EFFECTS OF PROBIOTICS *(Bacillus subtilis* and *Bacillus licheniformis*) ON PERFORMANCE AND ANTIMICROBIAL ACTIVITY OF BROILER

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*This is to certify that the thesis entitled, "**EFFECTS OF PROBIOTICS (Bacillus subtilis and Bacillus licheniformis) ON PERFORMANCE AND ANTIMICROBIAL ACTIVITY OF BROILER**" Submitted to the Department of Animal Nutrition, Genetics and Breeding, Faculty of Animal science and veterinary medicine, Sher-E-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) in Animal Nutrition** embodies the result of a piece of bonafide research work carried out by **Md. Imran Hossain, Registration No. 12-04826** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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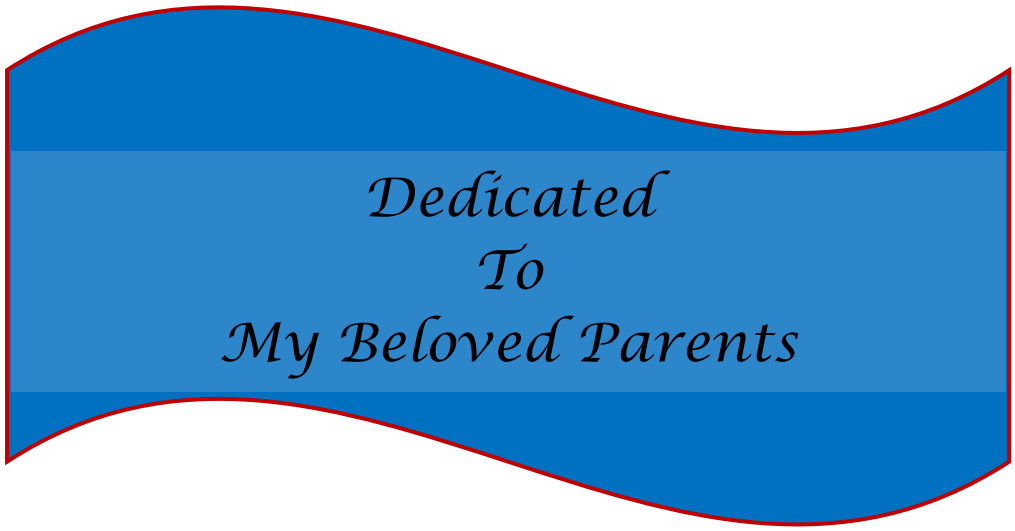
Dedicated
To
My Beloved Parents

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The Author

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LIST OF ACRONYMS AND ABBREVIATION

ABBREVIATION		FULL MEANING
A.M	=	Anti meridiem
ADG	=	Average daily gain
AGPs	=	Antibiotic growth promotors
ANOVA	=	Analysis of variance
Avg.	=	Average
BCR	=	Benefit cost ratio
BL	=	*Bacillus licheniformis*
BS	=	*Bacillus subtilis*
BWG	=	Body weight gain
CFU	=	Colony forming unit
cm^2	=	Square centimeter
CP	=	Crude protein
DOC	=	Day old chick
DP	=	Dressing percentage
DSM	=	Diagnostic and statistical manual
E. coli	=	*Escherichia coli*
e.g.	=	For example
EMB	=	Eosin methylene blue
et al.	=	And others/associates
EU	=	European union
FAO	=	Food and agricultural organization
FC	=	Feed consumption
FCR	=	Feed conversion ratio
FI	=	Feed intake
g	=	Gram
GIT	=	Gastro intestinal tract

ACRONYMS AND ABBREVIATION (CONT'D)

ABBREVIATION		FULL MEANING
i.e.	=	That is
IB	=	Infectious bronchitis
IBD	=	Infectious bursal disease
K Cal	=	Kilo calorie
Kg	=	Kilogram
L	=	Litre
M.S.	=	Master of science
ME	=	Metabolizable energy
ml	=	Mililitre
mm	=	Milimetre
MT	=	Metric ton
ND	=	Newcastle disease
No.	=	Number
NS	=	Non-significance
NSP	=	Non-starch polysaccharide
NSPs	=	Natural growth promotors
PPB	=	Profit per bird
RH	=	Relative humidity
SAU	=	Sher-e-bangla agricultural university
SC	=	*Saccharomyces cerevisiae*
SE	=	Statistical error
SPSS	=	Statistical package for social sciences
SS	=	*Salmonella-shigella*
Viz.	=	Such as
Vs.	=	Versus
WHO	=	World health organization
Wks.	=	Weeks

LIST OF SYMBOLS

SYMBOLS		FULL MEANING
*	=	5% level of significance
&	=	And
@	=	At the rate of
℃	=	Degree celcius
℉	=	Degree fahrenheit
$	=	Dollar
>	=	Greater than
<	=	Less than
/	=	Per
%	=	Percentage
±	=	Plus-minus
:	=	Ratio

EFFECTS OF PROBIOTICS *(Bacillus subtilis* and *Bacillus licheniformis*) ON PERFORMANCE AND ANTIMICROBIAL ACTIVITY OF BROILER

ABSTRACT

Antibiotics are used to fight against bacterial infection and bacterial resistance to antibiotic are increasing. Hence, this study was conducted to find out the efficacy of probiotics *Bacillus subtilis* (BS) and *Bacillus licheniformis* (BL) on performance and antimicrobial activity of broiler chicken. One-Day old of cobb-500 broiler chicks (n=150) were divided into 5 experimental groups with 3 replicates as 10 chicks in each replication. One of the 5 experimental group fed diet without probiotic was as control and the remaining four groups fed diet with 4 levels of commercial probiotics were T_1 (20g BS/metric ton feed), T_2 (50g BS/metric ton feed), T_3 (20g BL/metric ton feed) and T_4 (50g BL/metric ton feed). The group T_4 treated with 50g BL/ metric ton feed (MT) showed higher body weight (1607.50±30.98g) compared to control (1472.14±38.51). Feed consumption was significantly ($P<0.05$) higher in control group than probiotic treated groups. FCR was better in all probiotic treated groups compared to the control (1.52±0.04) and the best feed conversion ratio (FCR) result was found in T_4 group (1.33±0.02). Carcass percentage was significantly ($P<0.05$) higher in T_4 group (71.33±1.30) compared with the control (65.67±1.33). The weight of breast, thigh, drumstick and giblet was significantly ($P<0.05$) high in T_4 group as compared to others treatment groups and control (T_0). In addition, the present study showed that feeding dietary probiotics in different groups had no significant effect ($P>0.05$) on relative weight of neck, wing, intestine, gizzard, spleen and bursa. The numbers of intestinal microflora (*E. coli* and *Salmonella*) were significantly higher ($P<0.05$) in control group compared to other groups and among the treatment groups the number of *salmonella* bacteria was significantly lower ($P<0.05$) in T_4 group. Total expenditure per bird was significantly higher ($P<0.05$) in treated group (T_2) than control group (T_0). Feed cost was significantly higher ($P<0.05$) in control group (T_0) compared to different treated groups. BCR and profit per bird was significantly higher ($P<0.05$) in treatment groups than control (T_0) and among the treatment groups T_4 performed better than others. Overall, between these two probiotics (BS and BL), 50g BL/MT feed showed better results in terms of improved growth performance with better FCR, better carcass yield with net economic benefit.

CHAPTER-1
INTRODUCTION

CHAPTER 1

INTRODUCTION

1.1 General Background

Bangladesh is an agro-based country where 80 percent of the population depends on agriculture. Poultry plays a vital role in the income generating framework of the rural people of Bangladesh. The contribution of poultry sector towards promoting resources for improving the life style and livelihood of landless and marginal farmers is noted worthy. In large-scale rearing facilities where poultry are exposed to stressful conditions may lead to diseases or decrease the production potentials which in turn results in serious economic losses. Poultry such as chicken is one the main sources of animal protein for Bangladeshi people (Kamal and Shafiullah, 2016).

Due to increasing population, there is an increasing demand for meat and eggs which led to commercialization of poultry production, with a large number of farms now operating across the country (Raha, 2007). One of the major challenges this industry faces is the spreading of diseases among the poultry population due to bacterial pathogens which results in serious economic losses (Huque *et al*., 2011). As a result, the use of antimicrobial agents and growth promoters is substantially increasing in the poultry industry to prevent diseases and to promote faster growth (Islam *et al.,* 2016). An assortment of substances, such as growth promoters is added to the feed and the drinking water of poultry to improve its production and reduce or prevent the spread of diseases (Diarra and Malouin, 2014). These are substances used to increase the feed efficiency, average daily gain, eggs and meat production.

In poultry industry, antibiotic growth promotors (AGP) have been used as a feed additive to enhance gut health and control sub-clinical diseases. Synthetic growth enhancers and supplements in poultry nutrition are expensive, usually unavailable and possess adverse effects in bird and human. Sub-therapeutic levels of antibiotics given to poultry as growth enhancer may result to the development of antibiotic-resistant bacteria, which are hazardous to animal and human health (Sarica *et al.,* 2005). However, the use of antibiotics as feed additives is under severe criticism. Growth stimulating antibiotics, by the spread of antibiotic resistant bacteria, are a threat to human health (Wray and Davies, 2000; Turnidge, 2004). Sub-therapeutic levels of antibiotics are mixing in feed ingredient during processing of feed or mixing in drinking water also increasing the cost of feed.

1.2 State of the Problems

Restrictions or total bans on the use of growth promoting antibiotics in poultry feed are currently in place, to limit and prevent negative effects associated with over usage, such as the induction of microbial antibiotic resistance (Hooge *et al*., 2004). As such, alternatives are currently being proposed and sought out, of which probiotics have been specifically targeted for use in the poultry industry (Patterson and Burkholder, 2003; Zhang *et al*., 2012). As a general category, probiotics tend to refer to bacterial cultures capable of stimulating intestinal microflora, which in turn are capable of modifying the gastrointestinal environment in a positive manner, benefitting beneficial bacteria and improving the growth performance and feed efficiency of broilers (Tabidi *et al*., 2013).

Poultry are the cheapest source of animal protein, contributing significantly to supply the growing demand for animal food products around the world (Farrell, 2013). The consumption and trade in poultry products are increasing rapidly as the human population increases, making it the second largest source of meat after pork (FAO, 2014). The biggest challenge of commercial poultry production is the availability of quality feed on sustainable basis at stable prices. Probiotics (or direct fed microbials) are increasingly being popular as one of the alternatives to AGP. Probiotics can improve broiler chicken growth rates (Afsharmanesh and Sadaghi, 2014; Lei *et al.*, 2015), it also helps in maintenance and establishment of intestinal micro biota beneficially that may enhance beneficial colonization in the GIT against pathogens. Supplementation of probiotics enhanced the growth rate in broilers better than AGP (Zhang and Kim, 2014) and other substitutes for AGP, such as phytochemicals e.g. essential oils (Khaksar *et al.,* 2012). Probiotics are active against enteropathogens in several ways, including improved immunity-based elimination, competing for mucosal attachment and crucial nutrients, and producing antimicrobial complexes (Patel *et al.,* 2015).

Bacillus species are superior probiotic feed- additives for poultry and pigs due to their big genomes with relevant features; they are spore producers which makes the product stable for long time and enhancing the bird's intestinal integrity and growth performance (Vazquez, 2016). As a widely used probiotic strain, combination of *Bacillus subtilis* and *Bacillus licheniformis* are considered one of the most health-boosting bacteria because they have demonstrated a positive effect in aiding nutrient

digestion and absorption in the host's body (Scgarrd and Demark, 1990).

In recent times, there has been significant progress in scientific evaluation and studies on probiotic *Bacillus subtilis*, revealing possible mechanisms of action like antimicrobial effect by synthesis of antimicrobial substances, antidiarrheal effect, immune stimulatory effect, competitive exclusion of pathogens, prevention of intestinal inflammation, and normalization of intestinal flora (Suva *et al.,* 2016). Blanch *et al.* (2017) observed the addition of *Bacillus subtilis* DSM 17299 may efficiently compensate certain reductions of ME, CP and amino acid in broiler diets supplemented with NSP-enzymes and phytase.

1.3 Justification of the study

As a kind of green feed additive, probiotics has many advantages such as they improve livestock production, keep animals' intestinal healthy and enhance the animals' immunity without toxic side- effect or drug residues (Abdur-Rahman *et al.*, 2014; Dragana *et al.*, 2014). But most probiotics preparations are vulnerable to environment changes. As the holding time extends, viable bacterium will gradually die. Therefore, the amount of viable bacterium in the feed microorganism additives getting access to the intestinal tracts of animals is small, which significantly reduces the effect of additive (Xiang *et al.*, 2009). *Bacillus subtilis* can form spores in adverse environment that has some unique biological characters such as resistance to acid, alkali, and heat. They also grow fast. Thus, the spores can still plant in intestinal tracts to grow and breed on arrival after the extrusion process for granulating in feed processing and the expose to strong acidic environment in animals' stomach. Moreover, *B. subtilis* is aerobic bacteria, it takes a large amount of free oxygen while reproducing in the intestinal tract thus it can strongly restrain the growth of the majority of aerobic pathogen bacteria, enhance the growth of anaerobic probiotics such as *Lactobacillus*, yeast and *Bifidobacterium* (Wang *et al.*, 2006). Therefore, it is useful to restore and maintain the intestinal flora balance of animal, improve immune function, enhance animals' resistance to disease, and promote their growth (Zhou *et al*., 2012).

The global increase in demand from livestock sector for availability of high-quality protein for human consumption has prompted the need to explore cost efficient and faster means of increasing poultry performance and yield at the same time reducing

feed consumption. Over the years, antibiotics were used in the poultry industry for prophylactic and therapeutic purposes and also as growth enhancers. Nutrition and diseases are part of the challenges of the poultry industry (Aromolaran *et al*., 2013). Antibiotic usage as growth promoters leaves residues in poultry products (meat and eggs) which have deleterious effect on humans as the consumer and also shown to cause bacteria resistance (Donoghue, 2003). Consequently, this steered to the prohibition of sub-therapeutic use of antibiotics as growth promoters.

Poultry gut microflora plays the most vital role in its physiological performance. Feed supplementation is an important aspect of livestock nutrition, since it has been shown to increase the efficiency of feed utilization and significantly affect blood parameters (Vantsawa and Daramola, 2014).

1.4 Objectives

With this background, the work was planned to explore the possibilities of probiotic *(Bacillus subtilis* and *Bacillus licheniformis*) in broiler chicken feeds as a replacement for the antibiotic growth promoters, with the following specific objectives:

- ✓ To evaluate the growth performance and carcass characteristics of broiler chicken
- ✓ To find out the effect of probiotic *(Bacillus subtilis* and *Bacillus licheniformis*) on *E. coli* and *Salmonella spp*
- ✓ To estimate the cost benefit of using probiotics in broiler rearing under different probiotic treatment
- ✓ To recommend the inclusion level of probiotic *(Bacillus subtilis* and *Bacillus licheniformis*) in broiler ration as a supplement of growth promotors

CHAPTER-2

REVIEW OF LITERATURE

CHAPTER 2
REVIEW OF LITERATURE

Review of literature is advantageous and important for performing any type of survey or experiment which are linked to the proposed study for the amelioration of research work. During the last decade, different studies have been attempted to find nutrition-based health approaches and natural feed additives to improve performance and immunity of poultry, and strongly recommended the use of probiotics, prebiotics, phytogenic additives or organic acids. Residual side effects of antibiotics on human health among these feed supplement probiotics individually have drawn much great attention. Nowadays, there has been growing interest among researchers and the feed industry to prepare a probiotic feed supplement at a low cost that have beneficial effects on broiler growth performance, health status, and product quality of poultry. The literature reviewed here have been limited to these which are considered compatible and related to the objectives of the present study.

2.1 Antibiotics impacts

2.1.1 Impact on chicken growth, digestive tract and immune systems

In the poultry industry, antibiotics are used worldwide to prevent poultry pathogens and disease so as to improve meat and egg production. However, the use of dietary antibiotics resulted in common problems such as development of drug-resistant bacteria (Sorum and Sunde, 2001), drug residues in the body of the birds (Burgat, 1999) and imbalance of normal microflora (Andremont, 2000).

Animals including poultry are vulnerable to potentially pathogenic microorganism such as *Escherichia coli*, *salmonella ssp.*, *Clostridium perfringens* and *Campylobacter sputorum*. Pathogenic microbial flora in the small intestine compete with the host for nutrients and also reduce the digestion of fat and fat-soluble vitamins due to deconjugating effects of bile acids (Engberg *et al.*, 2000). This leads to depressed growth performance and to increased incidence of disease. Antibiotic feed additives as growth promoters have long been supplemented to poultry feed to stabilize the intestinal microbial flora and improve the general performances and prevent some specific intestinal pathologies (Truscott and Al-sheikhly, 1997).

Dono, (2014) reported that the commercially available antibiotics have been used in poultry feed to provide supplementary support to fight against harmful exogenous

pathogens. These antibiotics helps to overcome with the morbidity and mortality issues with poultry farming, however can affect the public health by developing drug resistant micro flora. It is reported that the use of antibiotics in poultry diet was completely banned in European countries since January 2006 (Casewell *et al.*, 2003).

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 (Barcelo, 2007; Chattopadhyay 2014; Engberg *et a*l., 2000; Harms *et al*., 1986; Khodambashi Emami, 2012; Rosen, 1996) and protect the health of birds by modifying the immune status of broiler chickens (Lee *et al*., 2012). This is mainly due to the control of gastrointestinal infections and microbiota modification in the intestine (Dibner and Richards, 2005; Singh *et al.*, 2013; Torok *et al.*, 2011). 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, meta-genome sequencing approaches have demonstrated that diets with salinomycin (60 ppm) has an impact on microbiome dynamics in chicken ceca (Fung *et al.*, 2013).

Similarly, the use of virginiamycin (100 ppm) as a growth promoter has been associated with an increased abundance of *Lactobacillus* species in broiler duodenal loop at proximal ileum. This indicates that virginiamycin alters the composition of chicken gut microbiota (Dumonceaux *et al.*, 2006). In addition, populations of *Lactobacillus* spp. in the ileum of chickens receiving feed containing tylosin, a bacteriostatic, are significantly lower than those in chickens receiving no tylosin (Lin *et al.*, 2013). This decrease in *Lactobacilli* species following the use of antibiotics has been demonstrated in other studies (Danzeisen *et al*., 2011; Lee *et al.*, 2012; Zhou *et al.*, 2007). For reminder, *Lactobacillus* are the primary commensal bacteria for the production of bile hydrolase salt. The decrease in the *Lactobacillus* population in antibiotic-treated animals probably reduces the intestinal activity of the bile hydrolase salts, which would increase the relative abundance of conjugated bile salts, thus promotes lipid metabolism and energy harvesting and increases animal weight gain (Lin *et al.*, 2013).

A change in the intestinal microbiota of chickens can influence their immunity and their health. However, changes in the intestinal microbiota of chickens can be

influenced by several factors. These factors include housing conditions, exposure to pathogens, diet composition and the presence of antibiotics in feed (Lee *et al.*, 2012).

2.1.2 Impact on meat quality

Campylobacter is a major cause of food-borne diarrheal diseases in humans. *Campylobacter* infections can be severe or fatal in immune compromised or elderly people and very young children. *Escherichia coli* bacteria are very common and can also cause diseases. The most common type of *E. coli* infection that causes illness in people is called *E. coli* O157:H7. Salmonellosis is one of the most common and widespread food-borne illnesses in the world. *Salmonella* infections usually cause mild gastroenteritis. These 3 bacteria and others are monitored by specialized agencies around the world, for example, public health agency of Canada in Canada, fda in USA, European food safety authority (EFSA) in EU. Tens of millions of cases of these bacterial infections occur in humans every year worldwide. 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.* 2014; Singh *et al*., 2010). Eggs are frequently implicated in *Salmonella* transmission (Singh *et al.*, 2010). This contamination is due mainly to the proliferation of pathogens in the intestines. There are secondary contaminations along the production line by resistant bacteria in foods of animal origin. Schwaiger *et al*. (2012) reported that the prevalence of multi-resistant of *Salmonella* was higher in retail samples compared to slaughterhouse samples.

2.2 Antibiotic and bacterial resistance

Scientific evidence suggests that the use of antimicrobials in livestock production can promote bacterial resistance in treated animals. Antibiotic resistance is defined as the ability of microorganisms to proliferate in presence of an antibiotic that generally inhibits or kills microorganisms of the same species. Chicken harbors large proportion of *Enterobacteriaceae* resistant to aminosides in its digestive tract and tetracycline in its meat (Guillot *et al*., 1977). Bacterial resistance to antibiotics has been the subject of several studies in the recent years (Diarra and Molium, 2014). In one study on *Salmonella enterica* isolates collected from poultry farms in British Columbia

(Canada), Diarra and Molium (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), *Mycoplasma gallisepticum* (Pakpinyo and Sasipreeyajan, 2007) 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, while 30% of isolates showed multi pharmacological phenotypic resistance to ampicillin, sulfamethoxazole and 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 and Molium, 2014). In addition, Hur *et al.* (2011) founded that isolates of *S. enterica* from egg and chicken carcasses were resistant to penicillins, sulfisoxazole, treptomycin, tetracycline and quinolones. *S. enterica* isolates were resistant to at least 21 antibiotics used by the authors. Most isolates harbored genes associated with SPI-1 and SPI-2 and the spv operon, which are known to be associated with human infections. This represents a threat to human health. This situation is mainly due to the misuse of certain antibiotics such as penicillins, tetracyclines, macrolides and aminoglycosides (Diarra and Malouin, 2014). The abusive use of antibiotics and the associated selection pressure have led to decreased therapeutic efficacy and created populations of antibiotic-resistant microorganisms. Antibiotic resistance may spread over time despite the suspension of antibiotic use. Indeed, strains of *E. coli* resistant to trimethoprim and streptomycin have been shown to persist for several weeks in a chicken farm without using the antibiotics mentioned above (Chaslus-Dancla *et al.*, 1987). On the other hand, antibiotic resistance is lower in organic farms (Hegde *et al*., 2016). Thus, it is imperative to determine the exact sources and ecology of resistant bacteria in order to develop strategies to stop their proliferation (Diarra and Malouin, 2014).

2.3 Antibacterial growth promoters (AGPs)

There is increasing interest in finding alternatives to antibiotics for poultry production. Because of the general problem of increased resistance of bacteria and the decreasing acceptance of the consumers for antibacterial growth promoters (AGPs), different substances, referred as natural growth promoters (NGPs), have been identified as effective and safe alternatives to AGPs. At present, there is a large number of NGPs available in the market, including probiotics, prebiotics and synbiotics. Antibiotic growth promoters (AGP) are used worldwide to prevent poultry pathogens and disease so as to improve meat and egg production. However, the use of AGP resulted in common problems such as development of drug-resistant bacteria, drug residues in the body of birds, and imbalance of normal microflora. As a consequence, it has become necessary to develop alternatives using either beneficial microorganisms or non-digestible ingredients that enhance growth (Awad *et al.,* 2009).

Substitution of conventional and prohibited AGPs with probiotics has received much attention in the recent years. One of the major reasons for increased interest in the use of probiotics is because they are natural alternatives to antibiotics for growth promotion in poultry. 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. Much research has been carried out to look for natural agents with similar beneficial effects of growth promoters. 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, immuno stimulants, bacteriocins, bacteriophages, phytogenic feed additives, phytocides, nanoparticles and essential oils.

2.3.1 Probiotics

Probiotics are live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989). In broiler nutrition, probiotic species belonging to *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces* have a beneficial effect on broiler performance (Ashayerizadeh *et al.,* 2009) modulation of intestinal microflora and pathogen inhibition (Mountzouris *et al.,* 2007) and promoting microbiological meat quality of broilers (Kabir, 2009). The mode of action

of probiotics in poultry includes maintaining normal intestinal microflora by competitive exclusion antagonism, lowering the pH through acid fermentation, competing for mucosal attachment and nutrients, producing bacteriocins, stimulating the immune system associated with the gut, increasing production of short-chain fatty acids (Ferket, 2011).

Kabir (2009) stated that probiotic effects on intestinal microflora and pathogen inhibition, intestinal histological changes, immuno-modulation, some haemato-biochemical parameters and subsequently improve growth performance of broilers. He also mentioned that probiotic improves sensory characteristics of dressed broiler meat and microbiological meat quality of broilers. However, it is mentioned that the main effect of probiotic is in the gastrointestinal tract and associated with its capacity to stimulate the immune response and to control the growth of pathogenic bacteria.

Mode of actions of probiotic is still unclear despite the suggestions given by Montes and Pugh (1993) 1) beneficial changes in gut flora with reductions in the population of Escherichia coli, 2) lactate production with subsequent changes in intestinal pH, 3) production of antibiotic type substances, 4) production of enzymes, 5) competition for adhesion receptors in the intestine, 6) competition for nutrients, 7) reduction of toxin release and immuno-stimulation.

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. 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). Probiotic bacteria produce molecules with antimicrobial activities such as bacteriocins which inhibits toxins' production and pathogens' adhesion (Pan and Zhongtang, 2014). On the other hand, probiotics stimulate the immune response and increase resistance to colonization of bacteria (Hassanein and Soliman, 2010).

2.3.2 Prebiotics

Prebiotics are nondigestible food ingredient that beneficially affects the host, selectively stimulating the growth or activity, or both, of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). Lactobacilli and enterococci are

among the wide variety of microbial species that have been used extensively as probiotics (Patterson and Burkholder, 2003). After feeding of probiotics, improvements in growth performance and feed efficiency have been reported in broiler chickens (Samli *et al.*, 2007). The proposed modes of action of probiotics in poultry are as follows: 1) maintaining a beneficial microbial population by competitive exclusion and antagonism (Fuller, 1989), 2) improving feed intake and digestion (Nahanshon *et al.*, 1993), and 3) altering bacterial metabolism (Jin *et al*., 1997). It has been shown that prebiotics encourages the growth of endogenous microbial population groups such as *Bifidobacteria* and *Lactobacilli* which are particularly stimulated, and these bacteria species are considered as beneficial to animal health. Furthermore, dietary supplementation of a fructo-oligosaccharides (0.3% dose) or oligo chitosan (0.1% dose) as prebiotic, showed growth-promoting effects similar to antibiotic treatments based on flavomycin or aureomycin (Li X, 2008).

2.3.3 Synbiotics (probiotic and prebiotic)

Synbiotics is a combination of probiotics and prebiotics (Ashraf, 2013). This product could improve the survival of the probiotic organism because its specific substrate is available for fermentation. This could result in advantages to the host through the availability of the live microorganism. The combination of a pre and probiotic in one product has been shown to confer benefits beyond those of either on its own. A way of potentiating the efficacy of probiotic preparations may be the combination of both prebiotics and probiotics as synbiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract.

2.3.4 Phytogenic feed additives

Phytogenic feed additives (PFA) derived from plants, herbs and spices are used to improve animal performance. They have been very successful because of their positive effects on growth, improved immune system and reduced stress response. Recent results showed that PFA were good alternatives to antibiotics (Frankic *et al.*, 2009; Ghasemi *et al.*, 2014; Toghyani *et al.*, 2011) and promoted broiler chicken growth (Ghasemi *et al*., 2014; Lei *et al*., 2015; Toghyani *et al.*, 2011). For example, inclusion of cinnamon 2 g/kg of the diet had a positive effect on growth performance at 28 days of age (974 vs. 850 g) and at 42 days of age (2,111 vs. 1,931 g) (Toghyani

et al., 2011). Also, inclusion of *Lippia javanica* at 5 g/kg in broiler feed had beneficial effects on ADG in the grower period (67 vs. 30 g), slaughter weight (2,213 vs. 1,967 g) and fatty acid profiles of broiler chicken meat (Mpofu *et al*., 2016). According to Mpofu *et al.* (2016), phytogenic extracts in *L. javanica* leaf meal can stimulate glycolysis and increase utilization of energy production and ultimately growth. In addition, a mixture of garlic (5 g/kg) and black pepper (1 g/kg) powder had positive effects on weight gain and broiler chicken consumption index (Kirubakaran *et al.*, 2016).

2.3.5 Amino acids and enzymes

The feed additive enzymes are produced through fungi and bacteria fermentations. They are used to maximize feed conversion. Enzymes facilitate components degradation such as proteins, phytates and glucans. For example, endo-b-1-4-xylanases and b-1-3, 1-4-glucanases have been used in wheat and barley diets of broilers to improve their digestion (Cowieson *et al.*, 2006). Also, phytase enzyme can increase villus width and decrease crypt depth which can improve ADG (Mohammadagheri *et al.*, 2016). Lysins are bacteriophage endolysins representing an innovative alternative therapeutic option of antibacterial. Lysins are phage-encoded peptidoglycan hydrolases which bring about the bacterial cell lysis when applied exogenously to Gram-positive bacteria (Fenton *et al.*, 2010; Rios *et al.*, 2016). According to Volozhantsev *et al*. (2011), administration of a combination of a group of lysins containing peptidases, amidases and lysozymes produces an antimicrobial effect against *C. perfringens* in poultry. For example, Ply3626 lysine is an enzyme which has been shown lytic activity against several strains of *C. perfringens*, which is an important cause of food poisoning and leads to economic losses in poultry production (Fenton *et al.*, 2010).

2.3.6 Organic acids

The antimicrobial action of organic acids is due to the fact that non-dissociated acids can diffuse through lipophilic bacteria membrane and disrupt enzymatic reactions and transport system (Cherrington *et al.*, 1991). Some studies (Hassan *et al.*, 2010; Nava *et al*., 2009) showed that organic acids addition to broiler feed promotes growth, feed conversion rate and feed utilization. Adding organic acids in drinking water gives young chicks a protective efficacy against *Campylobacter* infection (Chaveerach

*et a*l., 2004). These acids also have a protective action against *E. coli* (Izat *et al.*, 1990). Thus, it has been shown (Mohammadagheri *et al.*, 2016) that supplementation with citric acid (2%) can improve cell proliferation epithelial and villi height of gastrointestinal tract. Organic acid blend, formic and propionic acid supplementation (0.0525% in drinking water) generates more homogeneous and distinct populations in the intestinal microbiota and increases the colonization of *Lactobacillus* spp. in ileum of chicken (Nava *et al.*, 2009). These changes in the intestinal microbiota and the increase in *Lactobacillus* populations show that organic acid can be used as an alternative to antibiotics to reduce pathogenic bacteria in the gastrointestinal tract (Nava *et al*., 2009).

2.4 Supplemental effect of probiotic on poultry

Supplementing the ratio with antibiotics growth promoters could increase growth performance of animals. Various mechanisms have been proposed which are include: (a) the nutrients are more efficiently absorbed and less are utilized by the gut, (b) more nutrients are available to the host because of a reduced intestinal microflora, (c) there is a reduction in harmful gut bacteria, (d) production of growth suppressing toxins or metabolites is reduced, (e) microbial de-conjugation of bile acids is decreased (Ohimain *et al.*, 2012). But, with increasing concerns about antibiotic resistance, the ban on sub-therapeutic antibiotic usage, there is increasing interest in finding alternatives to antibiotics for poultry production and using probiotics is an approach that has potential to reduce enteric disease in poultry and subsequent contamination of poultry products (Patterson *et al.*, 2003). However, it is possible to promote growth of broiler chickens and achieving both enhanced performance and good health by using alternatives such as probiotics. Probiotics are live microorganisms that affect the host animal by improving its intestinal balance (Fuller, 2001). Furlan (2005) mentioned that the probiotic mode of action is related to the competition for attachment sites. The bacteria present in the probiotic attach to the intestinal mucosa and blocks the attachment of pathogenic bacteria by forming a physical barrier.

Kabir *et al*. (2004) reported that experimental birds were fed with commercial ration with the addition of 2gm probiotics (Protexin® Boost)/10 litres drinking water upto 6th week of age. The result evidenced that the live weight gains obtained were

significantly (P<0.01) higher in experimental birds as compared to control ones at all levels during the period of 2nd, 4th, 5th and 6th weeks of age, both in vaccinated and non-vaccinated bird.

2.4.1 Effect of probiotic on growth performance

Liu *et al.* (2012) reported that 1 ml *Bacillus licheniformis* supplementation showed a significant increase in the body weight compared with the control group (P<0.05) and this positive effect of probiotic on body weight persisted until 6 weeks of age (P<0.05). In addition, body weight of cocks treated by 2 ml *Bacillus licheniformis* supplement was significantly increased compared to the control group on 1, 3 and 5 wks. (P<0.05). It can be noticed that the two levels of probiotic groups showed significant increase in the body weight compared with the control group during the grower periods 3 to 6 wks. (P<0.05). The 1 ml *Bacillus licheniformis* treatment group showed a significant increase in the daily weight gain at 4 and 6 weeks of age (P<0.05), but had a decrease of BWG compared with controls on 5 wks. (P<0.05). Moreover, no significant differences in growth performance were found between the 1 ml *Bacillus licheniformis*-treated birds and the control birds in the entire experimental period. Whereas, the hens fed on the 2 ml *Bacillus licheniformis* had a greater average daily weight gain than control hens over weeks 0 to 4 and 0 to 6 (P<0.05).

Ahmad and Taghi (2006) reported that improvement in weight gain when broiler diet was supplemented with probiotics (*Bacillus subtilis* and *Bacillus licheniformis*) during 21-42 days' period.

Ignatova *et al.* (2009) conducted a research to evaluate effects of dietary inclusion of probiotics on chicken's performance. Two hundred one old male white plymouth rock-mini chickens were studied for this research purpose. However, results revealed that probiotic supplementation has positive effects on final body weight by 14.4% (P<0.001), increased feed intake by 7.7%, and improved feed utilization by 8.1%. Several studies have been conducted to evaluate the ability of probiotics to change the type and number of the microflora in the digestive tract and results show that dietary supplementation of probiotic have a positive effect on growth performance and would significantly increase ADG and FCR in broiler chicks receiving probiotics.

Cavazzoni *et al.* (1998) evaluated performance of broiler chickens supplemented with *Bacillus coagulans* as probiotic and found that feeding probiotic supplements increase the growth rate of broilers.

Kaoud (2010) who reported significant increase in BWG of broilers fed probiotic mixture containing *Lactobacillus acidophilus*, *Lactobacillus sporogenes* and *Saccharomyces cerevisiae* at 0.5g/ kg feed at 6 weeks of age.

Kabir *et al.* (2004) conducted a 6-week growth performance study with broilers and found that live weight gains and carcass yields were significantly higher in broilers fed probiotic supplementation. Probiotics are reported to prevent colonization gut by pathogens like *Escherichia coli* and *Salmonella.*They also prevent contamination of carcasses by intestinal pathogens during processing and promote higher growth rate and feed conversion efficiency in growing chickens.

Singh and Sharma (1999) studied the effect of different levels (0.02, 0.03 and 0.04%) of probiotics (*Lactobacillus sporogenes*) on commercial broilers and observed highest weight gain in broilers offered diet supplemented with 0.02 % probiotics.

Mahajan *et al*. (2000) studied the effect of probiotics feeding during summer and winter season on the growth performance and carcass quality of broilers and observed significantly ($P<0.05$) higher body weight for lacto-saccharide fed broilers as compared to control during summer season.

Naik *et al.* (2000) evaluated the effect of different probiotics (*Lactobacillus acidophilus, Saccharomycs cerevisiae* and their combination) on the performance of broilers and reported that supplementation of both *Lactobacillus* and *Saccharomyces* individually to the basal diet at 0.05% improved body weight gain in broilers.

Safalaoh *et al.* (2001) shown that effective microorganisms (probiotics) had growth promoting and hypo cholesteraemic effects as potential alternative to antibiotics in broiler diets.

Chitra *et al.* (2004) conducted an experiment to study the effect of probiotics and ascorbic acid supplementation independently either in feed or in drinking water on production performance of broilers in summer season and observed that inclusion of probiotic and ascorbic acid both independently and simultaneously either in feed or in

drinking water to broilers had made significant (P<0.01) improvement in body weight of commercial broilers.

Das *et al.* (2005) reported no significant (P>0.05) difference in dressed weight and blood parameters in broilers after supplementation of commercial probiotics preparation.

2.4.2 Effect of probiotic on feed consumption

Shim *et al.* (2012) reported that supplementation of probiotic decreased the feed intake significantly (P<0.05) as compared to control group. Increased villus height and crypt depth in the birds of probiotics supplemented group improved the nutrient absorption and this may be the possibly reason for lower feed intake with improved growth performance in the birds of treatment groups.

Panda *et al*. (2006) and Rada *et al*. (2013) did not found significant difference in feed intake between control and probiotic supplemented groups.

Manoj *et al.* (2018) reported that the supplementation of *Bacillus subtilis* (1miilion/g of finished feed) resulted in highly significant (P<0.01) increase in the body weight of birds as compare to control (T_1) during 4th and 5th weeks of experiment. The feed consumption recorded lower in T_4. The addition of *Bacillus subtilis* based probiotic and AGP showed highly significant (P<0.01) variation regarding weekly FCR during 3^{rd} and 4^{th} week.

Molnar *et al.* (2011) reported that the feed consumption of all experimental groups was high because a one-phase meal diet was fed throughout. There was a significant tendency towards a lower feed conversion ratio in the groups consuming *Bacillus subtilis* supplemented diets, compared with the control group.

Jin *et al.* (1996)) found that feed consumption and feed conversion were not improved with addition of *Bacillus subtilis* when it was combined with other probiotics in broilers.

Santoso *et al.* (2001) reported that 0.5% fermented product from *Bacillus subtilis* inclusion reduced feed consumption.

Babazadeh *et al*. (2011) indicated that probiotics did not have any significant positive effect on broilers FI, Body Weight (BW) and Feed Conversion Ratio (FCR).

Zhang and Kim (2014) reported an increase body in FI in chicken fed with multi strain probiotics compared with that in control group fed basal diet.

2.4.3 Effect of probiotic on FCR

Manoj *et al.* (2018) reported that the effect of supplementation of probiotics on weekly feed conversion ratio and cumulative FCR at the end of starter phase was significantly ($P<0.01$) better in groups fed diet supplemented with probiotics irrespective of concentration as compare to birds fed control and AGP supplemented groups.

Liu *et al.* (2012) reported that feed intake for 0 to 3, 3 to 6 and 0 to 6 weeks was not influenced by 1 ml *Bacillus licheniformis* provision, whereas, the broiler supplemented by 2 ml *B. licheniformis* consumed significantly more feed than other two groups ($P<0.05$). Probiotic treatment groups showed lower FCR than the control group over 3 to 6 and 0 to 6 wks. ($P<0.05$). However, there was no significant difference in means of FCR among three groups over 0 to 3 weeks. Improvement of feed conversion ratio was evident in *B. licheniformis* treated groups over control in growing period.

Shim *et al.* (2012) and Sabatkova *et al.*, (2008) reported that supplementation of broiler feed with *Bacillus subtilis* and *B. licheniformis* improved the feed conversion efficiency.

Panda *et al.* (2006) reported significantly better feed conversion efficiency in white leghorn breeders' stock during 25-40 weeks of age of birds with dietary inclusion of *Bacillus subtilis* and *B. licheniformis* at the rate of 6×10^8 spores per kg of diet.

Salim *et al.* (2013) reported that better feed conversion ratio in broiler chicken fed diets with probiotic as compared to birds of antibiotic and control groups. The inclusion of desirable microorganisms (probiotics) in the diet allows the rapid development of beneficial bacteria in the digestive tract of the host, improving its performance. As a consequence, there is an improvement in the intestinal environment, increasing the efficiency of digestion and nutrient absorption processes (Pelicano *et al.*, 2004).

Manickam *et al.* (1994) reported significantly (P<0.05) lower feed conversion efficiency for probiotics supplemented treatment (2.36±0.01) as compared to control (2.55±0.01).

Liu *et al.* (2012) reported that supplementation of broilers with *Bacillus licheniformis* increased the BWG and FCR during both grower and overall phases (P<0.05), suggesting an improved intestinal balance of microbial population in probiotic treatments.

2.4.4 Effect of probiotic on internal organ

Ghari *et al.* (2013) reported that the weight of small intestine was significantly greater (P<0.05) in the probiotic- supplemented group than that in the control group and other treatment groups. The weight of heart was increased (P<0.01) in the prebiotic-supplemented group compared with that of the control group and other treatment groups. In addition, the absolute weights of gizzard, proventriculus, spleen, cecum and bursa did not show any significant differences among the dietary treatments.

Ghari *et al*. (2013) reported that the weight of heart, liver, small intestine, pancreas relative to the BW tended to be lower (P<0.01) for synbiotic-fed birds than those of control group and other product-fed birds. The relative weight of heart, liver and small intestine were significantly greater (P<0.01) for probiotic compared with synbiotic-fed birds. In addition, the relative weights of proventriculus, cecum, spleen, and bursa remained unaffected by dietary supplementations**.**

Manoj *et al.* (2018) reported that the weight of liver, heart and intestine and the weight of different cuts (thigh, wing, and back) as percent of live weight accounted non-significant variations among different groups. However, the weight of breast as per cent of live weight was highly significant (P<0.01) between the groups and found maximum in T_4 group. Molnar *et al.* (2011) reported that the groups given *Bacillus subtilis* supplementation produced relatively bigger breasts, and smaller carcasses and thighs, compared with the control group.

Molnar *et al.* (2011) reported that the carcass yield was decreased by the *Bacillus subtilis* supplementation. The treated groups had relatively smaller carcasses compared with the control group. Breast yield was higher in the *Bacillus subtilis* supplemented groups, and the thigh meat yield was lower than in the control group,

but not significantly. There was no effect of supplementation on carcass, breast and thigh yields, or the abdominal fat content.

Awad *et al.* (2009) reported that the absolute and relative weight of spleen and thymus tended to be greater (P<0.1) for the probiotic-supplemented group compared with the synbiotic-supplemented group.

Awad *et al.* (2009) reported that spleen weight was higher in the probiotic supplemented group. Lymphoproliferative responses to phyto hemagglutinins-p were registered higher (P<0.05) in chickens treated with higher concentration of *Bacillus subtilis* compared with ZnB and control groups. On day 35, liver, spleen and thymus weighed more (P<0.05) in the supplemented groups compared with control. On day 35, the thymus cortical width in BS-0.1 and the medullary area in BS-0.05 improved (P<0.05) compared with control. Compared with control, the germinal centre area of spleen was increased (P<0.05) in BS-0.1 and BS-0.05 groups on day 35. The results showed enlarged (P<0.05) bursal follicular area on day 21, and bursal follicular length on day 35 in BS-0.1 group compared with ZnB and control. Compared with control, the bursal follicular area was greater in BS-0.1 and BS-0.05 groups on day 35. On day 21, in comparison with control and ZnB, the thymus cortical width and cortex/medulla ratio increased (P<0.05) in BS-0.1 group.

2.4.5 Effect of probiotic on carcass quality

Molnar *et al.* (2011) reported tha 20 ecies supplemented group had significantly (P<0.05) higher breast yield than the control group.

Kabir *et al.* (2004) reported that a significantly (P<0.01) higher carcass yield occurred in broiler chicks fed with the probiotics on the 2nd, 4th and 6th week of age both in vaccinated and non-vaccinated birds. The weight of leg was found significantly (P<0.01) greater for experimental birds as compared to control ones on the 2nd, 4th and 6th week of age. A significantly (P<0.01) higher breast weight in broiler chicks fed with the probiotics was observed on the 4th and 6th week of age. Analogously a significantly (P<0.05) higher breast portion weight was found in experimental birds as compared to control ones during 2nd week of age. The antibody production was found significantly (P<0.01) higher in experimental birds as compared to control ones.

Mahmoud *et al*. (2017) did not found statistically significant difference in carcass yield between birds of probiotic supplemented group and control.

Pelicano *et al*. (2003) observed that probiotic use in broiler diets lowered the dressed carcass and back yields and increased leg yield while wing and breast yield remained similar across treatment groups.

Kaoud (2010) and Swain *et al.* (2012) who found that the eviscerated yield and weight of cut up parts (breast yield) were increased ($P<0.05$) in chicks fed diet supplemented with probiotic-yeast mixture 1.0g/ kg feed.

Jensen and Jensen (1992) studied a positive effect of probiotics containing *Bacillus licheniformis* and *Bacillus subtilis* spores on the flavor of broiler meat after cooling for 5 days. However, Loddi *et al.* (2000) found out in his studies that probiotics fed with water and feed did not had any effect on sensory characteristics of meat.

Pelicano *et al.*, (2003) reported that significant ($P<0.05$) improvement in meat flavor feeded with probiotics.

Ceslovas *et al*. (2005) stated that probiotic supplementation significantly ($P<0.05$) increased the meat tenderness and meat quality. Most of the carcass characteristics are directly proportional to the increased body weight at the time of slaughter. Anna *et al.* (2005) observed no significant ($P>0.05$) difference in carcass % between probiotic treated and untreated treatments on the sensory parameter basis.

Ghari *et al*. (2013) reported that the carcass weight was significantly higher in synbiotic treated group compared with control and phosphomycin treated groups ($P<0.05$), and it was significantly increased for prebiotic and probiotic compared with that of control treatment ($P<0.05$). No significant differences on carcass weight were found between synbiotic, prebiotic and probiotic treatments with each other ($P>0.05$). Birds supplemented with the synbiotic had a greater ($P<0.01$) live weight compared with that of control and other treatments. Moreover, prebiotic supplemented birds had a greater ($P<0.01$) live weight than probiotic and phosphomycin supplemented birds. However, birds supplemented with the probiotic had a greater live weight than that of phosphomycin supplemented birds but the difference was not significant ($P>0.05$). Both probiotic and phosphomycin increased live weight ($P<0.01$) compared with that of the control group.

Awad *et al.* (2009) reported that the relative liver weight was greater ($P<0.05$) for probiotic-fed birds compared with synbiotic-fed birds. Additionally, the weight of small intestine was greater for either probiotic or symbiotic-fed birds than the controls. Dietary treatments influenced the histo-morphological measurements of small intestinal villi. The addition of either probiotic or synbiotic increased ($P<0.05$) the villus height: crypt depth ratio and villus height in both duodenum and ileum. The duodenal crypt depth remained unaffected ($P>0.05$). However, the ileal crypt depth was decreased by dietary supplementations compared with control.

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). 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. This hypothesis can be confirmed by other studies that showing the inclusion of *Aspergillus awamori* and *Saccharomyces cerevisiae* in chicken feed reduced blood saturated fatty acids and increased the polyunsaturated (Saleh *et al.*, 2012). Another similar study of Liu *et al.* (2012) showed that treatment with *Bacillus licheniformis* significantly increased the protein content and the respective essential and aromatic amino acids (Liu *et al*., 2012). Feed containing *Bacillus licheniformis* improves meat color, juiceness and flavor of broiler chickens (Liu *et al.*, 2012). These factors are very important in terms of consumer appreciation especially the color.

Liu *et al.* (2012b) reported that the chicken breast fillet of two *Bacillus licheniformis* treatments appeared to have higher protein and amino acid content. Treatment of 2 ml *Bacillus licheniformis* supplement had a lower fat percentage in both probiotic treatments, which suggested that *Bacillus licheniformis* intakes made the main contribution to chemical, nutritional and sensorial quality improvements.

2.4.6 Effect of probiotic on microbial load

Chichlowski *et al.* (2007) reported that a probiotic containing lactobacilli *Bifidobacterium thermophilum* and *Enterococcus faecium* increased the jejunal villus height and decreased the villus crypt depth compared with salinomycin and control.

Samanya and Yamauchi (2002) reported that longer villi were found in the ileum of adult male layers with slight improvement in feed efficiency after dietary addition of *Bacillus subtilis* var. *natto.*

Kabir (2009) stated that probiotic effects on intestinal microflora and pathogen inhibition, intestinal histological changes, immunomodulation, some haemato-biochemical parameters and subsequently improve growth performance of broilers. He also mentioned that probiotic improves sensory characteristics of dressed broiler meat and microbiological meat quality of broilers. However, it is mentioned that the main effect of probiotic is in the gastrointestinal tract and associated with its capacity to stimulate the immune response and to control the growth of pathogenic bacteria.

Panda *et al.* (2006) studied the effect of dietary supplementation of probiotics on growth and gut microflora of broilers and no significant ($P>0.05$) effect on body weight gain was reported, however a significant ($P<0.05$) decrease in *E. coli* count was reported.

Dibner and Richards, (2005) reported that there is a strong interaction between probiotics and the intestinal micro flora. Hence, this improvement in performance due to the action of probiotics on the micro flora can be interpreted in two ways: the first is related to the reduction in the utilization of nutrients by microorganisms and the second is the decrease of microbial metabolites that interfere with host growth. In addition, maintaining the integrity of the intestinal mucosa results in high energy requirements, and the decrease of pathogens and intestinal metabolites can also decrease intestinal cell turnover, resulting in more energy available for production. Finally, the reduction of opportunistic pathogens and subclinical infections can also be associated with the use of probiotics.

Song (2014) reported that probiotic mixture contained *Bacillus licheniformis, Bacillus subtilis,* and *Lactobacillus plantarum* increased ($P<0.05$) the viable counts of Lactobacillus and Bifidobacterium, decreased ($P<0.05$) viable counts of coliforms, and tended ($P<0.10$) to decrease viable counts of Clostridium.

Molnar *et al.* (2011) reported that the higher inclusion rate of *B. subtilis* did not increase Lactobacillus concentrations in the ileum or in the caecum, but decreased the *E. coli* population significantly. The appearance of increased diffuse lympho histiocytic infiltration and solitary lymphoid follicles in the mucosa, and a stronger

response to NDV vaccination, indicate increased immunological responses in chickens fed with the *Bacillus subtilis* supplemented diet.

Molnar *et al.* (2011) reported that there was no difference in the caecal populations of Lactobacillus, *E. coli* and Clostridium between the control and the *Bacillus subtilis* supplemented chickens. *Salmonella* could not be isolated from any birds. The *Lactobacillus* population in the ileum of broilers fed *Bacillus subtilis* incorporated feed was significantly ($P<0.05$) lower than that of the control. A significantly ($P<0.01$), two-fold lower population of intestinal *E. coli* was also found in chickens fed with the *Bacillus subtilis* supplemented diet. The ratio of *Lactobacillus* and *E. coli* was influenced positively by the administration of *Bacillus subtilis.*

Jin *et al.* (1996) found an increase in the number of *Lactobacillus*, and they did not find a decrease in the *E. coli* population in the intestine of broilers fed with a diet supplemented with *Bacillus subtilis*.

Sinolsena *et al*. (2012) stated that at day 35, birds supplemented with increasing levels of *B. subtilis* showed decrease in caecal *Clostridium* and *Coliform* count (linear, $P<0.05$). supplementation of *B. subtilis* increased (linear, $P<0.05$) villus height and villus height to crypt depth ratio in both duodenum and ileum.

CHAPTER-3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1 Statement of the experiment

The research work was conducted at Sher-E-Bangla Agricultural University, Poultry Farm, Dhaka, with 150-day-old chick for a period of 28 days from 11th April to 9th May, 2019 to assess the probability of using probiotic *(Bacillus subtilis* and *Bacillus licheniformis)* in commercial broiler diet on growth performance, carcass traits and antimicrobial activity of broilers. The experiment was performed by applying different concentration levels of probiotic.

3.2 Collection of experimental broilers

A total of 150-day old chicks of "Cobb-500" strain having 44±0.2g average body weight were obtained from Kazi farm limited hatchery, Gazipur, Dhaka.

3.3 Experimental materials

The collected chicks were carried to the university poultry farm after midnight at 1.30 a.m. They were kept in electric brooders equally for 3 days by maintaining standard brooding protocol. During brooding time only basal diet was given no probiotic was used as treatment. After three days, 120 chicks were selected from brooders and distributed randomly in 4 dietary treatments of probiotic; remaining 30 chicks were distributed randomly in one treatment for control. For proper handling and data collection, the chicks of each treatment group were divided into three replications and in each replication of dietary treatment, there were 10 birds (Table 1). After 28 days of nursing and feeding, data were collected for the following parameters: feed intake, live weight, body weight gain, feed conversion ratio, carcass characteristics, bacterial load in the caecum, profit per bird and benefit-cost ratio.

3.4 Experimental treatments

The probiotic was mixed properly with commercial dietary feed at four different inclusion level. The experimental treatments were followings:

T_0 = No probiotics in basal diets/ control group

T_1 = 20g *Bacillus subtilis* probiotic/metric ton of the feed

T_2 = 50g *Bacillus subtilis* probiotic/metric ton of the feed

T_3 = 20g *Bacillus licheniformis* probiotic/metric ton of the feed

T_4 = 50g *Bacillus licheniformis* probiotic/metric ton of the feed

Table 1. Lay out of the experiment

Treatment groups	No. of replications			Total
	R_1	R_2	R_3	
T_0	10	10	10	30
T_1	10	10	10	30
T_2	10	10	10	30
T_3	10	10	10	30
T_4	10	10	10	30
Total	50	50	50	150

3.5 Collection of probiotics

It was not easy to collect the probiotics *Bacillus subtilis* and *Bacillus licheniformis* because of its unavailability in the market of our country. The probiotics were collected from 11th international poultry show and seminar-2019 stall which was held in international convention city Bashundhara, Dhaka. Beijing challenge group which is the world-famous probiotic producing company were provided the probiotics during my research work.

3.5.1 Description about probiotics

The probiotic *Bacillus subtilis* and *Bacillus licheniformis* is brown in color. Its original package was 25kg/bag or 25kg/drum but the collected sachet containing probiotic were 30g/sachet and 50g/sachet. *Bacillus subtilis* and *Bacillus licheniformis* probiotic containing 1×10^{11} CFU/g. The type of collected probiotic was powdery in form.

3.6 Preparation of experimental house

The broiler shed was an open sided natural house. It was a tin shed house with concrete floor. The experimental room was properly cleaned and washed by using tap water. All the equipment of the broiler house was cleaned and disinfected. There was 1ft. side wall around the shed with no ceiling. The floor was above 1ft. from the ground and the top of the roof was above 15ft. from the floor. The house was

disinfected by n-alkyl dimethyl benzyl ammonium chloride (Timsen^{TM}) solution before starting the experiment. After proper drying, the house was divided into pens as per lay-out of the experiment by polythene sheet so that air cannot pass one pen to another. The height of pens was 5 ft. Before placement of chicks the house was fumigated by formalin and potassium permanganate @ 500 ml formalin and 250 g potassium permanganate (i.e. 2:1) for 35 m^3 experimental area. Rice husk was used as a litter material to keep free the floor from moisture.

3.7 Experimental diets

Starter Nourish and grower fresh commercial broiler feed were purchased from the local market (Table 2 and Table 3).

Table 2. Name of components present in starter ration

Starter diet	Minimum percentage (%)
Arginine	1.26
Ash	8.0
Cysteine	0.40
Fat	6.0
Fiber	5.0
Lysine	1.20
Methionine	0.49
Protein	21.0
Threonine	0.79
Tryptophan	0.19

Table 3. Name of components present in grower ration

Grower ration	Minimum percentage (%)
Ash	8.0
Cysteine	0.39
Fat	6.0
Fiber	5.0
Lysine	1.10

Grower ration	Minimum percentage (%)
Methionine	0.47
Protein	19.0
Threonine	0.75
Tryptophan	0.18
Arginine	1.18

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

3.8 Management procedures

Different aspects of the management of chicks, experimental events and management procedures are described in detail below:

3.8.1 Litter management

High absorbing bedding material was used as litter on floor. Fresh, clean and sun-dried rice husk was used as shallow litter to absorb moisture from fecal discharge of broiler chicken. The shallow litter was 5 cm (2 inch) in depth. About 250g calcium oxide powder was mixed with rice husk in every pen as disinfectant. At the end of each week the litter was harrowed to prevent accumulation of toxic gases and to reduce moisture and parasitic infection. At 3^{rd} and 4^{th} week of rearing period, droppings were cleaned from the surface level by removing a thin layer of litter and same amount new litter was placed in each pen.

3.8.2 Receiving of day-old chicks

Just after arrival of day-old chicks to the poultry house the initial weight of the chicks were recorded by a digital electronic balance, and distributed them under the hover for brooding. The chicks were supplied glucose water with vitamin-c to drink for the first 3 hours to overcome dehydration and transportation stress. Subsequently small feed particles were supplied on the newspapers to start feeding for the first 24 hours.

3.8.3 Brooding of baby chicks

Electric brooder was used to brood chicks. Due to hot climate brooding temperature was maintained as per requirement. Brooding temperature was adjusted (below 35^0C) with house temperature. So, when the environmental temperature was above the

recommendation, then no extra heat was provided. At day time only an electric bulb was used to stimulate the chicks to eat and drink. In brooding extra heat was not provided at day time except mid night to morning. Electric fans were used as per necessity to save the birds from the heat stress. Partitioning brooding was done due to different experimental treatment. Each brooder had one hover and a round chick guard to protect chicks and four portioning chambers. Sometimes day temperature was 31-37^{0}C. So, at that time there was no need of extra heat to brood the baby chicks, but at night a 100-watt bulb was used in each pen to rise up low temperature according to heat requirement of brooding schedule. The brooding temperature was checked every 2 hours later by digital thermometer to maintain the temperature of the brooder.

3.8.4 Room temperature and relative humidity

Daily room temperature (^{0}C) and humidity were recorded with a thermometer and a wet and dry bulb thermometer respectively. Daily of room temperature and percent relative humidity for the experimental period were recorded and presented in Appendix 1. Average of room temperature and percent relative humidity for the experimental period was recorded and presented in table 4.

Table 4. Average Temperature and Humidity

Week	Date	Temperature (^{0}C)		Humidity (%)	
		Avg. Maximum	Avg. Minimum	Avg. Maximum	Avg. Minimum
1st	11.04.19-18.04.19	36.45	28.2	71.5	50.75
2nd	19.04.19-25.04.19	39.64	26.03	75.57	32
3rd	26.04.19-02.05.19	38.49	28.03	91.14	47.14
4th	03.05.19-09.05.19	35.49	27.29	76.57	43

3.8.5 Feeding and drinking

Crumble feed was used as starter (0-2 wks.) and pellet feed for grower (3-4 wks.) ration. *Ad libitum* feeding was allowed for rapid growth of broiler chicks up to the end

of the four weeks. Fresh clean drinking water was also supplied *Ad libitum*. Feeds were supplied 3 times: morning, noon and night. Water was supplied two times daily: morning and evening. Left over feeds and water were recorded to calculate actual intake. Digital electronic balance and measuring plastic cylinder was used to take record of feed and water. Daily water consumption (ml) and weekly feed consumption (gm)/bird were calculated to find out weekly and total consumption of feed and water. All feeders and drinkers were washed and sun-dried before starting the trial. One plastic made round feeder and one drinker were kept in the experimental pen. Feeder and drinker size were changed according to the age of the birds. Feeders were washed at the end of the week and drinkers once daily.

3.8.6 Lighting

At night there was provision of light in the broiler house to stimulate feed intake and rapid body growth. Four (4) energy lights were provided to ensure 24 hours' light for first 2 weeks. Thereafter 23 hours' light and one-hour dark were scheduled up to marketable age. At night one-hour dark was provided in two times by half an hour.

3.8.7 Ventilation

The broiler shed was south facing and open-sided. Due to wire-net cross ventilation was easy to remove polluted gases from the farm. Besides, on the basis of necessity ventilation was regulated by folding polythene screen. The open space around the farm were favorable for cross ventilation.

3.8.8 Bio security measures

Bio-security is a set of management practices that reduce the potential for introduction and spread of diseases causing organisms. To keep disease away from the broiler, farm the following vaccination, medication and sanitation program was undertaken. All groups of broiler chicks were supplied Vitamin B-Complex, Vitamin-A, D, E, K, Vitamin-C, Ca and Vitamin-D enriched medicine and electrolytes.

3.8.9 Vaccination

The vaccines were collected from medicine shop (Ceva Company) and applied to the experimental birds according to the vaccination schedule. One ampoule vaccine was diluted with distilled water according to the recommendation of the manufacturer. The

cool chain of vaccine was maintained strictly up to vaccination. The vaccination schedule of broiler is shown in Table 5.

Table 5. Vaccination schedule

Age	Name of disease	Name of vaccine	Route of vaccination
0 day	Infectious Bronchitis + Newcastle Disease (IB+ND)	CEVAC BI L	One drop in eye
09 day	Gumboro (IBD)	CEVAC IBDL	Drinking water
17 day	Gumboro (IBD)	CEVAC IBDL	Drinking water

3.8.10 Medication

Vitamin-B complex, vitamin-A, D_3, and E were used against deficiency diseases. Electromin and Vitamin-C also used to save the birds from heat stress. The medication program is presented in the table 6.

Table 6. Medication programme

Medicine	Composition	Dose	Period
B-Com-Vit	Vitamin B-complex	2-5ml/1L water	3-5 days (all groups)
Renasol AD_3E (Vet)	Vitamin A, D & E	1 ml/5L water	3 -5 days (all groups)
Electromin powder	Electrolytes	1g/2L water	4 -5 days (all groups)
Revit-C	Vitamin-C Premix	1g/5L water	4 -5 days (all groups)
Calplex	Ca, P and Vit-D	10 ml/100 bird	3-5 days (all groups)

3.8.11 Sanitation

Proper hygienic measures were maintained throughout the experimental period. Cleaning and washing of broiler shed and its premises were under a routine sanitation work. Flies and insects were controlled by spraying phenol and lysol to the surroundings of the broiler shed. The attendants used farm dress and shoe. There was a provision of wearing polythene shoe at the entry gate of the broiler shed to prevent

any probable contamination of diseases. Strict sanitary measures were followed during the experimental period.

3.9. Recorded parameters

Weekly lives weight, weekly feed consumption and death of chicks to calculate mortality percent were taken during the study. FCR was calculated from final live weight and total feed consumption per bird in each replication. After slaughter carcass weight and gizzard, liver, spleen, bursa, intestine and heart were measured from each broiler chicken. Dressing yield was calculated for each replication to find out dressing percentage. Faecal sample was collected to measure microbial load in the gut.

3.10 Data collection

3.10.1 Live weight

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

3.10.2 Dressing yield

Dressing yield of bird was obtained from live weight subtracting blood, feathers, head, shank and inedible viscera.

3.10.3 Feed consumption

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

3.10.4 Survivability of chicks

Daily death record for each replication was counted up to 28 days of age to calculate mortality if occurred that indicated the survivability of the bird.

3.11. Dressing procedures of broiler chicken

Three birds were picked up at random from each replicate at the 28^{th} day of age and sacrificed to estimate dressing percent of broiler chicken. All birds to be slaughtered were weighed and fasted by halal method or overnight (12 hours) but drinking water was provided *ad-libitum* during fasting to facilitate proper bleeding. All the live birds were weighed again prior to slaughter. Birds were slaughtered by severing jugular

vein, carotid artery and the trachea by a single incision with a sharp knife and allowed to complete bleed out at least for 2 minutes. Outer skin was removed by sharp scissor and hand. Then the carcasses were washed manually to remove loose singed feathers and other foreign materials from the surface of the carcass. Afterward the carcasses were eviscerated and dissected according to the methods by Jones (1982). Heart and liver were removed from the remaining viscera by cutting them loose and then the gall bladder was removed from the liver. Cutting it loose in front of the proventiculus and then cutting with both incoming and outgoing tracts removed the gizzard. Giblet were collected after removing the gall bladder. All the carcasses were washed with cold water inside and out to remove traces blood, loosely attached tissue or any foreign materials. Then the eviscerated weight of carcasses was recorded. Thereafter the weight of carcass cuts such as breast, thigh (both), drumstick (both), back, neck, wing (both), heart, liver, gizzard was taken. Dressing yield was found by subtracting blood, feathers, head, shank, liver, heart and digestive system from live weight. Liver, heart, gizzard and neck were considered as giblet. Percent of breast, thigh, drumstick, back, wing, giblet and abdominal fat were found as DP by the following formula-

$$\text{DP} = \frac{\text{Dressing yield (g)}}{\text{Live weight (g)}} \times 100$$

Dressing yield = Breast, thigh, drumstick, back, wing, giblet, abdominal fat weight

3.12. Estimation of *Escherichia coli* (*E. coli*) population in broiler caecum

The population of *Escherichia coli* was estimated as CFU g^{-1} (colony forming unit). EMB agar (eosin methylene blue agar) was used to culture the *E. coli* bacteria. EMB (Company name- HIMEDIA EMB agar) agar was purchased from Hatkhola Scientific Market, Dhaka. The composition of HIMEDIA EMB agar is presented in table 7.

Table 7. Composition of EMB agar

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.00
Dipotassium phosphate	2.00
Lactose	5.00

Sucrose	5.00
Eosin – Y	0.40
Methylene blue	0.065
Agar	13.50

3.13. Estimation of *Salmonella* population in broiler caecum

The population salmonella was estimated as colony forming unit (CFU)/g. *Salmonella-shigella (SS)* agar was used to culture the salmonella bacteria. SS (Company name- HIMEDIA SS agar) agar was purchased from Hatkhola Scientific Market, Dhaka. The composition of HIMEDIA SS agar is given in table 8.

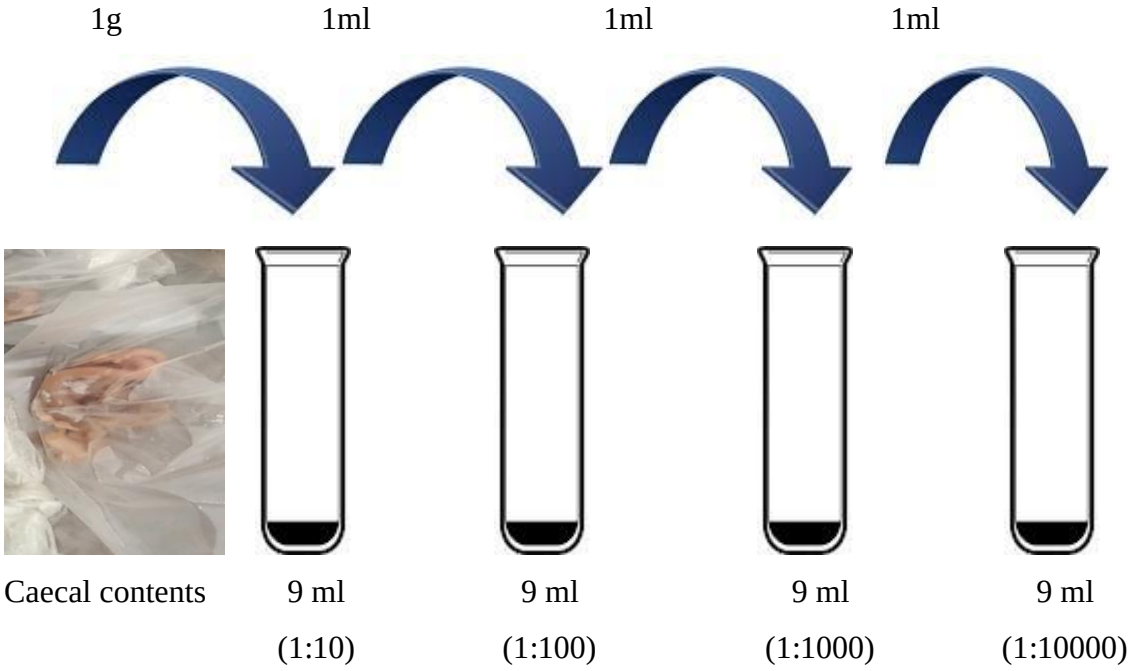
Table 8. Composition of SS agar

Ingredients	Gms / Litre
Beef extract	5.00
Enzymatic digest of casein	2.50
Enzymatic digest of animal tissue	2.50
Lactose	10.00
Bile salts	8.50
Sodium citrate	8.50
Agar	13.50
Sodium thiosulfate	8.50
Ferric citrate	1.00
Brilliant green	0.00033
Neutral red	0.025
Agar	13.50

3.14. Preparation of dilution

At the end of the experiment, 15 birds of each treatment group were slaughtered for extraction of caecal contents. Four sterilized test tubes with 9 ml of distilled water

were used. One gram of caecal content from each sample was mixed in 9 ml of sterilized distilled water in a test tube and shake well, its ratio was 1:10 and dilution factor was 10^1. Then 1 ml liquid was collected from 1:10 ratio in test tube and mixed in 9 ml of sterilized distilled water in a test tube. Its ratio was 1:100 and dilution factor was 10^2. Finally, 1:1000 and 1:10000 ration was made in same way and their dilution factor was 10^3 and 10^4 respectively. The dilution preparation is presented below:



3.15. Preparation of agar medium

Only 36 grams EMB and SS agar powder was mixed in 1000 ml distilled water. Mix until suspension was uniform. It was heated to dissolve the medium completely. Dispensed and sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. Then it was poured into the petri dish. It made cool to 50°C and shaked the medium in order to oxidize the methylene blue (i.e. to restore its blue colour) and to suspend the flocculent precipitate. One ml of liquid of 1:10000 ratio test tube was collected for each sample and poured to petri dish which was partially filled with EMB medium.

3.15.1 Incubation

Petri dishes were sent to bacterial growth chamber for 24 hours at 37^0C.

3.16 Calculations

Each data was collected by the following formulae:

3.16.1 Live weight gain

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

Body weight gain = Final weight – Initial weight

3.16.2 Feed intake

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

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

3.16.3 Feed conversion ratio

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

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

3.16.4 Dressing percentage

Dressing yield was found by subtracting blood, feathers, head, shank and digestive system from live weight. Liver, heart, gizzard and neck were considered as giblet. Dressing percentage of bird was calculated by the following formulae-

$$\mathbf{D}\text{P} = \frac{\text{Dressing yield (g)}}{\text{Live weight (g)}} \times 100$$

Dressing yield = Breast, thigh, drumstick, back, wing, giblet, abdominal fat weight

3.16.5 Bacterial colony count

After 24 hours *E. coli* and *salmonella* colonies were counted by colony counter and following formula was used to estimate *E. coli* and *salmonella* population-

$$\text{CFU/g} = \frac{\text{No. of colonies} \times \text{dilution factor}}{\text{Volume inoculated}}$$

3.16.6 Flock uniformity

Flock uniformity is a measure of the variability of bird size in a flock. Uniformity is differentiated between weak and healthy birds. At first individual weight of each bird was taken and then the flock uniformity was calculated by using the following formulae-

$$\text{Flock uniformity} = \frac{\text{Average weight -Total birds (Average weight of birds } \pm 10\%)}{\text{Average weight}} \times 100$$

Here, Average weight of birds = Birds weight/Total birds

3.17. Economic analysis

3.17.1 Profit per bird (PPB)

The benefit cost ratio was analyzed considering stocking density and feeding regime. The capital expenditure, recurring expenditure and depreciation cost were considered

to calculate total expenditure. The major expenditure included cost of chick, feed, litter, medicine, vaccine, and labor and electricity bill. The common expenditure per bird was found out from the total expenditure of one batch. The consumption of feed was not same in different replications, so feed expenditure was calculated for every individual replication. Similarly, due to differences of live weight gain, the sale value of birds was calculated for every individual replication. The sale value of poultry manure and feed bags were also considered to compute income. Number of live birds in each replication considered here to calculate average value. Finally, treatment wise production cost and income was calculated. Net profit per bird was found out by deducting the total expenditure from the total income according to replication under each treatment.

$$\text{PPB} = \text{Total income/b} - \text{total expenditure/b}$$

3.17.2. Benefit cost ratio (BCR)

The capital expenditure, recurring expenditure and depreciation cost were considered to calculate total expenditure. The major expenditure included cost of chick, feed, litter, medicine, vaccine, labor and electricity charges. The common expenditure per bird was found out from the total expenditure of one batch. The consumption of feed was not same in different replications, so feed expenditure was calculated for every individual replication. Similarly, due to differences of live weight gain, the sale value of birds was calculated for every individual replication. The sale value of poultry manure and feed bags were also considered to compute income. Number of live birds in each replication considered here to calculate average value. Finally, treatment wise production cost and income was calculated. Net profit per m^2 was found out by deducting the total expenditure from the total income according to replication under each treatment.

$$\text{BCR} = \frac{\text{Total income}}{\text{Total cost of production}}$$

3.18 Statistical analysis

Total data were complied, tabulated and analyzed in accordance with the objectives of the study. Excel Program was practiced for preliminary data calculation. The collected data was subjected to statistical analysis by applying one-way ANOVA using Statistical Package for Social Sciences (SPSS version 16.0) in accordance with the principles of completely randomized design (CRD). Differences between means were tested using Duncan's multiple comparison test, and significance was set at $P<0.05$.

Some photograph of chick management and experimental procedure is represented in plate 1-29 below:

Plate 1. Washing of floor by detergent

Plate 2. Washing of feeder and drinker

Plate 3. Preparation of broiler shed

Plate 4. Preparation of brooder

Plate 5. Collection of probiotic (*Bacillus subtilis* and *Bacillus licheniformis*)

Plate 6. Receiving of day old chick

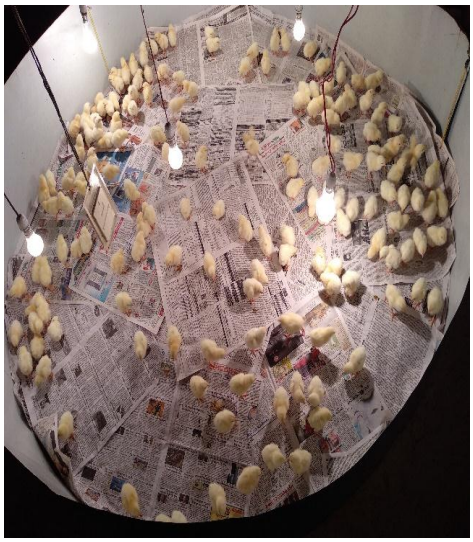


Plate 7. Arrival of day old chick

Plate 8. Collection and separation of broiler starter feed


Plate 9. Monitoring of research activities by the supervisor

Plate10. Vaccination of chick

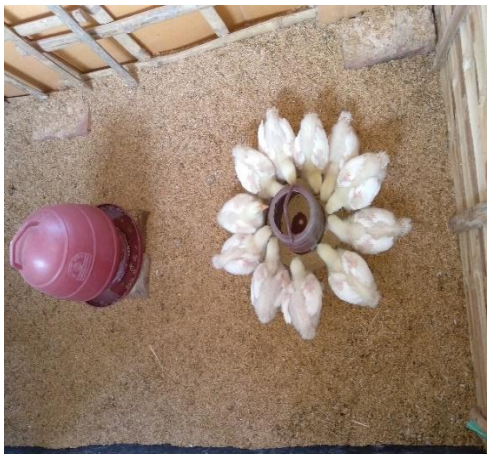


Plate 11. Giving starter feed of chicks

Plate 12. Taking weight of chicks at 2nd week

Plate 13. Taking weight of chicks at 3rd week

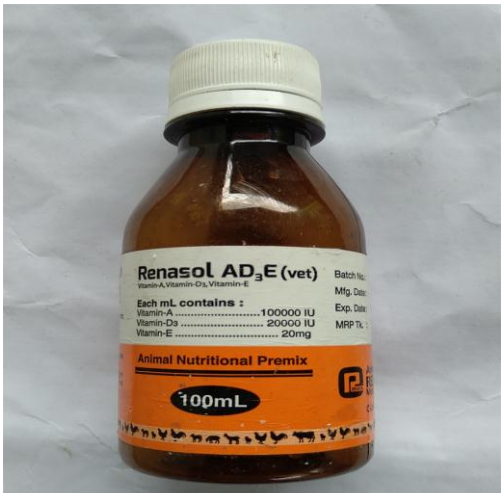


Plate 14. Vitamin B and vitamin AD_3E

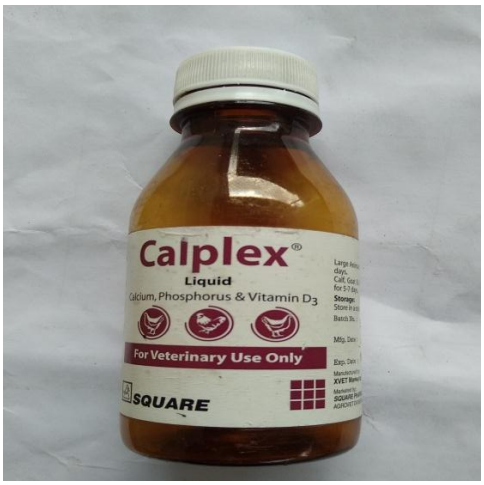


Plate 15. Electromin powder and calplex

Plate 16. IB and IBD vaccination vial

Plate 17. Collection of caeca from different treatment

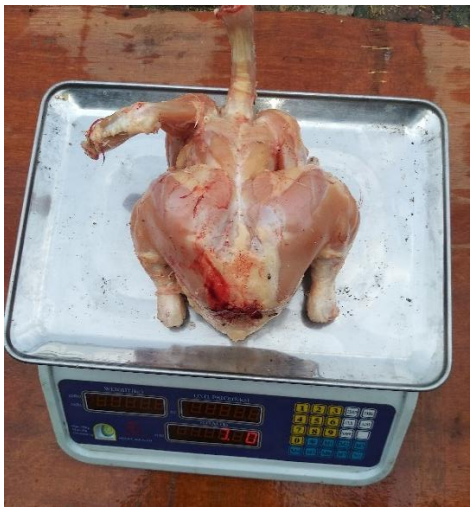


Plate 18. Taking weight of dressed broiler

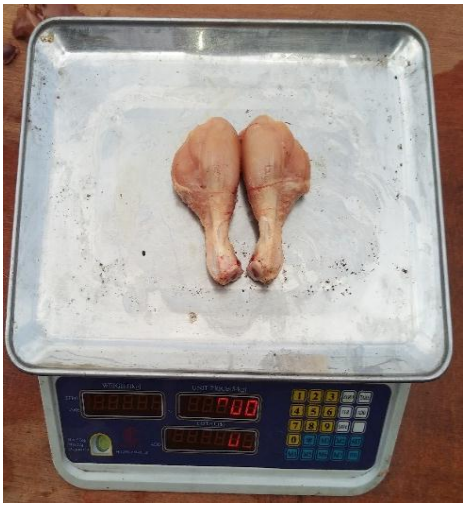


Plate 19. Taking weight of drumstick

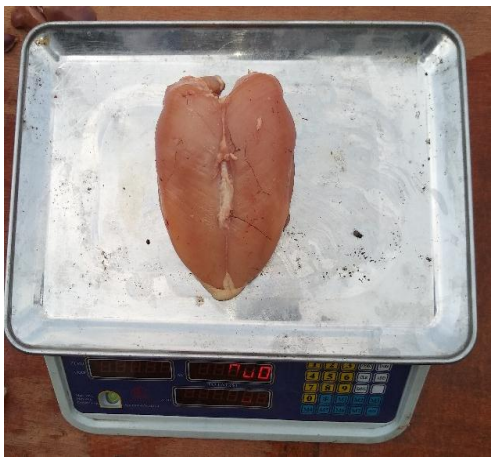


Plate 20. Taking weight of breast

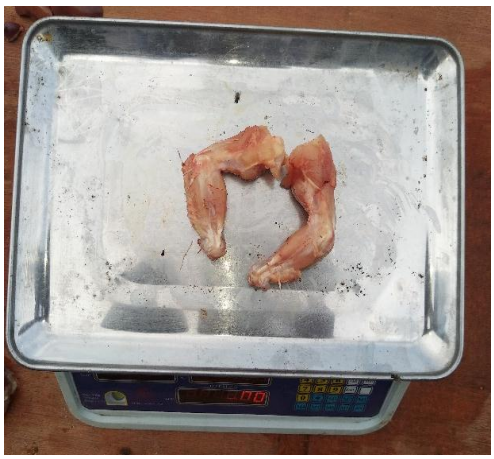


Plate 21. Taking weight of wings

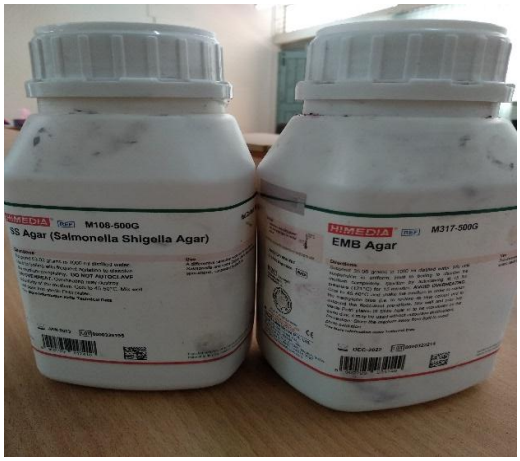


Plate 22. SS and EMB agar

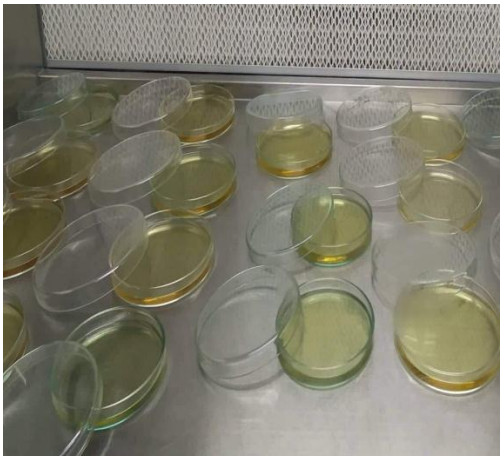


Plate 23. SS agar in petridishes

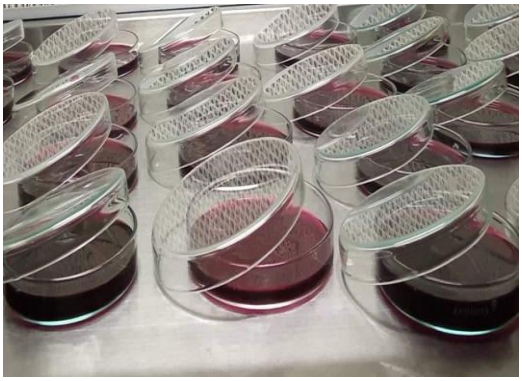


Plate 24. EMB agar in petridishes

Plate 25. Incubation of agar plate

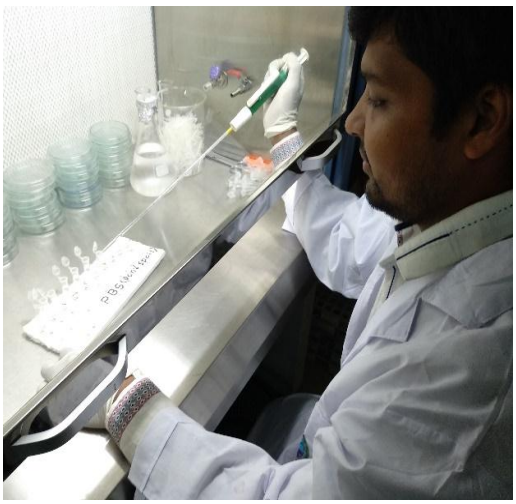


Plate 26. Dilution of the original sample

Plate 27. discarded sample from the last tube

Plate 28. Colonies of *E. coli* bacteria

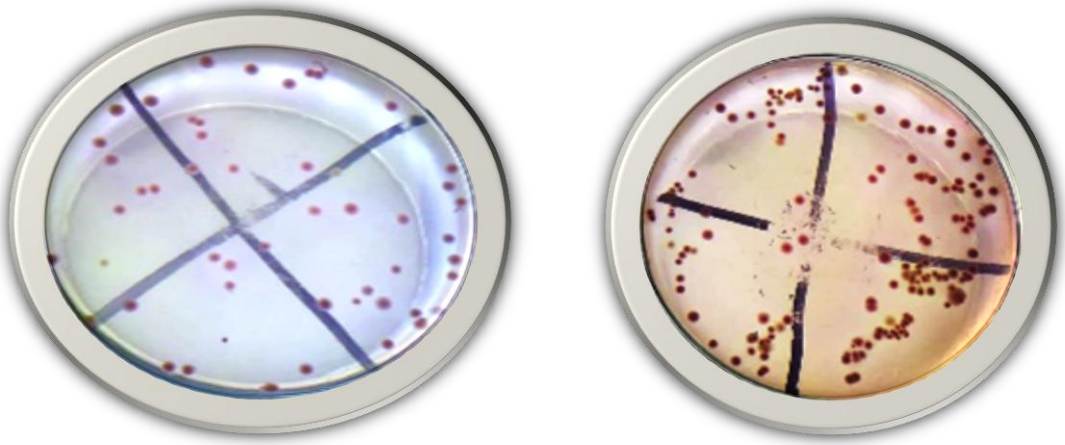


Plate 29. Colonies of *salmonella* bacteria

CHAPTER- 4

RESULTS AND DISCUSSION

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Production performances of broiler chicken

The health promoting effect of probiotic in the gastrointestinal tract has been mainly associated with their capacity to stimulate the immune response and to inhibit the growth of pathogenic bacteria. The chicks were randomly divided into five experimental treatment groups. The five groups were T_1 (20g BS/MT feed), T_2 (50g BS/MT feed), T_3 (20g BL/MT feed) and T_4 (50g BL/MT feed) and T_0 (control). The performance traits *viz.* final live weight, weight gain, feed consumption, FCR, dressed bird weight, relative giblet weight, survivability, flock uniformity, bacterial colony counts and economic impact on broiler rearing that includes production cost, profit per bird (PPB) and benefit cost ratio (BCR) were discussed in this chapter.

4.1.1 Final live weight

Data submitted in Table 9 and Figure 1. expressed that the effect of treatments on final live weight (gram per broiler chicken) was significant ($P<0.05$). The relative final live weight (g) of broiler chickens in the different groups T_0, T_1, T_2, T_3 and T_4 were 1472.14±38.51, 1592.07±35.81, 1560.00±26.70, 1601.57±29.18 and 1607.50±30.98 respectively. The highest result was found in T_4 (1607.50g) and lowest result was found in T_0 (1472.14g) control group and that was statistically significant ($P<0.05$). Results also expressed that the body weights also different among the treatment groups having statistical significance ($P<0.05$) and all the treated groups had higher body weight than control. The higher body weight in T_4 group might be due to the positive effect of probiotic (*Bacillus licheniformis*) supplementation.

These results are in agreement with those obtained by Deniz *et al*. (2011) who found that the probiotic supplementation had no significant ($P>0.05$) effect on body weight and mortality, but compared to the controls, the total body weight gain calculated for the whole experimental periods (6 weeks) was significantly increased in broilers supplemented with the *B. subtilis* spores ($P<0.05$) whereas the total feed intake was significantly reduced ($P<0.001$). Liu *et al.* (2012) reported that 1 ml *B. licheniformis* supplementation showed a significant increase in the body weight compared with the control group ($P<0.05$), and this positive effect of probiotic on body weight persisted

until 6 weeks of age (P<0.05). Another researcher Manoj Yadav *et al.* (2018) reported that the supplementation of *Bacillus subtilis* (1miilion/g of finished feed) resulted in highly significant (P<0.01) increase in the body weight of birds as compare to control (T_1) during 4^{th} and 5^{th} weeks of experiment.

4.1.2 Weekly body weight gains (BWG)

Body weight gains of broiler chicken at different weeks presented in Table 10 & Figure 2. The mean body weight gains (g) of broiler chicks in different groups T_0, T_1, T_2, T_3 and T_4 were 469.23±17.74, 529.63±29.11, 508.10±12.52, 504.50±27.85, and 561.80±11.03 respectively. The difference in average weekly body weight gain was highly significantly (P<0.05) amongst the treatment groups at 1^{st}, 3^{rd} and 4^{th} weeks than control group. At the 2^{nd} week there were also higher body weight gain value recorded in treatment groups than control, but was statistically insignificant (P>0.05). Higher weight gain was recorded in broiler chicks received higher concentration of *Bacillus licheniformis* (T_4) as compare to other treatment groups. Similarly, the difference in average weight gain was found to be highly significant (P<0.05) between treatment groups and control. Highest cumulative weight gain (1602.17g) was recorded in the birds of treatment group (T_4) and it was significantly (P<0.05) higher as compare to other groups. Weekly body weight gain increased gradually from 1^{st} to 4^{th} week and the possible cause of growth might be due to the influence of probiotics where the probiotics prevent colonization in gut from pathogen like *E. coli* and *salmonella*.

These results are in agreement with those obtained by Ahmad and Taghi (2006) who reported that improvement in body weight gain when broiler diet was supplemented with probiotics (*Bacillus subtilis and Bacillus licheniformis*) during 21-42 days' period. The improvement in body weight gain due to supplementation of *Bacillus licheniformis* based probiotics, in present experiment was associated with significantly better feed conversion ratio.

4.1.3 Feed consumption (FC)

All the treatment groups (Table 9) showed significant (P<0.05) differences in FC of broiler chicken and there was also significant difference (P<0.05) among the treatment group. Control (T_0) group consumed higher amount of feed (2173.93g±1.44) and T_4 (50g BL probiotic/MT of feed) treated group consumed

relatively lower amount of feed (2128.50g±9.04) whereas, T_1, T_2 and T_3 consumed 2150.97g±1.86, 2141.70g±1.21 and 2137.77g±1.57 feed respectively. Increased villus height and crypt depth in the birds of probiotic supplemented group improved the nutrient absorption and this might be the possible reason for lower feed intake.

Comparatively lower feed consumption observed in probiotics (*Bacillus subtilis* and *Bacillus licheniformis*) supplemented group in present experiment is in agreement with the results reported by earlier researchers (Shim *et al.,* 2012; Eseceli and Demir, 2010) that supplementation of probiotic decreased the feed intake significantly ($P<0.05$) as compared to control group. These results are in contradictory with panda *et al.* (2006) and Rada *et al.* (2013) who found that there was no significant difference in feed intake between control and probiotic supplemented groups. Another researcher Santoso *et al.* (2001) reported that 0.5% fermented product from *Bacillus subtilis* inclusion reduced feed consumption.

4.1.4 Weekly feed consumption (FC)

The average weekly feed intake due to supplementation of probiotics was significant ($P<0.05$) amongst the treatment groups at 1st and 4th week. During 2nd and 3rd week of experiment the effect of supplementation of probiotics on weekly feed intake was highly significant ($P<0.05$) as compare to control but there was no significant difference($P>0.05$) amongst the treatment group. The mean of weekly feed consumption of broiler chicks at the end of 4th week in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 829.90±1.42, 827.87±0.32, 823.50±0.58, 821.63±0.52, 813.97±6.57g correspondingly. The weekly feed intake at 4th week was significantly ($P<0.05$) low in group that fed probiotics *Bacillus licheniformis* in higher concentration (T_4) as compare to other groups.

These results are in harmony with those of previous researchers Manoj Yadav *et al.* (2018) who found that the average weekly feed intake due to supplementation of probiotics did not vary significantly amongst the treatment groups till 5th week of experiment. Salma *et al*. (2007) who found that feed intake of broilers did not differ significantly by dietary inclusion of probiotics.

4.1.5 Feed conversion ratio (FCR)

The feed conversion ratio (FCR) of broilers under different treatment groups have been shown in Table 9. The effect of supplementation of probiotics on cumulative

feed conversion ratio was significantly (P<0.05) better in groups fed diet supplemented with probiotics irrespective of concentration as compare to birds fed control. Feed conversion ratio (FCR) was significantly (P<0.05) lower (1.33±0.02) for bird supplemented with 5og BL/MT of feed at T_4 group than control birds (1.52±0.04). Statistically, there were no significant difference in feed conversion ratio (FCR) amongst the $T_{1,}$ $T_{2,}$ T_3 and T_4 group. The better FCR in probiotic supplemented group might be due to the rapid development of beneficial bacteria in the digestive tract of host and increasing the efficiency of digestion and nutrient absorption process.

These results are in harmony with those of previous researchers Shim *et al.* (2012) who had reported that supplementation of broiler feed with *Bacillus subtilis* and *B. licheniformis* improved the feed conversion efficiency. Similarly, Panda *et al.* (2008) reported significantly better feed conversion efficiency in white leghorn breeders stock during (25-40 wks. of age of birds) with dietary inclusion of *Bacillus subtilis* and *B. licheniformis* (at the rate of 6 x 10^8 spores per kg of diet).

4.1.6 Weekly feed conversion ratio (FCR)

The mean weekly FCR of broiler chicks in different groups were presented in Table 12 and Figure 4. The FCR of 1st, 3rd and 4th weeks were significantly (P<0.05) better than the control groups. The FCR of 2nd week was insignificant (P>0.05) among the treated groups with control also. At 4th week the FCR (1.45±0.04) was significantly (P<0.05) better in T_4 group fed higher concentration of *Bacillus licheniformis* probiotics as compare to control and others treatment 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. Liu *et al.* (2012) reported that feed intake for 0 to 3, 3 to 6 and 0 to 6 wks. was not influenced by 1 ml *B. licheniformis* provision, whereas, the broiler supplemented by 2 ml *B. licheniformis* consumed significantly more feed than other two groups (P<0.05).

4.1.7 Survivability

The survivability rate showed on Table 9. Survivability rate was statistically higher for the probiotics treated group (100±0.00) than the control group (86.67±3.33) but no significant (P>0.05) difference amongst them. The overall survivability (0-4 weeks) during the experimental period was higher in the treatment group. The variation in

mortality among the control group might be due to the seasonal influence of summer season. The another possible cause of survivability might be due to the development of immunity amongst the treatment group than control.

The mortality observed in the present study agreed with the report of Awad *et al*. (2009) who reported that lower mortality rate for probiotic supplemented group (3%) than the synbiotic supplemented group and control group (3.5%) in Ross 308 commercial broilers.

4.1.8 Dressing percentage (DP)

The 50g BL probiotic /MT of feed at T_4 supplemented group had significantly ($P<0.05$) higher carcass percentage (71.33±1.30%) compared with the control group (65.67±1.33%) whereas the T_1, T_2 and T_3 group were 68.73±0.82%, 70.07±2.07% and 68.97±0.42% respectively. The dressing percent of the carcass were ranged from 65.67% to 71.33%. The higher dressing percentage in T_4 groups might be due to the positive influence of probiotic (*Bacillus licheniformis*) by utilizing nutrients resulting in more energy available for production.

The present findings were higher than the report of Narasimha *et al*. who reported dressing yield (%) ranging from 63.67% to 66.67% in Cobb commercial broiler at 42 days of age. The carcass yield (%) in the present study ranged from 65.67% to 71.33% after 42 days of age which was similar with the value observed by Abdel-Raheem and Abd-Allah who reported 64.45 to 70.68% in Avian-48 broilers of 42 days of age.

4.1.9 Carcass characteristics

Carcass characteristics of the birds had shown in (Table 13). The weight of breast, thigh and drumstick was significantly ($P<0.05$) high in treatment group as compared to control. The weight (g) of breast was higher in T_4 group (392.83±7.86) compared to the other group which values were T_0 (318.90±15.81), T_1 (372.00±12.02), T_2 (351.13±5.84) and T_3 (365.10±11.80) correspondingly. But these values were also significantly different amongst the treatments. The weight (g) of thigh was higher in T_4 group (177.86±1.48) compared to the others group whose values were T_0 (164.16±2.20), T_1 (175.43±2.19), T_2 (170.13±0.99) and T_3 (170.10±1.66) correspondingly. Among the treatment the weight (g) of drumstick was significantly

($P<0.05$) high in T_4 group (176.33±2.07) as compared to other groups T_1 (164.10±4.60), T_2 (167.13±3.61), T_3 (162.76±3.04) and T_0 (162.73±0.77). On the other hand, the weight of wing in different groups showed that there were no significant ($P>0.05$) difference amongst the groups.

The findings corroborate with Molnar *et al.* (2013) who reported that *Bacillus* species supplemented group had significantly ($P<0.05$) higher breast yield than the control group. Increased carcass yield, drumstick and breast weight was also reported by Kabir *et al.* (2004), Farhoomand and Dadvend (2007). Whereas Mahmoud *et al.* (2017) did not found statistically significant difference in carcass yield between birds of probiotic supplemented group and control.

4.2 Relative giblet weight (liver, heart, neck and gizzard)

The relative weight of giblet in different groups were presented in Table 14. The relative weight (g) of liver of broiler chicken in different group T_0, T_1, T_2, T_3, and T_4 were 34.60±0.55, 37.73±0.84, 35.63±0.92, 36.20±1.03 and 41.80±0.92 respectively. The highest results were obtained in T_4 and lowest in T_0 group. There was statistically significant ($P<0.05$) difference in the relative weight of liver between the groups.

The relative weight of heart of broiler chicken in different group T_0, T_1, T_2, T_3, and T_4 were 7.13±0.06, 7.33±0.12, 6.87±0.08, 6.90±0.05 and 7.63±0.31 respectively. The highest results were obtained in T_4 and lowest in T_2 group. There was significant ($P<0.05$) difference in the relative weight of heart between the groups.

The relative weight (g) of neck was significantly ($P<0.05$) high in T_2 (40.83±1.41) and T_4 (43.13±0.21) group compared to T_1 (35.63±0.68), T_3 (37.90±0.35) and T_0 (34.73±1.12) groups. The result showed that there was significant difference ($P<0.05$) in neck weight amongst the treatment group.

The relative weight of gizzard in different groups showed no significant ($P>0.05$) difference. The relative weight (g) of gizzard in different groups were T_0 (38.00±2.25), T_1 (33.70±3.59), T2 (36.53±0.97), T_3 (42.33±3.40) and T_4 (41.67±4.82) respectively. The highest results were obtained in T_4 and lowest in T_1 group.

The present finding was in agreement with Ghari *et al.* (2013) reported that the weight of heart was increased ($P<0.01$) in the probiotic-supplemented group compared with that of the control group and other treatment groups. Another researcher Manoj Yadav *et al.* (2018) reported that the weight of liver, as percent of

live weight accounted non-significant (P>0.05) variations among different groups. Another researcher Abdel-Raheem *et al. (*2011) found that there was no significant (P>0.05) difference observed in the carcass traits with respect to carcass percentage, liver weight and gizzard weight in Cobb broilers under study.

4.3 Weight of intestine

The relative weight of intestine in different groups were presented in Table 14. The relative weight (g) of intestine of broiler chicken in group T_0, T_1, T_2, T_3, and T_4 were 91.29±5.60, 102.70±10.48, 98.60±10.57, 92.73±11.18 and 109.77±2.23 respectively. The highest results were obtained in T_4 and lowest in T_1 group. However, there was no significant (P>0.05) difference in the relative weight of intestine between the groups.

These results are contradictory with the findings of Ghari *et al.* (2013) reported that the weight of small intestine was significantly greater (P<0.05) in the probiotic-supplemented group than that in the control group and other treatment groups. Another researcher Manoj Yadav *et al.* (2018) reported that the weight of small intestine and the weight of different cuts (thigh, wing, and back) as percent of live weight accounted non-significant (P>0.05) variations among different groups.

4.4 Immune organs (spleen and bursa)

The relative weight of spleen and bursa in different groups were presented in Table 14. The relative weight (g) of spleen of broiler chicken in dietary groups T_0, T_1, T_2, T_3 and T_4 were 1.87±0.12, 1.97±0.14, 1.90±0.05, 1.87±0.21 and 1.90±0.10 respectively. The highest value was T_4 (1.90±0.10) and lowest value was T_0 (1.87±0.12) and T_3 (1.87±0.21) group. On the other hand, the relative weight of spleen in different groups showed that there was no significant difference (P>0.05) statistically among the groups.

The weight (g) of bursa was higher in T_0 group (1.97±0.40) compared to the other groups which values were T_1 (1.63±0.49), T_2 (1.90±0.10), T_3 (1.83±0.12) and T_4 (1.90±0.05) correspondingly. But these values were non-significant (P>0.05) among the treatments.

The present finding was in agreement with Awad *et al.* (2009) reported that the absolute and relative weight of spleen and bursa tended to be greater (P<0.1) for the probiotic-supplemented group compared with the synbiotic-supplemented group.

4.5 Intestinal microflora

The intestinal microflora mainly *E. coli* and *salmonella* were counted and the data were presented in Table 15. The microbial load in intestine was significantly (P<0.05) different in dissimilar group. *E. coli* count (*E. coli* (EMB) $\times 10^6$ (CFU/g)) was significantly (P<0.05) decreased in birds fed 20g BS/MT feed in T_1 group, 50g BS/MT feed in T_2 group, 20g BL/MT feed in T_3 group and 50g BL/MT feed in T_4 group (11.1±8.33, 11.1±8.14, 10.0±5.70 and 10.0±5.61 respectively) than the control birds (14.5±3.60) but there was no significant difference amongst the treatment group. *Salmonella sp.* count (*Salmonella* (SS) $\times 10^6$ (CFU/g)) was significantly (P<0.05) decreased in birds fed 20g BS/MT feed in T_1 group, 50g BS/MT feed in T_2 group, 20g BL/MT feed in T_3 group and 50g BL/MT feed in T_4 group (7.47±3.38, 6.93±6.17, 6.53±5.92 and 5.87±1.76 respectively) than the control birds (8.27±3.48) and there was significant difference amongst the treatment group. The s*almonella* spp. count was lowest in T_4 group compared to control (T_0). The lower bacterial colony counts in T_4 group might be due to the beneficiary effect of probiotics (*Bacillus licheniformis*) that decreases pathogenic bacteria in gut.

These results are in harmony with those of previous researchers Panda *et al.* (2001) found that the effect of dietary supplementation of probiotics significantly (P<0.05) decrease in *E. coli* bacteria. Another researcher J. Song (2014) reported that probiotic mixture contained *Bacillus licheniformis, Bacillus subtilis* decreased (P<0.05) viable counts of coliforms, and tended (P<0.10) to decrease viable counts of Clostridium. Sinol sen *et al*. (2012) found that at day 35, birds supplemented with increasing levels of *B. subtilis* showed decrease in caecal *Clostridium* and *Coliform* count (P<0.05).

4.6 Flock uniformity

Flock uniformity of broiler chicken were presented in table 9 and figure 7. The higher flock uniformity (88.67±5.67%) was found in control (T_0). The lower flock uniformity (66.67 ±12.02%) was found in T_4 group. A statistically insignificant

(P>0.05) difference was noted on flock uniformity of the broilers between the treatment groups and control. The flock uniformity was ranges from 66.67% to 88.67%. The flock uniformity was higher in control (T_0) because all the birds were uniform in weight and there was no influence of probiotic on control group. The flock uniformity was lower in T_4 group because some bird gained more weight due to the influence of probiotics and the others bird were not as like as the weighted bird. So, the variation of uniformity between T_4 and T_0 group might be due to the positive effect of probiotic.

4.7 Economics

The cost of different treatment groups and control group presented in Table 16 and Figure 5. Total expenditure per bird was significantly higher (P<0.05) in treated group T_2 (158.74TK±0.05) than control group T_0 (158.15TK±0.06). Feed cost was significantly higher (P<0.05) in control group (T_0) compared to different treated group.

The price of probiotics both the BS and BL were BDT 800/kg and the charge for incorporation in feeding was calculated. Profit per bird (PPB) and benefit cost ratio (BCR) also presented in Table 16 and figure 6, demonstrated the economic impact of the treatment groups compared with the untreated group. Return was calculated after selling the live birds per kg weight and profit was computed by subtracting the expenditure.

Profit per bird was significantly higher (P<0.05) in treatment groups T_4 (66.90±4.02 TK), T_3 (66.62±4.05 TK), T_1 (64.75±4.95 TK) and T_2 (59.66±3.79 TK) than T_0 (47.94±5.38 TK). Among the treatment groups T_4 performed better than others. BCR was also statistically higher (P<0.05) in treatment groups T_1 (1.40±0.03), T_2 (1.37±0.02) and T_3 (1.42±0.03), T_4 (1.42±0.02) compared with T_0 (1.30±0.04) (Table 16).

These results are in agreement with those of previous researchers Nayebpor *et al.* (2007) who found that, feeding broiler chickens on direct fed microbial probiotic was significantly (P<0.05) improved body weights and indicated that addition of Probiotic to broilers diet caused a higher improvement in broilers net profit than control which given diet without any feed additives. Another researcher panda *et al.* (2006) where they found a significant increase (P<0.05) in net income value of supplemented group with probiotic than control group.

Table 9. Effects of probiotics on production performances of broiler

Treatment	Final Live weight (g/bird)	Average BWG (g/bird)	Total FC (g/bird)	Final FCR	Flock Uniformity	Survivability (%)
T_0	1472.14^{b}±38.51	1430.13^{b}±38.54	2173.93^{a}±1.44	1.52^{a}±0.04	88.67±5.67	90.33^{b}±2.67
T_1	1592.07^{a}±35.81	1550.07^{a}±35.81	2150.97^{b}±1.86	1.39^{b}±0.03	73.33±8.82	100.00^{a}±0.00
T_2	1560.00^{ab}±26.70	1518.00^{ab}±26.70	2141.70^{bc}±1.21	1.41^{b}±0.03	76.67±6.67	100.00^{a}±0.00
T_3	1601.57^{a}±29.18	1559.57^{a}±29.18	2137.77^{bc}±1.57	1.37^{b}±0.02	80.00±5.77	100.00^{a}±0.00
T_4	1607.50^{a}±30.98	1602.17^{a} ±25.39	2128.50^{c}±9.04	1.33^{b}±0.02	66.67±12.02	100.00^{a}±0.00
Mean ± SE	1566.66±18.16	1531.99±19.47	2146.57±4.44	1.40±0.02	77.07±3.64	98.07^{a}±0.00

Here, T_0 = (Control), T_1 = (20g BS Probiotic/MT Feed), T_2 = (50g BS Probiotic/MT Feed), T_3 = (20g BL Probiotic/MT Feed), T_4 = (50g BL Probiotic/MT Feed). Values are Mean ± SE (n=15) one-way ANOVA (SPSS, Duncan method).

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

Table 10. Effects of probiotics on body weight gain (BWG) (g/bird) of broiler in different weeks

Treatment	1st Week BWG	2nd Week BWG	3rd Week BWG	4th Week BWG
T_0	196.10^{b}±5.03	278.17±13.72	486.63^{b}±19.69	469.23^{b}±17.74
T_1	222.10^{a}±4.86	297.90±4.80	500.43^{ab}±13.41	529.63^{ab}±29.11
T_2	219.57^{a}±3.19	285.83±4.02	504.50^{ab}±19.43	508.10^{ab}±12.52
T_3	217.80^{a}±7.64	293.20±9.54	544.07^{a}±5.07	504.50^{ab}±27.85
T_4	221.90^{a}±2.99	292.53±3.98	525.93^{ab}±14.73	561.80^{a}±11.03
Mean ± SE	215.49±3.24	289.53±3.60	512.31±7.96	514.65±11.39

Here, T_0 = (Control), T_1 = (20g BS Probiotic/MT Feed), T_2 = (50g BS Probiotic/MT Feed), T_3 = (20g BL Probiotic/MT Feed), T_4 = (50g BL Probiotic/MT Feed). Values are Mean ± SE (n=15) one-way ANOVA (SPSS, Duncan method).

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

Table 11. Effects of probiotics on feed consumption (g/bird) of broiler in different weeks

Treatment	1st Week FC	2nd Week FC	3rd Week FC	4th Week FC
T_0	229.23^{a}±0.61	422.17^{a}±0.88	692.63^{a}±1.57	829.90^{a}±1.42
T_1	228.53^{ab}±0.67	415.80^{b}±0.85	678.77^{b}±0.93	827.87^{a}±0.32
T_2	227.47^{ab}±0.94	415.97^{b}±1.28	674.77^{b}±2.55	823.50^{ab}±0.58
T_3	228.40^{ab}±0.25	413.03^{b}±0.94	674.70^{b}±1.99	821.63^{ab}±0.52
T_4	225.47^{b}±2.06	414.87^{b}±1.21	674.20^{b}±2.22	813.97^{b}±6.57
Mean ± SE	227.82±0.54	416.37±0.91	679.01±2.01	823.37±1.87

Here, T_0 = (Control), T_1 = (20g BS Probiotic/MT Feed), T_2 = (50g BS Probiotic/MT Feed), T_3 = (20g BL Probiotic/MT Feed), T_4 = (50g BL Probiotic/MT Feed). Values are Mean ± SE (n=15) one-way ANOVA (SPSS, Duncan method).

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

Table 12. Effects of probiotics on feed conversion ratio (FCR) of broiler in different weeks

Treatment	1st Week FCR	2nd Week FCR	3rd Week FCR	4th Week FCR
T_0	1.17^{a}±0.03	1.52±0.08	1.43^{a}±0.06	1.77^{a}±0.06
T_1	1.03^{b}±0.03	1.40±0.02	1.36^{ab}±0.04	1.57^{ab}±0.10
T_2	1.03^{b} 0.02	1.46±0.02	1.34^{ab}±0.06	1.62^{ab}±0.04
T_3	1.05^{b}±0.04	1.41±0.05	1.24^{b}±0.02	1.64^{ab}±0.09
T_4	1.02^{b}±0.02	1.42±0.02	1.28^{ab}±0.04	1.45^{b}±0.04
Mean ± SE	1.06±0.02	1.44±0.02	1.33±0.02	1.61±0.04

Here, T_0 = (Control), T_1 = (20g BS Probiotic/MT Feed), T_2 = (50g BS Probiotic/MT Feed), T_3 = (20g BL Probiotic/MT Feed), T_4 = (50g BL Probiotic/MT Feed). Values are Mean ± SE (n=15) one-way ANOVA (SPSS, Duncan method).

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

Table 13: Effects of probiotics on carcass characteristics of broiler

Treatment	Breast weight (g)	Thigh weight (g)	Wing weight (g)	Drumstick weight (g)	Dressing percentage
T_0	318.90^{c}±15.81	164.16^{c}±2.20	105.80±2.45	162.73^{b}±0.77	65.67^{b}±1.33
T_1	372.00^{ab}±12.02	175.43^{ab}±2.19	106.43±3.73	164.10^{b}±4.60	68.73^{ab}±.82
T_2	351.13^{bc}±5.84	170.13^{b}±0.99	107.70±4.50	167.13^{ab}±3.61	70.07^{ab}±2.07
T_3	365.10^{ab}±11.80	170.10^{b}±1.66	114.30±1.78	162.76^{b}±3.04	68.97^{ab}±0.42
T_4	392.83^{a}±7.86	177.86^{a}±1.48	115.43±0.69	176.33^{a}±2.07	71.33^{a}±1.30
Mean ± SE	359.99±7.81	171.54±1.43	109.93±1.56	166.61±1.80	68.95±0.71

Here, T_0 = (Control), T_1 = (20g BS Probiotic/MT Feed), T_2 = (50g BS Probiotic/MT Feed), T_3 = (20g BL Probiotic/MT Feed), T_4 = (50g BL Probiotic/MT Feed). Values are Mean ± SE (n=15) one-way ANOVA (SPSS, Duncan method).

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

Table 14. Effects of probiotics on giblet, intestine, spleen and bursa of broiler under different treatments Group

Treatment	Liver weight (g)	Heart weight (g)	Neck weight (g)	Gizzard weight (g)	Giblet weight (g)	Intestine weight (g)	Spleen weight (g)	Bursa weight (g)
T_0	34.60^{c}±0.55	7.13^{ab}±0.06	34.73^{c}±1.12	38.00±2.25	114.47^{b}±3.58	91.29±5.60	1.87±0.12	1.97±0.40
T_1	37.73^{b}±0.84	7.33^{ab}±0.12	35.63^{bc}±0.68	33.70±3.59	114.40^{b}±4.97	102.70±10.48	1.97±0.14	1.63±0.49
T_2	35.63^{bc}±0.92	6.87^{b}±0.08	40.83^{a}±1.41	36.53±0.97	119.87^{b}±1.31	98.60±10.57	1.90±0.05	1.90±0.10
T_3	36.20^{bc}±1.03	6.90^{b}±0.05	37.90^{b}±0.35	42.33±3.40	123.33^{ab}±3.03	92.73±11.18	1.87±0.21	1.83±0.12
T_4	41.80^{a}±0.92	7.63^{a}±0.31	43.13^{a}±0.21	41.67±4.82	134.23^{a}±5.78	109.77±2.23	1.90±0.10	1.90±0.05
Mean ± SE	37.19±0.74	7.17±0.09	38.45±0.90	38.45±1.51	121.26±2.48	99.02±3.77	1.90±0.53	1.85±0.11

Here, T_0 = (Control), T_1 = (20g BS Probiotic/MT Feed), T_2 = (50g BS Probiotic/MT Feed), T_3 = (20g BL Probiotic/MT Feed), T_4 = (50g BL Probiotic/MT Feed). Values are Mean ± SE (n=15) one-way ANOVA (SPSS, Duncan method).

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

Table 15. Effects of probiotics (BS and BL) on microflora [Flora numbers, (CFU/g)] in the cecum of broiler

Treatment	*E. coli* (EMB) × 10^6 (CFU/g)	Salmonella (SS) × 10^6 (CFU/g)
T_0	14.5^{a}±3.60	8.27^{a}±3.48
T_1	11.1^{b}±8.33	7.47^{ab}±3.38
T_2	11.1^{b}±8.14	6.93^{abc}±6.17
T_3	10.0^{b}±5.70	6.53^{bc}±5.92
T_4	10.0^{b}±5.61	5.87^{c}±1.76
Mean ± SE	11.4±5.04	7.03± 2.76

Here, T_0 = (Control), T_1 = (20g BS Probiotic/MT Feed), T_2 = (50g BS Probiotic/MT Feed), T_3 = (20g BL Probiotic/MT Feed), T_4 = (50g BL Probiotic/MT Feed). Values are Mean ± SE (n=15) one-way ANOVA (SPSS, Duncan method).

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

Table 16. Effects of BS and BL in economic impact on broiler rearing

Treatment	Feed cost (BDT) per bird	Cost of BS and BL probiotic (BDT) per bird	Common expenditure (BDT) per bird	Total expenditure (BDT) per bird	Receipt per bird when sold @ 140 TK/ Kg live weight	Profit per bird (BDT)	Benefit cost ratio
T_0	95.65^{a}±0.06	0±0.00	62.50	158.15^{ab}±0.06	206.10^{b}±5.39	47.94^{b}±5.38	1.30^{b}±0.03
T_1	94.64^{b}±0.08	1±0.00	62.50	158.14^{ab}±0.83	222.89^{a}±5.01	64.75^{a}±4.95	1.40^{a}±0.03
T_2	94.24^{bc}±0.05	2±0.00	62.50	158.74^{a}±0.05	218.40^{ab}±3.74	59.66^{ab}±3.79	1.37^{ab}±0.02
T_3	94.10^{bc}±0.06	1±0.00	62.50	157.60^{b}±0.06	224.22^{a}±4.09	66.62^{a}±4.05	1.42^{a}±0.03
T_4	93.65^{c}±0.40	2±0.00	62.50	158.15^{ab}±0.40	225.05^{a}±4.33	66.90^{a} ±4.02	1.42^{a}±0.02
Mean ± SE	94.46±0.19	1.2±0.20	62.50	158.16±0.12	219.33±2.54	61.17±2.54	1.38±0.02

Here, T_0 = (Control), T_1 = (20g BS Probiotic/MT Feed), T_2 = (50g BS Probiotic/MT Feed), T_3 = (20g BL Probiotic/MT Feed), T_4 = (50g BL Probiotic/MT Feed). Values are Mean ± SE (n=15) one-way ANOVA (SPSS, Duncan method).

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

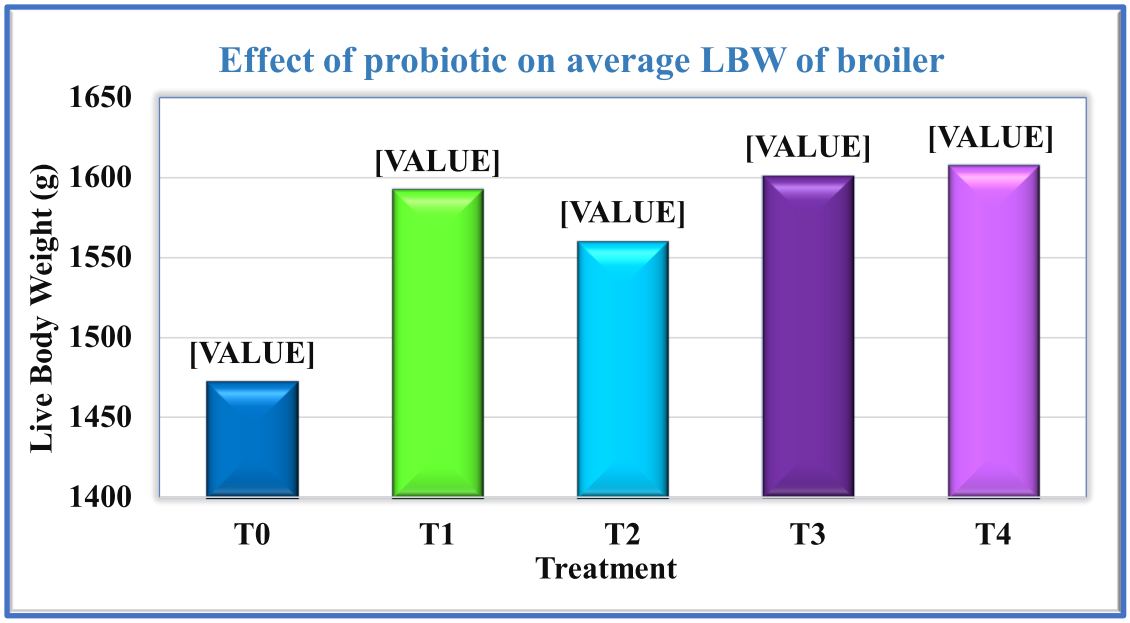


Figure 1. Effect of probiotic on average live body weight of broiler under different treatment

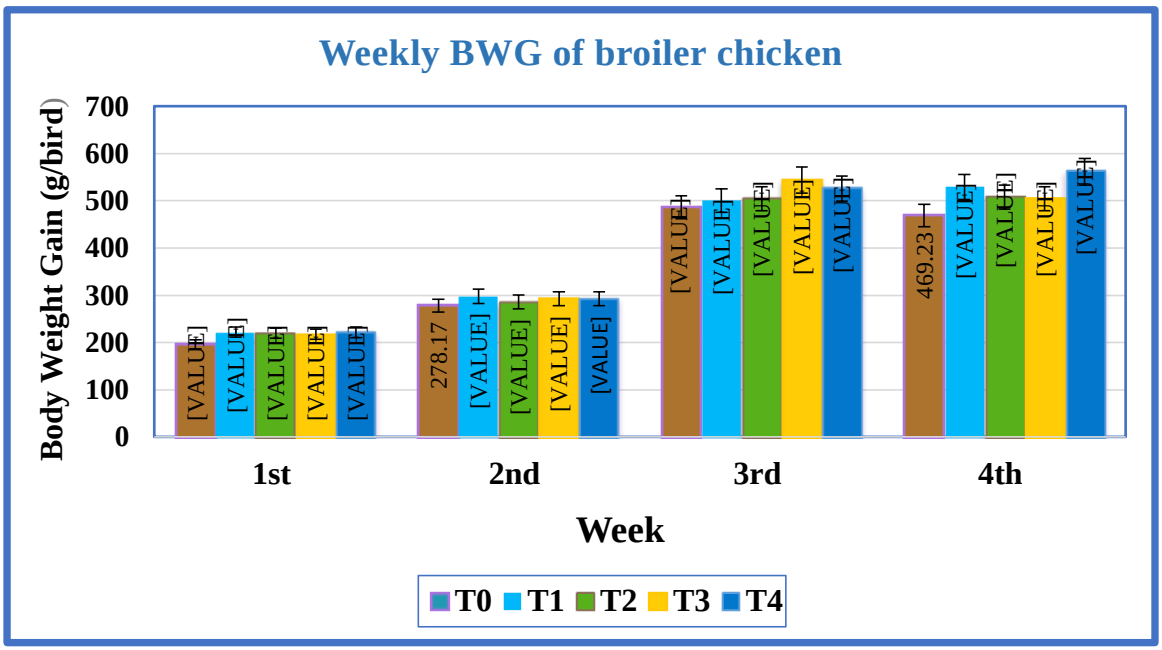


Figure 2. Effect of probiotic on weekly body weight gain of broiler

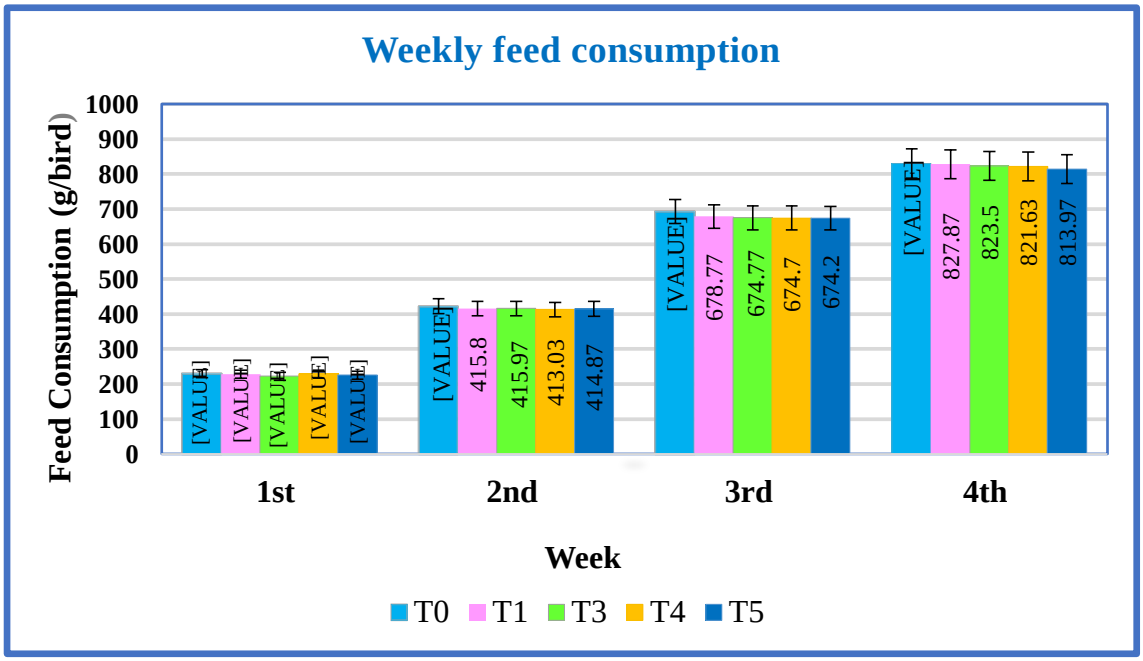


Figure 3. Effect of probiotic on weekly feed consumption of broiler

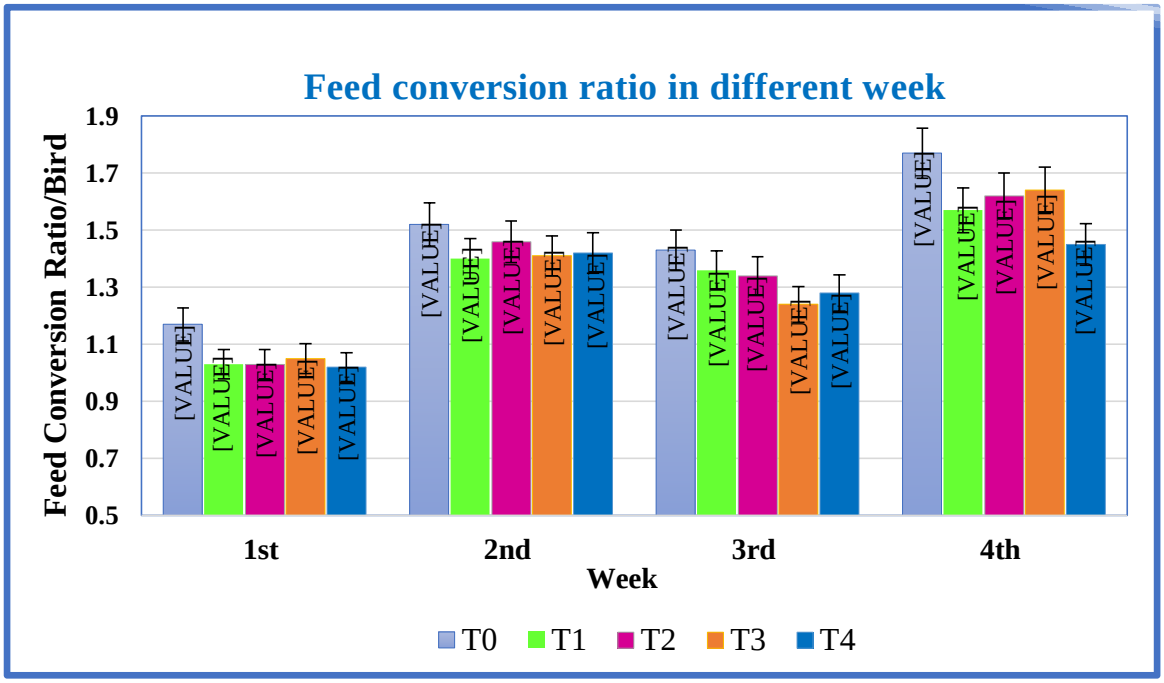


Figure 4. Effect of probiotic on weekly feed conversion ratio of broiler

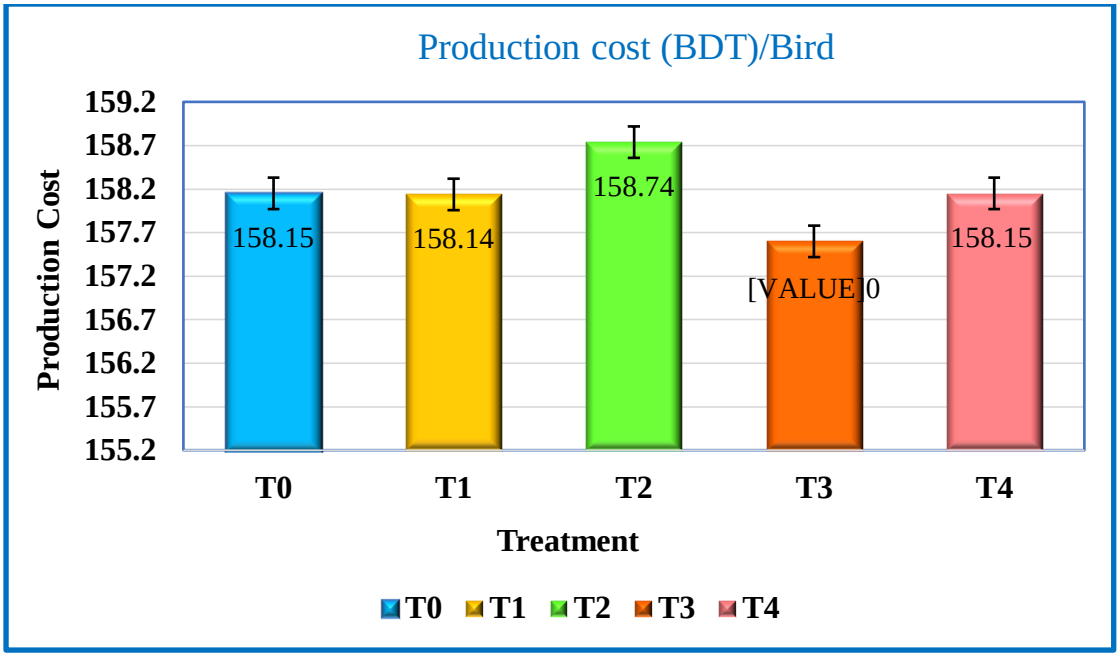


Figure 5. Effects of probiotic on production cost per broiler

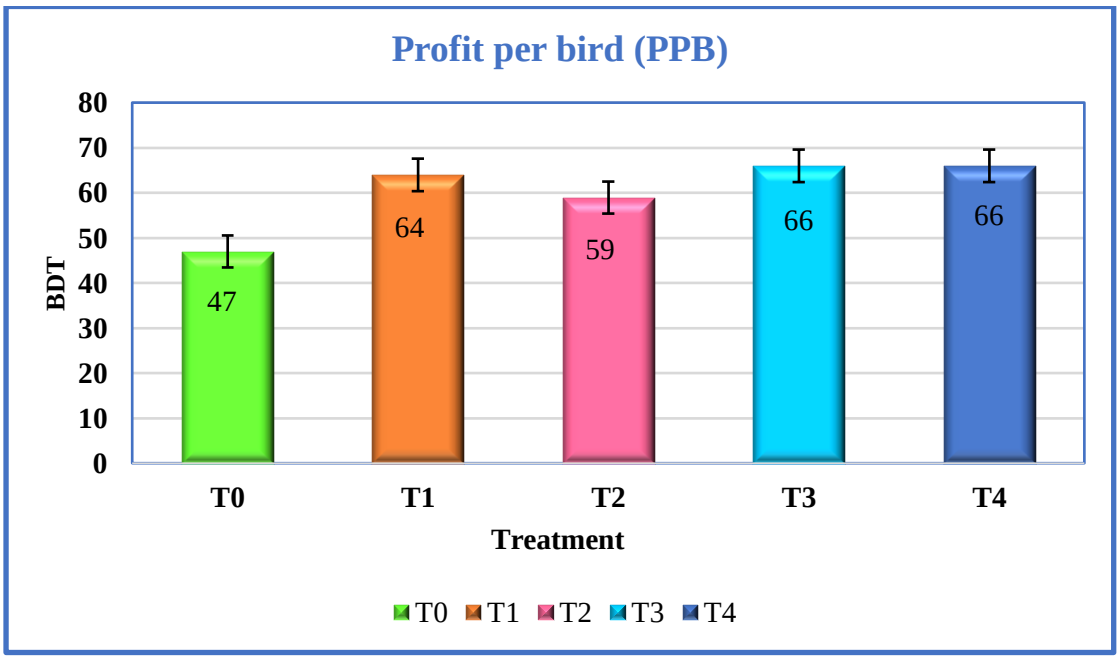


Figure 6. Effects of probiotic on profit per bird (ppb)

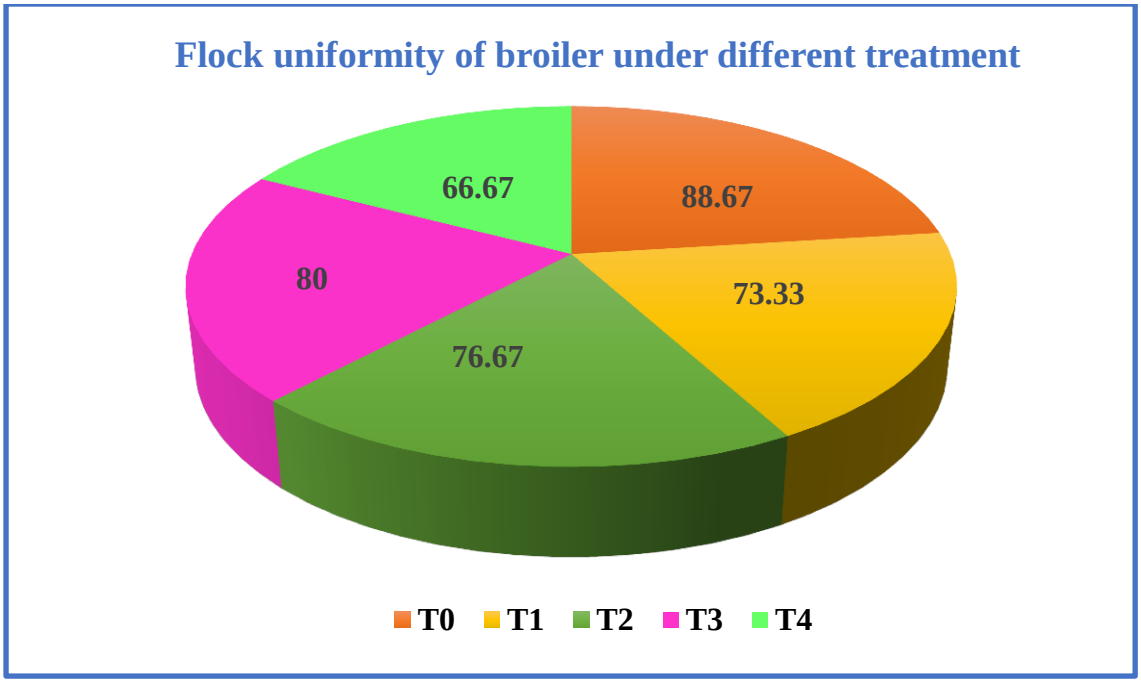


Figure 7. Flock uniformity of broiler under different treatment

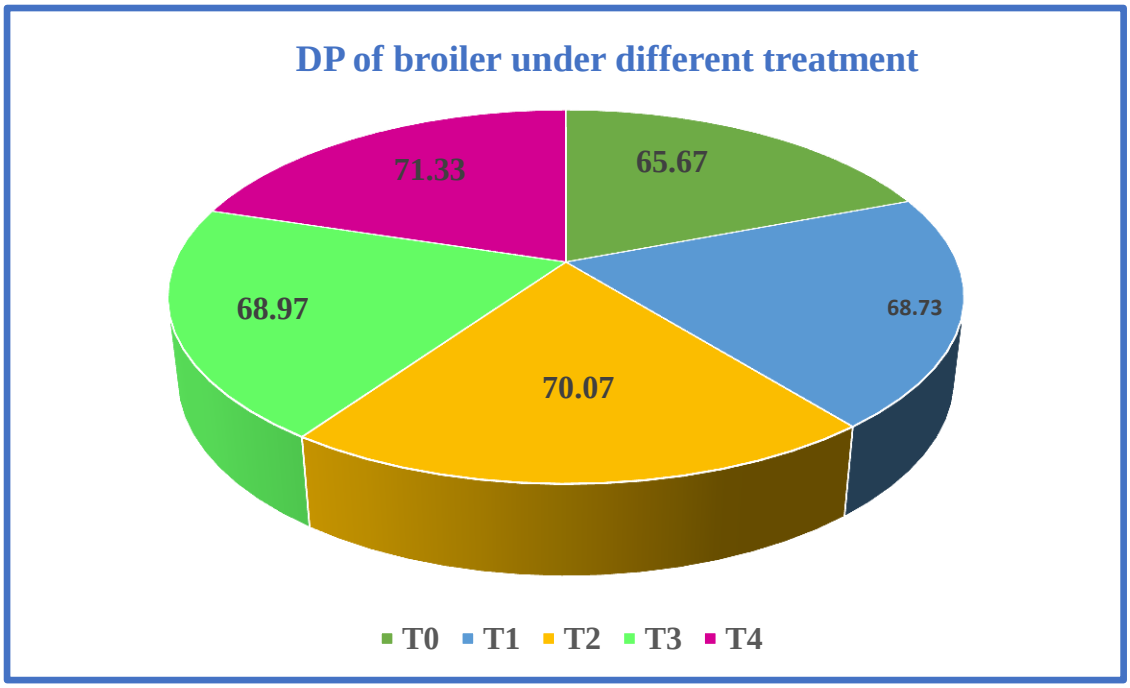


Figure 8. Dressing percentage of broiler under different treatment

CHAPTER- 5

SUMMARY, CONCLUSION AND RECOMMENDATION

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A total of 150-day old chicks of "Cobb-500" were reared in Sher-E-Bangla Agricultural University, Dhaka Poultry Farm for a period of four weeks using probiotics *Bacillus subtilis* and *Bacillus licheniformis*. Chicks were divided randomly into 5 experimental groups of 3 replicates (10 chicks were allocated in each treatment group). One of the 5 experimental group was fed diet without probiotic were considered as control while, the remaining four groups were fed diet with 20g BS/MT of feed, 50g BS/MT of feed, 20g BL/MT of feed and 50g BL/MT of feed. The specific objectives of this experiment were i) to evaluate the growth performance, and carcass characteristics of broiler chicken ii) to find out the effect of probiotic *(Bacillus subtilis* and *Bacillus licheniformis*) on *E. coli* and *Salmonella spp.* iii) to estimate the cost benefit of using probiotics in broiler rearing under different probiotic treatment iv) to recommend the inclusion level of probiotic in broiler ration as a supplement of growth promotors. The performance traits *viz.* body weight, weight gain, feed consumption, FCR, dressed bird weight, relative giblet weight, survivability, flock uniformity, bacterial colony count, meat yield and economic impact on broiler rearing that includes production cost, profit per bird (PPB) and benefit cost ratio (BCR) of broiler on different replication of the treatments were recorded and compared in each group.

A statistically significant difference ($P<0.05$) was noted on body weight, feed consumption, BWG, FCR, carcass weight, dressing percentage and bacterial colony count value of the birds treated with probiotics. The group T_4 showed higher body weight compared to the group T_3, group T_1, group T_2 and group T_0 followed in descending order. The difference in average weekly body weight gain was highly significantly ($P<0.05$) amongst the treatment groups at 1^{st}, 3^{rd} and 4^{th} weeks than control group. Feed consumption was significantly ($P<0.05$) higher in control group than probiotic treated groups. There was significant difference in weekly feed consumption at 1^{st} and 4^{th} weeks amongst the treatment groups and control but no significant ($P>0.05$) difference in weekly feed consumption at 2^{nd} and 3^{rd} weeks amongst the probiotics treated groups. The FCR was better in all probiotic treated groups compared to the control group and the best value found in T_4 group. The FCR of 1^{st}, 3^{rd} and 4^{th} weeks were significant ($P<0.05$) in treatment group but in 2^{nd} week

was insignificant (P>0.05). The survivability rate was significantly (P<0.05) higher in treatment groups than the control groups but no significant (P>0.05) difference amongst the treated groups. T_4 supplemented group had a greater carcass percentage (P<0.05) compared with the control group. The weight (g) of breast was significantly (P<0.05) high in T_4 group compared to the group T_1, group T_3, group T_2 and group T_0 followed in descending order. The relative weight of thigh and drumstick was higher in T_4 group and lower in T_0 group and there was significant difference (P<0.05) amongst the treatment groups. The relative weight of wing in different groups was insignificant (P>0.05). Giblet weight was significantly (P<0.05) higher in T_4 group than other treatment and control group. The relative weight of intestine in different groups were insignificant (P>0.05). The relative weight of spleen and bursa in different groups showed no significant (P>0.05) difference. The numbers of intestinal microflora (*E. coli* and *Salmonella*) were significantly higher in control group compared to other groups. The number of *salmonella* bacteria were significantly lower in T_4 group than control. Total expenditure per bird was significantly higher (P<0.05) in treated group T_2 than control group (T_0). Feed cost was significantly higher (P<0.05) in control group (T_0) compared to other treated group. Profit per bird was significantly higher (P<0.05) in treatment groups than control group (T_0) and among the treatment groups T_4 performed better than others. BCR was also statistically higher (P<0.05) in treatment groups compared with the control (T_0).

Analyzing the above research findings, probiotic (*Bacillus licheniformis*) was used in T_4 groups (50g BL/MT of feed) showed better results than control and other treatment groups in terms of improved growth performance with better FCR and increased carcass weight, giblet percentage with minimized the *E. coli* and *salmonella* bacteria in the gut of broiler. Among the four dietary treatment group T_4 (50g BL/MT of feed) showed better result than group T_3 (20g BL/MT of feed) and group T_1 (20g BS/MT of feed) showed better result than group T_2 (50g BS/MT of feed). So, probiotic 50g BL/MT of feed could be used as an alternative of antibiotics on broiler ration. These two types of probiotic (*Bacillus subtilis* and *Bacillus licheniformis*) were not available in our country but could be used as an alternative to antibiotics and therefore the study recommends for hematological parameters on birds' immunity and conducting feeding trial on commercial poultry farm to fix up inclusion level perfectly and safely used in broiler rearing for higher economical return without any adversity.

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APPENDICES

Appendix 1. Temperature and humidity

Date	Age of chicks (days)	Maximum tem. (^{0}C)	Minimum tem. (^{0}C)	Maximum humidity (%)	Minimum humidity (%)
11.04.19	0	34.9	30.1	71	62
12.04.19	1st	34.4	29.9	66	60
13.04.19	2nd	36.0	28.9	80	58
14.04.19	3rd	36.4	27.8	65	49
15.04.19	4th	36.3	28.4	79	45
16.04.19	5th	37.0	28.5	84	56
17.04.19	6th	38.1	26.0	87	40
18.04.19	7th	38.5	26.0	40	36
19.04.19	8th	40.1	25.0	82	22
20.04.19	9th	38.8	26.4	74	31
21.04.19	10th	39.3	25.4	76	37
22.04.19	11th	39.7	25.2	66	30
23.04.19	12th	41.6	25.1	77	31
24.04.19	13th	38.1	26.6	79	36
25.04.19	14th	39.9	28.5	75	37
26.04.19	15th	37.9	27.9	94	42
27.04.19	16th	35.9	27.2	85	56
28.04.19	17th	37.3	28.2	94	43
29.04.19	18th	37.5	29.0	96	53
30.04.19	19th	39.9	28.0	86	56
01.05.19	20th	41.2	28.9	96	39
02.05.19	21th	39.7	27.0	87	41
03.05.19	22th	37.5	26.8	95	52
04.05.19	23th	27.7	26.0	96	40
05.05.19	24th	35.0	25.4	59	36
06.05.19	25th	35.8	28.1	69	36
07.05.19	26th	37.3	28.3	71	40
08.05.19	27th	37.6	27.9	70	47
09.05.19	28th	37.5	28.5	76	50

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

Treatment	Replication	1st Week FC	2nd Week FC	3rd Week FC	4th Week FC	Total FC
T_0	R_1	229.2	420.5	690.1	832.5	2172.30
	R_2	230.3	422.5	692.3	827.6	2172.70
	R_3	228.2	423.5	695.5	829.6	2176.80
T_1	R_1	228.3	417.4	680.5	828.5	2154.70
	R_2	227.5	415.5	678.5	827.5	2149.00
	R_3	229.8	414.5	677.3	827.6	2149.20
T_2	R_1	225.6	416.6	679.4	822.5	2144.10
	R_2	228.3	417.8	670.6	823.5	2140.20
	R_3	228.5	413.5	674.3	824.5	2140.80
T_3	R_1	227.9	412.7	678.6	821.7	2140.90
	R_2	228.6	411.6	673.4	822.5	2136.10
	R_3	228.7	414.8	672.1	820.7	2136.30
T_4	R_1	228.4	416.5	678.6	815.5	2139.00
	R_2	226.5	412.5	672.5	824.5	2136.00
	R_3	221.5	415.6	671.5	801.9	2110.50

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

Treatment	Replication	1st Week	2nd Week	3rd Week	4th Week
T_0	R_1	198.3	300.5	488.8	499.1
	R_2	186.5	280.8	451.5	437.7
	R_3	203.5	253.2	519.6	470.9
T_1	R_1	226.5	300.5	526.5	548.5
	R_2	227.4	288.6	492.9	472.5
	R_3	212.4	304.6	481.9	567.9
T_2	R_1	222.4	293.6	466.0	483.1
	R_2	213.2	283.8	519.2	521.9
	R_3	223.1	280.1	528.3	519.3
T_3	R_1	222.8	301.2	534.2	555.5
	R_2	227.8	274.2	551.0	498.4
	R_3	202.8	304.2	547.0	459.6
T_4	R_1	226.5	293.8	550.9	570.5
	R_2	216.3	298.7	499.9	539.9
	R_3	222.9	285.1	527.0	575.0

Appendix 4. Feed conversion ratio (FCR) of 1st, 2nd, 3rd and 4th week under different treatments

Treatment	Replication	1st week	2nd week	3rd week	4th week
T_0	R_1	1.16	1.40	1.41	1.67
	R_2	1.23	1.50	1.53	1.89
	R_3	1.12	1.67	1.34	1.76
T_1	R_1	1.01	1.39	1.29	1.51
	R_2	1.00	1.44	1.38	1.75
	R_3	1.08	1.36	1.41	1.46
T_2	R_1	1.01	1.42	1.46	1.70
	R_2	1.07	1.47	1.29	1.58
	R_3	1.02	1.48	1.28	1.59
T_3	R_1	1.02	1.37	1.27	1.48
	R_2	1.00	1.50	1.22	1.65
	R_3	1.13	1.36	1.23	1.79
T_4	R_1	1.01	1.42	1.23	1.43
	R_2	1.05	1.38	1.35	1.53
	R_3	0.99	1.46	1.27	1.39

Appendix 5. Average live weight, eviscerated weight and dressing percentage of broiler chicken under different treatments

Treatment	Replication	Average Live weight (g)	Eviscerated Weight(g)	Dressing Percentage (%)
T_0	R_1	1615.3	1017.3	63.0
	R_2	1513.6	1013.6	67.0
	R_3	1472.5	986.5	67.0
T_1	R_1	1652.3	1149.3	69.6
	R_2	1600.2	1074.2	67.1
	R_3	1620.3	1125.3	69.5
T_2	R_1	1503.2	1000.2	66.5
	R_2	1557.1	1147.1	73.7
	R_3	1622.4	1136.4	70.0
T_3	R_1	1620.4	1130.4	69.8
	R_2	1513.3	1038.3	68.6
	R_3	1585.2	1085.2	68.5
T_4	R_1	1765.4	1305.4	73.9
	R_2	1695.3	1195.3	70.5
	R_3	1612.3	1122.3	69.6

Appendix 6. Production performance of broiler chicken under different treatments

Treatment	Replication	Final Live weight (g/bird)	Total FC (g/bird)	Total BWG (g/bird)	Final FCR	Survivability (%)
T_0	R_1	1528.67	2172.30	1486.7	1.46	90
	R_2	1398.58	2172.70	1356.5	1.60	90
	R_3	1489.17	2176.80	1447.2	1.50	80
T_1	R_1	1644.0	2154.70	1602.0	1.35	100
	R_2	1523.4	2149.00	1481.4	1.45	100
	R_3	1608.8	2149.20	1566.8	1.37	100
T_2	R_1	1507.1	2144.10	1465.1	1.46	100
	R_2	1580.1	2140.20	1538.1	1.39	100
	R_3	1592.8	2140.80	1550.8	1.38	100
T_3	R_1	1655.7	2140.90	1613.7	1.33	100
	R_2	1593.4	2136.10	1551.4	1.38	100
	R_3	1555.6	2136.30	1513.6	1.41	100
T_4	R_1	1665.7	2139.00	1641.7	1.30	100
	R_2	1596.8	2136.00	1554.8	1.37	100
	R_3	1560.0	2110.50	1610.0	1.31	100

Appendix 7. Weight (g) of giblet, intestine, spleen and bursa of broiler chicken under different treatment groups

Treatment	Replication	liver	Heart	Neck	Gizzard	Giblet	Intestine	Spleen	Bursa
T_0	R_1	35.7	7.2	36.7	42.0	121.6	89.66	2.1	2.7
	R_2	34.2	7.2	34.7	34.2	110.3	101.7	1.7	1.9
	R_3	33.9	7.0	32.8	37.8	111.5	82.5	1.8	1.3
T_1	R_1	38.9	7.4	37.0	40.8	124.1	110.1	2.2	2.5
	R_2	36.1	7.5	34.8	29.2	107.6	116.0	1.7	1.6
	R_3	38.2	7.1	35.1	31.1	111.5	82.0	2.0	0.8
T_2	R_1	34.1	6.9	43.2	36.1	120.3	77.9	1.8	2.0
	R_2	35.5	7.0	41.0	38.4	121.9	105.2	1.9	1.7
	R_3	37.3	6.7	38.3	35.1	117.4	112.7	2.0	2.0
T_3	R_1	38.0	7.0	38.3	40.2	123.5	83.5	2.3	1.6
	R_2	34.4	6.9	38.2	49.0	128.5	115.0	1.7	1.9
	R_3	36.2	6.8	37.2	37.8	118.0	79.7	1.6	2.0
T_4	R_1	43.1	8.0	42.7	50.7	144.5	113.4	2.1	1.9
	R_2	42.3	7.9	43.4	40.1	133.7	110.2	1.8	1.8
	R_3	40.0	7.0	43.3	34.2	124.5	105.7	1.8	2.0

Appendix 8. Weight (g) of carcass cut of broiler chicken under different treatment groups

Treatment	Replication	Breast	Thigh	Wing	Back	Drumstick
T_0	R_1	332.1	160.0	110.7	167.1	163.7
	R_2	337.2	165.0	103.7	155.3	161.2
	R_3	287.4	167.5	103.0	164.2	163.3
T_1	R_1	385.3	179.2	113.7	171.4	159.3
	R_2	348.0	171.6	101.3	162.7	173.3
	R_3	382.7	175.5	104.3	166.0	159.7
T_2	R_1	353.0	168.3	98.7	157.3	160.3
	R_2	360.2	171.7	111.7	165.7	168.5
	R_3	340.2	170.4	112.7	166.3	172.6
T_3	R_1	388.7	173.3	117.4	169.2	156.7
	R_2	353.9	167.7	111.2	161.7	166.3
	R_3	352.7	169.3	114.3	155.4	165.3
T_4	R_1	408.5	175.7	115.3	164.3	180.3
	R_2	386.3	180.7	116.7	159.7	175.4
	R_3	383.7	177.2	114.3	162.0	173.3

Appendix 9. Economic impact of treatments on broiler production

Treatment	Replication	Feed cost (BDT) Per Bird	Cost of *B.S* and *B.L* Probiotic (BDT) Per Bird	Common Expenditure (BDT) Per Bird	Total Expenditure (BDT) Per Bird
T_0	R_1	95.58	0	62.50	158.08
	R_2	95.60	0	62.50	158.10
	R_3	95.78	0	62.50	158.28
T_1	R_1	94.81	1.00	62.50	158.31
	R_2	94.56	1.00	62.50	158.06
	R_3	94.56	1.00	62.50	158.06
T_2	R_1	94.34	2.00	62.50	158.84
	R_2	94.17	2.00	62.50	158.67
	R_3	94.20	2.00	62.50	158.70
T_3	R_1	94.20	1.00	62.50	157.70
	R_2	93.99	1.00	62.50	157.49
	R_3	94.10	1.00	62.50	157.60
T_4	R_1	94.11	2.00	62.50	158.61
	R_2	93.98	2.00	62.50	158.48
	R_3	92.86	2.00	62.50	157.36

Appendix 10. Production cost of the birds at 28 days of rearing period

Parameter	Amount (BDT)
Day Old chick cost (150 chick)	8400.00
Feed cost (7 bag)	15400.00
Litter cost	1300.00
Cost of BS and BL Probiotic	384.00
Medicine cost	300.00
Vaccine cost	500.00
Others cost	2500.00
Total	**28,784.00**

Appendix 11. Selling price of the birds under different treatment group

Treatment	Replication	NO. of Bird	Live Body Weight (Kg)	Selling price (BDT) @ 140 TK/ Kg live weight)	Total Selling Price
T_0	R_1	9	13.758	1926.12	
	R_2	9	12.587	1762.18	
	R_3	8	11.913	1667.82	
T_1	R_1	10	16.440	2301.60	
	R_2	10	15.234	2132.76	32,072.88
	R_3	10	16.088	2252.32	
T_2	R_1	10	15.071	2109.94	
	R_2	10	15.801	2212.14	
	R_3	10	15.928	2229.92	
T_3	R_1	10	16.557	2317.98	
	R_2	10	15.934	2230.76	
	R_3	10	15.556	2177.84	
T_4	R_1	10	16.657	2331.98	
	R_2	10	15.968	2235.52	
	R_3	10	15.600	2184.00	

Appendix 12. Net return of the birds under different treatment groups

Treatment	Replication	Average LBW(Kg)/ Bird	Receipt per bird when sold @ 140 TK/ Kg Live weight)	Profit per bird (BDT)	Benefit Cost Ratio
T_0	R_1	1.52867	214.01	55.93	1.35
	R_2	1.39858	195.80	37.70	1.24
	R_3	1.4891	208.48	50.20	1.32
T_1	R_1	1.6440	230.16	71.85	1.45
	R_2	1.5234	213.28	55.22	1.35
	R_3	1.6088	225.23	67.17	1.42
T_2	R_1	1.5071	210.99	52.15	1.33
	R_2	1.5801	221.21	62.54	1.39
	R_3	1.5928	222.99	64.29	1.41
T_3	R_1	1.6557	231.80	74.10	1.47
	R_2	1.5934	223.08	65.59	1.42
	R_3	1.5556	217.78	60.18	1.38
T_4	R_1	1.6657	233.20	74.59	1.47
	R_2	1.5968	223.55	65.07	1.41
	R_3	1.5600	218.40	61.04	1.39

Treatment	Replication	Uniformity (%)	Average Uniformity (%)
T_0	R_1	100	
	R_2	83	88
	R_3	83	
T_1	R_1	90	
	R_2	70	73
	R_3	60	
T_2	R_1	70	
	R_2	70	76
	R_3	90	
T_3	R_1	80	
	R_2	70	80
	R_3	90	
T_4	R_1	90	
	R_2	50	67
	R_3	60	

Appendix 13. Effects of probiotics on flock uniformity of chickens

Appendix 14. Effects of BS and BL on microflora [Flora numbers, (CFU/g)] in the cecum of broilers

Treatment	Replication	No. of *E. coli* colony (average)	No. of Salmonella colony (average)
T_0	R_1	140	77
	R_2	152	82
	R_3	143	89
T_1	R_1	123	77
	R_2	115	68
	R_3	95	79
T_2	R_1	113	75
	R_2	96	76
	R_3	124	57
T_3	R_1	89	54
	R_2	107	74
	R_3	105	68
T_4	R_1	92	56
	R_2	98	62
	R_3	111	58