Full Length Article

EFFECTS OF DIETARY SUPPLEMENTATION OF BACTERIOPHAGE ON CARCASS CHARACTERISTICS OF *E. coli* CHALLENGED COMMERCIAL BROILERS

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ABSTRACT

A biological trial was conducted to investigate the effect of bacteriophage on carcass characteristics of commercial broilers with or without bacterial challenge. Totally 180 day old Vencobb broiler chicks were randomly allotted into five dietary treatments designated as T1 to T5 with each treatment replicated thrice. T1 and T2 served as negative and positive control respectively, while birds in T3, T4 and T5 were exposed to E. coli on different days during 35 days course. Dietary treatments included T1 - basal diet, T2 - basal diet with bacteriophage in feed (50 mg/bird/ day up to 7th day and 25 mg/bird/day up to 35th day), T3 without bacteriophage, T4-bacteriophage in feed (50 mg/bird/day up to 35th day) and T5 - bacteriophage in feed (50 mg/bird/day up to 7th day and 25 mg/bird/day up to 35th day). E. coli was introduced orally in groups T3 and T4 on day 10, while T5 on day 26 (10^{6} CFU/ bird). The bacteriophage supplementation to E. coli challenged birds had significantly increased the weights of eviscerated carcass, giblets, ready-to-cook meat; the cut-up parts like breast, back and drumstick compared to non-supplemented group. Results shed light on the potential benefits of bacteriophage as a natural alternate approach to antibiotic growth promotors in broiler production.

Key words: Poultry, bacteriophage, carcass yield, cut up parts

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INTRODUCTION

Over four decades, India's poultry industry has transformed from a small hobby to a significant commercial Agri-based sector, marked by the development of high yielding layer and broiler strains, driving annual growth rates of 4–6% in egg and 8–10% in

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broiler production (Chatterjee and Rajkumar, 2015). To meet global demand for meat, milk, and eggs, livestock producers globally have adopted large-scale, intensive farming systems, posing disease spread risks due to high stocking density and productivity (Upadhyaya *et al.,* 2021).

In developing countries, antimicrobial drugs are used not just for treatment but also to boost animal productivity, raising concerns about antibiotic resistance (Snary *et al.,* 2004; Levy, 2014). This global concern may lead to the rapid spread of antibiotic resistance from countries with strong surveillance to those without, impacting infectious disease treatment (Mamza *et al.,* 2010). Discovered independently by Twort and d'Herelle in 1915 and 1917, bacteriophages are considered potential antibiotic substitutes, offering a safer alternative to antibiotics without adverse effects on cells (El-Gohary *et al.,* 2014). Bacteriophage therapy, utilizing self-replicating lytic bacteriophages, is a novel approach for bacterial infections without inducing antibiotic resistance (Jamalludeen *et al.,* 2009; Huff *et al.,* 2006; Clark and March, 2006).

Avian colibacillosis, a significant poultry disease, causes high morbidity, mortality, and economic losses (Paixao *et al.,* 2016). Pathogenic *E. coli* strains, especially Avian Pathogenic *E. coli* (APEC), are associated with extra intestinal illnesses, leading to septicemia and high poultry mortality (Guabiraba *et al.,* 2015). Recent studies, including Eid *et al.* (2022), highlight bacteriophages as a valuable substitute for antibiotics, particularly in multidrug-resistant infections. The objective of the present study was to evaluate the effect of bacteriophage on carcass characteristics of *E. coli* challenged commercial broilers.

MATERIALS AND METHODS

Procurement and analysis of bacteriophage

Commercially available cocktail of four bacteriophages specifically against *E. coli* with a concentration of 20 million Plaque Forming Units (PFU) per g was used for this experiment. Bacteriophage cocktail was analysed for its effect on *E. coli* bacteria by using different dilutions of both the phage and the bacteria. Gel punch method was used for the same to check the zone of inhibition produced by the phage.

Fig 1. Zone of inhibition of bacteria produced by the bacteriophage

Challenging the birds with *E. coli*

Pathogenic *E. coli* was isolated from infected bird. All the birds in three treatment groups were introduced to the bacteria orally at a dose of 100 µl of inoculum per bird. The

concentration of the bacteria was 10^6 Colony Forming Units (CFU) per 100 µl. For treatment groups T3 and T4 the bacterial inoculation was done on 10th day whereas for treatment group T5 it was done on 26th day of the trial.

Design of experiment, sample collection and analysis

The feeding trial was carried out with 180 straight-run commercial broiler chicks (Vencobb 400) raised on deep litter system for 35 days. The chicks were randomly allotted to five dietary treatments designated as T1 to T5 with each treatment replicated thrice with 12 chicks in a Completely Randomized Design. T1 and T2 served as negative and positive control respectively, while birds in T3, T4 and T5 were exposed to *E. coli* on different days during the course of 35 days. Dietary treatments included T1- basal diet, T2- basal diet with bacteriophage in feed @ 50 mg/ bird/day up to 7th day and 25 mg/bird/day up to 35th day, T3 - without bacteriophage, T4 - bacteriophage in feed @ 50 mg/bird/day up to 35th day and T5 - bacteriophage in feed @ 50 mg/bird/day up to 7th day and 25 mg/bird/day up to 35th day. *E. coli* was introduced orally in groups T3 and T4 on day 10, while T5 on day 26 @ 10^6 CFU/ bird. The experimental period was divided into three phases; namely, Pre starter (0-7 days), Starter (8-21 days), and Finisher (22-35 days).

Sample collection

A total of six birds per treatment were selected and slaughtered humanly severing the jugular vein after stunning by cervical dislocation. Pre-slaughter weight, eviscerated carcass weight and ready-to-cook weight were recorded. The cut-up parts yield of breast, back, thigh, drumstick, neck, and wings were recorded. The data was analysed statistically by general linear model with univariate analysis using one-way ANOVA in SPSS software.

RESULTS AND DISCUSSION

Carcass characteristics

The results of present study revealed that the *E. coli* challenge in commercial broilers had produced deleterious effects on pre-slaughter, eviscerated carcass, ready-to-cook carcass and giblet weights (Table 1). Supplementation of bacteriophage as treatment and as preventative was found to alleviate the deleterious effects on the above parameters with varying degrees of efficiency. Supplementation of bacteriophage was found to have no effect on eviscerated, ready – to-cook and giblet yields; however, in terms of weight, bacteriophage supplemented birds had significantly ($p<0.05$) lower eviscerated carcass weight (1296.50 g in T2 Vs. 1365.83 g in T1). In contrast to this finding, Kim *et al.* (2014) reported improved carcass weight in the birds supplemented with bacteriophage at a dose of 0.5% compared to the birds fed with basal diet. Similarly, Upadhyaya *et al.* (2021) also reported increased carcass yield in the birds fed with bacteriophage cocktail at a dose of 0.05 or 0.10%. Mlambo *et al.* (2023) also observed significantly ($p<0.05$) increased carcass weight in birds fed with *Salmonella* specific phage cocktail. In contrast with the finding of the present study, Kim *et al.* (2013)

Table 1. Effect of dietary bacteriophage as preventative and treatment on carcass characteristics (g) and carcass yield (%) of *E. coli* challenged commercial broilers (n=6)

Treatment	Pre-slaughter weight (g)	Eviscerated carcass		Giblets		Ready-to-cook carcass	
		Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)
T1 (Control)	$1812.66^{a} \pm 17.27$	$1365.83^{a} \pm 13.73$	75.37 ± 0.79	$75.66^{a} \pm 2.98$	5.24 ± 0.19	$1441.50^{a} \pm 14.72$	79.53 ± 0.76
T2 (BP)	$1721.50^{b} \pm 45.65$	$1296.50^{b} \pm 29.14$	75.37 ± 0.72	$76.00^{a} \pm 1.93$	5.54 ± 0.09	$1372.50^{ab} \pm 30.52$	79.79 ± 0.74
T3 (EC)	$1577.66^{c} \pm 13.94$	$1151.66^{d} \pm 15.05$	73.01 ± 1.00	$63.33^{b} \pm 3.60$	5.20 ± 0.25	$1215.00^{d} \pm 17.37$	77.03 ± 1.12
T4 (EC - BP Treatment)	$1629.00^{c} \pm 32.11$	$1218.16^{cd} \pm 33.07$	74.73 ± 0.87	$69.00^{ab} \pm 4.15$	5.34 ± 0.21	$1287.16^{c} \pm 36.21$	78.96 ± 0.89
T5 (EC - BP Preventative)	$1718.50^{b} \pm 22.99$	$1281.33^{bc} \pm 17.51$	74.61 ± 1.28	$75.66^{a} \pm 2.21$	5.58 ± 0.19	$1357.00^{ab} \pm 17.12$	79.02 ± 1.23
F Value	10.02**	12.33**	0.41^{NS}	3.32**	0.73^{NS}	12.24**	0.34^{NS}

observed increased giblets weight in Arbor Acres and Ross 308 broilers supplemented with bacteriophage. Similarly, the increased giblet weight was reported by Upadhyaya *et al.* (2021) in Ross broilers fed with bacteriophage.

The *E. coli* challenge in commercial broilers has resulted in significantly ($p<0.05$) lower pre-slaughter (1577.66 g in T3 Vs 1812.66 g in T1), eviscerated (1151.66 g in T3 Vs. 1365.83 g in T1), giblets (63.33 g in T3 Vs, 75.66 g in T1) and ready-to-cook (1215.00 g in T3 Vs. 1441.50 g in T1) weights compared to control. Similar results were observed earlier by Russell (2003) in *E. coli* challenged birds when compared to healthy birds. However, the results in terms of yields in the present study revealed no significant change due to *E. coli* challenge on all these carcass parameters.

The *E. coli* challenged birds which received bacteriophage through feed as preventative (T5) registered increased pre-slaughter (1718.50 g in T5 Vs. 1577.66 g in T3), eviscerated, (1281.33 g in T5 Vs. 1151.66 g in T3), giblets (75.66 g in T5 Vs. 63.33 g in T3) and ready-to-cook (1357.00 g in T5 Vs 1215.00 g in T3) weights when compared to the birds challenged with *E. coli* without bacteriophage supplementation

Table 2. Effect of dietary bacteriophage as preventative and treatment on the cut-up parts weight (g) and yield (%) of *E. coli* challenged commercial broilers (n=6)

Treatment	Breast		Back		Thigh		Drumstick		Wings		Neck	
	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)
T1 (Control)	$526.33^{a} \pm 12.82$	$36.50^{a} \pm 0.71$	$237.00^{ab} \pm 6.96$	16.43 ± 0.43	176.83 ± 5.24	$12.26^{b} \pm 0.35$	$185.33^{a} \pm 8.25$	12.85 ± 0.55	138.00 ± 5.88	9.58 ± 0.42	102.33 ± 2.82	7.10 ± 0.23
T2 (BP)	$478.50^{b} \pm 14.31$	$34.84^{ab} \pm 0.45$	$240.00^{ab} \pm 9.04$	17.47 ± 0.49	171.00 ± 6.77	$12.44^{b} \pm 0.28$	$167.66^{ab} \pm 5.38$	12.20 ± 0.17	146.00 ± 5.44	10.68 ± 0.54	93.33 ± 6.43	6.80 ± 0.47
T3 (EC)	$413.16^{c} \pm 12.96$	$33.99^{b} \pm 0.85$	$201.00^{c} \pm 11.56$	16.51 ± 0.76	173.66 ± 5.52	$14.31^{a} \pm 0.53$	$158.66^{b} \pm 4.35$	13.05 ± 0.28	114.50 ± 7.34	9.46 ± 0.69	90.66 ± 5.46	7.46 ± 0.41
T4 (EC - BP Treatment)	$443.83^{bc} \pm 21.96$	$34.40^{ab} \pm 0.95$	$219.00^{bc} \pm 9.62$	16.99 ± 0.45	161.00 ± 4.64	$12.54^{b} \pm 0.41$	$159.50^{b} \pm 5.94$	12.43 ± 0.59	134.83 ± 10.05	10.50 ± 0.78	100.00 ± 11.1	7.76 ± 0.90
T5 (EC - BP Preventative)	$444.00^{bc} \pm 5.69$	$32.73^{b} \pm 0.44$	$249.83^{a} \pm 4.31$	18.42 ± 0.34	180.33 ± 5.19	$13.28^{ab} \pm 0.25$	$174.50^{ab} \pm 5.15$	12.85 ± 0.28	139.33 ± 7.42	10.25 ± 0.45	93.33 ± 3.48	6.88 ± 0.27
F value	8.84**	3.69**	5.00**	2.49^{NS}	1.77^{NS}	4.88**	3.47*	0.71^{NS}	2.58^{NS}	0.85^{NS}	0.57^{NS}	0.68^{NS}

Mean bearing different superscripts (a, b, c) within a column differ significantly ($p < 0.05$)

** Significant ($p < 0.01$); * Significant ($p < 0.05$); NS - Not significant;

BP: Dietary supplementation of bacteriophage from day 1

EC: *E. coli* challenge on day 10 and no dietary supplementation of bacteriophage

EC - BP Treatment: *E. coli* challenge on day 10 and dietary supplementation of bacteriophage from day 12

EC - BP Preventative: Dietary supplementation of bacteriophage from day 1 and *E. coli* challenge on day 26

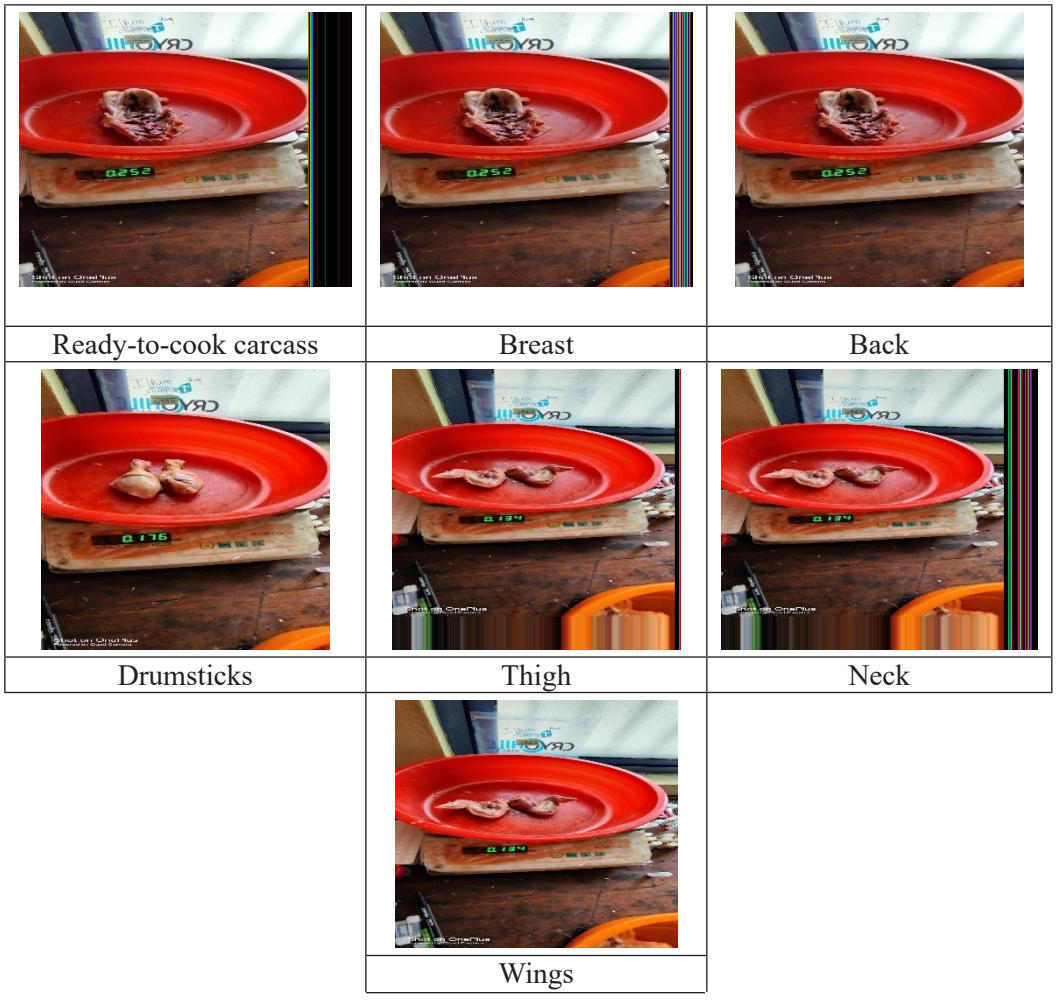

Fig 2. Ready-to-cook carcass and different cut-up parts

(T3). However, supplementing bacteriophage as treatment (T4) had beneficial effect only on ready-to-cook carcass weight (1287.16 g in T4 Vs. 1215.00 g in T3). These results of improved carcass characters in *E. coli* challenged bacteriophage supplemented birds are in agreement with the findings of Ngu *et al.* (2022b) in Vietnamese Noi chickens, who reported increased carcass proportion in birds challenged with *S. typhimurium* and treated with bacteriophage. In concurrence with the present findings, Ngu *et al*. (2022a) also reported highest giblets weight in *E. coli* challenged birds treated with phage compared to the birds not receiving phage as treatment.

Significantly ($p<0.05$) higher pre-slaughter, eviscerated carcass and ready-to-cook carcass weights were observed in

negative control without *E. coli* challenge (T1); whereas significantly ($p<0.05$) lower values of these parameters were recorded in the birds challenged with *E. coli* (T3). The birds received bacteriophage supplementation as preventative before *E. coli* challenge (T5) had significantly ($p<0.05$) higher pre-slaughter and eviscerated carcass and ready-to-cook carcass weights compared to *E. coli* challenged birds (T3). The birds received bacteriophage as treatment (T4) had significantly ($p<0.05$) higher ready-to-cook carcass weight compared to *E. coli* challenged birds. The yields of eviscerated and ready-to-cook carcass weights in all the treatment groups had statistically similar values. Significantly ($p<0.05$) higher giblet weights were recorded in *E. coli* unchallenged groups (T1 and T2) and *E. coli* challenged group with supplementation of bacteriophage as preventative (T5) compared to the birds of *E. coli* challenged group without bacteriophage supplementation (T3). The yields of eviscerated and ready-to-cook carcass and giblets did not reveal any significant difference among the treatment groups.

Cut-up Parts

The present study has not revealed any significant change in cut-up parts (Table 2) due to feeding of bacteriophage as probiotic in unchallenged birds except breast weight; which recorded significant ($p<0.05$) decrease from control (478.50 g in T2 Vs. 526.33 g in T1). Contrary to the present findings, Upadhyaya *et al*. (2021) observed increased ($p<0.05$) breast weight of 35 days old Ross broilers fed with bacteriophage cocktail at 0.05 or 0.10 per cent with basal diet. Similarly, contrasting results of increased breast weight in birds fed with *Salmonella* specific phage cocktail in Ross 308 broilers was observed by Mlambo *et al.* (2023). The weights and yields of back, thigh, drumstick wings and neck were almost similar in both negative and positive controls in the present study and were not affected by bacteriophage supplementation. In concurrence with the findings of the study, Kim *et al.* (2013) also found that the cut-up part weight was not influenced by the bacteriophage supplementation in Arbor Acres broilers.

The results of the present study indicated that the weight (413.16 g in T3 Vs. 526.33 g in T1) and yield (33.99 % in T3 Vs. 36.50 % in T1) of breast cut and weight of back cut (201.00 g in T3 Vs. 237.00 g in T1) were significantly ($p<0.05$) lower in the *E coli* challenged birds compared to control; whereas, the thigh cut showed a reverse trend of significant ($p<0.05$) increase (14.31 % in T3 Vs. 12.26 % in T1). Similarly, Ngu *et al.* (2022a) observed lower cut-up part weights in native Vietnamese Noi chickens after challenging with *E. coli* and feeding bacteriophage at 24 hour post-inoculation.

The weight and yield of breast and weights of back and drumstick were significantly ($p<0.05$) lower in *E. coli* challenged birds (T3) compared to control (T1). The *E. coli* challenged birds which received supplementation of bacteriophage as preventative (T5) had significantly

($p<0.05$) higher back weight compared to the birds challenged with *E. coli* alone. Significant ($p<0.05$) increase in thigh yield due to *E. coli* challenge was reversed with the supplementation of bacteriophage as treatment (T4). The yields of back and drumstick, weight of thigh, weights and yields of wings and neck were not influenced either by *E. coli* challenge or by bacteriophage supplementation.

The higher body weight registered in bacteriophage supplemented group might be resulted in the increased yield of carcass, ready-to-cook percentage and giblets weight. The increased body weight of broilers supplemented with bacteriophage might be because of the inhibitive or lytic effect on harmful pathogens (*E. coli*) in the gastrointestinal (GI) tract of the birds by bacteriophage. Since the bacteriophage is able to reduce the pathogenic bacteria in the GI tract, there will be increase in the population of beneficial bacteria (*Lactobacillus*) which will promote the gut health positively and enhance the nutrient absorption.

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