COMPARATIVE STUDY OF FEEDING PROBIOTIC (A-MAX®) AND GROWTH PROMOTER ON GROWTH PERFORMANCE OF COMMERCIAL BROILER

A THESIS

BY

MD. SHAHINUR RAHMAN

Registration No. 1505037

Session: 2015-2016

Semester: January-June, 2016

MASTER OF SCIENCE (MS)

IN

ANIMAL NUTRITION

DEPARTMENT OF GENERAL ANIMAL SCIENCE AND NUTRITION

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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[Submitted to the Department of General Animal Science and Nutrition, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur for partial fulfillment of the requirement of the degree]

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Approved as to style and content by

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DEPARTMENT OF GENERAL ANIMAL SCIENCE AND NUTRITION
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

JUNE, 2016

Dedicated
To
My Beloved Parents

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ABSTRACT

An experiment was conducted with 120 Cobb-500 Broiler day old chicks to evaluate the effect of feeding probiotic (*Saccharomyces cerevisiae*) with or without growth promoter (GP). Birds were reared in an open sided shed type house. Body weight and feed intake were collected and examined on day 0, 7, 14, 21, 28 and 32. The experiment was conducted in a Completely Randomized Design. One hundred twenty Cobb-500 day-old chicks were randomly distributed into four dietary groups having three replications. The number of birds in each replication was 10. Four diets were considered: control; probiotic (PB) at a level of 1ml/liter; GP at a level of 100g/50kg and GP plus PB (1ml/liter+ 100g/50kg). The records on kept of body weight, feed intake and mortality while weight gain, feed efficiency (FE) and survivability were calculated. Temperature and humidity were recorded four times daily. One broiler that was very close to the average of pen weight was sacrificed from each replication at the end of the experiment to determine carcass characteristics. Broiler chicks that received PB and a combination of PB+GP treatments showed significant improvement in performance ($p<0.01$) over control with respect to body weight gain, feed efficiency, carcass yield and cost-effectiveness. Feeding GP alone had comparatively less weight gain, net profit and almost similar feed efficiency compared with PB and GP+PB groups but it's performance was significantly better than that of control group. This study indicated that the diet containing GP+PB offered slightly increased benefits to the growth performance of broilers, and these benefits were almost equal to the PB. It is revealed that probiotic (A-MAX) supplementation with growth promoter is beneficial for broiler production and no hazard on human health.

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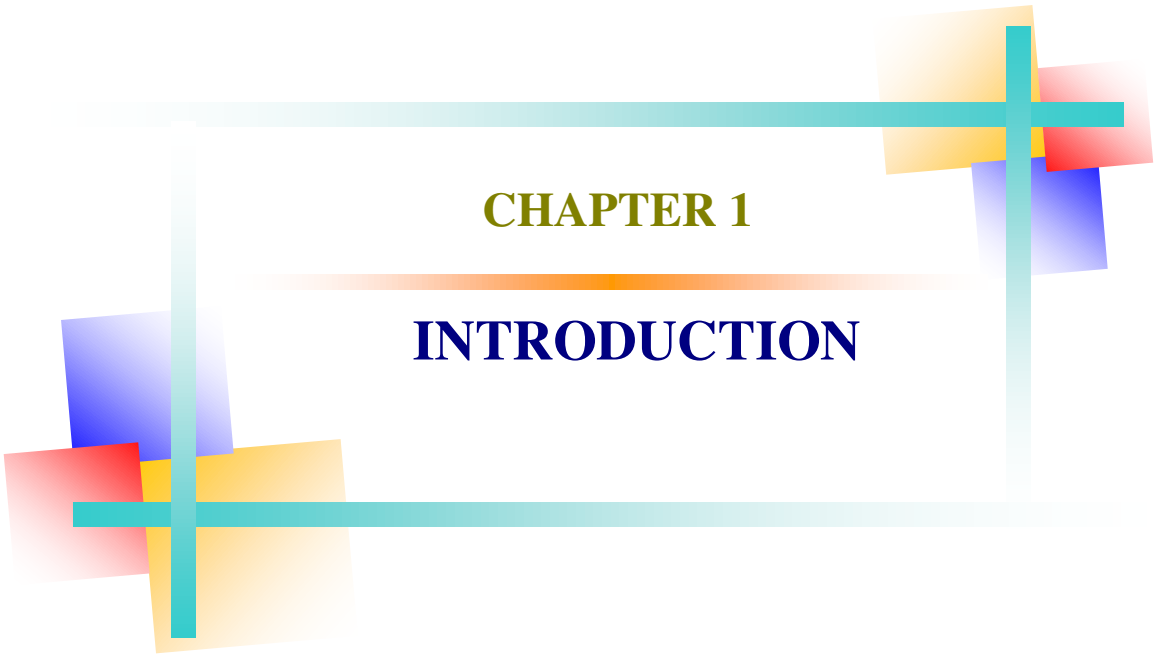
LIST OF ABBREVIATION

Abbreviations	Full meanings
ANOVA	Analysis of variance
AM	Ante Meridian
ADG	Average Daily Gain
BW	Body Weight
BSTI	Bangladesh Standard & Testing Institution
Ca	Calcium
CF	Crude Fiber
CFU	Colony Forming Unit
Contd.	Continued
CP	Crude Protein
CRD	Completely Randomized Design
DCP	Digestible Crude Protein
DFM	Direct Feed Microbial
EE	Ether Extract
e.g.	For Example
et al.	Associates
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration
FE	Feed Efficiency
FI	Feed Intake
Fig.	Figure
FLW	Final Live Weight
GP	Growth Promoter
g	Gram
g/b	Gram per bird
IB	Infectious Bronchitis
IBD	Infectious Bursal Disease
ILW	Initial Live Weight
K	Potassium
Kcal	Kilo Calorie
Kg	Kilogram
LWG	Live Weight Gain
Lys	Lysine
Ltd.	Limited
ME	Metabolizable Energy
ml	Milliliter

Abbreviations	Full meanings
MS	Master of Science
NA	Nutrient Agar
No.	Number
ND	New Castle Disease
NS	Non-significant
P	Phosphorus
PI	Performance Index
PB	Probiotic
PBS	Phosphate Buffered Saline
PM	Post Meridian
R	Replication
SEM	Standard Error Mean
SPSS	Statistical Package for the Social Services
Sq. ft.	Square Foot
WHO	World Health Organization
T	Treatment
TK	Taka
µl	Micro liter
1^{st}	First
2^{nd}	Second
3^{rd}	Third
4^{th}	Fourth
5^{th}	Fifth
Symbol	**Full meaning**
%	Percentage
®	Registered Trademark
@	At the Rate Of
°c	Degree Celsius
Symbol	**Full meaning**
/	Per
±	Plus-Minus
*	5% level of significant
**	1% level of significant
>	Greater than
<	Smaller than
:	Ratio
“”	Inverted Coma

Abbreviations	Full meanings
,	Coma
;	Semicolon
-	Hyphen
()	Parenthesis Marks
.	Full Stop



CHAPTER 1

INTRODUCTION

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INTRODUCTION

Poultry rearing is considered superior to the other agricultural sector in Bangladesh because of the quick economic return in a relatively short period of time. Poultry serves as one of the means of satisfying the increased demand for animal protein. The poultry industry during the past two decades has been one of the most dynamic and ever expanding sectors in the world (Alkhalf *et al*., 2010). The present population of poultry in Bangladesh is estimated to be 317.70 million including 266.07 million of chicken and 51.62 million of ducks (Bangladesh Economic Review, 2016). Presently, chicken meat is on demand as a cheap source of protein with low cholesterol value. Therefore, adaptation of broiler farming is increasing day by day by farmers. The biggest challenge of commercial poultry production is the availability of quality feed on sustainable basis at stable prices. In spite of this challenge, commercial poultry production ranks highest among the source of animal protein (Iyayi *et al*., 2008). The increase in the size of the poultry industry has been faster than the other food producing animal industries. The trade volume of poultry products has also increased parallel to the rapid growth of global poultry meat and egg production (Windhorst *et al*., 2006). Many factors may lead to alterations in meat quality. The most directly related to meat quality are pre and post-slaughter practices, bird age, strain, sex, environment, nutrition. Within the latter, antibiotics have been particularly considered by international health institutes, such as the Food and Drug Administration. As 70% of total cost of production is contributed by feed only, improvement of Feed Efficiency (FE) will significantly enhance the margin of profit. Antibiotics have long been used as growth promoters. In recent years, due to the residual effect of antibiotics on human health, the use of many antibiotics in food production is banned or going to be banned. The occurrence of cross resistance of antibiotic growth promoters with the human medicines has become an important issue at present. Moreover, the growing concern arising among the people about food safety, environment contamination, and general health issues due to the presence of residual antibiotics in poultry meat has driven a way to find out a solution to the use of antibiotic growth promoter. Considering these facts in mind the feeding of other non-antibiotic growth promoters such as probiotics, and synbiotics finds a potential substitute for antibiotics.

The term probiotic derived from Greek word "pro bios" which means "in favor of life" (Coppola & Turnes, 2004). According to the definition by FAO/WHO, probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (Fuller *et al*., 1989). The use of probiotic has shown many beneficial effects in broiler. These advantages include the improvement of general health, feed efficiency, and growth rate, as well as resistance to diseases (Ahmad, 2006) and positive response on mortality rate in broiler chickens (Anjum *et al*., 2005). Moreover, probiotics comprise a functional nutritional approach in which maintenance of a healthy gastrointestinal environment is achieved through the intake of adequate quantities of live beneficial microorganisms (Fuller, 1989; FAO, 2002). Probiotics have been regarded as good replacement of feed additives (Tomasik *et al*., 2003). Probiotics are responsible for the production of vitamin B complex and digestive enzymes, and for stimulation of intestinal immunity, increasing protection against toxins produced by pathogenic microorganisms (Kyriakis *et al*., 1999; Alexopoulos *et al*., 2004).

Probiotics are specific chemical agents produced by microorganisms containing *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus rhymnosus, Enterococcus faecium, Bifidobacterium befidum, Aspergillus oryzae, Saccharomyces cerevisiae, Streptococcus thermophiles* and *Torulopsis* (Mohon *et al*., 1996).

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. As a widely used probiotic strain, *Saccharomyces cerevisiae* is considered one of the most health boosting fungus because it have demonstrated a positive effect in aiding nutrient digestion and absorption in the host's body (Scgarrd and Demark, 1990). They have been broadly applied in livestock and poultry as a growth promoter and a competitive exclusion agent (Simon, 2010). The use of *Saccharomyces cerevisiae* spores as a probiotic or a direct fed microorganism could be an alternative to adding medicine to feed in the prevention and treatment of broiler chicken's necrotic enteritis under commercial like conditions (Knap *et al*. 2010). Therefore, when used as a poultry growth promoter, these spores added to feed could enhance broiler chicken's digestibility and performance parameters by creating the favorable conditions for

beneficial bacteria (Steiner *et al*., 2006). In the recent research, it has been revealed that probiotics affect gene expression of carrier proteins responsible for cholesterol absorption (Matur&Eraslan,2012).Since there have been a few investigations on effects of *Saccharomyces cerevisiae* in poultry, little information is available on its impact on nutrient metabolism and histological alterations to intestine in chickens. So, to further prove the potential of these fungal spore containing probiotic in improving broiler performance, this experiment investigated the effect of probiotic (*Saccharomyces cerevisiae*) supplemented in feed with or without growth promoter. Antibiotics are extensively used as growth promoters in poultry production or to control infectious disease. Anti-microbial exercise and/or especially abuse is considered to be the most vital selecting force to antimicrobial resistance of bacteria (Moreno *et al*., 2000, Okeke *et al*., 1999).

Keeping this view in mind, the present research work was undertaken to fulfill the following objectives:

- To determine the carcass characteristics of the experimental broiler (meat composition, meat yield, internal organ and bone development);
- To investigate the effect of probiotics on cecal microbial count of broiler;
- To investigate cost-effectiveness of different diets in broiler performance.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

Now, it is a great concern about the human health to get good quality animal products specially broiler meat. Therefore, animal production systems will have to focus not only on obtaining high production, but also on their impact on the environment as well as on human and animal health (Ferket, 2003).

A number of feed additives including antibiotics have been widely used in the poultry industry for several decades. The manipulation of gut function and microbial habitat of domestic animals with feed additives has been recognized as an important tool for improving growth performance and feed efficiency (Collington *et al*., 1990). The poultry sector is continuously searching for new feed additives in order to improve the feed efficiency and chicken health. The use of feed additives has mainly two objectives; the first one is to control of pathogen microorganisms such as *Salmonella* and Coliforms and the second one is to enhance the digestive microflora with beneficial microorganisms (Shane, 1999). Pharmaceutical or nutritional substances that are not natural feedstuffs are added to made-up and stored feeds for various purposes, chiefly to control infectious disease or to promote growth. Improper use may cause poisoning in the subject animals or undesirable residues in food for human consumption produced by the animals. The use of additives in this way is strictly controlled by legislation in most countries. Some of them require a prescription by a veterinarian to comply with local poisons laws.

2.1 Probiotics

The term "Probiotics" was firstly introduced by Lilly and Stillwell (1965) to describe the growth promoting factors produced by microorganisms. Probiotics, defined as live cultures of microorganisms administered orally, act beneficially on host health; inhibit pathogens; enhance intestinal immunity; and have a protective effect on the gut microflora.

Havenaar and Huisin't Veld (1992) defined Probiotics as "a mono or defined mixed culture of live microorganisms which, applied to animal or man beneficially affect the host by improving the properties of the indigenous gastrointestinal micro biota, but restricted to products that (a) contain live microorganisms (e.g., as freeze-dried cell or in

fresh or fermented product)(b) improve the health and well-being of animals or man (including growth promotion of animals) and (c) can have their effect on all host mucosal surface, including the mouth and gastrointestinal tract (e.g., applied in food, pill, or capsule form), the upper respiratory tract (e.g., applied as an aerosol), or in the urogenital tract (local application)."

Green and Sainsbury (2001) defined probiotics as living microorganism which, given to animals assist in the establishment of an intestinal population which is beneficial to the animal and antagonistic to harmful microbes.

Probiotics, containing lactic acid bacteria lowers the intestinal pH due to production of lactic acid and organic acid while cells adhere to intestinal cell wall and prevent colonization by pathogens. Probiotic microbes start competition for nutrient with pathogenic bacteria. Not only suppress the growth of pathogenic microorganisms in the intestine and incidence of diarrhea but also increases the bioavailability of dietary minerals and increases the growth rate and feed conversion efficiency.

2.2 Characterization of good probiotics

a. The culture should exert a positive effect on the host. It should be acid resistant, bile resistant and contain minimum 30×10^9 CFU per gram.

b. The culture should possess high survival rate and multiply faster in the digestive tract. It should be strain specific.

c. The cultured microorganisms should neither pathogenic nor toxic to the host.

d. The adhesive capability of microorganisms must be firm and faster.

e. Be durable enough to withstand the duress of commercial manufacturing, processing and distribution so that can be delivered alive to the intestine.

f. The cultured microorganisms should possess the ability to reduce the number of pathogenic microorganisms in intestine.

2.3 Effect of probiotics on performance of broiler

There are several types of probiotic preparations in the market. Many studies have been conducted to list the efficacy and effects of such preparations on broiler performances

like body weight, body weight gain, feed intake and feed conversion as well as hemato-biochemical parameters.

2.3.1 Effect on body weight

Kermanshahi and Rostami (2006), Thitarum *et al.* (2005) and Nayebpor *et al.* (2007) reported that probiotic and prebiotic can improve the weight of birds. Comparative study of antibiotics (zinc bacitracin or oxytetracycline) and *Lactobacillus* culture (probiotics) was investigated by Adbul Rahim *et al.* (1999) and Zulkifli *et al.* (2000) and found that combined effects of the treatment had better body weight achievement in broiler chickens. It had also been suggested by Zulkifli *et al.* (2000) and Jin *et al.* (1997) that probiotic supplementation was of the greatest benefit when birds are exposed to stressful condition. Ham *et al.* (1999) found average 159.9 gm higher body weight in broilers with Lactic acid bacteria and yeast. But yeast alone is a good substitute for antibiotics. On the other hand, Samanta and Biswas (1995) stated that feeding probiotics (lactic acid) at a level of 0.3% with drinking water, for the first 2 weeks of broilers did not promote weight gain.

Manickam *et al.* (1994) reported that *Lactobacillus sporogenes* had better final body weight than the control group, which were 1042.21 gm and 955.12 gm per bird respectively. However, Yadav *et al.* (1994) found no effect on body weight of broilers with probiotics.

2.3.2 Effect on body weight gain

The addition of either pure *Lactobacillus* cultures or mixtures of *Lactobacilli* and other bacteria or effective microorganisms produced variable results in body weight gain.

Kim *et al.* (1988) observed that supplementation of a commercial probiotic *(Lactobacillus sporogenes)* increased the weight gain of chicken given a diet containing 10 % moldy maize during 2 or 6 weeks of age. Jin *et al.* (1996) found that significantly higher body weight gain and feed efficiency, providing feed supplemented with commercial *Lactobacillus* in Arbor Acres broiler chicks.

Tarakanov *et al.* (1999) stated that lactoamylovorin, a new probiotic containing *Lactobacillus amylovorous,* receiving bird gained higher body weight (1660 g) than that

of control (1540 g). Singh *et al.* (1999) reported highest weight gain and feed efficiency of broiler chicks with diet containing 0.02 % probiotics.

Erdogan (1999) stated that probiotics treated groups of Ross broilers were higher in live weight gains, feed consumption and feed efficiency than control. Shocib *et al.* (1997) reported probiotic promoter enhanced growth and immune response in chicken. Feeding a diet containing *L. casei* significantly increased average daily weight gain of broiler chicks during first 3 weeks but not during 4-6 days reported by Yeo and Kim (1997).

Mohan *et al.* (1996) reported that body weight gain could be improved from 5-9% when chicken were supplemented with l00 mg/kg of probiotics. It's containing a mixture of *L. acidophilus*, *L. casei, Bifidobacterium bifidum,* and *Aspergillus orygae.*

Holoubek (1993) worked with Lactigerm, Proma, Biostrong 500, Bioalginates and Micromist. It has found that Bioalginates caused a reduced growth but improved feed conversion whereas others improved weight gain in broilers. In commercial broilers the inclusion of *L. sporogenes* @ 100 mg/kg feed resulted in increased body weight gain, improved FCR and humoral immune response in broiler chicks during 0-6 weeks of age (Panda *et al*., 2005). Live yeast culture *(S. cerevisiae)* plus lactic acid producing bacteria *(L. acidophilus* and *S. faecium)* supplemented in broiler feed (1kg/ton) showed improved weight gain and feed conversion (Mohan *et al*., 1996).

2.3.3 Effect on feed intake

The microbial flora has an important role in the digestion and feed intake of broilers (Nahashon *et al*., 1992, 1993, 1994 & 1996) by stimulation of appetite or increasing rate of the digestion of nutrients especially at the lower intestine (March, 1979) or by producing digestive energy or bacterial enzymatic activity (Goldin and Gorbach, 1977). In addition, probiotics even could be able to increase the feed intake in case of moldy grain. Kim *et al.* (1988) found increased ($P<0.01$) feed intake in case of moldy maize treated with a probiotic product. Feed intake per chick was 3589.29, 3315.8, 3318.02, 3325.71 and 3331.76 gm when broilers fed with control, T.M. 50, Biovin-40 and Alback probiotics respectively from day old to 7 weeks of age (Khan *et al.,* 1992). Feed intake was significantly lower ($P<0.05$) when Cobb 500 broilers were fed antibiotics than probiotics at both 0-25 and 26-53 days (Fabris *et al.,* 1997).

In case of EM (Effective Micro-organism), Haq *et al.* (1997) found highly improved (P<0.01) feed intake and Chaiwatanasin *et al.* (1998) found significant (P<0.05) improvement of feed intake for broilers. On the other hand, Baidya *et al.* (1994) studied with *Lactobacillus sporogenes* and different antibiotics found no significant differences in feed intake between different antibiotics and probiotics group of broilers.

Opalinski *et al.* (2007) evaluated the effect of a probiotic (*Bacillus subtilis* strain *DSM 17299*) in broiler diets on the feed intake, live weight gain and feed conversion ratio. They compared the diets with probiotic as a growth promoter. The use of growth promoter did not improve the live weight gain at the studied ages. There were a marked improvement in the feed conversion ratio of broilers fed the diet with antibiotics and diet with added *B. subtilis*. It was concluded that the *B. subtilis* probiotic could be used as a growth promoter in broiler diets.

2.3.4 Effect on feed conversion

A variety of works with *Lactobacillus* spp. were done to understand the effect on feed conversion of broilers. Someone used single *Lactobacillus* and other used different type of microbes or energy levels also. Manickam *et al.* (1994) found a highly significant difference (P<0.01) between control and treatment group in respect of feed conversion of broiler using only *Lacbobacillus sporogenes* at the rate of l g/liter drinking water through out6 weeks experimental period.

Zinc bacitracin, an antibiotic, was used to compare the feed conversion with *Lactobacillus spp.* Alvarez *et al.* (1994) and Hamid *et al.* (1994) found an improved feed conversion of 1.9 for Indian River at 6 weeks and average 2.09 for 5 weeks respectively using *Lactobacillus* singly. Cho *et al.* (1992) used virginiamycin and found improved feed conversion by 0.3 to 3.1%.

Pumshothaman and Natanam (1999) indicated the significant improvements of feed conversion in broilers up to 8 weeks with yeast culture and enzymes. The similar result was also observed by Goh and Hwang (1999) using 1% yeast culture.

Chandra Sekaran and Reddy (2001) defined feed additives "As an ingredient or mixture of ingredients added to the basic feed mix or parts of there, usually in small quantities to fulfill a specific function, nutritive or non-nutritive. The antibiotic growth promoters have been under critical examination for many years and already banned in

many countries. It is the relationship with similar antibiotics used in human medicine and the possibility that their use may contribute to the pool of antibiotic resistance bacteria that cause concern. In light of that situation, probiotics and organic acids are now being used as feed additives in poultry diet as alternative approach of antibiotic growth promoters considering safety aspect of the products as well as human health. In case of EM (Effective Microorganism), Haq *et al.* (1997) found highly improved ($p<0.01$) feed intake and Chaiwatanasin *et al.* (1998) found significant ($p<0.05$) improvement of feed intake for broilers. On the other hand, Baidya *et al.* (1994) studied with *Lactobacillus sporogenes* and different antibiotics, Yadav *et al.* (1996) with Yeast, Samanta and Biswas (1995b) with *L. acidophillus* and *L. bulgaricus* or a mixture of it, and found no significant differences in feed intake between different antibiotics and probiotics group of broilers.

It has been shown by several scientific studies that antibiotics added to animal feeds as growth promoters allow better live performance (Dibner & Richards, 2005). However, the growing concern with the possible relation between in feed antibiotics and bacterial resistance in livestock and humans has driven the adoption of new measures to control those compounds (Ferket, 2003; Fuller, 1989; Jin, 1997), despite the lack of evidences (Jones *et al.*, 2005). This situation has driven much research on the search for alternatives that are able to maintain high productivity and to be economically feasible, as well as not being harmful to human and animal health. Thereby complying with the requirements of consumers and foreign markets. Among these alternatives probiotic is currently used for the industrial synthesis of products with biotechnological interest.

Salim *et al.* (2013) investigated the effects of supplementation of direct-fed microbials (DFM) as an alternative to antibiotics on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. They used 4 dietary treatments a cornmercial soybean meal basal diet (control); control plus 0.1% virginiamycin, as an antibiotic growth promoter (AGP); control plus 0.1% direct-fed microbials that contained *Lactobacillus reuteri* (DFM 1); and control plus 0.1% direct-fed microbials that contained a mixture of *Bacillus subtilis, B. licheniformis* and *Saccharomyces cerevisiae* (DFM 2). Their results showed that dietary AGP and DFM supplementation significantly increased ($p< 0.05$) the BW gain of broilers. The feed intake was reduced, whereas the feed conversion was improved significantly when birds

were fed DFM 2. The white blood cell, monocyte levels and plasma immunoglobulin were significantly higher in the DFM 2 group compared with the control.

Shim *et al.* (2012) conducted an experiment to evaluate the effect of multi-microbes probiotic on growth performance, nutrient retention and caecal microbiology of broilers. They fed the broiler a probiotic mixture (*B. subtilis, B. licheniformis and S. cerevisiae*) and compared with a control and an antibiotic (avilamycin) treated group. Finally, they reported that feeding probiotic mixture improved overall weight gain, CP retention and reduced *Clostridium* and coliforms in the caecum compared to the control group.

Bai *et al.* (2013) investigated the effects of a probiotic product incorporating *Lactobacillus fermentum, B. subtilis, B. licheniformis* and *Saccharomyces cerevisiae* on the growth performance and intestinal immune status in broiler chickens. They compared the probiotic treated group with a control, an antibiotic and (a probiotic + antibiotic) treated group and found that body weight gain and feed efficiency were improved ($P<0.05$) in broilers fed the probiotic diet compared with control, and were similar to the probiotic plus antibiotic treated group. Chicks fed probiotics had higher proportions of CD3+, CD4+, and CD8+ T-lymphocytes, whereas the antibiotic diet decreased the proportion of CD8+ T-lymphocytes in the foregut of broilers. These results indicated that the probiotic product incorporating *L. fermentum, B. subtilis, B. licheniformis* and *S. cerevisiae* could stimulate intestinal T cell immune system without decreasing growth performance in broilers.

Luiz *et al.* (2012) conducted an experiment to study the effects of biotic additives on growth performance and meat qualities in broiler chickens .They treated the broiler with five diets containing probiotics (*Bacillus licheniformis, B. subtilis*), prebiotics (mannan oligosaccharide-MOS), synbiotics (Saccharomyces *cerevisiae*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum),* MOS and FOS (fructooligosaccharides), Avilamycin, or a control treatment (no additives). Performance parameters including total weight, daily weight gain, feed intake, viability production efficiency index and yield of carcasses and cuts were evaluated. The results indicated that the biotic treatments caused significant differences in the parameters and these additives are therefore nutritionally feasible to replacement growth promoters and the animal husbandry indices of animals treated with these additives were similar to those of

animals fed the normal rations and the use of additives contributed to improvements in the meat quality.

Ashkan *et al*. (2012) investigated the effects of dietary supplementations of multi-strain probiotics (protexin) on broiler performance, carcass yield and organ weights of broiler chicks. A total of 96 seven-d-old mixed sex broiler chicks (Ross 308) were weighed and randomly allocated to two treatment groups, each with 4 replicate pens of 12 chicks. The experimental diets consisted of a basal diet without additive (control) and 0.2 g kg-1 probiotics (protexin). At day 42, two birds per replicate were slaughtered for the determination of carcass traits. Organ weights, body weight, feed intake and feed conversion ratio index were not markedly affected by dietary treatments. Carcass yield increased in broilers fed diets containing probiotics ($P<0.05$). In conclusion, our results suggest that protexin could be used to increase carcass yield of broiler chicks.

Fanelli *et al.* (2011) conducted an experiment to investigate the effects of the inclusion of a probiotics mix (*Bacillus licheniformis*, *B. subtilis and Cl. butyricum)* on growth performance, health status, and intestinal morphology of broiler chickens infected with *Eimeria spp.* He investigated that birds fed the diets containing probiotics (PC and P) had significantly higher body weight (BW), average daily gain (ADG) and feed intake (FI) compared to animals fed the diet containing only a coccidiostat (C) and the feed conversion ratio (FCR) was significantly lower in the groups fed the diets containing probiotics (P and PC). In addition to these, administration of probiotics plus coccidiostat (PC) positively affected the number of intestinal fold goblet cells (F) in the duodenum and ileum.

Haoshen *et al.* (2004) conducted an experiment to study the impact of probiotic on broiler production, blood biochemical parameter and intestinal microflora. They used different bacterial culture (*Bacillus subtilis, B. licheniformis, Streptococcus lactis)* and demostrated that *B. licheniformis* increased production performance, decreased diarrhoea and mortality rates of experimental group.

Pelicano *et al.* (2003) evaluated the effect of different probiotics *(Bacillus subtilis, Bacillus licheniformis*; and *Saccharomyces cerevisiae)* on carcass and meat quality of broilers. They found that the groups fed with probiotics showed higher ($P<0.01$) leg yield and there was a significant decrease in color (lightness) and increase in pH of breast muscle 5 hours after slaughter in the probiotics treated birds. In the sensory

analysis, meat quality was better when probiotics were fed in the water and diet instead of only in the diet.

Mutus *et al.* (2006) conducted a study to investigate the effects of a dietary supplemental probiotic on morphometric parameters and yield stress of the tibia. 50 day-old broiler chicks were assigned to a control or an experimental diet containing *Bacillus licheniformis* and *Bacillus subtilis*. They concluded that tibio-tarsi weight, length, and weight/length index, robusticity index, diaphysis diameter, modulus of elasticity, yield stress parameters, and percentage Ca content were not affected by the dietary supplementation of probiotic, whereas thickness of the medial and lateral wall of the tibia, tibio tarsal index, percentage ash, and P content were significantly improved by the probiotic. Medullary canal diameter of the tibia of the birds fed the control diet was significantly greater than that of birds fed the probiotic diet.

Novak *et al.* (2011) conducted an experiment to evaluate effects of two commercially available probiotic additives, containing *Bacillus subtilis, Bacillus licheniformis* (group A),*Bacillus cereus* (group B), on carcass and meat characteristics, serum lipids and concentration of cecal volatile fatty acids of meat type chickens. Results showed that birds in group B had higher ($P< 0.05$) final body weight compared to birds from group A and higher carcass weights and yield percentages compared with control. Breasts and whole legs were also heavier in group B, compared to control, but not the yield. Group A had higher yield of wings and lower abdominal fat weight compared to group B ($P< 0.05$). Both probiotics influenced the cecal fermentation, which was observed as decrease in cecal concentrations of propionic, butyric, n-butyric and n-valeric acids, but the differences compared to control group were statistically significant for group A only.

Rahimi (2009) conducted a study to investigate the effects of a commercial probiotic mixture (Bio-Plus 2B® containing *Bacillus subtilis* and *Bacillus licheniformis*) supplementation to the diet of broiler chickens on the growth performance and humoral immune response. Total of 180 one-day old Ross 208® broiler chicks were randomly divided into two experimental groups; group 1 as the control group and group 2 as the treatment group. He observed that, the probiotic mixture supplementation to the broilers diet could improve the live body weight; feed conversion ratio and antibody response to Newcastle disease virus.

2.4 Effect of growth promoters on the performance of broilers

Ashayerizadeh *et al.* (2009) observed the effects of antibiotic (flavomycin), probiotic (primalac containing *Bacillus subtilis, B. licheniformis, Streptococcus lactisetc*), prebiotic (Biolex-MB) and a mixture of probiotic and prebiotic (primalac plus Biolex-MB) as dietary growth promoter on growth performance and carcass characteristics of broiler. The highest significant values ($p<0.05$) of carcass and thigh were recorded for broiler fed diet supplemented with flavomycin. The highest breast weight was recorded for broiler fed the diet supplemented with primalac, meanwhile the lower value were showed for broiler fed diet supplemented with either Biolex-MB orprimilac plus Biolex-MB($P<0.05$). The result of present study revealed that probiotic and prebiotic as a growth promoter can be used as alternatives to antibiotics due to their beneficial effects on the consumer and to improve broiler growth indices.

Ali *et al.* (1994) used livol as a herbal growth promoter. They reported that livol at a level of 0.5% of the diet in broiler improved survival rate, dressing percentage and eviscerate meat yield percentage, but had no effect on abdominal fat.

Ahmad and Taghi (2006) conducted an experiment with 300 broilers to evaluate the influence of dietary supplementation of probiotic (*Bacillus subtilis and B. licheniformis*) on performance and immune competence. Body weight gain of broiler, fed diet supplemented with 50 mg/kg of probiotic was significantly higher during 1-21 and 22-42 days than broiler fed the control diets.

Sabatkova *et al.* (2008) conducted an experiment to compare the stimulatory effect of the probiotic BioPlus 2B (*Bacillus subtilis and B. licheniformis*) with that of the antibiotic Avilamycin on the growth and feed conversion in broiler chickens. They reported that the supplementation of the diets used in broiler prefattening and fattening with BioPlus 2B resulted in a 4–5% weight gain ($p<0.01$) and in a 4–5% improvement ($p<0.01$) in feed conversion. Mean slaughter yields were higher ($p< 0.01$) in chickens fed diets containing probiotic and antibiotic products compared to the group of chickens fed the diet not supplemented with a growth promotor. The weight of inner fat in chickens under study did not differ significantly either between experimental groups or between sexes.

O'Dea *et al.* (2006) examined probiotic mixtures (*Bacillus subtilis, B. licheniformis* and *Aspergillus oryza*) using different regimes and concluded that weight gain improve significantly in broilers produced by 43 and 57 week old breeder flocks fed the supplemented diet.

Zhou *et al.* (2010) found that *Bacillus spp.,* improved growth performance, FCR, breast chemical composition and meat quality of Guangxi Yellow chickens.

Panda *et al.* (2008) reported that dietary preparation of *Bacillus subtilis* and *B. licheniformis* (at the rate of 6×10^8 spore per kg of diet) significantly enhanced feed efficiency in White Leghorn Breeders, which was ascribed to the beneficial effects of probiotic feeding on digestion and utilization of nutrient. In the same study no positive effect of this probiotic was recorded on body weight gain and feed intake.

Erdogan (2007) studied the effects of Zinc bacitracin (antibiotic) and two different probiotics on body weight gain, feed conversion and dressing percentage of broiler. A total of 250 Ross broilers were divided into control and experimental groups. The control group was fed on supplemented basal diet, where experimental birds were given different amounts of probiotics. He suggested that the probiotic treated groups were higher in live weight gain, feed consumption and feed efficiency.

Faria *et al.* (2009) observed the effects of different antibiotics, probiotics and their combination on the performance and carcass yield of the broiler. It was observed that the tested probiotic could be used together with coccidiostat (sodicmonensin) and avilamycin (growth promoter). However, the presence of virginiamycin could impair the viability of probiotic. There was no significant interaction between antibiotic and probiotic for the evaluated variables. The performance parameter, carcass and yield characteristics were not influenced by the administration of probiotic, antibiotic or by the combination of such products in the diets.

Lei *et al.* (2013) evaluated the effects of dietary inclusion of *Bacillus licheniformis* on broiler performance, antioxidant enzyme activities, and intestinal barrier function. He randomized 540 broiler chicks into 6 groups. The control group received the basal diet formulated with maize and soybean meal. The treatment groups received the same basal diets supplemented with 0.01, 0.02, 0.03, 0.06, and 0.09% *Bacillus licheniformis* powder (2×10^{10} cfu/g) for an 8weeks trial. The results showed that dietary supplementation with

0.01 and 0.03% *B. licheniformis* significantly increased meat yield. He also reported that dietary supplementation with *B. licheniformis* increased the intestinal villus height.

Rada *et al.* (2013) conducted an experiment to evaluate the effect of addition of exogenous protease (a mono-component serine protease expressed in *Bacillus licheniformis*) into broiler grower diets on growth parameters (body weight and feed conversion ratio) and carcass characteristic (carcass weight and yield). The results of the experiment showed that the exogenous mono-component protease added into low protein broiler diet had no significant effect on both observed growth parameters and carcass characteristics.

Jinmo *et al.* (1997) reported that feeding 0:1% *Lactobacillus casei* (probiotics) in the diet of broiler chicken increased average daily gain during the first 3 weeks period compared to control. They further stated that the probiotic decreased bacterial activity in the small intestine of young chickens and may be beneficial for improving animal health and growth especially during early period of life.

Deng *et al.* (2012) investigated the effect of the probiotic *Bacillus licheniformis* on the egg production, gut morphology, and intestinal mucosal immunity of laying hens and observed that inclusion of 10^7cfu/g of *B. licheniformis* in the diet of heat- stressed hens was effective in overcoming decline in egg production and feed intake, restoring the impaired villus structure, and sustaining a balanced mucosal immune response. Therefore, they concluded that the probiotic *B. licheniformis* may be useful for ameliorating the adverse influence of heat on the egg production and gut health of laying hens.

Xiaolu *et al.* (2012) conducted a feeding trial to investigate effects of *Bacillus licheniformis* on growth performance and meat quality of broilers. Nine hundred one-day-old broiler chicks were randomly assigned to 3 experimental groups with three replicate pens of 100 broiler chicks. Three treatments were i) control, ii) basal diets supplemented with 1 ml of *B. licheniformis* for each in feed water per day iii) basal diets supplemented with 2 ml of *B. licheniformis* per chick in feed water per day. He reported that supplementation of *B. licheniformis* significantly increased body weight in grower chickens ($p<0.05$), and significantly improved the feed conversion in 3 to 6 and 0 to 6 wk feeding period compared with the control group ($p<0.05$). Additionally, the supplement also resulted in increased protein and free amino acid contents, and

decreased fat content in chicken breast fillet ($p<0.05$). Furthermore, improvement in sensory attributes was observed in broilers fed with the probiotic. Finally he concluded that, *B. licheniformis* can be used as a growth promoter and meat quality enhancer in broiler poultry.

Eseceli and Demir (2010) evaluated the effect of an antibiotic growth promoter (Avilamycin) and probiotic containing mannan oligosaccharide (Bio-MosReg) on performance of broiler. Live weight, live weight gain, feed intake, feed conversion ratio, mortality rate were not affected significantly by dietary treatments throughout the experiment ($p>0.05$). Mannan oligosaccharide (Bio-MosReg) has the potential to be an alternative to antibiotic growth promoter in broiler diets.

Sabiha *et al.* (2005) reported that the effects of different levels of probiotic (*Lactobacillus acidophilus, Streptococcus faecium* and Yeasacc 1026) supplementation on the performance of broiler chicken were evaluated using 144, one-day-old, commercial broiler chicks for a period of eight weeks. The 0.025 percent probiotic supplemented birds showed a significantly higher ($p<0.05$) body weight and weight gain upto six weeks of age. The feed intake, feed efficiency and protein efficiency were statistically non-significant at sixth and eighth weeks of age among the treatment groups. The mortality percentage was not affected by treatments. Cost of production of broilers was lower in the 0.025 and 0.05 per cent probiotic supplemented groups at six and eight weeks of age respectively. It was concluded that the probiotic supplementation in standard broiler ration at a lower level was beneficial in the early stages of growth.

Zhang Ren Yi (2010) conducted an experiment to study the effect and mechanism of *Bacillus licheniformis* on broiler growth performance. Three hundred one-day-old broiler chicks were randomly divided into 5 treatments to compare different level of *Bacillus licheniformis* preparation with a control and an antibiotic group. They concluded that Bacillus licheniformis preparation can improve broiler growth performance, immune function, antioxidant function and intestinal antibacterial capabilities and improve the organizational structure of the intestinal duodenal total protease activity, and has the function of promoting growth and increase resistance to disease.

Knap *et al.* (2011) evaluated the effect of *Bacillus licheniformis* to prevent necrotic enteritis in broiler chickens. Three studies were conducted using *Clostridium perfringens*

as an intestinal challenge to produce necrotic enteritis (NE). In all three studies *B. licheniformis* (1.6×10^6–8×10^7 CFUs/g) showed a significantly decreased feed conversion ratio, increased weight gain, reduced NE lesion score, and NE- reduced mortality compared to the non-medicated *C. perfringens*–challenged group. The present data indicate that the use of *B. licheniformis* spores as a probiotic or direct-fed microbial could be an alternative to adding medication to the feed to overcome NE under commercial conditions and could therefore be of direct use in preventing antibiotic-resistant pathogens in chickens.

Poovendran *et al.* (2011) conducted an experiment to study the effect of *Bacillus licheniformis* on feather keratine degradation and quantification of keratinase enzyme produced. They used *B. licheniformis* for degrading keratine substrate such as sheep, goat hair and poultry feather and found that after degration the final product contained about 70% protein and 20% carbohydrate. They concluded that this process reduces the feed cost and produce industrially important enzyme and at the same time protect the environment from pollution.

Tamilmani *et al.* (2008) conducted an experiment to study the effects of *Bacillus licheniformis*on degradation of extra cellular feather of poultry. They isolated unidentified bacterial strain from the soil and found that *Bacillus licheniformis* was responsible for keratinase production by shake flask fermentation in a basal medium containing 1% feather.

Lee *et al.* (2007) found that in-feed probiotic can be effective against *Eimeria tenella* and *E. acervulina* infections. They found that feeding 0.1% probiotics orally reduced oocyte shedding in broilers infected with 5000 CFU *E. aecrvulina,* resulting in improved weight gain. In the same experiment, eimeria-specific antibody levels were significantly higher compared to the unsupplemented control birds. These results suggest that probiotics effectively enhance the resistance of broiler and protect them against the negative growth effects associated with coccidiosis.

Jayaraman *et al.* (2012) investigated the influence of a dietary supplement, *Bacillus subtilis*PB6, on performance, intestinal health, and gut integrity against *C. perfringens*-induced NE in broiler birds. They found that supplementation of *B. subtilis* PB6 reduced the FCR ($p<0.05$) and intestinal *C. perfringens* counts significantly ($p<0.05$)

compared with the infected control group and improved villi length by 10.88 and 30.46% ($p<0.05$) compared with uninfected and infected control groups respectively.

2.5 Research gaps

The beneficial effects of probiotics are the of improved growth performance, immune function, better feed utilization, absorption of nutrients, resistance to infectious bacteria and beneficial changes in the intestinal architecture. As such, probiotics may serve as alternative to growth promoting antibiotics. Although the intake of probiotics has been associated with many beneficial effects in poultry production, there is no consensus on the exact dose or type or bend of probiotic(s) to be fed and the duration of feeding. Moreover, literature on the effect of feeding probiotic, growth promoter and their combination on broiler performance as well as its cost- effectiveness under Bangladesh condition are scanty. Many works have been done on probiotics and antibiotics on broiler performance but the use of growth promoter (not antibiotic growth promoter) in broiler diet is new in Bangladesh. Keeping these views in mind, an attempt is undertaken to investigate the effect of growth promoter and probiotics as an alternative to antibiotic growth promoter in broiler production. Therefore the proposed study was attempted to generate more information on the effects of probiotic with or without growth promoter on broiler performance. To get the safe poultry products, the poultry feed industry needs adequate information on this aspect to augment commercial broiler production in Bangladesh.

CHAPTER 3

MATERIALS AND METHODS

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3.1 Statement of the experiment

The experiment was conducted at Kader Poultry Farm, Cornai, Dinajpur to study the effect of feeding probiotic *(*A-MAX®*)* with or without growth promoter (Ami vet®) on broiler performance. A total of 120 one-day-old straight run Cobb-500 commercial broiler chicks were used for this research work. The duration of the research work was 32 days (from 03 May to 04 June, 2016).

3.2 Preparation of the experimental house

A gable type open sided house was used for experimental purpose. The room was 70 cm above from the ground surface on the bamboo made floor. A wire net was used on the floor. The room area was 147 sq. ft. The room was partitioned into 12 pens of equal size by using wire net and bamboo materials. Area of each pen was 12.25 square feet (3.5ftx 3.5ft). The room was divided into three parts. The two parts were used as replication. The experimental room was thoroughly brushed, swiped and properly washed by water after that bleaching powder @ 1kg/500 sq.ft was spread over the floor and it was kept 24 hours without any further attention. The bleaching powder was cleaned by using forced tap water. After that the room was disinfected by GPC 8®solution (Manufactured by Animal Health Divisionl, Reneta Ltd, Bangladesh). Feeders, waterers, buckets and all other necessary equipments were also properly, washed and disinfected by GPC 8®solution (5 ml/L). Then fresh and dry rice husks were spread on the floor of the pens as a litter material. Proper managemental procedures were followed during experimental period and identical management practices were maintained.

3.3 Collection of the experimental broiler

The chicks were collected from a reputed hatchery (Kazi Farms Ltd., East Goalpara, Thakurgaon Town, Thakurgaon) of Bangladesh.

3.4 Design of the Experiment

The experimental broiler chicks were equally and randomly divided and distributed into 4 dietary groups and each group was replicated to 3 subgroups. Each dietary group consists of 30 chicks distributed into 3 replicated pens having 10 chicks. The layout of the experiment is shown in Table 1.

Table 1: Layout of the experiment

Treatments	No. of chicks per Replication			Total Number
	R1	R2	R3	
T_0 (Control)	10	10	10	30
T_1 (Control+ PB)	10	10	10	30
T_2 (Control+ GP)	10	10	10	30
T_3 (Control + GP + PB)	10	10	10	30
Total	40	40	40	**120**

GP= Growth promoter, PB= Probiotic, R= Replication

Figure 3.1: Design of experimental house

3.5 Collection of probiotic

The trade name of the probiotic product used in the experiment was "A-MAX®". It was manufactured by one of the USA Company named "Varied Industries Corporation (VI-

COR)" and imported in Bangladesh. According to manufacturer's instruction the inclusion rate of the product for commercial broiler was 100g/50 kg feed.

Table 2: Composition of the probiotic (A-MAX®)

Name of the ingredients	**Amount**
Saccharomyces Cerevisiae	$1.5x10^{12}$ CFU/kg
Amino Acids: Alanine, Argine, Aspertic Acid, Cystine, Glutamic Acid, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phnylalanine, Proline, Serine, Threonine, Valine	21.0% Minimum
Vitamin B_{12}	8.0% Minimum
Minerals & Other Vitamins	5.5% Minimum
Total Amino Acid, Vitamin & Minerals	34.5% Minimum

3.6 Collection of Growth Promoter

The trade name of the growth promoter used in this study was "Ami vet®" (multivitamin) and manufactured by a Bangladeshi Company named "Gentry Pharmaceuticals Ltd". This product was collected from a pharmacy named "Meysers Poshu Sastho." Dinajpur, Bangladesh, who is responsible for marketing this product in Dinajpur district.

Figure 3.2: Probiotic (A-MAX®), Therometer and Growth promoter (Ami vet®)

3.7 Management during brooding period

The chicks were brooded up to 7 days. For conducting the experiment in winter season at early period the temperature was lower than the required brooding temperature. Temperature was maintained at 30℃ as brooding temperature which decreased gradually in subsequent weeks @ 2.5℃/week until the birds were adjusted to environmental temperature. To maintain lighting program and brooding, electric bulb (100 watt, 1 in each pen) were used up to 14 days of age of broilers. Broilers were exposed to 24 hours continuous light in first 14 days. Next 9 days 1 hour dark then 5 days 2 hr dark and last 4 days 4 hour dark was provided. To maintain optimum temperature one 60 watt bulb was replaced between two cages instead of 100 watt bulb.

Figure 3.3: Management of chicks during brooding period

3.8 Experimental diet

Completely pellet form diet was given to the broiler. All the feed ingredients were fresh and of good quality. Chemical analysis of the experimental ration was done in Degussa Lab, Germany (Courtesy of Evonik Degussa GmbH) by the Aftab feed Ltd., Bangladesh.

3.9 Formulation of broiler ration

The broiler diet was formulated for two phases (starter and grower). Starter diet was provided from 1st day to 12th days and grower diet was provided from 13th days to 32th days. Both types of diet were supplied in pellet form. The nutrient requirements (ME, CP, CF, EE, Ca, P, Lysine and Methionine) were satisfied as per requirement as recommended for Cobb-500 broiler strain diet and also same for all treatment except growth promoter (GP), probiotic (PB) and their combination.

After weighing according to requirement, pellets were mixed with the proper amount of probiotics by hand mixing using protected separate hand gloves. Then required amount of probiotics were mixed with a small quantity (about 250gm) of pelleted feed. Eventually this amount was mixed with total feed using hand mixing of 1 kg capacity. Diets for each treatment were supply separately and distributed into three replicates with the help of plastic containers for each treatment. Four plastic containers were needed for 12 replicates. The nutrients requirement of broilers was satisfied according to BSTI standard. The composition of the diet is shown in Table 3; 4; 5.

Table 3: Ingredient composition of broiler starter ration

Ingredients (%)	Treatments			
	T_0	T_1	T_2	T_3
Corn	51.16	51.16	51.16	51.16
Soybean meal 44%	41.71	41.71	41.71	41.71
Soya oil	3.38	3.38	3.38	3.38
DCP	1.63	1.63	1.63	1.63
Calcium Carbonate	0.953	0.953	0.953	0.953
NaCl	0.273	0.273	0.273	0.273
$NaHCO_3$	0.23	0.23	0.23	0.23
DL-Methionine	0.305	0.305	0.305	0.305
L-Threonine	0.03	0.03	0.03	0.03
Vit-Min-Premix	0.25	0.25	0.25	0.25
Probiotic(PB)	-	-	0.20	0.20

T_0= Control, T_1 = Control + Probiotic, T_2 = Control + Growth promoter,

T_3= Control + Growth promoter + Probiotic

Table 4: Ingredient composition of broiler grower ration

Ingredients (%)	Treatments			
	T_0	T_1	T_2	T_3
Corn	61.45	61.45	61.45	61.45
Soybean meal 44%	31.63	31.63	31.63	31.63
Soya oil	3.10	3.10	3.10	3.10
DCP	1.725	1.725	1.725	1.725
Calcium Carbonate	0.94	0.94	0.94	0.94
NaCl	0.27	0.27	0.27	0.27
$NaHCO_3$	0.23	0.23	0.23	0.23
DL-Methionine	0.25	0.25	0.25	0.25
L-Threonine	0.04	0.04	0.04	0.04
Vit-Min-Premix	0.25	0.25	0.25	0.25
Probiotic(PB)	-	-	0.30	0.30

T_0= Control, T_1 = Control + Probiotic, T_2 = Control + Growth promoter,

T_3= Control + Growth promoter + Probiotic

Table 5: Nutrient composition of broiler feed

Parameter	Starter diet (0-12days)	Grower diet (13-32days)
ME (kcal/kg)	3025	3100
Crude Protein (%)	22.00	21.00
Crude Fat (%)	5.00	5.50
Crude fiber (%)	2.50	2.50
Lysine (%)	1.20	1.10
Methionine (%)	0.50	0.48
Calcium (%)	0.90	0.88
Phosphorus (%)	0.45	0.42
Moisture (%)	11.00	11.0

3.10 Uses of experimental feeds

Starter diet was provided for the first 13th days and grower diet was provided to the broiler up to 32 days of age. In all cases, feeds were offered to the broiler chicks *adlibitum*.

3.11 Routine management

Following routine management procedures are followed during experiment-

- Litter management
- Floor space management for birds
- Temperature management (Lighting for broiler)
- Feed and water management

Fresh and dry rice husk was used as litter materials at a depth of about 3cm. After 14 days, all old litter was replaced by fresh rice husk. The floor space allowed for each bird was 1 sq. ft. to ensure comfort of the birds. Since the research was conducted during summer when the environmental temperature was more than 35°C, supply of extra heat was not necessary. Even though the provision of brooding was kept ready for emergency meet up. Gunny bags and jute cloth were provided around the broiler room to prevent adverse environmental conditions. The broiler was exposed to a continuous lighting of 23 hours and a dark period of 1 hour in each 24 hours of photoperiod. One round tube feeder and one round drinker with a capacity of eight liter were provided in each pen. The feeder and drinker were fixed in such a way that the broilers were able to eat and drink conveniently. Feeders were cleaned everyday while waterers were cleaned every day at morning and afternoon. From day 1 to 21 days of age feed was supplied on *ad libitum* basis4 times daily (dawn, morning, afternoon and early night) after that feed was supplied three times daily (morning, afternoon and early night). Fresh and clean drinking water was also supplied *ad libitum* basis with growth promoter three times daily (morning, afternoon and early night).

3.12 Immunization of birds

Birds were vaccinated against common infectious diseases as a part of disease prevention program. All of the experimental broilers were vaccinated against New Castle disease (Ranikhet) and Infectious Bursal Disease (IBD) (Gumboro) at the age of day 5^{th}, $10^{th,}$ 17^{th} and 21^{th} respectively. All the vaccines were administered as per recommendation of the manufacturer (one drop in each eye) at the cooler part of the day (morning).Vaccines were used as per manufacturer's instruction and following schedule was followed.

Table 6: Vaccination schedule

SL. No	Age of vaccination	Name of vaccines	Trade Name	Company	*Doses	Method of vaccination
1	5th day	IB+ND	Cevac IBD L	ACI	1000	Eye drop
2	10th day	Gumboro	Hipra GM97	Hipra	Do	Drinking water
3	17th day	ND	Cevac IBD L	ACI	Do	Do
4	21st day	Gumboro	Hipra GM97	Hipra	Do	Do

*As per manufacturer's instructions

3.13 Processing of broilers

At the end of the trial, to determine meat yield characteristics of the birds, 12 broilers; one broiler from each replicate group weighing average of pen weight were selected to facilitate processing. All broilers feed was withdrawn 12 hours prior to killing the birds. The birds were killed and allowed to bleed for 2 minutes and immersed in hot water (51-55°C) for 120 seconds in order to lose the feathers and this procedure was called semi-scalding. The feathers were removed by hand pinning. This was done manually. Then head, shank, viscera, giblet (heart, liver and gizzard) and abdominal fat were removed for determination of meat yield parameters. Dressed broilers were cut into different parts such as breast, thigh, drumstick, wing and back. Finally, every cut up parts were weighed and recorded for broiler of all replications.

The broiler processing data were calculated and recorded as follows:

i) **Blood weight:** Blood weight was calculated by deducting the slaughtered weight from the live weight of broilers after complete bleeding.

ii) **Feather weight:** Feather weight was calculated by deducting the complete de-feathering broiler weight from the slaughtered weight of broilers.

iii) **Cut-up parts weight:** The weight of head, neck, viscera, heart, liver, gizzard, thigh meat, drumstick meat, back meat and breast meat were determined individually by weighing in a sensitive digital balance.

iv) **Dressing yield:** Dressing yield was calculated by subtracting the weight of blood, feathers, viscera and shank from the live weight.

v) **Giblet weight of broiler:** Giblet weight was the total weight of liver, heart, gizzard, lungs and spleen.

vi) **Dressed weight of broiler:** Dressed weights of broilers were calculated deducting the weights of head, neck and giblet

3.14 Data collection and record keeping

A standard record book was maintained throughout the experimental period.

Following parameters were recorded in the record book-

- Body weight of the broiler(in each week)
- Body weight gain
- Daily supplied amount of feed and feed residue
- Feed Efficiency (FE)
- Performance index (PI)
- No. of dead birds (mortality)
- Temperature and humidity (on regular basis)
- Record of vaccination
- Any disease or abnormal condition of the broiler
- Cost of production

Body weight of the experimental birds was recorded initially and weekly basis for all birds from each replication. The average body weight gain of the broiler in each replication was calculated by deducting initial body weight from the final body weight. Feed consumption was calculated as the total feed consumed in each replication divided by the number of birds. The amount of feed consumed per unit of weight gain was calculated and shown as Feed Efficiency (FE). Performance index (PI) was calculated as the live weight (kg) of the bird by the Feed efficiency and multiplied by 100. Mortality was calculated on the basis of total number of birds housed and number of birds died during the experimental period. During the experimental period, the temperature and relative humidity of the experimental house were recorded four times in a day (6.00AM, 2.00PM, 6.00PM, 11.00PM) with the help of a thermometer and recording regular local humidity. At the end of the experiment the dressing percentage of the broiler was calculated as the dressed weight divided by final body weight of the broiler.

3.14.1 Records of dressing yield

During processing following meat yield data were recorded for some parameters from each pen. The recorded data were on live weight, selection of broiler. Individual broiler weighing average of the pen weight was collected, slaughtered, blade, de-feathered, eviscerated, dressed and dissected to determine meat yield.

Parameters are: blood loss, feather loss, head weight, neck weight, shank weight, viscera weight, dressed weight, breast weight, thigh meat, drumstick weight, wing meat, heart weight, abdominal fat weight, trimmed meat, total meat and dressing percentage.

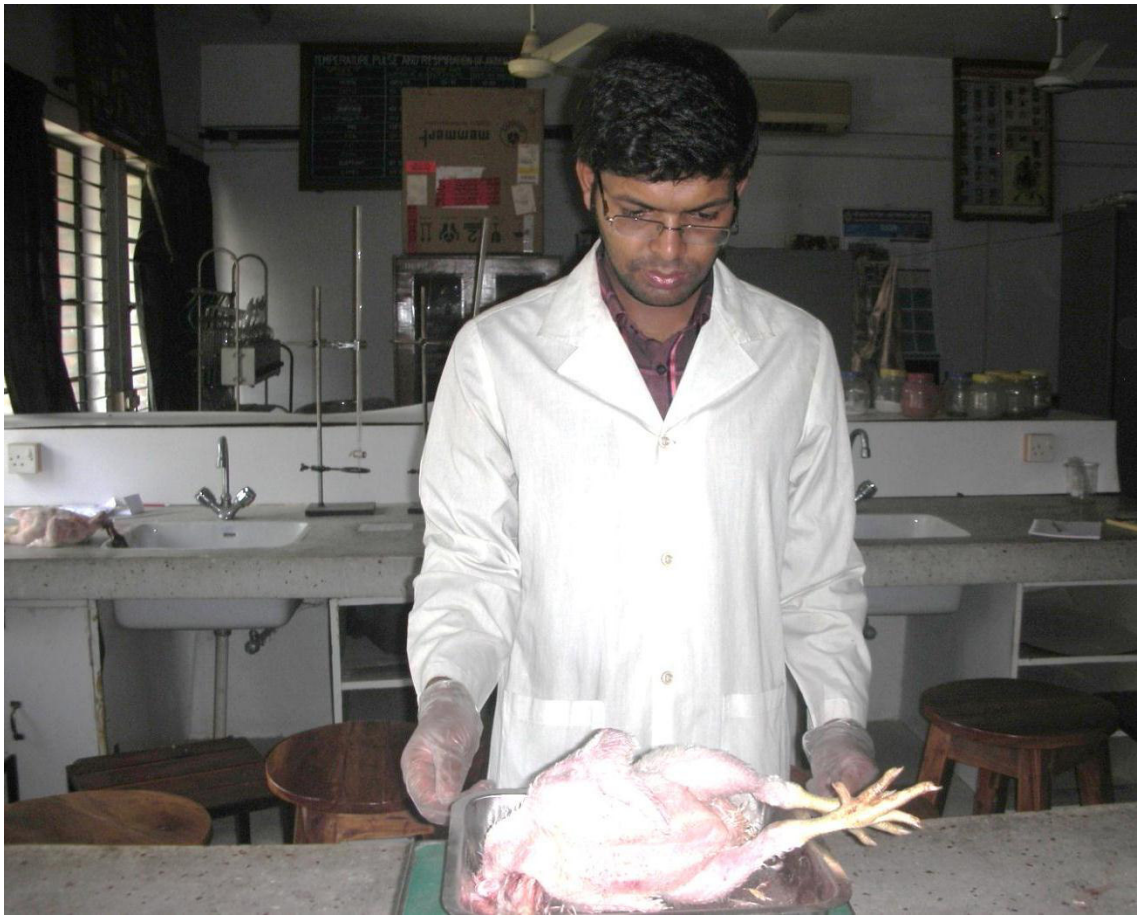

Figure 3.4: Record of weight after defeathering of broiler

3.15 Microbial assessment of cecal digesta

For the determination of total viable counts (TVC), 1 gm. of sample from the cecum through the eppendorf tube was transferred in to a test tube containing 10 ml of PBS (phosphate buffer solution). 1 ml of diluted solution from first tube was taken into another test tube contains 9 ml of PBS and mixed well. Then 50 µl of sample from

2nd test tube spread on two NA agar plates using a micropipette. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile glass spreader. One sterile spreader was used for each plate. The plates were then kept in an incubator at 37^0C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies was multiplied by the dilution factor to obtain the total viable count and average TVC of two plates was calculated. The total viable count was calculated according to ISO (1995). The results of the total bacterial counts were expressed as the number of organism of colony forming units per gram (CFU/gm.) of samples.

3.16Production cost

Cost of production per broiler was calculated by considering chick cost, feed cost, adding all vaccination cost, labor cost, litter cost and transportation cost.

3.17Biosecurity

A strict biosecurity program was maintained inside and outside of the research shed as a most effective part of the disease prevention program. Entry to the experimental shed was highly restricted. At the entrance, foot Spray was maintained, where virucid® solution (highly effective against viral, bacterial and fungal) was used as a disinfectant. A separate footwear and apron were used in the experimental shed to prevent contamination. To prevent the rodents and wild animals fencing was done and additional care was taken. In addition to, the following measures were taken to maintain bio security-

- Visitors were not allowed to enter into the house.
- All equipment used in the experimental house was kept clean.
- Dead birds were disposed of properly.

3.18 Statistical analysis

The raw data were entered and sorted into MS Excel spread Sheet software. Data on body weight, body weight gain, feed intake, Feed Efficiency (FE), livability and edible meat characteristics of broilers were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD) employing Statistical Package for the Social Sciences (SPSS, version16) for descriptive analysis.

CHAPTER 4

RESULTS

CHAPTER 4

RESULTS

4.1 Productive Performance of broiler

The productive performances of broiler after feeding probiotic with or without growth promoter are presented in the following sub-headings:

4.1.1 Live weight and live weight gain

The productive performance of broiler receiving feed supplemented with probiotic or growth promoter or their combination is shown in Figure 4.1. In respect to initial body weight, there was no significant difference among the dietary groups. At the end of 32 days of age, the highest live weight (1916.21 g/b) was found in broilers fed with both probiotic and growth promoter (GP+PB). This was followed by broiler belonging to probiotic (1890.43g/b), growth promoter (1828.13g/b) and control group (1788.27 g/b) respectively. However, broiler receiving either probiotic or growth promoter or both weighed were highly significant than that of control ($p<0.01$). The difference with regard to live weight and live weight gain was highly significant ($p<0.01$) in GP+PB.

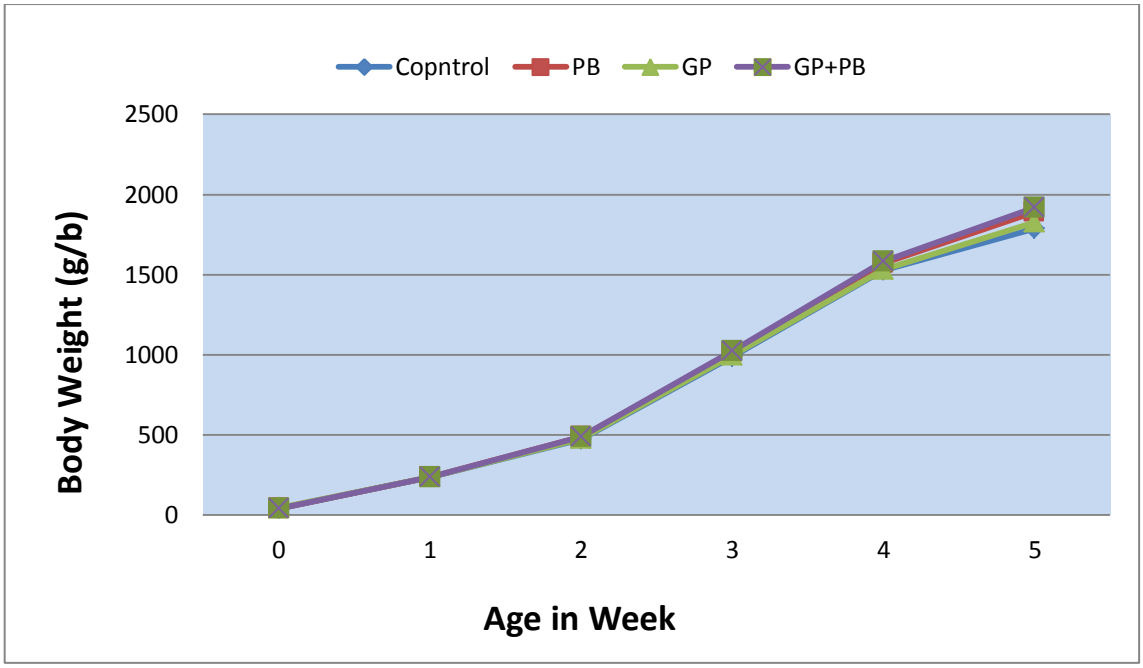

Figure 4.1: Effects of feeding probiotic on body weight of broiler chickens with or without growth promoter

Figure 4.1 shows the trend in growth pattern of birds receiving different dietary treatments. In the figure, it is very clear that test ingredients had no effect on weight gain up to four weeks of age. At fifth week, body weight was significantly differed among different dietary groups.

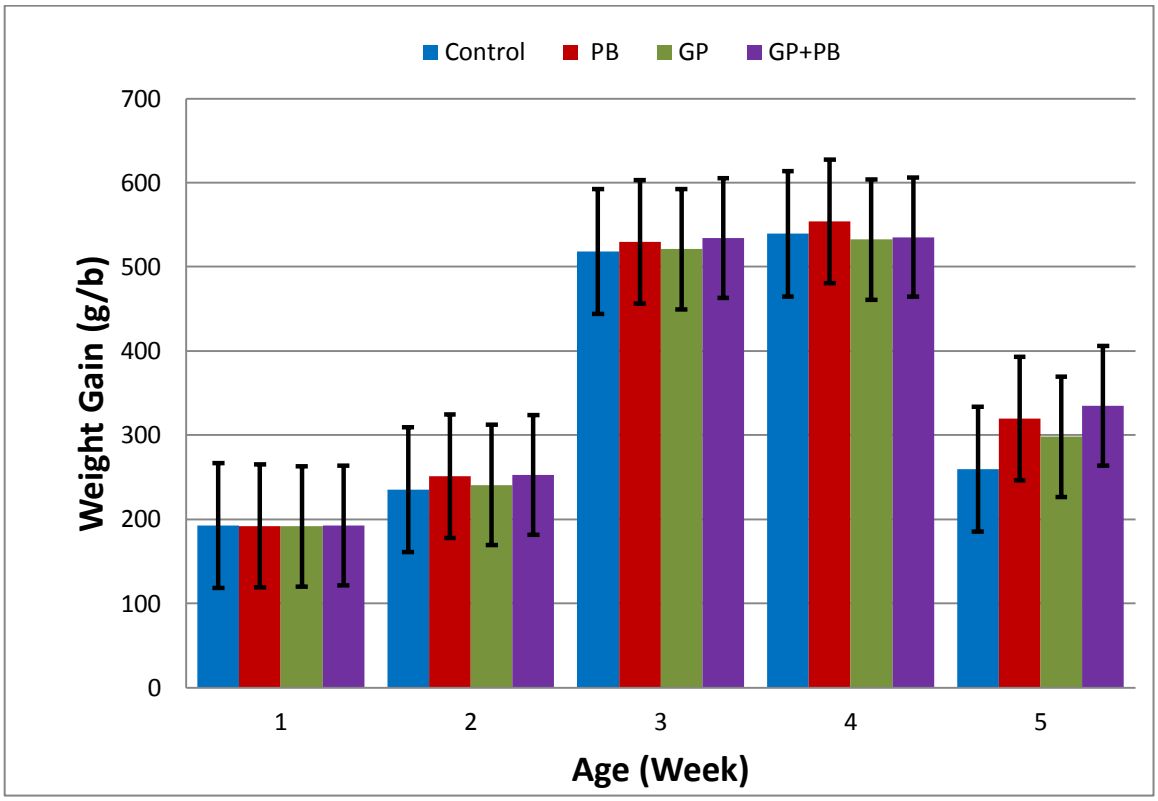

Figure 4.2: Effect of feeding probiotic on live weight gain broiler with or without growth promoter

Figure 4.2 shows the trend in growth pattern of birds receiving different dietary treatments. In the figure, it is very clear that test ingredients had effect on weight gain from second week to fifth weeks of age. Both probiotic (PB) and combination of probiotic with growth promoter(PB+GP) enriched groups showed increased growth gain at 2nd, 3rd and 5th week of age. At 4th week of age, probiotic group was higher to weight gain than the other groups, but the differences were not significant.

4.1.2 Feed Intake

Figure 4.3 show the average feed consumption pattern of the broilers of different treatment groups. Both PB and GP containing groups consumed similar amounts of feed which were significantly lower than that of control and PB + GP ($p<0.01$). Weekly feed consumption data revealed that birds of all groups consumed more or less similar

amounts of feed up to 28 days of age but GP and PB + GP groups had an increased feed intake during the last week of the trial. For this reason, variation was observed in cumulative feed consumption and therefore it differed significantly from remaining dietary groups ($p<0.01$).

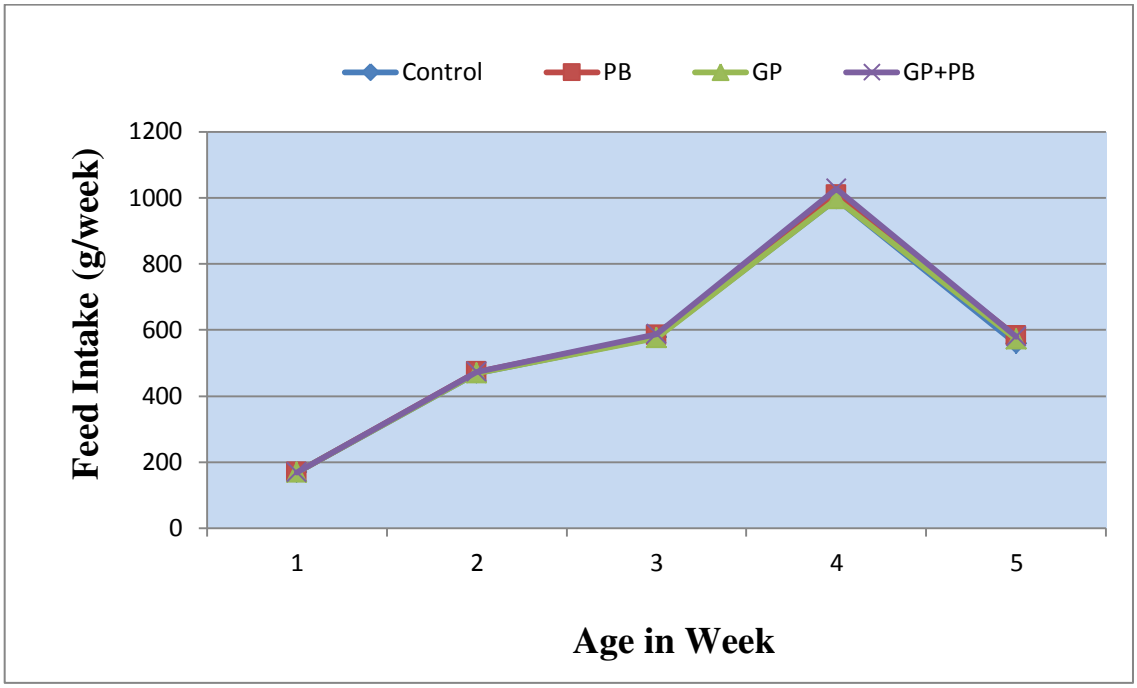

Figure 4.3: Weekly feed consumption patterns of different treatments

4.1.3 Feed Efficiency (FE)

Difference in cumulative Feed Efficiency (FE) of the broiler of different dietary groups differed significantly ($p<0.05$). The lowest value was obtained for birds that received GP+PB. PB and GP supplemented groups showed almost similar but higher efficiency was found from control group ($p<0.05$). The results presented in Figure 4.4 clearly exhibits an impression that the broiler receiving GP+PB was the best converters of feed into live weight and the effect of probiotic was more prominent after 28 days and onwards.

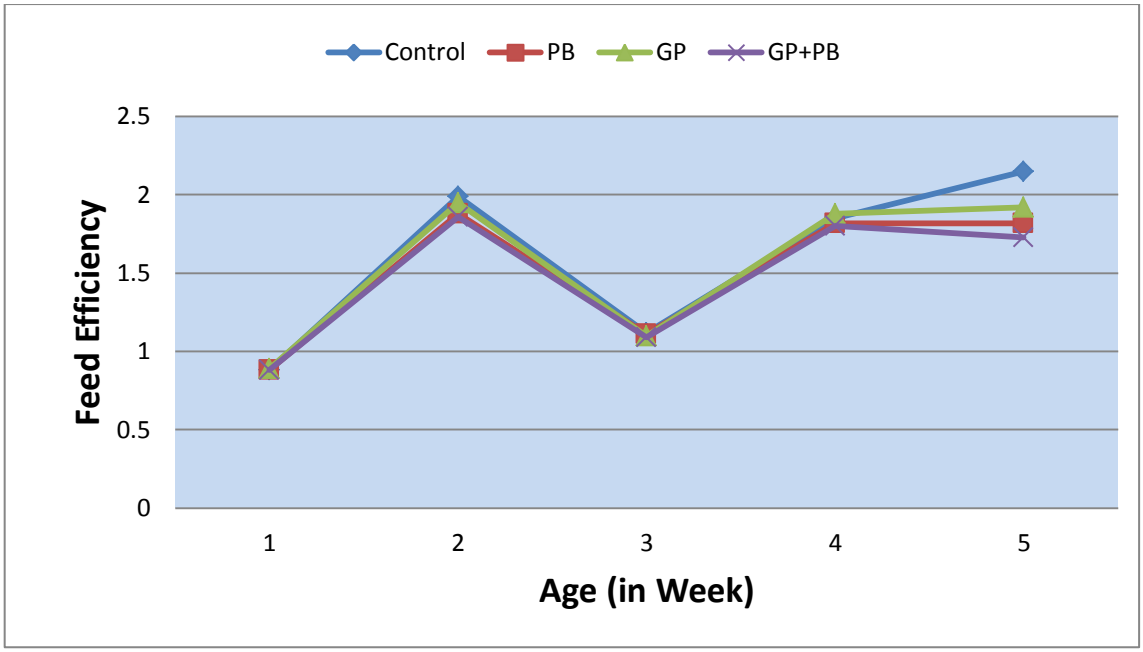

Figure 4.4: Weekly Feed Efficiency (FE) of broiler chicks receiving diets containing PB with or without GP.

4.1.4 Survivability

PB and GP+PB receiving groups had no mortality while the survivability of the control group and GP were 97.33% and 96.67%. However, it is clear that the control group suffered much as compared to remaining groups.

4.2 Edible meat yield characteristics

Edible meat yield characteristics of broiler receiving probiotic supplemented diet with or without growth promoter are shown in Table 7.

Table 7: Some edible meat yield characteristics of broilers fed on Probiotic (A-MAX) with or without growth promoter (0-32 days)

Variable	Dietary treatments				Level of significance
	Control	PB	GP	GP+PB	
Live Weight (g/b)	1491.67±21.54	1533.67±23.14	1526.67±14.53	1580.67±20.08	NS
Dressing weight (g)	1117.67^{b}±15.72	1136.33^{ab}±18.12	1126.67^{ab}±12.98	1220.00^{a}±31.21	*
Dressing percentage (%)	74.91^{ab}±0.04	74.09^{b}±0.10	73.8^{b}±0.17	77.15^{a}±0.99	**
Thigh (g)	111.33±5.81	112.67±1.76	113±1.53	107±4.36	NS
Drumstick (g)	56.67±4.98	56.33±1.20	65.67±0.88	54±2.89	NS
Breast meat (g)	303.33^{b}±4.41	386.67^{a}±6.07	365^{a}±2.89	376.67^{a}±7.26	**
Wing meat (g)	72.67±1.76	68.67±1.33	70.67±2.96	75.67±1.20	NS
Head (g)	34.67±1.45	35.67±0.88	37±0.58	35.33±0.67	NS
Neck (g)	36±3.05	43.33±4.81	42.33±4.26	46.33±0.33	NS
Liver (g)	37.67^{b}±0.33	37.33^{b}±1.45	38.33^{b}±0.67	45.67^{a}±1.10	**
Gizzard (g)	21.33^{b}±0.67	22.33^{b}±0.33	23.33^{b}±0.88	27.00^{a}±1.00	**
Abdominal fat (g)	16.33^{a}±0.88	7^{b}±0.58	10.33^{b}±1.45	7.33^{b}±0.67	**

a, b, c Means bearing dissimilar superscript in a row differ significantly, **=($p<0.01$), *=($p<0.05$), GP= Growth promoter, PB=Probiotic.

Meat yield data are presented in Table 7. The analyzed data in the table indicates that the treatments had no significant effect ($p>0.05$) on live weight, thigh, drumstick, wing, head and neck weight of the experimental birds. On the other hand, highly significant ($p<0.01$) differences were obtained in dressing percentage, breast meat, abdominal fat content, liver and gizzard weight among different treatments. Highest and lowest breast meat weight was recorded in PB and control group respectively. There was a tendency of increased breast meat content among PB, PB+GP and GP groups which had highly significant ($p<0.01$) effect compared to control group. Dressing percent, liver and gizzard weight was higher in GP+PB group than the control group. Abdominal fat was

higher in control group than others. Also significant differences ($p<0.05$) were found on heart and dressed weight among the dietary groups.

4.3 Cost-effectiveness of production

The total cost of production in terms of per bird and per kg broiler was TK. 203.33 and TK. 113.72 for control diet, TK. 207.09 and TK. 110.80 for probiotic (PB) group, TK. 206.81 and TK. 111.79 for growth promoter (GP) group, TK. 211.17 and TK. 110.21 for (GP+PB) group respectively. The profit in terms of per bird and per kg of broiler were highest in PB+GP group followed by probiotic (PB), growth promoter (GP) and control group respectively. It is therefore clear that additional supplementation of PB+GP and PB is profitable over GP and control group.

Table 8: Cost of production and profit in different dietary treatment groups of broilers

Variables	Dietary treatments			
	T_0	T_1	T_2	T_3
Feed intake (gm/broiler)	2770.83	2813.93	2780.64	2835.83
Final weight (kg/broiler)	1.788	1.869	1.850	1.916
Feed price TK. 43 per kg	43	43	43	43
GP@ Tk.110/-per Liter, 1 ml/1 L	---	---	1.10	1.10
A-MAX@340/-per kg, 200g/100kg	----	0.68	----	0.68
Feed cost (with or without test ingredients)/kg	43	43.68	44.1	44.78
Feed cost/bird	119.15	122.91	122.63	126.99
Others (Chicks, vaccines, disinfectants, transport, bedding materials, labor etc.)	84.18	84.18	84.18	84.18
Total cost of production /bird	203.33	207.09	206.81	11.17
Total cost of production Tk. /kg	113.72	110.80	111.79	110.21
Sale price Tk.155/ per kg	277.14	289.69	286.75	296.98
Profit Tk./broiler	73.81	82.6	79.94	85.81
Profit Tk./kg	41.28	44.2	43.21	44.79
Profit Tk. /kg (over control)	--	2.92	1.93	3.51

T_0= Control, T_1= Control +Probiotic, T_2= Control + Growth promoter,

T_3= Control + growth promoter + Probiotic.

The additional costs incurred for growth promoter (GP) over control was Tk. 1.10/liter water, Tk. 0.68/kg for probiotic (PB) and Tk. 1.78/kg for PB+GP group. In contrast to the additional cost incurred for PB and GP supplementation, the profit over control was Tk. 2.92/kg, Tk. 1.93/kg and 3.51/kg broiler in PB, GP and GP+PB group respectively. Consequently, supplementation of GP+PB in broiler diet was more cost effective followed by PB and GP respectively.

CHAPTER 5

DISCUSSION

CHAPTER 5

DISCUSSION

The research status was carried out to determine the actual status of live weight, live weight gain, feed intake, feed efficiency, livability, edible meat characteristics and cost of production.

5.1 Live weight and live weight gain

The research results obtained in this study are consistence with the findings of Ahmad and Taghi (2006) also found that body weight gain of broiler, fed supplemented with probiotic (*Bacillus subtilis and B. licheniformis*) were significantly higher during the grower phase (21-42 days) than broiler fed the control diets.

Other author Sabatkova *et al.* (2008) compared the efficacy of Avilamycin (GP) and probiotic Bio Plus 2B (*Bacillus subtilis and B. licheniformis*) to investigate the performance and slaughter yields. They finally reported that the supplementation of Probiotic improved 4–5% weight gain ($P<0.01$).

Bai *et al.* (2013). They compared the probiotic treated group with a control, an antibiotic and (probiotic + antibiotic) treated group and found that both antibiotic, probiotic and their combination improved average body weight in broilers during grower period (21-42days) compared with control, but there was no difference ($p> 0.05$) in the weight gain of broilers in starter phase.

Not only that, the finding of this trial is also agreed with Salim *et al.* (2013); Shim *et al.* (2012); HaoShen *et al.* (2004); Ashayerizadeh *et al.* (2009); O'Dea *et al.* (2006); Pelicano *et al.* (2003); Rahimi (2009). They also reported that supplementation of probiotic in broiler feed improved body weight and body weight gain significantly.

In this study, both the live weight and live weight gain of the broiler of both PB and PB+GP groups are very close to the Cobb-500 commercial broiler's productive performance (Cobb 500 Management Guide, 2010).

5.2 Feed Intake

Comparatively lower feed intake in probiotic supplemented group was an agreement with the results of Shim *et al.* (2012). They found that birds fed 10 mg/kg avilamycin consumed more (P<0.05) feed during the finisher and overall periods than birds fed diets containing probiotic without avilamycin while others have found non-significant variation in feed intake between control and probiotic group (Panda *et al.* 2008; Faria *et al.* 2009; Rada *et al.,* 2013). But the result was consistence with Eseceli and Demir, 2010 and Erdogan, 2007. They also reported that supplementation of probiotic decreased feed intake significantly (P<0.05) compared to control group. In the present study feed intake of probiotic treated group was significantly lower (P<0.01) than control and PB+GP treated groups.

5.3 Feed Efficiency (FE)

The significant effect of probiotic on Feed Efficiency (FE) of broiler was in close agreement with Shim *et al.* (2012); HaoShen (2004); Ashayerizadeh *et al.* (2009); O'Dea *et al.* (2006); Pelicano *et al.* (2003); Rahimi (2009); Sabatkova *et al.* (2008); Zhou *et al.* (2010); Hassanein and Soliman (2010). They found that supplementing with *Bacillus subtilis* and *B. licheniformis* improved Feed conversion efficiency in broiler.

Panda *et al.* (2008) reported that dietary preparation of *Bacillus subtilis* and *B. licheniformis* (at the rate of 6 x 108 spore per kg of diet) significantly enhanced feed efficiency in White Leghorn Breeders.

In another study Salim *et al.* (2013) also reported the lowest Feed Efficiency (FE) with probiotic (1.49) compared to antibiotic (1.50) and control (1.52) group respectively. This result was almost similar to the present study where the FE for probiotic, growth promoter and control group was 1.53, 1.56 and 1.59 respectively.

5.4 Livability

The research results obtained in this study are positively consistent with the observation of Knap *et al.* (2011); Zhang Ren Yi (2010) and Lee *et al.* (2007). They also found that feeding probiotics (*Bacillus spp.*) supplemented diet effectively enhance the resistance of broiler and protect them against the negative growth effects and mortality.

However, HaoShen (2004) and Rahimi (2009) demostrated that *B. licheniformis* significantly ($p<0.05$) decreased diarrhea and mortality rates of experimental group in broiler.

But Faria *et al.* (2009) and Eseceli and Demir (2010) revealed that there was no statistically significant difference ($p>0.01$) in the livability of birds reared with or without adding probiotic in diet.

5.5 Edible meat characteristics

In the present study, it was clearly indicate the effect of dietary probiotic towards some important meat yield characteristics of broiler. This result was particularly similar to the result of Molnar *et al.* (2013) who reported that *Bacillus spp.* supplemented group had significantly higher ($p<0.05$) breast yield (549g) and lower thigh meat yield than the control group (474g) where the breast weight of the broiler of this experiment for control and probiotic supplementation was 303.33 g and 386.67 g respectively. Novak *et al.* (2011) conducted a study and found that supplementation of *Bacillus subtilis* and *Bacillus licheniformis* had higher yield of wings and lower abdominal fat weight compared to control and *Bacillus cereus* supplemented group ($p<0.05$).

The result of this study was also particularly consistent with the findings of Xiaolu *et al.* (2012), who reported that the supplementation of *Bacillus licheniformis* resulted in increased protein and free amino acid contents, and decreased fat content in chicken breast fillet ($p<0.05$).

Luiz *et al.* (2012) compared the efficacy of antibiotic with probiotic in meat yield characteristics of broiler and finally reported that probiotic group have lower abdominal fat content (5.2g) compared to antibiotic (9.6g) and control (22.9 g) group respectively where the abdominal fat content of the experimental broiler of the present study was 7 g for probiotic group, 10.33 g for growth promoter and 29.6 g for control group respectively.

Other authors (Moreira *et al.* 2001, Loddi *et al.* 2002, Vargar *et al.* 2000) found no significant difference in carcass yield between birds that were fed probiotic and control diet.

However, the result of this study agreed well with the findings of Pelicano *et al.* (2003), Mutus *et al.* (2006), Sabatkova *et al.* (2008), Zhou *et al.* (2010), Lei *et al.* (2013).

5.6 Cost effectiveness of production

The result of present study, it was clearly indicated that the feeding of PB, GP and their combination had beneficial effect on the profitability of broiler. The combination of GP+PB provided highest profit which is almost similar to PB group but higher than the control and GP group. This result was particularly similar to the results of Roy *et al.* (2013) who reported that the feeding of probiotic to broiler was either similar or more profitable than combination of PB+GP while better than GP alone. So it can be concluded that combined feeding may give more profit than others in case of commercial broiler farming.

CHAPTER 6

SUMMARY AND CONCLUSIONS

CHAPTER 6

SUMMARY AND CONCLUSIONS

The present research was undertaken to study the growth performance of broilers fed with probiotics (A-MAX®) and growth promoter (Ami vet®) through feed and drinking water respectively. An experiment was conducted with 120 one-day-old Cobb 500 broiler chicks for a period of 32 days of age at Kader Poultry Farm, Cornai, Dinajpur to study the effect of probiotic with or without growth promoter on broiler performance and their cost-effectiveness in broiler production. The broiler chicks were divided into four groups each of 30, replicated to three sub-groups each of 10 birds. First, second, third and remaining group of chicks was considered as control, probiotic (A-MAX®), growth Promoter (Ami vet®) and combination of growth promoter and probiotic respectively. Live weight, feed intake, feed efficiency, livability, edible meat yield, temperature and humidity, and production cost of broiler on different treatments were recorded.

The live weight of broiler among different dietary groups was significantly different ($P<0.01$). The final body weight of broiler was highest in broilers supplemented with (GP+PB) followed by probiotic, growth promoter and control group respectively. At end of the experiment (32 days of age), the live weight of broilers among the dietary groups were 1788.27, 1890.43, 1828.13 and 1916.21 g for control, probiotic (PB), growth promoter (GP) and (GP+PB) group respectively. Significant differences were observed in GP+PB from the control groups ($p<0.01$).

The cumulative feed intake during the period of 32 days was found highest in the GP+PB group (2.84 kg/bird). It was more or less similar to the PB and GP groups but higher than the control group. Chicks belonging to probiotic group, consumed 2.83 kg feed per bird while those of growth promoter group, consumed 2.78 kg feed per bird.

This cumulative feed consumption of birds receiving GP and PB up to 32 days of age differed significantly as compared to control group ($p<0.01$).

The differences in feed consumption in relation to body weight of the broilers among different groups, resulted a significant differences in feed efficiency ($p<0.05$). Significant difference was observed between GP+PB and control group. Cumulative FE

was 1.59, 1.53, 1.56, 1.51 for control, probiotic (PB), growth promoter (GP) and GP+PB groups respectively.

Over all livability was good for probiotic and probiotic with growth promoter. Slightly lower in growth promoter group but the lowest livability found in control group. Livability percentage was 100% for PB and GP+PB groups while 97% and 93% for GP and control groups respectively.

Meat yield parameters of broiler showed highly significant differences ($p<0.01$) for the dressing percentage, breast meat, liver, gizzard and abdominal fat due to addition of either probiotic or growth promoter or their combination in diets. On the other hand, there were no appreciable differences in live weight, thigh, wing meat, head and neck of the broilers among the treatments. Dressing weight of GP and PB groups and heart meat yield of GP and GP+PB groups were more or less similar which differed significantly ($P<0.05$) from the control group. Again the control group had highest abdominal fat percentage which differed significantly ($P<0.01$) from the other treatment groups. The probiotic containing diet had the special activity to improve breast meat weight. The probiotic reduced abdominal fat.

The highest profit per kg live weight was obtained in probiotic plus growth promoter group followed by probiotic, growth promoter and lowest in control group respectively. Additional cost for growth promoter supplementation was Tk. 1.10/bird, for probiotic supplementation was Tk. 0.68/bird and for combination of these two products was Tk. 1.780/bird. The net profit over control was TK. 2.92/kg, TK. 1.93/kg and Tk. 3.51/kg of broiler for probiotic, growth promoter and their combination respectively.

The experiment revealed that performance and effectiveness of broiler was better with the supplementation of probiotic and growth promoter than the group of others. With regards to profit, combination effect of GP and PB had more profit. At present, world health leaders have described antibiotic-resistant microorganisms as “nightmare bacteria” that “pose a catastrophic threat” to people in every country in the world. In the context of this study, it may be concluded that combination of probiotic (A-MAX®) and growth promoter may be considered as an effective methods of increasing broiler productive performance.

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APPENDICES

APPENDICES

Appendix 1: Body weight (gm/bird) of broilers fed on different dietary treatments at different ages

Treatment	Replication	Age (week)					
		Day-old	1st	2nd	3rd	4th	5th
Control	R-1	43.39	236	476	995.5	1514.4	1762.28
	R-2	44.31	236	474.1	989.4	1549.5	1803.64
	R-3	43.58	236	464.5	984.2	1522.9	1798.9
Mean		**43.76**	**236**	**471.53**	**989.7**	**1528.9**	**1788.27**
PB	R-1	44.11	236	486.6	1020.9	1567.8	1866.9
	R-2	43.85	235.9	491.9	1015.2	1592.6	1929
	R-3	43.73	236	483.6	1014.4	1552.2	1875.4
Mean		**43.9**	**235.97**	**487.37**	**1016.833**	**1570.9**	**1890.43**
GP	R-1	44.85	236	479.4	996.1	1530.3	1842.1
	R-2	44.76	236.1	479.1	1000.3	1557.2	1856.5
	R-3	43.8	236	471.6	996.8	1502.8	1785.8
Mean		**44.47**	**236.03**	**476.7**	**997.73**	**1530.1**	**1828.13**
GP+PB	R-1	43.5	236	487.7	1026.3	1572.3	1909.18
	R-2	44.43	236	492.2	1022.1	1594.3	1928.95
	R-3	43.08	236	485.8	1022	1577.8	1910.49
Mean		**43.67**	**236**	**488.57**	**1023.47**	**1581.5**	**1916.49**

GP=Growth promoter, PB=Probiotic

Appendix 2: Weekly body weight gain (gm/bird) of broilers fed on different dietary treatments at different ages

Treatment	Replication	Age (week)					
		Day-old	1st	2nd	3rd	4th	5th
Control	R-1	192.61	240	519.5	518.9	247.88	1762.28
	R-2	191.69	238.1	515.3	560.1	254.14	1803.64
	R-3	192.42	228.5	519.7	538.7	276	1798.9
Mean		**192.24**	**235.4**	**518.27**	**539.23**	**259.34**	**1788.27**
PB	R-1	191.89	250.6	534.3	546.9	299.1	1866.9
	R-2	192.05	256	523.3	577.4	336.4	1929
	R-3	192.27	247.6	530.8	537.8	323.2	1875.4
Mean		**192.07**	**251.4**	**529.47**	**554.03**	**319.53**	**1890.43**
GP	R-1	191.15	243.4	516.7	534.2	311.8	1842.1
	R-2	191.34	243	521.2	556.9	299.3	1856.5
	R-3	192.2	235.6	525.2	506.0	283	1785.8
Mean		**191.56**	**240.67**	**521.03**	**532.37**	**298.03**	**1828.13**
GP+PB	R-1	192.5	251.7	538.6	546	336.88	1909.18
	R-2	191.57	256.2	529.9	572	334.65	1928.95
	R-3	192.92	249.8	536.2	555.8	332.69	1910.49
Mean		**192.33**	**252.57**	**534.25**	**535.2**	**334.74**	**1916.49**

GP=Growth promoter, PB=Probiotic

Appendix 3: Feed Intake (gm/bird) of broilers fed on different dietary treatments at different ages

Treatment	Replication	Age (days)				
		0-7	**0-14**	**0-21**	**0-28**	**0-32**
Control	R-1	169.75	640.88	1230.88	2227.08	2787.28
	R-2	169.75	639.75	1209.75	2213.75	2760.15
	R-3	169.75	634.75	1209.75	2197.75	2763.55
Mean		**169.75**	**638.46**	**1216.79**	**2212.86**	**2770.33**
PB	R-1	169.75	640.88	1220.88	2236.48	2806.98
	R-2	169.75	641.25	1241.25	2247.25	2839.85
	R-3	169.75	644.75	1216.75	2214.75	2794.95
Mean		**169.75**	**642.29**	**1226.29**	**2232.83**	**2813.93**
GP	R-1	169.75	640.13	1235.13	2219.63	2781.93
	R-2	169.75	640.13	1200.13	2210.13	2788.73
	R-3	169.75	634.75	1204.751213	2199.25	2771.25
Mean		**169.75**	**638.34**	**1213.34**	**2209.67**	**2780.64**
GP+PB	R-1	169.75	640.88	1235.88	2261.38	2844.18
	R-2	169.75	645.25	1225.25	2237.25	2812.85
	R-3	169.75	640.25	1226.25	2268.25	2850.45
Mean		**169.75**	**642.13**	**1229.13**	**2255.63**	**2835.83**

GP=Growth promoter, PB=Probiotic

Appendix 4: Weekly feed intake (gm/bird) of broilers fed on different dietary treatments at different ages

Treatment	Replication	Age (week)				
		1st	**2nd**	**3rd**	**4th**	**5th**
Control	R-1	169.75	471.13	590	996.2	560.2
	R-2	169.75	470	570	1004	546.4
	R-3	169.75	465	575	988	565.8
Mean		**169.75**	**468.71**	**578.33**	**996.07**	**557.47**
PB	R-1	169.75	471.13	580	1015.6	570.5
	R-2	169.75	471.5	600	1006	592.6
	R-3	169.75	475	572	998	580.2
Mean		**169.75**	**472.54**	**584**	**1006.53**	**581.1**
GP	R-1	169.75	471.13	595	984.5	562.3
	R-2	169.75	471.13	560	1010	578.6
	R-3	169.75	465	570	994.5	572
Mean		**169.75**	**469.09**	**575**	**996.33**	**570.97**
GP+PB	R-1	169.75	471.13	595	1025.5	582.8
	R-2	169.75	475.5	580	1012	575.6
	R-3	169.75	470.5	586	1042	582.2
Mean		**169.75**	**472.38**	**587**	**1026.5**	**580.2**

GP=Growth promoter, PB=Probiotic

Appendix 5: Weekly feed efficiency (FE) of broilers fed on different dietary treatments at different ages

Treatment	Replication	Age (week)				
		1st	**2nd**	**3rd**	**4th**	**5th**
Control	R-1	0.88	1.96	1.14	1.92	2.26
	R-2	0.89	1.97	1.11	1.79	2.15
	R-3	0.88	2.04	1.11	1.83	2.05
Mean		**0.88**	**1.99**	**1.12**	**1.85**	**2.15**
PB	R-1	0.88	1.88	1.09	1.86	1.91
	R-2	0.88	1.84	1.15	1.74	1.76
	R-3	0.88	1.92	1.08	1.86	1.80
Mean		**0.88**	**1.88**	**1.11**	**1.82**	**1.82**
GP	R-1	0.89	1.94	1.15	1.84	1.80
	R-2	0.89	1.94	1.07	1.82	1.93
	R-3	0.88	1.98	1.09	1.97	2.02
Mean		**0.89**	**1.95**	**1.10**	**1.88**	**1.92**
GP+PB	R-1	0.88	1.87	1.10	1.87	1.73
	R-2	0.89	1.86	1.09	1.76	1.72
	R-3	0.88	1.86	1.09	1.87	1.75
Mean		**0.88**	**1.86**	**1.09**	**1.80**	**1.73**

GP=Growth promoter, PB=Probiotic

Appendix6: Mortality and livability percentages during the entire experimental periods (0-32 days)

Treatment	Replication	No. of birds	No. of dead birds	Day of mortality	Mortality (%)	Livability (%)
Control	R-1	10	0	--	0	100
	R-2	10	0	--	0	100
	R-3	10	2	28^{th} 28t	20	80.00
Mean		**30**	**2**	**--**	**6.67**	**93.33**
PB	R-1	10	0	--	0	100
	R-2	10	0	--	0	100
	R-3	10	0	--	0	100
Mean		**30**	**0**	**--**	**0**	**100**
GP	R-1	10	0	--	0	100
	R-2	10	0	--	0	100
	R-3	10	1	30^{th}	10	90
Mean		**30**	**1**	**--**	**3.33**	**96.67**
GP+PB	R-1	10	0	--	0	100
	R-2	10	0	--	0	100
	R-3	10	0	--	0	100
Mean		**30**	**0**	**--**	**0**	**100**

GP=Growth promoter, PB=Probiotic

Appendix 7: Edible meat yield characteristics of broiler of supplemented with Control

Variables	Replication			
	R-1	**R-2**	**R-3**	**Mean**
Live Weight (g/b)	1450	1522	1503	11491.67
Blood Weight (g)	170	182	176	**176**
Feather Weight (g)	55	56	56	**55.67**
Head (g)	32	37	35	**34.67**
Skin Weight (g)	84	88	89	**87**
Thigh (g)	102	110	122	**111.33**
Shank (g)	28	29	30	**29**
Drumstick (g)	49	66	55	**56.67**
Neck (g)	34	48	45	**42.33**
Heart (g)	9	9	8	**35.33**
Liver (g/b)	47	45	45	**45.67**
Spleen (g)	3	3	3	**3**
Gizzard (g)	25	23	22	**23.33**
Abdominal fat (g)	18	16	15	**16.33**
Dressing weight (g)	1087	1139	1127	**1117.67**
Dressing percent (%)	74.97	74.84	74.98	**74.93**

GP=Growth promoter, PB=Probiotic

Appendix 8: Edible meat yield characteristics of broiler of supplemented with Probiotic (A-Max®)

Variables	Replication			
	R-1	**R-2**	**R-3**	**Mean**
Live Weight (g/b)	1550	1488	1563	1533.67
Blood Weight (g)	202	178	190	**190**
Feather Weight (g)	55	62	65	**60.67**
Head (g)	34	36	37	**35.67**
Skin Weight (g)	86	85	88	**86.33**
Thigh (g)	112	110	116	**122.67**
Shank (g)	30	29	31	**30**
Drumstick (g)	59	56	60	**50.33**
Neck (g)	30	50	46	**42**
Heart (g)	10	9	10	**9.67**
Liver (g/b)	37	40	35	**37.33**
Spleen (g)	4	3	3	**3.37**
Gizzard (g)	23	22	22	**22.33**
Abdominal fat (g)	7	8	6	**7**
Dressing weight (g)	1147	1101	1161	**1336.33**
Dressing percent (%)	74	73.99	74.28	**74.09**

GP=Growth promoter, PB=Probiotic

Appendix 9: Edible meat yield characteristics of broiler of supplemented with growth promoter (Ami vet®)

Variables	Replication			Mean
	R-1	R-2	R-3	
Live Weight (g/b)	1550	1500	1530	1526.67
Blood Weight (g)	192	184	190	**188.67**
Feather Weight (g)	66	70	66	**67.33**
Head (g)	38	37	36	**37**
Skin Weight (g)	90	88	90	**89.33**
Thigh (g)	114	110	115	**113**
Shank (g)	30	30	29	**29.67**
Drumstick (g)	66	64	67	**65.67**
Neck (g)	47	46	46	**46.33**
Heart (g)	7	7	7	**7**
Liver (g/b)	38	38	37	**37.67**
Spleen (g)	3	3	3	**3**
Gizzard (g)	22	20	22	**21.33**
Abdominal fat (g)	13	8	10	**10.33**
Dressing weight (g)	1146	1102	1132	**1126.67**
Dressing percent (%)	73.94	73.47	73.99	**73.8**

GP=Growth promoter, PB=Probiotic

Appendix 10: Edible meat yield characteristics of broiler of supplemented with growth promoter and Probiotic

	Replication			
Variables	**R-1**	**R-2**	**R-3**	**Mean**
Live Weight (g/b)	1620	1568	1554	1580.67
Blood Weight (g)	140	168	170	**1599.33**
Feather Weight (g)	46	52	60	**62.67**
Head (g)	34	36	36	**35.33**
Skin Weight (g)	98	88	86	**90.67**
Thigh (g)	99	108	114	**107**
Shank (g)	27	29	30	**28.67**
Drumstick (g)	49	54	59	**54**
Neck (g)	32	34	42	**36**
Heart (g)	7	8	10	**41.67**
Liver (g/b)	39	38	38	**38.33**
Spleen (g)	3	4	3	**3.33**
Gizzard (g)	25	24	22	**23.67**
Abdominal fat (g)	6	8	8	**7.33**
Dressing weight (g)	1281	1201	1178	**1220**
Dressing percent (%)	79.074	76.59	75.8	**77**

GP=Growth promoter, PB=Probiotic