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ISOLATION AND IDENTIFICATION OF BACTERIA FROM THE INTESTINE OF CHICKEN

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ABSTRACT

Chicken being white meat is superior to red meat for a number of reasons, such as its health benefits due to its low fat and cholesterol content. In the gastrointestinal tracts of chickens, there is a high concentration of complex microbial populations, mostly composed of bacteria but also includes fungus, viruses, bacterial, protozoa and microorganisms. The proposed study was conducted for Isolation and identification of bacteria from the intestine of chicken. For this purpose, ten samples of chicken's intestine were collected and samples were divided into three experimental groups such as Control, T1 and T2. Each group contained ten specimens. The control group showed less numbers of colonies while T1 and T2 showed high colony count. Proper disinfection was maintained. During the whole trial. The bacterial colonies were counted from the both control and experimental groups T1 and T2 as control was outside while T1 and T2 were kept inside the incubator for 24 hours. T3 demonstrated the highest colony count as compared to T1 and control group. The minimum number of colonies observed on control group samples was 33 x 10 CFU/ml and maximum number of colonies were observed was 330 x 10 CFU/mL in control group. The number of colonies was observed in experimental group 1 was 180 x 10 CFU/mL and maximum number of colonies was 10 CFU/mL. In the experimental group 2 the minimum number 218 x 10 CFU/mL and maximum colonies were 510 x 10 CFU/mL. The data was obtained and subjected to statistical analysis by applying one-way ANOVA that showed highly significant differences as the p-value was found to be equal to 0.01 ($p<0.05$). Hence, after performing the following experiment, we isolated and identified the different bacterial species from intestine of *Gallus gallus domesticus*.

INTRODUCTION

The word "micro biota" commonly refers to the microbial population, which includes microorganisms that are harmful, commensal and symbiotic (Sender *et al*., 2016). The entire genotype of these microorganisms is referred to as the micro biome. Any microorganism that is composed of both recipient and bacterial parts is commonly known by the term "supraorganism" (Turnbaugh *et al*., 2007).

Chicken being white meat is superior to red meat for a number of reasons, such as its health benefits due to its low fat and cholesterol content (Liu *et al*., 2012). A few rapidly expanding commercial broilers are essential for providing the world's population with the necessary quantity of chicken meat. Meat and its derivatives are more likely to be consumed to persist if the meat is tasty, healthy, and secure for the customers. It was long believed that broiler chicken, a concentrated source of nutrition, was essential for optimal human development and growth (Higgs, 2000).

The digestive system (GIT) is an essential component of poultry health because it serves as initial line of defenses against outside infections and facilitates nutrition absorption. Broiler chicken's gastrointestinal tract (GIT) are home to micro biomes, or bacteria communities that are crucial to their development and growth, including the synthesis of small chain fatty acids, which are an excellent source of energy (Dunkley *et al*., 2007). The intestinal tract of broiler chicken has inhabitants by different microorganisms like Gram-negative bacteria and Gram-positive bacteria, methylogenic bacteria (Saengkerdsub *et al*., 2007), fungi, and viruses (Qu *et al*., 2008).

The gastrointestinal micro biome makeup represents the interaction of the host's DNA, immune response and metabolism with the microbial inhabitants as well as external variables (Yeoman *et al*., 2011). As soon as the chickens hatch, environmental germs start colonizing their guts with bacteria. The environment, dietary variety, clinical circumstances, antibiotic medication, and other factors all affect the composition of chicken intestinal microbiota. These gut microbes combined genomes make up a microbiome that is far larger than the genome of the host. Moreover, bacteria and the lining of the digestive tract can interact directly, changing the immunological and physiological condition of the bird (Danzeisen *et al*., 2011). The taxa that make up the gut microbiota include fungi, bacteria, and archaea (Permin *et al*., 2006).

A single chicken's gut microbiota may include between two hundred and three hundred and fifty distinct bacterial species, even though six hundred and forty different bacterial species have so far been found in the chicken's digestive system (Apajalahti *et al*., 2004). In recent years, it has become clear that these wide variety of bacteria are not just harmless bystanders but rather play a variety of roles in the host, ranging from metabolism to immunological development (Oakley *et al*., 2014). With this realization came the desire to pinpoint helpful bacteria and control their population to enhance their effects. The Enterobacteriaceae, Lactobacillus, and Bifidobacterium families made up the microbial community. Over time, these groups dissipate, resulting in a micro biota that is primarily made up of Clostridiales (Zhu *et al*., 2003). Lactobacilli bacteria were found in the caecum and crop of chicks. In contrast, lactic acid bacteria (LAB) quickly develop in the duodenum, ileum, and caecum (Gong *et al*., 2002). Experiments utilizing both culture-dependent and culture-independent techniques have shown that the intestinal bacterial community of broilers differs with age (Zhu *et al*., 2002). According to earlier research, the normal bacterial population of the small intestine and duodenum appears to be developed within the first two weeks of life (Wise *et al*., 2007).

Only small intestine and not the caeca appear to undergo a steady drop in streptococci and enterococci. The intestinal micro biota of chickens is complicated, as it is in all homoeothermic species, and each intestinal compartment has a physicochemical microenvironment that varies greatly. The presence of adequate growth substrates, the current pH and redox potential, as well as the host's antibacterial secretions in a particular intestine segment, are the key variables that affect the microbes' fitness and colonization efficiency (Lu *et al*., 2003).

When bacteria progress through the gastrointestinal tract, there are fewer simple substrates for their development available. Because of this, lower intestinal bacteria frequently available for utilizing feed components that the host's endogenous digestive system cannot break down, such as resistant starch, resistant protein, and non-starch polysaccharides. Low pH in inhibiting the growth of many other species is the proximal gastrointestinal tract (crop, proventriculus, and gizzard) (Morgan *et al*., 2014).

The horizontal propagation of genetic components that are mobile like transposons and plasmids has the potential to introduce genes associated with antimicrobial resistance (ARGs) into the gut micro biota. By lowering the number of dangerous bacteria, promoting gastrointestinal absorption of nutrients, and ultimately raising growth indices, antibiotics can change the gut microbiome of chickens (Islam *et al*., 2019).

For the nutrition and defense of animal, these bacteria produce short-chain fatty acids like propionic acids, butyric, acetic acid and organic acids like lactic acid, and antimicrobial compounds like bacteriocins. Vitamins produced by these bacteria include vitamin K and vitamin B groups. They also reduce triglyceride levels and stimulate immunological responses that are not harmful. Contrarily, the gastrointestinal (GI) microbiome *Salmonella* and *Campylobacter* can be the source of bacterial diseases which can spread to humans or serve as a hotspot for the spread of antibiotic resistance, raising significant issues for public health (Hegde *et al*., 2016).

Commercial products have been created for distribution to 1-day-old chicks at chicken farms in order to better colonize their intestines with a bacteria. Adult birds are used to make the products that are most effective at competitively excluding diseases; but, because of their generally ill-defined microbial composition, their commercial accessibility in some nations is constrained (Ferreira *et al*., 2003).

Goblet cells are found within the small intestine's epithelium, with the jejunum serving as the primary site for nutrition absorption and processing. One of the earliest methods for identifying goblet cells is mucicarmine. Mucicarmine staining, albeit less frequently used for birds, is a useful method for assessing goblet cells. The AB-PAS staining approach for detecting goblet cells is a dual-purpose technique that stains both neutrality and acidic mucins (Contreras-Ruiz *et al*., 2013).

MATHODOLOGY

Experimental station

The study was conducted to estimate "Isolation and identification of bacteria from the intestine of chicken *(Gallus gallus domesticus*)".

Experimental species

Broiler Chicken *(Gallus gallus domesticus)*

Apparatus

Flasks, cotton plugs, micropipette tips (100µl, 1000µl), flame, match box, autoclave, compound microscope, incubator, measuring cylinders, aluminium foil paper, beakers, cover slips, dissecting box, dropper, eppendorf tubes, glass slides, glass spreader, inoculating needle, laminar air flame, petri-dishes, and shaker.

Chemicals

Ethanol, nutrient agar, saline solution and distilled water.

Cleaning and Disinfection

Glassware, tools, sampling bags and working areas were completely cleaned and surface sterilized before being used for microbiological work. Tap water was utilized for washing, followed by distilled water for rinsing and ethanol was used to disinfect working areas, hands, and glassware. Because 70 percent ethanol takes longer to evaporate off the surface and keeps things wet longer, it works as a disinfectant by dissolving all surface bacterial cell walls and cell membranes. Alcohol quickly dissolves the thin peptidoglycan layer that covers the plasma membrane of gram-negative bacteria. Water plays a vital role in the reaction by denaturing the protein in the cell membrane and acting as a catalyst. On the other hand, 100% ethanol quickly coagulates the protein by forming a protein layer that prevents further coagulation. Germs are not destroyed by remain dormant as a result of this, whereas 70% ethanol enters the cell wall more slowly and coagulates the cell wall's protein, killing the microorganisms.

Sterilization

The method of removing all varieties of microbial existence to generate sterile objects and a greater environment for microbiological research. For microbiological work, many sterilizing procedures were utilized. These following procedures are widely used:

Dry sterilization
Moist sterilization
Chemical sterilization

Dry sterilization

Dry heat sterilization uses extreme temperatures to kill bacterial spores and other germs and longer exposure times than moist heat sterilization (about 1.5-3). Glassware and other comparable items should be dry sterilized in an oven at the temperature of 70°C for only 2 hours.

Moist sterilization

A process called moist heat sterilization uses heated, steam with high pressure to kill bacteria on an object. This method of sterilization kills and eradicates potentially contagious bacteria, viruses, and spores without using any hazardous chemicals or gases, and it is economical, rapid, and effective. Moist sterilization culture media and glassware were sterilized for 20 minutes at 121°C in an autoclave at 2001b pressure. The inoculating needles were red-hot sterilized in a flame.

Chemical sterilization

The process of removing bacteria by the application of chemical bactericidal substances is known as chemical sterilization such as ozone, formaldehyde and ethylene oxide. Chemical sterilization is commonly utilized for devices that are sensitive to the intense heat used in steam sterilization, as well as those that could be destroyed by irradiation (rubbers and plastic can become more brittle after irradiation)

Collection of Sample

For sampling, different locations were visited like Jhang Bazar, Jinnah Colony, Samundri Road, Abdullah Pull, Ghulamabad, Millat Town, Ali Town, Lasani Town, Islam Nagar and Daewoo Road of Faisalabad, samples of broiler chicken *(Gallas gallus domesticus*) were collected. About 10 samples of chicken's intestine were collected in the eppendorf tubes. Eppendorf tubes were filled about 1.5 ml with saline solution.

Preparation of microbiological media

To lessen the possibility of contamination and chemical reactions, culture media were prepared with distilled water and instruments that were wrapped in foil paper. These instruments were cleaned and properly dried. Different media, such as Brilliant Green Agar (BGA), Xylose-Lisine-Deoxycholate, Salmonella Shigella Agar (SS Agar), Nutrient Agar (NA), Bismuth Sulfite Agar and Tryptic Soya Agar (TSA) were used to develop the bacterial colonies.

Culturing of bacteria

For culturing of bacteria, following steps were performed:

Sample preparation

The dissected organ were placed in a flask containing a 9% saline solution and thoroughly shaken using an electric shaker as part of the sample preparation process. The culture media solution were removed from the autoclave, allowed to sit for some time until it becomes a semi-solid gel-like consistency, and then to solidify poured on to Petri dishes. All the Petri dishes have around 20 milliliter of culture media inside for improved growth of microorganisms. For the isolation of bacteria saline solution filled in eppendorf tubes.

In the second dilution, 100 µl of solution were taken from the tube and transferred to the next. The agar culture media were dispersed by using a micropipette. After that, a 37°C incubator were used to keep the inoculated Petri dishes. After 24-48 hours of incubation, bacterial colonies were well cultivated and clear to see.

Nutrient agar media colony

After 24 hours of incubation, the media develops a bright yellow transparent to slightly opalescent gel in Petri plates. *Aeromonas*, *Escherichia coli*, *Lactobacillus* and *Bacillus subtilis*, *Pseudomonas aeruginosa*, were among the microorganisms cultivated on nutritional agar media.

Escherichia coli **colonies on nutrient agar**

Morphology

Escherichia coli is a gram negative (-ve) bacteria with a rod form. Its dimensions are 1-3 x 0.4–0.7 m, and its capacity is 0.6–0.7 m3. Both alone and in pairs, it can be employed. It is motile because of peritrichous flagella. Certain strains are not mobile. There is a chance of fibricated strains. Both motile and non-motile strains can contain type 1 fimbriae (hemaglutinating mannose-sensitive). Several E. coli strains isolated from extraintestinal infections contained a polysaccharide capsule. They do not disseminate spores. With one or two layers of peptidoglycan, their cell wall is extremely thin. They can survive in the absence of oxygen because they are a facultative anaerobes. Temperature between 15-45°C are ideal for growth.

Bacillus subtilis **colonies on nutrient agar**

Morphology

The typical germ *Bacillus subtilis* is rod-shaped, with gram-positive, straight- or round-ended vegetative cells. The circular colony of this bacteria measures 0.5 to 1.2 by 2.5 to 10.0 micrometres when it is cultivated on nutrient agar. The majority of bacillus bacteria exhibit peritrichus flagella-based movement. Bacilli don't have capsules. This genus has numerous species that display a broad range of physiological traits that enable them to survive in various kinds of natural environments. Per cell, just one endospore forms. The spores are immune to radiation, desiccation, heat, cold, and cleaning agents.

Aeromonas **colonies on nutrient agar**

Morphology

Aeromonas are gram-negative, rod-shaped facultative anaerobes and non-sporing bacterium. The size range for the genus Aeromonas is 0.3-1.0 m and 1.0-3.5 m. They normally show up as buff-colored, fluid, conical colonies 3-5 mm in size on not specific agar media following an overnight incubation at 35–37°C. Some strains of Aeromonas have an outermost layer called the S layer, which is made up of a single surface array protein (Sap), a 49–55 kDa acidic protein. Aeromonas is a motile bacterium. Swarming motility. Numerous Aeromonas species have been shown to contain capsules. In Aeromonas, two main kinds of fimbriae have also been identified.

- Rigid, contains protein subunits with a size range of 17–21 kDa and has a 9 nm diameter.
- Flexible, protein subunits with a size range of 19–23 kDa, each measuring 7 nm in diameter.

Most species have a single polar flagellum. Maximal growth is seen when the temperature is between 37°C and 44°C. All Aeromonas are capable of surviving in pH ranges of 4.5 to 9, but 5.5 to 9 is the ideal range.

***Lactobacillus* colonies on nutrient agar**

Morphology

Lactobacillus are anaerobic, positive, long, slender rods to short, coryne form *coccobacilli*. *Lactobacillus* has a size range of 0.5-1.2 micrometres by 1.0-10.0 micrometres. A non-sporing, non-motile bacteria that only occasionally exhibits movement by peritrichous flagella. Capsules are absent in *Lactobacillus*. Maximal growth is seen when the temperature is between 28-30°C.

Microbial count

In order to properly distribute the petri dish floor is covered in liquid culture medium. Nutrient media agar was evenly spread over the petri dish. Sterilized loops were also used for this purpose. For reliable bacterial counts, Petri dishes were prepared and the average of these were evaluated. To prevent any kind of condensation, each petri dish were kept upside down in the incubator. This approach were used repeatedly throughout the research project for various cultural media. The overall viable count for each sample were checked. By multiplying the average number of colonies in triplicate petri-plates of a sufficient dilution by the dilution factor, the total viable count per ml of material was obtained.

Total viable count = Average no. of colonies × Dilution factor

Colony morphology

On solid medium, bacterial colonies grow in small, medium, large, and pinpoint sizes; their shapes are spherical, irregular, filamentous, biconcave and rhizoid; their margins are entire, undulate, lobate, wavy, dentate and filamentous; their optical characteristics are milky and transparent and their coloration is yellow, purple, white, off-white and transparent support in identification of specific bacteria. After 24 and 48 hours of growth, microscopic morphologies if bacterial colonies were discovered. The following formula will be used to determine the number of colonies that will comprise a unit:

CFU = Total number of colonies × Dilution factor

Preparation of slides for staining

The given slides were prepared for staining.

Staining characteristics

Bacterial cells were stained under a compound microscope to reveal their cell morphological characteristics, such as morphology (bacilli, spiral, filamentous), and arrangement, which emerged in chains and clusters. Staining is a process in which colored chemicals called dyes are given to a bacterial specimen and entire or a portion of the bacterial cells is stained according to the dyes nature. Under the compound microscope, chosen slides were inspected.

Gram staining

The bacterial colony was identified using the gramme staining technique. Two types of bacterial cells were distinguished. Gram-positive bacteria can be distinguished from gram-negative bacteria by their cell walls.

On gram-positive bacteria, the peptidoglycan coating is thicker. Certain microbes are able to maintain the primary stain after being stained with the basic stain Crystal Violet and attached by the mordant, while other bacteria are decolored by alcohol.

Gram-positive bacteria have low levels of lipid and a thick covering of peptidoglycan, a protein-sugar complex. The cell's thick cell wall becomes dehydrated and shrinks as a result of decolorization, which seals the cell wall's pores and keeps the stain from leaving the cell. As a result, the Crystal Violet-lodine complex, which is colored blue or purple and linked to the gram-positive bacteria's thick layer of peptidoglycan, cannot be removed by ethanol.

Gram-negative bacteria also take up the Crystal Violet-lodine compound in their cell walls, but this complex is washed off because of a thin coating of peptidoglycan and the thick outer layer made of lipids.

Take a clean, grease-free slide for decolonization purposes. Prepare a slide that is clean with a loop of material full of sample for the smear of suspension. Heat fix and air drying after adding the crystal violet, one minute was given before washing with water. Afterward, examine under a microscope.

Physiochemical parameters

HANNA HI-5424 is an electronic meter which was used to calculate pH.

Temperature

The samples were kept in lab at room temperature.

pH

The Microprocessor pH meter (HANNA-HI, 9023) was used to measure the pH after having its range set to "pH" point.

Electric conductivity

Electric Conductivity Metre was used to measure electrical conductivity.

Statistical analysis

Analytical statistics using one-way analysis of variance, statistical analysis was used to compare the quantitative data (ANOVA). The data of the trial will be statistically examined using Mean ± SEM and ANOVA, followed by Tukey's test.

RESULTS

The research entitled as isolation and identification of bacteria from the intestine of chicken was conducted at Microbiology and Immunology lab, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. In control group, standard feed was given according to 6% of body weight of broiler chicken. In experimental groups, supplemented feed was given with 0.6% of bacterial feed.

CFU (colony forming unit) and total microbiological content of intestine sample of *Gallus gallus domesticus* on Nutrient Agar Media (control group)

The intestinal samples of chicken's intestine were cultured on Nutrient agar. The minimum number of colonies were observed on samples was 33 x 10 CFU/mL and maximum number of colonies was observed was 330 x 10 CFU/mL in control group.

CFU (colony forming unit) and total microbiological content of intestine sample of *Gallus gallus domesticus* on Nutrient Agar media (experimental group supplemented feed)

The intestinal samples of broiler chicken were cultured on Nutrient agar. The minimum number of colonies was absorbed in experimental group 1 supplemented feed with 2% *Bacillus subtilis* was 180 x 10 CFU/mL and maximum number of colonies was 490 x10 CFU/mL.

CFU (colony forming unit) and total microbiological content of intestine sample of *Gallus gallus domesticus* on Nutrient Agar media (experimental group supplemented feed with 3% *Bacillus subtilis*)

The intestinal samples of broiler chicken were cultured on Nutrient agar. In experimental group 2 supplemented feed with 3% *Bacillus subtilis*, the minimum number of colonies were 218 x 10 CFU/mL and maximum colonies were 510 x 10 CFU/mL.

One way analysis of variance (ANOVA) for the viable count of intestinal samples of *Gallus gallus domesticus* on Nutrient agar is shown. We can see the result is significant because the value of P<0.05 in the table and the difference in the values are shown in the table.

	Nutrient agar	
Sample no.	Colony count	CFU106
1	247	247000000
2	229	229000000
3	330	33000000
4	275	275000000
5	33	330000000
6	76	760000000
7	310	310000000
8	280	280000000
9	236	2360000000
10	250	250000000

Table 1- Bacterial culture in the intestine sample of *Gallus gallus domesticus* by using nutrient agar for the control group.

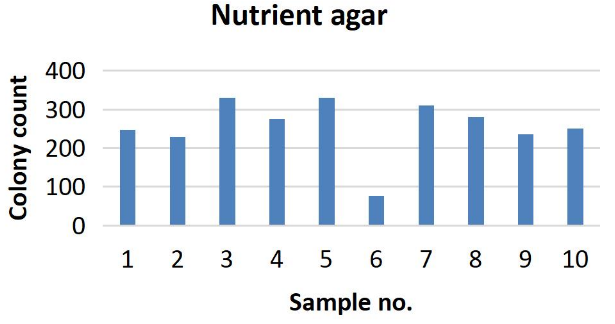

Fig. 1- Graphical representation of colony count under the influence of nutrient agar for control group.

Interpretation:

The intestinal samples of *Gallus gallus domesticus* were cultured on Nutrient agar. The A number of colonies were observed on Nutrient agar. The minimum number of colonies were observed on samples was 33 x 10 CFU/ml and maximum number of colonies were observed was 330 x 10 CFU/mL in control group.

	Nutrient agar	
Sample no.	Colony count	CFU106
1	470	470000000
2	490	490000000
3	210	21000000
4	340	340000000
5	180	180000000
6	280	280000000
7	200	200000000
8	250	250000000
9	460	460000000
10	250	250000000

Table 2- Bacterial culture in the intestine sample of *Gallus gallus domesticus* by using nutrient agar for Experimental group 1.

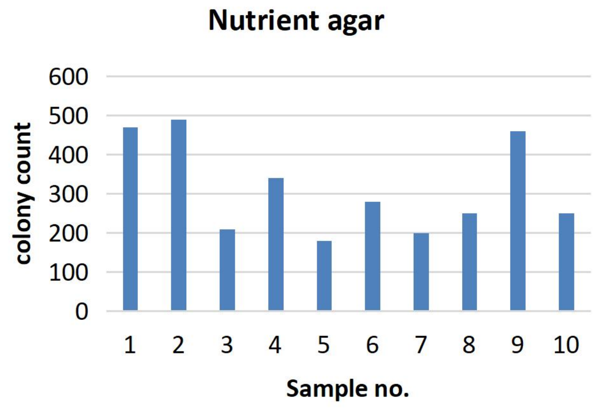

Fig. 2- Graphical representation of colony count under the influence of nutrient agar for Experimental group 1.

Interpretation:

The intestinal samples of *Gallus gallus domesticus* were cultured on Nutrient agar. The number of colonies was absorbed in experimental group 1 supplemented feed with 2% *Bacillus subtilis* was 180 x 10 CFU/mL and maximum number of colonies was 10 CFU/mL.

Nutrient agar		
Sample no.	Colony count	CFU106
1	500	500000000
2	510	510000000
3	315	315000000
4	450	450000000
5	218	218000000
6	400	400000000
7	267	267000000
8	344	344000000
9	471	471000000
10	310	310000000

Table -: Bacterial culture in the intestine sample of *Gallus gallus domesticus* by using nutrient Agar for Experimental group 2.

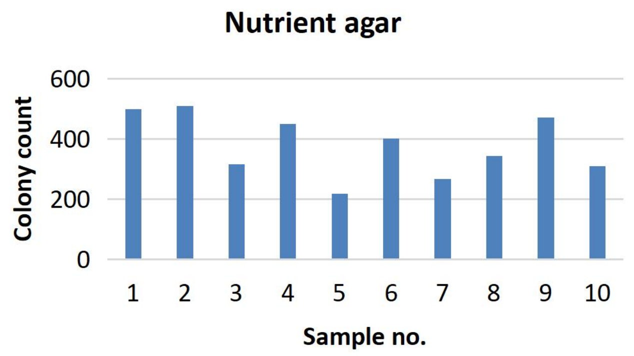

Fig. 4- Graphical representation of colony count under the influence of nutrient agar for Experimental group 2.

Interpretation:

The intestinal samples of *Gallus gallus domesticus* were cultured on Nutrient agar. And in experimental group 2 supplemented feed with 3% *Bacillus subtilis*, the minimum number 218 x 10 CFU/mL and maximum colonies were 510 x 10 CFU/mL.

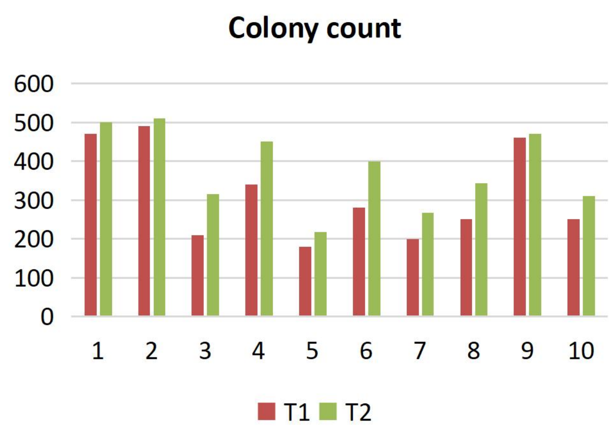

Fig. 5-Graphical representation of total microbiological count of intestinal part of *Gallus gallus domesticus*

ANOVA					
Source of Variation	SS	Df	MS	F	P-value
Betwee	116096	2	58048.	5.1091	0.01*

n Groups	.1		03	47	
Within Groups	306762.9	27	11361.59		
Total	422859	29			

Table: 5- Analysis of variance of microbiological contents in intestinal parts of *Gallus gallus domesticus* of control group and 2 other experimental groups.

*= Significant

**= Highly significant

ns= Non-significant

One way analysis of variance (ANOVA) for the viable count of intestinal samples of *Gallus gallus domesticus* on nutrient agar is shown. We can see the result is significant because the value of $P<0.05$ in the table and the difference in the values are shown in the table. From the above table there is different type of variations. The first source of variation is replication; the second source of variation is treatment where we check the equality of means of means of treatment. The second last variation is error which is also called unexplained variation and last source of variation is total variation. Second term is sum of squares, degree of freedom. 4th column in ANOVA table is mean sum of squares then from mean sum of squares we calculate statistics, we use the analysis of variance technique to determine the significance of the treatment means. The p-value in an analysis of variance table indicates the treatment's significance. If the p-value is less than 0.05, our findings are significant. The p-value in the above ANOVA table indicates a significant results because it is 0.01 which is less than 0.05, indicating that treatment is significantly different.

DISCUSSION

The research conducted for the isolation and identification of bacteria from the intestine of chicken (Gallus gallus domesticus). So, it was cleared from the results, Bacillus subtilis was high with specific bacterial probiotic and colonies of other pathogenic microbiota like Enterobacter cloacae, Plesimonas Shigelloides, A. hydrophila were less observed. This showed that addition of respective bacteria in the form of probiotic could enhance the microbial count of that bacteria low in intestine which showed the increased immunity. In the context of study in the intestine of chicken increase of beneficial bacteria detected the positive effect of probiotic on chicken health. So, in present study replacement of beneficial bacteria was recorded with addition of microbes contain probiotics.

The gastrointestinal system is a dynamic habitat that is home to a highly developed microbial community. In particular, short-chain fatty acids, ammonia concentrations, and lactate, as well as the microbial fermentation profile, are included in this paper's assessment of the native gut bacteria. The small intestine contains fusobacteria, lactobacilli, enterobacteria, streptococci, and eubacteria, its bacterial density rises with aging. Young broilers are predominately colonized by strict anaerobes. Our results are similar to Rehman *et al*. (2007) investigated the gastrointestinal system of broiler chicken's native bacteria and metabolic products of bacteria

The majority of the bacteria in the gastrointestinal system of broiler chickens are lactic acid bacteria, which have specific nutritional needs and depend on the availability of amino acids in their environment for growth. Domestication genomics has benefited from the use of chickens as model organisms. These findings correlates with the study of Apajalahti et al. (2016) studied the protein digestion and the intestinal microbiota of chickens.. This study emphasizes the value of using chickens as an animal model in fundamental and pre-clinical research, including investigations on poultry epigenetics and ancestor-based genomics.

It is unknown; however, which adult microbiota individuals are able to colonize the caecum of freshly hatched chickens. As a result, on the first day of life, we mechanically inoculated chicks with just one culture of 76 different isolates of bacteria from chicken caecum, and seven days later we evaluated their ability to colonize. Bacteria from the phyla Bacteroidetes, Proteobacteria, Synergistetes, or Verrucomicrobia, as well as isolates from the class Negativicutes (phylum Firmicutes), may colonize the caecum of freshly hatched chickens. The chicken digestive tract did not become colonized by members of probiotic genera like Lactobacillus, Enterococcus Bacillus subtilis after the infusion of a single dosage. These results are also compatible to Kubasova et al. (2019) studied that the commercially raised chicks are extremely susceptible to enteric infections; but, by giving them complex adult microbiota, immunity can be boosted.

The intestinal samples of Gallus gallus domesticus for control group were cultured on Nutrient agar. A number of colonies were observed on Nutrient agar. The minimum number of colonies was observed on samples were 33 x 10 CFU/ml and maximum number of colonies were observed was 330 x 10 CPU/mL in control group. The intestinal samples of Gallus gallus domesticus were cultured on Nutrient agar. The number of colonies was absorbed in experimental group 1 supplemented feed with 2% Bacillus subtilis was 180 x 10 CFU/mL and maximum number of colonies was 10 CFU/mL. And in experimental group 2 supplemented feed with 3% Bacillus subtilis, the minimum number 218 x 10 CFU/mL and maximum colonies were 510 x 10 CFU/mL.

Conclusion

This study concludes that,there was valuable increase in the intestinal microbiota of Poultry Chicken. *Escherichia coli*, *Bacillus subtilis* enhanced the digestion by secreting different enzymes in chicken's intestine. Chicken showed strong immune response and disease resistance.The synthesis of total volatile basic nitrogen and the consecration and colonization of *Pseudomonas spp*. in broiler chicken gut and flesh both significantly decreased. By adding, *Bacillus subtilis* as probiotics the feed digestibility was increased. It proved helpful to prevent the chicken from various microbial diseases.Application of *Escherichia coli*, *Bacillus subtilis*, proved efficient in improving water quality by reducing ammonia and nitrate toxicity. Water quality parameters like pH, temperature, dissolve oxygen and alkalinity showed a great influence on immune system of chicken.

REFERENCES

Sender, R., S. Fuchs and R. Milo. 2016. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol. 14:1-14.

Turnbaugh, P. J., R. E. Ley, M. Hamady, C. M. Fraser-Liggett, R. Knight and J. I. Gordon. 2007. The human microbiome project. Nature. 449:804-810.

Liu, X. D., D.D. Jayasena, Y. Jung, S. Jung, B.S. Kang, K.N. Heo, J. H. Lee and C. Jo. 2012. Differential proteome analysis of breast and thigh muscles between Korean native chickens and commercial broilers. Asian Australas. J. Anim. Sci. 25:895-902.

Higgs, J. D. 2000. The changing nature of red meat: 20 years of improving nutritional quality. Trends Food Sci. Technol. 11:85-95.

Dunkley, K.D., C.S. Dunkley, N.L. Njongmeta, T.R. Callaway, M.E. Hume, L.F. Kubena, D.J. Nisbet and S.C. Ricke. 2007. Comparison of in vitro fermentation and molecular microbial profiles of high-fiber feed substrates incubated with chicken cecal inocula. Poult. Sci. 86:801-810.

Saengkerdsub, S., R.C. Anderson, H.H. Wilkinson, W.K. Kim, D.J. Nisbet, and S.C. Ricke. 2007. Identification and quantification of methanogenic archaea in adult chicken ceca. Appl. Environ. Microbiol. 73:353-356.

Qu, A., J.M. Brulc, M.K. Wilson, B.F. Law, J.R. Theoret, L.A. Joens, M.E. Konkel, F. Angly, E.A. Dinsdale, R.A. Edwards and K.E. Nelson. 2008. Comparative metagenomics reveals host specific metavirulomes and horizontal gene transfer elements in the chicken cecum microbiome. 3:1-19.

Yeoman, C.J., N. Chia, S. Yildirim, M.E.B. Miller, A. Kent, R. Stumpf, S.R. Leigh, K.E. Nelson, B.A. White and B.A. Wilson. 2011. Towards an evolutionary model of animal-associated microbiomes. Entropy 13:570-594.

Permin, A., J.P. Christensen, M. Bisgaard. 2006. Consequences of concurrent Ascaridia galli and Escherichia coli infections in chickens. Acta. Vet. Scand. 47:43-54.

Danzeisen, J. L., H. B. Kim, R. E. Isaacson, Z. J. Tu and T. J. Johnson. 2011. Modulations of the chicken cecal microbiome and metagenome in respons to anticoccidial and growth promoter treatment. 6:1-14.

Apajalahti, J., A. Kettunen and H. Graham. 2004. Characteristics of the gastrointestinal microbial communities with special reference to the chicken. Poult. Sci. J. 60:223-232.

Oakley, B.B., H.S. Lillehoj, M.H. Kogut, W.K. Kim, J.J. Maurer, A. Pedroso, M.D. Lee, S.R. Collett, T.J. Johnson and N.A. Cox. 2014. The chicken gastrointestinal microbiome. FEMS Microbiol. Lett. 360:100-112.

Zhu, X.Y. and R.D. Joerger. 2003. Composition of microbiota in content and mucus from caeca of broiler chickens as measured by fluorescent in situ hybridization with group-specific, 16S rRNA-targeted oligonucleotide probes. Poult. Sci. 82:1242-1249.

Zhu, X.Y., T. Zhong, Y. Pandya and R.D. Joerger. 2002. 16S rRNA-based analysis of microbiota from the cecum of broiler chickens. Appl. Environ. Microbiol. 68:124-137.

Wise, M.G. and G.R. Siragusa. 2007. Quantitative analysis of the intestinal bacterial community in one to three week old commercially reared broiler chickens fed conventional or antibiotic free vegetable based diets. J. Appl. Microbiol. 102:1138-1149.

Morgan, N.K., C.L. Walk, M.R. Bedford and E.J. Burton. 2014. The effect of dietary calcium inclusion on broiler gastrointestinal pH:

Quantification and method optimization. Poult. Sci. 93:354-363.
Lu, J., U. Idris, B. Harmon, C. Hofacre, J.J. Maurer and M.D. Lee. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl. Environ. Microbiol. 9:6816-6824.
Islam, M.R., D. Lepp, D.V. Godfrey, S. Orban, K. Ross, P. Delaquis and M.S. Diarra. 2019. Effects of wild blueberry (*Vaccinium angustifolium*) pomace feeding on gut microbiota and blood metabolites in free-range pastured broiler chickens. Poult. Sci. 98:3739-3755.
Hegde, N.V., S. Kariyawasam and C. DebRoy. 2016. Comparison of antimicrobial resistant genes in chicken gut microbiome grown on organic and conventional diet. Vet. Sci. 1:9-16.
Hegde, N.V., S. Kariyawasam and C. DebRoy. 2016. Comparison of antimicrobial resistant genes in chicken gut microbiome grown on organic and conventional diet. Vet. Sci. 1:9-16.
Ferreira, A.J.P., C.S.A. Ferreira, T. Knobl, A.M. Moreno, M.R. Bacarro, M. Chen, M. Robach, and G.C. Mead. 2003. Comparison of three commercial competitive-exclusion products for controlling Salmonella colonization of broilers in Brazil. J. Food Prot. 66:490-492.
Contreras-Ruiz, L., A. Ghosh-Mitra, M. A. Shatos, D. A. Dartt and S. Masli. 2013. Modulation of conjunctival goblet cell function by inflammatory cytokines. Mediat. Inflamm. 1:1-11.
Kubasova, T., M. Kollarcikova, M. Crhanova, M. Karasovax, D. Cejkova, A. Sebkova, J. Matiasovicova, M. Faldynova, F. Sisak, V. Babak and A. Pokorna. 2019. Gut anaerobes capable of chicken caecum colonisation. Microorganisms 7:1-11.
Rehman, H.U., W., Vahjen, W.A. Awad and J., Zentek. 2007. Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. Arch. Anim. Nutr. 61:319-335.