Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2022 / v.24 / n.3 / 001-008

http://dx.doi.org/10.1590/1806-9061-2021-1561

Original Article

The Efficiency of Bacteriophages Against Salmonella Typhimurium Infection in Native Noi Broilers

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■Keywords

Growth, indigenous chickens, mortality, phage therapy, *Salmonella*.

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Submitted: 27/October/2021
Approved: 03/February/2022

ABSTRACT

Though recently considered a therapeutic treatment for commercial broilers, little is known about the effects of bacteriophages on native, slow-growing birds. This study evaluated their efficacy against *Salmonella enterica* subsp. *enterica* serovar Typhimurium infected Noi chicken, a native Vietnamese broiler breed. In total, 420 birds were used in a completely randomized design consisting of seven treatments and four replicates of 15 birds. The treatments were NC (negative control), PC (positive control, *S*. Typhimurium challenged); NC+B1 and NC+B2 (negative control plus B1 or B2 bacteriophage, respectively); PC+B1, PC+B2 (positive control plus B1 or B2 bacteriophage, respectively) and PC+B1B2 (positive control plus both B1 and B2 bacteriophages). After four weeks of infection, the mortality rate in the PC group was 51.1% compared with 11.1% in the PC+B1B2 treatment. Bacteriophage administration had resulted in increased weight gain and decreased feed conversion ratio, particularly when both phages were included in the treatment ($p<0.001$). Moreover, the relative percentage of carcass weight was lowest in the PC treatment (66.9%) ($p<0.001$), whereas the other treatments registered similar carcass weight values. Regarding the internal organs, liver weight percentage was higher in the non-treated *Salmonella* group, and enlarged spleens were also noted in infected chickens even when treated with bacteriophages. The correlation between phage administration and blood parameters was unclear. Although the use of two bacteriophages for therapy was determined to be preferable for the majority of the criteria examined, further genetic characterization of the phages will be required before they can be widely used in chicken farms.

INTRODUCTION

Salmonella is a significant poultry pathogen that is of concern for public health and can potentially cause losses in poultry production (Berchieri Jr *et al.*, 2001). In Vietnam, salmonellosis is one of the most frequent bacterial infections in poultry farms, with 8.81 percent of chickens in the Mekong Delta being infected with the disease in 2010 (Khai *et al.*, 2010). So far, antibiotics have been used extensively to treat and prevent bacterial infections, resulting in antibiotic-resistant microorganisms. It is estimated that roughly 148 mg of antimicrobial are required to raise one kilogram of live chicken worldwide; however, in the Mekong Delta region of Vietnam, a higher level of antimicrobials in chicken production (260 mg/kg of chicken, excluding medicated feed) has been documented (Van Boeckel *et al.*, 2015; Nguyen *et al.*, 2015; Carrique-Mas *et al.*, 2015). Salmonellosis control at the farm level can be accomplished through a multi-factorial approach, and when broilers get *Salmonella*, several simultaneous or sequential procedures may be applied (Clavijo *et al.*, 2019), of which bacteriophage therapy has been described as a promising biological control for salmonellosis in poultry (Thanki *et al.*, 2021).

Bacteriophages, or phages, are predators of bacteria that are naturally present in the environment. According to some estimates, they are abundant in the environment, with an estimated 10:1 ratio compared to their bacterial counterparts (O'Flaherty *et al.*, 2009). Bacteriophages are defined as bacterial viruses that can infect and subsequently kill their host bacteria via bacteriolysis. Due to their availability, non-toxicity, and specificity to the bacterial hosts, lytic phages have been utilized as efficient tools for various purposes such as improving food safety or preventing and treating bacterial pathogens (Brovko *et al.*, 2012; Sulakvelidze, 2013). In addition, bacteriophages are extremely specific by infecting only a single species of bacteria, and phage therapy is regarded as safer and more effective than antibiotics (Upadhaya *et al.*, 2021). Previously, several reports have mentioned the positive effects of bacteriophage in reducing pathogens and improving the production performance of broiler chickens (Atterbury *et al.*, 2007; Lim *et al.*, 2012; Wang *et al.*, 2013). According to a recent study (Nabil *et al.*, 2018), bacteriophage treatment can rapidly reduce *S.* Typhimurium and *S.* Enteritidis in broiler chickens and could be used instead of antibiotics. The investigation of Clavijo *et al.* (2019) also demonstrated that phage treatment on commercial broiler farms had no adverse effects on production parameters and significantly reduced *Salmonella* infection in the chickens.

The majority of the research appears to have been concentrating on commercial broilers, with little information on the use of bacteriophage for indigenous breeds with slower growth and superior meat quality, which is an important component in the economies of rural and underdeveloped countries (Padhi, 2016). Therefore, the present study was undertaken in Noi chickens, one of the native breeds mainly reared in the Southern regions of Vietnam, aiming to evaluate the effects of bacteriophage on treating *S.* Typhimurium infection and further identify its effectiveness in the growth and carcass characteristics of the birds.

MATERIALS AND METHODS

Bacteria strains and bacteriophage preparation

In this study, the virulent strain *S.* Typhimurium ATCC®14028™ (American Type Culture Collection) was used for the *in vivo* infection in Noi chickens. Moreover, two bacteriophages, namely B1 and B2, were isolated and screened from chicken intestines and environmental sources at various poultry farms in the Mekong Delta of Vietnam (Souvannaty *et al.*, 2019). Briefly, for phage isolation and pure culture isolation, the double agar-plaque assay (Kropinski *et al.*, 2009) and the method of Poxleitner *et al.* (2017) were applied. The phages were then tested for host spectrum, pH tolerance (Verma *et al.*, 2009), and lysis capacity *in vitro* (Kropinski *et al.*, 2009; Verma *et al.*, 2009). Based on the criteria for host spectrum, pH tolerance, and ability to lyse *S*. Typhimurium, the B1 and B2 bacteriophages were selected for the experimental infection of chickens.

Phage proliferation: from the isolates available in the laboratory, the phages were proliferated in the ratio of phage: *Salmonella*: TSB being 1: 2: 30 with a *Salmonella* (*Salmonella* Typhimurium - ATCC®14028™) population of 10^8 CFU/ml. The phages were incubated at 37°C for 24 hours. After that, chloroform solution was added to the proliferated phage biomass at the rate of 1 chloroform: 10 phages. The solution was then vortexed, incubated for 2 hours followed by centrifuging at 6,000 rpm at 4°C for 15 minutes for phage collection (Silva *et al.*, 2014). The concentration of phages was determined as described Poxleitner *et al.* (2017) and Gonzalez-Menendez *et al.* (2018).

Chickens care and experimental design

The study was carried out on the experimental farm of Can Tho University located at Campus IV, Phung Hiep district, Hau Giang province. The care and handling of chickens were performed following guidelines of Animal Husbandry Law (32/2018/QH14), and the experimental procedure was approved by the Council for Science and Education, College of Agriculture (A10-02-2019/KNN), Can Tho University. The indigenous Noi chickens were obtained from a breeding company based in Soc Trang near Hau Giang province. During the rearing period, chickens were vaccinated against Marek disease (one-day-old), Newcastle disease and infectious bronchitis (days 4, 14, and 60th), Gumboro (days 7 and 12th), Fowl Pox (day 10th), and H5N1 avian influenza (day 45th). All birds were kept in confinement houses at the density of 10 birds/m^2 in the finishing stage. Drinking water was available, and chickens were fed *ad libitum* of commercial broiler diets: starter from 1 to 28 days (16% crude protein, 4% crude fiber, metabolizable energy 2,800 kcal/kg) and grower and finisher from 29 to 98 days (14% crude protein, 5% crude fiber, metabolizable energy 2,800 kcal/kg).

In total, 420 birds were arranged in a completely randomized design with 7 treatments and 4 replicates of 15 birds. The treatments were NC (negative control, without *S*. Typhimurium, without bacteriophage); PC (positive control, *S*. Typhimurium challenged, without bacteriophages); NC+B1 and NC+B2 (negative control plus B1 or B2 bacteriophage, respectively); PC+B1, PC+B2 (positive control plus B1 or B2 bacteriophage, respectively) and PC+B1B2 (positive control plus both B1 and B2 bacteriophages). The chicks were challenged with 1 ml of *S*. Typhimurium on the 2nd day at the dose of $10^{8.5}$ cfu/ml via oral administration. In treatments with bacteriophage therapy, the phages were orally inoculated at the dose of 10^{10} pfu/ml starting at 24 hours after infection, followed for three consecutive days, and repeated weekly until day 63rd.

Data collection and measurements

The experiment lasted for 100 days till the Noi chickens reached their mature weight. Feed intake and body weight were recorded weekly for each replicate, and feed conversion ratio (FCR) was calculated as g feed intake/g weight gain after correcting mortality. The mortality was recorded in the first four weeks after infection. During the experimental period, four birds per treatment (sex-balanced when applicable) were randomly selected for re-isolation of *S.* Typhimurium on days 7, 21, and 35 (Gomes *et al.*, 2014), and organ relative weight calculation on days 7, 35, and 63.

At the end of the experiment, 56 chickens (8 birds/treatment, sex-balanced) with weights around the means were selected for slaughtering to evaluate carcass characteristics and organ measurements. The broilers were individually weighed and then killed via cervical dislocation and exsanguination. The breast, thigh and drumstick muscle and wings were collected as described by Goliomytis *et al.* (2003). In addition, the liver, spleen, heart, and gizzard were then removed and weighed, and the organ weights were expressed as a percentage of the body weight.

Statistical analysis

The data were subjected to analysis of variance using the General Linear Model procedure of the Minitab version 16.2.1 software (State College, PA, USA) (Minitab, 2010). The difference of means among treatments was measured through Tukey's comparison test with $p \leq 0.05$.

RESULTS AND DISCUSSION

Mortality of chickens during four weeks after infection

The effect of treatment on mortality is shown in Table 1. In the negative control and treatments without *S.* Typhimurium challenged, no death were recorded. In contrast, the mortality was the highest in the positive control during four weeks post-infection, with an accumulative value of 51.1%. The bacteriophage administered treatments either in a single phage or cocktail phage demonstrated a reduction in mortality down to 11.1% over four weeks. It was also noted that a majority of birds' death happened during the first week of inoculation. There is little evidence available on the effects of *S.* Typhimurium on the death rate of native chickens. However, in broiler chickens, Nabil *et al.* (2018) reported on two-day old chicks infected with *S.* Typhimurium (10^5 cfu/ml) that mortality appeared after three days of infection with an overall rate of 20%, which is lower than the dead rate of Noi chickens in the current study, most likely due to the different breeds and higher dose of bacteria used in our study ($10^{8.5}$ cfu/ml). When bacteriophages were added, the effectiveness of reducing diarrhea after two days of infection was demonstrated, and no dead chickens were recorded in these treatments (Nabil *et al.*, 2018). In the present study, the death were also investigated for lesions, and the nine internal organs (liver, spleen, and heart) were used for bacteria re-isolation, and their sequence was confirmed as *Salmonella* enterica subsp. enterica serovar Typhimurium str. 14028S in BLAST, NCBI (https://blast.ncbi.nlm.nih.gov) (data not shown).

In the current study, there were no death in the treatments inoculated only bacteriophages after four

Table 1 – Mortality rate (%) of chickens after four weeks of infection.

Week	Treatments*						
	NC	PC	NC+B1	NC+B2	PC+B1	PC+B2	PC+B1B2
1	0	28.9	0	0	11.1	13.3	8.90
2	0	13.5	0	0	2.20	6.70	2.20
3	0	4.40	0	0	4.50	2.20	0.00
4	0	4.40	0	0	0.00	0.00	0.00
1-4	0	51.1	0	0	17.8	22.2	11.1

*NC: negative control, without *S.* Typhimurium, without bacteriophage; PC: positive control, S. Typhimurium challenged, without bacteriophages; NC+B1 and NC+B2: negative control plus B1 or B2 bacteriophage, respectively; PC+B1, PC+B2: positive control plus B1 or B2 bacteriophage, respectively; PC+B1B2: positive control plus both B1 and B2 bacteriophages.

weeks of infection, showing that the existing phage application was practically safe for chickens or did not cause any other health problems. Similar findings were also reported elsewhere by Wójcik *et al.* (2020). Moreover, in our study, the therapeutic effectiveness has been shown with reduced mortality in treatments with bacteriophage usage. The clinical manifestations of salmonellosis were comparably mild, and the combination of the two phages received a better response from chicken. It was reported by Fischer *et al.* (2013) that combinations of multiple phages might be more effective at phage bio-control because they provide a wider range of hosts and, as a result, help to keep resistances under control. In terms of reducing the *Campylobacter jejuni* population, the phage cocktails performed slightly better than the single phage following phage delivery. On the other hand, the cocktail slowed and delayed the formation of phage resistance (Fischer *et al.*, 2013), and the use of a phage cocktail for the treatment of *S.* Typhimurium in chickens might bring desirable results.

The high mortality rate in the first week after infection could be attributed to the high concentration of bacteria in internal organs, as well as the fact that the birds were still young, with an immature immune system (Akhtar *et al.*, 2013). Previously, Berchieri Jr *et al.* (1991) also mentioned that when newly hatched chicks were infected with *S.* Typhimurium, giving phage lytic solutions soon after infection was shown to reduce mortality. Still, the efficacy was reduced when offering phages a few hours later or in lower numbers. This is important because controlling *Salmonella* from its origin has been difficult since *S.* Typhimurium infection in young chicks generally causes no symptoms, and as a result, environmental contamination occurs, which contributes to the organism's proliferation among the flock (Hooton *et al.*, 2011). In addition, Huff *et al.* (2006) concluded that to treat a systemic bacterial infection successfully, a sufficient number of bacteriophages need to be present when the infection begins. Taken together, a two-phage cocktail in this study could reduce the *S.* Typhimurium invasion in the internal organs, as well as the mortality of chickens.

Re-isolation of *S.* Typhimurium in the internal organs

Among the organs used to re-isolate *S.* Typhimurium, a slightly lower concentration of bacteria was found in the heart over 7, 21, and 35 days post-infection (Figure 1). As expected, no bacteria were found in the negative treatments and those without *S.* Typhimurium inoculation. The bacterial invasion decreased over time, indicating that bacteriophages aided in eradicating *S.* Typhimurium colonization in the birds' liver, spleen, and heart. Moreover, a combination of two phages consistently outperformed a single phage therapy regardless of the checking point.

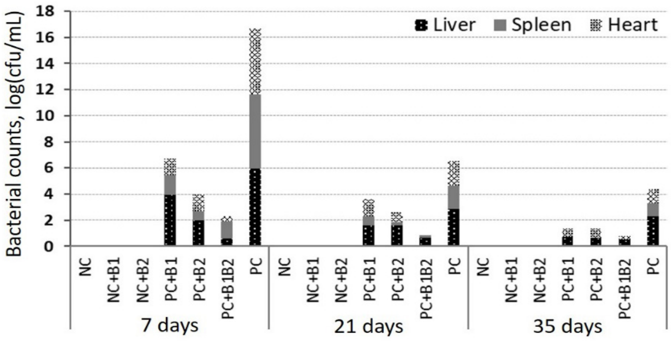


Figure 1 – Number of *S.* Typhimurium re-isolated from organs at different days post--infection.

NC: negative control; PC: positive control: B1: Bacteriophage 1; B2: Bacteriophage 2.

In the absence of *Salmonella* infection, re-isolated organs were free of *S*. Typhimurium, indicating that the control group from the current study was not infected either from other chickens or the feed used in the experiment, which is consistent with the report of Goliomytis *et al.* (2003). The presence of infected bacteria in the organs was expected because when predominant *Salmonella* serovars infect chickens, the germs can be found in the spleen, liver, heart, gallbladder, intestinal tissues, ceca, and various portions of the ovary and oviduct (Gast *et al.*, 2007; Keller *et al.*, 1995). Although the bacterial counts were reduced in the current investigation, their presence was still observed after 35 days. The phages applied in our work were not as effective as reported by Wong *et al.* (2013) that within 12 hours, the *Salmonella* was wholly eradicated from the chicks with phage by a single intracloacal inoculation an hour after *S.* Typhimurium inoculation. The supplied route could be one reason, as intracloacal inoculation ensures caecal wall lining establishment and eliminates passage through the acidic conditions of the crop and gizzard (Wong *et al.*, 2013). This intracloacal application, however, is impractical at the farm level. Another reason for the effectiveness of bacteriophage treatment may be the low ratio of bacteriophage to bacteria. Supporting this conclusion was the significant reduction in *S.* Typhimurium concentration achieved by first administering the phage cocktail before or just after bacterial infection, then twice daily for four days, and then weekly (Bardina *et al.*, 2012).

Effects of bacteriophage treatments on production performance of broilers

Body weight, feed intake, and feed conversion ratio (FCR) of chickens were affected by treatments during the experimental period (Table 2). Bacteriophage administration has contributed to better weight gain and reduced FCR, especially in the treatment with both phages included. Chickens in the positive control treatment grew slowly (265 g/bird) in the first 35 days, which was significantly different (p<0.001) from the ones in other treatments without *S.* Typhimurium infection and tended to be lower than those with bacteriophage inoculation. In the next stages, although not significant, a superior growth rate was also documented in bacteriophage treatments, and this contributed to the difference in overall weight gain with a respective increase per bird of 1,117 g, 1,263 g, and 1,217 g for PC, NC, and PC+B1B2 (p<0.005). As a consequence of higher intake but low efficiency in feed conversion, the most considerable FCR increased was noted in the PC treatment. Among the infected chickens, those with phage applied provided lower FCR, ranging from 3.56 (phage cocktail) to 3.86 (phage B2). According to Khoa *et al.* (2019), the BW of a Noi broiler at 84 days of age was 1,196 g for females and 1,424 g for males, with an average FCR of 3.52. Thus, the chickens used in this experiment developed normally, with an average body weight of 1.263 g in the NC treatment.

Body weight is an important characteristic in broiler production since lower body weight correlates to higher broiler meat production costs. A clear tendency of improving the growth rate of infected chickens treated with bacteriophages was identified in our study. The present findings corroborated the research outputs of a previous study (Toro *et al.*, 2005) that *S.* Typhimurium phage treatment resulted in a favorable effect on weight gain. These authors also pointed out that chicks gained less weight following the challenge and maintained lower weights throughout the study, which parallels our results.

Apart from being used as therapeutic bioagents to eradicate bacteria, bacteriophages have been used as feed additives to prevent pathogens, resulting in productivity advantage. For example, in experiments using diets containing phages against *S.* Gallinarum, *S.* Typhimurium, and *S.* Enteritidis, where broiler chickens were raised under normal physiological conditions (without bacterial challenge), improvements in body weight and FCR were observed (Lim *et al.*, 2010; Kim *et al.*, 2014; Wójcik *et al.*, 2020). This is due to the fact that the bacteriophage inhibits the growth of bacteria in the broilers' gastrointestinal tract (Lim *et al.*, 2010), and the increase in body weight may be related to an increase in feed intake (Upadhaya *et al.*, 2021). However, not all cases of phage therapy or phage supplementation in diets resulted in enhanced broiler production traits. Kim *et al.* (2013) found that feeding S. enteritidis-targeted phages had little influence on

Table 2 – Effects of bacteriophage on body weight gain, feed intake and feed conversion ratio.

Parameters*	Treatments							SEM	*p*
	NC	PC	NC+B1	NC+B2	PC+B1	PC+B2	PC+B1B2		
1-35 days									
BWG	327^{ab}	265^{c}	352^{ab}	356^{a}	305^{bc}	302^{bc}	314^{abc}	10.3	0.000
FI	796	859	857	839	867	860	846	15.4	0.075
FCR	2.45^{bc}	3.25^{a}	2.44^{bc}	2.36^{c}	2.84^{ab}	2.84^{ab}	2.71^{bc}	0.10	0.000
36-70 days									
BWG	518	474	552	555	539	505	525	21.2	0.175
FI	$1,750^{c}$	$1,930^{ab}$	$1,870^{c}$	$1,870^{abc}$	$1,899a^{bc}$	$1,982^{a}$	$1,872^{abc}$	32.0	0.003
FCR	3.39	4.10	3.40	3.33	3.53	3.92	3.58	0.18	0.051
71-98 days									
BWG	400	376	414	388	364	362	377	15.7	0.257
FI	$1,578^{bc}$	$1,742^{a}$	$1,577^{bc}$	$1,502^{c}$	$1,581^{bc}$	$1,675^{ab}$	$1,611^{b}$	22.3	0.000
FCR	3.79^{b}	4.63^{a}	3.80^{b}	3.92^{ab}	3.35^{ab}	4.63^{a}	4.27^{ab}	0.15	0.003
1-98 days									
BWG	$1,263^{ab}$	$1,117^{c}$	$1,319^{a}$	$1,301^{ab}$	$1,209^{abc}$	$1,171^{bc}$	$1,217^{abc}$	30.4	0.005
FI	$4,124^{e}$	$4,533^{a}$	$4,304^{cd}$	$4,145^{de}$	$4,348^{bc}$	$4,518^{ab}$	$4,330^{c}$	36.7	0.000
FCR	3.27^{c}	4.07^{a}	3.26^{c}	3.20^{c}	3.60^{bc}	3.86^{ab}	3.56^{bc}	0.08	0.000

*BWG: Body weight gain (g); FI: Feed intake (g); FCR: Feed conversion ratio (feed/gain). NC: negative control, without *S.* Typhimurium, without bacteriophage; PC: positive control, *S.* Typhimurium challenged, without bacteriophages; NC+B1 and NC+B2: negative control plus B1 or B2 bacteriophage, respectively; PC+B1, PC+B2: positive control plus B1 or B2 bacteriophage, respectively; PC+B1B2: positive control plus both B1 and B2 bacteriophages.

^{a,b,c,d,e}: Within a row, values with different superscripts differ statistically at p<0.05.

broiler performance and it was additionally suggested that phages targeting several pathogenic bacteria have more favorable effects than phages targeting single pathogenic bacteria in boosting broiler performance (Kim *et al.*, 2014). In another study, Noor *et al.* (2020) concluded that antibiotics and bacteriophages did not affect body weight gain, feed intake, or FCR during the 15-32 days of commercial broilers. The phage-host interaction in production traits of chickens might be dependent on phage types and genotypes, which can be found from poultry and environmental isolates.

Carcass characteristics and internal organs of Noi chickens

Carcass composition and internal organ measurements of chickens are presented in Table 3. The relative percentage of carcass weight was lowest at the PC treatment (66.9%) ($p<0.001$), while in the other treatments, these values were similar. The greatest breast muscle and thigh and drumstick ratios were shown in NC+B1 and NC+B2 treatments ($p<0.001$). As a consequence of low carcass content in the body, the percentage of breast, thigh, and drumstick in chickens from PC was significantly lower than those from others. There were also differences in wing weight ratio among treatments, with the highest being 11.0% in the PC+B1B2 treatment. The improved carcass weight percentage may benefit from the superior live weight as these two criteria are closely related (Tuoi *et al.*, 2021).

As for leg muscle percentage, the current outputs are in line with the report of (Kim *et al.*, 2013), according to which the anti-*S.* Enteritidis bacteriophage treatments had higher relative leg muscle weights than the control treatments. In the NC treatment, values of carcass cuts agreed with the report by Hung *et al.* (2020) in Noi chicken at the slaughter of 84 days. There is a scarcity of comparable data on the impact of bacteriophages on other carcass traits; however, positive responses to bacteriophage treatments have been demonstrated. Most internal organs were numerically higher in the PC treatment ($p<0.005$), mainly the relative weights of small intestines, liver, and gizzard. For spleen percentage, the highest values were on PC treatment for most of the time points (Table 3). In addition, throughout the experiment, a tendency to decrease liver weight percentage was shown, which could be attributed to an increase in final body weight (Figure 2a). In contrast, these figures demonstrated an upward trend in spleen to body weight ratio, with a notable figure in the PC treatment and other treatments with *Salmonella* infection (Figure 2b).

In the present study, liver weight percentage was higher in the non-treated Salmonella group, similar to that shown by Wang *et al.* (2013). The mechanism for this is unknown, but it could be hypothesized that bacteriophages may alter the population of gut intestinal bacteria, which are required for gut and liver immune system development (Wang *et al.*, 2013). Regarding spleen weight ratio, the benefit of treating

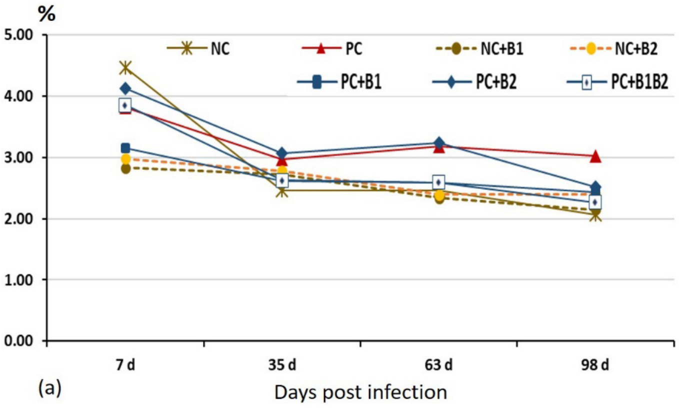
Table 3 – Means weights of the carcass, carcass parts, and organs of chickens.

Parameters*	Treatments**							SEM	*p*
	NC	PC	NC+B1	NC+B2	PC+B1	PC+B2	PC+B1B2		
LW, g	1.280^{bc}	1.148^{e}	1.344^{a}	1.337^{ab}	1.228^{cd}	1.193^{de}	1.240^{cd}	13.5	0.000
Carcass parts relative to LW (%)									
KW	95.8	96.4	97.0	96.6	96.2	96.6	96.5	0.30	0.209
DFW	85.6^{a}	82.0^{b}	85.6^{a}	86.1^{a}	85.4^{a}	84.8^{a}	85.0^{a}	0.45	0.000
CW	71.0^{ab}	66.9^{c}	71.4^{ab}	71.5^{a}	70.9^{ab}	70.0^{b}	70.8^{ab}	0.32	0.000
BW	12.5^{bc}	10.9^{d}	14.7^{a}	13.2^{b}	11.5^{cd}	12.0^{cd}	11.7^{cd}	0.26	0.000
TDW	16.2^{a}	13.9^{b}	15.8^{ab}	17.2^{a}	13.9^{b}	15.9^{ab}	15.6^{ab}	0.47	0.000
WW	10.5^{abc}	9.89^{c}	10.7^{ab}	10.8^{a}	9.96^{bc}	10.7^{ab}	11.0^{a}	0.17	0.000
Internal organs relative to LW (%)									
SM	7.70^{ab}	9.35^{a}	6.75^{b}	7.52^{b}	8.19^{ab}	7.52^{b}	7.23^{b}	0.38	0.002
Caecum	0.72^{b}	1.02^{a}	0.69^{b}	0.75^{b}	0.87^{ab}	1.03^{a}	0.82^{ab}	0.05	0.000
Liver	2.06^{b}	3.02^{a}	2.14^{b}	2.40^{b}	2.43^{b}	2.51^{ab}	2.26^{b}	0.12	0.000
Spleen	0.18^{b}	0.32^{a}	0.24^{ab}	0.30^{ab}	0.29^{ab}	0.31^{a}	0.32^{a}	0.03	0.000
Gizzard	2.51^{b}	3.95^{a}	2.74^{b}	2.81^{b}	2.52^{b}	2.44^{b}	2.90^{b}	0.14	0.000
Heart	0.49^{abc}	0.67^{ab}	0.46^{c}	0.48^{bc}	0.54^{abc}	0.58^{a}	0.44^{c}	0.02	0.000

*LW: live weight; KW: killed weight; DFW: De-feather wight; CW: Carcass weight; BW: Breast muscle weight; TDM: Thigh and drumstick muscle weight; WW: Wing weight; SM: Small intestine.

**NC: negative control, without *S.* Typhimurium, without bacteriophage; PC: positive control, *S.* Typhimurium challenged, without bacteriophages; NC+B1 and NC+B2: negative control plus B1 or B2 bacteriophage, respectively; PC+B1, PC+B2: positive control plus B1 or B2 bacteriophage, respectively; PC+B1B2: positive control plus both B1 and B2 bacteriophages.

[a,b,c]: Within a row, values with different superscripts differ statistically at $p<0.05$.



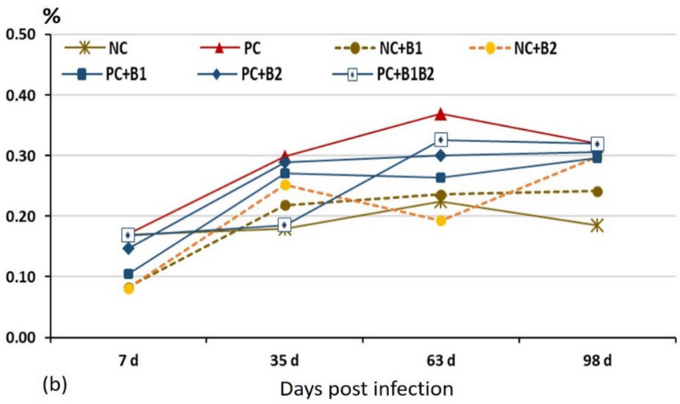


Figure 2 – Relative weight percentage of chicken liver (a) and spleen (b) during the experimental period.

chicken with phages was not demonstrated compared to those from Kim *et al.* (2013), whose results showed a drop in spleen weight relative to body weight, indicating that phage inclusion was better. The current outputs are consistent with the findings of Upadhaya *et al.* (2021), who discovered that the relative weight of the gizzard increased more substantially in the positive control group than the control and phage groups, which could be explained by an increase in feed intake in the positive control group. On the other hand, supplementation with anti-S. Enteritidis bacteriophage did not affect the relative weight of organs after 35 days (Kim *et al.*, 2013). Moreover, in E. coli-infected birds treated with bacteriophages, the heart, liver, and spleen sizes were not significantly different from the control groups (Lau *et al.*, 2010). Nonetheless, on days 2, 3, and 21 post-infection, the livers of untreated E. coli-challenged birds were enlarged. These birds' spleens were also enlarged on days 2, 3, and 7 post-infection but subsequently experienced a gradual reduction in size (Lau *et al.*, 2010).

CONCLUSION

In this study, bacteriophage treatment resulted in a decrease in mortality in broilers infected with *S.* Typhimurium. Additionally, they tended to facilitate the growth of chickens and the improvement of feed conversion ratio. When bacteriophages were applied, the carcass proportion increased, yet no evident benefits were observed in internal organ weight. The use of two bacteriophages for therapy was preferable for most of the criteria investigated; nevertheless, a comprehensive genetic characterization of the phages and their host range needs to be undertaken as the next stage before they can be extensively used in chicken farms.

ACKNOWLEDGMENTS

This study is funded in part by the Can Tho University Improvement Project VN14-P6, supported by a Japanese ODA loan.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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