EFFECTS OF DIETARY SUPPLEMENTATION OF PAPAYA LEAF (*Carica papaya*) AND BLACK CUMIN (*Nigella sativa*) AS THE ALTERNATIVE TO ANTIBIOTIC ON GROWTH PROMOTION AND HEALTH STATUS OF BROILER CHICKEN

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CERTIFICATE

This is to certify that the thesis entitled "EFFECTS OF DIETARY SUPPLEMENTATION OF PAPAYA LEAF (Carica papaya) AND BLACK CUMIN (Nigella sativa) AS THE ALTERNATIVE TO ANTIBIOTIC ON GROWTH PROMOTION AND HEALTH STATUS 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 MD. MONIRUL HAQUE, Registration No. 17-08292 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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The Author

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ACRONYMS AND ABBREVIATIONS

Abbreviation		Full meaning
A.M	=	Ante meridiem
AGPs	=	Antibiotic growth promoters
ANOVA	=	Analysis of Variance
BANSDOC	=	Bangladesh National Scientific And Technical Documentation Centre
BARC	=	Bangladesh Agricultural Research Council
BBS	=	Bangladesh Bureau of Statistics
BLRI		Bangladesh Livestock Research Institute
Ca	=	Calcium
CBC	=	Complete Blood Count
CF	=	Crude Fiber
CFU	=	Colony Forming Units
Cm	=	Centimeter
cm^2	=	Square Centimeter
CONTD.	=	Continued
CP	=	Crude Protein
CRD	=	Complete Randomized Design
Dr.	=	Doctor
e.g.	=	For Example
EDTA	=	Ethylene Diamine Tetraacetic Acid
et al.	=	And others/Associates
FC	=	Feed Consumption
FCR	=	Feed Conversion Ratio
Hb	=	Hemoglobin
i.e.	=	That is
IBV	=	Infectious Bronchitis Vaccines
kcal	=	Kilo-calorie
Kg	=	Kilogram
Ltd.	=	Limited
M.S.	=	Master of Science
MCHC	=	Mean Corpuscular Hemoglobin Concentration
ml	=	Milliliter
mm	=	Millimetre
mmol	=	Millimoles
MT	=	Metric ton
N	=	Nitrogen
NDV	=	Newcastle Disease Vaccine
No.	=	Number
NS	=	Non-significant
P	=	Phosphorus
PCV	=	Packed Cell Volume

ACRONYMS AND ABBREVIATIONS (CONT'D)

Abbreviation		Full meaning
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
WPSA	=	World's Poultry Science Association

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

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ABSTRACT

A feeding trial was conducted on 150 day-old Cobb 500 broiler chicks for a period of 28 days in the Poultry Farm of Sher-e-Bangla Agricultural University, Dhaka. The use of locally available feed ingredients has received particular attention as a viable alternative to the use of antibiotic and conventional feedstuffs. The aim of the study was to assess the efficiency of dietary Papaya Leaf (*Carica papaya*) Meal and Black Cumin (*Nigella sativa*) Seed supplementation on the production index and health status of commercial broiler chicken. The chicks were assigned to 5 treatment groups comprising of T_1 (Control), T_2 (antibiotic), T_3 (2% Papaya Leaf Meal), T_4 (1% Black Cumin Seed) and T_5 (1% of each Papaya leaf meal & Black Cumin Seed) randomly. Treatments were replicated thrice with 10 chicks per replicate. The results showed that dietary supplementation of Papaya Leaf Meal (PLM) and Black Cumin Seed (BCS) had no significant (P>0.05) difference on feed consumption, body weight gain, final live weight, dressing percentage of broiler compared to control group. Higher feed consumption found in T_1 (2212.60±11.320g) group compared to other groups. However, superior final live weight (1559±26.404g) obtained in T_3 group where birds fed with 2% PLM compared to those of antibiotic and control group. Improved FCR value (1.41±.026) found in T_3 group which is statistically non-significant (P>0.05) with the values of other groups. Dressing percentage is also higher in T_3 (72.34±1.131) group where birds fed with 2% Papaya leaf meal. Dietary supplementation of PLM and BCS had no significant (P>0.05) effect on the relative weight of spleen in different groups. Birds supplemented with 1% Black cumin seed (T_4) showed insignificantly (P>0.05) higher spleen weight (2.28±.169g). But there was a significant (P<0.05) increased on the weight of bursa compared to antibiotic and control group. Higher bursa weight 2.50±.408g was found in T_3 (2% Papaya Leaf Meal) group. The relative weight of liver, gizzard, heart and intestine of different groups showed that there was no significant (P>0.05) difference among the groups. The average weight of liver, gizzard and heart were 36.32±.796g, 35.11±.557g and 7.58±.133g respectively. The glucose and cholesterol concentration had no significant (P>0.05) difference among all groups but comparatively lower cholesterol level 169.11±5.308mg/dl was found in 2% papaya leaf meal supplemented group. In addition, the hematological parameters including Red blood cell (RBC), White blood cell (WBC), Lymphocyte and Packed cell volume (PCV) which were significantly (P<0.05) increased in Papaya leaf meal and Black cumin seed supplemented groups compared to antibiotic and control group. Diets supplemented with PLM and BCS had leads to significant (P<0.05) reduction in bacterial colony count compared to control group but statistically no difference with T_2 (antibiotic) group. Birds fed with 2% Papaya Leaf Meal supplemented diet achieved superior result.

CHAPTER 1

INTRODUCTION

Poultry industry has become a specialized and speedy business sector at present time in all over the world. 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. Commercial poultry could also serve as a tool for employment generation and poverty alleviation. Recently, broiler industry has become take the leading position among the other sector of poultry production.

Intensive poultry production in developing countries could be further enhanced through feeding strategies that promote feed utilization in relation to bird performances. A variety of synthetic feed additives including antibiotic growth promoters (AGPs) have been used in poultry feeds to maximize the efficiency of production, product quality and to control diseases. Before July 1999 in the European Union, the inclusion of antibiotics as growth promoters in animal feed was widely adopted and better growth stimulation, uniformity was observed (Bedford, 2000). Antibiotics have the ability to decrease feed usage per production unit with concomitant increase in production performance (Falçao-e-Cunha *et al*., 2007). Beside these advantages, antibiotic exerts several fatal hazards which compromise human and animal health (Diarra *et al*., 2010).

Antibiotic growth promoters are used to designate the sub therapeutic administration of antibiotic in farm animals to improve the growth performance of the animals through destroying the harmful pathogen, improve nutrient absorption by thinning the gastro-intestinal tract wall. Administering antibiotics to poultry, cattle and pigs in their feed or drinking water has had a major impact on the commercial production of meat for human consumption since the early 1950s (Jukes, 1972). Growth enhancement was first observed by using sulphonamides in farm animals (Moore *et al*., 1946).

The administration of antibiotic in food animals results in the emergence and spread of antimicrobial resistant bacteria, which is a cause of worldwide concern (Garcia-Migura *et al*., 2014). Scientific evidence suggests that the unregulated massive use of antibiotic has led to increased problem of antibiotic resistance (Diarra *et al*., 2007; Furtula *et al*., 2010; Forgetta *et al*., 2012) which causes spread of resistant microbes and presence of antibiotics residues in feed and environment (Carvalho and Santos, 2016; Gonzalez Ronquillo *et al*., 2017).

Over potent misuse of antibiotic application is considered the most important factor promoting the emergence, selection and dissemination of antibiotic resistant microorganisms in both veterinary and human medicine (Witte, 1998). Resistances occur not only in pathogenic microbes but also in the endogenous flora of exposed individuals (animals and humans) or populations (Baldwin *et al*., 1976; Hinton *et al*., 1982; Piddock, 1996). Application of such synthetic drugs and growth promoters into broiler diets are done to obtain rapid growth, but their use have shown many disadvantages like high cost, adverse side effect on health of birds and long residual properties and carcinogenic effect in humans (Butaye *et al*., 2003).

Now a day, antimicrobial resistance is a great concern in human and animals. This concern is also increasing due to the potential misuse of antibiotics in poultry feeds these shown many disadvantages like high cost, adverse side effect on health of birds and long residual properties etc. For this reason the inclusion of antibiotics in the diets of animal has been prohibited in the EU countries since January 2006. Very recently in Bangladesh, Use of antibiotics, steroids and hormones as a growth promoter in animal feeds are completely banned in the Fish and Animal Feed Act 2010; section 14(1) & (2) to ensure the safety of livestock products for the betterment of the consumers. However, the ban on the use of antibiotics in animal feed has fueled the search for alternative animal growth promoters in the form of phytogenic feed additives (Oloruntola *et al*., 2018), probiotics, prebiotics, enzymes, acidifiers, organic acids, antimicrobial peptides, single cell protein, dietary amino acids, immune stimulants, antioxidants and immunoglobulin which obtain the major attention through the blessings of biotechnology.

Phytogenic feed additive are a group of natural growth promoters or non-antibiotic growth promoters used as feed additives, derived from herbs, spices and different plants. Herbs and medicinal plants can be used as potential alternative to AGP. Herbs contain some complicated mixture of organic chemicals having antimicrobial, antipyretic, anti-inflammatory and immune reactive properties (Guo *et al*., 2003). Plant derived ingredients are used as digestive stimulants, anti-diarrheic, antiseptic, anti-inflammatory, anti-parasitic and appetite stimulants in human beings as well as animals. The primary mode of action of Phytogenic feed additives (PFA) is controlling potential pathogens and beneficially modulating the intestinal microbiota. Phytogenic feed additives (PFAs) contain natural, plant-based bioactive molecules. There are four major families of phytogenics: essential oils, saponins, tannins and flavonoids and their efficacy as a protective mechanism for poultry health depends largely on the plant from which they're derived. Essential oils, tannins and saponins are most commonly used in poultry production. Studies conducted in broilers have demonstrated the antimicrobial efficacy of PFA against pathogenic bacteria, such as *Escherichia coli* and *Clostridium perfringens* (Jamroz *et al*., 2005; Mitsch *et al*., 2012).

Medicinal plants have been proficiently used for curing diseases for many centuries in the form of different indigenous medicine system as well as folk medicines (Prasad *et al*., 2011). Researchers are focusing on medicinal plants on the basis of pharmacological properties and source of nutrients possessing by those plants such as acarotenoids, vitamin C, folate and dietary fiber (Ali and Blunden, 2003). Papaya (*Carica papaya*) is a medicinal plant contains a variety of phytochemicals, including lycopene and polyphenols (Kale *et al*., 2003). Papaya leaves are rich source of the proteolytic enzymes papain and chymopapain which have protein digesting properties and are useful in controlling digestive problems and intestinal worms (Burkill, 1985). Papaya leaves also contain carotene, provitamin A, which serves as many as 18-50 IU and can be used as a promising source of natural xanthophyl. Papaya leaves contain vitamin C, vitamin E, calcium, phosphorous and iron. Beside that the leaves contain 20.88% crude protein, 0.99% calcium, 0.47% phosphorous and 2912 kcal / kg gross energy. Papaya leaves are used for the treatment of malaria and dengue fever. It is showed that papaya leaves significantly inhibit coccidiosis (AL-Fifi, 2007; Nghonjuyi, 2015). It has been found that papaya leaf contributed to the pigmentation of the blood, which recorded highest concentration of RBC, hemoglobin, platelets and PCV in the supplemented trial (Agboola *et al*., 2018). Several studies shows that better growth performance, immune response could be achieved in broilers supplemented with papaya leaf (Sorwar *et al*., 2016; Oloruntola *et al*., 2018).

Black cumin (*Nigella sativa*) locally known as 'Kalo jeera' is emerging as a miracle herb. Due to its miraculous power of healing, *N. sativa* has got the top ranked place of herbal medicines. The black cumin seeds contains volatile oil (0.5- 1.6%), alkaloids, sterols, saponins and quinines and the seeds are used for traditional medicine (Al-Homidan *et al*., 2002). Various beneficial properties have been attributed to black cumin, including its antioxidant potential. Black cumin also having various therapeutic potentials and it possess wide spectrum of activities like antibacterial, antihypertensive, anticancer (Padhye *et al*., 2008) and immune-modulatory, analgesic, antimicrobial, antihelminthics, anti-inflammatory, gastro protective, and renal protective properties (Zaoui *et al*., 2000). Several studies mentioned that black cumin seed had positive effect on weight gain and feed conversion ratio (Al-Harthi, 2004; Mansoori *et al*., 2006; Khan *et al*., 2012), feed intake, dressing percentage and weight of different internal organs (Durrani *et al*., 2007). The seeds of *N. sativa* and their oil have been widely used in the treatment of various diseases for centuries throughout the world. It was showed that black seed modulate cholesterol level in broiler serum which reflects the level in broiler meat and consequently improve its quality for human nutrition (Khadr and Abdel-Fattah, 2006).

On the basis of this background, the experiment was planned to explore the possibilities of Papaya leaf (*Carica papaya*) meal and Black cumin (*Nigella sative*) seed as the replacement of antibiotic growth promoters, with the following objectives:

1. To evaluate the effect of Papaya leaf meal and Black cumin seed on growth performance, hematological properties and internal organ characteristics of broiler chicken in comparison with antibiotic and basal diet.
2. To determine the effect of dietary supplementation of Papaya leaf meal and Black cumin seed on immune system of broiler chicken.

CHAPTER 2

REVIEW OF LITERATURE

Sources of literature

1. Book and journal in different libraries as mentioned below:
    - ✓ Sher-e-Bangla Agricultural University (SAU) Library, Dhaka
    - ✓ Bangladesh Agricultural Research Council (BARC) Library, Farmgate, Dhaka
    - ✓ Bangladesh National Scientific And Technical Documentation Centre (BANSDOC) Library, Agargaon, Dhaka
    - ✓ Bangladesh Livestock Research Institute (BLRI) Library, Savar, Dhaka
2. Abstract searching at BARC, Farmgate, Dhaka, BANSDOC, Agargoan and Dhaka.
3. Internet browsing.

A total of about 80 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 24 were full article and 40 abstracts, 16 were only titles and some were miscellaneous. A brief account is given below depending on 8 main headlines viz, Antibiotics in poultry production, Antibiotic growth promoters (AGPs), Antibiotic resistance, Antimicrobial residues, Alternative to antibiotic growth promoters, Phytogenic feed additives, Papaya leaf and Black cumin seed.

The traditional system or sequences in stating the references is avoided. Crucial 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 (FAO Statistics Division, 2009). 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 a lot of synthetic feed additives including antibiotic growth promoters are used in poultry feeds to maximize the efficiency of production.

2.1 Antibiotics in poultry production

Antibiotics are any substances or semisynthetic substances derived from a micro-organism that inhibits the growth or kills other micro-organisms. Alexander Fleming discovered penicillin, the first natural antibiotic, in 1928. There are four applications of antibiotics: Therapeutic (for the treatment of disease), Prophylaxis, Metaphylaxis (for disease prevention), and for Growth promotion (Poole and Sheffield, 2013). Antimicrobials used in animal production in 1910 when due to shortage of meat products, workers carried out protests across America (Ogle, 2013). At that period scientists were searched for means of producing more meat at relatively cheaper costs; resulting in the use of antibiotics and other antimicrobial agents (Dibner and Richards, 2005).

Antibiotics mainly used for disease prevention in poultry production all over the world. Entrepreneurs and farmers applied antibiotics for the treatment of intestinal infections such as colibacillosis, necrotic enteritis, and other common diseases generally caused by *E. coli*, *Salmonella*, *Clostridium* species. These infections are marked as major threat in poultry production leading to huge economic losses. The sub-therapeutic concentrations of antibiotic stabilize the bacterial population which helps the bird to obtain more nutrients from the diets thus increases animal production (Hofacre *et al.*, 2008).

In 1950s, the poultry industry first applied antibiotic at sub-therapeutic levels as feed additives to improve performance of birds in terms of feed conversion and weight gain in the United States. In 1965, scientists found improved FCR in food animals fed with small amounts of antibiotics. They found 2.5 pound chicken produced with a 4.7

feed conversion ratio after a raising period of 112 days and consequently 6 pound chicken with a feed conversion ratio of 1.8 in only 42 days (Diaz-Sanchez, 2015). This significant improvement in growth promotion rate and feed efficiency was partly due to the application of antibiotic as feed additive. Afterwards, unregulated application of antibiotic in poultry production disseminate the emergence of antibiotic resistance pathogens which gained a concerning momentum in 1980s. Because of consumer opposition and concerns over antibiotic resistant bacteria, many countries all over the world have banned or restricted the inclusion of antibiotics in animal diets for growth promotion purposes.

The use of antibiotics has favorable impact in poultry and livestock production both for the farmers and entrepreneurs. It has generally improved poultry production effectively and economically. But at the same time, this causes emergence and spread of antibiotic resistant strains of pathogenic and non-pathogenic organisms into the environment and their further transference to humans via the poultry meat, egg and other poultry products which lead to fatal consequences on public health (Apata, 2009).

2.2 Antibiotic growth promoters (AGPs)

The term "Antibiotic growth promoter" is used to designate the administration of antibiotic at non-therapeutic concentrations in the feed of food animals to stimulate growth and improve feed efficiency (Costa *et al*., 2011; Lin *et al*., 2013). Growth enhancement with sulphonamides (Antibiotic) was first observed by Moore *et al*. (1946). Jukes and his colleagues found that Aureomycin stimulated significant growth in chickens at Lederle Laboratories (Jukes, 1985).

Antibiotics have been used in animal feed not only as an anti-microbial agent, but also as a growth-promoting agent. The benefits of using antibiotics in animal feed include increasing feed efficiency and growth rate, treatment of sick animals and preventing or decreasing the incidence of various infectious diseases.

The mode of action of antibiotic growth promoters and how antibiotic enhances growth are not clearly defined. However, many scientists have mentioned that antibiotics have the capability to produce immune-modulatory and anti-inflammatory effects. Scientist also demonstrated that antibiotics are capable to inhibit one or more functions of inflammatory cells and pro-inflammatory cytokine production. Consequently, AGPs may prevent immunologic stress that is associated with metabolic and gut microbial environment changes, allowing more energy to be available for muscle development which consequently improving growth.

There are four common hypotheses on how antibiotic growth promoters enhance growth: 1) antibiotic reduces the short chain fatty acid in intestinal lumen through microbial fermentation which leads to thinning of the intestinal wall and villi by losing of mucosal cell and accelerate nutrient absorption, 2) restrict bacteria from using nutrients, 3) reduces bacteria and toxins produced by them, 4) increase immunity and reducing the incidence of subclinical infections (Gaskins *et al*., 2002; Butaye *et al*., 2003; Dibner and Richards, 2005; Niewold, 2007; Costa *et al*., 2011).

Poultry industry used several antibiotics which are also important in terms of public health aspects such as virginiamycin, oxytetracycline, chlortetracycline, neomycin, tylosin, bacitracin, avopracin etc. outpatient antibiotic overuse is a specified problem that causes antibiotic resistance. After animals have been fed antibiotics over a period of time, they retain the strains of antibiotic resistant bacteria. These bacteria proliferate in the gut of animal and transmitted to the other animals through interactions and become colonized (McDonald, 1997). The bacteria flourish in the intestinal flora as well as, in the muscle of the animals. Those resistant bacteria spread to human mainly through food chain, environment, trades and travel.

2.3 Antibiotic resistance

The term "Antibiotic resistance" is used to describe the ability of bacteria and other microorganisms to resist the effects of an antibiotic to which once they were sensitive (William, 2018). Bacteria counteract the actions of antibiotics by four well-known mechanisms, including 1) decreased permeability of bacterial membrane, 2) alteration in target binding sites, 3) enzyme modification, 4) efflux activity (Bassetti *et al*., 2013).

Alexander Fleming told in his Nobel Prize acceptance speech in 1945 about the resistance of antibiotic. He mentioned that:

"Then there is the danger that the ignorant man may easily under dose himself and by exposing his microbes to non-lethal quantities of drug, make them resistant."

Starr and Reynolds's (1951) report on turkeys mentioned that after they had been fed with streptomycin antibiotic, resistant bacteria found in those turkeys. This report may have been the first report of antibiotic resistance in food animals. The bacteria had not caused disease in the turkeys, but the researchers mentioned its possibility of spread of resistant *Salmonella* from poultry to humans. Dierikx *et al*. (2013) and Kemmett *et al*. (2013) documented horizontal transmission and vertical transmission of some resistant bacteria in poultry, especially *Escherichia coli*, from breeder flocks to poultry house. Resistant bacteria transfer their genetically modified resistance criteria to the next generation through mutation and plasmid transmission (Gould, 2008). Kemmett *et al*. (2014) mentioned that infection can caused by the spread of such resistant strains in young broiler chicks. Another report revealed that spread of resistant *Salmonella* bacteria occurred from animal to human due to feeding AGPs causing disease in human (Anderson, 1968). Antibiotic applications in poultry production bring about an increase in resistance of antibiotic not only in pathogenic organisms, but also in beneficial organisms (Lukasova and Sustackova, 2003).

2.4 Antimicrobial residues

The over potent misuse of antimicrobial agents in food animals has become a very important public health issue. The abuse or misuse of veterinary drugs is one of the causes of drug residues in animal products (Salehzadeh *et al*., 2006; Pavlov *et al*., 2008).

Reyes-Herrera *et al*. (2005) conducted an experiment to evaluate the presence of antibiotic residues in different edible muscle tissues in poultry. Chickens were dosed with enrofloxacin (Baytril) at either 25 ppm for 3 days, 25 ppm for 7 days, 50 ppm for 3 days. Muscle tissues of breast and thigh were collected from each bird during the dosing and withdrawal period and determine fluoroquinolone concentration. The results mentioned higher ($P<0.05$) enrofloxacin concentrations in breast muscle for each treatment group.

Tetracycline in meat may potentially stain the teeth of young children. Penicillin in chicken was reported to have caused severe anaphylactic reaction in a consumer (The and Rigg, 1992). Skin allergies in eggs containing sulfonamide residues have also been reported (WHO, 1989).

2.5 Alternative to antibiotic growth promoters

The banned on all growth-promoting antibiotics triggers the search for non-antibiotic growth promoters or natural growth promoters for improving the production performance and health status of farm animals. To achieve this goal several approached can be taken such as maintain good housing, hygienic protocols and better feeding strategies with the best possible application of antibiotic alternatives, including probiotics, prebiotics, organic acids, dietary fiber, phytogenic feed ingredients (plant derived ingredients, herbs, spices), feed enzymes, antimicrobial peptides, single cell protein, dietary amino acids, immune stimulants, antioxidants, immunoglobulin and many more.

2.6 Phytogenic feed additives

Phytogenic feed additives are known to have a range of biologically active properties that are beneficial in modern livestock & poultry production, including: anti-oxidative, anti-inflammatory, anti-microbial and digestion enhancing effects. For example, Herbs and medicinal plant leaf like Papaya leaf (*Carica papaya*), Neem leaf (*Azadirachta indica*), Tulsi (*Ocimum tenuiflorum*), Moringa leaf (*Moringa oleifera*) etc. Seeds of Black cumin (*Nigella sativa*), Fenugreek (*Trigonella foenum graecum*) etc. have beneficial potentiality to perform as a natural growth promoter. Achieving consistent and reliable results with plant-based substances in animal diets requires a well-defined formulation, standardized raw materials and effective quality control.

Nutritionists, business owners, veterinarians and consultants located in over 100 countries provided their views on the use of plant-derived compounds in farm animal nutrition within the framework of the 2017 BIOMIN Phytogenic Feed Additives Survey. Digestibility enhancement ranked as the number one reason that the livestock industry uses phytogenic feed additives followed by their perceived antimicrobial effects, their use within an AGP replacement strategy and growth promotion. Respondents also cited PFAs anti-inflammatory effects, good past experience with PFAs, an optimized feed conversion ratio (FCR), and higher feed intake as reasons that they use phytogenic feed additives in farm animal production.

2.7 Papaya leaf

Papaya (*Carica papaya*) is an invaluable medicinal plant, used in the West and Asian countries to cope several diseases. It belongs to the family Caricaceae. It was originally derived from the southern part of Mexico. Different parts of the papaya plant including leaves, seeds, and fruit have been shown to have excellent nutritional and medicinal values (Krishna *et al*., 2008; Afolabi *et al*., 2011; Pradeep *et al*., 2014). Papaya leaves used as an alternative protein source for livestock feed (Adewolu, 2008; Onyimonyi and Onu, 2009; Ebenebe *et al*., 2011). Seeds and leaves of papaya were reported to neutraceutical and antioxidant properties (Kadiri *et al*., 2016). Papaya contains a high content of vitamin A, B, and C; papain; and chymopapain. The leaves of papaya contain 2-2-diphenyl-1-piccrlhydrazyhydrate (7.8 mg/ml), phenol (424.89 mg/100g dry weight), and flavonoid (333.14 mg/100 g dry weight), respectively (Maisarah *et al*., 2014). In addition, the papaya leaf contains broad spectrum phytochemicals including alkaloids and phenols. Phenolic compounds have high antioxidant activity and free radical scavenging capacity, with the mechanism of inhibiting enzymes responsible for reactive oxygen species production (Kahkonen *et al*., 2001).

2.7.1 Effect of Papaya leaf on live weight and live weight gain

Unigwe *et al*. (2014) conducted an experiment on Anak broiler chicks to assess the effect of sun dried Papaya leaf meal (PLM) supplementation on their growth performance. The experiment was designed with three inclusion level of PLM (5%, 10% and 15%) and control group (0% PLM). The result showed that there was no significant difference ($P>0.05$) among all groups with respect to average final body weight, average daily body weight gain. But the weight performance was numerically increased as the inclusion levels of Papaya leaf meal increased.

Fouzder (2010) concluded that different concentrations of dried papaya (*Carica papaya*) skin incorporation in growing pullets diets did not produces any significant difference in weight gain, food consumption, food conversion and protein efficiency of the treated birds when compared with birds that received the control diet.

Opara (1996) found that when the levels of inclusion of Papaya Leaf Meal increased progressively from 1% through 1.5% to 2% then there was a gradual decrease in average weight gains of the birds.

Ebenebe *et al*. (2011) who found that increased weight gain associated with the supplementation of Papaya Leaf Meal in the diet of broiler chicks compared to that of control groups.

2.7.2 Effect of Papaya leaf on feed consumption

Bolu *et al*. (2009) reported that broiler chickens were randomly divided into four treatments groups of diets containing 0%, 5%, 10% and 15% Dried Papaya seed (DPS). Feed intake, average weekly feed intake and weight gain had no significant effect ($p>0.05$) by the dietary DPS supplementation. Highest feed intake was observed in the 5% DPS supplemented groups than other groups.

Rumokoy *et al*. (2016) conducted an experiment evaluate the efficacy of papain crude extract addition in the feed of broiler chickens on production performance. Four treatment levels of Papaya extract (0, 0.03, 0.05, and 0.07 %) were used. The results of the experiment showed that the interaction was significantly ($P<0.01$) higher for feed intake, body weight, carcass percentage respectively while feed conversions had significant interaction ($P<0.05$).

2.7.3 Effect of Papaya leaf on FCR

Onyimonyi and Onu (2009) evaluated the efficacy of dietary papaya leaf supplementation in finisher broiler for a period of 28 days. Diet was incorporated with 0%, 0.5%, 1.5% and 2% Papaya leaf meal. They found that birds treated 2% Papaya leaf meal showed superiority in all research parameters like final body weight (g/birds), weight gain (g/birds), feed conversion ratio and feed cost/kg gain. The result showed that feed conversion ratio 2.30 was significantly ($P<0.05$) better than other treated groups and compared to that of control.

Mahejabin *et al*. (2015) observed that supplementation of 2% neem, turmeric and papaya leaf extract 1 ml per liter of drinking water had significantly increased

(P<0.05) body weight. The treatment group caused better improvement in the feed conversion ratio as compared to that of control group.

Wanker *et al*. (2009) reported that birds supplemented with papaya leaf with basal diet had increased feed efficiency compared to that of control groups.

Unigwe *et al*. (2014) concluded that feed conversion ratios (FCR) in broiler supplemented with dried papaya leaf meal (PLM) at 5%, 10% and 15% inclusion level were not significantly different (P>0.05) among all treatment groups and compared to that of control group.

2.7.4 Effect of Papaya leaf on immune organs

Haruna and Odunsi (2018) performed an experiment by using 0%, 0.1%, 0.15% and 0.2% Papaya leaf extract with basal diet in day-old Arbor Acre broiler chicken. The result showed significant (P<0.05) increase in the weight of spleen and bursa in 0.2% Papaya leaf extract supplemented group than other papaya leaf inclusion level. The bursa and spleen index was also significantly (P<0.05) higher in all papaya leaf supplemented groups compared to that of control group.

Battaa *et al*. (2015) evaluated the efficiency of diets divided into control (basal diet without any addition of PL_0) and 3 treatment groups where basal diet containing papaya latex ground powder at levels 0.01% (PL_1), 0.03% (PL_2) and 0.05% (PL_3). They observed that immune response was increased with increase in dietary levels of papain enzyme extracted from papaya leaf compared to that of control.

El-Kholly *et al*. (2008) observed the effect of *Carica papaya* as feed additive and enzymes complex on performance growing rabbits. Treatments were designed under 4 groups supplementing control diet with 0% papaya latex and other three with 0.1, 0.5 and 0.7% of papaya latex. The result showed that values of WBC, lymphocytes and total protein increased linearly due to increment of 1 unit of papaya latex inclusion in the basal diet. Cell mediated immunity also increased significantly (P<0.002) in a linear pattern as papaya latex inclusion increased in dietary supplemented groups. This is in agreement with the observations of Fu Chang *et al*. (2004) who observed that immune stimulation was increased with the increased level of *C. papaya* supplementation.

2.7.5 Effect of Papaya leaf on internal organs

Sorwar *et al*. (2016) concluded that broiler chicken supplemented with papaya leaf powder with drinking water did not show any difference between the relative liver weight, relative gizzard weight, spleen weight and relative pancreas weight of the birds of different feeding group.

Kamal *et al*. (2015) conducted an experiment where Papaya (*Carica papaya*) leaves, Neem and Nishyinda leaves powder were supplemented in drinking water as a growth promoter in broiler chicks. The result stated that there were significant differences in weight gain of heart, liver, spleen and pancreas except gizzard weight.

2.7.6 Effect of Papaya leaf on serum biochemical properties

Juárez-Rojop *et al*. (2012) reported that the aqueous extract of *Carica papaya* 0.75 g and 1.5 g/100 ml drinking water significantly decreased blood glucose levels ($p<0.05$) in diabetic rats. Supplementation of *Carica papaya* extract also decreased cholesterol, triacylglycerol and amino-transferases blood levels.

Zetina-Esquivel *et al*. (2015) found that daily doses of *C. papaya* extract at 0, 31, 62 and 125 mg/kg body weight were orally administered in 300 µl polyethylene glycol to hypercholesterolemic rats. The result assessed that *C. papaya* extract produced a significant decrease of serum and liver cholesterol concentrations in hypercholesterolemic rats. *C. papaya* treatment also decreased LDL and increased HDL in serum significantly.

2.7.7 Effect of Papaya leaf on blood parameter

Oloruntola *et al*. (2018) mentioned that broilers supplemented with dietary Papaya leaf meal and enzyme had no significant ($P>0.05$) difference on the blood profile of broiler chickens except platelets counts, RBC, WBC and lymphocyte concentration. Red blood cells tended to increase with the interaction of papaya leaf meal and enzyme.

NghoNjuyi *et al*. (2015) conducted an experiment to evaluating the efficacy of ethanolic leaf extract of *Carica papaya* in chicken infected with *Eimeria* oocysts. The result showed that papaya extract had a statistically significant difference in RBC and WBC counts at ($P\leq0.043$) and ($P\leq0.031$) respectively between experimental groups treated with ethanolic *Carica papaya* leaf extract. They mentioned that the level of RBC and WBC were higher in the *Carica papaya* extract supplemented group.

Bolu *et al*. (2009) observed that dietary *Carica papaya* leaf supplementation had no significant differences ($p>0.05$) in the WBC, Leucocyte and Neutrophil values in response to dietary papaya leaf incorporation. Inclusion of papaya leaf in the diets of broilers tended to improve their hematological components especially at 5% dietary level.

Agboola *et al*. (2018) found that broiler chickens diet incorporated with Papaya leaf meal showed highest values of packed cell volume, red blood cell, and hemoglobin in all the papaya leaf included treatments.

2.7.8 Effect of Papaya leaf on microbial load

El-Neney *et al*. (2013) reported that diets supplemented with papaya latex at levels 0.05, 0.07 and 0.09% had leads to significant ($P<0.05$) reduction of bacteria count *E. coli* and total anaerobic bacteria for the group rabbits received diets with papaya latex (0.07 and 0.09%). They mentioned that occurrence of such criteria was due to the decreasing of the cecum pH with increasing the level of papaya latex when compared to the control diet.

Osato *et al*. (1993) mentioned that *Carica papaya* latex had bacteriostatic mechanism against several microbes such as *E. coli, Salmonella typhi, B. subtilis, Enterobacter cloacae, Staphylococcus aureus, and Proteus vulgaris*.

2.7.9 Effect of Papaya leaf on carcass quality

Ebrahimi *et al*. (2015) carried out an experiment to evaluate the potential of papaya leaf to prevent lipid peroxidation and enhance the antioxidant activity of breast meat of broiler chicken. The results showed that supplementation of papaya leaf at 5%, significantly ($p<0.05$) reduced the lipid peroxidation compared to control group.

Sorwar *et al*. (2016) concluded that broiler chicken supplemented with papaya leaf powder with drinking water did not show any significant difference between the dressing percentages of the birds of different feeding group.

Abdalla *et al*. (2013) found that addition of dried papaya leaves powder to spent layer hens ration significantly ($P \leq 0.05$) increased dressing percentage of the birds. Ration containing 10% dried papaya leaves powder (DPLP) had significantly ($P \leq 0.05$) higher live body weight, carcass and abdominal fat pad weights. They also mentioned that supplementation of DPLP had significantly ($P \leq 0.05$) increased the level of meat tenderness. These results might be attributed to the effect of papain enzyme found in the papaya leaves which may lead to more degradation and availability of dietary protein to the treated groups.

Adeyemo and Akanmu (2012) conducted an experiment diets incorporated with papaya and neem leaf meal in broiler chicken. The result showed supplementation of PLM and NLM improved the dressing percentages where the highest values was obtained 89.29% and 87.55% respectively which were significantly different ($p<0.05$) when compared with the value obtained from control group.

2.7.10 Effect of Papaya leaf on survivability

Hema *et al*. (2015) carried out an experiment to assessed anti-coccidial efficacy of *Azadirachta indica* and *Carica papaya* with Salinomycin as a dietary feed supplement on the broiler chickens. They found the livability percentage (94 ± 0.0) was better in T_6 group supplemented with 0.1% Papaya leaves powder as compared to other infected groups.

2.8 Black cumin seed

Nigella (Nigella sativa) is an annual herbaceous plant belonging to the Ranunculaceae family (Al-Gaby, 1998). It is commonly known as black seed which is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Bangladesh, Syria, Turkey, Saudi Arabia (Satrija *et al*., 1994).The seeds of *N. sativa* and their oil have been widely used for centuries in the treatment of various ailments throughout the world. And it is an important drug in the Indian traditional system of medicine like Unani and Ayurveda. *N. sativa* has been extensively studied for its biological activities and therapeutic potential and shown to possess wide spectrum of activities viz. as diuretic, antihypertensive, anti-diabetic, anticancer and immune-modulatory, analgesic, antimicrobial, analgesics and anti-inflammatory, spasmolytic, bronchodilator, gastro protective, liver protective, renal protective and antioxidant properties (Sandhya and Veerannah, 1996).

2.8.1 Effect of Black cumin on live weight and live weight gain

Jahan *et al*. (2015) conducted an experiment with dietary supplementation of 0%, 0.5%, 1.0% and 1.5% of Black Cumin Seed Meal in broiler chicken. They found that broilers supplemented with 1.5% BCSM showed significant ($P<0.05$) improvement on live weight at 21, 28 and 35 days of age compared to that of control group.

Al-Betawi and El-Ghousein (2008) reported that diets containing crushed *N. sativa* seed had higher live body weight and higher body weight gain in comparison with that of control. The result showed that chicks supplied 1.5% crushed black cumin seed had significantly ($P<0.05$) higher live body weight and body weight gain than other black cumin treated group.

Ziad *et al*. (2008) concluded that lohman broilers fed with 1 and 1.5% black cumin seeds for a period of 4 weeks was significantly ($p<0.05$) increased the body gain by 10 and 14.5%, respectively.

Sarkar *et al*. (2015) evaluated the benefits of supplementation of Black cumin and Thankuni powder in drinking water. There was a significant effect on growth rate of the birds supplemented with Black cumin powder. The body weight of treatment group was 17.24% more significant ($p<1\%$) than that of control group.

Khan *et al*. (2012) reported that supplementation of Black cumin seed to the diets had variable effects on chicken performance. They indicated that birds fed diets supplemented with 2.5 or 5.0% BCS had significantly higher body weight gain than those fed with the 1.25% BCS diet and the control group.

2.8.2 Effect of Black cumin on feed consumption

Guler *et al*. (2006) reported that dietary supplementation of *N. sativa* had no significant differences ($P>0.05$) between the six treatments including 0.5%, 1%, 2%, 3% black cumin, avilamycin and control group in case of daily feed intake of the experimented broilers.

Abbas and Ahmed (2010) mentioned that broiler diet supplemented with 1% ground black cumin was significantly ($P < 0.05$) decreased feed intake of the birds.

Experiment conducted by Halle *et al*. (1999) narrated that the inclusion of essential oil from black cumin seed affected the feed intake of broiler chicken. The experiment was performed during high environmental temperature. This high temperature may be one of the reasons behind variation in feed intake of the experimental birds.

2.8.3 Effect of Black cumin on FCR

Al-Harthi (2004) reported that dietary Black cumin was significantly ($P<0.05$) improved feed conversion ratio by 16.2% of growing broiler chickens compared with the control groups. Supplementation of 0.15% *N. sativa* in broiler chickens diet was given the better result on feed efficiency.

Majeed *et al*. (2010); Amad *et al*. (2013) and Saeid *et al*. (2013) reported that feed conversion ratio in broiler chicken was not significantly influenced by the dietary supplementation of Black Cumin Seed.

Durrani *et al*. (2007) conducted an experiment with 160 broiler chicks where four experimental diets designed as control (A), 2% Black cumin (B), 3% Black cumin (C) and 4% Black cumin seed (D) for a period of 35 days. It is found that group D which received 4% *N. sativa* seed in the feed had a significantly ($P<0.05$) better feed conversion ratio than other groups. Mean feed efficiency was 2.68, 2.16, 2.08 and 1.93 respectively for the following A, B, C and D experimental group.

2.8.4 Effect of Black cumin on immune organs

Toghyani *et al*. (2010) recorded that day-old Ross 308 male broilers supplemented with dietary treatments consisted of the basal diet as control, 2% and 4% black cumin seed and 4% and 8% peppermint. The result showed a marked ($P < 0.05$) increase in the weight of lymphoid organs at 42 days in the black cumin supplemented groups compared with control and other treated groups.

Sideeg (2000) mentioned that birds supplemented with dietary 0.25% *Nigella sativa* showed significantly higher value of spleen weight than the birds supplemented with no *N. sativa* seed. On the other hand, It had also significant effect on Bursa weight

(P<0.01) where 0.25% level supplemented birds showed significantly lower value of Bursa weight than other groups.

2.8.5 Effect of Black cumin on internal organs

Mansoori *et al*. (2006) evaluated the nutritional value of cumin seed meal as broiler feedstuff. 288 Male broiler chicks were fed diets containing 0%, 2.5%, 5% BCSM with and without polyethylene glycol enzyme for a period of 28 days. The result showed that inclusion of BCSM in broiler diets was increase relative weight of gizzard significantly (P<0.01) when the inclusion level of BCSM in the diet was increased.

Durrani *et al*. (2007) reported that dietary supplementation of Black cumin seed at 2%, 3% and 4% inclusion level had significantly higher mean liver weight in the supplemented groups compared with that of control group. But mean weight of gizzard, intestine and abdominal fat were not significantly (P>0.05) differ among all groups.

2.8.6 Effect of Black cumin on carcass quality

Amad *et al*. (2013) performed an experiment with three different levels of black cumin seeds powder was added to the basal diet at control 0% (Diet 1), 1.5% (Diet 2), 2.5% (Diet 3) and 3.5% (Diet4). Result showed no significant effects of dietary BCS supplementation on the dressing percentage of the birds.

Sorwar *et al*. (2016) concluded that dietary supplementation with Black cumin seed powder with drinking water did not show any significant difference on dressing percentages of the birds of different feeding group.

2.8.7 Effect of Black cumin on microbial load

Hanafy and Hatam (1991) reported that broiler diets incorporated with Black cumin had beneficial improvement in weight gain due to the concentration of ethyl ether extracts of black cumin seed. Ethyl ether extracts of BC inhibits the growth of harmful intestinal bacteria such as *E. coli* and *S. aureus* which accelerated the production performance of broiler chickens.

Abd El-Hack (2018) concluded that the Black cumin oil (BCO) showed highest antibacterial effect against *Escherichia coli* and *Salmonella enterica*. The bacterial populations, total bacterial count (TBC), coliform, Salmonella species, and *Escherichia coli* were decreased in meat type quail supplemented with Black cumin seed oil 0.5 and 1.0 BCO g/kg diet compared with the control group.

2.8.8 Effect of Black cumin on blood parameter

The findings of the experiment carried out by Jamroz and Kamel (2002) showed that birds treated with Black cumin and Thankuni extracts had no significant effect on general blood parameters. The results were near about equal in both treated and control group which was a sign of indication that there were no extraordinary side effects of these two extracts on normal physiology of the birds. But in case of treated broilers, birds show improvement of general blood parameters which may confer more nutrients transportation and better production performance of the broiler chickens.

Yatoo (2012) evaluated the effect of Fenugreek and Black Cumin Seeds as feed additives on blood biochemical profile and performance of broiler chickens. The experiment was designed with four treatment groups: control group with no feed additive (T_1), 1% fenugreek (T_2), 1% black cumin (T_3) and 0.5% each of fenugreek and black cumin (T_4). They found that hemoglobin (g/dl), PCV (%), total protein (g/dl) and albumin (g/dl) level were higher ($P<0.05$) in T_3 and T_4 group as compared to T_1 and T_2 groups respectively.

2.8.9 Effect of Black cumin on serum biochemical properties

Khadr and Abdel-Fattah (2006) found that addition of *Nigella sativa* seed as supplementation with diets of broiler chickens at 1% and 2% level had significant difference ($p<0.05$) on serum glucose and cholesterol level. The results showed a significant decrease ($p<0.05$) in serum glucose and cholesterol concentration by dietary supplementation of black cumin seed 1% and 2% compared to the control group.

Akhtar *et al*. (2003) found that addition of 0, 0.5, 1 and 1.5% *N. sativa* seed powder with commercial layer ration significantly reduce yolk cholesterol concentration. The level of serum triglycerides, low density lipoprotein, cholesterol and total cholesterol were also reduced and on the other side the level of high density lipoprotein cholesterol was significantly increased by the inclusion of Black cumin seed powder with layer ration.

El-Dakhakhny (2000) mentioned that *Nigella sativa* seed oil showed a beneficial effect on the serum lipid pattern causing a decrease in serum total cholesterol, low density lipoprotein, triglycerides and a significant elevation of serum high density lipoprotein level.

2.8.10 Effect of Black cumin on survivability

Jahan *et al*. (2015) performed an experiment in broiler chicken with dietary supplementation of 0, 0.5, 1.0 and 1.5% of Black Cumin Seed Meal. They mentioned that only one bird from control and one from 0.5% dietary BCSM group died during the whole working period. They also concluded that supplementation of BCSM at 1.5% had the highest significant effect ($P<0.01$) on profit (Tk/kg/bird) and lower mortality.

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 Cobb 500 straight run commercial broiler chicks from a single hatch for a period of 28 days from **19th February to 19th March, 2019** to assess the feasibility of using Papaya leaf and Black cumin in commercial broiler diet on growth performance, meat yield characteristics and immune status of broiler chickens. This research helps to make a conclusion about Papaya leaf and Black cumin as the alternative of antibiotic.

3.2 Collection of experimental broiler chickens

A total of 150 day-old Cobb 500 commercial broiler chicks were collected from Kazi hatchery, Gazipur, Dhaka.

3.3 Experimental materials

The collected chicks were carried to the university poultry farm at midnight. They were kept in electric brooders equally for 4 days by maintaining standard brooding protocol. During brooding time only basal diet was given. Treatments were not used at that period. After four 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 Papaya leaf and Black cumin. 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

T_1 = Basal diets (control)

T_2 = Basal diets + Antibiotics

T_3 = 2% Papaya Leaf Meal (2 kg of Papaya Leaf Meal/100 kg of the feeds)

T_4 = 1% Black Cumin Seed (1 kg of Black Cumin Seed/100 kg of the feeds)

T_5 = 1% PLM & 1% BCS (1 kg PLM & 1 kg Black Cumin seed/100 kg of the feeds)

Table 1. Layout of the experiment

Treatments with Replication (10 birds/Replication)			No. of birds
T_3R_3 (n=10)	T_1R_2 (n=10)	T_4R_3 (n=10)	30
T_4R_1 (n=10)	T_2R_2 (n=10)	T_5R_2 (n=10)	30
T_5R_3 (n=10)	T_1R_3 (n=10)	T_3R_1 (n=10)	30
T_2R_3 (n=10)	T_4R_2 (n=10)	T_2R_1 (n=10)	30
T_1R_1 (n=10)	T_3R_2 (n=10)	T_5R_1 (n=10)	30
		Grand Total	**150**

3.5 Preparation of experimental house

Proper cleaning and washing of the experimental house was performed by 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 $1m^2/10$ birds.

3.6 Experimental diets

The chicks were supplemented with starter and grower commercial Kazi broiler feed which were purchased from the market.

Table 2. Name and minimum percentage of ingredients present in Starter and Grower ration.

Name of ingredients in Starter ration	Minimum percentage Present
Protein	21.0 %
Fat	6.0%
Fiber	5.0%
Ash	8.0%
Lysine	1.20%
Methionine	0.49%

Table 2 (cont'd).

Cysteine	0.40%
Threonine	0.79%
Arginine	1.26%
Name of ingredients in Grower ration	**Minimum percentage Present**
Protein	19.0 %
Fat	6.0%
fiber	5.0%
Ash	8.0%
Lysine	1.10%
Methionine	0.47%
Cysteine	0.39%
Tryptophan	0.18%
Threonine	0.75%
Arginine	1.18%

Feed were supplied 4 times daily by following Cobb 500 Manual and *ad libitum* drinking water supplied 2 times daily.

3.6.1 Collection of Papaya Leaf and Black Cumin

Papaya leaf and Black cumin was mixed in commercial basal diets according to treatment level. Good quality papaya leaves were harvested from local papaya garden and Black cumin from the local market. After collection, papaya leaves were washed properly with fresh water and air dried properly under shed for 7 days. Then dried leaves were crushed in wooden mortar and pestle. After that veins of leaves were separated by using sieve. Black cumin were also washed with fresh drinking water and dried properly. Both the ingredients were contained in air tight container until used. Photographs of Papaya leaf and Black cumin were given in Plate 1.

Plate 1: Papaya Leaf and Black Cumin seed

Table 3. Nutritional composition of Papaya Leaf

Nutrient Component	Amount per 100 gram
Calories	74 g
Water	77.5 g
Protein	7 g
Fat	2 g
Total carbohydrates	11.3 g
Fiber	1.8 g
Ash	2.2 g
Calcium	344 mg
Phosphorous	142 mg
Iron	0.8 mg
Sodium	16 mg
Potassium	625 mg
β-carotene	11,565 ug
Thiamine	0.09 mg
Riboflavin	0.48 mg
Niacin	2.1 mg
Ascorbic acid	140 mg
Vitamin E	136 mg

(**Source:** Arshad *et al*., 2012)

Table 4. Nutritional composition of Black Cumin

Nutrient Component	Amount
Proximate composition (%)	
Moisture	6.46 ± 0.17
Crude Protein	22.80 ± 0.60
Crude Fat	31.16 ± 0.82
Crude Fiber	6.03 ± 0.16
Ash	4.20 ± 0.11
NFE	29.36 ± 0.78
Mineral (mg/100gm)	
Potassium	808 ± 6.61
Calcium	570 ± 21.5
Phosphorus	543 ± 10.04
Magnesium	265 ± 4.87
Sodium	17.6 ± 2.21
Iron	9.70 ± 0.65
Manganese	8.53 ± 0.11

(**Source:** Sultan *et al*., 2009)

3.7 Management procedures

Feed intake and body weight were recorded 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 management of baby chicks

The experiment was conducted during **19th February to 19th March, 2019**. The average temperature was near about 29ºC and the RH was 73% in the poultry house. Common brooding was done for four days. After four 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 to morning. 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 22 hours light and 2 hours dark 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. Footbath was used in front of the shed door to avoid the risk of pathogen transmission. To prevent diseases in the farm, chicks were vaccinated as per standard vaccination schedule. Proper hygienic and sanitation program was undertaken in the farm and its premises. Several vitamins like Vitamin B-Complex, Vitamin-ADEK, Vitamin-C, Calcium and electrolytes were supplied to the birds.

3.7.7 Vaccination

The vaccines were collected from medicine shop (Ceva Company) and administered to the experimental birds according to the standard vaccination schedule. The vaccination schedule is shown in Table 5.

Table 5. vaccination schedule

Age of birds	Name of Disease	Name of vaccine	Route of administration
3 days	IB + ND	MA-5 + Clone-30	One drop in each eye
9 days	Gumboro	G-228E (inactivated)	Drinking Water
17 days	Gumboro	G-228E (inactivated) booster dose	Drinking Water
21 days	IB + ND	MA-5 + Clone-30	Drinking Water

3.7.8 Proper 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.

3.7.9 Sanitation

Strict sanitary measures were taken during the experimental period. Disinfectant (Virkon) was used to disinfect the feeders and drinkers and the house also.

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 each 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 glucose and cholesterol level, hemoglobin, RBC, WBC, PCV level and other concentration. Feces sample was collected to measure microbial load in the gut.

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:

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

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:

Three 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 gizzard content. 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 ethylenediamine tetraacetic acid (EDTA) tubes from the wing veins. Samples were transferred to the laboratory for analysis within 1 hour of collection. Glucose, Cholesterol and CBC was measured from Rainbow diagnosis Centre, Dhanmondi, Dhaka by maintaining standard protocol.

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 Feed intake:

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 conversion ratio:

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 Weekly Feed Consumption (FC)

The mean weekly feed consumption (g) of broiler chicks at the end of 4^{th} week in the dietary group T_1, T_2, T_3, T_4 and T_5 were 854.57±11.364, 836.47±17.086, 838.93±10.228, 816.17±4.899 and 826.53±10.135 accordingly. The overall mean feed consumption of different groups showed that there was no significant difference ($P>0.05$) among control (T_1), antibiotic (T_2), 2% Papaya leaf meal (T_3), 1% Black cumin seed (T_4) and 1% of each papaya leaf meal and black cumin seed (T_5) supplementation group (Table 6 and Figure 1).

These results are in agreement with the findings of Bolu *et al*. (2009) and Guler *et al*. (2006) reported that dietary Papaya leaf and Black cumin incorporation respectively had non-significant effect on weekly feed consumption in broiler chickens at different inclusion level compared to control group. These results are contradictory with the findings of Abbas and Ahmed (2010) mentioned that broiler diet supplemented with ground black cumin was significantly ($P< 0.05$) decreased feed intake of the birds.

Table 6. Effects of feeding different level of Papaya leaf meal, Black cumin seed and antibiotic on feed consumption (g/bird) of broiler chickens at different week.

Treatment	1st week FC	2nd week FC	3rd week FC	4th week FC
T_1	175.03±1.244	435.03±8.490	747.97±8.490	854.57±11.364
T_2	174.90±.737	447.50±7.337	734.83±6.938	836.47±17.086
T_3	176.50±.451	451.27±3.788	731.73±3.788	838.93±10.228
T_4	174.40±1.343	435.87±7.680	748.80±6.208	816.17±4.899
T_5	175.00±.723	452.70±2.250	729.80±1.750	826.53±10.135
Mean ± SE	175.17^{NS}±.409	444.47^{NS} ±3.151	738.63^{NS} ±3.123	834.53^{NS} ±5.517

Here, T_1=(Control), T_2=(Antibiotic), T_3=(2% Papaya leaf meal supplementation), T_4=(1% Black cumin seed supplementation) and T_5=(1% Papaya leaf meal & 1% Black cumin seed supplementation). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

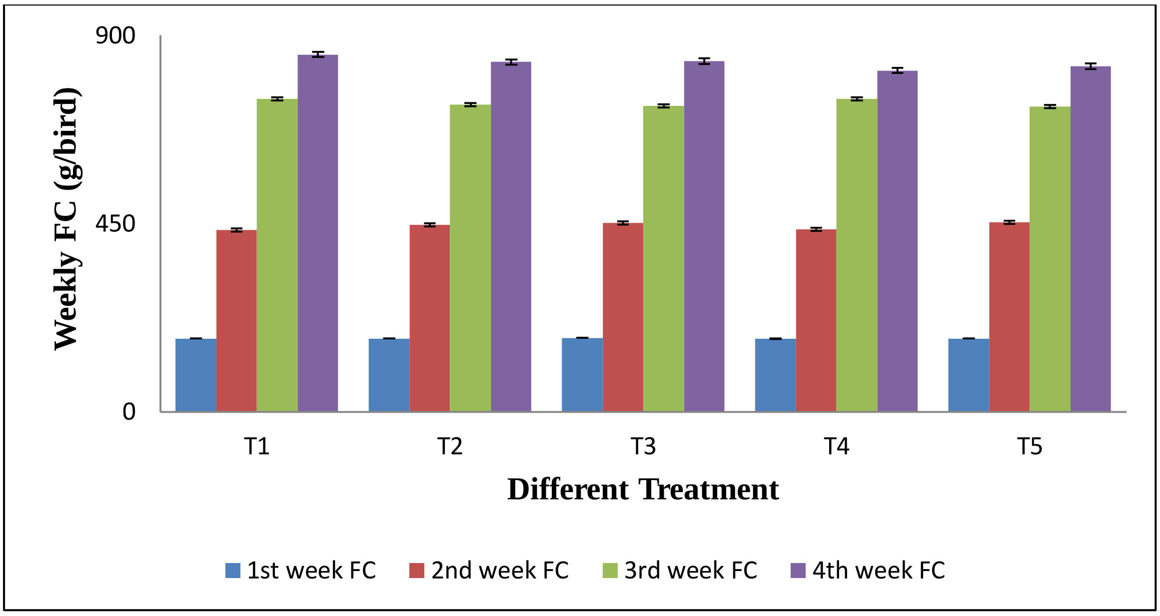

Figure 1. Effects of dietary supplementation of Papaya leaf meal, Black cumin seed and antibiotic on feed consumption (g/bird) of broiler chickens at different week.

4.1.2 Weekly Body weight gains

The mean body weight gains (g) of broiler chicken at the end of 4^{th} week in different groups were 459.87±14.410, 477.96±16.464, 474.60±8.314, 460.53±13.248 and 442.00±7.312 respectively. The overall mean body weight gain of different groups showed that there was no significant ($P>0.05$) difference in groups T_3, T_4 and T_5 compared to control (T_1) and antibiotic (T_2) group (Table 7 and Figure 2).

These results are in agreement with those of previous researcher's findings which showed that dietary supplementation of papaya leaf had no significant difference ($P>0.05$) on average daily body weight gain (Fouzder, 2010; Unigwe *et al.*, 2014). But in terms of dietary Black cumin supplementation these results are contradictory with the findings of previous researchers (Al-Betawi and El-Ghousein, 2008; Ziad *et al.*, 2008; Khan *et al.*, 2012) reported that birds fed with black cumin seed had significantly higher body weight gain than compared to control group.

Table 7. Effects of feeding different level of Papaya leaf meal, Black cumin seed and antibiotic on body weight gain (BWG) (g/bird) of broiler chickens at different week.

Treatment	1^{st} week BWG	2^{nd} week BWG	3^{rd} week BWG	4^{th} week BWG
T_1	198.10±3.853	354.13±2.188	499.80±20.850	459.87±14.410
T_2	196.93±1.224	352.57±6.328	513.88±2.083	477.96±16.464
T_3	204.10±2.084	345.50±5.254	535.37±16.871	474.60±8.314
T_4	191.57±2.233	351.00±9.831	529.67±10.798	460.53±13.248
T_5	203.43±.491	356.07±1.970	518.77±3.518	442.00±7.312
Mean ± SE	198.83^{NS}±1.501	351.85^{NS}±2.422	519.50^{NS}±5.953	462.99^{NS}±5.817

Here, T_1=(Control), T_2=(Antibiotic), T_3=(2% Papaya leaf meal supplementation), T_4=(1% Black cumin seed supplementation) and T_5=(1% Papaya leaf meal & 1% Black cumin seed supplementation). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

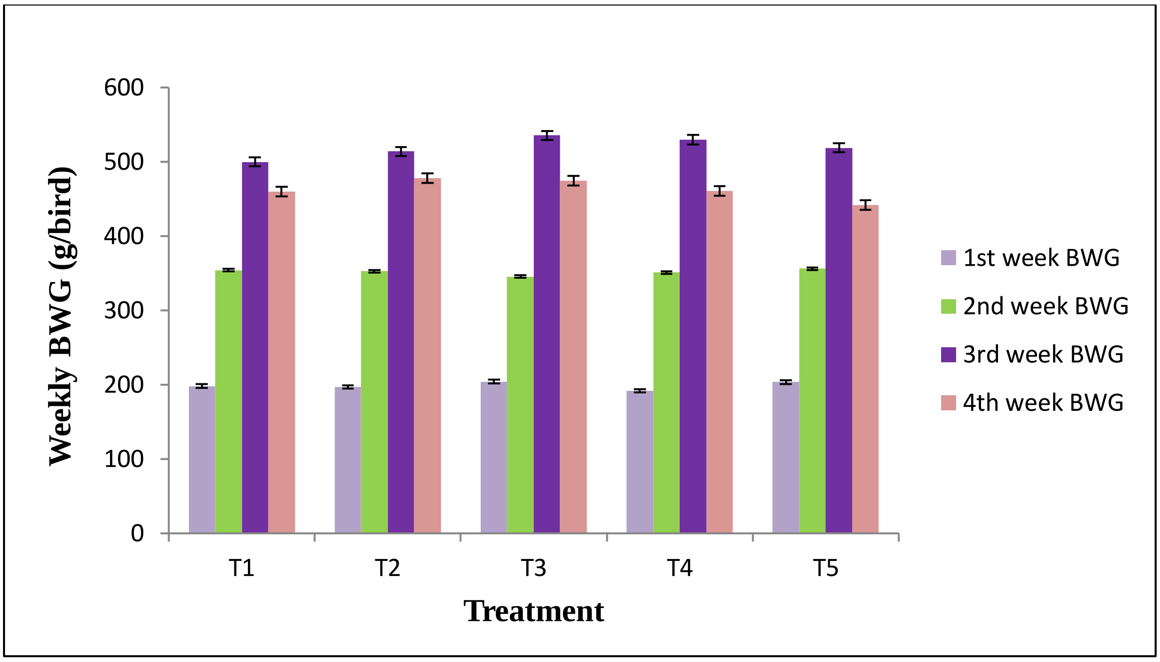

Figure 2. Effects of dietary supplementation of Papaya leaf meal, Black cumin seed and antibiotic on body weight gain (g/bird) of broiler chickens at different week.

4.1.3 Weekly Feed Conversion Ratio (FCR)

The mean weekly FCR of broiler chicks at the end of 4^{th} week in different groups were 1.86±.081, 1.75±.070, 1.77±.029, 1.77±.041 and 1.87±.053 respectively. 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 (Table 8).

These results are coincided with the findings of previous researchers (Majeed *et al*., 2010; Amad *et al*., 2013; Saeid *et al*., 2013; Unigwe *et al*., 2014) who concluded that dietary papaya leaf and black cumin supplementation respectively had non-significant effect on weekly feed conversion ratio (FCR). On the other hand, Mahejabin *et al*. (2015) observed that supplementation of 1 ml papaya leaf extract per liter of drinking water had significantly increased (P<0.05) body weight and the treatment group caused better improvement in the feed conversion ratio as compared to that of control group.

Table 8. Effects of feeding different level of Papaya leaf meal, Black cumin seed and antibiotic on FCR of broiler chickens at different week.

Treatment	1^{st} week FCR	2^{nd} week FCR	3^{rd} week FCR	4^{th} week FCR
T_1	0.88±.011	1.23±.020	1.50±.063	1.86±.081
T_2	0.89±.002	1.27±.007	1.43±.017	1.75±.070
T_3	0.86±.008	1.31±.021	1.37±.040	1.77±.029
T_4	0.91±.015	1.24±.028	1.42±.040	1.77±.041
T_5	0.86±.003	1.27±.007	1.41±.013	1.87±.053
Mean ±SE	0.88^{NS}±.006	1.26^{NS}±.010	1.42^{NS}±.019	1.81^{NS}±.026

Here, T_1=(Control), T_2=(Antibiotic), T_3=(2% Papaya leaf meal supplementation), T_4=(1% Black cumin seed supplementation) and T_5=(1% Papaya leaf meal & 1% Black cumin seed supplementation). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

4.1.4 Feed Consumption (FC)

Different treatment groups (Table 9) showed no significant (P>0.05) differences in feed consumption (g) of broiler chicken. Control group consumed higher amount of feed (2212.60±11.320) and 1% Black cumin seed (T_4) treated group consumed lower amount of feed (2175.23±7.043).

These results are in agreement with the findings of Bolu *et al*. (2009) who recorded that dietary supplementation of Papaya leaf in broiler diets had no significant effect (p>0.05) on feed intake and Guler *et al*. (2006) also mentioned that supplementation of black cumin seed in broiler diets had non-significant (P>0.05) differences on feed consumption of broiler chicken. In contrast, other researchers (Rumokoy *et al*., 2016) concluded that dietary papaya extract supplementation had higher significant (P<0.01) interaction on feed intake and Halle *et al*. (1999) narrated that the inclusion of essential oil from black cumin seed significantly affected the feed intake of broiler chicken. However, Abbas and Ahmed (2010) assessed that 1% ground black cumin was significantly (P< 0.05) decreased feed intake of the broiler chicken.

Table 9. Production index of broiler chicken supplemented with Papaya leaf meal, Black cumin seed and antibiotic.

Treatment	T_1	T_2	T_3	T_4	T_5	Mean ± SE
Final Live Weight (g/Broiler)	1511.90±10.106	1541.33±22.246	1559.57±26.404	1532.77±27.163	1520.27±10.581	1533.17^{NS}±9.000
FC (g)	2212.60±11.320	2193.70±17.495	2198.43±10.449	2175.23±7.043	2184.03±10.518	2192.80^{NS}±5.628
FCR	1.46±.006	1.42±.020	1.41±.026	1.42±.022	1.44±.016	1.43^{NS}±.009
DP% (Skinless)	70.93±2.637	69.47±2.115	72.34±1.131	71.09±1.947	66.31±1.877	70.03^{NS}±.937
Survivability (%)	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100^{NS}±0.00

Here, T_1=(Control), T_2=(Antibiotic), T_3=(2% Papaya leaf meal supplementation), T_4=(1% Black cumin seed supplementation) and T_5=(1% Papaya leaf meal & 1% Black cumin seed supplementation). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts don’t differ (P>0.05) significantly
- ✓ SE= Standard Error

4.1.5 Final Body Weight

Data presented in Table (9) showed that the effect of treatments on final live weight (gram per broiler chicken) was not 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 1511.90±10.106, 1541.33±22.246, 1559.57±26.404, 1532.77±27.163 and 1520.27±10.581 respectively. The highest result was found in T_3 (1559.57±26.404) and lowest result was in T_1 (1511.90±10.106) group. However, Final live weight of broiler fed with Papaya leaf meal diet increased but that was not significant (P>0.05) compared to that of the control and antibiotic treated groups. The final live weight of T_4 and T_5 group was also higher than the control group (T_1).

These results are in agreement with those obtained by Unigwe *et al*. (2014) who found that there was no significant difference (P>0.05) of dietary Papaya leaf supplementation on average final body weight of broiler chicken. But the weight performance was numerically increased as the inclusion levels of Papaya leaf meal increased. In addition, these results are in contradictory with the findings of Ebenebe *et al*. (2011) who mentioned that increased weight gain associated with the supplementation of Papaya Leaf Meal in the diet of broiler chickens compared to that of control group.

The result shows live weight of T_4 (1532.77±27.163) was insignificantly (P>0.05) higher than the T_1 (1511.90±10.106) group. It is contradictory with those of previous researchers (Al-Betawi and El-Ghousein, 2008; Ziad *et al*., 2008; Jahan *et al*., 2015) who reported that dietary supplementation of Black Cumin Seed Meal significantly (P<0.05) improved live weight of broiler chicken compared with the control group.

4.1.6 Feed Conversion Ratio (FCR)

Data presented in Table (9) showed that the effect of treatments on feed conversion ratio (FCR) was not significant (P>0.05) in broiler chicken. The lower FCR (1.41±.026) was found in birds supplemented with 2% papaya leaf meal (T_3) and higher FCR (1.46±.006) in the control group (T_1). However, Feed conversion ratio of T_4 (1.42±.022) and T_5 (1.44±.016) was lower than the control group.

These results are in agreement with the findings of Unigwe *et al*. (2014) who concluded that feed conversion ratios (FCR) in broiler supplemented with dried papaya leaf meal (PLM) at 5%, 10% and 15% inclusion level were not significantly different ($P>0.05$) among all treatment groups and compared to that of control group.

In addition, the findings of previous researchers (Majeed *et al*., 2010; Amad *et al*., 2013; Saeid *et al*., 2013) reported that feed conversion ratio in broiler chicken was not significantly influenced by the dietary supplementation of Black Cumin Seed.

These results are in contradictory with those of previous researchers (Onyimonyi and Onu, 2009; Wanker *et al*., 2009; Mahejabin *et al*., 2015) who recorded that birds fed dietary papaya leaf meal had significant ($P<0.05$) increase in feed conversion ratio. In terms of Black cumin seed (BCS) supplementation, Al-Harthi (2004) and Durrani *et al*. (2007) reported that feed conversion ratio (FCR) was significantly improved by BCS supplementation in broiler chicken.

4.1.7 Dressing Percentage (DP)

The dressing percentage of broiler chicks at 28^{th} days presented in Table (9) 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 2% papaya leaf meal group (T_3) had a greater ($P>0.05$) carcass quality and dressing percentage (72.34±1.131) compared with the antibiotic (69.47±2.115) and control (70.93±2.637) group. Dressing percentage of 1% Black cumin seed supplemented group T_4 was 71.09±1.947 and 1% of each papaya leaf meal and black cumin seed supplemented group T_5 was 66.31±1.877.

These results of average dressing percentage (DP) are in agreement with Sorwar *et al*. (2016) reported that broiler chicken supplemented with papaya leaf and Black cumin seed powder with drinking water did not show any significant difference on the dressing percentages of the birds of different feeding group. Further, Amad *et al*. (2013) reported that three different levels of black cumin seeds powder was added to the basal diet showed no significant effect on the dressing percentage of the birds.

In contrast, the results of previous researchers (Adeyemo and Akanmu, 2012; Abdalla *et al*., 2013) concluded that addition of dried papaya leaves powder significantly ($P\leq0.05$) increased dressing percentage of the birds compared with the value obtained from control group. However, Ebrahimi *et al*. (2015) reported that supplementation of papaya leaf in broiler diet significantly ($p<0.05$) reduced the lipid peroxidation of broiler breast meat compared to that of control group.

4.1.8 Survivability

Data presented in Table (9) showed that dietary supplementation of Papaya leaf meal and Black cumin seed had no adverse effect on the survivability rate (100±0.00) of broiler chicken. Mortality rate was 0% during the time of research period.

These results are similar with the findings of Hema *et al.* (2015) who assessed that livability percentage (94 ± 0.0) was better in T_6 group supplemented with 0.1% Papaya leaves powder as compared to other coccidiosis infected groups. Jahan *et al.* (2015) concluded that supplementation of BCSM at 1.5% had the highest significant effect ($P<0.01$) on lower mortality which confer better survivability.

4.2 Serum Biochemical Parameters

4.2.1 Glucose

Effects of dietary supplementation of Papaya leaf meal and Black cumin seed on concentration of glucose of broiler chickens are presented in Table (10) and Figure (3). Dietary incorporation of Papaya leaf meal and Black cumin seed had no significant ($P>0.05$) difference among the treatment group. The lowest amount (192.11±1.467) of plasma glucose found in T_4 (1% Black cumin seed) and supplementation of 2%Papaya leaf meal (T_3) also showed lower glucose level (196.11±2.118) compared to that of antibiotic and control group. But there was no statistical difference among the values.

The present study shows no significant difference on glucose concentration among all group which is contradictory with those of previous researchers (El-Dakhakhny, 2000; Akhtar *et al*., 2003; Khadr and Abdel-Fattah, 2006; Juárez-Rojop *et al*., 2012) who reported that dietary Papaya leaf and Black cumin supplementation significantly decrease serum glucose level. The decrease in serum glucose may be firstly, attributed to the stimulatory effect on beta cell function with consequent increase in serum insulin level (Fararh *et al*., 2002; Rchid *et al*., 2004), Secondly, decrease in hepatic gluconeogenesis through the action of thymoquinone (Fararh *et al*., 2004) and thirdly, by decreasing oxidative stress and preserving pancreatic beta-cell integrity (Kanter *et al*., 2004).

Table 10. Effects of Papaya leaf meal, Black cumin seed and antibiotic supplementation on serum biochemical level of different broiler chicken under different treatment.

Parameters	T_1	T_2	T_3	T_4	T_5	Mean ± SE
Glucose mg/dl	199.11±3.653	202.11±5.181	196.11±2.118	192.11±1.467	195.00±2.062	196.89^{NS}±1.458
Cholesterol mg/dl	176.00±3.859	172.11±4.718	169.11±5.308	170.67±5.207	173.22±5.387	172.22^{NS}±2.131

Here, T_1=(Control), T_2=(Antibiotic), T_3=(2% Papaya leaf meal supplementation), T_4=(1% Black cumin seed supplementation) and T_5=(1% Papaya leaf meal & 1% Black cumin seed supplementation). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts don’t differ (P>0.05) significantly
- ✓ SE= Standard Error

4.2.2 Cholesterol

Total cholesterol concentration (mg/dl) in the serum of different groups ranged from 169.11±5.308 to 176.00±3.859. Statistical analysis revealed that non-significant ($P>0.05$) difference among the groups (Table 10 and Figure 3). The lower amount (169.11±5.308) of cholesterol found in 2% Papaya leaf meal supplementation group (T_3) comparable to antibiotic and control group but there was no statistical difference. These results are in agreement with the findings of previous researchers (Juárez-Rojop *et al.*, 2012; Zetina-Esquivel *et al.*, 2015) reported that *C. papaya* supplementation decrease serum cholesterol level in hypercholesterolemic rats and diabetic rat. El-Dakhakhny (2000) mentioned that *Nigella sativa* seed oil showed a beneficial effect on the serum lipid pattern causing a decrease in serum total cholesterol, low density lipoprotein, triglycerides levels.

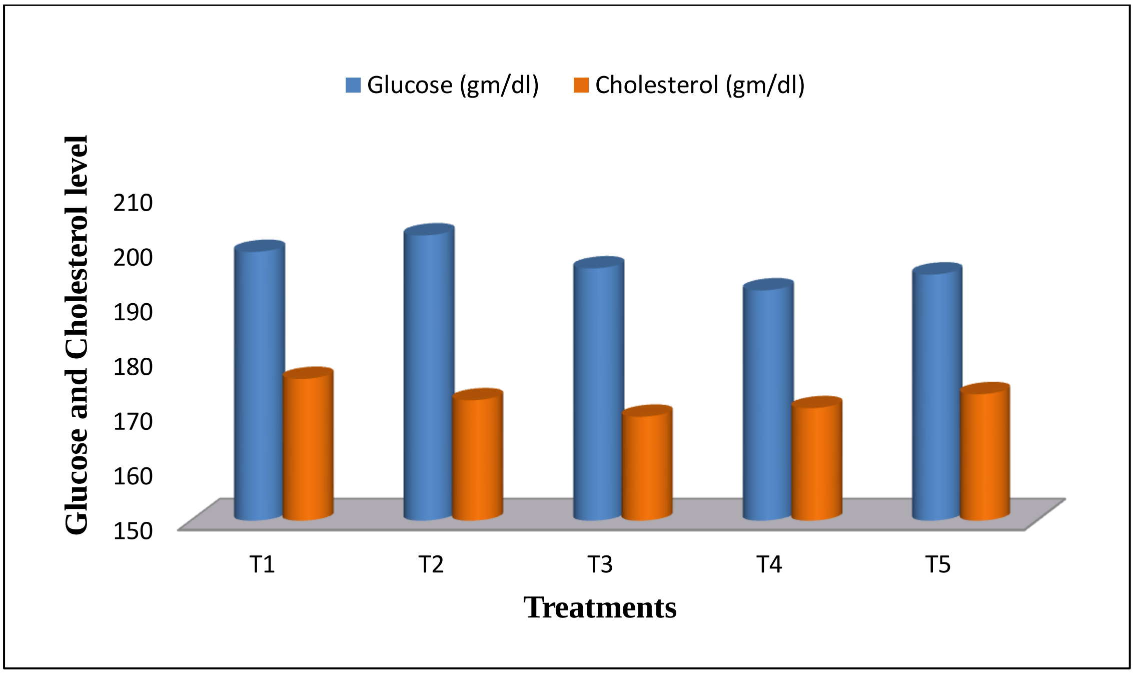

Figure 3. Effects of dietary supplementation of Papaya leaf meal, Black cumin seed and antibiotic on serum biochemical level of broiler chickens under different treatment.

4.3 Internal organs

4.3.1 Relative giblet weight (liver, gizzard and heart)

The relative weight of liver (g) of broiler chicks in dietary group T_1, T_2, T_3, T_4 and T_5 were 35.33±1.633, 35.00±2.327, 37.61±1.369, 36.78±2.172 and 36.89±1.457 respectively. The highest result was obtained in T_3 (2% Papaya leaf meal) and lowest was in T_2 group. However, there was no significant (P>0.05) difference in the relative weight of liver among the groups (Table 11).

The comparative weight of gizzard (g) presented in Table (11) did not show any 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 34.78±.846, 34.67±1.607, 34.89±1.195, 35.67±1.323 and 35.56±1.396 respectively.

The relative weight of heart (g) of broiler chicken in the dietary groups were T_1 (7.61±.217), T_2 (7.28±.409), T_3 (7.61±.341), T_4 (7.72±.313) and T_5 (7.67±.204). The results shows that there was no significant (P>0.05) difference of values among groups (Table 11).

These results are in agreement with the findings of Sorwar *et al*. (2016) who concluded that broiler chicken supplemented with Papaya leaf and Black cumin seed powder did not show any difference between the relative weight of liver, gizzard and pancreas of the birds of different feeding group. In contrast, Kamal *et al*. (2015) reported that incorporation of Papaya leaves had significant differences in weight gain of heart, liver and pancreas. Mansoori *et al*. (2006) mentioned that inclusion of Black Cumin Seed Meal (BCSM) in broiler diets was increase relative weight of gizzard significantly (P<0.01) when the inclusion level of BCSM in the diet was increased.

4.3.2 Intestine weight

Data presented in Table (11) showed that the relative weight of intestine (g) had no significant (P>0.05) difference among all treatment groups. The relative weight of intestine (g) of broiler chicken in the dietary groups were T_1 (70.78±3.117), T_2 (74.78±5.790), T_3 (75.78±3.943), T_4 (71.78±2.425) and T_5 (74.22±2.314). The results of this present study are coincided with that of Durrani *et al*. (2007) and Sorwar *et al*. (2016) reported that dietary papaya leaf and black cumin supplementation had no significant difference on intestine weight.

Table 11. Effects of dietary supplementation of Papaya leaf meal, Black cumin seed and antibiotic on Liver, Gizzard, Heart and Intestine weight of different treatment.

Parameters	T_1	T_2	T_3	T_4	T_5	Mean ± SE
Liver weight (g)	35.33±1.633	35.00±2.327	37.61±1.369	36.78±2.172	36.89±1.457	36.32^{NS}±.796
Gizzard weight (g)	34.78±.846	34.67±1.607	34.89±1.195	35.67±1.323	35.56±1.396	35.11^{NS}±.557
Heart weight (g)	7.61±.217	7.28±.409	7.61±.341	7.72±.313	7.67±.204	7.58^{NS}±.133
Intestine weight (g)	70.78±3.117	74.78±5.790	75.78±3.943	71.78±2.425	74.22±2.314	73.47^{NS}±1.621

Here, T_1=(Control), T_2=(Antibiotic), T_3=(2% Papaya leaf meal supplementation), T_4=(1% Black cumin seed supplementation) and T_5=(1% Papaya leaf meal & 1% Black cumin seed supplementation). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

4.4 Immune organs

Data presented in Table (12) and Figure (4) shows the effect of dietary Papaya leaf meal and Black cumin seed supplementation on immune organs of Cobb 500 strain broiler during the period from day 0 to 28 days of age. The comparative weight of spleen (g) of broiler chicks in the dietary groups were T_1 (1.78±.121), T_2 (1.83±.144), T_3 (1.94±.294), T_4 (2.28±.169) and T_5 (1.94±.155). The highest value was found in T_4 (2.28±.169) and lowest value was in T_1 (1.78±.121). The relative weight of spleen of different groups showed that there were no significant ($P>0.05$) difference among the treatments.

The present study shows that dietary supplementation of Papaya leaf meal and Black cumin seed in broiler ration had significant ($P<0.05$) difference on bursa weight of broiler chicken (Table 12). The highest bursa weight (2.50±.408) was found in the 2% papaya leaf meal supplementation (T_3) group and lowest (1.67±.167) in the control (T_1) group.

These results reveal that supplementation of Papaya leaf meal and Black cumin seed in broiler ration improved the weight of spleen and bursa compared with the control group. But there were no statistical differences among the values. Pursuant to these results several researchers reported that Papaya leaf (Battaa *et al*., 2015; Haruna and Odunsi, 2018) and Black cumin seed (Sideeg, 2000; Toghyani *et al*., 2010) supplementation improved spleen, bursa and thymus weight compared to control group.

Papaya improves spleen, bursa and thymus index, which lead to the production of T-cells. T-cells play a major role in cell-mediated immunity (Stephen, 2007). Papaya extract also has antibacterial and anti-fungal properties (Afolabi *et al*., 2011) which may further enhance the activity of the immune system. Fu Chang *et al*. (2004); El-Kholly *et al*. (2008) and Battaa *et al*. (2015) also reported that the index of bursa, spleen and the thymus determines the immunity strength. The bigger the immunity index the stronger the immune response.

Table 12. Effects of Papaya leaf meal, Black cumin and antibiotic supplementation to broiler diet on some immune organs.

Parameters	T_1	T_2	T_3	T_4	T_5	Mean ± SE
Spleen weight (g)	1.78±.121	1.83±.144	1.94±.294	2.28±.169	1.94±.155	1.96^{NS}±.084
Bursa weight (g)	1.67^{b}±.167	2.22^{ab}±.252	2.50^{a}±.408	1.72^{ab}±.222	2.22^{ab}±.121	2.07*±.118

Here, T_1=(Control), T_2=(Antibiotic), T_3=(2% Papaya leaf meal supplementation), T_4=(1% Black cumin seed supplementation) and T_5=(1% Papaya leaf meal & 1% Black cumin seed supplementation). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts 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 ($P<0.05$)

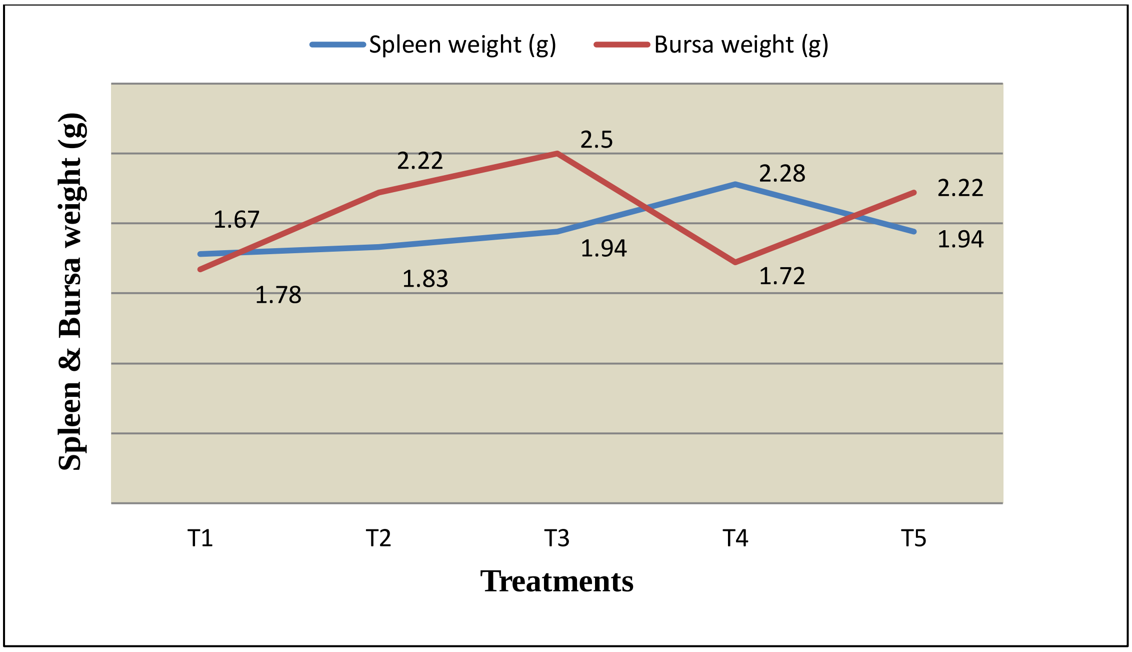

Figure 4. Effects of dietary supplementation of Papaya leaf meal, Black cumin seed and antibiotic on immune organs of broiler chickens under different treatment.

4.5 Hematological parameters

Data presented in Table (13) show the effect of dietary supplementation of Papaya leaf meal and Black cumin seed on some blood parameters of broiler chicken. Considering the treatment impact on blood components, the results indicated no significant (P>0.05) differences due to supplementation of Papaya leaf meal and Black cumin seed, except RBC, WBC, Lymphocyte and PCV which were significantly affected (P<0.05). The values of RBC, WBC, Lymphocyte and PCV had higher in T_3 (2% Papaya leaf meal), T_4 (1% Black cumin seed), T_5 (1% of each Papaya leaf meal and Black cumin seed) group compared to antibiotic (T_2) and control (T_1) group. The highest value of RBC (4.51±.156), WBC (9533.33±78.1), Lymphocyte (35.22±2.602) and PCV (42.00±1.181) were found in T_3 group. Other blood parameters like Hb, Neutrophil, Monocyte, Eosinophil, MCV, MCH and MCHC were near about equal in both treated and control group which was a sign of indication that there were no extraordinary side effects of Papaya leaf and Black cumin supplementation on normal physiology of the birds.

These results are in agreement with the findings of previous researchers who observed that dietary *Carica papaya* leaf and Black cumin supplementation had no significant differences (p>0.05) on general blood parameters (Jamroz and Kamel, 2002; Bolu *et* *al*., 2009). However, Agboola *et al*. (2018) reported that broiler chickens diet incorporated with Papaya leaf meal showed highest values of packed cell volume, red blood cell, and hemoglobin in all the papaya leaf included treatments. Yatoo (2012) mentioned that Hemoglobin (g/dl), PCV (%), total protein (g/dl) level were higher in Black cumin supplemented groups compared to that of control.

Hematological values are indirect pointers to the health of livestock (Jain, 1986; Kecceci *et al*., 1998) and are further modified by several other factors including diets (Talebi *et al*., 2005). Papaya leaf, fruits, seeds are rich in antioxidant, nutrients like carotene, vitamin C, vitamin B, flavonoids, folate, panthotenic acids and minerals such as iron, potassium and magnesium, all these are reported to promote the better functions of cardiovascular system and proper nutrient absorption (Franco *et al*., 1993; Fischer, 1998). Thus consequently improve the production performance of broiler chicken.

Table 13. Effects of Papaya leaf meal, Black cumin seed and antibiotic on blood parameters of broiler chickens.

Parameters	T_1	T_2	T_3	T_4	T_5	Mean ± SE
Hemoglobin (g/dl)	10.63±.302	10.97±.356	11.93±.567	11.53±.438	11.32±.396	11.28^{NS}±.192
RBC (million/mm^3)	3.61^{c}±.096	3.98^{b}±.083	4.51^{a}±.156	4.39^{a}±.102	4.28^{ab}±.085	4.15*±.067
WBC (10^3/mm^3)	7477.78^{c}±443.7	7766.67^{bc}±494.4	9533.33^{a}±78.1	8033.33^{bc}±334.9	8855.56^{ab}±411.6	8333.33*±198.7
Neutrophil (%)	60.00±2.421	63.67±3.240	58.89±2.226	61.11±2.348	59.44±3.163	60.62^{NS}±1.185
Lymphocyte (%)	23.56^{b}±.626	24.00^{b}±.816	35.22^{a}±2.602	31.11^{a}±2.137	32.67^{a}±1.848	29.31*±1.039
Monocyte (%)	4.22±.434	4.78±.572	4.67±.333	4.89±.512	4.33±.441	4.58^{NS}±.202
Eosinophil (%)	3.67±.441	3.56±.556	2.33±.408	3.00±.373	3.44±.503	3.20^{NS}±.210
PCV (%)	36.99^{c}±.316	35.60^{c}±.450	42.00^{a}±1.181	38.41^{bc}±1.106	41.00^{ab}±1.939	38.80*±.611
MCV (FI)	82.25±.422	84.46±1.028	82.46±.586	83.49±.762	83.29±.737	83.19^{NS}±.335
MCH (pg)	31.32±.322	31.61±1.194	30.94±.225	30.61±.248	30.09±.291	30.91^{NS}±.263
MCHC (g/dl)	32.59±.232	32.29±.610	32.73±.188	33.39±.321	32.32±.447	32.66^{NS}±.177

Here, T_1=(Control), T_2=(Antibiotic), T_3=(2% Papaya leaf meal supplementation), T_4=(1% Black cumin seed supplementation) and T_5=(1% Papaya leaf meal & 1% Black cumin seed supplementation). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts 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)

4.6 Intestinal microbial load

Data presented in Table (14) show the effect of dietary supplementation of Papaya leaf meal and Black cumin seed on microbial load (total count of *E. coli* & *Salmonella* for its beneficial effect) in broilers under different treatment. *E. coli* count significantly ($P<0.05$) decreased in T_2, T_3, T_4 and T_5 (4.10±.115, 4.43±.328, 4.80±.346 and 5.27±.203) group compared to T_1 (control) group. *Salmonella* count also had significant ($P<0.05$) decrease in T_2, T_3, T_4 and T_5 group (6.13±.406, 6.40±.416, 6.50±.115 and 6.83±.426) than control group.

The results of the present study are in agreement with the findings of previous researchers (Osato *et al.*, 1993; El-Neney *et al.*, 2013) who reported that papaya leaf supplementation significantly reduced *E. coli, Salmonella* spp, *Staphylococcus aureus* count by bacteriostatic mechanism. Abd El-Hack (2018) mentioned that the Black cumin oil showed highest antibacterial effect against *Escherichia coli* and *Salmonella enterica*.

Table 14. Effects of dietary supplementation of Papaya leaf meal, Black cumin seed and antibiotic to broiler diets on bacterial colony count.

Treatment	*E. coli* (EMB) × 10^6 (CFU/ml)	*Salmonella* (SS) × 10^6 (CFU/ml)
T_1	6.97^{a}±.145	7.67^{a}±.233
T_2	4.10^{c}±.115	6.13^{b}±.406
T_3	4.43^{c}±.328	6.40^{b}±.416
T_4	4.80^{bc}±.346	6.50^{b}±.115
T_5	5.27^{b}±.203	6.83^{ab}±.426
Mean ±SE	5.11*±.284	6.71*±.192

Here, T_1=(Control), T_2=(Antibiotic), T_3=(2% Papaya leaf meal supplementation), T_4=(1% Black cumin seed supplementation) and T_5=(1% Papaya leaf meal & 1% Black cumin seed supplementation). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts 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 ($P<0.05$)

CHAPTER 5

SUMMARY AND CONCLUSION

A feeding trial was conducted on 150 day-old Cobb 500 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 comprising of T_1 (Control), T_2 (antibiotic), T_3 (2% Papaya Leaf Meal), T_4 (1% Black Cumin Seed) and T_5 (1% of each Papaya leaf Meal & Black Cumin Seed) randomly. Treatments were replicated thrice with 10 chicks per replicate.

At 28 days of age, 45 broilers were sacrificed in halal method to evaluate the efficacy of dietary Papaya Leaf Meal, Black Cumin seed and antibiotic supplementation. The production indexes viz. feed consumption, body weight, body weight gain, FCR, dressed weight, dressing percentage, relative internal organs weight, relative immune organs weight; Hematological and blood biochemical parameters and survivability of broiler on different replication of different treatments was recorded and compared. All collected data were subjected to one-way analysis of variance using statistical package for social science (SPSS) version 16 and differences in compare means using Duncan method.

There was no significant difference (P>0.05) on the live body weight among different treatment groups. The higher body weight found in T_3 treatment group compared to other groups and values were followed in an ascending order in T_2, T_4, T_5 and T_1 group. The feed consumption, weight gain and FCR showed no significant (P>0.05) difference among all treatment groups. The better FCR found in birds fed diets with 2% Papaya leaf meal supplementation compared to that of control group. The dressing percentage in T_3 (72.34±1.131) and T_4 (71.09±1.947) group was non-significantly (P>0.05) higher than compared to control and antibiotic group.

The relative weight of liver, gizzard, heart and intestine did not show any significant difference among the treatment groups. The serum biochemical parameters viz. glucose and cholesterol concentration was measured. The result showed that level of glucose and cholesterol decreased non-significantly (P>0.05) in the Papaya Leaf meal and Black Cumin seed supplemented groups compared to antibiotic and control group.

The result indicated that effects of supplementation of Papaya Leaf Meal and Black Cumin seed in blood constituents had no significant alteration, except Red blood cell (RBC), White blood cell (WBC), Lymphocyte and Packed cell volume (PCV) which were significantly ($P<0.05$) increased compared to antibiotic and control group. The 2% Papaya Leaf Meal supplemented group (T_3) showed higher values of Red blood cell (RBC), White blood cell (WBC), lymphocytes and Packed cell volume (PCV) than T_4 and T_5 treatment group. The relative weight of spleen had no significant ($P>0.05$) difference due to Papaya Leaf Meal and Black Cumin Seed supplementation. The higher spleen weight (2.28±.169) found in T_4 (1% BCS) treatment group which was higher than T_3, T_5, T_2 and T_1 group consequently. The result showed significant ($P<0.05$) effect on the relative weight of bursa due to papaya leaf Meal and black cumin seed supplementation. The T_3 group supplemented with 2% Papaya Leaf meal diets showed higher bursa weight (2.50±.408) than compared to antibiotic and control group. The result showed that the numbers of intestinal pathogens (*E. coli* and *Salmonella*) were significantly decreased in T_2, T_3, T_4 and T_5 group compared to T_1 (control) group. However, the intestinal microbial counts in T_3 (2% Papaya Leaf Meal), T_4 (1% Black Cumin Seed) and T_5 (1% of each Papaya Leaf Meal and Black Cumin Seed) group had no statistical difference with T_2 (antibiotic) group.

On the basis of analysis of the above mentioned research findings, it can be concluded that Papaya Leaf Meal and Black Cumin Seed supplementation had very effective impact on production performance, serum biochemical and hematological parameters, immune stimulation and microbial state of broiler chicken. Birds fed 2% Papaya Leaf Meal supplemented diet achieved superior result. So, Papaya Leaf Meal and Black Cumin Seed could be used as natural feed additive for the replacement of antibiotic in broiler production. Therefore, the present study recommends that implementation of these formulations in the field aspect for commercial broiler production which is safe, sound, and economically viable and environmentally suitable for our country. However, further more experimental trials are required to assess the impact of these medicinal plants on the better quality of broiler meat production to ensure the safety of human consumption.

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APPENDICES

Appendix 1. Recorded temperature (⁰C) during experiment

Weeks	**Room temperature (⁰C)**						**Average**
	Period	7 A.M.	11 A.M.	3P.M.	7 P.M.	11 P.M.	
1st	19.02.2019-25.02.2019	29.3	30.5	31.2	30.8	28.3	30.02
2nd	26.02.2019-04.03.2019	27.7	29.8	30.5	29.7	27.9	29.12
3rd	05.03.2019-11.03.2019	26.1	29.2	28.6	27.6	26.8	27.66
4th	12.03.2019-19.03.2019	25.3	27.6	28.3	27.2	26.7	27.02

Appendix 2. Recorded relative humidity (%) during experiment

Weeks	**Relative humidity (%)**						**Average**
	Period	7 A.M.	11 A.M.	3P.M.	7 P.M.	11 P.M.	
1st	19.02.2019-25.02.2019	67	71	60	72	74	68.8
2nd	26.02.2019-04.03.2019	68	72	63	71	77	70.2
3rd	05.03.2019-11.03.2019	71	75	65	74	81	73.3
4th	12.03.2019-19.03.2019	74	79	70	78	84	77.2

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

Treatment	Replication	1st Week Feed Consumption/Bird(g)	2nd Week Feed Consumption/Bird(g)	3rd Week Feed Consumption/Bird(g)	4th Week Feed Consumption/Bird(g)
T_1	R1	176.7	440.9	742.1	870
	R2	172.6	418.3	764.7	861.3
	R3	175.8	445.9	737.1	832.4
T_2	R1	173.8	438.3	742.7	815.4
	R2	176.3	442.2	740.8	823.7
	R3	174.6	462	721	870.3
T_3	R1	177	458.5	724.5	859.3
	R2	175.6	449.6	733.4	830.4
	R3	176.9	445.7	737.3	827.1
T_4	R1	171.9	434.5	748.5	809.8
	R2	176.5	423.3	759.7	812.9
	R3	174.8	449.8	738.2	825.8
T_5	R1	176.2	454.9	728.1	835.6
	R2	175.1	448.2	733.3	837.7
	R3	173.7	455	728	806.3

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

Treatment	Replication	1st Week Body Weight/Bird(g)	2nd Week Body Weight/Bird(g)	3rd Week Body Weight/Bird(g)	4th Week Body Weight/Bird(g)
T_1	R1	203	561.2	1100.7	1532.1
	R2	190.5	541.2	1032.2	1502.4
	R3	200.8	554.3	1023.2	1501.2
T_2	R1	195	536.9	1046.63	1500.4
	R2	199.2	551.2	1067.5	1576.9
	R3	196.6	560.4	1076	1546.7
T_3	R1	202.7	546.3	1062.5	1542.4
	R2	201.4	556.8	1125.8	1611.4
	R3	208.2	545.7	1066.6	1524.9
T_4	R1	192	558.2	1085.6	1524.5
	R2	187.5	520.1	1032.3	1490.4
	R3	195.2	549.4	1098.8	1583.4
T_5	R1	204.3	564.3	1083.9	1521.4
	R2	202.6	556.9	1069.2	1501.4
	R3	203.4	557.3	1081.7	1538

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	1642.5	1079	65.69254
	R2	1453	1061	73.02134
	R3	1522	1127.5	74.08016
T_2	R1	1425	1038	72.84211
	R2	1612	1057	65.57072
	R3	1471	1029.5	69.9864
T_3	R1	1436	1049	73.05014
	R2	1523	1068	70.12475
	R3	1472	1087	73.84511
T_4	R1	1544	1068.5	69.20337
	R2	1586.5	1096	69.08289
	R3	1469	1101.5	74.98298
T_5	R1	1561.5	1014	64.93756
	R2	1668	1067	63.96882
	R3	1486	1040.5	70.02019

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

Treatment	Replication	Liver Weight (g)	Gizzard Weight (g)	Spleen Weight (g)	Bursa Weight (g)	Intestine Weight (g)	Heart Weight (g)
T_1	R1(1)	29	37	1	2	85	8
	R1(2)	32	32	2	2.5	57	7
	R1(3)	31	31	2	1	71	7.5
	R2(1)	39	34	1.5	2	69	7
	R2(2)	35	36	2	1.5	84	7
	R2(3)	32	33	2	2	76	7.5
	R3(1)	36	36	2	1.5	66	7.5
	R3(2)	44	35	1.5	1	64	9
	R3(3)	40	39	2	1.5	65	8
T_2	R1(1)	39	34	2	1.5	84	6
	R1(2)	41	30	2.5	2	100	7
	R1(3)	40	32	2	2	92	6.5
	R2(1)	28	33	1	3.5	53	7
	R2(2)	30	43	2	3	70	10
	R2(3)	29	38	2	3	62	8.5
	R3(1)	26	28	1.5	1.5	52	6.5
	R3(2)	46	40	1.5	1.5	89	7
	R3(3)	36	34	2	2	71	7
T_3	R1(1)	47	30	2	1	54	8
	R1(2)	36	34	1	1	100	6
	R1(3)	41	33	1.5	1	77	7
	R2(1)	36	35	1.5	4	80	8.5
	R2(2)	38	41	1	2.5	75	9
	R2(3)	37	37	1.5	3	78	8.5
	R3(1)	34	31	2.5	3	71	6.5
	R3(2)	34.5	39	3.5	4	74	8
	R3(3)	35	34	3	3	73	7

Appendix 6 (Cont'd)

Treatment	Replication	Liver Weight (g)	Gizzard Weight (g)	Spleen Weight (g)	Bursa Weight (g)	Intestine Weight (g)	Heart Weight (g)
T_4	R1(1)	46	30	2	2	64	8.5
	R1(2)	28	36	1.5	2	81	8
	R1(3)	37	33	2	2	73	9
	R2(1)	41	40	3	1	80	8.5
	R2(2)	43	37	2.5	1	64	8
	R2(3)	42	38	3	1.5	72	6
	R3(1)	30	30	2	1	79	7.5
	R3(2)	33	41	2	3	62	7
	R3(3)	31	36	2.5	2	71	7
T_5	R1(1)	35	37	2.5	2	73	8.5
	R1(2)	33	42	2	3	78	6.5
	R1(3)	34	40	2	2.5	75	8
	R2(1)	41	36	1	2	67	8
	R2(2)	32	30	2.5	2	65	7
	R2(3)	34	33	2	2	66	7.5
	R3(1)	38	30	1.5	2	79	7.5
	R3(2)	40	38	2	2	84	8
	R3(3)	45	34	2	2.5	81	8

Appendix 7. Serum biochemical data in different treatment groups.

Treatment	Replication	Glucose (mg/dl)	Cholesterol (mg/dl)
T_1	R1(1)	188	181
	R1(2)	206	175
	R1(3)	190	180
	R2(1)	192	157
	R2(2)	201	197
	R2(3)	218	163
	R3(1)	191	175
	R3(2)	213	183
	R3(3)	193	173
T_2	R1(1)	212	191
	R1(2)	191	163
	R1(3)	189	168
	R2(1)	203	179
	R2(2)	228	148
	R2(3)	185	182
	R3(1)	196	180
	R3(2)	192	156
	R3(3)	223	182
T_3	R1(1)	208	158
	R1(2)	193	146
	R1(3)	197	196
	R2(1)	189	176
	R2(2)	205	153
	R2(3)	195	186
	R3(1)	193	167
	R3(2)	194	175
	R3(3)	191	165
T_4	R1(1)	195	168
	R1(2)	188	170
	R1(3)	192	156
	R2(1)	186	187
	R2(2)	196	163
	R2(3)	197	182
	R3(1)	186	193
	R3(2)	193	174
	R3(3)	196	143
T_5	R1(1)	188	141
	R1(2)	198	182
	R1(3)	187	176
	R2(1)	194	198
	R2(2)	204	159
	R2(3)	189	180
	R3(1)	199	168
	R3(2)	194	182
	R3(3)	202	173

Appendix 8. Data of Complete Blood Count (CBC) under different treatment groups.

Treatment	Replication	Hb (gm/dl)	RBC (10^6/mm^3)	WBC (mm^3)	Nutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	PCV (%)	MCV (FI)	MCH (pg)	MCHC (g/dl)
T_1	R1(1)	9.4	3.2	7000	46	22	5	5	36.4	81.5	32.52	33.24
	R1(2)	10	3.5	5000	56	24	4	4	38.45	84.4	30.42	32.27
	R1(3)	9.8	3.4	6000	52	25	5	6	35.8	83.6	31.61	31.37
	R2(1)	11.4	4	7000	66	23	3	3	37.45	81.1	31.42	32.28
	R2(2)	11.2	3.6	8500	62	21	2	3	36.48	82.6	32.42	32.78
	R2(3)	11.6	4	7800	64	27	3	2	36.43	81.65	30.36	31.87
	R3(1)	11.4	3.8	8700	61	22	5	4	36.46	82.7	30.35	33.36
	R3(2)	9.6	3.3	8700	68	23	5	2	38.48	80.3	32.38	33.36
	R3(3)	11.3	3.7	8600	65	25	6	4	37	82.4	30.37	32.76
T_2	R1(1)	9.6	3.6	8700	65	29	2	6	35	85.22	30.2	30.32
	R1(2)	11.2	4	9700	61	21	5	5	35	85.12	30.22	30.2
	R1(3)	10.4	3.9	9200	63	24	6	5	36.02	84.26	31	30.24
	R2(1)	12.6	4.3	7700	72	26	3	2	35.7	85.25	28.25	33.15
	R2(2)	11.3	4.1	5700	71	24	3	1	37.7	87.26	29.24	32.15
	R2(3)	10	3.8	6700	70	23	6	2	36	86.23	29.07	31.71
	R3(1)	9.8	3.7	5800	72	22	7	3	37	77.16	39.22	35.14
	R3(2)	11.7	4.1	9000	42	25	6	4	33	87.06	31.52	33.54
	R3(3)	12.1	4.3	7400	57	22	5	4	35	82.6	35.74	34.17

Appendix 8 (Cont'd)

Treatment	Replication	Hb (gm/dl)	RBC (10^6/mm^3)	WBC (mm^3)	Nutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	PCV (%)	MCV (FI)	MCH (pg)	MCHC (g/dl)
T_3	R1(1)	11.6	4.5	9900	61	23	3	4	40	80.6	29.7	32.56
	R1(2)	12	4.3	9400	63	29	4	4	47	84.6	31.7	32.06
	R1(3)	10.6	4.1	9600	51	26	4	3	43.5	82.4	30.8	32
	R2(1)	9.6	3.9	9800	65	39	5	1	42	85.6	31.77	33.06
	R2(2)	12.7	4.4	9300	46	34	4	1	46	81.6	30.77	33.56
	R2(3)	9.8	4.2	9500	60	36	5	1	44	83.4	30.56	32.86
	R3(1)	13.7	5.1	9700	56	44	5	2	41	81.2	30.7	33.5
	R3(2)	14.5	5.3	9200	66	43	6	3	36	82.2	31.7	32.5
	R3(3)	12.9	4.8	9400	62	43	6	2	38.5	80.57	30.8	32.47
T_4	R1(1)	10.8	4.2	8900	54	44	7	2	37.2	81.2	30.7	31.5
	R1(2)	12.4	4.5	7900	48	23	3	1	37	85.2	30.7	34.5
	R1(3)	11.5	4.3	8400	55	34	5	2	36.82	83.3	31	33.6
	R2(1)	9.7	4.1	8900	63	28	6	4	37.5	85.29	30.73	34
	R2(2)	11.8	4.3	5900	65	27	3	4	36.4	87.29	31.73	34
	R2(3)	13.4	5.1	7400	66	28	6	3	36.91	84.27	30.54	32.53
	R3(1)	9.5	4.1	8900	64	37	5	4	46.4	80.29	31.03	34.3
	R3(2)	12.6	4.5	8600	66	27	3	3	36.4	83.29	29.03	33
	R3(3)	12.1	4.4	7400	69	32	6	4	41.08	81.25	30.04	33.04

Appendix 8 (Cont'd)

Treatment	Replication	Hb (gm/dl)	RBC ($10^6/mm^3$)	WBC (mm^3)	Nutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	PCV (%)	MCV (FI)	MCH (pg)	MCHC (g/dl)
T_5	R1(1)	13	4.6	9400	66	26	6	1	32	82.29	28.02	33.02
	R1(2)	9.5	3.9	9500	76	28	3	5	35	85.29	31.02	33.82
	R1(3)	11.4	4.1	9400	71	26	2	3	33	83.04	30.03	32.81
	R2(1)	9.7	4.1	9900	50	38	4	5	45	85.29	30.02	33.8
	R2(2)	10.5	4.1	9000	51	40	5	2	45.2	85.22	30.22	32.8
	R2(3)	11.6	4.2	9400	50	39	4	4	44.12	86	31	32.6
	R3(1)	11.8	4.4	9400	57	30	6	2	45.2	81.22	30.29	30
	R3(2)	12.1	4.5	6000	58	35	4	5	45.2	81.22	30.28	30.8
	R3(3)	12.3	4.5	7700	56	32	5	4	44.31	80.06	29.93	31.23

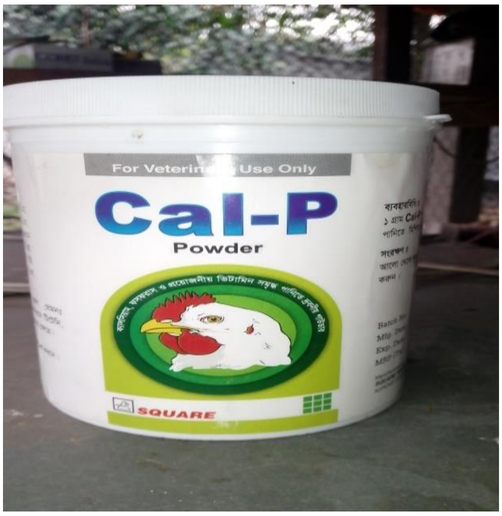
Appendix 9. Some photographs during the period of experiment conducted at SAU poultry farm.

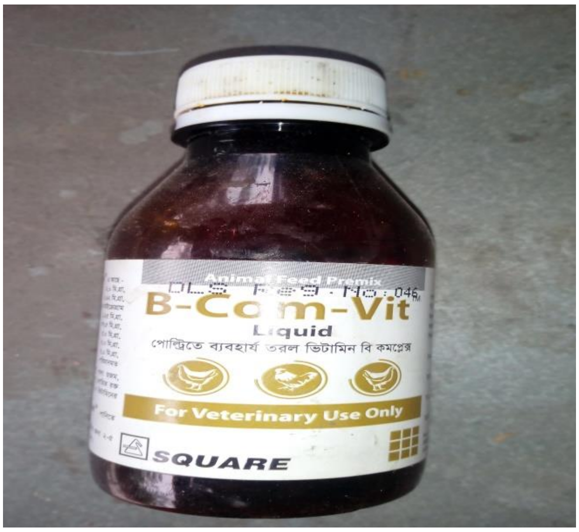
Activities performed before and after arrival of day old broiler chicks

Appendix 9. Cont'd

Monitoring of research activities by the honorable supervisor

Appendix 9. Cont'd





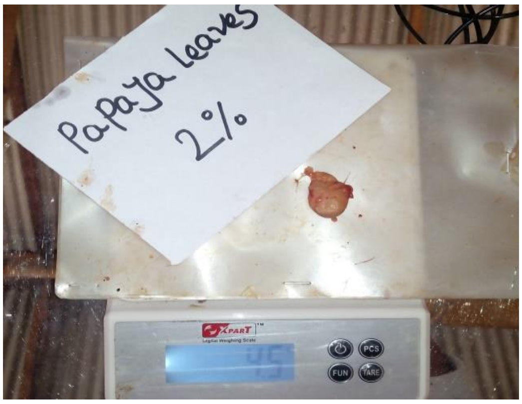
Different types of medication and vaccine used in the experiment

Appendix 9. Cont'd

Feeding and watering management of broiler chicken

Collection of blood sample at the age of 26 days of old

Appendix 9. Cont'd

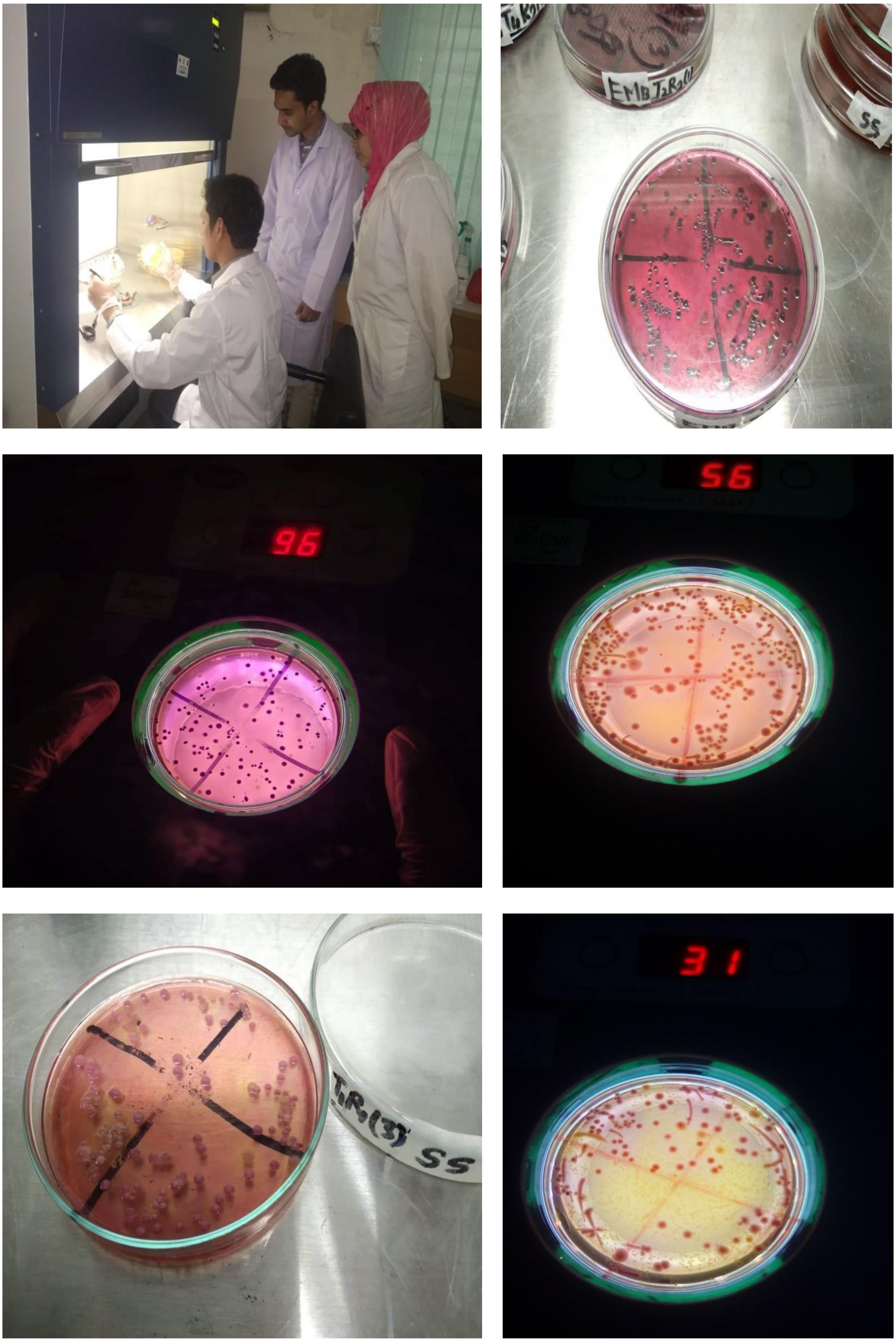


Monitoring and weighing of internal organs and dressed broiler chicken

Appendix 9. Cont'd

Placement of microbial sample in the incubator for incubation

Appendix 9. Cont'd



Bacterial colony count by colony counter