D 78 (Booster dose) freeze dried live vaccine	As used in day 11	One drop in each eye

Source: BCRDV- Livestock Research Institute (LRI), Mohakhali, Dhaka, Nobilisgumboro D 78®- Intervet, International B.V.,Boxmeer, Holland.

The following measures were taken during the experimental period to prevent diseases.

i) Entrance of personnel was restricted except farm owner, researcher, supervisor and co-supervisor who visited the farm following special care.

ii) Hands and feet were washed with soap and GPC-8® (Renata, Bangladesh) was sprayed thoroughly just prior to entrance at shed.

iii) Hygienic measurements were taken for vaccination, weighing of birds, feeds, feed storage and during administration.

iv) Fencing was performed around shed with electric to keep the experimental areas free from rats, rodents and wild birds.

v) Dead birds were removed instantly.

3.6 Measurement of live weight

The live weight of each bird was measured with the help of digital balance on 07 days of age (0 day of experiment) and subsequently at every 7 days interval the birds were weighed and recorded up to 35 days of bird age. Total 16 birds were sacrificed, processed and then weights (live and dressed weight, weights of breast, liver, skin, legs, visceral) were taken by electric balance to study the meat yield and hematological characteristics of birds at the end of experiment (35 days of age).

Photo 3: Weighing of birds at different weeks

3.7 Organ weight of birds

The weight of the organs (liver, heart, spleen, viscera, breast meat and leg meat) were measured with the help of electric balance on the day 35 of age (28 days of experiment) of each group.

Photo 4: Weighing of different parts of carcass

3.8 Reagents used for hematological studies

Anticoagulant (EDTA) was used to prevent coagulation of blood. Normal physiological saline composed of 8.5 g sodium chloride (NaCl) and 1000ml distilled water was used for TEC, 1% hydrochloric acid (HCl) was used to estimate TLC and for estimation of hemoglobin.

3.9 Blood collection

Blood was collected in sterile test tubes containing anticoagulant (EDTA) from each group through slaughtering. The hematological measurements were performed as soon as possible of blood collection.

3.10 Methods

Following hematological parameters were analyzed.

3.10.1 Total erythrocytes count (TEC)

a) The tip of the dry clean red pipette was dipped into the blood sample and blood was sucked up to 0.5 mark of the pipette.

b) The tip of the pipette was wiped with cotton. Then the tip immediately placed into the red cell diluting fluid and the pipette was filled with the fluid up to 101 marks.

c) The contents of the pipette were mixed manually by 8-knot methods for 5 minutes.

d) The counting chamber was placed with cover glass under microscope using low power (10×) objectives.

e) After discarding 2 or 3 drops of fluid from the pipette a small drop was placed to the edge of the cover glass placed on the counting chamber and the area under the cover glass was filled by the fluid introduced.

f) One minute time was allowed to settle the cells uniformly into the chamber.

g) The cells were counted from the recognized 80 small squares under high power objective (40×) and were calculated accordingly. The result was expressed in million/mm^3.

3.10.2 Total Leukocyte Count (TLC)

a) At first, well-mixed capillary or venous blood exactly to the 0.5 mark was drawn in a white blood cell diluting pipette.

b) The excess blood was wiped from the outside of the pipette to avoid transfer of cells to the diluting fluid.

c) Then diluting fluid was immediately drawn up to the "11" mark while rotating the pipette between the thumb and forefinger to mix the specimen and diluents.

d) The contents of the pipette was mixed thoroughly for 3-5 minutes to ensure even distribution of cells then unmixed and relatively cell free fluid was expelled from the capillary portion of the pipette.

e) After that, the forefinger was placed over the top (short end) of the pipette, the pipette was held at a 450 degree angle, and then the pipette tip was touched to the junction of the cover glass and the counting chamber.

f) The mixture then allowed to flow under the cover glass until the chamber is completely charged.

g) The cells were allowed to settle for about 3 minutes. Under low power magnification and reduced light, the ruled area was focused and observes for even distribution of cells.

h) The white cells then counted in the four 1 square mm corner areas.

i) All the white cells lying within the square and those touching the upper and right-hand center lines were counted. The white cells that touch the left-hand and bottom lines did not count. In each of the four areas, conduct the count as indicated by the "snake-like" line.

3.10.3 Determination of hemoglobin (acid-hematin method)

a) N/10 HCI solution was taken in a graduated diluting tube up to 2 marks with the help of a dropper.

b) EDTA mixed well-homogenized blood was then drawn into the Sahli’s pipette up to 20 cmm mark.

c) The tip of the pipette was wiped with sterile cotton to get rid of unwanted blood and the blood of the pipette was immediately transferred into the diluting containing HCI solution.

d) The pipette was rinsed 2-3 times by sucking fluid from the top of the tube. This blood and acid were thoroughly mixed by a glass stirrer into the diluting tube. There was the formation of acid hematin in the tube by the hemolysed RBC and HCI.

e) This tube containing acid hematin mixture was kept standing in the comparator for 5 minutes. After that distilled water was added drop by drop.

f) The solution was mixed well with a stirrer until the color of the mixture resembled the standard color of the comparator.

g) The result was read in daylight by observing the height of the liquid in the tube considering the lower meniscus of the liquid column.

h) The result was then expressed in g/dl.

3.10.4 Determination of Packed cell volume (PCV)

The citrated blood was drawn into the special loading pipette (Wintrobe pipette).

a) The tip of the pipette was inserted (placed) to the bottom of a clean, dry Wintrobe hematocrit tube.

b) The rubber bulb of the pipette was pressed continuously to expel the blood out of the pipette.

c) The Wintrobe hematocrit tube was filled from the bottom.

d) As blood came out, the pipette was slowly withdrawn and pressure was continued on the rubber bulb as the tube fills.

e) The tube was filled exactly up to 10 mark of the right sided scale.

f) The tube was placed in a centrifuge machine and was centrifuged for 30 minutes at 3000 rpm.

g) After 30 minutes the tube was taken out and the reading was recorded from the right sided scale.

h) The result was expressed in percentage (%).

Photo 5: Estimation of different hematological parameters.

3.11 Calculation

Performance & carcass characteristics:

$$\textbf{Total weight gain (g)} = \text{Average final wt} - \text{Average initial wt.}$$

$$\textbf{Average daily gain (g)} = \frac{\text{Finish live weight} - \text{Initial live weight}}{\text{Age (days)}}$$

$$\textbf{Feed Effciency (FE)} = \frac{\text{Feed Intake}}{\text{Average daily gain}}$$

TLC: The formula is as follows-

$$\textbf{WBC}\left(\frac{\text{thou}}{\text{mm3}}\right) = \frac{\text{No. of WBC counted X 1 large Sq. X Dilution factor(20)}}{\text{4 large sq. X 0.1 mm3}}$$

TEC: The formula is as follows-

$$\textbf{RBC}\left(\frac{\text{million}}{\text{mm3}}\right) = \frac{\text{No. of RBC counted X 25 Small Sq. X Dilution factor(200)}}{\text{5 Small sq. X 0.1 mm3}}$$

Photo 6: Respected supervisor and co-supervisor were visited to guide our research work.

CHAPTER IV

RESULTS

4.1 Performance of broiler

The results of feeding copper as copper sulfate pentahydrate ($CuSO_4.5H_2O$), zinc as zinc sulfate ($ZnSO_4$) and their combination on broiler are presented in the following sub-headings:

Table 3: Production performance with the dietary supplementation of copper sulfate pentahydrate ($CuSO_4.5H_2O$), zinc sulfate ($ZnSO_4$) and their combination.

Parameter	Dietary treatment				Level of significance
	Control	$CuSO_4.5H_2O$	$ZnSO_4$	$CuSO4.5H_2O$ + $ZnSO_4$	
ILW at 7 days (g/bird)	166.5 ± 0.52	167.5 ± 0.96	167.5 ± 0.97	168.8 ± 0.89	NS
FLW at 35 days (g/bird)	1926.0 ± 6.53^a	2312.0 ± 5.23^b	2440.0 ± 30.82^c	2415.0 ± 17.09^c	*
Total LWG (g/bird)	1759.35 ± 6.88^a	2144.95 ± 5.32^b	2272.90 ± 30.89^c	2246.65 ± 17.93^c	*
Total FI (g/bird)	4006.0 ± 13.59	4081.0 ± 37.98	4073.0 ± 42.39	4161.0 ± 14.34	NS
FE	2.077 ± 0.01^c	1.767 ± 0.01^b	1.668 ± 0.01^a	1.722 ± 0.01^b	*

*$CuSO4.5H_2O$= Copper sulfate pentahydrate, $ZnSO_4$= Zinc sulfate, ILW = Initial live weight, FLW = Final live weight, LWG=Live weight gain, FI=Feed intake, FE= Feed efficiency, NS= Non-significant at 5% level of probability, Values indicate average ± Standard error mean (SEM). $^{a, b, c}$ means bearing different superscripts in a row differ significantly. *= ($P<0.05$).*

4.1.1 Live weight and live weight gain

Table 3 represents the productive performance of broiler received feed supplemented with $CuSO_4.5H_2O$, $ZnSO_4$ and their combination.

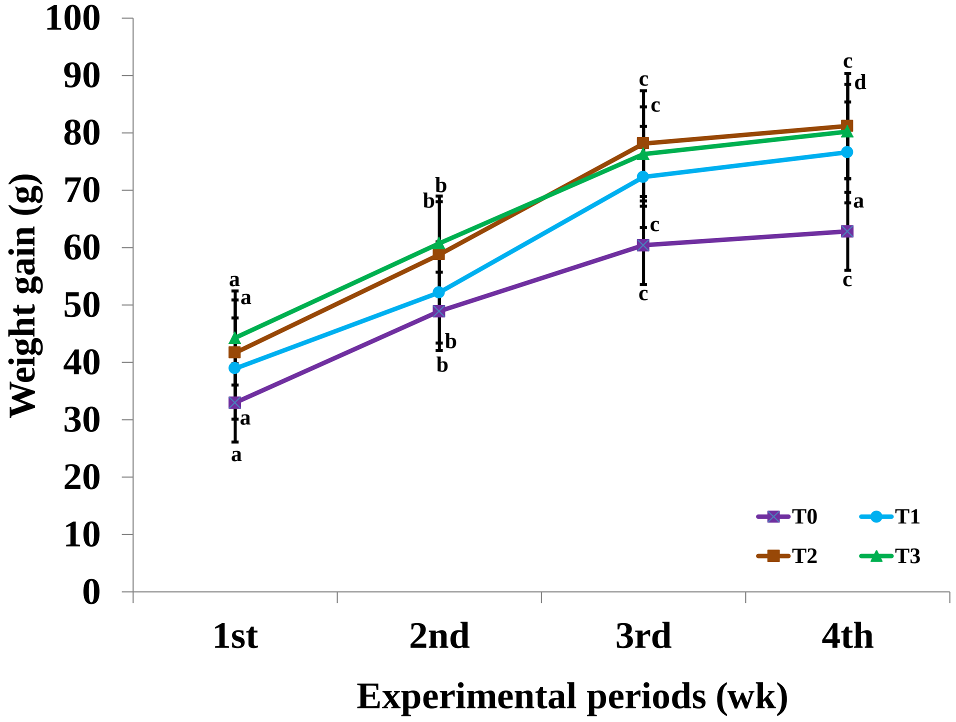

Figure 7. Live weight gain of broilers fed diet supplemented with $CuSO_4.5H_2O$, $ZnSO_4$ and their combination (*T_0, Control; T_1, containing $CuSO_4.5H_2O$ 150 mg/kg; T_2, containing $ZnSO_4$ 100 mg/kg and T_3, containing $CuSO_4.5H_2O$ 150 mg and $ZnSO_4$ 100 mg/kg)*. Each line with error bar represents Mean ± Standard error means (SEM). Differences were significant ($P<0.05$) among the groups. $^{a, b, c, d}$ means bearing different superscripts in a row differ significantly($P<0.05$).

In respect to initial live weight, there was no significant ($P>0.05$) difference among the experimental groups. At 35 days of age, the highest live weight (T_2- 2440 g/bird) was found in broilers fed diet with $ZnSO_4$.

This was followed by the group of broiler birds belonging to $CuSO_4.5H_2O+ZnSO_4$ (T_3-2415 g/bird), $CuSO_4.5H_2O$ (T_1-2312 g/bird) and control group (T_0-1926 g/bird), respectively. However, broiler receiving $ZnSO_4$ weighed significantly ($P<0.05$) higher than that of other experimental groups. In along with this, the difference on total live weight gain with regard to $ZnSO_4$(T_2-2272.90 g/bird) compared with the broiler group fed diet supplemented with $CuSO_4.5H_2O$ (T_1-2144.95 g/bird), control (T_0-1759.35 g/bird) and combination of $CuSO_4.5H_2O$ and $ZnSO_4$ (T_3-2246.65 g/bird) were also significant ($P<0.05$).

The effect of feeding diet supplemented with $CuSO_4.5H_2O$, $ZnSO_4$ and their combination on weekly live weight gain at different bird groups are shown in Figure 7. During 1st week of experiment, the highest live weight gain was observed at group supplemented with $CuSO_4.5H_2O$ and $ZnSO_4$ combindly(T_3-44.26 g/d/bird) than those of the other three experimental groups i.e. $ZnSO_4$(T_2-41.68 g/d/bird), $CuSO_4.5H_2O$ (T_1-38.94 g/d/bird) and control (T_0-32.95 g/d/bird), respectively. The live weight gain pattern was similar for the 2nd week also. But in 3rdand 4th week, the live weight gain of broilers fed diet supplemented with $ZnSO_4$ (T_2) observed significantly ($P<0.05$) higher among the experimental groups. The highest weight gain at 3rd and 4th week of experiment was recorded in T_2 (78.14, 81.18 g/d/bird) followed by combined (T_3-76.32, 80.24 g/d/bird), $CuSO_4.5H_2O$ (T_1-72.29, 76.60 g/d/bird) and control (T_0-60.40, 62.83 g/d/bird), respectively.

4.1.2 Feed intake

The average feed intake by the broilers of different experimental groups was shown in Table 3 and Figure 8.

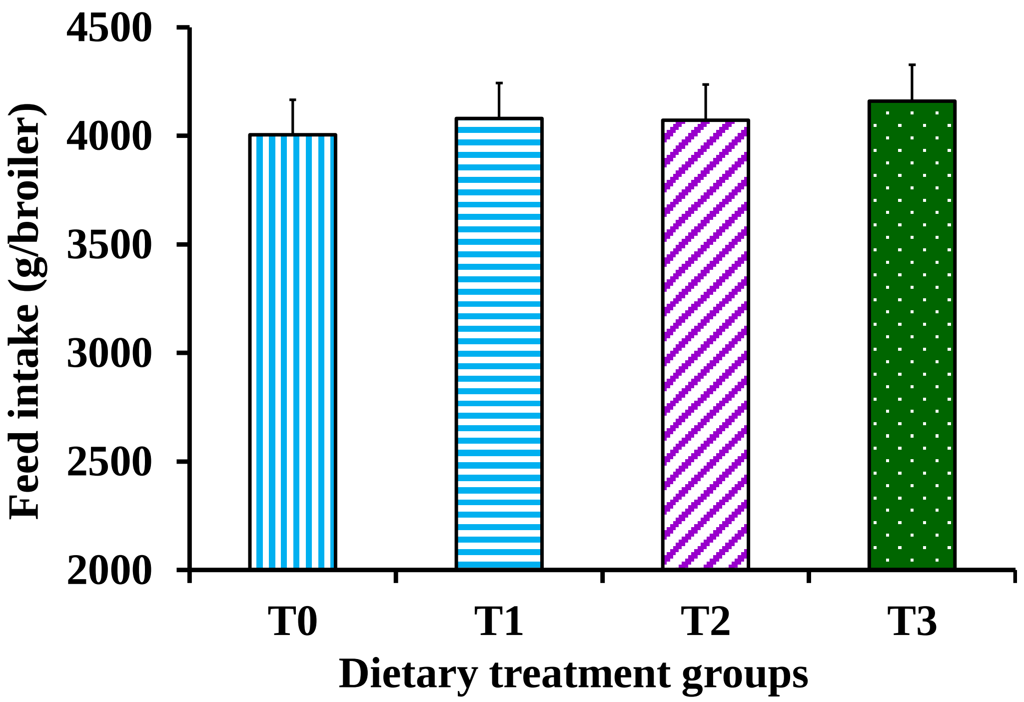

Figure 8. Feed intake of broilers fed $CuSO_4.5H_2O$, $ZnSO_4$ and their combination (*T_0, Control; T_1, containing $CuSO_4.5H_2O$150 mg/kg; T_2, containing $ZnSO_4$ 100 mg/kg and T_3, containing $CuSO_4.5H_2O$150 mg and $ZnSO_4$ 100 mg/kg).* Each bar with error bar represents Mean ± Standard error means. Differences were significant ($P<0.05$) among the groups.

It was found that feed intake of experimental broilers was not significantly ($P>0.05$) differed among the groups during the experimental period. Although a little higher feed intake was observed in group diet supplied with $CuSO_4.5H_2O$ (T_1-4081 g/bird), $ZnSO_4$ (T_2-4073 g/bird) and combined (T_3-4161 g/bird) group of broilers fed $CuSO_4.5H_2O$ and $ZnSO_4$, respectively but it was not significantly ($P>0.05$) higher than that of the control group (T_0-4006 g/bird).

4.1.3 Feed efficiency (FE)

The effect of $CuSO_4.5H_2O$, $ZnSO_4$ and their combination on feed efficiency of broiler in different experimental groups is presented in Figure 9.

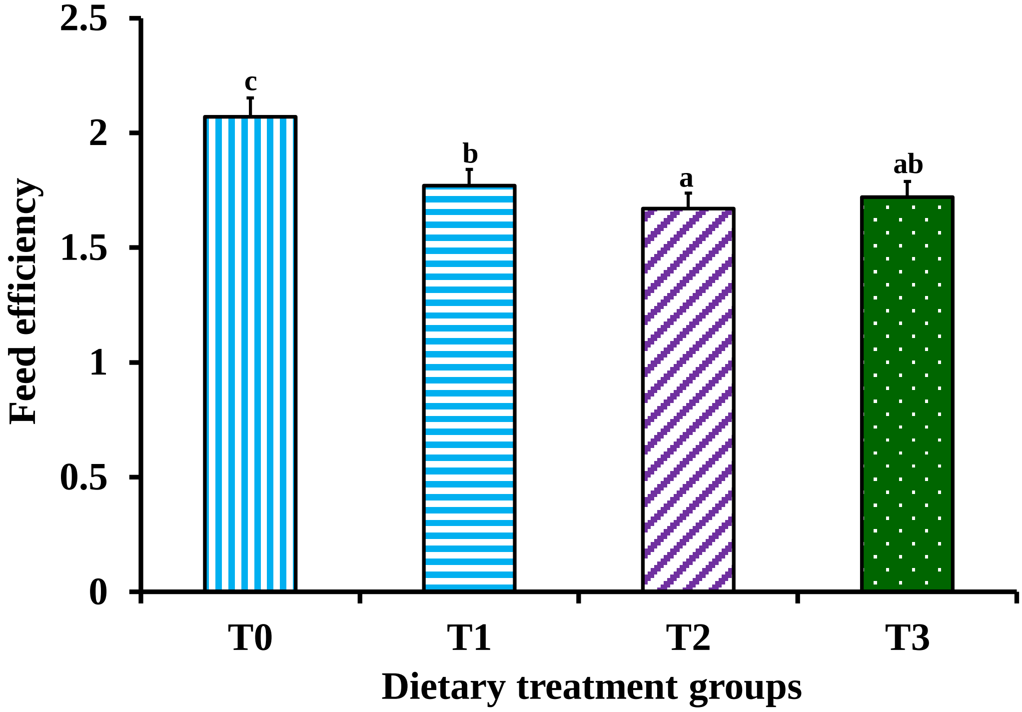

Figure 9. Feed efficiency (FE) of broilers fed diet supplemented with $CuSO_4.5H_2O$, $ZnSO_4$ and their combination (*T_0, Control; T_1, containing $CuSO_4.5H_2O$150 mg/kg; T_2, containing $ZnSO_4$ 100 mg/kg and T_3, containing $CuSO_4.5H_2O$150 mg and $ZnSO_4$ 100 mg/kg).* Each bar with error bar represents Mean ± Standard error means. Differences were significant ($P<0.05$) among the groups. $^{a, b, c}$ means bearing different superscripts in a row differ significantly ($P<0.05$).

It was found that feed efficiency was significantly ($P<0.05$) higher in the broilers fed diet supplemented with $CuSO_4.5H_2O$, $ZnSO_4$ and combination of $CuSO_4.5H_2O$and $ZnSO_4$ (T_1-1.77, T_2-1.67 and T_3-1.72), respectively than the control group (T_0-2.07).

Broilers fed diet supplemented with $CuSO_4.5H_2O$ (T_1) and $ZnSO_4$ (T_2) significantly ($P<0.05$) differed in their feed efficiency but broilers fed diet supplemented with combination of $CuSO_4.5H_2O$ and $ZnSO_4$ (T_3) was not significantly ($P>0.05$) differed with T_1 and T_2.

4.2 Carcass characteristics

Effect of feeding $CuSO_4.5H_2O$, $ZnSO_4$ and their combination on carcass characteristics of broiler represent in Table 4.The analyzed data in the table indicates that the treatments had no significant (P>0.05) effect on defeathering percentage, liver, heart and abdominal fat weight among the experimental birds.

Table 4: Carcass characteristics of broilers fed diet with $CuSO_4.5H_2O$, $ZnSO_4$ and their combination.

Variables	Dietary treatments				Level of significance
	Control	**$CuSO_4.5H_2O$**	**$ZnSO_4$**	**$CuSO_4.5H_2O$ + $ZnSO_4$**	
Defeathering percentages (%)	93.49 ± 0.45^a	93.99 ± 0.77^a	92.63 ± 0.29^a	92.87 ± 0.40^a	NS
Carcass weight (g)	1398.87 ± 14.93^a	1712.50 ± 9.87^b	1758.62 ± 20.15^b	1876.25 ± 44.37^c	*
Thigh weight (g)	233.81 ± 6.81^a	290.88 ± 3.30^b	300 ± 4.39^b	304.5 ± 2.95^b	*
Breast weight (g)	376.3 ± 3.38^a	466.8 ± 4.91^b	439.0 ± 23.80^b	503.9 ± 8.87^c	*
Liver weight (g)	49.75 ± 0.52	49.00 ± 0.57	51.37 ± 0.71	51.87 ± 1.7	NS
Heart weight (g)	11.62 ± 0.13	11.62 ± 0.24	11.75 ± 0.14	12.12 ± 0.24	NS
Abdominal fat weight (g)	49.00 ± 0.73	49.75 ± 0.52	50.75 ± 1.01	50.25 ± 1.05	NS

*$CuSO_4.5H_2O$= Copper sulfate pentahydrate, $ZnSO_4$= Zinc sulfate, NS= Non-significant at 5% level of probability, values indicate Mean ± Standard error mean (SEM). ^{a,b,c} means bearing different superscripts in a row differ significantly. *= (P<0.05).*

Although the defeathering percentages is slightly higher in group treated with dietary supplementation of $CuSO_4$ (T_1- 93.99 %) compared to other 3 experimental group i.e. control (T_0- 93.49 %), combination of $CuSO_4$ and $ZnSO_4$ (T_3- 92.87 %) and $ZnSO_4$ (T_2- 92.63 %). But carcass weight was significantly (P<0.05) differing among the experimental group. Both T_1 and T_2 was not significantly differ between themselves but they differed from the other 2 experimental groups i.e. T_3 and T_0 where the highest value was recorded in groups fed diet supplemented with combination of $CuSO_4.5H_2O$ and $ZnSO_4$ (T_3- 1876.25 g/bird) followed by $ZnSO_4$ (T_2- 1758.62 g/bird), $CuSO_4.5H_2O$ (T_1- 1712.50 g/bird) and the lowest was found in control (T_0- 1398.87 g/bird) groups. The thigh weight value was found significantly (P<0.05) differ in treatment group i.e. (T_1-

290.88 g/bird), (T_2- 300 g/bird) and (T_3- 304.5 g/bird) when compared with control (T_0- 233.81 g/bird). But the treatment group was found non-significant (P>0.05)within themselves where highest value was recorded in group fed diet supplemented with combination of $CuSO_4.5H_2O$ and $ZnSO_4$ and the lowest thigh was recorded in control group. On the other hand, breast weight was significantly (P<0.05) differing among the experimental group. Both T_1 and T_2 was not significantly (P<0.05) differ between themselves but they differed from the other experimental groups i.e. T_3 and T_0 where the highest value was recorded in groups fed diet supplemented with combination of $CuSO_4.5H_2O$ and $ZnSO_4$ (T_3- 503.9 g/bird) followed by $ZnSO_4$ (T_2- 439 g/bird), $CuSO_4.5H_2O$ (T_1-466.8 g/bird) and the lowest was found in control (T_0- 376.3 g/bird) groups.

4.3 Effect on hematological parameters

The effect of $CuSO_4.5H_2O$, $ZnSO_4$ and their combined effect on blood parameters of experimental broilers are shown in Table 5. In the present study, there was no significant (P>0.05) difference found in TEC and TLC. But, significant (P<0.05) different was observed in hemoglobin content. The hemoglobin content of control (T_0-6.55 g/dl) and group fed diet supplemented with $ZnSO_4$ (T_2- 6.65 g/dl) does not significantly (P>0.05) differ but they significantly (P<0.05) differ from dietary supplementation of $CuSO_4.5H_2O$ group (T_1-8.07 g/dl) and combination of $CuSO_4.5H_2O$ and $ZnSO_4$ (T_3-7.08 g/dl) where highest hemoglobin content was recorded in $CuSO_4.5H_2O$ dietary supplementation group compared to the other experimental groups. The PCV % of group fed diet supplemented with combination of $CuSO_4.5H_2O$ and $ZnSO_4$ (T_3-29.03 %) does not significantly (P>0.05) differ from group fed diet supplemented with $ZnSO_4$ (T_2- 28.48 %) and group fed diet supplemented with $CuSO_4.5H_2O$ (T_1- 29.50 %) but it differ significantly (P<0.05) from control group (T_0- 27.38 %).

Table 5. Blood parameters of broilers fed diet with $CuSO_4.5H_2O$, $ZnSO_4$ and their combination.

Parameter	Dietary treatment				Level of significance
	Control	$CuSO_4.5H_2O$	$ZnSO_4$	$CuSO_4.5H_2O$ + $ZnSO_4$	
TEC (millions/mm^{3})	2.63 ± 0.02	2.74 ± 0.02	2.69 ± 0.03	2.73 ± 0.03	NS
TLC (thousands/mm^3)	23.08 ± 0.09	24.55 ± 0.17	24.08 ± 0.11	24.17 ± 0.14	NS
Hb (g/dl)	6.55 ± 0.13^a	8.07 ± 0.05^c	6.65 ± 0.11^a	7.08 ± 0.09^b	*
PCV (%)	27.38 ± 0.19^a	29.50 ± 0.23^c	28.48 ± 0.20^b	29.03 ± 0.08^{bc}	*

*TEC= Total erythrocytes count, TLC= Total leucocytes count, Hb = Hemoglobin, PCV= Packed cell volume, $CuSO_4.5H_2O$= Copper sulfate pentahydrate, $ZnSO_4$= Zinc sulfate, NS= Non-significant at 5% level of probability, values indicate Mean ± Standard error mean (SEM). $^{a,b,\,c}$ means bearing different superscripts in a row differ significantly. *= ($P<0.05$).*

4.4 Cost-benefit analysis of production

Table 6 shows the cost effective analysis for broiler production fed on $CuSO_4.5H_2O$, $ZnSO_4$ and their combination. Total production cost in terms of per bird was 239 Tk. for control, 251.36 Tk. for $CuSO_4.5H_2O$, 267.94 Tk. for $ZnSO_4$ and Tk. 280.72 for the group fed $CuSO_4.5H_2O$ and $ZnSO_4$ combindly. The profit in terms of per bird of broiler were slightly highest in $CuSO_4.5H_2O$ (54.14 Tk.) group followed by combined (31.06 Tk.), $ZnSO_4$ (37.78 Tk.) and the lowest in control (9.30 Tk.).

Table 6. Cost effective analysis of dietary effect of $CuSO_4.5H_2O$, $ZnSO_4$ and their combination on broilers.

Description	**To (Control)**	**T_1 ($CuSO_4.5H_2O$ @150 mg/kg feed)**	**T_2 ($ZnSO_4$ @ 100mg/kg of feed)**	**T_3 ($CuSO_4.5H_2O$ @ 150 mg/kg feed + $ZnSO_4$@ 100mg/kg feed)**
Cost/chick (Taka)	55	55	55	55
Average feed consumed kg/birds	4	4.08	4.07	4.16
Feed price/kg (Taka)	42	42	42	42
Cost of $CuSO_4.5H_2O$ (Tk./bird)	0	9	0	9
Cost of $ZnSO_4$ (Tk./ bird)	0	0	26	26
Feed cost (Tk./ bird)	168	171.36	170.94	174.72
Miscellaneous (Tk./ bird)	16	16	16	16
Total cost/broiler (Taka)	239	251.36	267.94	280.72
Average live weight (kg)	1.91	2.35	2.3	2.45
Sale price/Kg live wt. (Taka.)	130	130	130	130
Sale price/broiler (Taka)	248.3	305.5	299	318.5
Net profit/broiler (Taka.)	9.3	54.14	31.06	37.78
Benefit over control/ broiler (Taka)	0	44.84	21.76	28.48

Net profit over control was highest for the group fed $CuSO_4.5H_2O$ (44.84 Tk.), this might be due to the minimum cost of $CuSO_4.5H_2O$, followed by combined group (28.48 Tk.) and $ZnSO_4$ (21.76 Tk.). It is therefore distinct that additional supplementation of $CuSO_4.5H_2O$ and $ZnSO_4$ is profitable over control group.

CHAPTER V

DISCUSSION

5.1 Live weight and live weight gain

In the present study, the final live weight at 35 days of age was significantly higher in the broilers fed diet supplemented with $ZnSO_4$ (2440 g/bird), compared to control (1926 g/bird), $CuSO_4.5H_2O$ (2312 g/bird) and combination of $CuSO_4.5H_2O$ and $ZnSO_4$ (2415 g/bird). This finding is agreed with Burrell *et al.* (2004) who conduct a study in broiler chickens with the supplementation of 110mg/kg zinc and reported optimum live weight achievement. Another author Mohanna and Nys (1999) reported that up to 25 mg/kg of zinc supplementation on diet has the effect to increase the live weight. Midilli *et al.* (2014) found increasing live weight, while they supplied inorganic and organic form of zinc alone or combination with microbial phytase. Whereas Abhishek *et al.* (2016) reported with a significant effect on increase in live weight in groups fed with 80 mg/ kg of zinc from 21-45 days of age, the author found the lowest live weight of birds in control group then the other 4 treatment groups which prove that zinc was essential for growth.

The effect of feeding diet supplemented with $CuSO_4.5H_2O$, $ZnSO_4$ and their combination on weekly live weight gain at different bird groups was significantly differed. During 1st week of experiment, the highest live weight gain was observed at group supplemented with $CuSO_4.5H_2O$ and $ZnSO_4$ combindly(T_3- 44.26 g/d/bird) than those of the other three experimental groups i.e. $ZnSO_4$ (T_2- 41.68 g/d/bird), $CuSO_4.5H_2O$ (T_1- 38.94 g/d/bird) and control (T_0- 32.95 g/d/bird), respectively. The live weight gain pattern was similar for the 2nd week also. Hall *et al.* (1979); Gonzalez *et al.* (2005) and Fischer *et al.* (1983) showed that high zinc intake induced high metallothionein in the intestinal mucosa that has high binding affinity for copper. Another author Burnell *et al.* (1988) reported antimicrobial activity along with the growth promoting action of copper. But this result is not agreed with Ao *et al.* (2007) who reported that chicks fed both zinc and copper provided as the inorganic form had lower ($P<0.01$) live weight gain. The author found the antagonistic behavior between copper and zinc in inorganic form. But in 3rd and 4th week, the live weight gain of broilers fed diet supplemented with $ZnSO_4$ (T_2) observed significantly ($P<0.05$) higher among the experimental groups. The highest weight gain at 3rd and 4th week of experiment was recorded in T_2 (78.14, 81.18 g/d/bird) followed by combined (T_3- 76.32, 80.24 g/d/bird), $CuSO_4.5H_2O$ (T_1- 72.29, 76.60 g/d/bird) and control (T_0- 60.40, 62.83 g/d/bird), respectively. This finding is similar with Midilli *et al.* (2014) and Salabi *et al.* (2011) who found improves live weight gain when they added zinc at the level of 90 mg/kg of diet. Liu *et al.* (2011) reported average daily weight gain ($P<0.04$) with the addition of 60, 120, or 180 mg/kg of zinc in diet.

The difference on total live weight gain with regard to $ZnSO_4$ (T_2- 2272.90 g/bird) compared with the broiler group fed diet supplemented with $CuSO_4.5H_2O$ (T_1-2144.95 g/bird), control (T_0- 1759.35 g/bird) and combination of $CuSO_4.5H_2O$ and $ZnSO_4$ (T_3- 2246.65 g/bird) were also significant ($P<0.05$).The result is similar with Ezzati *et al.* (2013) who explained live weight gain was improved with the supplementation of 100 mg/kg zinc on diet but not similar with Liu *et al.* (2011) who reported no differences in average daily weight gains during either experimental period.

5.1.1 Feed intake

It was found that feed intake of experimental broilers was not significantly ($P>0.05$) differed among the groups during the experimental period. Although a little higher feed intake was observed in group diet supplied with $CuSO_4.5H_2O$ (T_1- 4081 g/bird), $ZnSO_4$ (T_2- 4073 g/bird) and combined (T_3- 4161 g/bird) group of broilers fed $CuSO_4.5H_2O$ and $ZnSO_4$, respectively but it was not significantly ($P>0.05$) higher than that of the control group (T_0- 4006 g/bird).The findings of the present study is partially agreed result of Anil *et al.* (2012)who concluded as the supplementation of zinc in the form of inorganic and organic at 20, 40, 60, 80 mg/kg on diet did not influence much with regard to the feed intake. But Ezzati *et al.* (2013) reported decreased feed intake without changing the feed efficiency when zinc was added at the level of 100 mg/kg supplementation on diet.

5.1.2 Feed efficiency (FE)

The effect of $CuSO_4$, $ZnSO_4$ and their combination in feed efficiency on broiler in different experimental group is differed significantly. It was found that FE was higher in the groups fed diet supplemented with $ZnSO_4$ (T_2- 1.67) compared to other treatment groups i.e. $CuSO_4.5H_2O$ (T_1- 1.77) and the combination of $CuSO_4.5H_2O$ and $ZnSO_4$ (T_3- 1.72), respectively and lowest in control group (T_0- 2.07). This finding is similar with the observation of Midilli *et al.* (2014) who found improving feed efficiency while supplied inorganic and organic form of zinc separately or combination with microbial phytase. Ao *et al.* (2007) found improved weight gain: feed intake ratio in broilers by dietary supplementation of zinc compared to the control (basal diet) group. Huang *et al.* (2007) reported highest weight gain: feed intake ratio was observed when zinc was added at the level of 20 mg/kg in diet. Another form of zinc is reported to beneficial for broilers that is zinc-methionine which improves the feed efficiency significantly rather than zinc oxide (Sanford and Kawchumnong, 1972).

5.2 Carcass characteristics

In the present study, the treatments shows no significant (P>0.05) effect on defeathering weight percentage, liver, heart and abdominal fat weight among the experimental birds. Although the defeathering weight percentages is slightly higher in group treated with dietary supplementation of $CuSO_4.5H_2O$ (T_1- 93.99 %) compared to other 3 experimental group i.e. control (T_0- 93.49 %), combination of $CuSO_4.5H_2O$ and $ZnSO_4$ (T_3- 92.87 %) and $ZnSO_4$ (T_2- 92.63 %). The finding is agreed with Barber *et al.* (1960) who showed increased dressing percentage when the broiler groups were treated with copper in diet. Prajapati *et al.* (2010) observed improvement on dressing weight of treatment group as compared to the control by copper supplementation. Neethu *et al.* (2015) also reported that relative dressed weights also increase within the different group when treated with different level of $CuSO_4.5H_2O$ compared to control group. Whereas Waldroup *et al.* (2003) tested with the supplementation of 55 and 250 mg/kg copper as copper sulfate and found unaffected dressing percentage in broiler chicken. On the other hand, Arias and Koutsos (2006) did not observe any change on weight of liver when supplemented copper at 188 mg/kg through tri-basic copper chloride or copper sulfate and 128 mg/kg of copper through copper sulfate (Sunder *et al.*, 2009). Liu *et al.* (2011) reported unaffected percentages of abdominal fat with the dietary supplementation of zinc.

The carcass weight was significantly differing among the experimental group where the highest value was recorded in groups fed diet supplemented with combination of $CuSO_4.5H_2O$ and $ZnSO_4$ (T_3- 1876.25 g/bird) followed by $ZnSO_4$ (T_2- 1758.62 g/bird), $CuSO_4.5H_2O$ (T_1-1712.50 g/bird) and the lowest was found in control (T_0- 1398.87 g/bird) groups. The thigh weight value was found significantly differ in treatment group i.e. T_1 (290.88 g/bird), T_2 (300 g/bird) and T_3 (304.5 g/bird) when compared with control (T_0- 233.81 g/bird). On the other hand, breast weight was significantly differing among the experimental group. The highest value was recorded in groups fed diet supplemented with combination of $CuSO_4.5H_2O$ and $ZnSO_4$ (T_3- 503.9 g/bird) followed by $ZnSO_4$ (T_2- 439 g/bird), $CuSO_4.5H_2O$ (T_1-466.8 g/bird) and the lowest was found in control (T_0- 376.3 g/bird) groups. The result is partially similar with the findings of Arias and Koustos (2006) who reported an improvement in the carcass weight when used either recycled or fresh litter with the supplemented 188 mg/kg of copper in broiler birds. Liu *et al.* (2011) reported a positive significant (P<0.07) effect of dietary supplementation of $ZnSO_4$ on percentage of eviscerated yield and also found numerically higher (P=0.17) percentage of breast muscle than broilers fed with basal diets (control). But Waldroup *et al.* (2003) did not found any significant effect among the experimental group on edible carcass yield when treated with the supplementation of 55 and 250 mg/kg copper as copper sulfate. Similarly Mondal *et al.* (2007) was also unable to found any improvement in carcass weight with the supplementation of copper at 200 and 400 mg/kg as copper sulfate and 128 mg/kg in diet (Sunder *et al.*, 2009).

5.3 Effect on hematological parameters

In the present study, there was no significant (P>0.05) difference found in TEC and TLC. These findings shows that the birds persisted healthy because the value of TEC and TLC were within the normal range being a hint of without any allergic conditions, presence of foreign body in circulating system. But, significant (P<0.05) different was observed in hemoglobin content and PCV %.

The highest hemoglobin content was recorded in $CuSO_4.5H_2O$ (T1- 8.07 g/dl) and the lowest was found in control (T_0-6.55 g/dl). On the other hand, the PCV % of group fed diet supplemented with of $CuSO_4.5H_2O$ (T_1- 29.50 %)and lowest value was found in control (T0- 27.38 %)group. The findings is agreed with the finding of Chiou *et al.* (1999) who describes that copper plays a major role as cofactor in hematogenesis when supplemented in commercial broiler rations. Similarly Mpofu *et al.* (1999) explain the importance of copper as this the most compulsory trace elements in livestock because it is necessary for hemoglobin formation, iron absorption from GI-tract and iron mobilization from tissue stores. Zia-Ur-Rahman and Akhtar (2001) also found increased packed cell volume, and hemoglobin when birds were treated with copper sulfate.

5.4 Cost benefit analysis of production

Table 6 shows the cost effective analysis for broiler production fed on $CuSO_4.5H_2O$, $ZnSO_4$ and their combination. Total production cost in terms of per bird was 239 Tk. for control, 251.36 Tk. for $CuSO_4.5H_2O$, 267.94 Tk. for $ZnSO_4$ and Tk. 280.72 for the group fed $CuSO_4.5H_2O$ and $ZnSO_4$ combindly. The profit in terms of per bird of broiler were slightly highest in $CuSO_4.5H_2O$ (54.14 Tk.) group followed by combined (37.78 Tk.), $ZnSO_4$ (31.06 Tk.) and the lowest in control (9.30 Tk.). Net profit over control was highest for the group fed $CuSO_4.5H_2O$ (44.84 Tk.), this might be due to the minimum cost of $CuSO_4.5H_2O$, followed by combined group (28.48 Tk.) and group fed diet supplemented with $ZnSO_4$ (21.76 Tk.). It is therefore clear that additional supplementation of $CuSO_4.5H_2O$ and $ZnSO_4$ is profitable over control group. The result is agreed with the findings of Abdallah *et al.* (2009) who showed the comparatively better economic efficiency compared to the control when treated with organic copper 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 (Roy and Chowdhury, 2013).

CHAPTER VI

SUMMARY AND CONCLUSION

Resistance to antimicrobials is one of the most severe universal problems as we move in the 21st century. There are two main parts of alarm over the presence of residues of antimicrobials in animal-derived foodstuff with concern to public health. The major is allergic response and the next is development of antimicrobial resistance in human. Antimicrobial resistance is now a most important human health issue. In order to specifically identify the problem, the livestock productions and their consultants must decrease and limited the use of antimicrobials in livestock production and substitute antibiotics growth promoter with alternative compound that has positive effect on growth in terms of 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. By focusing these issues, a study was conducted to investigate the effect of copper sulfate pentahydrate and zinc sulfate as growth promoter alternative to antibiotic in commercial broiler performances. The study was conducted in a private farm at Kornai, adjacent to the Hajee Mohammad Danesh Science and Technology University (HSTU), Basher Hat, Dinajpur. The name of the farm owner is Md. Mustakim Mia. The relevant laboratory works were done with proper authentication at Physiology and Pharmacology Department Laboratory, Faculty of Veterinary & Animal Science, HSTU, Dinajpur, Bangladesh.

In present study, total 80 of 7 days old chicks were divided into 4 dietary treatment groups (T_0, T_1, T_2 and T_3 fed 0, $CuSO_4.5H_2O$ ate the rate of 150 mg/kg of diet, $ZnSO_4$ at the rate of 100 mg/kg of diet and combination of $CuSO_4.5H_2O$ + $ZnSO_4$ at the rate of 150 mg/kg + 100 mg/kg of diet, respectively) to examine the effect of copper and zinc on production performance such as live weight gain, feed intake, feed efficiency, carcass weight, defeathering percentages, heart, liver and abdominal fat weight and some hematological parameters such as total erythrocytes count, total leucocytes count, hemoglobin content and packed cell volume on commercial broiler at a 28 days of experiment. Weekly data was collected on live weight gain and weekly feed intake and finally 35 days of ages, the live weight and feed intake were calculated and recorded. After that, two birds from each replication were sacrificed for estimation of different carcass characteristics and evaluation of effect of copper and zinc on blood profile of broilers. The recorded data were analyzed with SPSS software at 5% level of probability. The analyzed data shows the following result that supplementation of $CuSO_4.5H_2O$ and $ZnSO_4$ had effect on different parameters. Dietary supplementation of copper results the highest value in hemoglobin content and packed cell volume (PCV) percentages. But zinc sulfate shows the highest value in final live weight, feed efficiency, and daily live weight gain. Whereas the combination of $CuSO_4.5H_2O$ and $ZnSO_4$ shows the best value for carcass weight, individual thigh and breast weight. But these compounds fail to reveal any effect on total feed intake, defeathering percentages, liver, heart and abdominal fat weight.

During the comparison of cost benefit of these treatments over control, it was found that dietary copper sulfate pentahydrate shows the best value compared to zinc sulfate and combined group in terms of taka. Although zinc sulfate supplementation resultant the highest live weight gain in broiler but it is costly than copper sulfate pentahydrate. By calculating the cost benefit, it concluded that copper sulfate pentahydrate might be profitable to use as a growth promoter in commercial broiler ration.

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