Project ID: 532

Competitive Research Grant

Sub-Project Completion Report

On

Development of probiotic food products for human and feed products for poultry using native isolates

Project Duration
May 2016 to September 2018

Biotechnology and Genetic Engineering Discipline
Khulna University; Khulna-9208; Bangladesh

Submitted to
Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

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Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka – 1215
Bangladesh

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Acronyms

ADD	: Antibiotic associated diarrhea
AGP	: Antibiotic growth promoter
ANOVA	: Analysis of variance
B	: Bifidobacterium
bw	: Body weight
CFU	: Colony forming unit
CTAB	: Cethyl Trimethyl Ammonium Bromide
DNA	: Deoxyribonucleic Acid
E	: Enterococcus
ELISA	: Enzyme Linked Immune Sorbent Assay
e.g.	: Exempli gratia (for example)
et al.	: et alliori (and others)
etc.	: et cetra (and the rest)
FAO	: Food and Agriculture Organization
FCR	: Feed Conversion Ratio
FMF	: Fermented Moist Feed
g	: Gram
GIT	: Gastrointestinal Tract
GLP	: Glucagon like peptide
GPA	: Growth Promoting Antibiotics
Hb	: Hemoglobin
HDL	: High Density Lipoprotein
i.e.	: id est (That is)
ip	: Intraperitoneal
ITS	: Internal Transcribed Spacer
L/l	: Liter
LAB	: Lactic Acid Bacteria
LDL	: Low Density Lipoprotein
L/Lb	: Lactobacillus
mg	: milligram
µg	: Microgram
ml	: milliliter
µl	: Micro liter
mmol/l	: Milimole per liter
µmol	: Micromole
MRS	: de Man, Rogosa and Sharpe
ng	: Nano gram
OD	: Optical Density
OGTT	: Oral Glucose Tolerance Test
PBS	: Phosphate Buffered Saline
PCR	: Polymerase Chain Reaction
%	: Percent
PYT	: Probiotic yogurt treated

rDNA	: Ribosomal DNA
RFLP	: Restriction Fragment Length Polymorphism
RNA	: Ribonucleic Acid
rpm	: Rotation per minute
rRNA	: Ribosomal RNA
SCFA	: Short-Chained Fatty Acids
SD	: Standard Deviation
SDS	: Sodium Dodecyl Sulfate
TD	: Traveler's diarrhea
TE	: Tris-EDTA
TPY	: Typticase Phytone Yeast
v/v	: volume/volume
w/v	: weight/volume
WHO	: World Health Organization

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Executive Summary

Probiotics are live microorganisms that confer health benefit on the host when administered in adequate amounts. The major objectives of the project were to isolate and characterize the native Probiotic isolates and to develop Probiotic food products for human and Probiotic supplemented feed for poultry and finally to study their effects on mice and poultry through *in vivo* trials. For isolation of probiotic bacteria yogurt and poultry samples were collected from seven Divisions (Dhaka, Rajshahi, Chattogram, Khulna, Sylhet, Kumilla and Mymensingh) of the country. Isolated probiotic bacteria were characterized morphologically (by gram staining, colony morphology, motility activity tests and growth patterns) and biochemically (by catalase test, coagulase activity test, pH tolerance test, bile salt tolerance test, sodium chloride tolerance test, phenol tolerance and sugar fermentation tests). From the yogurt samples, forty two (42) and from the gastrointestinal tracts of poultry, thirty (30) native probiotic isolates (presumptive *Lactobacillus* spp.) were obtained. Probiotic yogurt was prepared using the probiotic bacteria (5% v/v liquid culture) isolated from yogurt samples. Starter poultry feeds for layer, broiler and ducklings were prepared supplemented with 40-50 ml/kg feed of probiotic bacteria, at a concentration of 2-4x10^9 cfu and 8.8-9.9x10^9 cfu respectively, isolated from the gut of poultry. Effects of native Probiotic isolates on mice (using the probiotic yogurt) and poultry (using the probiotic supplemented starter feed) were studied through *in vivo* trials to determine the properties like anti-hypercholesterolemic, anti-diabetic, antimicrobial, anti-diarrheal, anti-allergic, effects against enteric pathogens, effects as layer starter feed supplement, effects as broiler starter feed supplement and effects as duck starter feed supplement. The experimental results showed significant effects of native Probiotic isolates in relation to anti-hypercholesterolemia, anti-diabetic, anti-diarrheal, anti-allergic and anti-microbial activities against human enteric pathogens through *in vivo* mice/rats trials. Besides, poultry isolates showed significant positive effects as layer, broiler and duck starter feed on body weight, daily weight gain, carcass yield, total serum cholesterol, serum LDL, serum calcium, meat protein content, decreased mortality rate, feed intake, feed conversion ratio, dressing percentage and proportion of internal organs, decreased uric acid, albumin, and total protein content. It is expected that the project outputs will help to develop different Probiotic food products for human and feed supplement for poultry, which in turn will help preventing severe diseases like liver disease, ulcer, diabetes and antibiotic associated diarrhea in human. It will also help to increase the body weight gain, growth rate and disease prevention in animals and poultry. Moreover, it is hoped that Probiotic supplemented poultry feeds will substitute the existing antibiotic based feed additives.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the CRG sub-project:
Development of probiotic food products for human and feed products for poultry using native isolates

2. Implementing organization:
Biotechnology and Genetic Engineering Discipline
Khulna University, Khulna-9208, Bangladesh

3. Name and full address with phone, cell and E-mail of PI/Co-PI :

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4. Sub-project budget (Tk):

a. Total: Tk. 25,00,000.00
b. Revised (if any): None

5. Duration of the sub-project:

a. Start date (based on LoA signed) : May 2017
b. End date : 30 September 2018

6. Justification of undertaking the sub-project:

Probiotic are live microorganisms that confer health benefit on the host when administered in adequate amounts (FAO/WHO, 2011). The beneficial effects of Probiotic strains are: cholesterol lowering, anti-diabetic, hepatoprotective, preventing antibiotic resistance, anti-diarrheal, anti-cancer, good immunity, disease prevention, etc. Probiotic have been extensively studied over the past several years in the prevention and to a larger extent, in the treatment of diarrheal diseases, especially in pediatric populations (Stefano, 2011). The prevention of atopic eczema in high-risk infants is possible by modulating the infant's gut microbiota with Probiotics and Prebiotics (Kaarina et al., 2007). The prospect of using bacteriocins from lactic acid bacteria, primarily used as bio-preservatives, represents a perspective, alternative antimicrobial strategy for continuously increasing problem with antibiotic resistance. Another strategy in resolving this problem is an application of probiotics for different gastrointestinal and urogenital infection therapies (Jagoda et al., 2010). The commercial imported probiotic cultures/products currently being used in Bangladesh are of foreign origin. Therefore, it is imperative to isolate, characterize and document the efficacy of our native probiotic bacteria from our regional yoghurt and poultry samples. Dairy and poultry sub-sectors are crucial for poverty alleviation in Bangladesh. It is the need of time to develop safe food for human and feeds for poultry using native probiotic isolates that will exert beneficial health effects. It would also be attractive for the young entrepreneurs to invest in these promising sectors. Therefore, this study would be worthy to recognize the native probiotic isolates and subsequently help to develop safe and efficacious probiotic food products for human and poultry.

7. Sub-project goal:

Development of commercially feasible probiotic food products for human and feed products for poultry using native isolates

8. Sub-project objective (s):

a) To isolate, characterize and studies of the properties of native probiotic isolates.
b) To develop probiotic food/feed products for human/poultry and to study the effects of native probiotic isolates on mice and poultry through *in vivo* trials

9. Implementing location (s):

Food Biotechnology and Animal Cell Culture laboratory, Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh

10. Methodology:

10.1 Isolation, characterization and studies of the properties of native probiotic isolates:

10.1.1 Isolation of native probiotic bacteria from yogurt and poultry samples

Sample collection

For isolation of native probiotic bacteria, the yogurt and poultry samples were collected from Dhaka, Rajshahi, Chittagong, Khulna, Sylhet, Cumilla and Mymensingh Divisions of the country. The yogurt samples were collected, from the different dairy sweetmeat shops, yoghurt corners and restaurants in plastic containers labeled with the name of the shops, locations and dates. The samples were immediately brought to the laboratory to place them into refrigerator at 4^0C for short time and in freezer at -20^0C for long time preservation and experimental uses. The poultry samples were collected from the two age groups (15 and 30 days old) of healthy broiler chickens. After sacrificing the chickens and dissecting different parts of gastrointestinal tract (GIT) the crop, small intestine and caeca of the chickens were collected aseptically by using sterile scalpel in sterile Petridishes (Figure 1). The collected samples of caeca, small intestine and crop were cut into smaller sections and the tissues were grinded until homogenized. Then each of the samples from the yoghurt and poultry were dissolved with its content in 9 ml of 0.15% buffered peptone water solution and diluted up to 10^{-10} fold. The diluted samples were inoculated into the MRS agar plate by ensuring 6.5 pH value and 37°C incubation temperature. Then each of the samples was sub-cultured for three times to obtain pure colonies with homogenous morphology. The isolated cultures were maintained in MRS agar medium refrigerated at 4°C temperature.

(A) (B) (C)

Figure 1: Caeca, (A); small intestine, (B) and crop, (C) from GI tract of poultry

Isolation of probiotic bacteria

Media preparation

Nutrient agar media was preferred for the purpose of the project to get a number of isolates besides *Lactobacillus* spp., where MRS media was selectively used.

Media composition

1. 0.3% yeast/beef extract,
2. 0.5% peptone,
3. 0.5% NaCl (Molecular biology grade),
4. 1.8% agar.

All the constituents were weighed properly in a conical flask using electronic balance and 100 ml distilled water was poured into the flask to make 100 ml solution. The conical flask containing the ingredients was autoclaved at 121^{0}C for 15min. After autoclaving, the media was poured into Petri dishes bringing into laminar air flow (LAF) system. Total five plates were prepared for a single sample to make replica of the work. In case of nutrient broth preparation, agar was excluded.

Serial dilution

Each sample was thawed bringing out of the freezer/refrigerator before commencing the isolating procedure. Total six test tubes were used to prepare a single isolate from a single sample each time to avoid undesired contamination and loss of product viability. Total 53.8 ml peptone water was prepared from where 9.8 ml was transferred to the first test tube and 9 ml for the rests. Afterward, 2 mg yoghurt sample was taken into the first test tube containing 9.8 ml peptone water and vortexed properly to homogenize them. Following this, 1ml of the solution was pipetted to the second test tube containing 9 ml peptone water and vortexed for 30 sec. Then, 1ml vortexed solution was transferred to the next test tubes respectively, from each test tube.

Preparation of primary culture

The 1ml vortexed solution from the final test tube was poured into each Petri dishes containing nutrient agar and spread on the surface of the medium properly with a glass rod spreader. The plates were marked with the sample name, date and the name of the student separately. Finally, the plates were incubated at 37^{0}C for 24to 48 hours.

Preparation of primary subculture

After 48hr of incubation at controlled anaerobic condition, the culture plates were brought out into laminar air flow system and the single colonies were observed. Then, a single colony from each Petri dish was sub-cultured into another fresh nutrient agar plates using sterilized sticking loops. Afterwards, the plates were re-incubated at the incubator (INQUCELL) at 37^0C for another 24 to 48 hours.

Pure isolates preparation

The aforementioned steps of sub-culturing were done another 5-6 times to get pure isolates from each single colony from each culture plate.

Preparation of stock isolates and *in vitro* conservation

The single colony from the final sub-culture plate was taken for nutrient broth culture individually. After incubation at 37^0C for overnight period, the broth cultures were placed separately inside laminar air flow to pour 5µl broth into sterilized plastic vial (screw capped) containing 5µl sterilized glycerol for long term preservation in the refrigerator at -20^0C as stock isolates.

Preservation and maintenance of probiotic bacterial isolates

The lactic acid bacteria were maintained by sub-culturing for daily or weekly use. The purified bacteria were stored with homogeneous cell morphology in a -20°C deep freeze in MRS medium in 20% (v/v) glycerol for preservation. The glycerol stocks were prepared by mixing 0.5 ml of active cultures with 0.5 ml of fresh, sterile MRS medium with 40% glycerol in dram vials. The frozen stocks were prepared in triplicate and were used only one set for the test for avoiding any contamination or loss of activity.

10.1.2. Characterization of native probiotic isolates

10.1.2.1 Morphological tests

Motility assay

Broth cultures of the pure culture isolates were prepared separately in nutrient broth through overnight incubation at the incubator. A single test tube containing 10 ml nutrient agar was prepared for a single isolate at a time. A sterilized needle was placed inside the nutrient broth containing pure culture isolate and stabbed into the nutrient agar containing test tubes three times individually to each other. This technique was repeated for each of the pure isolates. Finally, the test tubes were labeled properly with the sample name and date, covered with aluminum foil paper and placed them inside incubator at 37^0C for another 24 hr to 48 hr.

Colony morphology

In the pure culture plates, isolated colonies were observed properly to study the colony morphology. Factors considered in the very cases were

1. Number of single colonies per plate
2. Number of joint colonies per plate
3. Color of the colonies (both single and joint colonies) and
4. Number of different looking colonies per plate considering the majority of similar looking colonies.

10.1.2.2 Biochemical tests

Gram Staining

A single colony was taken and smeared onto a clean dry slide to be heat fixed. The heat-fixed smear was flooded with crystal violet solution for 30 seconds and was rinsed with water for 5 seconds. The grams iodine solution was used to cover over the slide for one minute and rinsed with tap water for 5 seconds. Finally, Safranine was used as counterstaining agent for 60 to 80 seconds and was rinsed with water. After that, the gram positive and negative bacteria were detected under the light microscope.

Catalase test

A sterilized transparent looking glass slide was used for a single isolate to commence catalase test. A drop of water and a drop of bacterial culture broth were placed at the two opposite ends of the glass slide. Another drop of H_2O_2 was dropped on both the ends. The bacterial culture containing H_2O_2 generates bubbles (if Catalase positive) or no bubble (if Catalase negative).

Coagulase test

A test tube containing 9.8 ml fresh cow milk was inoculated with 200 µl of a pure single isolate at a time. Then the clotting of the solid from liquid was observed in every two-hour interval and necessary snaps were taken. This technique was followed for each of the isolates form different yoghurt samples collected.

Antimicrobial activity test

Each of the isolates was co-cultured with a particular pathogen at a time and the zone of inhibition was observed whether or not the presumptive probiotics isolates have antimicrobial activity. Total eight different pathogens were used for the antimicrobial activity test of each probiotic isolate separately. Stick plate co-culture was preferred for the very purpose. All the necessary snaps were taken to further analyze and documentation.

Resistance to Low pH

It is considered that, the pH of human stomach is1.5 to 3.5. Therefore, resistance to pH 3 is often used for *in vitro* assay to determine the resistance of stomach pH. For this purpose, MRS broth medium was prepared with pH 3 by using 5N HCl. Then 20µL overnight grown bacterial cultures were inoculated in the 15 ml broth medium. Afterwards, the test tubes were incubated at 37℃ and absorbance was taken every four hour at 620nm to determine the low pH tolerance and viability of the bacteria.

Bile salt tolerance

It is believed that, the intestinal bile concentration of human is 0.3%. Therefore, the experiment was done at this concentration. Sterilized 15ml MRS broth medium containing 0.3% bile was inoculated with 20 µL overnight grown cultures. Then the tubes were incubated at 37℃ and optical density was measured at 620nm for every four hour for 16 hour.

NaCl tolerance test

Test tubes containing MRS broth were adjusted with different concentrations (1-10%) of NaCl. After sterilization, each test tube were inoculated with 1% fresh overnight culture of LABs and incubated at 37ºC for 24 hr. After 24 hr of incubation, growth of the microorganism was determined by observing their turbidity. Maximum growth rate were indicated as double positive (++), normal growth as (+) positive and no growth were indicated as negative (-) sign.

Phenol tolerance

Four different concentrations of phenol (0.1%, 0.2%, 0.3% and 0.4%) were used for this test. MRS broth containing these different concentrations of phenol were inoculated with 20 µl overnight grown bacterial cultures and incubated at 37°C. Then the optical density was measured at 620 nm for determining the tolerance against phenol.

Sugar fermentation

MRS broth (pH 6.5) was taken into the screw-capped test tube and phenol red (.01 gm/l) was added to the tube as pH indicator. The medium was autoclaved at 121ºC for 15 min. After autoclaving, 1ml different sugar solutions (5%) (filtered/sterilized) were inoculated into different tubes. Then 200 µl overnight liquid cultures were inoculated into the broth. A Durham tube was placed inversely to each of the test tube to determine the gas production.

Endospore test

Single colony was aseptically taken by using inoculating loop, and then smeared onto a clean dry slide, later air dried and heat fixed. The slide was covered using blotting paper and then soaked with malachite green and was heated for 5 minutes to steam the stain and then more dye was added as required. The blotting paper was removed and allowed the slide to cool and rinsed with tap water for 30 sec. Finally, safranin was used for 60 to 80 sec and then was examined under light microscope. The vegetative cells should be stained as red and both, endospore and free spore as green.

Growth at various temperatures

For the determination of growth on various temperatures, MRS broth was inoculated with one colony of fresh over-night culture of LAB and was incubated at 25°C, 35°C and 45°C for 24 h. At the time points evaluated, each sample was streaked into MRS agar to monitor growth. The turbidity of each tube was also noted as an indication of growth or no growth. The test was performed in triplicates.

Growth at different pH

Tolerance of LABs to different pH was determined by growing of bacteria in MRS broth having pH 3.5, 4.0, 5.0, 8.0 and 9.0 .The pH was adjusted with 1 N HCL and 0.5 N NaOH, 1% fresh overnight culture of LABs was inoculated into MRS broth having pH 3.5, 4.0, 5.0, 8.0 and 9.0 and incubated at 37°C for 24 h. After 24h incubation, each sample was streaked into MRS agar to determine the presence and absence of growth, which was used to confirm livability of strains. The turbidity of each tube also was noted as an indication of growth or on growth.

Assay for determination of Gastric Juice Tolerance

The gastric juice tolerance of identified lactic acid bacteria was determined using protocol described by Graciela and Maria (2001). The gastric juice tolerance of identified lactic acid bacteria was determined. The artificial gastric juice having pH 2.2 and pH 6.6 was inoculated with the 1% (v/v) bacterial overnight culture. Cell concentration was determined as CFU/ml.

Antimicrobial susceptibility test

Preparation of dried paper disc: Whatman filter paper was used to prepare disc approximately 6mm in diameter using paper puncher and sterilized by autoclaving. Using a micropipette the discs was loaded with 0.005 ml or 5 µl antibiotic solutions one by one. Then the antibiotic discs were dried at room temperature in a sterile laminar flow cabinet which took up to 2 to 3 hours.

Disc diffusion method: Antimicrobial susceptibility was studied by employing the method described by Bauer et al. (1966). Each LAB isolate was spread evenly on MRS agar medium to make bacterial lawn. The plates were allowed to dry for 5-15 minutes. 4 antibiotic discs were placed on the surface of each agar in center of sectioned area and the plates were incubated for 24-48 hours at 37°C anaerobically. After incubation the diameter of zone of inhibition was measured and recorded.

10.2. Study of the effects of native probiotic isolates in mice and poultry through:

10.2.1 Study the effects of native probiotic bacteria isolated from yogurt

Preparation of probiotic yogurt
For the preparation of probiotic yogurt (Figure 2) cow milk was collected from a regional farm and was heated at 100°C for 15 min and then cooled to 40°C. Then the milk was inoculated with a 5% (v/v) liquid culture of isolated Probiotic yogurt culture, which was characterized during biochemical assay. Then the inoculated milk were poured into containers and incubated under anaerobic condition at 37°C for 12 hours. After coagulation fresh yogurt were preserved at 4°C for further usage.

Figure *2*: Preparation of yogurt at laboratory

Experiment No: 01

Effects of native Probiotic isolates on Hypercholesterolemic mice

Thirty six young Swiss-albino mice aged 3-4 weeks, average weight of15-20 gm were used for the experiment. The mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (icddr,b). They were kept in standard environmental condition for ten days in the animal house of the Biotechnology and Genetic Engineering Discipline, Khulna University, Bangladesh for acclimatization. The mice were provided with standard laboratory food and distilled water and maintained at natural day night cycle. All the experiments were conducted on an isolated and noiseless condition.

Prednisone induced Hypercholesterolemia: Prednisone contains high amount of cholesterol or steroids. In this case, mice were induced using 2mg of prednisone per mice by intra peritoneal (IP) injection (Figure 3). It was hoped that within 48- 72 hrs the mice would be induced with high level of cholesterol.

Figure 3: Administration of Prednisone in mice by IP injection

Hypercholesterolemia potential: All mice were kept for 7 days into animal house for their proper adaptation and 3 days for hypercholesterolemia induction. The mice were then divided into following groups as shown in Table 1.

Table 1: Grouping of experimental mice

Control Group	Negative Control Group	Standard Group	Treatment Groups
Normal feed, Distilled Water (Day 11-38) (6 Normal mice)	Normal feed, Distilled Water.(Day 11-38) (6 Hypercholesterolemia induced mice)	Normal feed, Distilled Water (Day 11-38) + Simvastatin (1.5 mg/mice/day) (From day 15) (6 Hypercholesterolemia induced mice)	1. Normal feed (Day 11-38) + Selective probiotics Yogurt about .5 ml/mice/day (6 Hypercholesterolemia induced mice)
			2.Normal feed (Day 11-38) + Selective probiotics Yogurt 1ml/mice/day (6 Hypercholesterolemia induced mice)
			3.Normal feed (Day 11-38) + Selective probiotics Yogurt 1.5ml/mice/day (6 Hypercholesterolemia induced mice)

Total mice=36

Parameter of hypercholesterolemia analysis: Measurement of body weight; serum lipids, alanine transaminase (ALT), and aspartate transaminase (AST); total cholesterol (TC) and total glyceraldehyde (TG) level; serum HDL level and LDL level; total protein, total albumin and total globulin levels were performed.

Body Weight

Body weight of all mice was measured in every week.

Measurement of Serum Lipids, Alanine Transaminase (ALT) and Aspartate Transaminase (AST)

At the end of 4 weeks, all rats were sacrificed after 12 h fasting and blood was collected from the abdominal vein using EDTA tubes. Serum was obtained by centrifugation at 1500×g for 15 min. A Hitachi 7020 system (Hitachi, Tokyo, Japan) was used for analysis of serum LDL, HDL, ALT and AST levels.

Measurement of Hepatic TC and TG

The hepatic lipids were extracted from the liver tissue using a chloroform/methanol mixed solution (2:1, v/v). Samples were then centrifuged at 12,000 g for 10 min. After obtaining supernatants, hepatic TC and TG levels were quantified by using commercial enzymatic kits (Asan Pharmaceutical Co., Asan, Korea).

Measurement of total protein, albumin and globulin

Blood samples were centrifuged at 12,000xg for 10 min and serum was collected. For sample analysis (total protein by Biuret method), a kit supplied by Transasia Bio-medicals Ltd, Baddi, Himachal Pradesh, India was used. With the twenty microlitre of the serum, one ml of total protein reagent was added and incubated for 10 minutes at 37^{o} C. The principle involved for this reaction is that the peptide bonds of protein react with copper II ions in alkaline solution to form blue-violet complex (Biuret reaction) and each copper ion complexes with 5 or 6 peptide bonds. Tartrate was added as a stabilizer whilst Iodide was used to prevent auto-reduction of the alkaline copper complex. The color formed was proportional to the protein concentration.

For sample analysis (albumin-BCG method), a kit supplied by Transasia Bio-medicals Ltd, Baddi, Himachal Pradesh, India) was used. To 10 microlitre of the serum, 1 ml of albumin reagent was added and incubated for 1 minute at 37 ^{o} C. The principle involved in this reaction was that the albumin binds with Bromocresol Green (BCG) at pH 4.2 causing a shift in absorbance of the yellow BCG dye. The Blue green color formed was proportional to the concentration of albumin.

Statistical Analysis
The data was analyzed using Dunnett's Multiple Comparison Test and One way ANOVA through Graph Pad Prism 6.0.

Experiment No: 02

Effects of native Probiotic isolates on diabetic induced model rats

Thirty six adult Long Evans rats weighing 170-360g were included in the study. The animals were bred at Bangladesh University of Health Sciences (BUHS) animal house, Dhaka, Bangladesh. Rats were maintained at a constant room temperature of 22±5^{0}C with humidity of 40-70 % and the natural 12 hours day-night cycle. The rats were fed on a standard laboratory pellet diet and water was supplied *ad libitum*. Standard rat pallet containing wheat (40%), wheat bran (20%), rice polish (5%), fish meal (10%), oil cake (10%), gram (3.9%), pulses (3.9%), milk (3.8%), soyabean oil (1.5%), molasses (0.95%) and salt (0.95%). Embavit GS (vitamin mixture) 250g was added per 100 kg of rat feed.

Preparation of type 2 diabetes model rats: Type 2 diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) in citrate buffer (10 ml), at a dose of 90 mg/kg of body weight into the rat pups (48 hours old, average weight 7 gm) as shown in Figure 4 (Bonner-Weir et.al., 1981). Following 3 months of STZ injection, rats were examined for their blood glucose level by oral glucose tolerance test (OGTT). Diabetic model rats with blood glucose level >7.00 mmol/l at fasting condition were selected for studying the effects of probiotic yogurt.

Figure 4: IP injection of Streptozotocin in model rats for inducing type 2 diabetes

Formulation of gliclazide and probiotic yoghurt doses: For all the pharmacological studies, the drug gliclazide was prepared at a dose of 20 mg/5ml/kg body weight of Type 2 model rats. The probiotic yogurt was given at 2 gm, 4 gm and 6 gm, respectively.

Dose and route of administration: For evaluation of the anti-diabetic activity, yogurt enriched with the probiotic isolates were administrated orally to the rats for 21 days at the doses of 2 gm, 4 gm and 6 gm, respectively in 3 groups. For all the pharmacological studies, the drug gliclazide was administered orally at a dose of 20 mg/5ml/kg body weight of Type 2 model rats. For the control groups, 10 ml water was administrated per kg body weight.

Experimental design

Six normal rats and 30 type 2 diabetic model rats, total 36 long Evans rats were used in this 21 days experimental period. They were divided into 6 groups as shown in Table 2.

Table 2: Experimental rat groups

Group-1 (n=6)	Normal group
Group-2 (n=6)	Type 2 diabetic group (10 ml water/kg body weight)
Group-3 (n=6)	Type 2 gliclazide group 20 mg/5ml/kg body weight of Type 2 model rats
Group-4 (n=6)	Probiotic yoghurt treated group at low dose (2 gm)
Group-5 (n=6)	Probiotic yoghurt treated group at medium dose (4 gm)
Group-6 (n=6)	Probiotic yoghurt treated group at high dose (6 gm)

Collection of blood sample for biochemical analysis:

At day 0, blood samples were collected from rats, kept under fasting conditions, by amputation of the tail tip under diethyl ether anesthesia (Figure 5). Just before the amputation, the tail was immersed into warm water (about 40˚C) for approximately 20 - 30 seconds for vasodilatation. After cutting the tail tip, about 0.2 ml blood was taken out cautiously in eppendorf tube to avoid haemolysis. On the 21st day, the animals were decapitated and their blood was collected from heart.

Figure 5: Blood collection from tail tip of rats after amputation.

The collected blood samples were centrifuged at 2500 rpm for 15 minutes and finally the sera samples were separated (Figure 6) for biochemical analysis and 100 µl of the sera were kept frozen at -20˚C until analysis of fasting serum insulin.

Figure 6: Separation of serum after centrifugation from the blood.

Recording of body weight

All the rats were ensured constant environmental condition and were provided with enough food and water throughout the experiment. The body weights of each rat were measured at seven days interval and accordingly they were provided with their respective treatment.

Biochemical analysis

The following parameters of type 2 diabetic model rats were measured for the anti-diabetic and antioxidant effects of probiotic yogurt.

1. Serum glucose was measured by Glucose Oxidase (GOD-PAP) method using micro-plate reader (Bio-Tec, ELISA).
2. Serum total cholesterol was measured by enzymatic colorimetric (Cholesterol Peroxidase/Oxidase, CHOD-PAP) method (Randox Laboratories Ltd., UK), using auto analyzer, Auto Lab.
3. Serum triglyceride (TG) was measured by enzymatic colorimetric (GPO-PAP) method (Randox Laboratories Ltd., UK) using auto analyzer, Auto Lab.
4. Measurement of glycogen was done from rat liver (standard Method).

Statistical Analysis

Data from the experiments were analyzed using the Statistical Package for Social Science (SPSS, 2016).

Experiment No: 03

Effects of native probiotic isolates on selective enteric pathogens in mice

Principles

Antimicrobial agents are the most widely used therapeutic drugs worldwide. To determine the efficacy, probiotic yogurt was supplied as a probiotic feed. Thirty six mice were divided into six group viz. positive control, negative control, standard group, Treatment group 1, Treatment group 2, and Treatment group 3. All the treatment groups were supplied with three different concentrations of probiotic yogurt till 4 weeks and at the fifth week, treatment groups were induced with different pathogens. At the fifth week, standard group was induced by ciprofloxacin as a standard drug and single pathogen. By comparing the result among the treatment groups, the best one were picked to compare with the standard for measuring the efficacy of selective probiotic isolates over antimicrobial activity.

Experimental animal

To conduct this research work, Swiss albino experimental mice were used. These mice were weighing 15-20 gram at 5-weeks age. These mice bought from the Animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (icddr,b). The animals were kept in cages at room temperature and maintained aseptically in the animal maintaining room of Biotechnology and Genetic Engineering Discipline, Khulna University. All the animals were provided standard mice pellet diet and water adlibitum as their feed. All the mice were adopted about one week prior to the experiment. The care and handling was according to the ethical guidelines approved by Bangladesh Association for Laboratory Animal Science.

Grouping and Dosing

Thirty six mice (18 male and 18 female) were assigned for the study. Male and female mice were separately grouped into six groups each having six mice. The groups were randomly assigned for the study design. All groups were provided their respective treatment orally. The first group was assigned as negative control group and received only basal diet with water to the whole study period. The second group was assigned as positive control group and received basal diet and pathogen was administered. The third group was assigned as standard group and standard drug, ciprofloxacin (500 mg) was administered orally (40mg/ kg body weight) for all the test. For the three test groups, three dose levels of yogurt (50 ml, 100 ml, 150 ml/kg body weight) per day were administered for the Treatment groups 1, 2 and 3, respectively. Yogurt was started to administer from the day of experiment until the 35th day of experiment and at 28^{th} day, single pathogen was induced among the three treatment groups. Details of grouping and dosing are shown in Tables 3 and 4.

Feeding system during trial

Table 3: Feeding of experiential mice from week 1 to week 4

Group	Week 1	Week 2	Week 3	Week 4
Negative control (6 mice)	Pure drinking water + basal feed	Pure drinking water + basal feed	Pure drinking water + basal feed	Pure drinking water + basal feed
Positive control (6 mice)	Pure drinking water + basal feed	Pure drinking water + basal feed	Pure drinking water + basal feed	Pure drinking water + basal feed
Standard Group (6 mice)	Pure drinking water + basal feed	Pure drinking water + basal feed	Pure drinking water + basal feed	Pure drinking water + basal feed
Treatment group 1 (6 mice)	Pure drinking water + basal feed	Pure drinking water + basal feed + probiotic yogurt (50ml)	Pure drinking water + basal feed + probiotic yogurt (50ml)	Pure drinking water + basal feed + probiotic yogurt (50ml)
Treatment Group 2 (6 mice)	Pure drinking water + basal feed	Pure drinking water + basal feed+ probiotic yogurt (100ml)	Pure drinking water + basal feed+ probiotic yogurt (100ml)	Pure drinking water + basal feed+ probiotic yogurt (100ml)
Treatment group 3 (6 mice)	Pure drinking water + basal feed	Pure drinking water + basal feed+ probiotic yogurt (150ml)	Pure drinking water + basal feed+ probiotic yogurt (150ml)	Pure drinking water + basal feed+ probiotic yogurt (150ml)

Table 4: Feeding of experimental mice during week 5

Group	Week 5
Negative Control (mice=6)	Pure drinking water + basal feed
Positive Control (mice=6)	Pure drinking water + basal feed
Standard (mice=6)	Pure drinking water + basal feed + Antibiotic (ciprofloxacin, 40mg/kg body weight) + pathogenic microbes : *E. coli*
Treatment group1 (mice= 6)	Pure drinking water + basal feed + Probiotic yogurt (50ml) + pathogenic microbes *E. coli*
Treatment group 2 (mice=6)	Pure drinking water + basal feed + Probiotic yogurt (100ml) + pathogenic microbes *E. coli*
Treatment group 3 (mice=6)	Pure drinking water + basal feed + Probiotic yogurt (150ml) + pathogenic microbes *E. coli*

Blood Collection

At the end of 5 weeks, all rats were sacrificed after fasting for 12 hours and blood was then collected (Figure 7) from the abdominal vein by heparinized syringe. Serum was collected by centrifugation at 1500×g for 15 min.

Figure 7: Blood taken from rats

Parameter of Antimicrobial activity analysis

- Body weight
- Complete blood count (CBC)
- Total protein count
- A/G ratio determination
- Immunoglobulin (IgG) test
- Leishman staining
- Inhibition of the growth of pathogenic microorganisms *in vivo*

Experiment No: 04

Effects of native Probiotic isolates on diarrhea induced mice

Grouping and Dosing

Thirty six mice (18 male and 18 female) were assigned for the study. Male and female mice were grouped separately into six, having six mice in each group. All groups were provided their respective treatment orally as shown in Table 5. The first group was assigned as negative control and received only basal diet with water for the whole study period. The second group was assigned as positive control and received basal diet and castor oil was administered. The third group was assigned as standard group and standard drug, loperamide (3 mg/Kg) was administered orally for the entire test. For the three test groups, three dose levels of yogurt (0.5 ml, 1 ml and 1.5 ml) per mice per day were determined for the Treatment groups 1, 2 and 3 respectively. Yogurt was started to administer from the day of experiment until the 27th day of experiment.

Table 5: Grouping of mice according to treatment

Animal Groups (No.)	Treatment	Doses (Per kg body weight)	Duration of administration (Days)	Route of administration
Negative Control(I)	Water	10 ml	1-28	Orally
Positive Control (II)	Castor oil	25 ml	1-28	Orally
Standard (III)	Loperamide	3 mg	1-28	Orally
Treatment Group-1(IV)	Probiotic Yogurt	0 5 ml	1-27	Orally
Treatment Group-2(V)	Probiotic Yogurt	1.0 ml	1-27	Orally
Treatment Group-3(VI)	Probiotic Yogurt	1.5 ml	1-27	Orally

Castor oil induced diarrhea

The method was used in this study was described by Umer et al. (2013) with slight modifications. Briefly, Swiss albino mice of the described group was fasted for 24 hours but had access to water. Mice in the standard group were administered (3 mg/kg) loperamide 1 hour before administration of castor oil. Then all the mice were given 0.5 ml of castor oil orally and white blotting paper was lined under each of the cage. During the observation period of four hour, latent period (time interval between the administration of castor oil and the first defecation in minute), total fecal output and fecal water content were recorded for individual mouse. Percentages of diarrhea inhibition as well as the weight of total and wet fecal output were determined according to the formula 1and 2**.**

1. Inhibition % = $\frac{\mathbf{ATFPC-ATFT}}{\mathbf{ATFNC}}$ x100
   ATFPC= Average number of wet feces in positive control group
   ATFT= Average number of total feces in test group

2. Total fecal output % =$\frac{\mathbf{Meanfaecalweightofeachgroup}}{\mathbf{Meanfaecalweightofpositivecontrol}}$ x100

Fecal output and fecal water content in mice

After administration of castor oil, when the feces became unformed; muddy or watery was considered as diarrheic. Feces samples were collected after each defecation and put into a covered vessel for each animal to prevent the feces from drying. All the feces collected over 4 hour period of time dried for 1 hour at 100°C in a ventilated oven.

Fecal water content was determined according to the following formula:
Fecal water content = wet fecal weight - dry fecal weight.

Effect in serum metabolites

After four hour of observation, all the mice were sacrificed and blood was obtained by heart puncture. Then the blood was kept in anticoagulant tube and analyzed for total protein, albumin, sodium, potassium, calcium, phosphate, WBC and hemoglobin.

Statistical Analysis

Data were analyzed using Statistical Analysis System (SAS) and Graph Pad Prism Version 5.0.

Experiment No: 05

Effects of native Probiotic isolates on allergic induced mice model

Identified presumptive strains were used in various concentrations (ml) after subdividing the mice into six groups namely negative control group (NCG), positive control group (PCG), standard group (SG),Treatment group 1 (TG 1), Treatment group 2 (TG 2) and Treatment group 3 (TG 3) for a 4-week trial period.

Feeding procedure used in the mice trial

1. In the negative control group, only basal feed was provided from week 1 to week 4.
2. In case of positive control group, basal feed was given from week 1 to week 2 and histamine (minarin) along with basal feed was given from week 3 to week 4.
3. For the standard group, basal feed was given from week 1 to week 2 and a histamine (minarin, at the rate of 1% of body weight) and an anti-histamine (fenadin, at the rate of 1% of body weight) were provided parallel with basal feed from week 3 to week 4.
4. For the Treatment group 1, basal feed was given from week 1 to week 2 and a histamine (minarin, at the rate of 1% of body weight) and probiotic yoghurt (at the rate of 0.5ml/mouse) were provided parallel with basal feed from week 3 to week 4.
5. For the Treatment group 2, basal feed was given from week 1 to week 2 and a histamine (minarin) and probiotic yoghurt (at the rate of 1ml/mouse) were provided parallel with basal feed from week 3 to week 4.
6. For the Treatment group 3, basal feed was given from week 1 to week 2 and a histamine (minarin) and probiotic yoghurt (at the rate of 1.5 ml/mouse) were provided parallel with basal feed from week 3 to week 4.

Table 6: Grouping of mice for the trial

Group	No. of mice/group
Negative Control Group	6
Positive Control Group	6
Standard Group	6
Treatment Groups	18 (three subgroups were made, each having 6 mice)
Total	36

Table 7: Feeding plan of experimental mice during trials

Group	Week 1	Week 2	Week 3	Week 4
Negative Control Group	Basal feed + Pure drinking water	Basal feed + Pure drinking water	Basal feed + Pure drinking water	Basal feed + Pure drinking water
Positive Control Group	Basal feed + Pure drinking water	Basal feed + Pure drinking water	Basal feed + Pure drinking water + Minarin	Basal feed + Pure drinking water + Minarin
Standard Group	Basal feed + Pure drinking water	Basal feed + Pure drinking water	Basal feed + Pure drinking water + Minarin + Fenadin	Basal feed + Pure drinking water+ Minarin + Fenadin
Treatment Group 1	Basal feed + Pure drinking water	Basal feed + Pure drinking water	Basal feed + Pure drinking water + Minarin + probiotic yogurt (0.5 ml/mouse)	Basal feed + Pure drinking water+ Minarin + probiotic yogurt (0.5 ml/mouse)
Treatment Group 2	Basal feed + Pure drinking water	Basal feed + Pure drinking water	Basal feed + Pure drinking water+ Minarin + probiotic yogurt (0.5 ml/mouse)	Basal feed + Pure drinking water+ Minarin + probiotic yogurt (0.5 ml/mouse)
Treatment Group 3	Basal feed + Pure drinking water	Basal feed + Pure drinking water	Basal feed + Pure drinking water+ Minarin + probiotic yogurt (0.5 ml/mouse)	Basal feed + Pure drinking water+ Minarin + probiotic yogurt (0.5 ml/mouse)

After then several physical symptoms were observed and the readings for the indication of allergy were taken.

Physical symptoms recorded were:

1. Fatigue
2. Loss of appetite
3. Decreased motility
4. Increased prone to any disease
5. Death rate

Data measured were:

1. Eosinophil level count
2. IgE level count
3. Peripheral lymphocyte count

10.2.2 Study the effects of native probiotic bacteria isolated from poultry

Experiment No. 6

Effects of native Probiotic isolates as layer starter feed supplement

Probiotic feed development

Bacteria, growth conditions and preparation of cell suspension

Previously isolated Probiotic bacteria were cultured in MRS media for 24 h at 37˚C. Cells were harvested by centrifugation at 3000xg for 20 min and washed twice with phosphate buffer saline (PBS) at pH 7. Cell counts were determined by standard plate method after 48 h under anaerobic conditions at 37˚C on MRS agar media.

Bacterial enumeration

To determine the viable counts of the probiotics, 1 ml of probiotic culture was added with 9 ml of phosphate buffer (0.1 M, pH 7.4), followed by gentle shaking for 30 min at room temperature. Seven fold serial dilutions were done. The colony forming units (CFU/ml) were determined by plating on MRS agar plates and incubation for 48 h at 37˚C. Isolated bacterial colonies were enumerated on MRS agar. Peptone water was used to prepare the serial dilutions. The culture was plated using the pour plate technique and incubated at 30˚C for 48 hours.
So, the dilution factor = Final Volume / Sample volume × diluted concentration

$$=10/1 \times 10^7 \text{ or } 10 \times 10^7$$

Then 1 ml of diluted probiotic solution was inoculated and cultured for 24 hours at 37˚C. The colony counted for each plate was 88.

In this case CFU/ml = (no. of colonies x dilution factor) / volume of culture plate

$$= (88 \times 10 \times 10^7) / 1$$
$$= 8.8 \times 10^9 \text{ CFU/ml}$$

Broth cultures (mixed) were centrifuged at 10,000 rpm for 5 min and pellets were collected. Which were properly mixed with the starter feed daily in an amount according to their fixed CFU.

Preparation of layer feed

All the ingredients, mentioned in Table 8, were mashed through grinding and properly mixed according to their proper nutrient composition.

Table 8: Feed composition for starter diet of layer chick (0-9 weeks)

Ingredients	Quantity (kg)	Ingredients	Quantity (kg)
Maize	60.0	Oyster Shell	2.0
Fishmeal	10.0	Vitamin/Mineral Premix*	0.5
Soybean Meal	15.0	Sodium chloride	0.5
Wheat Bran	12.0	Probiotics	40 ml/kg and 50 ml/kg
Total = 100 kg			

*Source: J. Bawah, 2015; *Composition of premix is shown in Table 10*

Inoculation of probiotic into feed

Probiotic cells obtained from centrifugation were mixed with starter mash according to their specific dose. In the preparation of layer starter feed, 40 ml and 50 ml of probiotic culture were added to 1 kg of starter feed.

Determination of the effect of probiotic feed on layer chick

Experimental birds

Fifty day old female ISA Brown layer chicks were used to conduct the experiment. The chicks were collected from Islamia Traders, Moylapota, Khulna.

Experimental design

The experiment was conducted in the Regional Youth Training Center, Khulna. A chicken shade was provided to rare the chicks for the experimental purpose. The experiment was designed for three weeks of time and additional 3 days were also added for acclimatization of the chicks. After three days of arrival of the chicks, they were divided into five groups. Among them four were treatment groups consisting of two probiotic treatment groups (T1 and T2) and two antibiotic groups (T3 and T4) and the remaining one was the control group (C) with no probiotic or antibiotic.

C= Control Group with no probiotic or antibiotic
T1= Treatment Group supplemented with 50 ml/kg probiotic feed
T2=Treatment Group supplemented with 40 ml/kg probiotic feed
T3=Treatment Group with 1 gm/L of oxy-tetracycline
T4=treatment Group with 1gm/ L of Ciprofloxacin

Bird management

The birds were kept in houses with a dimension of 80 cm×90 cm providing 7200 cm^2 area for ten layer chicks. For the flooring of the house 6 cm deep litter mixed with quick lime was used. Cleaned chick feeder and drinker was used for the experiment. After arrival of the chicks, they were provided with glucose to overcome their journey stress. The basal diet for layer starter was given to all chicks for three days of acclimatization. During the treatment period, the Treatment Group 1 and 2 were provided with starter feed supplemented with specific probiotic does and the Treatment Group 3 and 4 were provided with additional antibiotic selected for the experiment. Only the basal diet was provided to the control group. Feed and water was given four times a day and the chick house was facilitated with electric bulb and brooder for gaining the optimum temperature for the chicks.

Data Recording

The following parameters were measured weekly:

Body weight gain

The initial weight of the chicks was measured which was indicated by week 0 weight. The body weight of the chicks of all groups was determined by using an electric balance and documented to analyze the data. The difference of the weight of the 1st and last day of the week was considered as the weekly body weight gain.

Feed intake

To determine the feed intake ratio, the feed consumed per week in a group was divided by the number of chick number of that group.

Feed conversion ratio (FCR)

Feed conversion ratio of the chicks was determined by dividing the feed intake by the body weight gain.

Feed conversion ratio of chick =$\frac{daily feed intake}{daily \text{weight gain}}$

Daily weight gain

Daily weight was obtained by dividing the weight gain by the particular days that was required for that weight gain.

Mortality rate

The number of chicken died in the different group during the experimental period was considered to determine the mortality rate of the chicks of that specific group.

Mortality rate of chick = $\frac{number of chick died \times 100}{total chicks}$

Collection of blood sample

Blood samples of the chicks from different treatment and control groups were collected from the wing vein using a sterile syringe (Figure 8) to analyze the serum cholesterol level and serum Ca level. Blood were stored in Vacutainer blood collection tube and then sent to Science Diew diagnostic and Research Centre, Sonadanga, Khulna to test the samples.

Figure 8: Blood sample collection from wing vein of layer chick

Experiment No. 7

Effects of native Probiotic isolates as broiler starter feed supplement

Probiotic feed development, Probiotic bacteria, growth conditions, preparation of cell suspension and bacterial enumeration were same as described under the Experiment 6.

Preparation of probiotic based poultry starter feed

All the ingredients (Table 9) were mashed through grinding (Figure 9) and properly mixed according to their proper nutrient composition. With the starter mash Square premix broiler (Table 10) was added at the rate of 250 gm/100 kg feed. Probiotic cells obtained from centrifugation were mixed with starter mash according to their specific doses.

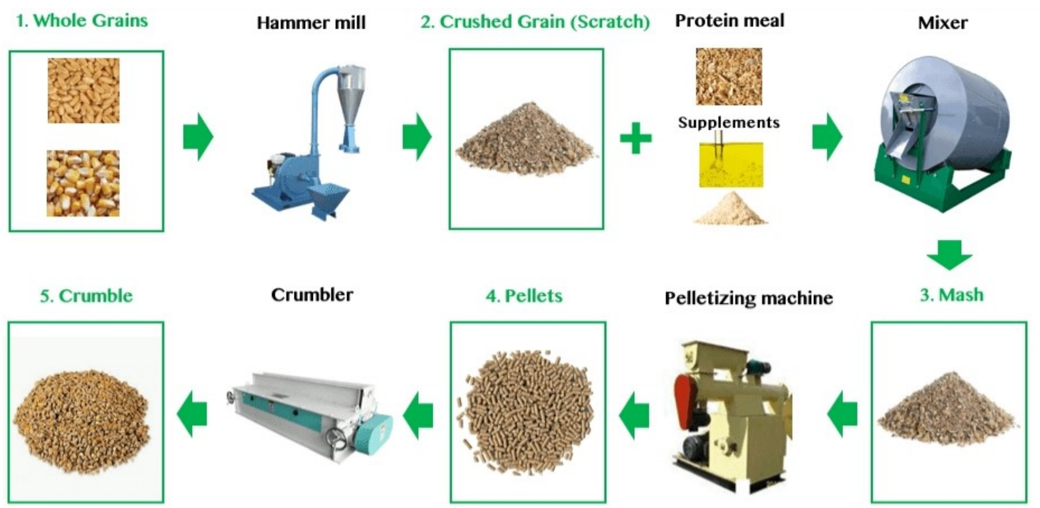


Figure 9: Flow chart of preparation of Starter mash feed for broiler

Table 9: Ingredients used in broiler starter mash

Ingredients	Quantity (gm)	Ingredients	Quantity (gm)
Maize	212	Rice bran	50
Wheat	270	Premix*	4
Soybean meal	300	DCP	10
Fish meal	100	NaCl	4
Wheat bran	50	Probiotics	2×10^9CFU and 4×10^9CFU
Total = 1 Kg			

*Probiotic dose: 35gm/kg and 50gm/kg; *Composition of premix is shown in Table 10*

Table 10: Composition of Square premix

Ingredients	Quantity (mg)	Ingredients	Quantity (mg)	Ingredients	Quantity
Vitamin E	20,000	Biotin	100	Anti-Oxidant	5,000 mg
Vitamin K_3	4,000	Iron	40,000	Nicotinic acid	40,000 mcg
Vitamin B_1	2,500	Iodine	400	Vitamin B_{12}	12,000 mcg
Vitamin B_2	5,000	Manganese	60,000	Di-Calcium Phosphate	380 gm
Pantothenic acid	12,500	Zinc	50,000	Carrier (lime stone)	q. s. to make 2.5 kg
Vitamin B_6	4,000	Selenium	150		
Folic acid	800	Zinc-bacitracin	4,000		

Experimental house and the birds

The experiment was conducted in a room with an area of 5 sq. ft. The room was partitioned into 5 parts of equal size using hard paper (Figure 10). Area of each pen was nearly 0.5 sq. ft. A total of 40 day-old Cobb 500 broilers (mixed sex) commercial broiler chicks were purchased from the CP Poultry Co. Khulna to carry out this research work. The chickens were randomly divided into a control group and 4 experimental groups (treatment). The experiment was conducted for a period of 22 days from 03 September 2018 to 26 September 2018.

Figure 10: Experimental chickens in five divided spaces

Management of the experimental birds

The chicks were brooded in the respective pens using one 100-watt electric bulb per pen. The chicks were provided with a temperature of 35°C at first week of age, decreasing gradually at a rate of 3°C per week and continued up to 3 weeks of age to 25°C. Fresh and dried rice husk was used as litter material and spread over the floor at a depth of 3 cm. After first two weeks, upper part of the litter mixed with droppings was removed and replaced with new litter. At the end of 2 weeks, old litter was totally replaced by new litter. Litter was stirred in every alternative day to dry up quickly and to remove harmful gases. The broilers were exposed to a continuous lighting period of 23 hours and a dark period of 1 hour in each 24 hours. During the 22 days of experimental period, environmental factors (lightning, temperature, humidity, ventilation) maintained at optimal levels recommended for Cobb 500 broiler chickens.

Feeding program

Starter diet was provided in all cases, feeds were offered adlibitum to all broilers. Feed was supplied four times daily; once in the morning, noon, afternoon and again at night in such a way that feeder was not kept empty. Fresh and clean water was made available at all times.

Vaccine

The vaccination schedule that was followed during the experimental period is given in Table 11.

Table 11: Vaccination schedule of broiler chicks

Age of broilers (day)	Name of Vaccine	Dose	Route of vaccination
1	ICDRB	One drop	Eye
14	GUMBORO	One drop	Eye

Experimental design

The experiment was conducted in the Regional Youth Training Center, Khulna. A chicken shade was provided to rare the chicks for the experimental purpose. The experiment was designed for three weeks of time and additional 3 days were also added for acclimatization of the chicks. The chicks were divided into five groups after three days of arrival. There were four treatment groups consisting of two probiotic groups (T1 and T2) and two antibiotic groups (T3 and T4) and the remaining one was the control group (C) with no probiotic or antibiotic (Table 12).

Table 12: Experimental design of chickens in five groups

Control (C)	Treatment 1 (T1)	Treatment 2 (T2)	Treatment 3 (T3)	Treatment 4 (T4)
	GPA		**Probiotic**	
BD + Premix	BD + Premix +Oxytetracycline (Renamycin) 400-800mg	BD + Premix +Chlortetracycline (Auriomycin) 400-800mg	BD + Premix +Probiotic supplement (2×10^{9} CFU)	BD + Premix +Probiotic supplement (4×10^{9} CFU)

Where, BD = Basal Diet (70-100 g /chicks/day); GPA = Growth promoting antibiotics (500 mg/day); Probiotics: 35gm/kg and 50gm/kg mixed with basal feed in T3&T4 group respectively.

Processing of broilers

At the end of feeding trial, average weight were taken from each pen for recording growth and meat yield parameters. At the end of experiment, 3 birds per treatment (total 15 birds) were randomly selected, slaughtered and blood samples collected. The carcasses were then immersed in hot water (semi-scalding; at 51-55°C) for 120 seconds in order to loosen feathers followed by removal of feathers by hand pinning. Then head, shank, viscera, giblet (heart, liver and gizzard) and abdominal fat were removed for determination of meat yield parameters. Dressed broilers were cut into different parts such as breast, thigh, drumstick and wing. Finally, every cut up parts were weighed and recorded for male and female broilers of all the treatments.

Data recording for performance study

Body weight gain (BWG), Average daily gain (ADG), Feed intake, Feed conversion ratio (FCR) and Mortality rate was measured as described under the experiment 6. However, Carcass yield, Meat quality and Serum biochemical test was conducted as described below:

Carcass yield

a. Dressed yield (g)
b. Breast meat (g)
c. Drumstick (g)
d. Wing (g)
e. Abdominal fat (g)

Calculation: (weight of the carcass/weight of the live chicks) x 100

Meat quality

Evaluation of protein content in the different treatment groups

Method

Sample collection

Meat was in vitro digested according to the procedures of Escudero et al. (2010) with some modifications. Five (5) gm of meat sample was blended using blender. Trypsin was added based on the mass of meat (5g in 100 ml DW). After 2 h of trypsin digestion, enzyme activity was terminated by heating at 95℃ for 5 min followed by storing for 12 hours at -20℃ for further analysis. The test was conducted at Science view diagnostics and research center, KDA outer by pass, Sonadanga, Khulna.

Serum biochemical test

Collected serum was tested for cholesterol content among the different treatment groups. The parameters studied were:

I. HDL (mg/dL)
II. LDL (mg/dL)

Mortality rate (%)

Mortality of chicks was observed during each day of trial.

Statistical analysis

Data of body weight gain, carcass yield, ADG of broilers were subjected to analysis of variance (One Way ANOVA) in a completely randomized design (CRD). Graph pad Software was used to perform F test in analysis of variance. Probability values P<0.05 were considered as significant values & P<0.01 were considered as highly significant.

Experiment No. 8

Effects of native Probiotic isolates as duck starter feed supplement

Probiotic feed development, Probiotic bacteria, growth conditions, preparation of cell suspension and bacterial enumeration were same as describe under the Experiment 6.

Preparation of duck starter mash

All the ingredients (Table 13) were mashed through grinding (Figure 8) and properly mixed according to their proper nutrient composition. With the starter mash the premix was added as required. For feeding the probiotic Treatment Groups, the serially diluted potential probiotics were centrifuged and the pelleted cells were mixed with the mashed feed. For feeding the antibiotic Treatment Groups, the selected antibiotics were mixed with the mashed starter feed. The Control Group was supplied only with the mashed feed and the premix.

Table 13: Composition of duck starter feed (Wang, 2007)

Ingredients	Percentage (%)	Ingredients	Percentage (%)
Maize	51.0	Talcum powder	1.2
Soybean meal	29.0	Dicalcium phosphate	1.7
Fish meal	2.0	NaCl	0.3
Wheat shorts	9.3	Carrier (Bentonite)	1.0
Chaffed straw	3.5	Premix	1.0
	Total		100

***In vivo* trial of Duck**

Experiment site

The animal trial section of this study was performed in the poultry shed of Regional Youth Development center, Khulna.

Experimental Ducks

Day old 40 male Khaki Campbell (*Anas platyrhynchos domesticus)* ducklings were collected from "Rupali duck farm, Daulatpur, Khulna".

Duck model management

Brooder house was constructed for rearing the day old ducklings for the first few days (Figure 11). A system with proper ventilation, humidity and temperature was ensured for the safety of the duckling during brooding period. The bed was prepared with husk mixed with lime and almost 8 cm deep bed was prepared. Heating devices were uniformly distributed and 100W bulb was mainly used for heat generation. Drinkers and feeders were well washed and were kept ready. The light supply was continued for 24 hours for helping the ducklings to easily recognize the feeders and drinkers.

Figure 11: Ducklings in brooding condition prior to grouping

After receiving the ducklings they were immediately supplied with 10% Dextrose. After day three, Rena WS multivitamin 1g/litter water was supplied to all the ducklings. The bed was kept dry by altering the litter twice a day and the ducklings were supplied with normal basal diet for first two days prior to Treatment and dosing period. The body weight of the ducklings were taken daily and noted.

Treatment and duck trial design

After the initial two days, the 3 week treatment period started. The ducklings were grouped into five groups with 8 ducklings per group (Table 14 and Figure 12). One group was the Control and they were supplied with only basal diet and premix. The two antibiotic Treatment Groups (T1 and T2) were supplied with basal diet, premix and the specific antibiotic for the first seven days of trial. T3 and T4 were the other two groups where two different concentrations of probiotics were given with the basal diet. The water supply, temperature, humidity, light supply, ventilation and all other things were provided evenly to all the groups. All the experimental ducklings were fed ad libitum.

Table 14: Experimental duckling grouping and dosing

Groups (Number)	Diet	Treatment and Dose	Dosing Period
Control (8)	Basal diet +Premix	-	-
Treatment Group 1 (8)	Basal diet +Premix	Tylosin (1g/L drinking water)	For first 7 days
Treatment Group 2 (8)	Basal diet +Premix	Oxytetracycline (1g/L drinking water)	For first 7 days
Treatment Group 3 (8)	Basal diet +Premix	Probiotics (8.8×10^{9} CFU/ml) 15 ml/kg feed	For all three weeks
Treatment Group 4 (8)	Basal diet +Premix	Probiotics (9.9×10^{9} CFU/ml) 15 ml/kg feed	For all three weeks

Figure 12: Experimental ducklings

Data recording for performance study, Carcass status determination and serum biochemical tests
Performance characteristics, carcass status determination and serum biochemical tests were conducted as described under the Experiment 7.

11. Results and Discussions

11.1 Results

11.1.1 Isolation and characterization of presumptive probiotic isolates from yogurt and poultry

11.1.1.1 Isolation and characterization of presumptive probiotic isolates from yogurt

Table 15: Isolation and characterization of presumptive Probiotic isolates from yogurt

Isolates from different Divisions with locations			Biochemical Tests			Physiological Tests
Dhaka Division, Dhaka	**Short names**	**Exact locations**	**Gram Staining**	**Catalase Test**	**Coagulase Test**	**Motility**
Prince Sweets	PS	Mirpur1, Bus stand	+	-	+	-
Vikrampur	VKP	119, Lake Circus, Kolabagan	+	-	+	-
Moronchand	MOC	Section-1, Mirpur	+	-	+	-
Vaggokul	VGK	67, Lake Circus, Kolabagan	+	-	+	-
Mohonchand	MHC	22/Mirpur road, New Market	+	-	+	-
Muslims Sweets and Confec.	MSC	23/8 Mirpur Road, Shaymoli	+	-	+	-
Cumilla						
Dodhi Mella	DM	Rajgon, Deshwalipatti	+	-	+	-
Mithai	MTI	Rajgon, Deshwalipatti	+	-	+	-
Vikrampur Ghee Store	VGS	Rajgon, Deshwalipatti	+	-	+	-
Mattri Vander	MV	Monohorpur	+	-	+	-
Amrita Vander	AV	Rajgon, Deshwalipatti	+	-	+	-
Shitol Vander	SV	Monohorpur	+	-	+	-
Khulna Division, Khulna						
Tala Adi Ghosh Dairy	TGD	Sher-E-Bangla Road, Moilapota Bottala	+	-	+	-
Sathkhira Ghosh Dairy (PKG)	SGD	Sher-E-Bangla Road, Moilapota Bottala	+	-	+	-
Sathkhira Ghosh Dairy (KG)	SGDKG	147, Sher-E-Bangla Road, (Haider Complex), Moilapota More	+	-	+	-
New Sathkhira Ghosh Dairy (PKG)	SGDAAR	15/1 Ahsah Ahmed Road	+	-	+	-
Misti Mohol	MM	105, BIDC Road Khalishpur	+	-	+	-
Dodhi Ghor	DG	Shaheber Kobor Khana, Symmentry Road	+	-	+	-
Chattogram Division, Chattogram						
Food Fair	FF	O.R. Nizam Road	+	-	+	-
Well Foods LTD.	WF	O.R. Nizam Road	+	-	+	-
Flavors	FVS	O.R. Nizam Road, GEC More	+	-	+	-
Moo Royale	MR	O.R. Nizam Road, GEC More	+	-	+	-
Sweet Kings	SK	O.R. Nizam Road	+	-	+	-
K-Bakery	KB	O.R. Nizam Road	+	-	+	-

(Table 15 continued)

Rajshahi Division, Bogra and Pabna						
Doi Ghor	DG	Station Road, Sathmatha, Bogra	+	-	+	-
Moharram Ali Doi Ghor	MADG	Station Road, Sathmatha, Bogra	+	-	+	-
Asia Sweet Meat & Cold Drinks	ASMCD	Kobi Kai Nazrul Islam Road, Bogra	+	-	+	-
Bonolota Sweets & Bakery	BSB	Central Bus A. Hamid Road, Pabna	+	-	+	-
Akbaria Sweets	AS	Kobi Kai Nazrul Islam Road, Bogra	+	-	+	-
Shamol Doi Vander	SDV	A.R. Corner, A. Hamid Road, Pabna	+	-	+	-
Sylhet Division, Sylhet						
Rifat and Co.	RC	Sunamgang Road, Kumargaon	+	-	+	-
Fizza and Co.	FC	Sattar Manshon, Mendibagh	+	-	+	-
Modhubon	MB	Rose View Complex, Subhani Ghat	+	-	+	-
Shaad and Co.	SC	898 Sheikh Ghat	+	-	+	-
Bonoful and Co.	BC	Golden Plaza, Subhani Ghat	+	-	+	-
Fulcoli Sweets and Pure Foods	FS	9-10 Bisic Industrial Estate, Khadimnagar	+	-	+	-
Mymensingh Division, Mymensingh						
Krishna Cabin	KC	55 Station Road, Asad Market	+	-	+	-
Anil Ghosh Sweets	AG	7, Swadeshi Bazar	+	-	+	-
Dayamay Mistanno Bhandar	DM	19, A.B Guho Road	+	-	+	-
Misti Kanon	MK	14, Hossain Plaza, Ganginapar	+	-	+	-
Sudhir Ghosh	SG	2, Swadeshi Bazar	+	-	+	-
Mamoni Sweets	MS	1/A GC Guho Road	+	-	+	-

Legends: (+) means reaction positive; (-) means reaction negative

According to Table 15, all the isolates collected from the different sources of yogurt were Gram positive, Catalase negative, Coagulase positive and Motility negative. Most of the isolates were found to have positive antimicrobial activity against the common pathogens *Vibrio cholera*, *Streptococcus aureus*, *Micrococcus*, *Salmonella paratyphi*, *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* (Table 16).

Table 16: Antimicrobial activity of selective presumptive probiotic isolates from yogurt

Isolates from different Divisions		Name of pathogens							
Dhaka	**Short forms**	***Vibrio cholera***	***Streptococcus aureus***	***Micrococcus***	***Salmonella paratyphi***	***Bacillus megaterium***	***Pseudomonas aeruginosa***	***Salmonella typhi***	***Escherichia coli***
Prince Sweets	PS	+	+	+	+	+	+	+	+
Vikrampur	VKP	-	+	+	+	+	+	+	+
Moronchand	MOC	+	+	+	+	+	+	+	+
Vaggokul	VGK	+	+	+	-	+	+	-	-
Mohonchand	MHC	+	+	+	+	+	+	+	+
Muslims Sweets and Confectionary	MSC	+	+	+	+	+	+	+	+
Cumilla									
Dodhi Mella	DM	+	+	+	+	+	+	+	+
Mithai	MTI	-	+	+	+	+	+	+	+
Vikrampur Ghee Store	VGS	+	+	+	+	+	+	+	+
Mattri Vander	MV	+	+	+	+	+	+	+	+
Amrita Vander	AV	+	+	+	+	+	+	+	+
Shitol Vander	SV	+	+	-	+	+	+	-	-
Khulna									
Tala Ghosh Dairy	TGD	+	-	+	+	+	+	+	+
Sathkhira Ghosh Dairy	SGD	+	+	+	+	+	+	+	+
Sathkhira Ghosh Dairy (KG)	SGDKG	+	+	+	+	+	+	+	+
Sathkhira Ghosh Dairy (AAR)	SGDAAR	+	+	+	+	+	+	+	+
Misti Mohol	MM	+	+	-	+	+	+	-	+
Dodhi Ghor	DG	+	+	+	+	+	-	+	+
Chattogram									
Food Fair	FF	+	+	+	+	+	-	+	+
Well Foods LTD.	WF	-	+	+	+	+	+	-	+
Flavors	FVS	+	+	+	+	+	+	+	+
Moo Royale	MR	+	+	+	+	+	+	+	-
Sweet Kings	SK	-	+	+	+	+	+	-	+
K-Bakery	KB	+	+	-	+	-	-	+	+
Rajshahi									
Doi Ghor	DG	-	+	+	+	+	+	+	+
Moharram Ali Doi Ghor	MADG	+	+	+	+	+	+	+	+
Asia Sweet Meat & Cold Drinks	ASMCD	+	+	+	+	+	+	+	+
Bonolota Sweets & Bakery	BSB	+	+	+	-	+	+	+	+
Akbaria Sweets	AS	-	+	+	+	+	+	+	+
Shamol Doi Vander	SDV	+	+	-	+	+	+	+	+

(Table 16 continued)

Sylhet									
Rifat and Co.	RC	-	+	+	+	+	+	+	+
Fizza and Co.	FC	+	+	+	+	+	+	+	+
Modhubon	MB	+	+	+	+	+	+	+	+
Shaad and Co.	SC	+	+	+	-	+	+	+	+
Bonoful and Co.	BC	-	+	+	+	+	+	+	+
Fulcoli Sweets	FS	+	+	-	+	+	+	+	+
Mymensingh									
Krishna Cabin	KC	+	+	+	+	+	-	+	+
Anil Ghosh Sweets	AG	-	+	+	+	+	+	-	+
Dayamay Mistanno Bhandar	DM	+	+	+	+	+	+	+	+
Misti Kanon	MK	+	+	+	+	+	+	+	-
Sudhir Ghosh	SG	-	+	+	+	+	+	-	+
Mamoni Sweets	MS	+	+	-	+	-	-	+	+

Legends: (+) means pathogens are sensitive to the probiotics antimicrobial activity, (-) means pathogens are resistant to the probiotics antimicrobial activity

Table 17 shows that all the presumptive probiotic isolates from yogurt had probiotic properties of Low pH tolerance (pH 3), Bile salt tolerance (0.3%), NaCl tolerance (up to 4%) and Phenol tolerance (up to 0.4%).

Table 17: Probiotic properties of the isolates from yogurt

Name of isolates		**Probiotic Properties**									
Dhaka Division	**Short names**	**Low pH Tolerance**	**Bile salt Tolerance (%)**	**NaCl Tolerance (%)**				**Phenol Tolerance (%)**			
		pH 3	**0.3**	**2**	**4**	**6**	**8**	**0.1**	**0.2**	**0.3**	**0.4**
Prince Sweets	PS	+	+	+	+	-	-	+	+	+	+
Vikrampur	VKP	+	+	+	+	-	-	+	+	+	+
Moronchand	MOC	+	+	+	+	-	-	+	+	+	+
Vaggokul	VGK	+	+	+	+	-	-	+	+	+	+
Mohonchand	MHC	+	+	+	+	-	-	+	+	+	+
Muslims Sweets and Confectionary	MSC	+	+	+	+	-	-	+	+	+	+
Cumilla Division											
Dodhi Mella	DM	+	+	+	+	-	-	+	+	+	+
Mithai	MTI	+	+	+	+	-	-	+	+	+	+
Vikrampur Ghee Store	VGS	+	+	+	+	-	-	+	+	+	+
Mattri Vander	MV	+	+	+	+	-	-	+	+	+	+
Amrita Vander	AV	+	+	+	+	-	-	+	+	+	+
Shitol Vander	SV	+	+	+	+	-	-	+	+	+	+

(Table 17 continued)

Khulna Division											
Tala Ghosh Dairy	TGD	+	+	+	+	-	-	+	+	+	+
Sathkhira Ghosh Dairy	SGD	+	+	+	+	-	-	+	+	+	+
Sathkhira Ghosh Dairy (KG)	SGDKG	+	+	+	+	-	-	+	+	+	+
Sathkhira Ghosh Dairy (AAR)	SGDAAR	+	+	+	+	-	-	+	+	+	+
Misti Mohol	MM	+	+	+	+	-	-	+	+	+	+
Dodhi Ghor	DG	+	+	+	+	-	-	+	+	+	+
Chattogram Division											
Food Fair	FF	+	+	+	+	-	-	+	+	+	+
Well Foods LTD.	WF	+	+	+	+	-	-	+	+	+	+
Flavors	FVS	+	+	+	+	-	-	+	+	+	+
Moo Royale	MR	+	+	+	+	-	-	+	+	+	+
Sweet Kings	SK	+	+	+	+	-	-	+	+	+	+
K-Bakery	KB	+	+	+	+	-	-	+	+	+	+
Rajshahi Division											
Doi Ghor	DG	+	+	+	+	-	-	+	+	+	+
Moharram Ali Doi Ghor	MADG	+	+	+	+	-	-	+	+	+	+
Asia Sweet Meat & Cold Drinks	ASMCD	+	+	+	+	-	-	+	+	+	+
Bonolota Sweets & Bakery	BSB	+	+	+	+	-	-	+	+	+	+
Akbaria Sweets	AS	+	+	+	+	-	-	+	+	+	+
Shamol Doi Vander	SDV	+	+	+	+	-	-	+	+	+	+
Sylhet Division											
Rifat and Co.	RC	+	+	+	+	-	-	+	+	+	+
Fizza and Co.	FC	+	+	+	+	-	-	+	+	+	+
Modhubon	MB	+	+	+	+	-	-	+	+	+	+
Shaad and Co.	SC	+	+	+	+	-	-	+	+	+	+
Bonoful and Co.	BC	+	+	+	+	-	-	+	+	+	+
Fulcoli Sweets and Pure Foods	FS	+	+	+	+	-	-	+	+	+	+
Mymensingh Division											
Krishna Cabin	KC	+	+	+	+	-	-	+	+	+	+
Anil Ghosh Sweets	AG	+	+	+	+	-	-	+	+	+	+
Dayamay Mistanno Bhandar	DM	+	+	+	+	-	-	+	+	+	+
Misti Kanon	MK	+	+	+	+	-	-	+	+	+	+
Sudhir Ghosh	SG	+	+	+	+	-	-	+	+	+	+
Mamoni Sweets	MS	+	+	+	+	-	-	+	+	+	+

Legends: (+) means tolerance; (-) means sensitive

Table18: Sugar fermentation pattern of the isolates from yogurt

Name of isolates		Name of Sugar									
Dhaka Division	**Short Names**	**Glucose**	**Sucrose**	**Lactose**	**Xylose**	**Raffinose**	**Manitol**	**Glactose**	**Maltose**	**Sorbitol**	**Fructose**
Prince Sweets	PS	+	+	+	+	+	-	+	+	-	+
Vikrampur	VKP	+	+	+	+	+	-	+	+	-	+
Moronchand	MOC	+	+	+	+	+	-	+	+	-	+
Vaggokul	VGK	+	+	+	+	+	-	+	+	-	+
Mohonchand	MHC	+	+	+	+	+	-	+	+	-	+
Muslims Sweets and Confectionary	MSC	+	+	+	+	+	-	+	+	-	+
Cumilla Division											
Dodhi Mella	DM	+	+	+	+	+	-	+	+	-	+
Mithai	MTI	+	+	+	+	+	-	+	+	-	+
Vikrampur Ghee Store	VGS	+	+	+	+	+	-	+	+	-	+
Mattri Vander	MV	+	+	+	+	+	-	+	+	-	+
Amrita Vander	AV	+	+	+	+	+	-	+	+	-	+
Shitol Vander	SV	+	+	+	+	+	-	+	+	-	+
Khulna Division											
Tala Ghosh Dairy	TGD	+	+	+	+	+	-	+	+	-	+
Sathkhira Ghosh Dairy	SGD	+	+	+	+	+	-	+	+	-	+
Sathkhira Ghosh Dairy (KG)	SGDKG	+	+	+	+	+	-	+	+	-	+
Sathkhira Ghosh Dairy (AAR)	SGDAAR	+	+	+	+	+	-	+	+	-	+
Misti Mohol	MM	+	+	+	+	+	-	+	+	-	+
Dodhi Ghor	DG	+	+	+	+	+	-	+	+	-	+
Chattogram Division											
Food Fair	FF	+	+	+	+	+	-	+	+	-	+
Well Foods LTD.	WF	+	+	+	+	+	-	+	+	-	+
Flavors	FVS	+	+	+	+	+	-	+	+	-	+
Moo Royale	MR	+	+	+	+	+	-	+	+	-	+
Sweet Kings	SK	+	+	+	+	+	-	+	+	-	+
K-Bakery	KB	+	+	+	+	+	-	+	+	-	+
Rajshahi Division											
Doi Ghor	DG	+	+	+	+	+	-	+	+	-	+
Moharram Ali Doi Ghor	MADG	+	+	+	+	+	-	+	+	-	+
Asia Sweet Meat & Cold Drinks	ASMCD	+	+	+	+	+	-	+	+	-	+
Bonolota Sweets & Bakery	BSB	+	+	+	+	+	-	+	+	-	+
Akbaria Sweets	AS	+	+	+	+	+	-	+	+	-	+
Shamol Doi Vander	SDV	+	+	+	+	+	-	+	+	-	+
Sylhet Division											
Rifat and Co.	RC	+	+	+	+	+	-	+	+	-	+
Fizza and Co.	FC	+	+	+	+	+	-	+	+	-	+
Modhubon	MB	+	+	+	+	+	-	+	+	-	+
Shaad and Co.	SC	+	+	+	+	+	-	+	+	-	+
Bonoful and Co.	BC	+	+	+	+	+	-	+	+	-	+
Fulcoli Sweets and Pure Foods	FS	+	+	+	+	+	-	+	+	-	+

(Table 18 continued)

Mymensingh Division											
Krishna Cabin	KC	+	+	+	+	+	-	+	+	-	+
Anil Ghosh Sweets	AG	+	+	+	+	+	-	+	+	-	+
Dayamay Mistanno Bhandar	DM	+	+	+	+	+	-	+	+	-	+
Misti Kanon	MK	+	+	+	+	+	-	+	+	-	+
Sudhir Ghosh	SG	+	+	+	+	+	-	+	+	-	+
Mamoni Sweets	MS	+	+	+	+	+	-	+	+	-	+

Legends: (+) means reaction positive; (-) means reaction negative

Table18 shows the sugar fermentation pattern of the isolates from yogurt. All of the isolates were positive to Glucose, Sucrose, Lactose, Xylose, Raffinose, Galactose, Maltose and Fructose. But, they were negative to Manitol and Sorbitol.

11.1.1.2 Isolation and characterization of presumptive probiotic isolates from poultry

Tables 19, 20 and 21 show the Morphological and Biochemical characteristics of the isolated bacteria obtained from chicken samples collected from Dhaka, Kumilla, Sylhet, Rajshahi, Rangpur, Khulna and Barisal Divisions. All the isolates were Gram positive but were negative to Catalase, Endospore and Motility tests.

Table 19: Morphological and Biochemical characteristics of the isolated bacteria from chicken samples collected from Dhaka and Kumilla Divisions

Sources of Isolates	Morphological and Biochemical Characteristics				
	Colony Morphology	Gram Staining	Catalase	Endospore	Motility
C1	Medium, Irregular	+	-	-	-
CR1	Round	+	-	-	-
SI1	Small, Irregular	+	-	-	-
CR2	Round	+	-	-	-
SI2	Small, Irregular	+	-	-	-
CR3	Round	+	-	-	-
SI3	Round	+	-	-	-
C3	Medium, Irregular	+	-	-	-
CR4	Round	+	-	-	-
SI4	Medium, Irregular	+	-	-	-

C1 = Caeca of the chicken from Dhaka Division 1, CR1 = Crop of the chicken from Dhaka Division 1, SI1 = Small Intestine of the chicken from Dhaka Division 1, CR2 = Crop of the chicken from Dhaka Division 2, SI2 = Small Intestine of the chicken from Dhaka Division 2, CR3 = Crop of the chicken from Kumilla Division 3, SI3= Small Intestine of the chicken from Kumilla Division 3, C3 = Caeca of the chicken from Kumilla Division 3, CR4 = Crop of the chicken from Kumilla Division 4, SI4 = Small Intestine of the chicken from Kumilla Division 4. (+) sign means Positive Response and (-) sign means Negative Response

Table 20: Morphological and Biochemical properties of isolated bacteria from poultry samples collected from Sylhet, Rajshahi and Rangpur Divisions

Sources of Isolates	Morphological and Biochemical Characterization Results				
	Colony Morphology	Gram Staining	Catalase test	Endospore test	Motility test
CS2	Round	+	-	-	-
SS1	Round	+	-	-	-
CS1	Irregular	+	-	-	-
CR1	Circular	+	-	-	-
SR2	Round	+	-	-	-
CR2	Circular	+	-	-	-
CR3	Round	+	-	-	-
SR1	Round	+	-	-	-
CR4	Circular	+	-	-	-
CS3	Round	+	-	-	-

CS2 = Crop of chicken from Sylhet Division 2, SS1 = Small Intestine of chicken from Sylhet Division 1, CS1 = Crop of chicken from Sylhet Division 1, CR1 = Crop of chicken from Rajshahi Division 1, SR2 = Small Intestine of chicken from Rajshahi Division 2, CR2 = Crop of chicken from Rajshahi Division 2, CR3 = Crop of chicken from Rangpur Division 3, SR1 = Small Intestine of chicken from Rajshahi Division 1, CR4 = Crop of chicken from Rangpur Division 4, CS3 = Crop of chicken from Sylhet Division 3. (+) sign means Positive Response and (-) sign means Negative Response

Table 21: Morphological and Biochemical characteristics of the isolated bacteria from poultry samples collected from Khulna and Barisal Divisions

Sources of Isolates	Morphological and Biochemical Characteristics				
	Colony Morphology	Gram Staining	Catalase test	Endospore test	Motility test
Cr1	Circular	+	-	-	-
Si1	Circular	+	-	-	-
Cr2	Circular	+	-	-	-
C2	Round	+	-	-	-
Si2	Round	+	-	-	-
C3	Round	+	-	-	-
Cr4	Round	+	-	-	-
C4	Round	+	-	-	-
Si5	Triangular	+	-	-	-
Cr5	Triangular	+	-	-	-

Cr1 = Crop of the chicken from Khulna Division 1, Si1 = Small Intestine of the chicken from Khulna Division 1, Cr2 = Crop of the chicken from Khulna Division 2, C2 = Caeca of the chicken from Khulna Division2, of the chicken from Khulna Division 1, Si3 = Small Intestine of the chicken from Khulna Division 3, C3 = Caeca of the chicken from Khulna 3, Cr4 = Crop of the chicken from Barisal Division 4, C4 = Caeca of the chicken from Barisal Division 4, Si5 = Small Intestine of the chicken from Barisal Division 5, Cr5 = Crop of the chicken from Barisal Division 5. (+) sign means Positive Response and (-) sign means Negative Response

Table 22: Physicochemical and Biochemical property analysis of the isolated bacteria from chicken samples collected from Dhaka and Kumilla Divisions

Sources of Isolates	Physicochemical and Biochemical Properties of the Isolates						
	pH Tolerance	Bile Salt Tolerance	Nacl Tolerance				Gastric Juice
	pH 3.5	0.3%	2 %	4 %	6 %	8 %	pH 2.2
C1	++	++	++	++	+	-	+
CR1	++	++	++	++	-	-	+
SI1	++	++	++	++	-	-	+
CR2	+	++	++	++	-	-	+
SI2	++	++	++	++	-	-	+
CR3	++	++	++	++	-	-	+
SI3	+	++	++	++	-	-	+
C3	+	++	++	++	-	-	+
CR4	++	++	++	++	-	-	+
SI4	++	++	++	++	-	-	+

C1 = Caeca of the chicken from Dhaka Division 1, CR1 = Crop of the chicken from Dhaka Division 1, SI1 = Small Intestine of the chicken from Dhaka Division 1, CR2 = Crop of the chicken from Dhaka Division 2, SI2 = Small Intestine of the chicken from Dhaka Division 2, CR3= Crop of the chicken from Kumilla Division 3, SI3= Small Intestine of the chicken from Kumilla Division 3, C3 = Caeca of the chicken from Kumilla Division 3, CR4 = Crop of the chicken from Kumilla Division 4, SI4 = Small Intestine of the chicken from Kumilla Division 4. (++) sign means excellent growth, (+) means moderate growth, (-) sign means no growth

Table 23: Physico-chemical and Biochemical property analysis of the isolates from poultry samples collected from Sylhet, Rajshahi and Rangpur Divisions

Sources of Isolates	Physico-chemical and Biochemical Property Analysis						
	pH Tolerance	NaCl Tolerance				Bile Salt Tolerance	Gastric Juice Tolerance
	pH 3.5	2%	4%	6%	8%	0.3%	pH 2.2
CS2	++	++	++	+	-	++	+
SS1	++	++	++	+	-	++	+
CS1	++	++	++	-	-	++	+
CR1	++	++	++	-	-	++	+
SR2	++	++	++	-	-	++	+
CR2	++	++	++	-	-	+	+
CR3	++	++	++	-	-	++	+
SR1	++	++	++	-	-	++	+
CR4	++	++	++	-	-	++	+
CS3	++	++	++	+	-	++	+

CS2 = Crop of chicken from Sylhet Division 2, SS1 = Small Intestine of chicken from Sylhet Division 1, CS1 = Crop of chicken from Sylhet Division 1, CR1 = Crop of chicken from Rajshahi Division 1, SR2 = Small Intestine of chicken from Rajshahi Division 2, CR2 = Crop of chicken from Rajshahi Division 2, CR3 = Crop of chicken from Rangpur Division 3, SR1 = Small Intestine of chicken from Rajshahi Division 1, CR4 = Crop of chicken from Rangpur Division 4, CS3 = Crop of chicken from Sylhet Division 3. (++) sign means excellent growth, (+) means moderate growth, (-) sign means no growth

Table 24: Physico-chemical and Biochemical property analysis of the isolated bacteria from poultry samples collected from Khulna and Barisal Divisions

Sources of Isolates	Physico-chemical and Biochemical Properties of the Isolates						
	pH Tolerance	Bile Salt Tolerance	Nacl Tolerance				Gastric Juice
	pH 3.5	0.3%	2 %	4 %	6 %	8 %	pH 2.2
Cr1	++	++	++	++	+	-	++
SI1	++	++	++	++	-	-	++
Cr2	++	++	++	++	-	-	++
C2	++	++	++	++	-	-	++
SI2	++	++	++	++	-	-	++
C3	++	++	++	++	-	-	++
Cr4	++	++	++	++	-	-	++
C4	++	++	++	++	-	-	++
SI5	++	++	++	++	-	-	++
Cr5	++	++	++	++	-	-	++

Cr1 = Crop of the chicken from Khulna Division 1, Si1 = Small Intestine of the chicken from Khulna Division 1, Cr2 = Crop of the chicken from Khulna Division 2, C2 = Caeca of the chicken from Khulna Division2, of the chicken from Khulna Division 1, Si3 = Small Intestine of the chicken from Khulna Division 3, C3 = Caeca of the chicken from Khulna 3, Cr4 = Crop of the chicken from Barisal Division 4, C4 = Caeca of the chicken from Barisal Division 4, Si5 = Small Intestine of the chicken from Barisal Division 5, Cr5 = Crop of the chicken from Barisal Division 5. (++) sign means excellent growth, (+) means moderate growth, (-) sign means no growth

Tables 22, 23 and 24 show the Physicochemical and Biochemical property analysis of the isolated bacteria obtained from chicken samples collected from Dhaka, Kumilla, Sylhet, Rajshahi, Rangpur, Khulna and Barisal Divisions . All the isolates had pH tolerance of 3.5, Bile salt tolerance of 0.3%, NaCl tolerance up to 4% and Gastric juice tolerance of pH 2.2.

Table 25: Sugar fermentation patterns of the isolates from poultry samples collected from Dhaka and Kumilla Divisions

Sources of Isolates	D-Glucose	D-Sorbitol	Maltose	Lactose	Sucrose	D-Fructose	D-Xylose	D-Galactose	D-Raffinose	D-Mannose
C1	+	-	+	+	+	+	-	+	+	+
CR1	+	-	+	+	+	+	-	+	+	+
SI1	+	-	+	+	+	+	-	+	+	+
CR2	+	-	+	+	+	+	-	+	+	+
SI2	+	-	+	+	+	+	-	+	+	+
CR3	+	-	+	+	+	+	-	+	+	+
SI3	+	-	+	-	+	+	-	+	+	+
C3	+	-	+	+	+	+	-	+	-	+
CR4	+	-	+	+	+	+	-	+	+	+
SI4	+	-	+	+	+	+	-	+	-	+

C1 = Caeca of the chicken from Dhaka Division 1, CR1 = Crop of the chicken from Dhaka Division 1, SI1 = Small Intestine of the chicken from Dhaka Division 1, CR2 = Crop of the chicken from Dhaka Division 2, SI2 = Small Intestine of the chicken from Dhaka Division 2, CR3= Crop of the chicken from Kumilla Division 3, SI3= Small Intestine of the chicken from Kumilla Division 3, C3 = Caeca of the chicken from Kumilla Division 3, CR4 = Crop of the chicken from Kumilla Division 4, SI4 = Small Intestine of the chicken from Kumilla Division 4. (+) sign means isolates had the ability to ferment particular sugar, (-) sign means isolates did not have the ability to ferment particular sugar

Table 26: Sugar fermentation patterns of the isolates from poultry samples collected from Sylhet, Rajshahi and Rangpur Divisions

Sources of Isolates	Glucose	Sorbitol	Maltose	Lactose	Sucrose	Fructose	Xylose	Galactose	Raffinose	Mannose
CS2	+	-	+	+	+	+	-	+	+	+
SS1	+	-	+	+	+	+	-	+	+	+
CS1	+	-	+	+	+	+	-	+	+	+
CR1	+	-	+	+	+	+	-	+	+	+
SR2	+	-	+	+	+	+	-	+	+	+
CR2	+	-	+	+	+	+	-	+	+	+
CR3	+	-	+	+	+	+	-	+	+	+
SR1	+	-	+	+	+	+	-	+	+	+
CR4	+	-	+	+	+	+	-	+	+	+
CS3	+	-	+	+	+	+	-	+	+	+

CS2 = Crop of chicken from Sylhet Division 2, SS1 = Small Intestine of chicken from Sylhet Division 1, CS1 = Crop of chicken from Sylhet Division 1, CR1 = Crop of chicken from Rajshahi Division 1, SR2 = Small Intestine of chicken from Rajshahi Division 2, CR2 = Crop of chicken from Rajshahi Division 2, CR3 = Crop of chicken from Rangpur Division 3, SR1 = Small Intestine of chicken from Rajshahi Division 1, CR4 = Crop of chicken from Rangpur Division 4, CS3 = Crop of chicken from Sylhet Division 3. (+) sign means isolates had the ability to ferment particular sugar, (-) sign means isolates did not have the ability to ferment particular sugar

Table 27: Sugar fermentation patterns of the isolates from poultry samples collected from Khulna and Barisal Divisions

Sources of Isolates	Glucose	Sorbitol	Maltose	Lactose	Sucrose	Fructose	Xylose	Galactose	Raffinose	Mannose
Cr1	+	-	+	+	+/-	+	-	+	+	+
Si1	+	-	+	+	+	+	-	+	+	+
Cr2	+	-	+	+	+	+	-	+	+	+
C2	+	-	+	+	+	+	-	+	+	+
Si2	+	-	+	+	+	+	-	+	+	+
C3	+	-	+	+	+	+	-	+	+	+
Cr4	+	-	+	-	+	+	-	+	+	+
C4	+	-	+	+	+	+	-	+	-	+
Si5	+	-	+	+	+	+	-	+	+	+
Cr5	+	-	+	+	+	+	-	+/-	-	+

Cr1 = Crop of the chicken from Khulna Division 1, Si1 = Small Intestine of the chicken from Khulna Division 1, Cr2 = Crop of the chicken from Khulna Division 2, C2 = Caeca of the chicken from Khulna Division2, of the chicken from Khulna Division 1, Si3 = Small Intestine of the chicken from Khulna Division 3, C3 = Caeca of the chicken from Khulna 3, Cr4 = Crop of the chicken from Barisal Division 4, C4 = Caeca of the chicken from Barisal Division 4, Si5 = Small Intestine of the chicken from Barisal Division 5, Cr5 = Crop of the chicken from Barisal Division 5. (+) sign means isolates had the ability to ferment particular sugar, (-) sign means isolates did not have the ability to ferment particular sugar

Tables 25, 26 and 27 show Sugar fermentation patterns of the isolates obtained from poultry samples collected from Dhaka, Kumilla, Sylhet, Rajshahi, Rangpur, Khulna and Barisal Divisions. All most all the isolates were found positive to Glucose, Maltose, Lactose, Sucrose, Fructose, Galactose, Raffinose and Mannose but negative to Sorbitol and xylose.

Table 28 shows the antimicrobial activity of the isolates obtained from chicken samples collected from Dhaka and Kumilla Divisions. All most all of the isolates had antimicrobial activity against the indicator microorganisms *Vibrio cholera, Streptococcus aureus, H. pylori, Salmonella typhi* and *E. coli*.

Table 28: Antimicrobial activity of the isolates from chicken samples collected from Dhaka and Kumilla Divisions

Sources of isolates	**Name of Indicator Microorganisms**				
	Vibrio cholera	***Streptococcus aureus***	***H. pylori***	***Salmonella typhi***	***E. coli***
C1	+	+	+	+	+
CR1	+	+	+	+	+
SI1	+	+	+	-	+
CR2	-	+	+	+	+
SI2	+	+	+	+	+
CR3	+	+	+	+	+
SI3	+	+	+	+	+
C3	+	+	+	+	+
CR4	+	+	+	+	+
SI4	+	+	+	+	+

C1 = Caeca of the chicken from Dhaka Division 1, CR1 = Crop of the chicken from Dhaka Division 1, SI1 = Small Intestine of the chicken from Dhaka Division 1, CR2 = Crop of the chicken from Dhaka Division 2, SI2 = Small Intestine of the chicken from Dhaka Division 2, CR3= Crop of the chicken from Kumilla Division 3, SI3= Small Intestine of the chicken from Kumilla Division 3, C3 = Caeca of the chicken from Kumilla Division 3, CR4 = Crop of the chicken from Kumilla Division 4, SI4 = Small Intestine of the chicken from Kumilla Division 4. (+) signs means isolates show antimicrobial activity and (-) means isolates don't show antimicrobial activity

Tables 29, 30 and 31 show the susceptibility patterns of isolated bacteria obtained from poultry samples collected from Khulna, Barisal, Dhaka, Kumilla, Sylhet, Rajshahi and Rangpur Divisions against particular antibiotics. Majority of the isolates were found to be resistance against the antibiotics tested for susceptibility pattern with the Nalidixic acid showing the highest resistance. However, most of the isolates were found to be either moderately susceptible or susceptible against Clindamycin and few were moderately susceptible against Penicillin and Amoxicillin.

Table 29: Susceptibility patterns of isolated bacteria from poultry samples collected from Khulna and Barisal Divisions against particular antibiotics

Sources of Isolates	Antibiotics Name							
	Cefuroxime	Clindamycin	Azithromycin	Clavulanic acid	Nalidixic acid	Amoxicilin	Penicillin	Tetracycline
Cr1	S	MS	R	S	R	S	S	R
SI1	S	MS	R	S	R	S	S	R
Cr2	S	MS	R	S	R	S	S	R
C2	S	MS	S	R	R	R	MS	R
SI2	R	S	S	R	R	R	R	R
C3	R	S	S	R	R	R	R	R
Cr4	S	S	S	S	R	S	S	R
C4	S	S	R	S	R	S	S	R
SI5	S	MS	MS	S	R	S	S	R
Cr5	R	MS	S	R	R	S	S	R

Cr1 = Crop of the chicken from Khulna Division 1, Si1 = Small Intestine of the chicken from Khulna Division 1, Cr2 = Crop of the chicken from Khulna Division 2, C2 = Caeca of the chicken from Khulna Division2, of the chicken from Khulna Division 1, Si3 = Small Intestine of the chicken from Khulna Division 3, C3 = Caeca of the chicken from Khulna 3, Cr4 = Crop of the chicken from Barisal Division 4, C4 = Caeca of the chicken from Barisal Division 4, Si5 = Small Intestine of the chicken from Barisal Division 5, Cr5 = Crop of the chicken from Barisal Division 5. (S) means susceptible, (MS) means moderately susceptible and (R) means resistance

Table 30: Susceptibility Patterns of isolated bacteria from chicken samples collected from Dhaka and Kumilla Divisions against particular antibiotics

Sources of Isolates	Name of Antibiotics					
	Clindamycin	Nalidixic acid	Amoxicillin	Penicillin	Tetracycline	Azithromycin
C1	S	R	R	MS	R	R
CR1	S	R	R	MS	R	R
SI1	S	R	R	MS	R	R
CR2	S	R	R	MS	R	R
SI2	S	R	R	MS	R	R
CR3	S	R	R	MS	R	R
SI3	S	R	R	MS	R	R
C3	S	R	R	MS	R	R
CR4	S	R	R	MS	R	R
SI4	S	R	R	MS	R	R

C1 = Caeca of the chicken from Dhaka Division 1, CR1 = Crop of the chicken from Dhaka Division 1, SI1 = Small Intestine of the chicken from Dhaka Division 1, CR2 = Crop of the chicken from Dhaka Division 2, SI2 = Small Intestine of the chicken from Dhaka Division 2, CR3= Crop of the chicken from Kumilla Division 3, SI3= Small Intestine of the chicken from Kumilla Division 3, C3 = Caeca of the chicken from Kumilla Division 3, CR4 = Crop of the chicken from Kumilla Division 4, SI4 = Small Intestine of the chicken from Kumilla Division 4.(S) means susceptible, (MS) means moderately susceptible and (R) means resistance

Table 31: Susceptibility Patterns of isolated bacteria from chicken samples collected from Sylhet, Rajshahi and Rangpur Divisions against particular antibiotics

Name of antibiotics	Susceptibility patterns of isolated bacteria									
	CS2	SS1	CS1	CR1	SR2	CR2	CR3	SR1	CR4	CS3
Erythromycin	R	R	R	MS	MS	R	R	R	R	R
Clindamycin	MS	MS	MS	S	S	S	S	MS	MS	MS
Cefixime	R	R	R	R	R	R	R	R	R	R
Azithromycin	R	R	R	R	R	R	R	R	R	R
Tetracycline	R	R	R	MS	MS	R	R	R	R	R
Penicillin	R	R	R	MS	MS	MS	MS	R	R	R
Amoxicillin	R	R	R	MS	MS	MS	R	R	R	R
Nalidixic acid	R	R	R	R	R	R	R	R	R	R

CS2 = Crop of chicken from Sylhet Division 2, SS1 = Small Intestine of chicken from Sylhet Division 1, CS1 = Crop of chicken from Sylhet Division 1, CR1 = Crop of chicken from Rajshahi Division 1, SR2 = Small Intestine of chicken from Rajshahi Division 2, CR2 = Crop of chicken from Rajshahi Division 2, CR3 = Crop of chicken from Rangpur Division 3, SR1 = Small Intestine of chicken from Rajshahi Division 1, CR4 = Crop of chicken from Rangpur Division 4, CS3 = Crop of chicken from Sylhet Division 3.R=Resistant & MS=Moderately susceptible S= Susceptible against particular antibiotics

11.1.2 Study of the effects of the probiotic isolates

11.1.2.1 Study of the effects of the probiotics isolated from yogurt

Experiment No: 01

Effects of native Probiotic isolates on Hypercholesterolemic mice

Body weight gain

Individual body weight of mice was measured at day 0, day 7, day 14 day 21 and day 28. Due to sudden fluctuation of environmental condition (temperature, weather etc.), weights of the mice showed variation time to time. However, after the trial all the treatment groups achieved significant weight gain compared to control group.

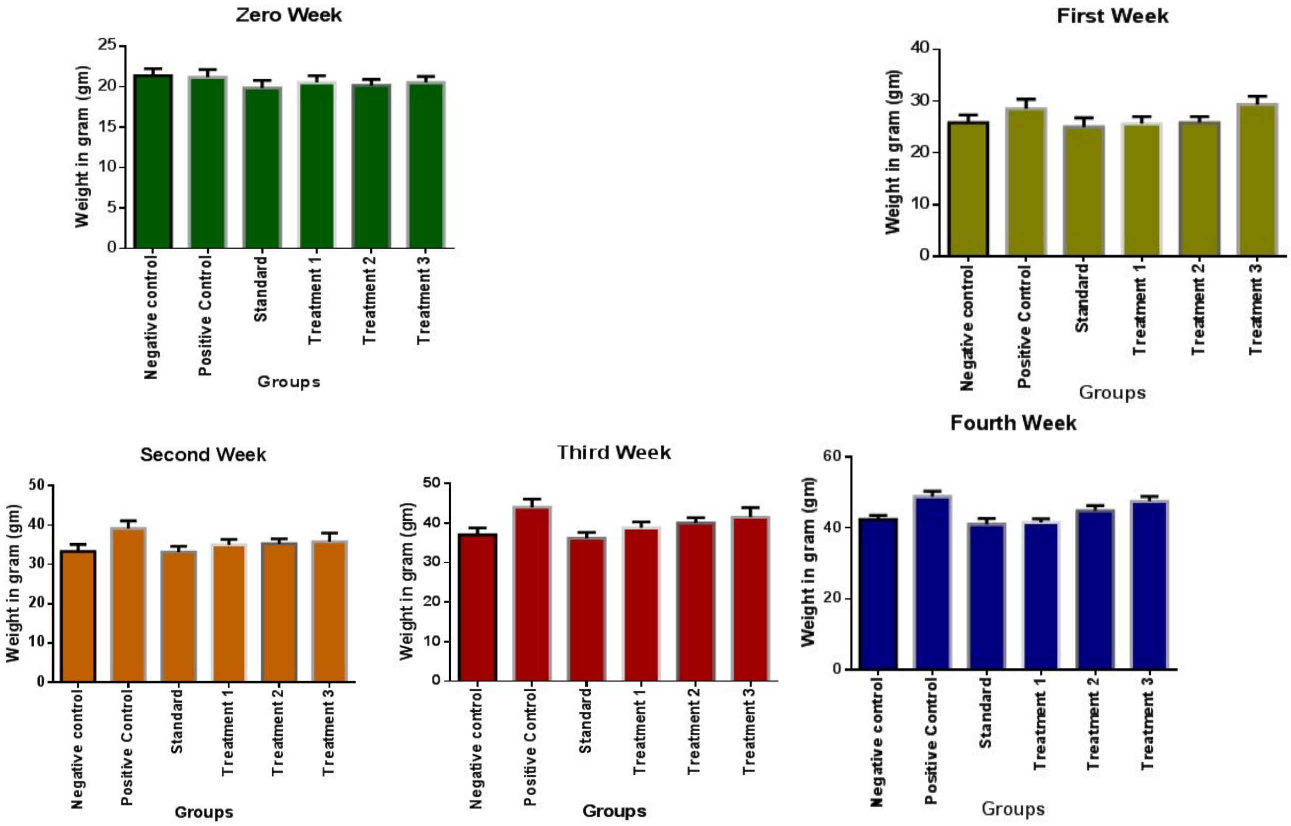


Figure 13: Total body weight gain of all groups of mice

Figure 13 represents the average body weight among all the treatment groups. The average body weight gain of control group was nearly 20 gm in twenty eight days trails. The positive control was induced by cholesterol and their body weight was higher than other groups and results become 30 gm weight gains. However, comparing among the Treatment groups & standard group, Treatment group 3 gained highest average body weight and were significantly different with the control and standard groups.

AST measurement

Tables 32, 33, 34, 35 and Figure 14 show the results of Aspartate Aminotransferase (AST) analysis. After twenty eight days of trails, probiotic Treatment groups showed significantly decreased AST level. In One way ANOVA, positive control group mice was compared with rest of mice groups where there was significant difference (P>0.0001) among the Treatment groups and standard group. Probiotic Treatment group 2 showed the highest mean difference (99.67). In 95% Confidence level of difference, Probiotic Treatment group 2 showed 86.30 to 113.0 which was the highest value compared with others. Regarding q value, Probiotic Treatment group 2 showed 19.81 which was highest compared with others. In case of Negative control comparison with others by using one way ANOVA, no significant variation was observed in the Treatment groups and standard group but in case of positive control group, there was significant variation (P>0.0001) compared with negative control. In all cases, it was clearly indicated that probiotic yogurt significantly reduced the AST level which was slightly higher than the standard group.

Table 32: Dunnett's multiple comparison tests in positive control group versus other groups for Aspartate Aminotransferase (AST) test

Dunnett's multiple comparison tests	Mean Diff.	95% CI of diff.	Significant	Summary
Positive Control vs. Negative control	98.33	84.96 to 111.7	Yes	****
Positive Control vs. Standard	93.50	80.13 to 106.9	Yes	****
Positive Control vs. Treatment 1	96.83	83.46 to 110.2	Yes	****
Positive Control vs. Treatment 2	99.67	86.30 to 113.0	Yes	****
Positive Control vs. Treatment 3	94.33	80.96 to 107.7	Yes	****

Table 33: One way ANOVA and Dunnett's multiple comparison tests in positive control group versus other groups for AST test

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Positive Control vs. Negative control	119.2	20.83	98.33	5.032	6	6	19.54	30
Positive Control vs. Standard	119.2	25.67	93.5	5.032	6	6	18.58	30
Positive Control vs. Treatment 1	119.2	22.33	96.83	5.032	6	6	19.24	30
Positive Control vs. Treatment 2	119.2	19.5	99.67	5.032	6	6	19.81	30
Positive Control vs. Treatment 3	119.2	24.83	94.33	5.032	6	6	18.75	30

Table 34: Dunnett's multiple comparison tests in negative control group versus other groups for AST test

Dunnett's multiple comparison tests	Mean Diff.	95% CI of diff.	Significant	Summary
Negative control vs. Positive Control	-98.33	-111.7 to -84.96	Yes	****
Negative control vs. Standard	-4.833	-18.20 to 8.536	No	ns
Negative control vs. Treatment 1	-1.500	-14.87 to 11.87	No	ns
Negative control vs. Treatment 2	1.333	-12.04 to 14.70	No	ns
Negative control vs. Treatment 3	-4.000	-17.37 to 9.369	No	ns

Table 35: One way ANOVA and Dunnett's multiple comparison tests in negative control group versus other groups for AST test

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Negative control vs. Positive Control	20.83	119.2	-98.33	5.032	6	6	19.54	30
Negative control vs. Standard	20.83	25.67	-4.833	5.032	6	6	0.9606	30
Negative control vs. Treatment 1	20.83	22.33	-1.5	5.032	6	6	0.2981	30
Negative control vs. Treatment 2	20.83	19.5	1.333	5.032	6	6	0.265	30
Negative control vs. Treatment 3	20.83	24.83	-4	5.032	6	6	0.795	30

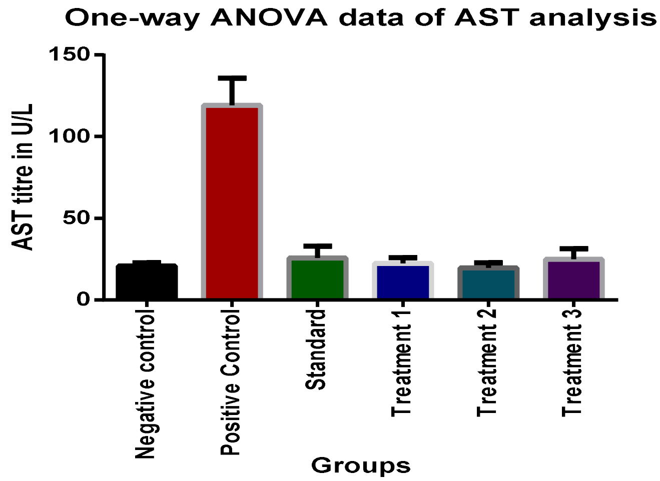


Figure 14: Aspartate Aminotransferase (AST) analysis

ALT analysis

Tables 36, 37, 38, 39 and Figure 15 show the results of Alanine Aminotransferase (ALT) analysis. After twenty eight days trails, probiotic Treatment groups showed significantly decreased ALT level. In One way ANOVA, positive control group mice was compared with rest of the mice groups where there was significant difference (P>0.0001) among the Treatment groups and standard group. Probiotic Treatment group 1 showed highest mean difference (107.5). In 95% Confidence level of difference, Probiotic Treatment group 1 was 93.53 to 121.5 which was the highest value compared with others. Regarding q value, Probiotic Treatment group 1 showed 20.45 which was highest compared with others. In case of Negative control comparison with others by using one way ANOVA no significant variation was observed in the Treatment groups and standard group but in case of positive control group, there was significant variation (P>0.0001) compared with negative control. In all cases, it was clearly indicated that probiotic yogurt significantly reduced the ALT level which was slightly higher than the standard group.

Table 36: Dunnett's multiple comparison tests in positive control group versus other groups for Alanine Aminotransferase (ALT) test

Dunnett's multiple comparison tests	**Mean Diff.**	**95% CI of diff.**	**Significant**	**Summary**
Positive Control vs. Negative control	105.2	91.20 to 119.1	Yes	****
Positive Control vs. Standard	101.0	87.03 to 115.0	Yes	****
Positive Control vs. Treatment 1	107.5	93.53 to 121.5	Yes	****
Positive Control vs. Treatment 2	103.0	89.03 to 117.0	Yes	****
Positive Control vs. Treatment 3	103.3	89.37 to 117.3	Yes	****

Table 37: One way ANOVA and Dunnett's multiple comparison tests in positive control group versus other groups for ALT test

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Positive Control vs. Negative control	137.5	32.33	105.2	5.256	6	6	20.01	30
Positive Control vs. Standard	137.5	36.5	101	5.256	6	6	19.21	30
Positive Control vs. Treatment 1	137.5	30	107.5	5.256	6	6	20.45	30
Positive Control vs. Treatment 2	137.5	34.5	103	5.256	6	6	19.6	30
Positive Control vs. Treatment 3	137.5	34.17	103.3	5.256	6	6	19.66	30

Table 38: Dunnett's multiple comparison tests in negative control group versus other groups for ALT test

Dunnett's multiple comparison tests	Mean Diff.	95% CI of diff.	Significant	Summary
Negative control vs. Positive Control	-105.2	-119.1 to -91.20	Yes	****
Negative control vs. Standard	-4.167	-18.13 to 9.799	No	ns
Negative control vs. Treatment 1	2.333	-11.63 to 16.30	No	ns
Negative control vs. Treatment 2	-2.167	-16.13 to 11.80	No	ns
Negative control vs. Treatment 3	-1.833	-15.80 to 12.13	No	ns

Table 39: One way ANOVA and Dunnett's multiple comparison tests in negative control group versus other groups in ALT test

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Negative control vs. Positive Control	32.33	137.5	-105.2	5.256	6	6	20.01	30
Negative control vs. Standard	32.33	36.5	-4.167	5.256	6	6	0.7927	30
Negative control vs. Treatment 1	32.33	30	2.333	5.256	6	6	0.4439	30
Negative control vs. Treatment 2	32.33	34.5	-2.167	5.256	6	6	0.4122	30
Negative control vs. Treatment 3	32.33	34.17	-1.833	5.256	6	6	0.3488	30

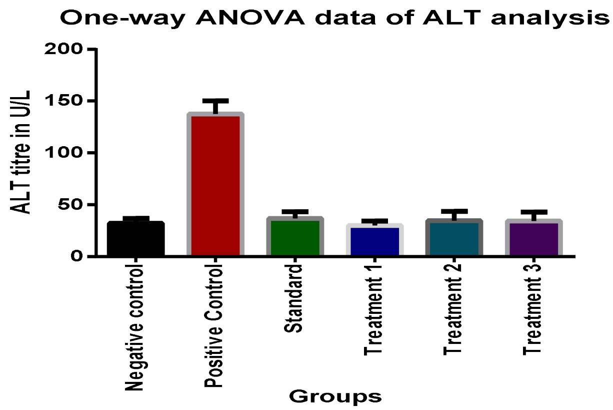


Figure 15: Alanine Aminotransferase (ALT) analysis

Total Cholesterol Level

Tables 40, 41, 42, 43 and Figure 16 show the results of total cholesterol analysis. After twenty eight days trails, probiotic Treatment groups showed significantly decreased level of total cholesterol. In One way ANOVA, positive control group mice was compared with rest of mice groups where there was significant difference (P>0.0001) among the Treatment groups and standard group. Probiotic Treatment group 3 showed highest mean difference (135.8). In 95% Confidence level of difference, Probiotic Treatment group 3 was 99.49 to 172, which was the highest value compared with others. Regarding q value, Probiotic Treatment group 3 showed 9.931 which was the highest compared with others. In the case of Negative control comparison with others by ANOVA, no significant variation showed in the treatment groups and standard group but in the case of positive control, negative control was significantly varied and it showed (P>0.0001) significant level of variation. The findings clearly indicated that probiotic yogurt significantly reduced the total cholesterol level which was slightly higher than the standard groups.

Table 40: Dunnett's multiple comparison tests in positive control group versus other groups for total cholesterol analysis

Dunnett's multiple comparison tests	**Mean Diff.**	**95% CI of diff.**	**Significant**	**Summary**
Positive Control vs. Negative control	111.7	75.33 to 148.0	Yes	****
Positive Control vs. Standard	72.83	36.49 to 109.2	Yes	****
Positive Control vs. Treatment 1	89.50	53.16 to 125.8	Yes	****
Positive Control vs. Treatment 2	91.67	55.33 to 128.0	Yes	****
Positive Control vs. Treatment 3	135.8	99.49 to 172.2	Yes	****

Table 41: One way ANOVA and Dunnett's multiple comparison tests in positive control group versus other groups for total cholesterol analysis

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Positive Control vs. Negative control	306	194.3	111.7	13.68	6	6	8.164	30
Positive Control vs. Standard	306	233.2	72.83	13.68	6	6	5.325	30
Positive Control vs. Treatment 1	306	216.5	89.5	13.68	6	6	6.544	30
Positive Control vs. Treatment 2	306	214.3	91.67	13.68	6	6	6.702	30
Positive Control vs. Treatment 3	306	170.2	135.8	13.68	6	6	9.931	30

Table 42: Dunnett's multiple comparison tests in negative control group versus other groups for total cholesterol analysis

Dunnett's multiple comparison tests	Mean Diff.	95% CI of diff.	Significant	Summary
Negative control vs. Positive Control	-111.7	-148.0 to -75.33	Yes	****
Negative control vs. Standard	-38.83	-75.17 to -2.493	Yes	*
Negative control vs. Treatment 1	-22.17	-58.51 to 14.17	No	ns
Negative control vs. Treatment 2	-20.00	-56.34 to 16.34	No	ns
Negative control vs. Treatment 3	24.17	-12.17 to 60.51	No	ns

Table 43: One way ANOVA and Dunnett's multiple comparison tests in negative control group versus other groups for total cholesterol level analysis

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Negative control vs. Positive Control	194.3	306	-111.7	13.68	6	6	8.164	30
Negative control vs. Standard	194.3	233.2	-38.83	13.68	6	6	2.839	30
Negative control vs. Treatment 1	194.3	216.5	-22.17	13.68	6	6	1.621	30
Negative control vs. Treatment 2	194.3	214.3	-20	13.68	6	6	1.462	30
Negative control vs. Treatment 3	194.3	170.2	24.17	13.68	6	6	1.767	30

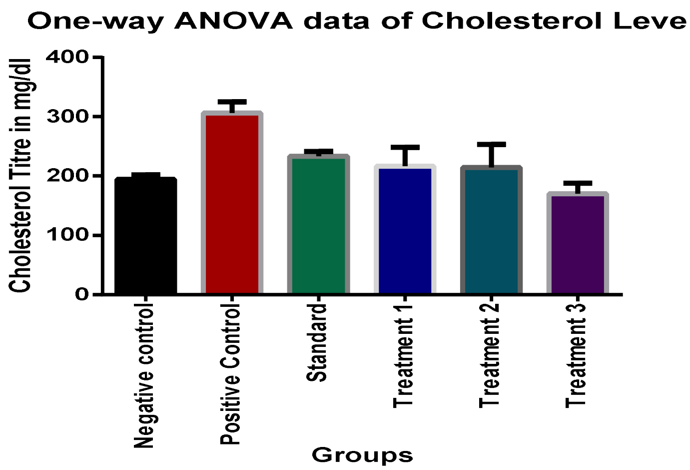


Figure 16: Total cholesterol analysis

Total triglyceride level

Tables 44, 45, 46, 47 and Figure 17 show the results of total triglyceride analysis. After twenty eight days trails, probiotic Treatment groups showed significantly decreased level of total triglyceride. In One way ANOVA, positive control group mice was compared with rest of mice groups where there was significant difference (P>0.0001) among the Treatment groups and standard group. Standard group showed highest mean difference (253.5) which was almost similar with probiotic treatment groups. In 95% Confidence level of difference, standard group was about 193.0 to 314.0 1 which was the highest compared with others whereas, probiotic treatment group 2 & 3 were 187.2 to 308. In the q value of standard group was 11.14 which was the highest compared with others whereas, probiotic treatment group 2 & 3 were 9.931 (Table 4.17 & Table 4.18). In case of Negative control comparison with others by ANOVA no significant variation showed in the treatment groups and standard group but in the case of positive control, negative control varied significantly (P<0.0001). The findings show clearly that probiotic yogurt significantly reduced the total triglyceride level which was slightly higher than the standard groups.

Table 44: Dunnett's multiple comparison tests in positive control group versus other groups for total triglyceride analysis

Dunnett's multiple comparison tests	Mean Diff.	95% CI of diff.	Significant	Summary
Positive Control vs. Negative control	270.0	209.5 to 330.5	Yes	****
Positive Control vs. Standard	253.5	193.0 to 314.0	Yes	****
Positive Control vs. Treatment 1	246.2	185.7 to 306.6	Yes	****
Positive Control vs. Treatment 2	247.7	187.2 to 308.1	Yes	****
Positive Control vs. Treatment 3	247.7	187.2 to 308.1	Yes	****

Table 45: One way ANOVA and Dunnett's multiple comparison tests in positive control group versus other groups for total triglyceride analysis

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Positive Control vs. Negative control	390.5	120.5	270	22.76	6	6	11.86	30
Positive Control vs. Standard	390.5	137	253.5	22.76	6	6	11.14	30
Positive Control vs. Treatment 1	390.5	144.3	246.2	22.76	6	6	10.81	30
Positive Control vs. Treatment 2	390.5	142.8	247.7	22.76	6	6	10.88	30
Positive Control vs. Treatment 3	390.5	142.8	247.7	22.76	6	6	10.88	30

Table 46: Dunnett's multiple comparison test in negative control group versus other groups for total triglyceride analysis

Dunnett's multiple comparison tests	Mean Diff.	95% CI of diff.	Significant	Summary
Negative control vs. Positive Control	-270.0	-330.5 to -209.5	Yes	****
Negative control vs. Standard	-16.50	-76.98 to 43.98	No	ns
Negative control vs. Treatment 1	-23.83	-84.31 to 36.65	No	ns
Negative control vs. Treatment 2	-22.33	-82.81 to 38.15	No	ns
Negative control vs. Treatment 3	-22.33	-82.81 to 38.15	No	ns

Table 47: One way ANOVA and Dunnett's multiple comparison tests in negative control group versus other groups for total triglyceride analysis

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Negative control vs. Positive Control	120.5	390.5	-270	22.76	6	6	11.86	30
Negative control vs. Standard	120.5	137	-16.5	22.76	6	6	0.7249	30
Negative control vs. Treatment 1	120.5	144.3	-23.83	22.76	6	6	1.047	30
Negative control vs. Treatment 2	120.5	142.8	-22.33	22.76	6	6	0.9811	30
Negative control vs. Treatment 3	120.5	142.8	-22.33	22.76	6	6	0.9811	30

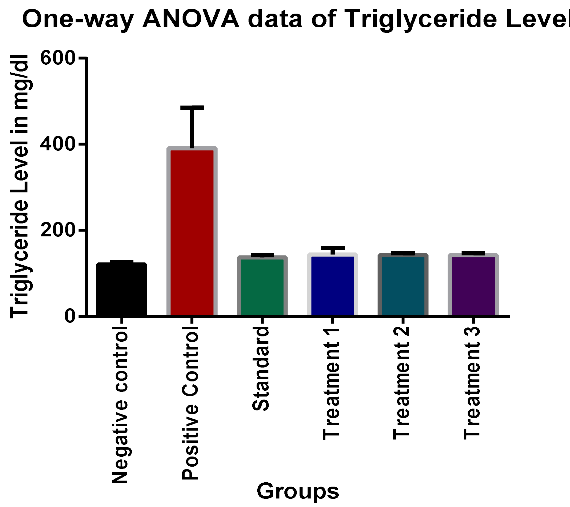


Figure 17: Total triglyceride analysis

Total HDL level

Tables 48, 49, 50, 51 and Figure 18 show the results of total HDL analysis. After twenty eight days trails, probiotic Treatment groups showed significantly increased level of total HDL. In One way ANOVA, positive control group mice was compared with rest of mice groups where there was significant difference (P>0.0001) among the Treatment groups and standard group. Standard group showed highest mean difference (-12.33) which is almost similar with probiotic treatment groups. In 95% Confidence level of difference, standard group was about -16.52 to -8.148 which were highest compared with others whereas, Probiotic Treatment group 3 is -15.19 to -6.815. In the q value of standard group was 7.829 which was also highest compared with others whereas, probiotic treatment group 3 was 6.983. In case of Negative control comparison with others by ANOVA no significant variation showed in the treatment groups and standard group but in the case of positive control, negative control varied significantly at the level of P<0.0001. The findings thus clearly indicate that probiotic yogurt significantly increased the total HDL level which was slightly higher than the standard groups.

Table 48: Dunnett's multiple comparison tests in positive control group versus other groups for total HDL analysis

Dunnett's multiple comparison tests	**Mean Diff.**	**95% CI of diff.**	**Significant**	**Summary**
Positive Control vs. Negative control	-13.33	-17.52 to -9.148	Yes	****
Positive Control vs. Standard	-12.33	-16.52 to -8.148	Yes	****
Positive Control vs. Treatment 1	-7.167	-11.35 to -2.981	Yes	***
Positive Control vs. Treatment 2	-8.500	-12.69 to -4.315	Yes	****
Positive Control vs. Treatment 3	-11.00	-15.19 to -6.815	Yes	****

Table 49: One way ANOVA and Dunnett's multiple comparison tests in positive control group versus other groups for total HDL analysis

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Positive Control vs. Negative control	33.17	46.5	-13.33	1.575	6	6	8.464	30
Positive Control vs. Standard	33.17	45.5	-12.33	1.575	6	6	7.829	30
Positive Control vs. Treatment 1	33.17	40.33	-7.167	1.575	6	6	4.549	30
Positive Control vs. Treatment 2	33.17	41.67	-8.5	1.575	6	6	5.396	30
Positive Control vs. Treatment 3	33.17	44.17	-11	1.575	6	6	6.983	30

Table 50: Dunnett's multiple comparison tests in negative control group versus other groups for total HDL analysis

Dunnett's multiple comparison tests	Mean Diff.	95% CI of diff.	Significant	Summary
Negative control vs. Positive Control	13.33	9.148 to 17.52	Yes	****
Negative control vs. Standard	1.000	-3.185 to 5.185	No	ns
Negative control vs. Treatment 1	6.167	1.981 to 10.35	Yes	**
Negative control vs. Treatment 2	4.833	0.6479 to 9.019	Yes	*
Negative control vs. Treatment 3	2.333	-1.852 to 6.519	No	ns

Table 51: One way ANOVA and Dunnett's multiple comparison tests in negative control group versus other groups for total HDL analysis

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Negative control vs. Positive Control	46.5	33.17	13.33	1.575	6	6	8.464	30
Negative control vs. Standard	46.5	45.5	1	1.575	6	6	0.6348	30
Negative control vs. Treatment 1	46.5	40.33	6.167	1.575	6	6	3.915	30
Negative control vs. Treatment 2	46.5	41.67	4.833	1.575	6	6	3.068	30
Negative control vs. Treatment 3	46.5	44.17	2.333	1.575	6	6	1.481	30

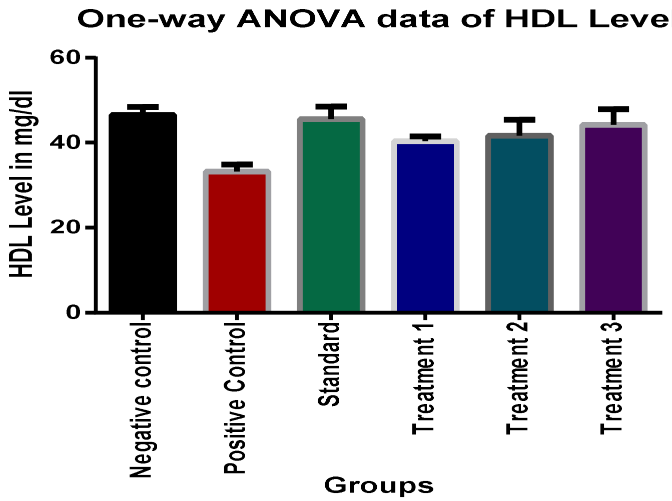


Figure 18: Total HDL analysis

LDL level analysis

Tables 52, 53, 54, 55 and Figure 19 show the results of total LDL analysis. After twenty eight days trails, probiotic Treatment groups showed significantly decreased level of total LDL. In One way ANOVA, positive control group mice was compared with rest of mice groups where there was significant difference (P>0.0001) among the Treatment groups and standard group. In probiotic treatment group 3 showed the highest mean difference (97.67). In 95% Confidence level of difference, probiotic treatment group 3 was 64.34 to 131.0 which was the highest compared with others In the q value of probiotic Treatment group 3 was 7.786 which was the highest compared with others. In case of Negative control comparison with others by ANOVA no significant variation showed in the treatment groups. In standard group there was significant (p<0.01) and in the case of positive control, negative control varied significantly at the level of P<0.0001. The findings clearly indicate that probiotic yogurt significantly reduced the total cholesterol level and standard groups could not sufficiently reduce the LDL level.

Table 52: Dunnett's multiple comparison tests in positive control group versus other groups for total LDL analysis

Dunnett's multiple comparison tests	Mean Diff.	95% CI of diff.	Significant	Summary
Positive Control vs. Negative control	66.00	32.67 to 99.33	Yes	****
Positive Control vs. Standard	32.17	-1.164 to 65.50	No	ns
Positive Control vs. Treatment 1	47.33	14.00 to 80.66	Yes	**
Positive Control vs. Treatment 2	49.50	16.17 to 82.83	Yes	**
Positive Control vs. Treatment 3	97.67	64.34 to 131.0	Yes	****

Table 53: One way ANOVA and Dunnett's multiple comparison tests in positive control group versus other groups for total LDL analysis

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Positive Control vs. Negative control	194.8	128.8	66	12.54	6	6	5.261	30
Positive Control vs. Standard	194.8	162.7	32.17	12.54	6	6	2.564	30
Positive Control vs. Treatment 1	194.8	147.5	47.33	12.54	6	6	3.773	30
Positive Control vs. Treatment 2	194.8	145.3	49.5	12.54	6	6	3.946	30
Positive Control vs. Treatment 3	194.8	97.17	97.67	12.54	6	6	7.786	30

Table 54: Dunnett's multiple comparison tests in negative control group versus other groups for total LDL analysis

Dunnett's multiple comparison test	Mean Diff.	95% CI of diff.	Significant	Summary
Negative control vs. Positive Control	-66.00	-99.33 to -32.67	Yes	****
Negative control vs. Standard	-33.83	-67.16 to -0.5031	Yes	*
Negative control vs. Treatment 1	-18.67	-52.00 to 14.66	No	ns
Negative control vs. Treatment 2	-16.50	-49.83 to 16.83	No	ns
Negative control vs. Treatment 3	31.67	-1.664 to 65.00	No	ns

Table 55: One way ANOVA and Dunnett's multiple comparison tests in negative control group versus other groups for total LDL analysis

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Negative control vs. Positive Control	128.8	194.8	-66	12.54	6	6	5.261	30
Negative control vs. Standard	128.8	162.7	-33.83	12.54	6	6	2.697	30
Negative control vs. Treatment 1	128.8	147.5	-18.67	12.54	6	6	1.488	30
Negative control vs. Treatment 2	128.8	145.3	-16.5	12.54	6	6	1.315	30
Negative control vs. Treatment 3	128.8	97.17	31.67	12.54	6	6	2.524	30

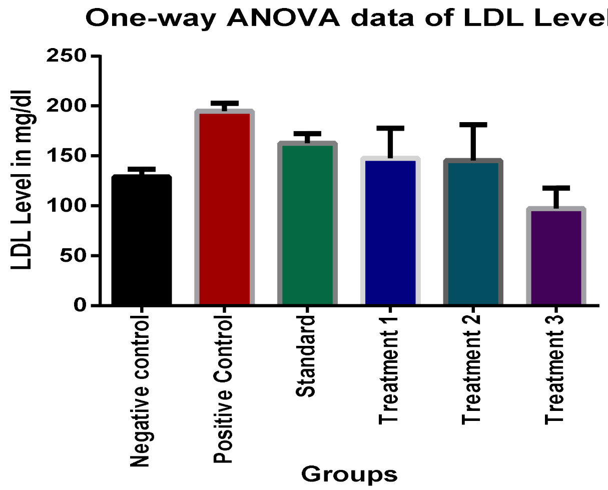


Figure 19: Total LDL level analysis

Total protein level

Tables 56, 57, 58, 59 and Figure 20 show the results of total protein analysis. After twenty eight days trails, probiotic Treatment groups showed significantly decreased level of total protein. In One way ANOVA, positive control group mice was compared with rest of mice groups where there was significant difference (P>0.0001) among the Treatment groups and standard group. Probiotic Treatment group 1 showed highest mean difference (127.7). In 95% Confidence level of difference, probiotic treatment group 1 was 122.2 to 133.1 which were the highest compared with others. In the q value probiotic treatment group 1 was 62.39 which was also the highest compared with others. In the case of Negative control comparison with others by ANOVA no significant variation showed in the treatment groups and standard group but in the case of positive control, negative control varied significantly (P>0.0001). All the results clearly indicate that probiotic yogurt significantly reduced the extra protein level which was slightly higher than the standard groups.

Table 56: Dunnett's multiple comparison test in positive control group versus other groups in total protein test

Dunnett's multiple comparison test	Mean Diff.	95% CI of diff.	Significant?	Summary
Positive Control vs. Negative control	122.5	117.1 to 127.9	Yes	****
Positive Control vs. Standard	124.7	119.2 to 130.1	Yes	****
Positive Control vs. Treatment 1	127.7	122.2 to 133.1	Yes	****
Positive Control vs. Treatment 2	124.0	118.6 to 129.4	Yes	****
Positive Control vs. Treatment 3	120.3	114.9 to 125.8	Yes	****

Table 57: One way ANOVA and Dunnett's multiple comparison tests in positive control group versus other groups in total protein test

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Positive Control vs. Negative control	192.2	69.67	122.5	2.046	6	6	59.87	30
Positive Control vs. Standard	192.2	67.5	124.7	2.046	6	6	60.93	30
Positive Control vs. Treatment 1	192.2	64.5	127.7	2.046	6	6	62.39	30
Positive Control vs. Treatment 2	192.2	68.17	124	2.046	6	6	60.6	30
Positive Control vs. Treatment 3	192.2	71.83	120.3	2.046	6	6	58.81	30

Table 58: Dunnett's multiple comparison tests in negative control group versus other groups in total protein analysis

Dunnett's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant	Summary
Negative control vs. Positive Control	-122.5	-127.9 to -117.1	Yes	****
Negative control vs. Standard	2.167	-3.270 to 7.603	No	ns
Negative control vs. Treatment 1	5.167	-0.2701 to 10.60	No	ns
Negative control vs. Treatment 2	1.500	-3.937 to 6.937	No	ns
Negative control vs. Treatment 3	-2.167	-7.603 to 3.270	No	ns

Table 59: One way ANOVA and Dunnett's multiple comparison tests in negative control group versus other groups in total protein analysis

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Negative control vs. Positive Control	69.67	192.2	-122.5	2.046	6	6	59.87	30
Negative control vs. Standard	69.67	67.5	2.167	2.046	6	6	1.059	30
Negative control vs. Treatment 1	69.67	64.5	5.167	2.046	6	6	2.525	30
Negative control vs. Treatment 2	69.67	68.17	1.5	2.046	6	6	0.7331	30
Negative control vs. Treatment 3	69.67	71.83	-2.167	2.046	6	6	1.059	30

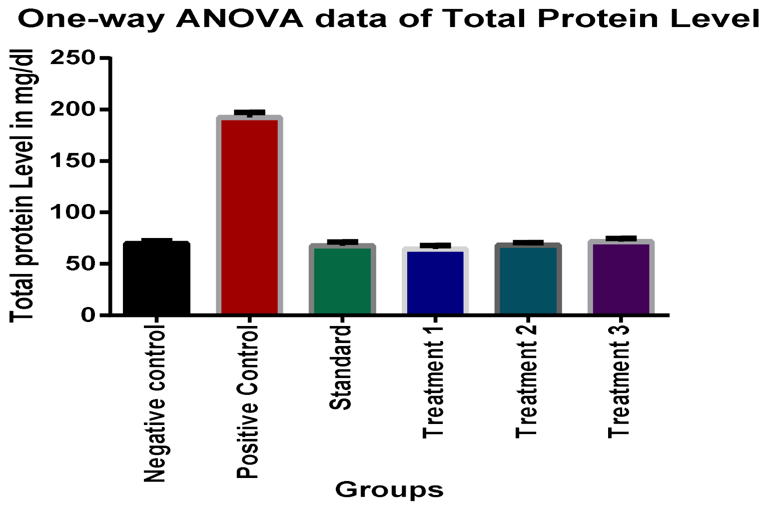


Figure 20: Total protein analysis

Total Albumin Level

Tables 60, 61, 62, 63 and Figure 21 show the results of total albumin level analysis. After twenty eight days trails, probiotic Treatment groups showed significantly decreased total albumin level. In One way ANOVA, positive control group mice was compared with rest of mice groups where there was significant difference (P>0.0001) among the Treatment groups and standard group. Probiotic Treatment group 1 showed highest mean difference (46.17). In 95% Confidence level of difference, probiotic treatment group 1 was 41.49 to 50.84 which was the highest compared with others. In the q value probiotic treatment group 1 was 26.25 which was also the highest compared with others. In the case of Negative control comparison with others by ANOVA no significant variation showed in the treatment groups and standard group but in the case of positive control, negative control varied significantly (P>0.0001). All the results clearly indicate that probiotic yogurt significantly reduced the extra Albumin level which was slightly higher than the standard groups.

Table 60: Dunnett's multiple comparison tests in positive control group versus other groups for total albumin level analysis

Dunnett's multiple comparison tests	**Mean Diff.**	**95% CI of diff.**	**Significant?**	**Summary**
Positive Control vs. Negative control	41.83	37.16 to 46.51	Yes	****
Positive Control vs. Standard	43.50	38.83 to 48.17	Yes	****
Positive Control vs. Treatment 1	46.17	41.49 to 50.84	Yes	****
Positive Control vs. Treatment 2	43.00	38.33 to 47.67	Yes	****
Positive Control vs. Treatment 3	40.83	36.16 to 45.51	Yes	****

Table 61: One way ANOVA and Dunnett's multiple comparison tests in positive control group versus other groups in total albumin level analysis

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Positive Control vs. Negative control	83.83	42	41.83	1.759	6	6	23.79	30
Positive Control vs. Standard	83.83	40.33	43.5	1.759	6	6	24.74	30
Positive Control vs. Treatment 1	83.83	37.67	46.17	1.759	6	6	26.25	30
Positive Control vs. Treatment 2	83.83	40.83	43	1.759	6	6	24.45	30
Positive Control vs. Treatment 3	83.83	43	40.83	1.759	6	6	23.22	30

Table 62: Dunnett's multiple comparison tests in negative control group versus other groups for total albumin level analysis

Dunnett's multiple comparison tests	Mean Diff.	95% CI of diff.	Significant	Summary
Negative control vs. Positive Control	-41.83	-46.51 to -37.16	Yes	****
Negative control vs. Standard	1.667	-3.006 to 6.339	No	ns
Negative control vs. Treatment 1	4.333	-0.3392 to 9.006	No	ns
Negative control vs. Treatment 2	1.167	-3.506 to 5.839	No	ns
Negative control vs. Treatment 3	-1.000	-5.672 to 3.672	No	ns

Table 63: One way ANOVA and Dunnett's multiple comparison tests in negative control group versus other groups in total albumin analysis

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Negative control vs. Positive Control	42	83.83	-41.83	1.759	6	6	23.79	30
Negative control vs. Standard	42	40.33	1.667	1.759	6	6	0.9477	30
Negative control vs. Treatment 1	42	37.67	4.333	1.759	6	6	2.464	30
Negative control vs. Treatment 2	42	40.83	1.167	1.759	6	6	0.6634	30
Negative control vs. Treatment 3	42	43	-1	1.759	6	6	0.5686	30

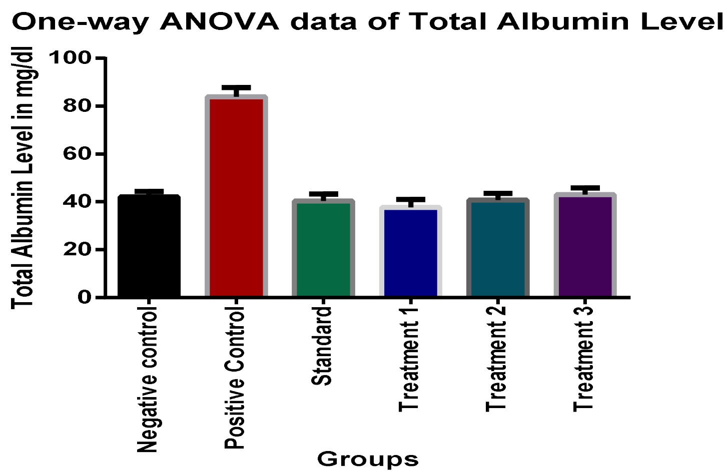


Figure 21: Total albumin level analysis

Total Globulin Level

The tables 64, 65, 66, 67 and Figure 22 show the results of total globulin level analysis. After twenty eight days trails, probiotic Treatment groups showed significantly decreased total globulin level. In One way ANOVA, positive control group mice was compared with rest of mice groups where there was significant difference (P>0.0001) among the Treatment groups and standard group. Probiotic Treatment group 1 showed highest mean difference (82.00). In 95% Confidence level of difference, probiotic treatment group 1 is 76.29 to 87.71 which was the highest compared with others. In the q value probiotic treatment group 1 was 38.15 which was the highest compared with others. In the case of Negative control comparison with others by ANOVA no significant variation showed in the treatment groups and standard group but in the case of positive control, negative control varied significantly at the level of P>0.0001. All the results clearly indicate that probiotic yogurt significantly reduced the extra globulin level which was slightly higher than the standard groups.

Table 64: Dunnett's multiple comparison tests in positive control group versus other groups for total globulin level analysis

Dunnett's multiple comparison tests	Mean Diff.	95% CI of diff.	Significant	Summary
Positive Control vs. Negative control	81.67	75.96 to 87.38	Yes	****
Positive Control vs. Standard	72.17	66.46 to 77.88	Yes	****
Positive Control vs. Treatment 1	82.00	76.29 to 87.71	Yes	****
Positive Control vs. Treatment 2	81.50	75.79 to 87.21	Yes	****
Positive Control vs. Treatment 3	78.83	73.12 to 84.54	Yes	****

Table 65: One way ANOVA and Dunnett's multiple comparison tests in positive control group versus other groups for total globulin analysis

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Positive Control vs. Negative control	110.7	29	81.67	2.15	6	6	37.99	30
Positive Control vs. Standard	110.7	38.5	72.17	2.15	6	6	33.57	30
Positive Control vs. Treatment 1	110.7	28.67	82	2.15	6	6	38.15	30
Positive Control vs. Treatment 2	110.7	29.17	81.5	2.15	6	6	37.92	30
Positive Control vs. Treatment 3	110.7	31.83	78.83	2.15	6	6	36.68	30

Table 66: Dunnett's multiple comparison tests in negative control group versus other groups for total globulin analysis

Dunnett's multiple comparison tests	Mean Diff.	95% CI of diff.	Significant?	Summary
Negative control vs. Positive Control	-81.67	-87.38 to -75.96	Yes	****
Negative control vs. Standard	-9.500	-15.21 to -3.789	Yes	***
Negative control vs. Treatment 1	0.3333	-5.378 to 6.045	No	ns
Negative control vs. Treatment 2	-0.1667	-5.878 to 5.545	No	ns
Negative control vs. Treatment 3	-2.833	-8.545 to 2.878	No	ns

Table 67: One way ANOVA and Dunnett's multiple comparison tests in negative control group versus other groups for total globulin analysis

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Negative control vs. Positive Control	29	110.7	-81.67	2.15	6	6	37.99	30
Negative control vs. Standard	29	38.5	-9.5	2.15	6	6	4.42	30
Negative control vs. Treatment 1	29	28.67	0.3333	2.15	6	6	0.1551	30
Negative control vs. Treatment 2	29	29.17	-0.1667	2.15	6	6	0.07754	30
Negative control vs. Treatment 3	29	31.83	-2.833	2.15	6	6	1.318	30

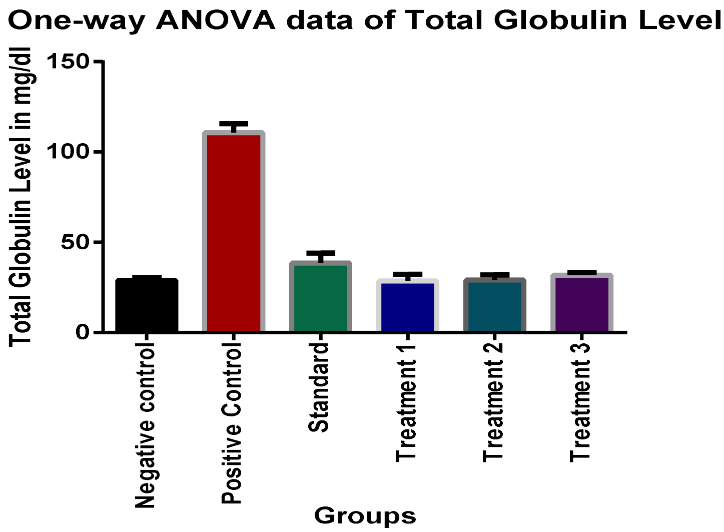


Figure 22: Total globulin analysis

Experiment No: 02

Effects of native Probiotic isolates on diabetic induced model rats

Effect of Probiotic Yogurt on the body weight of type 2 diabetic model rats

Changes in body weight in different groups of rat are shown in Table 68. Initial body weight (g) were 238±10, 259±42, 254±30, 208±19, 205±35, and 208±16 in normal water control, diabetic water control rats, Gliclazide treated and probiotic yogurt (2 g, 4 g, 6 g) treated diabetic model rats respectively.

Table 68: Body weight of different groups of type 2 diabetic model rats during the experimental period

1 Group	2 Body weight (Gram) from 0 day to 21^{1st} day (M±SD)			
	3 0 day 4	**5 7day**	**6 14 day**	**7 21day**
8 NWC (n=6)	9 238 ± 10 10 100%	11 270 ± 8	12 283±10	13 304±13 14 127%
15 DWC(n=6)	16 259±42 17 100%	18 252±46	19 257±42	20 264±42 21 101%
22 GT(n=6)	23 254±30 24 100%	25 251±32	26 255±29	27 257±27 28 101%
29 PYT (2g)(n=6)	30 208±19 31 100%	32 207±20	33 214±20	34 206±20 35 99%
36 PYT(4g)(n=6)	37 205±35 38 100%	39 203±32	40 211±34	41 206±27 42 100.4%
43 PYT (6g)(n=6)	44 208±16 45 100%	46 215±14	47 218±13	48 210±14 49 100.9%
50 One way ANOVA				

(Table continued)

51 NWC Vs DWC	52 1.000	53 1.000	54 1.000	55 0.593
56 NWC Vs GT	57 1.000	58 1.000	59 1.000	60 0.234
61 NWC Vs PYT (2g)	62 1.000	63 0.024	64 0.009	65 0.000
66 NWC Vs PYT (4g)	67 1.000	68 0.014	69 0.006	70 0.000
71 NWC Vs PYT (6g)	72 1.000	73 0.118	74 0.026	75 0.000
76 DWC Vs GT	77 1.000	78 1.000	79 1.000	80 1.000
81 DWC Vs PYT (2g)	82 0.336	83 0.437	84 0.532	85 0.057
86 DWC Vs PYT (4g)	87 0.225	88 0.274	89 0.397	90 0.057
91 DWC Vs PYT (6g)	92 0.417	93 1.000	94 1.000	95 0.130
96 GT Vs PYT (2g)	97 0.599	98 0.494	99 0.679	100 0.158
101 GT Vs PYT (4g)	102 0.409	103 0.311	104 0.510	105 0.158
106 GT Vs PYT (6g)	107 0.719	108 1.000	109 1.000	110 0.329
111 PYT (2g) Vs PYT (4g)	112 1.000	113 1.000	114 1.000	115 1.000
116 PYT (2g) Vs PYT (6g)	117 1.000	118 1.000	119 1.000	120 1.000
121 PYT (4g) Vs PYT (6g)	122 1.000	123 1.000	124 1.000	125 1.000

Group NWC, DWC, GT and PYT represent normal water control, diabetic water control, Gliclazide treated and probiotic yogurt treated diabetic rat respectively. Data presented as mean ± standard deviation (M±SD). Statistical comparison between groups was performed using one way ANOVA.

Effect of Probiotic Yogurt on serum glucose level of type 2 diabetic model rats

To evaluate the effect of Probiotic Yogurt on glucose metabolism, fasting serum glucose levels of different experimental groups were measured. Table 69 summarizes the results of serum glucose level.

Table 69: Fasting serum glucose level (mmol/l) in different groups of type 2 diabetic model rats on day 0 and 21^{1st} day of the experimental period

Group	**Fasting serum glucose, mmol/l (M±SD)**	
	Day 0	Day 21
NWC (n=6)	6.12 ± 0.61 (100%)	6.26± 0.47 (102.2%)
DWC (n=6)	7.17 ± 1.13 (100%)	7.73 ± 1.14 (107.8%)
GT (n=6)	7.71 ± 0.75 (100%)	7.36 ± 1.34 (95.4%)
PYT (2 g)(n=6)	6.69 ± 0.95 (100%)	5.95 ±1.57 (88.9%)
PYT (4 g)(n=6)	7.64 ± 1.18 (100%)	5.75 ±1.44 (75.2%)
PYT (6 g)(n=6)	8.93 ± 0.18 (100%)	6.87 ± 1.19 (76.9%)
One way ANOVA		
NWC Vs DWC	1.000	1.000
NWC Vs GT	0.411	1.000
NWC Vs PYT (2 g)	1.000	1.000
NWC Vs PYT (4 g)	0.412	1.000
NWC Vs PYT (6 g)	0.003	1.000
DWC Vs GT	1.000	1.000
DWC Vs PYT (2 g)	1.000	0.622
DWC Vs PYT (4 g)	1.000	0.350
DWC Vs PYT (6 g)	0.217	1.000

(Table continued)

GT Vs PYT (2 g)	1.000	1.000
GT Vs PYT (4 g)	1.000	1.000
GT Vs PYT (6 g)	1.000	1.000
PYT (2 g) Vs PYT (4 g)	1.000	1.000
PYT (2 g) Vs PYT (6 g)	0.022	1.000
PYT (4 g) Vs PYT (6 g)	0.924	1.000
Paired sample t test (O day Vs 21st day)		
NWC (n=6)	0.451	
DWC(n=6)	0.447	
GT(n=6)	0.766	
PYT (2 g)(n=6)	0.234	
PYT (4 g)(n=6)	0.86	
PYT (6 g) (n=5)	**0.027**	

Group NWC, DWC, *GT and* PYT *represent normal water control, diabetic water control, Gliclazide treated and probiotic yoghurt treated diabetic rat respectively. Data presented as mean ± standard deviation (M±SD). Statistical comparison between groups was performed using one way ANOVA and paired sample t test.*

Effects of Probiotic Yogurt on lipid profile of type-2 diabetic model rats

Table 70 shows the effect of Probiotic Yogurt on lipidemic status of type 2 diabetic rats.

Table 70: Effects of Probiotic Yoghurt on lipid profile of type-2 diabetic model rats

Groups (n=6 in each group)	**TG (mg/dl)**		**Cholesterol (mg/dl)**		**HDL (mg/dl)**		**LDL (mg/dl)**	
	0 Day	21st Day	0 Day	21th Day	0 Day	21th Day	0 Day	21th Day
NWC	87.2±16.9 100%	56.6±12.8 64.9%	51.0±2.5 100%	38.4±4.9 75.2%	16.4±4.6 100%	17.9±5.4 109.1%	17.1±6.2 100%	16.2±3.2 94.7%
DWC	51.1±12.6 100%	82.9±15.9 162.2%	50.7±10.1 100%	52.7±10.1 103.9%	21.4±6.1 100%	21.3±4.1 99.5%	21.0±12.0 100%	17.9±6.1 85.2%
GT	68.3±29.4 100%	93.6±18.6 137.0%	50.9±5.5 100%	58.3±10.5 114.5%	20.1±4.5 100%	20.9±3.1 103.9%	20.1±6.9 100%	20.2±7.6 100.4%
PYT (2 g)	99.8±45.3 100%	74.2±21.5 74.3%	51.1±4.9 100%	62.8±6.2 122.8%	20.0±4.0 100%	25.7±3.2 128.5%	17.5±5.1 100%	24.6±7.5 140.5%
PYT (4 g)	94.2±33.6 100%	79.6±11.2 84.5%	54.4±3.0 100%	57.9±7.3 106.4%	23.0±4.2 100%	28.0±5.6 121.7%	18.7±3.2 100%	15.6±3.1 83.4%
PYT (6 g)	105.4±29.9 100%	89.3±22.3 84.7%	50.2±6.0 100%	60.1±7.6 119.7%	24.5±4.7 100%	22.5±5.9 91.8%	10.1±4.3 100%	17.5±2.6 173.2%
Paired sample t test (O day Vs 21st day)								
NWC	0.006		0.004		0.752		0.803	
DWC	0.118		0.995		0.813		0.347	
GT	0.274		0.33		0.786		0.968	
PYT (2 g)	0.102		0.002		**0.017**		0.099	
PYT (4 g)	0.301		0.15		**0.053**		**0.033**	
PYT (6 g)	0.49		0.062		0.638		0.638	

Group NWC, DWC, *GT and* PYT *represent normal water control, diabetic water control, Gliclazide treated and probiotic yoghurt treated diabetic rat respectively. Data presented as mean ± standard deviation (M±SD). Statistical comparison between groups was performed using and paired sample t test.*

Effects of Probiotic Yogurt on the Liver Glycogen of Type-2 Diabetic Model Rats

The effect of probiotic yoghurt on hepatic glycogen content of type 2 diabetic model rats was observed. After 21 days of chronic feeding, all the rats from different test groups were sacrificed and their hepatic glycogen content was measured. The results are shown in Table 71.

Table 71: Effects of Probiotic Yoghurt on the liver glycogen of type-2 diabetic model rats

Groups	Glycogen (mg/g) 21th Day
NWC (n=6)	14.2±1.8
DWC(n=6)	17.1±4.7 (100%)
GT(n=6)	17.3±3.7 (101.1%)
PYT (2 g)(n=6)	23.0±1.0 (134.5%)
PYT (4 g)(n=6)	22.4±1.1 (130.9%)
PYT (6 g)(n=6)	23.8±2.3 (139.1%)
***Bonferronip* value**	
NWC Vs DWC	1.000
NWC Vs GT	1.000
NWC Vs PYT (2 g)	**0.002**
NWC Vs PYT (4 g)	**0.003**
NWC Vs PYT (6 g)	**0.001**
DWC Vs GT	1.000
DWC Vs PYT (2 g)	0.318
DWC Vs PYT (4 g)	0.541
DWC Vs PYT (6 g)	0.173
GT Vs PYT (2 g)	0.142
GT Vs PYT (4 g)	0.271
GT Vs PYT (6 g)	0.076
PYT (2gm) Vs PYT (4 g)	1.000
PYT (2gm) Vs PYT (6 g)	1.000
PYT (4gm) Vs PYT (6 g)	1.000

Group NWC*,* DWC, *GT and* PYT *represent normal water control, diabetic water control, Gliclazide treated and probiotic yoghurt treated diabetic rat respectively. Data presented as mean ± standard deviation (M±SD). Statistical comparison between groups was performed using Bonferronip value*

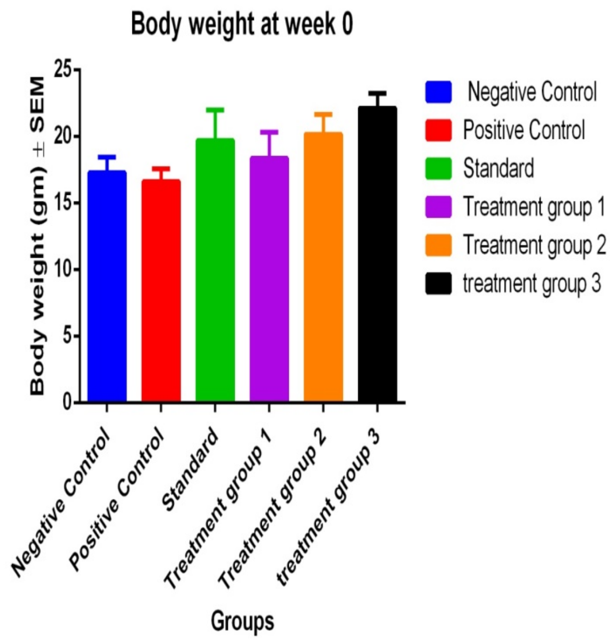
Experiment No: 03

Effects of native Probiotic isolates on selective enteric pathogens in mice

Effect of Probiotics on body weight

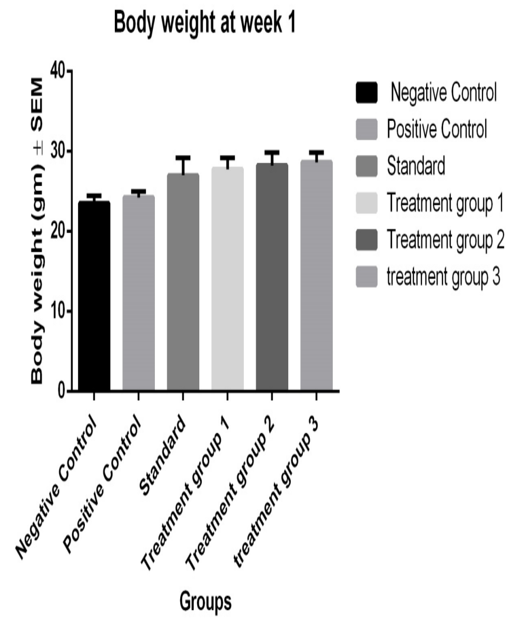
Body weight of mice was measured every week during the mice trail. At week 0, body weight of mice ranged 15-25 gm. At the starting of the treatment, the body weights were slightly increased in every group. This body weight was considered as body weight at week 1. Then, every week the body weight was measured and it was observed that the mean body weight of every group was increased. However, the highest mean body weight was observed in Treatment group 3 at the end of every week with significance level of $p<0.001$. The lowest mean body weight was observed in negative control group at the end of the treatment. At the end of the trail period all of the treatments was significantly ($p<0.001$)

varied compared with the negative control group. Weekly gained body weight results are shown in Figure 23 below.



±

(A)



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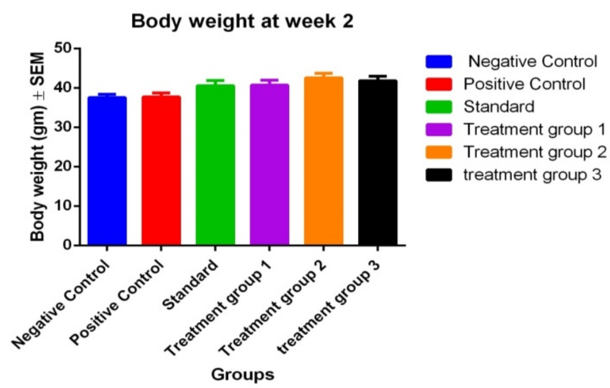
(B)



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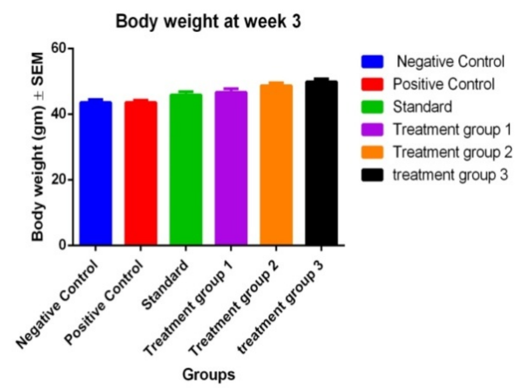
(C)



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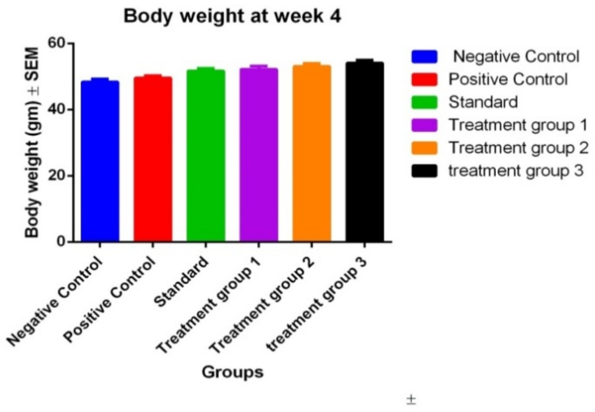
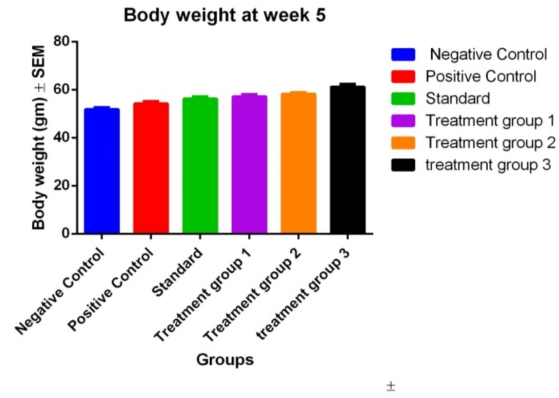
(D)





(E) (F)

Figure 23: Weekly body weight measurement (gm) in different experimental mice groups
(A) Body weight at week 0 (B) Body weight at week 1 (C) Body weight at week 2 (D) Body weight at week 3 (E) Body weight at week 4 (F) Body weight at week 5

Effects of Probiotic in pathogen induced mice

Effects of probiotic in pathogen induced mice model is shown in Table 72, 73 and 74. Probiotic Yogurt significantly prolonged the onset of antimicrobial activity and reduced the frequency of infection compared with positive control group. All the mice from probiotic fed treatment groups showed statistically significant (p<0.001) effects compared with positive control group.

In case of total protein, positive control group was compared with negative control group, Standard group, Treatment group 1, Treatment group 2 and Treatment group 3 where significant (p<05; p<001; p<01; p<01; p<05) differences were observed. Regarding negative control group comparison with standard group significant (p<001) result was obtained.

For albumin, Standard group, Treatment group 2 and Treatment group 3 showed significant (p<001; p<05; p<05) differences with positive control group. Standard group, Treatment group 2 showed significantly (p<01; P<05) increased level of the desired activity respectively with negative control group. So, here it proved that Standard group showed significantly (p<001) increased antimicrobial activity when compared with all the treatment groups. For the determination of globulin level, Negative control, Treatment group 2, Treatment group 3 showed significant (p<05; p<05; p<01) difference compared with positive control group where rests of other groups showed non-significant results.

For the measurement of IgG level, negative control group, Treatment group 3 showed significant (p<001; p<01) differences with positive control. In the case of standard group, Treatment group1, Treatment group 2 and Treatment group 3 with negative control group showed highly significant (p<001; p<001; p<001; p<001) difference in antimicrobial activity. In case of WBC measurement, Standard group, Treatment group 2, Treatment group 3 showed significant (p<01; p<01; p<05) increasing properties of antimicrobial activity respectively compared with positive control group. Regarding standard group, Treatment group 1, Treatment group2, Treatment group 3 showed highly significance (P<001; p<05; p<001) increasing level of antimicrobial activity compared with negative control group.

For the determination of HB level among the experimental animal model, Negative control and Treatment group 1 showed significant level (p<05; p<05) of difference compared with positive control group whereas in negative group comparison no significance variation was observed with rest of the groups.

Table 72: Antimicrobial effects on blood metabolites in pathogen induced mice

Groups	Total Protein (g/l)	Albumin (g/l)	Globulin (g/l)	IgG (g/l)
Negative Control	6.62±0.365^{a1}	3.48±0.36^{a}	3.13±0.197^{a1}	3.00±0.88^{a123}
Positive Control	5.83±0.216	3.27±0.15	2.57±0.15	7.68±1.40
Standard	7.20±0.489^{a123b1}	4.15±0.24^{a123b12}	8.60±0.4^{ab}	7.61±0.84^{ab123}
Treatment Group 1	7.05±0.476^{a12cf}	3.87±0.3^{a1cf}	3.18±0.68^{acf}	7.41±0.5^{ac123f}
Treatment Group 2	7.02±0.476^{a12dgi}	3.92±0.17^{a12d1gi}	3.10±0.44^{a1dgi}	8.51±0.99^{ad123gi1}
Treatment Group 3	6.78±0.577^{a1ehjk}	3.72±0.43^{a1ehjk}	3.07±0.24^{a1ehjk}	10.29±1.73^{a12e123h12j12k}

^{a}compared with positive control group, ^{b}standard compared with negative control, ^{c}Treatment group1 compared with negative control, ^{d}Treatment group2 compared with negative control, ^{e}Treatment group 3 compared with negative control, ^{f}Treatment group 1, ^{g}Treatment group 2, ^{h}Treatment group 3 compared with standard group. ^{i}Treatment group 2 compared with treatment group 1, ^{j}Treatment group 3 compared with Treatment group 1, ^{k}Treatment group compared with treatment group2. Values are mean ± SEM (n= 6). Here, 1p<0.05, 2p<0.01, 3p<0.001.

Table 73: Antimicrobial effects on WBC and Hemoglobin in pathogen induced mice

Group	WBC (cumm)	Hb (gm/dl)
Negative Control Group	7.91±0.59^{a}	10.44±0.46^{a1}
Positive Control Group	8.80±1.10	9.18±1.108
Standard	10.78±0.76^{a1b123}	12.30±4.165^{ab}
Treatment Group 1	9.73±0.87^{ac1f}	10.77±1.226^{a1cf}
Treatment Group 2	10.23±0.94^{a1d12gi}	9.93±1.650^{adgi}
Treatment Group 3	10.53±1.23^{a1e123hjk}	9.60±0.907^{aehjk}

^{a}compared with positive control group, ^{b}standard compared with negative control, ^{c}Treatment group1 compared with negative control, ^{d}Treatment group2 compared with negative control, ^{e}Treatment group 3 compared with negative control, ^{f}Treatment group 1, ^{g}Treatment group 2, ^{h}Treatment group 3 compared with standard group. ^{i}Treatment group 2 compared with treatment group 1, ^{j}Treatment group 3 compared with Treatment group 1, ^{k}Treatment group compared with treatment group2. Values are mean ± SEM (n= 6). Here, 1p<0.05, 2p<0.01, 3p<0.001.

Table 74: Antimicrobial effects on different fecal samples from pathogen induced mice

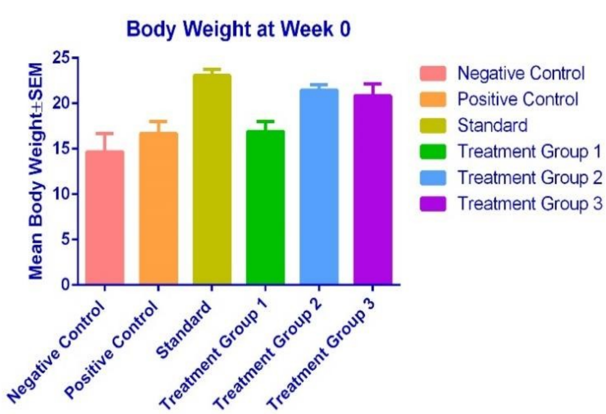
Group	Pathogens in fecal sample Millions (cfu)
Control Group	6.00 ±0.864
Standard	2.58 ± 0.47^{a1}
Treatment Group 1	4.41 ± 0.496^{a1f1d1}
Treatment Group 2	3.53 ± 0.499^{a1}
Treatment Group 3	2.60 ± 0.357^{a1}

Experiment No: 04

Effects of native Probiotic isolates on diarrhea induced mice

Effect of Probiotic on mice body weight

The total mean body weight data are presented as mean in Figure 24. Body weights of mice were measured every week during the period of Treatment. At week 0, body weight of mice ranged 15-25 gm. At the starting of the treatment, the body weight was slightly increased in every group. This body weight was considered as body weight at week 1. Then, every week the body weight was measured and it was observed that the mean body weight of every group was increased. However, the highest mean body weight was observed in Treatment group 3 at the end of every week. The lowest mean body weight was found in negative control group at the end of the treatment. After one week of probiotic yogurt Treatment, the mean body weight of the negative control, positive control, standard, Treatment group 1, Treatment group 2 and Treatment group 3 was 26.80 gm, 29.15 gm, 30.47 gm, 31.93 gm, 31.92 gm and 38.58 gm respectively. After 4 week period of treatment the body weight was significantly increased in all treatment groups. At the end of the trial period the mean weights of negative control, positive control, standard, Treatment group 1, Treatment group 2 and Treatment group 3 were 31.80 gm, 34.15 gm, 35.47 gm, 36.93 gm, 36.92 gm and 43.58 gm respectively.



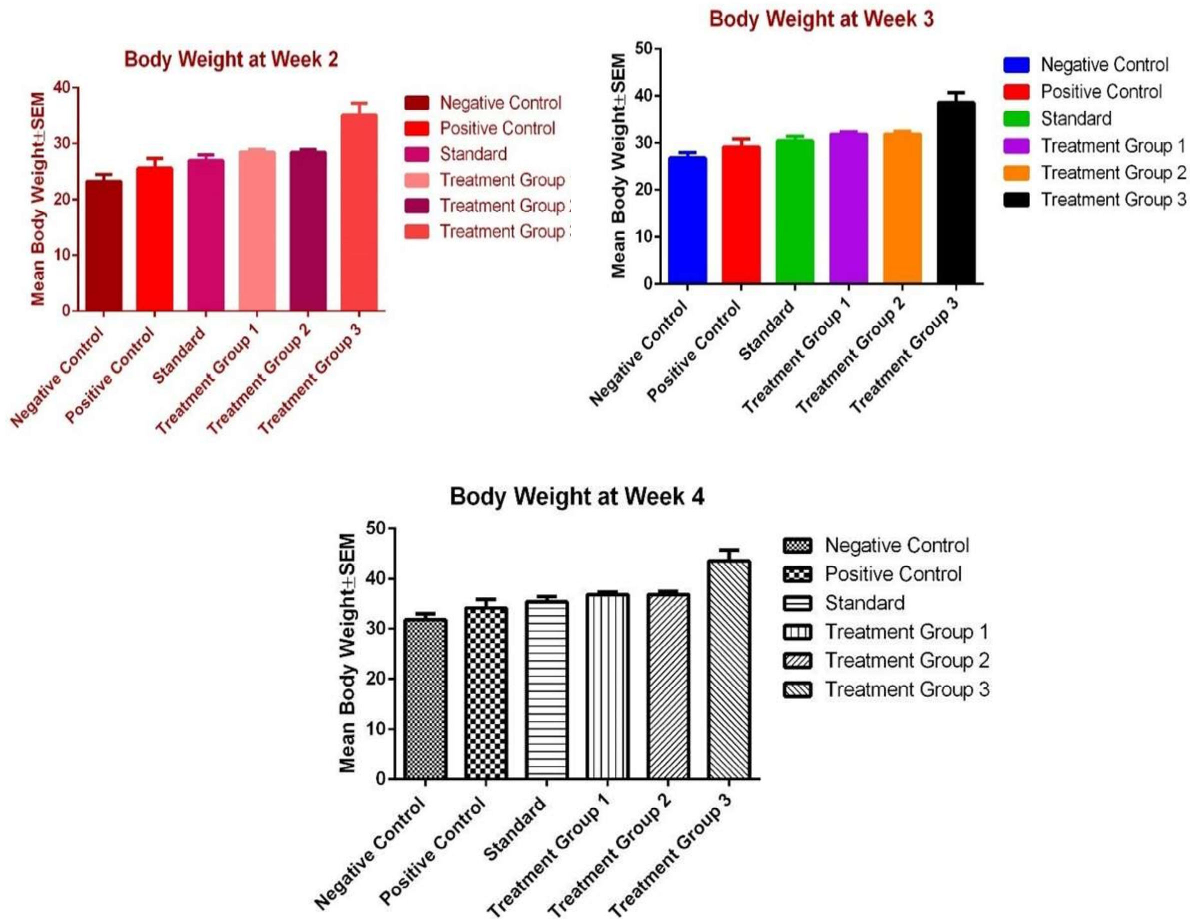


Figure 24: Weekly body weight measurement (gm) in different experimental mice groups

Effects of Probiotic on castor oil induced diarrheal model

In the castor oil induced diarrheal model probiotic yogurt significantly prolonged the onset of diarrhea and reduced the frequency and weight of total stool compared with positive control. All the probiotic fed treatment group showed statistically significant ($p<0.05$, $p<0.01$, $p<0.001$) effects on onset of diarrhea, total number of feces, average weight of total feces and average fecal water content compared with positive control group. Hence, Treatment group 2 (100 ml/kg body weight) and Treatment group 3 (125 ml/kg body weight) showed significant ($p<0.05$, $p<0.01$, $p<0.001$) differences with Treatment group 1 (75 ml/kg body weight) regarding onset of diarrhea. Moreover, a significant difference ($p<0.05$) was observed in case of onset of diarrhea when compared with Treatment group 3 and Treatment group 2. In case of total number of feces it was significantly ($p<0.05$, $p<0.01$, $p<0.001$) reduced in all the Treatment groups when compared with positive control group. However, no significance difference was observed in case of total number of feces in the all Treatment groups when compared with standard group. In case of the weight of total stools, Treatment group 3 and standard group showed significant ($p<0.05$, $p<0.01$, $p<0.001$) reduction of total stool weight compared with

Treatment group 1 and Treatment group 2. However, in case of total fecal weight, significant difference was observed between the Treatment group 3 and the standard group. When total fecal weight was compared within the Treatment groups as well as with standard group, no significant differences were observed. Besides, the data revealed that, the percentage of diarrheal inhibitions were 56.84%, 61.05%, 67.37% and 73.68% respectively in Treatment group1, Treatment group 2, Treatment group 3 and standard group. Even the percentages of total fecal output was 56.29%, 50.14%, 40.48% and 33.99% respectively in Treatment group1, Treatment group 2, Treatment group 3 and standard group.

Effects of Probiotic on Blood Metabolites in Castor oil Induced Diarrheal Model

Table 75 shows the effects of probiotic yogurt on serum electrolytes and metabolites including serum total protein level, albumin, globulin, WBC and hemoglobin. In case of sodium all of the groups were significantly ($p<0.05$, $p<0.01$, $p<0.001$) different when compared with positive control group. Treatment group 2 was significantly ($p<0.05$, $p<0.01$) different when compared with negative control group and Treatment group was different with negative control group at significant level of $p<0.05$, $p<0.01$, $p<0.001$. But all the values were in normal level. Moreover Treatment group 3 was significantly ($p<0.05$, $p<0.01$, $p<0.001$) different with Treatment group 1 and with standard group at the significance level of $p<0.05$.

Serum potassium level was also compared with positive control group and all the Treatment groups and negative control groups. Treatment group 3 and standard group had statistically significant ($p<0.05$, $p<0.01$, $p<0.001$) difference with the positive control group. But Treatment group 1 and Treatment group 2 showed difference at the level of significance which was $p<0.01$ and $p<0.05$ respectively. Difference was found between the Treatment group 3 and Treatment group 2 at $p<0.05$ level of significance. No differences were found between the three Treatment groups and standard group. Moreover probiotic yogurt showed no effects on serum chloride level. When total protein levels of all the groups were compared with the positive control group there were significant differences between the groups. The level of significance was $p<0.01$, $p<0.01$ and $p<0.05$ for Treatment group 1, Treatment group 2 and Treatment group 3 respectively. The standard group showed the highest level of significance ($p<0.001$) when compared with the positive control group. There were no statistically significant difference between the Treatment groups and standard group. In case of total albumin, standard group, Treatment group 1 and Treatment group 2 showed significant difference with positive control group and the level of significance were $p<0.001$, $p<0.05$ and $p<0.01$ respectively. However, there were no significant variation between the groups in case of globulin and hemoglobin. When WBC count was compared to the positive control group, standard group showed significant ($p<0.05$) difference with the positive control group. There were no significant differences in Treatment groups compared to the negative control groups in case of the above mentioned parameters. All the parameters were in normal level.

Table: 75: Effects of Probiotics on blood Serum electrolytes and metabolites

Groups	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Total Protein (m g/dl)	Albumin (mg/dl)	Globulin (mg/dl)	WBC $10^3/mm^3$	Hemoglobin (mg/dl)
Negative Control	135.833± 0.7032^{a123}	3.6000± 0.05774^{a1}	98.2000± 0.6218^{A}	6.617± 0.1493^{a}	3.48± 0.1470^{a}	3.13± 0.0802^{a}	7.90± 0.2530^{a}	10.46± 0.1980^{a}
Positive Control	129.67± 1.453	3.117± 0.05426	95.67± 1.145	5.83± 0.08819	3.27± 0.06146	2.57± 0.06146	8.80± 0.4509	9.18± 0.4527
Standard	141.50± 1.310^{a123e}	4.47± 0.1856^{a123e}	99.67± 1.430^{ae}	7.20± 0.2000^{a12e}	4.15± 0.09916^{a123e}	3.05± 0.1688^{ae}	10.78± 0.3114^{a1e}	12.30± 1.701^{ae}
Treatment Group 1	138.17± 1.424^{a123bE}	4.07± 0.1838^{a12be}	100.50± 1.478^{aBe}	7.05± 0.1945^{a12be}	3.87± 0.1256^{a1Be}	3.18± 0.2774^{abe}	9.73± 0.3584^{abe}	10.78± 0.5005^{abcde}
Treatment Group 2	142.67± 1.282^{a123bce12}	3.77± 0.04944^{a1bc1e}	100.50 ± 1.204^{aBce}	7.017± 0.2358^{a12bce}	3.917± 0.07032^{a12bce}	3.10± 0.1789^{abce}	10.23± 0.3861^{abce}	9.93± 0.6736^{abce}
Treatment Group 3	147.67± 0.8433^{a123b1c123de123}	4.55± 0.2405^{a123bcd1e}	99.83± 1.493^{aBcde}	6.78± 0.2428^{a1bcde}	3.72± 0.1778^{abCde}	3.07± 0.09888 abcde	10.53± 0.5057^{a1bcde}	9.60± 0.3706^{abe}

Values are mean ±SEM (n= 6); analysis was performed using one way ANOVA followed by Tukey's adjustment.[a]Compared with positive control, [b]Compared with standard group, [c]Compared Treatment group 1, [d]Compared with Treatment group 2 and [e] Compared with negative control group. Here, [1]p<0.05, [2]p<0.01, [3]p<0.001.

Experiment No: 05

Effects of native probiotic isolates on allergic induced mice model

All the data generated from the samples of animal trials were properly collected and is presented graphically in the Figures 25, 26, 27, 28, 29, 30, 31, 32 and 33.

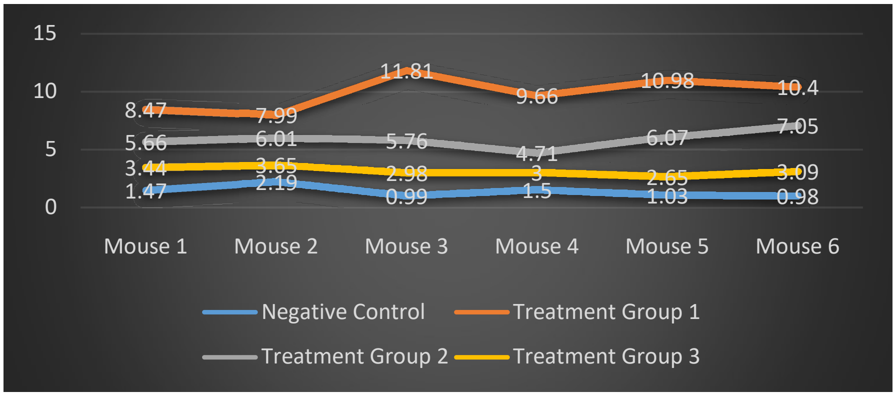


Figure 25: Comparison of IgE levels of the treatment groups with the negative control group.

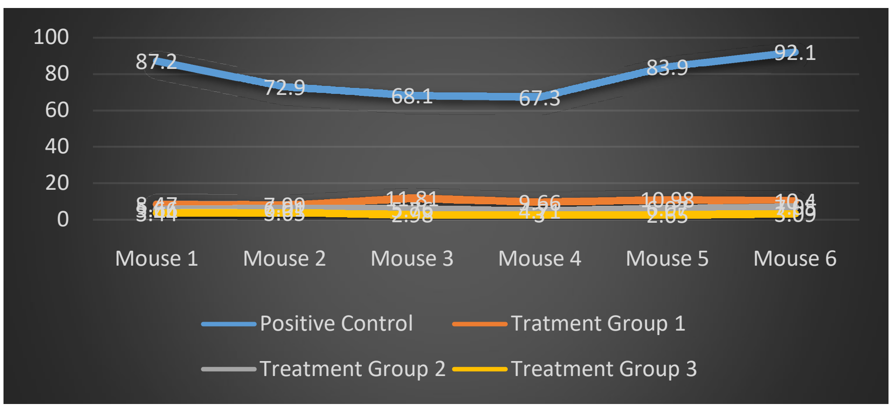


Figure 26: Comparison of IgE levels of the treatment groups with the positive control group.

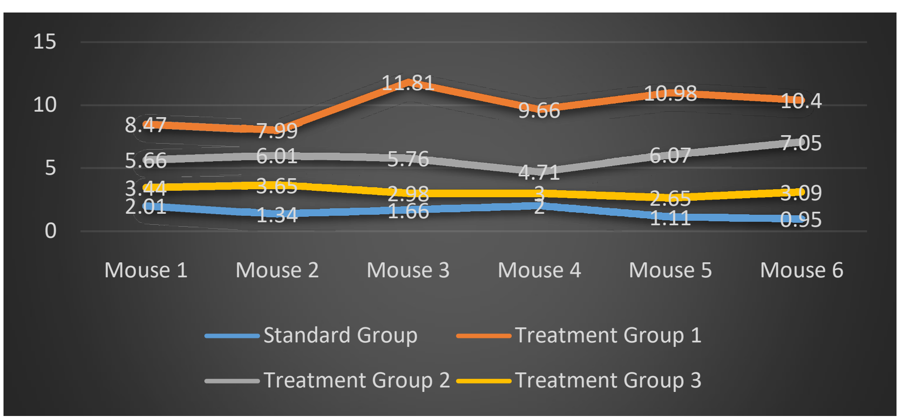


Figure 27: Comparison of IgE levels of the treatment groups with the standard group

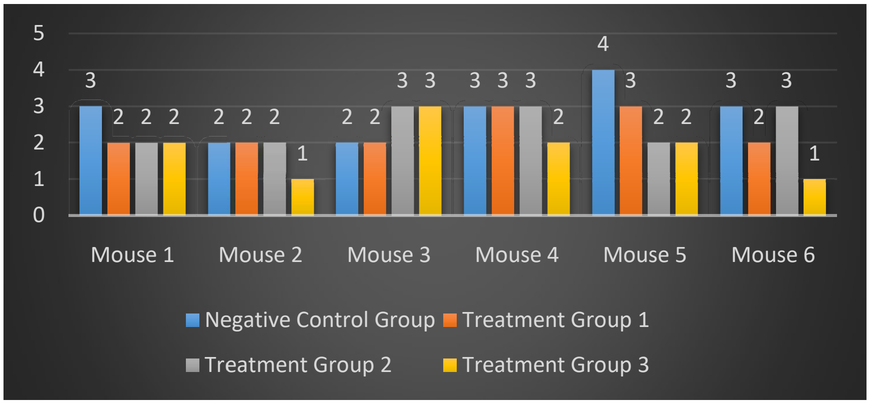


Figure 28: Comparison of eosinophil count of the treatment groups with the negative control group

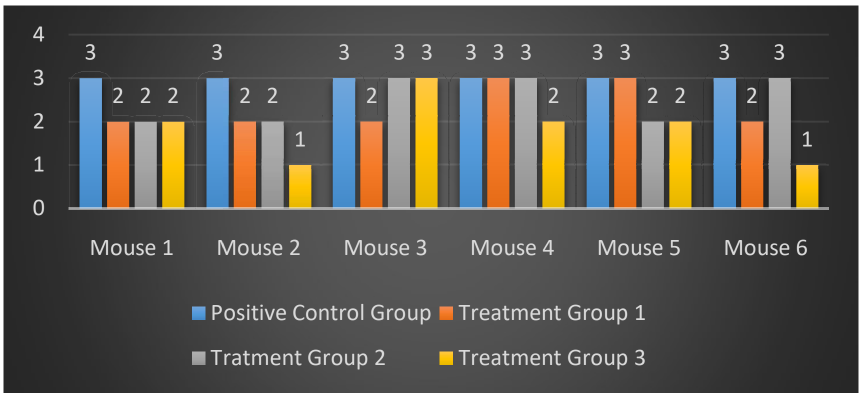


Figure 29: Comparison of eosinophil count of the treatment groups with the positive control group

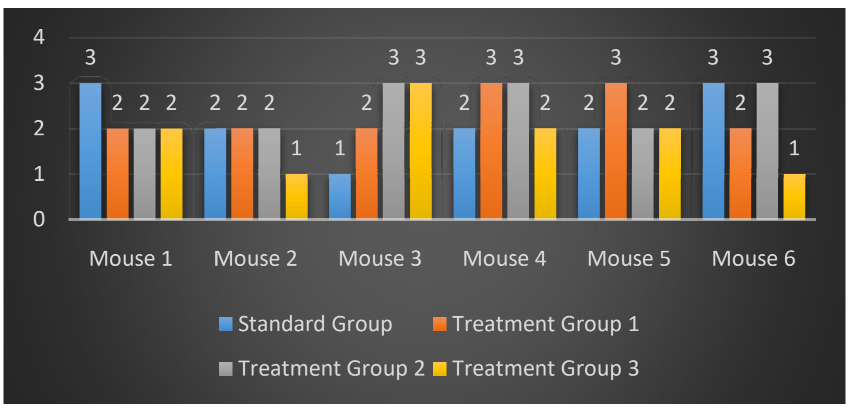


Figure 30: Comparison of eosinophil count of the treatment groups with the standard group

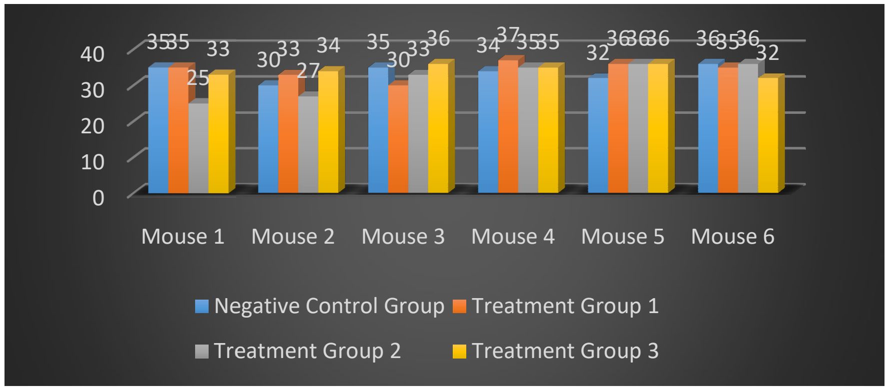


Figure 31: Comparison of peripheral lymphocyte count of the treatment groups with the negative control group

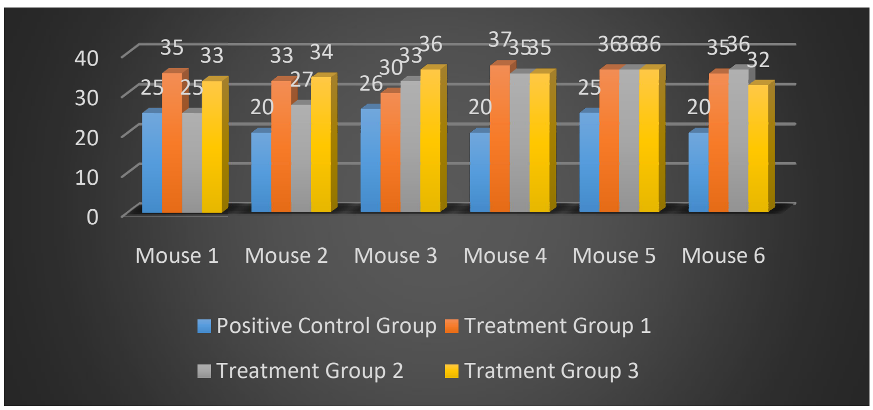


Figure 32: Comparison of peripheral lymphocyte count of the treatment groups with the positive control group

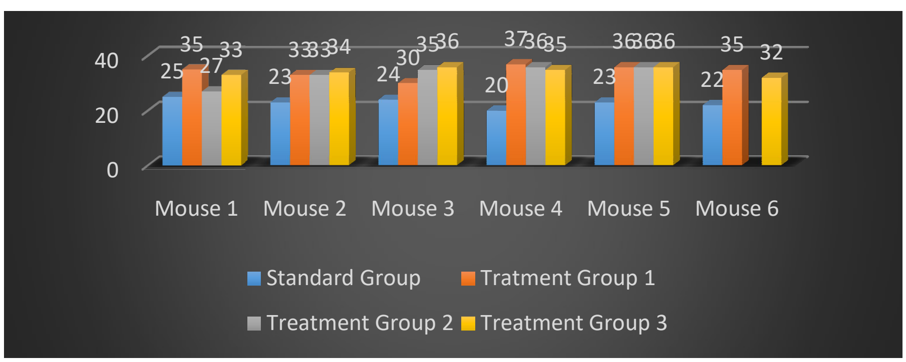


Figure 33: Comparison of peripheral lymphocyte count of the treatment groups with the standard group

Experiment No. 6

Effects of native Probiotic isolates as layer starter feed supplement

Effects of probiotic feed on layer chick

Body weight

Body weights of chicks were measured every week during the experimental period. The starting day of the treatment was considered as week 0. The body weight of that day was the body weight of week 0. Then, the body weight was measured every week to examine their probiotic effect on growth of layer chicks. The highest mean body weight was observed in treatment group 1 at the end of every week. The lowest mean body weight was in control group at the end of the treatment. Though the Treatment Group 1 showed the highest growth of the chicks but the probiotic effect was not significant in that study. The total mean body weight data are presented as mean ±SEM in the Table 76.

Table 76: Weekly body weight (gm) gain of different groups of experimental layer chicks

Period of time	Probiotic Group-1 (T1)	Probiotic Group-2 (T2)	Oxytetracycline Group (T3)	Ciprofloxacin Group (T4)	Control Group (C)
Day 0	30.4±.82	29.5±.3	30±.8	30.25±.96	29.25±.26
Week 1	68±.74	58.2±.63	61.5±.69	60.75±.93	56.6±.83
Week 2	131.5±1	121.25±.86	112.5±.94	108.25±1.5	111±1.6
Week 3	185.5±.7	173.5±.32	172.25±.65	168.75±1.53	151±.8

Feed intake

The total feed that was consumed by the chicks of a particular treatment group was measured in weekly basis and then the amount of feed was divided by the chick number. Total 22 kg feed was consumed by the chicks during the whole experimental period. In the first week, the chicks of Treatment Group 1, 2, 3, 4 and control group consumed 105 gm, 95 gm, 100 gm, 105 gm and 95 gm of starter feed and in the 3rd week they consumed 175 gm, 150 gm, 150 gm, 225 gm and 200 gm of starter feed, respectively. However, the feed intake was not affected significantly by the probiotics. The amount of feed consumed by the chicks is presented in the Table 77.

Table 77: Weekly feed intake (gm/chick/week) of different groups of experimental layer chicks

Period of time	Probiotic Group-1 (T1)	Probiotic Group-2 (T2)	Oxytetracycline Group (T3)	Ciprofloxacin Group (T4)	Control Group (C)
Week 1	105	95	100	105	95
Week 2	140	135	145	135	145
Week 3	175	150	150	225	200

Feed conversion ratio (FCR)

Table 78 shows the weekly feed conversion ratio of different groups of experimental layer chicks. FCR values among the layers kept under the four treatments did not differ significantly. However, the numerical differences showed that the feed conversion ratio was lowest in Treatment Group 1 in the 1st week which was provided with layer starter having a probiotic dose 50 gm/kg. Even in the 2nd week the feed conversion ratio was lowest in Treatment Group 2 which was a probiotic supplemented group with a dose of 40 gm/kg. However, in case of third week the feed conversion ratio was lowest in Treatment

Group 3 which was supplemented with antibiotic, oxytetracycline. Like body weight gain and feed intake, feed conversion ratio also did not differ significantly due to probiotic feed.

Table 78: Weekly feed conversion ratio of different groups of experimental layer chicks

Period of time	Group 1 (T1) 50 ml/kg probiotic	Group 2 (T2) 40 ml/kg probiotic	Group 3 (T3) Oxytetracycline	Group 4 (T4) Ciprofloxacin	Control group (C)
Week 1	2.82	3.31	3.17	3.44	3.47
Week 2	2.2	2.14	2.9	2.83	2.66
Week 3	3.24	2.85	2.52	3.73	5

Average daily gain

Average daily gain was calculated by dividing the body weight gained in a week by the number of days. Though the average daily gain was not significant but the average daily gain for first one and two week remained the highest for the Treatment Group 1 which was a probiotic group supplemented with 50 ml/kg of probiotic. Though the highest daily weight gain was observed in the Treatment Group 4, an antibiotic supplemented group. Results are shown in Table 79.

Table 79: Daily weight gain (gm) of different groups of experimental layer chicks

Period of time	Group1 (T1) 50 ml/kg probiotic	Group2 (T2) 40 ml/kg probiotic	Group3 (T3) Oxytetracycline	Group4 (T4) Ciprofloxacin	Control Group (C)
Week 1	5.31±.6	4.1±.11	4.5±.09	4.36±.10	3.91±.11
Week 2	9.1±.09	9.01±.10	7.2±.09	6.8±.11	7.77±.10
Week 3	7.7±.06	7.5±.08	8.5±.1	8.6±.09	5.71±.10

Mortality rate

No chicks died during the experimental period. Therefore, the mortality rate was calculated to be zero.

Serum cholesterol level

There was significant (p<0.0001) difference among the different treatment groups regarding serum cholesterol (Figures 34 and 35). When compared with probiotic supplemented feed groups viz. Treatment Groups 1 and 2, and the Control Group then the serum cholesterol level was significantly different (p<0032). When Treatment Groups 1 and 2 were compared with Treatment Groups 3 and 4 the difference in serum cholesterol level was highly significant (p<0.0001).

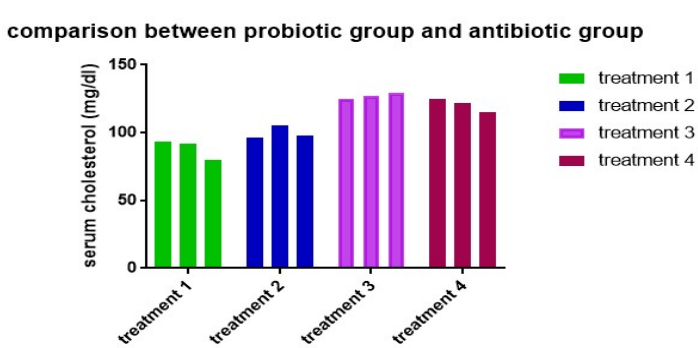


Figure 34: Serum cholesterol level (gm/dl) in different experimental layer chicks

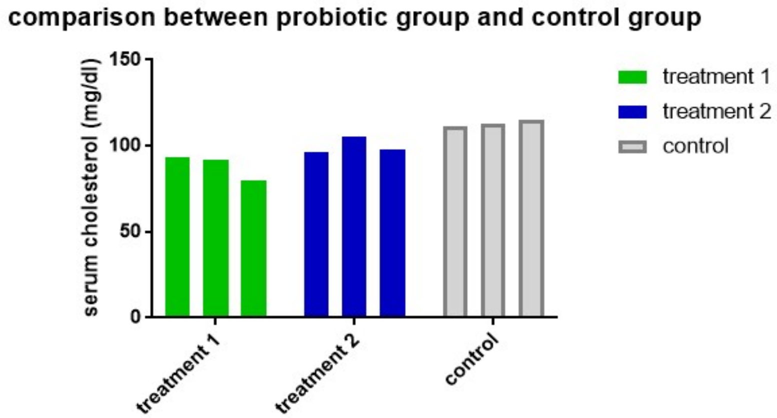


Figure 35: Serum cholesterol level (gm/dl) in probiotic vs. Control Groups of layer chicks

Serum Ca level

The serum calcium levels in the chicks of different groups had significant difference (p<0.05). From the value presented in Table 80 and Figures 36 and 37, it is clear that the highest Ca level was observed in the Treatment Group 1 while the lowest was observed in the Treatment Group 3. When compared with different treatment groups, the serum Ca level was significantly different. There was a significant increase in serum Ca level of Treatment Group 1 and Treatment Group 2 compared with Treatment Group 3 and Treatment Group 4.

Table 80: Serum calcium level (g/dl) in chicks of different experimental groups

Parameters	Group (T1) 50 ml/kg probiotic	Group (T2) 40 ml/kg probiotic	Group (T3) Oxytetracycline	Group (T4) Ciprofloxacin	Control Group (C)
Serum Ca level (g/dl)	13.5	13.33	8	10.7	9.4
SEM	0.25	0.3	2.6	0.5	0.25

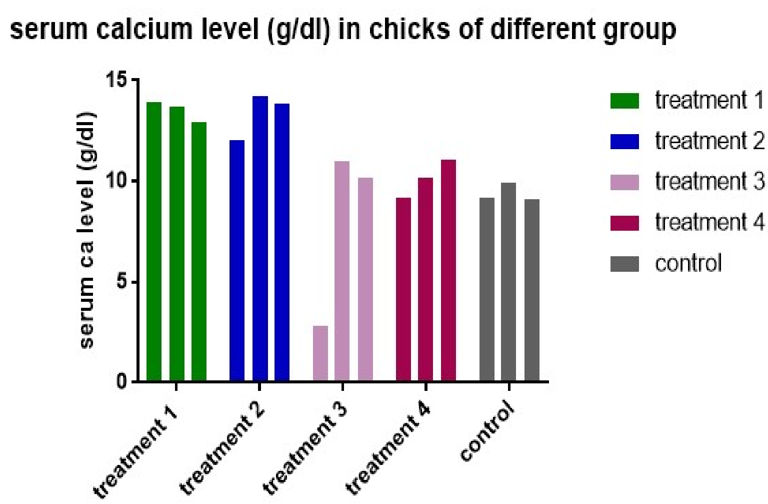


Figure 36: Serum calcium level (g/dl) in chicks of different experimental groups

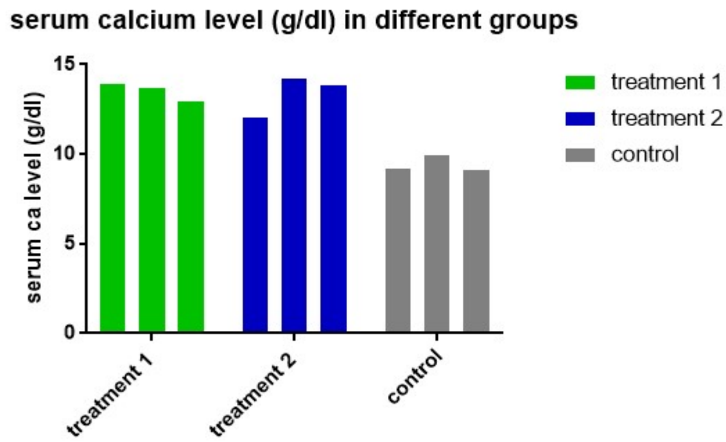


Figure 37: Serum calcium level (g/dl) in chicks of probiotic (T1 and T2) vs. Control group

Experiment No. 7

Effects of native Probiotic isolates as broiler starter feed supplement

Experimental trial on Broiler Chicks

Mean body weight (MBW)

Body weight of chicks was measured every week during the period of trial. The mean body weight ± SEM values of each experimental group are shown in Table 81 with the level of significance among all the groups which was calculated by one way ANOVA. In week zero, there were no significant differences among all the groups meaning that each group contained chicks with very low level of body weight differences at the start of 3 week treatment period. At the end of week one, all the groups showed excellent level of differences ($p<0.001$) when compared with the control. There was significant differences ($p<0.001$) of mean body weight between probiotic (T3 and T4) and control groups when compared with. Antibiotic groups (T1 and T2) also showed significant differences ($p<0.001$) when compared to the control group as well as when compared to the probiotic (T3 and T4) groups. Bothe the T3 and T4 groups showed significant increase ($p<0.001$) in mean body weight compared to control, T1 and T2 groups.

At the end of week two, all the groups showed excellent level of differences ($p<0.001$) when compared with the control. There was significant differences ($p<0.001$) between the T3 and T4 groups when compared with the control group. T1 and T2 showed significant differences ($p<0.01$) when compared to the control group and when compared to the T3 and T4 groups. T1 and T2 were both significantly lower in mean body weight with varying level of significance ($p<0.05$ and $p<0.01$ respectively). Both the T3 and T4 groups showed significant increase ($p<0.001$) in mean body weight compared to control, T1 and T2 groups.

At the end of the trial, all the groups showed excellent level of differences ($p<0.001$) when compared with the control. There was significant differences ($p<0.001$) between the T3 and T4 groups when compared with the control group. T1 and T2 showed significant differences ($p<0.001$) when compared to the control group as well as when compared to the T3 and T4 groups. Both the T3 and T4 groups showed significant increase ($p<0.001$) in mean body weight compared to control, T1 and T2 groups.

Table 81: Mean body weight (g) of chicks of different treatment groups in experimental trial

Experimental Groups	Mean body weight (g) ± SEM			
	Week 0	**Week 1**	**Week 2**	**Week 3**
Control	50±0.88^{d0ef0}	97.3±1.16^{d123ef123}	221.33±1.45^{d123ef123}	469.8±2.503^{d123ef123}
T1 (Ciprofloxacin)	50.1±1.2^{ac0ef0}	112.2±1.35^{ac123ef123}	253.2±2.906^{ac12ef1}	500.2±1.734^{ac123ef123}
T2 (Oxytetracycline)	50.2±0.78^{ab0ef0}	110.3±0.782^{ab123ef123}	250.5±1.732^{ab12ef12}	499.8±1.556^{ab123ef123}
T3 (Probiotic 2×10^9 CFU)	50.1±0.88^{abc0}	113.4±0.682^{abc123}	253.8±2.603^{abc123}	507.6±1.548^{abc123}
T4 (Probiotic 4×10^9 CFU)	50±0.78^{abc0}	116.8±1.3^{abc123}	259.3±1.202^{abc123}	519.8±2.387^{abc123}

Values are mean ±SEM (n= 8); analysis was performed using one way ANOVA.
^{a}Compared with control, ^{b}Compared treatment group 1, ^{c}Compared with treatment group 2,^{d}Compared with treatment group 1, treatment group 2, treatment group 3 and treatment group 4, ^{e}compared with treatment group 3, ^{f}compared with treatment group 4. Here, $^{1}p<0.05$, $^{2}p<0.01$, $^{3}p<0.001$. $^{0}p>0.05$

Average daily gain (ADG)

The average daily gains of chicks are shown in Table 82 and Figure 38. Here, in case of treatment group 4, body weight was found to increase from week 1. The increased level can be compared to the other groups. Between T4 (probiotic) and T2 (Oxytetracycline) group, a sort of similar increased level was observed. T4 group when compared with other groups, a positive increase was observed. Control group when compared with the 4 treatment groups, treatment groups showed increased average daily gain. T4 group obtained highest average body weight gain among the other groups. So, it can be concluded that probiotics have positive effect on the average daily gain of chicks and thus could be compared to antibiotic groups.

Table 82: Average daily gain (g) of chicks of different treatment groups in experimental trial

Days	C	T1	T2	T3	T4
1-7	7.00	8.90	8.60	9.00	9.50
7-14	17.30	18.44	18.35	20.33	21.48
14-21	29.30	31.80	31.60	35.80	36.20

C = Control, T1 = Ciprofloxacin, T2 = Oxytetracycline, T3 = Probiotic (2×10^9 CFU) and T4 = Probiotic (4×10^9 CFU)

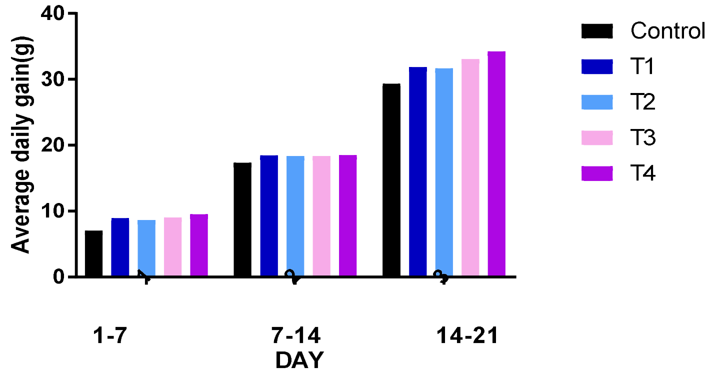


Figure 38: Average daily gain (g) of chicks of different treatment groups in experimental trial

Feed intake

Feed intake of chicks is shown in Table 83 and Figure 39. Here, in case of all treatment groups feed intake was found to increase from week 1. The increased level can be compared to the other treatment groups. All the treatment groups showed increased feed intake compared to control group.

Table 83: Feed intake (g) of chicks of different treatment groups in experimental trial

Week	C	T1	T2	T3	T4
1	14.34	17.10	17.33	17.20	16.00
2	49.54	52.90	50.00	52.41	52.40
3	68.25	71.00	70.50	73.19	72.05

C = Control, T1 = Ciprofloxacin, T2 = Oxytetracycline, T3 = Probiotic (2×10^9 CFU) and T4 = Probiotic (4×10^9 CFU)

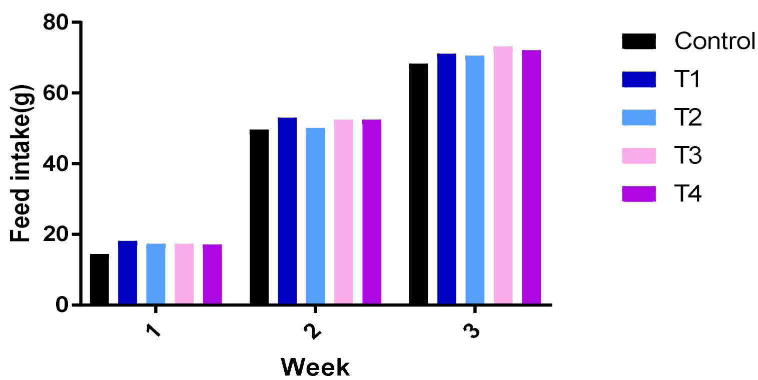


Figure 39: Feed intake (g) of chicks of different treatment groups in experimental trial

Feed conversion ratio (FCR)

The FCR of chicks is shown in Table 84 and Figure 40. Here in case of treatment group 4, FCR was decreased from week 1. The decreased level can be compared to the other treatment groups. Between T4 (Oxytetracycline) and T2 (probiotic) groups, similar level of decrease was observed. T4 group when compared with other groups, a positive decrease level was observed. Control group showed increased FCR compared to other treatment groups. Among the treatment groups T4 group obtained lowest FCR. Therefore, it can be concluded that probiotics has significant effect on the FCR of chicks and can be compared to the antibiotic groups.

Table 84: Feed conversion ratio of different treatment group chiks in experimental trial

Week	C	T1	T2	T3	T4
1	2.40	2.03	2.01	1.91	1.89
2	2.89	2.86	2.72	2.85	2.74
3	2.64	2.23	2.23	2.21	2.22

C=Control, T1 = Ciprofloxacin, T2 = Oxytetracycline, T3 = Probiotic (2×10^9 CFU) and T4 = Probiotic (4×10^9 CFU)

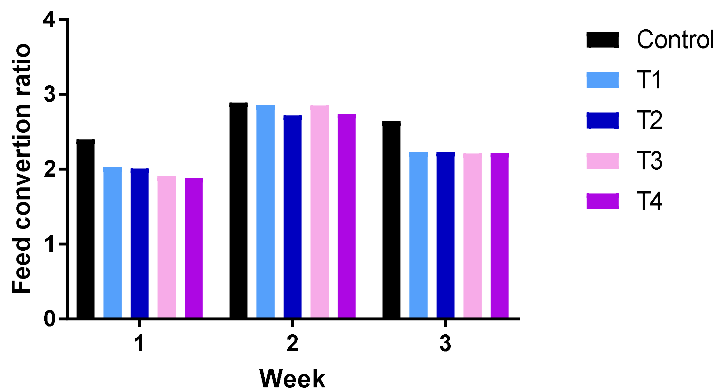


Figure 40: Feed conversion ratio of different treatment groups in experimental trial

Carcass yield

The carcass yield of chicks is shown in Table 85 and Figures 41-45. Here in case of treatment group 4, carcass yield was increased from week 1. The increased level can be compared to the other treatment groups. Between T4 (probiotic) and T2 (Oxytetracycline) group, some sort of similar increased level was observed. T4 group, when compared with other treatment groups a positive increased level was observed for T4 group. Control group showed decreased carcass yield when compared with other 4 treatment groups, excepting the abdominal fat that was highest in the control group. Among the treatment groups T4 group showed highest carcass yield. Therefore, it can be concluded that probiotics has significant effect on the carcass yield of chicks that can be compared to the antibiotic groups.

Table 85: Carcass yield (g) of birds of different treatment groups

Yield (g)	C	T1	T2	T3	T4
Dressing yield	348.59±2.67	381.35±3.27	376.23±2.13	404.40±2.12	418.67±3.89
Breast	143.30±1.67	170.80±1.32	169.71±1.64	170.52±0.97	174.27±1.07
Drumstick	128.21±1.12	153.48±1.45	151.50±1.34	162.91±1.12	168.47±1.07
Wings	31.01±0.67	36.5±0.57	35.59±0.59	37.8±0.47	39.7±0.46
Abdominal fat	5.32±0.04	5.26±0.09	5.17±0.06	5.15±0.07	5.05±0.07

C = Control, T1 = Ciprofloxacin, T2 = Oxytetracycline, T3 = Probiotic (2×10^9 CFU) and T4 = Probiotic (4×10^9 CFU)

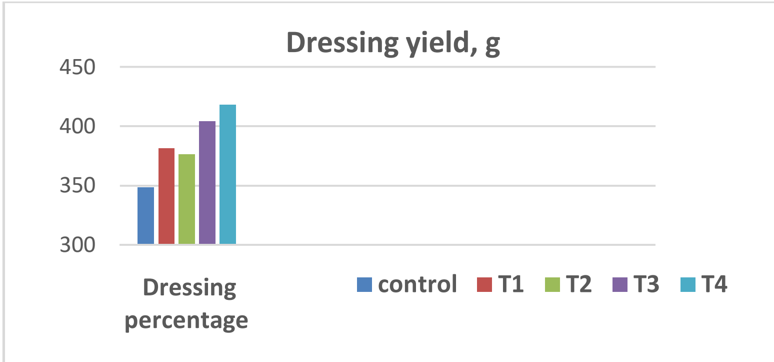


Figure 41: Dressing yield (g) of broiler chicks within different treatment groups

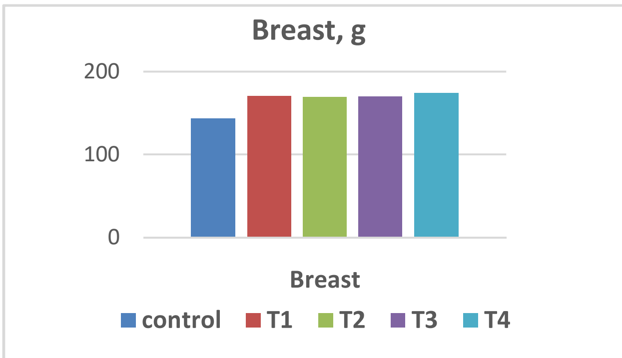


Figure 42: Breast meat yield (g) of broiler chicks within different treatment groups

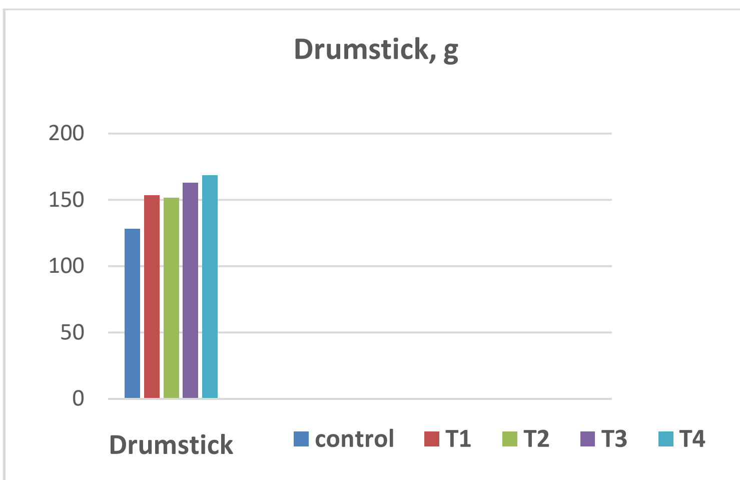


Figure 43: Weights of Drumstick (g) of broiler chicks within different treatment groups

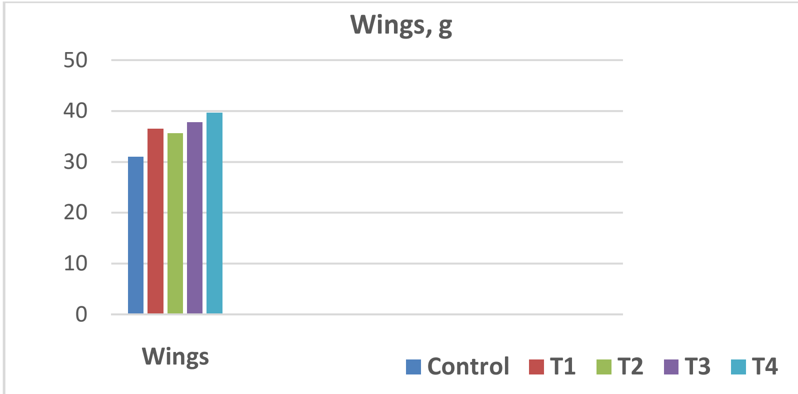


Figure 44: Weights of wings (g) of broiler chicks within different treatment groups

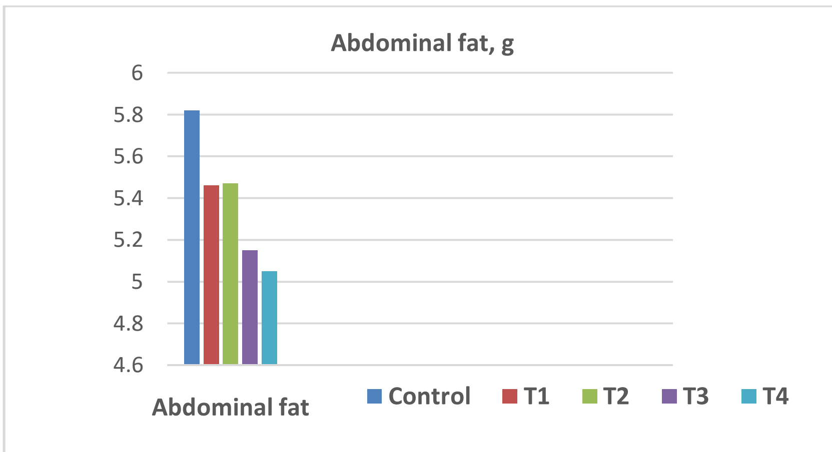


Figure 45: Weights of abdominal fat (g) of broiler chicks within different treatment groups

Serum biochemical test

In case of HDL level, there was significant differences ($p<0.05$) among all the groups when compared with the control group (Table 86). T3 and T4 groups showed significant increase ($p<0.05$) in HDL level when compared with the control group as well as with the T1 group and T2 groups. In case of LDL level, there was no significant difference among all the groups when compared with the control group. T3 and T4 groups had no significant decrease in LDL level when compared with the control group as well as with the T1 group and T2 group.

Table 86: Serum HDL and LDL level of broiler chicks within different treatment groups

Groups	HDL level (mg/dl)	LDL level (mg/dl)
Control (C)	32.33±0.5819^{d12ef12}	103.23±0.57^{d0ef0}
T1 (Ciprofloxacin)	34±0.67^{ac12ef12}	102.2±0.15^{ac0ef0}
T2 (Oxytetracycline)	35.67±0.68^{ab12ef12}	103.9±0.15^{ab0ef0}
T3 (Probiotic 2×10^{9} CFU)	40.3±0.78^{abc12}	101.27±0.33^{abc0}
T4 (Probiotic 4×10^{9} CFU)	42±0.37^{abc12}	103.25±0.33^{abc0}

Values are mean ±SEM (n= 8); analysis was performed using one way ANOVA.

^{a}Compared with control, ^{b}Compared treatment group 1, ^{c}Compared with treatment group 2,^{d}Compared with treatment group 1, treatment group 2, treatment group 3 and treatment group 4, ^{e}compared with treatment group 3, ^{f}compared with treatment group 5. Here, ^{1}p<0.05, ^{2}p<0.01, ^{3}p<0.001; ^{0}p>0.05

Protein content in meat

The meat protein content of chicken meat is shown in Table 87. In case of total protein level, there was significant differences (p<0.05) among all the groups when compared with the control group. T3 and T4 groups had significant increase (p<0.05) in total protein level when compared with the control group as well as with the T1 group. There was significant (p<0.05) increase in total protein level when T3 and T4 were compared with T2 group although some sorts of increase were observed in the mean value.

Table 87: Protein content (g) in meat of broiler chicks within different treatment groups

Groups	Meat protein content/5 gram of breast meat (g)
Control (C)	1.30±0.048^{d1ef1}
T1 (Ciprofloxacin)	1.67±0.038^{ac1ef1}
T2 (Oxytetracycline)	1.73±0.038^{ab1ef1}
T3 (Probiotic 2×10^{9} CFU)	2.03±0.03^{abc1}
T4 (Probiotic 4×10^{9} CFU)	2.2±0.07^{abc1}

Values are mean ±SEM (n= 8); analysis was performed using one way ANOVA.

^{a}Compared with control, ^{b}Compared treatment group 1, ^{c}Compared with treatment group 2,^{d}Compared with treatment group 1, treatment group 2, treatment group 3 and treatment group 4, ^{e}compared with treatment group 3, ^{f}compared with treatment group 5. Here, ^{1}p<0.05, ^{2}p<0.01, ^{3}p<0.001; ^{0}p>0.05

Mortality rate

The Mortality rate of chicks is shown in Table 88. During the trial period Probiotic feed groups (T3 and T4) did not have any mortality. On the other hand, control group suffered from 25% mortality followed by antibiotic (T1 and T2) groups, where there was 12.50% mortality in each group.

Table 88: Mortality rate of broiler chicks within different treatment groups

Mortality Rate	C	T1	T2	T3	T4
(%)	25.00	12.50	12.50	0.00	0.00

C = Control, T1 = Ciprofloxacin, T2 = Oxytetracycline, T3 = Probiotic (2×10^{9} CFU) and T4 = Probiotic (4×10^{9} CFU)

Experiment No.8

Effects of native Probiotic isolates as duck starter feed supplement

Body weight

Body weight of ducklings was measured every week during the period of the trial. At week 0, body weights of ducklings ranged between 41 and 46 gm. After 3 week period of treatment, the body weight was significantly increased in Treatment Groups. The highest mean body weight was observed in Treatment Group 4. The lowest mean body weight was in Control Group. The mean body weight ± SEM values of each experimental group are shown in Table 89 with the level of significant variation among all the groups which was calculated by one way ANOVA.

In week zero, there were no significant differences among all the groups meaning that each group contained ducklings with very low level of body weight differences at the start of 3 week Treatment period.

At the end of the trial, all the Groups showed significant level of differences ($p<0.001$) when compared with the control. There was significant differences ($p<0.001$) between Probiotic Groups (T3 and T4) and Control Group. Antibiotic Groups (T1 and T2) also showed significant differences ($p<0.001$) when compared with the Control Group as well as when compared with the T3 and T4 Groups. Both T3 and T4 Groups showed significant increase ($p<0.001$) in mean body weight compared to control, T1 and T2 Groups.

Table 89: Weekly body weight of each experimental group of ducklings

Groups	Mean body weight (g) ± SEM			
	Week 0	**Week 1**	**Week 2**	**Week 3**
C	$44.33±0.88^{d0ef0}$	$109±1.15^{d123ef123}$	$193.33±1.45^{d123ef123}$	$280.33±2.603^{d123ef123}$
T 1	$42.67±1.2^{ac0ef0}$	$118.67±1.45^{ac123ef123}$	$205.67±2.906^{ac12ef1}$	$299.33±1.764^{ac123ef123}$
T 2	$42.67±0.88^{ab0ef0}$	$124.67±0.882^{ab123ef123}$	$206±1.732^{ab12ef12}$	$309.67±1.856^{ab123ef123}$
T 3	$41.67±0.88^{abc0}$	$139.67±0.882^{abc123}$	$215.33±2.603^{abc123}$	$324±1.528^{abc123}$
T 4	$41.33±0.88^{abc0}$	$144.33±1.2^{abc123}$	$220.67±1.202^{abc123}$	$335±2.887^{abc123}$

Values are mean ±SEM (n= 8); analysis was performed using one way ANOVA.

C = Control, T1 = Ciprofloxacin, T2 = Oxytetracycline, T3 = Probiotic ($8.8×10^9$ CFU) and T4 = Probiotic ($9.9×10^9$ CFU)

^{a}Compared with Control, ^{b}Compared Treatment Group 1, ^{c}Compared with Treatment Group 2, ^{d}Compared with Treatment Group 1, Treatment Group 2, Treatment Group 3 and Treatment Group 4, ^{e}compared with Treatment Group 3, ^{f}compared with Treatment Group 5. Here, $^1p<0.05$, $^2p<0.01$, $^3p<0.001$; $^0p>0.05$

Feed intake

Feed intake average of the ducklings for all the experimental groups is shown in Table 90 and Figure 46. At the end of every week, all the groups had high feed intake amount compared to the Control group. Probiotic supplemented groups (T4 and T3) have the highest feed intake value compared to the control and Antibiotic groups (T1 and T2). T4 has higher feed intake value compared to that of the T3 meaning high probiotic dose may have higher impact on increasing feed intake value.

Table 90: Weekly feed intake in all the experimental groups of ducklings

Groups	Feed intake (g/bird/week)		
	Week 1	Week 2	Week 3
Control (C)	440.25	500.375	600.25
T1 (Ciprofloxacin)	500.125	610.875	718.75
T2 (Oxytetracycline)	510.75	620.25	730
T3 (Probiotic 8.8×10^9 CFU)	637.5	650.625	805.5
T4 (Probiotic 9.9×10^9 CFU)	671	655.5	820.23

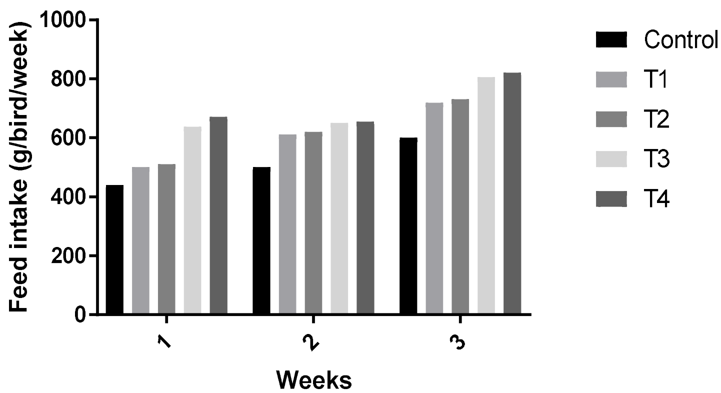


Figure 46: Feed intake comparison among the experimental Groups of ducklings

Feed conversion ratio

Feed conversion ratio achieved by the experimental ducklings is shown in Table 91 and Figure 47. At the end of week one, all the Groups had lower feed conversion ratio compared to the Control Group. T4 and T3 Groups had higher feed conversion ratio compared to the T2 Group but lower feed conversion ratio compared to Control and T1 Group. At the end of second and third week, all the Groups had high feed conversion ratio compared to the Control Group. T4 and T3 Groups had the highest feed intake value compared to the control, T1 and T2 Groups.

Table 91: Weekly feed conversion ratio in all the experimental groups of ducklings

Experimental Groups	Feed conversion ratio		
	Week 1	Week 2	Week 3
Control (C)	6.86	5.93	6.89
T1 (Ciprofloxacin)	6.58	7.02	7.70
T2 (Oxytetracycline)	6.22	7.62	7.04
T3 (Probiotic 8.8×10^9 CFU)	6.5	8.59	7.41
T4 (Probiotic 9.9×10^9 CFU)	6.51	8.58	7.17

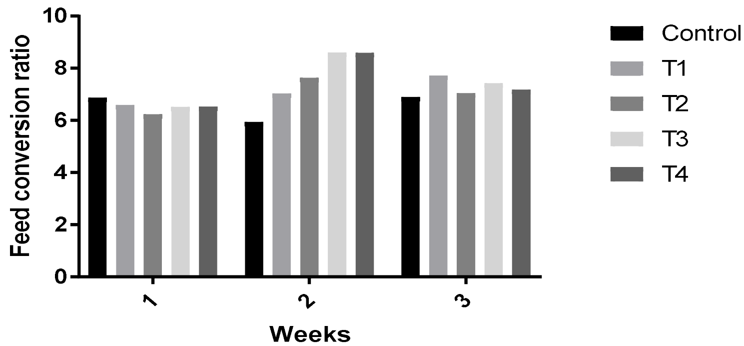


Figure 47: Comparison of feed conversion ratio among the experimental Groups of ducklings

Carcass status

Carcass status of the experimental ducklings is shown in Table 92. At the end of the 3 week trial, different carcass characteristics were measured by determining the percentage of pre-slaughter weight. In case of dressing% there was significant differences (p<0.001) among all the Groups when compared with the Control Group. Probiotic supplemented feed groups (T3 and T4) showed significant increase (p<0.01) in dressing% when compared with the Control proup as well as with the antibiotic supplemented groups (T1 and T2). There was no significant (p>0.05) increase in dressing % when T1 and T2 were compared with Control group although some sorts of increase were observed in the mean value. There was significant increase in dressing % in T3 and T4 Groups when compared with antibiotic Treatment groups.

Regarding liver %, there was significant differences (p<0.001) among all the Groups when compared with the Control Group. T3 and T4 Groups had significant increase (p<0.01) in liver % when compared with the Control Group as well as with the T1 and T2 Groups. There was significant (p<0.001) increase in liver % when T1 and T2 were compared with Control Group. There was significant increase in liver % in T3 and T4 Groups when compared with antibiotic Treatment Groups.

In terms of heart %, there was significant differences (p<0.001) among all the Groups when compared with the Control Group. T3 and T4 Groups had significant increase (p<0.01) in heart % when compared with the Control Group as well as with the T1 and T2 Groups. There was significant (p<0.05) increase in heart % when T1 and T2 were compared with Control Group. There was significant increase in liver % in T3 and T4 Groups when compared with antibiotic Treatment Groups.

With respect to gizzard %, there was significant differences (p<0.001) among all the Groups when compared with the Control Group. T3 and T4 Groups had significant increase (p<0.01) in gizzard % when compared with the Control Group as well as with the T1 and T2 Groups. There was significant (p<0.01) increase in gizzard % when T1 and T2 were compared with Control Group. There was significant increase in gizzard % in T3 and T4 Groups when compared with antibiotic Treatment Groups.

Table 92: Carcass characteristics among the experimental groups of ducklings

Groups	Carcass Status (Expressed as percentage of pre-slaughter weight ± SEM)			
	Dressing (%)	Liver (%)	Heart (%)	Gizzard (%)
Control	$21±1^{d123ef12}$	$3.21±0.01^{d123ef123}$	$0.51 ±0.005^{d123ef123}$	$5.03±0.02^{d123ef123}$
T 1	$21.82±0.32^{ac0ef123}$	$3.35±0.008^{ac123ef123}$	$0.54 ±0.005^{ac1ef123}$	$5.11±0.008^{ac12ef123}$
T 2	$22.37±0.37^{ab0ef12}$	$3.39±0.008^{ab123ef123}$	$0.54±0.005^{ab1ef123}$	$5.13±0.01^{ab12ef123}$
T 3	$23.5±0.5^{abc12}$	$3.65±0.01^{abc123}$	$0.6±0.005^{abc123}$	$5.26±0.02^{abc123}$
T 4	$24±0.2^{abc12}$	$3.72±0.008^{abc123}$	$0.6±0.005^{abc123}$	$5.32±0.008^{abc123}$

Values are mean ±SEM (n= 8); analysis was performed using one way ANOVA.
C = Control, T1 = Ciprofloxacin, T2 = Oxytetracycline, T3 = Probiotic ($8.8×10^9$ CFU) and T4 = Probiotic ($9.9×10^9$ CFU)
^{a}Compared with Control, ^{b}Compared Treatment Group 1, ^{c}Compared with Treatment Group 2,^{d}Compared with Treatment Group 1, Treatment Group 2, Treatment Group 3 and Treatment Group 4, ^{e}compared with Treatment Group 3, ^{f}compared with Treatment Group 5. Here, $^1p<0.05$, $^2p<0.01$, $^3p<0.001$;$^0p>0.05$

Serum metabolites

Effects on different serum metabolites in all the experimental groups are shown in Table 93. In case of total protein level, there was significant differences ($p<0.001$) among all the groups when compared with the Control group. Probiotic supplemented groups (T3 and T4) showed significant increase ($p<0.01$) in total protein level when compared with the Control group as well as with the T1 group but the difference was slightly lower ($p<0.05$) in case of comparison with T1 Group. There were no significant differences in total protein level when T3 and T4 were compared with T2 group although some sorts of increase were observed in the mean value.

In terms of albumin level, there was significant differences ($p<0.001$) among all the groups when compared with the Control group. T3 and T4 groups had significant increase ($p<0.01$) in albumin level when compared with the Control group as well as with the T1 group but the difference was slightly lower ($p<0.05$) in case of comparison with T1 group. There was significant differences ($p<0.05$) in albumin level when T3 and T4 were compared with T2 group.

Regarding globulin level, there was significant differences ($p<0.05$) among all the groups when compared with the Control group. T3 group had significant increase ($p<0.05$) in albumin level when compared with the Control group. But there were no significant differences in globulin level when T3 and T4 both were compared with T1 and T2 groups although from the mean value it was obvious that T3 and T4 had higher globulin level but the difference was not significant.

Again with respect to albumin and globulin ratio, there were no significant differences among all the groups. However, T3 and T4 groups had comparatively higher albumin globulin ratio than the other three groups.

In respect of uric acid level, there was significant differences ($p<0.05$) among all the groups when compared with the Control group. T3 and T4 groups had significant decrease ($p<0.001$) in uric acid level when compared with the Control group as well as with the T1 and T2 groups but the differences were slightly lower ($p<0.05$) in case of comparison with T1 and T2 groups.

In case of HDL level, there was significant differences ($p<0.001$) among all the groups when compared with the Control group. T3 and T4 groups had significant increase ($p<0.001$) in HDL level when compared with the Control group as well as with the T1 and T2 groups.

In case of LDL level, there was significant differences ($p<0.01$) among all the groups when compared with the Control group. T3 and T4 groups had significant decrease ($p<0.05$) in LDL level when compared with the Control group as well as with the T1 and T2 groups.

Table 93: Effects of probiotics on different serum metabolites in experimental ducklings

Groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin Globulin ratio	Uric acid (mg/dl)	HDL level (mg/dl)	LDL level (mg/dl)
Control	3.30 ± 0.058^{d123ef12}	0.83 ± 0.03^{d123ef12}	2.53 ± 0.12^{d1ef0}	0.32 ± 0.05^{d0ef0}	3.3 ± 0.058^{d1ef123}	94.33 ± 0.8^{d123ef123}	61 ± 0.57^{d12ef12}
T 1	3.6 ± 0.058^{ac1ef1}	0.93 ± 0.03^{ac0ef1}	2.66 ± 0.06^{ac0ef0}	0.34 ± 0.03^{ac0ef0}	3.767 ± 0.12^{ac0ef123}	86 ± 0.57^{ac123ef123}	61 ± 1.15^{ac0ef1}
T 2	3.73 ± 0.088^{ab1ef0}	0.9 ± 0^{ab0ef1}	2.83 ± 0.08^{ab0ef0}	0.31 ± 0.015^{ab0ef0}	4.83 ± 1.05^{ab0ef1}	$91.67\pm0.88^{ab123ef123}$	62 ± 1.15^{ab0ef12}
T 3	4.03 ± 0.03^{abc12}	1.067 ± 0.06^{abc12}	2.93 ± 0.03^{abc1}	0.37 ± 0.04^{abc0}	2.4 ± 0.05^{abc0}	106.3 ± 0.88^{abc123}	57.6 ± 0.33^{abc1}
T 4	4.2 ± 0.173^{abc12}	1.20 ± 0.058^{abc123}	3 ± 0.26^{abc0}	0.39 ± 0.04^{abc0}	1.9 ± 0.15^{abc1}	109 ± 0.57^{abc123}	57.3 ± 0.33^{abc1}

Values are mean ±SEM (n= 8); analysis was performed using one way ANOVA.
C = Control, T1 = Ciprofloxacin, T2 = Oxytetracycline, T3 = Probiotic (8.8×10^9 CFU) and T4 = Probiotic (9.9×10^9 CFU)
[a]Compared with control, [b]Compared Treatment Group 1, [c]Compared with Treatment Group 2,[d]Compared with Treatment Group 1, Treatment Group 2, Treatment Group 3 and Treatment Group 4, [e]Compared with Treatment Group 3, [f]Compared with Treatment Group 5. Here, $^1p<0.05$, $^2p<0.01$, $^3p<0.001$;$^0p>0.05$

11.2 Discussion

Effects of native Probiotic isolates on Hypercholesterolemic mice

Dairy products contain substantial numbers of Probiotic bacteria which helps to alter the nutrient properties of the food to exert beneficial health effects. Many researchers showed the fermented milk as yogurt or dairy products to have a low cholesterol effects (Agerback et al., 1995; Xiao et al., 2003). In a trial conducted by feeding yogurt varying strains of Probiotic bacteria revealed that Probiotics has an effect on reducing LDL cholesterol (Agerholm-Larsen et al., 2000). Another research group observed serum lipid profile and two transaminase enzyme activities (ALT and AST) of rats received bacterial culture along with food supplements. The experimental results showed significant increase in HDL levels and reduction in triglyceride, VLDL and LDL levels (Ngongang et al., 2016).

One of the studies on the effect of fermented milk on cholesterol concentration was conducted in Maasai Tribes (Mann and Spoerry, 1974). This initiated more studies to investigate the possible relation between cholesterol and fermented milk. Several *in vitro* studies have shown that some strains of LABS such as *bifidobacteria* and *lactobacilli,* in the presence of bile acids, are capable to assimilate cholesterol. Evidence from some animal studies, proposes that some fermented milk products are capable to reduce cholesterol moderately. However, studies on human have not been decisive on their effect on reducing cholesterol or low density lipoprotein (Lewis and Burmeister 2005; Greany et al, 2008; Klein et al, 2008).

Other group of researchers examined the effects of Probiotic enriched milk products on blood lipids in rats. They used fermented milk containing *bifidobacterium longum* strain *BL1*. Rats were given cholesterol-enriched food. Their diet was enriched with lyophilized milk powders as follows: (i) acid milk, (ii) milk fermented with a culture of normal yoghurt made up of *thermophilus, streptococcus, and lactobacillus delbueckii subsp. Bulgaricus,* and (iii) *bifidobacterium* milk fermented with *B. Longum* strain *BL1*. In rats, consumption of *bifidobacterium* milk led to a meaningful reduction in their triglyceride, low density lipid, and total cholesterol compared to the control group. The group that was fed ordinary fermented milk, their HDL cholesterol level remained unchanged. This group showed a minor drop in its lipid levels (Xiao et al., 2003).

In a randomized, double blind, placebo controlled trial, a milk product (Gaio), fermented with two strains of *S. thermophilus* and *Enterococcum faecium*, and was examined for its effects on lowering cholesterol. Healthy men with normal serum cholesterol levels were told to add 200 ml/day of FM to their usual diet over a six-week period. The placebo group consumed milk acidified with an organic acid. In the fermented milk consuming group, there was a significant decrease in total cholesterol of 3% and 6% after 3 and 6 week of administering the product, respectively, and LDL cholesterol levels decreased by 10% after 6 week of consumption (Agerbaeck et al., 1995).

Two controlled clinical trials were conducted to study the effects of eating a single daily serving (200 ml) of fermented milk containing *lactobacillus acidophilus L1* taken from humans or fermented milk with *L. acidophilus ATCC43211* from swine on lipids (Anderson and Gilliland, 1999). The first study was single-blind and lasted for 21 days. The results of first trial showed a significant (2.4%) decrease in blood cholesterol for FM containing *lactobacillus acidophilus L1*. The second part was a placebo-controlled, double-blinded, cross-over study. In these studies, subjects consumed either fermented milk or FM containing *L. acidophilus L1* over 4-weeks. In the second trial, *L. acidophilus L1*caused a 3.2% ($P<0.05$) decrease in blood cholesterol during the first interval. In the second interval, cholesterol remained unchanged. Collective analysis of the two *L1* treatment trials revealed 2.9% reduction in blood cholesterol level (Anderson and Gilli, 1999).

In a randomized, 2-month, double-blind clinical study, effects of fermented yoghurt on serum cholesterol of over-weight individuals was examined. They consumed 450 ml/day yoghurt fermented with *Strep. thermophilus* and *E. faecium*. Results showed an 8.4% decrease in low density lipid cholesterol and an increase in fibrinogen concentrations (Agerholm-Larsen et al., 2000). In a randomized clinical trial, 32 subjects with serum cholesterol 220-280 mg/dl were recruited. After a four-week consumption of 300 ml/day ordinary yoghurt or ordinary yoghurt starters plus *Bifidobacterium longum* strain *BL1*, sixteen of the participants in the Probiotic group showed a decrease in their total cholesterol. Participants with relatively high total cholesterol (> 240 mg/dl) showed a particularly significant reduction in their blood lipid. Serum cholesterol levels in the rest of the Probiotic group remained almost stable during the experiment (Xiao et al.,2003). In a randomized, 56-week, double blind, placebo controlled clinical trial, the effect of *faecium*, enriched with selenium on plasma lipids was studied (Hilvak et al., 2005). The result demonstrated that *E. faecium M-74* Probiotic strain reduced cholesterol levels by 12%. As high density lipid and triglyceride concentrations showed no significant change, the decrease in serum cholesterol must be mainly attributed to a drop in low density lipid cholesterol. In a cross-over clinical trial, the effects of Probiotic and conventional yoghurt on serum lipids of women with normal cholesterol concentrations were studied (Fabianand, 2006). Volunteers were given 100g/day of Probiotic enriched yoghurt (*L. casei*) or conventional yoghurt for 14 days and without a washout period, another two weeks of 200g/day. A number of lipid levels were affected in both the conventional and the Probiotic yoghurt groups, but no meaningful difference was seen between the two. Twenty-six volunteers were recruited for a placebo-controlled, double-blind, randomized crossover study (Klein et al., 2008). After a 21-day run-in period, thirteen of them were given 300 g/day of Probiotic yoghurt with *lactobacillus acidophilus 74-2* and *Bifidobacterium animalis subsp. lactis GCC420*. The rest of the group was given placebo for five weeks. The two groups switched in the next five-week trial. Even though *L. acidophilus* and *B. lactis* were recovered in feces in significantly elevated number after supplementation, fecal SCFA and serum cholesterol levels were not changed. A significant reduction (11.6%) in serum triglyceride levels were observed, however, during the period, Probiotic was consumed.

In the present study, almost similar results were obtained in line with the above studies. Considering the effects of Probiotic isolates on hypercholesterolemia study in experimental mice, body weight, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), total protein, total albumin and total globulin levels were measured. Probiotic treatment groups showed significant results compared to the control group. Probiotic treatment groups showed significant results regarding body weight gain, reduction of LDL, HDL, AST and ALT compared with the standard groups. In case of total protein level, albumin and globulins level showed no significant variation among the treatments and standard groups. However, when compared between negative and positive control groups, there was significant variation.

Effects of native Probiotic isolates on diabetic induced model rats

Probiotics are effective for the diabetic patients especially for lowering blood glucose level in case of diabetes mellitus by balancing microbial gut flora. It is reported that low-fat (2.5%) yoghurt containing Probiotic *Lactobacillus acidophilus* and *Lactobacillus casei* when tested in rats against high fructose-induced type-2 diabetes and both of these bacteria proved beneficial effect in lowering blood glucose level by decreasing insulin resistance (Yadavet et al., 2006). *Bifidobacterium spp* is an important Probiotic and it is reported that this bacteria delivers pharmaco nutritional support in treating insulin resistance (Cani and Delzenne, 2011).

Some Probiotics i.e., *Lactobacillus* and *Bifidobacterium* have effect on type 2 diabetes. They show satinogenic and insulionotropic effect and they also secrete gut hormone like GLP1, GLP2 etc. that help lowering blood glucose level in type 2 diabetes patients (Panwar et al., 2012). Yoghurt is a fermented food and feeding of yogurt shows anti-diabetic property in type 2 diabetic individuals. It is reported that oral feeding of Probiotic dahi (15 g/day/rat) significantly delayed reduction of insulin secretion during oral glucose tolerance test using skim milk or control dahi and it also reduced the total cholesterol, triglycerides, LDL and VLDL cholesterol and increased HDL cholesterol levels in Streptozotocin (STZ) induced type 2 diabetic rats (Yadavet et al., 2008).

In the present study, regarding the effects of Probiotic on streptozotocin induced type 2 diabetic rats, there was a significant decrease in the LDL cholesterol level by Probiotic yogurt treated group, and significantly increased HDL cholesterol level when treated with Probiotic yogurt. Hepatic glycogen content was increased among the Probiotic yogurt feed groups compared to the control group. However, no significant results were found in case of body weight gain and total cholesterol level. It was observed that administration of Probiotic yogurt improved glycemic and lipidemic status in type 2 diabetic rats. The findings did corroborate with the findings of the most authors.

Effects of native Probiotic isolates on selective enteric pathogens in mice

The isolated presumptive Probiotic bacteria were assayed for their anti-microbial activity *in vitro* against eight human and animal enteric pathogens. Most of the isolated lactic acid bacteria showed their antimicrobial activity against the selected pathogens. The diameter of inhibition zones showed that, most of the isolates had antimicrobial activity. However, one particular isolate did not show inhibitory effect against *Pseudomonas aeruginosa*. Similarly, another isolate did not show antimicrobial activity against *Vibrio cholera* and *Salmonella typhi*. Two isolates did not show any antimicrobial activity against *Vibrio* cholera and *Micrococcus*, respectively. Rest of the isolates showed antimicrobial activity against all the pathogens. It was reported that *Lactobacillus reuteri* resides in human GI tract and convert glycerol into reuterin which have potent antimicrobial activity against broad spectrum gram positive and gram negative bacteria (Spinler et al., 2008).

In vitro antibacterial activity of selected strains belonging to probiotic genera, *Lactobacillus,* was investigated. In this study, agar spot test showed that all the selected strains was antagonistic against *Salmonella Typhimurium, Escherichia coli, Enterococcus faecalis, Staphylococcus aureus* and *Clostridium difficile* (Tejero-Sariñena et al., 2012) which indicates the similarity of the findings of the present study. In another study, antimicrobial activity was examined for different *Lactobacillus* strains (*L.reuteri, L. plantarum, L.mucosae, L. rossiae*) isolated from pig feaces against *Salmonella typhimurium ATCC 27164, E. coli, C. perfringens 22G, S. aureus ATCC 25923, B. megaterium F6, L. innocua DSM 20649* and *B. hyodysenteriae ATCC 27164*. The results showed that all of the *Lactobacillus* strains had an inhibitory effect against all of the pathogens except *B. hyodysenteriae ATCC 27164* (Angelis et al., 2006) which directly indicates the similarity of the findings ofpresent study. In another study, four Lactobacillus strains (*L. salivary CECT5713, L. gasseri CECT5714, L.gasseri CECT 5715 and L.fermentum CECT5716*) was isolated from human milk and investigated the antimicrobial potentiality against pathogenic bacteria (*Salmonella choleraesuis CECT4155, CECT409 and CECT443, Escherichiacoli CECT439 and E. coli O157:H7 server CECT4076, Staphylococcus aureus CECT4013 and CECT9776, Listeria monocytogenes Scott A and the spoilage strain Clostridium tyrobutyricum CECT4011*). All of the probiotic strains showed antagonistic effects against all of the pathogens but *L. salivary CECT5713* showed best antimicrobial activity and highest protective effect against *Salmonella choleraesuis CECT4155* (Olivares *et al*., 2005).

Effects of native Probiotic isolates on diarrheal induced mice

Regarding the effects of Probiotic in diarrheal induced mice, a four week mice trail using Probiotic yoghurt was conducted. It was observed that Probiotic yoghurt significantly reduced the percentage of diarrhea in experimental mice groups. All the treatment group showed significant increase in the latent period, reduced the total fecal output, frequency of defecation and fecal water content compared to the positive control group. Serum electrolytes (Na^+ and K^+) and total protein levels were also significantly higher in the Probiotic fed Treatment group compared to the positive control group.

Diarrhea is characterized by fecal urgency and inconsistence (WHO, 2012). Probiotic exhibiting anti-diarrheal activity may have a potential to retard the onset of diarrhea significantly as observed in the present study. According to WHO (2013) criteria, a decrease in consistency and an increase in frequency in bowel movements to greater than three stools per day generally describes diarrhea. Even though diarrhea has been defined over time by various scientific groups in different ways, emphasis is given on the consistency of stools rather than the numbers. Gidudu *et al*. (2011) defined diarrhea as: when the water percentages exceed 90%, whereas the water percent of stools normally 60-90%. However, the percentages of inhibition are mainly focused on the frequency and total stools as a good marker of anti-diarrheal activity.

Diarrhea is also presented as an increase of weight of defecations (Thoamas *et al*., 2003). Accordingly, the present study observed a dose dependent reduction in percentages of total fecal output ($p<0.001$) indicating the anti-diarrheal potential of the study models. This study is concordant with other studies in which *Lactobacillus sporogenes* significantly reduced the episodes ($p<0.002$) and shortened the duration ($p<0.001$) of acute rotavirus diarrhea in infants than placebo (Chandra, 2002).. Another double-blind randomized study investigated that *L. reuteri DSM 17938* significantly reduced watery diarrhea than placebo (2.1 ± 1.7 days vs. 3.3 ± 2.1 days; $P < 0.03$) as well as relapsed rate of diarrhea (15% vs. 42%; $P < 0.03$) (Francavilla *et al.*, 2012). Foster et al.(1980) reported that injection of *Lactobacillus* preparation in infected ilea loop significantly reduced the enterotoxin response against *E. coli* enterotoxin-induced diarrhea in the rabbit. Another study reported that, *Lactobacillus GG* reduced the duration of non-bloody diarrhea compared to the control (31% vs 75% at 48 hours) admitted for severe diarrhea and malnutrition (Raza *et al*., 1995) which explains the similarity of the findings of the present study.

Milk drink fermented with *Lactobacillus rhamnosus GG (LGG), Lactobacillus acidophilus La-5 and Bifidobacterium Bb-12* were administered in a treatment group in a double-blind manner. It was observed that, only 5.9% ADD developed patient had a significant reduction compared to the placebo group (27.6%), Wenus *et al*. (2008). Another randomized double-blind study was conducted to investigate the anti-diarrheal efficacy using a mixture of *Lactobacillus rhamnosus GG (LGG), Bifidobacterium lactis (Bb-12)* and *Lactobacillus acidophilus (La-5)* in case of ADD developed patient. The results showed no incident of severe diarrhea in the probiotic group compared to 6 cases in the placebo group though there was only one episode of minor diarrhea compared to 21 in the placebo group ($p<0.001$) (Fox et al., 2015).

Regarding serum electrolytes and other metabolites study of the present experiment, it was observed that Probiotic significantly increased the serum sodium and potassium levels. However, there were no effects on chloride levels. Serum sodium levels significantly ($p<0.001$) increased in the entire treatment group. Potassium levels was also increased in the treatment groups but the highest significant ($p<0.001$) results were obtained in high dose Probiotic fed treatment group. The total proteins levels were also significantly increased in the Probiotic fed treatment group.

Effects of native Probiotic isolates on allergic induced mice model

In the present experiment, three parameters viz. IgE level, eosinophil count and peripheral lymphocyte count were studied. It was observed that, the highest value of the IgE level in negative control group was 2.19 IU/ml and the lowest value of this group was 0.98 IU/ml. The highest value of the IgE level in positive control group was 87.2 IU/ml and the lowest value of this group was 67.3 IU/ml. The highest value of the IgE level in the standard group was 2.01 IU/ml and the lowest value of this group was 0.95 IU/ml. The highest value of the IgE level in the Probiotic fed treatment group 1 was 11.81 IU/ml whereas, the lowest value of this group was 7.99 IU/ml. The highest value of the IgE level in the Probiotic fed treatment group 2 was 7.05 IU/ml whereas, the lowest value of this group was 4.71 IU/ml. The highest value of the IgE level in the Probiotic fed treatment group 3 was 3.65 IU/ml whereas, the lowest value of this group was 2.65 IU/ml. The results revealed that, the Probiotic yoghurt had significant effect on the IgE level of the mice. There are some variations in the results of this current study with the result of similar studies and these variations may be due to different sample size, Probiotic content of the yoghurt and the process of Probiotic ingestion.

In the present study, the highest value of the eosinophil count in negative control group was 4% and the lowest value of this group was 2%. In the positive control group, the value of eosinophil count was same for all the samples (3%). The highest value of the eosinophil count in the standard group was 3% and the lowest value of this group was 1%. The highest value of the eosinophil count in the Probiotic fed treatment group 1 was 3% whereas, the lowest value of this group was 1%. The highest value of the eosinophil count in the Probiotic fed treatment group 2 was 3% whereas, the lowest value of this group was 2%. The highest value of the eosinophil count in the Probiotic fed treatment group 3 was 3% whereas, the lowest value of this group was 1%. It was found that, the Probiotic yoghurt had no significant effect on the eosinophil count of the mice used n this experiment. There are some variations in the results of this current study with the result of similar studies and these variations may be due to different sample size, different Probiotic content of the yoghurt, difference in the process of Probiotic ingestion and the difference in the process of blood sample handling.

In the present study, the highest value of the peripheral lymphocyte count in negative control group was 36% and the lowest value of this group was 30%. The highest value of the peripheral lymphocyte in positive control group was 26% and the lowest value of this group was 20%. The highest value of the peripheral lymphocyte count in the standard group was 25% and the lowest value of this group was 20%. The highest value of the peripheral lymphocyte count in the Probiotic fed treatment group 1was 37% whereas, the lowest value of this group was 30%. The highest value of the peripheral lymphocyte count in the Probiotic fed treatment group 2 was 36% whereas, the lowest value of this group was 25%. The highest value of the peripheral lymphocyte count in the Probiotic fed treatment group 3 was 36% whereas, the lowest value of this group was 32%. Here, it has been observed that, the Probiotic yoghurt had no significant effect on the eosinophil count of the mice used. There are some variations in the results of this current study with the result of similar studies and these variations may be due to different sample size, different Probiotic content of the yoghurt, difference in the process of Probiotic ingestion and the difference in the process of blood sample handling.

Effects of native Probiotic isolates as layer starter feed supplement

The aim of the present study was to develop Probiotic layer starter feed and to examine its effect as layer starter. Therefore, the Probiotic isolates were obtained from the gastrointestinal tract of poultry from two different Divisions viz. Dhaka and Kumilla. Then the isolates were identified according to their morphological and biochemical tests. Moreover, the presumptively Probiotic isolates were assayed for their potential Probiotic properties by several biochemical tests along with antimicrobial and antibiotic sensitivity test.

Isolation of presumptive Probiotic from gastrointestinal tract of chicken

In the present study, ten bacteria were isolated from the gastrointestinal tract of 15 and 30 days old chickens of Dhaka and Kumilla Divisions. More specifically, the bacteria were isolated from crop, small intestine and ceca using MRS agar media which is selective media for lactobacillus species. Similar work was done by Kabir et al. (2016) where eighty two isolates were isolated from the gastrointestinal (GI) tract of broiler chickens. In that experiment, the caecum and cloacal contents of broiler chickens were collected and cultured on MRS broth and agar. The isolates were identified as Lactobacillus based on morphological, physiological and biochemical tests which are specific for Lactobacillus genus (Kabir et al., 2016). In another study, 13 strains of Lactobacillus were isolated by Rajoka et al. in 2018 from poultry intestine using MRS media (Rajoka et al., 2018). Different studies aimed to identify the microbiota of the gastrointestinal tract of poultry pointed out the predominance of lactobacilli such as *Lactobacillus crispatus*, which was isolated from chicken crops and intestine (Beasley et al., 2004). Similarly Mojgani et al. (2007) screened total 250 chicken intestinal specimens by routine cultural, morphological and biochemical reactions and found 75 LAB isolates.

Identification and characterization of isolated bacteria

A total ten isolates were identified as Probiotic bacteria belonging to the *Lactobacillus* genus. The isolates were found gram positive, endospore negative, catalase negative, non-motile, had the ability to ferment 8 out of 10 sugars used for carbohydrate profile determination. The isolates were also resistance to inhibitory substances such as pH 2.2, 0.3% bile acid and 1-4% NaCl concentration. Therefore, it was assumed that all the ten isolates were identified as *Lactobacillus* species. Shokryazdan et al. in 2014 found the similar results with 42 isolates. He observed that the isolates were rod shaped, varying from short to long (1.1 to 5.7 µm long), and straight to crescent rods, arranged singly, in pairs or in short or long chains. In Gram's staining, the morphological characteristics of isolated Lactobacillus species exhibited Gram-positives, rods, arranged in single or chain or in palisade shaped, non-motile, non-spore forming bacteria under microscope (Shokryazdan et al., 2014) which was supported by other researchers (Felies et al., 2007; Belkacem, B. et al., 2009; Kim H. J. et al., 2011; Jothi et al., 2012). The isolates of the present study also showed catalase negative activity which was supported by the study of Shokryazdan et al. in 2014 in where 37 isolates were catalase negative among 42 isolates of chicken GI tract (Shokryazdan et al., 2014).

NaCL tolerance test

While sodium chloride is growth inhibitory to several other types of bacteria, the Probiotic organisms withstand high salt concentration in the human gut (Halder and Mandal, 2015). The isolates of the current study showed tolerance to 1%, 2% and 4% sodium chloride but could not survive well in 6% NaCl and showed no growth in 8% NaCl. Similarly, in a study of (Zinigul et al., 2016), it was found thatthe *Lactobacillus* strains had good growth up to 2-6% concentration in the culture medium, with the exception of *L. sakei*. The results also have the similarities with the findings of (Pundir et al., 2013), that the LAB isolates were tolerant to 1-6% NaCl. Bhardwaj et al. demonstrated that no growth even for a single *Lactobacillus* strain was noticed at the sodium chloride concentration of 6.5% (Sharma et al., 2016). Wang et al. (2016) established that increase in the salt concentrations above 6% resulted in decrease of bacterial density *L. plantarum* ATCC 14917 71.

Bile salt tolerance test

The ability to survive under high bile salt concentration and low pH are the important features for successful passage through the gastrointestinal tract (Mandal et al., 2015; Oh and Jung, 2015). In the present study, ten isolates had maximum growth in 0.3% bile salt after 24 hours of incubation. Similar result were found by Verdenelli et al., 2009 in which *Lactobacillus rhamnosus* IMC 501 and *Lactobacillus paracasei* IMC 502 were tested at 0.3% (m/v) bile concentration. The results of resistance against bile salt are supported by the findings of Gilliland & Speck, 1977 who reported that Lactobacilli which were isolated from animal intestines showed high tolerance to bile salts than species isolated from milk products. Similar results were found in another study conducted by Patel et al., 2004. In their study, *Lactobacillus reutrei* was isolated from human intestines and found highly resistant to 2.0% bile salt. A similar finding was reported by Koll et al., 2008 who found that all 67 Lactobacillus strains showed bile tolerance at 0.3%. Jin et al., 1998 also found that all 12 Lactobacillus strains were able to tolerate 0.3% of bile salt, while Jacobsen et al., 1999 reported that 41 of 42 tested Lactobacillus strains could tolerate bile at this concentration.

Growth at different pH

In the present study, most of the isolates survived well in pH 4, 5, 8 and 9.5 and six isolates survived in pH 3.5. Similar result was found by Shokryazdan et al., 2014 where 14 of the 26 isolates showed 95.6 to 107.0% growth at pH 3. Sahadeva et al., 2011 had found that Probiotic strains in their study, which included L. acidophilus, *L. casei, L. casei Shirota*, *Streptococcus thermophilus* and *Bifidobacterium*, could survive for 3 h at pH 1.5. Earlier, (Chan et al., 2005) also demonstrated that aciduric Lactobacillus strains such as *L. acidophilus* could not survive after 2 h of exposure to pH 2.

Antimicrobial activity test

All of the isolates of current study had antibacterial effect on enteropathogenic microorganisms viz *Salmonella typhi, Escherichia coli, Vibrio cholera, Streptococcus aureus* and *Helicobacter pylori*. The zone of inhibition varied from 15.73 mm to 24.42 mm for all isolates. The maximum inhibition zone was obtained from isolate no 2 for *E. coli* and that was 24.42 mm. similar result was found by Zinigul et al., 2016 where *L. casei*, *L. sakei* and *L. plantarum* inhibited growth of pathogenic bacteria, such *E. coli*, *S. aureus*, *Ser. marcescens*. Therefore, they were able to prevent the growth of other pathogenic microorganisms in gut system. Georgieva *et al*., 2015 also reported antagonistic effect of probiotic against *S. aureus*, *E.coli, B. cereus* and *C. albicans.* Garriga et al., 1998 reported that *Lactobacillus salivarius* was antagonistic against *Escherichia coli* and *Salmonella Enteritidis* which were found in gastrointestinal tracts of chicks. A number of lactobacilli isolates had the ability to inhibit the growth of salmonella spp and serotypes of *Escherichia coli* (Jin et al., 2000).

Antibiotic sensitivity test

One of the most important characteristics of Probiotic was their resistance against antibiotics particularly when used after antibiotic treatment. All the isolates of the present study were resistant to nalidixic acid, amoxicillin, tetracycline and azithromycin. However, isolates were susceptible to clindamycin and moderately susceptible to penicillin. These results were supported by many research findings indicating that lactic acid bacteria are resistant to commonly used principal antibiotics (Halami et al., 2000; Nawaz et al., 2011; Bobcek et al., 2011; Coppola et al., 2005). A contrasting result was found in case of tetracycline where *Lactobacillus i*solates were sensitive to gentamicin and tetracycline (Kabir et al., 2016). However, a similar result was found by Rajoka et al. in 2018 where isolates were resistant to tetracycline but were susceptible to amoxicillin which contradicts with the findings of present study. Although lactobacilli have acquired 'generally regarded as safe' status, presence of transferable antibiotic resistance may result in transmission of antibiotic resistance genes topathogenic bacteria in the poultry gut and the environment (Casado et al., 2014; Klose et al., 2014; Nawaz et al., 2011a; Thumu and Halami, 2012). Acquired resistance to erythromycin and ciprofloxacin has also been reported previously (Drago et al., 2011; Nawaz et al., 2011a; Thumu and Halami, 2012). Again, Nemcova et al. (1997) investigated the antibiotic susceptibility of 13 lactobacilli strains isolated from suckling piglets and found they were susceptible to many common feed additive antibiotics which contradicts with the results of the present study. Although it is clear that simultaneous application of susceptible proniotic with oral antibiotics is generally unreasonable, in case of microbial infection it might be possible to use these resistance Probiotic in combination with appropriate antibiotics (Marounec and Rada, 1995).

Effect of Probiotic feed on layer performance

In the present study, Treatment Group 1, supplemented with 50 gm/kg of Probiotic showed the highest growth rate, better feed conversion ratio and higher average daily gain of the chick but the feed intake was not affected much by the Probiotic. Though the Probiotic supplemented feed improved the performance characteristics of layer chicks but no significant change were observed between the Treatment Groups. A similar result was found by Juliana Bawah in 2014, where no significant difference were observed regarding initial weight, daily weight gain, daily feed intake and feed conversion ratio among the treatment groups. The findings are also supported by another study (Nahashon et al., 1994) in which no significant difference was observed between the hens that consumed diets with or without Probiotic. Samad et al. (2011) also obtained a non significant difference in feed intake and FCR between birds on basal and treatment diet when Probiotic was supplemented in the diet of birds. The current study contrasts to the findings of Fritts et al., (2000), Gil de los Santos *et al*., (2005) and Samad et al. (2011) who concluded that the feeding of Probiotic-supplemented diets to chicken increased the final body weight of chickens by 16, 5 and 7 per cent, respectively. Many studies have shown that Probiotic improved feed conversion ratios in layer chickens (Nahashon et al., 1994; Nahashon et al., 1996; Tortuero and Fernández, 1995). Probiotic improve feed conversion efficiency through several mechanisms including alteration in intestinal flora, enhancement of growth of nonpathogenic facultative anaerobic and gram positive bacteria forming lactic acid and hydrogen peroxide, suppression of growth of intestinal pathogens, and enhancement of digestion and utilization of nutrients (Yeo, 1997). Therefore, the major outcomes from using Probiotic include improvement in growth (Yeo, 1997), reduction in mortality (Kumprecht and Zobac, 1998), and improvement in feed conversion efficiency (Yeo, 1997).

Serum cholesterol and Serum calcium level

After three weeks of trial, the blood sample of chicks under different treatment groups was tested to analyze serum cholesterol and serum calcium level. Serum cholesterol level was found to be decreased at a significant level while serum calcium level was significantly increased in Probiotic supplemented groups. Similar result was found by Panda et al. in 2014 that the serum concentration of cholesterol decreased significantly due to feeding of Probiotic at either 100 or 150 $mgkg^{-1}$ diet. He also suggested that feeding of *L. sporogenes* had helped to assimilate more calcium which was evident by increased concentration of Ca in serum with Probiotic supplementation. A similar trend was also observed in their earlier studies (Panda et al., 2003). According to a study, no significant difference was observed in serum Ca levels except a significant decrease ($P < 0.05$) in total cholesterol (Mohan et al., 1995; Tortuero and Fernandez, 1995; Yesilbag and Colpan, 2006).

Effects of native Probiotic isolates as broiler starter feed supplement

A total of ten isolates were isolated and identified as Probiotic bacteria. Ten isolates were found gram positive, endospore negative, catalase negative, non-motile and were able to ferment eight sugars. Isolates showed tolerance to 1-4% NaCl, were able to grow low pH (pH 3.5), survived in artificial gastric juice at both low pH (pH 2.2) and favorable pH (pH 6.6) and were also able to survive and multiply in 0.05%, 0.15% and 0.3% bile salt. Isolated bacteria showed inhibitory activities against five pathogenic test microorganisms and exhibited different susceptibility patterns against eight tested antibiotics. The isolates showed significant difference in body weight, meat protein content, serum HDL level on the experimental broiler chicks. The isolates showed increase average daily gain and carcass yield. The isolates also showed decrease feed conversion ratio and mortality rate. The isolates exhibited decrease uric acid level in the experimental broilers. There were no significant differences in serum LDL level.

Carbohydrate/Sugar Fermentation Profiles

In the present study, sugar fermentation pattern of 10 isolates were determined using 10 sugars. All isolates were able to ferment D-Glucose, Maltose, Lactose, Sucrose, D-fructose, D-galactose, D-raffinose, D-mannose. However, all the isolate did not ferment D-sorbitol and D-xylose. The results have correlation with the study of Cenesz et al. (2008), which have similarly with the findings of Champagne et al. (2007). Thus, the present study results have the similarities with other investigators findings.

Sodium Chloride (NaCl) Tolerance Test

In the present study, the identified isolated Probiotic bacteria were tested for different concentration of NaCl (1-10%) and they showed excellent growth at 1-4% NaCl concentration, moderate growth at 6% NaCl concentration and no growth at 8% NaCl concentration. These test results were found similar to Bailey et al.(1994) who isolated Lactobacillus species from intestinal tract of indigenous Algerian chickens and were unable to grow at 6.5% NaCl. Devirgiliis et al. (2009) found *Lactobacillus fermentum* strain which were able to tolerate at 4% but not 8% of NaCl concentrations.

Growth at Different pH

In the present study, the tolerance of the isolated LAB's was determined by growing them at pH 3.5, 4.0, 5.0, 8.0 and 9.5 for 24 hours at 37 C. All the presumptively identified Probiotic isolates did grow at pH 3.5; however, all isolate failed to grow at pH 4.0 and 5.5. The test results of this study were similar with the findings of Smith (2013) who obtained growth at pH 3.7 and all isolate obtained from duodenum and caeca of chickens did grow at pH 3.5.

Gastric Juice Tolerance

In the present study, the survivor and multiplication of the isolates in gastric juice environment was observed from the absorbance data at 620 nm after 0, 2, 4 and 24 hours of incubations. The gastric juice resistance test results of the present study were found nearly similar with the results obtained by Jenke et al. (2011) who showed that the *Lactobacillus* strain isolated from chicken caecum had better tolerance to acid than those from the ilium; where most caecal *Lactobacillus* strains did survive even at a low pH of 2.0 for up to 2 h of incubation.

Bile Salt Tolerance

In the present study among 10 isolates, isolate no. 1 and 2 exhibited the highest resistance whereas isolate no. 2 showed maximum resistance against 0.05% and 0.15% bile but in case of 0.3% bile acid concentration, the resistance capacity of isolate no.1 was better after 24 hours of incubation. Isolate no. 8 showed the poor growth and multiplication and isolate no.9 and 10 exhibited slightly better tolerance then isolate no. 3 in bile salt. Isolate no. 6 showed better resistance than isolate no. 4 and 5. Isolate no. 7 had better resistance than isolate no. 8. In the present research, after 24 hours of incubation all 10 isolates at 0.05% and 0.15% concentrations of bile salt showed better result and less inhibition than 0.3% that agreed with the findings of Kotiranta et al. (2000).

Antimicrobial Activity Test

In the present study, against 5 enteric pathogens all the isolates showed different zone of inhibition. They showed inhibitory characteristics against them. Lilly et al. (1965) detected the antibacterial activity of LAB isolated from chicken intestinal specimens against E. coli and Salmonella (Serotypes Enteritidis, Pullorum and Typhimutium) in which the highest inhibitory activity was found against Salmonella pullorum (20.3 mm). Marshall et al. (2011) tested antimicrobial activity of probiotic organisms isolated from gastrointestinal tracts of broiler chicken against E. coli where the maximum zone diameter wad 26 mm. The present experimental result matches all these researchers' findings.

Antibiotic Susceptibility

In the present research, 10 identified LAB isolates were assayed for their susceptibility to eight antibiotics such as amoxicillin (30 µg), penicillin (6 µg), erythromycin (15 µg), clindamycin (2 µg), nalidixic acid (30 µg), tetracycline (30 µg), cephalexin (30 µg) and cefixime (10 µg) using the disc diffusion method. All the isolate showed resistance against cefixime, nalidixic acid and cephalexin. This results has the significant relation to the findings of other works, such as:Lactobacilli are usually susceptible to chloramphenicol, erythromycin and clindamycin (Ongena et al., 2008). *Lactobacilli* are generally susceptible to antibiotics that inhibit the synthesis of protein such as erythromycin and tetracycline (Payne et al., 2007; Essid et al., 2009).

Mean body weight (MBW)

At the end of the trial, all the groups showed significant level of bodyweight differences (p<0.001) when compared with the control. There was significant differences (p<0.001) between the T3 and T4 groups when compared with the control group. T1 and T2 showed significant differences (p<0.001) when compared to the control group as well as when compared to the T3 and T4 groups. **B**oth the T3 and T4 groups showed significant increase (p<0.001) in mean body weight compared to control, T1 and T2 groups. These results have the significant relation to the findings of other work such as: Skinner et al. (2010) reported no significant (P≤ 0.05) difference in dressed weight and blood parameters in broilers after supplementation of commercial Probiotic preparation. Sabiha et al. (2005) studied the effects of different levels (0.025% and 0.05%) of Probiotic (*Lactobacillus acidophilus*, *Streptococcus faecium* and Yeasacc 1026) supplementation on the performance of broiler chicken and observed statistically (P≤ 0.05) higher body weight gain up to 6 weeks of age in 0.025 % Probiotic supplemented birds. Cao et al. (2013) found that *E. faecium* (HJEF005) at 109 cfu/kg of feed improved growth rate in male Cobb broilers challenged with *E. coli.* Mountzouris et al. (2010) observed that diets containing 108 cfu Probiotic/kg increased body weight of broilers significantly compared to control group. Between days 15-28, feed intake of broilers in Probiotic group was significantly (P<0.05) higher than control group.

Feed intake (g/bird/day)

All treatment group feed intake was increased from week 1. When compared control group with other 4 groups, it was observed that other groups had increased feed intake. Therefore, it can be concluded that Probiotic have no significant effect on the feed intake of chicks and which can be compared to antibiotic group or Antibiotic Growth Promoter (AGP) group. In case of feed intake, Probiotic supposed to show decreased feed intake level than control group. This result has the significant relation to the findings of other work. In many cases the improvement in growth rate in the Probiotic treated birds was associated with increased feed intake (Abdel-Raheem, Abd-Allah and Hassanein, 2012; Landy and Kavyani, 2013; Lei et al., 2015) and improved feed use efficiency (Mountzouris et al., 2010; Shim et al., 2012; Zhang and Kim, 2014) compared with untreated birds.

Therefore, increased digestibility of feed resulting in improved feed efficiency and could be one of mode of actions for improved growth rate. Also, the differences in performance between treated and untreated birds may be due to a change in microbial populations in the GIT resulting increased production of SCFA and immuno-modulation (Zhao et al., 2013).

Carcass yield

In this present study, Probiotic showed significant effect on the carcass yield of chicks and which can be compared to antibiotic group or Antibiotic Growth Promoter (AGP) group. Therefore, it can be concluded that Probiotic has shown better result than AGP and of course from control group. Besides, the higher dose rate have positive effect on the carcass yield because when compared between T3 and T4 groups,T4 group had been shown high performance than T3 group and it was T4 group which has shown higher performance over antibiotic (T1 and T2) groups. This result has the significant relation to the findings of other worker. Moser et al. (2001) stated in their study that the scores for the sensory attributes of the meat balls i.e. appearance, texture, juiciness and overall acceptability were significantly (P≤ 0.05) higher and those for flavor were lower in the Probiotic-Lacto-Sacc fed treatment. Pelicano et al. (2003) reported that significant(P≤ 0.05) improvement in meat flavor fed with Probiotic apart from this studies conducted on effect of Probiotic on meat characteristics of other species of animals also revealed contradicting results on sensory characteristics of meat. In a study by Ceslovas et al. (2005) it was observed that Probiotic supplementation significantly (P≤ 0.05) increased the meat tenderness and meat quality. Most of the carcass characteristics are directly proportional to the increased body weight at the time of slaughter. Anna et al. (2005) observed no significant ($P \leq 0.05$) difference in carcass % between Probiotic treated and untreated treatments on the sensory parameter basis.

Serum HDL and LDL level

In the present research work, in case of HDL level, there was significant differences ($p<0.05$) among all the groups when compared with the control group. T3 and T4 groups showed significant increase ($p<0.05$) in HDL level when compared with the control group as well as with the T1 group and T2 group. In case of LDL level, there was no significant difference among all the groups when compared with the control group. T3 and T4 groups had no significant decrease in LDL level when compared with the control group as well as with the T1 group and T2 group. Not so much work has been conducted on this topic so far.

Mortality rate

In this present study, when compared the control group with other 4 groups, mortality rate has been decreased to 0% in T3 and T4 groups (Probiotic groups). Therefore, Probiotic group showed decreased mortality rate when compared to the control and antibiotics groups. This result has the significant relation to the findings of other workers. Ohya et al. (2000) reported zero mortality in broilers fed *Lactobacillus acidophilus* and *L. salvarius* and 12.7% mortality in non-supplemented control group, mostly due to bacterial enteropathogens. Upendra and Yatiraj (2002) recorded significant ($P \leq 0.05$) reduction (54.25%) in chick mortality when chicks were fed diets supplemented with Lacto-Sacc. Gupta (2004) concluded that Probiotic supplementation decreased mortality percentage in broilers.

Effects of native Probiotic isolates as duck starter feed supplement

In the present study, a total ten isolates were identified as Probiotic bacteria belonging to the *Lactobacillus* genus. The isolates were found gram positive, endospore negative, catalase negative, non-motile, had the ability to ferment 8 out of 10 sugars used for carbohydrate profile determination. The isolated bacteria showed antimicrobial activity against five enteric pathogens and showed antibiotic resistance to nalidixic acid and tetracycline. The isolates showed significant increase in body weight, total protein level, albumin level in the experimental ducklings used for *in vivo* trial purpose. The isolates also showed increase in feed intake amount, feed conversion ratio, globulin level and albumin/globulin ratio. They did exhibit decrease in uric acid level in the experimental ducklings.

Bacterial characterization

In the present study, at first colony morphology of the isolates was obseved and then different *in vitro* tests were carried out for the potentiality of the isolates as probiotics. Initial isolation and identification were based on the morphological appearance and on the catalase test. After performing some preliminary tests (Gram staining and catalase), a total of 10 isolates were selected for further identification. It was found that the 10 isolates were cocci and gram-positive, catalase-negative rods. The catalase test is one of the most useful diagnostic tests for the recognition of bacteria due to its simplicity. During the catalase test, no bubbles were observed indicating that the isolated bacteria are catalase negative and could not mediate the decomposition of hydrogen peroxide (H_2O_2) to produce carbon dioxide (O_2). All the isolates were also characterized by biochemical and physiological tests. In the *in vitro* bacteriological tests, all potential probiotic isolates were endospore negative, non-motile, catalase negative, gram positive and rod or coccoid shaped. These findings were similar to that of the findings of the research done by (Noohi et al., 2014).

Sugar fermentation profile

The ability of LAB isolates to ferment oligosaccharides is one of the desirable probiotic characteristics because the mono-saccharin that exists in the gastrointestinal tract influences the life of microorganisms in the intestine (Kaplan & Hutkins, 2000). The ability of the isolates to ferment carbohydrates has been demonstrated by the discoloration of the red basal medium in a yellow color. It was observed that, not all carbohydrates could be fermented from selected isolates. In the present study, all 10 isolate were unable to ferment the D-xylose and D-sorbitol. The sugars used in this test were D-Glucose, D-Sorbitol, Maltose, Lactose, Sucrose, D-Fructose, D-Xylose, D-Galactose, D-Raffinose and D-Mannose. This fermentation pattern of the potential probiotic isolates were similar to the findings of the study of Karna et al. (2007), Erkus (2007) and Belkacem et al. (2009). The incompatibility in two sugars may be due to the involvement of some factors such as the availability of sufficient D-xylose and D-sorbitol as well as may be due to the lack of ability of the enzyme produced by the isolates to decompose the sugars in the basal medium.

Growth on different temparature

In the digestive tract of poultry, the temparature is 42°C (Dawson & Whittow, 2000). Therefore, the isolates that will be able to grow at that temperature should be selected for poultry feed development. The test to examine the influence of temperature was aimed at understanding the type of bacteria, belonging to mesophilic or thermophilic Groups. The results of the present study indicated that only one isolate was not able to grow at 45°C. The findings of the temperature survivality test was in accordance with a study that concluded from the results of 24 h observation, all 17 isolates from poultry GIT can grow at 25°, 37°, and 45° C (Powthong & Suntornthiticharoen, 2013). In the present study most of the isolates had high viability at high temparature condition.

Optimal growth at different pH

Probiotic bacteria do show resistance to different acidic conditions (Fontana et al., 2013). In the present study, pH 3.5, 4.0, 5.0, 8.0 and 9.0 were maintained in the culture media to obtain the growth of the isolates at different pH . They were not able to multiply at pH 4.0 and below.The tests results were similar with the findings of a research where all isolates from duodenum and ceca of chicken grew but could not survive at pH 3.5 for 48h (Sangsoponjit & Soytong, 2013). Another similar study was conducted with 13 strains of *Lactobacillus plantarum*, among the 7 strains showed a low tolerance against low pH (Cebeci & Gürakan, 2003).

NaCl tolerance test

NaCl is an inhibitory substance which may inhibit growth of certain types of bacteria. In the present study, all of the isolates showed tolerance against 1%, 2% and 4% NaCl concentrations after 24hours of time. The results obsereved in a study by Pancheniak and Soccol (2005) which summarized that all the isolated strains were able to tolerate 1-6% NaCl which correlates the findings of the present study.

Bile tolerance test

In assessing the potential use of lactic bacteria as an effective Probiotic, it is generally considered necessary to evaluate their ability to resist the effects of bile salts and acid. In the present study, isolated bacteria were able to survive in 0.05%, 0.15% and 0.3% inhibitory substance; bile acids as well as they were also able to multiply in above mentioned concentrations of bile acid. After 24 hours of incubation, all ten isolates showed better result and less inhibition at 0.05% and 0.15% concentrations of bile salt than 0.3%, which was similar to the findings of Walker (2000).

Antimicrobial activity test

The inhibitory properties of LAB due to the accumulation of primary metabolites, including lactic acid and acetic acid, ethanol and diacetyl carbon dioxide. Many of these metabolites are antimicrobial compounds such as formic acid and benzoic acid, hydrogen peroxide, siderophore, reuterine, acetoin and bacteriocins (Delgado, 2001; Helander et al., 1997).

In the present study, five different pathogenic bacteria viz. *Salmonella typhi, Salmonella para-typhi, Escherichia coli, Vibrio cholera* and *Streptococcus aureus* were used. The diameter of inhibition zones showed that, most of the isolates have antimicrobial activity. In the *in vitro* antibacterial activity of selected strains belonging to probiotic genera, *Lactobacillus* was investigated. In a study, agar spot test showed all the selected strains were antagonistic against *Salmonella Typhimurium, Escherichia coli, Enterococcus faecalis, Staphylococcus aureus* and *Clostridium difficile* (Tejero-Sariñena et al., 2012) which indicates the similarity of the findings of the present study. Another study summarized that most isolated LABs showed a large zone of inhibition for *Salmonella para-typhi, Escherichia coli* and *Shigella flexneri,* respectively (Powthong & Suntornthiticharoen, 2013) which is also similar to the findings of the present study.

Antibiotic susceptibility test

Now a days in a country like Bangladesh, use of antibiotic is a common practice in poultry production. Therefore, if the objective is to provide probiotic at the time of antibiotic treatment in poultry feed, then antibiotic resistance probiotics can be provided. In the present study, all the potential LAB isolates were resistant to tetracycline and nalidixic acid. Isolate 5, 6 and 10 were resistant to cefuroxime. Isolate no 1, 2, 3, 4, 9 and 10 were medium susceptible to clindamycin. Isolate no 1, 2, 3 and 8 were resistant to azithromycin. Isolate no. 4, 5, 6 and 10 were resistant to clavulanic acid. Isolate no 4 was resistant to amoxicillin but medium susceptible to penicillin whereas isolate no 5 and 6 were resistant to both amoxicillin and penicillin. This variation in antibiotic sensitivity pattern is similar to the findings of a study by Toomey et al. (2009) who reported that resistance *among L actobacilli* isolates appeared to vary between species.

In the present study, all the isolates were resistant to tetracycline which correlates the findings of Roberts (2005) and Korhonnen et al., (2008) who showed that resistance to tetracycline has been observed more often among *Lactobacilli*. The susceptibility pattern of the isolates in this study to azithromycin and clindamycin have similarities with the findings of Powthong and Suntornthiticharoen (2013) and Majhenic and Matijasic (2001). Almost all the present study isolates were sussceptible to penicillin and amoxicillin which have similarities with the findings of Danielsen and Wind (2003) and Bakari et al. (2011) who reported that generally *Lactobacilli* seem to be sensitive to these two types of antibiotics. In the present experiment, isolate no 1, 2, 3, 7, 8 and 9 were susceptible to clavulanic acid and cefuroxime which were similar to the findings of Bakari et al. (2011). Resistance to clavulanic acid, cefuroxime, penicillin and amoxicillin of some isolates is not intrinsic to *Lactobacilli*; therefore, it might be important to study for the presence of known genes providing such resistance in these strains.

Performance characteristics of the experimental ducklings

In the present study, the body weight was measured in every week and it was observed that the mean body weight of every Group was increased. However, the highest mean body weight was observed in Treatment Group 4 at end of the every week. The lowest mean body weight was in Control Group at the end of each week. After 3-weeks period of treatment, the body weight was significantly increased in Treatment Groups. Probiotic fed Treatment Group 3 and Treatment Group 4 ducklings had significantly higher body weight in every week which has the similarities with the findings of Wang and Zhou (2007) and Weis et al. (2010) who reported that the addition of Probiotic ducklings' diet significantly improved the increase in body weight compared to the Control Group. At the end of the experimental trial, Probiotic fed T4 and T3 Groups had the highest feed conversion ratio compared to the Control Group. T1 and T2 Groups results had the similarities with the findings of studies conducted by Djouvinov et al. (2005) and Talebi et al. (2008) who reported that the addition of Probiotic for meat production significantly improves the feed conversion rate.

Carcass status of the experimental ducklings

In the present study, it was observed that there were slight increase in all the carcass characteristics in T3 and T4 Groups compared to the other three Groups, where T3 and T4 were Probiotic fed Groups. The carcass characteristics studied were the dressing % and different internal organ percentage. The findings have similarities with the findings of Kumar et al. (2003) and Hassan et al. (2015) who reported that there were significant improvements in carcass characteristics of the Probiotic fed ducks. In the present study, the improvement in carcass characteristics were not that significant which might be due to the number of birds in each Group, geographic location or the characteristics of the potential native Probiotic isolates.

Effects of isolated Probiotic on serum metabolites of the experimental ducklings

At the end of the trial, blood samples were obtained from the experimental ducklings and tested for total protein, albumin, globulin levels and albumin globulin ratio, HDL and LDL levels. There was significant increase in total protein and albumin levels in the Probiotic fed Treatment Groups compared to the Control and antibiotic Treatment Groups. These findings have the similarities with the findings of studies conducted by Panda et al. (2006) and Hassan et al. (2015) who reported that the concentration of serum protein and albumin level increased significantly due to the administration of Probiotic supplements. In case of globulin level and albumin/globulin ratio (the mean value), it was obvious that Probiotic supplemented Groups had higher globulin level and albumin/globulin ratio but the differences were not significant. There was significant decrease in uric acid level in the Probiotic supplemented Groups compared to the Control and antibiotic fed Groups. These findings have the similarities with the findings of Hassan et al. (2015) who reported that there were significant differences in serum albumin, globulin, albumin/globulin ratio and uric acid level between the Control Group and other Treatment Groups. The low level of differences among the Probiotic fed Treatment Groups and other experimental Groups in case of globulin and albumin/globulin ratio might be due to the differences in the amount of antibiotic feed, composition of the diet, age of the poultry, race, geographical location and section of the intestinal tract such as small intestine, ileum and ceca that are responsible for influencing the composition of normal intestinal bacterial content in particular *Lactobacillus* species. In case of serum cholesterol, there was significant increase in HDL value and significant decrease in LDL value in Probiotic fed Treatment Groups compared to that of the Control Group and Antibiotic Treatment Groups in the present study.

12. Research highlight/findings:

- Forty two native probiotic isolates (presumptive *Lactobacillus* spp.) were obtained from yogurt samples of seven Divisions of the country (six from each Division).
- Thirty native probiotic isolates (presumptive *Lactobacillus* spp.) were obtained from gastrointestinal tracts of poultry of five Divisions of the country (six from each Division).
- Probiotic yogurt was prepared using the probiotic bacteria (5% v/v liquid culture) isolated from yogurt samples
- Starter poultry feeds for layer, broiler and ducklings were prepared supplemented with 40-50 ml/kg feed containing 2-4x10^9 cfu and 8.8-9.9x10^9 cfu respectively of probiotic bacteria isolated from the gut of poultry.
- Native probiotics isolates showed significant anti-hypercholesterolemic and anti-diabetic effects on mice and rat, respectively.
- The isolated bacteria showed antimicrobial activity against five enteric pathogens and showed antibiotic resistance to several antibiotic drugs. The isolates also showed significant anti-diarrheal and anti-allergic effects on mice.
- Isolated probiotics showed significant effects as layer starter feed supplement on increasing body weight gain, reducing serum cholesterol (LDL) level and increasing serum calcium level.
- Isolated probiotics showed significant effects as broiler starter feed supplement on body weight gain, meat protein content, serum HDL level, increasing average daily gain, carcass yield, decreasing uric acid level and decreasing mortality rate.
- The poultry isolates showed significant increase in body weight gain, total protein and albumin content in the experimental ducklings. The isolates also showed increased feed intake, feed conversion ratio, globulin level and albumin/globulin ratio. On the other hand, the uric acid level was significantly decreased.

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	a) Laptop b) Scanner c) Laser Printer d) USP (Offline)	100000.00	a. Laptop b. Scanner c. Laser Printer d.USP (Offline)	99500.00	
(b) Lab &field equipment	1. Polymerase Chain Reaction (PCR) Machine 2. laboratory Tools/Equipments 3. Chemicals reagents and kits	500000.00 106000.00 352265.00	1. Polymerase Chain Reaction (PCR) Machine 2. laboratory Tools/Equipments 3. Chemicals reagents and kits	499500.00 105150.00 351851.00	
(c) Other capital items	Furniture	44000.00	Furniture	39600.00	

2. Establishment/renovation facilities: N/A

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

3. Training/study tour/ seminar/workshop/conference organized: N/A

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Femal e	Total		
(a) Training					
(b) Workshop					

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	441735.00	418550.00	413411.00	5139.00	93.59	-
B. Field research/lab expenses and supplies	845050.00	806455.00	791832.00	14623.00	93.70	
C. Operating expenses	330000.00	269800.00	269307.00	493.00	81.61	-
D. Vehicle hire and fuel, oil & maintenance	-	-	-	-	-	-
E. Training/workshop/seminar etc.	-	-	-	-	-	-
F. Publications and printing	-	-	-	-	-	-
G. Miscellaneous	55065.00	56050.00	55000.00	1050.00	99.88	-
H. Capital expenses	748150.00	727187.00	743750.00	-16563	99.41	-

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	**Major technical activities performed in respect of the set objectives**	**Output (i.e. product obtained, visible, measurable)**	**Outcome (short term effect of the research)**
To isolate, characterize and properties studies of native probiotic isolates.	Collection of yogurt and poultry (gastrointestinal tracts) samples from seven Divisions of Bangladesh. Isolation and identification of presumptive probiotic bacteria. Antimicrobial,	Forty two native probiotic isolates were obtained from yogurt samples. Thirty native probiotic isolates were obtained from gastrointestinal tracts of poultry.	The native probiotic isolates can be preserved as seed stock for future application in food, feed and new drug discovery. The isolates can also be used for the production of different probiotic

	physiological and biochemical properties study of the isolated probiotic bacteria.		food products for human and feed products for poultry and other mammals.
To develop probiotic food/feed products for human/poultry and to study the effects of native probiotic isolates on mice/poultry through *in vivo* trials.	Development of probiotic yogurt. Development of probiotic based broiler starter, layer starter and duckling starter feeds. Trials to determine the effects of probiotic yogurt on mice and rats. Trials to determine the effects of probiotic feed on layer, broiler and ducklings.	Probiotic yogurt using isolated probiotic bacteria was prepared in the laboratory. Probiotic feeds (broiler starter, layer starter and duckling starter) using isolated probiotic bacteria were prepared in the laboratory. Native probiotics isolates showed significant anti-hypercholesterolemic, anti-diabetic, antimicrobial, anti-diarrheal and anti-allergic effects on Swiss-Albino mice and rats. Feeds supplemented with native probiotic isolates showed significant effects on layer birds in relation to increasing body weight gain, reducing serum cholesterol level and increasing serum calcium level. Feeds supplemented with native probiotic isolates showed significant effects on broiler in relation to body weight gain, meat protein content, serum HDL level, increasing average daily gain, carcass yield, decreasing uric acid level and mortality rate. Feeds supplemented with native probiotic isolates showed significant increase in body weight gain, total protein and albumin content in the experimental ducklings. The isolates also showed increased feed intake, feed conversion ratio, globulin level and albumin/globulin ratio. On the other hand, the uric acid level was significantly decreased.	Probiotic based food products can exert several health beneficial effects viz. Anti-hypercholesterolemic, anti-diabetic, anti-microbial, anti-diarrheal and anti-allergic effects on human. As poultry feed supplements/feed additives, isolated probiotic bacteria would be able to provide certain health beneficial effects including increased daily weight gain, carcass yield, serum calcium, meat protein content, feed intake, feed conversion ratio and dressing percentage. They would also be able to decrease uric acid, serum cholesterol, LDL levels and mortality rate of poultry.

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.	-	-	-
Journal publications		3	1. A study on the anti-allergic effects of the native probiotic isolates on $\beta - histine$ hydrochloride induced mice model. *B. Journal of Livestock Research,* Special Volume 21-25:36-44. **2.** Isolation and biochemical characterization of probiotic bacteria obtained from yoghurt samples of Rajshahi and Chittagong Divisions of Bangladesh and their antimicrobial activity against enteric pathogens. *B. Journal of Livestock Research,* Special Volume 21-25:142-152. **3.** Antidiarrheal efficacy of Probiotic bacteria in castor oil induced diarrheal mice. *Preventive Nutrition and Food Science, 23(4):294-300.*
Information development	-	-	-
Other publications, if any (Thesis)	-	7	a) Characterization of native Probiotic isolates and their effects in diarrheal induced mice. b) Biochemical characterization of native Probiotics and their effects on interperitoneally prednisone induced hypercholesterolemic mice. c) A study on characterization of native Probiotics and their effect on streptozotocin induced type-2 diabetic model rats. d) Biochemical characterization of Probiotic isolates and their effects against selective enteric pathogen in mice. e) Biochemical characterization of native probiotic isolates from gastrointestinal tract of chicken and their effects as layer starter feed supplement. f) Study on biochemical properties of native isolated probiotics and their effects as feed supplement on broiler chicks. g) Biochemical property analysis of native probiotic isolates from selective poultry and their effects as duck starter feed supplement.

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity):

a) Probiotic yogurt using native probiotic isolates has been developed for human consumption.
b) Broiler starter, layer starter and duckling starter feeds have been developed using native probiotic isolates.

ii. Generation of new knowledge that help in developing more technology in future:

Native probiotic isolates from yogurt would help in developing more probiotic food products for human consumption and the probiotic bacteria isolated from poultry gut would help in developing different types of probiotic based feeds for animals to substitute antibiotic feed additives.

iii. Technology transferred that help increased agricultural productivity and farmers' income:

The technologies developed through this project need further verification before transferring to the appropriated stakeholders/farmers.

iv. Policy Support

Government policy makers may consider taking bigger projects on the development of probiotic based feeds for animals to substitute antibiotic feed additives.

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring description & output of consultation meeting, monitoring workshops/seminars etc.):

The findings of the research and thesis were presented in the Thesis Defense Session in front of all the Academics of Biotechnology and Genetic Engineering Discipline of Khulna University and concerned Expert Members and their suggestions/advice were considered as per necessary.

ii) Field Monitoring (time& No. of visit, Team visit and output):

Monitoring team	Date(s) of visit	Total visit till date (No.)	Output
Technical Division/ Unit, BARC	20-02-2018	01	Did appreciate the project activities and advised to continue the research work as per schedule of the project.
PIU-BARC, NATP-2	08-04-2018	01	Did appreciate and advised to continue the research activities as per schedule of the project.

H . Lesson Learned

Collaboration with respective food and pharmaceutical industries would be necessary to develop probiotic based human foods and feeds for poultry.

I. Challenges

i) Time constraints and allocation of insufficient fund in order to execute the research properly.

ii) Lack of providing sophisticated instruments especially Lyophilizer and Sequencer

Signature of the Principal Investigator
Date …………………………
Seal

Counter signature of the Head of the organization/authorized representative
Date ………………………….
Seal