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In-feed *Salmonella*-specific phages alter the physiology, intestinal histomorphology, and carcass and meat quality parameters in broiler chickens

Sicelo Beauty Dlamini[a,d], Caven Mguvane Mnisi[a,b], Collins Njie Ateba[b,c], Chidozie Freedom Egbu[a,b], Victor Mlambo[d,*]

[a] Department of Animal Science, School of Agricultural Science, North-West University, Mafikeng, South Africa
[b] Food Security and Safety Focus Area, Faculty of Natural and Agricultural Science, North-West University, Mafikeng, South Africa
[c] Department of Microbiology, Faculty of Natural and Agricultural Sciences, North-West University, Mafikeng, South Africa
[d] School of Agricultural Sciences, Faculty of Agriculture and Natural Sciences, University of Mpumalanga, Nelspruit, South Africa

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ABSTRACT

The use of antibiotic growth promoters (AGP) in broiler production leads to the emergence of antibiotic-resistant bacterial pathogens. To mitigate this challenge, biocontrol agents such as phages are being investigated as sustainable alternatives to AGP in commercial broiler production systems. This study aims to investigate the effect of different levels of an in-feed encapsulated *Salmonella*-specific phage cocktail (SPC) on various parameters, including growth performance, blood indices, carcass characteristics, intestinal histomorphology, and meat quality traits in broiler chickens. A total of 400-day-old Ross 308 male chicks (49.84 ± 1.03 g live weight) were reared on five experimental diets. The diets were formulated as follows: 1) a negative control diet without zinc bacitracin and SPC (NC), 2) a positive control diet with 0.5 g/kg zinc bacitracin but without SPC (PC), and 3) NC with 0.075 (SP75), 0.1 (SP100), and 0.175 g/kg SPC (SP175). During the feeding trial, phages were added on days 1 – 2; 11 – 12; 21 – 22; and 29 – 30 for the SPC treatments. Feed intake, bird weight, and blood parameters were determined during the feeding trial. Birds were slaughtered at the end of the feeding trial (5 weeks) to evaluate carcass characteristics, intestinal histomorphology, and meat quality traits. Five-week-old birds reared on SP100 had lower weight gains (555.7 g/bird) than those reared in the other treatment groups ($p < 0.05$). The inclusion of SPC in diets induced positive quadratic effects on overall feed intake ($R^2 = 0.169$; $p = 0.048$) and meat chroma ($R^2 = 0.184$; $p = 0.024$) but resulted in negative quadratic effects for breast weight ($R^2 = 0.418$; $p = 0.046$) and linear effects for meat pH 1-h post-mortem ($R^2 = 0.161$; $p = 0.040$) and proventriculus weight ($R^2 = 0.195$; $p = 0.024$). A positive quadratic effect was noted for duodenal villus height ($R^2 = 0.935$; $p = 0.003$), width ($R^2 = 0.882$; $p = 0.009$), and area ($R^2 = 0.929$; $p = 0.001$); jejunal villus height ($R^2 = 0.914$; $p = 0.001$), width ($R^2 = 0.917$; $p = 0.002$), area ($R^2 = 0.903$;

* Corresponding author.
E-mail address: Victor.Mlambo@ump.ac.za (V. Mlambo).

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p = 0.001), and muscle thickness (R^2 = 0.970; p = 0.001); ileal villus width (R^2 = 0.747; p = 0.006), and crypt depth (R^2 = 0.916; p = 0.001). Compared to the negative control, SPC induced positive changes in the jejunal and ileal villus height and VH:CD, however, this did not improve growth performance of broiler chickens. It can be concluded that periodic SPC inclusion has the potential to replace subtherapeutic antibiotic use in poultry production.

Introduction

The emergence and proliferation of antimicrobial-resistant pathogens such as non-typhoid *Salmonella* species due to the use of in-feed antibiotics has become a major global concern [1]. Indeed, the World Health Organization (WHO) classified antimicrobial drug resistance (AMR) as a global public health concern in 2000. The WHO also identified *Salmonella*, a genus of Gram-negative bacteria, as one of the most common causes of foodborne illness worldwide, with an estimated 93.8 million cases of gastroenteritis and 155,000 deaths annually [2]. These infections occur through the consumption of contaminated food, particularly meat, poultry, eggs, and dairy products, as well as through contact with infected animals or their environments [3]. In broilers, *Salmonella* colonizes the intestinal tract leading to reduced productivity and increased mortality rates. Consequently, broiler producers have traditionally relied on growth-promoting antibiotics [4], also termed antibiotic growth promoters (AGP) to control *Salmonella* and other pathogens and thus boost bird health and productivity. Antibiotic growth promoters are medicines or drugs administered at low subtherapeutic dosages to destroy or inhibit bacterial growth [5]. However, the use of AGP in livestock production has been identified as one of the major drivers of antimicrobial resistance resulting in unfavourable treatment outcomes in infected humans and animals.

In response, the European Union has banned in-feed antibiotics in livestock production [6] while other regions have severely restricted their use. However, the complete withdrawal of AGP without effective alternatives has resulted in increased enteric infections in chickens, poor bird productivity, and compromised consumer health [7]. Effective alternative biocontrol strategies such as bacteriophages are, therefore, necessary to reduce the emergence of antimicrobial-resistant pathogens and improve public confidence in animal-derived food products, such as meat, milk, and eggs [8]. Phage therapy could be used in place of AGP to enhance feed utilization efficiency and growth performance of birds while mitigating the emergence of antimicrobial-resistant pathogens. The mechanism of action of lytic phages includes infecting susceptible bacterial cells to produce phage virions, which then mature and lyse bacterial cells to release more phages capable of infecting other bacterial cells. Lytic phages are host-specific, self-replicating, non-toxic, and inexpensive compared to the development of new antibiotics [9]. However, the use of bacteriophages as feed additives or directly on ready-to-eat food still faces several technical, environmental, and administrative challenges, which remain unresolved [10]. In addition, bacteria can develop resistance to phages, which negatively affects phage therapy outcomes. Phage resistance occurs when the target bacteria lose phage receptors, acquiring restriction-modification system that degrades injected phage nucleic acid or through gene mutation [11]. However, phage resistance can be overcome using phage cocktails. Despite phages being capable of reducing pathogens in food and live animals [12], their use in broiler chicken feeds still requires the optimization of several factors such as manufacturing and formulation, concomitant preparations (competitive exclusion), dosage, the timing of dosage, and route of administration [13]. Although studies have reported that some phages survive oral administration in poultry [14], phages differ in terms of their tolerance of physico-chemical factors such as pH, ions, and temperature [15,11,16]. While oral administration of phages in poultry is a more practical approach, the low pH levels in the proventriculus may compromise their stability and infectivity [17]. Encapsulating phages can protect them from harsh conditions, such as low pH levels and the host immune system, thereby improving their delivery to the intestines where they can reduce pathogenic microbes [18,19]. This study compared the effect of encapsulated dietary SPC and zinc-bacitracin on growth performance, physiological responses, intestinal histomorphology, and meat quality traits of Ross 308 chickens. We tested the hypothesis that in-feed encapsulated SPC would have similar positive effects on these parameters as the commonly used zinc-bacitracin antibiotic.

Materials and methods

Bacteria strains

Salmonella enteriditis (ATCC: 13076TM) and *Salmonella typhimurium* (ATCC:14028TM) reference strains obtained from Sigma Aldrich were used to isolate phages. Using a double-layer method, a total of five *Salmonella*-specific phages were isolated from sewage water samples around the study area using the *Salmonella* reference strains. All isolated phages were purified and characterized by determining their virulence potential, host range, and stability under different physiochemical conditions [20] before their use. The five phages were subjected to whole genome sequencing analysis, which revealed

Table 1
Gross ingredient and nutrient composition (g/kg, as fed basis) of the experimental diets.

Ingredients	Starter (0-10 days)	Grower (11-28 days)	Finisher (29-35 days)
Maize	44.20	49.00	53.00
Wheat	9.50	9.50	9.50
Soybean meal 46% CP	37.70	32.5	28.7
L-Lysine HCl	0.23	0.23	0.21
DL-Methionine	0.31	0.29	0.24
L-Threonine	0.09	0.09	0.08
Limestone fine	0.96	0.72	0.71
Monocalcium phosphate	1.96	1.73	1.49
Salt	0.27	0.27	0.27
Sodium bicarbonate	0.12	0.13	0.13
[1]Vitamin and mineral premix	1.00	1.00	1.00
Soy oil	3.30	4.06	4.19
Nutritional composition (g/kg DM, unless stated otherwise)			
Calculated ME (MJ/kg)	12.25	12.87	13.02
Crude protein	220.6	203.5	188.7
Dry matter	876.3	881.5	882.7
Ether extract	485.6	567.6	658.7
Neutral detergent fibre	261.4	298.3	323.1
Phosphorus	7.70	7.50	6.00
Calcium	8.30	6.30	5.20
Potassium	10.10	9.00	7.60
Sodium	1.50	1.30	1.30
Magnesium	1.80	1.80	1.40

[1] Vitamin and mineral premix: copper sulphate 8.0 mg; zinc sulphate 79 mg; ferrous sulphate 80 mg; niacin 30 mg; magnesium sulphate 100 mg; vitamin A 11,000 IU; potassium iodide 0.34 mg; pantothenic acid 10 mg; folic acid 0.7 mg; biotin 0.12 g; vitamin B6 5.1 mg; vitamin B1 2.5 mg; vitamin B2 4.5 mg; vitamin D3 2,500 IU; vitamin E 25 IU; vitamin K3 2.0 mg; and sodium selenite, 0.25 mg.

that they did not harbor any virulent or antimicrobial-resistant genes and thus considered to be safe for use in the feeding trial described below. The concentration of the phages was adjusted to 1×10^8 PFU/mL before being subjected to micro-encapsulation using a pH-dependent polymer (Eudragit® S100) and alginate [21]. To improve the outcome of the phage therapy, all five phages were encapsulated as a cocktail in equal titers using equal volumes of phage lysates [22]. Encapsulated phages were prepared by adding 1 % calcium carbonate and 1.8 % alginate with 5 % Eudragit® S100 to 50 ml of phage filtrate. The mixture was extruded into 1.8 % calcium chloride and washed in distilled water. The resulting particles were further coated with 0.4 % chitosan and stored at 4°C. The resultant pH-responsive micro-particles of *Salmonella*-specific phages were used to produce experimental diets.

Experimental diets

The dietary treatments were formulated (Table 1) by hand to meet the nutritional requirements of chickens [23] for the starter (1 – 10 d), grower (11 – 28 d), and finisher (29 – 35 d) phases as follows: 1) a negative control diet without phage and zinc bacitracin antibiotic (NC), 2) a positive control diet without phage but with 0.5 g/kg zinc bacitracin (PC), 3) a negative control diet with 0.075 g/kg of encapsulated *Salmonella*-specific phages (SP75), 4) a negative control diet with 0.1 g/kg of encapsulated *Salmonella*-specific phages (SP100), and 5) a negative control diet with 0.175 g/kg of encapsulated *Salmonella*-specific phages (SP175). The starter, grower, and finisher diet samples were then ground to a consistent 2 mm size (Retsch Cutting Mill BSM 100, Retsch, Germany). The dry matter, ash, organic matter (OM), and crude protein were determined using triplicate samples from each phase [24]. The neutral detergent fiber (NDF) was tested using the ANKOM^{2000} Fiber analyzer (ANKOM Technology, New York, NY) in accordance with the detergent methods by Van Soest *et al.* [25]. The mineral content was determined using the Agri-Laboratory Association of Southern Africa's guidelines [26]. The metabolizable energy (ME) of the diets was estimated using the formula by Carpenter and Clegg [27]:

$$[ME\ (kcal/kg) = (35\ \times CP\ \%) + (79.5\ \times EE\ \%) + (40.6 + NFE\ \%) + 190]$$

Feeding trial and bird management

A total of 400, one-day-old Ross 308 male chicks (49.84 ± 1.03 g live weight) were purchased from Superior Chicks (Pty) Ltd, (Pretoria, South Africa). The experiment was conducted in winter (May – June 2022) at North-West University's Molelwane Farm (25°86′00" S; 25°64′32" E), South Africa When they arrived, the chicks' body temperature was measured using a digital rectal thermometer (PRO RS-6070, ©RS Components (SA) Midrand, South Africa). Within the first three days of arrival, the birds were immunized against Newcastle and Gumboro diseases and offered drinking water in which a stress pack containing vitamins and minerals had been dissolved. The chicks were thereafter randomly allocated to one of five

Table 2
Mortality (%) of chickens reared for five weeks on diets containing encapsulated *Salmonella*-specific phages.

	[1]Diets					
	NC	PC	SP75	SP100	SP175	P-value
Week 1	3.13	2.27	0.00	1.04	2.08	0.5996
Week 2	0.00	3.23	0.00	0.00	0.00	0.1299
Week 3	0.96	3.52	3.04	3.31	4.18	0.7854
Week 4	0.00	0.00	0.00	0.00	1.14	0.4207
Week 5	0.00	0.00	0.00	0.00	0.00	-
Total	4.09	9.02	3.04	4.35	7.40	0.4267

[1] Diets: NC = a negative control diet without phage and zinc bacitracin antibiotic; PC = a positive control diet without phage but with 0.5 g/kg zinc bacitracin; SP75 = a negative control diet with 0.075 g/kg encapsulated *Salmonella*-specific phages; SP100 = a negative control diet with 0.10 g/kg encapsulated *Salmonella*-specific phages; SP175 = a negative control diet with 0.175 g/kg encapsulated *Salmonella*-specific phages.

experimental diets replicated 8 times and reared for 35 days. Each replicate pen (experimental unit), measuring 3.5 m in length, 1.0 m in width, and 1.85 m in height, held 10 birds translating to a density of 10 birds/3.5 m^2, and the floors were covered with wood shavings. For the SP75, SP100, and SP175 treatments, phages were only added to rations offered on days 1 – 2; 11 – 12; 21 – 22; and 29 – 30 of the feeding trial. These days were selected because they were identified as transition phases with 1 – 2 d being the beginning of the starter phase, 11 – 12 d being the transition to the grower phase, 21 – 22 being the second week of the grower phase, and 29 – 30 d being the transition to the finisher phase. Clean fresh water and feeds were given to the birds without any constraints via chicken drinkers and feeders, respectively. The house curtains were opened at 0900 h and closed at 1700 h to allow for ventilation. For the first two weeks, infrared electric lights were used to maintain the temperature at 34 ± 4°C. The temperature was then subsequently decreased by 2°C every week while the humidity was maintained between 55 ± 5% throughout the study. The birds were reared under natural lighting conditions (12 h of daylight).

Growth performance measurements

The difference between the feed offered and the feed declined was used to compute daily feed intake. The average weekly feed intake (AWFI) was determined using the daily feed intake data. The chicks' initial body weights (g) were recorded before treatment commenced and then weighed weekly to ascertain average weekly body weight gain (ABWG). The feed conversion efficiency (FCE) was computed using the weekly feed intake and weight gain data. The growth performance data were adjusted using the mortality values (Table 2) randomly observed from the treatment groups during the feeding trial.

Blood collection and evaluation

On day 35 of age, blood (4 mL) samples were drawn from 2 randomly picked birds per pen. The blood was drawn by puncturing the brachial vein using sterilized 23-gauge needles and 5 mL syringes. The blood samples were drawn into whole blood (with an anticoagulant) and serum (without an anti-coagulant) tubes. The whole blood tubes were kept in a cooler box and analysed within 48 hours of collection. Haematological parameters (haematocrits, white cell counts (WCC), platelets, and monocytes) were analysed with an automated LaserCyte Haematology Analyser (model no. 93−30001 −01, IDEXX Laboratories (Pty) Ltd., Gauteng, South Africa). The collected blood in serum tubes was centrifuged at 3500 rpm for 10 min (Centrifuge Z 206 A Hermle Labortechnik GmbH, Lasec SA (Pty) Ltd, Midrand, South Africa) to obtain sera. Serum biochemical indices (glucose, symmetric dimethyl arginine (SDMA), urea, phosphorus, calcium, total protein, albumin, globulin, albumin/globulin ratio, alanine aminotransferase (ALT), alkaline phosphatase (ALKP), gamma-glutamyl transferase (GGT), bilirubin, cholesterol, amylase, and lipase) were analysed with an automated IDEXX Catalyst One Chemistry Analyzer (model no. 89−92525−00, IDEXX Laboratories (Pty) Ltd., Gauteng, South Africa).

Slaughter, carcass characteristics, and internal organs

At 35 days, all the birds in each experimental unit were weighed to determine slaughter weight before being sent to a nearby abattoir. After a 2-hour rest period at the abattoir, the birds were stunned electrically and slaughtered by severing the jugular vein with a sharp knife. To measure hot carcass weight (HCW), the feathers, head, and feet were removed then carcasses were eviscerated by hand and promptly weighed on a digital scale (Model 330 Weighing, Richter Scale (Pty) Ltd., Gauteng, South Africa). To obtain cold carcass weight (CCW), the carcasses were reweighed after 24 hours of chilling at 4°C. The dressing percentage was calculated as the proportion of HCW on slaughter weight. The weights of carcass cuts (breast,

drumstick, thigh, and wing) were determined using the above-mentioned weighing scale and expressed as a proportion of CCW (%CCW). A digital weighing scale (Explorer EX224, OHAUS Corp, NJ, USA) was used to determine the weights of the internal organs (gizzard, liver, proventriculus, spleen, duodenum, jejunum, ileum, caecum, and colon). The intestinal lengths were measured using a tape measure (cm).

Intestinal morphological traits

The duodenum, jejunum, and ileum sections of the small intestine were placed into separate microcentrifuge tubes with 10% neutral buffered formalin. Samples were placed in the Leica HistoCore Pearl Tissue Processor (Leica Microsystems, Wetzlar, Germany) for tissue processing and paraffin wax infiltration. After processing the samples were then embedded using the Leica EG1150H embedder (Leica Microsystems, Wetzlar, Germany) and sectioned using the Leica RM2125RT microtome (Leica Microsystems, Wetzlar, Germany). Samples section sizes were between 3 and 5 µm. To evaluate general tissue morphology and for morphometric measurements, Haematoxylin and Eosin staining were done. Finally, the slides were dipped in xylene before the coverslip was mounted using a DPX mounting medium. The slides were viewed with a Nikon Eclipse E400 transmitted light microscope (Nikon Instruments Inc., Amsterdam, Netherlands). An image analysis software Digimizer, version 6 (Medcalc Software, Oostende, Belgium) was used for measurements of villus height, width, area, crypt depth, and muscle thickness. The villus height was measured from the villus tip to the junction of the villus crypt, while crypt depth was measured from the base to the transition region between the villus and the crypt [28]. The villus height/crypt depth ratio (VH:CD) was calculated [29].

Meat quality traits

The pH and temperature of breast meat were measured at 1 hour and 24 hours (pH_1, pH_{24}, $Temp_1$, and $Temp_{24}$) after slaughter using a digital meter fitted with a spear-piercing electrode (HI98163 Professional Portable pH-temperature meter, Hanna instruments (Pty) Ltd, JHB, South Africa). Standard solutions (pH 4, 7, and 10) were used to calibrate the meter for each replicate pen. At 1 and 24 h post-mortem, the lightness (L^*), redness (a^*), yellowness (b^*), hue angle, and chroma of breast meat were analysed using a colour spectrophotometer (Konica Minolta Chroma Meter CR-400, Narich (Pty) Ltd, Japan) with a 20 mm diameter measuring area, an illuminant D65-daylight, and a 10° observation angle [30]. The water-holding capacity (WHC) of the breast meat was determined using the filter-paper method [31], in which 60 kg pressure was applied for 5 minutes to samples (8 g) held in between two filter papers. Drip loss was assessed using the procedure by Honikel [31], in which breast meat samples (2 g; wet weight, w1) were hooked and suspended in a cold chamber (4°C) after 72 hours. Cooking losses in breast meat were determined after the samples were cooked to a core temperature of 75°C [31]. In accordance with the procedure outlined in our prior research [32], raw chicken breast samples were subjected to shear force testing as a means of evaluating their tenderness.

Data analysis

The repeated measures analysis option in the general linear model (GLM) procedure of SAS [33] was used to examine the interaction effects of time and diet on AWFI, ABWG, and FCE data. A one-way analysis of variance [33] was used to account for dietary differences in terms of growth performance, haemato-biochemical indices, internal organs, carcass characteristics, intestinal morphology, and meat quality traits data. Response surface regression analysis procedure [33] was used to evaluate the data (apart from the PC data) for linear and quadratic effects using the following non-linear model:

$$y = ax^2 + bx + c$$

where y is the dependent variable; a and b are the coefficients of the model; c is the intercept; x is dietary SB dosage; and $-b/2a$ is the x value that maximises or minimises a response parameter. For all the parameters, significance was set at $p < 0.05$ and the least square means were separated using the probability of difference option in SAS.

Results

Mortality of chickens

The total mortality (Table 2) in the present study was numerically highest in PC (9.02%) and the lowest in SP75 (3.04%). However, these differences were not statistically significant ($p > 0.05$).

Growth performance

Repeated measures analysis showed significant diet × week (age) interaction effects on ABWG ($p = 0.024$), but not on FI ($p = 0.509$) and FCE ($p = 0.460$). The overall FI showed a positive quadratic response [$y = 1.43$ (±0.69) x^2 – 25.45 (±10.87) x + 3048.3 (±33.85); $R^2 = 0.169$; $p = 0.048$] to increasing dosage of SPC (Table 3). The GLM result indicated that five-week-old birds reared on SP100 had lower body weight gains than those reared on NC, PC, SP75, and SP175, whose BWG did not vary ($p > 0.05$). Neither linear nor quadratic effects ($p > 0.05$) were observed for overall FCE.

Table 3
Growth performance in Ross 308 chickens (n = 400) fed diets containing encapsulated *Salmonella*-specific phages.

	[1]Diets						Significance		
	NC	PC	SP75	SP100	SP175	SEM	P-$_{GLM}$	P-$_{Linear}$	P-$_{Quadratic}$
	Body weight gain (g/bird)								
Week 1	122.0	122.2	120.1	118.4	131.5	5.318	0.469	0.230	0.198
Week 2	257.7	252.6	252.0	255.0	247.7	11.29	0.979	0.587	0.942
Week 3	430.5	445.6	429.5	416.4	423.7	12.49	0.569	0.543	0.736
Week 4	531.1	547.5	560.1	530.1	550.1	13.54	0.470	0.676	0.756
Week 5	645.5[a]	656.4[a]	634.6[a]	555.7[b]	611.2[a]	19.59	0.007**	0.070	0.136
	Overall growth performance								
Overall FI (g/bird)	3043	3089	2972	2921	2994	33.08	33.08	0.212	0.048
Overall FCE	0.65	0.66	0.67	0.64	0.66	0.009	0.255	0.634	0.792

[a,b] Means within a row without a common superscript are significantly different ($p < 0.05$).
[1] Diets: NC = a negative control diet without phage and zinc bacitracin antibiotic; PC = a positive control diet without phage but with 0.5 g/kg zinc bacitracin; SP75 = a negative control diet with 0.075 g/kg encapsulated *Salmonella*-specific phages; SP100 = a negative control diet with 0.10 g/kg encapsulated *Salmonella*-specific phages; SP175 = a negative control diet with 0.175 g/kg encapsulated *Salmonella*-specific phages.FI, feed intake; FCE, feed conversion efficiency.

Table 4
Haemato-biochemical parameters of five-week-old Ross 308 chickens (n = 80) fed with diets containing encapsulated *Salmonella*-specific phages.

	[1]Diets						Significance		
[2]Parameters	NC	PC	SP75	SP100	SP175	SEM	P-$_{GLM}$	P-$_{Linear}$	P-$_{Quadratic}$
Haematocrit (%)	35.13	32.94	34.13	34.81	34.75	0.636	0.071	0.875	0.454
WCC ($\times 10^9$/L)	12.84	14.60	14.21	15.24	14.20	0.842	0.376	0.167	0.141
Platelets ($\times 10^9$/L)	39.01	52.74	41.81	44.06	44.31	3.341	0.072	0.108	0.606
Monocytes (%)	0.81	1.10	0.80	1.30	1.34	0.182	0.115	0.020	0.874
Glucose (mmol/L)	5.58	5.60	5.83	5.50	5.68	0.409	0.985	0.963	0.953
SDMA (μg/dL)	26.44	23.25	21.13	23.19	23.75	2.505	0.681	0.619	0.280
Urea (mmol/L)	1.28	0.60	0.60	0.64	0.78	0.305	0.481	0.326	0.243
Phosphorus (mmol/L)	2.89	2.78	2.80	2.93	3.13	0.144	0.459	0.204	0.328
Calcium (mmol/L)	1.38	1.48	1.51	1.43	1.36	0.088	0.711	0.714	0.330
Total protein (g/L)	29.81	31.94	30.94	32.19	33.13	1.204	0.369	0.052	0.940
Albumin (g/L)	12.69	13.19	13.00	13.19	13.69	0.337	0.345	0.047	0.787
Globulin (g/L)	17.00	18.69	17.75	18.88	19.94	1.000	0.304	0.043	0.883
ALB/GLOB	0.76	0.74	1.15	0.70	0.79	0.182	0.416	0.633	0.463
ALT (U/L)	36.94	30.88	31.56	35.63	39.56	2.351	0.066	0.307	0.080
ALKP (U/L)	743.6	803.1	822.9	763.9	657.4	96.22	0.773	0.387	0.260
GGT (U/L)	12.38	8.38	12.38	7.00	9.75	2.359	0.404	0.248	0.588
Bilirubin (μmol/L)	6.31	7.13	7.58	8.63	10.25	1.543	0.436	0.070	0.906
Cholesterol (mmol/L)	2.80	2.71	2.90	2.94	2.78	0.137	0.771	0.728	0.347
Amylase (U/L)	470.5	486.9	492.7	480.9	526.6	49.48	0.945	0.453	0.780
Lipase (U/L)	196.6	162.4	185.3	163.3	178.5	17.69	0.607	0.372	0.487

[1] Diets: NC = a negative control diet without phage and zinc bacitracin antibiotic; PC = a positive control diet without phage but with 0.5 g/kg zinc bacitracin; SP75 = a negative control diet with 0.075 g/kg encapsulated *Salmonella*-specific phages; SP100 = a negative control diet with 0.10 g/kg encapsulated *Salmonella*-specific phages; SP175 = a negative control diet with 0.175 g/kg encapsulated *Salmonella*-specific phages.
[2] Parameters: ALB/GLOB, Albumin/globulin ratio; WCC, white cell count; SDMA, symmetric dimethylarginine; ALT, alanine aminotransferase; ALKP, alkaline phosphatase; GGT: gamma-glutamyl transferase.

Haemato-biochemical indices

The inclusion of varying SPC dosages caused a linear increase on monocytes [y = 0.03 (±0.057) x + 0.77 (±0.179); R^2 = 0.173; p = 0.020], albumin [y = 0.04 (±0.108) x + 12.71 (±0.335); R^2 = 0.131; p = 0.047], and globulin [y = 0.15 (±0.329) x + 16.98 (±1.024); R^2 = 0.134; p = 0.043] (Table 4).

Carcass characteristics and internal organs

The inclusion of SPC in the diet, induced neither linear nor quadratic effects ($p > 0.05$) for carcass characteristics and internal organs except for breast and proventriculus weights (Table 5). A negative quadratic response was noted for breast weight [y = 15.27 (±0.587) + 0.60 (±0.188) x - 0.03 (±0.012) x^2; R^2 = 0.418; p = 0.046], while a negative linear effect was recorded for proventriculus size [y = 0.57 (±0.048) – 0.03 (±0.015) x; R^2 = 0.195; p = 0.024]. There was a treatment effect ($p < 0.05$) on breast weight only, where birds reared on SPC (SP75, SP100, and SP175) had heavier ($p < 0.05$) breast weights than the birds reared on NC but did not differ ($p > 0.05$) from the birds in PC.

Table 5
Carcass characteristics and internal organs (%CCW, unless stated otherwise) of five-week-old Ross 308 chickens (n = 320) fed diets containing encapsulated *Salmonella*-specific phages.

	[1]Diets						Significance		
[2]Parameters	NC	PC	SP75	SP100	SP175	SEM	P-$_{GLM}$	P-$_{Linear}$	P-$_{Quadratic}$
Slaughter weight (g)	2038	2075	2046	1925	2013	37.31	0.074	0.254	0.287
HCW (g)	1488	1538	1475	1438	1475	33.61	0.350	0.776	0.292
CCW (g)	1388	1425	1400	1350	1450	34.00	0.311	0.331	0.286
Dressing (%)	72.76	73.17	72.00	74.20	73.85	1.007	0.564	0.262	0.849
Breast	15.18[b]	17.65[a]	17.89[a]	18.45[a]	18.64[a]	0.638	0.004	0.003	0.046
Wing	5.14	5.20	5.19	5.36	5.11	0.133	0.725	0.863	0.230
Drumstick	5.95	6.29	5.87	6.17	5.89	0.152	0.230	0.848	0.533
Thigh	6.39	6.48	6.64	6.84	6.78	0.193	0.425	0.067	0.331
Gizzard	2.32	2.38	2.41	2.34	2.32	0.089	0.943	0.863	0.546
Liver	2.93	3.06	2.94	2.98	2.85	0.101	0.685	0.560	0.430
Proventriculus	0.58	0.50	0.45	0.43	0.41	0.048	0.143	0.024	0.260
Spleen	0.20	0.19	0.22	0.23	0.21	0.012	0.211	0.690	0.128
Duodenum	1.36	1.32	1.35	1.34	1.35	0.043	0.964	0.884	0.787
Jejunum	2.24	2.19	2.17	2.14	2.29	0.089	0.780	0.770	0.177
Ileum	1.82	1.91	1.85	1.95	2.88	0.415	0.340	0.116	0.327
Caecum	0.91	0.94	0.99	1.28	0.98	0.149	0.437	0.517	0.267
Colon	0.13	0.13	0.13	0.14	0.14	0.006	0.373	0.121	0.815
Duodenum (cm)	21.90	23.20	21.60	23.50	22.10	0.086	0.451	0.552	0.593
Jejunum (cm)	58.30	54.10	57.90	59.10	57.50	0.169	0.286	0.898	0.744
Ileum (cm)	54.00	57.20	55.90	58.40	54.90	0.548	0.142	0.492	0.127
Caecum (cm)	14.70	13.50	14.60	15.50	14.30	0.046	0.057	0.908	0.310
Colon (cm)	3.70	3.50	4.30	6.30	4.20	0.118	0.468	0.558	0.312

[a,b] Means within a row without a common superscript are significantly different ($p < 0.05$).
[1] Diets: NC = a negative control diet without phage and zinc bacitracin antibiotic; PC = a positive control diet without phage but with 0.5 g/kg zinc bacitracin; SP75 = a negative control diet with 0.075 g/kg encapsulated *Salmonella*-specific phages; SP100 = a negative control diet with 0.10 g/kg encapsulated *Salmonella*-specific phages; SP175 = a negative control diet with 0.175 g/kg encapsulated *Salmonella*-specific phages
[2] Parameters: HCW, hot carcass weight; CCW, cold carcass weight.

Intestinal morphological traits

Except for crypt depth and VH:CD in the jejunum and villus height and villus area in the ileum, the addition of SPC in the diet resulted in either linear or quadratic effects (Table 6). A positive quadratic effect was noted for duodenal villus height [$R^2 = 0.935$; $p = 0.003$], width [$R^2 = 0.882$; $p = 0.009$], and area [$R^2 = 0.929$; $p = 0.001$]; jejunal villus height [$R^2 = 0.914$; $p = 0.001$], width [$R^2 = 0.917$; $p = 0.002$], area [$R^2 = 0.903$; $p = 0.001$], and muscle thickness [$R^2 = 0.970$; $p = 0.001$]; ileal villus width [$R^2 = 0.747$; $p = 0.006$], and crypt depth [$R^2 = 0.916$; $p = 0.001$]. A negative quadratic effect was observed for duodenal muscle thickness [$R^2 = 0.968$; p = 0.013], ileal muscle thickness [$R^2 = 0.858$; $p = 0.029$], and VH:CD [$R^2 = 0.596$; $p = 0.040$]. In addition, a positive and negative linear effect was noted for duodenal crypt depth [$R^2 = 0.753$; $p = 0.005$] and VH:CD [$R^2 = 0.908$; $p = 0.001$], respectively.

There was a treatment effect ($p < 0.05$) on intestinal morphological traits. In the duodenum, the SPC group (SP75, SP100, and SP175) had lower villus height than those in PC. Birds on SP175 had the lowest villus width, followed by those in PC, SP75, and SP100, and the highest villus width was from those in NC. The SPC treatment group (SP75, SP100, and SP175) promoted lower villus area and crypt depth than the PC. The PC promoted lower muscle thickness than the SPC group (SP75, SP100, and SP175). The PC promoted lower VH:CD compared to SP75. At the jejunum, birds reared on SP175 had the highest villus height, followed by those in NC, SP100, and PC, and the lowest was the SP75 group. PC promoted lower villus width and area compared to those in the SPC group (SP75, SP100, and SP175). Birds reared on SP100 had the highest crypt depth, followed by those in NC, SP175, and PC, and the lowest was from SP75. The PC promoted higher muscle thickness compared to the SPC group (SP75, SP100, and SP175). Birds reared on SP100 had the lowest VH:CD, followed by those in PC, NC, and SP175, and the highest was from SP75. At the ileum, PC promoted lower villus height, width, area, muscle thickness, and VH:CD compared to those in the SPC group (SP75, SP100, and SP175). Birds reared on PC had higher crypt depth than those reared in the SPC group (SP75, SP100, and SP175).

Meat quality traits

Table 7 shows that the inclusion of SPC in the diets resulted in a negative linear effect for pH_1 [$y = 5.75$ (± 0.048) – 0.01 (± 0.015) x; $R^2 = 0.161$; $p = 0.040$] and positive quadratic response for chroma [$y = 0.01$ (± 0.002) x^2 – 0.08 (± 0.033) $x + 4.27$ (± 0.103); $R^2 = 0.184$; $p = 0.024$]. The GLM results showed that birds reared on the SPC treatments (SP75, SP100, and SP175) had lower ($p < 0.05$) WHC than those reared on the PC treatment but did not vary ($p > 0.05$) with the birds reared on the NC treatment.

Table 6
Intestinal morphological traits (μm, unless stated otherwise) of five-week-old Ross 308 chickens (n = 320) fed diets containing encapsulated *Salmonella*-specific phages.

[2]Parameters	[1]Diets					SEM	Significance		
	NC	PC	SP75	SP100	SP175		P-$_{GLM}$	P-$_{Linear}$	P-$_{Quadratic}$
	Duodenum								
Villus height	640.7[a]	621.0[b]	574.7[c]	554.7[d]	558.3[d]	5.527	0.001	0.001	0.003
Villus width	87.67[a]	62.00[b]	63.00[b]	64.00[b]	57.33[c]	1.022	0.001	0.001	0.009
Villus area (mm)	178.0[a]	119.0[b]	112.0[c]	110.0[d]	97.67[e]	0.649	0.001	0.001	0.001
Crypt depth	135.0[d]	176.3[a]	134.3[d]	166.0[b]	163.3[c]	0.447	0.001	0.005	0.846
Muscle thickness	258.3[a]	239.7[e]	257.3[b]	251.3[c]	246.7[d]	0.333	0.001	0.001	0.013
VH:CD	4.75[a]	3.52[c]	4.28[b]	3.34[e]	3.41[d]	0.045	0.001	0.001	0.050
	Jejunum								
Villus height	547.3[b]	485.3[d]	473.3[e]	502.7[c]	551.0[a]	0.537	0.002	0.187	0.001
Villus width	80.33[a]	47.33[d]	57.33[c]	58.00[b]	57.33[c]	0.394	0.012	0.001	0.002
Villus area (mm)	137.3[a]	71.67[e]	85.33[d]	91.33	99.00[b]	0.394	0.004	0.003	0.001
Crypt depth	190.3[b]	171.0[d]	155.3[e]	213.3[a]	185.7[c]	0.394	0.004	0.479	0.789
Muscle thickness	298.3[a]	258.3[b]	241.3[c]	233.3[d]	232.3[e]	0.333	0.003	0.001	0.001
VH:CD	2.88[c]	2.84[d]	3.05[a]	2.36[e]	2.97[b]	0.006	0.004	0.579	0.206
	Ileum								
Villus height	390.4[c]	354.3[e]	369.3[d]	476.2[a]	409.1[b]	0.803	0.003	0.138	0.334
Villus width	75.34[a]	44.32[e]	51.31[d]	58.33[b]	56.30[c]	0.667	0.001	0.005	0.006
Villus area (mm)	91.34[a]	48.30[e]	59.29[d]	86.33[e]	71.32[c]	0.668	0.001	0.354	0.289
Crypt depth	196.2[a]	174.4[b]	166.3[c]	135.5[e]	159.3[d]	0.665	0.001	0.001	0.001
Muscle thickness	214.2[e]	231.1[d]	251.4[b]	247.3[c]	261.0[a]	0.669	0.002	0.001	0.029
VH:CD	1.99[e]	2.03[d]	2.22[c]	3.52[a]	2.57[b]	0.007	0.004	0.023	0.040

[a,b] Means within a row without a common superscript are significantly different ($p < 0.05$).
[1] Diets: NC = a negative control diet without phage and zinc bacitracin antibiotic; PC = a positive control diet without phage but with 0.5 g/kg zinc bacitracin; SP75 = a negative control diet with 0.075 g/kg encapsulated *Salmonella*-specific phages; SP100 = a negative control diet with 0.10 g/kg encapsulated *Salmonella*-specific phages; SP175 = a negative control diet with 0.175 g/kg encapsulated *Salmonella*-specific phages.

Discussion

Mortality of chickens

In the poultry industry, bacterial infections can cause significant economic losses due to reduced productivity and increased mortality rates. Phage cocktails offer a promising alternative to antibiotics, as they are highly specific to the bacteria they infect and do not harm beneficial bacteria or the host animal. They can be used to prevent and/or treat bacterial infections in poultry, including those caused by *Salmonella*. Studies have shown that phages can significantly reduce mortality rates in poultry, as well as improve feed conversion, growth rates, and overall health [34,6], and their use may help to reduce the reliance on antibiotics in the industry. In this study, birds on SPC treatments (SP75, SP100, and SP175) had numerically lower mortalities (3.04, 4.35, and 7.40%) compared to birds reared on the zinc-bacitracin-containing positive control (9.02%) during the five weeks feeding trial. However, these differences were not statistically significant.

Growth performance

The administration of encapsulated SPC in diets of broiler chickens is aimed at reducing *Salmonella* pathogens in the gut to enhance the proliferation of beneficial microbes and, thus improve gut health, nutrient absorption, and bird performance [35]. Repeated measures analysis indicated significant interaction effects between diet and week (age) on average weekly weight gain, demonstrating that the relative dietary effects on body mass depended on the birds' age. The noted treatment effect on weight gain for five-week-old birds contradicts the report by Wang *et al.* [36], who observed that the BWG of Arbor Acres chickens was not influenced by the inclusion of 0.5 g/kg phage (*Salmonella gallinarum, S. typhimurium*, and *S. enteritidis* at 3:3:4; containing 10^8 pfu/g) under normal physiological conditions. These differences could be because the phages used in this study were encapsulated, suggesting that they were still viable when delivered in the lower GIT. However, current results corroborate Upadhaya *et al.* [35], who observed a linear increase in BWG when a phage cocktail (consisting of *Salmonella gallinarum, S. typhimurium, S. enteritidis and Escherichia coli* at 10^8 pfu/g each, and *Clostridium perfringens* at 10^6 pfu/g) was supplemented in diets of Ross 308 chickens. Increased BWG is an essential characteristic in broiler production because increased BWG correlates to high-profit margins from saleable meat products. The similarities in BWG of SPC-reared birds and the PC group was a desirable outcome because it shows that phages may have had a prohibitive impact on pathogens in the GIT comparable to that of the commercial antibiotic, zinc-bacitracin.

Contrary to the findings of Kim *et al.* [37] where FI in broilers was unaffected by dietary anti-*Salmonella enteritidis* phage (0.05, 0.1, and 0.2; 10^9 pfu/g) that were not encapsulated, the inclusion of SPC at 0.075, 0.1, and 0.175 g/kg levels showed

Table 7
Breast meat quality traits of five-week-old Ross 308 chickens (n = 320) fed diets containing encapsulated *Salmonella*-specific phages.

[2]Parameters	[1]Diets					SEM	Significance		
	NC	PC	SP75	SP100	SP175		P-$_{GLM}$	P-$_{Linear}$	P-$_{Quadratic}$
pH_1	5.75	5.81	5.76	5.79	5.89	0.054	0.336	0.040	0.342
$Temp_1$	23.48	24.53	23.12	25.12	24.80	0.979	0.542	0.192	0.987
L^*_1 (Lightness)	44.98	46.60	46.08	45.86	44.90	0.684	0.353	0.880	0.115
a^*_1 (Redness)	2.84	2.26	2.52	2.31	2.41	0.256	0.518	0.136	0.351
b^*_1 (Yellowness)	1.94	1.69	1.88	1.48	1.78	0.167	0.353	0.171	0.208
Hue $angle_1$	1.15	1.14	1.18	1.19	1.17	0.044	0.472	0.216	0.307
$Chroma_1$	4.06	4.15	4.21	4.00	4.20	0.142	0.473	0.563	0.853
pH_{24}	6.03	5.85	5.88	5.91	5.94	0.061	0.420	0.185	0.318
$Temp_{24}$	12.92	12.11	12.67	12.36	13.03	0.291	0.167	0.997	0.134
L^*_{24} (Lightness)	43.27	44.00	44.47	43.76	43.59	0.446	0.410	0.895	0.111
a^*_{24} (Redness)	3.42	3.27	3.14	3.13	3.43	0.166	0.566	0.976	0.059
b^*_{24} (Yellowness)	2.51	2.67	2.49	2.26	2.32	0.126	0.181	0.114	0.709
Hue $angle_{24}$	0.93	0.88	0.90	0.94	0.97	0.038	0.501	0.361	0.392
$Chroma_{24}$	4.26	4.25	4.02	3.89	4.16	0.138	0.279	0.362	0.024
Drip loss (%)	14.90	15.54	13.27	13.54	16.26	1.182	0.340	0.447	0.097
Cooking loss (%)	14.08	13.80	12.63	12.72	14.95	1.546	0.811	0.703	0.248
WHC (%)	91.61^b	93.51^a	89.79^b	90.74^b	91.10^b	0.746	0.018	0.870	0.175
Shear force	2.62	2.43	2.31	2.42	2.56	1.364	0.978	0.810	0.976

[a,b] Means within a row without a common superscript are significantly different ($p < 0.05$).
[1] Diets: NC = a negative control diet without phage and zinc bacitracin antibiotic; PC = a positive control diet without phage but with 0.5 g/kg zinc bacitracin; SP75 = a negative control diet with 0.075 g/kg encapsulated *Salmonella*-specific phages; SP100 = a negative control diet with 0.10 g/kg encapsulated *Salmonella*-specific phages; SP175 = a negative control diet with 0.175 g/kg encapsulated *Salmonella*-specific phages.
[2] Parameters: pH_1, pH 1-hour post-mortem; pH_{24}, pH 24-hours post-mortem; WHC, water holding capacity.

positive quadratic effects on overall FI in the present study. This might be attributed to the survivability of phages through the GIT to the site of interest because of the microencapsulation [27]. Inconsistent with our study, Ngu *et al.* [6] demonstrated that the overall FI was reduced.

Despite their much-touted lytic effect on pathogens that are reported to have growth-stimulating activities, the inclusion of SPC up to 0.175 g/kg did not have any significant effect on the overall FCE of the Ross 308 birds. The lack of treatment effects on FCE in the current study suggests the need to investigate phage inclusion levels higher than 0.175 g/kg and/or the use of mixtures of phages to target a wider range of gut pathogen species. Comparable to the present results, Upadhaya *et al.* [35] reported that inclusion levels of dietary phage between 0.05 and 0.1 g/kg did not influence the feed conversion ratio in Ross 308 chickens. Similarly, Wang *et al.* [36] and Ngu *et al.* [6] found that the inclusion of phage in broiler chicken diets had no influence on the feed conversion ratio. Research reports suggest that phage mixtures targeting several pathogenic organisms have a more effective impact than phage targeting a single pathogenic organism in enhancing broiler performance hence the benefit of using phage cocktails over a single phage [38,22].

Haemato-biochemical parameters

Hemato-biochemical parameters are the most reliable indicators of bird health and pathophysiological status [39]. Blood evaluation allows for the clinical analysis of different metabolites to measure nutritional, pathological, and physiological responses in animals [40]. The inclusion of SPC in the diets elicited a positive linear effect on monocytes, which are a critical component of the innate immune system that can differentiate into macrophages in response to an infection or accumulation of damaged bacterial cells. Studies have shown that immune cell production can be stimulated directly by phages and indirectly by the massive pathogen-associated patterns released during bacterial cell lyses or phage-infected bacteria [41]. Monocytes become activated by the presence of phage-infected bacteria and release their own cytokines in response [42]. This activation of monocytes can lead to increased phagocytosis (engulfing and digesting of foreign particles) and other immune responses, which can help to control bacterial infections [43]. The current study findings vary with those of Wang *et al.* [36], who reported that the usage of phages in broiler diets did not affect the haematological values. Albumin and globulin are a group of proteins in the blood produced by the liver to help fight infections and move nutrients throughout the body. Hence, the positive linear effect observed for albumin and globulin in response to SPC inclusion could be ascribed to the interaction between phage and the immune system. Phages can stimulate the immune system to produce more antibodies, which may indirectly increase the levels of serum albumin and globulin [44]. Furthermore, the observed modulation of the immune system by SPC indicates its potential to protect birds from liver inflammation. Standard indicators for hepatocellular damage include alanine transaminase (ALT), alkaline phosphatase (ALKP), and gamma-glutamyl transferase (GGT). In situations of hepatic injury, the ALT, ALKT, and GGT enzymes found in the cytoplasm of hepatocytes are released into the blood. Thus, increased concentrations of ALT, ALKT, and GGT in the bloodstream imply hepatocellular malfunction

[45]. Therefore, the comparable ALT, ALKT, and GGT values among treatments further indicate that the inclusion of SPC did not compromise the health of the birds.

Carcass characteristics and internal organs

The addition of the SPC in the diets of Ross 308 chickens did not elicit any variation in carcass characteristics except for breast weight. The current results were consistent with those of Ngu *et al.* [6], who reported higher breast weight in Noi broilers reared on diets containing *S. typhimurium*, the B1 and B2 bacteriophages. However, Wang *et al.* [36] and Upadhaya *et al.* [35] reported that the incorporation of phage up to 0.5 g/kg and 0.1 g/kg, respectively did not affect the breast weight of broiler chickens. The same authors also observed no changes in the sizes of internal organs measured when phage was supplemented in the diets of broiler chickens (36, 35]. Unlike in the current study, Ngu et al. [6] observed that dietary phages influenced the liver, caecum, small intestine, spleen, and gizzard weights in Noi broiler diets. The inconsistencies in bacteriophage feeding studies could be attributed to phage dosage, multiple or single phage administration, route of supplementation, and species differences. In the present study, negative linear responses were observed for proventriculus weight as SPC dosage increased. However, the weights of the gizzard, liver, spleen, caecum, duodenum, and jejunum were not affected, implying that SPC inclusion did not compromise the GIT and internal organs of the birds.

Intestinal morphological traits

The intestinal morphological traits are excellent predictors of GIT health and feed utilization. A higher villus height is correlated to higher nutrient absorption since a larger villus area is required for enhanced nutrient uptake [46]. The villus crypt has been identified as a key spot that provides stem cells for renewal of the intestinal epithelium, which turns over every 3 to 4 days [47]. In the current study, the duodenal morphological traits were not enhanced with SPC dosage. However, the jejunal and ileal villus height and VH:CD was higher in birds on SPC treatments. This corroborates the findings of Sarrami *et al.* [47], who reported increased jejunal villus height and VC:CD when phages were included in the diets of Ross 308 chickens. However, in this current study, there was no resultant improvement in the growth performance contrary to findings by Sarrami *et al.* [47]. Despite the positive changes in the jejunal and ileal villus height and VH:CD, the lack of improved growth performance in broiler chickens could be due to the lack of influence of phages on other key intestinal parameters (duodenal, jejunal, or ileal villus width and area). In addition, this outcome can also be attributed to the presence of other bacterial pathogens found in the gut of chickens, which means that preventing the colonization of *Salmonella* species alone could have left other pathogens to negatively affect the duodenal, jejunal, or ileal villus and growth performance of the chicken. Future studies can, therefore, investigate the efficacy of phage cocktails targeting various bacterial pathogens in the gut instead of *Salmonella* pathogens only.

Meat quality

A combination of sensory attributes used to determine meat quality includes appearance, odour, colour, texture, tenderness, taste, juiciness, and water-holding capacity [48]. These characteristics are used to evaluate meat's biological, chemical, and physical qualities [49]. Feeding varied doses of SPC had no effect on meat quality measures but decreased the WHC, which indicates that the use of encapsulated SPC compromised the ability of the meat to retain water. WHC measures meat's ability to retain water during processing, cooking, and storage. It impacts juiciness, tenderness, and sensory attributes. The decrease in WHC observed in this study could be attributed to the action of SPC on the bacterial populations in the broiler gut. The encapsulated SPC target and kill *Salmonella sp*, and by doing so, they can potentially alter the microbial composition in the gut. This alteration may affect the gut environment and the processes involved in water retention in the meat [50]. The current results were inconsistent with those of Upadhaya *et al.* [35], who recorded no influence in breast meat WHC in broiler chickens reared on phage-containing diets. Furthermore, the lack of effects on cooking loss and drip loss values among the treatments suggests that the encapsulated phages did not interfere with the normal oxidative stability levels of the meat. According to Wang *et al.* [51] and Chen *et al.* [52], oxidative stress is a biological phenomenon that negatively impacts meat quality by hastening the pace at which meat pH drops and lowering meat WHC. Furthermore, Bowker and Zhuang [53] observed that post-mortem metabolism promotes a drop in muscle pH, which lowers the overall ions of the muscle proteins, resulting in sarcoplasmic protein denaturation and consequently poor meat WHC. The observed differences in WHC could be explained by the negative linear effect on the pH_1 values across the treatments since there is a significant correlation between muscle pH and WHC.

The meat colour of breast muscle did not vary in the present study even with the linear effect on pH_1 suggesting that the similarity in meat colour was not due to pH changes. Nonetheless, Wang *et al.* [36] revealed that the addition of phages to the standard diet of broiler chickens had no effect on either meat colour or meat pH. Furthermore, there is no clear explanation for the observed positive quadratic effect in the breast meat chroma.

Conclusions

The administration of encapsulated *Salmonella*-specific phages cocktails in the diets of Ross 308 chicken diets enhanced overall feed intake, and breast meat chroma but had a negative quadratic effect on breast meat weight. Furthermore, it

induced changes in intestinal morphology but had no effect on the haemato-biochemical parameters of the birds. Compared to the negative control, SPC induced positive changes in the jejunal and ileal villus height and VH:CD, however, this did not result in improved growth performance of broiler chickens. Based on the lack of statistical differences between the positive control and SPC-containing diets on key response parameters, it can be concluded that periodic SPC inclusion has the potential to replace subtherapeutic antibiotic use in poultry production.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Sicelo Beauty Dlamini: Conceptualization, Data curation, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Caven Mguvane Mnisi:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Collins Njie Ateba:** Conceptualization, Supervision, Validation, Visualization, Writing – review & editing. **Chidozie Freedom Egbu:** Validation, Visualization, Writing – original draft, Writing – review & editing. **Victor Mlambo:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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