THE EFFECTS OF DIETARY SUPPLEMENTION OF FENUGREEK (*Trigonella foenum-graecum*) SEED AS AN ALTERNATIVE TO ANTIBIOTIC ON GROWTH PERFORMANCE AND HEALTH OF BROILER CHICKEN

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*This is to certify that the thesis entitled "**THE EFFECTS OF DIETARY SUPPLEMENTATION OF FENUGREEK (Trigonella foenum-graecum) SEED AS AN ALTERNATIVE TO ANTIBIOTIC ON GROWTH PERFORMANCE AND HEALTH 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 **Sadik Ahmed**, Registration No. **13-05572** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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TO

MY PARENTS AND TEACHERS

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The Author

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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
CDIL	=	Central Disease Investigation Laboratory
CF	=	Crude Fibre
CFU	=	Colony Forming Units
Cm	=	Centimeter
cm^2	=	Square Centimeter
CONTD.	=	Continued
CP	=	Crude Protein
CRD	=	Complete Randomized Design
DMD	=	Dry Matter Digestibility
Dr.	=	Doctor
e.g.	=	For Example
EDTA	=	Ethylene Diethyl Tetra acetic Acid
et al.	=	And others/Associates
FC	=	Feed Consumption
FCR	=	Feed Conversion Ratio
FSP	=	Fenugreek Seed Powder
FOS	=	Fructos-oligosaccharides
FS	=	Fenugreek Seed
FR	=	Fenugreek Residue
gGSH	=	gGram Glutathione
Hb	=	Hemoglobin
HETE	=	Hydroxy Eicosatetraenoic Acid
HPA	=	Hypothalamus Pituitary Axis
i.e.	=	That is
IBV	=	Infectious Bronchitis Vaccines

ACRONYMS AND ABBREVIATIONS

Abbreviation		Full meaning
Kcal	=	Kilo-calorie
Kg	=	Kilogram
M.S.	=	Master of Science
MDA	=	Malondialdehyde
ME MOS	=	Metabolizable Energy Mannan-oligosaccharides
Ml	=	Mililitre
MCHC	=	Mean Corpuscular Hemoglobin Concentration
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

ACRONYMS AND ABBREVIATIONS

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

THE EFFECTS OF DIETARY SUPPLEMENTION OF FENUGREEK (*Trigonella foenum-graecum*) SEED AS AN ALTERNATIVE TO ANTIBIOTIC ON THE GROWTH PERFORMANCE AND HEALTH STATUS OF BROILER CHICKEN

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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 graded levels of FS (Fenugreek Seed) compared to antibiotic based diet. Chicks were distributed randomly in a complete randomized design 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 FS (1, 1.5 and 2%) and antibiotic. The results obtained revealed that the body weight was significant ($P<0.05$) and the dressing percentage was also significant ($P<0.05$) by the dietary inclusion of FS as compared to control fed broilers. However, a linear increase in body weight was found with the increase in FS level in the diet. Birds fed 1.5% FS diets achieved superior body weights ($1528.33^{a}\pm57.468$) compared to those of the control and antibiotic group. Feed Conversion Ratio (FCR) was non-significant but feed consumption was significantly higher ($2289.67^{a}\pm2.603$) in 1% FS treated group in comparison to others. 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 Fenugreek seed had no significant ($P>0.05$) effects on liver, gizzard, intestine and heart weight among the treatments. The level of Cholesterol was significantly ($p<0.05$) lower in fenugreek treated group but no significant differences in glucose level. The results of hematological studies showed no significant ($P>0.05$) differences due to supplementation of Fenugreek seed, except Hemoglobin, Red blood cell (RBC), White blood cell (WBC), Lymphocyte which were significantly affected ($p<0.05$) compared with control and antibiotic. However, addition of FS to broiler chicks diets showed significant ($p<0.05$) difference in bacterial colony count among the groups. The FS supplementing groups showed lower number of E. coli and Salmonella sp. and higher number of Lactobacillus sp. compared to control group. Treatments with FS significantly ($P<0.05$) increased Newcastle disease (ND) titre.

CHAPTER I

INTRODUCTION

The most important sources of animal protein in the world is poultry meat and therefore, contributing significantly in maintaining the health status of the people, especially in developing countries like Bangladesh. Poultry meat alone contributes 37% of the total meat production in Bangladesh (Hamid *et al.,* 2017*).* Overall poultry contributes about 22-27% of the total animal protein supply in the country (DLS, 2015).

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

The term "antibiotic growth promoter" is used to describe any medicine that destroys or inhibits bacteria which is administered at a low sub-therapeutic dose. The mechanism of action of antibiotics as growth promoters is related to interactions with intestinal microbial population (Dibner and Richards, 2005).
The antibiotic growth promotors have been used in poultry feed worldwide during the last 50 years (Yegani and Korver, 2008),but their ban has lead the world to restrict their use in animal feed as growth promotors (Nisha, 2008).Therefore 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 and their seeds have been used for medical treatment since prehistoric time (Dragland *et al.*, 2003). There are some important bioactive components such as alkaloids, bitters, flavonoids,

glycosides, mucilage, saponins, tannins (Vandergrift, 1998) phenols, phenolic acids, guinones, coumarins, terpenoids, essential oils, lectins and polypeptides (Cowan, 1999) in the structures of nearly all the plants. The use of various plant materials as dietary supplements may positively affect poultry health and productivity.

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

Generally plant extracts have no problem of resistance (Tipu *et al.*, 2006) and broilers fed on herbal feed additives were accepted well by the consumers (Hernandez *et al.*, 2004).

Herbs and plant extracts are being incorporated in poultry feed as growth promotors (Alloui *et al.*, 2013).Compared with synthetic antibiotics or inorganic chemicals, these plant derived products have been proven to be safe, less toxic, residue free and are thought to be ideal feed additives in food animal production (Hashemi and Davoodi, 2010).

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. Herbs and plant extracts used in animal feed are known as phytogenic feed additives. Phytogenics have been defined as plant-derived natural bioactive compounds

with positive effects on animal growth and health (Puvaca *et al.*, 2013). They are incorporated in the diet of animal feed in order to enhance productivity by improvement of digestibility, nutrient absorbtion and elimination of pathogens residence in the gut (Athanasiadou *et al.*, 2007).

Among the herbal flora available in Bangladesh, seeds of fenugreek (Trigonella foenum graecum) was utilized for the study in the diet of broiler chicken.

Fenugreek seeds have many therapeutical effects such as hypoglycaemic, anti- helminthic, anti-inflammatory and anti-microbial properties (Bash *et al.*, 2003). It also contains lecithin and choline that help to dissolve cholesterol and fatty substances. It also contains neurin, biotin, trimethylamine which tends to stimulate the appetite by their action on the nervous system (Michael and Kumawat, 2003).

Moreover enzyme supplementation in poultry diets has been reported to improve the performance (Yousuf *et al.*,2012) by degrading non starchy polysaccharides, improving the digestion and absorption of nutrients (Tufarelli *et al.*, 2007) and improving their intestinal morphology (Ayoola *et al.*, 2015). Fenugreek seeds can also improve immunity, ND titre, immunoglobulin, white blood cell, red blood cell and hematocrit counts (Motamedi and Taklimi, 2014). Elmahdi Elbushra noted that significant improvements in efficiency of energy utilization values in average feed consumption were recorded for the groups fed diets with 0.5 and 1.5 % during the experiment period (Elbushra, 2012).

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

1. To evaluate the growth performance and hematological properties of broiler fed FS based diet comparison with antibiotic and basal diet.

2. To find out the effect of FS on *E coli sp.*, *Salmonella sp*. and *Lactobacillus sp.*

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

4. To study the effect of FS on ND titre properties of broiler chicken.

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 150 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 60 were full article and 55 abstracts, 35 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 *Trigonella*.

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.

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

Specifically, these are raised for meat production under intensive production system using commercial feed ration. However, broiler production cost has gone up substantially in recent years due to the increase in price of feed ingredients. The search for cheap, locally available and equally nutritive feed sources to partially substitute commercial poultry diet has never been more pressing. Plant proteins are good sources of dietary fiber and essential amino acids in the diet. Unlike livestock farming, poultry farming is always intensive and hence the birds are more subjected to stressful conditions. Stress is an important factor that renders the birds vulnerable to potentially pathogenic microorganisms like E.coli, salmonella, clostridium, camphylobacter etc. These pathogenic 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 Alternatives to antibiotic growth promoters

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

2.4.1 Probiotics

Probiotics are individual microorganisms or groups of microorganisms, which have favourable effect on host by improving the characteristics of intestinal 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 cerevisiaes) (Zikic *et al.*, 2006).

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

In this way, they create conditions in intestines, which favour growth of useful bacteria and inhibit the development of pathogenic bacteria (Line *et al.*, 1998). They improve the function of the immune system (Zulkifli *et al.*, 2000; Kabir *et al.*, 2004) and exhibit significant influence on morpho-functional characteristics of intestines (Yang *et al.*, 2009). These effects lead to growth of broiler chickens (Jin *et al.*, 1997; Li *et al.*, 2008), improvement of feed conversion (Li *et al.*, 2008; Zulkifli *et al.*, 2000; Kabir *et al.*, 2004) and reduced mortality (Mohan *et al.*, 1996).

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

Nutrition plays a key role in maintaining the prooxidant-antioxidant balance (Cowey, 1986). Under physiological conditions the reactive species figure a crucial role in primary immune defense (Diplock *et al.*, 1998). But prolonged excess of reactive species is highly damaging for the host biomolecules and cells, resulting in dysbalance of the functional antioxidative network of the organism and leading to substantial escalation of pathological inflammation (Petrof *et al.*, 2004).

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

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

2.4.2 Prebiotics

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

The most significant compounds, which belong to group of prebiotics, are 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.

2.4.3 Synbiotics

This is relatively recent term among additives used in poultry nutrition. Synbiotics are combination primarily of probiotics and prebiotics, as well as other promoting substances which together exhibit joint effect with regard to health of digestive tract, digestibility and performances of broilers. Investigations showed that combinations used in synbiotics are often more efficient in relation to individual additives (Ušćebrka *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 *et al.*, 2011).

2.4.4 Enzymes

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

It is obvious that the positive effect of application of additives depends on the quantity and quality of feeds included in the mixture, type of enzyme, as well as fattening conditions (Acamovic, 2001; Lukic *et al.*, 2002). Obtained results in some researches indicate that better effect is realized with utilization of two or more enzymes in food (Silversides and Bedford, 1999; Chesson, 2001). Therefore, new enzyme combinations are constantly analyzed, as well as their optimum doses, in order to realize positive financial effect through improved utilization of feeds. The main reasons for supplementing wheat- and barley-based poultry diets with enzymes is to increase the available energy content of the diet. Increased availability of carbohydrates for energy utilization is associated with increased energy digestibility (Partridge and Wyatt, 1995; Van der Klis *et al.*, 1995).

Enzymes have been shown to improve performance and nutrient digestibility when added to poultry diets containing cereals, such as barley and wheat (Friesen *et al.,* 1992; Marquardt *et al.*, 1994), maize (Saleh *et al.*, 2003), oats and rye (Friesen *et al.*, 1991, 1992; Bedford and Classen 1992; Marquardt *et al.*, 1994) and to those containing pulses, such as lupins (Brenes *et al*., 1993). The effect of enzyme supplementation on dry matter digestibilities (DMD) in pigs and poultry depends on the type of diet and the type of animal: increases in DMD range from 0.9 (Schutte *et al.,* 1995) to 17% (Annison and Choct, 1993) in poultry. Morgan and Bedford (1995) reported that coccidiosis problems could be prevented by using enzymes. According to Bharathidhasan *et al.* (2009) when Broilers were supplemented with enzyme level at 0, 250, 500, 750 and 1000 g/ton of feed there was no significant difference in carcass yield, dressing percentage, giblet weight, carcass weight, intestinal length and organoleptic characteristics of the meat.

2.4.5 Acidifiers

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

2.4.6 Antioxidants

Antioxidants are the agents, which donate free electron to reactive oxygen species (ROS) and reactive nitrogen species (RNS) and convert them to harmless substances and break the chain reaction (Dekkers *et al.*, 1996).

After donating an electron, an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive. Antioxidants are synthesized within the body and can also be extracted from the food that humans and animals eat, such as fruits, vegetables, seeds, nuts, meat, oil, leaves and grass (natural antioxidants). There are two lines of antioxidant defense within the cell. The first line, found in the fat-soluble cellular membrane consists of vitamin E, *beta*-carotene and coenzyme-Q (Kaczmarski, 1999). Of these, vitamin E is considered to be the most potent chain-breaking antioxidant within the membrane of the cell. The second line, inside the cell consists of water soluble antioxidant scavengers that include vitamin C, glutathione peroxidase, superoxide dismutase (SOD) and catalase (CAT) (Dekkers *et al.*, 1996). To maximize the oxidative stability of meat, antioxidants, mostly α-tocopheryl acetate (ATA), are added to feeds.

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

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

Therefore, selenium and 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).

2.4.7 Herbal adaptogens

An adaptogen is a substance that shows some nonspecific effect, such as increasing body resistance to physical, chemical, or biological noxious agents and have a normalizing influence on pathological state, independent of the nature of that state. A vast number of plants have been recognized as valuable sources of natural antimicrobial compounds (Mahady, 2005). A wide range of phytochemicals present in plants are known to inhibit bacterial pathogens (Cowan, 1999; Medina *et al.*, 2005).

Successful determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone and methanol are often used to extract bioactive compounds (Eloff, 1998). Ethanol is the most commonly used organic solvent by herbal medicine manufactures because the finished products can be safely used internally by consumers (Low Dog, 2009) In terms of active ingredients, adaptogenic preparations

can be divided into three groups.

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 Those that contain tetracyclic triterpenes, such as cucurbitacin R diglucoside, an effect in the early stages of the stress response (Kochetkov *et al.*, 1962; Wagner, 1995).

b. 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 clearer when it is recalled the stress is a defensive response to external factors and that it stimulates the formation of endogenous messenger substances such as catecholamines, prostaglandins, cytokines, NO and platelet-activating factor, which inturn activate other factors that may either counteract stress or conversely, induce or facilitate disease. According to this concept, the “stress-executing” or „“switch- on““ mechanism activates the sympathoadrenal system (SAS) and over the longer

term also activates the HPA, together with various regulators of cell and organ function (Panossian, 1999).

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

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

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

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

In the past two decades a number of ayurvedic preparations have been extensively used in poultry industry in India. Preparations like Livol® and Zeestress® have been found to possess 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).

2.5 Fenugreek Seed

The fenugreek is an erect small annual leguminous herb belonging to the family of fabaceae of the genus Trigonella. The plant grows up to about 2 feet high, similar in habit to Lucerne, with light green color trifoliate

leaves and white flowers, it bear long slender, yellow-brown pods containing 10-20 golden-yellow color seeds. The seeds are brownish, about 1/8 inches long, rhomboidal, with a deep furrow dividing them into two unequal lobes.

They are contained, ten to twenty together, in long narrow, sickle, like pods. Raw seeds have maple flavor and bitter taste. However, their taste become more acceptable once they were gently dry-roasted under light heat. It is self -pollinating crop (Petropoulos, 2002).

Fenugreek is used both as herb (the leaves) and as spice (the seed). It is cultivated worldwide as semi-arid crop. Trigonella foenum-graecum (fenugreek) is an annual herb cultivated worldwide. It originated from southeastern Europe and western Asia.

The seeds have many uses especially in folk medicine. It is a good source of dietary protein for consumption by human and animals. It is having properties of lowering blood sugar level, anthelmentic, antibacterial, anti–inflammatory, antipyretic, and antimicrobial.

It contains minerals, B complex, iron, Phosphates, (Para Amino Benzoic Acid), A and D vitamins, lecithin and choline that help to dissolve cholesterol and fatty substances (Dixit *et al.,* 2005, Caunii *et al.;* 2015; Ianculov *et al.;* 2004).

It also contains neurin, biotin, trimethylamine which tends to stimulate the appetite by their action on the nervous system (Ahmadiani *et al.,* 2001, Samfira *et al.;* 2014; Butnariu; 2012; Butu *et al.;* 2014a).

Gacche and collab reported moderate level of anti–proteolytic activity in fenugreek [Gacche *et al.* 2010].

Fenugreek contains coumarins and other constituents that might affect platelet aggregation, but this might not be significant clinically. It contains

different alkaloids, flavonoids and saponins but out of all these, saponins are found to be in maximum concentration in the fenugreek [Tariq *et al.*, 2016; Kumari *et al.*, 2012].

2.5.1 Chemical composition of Fenugreek seed:

Chemical composition (Table 1) and antioxidant activity of husk (seed coat) and endosperm of fenugreek seeds have revealed that endosperm has the highest content of saponin (4.63%) and protein (43.8%) (Madhava Naidu et al., 2010). In contrast, husk (seed coat) contains higher amount of polyphenols (103.8 mg of garlic acid equivalent) and total dietary fiber (77.1%).

Schryver, (2002) reported that fenugreek is a good source of dietary protein (2030%), the fatty acids from 5-10% which are predominantly linoleic, linolenic, oleic and palmatic acids. It had 45-65% total carbohydrates with 15% galactomannan (a soluble fiber).

The seeds contain many phytochemical compounds such as choline trigonelline, diosgenin, vamogenin, gitogenin, tigogenim and neotigogens . The fenugreek seed is an excellent source of minerals like copper, potassium, calcium, iron, selenium, zinc, manganese and magnesium. It also rich in many vital vitamins that are essential nutrients for optimum health including thiamin, pyridoxine (vit B6),linolic acid, riboflavin, niacin, vitamin A and vitamin C, (Michael and kumawat, 2003). Rao and Sharma (1987) found that the seeds of fenugreek contained 4.8% saponins. Fenugreek seeds contained 27%protein, 7-10% oil (Akgul, 1973) also, Abd El-Aal and Rahma, (1986)reported that fenugreek is considered to be a good source of crude protein, crude fat and total carbohydrates .Srinivasan (2006) reported that fenugreek mature seeds (100g)contained protein 30g, fat 7.5g, fiber 50g, sapogenins, diosgenins,yamogenin, gitogenin, neogitogenin, yuccagenin, tigogenin ,sarsasapogenin, smilagenin 2g, trigonelline 380 mg, Ca 160mg, Mg160mg, P 370mg, Fe 14mg, Na 19mg, K 530mg, Cu 33mg, S16mg, CI 165mg, Mn 1.5g, Zn 7.0mg, Cr 0.1mg,

Choline 50mg, vitamin C 50mg, B-carotene 90mg, Thiamine 340mg, Riboflavin 290 mg, Nictoinic acid 1.1mg, folic acid 84mg .Fenugreek seed contains approximately 4-10% moisture, 6-8 fat,18-30% protein and 4855% fibers (Sauvaire et al,. 1976; Sharma,1986b; Vats et al., 2003 and Srinivasan, 2006) depending on varietal and ecological factors. Hemavathy and Prabhakar (1989) reported the lipid composition of fenugreek seeds that total lipids extracted from dry seeds were 7.5% (neutral lipid 84.1%, 5.4% glycolipids and 10.5%phospholipids).

Plate 1. Fenugreek seed (FS)

Table1. Percent composition of fenugreek seeds (dry matter basis)

Item	percent
Moisture	4.1
Dry matter	95.9
Crude protein	25.68
Ether extract	27.6
Crude fibre	0.4
Nitrogen free extract	34.83
Ash	7.3
ME (Mcal/kg)	2.3896

Lodhi et al., (1976)

2.5.2 Mechanism of action of Fenugreek seed:

The effect of the fiber content of fenugreek has been attributed to its ability to inhibit lipid and carbohydrate-hydrolyzing enzymes in the digestive

system (Hannan et al., 2007, Srechamroen et al., 2009, Hamden et al., 2010), which is a well-established mechanism by which fiber has been shown to inhibit lipid and glucose absorption (Eastwood *et al.*, 2005) and thereby decrease postprandial hyperglycemia and hyperlipidemia (Ku *et al.*, 2009).

Dietary fiber from fenugreek blunts glucose and cholesterol after a meal and regulates the production of cholesterol in the liver. Fenugreek seeds contain 45.4% dietary fiber (32% insoluble and 13.3% soluble), and the gum is composed of galactose and mannose. The latter compounds are associated with reduced glycemia and cholesterolemia. Fenugreek's hypoglycemic effect has been especially documented in humans and animals with type 1 and type 2 diabetes mellitus. In addition, this dietary fiber has potential for widespread use in the food industry because its galactomannan composition has emulsifying and stabilizing properties (Keisha T Roberts., 2011).

2.5.3 Antioxidant properties of Fenugreek seed:

Fenugreek (*Trigonella-Foenum Graecum*) is known as one of the traditional and most promising medicinal herbs belongs to the leguminous family. The seeds of fenugreek have been extensively studied for the treatment of inflammation, cancer and diabetes.

The antioxidant activity of fenugreek against 2,2-diphenyl-picrylhydrazyl (DPPH) and 2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+\bullet}$) radicals is higher due to its higher total phenolic content (TPC) and total flavonoid content (TFC) presence in it (Sweeta *et al.*, 2018). According to Shimon et al. (1995) the fenugreek has volatile oil, phenolic acids and flavonoids; therefore it is a potent source of antioxidants.

Syeda *et al,* (2008) reported that Crude extracts of fenugreek were prepared by soxhelt extraction method with different solvents such as methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate. Extracts were subjected for the measurement of total phenolic content (TPC) by Folin-

Ciocalteu method as well as flavonoid content, chelating activity, reducing power and antioxidant/radical scavenging activity [1, 1-diphenyl-2-picryl-hydrazyl (DPPH°) free radical scavenging activity]. Results from different parameters were in agreement with each other. The results reveal that all extracts of the fenugreek exhibit antioxidant activity. These findings suggest that the fenugreek extracts could act as potent source of antioxidants.

2.5.4 Anti-inflammatory activities of Fenugreek seed

Petroleum ether extract of fenugreek seeds has significant anti-inflammatory and anti-arthritic activities which are due to the presence of linolenic and linoleic acids.It contains oleic (33.61%), linoleic (40.37%), and linolenic (12.51%) acids in it (Pundarikakshudu *et al.*, 2016).

Mostafa *et al.*, (2018) reported success in separating flavonoid-rich fractions with anti-inflammatory effect from fenugreek seeds (Trigonella foenum-graecum L.). They carried out further fractionation to find active anti-inflammatory subfractions. Trigonelline content of the plant was determined by spectrophotometric method. Fenugreek seeds were extracted consecutively with petroleum ether, acidified chloroform (ACC), alkaline chloroform (AKC), methanol, and water. ACC fraction, which had exhibited the highest anti-inflammatory effect, was further fractionated using column chromatography. Obtained subfractions were evaluated using carrageenan-induced paw edema (CIPE) method. Animals were pretreated by test compounds, and after 30 minutes edema was induced by subcutaneous injection of 100 µl of 1% w/v carrageenan into the right paw of animals. Volume difference of both paws was measured at different times after carrageenan injection. The concentration of trigonelline was determined as 16.2%. ACC fraction inhibited paw edema significantly in comparison to control ($p < 0.05$).

2.5.5 Effect of Fenugreek seed on live weight and live weight gain

Tariq *et al.,* (2014) showed that supplementation of fenugreek seed powder in various levels improved significantly ($p<0.05$) live body weight. This might be due to the presence of the fatty acids, or due to stimulating effect on the digestive system of broilers (Hernandez *et al.,* 2004). These findings were also in agreement with those of Alloui *et al.,* (2012) who noted that addition of fenugreek seed in broiler diets increased live body weight. Tariq *et al.,* (2014) also revealed a significant improvement in body weight gain of the chicks on treated groups compared to those fed on the basal diet. This may be attributed to increase of feed intake or to the fenugreek contents of active compounds such as antibacterial, antifungal, anti-inflammatory, carminative and antioxidant activities. The result was in line with findings Abou EL-Wafa *et al.,* (2003) and Hamden *et al.,* (2010).

Alloui *et al.,* (2012) showed that broiler chicks fed diet supplemented with Fenugreek seeds at 3g/kg of feed, had the highest values ($p<0.05$) of live body weight (LBW) at 21 and 42 days of age. The improvement in body weight may be due to the presence of the fatty acids (Murray *et al,* 1991).

This finding was in agreement with those of Azoua (2001) who noted that adding Fenugreek to broiler diet resulted in an increased body weight.

Butnariu and Samfira (2012) showed that the improvement in body weight gain may be due to antibacterial related to flavonoids in fenugreek that led to maintaining normal intestine microflora population.

Hind *et al.,* (2013) reported that the effect of feeding 2% level of different spices on the performance of broiler chicks revealed the presence of significant differences ($P<0.05$) among the treatment groups for body weight gain.Weight gain was significantly ($P<0.05$) affected by treatment.Group D (Fenugreek) reported the highest weight gain (720.13 g) while the control group showed the lowest weight gain (528.40 g).

El-Gharmy *et al.,* (2004) found that, addition of fenugreek chickens at 1.5% level had significantly ($p< 0.05$) heavier live body weight and body weight gain than those fed on control diet.

According to Elbushra (2012) BW gain and live weight improved for chicks fed diets supplemented with 0.5% or 1.5% fenugreek seeds.

2.5.6 Effect of Fenugreek seed on feed consumption

Alloui *et al.,* (2012) reported that that feeding of Fenugreek seeds supplemented diet significantly ($p<0.05$) affected feed intake (FI) value during 42 days of age, while there appeared no significant differences ($P>0.05$) when broiler chicks fed fenugreek seed during the 21 days of age as compared with control group. The improvement in feed intake with the addition of fenugreek seed could be attributed to the carbohydrates and their main component (galactomannan) which stimulated the appetizing and digestive process in animals (Steiner, 2009).

Tariq *et al.,* (2014) also showed that broiler chicks feed on diets containing fenugreek seeds flour (FSF) recorded significantly ($p<0.05$) higher values for growth performance. Groups fed on diets containing FSF observed significantly ($p<0.05$) high feed intake compared to the control group.

Hind *et al.,* (2013) reported that feed intake reported its maximum value in group D (Fenugreek) followed by group A (control), B (control+ antibiotic) and F (Cinnamon) while the lowest estimate was recorded by group C (Cumin). Fenugreek supplementation resulted in improvement in feed intake which could be attributed to the carbohydrates and their main component (galactomannan) which stimulated the appetizing and digestive process in animals.

Shah *et al.,* (2016) showed broilers received diet containing 2% FGS consumed the highest amount of feed at all ages followed by the broilers

received 1% FGS, AGP in the diet and basal diet alone.

2.5.7 Effect of Fenugreek seed on FCR

Alloui *et al.,* (2012) showed that fenugreek seeds significantly ($p<0.05$) affected Feed Conversion Ratio during the 42 days of age. This is related to the development of the broiler chicks' gut.

Weerasingha and Atapattu conclude that use of fenugreek powder improved the (FCR) by 13.8 % (Weerasingha and Atapattu 2013), compared to control group.

Weerasingha and Atapattu (2013) reported that FCR of the birds given 1% fenugreek was significantly best than that of control.

Magda *et al.,* (2012) reported the best feed conversion ratio was obtained by birds given 0.5 Fenugreek diets while the lowest was obtained by the control group.

Similar trend was observed by Abdel – Latif *et al.*, (2002) in Japanese quail when reported that adding FK to the control diet at a level of 1000g Fk/ton diet improved feed conversion ratio.

Tariq *et al.,* (2014) reported that broiler chicks feed on diets containing fenugreek seeds flour (FSF) recorded significantly ($p<0.05$) higher values for growth performance. Groups fed on diets containing FSF observed significantly ($p<0.05$) higher feed conversion ratio (FCR) compared to the control group.

Upper results also matched with the finding of El-Gendi *et al.* (1996) who indicated that there was an improvement in feed conversion with feeding herbal products (Fenugreek,parsley) as feed additives that could be attributed to their effect on improving the digestibility of dietary protein in the small intestine.

Shah *et al.*, (2016) reported inclusion of FS in broiler diet resulted in lower FCR compared to the AGP inclusion in feed throughout the experiment. Broilers fed on basal diet alone exhibited the highest FCR at all ages. The differences in FCR were significant (P<0.05) between the dietary treatment groups.

J. Abo Omar *et al.*, (2016) showed there were a significant effect on FCR of treated chicks (P < 0.05). The highest amount of feed conversion were in birds consuming the chicken plus diet (Fenugreek, Chamomile, Thyme, Black seed) compared to the control birds consuming the regular broiler diets.

2.5.8 Effect of Fenugreek seed on dressing percentage

Tariq *et al.*, (2014) showed that the dressing percentages of chicks fed on 1% and 2% FSP showed significantly (p<0.05) heavy weights compared to unsupplemented group.

Azoua (2001); El-Husseiny *et al*. (2002) and Hassan et al. (2004) found that addition of MAP had significantly higher dressing percent in broiler than those fed control diets.

J.abo omar *et al.*, (2016) found that feeds supplemented by the herb extract resulted in higher dressing proportions compared to control birds. The dressing proportions were 70.5%, 77.0% and 75% for the control, birds fed regular or growth promoter deficient feeds, respectively.

The result of the experiment of Saim *et al.*, (2016) showed that the dressing percentage was highest (73.97±0.21%) in the group fed combination of enzyme treated dandelion and fenugreek group.

2.5.9 Effect of Fenugreek seed on immunity and Antiviral activity

Abed *et.al.*, (2014) showed that treatment that supplemented with 1 %

fenugreek recorded high antibody titter against Newcastle disease virus virus at 21 day of broilers age.

Abed *et al.,* (2014) also demonstrated that the fenugreek increasing the immunity of birds at 24 and 34 day and because fenugreek increases the cellular ties of thymus gland and bone marrow.

The weight of the immunological organs (thymus, bursa and spleen) was not significantly ($p>0.05$) affected by the treatment differences (Hind *et al.,* 2013).

Bin *et al.,* (2003) reported that the addition of Fenugreek to boiler feeds lead to increased bursal weight.

S. Waheed *et al.,* (2017) found remarkably better titres of ND achieved in blood in fenugreek treated. Natural extracts in general had significantly better ($p\leq0.05$) titers as compared to control.

The mean value of antibody titer against ND was higher ($P\leq0.05$) in birds fed diets T3 (1% FS) and T4 (2%FS) at 21st sampling day. The ND antibody titer of birds fed fenugreek at 2% was significantly higher ($P\leq0.05$) than the control birds (Yonatan kassu yesuf, 2018).

2.5.10 Effect of Fenugreek seed on viscera

Yaser *et al.,* (2018) showed that there were no significant differences between liver weight percentages among fenugreek treated group and control group.

Khan and collab reported that fenugreek seed extract had no impact on visceral organs (liver, heart, gizzard, and intestines) of broiler chicks (Khan *et al.*, 2011).

Yaser *et al.,* (2018) showed feeding fenugreek powder significantly

decreased gizzard weight, and significant effect on intestine weight and liver.

Farman *et al.,* (2009) reported no difference (P>0.05) was observed in mean weights of giblet (heart, liver, gizzard) and intestine in all groups of the research study. It means that fenugreek infusion having antimicrobial and antibiotics like properties have no influence on either increasing or decreasing the relative weights of giblet.
Upper findings of Farman are in contrast to the results of the Fairley *et al.,* (1985). Who reported that an increase (P<0.05) occurred in the relative proportions of giblet, when broiler chicks were fed an antibiotic avoparcin. The result of liver weight of this study was not effected significantly in either treated or control groups.

Guo *et al.,* (2004) reported that a Chinese herbal medicine containing fenugreek and an antibiotic virginiamycin did not influence (P>0.05) the liver weight in broiler chicks. Mohsen *et al.,* (2015) reported that Trigonella foenum-graecum level had not significant effect on liver and bile weight (P>0.05).

Tariq *et al.,* (2014) showed that feeding fenugreek seed powder significantly decreased gizzard weight, no significant effect on intestine weights and non-carcass components (liver, gizzard, heart).

J.abo omar *et al.,* (2016) there were no significant (P>0.05) differences in the carcass characters and visceral organ relative weights among the treatment groups.

The result of the experiment of Saim *et al.,* (2016) showed that there were no significant (P>0.05) difference in the yield characteristics of giblets viz. gizzard weight, heart weight and liver weight among different treatment groups. Results obtained for non-carcass components revealed no significant difference in (gizzard, neck, heart, liver, and legs) weights recorded between tested groups (Hiba Hamza ALSedig Hamid, 2018).

2.5.11 Antimicrobial effect of Fenugreek seed

Supplementation of 0.5% Fenugreek seed in the diet of broilers significantly lowered total bacterial count, gram negative and coliform bacteria and was ineffective on Salmonella (Pajouhesh and Sazandegi 2010).

M R Purushothaman *et al.,* (2014) reported the jejunum bacterial count - E.coli and Clostridium sp. of birds fed with FR at different levels with and without antibiotic suggested that numerical reduction in E. coli due to FR supplementation.

G.Attia *et al.,* (2017) observed a significant difference in the caecal microflora count due to dietary intake of the plant extract blend (FS, Oregano and Chamomile). The beneficial bacteria count (Lactobacillus spp.) was linearly increased ($p<0.001$) and the harmful bacteria count (Coliforms) was linearly decreased ($p<0.001$) by increasing the level of the plant extract blend.

Dash *et al*. (2011) showed that the botanical extracts of Coriandrumsativum L. and Trigonellafoenum L. contain effective antimicrobial agents. The antibacterial and antifungal role of fenugreek seed has recently been shown. The extracts contain effective antimicrobial agents.

Study by Ahmad (2016) showed that fenugreek oil has a positive effect on microbial health by lowering the total bacterial count, Salmonella and E. coli of the laying hens and thus can be used instead of commercial antibiotics. However, not much literature reported regarding the in vivo effect of fenugreek in poultry.

Qureshi *et al.* (2015) investigated the in vitro antibacterial activity of fenugreek and reported the 2.1 mm of zone of inhibition for the concentration of 0.05 mg/ml of extract against E. coli.

Similarly, in vitro antibacterial activity of methanolic extract of fenugreek against E. coli has been reported by Dash *et al.* (2011) and ascribed to the flavonoids, saponins and phenols present in it (Schryver, 2002).

In a study by Haouala *et al.* (2008) found that all parts of the fenugreek plant showed antifungal potential and the magnitude of effect varies with plant parts and species of fungus such as Fusarium graminearum, Botrytis cinerea, Alternaria sp., Rhizoctonia solani and Pythium aphanidermatum (Haouala *et al.,* 2008). It could suggest that fenugreek is an important source of biologically active compounds useful for developing better and novel antifungal drugs.

The cloacae fecal samples analysis of anaerobic bacteria, the first week of herbal feeding showed 50% reduction of anaerobic bacteria when compared with untreated control group. 2nd and 3rd week of herbal feeding showed significance reduction of anaerobic bacterial excretion in dropping materials compared with untreated control group (Kalaiselvi *et al.,* 2017).

2.5.12 Effect of Fenugreek seed on Serum biochemical properties

Tariq *et al.,* (2014) reported that the results of serum metabolite showed reduction in total cholesterol in groups of chicks fed on FSP compared to control group. The hypocholesterolemic effect of FSP due to its active ingredients such as saponins, hemicelluloses, mucilage, tannin and pectin and these compounds help lower blood LDL-cholesterol levels by inhibiting bile salts.

Rabia (2010) found the serum constituents indicated that feeding fenugreek, parsley and basil seeds were not significantly affected to total protein, albumin, globulin, albumin/globulin ratio and glucose contents, but serum cholesterol was significantly affected ($p<0.05$) by adding these materials. Al-Habori et al. (1998) also reported on rabbits who found that plasma cholesterol was significantly reduced in fenugreek groups compare

to control group.

Moreover, Sowmya and Rajyalakshmi (1999) found that the germinated fenugreek seeds (12.5 and 18g/day for 1 month) significantly reduced total cholesterol levels in human. Abdel and Yousif (2003) also showed that fenugreek seed powder as capsules (750mg/kg body weight) decreased blood cholesterol in broiler chickens.

Similar results were observed by El-Ghamry *et al*. (2002) with Muscovi duckling who demonstrated that total cholesterol value in plasma of Fenugreek seeds (1.5%) treatments were significantly lower than those of the control group.

A number of studies (Abbas, 2010; El-Ghamry *et al.,* 2002) have shown that serum cholesterol levels were reduced when fenugreek was added. The defatted seeds material of fenugreek may reduce gastrointestinal absorption of glucose and cholesterol and increase bile acid secretion (Dash et al. 2011).

M R Purushothaman *et al.,* (2014) reported the level of cholesterol was found to reduce at 2% FR in both groups. The hypocholestermic effects of fenugreek have mainly been attributed to its fiber, gum, saponin and amino acid content (Mathur and Choudhry 2009).

Dietary 1% garlic + 1% fenugreek from 30 to 38 weeks of age resulted in a reduction in both serum and egg yolk chlesterol concentrations during treatments and for 8 weeks after switching to the basal diet in White Bovans laying hens (Hassan, 2000 and El-Kaiaty *et al.,* 2002a).

Hassan (2000) and El-Kaiaty *et al*. (2002b) indicated that feeding White Bovans laying hens either 2% garlic or 2% fenugreek from 30 to 38 weeks of age decreased significantly serum cholesterol by 18 and 7% and egg yolk cholesterol by 20 and 9%, respectively.

Many researchers reported that incorporation of dietary fenugreek seeds in broilers diets reduced serum glucose level of broiler chicken (Qureshi *et al.,* 2015; Mamoun *et al.,* 2014; Safaei *et al.,* 2013; Abdul-Rahman, 2012; Eman, 2011).

The hypoglycemia effects of fenugreek seed mode of action had not been fully elucidated; however, Schryver, (2002) ascertained that the reduction in the serum glucose levels may be related to the presence of special amino acid (4-hydroxyisoleucine), which found abundantly in fenugreek seed that stimulate directly β-cell stimulation in the pancreases to increases insulin secretions, thus improves glucose uptake by the body rather than stay in the blood.

2.5.13 Effect of Fenugreek seed on blood parameter

Waheed *et al.,* (2017) showed Effect of supplementation spice extracts in feed resulted in increased haemoglobin in fenugreek (F3), Black cumin seed (F4), sweet violet (F5) and F8 group ($p \leq 0.05$) as compared to negative control group (F2). Hemoglobin levels were increased from 11.35, 10.5, 10.45, and 11.15 g/dl respectively in F3, F4, F5, and F8 groups of broilers.

There was a highly significant ($P<0.01$) difference for haemoglobin content at fourth and sixth week of age with highest haemoglobin content in fenugreek and cumin supplemented group were evident. Haemoglbin content in overall and males at eighth week of age were significant ($P<0.05$) with highest values recorded in fenugreek and Cumin (Darshana *et al.,* 2012).

WBC counts were higher in birds fed fenugreek when compared to control. This significant difference ($p \leq 0.05$) justifies the presence of immunity boosting polyphenols extracts in fenugreek (Waheed *et al.,* 2017).

RBC, Lymphocytes, Neutrophils, MCH, MCHC, MCV: There was no significant difference ($P>0.05$) in RBC, lymphocyte, MCH and MCV counts

of birds in different groups (S. Waheed *et al.*, 2017). Platelets were significantly improved ($p \leq 0.05$) by addition of fenugreek, black cumin seed or sweet violet extracts (Waheed *et al.*, 2017).

The inclusion of fenugreek seeds at 10 g/kg of diet in broiler breeder chicken significantly improved the PCV, RBC and Hb concentration and attributed this improvement in erythropoiesis to the enhancement of antioxidant activities in RBCs, which decreases the production of free radicals that destroy Hb and cause hemolysis of RBCs (Abdul-Rahman, 2012).

According to Bhaisare and Thyagarajan (2014), the Hb content were significantly higher when fenugreek seeds used in turkey poults, indicating that certain bioactive principles in fenugreek seeds have positive effect on haemopoietic process in the body.

Yonatan kassu yesuf (2018) findings shown no significant difference ($P \geq 0.05$) among treatments (black cumin, fenugreek and turmeric) regarding the white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values. But the RBC count was higher for T6 and T3 (FS) as compared to the control ($P \leq 0.05$).

Yonatan kassu yesuf (2018) findings also shown the serum glucose was significantly reduced ($P \leq 0.05$) in birds which were fed treatment 1% FS, 2% FS, respectively than the control diet fed birds.

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 **08th May to 5th June, 2019** to assess the feasibility of using Fenugreek Seed (FS) in commercial broiler diet on growth performance, dressing characteristics, hematological and immune status of broilers. This research will help to make a conclusion about FS 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 Fenugreek Seed was used as treatment. After two days 90 chicks were selected from brooders and distributed randomly in three (3) dietary treatments of FS; 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: Basal diet / Control

T_2: Basal Diets + Antibiotics (Doxivet)

T_3: 1% of Fenugreek seed (1 kg FS/100 kg of the feeds)

T_4: 1.5% of Fenugreek seed (1.5 kg FS/100 kg of the feeds)

T_5: 2% of Fenugreek seed (2 kg FS/100 kg of the feeds)

Table 2. Layout of the experiment

Treatments with Replications (10 birds/ replication)			**No. of birds**
T_4R_2 (n=10)	T_3R_1 (n=10)	T_1R_3 (n=10)	30
T_1R_1 (n=10)	T_2R_2 (n=10)	T_5R_2 (n=10)	30
T_3R_3 (n=10)	T_4R_1 (n=10)	T_3R_2 (n=10)	30
T_5R_1 (n=10)	T_2R_1 (n=10)	T_1R_2 (n=10)	30
T_2R_3 (n=10)	T_5R_3 (n=10)	T_4R_3 (n=10)	30
Total			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 3. 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%
tryptophan	0.19%
lysine	1.20%
methionine	0.49%
cystine	0.40%
threonine	0.79%
arginine	1.26%
Name of ingredients in Grower ration	**Minimum percentage Present**
protein	19.0 %
fat	6.0%
fiber,	5.0%
ash	8.0%
lysine	1.10%
methionine	0.47%
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 *Trigonella foenum-graecum*

The medicinal plants, namely Trigonella foenum-graecum L. (fenugreek) seeds purchased from the vicinity local markets for incorporate in the diets of broiler chickens as phytobiotic feed additives. The fenugreek seeds was washed with tap water and sun dried under shade. Then, the dried fenugreek seeds were stored in polythelene bags until required for the formulation of experimental rations.

Table 4. Nutritional composition of *T. foenum-graecum* (per 100 g)

Nutrient Component	Amount
Water	8.8 g
Energy	323 kcal
Carbohydrates	58 g
Dietary fibre	25 g
Fat	6.4 g
Protein	23 g
Thiamine (B_1)	0.322 mg
Riboflavin (B_2)	0.366 mg
Niacin (B_3)	1.64 mg
Vitamin B_6	0.6 mg
Folate (B_9)	57 µg
Vitamin C	3 mg
Calcium	176 mg
Iron	34 mg
Magnesium	191 mg
Manganese	1.23 mg
Phosphorus	296 mg
Potassium	770 mg
Sodium	67 mg
Zinc	2.5 mg

Source: USDA Nutrient Database, SR Legacy, FDC Published:January 2019

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, 2019**. 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^{0}C) 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 18 hours light and 6 hours dark was scheduled up to 28 days.

3.7.6 General preventive measures

a. Medication

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.

b. 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 5.

Table 5. Vaccination schedule

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

c. Sanitation

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

3.7.7 Ventilation

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

3.8 Study Parameters

3.8.1 Recorded parameters

Weekly live weight, weekly feed consumption and death of chicks to calculate mortality percent. FCR was calculated from final live weight and total feed consumption per bird in each replication. After slaughter gizzard, liver, spleen, intestine, heart and bursa 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) and sugar and cholesterol level. Feces sample was collected to measure microbial load in the gut.

3.9 Data collection

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

3.9.2 Dressing yield

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

3.9.3 Feed consumption

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

3.9.4 Mortality of chicks

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

3.9.5 Dressing procedures of broiler chicken

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

3.9.6 Blood sample analysis

Blood samples (1 ml/bird) were collected into ethylene diethyle tetraacitic acid (EDTA) tubes from the wing veins. Samples were transferred to the laboratory for analysis within 1 hour of collection. Glucose, Cholesterol and Complete blood count was measured from Rainbow diagnosis centre Dhanmondi Dhaka by maintaining standard protocol.

3.10 Calculations

3.10.1 Live weight gain

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

Body weight gain = Final weight – Initial weight

3.10.2 Feed intake

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

3.10.3 Feed conversion ratio

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

3.11 Statistical analysis

The data was subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version 16. Differences between means were tested using Duncan‟s multiple comparison test and significance was set at $P<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Production performances of broiler chicken

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

4.1.1 Final Live weight

The effect of dietary inclusion of Fenugreek seed on the production performances of broiler chickens was significant ($p<0.05$) and good fluctuation was observed among the different treatment groups (Table 6). Data presented in Table 6 showed that the effect of treatments on final live weight (gram per broiler chicken) was significant ($P<0.05$). The relative final live weight (g) of broiler chickens in the dietary group T_1, T_2, T_3, T_4, and T_5 were $1344.22^{b}\pm70.429$, $1312.44^{b}\pm45.815$, $1527.11^{a}\pm7.333$, $1528.33^{a}\pm57.468$ and $1302.07^{b}\pm3.771$ respectively. The higher result was found in T_3 and T_4 ($1528.33^{a}\pm57.468$) than result was in T_5 ($1302.07^{b}\pm3.771$) group that may be due to the presence of saponin which hamper the digestion and utilization of feed into the body. The final live weight of broiler fed fenugreek diets was also higher than control and antibiotic treated group and the difference was significant ($P<0.05$). The present findings are in accordance with Tariq et al., (2014) who showed that supplementation of fenugreek seed powder in various levels improved significantly ($p<0.05$) live body weight. This might be due to the presence of the fatty acids, or due to stimulating effect on the digestive system of broilers (Hernandez et al., 2004). This may be attributed to increase of feed intake or to the fenugreek contents of active compounds such as anti-bacterial, antifungal, anti-inflammatory, carminative and antioxidant activities. The result was in line with findings Abou EL-Wafa *et al.*, (2003) and Hamden *et al.*, (2010).

El-Gharmy *et al.*, (2004) found that, addition of fenugreek chickens at 1.5% level had significantly ($p< 0.05$) heavier live body weight and body weight gain than those fed on control diet.

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

Parameters	T1	T2	T3	T4	T5	Mean±SE
Final live wt. g/broiler	1344.22^{b} ±70.42	1312.44^{b} ±45.81	1527.11^{a} ±7.33	1528.33^{a} ±57.46	1302.07^{b} ±3.77	1402.83* ±32.47
FC(g)	2244.67^{b} ±5.04	2243.00^{b} ±25.81	2289.67^{a} ±2.60	2284.67^{ab} ±10.58	2248.00^{ab} ±5.29	2262.00* ±7.38
FCR	1.51±0.02	1.47±0.02	1.38±0.00	1.45±0.03	1.50±0.08	1.46^{NS}±0.02
DP%(skinless)	57.27^{b}±4.67	61.00^{ab}± 0.75	63.67^{ab}± 0.67	65.55^{ab}± 0.32	68.08^{a}±2.92	63.11*±1.37

Here, T_1 = (Control), T_2 = (Antibiotic), T_3 = (1% FS), T_4 = (1.5% FS) and T_5 = (2% FS). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

4.1.2 Feed consumption (FC)

Different treatment groups (Table 6) showed significant (P<0.05) differences in FC of broiler chicken. Fenugreek treated T_3 group consumed

higher amount of feed (2289.67^{a}±2.603) and antibiotic treated group consumed lower amount of feed (2243.00^{b}±25.813) significantly ($P<0.05$). Antibiotic treated group showed no significant difference among $T_{1,}$ $T_{4,}$ T_{5}. But FS treated groups were not affected by different levels of doses.
These results are in agreement with those of previous researchers (N alloui et al., 2012; Tariq et al., 2014; Shah et al., 2016; Hind A.A Elagib et al., 2013) who recorded that dietary fenugreek significantly ($P<0.05$) improved Feed consumption (FC) of broiler chickens in different FS inclusion levels.

The improvement in feed intake with the addition of fenugreek seed could be attributed to the carbohydrates and their main component (galactomannan) which stimulated the appetizing and digestive process in animals (Steiner, 2009).

4.1.3 Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) was non-significant ($P>0.05$) and the FCR of different groups showed 1.51±.021; 1.47±.027; 1.38±.003; 1.45±.037; 1.50±.084 respectively (Table 6). No significant ($P>0.05$) difference were found in FCR data of broiler chicken among different treatment groups but better FCR were found in most of the FS supplemented groups than antibiotic and control groups. T_3 group showed best FCR. J. Abo Omar *et al.,* (2016) showed that there were a significant effect on FCR of treated chicks ($P < 0.05$). The highest amount of feed conversion were in birds consuming the chicken plus diet (Fenugreek, Chamomile, Thyme, Black seed) compared to the control birds consuming the regular broiler diets.

Present study results are in contradictory with those of previous researchers (Weerasingha and Atapattu 2013; A.S. Weerasingha et al., 2013; Magda *et al.,* 2012) who recorded significant ($P>0.05$) effects of dietary Fenugreek supplementation on feed conversion.
Contradictory results are possibly due to the different FS 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.

4.1.4 Dressing Percentage

The DP of different treatment groups T_1, T_2, T_3, T_4 and T_5 were 57.27^{b}±4.678, 61.00^{ab}±.752, 63.67^{ab}±.675, 65.55^{ab}±.329 and 68.08^{a}±2.923 Respectively (table 6). 2% (T_5) FS (68.08^{a}±2.923) supplemented group had a greater ($P > 0.05$) dressing percentage compared with the control group (57.27^{b}±4.678). Although T_5 showed no significant difference with antibiotic and other FS supplemented groups.

This findings are in accordance with the findings of Tariq et al., (2014) who showed that the dressing percentages of chicks fed on 1% and 2% FSP showed significantly ($p<0.05$) heavy weights compared to unsupplemented group.

Azoua (2001); El-Husseiny *et al*. (2002) and Hassan *et al.* (2004) also found that addition of hertbal extracts including FS had significantly higher dressing percent in broiler than those fed control diets.

4.1.5 Weekly Body Weight Gain

The mean body weight gains (g) of broiler chicks at the end of 4th week in different groups were 600.73^{a} ± 21.82, 448.43^{b} ± 17.11, 352.23^{b} ± 21.79, 440.57^{b} ± 47.17 and 425.40^{b} ± 62.23 respectively (Table 7 and Figure 1). The highest body weight was gained by control group and lowest in T_3 group. No significant difference was found among antibiotic and FS treated groups.

At the end of 1^{st} week the body weight gain in different groups were significantly different ($P<0.05$). T_4 group had the higher body weight gain than other group. According to Elbushra (2012) BW gain, live weight improved for chicks fed diets supplemented with 0.5% or 1.5% fenugreek seeds.

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

Treatment	1st w. BWG	2nd w. BWG	3rd w. BWG	4th w. BWG
T1	203.33^{b} ± 2.52	293.40 ± 11.48	453.53 ± 11.08	600.73^{a} ± 21.82
T2	202.23^{b} ±0 .67	307.63 ± 2.18	461.23 ± 17.32	448.43^{b} ± 17.11
T3	209.67^{ab} ± 2.48	338.47 ± 18.40	495.47 ± 13.47	352.23^{b} ± 21.79
T4	215.07^{a} ± 2.08	336.90 ± 16.54	500.03 ± 34.84	440.57^{b} ± 47.17
T5	202.40^{b} ± 3.64	314.67 ± 15.09	488.73 ± 30.56	425.40^{b} ± 62.23
Mean± SE	206.54^{*} ± 1.64	318.21^{NS} ± 7.02	479.80^{NS} ± 10.19	453.47^{*} ± 26.07

Here, T_1 = (Control), T_2 = (Antibiotic), T_3 = (1% FS Supplementation), T_4 = (1.5% FS Supplementation) and T_5 = (2% FS Supplementation). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

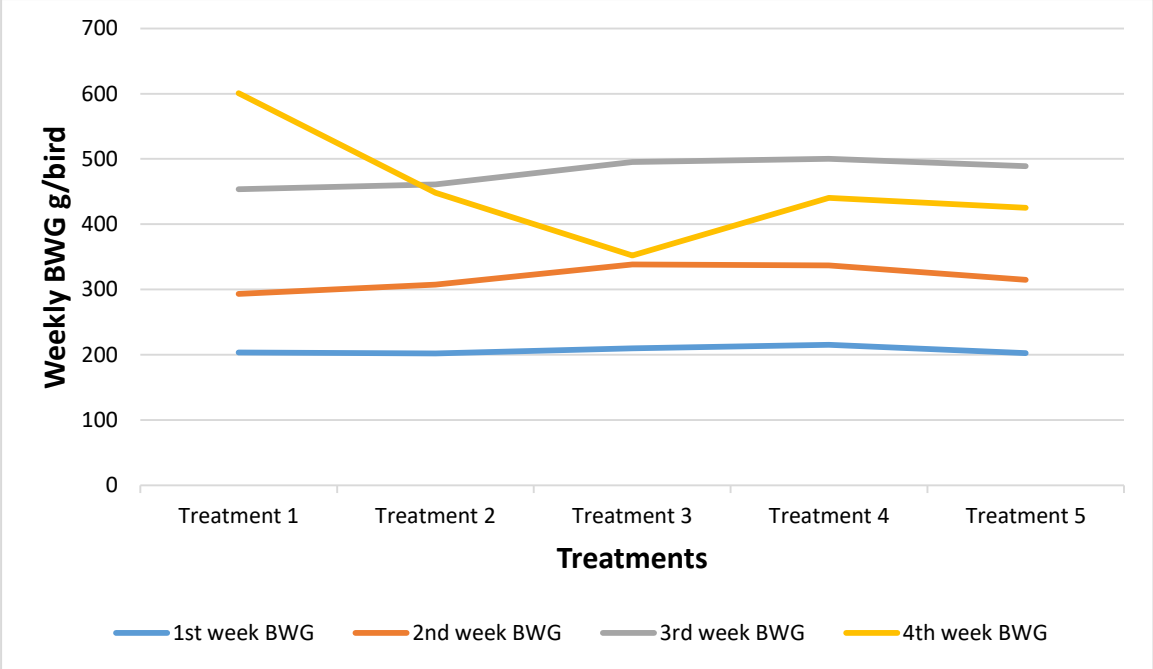

Figure 1. The Effect of supplementation Fenugreek to broiler diets on Body Weight Gain (g/bird) of broiler chickens at different week

4.1.6 Weekly Feed consumption (FC)

On perusal of the mean weekly feed intake of the present study (Figure 2), it could be seen that during the first week of age the feed intake was lowest in T_4 (179.33±2.84g) group and highest in T_2 (185.00±1.15g) group.

During the second week, feed intake was highest in T_5 (447.67±8.81g) group and lowest in T_4 (431.00±5.77g) group. Similarly in third week of age feed intake was highest in T_4 (768.67±6.66g) group and lowest in T_2 (755.33±3.33g) group, except that feed intake was lowest in T_2 group.At the end of the four week of age higher feed intake was found in T_1 group (903.00±3.21g) and lower in T_5 group (857.67±2.40g).

N alloui *et al.*, (2012) reported that feeding of Fenugreek seeds supplemented diet significantly ($p<0.05$) affected feed intake (FI) value during 42 days of age, while there appeared no significant differences ($P>0.05$) when broiler chicks fed fenugreek seed during the 21 days of age

as compared with control group. The improvement in feed intake with the addition of fenugreek seed could be attributed to the carbohydrates and their main component (galactomannan) which stimulated the appetizing and digestive process in animals (Steiner, 2009).

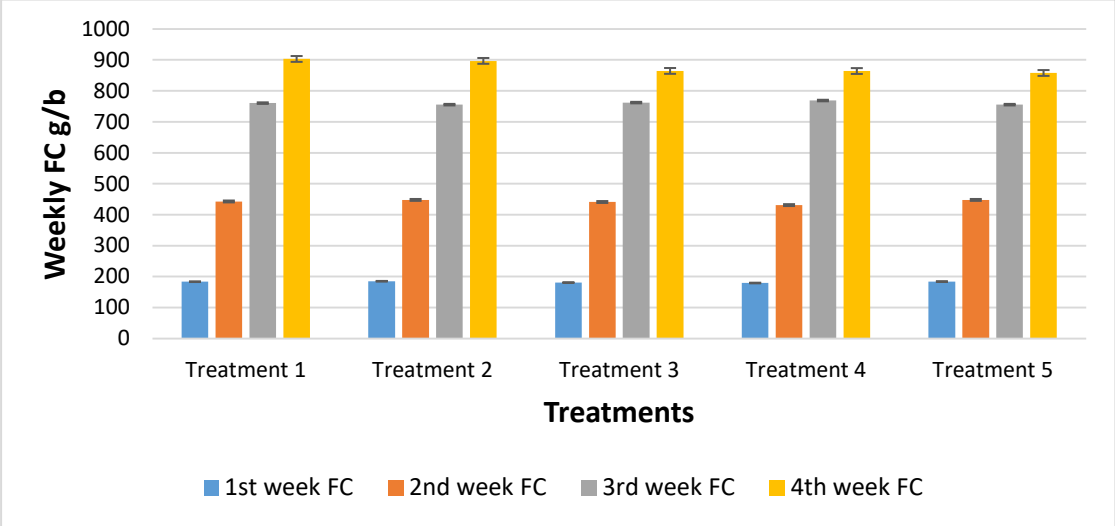

Figure 2. The Effect of supplementation of Fenugreek to broiler diets on feed consumption (g/bird) of broiler chickens at different week.

4.1.7 Weekly Feed Conversion Ratio (FCR)

The mean body FCR of broiler chicks at the end of 4th week in different groups were 1.51^{b} ± 0.04, 2.00^{ab} ± 0.07, 2.47^{a} ± 0.14, 1.99^{ab} ± 0.15 and 2.12^{ab} ± 0.35 respectively. The overall mean FCR of different groups showed that there were a significant ($P<0.05$) difference in groups. T_3 showed the highest FCR compared to control and antibiotic group (Table 8). Weerasingha and Atapattu conclude that use of fenugreek powder improved the (FCR) by 13.8 % [Weerasingha and Atapattu 2013], compared to control group.

N alloui et al., (2012) found similar result and showed that fenugreek seeds significantly ($p<0.05$) affected Feed Conversion Ratio during the 42 days of age. This is related to the development of the broiler chicks' gut. Tariq et al., (2014) reported that broiler chicks feed on diets containing fenugreek

seeds flour (FSF) recorded significantly ($p<0.05$) higher values for growth performance. Groups fed on diets containing FSF observed significantly ($p<0.05$) higher feed conversion ratio (FCR) compared to the control group.

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

Treat	1st w. FCR	2nd w. FCR	3rd w. FCR	4th w. FCR
T1	$0.90^{ab} \pm 0.00$	1.51 ± 0.03	$1.67 \pm .04$	$1.51^{b} \pm 0.04$
T2	$0.91^{a} \pm 0.00$	1.46 ± 0.02	$1.64 \pm .06$	$2.00^{ab} \pm 0.07$
T3	$0.86^{bc} \pm 0.01$	1.31 ± 0.09	$1.54 \pm .05$	$2.47^{a} \pm 0.14$
T4	$0.83^{c} \pm 0.01$	1.29 ± 0.08	$1.55 \pm .09$	$1.99^{ab} \pm 0.15$
T5	$0.91^{a} \pm 0.02$	1.43 ± 0.07	$1.56 \pm .10$	$2.12^{ab} \pm 0.35$
Mean± SE	$0.88^{*} \pm 0.01$	$1.40^{NS} \pm 0.03$	$1.59^{NS} \pm .03$	$2.02^{*} \pm 0.10$

Here, T_1 = (Control), T_2 = (Antibiotic), T_3 = (1% FS), T_4 = (1.5% FS) and T_5 = (2% FS). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different ($P<0.05$)
- ✓ Mean within same superscripts don‟t differ ($P>0.05$) significantly
- ✓ SE= Standard Error
- ✓ * means significant at 5% level of significance ($p<0.05$)

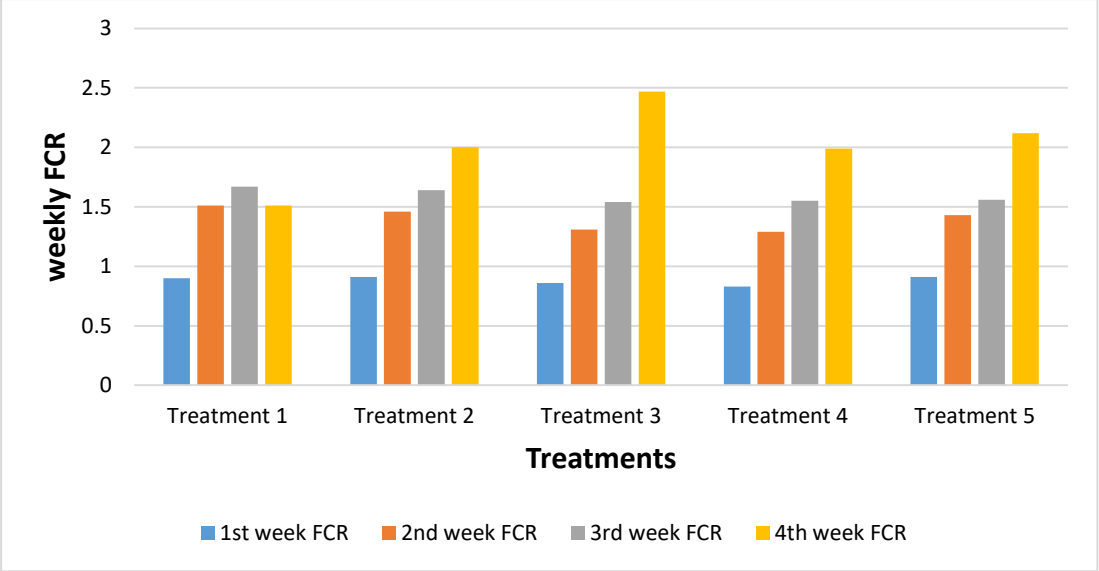

Figure 3. Effects of feeding different level of Fenugreek and antibiotic on FCR of broiler chickens at different weeks

4.2.1 Glucose

The effects of dietary fenugreek supplementation on concentration of glucose of broiler chickens are presented in Table 9. There was no significant (P>0.05) difference among the treatment. Although the highest amount (10.76±.55) of plasma glucose are found in T_5 (2% FS) and lowest in T_4 of 1.5% FS but this was not statistically difference with antibiotic, control and other groups. The results of the present study are compatible with those observed by (Qureshi *et al.*, 2015; Mamoun *et al.*, 2014; Safaei *et al.*, 2013; Abdul-Rahman, 2012; Eman, 2011) who observed incorporation of dietary fenugreek seeds in broilers diets reduced serum glucose level of broiler chicken.

4.2.2 Cholesterol

Total cholesterol concentration (mg/dl) in the serum of different groups ranged from 114.22±4.39 to 142.44± 5.06 (table 9 and figure 4). Statistical analysis revealed a significant (P<0.05) difference among the group. However the cholesterol level was lower in T_4 fed group (114.22^{b}±4.39) followed by T_3 (114.56^{b}±5.73), T2 (118.89^{b}±4.04), T_5 (125.22^{b}±2.21), and T_1 (142.44^{a}±5.06) respondingly. Similar results had also been observed by

Abdel-Rasoul and Yousif (2003) who showed that fenugreek seed powder as capsules (750mg/kg body weight) decreased blood cholesterol in broiler chickens. Other studies (Abbas, 2010; El-Ghamry *et al.*, 2002) had also shown that serum cholesterol levels were reduced when fenugreek was added. M R purushothaman *et al.*, (2014) reported the level of cholesterol was found to reduce at 2% FR in both groups. The hypocholestermic effects of fenugreek have mainly been attributed to its fiber, gum, saponin and amino acid content to reduce the level of cholesterol (Mathur and Choudhry 2009).

Table 9. The Effect of supplementation FS to broiler diets on serum Biochemical level

Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**Mean±SE**
Glucose (mmol/L)	10.07±0.41	10.08±0.53	10.18±0.37	9.37±.31	10.76±0.55	10.09^{NS}±0.20
Cholesterol(mg/dl)	142.44^{a} ±5.06	118.89^{b} ±4.04	114.56^{b} ±5.73	114.22^{b}± 4.39	125.22^{b} ±2.21	123.07^{*}±2.46

Here, T_1 =Control, T_2 = Antibiotic, T_3 = 1% FS, T_4 =1.5% FS and T_5 = 2% FS. Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- Mean with different superscripts are significantly different (P<0.05)
- Mean within same superscripts don't differ (P>0.05) significantly
- SE= Standard Error
- * means significant at 5% level of significance (p<0. 0)

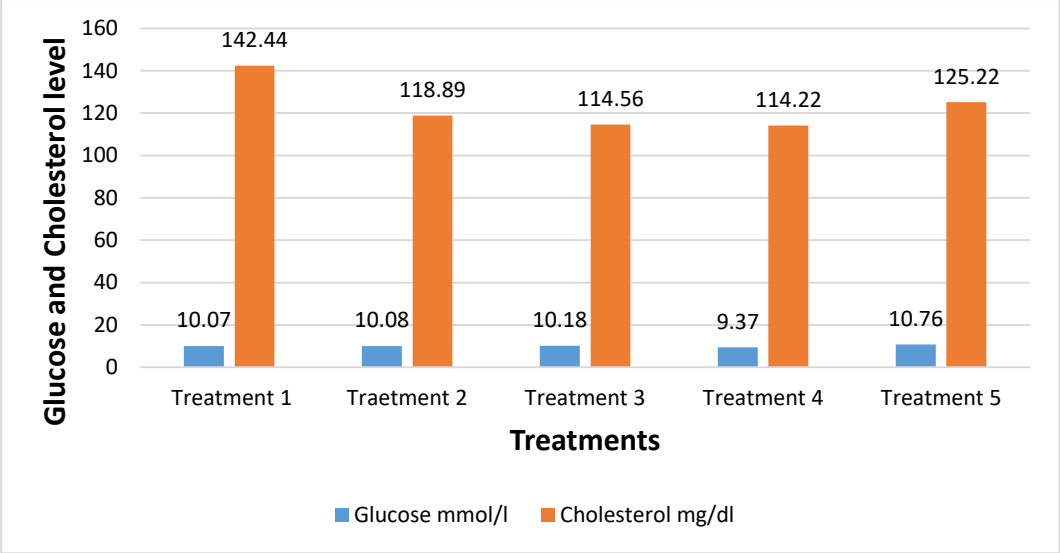

Figure 4. Effect of Fenugreek on Serum biochemical level of different broiler chicken under different treatment

4.3.1 Relative weight of liver, gizzard and heart

The relative weight of liver (g) of broiler chicks in the dietary group T1, T2, T3, T4 and T5 were 36.11±1.409, 35.78±2.197, 33.56±.944, 34.22±.983 and 32.33±1.179 respectively (table 10). The highest results were obtain in T_1 and lowest was in T_5 group. However, there was no significant (P>0.05) difference in the relative weight of liver between the groups.

Yaser Rahimian et al., (2018) showed that there were no significant differences between liver weight percentages among fenugreek treated group and control group.

The comparative weight of gizzard of different groups did not show any significant (P>0.05) difference in groups T1 (31.44±1.082), T2 (34.78±1.382), T3 (34.22±1.561), T4 (34.56±2.135) and T5 (31.78±1.498) (Table 10). The comparative weight of heart (g) of broiler chicks in the dietary group T1, T2, T3, T4 and T5 were 7.39±.389, 7.44±.256, 6.89±.351, 7.06±.429 and 6.67±.323 correspondingly. The qualified weight of heart of different groups showed that there was no significant (P>0.05) difference among the groups (Table 8). Khan and collab reported that fenugreek seed extract had no impact on visceral organs (liver, heart, gizzard, and

intestines) of broiler chicks (Khan et al., 2011). It means that fenugreek infusion having antimicrobial and antibiotics like properties have no influence on either increasing or decreasing the relative weights of giblet.

4.3.2 Weight of intestine

The results of different groups showed that there was no significant ($P>0.05$) difference among the groups and the values were ranged from 76.33 ±5.341 to 80.33 ±3.000 (Table 10).This finding is in the line of Khan and collab. Who reported that fenugreek seed extract had no impact on visceral organs (liver, heart, gizzard, and intestines) of broiler chicks (Khan *et al.*, 2011). Upper findings are in line with the findings of Tariq *et al.*, (2014) who showed that feeding fenugreek seed powder had no significant effect on intestine weights and non- carcass components (liver, gizzard, heart).

Table 10. Effect of dietary supplementation of Fenugreek Seed on Liver, Gizzard, Intestine and Heart weight of different treatment.

Parameters	T1	T2	T3	T4	T5	Mean±SE
Liver weight(g)	36.11±1.4	35.78±2.19	33.56±.944	34.22±.98	32.33±1.17	34.40±.64^{NS}
Gizzard weight(g)	31.44±1.08	34.78±1.38	34.22±1.56	34.56±2.13	31.78±1.49	33.36±.70^{NS}
Heart weight(g)	7.39±.38	7.44±.25	6.89±.35	7.06±.42	6.67±.32	7.09±.15^{NS}
Intestine (g)	77.89 ±1.73	76.33±5.34	77.89±4.26	80.33±3.00	78.11±2.23	78.11±1.53^{NS}

Here, T1 = (Control), T2 = (Antibiotic), T3 = (1% FS), T4 = (1.5% FS) and T_5 = (2% FS). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- Mean with different superscripts are significantly different (P<0.05)
- Mean within same superscripts don"t differ (P>0.05) significantly
- SE= Standard Error
- * means significant at 5% level of significance (p<0.05)

4.4 Immune organs

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

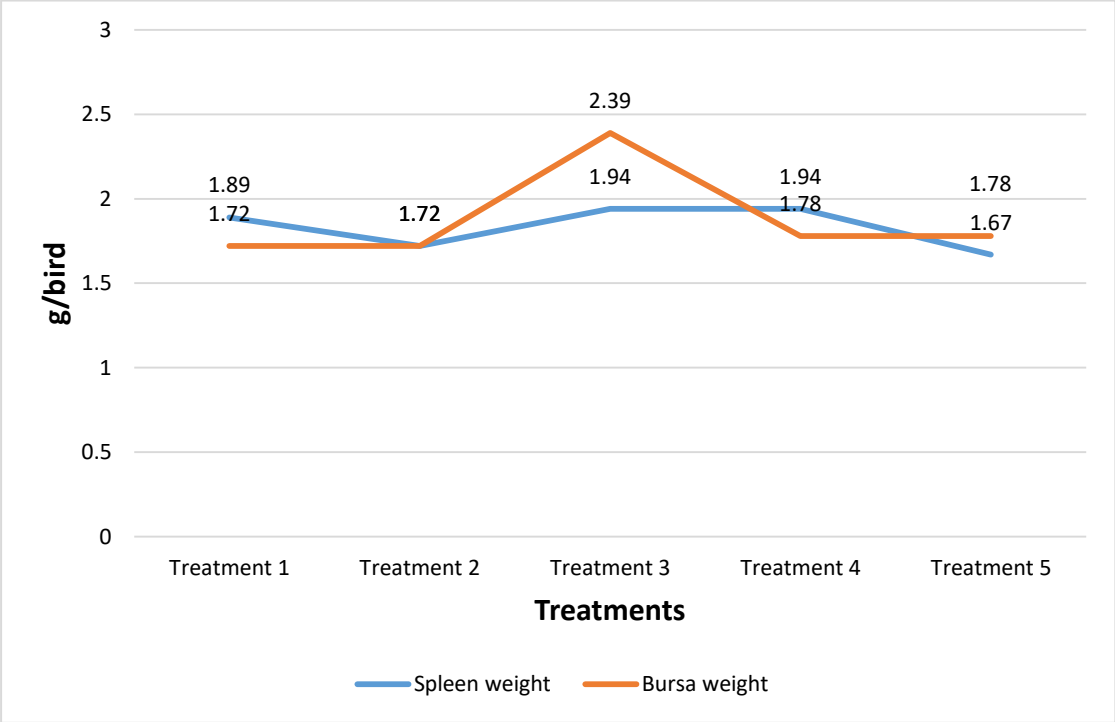

Figure 5: The Effect of supplementation of different level of FS to broiler diets on some immune organs.

The comparative weight of spleen (g) of broiler chicks in the dietary group T1, T2, T3, T4 and T5 were 1.89±0.16, 1.72±0.16, 1.94±0.25, 1.94±0.15 and 1.67±0.22 respectively. The highest value was T3 (1.94±0.25) and lowest value was T5 (1.67±0.22). But the relative weight of spleen of

different groups showed that there were no significant (P>0.05) difference. The weight of bursa was higher in T3 group (2.39±.371) compared to the other group which values were T1 (1.72±.188), T2 (1.72±.278), T4 (1.78±.222), and T5 (1.78±.278) correspondingly. But these values are not significantly different among the treatments (Figure-5). Other researchers found that the weight of the immunological organs (thymus, bursa and spleen) was not significantly (p>0.05) affected by FS treatment compare to control (Hind A.A et al., 2013).These results are in contrast with the findings of Bin et al., (2003) who reported that the addition of Fenugreek to boiler feeds lead to increased bursal weight.

4.5 Hematological parameters

Table 11 show the effect of dietary levels of fenugreek seed (1%, 1.5%, and 2%) 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 fenugreek seed, except Hemoglobin, RBC, WBC and Lymphocyte which were significantly affected (p<0.05). The birds fed diets supplemented with fenugreek seed (at levels of 1%,1.5% and 2%) diet had higher values of Hemoglobin (9.25 to 9.78 g/dl), RBC (4.18 to 4.73), WBC (7.67 to 8.11) and lymphocyte (66.11 to 73.22). No significant difference was found in Neutrophil, Monocyte, Eosiniphil, PCV, MCV, MCH and MCHC. But highest level of these parameters were found in FS treated diets of broiler chicken except MCHC.

Table 11. Effect of supplementation of Fenugreek Seed (FS) to broiler diets on blood parameters

Parameters	T1	T2	T3	T4	T5	Mean±SE
Hemoglobin(g/dl)	8.70^{b}±0.35	9.20^{ab}±0.19	9.78^{a}±0.11	9.25^{ab}±0.24	9.39^{ab}±0.19	9.26^{*}±0.11
RBC	3.28^{b}±0.17	3.36^{b}±0.24	4.18^{a}±0.12	4.73^{a}±0.16	4.49^{a}±0.28	4.01^{*}±0.12
WBC	6.78^{b}±0.70	7.44^{ab}±0.33	7.67^{ab}±0.28	8.11^{a}±0.26	8.00^{ab}±0.33	7.60^{*}±0.19
Neutrophil	66.56±2.24	69.67±1.52	70.89±1.41	69.78±1.28	71.33±1.26	69.64^{NS}±0.72
Lymphocyte	58.67^{b}±4.24	62.33^{ab}±4.06	73.22^{a}±3.75	66.11^{ab}±4.72	70.22^{ab}±4.36	66.11^{*}±1.97
Monocyte	1.26±0.10	1.50±0.07	1.58±0.11	1.55±0.10	1.44±0.13	1.46^{NS}±0.04
Eosinophil	1.51±0.07	1.52±0.05	1.51±0.08	1.59±0.05	1.55±0.06	1.54^{NS}±0.02
PCV	27.96±1.25	28.66±0.94	30.09±0.92	30.01±0.94	30.06±0.95	29.35^{NS}±0.45
MCV	78.89±0.44	78.46±2.77	81.70±0.93	81.81±1.49	81.52±1.330	80.48^{NS}±0.71
MCH	30.11±0.30	30.43±0.37	30.11±0.50	31.15±0.48	30.76±0.59	30.51^{NS}±0.20
MCHC	30.17±0.63	31.53±0.33	31.20±0.32	31.14±0.44	31.27±0.36	31.06^{NS}±0.20

Here, T_1 = (Control), T_2 = (Antibiotic), T_3 = (1% FS Supplementation), T_4 = (1.5% FS supplementation) and T5 = (2% FS supplementation). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts don‟t differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference
- ✓ * means significant at 5% level of significance (p<0.0)

These results are in agreement with the earlier findings of S. Waheed et al., (2017) who showed effect of supplementation spice extracts in feed resulted in increased hemoglobin in fenugreek, Black cumin seed, sweet violet as compared to negative control group. These results are in line with the findings that WBC counts were higher in birds fed fenugreek when compared to control. This significant difference ($p \leq 0.05$) justifies the presence of immunity boosting polyphenols extracts in fenugreek (S. Waheed et al., 2017).

Other researchers also found that the inclusion of fenugreek seeds at 10 g/kg of diet in broiler breeder chicken significantly improved the PCV, RBC and Hb concentration and attributed this improvement in erythropoiesis to the enhancement of antioxidant activities in RBCs, which decreases the production of free radicals that destroy Hb and cause hemolysis of RBCs (Abdul-Rahman, 2012).

These results are in accordance with Yonatan kassu yesuf (2018) findings who also shown no significant difference ($P \geq 0.05$) among treatments (black cumin, fenugreek and turmeric) regarding the white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values. But the RBC count was higher for FS as compared to the control ($P \leq 0.05$).

4.6 Intestinal micro flora

The microbial load (total count, E. coli, Salmonella, Lactobacilllus for its beneficial effect) in broilers fed different levels of Fenugreek seed is given in Table 12, E. coli count was significantly (P<0.05) decreased in birds fed 1%, 1.5%, 2% fenugreek seed and antibiotic ($12.66^{b} \pm 0.59$, $12.23^{b} \pm 0.58$,

12.21^{b} ± 0.47 and 12.12^{b} ±0.35 respectively) than the control birds (16.64^{a}±.79). Salmonella sp. count was significantly (P<0.05) decreased in birds fed 1% (0.00^{b} ± 0.00), 1.5% (0.00^{b} ± 0.00), 2% (0.00^{b} ± 0.00) fenugreek seed and antibiotic (0.00^{b} ± 0.00) than the control birds (1.33^{a}±0.89). Lactobacillus count was significantly (P<0.05) increased in birds fed 1%, 1.5%, 2% fenugreek seed. The highest number of lactobacillus was counted in T_5 group (19.43^{a} ± 0.35) and the lowest in T_1 group (11.82^{d} ± 0.49).

Table 12. Bacterial colony count in Fenugreek experiment in broiler chicken

Parameters	E.coli ×10^6 (CFU/ml)	Salmonella × 10^6 (CFU/ml)	Lactobacillus ×10^6 (CFU/ml)
T_1	16.64^{a} ± 0.79	1.33^{a} ± 0.89	11.82^{d} ± 0.49
T_2	12.12^{b} ± 0.35	0.00^{b} ± 0.00	15.02^{c} ± 0.77
T_3	12.66^{b} ± 0.59	0.00^{b} ± 0.00	17.40^{b} ± 0.87
T_4	12.23^{b} ± 0.58	0.00^{b} ± 0.00	18.28^{ab} ± 0.58
T_5	12.21^{b} ± 0.47	0.00^{b} ± 0.00	19.43^{a} ± 0.35
Mean±SE	13.17^{*} ± 0.36	0.27^{*} ± 0.18	16.39^{*} ± 0.49

Here, T_1 = (Control), T_2 = (Antibiotic), T_3 = (1% FS Supplementation), T_4 = (1.5% FS Supplementation) and T5 = (2% FS Supplementation). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

These results are in accordance with the earlier findings of M R purushothaman et al., (2014) who reported that the jejunum bacterial count

- E.coli and Clostridium sp. of birds fed with FR at different levels with and without antibiotic suggested that numerical reduction in E. coli due to FR supplementation. G.Attia et al., (2017) also observed a significant difference in the caecal micro flora count due to dietary intake of the plant extract blend (Fenugreek, Oregano and Chamomile). The beneficial bacteria count (Lactobacillus spp.) was linearly increased ($p<0.001$) and the harmful bacteria count (Coliforms) was linearly decreased ($p<0.001$) by increasing the level of the plant extract blend.

Qureshi et al. (2015) also investigated the in vitro antibacterial activity of fenugreek and reported the 2.1 mm of zone of inhibition for the concentration of 0.05 mg/ml of extract against E. coli. Upper findings are also in the line with the Study of Ahmad (2016) who showed that fenugreek oil has a positive effect on microbial health by lowering the total bacterial count, Salmonella and E. coli of the laying hens and thus can be used instead of commercial antibiotics.

4.7 Antiviral activity

Tables (13) show the effect of dietary levels of fenugreek seed (1%, 1.5%, and 2%) in feed, and their impact on haemagglutination inhibition titre against Newcastle disease (ND). Concerning the treatment effect on HI titre the results indicated significant ($p<0.05$) differences due to supplementation of fenugreek seed. Remarkably better titres of ND achieved in blood in the T_5 (day 15- 5.78, day 20- 4.00 and day 29- 6.56) treatments compare to control group.

Table 13. Effect of Fenugreek seed on immune response in broiler chicken

Parameters	Day 15 (log^2)	Day 20 (log^2)	Day 29 (log^2)
T_1	$4.00^{b} \pm 0.28$	$2.89^{b} \pm 0.26$	$5.33^{b} \pm 0.28$
T_2	$4.44^{b} \pm 0.37$	$3.78^{a} \pm 0.22$	$5.89^{ab} \pm 0.20$
T_3	$5.44^{a} \pm 0.29$	$3.89^{a} \pm 0.26$	$6.44^{a} \pm 0.17$
T_4	$5.89^{a} \pm 0.26$	$3.89^{a} \pm 0.26$	$6.56^{a} \pm 0.29$
T_5	$5.78^{a} \pm 0.32$	$4.00^{a} \pm 0.23$	$6.56^{a} \pm 0.24$
Mean±SE	$5.11^{*} \pm 0.17$	$3.69^{*} \pm 0.12$	$6.16^{*} \pm 0.12$

Here, T_1 = (Control), T_2 = (Antibiotic), T_3 = (1% FS Supplementation), T_4 = (1.5% FS Supplementation) and T5 = (2% FS Supplementation). Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

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

These results are in accordance with the earlier findings of Abed and collab (2014) showed that treatment that supplemented with 1 % fenugreek recorded high antibody titter against Newcastle disease virus at 21 day of broilers age. Abid and collab (2014) also demonstrated that the fenugreek increasing the immunity of birds at 24 and 34 day and because fenugreek increases the cellular ties of thymus gland and bone marrow.

Other researchers S. Waheed et al., (2017) also found remarkably better titres of ND achieved in blood in fenugreek treated. Natural extracts in general had significantly better (p≤0.05) titers as compared to control.

CHAPTER 5
SUMMARY AND CONCLUSION

A study was conducted with broilers to investigate the effects of herbal natural feed additives as alternative to an antibiotic growth promoter. The study was planned to determine the comparative efficacy of Fenugreek seed and antibiotic on the productive performance, haematology and health status of commercial broilers. A total of 150 day-old Cobb 500 broiler chicks were reared in Sher-e-Bangla Agricultural University Poultry Farm, Dhaka. Chicks were divided randomly into 5 experimental groups of 3 replications and each replication contains 10 chicks. These groups were allotted to five treatment designated as T_1, T_2, T_3, T_4 and T_5 Group. T_1 was offered basal feed without any supplementation and served as a control. Whereas, group T_2, T_3, T_4 and T_5 were offered basal feed supplemented with Antibiotic, Fenugreek seed (FS) 1%, Fenugreek seed 1.5% and Fenugreek seed 2% respectively. The results showed that the weekly body weight gain in 4^{th} week was significantly higher in control group (T_1) than other groups. Final live weight was significantly higher in 1.5% FS ($1528.33^{a}\pm57.468$) group than control (T1) group. Weekly feed consumption (FC) was insignificant in different group but total FC significantly lower in Antibiotic than T_3 FS treated group. Weekly FCR was significantly lower in T_1 group than T_3 group in 4^{th} week. In case of final FCR no significant difference were seen between the groups. Dressing percentage was significantly ($P>0.05$) higher in T_5 ($68.08^{a}\pm2.923$) group by the dietary inclusion of 2% FS as compared to control fed broilers. The relative weight of spleen and bursa of different groups showed that there were no significant ($P>0.05$) difference between the groups. In addition, the present study showed that feeding dietary FS and antibiotics had no significant ($P>0.05$) effects on liver, gizzard, heart and intestine weight

among the treatment groups compared with control and antibiotic. The results of glucose showed no significant differences but Cholesterol studies showed a significant ($P<0.05$) difference due to supplementation of FS. Comparatively lowest cholesterol found in 1.5% FS treated group than control and antibiotic. Concerning the treatment effect on blood constituents, no significant differences were found in Neutrophil, monocyte, Eosinophil, PCV, MCV, MCH and MCHC. But higher level of these constituents were found in FS treated diets except MCHC. Other parameters Hemoglobin, RBC, WBC and Lymphocyte which were significantly affected ($p<0.05$). Birds fed diets supplemented with FS (at levels of 1%, 1.5% and 2%) diet had higher values of Hemoglobin, RBC, WBC and lymphocyte. The numbers of intestinal micro flora (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 FS and antibiotic supplementing groups. The number of Lactobacillus were significantly ($p<0.05$) higher in 2% FS supplemented (T_5) group compared to control and antibiotic group. Treatment with 1%, 1.5% and 2% FS significantly ($p<0.05$) increases the ND titre level in T_3, T_4 and T_5 group compared to control and antibiotic group. Therefore, it could be concluded that the FS have the positive feedback and can significantly affect the productive performance and health status of broiler chicken.

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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	14.05.19-20.05.19	28.9	29.5	31.6	31.5	30.0	29	30.08
2nd	21.05.19-27.05.19	28.3	28.5	32.1	31.6	30.2	28.5	29.87
3rd	28.05.19-03.06.19	27.0	27.2	28.8	27.2	26.0	25.8	27.00
4th	04.06.19-10.06.19	26.8	27.0	28.6	28.5	27.4	27.2	27.58

Appendix 4. Relative humidity (%) during experiment

Age in weeks		**Relative humidity (%)**						
	Period (day)	8 A.M	12A.M	4 P.M.	8 P.M.	12 P.M.	4 A.M	Average
1st	14.05.19-20.05.19	85	82	73	74	78	80	78.67
2nd	21.05.19-27.05.19	85	83	71	72	77	79	77.83
3rd	28.05.19-03.06.19	86	85	74	75	81	83	80.67
4th	04.06.19-10.06.19	87	86	83	77	84	86	83.83

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

Treatment	Replication	Live Weight (g)	Eviscerated Weight (g)	Dressing Percentage (%)
T_1 (C)	R_1	1209	754.33	62.3928867
	R_2	1377.66	847	61.4810621
	R_3	1446	693	47.9253112
T_2 (A)	R_1	1432.66	929.66	64.8904834
	R_2	1521	1001.33	65.8336621
	R_3	1631.33	1075.33	65.9173803
T_3	R_1	1309.6	879.3	67.142639
	R_2	1298	954.66	73.5485362
	R_3	1298.6	825.33	63.5553673
T_4	R_1	1274.33	793.33	62.254675
	R_2	1403.66	837.33	59.6533349
	R_3	1259.33	769.33	61.0904211
T_5	R_1	1526	990.67	64.9193971
	R_2	1515	961.67	63.4765677
	R_3	1540.33	964.33	62.6054157

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

Treatment	Replication	Liver weight	Spleen weight	Gizzard weight	Bursa weight	Intestine weight	Heart Weight
T_1 (c)	R_1(1)	29	2	30	1	72	6
	R_1(2)	32	1	32	1	81	7
	R_1(3)	36	2	30	2	75	8

	Appendix 6 (Cont'd)						
	$R_2(1)$	39	1.5	34	4	80	7
	$R_2(2)$	35	1	32	2.5	85	10
	$R_2(3)$	36	2	33	2	70	8
	$R_3(1)$	36	2.5	24	3	79	6.5
	$R_3(2)$	44	3.5	35	4	84	7
	$R_3(3)$	38	2	33	2	75	7
$T_{2\ (a)}$	$R_1(1)$	39	2.5	37	2	84	8
	$R_1(2)$	41	2	42	2	100	7
	$R_1(3)$	39	2	35	2	86	6.5
	$R_2(1)$	28	1	36	1	53	7
	$R_2(2)$	30	2.5	30	1	70	7
	$R_2(3)$	35	2	30	2	75	7
	$R_3(1)$	26	1.5	30	1	52	7.5
	$R_3(2)$	46	2	38	3	89	9
	$R_3(3)$	38	2	35	2	78	8
T_3	$R_1(1)$	33	3	40	1	71	7
	$R_1(2)$	32	1	39	1	103	6
	$R_1(3)$	35	2	38	2	95	6
	$R_2(1)$	29	2	32	3	78	7
	$R_2(2)$	34	1.5	32	3	65	9
	$R_2(3)$	37	1	33	2	67	6
	$R_3(1)$	30	1.5	38	2	72	8
	$R_3(2)$	37	2	27	1	74	7
	$R_3(3)$	35	1	29	1	76	6
T_4	$R_1(1)$	30	2	25	1	78	6
	$R_1(2)$	32	2	31	1	76	9
	$R_1(3)$	35	1	30	1	85	7
	$R_2(1)$	36	1.5	44	3	83	7
	$R_2(2)$	34	2.5	35	1	88	7
	$R_2(3)$	37	2	33	2	85	8
	$R_3(1)$	30	1.5	33	3	59	4.5

	Appendix 6 (Cont'd)						
	$R_3(2)$	38	1	45	1.5	81	8
	$R_3(3)$	36	2	35	2	88	7
T_5	$R_1(1)$	33	2.5	38	2.5	90	6
	$R_1(2)$	33	1.5	26	1	67	7.5
	$R_1(3)$	38	2	32	2	75	6
	$R_2(1)$	32	1.5	24	1	80	8
	$R_2(2)$	33	1	34	2	81	8
	$R_2(3)$	35	2	31	2	80	7
	$R_3(1)$	32	2.5	31	2	83	6
	$R_3(2)$	25	2	34	1	75	5.5
	$R_3(3)$	30	2	36	2	72	6

Appendix 7. Biochemical data in different treatment groups

Treatment	Replication	Sugar mmol/L	Cholesterol mg/L
$T_{1\ (c)}$	R_1 (1)	8.8	138
	R_1 (2)	11	170
	$R_1(3)$	10.6	156
	$R_2(1)$	10.4	155
	$R_2(2)$	11.2	132
	$R_2(3)$	8.9	145
	$R_3(1)$	8.8	125
	$R_3(2)$	12	127
	$R_3(3)$	8.9	134
$T_{2\ (a)}$	$R_1(1)$	9.9	135
	$R_1(2)$	8.9	99
	$R_1(3)$	7.9	135
	$R_2(1)$	10.8	129
	$R_2(2)$	11.4	115
	$R_2(3)$	9	120
	$R_3(1)$	8.8	112
	$R_3(2)$	13	115
	$R_3(3)$	11	110
T_3	$R_1(1)$	11.2	110

	Appendix 7 (Cont'd)		
	$R_1(2)$	8.4	140
	$R_1(3)$	9.9	97
	$R_2(1)$	9	135
	$R_2(2)$	11.2	112
	$R_2(3)$	9.9	98
	$R_3(1)$	10.1	112
	$R_3(2)$	9.9	95
	$R_3(3)$	12	132
T_4	$R_1(1)$	8.5	111
	$R_1(2)$	9.2	98
	$R_1(3)$	10	112
	$R_2(1)$	10.9	100
	$R_2(2)$	10.2	102
	$R_2(3)$	8.6	123
	$R_3(1)$	9.5	122
	$R_3(2)$	7.9	122
	$R_3(3)$	9.5	138
T_5	$R_1(1)$	10.2	122
	$R_1(2)$	9.9	134
	$R_1(3)$	9.8	132
	$R_2(1)$	11	130
	$R_2(2)$	14.2	126
	$R_2(3)$	8.9	112
	$R_3(1)$	10.4	126
	$R_3(2)$	9.8	124
	$R_3(3)$	12.6	121

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

Treatments	Replications	Hb (g/dl)	RBC (Million /Cumm)	WBC	Neut Rophil/Cumm	Lymphocyte	Mono Cyte	Eosinophil	HCT/ PCV	MCV	MCH	MCHC
T_1	$R_1(1)$	10.01	3.40	4,200	62	52	1	1.70	26.50	80.21	30.19	31.48
	$R_1(2)$	6.50	3.40	6,200	76	45	1.80	1.20	30.50	78.21	28.19	30.48
	$R_1(3)$	8.40	2.70	3,800	62	74	1.52	1.45	24.60	80.20	30.25	30.22
	$R_2(1)$	9.50	3.20	9,200	72	70	1.20	1.72	30.50	78.21	30.19	32.48
	$R_2(2)$	8.50	4.40	7,200	65	51	1.08	1.65	22.50	79.28	31.15	28.48
	$R_2(3)$	8.10	3.10	8,000	54	69	1.02	1.56	25.50	76.25	29.38	26.18
	$R_3(1)$	9.50	3.40	8,200	70	40	1.60	1.06	30.50	78.21	30.19	31.48
	$R_3(2)$	8.25	3.40	7,200	72	55	1.03	1.70	34.50	79.18	31.19	29.48
	$R_3(3)$	9.50	2.50	9,100	66	72	1.05	1.58	26.50	80.30	30.22	31.24
T_2	$R_1(1)$	10.00	3.30	8,200	59	45	1.50	1.70	29.50	59.21	31.19	30.40
	$R_1(2)$	8.60	4.10	8,200	74	55	1.60	1.74	25.50	82.21	29.19	32.48
	$R_1(3)$	9.50	2.65	6,200	72	62	1.06	1.50	32.60	80.52	31.25	32.22
	$R_2(1)$	8.40	3.20	8,200	69	72	1.45	1.62	28.50	79.21	29.19	32.11
	$R_2(2)$	9.10	3.50	9,200	73	85	1.40	1.45	27.50	86.21	29.09	31.51
	$R_2(3)$	8.80	4.80	7,200	70	52	1.33	1.66	26.10	80.25	32.25	29.85
	$R_3(1)$	9.40	3.50	8,700	67	57	1.85	1.20	28.50	86.21	31.09	30.51
	$R_3(2)$	10.02	2.50	6,700	73	61	1.76	1.35	33.50	72.21	30.09	32.51
	$R_3(3)$	9.02	2.65	7,800	70	72	1.54	1.48	26.20	80.15	30.50	32.18
T3	$R_1(1)$	9.95	3.90	8,500	72	75	1.62	1.75	33.40	84.27	32.04	31.58
	$R_1(2)$	10.03	3.98	9,200	74	63	1	1.45	26.60	78.27	27.07	32.12
	$R_1(3)$	10.01	4.28	8,700	77	84	1.65	1.70	29.50	80.25	31.15	31.32
	$R_2(1)$	10.00	4.20	7,900	71	52	1.05	1.50	34.50	85.62	29.23	30.25
	$R_2(2)$	10.02	4.14	8,600	76	74	1.85	1.65	29.36	82.95	30.24	30.12
	$R_2(3)$	9.40	3.75	7,500	68	83	1.56	1.30	28.10	80.15	30.25	31.46
	$R_3(1)$	9.25	4.25	6,600	69	65	1.76	1.58	27.25	78.50	30.22	32.12
	$R_3(2)$	10.01	4.22	7,500	65	86	1.90	1.45	29.32	80.25	31.65	32.25
	$R_3(3)$	9.35	5.10	8,500	66	77	1.86	1.68	33.25	85.63	29.35	29.80

	Appendix 8 (Cont'd)											
T_4	$R_1(1)$	9.85	4.86	9,200	76	84	1.75	1.65	30.28	84.23	28.69	32.50
	$R_1(2)$	9.75	4.76	8,900	65	85	1.05	1.75	26.85	84.22	31.86	31.74
	$R_1(3)$	9.52	4.85	8,700	69	55	1.64	1.20	34.45	79.16	32.45	28.22
	$R_2(1)$	9.01	4.52	7,600	72	65	1.42	1.64	32.86	79.52	28.76	31.62
	$R_2(2)$	8.65	5.06	9,300	66	52	1.58	1.75	29.56	72.16	32.06	32.32
	$R_2(3)$	9.20	5.12	8,500	68	48	1.78	1.70	27.87	80.56	31.86	30.32
	$R_3(1)$	9.86	5.01	8,600	75	78	1.06	1.50	32.82	85.65	32.25	31.88
	$R_3(2)$	9.74	4.83	9,200	67	72	1.86	1.48	26.62	84.21	31.64	31.42
	$R_3(3)$	7.68	3.54	7,800	70	56	1.78	1.67	28.75	86.56	30.78	30.28
T_5	$R_1(1)$	9.01	2.56	6,800	68	85	1.05	1.20	29.35	79.26	29.62	31.26
	$R_1(2)$	9.85	3.85	8,800	72	76	1.85	1.38	28.42	78.21	32.33	29.56
	$R_1(3)$	10.02	4.56	9,500	77	82	1.76	1.45	30.24	86.76	30.46	32.50
	$R_2(1)$	10.01	4.95	9,600	75	56	1.05	1.75	32.50	85.37	27.22	32.48
	$R_2(2)$	9.95	5.12	8,700	66	49	1.06	1.67	34.28	86.95	29.64	30.42
	$R_2(3)$	8.52	5.13	7,900	69	58	1.05	1.56	33.62	82.45	32.47	31.82
	$R_3(1)$	8.76	4.21	8,700	75	68	1.88	1.75	27.00	76.28	30.38	32.42
	$R_3(2)$	9.45	4.88	9,500	72	82	1.45	1.66	26.06	78.64	32.25	30.15
	$R_3(3)$	8.90	5.11	8,200	68	76	1.85	1.52	29.08	79.80	32.46	30.84

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

Treatment	Replication	1st Week Feed Consumption/ Bird (g)	2nd Week Feed Consumption/ Bird (g)	3rd Week Feed Consumption/ Bird (g)	4th Week Feed Consumption/ Bird (g)
T_1	R1	185	451	752	897
	R2	187	451	752	904
	R3	179	426	777	908
T_2	R1	187	451	752	901
	R2	185	441	762	911
	R3	183	451	752	878
T_3	R1	175	421	782	868
	R2	183	451	752	872
	R3	184	451	752	853

	Appendix 9 (Cont'd)				
T_4	R1	185	421	782	813
	R2	177	431	762	868
	R3	176	441	762	911
T_5	R1	185	451	752	859
	R2	179	461	742	853
	R3	188	431	772	861

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

Treatments	Replications	1st week Body Weight/Bird(g)	2nd Week Body Weight/Bird(g)	3rd Week Body Weight/Bird(g)	4th Week Body Weight/Bird(g)
T_1	R_1	206.7	506.5	977.0	1545.4
	R_2	204.9	514.2	971.6	1563.1
	R_3	198.4	469.5	902.2	1544.5
T_2	R_1	203.1	508.5	997.5	1413.5
	R_2	202.7	514.7	980.0	1454.1
	R_3	200.9	506.4	935.8	1391.0
T_3	R_1	209.6	560.2	1048.3	1417.3
	R_2	205.4	507.7	1029.3	1408.0
	R_3	214.0	576.5	1053.2	1362.2
T_4	R_1	217.4	582.5	1145.2	1502.3
	R_2	210.9	548.7	991.0	1435.2
	R_3	216.9	524.7	1019.8	1540.2
T_5	R_1	196.3	481.7	1030.4	1333.0
	R_2	208.9	544.6	1013.6	1518.0
	R_3	202.0	524.9	973.4	1442.6

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

Treatment	Replication	E.coli×10^6 (CFU/ml)	Salmonella×10^6 (CFU/ml)	Lactobacillus×10^6 (CFU/ml)
T_1	R_1 (1)	16.8	Absent	13.4
	R_1(2)	17.5	5	12.0
	R_1	12.0	Absent	11.2
	R_2	18.6	Absent	14.7
	R_2	15.7	7	10.2
	R_2	16.9	Absent	12.1
	R_3	14.1	Absent	10.8
	R_3	19.5	Absent	10.2
	R_3	18.7	Absent	11.8
T_2	R_1	10.8	Absent	14.0
	R_1	11.2	Absent	15.6
	R_1	12.7	Absent	17.7
	R_2	13.3	Absent	13.8
	R_2	10.5	Absent	12.1
	R_2	13.3	Absent	18.8
	R_3	11.9	Absent	16.3
	R_3	12.4	Absent	12.0
	R_3	13.0	Absent	14.9
T_3	R_1	11.2	Absent	17.9
	R_1	13.6	Absent	16.7
	R_1	14.7	Absent	19.8
	R_2	15.9	Absent	14.3
	R_2	12.2	Absent	13.2
	R_2	10.9	Absent	20.6
	R_3	12.9	Absent	15.6
	R_3	11.7	Absent	18.4

	Appendix 11 (Cont'd)			
	R_3	10.8	Absent	20.1
T_4	R_1	11.5	Absent	20.5
	R_1	12.6	Absent	19.6
	R_1	10.8	Absent	15.8
	R_2	13.4	Absent	19.9
	R_2	14.6	Absent	16.6
	R_2	10.5	Absent	18.9
	R_3	10.4	Absent	16.8
	R_3	15.0	Absent	16.8
	R_3	11.3	Absent	19.6
T_5	R_1	11.6	Absent	18.7
	R_1	10.9	Absent	18.4
	R_1	12.2	Absent	19.3
	R_2	11.3	Absent	18.7
	R_2	11.7	Absent	21.6
	R_2	12.9	Absent	18.7
	R_3	10.6	Absent	19.3
	R_3	13.8	Absent	19.6
	R_3	14.9	Absent	20.6

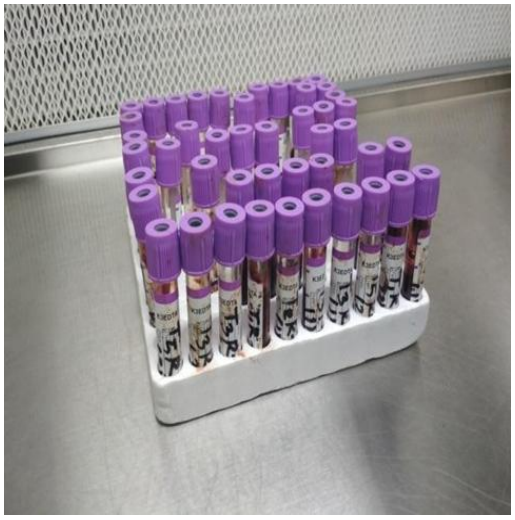
Appendix 12: ND HI titre level of broiler under different treatment groups at 15^{th}, 20^{th} and 29^{th} day of age.

Treatment	Replication	Day 15	Day 20	Day 29
T1	R1	2^3	2^2	2^5
	R1	2^4	2^3	2^6
	R1	2^3	2^4	2^7
	R2	2^5	2^3	2^7
	R2	2^5	2^2	2^4
	R2	2^4	2^2	2^5
	R3	2^4	2^3	2^5
	R3	2^3	2^4	2^6
	R3	2^5	2^3	2^7
T2	R1	2^4	2^4	2^7

	Appendix 12 (Cont'd)			
	R1	2^6	2^4	2^6
	R1	2^5	2^3	2^6
	R2	2^3	2^3	2^5
	R2	2^4	2^3	2^6
	R2	2^5	2^4	2^5
	R3	2^4	2^5	2^6
	R3	2^6	2^4	2^5
	R3	2^3	2^4	2^6
T3	R1	2^6	2^3	2^6
	R1	2^5	2^5	2^6
	R1	2^7	2^4	2^7
	R2	2^5	2^4	2^7
	R2	2^6	2^3	2^6
	R2	2^5	2^4	2^6
	R3	2^4	2^5	2^7
	R3	2^6	2^4	2^6
	R3	2^5	2^3	2^7
T4	R1	2^5	2^4	2^7
	R1	2^6	2^3	2^7
	R1	2^5	2^4	2^6
	R2	2^6	2^4	2^6
	R2	2^6	2^5	2^8
	R2	2^7	2^3	2^5
	R3	2^7	2^4	2^7
	R3	2^6	2^5	2^6
	R3	2^5	2^3	2^7
T5	R1	2^4	2^3	2^6
	R1	2^5	2^4	2^6
	R1	2^7	2^4	2^8
	R2	2^6	2^5	2^6
	R2	2^5	2^4	2^7
	R2	2^6	2^4	2^7
	R3	2^6	2^4	2^6
	R3	2^7	2^3	2^7
	R3	2^6	2^5	2^6

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

Activities after arrival of day old broiler chicks.



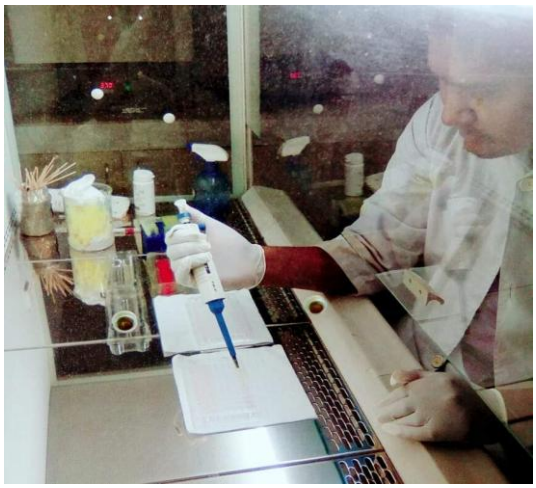
Collection of blood at the age of 27 days of old

Appendix 13 (Cont'd)

Bacterial culture preparation and colony count by colony counter

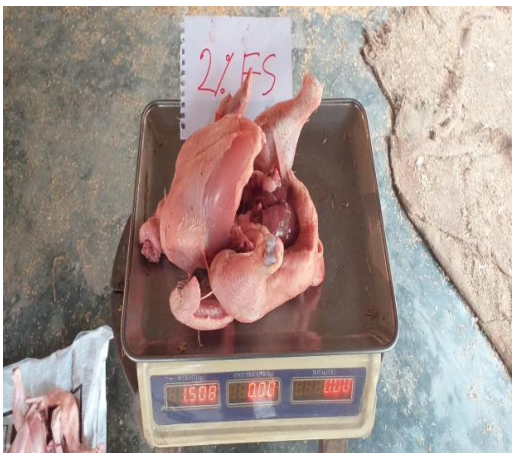
Appendix 13 (Cont'd)



Determination of ND HI titre at CDIL

Appendix 13 (Cont'd)



Monitoring and weighing of dressed broiler chicken with internal organs

Appendix 13 (Cont'd)

Different types of Medication and vaccine used in experiment