EFFECTS OF LOCALLY ISOLATED PROBIOTICS AS ALTERNATIVE TO ANTIBIOTIC ON GROWTH PERFORMANCE, HEMATOLOGICAL TRAITS AND CECAL MICROFLORA OF BROILER CHICKEN

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CERTIFICATE

This is to certify that the thesis entitled, "EFFECTS OF LOCALLY ISOLATED PROBIOTICS AS ALTERNATIVE TO ANTIBIOTIC ON GROWTH PERFORMANCE, HEMATOLOGICAL TRAITS AND CECAL MICROFLORA OF BROILER CHICKEN" submitted to the Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (MS) in POULTRY SCIENCE embodies the result of a piece of bona fide research work carried out by LITA BISWAS, Reg. No. 12-04868 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly acknowledged by the Author.

Dated: June, 2019
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Dedicated To

My Beloved Parents and Teachers

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ABSTRACT

Increasing knowledge of bacterial resistance to antibiotics used in both human and veterinary medicine has contributed to development of perceptions in consumers that suitable alternatives to antibiotics must be identified. Probiotics have been recently documented to have beneficial effects on animal health and thus stand for a better alternative to antibiotics. Therefore, the aim of the study was to evaluate the effect of drinking water supplementation of locally isolated probiotics on broiler growth performance, organ development, hematological profile and cecal microbial composition in replacing antibiotic growth promoters (AGPs). A total of 320 one day old mixed sexed "Cobb 500" broiler chicks were allocated to 4 treatment groups in a complete randomized design (CRD) and each treatment was replicated 4 times with 20 chicks in each replicate. Treatments included (T1) the basal diet as a negative control; (T2) basal diet + doxycycline antibiotic via drinking water (2g/L) as a positive control; (T3) basal diet + probiotic I (*Lactobacillus spp*); (T4) basal diet + probiotic II (*Bifidobacterium spp.*). The results revealed that supplementation of probiotic I (*Lactobacillus spp.*) increased ($P<0.05$) body weight and decreased FCR but had no significant difference ($P>0.05$) in feed consumption, dressing percentage and survivability percentage. None of the weights of the heart, gizzard, spleen, bursa, and abdominal fat were influenced by supplemental probiotics ($P>0.05$), although improved liver weight was found by supplemented with probiotic I ($P<0.05$). Significant ($P<0.05$) improvements were also observed in Hb, RBC and WBC counts in probiotic supplemented group than the antibiotic and control group. In addition, DLCs, PCV, MCH, MCHC were not affected ($P>0.05$) by the treatments. The microbiological analysis indicated that the lactic acid bacterial population boosted predominantly. The total coliform and Salmonella counts were significantly reduced and *Lactobacillus spp* was significantly ($P<0.05$) increased supplemented with probiotic I and probiotic II compared with antibiotic and control group in the cecal contents of birds at day 28. The best performance was detected in birds of probiotic I group followed by the probiotic II, antibiotic and control group. Because of its remarkable efficacy it is concluded that, the use of mixed culture of *Lactobacillus spp.* could be considered as a good potential probiotic for broiler chickens and viable alternative to antibiotics in broiler diet at finisher stage and its benefits should be further evaluated on a commercial scale.

TABLE OF CONTENTS

LIST OF CONTENTS

LIST OF CONTENTS (Cont'd)

LIST OF CONTENTS (Cont'd)

LIST OF CONTENTS (Cont'd)

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATION	FULL MEANING
ANOVA	Analysis of Variance
AM	Anti- Meridian
AB	Antibiotic
AGPs	Antibiotic Growth Promoters
ADG	Average Daily Gain
AFW	Abdominal Fat Weight
BSA	Bifidobacterium Selective Agar
BSC	Bifidoselective Media
Ca	Calcium
CSCRA	Canadian Securities Regulatory Authority
Cm	Centimeter
Cl	Chlorine
CFU	Colony Forming Unit
CBC	Complete Blood Count
Contd.	Continued
CF	Crude Fiber
CP	Crude Protein
cumm	Cubic Millimeter
DCP	Dicalcium Phosphate
EDTA	Diethyle Tetraacitic Acid
DLCs	Differential Leukocytes Counts
DFM	Direct Feed Microbials
DM	Dry Matter
EMB	Eosine Methylene Blue agar
E.coli	*Escherichia coli*
EE	Ether Extract
et al.	and others
FCR	Feed Conversion Ratio
FI	Feed Intake
Fig.	Figure
FLW	Final Live Weight
GIT	Gastrointestinal Tract
g/gm	Gram
Hb	Hemoglobin
IBD	Infectious Bursal Disease
Kcal	Kilo-calorie

LIST OF ABBREVIATIONS AND SYMBOLS (Cont'd)

MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
ME	Metabolizable Energy
mm	Millimeter
mm^3	Cubic Millimeter
mg	Milligram
mL	Milliliter
Mm	Millimeter
mmol	Millimol
MRS-Cys	De Man, Rogosa, and Sharpe Media -Cystine
ND	Newcastle Disease
No.	Number
NA	Nutrient Agar
PCV	Packed Cell Volume
P	Phosphorus
PM	Post- Meridian
PB I	Probiotic I
PB II	Probiotic II
RH	Relative Humidity
R.	Replication
RBC	Red Blood Cell
SAU	Sher-e- Bangla Agricultural University
Sq. ft.	Square Feet
SE	Standard Error
TVC	Total Viable Count
Treat.	Treatment
WBC	White Blood Cell
@	At the rate of
+	Plus
>	Less than
<	Greater than
/	Per
˚C	Degree Celsius
%	Percentage
+	Positive
µg	Microgram
µl	Microliter

CHAPTER 1

INTRODUCTION

Poultry industry is one of the most important and promising industrial sector for the economic development of Bangladesh. The contribution of poultry to the Gross Domestic Production (GDP) is approximately 1% (Rahman *et al.*, 2017). Poultry meat is an important source of first class protein in developing countries since it is less expensive to produce than other meats and is acceptable in virtually all cultures and religions. It has been estimated that poultry meat alone contributes 37% of the total meat production in Bangladesh. Poultry contributes about 22-27% of the total animal protein supply in the country (Prabakaran, 2003).

In Bangladesh commercial broiler farming has got industrial shape during the journey of last 4-5 decades. Volumes of production have been increased manifolds due to mechanization of poultry industry. In addition, intensive rearing of broiler chicken has raised a particular issue with disease. Diseases are now considered by many to be the most important obstacle for poultry sector itself and for public health (Asselt *et al*., 2018). Among these conditions, the major economic losses are mainly due to infectious diseases which could be caused by viruses, bacteria, fungi, protozoa and the cost of preventive medication. The fasting growing poultry sector has elicited the discovery and widespread use of number of "feed additives". The main objective of adding feed additives is to boost animal performance by increasing animal growth rate, better feed conversion ratio, greater livability and lowered mortality in poultry birds.

In poultry industry, antibiotic growth promoters (AGPs) have been used as a feed additive to enhance gut health and control sub-clinical diseases. The term AGP is used to describe the medicine that destroys or inhibits bacteria which is administered at a low sub therapeutic dose .The mechanism of action of antibiotics as growth promoter is related to interactions with intestinal microbial populations (Dibner and Richards, 2005; Niewold, 2007). There are four hypothesis have been proposed to explain their action: (i) Nutrients are more efficiently absorbed because of a thinner small-intestinal epithelium; (ii) nutrients are spared since competing microorganisms are reduced; (iii) microorganisms

responsible for subclinical infections are reduced or eliminated and (iv) production of growth-depressing toxins or metabolites by intestinal microflora is reduced. Using of antibiotics in feed led to improved feed conversion efficiency and reduced pathological load associated with poultry production. The greatest problem with antibiotics for poultry as well as for human is antibiotic–resistant bacteria (Nhung *et al.,* 2017).

Now-a-days the quality food a component of food security imparting a major concern throughout the world even in Bangladesh. Unfortunately, farmers are using antibiotics in broiler feed to improve growth and feed efficiency which adversely affects on human health. Antibiotics used as growth promoters are incorporated in feed at sub-therapeutic levels over extended periods to entire flocks. Giving animals antibiotics in their feed can cause microbes in the livestock to become resistant to the drugs. People can then become infected with the resistant bacteria by eating or handling meat contaminated with the pathogens. Infections caused by resistant strains of enterococci, streptococci, *Salmonella*, *Campylobacter*, *E. coli*, etc. are the current treatment problems originating from the use of antimicrobials in animal production (Wegener *et al*., 2006). For this reason, European Union has banned regulation the use of antibiotics in animal production from 2006 and its use has become limited in other developed countries.

In many countries of the world, including Bangladesh the use of most AGP has been banned to preserve the effectiveness of important human drugs (Casewell *et al*., 2003). However part of our consumers have still the dilemma regarding the safety and quality of broiler meat during broiler production, processing and marketing. So, it is the pertinent time for poultry experts, scientists and relevant sector of the government to work together in a collaborative manner to ensure quality and safety of broiler meat not only for changing the notion of consumers but also to build up a healthy nation.

Under such circumstances, poultry nutritionists have taken various attempts for production of safe and quality broiler meat by supplementations of probiotics, prebiotics, acidifiers, various medicinal plants, and herbs etc. as alternatives to traditional antibiotics, hormone, enzyme or any other chemicals. Therefore, there is a need to reduce the usage of antibiotics as growth promoters and alternative substance that

environmentally friendly and ease in farm application need to be explored. Recently, alternatives for substituting these traditional growth promoters have been evaluated and probiotics feeding have been the area of interest. A great deal of attention has recently been received from nutritionists and veterinary experts for proper utilization of nutrients and the use of probiotics for growth promotion of poultry.

The term probiotic derived from Greek word "pro bios" which means "in favor of life" (Coppola and Turnes, 2004). According to the definition by FAO/WHO, probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (Fuller *et al*., 1989). These organisms have been widely reported to exert many beneficial health effects, such as activation of the immune system, maintenance of mucosal integrity and presentation of an antagonistic environment for pathogens (Rashmi and Gayathri, 2014). Currently, probiotics seem to be good alternatives to the use of antibiotics as growth promoters which have been used on poultry and livestock in an attempt to increase mean weight gain (Tomasik, 2003). Probiotics are responsible for the production of vitamin B complex and digestive enzymes and for stimulation of intestinal immunity, increasing protection against toxins produced by pathogenic microorganisms (Alexopoulos *et al*., 2004). In broiler nutrition, probiotic species such as *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Bacillus*, *Enterococcus*, *Aspergillus* and *Saccharomyces* are widely used to prevent poultry pathogens and diseases and improve broiler's growth performance (Kalavathy *et al*., 2003; Kabir *et a*l., 2004; Timmerman *et. al*., 2006; Mountzouris *et al.*, 2007; Awad *et al.*, 2009), immunomodulation (Zulkifli *et al*., 2000; Khaksefidi *et al*., 2006; Haghighi *et al.*, 2006; Nayebpor *et al*., 2007), certain hematological parameters (Jin *et al*., 1998; Islam *et al*., 2004; Ashayerizadeh *et al.*, 2009) and promoting microbiological meat quality of broilers (Kabir *et al*., 2005).

Two genera of bacteria are mostly reported including lactic acid bacteria of the genus *Lactobacillus* (Dalloul *et al*., 2005 ; Higgins *et al*., 2008; Lee *et al*., 2010) and *Bifidobacteria* (Patterson and Burkholder, 2003; Willis *et al*., 2010a, 2010b). Probiotics have become a major focus of lactic acid bacteria research over the past 10 years with most attention drawn to the genera *Lactobacillus* and *Bifidobacterium* for improving chicken health in natural way (Fernandez *et al*., 2003). They have been

broadly applied in livestock and poultry as a growth promoter and a competitive exclusion agent (Simon, 2010). Therefore, when used as a poultry growth promoter, these spores added to feed could enhance broiler chicken's digestibility and performance parameters by creating the favorable conditions for beneficial bacteria (Steiner *et al*., 2006) and affect gene expression of carrier proteins responsible for cholesterol absorption (Matur and Eraslan, 2012). Thus the use of defined probiotic cultures in the poultry industry has recently become more common for obtaining better productivity and health benefits (Dhama and Singh, 2010; Hajati and Rezaei, 2010).

The importance of probiotics as an appropriate alternative has increased more than ever due to the possible hazards and risks of antibiotics in poultry production. However, the actions of these two probiotics from native chicken have not yet been investigated in detail in Bangladesh. Since the indigenous chicken of Bangladesh are very much disease resistance than the commercial chicken it would be better to find a suitable probiotic isolated from health gut of local chicken. Moreover, every year most of the company of our country imports feed additives as probiotics. This imported probiotics cost is very high. If it is possible to prepare this probiotic from local chicken isolation, it will be very suitable for our climate condition to combat diseases as well as it would be possible to save huge foreign currency. When this probiotic is available and convenient, new entrepreneur may encourage involving in this sector. But the system of using this probiotic isolating from indigenous chicken and its commercial utilization is almost new in our country. Therefore, the present study is undertaken to evaluate the effects of these two probiotics as alternative to AGPs on the growth performance, internal organ development, hematological traits and cecal microbial population of broilers. The objectives centered on the study comprise the target points:

- To determine the effects of locally isolated probiotics on growth performance and carcass characteristics of broilers.
- To evaluate the effects of locally isolated probiotics on serum biochemistry and hematological traits (Hb, RBC, WBC, DLCs, PCV, MCH, MCHC) of broilers.
- To investigate the effects of locally isolated probiotics on cecal microbial population of broilers.

CHAPTER 2

REVIEW OF LITERATURE

Poultry is one of the fastest growing segments of agriculture and veterinary sector. Like other sector of agricultural industry, major aim of this industry is also to produce maximum with minimum input. Feed is one of the largest items of expenditure in poultry production and it alone accounts to 70% of total poultry production. The constant increase in the cost of poultry feed ingredients and compounded feed is making the profit less for poultry farmers. Therefore, balanced and effective feeding is most important requisite for economic poultry production.

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 (as growth promoter) have been extensively used for enhancing poultry production but due to development of antibiotic resistant bacterial strains and residual effects of these feed additives in meat, they lead to various health hazards to consumers.

However, scientific evidence suggests that the massive use of these compounds has led to increased problem of antibiotic resistance (Furtula *et al*., 2010; Forgetta *et al*., 2012,), presence of antibiotics residues in feed and environment (Carvalho and Santos, 2016; Gonzalez Ronquillo *et al*., 2017) and compromises human and animal health (Diarra *et al*., 2010). However, the literatures which are most relevant to the present study are reviewed and cited here under the following headings-

2.1 Uses of antibiotics in broiler chicken production

Over the past 50 years, the use of antibiotics combined with strict biosecurity and hygiene measures has helped the poultry industry to grow by preventing the negative impacts of many avian diseases (Bermudez, 2003).

In intensive poultry farming, especially in North America, antibiotics such as tetracycline, bacitracin, tylosin, salinomycin, virginiamycin and bambermycin are often used (Diarra and Malouin, 2014). In the United States, tetracyclines represent more than two-thirds of antimicrobials administered to animals (Gonzalez Ronquillo

and Angeles Hernandez, 2017), while in European Union (EU) they represent only 37% (Carvalho and Santos, 2016). The use of antibiotics as growth factors is not allowed in the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) participating countries (ESVAC, 2017). In 2014, 81% of the antimicrobials used in Canada on broiler farms were for prevention purposes. They were primarily intended to prevent necrotic enteritis caused by C*lostridium perfringens* and coccidiosis (CSCRA, 2016).

2.2 Impacts of antibiotic on chicken growth, digestive tract and immune systems

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) and protect the health of birds by modifying the immune status of broiler chickens (Lee e*t al.*, 2012). This is mainly due to the control of gastrointestinal infections and microbiota modification in the intestine (Singh *et al.*, 2013). The mechanism remains unclear, but antibiotics are likely to act by remodeling microbial diversity and relative abundance in the intestine to provide an optimal microbiota for growth (Dibner and Richards, 2005). For example, 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.

2.3 Impacts of antibiotic on meat quality

Escherichia coli bacteria are very common and can also cause diseases. Salmonellosis is one of the most common and widespread food-borne illnesses in the world. Salmonella infections usually cause mild gastroenteritis.

According to CSCRA (2016) report, chicken contamination rates for *E. coli,* Campylobacter and Salmonella spp. are respectively 96%, 25% and 34% in Canada. In addition, antibiogram test revealed multi-pharmacological resistance in

Enterobacteriaceae isolates from eggs and broiler meat (Diarra *et al.*, 2010 and Yulistiani *et al.*, 2017). Schwaiger *et al.* (2012) reported that the prevalence of multi-resistant of Salmonella was higher in retail samples compared to slaughterhouse samples.

2.4 Impacts of antibiotic on consumer health and the environment

In addition to bio-resistance, antibiotics abuse has resulted in drug residues in animal products (Gonzalez Ronquillo and Angeles Hernandez, 2017). Several antibiotics such as penicillin, tetracycline, macrolide and aminoglycoside have been detected in foods (Diarra and Malouin, 2014). Residues in livestock production can actually have adverse impact on human health. Gassner and Wuethrich (1994) have demonstrated the presence of chloramphenicol metabolites in meat products. These authors concluded a possibility link between the presence of these antibiotic residues in meat and the occurrence of aplastic anemia in humans.

The global consumption of antibiotics in human and animal production is estimated between 1×10^5 and 2×10^5 t (Manzetti and Ghisi, 2014). Releasing thereby large quantities of antibiotics into the environment. Large amounts of antibiotics administered to animals are excreted into the environment via urine and faeces (Carvalho and Santos, 2016). After metabolic changes in animals, 30% to up 90% of the dose consumed is found in the urine and feces as parent compounds and/or metabolite compounds (Carvalho and Santos, 2016). This makes sewage disposal systems one of the most important routes by which antibiotics can enter in the environment (Gonzalez Ronquillo and Angeles Hernandez, 2017) and contaminate even coastal waters (Chen *et al.*, 2015). Liu *et al.* (2012a) have shown that airborne transmission causes the spread of epidemic diseases and also poses impend over public health.

2.5 Antibiotic and bacterial resistance

Scientific evidence suggests that the use of antimicrobials in livestock production can promote bacterial resistance in treated animals (O'Brien, 2002). In one study on Salmonella enterica isolates collected from poultry farms in British Columbia (Canada). Diarra *et al.* (2014) showed that more than 43% of the isolates were

simultaneously resistant to ampicillin, amoxicillin-clavulanic acid, ceftiofur, cefoxitim and ceftriaxone. Another Canadian study (Diarra and Malouin, 2014) highlights the existence of different stereotypes of Salmonella, isolated from broiler farms, resistant and multi-resistant to antibiotics. In addition, antibiotic resistance in Enterococci (Silbergeld *et al.*, 2008) and Salmonella spp. (Manning *et al.*, 2015) isolated in broilers have been reported. A study in Germany (Schwaiger *et al.*, 2012) showed that resistant and multi-resistant isolates are very common in chicken meat. Another study in Italy (Bacci *et al*., 2012) reported that 86% of *S. enterica* isolated from chicken carcasses were resistant to tetracycline. Bacterial resistance to animal antibiotics is a public health issue. In Canada, for example, poultry meat may play a role in human infections (Diarra *et al.*, 2010).This represents a threat to human health. On the other hand, antibiotic resistance is lower in organic farms (Hegde *et al*., 2016).

2.6 Alternative strategies to AGPs in poultry production

Consumers' pressure and worries towards harmful effects of antibiotic use and the ban of antibiotics in EU have prompted researchers to think about alternatives to antibiotics (Diarra and Malouin, 2014). The aim of these alternatives is to maintain a low mortality rate, a good level of animal yield while preserving environment and consumer health. There are indeed a number of non-therapeutic alternatives that can substitute antibiotics use. Among these, the most popular are probiotics, prebiotics, enzymes, organic acids, immunostimulants, bacteriophages, phytogenic feed additives, phytocides, nanoparticles and essential oils.

2.6.1 Probiotics in poultry production

The most important advantage of probiotics is that unlike antibiotics, they leave no residues in meat, which may have serious implications for consumers' health. Probiotics, a name which means 'for life', has been defined in several ways. In the beginning it was defined as those substances produced by microbes that stimulate one another (Houndonougbo *et al*., 2011), but later this term was used for animal feed supplements which produce beneficial effects on the host animal (Saleh and Hayashi, 2011). Later still the definition was refined to refer to live microbial cultures which beneficially affect the host by improving its intestinal microbial balance (Fuller, 1989). The experts of the joint Food and Agriculture Organization of the United

States/World Health Organisation (FAO/WHO) define probiotics as 'live microorganisms which, when administered in adequate amounts, confer a health benefit to the host' (Anonymous, 2001). Today it is well recognized that probiotics are strain-specific, living microbial cultures that produce beneficial effects on the host's body (O'Dea *et al*., 2006). These living organisms may be bacteria, fungi or yeasts (Fox, 1988). They are isolated from the gut of a healthy adult animal typically of the same species to which the probiotics will be given (O'Dea *et al*., 2006).

The success of probiotics depends upon the survival and stability of the probiotics, the strain, specificity of the strain to the host, dose frequency, health and nutritional status of the bird as well as the age, physiological stress level and genetic make-up of the host (Chichlowski *et al*., 2007). Enumerating several useful points regarding probiotics, Chichlowski *et al.* (2007) described probiotic bacteria as either anaerobic or facultative .The small intestine contains a large number of facultative anaerobes such as Lactobacillus, Streptococci and anaerobes like Bacteroides and *Bifadobacterium spp.* In poultry, probiotic bacterial colonisation is traditionally measured by colony forming units (CFU). The most heavily colonised region of the GIT is the colon and caecum, with 10^{10} to 10^{13} CFU/ml (Heczko *et al*., 2000). The species that are used in probiotic preparations are *Lactobacillus bulgaricus, L. acidophillus, L. casei, L. helveticus, L. salvarius, L. plantarum, L. faecalis, Bifidobacterium spp. Bacillus subtilis, Enterococcus faecium,Streptococcus thermophilus, Enterococcus faecium, Saccharomyces cerevisiae ,Aspergillus oryzae and Toulopsis sphaerica. Lactobacilli and Streptococci a*re most commonly used treatments of bacteria in the production of probiotics (Mohan *et al*., 1996; Yoruk *et al*., 2004; O'Dea *et al*., 2006; Choudhari *et al*., 2008; Hassanein and Soliman, 2010; Kapil *et al*., 2015).

2.6.2 Criteria for selecting probiotics in the poultry industry

The effectiveness of a probiotic supplement depends upon what it contains. A good probiotic should have the following characteristics:

- The culture should be acid and bile resistant and should contain a minimum of 30,109 CFU (Patterson and Burkholder, 2003; Choudhari *et al*., 2008).

- It should be strain specific. The culture should possess high survival ability and multiply fast in the conditions within the poultry gut (Choudhari *et al*., 2008).
- The culture should not have any side effects. It should be neither pathogenic nor toxic to the host (Patterson and Burkholder, 2003; Choudhari *et al*., 2008).
- The culture should have a strong adhesive capability with the digestive tract of the poultry (Patterson and Burkholder, 2003).
- Be durable enough to withstand the duress of commercial manufacturing, processing and distribution (Patterson and Burkholder, 2003).
- The culture should have the ability to reduce pathogenic microorganisms (Patterson and Burkholder, 2003; Choudhari *et al*., 2008).
- Capability of exerting beneficial effects on the host animal viz. increased growth or resistance to disease.
- Should be present as viable cells, preferably in large numbers although the minimum effective dose is not fully defined.
- Persistency in intestinal tract.
- Ability to modulate immune response.

2.6.3 Mode of actions of probiotics in poultry

i. Maintaining normal intestinal microflora by competitive exclusion and antagonism (Kabir *et al*., 2005; Fuller, 1989; Kizerwetter *et a*l., 2009).
ii. Altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production (Jin *et al*., 2000 ; Yoon *et al*., 2004)
iii. Improving feed intake and digestion (Yeo and Kim , 1997; Awad *et al.*, 2006)
iv. Stimulating the immune system making birds less vulnerable to disease a) By increasing antibody level ,b) By increasing microphage activity and c) By improving production of immunoglobulin-IgA, IgM and IgG and also cytokine (Huang *et al*., 2004; Haghighi *et al*., 2005; Dalloul *et al*., 2005; Nayebpor *et al.*, 2007; Apata, 2008; Brisbin *et al*., 2008)

2.6.4 Probiotic properties in the genus *Lactobacillus*

Lactobacilli are a heterogeneous, non-sporing, rod shaped, catalase negative gram-positive bacteria and several species viz. *Lactobacillus casei, L. zeae, L. paracasei, and L. rhamnosus* have been commonly used as probiotic. As their population increases the entity of other undesirable microflora decreases in the gut (Fathabad & Eslamifar, 2011). Lactic acid bacteria (LAB) are in the habitats of dairy and meat products, sewage, humans, plants and animals (Pelinescu *et al.,* 2009) and few viz. *L. gallinarum* and *L. Johnsonii* from chicken faeces (Fujisawa *et al*., 1992) have been isolated from poultry farming, gastro intestinal tracts and faces of chicken. Strengthening research on LAB and their relationship in the environments of poultry farms would help developing better probiotic feed. Lactobacilli species are commonly selected as probiotics since they express many crucial properties such as: high tolerance to acid and bile, capability to adhere to intestinal surfaces, withstanding low pH, gastric juice, inhibiting potentially pathogenic species (antimicrobial activity), resisting antibiotics and removing cholesterol. Garriga *et al.* (1998) lactobacilli strains showing inhibition against one or more enteric indicator strains (*E. coli, Salmonella enteritidis*).

2.6.5 Probiotic properties in the genus *Bifidobacterium*

The genus *Bifidobacterium* includes various Gram positive non-motile anaerobic bacteria. Strains of the genus *Bifidobacterium* are also often used as probiotic bacteria as they are known for their variety of resistance mechanisms to bile salts, which is important since the beneficial effects of probiotic bacteria must be generated in the presence of this biological fluid.

Several strains of bifidobacteria are considered as important probiotics including: *Bifidobacterium infantis, B. adolescentis, B. animalis* subsp *animalis, B. animalis* subsp *lactis, B. bifidum, B. longum, B. breve*. There are only a few reports on the use of bifidobacteria as probiotics for animals (Abe *et al*., 1995). The mechanisms likely to explain the favorable effects of bifidobacteria are linked to a change in the pattern of bacterial populations, such as reduction of certain potential pathogenic bacteria, or to a modification of the intestinal environment, *i.e*., intestinal pH. An important role of the normal intestinal microflora is to exert a barrier against attachment and colonization of the intestinal epithelium by pathogenic bacteria (Hentges, 1992).

Bifidobacterium species together with other probiotics have been proven to cholesterol-lowering capacities (Ruiz *et al*., 2013).

2.6.6 Delivery methods of probiotic

There are several factors that can negatively affect the viability of probiotic bacteria during manufacture and/or storage, for example temperature, water activity and other food ingredients. However the main reason for reduced viability is the high temperature during manufacturing processes, this is because of most probiotics have low thermo-resistance (Vesterlund *et al*., 2012). Hence, an ideal delivery system is needed which can protect probiotic bacteria from adverse conditions during production and storage and in the acidic gastric environment, that finally make sufficient amount of probiotics available at the site of action (Kim *et al*., 2016). In terms of delivery, there are several different ways of supplying probiotics to broiler chickens such as, mist spraying, via feed, oral gavage, application to vent lip, and via drinking water (Olnood *et al*., 2015) and even delivering probiotic by injection of the egg at the end of incubation (de Oliveira, *et al*., 2014) and spraying the litter that broiler chickens reared on (Olnood *et al*., 2015).

2.7 Probiotics in the diets of broilers

2.7.1 Effects on growth rate and body weight gain

Shome *et al*. (2000) reported that when mixture of *Lactobacillus acidophilus* and *L. salivarius* was fed to broilers, the live weight of chicken was higher during starter phase in experimental groups compared to control. Naik *et al*. (2000) evaluated the effect of different probiotics (*Lactobacillus acidophilus, Saccharomycs cerevisiae a*nd their combination) on the performance of broilers and reported that supplementation of both *Lactobacillus* and *Saccharomyces* invidually to the basal diet at 0.05% improved body weight gain in broilers. Katoch *et al*. (2000) reported that the 'Vencobb' broilers out of three strains of commercial broilers gave significantly higher gain in body weight when they were fed diet supplemented with combination of *Lactobacillus acidophilus, Streptococcus faecalis* and *Saccharomyces carlsbergensis* isolated from leopard excreta and combination of their respective standard counterpart i.e. *L. bulgaricus* (L4), *S. lactis* (S1) and *Saccharomycs cerevisiae* (Y3) up to six weeks of age. Shome *et al*. (2000) reported that when

mixture of Lactobacillus acidophilus and *L. salivarius* was fed to broilers, the live weight of chicken was higher during starter phase in experimental groups compared to control. Balevi *et al.* (2001) found that supplementation of the diet with a commercial probiotic (Protexin™) at 500 g/tonne resulted in improved body weight gain. Safalaoh *et al.* (2001) shown that effective microorganisms (probiotics) had growth promoting and hypocholesteraemic effects as potential alternative to antibiotics in broiler diets. Bandy and Risam (2001) conducted an experiment to determine the efficiency of probiotic at three different levels- 25, 50 and 75 g/100kg feed respectively and observed that chicks fed with probiotics grew faster than control and highest live weight was obtained in the treatment fed probiotics at 75/100kg feed. Kumar *et al.* (2002) observed that the supplementation of EY Micromix at 30 g and 40 g per quintal of feed showed significantly higher gain in body weight at marketable age. Upendra and Yathiraj (2002) observed that supplementation of Lacto-sacc (a combination of, *Saccharomycs cerevisiae, Lactobacillus acidophilus and Streptococcus faecium*) at 250g/ton of feed resulted in numerical increase in body weight gain by 1.7 % as compared to control. Panda *et al*. (2003) reported that the inclusion of *L. sporogens* (100 mg/kg feed) resulted in an increased body weight in commercial broilers. Gupta (2003) supplemented different strains of Lactococcus isolated from excreta of Sambhar, Himalayan Black bear and Monal and their standard counterpart- *L. lactis* (CFTRI, Mysore) and Bacitracin. He observed that differences in body weight gain were significant ($P \leq 0.05$) in all treatment treatments as compared to unsupplemented control and also concluded that treatment s fed with Bacitracin and Lactococcus species isolated from Monal showed highest % increase in body weight gain *i.e*., 6.80 and 5.44 %over control. Arslan *et al.* (2004) reported that probiotics had no significant ($P \leq 0.05$) effect on growth in broilers. Huang *et al.* (2004) reported that inactivated probiotics, after disruption with a high pressure homogeniser, have beneficial effects on the productivity of broiler chicks when used at a certain concentration. They also found that body weight gain was improved with disrupted, cobalt-enriched lactic acid bacteria (*L. acidophilus* and *L. casei*) and fungal mycelium (*S. acidophilum*), when sprayed onto a mash basal diet. Kabir *et al.* (2004) reported the occurrence of a significantly ($P<0.01$) higher carcass yield in broiler chicks fed with the probiotics on the 2^{nd}, 4^{th} and 6^{th} week of age both in vaccinated and nonvaccinated birds. Anjum *et al*. (2005) reported that multi-strain probiotics (protexin) supplementation in the diet significantly ($P \leq 0.05$) improved body weight

gain in broilers. Das *et al.* (2005) reported no significant (P≤ 0.05) difference in dressed weight in broilers after supplementation of commercial probiotics preparation. O'Dea *et al*. (2006) examined probiotic mixtures (*Lactobacillus acidophilus, Lactobacillus bifidus*, and *Streptococcus faecalis*) using different regimes and concluded that weight gain improved significantly in broilers produced by 43 and 57 week old breeder flocks fed the supplemented diet. Accumulated evidence suggests that inactivated probiotics could have similar beneficial effects to those of live probiotics. The addition of probiotic (*L. acidofillus* and *S. faecium*) to broiler feed significantly improved the growth rate (Mohan *et al.,* 1996; Choudhary *et al*., 2008). The live yeast culture of *S. cerevisiae* along with *L. acidophillus* and *S. faecium* were supplemented in broiler feed (1 kg/ton) and the results showed an improved weight gain (Choudhary *et al.,* 2008). Eckert *et al*. (2010) also reported that Lactobacillus via drinking water can improve the body weight of broiler chickens within commercial environments. Luiz *et al.* (2012) examined the effects of probiotic (*Bacillus licheniformis, B. subtilis*), prebiotic (mannan oligosaccharide-MOS), synbiotic (*Saccharomyces cerevisiae, Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum*), MOS and fructoligosaccharides (FOS), Avilamycin on growth performance and meat qualities in broiler chickens. The results indicated that the biotic treatments caused significant differences in the parameters and these additives are nutritionally feasible to replace AGP for the improvement in the meat quality. Salim *et al.* (2013) investigated the effects of supplementation of 0.1% as direct-fed microbials (DFM) containing either *Lactobacillus reuteri* or a mixture of *Bacillus subtilis, B. licheniformis* and *Saccharomyces cerevisiae* as alternative to antibiotic growth promoter (AGP) (0.1% virginiamycin) on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. It has been shown that the DFM supplementation increases body weight gain of broilers. Sarker *et al*. (2017) concluded that probiotic fed Cobb broiler chickens had higher body weight, dressing percentage and higher European Broiler Index.Broiler chickens diet inoculated with Lacto feed which consists of 2.5×10^{10} CFU/kg of each Lactobacillus acidophilus, *Lactobacillus casei, Bifidobacterium thermophilum* and *Enterococous faecium* improved the body weight at day 42 of broiler (Zarei *et al*., 2018).

2.7.2 Effects on feed intake and feed conversion efficiency

Naik *et al*. (2000) valuated the effect of different probiotics *Lactobacillus acidophilus, Saccharomycs cerevisiae* and their combination) on the performance of broilers and reported that supplementation of Lactobacillus to the basal diet at 0.05% improved feed efficiency in broilers as compared to supplemented controls. Safalaoh *et al*. (2001) showed that effective microorganisms (probiotics) improved feed efficiency in broilers alone or with antibiotics, which is more pronounced at the higher dosage (30g/kg feed). Balevi *et al*. (2001) found that supplementation of the diet with a commercial probiotic (Protexin™) at 500 g/tone resulted in improved feed intake, body weight gain and FCR. Upendra and Yathiraj (2002) observed that supplementation of Lacto-sacc at 250g/ton of feed resulted in an improvement of FCR, which was 10.8% better over that of control. Gupta (2003) supplemented broiler diets with different strains of Lactococci and Bacitracin. He observed that all the diets showed lower ($P \leq 0.05$) FCR than control. Panda *et al*. (2003) reported that the inclusion of *L. sporogens* (100 mg/kg feed) resulted in an increased body weight and improved FCR in commercial broilers. Yoruk *et al.* (2004) reported that FCR improved linearly with increasing level (0.1% and 0.2%) of probiotics (*Lactobacilli spp. + Enterococcus faecium + Bifadobacterium bifidum + Aspergillus oryza*) in hens during the late laying period. Chitra *et al.* (2004) reported that inclusion of probiotics and ascorbic acid both independently and simultaneously either in feed or in drinking water to broilers had made significant improvement in total feed consumption and feed efficiency during summer season. Using two commercial probiotics, the first composed with *Bacillus subtilis* (150 g/ton feed) and the second with *Lactobacillus acidophilus* and *casei, Streptococcus lactis* and *faecium, Bifidobacterium bifidum* and *Aspergillus oryzae* (1 kg/ton feed) for broilers in the period of one to 14 days of age. Pelicano *et al*. (2004) observed an improvement in feed conversion up to 21 days of age in animals receiving probiotics, regardless of the composition, in relation to the group without any addition. Anjum *et al*. (2005) observed that there was significant ($P \leq 0.05$) improvement in feed conversion ratio after supplementation of multi-strain probiotics (protexin) in broilers, however, no improvement in feed intake was observed. The live yeast culture of *S. cerevisiae* along with *L. acidophillus* and *S. faecium* were supplemented in broiler feed (1 kg/ton) and the results showed an improved weight gain and FCR (Choudhary *et al*., 2008). Panda *et al*. (2008)

reported that dietary preparation of *L. sporogenes* at 100 mg (6×10^8 spore) per kg of diet significantly enhanced feed efficiency in White Leghorn breeders, which was ascribed to the beneficial effects of probiotic feeding on digestion and utilization of nutrients. In the same study, no positive effect of this probiotic was recorded on body weight gain and feed intake. Zhu *et al*. (2009) described that the degree of a probiotics effect depends upon species, bacterial strain, application method, bird's age, overall hygiene conditions on farm and environmental factors. Eckert *et al.* (2010) also reported that Lactobacillus via drinking water can improve the feed conversion of broiler chickens within commercial environments. Hassanein and Soliman (2010) found that supplementing with a live yeast culture of *Saccharomyces cerevisiae* at the level of 0.4% and 0.8% improved FCR in White Leghorn birds. Salim *et al*. (2013) investigated the effects of supplementation of 0.1% as direct-fed microbials (DFM) containing either *Lactobacillus reuteri* or a mixture of *Bacillus subtilis, B. licheniformis* and *Saccharomyces cerevisiae* as alternative to antibiotic growth promoter (AGP) (0.1% virginiamycin) on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. It has been shown that the DFM supplementation reduces feed intake and improves feed convertion ratio of broilers. Bai *et al.* (2013) investigated the effects of a probiotic product incorporating *Lactobacillus fermentum, B. subtilis, B. licheniformis* and *Saccharomyces cerevisiae* on the growth performance and intestinal immune status in broiler chickens. They compared the probiotic treated group with an antibiotic, and a probiotic plus antibiotic treated group. They reported improved the body weight gain and feed efficiency in broilers fed on diet containing probiotic. De Cesare *et al*. (2017) investigated the effects of the probiotic dietary supplementation of *Lactobacillus acidophilus* D2/CSL (CECT 4529) on productive performance, and found that this supplementation improved the FCR of the broiler. Broiler chickens diet inoculated with LactoFeed which consists of 2.5 × 1010 CFU/kg of each Lactobacillus acidophilus, *Lactobacillus casei, Bifidobacterium thermophilum* and *Enterococous faecium* improved the FCR at day 42 of broiler (Zarei *et al*., 2018).

2.7.3 Effects on the carcass quality and hematological traits

Kim *et al*. (2000) reported that there was significant lowered blood cholesterol levels in the broiler birds supplemented with probiotics. Bailey *et al.* (2000) proposed that

competitive exclusion cultures for broilers can be used to reduce contamination by Salmonella *enteritidis* in processed carcasses, reducing therefore the exposure of consumers to food-borne infections. Estrada *et al*. (2001) observed a tendency to reduce total aerobic bacteria, coliforms and clostridia in broilers receiving *Bifidobacterium bifidum*, and proven a reduction in the number of carcass condemnation by cellulites in animals supplemented. Safalaoh *et al.* (2001) shown that effective microorganisms (probiotics) had growth promoting and hypocholesteraemic effects as potential alternative to antibiotics in broiler diets. Pietras (2001) reported that protein content of chicken given probiotics is higher, while their crude fat and serum cholesterol is lower than control treatment. Supplementation of probiotics based on *Lactobacillus spp*. demonstrated similar results, with reduction in the total cholesterol and low density lipoprotein (LDL) cholesterol levels (Kalavathy *et al.*, 2003; Taherpour *et al.*, 2009) and triglycerides (Kalavathy *et al*. 2003) in blood serum of broilers. Chitra *et al*. (2004) also reported that supplementation of probiotics showed highly significant reduction of serum cholesterol level. Das *et al.* (2005) reported no significant ($P \leq 0.05$) difference in dressed weight and blood parameters in broilers after supplementation of commercial probiotics preparation. Cenesz *et al*. (2008) also reported that supplementation of probiotic in broiler birds significantly reduced total cholesterol serum level. Lilly *et al*. (2011) observed 86% reduction in contamination by *Salmonella* before slaughtering in broilers receiving probiotic with combination of *Lactobacillus acidophilus*, *Enterococcus faecium*, *Lactobacillus plantarum* and *Pediococcus acidilactici.* According to Matur & Eraslan (2012), hypocholesterolemic effect of probiotics depends on the species of the bacteria, and can occur by the assimilation of cholesterol from either endogen or hexogen origin in the intestinal tract, or de-conjugating bile acids by lactic acid bacteria (Gilliland *et al.*, 1990). Salim *et al*. (2013) investigated the effects of supplementation of 0.1% as direct-fed microbials (DFM) containing either *Lactobacillus reuteri* or a mixture of *Bacillus subtilis*, *B. licheniformis* and *Saccharomyces cerevisiae* as alternative to antibiotic growth promoter (AGP) (0.1% virginiamycin) on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. It has been shown that the DFM supplementation increases white blood cell, monocyte levels and plasma immunoglobulin of broilers. The probiotics affect the protein and fat contents of meat and thus the meat quality. Abdurrahman *et al.* (2016) reported that lipid

oxidation is one of the main causes of deterioration in feed quality. Probiotics have positive effects on poultry meat quality (Hassanein and Soliman, 2010, Popova, 2017). They improve pH, color, fatty acid profile, chemical composition, water retention capacity and oxidation stability (Popova, 2017).

2.7.4 Effects on reduction of coliform bacteria, stimulation of immune system and gut health

Panda *et al*. (2001) studied the effect of dietary supplementation of probiotics on growth and gut microflora of broilers and no significant ($P \leq 0.05$) effect on body weight gain was reported, however a significant ($P \leq 0.05$) decrease in *E.coli* count was reported. According to (Menten and Loddi, 2003), the bacterium genera present in probiotics that are directly related to the increase in immunity of poultry are *Lactobacillus* and *Bifidobacterium*, mainly when related to diseases affecting the gastrointestinal tract. Kabir *et al.* (2005) attempted to evaluate the effect of probiotics with regard to clearing bacterial infections and regulating intestinal flora by determining the total viable count (TVC) and total lactobacillus count (TLC) of the crop and cecum samples of probiotics and conventional fed groups at the 2nd, 4th and 6th week of age. Haghighi *et al*. (2006) shown that a commercial probiotic containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Streptococcus faecalis* stimulated the production of antitoxin α IgA from *C. perfringens* in the intestine of non-vaccinated chicks. Mountzouris *et al.* (2006) and Higgins *et al.* (2007) demonstrated that probiotic species belonging to *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces* have a potential effect on modulation of intestinal microflora and pathogen inhibition. Higgins *et al.* (2007) suggested that macrophages are directly or indirectly involved in the diminution of salmonella colonization caused by the administration of probiotics. Callaway *et al*. (2008) stated that the 'link between human salmonella and host animals is most clear in poultry' and that raw eggs and undercooked poultry are considered to be hazardous. Cox and Pavic, (2009) reported that increased numbers of *Lactobacillus* and *Bifadobacterium spp*. correlated with reduced *Salmonella spp*. He also reported that competitive exclusion through probiotics may provide the best tool to exclude *Salmonella spp*., however, under commercial conditions, degree of exclusion of *Salmonella spp*. has been highly variable as the efficacy of competitive

exclusion requires salmonella-free chicks, good biosecurity and low stress levels during the first few days of treatment, which may not be practical or possible. Kizerwetter-Swida and Binek (2009) demonstrated that *L. salivarius* 3d strain reduced the number of *Salmonella enteritidis* and *Clostridium perfringens* in the group of chickens treated with *Lactobacillus*. Mechanisms of action of probiotics include stimulation of endogenous enzymes, reduction of metabolic reactions that produce toxic substances, and production of vitamins or antimicrobial substances (Hassanein and Soliman, 2010). Santini *et al.* (2010) suggested that *Bifadobacterium longum* PCB 133, possesses high probiotic properties and marked anti-campylobacter activities both in vivo and in vitro, and is an excellent candidate as a feed additive for poultry for the reduction of food-borne campylobacteria in humans. Mountzouris *et al.* (2010), studying inclusion levels of a probiotic composed by *Lactobacillus reuteri, Bifidobacterium animalis, Pediococcus acidilactici* and *Lactobacillus salivarius,* found that the inclusion of 10^9 and 10^{10} CFU/kg feed provided benefit in modulation of the composition of cecal microflora. Particularly, they reduced the concentration of coliforms in the cecum (log CFU/g of wet digesta) at 14 and 42 days of age in broilers. Also, the authors have found an increase in the concentration of *Bifidobacterium* and *Lactobacillus* at 42 days of age. Results of Giannenas *et al.* (2012) suggest that treatment with probiotics may mitigate the impact of parasitic infection on chickens in the absence of anticoccidial infections. The use of probiotics exerted coccidiostatic effect against *Eimeria tenella*. This can help to minimize the risk and spread of coccidiosis and maintain intestinal health. Salim *et al.* (2013) investigated the effects of supplementation of 0.1% as direct-fed microbials (DFM) containing either *Lactobacillus reuteri* or a mixture of *Bacillus subtilis, B. licheniformis* and *Saccharomyces cerevisiae* as alternative to antibiotic growth promoter (AGP) (0.1% virginiamycin) on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. It has been shown that the DFM supplementation increases white blood cell, monocyte levels and plasma immunoglobulin of broilers. Bai *et al.* (2013) investigated the effects of a probiotic product incorporating *Lactobacillus fermentum, B. subtilis, B. licheniformis* and *Saccharomyces cerevisiae* on the growth performance and intestinal immune status in broiler chickens. They compared the probiotic treated group with an antibiotic, and a probiotic plus antibiotic treated group. They reported improved the body weight gain and feed efficiency in broilers fed on diet containing probiotic

Chicks fed on probiotic had high proportions of CD3+, CD4+, and CD8+ T-lymphocytes, whereas chicks fed on diet containing AGP had a low proportion of CD8+ T-lymphocytes in the foregut of broilers. These results indicated that the probiotic product incorporating *L. fermentum, B. subtilis, B. licheniformis* and *S. cerevisiae* could stimulate intestinal T cell immune system without decreasing growth performance in broilers. Probiotic bacteria produce molecules with antimicrobial activities such as bacteriocins which inhibits toxins' production and pathogens' adhesion (Pan and Yu, 2014). Probiotic feed supplementation improves growth, feed efficiency and intestinal health (Ghasemi *et al.*, 2014). This improvement is achieved by reducing intestinal pH, intestinal bacteria composition and digestive activity. Deraz *et al*. (2019) concluded that the total coliform and Salmonella counts were significantly reduced and/or totally eliminated in broiler groups supplemented with lactic acid bacteria via drinking water at 28 and 42 days of age in Hubbard commercial broiler chicks.

CHAPTER 3

MATERIALS AND METHODS

3.1 Statement of the experiment

The study was conducted under a probiotic development project from Ministry of Education entitled as "Development of multispecies/multistrains probiotic mixture from Bangladeshi local isolates and their validation for potential use in commercial poultry industry (Project ID; LS-1477)". The research activities were carried out at Laboratory of Medicine and Public Health and Central Poultry Farm of Sher-e-Bangla Agricultural University, Dhaka. The main objective of the research was to observe the potential effects of two locally isolated probiotics supplementation with drinking water as an alternative to antibiotic growth promoters on broiler production. A total of 320 day-old mixed sexed "Cobb 500" commercial broilers was reared for a period of 28 days from 6th march 2018 to 3th April, 2018 to assess the impact of locally isolated probiotics in broiler on growth performance, internal organ characteristics, hematological traits and cecal microbial population. The determination of various blood parameters of experimental broilers were determined to understand the lipid profile and immune status. Total cholesterol and CBC analysis were done at the ACI Diagnostic Center, Gulshan, Dhaka, Bangladesh.

3.2 Preparation of experimental house

A gable type open-sided house was used for the experiment. The experimental room was thoroughly brushed, swiped and properly washed by tap water. Then bleaching powder at the rate of 1kg/400 square feet was spread over the floor and ceiling walls and kept for 24 hours. Bleaching powder was cleaned by using forced tap water. The room was disinfected by 1% TH4+ solution (0.1 litter diluted solution per square feet), manufactured by Sogeval, France, Marketed by-Century Agro Ltd, Bangladesh. Feeders, drinkers, buckets and all other necessary equipment were also properly washed and disinfected by 0.5% TH4+ solution.

After proper drying the house was divided into 16 pens of equal size using wood material and wire net. The height of wire net was 36 cm. A group of 20 birds were randomly

allocated to each pen (replication) of four treatments. The stocking density was $1m^2$/10 birds.

3.3 Collection of experimental birds

A total of 320 day-old mix sexed "Cobb 500" broiler chicks were collected from a renowned hatchery, Gazipur, Dhaka.

3.4 Experimental materials

The collected chicks were carried to the university poultry farm early in the morning. Then they were kept in electric brooders equally for 7 days by maintaining standard brooding protocol. During brooding only basal diet was given. From 7^{th} day the experimental broiler chicks were equally and randomly divided and distributed into 4 dietary treatment groups. Each group was replicated into 4 sub-groups. Each dietary group consists of 80 chicks distributed into 4 replicated pens having 20 chicks in each replication. Two probiotic and one antibiotic were used in the experiment as treatment from 14^{th} to 28^{th} days of age.

3.5 Preparation of probiotic as broiler feed additive

Previously isolated glycerol stock of *Lactobacillus* and *Bifidobacterium* strains were used to prepare mixed probiotic I and probiotic II. These Lactobacillus and *Bifidobacterium* strains were collected from indigenous native chicken under the project "Development of multispecies/multistrains probiotic mixture from Bangladeshi local isolates and their validation for potential use in commercial poultry industry". The isolated strains were stored at -20^oC which were further tested for their viability as probiotic.

3.5.1 Viability test of stock culture

3.5.1.1 Growth on selective media

The selective isolates were inoculated on MRS (Hi Media, India) agar plate by ensuring the criteria of P^H 6.5, incubated anaerobically for 48 hours at 37°C and bifidoselective media, BSC propionate agar base (Hi media, India) was used.

3.5.1.2 Cultural characteristics

Colony morphologies (color, shape and size) were examined in physically on the selective media for each species. Microscopic observation was performed to separate one colony to another. Cell morphology and colony characteristics on selective agar were tested by gram staining.

At first single colony was taken aseptically to smear on to a clean dry slide and heat-fixed. The smear was flooded with crystal violet solution for 30 sec and rinsed with tap water for 5 sec. Then grams iodine solution was used to cover over the slide for 1minute and rinsed with tap water for 5 sec. The slide was then decolorized with 95% ethanol for 15 to 30 sec and rinsed with 5 sec. Finally saffranin was used as counter stains for 60-80 sec and rinsed with water, and then scrutinizes the isolates under light microscope. Gram-positive, catalase-negative, non-spore-forming and rod-shaped isolates were examined for *Lactobacillus* strains confirmation.

Colonies formed on BSA agar plates are convex, creamy or white, glossy, smooth, sticky and soft, short and thin with pointed and irregular ends, long cells with many branches, single cells, chains, V-shaped were tested by gram staining for checking nonmotile, gram-positive, nonsporulating *Bifidobacterium* strains. The selected isolates with their viability results are presented in **plate 1**.

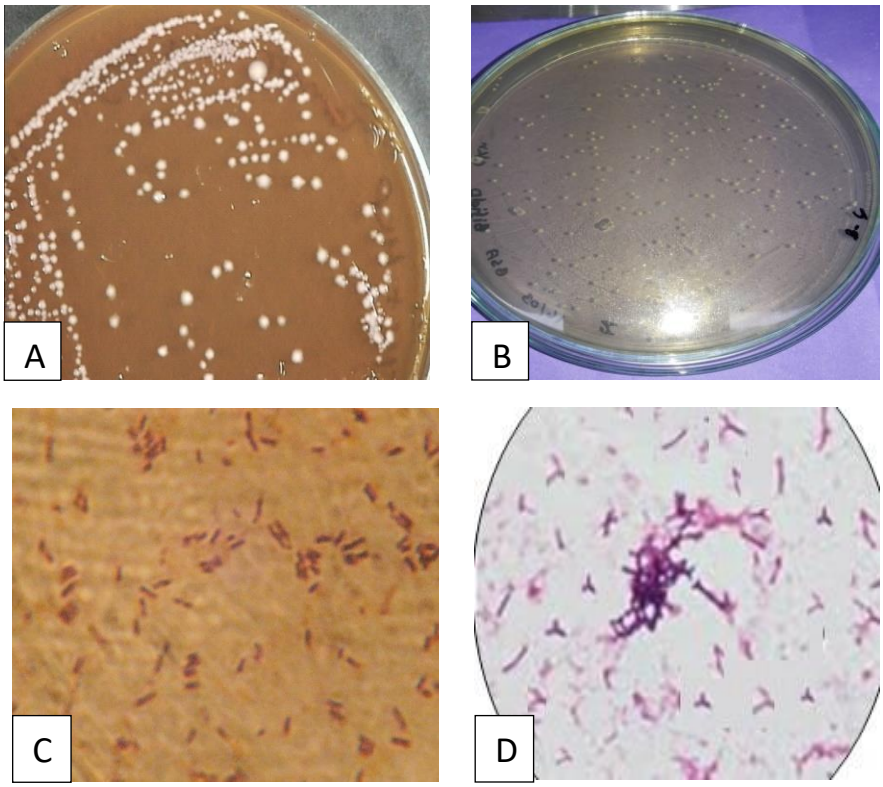

Plate 1. Viability test of bacterial isolates. (A) *Lactobacillus* growth in MRS. (B) *Bifidobacteria* growth in BSA. (C) Microscopic view (100X) of *Lactobacillus* after gram staining. (D) Microscopic view (100X) of *Bifidobacteria* after gram staining.

3.5.1.3 Biochemical test

Slide method is used to perform catalase test. In this method a clean glass slide was divided into two sections with lubricant pencil, one should be labeled as test and the other as control. A small drop of normal saline on each area was placed with a sterilized and cooled inoculating loop; a small amount of the culture from the petri plate was picked up. One or two colonies were emulsified on each drop to make a level suspension. One drop of hydrogen peroxide was given over the test smear and the other drop on control part. The fluid over the smears was observed for the appearance of gas bubbles. In this, *Lactobacillus* isolates gave catalase negative and *Bifidobacterium* isolates gave catalase negative results.

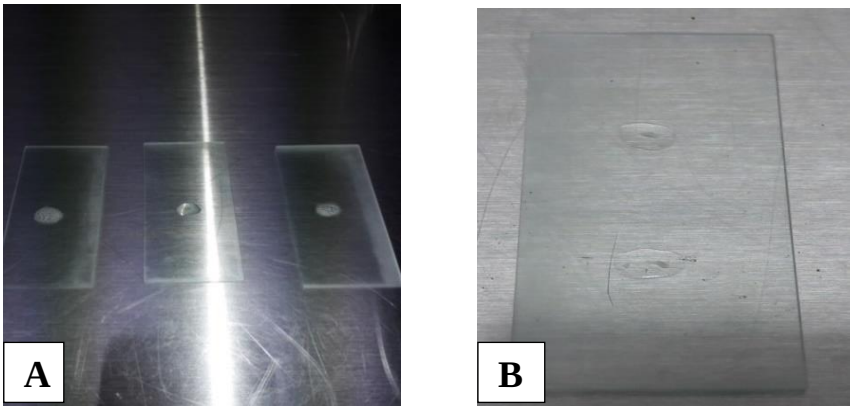

Plate 2. Biochemical test for selected bacterial strains. (A) Catalase positive test (B) Catalase negative test

3.5.2 Preparation of probiotic mixture

Gram positive, catalase negative, rod shaped *Lactobacillus* bacterium and convex, creamy or white, glossy, short, thin with pointed and irregular ends, long cells with many branches, V-shaped, gram-positive, nonsporulating, catalase negative *Bifidobacterium* were used to prepare two probiotic mixture (probiotic I & Probiotic II). Experimental organisms were inoculated in MRS broth (in 15 ml screw cap tube) for 48 hours at 37^{o}C and the turbidity were checked. The tubes were centrifuged at 5000 rpm for 5 minutes and the supernatants were discarded. The cells were harvested from 15 mL MRS broth and washed thrice with 1ml Phosphate Buffered Solution (PBS) in the tube. Then 1 ml PBS was added to the pellet in 1.5 ml ependroff tube and dissolve. The tubes were centrifuged at 10000 rpm for 5 minutes and the supernatant was discarded. An aliquot of 300 µl of 30% glycerol was added to the tube. All tubes are stored at -80^{0}C. All strains were screened for useful properties to produce a liquid probiotic supplement. The selection of strains was then checked for growth and stability, as assessed by viable cell count after 1 week of refrigerated storage, in a liquid fermentation medium. The isolated mixed culture of Lactobacillus strains are used as probiotic I. Similarly, isolated mixed culture of *Bifidobacterium* strains are used as probiotic II

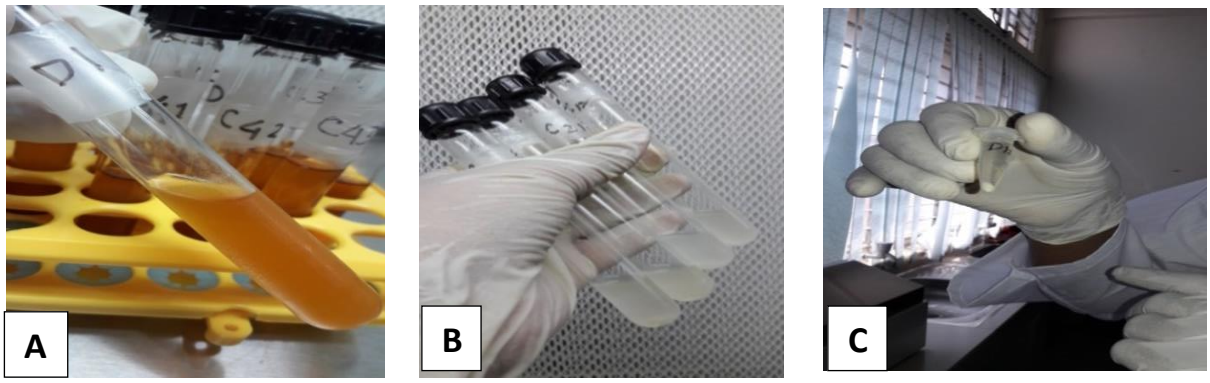

Plate 3. Growth and harvesting of bacterial isolates (A) Bacterial isolates growth in MRS broth. (B) and (C) Harvesting of bacterial isolates.

3.6 Layout of the experiment

The layout of the experiment is shown in Table 3.1

Table 1. Layout of the Experiment

T_4R_1 (n=20)	T_3R_3 (n=20)	T_1R_2 (n=20)	T_1R_4 (n=20)
T_2R_2 (n=20)	T_1R_1 (n=20)	T_3R_4 (n=20)	T_2R_1 (n=20)
T_3R_1 (n=20)	T_2R_3 (n=20)	T_2R_4 (n=20)	T_4R_2 (n=20)
T_4R_2 (n=20)	T_1R_3 (n=20)	T_3R_2 (n=20)	T_4R_3 (n=20)
Grand Total = 320			

T_1: Negative Control (NC); Basal Diet (BD)

T_2: Positive Control (PC); Basal Diet (BD) +Antibiotic

T_3: Experimental Diet (ED) I; Basal Diet (BD) + Probiotic I (*Lactobacillus spp.*)

T_4: Experimental Diet (ED) II; Basal Diet (BD) + Probiotic II (*Bifidobacterium spp)*

R_1: Replication 1

R_2: Replication 2

R_3: Replication 3

R_4: Replication 4

The Experimental design

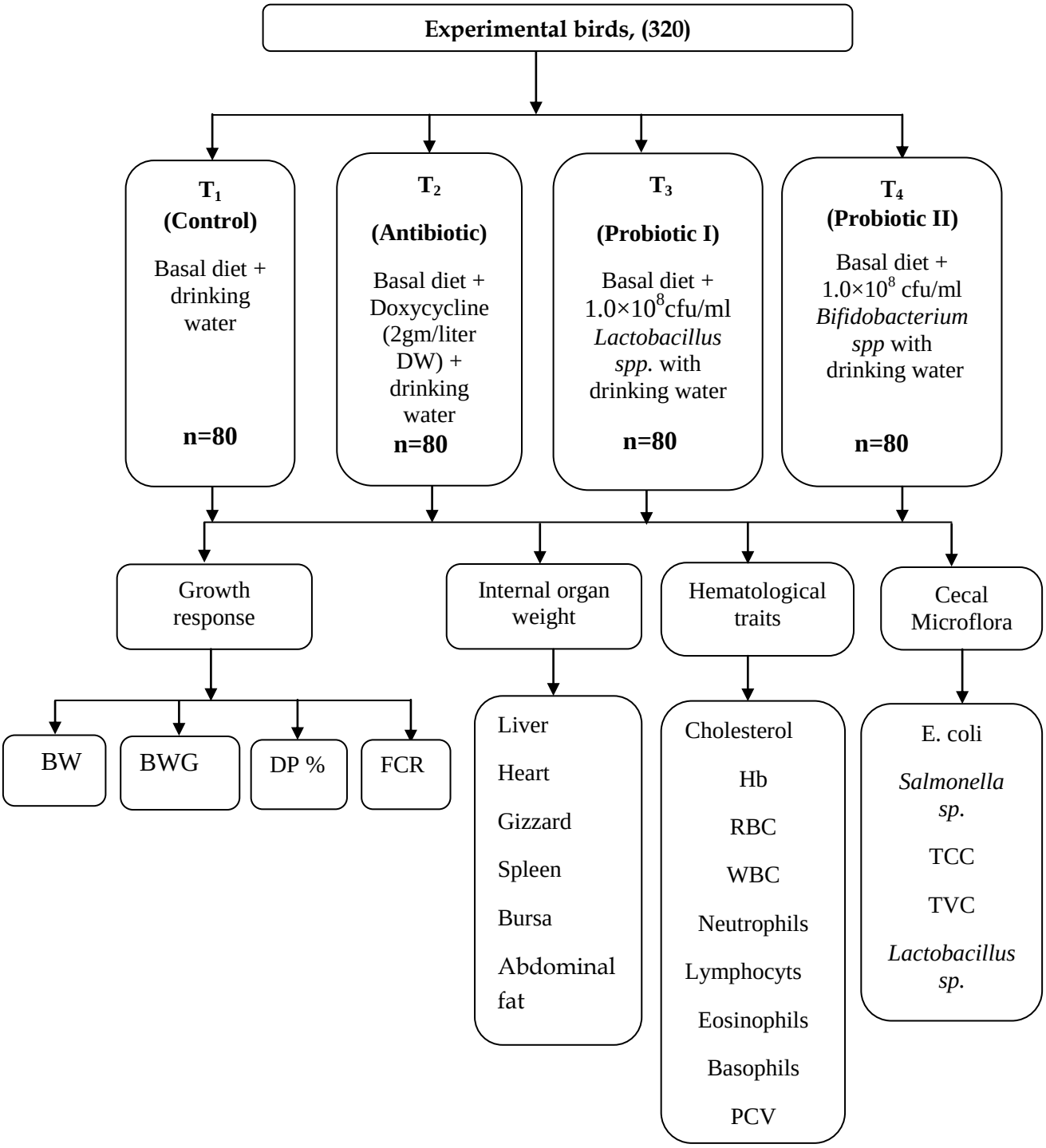

Figure 1. The experimental design

3.7 Treatments of the experiment

A total of 320 one-day old Cobb-500 chicks were assigned randomly to four treatment groups. Four replicates of 20 chicks were considered for each treatment. The dietary treatments were :T_1=basal diet as a control; T_2 = basal diet + antibiotic (doxycycline 2gm/liter DW); T_3= basal diet + probiotic I (minimum 1.0×10^8 cfu/ml *Lactobacillus spp*) in drinking water; T_4 = basal diet+ probiotic II (minimum 1.0×10^8 cfu/ml *Bifidobacterium spp*) in drinking water. Once prepared the probiotic mix was used within one week. Working solution of probiotic mix was prepared from the stock solution following the standard protocol so that 1.0×10^8 cfu/ml concentration is achieved. Each probiotic solution was further mixed with a total of 250 ml 5% dextrose solution. An aliquot of 60 ml of the probiotic solution was added to the drinking water of each replicate once in the morning at 6 AM daily from 14 to 28 days of age.

Table 2. Name and percentage of nutrients present in the commercial starter ration (1-14 days of age).

Name of the nutrients in broiler Starter ration	Minimum percentage(%) present
ME	3000 kcal/kg
Crude protein	21
Fat	5.0
Fiber	5.0
Ash	8.0
Lysine	1.20
Metionine	0.50
Cystine	0.40
Tryptophan	0.19
Therionine	0.79
Arginine	1.26

Table 3. Name and percentage of nutrients present in the commercial grower ration (15-28 days of age).

Name of the nutrients in broiler Grower ration	Minimum percentage (%) present
ME	3100 kcal/kg
Crude protein	19.0
Fat	6.0
Fiber	5.0
Ash	8.0
Lysine	1.10
Metionine	0.47
Cystine	0.39
Tryptophan	0.18
Therionine	0.75
Arginine	1.18

Feed was supplied 3 times daily by following Cobb 500 manual and adlibitum drinking water 2 times daily. (**Appendix 1. and Appendix 2.).**

3.8 Management of experimental birds

The following management procedures were followed during the entire experimental period and these management practices were identical for all the treatment groups.

3.8.1. Feed and water management

For the first 2 days, feeds were given on newspaper and then on small feeder. One round feeder and one round drinker with a capacity of 5-liter water were provided in each pen, so that the birds can accustomed with feeders and drinkers. The feeder and drinker were placed in such a way that the broilers were able to eat and drink conveniently. Feeders were cleaned in every week and drinkers were cleaned twice daily, once in morning and again at afternoon prior to water supply. Starter diet was provided for the first 0 to 14 days and grower diet was provided from 15 to 28 days of age. In all cases, ad libitum feeds were offered to the broilers. Feed was supplied three times daily, once in the morning, afternoon and again at night in such a way that feeders were not kept empty.

Fresh and clean water was made available at all times. All groups of broiler birds were supplied vitamin B-Complex, Vitamin -ADEK, Vitamin C, Ca and Vitamin D enriched medicine and electrolytes**.**

3.8.2 Litter management

Fresh and dried rice husk was used as litter material and spread over the floor at a depth of about 6 cm. Every day the litter was stirred to remove harmful gases, make the liter dry and prevent cake formation. At the 3rd weeks of age upper part of the litter with droppings was removed and replaced with new litter.

3.8.3 Brooding of baby chicks.

The first experiment was conducted during March to April in 2018. During this time environmental temperature was lower than the brooding temperature. So, additional heat was provided to chicks. The chicks were provided with a temperature of 35°C at first week of age, decreasing gradually at the rate of 3°C per week up to 4 weeks of age. The temperature was adjusted to keep the bulb up at down depending on situation. Common brooding was done for one week. After one week the chicks were distributed in the pen randomly. There were 20 chicks in each pen and pen space was 2 m^2. The average temperature was 29^oC and RH was 63% in the poultry house. Due to hot climate brooding temperature was maintained as per requirement. At day time only one electric bulb was used to stimulate the chicks to eat and drink. Electric fans were to as per necessity to save the birds from the heat stress.

3.8.4 Room temperature and relative humidity

Daily room temperature and relative humidity were recorded with a thermometer and wet and dry bulb thermometer respectively. Room temperature and humidity also measured by automatic thermo hygrometer. Polythene was used on two sides of the house and in ventilators to protect cold and stormy wind. These were removed partly or whole particularly at the later stage of finishing period when room temperature was found favorable. The temperature was recorded five times daily (6 A.M., 12 P.M.,4 P.M., 6 P.M.,12 P.M.) and relative humidity was recorded four times daily (6 A.M., 12 P.M.,4 P.M.,8P.M) using an automatic thermo-hygrometer during the whole experimental

period. The recorded housing temperature (˚C) and relative humidity (%) during the experimental period are shown in **Appendix Table 3 and Appendix Table 4**

3.8.5 Lighting

The birds were exposed to a continuous lighting period of 23 hours and 30 minutes and a dark period of 30 minutes in each 24 hours. The dark period provision was done to make broilers familiar with darkness to acclimatize with failure of electricity, if any.

3.8.6 Vaccination

The following vaccination schedule was followed during the experimental period.

3.8.7 Vaccination schedule

Table 4. Vaccination schedule

SL. No.	Age of birds	Name of Diseases	Trade Name	Doses	Methods of vaccination
1.	3rd	IB+ND	CEVAC® BIL	500	Eye drops
2.	9th	IBD (Gumboro)	Gumboro D78	1000	Drinking water
3.	17th	IBD (Gumboro)	Gumboro D78	1000	Drinking water

At 3rd day of age the birds were vaccinated by Infectious Bronchitis and Newcastle disease vaccine (CEVAC® BIL - contains the Massachusetts B48 strain of Infectious Bronchitis virus and the Hitchner B1 strain of Newcastle Disease virus in live, freeze dried form). The embryonated hen eggs used in the production of the vaccine are obtained from specified-pathogen-free flocks (SPF) produced in Hungary. The following procedure was followed for the administration of that particular vaccine.

One ampule of vaccine was diluted with 100 ml of distilled water. After that, the vaccine was put into a dropper and one drop of diluted vaccine was applied in intra ocular route (one drop vaccine in one eye/bird). At 9th days of age birds were again vaccinated against Gumboro (Infectious Bursal Disease) disease. The name of vaccine was **Gumboro D78, which** is a live freeze-dried vaccine containing live Infectious Bursal Disease (Gumboro) virus strain D78 with stabilisers. Each dose contains at least 4, 0 $\log^{10}$ $TCID_{50}$ of Infectious Bursal Disease (Gumboro) virus strain D78. The freeze-dried vaccine pellet

contains stabilizers and gentamycin. The following procedure was followed for the administration of that particular vaccine.

At first one ampule of vaccine was diluted with 100 ml of distilled water and further mixed with more water to supply the birds at 5.30 AM. At 19th day booster dose was applied.

3.8.8. Ventilation

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

3.8.9 Sanitation

Strict sanitary measures were taken during the experimental period. Disinfectant was used to disinfect the feeders and waters and the house also.

3.8.10 Bio-security

A strict bio-security program was maintained inside and outside of the research shed as a most effective part of disease prevention program. Entry to the experimental shed was highly restricted. A separate footwear and apron were used in the experimental shed to prevent contamination. Necessary fencing was done around the experimental shed and other additional cares were taken so that the birds could be kept free from rodents and wild birds, small reptiles or any other animals.

3.9 Study parameters

3.9.1 Recorded parameters

Data on weekly live weight, weekly feed consumption and birds mortality were recorded. On d 1, individual weights (g) of all chicks were measured. Afterward, weekly BW were measured and initial BW were subtracted to obtain final BWG. Feed consumption (g) was calculated by subtracting residual feed from the offered feed. Data of feed consumption and BWG were used to calculate FCR. FCR was calculated from final live weight and total feed consumption per bird in each replication. After slaughter the eight of cut parts such as gizzard, liver, spleen, and bursa were measured from each bird. Dressing yield was calculated for each replication to find out dressing percentage .Blood

sample was analyzed from each replication to measure Complete Blood Count (CBC) and total cholesterol level. Caecal sample was collected to measure microbial composition in the gut.

3.9.2 Data collection and record keeping

Following records and calculated data were kept throughout the experimental period.

3.9.2.1 Body /live weight

Birds were weighed at the first day of experiment (initial body weight) and weekly basis for all birds from each replication. Average body weight of the broiler in each replication was calculated by deducting initial body weight from the final body weight.

Body weight gain=Final body weight- Initial weight

3.9.2.2 Weekly Body weight gain

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

3.9.2.3 Feed intake

The amount of feed consumed by the birds in a particular replication of each treatment groups were calculated for every week by deducting the amount of left over feed from the amount supplied for that particular week. Feed intake was calculated as the total feed consumption in a replication divided by number of birds in each replication.

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

3.9.2.4 Feed conversion ratio (FCR)

Feed conversion ratio was calculated as the unit of feed consumed per unit of body weight gain.

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

3.9.2.5 Survivability (%)

Survivability per cent of the birds were calculated by dividing the number of birds alive by the total number of the birds and multiplying by 100.

$$\text{Survivability} = \frac{\text{No. of birds survival}}{\text{Total no. of birds considered}} \times 100$$

3.9.2.6 Temperature and humidity

Both temperature and humidity were recorded four times daily (6 A.M., 12 P.M., 4 P.M., and 8P.M) using an automatic thermo-hygrometer during the whole experimental period. The housing temperature (^{0}C) and relative humidity (%) during the experimental period are shown in **Appendix Table 3 and 4.**

3.9.2.7 Dressing yield

At the end of the experiment, three broilers were slaughtered from each replication per treatment to estimate the dressing yield.

Live weight – (blood + feathers + head + shank +all visceral organs)

3.9.2.8 Blood collection and separation of serum: Blood serum was collected at 28 days of ages

3.10 Dressing procedures of broiler chicken

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

3.11 Determination of hemato-biochemical parameters

Blood sample 1.5 ml /bird (3 bird /replication) were collected into EDTA tubes from the wing veins. Samples were transferred to the laboratory for analysis within 1 hour of

collection. For serum sample preparation, blood samples were collected during slaughtering from 12 birds/treatment group into dry clean centrifuge tubes. Then blood samples were centrifuged for 15 minutes at 3,500 rpm to obtain serum, and stored at −20°C for later analysis. Serum cholesterol and CBC were analyzed from ACI Diagnostic Center, Gulhsan, Dhaka by maintaining standard protocol.

Plate 4. Sample preparation for hemato-biochemical test. (A) Collection of blood. (B) Blood containing EDTA tube. (C) Centrifugation of serum sample (D) Serum samples for biochemical test.

3.12 Enumeration of Cecum microflora

At the end of experiment, three birds were randomly selected and sacrificed by decapitation. The cecal contents of each bird were transferred to sterile bags and stored -80°C for microbial enumeration. One milliliter of the homogenized suspension was then transferred into 9 mL of anaerobic broth and serially diluted from 10^{-1} to 10^{-6}, in phosphate buffer solution out of which 100 µL were plated on agar plates. In order to evaluate *Lactobacillus spp., Salmonella spp, E. coli,* total coliform count and total viable

count populations, the diluted samples were seeded on MRS agar (anaerobic conditions at 39°C for 48 h), SS, EMB, MacConkey agar and NA respectively, and incubated for 48 h at 37°C. Results were expressed as log10 colony forming units per gram of cecum digesta (log10 cfu/g) (Hashemi *et al.*, 2012).

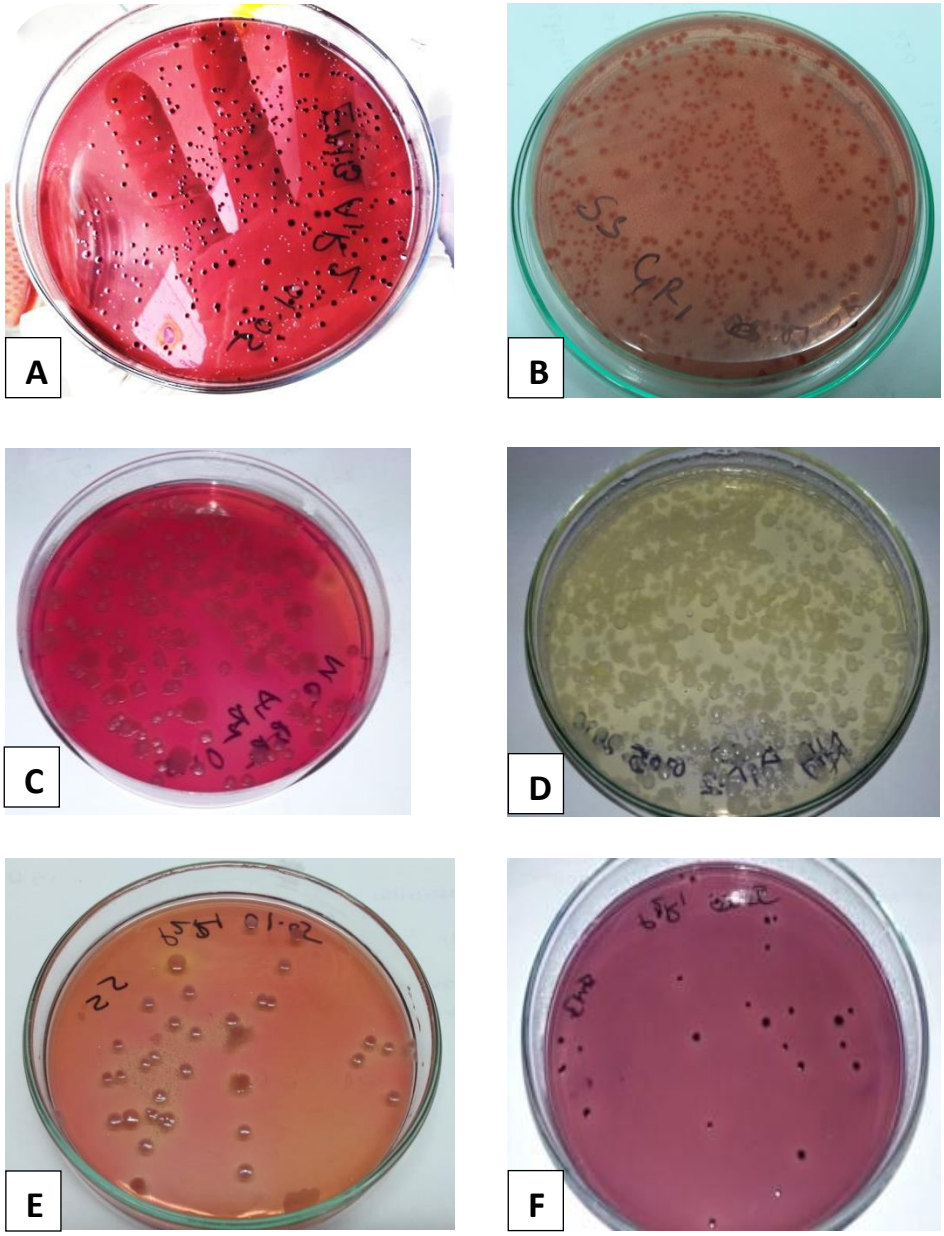

Plate 5. Enumeration of coliform bacteria in different treatments. (A) Inoculum from AB group in EMB (B) Inoculum from control group in SS plate. (C) Inoculum from AB group in MC (D) Inoculum from AB group in NA Plate (E) Inoculum from PB I group in SS. (F) Inoculum from PB II group in EMB.

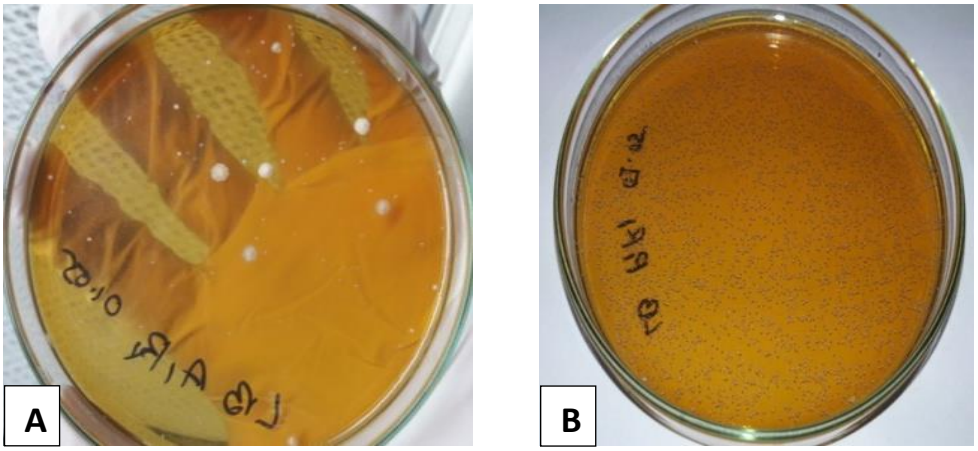

Plate 6. Enumeration of *Lactobacillus spp* in different treatments. (A) Inoculum from AB group in MRS. (B) Inoculum from PB I group in MRS.

3.13 Statistical analysis

All recorded and calculated data were statistically analyzed using analysis of variance (ANOVA) technique by computer using a SPSS (16.0 version) Statistical Computer Package Program in accordance with the principles of Completely Randomized Design (CRD). Differences between means were tested using Least Significant Difference (LSD) test and significance was set at $p < 0.05$.

CHAPTER 4
RESULTS AND DISCUSSION

This experiment was conducted to observe the effects of two probiotics and antibiotic on growth performance (BW, BWG, FC, DP, visceral organ weight, FCR, hemato-biochemical traits (total cholesterol, Hb, RBC, WBC, DLCs, PCV, MCH and MCHC) and cecum microflora composition in experimental broiler chickens. The results of feeding broilers on diet containing probiotics and antibiotic are presented in the following sub-headings:

4.1 Effects of probiotics and antibiotic on weekly body weight of broilers

The body weights of experimental birds fed with two probiotics and antibiotic are presented in table 5 and figure 2. The mean final body weight of T_1, T_2, T_3 and T_4 were 1509.25, 1530.75, 1572.50, 1548.75 and 1540.31g/bird respectively. The highest body weight was resulted in T_3 (1572.50 g/bird). This was followed by broiler belonging to T_4 (1548.75g/b), T_2 (1530.75g/b) and T_1 (1509.25 g/b). In respect to initial, 1st, 2nd and 3rd week body weight, there were no significant differences ($P>0.05$) among the treatment groups. Results showed that there were significant ($P<0.05$) differences were found in the end of 4th week (28 days) in weekly body weight among the different treatment groups during 28 days of rearing but significantly similar results were found in T_2 and T_4 groups. Melluzi *et al*. (1986) studied the effect of lactic acid bacteria and Bifidobacteria in broiler chicks and observed that birds fed with 2% of lactic acid bacteria culture gave significantly ($P\leq 0.05$) higher body weight than that of control given reconstituted sterile milk.

A number of studies had also shown similar agreements with the improvements in body weight of broiler chickens diets supplemented with a mixture of Lactobacillus strains (Kalavathy *et al.*, 2006; Timmerman *et al*., 2006; Kalavathy *et al*., 2008; Kalavathy *et al*., 2009; Mansoub, 2010; Saminathan *et al*., 2014; Shokryazdan *et al*., 2017; Zarei *et al*., 2018) or with preparations of lactobacilli.

There were also some studies which reported no positive results in performance of broilers fed probiotic Lactobacillus supplemented feeds (Yeo *et al.*, 1997). The variations

in the results from different studies could be due to differences in the strains, sources, viability and concentrations of used bacteria, methods of administration, and conditions of chickens.

However, the results obtained in this study revealed that broilers receiving either mixed culture of Lactobacillus strains or Bifidobacterium strains had significantly higher or similar weight compared with control or antibiotic ($P<0.05$).

Table 5. Weekly body weight of broiler in different treatments (g/bird) groups

Treat. / Age	T_1 (Control)	T_2 (Antibiotic)	T_3 (Probiotic I)	T_4 (Probiotic II)	Mean ±SE	LSD (0.05)
Initial	42.00 ± 0.41	42.00 ± 0.41	41.50 ± 0.65	41.00 ± 0.71	41.02 ± 0.28	0.791^{NS}
1st wk	218.25 ± 3.49	224.75 ± 2.06	223.50 ± 2.72	225.50 ± 4.18	223.00 ± 1.62	4.544^{NS}
2nd wk	483.00 ± 4.72	485.50 ± 4.17	483.75 ± 5.65	490.75 ± 2.02	485.75 ± 2.09	6.153^{NS}
3rd wk	934.00 ± 11.71	941.75 ± 10.48	957.50 ± 9.32	960.12 ± 4.39	948.34 ± 5.05	13.287^{NS}
4th wk	1509.25^{b} ± 11.45	1530.75^{ab} ± 17.61	1572.50^{a} ±17.207	1553.75^{ab}±18.19	1541.56 ±9.56	23.129*

Where, T_1= Control (Basal Diet); T_2 = Basal Diet (BD) + Antibiotic (Doxycycline pow. 2gm /liter DW.); T_3= Basal Diet (BD) + Probiotic I (*Lactobacillus spp*); T_4= Basal Diet (BD) + Probiotic II (*Bifidobacterium spp)*. Values are mean ± S.E (n=16) 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
- ✓ NS= Non-significant
- ✓ S.E.= Standard Error
- ✓ LSD =Least Significant Difference
- ✓ * means significant at 5% level of significance ($P<0.05$)

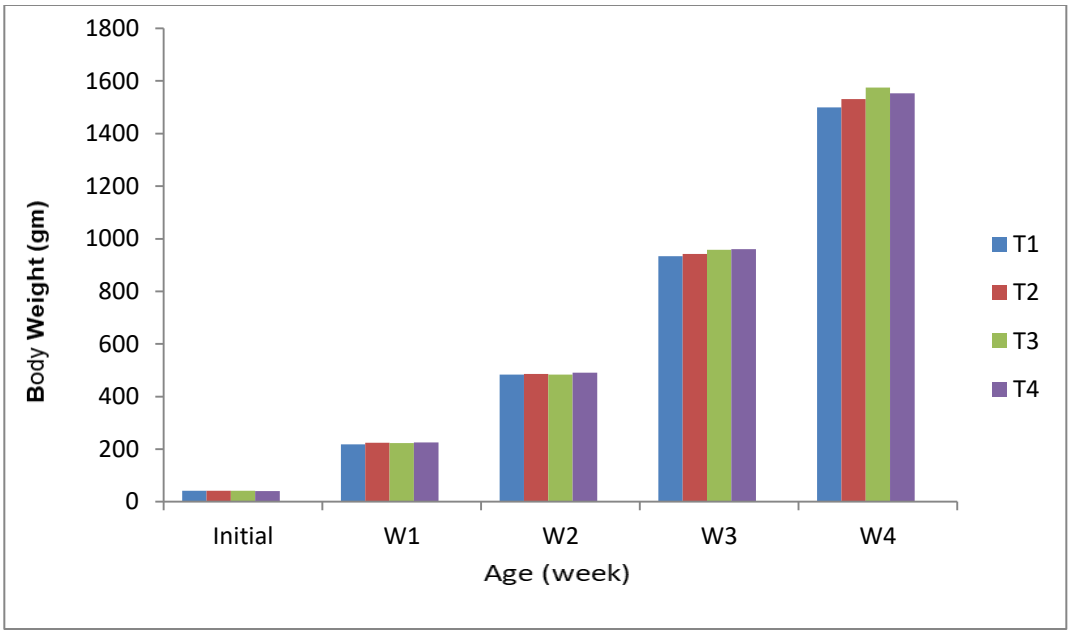

Figure 2: Effects of supplementation of probiotics and antibiotic on weekly body weight (g/bird) of broilers at different weeks.

4.2 Effects of probiotics and antibiotic on weekly body weight gain

Body weight gains of broiler in different dietary groups with the basal diet are presented in the table 6. All the birds were in very good health during the experimental period of 4 weeks. At the end of 4th week of age, the mean body weight gain of T_1, T_2, T_3 and T_4 were 575.25, 589.00, 615.00 and 593.50 g/bird respectively. The highest live weight gain was found in group T_3 (615.00g/b) broilers supplementation with *Lactobacillus* strains. This was followed by broiler belonging to group T_4 (593.50g/bird), T_2 (589.00g/b), T_1 (575.25g/b) respectively. In addition, the average weekly weight gain 3-4 week the birds supplemented with the probiotics showed higher body weight gains than the control group although the differences were statistically non-significant ($P>0.05$) during 0-4 weeks of age and it was in accordance with Mokhrati *et al*. (2010) who studied the efficiency of different growth promoters and reported no significant difference between treatments in body weight gain but all of them had a beneficial effect as compared to control. The present findings were in agreement with Awad *et al*. (2009) and Ashayerizadeh *et al*. (2011) who reported that addition of probiotic containing a mixture of *Lactobacillus* cultures and other bacteria to broilers did not show any significant effect

(P>0.05) on body weight gain compared with control. Also in accordance with Kabir *et al*. (2004), who obtained numerically higher body weight gain in broilers fed probiotic product.

Results of the present study differed from Song *et al*., (2014) who reported significant increase in body weight gain in broilers fed probiotic *Lactobacillus, Bifidobacterium* strains which were also supported by Samanta and Biswas (1995). They recorded a highly significant (P≤ 0.01) difference in weight gain between control and experimental group of broiler when probiotics (*Lactobacillus* spp) was in drinking water for a period of 0-6 weeks at starter and finisher phase.

Moreover, the birds supplemented with probiotic I (*Lactobacillus* strains) showed numerically best weight gain than the other probiotic II (*Bifidobacterium* strains), antibiotic as well as control group. However, non-significant improvement of body weight gain in birds supplemented with Lactobacillus strains or Bifidobacterium strains than that of control and AGP group.

Table 6.Weekly body weight gain of broiler in different treatments (g/bird)

Treat. / **Age**	T_1 **(Control)**	T_2 **(Antibiotic)**	T_3 **(Probiotic I)**	T_4 **(Probiotic II)**	**Mean ±SE**	**LSD (0.05)**
1st wk	176.25±3.3	182.75 ± 2.13	182.00± 2.27	184.50± 3.66	181.38± 1.53	4.126^{NS}
2nd wk	306.00±1.87	301.75±2.66	301.25±8.32	306.25±4.69	303.81±2.34	7.136^{NS}
3rd wk	451.00±10.96	456.25±8.59	473.75±5.33	469.50±3.30	462.62±4.18	10.79^{NS}
4th wk	575.25±8.15	589.00±12.17	615.00±23.01	593.50±14.64	593.13±7.84	21.892^{NS}

Where, T_1= Control (Basal Diet); T_2 = Basal Diet (BD) + Antibiotic (Doxycycline pow. 2gm /liter DW.); T_3= Basal Diet (BD) + Probiotic I (*Lactobacillus spp*); T_4= Basal Diet (BD) + Probiotic II (*Bifidobacterium spp)*. Values are mean ± S.E (n=16) 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
- ✓ NS= Non-significant
- ✓ S.E.= Standard Error
- ✓ LSD =Least Significant Difference
- ✓ * means significant at 5% level of significance (P<0.05)

4.3 Effects of probiotics and antibiotic on feed intake

The feed consumption of broilers at different ages in different treatment groups are shown in table 7. Results showed that there were no significant (P>0.05) effect on 1st, 2nd, 3rd, 4th week and total feed of broiler in different treatments groups. The mean total feed consumption of T_1, T_2, T_3 and T_4 were 2071.25, 2065.00, 2076.25 and 2075.12 g/bird respectively. In the current study, a tendency of higher feed consumption was observed in probiotics group than antibiotic group.

The result was in consistence with the previous report of Rada *et al*. (2013) and Shokryazdan *et al*. (2017). They reported that no significant difference was found in feed intake of broilers in the dietary treatments containing mixture of *Lactobacillus* strains throughout the experimental period. Similar results were also obtained by Jung *et al*. (2008) who found that addition of probiotic (*Bifidobacteria lactis*) did not have any significant effect on feed intake of broiler chickens. Hosseini *et al.* (2012) examined the effect of adding probiotics containing *Streptococcus* and *Bifidobacterium* on the performance of broilers. In terms of feed intake, carcass percentage no significant difference was observed between treatments.

The results are partially consistent with the other studies where it was shown that feed intake of chickens was not affected by supplementation of *Lactobacillus* or other bacteria (Kalavathy *et al*., 2003; Kalavathy *et al.*, 2006; Timmerman 2006; Nayebpor *et al*., 2007; Kalavathy *et al*., 2008; Ramasamy *et al.*, 2010 and Saminathan *et al*., 2014)

In contrast, others have found significant variation in feed intake between control and probiotic group (Zulkifli *et al*., 2000).

Several health benefits, resulting from improved digestion, have been claimed for both *Lactobacillus spp.* and *Bifidobacterium spp* reported by Olnood *et al*., (2015). Variation in the effects of probiotics on growth performance of broiler chickens might be attributed to differences in the strains of bacteria used as the dietary supplements.

Table 7.Weekly feed intake (g/bird) of broiler in different treatments

Treat. / Age	T_1 (Control)	T_2 (Antibiotic)	T_3 (Probiotic I)	T_4 (Probiotic II)	Mean ± SE	LSD (0.05)
1st week	158.00 ± 0.000	158.00 ± 0.000	158.00 ± 0.000	158.00 ± 0.000	158.00 [a] ± 0.000	0.00 [NS]
2nd week	370.25 ± 5.48	359.50 ± 5.57	365.50 ± 4.87	373.50 ± 9.90	367.19 ± 3.31	9.565 [NS]
3rd week	620.75 ± 5.45	622.75 ± 7.25	625.25 ± 7.49	626.50 ± 12.61	623.81 ± 3.89	12.195 [NS]
4th week	922.25 ± 3.42	924.75 ± 10.64	927.50 ± 12.69	930.00 ± 11.14	926.12 ± 4.59	14.319 [NS]
Total	2071.25 ± 11.52	2065.00 ± 14.45	2076.25 ± 20.57	2088.00 ± 31.15	2075.12 ± 9.57	29.454 [NS]

Where, T_1= Control (Basal Diet); T_2 = Basal Diet (BD) + Antibiotic (Doxycycline pow. 2gm /liter DW.); T_3= Basal Diet (BD) + Probiotic I (*Lactobacillus spp*); T_4= Basal Diet (BD) + Probiotic II (*Bifidobacterium spp)*. Values are mean ± S.E (n=16) 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
- ✓ NS= Non-significant
- ✓ S.E.= Standard Error
- ✓ LSD =Least Significant Difference
- ✓ * means significant at 5% level of significance (P<0.05)

4.4 Effects of probiotics and antibiotic on weekly feed conversion ratio (FCR)

Result from table 8 indicates that there were no significant variations in 1st, 2nd, 3rd and 4th week FCR among the different treatment groups (P<0.05). The mean FCR of broiler for the 4th week in T_1, T_2 T_3 and T_4 treatment groups were 1.56, 1.57, 1.50, and 1.57 respectively. However, T_3 (*Lactobacillus sp*) group showed numerically better with respect to FCR among different treatments groups.

Performance results in the present study corroborate findings reported by Dionizio *et al.* (2002) who observed no effects of the addition of different probiotic in broiler diets on feed conversion although numerically lower FCR was seen in birds fed probiotics compared to the birds fed the control diet without additives .

The results are in disagreed with Saminathan *et al.* (2014) and Shokryazdan *et al.* (2017) who recorded improvements in FCR of broiler chickens fed diets supplemented with a mixture of Lactobacillus strains or with preparations of lactobacilli.

The variations in the results from different studies could be due to differences in the strains, sources, viability and concentrations of used bacteria, methods of administration, and conditions of chickens.

Table 8: Weekly FCR of broiler in different treatments groups

Treat. / Age	T_1 (Control)	T_2 (Antibiotic)	T_3 (Probiotic I)	T_4 (Probiotic II)	Mean ± SE	LSD (0.05)
1st wk	1.11 ± 0.02	1.11 ± 0.05	1.14 ± 0.01	1.16 ± 0.02	1.13 ± 0.01	0.041 ^{NS}
2nd wk	1.21 ± 0.02	1.19 ± 0.02	1.21 ± 0.02	1.22 ± 0.48	1.21 ± 0.01	0.044 ^{NS}
3rd wk	1.38 ± 0.04	1.36 ± 0.02	1.32 ± 0.02	1.33 ± 0.02	1.35 ± 0.01	0.033 ^{NS}
4th wk	1.56 ± 0.02	1.57 ± 0.05	1.50 ± 0.05	1.57 ± 0.05	1.55 ± 0.02	0.059 ^{NS}

Where, T_1= Control (Basal Diet); T_2 = Basal Diet (BD) + Antibiotic (Doxycycline pow. 2gm /liter DW.); T_3= Basal Diet (BD) + Probiotic I (*Lactobacillus spp*); T_4= Basal Diet (BD) + Probiotic II (*Bifidobacterium spp)*. Values are mean ± S.E (n=16) 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
- ✓ NS= Non-significant
- ✓ S.E.= Standard Error
- ✓ LSD =Least Significant Difference
- ✓ * means significant at 5% level of significance (P<0.05)

4.5 Effects of probiotics and antibiotic on FCR, DP and survivability

4.5.1 Feed Conversion Ratio (FCR)

Differences in cumulative feed conversion ratio (FCR) of the broiler of different dietary groups differed significantly (P<0.05) showed in table 9. The mean FCR of broiler in T_1, T_2 T_3 and T_4 groups were 1.42, 1.38, 1.35, and 1.38 respectively. Significantly (P<0.05) improved FCR value was obtained for birds of group T_3 (1.35) followed by the group T_4 (1.38), T_2 (1.38) and T_1 (1.42) but similar significant result was found in T_4 and T_2 group. The present finding was in agreement with Talebi *et al*. (2008) who reported that addition of probiotic to broiler chicken diets decreased FCR significantly and also strongly supported by Shokryazdan *et al.* (2017), reported that from 22 to 42 and 1 to 42 d of age, broiler chickens fed 0.5 or 1 g kg-1 Lactobacillus culture had significantly (P < 0.01)

better FCR than control chickens. The results presented in Table 9 clearly exhibits an impression that the broiler receiving probiotic (*Lactobacillus sp*) the best converters of feed into live weight and the effect of probiotic was more prominent.

The results of the present experiment confirmed the probiotic potential of the selected strains, and were consistent with those obtained in other studies, showing that the presence of probiotic additives enhances the performance (FCR, BWG and BW) of broilers (Ashayerizadeh *et al.,* 2009; Babazadeh *et al*., 2011). Addition of probiotics to drinking water also functions to maintain the balance of the microflora ecosystem in the digestive tract and provides enzymes that can digest crude fiber, protein, fat and detoxify toxins or their metabolites (Gaggìa *et al*., 2010).

However the significantly improvement in FCR of birds fed diets containing the tested probiotic shows that the use of these products is a feasible alternative to antibiotics used as growth promoters (Pelícia *et al*., 2004).

4.5.2 Dressing Percentage (skinless)

Dressing percentage of broiler receiving two different probiotics and antibiotic are shown in table 9. The table shows that there were no significant differences among the treatment groups. The highest value was observed in T_3 (70.05%), where the remaining group T_1 group (68.38%), T_2 (68.04%) and T_4 (69.54%) didn't show any significant difference among themselves ($P>0.05$). Though dressing % was not different significantly but numerically higher number was observed in T_3 group than other treatments. The dressing percent of the carcasses were ranged from 77.81% to 80.18%. The present study findings were in fully agreement with Sarangi *et al. (*2016) who recorded that there was no significant difference observed in the dressing percentage, heart weight and gizzard weight in Cobb broilers under study.

Furthermore, Hosseini *et al.* (2012) examined the effect of adding probiotics containing *Streptococcus* and *Bifidobacterium* on the performance of broilers. In terms of feed intake, carcass percentage no significant difference was observed between treatments.

However, supplementation of *Lactobacillus spp* and *Bifidobacterium spp* with drinking water displayed a greater growth promoting effect although no significant effect on

dressing percentage in broiler and finally could be used as a suitable alternative to antibiotic growth promoters.

4.5.3 Survivability (%)

The survivability rate was showed in the table 9. No mortality was found up to end of the trial and no significant difference (P>0.05) was found among the treatment groups. However, the Survivability of all the treatment groups was 100%. With similar trials with broilers given *Lactobacillus* preparations, the effects on mortality were inconsistent (Zulkifli *et al.*, 2000). O'Dea *et al*. (2006) reported that there were no significant differences (P>0.05) in broiler mortality between the probiotic treatments and the control group in any of the trials. However, this could be due to the proper probiotic or direct fed microbial (DFM) supplementation promoting favorable condition in the intestine for the colonization of beneficial microflora, which in turn facilitated better growth performance of broiler chicks

Table 9. Production performances of broiler chicken in different treatments groups

Treat. / Criteria	T_1 (Control)	T_2 (Antibiotic)	T_3 (Probiotic I)	T_4 (Probiotic II)	Mean ± SE	LSD (0.05)
FCR	1.42^{a} ± 0.02	1.38^{ab} ± 0.02	1.35^{b} ± 0.01	1.38^{ab} ± 0.024	1.38 ± 0.01	0.026*
DP % (skinless)	68.38 ± 0.33	68.04 ± 0.97	70.05 ± 0.58	69.54 ± 0.54	69.00 ± 0.36	0.911^{NS}
Survivality (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	0.00^{NS}

Where, T_1= Control (Basal Diet); T_2 = Basal Diet (BD) + Antibiotic (Doxycycline pow. 2gm /liter DW.); T_3= Basal Diet (BD) + Probiotic I (*Lactobacillus spp*); T_4= Basal Diet (BD) + Probiotic II (*Bifidobacterium spp)*. Values are mean ± S.E (n=16) 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
- ✓ NS= Non-significant
- ✓ S.E.= Standard Error
- ✓ LSD =Least Significant Difference
- ✓ * means significant at 5% level of significance (P<0.05)

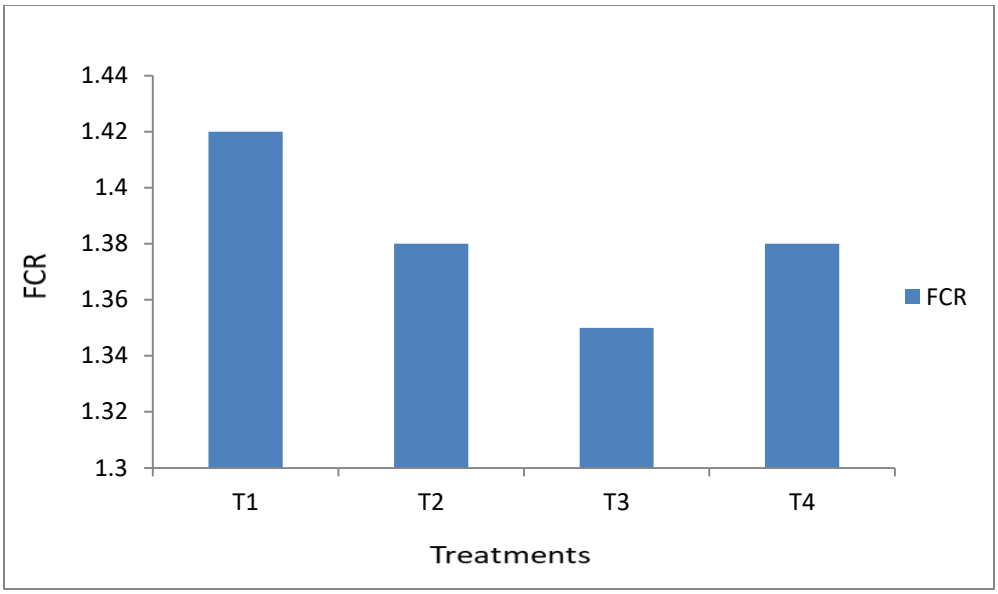

Figure 3. Effects of supplementation of probiotics and antibiotic on FCR of different treatment group to broiler chickens

4.6 Effects of probiotic and antibiotic on giblet weight and AFW weight of broilers

4.6.1 Effects of probiotic and antibiotic on liver weight

The mean relative weight of liver in the $T_{1,}$ T_2 T_3 and T_4 groups were 37.58, 37.33, 51.25 and 44.00 (g/bird) respectively. The highest result was obtained in T_3 group (51.25g/b) and lowest was in T_4 group (37.33g/b). Data in table 10 showed that, the relative weight of liver, significantly increased ($P<0.05$) in the treated group as compared to the control at finisher stage which was totally coincided with Hatab *et al*. (2016) stated that liver weight was significantly increased in the treated group containing different strains of lactic acid bacteria as compared to the control during overall experimental period.

In contrast, Olnood *et al*. (2015) reported that the relative weights of the liver were not affected by the probiotic *L. johnsonii* administrated by routes with drinking water.

4.6.2 Effects of probiotic and antibiotic on heart and gizzard weight

The mean relative weight of heart in the $T_{1,}$ T_2 T_3 and T_4 groups were 8.75, 8.75, 11.42 and 9.3(g/b) respectively. Again, the average gizzard weight in the different groups were T_1 (42.50g/b), T_2 (41.25g/b), T_3 (44.17 g/b) and T_4 (45.67g/b). The organ weight like heart, gizzard did not influenced ($P>0.05$) by the treatments compared with control and

antibiotic groups. But the organ weight likes heart, gizzards were numerically greater for the probiotic-supplemented birds compared with that for the antibiotic and control group birds. This results were in harmony with the results of Deraz *et al.* (2019) stated that supplementation of lactic acid bacteria with drinking water resulted in nonsignificant improvements in heart and gizzard weight compared to control group and also Sarangi *et al*. (2016) who recorded that there was no significant difference observed in the heart and gizzard weight in Cobb broilers .

But the results differed with Valentim *et al*. (2017) who reported that liver yield was not influenced (P>0.05) by the supplementation with probiotic on Peeled Neck chicken's ration at 90 days of age.

4.6.3 Effects of probiotic and antibiotic on AFW of broilers

Abdominal fat also did not influenced (P>0.05) by the treatments but in this study T_1 (29.25g/bird) group found a numerically higher abdominal fat weight compared with other treatments. Moreover T_3 (28.75**)** and T_4 (26.50*)* group showed numerically lower abdominal fat than the T_1 (29.25) group. This result is fully agreed with Hosseini *et al.* (2012) who examined the effect of adding probiotics containing Streptococcus and *Bifidobacterium* on the performance of broilers. In terms of abdominal fat weight, no significant difference (P > 0.05) was observed between treatments. Similarly, Haščík *et al*. (2016) did not find any effect of probiotics (*Lactobacillus fermentum*) on abdominal fat and carcass characteristics of broiler chicks. It may be happened that the different types of probiotic microorganisms, strains of broiler chicks, and conditions of trials seemed to be responsible for the above divergent results.

Table 10. Relative giblet and abdominal fat weight (g/bird) of broilers (d-28)

Treat. / Criteria	T_1 (Control)	T_2 (Antibiotic)	T_3 (Probiotic I)	T_4 (Probiotic II)	Mean ± SE	LSD (0.05)
Liver (g/Bird)	37.58^{b} ± 1.272	37.33^{b} ± 3.226	51.25^{a} ± 4.385	44.00^{ab} ± 4.021	42.54 ± 2.129	4.869*
Heart (g/b)	8.75 ± 0.896	8.75 ± 0.567	11.42 ± 1.663	9.33 ± 0.527	9.56 ± 0.537	1.444^{NS}
Gizzard filled (g/b)	42.50 ± 2.255	41.25 ± 0.896	44.17±3.357	45.67 ± 0.653	43.40 ± 1.032	2.965^{NS}
AFW (g/b)	29.25 ± 5.833	25.67 ± 1.368	28.75 ± 3.041	26.50 ± 2.882	27.54 ± 1.680	5.17^{NS}

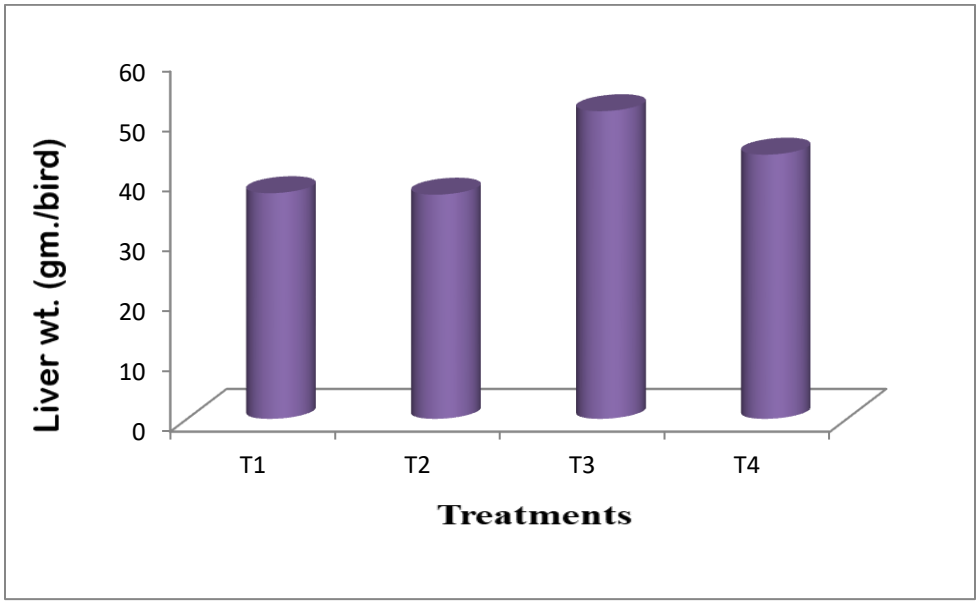

Figure 4. Effects of supplementation of probiotics and antibiotic on liver weight of broilers

4.7 Effects of supplementation of probiotic and antibiotic on immune organs

Effects of supplementation of two different probiotics and antibiotic on immune organs of Cobb 500 strain broiler chicken are summarized in the table 11. The comparative mean weight of spleen in the T_1, T_2, T_3 and T_4 were 2.83, 2.17, 3.83, 3.08 g/bird respectively. The highest weight was found in T_3 (3.83 g/bird) and lowest weight was found in T_2 (2.17 g/bird) group.

In case of bursa of fabricius, mean weight in the T_1, T_2, T_3 and T_4 groups were 2.50, 2.00, 3.92 and 4.25 g/bird respectively. Results were revealed that there was no significant difference ($P>0.05$) in spleen and bursa weight in the different treatment groups. Interestingly, the treated groups received the probiotic preparations individually had relatively higher immune organ weights compared to control and antibiotic group. These results were in harmony with Olnood *et al*. (2015) and Abdel-Hafeez *et al*. (2017) reported that the Lactobacillus strains probiotic feed additives did not influence ($P>0.05$) the spleen and bursa of fabricius.

These results inconsistent with the observations of Hatab *et al*. (2016) who reported that spleen relative weight were significantly increased in the probiotic-fed broilers as compared to the control.

Measurement of immune organs weight is a common method for evaluation of immune status in chickens (Heckert *et al*., 2002). Such related organs include bursa of Fabricius, liver and spleen. Good development of these organs is crucial for optimal immunoglobulin synthesis. Therefore, beneficial effects of mixed culture of Lactobacillus and *Bifidobacterium* strains supplementation in the gastrointestinal tract could result in an improvement of overall health, performance and immune response of broiler bird although the variations of result depends on the uses of different probiotic strains, broiler bird strains, management and climate condition etc.

Table 11. Immune organs weight (g/bird) of broiler chicken (d-28)

Treat. / **Criteria**	T_1 **(Control)**	T_2 **(Antibiotic)**	T_3 **(Probiotic I)**	T_4 **(Probiotic II)**	**Mean ± SE**	**LSD (0.05)**
Spleen weight (g/b)	2.83 ± 0.39	2.17 ± 0.29	3.83 ± 1.18	3.08 ± 0.48	2.98 ± 0.34	0.962^{NS}
Bursa weight (g/b)	2.50 ± 0.55	2.00 ± 0 .47	3.92 ± 1.25	4.25 ± 0.59	3.17 ± 0.43	1.102^{NS}

Where, T_1= Control (Basal Diet); T_2 = Basal Diet (BD) + Antibiotic (Doxycycline pow. 2gm /liter DW.); T_3= Basal Diet (BD) + Probiotic I (*Lactobacillus spp*); T_4= Basal Diet (BD) + Probiotic II (*Bifidobacterium spp)*. Values are mean ± S.E (n=16) 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
- ✓ NS= Non-significant
- ✓ S.E.= Standard Error
- ✓ LSD =Least Significant Difference
- ✓ * means significant at 5% level of significance (P<0.05)

4.8 Effects of probiotic and antibiotic on serum biochemical profile of broiler

The result of this experiment for total cholesterol level in different treatments is summarized in table 12. The level of mean total cholesterol of different treatments are T_1 (3.48 mmol/L), T2 (3.75 mmol/L), T3 (2.92 mmol/L) and T_1 (3.02 mmol/L) correspondingly. Although, the result was not significantly different (P>0.05), it was shown that the level of total cholesterol marked reduction in T_3 (Probiotic I) and T_4

(Probiotic II) groups than T_1 (control) and T_2 (antibiotic) group at 28 days of age. The present findings were fully agreement with Mokhrati *et al*. (2010) who studied the efficiency of different growth promoters and reported no significant effect for the additives addition on serum total cholesterol level but dietary supplemented treatments were numerically low. And partial agreement with Abdel-Hafeez *et al*. (2017) the values of the tested total cholesterol in the additive groups (prebiotic, probiotic, and symbiotic) were similar to that of the control, resulting in no statistical differences (P>0.05) among the treatments. While dissimilar result was found with Shokryazdan *et al*. (2017) reported that, serum total cholesterol, LDL-cholesterol and triglyceride concentrations were significantly (P < 0.05) reduced in broiler chickens fed 0.5 or 1 g kg-1 Lactobacillus culture when compared to control broilers at 21 and 42 d of age.

The variations in the results from different studies could be due to differences in the strains, sources, viability and concentrations of used bacteria, methods of administrations and trial period of chickens.

In general, the effective microorganisms such as Lactobacillus and Bifidobacterium strains mixed culture non-significant hypocholesteraemic effects could be a potential alternative to antibiotics in broiler diets (Safalaoh *et al*., 2001)

Table 12: Serum total cholesterol level of broiler in different dietary treatment group (d-28)

Treat. / Criteria	T_1 (Control)	T_2 (Antibiotic)	T_3 (Probiotic I)	T_4 (Probiotic II)	Mean ± SE	LSD (0.05)
Cholesterol (mmol/L)	3.48 ± 0.37	3.75 ± 0.29	2.92 ± 0.31	3.02 ± 0.383	3.29 ± 0.18	0.483^{NS}

Where, T_1= Control (Basal Diet); T_2 = Basal Diet (BD) + Antibiotic (Doxycycline pow. 2gm /liter DW.); T_3= Basal Diet (BD) + Probiotic I (*Lactobacillus spp*); T_4= Basal Diet (BD) + Probiotic II (*Bifidobacterium spp)*. Values are mean ± S.E (n=16) 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
- ✓ NS= Non-significant
- ✓ S.E.= Standard Error
- ✓ LSD =Least Significant Difference
- ✓ * means significant at 5% level of significance (P<0.05)

4.9 Effects of probiotics and antibiotic on hematological profile of broiler

The data on hematological profile of broilers are presented in table 13.

4.9.1 Effects of probiotics and antibiotic on Hb, RBC and WBC of broiler

In the present study, hemoglobin level was significantly increased in T_3 (11.66 g. /dL) followed by the T_4 (10.93 g/dL), T_2 (10.77 g/dL) and T_1 (10.34 g/dL) group. The results were in line with the findings of Beski and Sardary (2015) reported that probiotics resulted in a significant increase in the concentration of Hb. The higher Hb concentration in the chicks receiving probiotics may be due to the acidic media of the alimentary tract caused by probiotic fermentation which resulted in better iron salt absorption from the small intestine. This may also cause better vitamin B complex production by useful bacteria, which may result in positively affecting blood-forming processes. Absorption of the nutrient elements such as proteins, mineral elements and vitamins may elevate the concentration of hemoglobin. In contrast, Alkhalf *et al*. (2010) and Abdel-Hafeez *et al*. (2017) who found that no differences ($P>0.05$) in hemoglobin (Hb) concentration due the addition of probiotic to the diet of broiler.

In case of RBC count, tended to increase ($P>0.05$) in the probiotics supplemented groups (T_3 and T_4) than the control (T_1) and antibiotic (T_2) groups. This result is strongly supported by Thongsong and Chavananikul (2008) demonstrated that probiotics significantly increased red blood cell counts, mean hemoglobin concentration, concentration of broilers. This improvement in RBC count could be attributed to improved health status and physiological well-being of the birds administered with probiotic.

The results of WBC count (thousand/cumm) in the probiotic treated group T_4 (*Bifidobacterium spp*) were significantly ($P<0.05$) increased compared T_3 (*Lactobacillus spp)* and T_1 (control) group but statistically similar result was found in T_3 (*Lactobacillus spp*) and T_2 (control) group on day 28 of age. The present results were in correspondence with the findings of Zare *et al*. (2007) and Fathi (2013), who obtained significantly higher WBC counts in broilers fed probiotics than in those fed a control diet. The manipulation of intestinal microbiota via the utilization of probiotics influences the development of the immune response. In contrast, Strompfova *et al.* (2005) showed no significant differences

among the treatments in RBC, leucocyte count, hematocrit, hemoglobin concentration in Japanese quail fed *Lactobacillus fermentum*. However, it was shown that probiotics stimulate several subsets of immune system cells which in turn play an important role in the regulation of the immune response (Kabir *et al*., 2009).

4.9.2 Effects of probiotics and antibiotic on DLCs of broiler

No significant differences (P>0.05) were found on the DLCs such as neutrophils, lymphocytes, monocytes and eosinophils among the probiotics supplemented, antibiotic and control group at day 28 of age (table 12). However, there is a tendency to increase lymphocytes and monocytes count in the probiotic supplemented groups (T_3 and T_4) than the antibiotic (T_2) and control (T_1) group which indicates may had more antigenic effects than the remaining two since probiotics generally have been reported to stimulate the immunity of the chickens (Spellberg and Edwards, 2001; Toms and Prowrie, 2001). The present study findings were strongly supported by Cetin *et al.* (2005) reported that dietary supplementation of DFM significantly increased (P<0.05) the erythrocyte count, hemoglobin concentration, and hematocrit values in turkeys, but the total leucocyte and differential leucocyte counts were not affected by dietary DFM supplementation.

4.9.3 Effects of probiotics and antibiotic on PCV, MCH and MCHC of broiler

In the current study, the mean PCV count of T_1, T_2.T_3 and T_4 were 39.88 %, 32.36 %, 35.14% and 41.34% respectively. No significant effects (P>0.05) observed in PCV of different treatment groups were in line with Hanamanta *et al*. (2009) and Abdel-Hafeez *et al*. (2017) who found that the addition of probiotic to broiler diet had no significant (P>0.05) effects on PCV compared to control group. However this present findings are contrary to the finding of with the work of Islam *et al*. (2004) who reported that the PCV of different treatments of broiler chickens with probiotics were significantly different (P<0.01). In the overall, the PCV and the differential leukocytes count values of all the treatments were within the normal range for healthy chickens as outlined by Wakenell, (2010).

Similarly in case of MCV and MCHC no differences (P>0.05) were found in the in probiotic strains (T_3 and T_4) with the control (T_1) and antibiotic (T_2) group. But the birds fed the locally isolated probiotic strains had numerically the highest MCH and MCHC

compared to the other treatments, and there was no statistically significant difference between the two probiotics groups also (Siadati *et al.,* 2017).

Table 13: Hematological traits of broiler in different treatment groups (d- 28)

Treat. / Criteria	T_1 (Control)	T_2 (Antibiotic)	T_3 (Probiotic I)	T_4 (Probiotic II)	Mean ± SE	LSD (0.05)
Hb (g/dL)	10.34^{b} ± 0.45	10.77^{ab} ± 0.25	11.66^{a} ± 0.13	10.93^{ab} ± 0.26	10.93 ± 0.18	0.416*
RBC (million /cum)	4.00^{b} ± 0.13	4.04^{ab} ± 0.06	4.28^{a} ± 0.04	4.14^{ab} ± 0.04	4.11 ± 0.04	0.108*
WBC (thousand/cum)	6783.33^{b} ± 301.39	8083.33^{ab} ± 328.15	7925.00^{ab} ± 647.84	8708.33^{a} ± 703.74	7875.00 ± 296.44	746.148*
Neutrophils (%)	66.17 ± 3.22	65.83 ± 0.78	58.60 ± 3.38	62.42 ± 3.42	63.23 ± 1.53	4.130^{NS}
Lymphocytes (%)	29.08 ± 2.85	28.50 ± 0.78	35.42 ± 3.14	32.33 ± 3.26	31.33 ± 1.40	3.819^{NS}
Monocytes (%)	2.17 ± 0.22	2.83 ± 0.44	2.67 ± 0.14	2.50 ± 0.17	2.54 ± 0.14	0.379^{NS}
Eosinophil (%)	2.58 ± 0.44	2.83 ± 0.50	3.33 ± 0.24	2.75 ± 0.25	2.88 ± 0.18	0.529^{NS}
PCV (%)	39.88 ± 7.64	32.36 ± 0.89	35.14 ± 0.43	41.34 ± 8.61	37.18 ± 2.75	8.170^{NS}
MCH (FI)	30.02 ± 0.08	30.20 ± 0.14	30.10 ± 0.07	30.05 ± 0.08	30.09 ± 0.05	0.136^{NS}
MCHC (g/dL)	32.42 ± 0.33	32.25 ± 0.39	32.98 ± 0.19	32.95 ± 0.16	32.65 ± 0.15	0.401^{NS}

Where, T_1= Control (Basal Diet); T_2 = Basal Diet (BD) + Antibiotic (Doxycycline pow. 2gm /liter DW.); T_3= Basal Diet (BD) + Probiotic I (*Lactobacillus spp*); T_4= Basal Diet (BD) + Probiotic II (*Bifidobacterium spp)*; RBC = Red Blood Cell; WBC = White Blood Cell; DLCs = Differential Leukocytes Counts; PCV = Packed Cell Volume; MCH= Mean Corpuscular Hemoglobin. Values are mean ± S.E (n=16) 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
- ✓ NS= Non-significant
- ✓ S.E.= Standard Error
- ✓ LSD =Least Significant Difference
- ✓ * means significant at 5% level of significance (P<0.05)

4.10 Effects of probiotics and antibiotic on cecal microflora of broiler

As is shown in table 13, the probiotics supplemented groups in this study influenced ($P<0.05$) both *Lactobacillus* bacteria and coliform populations in the cecal samples. The salmonella, total coliforms and total viable count showed significantly ($P<0.05$) lower counts in the cecum of probiotic-treated groups (T_3 and T_4) than in the T_2 (antibiotic) group, with no differences ($P>0.05$) between T_3 and T_4 group whereas the T_1 (control) group showed the highest value (table 14).

The largest Lactobacillus bacterial population was observed in the cecal contents of T_3 birds (5.47 cfu/g) followed by the T_4 (4.76 cfu/g), T_1 (4.52 cfu/gm), T_2 (4.50 cfu/g). Interestingly, the lowest *E. coli* populations were observed in the probiotic-treated groups T_3 (4.49 cfu/g), T_4 (4.52 cfu/g) than T_1 (5.38 cfu/g) and T_2 (5.40 cfu/gm groups. The number of unwanted bacteria was lower and the number of lactobacilli was higher in probiotic treated groups. The results in accordance with Deraz *et al*. (2019) concluded that the total coliform and *Salmonella* counts were significantly reduced and/or totally eliminated in broiler groups supplemented with lactic acid bacteria via drinking water at 28 and 42 days of age in Hubbard commercial broiler chicks. Other studies have also shown that there was a higher percentage reduction in *Salmonella* colonization and suppress the growth potentially pathogenic bacteria in birds fed diets with antibiotic and probiotics (Alonge *et al.*, 2017).

The present study, showing a decrease in the *E. coli* intestinal population associated with a higher count of *Lactobacillus* spp. It supports the hypothesis that lactobacilli could compete with *E. coli* for intestinal colonization. The antagonistic abilities of probiotics towards several pathogenic bacteria, such as *E. coli*, *Salmonella* spp. have been well documented (Patterson and Burkholder, 2003). Zhang and Kim (2013) reported that dietary supplementation of the probiotic increased excreta *Lactobacillus* counts and decreased *Escherichia coli* counts compared with hens fed the diets without the probiotic.

Likewise, Estrada *et al*. (2001) observed a tendency to reduce total aerobic bacteria, coliforms and clostridia in broilers receiving *Bifidobacterium bifidum*, and proven a reduction in the number of carcass condemnation by cellulites in animals supplemented.

Higgins *et al.* (2007) also showed that probiotic microorganisms, such as *Lactobacillus* spp. and *Bifidobacterium* spp., played a role in the modulation of intestinal microflora and pathogen inhibition. Salim *et al*. (2013) stated that the dietary supplementation of DFM decreases the number of *E. coli* and improves the ileal morphology of broiler chickens. Jin *et al.* (1998) observed that *L. acidophilus* and a mixture of *Lactobacillus spp.* increased the concentration of volatile fatty acids in the ileum and cecum in broiler chickens and reduced the pH value, which may be responsible for a decline of intestinal coliforms.

The proper DFM supplementation may provide a favorable condition in the intestines for the colonization of beneficial microflora, which in turn facilitates better growth performance of broiler chicks (Mohnl, 2011).

By contrast to Mountzouris *et al*. (2007) it was found that DFM supplementation had no effect on *Salmonella* and *Lactobacillu*s contents of the cecal digesta. Also Jin *et al.* (1998) reported no significant increase of the *Lactobacillus* spp. count in chickens fed *L. acidophilus* or a mixture of *Lactobacillus* spp.

One of the prime objectives to use DFM in broiler diets is to increase the beneficial organism for the host and to reduce the pathogenic organism which causes infectious diseases (Higgins *et al*., 2010; Mountzouris *et al*., 2010). The observed improvement in BW, BWG and FCR in the T_3 and T_4 groups may be attributed to the total effects of probiotic bacterial actions, including improved intestinal absorption, resistance to disease, and promotion of host-beneficial bacteria. The use of probiotic spray for newly hatched chicks through administration in the first drinking water is a very efficient method for controlling the colonization of Salmonella in poultry intestine. This shows that chicks treated with probiotics can serve as a useful means for reducing *Salmonella* contamination.

As a consequence, there is an improvement in the intestinal environment, increasing the efficiency of digestion and nutrient absorption processes (Pelicano *et al*., 2004), which may explain the improvement in feed conversion ratio observed in the present study. Therefore, the ability of *Lactobacillus spp*. and *Bifidobacterium spp* bacteria to increase

the immune system is a viable reason for supporting their use as an alternative to antibiotics for improving animal health and productivity.

Table 14: Viable cell counts in the cecal contents of broilers (d 28; log_{10} cfu/g) in different treatment groups

Treat. / Criteria	T_1 (Control)	T_2 (Antibiotic)	T_3 (Probiotic I)	T_4 (Probiotic II)	Mean ± SE	LSD(0.05)
E. coli (cfu/gm)	5.38^{a} ± 0.014	5.40^{a} ±0.025	4.49^{c}±0.004	4.55^{b} ±0.027	4.96±0.113	0.028*
***Salmonella spp.* (cfu/gm)**	5.33^{a} ±0.007	5.20^{b} ±0.021	4.58^{c} ±0.022	4.51^{c} ±0.049	4.91±0.095	0.041*
TCC (cfu/gm)	5.28^{a} ±0.018	5.20^{b} ±0.025	4.49^{c} ±0.007	4.48^{c} ±0.005	4.86±0.098	0.022*
TVC (cfu/gm)	5.46^{a} ±0.004	5.44^{a} ±0.010	4.89^{b} ±0.020	4.92^{b} ±0.027	5.18±0.070	0.025*
***Lactobacillus spp.* (cfu/gm)**	4.52^{c} ±0.030	4.50^{c} ±0.024	5.47^{a} ±0.005	4.76^{b} ±0.040	4.81±0.102	0.039*

Where, T_1= Control (Basal Diet); T_2 = Basal Diet (BD) + Antibiotic (Doxycycline pow. 2gm /liter DW.); T_3= Basal Diet (BD) + Probiotic I (*Lactobacillus spp*); T_4= Basal Diet (BD) + Probiotic II (*Bifidobacterium spp)*; TCC = Total Coliform Count; TVC = Total Viable Count. Values are mean ± S.E (n=16) 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
- ✓ S.E.= Standard Error
- ✓ LSD =Least Significant Difference
- ✓ * means significant at 5% level of significance (P<0.05)

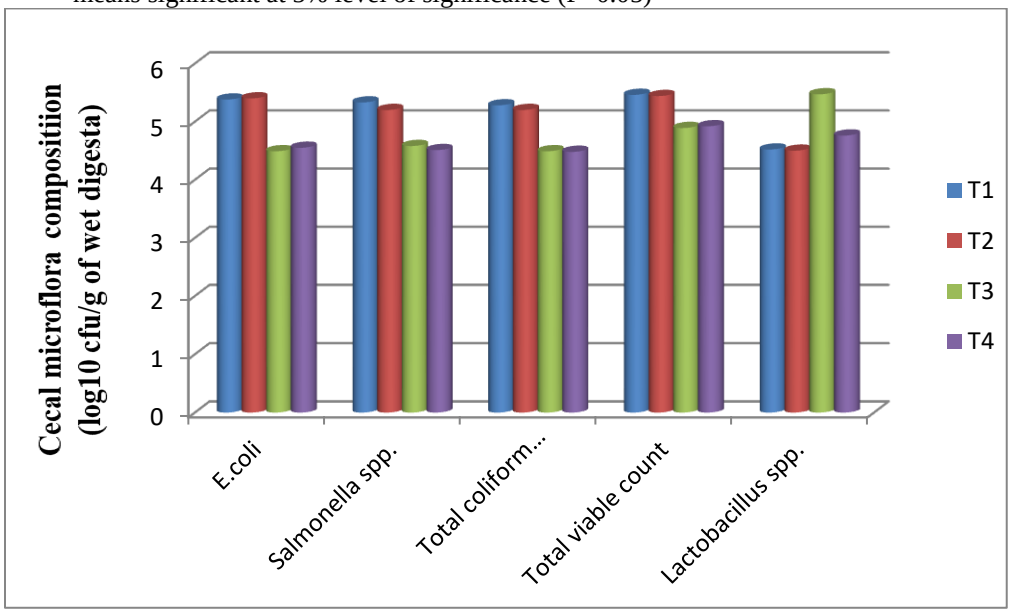

Figure 5. Effect of supplementation of probiotics and antibiotic on viable cell counts in the cecal contents of broiler

CHAPTER 5

SUMMARY AND CONCLUSION

The ban of using antibiotic growth promoters in many countries in order to satisfy the consumers demands for healthy and safe meat leads increasing researchers interest in finding strategies to maintain chicken health and production. Probiotics which are live microbial compounds are considered a good alternative to antibiotics, as their use in poultry diets has been associated with positive effects on health and growth in birds.

The present research was conducted with 320 day-old mixed sexed "Cobb 500" broiler chicks for a period of 28 days at Sher-e-Bangla Agricultural University (SAU) Poultry Farm, Dhaka to study the effect of supplementation of two different locally isolated probiotics and antibiotic on growth performance, carcass characteristics, hematological traits and cecum microflora composition of broilers. The chicks were randomly divided into four equal treatment groups and each having 20 birds. First group of chicks received basal diet as control, the 2nd group of chicks received antibiotic (Doxycycline: 1gm/2L drinking water), the 3rd group of chicks received probiotic I (*Lactobacillus spp*.) and the 4th group received probiotic II (*Bifidobacterium spp*). Body weight, feed intake, FCR, survivability, carcass weight, temperature and humidity, internal organs weight, hematological traits and cecal microbial population of broiler on different treatments were recorded.

The body weight of broiler among different treatments groups were significantly differed ($P<0.05$). At the end of the experiment (d-28), body weight of experimental birds were significantly differed ($P<0.05$) among the treatment groups. The highest body weight (1572.50g/b) was observed in the birds received probiotic I i.e. *Lactobacillus spp*. followed by probiotic II i.e. *Bifidobacterium spp* (1553.72g/b) and antibiotic group (1530.75 g/b). However the lowest body weight (1509.25 g/b) was found in the birds that supplied only basal diet. These results suggest that either of the probiotics (I and II) independently attributed better results in growth of experimental broiler chickens as compared to traditional AGPs. It has been observed that broiler chicks fed with probiotic

I showed significantly (P<0.05) highest and the second best FLW was observed in probiotic II compared to antibiotic and control group.

The cumulative feed consumption during the period of 28 days was highest in the group probiotic II (2088.00 g/b) and lowest in the antibiotic group (2065.00g/b). Chicks belonging to probiotic I group, 2076.25 g/b and control group consumed 2071.25 g/b. No significant effects among the treatment groups for FC (P>0.05).

In case of feed conversion ratio (FCR) significant (P<0.05) effects were observed between the treatment and control groups. Cumulative FCR was 1.35, 1.38, 1.38 and 1.42 for probiotic I, probiotic II, and antibiotic and control group, respectively.

There was no significant difference (P>0.05) on dressing and survivability percentage among the treatments but it was noticed that there was a tendency to increase in DP % in probiotic I (70.05 %) followed by the probiotic II (69.54%), control (68.38%) and antibiotic (68.04%) group.

The relative giblet weight and immune organs did not show any difference either between any of treatment or control group except liver weight in which significantly higher weight was found in the probiotic I than the control and AGP group. In the same time numerically higher giblet and immune organ weight and also lowered abdominal fat were found in the probiotics treatments group than in the control and antibiotic group.

There was no significant effect on serum total cholesterol among the treatments but it was noticed that there was a marked reduction of total cholesterol level in probiotic I (2.92 mmol/L) and the probiotic II (3.02mmol/L) than the control (3.48mmol/L) and antibiotic (3.75mmol/L) group.

Concerning the treatment effect on blood constituents, the results indicated no significant differences (P>0.05) were found with probiotics supplementation except of hemoglobin, RBC and WBC. In case of Hb and RBC significantly higher values were found in probiotic I than in the probiotic II, control and AGP group. In the same time WBC was significantly higher in probiotic II (8708.33 thousands/cum); where probiotic I (7925.00 thousands/cum) and antibiotic (8083.33 thousands/cum) group showed significantly

similar result and control (6783.33 thousand/cum) showed significantly lower result. In the present study, it is concluded that the increase in total leukocyte count in probiotics supplemented groups may be indicative of gut microbiota stimulating intestinal immune system and leukopoietic effect.

Supplementation of probiotic I and probiotic II in drinking water of finishing broilers has significant ($P<0.05$) effects on the *E. coli, Salmonella spp,* total coliform count, total viable count of cecal contents when compared to the control group but no significant difference ($P>0.05$) when they were compared among themselves except on the *E. coli* count. In addition, significantly higher ($P<0.05$) number of these counts were observed in the antibiotic group compared with the probiotic I and probiotic II group.

Supplementation of probiotics have a significant effect on improve health , reduce enteric pathogens and increase beneficial microbes as the present study showed that total count of *Lactobacillus spp.* was significantly ($P<0.05$) increased in probiotic I group compared with other groups. The reason for the improvements in FCR of the broiler with probiotics fed in the present study was probably an increase in the population of beneficial intestinal bacteria and reduction of the population of pathogenic bacteria, and consequently, better nutrient digestibility and absorption.

Considering these results, it is clearly indicated that probiotic I (*Lactobacillus spp*.) and probiotic II (*Bifidobacterium spp.*) at a concentration of 1×10^{8}cfu/ml effectively enhanced the growth performance (BW and FCR), giblet weight (liver), blood parameters and modulation of intestinal microflora and pathogen inhibition of broiler at the finisher stage. These probiotics may be recognized as safe without any reported side effects; show no residue carry-over in meat and thereby no human health hazards unlike use of antibiotics as growth promoters and reduces expenditure on therapeutics (medicines/antibiotics).

However, the growth-promoting effects of probiotics are dependent on the specific probiotics, the application level of probiotics, the age of birds as well as the delivery method (i.e. via water and/or feed). Moreover, there are many factors from nutrition, environment (sanitary condition), to management that could compromise the effectiveness of probiotics

So, finally it can be concluded that locally isolated probiotics could be a viable alternative to antibiotics in broiler diets where probiotic I (*Lactobacillus spp*.) is better than probiotic II

(*Bifidobacterium spp.*). Before application of these two probiotics at commercial basis as an alternative to antibiotic growth promoter's (e.g. Doxycycline) further trial can be designed to make comparative study with few commercial probiotics with special reference to the efficacy of locally isolated product and their economic feasibility as well (i.e. considering cost-benefit analysis) to make the findings more accurate and effective.

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Appendix 1. Recommended level of nutrients for broiler

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

Source : Cobb 500 Broiler Management Guide,2016

Appendix 2. Nutrient composition of the ingredients used in formulate experimental diet

Ingredients	DM (%)	ME (k.cal/kg)	CP (%)	CF (%)	Ca (%)	P (%)	Lysine (%)	Methionine (%)	Tryptophan (%)
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	91.64	2860	63.30	8.1	6.37	3.24	3.87	1.78	0.53
Meat and Bone meal	95.5	1044	14.6	2.5	7.0	12.11	0.66	0.24	0.12

Source : Cobb 500 Broiler Management Guide,2016

Appendix 3. Recorded temperature (^{o}C) during the experimental period

Age (days)	Date	Room temperature (^{o}C)					
		6 A.M.	12P.M.	4P.M.	6P.M.	12P.M.	Average
0	06.03.2018	30	35	33	32	28	31.6
1	07.03.2018	31	33	34	29	28	31
2	08.03.2018	30	31.5	33	28	26	29.7
3	09.03.2018	31	32	32	27	26	29.6
4	10.03.2018	31	32	33	29	27	30.4
5	11.03.2018	30	33	34	28	27	30.4
6	12.03.2018	30	33	35	29	25	30.4
7	13.03.2018	29	32	33	27	26	29.4
8	14.03.2018	28	30	33	26	25	28.4
9	15.03.2018	28	30	32	27	26	28.6
10	16.03.2018	27	33	35	28	24	29.4
11	17.03.2018	23	32	34	26	25	28
12	18.03.2018	24	30	32	26	25	27.4
13	19.03.2018	22	29	33.4	27	26	27.4
14	20.03.2018	20.5	33	35	26	25	27.9
15	21.03.2018	24	29	35	26	25	27.8
16	22.03.2018	23.5	30	35	28	27	28.5
17	23.03.2018	24	32	34	27	25	28.6
18	24.03.2018	26	32.5	35.5	26.5	25	29.1
19	25.03.2018	26	33	35	27	26	29.4
20	26.03.2018	21	32	36	29	26	28.8
21	27.03.2018	20.5	33	35	26	25	27.9
22	28.03.2018	26.5	32	34	28	25	29.1
23	29.03.2018	24.5	30	35	26	25	28.1
24	30.03.2018	23	33	34	26	22	27.6
25	31.04.2018	21	29	32	27	23	26.4
26	01.04.2018	23	30	33	26	24	27.2
27	02.04.2018	24	30	33	25	22	26.8

Appendix 4. Recorded relative humidity (%) during the experimental period

Age (days)	Date	Relative Humidity (%)				
		6 A.M.	12P.M.	4P.M.	8P.M.	Average
0	06.03.2018	78	63	32	62	58.75
1	07.03.2018	86	71	30	55	60.5
2	08.03.2018	80	65	40	60	61.25
3	09.03.2018	84	67	30	70	62.75
4	10.03.2018	76	61	31	59	56.75
5	11.03.2018	90	73	29	55	61.75
6	12.03.2018	81	60	40	67	62
7	13.03.2018	89	65	37	66	64.25
8	14.03.2018	90	56	35	70	62.75
9	15.03.2018	89	67	30	75	65.25
10	16.03.2018	90	63	29	79	65.25
11	17.03.2018	87	61	32	61	60.25
12	18.03.2018	79	56	27	63	56.25
13	19.03.2018	82	55	42	55	58.75
15	21.03.2018	79	52	27	50	52
16	22.03.2018	76	50	25	49	50
17	23.03.2018	87	55	47	60	62.25
18	24.03.2018	85	49	39	50	55.75
19	25.03.2018	92	59	42	57	62.5
20	26.03.2018	92	60	45	70	66.75
21	27.03.2018	87	50	47	66	62.5
22	28.03.2018	91	49	52	75	66.75
23	29.03.2018	92	54	49	80	68.75
24	30.03.2018	87	48	54	79	67
25	31.04.2018	83	63	56	81	70.75
26	01.04.2018	86	70	60	84	75
27	02.04.2018	89	71	62	80	75.5

Appendix 5. Body weight of broiler in different treatments (g/bird) groups at different age

Treatment	Replication	Initial	1st week	2nd week	3rd week	4th week
T_1	R_1	42	217	476	942	1531
	R_2	43	219	484	956	1527
	R_3	41	210	476	901	1488
	R_4	42	227	496	937	1450
T_2	R_1	42	230	497	967	1541
	R_2	42	220	481	920	1491
	R_3	41	225	486	930	1517
	R_4	43	224	478	950	1574
T_3	R_1	40	222	485	945	1548
	R_2	41	217	499	985	1558
	R_3	43	230	473	947	1621
	R_4	42	225	478	953	1573
T_4	R_1	41	224	485	953	1518
	R_2	40	215	494	965	1552
	R_3	40	228	491	952	1541
	R_4	43	235	493	970	1604

Appendix 6. Body weight gain of broiler in different treatments (g/bird) at different age

Treatment	Replication	1st week	2nd week	3rd week	4th week
T_1	R_1	175	301	466	589
	R_2	176	306	472	571
	R_3	169	307	425	587
	R_4	185	310	441	613
T_2	R_1	188	308	470	574
	R_2	178	302	439	571
	R_3	184	302	444	587
	R_4	181	295	472	624
T_3	R_1	182	304	460	603
	R_2	176	323	486	573
	R_3	187	284	474	674
	R_4	183	294	475	620
T_4	R_1	183	302	469	564
	R_2	175	320	471	587
	R_3	188	304	461	589
	R_4	192	299	477	634

Appendix 7. Feed intake of broiler in different treatments groups (g/bird) at different age

Treatment	Replication	1st week	2nd week	3rd week	4th week	Total Feed Consumption
T_1	R_1	158	380	635	930	2103
	R_2	158	355	610	925	2048
	R_3	158	370	623	914	2065
	R_4	158	376	615	920	2069
T_2	R_1	158	350	640	955	2103
	R_2	158	360	605	920	2043
	R_3	158	375	620	919	2072
	R_4	158	353	626	905	2042
T_3	R_1	158	357	605	902	2022
	R_2	158	379	624	934	2095
	R_3	158	360	640	960	2118
	R_4	158	366	632	914	2070
T_4	R_1	158	385	646	960	2149
	R_2	158	350	600	920	2028
	R_3	158	365	610	908	2041
	R_4	158	394	650	932	2134

Appendix 8. FCR of broiler different treatments groups in different age

Treatment	Replication	1st week	2nd week	3rd week	4th week	Total
T_1	R_1	1.1	1.26	1.36	1.57	1.41
	R_2	1.11	1.16	1.29	1.61	1.38
	R_3	1.06	1.2	1.46	1.55	1.43
	R_4	1.17	1.21	1.39	1.5	1.47
T_2	R_1	1.18	1.14	1.36	1.66	1.4
	R_2	1.12	1.19	1.37	1.61	1.41
	R_3	1.16	1.24	1.39	1.56	1.4
	R_4	0.98	1.19	1.32	1.45	1.33
T_3	R_1	1.14	1.17	1.31	1.49	1.34
	R_2	1.11	1.17	1.28	1.63	1.38
	R_3	1.18	1.27	1.35	1.42	1.34
	R_4	1.15	1.24	1.33	1.47	1.35
T_4	R_1	1.15	1.27	1.37	1.7	1.45
	R_2	1.1	1.09	1.27	1.56	1.34
	R_3	1.18	1.2	1.32	1.54	1.36
	R_4	1.21	1.31	1.36	1.47	1.37

Appendix 9. Production performance of broiler in different treatments groups

Treatments	Replications	Sample no.	Dressed weight (g/b)	Dressing (%)	Survivability (%)
T_1	R_1	**1**	1276	69.08	100
		2	1210	66.91	100
		3	1438	68.49	100
	R_2	**1**	1190	72.2	100
		2	1290	68.76	100
		3	1221	67.06	100
	R_3	**1**	1470	70.18	100
		2	1290	68.4	100
		3	1240	65.8	100
	R_4	**1**	1350	68.61	100
		2	1270	65.22	100
		3	1278	69.82	100
T_2	R_1	**1**	1216	69.53	100
		2	1372	69.57	100
		3	1130	69.35	100
	R_2	**1**	1435	70.84	100
		2	1240	69.12	100
		3	1220	69.72	100
	R_3	**1**	1283	67.19	100
		2	1221	65.82	100
		3	1320	65	100
	R_4	**1**	1298	65.95	100
		2	1306	67.81	100
		3	1450	66.64	100

Appendix 9 (Contd.)

Treatments	Replications	Sample no.	Dressed weight (g/b)	Dressing (%)	Survivability (%)
T_3	R_1	**1**	1352	67.96	100
		2	1193	67.82	100
		3	1201	71.52	100
	R_2	**1**	1128	72.86	100
		2	1070	70.41	100
		3	1146	69.81	100
	R_3	**1**	1255	71.51	100
		2	1204	67.56	100
		3	1170	67.95	100
	R_4	**1**	1230	70.46	100
		2	1120	72.34	100
		3	1150	70.41	100
T_4	R_1	**1**	1254	65.56	100
		2	1369	74.6	100
		3	1307	66.71	100
	R_2	**1**	1320	71.76	100
		2	1274	68.45	100
		3	1254	69.29	100
	R_3	**1**	1316	70.83	100
		2	1067	73.05	100
		3	1165	68.83	100
	R_4	**1**	1050	69.39	100
		2	1248	68.05	100
		3	1305	67.97	100

Appendix 10. Relative giblet and abdominal fat weight of broiler in different treatments groups (d-28)

Treatments	Replications	Sample no.	Liver wt. (g/b)	Spleen wt. (g/b)	Heart wt. (g/b)	Gizzard wt. (g/b)	Bursa wt. (g/b)	AFW (g/b)
T_1	R_1	**1**	31	2	8	47	2	28
		2	46	5	11	45	3	31
		3	36	3	8	44	2	18
	R_2	**1**	42	5	13	51	5	63
		2	40	2	10	39	3	39
		3	38	3	9	49	4	37
	R_3	**1**	48	3	10	37	1	22
		2	28	2	8	39	3	16
		3	39	4	9	50	3	22
	R_4	**1**	27	1	6	32	1	18
		2	43	2	7	41	2	23
		3	32	2	6	36	1	34
T_2	R_1	**1**	41	2	9	44	3	19
		2	42	2	9	42	2	33
		3	44	3	11	45	5	26
	R_2	**1**	35	4	12	47	1	21
		2	30	2	7	36	2	18
		3	38	2	10	40	1	30
	R_3	**1**	51	2	9	42	1	31
		2	44	3	8	43	3	23
		3	34	2	8	38	2	19
	R_4	**1**	27	1	5	37	1	22
		2	32	2	8	38	2	37
		3	30	1	9	43	1	29

Appendix 9 (Cont'd)

Treatments	Replications	Sample no.	Liver wt. (g/b)	Spleen wt. (g/b)	Heart wt. (g/b)	Gizzard wt. (g/b)	Bursa wt. (g/b)	AFW (g/b)
T_3	R_1	**1**	56	4	14	52	4	37
		2	44	2	9	47	2	36
		3	68	10	16	50	10	40
	R_2	**1**	35	1	9	37	1	24
		2	42	2	8	40	4	25
		3	41	2	8	44	2	23
	R_3	**1**	71	10	18	52	10	34
		2	62	5	16	50	6	27
		3	45	4	12	48	4	20
	R_4	**1**	47	1	9	34	1	22
		2	49	2	9	39	1	32
		3	55	3	9	37	2	25
T_4	R_1	**1**	36	3	10	47	5	26
		2	38	1	7	62	3	29
		3	33	2	9	33	3	22
	R_2	**1**	55	6	14	55	7	42
		2	47	4	10	42	6	26
		3	54	3	8	41	4	24
	R_3	**1**	45	4	12	59	5	18
		2	33	3	9	39	3	20
		3	38	2	8	35	1	18
	R_4	**1**	54	2	7	43	3	25
		2	45	2	9	38	5	26
		3	50	5	9	54	6	42

Appendix 11. Hematological traits of broiler in different treatments groups (d- 28)

T.	R.	S. no.	Chol. (mmol/L)	Hb. (g/dl)	RBC (million /cumm)	WBC (thousand /Cumm)	Neu. (%)	Lymp. (%)	Mono. (%)	Eosi. (%)	PCV (%)	MCH (pg/dl)	MCHC (g/dl)
T_1	R_1	1	2.42	10.23	4.12	3,000	62	30	2	6	31.5	32.14	33.17
		2	2.41	11.16	4.20	5,500	58	38	2	2	34.5	30.19	33.27
		3	2.72	11.6	4.25	8,000	51	44	3	2	34.5	30.16	33.28
	R_2	1	4.12	11.6	4.20	5,000	63	31	4	2	34.49	30.14	33.27
		2	3.17	12	4.40	10,000	78	15	2	5	36.51	30.12	33.27
		3	3.28	11.16	4.24	7,000	64	32	1	3	34.5	30.19	33.26
	R_3	1	4.85	11.6	4.20	6,100	70	27	1	2	34.5	30.16	33.29
		2	1.92	10.23	4.21	6,000	60	36	2	2	31.2	30.17	33.26
		3	3.89	9.3	3.99	9,000	69	27	3	1	29.3	29.34	31.27
	R_4	1	4.39	10.25	4.3	5500	78	18	2	2	31.5	30.14	33.17
		2	4.63	9.2	3.15	7000	66	29	2	3	29.3	29.3	31.32
		3	3.92	9.5	3.6	8000	74	24	1	1	29.5	30.17	31.5
T_2	R_1	1	3.43	9.8	3.96	7,000	58	36	2	4	29.34	30.12	32.5
		2	4.89	9.6	3.9	5,400	55	41	3	1	129.11	29.37	30.18
		3	3.11	9.8	3.91	5,400	59	33	3	5	29.3	31	32.7
	R_2	1	3.11	9.8	3.95	7,000	64	29	6	1	29.34	30.16	32.15
		2	3.04	10.23	4.12	8,000	71	23	4	2	31.2	30.14	33.28
	R_3	3	4.95	10.7	4.15	11,000	58	38	2	2	31.5	30.16	33.25
		1	3.22	9.8	3.99	4,000	59	36	2	3	30.12	30.16	32.49
		2	3.03	11.6	4.2	10,000	78	15	3	4	34.5	30.16	32.45
		3	2.82	12	3.5	8000	67	28	1	4	36.5	30.16	33.29
	R_4	1	4.85	10.6	4.11	9300	62	32	3	3	31.5	31.5	30.25
		2	4.72	9.5	3.66	8000	64	32	2	2	30.25	30.14	30.14
		3	3.87	11.2	4.2	8500	71	23	4	2	31.20	30.12	30.15

Appendix 11. (Cont'd)

T.	R.	S. no.	Chol. (mmol/L)	Hb. (g/dl)	RBC (million /cumm)	WBC (thousand /Cumm)	Neu. (%)	Lymp. (%)	Mono. (%)	Eosi. (%)	PCV (%)	MCH (pg/dl)	MCHC (g/dl)
T_3	R_1	1	3.62	12	4.3	8,500	69	26	3	2	36.41	30.24	33.12
		2	3.00	9.8	3.95	4,800	56	38	2	4	29.3	29.34	30.22
		3	2.43	12	4.35	9,900	71	22	2	5	36.5	30.16	33.24
	R_2	1	3.65	11.16	4.25	3,200	48	44	3	5	34.44	30.17	32.13
		2	3.8	12.60	4.4	11,500	81	15	2	2	37.5	30.24	33.16
		3	3.8	10.23	4.21	4,500	46	49	3	3	31.2	30.14	32.17
	R_3	1	2.05	11.6	4.3	8,400	65	28	4	3	34.5	30.17	33.28
		2	3.21	12.6	4.42	6,000	56	39	3	2	37.52	30.16	33.23
		3	1.95	11.6	4.3	8,000	62	33	2	3	34.5	30.12	33.27
	R_4	1	2.41	12.5	4.52	10500	51	42	2	3	37.5	30.25	33.28
		2	3.05	11.60	4.25	9300	49	44	3	4	36.5	30.16	32.27
		3	2.09	11.26	3.75	8500	48	45	3	4	34.5	29.31	33.19
T_4	R_1	1	2.45	12.6	4.4	8,500	58	38	2	2	37.51	30.28	33.19
		2	3.63	11.6	4.25	10,000	78	16	2	4	134.48	30.14	33.27
		3	3.08	9.8	3.99	8,600	61	33	3	3	29.3	29.31	32.16
	R_2	1	2.11	10.7	4.12	10,000	76	18	4	2	31.52	30.21	33.19
		2	1.91	12	4.36	8,000	69	27	2	2	36.5	30.27	33.18
	R_3	3	2.95	9.8	3.98	3,200	48	46	3	3	29.31	30.14	33.24
		1	4.30	11.16	4.2	9,300	71	24	2	3	34.5	30.24	33.17
		2	4.55	9.8	3.99	4,200	49	47	2	2	30.16	30.16	32.27
		3	3.39	9.8	3.94	11,400	82	13	3	2	29.34	29.34	32.17
	R_4	1	3.24	11.25	4.15	9500	52	44	2	2	37.5	30.24	33.25
		2	1.99	12.11	4.45	11500	47	45	3	5	34.5	31.00	33.17
		3	2.65	10.60	3.80	10300	58	37	2	3	31.45	29.31	33.15

Appendix 12. Microbial composition/count (log_{10} cfu/gm) of broiler in different treatment groups (d-28)

Treatment	Replication	*Escherichia coli*	*Salmonella sp*	TCC	TVC	*Lactobacillus sp*
T_1	R1	5.38	5.32	5.29	5.46	4.54
	R2	5.36	5.33	5.23	5.47	4.48
	R3	5.42	5.35	5.31	5.45	4.47
	R4	5.36	5.32	5.30	5.46	4.60
T_2	R1	5.39	5.23	5.14	5.46	4.47
	R2	5.42	5.18	5.26	5.42	4.5
	R3	5.46	5.26	5.20	5.44	4.47
	R4	5.34	5.17	5.21	5.42	4.57
T_3	R1	4.49	4.6	4.51	4.85	5.46
	R2	4.48	4.6	4.48	4.87	5.48
	R3	4.49	4.52	4.48	4.91	5.47
	R4	4.50	4.62	4.48	4.94	5.48
T_4	R1	4.49	4.55	4.48	4.9	4.84
	R2	4.62	4.38	4.48	4.86	4.78
	R3	4.54	4.50	4.48	4.95	4.77
	R4	4.56	4.61	4.50	4.98	4.65

Appendix 13. Photographs during conducting the experiment

Receiving day old chicks

Weighing day old chicks

Common brooding for one week

Vaccination of birds

Feeding management

Appendix 13. (Contd.)

Water management

Routine Checking of birds

Recorded body weight

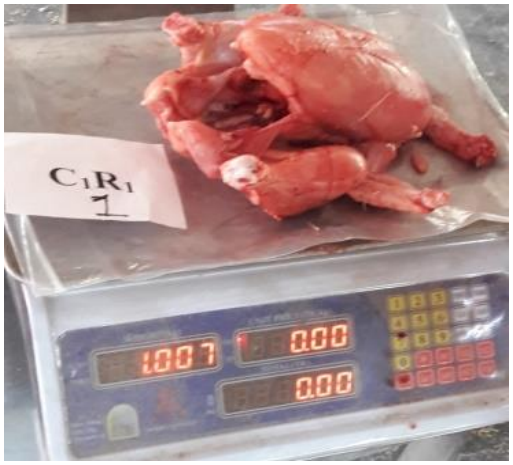
Recorded feed weight

Checking feed condition

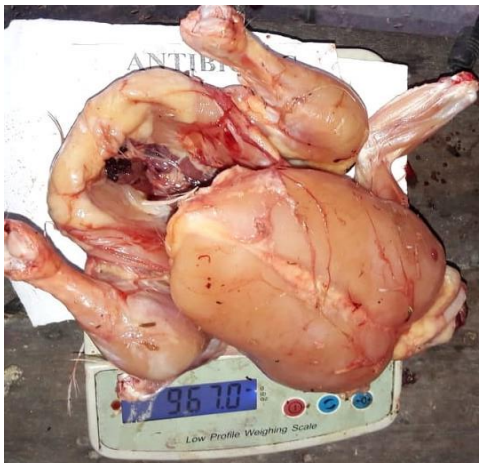
Recorded temp. & RH

Management practices of experimental birds

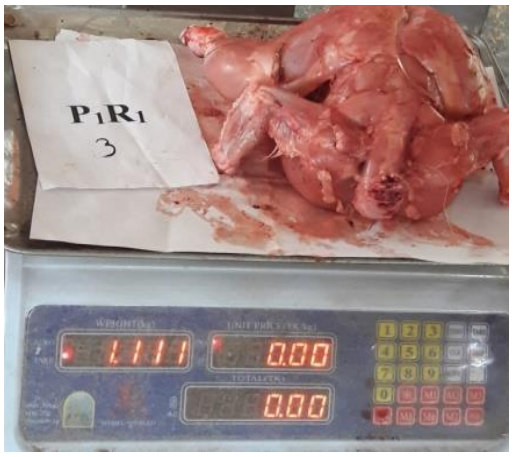
Appendix 13. (Contd.)



Dressed wt. in control group



Dressed wt. in antibiotic group



Dressed wt. in probiotic I group

Dressed wt. in probiotic II group

Liver wt. in control group

Liver wt. in probiotic I group

Weighing of dressed birds and internal organs