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Assessing the impact of supplementing bacteriophages to increase laying hen production performance

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Abstract
A study was conducted to evaluate the effect of supplementation of Bacteriophage on the production performance of Rhode Island Red Laying Hens. A total of 120 Rhode Island Red laying hens were randomly divided into 5 treatment groups of 24 each, with three replicates in a completely randomised design. The included treatments were (i) T_1 (Control) without antibiotics and bacteriophages, (ii) T_2 was supplemented with antibiotic (Oxytetracycline@50g/q of feed) and T_3, T_4 and T_5, bacteriophage was supplemented at @0.20, 0.25 and 0.30 and ml/litre of drinking water, of drinking water, respectively. Dietary supplementation of antibiotics (oxytetracycline @ 25mg/kg diet) significantly (*P*<0.05) increased the HHEP (*P*<0.05) compared to the control group. Similarly, supplementation of bacteriophage (BAFACOL) @0.2 ml/L of drinking water to the laying hens significantly (*P*<0.05) increased egg production (*P*<0.05). Dietary supplementation of bacteriophage at all the levels of supplementation (0.2, 0.25 and 0.3ml/L) significantly (*P*<0.05) improved the FCR (g feed/g egg) compared to the control group. The findings of the present study suggested that supplementation of bacteriophage in the drinking water of laying hens can serve as a potent alternative to antibiotics.

Keywords: Poultry, hen housed egg production, feed conversion ratio, antimicrobial resistance

Introduction
India is the second leading country in the world in terms of egg production. The total Egg production in the country is estimated as 142.77 billion nos. during 2023-24 and registered a growth of 6.8% growth over the past 10 years as compared to the estimates of 78.48 billion numbers during 2014-15. Further, the production has increased annually by 3.18% during 2023-24 over 2022-23. The major contribution in the total Egg production comes from Andhra Pradesh with a share of 17.85 % of total Egg production followed by Tamil Nadu (15.64 %), Telangana (12.88%), West Bengal (11.37%) and Karnataka (6.63 %). In terms of AGR, the highest growth rate was recorded by Ladakh (75.88%) and followed by Manipur (33.84%) and Uttar Pradesh (29.88%) (Basic animal husbandry statistics,2024). Due to the higher increase in the human population and increased demand of quality animal protein the intensive rearing of poultry has been rapidly excavating. In the Intensive farming system because of its systematic feeding systems, selective breeding techniques and lack of proper ventilation, animal health is compromised causing unnecessary stress and discomfort. This is one of the major factors responsible for clinical and subclinical infections in the poultry farming.

Researchers are to be encouraged to find the safe alternatives like prebiotics, probiotics, phytogenics and bacteriophages. bacteriophages self-replicate, thus they do not need to be applied frequently. Another essential characteristic is that phages are non-toxic since they are often made up mostly of proteins and nucleic acids (Wernicki *et al.*, 2017) [1]. The increasing usage of bacteriophage therapy is due to the phage's great specificity for a given bacterial species as well as their capability to lyse infected bacteria and mutate resistant bacteria (L, O, Sullivan *et al.*,2016) [13].

The viruses that destroy bacteria are called bacteriophages. The most prevalent and varied living entities in the biosphere contain phages (Díaz-Muñoz *et al.*, 2014) [6]. They may be readily observed in any kind of environment where bacteria can proliferate and multiply themselves (D'Accolti *et al.*,2021) [4]. When it comes to bacterial illnesses, phages are effective (Jiang *et al.*, 2022) [10]. The primary function of phages is to create endolysins, which tear down the peptidoglycan layer in bacterial cell walls and allow the phage genome to enter the host (Fey *et al.*,2010) [8]. According to (Loessner *et al.*, 2005) [14], these endolysins are often effective against all kinds of bacteria. They break down all of the peptidoglycan linkages and produce holes in the bacterial cell wall. The anti-microbial properties of this bacteriophage might be used to combat a variety of bacterial illnesses in plants, animals, people, and marine life (Jamal *et al.*, 2019) [9].

Material and methods

A total of 120 Rhode Island Red laying hens were randomly divided into 5 treatment groups of 24 each, with three replicates in a completely randomized design. The included treatments were (i) T_1 (Control) without antibiotics and bacteriophages, (ii) T_2 was supplemented with antibiotic (Oxytetracycline@50g/q of feed) and T_3, T_4 and T_5, bacteriophage was supplemented at @0.20, 0.25 and 0.30 and ml/litre of drinking water, of drinking water, respectively. Bacteriophage utilised in our experiment comprised 5 Lytic bacteriophages with a concentration of 10^8 plaque-forming units (PFU)/ml against Avian Pathogenic E. coli (APEC). The different feed ingredients like maize, soyabean meal, deoiled rice bran, shell grit, dicalcium phosphate, salt, vitamins and mineral mixture were balanced to get the ME content of 2602 Kcal/kg (calculated) and CP content of 16.01% in all experimental diets. Birds were allowed to have restricted feeding in which 110 grams of feed per bird was measured and given daily for 8 weeks. Feed was provided once a day in the forenoon, and fresh drinking water was provided twice a day.

The weight of birds was noted at the 28th week and the 36th week. Body weight gain was calculated by subtracting the initial weight from the final weight. The housed egg production (HHEP) percentage was calculated by dividing the egg lay per bird by the start number. The Average feed consumption is noted as feed intake (grams)/hen per day. The feed conversion ratio is determined as the feed requirement per unit of body weight gain. Egg weight is calculated by calculating egg mass divided by HHEP**.**

The experimental data recorded were statistically analysed according to Snedecor and Cochran (1994) [17]. Data was subjected to analysis of variance (ANOVA) and Duncan Multiple Range (DMR) Test (Duncan, 1955) to test the difference between treatment means, wherever necessary.

Results: All dietary groups had weight gain throughout the experiment, but supplementation with antibiotics or bacteriophage did not influence this weight gain. The study found that dietary supplementation of antibiotics (oxytetracycline @ 25mg/kg diet) and bacteriophage (BAFACOL) @0.2 ml/L of drinking water significantly increased hen housed egg production (HHEP%) (p<0.05) in laying hens. The control group had the lowest HHEP percentage. However, supplementation of antibiotics (oxytetracycline) and bacteriophage (BAFACOL) increased egg production. The egg production between the antibiotic-supplemented and bacteriophage groups was comparable (0.2, 0.25 and 0.3ml/L) over 8 weeks. The egg mass per bird per day and feed consumed per day were also comparable across the dietary groups. The feed conversion ratio (FCR) increased in the control group, while supplementation of bacteriophage (0.2, 0.25 and 0.3ml/L) significantly(P<0.05) increased it compared to the group supplemented with neither antibiotic nor bacteriophage. The FCR in the antibiotic-supplemented group was intermediate.

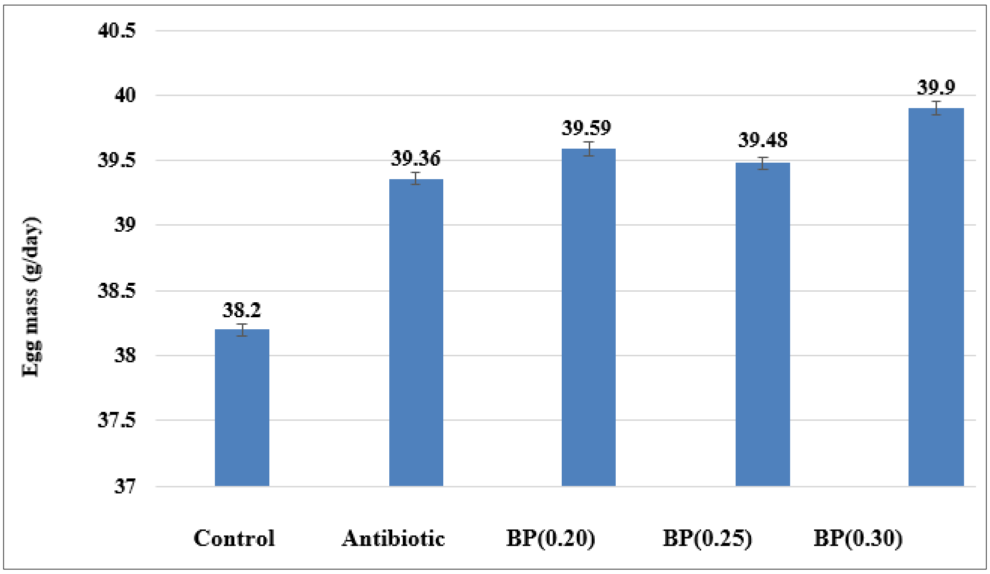

Fig 1: Effect of bacteriophage supplementation on production performance (egg mass) of RIR laying hens

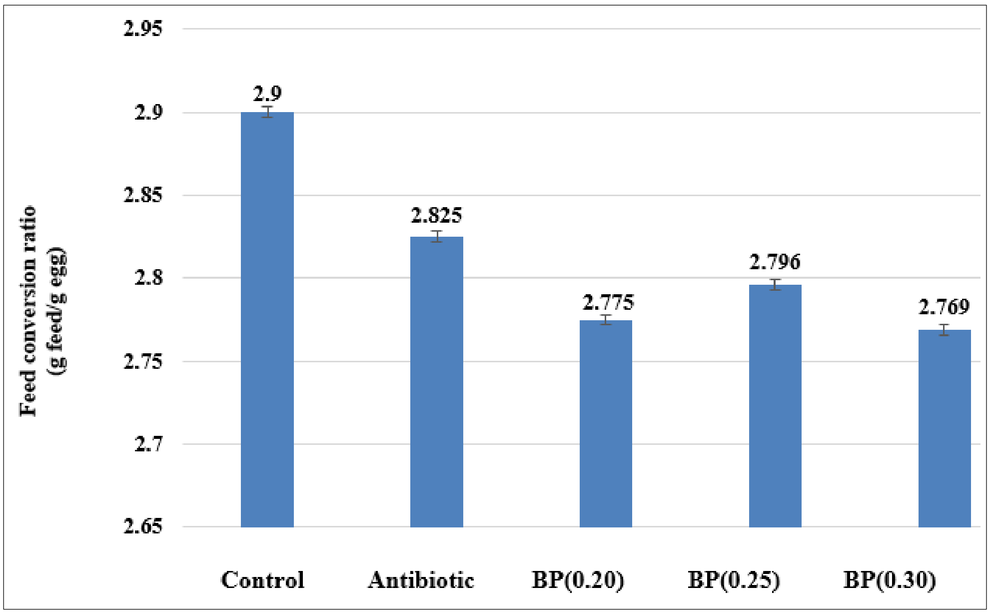

Fig 2: Effect of bacteriophage supplementation on production performance (feed conversion ratio) of RIR laying hens

Discussion

The final body weight and the bodyweight gain during the experimental period of 8 weeks (28-36 weeks) were not influenced by the supplementation of bacteriophage in the present study. Most of the study on bacteriophage usage in poultry is limited to broiler chickens, and higher weight gain has been observed by dietary supplementation of bacteriophage in young broilers (Noor *et al.*, 2020 [15] and Upadhaya *et al.*, 2021) [18]. 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 of nutrients and absorbing them (Zahra Sarrami *et al*., 2022) [16]. The present study was conducted in adult laying hens (28 wk of age), in which the growth rate is negligible. To our knowledge, no information on the literature is available to support the observations of the present experiment on the influence of bacteriophages on body weight changes. But the findings suggested that bacteriophage supplementation does not influence the body weight of laying hens.

Increased Lactobacillus concentrations and reduced *E. coli* concentrations in the ileum and caecum have been reported with bacteriophage inclusion in the diet (Zahra Sarrami *et al*., 2022) [16]. 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. In the present experiment, the hen housed egg production, egg mass (g/day) and feed conversion ratio (g feed/g egg) were influenced due to supplementation of bacteriophage. *Salmonella enteritidis* is very invasive in laying hens, infecting several tissues, including blood, liver, spleen, kidney, ovary/ovules, oviduct, and lung (Atterbury *et al*., 2009; Sieiro *et al.*, 2020) [2, 20].

(Miller *et al.*, 2010) found that *Salmonella enteritidis* may enter the lower oviduct, travel to the infundibulum, and then reach the ovary and other organs via the peritoneal cavity and may induce egg production issues, resulting in reduced egg output. Similar to the findings of the present experiment (Zhao *et al.*, 2012) [19] observed an overall improvement in egg production due to feeding of diets containing bacteriophage mixture targeting *Salmonella* spp. to laying hens during a period of 6-week study. The author suggested that the increase in egg production was due to reduced *Salmonella* concentration in the gastrointestinal tract due to dietary supplementation of a bacteriophage mixture. In another study conducted in layers, (Noor *et al.*, 2020) [15] observed a significant increase in egg production due to bacteriophage supplementation. In contrast to the observations of the current experiment (Kim *et al.*, 2015) [12] did not report any significant difference in laying performance (hen day egg production, egg weight, egg mass, feed intake and feed conversion ratio) by dietary supplementation of bacteriophage cocktail against *Salmonella* spp. and *Staphylococcus aureus*. There is limited data available on the effects of dietary bacteriophage on laying performance, and the variation could be attributed to the health status, age of laying hens and experimental conditions such as hygiene, dietary components, levels and composition of bacteriophage mixtures (Joerger *et al.*, 2003) [21].

Conclusion

Bacteriophage supplementation in adult laying hens (28-36 weeks of age) did not influence final body weight or bodyweight gain, corroborating previous findings that mature hens exhibit minimal growth response to phage inclusion. Importantly, hens receiving phage-enriched diets showed significant improvements in hen-house egg production, daily egg mass, and feed conversion ratio, indicating enhanced production performance. The supplementation also led to marked reductions in *E. coli* and *Salmonella* levels in excreta, while boosting beneficial microbial populations, thus suggesting targeted pathogen control within the gut environment. These microbial shifts likely supported improved intestinal morphology and nutrient absorption, which underlie observed gains in feed efficiency and egg output. While bacteriophage supplementation does not affect body weight in mature laying hens, it meaningfully enhances egg production performance and feed efficiency through modulation of the gut microbiota and pathogen reduction.

In conclusion, Bacteriophage supplementation seems to be a potential alternative to the antibiotics in maintaining the health and productivity of the laying hens.

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