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Effect of Bacteriophage Cocktail on Growth Performance, Diarrhea Score, and Gut Bacteria of Broilers in Comparison with *Bacillus subtilis*

Research Article

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ABSTRACT

This study investigated the efficacy of a bacteriophage cocktail on growth parameters, diarrhea score, and ileal and cloacal bacterial counts of broilers, compared to *Bacillus subtilis*. A total of 4131 mixed-sex broiler day old chicks (DOCs) were procured from a commercial company and reared for 42 days in cages within a controlled broiler shed. Birds were assigned to three treatment groups: T1 (supplemented with *B. subtilis* at 200 g/kg of feed), T2 (bacteriophage added to basal feed at 300 g/kg of feed), and T3 (feed supplemented with bacteriophage at 500 g/ton of feed). Each treatment was replicated into 81 cages, with 17 broilers per cage. Feed conversion ratio (FCR) was lowest, and the survival rate and European production efficiency factor (EPEF) were highest in the T3 group, with T2 showing intermediate performance. Bacteriophage-supplemented groups exhibited lower diarrhea scores, as feces appeared normal. *Escherichia coli* and *Salmonella* counts were lowest in the T3 group throughout the experiment, followed by T2, while these groups had higher counts of *Lactobacillus and Bifidobacterium.* The T3 group demonstrated superior overall performance in comparison with the other two experimental groups. In essence, supplementing a bacteriophage cocktail at a rate of 500 g/ton of feed could serve as an effective feed additive for enhancing growth performance, overall welfare, and modulation of gut bacteria by increasing beneficial bacteria and reducing targeted pathogens.

KEY WORDS *Bacillus subtilis*, bacteriophage cocktail, broiler, growth performance, health condition.

INTRODUCTION

The poultry industry is the top contributor of efficient, high-quality animal proteins globally. Fresh eggs and meat from poultry are advantageous options compared to other animal-based dietary sources, as these nutritionally superior products have no cultural or religious restrictions (Korver, 2023). Despite significant advancements in recent decades, the poultry industry is facing challenges following the ban on antibiotic growth promoters (AGPs), as the industry had heavily relied on them (Abreu *et al.* 2023). In the 1950s, AGPs significantly improved poultry production and were used extensively in poultry feed from then on. However, concerns such as the development of antimicrobial resistance (AMR) and the potential transfer of antibiotic resistance microbiome from animals to the human led to a ban on AGPs (Castanon, 2007). For this reason, finding alternatives to AGPs is mandatory for poultry producers. Lytic bacteriophages can be a great next-generation substitute for combating antibiotic-resistant microbiota and also serve as a growth promoter (Upadhaya *et al.* 2021).

"Bacteriophages, or phages", which can be referred to as 'bacteria eaters', are a group of viruses that infect and multiply within target bacteria or archaea through either a lytic or lysogenic cycle (Abd-El Wahab *et al*. 2023). With a vast community of about 4.8 × 10^{31} phage particles, they are abundant in every sphere on Earth, colonizing water, soil, humans, plants, and the rest of the biosphere (Gómez-Gómez *et al*. 2019). This suggests a population 10 times larger than that of bacteria make them powerful candidates against bacterial infection.

Specifically, lytic bacteriophages selectively kill bacteria by lysis in a species-specific manner and replicate their own progeny without causing any genetic mutations of bacteria or harm to the commensal microbiota (Abbas *et al*. 2022). The U.S. FDA approved bacteriophages as a Generally Recognized as Safe (GRAS) product due to their non-toxic nature (Gindin *et al*. 2019). Research has reported that supplementation with bacteriophages enhances growth performance by increasing body weight gain, improving feed efficiency, and positively modulating gut microbiota by killing targeted pathogens and promoting beneficial commensal bacteria (Upadhaya *et al*. 2021; Sarrami *et al*. 2022).

Among the other approaches for dietary supplements, probiotics, prebiotics, and synbiotics are the most commonly used in broilers (Habteweld and Asfaw, 2023). In the case of probiotics, *Bacillus subtilis* is the most widely used and proven species. Over the last decade, research has shown that *B. subtilis* not only enhances growth but also prevents disease and improves gut health by boosting mucosal immunity, strengthening intestinal barrier integrity, increasing digestive enzyme activity, and optimizing nutrient absorption through gut microbiota modulation (Ningsih *et al*. 2023). Further research on *B. subtilis* has indicated it to be a more effective natural growth promoter than synthetic antibiotics (Jayaraman *et al*. 2017; Liu *et al*. 2020; Ramlucken *et al*. 2020; Qiu *et al*. 2021).

The precise ability of 'Bacteriophages' to combat specific bacterial challenges without disrupting commensal bacterial colonies makes them a potential candidate as feed additives (Huff *et al*. 2003; Miller *et al*. 2010; Mosimann *et al*. 2021).

However, research on their dietary usage in chickens without bacterial challenges remains insufficient to demonstrate their effectiveness (Upadhaya *et al*. 2021). Since phages are strain- or species-specific, a cocktail of phages can be more effective as dietary use. We designed the current study to demonstrate the effects of bacteriophages, administered at two different doses, as feed additives on growth parameters, diarrhea scores, and gut bacteria in broilers reared under normal physiological conditions (without inducing bacterial challenges). The effects were compared with birds that were given *B. subtilis* as a supplement, as it is an effective natural substitute for AGPs.

MATERIALS AND METHODS

Ethical statement and approval

A prior approval was granted by the Animal Protocol Review Committee of Linyi University (LU202403, Lanshan District, Linyi City, China) for this experiment. The birds were handled and reared with intensive care, strictly adhering to the guidelines established by the Animal Care and Use Committee of Linyi University.

Bacteriophages solution

In this study, the ProBe-Bac PE® bacteriophage solution was used. ProBe-Bac PE ® is a bacteriophage cocktail composed of *Salmonella typhimurium* bacteriophage, *S. gallinarum* bacteriophage, *S. enteritidis* bacteriophage, *S. pullorum* bacteriophage, *Escherichia coli K88* (ETEC=Entero-toxigenic *E. coli*) bacteriophage, *E. coli O78* (APEC=Avian Pathogenic *E. coli*) bacteriophage*, and Clostridium perfringens* bacteriophage. It was co-developed by Optipharm, a company affiliated with Pathway Intermediates.

Broilers, study design, and diets

A total of 4131 day-old broilers with an average initial weight of 42 ± 2 g were procured from Boone Farm & Livestock Group, a commercial company, and reared for 42 days. The birds were randomly assigned according to their weights to three treatments, namely: Treatment 1 (T1), where birds were fed a basal diet supplemented with 200 g/ton of *Bacillus subtilis*; Treatment 2 (T2), where birds were fed 300 g/ton of bacteriophage with the basal diet; and Treatment 3 (T3), where birds were given the basal diet including 500 g/ton of bacteriophage. Each treatment group was further replicated in 81 cages, with each cage housing 17 broilers. A basal diet based on corn-soybean-canola meal was formulated to fulfill or surpass the nutrient requirements for broilers as set by the NRC (1994) and was applied in a three-phase feeding approach. Starter feed was provided from days 1–14, followed by grower feed from days 15–33, and finisher feed from days 34–42 (Table 1). Throughout the experimental period, the broilers were provided with an adequate amount of feed and clean drinking water.

Study site and management

Broiler chickens were housed at Backbone Company and managed using an all-in, all-out production system. A temperature-controlled shed equipped with three floors of stainless-steel cages, each identical in size (1.8 m×1.8 m), was used for the experiment. For the first three days, the temperature of the room was maintained at 33 ± 1 °C.

Table 1 Ingredient composition of experimental diets as-fed basis

Ingredients (%)	Starter	Grower	Finisher
Corn	54.05	55.11	56.51
Soybean meal	33.98	26.45	18.57
Canola meal	5	10	15
Soybean oil	2.14	3.71	5.15
DCP	1.7	-	-
MDCP	-	1.28	1.11
Limestone	1.15	1.33	1.22
L-lysine	0.43	0.51	0.67
DL-methionine	0.41	0.46	0.51
L-threonine	0.19	0.24	0.32
L-tryptophan	-	0.01	0.04
$NaHCO_3$	0.1	0.1	0.1
Salt	0.3	0.3	0.3
Choline	0.1	0.1	0.1
Vitamin premix[1]	0.2	0.2	0.2
Mineral premix[2]	0.2	0.2	0.2
Total	100	100	100
Analyzed composition			
ME (kcal/kg)	3000	3100	3200
CP %	23	21.5	20
Lys %	1.45	1.3	1.2
Met + Cys %	1.08	0.99	0.94
AP %	0.48	0.44	0.41
Ca %	0.96	0.87	0.81

[1] Provided per kg of complete diet: vitamin A: 11025 IU; vitamin D3: 1103 IU; vitamin E: 44 IU; vitamin K: 4.4 mg; Riboflavin: 8.3 mg; Niacin: 50 mg; Thiamine: 4 mg; D-pantothenic: 29 mg; Choline: 166 mg and vitamin B12: 33 µg.
[2] Provided per kg of complete diet: Cu (as $CuSO_4.5H_2O$): 12 mg; Zn (as $ZnSO_4$): 85 mg; Mn (as MnO_2): 8 mg; I (as KI): 0.28 mg and Se (as $Na_2SeO_3.5H_2O$): 0.15 mg.
MDCP: mono-dicalcium phosphate; DCP: dicalcium phosphate; ME: metabolizable energy; CP: crude protein and AP: available phosphorus.

After that, it was gradually lowered by 3 °C per week to 24 °C until the end of the experiment. Humidity was maintained at about 60% throughout the experimental period. The birds were provided with artificial light for 24 hours a day using fluorescent lights. The room was cleaned once a week and disinfected regularly.

Growth parameters measurement

Growth parameters data of the broilers were collected from 17 broilers per cage in each treatment. Growth parameters data included body weight, average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), rate of survival, and the European index. The equations for measuring growth performance are as follows:

ADG= (total weight gain measured per replicate in given number of days; g) / (total number of broilers × total number of days)

ADFI= (feed offered to the broilers per replicate; g) – (residual feed on the feeder; g+feed waste; g) / (total number of broilers per replicate×total number of days)

FCR= ADFI (Avg. daily feed intake) / ADG (Avg. daily gain)

Rate of survival= (number of broilers surviving/total number of broilers at the start of experiment) × 100

European production efficiency factor (EPEF)= (rate of survival×body weight/FCR×marketing age) × 100

Sampling and chemistry analysis

Rectal feces were collected completely randomly from 5 birds per 27 cages per treatment at 8:00 a.m. on days 14, 28, and 42 and scored for diarrhea. The degree of diarrhea, feces appearance and diarrhea score were assessed according to Table 2.

5 broilers per cage in each treatment were randomly selected to collect feces. The quantity of *Salmonella*, *E. coli lactobacillus* and *Bifidobacterium* in feces was measured by the method of marking plates at 14d, 28d and 42d.

At the end of experiment, 5 broilers per cage in each treatment were randomly selected to collect intestinal chyme from ileum, after chicks are euthanized under the institutional animal care guidelines. The quantity of *Salmonella*, *E. coli lactobacillus* and *Bifidobacterium* in chyme was measured by the method of marking plates.

Statistical method

This study was structured using an randomized complete block design (RCBD) model, with each cage serving as the experimental unit. The data were collected in an Excel spreadsheet (Microsoft, 2013) and then analyzed using the

General Linear Model (GLM) procedure in SAS (SAS, 1996). Linear and quadratic polynomial contrasts were calculated to evaluate the trends in how bacteriophage supplementation affected the experimental outcomes. Variability in the data is presented as the standard error of the mean (SEM). A significance level of P < 0.05 was used to determine statistical significance.

RESULTS AND DISCUSSION

Data related to the growth parameters of broilers, including body weight, average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), survival rate, and EPEF, are presented in Table 3.

In this study, a significantly lower (P<0.05) FCR was found in the T3 group on days 15–28 compared to T1 and T2. On days 29-42 and 1-42 (overall experimental period), T3 had a significantly lower (P<0.05) FCR than T1, with T2 being intermediate. The birds in the T3 group had a significantly higher (P<0.05) rate of survival over the entire experimental period (1–42 days) compared to T1, with T2 being intermediate. The EPEF of the experimental broilers in the bacteriophage-fed groups (T3 and T2) was significantly higher (P<0.05) than that of T1, with T3 being the highest. T3 showed an improved EPEF by 6.89% in comparison with the T1 group. However, feeding bacteriophage did not cause any significant changes in ADG, ADFI, or body weight of broilers among the groups throughout the experimental period.

Table 4 shows data about the rectal feces score of experimental broilers at three different stages. At 28 and 42 days of age, diarrhea scores were significantly different (P<0.05) among the groups, with T3 and T2 showing lower scores than T1. The linear trends were significant (P<0.05), indicating an increasing score from T3 to T1 (T3<T2< T1).

The bacterial counts of four species, namely, *Lactobacillus*, *Bifidobacterium*, *E. coli*, and *Salmonella*, from rectal swabs of experimental broilers at three different stages of age (14, 28, and 42 days) are shown in Table 5.

On day 14, the *Lactobacillus* and *Bifidobacterium* counts were significantly higher (P<0.05) in the bacteriophage-fed groups (T2 and T3) compared to the probiotic-supplemented group (T1). Among the groups, the counts were highest in T3. On the other hand, the *Salmonella* count on days 14, 28, and 42 was significantly lower (P<0.05) in the bacteriophage-supplemented groups than in T1 (T3<T2<T1). Similarly, the *E. coli* count on days 28 and 42 was significantly lower (P<0.05) in T3 compared to T1, while T2 showed an intermediate count. However, the differences in *E. coli* counts among the groups on day 14 were not significant.

The ileal bacterial counts of four species of experimental broiler chickens at 42 days of age are presented in Table 6. The *E. coli* count was significantly lower (P<0.05) in the T3 group than in T1, while T2 showed an intermediate result. In the case of *Salmonella*, the bacteriophage-treated groups (T2 and T3) showed significantly lower (P<0.05) bacterial counts than T1. A significant linear trend (P=0.01) indicated an increasing pattern from T3 to T1 (T3<T2< T1).

Bacteriophages hold potential as a superior substitute for antibiotics in combating multidrug-resistant bacterial species, as antimicrobial resistance (AMR) is the greatest menace to both animal and human health (Ling *et al.* 2022; Asghar *et al.* 2024). According to the EU report on AMR in zoonotic and indicator bacteria isolated from humans, animals, and food (2021–2022), two of the most common zoonotic bacterial species found in food-producing animals are *Salmonella* spp. and *E. coli* (EFSA and ECDC, 2024), while *Clostridium perfringes* associated with necrotizing enteritis in poultry and foodborne infections in humans (Tian *et al.* 2023). Scientific investigations on using a bacteriophage cocktail in broilers suggest that it could be an effective feed additive to counteract salmonellosis, pathogenic *E. coli*, *C. perfringens*, and other pathogens, reducing the impact of infections in birds and ultimately resulting in better growth and production (Wernicki *et al.* 2017; Upadhaya *et al.* 2021).

This study explored the effects of a commercial bacteriophage cocktail solution on growth parameters, diarrhea score, rectal bacterial count, and ileal bacterial count in broilers to assess its potential as a growth promoter. The bacteriophage solution was designed to target seven specific pathogen strains, namely *Salmonella typhimurium*, *S. gallinarum*, *S. enteritidis*, *S. pullorum*, *E. coli* K88 (ETEC), *E. coli* O78 (APEC), and *Clostridium perfringens* under normal physiological conditions of broilers (without inducing bacterial infection).

The improved outcomes of the growth parameters revealed that the overall growth performance was best in the T3 treatment group, which was supplemented with 500 g of BP solution per ton of the basal diet. Supplementation with BP significantly improved the FCR and EPEF of the experimental broilers. These improvements align with the findings of Sarrami *et al.* (2022) who also found that adding a BP cocktail at rates of 0.5g, 1g, and 1.5g/kg feed improved feed utilization by reducing the FCR and increasing the EPEF compared to control and antibiotic (colistin)-fed broilers. A different study conducted on pigs by Kim *et al.* (2014) observed findings parallel to our study, showing that supplementation with a BP cocktail can result in better FCR and overall growth performance compared to supplementation with *B. subtilis* and other probiotics.

Table 2 Diarrhea score according to feces appearance

The degree of diarrhea	Feces appearance	Diarrhea score
Almost normal	Hard bar or granulated	1
Normal	Normal, soft, formed	2
Mild diarrhea	Loose, un-formed	3
Moderate diarrhea	Semi-liquid, not separated feces and water	4
Severe diarrhea	Separated feces and water	5

Table 3 Effects of bacteriophage feeding on the growth parameters of broilers*

Growth parameters	T1	T2	T3	SEM	Linear	Quadratic
Body weights						
14 day (g/bird)	239.52	240.61	241.51	0.89	0.32	0.78
28 day (g/bird)	746.32	748.60	754.09	5.22	0.57	0.12
42 day (g/bird)	2610.74	2611.60	2613.33	2.94	0.82	0.71
Average daily gain (g/bird/day)						
1-14 day	14.11	14.19	14.25	0.06	0.31	0.97
15-28 day	36.20	36.28	36.61	0.39	0.73	0.27
29-42 day	133.17	133.07	132.80	0.49	0.86	0.20
1-42 day	61.16	61.18	61.22	0.07	0.53	0.71
Average daily feed intake (g/bird/day)						
1-14 day	16.67	16.39	16.07	0.10	0.23	0.57
15-28 day	54.63	54.21	48.09	1.48	0.06	0.86
29-42 day	226.26	219.54	219.71	1.94	0.08	0.26
1-42 day	99.19	96.41	94.62	0.82	0.19	0.38
Feed conversion ratio						
1-14 day	1.18	1.16	1.13	0.01	0.08	0.82
15-28 day	1.51[a]	1.49[a]	1.32[b]	0.03	0.01	0.02
29-42 day	1.69[a]	1.65[ab]	1.65[b]	0.02	0.01	0.22
1-42 day	1.62[a]	1.58[ab]	1.54[b]	0.01	0.04	0.38
Rate of survival						
1-42 day	96.52[b]	97.28[ab]	98.26[a]	0.41	0.03	0.18
European production efficiency						
1-42 day	370.26[c]	382.89[b]	395.78[a]	3.4	0.01	0.24

* The data represent the mean value of 17 broilers/cage per treatment.
T1: basal diet + 200 g/ton *B. subtilis*; T2: basal diet + 300 g/ ton BP; T3: Basal diet + 500 g/ ton Bp.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of bacteriophage on diarrhea score of broilers*

Age	T1	T2	T3	SEM	Linear	Quadratic
14 days	2.79	2.43	2.12	0.09	0.16	0.67
28 days	3.23[a]	2.83[b]	2.56[c]	0.07	0.05	0.91
42 days	3.27[a]	2.85[b]	2.26[c]	0.07	0.04	0.29

* The data represent the mean value of 5 broilers/cage per treatment.
Diarrhea score: A 5-point scoring system ranging from 1 (almost normal) to 5 (severe diarrhea), as presented in Table 2.
T1: basal diet + 200 g/ton *B. subtilis*; T2: basal diet + 300 g/ ton BP; T3: Basal diet + 500 g/ ton Bp.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The better FCR may result from a decrease in pathogens such as *E. coli* and *Salmonella* spp., which enhances intestinal morphology by increasing villus height and improving the height to crypt depth ratio of villus, leading to better digestion and absorption of the diet (Sarrami *et al.* 2023). A better EPEF value and higher survival rate in the bacteriophage supplemented group also indicates better economic efficiency, viability and welfare conditions for broilers (Curea *et al.* 2023). Final body weight, average daily gain, and feed intake did not differ significantly among BP-supplemented and probiotics-supplemented groups, suggesting that BP had no detrimental effect on broiler performance. The BP-supplemented groups showed a lower incidence of diarrhea, as indicated by the diarrhea score, which suggested approximately normal feces, whereas the group given *B. subtilis* exhibited a mild level of diarrhea.

Table 5 Effects of bacteriophage on rectal bacteria of broilers*

Age	Species	T1	T2	T3	SEM	Linear	Quadratic
14 days	*Lactobacillus*	8.42[c]	9.31[b]	9.73[a]	0.09	0.02	0.09
	Bifidobacterium	8.43[c]	9.34[b]	9.6[a]	0.09	0.12	0.05
	E. coli	8.60	7.55	6.3	0.24	0.07	0.18
	Salmonella	6.93[a]	5.81[b]	4.05[c]	0.27	0.01	0.34
28 days	*Lactobacillus*	8.84	9.41	9.91	0.08	0.06	0.12
	Bifidobacterium	9.05	9.48	9.79	0.05	0.23	0.46
	E. coli	7.92[a]	7.30[ab]	6.59[b]	0.13	0.01	0.69
	Salmonella	6.84[a]	5.79[b]	4.29[c]	0.14	0.01	0.22
42 days	*Lactobacillus*	8.72	9.38	9.85	0.09	0.53	0.45
	Bifidobacterium	8.84	9.46	9.82	0.08	0.51	0.21
	E. coli	8.47[a]	7.27[ab]	6.99[b]	0.24	0.01	0.14
	Salmonella	6.72[a]	5.62[b]	4.63[c]	0.27	0.01	0.89

* The data represent the mean value of 5 broilers/cage per treatment.
T1: basal diet + 200 g/ton *B. subtilis*; T2: basal diet + 300 g/ ton BP; T3: Basal diet + 500 g/ ton Bp.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effects of bacteriophage on ileal bacterial counts of broilers*

Age	Species	T1	T2	T3	SEM	Linear	Quadratic
42 days	*Lactobacillus*	8.63	9.35	9.85	0.09	0.18	0.35
	Bifidobacterium	8.64	9.30	9.74	0.08	0.61	0.39
	E. coli	8.40[a]	7.20[ab]	6.62[b]	0.28	0.01	0.36
	Salmonella	6.53[a]	5.71[b]	4.82[c]	0.24	0.01	0.90

* The data represent the mean value of 5 broilers/cage per treatment.
T1: basal diet + 200 g/ton *B. subtilis*; T2: basal diet + 300 g/ ton BP; T3: Basal diet + 500 g/ ton Bp.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Among these, the group fed a diet containing 500 g/ton of BP recorded the lowest diarrhea score. The reduction in the diarrhea score may be attributed to a decrease in the number of diarrhea-causing bacteria in the intestine, such as *E. coli* and *Salmonella* spp. (Hashem *et al*. 2022; Tariq *et al*. 2022). Those pathogenic bacteria can impair the digestive system by releasing proteolytic enzymes, which in turn deteriorate the enzymes associated with digestion or degrade the digestive system by damaging the villi of the intestine (Sarrami *et al*. 2022). Due to the lytic or inhibitory effect of bacteriophages (Fang *et al*. 2024), the number of pathogens decreased, ultimately leading to a lower incidence of diarrhea.

Gut bacteria play a major role in poultry by means of nutrient digestion, producing essential metabolic substrates (SCFAs, vitamins, amino acids), neurotransmitters, and influencing the immune system (Ducatelle *et al*. 2023). The manipulation of gastrointestinal microflora using bacteriophages as a feed additive to target pathogenic bacteria is a promising approach to enhance poultry production performance (Upadhaya *et al*. 2021). Unlike antibiotics, bacteriophages precisely infect only targeted strains, without leading to the development of resistant bacteria, and neither degrade commensal beneficial bacteria nor affect eukaryotic cells (Pelyuntha *et al*. 2022).

In the current study, rectal swabs revealed that bacteriophage cocktail supplementation significantly reduced pathogenic bacteria *(E. coli* and *Salmonella*) throughout the experiment, while significantly increasing beneficial bacteria (*Lactobacillus* and *Bifidobacterium*) at 14 days. However, during the remainder of the experiment, there was an increase in the beneficial bacteria count in the bacteriophage-supplemented groups, although the trend was not significant. Similarly, ileal swabs at the end of the experiment exhibited a parallel trend, with a significantly lower pathogen count and a non-significant increase in the beneficial bacteria count. Research findings by Wang *et al*. (2013) also support our results, as they observed that a diet supplemented with a BP cocktail at two levels (0.5 g/kg and 0.25 g/kg) significantly reduced *E. coli* and *Salmonella* counts compared to the control, while *Lactobacillus* counts increased in the BP-supplemented group in comparison with both the control and antibiotic-fed groups. This phenomenon can be ascribed to the selective pressure exerted by lytic bacteriophages on the targeted host bacterial strains, leaving the commensal beneficial bacteria unharmed and thereby modulating the bacterial niche in the broiler gut (Upadhaya *et al*. 2021). Previous research on bacteriophages has also shown a significant reduction in the colonization of harmful bacteria like *E. coli* and *Salmonella* in the gut of broilers, through their lytic cycle, where they directly infect and break apart the bacteria, leading to their death (Noor *et al*. 2020; Pelyuntha *et al*. 2022; Aljuhaishi and Albawi, 2024). This reduction, in turn, helps increase the colonization of beneficial bacteria as there was less competition for resources from pathogens in the gut (Pickard *et al*. 2017). Thus, balancing the bacterial population in the broiler gut by decreasing the pathogen load and increasing beneficial bacteria is advantageous for broiler growth (Sarrami *et al*. 2022). A decrease in pathogens indicates reduced vulnerability to diseases and improved resistance to stress, whereas increasing beneficial bacteria is believed to exert a positive impact on broiler health. Beneficial bacteria enhance the production of short-chain fatty acids (SCFAs), which serve as an excellent energy source for epithelial cells and lower the gut pH, thereby preventing the growth and proliferation of acid-sensitive pathogens, such as those from the *Enterobacteriaceae* family (Clavijo and Flórez, 2018). Increased SCFA production also positively influences gut morphology, ultimately leading to enhanced feed utilization by broilers (Sarrami *et al*. 2022). Furthermore, beneficial bacteria prevent the colonization of pathogenic bacteria by competing with them for nutrient sources and binding sites in the gut epithelium (Pickard *et al*. 2017). Additionally, beneficial bacteria enhance the immune response by increasing mucin production (Sarrami *et al*. 2022).

CONCLUSION

Bacteriophages demonstrate a higher safety and success rate compared to antibiotics, as they selectively target specific bacterial species, strains, or serotypes without harming commensal bacteria in the gut. In essence, our research findings suggest that a bacteriophage cocktail could serve as a promising feed additive, outperforming probiotics (*B. subtilis*). It effectively reduces targeted harmful bacterial strains while preserving commensal bacteria and increasing beneficial bacteria in the gut. This modulation results in a better feed conversion ratio, an increased survival rate, reduced occurrences of diarrhea, and improved growth parameters, leading to better production performance. Among the experimental groups, the T3 group showed the best results, with bacteriophage supplementation at 500 mg/ton of feed. Nevertheless, further research is necessary to explore synergistic effects when bacteriophages are combined with other natural additives, such as prebiotics, probiotics, and essential oils, as well as to assess their efficacy compared to different growth promoters. Additionally, the use of bacteriophages in broiler production across various modern production systems should be thoroughly evaluated to validate its superiority.

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