COMPARATIVE EFFICACY OF SYNTHETIC PROBIOTICS AND YOGURT ON EGG PRODUCTION, BODY WEIGHT AND HEMATOLOGICAL PARAMETERS OF LAYER

A THESIS
BY

SHYAMOL CHANDRA RAY
Registration No. 1405113
Session: 2014-15
Semester: January-June, 2016

MASTER OF SCIENCE (M.S.)
IN
PHARMACOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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Submitted to the Department of Physiology & Pharmacology
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Approved as to the style and content by

(Dr. Md. Mahmudul Hasan)	**(Dr. Fahima Binthe Aziz)**
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DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

JUNE, 2016

DEDICATED

TO MY

BELOVED PARENTS,

TEACHERS AND WELL

WISHERS

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ABSTRACT

The study was carried out to compare the efficacy of yogurt from synthetic probiotic on egg production, body weight and hematological parameter in layer at the about age of 80-85 weeks Hi Sex Brown breed. The study was conducted under the department of Physiology and Pharmacology. At 80 weeks of age, forty Hi Sex Brown breed were randomly divided into 4 groups (T_0, T_1, T_2 and T_3) and each group remain 10 hens. Group T_0 was kept for control, Group T_1 was treated with Probiotics (Protexin^R) with water at a dose of 1.5gm, Group T_2 were treated with Yogurt (Sour curd), Group T_3 was treated with combination of both probiotic(Protexin^R) and Yogurt. Over the course of the trial, observations were recorded for egg production, body weight and, hematological parameter. Egg production were increased significantly ($p<0.05$) in all treated groups in respect to the control group and highest was recorded in combine Group D treated with Probiotic and Yogurt Body weight gain were not significantly increase with compare to the treated group. Hematological parameter no significant ($p>0.05$) differences were observed among ESR (Erythrocyte Sedimentation Rate) and TLC (Total Leucocyte Count) of the treatment groups in case of hematological values in respect to the control group after treatment. But hematological parameter were significantly different between the PCV (Packed Cell Volume) at the level of ($p>0.05$) and Hb (Hemoglobin) level were significantly different ($p>0.01$). The present study reveals that combine Probiotic and Yogurt treatment (Group T_3) was the best effective on egg production and profitability without making any health hazard of aged breed among all the treated groups.

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LIST OF ABBREVIATIONS

B. wt.	:	Body weight
Conc.	:	Concentration
Cu mm	:	Cubic millimeter
d.w.	:	Drinking water
ESR	:	Erythrocyte Sedimentation Rate
et al.	:	Associates
Fig.	:	Figure
gm/g	:	Gram
Hb	:	Hemoglobin
HSTU	:	Hajee Mohammad Danesh Science and Technology University
i.e.	:	That is
J.	:	Journal
Kg	:	Kilogram
Lit/L	:	Litre
Ltd.	:	Limited
mg	:	Milligram
ml	:	Milliliter
mm^3	:	cubic millimeter
No.	:	Number
PCV	:	Packed Cell Volume
PM	:	Population Mean
SEM	:	Standard Error of mean
SM	:	Sample Mean
TEC	:	Total Erythrocyte Count
Vol.	:	Volume
µg	:	Microgram
%	:	Percent
&	:	And
@	:	At the rate of
<	:	Less than
>	:	Greater than
^0C	:	Degree centigrade
FCR	:	Feed Conversion Ratio
Tk.	:	Taka
NS	:	Non-significant
*	:	5% Level of significant
**	:	1% Level of significant

CHAPTER I

INTRODUCTION

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INTRODUCTION

Poultry is one of the most important and promising industrial sectors for the economic development of Bangladesh. Traditionally, poultry rearing was considered as a small scale operation and an additional source of income for the rural people. At the-doorstep of 21st century, there are many commercial sectors, which make the globalization concept to work and for strengthening the future economic development.

Although poultry industry is developing in Bangladesh, it is facing some constraints Feed is the main problem which influences the production cost. Feed additives, like antibiotics, hormones and coccidiostats are used in the diet of poultry to improve the efficiency of poultry production. However, antibiotics and hormones have harmful effects on poultry as well as human health. Recently, it is believed that probiotics have beneficial effects to improve the productive performance of poultry.

As defined by Crawford (1979) the term probiotic is a culture of specific living micro-organisms (Primarily Lactobacillus spp.) which implants in the animal to ensure the effective establishment of intestinal populations of both beneficial and pathogenic organisms. Probiotics are specific chemical agents produced by micro-organism containing Lactobacillus acidophilus *Lactobacillus casei*, *Bifidobacterium bifidum,*

Aspergillusoryzae and *Torulopsis* (Mohan *et al.,* 1995). Fuller (1988) defined probiotics as "A live microbial feed supplement, which beneficially affects the host by improving its intestinal microbial balance". The US National Food Ingredient Association defined, probiotic (direct fed microbial) as a source of live naturally occurring microorganisms and this includes bacteria, fungi and yeast (Miles and Bootwalla, 1991).

Probiotic is a microorganism or combination of microorganisms which selectively suppress the harmful bacteria (Salmonella, E. coil, etc.) in the gut of living beings. Probiotics also contain other substances to improve the intestinal microbial balance. The adverse effect of antibiotic feeding encouraged a shift in favors of feeding probiotics to boost up productive performance of chickens (Fuller, 1988). Impact of biotechnology in poultry nutrition has significant importance. Biotechnology plays a vital role for efficient utilization of feed and better production. So it is imperative to the poultry nutritionists to

use these resources in the diet of poultry to increase the efficiency of production. Improved egg production, feed conversion ratio, egg weight, specific gravity were observed by adding probiotic in the diet of layer (Nahashon *et al.*, 1994c, 1996a, 1996b, Mohan *et al.*, 1995; Nahashon *et al.*, 1992; 1993; 1996a; Tortucro and Fermander, 1995; Nahashon *et al.* 1994b). During the laying phase, supply of 153g CP/kg diet with Lactobacillus produced significantly larger eggs (P .05) than those given a similar diet without Lactobacillus (Nahashon *et al*., 1996a; Molauti *et al*., 1995; Tortuero and Fernander, 1995). But some authors found the reduced egg production and feed conversion efficiency in using probiotic in layer hen (Nahashon *et al*., 1994a; Goodling *et al.*, 1987).

All of the probiotics available in the market contain Lactobacilli and/or Streptococci. When the Lactobacilli are offered orally, they are able to migrate from the gut to the systemic circulation. They can translocate and survive many days in the spleen, liver and lungs. Cell wall products may have a co-stimulatory role on the induction of the systemic immune response (Fuller, 1988; Erickson and Hubbard, 2000).

Oral administration of *Lactobacillus casei* has been reported to enhance the activity of splenic NK cells and to stimulate phagocytic activity (Saito *et al*., 1981; Matsuzaki *al et*., 1998). But there is a scarcity reports on its usage of yogurt on laying hen. For this reason the present study was conducted to evaluate the potential of yogurt feeding in drinking water in laying hens in order to observe their influence on feed intake, laying performance, body weight gain and hematological parameter.

According to the information received from the manufacturer, probiotics have the following characteristics:

- Microorganism of probiotic can requisite and adjusts within a shortest possible time.
- Multiple species product is better than single species product.
- The stability of micro flora may be affected by many factors like change of feed, vaccination, intestinal pH, bile salt concentration in the gut and use of antibiotics.
- Many strains of lactic acid producing bacteria are resistant to antibiotics.
- It must have rapid colonizing ability and strong foothold in the gut. A probiotic available in the market with trade name protexin has the similar

characteristics. So, present study was undertaken to assess the effects of protexin(R) and yogurt on layer performance.

Objectives

- To study the efficacy of protexin(R) and yogurt on egg production.
- To study the efficacy of protexin(R) and yogurt on body weight and cost benefit.
- To study the effect of protexin(R) and yogurt on hematological parameters.
- To know the adverse effects related to treatment.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

With regard to the effect of probiotic, many research institutes, libraries were contacted and-internet web sites and computer databases were searched during the course of the experiment. It appeared that a lot of experiments were conducted in terms of probiotic using single or double species of bacteria but in terms of probiotic using multiple species of bacteria, yeast, and mold, limited information was available. However, the information collected through deliberate study are reviewed in this chapter.

2.1 Probiotics

Probiotics are feed supplement of live microbial origin which has beneficial effect on the host animal by improving the intestinal microbial balance. Although the word 'probiotic' was only used a few decades ago, but it has an aged and interesting history. The term probiotic was first introduced by Lilly and Stilwell, (1965) to describe the growth promoting factors produced by microorganisms. A real understanding of how probiotics function began when the Nobel Prize winner Russian physiologist, (Metchnikoff, 1907), introduced his intoxication theory. He stated that the main cause of aging is "toxicants" formed by intestinal putrefaction and fermentation and suggested drinking beverages such as yoghurt containing lactic acid bacteria would prevent aging. The concept of probiotics applied to preventive medicine. A he postulated that the longevity observed in the Balkan' person was due to the regular Consumption of soured milk containing *Lactobacillus bulgaricus.* Lactobacilli suddenly attracted world attention. In spite of unknown data or limited understanding on composition and physiology of gut microflora, from the results obtained with probiotic use has provided more knowledge and experience at least to understand how probiotics should be used for better performance. Several microorganisms have been identified as could be used as probiotics- such as different types of *Lactobacillus* e.g. *L. acidophilus, L. reuteri, Streptococcus faecium, S. lactis, Saccharomyces cerevisiae, Bacillus coagulans, Bacillus subtilis* and so on. Functional foods as a marketing term was initiated in Japan in the late 1980s and is used to describe food fortified with ingredients capable of producing health benefits. This concept is becoming increasingly popular with consumers because of a heightened awareness of the link between health, nutrition and diet. In Japan, a standard

was developed by the Fermented Milks and Lactic acid bacteria beverages association stipulating that a product should contain 1 x 10^7 viable bifidobacteria/gm or ml product to be considered a probiotic food (lshibashi and Shimamur, 1993).

2.1.1 Biotechnological aspect of probiotics

The biotechnology has a very important role in improving feed utilization capacity of birds and animals. Feed grade enzymes which are expected to utilize as feed additives to improve digestibility of broiler. Identification of microbes, which produce useful enzymes, identification of genes responsible for production of such enzymes and their manipulations has vast application in animal and broiler nutrition. Newer and newer species of beneficial microorganisms are identified as generally recognized as safe i.e. Generally Recognized As Safe (GRAS) listed. Some of these are native Organisms and support them in getting established in the gut. Specific strains are genetically to make them suitable for animal feed, e.g. feed processing viability, lactic acid production, enzyme production, shelf life etc. Lactobacilli were the first genus of bacteria proved to have health benefits. Lactobacilli have been shown to be present in the gastrointestinal tract of most of animals and birds. They lower the pH of gut by converting sugar to lactic acid, which inhibits the growth of enter pathogens. Lactobacillus sp. can get quickly colonized in the gut epithelium to deprive sites for attachment of pathogens. They have immunoregulatory actions by increasing macrophage activity and also by enhancing the production of immunoglobulins globally.

2.1.2 Different types of probiotics

- Synthetic probiotic
- Natural probiotic (Yogurt)
  i. Sweet yogurt
  ii. Sour yogurt

Synthetic probiotic of different company

Products	Company	Target Animal	Total CSU count	Bacterial Strain	Yeast Strain
Protexin	Ciba-Geigy	Pig	2×10^8/ GM	S. faecium	Apergillusoryzae
		Poultry		S. thermophilus	Torulopsis sp.
		Cattle		Lactobacillus easei	
		Horses		L plantarum	
		Sheep		L bulgaricus	
		Turkey		L acidophilus	
				B. bifidum	
Digester	ACI	poultry		L acidophilus	
		Fish		Bacillus polyfermenticus	
				Clostridium butyricum	
Lactosaac	All Tech	Pig	1.3×10^7	L acidophilus	Sacchoromycescere visiae
		Poultry		S. faecium	
Yeasacc	All Tech	Cattle			Sacchoromycescere visiae
		Sheep			
		Horses			
Toyocerin	Toyo Jazo	Pig	10×10^{10}	Bacillus toyoi	
		Poultry			
		Cattle			
		Fish			

2.1.3 Mode of Action of probiotic

Protexin^R has an effect on the small intestine by:

1. Suppression of viable count:

a) Production of antibacterial compounds
b) Competition for nutrients
c) Competition for adhesion sites

2. Alteration of microbial metabolism

a) Increased enzyme activity
b) Decreased enzyme activity

3. Stimulation of immunity

a) Increased antibody levels
b) Increased macrophage activity-

2.1.4 Type of probiotic and micro-organisms used

There are several types of probiotics available in the market are to be used in poultry, with arrange of micro-organisms present and, therefore, with different metabolic activities and action modes. Also, they present variations as to the capacity of colonizing the intestine or not, which justify variations on the results of their use. *Bacillus*, *Bifidobacterium*, *Enterococcus*, *E. coli*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus* species, and a range of yeast species and non-defined mixed cultures have been used (Fuller, 1992; Patterson and Burkholder, 2003; Kabir *et al*., 2004; Mountzouris *et al.*, 2007). However, even those belonging to the same species can have different strains and even these different strains from the same species can have different metabolic activities. These bacteria are used alone or in combination (Miles, 1993; Montes and Pugh, 1993). Non-defined mixed cultures, known as competitive exclusion cultures, are normally related to the treatment of one-day chicks with an indefinite microbiota derived from adult animals resulting in resistance to colonization against pathogenic micro-organisms. Among the colonizing species, *Lactobacillus sp.*, *Enterococcus sp.* and *Streptococcus sp*. Are worth mentioning, and among the non-colonizing species, *Bacillus spp.* (spores) and *Saccharomyces cerevisiae* (Zikic *et al*., 2006 and Peric *et al*., 2009). Another characteristic of probiotics is that some micro-organisms are constituted by microorganisms normal to the intestinal micro biota of poultry, and others by bacteria different from the ones from the digestive tract. According to Kabir (2009) the most commonly used species are: *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,* and except for *Lactobacillus bulgaricu s*and *Streptococcus*

thermophilus, all the remaining ones are intestinal strains. Recently, emphasis has been given to the selection, preparation and application of probiotic strains, especially lactic acid bacteria (Wang and Gu, 2010). Natural adaptation of lactic acid bacteria to intestinal environment and the lactic acid produced by them have provided advantages for these organisms over other microorganisms used as probiotic (Guerra *et al*., 2007).

2.2 Yoghurt

Yoghurt is the most popular fermented milk product in the world. Traditional yoghurt is also considered as probiotics. Generally, yoghurt are two types sweet and sour. Sweet yoghurt is generally prepared from mixed culture of *Streptococcus lactis*, *Streptococcus thermophilus*, *Streptococcus citrophilus*, and *Lactobacillus planterum*. Sour yoghurt is prepared by seeding milk with a combination of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Usually, the starter culture containing *Streptococcus lactis*, *Streptococcus thermophilus, Lactobacillus bulgaricus* and *Lactobacillus acidophilus* used for manufacture of yoghurt. In fresh yoghurt the amount of these in microorganisms (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) together are in a concentration of 10^8 cells/ ml.

2.2.1 Composition of Yogurt:

- *Streptococcus lactis*
- *Streptococcus thermophiles*
- *Streptococcus citrophilus*
- *Lactobacillus planterum.*
- *Lactobacillus bulgaricus*
- *Bifidobacteria*
- *Lactobacillus delbruecki subsp. bulgaricus*

2.2.2 Nutritional value per 100 g (3.5 oz) from USDA database

Energy	257 kJ (61 kcal)
Carbohydrates	4.7 g
Sugars	4.7 g (*)
Fat	3.3 g
Saturated	2.1 g
Monounsaturated	0.9 g
Protein	3.5 g
Vitamins	
Vitamin A equiv.	(3%) 27 µg
Riboflavin(B2)	(12%) 0.14 mg
Trace minerals	
Calcium	(12%) 121 mg

2.2.3 Comparison of Whole Dairy Milk and Plain Yogurt from Whole Dairy Milk, One Cup (245 g) Each from USDA database

Property	**Milk**	**Yogurt**
Calories	146	149
Total Fat	7.9 g	8.5 g
Cholesterol	24.4 mg	11 mg
Sodium	98 mg	113 mg
Total Carbohydrates	12.8 g	12 g
Protein	7.9 g	9 g
Vitamin A	249 IU	243 IU
Vitamin C	0.0 mg	1.2 mg
Vitamin D	96.5 IU	~
Vitamin E	0.1 mg	0.1 mg
Vitamin K	0.5 µg	0.5 µg
Thiamine	0.1 mg	0.1 mg
Riboflavin	0.3 mg	0.3 mg
Niacin	0.3 mg	0.2 mg
Vitamin B6	0.1 mg	0.1 mg
Folate	12.2 µg	17.2 µg
Vitamin B12	1.1 µg	0.9 µg
Choline	34.9 mg	1.0 mg
Biotin	1.5 mg	~
Water	215 g	215 g
Ash	1.7 g	1.8 g

2.2.4 Function of yoghurt

- ❖ Yoghurt could inhibit the growth of intestinal carcinoma through increased activity of Ig A, T cells and macrophages.
- ❖ Yoghurt increases the bioavailability of many essential nutrients such as Ca, Mg and Zn ions.
- ❖ Yoghurt allows the absorption of lactose in hydrolyzed form.

2.3 Protexin^{R} and Its mood of action

Ptotexin^{R} is a multistrain probiotic containing a selection of strains representative of the flora of several animals species and some micro-organisms. Such a preparation will be active against a wide range of conditions in several animal species. All the strains would not be expected colonise or be active in every case; different conditions in different animals. Protexin^{R} product is a freeze dried preparation containing live viable stains of naturally occurring micro- organisms.

- ➢ *Bacillus polyfermenticus n sp.*
- ➢ *Lactobacillus acidophilus*
- ➢ *Clostridium butyricum*
- ➢ *Streptococcus faecium*
- ➢ *Lactabacillusbulgaricus*
- ➢ *Streptococcus thermophilus*
- ➢ *Lactobacillus plantarum*

The product contains two yeasts: *Torulopsiss spp*. and *Aspergillusoryzae*, which produce protease, a-amylase, lipase, cellulase to enhance the digestion and able to digest of fiber.

Lactobacillus acidophilus

a) Stimulates immunity (Perdigon *et al*., 1990)
b) Suppresses microbial enzymes activity involved in production of carcinogens (Goldin *et al*., 1984)
c) Promotes growth of farm animals (Tortucro, 1973, King, 1968, benchman *et al*., 1977).
d) In gnotobiotic chicks antagonises Salmonella typhimurium, Staphylococcus aureus and E. coil (Watkins and Miller, 1983).

Lactobacillus plantarum

a) Characteristic lumen organism
b) Ferments wide range of carbohydrates
c) Acid tolerant(Jensen *et al.,* 1956)
d) Production of organic acids and bacteriocins (Konissky, 1982; West and Warner, 1988; Daesche, 1990; Anderson, 1981)

Lactobacillus rhamnosus

a) Stimulation of immunity (Perdigon *et al.,* 1990)
b) Macrophage activation (Kati and Mutai, 1981)
c) Prevents difficult diarrhea (Garbach *et al.,* 1987)

Lactobacillus delbrueckii sub sp. bulgaricus and

Streptococcus salivarius sub sp. Thermophilus

a) In vitro neutralization of E. coil (Mitchell and Kenworthy, 1976)
b) Protects against Salmonella enteritidies infection (Hitchins *et al.,* 1985)
c) Enhances immunity by stimulating interferon production

Bifidobacteriumbifidum

a) In gnotobiotic chicks it antagonises Proteus vulgaris and Klebsiella pneumonia (Timosshko *et al.,* 1981)
b) Prevents translocation of E. coil (Bianchi-salvadiri *et al.,* 1981)

Enterococcus faecium

a) Prevents E. coil diarrhea (Underdahal *et al.,* 1982)
b) Increases cellulytic activity in caecum of chicken
c) Antagonises Salmonella typhimurium in gnotobitic mice

Candida Pintolopessi

Attaches to secretary epithelium of stomach and prevents bacteria colonizing the Surface (1989).

2.3.1 Advantages of Protexin^{R}

The broad spectrum microorganisms in Protexin^{R} have the ability to rapidly colonies the entire gut for maximum probiotic activity; and enhance digestive efficiency.

- Proven effective in worldwide commercial use; reduce mortality; improve weight gains and feed efficiency.
- The yeast *Apergillusoryzae* in Protexin, produces cellulase which helps breakdown cellulose/fiber in feed ingredients.
- This is especially beneficial in young and monogastric animals.
- 1-leips prevent E. coil diarrhea through competition for adhesion sites and neutralization of E. coli toxin.
- Natural product - residue free and no selection for resistance.
- Probiotics protect against Salmonella infections including those caused by Salmonella enteritidis and Salmonella typhimurium.
- Probiotics stimulate immunity to infections by boosting interferon production, immunoglobulin in concentration and microphage activity.
- They suppress clostridial interaction, often associated with intensive livestock production.
- Probiotics have also been shown to be antagonistic to many other harmful bacteria such as Kiebsiellaproteus and Campylobacter.
- Probiotic also non to be effective against the development of cancers of animals.

2.4 Effect of probiotic on egg production

The study observed that increase egg production of laying hen Loh TC. *et al.* (2014). This study showed that Probiotics are beneficial bacteria that are able to colonize the host digestive system, increasing the natural flora and preventing colonization of pathogenic organisms and thus, securing optimal utility of the feed. Another major issue, has been highlighted in relation to the application of antibiotic resistant probiotics, the antibiotic resistant gene can be transferred between organisms. No significant difference ($P > 0.05$) was found among the treatment groups on overall feed intake, egg weight, egg mass and feed conversion efficiency and increases the egg production. Luo J. *et al.* (2010).

The effects of the probiotic, Propioni bacterium jensenii 702 (PJ 702), supplementation on egg productivity, egg shell thickness, fatty acid profile of eggs, and body weight in early layer hens were investigated. Twenty eight twenty-week-old starter pullets were evenly divided into a treatment and a control group for an eight week experiment. Each bird in the treatment group received 107 cfu PJ 702 daily in a total volume of 1 ml by oral administration increase the egg production. S. Mátéová, *et al.* (2009).

Probiotic bacteria are used to balance a disturbed intestinal microflora and dysfunctions of the gastrointestinal tract (GIT). They could be an effective alternative to the use of synthetic substances in nutrition and medicine. We investigated the effect of probiotics and potentiated probiotics on the productivity of laying hens. T. Balevi, *et al.* (2009).

In the present study, the effects of dietary supplementation of commercial probiotic (Protexin^{TM}) on daily 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 *Hysex Brown* layers were fed diets containing either 0, 250, 500 or 750 parts per million (ppm) for 90 days increase the egg production in relation of the control group. Kurtoglu V*et al.* (2004). 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 trigylceride in layer hens were investigated. Krugeret *et al.* (1977) reported that the addition of gentian violet and Lactobacillus culture to the diet of laying hens separately, and their combination at the rate of 454g and 2.27kg per ton increased egg production compared with control diet.

Addition of liquid non-viable Lactobacillus product in the diet of laying hens had no significant influence on hen day production (Cerniglia *et al.,* 1983 and Goodling *et al.,* 1987). Day *et al.* (1987) fed diets containing 0.25 or 0.5% live yeast culture (LYC) and 0.4 or 0.6% total phosphorus (TP) to individually caged hens for a period of 28 days. They observed statistically similar egg production on different LYC levels. Oishi *et al.* (1987) observed significantly lower egg production on diet containing Torula yeast with 0.06 mg/kg selenium for 15 weeks than the control diet with 0.24 mg/kg selenium. Gerendal *et al.* (1992) investigated that the addition of Lacto sacc and Yea sacc (1kg/1000 kg feed) to the laying hens diet increased egg production compared to control diet. Jadhav *et al.* (1992) found egg production rates of 77.14, 74.36, 78.41 and, 78.3 1%

on diet containing 0, 20, 40 and 60g/100g Lactobacillus sporogenes (LSP) respectively from 20-30 weeks of ago. Nahashon *et al.* (1994a) suggested that the supplementation of 1100mg Lacto/kg with 1 or 3% fat in the diet of Dekalb XL Single comb White Leghorn laying hens was better for hen day egg production than the 2200mg Lacto/kg diet with 1 or 3% fat. Nahashon *et al.* (1994b) used diet containing maize-soybean (CS; control), C-S plus condensed cane molasses soluble (CCMS) and C-S plus CCMS Lactobacillus (1100 mg/kg diet; 4.4 x $l0^7$ c.f.u/mg) with 0.25 or 0.45% available phosphorus (SF) in each diet to layers for a period of 28 days. They found lower hen day egg production on Lacto supplemented diet with 0.2.5 or 0.45% AP. Tortuero *et al.* (1995) carried out 3 experiment feeding control diets (Maize or barley based), and the mixture of different Lactobacillus species with control diets on laying hens from 6 to 10 months in the first, 12 to 16 months in the second and 15 to 19 months of age in the third experiment. They got the significantly higher hen day egg production on control diet with Lactobacillus species in all experiments. Moreover, the test diet of experiment 3 was the highest for hen day egg production followed by test diet of experiment 2 and 1.

Koudela *et al.* (1995) reported that pullets given Lactiferm to control diet reached the 50% egg production level by 155 to 166 days old, compared with 163 to 170 days for control pullets. Mohan *et al.* (1995) got better egg production in the diet with Probilac 100mg/kg compared with 0 md 150mg Probiolac/kg of feed for a period of 28 to 38 weeks.

Addition of bacterial culture to the laying hen diet increased egg production (Miles et al 1981 and Abdul Rahim *et al.* 1996) Haddadin *et al.* (1996) found significantly higher egg production on basal diet with Lactobacillus acidophilus at levels up to 4 million viable cells per kg of feed to laying hens for a period of 48 weeks of age than the basal diet.

Huthail Najib (1996) fed basal diets containing 0, 0.1, 0.2 and 0.3% yeast culture to 160, 20 weeks old Baladi (local) and White Leghorn hens for 30 weeks. He observed best hen day production with 0.3% yeast in Baladi and 0.2% in white Leghorn hens. Nahashon *et al.* (1996a) got better egg production on 15.3% CP containing Lactobacillus diet than those of 13.8, 14.3% CP Lacto and 15.3% CP condensed cane molasses soluble diet. Fuller (1997) reported that the use of probiotics under certain conditions has beneficial effect on egg production.

Supplementation of probiotic in the diet of laying hens significantly increased egg production, Crowford (1979), but it was contradicted with the findings of Balevi *et al.* (2001). He found statistically similar egg production when added probiotic in the layer diet.

2.5 Effect of probiotic on live weight gain

An experiment was carried out to evaluate during six weeks the bio-economic effects of tchoukoutou residue in broiler feeding. A total of 225 day-old of mix sex broiler chicks (Ross308) were divided into three dietary treatments of 75 chickens per treatment (three replicates of each). The control diet (Ro) was fed basal diet, whereas 3% of tchoukoutou (Rt) and 0.078% of a yoghourt (Ry) were supplemented to the other groups. The daily feed intake was similar. During the first 21 days, the daily weight gain was significantly higher in Rt treatment, and the feed conversion ratio was lower in Rt (1.89) compared to Ro (2.06) and Ry (2.00). The mortality rate was significantly reduced by the supplementation of both treatments M. F. Houndonougbo, *et al.* (2015).

Aftahi *et al.* (2006) studied the influence of yoghurt and protexin boost on broiler growth, feed intake, feed conversion ratio, livability and profitability production from 1 to 35 days of age of broiler chicks. She concluded that yoghurt (5g/liter of drinking water) and protexin boost (1g/litter of drinking water) could economize broiler production.

Hossain (2004) revealed that yoghurt and protexin boost could not show any beneficial effect on broiler performance at the level tested but was effective in reducing abdominal fat pad, total viable count (TVC) and total coliform count (TCC) while increased bursa weight and length of small intestine. Bhatt *et al.* (1995) fed 4 strains of Lactobacillus bulgaricus ($6.8x\ 10^{10}$ cells /kg feed) in diet of broiler up to 6 weeks of age and observed that only one strain improved survivability during finishing period. Jadhav *et al.* (1992) used diet having dried Lactobacillus sporogenes (LSP) 0, 20, 40 and 60 g/100kg of feed to pullet from 20 to 36 weeks of age. They reported that body weight gain was not affected by intake of LSP. Nahashon *et al.* (1994a) supplied 1100 and 2200 mg Lactobacillus per kg of diet with 1 or 3% fat to laying hens for a period of 28 days. They observed higher body weight gain on 2200 mg/kg diet with 1 or 3% fat as compared to control and 1100 mg/kg diet. Nahashon *et al.* (1994b) used diet containing maize soybean (C-S; control), C-S plus condensed cane molasses soluble (CCMS) and C-S plus

CCM-Lactobacillus with 0.25 or 0.45% available phosphorus (AP) in each diet to layers for a period of 28 days. They observed layers fed on 0.25% AP diets bad a lower weight gain than layers fed on 0.45% AP diet regardless of Lacto supplementation. Kaudela *et al.* (1995) reported that the supplementation of probiotic Lactiferm to the laying hens diet did not significantly increase live weight gain. Fuller (1997) reported that the use of probiotics under certain conditions has beneficial effects on growth rate.

2.6 Effects of probiotic on the external qualities of egg

Day *et al.* (1987) fed diet containing 0.25 live yeast culture (LYC) 0.4% total phosphorus (TP), 0.5% LYC 0.4% TP,0.25% LYC +0.6 TP and 0.5%LYC + 0.6% TP to 3 groups of 12 hens for a period of 28 days. They found highest egg breaking strength in birds fed diet with 0.4% TP. Goodling *et al.* (1987) reported that the supplementation of liquid or dried non viable Lactobacillus fermentater product (LAC) in the laying hen diet with similar protein level did not improve egg weight and the viable LAC product in the diet of laying hen with different protein level also did not improve egg size. Oishi *et al.* (1987) reported lower egg weight on 0.06 mg/kg selenium containing Torula yeast diet than in birds fed 0.24 mg/kg selenium basal diet. Gerendal *et al.* (1992) fed basal diet and basal plus Lacto Sacc and Yeas Sacc diet (l kg/l000kg) of fed to New Hampshire parent. They observed higher egg weight in Lacto Sacc and Yeas Sacc containing diet.

Jadhav *et al.* (1992) conducted an experiment with White Leghorn pullets fed diet supplemented with different levels (0, 20, 40 and 60g/l00g feed) of dried Lactobacillus supergenes power (LSP). They found statistically similar mean egg weight in said diet. Ahmed *et al.* (1994) provided diets el 180/0 CP and ME 2700 kcal /kg and drinking water with or without a probiotic. Lactobacillus sporogens, 1 g litter alone or acidified to pH 4.5 to White Leghorn layers from 33 to 42 weeks old. They found higher egg weight on Lactobacillus sporogens supplemented diet than that of control, and acidified diet.

Nahashon *et al.* (1994b) reported that the incorporation of condensed cane molasses solubles (CCMS) with Lactobacillus (1100mg/kg diet; 4.4 x 10^7 c fu/mg) in the diet produced lower egg mass and laid larger eggs than layers fed on unsupplemented diets. Nahashon *et al.* (1994a) used 1100 and 2200 mg Lactobacillus per kg- of diet with 1 or 3% fat to the Dekalb XL Single comb White Leghorn layer hens for a period of 28 days. The high egg mass, egg weight and egg size were observed on 1100 mg/kg diet with 1 or 3% fat as compared to control.

Huthail Najib (1995) fed control diets supplemented with 0, 0.1, 0.2 and 0.3%yeast culture (Saccharamycescerevisiae) to 20 weeks old Baladi (local) and White Leghorn hens for 30 weeks. He observed higher egg weight with 0.3% yeast in Baladi and 0.2% in White Leghorn hens.

Kaudela *et al.* (1995) reported that the addition of probiotic Lactiferm in the laying hen diet increased egg weight when compared with control diet. Mohan *et al.* (1995) studied effect of probiotic (Probiolac) on egg shell thickness in White Leghorn layers from 28 to 38 weeks of age at the levels of 0, 100 and 150 mg/kg of feed. They observed improved shell thickness on 100mg/kg probiotic supplemented diet compared to control. Nahashon *et al.* (1996a) observed higher egg weight and egg mass on 15.3% CP containing Lactobacillus diet than those of 13.8, 14.3 CP-Lacto and 15.3 condensed cane molasses soluble diet.

2.7 Effect of probiotic on internal qualities of egg

Nahashon *et al.* (1994a) fed basal and basal diet plus 1100 and 2200 mg/kg Lactobacillus with 1 or 3% fat to laying hens for a period of 28 days. They got improved internal qualities of egg with 1100 mg/kg Lactobacillus diet compared to 2200 mg/kg Lactobacillus diet. Tortuero *et al.* (1995) suggested that the basal diet with different Lactobacillus species mixture were better for albumen quality compared to the basal diet (maize or barley based). Abdul Rahim *et al.* (1996) reported that the addition of Lactobacillus acidophilus to the laying hens diet reduced cholesterol concentration in the eggs. Haddadin *et al.* (1996) used basal diet and basal diet supplemented with Lactobacillus acidophilus at levels up to 4 million viable cells per kg of feed to laying hens for a period of 48 weeks of age. They investigated significantly lower yolk cholesterol level on Lactobacillus acidophilus supplemented diet than basal diet.

Nahashon *et al.* (1996a) carried out an experiment using 13.8, 14.3 and 15.30% CP diets each supplemented with lacto 1100mg/kg and 15.3% CP diet supplemented with and without condensed cane molasses soluble (CCMS) to White Leghorn layers for 28 day periods. They observed that interior egg quality and egg shell thickness were not different with layers fed on CCMS-Lacto supplementation diets. They also found highest yolk color score on CCMS-Lacto diets.

2.8 Effect of probiotic on feed consumption and feed conversion

Krugeret *et al.* (1977) reported that the addition of gentian violet and Lactobacillus culture to the diet of laying hens separately and in combination increased feed conversion efficiency than control diets. Goodling *et al.* (1987) reported that the addition of liquid non-viable Lactobacillus fermenter product (LAC) or dried non-viable LAC product did not significantly improve feed conversion efficiency as compared to control. Oishi *et al.* (1987) used control diet with 0.24 mg/kg selenium and a diet containing Torula yeast with 0.06 mg/kg selenium to laying hens for 15 weeks period. They found significantly higher feed consumption in diet containing Torula yeast with 0.06mg/kg selenium. Gerendal *et al.* (1992) observed that the supplementation of Lacto-Sacc and Yea-Sacc (1 kg/1000 kg feed) to the New Hampshire parent fowls reduced fled consumption as compared to control diet.

Jadhav *et al.* (1992) conducted an experiment using diet containing as dried Lactobacillus sporogenes (LSP) 0. 20, 40 and 60 g/l00g of feed to pullet from 20 to 36 weeks of age and observed 2.969, 2.957, 2.829 and 2.906 kg feed intake /kg egg weight respectively. Abmed *et al.* (1994) investigated that the FCR (feed intake/egg mass) were 2.64, 2.29 and 2.39 on basal diet, diet with 1.0g/L Lacto and diet with acidified to pH 4.5 respectively in the White Leghorn layers from 33 to 42 weeks of age. Haddadin *et al.* (1996) used basal diet and test diet (basal diet with Lactobacillus acidophilus) at levels up to 4 million viable celli is /kg feed to laying hens. They got higher feed conversion efficiency on test diet than the basal diet. Nahashon *et al.* (1994b) fed basal diet, basal plus condensed cane molasses soluble (CCMS) and basal plus CCMS-Lactobacillus with 0.25 to 0.45% available phosphorous (AP) in each diet to lying hens. They observed poorer feed conversion efficiency on Lactobacillus supplemented diet with 0.25 or 0.45% AP than basal diet.

Nahashor *et al.* (1994a) used 1100 and 2200 mg Lactobacillus per kg of diet with 1 or 3% fat to laying hens for a period of 28 days. They observed lower feed consumption and higher feed con version efficiency on 11 mg /kg diet with 1 or 3% fat than without Lacto diet. Tortuero *et al.* (I995) conducted 3 experiments by feeding basal diets and the mixture of different Lactobacillus species with basal diets on laying hens from 6 to 10 months in the 1^{st} , 12 to 16 months in the 2^{nd} and 15 to 19 months of age in the 3^{th}

experiment. They observed significantly higher feed conversion efficiency on different Lactobacillus species supplemented diets than basal diet.

Huthail Najib (1995) investigated lowest feed intake and highest feed conversion efficiency on diet with 0.3% yeast culture than the diet with 0, 0.1 and 0.2 % yeast culture to Baladi local and White Leghorn hen during a period of 30 weeks Koudla *et al.* (W95) reported that the addition of probiotic Lactiferm decreased feed intake. Abdul Rahim *et al.* (1996) reported that the addition of Lactobacillus acidophilus to the laying hens diet improved feed conversion efficiency. Nahashon *et al.* (1996a) got higher feed conversion on 15.3% CP containing Lactobacillus diet than those of 13.8, 14.3% CP-Lacto and 15.3% CP condensed cane molasses soluble diet. Fuller (1997) and Crowford (1979) reported that the use of probiotics under certain conditions has beneficial effects on feed conversion efficiency.

2.9 Effect of probiotic on livability

Goodling *et al.* (1978) reported that the supplementation of viable Lactobacillus fermented product to the diet of laying hens with different protein levels did not improve livability.

Gerendal *et al.* (1992) reported that the supplementation of Lact Sacc and Year Sace (1 kg /1000kg feeds) To the New Hampshire parent foods reduced mortality (1%) in experimental groups when compared to controls. Kaudela *et al.* (1995) reported that the supplementation of probiotic Lactiferm to the laying hen diet slightly increased mortality. Huthail Najib (1996) used basal diets containing 0, 0.1,02 and 0.3% yeast culture (Saccharamycescerevisiae) to 160, 20 weeks of age Baladi (local) and White Leghorn hens for 30 weeks. He got better 1ivability on 0.3% yeast in Baladi and 0.2% in White Leghorn hens.

2.10 Effect of probiotic on blood parameter

Devegowda *et al.* (1994). State that probiotics treatment increase the blood Total Erytheocyte Count at the level of ($p<.005$) Dimcho *et al.*, (2005) also found no significant difference ($P>0.05$) in blood haemoglobin, total protein and total cholesterol concentrations when they fed probiotic as a supplement to Muskovy ducks. Baidya *et al*., 1994 and Mohan *et al.,* 1996) and it is attributed to improved health status and

physiological well-being of the birds administered with probiotic and increase the packed cell volume.

Islam *et al.*, (2004); Kundu *et al.* (1993) and Tabinda *et al.* (2012). Erythrocyte sedimentation rate values indicated slight decrease from the normal physiological range of between 3 and 12 mm as was suggested by Jain (1986). The non-significant decrease erythrocyte sedimentation rate mean values was also experienced by Islam *et al.* (2004) who did research on haematological Parameters of Fayoumi, Assil and Local chickens reared in Sylhet Region in Bangladesh.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental design

All the 40 chicken randomly divided into 4 groups (T_0, T_1, T_2 and T_3) for observing the effects of protexin and yogurt on egg production, body weight gain and hematological profile of aged layer.

Chickens of group 'T_0': was kept as control and was treated with normal feed and water.

Chickens of group 'T_1': was treated with probiotics (Protexin^R) @ 1.5 gm/litre in drinking water

Chickens of group 'T_2': was treated with Yogurt (sour curd) @ 15gm/litre in drinking water

Chickens of group 'T_3': was treated with @ 0.75gm probiotics (Protexin^R)/litre water and @ 7.5gm Yogurt/litre in drinking water

All the layer of treated groups were closely observed for 6 weeks and following parameter were studied:

LAYOUT OF THE EXPERIMENT

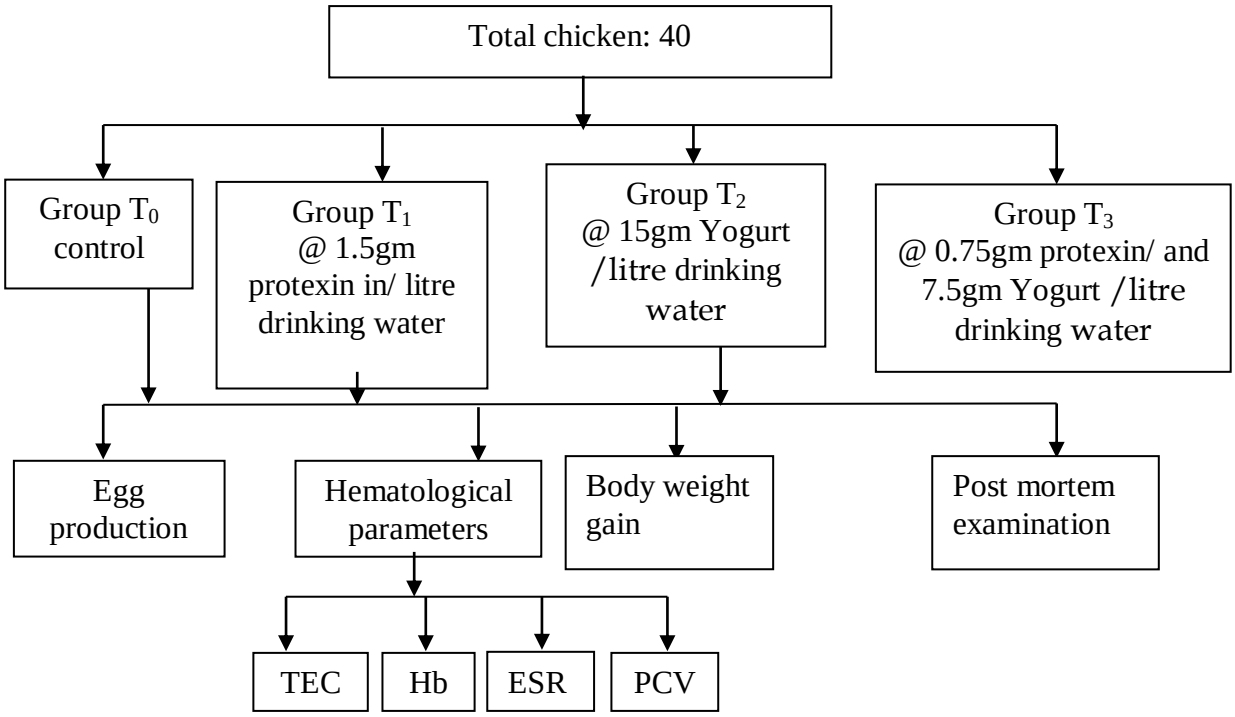


Fig. 1: Layout of the experimental design (each group consisting of ten birds)

The research was conducted for 6 weeks under the dept. of Physiology and Pharmacology at Hajee Mohammad Danesh Science and Technology University, Basherhat, Dinajpur. Poultry Farm Basherhat with 40 chickens to study the effects of a probiotic (Protexin^{R}) and Yogurt (Sour Curd).

3.2 Preparation of house

A cage house was used for rearing experimental birds and proper hygienic measures were taken in every step of cleaning, washing and disinfection of experimental house and all type of equipment's. The birds were randomly transferred to the clean laying cages. The test diets were given to the birds after one week for their adjustment to the new environment. The space given per bird was (12×18) inch.

Fig. 2: Poultry House

3.3 Collection of feed Ingredients, probiotic and Yogurt

Required layer crumble feed was bought from Dinajpur market. Probiotic and Yogurt was purchased from the local market of Dinajpur district town. The probiotic (Protexin^{R}) of Novertis Bangladesh company ltd. was considered for experimental purpose.

Fig. 3: Probiotics (Protexin^{R})

Fig. 4: Yogurt (Sour curd)

Fig. 5: Preparation of Probiotics (Protexin^{R})

Fig. 6: Preparation of Yogurt

3.4 Experimental birds

A total of 40 chicken of 80 weeks old were used in the study. They were kept on the cages in isolated pens and fed commercial ration and water adlibitum. The experimental chicks were randomly divided into four equal groups consisting of designated as group T_0, T_1, T_2, and T_3. Chicks in group T_0 were fed only basal diet supplement. Group T_1 were fed basal diet and probiotics (Protexin^R). Group T_2 were fed basal diet and Yogurt (Sour curd) and group T_3 were fed basal diet probiotics and Yogurt. Weekly feed consumption for each group was determined. Mean initial and weekly body weight of birds for each group were determined and then body weight gain was calculated. By the end of experimental period, three birds from each group were numbered, weighed and then slaughtered.

Fig. 7: Experimental birds

3.5 Collection and management of chickens

80 weeks aged birds (Hi Sex Brown) were purchases from Dinajpur district for experiment. The experiment was carried in poultry shed in local firm at HSTU. The chicken was 40 in number. The body weight of all selected chicken ranged from 1800 to 2000 gm respectively. Then the layer chickens were managed carefully.

3.6 Experimental diets

The commercial layer crumble and diets marketed by Nourish poultry feed and hatchery Ltd. was purchased from the local agent in Dinajpur.

Fig. 8: Experimental diet

3.7 Routine management

The commercial management procedures were followed during the whole experimental period.

3.8 Cage management

Layer was reared in cages, so regular cleaning and washing of cage were done.

3.9 Cage space

Each cage was 2.5 ft × 2 ft which was for ten birds. Therefore, the space given for each bird was one square ft.

3.10 Routine procedures

The experimental birds were exposed to similar care and management throughout the experimental period. Special care was taken to protect pullets from unhealthy condition. The day length was less than 16 hours during the experimental period. So a provision was made by using 60 waft electric bulb to meet up 16 hours light per day for maximum laying performance according to the recommendation of Shaver star Breeding Company. Feeder and waterer were cleaned and disinfected regularly to avoid microbial contamination. The floor of the experimental house was kept clean. The experimental diets were prepared weekly and stored in tin containers. Feed and water was offered twice daily, once in the morning and again in the afternoon in such a way that feeders and waterers were not kept empty.

3.11 Record keeping

The following performance characteristics were recorded during the experimental period.

i. Body weight: Individual body weight for each replication was recorded at the beginning of the experiment and at 6 weeks or at the termination-of experiment.
ii. Egg production: Recorded daily.
iii. Egg weight: individually once a week.
iv. Feed consumption: Weekly.
v. Mortality: Daily.

Fig. 9: Collection of Eggs

3.12 Biosecurity and sanitation

Proper hygienic and sanitation programs were followed during the experimental period. To prevent the outbreak of disease strict biosecurity was maintained during the experimental period. The following measures were taken to maintain the insecurity.

- Visitors were not allowed to enter in the house.
- All equipment's in the experimental house were kept clean.
- Dead birds were removed promptly.

All the chicken of treated and control groups were closely observed for six weeks after treatment and following parameter were studied.

3.13 Effect on body weight

i) The effect of the Probiotics and Yogurt on body weight gain, feed consumption was recorded before and during administration of treatment.

ii) Chickens under treatment and control groups were weighed with electric weighing machine. The weight of each chicken was taken weekly. The average of these weights was calculated and recorded.

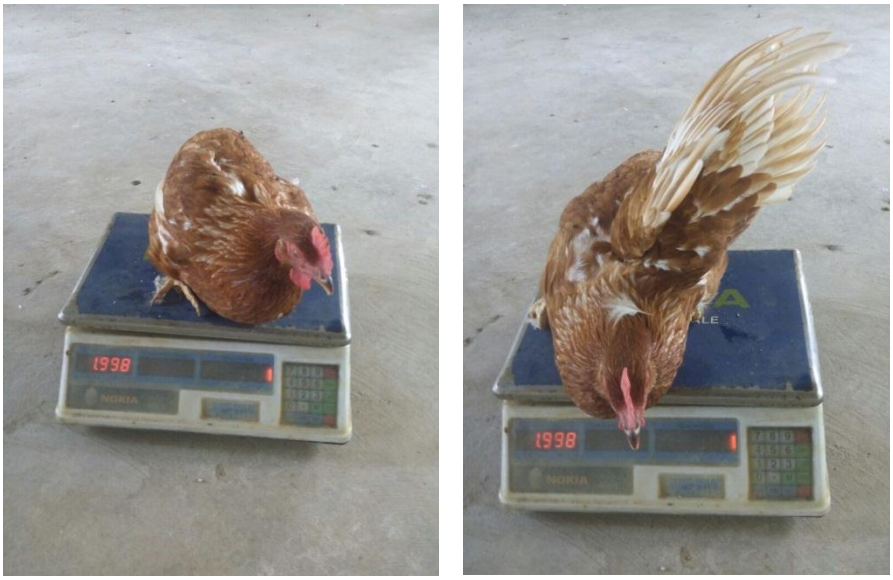


Fig. 10: Weighing procedure

3.14 Hematological parameters

Blood samples were collected from wing vein of chicken of both control and treated groups at 1st week and 6th weeks to study the effect of the Probiotics (Protexin^{R}) and Yogurt and the following parameters were observed:

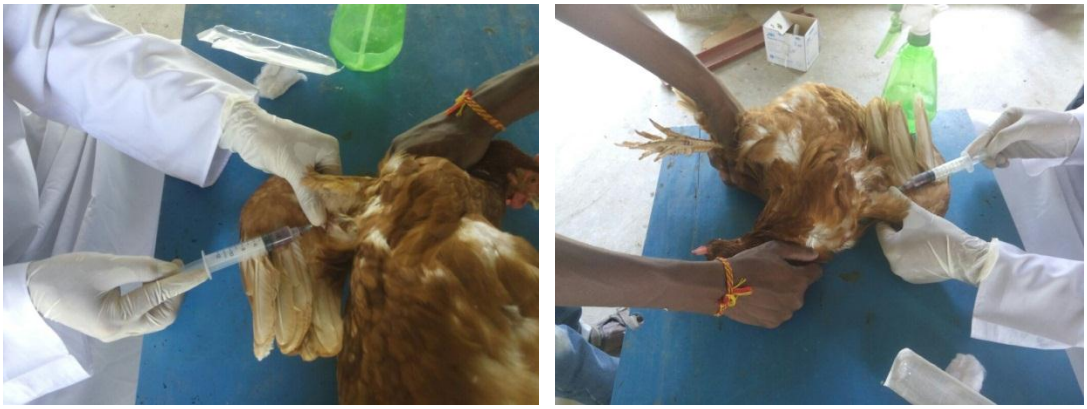


Fig. 11: Collection of Blood

(a) Total erythrocyte count (TEC)
(b) Hemoglobin estimation (Hb)
(c) Packed Cell Volume (PCV)
(d) Erythrocyte Sedimentation Rate (ESR)

3.14.1 Determination of total erythrocyte count (TEC)

Total erythrocyte count was done following the method described by Lamberg and Rothstein (1977). Well-mixed blood sample was drawn with red blood cell diluting pipette exactly up to 0.5 marks of the pipette. Outside of the tip of the pipette was wiped with cotton. Then the pipette was immediately filled with the red cell diluting fluid (Hayem's solution) up to 101 marks. The free end of the pipette was wrapped around with the rubber tube stretching to both the ends and held with thumb and middle finger. The content of the pipette was mixed thoroughly by shaking with 8-knot motion for 3-5 minutes. Then the counting chamber was placed with special cover glass under microscope using low power (10 x) objectives. After discarding 2 or 3 drops of fluid from the pipette, a small drop was placed to the edge of the cover glass on the counting chamber as the entire area under the cover glass was filled by the fluid. One-minute time was spared to allow the cells to settle on the chamber under the cover glass. Taking 5 larger squares (4 in the 4 corners and the central one) of the central large square, the cells

were counted from all the 80 small squares (16 x 5) under high power objectives (45 x). After completion of counting, the total number of RBC was calculated as number of cells counted x 10, 000 and the result was expressed in million/µl of blood.

3.14.2 Determination of hemoglobin concentrations (Hb)

The N/10 hydrochloric acid (HCl) was taken in a graduated tube up to 2 marks with the help of a dropper. Well-homogenized blood sample was then drawn into the Sahli pipette up to 20 cm. mark. The tip of the pipette was wiped with sterile cotton and the blood of the pipette was immediately transferred into the graduated tube containing hydrochloric acid. This blood and HCl acid were thoroughly mixed by stirring with a glass stirrer. There was a formation of acid hematin mixture in the tube by hemolysing red blood cells by the action of HCl. The tube containing acid hematin mixture was kept standing in the comparator for 5 minutes. After that distilled water was added drop by drop. The solution was mixed well with a glass stirrer until the color of the mixture resembled to the standard color of the comparator. The result was read in daylight by observing the height of the liquid in the tube considering the lower meniscus of the liquid column. The result was then expressed in g %. The above procedure was matched by the Helligehemometer method as described by Lamberg and Rothstein (1977).

3.14.3 Determination of Packed Cell Volume (PCV)

The citrated well mixed blood sample was drawn into special loading pipette (Wintrobe pipette). The tip of the pipette was inserted up to the bottom of a clean, dry Wintrobe hematocrit tube. Then the Wintrobe tube was filled from the bottom by pressing the rubber bulb of the pipette. As blood came out, the pipette was slowly withdrawn but pressure was continued on the rubber bulb of the pipette so as to exclude air bubbles. The tip of the pipette was tried to keep under the rising column of blood to avoid foaming and the tube was filled exactly to the 10 cm mark. Then the Wintrobe hematocrit tube was placed in the centrifuge machine and was centrifuged for 30 minutes at 3000 rpm. Then, the hematocrite or PCV was recorded by reading the graduation mark; the percent volume occupied by the hematocrit was calculated by using the following formula as described by Lamberg and Rothstein (1977).

$$\text{PCV\%} = \frac{\text{Height of the red cell volume in cm}}{\text{Height of total blood in cm}} \text{ X } 100$$

3.14.4 Determination of Erythrocyte Sedimentation Rate (ESR)

The fresh anticoagulant blood was taken into the Wintrobe hematocrit tube by using special loading pipette exactly up to 0 marks. Excess blood above the mark was wiped away by sterile cotton. The filled tube was placed vertically undisturbed on the wooden rack for one hour. After one hour the ESR was recorded from the top of the pipette. The result was expressed in mm/in 1st hour.

3.15 Postmortem examinations

There was no mortality in experimental birds during the experimental period. However, at the end of the experiment (i.e. 6 weeks) postmortem examinations were carried out but there was no significant change in any organ.

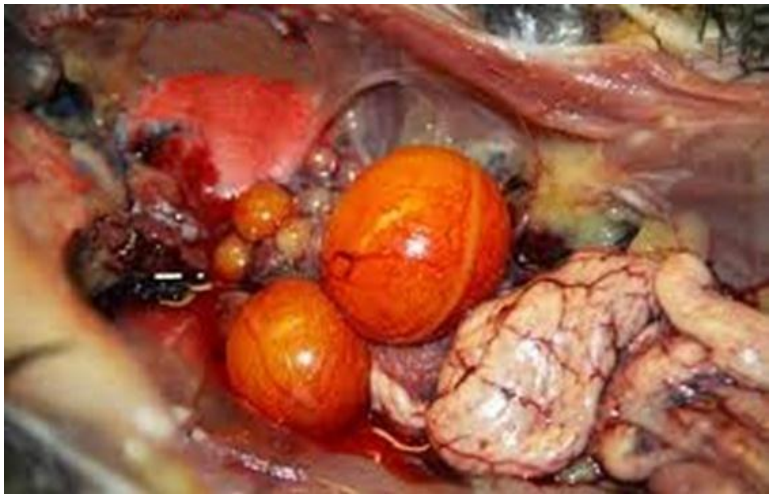


Fig. 12: Post mortem examination

3.16 Statistical analysis

Completely Randomized Design with factorial arrangement of time and treatments (Steel and Torrie, 1986). The experiment was design by following and data were analyzed by analysis of variance using Mstatc and SPSS program.

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSSION

This experiment was conducted to compare the effect of Yogurt from commercial Probiotic (Protecxin^{R}) on egg production body weight and hematological parameter of layer chicken. This experiment was conducted under the Department of Physiology and Pharmacology, Faculty of Veterinary and Animal Science.

4.1 Egg Production

Egg production of different groups of layer is recorded at 80 weeks to 85 weeks hen treated with probiotic, yogurt and combined probiotic and yogurt. The average egg production of different groups hen were recorded. Hens treated with probiotic showed average egg production 31.8± .32 within 80-85 weeks, yogurt treated groups showed average egg production 32.5± .50and combined treatment supplementation showed average egg production 33.3 ±.47within 80-85 weeks. (Table1).Control group showed average egg production 27.5±.34. Result showed highest egg production in combined treatment group (33.3 ±.47) and lowest in control group (27.5±.34). Moreover, probiotics increase egg production and quality (Kurtoglu *et al.,* 2004 and Panda *et al*., 2008) our study express the same results.

Table 1: Number of egg production (42days/bird)

Treatment Groups	Egg production(80-85)weeks
T_0 group (control)	$27.50^{c} \pm 0.34$
T_1 group(Protecxin^{R})	$31.80^{b} \pm 0.33$
T_2 group(Yogurt)	$32.50^{ab} \pm 0.50$
T_3 group(Protecxin^{R} and Yogurt)	$33.30^{a} \pm 0.47$

Values followed by different superscripts in the same column are statistically significant ($p<0.05$)

4.2 Body weight gain

Body weight of different groups of layer is recorded from 80 weeks to 85 weeks hen treated with protecxin^{R}, yogurt and combined probiotic and yogurt. The average body weight of different group's hen was recorded. Hens treated with probiotic showed

average Body weight gain $1754.0^{a} \pm 18.44$ to$1762.0^{a} \pm 21.37$ within 80-85 weeks, yogurt treated groups showed body weight gain $1732.0^{a} \pm 19.38$ to$1748.0^{a} \pm 16.01$ and combined treatment supplementation showed body weight $1749.0^{a} \pm 26.47$ to $1761.0^{a} \pm 31.08$ within 80-85 weeks (Table 2). Control group showed average body weight $1768.0^{a} \pm 14.70$ to $1737.0^{a} \pm 23.14$. Result showed that the bodyweight of different group same with the control group. Mahmud *et al.* (2014) shows that the probiotic increase the body weight and carcass quality at the level of ($p<0.05$) but our study shows no significant different from the control group. Results are contrary to that observed by Dei *et al.* (2010) and Bonsu *et al.* (2012) where broilers fed diets supplemented with RE-3 consumed less feed ($P<0.05$) compared to their counterparts on diets with no probiotic. This probably is because they used broilers for their studies whilst birds used in this experiment were layer. Bonsu *et al.* (2012) however attributed the reduction in intake to the improved nutrient retention and utilization arising 52 from a better gastrointestinal tract (GIT) environment enabled by the beneficial microorganisms. The results also are in contrast with Anukam *et al.* (2005) who recorded increased intake when rats were given feed supplemented with a DFM product containing *Lactobacillus* strains. It has been reported that probiotic-supplemented diets enhances digestion through the production of enzymes (Anukam *et al.,* 2005).

Table 2: Body weight gain

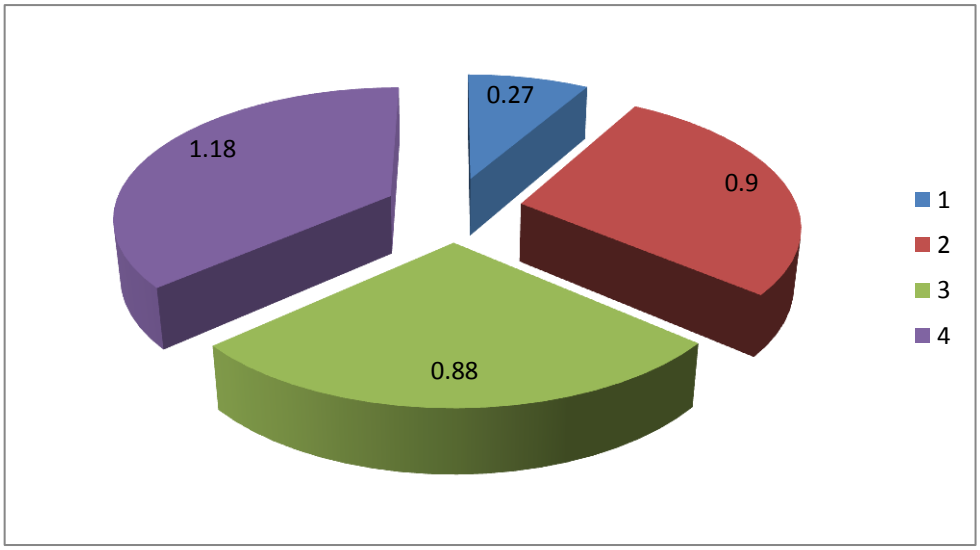
Group	Body weight (gm.)	
	80 weeks	85 weeks
T_0 (Control)	$1768.0^{a} \pm 14.70$	$1737.0^{a} \pm 23.14$
T_1 (Protexin^{R})	$1754.0^{a} \pm 18.44$	$1762.0^{a} \pm 21.37$
T_2(Yogurt)	$1732.0^{a} \pm 19.38$	$1748.0^{a} \pm 16.01$
T_3 (Protexin^{R} and Yogurt)	$1749.0^{a} \pm 26.47$	$1748.0^{a} \pm 31.08$

Values followed by same superscripts in the same column are not statistically significant ($p>0.05$)

Table 3: Cost benefit analysis (40 taka/kg feed)

Expenditure of Poultry				
Group	**T_0**	**T_1**	**T_2**	**T_3**
Poultry purchase	2750	2750	2750	2750
Feed cost (feed intake ×bird no. × days×40)	1990.80	2007.6	1976	1965.6
Protecxin^R		100		50
Yogurt			200	100
Miscellaneous	50	50	50	50
Total Cost	4790.8	4907.60	4976	4915.6
Total Income				
Poultry sell	2700	2750	2750	2750
Egg sell (no. of eggs× price per egg)	2208	2535.84	2599.68	2664
Total income	4908	5285.84	5349.68	5414
Benefit				
Total Benefit	117.2	378.25	373.68	498.4
Benefit/bird/day	0.27 taka	0.90 taka	0.88 taka	1.18 taka

Benefit in pie chart:



1=Group T_0, 2= Group T_1, 3= Group T_2, 4= Group T_3

4.3 Hematological parameter

Table 4: A. Total Erythrocyte Count (million/ mm^3)

Treatment group	TEC million/mm^3 (80 weeks) Mean ± SE of mean	TEC million/mm^3 (85 weeks) Mean ± SE of mean
T_0 (control)	3.050 [a] ± 0.026	3.053 [a] ± 0.028
T_1 (Protexin^R)	3.052 [a] ± 0.027	3.102 [a] ± 0.021
T_2 (Yogurt)	3.085 [a] ± 0.025	3.113 [a] ± 0.026
T_3 (Protexin^R and Yogurt)	3.010 [a] ± 0.018	3.128 [a] ± 0.023

Values followed by same superscripts in the same column are not statistically significant ($p>0.05$)

Total erythrocyte count is presented in (Table 4.A). The values of TEC in all treated groups and control group were no significantly different from the control group).These findings were not agreement with the observations made by Devegowda *et al.* (1994).

Table 4: B. Estimation of Hemoglobin (gm/dl)

Treatment group	Hemoglobin Hb (gm/dl) (80 weeks) Mean ± SE of mean	Hemoglobin Hb (gm/dl) (85 weeks) Mean ± SE of mean
T_0 (control)	11.86 [a] ± 0.156	11.90 [a] ± 0.164
T_1 (Protexin^R)	11.54 [a] ± 0.193	12.05 [a] ± 0.080
T_2 (Yogurt)	11.58 [a] ± 0.208	12.10 [a] ± 0.115
T_3 (Protexin^R and Yogurt)	11.89 [a] ± 0.210	12.17 [a] ± 0.108

Values followed by same superscripts in the same column are not statistically significant ($p>0.05$)

Hemoglobin content is presented in (Table 4.B). The values of Hb in all treated groups and control group were significantly different. These values show a little fluctuation they were statistically significant ($p>0.01$). Significantly ($P>0.05$). Dimcho *et al.* (2005) also found no significant difference ($P>0.05$) in blood haemoglobin, total protein and total cholesterol concentrations when they fed probiotic as a supplement to Muskovy ducks.

Table 4: C. Packed Cell Volume (%)

Treatment group	Packed cell volume (%) (80 weeks) Mean ± SE of mean	Packed cell volume (%) (85 weeks) Mean ± SE of mean
T_0 (control)	33.07 [a] ± 0.424	32.51 [b] ± 0.361
T_1 (Protexin[R])	33.19 [a] ± 0.323	33.52 [a] ± 0.304
T_2 (Yogurt)	32.26 [a] ± 0.397	33.52 [ab] ± 0.287
T_3 (Protexin[R] and Yogurt)	32.54 [a] ± 0.409	33.52 [a] ± 0.261

Values followed by same superscripts in the same column are not statistically significant ($p>0.05$) at 80 weeks and values followed by different superscripts in the same column are statistically significant ($p<0.05$) at 85weeks age birds.

Packed cell volume is presented in (Table 4.C). The values of PCV in all treated groups and control group were different from normal group. These values show a little fluctuation they were statistically significant ($p>0.05$). However, numerically increased PCV values were observed in treatment groups compared to control. This observation was in conformity with earlier workers (Baidya *et al*., 1994 and Mohan *et al.,* 1996) and it is attributed to improved health status and physiological well-being of the birds administered with probiotic.

Table 4: D. Erythrocyte Sedimentation Rate (mm/1st hour)

Treatment group	ESR (mm/1st hour) (80 weeks) Mean ± SE of mean	ESR (mm/1st hour) (85 weeks) Mean ± SE of mean
T_0 (control)	6.043 [a] ± 0.09177	6.030 [a] ± 0.062
T_1 (Protexin[R])	5.880 [a] ± 0.11119	5.791 [a] ± 0.122
T_2 (Yogurt)	5.933 [a] ± 0.03429	5.834 [a] ± 0.038
T_3 (Protexin[R] and Yogurt)	5.918 [a] ± 0.06166	5.836 [a] ± 0.057

Values followed by same superscripts in the same column are not statistically significant ($p>0.05$)

Erythrocyte sedimentation rate content is presented in (Table 4.D). The values of ESR in all treated groups and control group were more or less similar and the values were within the normal range. The highest ESR was recorded in Group T_3 and lowest in Group T_0. Although these values show a little fluctuation they were not statistically significant ($p>0.05$). The same findings were obtained by Islam *et al.* (2004); Kundu *et al.* (1993)

and Tabinda *et al.* (2012). Erythrocyte sedimentation rate values indicated slight decrease from the normal physiological range of between 3 and 12 mm as was suggested by Jain (1986). The non significant decrease erythrocyte sedimentation rate mean values was also experienced by Islam *et al.* (2004) who did research on haematological Parameters of Fayoumi, Assil and Local chickens reared in Sylhet Region in Bangladesh.

4.4 Postmortem Examination for Side Effect

After the experiment two chickens from every group were slaughtered to see if there were any pathological changes present on the period of treatment. There was no significant pathological change in any internal organs of the Hi Sex Brown chicken of treated groups. In case of treated birds increased the number of follicle in ovary other than control.

CHAPTER V

CONCLUSION

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CONCLUSION

This research work was conducted to study the effects of Probiotics, Yogurt and combination of Probiotics and Yogurt on egg production, Body weight and hematological values in commercial aged chickens. All the 40 chicken's entire period 6 weeks and randomly divided into 4 groups (n=10) to carry out this research work. Keeping one group as normal control group (T_0) and others three groups (T_1, T_2 and T_3) as group subjected to treatment with Probiotic (Protexin^R), Yogurt (Sour curd) and combination of Probiotics (Protexin^R) and Yogurt (Sour curd). Group T_1 supplemented with Probiotic (Protexin^R) @ 1.5gm/litre of water. The group of T_2 was supplemented with Yogurt (Sour curd) @ 15gm/litre of water. Group T_3 was supplemented with combination of Probiotic and Yogurt @ 0.75gm/litter of water and 7.5mg/Kg of feed. The treatment group T_1, T_2 and T_3 recorded statistically significant ($p<0.05$) increase for egg production than that of control group T_1. Egg production was increased in Probiotic ($31.80^b \pm 0.33$), Yogurt ($32.50^{ab} \pm 0.50$) and Probiotic + Yogurt ($33.30^a \pm 0.47$). Body weight were not significantly increase or decrease among the group and profit for egg production was Tk. 0.90, Tk. 0.88, Tk. 1.18 taka/bird/day in treatment group and in control group was TK. 0.27. It is concluded that supplementation of 0.75mg probiotic and Yogurt 7.5mg birds (T_3) of treatment groups caused significant increase in egg production .From this experiment we found that, between the control group and the treatment group of birds, the (T_3) groups 0.75mg/litter water probiotic and Yogurt 7.5mg/Kg feed laying hen are more profitable than any other groups. No significant ($p>0.05$) differences were observed the treatment groups in case of hematological values of ESR(Erythrocyte Sedimentation Rate) and TLC (Total Leukocyte Count) in respect to the control group after treatment. PCV (Packet Cell Volume) and there exist a significant ($P<0.05$) difference among the mean values and Hb (Hemoglobin) level significant different ($P<0.01$).

Recommendation

- Further studies should be conducted to confirm the results that probiotic can be incorporated in the diet of layers probiotic @1.5gm/litre of water and yogurt 15gm/litre of water increase eggs production.
- It is recommended that feeding trials be conducted to evaluate the effects of probiotic supplementation of the diet of poultry under conditions where environmental factors such as sanitation, stress, feeding and other management practices are difficult to control.
- Further research best supplementing probiotic at varying levels should be considered to assess its effect on production performance.
- Proper feeding of high quality feed should be done. Studies on feeding/feeds quality versus management systems are required.
- Disease, parasite control and management of chickens kept under different management systems should be taken into consideration.

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