Animal 18 (2024) 101324

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

# Effects of dietary propyl gallate and *Lactobacillus plantarum* addition on growth, intestinal morphology, antioxidant capacity, and immune functions of Pekin ducks

L. Dai [a], B.W. Wang [a], W.L. Fan [b], W. Qian [b], J. Zhang [a], B.H. Wang [c], B.B. Zhang [b], M.A. Zhang [b,*]

[a] College of Food Science and Engineering, Qingdao Agricultural University, Qingdao 266109, China
[b] College of Animal Science and Technology, Qingdao Agricultural University, Qingdao 266109, China
[c] Qingdao Huihe Biotechnology Co., Ltd, Qingdao 266109, China

ARTICLE INFO

*Article history:*
Received 24 March 2024
Revised 31 August 2024
Accepted 2 September 2024
Available online 11 September 2024

*Keywords:*
Antibiotic alternatives
Body health
Feed conversion ratio
Meat production
Serum

ABSTRACT

The interaction between probiotic bacteria and polyphenol antioxidants can potentially enhance animal health. The present study examined the effects of propyl gallate and *Lactobacillus plantarum* supplementation on the growth, intestinal morphology, antioxidant capacity, and immune functions of Pekin ducks. A total of 128 male Pekin ducks (7-day-old) were allocated to four treatment groups with four replicates of eight birds each. The ducks were fed the corn-soybean based diet (the control), supplemented with either propyl gallate (100 mg/kg), *Lactobacillus plantarum* ($4 \times 10^9$ CFU/kg), or both, for 5 weeks. Dietary supplementation with propyl gallate and *Lactobacillus plantarum* had no significant effect on feed intake ($P > 0.05$), but increased average daily gain ($P < 0.05$). *Lactobacillus plantarum* also reduced the feed/gain ratio ($P < 0.05$). Villus height (**VH**) in the duodenum and ileum was increased by supplementation, while only propyl gallate supplement increased VH in the jejunum ($P < 0.05$). Supplementation had no effect on small intestine crypt depth ($P > 0.05$). Enhanced total superoxide dismutase activity was observed with supplementation ($P < 0.05$), but no effects were seen on catalase, malondialdehyde, total antioxidant capacity, and glutathione peroxidase values ($P > 0.05$). Serum immunoglobulin G was increased with *Lactobacillus plantarum* ($P < 0.05$), but not with propyl gallate ($P > 0.05$). No change in IgA and IgM concentrations was observed with supplementation. In conclusion, dietary supplementation with propyl gallate, *Lactobacillus plantarum*, or both, enhanced the villus height of the small intestines, improving the growth rate of Pekin ducks. The synergistic effects of both propyl gallate and *Lactobacillus plantarum* on the villus height and serum total superoxide dismutase activity surpassed the individual effects of each supplement in Pekin ducks.



## Implications

The synergy between probiotic bacteria and polyphenolic antioxidants holds potential for enhancing animal health. This study demonstrated that dietary supplementation of propyl gallate, *Lactobacillus plantarum*, a combination of both increased the villus height in the small intestine, resulting in improved growth rates in Pekin ducks. Notably, birds that received both propyl gallate and *Lactobacillus plantarum* exhibited the most favourable outcomes. These findings introduce a promising feed additive option for investigating alternative approaches to growth promotion, potentially reducing reliance on antibiotics.

## Introduction

Intensive farming systems have met the rising demands of consumers for poultry products, yet it has also introduced challenges for avian health, including physiological stresses and digestive dysfunctions. These issues diminish the quality of animal products and threaten animal welfare (Averós and Estevez, 2018). Historically, antibiotics were added in feed to alleviate stresses and enhance bird growth performances. However, this practice has led to significant food safety and human health issues, resulting in the banning of antibiotic usage in poultry feed in many countries (Hayes et al., 2004). Consequently, there is an urgent requirement to develop additive feed alternatives to replace antibiotics in poultry diets.

*Lactobacillus plantarum* (**LP**) strains have shown significant promise as probiotics in animal feed. Previous studies have found that supplementing broilers' feed with LP improved growth perfor-

* Corresponding author at: College of Food Science and Engineering, Qingdao Agricultural University, No. 700 Changcheng Road, Qingdao 266109, China.
*E-mail address:* 13705320869@126.com (M.A. Zhang).

https://doi.org/10.1016/j.animal.2024.101324
1751-7311/

mance by enhancing immunity, promoting intestinal health, and modulating intestinal microflora (Wang et al., 2021a; b). Dietary supplementation of LP in Pekin duck has also shown considerable potential to enhance the growth performance and intestinal health (An et al., 2022). However, the viability and activity of probiotics often decline significantly during the long periods of processing, transport and storage due to oxidation (Guo et al., 2022). Fortunately, phenolic antioxidants have been proposed to improve the survival of probiotics under oxidative stress due to their beneficial effects in scavenging the reactive oxygen species and chelating metal ions (Hu and Kitts, 2001; Vodnar and Socaciu, 2012; Rishi et al., 2017; Zwolak, 2021).

Propyl gallate (**PG**) is a derivative of gallic acid recognised as an antioxidant by the U.S. Food and Drug Administration regulatory authority (Javaheri-Ghezeldizaj et al., 2023). Propyl gallate is widely used in cosmetics, food, pharmaceuticals, and various other industries (Nguyen et al., 2021). In addition to its antioxidant effect, PG also exhibits anti-inflammatory and anti-tumor properties in cells (Han et al., 2010; Jung et al., 2011). Karthikeyan et al. (2005) reported on the antioxidant properties of PG, highlighting its protective effects against oxidative stress in myocardial tissues of rats. Another study in rodents revealed the anti-inflammatory and analgesic properties of PG (McDonald-Gibson et al., 1976). Additionally, PG has shown substantial protective effects against toxin-induced liver injury in both *in vitro* and *in vivo* models (Wu et al., 1994). These studies provide a comprehensive view of the beneficial effects of PG on animal health, including its antioxidant, hepatoprotective, and anti-inflammatory properties. There are also reports showing that interactions between polyphenol antioxidants and probiotic bacteria can benefit animal health by the prevention of diet-induced metabolic disorders (Fang et al., 2019) and synergistic effects on delaying ageing process in mice (Sharma et al., 2019).

Therefore, we hypothesised that PG and LP in combination will enhance growth performance and improve the health of ducks. This hypothesis was tested by examining the effects of PG and LP dietary supplementation and their combination on the growth performance, intestinal morphology, antioxidant capacity, and immune functions of Pekin ducks.

## Material and methods

### *Ducks, experimental design, diets, and rearing conditions*

The study was conducted in accordance with the Chinese animal welfare guidelines and approved by the Animal Ethics Committee of Qingdao Agricultural University (File No. QAU20220510. Qingdao, China). A total of 128 seven-day-old male Pekin ducklings with similar BWs (261 ± 1.9 g) were used in this study. The ducklings were allocated to four treatment groups, with each group consisting of 32 birds housed in four cages (replicates) of eight birds each. After 1 week of acclimation to caging and a basal diet based on corn and soybean meal (Table 1), the four groups were randomly assigned to one of four diets: (1) basal diet as the control (CON), (2) basal diet supplemented with 100 mg PG per kg diet, (3) basal diet supplemented with $4 \times 10^9$ CFU LP per kg diet, and (4) basal diet supplemented with both 100 mg/kg of PG and $4 \times 10^9$ CFU/kg of diet (PG+LP). The PG product (Shandong Dexin Bio-Technology Co., Ltd., Qingdao, China) contained 98% propyl gallate. The *Lactobacillus plantarum* preparation was provided by the Waterfowl Research Institute of Qingdao Agricultural University, and the bacterial strain was isolated from the cecal content of Pekin ducks (Catalogue number CGMCC No. 26622, *Lactobacillus genus*). The basal diet was formulated to meet the nutrient requirements of growing ducks (NRC, 1994). The experiment lasted for 5 weeks (day 7–42) from June to July. The temperature in the duck shed was maintained at 18 ∼ 24 °C and 55 ∼ 65% relative humidity during the 5 weeks experimental period. The ducks were housed in plastic cages with wire-flooring (each cage had a floor space of 4 × 2 m) under natural daylight. The ducks had *ad libitum* access to feed and fresh water.

**Table 1**
Diet compositions and nutrition content of the basal diet for ducks (DM basis).

| Ingredients | % | Nutrient composition[3] | % |
|---|---|---|---|
| Corn | 62.13 | ME (MJ/kg) | 12.82 |
| Soybean meal | 25.85 | CP | 17.82 |
| Soybean oil | 5 | Lysine | 0.64 |
| Chrysanthemum stalks | 3.24 | Methionine | 0.47 |
| Dicalcium phosphate | 1.59 | Calcium | 0.85 |
| Limestone | 1.23 | Available phosphorus | 0.32 |
| *DL*-Methionine | 0.17 | Total phosphorus | 0.54 |
| *L*-Lysine | 0.06 | Crude fiber | 4.21 |
| Tryptophan | 0.01 | | |
| Trace mineral premix[1] | 0.15 | | |
| Vitamin premix[2] | 0.1 | | |
| NaCl | 0.3 | | |
| Choline chloride (70%) | 0.07 | | |
| Fungicide | 0.01 | | |
| Total | 100 | | |

[1] Trace element premix (provided per kilogram of feed) contains the following substances: Cu ($CuSO_4 \cdot 5H_2O$), 6 mg; Zn ($ZnSO_4 \cdot 7H_2O$), 40 mg; Fe ($FeSO_4 \cdot 7H_2O$), 80 mg; Mn ($MnSO_4 \cdot H_2O$), 100 mg; Se ($NaSeO_3$), 0.15 mg; I (KI), 0.35 mg.
[2] Vitamin premix (provided per kilogram of feed) contains the following substances: vitamin A, 12 500 IU; vitamin $D_3$, 3 500 IU; vitamin E, 20 IU; vitamin $K_3$, 2.65 mg; vitamin $B_1$, 2 mg; vitamin $B_2$, 6 mg; vitamin $B_{12}$, 0.025 mg; vitamin E, 30 IU; biotin, 0.0325 mg; folic acid, 12 mg; pantothenic acid, 50 mg; niacin,50 mg.
[3] CP, Ca, and P were determined by analysis based on triplicate determinations; others were calculated values according to the ingredient apparent metabolisable energy (AME) values for chickens. ME = metabolisable energy.

### *Growth performance and sampling*

On days 7, 21, and 42, all ducks in each cage were weighed after overnight fasting to calculate average daily gain (**ADG**). The amounts of feed offered and refusal for each cage were recorded daily to calculate the average daily feed intake for the entire period, which was also used to calculate the feed conversion ratio (kg feed per kg ADG, **F/G**). On day 42, blood samples (each 10 mL) were taken from the jugular vein of eight birds (two birds from each cage) with similar BWs from each treatment group. Blood samples were collected into tubes without anticoagulant and centrifuged at room temperature and 3 000 × g for 15 min, and the serum was collected and stored at −40 °C for subsequent analysis.

### *Intestinal morphology*

At the end of the experiment, following overnight food deprivation, two birds from each dietary treatment replicate were randomly selected for euthanasia through cervical dislocation. The small intestines (duodenum, jejunum and ileum) were isolated and sampled. Samples of the duodenum, jejunum and ileum were fixed with 4% paraformaldehyde for histological analysis. The fixed samples were dehydrated in ethanol, equilibrated in xylene, embedded in paraffin wax, and 5-μm sections cut. The sections were then mounted on glass slides, stained with hematoxylin and eosin, and 10 slides per segment of the intestine were examined under a microscope fitted with a digital camera (Olympus, Tokyo, Japan). Five well-oriented, distinct villi were identified on each slide and measured for villus height (**VH**) and crypt depth (**CD**), and their ratio (**VH/CD**) was calculated.

### Measurement of antioxidant indices and immunoglobulin

Catalase (#A007-1-1), total superoxide dismutase (#A001-3-2), glutathione peroxidase (#TE0700), total antioxidant capacity (#A015-3-1) and malondialdehyde (#A003-1-2) activity in serum was measured. Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) kits were used for Catalase, total superoxide dismutase, total antioxidant capacity and malondialdehyde analysis, and Beijing Leagene Biotechnology (Beijing, China) kits were used to determine glutathione peroxidase following the protocols provided by the manufacturers. The concentrations of immunoglobulin A (**IgA**, #CB10021-Du), immunoglobulin G (**IgG**, #CB10022-Du), and immunoglobulin M (**IgM**, #CB10087-Du) in serum samples were measured using ELISA kits following the manufacturer's protocols (Coibo Bio Technology, Shanghai, China). The detailed protocols were described in Supplementary Material S1.

### Statistical analysis

Data were subjected to a two-way analysis of variance (ANOVA) within a GLM in SPSS statistical software (version 26.0 for Windows; SPSS Inc., Chicago, IL). PG and LP were treated as factors, with their interactions assessed in a completely randomised design featuring a 2 × 2 factorial arrangement of treatments. Significance among treatments was determined using Duncan's multiple comparison test, with statistical significance set at $P < 0.05$. Pooled SEMs were calculated by averaging the SEMs determined with the LSD method to identify significant differences. The results are presented as the means and pooled SEMs.

## Results

### Growth performance

As shown in Table 2, supplementation with PG, LP, or their combination did not yield a significant effect on average daily feed intake ($P > 0.05$). However, the analysis of main effects results revealed that both PG and LP treatments increased ADG during days 21–42 and days 7–42 ($P < 0.05$) compared to the control group, while LP supplementation decreased F/G ($P < 0.05$). There was no interaction observed between PG and LP on growth performance ($P > 0.05$).

### Intestinal morphology

The histomorphology of the intestinal tissues for each group is shown in Fig. 1. As shown in Table 3, the analysis of main effects showed significant increases in VH in the duodenum and ileum with supplementation of PG and LP ($P < 0.05$). Specifically, an increase in VH in the jejunum was observed only with PG supplementation ($P < 0.05$). Furthermore, a significant interaction between PG and LP on VH was noted in the jejunum and ileum ($P < 0.05$), wherein combined supplementation of PG and LP increased VH compared to the other treatment groups. However, supplementation with PG, LP or their combination did not yield significant effects on CD in the small intestines ($P > 0.05$). The VH/CD ratio in the duodenum was increased by LP supplementation ($P < 0.05$), while the ratio in the ileum was increased by PG and LP supplementations ($P < 0.05$).

### Serum antioxidant indices

The antioxidant indices in serum are present in Table 4. The analysis of main effects revealed that supplementation with PG and LP significantly enhanced the activity of total superoxide dismutase compared to the control group ($P < 0.05$), with further enhancement observed with supplementation of both PG and LP ($P < 0.05$). However, supplementation with PG, LP or their combination had no significant effects on the values of Catalase, malondialdehyde, total antioxidant capacity, and glutathione peroxidase ($P > 0.05$).

### Serum immunoglobulins

As shown in Table 5, the analysis of main effects showed that LP supplementation significantly increased the IgG level in serum ($P < 0.05$), whereas there was no significant change observed with PG supplementation ($P > 0.05$). Neither PG nor LP supplementation significantly affected IgA and IgM concentrations in serum ($P > 0.05$), and there was no interaction between PG and LP on serum immunoglobulin levels ($P > 0.05$).

**Table 2**
Effects of PG and LP supplements on growth performance of Pekin ducks[1] from day 7–21, day 21–42, and day 7–42 (n = 4).

| Items[2] | Treatments[3] | | | | Pooled SEM | Main effects | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CON | PG | LP | PG+LP | | PG | | LP | | $P_{PG}$ | $P_{LP}$ | $P_{PG\times LP}$ |
| | | | | | | – | + | – | + | | | |
| ADFI (g/day) | | | | | | | | | | | | |
| Day 7–21 | 137 | 136 | 135 | 134 | 1.52 | 135.9 | 135.1 | 136.3 | 134.6 | 0.809 | 0.638 | 0.877 |
| Day 21–42 | 178 | 184 | 191 | 183 | 2.34 | 184.8 | 184.1 | 181.4 | 187.5 | 0.861 | 0.198 | 0.158 |
| Day 7–42 | 161 | 165 | 168 | 164 | 1.64 | 165.3 | 164.5 | 163.4 | 166.4 | 0.812 | 0.384 | 0.274 |
| ADG (g/day) | | | | | | | | | | | | |
| Day 7–21 | 59.5 | 61.0 | 62.4 | 64.0 | 0.81 | 60.9 | 62.6 | 60.2 | 63.3 | 0.281 | 0.060 | 0.938 |
| Day 21–42 | 71.7 | 77.1 | 80.7 | 81.8 | 1.11 | 76.2[b] | 79.4[a] | 74.4[b] | 81.2[a] | 0.008 | <0.001 | 0.062 |
| Day 7–42 | 66.8 | 70.7 | 73.4 | 74.8 | 0.91 | 70.1[b] | 72.7[a] | 68.8[b] | 74.1[a] | 0.026 | <0.001 | 0.256 |
| F/G | | | | | | | | | | | | |
| Day 7–21 | 2.30 | 2.22 | 2.15 | 2.10 | 0.02 | 2.23 | 2.16 | 2.26[a] | 2.12[b] | 0.058 | 0.002 | 0.804 |
| Day 21–42 | 2.48 | 2.39 | 2.37 | 2.24 | 0.03 | 2.42 | 2.31 | 2.43[a] | 2.30[b] | 0.072 | 0.037 | 0.807 |
| Day 7–42 | 2.42 | 2.33 | 2.29 | 2.19 | 0.03 | 2.36 | 2.26 | 2.37[a] | 2.24[b] | 0.283 | 0.029 | 0.616 |

[a,b] Mains with different superscript letters differ significantly ($P < 0.05$).
[1] Data represent the means of four replicate cages per treatment.
[2] Abbreviations: ADFI=average daily feed intake; ADG=average daily gain; F/G=the feed to gain ratio.
[3] Abbreviations: CON=the basal diet; PG=the basal diet supplemented with 100 mg/kg propyl gallate; LP=the basal diet supplemented with 1 × $10^9$ CFU/kg *Lactobacillus plantarum*; PG+LP, the basal diet supplemented with 100 mg/kg propyl gallate and 1 × $10^9$ CFU/kg *Lactobacillus plantarum*.

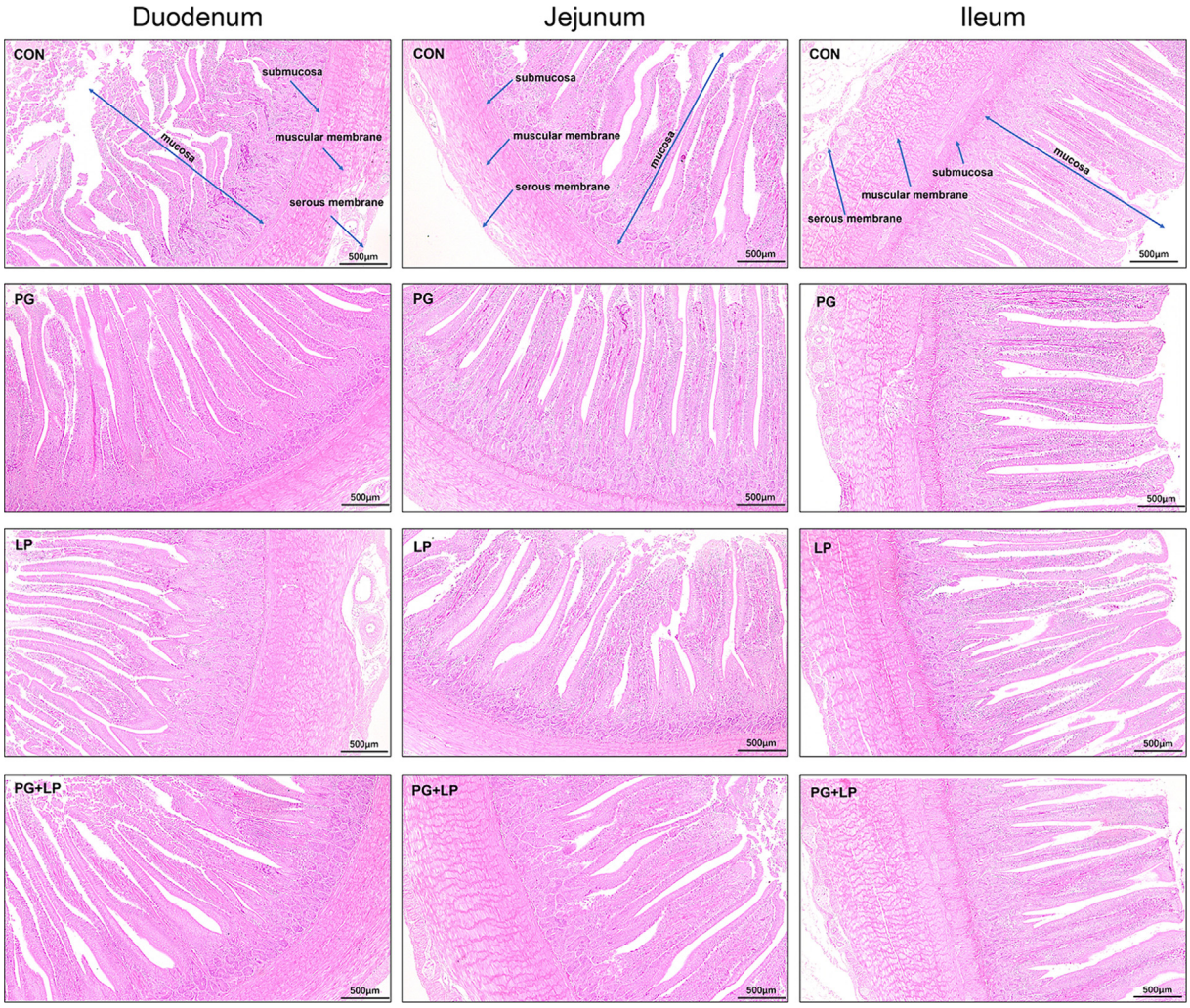


**Fig. 1.** Effects of PG and LP supplements on the morphology of small intestine in Pekin ducks (Scale bar, 500 μm). The mucosa, submucosa, muscularis, and serosa of the intestine are labeled in the figures of the CON group. Abbreviations: CON=the basal diet; PG=the basal diet supplemented with 100 mg/kg propyl gallate; LP=the basal diet supplemented with $1 \times 10^9$ CFU/kg *Lactobacillus plantarum*; PG+LP, the basal diet supplemented with 100 mg/kg propyl gallate and $1 \times 10^9$ CFU/kg *Lactobacillus plantarum*.

**Table 3**
Effects of PG and LP supplements on histomorphology of small intestines in Pekin ducks[1] (n = 4).

| Items[2] | Treatments[3] | | | | Pooled SEM | Main effects | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CON | PG | LP | PG+LP | | PG | | LP | | $P_{PG}$ | $P_{LP}$ | $P_{PG\times LP}$ |
| | | | | | | – | + | – | + | | | |
| Duodenum | | | | | | | | | | | | |
| VH (μm) | 877 | 948 | 976 | 1 021 | 17.07 | 927[b] | 984[a] | 912[b] | 999[a] | 0.005 | <0.001 | 0.425 |
| CD (μm) | 135 | 134 | 127 | 135 | 2.10 | 131 | 135 | 134 | 131 | 0.466 | 0.516 | 0.326 |
| VH/CD | 6.47 | 7.07 | 7.65 | 7.54 | 0.16 | 7.07 | 7.31 | 6.77[b] | 7.59[a] | 0.299 | 0.005 | 0.136 |
| Jejunum | | | | | | | | | | | | |
| VH (μm) | 825[b] | 841[b] | 823[b] | 888[a] | 8.94 | 824[b] | 865[a] | 833 | 855 | 0.003 | 0.053 | 0.036 |
| CD (μm) | 136 | 138 | 136 | 139 | 1.51 | 136 | 139 | 137 | 137 | 0.429 | 0.984 | 0.975 |
| VH/CD | 6.06 | 6.07 | 6.05 | 6.38 | 0.06 | 6.00 | 6.23 | 6.06 | 6.22 | 0.171 | 0.213 | 0.181 |
| Ileum | | | | | | | | | | | | |
| VH (μm) | 653[c] | 732[b] | 734[b] | 769[a] | 13.42 | 693[b] | 751[a] | 693[b] | 752[a] | <0.001 | <0.001 | 0.048 |
| CD (μm) | 128 | 132 | 132 | 123 | 2.05 | 130 | 127 | 130 | 128 | 0.436 | 0.578 | 0.128 |
| VH/CD | 5.08 | 5.55 | 5.53 | 6.28 | 0.16 | 5.31[b] | 5.92[a] | 5.32[b] | 5.90[a] | 0.025 | 0.030 | 0.546 |

[a-c] Means with different superscript letters differ significantly ($P < 0.05$).
[1] Data represent the means of four replicates, with two male ducks per replicate.
[2] Abbreviations: VH=villus height; CD=crypt depth; VH/CD=villus height and crypt depth ratio.
[3] Abbreviations: CON=the basal diet; PG=the basal diet supplemented with 100 mg/kg propyl gallate; LP=the basal diet supplemented with $1 \times 10^9$ CFU/kg *Lactobacillus plantarum*; PG+LP, the basal diet supplemented with 100 mg/kg propyl gallate and $1 \times 10^9$ CFU/kg *Lactobacillus plantarum*.

**Table 4**
Effects of PG and LP supplements on serum antioxidant capacity in Pekin ducks[1] (n = 4).

| Items[2] | Treatments[3] | | | | Pooled SEM | Main effects | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CON | PG | LP | PG+LP | | PG | | LP | | $P_{PG}$ | $P_{LP}$ | $P_{PG\times LP}$ |
| | | | | | | – | + | – | + | | | |
| CAT (U/mL) | 3.67 | 3.93 | 3.48 | 3.81 | 0.17 | 3.58 | 3.87 | 3.81 | 3.65 | 0.465 | 0.685 | 0.937 |
| MDA (nmol/mL) | 1.59 | 1.79 | 1.95 | 1.76 | 0.16 | 1.77 | 1.78 | 1.69 | 1.85 | 0.972 | 0.492 | 0.419 |
| T-SOD (U/mL) | 77.2[c] | 94.1[b] | 95.6[b] | 101.8[a] | 1.77 | 89.3[b] | 96.5[a] | 85.8[b] | 99.6[a] | 0.004 | 0.008 | 0.005 |
| T-AOC (U/mL) | 0.85 | 0.89 | 0.93 | 0.87 | 0.04 | 0.89 | 0.88 | 0.87 | 0.90 | 0.879 | 0.772 | 0.422 |
| GSH-Px (U/mL) | 188 | 192 | 193 | 193 | 1.49 | 191 | 192 | 190 | 193 | 0.577 | 0.265 | 0.544 |

[a-c] Means with different superscript letters differ significantly ($P < 0.05$).
[1] Data represent the means of four replicates, with two male ducks per replicate.
[2] Abbreviations: CAT=catalase; MDA=malondialdehyde; T-SOD=total superoxide dismutase; T-AOC = total antioxidant capacity; GSH-Px = glutathione peroxidase.
[3] Abbreviations: CON=the basal diet; PG=the basal diet supplemented with 100 mg/kg propyl gallate; LP=the basal diet supplemented with $1 \times 10^9$ CFU/kg *Lactobacillus plantarum*; PG+LP, the basal diet supplemented with 100 mg/kg propyl gallate and $1 \times 10^9$ CFU/kg *Lactobacillus plantarum*.

**Table 5**
Effects of PG and LP on serum immunoglobulins in Pekin ducks[1] (n = 4).

| Items[2] | Treatments[3] | | | | Pooled SEM | Main effects | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CON | PG | LP | PG+LP | | PG | | LP | | $P_{PG}$ | $P_{LP}$ | $P_{PG\times LP}$ |
| | | | | | | – | + | – | + | | | |
| IgM (μg/mL) | 484 | 482 | 483 | 508 | 10.26 | 483 | 495 | 483 | 489 | 0.604 | 0.588 | 0.555 |
| IgA (μg/mL) | 64.7 | 63.3 | 64.9 | 67.5 | 1.10 | 66.1 | 64.1 | 64.0 | 66.2 | 0.392 | 0.359 | 0.786 |
| IgG (μg/mL) | 720 | 741 | 856 | 855 | 23.95 | 788 | 798 | 731[b] | 856[a] | 0.811 | 0.008 | 0.785 |

[a,b] Means with different superscript letters differ significantly ($P < 0.05$).
[1] Data represent the means of four replicates, with two male ducks per replicate.
[2] Abbreviations: IgM = immunoglobulin M; IgA = immunoglobulin A; IgG = immunoglobulin G.
[3] Abbreviations: CON = the basal diet; PG = the basal diet supplemented with 100 mg/kg propyl gallate; LP=the basal diet supplemented with $1 \times 10^9$ CFU/kg *Lactobacillus plantarum*; PG+LP, the basal diet supplemented with 100 mg/kg propyl gallate and $1 \times 10^9$ CFU/kg *Lactobacillus plantarum*.

## Discussion

This study aimed to evaluate the efficacy of a combination of PG and LP as a feed additive for promoting growth in Pekin ducks. We found that supplementation with PG, LP or the PG+LP combination did not affect the feed intake of the duck, but increased BW gains, with LP supplementation also improving the feed conversion ratio. The positive effects of PG on growth performance have been documented in piglets (Lu et al., 2014), and gallic acid has been shown to increase ADG in broiler chickens (Biswas et al., 2023). LP has been reported to improve the feed conversion ratio, intestinal health, and digestion and absorption in chickens and ducks (Wang et al., 2018; An et al., 2022). These findings are consistent with our results. However, our hypothesis that there would be a synergistic effect of PG and LP on the growth performance of duck was not confirmed in this experiment. Therefore, additional research on multiple combinations of PG and LP is required to verify this hypothesis.

The VH, CD, and their ratio are key indices of intestinal morphology, reflecting the function, health, and absorptive capabilities of the intestine (Montagne et al., 2003; Cairo et al., 2018). Studies with broiler chickens have suggested that both gallic acid and LP have improved intestinal morphology and function (Viveros et al., 2011; Vineetha et al., 2017; Yang et al., 2017; Liu et al., 2023). In the present study, morphological analysis of the small intestines revealed that supplementation with PG or LP increased VH in all three intestinal segments. The combination of PG and LP synergistically increased VH in the jejunum and ileum. The exact mechanism by which probiotics and polyphenols improve intestinal morphology remained unclear. However, some researchers speculate that phenols benefit the growth of *Lactobacillus* by serving as substrates. *Lactobacillus* can utilise polyphenols during the growth process, providing energy for intestinal epithelial cells and nourishing the intestinal villi (García-Ruiz et al., 2008).

Poultry are often vulnerable to oxidative stress, which can detrimentally affect their health and productive performances. In this study, supplementation with PG or LP enhanced the total superoxide dismutase activity in serum, consistent with previous reports in chickens and pigs (Franco et al., 2012; Wu et al., 2019; Tang et al., 2021). PG can effectively scavenge free radicals and superoxide anions (Soares et al., 2003; Einali and Valizadeh, 2015). Several reports have suggested that LP exerts antioxidant protective effects by activating the Nrf2 signalling pathway, alleviating tissue oxidative stress by increasing SOD levels (Sahin et al., 2012; Gao et al., 2013). Additionally, *Lactobacillus* can interact with polyphenols to enhance antioxidant capacity. This interaction may explain the observed synergistic effect of PG and LP on total superoxide dismutase levels in ducks (Donkor and Shah, 2008; Uskova et al., 2010; Macedo et al., 2011).

IgG exhibits antibacterial and antiviral activities, serving as an indicator of overall health and immune status (Wu et al., 2021). LP supplementation has been shown to increase IgG concentration (Liu et al., 2023), with similar results observed in broilers and ducks (Maynard et al., 2012; An et al., 2022). However, the results in the present study indicate that PG may not regulate the production of immunoglobulins in ducks.

In summary, dietary supplementation with PG, LP, or both, increased VH of the small intestines, leading to an improved growth rate in Pekin duck. The synergistic effects of PG and LP on VH and serum total superoxide dismutase activity exceeded the individual effects of PG and LP. These results suggest that the combination of PG and LP may be a potential natural alternative to antibiotics.

## Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2024.101324.

## Ethics approval

All animal experimental and care procedures followed the Experimental Animal Care and Use Guidelines of China and were approved by the Animal Ethics Committee of the Qingdao Agricultural University, China (Licence no. QAU20220510).

## Data and model availability statement

The data were not deposited in an official repository. All data discussed in the study are available to readers on request to the corresponding author.

## Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

## Author ORCIDs

**Lin Dai:** https://orcid.org/0009-0008-1415-7953.
**Baowei Wang:** https://orcid.org/0000-0003-4367-2248.
**Wenlei Fan:** https://orcid.org/0000-0003-0092-0512.
**Wang Qian:** https://orcid.org/0009-0002-1253-8484.
**Jing Zhang:** https://orcid.org/0009-0008-1719-2025.
**Binghan Wang:** https://orcid.org/0009-0002-7490-9025.
**Beibei Zhang:** https://orcid.org/0000-0002-7538-7436.
**Mingai Zhang:** https://orcid.org/0009-0005-0843-4326.

## CRediT authorship contribution statement

**L. Dai:** Writing – review & editing, Writing – original draft, Project administration, Formal analysis, Data curation. **B.W. Wang:** Supervision, Funding acquisition, Conceptualization, Writing – review & editing. **W.L. Fan:** Project administration, Supervision, Writing – review & editing. **W. Qian:** Formal analysis, Data curation. **J. Zhang:** Methodology, Conceptualization, Writing – original draft. **B.B. Zhang:** Supervision, Project administration, Writing – review & editing. **M.A. Zhang:** Supervision, Funding acquisition, Conceptualization, Writing – original draft.

## Declaration of interest

The authors declare that they have no conflicts of interest.

## Acknowledgments

The authors would like to thank Institute of high-quality waterfowl in Qingdao Agricultural University for helping animal experiments.

## Financial support statement

This work was financially supported by China Agriculture Research System of MOF and MARA (CARS-42-14) and Key Technology Research and Development Program of Shandong (2022LZGC014).

## References

An, K., Gao, W., Li, P., Li, L., Xia, Z., 2022. Dietary *Lactobacillus plantarum* improves the growth performance and intestinal health of Pekin ducks. Poultry Science 101, 101844.
Averós, X., Estevez, I., 2018. Meta-analysis of the effects of intensive rearing environments on the performance and welfare of broiler chickens. Poultry Science 97, 3767–3785.
Biswas, S., Cho, S.B., Kim, I.H., 2023. An evaluation of gallic acid supplementation to corn-soybean-gluten meal-based diet in broilers. Poultry Science 102, 102738.
Cairo, P.L.G., Gois, F.D., Sbardella, M., Silveira, H., de Oliveira, R.M., Allaman, I.B., Cantarelli, V.S., Costa, L.B., 2018. Effects of dietary supplementation of red pepper (Schinus terebinthifolius Raddi) essential oil on performance, small intestinal morphology and microbial counts of weanling pigs. Journal of the Science of Food and Agriculture 98, 541–548.
Donkor, O.N., Shah, N.P., 2008. Production of β-Glucosidase and Hydrolysis of Isoflavone Phytoestrogens by Lactobacillus acidophilus, Bifidobacterium lactis, and Lactobacillus casei in Soymilk. Journal of Food Science 73, M15–M20.
Einali, A., Valizadeh, J., 2015. Propyl gallate promotes salt stress tolerance in green microalga Dunaliella salina by reducing free radical oxidants and enhancing β-carotene production. Acta Physiologiae Plantarum 37, 83.
Fang, C., Kim, H., Yanagisawa, L., Bennett, W., Sirven, M.A., Alaniz, R.C., Talcott, S.T., Mertens-Talcott, S.U., 2019. Gallotannins and Lactobacillus plantarum WCFS1 mitigate high-fat diet-induced inflammation and induce biomarkers for thermogenesis in adipose tissue in gnotobiotic mice. Molecular Nutrition & Food Research 63, 1800937.
Franco, J.G., Lisboa, P.C., Lima, N.S., Amaral, T.A.S., Peixoto-Silva, N., Resende, A.C., Oliveira, E., Passos, M.C.F., Moura, E.G., 2012. Resveratrol attenuates oxidative stress and prevents steatosis and hypertension in obese rats programmed by early weaning. The Journal of Nutritional Biochemistry 24, 960–966.
Gao, D., Gao, Z., Zhu, G., 2013. Antioxidant effects of Lactobacillus plantarum via activation of transcription factor Nrf2. Food & Function 4, 982–989.
García-Ruiz, A., Bartolomé, B., Martínez-Rodríguez, A.J., Pueyo, E., Martín-Álvarez, P. J., Moreno-Arribas, M.V., 2008. Potential of phenolic compounds for controlling lactic acid bacteria growth in wine. Food Control 19, 835–841.
Guo, Q., Li, T., Yuan, C., Liang, L., Gänzle, M.G., Zhao, M., 2022. Effects of protein fibrillation and antioxidants on probiotic survival during ambient storage. Food Chemistry 389, 133117.
Han, Y.H., Moon, H.J., You, B.R., Kim, S.Z., Kim, S.H., Park, W.H., 2010. Propyl gallate inhibits the growth of HeLa cells via caspase-dependent apoptosis as well as a G1 phase arrest of the cell cycle. Oncology Reports 23, 1153–1158.
Hayes, J.R., English, L.L., Carr, L.E., Wagner, D.D., Joseph, S.W., 2004. Multiple-antibiotic resistance of Enterococcus spp. isolated from commercial poultry production environments. Applied and Environmental Microbiology 70, 6005–6011.
Hu, C., Kitts, D.D., 2001. Evaluation of antioxidant activity of epigallocatechin gallate in biphasic model systems in vitro. Molecular and Cellular Biochemistry 218, 147–155.
Javaheri-Ghezeldizaj, F., Alizadeh, A.M., Dehghan, P., Ezzati Nazhad Dolatabadi, J., 2023. Pharmacokinetic and toxicological overview of propyl gallate food additive. Food Chemistry 423, 135219.
Jung, H.-J., Kim, S.-J., Jeon, W.-K., Kim, B.-C., Ahn, K., Kim, K., Kim, Y.-M., Park, E.-H., Lim, C.-J., 2011. Anti-inflammatory activity of n-Propyl Gallate through down-regulation of NF-κB and JNK pathways. Inflammation 34, 352–361.
Karthikeyan, K., Bai, B.R.S., Gauthaman, K., Devaraj, S.N., 2005. Protective effect of propyl gallate against myocardial oxidative stress-induced injury in rat. Journal of Pharmacy and Pharmacology 57, 67–73.
Liu, L., Li, L., Li, C., Wang, H., Zhang, X., Ren, Q., Zhang, H., Jin, N., Li, C., Zhao, C., 2023. Effects of Lactiplantibacillus plantarum LPJZ-658 Supplementation on the production, meat quality, intestinal morphology, and Cecal Microbiota of broilers chickens. Microorganisms 11, 1549.
Lu, T., Harper, A.F., Zhao, J., Estienne, M.J., Dalloul, R.A., 2014. Supplementing antioxidants to pigs fed diets high in oxidants: I. effects on growth performance, liver function, and oxidative status. Journal of Animal Science 92, 5455–5463.
Macedo, J.A., Battestin, V., Ribeiro, M.L., Macedo, G.A., 2011. Increasing the antioxidant power of tea extracts by biotransformation of polyphenols. Food Chemistry 126, 491–497.
Maynard, C.L., Elson, C.O., Hatton, R.D., Weaver, C.T., 2012. Reciprocal interactions of the intestinal microbiota and immune system. Nature 489, 231–241.
McDonald-Gibson, W.J., Saeed, S.A., Schneider, C., 1976. The local antinociceptive and topical anti-inflammatory effects of propyl gallate in rodents. British Journal of Pharmacology 58, 573–581.
Montagne, L., Pluske, J.R., Hampson, D.J., 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Animal Feed Science and Technology 108, 95–117.
Nguyen, V.H., Le, M.N., Nguyen, H.B., Ha, K.O., Pham, T.H.V., Nguyen, T.H., Dao, N.S. H., Nguyen, V.G., Nguyen, D.L., Trinh, N.T., 2021. Propyl Gallate. Molbank 2021, M1201.
NRC 1994. National Research Council Nutrient Requirements of Poultry - Ninth Revised Edition (1994). The Journal of Applied Poultry Research 3, 101–101.
Rishi, P., Arora, S., Kaur, U.J., Chopra, K., Kaur, I.P., 2017. Better management of alcohol liver disease using a 'Microstructured Synbox' system comprising L. plantarum and EGCG. PLOS ONE 12, e0168459.
Sahin, K., Orhan, C., Tuzcu, Z., Tuzcu, M., Sahin, N., 2012. Curcumin ameloriates heat stress via inhibition of oxidative stress and modulation of Nrf2/HO-1 pathway in quail. Food and Chemical Toxicology 50, 4035–4041.
Sharma, R., Kumari, M., Kumari, A., Sharma, A., Gulati, A., Gupta, M., Padwad, Y., 2019. Diet supplemented with phytochemical epigallocatechin gallate and probiotic Lactobacillus fermentum confers second generation synbiotic effects

by modulating cellular immune responses and antioxidant capacity in aging mice. European Journal of Nutrition 58, 2943–2957.
Soares, D.G., Andreazza, A.C., Salvador, M., 2003. Sequestering ability of Butylated Hydroxytoluene, Propyl Gallate, resveratrol, and vitamins C and E against ABTS, DPPH, and hydroxyl free radicals in chemical and biological systems. Journal of Agricultural and Food Chemistry 51, 1077–1080.
Tang, Q., Yi, H., Hong, W., Wu, Q., Yang, X., Hu, S., Xiong, Y., Wang, L., Jiang, Z., 2021. Comparative effects of L. plantarum CGMCC 1258 and L. reuteri LR1 on growth performance, antioxidant function, and intestinal immunity in weaned pigs. Frontiers in Veterinary Science 8, 728849.
Uskova, M.A., Kravchenko, L.V., Avrenjeva, L.I., Tutelyan, V.A., 2010. Effect of Lactobacillus casei 114001 probiotic on bioactivity of rutin. Bulletin of Experimental Biology and Medicine 149, 578–582.
Vineetha, P.G., Tomar, S., Saxena, V.K., Kapgate, M., Suvarna, A., Adil, K., 2017. Effect of laboratory-isolated Lactobacillus plantarum LGFCP4 from gastrointestinal tract of guinea fowl on growth performance, carcass traits, intestinal histomorphometry and gastrointestinal microflora population in broiler chicken. Journal of Animal Physiology and Animal Nutrition 101, e362–e370.
Viveros, A., Chamorro, S., Pizarro, M., Arija, I., Centeno, C., Brenes, A., 2011. Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. Poultry Science 90, 566–578.
Vodnar, D.C., Socaciu, C., 2012. Green tea increases the survival yield of Bifidobacteria in simulated gastrointestinal environment and during refrigerated conditions. Chemistry Central Journal 6, 61.
Wang, B., Gong, L., Zhou, Y., Tang, L., Zeng, Z., Wang, Q., Zou, P., Yu, D., Li, W., 2021a. Probiotic *Paenibacillus polymyxa* 10 and *Lactobacillus plantarum* 16 enhance growth performance of broilers by improving the intestinal health. Animal Nutrition 7, 829–840.
Wang, J., Ji, H., Wang, S., Liu, H., Zhang, W., Zhang, D., Wang, Y., 2018. Probiotic Lactobacillus plantarum promotes intestinal barrier function by strengthening the epithelium and modulating gut microbiota. Frontiers in Microbiology 9, 1953.
Wang, Y., Lv, X., Li, X., Zhao, J., Zhang, K., Hao, X., Liu, K., Liu, H., 2021b. Protective effect of Lactobacillus plantarum P8 on growth performance, intestinal health, and microbiota in eimeria-infected broilers. Frontiers in Microbiology 12, 705758.
Wu, T.-W., Fung, K.-P., Zeng, L.-H., Wu, J., Nakamura, H., 1994. Propyl gallate as a hepatoprotector *in vitro* and *in vivo*. Biochemical Pharmacology 48, 419–422.
Wu, Y., Wang, B., Zeng, Z., Liu, R., Tang, L., Gong, L., Li, W., 2019. Effects of probiotics *Lactobacillus plantarum* 16 and *Paenibacillus polymyxa* 10 on intestinal barrier function, antioxidative capacity, apoptosis, immune response, and biochemical parameters in broilers. Poultry Science 98, 5028–5039.
Wu, H., Yang, J., Wang, S., Zhang, X., Hou, J., Xu, F., Wang, Z., Xu, L., Diao, X., 2021. Effects of soybean isoflavone and astragalus polysaccharide mixture on colostrum components, serum antioxidant, immune and hormone levels of lactating sows. Animals 11, 132.
Yang, J.Y., Zhang, H.J., Wang, J., Wu, S.G., Yue, H.Y., Jiang, X.R., Qi, G.H., 2017. Effects of dietary grape proanthocyanidins on the growth performance, jejunum morphology and plasma biochemical indices of broiler chicks. Animal 11, 762–770.
Zwolak, I., 2021. Epigallocatechin Gallate for management of heavy metal-induced oxidative stress: mechanisms of action, efficacy, and concerns. International Journal of Molecular Sciences 22, 4027.