EFFECT OF COPPER SULPHATE AND ZINC SUPPLEMENTATION IN RATION ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF BROILER CHICKEN

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CERTIFICATE

*This is to certify that the thesis entitled "**EFFECT OF COPPER SULPHATE AND ZINC SUPPLEMENTATION IN RATION ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF BROILER CHICKEN**" submitted to the Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of Master of Science in Poultry Science, embodies the result of a piece of bona fide research work carried out by **RATAN KUMAR KARMAKAR**, Registration No. **18-09248** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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DEDICATED TO

MY BELOVED PARENTS,

TEACHERS AND FRIENDS

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ACRONYMS AND ABBREVIATIONS

Abbreviation	Full meaning
A.M	Ante meridiem
ANOVA	Analysis of Variance
CBC	Complete blood count
CF	Crude fiber
Cm	Centimeter
cm^2	Square centimeter
CONTD.	Continued
CP	Crude protein
CRD	Complete randomized design
e.g.	For example
EDTA	Ethylene diamine tetraacetic acid
et al.	And others/Associates
FC	Feed consumption
FCR	Feed conversion ratio

Abbreviation	Full meaning
Hb	Hemoglobin
i.e.	That is
IBD	Infectious bursal disease
kcal	Kilo –calorie
Kg	Kilogram
M.S.	Master of science
ml	Milliliter
mm	Millimeter
mmol	Millimoles
MT	Metric-ton
NS	Non-significant
P.M	Post meridiem
ppm	Parts per million
RBC	Red blood cell
SAU	Sher-e-Bangla Agricultural University
SE	Standard error
SPSS	Statistical package for social sciences
viz.	Such as
Vs	Versus
WBC	White blood cell
WHO	World health organization

LIST OF SYMBOLS

Symbols	Full meaning
@	At the rate of
+	Plus
<	Less than
>	Greater than
°C	Degree Celsius
%	Percentage
&	And
*	5% level of significance
/	Per

EFFECT OF COPPER SULPHATE AND ZINC SUPPLEMENTATION IN RATION ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF BROILER CHICKEN

ABSTRACT

A feeding trial was conducted on 150 day-old Lohman meat broiler chicks for a period of 28 days in the poultry farm of Sher-e-Bangla Agricultural University, Dhaka. The aim of the study was to assess the efficacy of copper sulphate and zinc supplementation. The production indexes viz. feed intake, body weight, body weight gain, FCR, dressed weight, dressing percentage, internal organs weight, hematological parameters and survivability of broiler on three replication of five treatments group containing 10 birds each group was recorded and compared.The chicks were assigned to 5 treatments groups of T_1 (control), T_2 (antibiotic), T_3 ($CuSo_4$ + Zn (8mg +30mg)) and T_4 ($CuSo_4$ + Zn (12mg +40mg)) and T_5($CuSo_4$ + Zn (16mg +50mg)) randomly.The results showed that there was significant difference ($P<0.05$) on the feed intake, final live weight, glucose, cholesterol, internal organs and hematological parameters among different treatment groups. The higher body weight found in $CuSo_4$ + Zn (8mg +30mg)T_3 treated group compared to other groups and values were followed in an ascending order in T_4,T_5 T_2, and T_1 group. The dressing percentage, body weight gain, feed conversion ratio, showed no significant ($P>0.05$) difference among all treatment groups. The better FCR (1.33±0.05) found inT_3group. The dressing percentage in T_5 (76.00±0.11) group was higher than other group. The result indicated that effects of supplementation of copper sulphate and zinc in blood constituents had significant effect except monocyte, eosinophil, basophil, and MCHC concentration which were non-significant ($P>0.05$). The control group (T_2) showed higher values of White blood cell (WBC) than other treated group. Higher feed intake found in T_5 (2499.62±8.83g) group compared to other groups. However, superior final live weight (1856.67±78.38g) obtained in T_3 group where birds fed with $CuSo_4$ + Zn (8mg +30mg) compared to those other treatment and control group. Birds fed with $CuSo_4$ + Zn (8mg +30mg)T_3 supplemented diet achieved superior result.

Key words: Broilers;Feed supplementation;Growth perforrmance;Immune response

CHAPTER 1

INTRODUCTION

Nowadays the poultry industry has become a specialized and vibrant business sector at present condition in all over the world. It has occupied a prominent position in the list of exports of products marketed in Bangladesh, producing broilers with high technology and very competitive costs. It is an integral part of farming system in Bangladesh and has created direct, indirect employment opportunity for the people. This sub-sector has proved as an attractive economic activity, thereby, indicating its importance for the entire economy. In Bangladesh malnutrition and unemployment problem are major of all problems. Poultry provides nutritious meats and eggs for human consumption within the shortest possible time which can efficiently and rapidly fulfill the shortage of protein requirements since it can be produced at shortest possible time as compared to meat of other meat producing animals.At present people consume about 44kg meat per year in Bangladesh where as in developed country people consume about 80kg meat per year .So more meat production is needed in this country. Recently, broiler industry has become take the leading position among the other sector of poultry production (DLS livestock Economy 2020-21).

The animal nutrition industry is searching for more suitable diet formulations and dietary supplements to provide better animal nutrition at lower production costs. Standard feed supplements have been used over the years, but they do not properly supply the requirements of many nutrients. Considering essential nutrients, trace minerals (like Copper Sulphate and Zinc) are critical to maintain poultry health and performance.

Zinc plays a useful role in the tissues of the pancreas that prevents oxidative damage and activates the secretions of the pancreas from digestive enzymes and thus stimulates the digestion of nutrients (Sahin *et al*., 2005).The addition of organic zinc had a positive influence on the immunological capacity by improving immunoglobulin (IgA, IgM, and IgG) levels and may also improve cellular response of broilers (Feng *et al*., 2010)Zinc contributes in the maintenance of the immune function, the growth performance (Liu *et al*., 2011). Scientist stated that Zn possesses many roles as an antioxidant agent, and associates in the hormone function including pancreatic (glucagon and insulin), growth and sex hormones. Zhang *et al*., (2018) clarified that supplementing Zn in the starter and grower diets at levels of 40 and 32 mg/kg feed, respectively, promoted the growth performance of broiler chickens and reduced excretion of Zn in the environment. Saleh *et al*., (2018) exhibited that dietary organic zinc supplements improved growth performance, humoral immunity, antioxidant properties, nutrient digestion and zinc content in raw meat, and reduced lipid peroxidation in broiler meat.Finally, zinc plays multiple roles in metabolism, immune response and antioxidant systems of poultry.

Copper is involved in both humoral (facilitates antibody production) and cell-mediated immunity (assists in eliminating invading bacteria). Copper as a feed additive has a helpful effect on the BWG, FCR and modification of the bacterial microflora in the gut (Ruiz *et al*., 2000). Xia *et al*., (2004) indicated that 150 mg copper sulfate/kg feed of broiler chicks had an affirmative impact on BWG that may be the result of the significant decline in the total pathogenic organism in the gut.). Copper has been used in poultry production as a nutritional supplement due to its microbiological activities and the ability to increase body weight (Wang *et al*., 2008). . Supplementation of copper sulfate (up to 200 mg/kg feed) to broiler diets had a beneficial influence on growth performance (Hashish *et al*., 2010). Samanta *et al*., (2011) described that supplementation of copper in broiler chickens' diet improved growth performance, as well as reduced plasma triglyceride and cholesterol, and meat cholesterol.

Samanta *et al*., (2011) confirmed that supplementation of copper is an effective way to improve hematological parameters in broiler chicken.Kumar *et al*., (2013) stated that the dietary addition of copper is useful for performance and blood biochemical parameters of broiler chicken. Copper sulphate shows immune stimulating action and supports maintaining appropriate microbiological balance in the digestive tract (Makarski *et al*., 2014). Recently, Yang *et al*., (2018) indicated that the dietary supplementation of Cu at levels of 8.77 and 11.6 mg/kg feed can improve growth and carcass yield in growing goslings from 28 to 70 days of age. Thus, copper is a micronutrient involved in many physiological processes and immunity, and it is necessary for optimal health and growth of poultry.

Copper (Cu) is an important mineral for chickens as it is essential for proper physiological functions.Cu plays an important role to inhibit inflammation by the synthesis of prostaglandins and converting arachidonic acid. Moreover, it affects the productive performance of animals as it acts as growth promoters. Also, it is used as an alternative for antibiotics that effects on chicken performance development by influencing the metabolism of nutrients as it affects the immune system which resulted in metabolic changes which known as immunological stress.

On the basis of this background, the experiment was planned to explore the possibilities of using copper sulphate and zinc supplementation with the following objectives:

- To determine the effect of copper sulphate and zinc powder on growth performance of broiler chicken.
- To determine the effect of copper sulphate and zinc powder on hematological parameters of broiler chicken.
- To determine the effect of copper sulphate and zinc powder on immune response of broiler Chicken.

CHAPTER 2

REVIEW OF LITERATURE

Sources of literature

1. Books and Journal of Sher-e-Bangla Agricultural University (SAU) Library, Dhaka

2.Internet browsing

A total of about 49 literatures were reviewed to assimilate the background, drawbacks and prospects of research, to understand previous findings and to answer the research status of this field.

Among them 27 were full article and 18 abstracts, 4 were only titles and some were miscellaneous. A brief account is given below depending on 7 main headlines viz, zinc in poultry production, copper sulphate in immune response, zinc and copper in poultry growth performance, zinc effect on body weight and temperature, effect of different sources and levels of zinc, copper sulphate effect on meat quality, copper effect on immune response. The traditional system or sequences in stating the references is avoided. Tactic inspection was made of each article and significant information was collected and compiled in a systematic arrangement according to specific title. For further higher research review attempts, it is look forward to be a pathfinder effort in Bangladesh.

The poultry sub-sector is the most commercialized agricultural sub-sector all over the world. Poultry are raised for their meat and eggs, and are an important source of edible animal protein. Poultry meat accounts for 30% of global meat consumption. The poultry sector was the most dynamic meat sector during the last decade, showing the greatest growth of all meat sectors as reflected in world consumption. The dynamism of the global poultry sector has been supported by a strong growth in demand. To fulfill this demand appropriate nutritional supplement like copper and zinc should be used in poultry feeds to maximize the rate of production.

2.1 History of zinc

After the discovery of Zn as a required nutrient in rats and mice, scientists found that farm animals required Zn in the 1930s and 1940s. The classic research of Tucker and Salmon, who reported that high Ca diets fed in swine production resulted in parakeratosis and could be cured by adding more Zn to the diets, stimulated others to study the Zn metabolism and physiology in farm animals.

Underwood noted in his classic review that there are many Zn deficient soils in the world that result in pastures and crops that are low in Zn. Hence, clinical signs of marginal Zn deficiency such as reduced growth and appetite, lesions of the skin and its outgrowths of hair, wool, and feathers, and poor reproduction exist in production agriculture. This is especially a problem when antagonists such as phytate found in plant proteins and excessive Ca and Fe are found in the diet.

As an element, Zn is relatively non-toxic and vomiting usually occurs after a high dose of Zn is ingested. Symptoms will depend on the Zn source and length of time and may include dehydration, electrolyte imbalance, abdominal pain, nausea, lethargy, dizziness, and muscular incoordination.

2.2 Metabolic Mechanisms and Rolesof zinc

Zinc can be found throughout the body and serves as a component of many enzymes from those involved in transcription, intra- and intercellular signals to the cell transcription machinery, protein carriers to binding of amino acids to maintain structure, etc. In enzymes, Zn is involved in structure as well as managing valance for the enzyme’s activity and is known to be essential in vitamin A transport. Zinc stimulates the production of MT that serves as storage and detoxification sites and plays a role in Cu/Zn interactions.

2.3 Zinc in poultry industry

It is reported the signs of Zn deficiency in chickens developed in 4 to 6 weeks of being fed 15 ppm in galvanized batteries. When the access to Zn in cage coating was removed, more severe deficiency symptoms developed. The reported symptoms included reduced growth, shortening and thickening of long bones, poor feather development, and rapid and labored breathing. The phytic acid content of soybeans makes Zn less available to non-ruminants and hence today the enzyme, phytase, is used in diets.

It is found that Zn deficiency in chicks has a direct effect on proliferation, differentiation, and apoptosis on growth plate chondrocytes; hence the shortened bones observed by the O'Dell and Savage laboratories. Alkaline phosphatase activity is reduced during Zn deficiency in the bones of turkey poults. It is suggested that extracellular Zn has an effect on water metabolism when studying Zn deficiency.

Large quantities of Zn (1000 ppm) from feed or gavage doses were compared relative to concentration in tissues of chicks. The Zn accumulations were greatest in bone, pancreas, kidney, and liver. It should be noted that high concentrations of Zn (3000 to 4000 ppm) can affect the adenohypophyseal gonadotropic hormone, adrenal glands, and exocrine and endocrine portions of the pancreas. After 2 weeks of adequate Zn, functions of these organs returned to normal. It is noted that up to 1200 ppm dietary Zn was managed homeostatically by the chick, but Zn metabolism was altered with 2400 ppm Zn.

2.3.1Zinc in Broiler Feeding and Nutrition

Zinc is an essential trace mineral for birds, functioning elaborately in enzyme systems and being involved in protein synthesis, carbohydrate metabolism, and many other biochemical reactions. Zinc is required for normal growth, reproduction, and glandular development of birds. A severe zinc deficiency causes numerous physical and pathological changes including skin lesions, decreased growth, general disability of bones and joints, very poor feathering, reproductive failure, and reduced immunity to infection of several diseases. In skin, it is five to six times more concentrated in the epidermis than the dermis. In addition, zinc is associated with wound healing because of its role in collagen and keratin syntheses. All proliferating cells, including inflammatory cells, epithelial cells, and fibroblast, require zinc. Furthermore, zinc is an essential element of more than 200 metalloenzymes and affects their conformity, stability, and activity. The superoxide dismutase, one of the zinc-containing antioxidant enzyme, has a critical role in keeping broiler skin healthy and increasing the shelf-life of broiler meat. However, a clear appreciation of the role of this element in broiler production is still limited. This article provides an overview on the role of zinc in broiler feeding and nutrition, immunity, reproduction, and meat quality in particular.

2.4 Effect of zinc on growth performance of broilers

2.4.1Feed intake and feed efficiency

Batal*et al*., (2001) reported that weight gain, feed intake, and feed efficiency (feedygain) responded quadratically to graded levels of supplemental zinc up to 20 mg/kg. The effect of zinc deficiency was reduced feed intake by animals which may be related to the role of zinc in inducing appetite (Berger, 2002).

Ao*et al*., (2006) showed that feed intake was increased quadratically with increasing levels of dietary zinc up to 10 mg/kg, and increased linearly when zinc was supplied as zinc sulfate (ZnSO4) at 40 mg/kg in broiler chicks, after which no further increase occurred. Huang *et al*., (2007) reported that weight gain and feed intake of broiler chicks were significantly increased with dietary zinc level, and the maximum weight gain and feed intake were observed in the diet supplemented with 20 mg/kg of zinc.

2.4.2 Growth rate

Growth retardation is universally observed in zinc deficiency, perhaps because of impairment of nucleic acid biosynthesis and amino acid utilisation or protein synthesis. Much research has been conducted regarding the effect of zinc on broiler growth performance. Batal*et al*., (2001) found the positive effect of zinc on the growth of broiler in optimum management system but other reports show no significant effect of zinc in broiler growth. Studies with inorganic zinc (Wang *et al.*, 2002), and with organic zinc (Hudson *et al*., 2004), indicated that growth performance, leg abnormalities, and meat yields were unaffected when dietary zinc were provided in excess of the recommendations of 40 mg/kg. By contrast, many investigators have added zinc in inorganic form (Edwards *et al.*, 2000), or in organic form (Yu *et al.*, 2005), to diets of broilers and observed an improvement in growth performance.Rossi *et al*., (2007) showed that body weight gain and carcass yield was not influenced by the addition of increasing levels of dietary organic zinc in broiler diets

2.4.3 Zinc and meat quality of broilers

Hess *et al*., (2001) showed that no significant difference was detected when birds supplemented with zinc (40 mg/kg) for whole carcass, abdominal fat, or different cut-up yields.The fatty acid composition and crude fat content of raw meat were not affected by zinc or selenium supplementation (Bou *et al*., 2004).Bou *et al*., (2004) also reported that zinc supplementation increased selenium content in chicken meat**.** Carcass characteristics Information about the effect of zinc on carcass characteristics, such as dressing percentage, carcass yield and carcass composition of broilers, is scarce. Rossi *et al*., (2007) stated that carcass and cut-up (drumstick, thigh, and breast) yield were not influenced by the addition of increasing levels of dietary organic zinc in broilers

2.4.4 Carcass quality

In a modern broiler production system skin quality is a crucial issue to produce healthier broiler meat for the consumers. Skin strength is highly correlated with the content of a strong, fibrous protein (collagen), which functions as an extracellular structural element in connective tissue. Therefore, skin with greater collagen content is less prone to tearing. Any nutritional factor that influences skin collagen content will, therefore, indirectly affect susceptibility to tearing. Zinc plays a role in collagen synthesis and deficiencies of this nutrient result in reduced production of skin collagen. In addition, zinc participates in the synthesis of keratin and nucleic acid of the skin which are responsible for the maintenance of skin quality (Downs *et al*., 2000). Tearing of carcass skin during the plucking process in the slaughter house has become one of the major economic problems during processing. Rossi *et al*., (2007) reported that, because of the higher number of epithelial cell layers, higher collagen content, and reduced inflammation of the skin, a reduction in skin tearing was observed by addition of organic zinc in broiler diet. Furthermore, collagen gives flexibility and resilience to the skin. Zinc deficiency decreased bone collagen turnover and probably accounted for the leg deformities seen in zinc deficient chicks.

2.4.5 Antioxidant activity of zinc

Zinc has an antioxidant capability that plays vital roles in avian nutrition. Zinc act as an antioxidant and its deficiencies in animals are alleviated by a-tocopherol and other antioxidants. Laying hens reared at a low temperature (6.8C) and given supplemental zinc (30 mg/kg) had higher serum a-tocopherol contents than those not receiving supplements. Zinc supplementation at 300 or 600 mg/kg did not affect the sensory quality, or lipid oxidation, and a-tocopherol content in raw meat. However, antioxidant role of zinc may provide an important defense system for skin and limit production of free radicals, such as superoxide and malondialdehyde content in broiler meat.

2.4.6 Tissue zinc concentration

Zinc content in chicken thigh is known to be higher than in breast (Mavromichalis *et al.,* 2000).Furthermore, zinc in meat products has a high bioavailability. Despite the fact that the recommended dietary allowances for zinc have recently been lowered, several developed and undeveloped countries have recommended daily intakes of zinc slightly higher or lower than these recommendations (Terree´ s *et al.,* 2001). Body tissues high in zinc include bone, liver, kidney, pancreas, retina, and the prostate. However, redistribution of zinc among body tissues can occur under stress, and also could be affected by the level of zinc supplementation in animal diet. Bou*et al.,* (2004) demonstrated that a zinc supplementation did not affect the content of zinc in mixed dark and white raw meat on a wet weight basis. The dark meat of a chicken has more zinc than the light meat (Health Line, 2007).

2.4.7 Bioavailability of zinc in broilers

Zinc is typically added to diets for poultry in a supplemental form, usually as inorganic feed grade zinc sulfate, chloride, oxide, or one of the organic forms complexed to amino acids, proteins, and carbohydrates. The nutritional value of dietary mineral sources depends upon their concentration in the feedstuff, the amount of the element that is absorbed and metabolised, andyor the bioavailability of the element to the bird. Several bioavailability studies of organic or inorganic zinc complexes were conducted for chicks or poults. Some studies showed small or no differences in bioavailability between inorganic and organic zinc sources**.**

2.4.8 Environmental implications of zinc supplementation

Modern broiler producers are faced with many challenging issues in reference to sustainable agriculture. Of these, environmental issues have begun to make an impact on production practices. One of the major environmental concerns associated with the poultry industry is emission of ammonia (NH3), which increases atmospheric acid deposition. Many studies have demonstrated that high levels of NH3 on the farm could reduce feed efficiency, growth rate, and egg production, damage the respiratory tract and impair immune responses. Thus, reduction of NH3 emission is very important to maintain human and animal health and a clean environment.

2.5Copper

Copper is involved in both humoral (facilitates antibody production) and cell-mediated immunity (assists in eliminating invading bacteria). Copper as a feed additive has a helpful effect on the BWG, FCR and modification of the bacterial microflora in the gut (Ruiz *et al.*, 2000). Xia *et al.*, (2004) indicated that 150 mg copper sulfate/kg feed of broiler chicks had an affirmative impact on BWG that may be the result of the significant decline in the total pathogenic organism in the gut. Copper has been used in poultry production as a nutritional supplement due to its microbiological activities and the ability to increase BW (Wang *et al.*, 2008). Supplementation of copper sulfate (up to 200 mg/kg feed) to broiler diets had a beneficial influence on growth performance (Hashish *et al.*, 2010).Samanta *et al.,* (2011) described that supplementation of copper in broiler chickens' diet improved growth performance, as well as reduced plasma triglyceride and cholesterol, and meat cholesterol.Samanta *et al*., (2011) confirmed that supplementation of copper is an effective way to improve haematological parameters in broiler chicken. Kumar *et al.*, (2013) stated that the dietary addition of copper is useful for performance and blood biochemical parameters of broiler chicken. Copper shows immune stimulating action and supports maintaining appropriate microbiological balance in the digestive tract (Makarski *et al.*, 2014). Recently, Yang *et al.*, (2018) indicated that the dietary supplementation of Cu at levels of 8.77 and 11.6 mg/kg feed can improve growth and carcass yield in growing goslings from 28 to 70 days of age. Copper is involved in iron transport and metabolism, and the formation of red blood cells. Thus, copper is a micronutrient involved in many physiological processes and immunity, and it is necessary for optimal health and growth of poultry.

2.6 Overall performanceof copper

Schone *et al.*, (1993) reported that the deleterious effects of glucosinolates on broiler performance could be normalized by pretreating rapeseed meal with Cu sulfate before feeding.It has been reported that supplementing 200 mg/kg of Cu in the form of Cu sulfate improves performance in broilers (Skirvan *et al.*, 2000).

The main effect of BWG and FCR were significantly affected ($P<0.05$) by 250 mg/kg copper treatment. The broiler chick's nutritional requirement for copper is approximately 8 mg/kg. Copper is usually fed commercially at much higher pharmacological levels (100 to 300 mg/kg) because of its growth promoting properties, which is caused by its antibacterial properties (Payvastegan *et al.* 2012). Various processing techniques were applied to remove glucosinolates in order to minimize their deleterious effects on animals. The Cu-sulfate supplementation may redirect glucosinolates to breakdown products, may react to form complex with, or to produce secondary breakdown products by rearrangement reactions. Payvastegan *et al.,* (2013) reported no significant effects of copper supplementation on feed intake, body weight gain and feed conversion ratio. Dietary Cu supplementation can affect the nutritive value and potential toxic effects of rapeseed meal.

2.7. Carcass traits of copper supplementation

The effects of copper sulfate solution on the relative weight of internal organs in broiler chickens.Main effect of carcass yields was significantly increased ($P<0.01$) with supplementation of Cu when added to the diets. An enhancement ($P<0.01$) in breast weight, as percentage of live BW, was observed due to addition of Cu ($P<0.05$) to diets. The growth stimulation effects of Cu could be attributed to shifting the gastrointestinal microbiota, thereby reducing the susceptibility of birds to disease, reducing intestinal lymphocyte recruitment and infiltration, and thus increasing nutrient absorption (Pang *et al.*, 2009).

2.8 Research gap and scope of present investigation

From the above literatures, it is clear that the supplementation of copper and zinc to poultry diet with appropriate doses is always favorable for better growth, meat quality and immune response. However, copper and zinc content in feed ingredients depends upon the concentration in soil. The copper and zinc concentration in Bangladeshi soil has been reported to be lower than the standard. So, it is obvious that the feed grains grown on Bangladeshi soil will be deficient of copper and zinc. Therefore, there is a scope of investigating the necessity of copper and zinc supplementation in poultry as well as in other animals. But, until recently no work has been done to study the effects of copper and zinc supplementation with the appropriate form and levels in poultry rations formulated from locally available ingredients.

CHAPTER 3

MATERIALS AND METHODS

3.1 Statement of the experiment

The research work was conducted in the experimental trial shed at **Sher-e-Bangla Agricultural University Poultry Farm, Dhaka,** includes 150 day-old straight run (Lohman meat) commercial broiler chicks from a single hatch for a period of 28 days from **13th October to 10th November, 2019** to assess the feasibility of using coppersulphate and zinc powder in commercial broiler diet on growth performance and immune response of broiler chickens. This research helps to make a conclusion about coppersulphate and zinc as nutrient and growth supplement.

3.2 Collection of experimental broilers

A total of 150 day-old Lohman meat commercial broiler chicks were collected from Kazi hatchery, Savar, Dhaka.

3.3 Experimental materials

The collected chicks were carried to the university poultry farm at early morning. They were kept in electric brooders equally for 7 days by maintaining standard brooding protocol. During brooding time only basal diet was given. Treatments were not used at that period. After 7 days chicks were selected from brooders and distributed randomly in five (5) dietary treatment groups. 30 chicks were distributed randomly in one group for control and other 30 chicks for antibiotic group. The rest 90 chicks were distributed randomly in three (3) groups treated with copper and zinc.

Each treatment had three (3) replications with 10 birds per replication. The total numbers of treatments were five (5) and their replications were 15.

3.4 Experimental treatments

T1 = Basal diets (control)

T2 = Antibiotics

T3 = $CuSo_4$ + Zn (8mg +30mg)

T4 = $CuSo_4$ + Zn (12mg +40mg)

T5 = $CuSo_4$ + Zn (16mg +50mg)

Table 1. Layout of the experiment

Treatments with Replication (10 birds / replication)			No. of birds
T1R1(n=10)	T1R2(n=10)	T1R3(n=10)	**30**
T2R1(n=10)	T2R2(n=10)	T2R3(n=10)	**30**
T3R1(n=10)	T3R2(n=10)	T3R3(n=10)	**30**
T4R1(n=10)	T4R2(n=10)	T4R3(n=10)	**30**
T5R1(n=10)	T5R2(n=10)	T5R3(n=10)	**30**
Total			**150**

3.5 Preparation of the experimental house

Proper cleaning and washing of the experimental house was performed using clean tap water. Ceiling, walls and floor were thoroughly cleaned and disinfected by spraying diluted Iodophor disinfectant solution (3 ml/liter water). After proper drying, the house was divided into 15 pens of equal size using wood materials and wire net. The height of wire net was 36 cm. A group of 10 birds were randomly allocated to each pen (replication) of the 5 (five) treatments. The stocking density was 1 m^2/10 birds.

3.6 Experimental diets

The composition of starter and grower diets are shown in the table 2

Table 2.Name of composition present in Starter and Grower ration.

Name of the Ingredient	Minimum percentage present in Starter diet	Minimum percentage present in Grower diet
ME	3000 kcal / kg	3050 kcal / kg
Crude protein	21.0%	19.0%
Crude Fat	6.0%	6.0%
Fiber	5.0%	5.0%
Ash	8.0%	8.0%
Lysine	1.20%	1.10%
Methionine	0.49%	0.47%

Feed were supplied 3 times daily by following Lohman meat Manual.

Adlibitum drinking water was supplied.

3.6.1 Collection of copper and zinc

Copper sulphate and zinc powder was purchased from Market.

Plate 1: Copper sulphate Plate 2: zinc powder

3.7 Management procedures

Body weight and feed intake were recorded in every week and survivability was recorded for each replication up to 28 days of age.The following management procedures were followed during the whole experiment period.

3.7.1 Brooding of baby chicks

The experiment was conducted during **13th October to 10th November, 2019**. The average temperature was near about 29ºC and the RH was 73% in the poultry house. Common brooding was done for seven days. After seven days the chicks were distributed in the pen randomly. There were 10 chicks in each pen and the pen space was $1m^2$. Due to cold climate brooding temperature was maintained as per requirement. Brooding temperature was adjusted (below 35ºC) with house temperature. When the environmental temperature was above the recommendation, then no extra heat was provided. At day time only few electric bulb was used to stimulate the chicks to eat and drink. In brooding extra heat was not provided at day time except mid night tomorning. Electric fans were used as per necessity to save the birds from the heat stress and its harmful effects. .

3.7.2 Room temperature and relative humidity

Daily room temperature (ºC) and humidity (%) were recorded every six hours with a thermometer and a wet and dry bulb thermometer respectively. Averages of room temperature and percent relative humidity for the experimental period were recorded and presented in **Appendix 1 & 2.**

3.7.3 Litter management

Litter was provided at a depth of 6 cm by using rice husk as litter material. At the end of each day, litter was stirred to prevent accumulation of harmful gases and to reduce parasite infestation. At 3 weeks of age, droppings on the upper layer of the litter were cleaned and for necessity fresh litter was added.

3.7.4 Feeding and watering

The birds were offered with ad libitum feed and clean fresh water. One large feeder and one big round drinker were provided in each pen for 10 birds. Feeders were cleaned at the end of each week and drinkers were washed daily in the morning before supplying water. Feces and dirt contamination in the feeder and drinker were avoided by raising the feeder and drinker at a manageable height by using brick.

3.7.5 Lighting

There was provision of light in the broiler farm to stimulate feed intake and body growth at night. For first 2 weeks 24 hours lighting schedule was used. Thereafter 1 hours dark period was scheduled up to 28 days.

3.7.6 Bio-security measures

Biosecurity components were properly maintained during the experimental period. Entry of wild birds and animals were prohibited. Foot bath (PPM) was used in front of farm gate to avoid the risk of pathogen transmission. Proper hygienic and sanitation program was undertaken in the farm and its premises. Strict sanitary measures were taken during the experimental period. Disinfectant (Virkon) was used to disinfect the feeders and waterers and the house also. Regular cleansing of the farm was done. Some vitamins like Vitamin B-Complex, Vitamin-AD_3K, Vitamin-C, Calcium and electrolytes were supplied to the birds.

3.7.7 Vaccination

To prevent diseases in the farm, chicks were vaccinated as per standard vaccination schedule. The vaccines collected from medicine shop (Hipraviar&Ceva Company) and applied to the experimental birds according to the vaccination schedule.

Table 3.Vaccination schedule

Age of birds	Name of Disease	Name of vaccine	Route of administration
3 days	ND + IB	Hipraviar(live)	One drop in each eye
9 days	Gumboro	G-228E (inactivated)	Drinking Water
17days	Gumboro	G-228E (inactivated) booster dose	Drinking Water
21 days	ND + IB	Hipraviar (live)	Drinking Water

3.7.8 Ventilation

The experimental shed was south facing and open-sided. Due to wire net cross ventilation, it was easy to remove polluted gases from the farm. Beside ventilation was regulated as per requirement by folding polythene screen.

3.8 Study Parameters

3.8.1 Recorded parameters

Data was recorded on weekly live weight, weekly feed consumption and death of chicks to calculate mortality percent. FCR was calculated from final live weight and total feed consumption per bird in each replication. After slaughter gizzard, liver, spleen, intestine, heart and bursa weight were measured from 30 broiler chicken. Dressing yield was calculated for each replication to find out dressing percentage. Blood sample was collected and analysis from each replication to measure blood parameters.

3.9 Data collection

3.9.1 Live weight:

The initial day-old live weight and weekly live weight of each replication was kept to get final live weight record per bird.

3.9.2 Dressing yield:

Live weight- (blood + feathers + head + shank+ digestive system + Liver+ Heart)

3.9.3 Feed consumption:

Daily feed consumption record of each replication was kept to get weekly and total feed consumption record per bird.

3.9.4 Mortality of chicks:

Daily death record for each replication was counted up to 28 days of age to calculate mortality.

3.9.5 Dressing procedures of broiler chicken:

Two birds were picked up randomly from each replicate at the 28th day of age and sacrificed in halal method to estimate dressing percentage of broiler chicken. All birds to be slaughtered were weighed and fasted for overnight (12 hours) but drinking water was provided ad-libitum during fasting to facilitate proper bleeding. All the live birds were weighed again prior to slaughter. Birds were slaughtered by severing jugular vein, carotid artery and the trachea by a single incision with a sharp knife and allowed to complete bleed out at least for 2 minutes. Outer skin was removed by sharp scissor and hand. Then the carcasses were washed manually to remove loose singed feathers and other foreign materials from the surface of the carcass. Afterward the carcasses were eviscerated and dissected according to the methods by Jones (1982). Heart and liver were separated from the remaining viscera by cutting them loose and then the gall bladder was removed from the liver. Cutting it loose in front of the proventriculus and then cutting with both incoming and outgoing tracts to remove the gizzardcontent. Dressing yield was found by subtracting blood, feathers, head, shank, liver, heart and digestive system weight from live weight.

3.9.6 Blood sample analysis

Blood samples (1 ml/bird) were collected into ethylenediethyltetraacetic acid (EDTA) tubes from the wing veins. Samples was calculated by Easy Touch meter for glucose & cholesterol.

3.10 Calculations

3.10.1 Live weight gain

The average body weight gain of each replication was calculated by deducting initial body weight from the final body weight of the birds.

Body weight gain = Final weight – Initial weight

3.10.2 Feedintake

Feed intake was calculated as the total feed consumption in a replication divided by number of birds in each replication.

$$\text{Feed intake (g/bird)} = \frac{\text{Feed intake in a replication}}{\text{Number of birds in a replication}}$$

3.10.3 Feed conversionratio

Feed conversion ratio (FCR) was calculated as the total feed consumption divided by weight gain in each replication.

$$\text{FCR} = \frac{\text{Feed intake (gm)}}{\text{Weight gain (gm)}}$$

3.11 Statistical analysis : The data was subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version 16. Differences between means were tested using Duncan's Multiple Comparison Test and significance was set at $P<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Production index of broiler chicken

Calculation of Production Index (PI) is one the major parameter to assess the successfulness of broiler chicken production which compare broiler results from different flocks, region and treatment groups. The performance of broiler chickens is measured through five factors. These factors are:

- The level of feed consumption
- The achievement of body weight
- Feed Conversion Ratio
- Dressing Percentage
- Survivability rate

Measurement and assessment of the five factors reflect the quality of maintenance and performance maintenance of broiler chickens.

4.1.1 Feed Intake (FI)

The mean weekly feed intake (g) of broiler chicks at the end of 4th week in the dietary group T_1, T_2, T_3, T_4 and T_5 were 2479.12±2.05, 2497.53±17.62,2482.06±4.32, 2491.43±6.60,2499.62±8.83 accordingly. The overall mean feed intake of different groups showed that there was significant difference ($P<0.05$) among control (T_1), antibiotic (T_2),$CuSo_4$ + Zn (8mg +30mg) (T_3), $CuSo_4$ + Zn (12mg +40mg)(T_4) and $CuSo_4$ + Zn (16mg +50mg) (T_5) supplementation group (Table 4).

This results are agreement with Huang *et al.,*(2007) reported that feed intake of broiler chicks were significantly increased with dietary zinc level and feed intake were observed in the diet supplemented with 50 mg/kg of zinc. Yang *et al.,* (2018) indicated that the dietary supplementation of Cu at levels of 8.77 and 11.6 mg/kg feed can improve feed intake in broiler birds**.**

Payvastegan *et al.,* (2013) reported no significant effects of copper and zinc supplementation on feed intake in broiler birds**.**

Table 4. Effects of different level of copper sulphate and zinc on feed intake (g/bird) of broiler chickens at different week

Treatment	1st week FI	2nd week FI	3rd week FI	4th week FI	Final FI
T1	$157.00^{a}\pm0.57$	$535.67^{ab}\pm0.78$	784.27 ± 8.09	$1002.17^{ab}\pm2.05$	$2479.12^{ab}\pm2.05$
T2	$160.67^{b}\pm0.88$	$534.37^{ab}\pm0.82$	781.87 ± 14.77	$1020.60^{ab}\pm17.62$	$2497.53^{ab}\pm17.62$
T3	$160.33^{b}\pm0.88$	$538.50^{b}\pm3.75$	776.17 ± 6.31	$1007.07^{ab}\pm4.32$	$2482.06^{ab}\pm4.32$
T4	$157.33^{a}\pm0.57$	$541.80^{b}\pm2.70$	803.03 ± 2.07	$989.60^{a}\pm6.60$	$2491.43^{a}\pm6.60$
T5	$158.00^{ab}\pm1.52$	$530.00^{a}\pm2.60$	784.57 ± 10.36	$1027.03^{b}\pm8.83$	$2499.62^{b}\pm8.83$
Mean ± SE	158.60*±0.55	536.07*±1.40	$785.98^{NS}\pm4.27$	1009.29*±5.06	2489.95*±5.06

Here, T_1=(Control), T_2=(Antibiotic), T_3=($CuSo_4$ + Zn (8mg +30mg), T_4=($CuSo_4$ + Zn (12mg +40mg) and T_5=($CuSo_4$ + Zn (16mg +50mg). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- Mean with different superscripts at the same column are significantly different (P<0.05)
- Mean within same superscripts don't differ (P>0.05) significantly
- SE= Standard Error
- *means significant at 5% level of significance (p<0.05)
- NS= Non significant

4.1.2 Body weight gains

The mean body weight gains (g) of broiler chicken at the end of 4^{th} week in different groups were 640.00±20.52, 529.33±8.29, 652.67±74.45, 576.00±32.90, 613.00±4.93 respectively. The overall mean body weight gain of different groups showed that there was no significant (P>0.05) difference in groups T_4 and T_5 compared to control (T_1) and antibiotic (T_2) group at the end of 4^{th} week except (Table 5).The highest body weight gain obtained at the end of 4^{th} week in $CuSo_4$ + Zn (8mg +30mg) supplementation (T_3) and the lowest body weight gain obtained in antibiotic supplementation (T_2) group.

Rossi *et al.*, (2007) showed that body weight gain was not influenced by the addition of increasing levels of dietary zinc in broiler diets. Dietary supplementationof copper and on body weight gain had no significant effects in broiler birds**.**(Payvastegan *et al.*, 2013).

In contrast some researchers said that copper and zinc used in feed has a helpful effect on the body weight gain by modification of the bacterial microflora in the gut (Ruiz *et al.*, 2000).Xia *et al.*, (2004) indicated that 150 mg copper sulfate/kg feed of broiler chicks had an affirmative impact on BWG that may be the result of the significant decline in the total pathogenic organism in the gut.

Table 5. Effects of feeding different level of copper sulphate and zincon body weight gain (BWG) (g/bird) of broiler chickens at different week

Treatment	1st wk BWG	2nd wk BWG	3rd wk BWG	4rd wk BWG	Final BWG
T1	139.67±4.17	371.33±9.52	575.33^{a}±13.34	640.00±20.52	1726.33±18.52
T2	141.33±2.40	378.00±4.93	620.33^{b}±13.04	529.33±8.29	1669.12±16.12
T3	153.33±0.88	377.33±3.48	634.33^{b}±10.58	652.67±74.45	1809.66±15.43
T4	147.33±4.17	370.33±3.48	646.67^{b}±6.17	576.00±32.90	1740.33±18.32
T5	148.00±1.15	388.00±4.04	530.67^{b}±0.66	613.00±4.93	1779.66±15.34
Mean ± SE	144.33^{NS}±1.41	377.00^{NS}±2.67	629.93^{*}±10.09	602.20^{NS}±18.63	1745.02^{NS}±18.22

Here, T_1=(Control), T_2=(Antibiotic), T_3=($CuSo_4$ + Zn (8mg +30mg), T_4=($CuSo_4$ + Zn (12mg +40mg) and T_5=($CuSo_4$ + Zn (16mg +50mg). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- Mean with different superscripts at the same column are significantly different (P<0.05)
- Mean within same superscripts don't differ (P>0.05) significantly
- SE= Standard Error
- NS= Non significant

4.1.3 Feed Conversion Ratio (FCR)

The mean FCR of broiler chicks at the end of 4th week in different groups were 1.39±0.05,1.45±0.04,1.33±0.02,1.39±0.10,1.36±0.01respectively. The overall mean FCR of different groups showed that there was no significant ($P>0.05$) increase in groups T_3, T_4 and T_5 compared to control (T_1) and antibiotic (T_2) group at the end of 4th week. (Table 6).

These results are coincided with the findings of previous research of Payvastegan *et al.*, (2013) reported no significant effects of copper supplementation on feed conversion ratio.*Rossi et al.*, (2007) showed that FCR was not influenced by the addition of increasing levels of dietary zinc in broiler diets.

In contrast some researchers stated that copper in feed has a helpful effect on the FCR and modification of the bacterial microflora in the gut (Ruiz et al. 2000).Batal*et al.*, (2001) also reported that feed consersion ratio responded quadratically to graded levels of supplemental zinc up to 20 mg/kg.

Table 6.Effects of feeding different level of copper sulphate and zincon FCR of broiler chickens at different week.

Treatment	1st week FCR	2nd week FCR	3rd week FCR	4th week FCR	Final FCR
T_1	1.12±0.03	1.44^{ab}±0.03	1.36^{b}±0.02	1.54±0.06	1.39±0.05
T_2	1.09±0.03	1.41^{ab}±0.01	1.26^{a}±0.01	1.92±0.03	1.45±0.04
T_3	1.11±0.01	1.42^{ab}±0.01	1.22^{a}±0.02	1.58±0.01	1.33±0.02
T_4	1.05±0.01	1.46^{b}±0.01	1.24^{a}±0.01	1.72±0.05	1.39±0.10
T_5	1.08±0.01	1.36^{a}±0.02	1.24^{a}±0.01	1.67±0.04	1.36±0.01
Mean ± SE	1.09^{NS}±0.01	1.42^{*}±0.01	1.26^{*}±0.02	1.68^{NS}±0.03	1.38^{NS}±0.05

Here, T_1=(Control), T_2=(Antibiotic), T_3=($CuSo_4$ + Zn (8mg +30mg), T_4=($CuSo_4$ + Zn (12mg +40mg) and T_5=($CuSo_4$ + Zn (16mg +50mg). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- Mean with different superscripts at the same column are significantly different ($P<0.05$)
- Mean within same superscripts don't differ ($P>0.05$) significantly
- SE= Standard Error

Table 7. Production index of broiler chicken supplemented with copper sulphate and zinc.

Treatment	Final Live Weight (g/Broiler)	Total Feed Intake(g)	Final FCR	DP% (Skinless)	Survivability (%)
T_1	$1773.33^{ab}\pm$ 11.79	$2479.12^{ab}\pm2.05$	1.39±0.05	74.43±0.10	100±0.00
T_2	$1716.00^{a}\pm$ 3.78	$2497.53^{ab}\pm17.62$	1.45±0.04	74.49±0.18	100±0.00
T_3	$1856.67^{b}\pm$ 78.38	$2482.06^{ab}\pm4.32$	1.33±0.02	75.15±0.82	100±0.00
T_4	$1787.33^{ab}\pm$ 36.22	$2491.43^{a}\pm6.60$	1.39±0.10	74.93±0.91	96.67±3.33
T_5	$1826.67^{ab}\pm$ 4.80	$2499.62^{b}\pm8.83$	1.36±0.01	76.00±0.11	93.33±3.33
Mean ± SE	$1792.00^{*}\pm19.56$	2489.95*±5.06	$1.38^{NS}\pm0.05$	$75.00^{NS}\pm$ 0.26	$98.00^{NS}\pm.1.07$

Here, T_1=(Control), T_2=(Antibiotic), T_3=($CuSo_4$ + Zn (8mg +30mg), T_4=($CuSo_4$ + Zn (12mg +40mg) and T_5=($CuSo_4$ + Zn (16mg +50mg). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- Mean with different superscripts at the same column are significantly different (P<0.05)
- Mean within same superscripts don't differ (P>0.05) significantly
- SE= Standard Error
- NS= Non significant

4.1.4 Final Body Weight

Data presented in (Table 7) showed that the effect of treatments on final live weight (gram per broiler chicken) was significant (P<0.05). The relative final live weight (g) of broiler chickens in the dietary group T_1, T_2, T_3, T_4 and T_5 were $1773.33^{ab}\pm$ 11.79, $1716.00^{a}\pm$ 3.78, $1856.67^{b}\pm$ 78.38, $1787.33^{ab}\pm$ 36.22 and $1826.67^{ab}\pm$ 4.80respectively. The highest result was found in T_3 ($1856.67^{b}\pm$ 78.38) and lowest result was in T_2($1716.00^{a}\pm$ 3.78) group. The final live weight of T_3 and T_5 group was also higher than the control group (T_1) and other group.

Saleh*et al.,* (2018) exhibited that dietary organic zinc supplements improved body growth performance. Copper has been used in poultry production as a nutritional supplement due to its microbiological activities and the ability to increase BW (Wang *et al.,* 2008).

Batal*et al.,* (2001) found the positive effect of zinc on the growth of broiler in optimum management system but other reports show no significant effect of zinc in broiler growth.

4.1.5 Dressing Percentage (DP)

The dressing percentage of broiler chicks at 28th days presented in (Table 7 and Figure 4) were not significantly (P>0.05) differ in T_1 (control), T_2 (antibiotic), T_3, T_4 and T_5 group. Broiler supplemented with $CuSo_4$ + Zn (16mg +50mg) (T_5) had a greater (P>0.05) dressing percentage (76.00±0.11) compared with the control T_1 (74.43±0.10) group. Dressing percentage of antibiotic T_2 was 74.49±0.18, $CuSo_4$ + Zn (8mg +30mg) supplemented group $T_3$was 75.15±0.82 and $CuSo_4$ + Zn (12mg +40mg) supplemented group T_4 was74.93±0.91.

These results are agreement with Hudson *et al.*,(2005)who stated that copper and zinc in feed did not affect the dressing percentage.

4.1.6 Survivability

The survivability rate showed on (Table 7 and Figure 4) different group was not significant (P>0.05). The survivability rate of different treatment groups T_1, T_2, T_3, T_4 and T_5 were 100±0.00, 100±0.00, 100±0.00, 96.67±3.33 and 93.33±3.33. Treatment had no significant (P>0.05) effect on survivability rate.

4.2 Serum Biochemical Parameters

4.2.1 Glucose

Effects of dietary supplementation of copper and zinc on concentration of glucose of broiler chickens presented in Table 8 and Figure 1.Dietary incorporation of copper and zinc powder had significant (P<0.05) difference among the treatment group. The lowest amount (16.83±0.27) of plasma glucose found in T_5 ($CuSo_4$ + Zn (16mg +50mg) and highest amount (18.10±0.28) of plasma glucose found in T_2 (antibiotic).

These results are in agreement with those obtained by Kumar *et al.,* (2013) stated that the dietary addition of copper is useful for performanceand blood biochemical parameters of broiler chicken. Zinc contributes in the maintenance of glucose level (Liu *et al.,* 2011).

On the other hand,Rossi *et al.,* (2007)concluded that blood glucose levels were not affected by zinc and copper supplementation.

4.2.2 Cholesterol

Total cholesterol concentration (mg/dl) in the serum of different groups ranged from 4.13±0.20 to 5.20±0.05. Statistical analysis revealed that significant ($P<0.05$) difference among the groups (Table 8 and Figure 1). The lower amount (4.13±0.20) of cholesterol found in $CuSo_4$ + Zn (16mg +50mg) (T_5) and highest amount of cholesterol found in $CuSo_4$ + Zn (8mg +30mg) T_3 group.

These results are in agreement with those obtained by Samanta *et al.*, (2011) described that supplementation of copper in broiler chickens' diet reduced plasma triglyceride and cholesterol, and meat cholesterol.Copper decreased cholesterol 7α-hydroxylase activity (Yang *et al.*, 2018).

4.2.3 Hemoglobin

Effects of dietary supplementation of copper sulphate and zinc on concentration of hemoglobin of broiler chickens presented in (Table 8 and Figure 1) different groups.Dietary incorporation of copper and zinc powder had significant ($P<0.05$) difference among the treatment group. The lowest amount (10.29^a±0.31) of hemoglobin found in T_2 (antibiotic) and highest amount (14.00^b±0.92) of hemoglobin found in $CuSo_4$ + Zn (8mg +30mg) T_3 group.

These results are in agreement with those obtained by Liu *et al.*, (2011) stated that the dietary addition of copper sulphate and zinc is useful for hemoglobin level increase.

On the other hand,Rossi *et al.,* (2007)concluded that blood hemoglobin levels were not affected by zinc and copper supplementation.

Table 8.Effects of copper sulphate and zincsupplementation on glucose, cholesterol and hemoglobin of different broiler chicken under different treatment.

Treatment	Glucose (mg/dl)	Cholesterol (mg/dl)	Hemoglobin(mg/dl)
T_1	17.38^{abc}±0.13	4.46^{ab}±0.12	11.91^{ab}±0.65
T_2	18.10^{c}±0.28	4.56^{b}±0.14	10.29^{a}±0.31
T_3	17.63^{bc}±0.18	5.20^{c}±0.05	14.00^{b}±0.92
T_4	17.23^{ab}±0.23	4.60^{b}±0.05	12.36^{ab}±0.58
T_5	16.83^{a}±0.27	4.13^{a}±0.20	13.76^{b}±0.53
Mean ± SE	16.91^{*}±0.20	4.59^{*}±0.10	12.46±0.43 *****

Here, T_1=(Control), T_2=(Antibiotic), T_3=($CuSo_4$ + Zn (8mg +30mg), T_4=($CuSo_4$ + Zn (12mg +40mg) and T_5=($CuSo_4$ + Zn (16mg +50mg). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- Mean with different superscripts at the same column are significantly different (P<0.05)
- Mean within same superscripts don't differ (P>0.05) significantly
- SE= Standard Error
- NS= Non significant

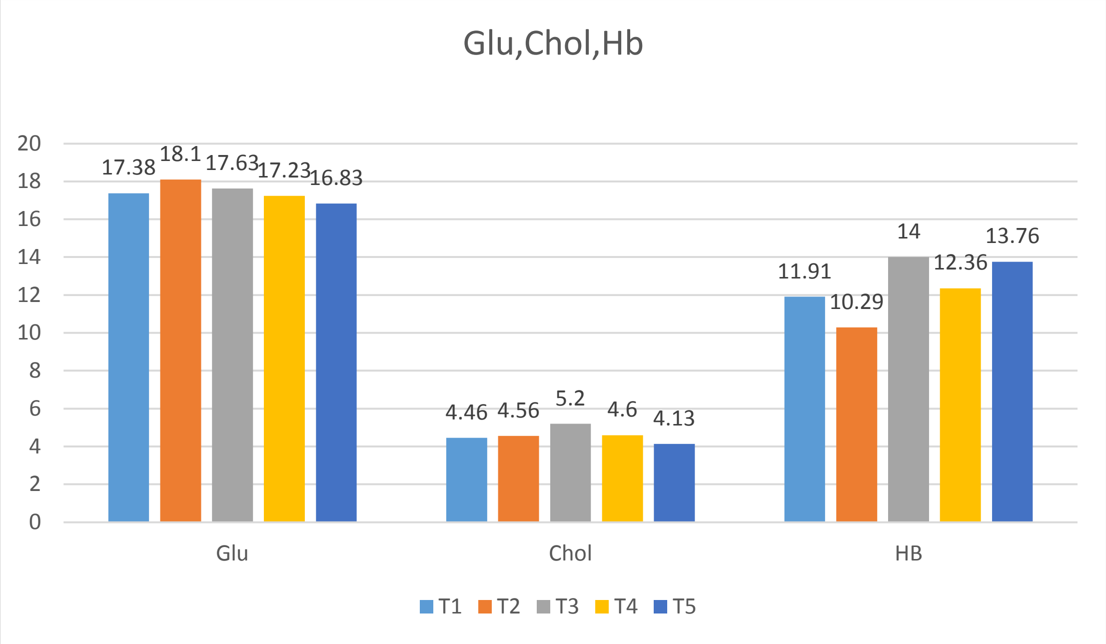


Figure 1.Effects of copper sulphate and zinc supplementation on glucose, cholesterol and hemoglobin of different broiler chicken under different treatment.

4.3 Internal organs

4.3.1 Relative weight (liver, gizzard, heart, spleen and bursa)

The relative weight of liver (g) of broiler chicks at the 28 days in dietary group T_1, T_2, T_3, T_4 and T_5 were 41.26±0.48,39.63±0.73,35.46±1.29,37.96±1.03and 38.20±0.61 respectively. The highest result was obtained in T_1 and lowest was in T_3 group. However, there was significant ($P<0.05$) difference in the relative weight of liver among the groups (Table 9 and Figure 2).

The comparative weight of gizzard (g) presented in (Table 10 and Figure 2)showed significant ($P<0.05$) difference among all treatment group. The relative weight of gizzard of broiler chicks in dietary group T_1, T_2, T_3, T_4 and T_5 were 40.43±0.97,45.00±0.57, 36.46±1.29, 37.83±0.72 and 40.66±0.88 respectively. The highest result (45.00±0.57) was obtained in T_1 (control) and lowest was in T_3 ($CuSo_4$ + Zn (8mg +30mg) group.

The relative weight of heart (g) of broiler chicken in the dietary groups were T_1 (9.70±0.60), T_2 (10.00±0.28), T_3 (10.16±0.60), T_4 (12.33±0.46) and T_5 (11.16±0.44). The results shows that there was significant ($P<0.05$) difference of values among groups. The highest result (12.33±0.46) was obtained in T_4 ($CuSo_4$ + Zn (12mg +40mg) and lowest was 10.00±0.28 in T_2(antibiotic) group (Table 9 and Figure 2).

In case of liver and heart weight, these results are in agreement with the findings of Pang *et al.,* (2009)who concluded that broiler chicken supplemented with copper and zinc had significant ($P<0.05$) effect on liver, gizzard and heart weight. But contradictory to results of gizzard weight. Similar results also found in the research of Payvastegan *et al.,* (2013) who reported that copper and zinc had no significant ($P>0.05$) differences on liver and heart weight in broiler compared to control group.

The comparative weight of spleen (g) of broiler chicks in the dietary groups were T_1 (2.33±0.32), T_2 (2.16±0.16), T_3 (2.33±0.16), T_4 (2.00±0.28) and T_5 (2.10±0.37). The highest value was found in T_1 (2.33±0.32) and lowest value was in T_4 (2.00±0.28) (Table 9 and Figure 6) shows. The relative weight of spleen of different groups showed that there were no significant ($P>0.05$) difference among the treatments.

The comparative weight of bursa (g) of broiler chicks in the dietary groups were T_1 (2.00±0.28), T_2 (1.93±0.29), T_3 (2.25±0.14), T_4 (1.83±0.16) and T_5 (1.73±0.12). The highest value was found in T_3 (2.25±0.14) and lowest value was in T_5 (1.73±0.12). The relative weight of spleen of different groups showed that there were no significant ($P>0.05$) difference among the treatments

Although these results reveal that supplementation of copper and zinc in broiler ration improved the weight of spleen compared with the antibiotic group.

The present study shows that dietary supplementation of copper and zinc in broiler ration had no significant ($P>0.05$)difference on bursa weight of broiler chicken (Table 9). The highest bursa weight (2.25±0.14) found in the $CuSo_4$ + Zn (8mg +30mg) supplementation (T_3) group and lowest (2.00±0.28) in the $CuSo_4$ + Zn (12mg +40mg) (T_4) group.

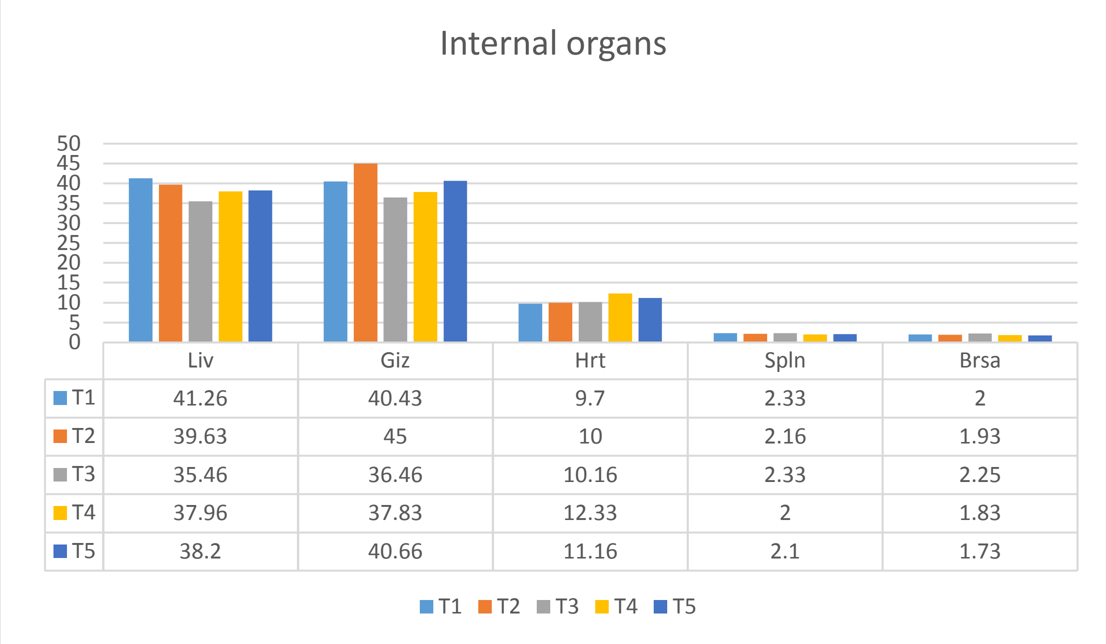
These results are contradictory to the findings of Saleh et al., (2018) who showed that dietary supplementation of zinc and copper had significant ($P<0.05$) differences in immune organ weight in case of broiler.

Table 9.Effects of dietary supplementation of copper sulphate and zinc on internal organs of different treatment.

Treatment	Liver	Gizzard	Heart	Spleen weight (gm)	Bursa weight (gm)
T_1	41.26^{c}±0.48	40.43^{b}±0.97	9.70^{a}±0.60	2.33±0.32	2.00±0.28
T_2	39.63^{bc}±0.73	45.00^{c}±0.57	10.00^{a}±0.28	2.16±0.16	1.93±0.29
T_3	35.46^{a}±1.29	36.46^{a}±1.29	10.16^{a}±0.60	2.33±0.16	2.25±0.14
T_4	37.96^{ab}±1.03	37.83^{ab}±0.72	12.33^{b}±0.46	2.00±0.28	1.83±0.16
T_5	38.20^{ab}±0.61	40.66^{b}±0.88	11.16^{ab}±0.44	2.10±0.37	1.73±0.12
Mean ± SE	38.50^{*}±0.61	40.08*±0.85	10.67^{*}±0.30	2.18^{NS}±0.11	1.95^{NS}±0.09

Here, T_1=(Control), T_2=(Antibiotic), T_3=($CuSo_4$ + Zn (8mg +30mg), T_4=($CuSo_4$ + Zn (12mg +40mg) and T_5=($CuSo_4$ + Zn (16mg +50mg). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- Mean with different superscripts at the same column are significantly different (P<0.05)
- Mean within same superscripts don't differ (P>0.05) significantly
- SE= Standard Error
- *means significant at 5% level of significance (p<0.05)



	Liv	Giz	Hrt	Spln	Brsa
T1	41.26	40.43	9.7	2.33	2
T2	39.63	45	10	2.16	1.93
T3	35.46	36.46	10.16	2.33	2.25
T4	37.96	37.83	12.33	2	1.83
T5	38.2	40.66	11.16	2.1	1.73

Figure 2.Effects of dietary supplementation of copper sulphate and zinc on inernal organs of broiler chicken under different treatment.

4.4 Hematological parameters

Data presented in (Table 10) showed the effect of dietary supplementation of copper sulphate and zinc on some blood parameters of broiler chicken. Considering the treatment impact on blood components, the results indicated significant ($P<0.05$) differences in supplementation of copper sulphate and zinc, except Monocyte, Eosinophil, Basophil and MCHC which were not significantly affected ($P>0.05$). The values had higher amount like RBC(5.03±0.10 in T_5), WBC($1.17e4^{b}$±1300.00 inT_2), Platelet (3.68e5±6.45e4 in T_2), Neutrophil (67.67^{d}±2.90 in T_2), Lymphocyte (74.00^{d}±1.00 inT_3), MCV (92.03^{b}±0.68 in T_5) and MCH (30.61^{b}±0.27 in T_5) treatment group.

Samanta *et al.*, (2011) confirmed that supplementation of copper is an effective way to improve hematological parameters in broiler chicken. Kumar *et al.*, (2013) stated that the dietary addition of copper is useful for performance and blood biochemical parameters of broiler chicken.On the contray Batal *et al.*, (2001) reports show no significant effect of zinc and copper in broiler hematological parameters. Rossi *et al.*, (2007) agreed to Batal *et al.*, (2001).

Table 10. Effects of feeding different level of copper and zinc on blood parameters of broiler chickens.

parameters	T_1	T_2	T_3	T_4	T_5	Mean ± SE
RBC(million/mm^3)	4.15^{ab}±0.15	3.90^{a}±0.12	4.81^{c}±0.24	4.68^{bc}±0.24	5.03^{c}±0.10	4.51±0.13 *
WBC(10^3/mm^3)	1.04e4^{ab}±208.16	1.17e4^{b}±1300.00	1.01e4^{a}±350.00	1.12e4^{ab}±305.50	1.07e4^{a}±884.40	1.08e4 ±392.43 *
Platelet(10^3/mm^3)	2.90e5^{ab}±5773.50	3.68e5^{b}±6.45e4	2.70e5^{ab}±1.80e4	2.82e5^{ab}±1.15e4	2.48e5^{a}±3.43e4	2.92e5±1.68e4 *
Neutrophil(%)	60.67^{cd}±2.72	67.67^{d}±2.90	20.67^{a}±2.02	35.00^{b}±8.50	51.00^{c}±3.00	47.00±4.88 *
Lymphocyte(%)	31.33^{ab}±3.84	27.00^{a}±2.88	74.00^{d}±1.00	59.33^{c}±7.83	42.33^{b}±2.66	46.80±4.97 *
Monocyte(%)	4.00±0.57	3.67±0.33	3.00±0.57	4.00±0.57	4.67±0.33	3.87±0.23
Eosonophil(%)	2.67±0.33	2.00±0.00	2.33±0.33	2.33±0.33	1.67±0.33	2.20±0.14
Basophil	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
HCT	36.26^{ab}±1.63	30.79^{a}±0.75	42.11^{b}±2.90	37.01^{b}±1.72	41.32^{b}±1.69	37.50±1.29 *
MCV(FI)	88.63^{ab}±1.53	86.77^{a}±1.18	91.43^{b}±0.79	91.17^{b}±0.57	92.03^{b}±0.68	90.00±0.65 *
MCH (pg)	30.42^{ab}±0.39	28.43^{a}±0.84	29.57^{ab}±0.73	30.59^{b}±0.58	30.61^{b}±0.27	29.92±0.32 *
MCHC(g/dl)	32.55±0.06	32.12±0.97	32.21±0.66	33.58±0.57	34.06±0.07	33.10±0.29

CHAPTER 5

SUMMARY AND CONCLUSION

A feeding trial was conducted on 150 day-old Lohman meat broiler chicks for a period of 28 days in the poultry farm of Sher-e-Bangla Agricultural University, Dhaka. The chicks were assigned to 5 treatment groups of T_1 (control), T_2 (antibiotic), T_3 ($CuSo_4$ + Zn (8mg +30mg)) and T_4 ($CuSo_4$ + Zn (12mg +40mg)) and T_5($CuSo_4$ + Zn (16mg +50mg)) randomly. Treatments were replicated three times with 10 chicks per replication. At 28 days of age, 30 broilers were sacrificed in halal method to evaluate the efficacy of copper sulphate and zinc diet supplementation. The production indexes viz. feed consumption, body weight, body weight gain, FCR, dressed weight, dressing percentage, internal organs weight, immune weight, hematological and blood biochemical parameters and survivability of broiler on different replication of different treatments was recorded and compared.

There was significant difference ($P<0.05$) on the feed intake, final live body weight, glucose, cholesterol, internal organs and hematological parameters among different treatment groups. The higher body weight found in $CuSo_4$ + Zn (8mg +30mg)T_3 treated group compared to other groups and values were followed in an ascending order in T_4,T_5 T_2, and T_1 group. The dressing percentage, body weight gain, feed conversion ratio, showed no significant ($P>0.05$) difference among all treatment groups. The better FCR (1.33±0.05) found inT_3 group. The dressing percentage in T_5 (76.00±0.11) group was higher than other group.

The serum biochemical parameters viz. glucose, cholesterol,hemoglobin concentration was measured.The level of glucose, cholesterol and hemoglobin significantly ($P<0.05$) in different treatment group. The result indicated that effects of supplementation of copper sulphate and zinc in blood constituents had significant effect except monocyte, eosinophil, basophil, and MCHC concentration which were non-significant ($P>0.05$). The control group (T_2) showed higher values of White blood cell (WBC) than other treated group.

The immune organs weight of trial birds was measured.Broiler chicken supplemented with copper sulphate and zinc had significant ($P<0.05$)effect on liver,gizzard spleen,bursa heart weight.The relative weight of spleen of different groups showed that there were no significant ($P>0.05$) difference among the treatments.The comparative weight of bursa (g) of broiler chicks in the dietary groups was T_3 (2.25±0.14), The relative weight of spleen of different groups showed that there were no significant ($P>0.05$) difference among the treatments.

On the basis of analysis of the above mentioned research findings, it can be concluded that copper sulphate and zinc had very effective impact on serum biochemical, hematological parameters and immune stimulation state of broiler chicken. So it can be said that birds fed with $CuSo_4$ + Zn (8mg +30mg)T_3 supplemented diet achieved superior result. So, copper sulphate and zinc can be used in broiler production. Thus the present study reveals that 8mg $CuSo_4$ and 30mg Zn improves the growth rate and immunity of broiler.The implementation of these formulations in the field of commercial broiler production can be said as productive for our country. However, further more experimental trials are required to assess the impact of these materials on the better quality of broiler meat production to ensure the safety of human consumption.

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APPENDICES

Appendix 1. Recorded temperature (^{0} C) during experiment

Weeks	Room temperature (^{0} C)						Average
	Period	7 A.M.	11 A.M.	3 P.M.	7 P.M.	11 P.M.	
1st	14.10.2019-20.10.2019	29.2	30.5	31.2	30.8	28.3	30.02
2nd	21.10.2019-27.10.2019	28.3	28.5	32.1	28.6	27.6	29.02
3rd	28.10.2019-03.11.2019	27.0	27.2	30.8	27.2	27.1	27.86
4th	04.11.2019-10.11.2019	28.2	30.2	30.0	28.6	26.8	28.76

Appendix 2. Recorded relative humidity (%) during experiment

Weeks	Relative humidity (%)						Average
	Period	7 A.M.	11 A.M.	3 P.M.	7 P.M.	11 P.M.	
1st	14.10.2019-20.10.2019	82	82	73	74	75	77.2
2nd	21.10.2019-27.10.2019	85	83	71	72	77	77.6
3rd	28.10.2019-03.11.2019	86	85	74	75	82	80.4
4th	04.11.2019-10.11.2019	87	86	83	77	81	82.8

Appendix 3.Feed consumption (g/bird) of 1st, 2nd, 3rd and 4th week under different treatment groups.

Treatment	Replication	1st week (g/bird)	2nd week (g/bird)	3rd week (g/bird)	4th week (g/bird)	Cumulative FC/bird (g)
T_1	R1	166	547.2	779.8	1043.5	2536.5
	R2	165	532.8	806.7	1026.9	2531.4
	R3	166	533.6	792.4	1047	2539
T_2	R1	167	534.8	802.2	1088	2592
	R2	162	537	814	1019.4	2532.4
	R3	166	533.9	761.1	1055	2516
T_3	R1	161	523.1	797.9	1027	2509
	R2	164	541.7	799.3	1026.5	2531.5
	R3	165	553.8	768.2	1016	2503
T_4	R1	157	550.8	814.2	1005	2527
	R2	156	546.7	811.6	1013.7	2528
	R3	157	551.6	818.4	1000	2527
T_5	R1	154	535.9	775.1	1048	2513
	R2	161	526.4	813.9	1032	2533.3
	R3	157	536.6	790.4	1046	2530

Appendix 4.Body weight (g/bird) of DOC, 1st, 2nd, 3rd and 4th week under different treatment groups.

Treatment	Replication	Weight of DOC (g)	1st week (g/bird)	2nd week (g/bird)	3rd week (g/bird)	4th week (g/bird)
T_1	R1	47	190	573	1185	1880
	R2	47	210	552	1200	1890
	R3	47	192	568	1160	1840
T_2	R1	47	197	581	1230	1870
	R2	47	200	589	1210	1930
	R3	47	191	579	1190	1910
T_3	R1	47	200	585	1200	1880
	R2	47	205	558	1240	1970
	R3	47	195	578	1210	1850
T_4	R1	47	201	566	1220	1877
	R2	47	210	576	1220	1880
	R3	47	190	564	1260	1830
T_5	R1	47	206	583	1220	1844
	R2	47	209	602	1130	1890
	R3	47	210	582	1210	2000

Appendix 5.Average Live Weight, Eviscerated Weight and Dressing Percentage of broiler chicken of different replication under different treatment groups.

Treatment	Replication	Live Weight (g)	Eviscerated Weight (g)	Dressing Percentage (%)
T_1	R1	1820.5	1351	74.21038
	R2	1872.5	1359	72.57677
	R3	1805	1289.5	71.44044
T_2	R1	1791	1308	73.03183
	R2	1641.5	1183.5	72.09869
	R3	1880	1451	77.18085
T_3	R1	1925	1421	73.81818
	R2	1820.5	1295	71.1343
	R3	1793.5	1260.5	70.28157
T_4	R1	1898.5	1329.5	70.02897
	R2	1890	1443	76.34921
	R3	1790	1223	68.32402
T_5	R1	1836	1312.5	71.48693
	R2	1841	1320.5	71.72732
	R3	1785	1255.5	70.33613

Appendix 6.Weight of internal organs (g/bird) of broiler chicken under different treatment groups.

Treatment	Replication	Liver Weight (g)	Gizzard Weight (g)	Heart Weight (g)	Spleen Weight (g)	Bursa Weight (g)
T_1	R1(1)	51.5	40	10	3.5	4.5
	R1(2)	52	33	12	2	1.5
	R2(1)	61	47	14	2.5	3
	R2(2)	42	31	12.5	2	2
	R3(1)	46	44.5	9.5	1.5	3
	R3(2)	41	34	10	2	3.5
T_2	R1(1)	38.5	33.5	10	1.5	2.5
	R1(2)	39	39	11	1.5	1
	R2(1)	35	44	10.5	1.5	2
	R2(2)	41	45	9.5	2.5	2
	R3(1)	59	36	12	2	3.5
	R3(2)	48	48	14	2	3.5
T_3	R1(1)	54	49	11	2.5	4.5
	R1(2)	38.5	39.5	11.5	2	3.5
	R2(1)	43.5	42.5	13	1.5	1.5
	R2(2)	40	34.5	9.5	1.5	1.5
	R3(1)	36	36	10.5	2	3
	R3(2)	35	42	9	2	4.5
T_4	R1(1)	38	46	11	1.5	2.5
	R1(2)	39	52	13.5	2	4.5
	R2(1)	47	44.5	14.5	2	3

T_5	**R1(1)**	**44**	**37**	**10.5**	**2**	**2.5**
	R1(2)	**37**	**44**	**11.5**	**2**	**2.5**
	R2(1)	**38**	**39**	**9**	**1.5**	**3**
	R2(2)	**43**	**48**	**13**	**3**	**3.5**
	R3(1)	**52**	**51**	**11**	**2.5**	**2.5**
	R3(2)	**42**	**47**	**10.5**	**2**	**3**

Appendix 7. Some photographs during the period of experiment conducted at SAU poultry farm

Some activities performed before and after arriving of DOC

Appendix 7. Cont'd

Monitoring of research activities

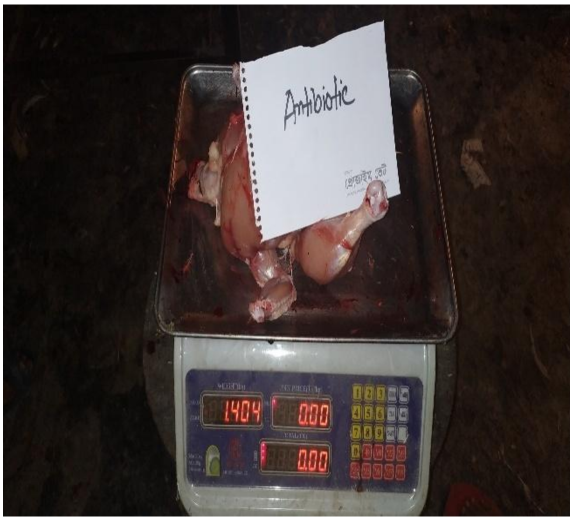
Appendix 7.Cont'nd

Different types of medication and vaccine used in the experiment

Appendix 7. Cont'd

Collection of blood sample at the age of 28 days

Appendix 7. Cont'd



Weight measurement of internal organs after dressing