THE EFFECTS OF DIETARY DRIED CHLORELLA POWDER SUPPLEMENTION AS AN ALTERNATIVE TO ANTIBIOTIC ON PRODUCTIVE PERFORMANCE AND HEALTH INDEX OF BROILER CHICKEN

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This is to certify that the thesis entitled ***"THE EFFECTS OF DIETARY DRIED CHLORELLA POWDER SUPPLEMENTION AS AN ALTERNATIVE TO ANTIBIOTIC ON PRODUCTIVE PERFORMANCE HEALTH INDEX 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* ***Noushin Angum Mow,*** *Registration No.* ***13-05258*** *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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TO

MY PARENTS AND TEACHERS

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The Author

LIST OF CONTENTS

LIST OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF APPENDICES

ACRONYMS AND ABBREVIATIONS

Abbreviation		Full meaning
A.M	=	Ante meridian
ACTH	=	Adreno Corticotropic hormone
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
CAT	=	Catalase
CBC	=	Complete Blood Count
CDIL	=	Central Disease Investigation Laboratory
CF	=	Crude Fibre
CFU	=	Colony Forming Units
Cm	=	Centimeter
cm^2	=	Square Centimeter
CONTD.	=	Continued
CP	=	Crude Protein
CRD	=	Complete Randomized Design
DMD	=	Dry Matter Digestibility
Dr.	=	Doctor
DCP	=	Dried Chlorella Powder
e.g.	=	For Example
EDTA	=	Ethylene Diethyl Tetraacitic Acid
et al.	=	And others/Associates
FC	=	Feed Consumption
FCR	=	Feed Conversion Ratio
FOS	=	Fructo-oligosaccharides
gGSH	=	gGram Glutathione
Hb	=	Hemoglobin
HETE	=	Hydroxy Eicosatetraenoic Acid
HPA	=	Hypothalamus Pituitary Axis
i.e.	=	That is
IBV	=	Infectious Bronchitis Vaccines

ACRONYMS AND ABBREVIATIONS

Abbreviation		Full meaning
Kcal	=	Kilo-calorie
Kg	=	Kilogram
M.S.	=	Master of Science
MDA	=	Malon di aldehyde
ME MOS	=	Metabolizable Energy Mannan-oligosaccharides
Ml	=	Mililitre
MCHC	=	Mean Corpuscular Hemoglobin Concentration
Mm	=	Milimeter
Mmol	=	Milimol
MT	=	Metric ton
N	=	Nitrogen
NC	=	Negative Control
NDV	=	Newcastle Disease Vaccine
No.	=	Number
NS	=	Non-significant
P	=	Phosphorus
PCV	=	Packed Cell Volume
Pp	=	Page to page
ppm	=	Parts per Million
PRP	=	Parboiled Rice Polish
WBC	=	White Blood Cell
SAU	=	Sher-e-Bangla Agricultural University
SED	=	Standard Error Difference
SOD	=	Superoxide dismutase
SPSS	=	Statistical Package for Social Sciences
UK	=	United Kingdom
USA	=	United States of America
viz.	=	Such as
Vs	=	Versus
WBC	=	White Blood Cell
WHO	=	World Health Organization
WPSA	=	World‟s Poultry Science Association

ACRONYMS AND ABBREVIATIONS

Symbols		Full meaning
:	=	Ratio
@	=	At the rate of
+	=	Plus
<	=	Less than
>	=	Greater than
°C	=	Degree Celsius
°F	=	Degree Fahrenheit
%	=	Percentage
&	=	And
*	=	5% level of significance
**	=	1% level of significance
/	=	Per

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ABSTRACT

The use of chlorella as an immune and growth stimulant to enhance nonspecific host defense mechanisms or as an antimicrobial to inhibit bacterial growth has been reported. Thus, a total of 120 day-old Cobb 500 broiler chicks were reared in Sher-e-Bangla Agricultural University Poultry Farm, Dhaka. The present study was designed to evaluate the productive performance and health status of commercial broiler chicks fed diet containing DCP (Dried Chlorella Powder) compared to antibiotic based diet. Chicks were divided randomly into 4 experimental groups of 3 replicates (10 chicks with each replications).One of the 4 experimental group was fed this diet as control while, the remaining three groups were fed diet with 2 levels of DCP (0.5% and 1.0%) and antibiotic. The results showed that the body weight (1665.13^{a}±8.81) was significant ($P<0.05$) highest at 1% DCP and the dressing percentage were also significantly higher ($P<0.05$) by the dietary inclusion of DCP as compared to control fed broilers. However, a linear increase in body weight had found with the increase of DCP level in the diet. Significantly less feed was consumed (2287.30^{c}±8.895) to gain better FCR (1.37^{c}±.010) at 1% DCP in comparison to others. The relative weight of spleen and bursa of different groups showed that there were no significant ($P>0.05$) difference between the groups. The present study showed that DCP had no significant ($P>0.05$) effects on liver, gizzard, intestine and heart weight among the treatments. The results of hematological studies showed no significant ($P>0.05$) differences except Hemoglobin, Red blood cell (RBC) which were significantly affected ($P<0.05$) by dried chlorella powder 1% compared with control and antibiotic. However, addition of DCP to broiler chicks diets showed significant ($P<0.05$) difference in bacterial colony count among the groups. The DCP supplementing T_3 (0.5%)and T_4 (1%)groups showed lower number of E. coli and Salmonella sp. and higher number of Lactobacillus sp. compared to control group. Treatments with DCP significantly ($P<0.05$) increased Newcastle disease (ND) titre level as compared to control group.

CHAPTER I
INTRODUCTION

Poultry farming has emerged as one of the fastest growing agribusiness industries in the world, even in Bangladesh. Poultry meat alone contributes 37% of the total meat production in Bangladesh (Hamid *et al.*, 2017*)*. Overall poultry contributes about 22-27% of the total animal protein supply in the country (DLS, 2015). Research on meat production globally indicates poultry as the fastest growing livestock sector especially in developing countries. It has triggered the discovery and widespread use of a number of "feed additives". The term feed additive is applied in a broad sense, to all products other than those commonly called feedstuffs, which could be added to the ration with the purpose of obtaining some special effects. The main objective of adding feed additives is to boost animal performance by increasing their growth rate, better-feed conversion efficiency, greater livability and lowered mortality in poultry birds. These feed additives are termed as "growth promoters" and often called as non-nutrient feed additives.

In poultry industry, antibiotic growth promoters (AGP) have been used as a feed additive to enhance gut health and control sub-clinical diseases. Synthetic growth enhancers and supplements in poultry nutrition are expensive, usually unavailable and possess adverse effects in bird and human (Mahady, G.B. 2005). Sub-therapeutic levels of antibiotics given to poultry as growth enhancer may result to the development of antibiotic-resistant bacteria, which are hazardous to animal and human health (Sarica *et al.*, 2005).

The term "antibiotic growth promoter" is used to describe any medicine that destroys or inhibits bacteria which is administered at a low sub therapeutic dose. The antibiotic growth promotors have been used in poultry feed worldwide during the last 50 years (Yegani and Korver, 2008),but their ban has lead the world to restrict their use in animal feed as growth promotors (Nisha*et al*, 2008).

The mechanism of action of antibiotics as growth promoters is related to interactions with intestinal microbial population (Dibner and Richards, 2005). Four hypotheses have been proposed to explain their action: (i) nutrients may be protected against bacterial destruction; (ii) absorption of nutrients may improve because of a thinning of the small intestinal barrier; (iii) the antibiotics may decrease the production of toxins by intestinal bacteria; and (iv) there may be a reduction in the incidence of subclinical intestinal infections and other pathogenic bacteria (Dafwang *et al.*, 1987; Feighner *et al*, 1987).

However, the use of antibiotics as feed additives is under severe criticism. Growth stimulating antibiotics, by the spread of antibiotic resistant bacteria, are a threat to human health (Wray and Davies, 2000; Turnidge *et al.*, 2004). Concerns were raised that the use of antibiotics as therapeutics and for growth promotion could lead to a problem of increasing resistance in bacteria of human and animal origin (Jensen, 1998), particularly regarding resistance in gram-negative bacteria (*Salmonella* spp. and *Escherichia coli*). In addition they also will have effect on gut flora composition, specifically in regard to increased excretion of food-borne pathogens (Neu, 1992; Williams *et al.*, 1975). The poultry industry is currently moving towards a reduction in use of synthetic antibiotics due to this reason (Barton, 1998).

Because of the growing concern over the transmission and proliferation of resistant bacteria via the food chain, the European Union (EU) banned antibiotic growth promoters to be used as additives in animal nutrition (Cardozo *et al.*, 2004). Alternative feed additives for farm animals are referred to as Natural Growth Promoters (NGP) or non-antibiotic growth promoters (Steiner*et al.*, 2006) which include acidifiers, probiotics, prebiotics, phytobiotics, feed enzymes, immune stimulants and antioxidants are gaining the attention. The NGPs, particularly some natural herbs have been used for medical treatment since prehistoric time (Dragland *et al.*, 2003).

There are some important bioactive components such as alkaloids, bitters, flavonoids, glycosides, mucilage, saponins, tannins (Vandergrift, 1998) phenols,

phenolic acids, guinones, coumarins, terpenoids, essential oils, lectins and polypeptides (Cowan, 1999) in the structures of nearly all the plants. The use of various plant materials as dietary supplements may positively affect poultry health and productivity.

The large number of active compounds in these supplements may therefore present a more acceptable defense against bacterial attack than synthetic antimicrobials. There is evidence to suggest that herbs, seeds, spices and various plant extracts have appetizing and digestion- stimulating properties and antimicrobial effects (Madrid *et al.*, 2003, Alçiçek *et al.*, 2004, Zhang *et al.*, 2005) which stimulate the growth of beneficial bacteria and minimize pathogenic bacterial activity in the gastrointestinal tract of poultry (Wenk, 2000). On the other hand, supplementing the diet with plant material that is rich in active substances with beneficial effects for the immune system can be used as an alternative to antibiotic growth promoters.

Beneficial effects of herbal extracts or active substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response, anti-bacterial, anti-viral, antioxidant and anti-helminthic actions.

Generally plant extracts have no problem of resistance (Tipu *et al.*, 2006) and broilers fed on herbal feed additives were accepted well by the consumers (Hernandez *et al.*, 2004). herbs and plant extracts are being incorporated in poultry feed as growth promoters (Alloui *et al.*, 2013).Compared with synthetic antibiotics or inorganic chemicals, these plant derived products have been proven to be safe, less toxic, residue free and are thought to be ideal feed additives in food animal production (Hashemi and Davoodi, 2010).

Herbs and plant extracts used in animal feed are known as phytogenic feed additives. Phytogenic have been defined as plant-derived natural bioactive compounds with positive effects on animal growth and health (Puvaca *et al.*, 2013).

They are incorporated in the diet of animal feed in order to enhance productivity by improvement of digestibility, nutrient absorption and elimination of pathogens residence in the gut (Athanasiadou *et al.,* 2007).

The beneficial effects of prebiotics are established, and prebiotics are widely used as an alternative to antibiotics in swine and poultry (Zhao *et al.,* 2013; Zhao *et al.,* 2016). Researchers have demonstrated that the dietary supplementation of broiler chickens with prebiotics leads to improved performance through enhancing growth performance and stimulation of the immune system (Vicente et al., 2008; Patel *et al.,* 2015).

The application of a wide range of plant extracts, especially sea plants and other natural substances, to enhance animal health and performance has been documented for a long time due to their anti-inflammatory, immuno-modulatory (Teas, J. *et. al.,* 1984) antioxidant, and antibacterial activities (Rhodes, M. J. 1996). Yoshizawa reported that algae extract activated the macrophages and increased the pro - inflammatory cytokine production of laboratory animals.

However, huge numbers of algae species are available that produce novel compounds. Among these, Chlorella (Chlorella vulgaris) is an important unicellular green microalgae that is used for human food, animal feed, bio-fertilizers, bio-fuels, and the development of pharmaceuticals (Borowitzka, M. A. 1988, and Becker, W. 2004). It provides most of the essential amino acids, minerals, vitamins, chlorophyll, and several bioactive substances (Schubert, L. E. 1988).

Chlorella vulgaris is generally regarded as rich source of protein, essential amino and fatty acids, vitamins and minerals. Traditionally Chlorella is in use since hundred years as part of human nutrition. Chlorella is also known to be rich in thiamin, riboflavin, pyridoxine, vitamin-B_{12}, vitamin C, gamma linoleic acid, phycocyanins, tocopherols, chlorophyll, β-carotenes and carotenoids (Abd El-Baky *et al.,* 2003; Khan *et al.,* 2005).

It has been reported that supplementation of Chlorella in human and animal diets performed numerous biochemical and physiological functions, such as growth promotion (Ishibashi, H. 1972), antioxidant functions (Lee, S. H. *et al.*, 2010), and immunomodulation (Guzmán*et et al.*, 2003). In addition, antimicrobial properties of Chlorella are considered to be an effective alternative to AGP in the diets to maintain optimum health and productivity of the animal.

So the study was conducted to investigate the effect of dried chlorella powder added to the diet in broiler chickens to evaluate the growth performance & immune response of commercial broiler. With this background, the work was planned to explore the possibilities of Chlorella in broiler chicken feeds as a replacement for the antibiotic growth promoters, with the following specific objectives:

1. To evaluate the growth performance, hematological properties and some organ characteristics of broiler fed Dried Chlorella Powder based diet comparison with antibiotic and basal diet.

2. To find out the effect of DCP diet on gut's microbial population and ND antibody titre.

3. To determine the inclusion level of DCP in broiler ration as a supplement of antibiotics.

CHAPTER 2

REVIEW OF LITERATURE

Sources of literature

(i) 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

(ii) Abstract searching at BARC, Farmgate, Dhaka, BANSDOC, Agargoan, Dhaka.

(iii) Internet browsing.

A total about 158 literature were reviewed to identify the background, drawbacks and prospects of research, understand previous findings and to answer the research status of this field.

Among them 48 were full article and 65 abstracts, 45 were only titles and some were miscellaneous. A brief account is given below depending on five main headlines viz, antibiotic impacts on poultry, Antibiotic growth promoters (AGPs), Antimicrobial resistance, Alternatives to antibiotic growth promoters and *Chlorella*.

Mentioning the references in a traditional way or sequence is avoided. A very critical enquires was made of each article and significant informations were collected and arranged according to specific title. It is expected to be pioneering efforts in Bangladesh for higher research review attempts.

In Bangladesh, the demand for broiler meat is increased rapidly, propelled by increased income and population growth and urbanization. Feed cost accounts for up to 80% of the total cost of production and is a very important component in determining the extent of poultry survival and profitability (Olugbemi *et al.,* 2010). Feed is a major component affecting net return from the poultry enterprise. Various strategies like feed supplements and additives are being used to ensure more net return and to minimize expenditure on feed. Economical broiler production largely depends on optimum utilization of feed, improved body weight, prevention of diseases and reduced mortality rate. Use of chemical feed additives as growth promoters has criticism due to adverse effects on consumer's health and there is increasing demand for organic meat and eggs. In view of this, herbal and plant derivatives would be a valuable alternative to promote growth and health in poultry as there is no residual toxicity (Agashe *et al.,* 2017).

Specifically, these are raised for meat production under intensive production system using commercial feed ration. However, broiler production cost has gone up substantially in recent years due to the increase in price of feed ingredients. The search for cheap, locally available and equally nutritive feed sources to partially substitute commercial poultry diet has never been more pressing. Plant proteins are good sources of dietary fiber and essential amino acids in the diet. Unlike livestock farming, poultry farming is always intensive and hence the birds are more subjected to stressful conditions. Stress is an important factor that renders the birds vulnerable to potentially pathogenic microorganisms like E.coli, salmonella, clostridium, camphylobacter etc. These pathogenic micro flora in the small intestine compete with the host for nutrients and also reduce the digestion of fat and fat-soluble vitamins due to de-conjugating effects of bile acids (Engberg *et al.,* 2000). This ultimately leads depressed growth performance and increase incidence of disease.

2. 1 Antibiotic impacts on poultry

The discovery of antibiotics was a success in controlling infectious pathologies and increasing feed efficiencies (Engberg *et al.*, 2000). Antibiotics, either of natural or synthetic origin are used to both prevent proliferation and destroy bacteria. Antibiotics are produced by lower fungi or certain bacteria. They are routinely used to treat and prevent infections in humans and animals. The poultry industry uses antibiotics to improve meat

Production through increased feed conversion, growth rate promotion and disease prevention. Antibiotics can be used successfully at sub-therapeutic doses in poultry production to promote growth (Chattopadhyay, 2014;Engberg *et al.*, 2000) and protect the health of birds by modifying the immune status of broiler chickens (Lee *et al.*, 2012). This is mainly due to the control of gastrointestinal infections due to microbiota modification and increase in the intestine (Singh *et al.*, 2013; Toro*ketal.*, 2011). The mechanism remains unclear, but antibiotics are likely to act by remodelling microbial diversity and relative abundance in the intestine to provide an optimal microbiota for growth (Dibner and Richards, 2005). For example, meta- genome sequencing approaches have demonstrated that diet with salinomycin (60 ppm) has an impact on microbiome dynamics in chicken ceca (Fung *et al*., 2013). Similarly, the use of virginiamycin (100 ppm) as a growth promoter has been associated with an increased abundance of Lactobacillus species in broiler duodenal loop at proximal ileum. This indicates that virginiamycin alters the composition of chicken gut microbiota (Dumonceaux *et al.*, 2006). In addition, populations of *Lactobacillus* spp. in the ileum of chickens receiving feed containing tylosin, a bacteriostatic, are significantly lower than in chickens receiving no tylosin (Lin *etal.*, 2013). This decrease in *Lactobacilli species* following the use of antibiotics has been demonstrated in other studies (Lee *et al.*, 2012). For reminder, *Lactobacillus* are the primary commensal bacteria for the production of bile hydrolase salt.

The decrease in the lactobacillus population in antibiotic-treated animals probably reduces the intestinal activity of the bile hydrolase salts, which would increase the relative abundance of conjugated bile salts, thus promotes lipid metabolism and energy harvesting and increases animal weight gain (Lin *et al.*, 2013).

A change in the intestinal microbiota of chickens can influence their immunity and their health. However, changes in the intestinal microbiota of chickens can be influenced by several factors. These factors include housing conditions, exposure to pathogens, diet composition and the presence of antibiotics in feed (Lee *et al.*, 2012).

2.2 **Antibiotic growth promoters (AGPs)**

Feed antibiotics were first applied in animal nutrition in 1946. The term "antibiotic growth promoter" is used to describe any medicine that destroys or inhibit bacteria and is administered at a low, sub therapeutic dose for the purpose of performance enhancement (Hughes and Heritage, 2002).

Antibacterial growth promoters are used to help the animals to digest their food more efficiently, get maximum benefit from it and allow them to develop in to strong and healthy individuals (Ellin, 2001). They may produce improved growth rate because of thinning of mucous membrane of the gut, facilitating better absorption, altering gut motility to enhance better assimilation, producing favorable conditions to beneficial microbes in the gut of animal by destroying harmful bacteria and partitioning proteins to muscle accretion by suppressing monokines (Prescott and Baggot, 1993). When used at sub- therapeutic levels, these antimicrobials improve overall performance (Falcao-e-Cunha *et al.,* 2007*)* through reduced normal intestinal flora (which compete with the host for nutrients) and harmful gut bacteria (which may reduce performance by causing sub clinical-diseases) (Jensen,1998).

But the antibiotics are specific to their spectrum of activity only in the active multiplying stage of bacteria and it will not provide overall protection. Large numbers of antimicrobials were banned due to residual effects on human health and cross-resistance to antimicrobial drugs used in human medicine (WHO, 1997).

Some antimicrobial agents (Virginiamanycin, Zn bacitracin, etc.), which are not absorbed in the systemic circulation and exert their action locally in the gut are still used as growth promoters (Ian phillips, 1999). Administration of drugs to food-producing animals requires not only consideration of effects on the animal but also the effects on humans who ingest food from these animals. In short, after food-producing animals have been exposed to drugs in order to cure or prevent disease or to promote growth, the effects of the residues of such treatment on humans should be known.

In view of the above the use of antibiotic growth promoters (AGPs) in poultry industry is under serious criticism by governmental policy makers and consumers because of the development of microbial resistance to these products and the potential harmful effects on human health. At present, only four AGPs are permitted for use in poultry nutrition. Thus, there is increasing public and government pressure in several countries to search for natural alternative to antibiotics (Botsoglou and Fletouris, 2001; Williams and Losa, 2001; McCartney, 2002).

2.3 Antimicrobial resistance

Bacterial resistance to antimicrobial drugs has become an issue of increased public concern and scientific interest during the last decade. This resulted from a growing concern that the use of antimicrobial drugs in veterinary medicine and animal husbandry may compromise human health if resistant bacteria develop in animals and are transferred to humans via the food chain or the environment.

While there is still no consensus on the degree to which usage of antibiotics in animals contributes to the development and dissemination of antimicrobial resistance in human bacteria, experiential evidence and epidemiological and molecular studies point to a relationship between antimicrobial use and the emergence of resistant bacterial strains in animals and their spread to humans, especially via the food chain (Moritz, 2001).

Bacitracin, chlortetracycline, tylosin, avoparcin, neomycin, oxytetracycline, virginiamycin, trimethoprim, lincosamides, cephalosporins etc are the commonly used antibiotics in poultry and some of which are of direct importance in human medicine. However, imprudent use of antibiotics in poultry production can lead to increased antibiotic resistant bacteria in poultry products.

In general, when an antibiotic is applied in poultry farming, the drug eliminates the susceptible bacterial strains, particularly at a therapeutic dose, leaving behind or selecting those variants with unusual traits that can resist it. These resistant bacteria thus become the predominant micro-organism in the population and they transmit their genetically defined resistance characteristics to subsequent progeny of the strains and to other bacterial species via mutation or plasmid-mediated (Gould, 2008).

According to WHO, the resistance to antibiotics is an ability of bacterial population to survive the effect of inhibitory concentration of antimicrobial agents (Catry *et al.*, 2003).

For example, the use of fluoroquinolone antibiotics in broiler chickens has caused an emergence of resistant *Campylobacter* in poultry (Randall *et al.*, 2003). Administration of avilamycin as a growth promoter resulted in an occurrence of avilamycin-resistant *Enterococcus faecium* in broiler farms (Aarestrup *et al.*, 2000).

Potential transfer of resistant bacteria from poultry products to human population may occur through consumption of inadequently cooked meat or

handling meat contaminated with the pathogens (Van den Bogaard and Stobberingh, 2000).

In turkeys fed vancomycin, there were concerns of glycopeptides resistance due to *enterococci* found in turkeys and humans (Stobbering *et al.*, 1999), which is an example of cross-resistance. Studies have shown that animal *enterococci* are mostly different from human colonizers, although concerns for transient transfers of resistance remain (Apata, 2009).

2.4 Alternatives to antibiotic growth promoters

In view of the concerns regarding the potential for selection of antibiotic resistant bacteria, residues and environmental effects attributed to the use of antimicrobial growth promoters, a host of non-antibiotic alternatives are available or under investigation.

2.4.1 Probiotics

Probiotics are individual microorganisms or groups of microorganisms, which have favourable effect on host by improving the characteristics of intestinal micro flora (Fuller, 1989). Certain species of bacteria, fungi and yeasts belong to the group of probiotics. Existing probiotics can be classified into colonizing species (Lactobacillus sp., Enterococcus sp. and Streptococcus sp.) and free, non-colonizing species (Bacillus sp and Saccharomyces cerevisiaes) (Zikic et al., 2006).

Probiotics acts by inhibiting bacterial growth by secretion of products, which inhibit their development, such as bacteriocins, organic acids and hydrogen peroxide. The other way by which probiotics act is competitive exclusion, which represents competition for locations to adhere to the intestinal mucous membranes and in this way pathogen microorganisms are prevented from inhabiting the digestive tract and the third way is competition for nutritious substances (Patterson and Burkholder, 2003).

In this way, they create conditions in intestines, which favour growth of useful bacteria and inhibit the development of pathogenic bacteria (Line *et al.*, 1998).

They improve the function of the immune system (Zulkifli *et al.*, 2000; Kabir *et al.*, 2004) and exhibit significant influence on morpho-functional characteristics of intestines (Yang *et al.*, 2009).

These effects lead to growth of broiler chickens (Jin *et al.*, 1997; Li *et al.*, 2008), improvement of feed conversion (Li *et al.*, 2008; Zulkifli *et al.*, 2000; Kabir *et al.*, 2004) and reduced mortality (Mohan *et al.*, 1996).

Majority of authors concluded that the effect of probiotics depended on the combination of bacterial strains contained in the probiotic preparation, level of its inclusion in the mixture, composition of mixture, quality of chickens and conditions of the environment in the production facility (Jin *et al.*, 1997; Patterson and Burkholder, 2003).

Nutrition plays a key role in maintaining the prooxidant-antioxidant balance (Cowey, 1986). Under physiological conditions the reactive species figure a crucial role in primary immune defense (Diplock *et al.*, 1998).

But prolonged excess of reactive species is highly damaging for the host biomolecules and cells, resulting in dysbalance of the functional antioxidative network of the organism and leading to substantial escalation of pathological inflammation (Petrof *et al.*, 2004).

Several studies reported the antioxidant activity of probiotic bacteria using assays in vitro (Shen *et al.*, 2011). Lactic acid bacteria are evaluated as beneficial bacteria by their product of acids (lactic acid), bacteriocin-like substances or bacteriocins (Strus *et al.*, 2001). Widely accepted probiotics contain different lactic acid producing bacteria: bifidobacteria, lactobacilli or enterococci (Mikelsaar and Zilmer, 2009).

Their efficiency was demonstrated for the treatment of gastrointestinal disorders, respiratory infections and allergic symptoms. In most cases, evidence for a beneficial effect was obtained by studies using animal models (Travers *et al.*, 2011).

2.4.2 Prebiotics

Prebiotics are defined as non-digestible food components, which have positive effect on host in their selective growth and activation of certain number of bacterial strains present in intestines (Gibson and Roberfroid, 1995).

The most significant compounds, which belong to group of prebiotics, are fructose-oligosaccharides (FOS), gluco-oligosaccharides and mannan-oligosaccharides (MOS).

Their advantage, compared to probiotics is that they promote growth of useful bacteria, which are already present in the host organism and are adapted to all conditions of the environment (Yang *et al.*, 2009). Similar to probiotics, Results of the effects of prebiotics on broiler performance are contradictory. A study was conducted to analyze the effects of incorporation of FOS on broiler performances and the results showed improvement in body weight gain by 5-8% and improvement of feed conversion by 2-6% (Li *et al.*, 2008; Yang *et al.*, 2009). But, Biggs *et al.* (2007) obtained results showing decrease of body weight gain by 2% in-group fed FOS in diet.

Application of MOS to fattening chicks resulted in improvement of body weight gain and feed conversion in fattening chickens by up to 6% (Roch, 1998; Newman, 1999). This proves that effect of application of prebiotics depends on the condition of animals, environment conditions, composition of food and level and type of prebiotic included in the mixtures.

2.4.3 Synbiotics

This is relatively recent term among additives used in poultry nutrition. Synbiotics are combination primarily of probiotics and prebiotics, as well as other promoting substances which together exhibit joint effect with regard to health of digestive tract, digestibility and performances of broilers. Investigations showed that combinations used in synbiotics are often more efficient in relation to individual additives (Uśćebrka *et al.*, 2005; Li *et al*., 2008). Maiorka *et al.* (2001) suggest that the substitution of antibiotics by symbiotics in broiler chicken diets is an alternative to poultry industry, since no negative effect was found on performance. According to Cristina *et al.* (2012) the usage of probiotic-prebiotic-ficofytic compounds as feed additive generated better results related to hens performance, feed valorization, eggs yield and their quality.

The administration of symbiotic to broiler chickens early in life increased significantly ($p<0.05$) the phagocytic activity, lysozyme activity and nitric oxide levels in a dose dependent manner and improved the oxidative state by increasing glutathione (GSH) and decreasing malondialdehyde(MDA).

High concentration of symbiotic improves the antibody response to Newcastle Disease Vaccine (NDV) and Infectious Bronchitis Vaccines (IBV) (El-Sissi and Mohamed, 2011).

2.4.4 Enzymes

Supplementation of broiler feed with enzymes is applied in order to increase the efficiency of production of poultry meat. This is especially interesting if enzymes, which enable utilization of feeds of poorer nutritive value, are used. Numerous authors have reported that administration of enzymes can improve the production performances by 10% (Cowieson *et al*., 2000, Cmiljanic *et al*., 2001), whereas in some studies no positive effect has been reported (Peric *et al.*, 2002).

It is obvious that the positive effect of application of additives depends on the quantity and quality of feeds included in the mixture, type of enzyme, as well as fattening conditions (Acamovic, 2001; Lukic *et al*., 2002). Obtained results in some researches indicate that better effect is realized with utilization of two or more enzymes in food (Silversides and Bedford, 1999; Chesson, 2001). Therefore, new enzyme combinations are constantly analyzed, as well as their optimum doses, in order to realize positive financial effect through improved utilization of feeds.

The main reasons for supplementing wheat- and barley-based poultry diets with enzymes is to increase the available energy content of the diet. Increased availability of carbohydrates for energy utilization is associated with increased energy digestibility (Partridge and Wyatt, 1995; Van der Klis *et al.*, 1995).

Enzymes have been shown to improve performance and nutrient digestibility when added to poultry diets containing cereals, such as barley and wheat (Friesen *et al*., 1992; Marquardt *et al.*, 1994), maize (Saleh *et al*., 2003), oats and rye (Friesen *et al*., 1991, 1992; Bedford and Classen 1992; Marquardt *et al.*, 1994) and to those containing pulses, such as lupins (Brenes *et al.*, 1993). The effect of enzyme supplementation on dry matter digestibilities (DMD) in pigs and poultry depends on the type of diet and the type of animal: increases in DMD range from 0.9 (Schutte *et al*., 1995) to 17% (Annison and Choct, 1993) in poultry.

Morgan and Bedford (1995) reported that coccidiosis problems could be prevented by using enzymes. According to Bharathidhasan *et al.* (2009) when Broilers were supplemented with enzyme level at 0, 250, 500, 750 and 1000 g/ton of feed there was no significant difference in carcass yield, dressing percentage, giblet weight, carcass weight, intestinal length and organoleptic characteristics of the meat.

2.4.5 Acidifiers

Acidifiers have been used in poultry nutrition for long time, in different forms and combinations, which are constantly changing. Organic acids reduce pH value of food and act as conserving agents and prevent microbial contamination of food in digestive tract of poultry (Freitag *et al.*, 1999). As a result of this there will be improved consumption of food, better- feed conversion and increased gain. Favourable effect of supplementation of individual organic acids to mixtures was established relatively long time ago for formic acid (Kirchgessner *et al.*, 1991).

Research published by Ao *et al.* (2009) it was established that citric acid in combination with α –galactosidase increased the effect of enzyme action, but also had negative effect on feed consumption and weight gain.

2.4.6 Antioxidants

Antioxidants are the agents, which donate free electron to reactive oxygen species (ROS) and reactive nitrogen species (RNS) and convert them to harmless substances and break the chain reaction (Dekkers *et al.*, 1996). After donating an electron, an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive.

Antioxidants are synthesized within the body and can also be extracted from the food that humans and animals eat, such as fruits, vegetables, seeds, nuts, meat, oil, leaves and grass (natural antioxidants). There are two lines of antioxidant defense within the cell. The first line, found in the fat-soluble cellular membrane consists of vitamin E, *beta*-carotene and coenzyme-Q (Kaczmarski, 1999). Of these, vitamin E is considered to be the most potent chain-breaking antioxidant within the membrane of the cell. The second line, inside the cell consists of water soluble antioxidant scavengers that include vitamin C, glutathione peroxidase, superoxide dismutase (SOD) and catalase (CAT) (Dekkers *et al.*, 1996). To maximize

the oxidative stability of meat, antioxidants, mostly α-tocopheryl acetate (ATA), are added to feeds.

The beneficial effect of dietary ATA supplementation for the enhanced stability of lipids in muscle foods has been extensively reported for poultry, beef cattle, veal calves and pigs (Gray *et al.*, 1996; Jensen *et al.*, 1998).

Selenium is component of enzyme glutathione peroxidase, which prevents formation of free radicals, which are very harmful to cells as they disrupt their integrity (Kanacki *et al*., 2008).

Therefore, selenium and otherantioxidants have favourable effect on quality of broiler meat (Surai, 2002; Tomovic *et al*., 2006; Peric *et al*., 2007a). Protective effect of selenium and vitamin E is also stated by Roch *et al*. (2000). One of the most accepted approaches for preservation of sensory properties of the meat is addition of antioxidants, such as selenium or vitamin E, directly to livestock food or during technological procedure of processing (Surai, 2002, Peric *et al.*, 2007b).

Beside positive effect on quality of meat, Edens *et al*. (2000) and Peric *et al.* (2006) established better feathering and body mass of chickens fed organic forms of selenium. Peric *et al.* (2008b) also stated that addition of organically bound selenium into feed for broiler parents significantly increases quality of one-day-old chickens. Lower plasma concentrations of antioxidant vitamins such as vitamin C, E and folic acid and minerals like zinc and chromium have been inversely correlated to increased oxidative damage in stressed poultry (Cheng *et al.*, 1990; Sahin *et al.*, 2002).

Super oxide dismutase (SOD), is a class of closely related enzymes that catalyze the breakdown of the highly reactive superoxide anion into oxygen and hydrogen peroxide. SOD proteins are present in almost all aerobic cells and in extra cellular fluids. Each molecule of superoxide dismutase contains atoms of copper, zinc, manganese or iron. SOD that is formed in the mitochondria contains manganese (Mn-SOD) and synthesized in the matrix

of the mitochondria. SOD that is formed in the cytoplasm of the cell contains copper and zinc (Cu/Zn-SOD). The SOD is a specific catalyst of the reaction and decreases concentration of O_2^-(Izumi *et al.*, 2002).

2.4.7 Herbal adaptogens

An adaptogen is a substance that shows some nonspecific effect, such as increasing body resistance to physical, chemical, or biological noxious agents and have a normalizing influence on pathological state, independent of the nature of that state.

A vast number of plants have been recognized as valuable sources of natural antimicrobial compounds (Mahady, 2005). A wide range of phytochemicals present in plants are known to inhibit bacterial pathogens (Cowan, 1999; Medina *et al.*, 2005).

Successful determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone and methanol are often used to extract bioactive compounds (Eloff, 1998).

Ethanol is the most commonly used organic solvent by herbal medicine manufactures because the finished products can be safely used internally by consumers (Low Dog, 2009) In terms of active ingredients, adaptogenic preparations can be divided into three groups.

- Those that contain phenolic compounds such as phenylpropanoids, phenylethane derivatives and lignans, which structurally resemble catecholamines that activates sympatho-adrenal system and possibly imply Those that contain tetracyclic triterpenes, such as cucurbitacin R diglucoside, an effect in the early stages of the stress response (Kochetkov *et al.*, 1962; Wagner, 1995).

- Which structurally resemble the specific corticosteroids that inactivate the stress system to protect against overreaction to stressors (Munck, 1984; Panossian et al., 1999).

- Those that contain unsaturated trihydroxy or epoxy fatty acids such as oxylipins structurally similar to leukotrienes and lipoxines (Panossian et al., 1999).

Mechanism of action of these additives is not completely clear. Some plant extracts influence digestion and secretion of digestive enzymes and besides, they exhibit antibacterial, antiviral and antioxidant action (Ertas *et al.*, 2005; Cross *et al.*, 2007).

There is extensive evidence that single-dose administration of adaptogens activates corticosteroid formation and repeated dosage with adaptogens normalizes the levels of stress hormones, such as adrenocorticotropic hormone (ACTH) (Panossian, 1999). The effects of adaptogens become somewhat clearer when it is recalled the stress is a defensive response to external factors and that it stimulates the formation of endogenous messenger substances such as catecholamines, prostaglandins, cytokines, NO and platelet-activating factor, which inturn activate other factors that may either counteract stress or conversely, induce or facilitate disease. According to this concept, the "stress-executing" or „"switch- on" mechanism activates the sympathoadrenal system (SAS) and over the longer term also activates the HPA, together with various regulators of cell and organ function (Panossian, 1999).

Results of research of application of phytobiotics in nutrition of broiler chickens are not completely consistent. Some authors state significant positive effects on broiler performance (Ertas *et al.*, 2005; Cross *et al.*, 2007, Peric *et al.*, 2008a), whereas another group of authors established no influence on weight gain and consumption or conversion of food (Cross *et al.*, 2007; Ocak *et al.*, 2008).

The differences in results are consequences of numerous factors, of which Yang *et al.* (2009) pointed out four:

1) Type and part of plant used and their physical properties, 2) time of harvest, 3) preparation method of phytogenic additive and 4) compatibility with other food components.

Tipakom, (2002) found that feeding of *Andrographis paniculatis* to broiler chickens resulted in improved feed conversion ratio, increased live weight and decreased mortality rate and opined that the plant feeding could be an alternative to chlortetracycline in the broiler diet.

In the past two decades a number of ayurvedic preparations have been extensively used in poultry industry in India. Preparations like Livol® and Zeestress® have been found to possess hepato protective and immune potentiative actions in vaccinated birds and reduced the stress in intensively housed chickens during summer (Parida *et al.*, 1995; Rao *et al.*, 1995).

2.5 Dried chlorella powder

For millennia, plants and micro- organisms from the sea and from fresh water have provided food and medicinal substances for humans. Marine microalgae have long been recognized for their valuable health effects for both human and animals. Among the marine algae, Chlorella has gained much attention in animal and veterinary use (Kang *et al.*, 2013).

C. vulgaris is a green eukaryotic microalgae in the genus *Chlorella*, which has been present on earth since the Precambrian period (Safi, C. *et al.*, 2014).This unicellular algae was discovered in 1890 by Martinus Willem Beijerinck as the first microalgae with a well-defined nucleus (Beijerinck, M. W. *et al.*, 1890). At the beginning of the 1990s, German scientists noticed the high protein content of *C. vulgaris* and began to consider it as a new food source.

Chlorella (Chlorella spp.) is a waterborne microorganism that have gained visibility as nutritional supplements that are touted for the concentrated packages of nutrients that it deliver. It is also known for its health-supporting, disease-prevention roles as detoxifying agent.

Chlorella spp. are simple, non-motile, unicellular, aquatic green microalgae. They were one of the first algae to be isolated as a pure culture. The *Chlorella* microalga measures between 5 and 10 micrometers and, under an optical microscope one, can observe its green color and spherical shape.

Compared to higher plants, *Chlorella* has a high concentration of chlorophyll and photosynthetic capacity. The microalga *Chlorella* is classified as a species according to the shape of the cells, characteristics of chlorophyll, and other variables.

There are 20–30 species, some of which are *Chlorella vulgaris, Chlorella pyrenoidosa,* and *Chlorella ellipsoidea*. The species are differentiated within the group, known as *strains* (Illman *et al.*, 2000).

This organism is nutrient-dense, have cleansing and detoxifying properties, and is yielding promising results in laboratory studies of its bioactivities and clinical trials on its possible benefits for people.

Biologically, Chlorella is classified as an algae and eukaryote. It is a complex plant with a nuclear membrane, well defined chromosomes, and well-differentiated cellular structures (Russ Mason *et al.*, 2001). Spherical shape and a size varying from 2 to 10 μm in diameter (Yamamoto, 2005).The species most commonly used in commercial production are Chlorella pyrenoidosa and Chlorella vulgaris. The cell walls of chlorella have three layers, of which the thicker middle layer contains cellulose micro fibrils and the outer layer a polymerized carotenoid material. It is this outer cellular material that most likely gives chlorella its detoxifying activity.

The material binds the heavy metals, pesticides, and toxins such as polychlorinated biphenyls (PCBs) and then carries these substances out of the body (Russ Mason *et al.*, 2001).

This microalga is known to have high contents of n-3 long-chain polyunsaturated fatty acids (LC-PUFAs) (Xue *et al.*, 2012), fiber and protein peptides (Kang *et al.*, 2013), which may act as health-promoting substances for birds (An *et al.*, 2008). In addition, Chlorella contains natural growth factors (Liang *et al.*, 2004) and high quality of protein (Kang *et al.*, 2013), which are essential for promoting the growth rate of broiler chickens.

Taking these beneficial properties of Chlorella into consideration, supplementation of broiler diets with this microalga seemed to be a desirable tool for ameliorating the depressed immune response and growth performance of broilers withheld from feed after hatching.

2.5.1 Chemical composition of Chlorella powder:

Generally 3 kinds of Chlorella materials are available in the market such as i) dried Chlorella powder (DCP), ii) Chlorella growth factor (CGF), and iii) fresh liquid Chlorella (FLC).

The nutritive value of open or indoor cultivated C. vulgaris depends upon the technological process used to treat the algal mass (Lubitz, 1963; Lin, 1969; Saleh *et al.*, 1985; Komaki *et al.*, 1998; Janczyk *et al.*, 2007a).

Plate 1. Dried chlorella powder (DCP)

Table1. Percent composition of Chlorella powder (dry matter basis)

Item	Percent (%)
Moisture	5.2
Dry matter	94.80
Crude protein	60.60
Ether extract	12.80
Crude fibre	13
Nitrogen free extract	9.1
Ash	4.5
ME (Kcal/kg)	3.00

(Daesang Corporation, Icheon, Korea**)**

2.5.2 Mechanism of action of Chlorella powder:

The 3D8 single-chain variable fragment (3D8 scFv) is an anti-nucleic acid antibody that can bind to and hydrolyze nucleic acids without sequence specificity (Kim *et al.,* 2006). Production and local delivery of genetically engineered antibody fragments by bacteria in the gastrointestinal tract could provide efficient therapy at a low cost (Pant *et al.,* 2006).

Many microalgae, particularly Spirulina and Chlorella, are good sources of β carotene, vitamin B12, and β-glucan, an immune regulatory polysaccharide component that plays a vital role in the body immune functions and inflammatory processes (Pesando, D. *et al.,* 1979; Qureshi, M. 1996; Mason, R. 2001).

Gamma-interferon is a protein produced by immune cells and protects the body from infections. Chlorella stimulates the activity of immune cells and macrophages by increasing interferon levels, thus enhancing the ability of the immune system to combat pathogens and foreign proteins.It was assumed that the fiber and protein peptides contained in the Chlorella stimulate immunoglobulin, producing B cells in the gut-associated lymphoid tissue and increase the IgA, IgM, and IgG concentration in the plasma of broiler chickens.

Chlorellin in chlorella fights only against pathogenic organisms without destroying beneficial microflora in the intestine, which in turn has the ability to improve the health and productivity of broiler chickens (Jensen, G. S., 2001).

2.5.3 Antioxidant properties of Chlorella powder:

Abou-zeid *et al.* (2015) reported that the alcohol extract of *chlorella* inhibited lipid peroxidation more significantly than the chemical antioxidants like α-tocopherol, BHA and β-carotene. It contains protein, a fraction of the beta-carotene, and more than double the amount of nucleic acid and chlorophyll.

In another study by Zhi-Gang *et al.*,(1997) the antioxidant effects of two fractions of a hot water extract were studied using three systems that generate superoxide, lipid and hydroxyl radicals. Both fractions showed significant capacity to scavenge hydroxyl radicals (the most highly reactive oxygen radical) but no effect on superoxide radicals. Miranda *et al.*,(1998) attributed the antioxidant effect to beta- carotene, tocopherol and phenolic compounds working individually or in synergy.

Chlorella algae as feed additives have been reported to improve growth, feed utilization, lipid metabolism, body composition, stress responses, liver function and disease resistance (Nakagawa *et al.,* 1983, Nakagawa *et al.,* 1984; Nematipour *et al.,* 1988; Nematipour *et al.,* 1990).

Beta-carotene concentration of *blue green algae* is ten times higher than that of carrot. Food rich in β-carotene can reduce the risk of cancer (Peto *et al.*, 1981).

It was found that the natural carotene of *Spirulina* could inhibit, shrink and destroy oral cancer cells. The beta-carotene in algae and leafy green vegetables has greater antioxidant effects than synthetic beta-carotene (Amotz, 1987).

2.5.4 Effect of Chlorella powder on live weight and live weight gain

Kang *et al.*, (2013) reported that several Chlorella-based supplements including DCP, liquid media or CGF added into the diets of broiler chicks enhanced body weight.

In contrast, in study of Kotrbáček *et al.*, (1994) they found that, dietary supplementation of 0.50 % biomass of chlorella did not affect final body weight in broiler chicks.

The above result was supported by Peiretti and Meineri (2008). They reported that final body weight (BW), weight gain were not affected by dietary supplementation of microalgae at different levels in the rabbit diet.

Takekoshi *et al.*, (2005) indicated that dietary supplementation of Chlorella (Chlorella pyrenoidosa) did not affect the weight gain of mice.

Thus, the improvement of chicken growth may be attributed to those essential nutrients contained in dried Chlorella powder.

H. Choi*et al.*, (2017) at 21 to 35 d old, the broilers fed the PC_2 treatment group (1.0% EFL with chlorella) ($P < 0.05$) exhibited higher BWG than in the primary NC treatment group, which showed increased BWG by 2.70%. During the total experimental period, the weight of the broilers in the PC_2 and T_2 (1.0% EFL with chlorella (anti-viral) treatment groups ($P < 0.01$) was significantly higher than that of those in the NC treatment group, which showed increased BWG by 3.00 and 2.55%, respectively.

Several researchers have reported that the use of chlorella as a prebiotic has a positive effect on the growth performance and immune characteristics of chickens and pigs (Yan, Lim and Kim, 2012; Kang *et al.,* 2013).

The final BW was linearly ($p = 0.001$) increased as the inclusion rate of fermented C. vulgaris into diets increased. Similarly, dietary C. vulgaris linearly increased the BW gain ($p = 0.001$) and the feed intake ($p = 0.001$), especially from 1 to 42 days post-hatch. However, there was no significant effect on feed efficiency between the treatment and control groups (S. T. Oh. *et al.,* 2015).

The increased growth performance of broiler chickens might be attributed to the high amount and high quality of protein inthe Chlorella, which enhances the weight gain of broilers (Ishibashi, H. 1972, Kay, R. A. 1991).

Abou-Zeid*et al.,* (2015) found that Body weight gain of broiler chicks fed different levels of dietary algae significantly ($P<0.5$) increased as compared to that of the control group during starter, finisher and the entire length of the experimental periods.

Byoung-Ki An*et al.,* 2016 reported that Chicks fed diets with 0.15 or 0.5 % DCP had a significantly higher final body weight compared with that of NC. During starter and grower periods, chicks in antibiotic and 0.15 or 0.5 % DCP showed a higher ($p < 0.05$) daily body weight gain as compared with NC. This increase in daily body weight gain, especially during the grower period, was reflected in improved feed conversion ratio in chicks fed diet with antibiotics or 0.5% DCP.

2.5.5 Effect of Chlorella powder on feed consumption

Takekoshi *et al.,* (2005) indicated that dietary supplementation of Chlorella (Chlorella pyrenoidosa) did not affect the feed intake of mice.

Chicks fed diets with 0.15 or 0.5 % DCP had no effect on feed intake between experimental groups compared with that of control group (Byoung-Ki An *et al.*, 2016).

Kang *et al.*, (2013) reported that several chlorella-based supplements including DCP, liquid media or CGF added into the diets of broiler chicks enhanced body weight, but did not affect feed consumption.

Abou-Zeid *et al.*, (2015) during the starter, finisher and throughout the experimental periods, it can be noticed that feed intake was not statistically ($P<0.05$) affected by spirulina and/ or chlorella levels supplementation.

In contrast, other researchers (Kharde *et al.*, 2012; Shanmugapriya & Saravana Babu, 2014) reported that dietary *Spirulina* significantly ($P<0.05$) improved Feed consumption (FC) of broiler chickens different *Spirulina* inclusion levels and quality in the present trials.

2.5.6 Effect of Chlorella powder on FCR

Peiretti and Meineri (2008) reported that final body weight (BW), weight gain, and feed efficiency were not affected by dietary supplementation of microalgae at different levels in the rabbit diet.

The increase in daily body weight gain, especially during the grower period, was reflected in improved feed conversion ratio in chicks fed diet with antibiotics or 0.5% DCP (Byoung-Ki An*et al.*, 2016).

Kang *et al.*, (2013) reported that several chlorella-based supplements including DCP, liquid media or CGF added into the diets of broiler chicks enhanced body weight, but did not affect feed conversion ratio.

M. Rezvani*et al.*, (2012) reported that Feed conversion ratio significantly decreased by adding the chlorella and commercial prebiotic to the control diet at 42 d of age ($p<0.05$).

Benites, V *et al.*, (2008) reported that broiler chickens fed a diet supplemented with prebiotic (manan-oligosaccharide) had a significant decrease in the feed conversion ratio in compare to control group.

Kaoud (2012), Mariey *et al.*, (2012) and Mariey *et al.* (2014), who reported that feed conversion ratio significantly improved by dietary inclusion of Spirulina platensis as compared to the control broilers.

Concerning the feed conversion ratio, Abou-Zeid *et al.*, (2015) dietary treatments improved feed conversion ratio compared to the birds fed control diet during starter, finisher and the whole experimental periods.

The improvement of feed conversion ratio in chlorella and prebiotic treated broilers could be related to better equilibrium in the intestinal flora (Bedford *et al.*, 2000).

However, there was no significant effect on feed efficiency between the chlorella treated and control groups (S. T. Oh. *et al.*, 2015).

2.5.7 Effect of Chlorella powder on dressing percentage

El Deek *et al.*, (1987) and El Deek and Brikaa (2009) found that using different levels of seaweed had insignificant effect on ducks carcass quality.

But dissimilar results were found by El Deek *et al.*, (2011).Regardless of thermal or enzymatic treatments, using different levels of algae in broiler finisher diets had significant effect on dressing percentages (ranged between 73.1 to 73.8%) at 39 days of age.

These results are in agreement in part with those reported by El Deek and Brikaa (2009) who found that the levels (0, 4, 8, and 12%) of seaweed did not affect the performance of the ducks.

Schaivone *et al.*, (2007) found that using of 5g algae / kg feed insignificantly affected on the slaughter characteristics, chemical structure,

color and stability of oxidation properties and sensory of the Muscovy ducks. These results are corresponding with El Deek *et al.*, (2011) experiment.

Venkatararaman *et al.,* (1994) found that broiler dressing percentage and the weights of different organs were not affected by the addition of Spirulina algae dried powder to broiler diet.

Abou-Zeid *et al.,* (2015) noticed that diet containing 2 g spirulina/kg diet (T3) improved carcass percentage by (4.9%), front part (6.4%),and edible parts (4.4%) compared to the control group.

Sameh A.*et al.,* 2019 repoted that supplementing the rabbit diets with CLV did not induce significant differences ($p > 0.05$) in dressing percentage as compared to the control animals.

2.5.8 Effect of Chlorella powder on immunity and Antiviral activity

The intestinal flora is in close contact with immune system cells which prevent from disease and improve performance.

Previous reports (Yamada *et al.*, 2003) have shown that dietary fiber enhances IgA production in the mesenteric lymphocytes.

The reason for the increased IgA concentration in the plasma of the broiler chickens was likely the increased production of B cells due to stimulating immunoglobulin in the auto-associated lymphoid tissue (Pugh *et al.,* 2001; Kang *et al.*, 2013).

Immurella, a polysaccharide compound in the Chlorella cells, is also an important factor to enhance the immune response of broilers fed Chlorella-supplemental diets (Pesando, D. *et al.*, 1979; Pugh *et al.*, 2001).

Byoung-Ki An *et al.,* (2016) found that the antibody titers against NDV and IBV in chicks were not affected by DCP and CGF. On the other hand, the concentrations of plasma IgG were elevated in chicks fed diets containing

0.05 and 0.5 % DCP compared with those in NC or PC groups. The chicks fed diet with 0.05 and 0.15 % DCP, or 0.15 % CGF had higher concentration of plasma IgM compared with control or antibiotic groups.

It is reported that either DCP or CGF improved immune functions in rodents (An *et al.*, 2008) and chickens (Kotrbáček *et al.*, 1994).

Dietary chlorella enhanced the antibody productions of IgM and IgG by splenocytes and mesenteric lymphocytes in male Sprague–Dawley rats (Kanouchi 2001).

kang*et al.*,(2013) reported that dietary supplementation of Chlorella significantly ($P < 0.05$) increased the plasma IgA, IgM and IgG concentration of chicks compared with AGP and control.

It has been shown that dietary Chlorella supplementation enhances the immune system in humans and animals (Guzmán, S.*et al.*, 2003; Queiroz, M. L. *et al.*, 2002; Halperin, S.A. 2002).

Previous reports (Yamada, K. *et al.*, 1998; Yamada, K. *et al.*, 2003) have shown that dietary fiber and vegetable proteins from plant sources enhance IgA production in the mesenteric lymphocytes.

Plasma IgA was not altered by dietary treatments. It has been reported that dietary spirulina or chlorella-based products modulated host immune responses.

Qureshi *et al.*, (1996) suggested that White Leghorn chicks fed a diet enriched with 1%spirulina had higher antibody levels against sheep red blood cells (SRBC) compared with the control group.

Furthermore, dietary spirulina increased serum concentration of IgG and stimulated phagocytic activity in broiler chicks (Qureshi *et al.*, 1996).

The addition of 0.5 or 1.5 g CLV/kg to the rabbit diet enhanced the serum values of IgG. Meanwhile, the addition of CLV at levels 0.5, 1.0, and 1.5

g/kg diet significantly ($p < 0.05$) enhanced IgM compared to the control group (Sameh A. *et al*., 2019).

2.5.9 Effect of Chlorella powder on viscera

El Deek *et al.,* (2011) accomplished that using different levels of algae in broiler finisher diets had insignificant effect on gizzard and spleen percentages (ranged between 2.12 to 2.35% and 0.12 to 0.15%, respectively), regardless of thermal or enzymatic treatments.

Venkatararaman *et al.,* (1994) found that broiler dressing percentage and the weights of different organs were not affected by the addition of Spirulina algae dried powder to broiler diet.

Schaivone *et al.,* (2007) found that using of 5g algae / kg feed insignificantly affected on the slaughter characteristics, chemical structure, color and stability of oxidation properties and sensory of the Muscovy ducks.

Relative weights of liver were significantly lowered by dietary fermented C. vulgaris (S. T. Oh. *et al.,* 2015).

In the study of Byoung-Ki An*et al.,* (2016), dietary chlorella did not affect relative organ weights including liver, spleen, bursa of Fabricius and abdominal fat.

Zheng *et al.,* 2012 showed no effect of C. vulgaris at the inclusion rate of 1,000 or 2,000 mg/kg in diet on the relative weights of liver in 80-wk-old laying hens compared with those of the control group.

On the other hand, spleen, abdominal fat, and the breast and leg muscles of ducks when adjusted to 100 g of BW were not affected by supplementation of the fermented C. vulgaris (S. T. Oh. *et al.*, 2015).

Sameh A. *et al.,* (2019) reported that supplementing the rabbit diets with CLV did not induce significant differences ($p > 0.05$) in giblets, heart, kidney, lung, and liver as compared to the control animals.

2.5.10 Antimicrobial effect of Chlorella powder

It is well known that increased beneficial micro flora concentration in the intestine of birds may help the host with better digestion and utilization of feed components. In addition, more beneficial bacterial communities in the intestinal tract are believed to positively affect host welfare, health, and productivity (Janczyk*et al.,* 2009, 2006).

In addition, a large amount of chlorophyll and fibrous cell walls in Chlorella is an important factor to increase the beneficial lactic acid bacteria in the gut of the broiler chicks.

Janczyk *et al.,* (2009) reported that feeding Chlorella vulgaris significantly increased the lactobacilli diversity in crop and ceca of laying hens with a stronger effect on the cecal bacterial population.

It has been suggested that prebiotics generally modulate the gut environment by increasing beneficial microorganisms and inhibiting the proliferation of pathogens in the intestine (Cho and Kim, 2014; Kotrb´aˇcek *et al.,* 2015; Wang *et al.,* 2016).

In addition, Baojiang (1994) who found that Spirulina is useful for the beneficial intestinal flora.

Supplemental AGP and various forms of Chlorella did not affect the E. coli and Salmonella population in the cecal microflora of broiler chicks, but the population of Lactobacillus was significantly increased ($P < 0.05$) when birds were fed FLC (kang *et al*., 2013).

In addition, CGF, the most powerful constituent of Chlorella, works as a probiotic that enhances the growth and quality of intestinal microflora life (Abeille d'Or Corporation, 2011).

The findings by Pratt *et al.,* (1944) who reported that chlorellin, the active component in Chlorella, has an antibiotic effect.

Amaro *et al.,* (2011) who reported that methanol extracts of C. vulgaris lowered E. coli and Salmonella.

Dietary fermented C. vulgaris did not affect total microbes andlactic acid bacteria in cecal contents. However, the population of cecal coliform bacteria in ducks fed diet with 2,000 mg/kg fermented C. vulgaris tended to be lower compared with their control-diet fed counterparts (linear effect at $p = 0.064$), indicating that C. vulgaris may have a positive effect on improving cecal microflora (S. T. Oh. *et al.*, 2015).

2.5.11 Effect of Chlorella powder on Serum biochemical properties

Ginzberg *et al.,* (2000) Supplementation of layer diets with Porphyridium (a red microalga) has been shown to reduce cholesterol and increase the omega-3 content of eggs.

The increase in plasma glucose concentration of hens fed dietary Chlorella may be attributed to its excellent nutritional profile and high carotenoid content. In this regard, El-Khimsawy (1985) reported that vitamin A plays an important role for synthesis glucose molecule in the body.

Sameh A. *et al.,* (2019) reported that most of the serum parameters were non-significantly different by CLV supplementation in rabbit diets.

The results of Kanagaraju and Omprakash (2016) and SweeWeng *et al.*, (2016) found that the addition of 1% Spirulina had significantly lower serum cholesterol level than that of the control group in quails.

These results are contradictory with the findings of Kannan *et al.,* (2005), Abdel-Daim *et al.,* (2013) and AbouGabal *et al.,* (2015).

Also, Spirulina platensis supplementation at level of 1% significantly improved the blood parameters (Shanmugapriya and Saravana Babu, 2014).

This contradictory result was found due to some adverse environmental effect and heat stress during the summer season. Furthermore, Jamil *et al.*, (2015) concluded that, ALT and AST decreased significantly ($P<0.05$) when fed with Spirulina platensis compared with the control group.

In the study of Byoung-Ki An*et al.*, (2016), Blood parameters including albumin, total protein, GOT, total cholesterol, HDL cholesterol, triacylglycerol were not altered by different dietary chlorella treatments.

Previous study by Kotrbáček *et al.*, (2013), who reported that dietary DCP did not affect the concentration of plasma triacylglycerol and cholesterol in laying hens.

2.5.12 Effect of Chlorella powder on blood parameter

An *et al.*, (2010) reported that total protein, albumin, glucose, and interferon-γ were increased in blood serum of mice fed hot water extract of Chlorella.

Similarly, Kotrbácek *et al.*, (1994) reported that 0.5% biomass of fresh water Chlorella significantly enhanced the phagocytic activity of leucocytes and lymphatic tissue development of broiler chickens. This results were not consistent with the report of An *et al.*, (2010) and Kotrbácek *et al.*, (1994).

However, the numbers of WBC and lymphocytes were higher in broilers fed with dried Chlorella powder (DCP) maybe due to the processing technique and the nutritional value of Chlorella forms (Robinson *et al.*1982 and Komaki *et al., 1998)*. These results are in accordance with the earlier findings of Wakwak *et al.*, (2003), Kabir *et al.*, (2004) and Kulshreshtha *et al.*, (2008).

Supplemental AGP and Chlorella had no effect on other blood leucocytes of broiler chickens (kang *et al.*, 2013).

Sameh A. *et al*., (2019) showed that CLV0.5 had significantly higher PLT, HCT, MCH, and MCHC compared to the control and the other CLV treated rabbits.Theyalso found that the experimental group CLV1.5% showed significant white blood cells (WBC) count compared with those other CLV treated and control groups.

CHAPTER 3

MATERIALS AND METHODS

3.1 Statement of the experiment

The research work was conducted at **Sher-e-Bangla Agricultural University Poultry Farm, Dhaka**, with 120-day-old straight run (Cobb 500) commercial broilers for a period of 28 days from **06th July to 5th August, 2018** to assess the feasibility of using dried chlorella powder (DCP) in commercial broiler diet on growth performance, dressing characteristics, hematological and immune status of broilers. This research will help to make a conclusion about DCP as the alternative of antibiotic.

3.2 Collection of experimental broilers

A total of 120 day-old Cobb 500 broiler chicks were collected from Kazi hatchery, Gazipur, Dhaka.

3.3 Experimental materials

The collected chicks were carried to the university poultry farm early in the morning. They were kept in electric brooders equally for 2 days by maintaining standard brooding protocol. During brooding time only basal diet was given no DCP was used as treatment. After two days 60 chicks were selected from brooders and distributed randomly in two (2) dietary treatments of DCP; another 60 chicks were distributed randomly in one treatment for antibiotic and another treatment for control. Each treatment had three (3) replications with 10 birds per replication. The total numbers of treatments were four (4) and their replications were twelve (12).

3.4 Experimental treatments

T_1: Basal diet / Control

T_2: Basal Diets + Antibiotics (Doxivet)

T_3: 0.5% of dried chlorella powder (0.5 kg DCP/100 kg of the feeds)

T_4: 1% of dried chlorella powder (1 kg DCP/100 kg of the feeds)

Table 2. Experimental design

Treatments with Replications (10 birds/ replication)			No. of birds
T_2R_1 (n=10)	T_4R_3 (n=10)	T_1R_1 (n=10)	30
T_1R_3 (n=10)	T_3R_1 (n=10)	T_1R_2 (n=10)	30
T_3R_2 (n=10)	T_4R_2 (n=10)	T_3R_3 (n=10)	30
T_4R_1 (n=10)	T_2R_3 (n=10)	T_2R_2 (n=10)	30
Total			120

3.5 Preparation of experimental house

The experimental room was properly cleaned and washed by using 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 $1m^2$/10birds.

3.6 Experimental diets

Starter and grower commercial Kazi broiler feed were purchased from the market. Starter diet was enriched with minimum:-

Table 3. Composition of the basal diet (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%**
Cystine	**0.40%**
Tryptophan	**0.19%**
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%**
Cystine	**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 2 times daily (**Appendix 1 and 2).**

3.6.1 Collection of *Dried Chlorella Powder*

Dried *Chlorella* powder (DCP) was used in commercial basal diets. Photographs of DCP was given (Plate 1). This *Chlorella* was imported directly from USA for conducting the research work.

Table 4. Nutritional composition of *C. vulgaris* (Dry Matter Basis)

Nutrient Component	Amount
DM (Dry matter)	94.80 %
ME	3kcal/kg
CP	60.60%
Fat	12.80 g
CF	13
Ash	4.50
Lysine	4.88
Methionine	1.20
Ca	0.01
P	1.06
K	1.12
Mg	0.36
Cu	1.40 mg/kg
Fe	224.00 mg/kg
Zn	33.70 mg/kg
Vitamin A	589 IU/kg
Vitamin E	207.48 IU/kg
Thiamine (B_1)	12.90 mg/kg
Riboflavin (B_2)	45.50 mg/kg
Vitamin C	740mg/kg

Source: Data were collected from the manufacturer (Daesang Corporation, Icheon, Korea)

3.7 Management procedures

Body weight and feed intake 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 of baby chicks

The experiment was conducted during **6th July to 5th August, 2018**. The average temperature was 31.5^0C and the relative humidity was 80% in the poultry house. Common brooding was done for one week. After one week the chicks were distributed in the pen randomly. There were 10 chicks in each pen and the pen space was $1m^2$. Due to hotclimate brooding temperature was maintained as per requirement. Brooding temperature was adjusted (below 35^0C) with house temperature. So when the environmental temperature was above the recommendation, then no extra heat was provided.

At day time only an 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.

3.7.2 Room temperature and relative humidity

Daily room temperature (^{0}C) 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 3 & 4.**

3.7.3 Litter management

Rice husk was used as litter at a depth of 6cm. 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

Feed and clean fresh water was offered to the birds *ad libitum.* One feeder and one round drinker were provided in each pen for 4 birds. Feeders were cleaned at the end of each week and drinkers were washed daily. All mash dry feed was fed to all birds *ad libitum* throughout the experimental period.

3.7.5 Lighting

At night there was provision of light in the broiler farm to stimulate feed intake and body growth. For first 2 weeks 24 hours light was used. Thereafter 20 hours light and 4 hours dark was scheduled up to 28 days.

3.7.6 Bio security measures

To keep disease away from the broiler farm recommended vaccination, sanitation program was undertaken in the farm and its premises. Visitors were restricted to enter into the farm. Fencing was done to prevent the predators. Footbath was prepared at the entrance of the farm with potassium permanganate solution for disinfection. Farm premises was sanitized by using insecticide to control fly and mosquitoes. All groups of broiler chicks were supplied Vitamin B-Complex, Vitamin-ADEK, Vitamin-C, Ca and Vitamin-D enriched medicine and electrolytes.

Vaccination

The vaccines collected from medicine shop (Cevac Company) and applied to the experimental birds according to the 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)	DrinkingWater
17days	Gumboro	G-228E (inactivated) booster dose	DrinkingWater
21 days	IB + ND	MA-5 + Clone-30	DrinkingWater

3.7.7 Ventilation

The broiler shed was south facing and open-sided. Due to wire-net cross ventilation it was easy to remove polluted gases from the farm. Besides ventilation was regulated as per requirement by folding polythene screen. It was summer season and electric cooling fans were used.

3.7.8 Sanitation

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

3.8 Study Parameters

3.8.1 Recorded parameters

Weekly lives weight, weekly feed consumption and mortality of chicks were calculated to calculate survivability 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 were measured from each broiler chicken. Dressing yield was calculated for each replication to find out dressing percentage. Blood sample was analysis from each replication to measure, Complete blood count, sugar and cholesterol level. 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)

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 at random from each replicate at the 28th day of age and sacrificed to estimate dressing percent of broiler chicken. All birds to be slaughtered were fasted 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 removed 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 proventiculus and then cutting with both incoming and outgoing tracts

removed the gizzard. Dressing yield was found by subtracting blood, feathers, head, shank, liver, heart and digestive system from live weight.

3.9.6 Blood sample analysis

Blood samples (1 ml/bird) were collected into ethylene diethyletetraacitic acid (EDTA) tubes from the wing veins. Samples were transferred to the laboratory for analysis within 1 hour of collection. Glucose, Cholesterol and Complete blood count 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.

3.10.3 Feed conversion ratio

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

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 performances of broiler chicken

Broilers are among the most efficient feed converting livestock in the world. During the selection process, intensive selection pressures placed on broiler performance traits, such as increased body weight and growth rate.

4.1.1 Final Live weight

Data presented in Table 6 showed that the effect of treatments on final live weight (gram per broiler chicken) was significant ($P<0.05$). The average final live weight (g) of broiler chickens in the dietary group T_1, T_2, T_3 and T_4 were $1610.47^{b}\pm4.667$, $1627.47^{b}\pm5.667$, $1631.80^{b}\pm5.774$ and $1665.13^{a}\pm8.819$ respectively. The highest result was found in T_4 ($1665.13^{a}\pm8.819$) and lowest result was in T_1 ($1610.47^{b}\pm4.667$) group. However, Final live weight of broiler fed *chlorella* diets increased significantly ($P<0.05$) compared to that of the control and antibiotic treated groups.

These results are in agreement with those obtained by Kang *et al.,* (2013), who found that several Chlorella-based supplements including DCP, liquid media or CGF added into the diets of broiler chicks enhanced body weight. In addition, these results are in contradictory with those of previous researchers (Kotrbáček *et al.,* 1994; Peiretti and Meineri 2008; Takekoshi *et al.,* 2005) reported that dietary *chlorella* did not significantly ($P>0.05$) improved weight gain of chickens compared with the control groups. However, H. Choi *et al.,* (2017) and Abou-Zeid *et al.,* (2015) reported that birds fed dietary *chlorella* had beneficial effects on productive performance.

Table 6: Production performance of broiler chicken treated with DCP and antibiotic

Treat-ment	T_1	T_2	T_3	T_4	Mean± SE
Final live weight (g/bird)	1610.47^{b}±4.66	1627.47^{b}±5.66	1631.80^{b}±5.77	1665.13^{a}±8.81	1633.72*±6.57
FC(g)	2338.33^{a}±3.16	2281.13^{c}±10.06	2312.47^{b}±1.27	2287.30^{c} ±8.89	2304.81^{*}±7.43
FCR	1.45^{a}±0.00	1.40^{b}±0.00	1.42^{b}±0.00	1.37^{c}±0.01	1.41^{*}±0.00
DP% (Skinless)	67.51^{b}±0.28	69.03^{ab}±1.48	71.09^{a}±0.44	71.39^{a}±0.54	69.76^{*}±0.58
Survivability	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 = (0.5% DCP), T_4 = (1% DCP). Values are Mean ± S.E (n=12) 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 of significant at level of significance (P>0.05)

4.1.2 Feed consumption (FC)

Different treatment groups (Table 6) showed significant (P<0.05) differences in FC of broiler chicken. Chlorella treated T_4 (2287.30^{c} ±8.89) and antibiotic treated T_2 (2281.13^{c}±10.06) group consumed lower amount of feed, and T_1 control group consumed the highest amount of feed (2338.33^{a}±3.16) significantly (P<0.05). Antibiotic treated group T_2 consumed numerically lower amount of feed compared to T_4 group.

These results are in contrast with the findings of previous researchers

(Takekoshi *et al.*, 2005, Kang *et al.*, 2013, Abou-Zeid *et al.*, 2015) who found that DCP had no effect on feed intake between experimental groups compared with that of control group. Finding of this experiment of FC are in agreement with those of previous researchers (Kharde *et al.*, 2012; Shanmugapriya & Saravana Babu, 2014) who recorded that dietary micro algae spirulina significantly ($P<0.05$) improved Feed consumption (FC) of broiler chickens in different inclusion levels.

4.1.3 Feed Conversion Ratio (FCR)

The FCR of different treatment groups were T_1 ($1.45^{a}\pm0.00$), T_2 ($1.40^{b}\pm0.00$), T_3 ($1.42^{b}\pm0.00$) and T_4 ($1.37^{c}\pm0.01$) in table (6). Feed conversion ratio (FCR) in different groups were significantly ($P<0.05$) different and the highest FCR was in T_1 ($1.45^{a}\pm0.00$) group and lowest FCR was in chlorella treated T_4 ($1.37^{c}\pm0.01$) group (Table 6). The lowest FCR indicates best feed efficiency and highest weight.

These results are in agreement with those of previous researchers M. Rezvani *et al.*, (2012); Benites, V *et al.*, (2008); Kaoud *et al.*, (2012); Mariey *et al.*, (2012) and Mariey *et al.*, (2014) who reported that dietary chlorella significantly ($P<0.05$) improved feed efficiency of broiler chickens compared with the control groups.

The improvement of feed conversion ratio in chlorella and prebiotic treated broilers could be related to better equilibrium in the intestinal flora (Bedford *et al.*, 2000).

These results are in contradictory with those of previous researchers Peiretti and Meineri (2008); Kang *et al.*, (2013) and S. T. Oh. *et al.*, 2015) who showed that there were no significant effect on feed efficiency between the chlorella treated and control groups.

4.1.4 Dressing Percentage

The percentage of different treatment groups T_1 ($67.51^{b}\pm0.28$), T_2 ($69.03^{ab}\pm1.48$), T_3 ($71.09^{a}\pm0.44$) and T_4 ($71.39^{a}\pm0.54$) are shown in Table 6.

The T_4 (71.39^{a}±0.54) and T_3 (71.09^{a}±0.44) DCP supplemented group had greater (P<0.05) dressing percentage compared with the control (67.51^{b}±0.28) group. This findings are in accordance with the findings of El Deek *et al.*, (2011) who showed that thermal or enzymatic treatments, using different levels of algae in broiler finisher diets had significant effect on dressing percentages (ranged between 73.1 to 73.8%) at 39 days of age. These results are in contradictory with those of El Deek *et al.*, (1987) and El Deek and Brikaa (2009); Schaivone *et al.*, (2007) and Sameh A. *et al.*,(2019)Venkatararaman *et al.*, (1994) who recorded non-significant (P>0.05) effects of dietary *chlorella* supplementation on dressing percentages as compared to control group.

4.1.5 Weekly Body Weight Gain

The mean body weight gains (g) of broiler chicks at the end of 4th week in different groups were 652.50^{b}±13.61, 726.83^{a}±3.33, 659.03^{b}±1.34 and 673.50^{b}±0.00 respectively (Table 7 and Figure 1).At the end of 1st week the body weight gain in different groups were non-significant (P>0.05). T_2 group had the higher body weight gain than other group. At the end of 4th week the body weight gain in different groups were significantly different (P<0.05). T_2 group had the higher body weight gain (726.83^{a}±3.33) than other group. According to H. Choi *et al.*, (2017) broilers fed the PC_2 treatment group (1.0% EFL with chlorella) (P < 0.05) exhibited higher BWG than in the primary NC treatment group.

Table 7. Effects of feeding different level of DCP and antibiotic on body weight gain (BWG) (g/bird) of broiler chickens at different week

Treatments	1st week b. w. g	2nd week b. w. g	3rd week b. w. g	4th week b. w. g
T1	196.23±2.02	337.57^{b}±0.33	466.83^{b}±11.83	652.50^{b}±13.61
T2	198.23±1.45	342.23^{a}±1.85	497.83^{a}±1.33	726.83^{a}±3.33

T3	195.90±4.00	335.57^{b}±0.88	419.97^{c}±1.27	659.03^{b}±1.34
T4	195.57±1.20	335.23^{b}±1.20	427.50^{c}±4.58	673.50^{b}±0.00
Mean±SE	196.48^{NS}±1.083	337.65^{*}±.986	453.03^{*}±9.853	677.97^{*}±9.307

Here, T_1 = (Control), T_2 = (Antibiotic), T_3 = (0.5% DCP Supplementation), T_4 = (1% DCP Supplementation). Values are Mean ± S.E (n=12) 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
- ✓ LSD= Least Significant Difference

□ *means significant at 5% level of significance (p<0.05)

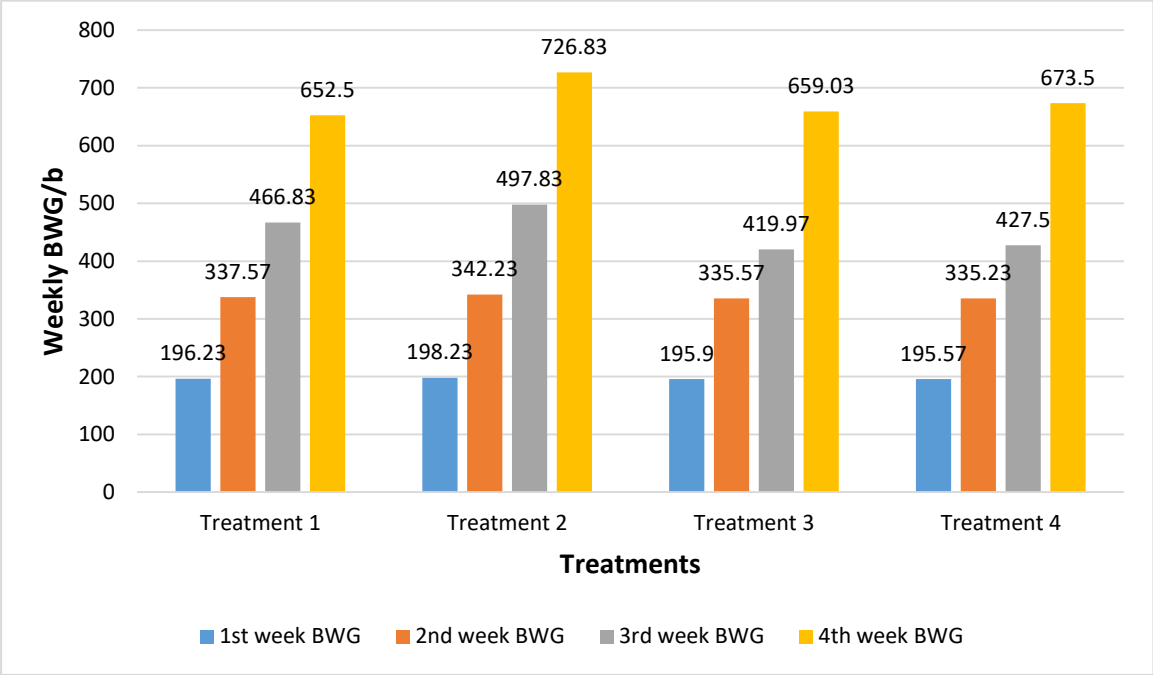

Figure 1. The Effect of supplementation DCP to broiler diets on Body Weight Gain (g/bird) of broiler chickens at different week

4.1.6 Weekly Feed consumption (FC)

The mean FC (g) of broiler chicks at the end of 4th week in different groups were 1004.80±5.56, 1000.90±15.08, 991.23±5.12 and 1008.00±4.36 respectively (figure 2). The overall mean FC of different groups showed that there were no significant (P>0.05) difference between different treatment groups. These findings are in accordance with Takekoshi *et al.*, (2005) who indicated that dietary supplementation of Chlorella (Chlorella pyrenoidosa) did not affect the feed intake of mice. Byoung-Ki An *et al.*, (2016) also showed Chicks fed diets with 0.15 or 0.5 % DCP had no effect on feed intake between experimental groups compared with that of control group.

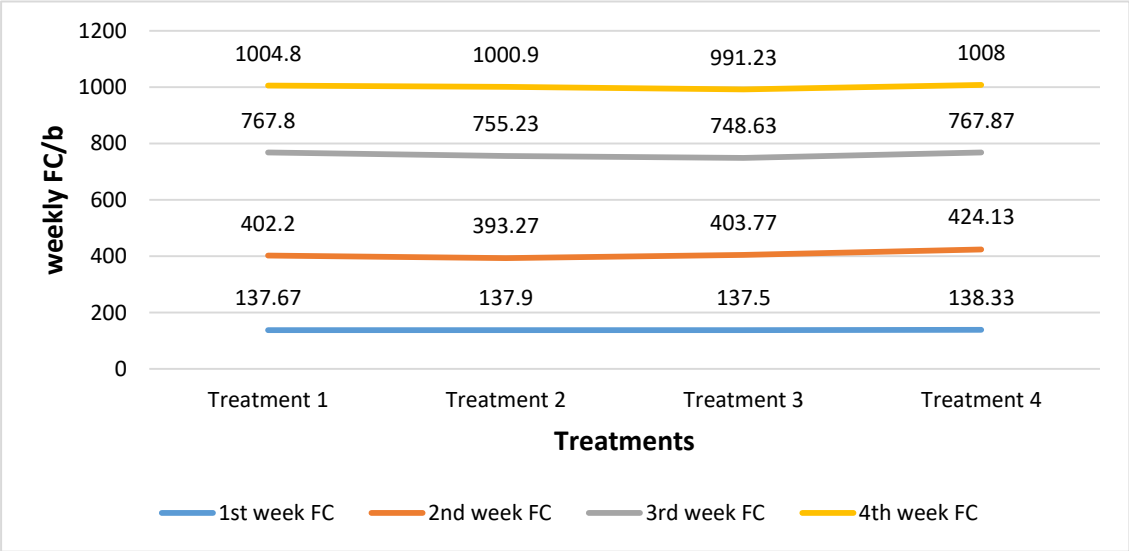

Figure 2. The Effect of supplementation of DCP to broiler diets on feed consumption (g/bird) of broiler chickens at different week.

4.1.7 Weekly Feed Conversion Ratio (FCR)

There were no significant difference found in 1st and 2nd week at different treatments. But 3rd and 4th week FCR were affected by treatments. The mean body FCR of broiler chicks at the end of 4th week in different groups were 1.55^{a}±0.02, 1.36^{b}±0.00, 1.52^{a}±0.00 and 1.49^{a}±0.02 respectively. The overall mean FCR of different groups showed that there were a significant (P<0.05) difference in groups. T_2 showed the lowest FCR compared to

control and other treatment groups (Table 8 and Figure 3).This findings are in line with the findings of Abou-Zeid *et al.*, (2015) dietary treatments improved feed conversion ratio compared to the birds fed control diet during starter, finisher and the whole experimental periods. In contrast these findings are opposite to the result of S. T. Oh. *et al.*, (2015) who reported that there were no significant effect on feed efficiency between the chlorella treated and control groups. Kang *et al.*, (2013) also reported that several chlorella-based supplements including DCP, liquid media or CGF added into the diets of broiler chicks did not affect feed conversion ratio.

Table 8. The Effects of feeding DCP and antibiotic on FCR of broiler chickens at different week.

Treatments	1st week FCR	2nd week FCR	3rd week FCR	4th week FCR
T_1	0.70 ± 0.00	1.26 ± 0.01	1.65^{b} ± 0.04	1.55^{a} ± 0.02
T_2	0.69 ± 0.01	1.18 ± 0.02	1.50^{c} ± 0.00	1.36^{b} ± 0.00
T_3	0.70 ± 0.01	1.20 ± 0.03	1.83^{a} ± 0.02	1.52^{a} ± 0.00
T_4	0.70 ± 0.00	1.17 ± 0.05	1.77^{a} ± 0.00	1.49^{a} ± 0.02
Mean± SE	0.70^{NS} ± 0.00	1.20^{NS} ± 0.01	1.69^{*} ± 0.03	1.48* ± 0.02

Here, T_1 = (Control), T_2 = (Antibiotic), T_3 = (0.5% DCP), T_4 = (1% DCP). Values are Mean ± S.E (n=12) 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)

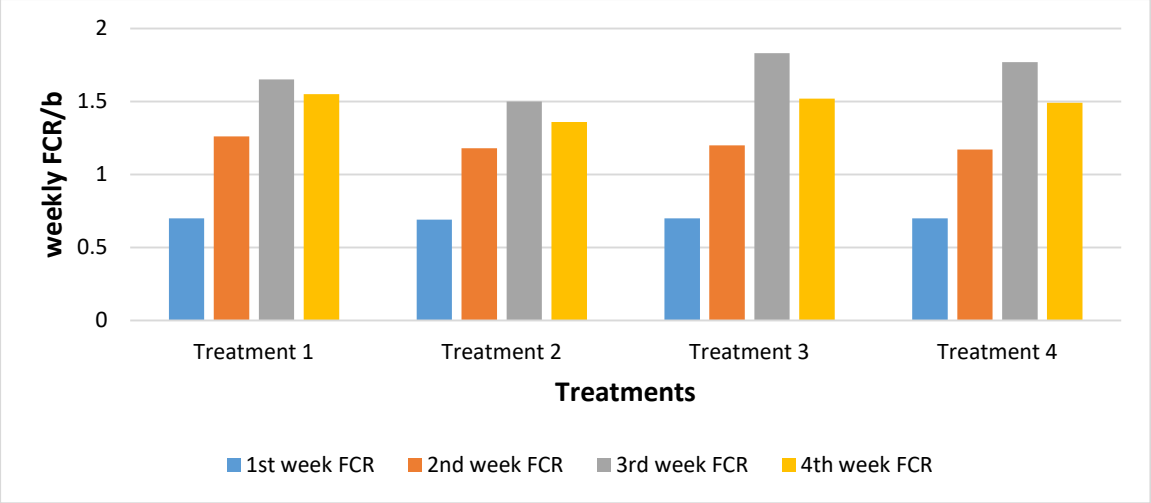

Figure 3. Effects of feeding different level of DCP and antibiotic on FCR of broiler chickens at different weeks

4.1.8 Survivability

The Survivability rate showed on table 6. There was no mortality in different groups, so survivability was (100±0.00) in all groups. no significant (P>0.05) difference was present between different group.

4.2.1 Glucose

The effects of dietary chlorella supplementation on concentration of glucose of broiler chickens are presented in Table 9 (figure 4). There were no significant (P>0.05) difference among the treatments. Although the highest amount (11.53±0.53) of plasma glucose are found in T_2 but this was not statistically different with control and other groups. The results of the present study are compatible with those observed by (Kotrbáček *et al.*,2013, Byoung-Ki An *et al.*,2016,Kannan *et al.*, 2005, Abdel-Daim *et al.*,2013) who observed incorporation of dietary chlorella in broilers diet had no significant (P>0.05)effect on serum glucose level of broiler chicken. These results are contradictory with the findings of El-Khimsawy (1985) who found an increase in plasma glucose concentration of hens fed dietary Chlorella. The increase in plasma glucose concentration of hens fed dietary Chlorella may be attributed to its excellent nutritional profile and high carotenoid content.

4.2.2 Cholesterol

Total cholesterol concentration (mg/dl) in the serum of T_1, T_2, T_3 and T_4 groups are 215.33±33.01, 214.67±10.17, 187.33±10.41 and 189.33±14.11 respectively. Statistical analysis revealed no significant (P>0.05) difference among the group (Table 9, figure 4). However the cholesterol level was lower in T_3 (187.33±10.41) and T_4 (189.33±14.11) fed group numerically but not statistically significant. Similar results had also been observed by Kotrbáček *et al.*, (2013), who reported that dietary DCP did not affect the concentration of plasma triacylglycerol and cholesterol in laying hens.

Other studies of (Ginzberg et al., 2000; Kanagaraju and Omprakash 2016) had shown contradictory result that chlorella reduce cholesterol and increase the omega-3 content of eggs.

Table 9. The Effect of supplementing DCP to broiler diets on serum biochemical level

Parameters	T1	T2	T3	T4	Mean±SE
Glucose(mmol/L)	11.13±0.59	11.53±0.53	10.80±0.40	10.63±0.44	11.02^{NS}±0.23
Cholesterol(mg/dl)	215.33±33.01	214.67±10.17	187.33±10.41	189.33±14.11	201.67^{NS}±9.18

Here, T_1 =Control, T_2 = Antibiotic, T_3 = 0.5% DCP, T_4 =1% DCP. Values are Mean ± S.E (n=12) 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)

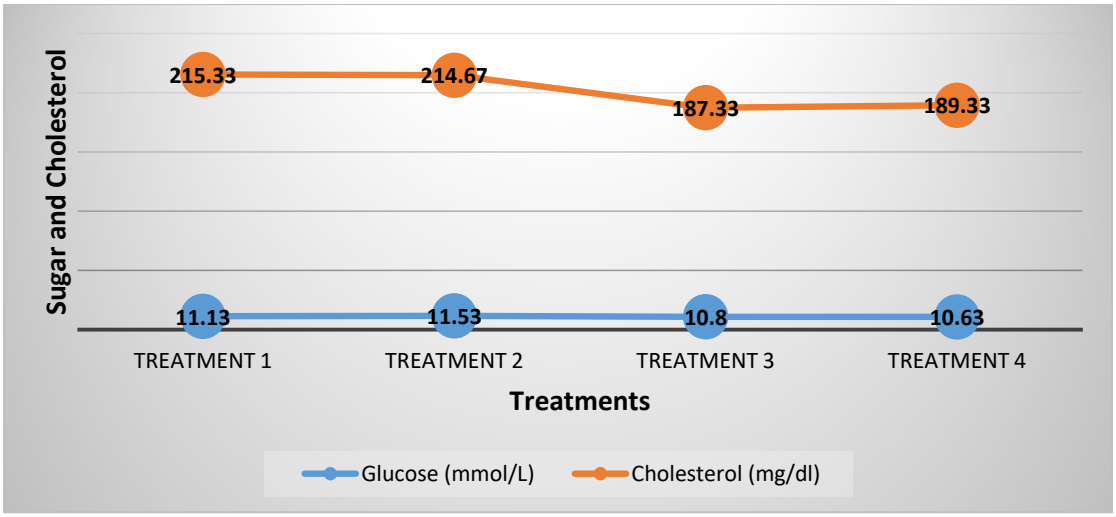

Figure 4. Effect of DCP on Serum biochemical level of different broiler chicken under different treatment

4.3.1 Relative weight of liver, gizzard, intestine and heart

The relative weight of liver (g) of broiler chicks in the dietary group T_1, T_2, T_3 and T_4 were 37.33±.167, 38.87±.318, 40.13±.913 and 38.50±1.528 respectively. The highest results were in T_3 and lowest was in T_1 group. However, there were no significant (P>0.05) difference in the relative weight of liver between the groups (Table 10). These results are in line with the findings of Byoung-Ki An et al., (2016) who reported that dietary chlorella did not affect relative organ weights including liver, spleen, bursa of Fabricius and abdominal fat.

The comparative weight of gizzard of different groups did not show any significant (P>0.05) difference in groups (Table 10). Other researchers Venkatararaman *et al.*, (1994), El Deek *et al.*, (2011) also accomplished that using different levels of algae in broiler finisher diets had insignificant (P>0.05) effect on gizzard weights.

The comparative weight of heart (g) of broiler chicks in the dietary group T_1, T_2, T_3 and T_4 were 9.33±0.60, 9.50±0.50, 10.50±0.00 and 9.17±0.72 correspondingly. The quantified weight of heart of different groups showed

that there were no significant (P>0.05) difference between the groups (Table 10). Sameh A. *et al.*, (2019) also reported that supplementing the rabbit diets with *Chlorella vulgaris* did not induce significant differences (p > 0.05) in giblets, heart, kidney, lung, and liver as compared to the control animals.

It means chlorella having antimicrobial and antibiotics properties have no influence on either increasing or decreasing the relative weights of some internal organ.

4.3.2 Intestine weight

The intestine weight of different groups showed that there were no significant (P>0.05) difference between the groups and the values were ranged from 84.67±.882 to 92.67±4.372 (Table 10). In the study of Byoung-Ki An*et al.*, (2016), they showed chlorella had no impact on visceral organs (liver, heart, gizzard, and intestines) of broiler chicks.

Table 10. Effect of dietary supplementation of DCP on Liver, Gizzard, Intestine and heart weight of different Treatment.

Parameters	T_1	T_2	T_3	T_4	Mean±SE
Liver weight(g)	37.33±.167	38.87±.318	40.13±.913	38.50±1.528	38.71^{NS}±.490
Gizzard Weight (g)	36.67±.333	38.33±.882	38.33±.441	38.50±.577	37.96^{NS}±.340
Intestine weight (g)	84.67±.882	90.00±3.786	91.00±4.000	92.67±4.372	89.58^{NS}±1.760
Heart(g)	9.33±.601	9.50±.500	10.50±.000	9.17±.726	9.62^{NS}±.276

Here, T_1 = (Control), T_2 = (Antibiotic), T_3 = (0.5% DCP), T_4 = (1% DCP). Values are Mean ± S.E (n=12) 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)

4.4 Immune organs

The effect of different level DCP supplementation on immune organs of Cobb 500 strain broiler chicks during the period from 0 to 28 days of age are summarized in Figure 5.

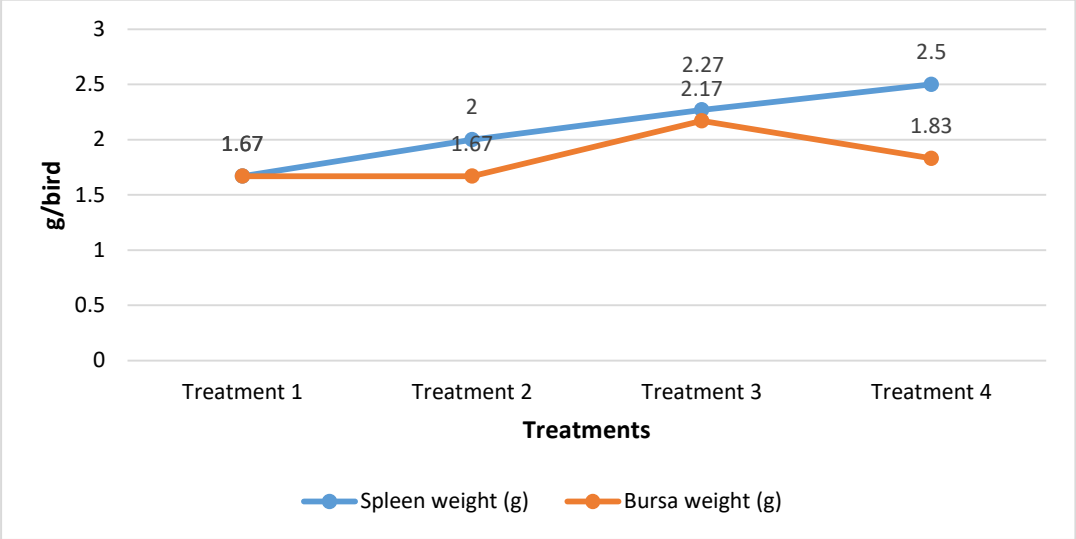

Figure 5: The Effect of supplementation different level of DCP to broiler diets on some immune organs.

The comparative weight of spleen (g) of broiler chicks in the dietary group T_1, T_2, T_3 and T_4 were 1.67±.441, 2.00±.500, 2.27±.267 and 2.50±.000 respectively. The highest value was T_4 (2.50±.000) and lowest value was T_1 (1.67±.441). On the other hand, the relative weight of spleen of different groups showed that there were no significant (P>0.05) difference.

The weight of bursa was higher in T_3 group (2.17±.333) compared to the

other group which values were T_1 (1.67±.167), T_2 (1.67±.167) and T_4 (1.83±.167) respectively. But these values are not significantly differing among the treatments (Figure-5). Other researchers Byoung-Ki An*et al.*, (2016) reported that dietary chlorella did not affect relative organ weights including spleen, bursa of Fabricius and abdominal fat.Schaivone *et al.*, (2007) also found that using of 5g algae / kg feed insignificantly affected on the slaughter characteristics of the Muscovy ducks.

4.5 Hematological parameters

Tables (11) show the effect of dietary levels of DCP (0.5% and 1%) in feed, and their impact on some blood parameters. Concerning the treatment effect on blood constituents, the results indicated no significant differences due to supplementation of DCP, except Hemoglobin and RBC which were significantly affected ($p<0.05$).Birds fed diets supplemented with chlorella (at levels of 0.5% and 1%) diet had higher values of Hemoglobin and RBC but in case of control group these trends are lower than chlorella treated groups.

Table 11. Effect of supplementation of DCP to broiler diets on blood parameters

Parameters	T1	T2	T3	T4	Mean±SE
Hemoglobin(g/dl)	8.98^{b}±.117	9.14^{ab}±.211	9.78^{a}±.245	9.39^{ab}±.254	9.32^{*}±.114
RBC	3.47^{b}±.277	4.39^{a}±.199	4.20^{a}±.127	4.49^{a}±.283	4.14^{*}±.129
WBC	7.44±.338	7.67±.167	7.56±.294	8.00±.333	7.67^{NS}±.144
Neutrophil	71.89±1.654	69.78±1.289	70.89±1.419	71.33±1.269	70.97^{NS}±.689
Lymphocyte	62.33±3.859	66.11±4.724	73.22±3.756	70.22±4.361	67.97^{NS}±2.122
Monocyte	1.52±.081	1.55±.103	1.58±.112	1.44±.131	1.52^{NS}±.052
Eosinophil	1.50±.056	1.59±.059	1.56±.049	1.55±.061	1.55^{NS}±.028
PCV	28.66±.940	30.01±.940	30.14±.958	30.06±.957	29.72^{NS}±.465
MCV	78.46±2.776	81.81±1.499	81.77±.969	81.52±1.330	80.89^{NS}±.883
MCH	30.43±.372	31.15±.484	30.13±.497	30.76±.599	30.62^{NS}±.245
MCHC	31.65±.443	31.14±.447	31.22±.313	31.27±.367	31.32^{NS}±.192

Here, T_1 = (Control), T_2 = (Antibiotic), T_3 = (0.5% DCP Supplementation), T_4 = (1% DCP supplementation). Values are Mean ± S.E (n=12) 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
- ✓ LSD= Least Significant Difference
- ✓ * means significant at 5% level of significance (p<0.05)

These results are in agreement with the earlier findings of An *et al.,* (2010) reported that total protein, albumin, glucose, and interferon-γ were increased in blood serum of mice fed hot water extract of Chlorella.

Findings of Kotrbácek *et al.,* (1994) reported that 0.5% biomass of fresh water Chlorella significantly enhanced the phagocytic activity of leucocytes and lymphatic tissue development of broiler chickens.These results are in accordance with the earlier findings of Wakwak *et al*., (2003), Kabir *et al.*, (2004) and Kulshreshtha *et al.,* (2008). In contrast, kang *et al.,* (2013) reported that supplemental AGP and Chlorella had no effect on blood leucocytes of broiler chickens.

4.6 Intestinal microflora

The microbial load in gut of broilers fed different levels of chlorella is given in Table 12. E. coli count was significantly (P<0.05) decreased in birds fed 0.5%, 1% chlorella and antibiotic (11.00^{b}±.299, 11.23^{b}±.438 and 11.68^{b}±.344 respectively) than the control birds (15.58^{a}±.879). Salmonella sp. count was also significantly (P<0.05) decreased in birds fed 0.5%, 1% DCP and antibiotic (5.70^{b} ±1.548, 4.66^{b}±1.672 and 9.03^{b} ±1.333 respectively) than the control birds(14.46^{a} ±1.247).Lactobacillus count was significantly (P<0.05)increased in birds fed 0.5% and 1% chlorella. The highest number of lactobacillus was counted in T_4 group (19.76^{a}±.382) and

the lowest in T_1 control group (11.70^{d} ±.331).

Table 12. Bacterial colony count in DCPexperiment in broiler chicken

Parameters	E.coli × 10^6 (CFU/ml)	Salmonella × 10^6 (CFU/ml)	Lactobacillus × 10^6 (CFU/ml)
T_1	15.58^{a}±0.87	14.46^{a} ±1.24	11.70^{d} ±0.33
T_2	11.68^{b}±0.34	9.03^{b} ±1.33	14.98^{c}±0.76
T_3	11.00^{b}±0.29	5.70^{b} ±1.54	18.07^{b}±0.48
T_4	11.23^{b}±0.43	4.66^{b}±1.67	19.76^{a}±0.38
Mean±SE	12.37^{*}±0.40	8.46*±0.95	16.12^{*} ±0.57

Here, T_1 = (Control), T_2 = (Antibiotic), T_3 = (0.5% DCP Supplementation), T_4 = (1% DCP Supplementation). Values are Mean ± S.E (n=12) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
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- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference
- ✓ * means significant at 5% level of significance (p<0.05)

These results of the experiment are in accordance with the earlier findings of Janczyk *et al.*, (2009) who reported that feeding Chlorella vulgaris significantly increased the lactobacilli diversity in crop and ceca of laying hens with a stronger effect on the cecal bacterial population. Amaro *et al.*, (2011) reported that methanol extracts of C. vulgaris lowered E. coli and Salmonella. The findings by Pratt *et al.*, (1944) reported that chlorellin, the active component in Chlorella, has an antibiotic effect. However, the population of cecal coliform bacteria in ducks fed diet with 2,000 mg/kg fermented C. vulgaris tended to be lower compared with their control-diet

fed counterparts (linear effect at p = 0.064), indicating that C. vulgaris may have a positive effect on improving cecal microflora (S. T. Oh. *et al.*, 2015).

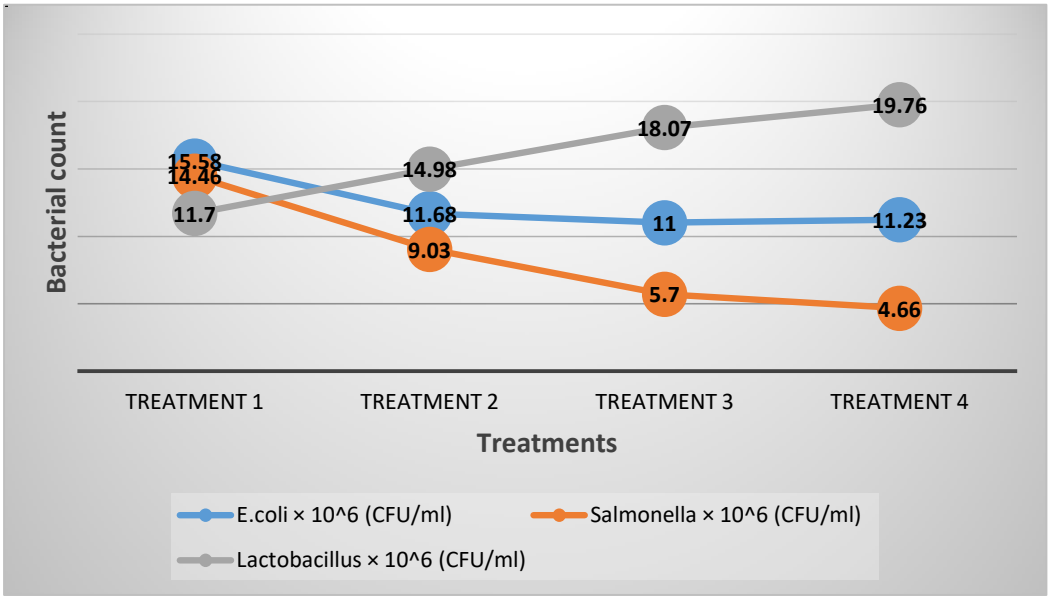

Figure 6: The Effect of supplementation different level of DCP to broiler diets on Intestinal microflora

4.7 Antiviral activity

Tables (13) show the effect of dietary levels of DCP (0.5%, and 1%) in feed, and their impact on haemagglutination inhibition titre against Newcastle disease (ND). Concerning the treatment effect on HI titre the results indicated significant ($p<0.05$) differences due to supplementation of DCP. Remarkably better titres of ND achieved in blood at day 15 T_3 ($5.89^{a}\pm$ 0.26) and T_4 ($5.56^{a}\pm$ 0.24), at day 20 T_3 ($4.00^{a}\pm$ 0.23) and T_4 ($3.78^{ab}\pm$ 0.22) and day 29 T_3 ($6.67^{a}\pm$ 0.23) and T_4 ($6.89^{a}\pm$ 0.26) treatments compare to control group.

It is reported that either DCP or CGF improved immune functions in rodents (An *et al.*, 2008) and chickens (Kotrbáček *et al.*, 1994). kang*et al.*, (2013) reported that dietary supplementation of Chlorella significantly ($P < 0.05$) increased the plasma IgA, IgM and IgG concentration of chicks compared with AGP and control.In contrast, these results are contradictory

with the earlier findings of Byoung-Ki An*et al.*, (2016) who found that the antibody titers against NDV and IBV in chicks were not affected by DCP and CGF. Qureshi *et al.*, (1996) suggested that White Leghorn chicks fed a diet enriched w ith 1% spirulina had higher antibody levels against sheep red blood cells (SRBC) compared with the control group.

Table 13. Effect of DCP on ND HI Titre in broiler chicken

Parameters	Day 15 (log^2)	Day 20 (log^2)	Day 29 (log^2)
T_1	3.78^{b}± 0.22	3.11^{b}± 0.26	5.22^{c}± 0.22
T_2	4.56^{b}± 0.37	3.44^{ab}± 0.17	5.89^{b}± 0.20
T_3	5.89^{a}± 0.26	4.00^{a}± 0.23	6.67^{a}± 0.23
T_4	5.56^{a}± 0.24	3.78^{ab}± 0.22	6.89^{a}± 0.26
Mean±SE	4.94^{*} ±0.19	3.58^{*}± 0.12	6.17^{*}± 0.15

Here, T_1 = (Control), T_2 = (Antibiotic), T_3 = (0.5% DCP Supplementation), T_4 = (1% DCP Supplementation). Values are Mean ± S.E (n=12) 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
- ✓ LSD= Least Significant Difference
- ✓ * means significant at 5% level of significance (p<0.05)

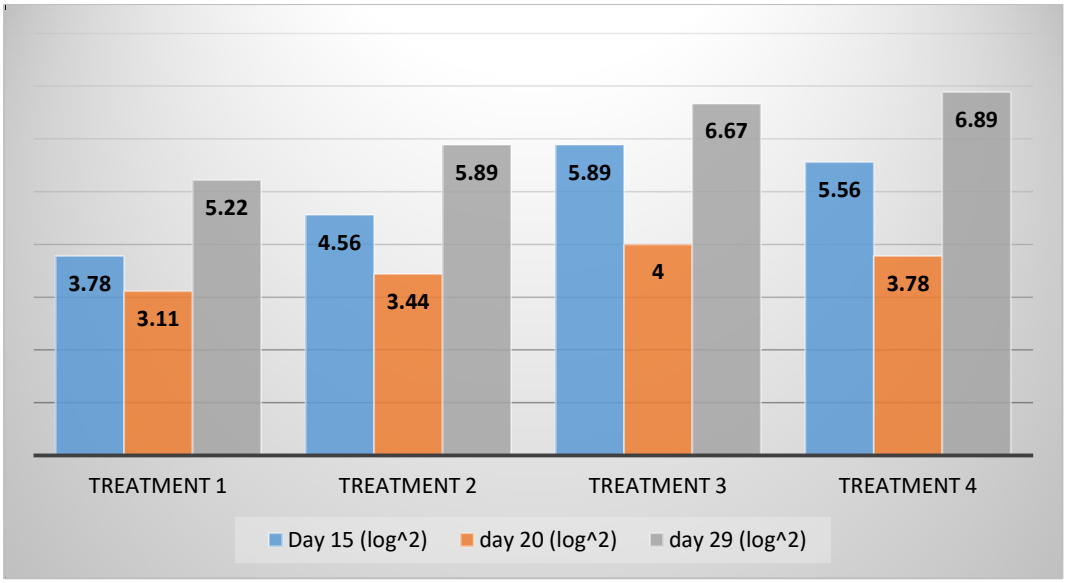

Figure 7: The Effect of supplementation different level of DCP to broiler diets on ND HI Titre

Immurella, a polysaccharide compound in the Chlorella cells, is also an important factor to enhance the immune response of broilers fed Chlorella-supplemental diets (Pesando, D. *et al.*, 1979; Pugh *et al.*, 2001).

CHAPTER 5

SUMMARY AND CONCLUSION

A total of 120 day-old Cobb-500 broiler chicks were reared in Sher-e-Bangla Agricultural University Poultry Farm, Dhaka. Chicks were divided randomly into 4 experimental groups of 3 replicates (10 chicks with each replications).One of the 4 experimental group was fed this diet as control while, the remaining three groups were fed diet with 2 levels of DCP (0.5%, and 1.0%) and antibiotic.

The effects of supplementation of DCP and antibiotic were measured. The performance traits *viz*. body weight, weight gain, feed consumption, FCR, dressed bird weight, relative giblet weight, survivability and meat yield of broiler on different replication of the treatments was recorded and compared in each group. At 28 days of age, 36 broilers were dissected to compare meat yield characteristics among different treatments. The group T_4 showed higher body weight ($1665.13^{a}\pm8.81$) compared to any other groups and group T_3, group T_2 and group T_1 followed in ascending order. The weekly body weight gain at the end of 4^{th} week was significantly higher in T_2 ($726.83^{a}\pm3.33$) group than other groups. Feed consumption was significantly higher ($2338.33^{a}\pm3.167$) in control group and lower in DCP treated group but weekly feed consumption was non-significant ($P>0.05$) between different groups. Final FCR was significant among different groups. FCR was better in T_4 DCP supplemented group compared to the T_1control group. The weekly FCR at 1^{st} and 2^{nd} week were insignificant but at the end of 3^{rd} and 4^{th} week T_2 group showed significantly ($P<0.05$) lower FCR than any other group. The relative giblet weight did not show any significant ($P>0.05$) difference between any of the treatment groups. The serum biochemistry parameters *viz*. sugar and total cholesterol was studied to evaluate the functional status of the body. The sugar in T_1 (11.13±0.59), T_2 (11.53±0.53), T_3 (10.80±0.40) and T_4 (10.63±0.44) and cholesterol level in T_1 (215.33±33.01), T_2 (214.67±10.17), T_3 (187.33±10.41) and T_4

(189.33±14.11) of different treatments were non- significant (P>0.05). The results indicated no alterations in biochemical parameters, except that a lower amount was observed in cholesterol levels in *Chlorella* supplemented groups. Weight of immune organs in different treatments were not significantly (P>0.05) affected by different treatments. Concerning the treatment effect on blood constituents, the results indicated no significant differences due to supplementation of dried Chlorella powder, except Hemoglobin and RBC, which were significantly affected (p<0.05). birds fed diets supplemented with dried *Chlorella* powder (at levels of 0.5% and 1%) diet had higher values of Hemoglobin (9.78^{a}±0.24 and 9.39^{ab}±0.25) and RBCs T_4 (4.49^{a}±0.28)but in case of antibiotic and control group this trends is lower than *Chlorella* treated groups.

The numbers of intestinal microflora (*E coli* and *Salmonella*) were significantly (P<0.05) higher in control group compared to other groups. However, *E coli* and Salmonella count had no significant difference between DCP and antibiotic supplementing groups. The number of Lactobacillus was significantly (P<0.05) highest in T_4 DCP supplemented group compared to control and antibiotic groups. ND HI titre level also significantly (P<0.05) higher in 1% DCP treated group as compared to control group.

Analyzing the above research findings the production performance, hematological parameter, weight of lymphatic organ, microbial load in feaces sample and ND HI titre, 1% dried chlorella powder was very effective. So Chlorella could be used as an alternative to antibiotics on broiler ration. The superior results were found at 1% inclusion level of DCP. The study therefore recommends conducting field trial on commercial poultry farm to fix up periodic examination of Chlorella.

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APPENDICES

Appendix 1. Recommended level of nutrients for broiler

Components	Starter	Grower
ME (kcal/kg)	3000	3100
% CP	22	20
% Ca	1.0	0.85
% P (Available)	0.5	0.4
% Lysine	1.2	1.0
% Methionine	0.5	0.45
% Tryptophane	0.21	0.18

Source: Cobb500 Broiler Management Guide, 2016

Appendix 2. Nutrient composition of the ingredients used to formulate experimental diets

Ingredients	DM (%)	ME (K. Cal/kg)	CP (%)	CF (%)	Ca (%)	P (%)	Lys (%)	Meth (%)	Tryp (%)
Soybean meal	90	2710	44.50	7.5	0.26	0.23	2.57	0.76	0.57
Maize	89.5	3309	9.2	2.4	0.25	0.40	0.18	0.15	0.09
DCP					22	17.21			
Soybean oil	100	8800							
Protein concentrate (Jeso-prot)	91.64	2860	63.30	8.1	6.37	3.24	3.87	1.78	.53
Meat and Bone meal	95.5	1044	14.6	2.5	7.0	12.11	.66	0.24	0.12

Source: Cobb500 Broiler Management Guide, 2016

Appendix 3. Recorded temperature (^{0}C) during experiment

Age in weeks	Room temperature (^{0}C)							
	Period	8 A.M	12A.M	4 P.M.	8 P.M.	12 P.M.	4 A.M	Average
1st	06.07.18-12.07.18	28.9	29.5	31.6	31.5	30.0	29	30.08
2nd	13.07.18-19.07.18	28.3	28.5	32.1	31.6	30.2	28.5	29.87
3rd	20.07.18-26.07.18	27.0	27.2	28.8	27.2	26.0	25.8	27.00
4th	27.07.18-02.08.18	26.8	27.0	28.6	28.5	27.4	27.2	27.58

Appendix 4. Relative humidity (%) during experiment

Age in weeks	Relative humidity (%)							
	Period (day)	8 A.M	12A.M	4 P.M.	8 P.M.	12 P.M.	4 A.M	Average
1st	06.07.18-12.07.18	85	82	73	74	78	80	78.67
2nd	13.07.18-19.07.18	85	83	71	72	77	79	77.83
3rd	20.07.18-26.07.18	86	85	74	75	81	83	80.67
4th	27.07.18-02.08.18	87	86	83	77	84	86	83.83

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

Treatment	Replication	Live Weight (g)	Eviscerated Weight (g)	Dressing Percentage (%)
T_1	R_1	1601.8	1080.7	67.46785
	R_2	1611.8	1080.5	67.03685
	R_3	1617.8	1100.5	68.02448
T_2	R_1	1621.8	1090.67	67.25059
	R_2	1638.8	1114.56	68.01074
	R_3	1621.8	1165	71.83376
T_3	R_1	1641.8	1181.5	71.9637
	R_2	1621.8	1148.6	70.82254
	R_3	1631.8	1150.34	70.49516
T_4	R_1	1661.8	1203.4	72.41545
	R_2	1681.8	1197.4	71.19753
	R_3	1651.8	1165.5	70.55939

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

Treatments	Replication	Liver weight	Spleen weight	Heart weight	Intestine Weight	Gizzard Weight	Bursa Weight
T_1	R_1	37.5	1	9	85	37	1.5
	R_2	37	2.5	8.5	86	37	1.5
	R_3	37.5	1.5	10.5	83	36	2
T_2	R_1	38.5	1	9	97	37	2
	R_2	39.5	2.5	10.5	84	38	1.5
	R_3	38.6	2.5	9	89	40	1.5
T_3	R_1	41.5	2	10.5	83	39	2.5
	R_2	38.4	2	10.5	95	38.5	2.5
	R_3	40.5	2.8	10.5	95	37.5	1.5
T_4	R_1	35.5	2.5	8	98	39.5	1.5
	R_2	39.5	2.5	9	84	38.5	2
	R_3	40.5	2.5	10.5	96	37.5	2

Appendix 7. Biochemical data in different treatment groups

Treatments	Replications	Glucose (mmol/L)	Cholesterol (mg/dL)
T_1	R_1	12.0	202
	R_2	11.4	278
	R_3	10	166
T_2	R_1	12.3	204
	R_2	11.8	205
	R_3	10.5	235
T_3	R_1	10	206
	R_2	11.1	170
	R_3	11.3	186
T_4	R_1	10.0	216
	R_2	11.5	184
	R_3	10.4	168

Appendix 8. Results of Complet blood count (CBC) under different treatment groups.

Treatments	Replications	Hb (g/dl)	RBC (Million /Cumm)	WBC	Neut Rophil/Cumm	Lymphocyte	Mono Cyte	Eosinophil	HCT/ PCV	MCV	MCH	MCHC
T_1	R_1(1)	9.00	4.30	7,100	69	45	1.70	1.50	29.50	59.21	31.19	31.40
	R_1(2)	8.60	4.10	8,100	84	75	1.60	1.77	25.50	82.21	29.19	33.46
	R_1(3)	9.50	3.65	6,200	72	62	1.06	1.50	32.60	80.52	31.25	32.30
	R_2(1)	8.40	3.20	8,200	69	72	1.45	1.62	28.50	79.21	29.19	32.11
	R_2(2)	9.10	3.50	9,200	73	75	1.40	1.45	27.50	86.21	29.09	31.51
	R_2(3)	8.80	4.80	8,200	70	52	1.33	1.66	26.10	80.25	32.25	28.85
	R_3(1)	9.40	2.50	8,800	67	57	1.85	1.20	28.50	86.21	31.09	30.51
	R_3(2)	9.02	2.50	6,600	73	51	1.76	1.35	33.50	72.21	30.09	32.51
	R_3(3)	9.02	2.65	7,800	70	72	1.54	1.48	26.20	80.15	30.50	32.18

	Appendix 8 (Cont'd)											
T_2	$R_1(1)$	8.85	3.86	8,200	76	84	1.75	1.65	30.28	84.23	28.69	32.50
	$R_1(2)$	9.75	3.76	7,900	65	85	1.05	1.75	26.85	84.22	31.86	31.74
	$R_1(3)$	9.52	4.85	7,700	69	55	1.64	1.20	34.45	79.16	32.45	28.22
	$R_2(1)$	8.01	4.52	8,600	72	65	1.42	1.64	32.86	79.52	28.76	31.62
	$R_2(2)$	8.65	4.06	8,300	66	52	1.58	1.75	29.56	72.16	32.06	32.32
	$R_2(3)$	9.20	5.12	8,500	68	48	1.78	1.70	27.87	80.56	31.86	30.32
	$R_3(1)$	9.86	5.01	8,600	75	78	1.06	1.50	32.82	85.65	32.25	31.88
	$R_3(2)$	9.74	4.83	8,200	67	72	1.86	1.48	26.62	84.21	31.64	31.42
	$R_3(3)$	8.68	3.54	7,800	70	56	1.78	1.67	28.75	86.56	30.78	30.28
T_3	$R_1(1)$	10.95	3.90	8,500	72	75	1.62	1.75	33.40	84.27	32.04	31.58
	$R_1(2)$	10.03	3.98	9,200	74	63	1	1.45	26.60	78.27	27.07	32.12
	$R_1(3)$	10.01	4.28	8,700	77	84	1.65	1.70	29.50	80.25	31.15	31.32
	$R_2(1)$	10.00	4.20	7,900	71	52	1.05	1.50	34.50	85.62	29.23	30.25
	$R_2(2)$	10.02	4.14	8,600	76	74	1.85	1.65	29.36	82.95	30.24	30.12
	$R_2(3)$	9.40	3.75	7,500	68	83	1.56	1.30	28.10	80.15	30.25	31.46
	$R_3(1)$	8.25	4.25	6,600	69	65	1.76	1.58	27.25	78.50	30.22	32.12
	$R_3(2)$	10.01	4.22	7,500	65	86	1.90	1.45	29.32	80.25	31.65	32.25
	$R_3(3)$	9.35	5.10	8,500	66	77	1.86	1.68	33.25	85.63	29.35	29.80
T_4	$R_1(1)$	8.01	2.56	6,800	68	85	1.05	1.20	29.35	79.26	29.62	31.26
	$R_1(2)$	9.85	3.85	8,800	72	76	1.85	1.38	28.42	78.21	32.33	29.56
	$R_1(3)$	10.02	4.56	9,500	77	82	1.76	1.45	30.24	86.76	30.46	32.50
	$R_2(1)$	10.01	4.95	9,600	75	56	1.05	1.75	32.50	85.37	27.22	32.48
	$R_2(2)$	9.95	5.12	8,700	66	49	1.06	1.67	34.28	86.95	29.64	30.42
	$R_2(3)$	8.52	5.13	7,900	69	58	1.05	1.56	33.62	82.45	32.47	31.82
	$R_3(1)$	8.76	4.21	8,700	75	68	1.88	1.75	27.00	76.28	30.38	32.42
	$R_3(2)$	9.45	4.88	9,500	72	82	1.45	1.66	26.06	78.64	32.25	30.15
	$R_3(3)$	9.90	5.11	8,200	68	76	1.85	1.52	29.08	79.80	32.46	30.84

Appendix 9. Feed consumption (g/bird) of 1st, 2nd, 3rd and 4th week under different treatments

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	R_1	137	413.5	769.5	1014
	R_2	138.5	426.7	768.8	1010.5
	R_3	139.5	432.2	765.3	999.5
T_2	R_1	134.5	392.1	752.4	982
	R_2	139.5	400.7	751.5	999.7
	R_3	138.5	418.5	742	992
T_3	R_1	136	375.9	783.1	1015.9
	R_2	139	415.3	758.7	998.5
	R_3	138	415.4	761.6	1000
T_4	R_1	137.2	362	777	1028.8
	R_2	138	399.7	742.3	996.9
	R_3	138.5	418.1	746.4	977

Appendix 10. Body weight (g/bird) of 1st, 2nd, 3rd and 4th week under different treatments.

Treatments	Replications	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	R_1	192.9	337.9	454.5	646.5
	R_2	199.9	337.9	455.5	678.5
	R_3	195.9	336.9	490.5	632.5
T_2	R_1	197.9	339.9	500.5	723.5
	R_2	195.9	345.9	496.5	723.5
	R_3	200.9	340.9	496.5	733.5
T_3	R_1	187.9	333.9	418.9	661.1
	R_2	199.9	336.9	418.5	656.5
	R_3	199.9	335.9	422.5	659.5
T_4	R_1	194.9	336.9	436.5	673.5
	R_2	193.9	332.9	421.5	673.5
	R_3	197.9	335.9	424.5	673.5

Appendix 11: Caecal microbial load (total viable count) of broiler under different treatment groups at 4th weeks of age.

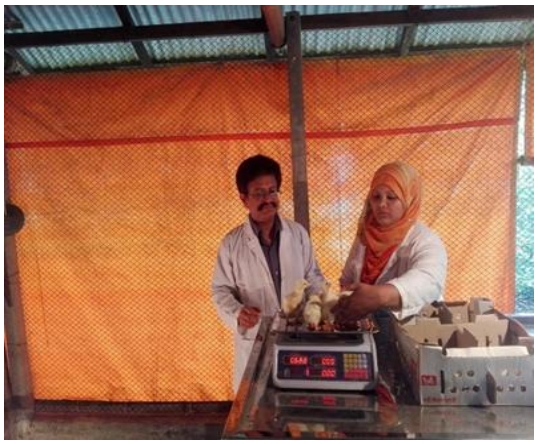
Treatment	Replication	E.coli×10^6 (CFU/ml)	Salmonella×10^6(CFU/ml)	Lactobacillus×10^6 (CFU/ml)
T_1	R_1 (1)	15.8	18.1	13.0
	R_1(2)	17.7	19.6	11.2
	R_1(3)	12.0	10.8	12.0
	R_2(1)	18.4	11.7	11.7
	R_2(2)	15.0	14.6	10.9
	R_2(3)	17.0	10.2	13.3
	R_3(1)	13.1	14.7	11.1
	R_3(2)	12.3	19.2	10.2

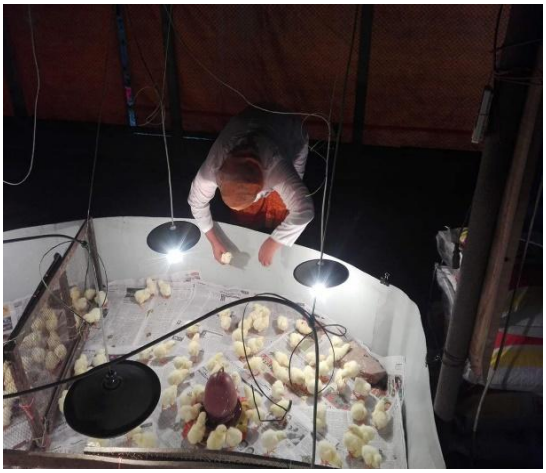
	Appendix 11 (Cont'd)			
	$R_3(3)$	18.9	11.2	11.9
T_2	$R_1(1)$	10.9	9.5	15.6
	$R_1(2)$	11.9	10.7	13.8
	$R_1(3)$	12.0	5.0	18.2
	$R_2(1)$	12.7	7.2	13.6
	$R_2(2)$	13.5	9.8	11.8
	$R_2(3)$	10.9	1.2	17.8
	$R_3(1)$	11.9	13.6	16.0
	$R_3(2)$	11.2	13.4	12.1
	$R_3(3)$	10.1	10.9	15.9
T_3	$R_1(1)$	10.8	10.2	20.3
	$R_1(2)$	12.6	9.2	18.3
	$R_1(3)$	11.3	3.4	16.8
	$R_2(1)$	12.0	0.0	15.6
	$R_2(2)$	10.8	9.6	17.5
	$R_2(3)$	10.5	0.0	18.9
	$R_3(1)$	9.6	1.9	17.6
	$R_3(2)$	11.1	5.0	19.8
	$R_3(3)$	10.3	12.0	17.8
T_4	$R_1(1)$	11.7	12.4	18.6
	$R_1(2)$	10.6	0.0	20.4
	$R_1(3)$	12.3	4.6	19.4
	$R_2(1)$	11.1	0.0	17.8
	$R_2(2)$	9.0	5.4	20.9
	$R_2(3)$	12.8	10.8	19.7
	$R_3(1)$	10.7	0.0	19.5
	$R_3(2)$	12.9	8.7	19.9
	$R_3(3)$	10.0	0.0	21.6

Appendix 12: ND HI titre level of broiler under different treatment groups at 15th, 20th and 29thday of age.

Treatment	Replication	Day 15	Day 20	Day 29
T_1	R_1 (1)	2^3	2^2	2^5
	R_1 (2)	2^4	2^3	2^6
	R_1 (3)	2^3	2^4	2^7
	R_2 (1)	2^5	2^3	2^7
	R_2 (2)	2^5	2^2	2^4
	R_2 (3)	2^4	2^2	2^5
	R_3 (1)	2^4	2^3	2^5
	R_3 ()2	2^3	2^4	2^6
	R_3 (3)	2^5	2^3	2^7
T_2	R_1 (1)	2^4	2^4	2^7
	R_1 (2)	2^6	2^4	2^6
	R_1 (3)	2^5	2^3	2^6
	R_2 (1)	2^3	2^3	2^5
	R_2 (2)	2^4	2^3	2^6
	R_2 (3)	2^5	2^4	2^5
	R_3 (1)	2^4	2^5	2^6
	R_3 (2)	2^6	2^4	2^5
	R_3 (3)	2^3	2^4	2^6
T_3	R_1 (1)	2^6	2^3	2^6
	R_1 (2)	2^5	2^5	2^6
	R_1(3)	2^7	2^4	2^7
	R_2(1)	2^5	2^4	2^7
	R_2 (2)	2^6	2^3	2^6
	R_2 (3)	2^5	2^4	2^6
	R_3 (1)	2^4	2^5	2^7
	R_3 (2)	2^6	2^4	2^6
	R_3 (3)	2^5	2^3	2^7
T_4	R_1 (1)	2^5	2^4	2^7
	R_1 (2)	2^6	2^3	2^7
	R_1 (3)	2^5	2^4	2^6
	R_2 (1)	2^6	2^4	2^6
	R_2 (2)	2^6	2^5	2^8
	R_2 (3)	2^7	2^3	2^5
	R_3 (1)	2^7	2^4	2^7
	R_3 (2)	2^6	2^5	2^6
	R_3 (3)	2^5	2^3	2^7

Appendix 13. Some photograph of Fenugreek experiment conducted at SAU poultry farm





Activities after arrival of day old broiler chicks

Appendix 13. Cont'd

Monitoring of research activities by the supervisor

Appendix 13. Cont'd

Different types of Medication and vaccine used in experiment

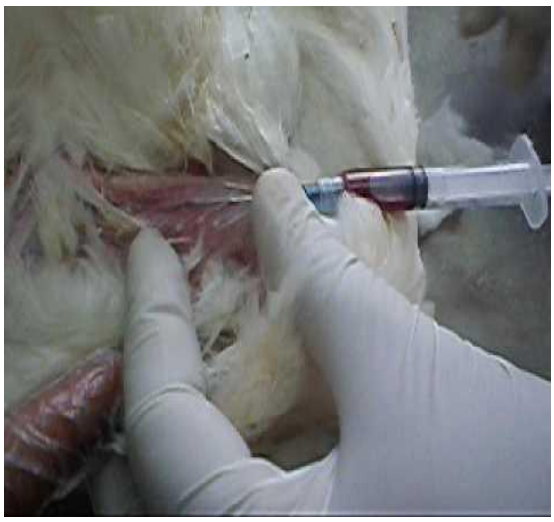
Appendix 13. Cont'd

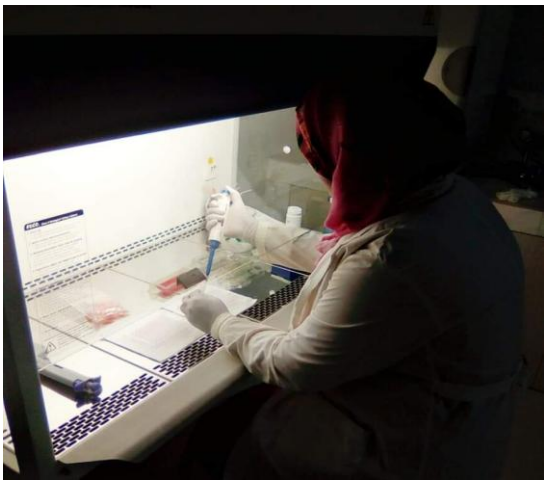
Monitoring and weighing of dressed broiler chicken with internal organs

Appendix 13. Cont'd

Bacterial colony count by colony counter

Appendix 13. Cont'd



Collection of blood at the age of 29 days of old and Determination of ND titer at CDIL