EFFECT OF PROBIOTICS INSTEAD OF ANTIBIOTICS AS GROWTH PROMOTERS IN BROILER PRODUCTION

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CERTIFICATE

THIS IS TO CERTIFY THAT THE THESIS ENTITLED **"EFFECT OF PROBIOTICS INSTEAD OF ANTIBIOTICS AS GROWTH PROMOTERS IN BROILER PRODUCTION"** SUBMITTED TO THE DEPARTMENT OF ANIMAL PRODUCTION AND MANAGEMENT, FACULTY OF ANIMAL SCIENCE &VETERINARY MEDICINE, SHER-E-BANGLA AGRICULTURAL UNIVERSITY, SHER-E-BANGLANAGAR, DHAKA-1207, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF **MASTER OF SCIENCE (MS) IN ANIMAL SCIENCE**, EMBODIES THE RESULT OF A PIECE OF BONA FIDE RESEARCH WORK CARRIED OUT BY **MD. RABIULHASAN**, REGISTRATION NO.: **12-04746,** UNDER MY SUPERVISION ANDGUIDANCE. NO PART OF THE THESIS HAS BEEN SUBMITTED FOR ANY OTHER DEGREE OR DIPLOMA.

I FURTHER CERTIFY THAT ANY HELP OR SOURCE OF INFORMATION, RECEIVED DURING THE COURSE OF THIS INVESTIGATION HAS BEEN DULY ACKNOWLEDGED

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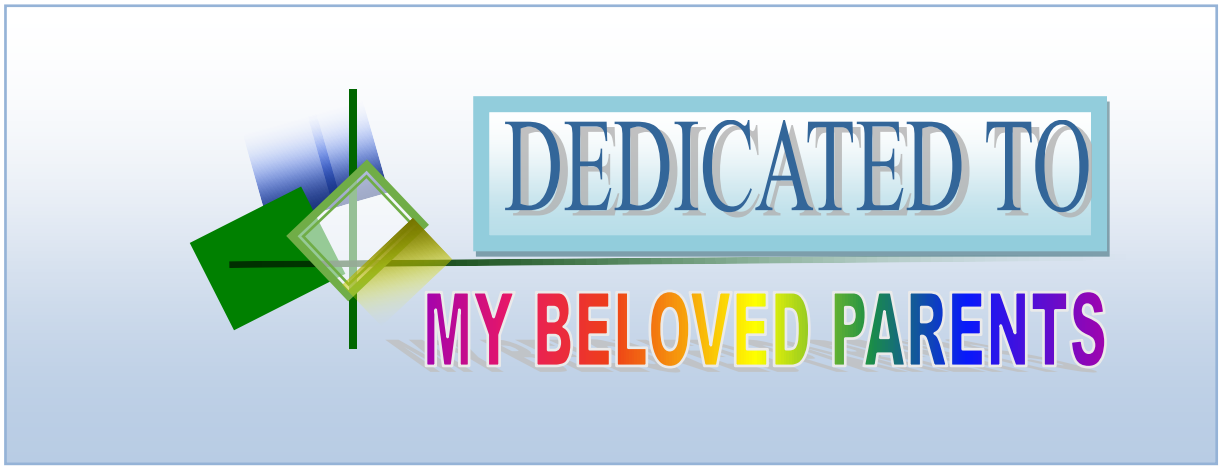
DEDICATED TO
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ACRONYMS AND ABBREVIATIONS

Abbreviation		Full meaning
A.M	=	Ante meridiem
ACTH	=	Adrenocorticotropic hormone
AGPs	=	Antibiotic growth promoters
ANOVA	=	Analysis of Variance
BANSDOC	=	Bangladesh National Scientific and Technical Documentation Centre
BARC	=	Bangladesh Agricultural Research Council
BBS	=	Bangladesh Bureau of Statistics
BLRI		Bangladesh Livestock Research Institute
Ca	=	Calcium
CAT	=	Catalase
CBC	=	Complete Blood Count
CF	=	Crude Fibre
CFU	=	Colony Forming Units
Cm	=	Centimeter
cm^2	=	Square Centimeter
CONTD.	=	Continued
CP	=	Crude Protein
CRD	=	Complete Randomized Design
DMD	=	Dry Matter Digestibility
Dr.	=	Doctor
DSP	=	Dried S*pirulina* Powder
e.g.	=	For Example
EDTA	=	Ethylene DiethyleneTetracitic Acid
et al.	=	And others/Associates
FC	=	Feed Consumption
FCR	=	Feed Conversion Ratio
FOS	=	Fructo-oligosaccharides
g	=	Gram
GSH	=	Glutathione

Hb	=	Hemoglobin
HETE	=	HydroxyEicosatetraenoic Acid
HPA	=	Hypothalamus Pituitary Axis
i.e.	=	That is
IBV	=	Infectious Bronchitis Vaccines
kcal	=	Kilo-calorie
K	=	Kilogram
LSD	=	Least Significant Difference
Ltd.	=	Limited
M.S.	=	Master of Science
MDA	=	Malondialdehyde
ME	=	Metabolizable Energy
MOS	=	Mannan-oligosaccharides
MCHC	=	Mean Corpuscular Hemoglobin Concentration
ml	=	Milliliter
mm	=	Millimeter
mmol	=	Millimole
MT	=	Metric ton
N	=	Nitrogen
NDV	=	Newcastle Disease Vaccine
No.	=	Number
NS	=	Non-significant
P	=	Phosphorus
PCV	=	Packed Cell Volume
MCHC	=	Mean Corpuscular Hemoglobin Concentration

ACRONYMS AND ABBREVIATIONS (CONT'D)

Abbreviation		Full meaning
Pp	=	Page to page
ppm	=	Parts per Million
PRP	=	Parboiled Rice Polish
RBC	=	White Blood Cell
SAU	=	Sher-E-Bangla Agricultural University
SED	=	Standard Error Difference
SOD	=	Superoxide dismutase
SPSS	=	Statistical Package for Social Sciences
UK	=	United Kingdom
USA	=	United States of America
viz.	=	Such as
Vs	=	Versus
WBC	=	White Blood Cell
WHO	=	World Health Organization
WPSA	=	World's Poultry Science Association

LIST OF SYMBOLS

Symbols		Full meaning
:	=	Ratio
@	=	At the rate of
+	=	Plus
<	=	Less than
>	=	Greater than
◦C	=	Degree Celsius
◦F	=	Degree Fahrenheit
%	=	Percentage
&	=	And
*	=	5% level of significance
/	=	Per

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ABSTRACT

A study was conducted on "Cobb-500" broiler chicks to evaluate the effects of probiotics as the growth promoter's supplementation on their growth performance, hematological parameter, microbiological load and meat composition. A total of 320, seventh days old broiler chicks were divided randomly into four groups. Each treatment group had four replications; each replication had twenty broiler chicks. Treatment Group 1 designated as the control group received standard broiler diet without any antibiotics or probiotics. Treatment group 2 received antibiotics at the rate of 14 mg Oxytetracycline®/ L of water), Treatment Group 3 received probiotics (only bacteria) at the rate of 1 x 10^8CFU/ ml / Lwaterand Treatment Group 4 had probiotics with bacteria and yeast 1 x 10^8CFU/ ml / Lof water 7^{th} to 28^{th} days of the study. It was observed that probiotics supplementation enhanced the body growth rate. Final body weight was significantly increased ($P<0.05$) in the treated groups in comparison with that of control group. The daily body weight gain at Treatment Group 4 was observed significantly ($P<0.05$) higher than others. The FCR were very much satisfactory at the probiotics providing groups.Total Erythrocyte Count (TEC), Hemoglobin (Hb) concentration, Packed Cell Volume (PCV) were significantly increased ($p<0.05$) in treated groups than the control group.Moreover, Inclusion of probiotics with water to broiler chicks found relative weight of liver, heart, gizzard and intestine weight which had no significant ($P>0.05$) differences among the treatments. Though the trends of weights were higher in probiotics supplementing group compared to the others. In addition supplementations to broiler water showed significant ($p<0.05$) difference in bacterial colony count among the groups. It is suggesting that the poultry farming may be benefited using probiotics.

CHAPTER 1

INTRODUCTION

Poultry is one of the fastest growing segments of agriculture and animal husbandry sector. Feed is one of the largest items of expenditure in poultry production. Like other sector of agricultural industry, major aim of this industry is also to produce maximum with minimum input. Poultry farming has emerged as one of the fastest growing agribusiness industries in the world, even in Bangladesh. Rapid growth rate in the poultry industry poses the problem of huge production of poultry excreta. Hence, utilization of this vast organic waste might be a critical issue in near future (*Yadav et. al., 2003*). Research on meat production globally indicates poultry as the fastest growing livestock sector especially in developing countries. It has triggered the discovery and widespread use of a number of "feed additives". The term feed additive is applied in a broad sense, to all products other than those commonly called feedstuffs, which could be added to the ration with the purpose of obtaining some special effects. The main objective of adding feed additives is to boost animal performance by increasing their growth rate, better-feed conversion efficiency, greater livability and lowered mortality in poultry birds. These feed additives are termed as "growth promoters" and often called as non-nutrient feed additives. Broilers are young chickens of either sex tender meat with soft pliable smooth texture skin and flexible breast bone cartilage. It is known as live machinery for quick return to edible meat and it can produce animal protein in the quickest possible time. The use of microbial feed supplements continuously may decrease the harmful effects of the pathogenic bacterial species in the ruminant digestive system and thereby improve the animal performance (*Frizzo et. al. 2010*). Broiler industry is playing a great role in agricultural economy. It gives maximum return with reasonable expense. It can be mentioned here that small area land can be well utilized for commercial broiler farming in a thickly populated country like Bangladesh. So, there is a wide scope for raising broiler production in Bangladesh. Broiler production is important in Bangladesh to meet up the protein requirements of the people. Poultry meats and eggs contribute approximately 41% of total animal protein in the country. There is a great

possibility of growth and expansion of this sector both domestic and commercial level. It is justified by the fact that farmers of this country are becoming more interested in broiler farming science last two decades. The beneficial effect of probiotic supplementation to broiler diet in terms of increased body weight and body weight gain is well documented in study of *Singh et. al. (1999) and Banday and Risam (2001)*. It provides a large part of increasing demand for animal protein, cash income and creates employment opportunities of the people. Probiotics are specific chemical agents produced by microorganism containing *Lactobacillus acidophilus, Lactobacillus casi, Bifido bacteriumbifidum and Aspergillu soryazae* etc. *Fuller et. al. (1989)* redefined probiotics as "A love microbial feed supplement which beneficially affects the host birds by improving it intestinal microbial balance. A probiotics is a microorganism or combination of microorganisms supposes to selectively suppress the harmful bacteria in the gut of the living beings". Probiotics also contain other substances to improve the intestinal microbial balance. Any probiotics, at a desired specific concentration per colony forming unit (per g) only can give effective results at the targeted level which is 30 x 10 to the power of nine per gram. Probiotics at the specific concentration stimulate the immune system. By being released as viable cells which destroy the invading or existing microorganism or absorbing the antigens from the dead pathogenic organism and thus stimulate the immune system. Impact of biotechnology in poultry nutrition has significant importance. The development of favorable micro flora in the gut of poultry can be enhanced by using probiotic especially during period of stress (*Krehbiel et. al. 2003*). Biotechnology plays a vital role in poultry feed industry. Nutritionists will put their effort for producing better and economical feed. Only good feed will not serves the purpose but it is better to utilize is also essential. So, it is imperative to give due to attention to proper utilization of feed. A great deal of attention has already been received for proper utilization of nutrients by probiotics. At presents, there are many probiotics available in the market and the indiscriminate use without experimental support is not justified. In assessing the value of probiotics following characteristics should be taken into consideration. Probiotics represents a single or mixed culture of live microorganisms which when applied to animals, affects the host beneficially by improving the

properties of indigenous micro flora (*Hong et. a., 2005*). Basically, it should naturally occurring microorganisms with a short reservation time. It is proved that a multiple species product is better than species product. The stability of micro-flora can easily be disturbed by many factors like change in feed, vaccination, intestinal pH, bile salt concentration in the gut and use of antibiotics. So, the Strains should be resistance to such antibiotics. It must have rapid colonizing ability and strong foothold in gut so that it can exclude by stable and have long self-life withstand in our environmental conditions. The addition of these substances to the feed or their introduction to animal body exploits the potential of utilization of feed and improves the efficiency of utilization of feed (*Nocek et. al. 2002*). One of such product available in the market in probiotics claimed that they considered all these facts. So, it is important to justify the statement before commercial use. Therefore the present study was conducted with the following objectives:

i. To determine the effect of feeding probiotics on the growth performance of broilers.
ii. To explore the effect of probiotics feeding on common blood parameters.
iii. To explore the impact of probiotics on immunization & gut health.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Sources of literature

(i) The Books and journals in different libraries as mentioned below:

- At Sher-E-Bangla Agricultural University (SAU) Library, Dhaka.
- At Bangladesh Agricultural Research Council (BARC) Library, Farmgate Dhaka.
- At Bangladesh National Scientific and Technical Documentation Centre (BANSDOC) Library, Agargaon, Dhaka.
- At Bangladesh Livestock Research Institute (BLRI) library, Savar, Dhaka.

(ii) Abstract searching at BARC, Farmgate, Dhaka, BANSDOC, Agargoan, and Dhaka.

(iii) Internet browsing

The value of probiotics in promotion growth food efficiency, egg production and egg mass in poultry has been well documented. The literature concerning the history of developments, mode of action and uses of different types of probiotics towards broilers welfare are reviewed in this chapter.

A total about 100 literature were reviewed to identify the backround, drawbacks and prospects of research, understand previous findings and to answer the research status of this field.

Mentioning the references in a traditional way or sequence is avoided. A very critical enquires was made of each article and significant information was collected and arranged according to specific title. It is expected to be pioneering efforts in Bangladesh for higher research review attempts.

Now-a-days, the Poultry farming has emerged as one of the fastest growing agribusiness industries in the world, even in Bangladesh. The Research on meat production globally indicates poultry as the fastest growing livestock sector especially in developing countries. It has triggered the discovery and widespread use of a number of "feed additives". Further, disease surveillance, monitoring and have power over will also decide the fate of this sector. Unlike live stock farming, poultry farming is always demanding and hence the birds are more subjected to stressful conditions. Stress is an important factor that renders the birds vulnerable to potentially pathogenic microorganisms like *E. coli*, *salmonella*, *clostridium*, *campylobacters* etc. These pathogenic micro floras in the small intestine contend with the host for nutrients and also reduce the digestion of fat and fat-soluble vitamins due to de-conjugating effects of bile acids (*Engberg et. al.* 2000). This ultimately leads depressed growth performance and increase incidence of disease.

2.2 Definition of probiotics

Probiotics are living microorganisms (microscopic organisms) that, when taken by mouth, benefit your health by improving the balance of bacteria in the intestines. These microorganisms are most often bacteria, but also include other kinds of organisms such as yeast. Probiotics are similar, or the same as the "good bacteria" already in your body, particularly those in your gut. The normal human intestinal tract contains 300-1,000 different kinds of bacterial species with about 10^{14} individual bacteria. Probiotics are dietary sugars that stimulate the growth of intestinal, protective bacteria. It has been most commonly used when the bacterial balance of the GI tract has been disrupted by the use of antibiotics (which deplete nearly all the bacteria in your GI tract) or when "bad" bacteria (such as *C. difficile)* that for various reasons have overgrown in your GI tract and can cause illness. When the body takes ANTIBIOTICS, they can disrupt the bacterial balance by not only killing the bad bacteria in the GI tract, but by also wiping out the beneficial bacteria. Probiotics help restore a healthy balance by adding "good" bacteria back to the gut and reducing the growth of any "bad" bacteria. FAO/WHO stated probiotic is a live microorganism which administered in adequate amounts, confers

a health benefit to the host. More recently, an expert committee has redefined probiotics as "living micro-organisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition" *(Gurner et. al. 1998; Schrezenmeier and de Vrese2001)*. A variety of microorganisms, mainly *lactobacilli, bifidobacteria, enterococci* and other microorganisms comprising *Bacillus subtilis, Bacillus cereus, Saccharomyces spp (Weese et. al. 2002)* and some species of yeast have also been used for probiotics (*Filho-lima et. al. 2000*). To be a probiotic that has efficacy in the intestines, organisms must survive passage though acid and bile environments adhere to intestinal epithelial cells, colonize the intestinal tract, produce an antimicrobial factor, and inhibit enteric pathogens (*Gibson and Fuller2000; Gorbach et. al. 2000; Dunne et. al. 2001*). Use of probiotics is becoming increasingly popular in the animal fields. Probiotics are currently widely available for the animal and regulated as nutritional supplements through the diet. Probiotics may also be considered as feed supplements to reduce the odor production. The probiotics are a class of feed additives consisting of living bacteria and/or yeast cultures fed to improve desirable micro-flora balance within the small and large intestine (*McKean et. al.2004*). Most common mixtures contain one or more of the *Lactobacillus species, Bacillus subtilis, Streptococcus faecium, Saccharomyces cerevisiae* and other commercial species. These mixtures are thought to work by either directly excluding harmful bacteria or by reducing intestinal pH to indirectly favor the development of other desirable health promoting microorganisms which compete with harmful bacteria to reduce their presence in the gut.

2.3 Antibiotic impacts on poultry

The finding of antibiotics was a success in controlling infectious pathologies and increasing feed efficiencies (*Engberg et. al.* 2000). Antibiotics, either of natural or synthetic origin are used to both avoid proliferation and destroy bacteria. Antibiotics are formed by lower fungi or certain bacteria. They are routinely used to treat and prevent infections in humans and animals. The poultry industry uses antibiotics to get better meat production through increased feed conversion, growth rate promotion and disease prevention. Antibiotics can be used successfully at sub-

therapeutic doses in poultry production to promote growth (*Engberg et. al. 2000*) and protect the health of birds by modifying the immune status of broiler chickens *(Lee et. al. 1193*). This is mainly due to the control of gastrointestinal infections due to macrobiotic modification and increase in the intestine (*Tabor et. al. 1985).*

2.4 Benefits Claimed for the Ingestion of Probiotics

Probiotics are used for multiple different types of digestive problems but since there are many different kinds of probiotics not all will have the benefit that are looking for as it relates to health. Possible beneficial effects of probiotics include:

- Absorbing and/or destroying toxins released by certain "bad" bacteria that can make the animal sick.
- Producing substances that prevent infection.
- Preventing harmful bacteria from attaching to the gut wall and growing there.
- Boosting the immune system.
- Sending signals to the cells to strengthen the mucus in the intestine, which helps it act as a barrier against infection.
- Production of B vitamins. Vitamin B is important in maintaining healthy skin, a healthy nervous system and preventing anemia.
- Decrease gas production and bloating.
- Improve digestion of lactose and reduce intestinal bloating, flatulence and discomfort.
- May prevent diarrhea.
- Prevent the potential outgrowth of spores of *Clostridium botulinum* in the GI-tract, the associated toxin production and a possible cause of sudden infant death syndrome (SID).

- Enhance the immune system, improve resistance to infection and improve well-being.
- Protect against certain types of cancer.
- Lower serum cholesterol levels and reduce the incidence of coronary heart disease.
- Prevent or help treat peptic ulcer disease.
- Treat intractable diarrhea following antibiotic therapy.
- Reduce allergic inflammation.

2.5Probiotic Bacteria and others Microorganisms

The main probiotic microorganisms used belong to the *Bifidobacterium* and *Lactobacillus* genera. Other bacteria and yeasts e.g. *Saccharomyces boulardii* have also been used. *Bifidobacterium* species and strains of *Lb. acidophilus* and *Lb. casei* are now used extensively. *Enterococci* are also used occasionally as probiotics. *Bifidobacterium* species have received particular attention and their study provides many insights into the potential therapeutic applications of probiotic bacteria. These organisms predominate in the GI- tract of babies fed with human milk where they account for some 95% of the flora. The predominance of these bacteria is due to selective agents in meconium (the sterile fluid in the GI-tract of human neonates), human colostrums and human milk. These selective factors are known as 'bifidus growth factors'. One of the best-studied enterococci used as a probiotic is *E. faecium*strain SF68. This strain is considered to be an alternative to antibiotics for the treatment of diarrhea (*Mullan et. al. 2002*).

2.6 EM (Effective Micro-organism) use as a Probiotics

Increased bacterial resistance to antibiotics in patients had caused an augented public and governmental interest in eliminating sub-therapeutic use of antibiotics in livestock. Such practice had urged to find alternatives to administration of antibiotics

for poultry production, which have probiotics action in these animals.Initially, probiotics are live microorganisms, which when consumed in adequate amounts; confer supporting healthy effects on the host. Recently, there are several researches on feeding of *Lactobacillus* spp. to livestock. Consequently, probiotics have different protective mechanisms; it may increase resistance to infection, or promote the growth, or having prophylactic effect. It has a role in promoting growth rates by improving feed efficiency with subsequent of animal health improvements. Furthermore, probiotics have positive effects on the main physiological functions of the gastrointestinal tract, reflected by better digestion, absorption and metabolism. Effective using of probiotics were widely investigated on the mucosal immune system, on the immune organs on the intestinal epithelium on the increased lymphocyte on the increase of the phagocyte activity of leukocytes and the phagocytes index in broilers. On the histological section of GIT of broiler chicks' probiotics act as crypt cells proliferation of small intestine increased the jejunely villus height ilea villus height and the number and depth of crypts. Many reports had supported the idea that the use of prebiotics can lengthen villi within the gut as well as their influence on the length of the gut. However, there is still lack of information regarding the efficacy and beneficial effects of EM in poultry. EM were innovated in Japan as a new technological advance constituting of 70 to 80 of different types of beneficial microorganisms contributing to the wide range of applications. The principal organisms of EM are usually five; photosynthetic bacteria (phototrophic bacteria), lactic acid bacteria, yeasts, actinomycets and fermenting fungi. There are several defensive proposed mechanisms of EM actions. The objectives of the current study were to evaluate the effects of supplementing broiler′s diet with EM as water probiotic additive on the performance, immunological and histological changes of intestinal linings of broilers.

2.7 Significance of Probiotics

Probiotics is versatile product that does not contain any organism imported from Japan or any other country to another, nor does it contain any genetically modified organism. Supplementing the ratio with antibiotics growth promoters could increase

growth performance of animals. Various mechanisms have been proposed which are include: (a) the nutrients are more efficiently absorbed and less are utilized by the gut, (b) more nutrients are available to the host because of a reduced intestinal micro-flora, (c) there is a reduction in harmful gut bacteria, (d) production of growth suppressing toxins or metabolites is reduced, (e) microbial de-conjugation of bile acids is decreased. But, with increasing concerns about antibiotic resistance, the ban on sub therapeutic antibiotic usage, there is increasing interest in finding alternatives to antibiotics for poultry production and using probiotics is an approach that has potential to reduce enteric disease in poultry and subsequent contamination of poultry products. However, it is possible to promote growth of broiler chickens and achieving both enhanced performance and good health by using alternatives such as probiotics and probiotics. Probiotics are live microorganisms that affect the host animal by improving its intestinal balance**.** *Fakhoury KJ and Ni JQ (2000)* mentioned that the probiotic mode of action is related to the competition for attachment sites (competitive exclusion). The bacteria present in the probiotic attach to the intestinal mucosa and blocks the attachment of pathogenic bacteria by forming a physical barrier. *Kumprech and Zobac (1998)* conducted an experiment with three hundred and twenty broiler chickens to measure the effects of probiotic on growth of chickens and results revealed that adding probiotic to the diet significantly improved the live weight and feed conversion rate of the chickens. *Cavazzoni et. al. (1998)* evaluated performance of broiler chickens supplemented with Bacillus coagulants as probiotic and found that feeding probiotic supplements increase the growth rate of broilers. There have been many previous studies to evaluate probiotics on broiler and to give good reason for its impact on broiler growth and health status different mechanisms have been proposed. *Kalavath*y *et. al.* (2003) stated that probiotic effects on intestinal micro-flora and pathogen inhibition, intestinal histological changes, immune modulation, some haemato-biochemical parameters and subsequently improve growth performance of broilers. He also mentioned that probiotic improves sensory characteristics of dressed broiler meat and microbiological meat quality of broilers. However, it is mentioned that the main effect of probiotic is in the gastrointestinal tract and associated with its capacity to stimulate the immune response and to control the growth of pathogenic

bacteria. In this study, effects of three probiotics include; Premalac, Calciporin, and Protexin on broilers growth performance were evaluated by measuring ADG, FCR and DFI.

2.8 Mode of Action of Probiotics

Probiotics are live microorganisms that provide health benefits to the host when ingested in adequate amounts. The strains most frequently used as probiotics include lactic acid bacteria and bifid bacteria. Major probiotics mechanisms of action include enhancement of the epithelial barrier, increased adhesion to intestinal mucosa, and concomitant inhibition of pathogen adhesion, competitive exclusion of pathogenic microorganisms, production of anti-microorganism substances and modulation of the immune system. Probiotics have demonstrated significant potential as therapeutic options for a variety of diseases, but the mechanisms responsible for these effects have not been fully elucidated yet.The gut microenvironment has an effect on the nutrition, feed conversion and disease of the host, thereby maintaining the microbial ecology of the gut (*Guarner et. al. 1998).* During the periods of stress, illness or antibiotic treatment, the gut flora is often changed in favor of harmful bacteria that may cause diarrhea and loss of appetite *(Cristiana and Simeanu 2012*). Over growth of the harmful bacteria and its subsequent invasion of the system lead to inflammatory, immunological, neurological and endrocrinological problems. Induction of the growth of beneficial bacteria is one of the possible solutions to normalize the health conditions. This could be achieved by the supplementation of viable bacterial cells into the host. Probiotics can help to build up the beneficial bacterial flora in the intestine and completely exclude the pathogenic bacteria. These bacteria also release some enzymes which help in the digestion of the feed (*Dunham et. al. 1993*). A daily intake of 10^9-10^{10} colony forming units (CFU) viable cells has been shown to have positive effect on the host health.

There are many microorganisms that could potentially function as probiotic, of which *Lactobacillus* and *Bifidobacterium* species are the most commonly used. Probiotics are live microorganisms thought to be beneficial to the host organism.

According to the currently adopted definition by FAO/WHO, probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit on the host. In addition, nonpathogenic species belonging to the class of *Saccharomyce*s, *Streptococcus* and *Lactococcus* are also used as probiotics. Probiotics affect the host beneficially, which may be direct or indirect, including enhanced barrier function, modulation of the mucosal immune system, production of antimicrobial agents, enhancement of digestion and absorption of food and alteration of the intestinal micro flora *(Santoso and Ohtani1995).*

The efficacy of a probiotic effect often depends on the mechanism by which they exert their activity. By and large, to treat a disease, the probiotics follow a set of mechanisms, which is discussed in this review. The effective performance of the probiotic depends on their strong adherence and colonization of the human gut, which in turn improves the host immune system *(Santoso and Ohtani1995*). The mechanism of adherence is still under investigation, but *Lactobacillus plantarum* 299v has been shown to exhibit a mannose specific adhesion by which it can adhere to human colonic cells. Once the probiotic adheres to the cell, various biological activities take place, which primarily include the release of cytokines and chemokines. These then exert their secondary activity such as stimulation of mucosal and systemic host immunity (*Dunham et. al. 1993; Sutton et. al. 1991).*

2.9 Effect of Probiotics on Growth

Antibiotics have been used in poultry industry for decades to promote growth and protect animals from diseases, followed by various side effects. In efforts of searching for a better alternative, probiotic is of extensive attention. We investigated the effects of *Bacillus subtitles, Rhodo-pseudomonas palustris, Candida utilize and Lactobacillus acidophilus* as 0.1% (W/W) feed additives on broiler growth performance and intestinal micro flora. The results showed the probiotics treatments significantly improved growth of broilers. Broilers supplemented with *B. subtilis* and *L. acidophilus* weighed 18.4% and 10.1% more than birds in control group at 42 days of age. Furthermore the feed conversion ratios of the birds in the two groups were also

improved, decreasing 9.1% and 12.9%, respectively. Further study indicated a significant increase of cecal *Lactobacilli* concentration in broilers supplemented with probiotics, expecially in *L. acidophilus* treatment group. Meanwhile, the count of cecal Actinomyces in birds treated with probiotics was significantly lower compared with the control group. In conclusion, probiotics such as *B. subtitles* and *L. acidophilus* are good alternatives to antibiotics in promoting growth resulting from a beneficial modulation of the intestinal micro flora, which leads to increased efficiency of intestinal digestion in the host animal.

2.10 Effects of Probiotics on Pathogen/ Diseases Suppression

The world as we have created it is a process of our thinking. It cannot be changed without changing our thinking-Albert Einstein. About 2000 years ago, Hippocrates quoted that-Let food be the medicine and medicine be the food is certainly the tenet of today (*Strus and Heczko 2001*). Currently there is an increased global interest due to the recognition that nutraceuticals ‘play a major role in health enhancement. The term-nutraceutical was coined by combining the terms-Nutrition and Pharmaceutical in 1989 by Dr. Stephen DeFelice, Chairman of Foundation for Innovation in Medicine. It can be defined as a food or nutrient, which provides health benefits, including the prevention and treatment of a disease, like joint problems, cardiovascular health, eye related problems and cancer prevention, such foods are commonly are referred to as functional foods‘, signifying they are/or their components may provide health benefit beyond basic nutrition. Nutraceuticals contain health promoting ingredients or natural components that have a potential health benefit for the body. For some decades now, bacteria known as probiotics have been added to various foods because of their beneficial effects for human health. The mechanism of action of probiotics is related to their ability to compete with pathogenic micro-organisms for adhesion sites, to antagonize these pathogens or to modulate the host's immune response. The potential application of probiotics includes prevention and treatment of various health conditions and diseases such as gastrointestinal infections, inflammatory bowel disease, lactose intolerance, allergies, urogenital infections, cystic fibrosis, various cancers, reduction of antibiotic side effects, in oral health such as prevention of dental

caries, periodontal diseases and oral malodor and many other effects which are under investigation. The results of many of these clinical investigations suggest that probiotics may be useful in preventing and treating various health conditions and diseases. However, many of these clinical studies require validation so as to apply these results to clinical realm. The role of clinical trials is instrumental in such investigations and in near future the results of such trials will decide the usefulness of probiotics in health and disease. This article strives to summarize the currently available data on the potential benefits of probiotics in health and disease. Probiotics are live bacteria and yeasts that are good for our health, especially our digestive system. We usually think of bacteria as something that causes diseases. But our body is full of bacteria, both good and bad. Probiotics are often called "good" or "helpful" bacteria because they help keep our gut healthy. Probiotics are naturally found in our body. We can also find them in some foods and supplements. It's only been since about the mid-1990s that people have wanted to know more about probiotics and their health benefits. Doctors often suggest them to help with digestive problems. And because of their newfound fame, we can find them in everything from yogurt to chocolate. Researchers are trying to figure out exactly how probiotics work. Here are some of the ways they may keep us healthy: When we lose "good" bacteria in our body, probiotics can help replace them. They can help balance our "good" and "bad" bacteria to keep our body working like it should. In present review, we have tried to focus on the role of probiotics in health in general and oral health in particular.

2.11 Antagonistic Activity of Probiotics

The antagonistic activity of five probiotic lactobacilli (*Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* La5, *Lactobacillus plantarum* 299v and *Lactobacillus paracasei*, *Lactobacillus fermentum* ME-38700:2) and two bifidobacteria (*Bifidobacterium lactis* Bb12, *Bifidobacterium longum* 46) against six target pathogens was estimated using different assays (solid and liquid media, anaerobic and micro-aerobic cultivation) and ranked (low, intermediate and high). Bacterial fermentation products were determined by gas chromatography, and the total anti-oxidative activity of probiotics was measured using linolenic acid test.

Pyelonephritic *Escherichia coli* were highly suppressed by GG and both bifidobacteria strains. Lactobacilli strains 8700:2, 299v and ME-3 were the most effective against *Salmonella enterica spp enterica* in microaerobic while ME-3 and both bifidobacteria expressed high activity against *Shigellasonnei* in anaerobic milieu. *Lactparacasei, Lactrhamnosus* & *Lactplantarum* strains showed intermediate antagonistic activity against *Helicobacter pylori* under micro-aerobic conditions on solid media. The highest anti-oxidative activity was characteristic for *Lactfermentum* ME-3 ($P < 0.05$). No efficient antagonist against *Clostridium difficile* was found. The positive correlations between the pH, lactic acid production and anti-microbial activity for all tested probiotics were assessed. Several bacterial strains, belonging to the genera *Lactobacillus, Enterococcus, Streptococcus* and *Bifidobacterium* are currently available as probiotics. Probiotics have been considered in developing a probiotic product for the *consumer (FAO and WHO Guidelines et. al.2002; Yumoto and Nakajima K 2004).*The evaluation of their functional validity, e.g. the beneficial effect of a particular probiotic strain, seems to be the most difficult aspect. One of the most frequent health claims for probiotics concerns the putative reduction and prevention of infectious disease in the gastrointestinal tract (GIT). The effect of probiotic strains depends on their ability to survive during passage through the stomach, as well as their ability to persist and compete with pathogens in GIT. In the case of Helicobacter pylori, this Gram-negative spiral bacterium has the ability to infect gastric and duodenal mucosa and is mainly associated with chronic gastritis and peptic ulcer (*Dunn et. al., 2001*). Moreover, enteric pathogens infect the host in different atmospheric conditions of GIT, causing diarrheal disease. Salmonella spp. and Clostridium difficult cause inflammation in ileum and colon while *Shigella spp.* clearly prefers the colonic mucosa (*Portejoie and Lebreton2004).* In addition, the colon has been considered the main reservoir of Escherichia coli strains causing urinary tract infections (*Gorbach et. al.2000).* In order to test the suppression of different pathogens by probiotic bacteria, it is necessary to consider their distinct environmental conditions in GIT. In order to find strains of probiotic bacteria, which are antagonistically active against selected enteric pathogens, their individual and distinct metabolic properties should be considered. Previous research has clearly

shown that the secreted compounds of particular species of lactic acid bacteria depend on the oxygen tension during growth, as well as their type of fermentation, e.g. obligatoryhomofermentative (OHOL), obligatoryheterofermentative (OHEL) and facultative hetero fermentative (FHEL) (*Annuk et. al. 2003*). However, at present there are few comparative studies in literature, which have elucidated a probiotic's anti-microbial effect under aerobic, micro-aerobic and anaerobic growth conditions (*Alvarez and Gonzalez1994*). Moreover, there is some evidence that lactic acid bacteria have an anti-oxidative potential (*Alkhalf et. al. 2010; Alwan et. al. 1997).* This property could be helpful in allowing lactobacilli to colonize the intestines, as well as in the course of inflammation to protect the intestinal mucosa against excessive oxidative stress *(Abdollahiv et. al. 2003).*

2.12 Mode of Action of Probiotics on Immune Stimulation

Probiotics play a role in defining and maintaining the delicate balance between necessary and excessive defense mechanisms including innate and adaptive immune responses. Points of interaction with the immune regulation for probiotics include bacteria direct interaction with intestinal epithelial cells, or following internalization by M cells through interaction with dendrite cells and follicle-associated epithelial cells, initiating responses mediated by macrophages and T and B lymphocytes. Regulation of gene expression and signaling pathways in the host cells are two major mechanisms underlying probiotics action leading to immune modulation. Metenomics analysis has expanded our understanding of the probiotics genes which are involved in the regulation of the host immune responses. Forty-two *Lactobacillus plantarum* strains isolated from diverse environmental and human sources were evaluated for their capacity to stimulate interleukin 10 (IL-10) and IL-12 produced by peripheral blood mononuclear cells. By comparison of the strain-specific cytokine responses and comparative genome hybridization profiles obtained using *L. plantarum* WCFS1 DNA microarrays, six candidate genes with immune modulator capacities were identified. These genes are involved in encoding an *N*-acetyl-glucosamine/galactosamine phosphor transferee system, the LamBDCA quorum-sensing system, components of bacteriocin biosynthesis and transport pathway.

Deletion of these genes in *L. plantarum* WCFS1 resulted in abolishing the capacity to stimulate cytokine production. Furthermore, the same bacteria and the methods were applied to study gene loci that regulate IL-10 and IL-12 production by dendrite cells. Several different genes from those involved in the regulation of cytokine production by peripheral blood mononuclear cells were identified, which include six genes involved in bacteria in production or secretion, one encoded a bile salt hydrolyses and another encoded a transcription regulator. Thus, these results suggest that regulation of responses by different immune cells is likewise probiotic gene specific Host factors have also been shown to exert effects on regulation of the transcription of probiotic genes. Genes associated with stress and adhesion in *Lactobacillus acidophilus* NCFM were studied in an in-vitro gastrointestinal tract model. Expression of the genes encoding the stress-related proteins, GroEL, DnaK, and ClpP, were unregulated in *L. acidophilus* NCFM preincubated with acidified milk during gastric digestion and declined upon subsequent duodenal digestion. Whereas genes encoding mucin-binding and fibronectin-binding proteins were not influenced by saliva or gastric juice, they were significantly increased during incubation in duodenal juice and bile. These results provide elegant examples of the complexity and functionality of probiotics during passage through the gastrointestinal tract.

2.13 Mode of Action of Microbes Presents in Probiotics

According to the supplied leaflet of Integrated Natural Farming (EM Donar), the mode of action of different microbes present in EM, are follows:

A. Lactic acid bacteria (*Lactobacillus spp*)

i. Produce lactic acid from sugar and other carbohydrates.
ii. Promotes the decomposition of material such as lignin or cellulose and ferments those materials.

Suppress diseases inducing microorganisms:

- Reduces nematode populations.
- Control propagation and spread of microbes.

B. Yeast (*Saccharomyces spp*)

i. Synthesize antimicrobial and other useful substances.
ii. Produce hormones and enzymes.

C. Photosynthetic Bacteria (*Rhodopseudomonasv spp*)

i. Photosynthetic bacteria support the activities of other microbes and considered as the pivot of EM activity.
ii. Synthesis useful substances by using and the heat as sources of energy. These include amino acids, nucleic acids, bio-active substances and sugars.

2.14 Use of Probiotics in Agriculture

EM becomes important in the agricultural sectors of all countries throughout the world which strive to enhance productivity while maintaining environmental quality. It boots the numbers and activities of beneficial microbes already presents in the soil and plan. EM enhances nutrients release and uptake as well as prevents putrefaction duration high moister products. Probiotics is used to make natural compost which is free of putrefaction products. It is used to inoculate sterile compost 16 provide competitive exclusion against pathogens. The more beneficial the bacteria and fungi are, the more "fertile" the soil is. These microorganisms break down organic matter in the soil into small, usable parts that plants can uptake through their roots. The healthier the soil, the lower the need for synthetic herb/pesticides and fertilizers. The concept that certain microorganisms 'probiotics' may confer direct benefit to the plant acting as bio-control agents for plants. The plant probiotic bacteria have been isolated and commercially developed for use in the biological control of plant diseases or bio fertilization. These microorganisms have fulfilled important functions for plant as they antagonize various plant pathogens, induce immunity, or promote growth. The interaction between bacteria and fungi with their host plants has shown their ability to promote plant growth and to suppress plant pathogens in several studies.

2.15 Safety Concerns of Probiotics Use

Newborn infants can develop infection from many species of resident micro-flora. The mechanisms for these infections and route of contamination are unclear. Many strains of Lactobacilli and Bifid bacteria are generally recognized as safe for use in the food supply. Documented correlations between systemic infections and probiotic consumption are few, and they have all occurred in patients with underlying medical conditions. Sporadic lactobacillemia from environmental, dietary, or fecal lactobacilli has been very rarely reported. Case reports of Lrhamnosus (GG) infections possibly associated with probiotic consumption, in immune compromised patients have been even less common. As opposed to the rarely reported episodes of lactobacillemia (some associated to ingested Lactobacilli), bifidobacteremia has not been sporadically reported, whether associated with consumption of commercial products containing Bifidobacteria or not. Bifidobacteria have also been consumed in infant formulas for more than 15 years worldwide and have not been associated with any pathologic or adverse event. Studies so far have documented safety and adequate growth with B. lactis in infants from birth and in vulnerable populations, including pret.erm infants, malnourished infants, and infants born to mothers with HIV disease.

From the safety point of view, according to current available information, Bifidobacteria, particularly B lactic, has a uniquely strong safety profile, making it a good probiotic candidate for newborns and young infants. Lactobacilli, particularly L rhamnosus (GG), also seems generally safe and be appropriate for older infants and children. Until adequate data are available for each specific probiotic bacterium, use of probiotics in general cannot be recommended in immune compromised populations. However, as safety is better documented for specific bacteria, we may be able to use them in certain populations that may benefit the most from probiotic use.

CHAPTER 3

MATERIALS AND METHODS

3.1 Place and duration of research work

To investigate the influence of a probiotic in the diet of broiler chicks, age 28 days feeding trial with 320 day-old Cobb-500 broilers was conducted in winter season at SAU Poultry Farm, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207. The trial period was continued from 22nd October to 17th November, 2018. This research helps to make a conclusion about Probiotics as the alternative of antibiotic.

3.2 Preparation of experimental house and equipments

An open-sided house was used for rearing the experimental birds. Experimental room was partitioned into 16 separate pens of equal size by using wire net and wood materials. The experimental rooms (ceiling, floor and wire net) were properly brushed with broom and then washed and cleaned by forced water using a hosepipe. After washing with clean water, the room was disinfected by bleaching powder solution. Then the room was left vacant for 15 days. Later, the room was again disinfected with virkon-s (Antec International Limited, England) and kept free to dry up properly. At the same time, all feeders, waters and other necessary equipments were also properly cleaned, washed and disinfected with bleaching powder solution, subsequently dried and left them empty for one week before the arrival of chicks. Ceiling, walls and wire net were also thoroughly disinfected by diluted virkon-s solution @ 10 g per 1 liter water. Three days before arrival of chicks, the rooms were enclosed with curtains made of jute materials and fumigated with potassium permanganate and formalin at double strength (2x). For 100 cubic feet area a mixture of 35g potassium permanganate and 70cc formalin, which is equal to double strength, was used for fumigation. The room was fumigated for a period of 48 hours to destroy pathogenic bacteria and virus. The fumigation was started in 18th October 3.00 PM and it was continued up to 20th October, 2018. The room was opened fully for proper aeration in

20th October, 2018 at 3.00 PM before 25 hours of the arrival of chicks. The chicks were allocated in the room on 22th October, at 8.00 AM.

3.3 Collection of the experimental birds

Three hundred's and twenty day-old Cobb-500 broiler chicks were procured from Savar, Dhaka. From the production house the chicks were carried through the car in the box. After receiving the chicks, the fresh water with little amount of lemon extract was provided to the chicks.

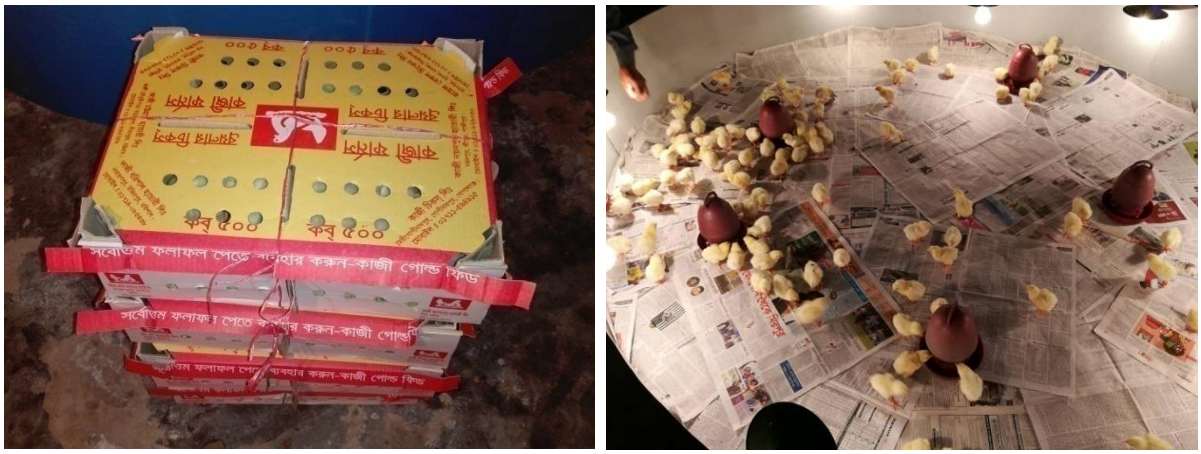

Figure 3.1: Day one experimental broiler chicks.

3.4 Layout of the experiment

The day-old Cobb-500 broiler chicks were first kept in the tin-shed surrounded chick guard with rice polish from first day to sixth day. The required waterers and feeders are provided for the new arrival chicks. At the seventh days, the chicks are randomly distributed into four dietary treatments, having four replicates in each treatment. The chicks were randomly selected and allotted to the respective replication pens. There were 20 chicks in each replication. The Table 3.1 is presenting layout which is showing the distribution of experimental birds from the very early till to the end of the experiments.

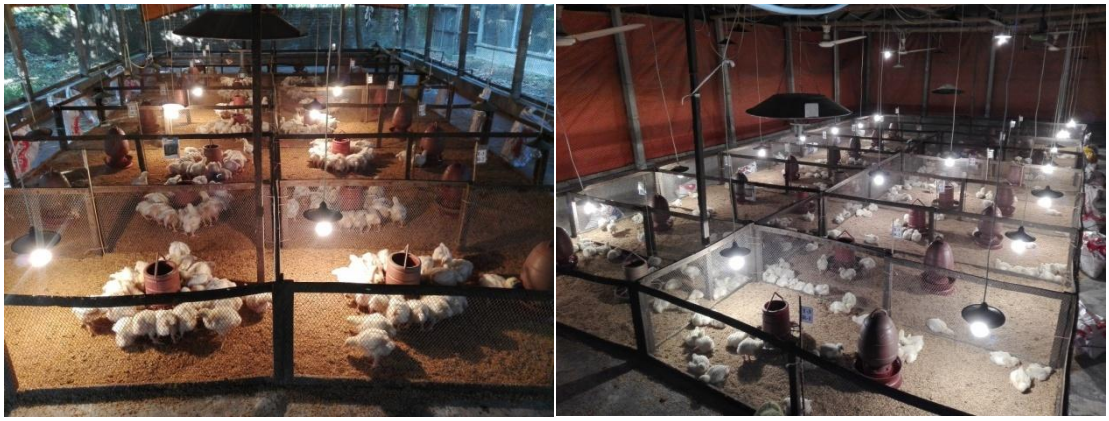

Figure 3.2: Distribution of the experimental birds for different treatment.

Table 3.1: Layout showing the distribution of experimental birds

Dietary Treatment	Age of the Birds (Days)	R-1	R-2	R-3	R-4
T-1	*07*	*20*	*20*	*20*	*20*
T-2	*07*	*20*	*20*	*20*	*20*
T-3	*07*	*20*	*20*	*20*	*20*
T-4	*07*	*20*	*20*	*20*	*20*

Here, T-1= (Control: No Antibiotic & Probiotics), **T-2**= (Antibiotic: 14 mg Oxytetracycline®/ L water), **T-3**= Only Bacterial Strain (*Lactobacillus spp)* Probiotics: 1 x 10^8CFU/ ml / L water), **T-4**=Bacterial (*Lactobacillus spp)* & Yeast Strain (*Saccharomyces spp*) Probiotics: 1 x 10^8CFU/ ml / L water).

3.5 Experimental diet

The experimental diets were divided into three phases (broiler-starter and broiler-finisher). Broiler starter diet was provided between 0 to 14 days, broiler-finisher phase consists of 15 to 28 days. The experimental diets were purchase from local market, the feed name was Usha feed and the company name is paragon. The required amount of probiotics was weighted treatment-wise and it was then mixed with the provided water for every treatment separately. Every morning the probiotics and probiotics with yeast were provided separately at every treatment group.

3.6 Source of Probiotics

Experimental bacterial strain (*Lactobacillus spp)* probiotics and bacterial (*Lactobacillus spp)* with yeast strain (*Saccharomyces spp*) probiotics mixed were obtained from prepared probiotics previously. The viability of probiotics mixture was characterized following the standard protocol (*Zulkifli et. al.,* 2000). Briefly, bacterial probiotics Strain were inoculated on LSM and incubated at 37◦C temperature overnight. The colony morphology, cultural characteristics, Straining properties and catalase test was performed to comply with the characteristics of Lactobacillus*Strain.* Once the Strain was few viable, they were further tested for their impacts on growth performances on broiler. Gram positive, catalase negative, rod shaped Lactobacillus bacterium were used to prepare probiotic mixture.Experimental organisms were inoculated in MRS broth (in 15 ml screw cap tube) for 48 hours at 37 °C and the turbidity were checked.

3.7 Routine management

The birds were uncovered to similar care and management in all treatment groups throughout through the experimental period. The following management practices were followed during the whole experimental period.

3.7.1 Litter management

The Fresh and dried rice husk was used as litter at a depth of about 3cm. At the end of each day, litter was stirred to prevent accumulation of harmful gases and to decrease parasite infestation. After 3 weeks of age, the old litter was totally removed and new litter was provided. Again it was practiced after 4 week of age.

3.7.2 Floor space

Each pen was 6 ft x 3.5 ft allocated for 20 birds. Therefore, each bird was provided a floor space of 1.75 sq. ft.

3.7.3 Brooding

Science experiment was done in autumn season (September to December); the environment temperature was some tikmes lower and sometimes higher than the requirements. In the first week of the experiment period, the environment temperature was lower than brooding temperature for all treatment groups, therefore, additional heat was provided to chicks during time. Brooding of chick's easy done by using 1 electric bulbin the respective pens. The bulbs were hanged just above the bird's level at the centre of each pen. Brooding temperature was kept 95°F at the beginning of the first week of age and decreased gradually as shown in Table 3.2.

3.7.4 Room temperature and relative humidity

Daily room temperature (°C) and humidity were recorded every six hours with a thermometer and a wet and dry bulb thermometer respectively. Averages of room temperature and percent relative humidity for the experimental period were recorded

Table 3.2: Brooding Temperature for experimental birds

Age of the Birds (Days)	Brooding Temperature °F	Brooding Humidity %
0-3	*95*	*73*
4-7	*93*	*71*
8-11	*91*	*68*
12-15	*86*	*67*
16-19	*84*	*65*
20-23	*81*	*64*
24-32	*79*	*63*

3.7.5 Lighting

At night there was provision of light in the broiler farm to stimulate feed intake and body growth. For first 2 weeks 24 hours light was used. Then the birds were exposed to 23 hours of lighting and a dark period of 1 hour per day throughout the experimental period. The dark period provision was practice to make broilers familiar with the possible darkness due to electricity failure. Ten 100 watt electric bulbs were satisfactory for lighting.

3.7.6 Feeder and waterer management

For the first 4 days, feeds were given on paper and water was supplied in ground water (small plastic pot). After 4 days of age, one round feeder and one round waterer were provided for each replication (20 birds). One additional round feeder was provided to each pen (replication) after 18 days of age. Required feeding and deinking space were provided according to the number and age of the birds in each replication. The feeders and waterer's were fixed in such a way that the birds were able to eat and drink conveniently. Feeders were cleaned at the end of each week and waterer's were washed twice a day.

3.7.7 Feeding and watering

Immediately after allocating the chicks in their respective pen, 5% glucose solution was provided to the chicks for 3 hours. Then crushed and fresh wheat, clean and cool drinking water was supplied to the chicks. For the first seven days, feeds were given to the birds at the two to three hours interval and water was provided four times a day. From the second week, feeds were supplied to the experimental birds three times every day, once in the morning, in the afternoon and again at night. Fresh cool drinking water was provided three times a day; once in the morning, in the noon, in night. Feeds and drinking water offered at ad libitumto the experimental birds. Feeders and waterer never kept empty.

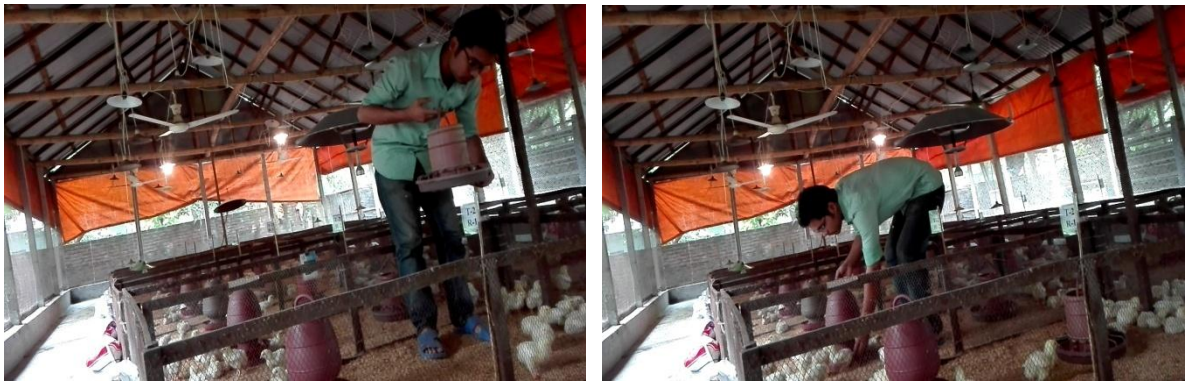

Figure 3.3: Feeding and watering of the experimental birds of different groups.

3.8 Bio security measures

To keep disease away from the broiler farm recommended vaccination, sanitation program was undertaken in the farm and its premises. All groups of broiler chicks were supplied Vitamin B-Complex, Vitamin-ADEK, Vitamin-C, Ca and Vitamin-D enriched medicine and electrolytes through the water for several times.

3.8.1 Immunization

The experimental birds were vaccinated to prevent Newcastle Disease (ND) and Infectious Bursal Disease (Gumbro). The vaccination schedule followed during the experimental period is given in the Table 3.3.

3.8.2 Vaccination schedule of experimental disease

The BCRD, Gumboro vaccine and Gumboro vaccine had been given to the birds at the very early age of the broilers chicks. The days of vaccination time of the broiler chicks and name of vaccine are given bellow the Table 3.3. This all vaccine has been Vaccination prepared by "Intervet International, Holland," was applied as per recommendation of the manufacturer.

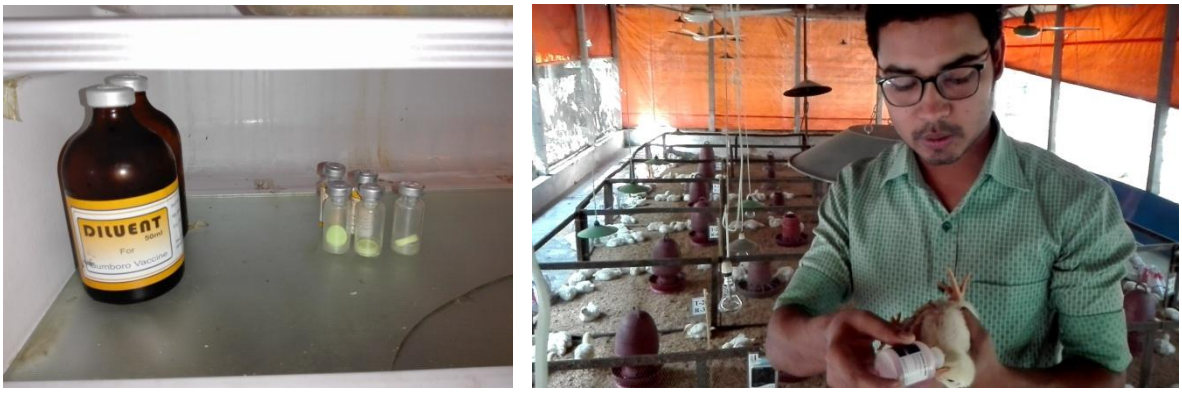

Figure 3.4: Vaccination schedule of experimental disease

Table 3.3: Vaccination schedule of experimental birds

Name of the vaccine	Age of the Birds (Days)	Dose and route of administration of diluted vaccine
BCRDV	*D O C*	*1 drop in Eye*
Gumboro vaccine	*10^{th} day*	*1 drop in Eye*
Gumboro vaccine	*17^{th} day*	*1 drop in Eye*

3.8.3 Sanitation

Proper hygienic measurement and strict sanitation programs were followed during the experimental period. The entrance point and baranda were kept clean and solution of bleaching power, savlon solution or potassium permanganate was sprayed alternatively. In addition, the service area of the experimental rooms, outside wall of the experimental house and the feed room were kept clean throughout the experimental period.

3.8.4 Postmortem examination of the broiler chick

All dead birds during the experiment were diagnosed tentatively. After postmortem examination, the results were collected and necessary measures were taken to remove the problem without applying medicines.

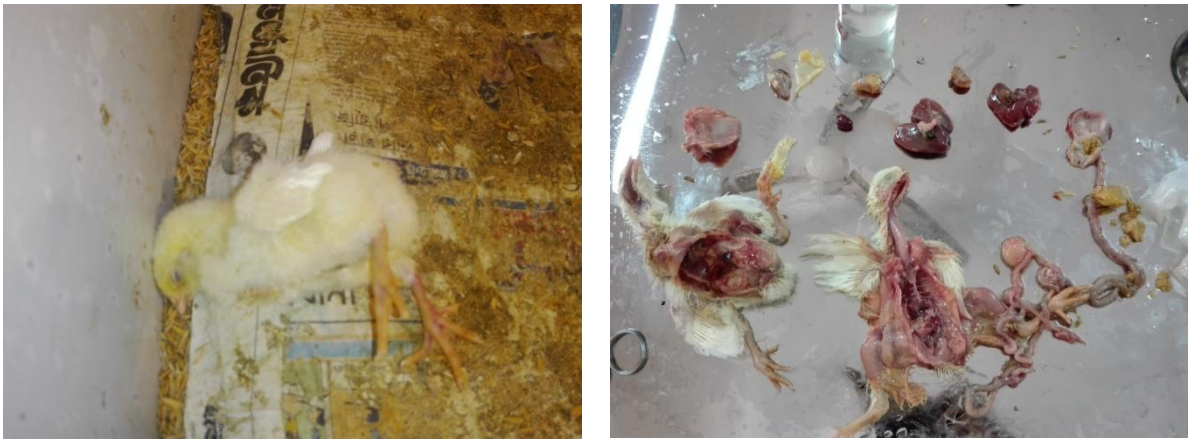

Figure 3.5: Postmortem examination of the broiler chick

3.9 Recorded parameters

Weekly lives weight, weekly feed consumption and death of chicks to calculate mortality percent. FCR was calculated from final live weight and total feed consumption per bird in each replication. After slaughter gizzard, liver, spleen, intestine, hear and bursa were measured from each broiler chicken.

Dressing yield was calculated for each replication to find out dressing percentage. Blood sample was analysis from each replication to measure, Complete blood count (CBC), sugar and cholesterol level. Feces sample was collected to measure microbial load in the gut.

3.10 Data collection

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

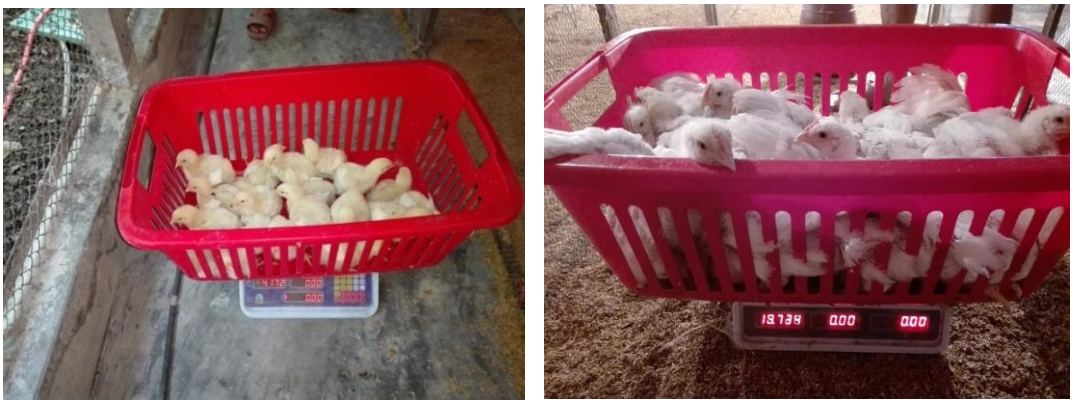

Figure 3.6: Live weight of broiler chicks had been measured by digital weight machine.

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

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

3.10. 4 Mortality of chicks: Daily death record for each replication was counted up to 28 days of age to calculate mortality.

3.10.5 Dressing procedures of broiler chicken: Three birds were picked up at random from each replicate at the 28th day of age and sacrificed to estimate dressing percent of broiler chicken. All birds to be slaughtered were weighed and fasted by halal method for fasting to facilitate proper bleeding. All the live birds were weighed again prior to slaughter.

3.11 Processing of broiler

The processing of broilers was done according to the procedure of *Krehbie and Gilliland (2003).* At the end of trial, the weight of the birds was taken and average body weight was calculated. At 35 days of age, two birds weighing average from each pen (replication) were randomly selected for determining carcess yield. To facilitate

slaughter, all birds from each treatment group were kept without feed for 12 hours period to killing, but water was supplied ad libitum. The birds were slaughtered and allowed to bleed for 2 minutes. After complete bleeding, birds were weighed individually. Then they are immersed in hot water (51°C to 55°C) for 2 minutes for proper de-feathering of carcass. The feathers were removed manually (by hand) and the birds were again individually weighed. Finally, processing was performed by removing head, shank, viscera, oil gland, kidney and giblets. As soon as these were removed, the gall bladder was removed from the liver and the pericardial sac and arteries were cut off from the heart. After removal of gizzard from the intestine, it was split open with knife and the fecal materials were removed. Then it was washed with clean water and lining was removed by hand.

3.12 Blood sample analysis

Blood samples (1 ml/bird) were collected into Ethylenediaminetetracetic acid (EDTA) tubes from the wing veins. Samples were transferred to the laboratory for analysis within 1 hour of collection. Sugar, Cholesterol and CBC was measured from Rainbow diagnosis Centre, Dhanmondi, Dhaka by maintaining standard protocol.

3.13 Record Keeping

Body weight of chicks was recorded initially and weekly replication wise for each individual or group of treatment. Feed intake was also recorded weekly replication wise for each treatment. Mortality was recorded daily of death occurred. During the whole experimental period, the temperature of the experimental house and pens were recorded four times a day at 6.00 AM, 12.00 PM, 6.00 PM, 12.00 AM with the help of an automatic digital thermometer. The relative humidity was also recorder four times a day by using a hygrometer. The different meat yield parameters like dressing weight, feather weight, liver weight, gizzard weight, heart weight, shank weight, breast meat weight, thigh weight, drumstick weight, wing weight and dark meat weight for individual bird were recorded after slaughtering.

3.14 Calculations

3.14.1 Live weight gain

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

Body weight gain = Final weight – Initial weight

3.14.2 Feed intake

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

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

3.14.3 Feed conversion ratio

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

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

3.14.4Statistical analysis

Data on performance were statistically analyzed by using analysis of variance (ANOVA) technique by a computer using SPSS (Statistical Package for the Social Sciences - version 20, Duncan method) program in accordance with the principles of Completely Randomized Design (CRD). The meat yield parameters were analyzed by using a 2 (sex) x 4 (diets) factorial experiment in a CRD. Least Significant Differences (LDS) were calculated to compare variation among treatments where ANOVA showed significant difference at 0.05 level of significance.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Performance of broiler

The performance in terms of live weight gain, feed intake and feed conversion of birds fed probiotics at different dietary levels is shown in table 4.2.

4.1.1 Body weight gain

Initial body weight of day-old broiler chicks fed on different dietary treatments was similar ($p>0.05$). From7 to 14 days of age and also from 07 to 28 days of age, the height body weight gain was attainted in birds that received the probiotics at the highest level (1 x 10^8CFU/ ml / Lwater). During 21 to 28 days of age,1 x 10^8CFU/ ml / LL of water group gained more weight than that of other treatment groups. From 07 to 21 days of age and also from 21 to 28 days of age, there was significant difference in weight gain of broilers among different dietary treatment ($p>0.05$). However, from 07 to 28 days of age, broiler chicks feed probiotics at 1 x 10^8CFU/ ml / Lof water group gained significantly more weight than other group consumed diet supplemented with probiotics at 1 x 10^8CFU/ ml / Lof water. There was significant improvement in treated groups compared to the control in the same period. The significant effect of probiotics on body weight gain was disagreement with the findings of some previous reports (*Ergun et. al. 2000; Ladukar et. al. 2001; Lima et. al. 2002; Priyankarage et. al. 2003*). But these findings were similar with the observation of *Jin at al., (2000); Bandy and Risam (2001); kalavathy et. al. (2003);* who found that supplementation of probiotics improved live weight gain of broilers. *Jin et. al. (1997*) explained that differences in the strains and forms of bacteria used and concentrations of viable cells could produce discrepancies in results. The effect of probiotics on body weight gain as obtained in this study might be due to some factors that affected the efficacy of probiotic such as composition of diet stress condition, Strain of microbes and concentration of microbes.

4.1.2 Feed intake

The average cumulative feed intake of broiler during the experimental period showed that except during the early period of rearing (7 to 14 days), probiotics supplemented groups tended to consume higher amounts of feed compared to control one in other stages of age (from 14 to 28 days). Among different dietary treatments, 1 x 10^8CFU/ ml / Lgroup had higher intake than that of other treatment groups from 14 to 28 days of age and also from 7 to 14 days of age. From 7 to 28 days of age the control group was consumed more feed than others group and the respectively consumption of feed are 2063.72 g, 1962.21 g, 1994.60 g and 1946.52 g. However, there was no significant difference ($p>0.05$) between the broilers fed on control diet and diets supplemented with probiotics at different levels. However, feed intake in probitics supplemented groups was in agreement with the results of some earlier studies (*Samanta and Biswas*1995; *Panda et. al.* 2002; *Ladukar et. al.* 2001). In those studies, feed intake of different broiler groups did no differ significantly due to addition of probiotics. However, similar to these observations, some workers have found that feed consumption differed significantly between the control and probiotics fed groups (*Mahajan et. al*. 1999; *Bandy and Risam*2001). *Mahajan et. al. (1999)* reported that the higher feed consumption in probiotics supplemented group might be due to an increase in digestive efficiency. *Mohan et. al.* (1996) also indicated that probiotic supplemented diets improved the feed intake irrespective of seasons. The higher amounts of feed consumption although no significant as found in the present study might be due to increased appetite and rate of enzymatic activity which enhances the digestive efficiency of the broilers. The Table 4.1 is presenting the chemical composition of broiler basal diet used during experimental feeding of broiler.

Table 4.1: Chemical composition of broiler basal diet used during experimental feeding of broiler.

Attributes	Broiler Starter (0-14 D)	Broiler Finisher (15-28 D)
Dry Matter (%)	*10.18*	*10.27*
Crude Protein (%)	*22.17*	*20.19*
Ether Extract (%)	*2.82*	*3.56*
Crude Fiber (%)	*4.92*	*4.79*
Total Ash (%)	*5.36*	*5.24*
Metabolic Energy (kcal/k)	*3025.40*	*3139.70*

4.1.3 Feed conversion ratio (FCR)

The feed conversion in different dietary treatment were very much close with each other in every stages of growth. At the end of the trail *i.e.* at 28 days of age, the feed conversion was better in treatment group T-4 (1 x 10^8CFU/ ml / Lprobiotic) was 1.237±2.11g followed by T-1, T-2 and T-3 were 1.372±1.21 g, 1.296±1.11 g and 1.300±0.11 g respectively. The data predating to the feed conversion ratio in different dietary treatments at different stages of age indicated that the addition of probiotic had significant effect on feed conversion ($p>0.05$) at any stage of treatment. The significant effect of probiotic on feed conversion was agreement with the observations of some researcher (*Mohan et. al. 1996; Yeo and Kim 1997; Lima et. al. 2002; Priyankarage et. al. 2003*). But this result was disagreed with *Ergun et. al., (2000)* reported that supplementation of probiotics with or without antibiotic in the rations had no significant effect on feed conversion of broilersin contrast, broiler feed, biospur (*Bandy and Risam*2001), Lacto-Sacc (*Mahajan et. al.* 1999). *Lactobacillus cultures* (*Zulkifli et. al.* 2000) and Pronifer or Biogen (*Mahajan et. al.* 1999) showed significant improvement in the food conversion when compare with control chicks. Probiotics supplemented groups consumed more food feed and could show a significant effect in body weight gain. It might be the reason for comparable feed conversion in the present study.

4.1.4 Survivability

Survivability of broiler fed on different dietary treatments was very much acceptable during the study period. The table 4.2 showssurvivability significant ($p>0.05$) effect among different treatment groups during the whole experiment period. Lower survivability of broiler fed diets supplemented with probiotics is available in the results of *Zulkifli et .al.* (2000). When broilers were given a dietary supplementation of probiotics (*Lactobacillus cultures*) and exposed to 35±1°C for 3 hours daily from day 14 to 28. But the results of present study were inconsistent with the findings of some earlier studies *(Samanta and Biswas 1995; Singh et. al. 1999; Hamid and Aijozuddin 2001).* In those studies, lower survivability in probiotics fed groups was found as compared to control ones. Since the result on survivability was quite acceptable in this study with little differences among the dietary groups, the beneficial effect could be detected over the control groups.

4.2 Growth Performance

The chemical composition of experimental rations is presented in table 4.1. The CP content of broiler starter and broiler finisher rations range between 17.93 to 22.18% and metabolism energy (ME) contents between 2978 to 3139 kcal/k. Day old chick and the weekly average live weight of broilers in different treatment groups are presented in table 4.2. It was observed from the results that the average live weights of broilers were increased gradually from beginning to 4^{th} week of age. Initial weight of chicks of different treatment was similar ($p>0.05$). The average body weight of the birds at the end of the 4^{th} week was higher ($p>0.05$) in all treatment than control. The morality was 2.50% in treatment T-1 group respectively presented at table 4.2. The data indicated that the percent of mortality was within the normal limit below the 5% limitation. Results obtained from experimental broiler chicks were shown in table 4.2. Results of the experimental showed that there were significant differences ($p>0.05$) in the body weight gain, feed conversion ratio (FCR) and final body weight. For feed intake is significant different among treatments but in the mortality rate there is no significant different among treatments ($p>0.05$). Results obtained in this study indicated that dietary inclusion of probiotics and antibiotic supported a superior performance of chicks and can be applied as antibiotics growth promoter substances in

broiler diet. However, many investigators reviewed the various benefits of feeding antibiotics growth promoters and reported that antibiotics may control and limit the growth and colonization of a variety of pathogenic and non-pathogenicspecies of bacteria in chicks' gut (*Bhuvnes et. al. 2002, Fakhoury et. al.2000*).

Table 4.2: Effects of probiotics on the performances of broilers

Variable	Name of Treatment				Level of significance
	T-1	**T-2**	**T-3**	**T-4**	
Initial body weight (g/broiler)	*37±06*	*37±07*	*37±05*	*37±03*	NS
Body weight (07 Days) (g/broiler)	*204±06*	*211±06*	*209±06*	*211±07*	NS
Body weight (28 Days) (g/broiler)	*1509.06±7.11*	*1513.67±9.14*	*1533.50±9.25*	*1572.37±8.14*	NS
Average daily weight gain (g/day)	*52.6±01*	*52.8±03*	*53.3±02*	*54.8±03*	NS
Feed intake (g/broiler)	*2064±1.11*	*1962±1.41*	*1995±1.26*	*1947±1.35*	*
Feed conversion ratio	*1.372±1.21*	*1.296±1.11*	*1.300±0.11*	*1.237±2.11*	NS
Mortality (%)	*2.50*	*00*	*00*	*00*	NS

Here, T-1= (Control: No Antibiotic & Probiotics), **T-2**= (Antibiotic: 14 mg Oxytetracycline®/ L water), **T-3**= Only Bacterial Strain (*Lactobacillus spp)* Probiotics: 1 x 10^8CFU/ ml / L water), **T-4**=Bacterial (*Lactobacillus spp)* & Yeast Strain (*Saccharomyces spp*) Probiotics: 1 x 10^8CFU/ ml / L water).

Data is presented as mean ± standard deviation. ^{ab}Means bearing different superscripts in a column differ significantly ($p<0.05$). * means significant at ($p<0.05$)& NS means Non-significant at ($p>0.05$).

4.3 Dressing percentage

Two birds from each replication under each treatment were randomly selected and slaughtered during the end of 4th week. The slaughtered birds were de-feathered, decapitated, eviscerated and two legs were removed beneath the hock joint, to observe the effect of various experimental diets on the dressing percentage, which was

calculated as the per cent of the carcass weight obtained after removing the feathers, neck, legs and internal viscera to its live body weight.

4.3.1 Meat to bone ratio of thigh portion

Thigh portion of the carcass from the carcass of 72 birds was separated weighed and preserved under frozen conditions. Later, the thigh portions were thawed and the bone and muscle were separated manually from each other and their individual weights were recorded to arrive at meat: bone ratio as:

$$\text{Meat: Bone} = \frac{\text{weight of the meat (g)}}{\text{Weight of bone (g)}}$$

4.3.2 Relative organ weight

Giblet weight

From the birds sacrificed on 28th day the heart, liver, Gizzard were carefully collected to know the effect of different dietary treatments on their weights which are briefed here under.

a) Heart: The average weight of the heart without pericardium from each replicate was recorded and expressed as per cent of average live body weight.

b) Liver: The average weight of the liver without gallbladder from each replicate was recorded and expressed as per cent of average body weight.

c) Gizzard: The average weight of the gizzard without the food contents and internal lining membrane from each replicate was recorded and expressed as percent of average live body weight.

4.3.3 Abdominal fat weight

The weight of the fat present in abdomen including fat surrounding gizzard, bursa, cloacae and adjacent muscles of each bird was recovered and expressed as per cent of live weight of the birds. The effects of diet, sex and interaction of diet and sex on different meat yield parameters are different. The table 4.3 indicates that there was no significant difference ($p>0.05$) in the presented weight if different organs and components of broilers except body weight and dressing % due to addition of probiotics in the diet of broiler. The differences in the presented abdominal fat of broiler fed diet supplemented with probiotics varied significantly ($p<0.05$) when compared with the control broiler chicks.

Table4.3: Weight of different body parts of Broiler

Parameters	Live weight (g)	Dressing Percentage (%)	Blood Weight (g)	Feather weight (g)	Drum stick Weight (g)	Shank weight (g)	Viscera weight (g)	Giblet weight (g)	Head weight (g)	Abdominal Fat weight (g)	Skin Weight (g)	Gizzard Weight (g)	Liver Weight (g)	Heart Weight (g)
T-1	1980[b]	84.34[b]	43[b]	274[b]	83.49[b]	63.86[b]	114.60[b]	135.90[b]	39.83[b]	28.72[b]	127.83[a]	43.80[b]	43.53[b]	9.30[b]
T-2	1950[b]	85.12[b]	32[b]	324[a]	88.44[b]	63.46[b]	119.84[b]	144.23[b]	40.25[b]	31.36[a]	125.48[b]	45.06[b]	39.72[b]	10.39[b]
T-3	2090[a]	85.16[a]	52[a]	248[b]	89.57[a]	75.52[a]	132.51[a]	158.02[b]	50.97[a]	30.15[b]	124.73[b]	53.32[a]	47.52[a]	11.90[a]
T-4	1980[b]	80.80[b]	39[b]	321[b]	86.40[b]	66.75[b]	126.22[b]	168.30[a]	24.79[b]	25.31[b]	102.20[b]	50.51[b]	42.12[b]	9.89[b]
Level of Significant	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS

Here,T-1= (Control: No Antibiotic & Probiotics), **T-2**= (Antibiotic: 14 mg Oxytetracycline®/ L water), **T-3**= Only Bacterial Strain (*Lactobacillus spp)* Probiotics: 1 x 10^8CFU/ ml / L water), **T-4**=Bacterial (*Lactobacillus spp)* & Yeast Strain (*Saccharomyces spp*) Probiotics: 1 x 10^8CFU/ ml / L water).

Data is presented as mean ± standard deviation. [ab]Means bearing different superscripts in a column differ significantly ($p<0.05$).

* means significant at ($p<0.05$)& NS means Non-significant at ($p>0.05$).

There was no significant influence of sex and interaction of sex with one percent weight of different organs of broilers. The observation of the present study with regard to meat yield was consistent with the findings of *Panda et. al. (2000); Ergun et. al. (2000); Kalavathy et. al. (2003)* who found o significant difference in the weights of organs between control and probiotic feed of broilers. But this result is inconsistent with the reports of *Bandy and Risamet.al. (2001).* They claimed that there was a significant improvement in the dressing, eviscerated and edible meat yields due to addition of probiotics. Significant reduction in the abdominal fat compared to control one agreed with well the results of some previous workers (*Chah et. al. 1975; Santoso et. al. 1995; Kalavathy et. al. 2003).* They found that diet supplemented with probiotics reduced abdominal fat significantly in broilers. But this finding consists with the observation of *Panda et.al. (2002*) who found no significant effect of probiotics on abdominal fat of broilers.

4.4 Composition of Leg and Breast Meat

The mean value for carcass and proximate composition (Moisture %, Protein %, Fat % and Ash %) of leg and breast meat (Table 4.5) exhibited different results. Protein % and Ash % were increased ($p<0.05$) in probiotic fed chickens together where antibiotic than control. Whereas the fat % and breast meat was lower ($p<0.05$) in probiotic and antibiotic fed chickens. This indicates a better retention of minerals especially Calcium, phosphorus, nitrogen and improved protein efficiency ratio (*Bhuvnes et. al. 2002, Fakhoury et. al. 2000*) in probiotic fed birds as compared to control birds Table 4.5.Effect of probiotic on proximal composition of leg and breast meat of broiler. Meat of chickens given probiotics (*Lactobacillus acidophilus and streptococcus faecium* bacteria) on the whole rearing period had significantly higher protein content. While crude fat and total cholesterol contents tended to decrease *(Sangakkara et. al.2002).* Addition of probiotic included species of *Bacillus, Lactobacillus, Streptococcus, Clostridium, Saccharomyces* and *Candida* to broiler diets, decreased cholesterol concentration in thigh meat and increased linolenic acid and unsaturated fatty acid / saturated fatty acid ratio in pectoral and thigh meat *(Dunne and Murphy 2001).*

Table 4.4: Effect of Probiotic on Proximal Composition of Leg and Breast Meat

Attributes		T1	T2	T3	T4	Level of Significance
Moisture%	**Breast**	*72.89±1.22^{b}*	*72.19±1.11^{b}*	*75.00±1.32^{a}*	*73.19±1.19^{b}*	NS
	Leg	*74.08±1.31^{b}*	*70.04±1.32^{b}*	*74.41±1.29^{b}*	*74.43±1.22^{a}*	NS
Dry matter%	**Breast**	*27.11±1.11^{b}*	*27.81±0.99^{a}*	*25.00±1.9^{b}*	*26.81±.74^{b}*	NS
	Leg	*26.92±1.50^{b}*	*29.96±0.98^{a}*	*25.59±1.6^{b}*	*25.57±1.11^{b}*	*
Crude Protein%	**Breast**	*88.79±1.26^{b}*	*90.84±1.23^{a}*	*89.47±1.12^{b}*	*89.36±1.22^{b}*	NS
	Leg	*90.07±1.32^{a}*	*89.66±1.19^{b}*	*86.47±1.26^{b}*	*84.78±1.25^{b}*	NS
Ash%	**Breast**	*4.46±0.65^{b}*	*4.90±0.65^{a}*	*4.44±0.33^{b}*	*4.73±0.37^{b}*	NS
	Leg	*4.79±0.53^{a}*	*4.03±0.56^{b}*	*4.53±0.47^{b}*	*3.99±0.62^{b}*	NS
Acid insoluble ash	**Breast**	*Nil*	*Nil*	*Nil*	*Nil*	NS
	Leg	*Nil*	*Nil*	*Nil*	*Nil*	NS
Crude fiber	**Breast**	*BDL*	*BDL*	*BDL*	*BDL*	NS
	Leg	*BDL*	*BDL*	*BDL*	*BDL*	NS
Crude fat	**Breast**	*4.27±0.57^{b}*	*4.60±0.65^{a}*	*4.36±0.46^{b}*	*4.18±0.32^{b}*	NS
	Leg	*4.90±0.48^{a}*	*4.88±0.25^{a}*	*5.10±0.36^{a}*	*4.95±0.84^{a}*	NS

Here,T-1= (Control: No Antibiotic & Probiotics), **T-2**= (Antibiotic: 14 mg Oxytetracycline®/ L water), **T-3**= Only Bacterial Strain (*Lactobacillus spp)* Probiotics: 1 x 10^{8}CFU/ ml / L water), **T-4**=Bacterial (*Lactobacillus spp)* & Yeast Strain (*Saccharomyces spp*) Probiotics: 1 x 10^{8}CFU/ ml / L water).

Data is presented as mean ± standard deviation. ^{ab}Means bearing different superscripts in a column differ significantly ($p<0.05$). * means significant at ($p<0.05$)& NS means Non-significant at ($p>0.05$).

4.5 Hematological parameters

A summary of hematological data is presented in table 4.6. Overall probiotics supplementation had no significant effect on any of the hematologic traits measured ($p<0.05$). According to *(Samanta and Biswas1995; Panda et. al. 2000; Ladukar et. al. 2001)* the addition of probiotics did not affect RBC, WBC, hemoglobin, haematocrit and platelet, total protein and total closterol concentrations significantly. However, values obtained from the hematological analysis were within the normal physiological

ranges and this is the conformity to blood and student data for gilts and layers. *Ladukar et. al. (2001)* reported that cholesterol level of serum significantly decreased in groups supplemented with probiotics in assimilation of cholesterol by Lactobacillus compared to control group fed with basal diet. The same study also reported that there is a significant decrease in the serum level of triglycerides between control group and groups treated with probiotics, probiotics with yeast supplement in broiler diet in combination with water or alone.

Name of Treatment	**RBC ($x10^6/mm^3$)**	**WBC ($x10^3/mm^3$)**	**HCT (%)**	**Hb (g/dl)**	**BGL (mmol/L)**	**BCL (mg/dl)**
T-1	2.4^b	138.11^b	30.13^b	9.62^b	15.22^b	140.64^b
T-2	2.90^b	139.08^a	30.69^b	10.67^a	14.98^b	138.30^b
T-3	3.58^a	136.91^b	30.82^a	10.34^b	14.98^b	141.29^a
T-4	2.86^b	138.21^b	30.23^b	10.67^a	15.27^a	140.50^b
Level of Significance	*	NS	NS	NS	NS	NS

Table 4.5: Blood Profile of Broiler

Here,T-1= (Control: No Antibiotic & Probiotics), **T-2**= (Antibiotic: 14 mg Oxytetracycline®/ L water), **T-3**= Only Bacterial Strain (*Lactobacillus spp)* Probiotics: 1 x 10^8CFU/ ml / L water), **T-4**=Bacterial (*Lactobacillus spp)* & Yeast Strain (*Saccharomyces spp*) Probiotics: 1 x 10^8CFU/ ml / L water).

Data is presented as mean ± standard deviation. ^{ab}Means bearing different superscripts in a column differ significantly ($p<0.05$). * means significant at ($p<0.05$)& NS means Non-significant at ($p>0.05$).

4.6 Immunological response

The antibody titers against NDV in the birds fed the experimental diets are presented in Table 4.7.The blood samples were collected randomly from two birds from each replicate group during the end of 4th week. Serum was separated individually and all the two replicates in each treatment were pooled and representative samples were subjected to antibody titer for ND and IBD microbiology lab, CDIL, Dhaka. The levels of NDV titer were significantly higher ($p<0.05$) in all the treated groups as

compared with that of control. The result agreed with *Kim and Lee et. al. (2004)* who previously reported that probiotics could modulate the systemic antibody response to antigens in broiler.

Table 4.6: Immunological response against newcastle disease of broilers

Group	Antibody titer (log_{10}) Antibody against ND at Day 32
T1	*00*
T2	6.00 ± 0.10^{a}
T3	2.00 ± 0.10^{b}
T4	3.00 ± 0.10^{b}
Level of Significance	**

Here,T-1= (Control: No Antibiotic & Probiotics), **T-2**= (Antibiotic: 14 mg Oxytetracycline®/ L water), **T-3**= Only Bacterial Strain (*Lactobacillus spp)* Probiotics: 1 x 10^{8}CFU/ ml / L water), **T-4**=Bacterial (*Lactobacillus spp)* & Yeast Strain (*Saccharomyces spp*) Probiotics: 1 x 10^{8}CFU/ ml / L water).

Data is presented as mean ± standard deviation. ^{ab}Means bearing different superscripts in a column differ significantly (P<0.05). * means significant at (P<0.05) & NS means Non-significant at (P>0.05).

4.7 Microbial Load

The results of microbial load were presented in table 4.8. The concentration of lactic acid bacteria in intestinal contents was significantly increased (p<0.05) in the groups fed diets containing antibiotic and probiotics than of control. Whereas the levels of total microbes and *E. coli* from bacteria were not changed by the dietary antibiotic and probiotics. Feeding probiotics resulted in a beneficial modulation of gut micro-flora as evidenced by the numerous increases in the concentration of lactic acid bacteria. These results concur with those of *Moeser and Kempen (2001)* who found hat broilers fed multispecies probiotics had higher number of *lactrobacillus spp* in cecalmicro flora. These results are in partial agreement with those of *Patterson and Burkholder 2003* who did not observe differences in *Lactobacilli*, *Enterococci* and total anaetobecountes but did observe lower *E. coli* counts in the caccum of broilers fed a probiotic supplemented diet compared with the control. This findings also supported

by *Patterson and Burkholder 2003* who observed that concentrations of cecal lactic acid bacteria in all groups fed BA-pro were significantly increased ($p<0.05$) compare to the control.

Table 4.7: Gut microbial load (log_{10}cfu/g) of broilers

Name of Treatment	Total bacterial count	Coliforms	Lactic acid bacteria
T-1	*5.43±0.11*^{b}	*3.83±0.16*^{a}	*4.11±0.01*^{b}
T-2	*5.12±0.16*^{b}	*3.81±0.09*^{b}	*4.02±0.11*^{b}
T-3	*5.00±0.19*^{b}	*3.51±0.11*^{b}	*5.09±0.16*^{a}
T-4	*4.93±0.21*^{a}	*3.59±0.13*^{b}	*4.99±0.10*^{b}
Level of Significance	**NS**	**NS**	*****

Here, T-1= (Control: No Antibiotic & Probiotics), **T-2**= (Antibiotic: 14 mg Oxytetracycline®/ L water), **T-3**= Only Bacterial Strain (*Lactobacillus spp)* Probiotics: 1 x 10^{8}CFU/ ml / L water), **T-4**=Bacterial (*Lactobacillus spp)* & Yeast Strain (*Saccharomyces spp*) Probiotics: 1 x 10^{8}CFU/ ml / L water).

Data is presented as mean ± standard deviation. ^{ab}Means bearing different superscripts in a column differ significantly ($P<0.05$). * means significant at ($P<0.05$) & NS means Non-significant at ($P>0.05$).

CHAPTER 5

SUMMARY AND CONCLUSION

A total of 320 day-old Cobb-500 broiler chicks were reared in Sher-e-Bangla Agricultural University Poultry Farm, Dhaka. All the Chicks were divided randomly into 4treatment groups and each treatment group was divided 4 replications (20 chicks with each replication group). Treatment Group 1 was designated as control group was given only normal broiler ration, no antibiotic, probiotics and probiotics with yeast was not given to the control group. The treatment group 2 from the rest of the groups was fed antibiotics, [Treatment Group 2 (antibiotics @ 14 mg Oxytetracycline®/ L of water), Treatment Group 3 (probiotics included only bacteria 1 x 10^8CFU/ ml / Lwater), and Treatment Group 4 (probiotics included bacteria with yeast 1 x 10^8 CFU / ml / L of water)] with drinking water respectively from 7^{th} to 28^{th} day of study.

The effects of supplementation of probiotics and antibiotic were measured. The performance traits *viz.* body weight, weight gain, feed consumption, FCR, dressed bird weight, relative giblet weight, survivability and meat yield of broiler on different replication of the treatments was recorded and compared in each group. At 28 days of age, 20 broilers were dissected to compare meat yield characteristics among different treatment groups. The group T-4 showed higher body weight compared to any other groups and group T-3 group T-2 and group T-1 followed in ascending order. The weight gain, feed consumption, and FCR followed similar trends with an exception that the difference is not significant among group T-1, group T-2 and group T-3 and similar result also found in group T-4. The FCR was better in the probiotics groups compared to the control group but significant ($p<0.05$) difference with the T-4 and T-2 groups. The relative giblet weight did not show any difference either between any of the treatment groups or the control. The serum biochemistry parameters *viz* sugar and total cholesterol was studied to evaluate the functional status body. The sugar and cholesterol level of different treatments were similar in all treatments compared to control one. The results indicated no alterations in biochemical parameters, except that a lower amount was observed in cholesterol levels in probioticssupplemented groups. Concerning the treatment effect on blood constituents, the results indicated no significant differences due to supplementation of probiotics, except, RBC,

Lymphocyte and MCHC which were significantly affected ($p<0.05$) birds fed diets supplemented with probiotics through the supply water had higher values of RBCs, lymphocyte and MCHC but in case of antibiotic and control group this trends are lower than probiotic provided groups. The numbers of intestinal micro-flora (*E coli* and *Salmonella spp*) were significantly higher in control group compared to other groups. Moreover, addition of probiotics to broiler chicks water showed significant ($p<0.05$) difference in bacterial colony count among the groups. Analyzing the above research findings the production performance, hematological parameter, weight of lymphatic organ and microbial load in feces sample was very effective. So, probiotics should be used as an alternative of antibiotics on broiler ration from the present study, it was appeared that the feeding of probiotics enhance body weight. Body weight increased significantly ($p<0.05$) in the treated group in the comparison with that of control groups in the all sampling days. Higher body weight was found in the provided group. It is suggested that the supplementation of probiotics in broiler production may be beneficiary for improve of broiler performance as well as the indirectly farmers will be benefited. In the all limitation, the scope of fresh meat availability will be satisfactory level if the government takes the step about probiotics.

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APPENDICES

Appendix 1: Recommended level of nutrients for broiler Components

Ingredients name	Starter	Grower
ME (kcal/g)	*3000*	*3100*
% CP	*22*	*20*
% Ca	*1.0*	*0.85*
% P (Available)	*0.5*	*0.4*
% Lysine	*1.2*	*1.0*
% Met.hionine	*0.5*	*0.45*
% Tryptophane	*0.21*	*0.18*

Source: Cobb500 Broiler Management Guide, (2016).

Appendix 2: Nutrient composition of the ingredients used to formulate experimental diets Ingredients

	DM (%)	ME (K. Cal/g)	CP (%)	CF (%)	Ca (%)	P (%)	Lys (%)	Meth (%)	Tryp (%)
Soybean meal	*90*	*2710*	*44.5*	*7.5*	*0.26*	*0.23*	*2.57*	*0.76*	*0.57*
Maize	*89.5*	*3309*	*9.2*	*2.4*	*0.25*	*0.40*	*0.18*	*0.15*	*0.09*
DCP	*22*	*17.21*							
Soybean oil	*100*	*8800*							
Protein concentrate (Jeso-prot)	*91.64*	*2860*	*63.3*	*8.1*	*6.37*	*3.24*	*3.87*	*1.78*	*0.53*
Meat and Bone meal	*95.5*	*1044*	*14.6*	*2.5*	*7.0*	*12.11*	*.66*	*0.24*	*0.12*

Source: Cobb500 Broiler Management Guide, (2016).

Appendix 3: Layout showing the distribution of experimental birds

Dietary Treatment	Age of the Birds (Days)	R-1	R-2	R-3	R-4
T-1	*07*	*20*	*20*	*20*	*20*
T-2	*07*	*20*	*20*	*20*	*20*
T-3	*07*	*20*	*20*	*20*	*20*
T-4	*07*	*20*	*20*	*20*	*20*

Source: Cobb500 Broiler Management Guide, (2016).

Appendix 4: Recorded temperature (°C) during experiment

		Room temperature (◦C)						
Age in Weeks	**Period**	**8 A.M**	**12A.M**	**4 P.M.**	**8 P.M.**	**12 P.M.**	**4 A.M**	**Average**
1st	22.10.18-27.10.18	*28.3*	*28.5*	*32.1*	*31.6*	*30.2*	*28.5*	*29.87*
2nd	28.10.18-03.11.18	*27.0*	*27.2*	*28.8*	*27.2*	*26.0*	*25.8*	*27.00*
3rd	04.11.18-10.11.18	*26.8*	*27.0*	*28.6*	*28.5*	*27.4*	*27.2*	*27.58*
4th	11.11.18-17.11.18	*25.9*	*26.2*	*27.5*	*27.0*	*26.5*	*26.4*	*26.58*

Source: Cobb500 Broiler Management Guide, (2016).

Appendix 5: Relative humidity (%) during experiment

		Relative humidity (%)						
Age in weeks	**Period**	**8 A.M**	**12A.M**	**4 P.M.**	**8 P.M**	**12 P.M**	**4 A.M**	**Average**
1st	22.10.18-27.10.18	*85*	*82*	*73*	*74*	*78*	*80*	*78.67*
2nd	28.10.18-03.11.18	*85*	*83*	*71*	*72*	*77*	*79*	*77.83*
3rd	04.11.18-10.11.18	*86*	*85*	*74*	*75*	*81*	*83*	*80.67*
4th	11.11.18-17.11.18	*87*	*86*	*83*	*77*	*84*	*86*	*83.83*

Source: Cobb500 Broiler Management Guide, (2016).

Appendix 6: Vaccination schedule of experimental birds

Name of the vaccine	**Age of the Birds (Days)**	**Dose and route of administration of diluted vaccine**
BCRDV	*D O C*	*1 drop in Eye*
Gumboro vaccine	*10^{th} day*	*1 drop in Eye*
Gumboro vaccine	*17^{th} day*	*1 drop in Eye*

Source: Cobb500 Broiler Management Guide, (2016).

Appendix 7: **Feed consumption (g/bird) of 1st, 2nd, 3rd and 4th week under different treatments**

Name of Treatment	1st Week Feed Consumption/ Bird (g)	2nd Week Feed Consumption/ Bird (g)	3rd Week Feed Consumption/ Bird (g)	4th Week Feed Consumption/ Bird (g)
T1R1	*169.3*	*282.5*	*650.3*	*895.3*
T1R2	*166.5*	*293.2*	*670.4*	*910.2*
T1R3	*170.3*	*298.1*	*658.2*	*909.4*
T1R4	*163.2*	*285.3*	*596.6*	*926.3*
T2R1	*159.3*	*272.5*	*598.2*	*975.6*
T2R2	*156.5*	*293.2*	*662.3*	*962.2*
T2R3	*170.3*	*287.1*	*648.1*	*969.3*
T2R4	*173.2*	*285.3*	*708.3*	*971.3*
T3R1	*179.3*	*292.5*	*676.6*	*939.4*
T3R2	*166.5*	*273.2*	*658.2*	*920.3*
T3R3	*170.3*	*282.5*	*662.3*	*955.6*
T3R4	*163.2*	*288.2*	*698.1*	*942.2*
T4R1	*180.3*	*357.1*	*713.3*	*903.4*
T4R2	*171.2*	*285.3*	*672.6*	*961.3*
T4R3	*170.3*	*292.5*	*651.2*	*938.4*
T4R4	*163.5*	*303.2*	*669.3*	*972.3*

Source: Cobb500 Broiler Management Guide, (2016).

Appendix8: Body weight (g/bird) of 1st, 2nd, 3rd and 4th week under different treatments

Name of Treatment	1st Week Body Weight /Bird(g)	2nd Week Body Weight /Bird(g)	3rd Week Body Weight /Bird(g)	4th Week Body Weight /Bird(g)
T1R1	202.3	286.3	483.3	539.3
T1R2	199.2	282.1	471.3	557.3
T1R3	206.1	314.0	488.6	531.5
T1R4	208.1	299.5	459.3	536.2
T2R1	210.6	280.3	483.2	521.3
T2R2	207.3	304.2	501.3	513.5
T2R3	213.3	282.3	500.2	534.1
T2R4	213.2	297.3	468.6	525.3
T3R1	202.1	286.3	467.3	547.3
T3R2	218.3	292.1	453.2	521.5
T3R3	207.3	304.0	451.3	536.2
T3R4	201.2	309.5	488.6	541.3
T4R1	212.2	299.3	449.3	563.5
T4R2	209.3	304.2	432.0	504.1
T4R3	208.3	292.3	431.3	545.3
T4R4	213.5	293.3	421.6	533.3

Source: Cobb500 Broiler Management Guide, (2016).

Appendix 9:Weight of different body parts of Broiler.

Parameters	Live weight (g)	Dressing Percentage (%)	Blood Weight (g)	Feather weight (g)	Drum stick Weight (g)	Shank weight (g)	Viscera weight (g)	Giblet weight (g)	Head weight (g)	Abdominal Fat weight (g)	Skin Weight (g)	Gizzard Weight (g)	Liver Weight (g)	Heart Weight (g)
T1R1	1980	84.34	43	274	83.49	63.86	114.60	135.90	39.83	28.72	127.83	43.80	43.53	9.30
T1R2	1935	84.90	42	273	83.00	63.02	114.30	135.60	40.60	28.20	127.90	43.01	43.80	8.53
T1R3	1992	83.65	41	269	82.10	64.10	112.90	136.00	38.99	28.99	126.90	43.26	43.01	9.11
T1R4	2010	84.00	39	272	83.60	63.90	114.60	135.99	38.99	28.65	128.01	41.99	43.26	10.01
T2R1	1950	85.12	32	324	88.44	63.46	119.84	144.23	40.25	31.36	125.48	45.06	39.72	10.39
T2R2	1995	84.90	33	269	83.00	63.02	119.01	152.01	40.60	33.10	127.90	43.01	43.80	8.53
T2R3	1980	83.65	39	272	82.10	64.10	117.30	136.00	38.99	28.20	126.90	43.26	43.01	9.11
T2R4	1910	84.00	34	350	83.60	63.91	121.30	135.99	38.99	28.99	125.69	41.99	36.49	10.01

Source: Cobb500 Broiler Management Guide, (2016).

Appendix 9 (Cont'd)

Parameters	Live weight (g)	Dressing Percentage (%)	Blood Weight (g)	Feather weight (g)	Drum stick Weight (g)	Shank weight (g)	Viscera weight (g)	Giblet weight (g)	Head weight (g)	Abdominal Fat weight (g)	Skin Weight (g)	Gizzard Weight (g)	Liver Weight (g)	Heart Weight (g)
T3R1	2090	85.16	52	248	89.57	85.52	132.51	158.02	50.97	30.15	124.73	53.32	47.52	11.90
T3R2	2010	84.90	53	269	83.00	89.57	138.96	135.99	48.94	33.10	127.90	59.32	43.26	8.53
T3R3	1950	83.65	44	272	82.10	83.04	126.46	144.23	52.64	28.20	126.90	49.25	53.72	9.11
T3R4	1995	84.00	59	226	95.64	82.10	130.32	152.01	50.34	28.99	125.69	42.34	43.80	10.01
T4R1	1980	80.80	39	321	86.40	66.75	126.22	168.30	44.79	25.31	102.20	50.51	42.12	9.89
T4R2	1992	79.90	40	320	83.10	70.64	129.01	164.23	50.97	28.20	114.73	52.32	43.26	8.53
T4R3	2010	83.65	36	326	82.10	70.35	127.30	152.01	48.94	28.99	117.90	49.25	53.72	9.11
T4R4	1950	81.00	44	312	94.64	61.42	121.30	176.00	52.64	22.94	106.90	55.34	43.80	10.01

Source: Cobb500 Broiler Management Guide, (2016).

Appendix 10: **Effect of probiotic on proximal composition of leg and breast meat of Broiler.**

Attributes		T1R1	T1R2	T1R3	T1R4	T2R1	T2R2	T2R3	T2R4
Moisture %	**Breast**	*72.82*	*72.88*	*72.23*	*72.47*	*72.94*	*72.77*	*72.12*	*72.09*
	Leg	*74.36*	*74.33*	*74.03*	*74.23*	*70.40*	*70.10*	*70.70*	*70.63*
Dry matter%	**Breast**	*27.02*	*27.06*	*27.84*	*27.74*	*27.26*	*27.42*	*27.11*	*27.12*
	Leg	*26.39*	*26.92*	*26.94*	*26.77*	*29.94*	*29.44*	*29.33*	*29.00*
Crude Protein %	**Breast**	*88.79*	*88.82*	*88.26*	*88.99*	*90.67*	*90.56*	*90.95*	*90.84*
	Leg	*90.67*	*90.62*	*90.00*	*89.62*	*89.00*	*89.66*	*89.63*	*89.22*
Ash%	**Breast**	*4.46*	*4.33*	*4.44*	*4.23*	*4.90*	*4.36*	*4.06*	*4.82*
	Leg	*4.79*	*4.66*	*4.23*	*4.35*	*4.90*	*4.36*	*4.62*	*4.36*
Acid insoluble ash	**Breast**	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
	Leg	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
Crude fiber	**Breast**	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*
	Leg	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*
Crude fat	**Breast**	*4.22*	*4.23*	*4.27*	*4.15*	*4.60*	*4.65*	*4.65*	*4.71*
	Leg	*4.90*	*4.78*	*4.99*	*4.96*	*4.78*	*4.99*	*4.96*	*4.78*

Source: Cobb500 Broiler Management Guide, (2016).

Appendix 11: **Effect of probiotic on proximal composition of leg and breast meat of Broiler.**

Name of Treatment		T3R1	T3R2	T3R3	T3R4	T4R1	T4R2	T4R3	T4R4
Moisture %	**Breast**	*75.23*	*75.63*	*75.12*	*75.00*	*73.62*	*75.60*	*72.33*	*73.00*
	Leg	*74.14*	*74.36*	*74.65*	*74.33*	*75.01*	*75.36*	*75.24*	*77.23*
Dry matter%	**Breast**	*25.32*	*25.12*	*25.00*	*25.02*	*26.81*	*26.34*	*26.12*	*26.34*
	Leg	*25.64*	*25.31*	*25.12*	*25.66*	*25.32*	*25.12*	*25.00*	*25.02*
Crude Protein%	**Breast**	*89.64*	*89.33*	*89.21*	*89.30*	*89.33*	*89.21*	*89.30*	*89.33*
	Leg	*86.10*	*84.99*	*86.33*	*85.99*	*84.01*	*84.36*	*84.10*	*83.99*
Ash%	**Breast**	*4.79*	*4.66*	*4.23*	*4.35*	*4.90*	*4.36*	*4.62*	*4.36*
	Leg	*4.46*	*4.33*	*4.44*	*4.23*	*4.90*	*4.36*	*4.06*	*4.82*
Acid insoluble ash	**Breast**	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
	Leg	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
Crude fiber	**Breast**	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*
	Leg	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*
Crude fat	**Breast**	*4..36*	*4.23*	*4.69*	*4.21*	*4.18*	*4.23*	*4.15*	*4.23*
	Leg	*5.00*	*5.05*	*5.20*	*5.22*	*4.93*	*4.95*	*4.99*	*4.88*

Source: Cobb500 Broiler Management Guide, (2016).

Appendix 12: Blood Profile of Broiler

Name of Treatment	RBC ($x10^6/mm^3$)	WBC ($x10^3/mm^3$)	HCT (%)	Hb (g/dl)	BGL (mmol/L)	BCL (mg/dl)
T1R1	*2.41*	*138.12*	*30.69*	*9.26*	*15.78*	*140.45*
T1R2	*2.62*	*138.18*	*30.66*	*9.24*	*15.64*	*140.12*
T1R3	*2.34*	*138.16*	*30.25*	*9.35*	*15.84*	*140.45*
T1R4	*2.39*	*138.09*	*30.34*	*9.64*	*15.04*	*140.10*
T2R1	*2.80*	*139.00*	*30.84*	*10.67*	*14.54*	*139.21*
T2R2	*2.92*	*139.09*	*30.24*	*10.55*	*14.64*	*138.00*
T2R3	*2.88*	*139.07*	*30.94*	*10.22*	*14.64*	*138.22*
T2R4	*2.91*	*139.05*	*30.36*	*10.46*	*14.87*	*138.21*
T3R1	*3.15*	*136.99*	*30.45*	*10.78*	*14.21*	*139.99*
T3R2	*3.71*	*136.95*	*30.24*	*10.49*	*14.01*	*141.01*
T3R3	*3.66*	*136.88*	*30.35*	*10.78*	*14.34*	*142.00*
T3R4	*3.55*	*136.71*	*30.24*	*10.91*	*15.74*	*140.95*
T4R1	*2.86*	*138.21*	*30.75*	*10.24*	*15.64*	*140.94*
T4R2	*2.91*	*138.20*	*30.94*	*10.34*	*15.85*	*140.45*
T4R3	*2.80*	*138.18*	*30.45*	*10.46*	*15.04*	*140.75*
T4R4	*2.70*	*138.11*	*30.54*	*10.35*	*15.78*	*140.24*

Source: Cobb500 Broiler Management Guide, (2016).

Appendix 13: Immunological response against Newcastle Disease of broilers

Name of Treatment	Antibody titer (log_{10}) Antibody against ND at Day 32
T1R1	*0*
T1R2	*0*
T1R3	*0*
T1R4	*0*
T2R1	*6*
T2R2	*7*
T2R3	*5*
T2R4	*6*
T3R1	*2*
T3R2	*0*
T3R3	*3*
T3R4	*3*
T4R1	*3*
T4R2	*3*
T4R3	*4*
T4R4	*2*

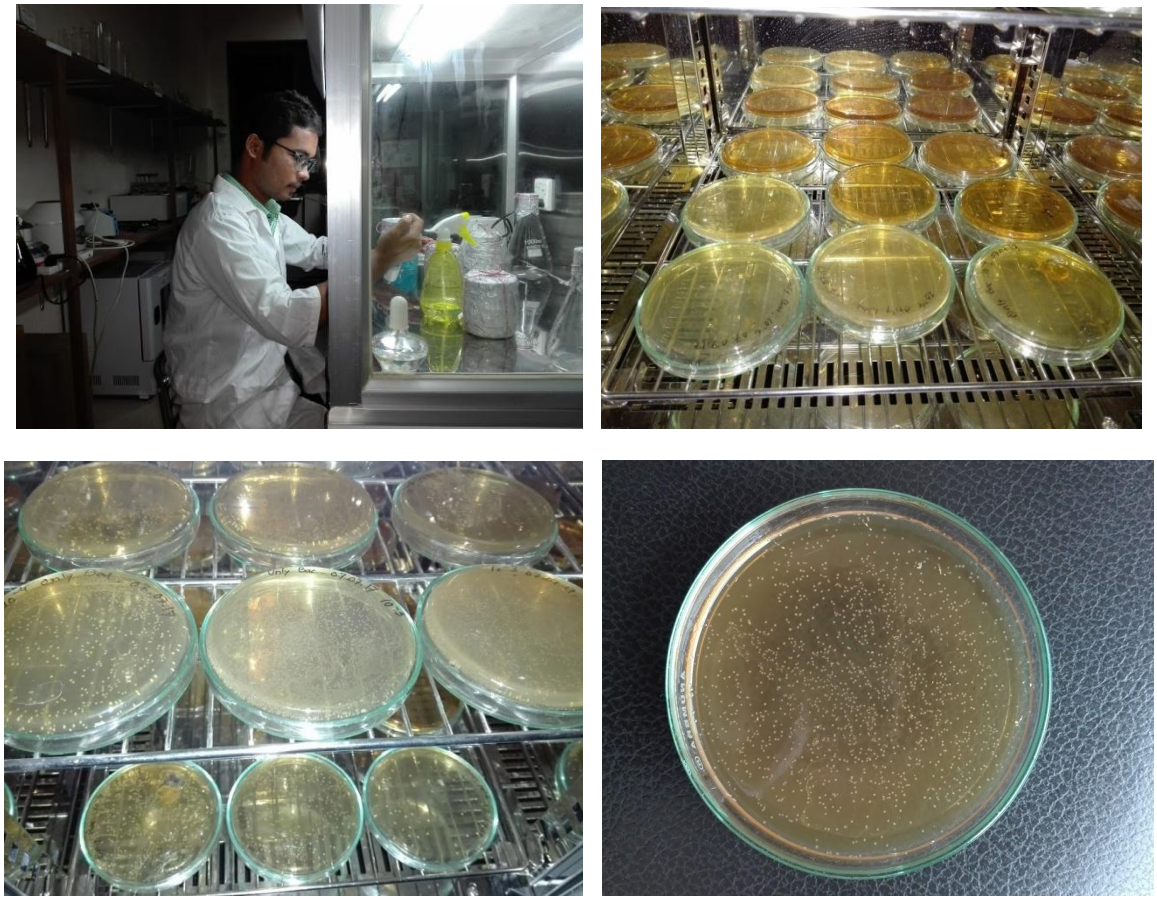
Source: Cobb500 Broiler Management Guide, (2016).

Appendix 14: Gut microbial load (log_{10}cfu/g) of broilers

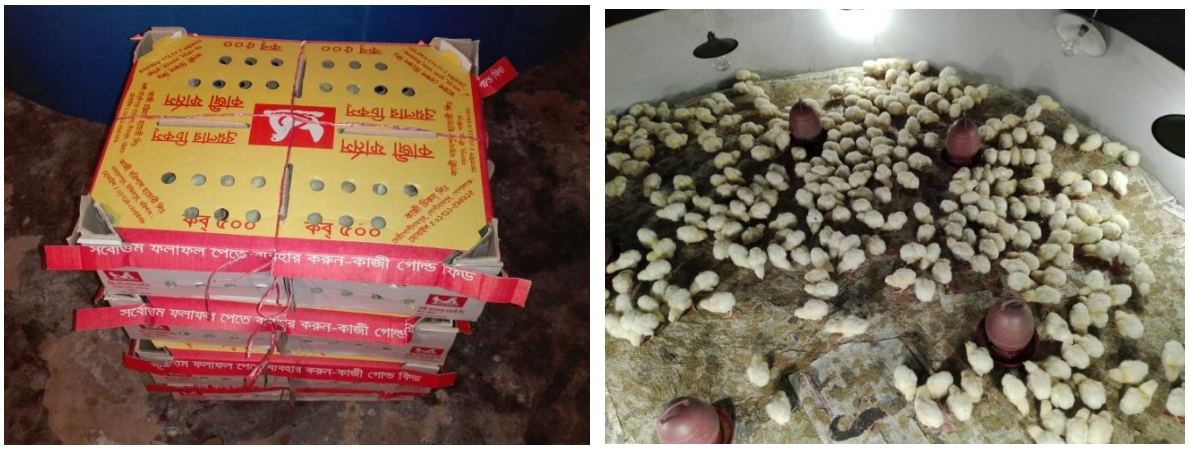
Name of Treatment	Total bacterial count	Coliforms	Lactic acid bacteria
T1R1	*5.43*	*3.83*	*4.11*
T1R2	*5.33*	*3.71*	*4.12*
T1R3	*5.36*	*3.77*	*4.09*
T1R4	*5.60*	*3.88*	*4.09*
T2R1	*5.12*	*3.81*	*4.02*
T2R2	*5.10*	*3.80*	*4.12*
T2R3	*5.09*	*3.76*	*4.09*
T2R4	*5.13*	*3.75*	*4.09*
T3R1	*5.06*	*3.99*	*5.16*
T3R2	*5.00*	*3.91*	*5.10*
T3R3	*4.96*	*3.78*	*4.11*
T3R4	*5.00*	*3.69*	*5.02*
T4R1	*4.92*	*3.94*	*4.99*
T4R2	*4.90*	*3.90*	*4.92*
T4R3	*4.91*	*3.96*	*4.82*
T4R4	*4.94*	*3.56*	*4.89*

Source: Cobb500 Broiler Management Guide, (2016).

Appendix 15: Some photographs of experiment conducted at Animal Science laboratory & SAU poultry farm.

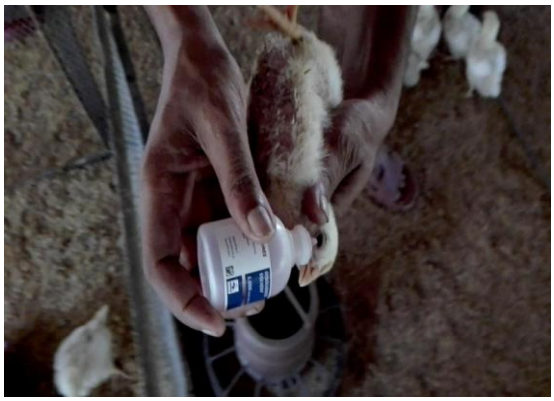
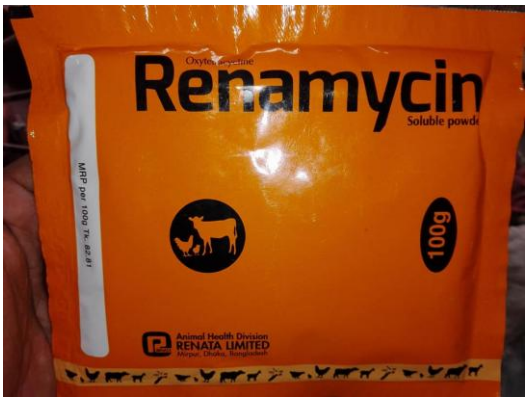


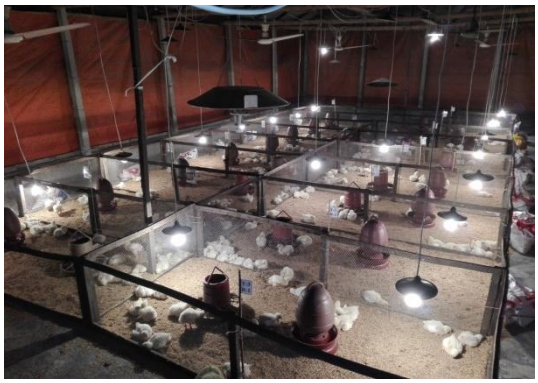
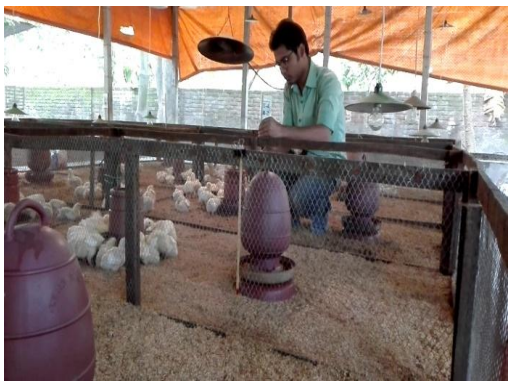
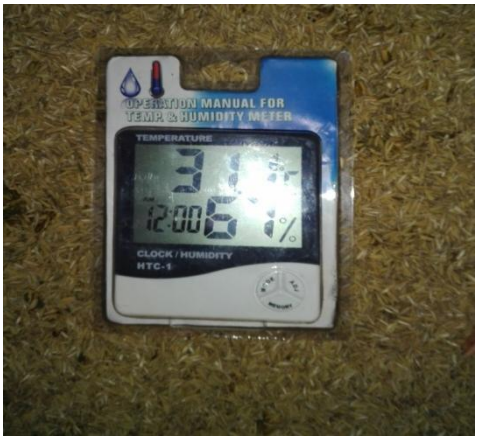
At the time of preparation of probiotics



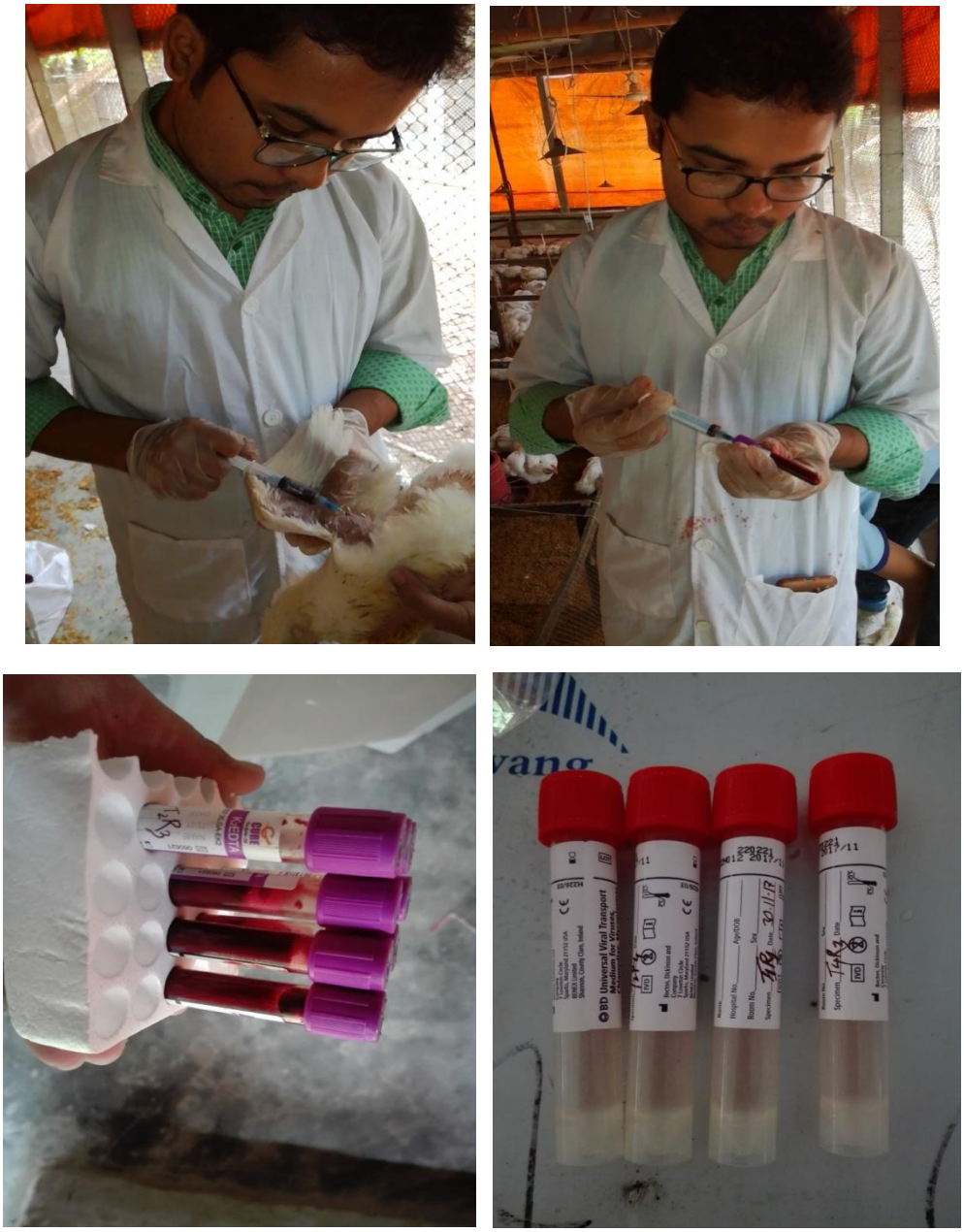
After receiving of Day-old experimental broiler chicks.



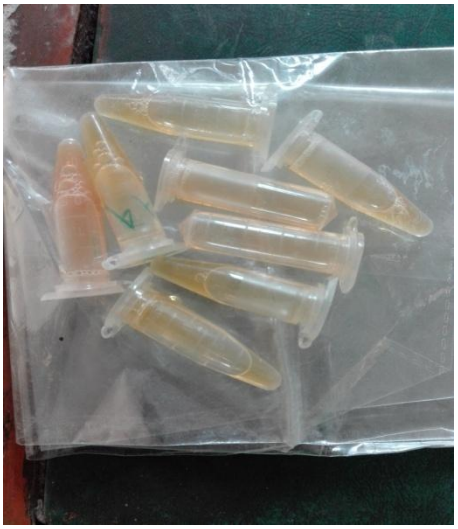



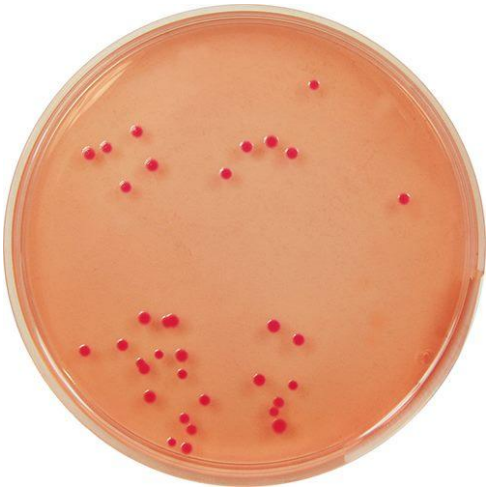
Different activities at the very early age of experimental broiler birds.



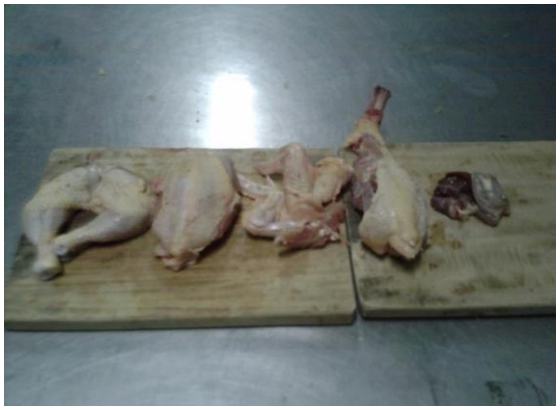
Blood collection and preservation of the collected blood.



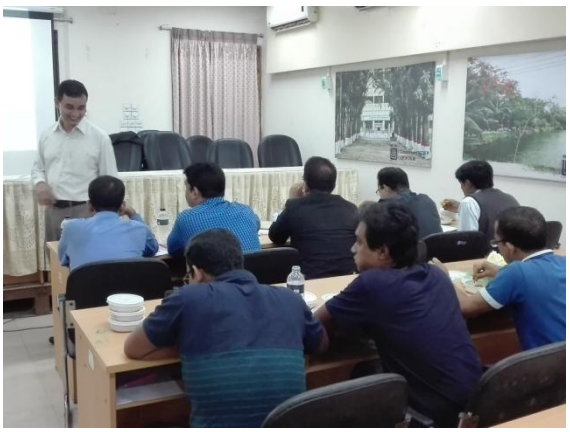
Collection serum for antibody titer examination

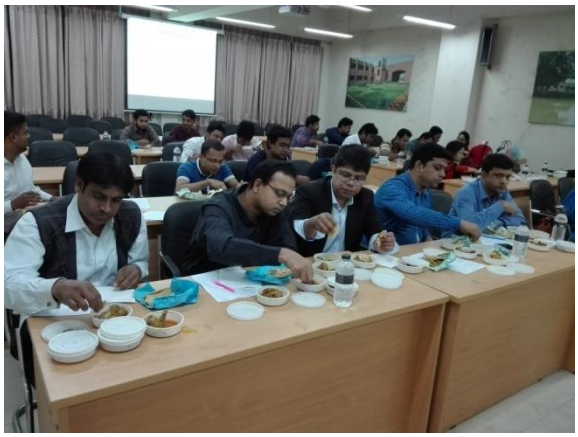


Microbial load examination



Dissection of different parts of the broilers for measurement of different body parts





Organoleptic test of broilers form different treatment groups