

PERFORMANCE, CARCASS CHARACTERISTICS AND BLOOD PARAMETERS OF BROILER USING PROBIOTICS



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the degree of Master of Science in Animal and Poultry Nutrition**

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February-2017

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***DEDICATED TO MY BELOVED
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Table of Contents

Contents	Page No
Authorization	ii
Acknowledgments	v
List of Tables	ix
List of Figure	x
List of Abbreviations	xi
Abstract	xii
Chapter 1 : INTRODUCTION	1-2
Chapter 2 : REVIEW OF LITERATURE	3-19
2.1 Probiotics	3
2.2 Historical development of probiotic	3
2.3 Criteria for an ideal probiotic	4
2.4 Commonly used microbes as probiotics	5
2.5 Applications of probiotics in poultry	5
2.6 Probiotic colonisation in the gastrointes-tinal tract	6
2.7 Improving digestion, nutrient metabolism and utilisation of nutrients	6
2.8 Combating harmful pathogens and disease conditions	7
2.9 Probiotics as growth promoter	8
2.10 Modes of action of probiotics	8
2.10.1 Competitive exclusion	8
2.10.2 Adhesion to the digestive tract wall to prevent colonization by pathogenic microorganisms	9
2.10.3 Competing with pathogenic bacteria for nutrients in the gut	9
2.10.4 Bacterial antagonism	10
2.10.5 Bactericidal activity	10
2.10.6 Neutralization of enterotoxins produced by pathogenic bacteria that cause fluid loss	10
2.10.7 Immune modulation	11
2.10.8 Probiotics in Poultry feeding	11
2.10.9 Microorganisms used as probiotics in Poultry nutrition	12
2.10.10 Examples of probiotic effects on the gut microbiota and immune system of birds	13
2.11 Manufacture of probiotics	15

2.12 Conclusion of the review of literature	19
Chapter 3: MATERIALS AND METHODOLOGY	20 – 31
3.1 Study area	20
3.2 Preparation of poultry shed	20
3.3 Experimental design	20
3.4 Collection of day-old chicks	21
3.5 Collection of Feed ingredients and Experimental feed ingredients	21
3.6 Feeding standard	22
3.7 Feed formulation and feeding diets	22
3.8 Managemental procedure	24
3.8.1 Brooding of the chicks	25
3.8.2 Maintaining room temperature	25
3.8.3 Brooder and cage spaces	25
3.8.4 Feeder and drinker spaces	25
3.8.5 Method of feeding, watering and lighting	26
3.8.6 Litter management	26
3.8.7 Vaccination and chemo prophylaxis/medication	26
3.8.8 Bio-security/Sanitation	27
3.9 Record keeping	27
3.9.1 Body weight	27
3.9.2 Feed intake	27
3.9.3 Mortality	27
3.10 Calculation of data	27
3.10.1 Body weight gain	27
3.10.2 Feed intake	28
3.10.3 Feed conversion (FC)	28
3.10.4 Mortality	28
3.11 Collection of blood and serum separation	28
3.12 Evaluation of carcass traits	28
3.13 Chemical analysis of RESM, RD, formulated feed and Meat	29
3.14 Cost-benefit analysis	29
3.15 Blood parameter estimation	30
3.16 Study design & statistical analysis	31

Chapter 4: RESULT	32-39
4.1 Effect of different organism used in probiotic on growth performance on weekly feed intake of broiler	32
4.1.1 Feed intake of broiler	32
4.1.1.1 Weekly feed intake of broiler among different dietary treatment groups (gm/broiler)	32
4.1.1.2 Cumulative feed intake of broiler among different dietary treatment groups (gm/broiler)	33
4.1.2 Body weight and weight gain	33
4.1.2.1 Weekly body weight of broiler among different dietary treatment groups (gm/broiler)	33
4.1.2.2 Cumulative body weight of broiler among different dietary treatment groups (gm/broiler)	34
4.1.2.3 Weekly body weight gain of broiler in different treatment groups (gm/broiler)	34
4.1.3 Feed Conversion	35
4.1.3.2 Cumulative FC of broiler in different treatment groups	36
4.2 Carcass characteristics of broiler	36
4.3 Effect of Probiotics on cost benefit analysis of broiler	38
4.4 Effect of Probiotics on blood parameters of broilers	39
Chapter 5: DISCUSSION	40-44
5.1 Effect of different organism used in probiotic on growth performance on weekly feed intake of broiler	40
5.2 Effect of different probiotic on weekly growth performance of broiler	40
5.3 Weekly feed conversion (FC) of different treatment groups	41
5.4 Effect of different probiotic on carcass characteristics of broiler	42
5.5 Cost benefit analysis	43
5.6 Effect of different probiotic on blood parameters of broiler	43
Chapter 6: CONCLUSION	45
Chapter 7: RECOMMENDATIONS AND FUTURE PERSPECTIVES	46
Chapter 8: REFERENCE	47-58
APPENDICES	59-63
Brief biography of the author	64

List of Tables

SL.NO.	Contents	Page No
1	2.10.10 Examples of different probiotic effects on the gut microbiota and immune system of birds	13
2	3.3.a Layout of the experiment (CRD) showing the distribution of DOC to the treatment group and replication	21
3	3.7.a Feed ingredients used in experimental broiler starter diets	22
4	3.7.b Feed ingredients used in experimental broiler grower diets	23
5	3.7.c Estimated chemical composition (DM basis) of the experimental broiler starter diets	24
6	3.7.d Estimated chemical composition (DM basis) of the experimental broiler grower diets	24
7	3.8.7.a Schedule of vaccination used during experiment period	26
8	3.8.7.b Schedule of chemo prophylaxis/medication	27
9	4.1 Effect of different organism used in probiotic on growth performance on weekly feed intake of broiler	32
10	4.2.1 Final body weight, eviscerated weight and Carcass yield of broiler among different treatment groups at 28 th day of age	36
11	4.2.2 Weight of primal parts and internal edible offal of broiler among different treatment groups at 28 th days of age (gm/broiler)	37
11	4.3.1 Cost of production and returns in different treatment	38
12	4.4 Effect of Probiotics on blood parameters of broilers	39

List of Figure

SL.NO.	Name of the figure	Page No
1	Figure 1: Preparation of feed	31
2	Figure 5: Box brooding of DOC	31
3	Figure 6: Broilers in cage	31
4	Figure 8 : Collected blood sample	31
5	Figure 9: Weighing of broiler	31

List of abbreviation

Abbreviation	Elaboration
°F	Degree Fahrenheit
et al.	And his associates
Etc.	Et cetera
FC	Feed Conversion
gm	Gram
kg	Kilogram
Wt.	Weight
gm	Gram
Kcal/kg	Kilocalorie per Kilogram
ME	Metabilizable Energy
CP	Crude Protein
CVASU	Chittagong Veterinary and Animal Sciences University
MS	Master of Science
Sq. Ft.	Square Feet
DOC	Day old chick
Tk.	Taka
BWG	Body weight gain
Bw	Body weight

Abstract

A three months long (October to December, 2016) study was conducted in experimental poultry farm and laboratories of Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong, Bangladesh to observe the effect of probiotics on performance, carcass quality and biochemical parameters of broiler Chicken. A total of 90 birds were allocated in completely randomized design (CRD) in three dietary treatment groups having three replications per treatment. The bird groups were treated either without probiotic (T_0 /control) or with probiotics (T_1 treated with PROBIO-5; T_2 treated with Polybiotic). Performance data (live weight, weight gain, feed intake, feed conversion) were taken for 4 weeks. After the end of 4th weeks 2 birds from each replication were slaughtered to know the carcass parameters and blood serum was also collected from birds for analyzing. Result showed that live weight, cumulative live weight, live weight gain was increased significantly ($P < 0.01$) at 3rd and 4th weeks of age of birds of T_1 and T_2 groups in comparison with control (T_0). However, T_2 (Polybiotic) group gained better weight than T_1 group. Feed intake was increased significantly ($P < 0.01$) in probiotic treatment groups (T_1 and T_2) compared to control. Feed conversion (FC) and cumulative feed conversion was significantly ($P < 0.01$) better in probiotic treatment groups at 3rd and 4th weeks compared to control. Best feed conversion was found in T_2 group. Difference in eviscerated weight was significant ($P < 0.05$) among the treatment groups. No significant differences ($P > 0.05$) were observed in weight of primal parts (i.e. drumstick, thigh, breast, back, neck, wing) and internal edible offal (i.e. gizzard and proventriculus, heart, liver, abdominal and neck fat) of birds of different treatment groups. Insignificant ($P > 0.05$) result was also found in different blood parameters (i.e. glucose, SGPT, SGOT, total protein, cholesterol, creatinine, triglyceride) of birds. Cost benefit analysis showed that Net profit (Tk./broiler), were increased in T_1 and T_2 groups than control (T_0). T_2 group showed highest profit among all the treatment groups. Mixing of probiotics at regular diet of broilers would be helpful in improving performance of broiler. Thus increase profitability of broiler farming.

Key words: Probiotics, performance, feed conversion (FC), carcass quality, blood parameters

Chapter 1

Introduction

Poultry provides animal protein in the form of meat and eggs. In Bangladesh commercial poultry production has been growing rapidly since the early 1990 by using improved genetics, manufactured feeds and management. This improvement is done mainly in the private sector as a device for additional source of income and employment opportunities particularly in rural area (Kabir et al., 2005). Indigenous chicken is widely reared throughout the country by rural people since time immemorial. Village poultry is still popular to millions, eight thousand years after domestication and play a vital role to poor rural households (Alders and Pym, 2009). The share of commercial strain of chicken and family poultry was 50:50 in egg production while for meat production it was 60:40 in Bangladesh (Bhuiyan, 2011).

Biotechnology plays a vital role in the poultry feed industry. Nutritionists are continually putting their efforts in producing better and more economical feed. Good feed alone will not serve the purpose but its better utilization is also essential. Dietary changes as well as lack of a healthy diet can influence the balance of the microflora in the gut thus predisposing to digestion upsets. A well-balanced ration sufficient in energy and nutrients is also of great importance in maintaining a healthy gut (Trafalska et al., 2004). Probiotics are microorganisms that are believed to provide health benefits when consumed (Rijkers et al., 2011). The term is currently used to name ingested microorganisms associated with benefits for humans and animals health care came into more common use after 1980 (Magdalena et al., 2006). The concept was introduced by the Nobel recipient Élie Metchnikoff, who postulated that yogurt-consuming Bulgarian peasants lived longer lives because of this custom (Brown et al., 2004). It was suggested that "the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes". A strikingly crucial event in the development of probiotics was the finding that newly hatched chickens could be protected against colonization by *Salmonella enteritidis* by dosing a suspension of gut contents derived from healthy adult chickens which is known as competitive exclusion (Élie et al., 2004).

Feed is the fuel of life. Broilers require energy and other nutrients including proteins, minerals, vitamins for their proper body maintenance, growth & production (Neto et al., 2011).

A great deal of attention has recently been received from nutritionists and veterinary experts for proper utilization of probiotics for growth promotion of poultry. In broiler nutrition, probiotic species belonging to *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida* and *Saccharomyces* have been proven to keep a beneficial effect on broiler performance, modulation of intestinal microflora and pathogen inhibition (Kabir et al., 2005; Ashayerizadeh et al., 2009). It also improves certain haemato-biochemical parameters, characteristics of dressed broiler meat and promotes microbiological meat quality of broilers (Pelicano et al., 2003; Chichlowski et al., 2007; Kabir et al., 2005).

Therefore the objectives of the present study were,

1. To determine the influences of different organisms used in probiotics on performance, carcass quality and blood parameters of commercial broilers.
2. To observe the economic benefit of using different probiotics and to select good quality probiotic for better performance of broiler.

Chapter 2

Review of literature

2.1 Probiotics

A significant expansion of the potential market for probiotics has led to higher requirements for scientific research to see the benefits conferred by the microorganisms.(Rijkers et al., 2011). Although there are numerous claimed benefits of using commercial probiotics, such as reduction of gastrointestinal discomfort or strengthening of the immune system, such claims are not concretely remarked by scientific evidence (Engle et al., 2011). Yet one systematic review of 15 human randomized controlled trials from July 2016 found that certain commercially available strains of probiotic bacteria from the *Bifidobacterium* and *Lactobacillus* genera (*B. longum*, *B. breve*, *B. infantis*, *L. helveticus*, *L. rhamnosus*, *L. plantarum*, and *L. casei*), when taken by mouth in daily doses of 10^9 – 10^{10} colony forming units (CFU) for 1-2 months, possess treatment efficacy (i.e., improved behavioral outcomes) in certain psychological disorders, e.g. anxiety, depression, autism spectrum disorder, obsessive compulsive disorder and improved certain aspects of memory. Probiotics are considered to be generally safe, but they may cause bacteria-host interactions and unwanted side effects in certain cases (Doron and Snyderman, 2015).

2.2 Historical development of probiotic

It was Tortuero who first employed living bacteria to replace antibiotics in poultry (Tortuero, 1973). The idea that intestinal bacteria plays a key role in maintenance of health was originated by Elie Metchnikoff when he found that lactic acid bacteria in fermented milk products are capable to increase longevity and maintenance of vigour in humans, and noted that consumption of fermented milk was closely related with longevity of Bulgarian peasants (Fuller, 1992). Later, many evidences supported that normal intestinal microflora inhibits the growth of intestinal pathogens. Lilly & Stillwell coined the term ‘probiotics’ in 1965 derived from Greek word “pro bios”, meaning “for life”. Probiotics, as per World Health Organisation (WHO) and Food and Agriculture Organisation (FAO), are “live microorganisms”, which when supplemented in adequate amounts affect the host intestinal microbial ecosystem by

providing a favourable balance between beneficial and harmful microbes in the intestinal microbial environment, help prevent the growth of pathogens resulting in improved health and prolonged life, which altogether confers a health benefit on the host (Fuller, 1989). Thus probiotics have been considered as “live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance” or “a live microbial feed that is beneficial to health” (Patterson & Burkholder, 2003; Isolauri et al., 2004). Probiotics may contain only one or a consortium of different bacterial species, and the mechanisms of action of different bacterial strains may differ (Bomba et al., 2002; Mai, 2004; Bouzaine et al., 2005). The commonly used microorganisms as probiotics are the strains of lactic acid producing bacteria which have specificity of adhering to the intestinal epithelium. Besides, other microbes viz. *Bacillus*, *Saccharomyces*, *Aspergillus oryzae* and many more along with their products are also classified as probiotics (Fuller, 2001; Dhama & Singh, 2010). The importance of probiotics as an appropriate alternative has increased more than ever due to the possible hazards and risks of antibiotics in poultry production (Hajati & Rezaei, 2010).

2.3 Criteria for an ideal probiotic

An ideal probiotic should contain sufficient number of viable microorganisms which can withstand the hostile gut environment like pH variations. It should be stable in large numbers, and be non-pathogenic, non-toxic and preferably host-specific strain(s) of beneficial microbes. Probiotics include gram-positive organisms, acid and bile resistant, and must be having a short generation time. These should adhere to intestinal epithelium, have the ability to rapidly and efficiently colonise the intestine and edge out the pathogenic microbes, and be persistently present among gut microflora. They should have the ability to produce antimicrobial compounds and modulate immune responses in a healthier way. Probiotics need to be free of diffusible antibiotic resistant genes, be sufficiently stable while manufacturing, processing and storage, should not undergo recombination with pathogenic strains and should not produce any side effects. These should preferably overcome pelleting temperatures and be compatible with most feed additives. Also these should have good sensory properties (Dhama et al., 2007; Dhama & Singh, 2010; Hajati & Rezaei, 2010).

2.4 Commonly used microbes as probiotics

Generally, live apathogenic bacterial strains belonging to genus *Lactobacillus*, *Streptococcus*, *Bacillus* or *Enterococcus* and the yeast *Saccharomyces*, are used in livestock and poultry. The strains of lactic acid producing bacteria, which have specificity of adhering to the intestinal epithelium, and *Aspergillus oryzae*, which confers beneficial impact on performance of poultry are frequently used in this industry. *Lactobacillus* and *Bifidobacterium* species have been used most exhaustively in humans. Since probiotics may also include fungi and yeast, besides bacteria, therefore the use of term “Direct Feed Microbials (DFM)” has been suggested. The most commonly used probiotics contain one or a mixture of harmless microbes (Patterson & Burkholder, 2003; Czerucka et al., 2007). The microbes generally considered for developing probiotic growth promoters are *Lactobacillus acidophilus*, *L. sporogenes*, *L. bulgaricus*, *L. casei*, *Lactobacillus paracasei*, *L. plantarum*, *L. cellobiosus*, *L. salivarius*, *L. reuteri*, *L. animalis*, *Streptococcus faecium*, *Streptococcus cristatus*, *S. thermophilus*; *Bacillus subtilis*, *Bacillus coagulans*, *Bifidobacterium bifidum*, *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Enterococcus faecium*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Escherichia spp.*, *Lactococcus spp.*, *Torulopsis spp.*, *Aspergillus oryzae* and *Hawaiian spirulina* (blue green algae) etc. (Dhama et al., 2008; Dhama & Singh, 2010; Hajati & Rezaei, 2010). Most commonly used among these are *Lactobacillus*-based probiotics. Yeast and *Lactobacillus sporogens* are highly resistant to pelleting temperatures and storage at different environmental conditions

2.5 Applications of probiotics in poultry

Probiotics maintain the proper balance of useful microbial population in the intestine of bird, which is important for the efficient feed conversion, growth, productivity and stimulation of birds' immune mechanisms to combat pathogens. The mechanism of action of probiotics in poultry production system includes establishing and maintaining healthy gut microflora, improving digestion and utilisation of nutrients, competitive exclusion of harmful bacteria/pathogens, decreases pH and releases various antibacterial substances, neutralisation of toxins, competition for nutrients with pathogens, reduction in ammonia production and stimulation of the immune system (Jin et al., 1997; Fuller, 1989; Rolfe, 2000; Patterson & Burkholder, 2003;

Boirivant & Strober, 2007; Ng et al., 2009; Dhama & Singh, 2010; Hajati & Rezaei, 2010).

2.6 Probiotic colonisation in the gastrointestinal tract

Establishing and maintaining healthy gut microflora is essential, which improves the microbial environment of a bird's intestinal tract by displacing harmful bacteria. Especially, in newly hatched chicks, the gut is sterile and starts acquiring microflora from environment. Since pathogenic microbes may multiply faster than the beneficial bacteria, chicks may get infected at this time. However, as the days progress post-hatch, the microflora stabilises and attains balance between the 'favorable' and 'harmful'. As the balance between these two gets affected or influenced by the surrounding environment like infectious pressure or internal factors like stress, the concept of probiotics supplementation emerges which is highly helpful. Successful colonisation of probiotics depends on the survival and stability of the microbial strain used, their relationship with the host, dose and usage frequency, and host health, nutritional status, age, stress and genetics (Mason et al., 2005). Measured as colony forming units (CFU), Colonisation increases at the beak and progresses distally to the colon (Simon et al., 2004). The small intestine is a reservoir of various bacteria including anaerobes with population ranging from 10^4 to 10^8 CFU/ml (*Lactobacilli*, *Streptococci* and *Enterobacteria*) (Gaskins, 2003). The maximum bacterial population, accounting to 10^{10} to 10^{13} CFU/ml, has been recorded in the colon and caecum (Heczko et al., 2000).

2.7 Improving digestion, nutrient metabolism and utilization of nutrients

Probiotics help improving digestion, nutrient metabolism and utilization of nutrients by offering digestible proteins, vitamins, enzymes and other important co-factors and by decreasing gut pH by production of lactic acids. As 'live enzyme factory' (amylase, protease, lipase) enhances digestion and absorption of carbohydrates, proteins and fats, which also increases the feed conversion efficiency. Probiotics help in metabolism of minerals and synthesis of vitamins (Biotin, Vitamin-B1, B2, B12 and K), which are responsible for proper growth and metabolism (Dhama & Singh, 2010). The facultative anaerobes (*Bifidobacterium* and *Ljptobacillus*) included in probiotic bacterial consortium reduce the redox potential in the gut and render the environment suitable for obligate anaerobes (Cummings & Macfarlane, 1997;

Chichlowski, 2007). An increase in the digestibility of dry matter is closely related to enzymes released by yeast (Lee et al., 2006). The effect of *Aspergillus oryzae* on macronutrients metabolism in laying hens might be of practical relevance (Han et al., 1999).

2.8 Combating harmful pathogens and disease conditions

The stress factors or pathogenic microbes existing in any animal or poultry rearing unit tend to create an imbalance in the intestinal microflora by lowering body defense mechanisms. This results in proliferation of pathogens, leading to physiological disturbances like diarrhea, loss of appetite, improper digestion and poor absorption of nutrients in host (Walker & Duffy, 1998; Pal & Chander, 1999; Nava et al., 2005; Dhama et al., 2008). The disturbances in balance of natural microflora will result in proliferation of harmful bacteria, which in turn reduces production performances and immunocompetence, causing heavy economic loss to poultry producers. To help prevent this malady, a probiotic should be used timely and regularly in feed in order to maintain a healthy balanced microflora to enhance health and productivity. Impact of probiotics on pathogen colonisation is reflected by the mechanism of competitive exclusion, by which these compete with harmful bacteria/pathogens for intestinal adhesive receptors that are necessary for the attachment and proliferation of the microorganisms. Thus rapid colonisation (dense layer of microflora) prevents the pathogens getting established in the gut. Antibacterial substances produced like bacteriocins, lactocin, lactocidin, acidolin, acidophilin, nisin, reuterin, organic acids (lactic and acetic acid), lactoferrin, hydrogen peroxide and lactoperoxidase inhibit pathogenic microbes. By releasing anti-enterotoxin substances (acidolin, acidophilin and lactin), probiotics help neutralise and/absorbing the enterotoxins released by pathogenic bacteria. Probiotics are also proved to bind mycotoxins present in feed. Probiotics make nutrients non-available to pathogens, by becoming successful colonisers in the intestine that efficiently utilise the available substrates. By antagonistic action towards ammonifying bacteria and reducing urease activity, probiotics decreases ammonia formation in litter and thus prevents keratoconjunctivitis which is a common problem as a result of excess ammonia in litter. Recently, an increased apoptosis has been suggested to be a mechanism by which the probiotic culture reduces Salmonella infection (Higgins et al., 2011).

2.9 Probiotics as growth promoter

As a group of growth promoters, the addition of probiotics to the diet of livestock (especially cattle and swine) and poultry has been found to improve growth performance, increase feed conversion efficiency and improve immune responses (Guillot, 2000; Balevi et al., 2001; Brashears et al., 2003; Mountzouris et al., 2007; Rowghani et al., 2007; Awad et al., 2009). Many studies have been undertaken to test the effect of probiotics on the production efficiency of broiler chickens (O’Dea et al., 2006). Researchers have also reported that some inactivated probiotics, similar to live ones has also been shown to have the beneficial effects on production performance and immune response in broiler chickens (Huang et al., 2004). A multispecies and chicken-specific probiotic preparation in fluid form, consisting of 7 *Lactobacillus* species isolated from the digestive tract of chickens was recently developed for application in broiler production. The *Lactobacillus* strains showed modulating effects on the immune system of layer and broiler chickens (Timmerman et al., 2006).

2.10 Modes of action of probiotics

It was assumed that the effect of probiotics was linked to the gastrointestinal tract and effects on incidence of diarrhea and other gut infections were expected. However, recent work in several different countries has indicated that the effects may be more general (Fuller, 1992). The reason for diverge mechanisms may be due to the different types of probiotics (Cho et al., 2011). Probiotics are believed to improve the overall health of an animal by improving the microbial balance in its gut. In general, the mode of action of probiotic feed additives is mainly based on competitive exclusion, bacterial antagonism, and immune modulation (Hughes et al., 2002).

2.10.1 Competitive exclusion

Competitive exclusion is defined by the ability of normal microflora to protect against the harmful establishment of pathogens. The concept of competitive exclusion indicates that cultures of selected, beneficial microorganisms, supplemented to the feed, compete with potentially harmful bacteria in terms of adhesion sites and organic substrates mainly carbon and energy sources. The adhesion to the digestive tract wall could be for different purposes: to prevent colonization by pathogenic microorganisms or to compete for nutrients (Steiner, 2009).

2.10.2 Adhesion to the digestive tract wall to prevent colonization by pathogenic microorganisms

Detrimental bacteria need to become attached to the gut wall to exert their harmful effects (McDonald et al., 2010). Therefore, an expected effect of the addition of probiotics to the gastrointestinal tract is an increase in normal microflora colonization with inhibition of the adhesion of harmful pathogens on the intestinal epithelium thereby blocking receptor sites and preventing the attachment of other bacteria including harmful species (Cho et al., 2011). By doing so, the probiotic bacteria exclude pathogens and thus prevent them from causing infection (Hughes et al., 2002). The mechanism of colonization is suggested to be associated with certain species within the microflora which can influence the expression of glycol conjugates on epithelial cells that may serve as receptors for the adhesion of bacteria.

Different studies have shown the potential of probiotics to decrease the risk of infections and intestinal disorders. The growth of *E. coli* was successfully inhibited by different strains of *Lactobacilli*. Moreover, it has been reported that, a combination of different lactic acid bacteria significantly reduced the levels of Salmonella in caecal contents of broilers which had been orally inoculated with the pathogen. In addition, in piglets, attachment of *E. coli* to the small intestinal epithelium has been reported to be inhibited by dietary supplementation with *Enterococcus faecium* (Hyronimus et al., 2000).

2.10.3 Competing with pathogenic bacteria for nutrients in the gut

Probiotics may compete for nutrients and absorption sites with pathogenic bacteria. In addition, competition for energy and nutrients between probiotic and other bacteria may result in a suppression of pathogenic species. The gut is such a rich source of nutrients that it may seem unlikely that microorganisms could not find sufficient food for growth. Probiotics possess a high fermentative activity and stimulate digestion. Lactobacilli are known to produce lactic acid and proteolytic enzymes which can enhance nutrient digestion in the gastrointestinal tract. Different studies demonstrated that probiotics maximized crude protein and energy digestibility compared with those in non-probiotic treatments (Yu et al., 2008). However, it should be noted be that the environment only has to be deficient in one essential nutrient in order to inhibit microbial growth. In addition, the ability to rapidly utilize an energy source may

reduce the log phase of bacterial growth and make it impossible for the organism to resist the flushing effect exerted by peristalsis (Cho et al., 2011).

2.10.4 Bacterial antagonism

Probiotic microorganisms, once established in the gut, may produce substances with bactericidal or bacteriostatic properties.

.2.10.5 Bactericidal activity

Lactobacilli ferment lactose to lactic acid, thereby reducing the pH to a level that harmful bacteria cannot tolerate. Hydrogen peroxide is also produced, which inhibits the growth of Gram-negative bacteria. These substances have a detrimental impact on harmful bacteria, which is primarily due to a lowering of the gut pH. A decrease in pH may partially offset the low secretion of hydrochloric acid in the stomach of weanling piglets. Moreover, live yeasts ferment sugars derived from the degradation of starch, thus competing with the lactic-acid-producing bacteria, and thereby stabilize rumen pH and reduce the risk of acidosis. Improvement in early digestion and intake is brought about by alterations in the numbers and species of microorganisms in the rumen (McDonald et al., 2010).

2.10.6 Neutralization of enterotoxins produced by pathogenic bacteria that cause fluid loss

Probiotic bacteria produce a variety of substances that include organic acids, antioxidants and bacteriocins (McDonald et al., 2010). These compounds may reduce not only the number of viable pathogenic organisms but may also affect bacterial metabolism and toxin production. Bacteriocins produced by lactic acid bacteria have been reported to be able to permeate the outer membrane of gram-negative bacteria and subsequently induce the inactivation of gram-negative bacteria in conjunction with other enhancing anti-microbial environmental factors such as low temperatures, organic acids and detergents. In addition, they can prevent amine synthesis (Alakomi et al., 2003). Coliform bacteria decarboxylate amino acids to produce amines, which irritate the gut, are toxic and are concurrent with the incidence of diarrhea. If desirable bacteria prevent the coliforms proliferating, then amine production will also be prevented (McDonald et al., 2010).

2.10.7 Immune modulation

Probiotics act as a stimulus for the immune system. Though, analysis and research into the ability of probiotics to influence the immune system of animals and humans is a recent development. Microbial communities can support the animal's defence against invading pathogens by stimulating gastrointestinal immune response. This may aid the development of the immune system by stimulation of the production of antibodies and increased phagocytic activity (McDonald et al., 2010). As the immune system is engaged following exposure to probiotic bacteria, any hostile bacteria are also noticed, following increased surveillance by leukocytes, and thus potential pathogens are eliminated (Hughes et al., 2002). Some probiotic strains such as spp. of *Lactobacillus* have proven to be capable of stimulating the immune system. (Fuller, 1992) explained the immune system to be stimulated in two ways. They can either migrate through the gut wall as viable cells or multiply to a limited extent or antigens released by the dead organisms can be absorbed and stimulate the immune system directly. It is the product of this change which induces the immune response. And currently, it appears to be some relationship between the ability of a strain to translocate and the ability to be immunogenic. However, it is difficult to completely conclude that probiotics contribute significantly to the immune system of the host as they are not intended to eradicate invasive pathogens in the gastrointestinal tract. Therefore, such observed improvements or positive effects are always somewhat compromised due to the animals' immune system status and the various applied situations (Cho et al., 2011). In addition to the above discussed, other postulated effects include beneficial interaction with bile salts, increased digestive enzyme production, more efficient absorption of nutrients, and greater vitamin production. Several mechanisms have been proposed to explain the effects of probiotics and it is likely that the positive results reported in the different animal studies are due to a combination of some, if not all, of these (McDonald et al., 2010).

2.10.8 Probiotics in poultry feeding

Modern rearing methods which include unnatural rearing conditions and diets induce stress and can cause changes in the composition of the microflora which compromise the animal's resistance to infection (Fuller, 1992). Thus, the aim of the probiotic

approach is to repair the deficiencies in the microflora and restore the animal's resistance to disease. Such a treatment does not introduce any foreign chemicals into the animal's internal environment and does not run the risk of contaminating the carcass and introducing hazardous chemicals into the food chain. Probiotics are now replacing the chemical growth promoters for farm animals and claims have also been made for increasing resistance to disease. Probiotics are preparations based on live microorganisms that are consumed as food and feed additives, and which have a beneficial effect on the health status of humans or animals. It is said that probiotics help prevent imbalances, and enhance the growth of the healthy microflora. Besides, probiotics are widely produced, promoted and marketed (Jiménez, 2012).

2.10.9 Microorganisms used as probiotics in poultry nutrition

Most probiotic products utilize one or more of several types of bacteria. The most commonly used bacterial probiotics are the strains of *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Bacillus*, *Pediococcus* and *Streptococcus*. Some products contain viable yeast and other fungi in addition to bacteria. There are marked differences between the various probiotic groups regarding their properties origin and mode of action. The following table shows probiotics that are commonly used in animal nutrition summarized from many literatures (Jiménez et al., 2012). The use of probiotics as farm animal feed supplements dates back to the 1970's. They were originally incorporated into feed to increase the animal's growth and to improve its health by increasing its resistance to disease (Fuller, 1992). Yet, use of probiotics in humans and animal species such as young pigs has been widely reported in the scientific literature. Numerous studies have shown that humans or animals fed probiotics have altered intestinal bacterial populations, improved resistance to disease, reduced shedding of pathogens when challenged orally, increased intestinal immunity, reduced disease symptoms, and improved health (Quigley, 2011). Certain microorganisms which are intentionally added to the feed (probiotics) counteract possible disruptions of the equilibrium and lead to eubiosis. Thus the colonisation of the intestine by undesirable microorganisms can be suppressed. As yet, not all actions of probiotics have been satisfactorily explained by science. Their overall positive effects, based on developing metabolic activity, comprise both direct and especially indirect effects. Probiotic is a generic term, and products can contain yeast cells, bacterial cultures, or both that stimulate microorganisms capable of modifying the

gastrointestinal environment to increase health status and improve feed efficiency. Administration of probiotic strains separately and in combination significantly improved feed intake, FCE, daily weight gain and total body weight in chicken, pig, sheep goat, cattle, and equine. In monogastric animals, strains of *Lactobacilli*, *Bacillus subtilis* and *Streptococci* have been used as probiotics. The use of these probiotics has been shown to help improve gain and feed efficiency in poultry and swine. However, other researchers have observed no significant response in swine. In ruminant animals, the application of yeast (*Saccharomyces cerevisiae*) in the form of live culture, or dead cells with culture extracts, has proved successful in beneficially modifying rumen fermentation (Cho et al., 2011). Probiotics have been used to potentially replace or decrease the use of antibiotics in neonatal and stressed calves, to enhance milk production in dairy cows, and to improve daily gain and feed efficiency in beef cattle. However, performance results of experiments where cattle were fed probiotics are generally inconsistent (Krehbiel et al., 2003).

2.10.10 Examples of different probiotic effect on the gut microbiota and immune system of birds

Strains of probiotic	Biological activities	References
<i>Effects on gut microbiota</i>		
Probiotic containing		
<i>L. acidophilus</i> , <i>L. casei</i> , <i>B. bifidum</i> , <i>A. oryzae</i> , <i>S. faecium</i> and <i>Torulopsis</i> spp.	Lowered numbers of <i>coliform</i> and <i>Campylobacter</i> in the gut	Khaksefidi and Rahimi, 2005
<i>L. agilis</i> JCM 1048 and <i>L. salivarius</i> sub sp. <i>Salicinius</i> JCM 1230	Enriched the diversity of <i>Lactobacillus</i> flora in jejunum and caecum by increasing the abundance and prevalence of <i>Lactobacillus</i> spp. inhabiting the intestine. Restored the microbial balance and maintained the natural stability of indigenous	Lan et al., 2004

Strains of probiotic	Biological activities	References
<i>L. salivarius</i>	bacterial microbiota in the gut Reduced the number of <i>S. enteritidis</i> and <i>C. perfringens</i> in the gut	Kizerwetter-Swida and Binek , 2005
<i>Lactobacillus</i> , <i>Bifidobacterium</i> , <i>Enterococcus</i> and <i>Pediococcus</i> strains	Increased the concentrations of bacteria belonging to <i>Bifidobacterium</i> spp., <i>Lactobacillus</i> spp. and gram-positive cocci	Mountzouris et al., 2007
<i>L. acidophilus</i>	Competed with pathogenic <i>E. coli</i> in the gut of gnotobiotic chicks	Watkins et al., 1982
<i>L. salivarius</i> CTC2197	Reduced <i>S. enteritidis</i> C-114 colonization of the gut <i>in vivo</i>	Pascual et al., 1999
<i>L. reuteri</i> C1, C10 and C16; <i>L. gallinarum</i> I16 and I26; <i>L. brevis</i> I12, I23, I25, I218 and I211, and <i>L. salivarius</i> I24	Increased the caecal populations of lactobacilli and <i>bifidobacteria</i> and decreased the caecal <i>E. coli</i>	Mookiah et al., 2014
<i>Effects on immune system</i>		
Commercial probiotic containing <i>L. acidophilus</i> , <i>B. bifidum</i> and <i>S. faecalis</i>	Increased production of antibodies at the systemic and local (intestinal) level	Haghighi et al., 2006
<i>L. acidophilus</i>	Induced T-helper-1 cytokines in caecal tonsil cells	Brisbin et al., 2010
<i>L. salivarius</i>	Induced anti-inflammatory responses (interleukin [IL]-10 and transforming growth factor [TGF]- β) in caecal tonsil cells	Brisbin et al., 2010
Commercial probiotics containing <i>L. plantarum</i> ,	Increased the production of antibodies. Increased the weight	Kabir et al., 2004

Strains of probiotic	Biological activities	References
<i>L. bulgaricus</i> , <i>L. acidophilus</i> , <i>L. rhamnosus</i> , <i>B. bifidum</i> , <i>B. thermophilus</i> , <i>E. faecium</i> , <i>A. oryzae</i> and <i>C. pintolopessi</i>	of spleen and bursa of the chicken	
<i>Lactobacillus</i> -based probiotic	Altered intestinal intraepithelial lymphocyte (IEL) subpopulations. Stimulated secretions of interferon [IFN]- γ and IL-2 against <i>E. acervulina</i>	Dalloul et al., 2003
Commercial probiotics containing <i>L. plantarum</i> , <i>L.</i> <i>bulgaricus</i> , <i>L. acidophilus</i> , <i>L.</i> <i>rhamnosus</i> , <i>B. bifidum</i> , <i>S.</i> <i>thermophilus</i> , <i>E. faecium</i> , <i>A.</i> <i>oryzae</i> and <i>C. pintolopessi</i>	Increased antibody titre against Newcastle disease (ND). Increased the geometric means of haemagglutination inhibition (HI) titres of birds.	Khan et al., 2011
<i>B. subtilis</i> Bs964, <i>C. utilis</i> BKM-Y74 and <i>L. acidophilus</i> LH1F	Enhanced intestinal mucosal immunity of the chicken at the early age.	Yurong et al., 2005

2.11 Manufacture of probiotics

Selection of microbial strains

In addition to being non-pathogenic to animals, micro-organisms used as probiotics are selected on the basis of their survival in the gastro-intestinal environment and ability to withstand low pH and high concentrations of bile acids. In addition, the chosen strain should tolerate the manufacturing, transportation, storage and application processes, maintaining its viability and desirable characteristics (Collins et al., 1998). The capacity of potential probiotic micro-organisms to withstand the gastrointestinal environment can be tested in vitro by challenging with low pH

(Collado and Sanz, 2006). The capacity to tolerate an acidic environment and bile varies among strains is different . Another desirable characteristic is the ability to adhere to the intestinal epithelium, enabling the probiotic strain(s) to colonize the intestine (Guarner and Schaafsma, 1998). In addition, ability to grow rapidly on inexpensive media is a requisite for economically viable production (Collins et al., 1998). Spore forming bacteria, particularly from the genus *Bacillus*, are increasingly being used as probiotics. *Bacillus* spores are resistant to physical and environmental factors, such as heat, desiccation and UV radiation (Mason and Setlow, 1986; Nicholson et al., 2000; Setlow, 2006; Cutting, 2011).It enables them to maintain their viability during feed pelleting, storage and handling.

Fermentation

Fermentation techniques are used either to produce microbial cells in large quantity or to produce extracellular microbial products (e.g. food-grade lactic acid), enzymes, amino acids, vitamins and other pharmaceutical compounds. Animal studies have used probiotics cultured in the laboratory or commercially available probiotics. Scaling up from the laboratory to a commercial product is not a trivial process, and quality control is paramount for a beneficial product outcome (Zhou et al., 2010; Shim et al., 2012).

Growth media

Micro-organism-specific growth media, either synthetic or dairy based, are generally used to grow probiotics in an economically viable way. Approximately 30% of the total cost of fermentation is media cost. Dairy based media have been preferred for production of human probiotics, with the use of dairy-based foods such as yoghurt as the carrier. Some countries have legal requirements preventing the use of synthetic media for the production of human probiotics but there are no such restrictions for fermentation media for the production of probiotics for animal use (Muller et al., 2009). Use of pure chemical substrates as carbon sources for fermentation generally results in high quality products. However, agricultural and other industrial by-products are preferred substrates for fermentation because of reduced cost (Hofvendahl and Hahn-Hägerdal, 2000). For example, popular substrates for industrial fermentation are whey (Timmer and Kromkamp, 1994; Øyaas et al., 1996), molasses (Montelongo, Chassy and McCord, 1993; Göksungur and Güvenç, 1997)

and starch n. Similarly, yeast extract and peptone are popular nitrogen sources in fermentation media (Chiarini et al., 1992). Yeast extract can be replaced with cheaper agricultural products (e.g. lentil flour) as nitrogen sources. Feed grade vegetable proteins and food grade carbohydrates have also been used for production of commercial probiotics. However, media information is not available for most commercial probiotics. The ideal growth medium that maximizes microbial growth can be very complex and expensive (Muller et al., 2009). Different probiotic strains generally require different media.

Growth condition

Temperature and pH affect fermentation growth rates, which are species and strain dependent. Optimum temperature for *Lactobacillus* strains varies between 25°C and 45°C (Hofvendahl and Hahn-Hägerdal, 2000). Similarly, optimal pH for the growth of probiotics also varies with microbial species and strain. In some cases, pH is set at the beginning of fermentation and allowed to drift (often decreasing due to the production of acids) while fermentation proceeds, while in other cases pH is kept fairly constant by adding buffer (Hofvendahl and Hahn-Hägerdal, 2000; Muller et al., 2009).

Fermentation method

Probiotics can be produced by either batch or continuous fermentation. In batch fermentation, all of the substrate (sterilized) and the inoculum are mixed together in the fermenter at the beginning and kept at the optimum temperature for the growth of the probiotic. In fed-batch fermentation, limiting nutrients can be added during the fermentation. The reduction of pH in the fermentation medium, to the level where it inhibits the rate of microbial growth, is one of the challenges with batch fermentation and is generally managed by adding a base or a buffer to the medium to maintain pH (Muller et al., 2009). After completion of the fermentation process, which is generally determined by measuring the concentration of probiotic in the fermenter, cells are recovered by centrifugation or filtration (Champagne et al., 2007). Obtaining a high cellular concentration while maintaining low viscosity is an important objective in optimizing the batch fermentation process, as high viscosity hinders the recovery of cells from the growth medium (Champagne et al., 2007). For spore-forming bacteria, vegetative cells are induced to sporulate, generally by limiting nutrient availability,

before harvesting. Reduction of pH is another method of triggering sporulation. With continuous fermentation, fresh growth medium is continuously added to the culture while bacterial cells and any inhibitory substances produced during fermentation are continuously removed so that continuous production of the probiotic can be maintained (Muller et al., 2009). Genetic drifts due to mutation(s) or to contamination with other bacteria occurring during the fermentation process are issues with continuous fermentation. Batch fermentation has been preferred because it is less costly than continuous fermentation (Muller et al., 2009). Developed a two-stage fermentation system as used in yoghurt production (Doleyres, Fliss and Lacroix, 2004). In their laboratory trial, the inoculum strain(s) was immobilized as a pure culture in carrageenan/locust bean gel beads, which then released bacteria at a controlled rate into the linked, continuous fermentation reactor to produce probiotics containing the required ratio of *Lactococcus lactis* subsp. *lactis* biovar, *Diacetylactis* MD and *B. longum* ATCC 15707 cells, but the ratio could not be maintained.

Drying

After fermentation the bacterial and yeast cells are usually dried for ease of transport and storage thus avoiding any need for specialized facilities for storage and transport of liquid inoculants or frozen cells. Probiotic micro-organisms are generally dried by freeze drying or spray drying but vacuum drying and fluidized bed drying are also used (Muller et al., 2009). Maintaining cell viability during drying is critical for successful probiotic production (Meng et al., 2008).

Freeze drying

A two-step process of freezing and drying is used. The bacteria are first frozen by using liquid nitrogen or dry ice, or refrigerated at -20°C and then dried under high vacuum to reduce the moisture level to 4% or below (Ananta et al., 2004). The freezing process should be fast enough to avoid the formation of ice crystals inside the cell (Mazur, 1976). Although this is the best method to dry bacteria, in terms of maintaining viability, the high cost associated with the process often hinders its application (Chávez and Ledebøer, 2007). Similarly, yeast cultures have also been preserved and stored by freeze drying. A modification of the standard freeze drying method involving evaporative cooling can preserve yeast cells for 30 years (Bond, 2007). In this method, a centrifugal head connected with a freeze dryer is used to

initially dry the yeast culture mixed with lyoprotectant, followed by secondary drying under vacuum using phosphorus pentoxide as a desiccant. Dehydration of yeast cells with successive reduction in pressure is a feasible alternative to freeze-drying (Rakotozafy et al., 2000).

Spray drying

Fine droplets of probiotic culture, atomized by spraying through a heated nozzle, are sprayed into the drying chamber against hot air. The micro-organisms (bacteria or yeast) are dried during the process and collected at the bottom of the chamber. The exposure to the high temperature during drying can kill a significant proportion of the vegetative cells, so this is a major constraint. However, the technique is popular because of the low cost of drying for the bulk production of probiotics. It is more suitable for drying spores as the probiotic product (Elizondo and Labuza, 1974)

2.12 Conclusion of the review of literature

Use of probiotics in animal nutrition is essential. Such guidelines would help prevent the use of inappropriate micro-organisms as probiotics and maintain the efficacy of probiotics in achieving the targeted benefits. Such guidelines would assist institutions involved in the production, marketing and regulation of probiotics and protect public health. Such guidelines should also give detailed instructions for the analysis of the risk associated with probiotics intended for use in animal production.

Chapter 3

Materials and Methodology

3.1 Study area

The current experiment was conducted from October to December, 2016 at the Experimental Poultry Farm and Laboratory of department of Animal Science and Nutrition, Chittagong Veterinary and Animal Sciences University (CVASU), Khulshi, Chittagong, Bangladesh.

3.2 Preparation of poultry shed

At first, the selected broiler shed was thoroughly washed and cleaned up by using tap water with caustic soda. Brooding boxes and broiler cages were also cleaned by using tap water caustic soda. Then copper sulphate solution was used as sprayer for 2 days. Formalin solution was also used as disinfectant for two days. After that potassium permanganate solution was used for two days. After cleaning and disinfecting the house was left for one week for drying. After one week lime was spread around the shed for bio-security.

3.3 Experimental design

The experiment was carried out for three month with 28 days rearing period where starter period was 1 to 14 days and grower period was 15 to 28 days. The statistical design used for the experiment was (completely randomized design (CRD)). In experiment, total 90 birds were allocated to three treatment groups with three replications in each. Chicks were equally and randomly distributed in three dietary treatment groups (T_0 , T_1 and T_2) with three replications in each. There were 30 birds per treatment group and 10 birds per replication. Diet T_0 was the control diet formulated without probiotic. Diets T_1 and T_2 were formulated with two different probiotics. All rations during starter (1-14 days) and grower periods (15-28 days) supplied in both cases were iso-caloric and iso-nitrogenous. Layout of the experiment is shown in Table 3.3.a.

Table 3.3.a: Layout of the experiment (CRD) showing the distribution of DOC to the treatment group and replication

Dietary treatment groups	No. of broilers/replications		Total no. of broilers per treatments
T ₀ (control)	R ₁	10	30
	R ₂	10	
	R ₃	10	
T ₁ (PROBIO-5)	R ₁	10	30
	R ₂	10	
	R ₃	10	
T ₂ (Polybiotic)	R ₁	10	30
	R ₂	10	
	R ₃	10	
Grand total			90

Composition: Polybiotic- *Lactic acid bacillus*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus magaterium*, *Bacillus mesentricus*, *Bacillus polymyxa*, Fortifiedwith *saccharomyces boulardil*.

PROBIO-5 –*Proteases*, *Chitosanase*, *Bacillus Coagulans*, *Lactobacillus Acidophilus*, *Bifidobacterium Longum*, *Lactobacillus Plantarum*, *Saccharomyces Boulardii*, Grape Seed Extract.

3.4 Collection of day-old chicks

A total of 90 unsexed Day-Old Chicks (Cobb 500 strain) were purchased from a sales centre of Nahar Agro., Chittagong, Bangladesh on 11 October, 2016. During purchasing all chicks were examined for uniform size and any kind of abnormalities.

3.5 Collection of Feed ingredients and Experimental feed ingredients

Feed ingredients and feed additives were collected from abattoir at Pahartoli Bazar, Khulshi, Chittagong Metropolitan.

3.6 Feeding standard

Feeding standard followed in the experiment was that of Bangladesh standard specification for poultry feed. The birds were provided with dry mash feed throughout the experimental period. Mash feed was prepared manually from raw feed ingredients, which were collected from retail and wholesale market. All the rations were iso-caloric and iso-nitrogenous. Feeds were supplied ad-libitum along with fresh clean drinking water for all the time. Rations were formulated according to the requirement of birds. The composition of different feed ingredients and nutritive value of starter and grower rations are given in Table 3.7.a and 3.7.b.

3.7 Feed formulation and feeding diets

Table 3.7.a Feed ingredients used in experimental broiler starter diets

Ingredients (Amount in kg)	Starter Period (0-14 days)		
	Control	T ₁ (PROBIO-5)	T ₂ (Polybiotic)
Maize	57.1	56.9	56.9
Rice Polish	6.5	6.5	6.5
Soyabean oil	2.0	2.0	2.0
Soybean meal	26.0	26.0	26.0
Protein Concentrate	5.2	5.2	5.2
Molasses	0.5	0.5	0.5
Common salt	0.3	0.3	0.3
Probiotic	-	0.2	0.2
Lime Stone	1.0	1.0	1.0
DCP	0.72	0.72	0.72
Vit Min Premix	0.25	0.25	0.25
Methionine	0.20	0.2	0.2
Lysine	0.1	0.1	0.1
Toxin binder	0.1	0.1	0.1
Enzyme	0.02	0.02	0.02
Anti oxidant	0.01	0.01	0.01
Total	100.0	100.0	100.0

Table 3.7.b Feed ingredients used in experimental broiler grower diet

Ingredients (Amount in kg)	Grower Ration (15-28 days)		
	Control	T₁ (PROBIO-5)	T₂ (Polybiotic)
Maize	60.2	60.0	60.0
Rice Polish	5.75	5.75	5.75
Vegetable oil	2.3	2.3	2.3
Soybean meal	26.0	26.0	26.0
Protein concentrate	3.078	3.078	3.078
Molasses	0.5	0.5	0.5
Common salt	0.3	0.3	0.3
Probiotic	-	0.2	0.2
Lime Stone	1.0	1.0	1.0
DCP	0.3	0.3	0.3
Vit Min Premix	0.25	0.25	0.25
Methionine	0.12	0.12	0.12
Lysine	0.05	0.05	0.05
Toxin binder	0.1	0.1	0.1
Enzyme	0.04	0.04	0.04
Anti oxidant	0.012	0.012	0.012
Total	100.0	100.0	100.0

Table 3.7.c Estimated Chemical composition (DM basis) of the experimental broiler starter diets

Parameters	T₀ (Control)	T₁ (PROBIO-5)	T₂ (Polybiotic)
ME (Kcal/kg)	3004.544	3004.43	3004.43
CP (gm/100gm)	20.181	20.157	20.157
CF (gm/100gm)	3.736	3.712	3.712
EE (gm/100gm)	5.064	5.039	5.039
Ca (gm/100gm)	0.686	0.686	0.686
P (gm/100gm)	0.615	0.613	0.613
Lysine (gm/100gm)	1.023	1.022	1.022
Methionine (gm/100gm)	0.469	0.468	0.468

ME= Metabolizable energy, CP = Crude protein, CF = Crude fibre, EE = Ether extract, Ca = Calcium, P = Phosphorus

Table 3.7.d Estimated chemical composition (DM basis) of the experimental broiler grower diets

Parameters	T₀ (control)	T₁ (PROBIO-5)	T₂ (Polybiotic)
ME (Kcal/kg)	3081.690	3081.545	3081.545
CP (gm/100gm)	20.975	20.935	20.935
CF (gm/100gm)	3.784	3.784	3.784
EE (gm/100gm)	5.790	5.590	5.590
Ca (gm/100gm)	0.932	0.932	0.932
P (gm/100gm)	0.707	0.707	0.707
Lysine (gm/100gm)	1.092	1.092	1.092
Methionine (gm/100gm)	0.413	0.413	0.413

ME = Metabolizable energy, CP = Crude protein, CF = Crude fibre, EE = Ether extract, Ca = Calcium, P = Phosphorus

3.8 Management procedure

The following management procedures were followed during the whole experimental periods and the uniformity in the management practices were maintained as much as possible.

3.8.1 Brooding of the chicks

The brooding boxes became ready for rearing broiler chicks after proper cleaning and drying. As the experiment was conducted in winter season, the ambient temperature was very low from the normal environmental temperature. Brooding box was pre-heated for few hours by using the electric bulb to reach the expected favorable temperature. Dry and clean newspaper was placed in the brooding box and changed for every 24-hours interval from the floor of the brooding box. After seven days later fresh dried rice husk litter materials was spread on the floor of the brooding box at a depth of about 4-5 inches.

3.8.2 Maintaining room temperature

Room temperature and humidity was maintained using 200 watt incandescent lamps and exhaust fans. The broilers were exposed to continuous lighting. During the brooding period chicks were brooded at a temperature of 95 °F, 90 °F, 85 °F and 80 °F for the 1st, 2nd, 3rd and 4th week, respectively with the help of electric bulbs. Basis on requirement temperature was increased and decreased in the brooding box as well as the whole house. The key concern was the comfort of broiler birds. The temperature was maintained by using thermometer.

3.8.3 Brooder and cage spaces

Each box brooder having 2.38 ft. × 2.08 ft. was owed for 30 birds. After 12 days later broiler birds were transferred to cage having 3.5 ft. × 1.63 ft. for 10 birds. Therefore, floor space for each bird in the brooding box was 0.17 sq. ft. and cage was 0.57 sq. ft. respectively.

3.8.4 Feeder and drinker spaces

In the early stage of brooding feed and water were given to birds on paper and small drinker. Feeding and watering were performed by using one small round plastic feeder and one round drinker with a capacity of 1.5 liter in each brooding box. The feeders and drinker were fixed in such a way so that the birds could eat and drink conveniently. After 5th day small round feeder was replaced by small liner feeder (2.21 ft. × 0.25 ft.) in each brooding box. During the period of cage rearing large liner

feeder (3.5 ft. × 0.38 ft.) and large round drinker with a capacity of three liters was used for feeding and drinking.

3.8.5 Method of feeding, watering and lighting

Formulated mash feed and fresh clean drinking water was supplied ad-libitum to the birds throughout the experimental period. Feed and drinking water were given three times a day. Starter ration was supplied for 0 to 14 days and grower ration for 15 to 28 days. During the early stage of growth feed and water were given to birds on paper and small drinkers. The birds were exposed to a continuous lighting of 24 hours of photo period.

3.8.6 Litter management

Dry newspapers were used as litter materials at a considerable depth during the brooding period. After the ends of brooding period birds were replaced in the cage for rearing until the end of experiment. Litter materials were cleaned by dandy brush from the tray and disinfected hygienically with detergent for four times in a day.

3.8.7 Vaccination and chemo prophylaxis/medication

All birds were vaccinated properly against Newcastle disease on the 4th days and booster dose again on 14th day according to the following schedule:

Table 3.8.7.a Schedule of vaccination used during experiment period

Age of birds	Name of diseases	Name of the vaccines	Route of administration
4 th days	New Castle Disease	BCRDV (Live)	One drop in one eye
14 th days	Infectious Bursal Disease	IBD(Live)	One drop in one eye

After each vaccination, Rena -WS multivitamin was supplied at 1g/5 liter of drinking water along with vitamin-C to overcome the stressed effect of vaccination and cold weather.

Chemo prophylactic measures/medication with water soluble vitamins, minerals and electrolyte were used at different ages of birds, details of which are given below:

Table 3.8.7.b Schedule of chemo prophylaxis/medication

Age of the birds (days)	Drugs used through water
1-7	Rena-WS +Electrolyte + Gluco-C
10-17	Rena-WS +Electrolyte + Gluco-C
18-28	Rena-WS +Electrolyte +Lemon+Gluco-C

3.8.8 Bio-security/Sanitation

Drinkers were washed with caustic soda and dried up daily in the morning, and feeders were also cleaned and washed with caustic soda every 3 days after. Potassium permanganate was used for washing the floor & nearer places of the shed. Lime powder and bleaching powder was also used for strict bio-security measures those were followed during the whole experimental period.

3.9 Record keeping

Following parameters were recorded throughout the experimental period.

3.9.1 Body weight

Body weight of the chicks was recorded at first day and then regular basis at the weekly intervals by a digital weighing balance for whole experimental period.

3.9.2 Feed intake

Weekly feed intake was calculated by deducting the left over feeds from the total amount of supplied feed to the broilers.

3.9.3 Mortality

Mortality was recorded throughout the experimental period when death occurred in any replication.

3.10 Calculation of data

3.10.1 Body weight gain

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

$$\text{Body weight gain} = \text{Final body weight} - \text{initial body weight}$$

3.10.2 Feed intake

Quantity of offered feed was weighed weekly. Refusal feed was recorded to determine the feed intake per week. Average feed intake was calculated weekly as gm/bird.

3.10.3 Feed conversion (FC)

The amount of feed intake per unit of weight gain is the feed conversion (FC). This was calculated by using following formula.

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

3.10.4 Mortality

It was calculated on the basis of total number of birds housed and number of birds died during the experimental period. The mortality was expressed in percent.

3.11 Collection of blood and serum separation

On the day 28, two birds were selected from each replication randomly for collection of blood. About 2.5 ml of blood was collected from every bird by sterile syringe and put those syringe in refrigerator vertically. After 6 hours serum was collected in sterile plastic vial to estimate serum parameters.

3.12 Evaluation of carcass traits

On day 28, five birds per experimental unit representative of average body weight were selected for the evaluation of carcass traits. Replicate groups were randomly selected for carcass and organ weight evaluation after fasting them over night but not without water. The birds were weighed, slaughtered by severing the jugular vein and allowed to bleed thoroughly. Birds were scalded at 75°C in a water bath for about 30 seconds before defeathering and then the birds were reweighed to calculate feathers weight by difference. The dressed chicks were later eviscerated. The wings were removed by cutting anteriorly severing at the humeoscapular joint, the cuts were made through the rib head to the shoulder girdle, the back were removed intact by pulling anteriorly. Thighs and drum stick were dissected from each carcass and weighed

separately. The measurement of the carcass traits (dressed weight %, eviscerated weight %, thigh, shank, chest, back, neck, wing, belly fat and head) were taken before dissecting out the organs. All the carcass traits except the dressed and eviscerated weight was expressed as percentages of the live weight while the organs will be expressed in g/kg body weight. The following traits were evaluated: carcass yield (CY), weight of primal parts (Drumstick, thigh, breast, back, neck, wing and feet) and weight of internal edible offal (gizzard and proventriculus, heart, liver, abdominal fat and neck fat). Carcass yield (CY) was calculated relative to live weight before slaughter.

$$\text{Carcass yield (CY) \%} = \frac{(\text{Carcass weight} \times 100)}{\text{Live weight}}$$

3.13 Cost-benefit analysis

In case of cost analysis, chick cost, total feed cost, management cost and finally total cost were calculated in Taka per bird. Total feed cost included to feed raw materials cost. Management cost included vaccination cost, labour cost, electricity cost, disinfectant cost and litter materials cost. In case of return, market sale price, total sale price and net profit were calculated in Taka per bird.

3.14 Blood parameter estimation

Blood was collected without anticoagulant from a total 6 birds from each group (2 birds from each replicate) at 28th days of age of broilers. Serum was separated after centrifugation at 3,000 rpm for 15 min. Serum enzyme like alanine aminotransferase/glutamic pyruvic transaminase (ALT/GPT), aspartate aminotransferase/glutamic oxalacetic transaminases (AST/GOT) activities were measured in the post graduate laboratory under the department of Physiology, Biochemistry and Pharmacology, CVASU using standard kits (BioMereux, France) and automatic analyzer (Humalyzer 300, Merck®, Germany) according to the manufacturer's instruction (FVMAAU; Addis Ababa, Ethiopia).

3.15 Study design & statistical analysis

Completely randomized design (CRD) was used as study design. All the data of live weight, weight gain, feed consumption and feed conversion, data related to carcass parameters and blood parameters were entered into MS excel (Microsoft office excel-2007, USA). Data were compared among the groups by one way ANOVA in STATA version-12.1 (STATA Corporation, College Station, Texas) and subsequent Duncan's Multiple Range Tests (DMART). Results were expressed as means and SEM. P values of either ≤ 0.05 or ≤ 0.01 were considered significant and highly significant, respectively.

3.16 Picture gallery related to methodology



Figure 1: Preparation of feed



Figure 2: Box brooding of DOC



Figure 3: Broilers in cage



Figure 4: Collected blood samples



Figure 5: Weighing of broiler

Chapter 4

Results

4.1 Effect of different organism used in probiotic on performance of broiler

Different parameters (feed consumption, body weight, body weight gain and feed conversion) of birds were recorded to observe the effect of different organisms used in probiotic. Cost benefit analysis was included in this experiment. At the end of the experiment carcass characteristics and different blood parameters in broilers serum were also recorded.

4.1.1 Feed intake of broiler

Feed consumption by birds was recorded daily and calculated at the end of week.

4.1.1.1 Weekly feed intake of broiler among different dietary treatment groups (gm/broiler)

Age of birds (Wks)	Mean			SEM	P value	Level of Sig.
	T ₀ (Control)	T ₁ (PROBIO-5)	T ₂ (Polybiotic)			
1 st	105.16	107.47	103.98	6.4	0.25	NS
2 nd	283.20	308.42	328.8	4.5	0.97	NS
3 rd	894.53 ^a	953.17 ^b	990.09 ^b	3.04	0.04	*
4 th	1033.82 ^a	1232.88 ^b	1282.96 ^b	5.2	0.00	**

Mean values having different superscripts differed significantly, NS= Non significant, * = Significant at 5% level, ** = Significant at 1% level

From the table 4.1.1.1, it was observed that the difference in feed intake of broilers of treatment were not significant ($P>0.05$) at first two weeks of age of broilers. Significantly higher ($P<0.05$) feed consumption was observed by birds of T₁ and T₂ groups at 3rd weeks of age. Highly significant ($P<0.01$) difference in feed consumption was observed at the end of the experiment (4th weeks of age). Feed intake by birds of control group was lower than other two groups (T₁ and T₂). However, birds of T₂ (Polybiotic) group showed highest feed consumption.

4.1.1.2 Cumulative feed intake of broiler among different dietary treatment groups (gm/broiler)

Age of birds (Wks)	Mean			SEM	P value	Level of Sig.
	T ₀ (Control)	T ₁ (PROBIO-5)	T ₂ (Polybiotic)			
1-2	388.36 ^a	415.89 ^b	432.78 ^c	5.84	0.04	*
1-3	1282.89 ^a	1369.06 ^b	1422.87 ^c	6.95	0.02	*
1-4	2316.71 ^a	2601.94 ^b	2705.83 ^c	9.04	0.04	*

NS= Non significant at 5% level, *=Significant at 5% level

From the table 4.1.1.2 it was noticed that the differences in cumulative feed intake of broiler of among different treatment groups were significant ($P < 0.05$) throughout the whole experiment. The bird treated with either PROBIO-5 (T₁) or polybiotic (T₂) showed higher feed intake than control group.

4.1.2 Body weight and weight gain

4.1.2.1 Weekly body weight of broiler among different dietary treatment groups (gm/broiler)

Age of birds (Wks)	Mean			SEM	P value	Level of Sig.
	T ₀ (Control)	T ₁ (PROBIO-5)	T ₂ (Polybiotic)			
Day 1	45.6	44.2	48.6	2.18	0.75	NS
1 st	139.5	141.9	144.0	3.84	0.14	NS
2 nd	324.6 ^a	362.2 ^b	377.2 ^b	4.95	0.03	*
3 rd	885.01 ^a	945 ^b	1010 ^c	5.04	0.00	**
4 th	1478.8 ^a	1691.1 ^b	1794.3 ^c	3.74	0.00	**

Mean values having uncommon superscripts differ significantly, NS= Non significant, *=Significant at 5% level, **=Significant at 1% level

Table 4.1.2.1 represents that initially no significant difference ($P > 0.05$) in live weight was observed among birds of three dietary treatment groups. At 1st week of age also the difference was not significant ($P > 0.05$), statistically. Significantly higher ($P < 0.05$) weight was found at 2nd weeks of age and weight of birds of T₂ group was higher than

birds of T₁ group. Lowest weight was found in control group (T₀). Similar results were observed both at 3rd and 4th weeks of age of birds but the difference in weight among the birds was highly significant (P<0.01)

4.1.2.2 Cumulative body weight of broiler among different dietary treatment groups (gm/broiler)

Age of birds (Wks)	Mean (gm)			SEM	P Value	Level of Sig.
	T ₀ (control)	T ₁ (PROBIO-5)	T ₂ (Polybiotic)			
1-2	464.1	504.1	521.2	4.19	0.75	NS
1-3	1349.11 ^a	1449.1 ^b	1531.2 ^c	3.37	0.04	*
1-4	2827.91 ^a	3140.2 ^b	3325.5 ^c	3.15	0.02	*

Mean values having uncommon superscripts differ significantly, NS= Non significant, *=Significant at 5% level

Table 4.1.2.2 represents the difference of cumulative body weight of birds which was insignificant (P>0.05) among the birds upto 2nd weeks of age. Significant differences (P<0.05) were observed both at 3rd and 4th weeks of age where probiotic treatment groups showed higher cumulative weight than control group (T₀).

4.1.2.3 Weekly body weight gain of broiler in different treatment groups (gm/broiler)

Age of birds (Wks)	Mean			SEM	P value	Level of Sig.
	T ₀ (Control)	T ₁ (PROBIO-5)	T ₂ (Polybiotic)			
1 st	93.9	97.7	95.4	3.84	0.24	NS
2 nd	185.1	220.3	233.2	4.95	0.69	NS
3 rd	522.81 ^a	582.7 ^b	632.8 ^c	5.04	0.00	**
4 th	593.8 ^a	746.1 ^b	784.3 ^b	3.74	0.00	**

Mean values having uncommon superscripts differ significantly, NS= Non significant at 5% level, **=Significant at 1% level

Table 4.1.2.3 demonstrates that no significant ($P>0.05$) differences were observed in body weight gain of the broiler among the groups at 1st and 2nd weeks of age. Birds of T₁ and T₂ groups gained significantly higher ($P<0.01$) weight than control group (T₀) both at 3rd and 4th weeks of age.

Table 4.1.2.4 Cumulative body weight gain of broiler in different treatment groups (gm/broiler)

Age of birds (Wks)	Mean			SEM	P Value	Level of Sig.
	T ₀ (control)	T ₁ (PROBIO-5)	T ₂ (Polybiotic)			
1-2	279	318	328.6	3.19	0.57	NS
1-3	801.81 ^a	900.7 ^b	961.4 ^b	2.53	0.02	*
1-4	1395.61 ^a	1646.8 ^b	1745.7 ^c	3.13	0.00	**

Mean values having uncommon superscripts differ significantly, NS = Non significant at 5% level, * = Significant at 5% level, ** = Significant at 5% level

Table 4.1.2.4 indicates that the differences in cumulative body weight gain among different dietary treatment groups were not significant ($P>0.05$) upto 2nd weeks of age. Significantly higher cumulative weights were noticed at 3rd ($P<0.05$) and 4th ($P<0.01$) of age of birds where control group gained lower weight than other two groups.

4.1.3 Feed Conversion

Table 4.1.3.1 Weekly feed conversion (FC) of broiler in different treatment groups

Age of birds (Wks)	Mean			SEM	P value	Level of Sig.
	T ₀ (Control)	T ₁ (PROBIO-5)	T ₂ (Polybiotic)			
1 st	1.12	1.10	1.09	0.14	0.76	NS
2 nd	1.53	1.40	1.41	0.35	0.53	NS
3 rd	1.71 ^a	1.64 ^b	1.56 ^c	0.20	0.01	**
4 th	1.74 ^a	1.65 ^b	1.63 ^b	0.15	0.01	**

Mean values having uncommon superscripts differ significantly, NS= Non significant, ** = Significant at 1% level

From the table 4.1.3.1, no significant differences ($P>0.05$) were observed in feed conversion of birds at 1st and 2nd weeks of age. The differences were significantly ($P<0.05$) better in T₁ and T₂ groups in comparison with T₀ group at last two weeks of age (3rd and 4th weeks).

4.1.3.2 Cumulative FC of broiler in different treatment groups

Age of birds (Wks)	Mean			SEM	P value	Level of Sig.
	T ₀ (Control)	T ₁ (PROBIO-5)	T ₂ (Polybiotic)			
1-2	1.39 ^a	1.31 ^b	1.31 ^b	0.01	0.03	*
1-3	1.60 ^a	1.52 ^b	1.48 ^c	0.05	0.00	**
1-4	1.66 ^a	1.58 ^b	1.55 ^c	0.04	0.00	**

Mean values having uncommon superscripts differ significantly, NS= Non significant, *=Significant at 1% level

Table 4.1.3.2 represents that the cumulative feed conversion of birds at different treatment groups were significantly better or lower in probiotic treatment groups (T₁ and T₂) than control at 2nd ($P<0.05$) 3rd and 4th weeks ($P<0.01$) of age of birds. Cumulative FC in T₂ group was better than T₁ group from 3rd to 4th weeks of age.

4.2 Carcass characteristics of broiler

Table 4.2.1 Final body weight, eviscerated weight and Carcass yield of broiler among different treatment groups at 28th day of age

Traits	Mean			SEM	P value	Level of Sig.
	T ₀ (control)	T ₁ (PROBIO-5)	T ₂ (Polybiotic)			
Final body weight (gm)	1454.7 ^a	1677.77 ^b	1785.37 ^c	0.42	0.04	*
Eviscerated weight (gm)	892.625 ^a	1100.52 ^b	1240.51 ^c	0.80	0.03	*
Carcass yield (CY) %	61.35	65.5	69.4	0.57	0.82	NS

Mean values having uncommon superscripts differ significantly, SEM = Standard error of mean, NS= Non significant, * = Significant at 5% level

The differences in final body weight and eviscerated weight were significant ($P < 0.05$) statistically though no significant difference ($P > 0.05$) was observed in carcass yield of birds of different groups. Significantly higher weight and carcass yield was found in T_2 group among all the groups (table 4.2.1).

Table 4.2.2 Weight of primal parts and internal edible offal of broiler among different treatment groups at 28th days of age (gm/broiler)

Traits	Mean (gm)			SEM	P value	Level of Sig.
	T ₀ (control)	T ₁ (PROBIO-5)	T ₂ (Polybiotic)			
Primal Parts						
Drumstick	65.6	70.5	69.8	9.8	0.33	NS
Thigh	23.15	23.91	24.04	4.78	0.58	NS
Breast	19.38	19.91	19.90	6.25	0.76	NS
Back	160.5	162.6	165.6	4.23	0.66	NS
Neck	34.3	38.7	39.4	3.43	0.69	NS
Wing	35.6	34.5	33.7	4.57	0.52	NS
Feet	55.7	60.2	63.7	3.57	0.87	NS
Internal Edible Offals						
Gizzard and Proventriculus	64.4	67.3	68.4	3.55	0.53	NS
Heart	7.9	8.2	8.9	1.1	0.87	NS
Liver	43.7	47.5	46.5	2.3	0.67	NS
Abdominal Fat	3.15	3.98	4.13	1.2	0.97	NS
Neck Fat	10.7	11.6	12.3	1.8	0.88	NS

SEM = Standard error of mean, NS= Non significant at 5% level.

The differences in weight of primal parts and internal edible offals of birds were not significant ($P > 0.05$), statistically.

4.3 Effect of Probiotics on cost benefit analysis of broiler

Table 4.3.1 Cost of production and returns in different treatment groups

Cost items					
Parameters	T ₀ (Control) Mean±SEM	T ₁ (5% RESM) Mean±SEM	T ₂ (10% RESM) Mean±SEM	P value	Level of sig.
Chick cost (Tk./Chick)	45.00	45.00	45.00	-	-
Total feed cost (Tk./Kg)	34.06	35.45	35.95	0.67	NS
Management cost (Tk./broiler)	16	16	16	-	-
Total feed cost (Tk./broiler)	78.85 ^a ±0.03	80.33 ^b ±0.15	80.64 ^b ±0.12	0.04	*
Total cost (Tk./broiler)	140.88 ^a ±4.03	144.9 ^b ±5.09	148.5 ^c ±4.15	0.02	*
Total cost (Tk./Kg live broiler)	118.75 ^a ±0.26	117.65 ^b ±0.25	117.13 ^b ±0.78	0.05	*

Income					
Market sale price (Tk./Kg broiler)	125	125	125	0.85	NS
Total sale price (Tk./broiler)	159.23 ^a ±0.52	165.8 ^b ±0.55	169.8 ^c ±0.41	0.01	**
Net Profit (Tk./broiler)	18.35 ^a ±0.61	20.9 ^b ±0.59	21.3 ^b ±0.56	0.05	*
Net Profit (Tk./Kg live broiler)	6.25 ^a ±0.30	7.35 ^b ±0.26	7.87 ^b ±0.38	0.03	*

Mean values having uncommon superscripts differ significantly, SEM = Standard error of mean, NS = Non significant, * = significant at 5% level, ** = significant at 1% level

N.B. Total feed cost included feed raw materials cost and probiotic cost, Management cost included vaccination cost, labour cost, electricity cost, disinfectant cost and litter material's cost.

Total feed cost (Tk./kg), market sale price of broiler (Tk./kg broiler) did not differ significantly ($P>0.05$) among the groups (T_0 , T_1 and T_2) in this experiment . However, there were significant differences in total feed cost (Tk/broiler), total cost (Tk/broiler), total cost (Tk/Kg live broiler), total sale price (Tk./broiler) of different groups. Net profit (Tk./broiler), net profit (Tk./Kg live broiler) increased significantly in probiotic treatment groups (T_1 and T_2) in comparison with control (T_0) group

4.4 Effect of Probiotics on blood parameters of broilers

Parameters	Mean			SEM	P value	Level of sig.
	T_0	T_1	T_2			
Blood glucose (mg/dl)	254.55	242.27	278.9	2.94	0.34	NS
SGPT (mmol/dl)	4.9	10.1	6.9	1.30	0.24	NS
SGOT (mmol/dl)	225.42	236.27	243.5	3.70	0.12	NS
Total Protein (gm/dl)	4.77	4.28	4.70	0.18	0.21	NS
Cholesterol (mg/dl)	108.6	100.3	102.1	3.98	0.78	NS
Creatinine (mg/dl)	0.45	0.40	0.37	0.03	0.10	NS
Triglyceride (mg/dl)	102.27	126.9	85.67	3.60	0.10	NS

T_0 = control, T_1 = PROBIO-5, T_2 = Polybiotic, SEM= Standard Error of mean, NS = Non significant

Table 4.4 indicates that there was no significant differences ($P>0.05$) in blood parameters of broilers among the different dietary treatment groups.

Chapter 5

Discussion

5.1 Effect of different organism used in probiotics on feed intake (FI) of broiler

The research showed that the difference in feed intake of broilers of treatment were not significant ($P>0.05$) at first two weeks of age of broilers. Significantly higher ($P<0.05$) feed consumption was observed by birds of T₁ and T₂ groups at 3rd weeks of age. Highly significant ($P<0.01$) difference in feed consumption was observed at the end of the experiment (4th weeks of age). Feed intake by birds of control group was lower than other two groups (T₁ and T₂). However, birds of T₂ (Polybiotic) group showed highest feed consumption. Dhama & Singh, (2010) reported that probiotics help in improving digestion, nutrient metabolism and utilisation of nutrients by offering digestible proteins, vitamins, enzymes and other important co-factors and by decreasing gut pH by production of lactic acids. As ‘live enzyme factory’ (amylase, protease, lipase) it also enhances digestion and absorption of carbohydrates, proteins and fats, which also increases the feed conversion efficiency. Several researchers in previous studies represented similar findings of increased feed consumption by birds with probiotic treatment (Cummings & Macfarlane, 1997; Fooks & Gibson, 2002; Jozefiak et al., 2004; Chichlowski, 2007). Probiotics help in metabolism of minerals and synthesis of vitamins (Biotin, Vitamin-B₁, B₂, B₁₂ and K) which are responsible for proper growth and metabolism. The facultative anaerobes (*Bifidobacterium* and *Lactobacillus*) included in probiotic bacterial consortium reduce the redox potential in the gut and render the environment suitable for obligate anaerobes. An increase in the digestibility of dry matter in feed is closely related to enzymes released by yeast (Jonvel, 1993; Lee et al., 2006). All these factors are related in increased feed consumption of probiotic treatment groups.

5.2 Effect of different organism used in growth performance of broiler

The research showed that initially no significant difference ($P>0.05$) in live weight was observed in live weight among birds of three dietary treatment groups. It indicates a higher possibility of having similar weighted birds in different groups prior the beginning of the experiment. Concordant result was also found at 1st week of age of birds. Live weight increased significantly ($P<0.05$) at 2nd weeks of age. Probiotic

treatment groups (T₁ and T₂) showed higher weight than control. Highly significant differences (P<0.01) in body weight of the broiler were observed both at 3rd and 4th weeks of age. The broiler of polybiotic treated group (T₂) gained better body weight than that of the control and PROBIO-5 treatment groups (T₁).

The mechanism of action of probiotics in poultry production system includes establishing and maintaining healthy gut microflora, improving digestion and utilisation of nutrients, competitive exclusion of harmful bacteria/pathogens, decreasing pH and releasing various antibacterial substances, neutralisation of toxins, competition for nutrients with pathogens, reduction in ammonia production and stimulation of the immune system (Jin et al., 1997; Fuller, 1989; Rolfe, 2000).

5.3 Weekly feed conversion (FC) of different treatment groups

At first two weeks, the differences in feed conversion (FC) of birds of different treatment groups were insignificant (P>0.05), statistically. However, highly significant (P<0.01) differences were observed at last two weeks of age (3rd and 4th weeks). Feed conversion was better or lower in T₁ and T₂ groups compared to control in these two weeks. Birds of T₂ group showed better feed conversion than T₁ group

Probiotics may compete for nutrients and absorption sites with pathogenic bacteria. In addition, competition for energy and nutrients between probiotic and other bacteria may result in a suppression of pathogenic species. The gut is such a rich source of nutrients that it may seem unlikely that microorganisms could not find sufficient food for growth. Probiotics possess a high fermentative activity and stimulate digestion. *Lactobacilli* are known to produce lactic acid and proteolytic enzymes which can enhance nutrient digestion in the gastrointestinal tract. Hence feed conversion by birds is improved (Yu et al., 2008). Improved feed conversion with application of probiotics has also been recorded previously by several researchers (Rowghani et al., 2007, Bansal et al., 2011). Different studies demonstrated that probiotics maximized crude protein and energy digestibility compared with those in non-probiotic treatments. In addition, the ability to rapidly utilize an energy source may reduce the log phase of bacterial growth and make it impossible for the organism to resist the flushing effect exerted by peristalsis (Cho et al., 2011).

5.4 Effect of different probiotic on carcass characteristics of broiler

The research showed significant differences ($P < 0.05$) among different dietary treatment groups in eviscerated weight, final weight of birds. The weights were significantly higher in T₁ and T₂ groups in comparison with control. Carcass yield(%) of birds did not differ significantly ($P > 0.05$) among the different dietary treatment groups.

No significant differences ($P < 0.05$) were observed in weight of different primal parts and internal edible organs. However, weight of breast, drumstick, back and thigh head, neck, wing etc were higher in probiotic treatment groups.

In general, the improvement in the body weight, daily weight gain, feed consumption and feed conversion ratio, carcass parameters in this study may be due to the increased efficiency of digestion and nutrient absorption processes due to presence of the probiotic bacteria. (Edens ,2003) reported that the inclusion of desirable microorganisms (probiotics) in the diet allows the rapid development of beneficial bacteria in the digestive tract of the host, improving its performance. As a consequence, there is an improvement in the intestinal environment, increasing the efficiency of digestion and nutrient absorption processes.(Edens et al., 1997) showed that *in vivo* and *ex vivo* administration of *Lactobacillus reuteri* resulted in an increased villus height,it indicates that probiotics are potentially able to enhance nutrient absorption and thereby improve growth performance and feed efficiency.

Probiotic bacteria produce a variety of substances that include organic acids, antioxidants and bacteriocins (McDonald et al., 2010). These compounds may reduce not only the number of viable pathogenic organisms but also affect bacterial metabolism and toxin production. Bacteriocins produced by lactic acid bacteria have been reported to be able to permeate the outer membrane of gram-negative bacteria and subsequently induce the inactivation of gram-negative bacteria in conjunction with other enhancing anti-microbial environmental factors such as low temperatures, organic acids and detergents (Alkhalif et al, 2003). In addition, they can prevent amine synthesis. Coliform bacteria decarboxylate amino acids to produce amines, which irritate the gut,they are toxic and are concurrent with the incidence of diarrhea. If desirable bacteria prevent the coliforms proliferating, then amine production will also

be prevented. *Lactobacilli* ferment lactose to lactic acid, thereby reducing the pH to a level that harmful bacteria cannot tolerate. Hydrogen peroxide is also produced, which inhibits the growth of Gram-negative bacteria. These substances have a detrimental impact on harmful bacteria, which is primarily due to a lowering the gut pH. A decrease in pH may partially offset the low secretion of hydrochloric acid in the stomach of weanling piglets. Moreover, live yeasts ferment sugars derived from the degradation of starch, thus competing with the lactic-acid-producing bacteria, and thereby stabilize rumen pH and reduce the risk of acidosis. Improvement in early digestion and intake is brought about by alterations in the numbers and species of microorganisms in the rumen (McDonald et. al, 2010).

5.5 Cost benefit analysis

Total feed cost (Tk./kg), market sale price of broiler (Tk./kg broiler) did not differ significantly ($P>0.05$) among the groups (T_0 , T_1 and T_2) in this experiment . However, there were significant differences in total feed cost (Tk/broiler), total cost (Tk/broiler), total cost (Tk/Kg live broiler), total sale price (Tk./broiler) of different groups. Net profit (Tk./broiler), net profit (Tk./Kg live broiler) increased significantly in probiotic treatment groups (T_1 and T_2) in comparison with control (T_0) group.

5.6 Effect of broiler in blood Parameters of Broilers

The research demonstrated no significant differences ($P>0.05$) were observed in serum concentrations of different parameters (i.e. blood glucose, SGPT, SGOT, total protein (TP), cholesterol, creatinine, triglyceride levels) among different levels of probiotic supplementation groups and control.

These findings are in agreement with Dimcho et al., (2005) who found that the probiotic supplementation did not affect the blood constituents comprising, haemoglobin concentrations. No significant changes ($P>0.05$) on haemoglobin and PCV levels among groups were recorded at all times of this trial. Probiotic supplementation decreased the cholesterol level in this experiment though the difference was not significant($P>0.05$).. Probiotics have cholesterol decreasing effect on broilers. At 28 days of age, chicken groups fed with various levels of probiotic showed a significant decrease in cholesterol concentrations when compared to the

control group. This observation is in agreement with numbers of previous literature. They also (Mohan et al., 1995) reported that probiotic supplementation resulted in lowering of the serum cholesterol level in white Leghorn layers serum from 176.5 to 114.3 mg/dl. Also, Mohan et al., (1996) mentioned that chickens that received 75, 100, and 125 mg probiotic/kg diets had lower serum cholesterol content (93.3 mg/100 ml) compared to the control birds (132.2 mg/100 ml).

Similar results were also reported by Arun et al. (2006) who found that serum total cholesterol and triglycerides were reduced significantly ($P < 0.01$) by dietary supplementation of probiotic containing *L. sporogene* at 100 mg per kg diet. The significant reduction in serum cholesterol of broiler chickens fed probiotic supplemented diet could be attributed to reduced absorption and/or synthesis of cholesterol in the gastro-intestinal tract by probiotic supplementation (Mohan et al., 1996). Also, it was speculated that *Lactobacillus acidophilus* reduces the cholesterol in the blood by deconjugating bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis (Abdulrahim et al., 1996; Surono, 2003).

In contrast, the findings disagree with Cetin et al., (2005) who observed that the probiotic supplementation caused statistically significant increase ($P > 0.05$) in different values of blood parameters of Turkeys. The differences may be attributed to difference in type and number of species of bacteria present in probiotics.

Chapter 6

Conclusion

Productivity in the poultry industry is hampered by various impacts, including emergence of a large variety of pathogens and bacterial resistance. This study provides a summary of the use of probiotics for improving performances, carcass quality and blood parameters of broiler. Supplementation of either PROBIO-5 or Polybiotic increased all those performances of broilers. The cost of rearing was also decreased and the profit was increased with using probiotics. It can be concluded that using probiotic in regular diet of broiler would be helpful in increasing performance of birds and reducing cost of broiler production.

The results of this experiment are evidencing consumer's protection by describing the way of preventing bacterial diseases in poultry with the use of probiotics, as well as demonstrating the potential role of probiotics in the growth performance, immune response of poultry, safety and wholesomeness of dressed poultry, improving blood parameters. This type of research work will be a new dimension for improving poultry industry in Bangladesh.

Chapter 7

Recommendations and future perspectives

According to this research work, the following recommendations may be done:

- ❖ Farmer may get increased performance of broiler with regular use of probiotics in diet.
- ❖ PROBIO-5, Polybiotic may be used in this regard.
- ❖ As it is a pilot study, further studies may be conducted on similar field to make a concrete remark.

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APPENDICES

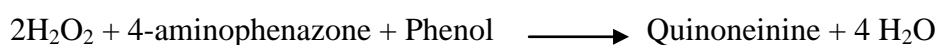
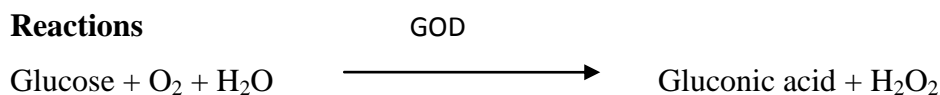
Methods of estimating different biochemical parameters (according to manufactures instruction)

Glucose assay

Principle

The principles outcome of glucose is based on the principle of competitive binding between glucose in the test specimen and GOD-PAP reagent of glucose. The glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone to a red-violet quinoneimine dye as indicator.

Reactions



Materials and reagents

1. Serum sample
2. Glucose conjugate reagent
3. Precision pipettes 10 μl , 1.0 ml
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

Procedure

The sterile eppendorf tubes were taken. 1000 μl of Glucose conjugate reagent was taken each into each eppendorf tube. Then 10 μl of Glucose standard was added in with the reagent in eppendorf tube and 10 μl of samples serum were taken in each sample eppendorf tube. The eppendorf tube was then incubated at 37°C for 10 minutes. Glucose standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum

with Glucose conjugate reagent was examined by Biochemical analyzer and the reading was taken. The standard value was used as a compared tool.

AST (Aspartate Aminotransferase) or SGOT in blood serum

Procedure:

Fresh ddH₂O was aspirated and a new Gain Calibration was performed in flow cell mode. AST is selected in the Run Test screen and a water blank is carried put as instructed.

Pipette into a test tube:	
Sample	0.05 ml
Reagent	0.5 ml

The sample is mixed and aspirated. Finally the test result is read.

Total protein assay

Principle

The principle outcome of total protein is based on the principle of competitive bindings between cupric ions react with protein in alkaline solution to form a purple complex. The absorbance of this complex is proportional to the protein concentration in the sample.

Materials and reagents

1. Serum sample
2. Total protein conjugate reagent
3. Precision pipettes: 20µl and 1.0ml
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

Procedure

This was a photometric colorimetric test for total proteins are called Biuret method. The sterile eppendorf tubes were taken. Then 20µl of total protein standards was taken in an eppendorf tube and 20µl of sample serums were taken in each 24 eppendorf tube. 1000µl of total protein conjugate reagent was then added to each eppendorf tube.

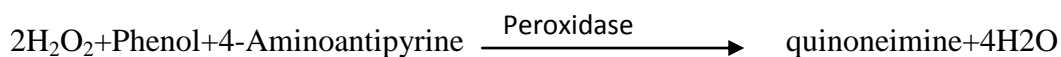
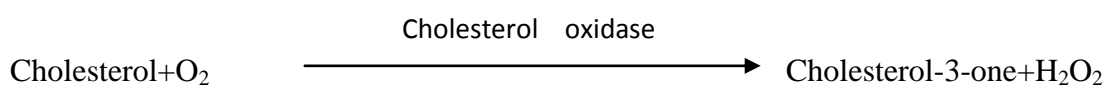
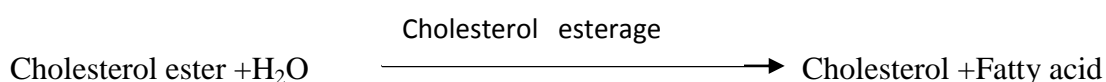
The eppendorf tube was then incubated at 37°C for 10 minutes. Total protein standards with conjugate.

Cholesterol assay

Principle

The principles outcome of cholesterol is based on the principle of competitive bindings between cholesterol and cholesterol reagent. The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. The absorbance of this complex is proportional to the cholesterol concentration in the sample.

Reactions



Materials and reagents

1. Serum sample
2. Cholesterol conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves.

Procedure

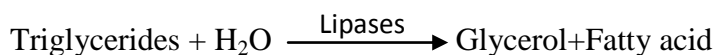
This was an enzymatic colorimetric test for cholesterol called the CHOD-PAP method. A sterile eppendorf tube was taken. Then 10 µl of cholesterol standards were taken in an eppendorf tube and 10 µl of sample serum was taken in each eppendorf tube. 1000 µl of cholesterol conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37°C for 10 minutes. Cholesterol standards with conjugate reagent were examined first to determine the standard value. Then all eppendorf tubes containing sample serum with cholesterol conjugate reagent were examined by an automated analyzer and the reading was taken. The standard value was used as a comparison tool.

Triglyceride assay

Principle

Triglycerides were determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone, and 4-chlorophenol under the catalytic influence of peroxidase.

Reactions



Materials and reagent

1. Serum sample
2. TG conjugate reagent
3. Precision pipettes
4. Eppendorf tube, eppendorf tube holder, disposable pipette tips, distilled water, 70% alcohol, absorbent paper or paper towel or cotton and gloves

Procedure

The sterile eppendorf tubes were taken. Then 1000µl TG standards was taken in an eppendorf tube and 10µl of sample serums were taken in each eppendorf tube. The eppendorf tube was then kept in room temperature for 10 minute. TG standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

Brief biography of the author

Mazharul Islam completed his graduation degree on veterinary medicine (DVM) from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. As an intern student he received clinical training from Madras Veterinary College and research institute of Tamilnadu Veterinary and Animal sciences University, India. Mazharul has a great enthusiasm in research and has done some microbiological research works. In order to detect the prevalence of *E. coli* in local dairy herd of Chittagong and dairy cows of bathan of sirajgonj distric, Bangladesh he collected the milk sample and studied the prevalence of *E. coli*. He wants to work more on poultry especially on broiler nutrition. His research interest is to produce quality broiler feed.