USE OF COMBINATION OF SELECTED BACTERIOPHAGES IN BROILER RATION: A SUSTAINABLE ALTERNATIVE TO ANTIBIOTIC GROWTH PROMOTERS

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CERTIFICATE

*This is to certify that the thesis entitled, "**USE OF COMBINATION OF SELECTED BACTERIOPHAGES IN BROILER RATION: A SUSTAINABLE ALTERNATIVE TO ANTIBIOTIC GROWTH PROMOTERS** Submitted to the Department of Animal Nutrition, Genetics and Breeding, Faculty of Animal science and veterinary medicine, Sher-E-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) in Animal Nutrition** embodies the result of a piece of bonafide research work carried out by **FATIMA YEASMIN, Registration No. 20-11131** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly acknowledged by him.

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Dedicated
To
My Parents

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LIST OF ACRONYMS AND ABBREVIATION

ABBREVIATION		FULL MEANING
ANOVA	=	Analysis of variance
AGPs	=	Antibiotic growth promotors
AMPs	=	Antimicrobial peptides
Avg.	=	Average
AWFI	=	Average weekly feed intake
AWG	=	Average weight gain
BCR	=	Benefit Cost Ratio
BMD	=	Bacitracin Methylene Disalicylate
BP	=	Bacteriophage
BW	=	Body weight
BWG	=	Body weight gain
CE	=	Competitive exclusion
Cm	=	Centimetre
CRD	=	Completely randomised design
CUF	=	Colony forming unit
DOC	=	Day old chicks
E. coli	=	*Escherichia coli*
e.g.	=	For example,
EMB	=	Eosin methylene blue
EFSA	=	European Food Safety Authority
et al.	=	And others/associates
FC	=	Feed consumption
FCR	=	Feed conversion ratio
FI	=	Feed intake
FDA	=	Food And Drug Administration
Ft	=	Feet
G	=	Gram
Gms	=	Grams
hrs.	=	Hours
i.e.	=	That is
IgY	=	Hyperimmune egg yolk antibodies

ACRONYMS AND ABBREVIATION (Cont'd)

ABBREVIATION		FULL MEANING
IB	=	Infectious bronchitis
IBD	=	Infectious bursal disease
IFA	=	In-feed antibiotic
K Cal	=	Kilo calorie
Kg	=	Kilogram
L	=	Litre
Lbs	=	Pound
Mg	=	Milligram
MS	=	Master of science
Ml	=	Millilitre
m^2	=	Square meter
ND	=	Newcastle disease
No.	=	Number
NS	=	Non-significance
Pfu	=	Plaque forming unit
pH	=	Potential of hydrogen
SAU	=	Sher-e-Bangla Agricultural University
SE	=	Standard Error
SPSS	=	Statistical package for social sciences
SS	=	Salmonella-shigella
Tk	=	Taka
Viz.	=	Such as
hrs.	=	Hours
WHO	=	World health organization
Wks	=	Weeks

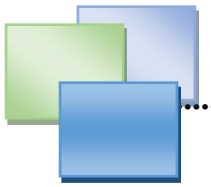
LIST OF SYMBOLS

SYMBOLS		FULL MEANING
*	=	5% level of significance
@	=	At the rate of
℃	=	Degree Celsius
>	=	Greater than
<	=	Less than
/	=	Per
%	=	Percentage
±	=	Plus-minus
:	=	Ratio

USE OF COMBINATION OF SELECTED BACTERIOPHAGES IN BROILER RATION: A SUSTAINABLE ALTERNATIVE TO ANTIBIOTIC GROWTH PROMOTERS

ABSTRACT

Antibiotic growth promoter alternatives are urgently needed in the poultry industry to maintain or improve poultry health and performance. Bacteriophage (BP) therapy mainly utilizes lytic phage to kill their respective bacterial hosts and exhibit no activity against animal and plant cells. They can be considered novel alternative solution to combating the emergence of antibiotic resistance in poultry. A total of 600-day-old mix broiler chicks (Hubbard Classic Efficiency Plus) with the initial body weight of 41.9±1.0 g were reared for 35-days experimental period. Birds were randomly allotted into 1 of 5 treatments according to a Completely Randomized Design (CRD). Dietary treatments consist T_0 Control (no antibiotics and no BP), T_1 (0.5 gm BP/kg of feed), T_2 (0.75 gm BP/kg of feed), T_3 (1.0 gm BP/kg of feed) and T_4 antibiotic control group (0.055 g antibiotic BMD/kg feed) (bacitracin methylene disalicylate). The group T_1 ($P < 0.05$) showed higher body weight (2251.58± 15.10 g) compared to T_0 (2027.78± 6.11 g) and T_4 (2093.93± 20.28 g). Best FCR result was found in T_1 ($P < 0.05$) (0.5g BP/kg) group (1.49) compared to the T_4 antibiotic treated group (1.54) and T_0 Control group (1.58). The group T_3 ($P < 0.05$) showed higher feed consumption compared to T_0 and T_4. *Escherichia coli* concentration in excreta is higher (6.84 log10CFU/g) in T_0 differ significantly ($P<0.05$) with other groups. *Salmonella* concentration is higher (4.28 log10 CFU/g) in T_0 and differ significantly ($P<0.05$) from other groups however not significantly different ($P = 0766.$) from T_4. The weight of the spleen in the control group T_0 is the highest and is differ significantly ($P<0.05$) with other groups. Similarly, the bursa of fabricus' weight is the highest in T_0 group and differs significantly ($P<0.05$) with other groups. Among the three-bacteriophage dietary treatment group T_1 showed better body weights and FCR than group T_2 and T_3. In conclusion, dietary supplementation of 0.5 g/kg BP reduced FCR and increased body weight with inhibiting of pathogens. Therefore, the research recommended inclusion of 0.5 g BP/kg of feed as an alternative to antibiotic growth promoters in broiler diets.



CHAPTER 1

INTRODUCTION

CHAPTER I

INTRODUCTION

1.1 General Background

The growing challenges to secure wholesome food of animal origin in quantities sufficient to feed the ever-increasing world population leads to the compelling need of search for newer means to enhance animal production. Such an endeavor often involves the use of substances with high biological potencies. In countries with large scale animal production, a high percentage of animals are exposed at one time or another during their lifespan to various antibiotic growth promotors or alternative growth promotors.

In response to the increase in the demand for livestock products such as meat, milk and eggs by a growing global population, livestock producers are compelled to significantly increase production of these products. Thus, large scale intensive farming systems are continuing to appear. Unfortunately, such production systems can promote disease transmission very easily due to their low genetic diversity and high stocking density, leading to concomitant production and economic losses (Nhung et al., 2017). Zoonotic pathogens associated with poultry and pigs such as Salmonella spp., E. coli, Campylobacter spp., Clostridium spp., and Listeria spp. have been reported by European Food Safety Authority (EFSA) to be often resistant to several antibiotics (EFSA 2017; EFSA 2017). In this context, alternative approaches have become imperative. One option is the application of lytic bacteriophage (BP) to combat the bacterial diseases in livestock (Brussow *et al*., 2005).

Bacteriophages are viruses that infect and use bacterial resources for their own reproduction. They are very common in all environments and have a high specificity for bacteria at infection (White *et al*., 2019). In a review, Domingo *et al*. (Domingo - Calp *et al*., 2016) suggested that BP have narrow spectrum activity against bacteria, in contrast to the broad-spectrum activity of antibiotics against bacteria. BP are specific for particular bacteria, and phage therapy is considered safe and effective in comparison to antibiotics partially because they infect one species, serotype or strain. This mechanism of action does not inhibit the proliferation of commensal intestinal flora (Wernichi *et al*., 2017; Cieplak *et al*., 2018). Fiorentin *et al*., 2005) noted that the

application of single oral cocktail of phages at a dosage of 10^{11} pfu decreased the occurrence of *Salmonella Enteritidis* strains by 3.5 log units.

In addition, other studies have also reported a successful reduction in the Salmonella spp. counts in chicken internal organs and excreta (Toro *et al*., 2005), and administering bacteriophage as an aerosol spray is effective in preventing *E. coli* respiratory infections in broiler chickens (Huff *et al*., 2002) as well as in poultry products (Whichard *et al*., 2013; Higgino *et al*., 2005) with BP application. Furthermore, it has been reported that BP supplementation improved body weight (Kim *et al*., 2014), feed efficiency, liver weight and reduced pathogens in broiler chickens (Wang *et al*., 2013) and improved egg production and egg quality in laying hens (Zhao *et al*., 2012).

The inclusion of Bacteriophages as a feed additive may potentially provide an integrated solution to modulate the gut microbiome in chicken by reducing specific pathogenic microbial populations, thereby promoting the proliferation of beneficial microbiota, resulting in improved gut health (Clavijo *et al*., 2018). Under bacterial challenge, bacteriophage has shown to be effective in several studies, which applied BP at different concentrations such as 0.1 mL containing 10^{11} pfu/mL, 1 mL containing 10^{10} pfu/mL or 1 mL containing 10^{7} pfu/mL respectively (Bardina *et al*., 2012; Fischer *et al*., 2013). Recently, it has been reported that the inclusion of bacteriophages in broiler ration could benefit the poultry farmers in terms of improved body weight and FCR in broilers. And also avoid the usage of antibiotics in feed. However, scanty data is available in Bangladesh on the use of bacteriophages in broiler feed.

1.2 Objectives

However, reports on the dietary usage of a bacteriophage cocktail in birds without bacterial challenge are scarce. Thus, the aim of the current study was to assess the effects of cocktail bacteriophage with following objectives:

- To assess the effects of three different concentrations of cocktail bacteriophage on the body weight, feed consumption and FCR in broilers.
- To evaluate the effect of bacteriophage on *Salmonella* Sp. and *Escherichia coli*
- To determine the effect of bacteriophage on organ weight.
- To evaluate the cost-effectiveness of using different levels of bacteriophage.

CHAPTER II

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

2.1 Antimicrobial growth promoters

The term “antimicrobial growth promoter (AGP)” is used to describe any medicine that destroys or inhibits bacteria and is administrated at a low, sub therapeutic dose for the purpose of performance enhancement. The use of antimicrobials for growth promotion has arisen with the intensification of livestock farming. Antimicrobial growth promoters are used to help the animals to digest their food more efficiently, get maximum benefit from it and allow them to develop into strong and healthy individuals. As prevention of diseases, enhancement of growth and feed efficacy are crucial to vital for animal husbandry business (Doyle, 2001).

The effect of antibiotics on improving performance was first reported by (Moore *et al*., 1946) when they observed that birds fed streptomycin exhibited increased growth responses. Many experiments conducted later in the early 1950s in chickens (Groschke and Evans, 1950; McGinnis, 1950; Whitehill *et al*., 1950), pigs (Jukes *et al*., 1950; Luecke *et al*., 1950 a, b), and calves (Rusoff *et al*., 1951) corroborated these results.

Several studies indicate that the use of antimicrobials has resulted in increased productivity and decreased cost for consumers (Ricke *et al*., 2012). The administration of AGPs at sub- therapeutic dosages has been shown to increase growth rate, feed conversion and consequently, broiler performance (Bbosa and Mwebaza, 2013). In-feed antibiotic (IFA) use soon became a common and well-established practice in the animal industry and rose with the intensification of livestock production.

In a review conducted by Rosen (1995), it was concluded that inclusion of antibiotics in the diets gave a positive response 72% of the time. It was also proposed that the net effect of using IFA in the poultry industry was a 3–5% increase in growth and feed conversion efficiency (Choct, 2001; Dahiya *et al*., 2006). Thus, it can be noted that IFA played a crucial role in contributing to the economic effectiveness of the livestock production (Wierup, 2000).

2.2 Antimicrobial growth promoters - mode of action

Orally ingested antibiotics promote growth and efficiency of poultry and other animals. The effect can include gain but often is limited to feed efficiency effects only. The mechanism of action must be focused on the gut because some of these antibiotics are not absorbed. Following early demonstrations that oral antibiotics do not have growth-promoting effects in germ-free animals (Coates *et al*., 1955; Coates *et al*., 1963), studies of the mechanism for growth promotion have focused on interactions between the antibiotic and the gut microbiota. Thus, direct effects of AGP on the microflora can be used to explain decreased competition for nutrients and reduction in microbial metabolites that depress growth (Visek, 1978a; Anderson *et al*., 1999). Additional AGP effects that also occur in germ-free animals include reduction in gut size, including thinner intestinal villi and total gut wall (Coates *et al*., 1955). This may be due, in part, to the loss of mucosa cell proliferation in the absence of luminal short chain fatty acids derived from microbial fermentation. The reduction in gut wall and villus lamina propria has been used to explain the enhanced nutrient digestibility observed with AGP (Jukes *et al*., 1956; Franti *et al*., 1972; Anderson *et al*., 1999).

Finally, a reduction in opportunistic pathogens and subclinical infection has also been linked to use of AGP. It should be noted that injection of bacterial metabolites such as lipopolysaccharides or immune mediators such as interleukin-1 can mimic the reduced efficiency of an animal with a conventional microflora and no antimicrobial in the diet (Roura *et al*., 1992), which illustrates the importance of the host response to the microflora as another factor limiting growth efficiency. The reduction in microflora, and its consequences, may be the underlying mechanism for beneficial effects of antibiotics.

2.3 Adverse effects of antimicrobial growth promoters

Despite their substantial contribution to the poultry industry, AGPs are under surveillance due to an increase in the incidence of drug resistance, caused majorly by the use of these drugs by livestock farmers without veterinary consultation or proper directions for dosage (Bbosa and Mwebaza, 2013). Despite the well- demonstrated beneficial effects their use was also known to be associated with some disadvantages

and challenges. Concerns exist that the use of IFA leads to development of antimicrobial resistance, posing a potential threat to human health (WHO, 2012).

In addition to bio-resistance, antibiotics abuse has resulted in drug residues in animal products (Gonzalez Ronquillo and Angeles Hernandez, 2017). Several antibiotics such as penicillin, tetracycline, macrolide, aminoglycoside and amphenicol have been detected in foods (Diarra and Malouin, 2014). Residues in livestock production can actually have antithetical impact on human health, this is the case for tetracyclines, which interfere with teeth development in young children (Kummerer, 2009). This is also the case with beta-agonists, such as clen buterol, leading sometimes to food poisoning and muscle tremors, palpitations and tachycardia (Chan, 1999).

Animal bedding contains residues of antimicrobial compounds. Residues of bacitracin, salino mycin, penicillin and Virginiamycin were detected in chicken litter at concentrations ranging from 0.07 to 66 mg/L (Furtula *et al*., 2010). When this bedding material is used as nitrogen amendment, the resistant bacteria can live in the soil for several months (Merchant *et al*., 2012) Bio-resistant bacteria (Staphylococcus xylosus) have also been reported in air in broiler farms (Vela *et al*., 2012; Liu *et al*. 2012) have shown that airborne transmission causes the spread of epidemic diseases and also poses an impede over public health.

According to (Manzetti and Ghisi *et al*., 2014), the most vulnerable ecosystems to antibiotic contamination are confined aquatic ecosystems such as ponds, lakes and soils close to urban sites. Large amounts of antibiotics administered to animals are excreted into the environment via urine and feces (Carvalho and Santos *et al*., 2016). Antibiotics risks in the aquatic environment and sediments are important because they can influence aquatic life behaviour (Kummerer, 2009). Continuous use of sub-therapeutic level of antibiotic growth promoters in animals caused consequent appearance of resistance to those antibiotics among several pathogenic bacteria, Resistance and cross resistance established in animal and human via food chain and scientific evidence of antibiotic resistance in food animals is associated with resistance infections in humans (Cervantes, 2004). Many scientists believe dependence on and misuse of antibiotics in human medicine is the primary cause of resistance (Zhao *et al*., 2003) which subsequently result in antibiotic-resistant bacteria, can be transferred between animals and between animals and people. The use of antibiotics as growth promoters is harmful in many ways especially for human health.

2.4 Prevalence of antimicrobial growth promoters in Bangladesh

Commercial poultry production in Bangladesh has emerged as one of the country's fastest growing industries in the last decades. Poultry meat and egg have become one of the major animal protein sources in Bangladesh due to their affordable price and availability (Saleque and Ansarey, 2020). Major bacterial diseases observed in broiler chicken in Bangladesh are Pullorum disease, Fowl Typhoid, Fowl Paratyphoid, Colibacillosis, Necrotic Enteritis and Omphalitis (Saleque *et al*., 2013; Hassaan *et al*., 2016; Mamun *et al*., 2019).

In Bangladesh, commercial poultry farmers extensively utilize antibiotics without any veterinary advice and often do not follow withdrawal period guidelines (Haque *et al*., 2020). A lack of both easily accessible veterinary facilities and adequate knowledge combined with a high-profit motive are some of the factors that drive local producers to inappropriate and at times, illegal use of antimicrobial agents (Saiful *et al*., 2016).

Sattar *et al*. (2014) reported antibiotic residues mostly in the liver, kidney, thigh meat, and lowest in breast meat of broilers. Screening of antibiotic residues in chicken meat in Bangladesh shows highest frequency in liver followed by thigh muscles and breast muscle. Among the antibiotics found in different organs where Ciprofloxacin, Doxycycline, Amoxicillin, Oxytetracycline and Enrofloxacin (Sarker *et al*., 2018). There has been a worldwide increase in the regulation or ban of the use of AGPs in poultry diets. Bangladesh Government too has banned the use of antibiotics by Bangladesh Gazette, Registered No. DA -1, Act No 2 of the year 2010 with a sub claws 14, dated 28th January 2010 (Bangladesh Gazette, 2010). Ban on AGP in feed resulted in lot of problems such as increase of production cost and reduced animal performance in Bangladesh.

2.5 Fate of antimicrobial growth promoters in future

The decline in the use of antibiotic growth promoters (AGPs) in the future seems inevitable, and the practice of using antimicrobials may prove economically impractical because of market limitations and export restrictions (Dibner and Richards, 2005).

With the increase in regulations regarding the use of antibiotic growth promoters and the rise, in consumer demand for poultry products from 'Raised Without Antibiotics' or 'No Antibiotics Ever' flocks, the quest for alternative products or approaches has intensified in recent years. A great deal of research has focused on the development of antibiotic alternatives to maintain or improve poultry health and performance. Since the discovery of antibiotics in the 1920's they have played a substantial role in the advancement and prosperity of the poultry industry. Antibiotics have been supplemented in animal feed at sub therapeutic doses to improve growth and feed conversion efficiency and to prevent infections for more than 60 years (Castanon, 2007).

The European Commission banned the use of avoparcin as a growth promoter on the grounds of unknown risk. Grosso *et al*. (2000) found that, after the ban, a decrease was observed in contamination of meat products by vancomycin-resistant enterococci. The reduction was statistically significant in poultry (from 18.8 percent to 9.6 percent). The European Commission no longer permits "medically important" antibiotics to be used as antibiotic growth promoters, due to possible risks of compromise of therapy. However, this needs to be a global effort as Fidler (1996) noted, bacteria do not respect international borders.

In the early 1970s, the UK banned the use of tetracycline and penicillin for growth promotional purposes, spurring other European countries to take the same precaution shortly after. In the mid-1970s, the Food and Drug Administration (FDA) proposed a ban in the USA, but Congress intervened and required the FDA to do more research before instituting a ban. Today, the European Commission, the World Health Organization, the Centres for Disease Control and the American Public Health Association all support the immediate prohibition of antibiotic growth-promoters that are the same as, or closely related to, antibiotics used in humans. In March 1999, the Centre for Science in the Public Interest, the Environmental Défense Fund, and others petitioned the FDA to ban, for purposes of growth promotion, six antibiotics used in or related to those used in human medicine, including penicillin, tetracycline, erythromycin, lincomycin, tylosin, and Virginiamycin. The FDA has recently launched a Task Force (FDA, 2001) to tackle the subject of the use of antimicrobials in agriculture but many politicians have greeted it with negativity. It is worth noting

that the Framework Document simply laid out a program for assessing the risk of antimicrobials on human health.

In view of the increasing concerns over AGP use, the quest for novel alternate replacements to mitigate antibiotic use in animal and agriculture has grown over the years. In the past two decades, a great deal of research has focused on the development of antibiotic alternatives to maintain or improve poultry health and performance (Gayatri *et al*., 2017). This phenomenon currently forces poultry nutritionists to search for new alternatives to AGPs.

2.6 Alternatives to antimicrobial growth promoters

An ideal alternative should have the same beneficial effects of AGP, ensure optimum animal performance, increase nutrient availability (Huyghebaert *et al*., 2011) and liveability (Dibner *et al*., 2005). Considering the proposed mechanism of action of AGPs (microbiome and immune-modulating activities), a practical alternative should possess both of these properties in addition to having a positive impact on feed conversion and/or growth (Huyghebaert *et al*., 2011; Seal *et al*., 2013). Several classes of alternatives have been proposed and tested in poultry production, including probiotics, prebiotics, synbiotics, organic acids, enzymes, phytogenics and metals. Novel alternatives such as hyper immune egg yolk IgY, (Gadde *et al*., 2015) antimicrobial peptides (AMP), bacteriophages, and clay have come into existence in recent years (Gadde *et al*., 2017).

2.6.1 Probiotics

Probiotics increase in body weight and feed conversion (Gheisar *et al*., 2016; Hatab *et al*., 2016) and decrease in pathogen count by competitive exclusion, increase of beneficial bacteria in gut by decrease of pH, and competing for nutrients and attachment sites (Olnood *et al*., 2015; Li *et al*., 2016) but do not exert a direct antimicrobial effect on pathogenic bacteria in the gut, rather they employ competitive exclusion (CE) to prevent pathogen colonization (Gayatri *et al*., 2017). However, several concerns with some probiotic-based products such as variations in the quality and dose of probiotics, poor survival rate in the stomach, inactivation during feed manufacturing, transport, or storage, allergenicity, potential crosstalk between

probiotics, pathogens and epithelial cells, and transmission of antibiotic-resistance genes can limit their use (Cheng *et al*., 2014; Joshi *et al*., 2018; Ramnani *et al*., 2012).

2.6.2 Prebiotics

Prebiotics increase in disease resistance, broiler efficiency and nutrient availability (Ganguly, 2015), increase in weight and population of beneficial bacteria (Arsi *et al*., 2015; Pourabedin and Zhao, 2015) and decrease in pathogen count (Kim *et al*., 2011; Shang *et al*., 2015) and Reversal of coccidial lesions (Chang *et al*., 2016). In contrast to the previous results, several authors reported that prebiotic supplementation had no effect on performance (Baurhoo *et al*., 2007; Józefiak *et al*., 2008; Geier *et al*., 2009; Corrigan *et al*., 2011; Houshmand *et al*.,2014). Despite their beneficial effects on the intestine, such as increased villi height and lower pH, the administration of a large amount of prebiotics might induce unwanted side effects such as bloating or diarrhoea due to the fermentation in the intestines (Joshi *et al*., 2018; Kridtayops *et al*., 2019; Roth *et al*., 2019)

2.6.3 Synbiotics

Synbiotics are a mixture of prebiotics and probiotics, they have the same strengths and weaknesses as probiotics and prebiotics as well as the same potential risks for bacterial resistance development. Like pre- and probiotics, synbiotics reduce diarrhoea, increase digestibility and daily weight gain, and promote beneficial bacterial strains, such as Lactobacillus and Bifidobacterium strains, leading to a more balanced gut microbiota (Cobb *et al*., 2019). The presence of prebiotics in the mixture assists probiotics in overcoming potential survival challenges (Kosznik – Kwasnicka *et al*., 2019). However, the majority of synbiotics used in animal feed have insufficient probiotic/prebiotic mixing ratios, and appropriate controls would need to be used in experiments for the development of symbiotic-supplemented animal feed (De Paepe *et al*., 2014).

2.6.4 Feed Acidifiers

Feed acidifiers decrease pathogen count (Koyuncu *et al*., 2013; Sultan *et al*., 2015), improvement in body weight gain and feed conversion ratio (Sohail et al. 2015; Reda *et al*., 2016), improvement of phytate phosphorus utilization (Rafacz-Livingston *et al*., 2005) and decrease in mortality and feed cost, increase in dressing percentage and liver weight (Khan *et al*., 2016). In spite of the demonstrated beneficial effects, using organic acids to improve performance lacks consistency. This can be attributed to various factors such as inclusion rates, the source of the organic acids, and the buffering capacity of other dietary ingredients (Dibner and Buttin, 2002; Kim *et al*., 2015).

Most acidifiers still show some weaknesses; the addition of acidifiers at an extreme level can negatively affect diet palatability, feed manufacturers can observe corrosiveness, which is harmful for feed processing equipment, and further research is needed to improve quality control and optimal dosage and to allow a better understanding of the potential threats (Ferronato *et al*., 2020; Nowak *et al*., 2021; Rhouma *et al*., 2017) Further research should address inconsistency issues and understand their mechanism of action to develop organic acids as effective antibiotic replacements (Gadde *et al*., 2017).

2.6.5 Phytogenic feed additives

Phytogenic feed additives increase in body weight (Bernard *et al*., 2016; Peng *et al*., 2016), improvement in feed conversion ratio and carcass yield (Jahan *et al*., 2016; Sadeghi *et al*., 2016), decrease in pathogen counts (Chang *et al*., 2016; Lan *et al*., 2016), improvement of fatty acid profile in egg yolk (Raza *et al*., 2016) and increased serum proteins and antioxidant status (Alzawqari *et al*., 2016). The mechanism of action of PFAs is not clearly understood and depends greatly upon the composition of the active ingredients in the product being used (Gadde *et al*., 2017). Although phytochemicals are considered “natural” items, they should be deeply evaluated for potential detrimental human and animal health effects as well as probable interactions with other dietary elements (Hashemipour *et al*., 2013). It also has drawbacks such as bad odours, need of high doses to obtain results, and toxicity have been observed in some of them (Alves-Santos *et al*., 2020; Pearlin *et al*., 2020).

2.6.6 Hyperimmune egg yolk antibodies (IgY)

Hyperimmune egg yolk antibodies (IgY), produced by repeated immunization of hens with specific antigens and collection of antibodies thereafter from their egg yolks, have been commonly employed in the prevention and treatment of various enteric diseases in humans and animals (Gadde *et al*., 2015). Limited research exists on the use of egg yolk antibodies as viable alternatives to AGP in improving growth and feed efficiency in poultry (Cook, 2004).

2.6.7 Antimicrobial peptides (AMPs)

Numerous studies on the use of antimicrobial peptides as growth promoters have shown their great potential as alternatives to antibiotics. Their abilities to improve growth performance and gut health, positively influence the microbiota, decrease the occurrence and severity of diarrhoea, and inhibit the expression of pro-inflammatory factors have been observed (Kurt *et al*., 2019). In addition, the degradation of antimicrobial peptides in the intestines prevents their release into the environment and reduces the risk of exposure that can lead to the development of resistance. However, this force is also having a weakness, as it decreases the half-life of the peptides in the intestine. Despite these attractive characteristics, the use of peptides has heretofore been limited by the problems associated with their large-scale production, their stability during feed preparation and storage, and their interactions with feed matrices (Assoni *et al*., 2020; Ioannou *et al*., 2017).The studies that have been done on Antimicrobial Peptides (AMPs) and their applications in poultry have been mostly focused on their protective potential against diverse pathogens causing infectious diseases rather than growth promoting activities The AMPs including bacteriocins have the potential to considerably enhance poultry health as alternatives to AGP and their potential might be improved when a number of obstacles such as high production cost, resistance development, and instability of the AMPs are addressed in the future. (Gadde *et al*., 2015).

2.6.8 Bacteriophages (BP)

The biblical Book of Kings relates how the prophet Elisha cured general Naaman's disease by commanding him to bathe seven times in the river Jordan. Since ancient times, there have been documented reports of river waters having the ability to cure infectious diseases such as leprosy (Keen, 2012). But, the British bacteriologist (Ernest Hankin, 1986) reported antibacterial activity against Vibrio cholerae, which he observed in the Ganges and Jumna rivers in India. He suggested that an unidentified substance was responsible for this phenomenon and for limiting the spread of cholera epidemics. Two years later, Gamaleya, the Russian bacteriologist, observed a similar phenomenon while working with Bacillus subtilis (Adhya Merril, 2006). It was not until 1914, however, that another British bacteriologist, Frederick Twort, advanced the hypothesis by proposing that it may have been due to, among other possibilities, a virus. For various reasons, including financial difficulties, Twort did not pursue this finding (Duckworth, 1976). A French-Canadian microbiologist, Felix d'Herelle, first observed in 1910 the bacteriophage phenomenon while studying microbiologic methods of controlling locusts in Mexico. In the lab, when he spread some cultures on agar, he observed round zones without growth, which he called plaques, and asserted they were caused by viral parasites. Six years later, he proposed the name "bacteriophage," or bacterium-eater (Duckworth, 1976).

BP therapy has advantages over antibiotic viz. BP are very specific to their hosts, so this minimizes the chance of secondary infections, but antibiotics do target both pathogens and normal flora of patients, which can cause the secondary infections or sometimes superinfections. Also, BP replicate at the site of infection where they are mostly needed to lyse the pathogens, but antibiotics travel throughout the body and do not concentrate at the site of infection. No side effects have been reported during or after phage application, but resistant bacteria, allergies (sometimes even fatal anaphylactic reaction), and secondary infections are the common side effects of antibiotics treatment (Sulakvelidze *et al*., 2001). BP are environmentally friendly and are based on natural selection, isolating and identifying bacteria in a very rapid process compared to new antibiotic development, which may take several years, may cost millions of dollars for clinical trials, and may also not be very cost effective (Weber-Dabrowska *et al*., 2000). Moreover, although bacteria can become resistant to phages, phage resistance is not nearly as worrisome as drug resistance. Like bacteria, phages

mutate and therefore can evolve to counter phage-resistant bacteria (Ho K, 2001; Matsuzaki *et al*., 2005). Furthermore, the development of phage resistance can be forestalled altogether if phages are used in cocktails (preparations containing multiple types of phages) and/or in conjunction with antibiotics. In fact, phage therapy and antibiotic therapy, when co-applied, are synergistic (Ho K, 2001; Kutateladze and Adamia, 2010).

Bacteriophages, which were discovered in the early 1900s (Twort, 1915; d'Herelle, 1917), are highly species-specific viruses that kill bacteria through the production of endolysins and the subsequent lysis of the bacterial cells (Joerger, 2003; Huff *et al*., 2005). BP can be considered safe antibiotic alternatives as they exhibit no activity against animal and plant cells. They have been used to prevent and treat various bacterial diseases in humans and animals (Huff *et al*., 2003; Miller *et al*., 2010). BP decrease in pathogen count (Koyuncu et al. 2013; Sultan et al., 2015), improvement in body weight gain and FCR (Sohail *et al*., 2015; Reda *et al*., 2016), improvement of phytate phosphorus utilization (Rafacz-Livingston et al., 2005), and decrease in mortality and feed cost, increase in dressing percentage and liver weight (Khan *et al*., 2016).

As bacteriophage exhibit no activity against animal and plant cells, they can be considered novel alternative to Antibiotic Growth Promoter. Thus, the aim of the current study was to assess the effects of cocktail bacteriophage at three (3) different concentration on the body weight, feed consumption and FCR. And also, to evaluate its effect on *Salmonella* and *Escherichia Coli* and organ weight. The cost analysis of different BP treatment addition levels will also be considered.

CHAPTER III

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MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Statement of the experiment

The research was conducted at Sher-e-Bangla Agricultural University poultry farm, Dhaka with 600-day-old commercial (Mix males and females) broiler chicks (DOC) (Hubbard Classic Efficiency Plus) for a period of 35 days from 8th November 2022 to 14th December 2022 to assess the effects of three different concentrations of cocktail bacteriophage in comparison with Control and antibiotic treated group,Bacitracin Methylene Disalicylate (BMD) on the body weight, feed consumption and FCR in broilers raised under open shaded broiler house. Similarly assessing the effect of BP on *Salmonella* and *Escherichia Coli* population, organ weight and cost analysis for Bacteriophage at different usage levels.

3.2 Collection of experimental birds

A total 600 DOC Hubbard Classic Efficiency Plus broiler chicks with initial body weight of 41.9±1.0g were collected from Paragon Hatcheries hatchery distribution point.

3.3 Collection of bacteriophages (ProBe-Bac PE)

The BP ProBe-Bac PE (Easy *Bio Inc*, Republic of South Korea,) used in this experiment was a mixture of individual BP targeting specifically at *Escherichia coli, Salmonella typhimurium, Salmonella enteritidis, Salmonella gallinarum, Salmonella Pullorum* and *Clostridium perfringens*.

3.4 Experimental materials

The chicks were collected from Paragon Poultry and Hatchery and carried to the university poultry farm early in the morning. The chicks were kept in the electric brooders for 7 days by maintaining standard brooding protocol. During brooding time, the chicks were distributed randomly in five (5) treatments viz. T_0, T_1, T_2, T_3 and T_4. Each treatment had four (4) replications viz. R_1, R_2, R_3 and R_4 where each replication

contains 30 birds. The total number of treatments were five (5) and their replications were twenty (20).

3.5 Experimental treatments

T_0: Control diet (Basal diet) commercial feed with no antibiotics and Bacteriophage.

T_1: 0.5 gm BP/kg of feed

T_2: 0.75 gm BP/kg of feed

T_3: 1.0 gm BP/kg of feed

T_4: Antibiotic control with Bacitracin Methylene Disalicylate (BMD) 0.055 gm/ of feed.

Table 1: Experimental layout: Distribution of treatment and birds

Treatment Groups	No. of replicates				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
Total	150	150	150	150	600

3.6 Preparation of experimental house

The broiler shed was an open sided natural house. It was a tin shed house with concrete floor. The experimental room was properly cleaned and washed by using tap water. All the equipment of the broiler house was cleaned and disinfected. There was 1ft. side wall around the shed with no ceiling. The floor was above 1ft. from the ground and the top of the roof was above 15ft. from the floor. The house was disinfected by n-alkyl dimethyl benzyl ammonium chloride (TimsenTM) solution before starting the experiment. After proper drying, the house was divided into pens as per lay-out of the experiment by polythene sheet so that air cannot pass one pen to another. The height of pens was 5 ft. Before placement of chicks the house was fumigated by formalin and potassium permanganate @ 500 ml formalin and 250 g potassium permanganate (i.e., 2:1) for 35 m3 experimental area. Rice husk was used as a litter material to keep free the floor from moisture. The house was divided into 20 pens of equal size using wood

materials after proper disinfection drying. A group of 30 birds were randomly shifted to each pen of the 5 treatments and 4 replications. One feeder and one waterer were distributed to each pen. The stocking density was 1 m^2/10 birds.

3.7 Experimental diets

The basal diet was formulated to meet the nutrient requirements of broilers as recommended by Hubbard Efficiency plus recommendation guide. Ekramul Haque Agro. Industries (Pvt.) Ltd. has supplied the Starter and Finisher broiler feeds as per the formulations provided to them in crumbs and pellet form respectively. The bacteriophage cocktail concentrations used in the present study was administrated by replacing the same amount of maize and procured from RS Poultry, Bangladesh.

Table 2: Experimental diet.

Items	Phase	
	Starter Kg	**Finisher Kg**
Ingredient		
Maize	500	570
Soya Meal	279	200
Rice Polish (Grade A)	24.85	26.85
Soya Oil	20	30
Poultry Meal	35	35
Full Fat Soya	100	100
DCP	10	8
LSP	11	10
Salt	3	3
Vitamin Mineral Premix Broiler	1.5	1.5
L Methionine	3.5	3
L Lysine	2.5	3
L Threonine	1	1
Toxin Binder	2	2
Choline Chloride 60%	0.8	0.8
Yeast Culture (Genikan)	2	2
Sodium Bicarbonate	0.8	0.8
MOS (Yeamune UP)	0.5	0.5
Emulsifier (Lipidol)	1	1
Anticoccidial (Coccilock)	1	1
Avemix (B-Gulcanase & Xylanase)	0.2	0.2
Hemicell (B- mannnanase)	0.2	0.2
Endophos (Phytase	0.15	0.15

Table 3: Calculated nutrient contents in starter broiler ration

Name of the Element	%
Metabolisable energy (kcal/kg)	3000.00
Protein	23.10
Fat	5.20
Fiber	4.00
Ash	7.80
Dig Lysine	1.27
Dig Methionine	0.54
Dig Methionine + Cysteine	0.95
Dig Tryptophan	0.22
Dig Threonine	0.84
Dig Arginine	1.40
Dig Valine	0.97
Dig Isoleucine	0.84
Calcium	0.98
Av. Phosphorus	0.48

Table 4: Calculated nutrient contents in finisher broiler ration

Name of the Element	%
Metabolisable energy (kcal/kg)	3150
Protein	20.50
Fat	5.80
Fiber	4.00
Ash	8.00
Dig Lysine	1.15
Dig Methionine	0.49
Dig Methionine + Cysteine	0.87
Dig Tryptophan	0.19
Dig Threonine	0.76
Dig Arginine	1.22
Dig Valine	0.88
Dig Isoleucine	0.76
Calcium	0.88
Av. Phosphorus	0.45

The experiment was divided in two nutritional phases, including starter (1 to 14 days), and finisher phase (15 to 35 days).

3.8 Management procedures

Different aspects of the management of chicks, experimental events and management procedures are described in detail below:

3.8.1 Litter management

High absorbing bedding material was used as litter on floor. Fresh, clean and sun-dried rice husk was used as shallow litter to absorb moisture from fecal discharge of broiler chicken. The shallow litter was 5 cm (2 inch) in depth. About 250 g calcium oxide powder was mixed with rice husk in every pen as disinfectant. At the end of each week the litter was harrowed to prevent accumulation of toxic gases and to reduce moisture and parasitic infection. At 3rd and 4th week of rearing period, droppings were cleaned from the surface level by removing a thin layer of litter and same amount new litter was placed in each pen.

3.8.2 Receiving of day-old chicks

Just after arrival of day-old chicks to the poultry house the initial weight of the chicks were recorded by a digital electronic balance, and distributed them under the hover for brooding. The chicks were supplied glucose water with vitamin-c to drink for the first 3 hours to overcome dehydration and transportation stress. Subsequently small feed particles were supplied on the newspapers to start feeding for the first 24 hours.

3.8.3 Brooding of baby chicks

Electric brooder was used to brood chicks. Brooding temperature was maintained as per requirement. Partitioning brooding was done due to different experimental treatment. Each brooder had one hover and a round chick guard to protect chicks and portioning chambers. The brooding temperature was checked every 2 hours by digital thermometer.

3.8.4 Room temperature and relative humidity

Daily room temperature (°C) and humidity were recorded with a thermometer and a wet and dry bulb thermometer respectively. Daily of room temperature and percent relative humidity for the experimental period were recorded.

3.8.5 Feeding and watering

Crumble feed was used as starter (0-2 wks.) and pellet feed for grower (3-5 wks.) ration ad- libitum feeding was allowed for rapid growth of broiler chicks up to the end of the five weeks. Fresh clean drinking water was also supplied ad-libitum. Feeds were supplied 3 times: morning, noon and night. Water was supplied two times daily: morning and evening. Left over feeds was recorded to calculate actual intake. Digital electronic balance was used to take record of feed. Daily and weekly feed consumption (gm/bird) were calculated to find out weekly and total consumption of feed. All feeders and drinkers were washed and sun-dried before starting the trial. One plastic made round feeder and one drinker were kept in the experimental pen. Feeder and drinker size were changed according to the age of the birds. Feeders were washed at the end of the week and drinkers once daily. One feeder and one drinker were provided in each pen for one group of birds.

3.8.6 Lighting

At night there was provision of light in the broiler house to stimulate feed intake and rapid body growth. Four (4) energy lights were provided to ensure 24 hours' light for first 2 wks. Thereafter 21 hours' light and three-hour dark were scheduled up to marketable age.

3.8.7 Ventilation

The broiler shed was south facing and open-sided. Due to wire-net cross ventilation was easy to remove polluted gases from the farm. Besides, on the basis of necessity ventilation was regulated by folding polythene screen. The open space around the farm were favorable for cross ventilation.

3.8.8 Bio security measures and sanitation

Recommended biosecurity and sanitation program was followed at the farm. Disinfectants were used to disinfect the feeders, waterers, house and surroundings of the house. Proper hygienic measures were maintained throughout the experimental period. Cleaning and washing of broiler shed and its premises were under a routine sanitation work.

3.8.9 Vaccination

Vaccines were collected from poultry medicine shop. The HIPRA company vaccines were administered to the birds as per the company recommendations.

Table 5: The vaccination schedule

Age of birds	Name of the disease	Name of vaccine	Route of administration
4 days	Infectious Bronchitis + Newcastle Disease (IB+ND)	HIPRAVIR B1/H120	One drop in each eye
9 days	Gumboro (IBD)	HIPRAGUMBORO GM97	Drinking water
17 days	Gumboro (IBD)	HIPRAGOMBORO GM97	Drinking water

3.9 Study parameters -Sampling and measurements

3.9.1 Body weight, Feed consumption and FCR

Body weight and feed consumption were recorded at day 7, 14, 21, 28 and 35. This information was then used to calculate body weight (BW), average feed intake (FI), and feed conversion ratio (FCR).

3.9.2 Excreta microbial count for *Salmonella* and *Escherichia coli*

For excreta microbial counts, excreta samples were collected from all 20 replications pens each treatment at day 35. Fresh droppings deposited within 2 hours were collected from each replicate pen per treatment and transferred into clean plastic containers. The excreta samples were immediately transferred to the laboratory in an ice box for the enumeration of *Salmonella* and *Escherichia coli* (*E. coli*). The viable counts of bacteria in the excreta were then determined by plating serial 10-fold dilutions (in 10 g/L peptone solution) in respective media. The selective medium used for isolation of *Salmonella* was Salmonella Shigella agar (HiMedia, India) and for *E. coli*, Eosin-methylene blue (EMB) agar (HiMedia, India). Eosin-methylene blue (EMB) agar and Salmonella Shigella agar plates were incubated for 24 h at 37 °C. The colony counts were then enumerated and results are presented as log10- transformed data.

3.9.3 Body organ weights

For body organ weight, 4 individual birds (n=4) per treatment from each pen were selected randomly, weighed (n = 20) at day 35 and killed by cervical dislocation and exsanguinated. The breast muscle (pectoralis major), liver, spleen, bursa of fabricius, gizzard and abdominal fat were then removed and weighed. Organ weights were expressed as a relative percentage to the whole-body weight.

3.10 Data collection

3.10.1. Live weight

The initial live weight of DOC and weekly live weight of each replication was recorded to find out the final live weight record per bird.

3.10.2 Feed consumption

Daily feed consumption was recorded for each replication to obtain weekly and total feed consumption.

3.10.3 Mortality of chicks

Daily death record for each replication was maintained till 35 days to calculate birds' mortality.

3.10.4 Estimation of *Escherichia coli* population in broiler excreta

The population of *Escherichia coli* was estimated as CFU g-1 (colony forming unit). EMB agar (eosin methylene blue agar) was used to culture the *E. coli* bacteria. EMB (Company name- HiMedia, India) agar was purchased from Hatkhola Scientific Market, Dhaka. The composition of HiMedia EMB agar is presented in table 6.

Table 6. Composition of EMB agar

Ingredients	Gms /Lit
Peptic digest of animal tissue	10
Dipotassium phosphate	2
Lactose	5
Sucrose	5
Eosin – Y	0.4
Methylene blue	0.065
Agar	13.5

3.10.5 Estimation of *Salmonella* population in broiler excreta

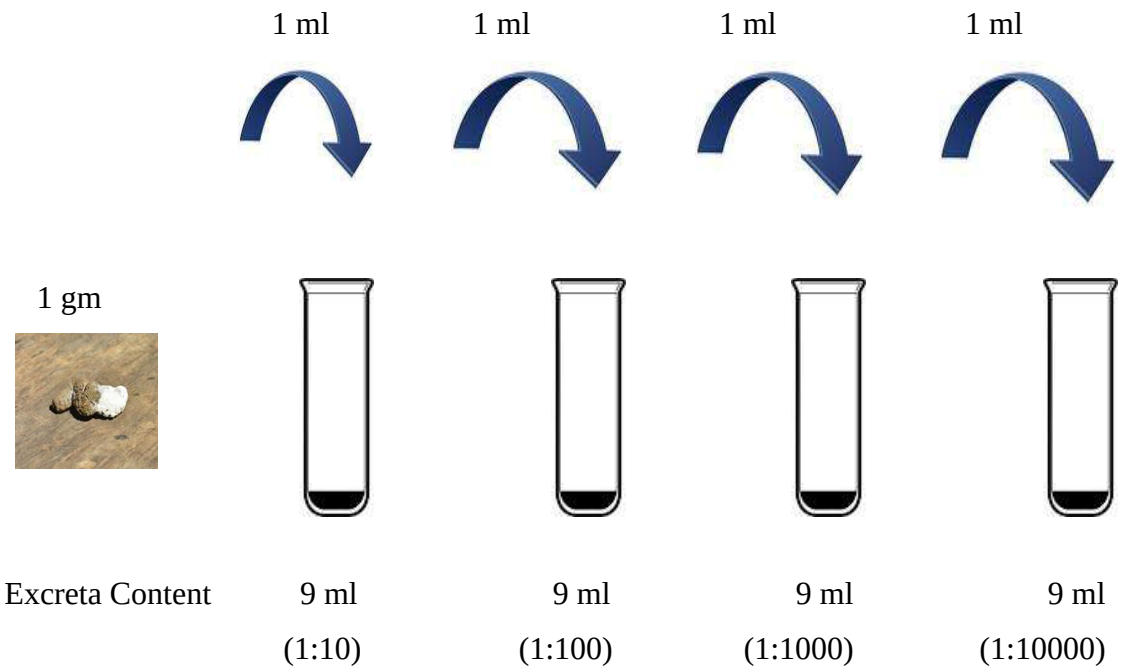
The population *salmonella* was estimated as colony forming unit (CFU)/g. *Salmonella shigella* (SS) agar was used to culture the salmonella bacteria. SS (Company name- HiMedia, India) agar was purchased from Hatkhola Scientific Market, Dhaka. The composition of HiMedia SS agar is given in table 7.

Table 7. Composition of *Salmonella Shigella* agar media

Ingredients	Gms /Lit
Beef extract	5
Enzymatic digest of casein	2.5
Lactose	10
Bile salts	8.5
Sodium citrate	8.5
Sodium thiosulfate	8.5
Ferric citrate	1
Brilliant green	0.00033
Neutral red	0.025
Agar	13.5

3.10.6 Preparation of dilution

At the end of the experiment, excreta samples were collected from broiler farm. Sterilized test tubes with 9 ml of distilled water were used. One gm of excreta content from each sample was mixed in 9 ml of sterilized distilled water in a test tube and shaked well, its ratio was 1:10 and dilution factor was 10^1. Then 1 ml liquid was collected from 1:10 ratio in test tube and mixed in 9 ml of sterilized distilled water in a test tube. Its ratio was 1:100 and dilution factor were 10^2. Finally, 1:1000 and 1:10000 ration was made in same way and their dilution factor was 10^3 and 10^4 respectively. The procedure is repeated to obtain the dilution factor of 10^{10} the dilution preparation is presented below:



3.10.7 Preparation of agar medium

36 grams EMB and SS agar powder was mixed in 1000 ml distilled water. Mixed until suspension was uniform. It was heated to dissolve the medium completely. Dispensed and sterilized by autoclaving at 15 lbs. pressure and temperature 121°C for 15 minutes. Then it was poured into the petri dish. It was cooled to 50°C and shacked in order to oxidize the methylene blue to restore its blue colour and to suspend the flocculent precipitate. One ml of liquid of 1:10000 ratio test tube was collected for each sample and poured to petri dish which was partially filled with EMB medium.

3.10.8 Incubation

Petri dishes were sent to bacterial growth chamber for 24 hrs at 37 °C.

3.10.9 Body organ weights

For organ weight, 4 birds (n=4) per treatment at day 35 were selected randomly and were individually weighed and killed by cervical dislocation and exsanguinated. The breast muscle (pectoralis major), liver, spleen, bursa of fabricius, gizzard and abdominal fat were removed and weighed on 35th day. Organ weights were expressed as a relative percentage to the whole-body weight.

3.11 Calculation

Each data was collected by the following formulae-

3.11.1 Live weight gain

The average body weight gain of each replication was calculated by deducting initial body weight from the final body weight of the birds.

Body weight gain = Final weight – Initial weight

3.11.2 Feed intake

Feed intake was calculated by dividing the total feed consumed in the replication by number of the birds in each replication.

$$\text{Feed intake(g/bird)} = \frac{\text{Feed intake in a replication (gm)}}{\text{Number of birds per replication}}$$

3.11.3 Growth performance and feed conversion ratio

Every week end birds of each replication pen were weighed by digital balance to calculate average weekly weight gain (AWG). The average weekly feed intake (AWFI) was calculated by calculating the difference of feed given to the birds and feed remained in the feeder. The feed efficiency or FCR was calculated in every week. Daily mortality of the birds were recorded to calculate and adjust the feed intake and feed efficiency.

Feed Conversion Ratio (FCR) was calculated as the total feed consumption by the birds divided by weight gain in each replication.

$$\text{FCR} = \frac{\text{Feed intake (kg)}}{\text{Weight gain (kg)}}$$

3.11.4 Benefit Cost Ratio

Benefit cost ratio (BCR) was calculated as the total income of the study divided by total cost of production.

$$\text{BCR} = \frac{\text{Total income}}{\text{Total cost of production}}$$

3.11.5 Bacterial colony count

After 24 hours *E. coli* and *Salmonella* colonies were counted by colony counter and following formula was used to estimate *E. coli* and *Salmonella* population-

$$\text{CFU/g} = \frac{\text{No. of colonies} \times \text{dilution factor}}{\text{Volume inoculated}}$$

3.12 Statistical analysis

Total data were complied, tabulated and analyzed in according to the objectives of the study. Excel program was used for organizing the preliminary data calculation. Then the data was subjected to the statistical analysis by applying one-way ANOVA using Statistical Package for Social Sciences (SPSS version 25.0) in according to the principles of completely randomized design (CRD). Differences between means were tested using the Duncan's multiple comparison test, and significance was set at $P<0.05$.

Some photograph of experimental farm and laboratory work were presented in Plates No. 1-20 below:

Plate 1. Feeding the Chicks

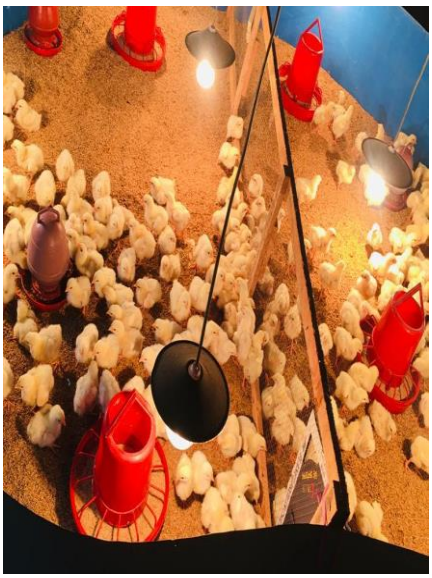


Plate 2. Brooding the chicks

Plate 3. Different treatment groups

Plate 4. Different treatment groups

Plate 5. Guidance from supervisor for farm work

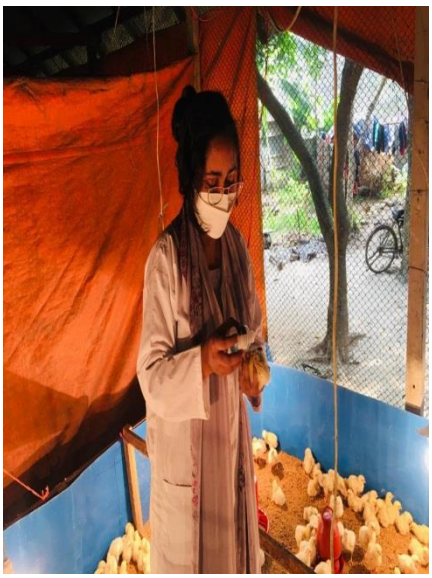


Plate 6. Vaccination of chicks

Plate 7. Farm record Keeping

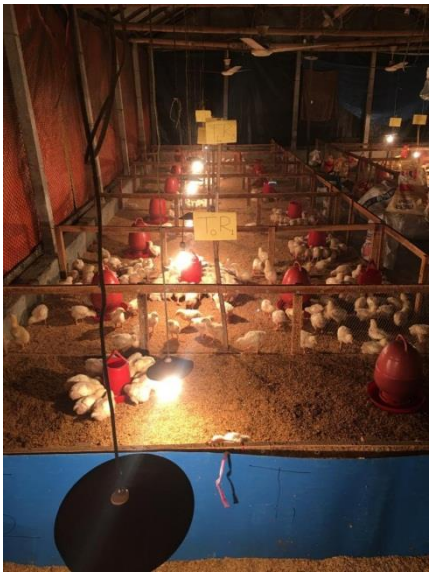


Plate 8. Night feeding to the broilers

Plate 9. Guidance from Supervisor in laboratory work

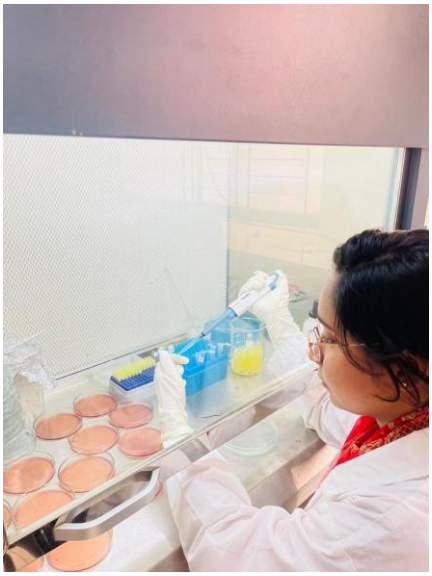


Plate 10. Dilution of the sample

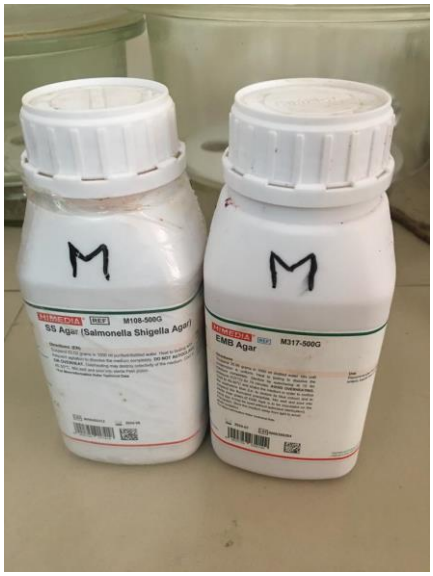


Plate 11. Media used for the work

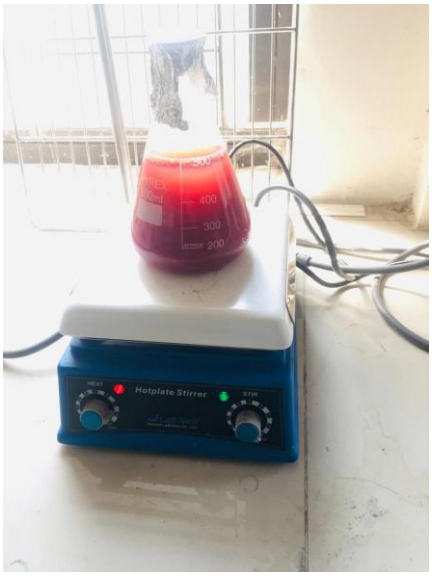


Plate 12. Media preparation

Plate 13. Guidance from Supervisor in laboratory

Plate 14. Monitoring the laboratory work by Supervisor

Plate 15. Colony counting by Supervisor

Plate 16. Colony counting in laboratory

Plate 17. SS media preparation

Plate 18. *Salmonella* colonies

Plate 19. EMB media preparation

Plate 20. *E. coli* colonies

CHAPTER IV

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RESULT AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

Results obtained from the present study on usage of bacteriophage have been presented and discussed in this chapter with a view to assess the effect of three different concentration of cocktail bacteriophage on average live weight, weekly live weight, average feed intake, weekly feed intake, FCR, weekly FCR, Organ weight and effect on *Salmonella* and *Escherichia coli* count in broiler production. The benefit cost ratio (BCR) also has been discussed. The data are given in different tables and figures. The results have been discussed and possible interpretations of the research are given under the following headings.

4.1 Production performances

The effect of different concentration of bacteriophage on live body weight, weekly live body weights, feed intake, weekly feed intake, FCR and weekly FCR of broiler chicken was monitored in this study. The chicks were randomly divided into five experimental treatment groups. The five groups were T_0 (control), T_1 (0.5 BP/kg of feed), T_2 (0.75 gm BP/kg of feed), T_3 (1.0 gm BP/kg of feed) and T_4 (0.05 gm BMD/kg of feed). The performance traits *viz*. average body weight, weekly body weights, feed intake, weekly feed intake, FCR, weekly FCR, different organ weight were analyzed along with estimation of *Escherichia coli*, *Salmonella* and benefit cost ratio were discussed in this chapter.

4.1.1 Body weight (BW)

Table 8 shows the effect of treatments on average live weight. The relative live weight (g) of broiler chickens at 35 days in the different treatment groups T_0, T_1, T_2, T_3 and T_4 were 2027.78±6.11, 2251.58±15.10, 2212.08±6.25, 2199.41±5.68 and 2093.93±20.28 respectively. The body weight was significantly ($P<0.05$) different. Based on Duncan Multiple range test, the highest body weight was found in T_1 and lowest in T_0. The body weight in the group T_0 differs significantly from group T_4 ($P=0.002$) and similarly group T_1 differs significantly from all other groups. However, the body weight in group T_2 and T_3 ($P=0.475$) does not differ significantly. The higher body

weight in T_1 ($P<0.05$) might be due to positive effect of bacteriophage supplementation.

4.1.2 Weekly Body weight gain (BWG)

Table 9 and figure 1 showed the effect of treatments on weekly body weight gain. The relative week 1 average body weight (g) of broiler chicken in different treatment groups T_0, T_1, T_2, T_3, and T_4 were 175.63±1.32, 182.75±0.94, 181.87±1.03, 179.73±1.83 and 185.39±0.51 respectively. The body weight was significantly ($P<0.05$) different. Based on the Duncan multiple range test, the highest body weight was found in T_4 and the lowest in T_0. T_0 differs significantly from every other group. However. body weight in group T_1 does not differ significantly from group T_2 ($P= 0.614$), group T3 ($P = 0.098$), and group T_4 ($P=0.144$), and similarly, body weight in group T_2 does not differ significantly from T_4 ($P =.057$).

Week 2: The average body weight for each of the treatment groups T_0, T_1, T_2, T_3, and T_4 were 482.98±3.37, 529.08±1.01, 516.68±0.75, 543.88±12.32 and 522.28±6.20 respectively. The body weight was significantly ($P<0.05$) different. Based on the Duncan multiple range test, the highest body weight was found T_3 and the lowest in T_0. The body weight in group T_0 differ significantly from group T_4 ($P=0.016$), However Group T_1 do not differ significantly from group T_2 ($P=0.061$) and T3($P=0.075$).

Week 3: The average body weight for each of the treatment groups T_0, T_1, T_2, T_3, and T_4 were 888.98±7.66, 966.08±8.06, 946.98±4.66, 947.98±7.46 and 914.48±4.66 respectively. The ANOVA showed that the body weight was significantly ($P<0.05$) different. Based on the Duncan multiple range test, the highest body weight was found T_1 and the lowest in T_0. T_0 ($P=0.016$) and T_4 differs significantly from every other group however T_1, T_2, and T_3 do not differ significantly.

Week 4: The average body weight for each of the treatment groups T_0, T_1, T_2, T_3, and T_4 were 1365.00±7.48, 1571.75±4.91, 1536.99±10.07, 1550.02±9.69 and 1428.67±14.28 respectively. The ANOVA showed that the body weight was significantly ($P<0.05$) different. Based on the Duncan multiple range test, the highest body weight was found T_1 and the lowest in T_0. T_0 differs significantly from every other group while T_1 ($P =0.137$) and T_3 do not differ significantly; similarly, T_2 ($P = 0.362$) and T_3 do not differ significantly.

Week 5: The average body weight for each of the treatment groups T_0, T_1, T_2, T_3, and T_4 were 2027.78± 6.11, 2251.58± 15.10, 2212.08± 6.25, 2199.41± 5.68 respectively. The ANOVA showed that body weight was significant (P<0.05). Based on the Duncan multiple range test, the highest body weight was found in T_1 and the lowest in T_0. T_0 differs significantly from every other group; similarly, T_1 differs significantly from every other group; T_2 (P=0.475) and T_3 do not differ significantly.

In this study the body weight gain (BWG) of experimental birds during 3rd (15 to 21 days) (p<0.05), 4th (22 to 28 days) (P<0.05) and 5th (29-35 days) (P<0.05) weeks of ages significantly differed in T_1 compared to control group. However, BWG of T_1 group does not differ significantly during 3rd week with T_2 (P = 0.061) and T_3 (P = 0.75).

These results are in agreement with those obtained by Upadhaya *et al*., (2021) who found bacteriophage supplementation has significant (P=0.089) linear effect on BWG from days 1-7, 22-35, and overall experiment. Noor et al. (2020) observed higher body weight gain (BWG) of experimental birds during 1-2 weeks (P=0.046) and 3-4 weeks (P=0.016) of ages with inclusion of bacteriophage at 0.5 g/kg level instead of 0.25 g/kg addition. However, these results are not in agreement with Wang *et al*., 2013 who reported that inclusion of BP at 0.5 g/kg did not affect the BWG during 15 to 32 days and overall experimental period.

In broiler production, an increase in body weight is an important parameter since lower body weight equates to an increased cost for broiler meat production (Kim *et al*., 2013). Feeding the diets containing a mixture of bacteriophage to broiler chickens improved growth performance (Kim *et al*., 2014). The increase in BWG when bacteriophage was used as a feed additive instead of antibiotics in animal feed might be due to the inhibitive or lytic effect on harmful bacteria in the gastrointestinal tract of broiler chickens (Yongsheng *et al*., 2008).

4.1.3 Feed intake (FI)

Table 8 showed the total feed consumption (g) of broiler chicken. The relative total feed consumption (g) of broiler chicken in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 3201.27±47.55, 3357.21±10.04, 3342.09±38.42, 3366.12±14.67 and 3229.77±54.52 respectively. The feed consumption was significantly (P<0.05) different. Based on Duncan Multiple range test, the highest feed consumption was

found in T_3 and lowest in T_0. There is no significant difference between T_0 (P=0.299) and T_4, Similarly, the food consumption in group T_1 does not differ significantly from groups T_2 (P=0.577) and T_3 (P=0.741).

4.1.4 Weekly feed Intake (FI)

Table 10 and figure 2 showed the effect of BP treatments on weekly feed intake. The week 1 average feed intake (g) of broiler chicken in different treatment groups T_0, T_1, T_2, T_3, and T_4 were 165.82±0.33, 169.05±0.29, 167.49±0.31, 164.25±0.48 and 165.28±.31 respectively. The ANOVA showed that feed intake was significantly (P<0.05) different. Based on the Duncan multiple range test, the highest feed intake was found in T_1 and the lowest in T_3. The food consumption in T4 does not differ significantly from T0 (P=0.295) and T3(P=0.055)

Week 2: The average feed intake for each of the treatment groups T_0, T_1, T_2, T_3, and T_4 were 533.75±2.12, 551.00±2.62, 543.08±2.72, 570.91±10.82 and 540.91±1.41 respectively. The ANOVA showed that feed intake was significantly (P<0.05) different. Based on the Duncan multiple range test, the highest feed intake was found in T_3 and the lowest in T_0. T_4 does not differs significantly from T_0 (P = 0.35), T_1 (P = 0.195) and T_2 (P= 0.775).

Week 3: The average feed intake for each of the treatment groups T_0, T_1, T_2, T_3, and T_4 were 1200.92±19.37, 1206.00±1.35, 1202.07±2.43, 1205.08±3.05 and 1218.61±5.41 respectively. The ANOVA showed that feed intake is not significantly (P>0.05) different. Based on the Duncan multiple range test, the highest feed intake was found in T_4 and the lowest in T_0. The inclusion of BP and antibiotic does not affect the feed intake (P= 0.673).

Week 4: The average feed intake for each of the treatment groups T_0, T_1, T_2, T_3, and T_4 were 2012.82±10.11, 2142.69±10.96, 2112.90±9.66. 2132.89±10.31and 2085.74±17.48 respectively. The ANOVA showed that feed intake was significantly (P<0.05) different. Based on the Duncan multiple range test, the highest feed intake was found in T_1 and the lowest in T_0. T_0 differs significantly from all other group. T_1 do not differ significantly from T_2 (P= 0.101) and T_3 (P= 0.574).

Week 5: The average feed intake for each of the treatment groups T_0, T_1, T_2, T_3, and T_4 were 3201.27±47.55, 3357.21±10.04, 3342.09±38.42, 3366.12±14.67 and

3229.77±54.52 respectively. The ANOVA showed that feed intake was significantly ($P<0.05$) different. Based on the Duncan multiple range test, the highest feed intake was found in T_3 and the lowest in T_0. T_1 does not differ significantly from T_2 ($P= 0.577$) and T_3 ($P= 0.741$).

In the currents study Comparatively less feed consumption occurred in the current experiment for the birds fed with antibiotic T_4 ($P<0.05$) and control diets T_0 ($P< 0.05$) compared to BP T_3. The highest feed consumption was observed in the birds fed with 1 g/kg Bp (T_3). The birds fed with bacteriophage T_3 has no significant difference in feed consumption compared birds fed with 0.5 g/kg Bp (T_1) ($P=0.741$) and 0.75 g/kg BP (T_2) ($P=0.379$).

These results are not in agreement with Upadhaya *et al*. (2021) who observed higher feed consumption ($P = 0.017$) in birds fed antibiotics during days 8-22 than control diets and FI tended to be higher ($P=0.0796$) in birds fed antibiotics than the diet supplemented with BP. Similarly, Wang *et al*., 2013 observed that the inclusion of antibiotic and bacteriophages did not affect the FI for overall experimental period. Noor *et al*., 2020 reported that inclusion of antibiotic and bacteriophages did not affect feed intake ($P=0.78$) throughout the experimental period (0-4 weeks).

4.1.5 Feed Conversion Ratio (FCR)

Table 8 showed the FCR of this experimental study. The FCR of the different treatment groups T_0, T_1, T_2, T_3 and T_4 were 1.58, 1.49, 1.51, 1.53 and 1.54 respectively. The FCR was significantly ($P<0.05$) different among the treatment groups. Based on Duncan Multiple range test, T_1 has the lowest while T_0 has the highest FCR. T_3 and T_4 ($P= 0.204$) did not differ significantly. However, T_1 treatment has better FCR among the groups treated with bacteriophages T_2 ($P= 0.047$) and T_3 ($P= 0.001$).

4.1.6 Weekly FCR

Table 11 and figure 3 showed the effect of BP treatments on weekly FCR. The week 1 average FCR for each of the treatment groups T_0, T_1, T_2, T_3, and T_4 were 0.944±0.007, 0.925±0.004, 0.921±0.004, 0.914±0.007 and 0.892±0.002 respectively. The ANOVA showed that FCR was significantly ($P<0.05$) different. Based on the Duncan multiple range test, the highest FCR was found in T_0 and the lowest in T_4. T_0

differs significantly from all other group; T_1 does not differ significantly from T_2 (P= 0.591), and T_3 (P=151).

Week 2: The average weekly FCR for each of the treatment groups T_0, T_1, T_2, T_3, and T_4 were 1.105±0.005, 1.042±0.005, 1.051±0.005, 1.05±0.005 and 1.036±0.015 respectively. The ANOVA showed that the FCR was significantly (P<0.05) different. Based on the Duncan multiple range test, the highest FCR was found in T_0 and the lowest in T_4. T_0 differs significantly from all other group; T_1 does not differ significantly from T_2 (P=0.420), T_3 (P=0.480).

Week 3: The average FCR for each of the treatment groups T_0, T_1, T_2, T_3, and T_4 were 1.351±0.020, 1.249±0.011, 1.27±0.006, 1.271±0.008 and 1.333±0.002 respectively. The ANOVA showed that FCR was significantly (P<0.05) different. Based on the Duncan multiple range test, the highest FCR was found in T_0 and the lowest in T_1. T_1, does not differ significantly from T_2 (P=0.205), and T_3 (P=0.176).

Week 4: The average FCR for each of the treatment groups T_0, T_1, T_2, T_3, and T_4 were 1.475±0.004, 1.363±0.009, 1.375±0.005, 1.376±0.005 and 1.460±0.004 respectively. The ANOVA showed that FCR was significantly (P<0.05) different. Based on the Duncan multiple range test, the highest FCR was found in T_0 and the lowest in T_1. T_1, does not differ significantly from T_2 (P=0.183), and T_3 (P=0.142).

Week 5: The average FCR for each of the treatment groups T_0, T_1, T_2, T_3, and T_4 were 1.58±0.009, 1.49±0.009, 1.51±0.005, 1.53±0.001 and 1.54±0.003 respectively. The ANOVA showed that FCR was significantly (P<0.05) different. Based on the Duncan multiple range test, the highest FCR was found in T_0 and the lowest in T_1. T_1 differ significantly from T_2 (P=0.047) and T_3 (p=0.001).

The results are in not agreement with Wang *et al*. (2013) who reported that the inclusion of bacteriophages did not affect the FCR during 15 to 32 days and overall experimental period. However, dietary supplementation of 0.5 g/kg bacteriophages reduced (p< 0.05) the FCR compared with the treatment from day 1 to 14 day. Similarly, Noor *et al*. (2020) found that there was no significant difference in FCR, no significant difference was observed at 0-1 weeks, 2-3 weeks and 3-4 weeks of ages among the four experimental groups whereas, during 1-2 weeks of age the FCR was found significantly higher in 0.5 g/kg BP group compared with control (P=0.011), antibiotic treated group and (P=0.022) and 0.25 g/kg BP groups (P=0.013)

The better FCR in 0.5 g/kg of BP supplemented group might be due to the rapid development of beneficial bacteria in the digestive tract. But there was no significant difference observed in FCR between T_1 and T_2 (P = 0.47) and T_2 and T_3 (P = 0.46) group fed with different level of bacteriophage in the diet.

4.2 *Escherichia* and *Salmonella*

Table 12 a and figure 4 showed the count of *E Coli* in this experimental study. The *E Coli* count in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 6.84, 6.20, 6.11, 6.21 and 6.35 (Log10 CFU/g) respectively. The *E. coli* count was significantly (P<0.05) different based on Duncan Multiple range test, T_0 (control) differs significantly (P< 0.05) from all other groups. The group T_1 does not differ significantly from groups T_2 (P=0.655) and T_3 (P=1.000). Similarly, group T_1 does not differ significantly with group T_4 (P=0.183). The highest *E. Coli* count was found in T_0 and lowest in T_2.

Similarly, table 12 a and figure 5 showed the *Salmonella* count in this experiment and was 4.28, 4.05, 3.91, 3.88 and 4.20 (Log10 CFU/g) respectively. There was significant (P<0.05) difference in the count of Salmonella. According to Duncan Multiple range test, T_1 does not differ significantly from T_2 (P=0.350) and T_3 (P=0.185) and T_4 (P= 0.231). The highest Salmonella count was found in T_0 and lowest in T_3.

In this study the *E. coli* count was higher and significantly (P< 0.05) different from all other groups. *E. coli* count was significantly (P<0.05) decreased in birds fed 0.5 g/kg BP in T_1 group, 0.75 g/kg BP and 1 g/kg BP in T_2 and T_3 group respectively.

Previous studies with *E. coli* demonstrated that phage therapy at concentrations of 10^6 pfu or 10^9 pfu could be as efficient as antibiotics (Huff *et al*., 2004; Barrow *et al*., 1998). Similarly, early works have indicated that *Salmonella* can be controlled by bacteriophages at a concentration of 1 mL containing 10^{10} pfu/mL, 0.1 mL containing 10^{10} pfu/mL, 0.1 mL containing 10^9 pfu/mL or 10^6 pfu/kg (Atterbury *et al*., 2007; Berchieri *et al*., 1991; Lim *et al*., 2011).

Wang *et al*. (2013) reported that the inclusion of antibiotic and bacteriophage significantly reduced the *E. coli* and *Salmonella* from the excreta of broilers compared with the control group. Similarly, Noor *et al*. (2020) found the inclusion of antibiotic and bacteriophage significantly reduced the *E. coli* (P<0.0001) and *Salmonella* (P<0.0001) counts in cecal content of broilers compared with the control group. These

results are in contradiction with the findings of Upadhaya *et al*., 2021 who reported that the dietary supplementation of BP did not have a significant effect on the pathogenic bacteria such as *E. coli*, *Salmonella* counts isolated from the caecal digesta.

4.3 Organ Weight

4.3.1 Relative organ weight (Breast Muscle, Liver, gizzard and abdominal fat)

Data presented in table 13 a showed the breast muscle, Liver, Gizzard and abdominal fat weight (g) of broiler chickens in different treatment groups. The relative weight (g) of breast muscle in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 22.185±0.115, 22.985±0.074, 23.058±0.098, 23.355±0.097 and 23.905±0.046 respectively. The weight of breast muscle was significantly ($P<0.05$) different based on Duncan Multiple range test, T_0 differs significantly from all other groups. T_1 does not differ significantly from, T_2 ($P = 0.574$). However, T_4 group has highest weight and differ significantly ($p <0.05$) from all other groups.

The relative weight (g) of liver in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 2.693±0.006, 2.695±0.010, 2.622±0.01, 2.640±0.004 and 2.723±0.009 respectively. The weight of liver was significantly ($P<0.05$) different. According to Duncan Multiple range test T_2 differs ($P< 0.05$) significantly from T_0 and T_1. T_4 has the highest liver weight and differs significantly from all the groups.

The relative weight of gizzard in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 1.675±0.003, 1.638±0.005, 1.673±0.006, 1.668±0.006 and 1.618±0.005 respectively. The weight of gizzard was significantly ($P<0.05$) different. According to Duncan Multiple range test, the weight in T_0 does not differ significantly from T_2 ($P=0.737$) and T_3 ($P=0.321$). T_4 differ significantly from all other groups and has lowest body weight.

The relative weight of abdominal fat in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 1.018±0.005, 1.118±0.005, 1.135±0.003, 1.195±0.006 and 1.100±0.004 respectively. The weight of abdominal fat was significantly ($P<0.05$) different. Based on Duncan Multiple range test. T_0 (control) has the lowest weight and differs significantly from all the groups. Similarly, T_3 has highest weight and differs significantly from all other groups.

In the current study it was observed that the weight of breast muscle and liver was highest in antibiotic treated group whereas gizzard weight was higher in control group. The abdominal fat has lowest weight in control T_1 group. However, the inclusion of bacteriophage at the level of 0.75 g/kg increased ($p<0.05$) the relative abdominal fat weight to the body weight. These finding are not in agreement with Wang *et al*. (2013), who observed the inclusion of bacteriophage at the level of 0.25 g/kg increased ($p<0.05$) the relative liver weight to the body weight and no difference was observed on the other relative organ weight among treatments. Similarly, Upadhaya *et al*. (2021), reported that none of the other weight parameters were affected between control and antibiotic diets. The relative weight of gizzard showed trends in increment in birds fed antibiotic than bacteriophage supplemented diets.

4.3.2 Immune organ weights (Spleen and Bursa)

The data presented in table 13 a and figure 6 showed relative weight (g) of spleen in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 0.193±0.003, 0.168±0.003, 0.173±0.003, 0.165±0.003 and 0.180±0.004 respectively. The weight of spleen was significantly ($P<0.05$) different. According to Duncan Multiple range test, group T_1 has no significant difference with T_2 ($P= 0.251$) and T_3 ($P= 0.559$). However, T_3 differ significantly ($P< 0.05$) from T_0 and T_4 group. T_0 has a highest spleen weight and differ significantly from all other groups.

The weight (g) of Bursa of fabricius in treatment T_0, T_1, T_2, T_3 and T_4 were 0.138±0.005, 0.108±0.005, 108±0.005, 0.103±0.003 and 0.120±0.004 respectively.

The weight of bursa fabricius was significantly ($P<0.05$) different from the control group. Based on Duncan Multiple range test, T_1 does not differ significantly from T_2 ($P= 1$) T_3 ($P= 0.422$) and T_4 (0.057). T_0 has the highest bursa weight.

In the current study it was observed that the weight of spleen and Bursa of fabricius was highest in control group T_0. These findings are not in agreement with Wang *et al.* (2013) who observed no significance difference on the relative organ weight among treatments in spleen and bursa of fabricius. Similarly, present findings are not in agreement with Upadhaya *et al*. (2021), reported significant reduction in relative weight of bursa of fabricius in birds fed antibiotics than control diets. Upadhaya *et al*., (2021) also observed a linear reduction in weight of bursa of fabricius ($P=0.026$) and

spleen (P=0.052) relative to body weight in birds fed diets supplemented with increasing level of bacteriophage.

4.4 Cost benefit ratio analysis

Cost benefit ratio analysis are presented in Table 14 a and figure 7. Benefit cost ratio (BCR) of the experimental study in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 1.24±0.005, 1.32±0.005, 1.30±0.000, 1.28±0.004 and 1.27±0.009 respectively. The cost benefit ratio was significantly (P< 0.05) different. Based on Duncan Multiple range test, BCR does not differ significantly in T_3 and T_4 (P=0.174).

Total cost analysis is presented in Table 14 a and figure 7. Total cost of the experimental study in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 277.07±1.31, 289.68±0.84, 289.45±0.89, 291.99±0.67 and 280.70±0.87 respectively. The total cost was significantly (P< 0.05) different. Based on Duncan Multiple range test, T_1 does not differ significantly from T_2 (P= 0.9040 and T_3 (P= 0.234). The total expenditure per bird was significantly higher (P<0.05) in treated group T_3 (291.99±0.67) than control group T_0 (277.07±1.31).

Sales analysis is presented in Table 14 a and figure and 7. Total revenue of the experimental study in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 344.72±0.78, 382.77±1.10, 376.05±0.73, 373.90±0.64 and 355.97±0.64 respectively. The sales were significantly (P< 0.05) different. According to Duncan Multiple range test T_2 does not differ significantly from T_3 (P=0.475). The highest revenue is represented by T_1.

Profit analysis is presented in Table 14 a and figure 7. Profit of the experimental study in different treatment groups T_0, T_1, T_2, T_3 and T_4 were 67.65±1.15, 93.10±0.99, 86.60±0.91, 81.91±1.16 and 75.27±1.43 respectively. The profit in each group differs (P<0.05) significantly. Based on Duncan Multiple range there is significant difference in all profit groups. The highest profit is shown by T_1 and the lowest by control group (T_0). Among the treatment groups T_1 performed better than others.

Table 8a: Effect of BP on 5th week body weight, feed intake and FCR

Treatnents	Parameter		
	Live Weight ±SE(g)	Feed Intake FI ±SE (g)	FCR±SE
T0	2027.78± 6.11	3201.27±47.55	1.58±0.02
T1	2251.58± 15.10	3357.21±10.04	1.49±0.02
T2	2212.08± 6.25	3342.09±38.42	1.51±0.01
T3	2199.41± 5.68	3366.12±14.67	1.53±0.00
T4	2093.93± 20.28	3229.77±54.52	1.54±0.01
Mean±SE	2156.96± 19.65	3299.29±17.58	1.53±0.01

Here, T_0= Positive control, T_1 = 0.5 gm BP /kg of feed, T_2 = 0.75 gm BP/kg of feed, T_3 = 1.0 gm BP/kg of feed, T_4 = Antibiotic control with BMD 0.055 gm/ of feed. Values: Mean±SE (n=20) Applying: One-way ANOVA (SPSS, Duncan's method), SE= Standard error

Table 8b: Effect of BP on 5th week body weight, feed intake and FCR

Parameters	Treatment					SEM	P-Value
	T_0	T_1	T_2	T_3	T_4		
BW	2027.78^{a}	2251.58^{d}	2212.08^{c}	2199.41^{c}	2093.93^{b}	17.29	<0.001
FI	3201.27^{a}	3357.21^{b}	3342.09^{b}	3366.12^{b}	3229.77^{a}	26.51	<0.001
FCR	1.58^{d}	1.49^{a}	1.51^{b}	1.53^{c}	1.54^{c}	0.01	<0.001

^{abc} means with the same superscript along a row are not significantly different.

Table 9a: Effects of bacteriophage on weekly body weight (BW) (g/bird)

Treatment	1st Wk. BW	2nd Wk. BW	3rd Wk. BW	4th Wk. BW	5th Wk. BW
T_0	175.63±1.32	482.98±3.37	888.98±7.66	1365.00±7.48	2027.78± 6.11
T_1	182.75±0.94	529.08±1.01	966.08±8.06	1571.75±4.91	2251.58± 15.10
T_2	181.87±1.03	516.68±0.75	946.98±4.66	1536.99±10.07	2212.08± 6.25
T_3	179.73±1.83	543.88±12.32	947.98±7.46	1550.02±9.69	2199.41± 5.68
T_4	185.39±0.51	522.28±6.20	914.48±4.66	1428.67±14.28	2093.93± 20.28
Mean ± SE	181.08±0.89	518.93±5.27	932.88±6.86	1490.49±18.73	2156.96± 19.65

Here, T_0= Positive control, T_1 = 0.5 gm BP /kg of feed, T_2 = 0.75 gm BP/kg of feed, T_3 = 1.0 gm BP/kg of feed, T_4 = Antibiotic control with BMD 0.055 gm/ of feed. Values: Mean±SE (n=20) Applying: One-way ANOVA (SPSS, Duncan's method), SE= Standard error

Table 9b: Effects of bacteriophage on weekly body weight (BW) (g/bird)

	Treatments						
Parameters	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**	**SEM**	**P-Value**
1st Wk. BW	175.63^{a}	182.75^{bc}	181.87^{bc}	179.73^{b}	185.39^{c}	1.71	<0.001
2nd Wk. BW	482.98^{a}	529.08^{bc}	516.68^{b}	543.88^{c}	522.28^{b}	9.02	<0.001
3rd Wk. BW	888.98^{a}	966.08^{c}	946.98^{c}	947.98^{c}	914.48^{b}	9.43	<0.001
4th Wk. BW	1365.00^{a}	1571.75^{d}	$1,536.99^{c}$	1550.02^{cd}	$1,428.67^{b}$	13.85	<0.001
5th Wk. BW	2027.78^{a}	2251.58^{d}	2212.08^{c}	2199.41^{c}	2093.93^{b}	17.29	<0.001

^{abc} means with the same superscript along a row are not significantly different.

Table 10a: Effects of bacteriophage on weekly feed intake (FI) (g/bird)

Treatment	1st Wk. FI	2nd Wk. FI	3rd Wk. FI	4th Wk. FI	5th Wk. FI
T_0	165.82±0.33	533.75±2.12	1200.92±19.37	2012.82±10.11	3201.27±47.55
T_1	169.05±0.29	551.00±2.62	1206.00±1.35	2142.69±10.96	3357.21±10.04
T_2	167.49±0.31	543.08±2.72	1202.07±2.43	2112.90±9.66	3342.09±38.42
T_3	164.25±0.48	570.91±10.82	1205.08±3.05	2132.89±10.31	3366.12±14.67
T_4	165.28±.31	540.91±1.41	1218.61±5.41	2085.74±17.48	3229.77±54.52
Mean ± SE	166.38±0.41	547.93±3.53	1206.54±3.93	2097.41±11.71	3299.29±17.58

Here, T_0= Positive control, T_1 = 0.5 gm BP /kg of feed, T_2 = 0.75 gm BP/kg of feed, T_3 = 1.0 gm BP/kg of feed, T_4 = Antibiotic control with BMD 0.055 gm/ of feed. Values: Mean±SE (n=20) Applying: One-way ANOVA (SPSS, Duncan's method), SE= Standard error

Table 10b: Effects of bacteriophage on weekly feed intake (FI) (g/bird)

	Treatments						
Parameters	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**	**SEM**	**P-Value**
1st Wk. FI	165.82^{b}	169.05^{d}	167.49^{c}	164.25^{a}	165.28^{ab}	0.49	<0.001
2nd Wk. FI	533.75^{a}	551.00^{b}	543.08^{ab}	570.91^{c}	540.91^{ab}	7.44	<0.002
3rd Wk. FI	1200.92^{a}	1206.00^{a}	1202.07^{a}	1205.08^{a}	1218.61^{a}	12.98	0.673
4th Wk. FI	2012.82^{a}	2142.69^{c}	2112.90^{bc}	2132.89^{c}	2085.74^{b}	17.06	<0.001
5th Wk. FI	3201.27^{a}	3357.21^{b}	3342.09^{b}	3366.12^{b}	3229.77^{a}	26.51	<0.001

^{abc} means with the same superscript along a row are not significantly different.

Table 11a: Effects of bacteriophage on Weekly FCR

Treatment	1st Week FCR	2nd Week FCR	3rd Week FCR	4th Week FCR	5th Week FCR
T0	0.944±0.007	1.105±0.005	1.351±0.020	1.475±0.004	1.58±0.009
T1	0.925±0.004	1.042±0.005	1.249±0.011	1.363±0.009	1.49±0.009
T2	0.921±0.004	1.051±0.005	1.27±0.006	1.375±0.005	1.51±0.005
T3	0.914±0.007	1.05±0.005	1.271±0.008	1.376±0.005	1.53±0.001
T4	0.892±0.002	1.036±0.015	1.333±0.002	1.460±0.004	1.54±0.003

Here, T_0= Positive control, T_1 = 0.5 gm BP /kg of feed, T_2 = 0.75 gm BP/kg of feed, T_3 = 1.0 gm BP/kg of feed, T_4 = Antibiotic control with BMD 0.055 gm/ of feed. Values: Mean±SE (n=20) Applying: One-way ANOVA (SPSS, Duncan's method), SE= Standard error

Table 11b: Effects of bacteriophage on Weekly FCR

	Treatments						
Parameters	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**	**SEM**	**P-Value**
1st Wk. FCR	0.944^{c}	0.925^{b}	0.921^{b}	0.914^{b}	0.892^{a}	0.01	<0.001
2nd Wk. FCR	1.105^{b}	1.042^{a}	1.051^{a}	1.050^{a}	1.036^{a}	0.01	<0.001
3rd Wk. FCR	1.351^{b}	1.249^{a}	1.270^{a}	1.271^{a}	1.333^{b}	0.02	<0.001
4th Wk. FCR	1.475^{b}	1.363^{a}	1.375^{a}	1.376^{a}	1.460^{b}	0.01	<0.001
5th Wk. FCR	1.58^{d}	1.49^{a}	1.51^{b}	1.530^{c}	1.540^{c}	0.01	<0.001

^{abc} means with the same superscript along a row are not significantly different.

12a: Effect of Bacteriophage on Escherichia coli and Salmonella (log_{10} CFU/g)

Treatment	Parameters	
	Escherichia	**Salmonella**
T_0	6.84±0.03	4.28±0.01
T_1	6.20±0.06	4.05±0.03
T_2	6.11±0.03	3.91±0.03
T_3	6.21±0.06	3.88±0.04
T_4	6.35±0.04	4.2±0.01
Mean±SE	6.34± 0.28	4.06± 0.04

Here, T_0= Positive control, T_1 = 0.5 gm BP /kg of feed, T_2 = 0.75 gm BP/kg of feed, T_3 = 1.0 gm BP/kg of feed, T_4 = Antibiotic control with BMD 0.055 gm/ of feed. Values: Mean±SE (n=20) Applying: One-way ANOVA (SPSS, Duncan's method), SE= Standard error

12b: Effect of Bacteriophage on *Escherichia coli* and *Salmonella* (log_{10} CFU/g)

Parameters	Treatment					SEM	P-Value
	T_0	**T_1**	**T_2**	**T_3**	**T_4**		
Escherichia	6.84^{c}	6.20^{ab}	6.11^{a}	6.21^{ab}	6.35^{b}	0.06	<0.001
Salmonella	4.28^{c}	4.05^{ab}	3.91^{a}	3.88^{a}	4.20^{bc}	0.07	<0.001

^{abc} means with the same superscript along a row are not significantly different.

Table 13a: Effect of Bacteriophage on organ weight

Treatments	Parameters					
	Breast Muscle	Liver	Spleen	Bursa of fabricus	Gizzard	Abdominal fat
T_0	22.185±0.115	2.693±0.006	0.193±0.003	0.138±0.005	1.675±0.003	1.018±0.005
T_1	22.985±0.074	2.695±0.010	0.168±0.003	0.108±0.005	1.638±0.005	1.118±0.005
T_2	23.058±0.098	2.622±0.01	0.173±0.003	0.108±0.005	1.673±0.006	1.135±0.003
T_3	23.355±0.097	2.640±0.004	0.165±0.003	0.103±0.003	1.668±0.006	1.195±0.006
T_4	23.905±0.046	2.723±0.009	0.180±0.004	0.120±0.004	1.618±0.005	1.100±0.004
Mean±SE	23.098±0.133	2.675±0.009	0.176±0.003	0.115±0.005	1.654±0.006	1.113±0.013

Here, T_0= Positive control, T_1 = 0.5 gm BP /kg of feed, T_2 = 0.75 gm BP/kg of feed, T_3 = 1.0 gm BP/kg of feed, T_4 = Antibiotic control with BMD 0.055 gm/ of feed. Values: Mean±SE (n=20) Applying: One-way ANOVA (SPSS, Duncan's method), SE= Standard error

Organ weights were expressed as a relative percentage to the whole-body weight.

Table 13b: Effect of Bacteriophage on organ weight

Parameters	T_0	T_1	T_2	T_3	T_4
Breast Muscle	22.19^{a}	22.99^{b}	23.06^{bc}	23.36^{c}	23.91^{d}
Liver	2.69^{b}	2.70^{b}	2.62^{a}	2.64^{a}	2.72^{c}
Spleen	0.19^{a}	0.17^{a}	0.17^{ab}	0.17^{a}	0.18^{b}
Bursa of fabricus	0.14^{a}	0.11^{a}	0.11^{a}	0.10^{b}	0.12^{a}
Gizzard	1.68^{c}	1.64^{a}	1.67^{c}	1.67^{c}	1.62^{b}
Abdominal fat	1.02^{a}	1.12^{bc}	1.14^{c}	1.20^{d}	1.10^{b}

^{abc} means with the same superscript along a row are not significantly different.

Table 14a: Cost benefit ratio analysis of different treatment groups

Treatment	Total Cost±SE (Tk./Bird)	Sales Price±SE (Tk./Bird)	Profit±SE (Tk./Bird)	BCR±SE
T_0	277.07±1.31	344.72±0.78	67.65±1.15	1.24±0.005
T_1	289.68±0.84	382.77±1.10	93.10±0.99	1.32±0.005
T_2	289.45±0.89	376.05±0.73	86.60±0.91	1.25±0.000
T_3	291.99±0.67	373.90±0.64	81.91±1.16	1.28±0.004
T_4	280.70±0.87	355.97±0.64	75.27±1.43	1.27±0.009
Mean±SE	285.77±1.38	366.683±3.25	80.91±2.08	1.27±0.007

Here, T_0= Positive control, T_1 = 0.5 gm BP /kg of feed, T_2 = 0.75 gm BP/kg of feed, T_3 = 1.0 gm BP/kg of feed, T_4 = Antibiotic control with BMD 0.05 gm/ of feed. Values: Mean±SE (n=20) Applying: One-way ANOVA (SPSS, Duncan's method), SE= Standard error

Table 14b: Cost benefit ratio analysis of different treatment groups

Parameters	Treatments					SEM	P-Value
	To	T_1	T_2	T_3	T_4		
Total Cost	277.07^{c}	289.68^{a}	289.45^{a}	291.99^{a}	280.70^{b}	1.33	<0.001
Sales Price	344.72^{d}	382.77^{a}	376.05^{b}	373.90^{b}	355.97^{c}	1.12	<0.001
Profit	67.65^{e}	93.09^{a}	86.60^{b}	81.91^{c}	75.27^{d}	1.61	<0.001
BCR	1.24^{a}	1.32^{d}	1.30^{ab}	1.28^{c}	1.27^{bc}	0.01	<0.001

abc means with the same superscript along a row are not significantly different.

Table 15: Effect of Bacteriophage on mortality%

Treatment	Mortality	Total Birds	Mortality %
T_0	5	120	4.17
T_1	1	120	0.83
T_2	2	120	1.67
T_3	2	120	1.67
T_4	3	120	2.50

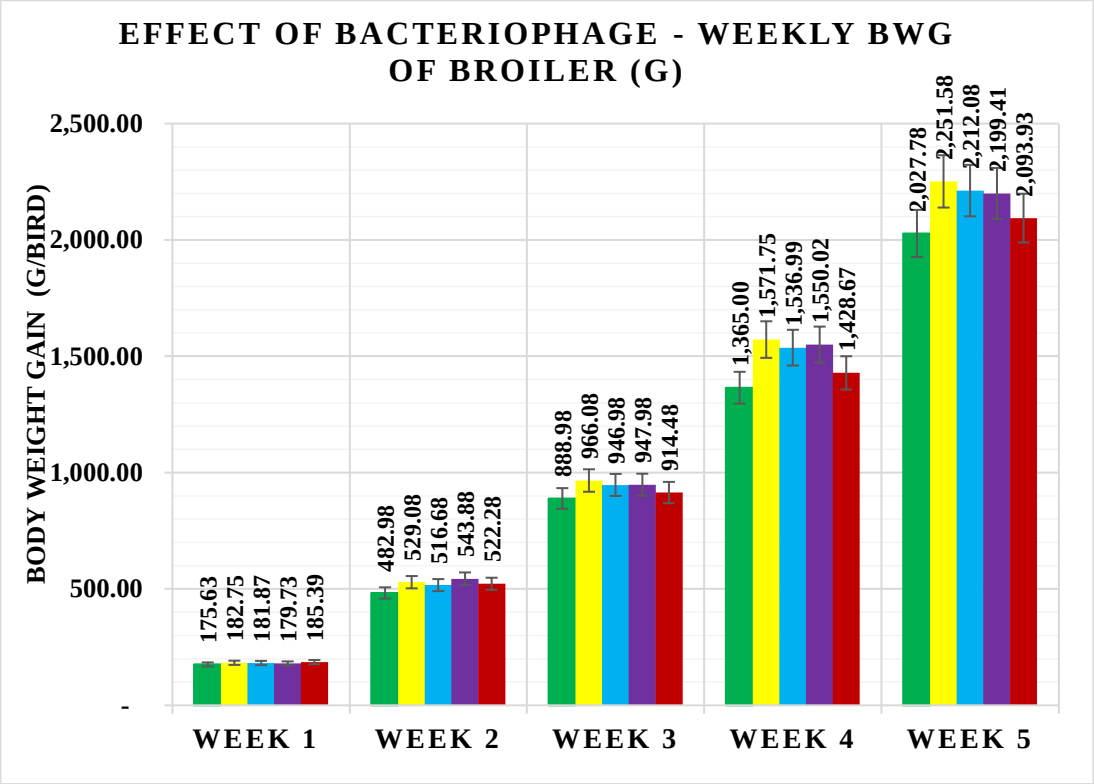

Figure 1. Effect of BP on weekly average live BW of broiler under different treatment

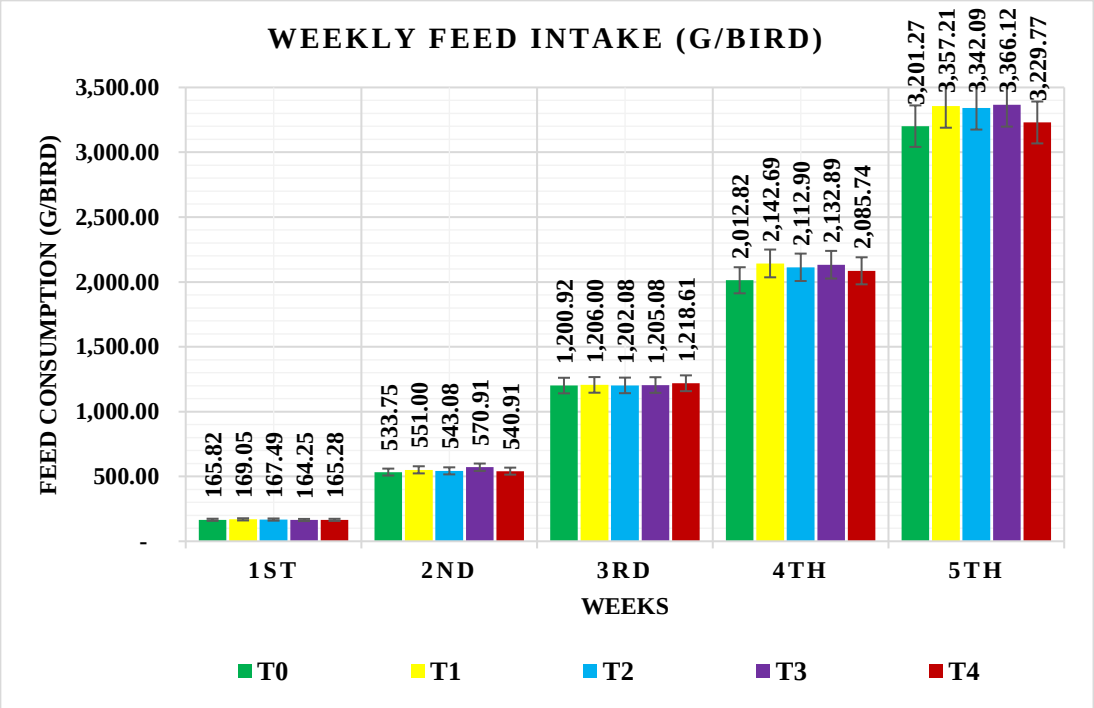

Figure 2. Effect of bacteriophage on weekly feed intake of broiler under different treatment

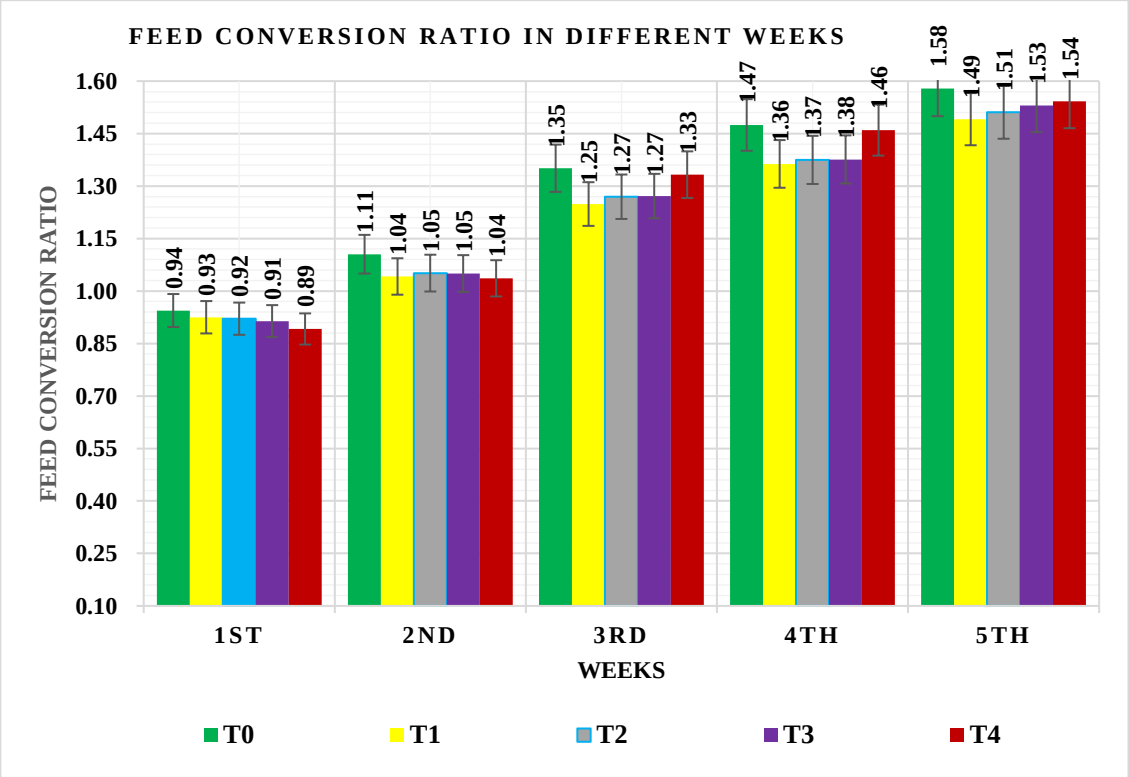

Figure 3. Effect of bacteriophage on weekly FCR of broiler under different treatment

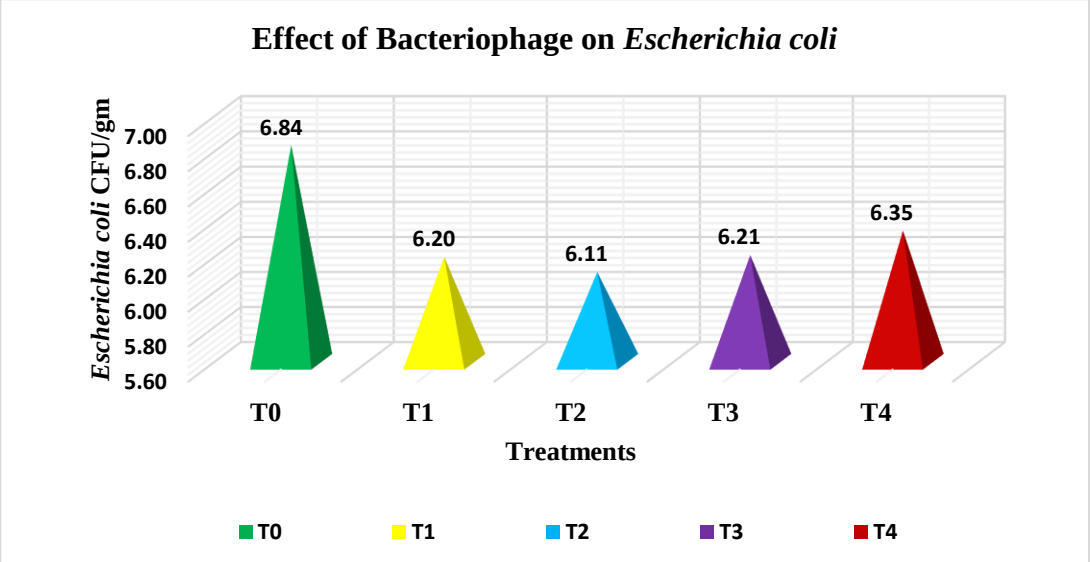

Figure 4. Effect of bacteriophage on *Escherichia coli.*

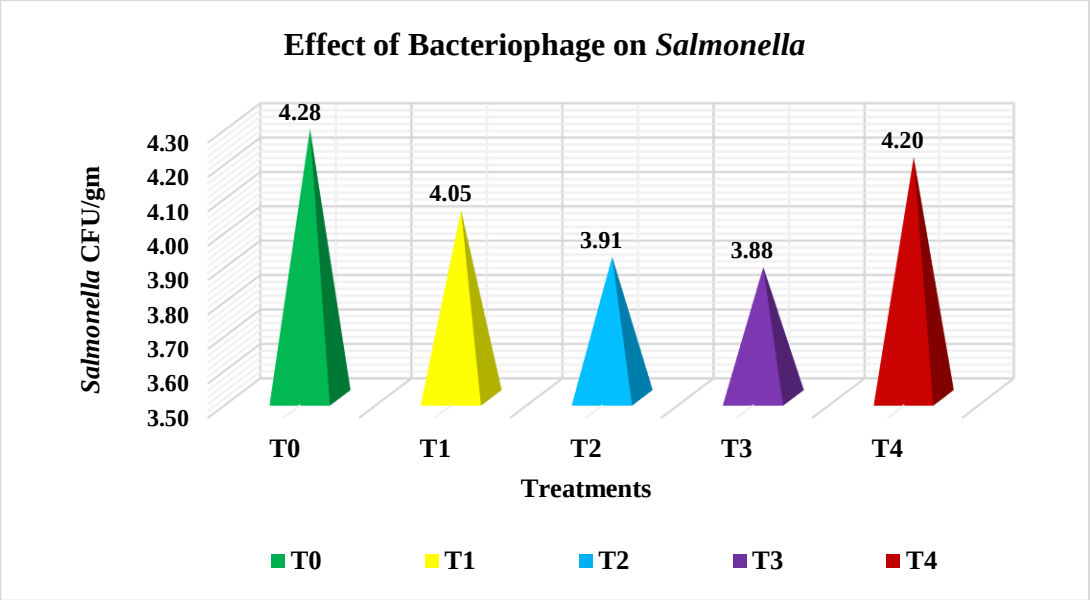

Figure 5. Effect of bacteriophage on *Salmonella*.

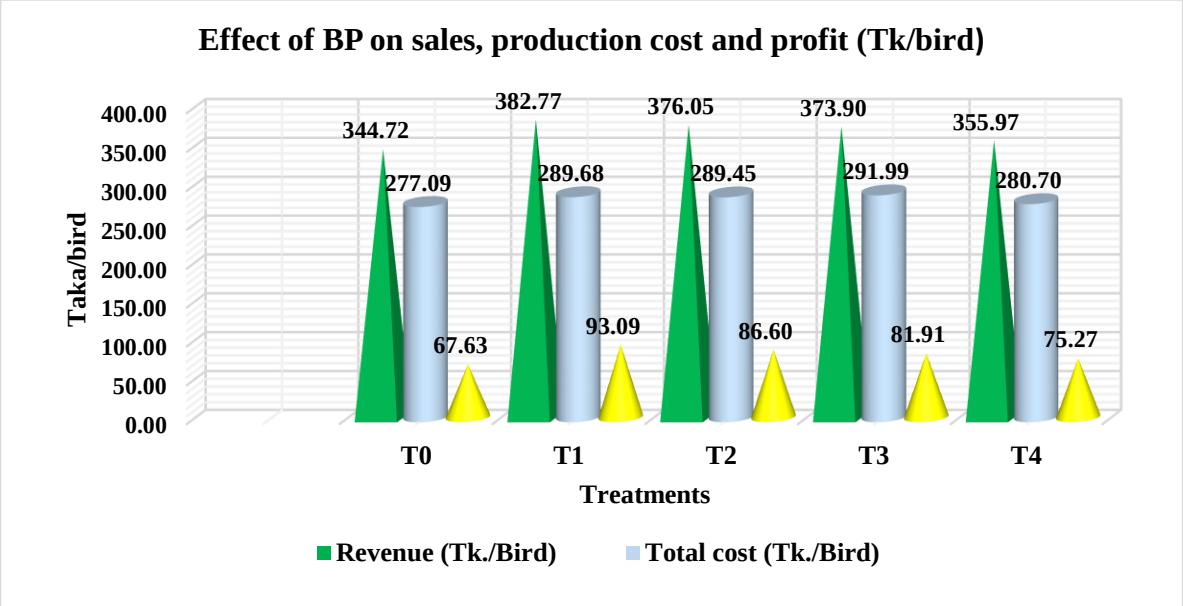

Figure 6. Effect of bacteriophage on sales, production cost and profit

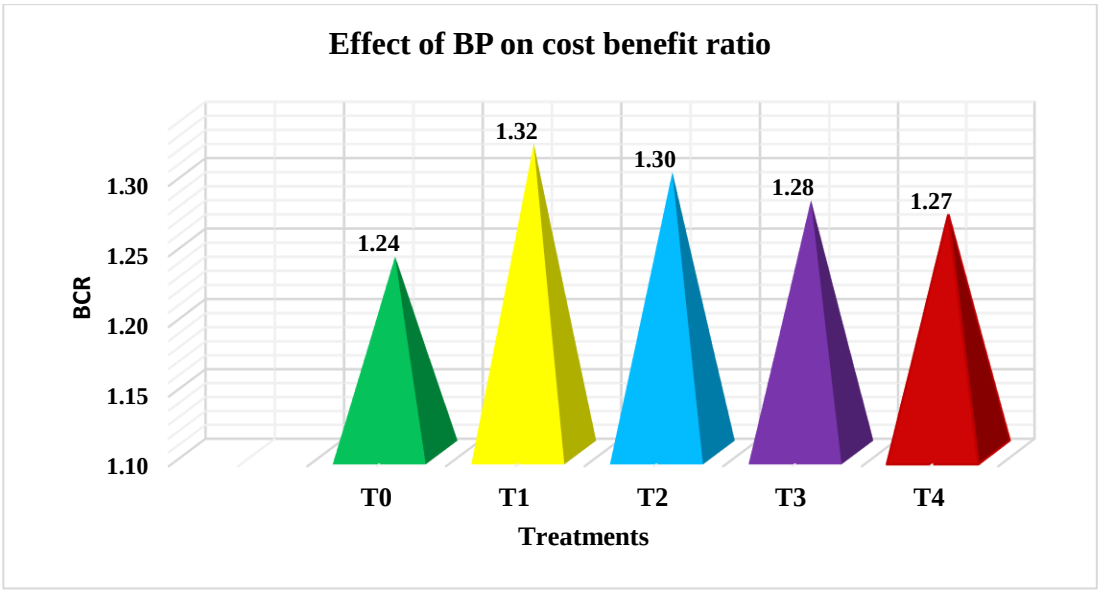

Figure 7. Effect of bacteriophage on cost benefit ratio.

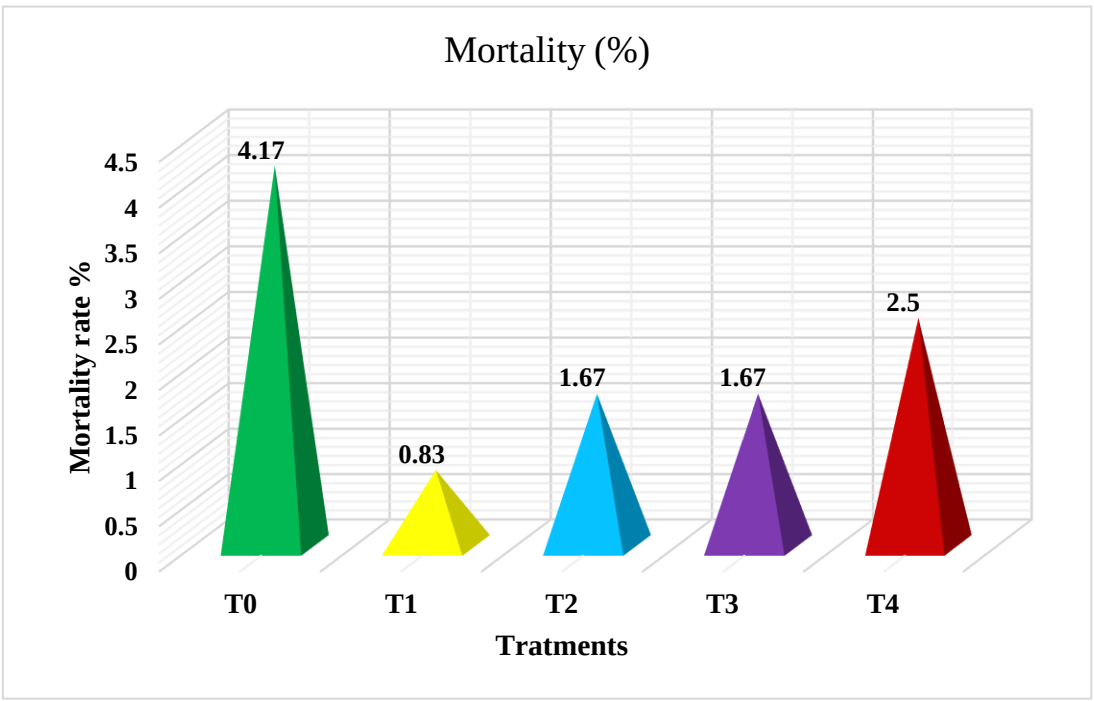

Figure 8. Mortality (%)

CHAPTER V

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CONCLUSION AND RECOMENDATIONS

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CONCLUSION AND RECOMENDATIONS

A total of 600-day old chicks of "Hubbard Classic Efficiency Plus" were reared at Sher-E- Bangla Agricultural University, Dhaka Poultry Farm for a period of five weeks using cocktail bacteriophage. The study was conducted with broilers to investigate the use of bacteriophage as a sustainable alternative to antibiotics. The specific objectives of this experiment were i) to evaluate the growth performance, body weight and FCR of broiler chickens raised with BP ii) to find out the effect of BP on *E. coli* and *Salmonella spp.* iii) to observe the effect of bacteriophage on organ weight iv) to estimate the cost benefit in broiler rearing under different bacteriophage treatment and v) to recommend the inclusion level of bacteriophage in broiler ration as an alternative to antibiotic supplement for growth promotors. Chicks were divided randomly into 5 experimental groups of 4 replications, R_1, R_2, R_3 and R_4, where each replication contains 30 birds. These five treatments groups were designated as T_0, T_1, T_2, T_3 and T_4. The performance traits *viz.* body weight, weight gain, feed consumption, FCR, relative organ weight, bacterial colony count, and economic impact on broiler rearing that includes production cost, profit per bird and benefit cost ratio (BCR) of broiler on different replication of the treatments were recorded and compared in each group. Collectively, the data from the present study indicate that the application of bacteriophage cocktail at the dosage of 0.5 g/kg of feed to the broiler ration is sufficient to be used in commercially raised broiler chickens. Dietary supplementation of bacteriophage improves body weight gain and FCR at 0.5 g/kg dosage and economically effective than using 0.75 and 1 g/kg dosage. Analyzing the above research findings, bacteriophage used in T_1 groups (0.5g/kg of feed) showed better results than other treatment groups in terms of improved growth performance with better FCR. Among the three-bacteriophage dietary treatment group T_1 (0.5 g/kg of feed) showed better result than group T_2 (0.75 g/kg of feed) and group T_3 (1 g/kg of feed). Collectively, the data from the present study indicate that the application of bacteriophage cocktail at concentrations of 0.5 g/kg and 0.75 g/kg of feed to the diet of commercially raised broiler chickens could increase body weight gain and improve FCR. Furthermore, it was observed that a 0.5 g/kg bacteriophage cocktail reduces the pathogenic organisms like *Escherichia coli* and *Salmonella* from excreta. These

findings suggest that a 0.5 g/kg bacteriophage cocktail dietary supplementation would be economical and effective as a safe alternative to antibiotics for raising broilers under open sledded farming systems. The study also recommends further investigation on the effect of bacteriophage on Lactobacillus, Clostridia, hematological parameters on birds' immunity and conducting feeding trial on commercial poultry farm to fix up inclusion level for higher economical return.

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CHAPTER VI

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APPENDICES

APPENDICES

Appendix I. Body weight (BW) (g/bird) of 1st, 2nd, 3rd, 4th & 5th week under different treatments.

Treatment	Replication	1st Wk.	2nd Wk.	3rd Wk.	4th Wk.	5th Wk.
T_0	R_1	172.55	474.53	878.18	1347.44	2038.11
	R_2	178.88	484.24	877.63	1364.00	2037.00
	R_3	176.22	490.91	910.36	1384.00	2012.33
	R_4	174.88	482.23	889.73	1364.56	2023.67
T_1	R_1	183.63	527.27	948.83	1562.00	2237.55
	R_2	184.84	527.27	982.16	1584.00	2294.00
	R_3	180.55	530.76	977.15	1566.00	2224.44
	R_4	182.00	530.77	956.05	1575.00	2250.33
T_2	R_1	180.88	515.91	950.03	1531.00	2203.00
	R_2	182.70	517.49	934.00	1566.77	2230.00
	R_3	184.30	518.18	955.97	1527.56	2204.00
	R_4	179.63	514.86	947.67	1522.67	2211.33
T_3	R_1	174.37	571.21	960.28	1530.44	2195.72
	R_2	182.48	511.38	956.06	1544.44	2209.58
	R_3	180.62	548.13	926.76	1548.52	2185.00
	R_4	181.49	544.57	948.77	1576.67	2207.33
T_4	R_1	186.79	510.78	900.91	1448.73	2090.94
	R_2	184.62	512.24	919.39	1457.05	2143.96
	R_3	185.51	532.42	921.43	1410.22	2044.76
	R_4	184.64	533.48	916.24	1398.67	2096.08

Appendix II. Feed intake (FI) (g/bird) of 1st, 2nd, 3rd, 4th & 5th week under different treatments.

Treatment	Replication	1st Wk.	2nd Wk.	3rd Wk.	4th Wk.	5th Wk.
T_0	R_1	166.00	530.00	1146.00	1995.57	3254.40
	R_2	166.61	540.00	1231.00	2018.45	3218.46
	R_3	165.59	532.00	1225.00	2039.00	3190.52
	R_4	165.06	533.00	1201.66	1998.24	3141.69
T_1	R_1	169.00	558.00	1204.00	2129.00	3366.73
	R_2	169.70	550.00	1205.00	2119.55	3365.03
	R_3	169.20	545.34	1205.00	2165.56	3348.88
	R_4	168.30	550.67	1210.00	2156.67	3348.20
T_2	R_1	168.00	539.00	1209.22	2093.00	3302.27
	R_2	167.21	538.00	1199.78	2139.19	3384.33
	R_3	168.00	549.00	1200.86	2112.22	3317.91
	R_4	166.74	546.33	1198.45	2107.19	3363.84
T_3	R_1	163.00	592.00	1208.22	2127.78	3357.91
	R_2	165.00	541.00	1212.12	2121.33	3376.47
	R_3	164.00	579.00	1199.45	2119.12	3349.75
	R_4	165.00	571.67	1200.55	2163.33	3380.38
T_4	R_1	166.00	541.00	1203.45	2115.15	3220.05
	R_2	165.45	545.00	1218.68	2112.77	3301.69
	R_3	165.13	538.00	1228.00	2073.02	3169.37
	R_4	164.53	539.67	1224.33	2042.05	3227.96

Appendix III. Feed conversion ratio (FCR) of 1st, 2nd, 3rd, 4th & 5th week under different treatments.

Treatment	Replication	1st Wk.	2nd Wk.	3rd Wk.	4th Wk.	5th Wk.
T_0	R_1	0.96	1.12	1.30	1.48	1.60
	R_2	0.93	1.12	1.40	1.48	1.58
	R_3	0.94	1.08	1.35	1.47	1.59
	R_4	0.94	1.11	1.35	1.46	1.55
T_1	R_1	0.92	1.06	1.27	1.36	1.50
	R_2	0.92	1.04	1.23	1.34	1.47
	R_3	0.94	1.03	1.23	1.38	1.51
	R_4	0.92	1.04	1.27	1.37	1.49
T_2	R_1	0.93	1.04	1.27	1.37	1.50
	R_2	0.92	1.04	1.28	1.37	1.52
	R_3	0.91	1.06	1.26	1.38	1.51
	R_4	0.93	1.06	1.26	1.38	1.52
T_3	R_1	0.93	1.04	1.26	1.39	1.53
	R_2	0.90	1.06	1.27	1.37	1.53
	R_3	0.91	1.06	1.29	1.37	1.53
	R_4	0.91	1.05	1.27	1.37	1.53
T_4	R_1	0.89	1.06	1.34	1.46	1.54
	R_2	0.90	1.06	1.33	1.45	1.54
	R_3	0.89	1.01	1.33	1.47	1.55
	R_4	0.89	1.01	1.34	1.46	1.54

Appendix IV. Effect of Bacteriophage on *Escherichia coli* and *Salmonella*

Treatment	Replication	E. coli	Salmonella
T_0	R_1	6.85	4.39
	R_2	6.79	4.40
	R_3	6.90	4.01
	R_4	6.80	4.33
T_1	R_1	6.32	4.01
	R_2	6.02	3.98
	R_3	6.21	4.12
	R_4	6.25	4.07
T_2	R_1	6.18	3.89
	R_2	6.06	3.88
	R_3	6.12	4.00
	R_4	6.09	3.87
T_3	R_1	6.28	3.80
	R_2	6.30	3.91
	R_3	6.22	3.82
	R_4	6.04	3.99
T_4	R_1	6.42	4.21
	R_2	6.40	4.22
	R_3	6.31	4.18
	R_4	6.27	4.19

Appendix V. Effect of Bacteriophage on organ weight.

Treatment	Replication	Breast Muscle	Liver	Spleen	Bursa of fabricus	Gizzard	Abdominal fat
T_0	R_1	22.12	2.71	0.19	0.13	1.68	1.01
	R_2	22.10	2.69	0.20	0.14	1.67	1.02
	R_3	22.52	2.68	0.19	0.15	1.67	1.03
	R_4	22.00	2.69	0.19	0.13	1.68	1.01
T_1	R_1	22.90	2.70	0.17	0.12	1.64	1.11
	R_2	23.12	2.72	0.16	0.11	1.65	1.13
	R_3	22.82	2.68	0.17	0.10	1.63	1.12
	R_4	23.10	2.68	0.17	0.10	1.63	1.11
T_2	R_1	23.05	2.63	0.17	0.10	1.69	1.13
	R_2	23.32	2.61	0.17	0.11	1.67	1.14
	R_3	23.01	2.62	0.18	0.12	1.66	1.13
	R_4	22.85	2.63	0.17	0.10	1.67	1.14
T_3	R_1	23.45	2.63	0.16	0.10	1.68	1.21
	R_2	23.32	2.64	0.17	0.11	1.67	1.19
	R_3	23.55	2.65	0.17	0.10	1.65	1.18
	R_4	23.10	2.64	0.16	0.10	1.67	1.20
T_4	R_1	23.92	2.72	0.19	0.12	1.61	1.09
	R_2	24.02	2.74	0.18	0.12	1.63	1.10
	R_3	23.80	2.70	0.17	0.13	1.61	1.11
	R_4	23.88	2.73	0.18	0.11	1.62	1.10

Appendix VI. Effect of bacteriophage on production cost

Treatment	Replications	Feed Cost (Tk/Bird)	BP Cost (Tk/bird)	Other Expenses (Tk/Bird)	Total Cost (Tk/bird)
T_0	R_1	227.81	0.00	53.00	280.81
	R_2	225.29	0.00	53.00	278.29
	R_3	223.34	0.00	53.00	276.34
	R_4	219.92	0.00	53.00	272.92
T_1	R_1	235.67	1.68	53.00	290.35
	R_2	235.55	1.68	53.00	290.23
	R_3	234.42	1.67	53.00	289.10
	R_4	234.37	1.67	53.00	289.05
T_2	R_1	231.16	2.48	53.00	286.64
	R_2	236.90	2.54	53.00	292.44
	R_3	232.25	2.49	53.00	287.74
	R_4	235.47	2.52	53.00	290.99
T_3	R_1	235.05	3.36	53.00	291.41
	R_2	236.35	3.38	53.00	292.73
	R_3	234.48	3.35	53.00	290.83
	R_4	236.63	3.38	53.00	293.01
T_4	R_1	225.40	1.61	53.00	280.01
	R_2	231.12	1.65	53.00	285.77
	R_3	221.86	1.58	53.00	276.44
	R_4	225.96	1.61	53.00	280.57

Appendix VII. Cost benefit ratio analysis

Treatment	Replications	Total Cost (Tk/bird)	Sales (Tk/bird)	Profit (Tk/bird)	BCR
T_0	R_1	280.81	346.48	65.67	1.23
	R_2	278.29	346.29	68.19	1.24
	R_3	276.34	342.10	70.14	1.24
	R_4	272.92	344.02	73.56	1.26
T_1	R_1	290.35	380.38	90.03	1.31
	R_2	290.23	389.98	99.75	1.34
	R_3	289.10	378.15	89.06	1.31
	R_4	289.05	382.56	93.51	1.32
T_2	R_1	286.64	374.51	87.87	1.31
	R_2	292.44	379.10	86.66	1.30
	R_3	287.74	374.68	86.94	1.30
	R_4	290.99	375.93	84.93	1.29
T_3	R_1	291.41	373.27	81.86	1.28
	R_2	292.73	375.63	82.90	1.28
	R_3	290.83	371.45	80.62	1.28
	R_4	293.01	375.25	82.24	1.28
T_4	R_1	280.01	355.46	75.45	1.27
	R_2	285.77	364.47	78.70	1.28
	R_3	276.44	347.61	71.17	1.26
	R_4	280.57	356.33	75.76	1.27