EFFECTS OF BACTERIOPHAGE SUPPLEMENTED DIET ON INTESTINAL BENEFICIARY MICROFLORA AND PRODUCTIVE PERFORMANCE OF BROILER.

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This is to certify that the thesis entitled "EFFECTS OF BACTERIOPHAGE SUPPLEMENTED DIET ON INTESTINAL BENIFICIARY MICROFLORA AND PRODUCTIVE PERFORMANCE OF BROILER."

Submitted to the Department of Poultry Science , Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University , Dhaka-1207, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (MS) in Poultry Science embodies the result of a piece of bonafide research work carried out by MD. ZULKARNAIN, Registration No. 20-11112 , Semester: January-June/2022 under my supervision and guidance . No part of the thesis has been submitted for any other degree ordiploma.

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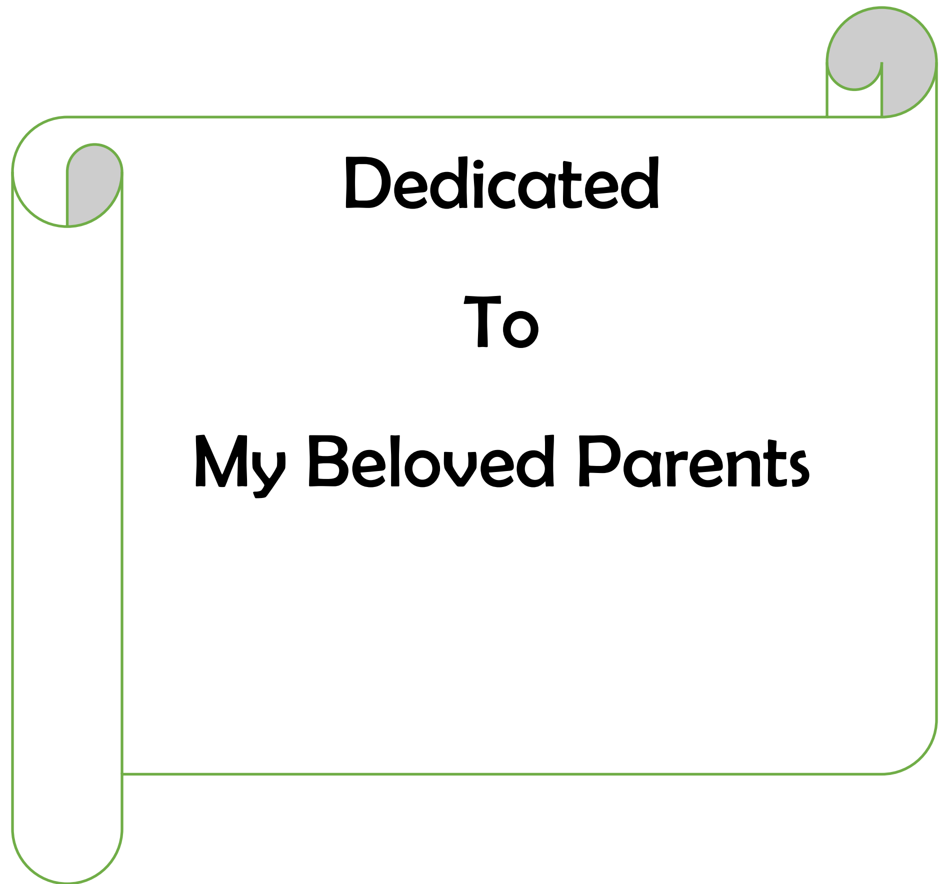
Dedicated
To
My Beloved Parents

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ACRONYMS AND ABBREVIATIONS

Abbreviation	Full meaning
AGPs	Antibiotic Growth Promoter
ANT	Antibiotic Treatment
ANOVA	Analysis of Variance
AMR	Antimicrobial Resistance
ARG	Antibiotic Resistant Gene
APEC	Asia Pacific Economic Co-operation
BP	Bacteriophage
BPICC	Bangladesh Poultry Industry Central Council
CFU	Colony forming Units
CONTD	Continued
C.	Campylobacteria
ETEC	Enterotoxigenic E. Coli
BMD	Bacitracin Methylene Disalicylate
E.	Escherichia
Et al	Associates
FDA	Food & Drug Administration
FC	Feed Consumption
FCR	Feed Conversion Ratio
g	Gram
i.e.	That is
Kg	Kilogram
LTD	Limited
M.S	Master of Science
NH_3	Ammonium
No.	Number
NS	Non-significant
PFU	Plaque forming Units
PPM	Parts per million
Phase	Bacteriophage
p.	Pseudomonas

CI	Confidence interval
rpsL	Ribosomal Protein Subunit
GRAS	Generally recognizes as safe
SAU	Sher-e-Bangla Agricultural University
SED	Standard Error Difference
SPSS	Statistical Package for Social Science
Viz.	Such As
T_{cc}	Total Colony Count
Pro-Be-Bac PE	Bacteriophage Trade Name
Vs.	Versus

LIST OF SYMBOLS

Symbols	**Full Meaning**
:	Ratio
@	At the rate of
&	And
*	5 % level of significance
**	1 % level of significance
/	Per
%	Percentage
<	Less than
>	Greater than

EFFECTS OF BACTERIOPHAGE SUPPLEMENTED DIET ON INTESTINAL BENEFICIARY MICROFLORA AND PRODUCTIVE PERFORMANCE OF BROILER.

ABSTRACT

The Experiment was done to investigate the effects of dietary bacteriophage (BP) supplementation on production performance and status of microflora in excreta along with carcass characteristics in broilers. A total of 600 day old broiler chicks were reared randomly allotted in 5 treatment groups having 4 replications R_1, R_2, R_3 and R_4 .Where each replication contain 30 birds for 5 weeks at SAU Poultry Farm, Dhaka-1207.These dietary treatments were designed as T_0 (Control diet), T_1 (Control+ 0.5g Bacteriophage per kg feed),T_2 (Control+ 0.75g Bacteriophage per kg feed) ,T_3 (Control+1g Bacteriophage per kg feed) and T_4(Control+ANT 0.05g per Kg Feed). In broilers the inclusion of antibiotic and bacteriophages directly affect the feed intake (P= 0.002) among the five groups.BWG (Body Weight Gain) in T_1 group was higher(2209.00 ±16.45 gm) which showed significant differences (P<0.05) among the other groups. LW (Live weight), FC(Feed Consumption) and FCR(Feed Conversion Ratio) at the end of 5th week were significant (P< 0.05) in different group, however better BWG, LW, FC were found in treated group T_1 where BWG, LW and FC were 2209.00 ±16.45 gm , 2251.00±16.45 gm & 3069.00±22.70 gm respectively. Whereas FCR performance was positively correlated with the levels of bacteriophage given in the diet. Highest FCR (1.39 ± 0.02) found in T_1 treatment. However, there are insignificant effect found in survivability (P >0.05) at 35 days of age of broiler .Bacteriophage group showed significant results in case of liver ,spleen, heart & breast weight compared to control and antibiotic except the gizzard weight . Results demonstrated that the average ammonia level was same at the end of the 1st & 2nd week , however it varied significantly (P<0.05) at the 3rd & 4th weeks of age (10.10±0.57 ppm & 13.75 ± 1.02 ppm). But at the end of 5th week T_2 & T_3 group showed highest ammonia emissions (18.50± 0.65 ppm & 18.25± 0.85 ppm respectively). Ammonia emissions were found lowest in T_1 (13.25±1.38 ppm).Flock uniformity demonstrated significant differences between the control and T_1 Group (67.72± 4.27 %.).The lactobacillus was significantly (P<0.05) lower in antibiotic and control group rather than the bacteriophage supplemented diet . T_1 showed highest lactobacillus (3.97 ± 0.13 CFU/ml) and T_4 showed the lowest (0.43± 0.07 CFU/ml). In conclusion, it can be said that , bacteriophage supplementation has beneficial effects on production performance of broiler and it can expand the beneficiary microbes in guts of commercial broiler chicken.

Keywords- Bacteriophage, Broiler ,Beneficial Microflora

1 Introduction

Poultry production is one of the most important ones of the livestock sector among all the subsectors in Bangladesh .This sector plays a vital role in rural economic growth and women's empowerment all over the Bangladesh . According to the Bangladesh Poultry Industry Central Council 2021-22 (BPICC) Bangladesh's Poultry sector currently produces 1.5 to 1.6 percent of the country's GDP. Protein requirement of the people is maintained by white meat rather than red meat due to some reason such as religious nutritious fact and cost. In considering all over the reasons , minimizing the production cost within ability , fulfillment of maximizing the protein requirement of human body,diseases of poultry industry is the important concern because of lost productivity , increased mortality and the associated contamination of poultry products for human consumption (human food safety).In broiler chicken , major bacterial diseases observed in Bangladesh are Pullorum, Fowl typhoid, Fowl para typhoid, Colibacillosis, Necrotic Enteritis , Omphalitis and bumble foot disease. InTwenty decades, it is the burning question of developing countries as like Bangladesh.

Antimicrobial resistance is a worldwide problem and themost serious threat to general public health as a major contributor to this owing to its poor health care standards, along with the misuse and overuse of antibiotics. Due to antimicrobial resistance occur when bacteria,viruses, fungi and parasites change over time and no longer respond to medicines making infections harder to treat and increasing the risk of disease spread, severe illness and death. In effort to reduce antibiotic resistance,action to reduce and eliminationsof the use of AGPs in poultry industry are increasing rapidly which is maintained by mandatorily and voluntarily.However such a movement have encouraged significant economic losses for producers and increasing uses of antibiotics for the therapeutic treatment. The demand for alternatives to AGPs is ever strong -for new solution that not only can directly counteract antibiotic resistant bacteria but enhance efficacy of therapeutic treatments.

However, there has been increasing concern over the impact of AGP on the emergence of antibiotic resistance in zoonotic bacterial pathogens in the microbial community of the poultry gut .Uses of antibiotics that claimed to improve feed conversion ,stimulate growth & reduction mortality used in feed additives has the most contribution resistance in the poultry farm can be transferred to other animals or humans through direct contact , food-produced animal products , or indirectly via environmental pathways.

About antibiotic resistance, there has been a world wide increase in the regulation or ban of the use of AGP in poultry diets , Bangladeshis one of the countries that currently Bangladesh Government has prohibited the use of antibiotics as a feed additive stated by Act No 2 of the year 2010 with a sub Claws 14 ,dated 28th January 2010, there is an increasing interest in finding alternatives to antibiotics for poultry production. Several potential alternatives such as probiotic, prebiotic and symbiotics have been developed. Probiotic as "a live microbial feed supplement that beneficially affects the host animal by improving its intestinal balance (Fuller , 1989; Markowiak, & Śliżewska, , 2017)"&prebiotics can be explained as " a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and the combination of prebiotics and probiotics are called as symbiotic(Gibson, , et al., 2017). But their efficacy has been inconsistent as compared to dietary AGPs. Banned on AGP in feed resulted in lot of problems such as increase of production cost and reduced animal performance . therefore further research is required to observe the efficacy of the bacteriophage supplemented diet as a sustainable alternatives to AGP/antibiotics on intestinal non pathogenic microflora and growth performance of broiler. Numerous in vivo and in vitro studies since then have shown that the commensal intestinal microflora inhibit pathogens, that disturbances of the intestinal microflora can increase susceptibility to infection and that in addition of prebiotics and probiotics increase resistance to infection. (Markowiak, & Śliżewska,, 2017.)

The bacteriophage or phage is an infectious virus that kills microbes by multiplying within their cells and subsequently destroy the host bacteria (Monk , et al., 2010).Bacteriophages have numerous advantages that make them effective instruments for treating bacterial infections and combating the emergence of bacterial resistance. One of the most serious concerns about antibiotics is their side effects, which can harm the microflora, which is related to a variety of imbalances and diseases. In this aspect, phage specificity can solve the problem because they will only replicate within their unique host (phages cannot infect eukaryotic cells). Unlike antibiotics, phages can proliferate rapidly inside the host (but only if they find a host), can be supplied in small doses with extended intervals of time between them, and are removed once their population is eliminated. (El-Shibiny, & El-Sahhar,, 2017) (Matsuzaki, , et al., 2014)

Implication of bacteriophages has been based on its specificity targeting at particular strains of pathogenic and nonpathogenic bacteria which has been increasing evidence to suggest that the applications of a single or mixture of specific Bacteriophage by aerosol spray , muscle

injection, or oral route to chickens challenged with specific pathogens enhance clinical symptoms of bacterial infection and decrease mortality (Domingo, et al., 2016) .

Among the all bacteriophages Lytic bacteriophage is a microorganism that selectively kills bacteria in a species-specific manner. The U.S. FDA has approved bacteriophage as GRAS substance (Generally Recognized As Safe) . ProBe- Bac is the latest bacteriophage solution from pathway Intermediates. It is created based on the real-time upgrade system, Powered by Optipharm. Pathogenic Bacteria population is constantly monitored and any significant disease outbreak will lead to solution upgrade in real time . It is a cocktail product containing mixture of bacteriophages precisely selected against E. coli(ETEC and APEC) ,Salmonella Typhimurium , Salmonella Enteritidis, S. Pullorum and Clostridium prefringens to tackle various diseases that are prevalent in poultry industry such as necrotic enteritis , Pullorum , Diarrhea and fowl typhoid . Long term use of phages in poultry has proved to be moderately effective in reducing the number of salmonella colonizing in the digestive tract . In broiler diet , anti-SE (anti-S. enetritidis) bacteriophages can be used as an alternative feed additive in lieu of antibiotics . . ProBe-Bac PE improves poultry performance by directly reducing pathogenic Bacteria counts in chicken feces , internal organs and poultry products and enhances effectiveness of therapeutic antibiotics by destroying and penetrating biofilm of pathogens.

Objectives

1. To evaluate the growth performance, and cost effectiveness in different levels of bacteriophage.
2. To assess the *Lactobacillus* in the gut of broiler.
3. To detect the ammonia levels in different level of bacteriophage supplemented diet.

2 Review of Literature

Broiler performance depends on the quality of the feed offered with considerable energy and protein content usually administered , the amount of nitrogen that meets the nutritional demands of the animals . Also makes the poultry feed the main source of nitrogen in the poultry waste and feedlot systems are a cost efficient form of animal production and characterized by relatively small production cycles, high technological levels, small production space, and the demand for fewer resources such as water and energy (Mendes & Nadege, January 2015) .

Toro , et al., 2005 demonstrated that bacteriophages are viruses that can infect and multiply in microbes, and are used to prevent and treat bacterial diseases making them an attractive alternative to drugs. Antibiotic in general, phages are intracellular parasites that multiply bacteria in the farm using some or all biosynthetic devices. When a phage enters a bacterial cell, it begins to replicate into 30-50 copies, causing the bacteria to degrade.

2.1 Genesis of Bacteriophage

Ernest Hanbury Hankin, a British bacteriologist who worked (Abedon, , et al., 2011)as the Chemical Examiner and Bacteriologist to the Government of the United Provinces and the Central Provinces of India, demonstrated in 1896 that the waters of the Indian rivers Ganga and Yamuna contained a biological principle that destroyed cultures of cholera-inducing bacteria. This material could pass through Millipore filters, which have been shown to be capable of retaining bigger microbes like bacteria. He reported his findings in the Annals of the Pasteur Institute in French. (Sorek, , et al., 2013) While investigating the growth of vaccinia virus on cell-free agar media in 1915, British microbiologist Frederick Twort noticed that "pure" cultures of bacteria might be coupled with a filter-passing transparent material that could completely break down bacteria in a culture into granules. (Twort,, 1915)This "filterable agent" was demonstrated in cultures of vaccinia micrococci: material from some colonies that could not be sub cultured was able to infect a fresh growth of micrococcus, and this condition could be transmitted to fresh cultures of the microorganism for an almost infinite number of generations. Twort described this transparent material, which was shown to be unable to grow in the absence of bacteria, as a ferment released by the microbe for some unknown reason.

Félix d'Herelle independently described a similar experimental discovery while observing patients suffering from or recovering from bacillary dysentery two years after this publication. He recovered a "anti-Shiga microbe" from the stools of recovering shigellosis patients by

filtering stools that had been cultured for 18 hours.When this active filtrate was put to a Shiga bacilli culture or emulsion, it was able to produce culture arrest, death, and finally lysis of the bacilli. (Abedon, , et al., 2011)In the early 1900 , bacteriophage was discovered by Twort,, 1915 that is a phage or bacterial virus that's any of a group of viruses that infect bacteria.

There are thousand types of varieties of phages exist in , each of which may infect only one or few types of bacteria . Phages are classified in a number of virus families, some examples include Inoviridae, Microviridae, Rudiviridae and Tectiviridae. Like all viruses , bacteriophages are simple organisms that consist of a core nucleic acid that's called genetic material surrounded by a protein capsid . These nucleic acid either DNA or RNA or may be double stranded or single stranded . There are three basic structural forms of phase ; an icosahedral (20 sided) head with a tail and an icosahedral without a tail and filamentous form. (Ackermann , 2011)

During infection a phage attaches to a bacterium and inserts the nucleic acid called as genetic material into the cell then that the phage follows one of two life cycles, lytic activity which is characteristic of Virulent phages and lysogenic activity ,involving integration of the genetic material of the bacteriophage with the bacterial chromosome and replication as part of the bacterial DNA. Lytic Phages take over the machinery of the cell to make phage components. They then destroy , or lyse the cell releasing new phage particles. Lysogenic phages incorporate their nucleic acid into the chromosome of the host cell and replicate with it as a unit without destroying the cell. Under certain conditions lysogenic phages can be induced to follow a lytic cycle. (Domingo, et al., 2016)

2.2 Mechanism of bacteriophage

(Young, 2013) proclaimed that bacteriophage structures must match strain-specific variants of bacterial receptors in order to initiate binding. Given the massive and constant alterations of both Bacterial production and bacterial structures, which are commonly mediated by bacteriophages, a single bacteriophage can only infect a small number of bacterial strains, and in certain situations, just a single strain. This explains the Bacteria production activity's perfect selectivity. The bacterial synthetic machinery is diverted to the production of virion and proteins after the bacteriophage enters the cell. Finally, bacteriophages are assembled and packed, and cells are lysed, introducing new pathogenic organisms that can infect additional bacterial cells. (Young, 2013).Lytic phages are preferred in phage therapy for two reasons. For starters, lytic phages will destroy their host bacterium, whereas temperate phages will not.

Second, temperate phages can transfer virulence and resistance genes due to their life cycle, in which the phage genome integrates into and replicates alongside bacterial genetic material. (Wittebole, , et al., 2013) (Criscuolo, et al., 2017)Lytic phages' intrinsic properties, such as high host-receptor specificity and bacterial cell lysis to release virions, make them ideal for therapeutic uses. (Gelman,, et al., 2018,) (Drulis-Kawa,, et al., 2012,)

The short replication time and ability to obtain a large number of viral progeny only in their specific hosts, the specificity to prevent damage in nonpathogenic bacteria (they are ecologically safe and have no known side effects), and the fast and low-cost production are some of the remarkable features of phage use. Furthermore, their small genomes allow us to better grasp the molecular pathways involved in managing resistant cells.(Gelman,, et al., 2018,)Some notable characteristics of phage utilization include their short replication period and ability to produce a large number of viral progeny solely in their specialized hosts, as well as their ability to minimize damage in nonpathogenic bacteria (they are environmentally safe). (Stern, & Sorek,, 2010,)

Bacteriophage as unidentified molecules that inhibit bacterial growth but in 1917 D'Herelle was the first to isolate and characterize phages and he also developed the first phage therapy against fowl typhoid induced by Salmonella Gallinarum in chickens . Scientist demonstrated that bacteriophages can be used to significantly reduce the cecal colonization of salmonella enterica serotypes Enteritidis and Typhimurium in commercial broiler chickens (Atterbury , 2006). Moreover , Huff , et al., 2002 confirmed that higher levels of bacteriophage is desirable to improve the feed efficiency , which is in agreement with Atterbury , et al., 2007 who demonstrated that higher dosage of bacteriophage is desirable to maintain growth performance by reducing the S. enteritidis and S. Typhimurium in cecal content . demonstrated the bacteriophage supplementation could reduce cecal salmonella colonization of broiler chickens & bacteriophage can increase lactobacillus and decrease E. coli and Salmonella concentration in excreta (Atterbury , et al., 2007).

2.3 Bacteriophage as AGPs

Bacteriophages have numerous advantages that make them effective instruments for treating bacterial infections and combating the emergence of bacterial resistance. One of the most serious concerns about antibiotics is their side effects, which can harm the microflora, which is related to a variety of imbalances and diseases. In this aspect, phage specificity can solve the problem because they will only replicate within their unique host (phages cannot infect

eukaryotic cells). Unlike antibiotics, phages can proliferate rapidly inside the host (but only if they find a host), can be supplied in small doses with extended intervals of time between them, and are removed once their population is eliminated. (El-Shibiny, & El-Sahhar,, 2017) (Matsuzaki, , et al., 2014) Phage action within the host is quite specialized, as phage replication occurs only inside bacteria. Antibiotics, on the other hand, are less precise and reach more places without the presence of bacteria in the organism. (Golkar,, et al., 2014,)Another advantage of phages is that they can be employed in difficult-to-reach regions of the body, such as treating central nervous system infections, which are a common cause of death. (Wittebole,, et al., 2013,)

Phage evolution is a noteworthy trait, since antibiotics are static compounds that cannot alter even if their environment changes. As previously said, another intriguing aspect of phages is their isolation and manufacturing costs. The expense of antibiotic manufacture is significant, both commercially and because antibiotics are not natural and have to be created in a laboratory. (Matsuzaki, , et al., 2014)It is worth emphasizing that the high specificity of phages is both a benefit and a drawback of phage therapy. Phages protect the microflora, but they must be tested in vitro to discover which bacteria are causing the sickness. This might be a tough process because identification must occur rapidly in order to apply the treatment to the patient. (Torres-Barceló, & Hochberg,, 2016,) (Kutateladze, & Adamia, , 2010,)

There are no bacteria that cannot be lysed by at least one bacteriophage, in theory. In this aspect, bacteriophages outperform antibiotics because, while some antimicrobial medicines have a broad spectrum of activity, no antibiotic exists that can kill all bacterial species. The most appealing feature of bacteriophages, however, is their specificity of action, or their ability to kill only the pathogen that they recognize. (Domingo-Calap & Delgado-Martínez, , 2018)

Bacteriophages are thought to offer several further advantages against antibiotics. Because bacteriophages replicate solely in the target bacterium and cannot infect mammalian cells, they are expected to be substantially safer and more tolerable. This result appears to be confirmed by all of the previous experiences gained in Eastern Europe, as well as all of the more recent research conducted in experimental animals and humans, which have not documented substantial adverse outcomes following bacteriophages treatment. (Kakasis & Panitsa, , (2018)) Because of the increase in bacteriophages concentration at the site of infection after the initial delivery, very few doses are required in general. Antibiotics, on the other hand, have an effect that is restricted to the site of infection that can be reached, even

when bacteria are located in a body organ or system that antimicrobials cannot penetrate. A lytic phage, EC200(PP), was tested in meningitis models with 100% mortality against S242, a fatal neonatal meningitis E. coli strain. Despite the fact that low bacteriophages titers were identified in the central nervous system, (Pouillot, , et al., 2012)

Edgar, , et al., 2012 demonstrated that It was possible to attack both the biofilm matrix and the bacterial cells at the same time. The modified bacteriophages decreased the number of bacterial biofilm cells by around 99.9%, which was a highly positive outcome. Additionally, bacteriophages genetic alterations can aid in the fight against bacterial drug resistance.

Finally, Miedzybrodzki, , Weber-Dabrowska, , Górski, , & Fortuna, , 2007 expressed into their paper using bacteriophages may be less expensive than using antibiotics that go after infections that are multidrug resistant. A small number of people with methicillin-resistant Staphylococcus aureus infections were examined.Potential advantages of bacteriophages compared to antibiotics are those specificity of action, Narrow spectrum of activity, Higher Safety, Higher tolerability ,Easy administration, Effect limited to the site of infection ,Possible additional benefits after engineering & less expensive.

Additionally, numerous findings of decreased intestinal E. coli, Campylobacter jejuni, and Salmonella spp. colonization of food-producing chickens and ruminants after bacteriophages treatment have been published. As a result, BPs are widely used to avoid food contamination. (Rosenquist, , et al., 2003) (Loc Carrillo, , et al., 2005) (Sheng, , et al., (2006).) (Carvalho, , et al., 2010)

Finally, there have been various attempts to treat animal respiratory infections with bacteriophages. For example , Hawkins,, et al., 2010 presented 10 dogs with persistent, unresponsive P. aeruginosa otitis media were treated with bacteriophages. The auditory canal of one animal received a single dosage of a topical preparation comprising about 1-105 plaque-forming units (PFU) of each of six bacteriophage that are specific for these infections. A clinical score based on five indicators was used to evaluate the status of the afflicted ear before treatment and 48 hours afterwards. Aural swabs were also collected to measure the concentrations of P. aeruginosa and blood pressure. Very safe and effective treatment was received. The pathogen mean count decreased by 67% (95% confidence interval (CI) 52-82, p 0.001), and the mean score was reduced by 30.1% (range 7.7-56.3%, p 0.0001).bacteriophage/swab counts significantly increased along with the improvement (mean 5.9 107 PFU/swab, range 1.7 106 to 2.6 108 PFU/swab). The bacteriophage counts were higher in all the animals than those given (mean increase: 99.1-fold, range: 2.8-433.3-fold),

indicating strong viral replication. No associated inflammation or other unfavorable local or systemic effects were brought on by the treatment. Additionally, administering bacteriophages by aerosol spray to broiler hens with an E. coli respiratory infection decreased their mortality rate. (Huff,, et al., 2003)

The inclusion of antibiotics and bacteriophage is partially effect the improvement in feed (Huff , et al., 2002)efficiency was observed during starter phase (from day 1 to 14) , and this effect diminished as age of chicks increased . This is likely because the younger chicks are more susceptible to pathogens and sensitive to the additives that can inhibit those potential pathogens in the environment . And the presence of bacteriophage enables the younger chicks to overcome potential pathogens that are not so detrimental to growing or finishing grower or finisher .In this way, it can work better in earlier age.

(Atterbury , et al., 2007) also reported that three broad host range phages against S. enteritidis, S. Typhimurium and S. Hadar as a pre-harvest treatment in broilers chickens, and suggested that high phages was potentially effective to reduce the levels of salmonella colonization in bacteriophages' significance has been founded on their ability to target certain species or strains of harmful bacteria.Bacteriophages' significance has been founded on their ability to target certain species or strains of harmful bacteria.

There is mounting evidence that treating hens exposed to particular infections with a single or combination of specific Bacteriophages by aerosol spray, muscle injection, or oral gavage reduces mortality and improves clinical symptoms of illness.The phenomena of co-evolution is one of the main downsides of extensive bacteriophage use, particularly in an agricultural environment. Bacteriophages and their hosts continue to engage in a tug-of-war in which the bacteriophage overcomes the resistance of the host bacterium to the bacteriophage of broilers. (Piniero , et al., 2008)

The primary requirements for any phage-based product used in poultry veterinarian care, poultry production, or the poultry business are security and effectiveness. The timing of the administration of phage-based products, together with the dosage, delivery strategy, and concomitant usage of medication, is crucial(Mills, et al., 2013;). The bacterial genome contains genetic material from lysogenic phages. As a result, they might act as food chain intermediaries for horizontal gene transfer between microorganisms, animals, or people. Because of research advances, it now seems conceivable to understand how genes transfer between phages and their hosts. Because of this, it would also be possible to stop potentially dangerous bacteriophages or redesign them so they are incapable of spreading any other kinds

of genes or undesirable traits (Garcia, et al., 2008).The interactions between the phage and the antibody do not always result in viral inactivation. However, different phages may respond to antibody neutralization in various ways. It is uncertain how a phage's immune system interacts with that of its host. Testing phage types' ability to prevent antibody neutralization. (Joerger & Ganguly , 2017;)To optimize the activity of phages that heal the illness of the inner cells and the covering and protection of those that are fed to animals, modern technical approaches have been developed. Phage encapsulation is one such innovation. (Yang , et al., 2009;)

2.4 Limitation of bacteriophage

Limitation of bacteriophage is the absence of specific activity for a given bacterial strain and difficulty in production of bacteriophage genome without integrase genes, antibiotic resistant genes ,genes for phage-encoded toxins or genes for other bacterial virulence factors .Another problems related to the formulation and stabilization of pharmaceutical preparations. Thereafter possible emergence of bacterial resistance against bacteriophages . Another contribution of bacteriophages in the development of antibiotic resistance . Reduced activity due to immune system response to bacteriophages finding a useful bacteriophage is quite difficult . Although there are distinctions between the different bacterial pathogens ,the initial stage involves isolating bacteriophages , which are typically found in sweage and waste water. For instance, it is much simpler if the bacteriophages target P. aeruginosa rather than S, aureus(Mattila, , et al., 2015)

A bacteriophage must first be shown to be specific for a particular bacterial strain before it can be considered a possible therapeutic agent. This is a somewhat challenging topic because the proof of a bacteriophage's lytic capacity might vary depending on the interactions between the bacteriophage and the bacteria, how those relationships change over time, the amount of virus utilized in the test, and other factors. The bacteriophage genome also needs to be sequenced and checked for the presence of integrase genes, such as those of the lysogenic type, antibiotic resistance genes (ARG), genes for toxins expressed by phages, or other genes for bacterial virulence. Finally, issues with the creation and preservation of pharmacological formulations for usage in clinical settings remain to be resolved. (Vandenheuvel, , et al., 2015)In this regard, it must be emphasized that studies appear to show that the stability of preparations for clinical use is solely dependent on bacteriophages, and stabilization tactics should be tailored specifically for each bacteriophage. (Miernikiewicz, et al., 2003)Factors that can limit the uses of bacteriophage is the absence of specific gravity for a given bacterial strain. Difficulty in production of bacteriophage genome without integrase genes , antibiotic resistant genes,

genes for phage encoded toxins or genes for other bacterial virulence factors. Problems related to the formulation and stabilization of pharmaceutical preparations . Possible emergence of bacterial resistance against bacteriophages . Contribution of bacteriophages in the development of Bacterial resistance and reduced activity due to immune system response to bacteriophages.The need to find nonantibiotic methods to prevent and treat bacterial disease has grown in importance as a result of the emergence of antibiotic resistance and the pressure to reduce the use of antibiotics in animal production. Research on the effectiveness of bacteriophages as an antibiotic substitute has increased as a result. Numerous diseases affecting plants, animals, and people can be effectively treated by bacteriophage therapy. . (Kutter & Sulakvelidze, 2005)

2.5 Bacteriophage against pathogenic bacteria

Our studies have shown that bacteriophages can be utilized to prevent and treat chicken colibacillosis (Huff , et al., 2002). Everywhere can be found Campylobacter spp., and they favor the bird's gut, where they coexist as commensals. A natural reservoir for Campylobacter spp., the primary cause of illnesses in humans, poultry has developed due to its favorable (optimum) body temperature (Young , et al., 2007) .Recent studies have shown that phage therapy is successful at reducing Campylobacter colonization in chicken and, consequently, the risk of it entering the food chain. A phage cocktail containing virulent Campylobacter phages was administered orally to broiler chickens that had Campylobacter jejuni (C. jejuni) infections (Atterbury , et al., 2003). Although Campylobacter spp. are widely distributed in the environment, it prefers to live commensally in the stomachs of birds. Poultry is a natural reservoir for bacteria due to Campylobacter's body temperature, which accounts for the majority of human infections. After hatching, Campylobacter spp. colonizes chicks in poultry farms in about seven days. When chickens are infected with Campylobacter, there are typically no visible symptoms or lesions. According to reports, chicken flocks have anywhere between 2% and 100% of Campylobacter spp. (Sahin , et al., 2015) .In some research, the presence of Campylobacter at the time of slaughter was detected in 100% of small intestine samples and 91.5% of carcass surface swabs. (Wysok , et al., 2015)Other authors pointed out that the frequency of campylobacter was lower in broiler chickens (34.3%, cecum samples). (Nowaczek , et al., 2019)

The prevalence of C. jejuni was specifically reduced by bacteriophages, but the microbiota did not change. According to studies, utilizing bacteriophage control to lower the number of C. jejuni in chickens may reduce human exposure and morbidity brought on by consuming

contaminated poultry products (Richards , et al., 2019;). Salmonella is a significant bacteria that affects commercial chicken and is the second-most significant zoonotic foodborne pathogen (after Campylobacter).In the 1990s, (Berchieri , et al., 1991)Following repeated oral delivery of the bacteriophage cocktail, there appears to have been a considerable reduction in the number of Salmonella cells in chicken cecumOther researchNabil , et al., 2018 revealed the presence of salmonella-specific bacteriophages in poultry sewage samples and infected broiler chickens. Bacterial phages from sewage water were used to treat salmonella infections before being administered orally to chicks and followed by four further phage therapies. Salmonella treatment for hens using phages was successful because no pathogen (bacteria) was discovered in the cecum after the fifth dose (at 15 dpi).

Commercial poultry is susceptible to numerous germs, but Salmonella is the second-most significant foodborne disease (after Campylobacter). (3) Nonmotile Salmonella infections come in three different forms: (1) The subspecies enterica serovar pullorum causes a host-specific Salmonella enterica infection (S. pullorum). Second, S. gallinarum is the agent that causes Salmonella enterica infections (subspecies enterica). Pullorum disease (PD), an acute systemic infection, is a disease of young birds caused by S. pullorum. Adults who are asymptomatic carriers are typically infected. S. gallinarum, a septicemic illness that mostly affects growing and mature birds, produces fowl typhoid. Nonhost-specific infections brought on by S. typhimurium and S. enteritidis are brought on by a motile Salmonella enterica serotype called paratyphoid salmonella (PT) , Although PT infections are common in chicken, acute systemic illness is only common in young, highly vulnerable birds under stressful conditions. These symptoms are typically seen in young birds (less than four weeks old). Unaffected internal organs and digestive tracts can become continuously colonized by PT infections in chicken, which could contaminate finished goods. Acute or chronic infection of birds called avian arizonosis (AA) is brought on by Salmonella enterica subspecies arizonae. The disease may still be present in the bird even if clinical signs often do not occur until the bird is older and has developed the condition. (Gast, 2013.) .

Although Nolan, et al., 2013 demonstrated various distinct Staphylococci strains have been discovered from chicken, Staphylococcus aureus is the most typical. Staphylococci are common in poultry habitats and can be found in healthy birds' skin and mucous membranes. According to Andreasen , 2013reports, S aureus infection causes a variety of conditions, including arthritic pain, synovitis, chondronecrosis, osteomyelitis, gangrenous dermatitis, subdermal abscesses (bumblefoot), and green liver-osteomyelitis complexes.

There are several instances of food poisoning where enterotoxin-producing bacteria are present. When poultry carcasses are contaminated with S aureus during processing, it might result in poultry-related food poisoning (especially when enterotoxin-producing strains are present). Additionally, poultry meat has been found to have Methicillin-resistant S aureus (MRSA). (Feßler, et al., 2011)

According to (Huff , et al., 2009), it is possible to prevent the onset of air sacculates command, a condition that affects a variety of microorganisms including S. aureus , (Marek, et al., 2019;) E. coli, and others. If an antibiotic is used in conjunction with bacteriophage therapy, the amount of antibiotics needed to treat bacterial illnesses may be decreased. (Żbikowska, et al., 2020;)Clostridium perfringens (C. perfringens) is a common member of the intestinal microbiota of chickens and is widely spread in the natural ecosystem. At low population levels (104 CFU), it is not harmful; however, the majority of its morbidity is caused by pathogens. According to several scientists, endolysin encoded by C. perfringens phages may be very useful for managing this disease. The results indicated that endolysin may be effective against all of the C. perfringens strains tested, while there may be variations in the sensitivity of the different strains. In poultry, C. perfringens causes necrotic enteritis (NE), which can be prevented by the bacteriophage (INT-401). The experimentally infected broiler chicken's phage therapy by food or water allowed them to gain weight more quickly, have a greater conversion of feed ratio (FCR), as well as reduce their mortality rate. (Żbikowska, et al., 2020;)Several different C. perfringens strains were resistant to infection by the phages. Additionally, phage activity seems to be restricted to a particular strain of this bacteria. According to several scientists, endolysin encoded by C. perfringens phages may be very useful for managing this disease.. (Monk , et al., 2010) (Wernicki , et al., 2017;)

According to recent publications (2019) from the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), campylobacteriosis, salmonellosis, Shiga toxin-producing E. coli, and yersiniosis are the most common zoonoses in Europe (EU). Lytic bacteriophages can only be used to treat bacterial illnesses with phage treatment because of their poor capacity to kill bacteria. Bacteriophages have far higher specificity than antibiotics. Antibiotics destroy harmful bacteria, but they also change the typical microbiota in the gastrointestinal system, leading to dysbiosis, immunosuppression, and subsequent infections. (Lin , et al., 2017)

Additionally, R. W. Miller, et al., (2010),showed that bacteriophages can effectively manage necrotic enteritis. However, the administration of bacteriophage must be feasible within the

chicken production system for bacteriophage therapy to be commercially effective in poultry production. When bacteriophages were given in drinking water and the birds were exposed to Escherichia coli via the air sac, we were unable to demonstrate any efficacy of bacteriophage therapy for colibacillosis. (Huff , et al., 2002). Bacteriophages are common in all environments and are thought to outnumber known bacteria by a factor of about 10, making them excellent candidates to eradicate infectious illnesses. (Brüssow , 2005)This mode of treatment does not harm the commensal gut flora. Bacteriophages self-replicate throughout treatment, therefore they don't need to be used regularly. A decrease in phage titer is caused by their failure to adhere to and grow in eukaryotic cells, and this is linked to a considerable reduction in the amount of harmful bacteria infecting the organism. Another crucial feature of phages is that they are non-toxic due to the fact that they frequently consist primarily of proteins and nucleic acids (Loc-Carrillo & Abedon , 2011) .Since bacteriophages can infect different species, serotypes, and strains, they are far more specialized than antibiotics. Antibiotics affect the microbiota of the gut in addition to killing pathogenic bacteria, which raises the danger of dysbiosis, immunosuppression, and subsequent infections. In order to cure bacterial infections in poultry, new bacteriophage therapies are quite successful. (Lin , et al., 2017;) When pathogenic germs are common in chicken houses, the probability for recovery on carcasses entering the processing facility increases. Through poultry products that have been carelessly handled, undercooked, or cross-contaminated in the kitchen, humans can contract Salmonella and Campylobacter. Salmonella has a negative effect on the chicken industry because it has the potential to damage consumer markets and raise production and processing costs (Payne & Kroger , 2005;).

These infections, which are typically detected in bird droppings, have the potential to remain for a very long time in the environment. When there are feces in the litter, disease populations might grow around the bird. The control and prevention of hazardous infectious diseases depends heavily on the cleanliness of chicken buildings. Growers can benefit from an efficient sanitation program by seeing an increase in bird performance and a decrease in the number of flocks that are ill. Any variety of best management practices, therapies, or disinfectants can be incorporated into a sanitation program. However, ineffective sanitation practices may have a negative effect on illness prevention and reduce bird numbers. performance (Payne, et al., 2005)

2.6 Importance of Anti-microbial resistance

Anti-microbial resistance is one of the foremost genuine risk to general public health. In exertion to play down hazard of antimicrobial resistance , activity to diminish and dispose of the utilize of antimicrobial resistance, activity to diminish and dispose of the utilize of anti-microbial development promoter in livestock industry are expanding mandatorily and deliberately. Be that as it may such a development have actuated critical financial misfortunes for makers and expanding utilization of antimicrobials for the restorative treatment. The request for options to AGP is ever solid for modern arrangement that not as it were can straightforwardly combat antimicrobial safe microbes but improve efficacy of restorative treatments. There has been a around the world increment within the direction or boycott of the utilize of AGP's in poultry diets.

According to the definition by FAO/WHO , Probiotics are , " Live micro-organisms which administered in adequate amounts confer a health benefit on the host' (Fuller , 1989). Bacillus licheniformis is a probiotic strain that is known to be beneficial for birds health. It helps to improve the absorption of nutrients from food and can help to improve digestion. (Sögaard & Suhr-Jessen, 1990)

2.7 Effect of bacteriophage on ammonia production

Existing production systems for broiler chicken production often use bedding materials to absorb moisture from bird droppings and to improve the health of the birds. The combination of bedding material and bird droppings is called litter. In this trial , both bedding & litter is used. Traditional bedding materials used around the world are generally organic (e.g. sawdust, tree bark, rice husks, peanut shells and seeds, straw, shredded paper) , but some inorganic materials have also been used (e.g. sand). (Watson & Wiedemann, 2018) The increase in demand and cost of chicken litter has stimulated interest in alternative sources of litter around the world. and agricultural by-products (e.g. cereal crop by-products, crops and seed husks) have been proposed (Kheravii, et al., 2017) (Garces, et al., 2017) (Villagra, et al., 2011). To date, there have been several studies on the potential hazards and contaminants of using used chicken manure to fertilize the soil. (Kyakuwaire, et al., 2019) However, very few studies have investigated potential contaminants harmful to animals and humans in pre-use bedding materials, and they are limited to recycled wood and paper (Fernandes, et al., 2019). Recently, there have been many examples of meat and egg contamination due to exposure of chickens to persistent organic pollutants through contaminated feed, housing materials and

litter in conventionally raised chickens. Uncharacterized alternative bedding materials may increase these risks. litter may also be associated with increased morbidity and mortality in chickens. (Garces, et al., 2017)

Table 1:Manure management system

Manure Management System	Description	Relative Emission		
		CH_4	N_2O	NH_4
Poultry with litter Broiler/Pullet/breeders	Enclosed Poultry houses utilize bedding material (Wood shavings, Rice hulls etc.) The bedding absorbs moisture & dilutes manure	Low	High	High
Poultry Without Litter Poultry Layers/Broiler breeders	In high rise cages or scrape-out/belt systems, manure is excreted onto the floor below with no bedding to absorb moisture. The ventilation system dries the manure as it is stored	Low	Low	Low

Source - (IPCC, 2006)

According to Angus , et al., 2006 several potential Intensive poultry production in the confined condition ,the use of reused litter which is used as bedding materials that is responsible for the emission of a significant amount of air pollutants . The most commonly farming produces the gases such as Carbon Dioxide(CO_2) , Carbon monoxide (CO) and ammonia (NH_3), with NH_3 being the main gas which negatively affects birds and workers which is generally found in high concentrations in poultry farms. These are the primary greenhouse gases to the atmosphere which is emitted by poultry production mechanically and non mechanically. In non mechanical way direct emissions occur from the decomposition and nutrification /de-nutrification of poultry waste (Manure & Urine) where CH_4 & N_2O are emitted . This reused waste is collected and stored also emits CH_4 & N_2O. Methane from enteric fermentation and manure management are the main sources of CH_4 that is emissions from agricultural activities and of all domestic livestock dairy and beef cattle are the largest

emitters of CH_4 . Poultry reared in management systems with litter and using solid storage have relatively high N_2O & low CH_4 emissions. Broiler, pullets and to an extent breeders, are reared using these manure management system. Commercial layers are typically reared in high rise cages or scrape-out/belt systems where the manure is excreted onto the floor below with no bedding to absorb moisture. The ventilation system dries the manure as it is stored. In some broiler breeder houses a part of the manure is collected under the slats in the houses making it similar to the commercial layers. In this type of manure management system both CH_4 & N_2O are relatively low (IPCC, 2006).Considerable waste generation , mixed with a significant ammonia emission potential, can negatively affect the air quality in the facilities and surroundings of the animal production facility. Waste in the avian litter causes gas production inside the facility and considered the main source of gas emissions (Nääs, et al., 2007; Lima, , et al., 2021) , mainly because almost half of the amount of the nitrogen in the feed is retained as animal protein, the rest is executed as waste. Of this, 35% has potential for emission by being converted to ammoniacal nitrogen, given the ammonia emission factor of waste excreted by bird. (de , et al., 2020.)

When birds ingest amino acids in the form of proteins, they are adsorbed and either synthesized into other amino acids or metabolized to generate vitality. Even though poultry diets frequently contain high levels of protein, a proportion of these proteins are excreted in their undigested manner, i.e., nitrogen that hasn't been metabolized as protein is excreted directly, and a portion of what has been processed outcome in the release of uric acid; though the microbial misconduct, alkali is released into the surrounding environment(Mgalula, , et al., 2021.).Animals excrete nitrogen in the form of uric acid, urea and ammonia, and birds mainly excrete uric acid(Swelum,, et al., 2021)which, when degraded, releases ammonium (NH4 +) a dominant form of nitrogen in poultry waste. Under certain conditions, such as high moisture levels and increased pH values, ammonium is rapidly converted into ammonia (NH3), an extremely volatile substance that negatively affects the quality of air inside the aviary (França , LGF, ; Tinoco, IFF;, (2014)) (Swelum,, et al., 2021). As mentioned before, ammonia volatilization is more common under alkaline conditions, with pH values around 9, coupled with high temperatures and increased ammonia concentrations in the waste (Insausti, , et al., 2020.)The degradation process of uric acid occurs according to

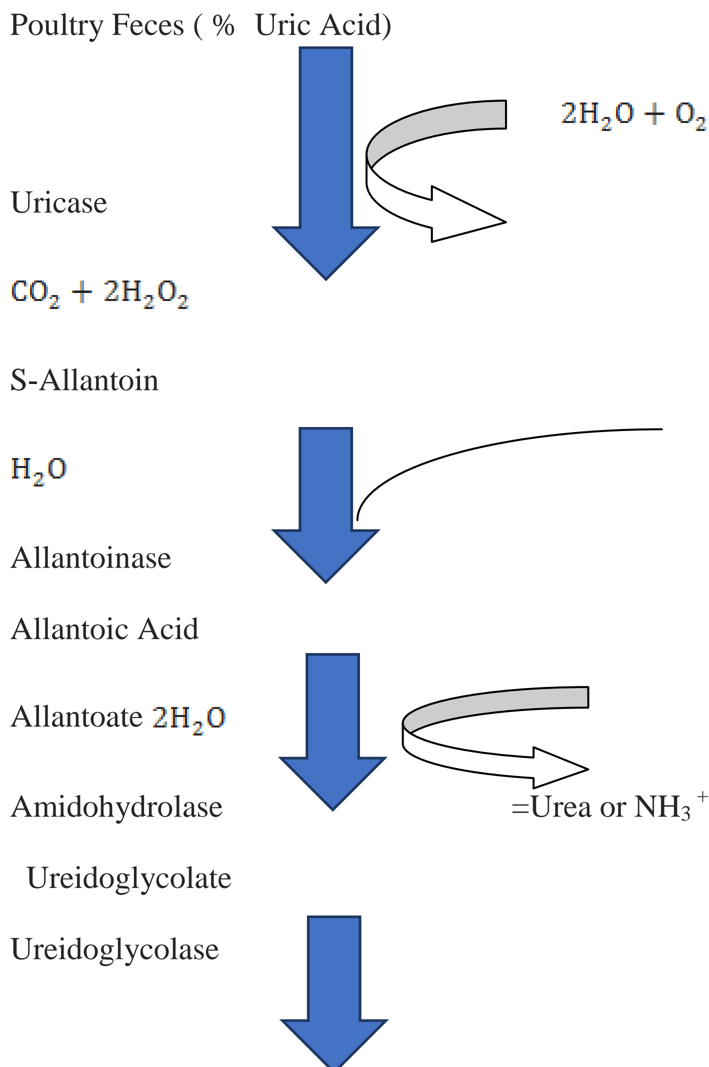
$$NH_4^+ + OH^- = NH_3 + H_2O$$

In summary, ammonia in broiler production environments is formed through chemical and microbial decomposition of uric acid excreted by birds. The decomposition process is carried

out by urease, an enzyme produced by microorganisms, which catalyzes the hydrolysis of urea into ammonia and carbon dioxide in aqueous medium, allowing ammonia volatilization, defined as nitrogen losses to the atmosphere (Oliveira , et al., 2004) . The decomposition of urea occurs according to :

$$(NH_2)_2CO + H_2O = 2NH_3 + CO_2$$

Five enzymatic steps are involved in the aerobic degradation of uric acid (Figure 1). First, uric acid ($C_5H_4N_4O_3$), the dominant form of nitrogen in the excreta, is converted into allantoin ($C_4H_6N_4O_3$) by the enzyme uricase. In the second step, allantoin is converted to allantoic acid by allantoinase. Subsequently, allantoic acid is converted to ureidoglycolate by allantoate amidohydrolase, and ureidoglycolate is then converted to glyoxylate and urea by ureidoglycolase. The last step consists of the hydrolysis of urea ($(NH_2)_2CO$) into ammonia (NH_3) and CO_2 by the enzyme urea (Behera, , et al., 2013.)



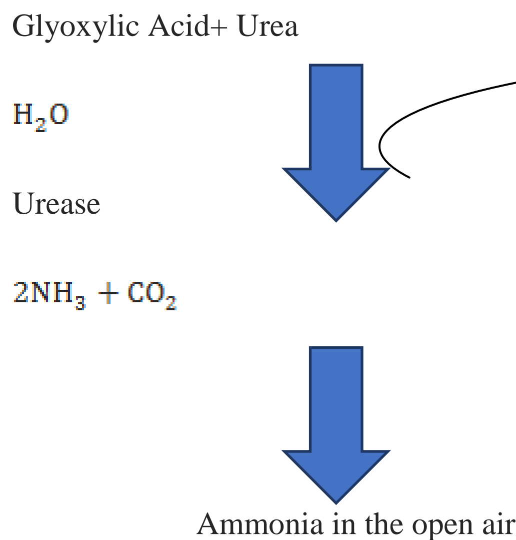


Figure--1:Stages of aerobic degradation of uric acid in ammonia. (Behera, , et al., 2013.)

2.7.1 Effects of ammonia in the environmental pollution

High levels of ammonia in the environment negatively impact both the health and production of animals and workers (Behera, , et al., 2013.) There are, basically, four risks arising from the production of pollutants in animal production environments: worker health, animal health, neighbor health, and deterioration of the facility and equipment (Lima, , et al., 2021) International standards for maximum ammonia concentration limits suggest that the concentration varies according to the period of exposure to the environment affected by ammonia; maximum limits are 25 ppm for 8 hours, 35 ppm for 15 min, and 50 ppm for 5 min (Jeevanandam,, et al., 2018)

The Brazilian legislation through the Ministry of Labor and Employment, according to Regulatory Norm NR15 (ABNT, 1978) established the maximum limit of 20 ppm of ammonia in work environments for a period of up to 48 hours per week. In the literature, 20 ppm of ammonia are recommended as the maximum tolerable value for continuous exposure in an animal production environment (Naseem, & King,, 2018).Excess ammonia in the environment can also cause various disorders and problems in animals, such as reduced appetite and respiratory rates, burns and calluses on the cushions of the feet, skin irritations, calluses in the chest, eye irritation, conjunctivitis, blindness, respiratory system issues, weight loss, low uniformity, and susceptibility to viral diseases and infections, thereby significantly decreasing productivity (Naseem, & King, , 2018.)

2.7.2 Effects of ammonia in broiler production

High concentrations of ammonia in broiler production facilities negatively influence the breeding environment, affecting animals and caretakers as well as locations close to these facilities (Medeiros , et al., 2008)Exposure to ammonia impairs weight gain , feed conversion ratio, and viability in the production of broilers, as it affects the average weight and feed intake and, for several reasons, can cause the death of the birds before the end of the productive cycle. In facilities with ammonia levels of 25 ppm during the entire growth period, a significant reduction of the final body weight was observed in the broilers produced, with an average total weight loss of 90 g per bird (Lott & Donald , (2015)) (Miles , et al., 2004). Continuous exposure to an ammonia-saturated environment, even at low levels, causes irritation of the respiratory mucosa of birds, increasing susceptibility to respiratory diseases. In humans, continuous exposure even to low ammonia levels can cause eye and lung irritations (Gay & Knowlton , 2009)In addition, ammonia is a highly corrosive compound that contributes to the deterioration of metal equipment and parts.Small particles, PM2.5, formed in the air by ammonia and other components, pose a health problem due to the impacts on the respiratory system. When inhaled, they can reach the lungs, and even short-term exposure can cause eye, nose, and throat irritation, in addition to coughing and sneezing. Long-term exposure can lead to a variety of respiratory and cardiovascular issues (Bittman & Mikkelsen , 2009) .Elevated levels of NH_3 in the premises can generate production losses by reducing feed efficiency and growth rates; excessive occurrence of certain particles can cause stress, affecting the immune system and generating vulnerability to diseases, thereby decreasing the productive performance of animals and workers (Osório , et al., 2009)). Ideally, ammonia levels are kept below a concentration of 25 ppm. Although the maximum acceptable limit of ammonia concentration is 20 ppm, but a maximum level of 10 ppm should always be the objective (Groot Koerkamp, et al., 1998)

Signs of lesions from ammonia intoxication vary according to the age of the bird, the degree of exposure, and the concentration of the gas. Prolonged exposure to high concentrations of ammonia, such as 50 to 100 ppm, decreases production due to the incidence of increased lacrimal secretion, catarrhal tracheitis, keratoconjunctivitis, and photophobia, which may result in more serious problems such as blindness (Egute, et al., (2010))

Ammonia emitted in concentrations higher than 60 ppm within animal production facilities leaves birds more susceptible to respiratory diseases, predisposing them to risks of infections secondary to vaccinations (Oliveira , et al., 2004) . In extreme cases, when concentrations

reach 100 ppm, immediate reduction of the respiratory rate can be the result, which leads to death even under short-term exposure (Groot Koerkamp, et al., 1998).Exposure to ammonia, pollutants, and aerial microorganisms significantly affects the growth of broilers, favors susceptibility to disease, reduces food consumption, alters feed conversion and growth rate, and increases mortality. Gas emissions in the production of broilers negatively influence the production environment and the surrounding region, causing considerable economic and financial losses(Medeiros , et al., 2008).High ammonia emissions can lead to weight losses of up to 250 grams per bird by current weight standards, with continuous exposure at 25 ppm levels, indicating losses of 90 grams per bird, and increased condemnation of carcasses to 500 birds per productive lot (Lott & Donald , (2015)).

2.7.3 Economical loss due to ammonia production

Even when exposure only occurs in the first weeks of breeding, the birds show significant weight reductions at the end of the production cycle (Miles , et al., 2004). In terms of financial losses resulting from high levels of ammonia in a shed with 20,000 birds with an ammonia concentration of 50 ppm, losses amount to around US$ 450.00 relative to weight losses of birds, about US$ 700.00 relative to the ration, because the increase of 8% in feed conversion, an average of US$ 160 with diseases and US$ 150 with condemnation of carcasses (Lott & Donald , (2015)) Ritz et al 2005). In a previous study about broiler production environments, ammonia reductions of 10% resulted in a final weight increase (at 42 days) of more than 45 grams (Miles , et al., 2004). Adequate management practices and efficient control of ammonia emissions may represent significant differences between profit and loss for producers; the costbenefit ratio of ammonia control is favorable when weight loss and increased feed efficiency are accounted for (Lott & Donald , (2015))

Table 2 : Different ammonia concentrations and the impacts on worker and bird health

Concentration (ppm)	Worker	Bird
5	Few can be detected by odor	
10	Most People can easily detect by odor	
20	Environmentally unhealthy	Initial discomfort
20-35	Respiratory system issues including coughing , salivary secretion, even urine retention	Maximum tolerable amount for birds at long term Exposure
35-40		Maximum tolerable amount for birds at short term exposure

50	Acute eye irritation	Acute eye irritation
80		Reduced feed consumption & growth
100	Eye Burns , temporary Blindness and skin irritation may occur	Drastic reduction of respiratory rate, consumption, growth
500	Violent attack of cough, severe irritation in the lungs , Pulmonary edema can lead to death	Lethal dose

Source from (Perry , (2003))

3 MATERIALS & METHODS

3.1 Statement of the experiment

The research work was conducted at Sher-e-Bangla Agricultural University Poultry farm, Dhaka , with 600 day-old straight run (Hubbard Efficiency Plus) commercial broilers for a period of 35 days from 8th November 2022 to 14th December to evaluate the performance of broiler, benefit-cost ratio& ammonia level of the farm using bacteriophage supplemented diet.The research helps to make a conclusion about bacteriophage as the alternative of antibiotic.

3.2 Collection of experimental broilers

A total of 600 one day old Hubbard classic broiler chicks were collected from Planet Agro, Sirajgonj.

3.3 Experimental materials

The collected chicks were carried to the University Poultry farm late at the night. Chicks were kept in the brooders equally for 2 days by maintaining standard brooding protocol. During brooding time only basal diet was given with no bacteriophage was used as treatment. After two days,360 chicks were selected from brooders and distributed randomly in three (3)dietary treatments of bacteriophage. Another 240 chicks were distributed randomly in one treatment for antibiotic and another treatment for control group. Each treatment had four

replications with 30 birds per replication . The total number of treatments were five and their replications were twenty (20).

3.4 Experimental treatments

T_0 – Negative Control, commercial feed with no antibiotics &bacteriophage

T_1–500 gm ProBe-Bac PE per metric ton of feed.

T_2–750 gm ProBe-Bac PE per metric ton of feed

T_3– 1000 gm ProBe-Bac PEper metric ton of feed

T_4– Positive Control with Bacitracin Methylene Disalicylate (BMD) 50/ton of feed

Table 3: Layout of the experiment

Treatment Groups	No. of Replication				Total
	R_1	R_2	R_3	R_4	
T_0	30	30	30	30	120
T_1	30	30	30	30	120
T_2	30	30	30	30	120
T_3	30	30	30	30	120
T_4	30	30	30	30	120

3.5 Preparation of experimental house

The experimental room was properly cleaned and washed by using tap water . Ceiling walls and floor were thoroughly cleaned and disinfected by spraying diluted iodophor disinfectant solution (3 ml/liter water) . After proper drying , the house was divided into 20 pens of equal

size using wood materials and wire net. The height of wire net was 36 cm. A group of 10 birds were randomly allocated to each pen (replication) of the five treatments. The stocking density was 1.076 ft^2/ birds

3.6 Experimental diets

Starter and grower commercial Haque feed were purchased from Joypurhat.

Table 4:Name and minimum percentage of ingredients present in starter ration

Name of ingredients in starter crumble(g)	Formulae T-0	Formulae T-1	Formulae T-2	Formulae T-3	Formulae T-4
Maize	480	480	480	480	480
Soya Meal	350	350	350	350	350
Rice Polish(Grade-A)	30.35	29.85	29.60	29.35	30.100
Soya Oil/ Palm Oil	15	15	15	15	15
Poultry Meal	25	25	25	25	25
F.F. Soya	60	60	60	60	60
DCP	8	8	8	8	8
LSP	12	12	12	12	12
Salt	3	3	3	3	3
Vitamin Premix broiler	1.5	1.5	1.5	1.5	1.5
L-Meth	3.5	3.5	3.5	3.5	3.5
L-lysine	2	2	2	2	2
Threonine	1	1	1	1	1
Toxin Binder	2	2	2	2	2
Choline Chloride	0.8	0.8	0.8	0.8	0.8
Genikan	2	2	2	2	2
Sodibicurb	0.8	0.8	0.8	0.8	0.8
YeamuneUP	0.5	0.5	0.5	0.5	0.5
Lipidol	1	1	1	1	1
Coccilock	1	1	1	1	1
Endopower Beta/ avemix	0.2	0.2	0.2	0.2	0.2
Hemicel ht	0.2	0.2	0.2	0.2	0.2
Endophos	0.15	0.15	0.15	0.15	0.15
BMD					0.05
ProBe Bac PE	0	0.5	0.75	1	
Total	1000	1000	1000	1000	1000

Table 5: Name and minimum percentage of ingredients present in grower ration

Name of ingredients in Grower (g)	Formulae T-0	Formulae T-1	Formulae T-2	Formulae T-3	Formulae T-4
Maize	570	570	570	570	570
Soya Meal	240	240	240	240	240
Rice Polish(Grade- A)	26.55	26.05	25.80	25.55	26.05
Soya Oil/ Palm Oil	25	25	25	25	25
Poultry Meal	20	20	20	20	20
F.F. Soya	80	80	80	80	80
DCP	8	8	8	8	8
LSP	12	12	12	12	12
Salt	3	3	3	3	3
Vitamin Premix broiler	1.5	1.5	1.5	1.5	1.5
L-Meth	3	3	3	3	3
L-lysine	1.8	1.8	1.8	1.8	1.8
Threonine	0.5	0.5	0.5	0.5	0.5
Toxin Binder	2	2	2	2	2
Choline Chloride	0.8	0.8	0.8	0.8	0.8
Genikan	2	2	2	2	2
Sodibicurb	0.8	0.8	0.8	0.8	0.8
YeamuneUP	0.5	0.5	0.5	0.5	0.5
Lipidol	1	1	1	1	1
Coccilock	1	1	1	1	1
Endopower Beta/ avemix	0.2	0.2	0.2	0.2	0.2
Hemicel ht	0.2	0.2	0.2	0.2	0.2
Name of ingredients in	Formulae	Formulae	Formulae	Formulae	Formulae

Grower (g)	T-0	T-1	T-2	T-3	T-4
Endophos	0.15	0.15	0.15	0.15	0.15
BMD					0.05
ProBe Bac PE	0	0.5	0.75	1	
Total	1000	1000	1000	1000	1000

Feed were supplied 4 times daily by following Hubbard Efficiency plusManual and adlibitum drinking water 2 times daily.

3.6.1 Collection of bacteriophage

Bacteriophage was used in commercial basal diets. This is collected fromOPTIPHARM an affiliate of pathway intermediates which is the global animal nutrition brand dedicated to research and development. This bacteriophages are sponsored by RS poultry , Dhaka , Bangladesh for conducting the research work

3.7 Managemental procedures

Body weight & feed intake were recorded every week & survivability was recorded for each replication up to 35 days of age . The following management procedures were followed during the whole experiment period.

3.7.1 Brooding of baby chicks

The experiment was conducted during `8thNovember 2022 to 14 December 2022. The average temperature was 31.5 ˚ C and the RH was 80 % in the poultry house. At the time of brooding it is divided into 5 treatment for one week . After one week the chicks were distributed in the pen according to replication randomly. There were 30 chicks in each pen each birds for 1.20 ft^2 . Due to cool climate brooding temperature was maintained as per requirement.Brooding temperature was adjusted (below 35˚C) with house temperature .So when the environmental temperature was above the recommendation ,then no extra heat was provided. At day time only an electric bulb was used to stimulate the chicks to eat & drink.In brooding extra heat was not provided at day time except midnight to morning. Due to hot weather at noon electric fans was used as per necessity to save the birds from heat stress.

3.7.2 Room temperature and relative humidity

Daily room temperature (˚C) and humidity were recorded with a thermometer and a wet and dry bulb thermometer respectively. Averages of room temperature and percent relative humidity for the experimental period were recorded that was given in appendix 2.

3.7.3 Litter management

Rice husk was used as litter at a depth of 6 cm . At the end of each day, litter was stirred to prevent accumulation of harmful gases and to reduce parasite infestation.At midnoon litter was stirred and electric fan was used for removal of excess gases of originate from litter and for drying the upper layer of bedding materials which is covered with bird droppings. At 3 weeks of age droppings on the upper layer of the litter were cleaned and for necessity fresh litter added.

3.7.4 Feeding & Watering

Feed and clean fresh water was offered to the poultry ad libitum. One feeder and one drinker were provided in each pen for 5 birds. Feeders were cleaned at the end of each week and drinkers were washed daily. All mash dry feed was fed to all birds ad libitum throughout the experimental period.

3.7.5 Lighting

For first 2 weeks 24 hours natural light was used& maintained the standard schedule

3.7.6 Bio security measures

To keep diseases away from the broiler farm recommended vaccination , sanitation program was undertaken in the farm and its premises as per requirement.

3.7.7 Vaccination

The vaccines applied to the experimental birds according to the vaccination schedule. Ther vaccination schedule is shown in table -6

Table 6:Vaccination schedule

Age of birds	Name of Disease	Name of vaccine	Route of administration
3 days	IB+ND	MA-5+ Clone 30	One drop in each eye
9 days	Gumboro	G-228 E (Inactivated)	Drinking water
17 days	Gumboro	G-228E (inactivated booster dose)	Drinking Water
21 days	IB+ ND	MA-5+ Clone 30	Drinking Water

3.7.8 Ventilation

The broiler shed was south facing open sided. Due to wire-net cross ventilation it was easy to remove polluted gases from the farm . Besides ventilation was regulated as per requirement by folding polythene screen.

3.7.9 Sanitation

During the experimental period strict sanitary measures were taken. Disinfectant (timsen) was used to disinfect the feeders and waterers and the house also.The following measures were taken during the experimental period to prevent diseases.

- Entrance of personnel was restricted except researcher , supervisor and co-supervisor who visited the farm following special care.
- Before entrance ,hands and feet were washed with soap and clean cloths wore while working.
- Footbath containing disinfectant (Iosan.) was used before entering the area .
- Adequate precautions were taken to vaccine storage, liquification and different methods of administration .
- New litter materials were dried and disinfected by using Virkon-s®, and mixed with lime powder before use.
- The experimental areas were kept free from rats, rodents wild birds & theft.

3.7.10 Medication

Table 7: Medication schedule. All are administered by water.

Day	Medication	Dose
0-1	Glucose Solution	1 gm/L
2-5	Electromin	1 gm /2L
6-8	Vitamin B complex with Calcium	1 gm/L
9-11	Vitamin AD_3E	1 ml / 4L
12-15	Cocci Cure (Gut mix)	1.5 gm/L
18-21	Vitamin B com	1 gm/L
22-25	Electromin	1 gm /2L
26-27	Electromin Vitamin B complex with calcium	1 gm/L 1 gm/L
28-35	Vitamin B complex with calcium Vitamin AD_3E	1 gm/L 1 ml / 4L

3.8 Collection of ammonia kit

The meter name is $P^{Hydrion\ TM}$ ammonia Meter . which is measured in PPM that is processing time to get results are 15 seconds

3.9 Study Parameters

3.9.1 Recorded parameters

Weekly live weight ,weekly feed consumption and death of chicks to calculate mortality percent were recorded . FCR was calculated from final live weight and total feed consumption per bird in each replication. After slaughter of broiler chicken from each treatment gizzard, liver , spleen , heart, proventriculus and bursa were measured from each bird. Dressing yield was calculated for each treatment to find out dressing percentage.

3.10 Data Collection

3.10.1 Live Weight

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

3.10.2 Dressing Yield

Live Weight – (blood + Feathers +Head+ Shank+Digestive System + Liver + Heart)

3.10.3 Feed Consumption

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

3.10.4 Mortality of chicks

Daily death record for each replication was counted upto 35 days of age to calculate mortality.

3.10.5 Dressing procedures of broiler chicken

One birds were picked up at random from each treatment at the 35th day of age and sacrifices to estimate dressing percent of broiler chicken. All birds to be slaughtered were weighed and fasted by halal method or overnight (12 hours) but drinking water was provided ad-libitum during fasting to facilitate proper bleeding. All the liver birds were weighed again prior to slaughter. Birds were slaughtered by severing jugular vein , carotid artery and the trachea by a single incision with a sharp knife and allowed to complete bleed out at least for 2 minutes . Outerskin was removed by sharp scissor and hand . Then the carcasses were washed manually

to remove loose feathers and other foreign materials from the surface of the carcass. Afterward the carcasses were eviscerated and dissected according to the methods by Jones(1982). Heart and liver were removed from the remaining viscera by cutting them loose and then the gall bladder was removed from the liver . Cutting it loose in front of the proventriculus and then cutting with both incoming and outgoing tracts removed the gizzard. Dressing yield was found by subtracting blood , feathers , head , shank , liver, heart and digestive system from live weight.

3.11 Calculation

3.11.1 Live weight gain

Average body weight gain in a replication was calculated by deducting initial feed intake in a replication. Body weight gain = Final weight- Initial weight

3.11.2 Feed Intake

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

3.11.3 Feed Conversion Ratio (FCR)

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

$$\text{FCR} = \frac{\text{Feed Intake (kg)}}{\text{Weight gain (kg)}} \times 100$$

3.11.4 Flock Uniformity

Uniformity can be calculated by individual weighting at least 100 birds . Individual bird weights are necessary to measure how much each bird’s body weight differs from the flock average weight . This calculation is called the deviation . A good quality sample is a selection of birds which represents the entire population.

$$s = \sqrt{\frac{\sum(x - x^{-})}{n - 1}}$$

X= The value in the data distribution

X^{-}= The sample mean

n= Total number of observations

$$C.V = \frac{\text{Standard deviation}}{\text{Average Body Weight (Kg)}} \times 100$$

C.V= Coefficiency of Variation

3.12 Microbial examination

In Poultry science lab this examination happened .

3.12.1 Fecal sample collection

Fecal content of birds of each treatment collected at the age of 7th ,14th,21th,28th,35th day age of bird and then preserve it normal temperature(0 to 4˚C)

3.12.2 Composition of Lactobacillus MRS agar media

Table 8: Composition of lactobacillus MRS agar media

Ingredients	Gm/Litre
Proteose peptone	10.00
HM peptone B#	10.00
Yeast Extract	5.00
Dextrose(Glucose)	20.00
Polysorbate 80(Tween 80)	1.00
Ammonium Citrate	2.00
Sodium acetate	5.00
Magnesium Sulphate	0.10
Manganese sulphate	0.05
Dipotassium Hydrogen phosphate	2.00
Agar	12.00
Final P^H(at 25˚C)	6.5±0.2
** Formula adjusted ,Standardized to suit performance parameters #Equivalent to beef Extract	

3.12.3 Preparation of Lactobacillus MRS agar media

- Suspend 67.15 g of the medium in one litter of deionize or distilled water.
- Mix well by digital mixing machine
- Heat with frequent agitation and boil for one minute.
- Donot autoclave the media
- Pour into plates
- Let the agar solidify and store in the refrigerator (avoid freezing). Prepared culture media can be kept for at least a week in refrigeration

3.12.4 Dilution

- If the count is expected more than 2.5×10^3 per ml or g , prepare decimal dilution as follows.
- Shake each dilution 25 times in 30 cm area.
- For each 10 fold dilution use fresh sterile pipette.
- Pipette 0.1 g fecal content homogenate into a tube containing 900 micro ml of the phosphate buffer solution.
- From the first dilution transfer 100 micro ml to second dilution tube containing 900 micro ml of the diluent
- Repeat using a third, fourth , more tubes until the desired dilution is obtained.

3.12.5 Pour plating

Label all petri plates with the sample number , dilution , date and another desired information. Pipette 0.1 g of 10 fold dilution of the fecal content of homogenate and of such dilution which have been selected for plating into a petri dish.Pour into 10-12 ml of MRS agar media separate petri dish before prepared . Mix the media and dilution by swirling anti-clock wise, to and fro trice and taking care that contents do not touch the lid. Allow to set

3.12.6 Incubation

Incubate the prepared dishes inverted at 35˚ C for 25±2 hours .(or the desired temperature as per fecal regulation).

3.12.7 Counting colonies

Following incubation count all colonies on dishes containing 30-300 colonies and recorder the result per dilution counted

3.12.8 Calculation

In dishes which contain 30-300 colonies count the actual number in both plates of a dilution as per the formula given below-

$$Tcc = \frac{\text{Number of colony}}{0.025} \times 10^n$$

n= which stage colony found.

4 Results & Discussion

4.1 Introduction

Calculation of different parameter were done to to evaluate the successfulness of body weight gain and significant effect on daily feed intake of broiler production which compare results from different treatment groups. The performance of broilers is measured through different factors was as below :

- Final Weight Gain
- Feed Consumption
- Feed Conversion Ratio
- Survivability rate
- NH_3 Level of production period
- Lactobacillus colony count
- Measurement and assessment of these factors reflect the maintenance and production performance of broiler .

The results of feeding broilers on supplementation of bacteriophage diet are presented in the following Sub-headings are-

4.2 Effect of bacteriophage supplemented diet on production performance of broiler chicken

Table 9 :Production performance of broiler chicken

Treatments	production performance of Broiler Chicken			
	Final Live weight (g/Bird)	Feed consumption (g/bird)	FCR	Survivability(%)
T_0	2157.00^{c} ±10.16	3536.00^{a}±52.85	1.61^{a}±0.01	99.18±0.83
T_1	**2251.00^{a}±16.45**	3069.00^{c}±22.70	1.39^{d}±0.02	98.35±0.95
T_2	2212.00^{b}±6.28	3165.00^{c}±32.01	1.46^{c}±0.02	97.50±1.60
T_3	2206.00^{b}±17.63	3282.00^{b}±65.41	1.52^{c}±0.02	98.35±0.95
T_4	2101.00^{d}±18.13	3283.00^{b}±33.49	1.60^{b}±0.00	97.52±0.83
Mean ± SE	2185.25±12.40	3256.87±37.61	1.53±0.01	98.18±0.922

P-value	0.003	0.002	0.003	0.009

T_0 (Control), T_1 (Con.+ 0.5g/kg feed),T_2 (CON+ 0.75 g/Kg feed) , T_3 (Con.+1g/kg feed) , T_4 (Con. + BMD 0.05g/Kg Feed). Values are Mean ±S.E (n=15) one way ANOVA (SPSS, Duncan Method). Mean with different superscripts at the same column are significantly different ($P<0.05$). Mean within same superscripts don't differ ($P>0.05$) significantly .S.E= Standard Error . * means significant at 5% level of significance ($P < 0.05$). NS= Non-Significant . $^{a. b.}$ = Means in the same row with different letters show significant differences.

4.2.1 Live weight

Data presented in Table 9 showed the productive performance of broiler receiving feed supplemented diet with antibiotic and bacteriophage .In case of live weight (g/bird) there were significant differences ($P< 0.05$) in different group. Among the all treatment $T_{0,}$ T_1 and T_4 are significantly different ($P<0.05$) where as T_2 &T_3 don't differ Significantly ($P>0.05$). Highest live weight was found in T_1 (2251.00±16.45) and T_2 (2212.00±6.28) group than the control T_0 (2157.00±10.16) and T_3 (2206.00±17.63) . Significantly ($P< 0.05$) lowest live weight was found in T_4 (2185.25±12.40).The higher body weight in T_1 group might be due to lower bacteriophage treatment which helps to regulate body digestion process and reduction of in the amount of harmful bacteria by infecting organism due to grow in eukaryotic cell and failure in adhere to. Similarly (Loc-Carrillo & Abedon , 2011) found that the final body weight in smaller phage which is significantly inclined by larger phage of bacteriophage . But contradiction with (Huff , et al., 2009) who showed that younger chicks to overcome potential pathogens that are not detrimental to finisher .

4.2.2 Feed Consumption

The result present in table 9 showed that , the effect of bacteriophage treatment on the relative total Feed Consumption (g/bird) of broiler chicken in different treatment groups were 3536.00±52.85 (T_0) , 3069.00±22.70 (T_1), 3165.00±32.01 (T_2), , 3282.00^b±65.41 (T_3),, 3283.00±33.49 (T_4), respectively. The highest feed consumption was found in T_0 and lowest in T_1 . The over all feed consumption of different treatment groups showed that there was significant effects P (< 0.05) . The feed consumption of bacteriophage treatment ascending from low amount of diet from T_1 to high amount in $T_{3.}$ Antibiotic groups don't differ with $T_{3,}$ there is same for T_1 & T_2 . But it may be trend to discuss that minimum level rather higher amount of BP significantly different for their lytic activity in the intestinal wall. According to the research of (Żbikowska, et al., 2020;) experimentally infected broiler chickens phage therapy by food allowed them to gain weight more quickly have a greater conversion of feed consumption.

4.2.3 FCR

The result present in Table 9 showed that , the FCR of this experimental study The FCR of different dietary treatment groups were 1.61^a±0.01 (T_0), 1.39^d±0.02(T_1), 1.46^c±0.02 (T_2), 1.52^c±0.02(T_3) and 1.60^b±0.00(T_4) respectively .There was significant difference (P<0.05) in the FCR of the research . However , T_2 treatment is better among different treatment groups , might be due to treat with body mechanism which help to maintain optimum temperature and digestibility inside the broiler . FCR shown into this experiment descending from T_1 to high bacteriophage diet T_3 & chronologically T_4 & $T_{0.}$(Wernicki , et al., 2017;) stated their investigation which is prolonged as low bacteriophage identified in the central nervous system. Another scientist revealed that possible to attack both the biofilms matrix and bacterial cell at the same time . The modified bacteriophages decreased the number of bacterial biofilm cells by around 99 % , which has a positively outcome that is presented by (Edgar, , et al., 2012)

4.2.4 Survivability

Table 10: Survivability (%) at the end of period

Treatments	Survivability		
	Number of birds	Survival number of birds	Survivability rate (%)
T_0	30.00±0.00	29.75±0.50	99.18±0.83
T_1	30.00±0.00	29.50±0.58	98.35±0.95
T_2	30.00±0.00	29.25±0.96	97.50±1.60
T_3	30.00±0.00	29.50±0.58	98.35±0.95
T_4	30.00±0.00	29.25±0.50	97.52±0.83
Mean ± SE	30.00±0.00	29.45±0.278	98.18±0.922
P-value	--	0.084	0.089

Here, T_0 (Control), T_1 (Con.+ 0.5g/kg feed),T_2 (CON+ 0.75 g/Kg feed) , T_3 (Con.+1g/kg feed) , T_4(Con. + BMD 0.05g/Kg Feed). Values are Mean ±S.E (n=15) one way ANOVA (SPSS, Duncan Method). Mean with different superscripts at the same column are significantly different (P<0.05). Mean within same superscripts don't differ (P>0.05) significantly . S.E= Standard Error . * means significant at 5% level of significance (P < 0.05). NS= Non-Significant . [a. b.] = Means in the same row with different letters show significant differences

Table 10 showed the survivability percentage of different dietary treatment groups were insignificant (P>0.05) . The survivability of different dietary treatment groups , ,T_4, are 99.18±0.83(T_0) , 98.35±0.95(T_1,) , 97.50±1.60(T_2) , 98.35±0.95(T_3) , 97.52±0.83(T_4), respectively .All treatment groups showed that survivability highest in control T_1, and lowest in T_2 . In Poultry there are many diseases causes mortality which can be prevented by the bacteriophage . The experimentally infected broiler chickens reduce mortality rate as their using phage therapy by feed or water . (Wernicki , et al., 2017;) (Monk , et al., 2010).

4.2.5 Weekly Body weight Gain

Table 11 : Effect of bacteriophage supplemented diet onweekly body weight Gain(BWG) (0-5 weeks)

Treatment	Body weight gain (BWG)(g/bird) of broiler chicken at different weeks					
	1st Week	2nd Week	3rd Week	4th Week	5th Week	Final gain
T_0	133.3^{c}±1.41	349.3^{c}±2.69	538.9^{b}±5.14	825.3^{c}±4.60	1332.0^{a}±13.38	2115.0^{c}±10.14
T_1	140.5^{b}±1.03	390.7^{ab}±4.08	524.8^{b}±6.36	950.7^{a}±5.96	1300.0^{ab}±10.5	2209.0^{a}±16.45
T_2	138.1^{b}±1.03	375.2^{b}±3.08	570.9^{a}±5.37	893.2^{b}±14.38	1319.0^{a}±13.52	2170.0^{b}±6.27
T_3	138.0^{b}±0.98	405.1^{a}±13.15	542.1^{b}±15.14	935.0^{a}±19.40	1271.0^{bc}±6.94	2164.0^{b}±17.62
T_4	143.1^{a}±0.67	369.9b^{c}±9.48	546.9^{b}±11.78	847.3^{c}±20.49	1254.0^{c}±5.95	2059.0^{d}±18.83
Mean ± SE	138.62±0.8	378.05±7.99	544.74±10.41	890.31±14.30	1295.04±11.1	2143.35±11.88
P-value	0.040	0.028	0.033	0.022	0.024	0.043

Here, T_0 (Control), T_1 (Con.+ 0.5g/kg feed),T_2 (CON+ 0.75 g/Kg feed) , T_3 (Con.+1g/kg feed) , T_4 (Con. + BMD 0.05g/Kg Feed). Values are Mean ±S.E (n=15) one way ANOVA (SPSS, Duncan Method). Mean with different superscripts at the same column are significantly different ($P<0.05$). Mean within same superscripts don't differ ($P>0.05$) significantly . S.E= Standard Error . * means significant at 5% level of significance ($P < 0.05$). NS= Non-Significant . [a. b.] = Means in the same row with different letters show significant differences

Body weight gain was differed by the addition of bacteriophage ($P<0.05$) diet .In 1st week comparatively T_4 shows the highest body weight gain 143.1±0.67 in Antibiotic significantly ($P< 0.05$) Control show the lowest weight 133.3±1.41 . in 2nd week T_3 shows higher body weight 405.1±13.15 at 1 % bacteriophage diet rather than another treatment T_1 ,T_2, T_4 but lowest in control stage 349.3±2.69 .The addition of 1 g/kg bacteriophage from 1-14 days its higher body weight gain .

(Huff , et al., 2002) presented that the body weight were not affected by the inclusion of bacteriophage under normal physiological states. It is noted that the the improvement in body weight gain during 1-14 days higher in Antibiotic and 1g/kg bacteriophage diet respectively

.This is likely because the younger chicken are more susceptible to pathogens than than the additives . Where as 3rd week highest in 0.75% bacteriophage diet that's are 570.9±5.37compared to the same lowest in Control T_0 538.9^b±5.14 . significantly ($P< 0.05$) .After 3rd week in general highest body weight gain in 0.5 % level of bacteriophage at T_1 950.7±5.96 compared to the lowest in control 825.3±4.60 . After 35th day the final stage there is highest Body weight In T_1 2209.0±16.45 gm than , 2115.0±10.14 gm(T_0), 2170.0±6.27 gm (T_2), 2164.0±17.62 gm (T_3), 2059.0±18.83 gm (T_4) Where as the lowest in T_4. In control its relatively low then the bacteriophage diet in minimum range it increased after next it descending chronologically from T_2 to T_4. It may happen for the favourable gut microflora which enhance the digestibility and to mechanize the body for growth and adaptation for very few terms of period . That addition of 0.5 g/kg bacteriophage seems have greater average daily weight gain than Control with statistical differences But these documents contradict with (J, et al., 2013) 0.5 g/kg bacteriophage included in applicable only for starter age.

4.2.6 Weekly Feed Consumption

Table 12 showed that , effect of different level of bacteriophage on Feed consumption (g/bird) were significant . At 14- 21 days of age lowest feed intake in control stage 368.0±1.98 gm , 833.3^{e}±17.97gm respectively that is the specially agreed with (Huff , et al., 2009) Feed efficiency was not a great factor which is at starter stage . where as the highest feed intake in T_3 & T_2 (406.5±11.21) (904.6±2.48) respectively . during 21 lowest feed intake for T_4 (1284.0±13.74) compared to the higher in T_1 1339.0±25.66. At the end of the 5th week the FC of different dietary groups were, T_1 ,T_2, T_3 ,T_4 1642.0±20.24(T_0) , 1730.0±21.22(T_1) , 1862.0±51.18 (T_2), 1884.0±79.45(T_3), 1999.0±21.85 (T_4) , respectively . whereas highest feed intake in control stage and lowest in T_1 . Every treatment is significantly differ from one another. This happen might be the lytic phages infect the cellular parts of organism that can prevent the broiler to take more movement and energy requirement comparatively lower than the control. These results showed a significance ($P<0.05$). (Atterbury , et al., 2007) treated to his experiment as highest feed consumption in T_3 due to highest digestibility and higher lactobacillus content .

Table 12 :Weekly Feed Consumption of the broiler supplemented with bacteriophage diet (0-5 Weeks)

Treatment	Feed consumption (FC) (g/bird)				
	1st Week	2nd Week	3rd Week	4th Week	5th Week
T_0	165.5±0.60	368.0^{c}±1.98	833.3^{e}±17.97	1291.0^{c}d±6.19	1642.0^{a}±20.24
T_1	168.8±0.52	392.6^{b}±6.56	848.5^{d}±9.14	1339.0^{b}±25.66	1730.0^{e}±21.22
T_2	167.2±0.54	377.6^{c}±2.33	904.6^{a}±2.48	1303.0^{c}±44.94	1862.0^{d}±51.18
T_3	163.5±0.65	406.5^{a}±11.21	864.0^{c}±8.99	1398.0^{a}±29.39	1884.0^{c}±79.45
T_4	165.0±0.53	373.0^{c}±3.07	881.4^{b}±8.33	1284.0^{d}±13.74	1999.0^{b}±21.85
Mean ± SE	166.02±0.25	383.52±6.46	866.35±9.41	1322.89±23.91	1943.29±31.62
P-value	0.062	0.047	0.034	0.029	0.044

Here , T_0 (Control), T_1 (Con.+ 0.5g/kg feed),T_2 (CON+ 0.75 g/Kg feed) , T_3 (Con.+1g/kg feed) , T_4 (Con. + BMD 0.05g/Kg Feed). Values are Mean ±S.E (n=15) oneway ANOVA (SPSS, Duncan Method). Mean with different superscripts at the same column are significantly different ($P<0.05$). Mean within same superscripts don't differ ($P>0.05$)

significantly . S.E= Standard Error . * means significant at 5% level of significance ($P < 0.05$). NS= Non-Significant . $^{a. b.}$ = Means in the same row with different letters show significant differences

4.2.7 Weekly Feed Conversion Ratio

Table 13 represents the FCR of broiler receiving feed supplemented with the bacteriophage or antibiotic . In respect to FCR up to 35 days there was significance difference ($P<0.05$) among the dietary groups . 0- 28 days of age FCR was no longer significant differences among different treatment group. At the end of 35 days Lowest FCR found in T_1 dietary treatment was 0.5 g/kg bacteriophage diet 1.39±0.02 rather than another treatment 1.61^a±0.01(T_0) , 1.46^c±0.029(T_2), 1.52^c±0.02($T_{3,}$) , 1.60^b±0.00 (T_4). Which may be ascending from the minimal stage of bacteriophage . It seems to be the feed consumption of broiler which is potentially effective to overcome potential pathogens that are not so detrimental .May be T_1 is more susceptible to pathogens and sensitive to the additives that can inhibit those potential pathogens in the environment according to (Huff,, et al., 2003). But when (Atterbury , et al., 2007) exclaimed effectively reduction level of salmonella colonization of in bacteriophages significance has been founded on their ability to target certain species or strains of harmful bacteria that enhance to ascending the level of FCR.

Table 13:Weekly Feed Conversion Ratio of the broiler supplemented with bacteriophage diet (0-5 Weeks)

Treatment	Feed Conversion Ratio (FCR) (g/bird)					
	1st Week	2nd Week	3rd Week	4th Week	5th Week	Final
T_0	0.94±0.01	1.11±0.01	1.35±0.02	1.56±0.00	1.58^a±0.01	1.61^a±0.01
T_1	0.93±0.00	1.06±0.01	1.36±0.01	1.48±0.02	1.36^d±0.02	1.39^d±0.02
T_2	0.93±0.00	1.06±0.00	1.36±0.00	1.51±0.05	1.43^c±0.02	1.46^c±0.02
T_3	0.91±0.00	1.05±0.00	1.35±0.01	1.52±0.03	1.51^b±0.01	1.52^c±0.02
T_4	0.89±0.00	1.05±0.00	1.37±0.01	1.56±0.01	1.56^b±0.00	1.60^b±0.00
Mean ± SE	0.92±0.004	1.07±0.01	1.37±0.01	1.53±0.02	1.50±0.01	1.53±0.01

P-value	0.063	0.071	0.057	0.072	0.002	0.004

Here , T_0 (Control), T_1 (Con.+ 0.5g/kg feed),T_2 (CON+ 0.75 g/Kg feed) , T_3 (Con.+1g/kg feed) , T_4 (Con. + BMD 0.05g/Kg Feed). Values are Mean ±S.E (n=15) one way ANOVA (SPSS, Duncan Method). Mean with different superscripts at the same column are significantly different (P<0.05). Mean within same superscripts don't differ (P>0.05) significantly . S.E= Standard Error . * means significant at 5% level of significance (P<0.05). NS= Non-Significant . $^{a.\ b.}$ = Means in the same row with different letters show significant differences

4.2.8 Flock Uniformity

Table 14 represents the uniformity of broiler supplemented with bacteriophage diet or antibiotic treatment. In respect to Uniformity after 35 days of age , there was significant differences (P< 0.05) among the dietary groups . At the end of 35 days of age , the better Uniformity was found in broilers feed on T_1 , 67.72±4.27% . comparatively higher than antibiotic T_4 (66.60^c±0.83) & Control T_0 (65.11±3.37). In case of the bacteriophage diet on, are respectively 67.72±4.27% (T_1), 64.12±1.52% (T_2), 63.74±3.99% (T_3) . Numerically Highest uniformity was in T_1 has a significant effect(P> 0.05) . This may be caused by minimum level of bacteriophage compared with antibiotic.

Table 14 : Flock Uniformity of the broiler supplemented with bacteriophage diet (0-5 weeks)

Treatments	Uniformity (%)
T_0	65.11^{ab}±3.37
T_1	**67.72^{a}±4.27**
T_2	64.12^{b}±1.52
T_3	63.74^{b}±3.99
T_4	66.60^{c}±0.83
Mean ± SE	62.86±3.43

Here , T_0 (Control), T_1 (Con.+ 0.5g/kg feed),T_2 (CON+ 0.75 g/Kg feed) , T_3 (Con.+1g/kg feed) , T_4 (Con. + BMD 0.05g/Kg Feed). Values are Mean ±S.E (n=15) one way ANOVA (SPSS, Duncan Method). Mean with different superscripts at the same column are significantly different (P<0.05). Mean within same superscripts don't differ (P>0.05) significantly . S.E= Standard Error . * means significant at 5% level of significance (P < 0.05). NS= Non-Significant . $^{a.\ b.}$ = Means in the same row with different letters show significant differences.

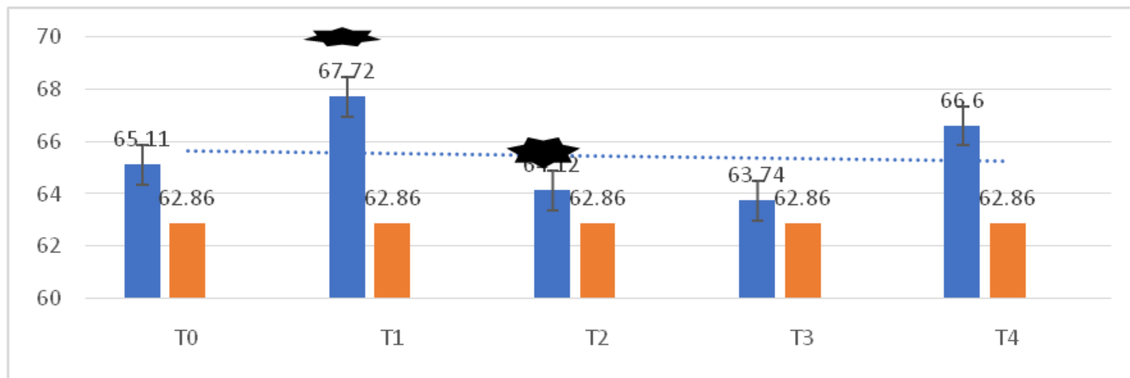


Figure 2- Flock Uniformity

4.3 Carcass characteristics

4.3.1 Dressing percentage(DP)

Table 15 :Dressing percentage of the broiler supplemented diet

Treatments	Dressing percentage
T_0	$65.15^{c}\pm2.19$
T_1	$68.98^{b}\pm3.59$
T_2	$71.94^{a}\pm2.61$
T_3	$70.27^{ab}\pm3.35$
T_4	$69.06^{b}\pm2.19$
Mean ± SE	69.08*±2.65

Here , T_0 (Control), T_1 (Con.+ 0.5g/kg feed),T_2 (Con.+ 0.75 g/Kg feed) , T_3 (Con.+1g/kg feed) , T_4 (Con. + BMD 0.05g/Kg Feed). Values are Mean ±S.E (n=15) one way ANOVA (SPSS, Duncan Method). Mean with different superscripts at the same column are significantly different ($P<0.05$). Mean within same superscripts don't differ ($P>0.05$) significantly . S.E= Standard Error . * means significant at 5% level of significance ($P < 0.05$). NS= Non-Significant . $^{a.\ b.}$ = Means in the same row with different letters show significant differences

Table 15 presented that the dressing percentage of broiler bacteriophage supplemented diet 65.15±2.19 (T_0), 68.98±3.59 (T_1) , 71.94±2.61 (T_2) 70.27±3.35 (T_3), 69.06±2.19 (T_4) which has significant difference among the dietary group. There was significant differences ($P<0.05$) in the dressing percentage research. However Highest dressing percentage found in 0.75g bacteriophage per kg feed T_2 than control group T_0. This is might be due to the effect of bacteriophage which is supplied in medium level of bacteriophage compared with control group.

4.3.2 Relative weight of giblet organs

Table 16 :Carcass characters of the broiler supplemented diet with bacteriophage

Treatment	Weight of internal organs of broiler chicken										
	Live weight	Dressed weight	Eviscerated weight	Liver weight	Heart weight	Intestine weight	Spleen weight	Breast weight	Wing weight	Bursa weight	Gizzard weight
T_0	$1605.0^{d}\pm112.62$	$1155.0^{d}\pm98.10$	$1051.0^{d}\pm104.21$	$32.50^{c}\pm1.55$	$8.75^{b}\pm0.85$	$80.25^{d}\pm2.29$	$2.00^{b}\pm0.00$	$502.5^{d}\pm34.27$	$72.00^{d}\pm2.55$	2.50 ± 0.29	$51.50^{b}\pm4.35$
T_1	$2207.0^{a}\pm182.44$	$1741.0^{a}\pm193.46$	$1593.0^{a}\pm195.19$	$52.25^{a}\pm9.42$	$13.50^{a}\pm1.71$	$99.25^{b}\pm33.34$	$3.25^{a}\pm0.25$	$562.8^{b}\pm32.86$	$107.3^{a}\pm15.04$	2.00 ± 0.00	$47.75^{c}\pm8.82$
T_2	$1967.0^{b}\pm169.40$	$1596.0^{b}\pm180.85$	$1428.0^{b}\pm172.68$	$54.25^{a}\pm5.88$	$12.25^{a}\pm1.31$	$129.0^{a}\pm17.69$	$3.25^{a}\pm0.25$	$548.0^{c}\pm5.89$	$95.25^{b}\pm3.97$	2.75 ± 0.25	$47.25^{c}\pm9.72$
T_3	$1904.0^{c}\pm88.04$	$1558.0^{b}\pm73.05$	$1340.0^{c}\pm101.82$	$49.00^{a}\pm5.55$	$11.00^{ab}\pm1.22$	$99.75^{b}\pm13.35$	$3.00^{a}\pm0.41$	$587.5^{a}\pm31.95$	$96.50^{b}\pm6.51$	2.25 ± 0.25	$55.50^{a}\pm1.19$
T_4	$1883.0^{c}\pm73.21$	$1480.0^{c}\pm88.61$	$1303.0^{c}\pm82.40$	$42.00^{b}\pm1.08$	$11.50^{a}\pm0.65$	$88.25^{c}\pm8.19$	$2.00^{b}\pm0.00$	$556.3^{b}\pm15.80$	$81.50^{c}\pm4.79$	2.25 ± 0.25	$52.50^{ab}\pm1.04$
Mean ± SE	1912.9*±135.01	1505.75*±130.17	1342.9*±143.53	46.00*±4.46	11.40**±1.17	99.3*± 17.03	2.70** ±0.19	551.4* ±28.31	90.50* ±7.91	2.35^{NS} ±0.22	50.9* ±6.46

Here , T_0 (Control), T_1 (Con.+ 0.5g/kg feed),T_2 (Con.+ 0.75 g/Kg feed) , T_3 (Con.+1g/kg feed) , T_4 (Con. + BMD 0.05g/Kg Feed). Values are Mean ±S.E (n=15) one way ANOVA (SPSS, Duncan Method). Mean with different superscripts at the same column are significantly different (P<0.05). Mean within same superscripts don't differ (P>0.05) significantly . S.E= Standard Error . * means significant at 5% level of significance (P < 0.05). NS= Non-Significant . [a. b.] = Means in the same row with different letters show significant differences .

Table 16indicates that bacteriophage had significant (P<0.05) effect ineviscerated, dressed, liver, heart , intestine ,spleen , breast, wing , Bursa and gizzard weight comparing to the control and antibiotic group. However, the treatments found no significant effect (P>0.05) on bursa weight , abdominal fat weight in relation to body weight. Inclusion level of bacteriophage will higher liver weight comparatively in 0.75 g/kg than 0.5 g/kg . 54.25±5.88 gand 52.25±9.42g ,and significant level of heart weight which is ascending from control. Relative weight of gizzard comparatively lower in T_1 than control and another antibiotic. Liver weight found higher in T_2 , 54.25 ± 5.88 g rather than control T_0 32.50± 1.55g . It may happen due to lytic phages of bacteriophage which is correlated with liver functioning. But heart weight found higher in T_1 13.50± 1.171 g whereas control is lowest in 8.75 ± 0.85. that has highly significance among the treatment. whereas intestine weight found higher in T_2 , 129.0± 17.69g but lower in T_4 , 80.25± 2.29 g . (J, et al., 2013) found in a research that heart weight & intestine weight highest in minimum range of bacteriophage .Spleen weight found lowest in T_0& T_4 (2.00± 0.00)plays significant differences (p< 0.05) whereas highest spleen weight found in T_1 , T_2 , T_3 (3.25 ± 0.25) ascending gradually.

(Santi , et al., 2021) observed a significant reduction in relative weight of bursa of fabricius in bacteriophage diet than control and gizzard shows increment than bacteriophage supplemented diet but contradiction in weight of bursa fabricius relative to body weight were seen in increasing level ofbacteriophage.

4.4 Effects of bacteriophage on microbial test of broiler fecal count

Table 17 : presented that Lactobacillus colony count of feces in control , antibiotic and supplemented bacteriophage . Lactobacillus spp. number found in 1.17±0.12 CFU/ml (T_0), 3.97±0.13 CFU/ml (T_1), 2.40±0.20 CFU/ml (T_2) , 2.90±0.14 CFU/ml (T_3), 0.43±0.07 CFU/ml (T_4). In this trial relatively higher in T_1 treatment group 3 and lowest in T_4 treatment . It has significant differences among the control and antibiotic .Higher lactobacillus in 0.5 g/kg that agreed with the (J, et al., 2013) who showed his study is noteworthy that bacteriophage supplementation at 0.5 g/kg resulted to a greater lactobacillus concentration; this difference is likely due to the lower levels of Salmonella and E. coli, which produced a better environment for the growth of the lactobacillus. It may happen due to the characteristics of bacteriophage self replication process throughout the treatment , whereas the considerable reduction in the amount of harmful bacteria .However they contain protein and nucleic acid.

Similarly (Lin , et al., 2017) showed in his experiment to teat bacteriophage , lytic bacteriophage can only be used to treat bacterial illness with phage treatment because of their poor capacity to kill bacteria & have far higher specify than antibiotics.

Table 17: Lactobacillus colony count of supplemented with bacteriophage diet

Treatments	Lactobacillus spp. colony in faces			
	Viable Count Plate 1	Viable Count Plate 2	Mean Viable Count	CFU/mL×10^8
T_0	28.25^c±4.59	30.75^b±2.63	29.50^c±2.98	1.17±0.12
T_1	23.50^c±1.55	25.75^c±5.25	24.63^c±3.36	**3.97±0.13**
T_2	55.00^b±4.74	68.25^a±7.59	60.38^b±4.75	2.40±0.20
T_3	80.75^a±4.77	65.25^a±3.38	73.00^a±3.46	2.90±0.14
T_4	11.75^d±1.25	9.750^d±3.57	10.75^d±1.74	0.43±0.07
Mean ± SE	39.85±2.75	39.95±5.12	39.65±3.26	1.57±0.49
P-value	0.005	0.038	0.006	0.173

Here , T_0 (Control), T_1 (Con.+ 0.5g/kg feed),T_2 (Con.+ 0.75 g/Kg feed) , T_3 (Con.+1g/kg feed) , T_4 (Con. + BMD 0.05g/Kg Feed). Values are Mean ±S.E (n=15) one way ANOVA (SPSS, Duncan Method). Mean with different superscripts at the same column are significantly different (P<0.05). Mean within same superscripts don't differ (P>0.05) significantly . S.E= Standard Error . * means significant at 5% level of significance (P < 0.05). NS= Non-Significant . $^{a.\ b.}$ = Means in the same row with different letters show significant differences .

4.5 Effects of bacteriophage onammonia production

The emissions of ammonia (ppm) from birds level of T_1 group is lower than T_0 , T_2 ,T_3& T_4 group. The emissions of ammonia (ppm) from birds level different treatment groups of the research was unsignificant . in days 21 were 8.750± 0.48 ppm (T_0) , 9.750 ± 0.063ppm (T_1), 10.25± 0.085ppm (T_2) , 11±1.29ppm (T_3) , 10.75 ±0.48ppm (T_4) . In 28 days are 14.25 ± 0.85ppm (T_0), 12.50 ±0.087ppm (T_1) , 14.25± 0.85ppm (T_2) , 13.75± 1.38ppm(T_3) , 14.00 ±0.71 ppm (T_4). In 35th days were 16.75 ±1.38 ppm (T_0), 13.25±1.38 ppm (T_1), 18.50±0.65ppm (T_2), 18.25±0.085 ppm (T_3), 17.50 ± 0.096ppm (T_4). The emissions of ammonia from birds level of T_2 group is higher than another treatment group. In 21 days T_0 - & T_3 groups significantly lower (p<0.05) , in days 28 & in 35 days only T_1 is lower . However, T_1 group showed less amount of ammonia than another treatment group. It might be due to the effect of minimum range of bacteriophage in T_1 group which helps to mechanize body that engulfs the pathogenic bacteria and stimulate the nonpathogenic bacteria.

Table 18 : Effects ofbacteriophage on NH_3 content

Treatments	NH_3 concentration weekly (PPM)				
	7 Days	14 Days	21 Days	28 Days	35 Days
T_0	6.00±0	8.25±0.63	8.750^{b}±0.48	14.25^{a}±0.85	16.75^{a}±1.38
T_1	6.00±0	8.00±0.91	9.750^{ab}±0.63	12.50^{b}±0.87	13.25^{b}±1.38
T_2	6.00±0	9.25±0.63	10.25^{ab}±0.85	14.25^{a}±0.85	18.50^{a}±0.65
T_3	6.00±0	8.50±1.04	11.00^{a}±1.29	13.75^{ab}±1.38	18.25^{a}±0.85
T_4	6.00±0	8.75±0.48	10.75^{a}±0.48	14.00^{ab}±0.71	17.50^{a}±0.96
Mean ± SE	6.00±0	8.55^{NS}±0.69	10.10±0.57	13.75±1.02	16.85±1.09
P Value	0	0.057	0.06	0.02	0.047

Here , T_0 (Control), T_1 (Con.+ 0.5g/kg feed),T_2 (Con.+ 0.75 g/Kg feed) , T_3 (Con.+1g/kg feed) , T_4 (Con. + BMD 0.05g/Kg Feed). Values are Mean ±S.E (n=15) one way ANOVA (SPSS, Duncan Method). Mean with different superscripts at the same column are significantly different (P<0.05). Mean within same superscripts don't differ (P>0.05) significantly . S.E= Standard Error . * means significant at 5% level of significance (P < 0.05). NS= Non-Significant . $^{a.\ b.}$ = Means in the same row with different letters show significant differences .

Data presented In table 18 showed that the ammonia emission (ppm) level from different types of broiler houses in this experiment study . At the end of 7 days in different treatment

groups was 6.00±0 ppm , as brooding period for all birds of the first week was common. Ammoniaemission (ppm) at the 14 days in different treatment groups were 8.25±0.63 ppm (T_0) , 8.00±0.91ppm (T_1) , 9.25±0.63 ppm (T_2), 8.50±1.04 ppm (T_3) , 8.75±0.48 ppm(T_4).

According to (Lott & Donald , (2015)) report, continuous exposure of 25 ppm levels indicating losses 90 grams per bird & increased condemnation of carcass. gas emissions in the production of broilers influences the production environment. Another scientist reported that ammonia level are kept below concentration of 25 ppm & maximum acceptable limit of ammonia concentration is 20 ppm & excessive occurrence of certain particles cause affecting immune system and general vulnerability to disease (Osório , et al., 2009) (Groot Koerkamp, et al., 1998). This study shows the partially agreed with the NH_3 influence production parameter (Miles , et al., 2004) also certified that high amount of NH_3 also influence the internal organ of the bird that's the causes of production losses.

4.6 Cost-benefit ratio analysis

Cost benefit ratio analysis are presented in Table 19. Benefit cost ratio/ m^2 were insignificant(P= 0.063). The benefit cost ratio showed that bacteriophage T_1 was higher (1.15 ±0.01) comparing the T_2 (1.09±0.02) and T_3 (1.08±0.01) . There was insignificant differences between (P<0.05) among the dietary groups.

Table 19: Cost-benefit analysis of broiler in different dietary treatment.

Treatments	Total income/m^2 (Tk)	Total Production Cost/m^2	Net profit Tk/m^2 (Tk)	Benefit Cost-ratio
T_0	9707.53^{d} ±45.65	9582.60 ±38.50	124.93^{e}±68.29	1.01 ±0.01
T_1	10128.38^{a}±74.02	8820.96± 42.01	1307.42^{a}±111.72	1.15 ±0.01
T_2	9953.25^{b} ±28.21	9138.33±29.06	814.92^{b}±147.54	1.09 ±0.02
T_3	9926.25^{c} ±79.31	9206.78±31.23	719.47^{c}±82.50	1.08 ±0.01
T_4	9454.87^{e} ±84.71	9210.00±32.06	244.87^{d}±25.06	1.03 ±0.00
Mean± SE	9834.05 ±24.33	9191.17±28.42	642.32 ±46.05	1.07 ±0.03

Here , T_0 (Control), T_1 (Con.+ 0.5g/kg feed),T_2 (Con.+ 0.75 g/Kg feed) , T_3 (Con.+1g/kg feed) , T_4 (Con. + BMD 0.05g/Kg Feed). Values are Mean ±S.E (n=15) one way ANOVA (SPSS, Duncan Method). Mean with different superscripts at the same column are significantly different (P<0.05). Mean within same superscripts don't differ (P>0.05) significantly . S.E= Standard Error . * means significant at 5% level of significance (P < 0.05).

NS= Non-Significant . [a. b.] = Means in the same row with different letters show significant differences .

5 Summary & Conclusion

The use of bacteriophage has been associated with many beneficial effects in poultry production. The study was planned to determine the comparative beneficial effects of commercial broilers in different rearing system. A total 600 one day old efficiency plus broiler chicks for a period of 35 days of age reared. Chicks were divided into 5 experimental groups each replication contains 30 birds. First group is T_0 as control, T_1, T_2, T_3 group of chicks was considered as bacteriophage diet in different ratio and T_4 antibiotic. Live weight ,feed intake , feed conversion ratio , livability , internal organ development , meat yield , bacteriophage parameters of broiler on different treatments were recorded and statistically analyzed . The body weight and body weight gain of broilers in 1st , 2nd,5th week and final body weight showed significant difference($P<0.05$) among the dietary groups . Result demonstrated that T_1 in 5th week higher body weight gain showed significant differences ($P<0.05$) among treatment group. Highest body weight was T_1 (2209.0±16.45), but in lowest in T_4 (2059.0±18.83) .In case of total feed intake there was significant differences among the treatment T_0, T_1, T_2, T_3, T_4 group. However ,there were insignificant effect ($P>0.05$) on 1st week of feed intake of broiler in different treatments. In case of feed intake was lowest in T_1 ($3069.00^c \pm 22.70$) rather than T_2 ($3165.00^c \pm 32.01$) ,T_3 ($3282.00^b \pm 65.41$),T_4 ($3283.00^b \pm 33.49$)& highest in T_0 ($3536.00^a \pm 52.85$).Feed Consumption (g/bird) were significant ($P< 0.05$) .

In this result showed total FCR value , showed better FCR in T_1 ($1.39^d \pm 0.02$) rather than T_0 ($1.67^a \pm 0.01$)and chronologically differ significance T_2($1.46^c \pm 0.02$), T_3 ($1.52^c \pm 0.02$) , T_4 ($1.60^b \pm 0.00$) .However there are insignificant effect in survivability ($P >0.05$) on 35 days of age of broiler .Bacteriophage group showed significantly ($P< 0.05$) in liver ,spleen, heart , breast weight compared to control and antibiotic but in low gizzard weight . The lactobacillus was significantly ($P<0.05$) lower in T_4 (0.43±0.07) and T_0 (1,17±0.12) rather than T_2 (2.40±0.20) & T_3 (2.90±0.14) respectively. However , T_1 showed the highest (3.97±0.13) and T_4 the lowest (0.43)

The uniformity showed the highest in T_1 ($67.72^{ab} \pm 4.27$) and lowest in T_3 ($53.60^c \pm 0.83$) have a significance ($P<0.05$) . There are significant differences among the different dietary treatment in dressing percentage in T_2 ($71.94^a \pm 2.61$) and lowest in T_0($65.15^c \pm 2.19$).

Result demonstrated that the NH_3 (ppm) level was same at the end of the first week, however it varied significantly ($p<0.05$) at the end of the 3rd , 4th& 5th week. At the end of 3^{rd}week , T_0 showed the lowest ($8.750^b \pm 0.48$), $4^{th}$and 5th week T_1 showed the lowest figure of NH_3 $12.50^b \pm 0.87$ & $13.25^b \pm 1.38$ whereas the highest amount of NH_3 in T_3 $11.00^a \pm 1.29$, $13.75^{ab} \pm 1.38$ & $18.25^a \pm 0.85$ that plays a different significant. With regards to profit , bacteriophage group T_1 showed higher profitability compared to another which has significantly differences among the treatment groups. However , considering the health safety concerned of supplemented groups if we increase the sell price up to 150 tk/kg then we will get higher profit from bacteriophage supplemented group T_1compare to thecontrol and antibiotic group.

So, finally it can be concluded that, addition of bacteriophage in the broiler diet positively affects the growth parameters . Moreover , upon supplementation of bacteriophage gizzard decreased in positively improved.. Considering these results it is clearly noticeable if we will use extensively bacteriophage in our country as a potential feed additives in poultry meat which will be safe food for human consumption and develops related industries in Bangladesh. More research is needed in this context.

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7 Appendices

Appendix 1:Effect of bacteriophage on production performance of broiler chicken

Treatment	Replication	Final Live Weight (g/Bird)	Total Feed Consumption (g/bird)	Total Body Weight Gain(g/Bird)	Final FCR	Survivability
T_0	R_1	2178	3417.601	2136	1.60	100.0
	R_2	2165	3502.950	2123	1.65	100.0
	R_3	2130	3369.610	2080	1.62	96.7
	R_4	2155	3359.670	2113	1.59	100.0
T_1	R_1	2246	3103.727	2204	1.38	96.7
	R_2	2294	3015.032	2252	1.31	96.7
	R_3	2214	3108.878	2172	1.40	100.0
	R_4	2249	3047.2	2207	1.35	100.0
T_2	R_1	2203	3190.272	2161	1.45	100.0
	R_2	2210	3071.837	2168	1.39	93.3
	R_3	2204	3217.906	2162	1.46	96.7
	R_4	2230	3178.333	2180	1.43	100.0
T_3	R_1	2256	3457.906	2214	1.53	96.7
	R_2	2178	3290.875	2136	1.51	96.7
	R_3	2185	3229.75	2143	1.48	100.0
	R_4	2204	3149.375	2162	1.43	100.0
T_4	R_1	2105	3283.187	2063	1.56	96.7
	R_2	2146	3366.363	2104	1.57	96.7
	R_3	2054	3202.303	2012	1.56	96.7
	R_4	2099	3281.951	2057	1.56	100

Appendix 2: Recorded temperature(˚C) &relative humidity (%) during experimental period

Date	Day	Temperature(°)		Humidity(%)	
		Max.	Min.	Max.	Min.
08-11-22	0				
09-11-22	1	38.2˚	35.1˚	60%	42%
10-11-22	2	35.1˚	32.8˚	62%	45%
11-11-22	3	34.0˚	28.8˚	66%	41%
12-11-22	4	33.9˚	28.5˚	68%	47%
13-11-22	5	33.6˚	28.9˚	52%	48%
14-11-22	6	34.6˚	28.2˚	56%	40%
15-11-22	7	33.6˚	28.6˚	66%	41%
16-11-22	8	34.6˚	28.7˚	58%	49%
17-11-22	9	30.7˚	27.0˚	64%	50%
18-11-22	10	30.9˚	26.4˚	62%	45%
19-11-22	11	30.8˚	25.4˚	69%	48%
20-11-22	12	30.7˚	25.4˚	64%	47%
21-11-22	13	30.3˚	25.6˚	62%	45%
22-11-22	14	29.1˚	25`.1˚	63%	45%
23-11-22	15	29.3˚	25.2˚	57%	48%
24-11-22	16	30.1˚	25.5˚	67%	47%
25-11-22	17	30.4˚	27.2˚	69%	46%
26-11-22	18	31.4˚	27.3˚	66%	48%
27-11-22	19	33.9˚	28.5˚	65%	45%
28-11-22	20	33.4˚	28.9˚	69%	40%
29-11-22	21	31.9˚	27.9˚	65%	43%
30-11-22	22	33.4˚	27.0˚	67%	49%
01-12-22	23	29.2˚	24.4˚	68%	47%
02-12-22	24	29.4˚	24.4˚	66%	46%
03-12-22	25	29.6˚	24.2˚	61%	46%
04-12-22	26	29.4˚	23.2˚	65%	46%
05-12-22	27	29.2˚	24.2˚	65%	41%
06-12-22	28	28.9˚	22.4˚	73%	49%
07-12-22	29	28.5˚	23.5˚	70%	48%
08-12-22	30	27.8˚	22.9˚	68%	53%

09-12-22	31	27.4°	22°	76%	52%
10-12-22	32	27.9°	20.9°	85%	51%
11-12-22	33	26.2°	20.2°	72%	53%
12-12-22	34	25.4°	20.6°	70%	52%
13-12-22	35	22.5°	21°	68%	47%

Appendix 3 :Effect of bacteriophage on dressing percentage of broiler chicken(g/bird)

	Replication	**Dressing Percentage (%)**	
T0	R1	62.55	65.14
	R2	70.95	
	R3	66.02	
	R4	61.07	
T1	R1	77.87	68.97
	R2	70.95	
	R3	66.02	
	R4	61.07	
T2	R1	77.82	71.94
	R2	73.44	
	R3	71.21	
	R4	65.30	
T3	R1	60.82	70.27
	R2	73.15	
	R3	76.31	
	R4	70.81	
T4	R1	64.47	69.06
	R2	72.39	
	R3	73.22	
	R4	66.17	

Appendix 4 :Effect of bacteriophage on internal organs of broiler chicken

	Replication	Live weight (gm)	Dressed Wt. (gm)	Eviscerated Wt. (gm)	Liver wt. (gm)	Heart wt. (gm)	Drum Stick(gm)	Gizzard (gm)	Intestine (gm)	Spleen Wt. (gm)	Breast (gm)	Thigh (gm)	Wing (gm)	Back (gm)	Bursa (gm)
T0	R1	1343	1012	840	34	7	117	39	75	2	406	252	70	108	3
	R2	1790	1334	1270	33	9	151	59	85	2	526	165	72	210	3
	R3	1795	1313	1185	35	8	157	53	83	2	511	167	79	198	2
	R4	1490	960	910	28	11	122	55	78	2	567	145	67	160	2
	R1	2711	2270	2111	80	18	203	68	171	4	649	448	152	328	2
T1	R2	1845	1344	1166	46	10	159	25	119	3	513	169	87	192	2
	R3	2090	1637	1513	45	12	191	50	11	3	579	201	96	244	2
	R4	2181	1712	1581	38	14	167	48	96	3	510	230	94	226	2
	R1	2412	2009	1877	71	16	205	74	179	4	556	445	106	332	3
T2	R2	1920	1610	1410	44	11	177	49	98	3	560	195	93	211	3
	R3	1952	1638	1390	49	10	180	31	127	3	536	187	87	212	3
	R4	1585	1127	1035	53	12	184	35	112	3	540	156	95	188	2
	R1	1932	1615	1175	62	10	177	54	129	4	667	329	96	190	2
	R2	1795	1445	1313	35	8	157	53	83	2	511	167	79	198	2
T3	R3	2140	1738	1633	51	13	189	57	115	3	592	207	101	245	3
	R4	1747	1433	1237	48	13	162	58	72	3	580	210	110	256	2
	R1	1748	1336	1127	41	10	157	53	86	2	592	334	71	155	2
T4	R2	2090	1715	1513	45	12	165	50	111	2	558	184	77	212	3
	R3	1830	1517	1340	42	11	177	52	84	2	515	195	93	211	2
	R4	1862	1350	1232	40	13	152	55	72	2	560	189	85	209	2

Appendix 5 :Effect of bacteriophage on uniformity of broiler chicken

Treatment	Replication	Uniformity(%)

T0	R1	60	65.10
	R2	75	
	R3	63.33	
	R4	**62.10**	
T1	R1	60.60	67.71
	R2	63.63	
	R3	66.66	
	R4	**79.98**	
T2	R1	66.66	64.11
	R2	66.66	
	R3	62.5	
	R4	**60.65**	
T3	R1	75	63.73
	R2	56.25	
	R3	62.5	
	R4	61.2	
T4	R1	67.12	66.60
	R2	69.54	
	R3	59.51	
	R4	70.23	

Appendix 6:Effect of bacteriophage on body weight Gain (BWG)(g/bird) of broiler chicken

Treatment		1st Week	2nd Week	3rd Week	4th Week	5th Week	Final Gain
T_0	T0R1	130.548	343.978	534.206	812.794	1365.206	2136
	T0R2	136.88	347.362	530.268	833.732	1331.268	2123
	T0R3	134.22	356.689	553.674	830.326	1299.674	2088
	T0R4	131.632	349.347	537.629	824.371	1331.544	2113.915
T_1	T1R1	141.63	385.643	543.187	948.813	1297.187	2204
	T1R2	142.84	384.233	517.927	966.073	1327.927	2252
	T1R3	138.55	402.208	514.943	937.057	1276.943	2172
	T1R4	139	390.768	523.279	950.721	1298.279	2207
T_2	T2R1	138.88	367.029	583.001	917.999	1285.001	2161
	T2R2	140.7	376.785	557.215	908.785	1321.215	2188
	T2R3	136.3	381.882	574.087	852.913	1351.087	2162
	T2R4	136.62	375.239	569.427	893.24	1317.09	2168.33
T_3	T3R1	136.365	434.847	525.434	984.566	1271.434	2214
	T3R2	140.482	370.893	585.169	889.831	1288.169	2136

	T3R3	138.621	409.504	517.253	930.747	1254.253	2143
	T3R4	136.489	405.081	540.619	935.048	1269.282	2162.33
T4	T4R1	144.792	365.989	534.917	835.083	1269.917	2063
	T4R2	142.62	369.722	539.671	897.329	1248.671	2104
	T4R3	143.51	348.914	572.315	799.485	1254.515	2012
	T4R4	141.64	394.842	520.401	837.266	1242.064	2057.33

Treatment		1st Week	2nd Week	3rd Week	4th Week	5th Week
T_0	T0R1	166	364.1	781.9	1300.1	1800.128
	T0R2	166.61	373.4	857.6	1274.4	1774.933
	T0R3	165.59	366.41	858.59	1300.41	1660.105
	T0R4	163.815	367.935	834.98	1287.35	1334.092
T_1	T1R1	169	386.51	837.49	1311.51	1792.217
	T1R2	169.7	380.51	874.49	1294.51	1720.522
	T1R3	169.2	410.86	834.14	1410.86	1698.018
	T1R4	167.3	392.37	847.96	1337.707	1709.493
T_2	T2R1	168	371	908	1185	2005.272
	T2R2	167.21	380.79	898.21	1320.79	1857.543
	T2R3	168	381	909	1403	1814.906
	T2R4	165.73	377.6	903.067	1302.933	1768.904

T_3	T3R1	163	429	863	1384	2073.906
	T3R2	165	376	887	1334	1956.875
	T3R3	164.05	414.95	843.05	1475.95	1753.8
	T3R4	162.01	405.99	863.01	1397.323	1752.052
T_4	T4R1	166	375	878	1268	2015.187
	T4R2	165.45	379.55	903.45	1323.55	2042.813
	T4R3	165.134	364.866	863.134	1262.866	1939.437
	T4R4	163.528	372.472	880.858	1283.472	1998.479

Treatment	Replication	1st Week	2nd Week	3rd Week	4th Week	5th Week	Final
T_0	T0R1	0.96	1.12	1.30	1.55	1.56	1.62

Appendix 7: Effect of bacteriophage on feed consumption (FC) (g/bird)

Treatment	Replication						
	T0R2	0.93	1.12	1.40	1.56	1.60	1.65
	T0R3	0.94	1.08	1.35	1.56	1.58	1.62
	T0R4	0.94	1.11	1.36	1.56	1.50	1.59
T_1	T1R1	0.92	1.05	1.32	1.44	1.38	1.41
	T1R2	0.92	1.04	1.39	1.46	1.31	1.34
	T1R3	0.94	1.07	1.36	1.55	1.40	1.43
	T1R4	0.92	1.06	1.36	1.48	1.35	1.38
T_2	T2R1	0.93	1.07	1.35	1.39	1.45	1.48
	T2R2	0.92	1.06	1.37	1.51	1.43	1.45
	T2R3	0.94	1.06	1.35	1.62	1.46	1.49
	T2R4	0.93	1.06	1.36	1.51	1.39	1.42
T_3	T3R1	0.91	1.04	1.35	1.49	1.53	1.56
	T3R2	0.90	1.06	1.32	1.51	1.51	1.54
	T3R3	0.91	1.06	1.36	1.60	1.48	1.51
	T3R4	0.91	1.04	1.35	1.49	1.53	1.46
T4	T4R1	0.89	1.06	1.39	1.57	1.56	1.59
	T4R2	0.90	1.06	1.38	1.53	1.57	1.60
	T4R3	0.89	1.08	1.33	1.55	1.56	1.59
	T4R4	0.89	1.00	1.37	1.57	1.56	1.60

Treatment	Replication	Feed Intake (gm/bird)	Feed cost 60TK/kg× amount of feed	Total Production Cost Tk/bird(Chick cost +Feed cost+ common cost 110 TK/bird)	No of Live Birds	Total Production Cost/m^2
T_0	T0R1	3613.228	216.792	316.792	30	9503.76
	T0R2	3484.333	209.058	319.058	30	9571.74
	T0R3	3490.515	209.43	319.43	29	9582.9
	T0R4	3540.442	212.4	322.4	30	9672
T_1	T1R1	3103.727	186.18	296.18	29	8885.4
	T1R2	3015.032	180.9	290.9	29	8727

Appendix 8: Effect of bacteriophage on Feed Conversion Ratio (FCR)

	T1R3	3108.878	186.528	296.528	30	8895.84
	T1R4	3047.2	182.52	292.52	30	8775.6
T_2	T2R1	3190.272	191.412	310.412	30	9312.36
	T2R2	3178.333	190.698	300.698	28	9020.94
	T2R3	3217.906	193.074	313.074	29	9392.22
	T2R4	3071.837	184.26	294.26	30	8827.8
T_3	T3R1	3457.906	207.42	317.42	29	9522.6
	T3R2	3290.875	197.448	307.448	29	9223.44
	T3R3	3229.75	193.74	303.74	30	9112.2
	T3R4	3149.375	188.962	298.962	30	8968.86
T4	T4R1	3283.187	196.98	306.98	29	9209.4
	T4R2	3366.363	201.968	311.968	29	9359.04
	T4R3	3202.303	192.138	302.138	29	9064.14
	T4R4	3281.951	196.914	306.914	30	9207.42

Treatment	Replication	Live Wt(gm/bird)	Sale Value 150Tk /kg live bird	No of Live birds	Total income/ m^2	Total Production cost/ m^2	Net Profit Tk/ m^2	Benefit Cost-ratio
T_0	T0R1	2178	326.7	30	9801	9503.76	297.24	1.0313
	T0R2	2165	324.75	30	9742.5	9571.74	170.76	1.0178

Appendix 9: Average production cost (TK.) of broilers at different treatments

Appendix 10: Average total income(TK) and benefit cost ratio (BCR)/m^2 of broilers at different treatment

	T0R3	2130	319.5	29	9585	9582.9	2.1	1.0002
	T0R4	2155.915	323.38725	30	9701.618	9672	29.6175	1.0031
T_1	T1R1	2246	336.9	29	10107	8885.4	1221.6	1.1375
	T1R2	2294	344.1	29	10323	8727	1596	1.1829
	T1R3	2214	332.1	30	9963	8895.84	1067.16	1.1200
	T1R4	2249	337.35	30	10120.5	8775.6	1344.9	1.1533
T_2	T2R1	2203	330.45	30	9913.5	9312.36	601.14	1.0646
	T2R2	2230	334.5	28	10035	9020.94	1014.06	1.1124
	T2R3	2204	330.6	29	9918	9392.22	525.78	1.0560
	T2R4	2210.33	331.5495	30	9946.485	8827.8	1118.685	1.1267
T_3	T3R1	2256	338.4	29	10152	9522.6	629.4	1.0661
	T3R2	2178	326.7	29	9801	9223.44	577.56	1.0626
	T3R3	2185	327.75	30	9832.5	9112.2	720.3	1.0790
	T3R4	2204.33	330.6495	30	9919.485	8968.86	950.625	1.1060
T4	T4R1	2105	315.75	29	9472.5	9209.4	263.1	1.0286
	T4R2	2146	321.9	29	9657	9359.04	297.96	1.0318
	T4R3	2054	308.1	29	9243	9064.14	178.86	1.0197
	T4R4	2099.33	314.8995	30	9446.985	9207.42	239.565	1.0260

Appendix 11: Effect of bacteriophage treatment on Lactobacillus Spp. colony in feces

Treat-ment	Replication	Dilution Factor	Viable Count		Mean Viable Count	CFU/mL
			Plate 1	Plate 2		
T_0	T0R1	10^{-5}	32	38	35	1.4×10^8
	T0R2	10^{-5}	18	26	22	$.88\times10^8$
	T0R3	10^{-5}	24	31	27.5	1.1×10^8
	T0R4	10^{-5}	39	28	33.5	1.3×10^8
T_1	T1R1	10^{-5}	20	11	15.5	$.62\times10^8$
	T1R2	10^{-5}	25	31	28	1.1×10^8
	T1R3	10^{-5}	27	35	31	1.2×10^8
	T1R4	10^{-5}	22	26	24	$.96\times10^8$
T_2	T2R1	10^{-5}	53	87	70	2.8×10^8
	T2R2	10^{-5}	44	52	48	1.9×10^8
	T2R3	10^{-5}	56	61	58.5	2.3×10^8
	T2R4	10^{-5}	67	73	65	2.6×10^8
T_3	T3R1	10^{-5}	84	74	79	3.1×10^8
	T3R2	10^{-5}	67	59	63	2.5×10^8
	T3R3	10^{-5}	83	67	75	3.0×10^8
	T3R4	10^{-5}	89	61	75	3.0×10^8
T4	T4R1	10^{-5}	11	2	6.5	$.26\times10^8$
	T4R2	10^{-5}	9	13	11	$.44\times10^8$
	T4R3	10^{-5}	12	18	15	$.60\times10^8$
	T4R4	10^{-5}	15	6	10.5	$.42\times10^8$

Appendix 12: Effect of bacteriophage treatment on survivability rate (%) of the research.

	Number of Birds	Survival number of birds	Survivability rate(%)
T0R1	30	30	100.0
T0R2	30	30	100.0
T0R3	30	29	96.7
T0R4	30	30	100.0
T1R1	30	29	96.7
T1R2	30	29	96.7
T1R3	30	30	100.0
T1R4	30	30	100.0
T2R1	30	30	100.0

T2R2	30	28	93.3
T2R3	30	29	96.7
T2R4	30	30	100.0
T3R1	30	29	96.7
T3R2	30	29	96.7
T3R3	30	30	100.0
T3R4	30	30	100.0
T4R1	30	29	96.7
T4R2	30	29	96.7
T4R3	30	29	96.7
T4R4	30	30	100

Appendix 13 :Effect of bacteriophage on NH_3 concentration (PPM)

Days	7 Days	14 Days	21 Days	28 Days	35 Days
T0R1	6	8	8	15	20
T0R2	6	7	8	16	18
T0R3	6	10	9	14	15
T0R4	6	8	10	12	14
T1R1	6	6	8	12	10
T1R2	6	7	10	15	16
T1R3	6	10	11	12	15
T1R4	6	9	10	11	12

T2R1	6	8	8	12	20
T2R2	6	9	10	16	18
T2R3	6	11	12	14	17
T2R4	6	9	11	15	19
T3R1	6	8	10	12	20
T3R2	6	6	8	11	18
T3R3	6	9	14	15	16
T3R4	6	11	12	17	19
T4R1	6	9	10	15	20
T4R2	6	10	11	15	16
T4R3	6	8	12	14	18
T4R4	6	8	10	12	16

Some activities in scenario

Figure 3: Brooder and shed preparation

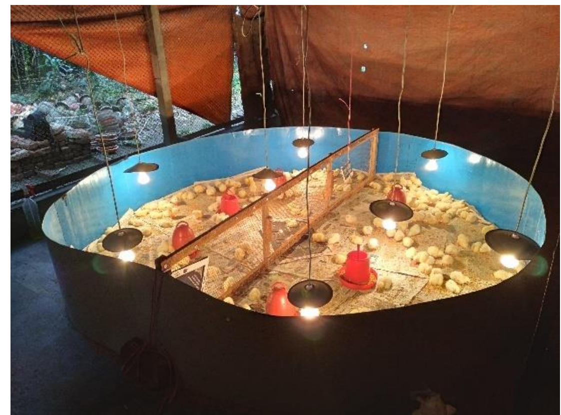

Figure 4 : Chick receiving & placement of chicks into brooding

Figure 5 : Vaccination

Figure 6 : Sample collection

Figure 8: Medication used in experiment

Figure 9 : Picture of determining internal organ characteristics

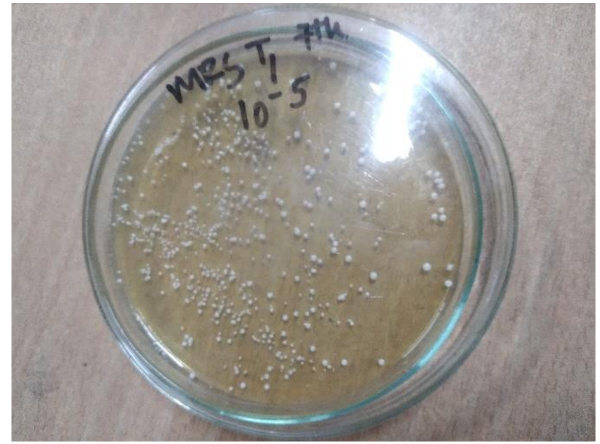

Figure 10 : Picture of Lactobacillus colony count in lab

Figure 11 :Monitoring by supervisor &co-supervisor Sir