DIETARY EFFECT OF PROBIOTICS AND ANTIBIOTIC ON PRODUCTIVE PERFORMANCE AND FAECAL MICROBIAL STATUS ON LAYING HEN

A THESIS

BY

Bikash Roy
Registration No.: 1505029
Session: 2015-2016
Semester: January-June, 2017

MASTER OF SCIENCE (M S)

IN

POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
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Submitted to the Department of Dairy and Poultry Science, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur for partial fulfillment of the requirement of the degree

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DEPARTMENT OF DAIRY AND POULTRY SCIENCE
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
DINAJPUR-5200

MAY, 2017

DEDICATED
TO MY
BELOVED PARENTS

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The Author

ABSTRACT

The study was carried out to assess, examine and quantify the effect of Probiotics (Protexin, Exolution) and Antibiotic (Renamycin) supplementation on egg production performance and microbial load of laying hens at the latter stage (56-63weeks) of production. The study was conducted at the poultry farm and Microbiology laboratory, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. Total 48 Hisex Brown hens of 56 weeks old were allocated to 4 treatments with 3replications, each containing 12 hens. The hens in individual cages were supplied feed 120 g/b/day (not fixed) containing 18.21% CP and 2762.21 ME KCal/kg diet. Laying hens were randomly allotted to 4 dietary treatments T_0 (control), T_1 (Protexin-30gm/100kg of feed), T_2 (Exolution--60gm/100kg of feed), T_3 (Renamycin -100gm/100kg of feed).There were no significant effect on body weight shown after the experiment. Egg production was more or less similar at first few weeks, but at later stage results showed increased production in T_1 and T_2 Treatment than the control T_0. The egg weight was gradually increased in T_2 (67.84g in 8^{th} week) treatment group. The highest egg weight was found in T_2 (67.84 gm). The feed intake of laying hens in different dietary treatments during experimental period was almost similar .The feed efficiency(FE) in different dietary treatments were statistically significant and the best FE was found in T_2 (1.75 in 5^{th} week) treatment group .Other treatment group like T_1, T_3 have also better FE than that of control group. Supplementation of Protexin, Exolution and Renamycin in the diets significantly decreased the population of harmful bacterium, *Escherichia coli,* and total culturable bacteria than those of control.

Key words: Protexin, Egg Production, Egg Weight, Bacteria.

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CHAPTER I

INTRODUCTION

Poverty alleviation is one of the most important challenges of the twenty first century in Bangladesh. Agricultural development is the main key to alleviate poverty from the country. Livestock is the most important agricultural component which alone contributes about 17.3% GDP to agriculture (DLS, 2014). Livestock population in Bangladesh is currently estimated about 25.7 million cattle, 0.83 million buffaloes, 14.8 million goats, 1.9 million sheep, 118.7 million chicken and 34.1 million ducks (DLS, 2014). The density of livestock population per acre of cultivable land is 7.37 (Banglapedia, 2012). In spite of a high density of livestock population, the country suffers from an acute shortage of livestock products like milk, meat and eggs. The shortage accounts for 85.9%, 88.1% and 70.7% for milk, meat and eggs, respectively (Banglapedia, 2012).Biotechnology plays a vital role in the poultry feed industry. Nutritionists are continually putting their efforts into producing better and more economical feed. Good feed alone will not serve the purpose but its better utilization is also essential. Dietary changes as well as lack of a healthy diet can influence the balance of the microflora in the gut thus predisposing to digestion upsets. A well-balanced ration sufficient in energy and nutrients is also of great importance in maintaining a healthy gut. A great deal of attention has recently been received from nutritionists and veterinary experts for proper utilization of nutrients and the use of probiotics for growth promotion of poultry (Kabir, 2009). Probiotics are live microbial complements that leave useful effects on the host through an improvement in the intestine's microbial equilibrium (Fuller, 1989). Different microbial species such as bacteria (Bacilli, Bifidobacteria, Enterococcus, Lactobacillus, and Streptococcus), yeasts (*Saccharomyces cerevisiae*), fungi (*Aspergillusoryzae* and *Aspergillusniger*) and indefinite mixed cultures have been used as probiotics (Simon *et al.*, 2001). Protexin® and AM Phi-Bact are a kind of commercial bacterial probiotic that contains entrococcus faceum and lactobacillus species respectively. For many years, antimicrobial compounds have been used in the poultry breeding industry for improvement in health status and performance of birds by reduction or correction of the population of the bacteria present in the gastrointestinal (GI) tract (El-Katcha1 *et al.*, 2012, Fairchild *et al.*, 2001). Microorganisms used as probiotics in animal nutrition: Probiotics are live microorganisms that, when administered through the digestive tract, have a positive

impact on the host's health. Microorganisms used in animal feed are mainly bacterial strains belonging to different genera, e.g. Lactobacillus, Enterococcus, Pediococcus and Bacillus. Other probiotics are microscopic fungi, including Saccharomyces yeasts. Some probiotic microorganisms are normal residents in the digestive tract, while others are not (Hassanein and Soliman, 2010, Guillot, 2009). The different mechanisms of action suggested are: (i) nutritional effect include: (1) reduction of metabolic reactions that produces toxic substances (2) stimulation of indigenous enzymes (3) production of vitamins or antimicrobial substances. (ii) sanitary effect include (1) increase in colonization resistance. (2) stimulation of the immune response (Hassanein and Soliman, 2010).Some experiments have demonstrated in vitro the effects of strains of Saccharomyces cerevisiae on the activity of anaerobic rumen microorganisms. The addition of S. cerevisiae live cells to cultures of some cellulolytic fungal species stimulated zoospores germination and cellulose degradation. The addition of yeasts stimulates also the growth of some anaerobic bacteria, including the cellulolytic and the lactic acid utilising bacteria (Hassanein and Soliman, 2010, Chaucheyras *et al*., 1995; Yoon and Stern, 1996). Kizerwtter and Binek, (2009) reported that probiotics have reduced the incidence and duration of diseases. Probiotic strains have been shown to inhibit pathogenic bacteria both in vitro and in vivo through several different mechanisms. The mode of action of probiotics in poultry includes: (i) maintaining normal intestinal microflora by competitive exclusion and antagonism (ii) altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production (iii) improving feed intake and digestion iv) stimulating the immune system (Apata, 2008; Kabir, 2009). The addition of probiotics to diets benefit the host animal by stimulating appetite (Nahashon *et al*., 1992), improve intestinal microbial balance (Fuller, 1989), stimulate the immune system (Toms and Powrie, 2001), decrease pH and release bacteriocins (Rolfe, 2000) that compete with other microbes for adhesive site, improve egg mass, egg weight, egg size in layers (Mohiti *et al*., 2007; Nahashon *et al*., 1992; Jin *et al*., 1997) and feed consumption in layers and also depress serum and egg yolk cholesterol concentrations in hens (Mohiti *et al*., 2007; Mohan *et al*., 1995; Kurtoglu *et al*., 2004).

Objectives:

1. To observe the effect of probiotics on productive performance of laying hen.
2. To observe the effect of probiotics and antibiotic on feed efficiency of laying hen.
3. To observe the bacterial load through dietary supplementation of probiotics and antibiotic.

CHAPTER II

REVIEW OF LITERATURE

2.1 Definition

The term ‘probiotics’ was first used by Lilly and Stillwell (1965) to designate unknown growth promoting substances produced by a ciliate protozoan that stimulated the growth of another ciliate. The term now covers a much broader group of organisms. Parker (1974) defined probiotics as “organisms and substances which contribute to intestinal microbial balance” thus including both living organisms and non-living substances. Fuller (1989) was critical of the inclusion of the word ‘substances’ and redefined probiotics as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”.

The joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) Working Group defined probiotics as “live micro-organisms which when administered in adequate amounts confer a health benefit on the host” (FAO/ WHO, 2001). This definition is widely accepted and adopted by the International Scientific Association for Probiotics and Prebiotics (Hill *et al.*, 2014).

Probiotic is a generic term, and products can contain yeast cells, bacterial cultures, or both that stimulate microorganisms capable of modifying the gastrointestinal environment to favor health status and improve feed efficiency (Kabir, 2009, Dierck, 1989).

Probioticsare microorganisms that are believed to provide health benefits when consumed (Hill *et al.*, 2014, Rijkers *et al.*, 2011). The term probiotic is currently used to name ingested microorganisms associated with benefits for humans and animals (Magdalena *et al.*, 2006). The term came into more common use after 1980. The introduction of the concept is generally attributed to Nobel laureate Élie Metchnikoff, who postulated that yogurt-consuming Bulgarian peasants lived longer lives because of this custom (Brown and Valiere, 2004). He suggested in 1907 that "the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes" (Elie, 2004). Although there are numerous claimed benefits of using commercial probiotics, such as

reducing gastrointestinal discomfort, improving immune health, relieving constipation, or avoiding the common cold, such claims are not backed by scientific evidence (Rijkers *et al.*, 2011, Slashinski *et al.*, 2012).

2.2 History

Probiotics have received renewed attention recently from product manufacturers, research studies, and consumers. The history of probiotics can be traced to the first use of cheese and fermented products that were well known to the Greeks and Romans who recommended their consumption (Gismondo *et al.,* 1999). The fermentation of dairy foods represents one of the oldest techniques for food preservation (A*zizpour et al.*, 2009).

The original modern hypothesis of the positive role played by certain bacteria was first introduced by Russian scientist and Nobel laureate Élie Metchnikoff, who in 1907 suggested that it would be possible to modify the gut flora and to replace harmful microbes with useful microbes (Elie, 2004). Metchnikoff, at that time a professor at the Pasteur Institute in Paris, proposed the hypothesis that the aging process results from the activity of putrefactive (proteolytic) microbes producing toxic substances in the large bowel. Proteolytic bacteria such as clostridia, which are part of the normal gut flora, produce toxic substances including phenols, indols, and ammonia from the digestion of proteins. According to Metchnikoff, these compounds were responsible for what he called "intestinal autointoxication", which would cause the physical changes associated with old age.

It was at that time known that milk fermented with lactic-acid bacteria inhibits the growth of proteolytic bacteria because of the low pH produced by the fermentation of lactose. Metchnikoff had also observed that certain rural populations in Europe, for example in Bulgaria and the Russian steppes, who lived largely on milk fermented by lactic-acid bacteria were exceptionally long lived. Based on these observations, Metchnikoff proposed that consumption of fermented milk would "seed" the intestine with harmless lactic-acid bacteria and decrease the intestinal pH, and that this would suppress the growth of proteolytic bacteria. Metchnikoff himself introduced in his diet sour milk fermented with the bacteria he called "Bulgarian Bacillus" and believed his health benefited. Friends in Paris soon followed his example and physicians began prescribing the sour-milk diet for their patients (Vaughan, 1965).

Bifido bacteria were first isolated from a breast-fed infant by Henry Tissier, who also worked at the Pasteur Institute. The isolated bacterium named *Bacillus bifiduscommunis* (Tissier, 1900) was later renamed to the genus *Bifidobacterium*. Tissier found that bifido bacteria are dominant in the gut flora of breast-fed babies and he observed clinical benefits from treating diarrhea in infants with bifido bacteria. The claimed effect was bifido bacterial displacement of proteolytic bacteria causing the disease.

During an outbreak of shigellosis in 1917, German professor Alfred Nissle isolated a strain of *Escherichia coli* from the feces of a soldier who was not affected by the disease (*Alfred, 1918*). Methods of treating infectious diseases were needed at that time when antibiotics were not yet available, and Nissle used the *E. coli* Nissle 1917 strain in acute gastrointestinal infectious salmonellosis and shigellosis.

In 1920, Rettger and Cheplin reported that Metchnikoff's "Bulgarian Bacillus", later called *Lactobacillus delbrueckii* subsp. *bulgaricus*, could not live in the human intestine (Cheplin and Rettger, 1920). They conducted experiments involving rats and humans volunteers, by feeding them with *Lactobacillus acidophilus*. They observed changes in composition of fecal micro biota, which they described as “transformation of the intestinal flora”(Cheplin and Rettger, 1920). Rettger further explored the possibilities of *L. acidophilus* and reasoned that bacteria originating from the gut were more likely to produce the desired effect in this environment. In 1935, certain strains of *L. acidophilus* were found to be very active when implanted in the human digestive tract (Rettger *et al.*, 1935).Trials were carried out using this organism, and encouraging results were obtained, especially in the relief of chronic constipation.

2.3 Microorganism in probiotics

The species currently being used in probiotic preparations are varied and many. These are mostly *Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus helveticus, Lactobacillus lactis, Lactobacillus salivarius, Lactobacillus plantarum, Streptococcus thermophilus, Enterococcus faecium, Enterococcus faecalis, Bifidobacterium spp.* and *Escherichia coli.* With two exceptions, these are all intestinal strains. The two exceptions, *Lactobacillus bulgaricus* and *Streptococcus thermophilus,* are yoghurt starter organisms. Some other probiotics are microscopic fungi such as strains of yeasts belonging to *Saccharomyces cerevisiae* species (Kabir, 2009).

In broiler nutrition, probiotic species belonging to *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces* (Kabir, 2009).

2.4 Mechanisms 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 producing bacteria and bifidobacteria. 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. Several important mechanisms underlying the antagonistic effects of probiotics on various microorganisms such as: modification of the gut micro biota, competitive adherence to the mucosa and epithelium, strengthening of the gut epithelial barrier and modulation of the immune system to convey an advantage to the host. Accumulating evidence demonstrates that probiotics communicate with the host by pattern recognition receptors, such as toll-like receptors and nucleotide-binding oligomerization domain-containing protein-like receptors, which modulate key signaling pathways, such as nuclear factor-κB and mitogen-activated protein kinase, to enhance or suppress activation and influence downstream pathways. This recognition is crucial for eliciting measured antimicrobial responses with minimal inflammatory tissue damage (Bermudez-Brito *et al.*, 2012).

The mode of action of probiotics in poultry includes maintaining normal intestinal microflora by competitive exclusion and antagonism, altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production, improving feed intake and digestion and neutralizing enterotoxins and stimulating the immune system (Král *et al.*, 2012).

The mode of action of probiotics in poultry includes: (i) maintaining normal intestinal microflora by competitive exclusion and antagonism; (ii) altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production; (iii) improving feed intake and digestion; and (iv) stimulating the immune system (Kabir, 2009).

Major probiotic 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 (fig.) (Bermudez-Brito *et al.*, 2012).

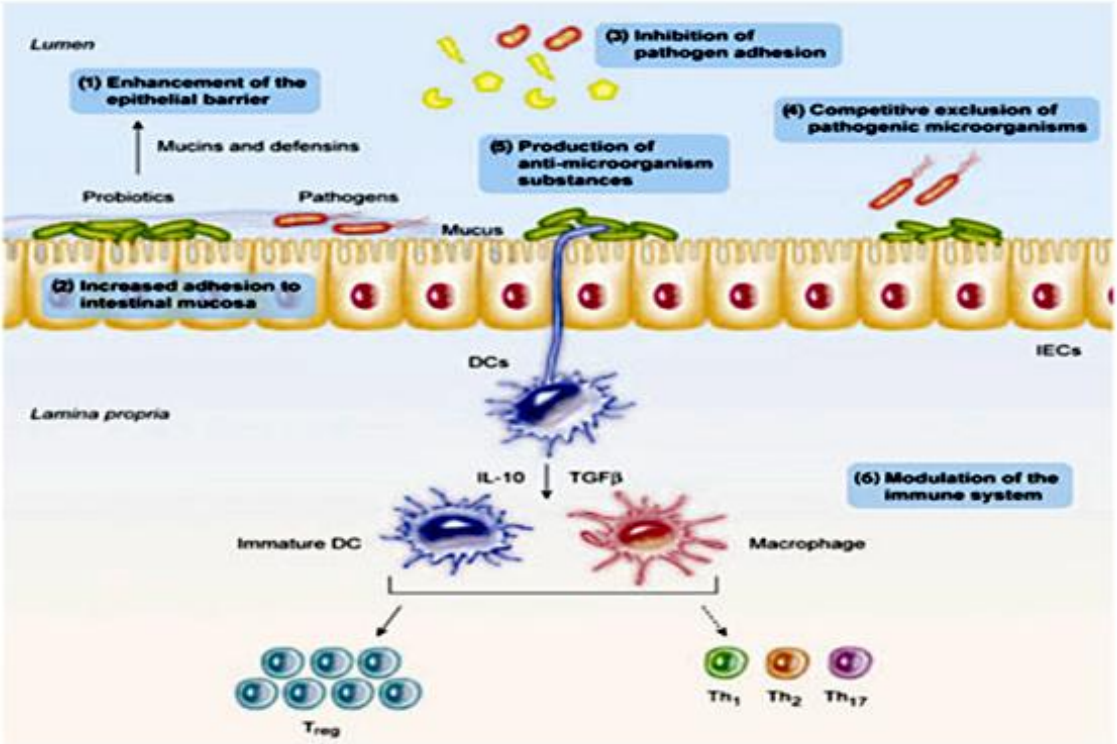

Figure 2.1: Major mechanisms of action of probiotics

2.5 Enhancement of epithelial barrier

The epithelium of intestine is in permanent contact with luminal contents and the variable enteric flora. The intestinal barrier, a major defense mechanism is used to maintain epithelial integrity and to protect the organism from the environment. The intestinal barrier defenses consist of the mucous layer, antimicrobial peptides, secretory IgA and the epithelial junction adhesion complex. If the function of the barrier is disrupted, bacterial and food antigens can reach the submucosa and can induce inflammatory responses resulting intestinal disorders. Consumption of non-pathogenic bacteria can contribute to intestinal barrier function, and probiotic bacteria have been extensively studied for their involvement in the maintenance of this barrier. However, the mechanisms by which probiotics enhance intestinal barrier function are not fully understood (Bermudez-Brito *et al.*, 2012).

2.6 Increased Adhesion to Intestinal Mucosa

The bacteria of the probiotic attach to the intestinal mucosa, thereby forming a physical barrier that blocks the attachment of pathogenic bacteria (Kral, 2012)

Adhesion to intestinal mucosa is regarded as a prerequisite for colonization and is important for the interaction between probiotic strains and the host. Adhesion of probiotics to the intestinal mucosa is also important for modulation of the immune system and antagonism against pathogens. (Bermudez-Brito *et al.*, 2012).

2.7 Production of antimicrobial substances

Some probiotics produce antimicrobial substances that may inhibit growth of pathogenic micro-organisms in the intestine (FAO, 2016).

Many bacterial species, including lactic acid bacteria (LAB) (Klaenhammer, 1988; Nes *et al.*, 1996; Flynn *et al.*, 2002), bifidobacteria (Cheikhyoussef *et al.*, 2008) and bacillus (Hyronimus *et al.*, 1998), can produce several types of thermostable bacteriocins (Cotter *et al.*, 2005) which have antimicrobial activity against a range of potential pathogens of animals including Bacillus, Staphylococcus, Enterococcus, Listeria, and Salmonella species (Flynn *et al.*, 2002; Corr *et al.*, 2007; Rea *et al.*, 2007).

2.8 Development of immune response

The GIT component of the immune system protecting the host from the different types of antigens in the lumen of the GIT is affected by probiotics. Both innate and adaptive immunity are affected by probiotics (FAO, 2016).

The introduction of gut micro biota via the administration of probiotics influences the development of the immune response (Kabir 2009, McCracken, 1999). The exact mechanisms mediating the immunomodulatory activities of probiotics are not clear. However, it has been shown that probiotics stimulate different subsets of immune system cells to produce cytokines, which play a role in the induction and regulation of the immune response (Kabir 2009, Christensen *et al.*, 2002, Lammers *et al.*, 2003, Maassen *et al.*, 2000).

Several studies have demonstrated immunostimulatory effects of probiotics. Bai *et al.* (2013) demonstrated that a probiotic containing *L. fermentum* and *S. cerevisiae*

stimulated the intestinal T-cell immune system, indicated by increased production of CD3+, CD4+ and CD8+ T-lymphocytes in the GIT of broiler chickens. Expression of CD3+, IL-2 and IFN-γ genes was significantly greater in the small intestine of neonatal chicks (day 3 and 7) fed with probiotics *L. jensenii* TL2937 and *L. gasseri* TL2919 than in the control without probiotics (Sato *et al.*, 2009).

2.9 Protexin:

Composition: 60 x 106 CFU/g as

Lactobacillus acidophilus,
L. delbrueckii subsp. bulgaricus,
L. plantarum,
L. rhamnosus,
Bifidobacteriumbifidum,
Enterococcus faecium,
Streptococcus salivariussubsp
Thermophilus

Actions Live microbial feed supplement (probiotic). Improves intestinal microbial balance by maintaining the digestive system, optimizing digestion of feed and naturally enhancing health, nontoxic and residue free.

Indications: An aid during intestinal dysfunction; treatment and control of scouring and diarrhoea; exclusion and suppression of pathogens such as *Escherichia coli*, Salmonella and *Aeromonas* sp; following antibiotic therapy in all animals to re-establish gut microflora; improvement in weight gains and feed conversion in growing pigs and cattle; productivity improvements and reduced mortality in poultry; an aid in the establishment of gastrointestinal micro flora in physiologically immature animals; periods of stress.

Protexin® and AM Phi- Bact are a kind of commercial bacterial probiotic that contains entrococcusfaceum and lactobacillus species respectively.

2.10 Exolution:

Composition:

Each gram powder contains

Bacillus subtilies- 1×10^6cfu

Bacteriophage-Q.S- 1 gm

Indication: To prevent and treat salmonellosis, collibacillosis and necrotic enteritis and maintain balance of beneficial microorganisms in the gut.

2.11 Effect on laying performance:

Balevi *et al.* (2001) were fed commercial multi strain regimen probiotic to 40-week-old layers and showed statistically significant differences in egg production and egg weight compared with the control.

Nahashon *et al.* (1992) and Tortuero and Fernandez, (1995) showed that using vital biomass of probiotic supplements affects the egg weight significantly ($P<0.05$).

Including a live yeast into laying hen diets improved egg production percentage (Kim *et al.*, 2002 and Shivani *et al.*, 2003), and egg weight (Han *et al.*, 1999; Park *et al.*, 2001 and Park *et al.*, 2002). Sharma *et al.* (2001); Kim *et al.* (2002) and Kabir (2009) reported that, adding a live yeast into laying hens diet improved feed intake and feed conversion ratio.

Inclusions of yeast into laying hen diets enhanced egg shell breaking strength (Park *et al.*, 2002) and reduced soft or broken eggs (Park *et al.*,2001).

Soliman (2003) studied the effect of supplementing a constant level of live yeast into laying hens diets, he observed an improvement in average egg weight, feed conversion values and nutrients utilization.

Kurtoglu *et al.* (2004) conducted a study to know the effects of dietary supplementation of a commercial probiotic (BioPlus 2B) on daily feed consumption, egg yield, egg weight, specific gravity, body weight, feed conversion ratio, serum and egg yolk cholesterol, and serum triglyceride in layer hens were investigated. In 12 replicates, 480 27-week-old Brown-Nick layers were fed with diets containing 0, 250, 500 or

750 mg kg^{-1} probiotic for 90 days. When compared with the controls, supplementation of 250, 500 and 750 mg kg^{-1} probiotic increased egg production, but decreased the damaged egg ratio ($P<0.05$), egg yolk cholesterol and serum cholesterol ($P<0.001$) levels. In addition, serum triglyceride levels were reduced by using 500 and 750 mg kg^{-1} probiotic supplementation ($P<0.001$). Feed conversion ratios were positively affected by supplementation of 250 and 500 mg kg^{-1} probiotic compared with controls ($P<0.05$). There was no statistically significant difference between the control and all treatment groups on feed consumption, egg weight, specific gravity, body weight, and egg yolk weight.

Balevi *et al*. (2010) studied the effects of dietary supplementation of a commercial probiotic (Protexin feed consumption, egg yield, egg weight, food conversion ratio and humoral immune response in layer hens were investigated. In 7 replicates, a total of 280 40-week-old layers were given diets containing either 0, 250, 500 or 750 parts per million (ppm) for 90 d. 2. When compared with the controls, the food consumption, food conversion ratio and the proportions of damaged eggs were lower in the group consuming 500 ppm probiotic ($p<0.05$). 3. There was no significant difference between the controls and the groups receiving 250 and 750 ppm probiotic in food consumption, food conversion ratio and proportion of damaged eggs. Similarly, the egg yield, egg weight, specific gravity, and peripheral immune response showed no statistically significant differences between the groups on daily.

Mohiti *et al*. (2007) to evaluate the effects of dietary probiotics, yeast, vitamin E and vitamin C supplementation on performance, serum and yolk cholesterol and immune response of heat stressed laying hens, a trial was conducted with sixty white layer hens of Hy-Line variety. Experiment was conducted by using completely randomized design with 5 treatments, 3 replicates and 4 hens in each replicate. The treatments involved: control, basal diet plus 50 mg multi strains probiotic, basal diet plus 1 g yeast of *Saccharomyces cerevisiae*, basal diet plus 200 mg vitamin C and basal diet plus 200 mg vitamin E per Kg of diet. Results indicated non-significant difference in hen performance, egg quality (shell thickness, shell resistance, shell percent and haugh unit) and serum and yolk cholesterol concentrations. Yolk percent was increased significantly and the highest yolk percent was observed in vitamin E treatment. Immune response of laying hens with multi strains probiotic and yeast supplementation was greater than

others. However, dietary vitamin E and C supplementation increased immune response, but differences were not significant compare with other groups.

Mahdavi *et al.* (2005) investigated the effect of probiotic supplements (0, 400, 1000 and 2000 gr Bioplus 2B ton-1 feed providing 0,1.28×106, 3.2×106 and 4.6×106 cfu gr-1 feed concentration) on egg quality and laying hen's performance on eighty white leghorn Hy-Line, W-36 strain. Evaluated traits were egg production, egg weight, egg mass, feed consumption, feed conversion ratio, shell thickness, shell hardness, Haugh unit, egg cholesterol, plasma cholesterol, plasma triglyceride and histological changes of duodenum. Although, using the different levels of probiotic caused highly significant increase ($P<0.01$) in goblet cell numbers, significant increase ($P<0.05$) in destroying apical cells of villus and significant decrease ($P<0.05$) in plasma cholesterol, plasma triglyceride and egg cholesterol (mg gr-1 of yolk), but it had no significant effects on other traits.

Zhang and Kim (2013) conducted a study to determine the effects of probiotic (*Enterococcus faecium* DSM 7134) supplementation on performance, egg quality, excreta microflora, excreta noxious gas emission, and serum cholesterol concentrations in laying hens. A total of 432 Hy-Line brown layers (40 wk old) were allotted into 4 dietary treatments with 2 levels of probiotic supplementation (0 or 0.01%) and 2 levels of energy (2,700 or 2,800 kcal ME/kg) and nutrient density. Weekly feed intake, egg quality, and daily egg production were determined. Eighteen layers per treatment (2layers/replication) were bled to determine serum cholesterol concentrations at wk 3 and 6. Excreta microbial shedding of *Lactobacillus*, *Escherichia coli*, and *Salmonella* and noxious gas emission were determined at the end of the experiment. Hens fed the high-energy and high-nutrient-density diets had less ($P< 0.01$) ADFI than those fed the low-energy and low-nutrient-density diets throughout the experimental period. During wk 4 to 6 and overall, hens fed the diets supplemented with the probiotic had greater ($P< 0.01$) egg production, egg weight, and eggshell thickness than hens fed the diets without the probiotic. Dietary supplementation of the probiotic increased ($P = 0.01$) excreta *Lactobacillus* counts and decreased ($P = 0.02$) *Escherichia coli* counts compared with hens fed the diets without the probiotic.

CHAPTER III

MATERIALS AND METHODS

3.1 Time period of the experiment

The experiment was conducted for a period of eight(8) weeks from 1st February to 5th April, 2015 to investigate the dietary effect of two probiotic namely Protexin (Novartis), Exolution (ACI), and an antibiotic namely Renamycin in different doses on production performance of laying hens and their microbial load at the latter stage of production.

3.2 Site of the Experiment

The trial was conducted at Hajee Mohammad Danesh Science and Technology University (HSTU) Poultry Shed, Basherhat, Dinajpur and the microbial load was analyzed in Microbiology Laboratory of the Department of Microbiology and Hygiene, HSTU.

3.3 Collection of experimental sample

Protexin, Exolution and Renamycin were purchased from local vet. shop of Dinajpur district.

3.4 Experimental birds (laying hen)

A total number of 48 egg laying hens (Hisex Brown) of 56 weeks of age having uniform body weight were selected as experimental birds. The birds were divided into four groups having 3 replications containing 4 birds in each replication. Four hens kept in each cage were considered as an experimental unit (Replication).Hens was randomly allocated to the cages.

3.5 Layout of the experiment

The layout of the experiment is shown in table 3.1. There were three replications in each dietary phase treatment. Thus total number of replicate was 12.

Table 3.1: Table showing the distribution of layer hens to different dietary probiotic and antibiotic in cage from 56 to 64 weeks of age

Replication (R)	Treatment (T)			
	T_0	T_1	T_2	T_3
R_1	4	4	4	4
R_2	4	4	4	4
R_3	4	4	4	4

* T_0 = Control (Basal diet)
* T_1 = (Control + *PRT-30gm/100kg feed)
* T_2 = (Control + *EXO-60gm/100kg feed)
* T_3 = (Control + *RNM- 100 gm/100kg feed)
* PRT means Protexin.
* EXO means Exolution
*RNM means Renamycin

3.6 Collection of feed ingredients

Various feed ingredients used for preparation of hand mixing diets to serve to the experimental hens were purchased from the local market of Dinajpur district. The ration was formulated according to nutrient requirements as specified by the 9th revised edition of National Research Council (NRC, 1994) for layer and was designated as the control diet.

Table 3.2: Ingredient amount and Chemical composition of diet

Ingredient (kg)	Treatment			
	T_0	T_1	T_2	T_3
Maize	55	55	55	55
Rice polish	8	8	8	8
Soybean meal	19.2	19.2	19.2	19.2
Protein concentrate(Propec)	7	7	7	7
Soybean oil	1.5	1.5	1.5	1.5
DCP	0.800	0.800	0.800	0.800
Limestone	8	8	8	8
Salt	0.500	0.500	0.500	0.500
Vitamin-mineral premix*	0.250	0.250	0.250	0.250
Lysine	0.060	0.060	0.060	0.060
Methionine	0.170	0.170	0.170	0.170
Toxin binder	0.060	0.060	0.060	0.060
Protexin	0	0.03	0	0
Exolution	0	0	0.06	0
Renamycin	0	0	0	.1
Chemical composition of control diet.				
Metabolizable Energy, ME (KCal/kg)	2762.21	2762.21	2762.21	2762.21
Crude Protein (%)	18.21	18.21	18.21	18.21
Crude Fibre (%)	3.67	3.67	3.67	3.67
Ether Extract (%)	5.13	5.13	5.13	5.13
Calcium (%)	3.64	3.64	3.64	3.64
Phosphorus (%)	0.78	0.78	0.78	0.78
Lysine (%)	1.38	1.38	1.38	1.38
Methionine(%)	0.36	0.36	0.36	0.36

* Added vitamin-mineral premix (Rena-Layer, Renata Animal Health Ltd) @ 250 g per 100 kg which contained: Vitamin A: 4800 IU; Vitamin D : 960 IU; Vitamin E: 9.2 mg; Vitamin k_3: 800 mg; Vitamin B_1: 600 mg; Vitamin B_2 :2 mg; Vitamin B_3: 12 mg; Vitamin B_5 :3.2 mg; Vitamin B_6 : 1.8 mg; Vitamin B_9: 2 mg;VitaminB_{12} :0.004 mg; Co:

0.3 mg; Cu: 2.6 mg; Fe: 9.6 mg; I : 0.6 mg ; Mn: 19.2 mg; Zn: 16 mg; Se:0.48 mg; DL-Methionine: 20 mg; L-lysine: 12 mg.

3.7 Preparation of diet

The probiotics and antibiotic were mixed with small amount of control feed and then thoroughly mixed with the total amount of feed accordingly to level. However, four diets were randomly distributed to four groups in a Completely Randomized Design (CRD). Dietary treatments consist of basal feed as T_0, a similar ration with Protexin (30gm/100kg of feed) as T_1, with Exolution (60gm/100kg of feed) as T_2, with Renamycin (100gm /100kg of feed) as T_3.

3.8 Feeding method

Two hens in a cage combining with another cage unit (Replication) were provided 480 g feed/day in two installments. Thus each hen was allocated 120g/day. Fresh clean drinking water was available at all times.

Figure 3.2: Experimental hens in cages

Figure 3.3: Sample of control diet

3.9 Lighting

During the entire experimental period all hens were exposed to a 16 hours photoperiod (natural light + artificial light) in an open sided house.

3.10 Routine management

Hens were provided to similar care and management in all replications throughout the study period. Adequate hygiene and sanitation were maintained properly.

3.11 Data collection and record keeping

The following data were collected per replication throughout the experimental period

3.11.1 Body weight change

The hens were weighed at start (initial body weight) and then at the end of the experiment (final body weight). Body weight gain/loss was calculated by the difference of initial body weight and final body weight.

Body weight change = Final body weight - initial body weight

3.11.2 Egg production

The following records were kept during the whole experimental period:

3.11.2.1 Hen day egg production Per cent

The hen day egg production percent was determined replication wise by the following formula.

$$\text{Hen day egg production (HDEP) (\%)} = \frac{\text{No.of eggs laid}}{\text{Total no.of days}} \times 100$$

3.11.3 Feed efficiency (FE)

The FE was calculated to dividing daily feed intake by daily egg mass and the relation was called FE.

$$\text{FE} = \frac{\text{Feed intake of the birds (g/b/d)}}{\text{Egg mass(g/b/d)}}$$

3.11.4 Egg weight

Egg weight was recorded before quality determination by using a digital balance.

3.12 Isolation of *E. coli* and salmonella from faeces sample

3.12.1. Faecal sample collection, transportation and preparation

Faecal samples of chicken were collected from healthy layer at the first and finally last week of experiment. All samples were collected with the help of sterile cotton buds and transferring the buds immediately to sterile nutrient agar. All the samples were transferred carefully to appropriate container. These were kept in box, wrapped with ice and transferred to laboratory for subsequent bacteriological examination.

3.12.2 Bacteriological media

a. **Cultural Media**

Commercially available media were used during this study. The commercial media were prepared according to the direction of the manufacturer's. The composition and the procedure for the preparation of media are presented in the Methods. The media used for bacteriological culture were Nutrient Agar (NA; HiMedia), Nutrient Broth (NB; HiMedia), Eosin-Methylene-Blue (EMB. Hi Media) Agar, MacConkey (MC; HiMedia) Agar.

b. Biochemical media

The following biochemical media were used for the bacteriological analysis: Triple Sugar Iron (TSI) medium, Methyl Red-Voges Proskauer Broth (MR-VP Broth; HiMedia), Motility Indole Urea medium (MIU, HiMedia), Indole test.

3.12.3 Bacteriological reagents

The reagents used were phenol red, phosphate buffered saline (PBS), mineral oil, normal physiological saline solution, peptone water, 3% tri sodium citrate solution and other common laboratory chemicals and reagents as and when required during the experiment.

3.12.4 Sugars

- Dextrose
- Sucrose
- Lactose

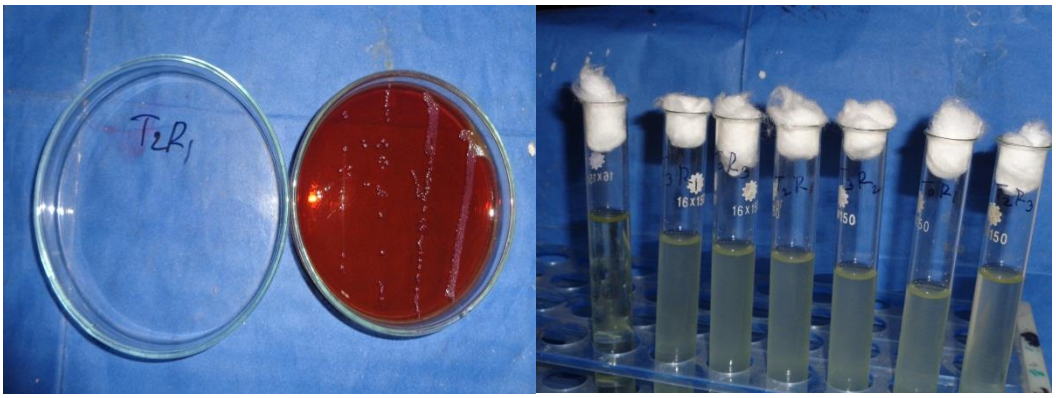

Figure 3.4: Bacteriological media with organisms (Agar and broth)

3.11.5 Bacteriological media preparation

a) Nutrient broth (NB)

Nutrient broth was prepared by dissolving 13 grams of dehydrated nutrient broth (Himedia, India) into 1000 ml of distilled water and was sterilized by autoclaving, at 121°C tinder 15 Ib pressure per square inch for 15 minutes. Then the broth was dispensed into tubes (10 mUtube) and stored at 4°C in the refrigerator until used.

b) MacConkey (MC) agar media

51.50 grams powder of MC agar base (HIMedia, India) was added to 1000 ml of distilled water in a flask and heated until boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at I21°C maintaining a pressure of 15 pounds/sq. inch for 15 minutes. After autoclaving, the medium was put into water bath of 45°C to decrease its temperature. After solidification of the medium in the petridishes, the petridishes were allowed for incubating at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

c) Eosin Methylene Blue (EMB) agar media

Thirty six (36) grams of EMB agar base (HiMedia, India) was added to 1000 ml of distilled water in a flask and heated until boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at 12 FC maintaining a pressure of 15 pounds/sq. inch for 15 minutes. After autoclaving, the medium was put into water bath of 45°C to decrease its, temperature. After solidification of the medium in the petridishes, the petridishes were allowed for incubation at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

d) Triple Sugar Iron (TSI) media.

A quantity of 65.0 gm of Bacto TSI medium (HiMedia) was dissolved in 1000 mlt1- of distilled water dispensed in 5 ml amount in each test tube and then the tubes were autoclaved at 121°C maintaining a pressure of 15 lb/sq. inch for 15 minutes. After autoclaving, the medium was put into water bath of 45°C to decrease its temperature. After solidification of the medium in the test tubes, the test tubes were allowed for incubation at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

e) Methyl-Red Voges-Proskauer (MR-VP) broth

A quantity of 17.0 gm of Bacto MR-VP medium (HiMedia) was dissolved in 250 ml of distilled water dispensed in 2 ml amount in each test tube and then the tubes were autoclaved at 121°C maintaining a pressure of 15 lb/sq. inch for 15 minutes. After autoclaving, the tubes containing medium were incubated at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

i) Motility Indole Urea (MIU) broth

18.00 grams powder of MIU agar base (HiMedia, India) was added to 950 ml of distilled water in a flask and heated until boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at 121°C maintaining a pressure of 15 pounds/sq. inch for 15 minutes. After autoclaving, the medium was put into water bath of 45(' C to decrease its temperature. After this the medium in the test tubes were allowed for incubating at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

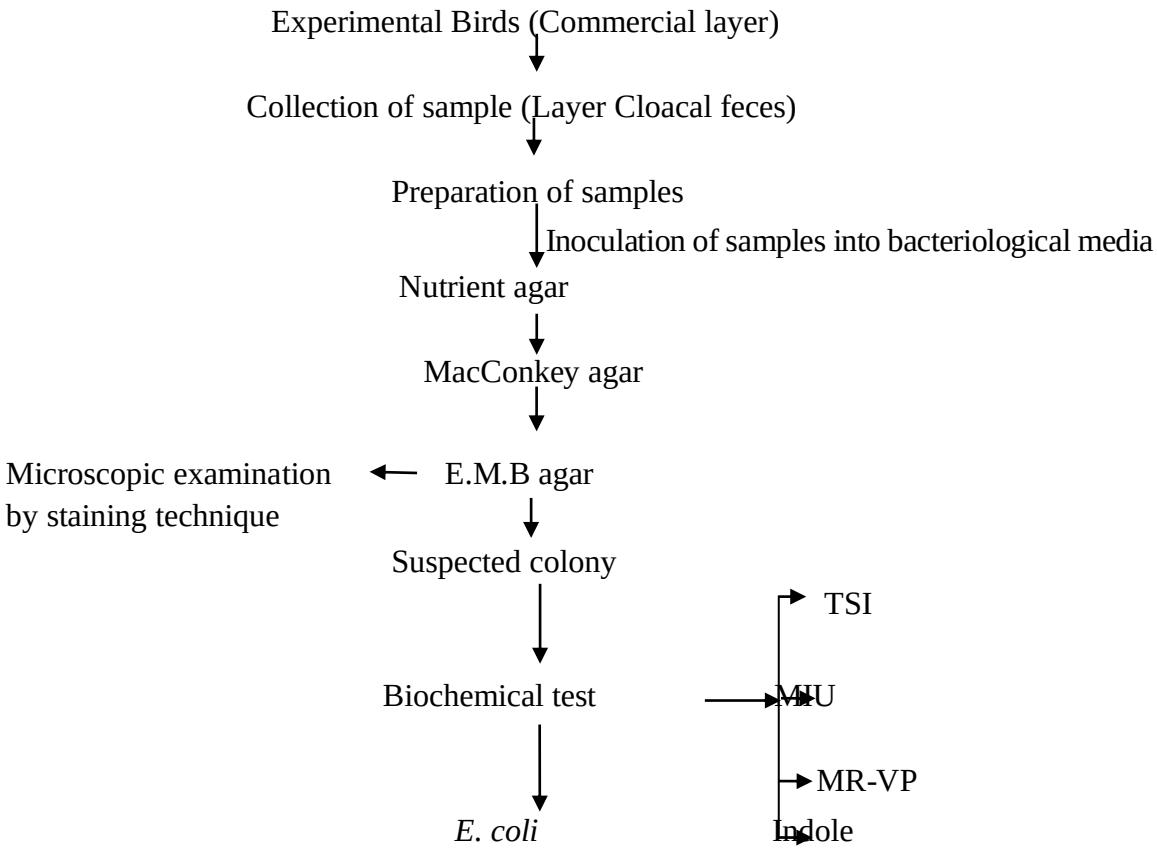

Figure 3.5: Schematic Illustration of Experiment

3.13 Isolation of E. coli in pure culture

All samples were cultured primarily in nutrient agar at 37°C for 24 h, and then subcultured onto the MacConkey and EMB agar and S-S agar by streak plate method to observe the morphology. The organism showing, characteristic colony morphology of *E. coli* was repeatedly subcultured onto EMB agar until the pure culture with homogenous colonies

3.14 Examination of Plates (Identification of the isolates)

a) Gross colony study

Morphological characteristics (shape. size, surface texture, edge, elevation, colour, opacity etc.) developed after 24 h of incubation were carefully studied as described by Marchant and Packer (1967) and recorded.

b) Microscopic study by staining method

Gram's staining method was done to study their morphology and staining character. Suspected colony from EMB agar were stained using Gram's stain as described by manual of Veterinary Investigation Laboratory Technique, 1984 (OIE, 2000).

The procedure was as follows:

A small colony was picked up with a bacteriological loop, smeared on a glass slide and fixed by gentle heating. Crystal violet solution was then applied on the smear to stain for two minutes and then washed with running water. Lugol's iodine was then added to act as mordant for one minute and then again washed with running water. Acetone alcohol was then added, which act as a decolourizer, for few seconds. After washing with water, safranine was added as counter stain and allowed to stain for two minutes. The slide was then washed with water, blotted and dried in air and then examined under microscope Is' with 10 X objectives and then with 100X objective using immersion oil. Gram negative rod shaped organisms were suspected for *E. coll.*

3.15 Biochemical test

The suspected isolated organism were subjected to different biochemical tests, such as sugar fermentation test for acid or acid and gas production, Indole production test, Methyl-red and Voges-proskauer (VP) test. Standard methods were followed for conducting these tests as described by Cowan (1985) during the experiment.

a) Sugar fermentation test

The sugar fermentation test was performed by inoculating a loop full of nutrient broth culture of the organisms into the tubes containing three basic sugars (dextrose, sucrose, and lactose) and incubated for 24 hours at 37°C to observe their sugar fermentation capability. Bacteria able to ferment all the five basic sugars were suspected for *E. coll*.

b) Indole production test

Two ml of peptone water was inoculated with 5 ml of bacterial culture and incubated for 48 hours. 0.5 ml of Kovac's reagent was added, shaked well and examined after I minute. A red colour in the reagent laver indicated indole.

c) Voges-Proskauer (V-P) test

2 ml of sterile glucose phosphate peptone water was inoculated with the 5 ml of test organism. It was incubated at 35-37°C for 48 hours. A very small amount (knife point) of creative was added and mixed. 3 ml of the sodium hydroxide reagent was added and shacked well. The bottle cap was removed left for an hour at room temperature. It was looked for the slow development of a pink-red colour.

d) Methyl Red Test:

The test was performed by inoculating a colony of the test organism in 0.5 ml of sterile glucose phosphate broth (as used in the V-P test). After overnight incubation at 35-37°C, a drop of methyl red solution was added. A positive methyl red test was shown by the appearance of a bright red colour, indicating acidity.

Procedure for total viable and *E. coli* count:

Nutrient agar media was used for total viable count and Eosin Methylene Blue (EMB) agar media for *E. coli* count in this study. The procedure was as follows-

At first 10% suspension of the collected fecal sample was prepared in 0.1% peptone water. Then serial 10 fold dilution of the suspension was prepared in 10 sterile test tubes using 0.1% peptone water as diluent. Then 1 ml of diluted sample from each test tube was taken and poured into a sterile petri dish. Three different petridishes were used for each dilution. Then 10ml of melted Glucose tryptone yeast agar was poured into each petridish when the temperature was reduced at 45 C. Then the petridishes were rotated clockwise and anticlockwise gently to mix the sample with the culture media. Then the petridishes were allowed for solidification of the media. After solidification of the media the petridishes were marked and incubated at 30 C for 72 hours. Then the colonies of each petridish were counted. The petridishes containing 30 to 300 colonies were taken in consideration. Then average numbers of colonies were counted. Then the result was obtained by using the following formula:

The number of total viable organisms per ml of sample = Average number of colonies x dilution factor. Therefore, The number of total viable organisms per gm of fecal sample = Average number of colonies × dilution factor × 10.

3.16 Statistical analysis

All data, either measured or calculated will be for a Completely Randomized Design (CRD) with three replications for each probiotic and antibiotic level. The results were expressed as mean ± standard deviation of mean. Means were analysed by one-way analysis of variance, followed by the Duncan post hoc test to determine significant differences in all the parameters among all groups using the SPSS computer program (Version 20.0; SPSS).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Effect on body weight

Body weight in different dietary treatments during experimental periods was almost similar and the differences were not significant (P> 0.01) (Table 4.1).These results indicate that the body weight slightly decrease in the dietary treatment at that in T_2 (Exolution, 60gm/100kg) in comparison to others showing no significant difference. These results are similar to the findings of Kurtoglu *et al.* (2007) who reported that there was no significant difference between the control and other treatment groups on body weight of laying hens using probiotics.

Table 4.1 Effect of Protexin, Exolution and Renamycin supplementation on body weight (Kg) of laying hens

Parameters	**Bodyweight (Kg)**				**Levels of Significance**
	T_0	T_1	T_2	T_3	
Initial body weight	2.00 ± 0.025	1.99 ± 0.044	2.01±0.064	1.85± 0.038	NS
Final body weight	2.01 ± 0.038	2.00 ± 0.055	1.99± 0.045	1.97± 0.074	NS
Mean±SEM	2.00 ± 0.017	1.998 ± 0.044	2.003 ± 0.087	1.91 ± 0.045	
Levels of Significance	NS	NS	NS	NS	

Diets were supplemented with Protexin(30gm/100kg), Exolution (60gm/100kg) and Renamycin (100gm/100kg) and were fed for 8 weeks; values are expressed as mean ± standard error of mean at least 3 replications each of which contains 4 birds; differences among the treatments most of the parameters were expressed as NS= Not significant, *= Significant at 5% level of significance, **= Significant at 1% level of significance

4.2 Laying performances

4.2.1 Egg production

The hen-day-egg production observed in different dietary treatments was almost similar and the differences were statistically non-significant (P> 0.01) at initial stage where it was nearly about 75% (Table 4.2). Result indicates that the feeding of Protexin, Exolution and Renamycin meal in the diet of laying hen has insignificant effect on egg production from first to fourth weeks but has significant effect on egg production from 5^{th} to 8^{th} weeks. Feeding of Protexin and Exolution mixed meal showed slightly higher egg production than others whereas the production was slightly increase (89% in 8^{th} week) when the birds received T_1 (Protexin) mixed meal in the diet. These results are closed with the previous report of Kurtoglu *et al.* (2004) showed that probiotic effect on egg production was not specific until day 60, but significant increase in egg production by probiotic supplementation were seen on days 60-90 of their experiment. However slightly differed from the observations of Mohiti *et al.* (2007), who found decreased egg production by using the probiotics.

Table 4.2 Effect of Protexin, Exolution and Renamycin on Egg production (%) of laying hens

Parameters	Egg production (%)				Levels of Significance
	T_0	T_1	T_2	T_3	
1st week	78.57 ± 3.13	88.10 ± 3.28	82.14 ±3.91	77.38± 1.75	NS
2nd week	78.57 ± 3.13	73.81 ± 3.65	79.76± 4.09	79.76 ± 4.44	NS
3rd week	76.19± 3.65	86.90 ± 3.28	75.00±3.45	79.76 ± 3.71	NS
4th week	72.62 ± 3.82	85.71 ± 3.26	84.52± 4.04	78.57± 3.97	NS
5th week	70.24 ± 4.44[a]	86.90 ± 4.09[b]	78.57± 3.97[ab]	70.24 ± 3.71[a]	*
6th week	69.05 ± 4.19[a]	85.71 ± 3.69[b]	83.33± 3.98[b]	73.81 ± 4.39[a]	*
7th week	77.38± 3.82[ab]	83.33± 2.63[b]	80.95± 2.38[b]	71.43± 3.12[a]	*
8th week	78.57 ±3.97[ab]	89.29± 2.77[b]	80.95± 3.40[ab]	75.00 ± 3.26[a]	*
Mean±SEM	75.14±1.34[a]	84.97±1.76[c]	80.65±1.05[b]	75.74±1.31[a]	**

Diets were supplemented with Protexin(30gm/100kg), Exolution(60gm/100kg) and Renamycin (100gm/100kg) and were fed for 8 weeks; values are expressed as mean ± standard error of mean at least 3 replications each of which contains 4 birds; differences among the treatments most of the parameters were expressed as NS= Not significant, *= Significant at 5% level of significance, **= Significant at 1% level of significance

4.2.2 Egg weight

The egg weights in different dietary treatments during experimental periods were statistically significant ($P<0.01$) and gradually increased in T_2 (67.84g in 8th week) treatment group compare to control group (63.80) (Table 4.3). These results indicate that inclusion of Protexin, Exolution and Renamycin in the diet of laying hens has effect on egg weight. The higest egg weight was found in T_2 (67.84 gm).The results agree with the findings of (Nahashonb *et al*., 1992; Tortuero and Fernandez, 1995) but differ with the report of Balevi *et al*. (2001).

Table 4.3 Effect of Protexin, Exolution and Renamycin supplementation on Egg weight (gm) of laying hens

Parameters	Egg weight (gm)				Levels of Significance
	T_0	T_1	T_2	T_3	
1st week	63.41 ± 0.68^{a}	62.19 ± 0.84^{a}	66.33 ± 0.90^{b}	65.80 ± 0.52^{b}	**
2nd week	63.35± 0.58^{a}	63.81± 0.58^{a}	67.48± 0.52^{c}	66.00± 0.30^{b}	**
3rd week	64.13± 0.66^{ab}	62.54± 0.59^{a}	66.73±0.68^{c}	64.76 ± 0.30^{b}	**
4th week	61.74 ± 0.80^{a}	62.54± 0.68^{a}	64.88± 0.67^{b}	64.52 ± 0.38^{b}	**
5th week	63.77 ± 0.55^{a}	63.44± 0.76^{a}	67.66 ± 0.47^{c}	65.98 ± 0.44^{b}	**
6th week	63.64 ± 0.57^{a}	64.56± 0.44^{ab}	67.57± 0.57^{c}	65.82± 0.29^{b}	**
7th week	61.84± 0.77^{a}	62.67± 0.36^{a}	65.09± 0.65^{b}	64.61± 0.33^{b}	**
8th week	63.80 ± 0.55^{a}	63.45± 0.76^{a}	67.84 ± 0.47^{b}	66.03 ± 0.44^{b}	**
Mean±SEM	63.21±0.32^{a}	63.15±0.28^{a}	66.69±0.41^{c}	65.44±0.24^{b}	**

Diets were supplemented with Protexin(30gm/100kg), Exolution (60gm/100kg) and Renamycin (100gm/100kg) and were fed for 8 weeks; values are expressed as mean ± standard error of mean at least 3 replications each of which contains 4 birds; differences among the treatments most of the parameters were expressed as NS= Not significant, *= Significant at 5% level of significance, **= Significant at 1% level of significance

4.2.3 Feed Intake

The feed intake of laying hens in different dietary treatments during experimental periods was almost similar and the differences were non- significant (P >.01) which is similar to the study of Mahdevi *et al.* 2005. Saada *et al.* (2010) who reported feed intake values of different treated groups were approximately similar with probiotic supplement. Only in 2nd and 6th week it become significant. Ramasami *et al.* 2008 shows that supplementation did not influenced feed intake. Yousefi and Karkoodi (2007) reported feed consumption was not affected by the dietary probiotic supplementation.

Table 4.4 Effect of Protexin, Exolution and Renamycin on feed intake (gm) of laying hens

Parameters	Feed intake (gm)				Levels of Significance
	T_0	T_1	T_2	T_3	
1st week	120.33 ± 0.74^{ab}	117.90± 0.74^{a}	119.07 ± 0.70^{ab}	121.18± 0.76^{b}	*
2nd week	122.62± 0.33^{c}	119.07± 0.57^{a}	118.98± 0.35^{a}	120.75± 0.59^{b}	**
3rd week	121.31± 0.51^{b}	119.38± 0.52^{a}	119.35±0.56^{a}	120.41 ± 0.61^{ab}	*
4th week	121.71± 0.60b	118.95± 0.63^{a}	120.09± 0.58^{b}	119.97 ± 0.77^{ab}	*
5th week	121.41± 0.63^{b}	118.54± 0.70^{a}	119.48 ± 0.70^{ab}	120.66 ± 0.75^{b}	*
6th week	122.65± 0.37^{b}	118.64± 0.49^{a}	119.49± 0.57^{ab}	120.79± 0.66^{ab}	**
7th week	121.83± 0.46^{b}	119.13± 0.67^{a}	119.50± 0.71^{a}	119.70± 0.67^{a}	*
8th week	121.57± 0.54^{b}	118.92± 0.64^{a}	119.63± 0.67^{a}	120.52 ± 0.65^{ab}	*
Mean±SEM	121.69± 0.26^{d}	118.82± 0.16^{a}	119.45± 0.12^{b}	120.50± 0.17^{c}	**

Diets were supplemented with Protexin(30gm/100kg), Exolution (60gm/100kg) and Renamycin (100gm/100kg) and were fed for 8 weeks; values are expressed as mean ± standard error of mean at least 3 replications each of which contains 4 birds; differences among the treatments most of the parameters were expressed as NS= Not significant, *= Significant at 5% level of significance, **= Significant at 1% level of significance

4.2.4 Feed Efficiency (FE)

The Feed Efficiency (FE) in different dietary treatments during experimental periods were statistically significant ($P<0.01$). The best FE was found in T_2 (1.75 in 5th week) treatment group (Table 4.5) and others treatment group like T_1, T_3 have also lower FE than that of control. These results indicate that inclusion of Protexin, Exolution and Renamycin in the diet of laying hens has effect on Feed Conversion Ratio. After overall it shows that best FE found in T_1. This study shows the similar result of Jagdish *et al.*, 1993, Alvarez *et al.*, 1994, Hamid *et al.*, 1994, Silva *et al.*, 2000 while other suggest no such effect on feed conversion ratio Samanta *et al.*, 1997,Gohain*et al.*, 1998,Panda *et al.*, 1999,Ahmad 2004 could not detect any difference in Feed efficiency.

Table 4.5 Effect of Protexin, Exolution and Renamycin on Feed Efficiency of laying hens

Parameters	Feed Efficiency				Levels of Significance
	T_0	T_1	T_2	T_3	
1st week	1.90 ± 0.02^{b}	1.90 ± 0.031^{b}	1.80 ± 0.031^{a}	1.85 ± 0.02^{ab}	*
2nd week	1.94 ± 0.016^{c}	1.87 ± 0.02^{b}	1.76 ± 0.015^{a}	1.83 ± 0.013^{b}	**
3rd week	1.89 ± 0.02^{b}	1.91 ± 0.017^{b}	1.78 ± 0.019^{a}	1.86 ± 0.012^{b}	**
4th week	1.98 ± 0.03^{b}	1.91 ± 0.023^{ab}	1.84 ± 0.022^{a}	1.86 ± 0.019^{a}	**
5th week	1.91 ± 0.018^{c}	1.87 ± 0.021^{bc}	1.75 ± 0.015^{a}	1.83 ± 0.019^{b}	**
6th week	1.92 ± 0.018^{c}	1.84 ± 0.016^{b}	1.77 ± 0.017^{a}	1.83 ± 0.012^{b}	**
7th week	1.97 ± 0.026^{b}	1.90 ± 0.026^{ab}	1.83 ± 0.022^{a}	1.85 ± 0.016^{a}	**
8th week	1.90 ± 0.018^{c}	1.88 ± 0.024^{bc}	1.88 ± 0.015^{a}	1.83 ± 0.018^{ab}	**
Mean±SEM	1.92 ± 0.012^{d}	1.88 ± 0.019^{c}	1.80 ± 0.09^{a}	1.84 ± 0.015^{b}	**

Diets were supplemented with Protexin(30gm/100kg), Exolution (60gm/100kg) and Renamycin (100gm/100kg) and were fed for 8 weeks; values are expressed as mean ± standard error of mean at least 3 replications each of which contains 4 birds; differences among the treatments most of the parameters were expressed as NS= Not significant, *= Significant at 5% level of significance, **= Significant at 1% level of significance

4.4 Bacterial colony count

Table 4.6 shows the effect of varying doses of Protexin (30gm/100kg) {T_1}, Exolution (60gm/100kg) {T_2} and Renamycin (100gm/100kg) {T_3} supplementation in diets on excreta cultivable bacterial colony counts. Supplementation of Protexin, Exolution and Renamycin in the diets significantly ($P<0.01$) decreased the population of harmful bacterium, *Escherichia coli,* and total culturable bacteria than those of control. The highest colony count was found in control group T_0 (295) and lowest found in T_3 (106) treatment group. This may partly explain the variation in experimental results. A non-significant decrease was found in colony-forming units of bacterial count in feces with the supplement of Renamycin. T Watkins and Kratzer reported that chicks dosed with *Lactobacillus* strains had lower numbers of coliforms in cecal macerates than the control. Francis *et al* also reported that the addition of *Lactobacillus* product at 75 mg/kg of feed significantly decreased the coliform counts in the ceca and small intestine of turkeys. Using gnotobiotic chicks, Fuller (2001) found that host-specific *Lactobacillus* strains were able to decrease *Escherichia coli* in the crop and small intestine. Kizerwetter-Swida and Binek demonstrated that *L. salivarius* 3d strain reduced the number of *Salmonella*

enteritidis and *Clostridium perfringens* in the group of chickens treated with Lactobacillus. Watkins *et al*. similarly observed that competitive exclusion of pathogenic *E. coli* occurred in the gastrointestinal tract of gnotobiotic chicks dosed with *L. acidophilus*. Recently Yaman *et al.*; Mountzouris *et al.* and Higgins *et al.* demonstrated that probiotic species belonging to *Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida,* and *Saccharomyces* have a potential effect on modulation of intestinal microflora and pathogen inhibition.

Table 4.6 Effect of Protexin, Exolution and Renamycin on bacterial load in faeces of laying hen (E. coli)

Parameters	Bacterial load in faeces				Levels of Significance
	T_0	T_1	T_2	T_3	
Initial	240.67± 7.45	257.67±6.39	245.33 ± 7.96	250.67 ± 5.68	NS
4th week	268.54^{a}±11.6	181.23^{b}± 13.4	151.38^{c}± 8.42	148.44^{c}± 18.46	*
8th week	295.54^{a}±16.3	136.33^{b}± 11.4	113.49^{c}± 8.42	106.49^{c}± 8.42	*

Diets were supplemented with Protexin (30gm/100kg), Exolution (60gm/100kg) and Renamycin (100gm/100kg) and were fed for 8 weeks; values are expressed as mean ± standard error of mean at least 3 replications each of which contains 4 birds; differences among the treatments most of the parameters were expressed as NS= Not significant, *= Significant at 5% level of significance, **= Significant at 1% level of significance

CHAPTER V

SUMMARY AND CONCLUSIONS

The study was carried out to assess, examine and quantify the effect of two types of Probiotics (Protexin and Exolution) and one Antibiotic (Renamycin) supplementation on production performance and microbial load of laying hens at the latter stage (56-63 weeks) of production. The field trial was conducted at the poultry farm and lab test was performed at Microbiology laboratory, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. 48 Hisex Brown laying hens of 56 weeks old were allocated to 4 treatments and three replications each containing 4 birds. Laying hens were randomly alloted to 4 dietary treatments T_0 (control), T_1 (Protexin-30gm/100feed), T_2 (Exolution--60gm/100kg of feed),T_3 (Renamycin -100gm/100kg of feed). Egg production, feed intake, feed efficiency, egg weight and microbial load were recorded and compared. We found that egg production, egg weight and feed efficiency were increased at supplementation of Probiotics and Antibiotic compared to control.

Therefore it is concluded that feeding of Protexin and Exolutions howed slightly higher egg production than control whereas the production was slightly increase (89% in 8^{th} week) when the birds received T_1 (Protexin).

The egg weights were gradually increased in T_2 (67.84g in 8^{th} week) treatment group. These result indicate that inclusion of Exolutionin the diet of laying hens has effect on egg weight.

The feed efficiency were gradually decreased by using Protexin and Exolution. The best feed efficiency was found in T_2 (1.75 in 5^{th} week) treatment group than others treatment group.

The present results indicate that probiotics could be successfully used as nutritional tools in poultry feeds for promotion of production, pathogen inhibition and a better feed efficiency of poultry.

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