EFFECTS OF DIETARY SUPPLEMENTION OF ALGAE (*Spirulina plantensis)* AS AN ALTERNATIVE TO ANTIBIOTIC ON GROWTH PERFORMANCE AND HEALTH STATUS OF BROILER CHICKEN

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*This is to certify that the thesis entitled "**EFFECTS OF DIETARY SUPPLEMENTION OF ALGAE (Spirulina plantensis) AS AN ALTERNATIVE TO ANTIBIOTIC ON GROWTH PERFORMANCE AND HEALTH STATUS OF BROILER CHICKEN**" submitted to the Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **Master of Science in Poultry Science**, embodies the result of a piece of bona fide research work carried out by **Md. Zahir Uddin Rubel**, Registration No. **12-04731** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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Dedicated
To
My Beloved Parents

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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
CF	=	Crude Fibre
CFU	=	Colony Forming Units
Cm	=	Centimeter
cm^2	=	Squre Centimeter
CONTD.	=	Continued
CP	=	Crude Protein
CRD	=	Complete Randomized Design
DMD	=	Dry Matter Digestibility
Dr.	=	Doctor
DSP	=	Dried S*pirulina* Powder
e.g.	=	For Example
EDTA	=	Ethylene Diethyle Tetraacitic Acid
et al.	=	And others/Associates
FC	=	Feed Consumption
FCR	=	Feed Conversion Ratio
FOS	=	Fructo-oligosaccharides
g	=	Gram
GSH	=	Glutathione
Hb	=	Haemoglobin
HETE	=	Hydroxy Eicosatetraenoic Acid
HPA	=	Hypothalamus Pituitary Axis
i.e.	=	That is
IBV	=	Infectious Bronchitis Vaccines
kcal	=	Kilo-calorie
Kg	=	Kilogram
LSD	=	Least Significant Difference
Ltd.	=	Limited
M.S.	=	Master of Science
MDA	=	Malondialdehyde
ME	=	Metabolizable Energy
MOS	=	Mannan-oligosaccharides

ACRONYMS AND ABBREVIATIONS (CONT'D)

Abbreviation		Full meaning
MCHC	=	Mean Corpuscular Hemoglobin Concentration
ml	=	Mililitre
mm	=	Milimeter
mmol	=	Milimol
MT	=	Metric ton
N	=	Nitrogen
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
RBC	=	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

LIST OF SYMBOLS

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

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ABSTRACT

A total of 150 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 DSP (Dried *Spirulina* Powder) compared to antibiotic based diet. Chicks were divided randomly into 5 experimental groups of 3 replicates (10 chicks with each replications).One of the 5 experimental group was fed this diet as control while, the remaining four groups were fed diet with 3 levels of DSP (0.5, 1.0 and 1.5%) and antibiotic. The results showed that the body weights, dressing percentage and survivability were non-significant (P>0.05) by the dietary inclusion of DSP as compared to control fed broilers. However, a linear increase in body weight had found with the increase in DSP level in the diet. Birds fed 1.5% DSP diets achieved superior body weights (1604.22± 62.88) compared to those of the control and antibiotic group. Feed Conversion Ratio (FCR) and feed consumption was significant in comparison to others. Though best FCR results found at 0.5% level of DSP but it was very close at 1.5% level of DSP. The relative weight of spleen and bursa of different groups showed that there was no significant (P>0.05) difference between the groups. In addition, the present study showed that feeding dietary *Spirulina* 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 due to supplementation of dried *Spirulina* powder, except Red blood cell (RBC), Lymphocyte and Mean Corpuscular Hemoglobin Concentration (MCHC) which were significantly affected (p<0.05) compared with control and antibiotic. Moreover, Inclusion of dried S*pirulina* powder to broiler chicks diets found relative weight of liver, heart, gizzard and intestine weight which had no significant (P>0.05) differences among the treatments. Although the trends of weights were higher in DSP supplementing group compared to the antibiotic and control. However, addition of DSP to broiler chicks diets showed significant (p<0.05) difference in bacterial colony count among the groups. The DSP supplementing groups showed low amount of *E coli* and *Salmonella sp* compared to control but statistically no deference with antibiotic group. Best results found at 1.5% inclusion level of DSP.

CHAPTER I

INTRODUCTION

Poultry farming has emerged as one of the fastest growing agribusiness industries in the world, even in Bangladesh. 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. 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 subtherapeutic dose. The mechanism of action of antibiotics as growth promoters is related to interactions with intestinal microbial population (Dibner and Richards, 2005; Niewold, 2007). 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 and Dashkevicz, 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, 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 and Tucker, 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, 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, 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, antibacterial, anti-viral,

antioxdant and antihelminthic 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). *Spirulina platensis* is a cyanobacterium, which is generally regarded as rich source of protein, essential amino and fatty acids, vitamins and minerals. Traditionally *spirulina* is in use since hundred years as part of human nutrition. *Spirulina* 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). Until very recently, the interest in *Spirulina* was mainly for its nutritive value. Over the past few years, however, it has been found to have many additional pharmacological properties. Many preclinical studies and a few clinical studies. suggest several therapeutic effects ranging from reduction of cholesterol and cancer to enhancement of immune system, an increase in intestinal *lactobacilli*, reduction of nephrotoxicity by heavy metals and drugs and radiation protection (Blinkova *et al.*, 2001; Kuhad *et al.*, 2006; Mohan *et al.*, 2006). Also, *Spirulina* is well known to have antioxidant properties, which are attributed to molecules such as phycocyanin, beta-carotene, tocopherol. It has been found that *Spirulina* is capable of inhibiting carcinogenesis and organ-specific toxicity due to its antioxidant properties (Upasani and Balaraman, 2003; Lu *et al.*, 2006). It was showed that *Spirulina* enhances immune function, reproduction and increases growth and has been used throughout the world as a feed component in quality broiler (Yoshida and Hoshii, 1980) and layer diets to enhance yolk colour and flesh (Toyomizu *et al.*, 2001).

With this background, the work was planned to explore the possibilities of *spirulina* 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 organ characteristics of broiler fed DSP based diet comparison with antibiotic and basal diet.

2. To find out the effect of DSP on *E coli* and *Salmonella sp* diet containing *Spirulina*.

3. To determine the inclusion level of DSP 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, and Dhaka.

(iii) Internet browsing.

A total about 100 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 22 were full article and 60 abstracts, 18 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 *Spirulina.*

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

Poultry farming has emerged as one of the fastest growing agribusiness industries in the world, even in Bangladesh. 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". Further, disease surveillance, monitoring and control will also decide the fate of this sector.

Unlike live stock 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 microflora 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; Torok *et al.*, 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 sequencingapproaches 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 *et al.*, 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 Antimicrobial residues

In poultry, antibiotic usage had facilitated their efficient production and also enhanced the health and wellbeing of poultry by reducing the incidence of disease, but unfortunately, edible poultry tissues may be contaminated with harmful concentrations of drug residues (Donoghue, 2003). Antibiotic residues in foods of animal origin are one of the sources of concern among the public and medical health professionals (Adams, 2001).

Many authors carried out investigations of antibiotic residues in poultry meat and products. Al-Ghamdi *et al.* (2000) reported that antibiotic residues were identified in the chicken muscle, liver and egg samples of 33 broiler and 5 layer poultry farms in

Saudi Arabia. Abdulsalam *et al.* (2000) reported that daily oral administration of two dose levels of 1 and 2 mg/kg body weight of ampicillin, 50 and 100 mg/kg body weight of oxytetracycline and 50 and 100 mg/kg body weight sulphadimidine, in broiler feed resulted in an immediate increase in concentrations of antibiotics in plasma and tissues from day 1 until day 40 of the treatment.

Schneider and Donoghue (2004) observed apparently higher concentrations of enrofloxacin residues in breast versus thigh muscle tissues in pooled samples collected from treated birds.

2.5 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. The currently available alternatives are reviewed here under.

2.5.1 Probiotics

Probiotics are individual microorganisms or groups of microorganisms, which have favourable effect on host by improving the characteristics of intestinal microflora (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 cerevisiae*s) (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).

2.5.2 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 fructo-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.

2.5.3 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).

2.5.4 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 (Friesen *et al.*, 1992; Marquardt *et al.*, 1994), maize (Saleh *et al.*, 2003), oats (Friesen *et al.*, 1992), rye (Friesen *et al.*, 1991, 1992; Bedford and Classen 1992; Marquardt *et al.*, 1994) and wheat (Friesen *et al.*, 1991; 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.

2.5.5 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) .n 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.

2.5.6 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 other antioxidants 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).

2.5.7 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.

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

b) Those that contain tetracyclic triterpenes, such as cucurbitacin R diglucoside, which structurally resemble the specific corticosteroids that inactivate the stress system to protect against overreaction to stressors (Munck, 1984; Panossian *et al.*, 1999).

c) 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 more clear 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. Tipakorn, (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 hepatoprotective and immunopotentiative actions in vaccinated birds and reduced the stress in intensively housed chickens during summer (Parida *et al.*, 1995; Rao *et al.*, 1995).

2.6 *Spirulina*

Spirulina, now named Arthrospira, is a microscopic and filamentous cyanobacterium (blue-green alga). It thrives in tropical and subtropical warm lakes with a high pH ranging from 9.4 to 11.0. There are two different species of *Spirulina*, *Spirulina maxima* and *Spirulina platensis*, with varying distribution throughout the world (Oliveira *et al.*, 1999). *S. platensis* is more widely distributed and found mainly in Africa, Asia and South America (Vonshak, 2002). *S. maxima* on the other hand is more confined to areas in Central America.

The blue-green algae (*Spirulina platensis*) have been used for hundreds of years as a food source for humans and animals due to the excellent nutritional profile and high carotenoid content. *Spirulina* is relatively high in protein with values ranging between 55-65% and includes all of the essential amino acids (Anusuya Devi *et al.*, 1981). The available energy content of *Spirulina* is estimated to be 2.50-3.29 kcal/g and its phosphorous availability is 41% (Yoshida and Hoshii, 1980; Blum *et al.*, 1976). In addition, it is rich in nutrients such as vitamins (thiamin, riboflavin, pyridoxine, vitamin-B12, vitamin-C), amino acids, gamma linoleic acid, phycocyanins, tocopherols, chlorophyll and β-carotenes (Abd El-Baky *et al.*, 2003 and Khan *et al.*,

2005), carotenoids and minerals especially iron.It has been reported that *Spirulina* has health benefits in conditions such as diabetes mellitus and arthritis (Parikh *et al.*, 2001; Rasool *et al.*, 2006). It hasalso been shown to have immuno-stimulatory effects and to have antiviral activity (Khan *et al.*, 2005).

2.6.1 Antioxidant properties of *Spirulina*

Manoj *et al.* (1992) reported that the alcohol extract of *Spirulina* inhibited lipid peroxidation more significantly (65% inhibition) than the chemical antioxidants like α-tocopherol (35%), BHA (45%) and β-carotene (48%). The water extract of *Spirulina* was also shown to have more antioxidant effect (76%) than gallic acid (54%) and chlorogenic acid (56%). An interesting aspect of their findings is that the water extract had a signicant antioxidant effect even after the removal of polyphenols.

In another study by Zhi-Gang *et al.* (1997) the antioxidant effects of two fractions of a hot water extract of *Spirulina* 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.

Beta-carotene concentration of *Spirulina* 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).

2.6.2 *Spirulina* as nutritional and therapeutic supplement in poultry

2.6.2.1 Effect of *Spirulina* on live weight and live weight gain

Ross *et al.* (1994), found that there was no adverse effect of dietary *Spirulina* on final body weight. Kharde *et al.*, 2012; Shanmugapriya & Saravana Babu, (2014) reported that dietary *Spirulina* significantly ($P<0.05$) improved weight gain of chickens compared with the control groups.

Ross and Dominy (1990) and Nikodémusz *et al.* (2010) reported that birds fed dietary *Spirulina* had benefit effects on productive performance. In this regard, Raju

et al. (2005) concluded that dietary inclusion of *Spirulina* at a level of 0.05% can partially offset the adverse effects of aflatoxin on growth rate of broiler chickens.

Effect of the dietary supplementation of *Spirulina* on the growth performance of the Japanese quail (*Coturnix japonica*) at Poultry Research Station, Chennai, India. The results revealed that the dietary supplementation of *Spirulina* in Japanese quails significantly ($P<0.05$) improved the body weight, gain.

Bonos *et al.* (2015) conducted an experiment and they showed that bodyweight gain (at 21 d and 42 d), differ among the groups. Therefore, *Spirulina* could be a promising functional ingredient in broiler chicken nutrition.

Zahroojian *et al.* (2013) concluded that no significant differences between the treatments with 2.0 and 2.5% of *Spirulina* in case of Mean live body weight of six weeks of the experiment and live weight at the end of experiment were found to be significantly ($P<0.05$) higher in *Spirulina* supplemented T_1 and T_2 groups of broilers than that of control (T_0) group. Comparatively better mean weekly weight gain and feed efficiency were also observed in *Spirulina* supplemented groups (T_1 and T_2) with decreased feed consumption as compared to control (T_0) group of broilers.

Zahroojian *et al.* (2013) concluded that no significant differences between the treatments with 2.0 and 2.5% of *Spirulina.* In conclusion, this study can suggest use of 2.0~2.5% of *Spirulina* in diet to produce an aesthetically pleasing yolk color. An experimental trial of six weeks was undertaken by Kharde *et al.* (2012) on 90 broiler chicks divided into three groups. Control (T_0) group was fed standard broiler diet and T_1 and T_2 groups were provided same broiler diet supplemented with 300 and 500 mg of *Spirulina* per kg feed, respectively.

2.6.2.2 Effect of *Spirulina* on FCR

Ross and Dominy, (1990) evaluated the nutritional value of dehydrated *Spirulina* in poultry. Male broiler chicks were fed *Spirulina* in the range of 1.5-12% for 41 days. It was concluded that dehydrated *Spirulina* at a diet content below 12% may be substituted for other protein sources in chick and broiler diets with good feed efficiency. The authors also found similar results with quail.

Sugiharto *et al.* (2017) conducted an experiment using 1% of *Spirulina platensis* and they come to a conclusion that the dose of *Spirulina* was 8g/kg and results were body weight was significantly ($P<0.05$) increased in the treatment groups fed with *Spirulina* diet from 7th days to 28th days old. FCR was also significantly ($P<0.05$) decreased among the treatment groups.

Kharde *et al.*, (2012); Shanmugapriya & Saravana Babu, (2014) reported that dietary *Spirulina* significantly (P<0.05) improved feed efficiency of broiler chickens compared with the control groups.

Ross & Dominy, (1990); Venkataraman *et al*., (1994), Qureshi *et al*., (1996), Gongnet *et al*., (2001) and Toyomizu *et al*.,(2001) recorded nonsignificant (P>0.05) effects of dietary *Spirulina* supplementation on performance parameters. However, Ross and Dominy (1990) and Nikodémusz *et al.* (2010) reported that birds fed dietary *Spirulina* had benefit effects on productive performance. Also, Sinai hens had significantly (P<0.05) a better value of feed conversion ratio than that of Gimmizah hens. In conclusion, taking the economical aspect into account, *Spirulina* algae could be safely used in laying hen diets with superior effects on their productive and reproductive performance.

2.6.2.3 Effect of *Spirulina* on feed consumption

Spirulina has been shown to enhance immune function and feed consumption. Less than 1% *Spirulina* added to chicken diets has been found to significantly enhance the defense systems *viz.* increased microbial killing, antigen processing and greater T-cell activity (Qureshi, *et al.*, 1994).

Ross and Dominy, (1990) reported that hens fed *Spirulina* containing diets achieved superior productive and reproductive performance compared to the control birds.

Spirulina is one of the high quality natural feed additives that can be used in animal and poultry nutrition and have been used throughout the world as a feed component in broiler and layer diets to enhance yolk color and flesh (Brune, 1982). Sakaida Takashi, (2003) also reported that egg yolk color was significantly improved by the addition of *Spirulina* to laying hen diets.

Ross & Dominy, 1990; Venkataraman *et al*., (1994), Qureshi *et al*., (1996), Gongnet *et al*., (2001) and Toyomizu *et al*.,(2001) recorded nonsignificant (P>0.05) effects of dietary *Spirulina* supplementation on performance parameters.

2.6.2.4 Effect of *Spirulina* on immune organs

Kaoud (2015) showed that the relative and absolute weights of thymus and bursa were induced for the groups fed diet containing *Spirulina* compared to the control group. These results may be considered as good indicator of healthy status of chicks fed dietary *Spirulina*.

Bennett and Stephens (2006) reported that the bursa functions are half of the immune system and its size reflects overall health status of bird. They added that stressed or sick birds have small size of bursa but, healthy or productive birds have large size. Bursa size is a biological indicator of how flocks are well-managed and preserved from disease.

Addition of less than 1% *Spirulina* in chicken diets significantly enhanced the defense systems for antigen processing, greater T-cell activity and increased microbial killing (Qureshi *et al.*, 1996). In addition, increased content of Zn concentration in *Spirulina* is playing a role to induce the cellular immunity of birds (Mohamed, 1998).

Mobarez *et al.* (2018) performed a study by using 2-3g/kg *Spirulina platensis* algae (SP) in laying hen and recommended that layer diets with *Spirulina platensis* algae (SP) or Turmeric Powder (TP) for better productive and reproductive performance as well as improved immune responses during the laying period.

Sugiharto *et al.* (2017) conducted an experiment using 1% of *Spirulina platensis* and they come to a conclusion that the administration of *S. platensis* for the first 21 days of broilers' life resulted in similar or even better responses than administration of *S. platensis* or in-feed antibiotics throughout the rearing period. The study suggests that, *Spirulina* is a good natural feed additive which has a tremendous effect to improve the broiler production and thereby may reduce the production cost. (Jamil *et al.* 2015).

Mariey *et al.* (2012) found that regardless of the effect of dietary inclusion of *Spirulina*, Gimmizah hens consume significantly more feed than that of Sinai hens,

while Sinai birds give significantly higher egg production performance compared with Gimmizah hens.

Hussein *et al.* (2015) concluded that the prebiotic and *Spirulina* platensis supplementation significantly increased body weight (BW) and decreased feed gain ratios and decreased the mortality. The *Spirulina platensis* offers a good alternative to improve poultry production.

Islam *et al.* (2009) found that *Spirulina* may be helpful in reducing the tissue burden of arsenic in ducks.

Raju *et al.* (2005) concluded that dietary inclusion of *Spirulina* at a level of 0.05% could partially offset the adverse effects of aflatoxin on growth rate and lymphoid organ weight of broiler chickens.

The cholesterol level in yolk and plasma was significantly decreased in birds fed with *Spirulina* diet. Fertility and hatchability of eggs produced by birds fed with *Spirulina*-diet were superior compared to control group (Mariey *et al.*, 2012). The color enhancement properties of *Spirulina* have been studied in poultry.

2.6.2.5 Effect of *Spirulina* on internal organs

Hernandez *et al.*, (2004), who observed no difference in the mean weight of proventriculus, gizzard, intestine, liver and pancreas in broilers fed on two herbal extracts. In another study by Zanu *et al.*, (2011) neem decoction was evaluated as a total replacement for antibiotics and coccidiostat in a 6 weeks feeding trail in broilers.

Ravi (2012) was competed a thesis and he suggested that Neem and *Spirulina* did not affect the functioning of liver and kidney as was indicated by unaffected serum biochemical profiles and histological architecture. Neem, *Spirulina* and their combinations were found to show cholesterol lowering capacity when compared to antibiotics group or control group. The study concludes that neem and *Spirulina* or their combinations can be used as an alternative to antibiotics as feed additive.

2.6.2.6 Effect of *Spirulina* on carcass quality

The effects of *Spirulina* on broiler performance parameters including average Dressing Percentage (DP) was in agreement with previous studies (Cavazzoni *et al.*,

1998; Jin *et al,* 1997; Zulkifli *et al.,* 2000; Kabir, *et al.,* 2004; Mountzouris *et al.,* 2007; Samli *et al.*, 2007).

Bellof & Alarcon (2013) reported that under organic farming, dietary *Spirulina* supplementation improved carcass performance parameters of broilers significantly ($P<0.05$). However, *Spirulina platensis* dried-supplement displayed a greater growth-promoting effect and increased the carcass yield percentage

2.6.2.7 Effect of *Spirulina* on Survivability

Ross and Dominy, (1990) have also observed a significant increase in egg yolk color in quail fed a diet containing 1.5% *Spirulina* compared to those fed the control diet. A recent Japanese patent (Sakakibara and Hamada, 1994) describes the use of *Spirulina* (0.1-2%) to reduce the death rate in quail. The death rate was reduced from 10% in the basal diet to 0.5-3.5% in the experimental diet containing 0.1-2.0% *Spirulina*. The lowest death rate was found with 0.2% *Spirulina*.

Qureshi *et al.*, 1995 has found significantly higher growth rate and lower non-specific mortality rate in turkey poults fed *Spirulina* at the level of 1000-10000 mg kg-1 compared to poults on a basal diet. Mortality was reduced from 12% in the control group to 3% in the 1000 ppm *Spirulina* group. Bacterial clearance rates were studied in chicken fed a control diet or a *Spirulina* supplemented diet.

2.6.2.8 Effect of *Spirulina* on microbial load

Wakwak *et al.* (2003), Kabir *et al.* (2004) and Kulshreshtha *et al.* (2008). In addition, Baojiang (1994) who found that *Spirulina* is useful for the beneficial intestinal flora. Injection of *Escherichia coli* or *Staphylococcus aureus* into chicks fed with *Spirulina* diet @ 1000-10000 mg/kg showed a significantly higher clearing rate at all levels but more at 1000 mg/kg. Time course studies of bacterial clearance in *Spirulina*- fed chicks also showed that the bacterial numbers were negligible even after a post-injection period of only 30 min. From these results the authors concluded that *Spirulina* supplementation improved the activity of the phagocytic cells, namely macrophages, heterophils and thrombocytes in chickens. They also proposed that 1000-10000mg kg-1 (0.1-1.0%) range of dietary *Spirulina* supplementation in chickens would be safe to use in terms of improved immune competence without compromising performance characteristics of chickens (Qureshi *et al.*, 1995).

2.6.2.9 Effect of *spirulina* on Serum biochemical properties

The increase in plasma glucose concentration of hens fed dietary Spirulina 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.

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

Kannan *et al.* (2005), Abdel-Daim *et al.* (2013) and Abou Gabal *et al.* (2015). Concluded that *Spirulina platensis* supplementation at level of 1% significantly improved the blood parameters (Shanmugapriya and Saravana Babu, 2014).

Jamil *et al.* (2015) concluded that, ALT and AST decreased significantly ($P<0.05$) when fed with *Spirulina platensis* compared with the control group.

2.6.2.10 Effect of *Spirulina* on blood parameter

The results Kamruzzaman (2005) study showed that the body weight gains differed significantly ($p < 0.05$) at the 2nd, 4th and 5th weeks of age in different treatment groups. The meat yield not differed significantly ($p > 0.05$). The drumstick, wing differed significantly ($p < 0.01$) and spleen weight differed at $p < 0.05$ among different groups. The mean haemato-biochemical values of Hb, ESR, PCV, heterophil, eosinophil, basophil, triglyceride, HDL, LDL, SGPT and SGOT were differed significantly ($p < 0.01$) in different groups. The present findings suggest that supplementation of probiotics has significant effect on growth performance and certain haemato-biochemical parameters of broiler chickens as compared to antibiotic supplementation. The study was conducted to evaluate the prebiotic effects of *Spirulina* as a growth and immunity promoter for broiler chickens. The dose of *Spirulina* was 8g/kg and results were body weight was significantly ($P<0.05$) increased in the treatment groups fed with *Spirulina* diet from 7th days to 28th days old.

Hematological parameters were significantly ($P<0.05$) increased except ESR which was decreased significantly ($P<0.05$) in the treatment group.Kannan *et al.* (2005), Abdel-Daim *et al.* (2013) and Abou Gabal *et al.* (2015). Concluded that *Spirulina platensis* supplementation at level of 1% significantly improved the blood parameters (Shanmugapriya and Saravana Babu, 2014).

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 150-day-old straight run (Cobb 500) commercial broilers for a period of 28 days from **8th May to 5th June, 2018** to assess the feasibility of using dried *Spirulina* powder (DSP) in commercial broiler diet on growth performance, meat yield characteristics and immune status of broilers. This research helps to make a conclusion about DSP as the alternative of antibiotic.

3.2 Collection of experimental broilers

A total of 150 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 DSP was used as treatment. After two days 90 chicks were selected from brooders and distributed randomly in five (5) dietary treatments of DSP; 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 five (5) and their replications were fifteen (15).

3.4 Experimental treatments

T_1: 0.5% of Dried *Spirulina* Powder (0.5 kg DSP/100 kg of the feeds)

T_2: 1.0% of Dried *Spirulina* Powder (1.0 kg DSP /100 kg of the feed)

T_3: 1.5 % of Dried *Spirulina* Powder (1.5 kg DSP / 100 kg of the feed)

T_4: Basal Diets + Antibiotics

T_5: Basal Diets/ Control

Table 1. Layout of the experiment.

Treatment groups	No. of replications			Total
	R_1	R_2	R3	
T_1	10	10	10	30
T_2	10	10	10	30
T_3	10	10	10	30
T_4	10	10	10	30
T_5	10	10	10	30
Total	50	50	50	150

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 $1m^2$/10 birds.

3.6 Experimental diets

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

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

Name of ingredients in Starter ration	Minimum percentage Present
protein	21.0 %
fat	6.0%
fiber	5.0%
ash	8.0%
lysine	1.20%
methionine	0.49%
cystine	0.40%

Table 2. Continued

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 *Spirulina*

Dried *Spirulina* powder (DSP) was used in commercial basal diets. Photographs of DSP were given in below (Plate 1). This *Spirulina* was manufactured by applied botany section of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh for conducting the research work.

Plate 1. Dried *Spirulina* Powder (DSP).

Table 3. Nutritional composition of *S. platensis*

Nutrient Component	Amount
Dry weight	92.76+0.26%
Lipids	30.12+1.19%
Proteins	37.55+0.07%
Fibers	31.32+7.95%
Sugars	24.39+0.99%
Energy	518.84 kcal
Iron	256.56+ 0.01 mg /Kg
Manganese	23.38+0.00 mg /Kg
Copper	28.95+0.00 mg /Kg
Zinc	25.01+0.01 mg /Kg
Selenium	1.24+0.01 mg /Kg
Ash	07.93+0.20 mg /Kg
Vitamin A	589 IU/kg
Vitamin E	207.48 IU/kg
Vitamin B1	12.90 mg /Kg
Vitamin B2	45.50 mg /Kg
Vitamin C	740.00 mg /Kg

Source: Annals. Food Science and Technology, Volume 17, Issue 2, 2016.

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 **8th May to 5th June, 2018**. The average temperature was 31.5^{0}C and the RH 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 hot

climate 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 22 hours light and 2 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. All groups of broiler chicks were supplied Vitamin B-Complex, Vitamin-ADEK, Vitamin-C, Ca and Vitamin-D enriched medicine and electrolytes.

3.7.7 Vaccination

The vaccines collected from medicine shop (Ceva Company) and applied to the experimental birds according to the vaccination schedule. The vaccination schedule is shown in Table 4.

Table 4. Vaccination schedule

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

3.7. 8 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.

3.7.9 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 death of chicks to calculate mortality percent. FCR was calculated from final live weight and total feed consumption per bird in each replication. After slaughter gizzard, liver, spleen, intestine, hear 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 (CBC), 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 = 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 28^{th} day of age and sacrificed to estimate dressing percent of broiler chicken. All birds to be slaughtered were weighed and fasted f by halal method or overnight (12 hours) but drinking water was provided *ad-libitum* during fasting to facilitate proper bleeding. All the live birds were weighed again prior to slaughter. Birds were slaughtered by severing jugular vein, carotid artery and the trachea by a single incision with a sharp knife and allowed to complete bleed out at least for 2 minutes. Outer skin was removed by sharp scissor and hand. Then the carcasses were washed manually to remove loose singed feathers and other foreign materials from the surface of the carcass. Afterward the carcasses were eviscerated and dissected according to the methods by Jones (1982). Heart and liver were 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 ethylenediethyletetraacitic acid (EDTA) tubes from the wing veins. Samples were transferred to the laboratory for analysis within 1 hour of collection. Sugar, Cholesterol and CBC was measured from Rainbow diagnosis centre Dhanmondi Dhaka by maintaining standard protocol.

3.10 Calculations

3.10.1 Live weight gain

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

Body weight gain = Final weight – Initial weight

3.10.2 Feed intake

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

$$\text{Feed intake (g/bird)} = \frac{\text{Feed intake in a replication}}{\text{No. of birds in a replication}}$$

3.10.3 Feed conversion ratio

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

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

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, LSD and significance was set at $P<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Production performances of broiler chicken

4.1.1 Final Life weight

Data presented in Table 8 showed that the effect of treatments on final live weight (gram per broiler chicken) was not significant (P>0.05). The relative final live weight (g) of broiler chickens in the dietary group T_1, T_2, T_3, T_4 and T_5 were 1538.89±18.69, 1522.22±55.68, 1604.22±62.88, 1461.11±36.12 and 1550.00±63.45 respectively. The highest result was found in T_3 (1604.22±62.88) and lowest result was in T_4 (1461.11±36.12) group. However, Final live weight of broiler fed *Spirulina* diets increased but that was insignificant (P>0.05) compared to that of the control and antibiotic treated groups.

These results are in agreement with those obtained by Ross *et al.* (1994), who found that there was no adverse effect of dietary *Spirulina* on final body weight. In addition, these results are in contradictory with those of previous researchers (Kharde *et al.*, 2012; Shanmugapriya & Saravana Babu, 2014) reported that dietary *Spirulina* significantly (P<0.05) improved weight gain of chickens compared with the control groups. However, Ross and Dominy (1990) and Nikodémusz *et al.* (2010) reported that birds fed dietary *Spirulina* had benefit effects on productive performance. In this regard, Raju *et al.* (2005) concluded that dietary inclusion of *Spirulina* at a level of 0.05% can partially offset the adverse effects of aflatoxin on growth rate of broiler chickens.

4.1.2 Weekly Body weight gains

The mean body weight gains (g) of broiler chicks at the end of 4th week in different groups were 442.33±6.17, 451.67±11.26, 482.33±4.25, 458.33±7.26, and 444.00±15.01 respectively. The overall mean body weight gain of different groups showed that there was significant (P<0.05) increase in groups T_1, T_2, and T_3 compared to control and antibiotic (Table 6 and Figure 2).

These results are in agreement with those of previous researchers (Kharde *et al.*, 2012; Shanmugapriya & Saravana Babu, 2014) reported that dietary *Spirulina*

significantly (P<0.05) improved weight gain of chickens compared with the control groups.

4.1.3 Feed consumption (FC)

Different treatment groups (Table 8) showed significant (P<0.05) differences in FC of broiler chicken. Control group consumed higher amount of feed (1928.43±37.35) and 1% (T_2) dried *Spirulina* powder treated group consumed lower amount of feed (1778.80±28.93). Antibiotic treated group T_4 (1867.53±21.98) showed no significant (P>0.05) difference in total FC and weekly FC with all other treatment groups.

These results are in agreement with those of previous researchers (Ross & Dominy, 1990; Venkataraman *et al.*, 1994; Qureshi *et al.*, 1996; Gongnet *et al.*, 2001; Toyomizu *et al.*, 2001), who recorded nonsignificant (P>0.05) effects of dietary *Spirulina* supplementation on performance parameters. 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.

4.1.4 Weekly Feed consumption (FC)

The mean body FC (g) of broiler chicks at the end of 4^{th} week in different groups were 716.67 ±16.66, 683.33 ±14.53, 773.33 ±20.00, 790 ±5.77, and 826.67 ±29.05 correspondingly. The overall mean FC of different groups showed that there was significant (P<0.05) increase in groups T_3, T_4, and T_5 compared to T_1 and T_2 antibiotic (Table 5 and Figure 1).

These results are in harmony with those of previous 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

4.1.5 Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) was significantly (P<0.05) lower for birds supplemented with 0.5% (1.26±0.03) dried *Spirulina* powder than control birds ((1.45±0.01)). However, Feed conversion ratio (FCR) was significantly (P<0.05) higher in T_4 group (1.42±0.01) (supplemented with antibiotic) compared to T_2 (1.29±0.02) and T_3 (1.27±0.02) groups respectively (Table 8).

These results are in agreement with those of previous researchers (Kharde *et al.*, 2012; Shanmugapriya & Saravana Babu, 2014) reported that dietary *Spirulina* significantly (P<0.05) improved feed efficiency of broiler chickens compared with the control groups. These results are in contradictory with those of previous researchers (Ross & Dominy, 1990; Venkataraman *et al*., 1994; Qureshi *et al*., 1996; Gongnet *et al*., 2001; Toyomizu *et al*., 2001), who recorded nonsignificant (P>0.05) effects of dietary *Spirulina* supplementation on performance parameters. However, Ross and Dominy (1990) and Nikodémusz *et al.* (2010) reported that birds fed dietary *Spirulina* had benefit effects on productive performance. Contradictory results are possibly due to the different *Spirulina* inclusion levels and quality in the present trials. In addition, secondary parameters, such as feed composition, housing conditions and production systems, might be reasons for the variation in the results of the present study.

4.1.6 Weekly Feed Conversion Ratio (FCR)

The mean body FCR of broiler chicks at the end of 4^{th} week in different groups were 1.62±0.06, 1.51±0.02, 1.66±0.04, 1.72±0.02, and 1.56±0.28 respectively. The overall mean FCR of different groups showed that there was no significant (P>0.05) increase in groups T_1, T_2, and T_3 compared to control and antibiotic (Table 7 and Figure 3).

These results are in agreement with those of previous researchers (Ross & Dominy, 1990; Venkataraman *et al*., 1994; Qureshi *et al*., 1996; Gongnet *et al*., 2001; Toyomizu *et al*., 2001), who recorded nonsignificant (P>0.05) effects of dietary *Spirulina* supplementation on FCR parameters.

4.1.7 Survivability

The Survivability rate showed on table 8. Was higher for the *Spirulina* -supplemented group (100±0.00) than the antibiotic supplemented group but no significant (P>0.05) difference with control group (100±0.00).

These results are in agreement with Qureshi *et al.*, 1995 has found lower non-specific mortality rate in turkey poults fed *spirulina* at the level of 1000-10000 mg kg-1 compared to poults on a basal diet. These results are also supported by other researchers (Ross & Dominy, 1990; Venkataraman *et al.*, 1994; Qureshi *et al.*, 1996; Gongnet *et al.*, 2001; Toyomizu *et al.*, 2001), who recorded nonsignificant (P>0.05) effects of dietary *Spirulina* supplementation on performance parameters.

4.1.8 Dressing Percentage (DP)

The 1.5% (T_3) *Spirulina* supplemented group had a greater (P > 0.05) carcass percentage (71.67±2.01%) compared with the antibiotic group (69.19±1.90%) and 0.5% (T_1), 1% (T_2) and control (T_5) group DP % were 68.66±1.66, 71.54±3.58 and 69.91±1.09 respectively (Table 8).

In the present study, the effects of *Spirulina* on broiler performance parameters including average Dressing Percentage (DP) was in agreement with previous studies (Cavazzoni *et al.,* 1998; Jin *et al*, 1997; Zulkifli *et al.,* 2000; Kabir, *et al.,* 2004; Mountzouris *et al.,* 2007; Samli *et al.,* 2007). Furthermore, Bellof & Alarcon (2013) reported that under organic farming, dietary *Spirulina* supplementation improved carcass performance parameters of broilers significantly (P<0.05). However, *Spirulina platensis* dried-supplement displayed a greater growth-promoting effect and increased the carcass yield percentage.

Table 5. Effects of feeding different level of *Spirulina* and antibiotic on feed consumption (g/bird) of broiler chickens at different week.

Treatment	1st week FC	2nd week FC	3rd week FC	4th week FC
T_1	136.63 ±4.31	326.33 ±9.50	593 ±3.51	716.67^{b} ±16.66
T_2	135.47 ±2.82	367 ±5.68	593 ±15.88	683.33^{b} ±14.53
T_3	132.33 ±2.74	354.33 ±2.84	587 ±6.11	773.33^{a} ±20.00
T_4	129.53 ±2.16	361.67 ±4.41	586.33 ±9.38	790^{a} ±5.77
T_5	136.1 ±3.26	365.33 ±7.05	600.33 ±9.83	826.67^{a} ±29.05
Mean ± SE	134.01±1.38	354.93±9.50	591.92±3.97	758±15.28
$LSD_{(0.05)}$	4.43^{NS}	32.31^{NS}	13.95^{NS}	24.85*

Here, T_1 =(0.5% DSP Supplementation), T_2 =(1% DSP Supplementation), T_3 =(1.5% DSP Supplementation), T_4 =(Antibiotic) and T_5 =(Control). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

Table 6. Effects of feeding different level of *Spirulina* and antibiotic on body weight gain (BWG) (g/bird) of broiler chickens at different week.

Treatment	1st week BWG	2nd week BWG	3rd week BWG	4th week BWG
T_1	164.50 ±2.550	296.50±6.95	461±9.50	442.33^{b}±6.17
T_2	160.73±6.664	304.87±11.07	465±5.68	451.67^{ab}±11.26
T_3	168.73±0.888	307.00±2.88	465.67±2.84	482.33^{a}±4.25
T_4	158.77±5.617	302.90±1.98	461.67±4.41	458.33^{ab}±7.26
T_5	164.90±2.108	297.77±7.27	462.67±7.05	444.00^{b}±15.01
Mean ± SE	163.53±1.83	301.81±2.81	463.20±2.44	453.73±5.30
$LSD_{(0.05)}$	5.92^{NS}	9.72^{NS}	8.94^{NS}	3.58*

Here, T_1 =(0.5% DSP Supplementation), T_2 =(1% DSP Supplementation), T_3 =(1.5% DSP Supplementation), T_4 =(Antibiotic) and T_5 =(Control). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

Table 7. Effects of feeding different level of *Spirulina* and antibiotic on FCR of broiler chickens at different week.

Treatment	1st week FCR	2nd week FCR	3rd week FCR	4th week FCR
T_1	0.84±0.66	1.10±0.16	1.29±0.02	1.62±0.06
T_2	0.84±0.05	1.21±0.05	1.28±0.03	1.51±0.02
T_3	0.78±0.02	1.15±0.01	1.26±0.01	1.66±0.04
T_4	0.82±0.02	1.19±0.03	1.27±0.01	1.72±0.02
T_5	0.83±0.03	1.23±0.02	1.30±0.01	1.56±0.28
Mean ± SE	0.82±0.01	1.18±0.23	1.28±0.02	1.62±0.53
$LSD_{(0.05)}$	0.03^{NS}	0.11^{NS}	0.02^{NS}	0.18^{NS}

Here, T_1 =(0.5% DSP Supplementation), T_2 =(1% DSP Supplementation), T_3 =(1.5% DSP Supplementation), T_4 =(Antibiotic) and T_5 =(Control). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

Table 8: Production performance of broiler chicken treated with *Spirulina* and antibiotic.

Treatment	T_1	T_2	T_3	T_4	T_5	Mean±SE	$LSD_{(0.05)}$
Final Live weight (g/broiler)	1538.89±18.69	1522.22±55.68	1604.22±62.88	1461.11±36.12	1550.00±63.45	1535.29 ± 22.71	71.374^{NS}
FC (g)	1819.63^{bc}±20.74	1778.80^{c}±28.93	1873.67^{ab}±25.73	1867.53^{abc}±21.98	1928.43^{a}±37.35	1853.61±17.13	39.028^{*}
FCR	1.28^{b}±0.03	1.30^{b}±0.02	1.29^{b}±0.02	1.42^{a}±0.01	1.45^{a}±0.01	1.34±0.023	0.026^{*}
DP% (Skinless)	68.66±1.66	71.54±3.58	71.67±2.01	69.19±1.90	70.50±3.15	69.91±1.09	3.64^{NS}
Survivability (%)	100±0.00	99±0.01	100±0.00	99±0.01	100±0.00	99.6±0.01	0.002^{NS}

Here, T_1 =(0.5% DSP Supplementation), T_2 =(1% DSP Supplementation), T_3 =(1.5% DSP Supplementation), T_4 =(Antibiotic) and T_5 =(Control). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

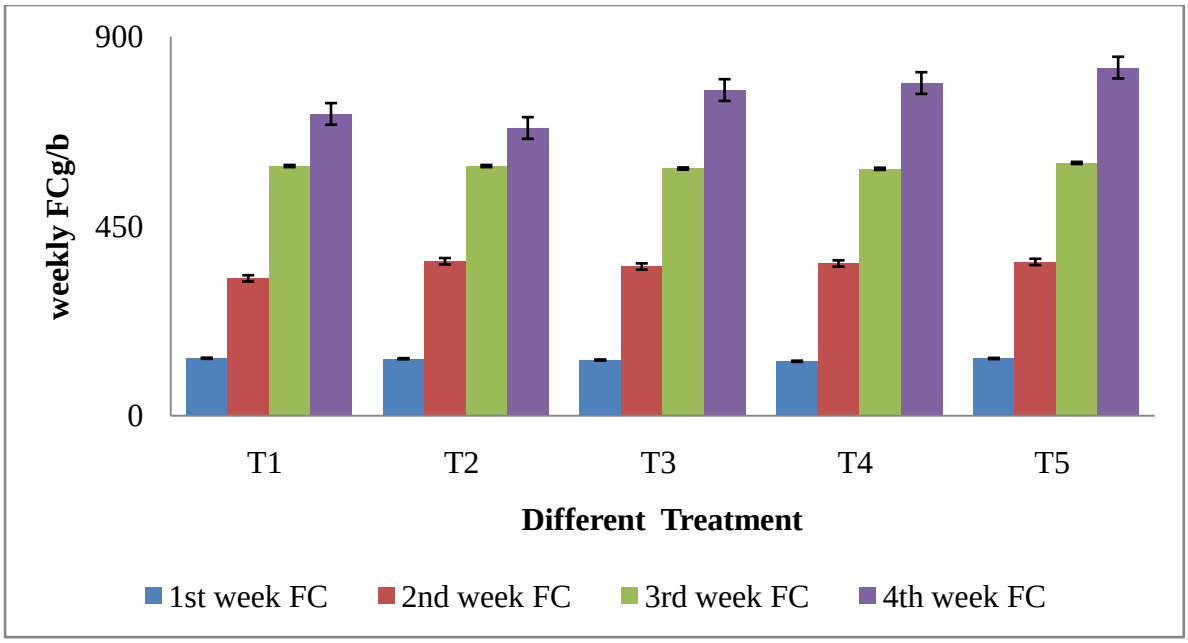

Figure 1. Effects of feeding different level of *Spirulina* and antibiotic on feed consumption (g/bird) of broiler chickens at different week.

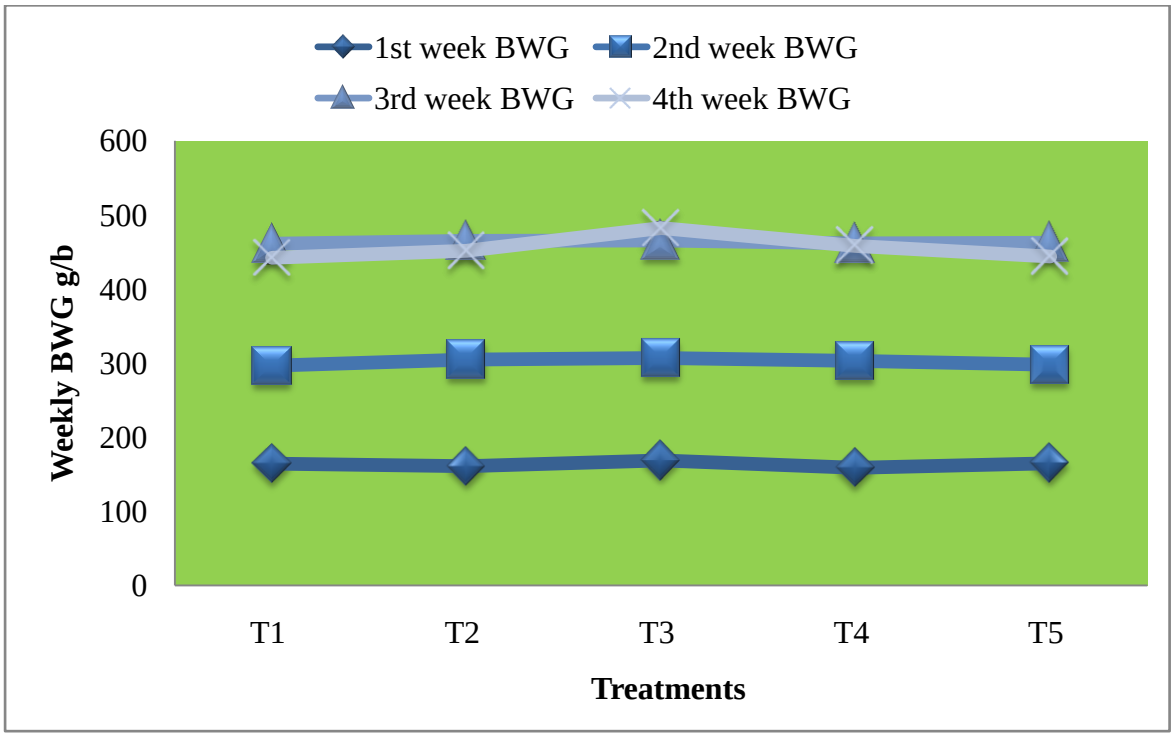

Figure 2. Effects of feeding different level of *Spirulina* and antibiotic on body weight gain (BWG) (g/bird) of broiler chickens at different week.

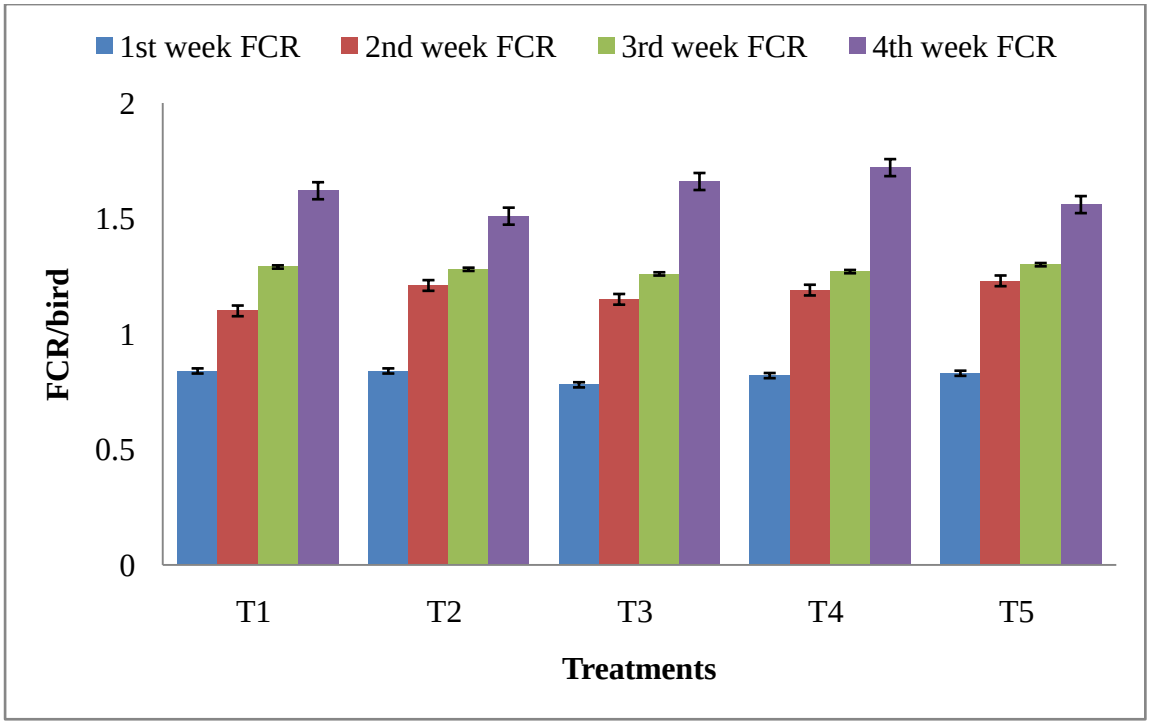

Figure 3. Effects of feeding different level of *Spirulina* and antibiotic on FCR of broiler chickens at different week.

Table 9: Effect of *spirulina* on Serum biochemical level of different broiler chicken under different treatment.

Parameters	T_1	T_2	T_3	T_4	T_5	Mean ±SE	LSD $_{(0.05)}$
Sugar mmol/L	10.18± 0.25	9.37±0.27	10.40±0.85	10.08± 0.60	10.33± 0.27	10.07±0.218	o.726^{NS}
Cholesterol mg/dl	119.00 ±1.07	117.44± 4.74	132.00± 12.81	130.00 ±11.64	132.89 ±4.81	126.27±3.615	11.775^{NS}

Here, T_1 = (0.5% DSP Supplementation), T_2 = (1% DSP Supplementation), T_3 = (1.5% DSP Supplementation), T_4 = (Antibiotic) and T5 = (Control). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

4.2.1 Sugar

Effects of dietary dried *Spirulina* powder supplementation on concentration of sugar of broiler chickens are presented in Table 9 and Figure 4. Feeding dietary *Spirulina* had no significant (P>0.05) difference among the treatment. Although the highest amount (10.40±0.85) of plasma sugar are found in T_3 (1.5% *Spirulina*) but this was not statistically difference with antibiotic, control and other groups.

The increase in plasma glucose concentration of hens fed dietary Spirulina 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.

4.2.2 Total Cholesterol

Total cholesterol concentration (mg/dl) in the serum of different groups ranged from 117.44±4.74 to 132.89±4.81. Statistical analysis revealed a nonsignificant (P>0.05) deference among the group. The cholesterol level of different treatments were T_1 (119.00±1.07), T_2 (117.44±4.74), T_3 (132.00±12.81), T_4 (130.00±11.64) and T_5 (132.89±4.81) correspondingly. While the concentration in T_5 (132.89±4.81) was comparable to that of T_3 (132.00±12.81) and T_4 (130.00±11.64) (Table 9 and Figure 4).

The present study give similar findings with the results of Kanagaraju and Omprakash (2016) and Swee Weng *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 Abou Gabal *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.

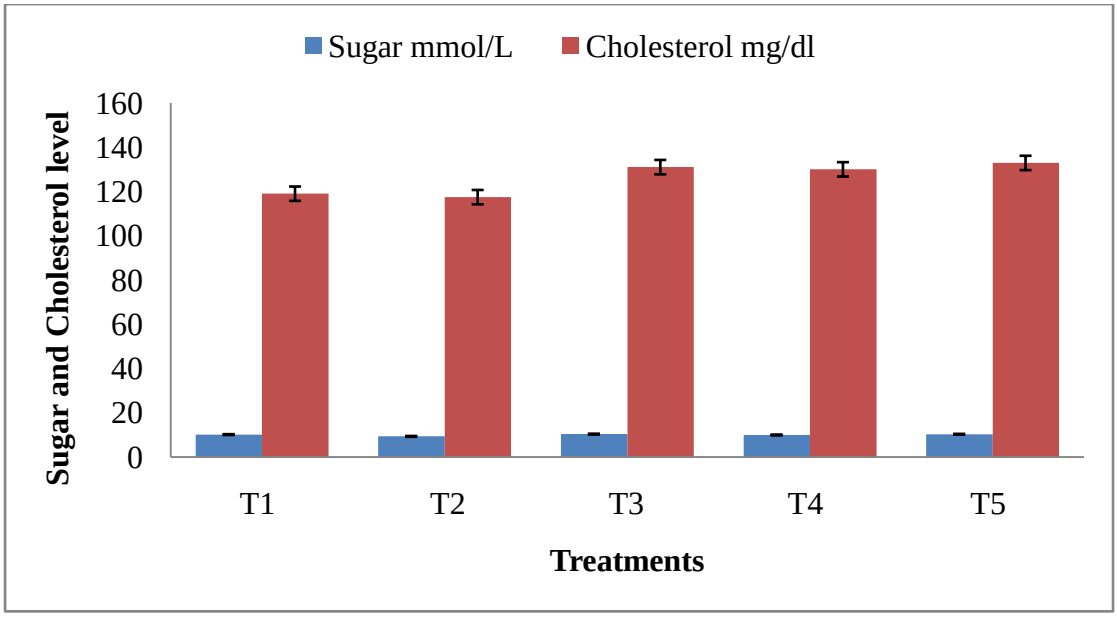

Figure 4. Effect of *Spirulina* on Serum biochemical level of different broiler chicken under different treatment.

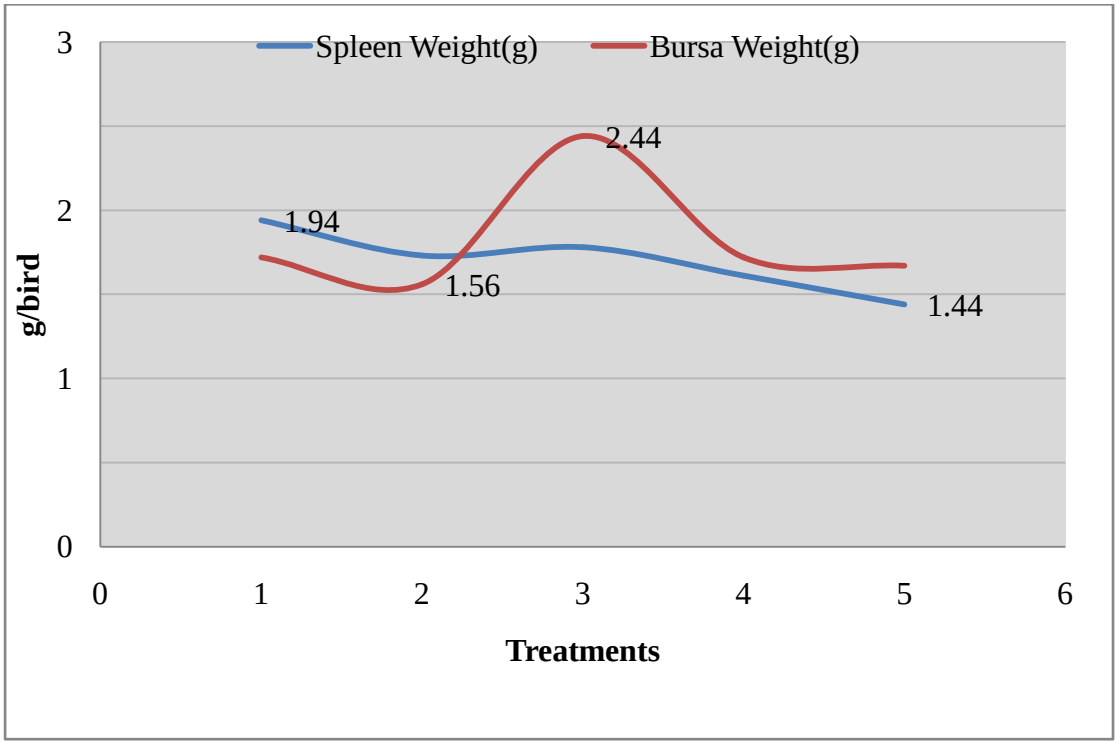

Figure 5. Effects of supplementation of dried *Spirulina* powder to broiler diets on some immune organs.

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

Parameters	T_1	T_2	T_3	T_4	T_5	Mean ±SE	LSD (0.05)
Liver weight (g)	37.89±3.13	37.33±1.61	35.56±2.25	34.00±2.50	35.83±1.62	36.12±0.944	3.252^{NS}
Gizzard Weight(g)	41.28±1.64	41.00±3.12	36.33±1.72	35.83±2.03	37.61±3.64	38.41±1.145	3.612^{NS}
Intestine Weight(g)	101.78±0.22	103.67±7.98	116.83±6.75	108.11±2.14	101.78±7.21	107.04±2.599	8.148^{NS}
Heart Weight (g)	7.39±0.72	6.11±0.56	7.44±0.27	6.52±0.36	6.72±0.05	6.84±0.221	0.650^{NS}

Here, T_1 = (0.5% DSP Supplementation), T_2 = (1% DSP Supplementation), T_3 = (1.5% DSP Supplementation),

T_4 = (Antibiotic) and T_5 = (Control). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

4.3.1 Relative giblet weight (liver, heart and gizzard)

The relative weight of liver (g) of broiler chicks in the dietary group T_1, T_2, T_3, T_4 and T_5 were 37.89±3.13, 37.33±1.61, 35.56±2.25, 34.00±2.50 and 35.83±1.62 respectively. The highest results were obtain in T_1 and lowest was in T_4 group. However, there was no significant (P>0.05) difference in the relative weight of liver between the groups. (Table 10).

The comparative weight of liver (g) of broiler chicks in the dietary group T_1, T_2, T_3, T_4 and T_5 were 7.39±0.72, 6.11±0.56, 7.44±0.27, 6.52±0.36, 6.72±0.05 correspondingly. The qualified weight of heart of different groups showed that there was no significant (P>0.05) difference between the groups and the values were ranged from 6.11±0.56 to 7.44±0.27 (Table 10).

The comparative weight of gizzard of different groups did not show any significant (P>0.05) difference in groups T_1 (41.28±1.64), T2 (41.00±3.12), T_3 (36.33±1.72), T_5 (37.61±3.64) when compared to group T_4 (35.83±2.03) (Table 10).

Relative weights of giblet organs viz. liver, heart and gizzard revealed no increase in any group. The present results are akin to that of Hernandez *et al.*, (2004), who observed no difference in the mean weight of proventriculus, gizzard, intestine, liver and pancreas in broilers fed on two herbal extracts. In another study by Zanu *et al.*, (2011) neem decoction was evaluated as a total replacement for antibiotics and coccidiostat in a 6 weeks feeding trail in broilers.

4.3.2 Weight of intestine

The results of different groups showed that there was no significant (P>0.05) difference between the groups and the values were ranged from 101.78a±0.22 to 116.83a±6.75 (Table 10).

The present results are akin to that of Hernandez *et al.* (2004), who observed no difference in the mean weight of proventriculus, gizzard, intestine, liver and pancreas in broilers fed on two herbal extracts.

Table 11: Effects of supplementation of dried *Spirulina* powder to broiler diets on some immune organs.

Parameters	T_1	T_2	T_3	T_4	T_5	Mean ±SE	LSD $_{(0.05)}$
Spleen Weight(g)	1.94±0.27	1.73±0.19	1.78±0.36	1.61±0.20	1.44±0.14	1.70±0.104	0.351^{NS}
Bursa Weight(g)	1.72±0.30	1.56±0.31	2.44±0.11	1.72±0.64	1.67±0.00	1.82±o.157	0.496^{NS}

Here, T_1 = (0.5% DSP Supplementation), T_2 = (1% DSP Supplementation), T_3 = (1.5% DSP Supplementation), T_4 = (Antibiotic) and T_5 = (Control). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

4.4 Immune organs

Effect of dried *Spirulina* powder supplementation on immune organs of Cobb 500 strain broiler chicks during the period from 0 to 28 days of age are summarized in Table 11. and Figure 5. The comparative weight of spleen (g) of broiler chicks in the dietary group T_1, T_2, T_3, T_4 and T_5 were 1.94±0.27, 1.73±0.19, 1.78±0.36, 1.61±0.20, 1.44±0.14 respectively. The highest value was T_1 (1.94±0.27) and lowest value was T_5 (1.44±0.14). On the other hand, the relative weight of spleen of different groups showed that there were no significant ($P>0.05$) difference among the groups and the values were ranged from 1.44±0.14 to 1.94±0.27.

The weight of bursa was higher in T_3 group (2.44±0.11) compared to the other group which values were T_1 (1.72±0.30), T_2 (1.56±0.31), T_4 (1.72±0.64) and T_5 (1.67±0.00) correspondingly. But these values are not significantly differing among the treatments (Table 11).

It can be concluded that addition of *Spirulina platensis* to broiler diets improved weight of bursa, spleen compared with the control. But these values were not differing among the groups. In accordance with the present results, Kaoud (2015) showed that the relative and absolute weights of thymus and bursa were induced for the groups fed diet containing *Spirulina* compared to the control group. These results may be considered as good indicator of healthy status of chicks fed dietary *Spirulina*. In this respect, Bennett and Stephens (2006) reported that the bursa functions are half of the immune system and its size reflects overall health status of bird. They added that stressed or sick birds have small size of bursa but, healthy or productive birds have large size. Bursa size is a biological indicator of how flocks are well-managed and preserved from disease. Also, Addition of less than 1% *Spirulina* in chicken diets significantly enhanced the defense systems for antigen processing, greater T-cell activity and increased microbial killing (Qureshi *et al.*, 1996). In addition, increased content of Zn concentration in *Spirulina* is playing a role to induce the cellular immunity of birds (Mohamed, 1998).

Table12. Effect of supplementation of Dried Spirulina Powder (DSP) to broiler diets on blood parameters.

Parameters	T_1	T_2	T_3	T_4	T_5	Mean ±SE	LSD $_{(0.05)}$
Hemoglobin (g/dL)	8.76±0.79	8.53±0.43	8.45±0.33	7.22±0.37	8.03±0.25	8.20±0.231	0.673^{NS}
RBC(million/mm^3)	3.23^{a}±0.35	3.07^{ab}±0.17	2.72^{ab}±0.23	2.48^{b}±0.01	2.51^{b}±0.04	2.81±0.113	0.292*
WBC(10^3/mm3)	6277.78±116	6155.56±908	5922.22±800.3	8833.33±1291.1	7366.67±1578.4	6911.11±494.635	1501.605^{NS}
Neutrophil(%)	62.78±2.35	62.89±0.80	$62.00^{\pm}$2.03	68.78±2.39	67.00±1.89	64.69±1.038	2.802^{NS}
Lymphocyte(%)	33.78^{a}±2.69	33.00^{ab}±0.69	33.89^{a}±2.04	26.67^{b}±2.51	28.56^{ab}±1.45	31.18±1.105	2.854^{*}
Monocyte(%)	1.78±0.11	1.67±0.19	1.67±0.19	1.78±0.11	1.56±0.22	1.69±0.069	0.243^{NS}
Eosinophil(%)	2.67±0.13	2.44±0.11	2.44±0.15	2.78±0.17	2.89±0.19	2.64±0.124	0.427^{NS}
PCV(%)	26.46±2.36	26.67^{a}±1.22	26.07±1.28	23.58±1.15	24.56±0.65	25.47±0.634	2.047^{NS}
MCV (FI)	80.45±2.27	81.85±1.5	78.18±2.57	76.97±2.02	78.87±1.01	79.26±0.871	2.773^{NS}
MCH (pg)	30.12±0.12	29.84±0.13	29.53±0.30	29.82±0.28	30.16±0.32	29.89±0.112	0.352^{NS}
MCHC(g/dl)	31.41^{a}±0.43	31.46^{a}±0.10	30.72^{ab}±0.29	30.24^{b}±0.19	30.22^{b}±0.17	30.81±0.177	0.378^{*}

Here, T_1 = (0.5% DSP Supplementation), T_2 = (1% DSP Supplementation), T_3 = (1.5% DSP Supplementation), T_4 = (Antibiotic) and T5 = (Control).
Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts don't differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference
- ✓ * means significant at 5% level of significance (p<0.0)

4.5 Haematological parameters

Tables (12) show the effect of dietary levels of dried *spirulina* powder (0.5%, 1%, and 1.5%) 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 dried *spirulina* powder, except, RBC, Lymphocyte and MCHC which were significantly affected ($p<0.05$). birds fed diets supplemented with dried *spirulina* powder (at levels of 0.5%,1% and 1.5%) diet had higher values of RBCs, lymphocyte and MCHC but in case of antibiotic and control group this trends is lower than *spirulina* treated groups.

These results are in line with the findings of Kannan *et al.* (2005), Abdel-Daim *et al.* (2013) and Abou Gabal *et al.* (2015). The increment in the blood indices may be related to the rich mineral content in *Spirulina* of Fe, Cu, and zinc (Tokuşoğlu *et al.,* 2003; Babadzhanov *et al.,* 2004). It is well known that iron plays an important role in hemoglobin and red blood cells biosynthesis to prevent anemia and is essential for metabolic enzymes biosynthesis such as cytochromes, superoxide dismutase and glutathione reductase (Bartove and Kanner, 1996; Mohamed, 1998; Badway, 1998). These results are in agreement with the previous reported by Bartove and Kanner, 1996; Mohamed, 1998; Badway, 1998. But 1% of *Spirulina platensis* supplementation significantly ($P<0.05$) improves the Blood Parameters **(**Shanmugapriya *et al.,* 2014). However Kamruzzaman (2005) concluded that the mean haemato-biochemical values of Hb, ESR, PCV, heterophil, eosinophil, basophil, triglyceride, HDL, LDL, SGPT and SGOT were differed significantly ($p < 0.01$) in different groups supplemention with probiotics in broiler ration.

Table 13. Bacterial colony count in *Spirulina* experiment in broiler chicken.

Treatment	E. coli (EMB) $\times 10^4$ (CFU/g)	Salmonella (SS) $\times 10^4$(CFU/g)
T_1	13.07^{ab}±2.78	12.40^{b}±1.55
T_2	14.88^{ab}±2.85	12.25^{b}±2.37
T_3	11.98^{b}±0.71	15.70^{b}±0.20
T_4	10.57^{b}±0.46	13.65^{b}±0.90
T_5	20.25^{a}±3.19	22.50^{a}±2.18
Mean ±SE	14.15±1.25	15.30±1.19
$LSD_{(0.05)}$	3.278^{*}	2.338^{*}

Here, T_1 = (0.5% DSP Supplementation), T_2 = (1% DSP Supplementation), T_3 = (1.5% DSP Supplementation), T_4 = (Antibiotic) and T5 = (Control). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

4.6 Intestinal microflora

The microbial load (total count, E. coli salmonella for its beneficial effect) in broilers fed different levels of dried *Spirulina* powder is given in Table 13, *E. coli* count was significantly (P<0.05) decreased in birds fed 0.5%, 1%, 1.5% dried *Spirulina* powder and antibiotic (13.07±2.78, 14.88±2.85, 11.98±0.71 and 10.57±0.46 respectively) than the control birds (20.25±3.19). *Salmonella sp.* count was significantly (P<0.05) decreased in birds fed 0.5%, 1%, 1.5% dried *Spirulina* powder and antibiotic (12.40±1.55, 12.25±2.37, 15.70±0.20 and 13.65±0.90) than the control birds (22.50±2.18).

These results are in accordance with the earlier findings of Wakwak *et al.* (2003), Kabir *et al.* (2004) and Kulshreshtha *et al.* (2008). In addition, the current results confirmed those of Baojiang (1994) who found that *Spirulina* is useful for the beneficial intestinal flora.

CHAPTER 5

SUMMARY AND CONCLUSION

A total of 150 day-old Cobb-500 broiler chicks were reared in Sher-e-Bangla Agricultural Unoversity Poultry Farm, Dhaka. Chicks were divided randomly into 5 experimental groups of 3 replicates (10 chicks with each replications).One of the 5 experimental group was fed this diet as control while, the remaining four groups were fed diet with 3 levels of DSP (0.5%, 1.0% and 1.5%) and antibiotic.

The effects of supplementation of DSP 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, 45 broilers were dissected to compare meat yield characteristics among different treatments.The group T_3 showed higher body weight compared to any other groups and group T_5, group T_1, group T_2 and group T_4 followed in ascending order. The weight gain, feed consumption, and FCR followed similar trends with an exception that the difference is not significant among group T_1, group T_2 and group T_3 and similar result also found in group T_4 and groupT_5. The FCR was better in all the DSP groups compared to the control group but significant ($p<0.05$) difference with the T_4 and T_5 groups. The relative giblet weight did not show any difference either between any of the treatment groups or the control. The serum biochemistry parameters *viz*. sugar and total cholesterol was studied to evaluate the functional status body. The sugar and cholesterol level of different treatments were similar in all treatments compared to control one. The results indicated no alterations in biochemical parameters, except that a lower amount was observed in cholesterol levels in *Spirulina* supplemented groups. Concerning the treatment effect on blood constituents, the results indicated no significant differences due to supplementation of dried *Spirulina* powder, except, RBC, Lymphocyte and MCHC which were significantly affected ($p<0.05$). birds fed diets supplemented with dried *Spirulina* powder (at levels of 0.5%,1% and 1.5%) diet had higher values of RBCs, lymphocyte and MCHC but in case of antibiotic and control group this trends is lower than *Spirulina* treated groups. The numbers of intestinal microflora (*E coli* and *Salmonella*) were significantly higher in control group compared to other groups. However, *E coli*

and *Salmonella* count had no significant difference between DSP and antibiotic supplementing groups.

Analyzing the above research findings the production performance, hematological parameter, weight of lymphatic organ and microbial load in feaces sample 1.5% *Spirulina platensis* powder was very effective. So *Spirulina platensis* could be used as an alternative of antibiotics on broiler ration. The superior results were found at 1.5% inclusion level of DSP. The study therefore recommends conducting field trial on commercial poultry farm to fix up periodic examination of *Spirulina platensis*.

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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	14.05.09-20.05.09	28.9	29.5	31.6	31.5	30.0	29	30.08
2nd	21.05.09-27.05.09	28.3	28.5	32.1	31.6	30.2	28.5	29.87
3rd	28.05.09-03.06.09	27.0	27.2	28.8	27.2	26.0	25.8	27.00
4th	04.06.09-10.06.09	26.8	27.0	28.6	28.5	27.4	27.2	27.58
5th	11.06.09-17.06.09	25.9	26.2	27.5	27.0	26.5	26.4	26.58

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	14.05.09-20.05.09	85	82	73	74	78	80	78.67
2nd	21.05.09-27.05.09	85	83	71	72	77	79	77.83
3rd	28.05.09-03.06.09	86	85	74	75	81	83	80.67
4th	04.06.09-10.06.09	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 (%)
T1	R1	1566.67	1048	66.89347
	R2	1680	1069.67	63.67083
	R3	1503	1043.33	69.4165
T2	R1	1633.33	1060.33	64.9183
	R2	1506.67	1092	72.47772
	R3	1460	1127.66	77.23699
T3	R1	1683.33	1173	69.68331
	R2	1649.33	1149	68.45204
	R3	1480	1120	76.14865
T4	R1	1533	1025	70.12394
	R2	1426	1017.66	73.46844
	R3	1423.33	1043	75.38659
T5	R1	1676	1054	62.88783
	R2	1483	1069	72.08361
	R3	1493.33	1084	72.58945

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

Treatment	Replication	Liver weight (g)	Spleen Weight(g)	Gizzard Weight(g)	Bursa Weight(g)	Intestine Weight(g)	Heart weight (g)
T_1	R1(1)	40	2.5	41.5	2.5	92	4.5
	R1(2)	36	3.5	44.5	0.5	124	8
	R1(3)	56	1.5	38.5	1.5	90	7.5
	R2(1)	36.5	1.5	50	1	127	8.5
	R2(2)	30	1	44	3	80	7
	R2(3)	34.5	2.5	38	3	99	11
	R3(1)	34	1.5	49	1	83	6
	R3(2)	39	1.5	25	1	143	7.5
	R3(3)	35	2	41	2	78	6.5
T_2	R1(1)	53	3	47.5	4	115	7
	R1(2)	36.5	1.5	43	1.5	104	6.5
	R1(3)	32	1.5	51	1	107.5	7
	R2(1)	33	2	33	0.5	85.5	7
	R2(2)	38	2	39.5	2	91.5	5.5
	R2(3)	34.5	1.5	43	1	87	7
	R3(1)	46	1.6	40	1.5	94	5
	R3(2)	34	1.5	36	1.5	139	6
	R3(3)	29	1	36	1	109.5	4
	R1(1)	42.5	3.5	29.5	3	136	9

Appendix 6 (Cont'd)

Treatment	Replication	Liver weight (g)	Spleen Weight(g)	Gizzard Weight(g)	Bursa Weight(g)	Intestine Weight(g)	Heart weight (g)
T_3	R1(2)	33.5	2	34.5	3	87	6.5
	R1(3)	40	2	53	1	122	8.5
	R2(1)	39.5	1.5	32	2.5	136	6
	R2(2)	30	1	28.5	2.5	134	7
	R2(3)	41	2	39	2	118	8.5
	R3(1)	40	1.5	29	3	129.5	9
	R3(2)	23.5	1.3	34.5	2.5	95	6
	R3(3)	30	1.2	47	2.5	94	6.5
T_4	R1(1)	42	1.5	46	2.5	110	7
	R1(2)	41	3	33.5	4	131	8
	R1(3)	32	1.5	32	2.5	96	6.7
	R2(1)	32	2.5	38.5	1	84	6.5
	R2(2)	29	1	42	1.5	117	6.5
	R2(3)	28	1	35	1	115	5
	R3(1)	32	1.5	37	2	86	6
	R3(2)	34	0.5	22.5	0.5	127	6.5
	R3(3)	36	2	36	0.5	107	6.5
T_5	R1(1)	37.5	1.5	43.5	2.5	114	7.5
	R1(2)	38	1.5	37	1.5	103	6.5
	R1(3)	40.5	1.5	44	1	130	6.5
	R2(1)	33.5	1	42.5	2	125	5
	R2(2)	34.5	1	20.5	2.5	129	8
	R2(3)	31	3	28	0.5	69	7
	R3(1)	27.5	0.5	37.5	1	81.5	6.5
	R3(2)	37	1	39.5	1.5	76	5.5
	R3(3)	43	2	46	2.5	116	8

Appendix 7. Biochemical data in different treatment groups.

Treatment	Replication	Sugar mmol/L	Cholesterol mg/dl
T_1	R1(1)	11.2	138
	R1(2)	8.4	98
	R1(3)	9.9	120
	R2(1)	9	155
	R2(2)	11.2	110
	R2(3)	9.9	98
	R3(1)	10.1	125
	R3(2)	9.9	127
	R3(3)	12	100
T_2	R1(1)	8.5	135
	R1(2)	9.2	99
	R1(3)	10	135
	R2(1)	10.9	129
	R2(2)	10.2	115
	R2(3)	8.6	120
	R3(1)	9.5	99
	R3(2)	7.9	115
	R3(3)	9.5	110
T_3	R1(1)	10.2	110
	R1(2)	9.9	140
	R1(3)	9.8	166
	R2(1)	11	135
	R2(2)	14.2	162
	R3(1)	8.9	145
	R2(3)	10.4	154
	R3(2)	9.8	95
	R3(3)	12.6	175
T_4	R1(1)	9.9	111
	R1(2)	8.9	99
	R1(3)	7.9	112
	R2(1)	10.8	142
	R2(2)	11.4	145
	R2(3)	9	123
	R3(1)	8.8	122
	R3(2)	13	178
	R3(3)	11	138
T_5	R1(1)	8.8	122
	R1(2)	11	146
	R1(3)	10.6	132
	R2(1)	10.4	130
	R2(2)	11.2	126
	R2(3)	8.9	190
	R3(1)	8.8	126
	R3(2)	12	177
	R3(1)	8.9	121

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

Treatments	Replications	Hb (gm/dl)	RBC (Milion /Cumm)	WBC	Neutrophil/Cumm	Lymphocyte	Monocyte	Eosinophil	HCT/PCV	MCV	MCH	MCHC
						%	%	%	%	(FI)	pg	g/dl
T_1	R1(1)	9.3	3.58	6000	65	32	1	2	28.3	81.46	31.18	32.5
	R1(2)	5	2.14	5,400	68	26	2	4	16.2	70.12	28.31	30.27
	R1(3)	7.4	2.18	8,000	66	29	2	3	21.5	80.24	30.16	29.34
	R2(1)	9.8	3.25	4,500	60	36	2	4	28.3	80.24	30.16	32.57
	R2(2)	10.23	4.12	4,200	61	34	2	3	31.5	85.24	30.14	32.57
	R2(3)	7.4	3.21	5,000	63	33	3	1	22.1	80.14	30.25	31.46
	R3(1)	10.23	4.25	5,100	59	35	2	4	31.5	89.21	30.24	33.16
	R3(2)	8	2.81	5,300	61	34	2	3	25.1	80.16	28.34	29.31
	R3(3)	8.4	2.95	5,000	64	33	2	1	25.3	81.24	30.16	32.54
T_2	R1(1)	7	2.46	8,000	64	30	2	4	21.5	80.16	30.28	31.47
	R1(2)	8	2.9	9,300	65	31	1	3	25.1	81.24	30.16	32.57
	R1(3)	7.4	2.5	6,800	70	27	2	1	22.16	80.26	30.16	32.57
	R2(1)	9.3	3.14	8,400	62	34	2	2	28.3	81.46	28.34	31.49
	R2(2)	11.16	4.12	4,000	63	34	1	2	34.5	89.21	30.46	32.54
	R2(3)	8	2.8	5,100	61	36	2	1	25.6	80.13	29.3	30.16
	R3(1)	7.4	2.15	4,500	56	40	1	3	22.5	70.16	28.34	30.16
	R3(2)	8	2.5	5,000	58	39	1	2	25.6	80.16	28.34	30.15
	R3(3)	7.2	2.41	9,600	69	26	3	2	22.16	80.24	30.18	32.49
T_3	R1(1)	6	2.1	18,500	77	19	1	3	29.3	70.16	29.34	29.1
	R1(2)	6.1	2.05	10,000	71	23	1	5	19.2	60.24	30.16	29.34
	R1(3)	7.4	2.8	8,000	72	25	1	2	22.16	80.13	30.25	31.27
	R2(1)	7.4	2.6	6,000	70	26	3	1	22.5	80.16	30.27	30.14
	R2(2)	7.4	2.4	7,000	65	31	2	2	24.1	80.16	30.25	29.34
	R2(3)	8.1	2.5	8,000	65	31	2	2	25.6	81.24	29.34	30.16
	R3(1)	7	2.41	9,600	69	26	3	2	22.16	80.24	30.18	29.34

Appendix 8 (Cont'd)

Treatments	Replications	Replications	Hb (gm/dl)	RBC (Milion /Cumm)	WBC	Neutrophil/Cumm	Lymphocyte	Monocyte	Eosinophil	HCT/PCV	MCV	MCH
							%	%	%	%	(FI)	pg
	R3(2)	6	2.85	6,000	68	26	2	4	22.1	80.24	28.31	31.27
	R3(3)		2.1	18,500	77	19	1	3	29.3	70.16	29.34	30.14
T_4	R1(1)	7.4	2.05	10,000	71	23	1	5	19.2	60.24	30.16	29.34
	R1(2)	7.4	2.8	8,000	72	25	1	2	22.16	80.13	30.25	29.34
	R1(3)		2.6	6,000	70	26	3	1	22.5	80.16	30.27	31.27
	R2(1)	7.4	2.4	7,000	65	31	2	2	24.1	80.16	30.25	29.34
	R2(2)	8.1	2.5	8,000	65	31	2	2	25.6	81.24	29.34	30.16
	R2(3)	8.4	2.6	6,400	62	33	1	4	25.1	80.16	30.24	30.18
	R3(1)	8	2.41	9,200	72	25	1	2	26.5	84.21	30.16	30.25
	R3(2)	7.4	2.5	5,600	61	34	2	3	21.5	75.16	30.25	30.14
	R3(3)	8	2.5	7,500	65	30	1	4	25.3	80.16	30.24	30.19
T_5	R1(1)	8.4	2.58	4,000	63	32	2	3	25.6	80.16	32.47	31.29
	R1(2)	6.5	2.1	6,000	69	25	2	4	19.3	70.25	29.34	30.12
	R1(3)	8.4	2.7	3,800	61	34	2	3	25.6	80.13	30.27	30.19
	R2(1)	9.8	2.8	11,200	76	20	2	2	29.3	80.12	30.1	30.27
	R2(2)	7.4	2.4	7,000	65	31	2	2	24.1	80.16	30.25	29.34
	R2(3)	8.1	2.5	8,000	65	31	2	2	25.6	81.24	29.34	30.16
	R3(1)	9.8	2.7	10,200	75	20	2	2	29.3	80.12	30.1	30.27
	R3(2)	7.4	2.4	8,000	65	30	2	2	24.1	80.16	30.25	29.34
	R3(3)	9.1	2.5	9,000	66	31	2	2	25.6	81.24	29.34	30.16

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	R1	141	226	590	700
	R2	140.8	370	589	700
	R3	128.1	383	600	750
T_2	R1	134.1	370	620	710
	R2	131.4	360	565	680
	R3	140.9	371	594	660
T_3	R1	128.3	360	575	760
	R2	135.7	343	591	820
	R3	133	360	595	820
T_4	R1	134.9	372	604	800
	R2	129.1	360	583	780
	R3	124.6	353	572	790
T_5	R1	130.2	369	620	880
	R2	137.8	365	591	820
	R3	140.3	362	590	780

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

Treatment	Replication	1st Week Body Weight /Bird(g)	2nd Week Body Weight /Bird(g)	3rd Week Body Weight /Bird(g)	4th Week Body Weight /Bird(g)
T_1	R1	162	452	900	1480
	R2	161.9	451	900	1450
	R3	169.6	480	910	1420
T_2	R1	153	476	950	1430
	R2	155.2	462	900	1380
	R3	172.2	457	900	1340
T_3	R1	151.8	451	920	1460
	R2	167.70	446	920	1520
	R3	170.5	469	930	1450
T_4	R1	170	470	940	1350
	R2	153	455	900	1300
	R3	153.3	460	920	1300
T_5	R1	163.7	476	950	1380
	R2	169	460	890	1320
	R3	162	452	880	1300

Appendix 11. Some photograph of dried *Spirulina* experiment conducted at SAU poultry farm.

Activities after arrival of day old broiler chicks.

Appendix 11. Cont'd

Monitoring of research activities by the supervisor.

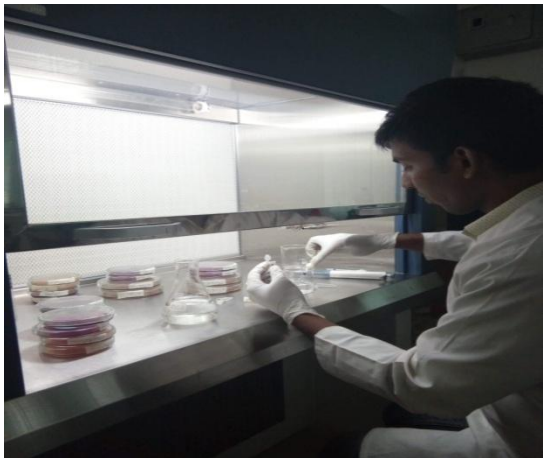
Appendix 11. Cont'd

Different types of Medication and vaccine used in experiment.

Appendix 11. Cont'd

Monitoring and weighing of dressed broiler chicken with internal organs.

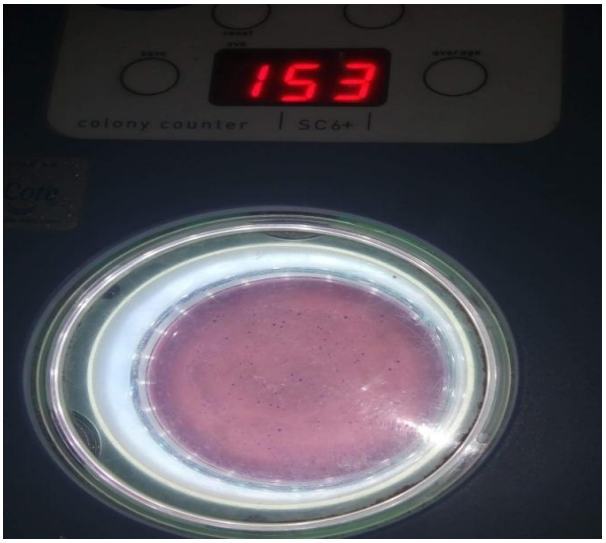
Appendix 11. Cont'd

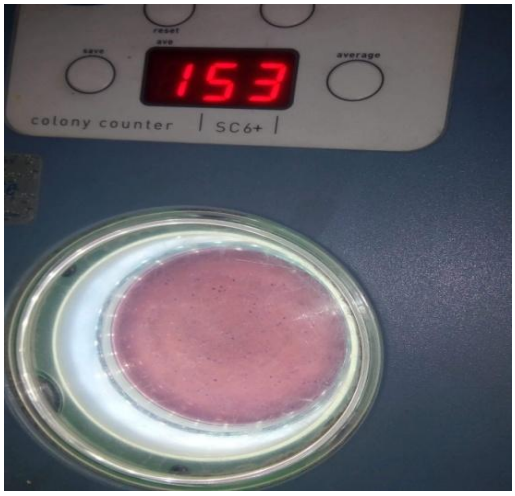


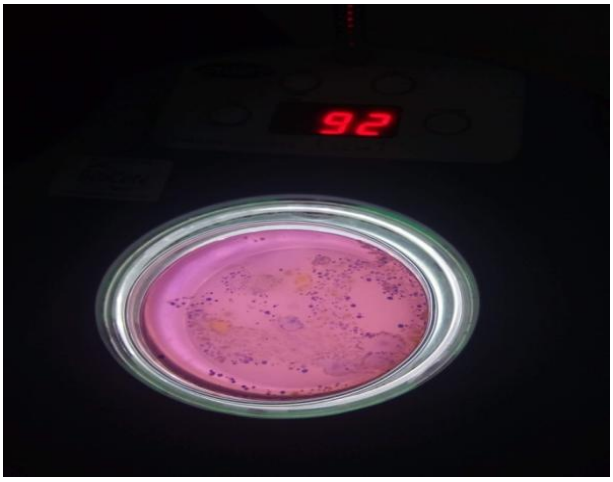

Transfer microbial sample to the incubator for incubation.


Centrifuge of feces sample for bacterial colony count.

Appendix 11. Cont'd







Bacterial colony count by colony counter.

Appendix 11. Cont'd

Collection of blood at the age of 25 days of old.