Research Article
Open Access

Evaluation of the effect of dietary supplementation of bacteriophage on production performance and excreta microflora of commercial broiler and layer chickens in Bangladesh

Abstract

The study was conducted to evaluate the effects of dietary bacteriophage (BP) supplementation on production performance, excreta microflora, and moisture content as well as blood profiles in broilers and layers. A total of 80 broilers (DOC) and 80 layers(36 weeks old) were randomly allotted in 4 treatment groups (20 birds in each group) in a completely random block designfor 4 weeks and 6 weeksfeeding trials, respectively. Dietary treatments for broilers included: i) CON (basal diet), ii) ANT (CON+0.5 g antibiotics/kg feed), iii) BP1 (CON+0.25 g bacteriophage/kg feed) and iv) BP2 (CON+0.5g bacteriophage/kg feed). Dietary treatments for layers included: i) CON (basal diet), ii) BP1 (CON+0.2 g bacteriophage/kg feed), iii) BP2 (CON+0.35g bacteriophage/kg feed), and iv) BP3 (CON+0.5 g bacteriophage/kg feed). In broilers, the inclusion of antibiotic and bacteriophages did not affect feed intake (P=0.78) among the four groups but BWG in BP2 group significantly differ during 1-2 weeks (P=0.046) and 3-4 weeks (P=0.016) of ages compared with the CON group. FCR of broilers was found significantly higher compared with CON (P=0.011), ANT (P=0.022) and BP1 groups (P=0.013). In case of laying hens, BP2 (P=0.001) and BP3 (P=0.0004) groups had significantly higher egg production than those of CON group during 36-39 weeks. In addition, egg production in BP1 (P=0.02), BP2 (P<0.0001), and BP3 (P=0.0003) groups increased significantly at 40-42 weeks compared with CON group. However, in case of egg weight, no significant difference was observed among the four treatment groups (P=0.86). In case of excreta microflora, inclusion of antibiotic and bacteriophages significantly reduced the *E. coli* (P<0.0001) and *Salmonella* (P<0.0001) concentrations in excreta of the broilers and layers compared with the CON group. However, on excreta moisture content of layers and blood profiles (RBC, PCV, HB, WBC and lymphocyte) of broilers, no significant effects of dietary antibiotic and bacteriophages supplementation was observed throughout the whole experiment (P>0.05). In conclusion, bacteriophage supplementation has beneficial effects on production performance of broilers and layers and can reduce excreta microflora concentration in commercial broiler and layer chicken.

Keywords: broiler, layer, growth performance, egg performance, excreta microflora

Volume 9 Issue 2 - 2020

MoniraNoor,[1] Nurjahan Yasmin Runa,[1] Asmaul Husna,[1] Marzia Rahman,[2] Dewan MM Rajib,[3] ATM Mahbub-e-Elahi,[3] Md Masudur Rahman[1]

[1]Department of Pathology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Bangladesh

[2]Department of Microbiology, Faculty of Veterinary Science, Bangladesh Agricultural University, Bangladesh

[3]Department of Microbiology and Immunology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Bangladesh

Correspondence: Md Masudur Rahman, Department of Pathology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet-3100, Bangladesh, Tel +8801712056240,
Email rahmanmm.dpp@sau.ac.bd, mmrahman.sau@gmail.com

Received: February 11, 2020 | **Published:** March 20, 2020

Introduction

In Bangladesh, poultry industry is an important part of its national economy. This industry has been engaging to supply quality protein to the Bangladeshi people at the lowest price in the world. The major crisis of this sector is disease problems that cause around 30-40% mortality of poultry as well as loss of production.[1] Now-a-days, food safety is a major concern in food production worldwide whereas, poultry meat and eggs are one of the major sources of food borne pathogens. In this situation, antibiotics are used randomly for the treatment, control and prevention of diseases in poultry and also as a growth promoter. For instance, antimicrobial resistant as well as multidrug resistant bacteria or superbugs are emerging spontaneously and persisting in human and animal food chains.[2] These resistant bacteria are causing huge clinical and economic losses and loss of life. To overcome this situation use of antibiotic should be kept under control in poultry production and alternatives to antibiotics need to be searched.[3]

Bacteriophages are bacteria-specific viruses that multiply inside bacteria by making use of some or all of the host biosynthetic machinery and, as such, are not pathogenic for animals, including humans.[4] Bacteriophages have been used widely as therapeutic agents in place of antibiotics since 1918.[5] The use of bacteriophages to eliminate pathogens, mostly food borne pathogens like *Salmonellae, Campylobacter* and *E. coli*, seems quite promising as bacteriophages are present in every ecosystem and their number is 10 times more than the number of characterized bacteria.[6]

Long-term use of phages in poultry has proved to be moderately effective in reducing the number of *Salmonella* colonizing in the digestive tract.[7] In broiler diet, anti-SE (anti- *S. enteritidis*) bacteriophages can be used as an alternative feed additive instead of antibiotics.[8] In laying hens, a positive effect of phage therapy was also observed in combating horizontal infections induced by strains of S. gallinarum.[9] According to previous studies, the inclusion of

bacteriophages could successfully reduce the *Salmonella* and *E. coli* counts in chicken internal organs and feces[10] or poultry products.[11–13] In future, there might be a possibility of launching phage mediated-therapy in the poultry industry to reduce or eliminate antibiotic use with increased consumer demand.

Recently, it has been reported that the inclusion of bacteriophages in poultry ration could benefit the poultry farmers in terms of improved feed conversion ratio in broilers and increased quality egg production in layer chickens.[14] However, no data is available in Bangladesh on the use of bacteriophages in poultry both at challenge condition and at normal physiologic state. Additionally, very few experiments investigating the effect of dietary supplementation of bacteriophages targeting multiple pathogenic *Salmonellae* species in laying hens have been conducted. Therefore, the objectives of the present study was to evaluate the effects of dietary supplementation of bacteriophages on the production performance and intestinal and excreta *Salmonella* and *E. coli* count in commercial broiler and layer chickens under normal physiological state. We also assessed the effects of dietary supplementation of bacteriophages on excreta moisture content of layer and blood profiles of broiler.

Results

Effects of dietary supplementation of bacteriophage on production performance of broiler and layer chickens

Effects on growth performance of broiler chickens: In this study, the inclusion of antibiotic and bacteriophages did not affect feed intake (P=0.78) throughout the experimental period (0-4 weeks). However, the body weight gain (BWG) of experimental birds during 1-2 weeks (P=0.046) and 3-4 weeks (P=0.016) of ages significantly differed in BP2 group compared with the CON group (Fig-1). In case of 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 BP2 group compared with CON (P=0.011), ANT (P=0.022) and BP1 groups (P=0.013) (Figure 1).

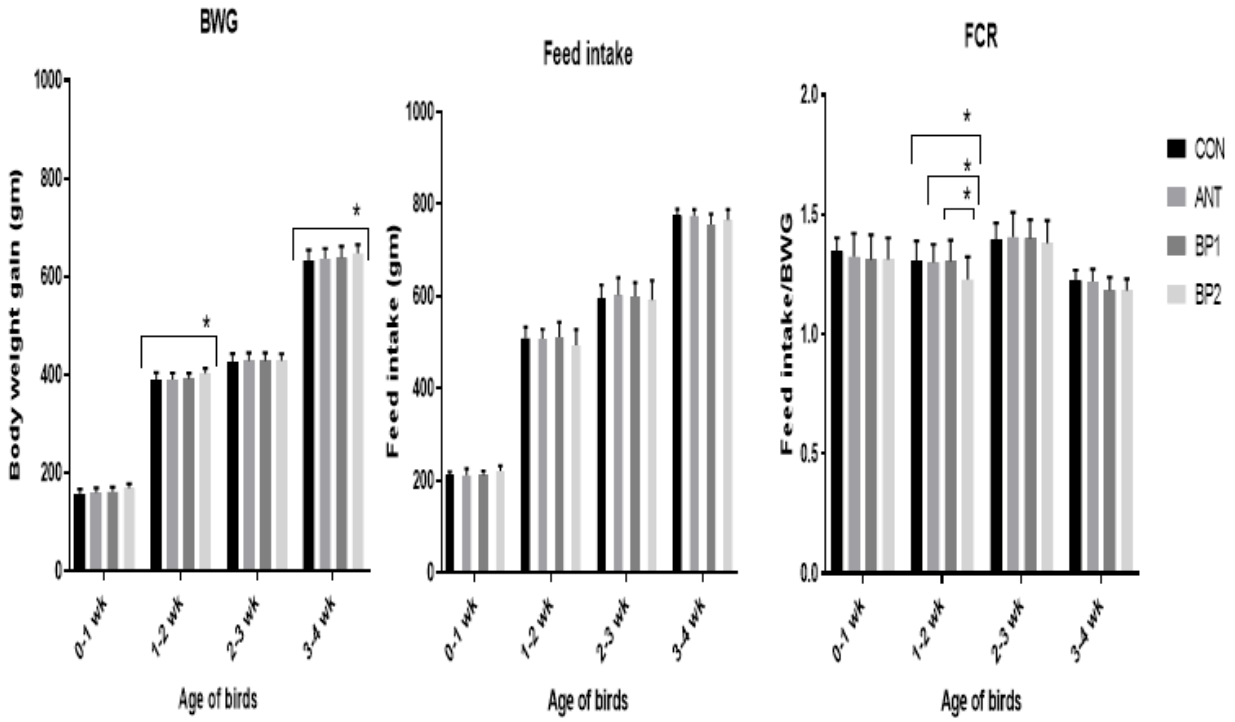

Figure 1 Effects of bacteriophage supplementation on growth performance (BWG, feed intake and FCR) in broilers. CON, basal diet; ANT, CON+0.5g antibiotics/kg feed; BP1, CON+0.25g bacteriophage/kg feed; BP2, CON+0.5g bacteriophage/kg feed. P<0.05 were considered statistically significant.

Effects on egg production performance in layer chickens: The effects of dietary supplementation of bacteriophage on egg production and egg weight in laying hens are shown in Figure 2. Laying hens of BP2 (P=0.001) and BP3 (P=0.0004) groups had significantly higher egg production than those of CON group during 36 to 39 weeks. In addition, egg production in BP1 (P=0.02), BP2 (P<0.0001), and BP3 (P=0.0003) groups increased significantly at 40 to 42 weeks compared with CON group. However, in case of egg weight, no significant difference was observed among the four treatment groups (P=0.86) throughout the experimental period (Figure 2).

Citation: Noor M, Runa NY, Husna A, et al. Evaluation of the effect of dietary supplementation of bacteriophage on production performance and excreta microflora of commercial broiler and layer chickens in Bangladesh. *MOJ Proteomics Bioinform.* 2020;9(2):27–31. DOI: 10.15406/mojpb.2020.09.00274

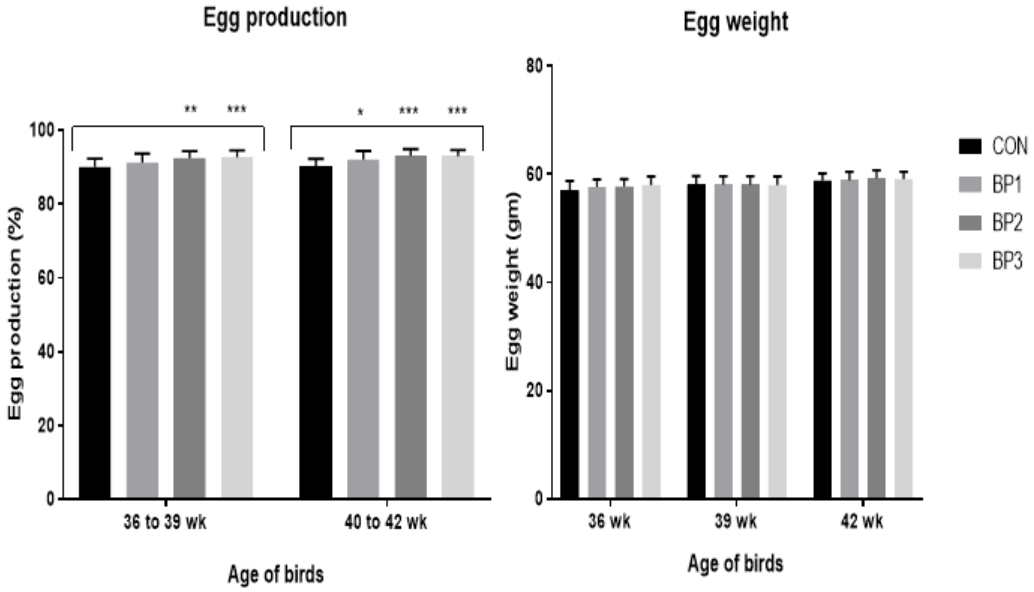

Figure 2 Effects of bacteriophage supplementation on egg production performance (egg production and egg weight) in layer chickens. CON, basal diet; BP1, CON+0.2g bacteriophage/kg feed; BP2, CON+0.35g bacteriophage/kg feed; and BP3, CON+0.5 g bacteriophage/kg feed. *P*<0.05 were considered statistically significant.

Effects of bacteriophage supplementation on Intestinal and Excreta Salmonella and E. coli shedding in broiler and layer chickens

Effects on intestinal Salmonella and E. coli shedding in broiler chickens: 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 CON group (Table 1).

Effects on excreta Salmonella and E. coli counts and moisture content in layer chickens: In case of layers, the dietary supplementation of bacteriophage significantly reduced the *E. coli* (*P*<0.0001) and *Salmonella* (*P*<0.0001) counts in BP1, BP2 and BP3 groups compared with CON group (Table 2). However, bacteriophage had no significant effect (*P*=0.613) on excreta moisture content in layers.

Effects of bacteriophage supplementation on blood profiles in broiler chickens

Dietary antibiotic and bacteriophage supplementation had no significant effect (*P*>0.05) on blood profiles (RBC, PCV, HB, WBC and lymphocyte) of the experimental broiler chickens (Table 3).

Table 1 The effects of bacteriophage supplementation on intestinal microflora in broilers

Items, Log_{10} CFU/ML	CON	ANT	BP1	BP2	SEM	P-value
Escherichia coli	8.2355	7.1625	7.113	7.275	0.265	P<0.0001***
Salmonella	7.585	6.7835	6.6785	6.5215	0.237	P<0.0001***

CON=(basal diet); ANT=(CON+0.5g antibiotics/kg feed); BP1=(CON+0.25g bacteriophage/kg feed); BP2=(CON+0.5g bacteriophage/kg feed); SEM, standard error of mean

Table 2 Effects of bacteriophage supplementation on excreta *Salmonella* and *E. coli* counts and moisture content in layer chickens

Items, Log_{10} CFU/ML	CON	BP1	BP2	BP3	SEM	P-value
Escherichia coli	7.13	6.447	6.2345	6.224	0.213	P<0.0001***
Salmonella	4.7585	3.675	3.5515	3.6115	0.287	P<0.0001***
Excreta moisture	66.785	66.535	66.315	66.485	0.097	0.613(NS)

CON=Basal diet; BP1=CON+0.020% bacteriophage; BP2=CON+ 0.035% bacteriophage; BP3, CON+0.050% bacteriophage; SEM, standard error of mean; NS, not significant

Citation: Noor M, Runa NY, Husna A, et al. Evaluation of the effect of dietary supplementation of bacteriophage on production performance and excreta microflora of commercial broiler and layer chickens in Bangladesh. *MOJ Proteomics Bioinform.* 2020;9(2):27–31. DOI: 10.15406/mojpb.2020.09.00274

Table 3 Effects of bacteriophage supplementation on blood profiles in broiler chickens

Items	CON	ANT	BP1	BP2	SEM	P value
RBC(10^6/µl)	4.1	3.75	4.72	4.69	0.237	0.42 (NS)
PCV%	25	23.25	32.92	35.28	2.941	0.61(NS)
HB%	8.42	7.77	10.82	10.19	0.719	0.28(NS)
WBC(103/µl)	16.5	17.46	16.44	16.09	0.29	0.05(NS)
Lymphocyte(%)	56.15	55.3	60.45	60.11	1.328	0.28(NS)

CON=(basal diet); ANT=(CON+0.5g antibiotics/kg feed); BP1=(CON+0.25g bacteriophage/kg feed); BP2=(CON+0.5g bacteriophage/kg feed); SEM, standard error of mean; NS, not significant

Discussion

In the current study, the effect of dietary supplementation of bacteriophage on the growth performance of broilers and egg production performance of layers were investigated under normal physiological situation. Results of our study suggested that the inclusion of antibiotics and small amount of bacteriophages in broilers' diet did not affect significantly on the feed intake, BWG and FCR throughout the experimental period which is more or less similar with the previous findings i.e. the body weight was not affected by the inclusion of bacteriophages in diet under normal physiological states.[12] However, it was noted that the improvement in feed efficiency was observed in 0.5 g/kg bacteriophage group (BP2) which suggest that inclution of bacterophages at higher dosage might improve the feed efficiency of broilers. Our findings are in agreement with previous findings[13,15] where mentioned that higher dosage of bacteriophage was desirable to maintain growth performance by reducing the *S. enteritideis* and *S. typhimurium*in cecal content. In case of layers, we found significant effect of bacteriophage on egg production but not in egg weight which is corroborated with the previous findings.[14] When bacteriophages were used in the current study, they elicited a beneficial effect on egg production. This was probably due to a decrease in the number of *Salmonella spp* by the effect of bacteriophage supplementation.

In case of excreta microbial shedding, we found that supplementation of bacteriophage significantly decreased *E. coli* and *Salmonella* counts in excreta of broiler and layer chickens. Similarly, many researchers[12,14,16,17] also suggested that the inclusion of bacteriophage could reduce the *E. coli* colonization in broilers and layers. They also reported that *Salmonella*-specific lytic bacteriophage can be used as a potential method to reduce *Salmonella* in livestock.[17] Therefore, we hypothesized that the beneficial effect of the bacteriophages could be attributed to the improved microbial ecosystem in the broilers and layers. Our results on the fecal microbial shedding also support this hypothesis, whereas the inclusion of bacteriophage led to a lower *E. coli* and *Salmonella* concentrations compared with basal diet.

Materials and methods

The study was conducted at the Department of Pathology, and the Department of Microbiology and Immunology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Bangladesh. The handling of animals in the study was performed in accordance with the current Bangladesh legislation (Cruelty to Animals Act 1920, Act No. I of 1920 of the Government of the People's Republic of Bangladesh). The specific experiments were approved by the Ethics Committee of the Sylhet Agricultural University, Bangladesh.

Experimental design, animals and diets

Bacteriophages cocktail containing *Salmonella* (*S.*) *gallinarum*, *S. typhimurium*, and *S. enteritidis* at the ratio of 3:3:4 were used in this study. The concentration of bacteriophages was 10^8 plaque forming unit per gram. Two individual feeding trial experiments were performed using broiler and layer chickens. In case of broiler experiment (n=80), feeding trial was performed using day old broiler chicks (DOC) for a period of 4 weeks and in case of layer experiment (n=80), feeding trial was performed using 36 weeks old commercial layers for a period of 6 weeks. In both the experiments, birds were randomly allotted in 4 treatment groups (20 birds in each group) in a completely random block design. Dietary treatments for broiler experiment included: i) positive control (basal diet), ii) antibiotic treated group (basal diet+0.5 g antibiotics/kg feed), iii) Bacteriophage 1 (basal diet+0.25 g bacteriophage/kg feed) and iv) Bacteriophage 2 (basal diet+0.5g bacteriophage/kg feed). Dietary treatments for layer experiment included: i) positive control (basal diet), ii) Bacteriophage 1 (basal diet+0.2 g bacteriophage/kg feed), iii) Bacteriophage 2 (basal diet+0.35g bacteriophage/kg feed), and iv) Bacteriophage 3 (basal diet+0.5 g bacteriophage/kg feed). All diets were formulated to meet or exceed requirements for broilers and laying hens and provided in mash form. Before the beginning of layer experiment, hens were provided with a basal diet for 7 days adjustment period. All cages were provided with free access to water and feed. The birds were maintained in same housing condition throughout the experimental period.

Sampling and measurements

Observing the production performance of broiler and layer chickens: Feed intake and body weight of broilers were recorded weekly by using pen to calculate the BW gain (BWG) and feed conversion ratio (FCR) after correcting mortality. In case of laying hens, daily records of egg production and egg weight were recorded to know the egg performance. The egg productions were expressed as an average hen-day production.

Observing the intestinal and excreta microbial shedding in broiler and layer chickens: In case of broilers, after blood collection, the same birds were weighed individually and sacrificed by cervical dislocation and exsanguinated. Cecal contents were collected aseptically from each sacrificed broilers into a plastic vial containing buffered peptone broth (Becton, Dickinson and Co.) to evaluate the effects of inclusion of bacteriophages on the intestinal *Salmonella* and *E. coli* count. Then the intestinal samples were homogenized and filtered.

In case of layers, excreta samples were collected directly from each cage and then pooled and placed on ice for transportation to

the lab. The identification of excreta microfloras were carried out at the end of wk 6 (42 wk). One gram of the composite excreta sample from each cage were diluted with 9 ml of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and then homogenized.

Viable counts of bacteria in the samples collected from both broilers and layers were determined by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) to isolate *Escherichia coli* (*E. coli)*. Then the MacConkey agar plates were incubated for 24 h at 37ºC. *Escherichia coli* colonies were counted immediately after removal from the incubator. For isolation of *Salmonella*, the serially diluted peptone broth tubes were incubated overnight at 37ºC, from which 1 ml was transferred to 9 ml of tetratinate broth (Neogen Corporation, Lansing, MI, USA) and then incubated for 48 h at 42ºC. From each tube, 1 ml was used to inoculate into 9 ml of Rappaport Vassiliadis broth (Neogen Corporation, Lansing, MI, USA) and incubated for 48 h at 42^{0}C. The Rappaport was used to inoculate XLT4 plates for *Salmonella* isolation. Finally, the *Salmonella* were presumably identified by using LIS and TSI agar tube (Difco Laboratories, Detroit, MI, USA).

Observing the excreta moisture content in layer experiment: To determine the moisture content, excreta samples from layers were placed in aluminum foil cups. The aluminum foil cups were weighed and placed in a drying oven at 100ºC for 24h and then reweighed to calculate moisture loss.

Observing the blood profiles in broiler experiment: On day 32, five broilers from each treatment group were randomly selected for blood collection. Blood samples were collected from the brachial vein with sterile syringe and kept in vials containing anticoagulant sodium- EDTA. The samples were stored at -4ºC. For analysis of hematological parameter such as TLC, TEC and DLC, sera were separated from the blood samples by centrifuging at 3,000rpm for 15 min and stored at -4ºC. Then the white blood cells (WBC), red blood cells (RBC), and lymphocyte percentage were analyzed manually under light microscope using hemocytometer slide.

Statistical analysis

All data were analyzed by computer using GraphPad Prism version 7.01 (Trial version). *P* values were detected by two-way ANOVA and multiple comparisons between the treatment groups were detected by using Tukey's multiple comparisons test where $P<0.05$ were considered statistically significant.

Conclusion

According to our findings, feed efficiency in broilers was increased significantly with dietary supplementation of bacteriophage @0.5g/kg feed. Also, significant effect on inhibiting excreta pathogen shedding in broilers was found. In case of layers, dietary supplementation of bacteriophages significantly increased egg production and decreased *E. coli* and *Salmonella* colonization in intestine of laying hens. Taken together, it may be concluded that dietary supplementation of bacteriophages might be used as a possible alternative to dietary antibiotic as growth promoters and improved egg production performance by reducing intestinal microbial load.

Acknowledgments

The research was funded by the University Grants Commission (UGC) of Bangladesh through Sylhet Agricultural University Research System (SAURES), Sylhet 3100, Bangladesh. The funder has no influence on research design, data generation, manuscript preparation and decision to publish data.

Conflicts of interest

Author declares that there are no conflicts of interest.

References

1. Hamid MA, Rahman MA, Ahmed S et al. Status of Poultry Industry in Bangladesh and the Role of Private Sector for Its Development. *Asian Journal of Poultry Science*. 2017;11:1–13.
2. Aslam B, Wang W, Arshad MI, et al. Antibiotic Resistance: A Rundown of a Global Crisis. *Infect Drug Resist*. 2018;11:1645–1658.
3. Joerger RD. Alternatives to Antibiotics: Bacteriocins, Antimicrobial Peptides and Bacteriophages. *Poult Sci*. 2003;82:640–647.
4. Runa NY, Husna A, Badruzzaman AM, et al. Bacteriophages (the Living Drugs): Novel Applications and Alternative to Antibiotics in Poultry Production. *Food Safety and Health*. 2018;1:1–14.
5. Hanlon GW. Bacteriophages: An Appraisal of Their Role in the Treatment of Bacterial Infections. *Int J Antimicrob Agents*. 2007;30(2):118–128.
6. Urban-Chmiel R, Wernicki A, Stegierska D, et al. Isolation and Characterization of Lytic Properties of Bacteriophages Specific for M. Haemolytica Strains. *PLoS One*. 2015;10:e0140140.
7. Sklar IB, Joerger RD. Decreasing the Load of C. Jejuni in Poultry Products Decreases the Risk of People Getting Sick, and in That Sense Phage Therapy May Also Be Considered to Have an Indirect "Probiotic" Activity. *Journal of Food Safety*. 2001;21:15–29.
8. Kim KH, Lee GY, Jang JC, et al. Evaluation of Anti-Se Bacteriophage as Feed Additives to Prevent Salmonella Enteritidis (Se) in Broiler. *Asian-Australas J Anim Sci*. 2013;26(3):386–393.
9. Lim TH, Lee DH, Lee YN, et al. Efficacy of Bacteriophage Therapy on Horizontal Transmission of Salmonella Gallinarum on Commercial Layer Chickens. *Avian Dis*. 2011;55:435–438.
10. Toro H, Price SB, McKee AS, et al. Use of Bacteriophages in Combination with Competitive Exclusion to Reduce Salmonella from Infected Chickens. *Avian Dis*. 2005;49(1):118–124.
11. Whichard JM, Sriranganathan, N Pierson. Suppression of Salmonella Growth by Wild-Type and Large-Plaque Variants of Bacteriophage Felix O1 in Liquid Culture and on Chicken Frankfurters. *J Food Prot*. 2003;66(2):220–225.
12. Huff WE, Huff GR, Rath NC, et al. Prevention of Escherichia Coli Respiratory Infection in Broiler Chickens with Bacteriophage (Spr02). *Poult Sci*. 2002;81(4):437–441.
13. Atterbury RJ, Van Bergen MA, Ortiz F, et al. Bacteriophage Therapy to Reduce Salmonella Colonization of Broiler Chickens. *Appl Environ Microbiol*. 2007;73(14):4543–4549.
14. Zhao PY, Baek HY, Kim IH. Effects of Bacteriophage Supplementation on Egg Performance, Egg Quality, Excreta Microflora, and Moisture Content in Laying Hens. *Asian-Australas J Anim Sci*. 2012;25(7):1015–1020.
15. Kim KH, Ingale SL, Kim JS, et al. Bacteriophage and Probiotics Both Enhance the Performance of Growing Pigs but Bacteriophage Are More Effective. *Animal Feed Science and Technology*. 2014;196:88–95.
16. Fiorentin L, Vieira ND, W BJ. Use of Lytic Bacteriophages to Reduce Salmonella Enteritidis in Experimentally Contaminated Chicken Cuts. *Braz J Poult Sci*. 2005;7(4):255–260.
17. Lee N, Harris DL. The Effect of Bacteriophage Treatment as Preharvest Intervention Strategy to Reduce the Rapid Dissemination of Salmonella Typhimurium in Pigs. *Proc Am Assoc Swine Vet*. 2001;555–557.