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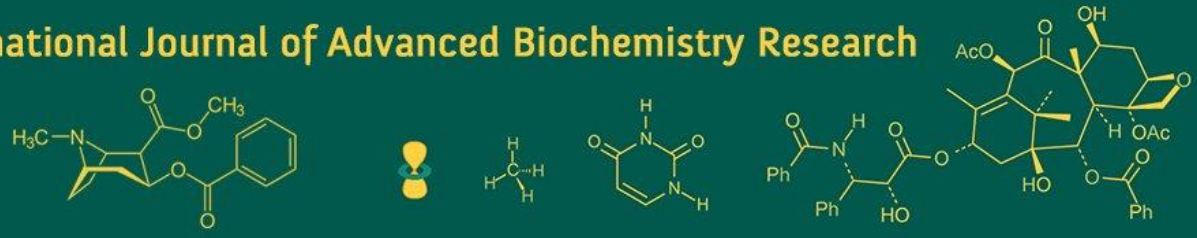

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Evaluating bacteriophage inclusion as a strategy to enhance layer production efficiency

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Abstract
A total of 120 Rhode Island Red laying hens were randomly selected. They were divided into 5 groups of 24 each with three replicates of 8 in each group in a Completely Randomized Design. The included treatments were (i) T_1 (Control group) diet was supplemented without antibiotics and bacteriophages, (ii) T_2 group the diet was supplemented with antibiotic (Oxytetracycline @ 50g/q of feed) and (iii) T_3, T_4 and T_5, bacteriophage was supplemented at different concentrations @ 0.20ml/litre of drinking water, @ 0.25ml/litre of drinking water, @ 0.30ml/litre of drinking water, respectively. Among the serum biochemical parameters in laying hens, concentrations of albumin, globulin, total cholesterol, triglycerides, phosphorus, and only total protein and calcium were significantly affected by bacteriophage supplementation. These shifts in biochemical markers suggest that bacteriophages influence key aspects of metabolism and nutrient balance. This study's findings indicate that adding bacteriophage to the diet of laying hens measurably modulates their serum profile. These outcomes support the conclusion that bacteriophage inclusion could serve as a viable alternative to antibiotic use. Overall, the results suggest that bacteriophage-enriched diets can positively influence both biochemical health and production performance in laying hens.

Keywords: Poultry, bacteriophage, antibiotics, antimicrobial resistance and serum biochemical profile

Introduction
The country's total egg production is projected to reach 142.77 billion eggs in 2023-2024, representing a 6.8% increase over the previous decade compared to predictions of 78.48 billion eggs in 2014-2015. Additionally, compared to 2022-2023-2024, the production grew by 3.18% yearly. Since more than 60% of infections are zoonotic across the food chain and animal diseases can result in 20% production losses, antimicrobials support public food animal health and welfare worldwide (Kasimanickam *et al.*, 2021) [8].

In India, chicken has a 69.7% ampicillin resistance rate, compared to 16.5% for Salmonella and *E. coli*. 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 *et al.*, 2018) [11]. 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 would bring together the several 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) [7].

Bacteriophages, commonly known as phage's, are tiny viruses that range in size from 20 to 200 nm (Fey *et al.*, 2010) [16]. They are thought to be safe for humans and mostly for all eukaryotic cells since they may specifically infect prokaryotic bacterial cells (L O'Sullivan *et al.*, 2016) [15]. According to (Jones *et al.*, 2013) [10], most phages are extremely selective and can only infect a small number of closely related bacteria. 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) [6]. As a result, they could be a more ecologically friendly way to manage and eradicate harmful bacteria than antibiotics (Keen *et al.*, 2015) [9].

Materials and Methods

For this experiment, a commercially available combination of five lytic bacteriophages with a concentration of 10^8 PFU/ml was employed. The biochemistry autoanalyzer is used to analyze the serum biochemical parameters listed below. respective procedures were given in all of the corresponding reagent kits provided by a division of Coral Clinical Systems, Crest Biosystems located at Goa, India. The total protein concentration (g/dl) was analysed by the Biuret method. Serum albumin (g/dl) by BCG (Bromo-Cresol Green), Serum globulin by substracting the serum albumin value from the total protein value and is denoted as g/dl, phosphorous concentration (g/dl) was estimated (Molybdate U.V. method), calcium (g/dl)-(O-Cresol phtaleincromogenic (OCPC) method), triglyceride concentration (g/dl)-(GPO-method), cholesterol concentration (g/dl)-(CHOD/ PAP method, uric acid concentration g/dl)-(Modified Berthelot method), creatinine concentration (mg/dl)-(Modified Jaffe's Kinetic method).

In accordance with Snedecor and Cochran (1994) [2], the experiment's results were statistically examined. When appropriate, the data were run through the Duncan Multiple Range (DMR) Test (Duncan, 1955) [1] and analysis of variance (ANOVA) to see if there was a difference between the means of the treatments.

Results

Out of all serum biochemical parameters total protein and calcium (Ca) concentrations in serum were influenced by the supplementation of bacteriophage. The serum concentrations of protein and Ca were significantly increased in every bacteriophage supplemented groups as compared to either control or antibiotic supplemented group. However, no difference in these above parameters could be noticed due to the level of supplementation of bacteriophage (0.20 to 0.30 ml/L of drinking water). The concentrations of other serum biochemical parameters albumen, globulin, uric acid, phosphorus, triglyceride and cholesterol were not influenced due to either supplementation of bacteriophage or supplementation of antibiotic.

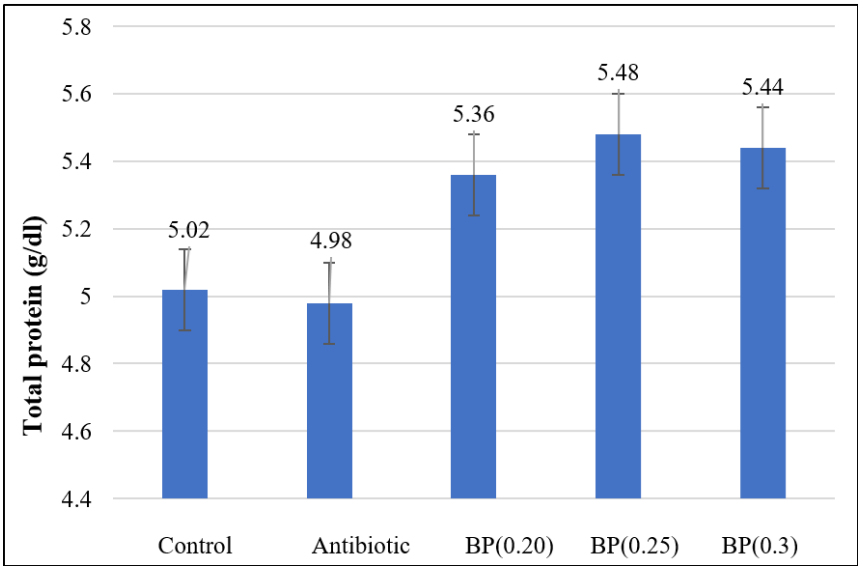

Fig 1: Effect of bacteriophage supplementation on serum biochemical (total protein) parameters of RIR laying hens

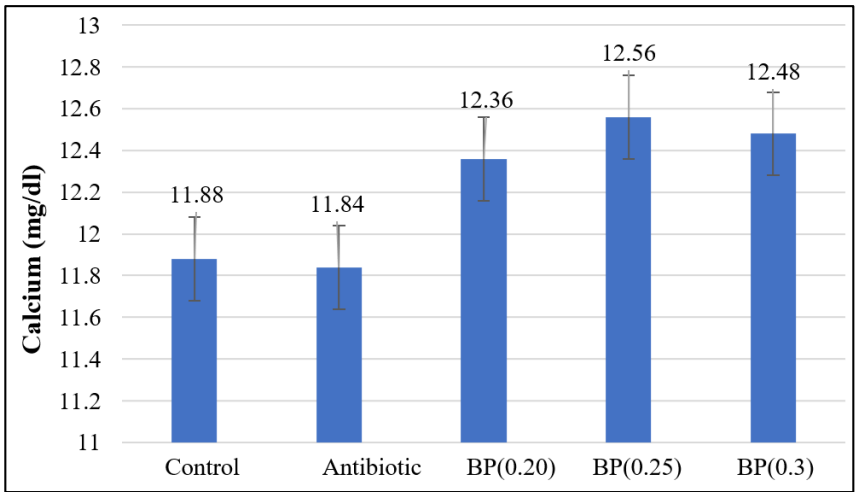

Fig 2: Effect of bacteriophage supplementation on serum biochemical parameters (Calcium) of RIR laying hens

Discussion

In the present study, the total protein concentration in serum was influenced due to the supplementation of bacteriophage. The total protein of serum indicates the measure of two major proteins like albumin and globulin (Eisen *et al.*, 1962) [5]. According to (Melillo *et al.*, 2013) [3] most of the plasma proteins in blood circulation are synthesized by the liver. In a study conducted by (Wang *et al.*, 2013) [17],

supplementation of bacteriophage increased the relative weight of the liver when compared to body weight. Therefore, it is hypothesised that supplementation of bacteriophage improved the absorption of nutrients from the gut, thereby enhancing the total protein levels in the serum. Supplementation of bacteriophages improved the serum calcium levels of laying hens (Castonon *et al.*, 2007) [13]. This might be due to the relationship between serum calcium and total protein. According to (Yu & Sharma *et al.*, 2021) [4], 40% of calcium is comprised of protein-bound calcium which is not utilized by the internal tissues (Vaarst *et al.*, 2019) [12]. The result of the present study supports the hypothesis, as the supplementation due to bacteriophage led to increased total protein and calcium levels in serum. (Wang *et al.*, 2013) [17] reported that dietary antibiotic and bacteriophage supplementation did not affect blood biochemical profiles like RBC, WBC, and Lymphocyte percentage. However, in the present study, the above parameters were not evaluated but serum concentrations of triglycerides, cholesterol, phosphorus, uric acid and creatinine were not influenced by the bacteriophage supplementation in laying hens.

Conclusion

The current investigation found that adding bacteriophage to layers diets significantly altered their serum biochemical profile. In particular, birds receiving phage supplementation exhibited notable increase in total protein and serum calcium levels compared to the control flock. These biochemical shifts suggest enhanced protein metabolism and improved mineral homeostasis in the birds. Given the essential roles of high serum protein and calcium in egg formation and overall health, these findings are especially relevant for productivity. Consequently, the study supports the practical use of bacteriophage supplementation in layer feed. By producing such physiological benefits, bacteriophages present a strong, antibiotic-free strategy to support both bird well-being and performance.

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