

Impact of Bacteriophage Supplementation on Laying Hens' Egg Quality and Gut Microbiota

Harika Mundrathi^{1*}, Laxman Kumar Babu², Arun Kumar Panda¹, Bhagirathi Panigrahi², Sumanth Kumar Mishra³

ABSTRACT

The study investigated the effect of bacteriophage supplementation on egg quality and gut microflora in laying hens. One hundred and twenty Rhode Island Red laying hens were chosen at random. In a completely randomised design, they were split up into five groups of 24, each with three replicates of eight, and were treated as T₁ group (Control), that received a diet supplemented with no antibiotics or bacteriophages; T₂ group, received a diet supplemented with antibiotics (Oxytetracycline @ 50 g/quintal of feed), while T₃, T₄, and T₅ groups received bacteriophages at varying concentrations of 0.20 mL, 0.25 mL and 0.30 mL per litre of drinking water, respectively. The addition of either antibiotic or bacteriophage did not affect any of the egg quality metrics (albumen percentage, yolk percentage, eggshell percentage, egg shape index, Haugh unit, and eggshell thickness). In comparison to the control and antibiotic-supplemented groups, bacteriophage supplementation significantly ($p < 0.05$) reduced the gut ileal and caecal *E. coli* counts. Thus, introducing bacteriophage to laying hens' diets can be an effective substitute for antibiotics.

Key words: Albumen, Anti-microbial resistance, Bacteriophage, Gut microbiota, Poultry.

Ind J Vet Sci and Biotech (2025): 10.48165/ijvsbt.21.6.33

INTRODUCTION

The intestinal microbiota is a crucial component of the digestive tract (GIT), regulating anatomy, nutrient absorption, structure, the body's defence and microbial resistance (Castanon, 2007). Lowering the number of undesirable microbes is a viable way to boost helpful bacteria. This might be due to decreased rivalry with pathogenic bacteria for gut-binding sites and nutrients (Bardy *et al.*, 2016). As a result, lowering harmful bacteria like *E. coli* and *Salmonella* allows beneficial microbes (such as *Lactobacillus*) to proliferate and multiply in a particular population. In-feed antibiotics have favourable benefits on the host, such as raising growth rate, decreasing mortality, and enhancing immunity against infections caused by bacteria. Nevertheless, the ingestion of in-feed antibiotics is currently associated with antimicrobial resistance advancement, which poses a healthcare threat (Miller *et al.*, 2010).

Antimicrobial usage in India is predicted to reach 2236.74 tonnes by 2030, up from 2160.02 tonnes in 2020. To reduce antimicrobial resistance, new alternative approaches must be looked for (Dy Ron *et al.*, 2018). Another tactic is to add bacteriophages as antibacterial agents to the diet in place of antibiotics (Shasha *et al.*, 2004). Currently, the world needs a one-health strategy that brings together the various sectors and stakeholders engaged in the production of food and feed, as well as the health of humans, animals, and plants (Bradley *et al.*, 2017).

Bacteriophages, commonly known as phages, are tiny viruses that range in size from 20 to 200 nm. They are thought to be safe for humans and mostly for all eukaryotic cells

¹ICAR-Central Institute for Women in Agriculture, Bhubaneswar-751003, Odisha, India

²Department of Livestock Production and Management, College of Veterinary Sciences and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar-7510038, Odisha, India

³Department of Animal Nutrition, College of Veterinary Sciences and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar-7510038, Odisha, India

Corresponding Author: Dr. Harika Mundrathi, Ph.D. Scholar, Department of Livestock Production and Management, College of Veterinary Sciences, PVNRTVU, Rajendranagar, Hyderabad-30, Telengana, India. e-mail: harikamundrathi07@gmail.com

How to cite this article: Mundrathi, H., Babu, L. K., Panda, A. K., Panigrahi, B., & Mishra, S. K. (2025). Impact of Bacteriophage Supplementation on Laying Hens' Egg Quality and Gut Microbiota. *Ind J Vet Sci and Biotech*, 21(6), 171-174.

Source of support: Nil

Conflict of interest: None

Submitted 21/07/2025 **Accepted** 25/08/2025 **Published** 10/11/2025

since they may specifically infect prokaryotic bacterial cells (O'Sullivan *et al.*, 2016). Therefore, according to biological criteria and ecological relationships between different forms of life, bacteriophages should be considered parasites rather than predators (Jiang *et al.*, 2022). Many studies (Korf *et al.*, 2020) have confirmed that the majority of bacteriophages have double-stranded, or less frequently single-stranded, DNA and that they only contain one kind of nucleic acid. Additionally, some species have either single-stranded or double-stranded RNA. While antibiotics frequently only function as bacteriostatic agents, lytic bacteriophages are

required to kill bacteria (Kittler *et al.*, 2020). As a result, they could be a more ecologically friendly way to manage and eradicate harmful bacteria than antibiotics (Keen, 2015). There are two types of phages: "lytic" or "virulent" phages, which cause host cell lysis soon after infection, and "temperate" or "lysogenic" phages, which cause a lysogenic cycle and live as prophages in the target cell without immediately causing host lysis (Miller *et al.*, 2010). Hence the objective of this study was to have an alternative approach to antibiotics, *i.e.*, bacteriophage, which could maintain the health of the bird and improve its egg quality and gut microbiota.

MATERIALS AND METHODS

Experimental Design and Birds

The study was conducted during February to July 2021 at ICAR-Central Institute for Women in Agriculture, Bhubaneswar, Odisha (India) following approval of the protocol by the Institutional Animal Ethics Committee. For the experiment, 120 laying hens (Rhode Island Red) of 28 weeks of age were chosen randomly. In a Completely Randomized Design, they were distributed into 5 groups of 24 birds with 3 replicates of 8 birds in every group. The control group (T₁) was fed with diets without supplementation of antibiotics and bacteriophage. Group 2 (T₂) was fed with a diet supplemented with oxytetracycline @ 50 g/quintal of feed (each gram of oxytetracycline hydrochloride contains 50 mg, *i.e.*, 25 mg/kg feed). The Groups T₃, T₄, and T₅ were given the feed with bacteriophage (BAFACOL, Proteon Pharmaceuticals, India), 5 lytic bacteriophages with a concentration of 10⁸ CFU/mL supplementation @ 0.20 mL, 0.25 mL, and 0.30 mL per litre of drinking water, respectively. Birds were allowed to have restricted feeding in which 110 grams of feed per bird was measured and given daily for 8 weeks. Birds were provided with feed once a day in the forenoon. Fresh drinking water was provided twice a day.

Sampling and Egg Quality Measurements

The egg quality parameters, *viz.*, egg weight, albumen height, albumen weight, albumen per cent, Haugh unit, yolk weight, yolk per cent, shape index, albumen index, yolk index, shell thickness, shell per cent, shell weight, were measured on 12

eggs randomly selected from each group on the fourth and eighth weeks of the experimental period using the routine standard procedures. To estimate egg composition, 12 eggs were randomly selected from a single dietary treatment. The fat and egg protein were estimated by following the method of AOAC (1995). The amino acid analysis of eggs was conducted at Evonik, Mumbai, India.

Gut *E. coli* Counts

One bird was chosen from each replicate (3 birds per treatment) at the end of the experiment and was sacrificed by stunning the head. The ileum and caecum were removed and preserved at 4°C for *E. coli* counts. Ileal and caecal contents (around 1 gm from each bird were taken and kept in 9 mL of nutrient broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA). Serial dilution of every sample was made in the nutrient broth. On MacConkey's agar (Difco Laboratories, Detroit, MI, USA) and EMB agar, the *E. coli* counts were made by means of the surface spread method. All measurements were done on a fresh basis and results were expressed as log₁₀cfu/g.

Statistical Analysis

The data was subjected to analysis of variance and the Duncan's multiple range test according to Snedecor and Cochran (1994) to examine the differences between treatment means, if any, at $p < 0.05$.

RESULTS AND DISCUSSION

Egg Quality Parameters

None of the egg quality parameters (Table 1) were affected due to supplementation of either antibiotic or bacteriophage in the present study. Concomitant to the findings of the present study, Kim *et al.* (2015) observed no difference in egg quality parameters by supplementation of bacteriophage mixture (*Salmonella* spp.). Similarly, Zhao *et al.* (2012) also did not observe any difference in egg quality parameters, except Haugh units, which was higher in the bacteriophage-supplemented group compared to the control, since bacteriophage supplementation reduced the *Salmonella* concentration, which reduces the egg quality. Wagenaar *et al.* (2005) found that *Salmonella* found in the oviduct might

Table 1: Effect of bacteriophage (BP) supplementation on egg quality parameters of RIR laying hens at the end of the experiment (36 weeks)

Parameters	Control	Oxytetracycline (25 mg/kg diet)	BP(mL/L of water)			SEM	p-value
			0.2	0.25	0.3		
Egg weight (g)	52.54	52.58	52.60	52.64	52.56	0.044	0.648
Albumen (%)	56.79	56.97	57.04	56.90	57.02	1.042	0.346
Yolk (%)	31.92	31.90	31.88	31.98	31.78	0.642	0.714
Egg shell (%)	11.29	11.13	11.08	11.12	11.20	0.222	0.642
Egg shape index	76.54	76.32	76.48	76.60	76.56	1.02	0.724
Haugh unit	78.84	78.64	78.98	78.88	78.90	2.24	0.654
Egg shell thickness (mm)	0.342	0.344	0.348	0.344	0.346	0.002	0.292



Table 2: Effect of bacteriophage (BP) supplementation on fresh ileal and caecal *E. coli* counts (\log_{10} cfu/g) of RIR laying hens

Parameters	Control	Oxytetracycline (25 mg/kg diet)	BP (mL/L of water)			SEM	p-value
			0.2	0.25	0.3		
Ileum	5.63 ^c	5.37 ^b	5.08 ^a	5.11 ^a	5.03 ^a	0.103	0.014
Caecum	6.92 ^c	6.48 ^b	6.28 ^a	6.16 ^a	6.19 ^a	0.091	0.012

Means with different superscripts in a row differ significantly ($p < 0.05$).

affect the albumen and shell membranes and reduce the quality of the eggs. This indicates that the bacteriophage used in the present study did not influence, but maintained the egg quality.

Gut Microbiota

The highest intestinal (both ileal and caecal) *E. coli* counts were observed in the birds fed control diet without antibiotics and bacteriophage. Dietary supplementation of antibiotics significantly reduced ($p < 0.01$) the *E. coli* counts compared to the control group. Supplementation of bacteriophage at 0.2 mL/L of drinking water further lowered the *E. coli* counts significantly in both organs (Table 2). However, no significant difference was found among three different levels of bacteriophage supplementation (0.2, 0.25 and 0.3 mL/L). The *E. coli* count of the bacteriophage-supplemented group was significantly lower when compared to either the control or antibiotic-supplemented group. Reducing the quantity of harmful bacteria is a viable technique for increasing healthy microbes, possibly as it reduces the competitors for gut binding sites and nutrients. As a result, lowering harmful bacteria such as *E. coli* and *Salmonella* allows beneficial bacteria (such as *Lactobacillus*) to thrive and multiply in a particular population (Sarrami *et al.*, 2022).

Significant reduction in the gut microflora count, *i.e.*, *E. coli* count, in the ileum and caecum observed following supplementation of bacteriophage in the current study concurred well with the report of Noor *et al.* (2020). Increased *Lactobacillus* concentrations and reduced *E. coli* concentrations in the ileum and caecum have also been reported with bacteriophage inclusion in the diet by Sarrami *et al.* (2022). Bacteriophage supplementation might have altered the gut microbiome through competitive exclusion, thus improving the balance between pathogenic and non-pathogenic bacteria, thereby enhancing the uptake of nutrients by the absorptive epithelial cells, improving the production performance. Enhancement in the intestine at the cellular level, such as improved length of villi and villus heights to crypt depth ratio, improves bird efficiency by boosting the breakdown and absorption of nutrients (Sarrami *et al.*, 2022). Unlike antibiotics, bacteriophages probably did not affect the non-pathogenic bacteria in the gut.

In general, bacteriophage supplementation seems to be a potential alternative to antibiotics in maintaining the health and productivity of the laying hens. Thus, the observations of the present study recommend that supplementation of

bacteriophages @ 0.2 to 0.3 mL/L of drinking water could be done in the laying hens as a potent alternative to antibiotics.

ACKNOWLEDGEMENT

The authors are grateful to the ICAR-Central Institute for Women in Agriculture, Bhubaneswar, for providing the necessary facilities and support for carrying out this study. Special thanks to the Department of LPM, College of Veterinary Sciences and Animal Husbandry, OUAT, Bhubaneswar, Odisha, for their technical assistance and guidance throughout the research period.

REFERENCES

- AOAC (1995). *Official Methods of Analysis*. 16th edn., Association of Official Analytical Chemists, Washington DC, USA.
- Bardy, P., Pantůček, R., Benešik, M., & Doškař, J. (2016). Genetically modified bacteriophages in applied microbiology. *Journal of Applied Microbiology*, 121(3), 618-633.
- Bradley, B.D., Jung, T., Tandon Verma, A., Khoury, B., Chan, T.C.Y., & Cheng, Y.L. (2017). Operations research in global health: A scoping review with a focus on the themes of health equity and impact. *Health Research Policy and Systems*, 15(1), 32.
- Castanon, J.I.R. (2007). History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Science*, 86(11), 2466-2471.
- Dy Ron, L., Rigano, L.A., & Fineran, P.C. (2018). Phagebased biocontrol strategies and their application in agriculture and aquaculture. *Biochemical Society Transactions*, 46(6), 1605-1613.
- Jiang, L., Jiang, Y., Liu, W., Zheng, R., & Li, C. (2022). Characterization of the lytic phage flora with a broad host range against multidrugresistant *Escherichia coli* and evaluation of its efficacy against *E. coli* biofilm formation. *Frontiers in Veterinary Science*, 9, 906973.
- Keen, E.C. (2015). A century of phage research: Bacteriophages and the shaping of modern biology. *BioEssays*, 37(1), 6-9.
- Kim, J.H., Kim, J.W., Shin, H.S., Kim, M.C., Lee, J.H., Kim, G.B., & Kil, D.Y. (2015). Effect of dietary supplementation of bacteriophage on performance, egg quality and caecal bacterial populations in laying hens. *British Poultry Science*, 56(1), 132-136.
- Kittler, S., Mengden, R., Korf, I.H.E., Bierbrodt, A., & Wittmann, J. (2020). Impact of bacteriophagesupplemented drinking water on the *E. coli* population in the chicken gut. *Pathogens*, 9(4), 1-21.
- Korf, I.H.E., Kittler, S., Bierbrodt, A., Mengden, R., Rohde, C., Rohde, M., Kroj, A., Lehnher, T., Fruth, A., Flieger, A., Lehnher, H., & Wittmann, J. (2020). *In vitro* evaluation of a phage cocktail controlling infections with *Escherichia coli*. *Viruses*, 12(12), 1470.
- Miller, E.J., Skinner, A., Sulakvelidze, A., Mathis, G.F., & Hofacre, C.L. (2010). Bacteriophage therapy for control of necrotic enteritis

- of broiler chickens experimentally infected with *Clostridium perfringens*. *Avian Diseases*, 54(1), 33-40.
- Noor, M., Runa, N.Y., Husna, A., Rahman, M., Rajib, D.M.M., Mahbubeelahi, A.T.M., & Rahman, M. (2020). Evaluation of the effect of dietary supplementation of bacteriophage on production performance and excreta microflora of commercial broiler and layer chickens in Bangladesh. *MOJ Poultry Biology*, 9(2), 274.
- O'Sullivan, L., Buttimer, C., McAuliffe, O., Bolton, D., & Coffey, A. (2016). Bacteriophage based tools: Recent advances and novel applications. *F1000 Research*, 5, 2782.
- Sarrami, Z., Sedghi, M., Mohammadi, I., Kim, W.K., & Mahdavi, A.H. (2022). Effects of bacteriophage supplement on the growth performance, microbial population, and PGC1 α and TLR4 gene expressions of broiler chickens. *Science Report*, 12, 14391.
- Shasha, S.M., Sharon, N., & Inbar, M. (2004). Bacteriophages as antibacterial agents. *Harefuah*, 143(2), 121-125.
- Snedecor, G.W., & Cochran, W.G. (1994). *Statistical Methods*. 8th edn., Iowa State University Press, Ames, Iowa, USA.
- Wagenaar, M.A., Van Bergen, M.A., Mueller, M.A., Wassenaar, T.M., & Carlton, R.M. (2005). Phage therapy reduces *Campylobacter jejuni* colonization in broilers. *Veterinary Microbiology*, 109(3-4), 275-283.
- Zhao, P.Y., Baek, H.Y., & Kim, I.H. (2012). Effects of bacteriophage supplementation on egg performance, egg quality, excreta microflora, and moisture content in laying hens. *Asian Australas Journal of Animal Science*, 25(7), 1015-1020.

