

Dietary Bacteriophage Supplementation on Laying Performance and Antioxidant Capacity of Hy-Line Brown Hens During the Late Laying Period

Zhaoxin Zhong^{1,2}, Kai Shi^{1,2}, Zhiqing Yang^{1,2*}, Chongfu Jin^{1,2}, Fuyin Hou^{1,2}, Chen An^{1,2}, Hua Sun^{1,2} and Xia Xiao^{1,2}

¹Institute of Agricultural Sciences in the Coastal Area in Jiangsu, Jiangsu Academy of Agricultural Sciences, Yancheng 224002, Jiangsu, China.

²Yancheng Livestock Breeding Equipment Engineering Technology Research Center, Yancheng 224002, Jiangsu, China

ABSTRACT

In this study, the effects of 3 graded dietary levels (0.1%, 0.3%, and 0.5%) of bacteriophage were studied in Hy-line brown hens during the late laying period (72–79 week). A total of 240 Hy-line brown hens (70-week-old) were randomly divided into 4 groups (3 replicates of 20 hens): basal diet group (Con), and basal diets supplemented with 0.1%, 0.3%, and 0.5% of bacteriophage (B1, B2, and B3), respectively. Results showed that dietary supplementation with 0.1% and 0.5% of bacteriophage increased laying rate and egg mass at 72–75week ($P < 0.05$). Meanwhile, dietary bacteriophage had significant effects on egg-feed ratio ($P < 0.05$). At 76–79week, dietary bacteriophage had significant effects on laying rate and egg mass and egg-feed ratio ($P < 0.05$). At 72–75week, dietary 0.1% bacteriophage had improved the content of glutathione (GSH) in jejunum and liver ($P < 0.05$), increased the activity of total antioxidant capacity (T-AOC), superoxide dismutase (SOD) in ileum ($P < 0.05$) and increased the activity of T-AOC in liver ($P < 0.05$). Dietary 0.3% bacteriophage had improved the activity of T-AOC in liver ($P < 0.05$). Dietary 0.5% bacteriophage had improved the activity of T-AOC in jejunum and the content of GSH in liver ($P < 0.05$). At 76–79week, the 3 treatment groups showed decreased the concentration of malondialdehyde (MDA) in liver ($P < 0.05$). At 72–75week, dietary bacteriophage supplementation increased the expression levels of *CAT*, *SOD1* and *GPXI* ($P < 0.05$). At 76–79week, the expression level of *CAT* was increased in B1 group and B2 group in liver ($P < 0.05$). In conclusion, supplementation with 0.1% and 0.3% bacteriophage both could improve the laying rate, jejunum ileum and liver antioxidant capacity of Hy-line brown hens during the late laying period.

Article Information

Received 06 March 2024

Revised 05 May 2025

Accepted 21 May 2025

Available online 22 September 2026 (early access)

Authors' Contribution

ZZ, ZY: Conceptualization; ZZ, CJ, FH, CA, HS, XX, ZY: Investigation; ZY, CJ: Methodology; KS, ZZ, FH, XX: data curation; KS, CA: Validation; ZZ: Formal analysis, supervision, funding acquisition; ZZ, KS, ZY: Writing – original draft; HS, FH, XX, CJ: Resources; HS: Visualization; CA: Software. All authors have read and agreed to the published version of the manuscript.

Key words

Bacteriophage, Catalase gene, Hy-line brown hens, Laying performance, Antioxidant capacity, Late laying period, Superoxide dismutase gene, Malondialdehyde gene, Glutathione gene

INTRODUCTION

For humans, poultry eggs are a valuable source of proteins, lipids, amino acids, and minerals (Yao *et al.*, 2022). To meet market demand, longer egg laying cycles can reduce production costs for laying hens. The feeding cycle of laying hens has been extended, from the traditional 72 weeks production cycle to 100 weeks, resulting in a longer duration of egg laying in the later stages (Bain *et al.*, 2016).

However, the main problems of physiological decline in laying hens during the later stages of egg production include decline in the reproductive system, disruption of intestinal function, decreased antioxidant capacity and immunity, which leads to a decline in egg production performance, gradual thinning of eggshells, and an increasing rate of breakage (E *et al.*, 2023; Yao *et al.*, 2023). Therefore, improving the egg production rate and antioxidant function of laying hens in the later stage of egg production is a research hotspot and scientific challenge in the laying hens breeding industry.

Bacteriophage, known as the natural predators of bacteria, may be the most numerous biological entities on earth (Brussow, 2005). It caused specific infection of bacteria, so can achieve the effect of sterilization (Merabishvili *et al.*, 2012). Phages have several advantages over antibiotics, including a strong antibacterial effect, ease of isolation and preparation,

* Corresponding author: 1182263434@qq.com
0030-9923/2025/0001-0001 \$ 9.00/0



Copyright 2025 by the authors. Licensee Zoological Society of Pakistan.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

high efficiency in infection without affecting normal flora, and few side effects (Kortright *et al.*, 2019; Styles *et al.*, 2022). In the research and application of poultry production, bacteriophage can be used as a substitute for antibiotics to promote healthy breeding of laying hens, and have advantages such as high efficiency, safety, and environmental protection. In the research and application of poultry production, bacteriophage can be used as a substitute for antibiotics to promote healthy breeding of laying hens, and have advantages such as high efficiency, safety, and environmental protection.

In present study, the results suggested that this intervention has the potential to extend the laying lifespan and enhance egg production. This study demonstrates that bacteriophage supplementation can improve the laying performance and antioxidant capacity of Hy-line brown hens. These findings provide a theoretical foundation for developing dietary interventions that can improve the health and productivity of poultry in the poultry industry. The practical implications of this study could lead to reduced production costs and increased economic benefits for poultry farming.

MATERIALS AND METHODS

Birds and experimental design

Randomly divide all 70-weeks old hens into a control group and a phage group (0.1%, 0.3%, and 0.5%) for 3 replicates, with 20 chickens per replicate. Prior to this, a 2-week pre-experiment was conducted to ensure that all hens used in this study were in the same healthy state and egg laying rate. The control group (Con) of hens were fed with a basic diet (Table 1), which met all NRC requirements. The hens of three bacteriophage groups (B1, B2, B3) were supplemented with 0.1%, 0.3%, and 0.5% bacteriophage powder in the basal diet, respectively. The bacteriophage used in this study were provided by Jiangsu Provincial Key Laboratory of Food Quality and Safety and the cultivation base of State Key Laboratory jointly built by the province and ministry.

Laying performance

The metrics for laying performance were the average daily feed intake (ADFI), egg production, weight and number of eggs laid. The mean weight of all the eggs from each duplicate was used to compute the average egg weight, and weekly measurements of the egg-feed ratio were made. Every week, the AFDI was calculated by deducting each replicate's final feed weight from its beginning feed weight.

Sample collection

At the middle and end of the experiment, three hens

(9 per group) were randomly selected and weighed in each replicate. Liver, jejunum and ileum samples were collected and stored at -80°C for analysis of antioxidant parameters.

Table 1. Dietary ingredients and nutrient levels.

Items	Content (%)
Ingredients	
Corn	60.5
Soybean meal	23.5
Shellac	10
Bran	2
Pepper seed powder	2
Premix ¹	2
Total	100
Nutrient levels²	
Metabolizable energy (Mcal/kg)	10.87
Crude protein	15.5
Calcium	3.5
Total phosphorus	0.6
Methionine	0.32
Lysine	0.7
Trp	0.15

Premix provided the following per kilogram of diet: vitamin A, 12 000 IU; vitamin D₃, 4 000 IU; salt, 3 g; Methionine, 1 g; choline chloride, 1.25g; complex enzyme, 200 mg; pyridoxine 6 mg; folic acid, 1.5 mg; chitin 250 mg; Fe, 80 mg; Zn, 80 mg; Cu, 8 mg. Metabolizable energy is a theoretical value; calcium, phosphorus, and amino acid amino acid content are measured.

Measurement of liver and intestine antioxidant indicators

Liver, jejunum and ileum were separated on an ice tray and homogenized in cold saline with a weight to volume ratio of 1:9. The homogenate was centrifuged at 9000 g for 10 min at 4°C with agitation, and the supernatant was collected for concentration analysis using a kit purchased from BUILT (Nanjing, China).

Quantitative real-time PCR (qRT-PCR) analysis

Invitrogen Trizol (ThermoFisher-15596026) and FastKing gDNA Dispelling RT SuperMix (TIANGEN-KR118) were used to RNA extraction and cDNA synthesis. 1 µg RNA was used for cDNA synthesis. The kit method extracted RNA and reverse transcribed cDNA were stored frozen at -20°C. Primers for ferroptosis, apoptosis, PI3K-Akt-mTOR, and cell cycle-related genes were designed according to GenBank using BLAST software in NCBI (Table 2), and ACTIN was used as an internal reference gene. The qRT-PCR technique was used to detect the

mRNA expression levels of each gene in PGCs after the treatment of insulin at different concentrations. 10 μ L qRT-PCR reaction system consists of 5 μ L 2 $\times$ Universal SYBR Green Fast qPCR Mix (YEASEN-11201ES03), 0.4 μ L upstream primer (10 μ M), 0.4 μ L downstream primer (10 μ M), 2 μ L cDNA, ddH₂O to 10 μ L. Reaction procedure: 95°C for 3 min; 95°C for 5 sec and 60°C for 30 sec with 40 cycles in total. Three biological replicates were set for the culture of PGCs, and 3 technical replicates were set for each sample. Expression levels were quantified using the 2 $^{-\Delta\Delta CT}$ method.

Table II. Sequences of primer pairs of mRNA.

Gene	Primer sequences (5'–3')	Accession number	Product length
<i>SOD1</i>	TGACCTCGGCAATGTGACTG CATGGTACGGCCAATGATGC	NM-000454.5	106
<i>CAT</i>	AGCAGGTGCCTTTGGCTATT TCCAGCAACAGTGGAGAACC	NM-001752.4	121
<i>GPX1</i>	AACTGCAGGACGACATCGAG CTTTGAAAACATCGGGCGCA	NM-204211.1	182
<i>Nrf2</i>	AACGCACCAAGAAAGACCC ACTGAACTGCTCCTTCGACA	NM-205117.1	147
β -actin	CAGCCATCTTTCTTGGGTAT CTGTGATCTCCTTCTGCATCC	NM-205518.1	167

CAT, catalase; *GPX1*, glutathione peroxidase 1; *SOD1*, superoxide dismutase 1; *Nrf2*, NF-E2-related factor 2.

RESULT

Laying performance

Table III shows that supplementing with bacteriophage did not affect average egg weight or ADFI ($P > 0.05$). At 72-75 week, the dietary supplementation of 0.1% and 0.5% of bacteriophage increased ($P < 0.05$) laying rate and egg mass compared with group Con. Meanwhile, dietary bacteriophage had significant effects on egg-feed ratio ($P < 0.05$). At 76-79 weeks, dietary bacteriophage had significant effects on laying rate and egg mass and egg-feed ratio ($P < 0.05$).

Antioxidant parameters in jejunum tissues of hens

As shown in Table IV, the content of GSH in jejunum of group B1 was higher than group Con at 72-75 weeks ($P < 0.05$). Moreover, group B3 had increased T-AOC activity in the jejunum compared to group Con after 72-75 weeks ($P < 0.05$). Dietary bacteriophage did not significantly affect ileal antioxidant markers during 76–79 weeks.

Antioxidant parameters in ileum tissues of hens

At 72-75 weeks, compared with Con group, B1 group

increased activities of SOD and T-AOC in ileum ($P < 0.05$) (Table V). At 76-79 week, dietary bacteriophage had no significant effects on Antioxidant parameters in ileum ($P > 0.05$).

Table III. Effect of dietary bacteriophage supplementation on laying performance of late laying period hens.

Items	Con	B1	B2	B3	SEM	P value
72-75 week						
Laying rate (%)	85.36 ^b	91.01 ^a	89.11 ^{ab}	91.43 ^a	0.85	0.014
Egg mass (g/day/hen)	56.29 ^b	59.45 ^a	57.88 ^{ab}	59.20 ^a	0.47	0.034
ADFI (g/d)				120		
Average egg weight (g)	65.97	65.32	64.95	64.75	0.29	0.519
Egg-feed ratio	2.13 ^a	2.02 ^b	2.07 ^b	2.03 ^b	0.02	0.032
76-79 week						
Laying rate (%)	80.64 ^b	85.90 ^a	86.40 ^a	85.46 ^a	0.78	0.004
Egg mass (g/day/hen)	53.43 ^b	56.68 ^a	56.32 ^a	56.55 ^a	0.46	0.004
ADFI (g/d)				120		
Average egg weight (g)	66.27	68.98	65.19	66.18	0.23	0.346
Egg-feed ratio	2.25 ^a	2.12 ^b	2.13 ^b	2.12 ^b	0.02	0.003

^{a,b} Mean values with different superscripts letters denote statistical significantly difference ($P < 0.05$). Con, basal diet group; B1, B2, and B3, basal diet supplemented with 0.1%, 0.3%, and 0.5% of bacteriophage, respectively.

Table IV. Effect of dietary bacteriophage supplementation on antioxidant parameters in jejunum tissues of hens.

Item	Con	B1	B2	B3	SEM	P value
72-75 week						
MDA (nmol/mg prot)	2.21	2.57	1.25	1.59	0.27	0.324
GSH (μ mol/g prot)	5.92 ^b	13.89 ^a	5.18 ^b	9.03 ^{ab}	1.18	0.006
SOD (U/mg prot)	96.96	81.27	98.21	76.15	6.23	0.558
T-AOC (U/mg prot)	1.73 ^b	1.83 ^{ab}	1.43 ^b	2.33 ^a	0.12	0.034
76-79 week						
MDA (nmol/mg prot)	2.97	2.87	2.81	2.76	0.12	0.954
GSH (μ mol/g prot)	7.58	8.82	8.75	8.31	0.56	0.891
SOD (U/mg prot)	98.99	94.27	94.24	96.68	3.72	0.975
T-AOC (U/mg prot)	1.78	1.88	1.85	1.82	0.03	0.628

GSH, reduced glutathione; MDA, malondialdehyde; SOD, superoxide dismutase; T-AOC, total antioxidant capacity.

For other abbreviations, see Table III.

Table V. Effect of dietary bacteriophage supplementation on antioxidant parameters in ileum tissues of hens.

Item	Con	B1	B2	B3	SEM	P value
72-75 week						
MDA (nmol/mg prot)	1.70	2.45	3.08	3.12	0.36	0.511
GSH (μ mol/g prot)	28.27	26.20	30.62	23.28	2.70	0.849
SOD (U/mg prot)	89.04 ^b	137.76 ^a	98.92 ^b	89.44 ^b	7.63 ^b	0.041
T-AOC (U/mg prot)	2.19 ^b	3.26 ^a	2.18 ^b	1.77 ^b	0.20	0.015
76-79 week						
MDA (nmol/mg prot)	1.90	1.97	2.19	2.19	0.12	0.827
GSH (μ mol/g prot)	5.93	6.06	6.13	5.81	0.36	0.993
SOD (U/mg prot)	111.85	113.44	104.70	106.71	3.72	0.862
T-AOC (U/mg prot)	2.10	2.13	2.07	1.98	0.04	0.491

For abbreviation, see Table IV.

Table VI. Effect of dietary bacteriophage supplementation on antioxidant parameters in liver tissues of hens.

Item	Con	B1	B2	B3	SEM	P value
72-75 week						
MDA (nmol/mg prot)	2.52	1.78	2.74	3.04	0.28	0.508
GSH (μ mol/g prot)	12.53 ^c	19.46 ^{ab}	25.31 ^a	16.46 ^b	1.84	0.029
SOD (U/mg prot)	46.66	43.43	54.89	48.50	3.43	0.747
T-AOC (U/mg prot)	3.33 ^b	4.99 ^a	4.97 ^a	3.79 ^b	0.24	0.003
76-79 week						
MDA (nmol/mg prot)	4.37 ^a	2.96 ^b	2.97 ^b	3.39 ^b	0.22	0.034
GSH (μ mol/g prot)	8.37	9.02	8.33	8.94	0.37	0.902
SOD (U/mg prot)	103.36	106.57	98.16	102.21	1.95	0.56
T-AOC (U/mg prot)	2.69 ^b	2.18 ^b	2.57 ^{ab}	3.99 ^a	0.22	<0.001

For abbreviation, see Table IV.

Antioxidant parameters in liver tissues of hens

The antioxidant parameters in liver of hens are presented in Table VI. At 72-75 weeks, the 3 treatment groups increased GSH content compared to the Con group ($P < 0.05$). B1 and B2 groups increased the activity of T-AOC in liver ($P < 0.05$). At 76-79 week, the 3 treatment groups showed decreased the concentration of MDA ($P < 0.05$). Moreover, the B3 group enhanced the activity of T-AOC significantly compared with those in Con and B1 groups ($P < 0.05$).

Jejunum antioxidant gene expression

Compared with Con group, expression levels of *SOD1* and *CAT* in B2 group and B3 group enhanced significantly at 72-75 week ($P < 0.05$). Furthermore, *GPX1* was expressed higher in 3 treatment groups than Con group ($P < 0.05$). At 76-79 week, *CAT* in B1 group and B2 group was expressed higher than Con group ($P < 0.05$).

Ileum antioxidant gene expression

As shown in Figure 1, at 72-75week the expression levels of *SOD1* and *CAT* in 3 treatment groups enhanced significantly than Con group ($P < 0.05$). Compared with Con group, expression levels of *GPX1* in B2 and B3 group enhanced dramatically ($P < 0.05$). At 76-79 weeks, there were no significant difference among the groups ($P > 0.05$).

Liver antioxidant gene expression

At 72-75 weeks, compared with C group, the expression levels of *CAT*, *SOD1* and *GPX1* in B2 group enhanced significantly ($P < 0.05$); the expression levels of *CAT* and *GPX1* in B3 group enhanced significantly ($P < 0.05$); the expression levels of *CAT* in B1 group enhanced significantly ($P < 0.05$). At 76-79 week, *CAT* in B2 group and B2 group was expressed higher than Con group ($P < 0.05$).

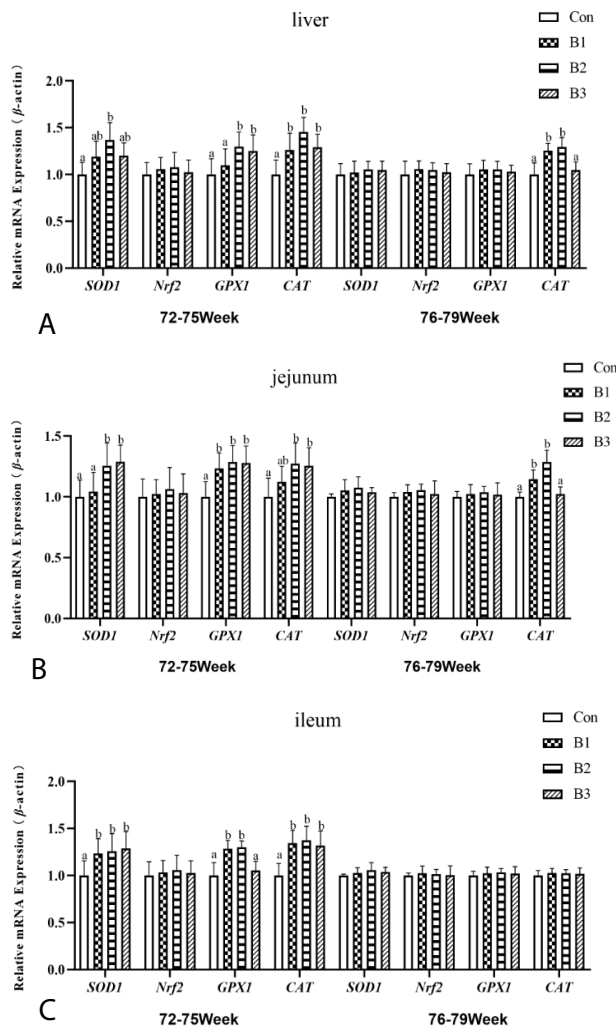


Fig. 1. Effect of dietary bacteriophage supplementation on relative mRNA expression of *SOD1*, *Nrf2*, *GPX1*, *CAT* genes after 72-76 week and 76-79 week in liver, jejunum and ileum of late layering period hens.

a,b Mean values with different superscripts letters denote statistical significantly difference ($P < 0.05$).

Con, basal diet group; B1, B2, and B3, basal diet supplemented with 0.1%, 0.3%, and 0.5% of bacteriophage, respectively; CAT, catalase; SOD1, superoxide dismutase 1; GPX1, glutathione peroxidase 1; Nrf2, NF-E2-related factor 2.

DISCUSSION

Maintaining high production performance of laying hens is the key to obtaining high economic benefits. Laying performance and feed conversion rate of laying hens will be reduced in the late stage of laying due to the effects of body degradation, multiple stresses and declining immune

function (Lv *et al.*, 2019). In addition, the oxidative stress of the organism seriously affects the reproductive performance of the animal. In the late egg-laying stage of laying hens, the antioxidant capacity of the organism is weakened, which accelerates the decline of the egg-laying rate. Bacteriophage is a natural organism widely distributed in nature, capable of producing specific infections against host bacteria, thereby achieving sterilization effects (Brussow, 2005). Bacteriophage are bactericidal and can target and destroy harmful bacteria in the poultry gut, thus ensuring intestinal health and improving antioxidant function and collective health (Upadhaya *et al.*, 2021).

This study found that dietary bacteriophage supplementation significantly increased laying performance, similar to previous results (Zhao *et al.*, 2012), indicating that dietary bacteriophage supplementation could enhance the economic production of laying hens in the late stage. We found no significant difference in egg production between the Con and B2 groups at 72-75 week, but the B2 group had a higher egg production rate than the Con group. Previous studies had found that dietary addition of phage did not significantly improve laying performance of laying hens, including laying rate, egg weight, egg mass and feed intake (Kim *et al.*, 2015). We think it was related to the phage we added, which isolated from chicken and well characterized regarding physiological and genetic aspects. This lytic phage was possessed of broad host range, rapid lytic cycle (Zhong *et al.*, 2024).

The level of antioxidant enzyme activity in the intestine and liver indirectly reflects the strength of the immunity level of the body of livestock and poultry (Hernandez *et al.*, 2004). Decreased antioxidant enzyme activity and increased free radicals can lead to avian dysfunction, causing the body to induce inflammation (Huiyun *et al.*, 2016). In the production of laying hens, the addition of phage to the diet or reference water is mainly used to protect the intestinal health and inhibit harmful bacteria, reduce the occurrence of organ inflammation, and less research has been conducted on the antioxidant activity of intestinal liver tissues (Lim *et al.*, 2012; Huang *et al.*, 2021). In the present study, we found that dietary addition of phage had a significant effect on hepatic and intestinal antioxidant enzyme activities at 72-75 weeks. However, the 3 treatment groups showed decreased the concentration of MDA and only B3 group enhanced the activity of T-AOC. We could tentatively conclude that dietary addition of phage promotes antioxidant enzyme activities in the liver and intestine at the early part of the late laying period. Therefore, we further measured the antioxidant genes expression in the intestine and liver to confirm our hypothesis.

We discovered that antioxidant gene expression was

higher in the groups with phage addition than in the Con group from weeks 72 to 75. However, from weeks 76 to 79, *CAT* expression was higher than in the Con group only in the liver and jejunum. This is generally consistent with our prior findings on antioxidant enzyme activity in liver and intestinal tissues. Although there was no significant changes in the expression of additional antioxidant genes between weeks 76 and 79, we may conclude that the test group had higher antioxidant gene expression than the control group. We could infer that the addition of phage was primarily beneficial to the liver's antioxidant function in later feeding sessions.

In poultry production, the addition of bacteriophage to the diet was mainly used as a substitute for antibiotics in poultry, which can promote the growth performance of broilers (Wang *et al.*, 2013), reduce the feed to weight ratio (Thanki *et al.*, 2023), improve feed utilization, and promote immunity (Vaz *et al.*, 2020). There was relatively little research on antioxidant function, especially in laying hens in the late stage of egg production. Based on previous studies on other poultry or other livestock, we initially investigated the effect of phages on egg production performance and antioxidant capacity of laying hens in the late stage of laying. In this article, we could conclude that adding bacteriophage to the diet could improve the antioxidant capacity and related gene expression of laying hens in the later stage of egg laying, especially in the early stage of egg laying. provided a reliable research foundation for the healthy production of laying hens in the late laying period.

CONCLUSION

In conclusion, the addition of phage to the diet could significantly enhance the egg production rate and egg mass of laying hens in the late stage of laying while decreasing the feed-to-egg ratio. In the early stages of the late laying phase, it could boost antioxidant enzyme activity and gene expression in the jejunum, ileum and liver. Among them, basal diets supplemented with 0.1% and 0.3% had the best effect.

DECLARATIONS

Funding

This study was supported by the Foundation of Coastal Agricultural Science Observation and Experimental Station of Ministry of Agriculture and Rural Affairs (No. YHS202109).

Supplementary material

There is supplementary material associated with

this article. Access the material online at <https://doi.org/10.1186/s43014-023-00193-6>. The complete genome sequence of phage vE20 was deposited at GenBank under accession number OP293233.

Ethical statement and IRB approval

Animal experiments were conducted in strict accordance with the principles of animal welfare and scientific ethics, and were approved by the Experimental Animal Ethics Committee of the Jiangsu Academy of Agricultural Sciences (Approval No.: IACUCRE-2024-01-021).

Consent for publication

All authors gave their consent for publication.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

- Bain, M.M., Nys, Y. and Dunn, I.C., 2016. Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges? *Br. Poult. Sci.*, **57**: 330-338. <https://doi.org/10.1080/00071668.2016.1161727>
- Brussow, H., 2005b. Phage therapy: The *Escherichia coli* experience. *Microbiology (Reading)*. **151**: 2133-2140. <https://doi.org/10.1099/mic.0.27849-0>
- E, X., Shao, D., Li, M., Shi, S. and Xiao, Y., 2023. Supplemental dietary genistein improves the laying performance and antioxidant capacity of Hy-Line brown hens during the late laying period. *Poult. Sci.*, **102**: 102573. <https://doi.org/10.1016/j.psj.2023.102573>
- Hernandez, F., Madrid, J., Garcia, V., Orengo, J. and Megias, M.D., 2004. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. *Poult. Sci.*, **83**: 169-174. <https://doi.org/10.1093/ps/83.2.169>
- Huang, P., Cui, X., Wang, Z., Xiao, C., Ji, Q., Wei, Q., Huang, Y., Bao, G. and Liu, Y., 2021. Effects of clostridium butyricum and a bacteriophage cocktail on growth performance, serum biochemistry, digestive enzyme activities, intestinal morphology, immune responses, and the intestinal microbiota in rabbits. *Antibiotics (Basel)*, **10**. <https://doi.org/10.3390/antibiotics10111347>
- Huiyun, Z., Jingjuan, W. and Xinyu, G., 2016. Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality. *Fd. Sci.*

- Hum. Wellness*, **5**: 39-48. <https://doi.org/10.1016/j.fshw.2015.11.003>
- Kim, J.H., Kim, J.W., Shin, H.S., Kim, M.C., Lee, J.H., Kim, G.B. and Kil, D.Y., 2015. Effect of dietary supplementation of bacteriophage on performance, egg quality and caecal bacterial populations in laying hens. *Br. Poult. Sci.*, **56**: 132-136. <https://doi.org/10.1080/00071668.2014.991272>
- Kortright, K.E., Chan, B.K., Koff, J.L. and Turner, P.E., 2019. Phage therapy: A renewed approach to combat Antibiotic-Resistant bacteria. *Cell Host Microbe*, **25**: 219-232. <https://doi.org/10.1016/j.chom.2019.01.014>
- Lim, T.H., Kim, M.S., Lee, D.H., Lee, Y.N., Park, J.K., Youn, H.N., Lee, H.J., Yang, S.Y., Cho, Y.W., Lee, J.B., Park, S.Y., Choi, I.S. and Song, C.S., 2012. Use of bacteriophage for biological control of *Salmonella enteritidis* infection in chicken. *Res. Vet. Sci.*, **93**: 1173-1178. <https://doi.org/10.1016/j.rvsc.2012.06.004>
- Lv, Z.P., Yan, S.J., Li, G., Liu, D. and Guo, Y.M., 2019. Genistein improves the reproductive performance and bone status of breeder hens during the late egg-laying period. *Poult. Sci.*, **98**: 7022-7029. <https://doi.org/10.3382/ps/pez367>
- Merabishvili, M., De Vos, D., Verbeken, G., Kropinski, A.M., Vandenheuvel, D., Lavigne, R., Wattiau, P., Mast, J., Ragimbeau, C., Mossong, J., Scheres, J., Chanishvili, N., Vaneechoutte, M. and Pirnay, J.P., 2012. Selection and characterization of a candidate therapeutic bacteriophage that lyses the *Escherichia coli* O104:H4 strain from the 2011 outbreak in Germany. *PLoS One*, **7**: e52709. <https://doi.org/10.1371/journal.pone.0052709>
- Styles, K.M., Locke, R.K., Cowley, L.A., Brown, A.T. and Sagona, A.P., 2022. Transposable element insertions into the *Escherichia coli* polysialic acid gene cluster result in resistance to the K1F bacteriophage. *Microbiol. Spectr.*, **10**: e211221. <https://doi.org/10.1128/spectrum.02112-21>
- Thanki, A.M., Hooton, S., Whenham, N., Salter, M.G., Bedford, M.R., O'Neill, H. and Clokie, M., 2023. A bacteriophage cocktail delivered in feed significantly reduced *Salmonella* colonization in challenged broiler chickens. *Emerg. Microbes Infect.*, **12**: 2217947. <https://doi.org/10.1080/22221751.2023.2217947>
- Upadhaya, S.D., Ahn, J.M., Cho, J.H., Kim, J.Y., Kang, D.K., Kim, S.W., Kim, H.B. and Kim, I.H., 2021. Bacteriophage cocktail supplementation improves growth performance, gut microbiome and production traits in broiler chickens. *J. Anim. Sci. Biotechnol.*, **12**: 49. <https://doi.org/10.1186/s40104-021-00570-6>
- Vaz, C., Voss-Rech, D., Alves, L., Coldebella, A., Brentano, L. and Trevisol, I.M., 2020. Effect of time of therapy with wild-type lytic bacteriophage on the reduction of *Salmonella enteritidis* in broiler chickens. *Vet. Microbiol.*, **240**: 108527. <https://doi.org/10.1016/j.vetmic.2019.108527>
- Wang, J.P., Yan, L., Lee, J.H. and Kim, I.H., 2013. Evaluation of bacteriophage supplementation on growth performance, blood characteristics, relative organ weight, breast muscle characteristics and excreta microbial shedding in broilers. *Asian Austral. J. Anim. Sci.*, **26**: 573-578. <https://doi.org/10.5713/ajas.2012.12544>
- Yao, H., Hu, Y., Wang, Q., Zhang, Y., Rao, K. and Shi, S., 2022. Effects of dietary dimethylglycine supplementation on laying performance, egg quality, and tissue index of hens during late laying period. *Poult. Sci.*, **101**: 101610. <https://doi.org/10.1016/j.psj.2021.101610>
- Yao, W., Wang, E., Zhou, Y., Han, Y., Li, S., Yin, X., Huang, X. and Huang, F., 2023. Effects of garcinol supplementation on the performance, egg quality, and intestinal health of laying hens in the late laying period. *Poult. Sci.*, **102**: 102939. <https://doi.org/10.1016/j.psj.2023.102939>
- Zhao, P.Y., Baek, H.Y. and Kim, I.H., 2012. Effects of bacteriophage supplementation on egg performance, egg quality, excreta microflora, and moisture content in laying hens. *Asian Austral. J. Anim. Sci.*, **25**: 1015-1020. <https://doi.org/10.5713/ajas.2012.12026>
- Zhong, Z., Wang, Y., Li, H., Zhang, H., Zhou, Y., Wang, R. and Bao, H., 2024. Characterization and genomic analysis of a novel *E. coli* lytic phage with extended lytic activity against *S. Enteritidis* and *S. Typhimurium*. *Fd. Prod. Proc. Nutr.*, **6**. <https://doi.org/10.1186/s43014-023-00193-6>