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Effect of Dietary Puerarin Supplementation on Growth Performance, Immune Response, Antioxidant Capacity, and Intestinal Morphology in Domestic Pigeons (*Columba livia*)

Runzhi Wang[1], Tingting Li[2], Zaixu Pan[3], Hui Chen[3], Shanjin Xu[3], Xixue Lu[4], Kai Shi[4], Bang Bian[1] and Guansuo Wu[1]

[1]Nanjing Institute of Animal Husbandry and Poultry Science, Nanjing 210036, China
[2]Wenzhou Institute, University of Chinese Academy of Sciences, Wenzhou 325011, China
[3]Nanjing Dongchen Pigeon Industry Co., Ltd., Nanjing 210000, China
[4]College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China

Puerarin is an isoflavone extracted from Gegen (*Pueraria lobata*) and has been widely utilized to treat various human diseases; however, information regarding its benefits in animal production is limited. In this study, we aimed to investigate the influence of dietary puerarin supplementation on growth performance, immune organ index, immunoglobulin profile, antioxidant capacity, and intestinal morphology in pigeons. In total, 375 healthy 28-day-old White King pigeons were randomly divided into five groups, each consisting of five replicates and 15 pigeons per replicate. Each group was administered one of five dietary treatments: the basal diet, or the basal diet supplemented with 40, 80, 120, or 160 mg/kg puerarin. Treatment duration was 30 days following a 7-day acclimation period. Puerarin treatment did not significantly alter the growth performance of pigeons but afforded a significant linear enhancement in the thymus index ($P < 0.05$). Additionally, puerarin supplementation significantly increased serum immunoglobulin A and immunoglobulin M levels in pigeons in a linear manner ($P < 0.05$). Similarly, puerarin significantly and linearly increased the activities of total antioxidant capacity, superoxide dismutase, glutathione, and catalase in the serum and liver, and decreased the malondialdehyde content ($P < 0.05$). Moreover, the villus height (VH), crypt depth (CD), and VH/CD ratio of the small intestine (including the duodenum, jejunum, and ileum) increased linearly upon puerarin supplementation ($P < 0.05$). Collectively, these results indicate that puerarin supplementation could improve the immune response, antioxidant capacity, and intestinal morphology of pigeons.

Key words: antioxidant capacity, immune response, intestinal morphology, pigeon, puerarin

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Introduction

Pigeons (*Columba livia*) represent an alternative and significant source of meat and eggs, ranking after chickens, ducks, and geese in importance within the poultry industry, as stated in Announcement No. 303 of the Ministry of Agriculture and Rural Affairs of the People's Republic of China (http://www.moa.gov.cn/). Pigeon meat is characterized by its low fat content, high protein levels, and rich amino acid composition, making it particularly favored by consumers, especially in China[1]. Currently, pigeon farming in China occurs at the largest scale worldwide, accounting for over 80% of the global market share and highlighting the promising potential of pigeon breeding[2,3]. However, the altricial nature of pigeons presents specific feeding challenges during production. Unlike chicks, which can self-feed within 24 hours of birth, newly hatched squabs are incapable of walking and feeding independently, requiring parental crop milk to obtain nourishment for a duration of 28 days after birth[4,5]. At 28 to 30 days of age, squabs can be either marketed or raised separately as breeding stock[6]. Squabs, when prematurely separated from their parents, are susceptible to stress, leading to a decline in their disease resistance and immunity, ultimately culminating in irreversible mortality, with consequential implications for economic gains[7]. Various factors contribute to pigeon stress, including

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Correspondence: Prof. Guansuo Wu, Nanjing Institute of Animal Husbandry and Poultry Science, Nanjing 210036, China. (E-mail: abnanjing@foxmail.com)

increased flock exposure, changes in the rearing environment, and alterations in feeding methods and nutrition. Among these, altering the feeding regime and diet of pigeons is akin to the weaning process observed in other animals. For example, upon weaning, piglets exhibit an underdeveloped gastrointestinal tract, imperfect immune function, susceptibility to diarrhea, and reduced feed intake, thereby seriously impeding their growth and developmental processes[8]. Similarly, the weaning process in pigeons places additional demands on their immune and digestive systems, reducing adaptability and resistance, elevating oxidative stress, impairing growth and development, and hindering the healthy development of the pigeon industry[9].

In order to mitigate the adverse effects of weaning, supplementation with plant extracts has been shown to constitute an effective strategy for alleviating stress and enhancing growth performance in animals[10,11]. Isoflavones, a type of flavonoid mainly found in legumes, have been reported to assuage oxidative stress in broilers[12], finishing pigs[13], and lactating sows[14], improve the intestinal health of newborn and weaned piglets[15,16], and enhance the immunity of broilers treated with lipopolysaccharide or virus[17,18], thereby playing a crucial protective role in animal production. In particular, *Pueraria lobata*, known as Gegen in China, originated in Southeast Asia and has been widely used for fodder and medicinal purposes for centuries[19]. It has been employed to treat various conditions such as cardiac dysfunction, liver injury, and toxicosis[20]. Puerarin, a natural isoflavone compound extracted from Gegen, possesses significant antioxidant properties and can effectively clear free radicals, diminish the expression of inflammatory genes, and enhance the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway activation[21,22]. In addition, puerarin exerts beneficial functions in the treatment of cardiovascular diseases, cerebrovascular disorders, cancer, and diabetes, and has been widely used in the clinic[23,24]. Recent studies have demonstrated that puerarin can relieve oxidative stress and provide protective effects in the liver and intestines[25,26]. However, despite its potential, limited information is available regarding the application of puerarin in pigeon production. The primary aim of this study was therefore to assess the protective effects of puerarin supplementation on antioxidant function, immune response, and intestinal morphology in newly weaned pigeons.

Materials and Methods

Ethical statement

All experimental design and sample collection procedures complied with the Chinese Guidelines for Animal Welfare and were approved by the Animal Care and Use Committee of Nanjing Institute of Animal Husbandry and Poultry Science (SYXK 2023-0013).

Animals, diets, and management

This study was conducted at Dongchen Pigeon Industry in Nanjing (Jiangsu, China). In total, 375 healthy 28-day-old White King pigeons were selected and randomly assigned to five treatment groups, with each group comprising five replicates of 15 pigeons per replicate. The five treatment diets comprised the basal diet (Control; Table 1) and the basal diet supplemented with puerarin at the following concentrations: 40, 80, 120, and 160 mg/kg. The puerarin (98% purity) used in this experiment was purchased from Shengqing Biotechnology Co., Ltd. (Shaanxi, China). The pigeons were raised in stacked cages with dimensions of 50 × 50 × 55 cm, accommodating three pigeons per cage. Feeding occurred three times daily at 8:00, 13:00, and 17:00. The pigeons had unrestricted access to water and food. The immunization program followed the standard procedure of the factory. The pre-feeding period lasted for seven days, followed by the formal experimental period spanning 30 days. On the 15th and 30th days following treatment initiation, one pigeon was randomly chosen from each replicate of each treatment group for sample collection. Growth performance, including average daily gain (ADG) and average daily feed intake (ADFI), was calculated for the periods of 1 to 15 days and 16 to 30 days.

Table 1. **Formulation and nutrient levels of the basal diet**

Item	Content (%)	Percentage (%)
Composition of granulated meal		50.00
Corn	40.88	
Soybean meal	24.90	
Fermented soybean	8.00	
Wheat	10.00	
Soybean oil	6.86	
Lysine	0.43	
Methionine	0.78	
Threonine	0.15	
Premix[a]	8.00	
Total	100.00	
Composition of whole-grain form feed		50.00
Corn	50.00	
Pea	50.00	
Total	100.00	
Calculated nutrient levels		
Metabolizable energy (Mcal/kg)	2.97	
Crude protein	17.29	
Calcium	0.95	
Available phosphorus	0.38	
Lysine	1.10	
Methionine + cysteine	0.76	

[a]Premix provided the following per kilogram of diet: vitamin A, 8000 IU; vitamin D_3, 3000 IU; vitamin E, 40 mg; vitamin K_3, 1.6 mg; vitamin B_1, 5 mg; vitamin B_2, 22 mg; vitamin B_6, 4 mg; vitamin B_{12}, 50 µg; niacin, 30 mg; folic acid, 0.6 mg, pantothenic acid, 6.4 mg; biotin, 0.2 mg; sodium chloride, 760 mg; copper, 24 mg; iron, 76 mg; zinc, 80 mg; manganese, 116 mg; selenium, 0.4 mg.

Sample collection

At 15 and 30 days following experimental diet administration, five pigeons from each treatment group close to the average

Table 2. **Effect of dietary puerarin on growth performance in pigeons**

Item	Puerarin level (mg/kg)					SEM	*P*-value	
	0	40	80	120	160		ANOVA	Linear
Days 1–15								
ADG (g)	0.51	0.51	0.51	0.49	0.52	0.09	0.877	0.794
ADFI (g)	45.97	46.08	45.75	45.86	45.22	0.20	0.688	0.225
Days 16–30								
ADG (g)	0.48	0.51	0.51	0.53	0.50	0.10	0.467	0.295
ADFI (g)	46.27	46.22	45.86	46.31	45.92	0.27	0.974	0.743

Data represent the means of five replicates (13–15 individuals per replicate) per treatment. ADG = average daily weight gain; ADFI = average daily feed intake; SEM = standard error of the mean; ANOVA = analysis of variance.

body weight were selected, used to obtain weight measurements and blood samples collected from wing veins, and euthanized by cervical dislocation. The whole blood was placed at room temperature (25 °C) for more than 30 minutes to allow for serum separation. Subsequently, the spleen, thymus, and bursae of Fabricius were separated and weighed to calculate the organ index using the following formula: organ index (g/kg)=organ weight (g)/body weight (kg)×1000. The liver samples were obtained and preserved in liquid nitrogen. After carefully isolating the duodenum, jejunum, and ileum, 1 cm segments from each of these intestinal sections were rinsed with phosphate-buffered saline and fixed in 4% paraformaldehyde for subsequent staining analysis.

Immune analysis of serum

The serum samples were applied to determine the levels of immunoglobulin A (IgA), IgM, and IgG using pigeon-specific enzyme-linked immunosorbent assay kits (Hongsheng, Jiangsu, China). Subsequently, the absorbance of the samples was detected using a microplate reader (Tecan, Männedorf, Switzerland).

Analysis of antioxidant activities in the serum and liver

An automatic sample rapid grinding machine was used to homogenize the liver tissue, followed by centrifugating at 1200×g (4°C) for 10 min to obtain the supernatant. The supernatants obtained from tissue and serum were applied for the detection of total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), and malondialdehyde (MDA) levels using commercial kits from Jiancheng (Jiangsu, China). Absorbance measurements of the tissue and serum were obtained using the microplate reader (Tecan, Männedorf, Switzerland).

Intestinal morphological measurements

The intestinal specimens (including the duodenum, jejunum, and ileum) fixed in paraformaldehyde were further embedded in paraffin after dehydrating using graded ethanol. The tissue segments were transversely sectioned (5 μm thickness) and then stained with hematoxylin and eosin. The images of the sections were obtained and analyzed using a slide scanner system (Pannoramic Midi, Budapest, Hungary). A minimum of 10 villi or crypts were recorded for each sample to measure villus height (VH) and crypt depth (CD).

Statistical analysis

The results were analyzed using the one-way analysis of variance (ANOVA) function within the SPSS 23.0 software package (IBM, Armonk, NY, USA) subsequent to confirming normality and homogeneity of variance. Tukey's multiple comparison test was employed to discern statistical distinctions among groups. Polynomial contrast was performed to assess the linear responses to varying levels of puerarin in the diet. The results are expressed as the means and standard error of the mean (SEM), with significance denoted at $P < 0.05$.

Results

Growth performance and immune organ index

The effect of puerarin on the growth performance of pigeons is shown in Table 2. Dietary supplementation with puerarin did not significantly affect the ADG and ADFI of pigeons ($P > 0.05$). Calculation of the immune organ index revealed that dietary puerarin significantly enhanced thymus development in pigeons on day 15 but not on day 30 ($P < 0.05$; Table 3). Specifically, the increase of the thymus index exhibited a linear trend with dosage ($P < 0.05$). Conversely, no significant difference in the spleen or bursa of Fabricius index was observed at either 15 or 30 days after puerarin treatment ($P > 0.05$; Table 3).

Serum immunoglobulin levels

To investigate whether the immune function of pigeons was affected by dietary puerarin supplementation, we measured the serum immunoglobulin levels. As shown in Table 4, puerarin supplementation at doses of 120 and 160 mg/kg led to a significant linear increase in serum IgM levels on day 15 compared to those of the Control group ($P < 0.05$). In comparison, a significant linear enhancement in the serum levels of both IgA and IgM was observed on day 30 ($P < 0.05$).

Antioxidant capacity of the serum and liver

To evaluate the antioxidant capacity of pigeons following puerarin supplementation, the levels of antioxidant indices were evaluated in the serum and liver. As shown in Table 5, we observed a linear elevation in serum T-AOC, SOD, and GSH activities in pigeons fed a puerarin-supplemented diet for 15 days compared to the levels in the Control group ($P < 0.05$). Similarly, the supplementation of dietary puerarin led to a linear increase in serum SOD, GSH, and CAT activities on day 30 ($P < 0.05$).

Table 6 shows the hepatic antioxidant indices. On day 15,

Table 3. **Effect of dietary puerarin on immune organ indices in pigeons**

Item	Puerarin level (mg/kg)					SEM	*P*–value	
	0	40	80	120	160		ANOVA	Linear
Day 15								
Thymus index[a]	2.69[c]	2.18[c]	2.43[c]	4.18[b]	4.13[b]	0.20	<0.001	<0.001
Spleen index	2.02	2.04	1.96	2.04	2.14	0.08	0.974	0.688
Bursa of Fabricius index	3.17	3.38	3.19	3.05	3.24	0.16	0.984	0.878
Day 30								
Thymus index	2.69	2.36	2.63	2.51	2.41	0.11	0.860	0.599
Spleen index	1.08	1.06	1.13	1.08	1.03	0.07	0.995	0.884
Bursa of Fabricius index	1.63	1.55	1.66	1.66	1.69	0.07	0.975	0.653

[a]Organ index (g/kg)=organ weight (g)/body weight (kg)×1000. Data represent the means of five individuals per treatment. [b, c]Means with different superscripts in a row differ significantly ($P < 0.05$). SEM = standard error of the mean; ANOVA = analysis of variance.

Table 4. **Effect of dietary puerarin on serum immunoglobulin concentration in pigeons**

Item	Puerarin level (mg/kg)					SEM	*P*–value	
	0	40	80	120	160		ANOVA	Linear
Day 15								
IgA (μg/mL)	56.94	52.14	58.45	57.96	62.42	1.81	0.527	0.204
IgM (μg/mL)	151.80[b]	149.93[b]	164.80[ab]	187.87[a]	191.87[a]	4.34	<0.001	<0.001
IgG (μg/mL)	478.96	490.93	476.94	490.44	479.98	9.00	0.983	0.982
Day 30								
IgA (μg/mL)	46.43[c]	51.54[c]	52.63[c]	66.20[a]	59.63[b]	1.44	<0.001	<0.001
IgM (μg/mL)	114.67[b]	143.80[a]	149.80[a]	159.07[a]	144.13[a]	3.21	<0.001	<0.001
IgG (μg/mL)	410.22	413.24	411.87	418.94	412.75	4.989	0.989	0.778

Data represent the means of five individuals per treatment. [a, b, c]Means with different superscripts in a row differ significantly ($P < 0.05$). IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; SEM = standard error of the mean; ANOVA = analysis of variance.

pigeons fed with puerarin exhibited a significant and linear increase in the activities of hepatic SOD and GSH, whereas the concentrations of hepatic MDA were decreased ($P < 0.05$). On day 30, the hepatic activities of T-AOC, SOD, and GSH showed a linear enhancement following puerarin treatment, whereas the concentrations of MDA exhibited a linear decrease ($P < 0.05$).

Intestinal histology

To assess the influence of puerarin on intestinal development, morphological analyses of the duodenum, jejunum, and ileum were conducted at days 15 and 30. As shown in Table 7, puerarin linearly increased the duodenal VH, CD, and the VH/CD ratio at day 15 ($P < 0.05$). Similarly, puerarin supplementation linearly increased VH and CD in the duodenum on day 30 ($P < 0.05$). Furthermore, puerarin supplementation significantly and linearly increased jejunal VH, CD, and the VH/CD ratio at day 15, and jejunal VH and CD at d 30 ($P < 0.05$). In turn, puerarin significantly increased the VH and the VH/CD ratio of the ileum on day 15, along with the VH, CD, and VH/CD ratio on day 30 in a linear manner ($P < 0.05$).

Discussion

In this study, we evaluated the effects of puerarin supplementation at varying levels on the growth performance, immune response, antioxidant capacity, and intestinal health of pigeons. Previous studies conducted on beef cattle demonstrated the growth performance-enhancing effects of puerarin[27]. However, in the present study, puerarin supplementation did not significantly alter the growth performance of pigeons. This discrepancy is likely due to the timeframe evaluated in the present study because the rapid growth period of pigeons occurs within the first month after birth and their body weight gradually stabilizes after 28 days of age, after which the potential benefits of puerarin on pigeon growth performance are not likely to be evident.

The development of immune organs is crucial for establishing a robust immune response in young animals[28]. Owing to the lack of independent feeding ability and compromised disease resistance following dietary changes during weaning, young pigeons are highly vulnerable to diseases commonly found in pigeon populations, such as salmonellosis and Newcastle disease, often resulting in death[29,30]. Alternatively, our results revealed that puerarin supplementation facilitated thymus development in pigeons during the early rearing phase. The thymus serves as the site for the proliferation and maturation of T cells, which are crucial participants in the immune response[31]. Accordingly, augmentation of the relative weight of immune organs contributes to the enhancement of immune functions in pigeons. Immuno-

Table 5. **Effect of dietary puerarin on serum antioxidant indices in pigeons**

Item	Puerarin level (mg/kg)					SEM	*P*–value	
	0	40	80	120	160		ANOVA	Linear
Day 15								
T-AOC (U/mL)	1.06^{b}	1.06^{b}	1.35^{ab}	1.51^{a}	1.14^{b}	0.05	0.003	0.026
SOD (U/mL)	141.64^{b}	182.79^{a}	192.64^{a}	182.06^{a}	182.53^{a}	4.60	0.001	0.003
MDA (nmol/mL)	4.44	4.68	4.05	3.34	3.89	0.18	0.133	0.049
GSH (mg/L)	4.48^{b}	5.37^{b}	4.53^{b}	7.86^{a}	5.86^{ab}	0.34	0.002	0.006
CAT (U/mL)	14.72	14.60	16.30	17.96	16.08	0.44	0.079	0.040
Day 30								
T-AOC (U/mL)	1.10	1.06	1.11	1.25	1.37	0.06	0.406	0.081
SOD (U/mL)	133.62^{b}	154.98^{ab}	167.39^{a}	171.51^{a}	181.29^{a}	4.19	0.001	<0.001
MDA (nmol/mL)	4.96	5.99	4.90	4.38	4.68	0.22	0.175	0.151
GSH (mg/L)	4.59^{a}	5.29^{ab}	6.87^{a}	6.91^{a}	6.21^{ab}	0.26	0.005	0.003
CAT (U/mL)	13.79^{b}	14.08^{b}	18.83^{a}	21.28^{a}	19.76^{a}	0.74	<0.001	<0.001

Data represent the means of five individuals per treatment. $^{a, b}$Means with different superscripts in a row differ significantly ($P < 0.05$). T-AOC = total antioxidant capacity; SOD = superoxide dismutase; MDA = malonaldehyde; GSH, glutathione; CAT = catalase; SEM = standard error of the mean; ANOVA = analysis of variance.

Table 6. **Effect of dietary puerarin on liver antioxidant indices in pigeons**

Item	Puerarin level (mg/kg)					SEM	*P*–value	
	0	40	80	120	160		ANOVA	Linear
Day 15								
T-AOC (U/mg prot)	1.16	1.41	1.37	1.38	1.34	0.05	0.512	0.336
SOD (U/mg prot)	341.23^{b}	343.11^{b}	464.99^{a}	470.07^{a}	323.93^{b}	12.30	<0.001	<0.001
MDA (nmol/mg prot)	2.32^{a}	1.73^{ab}	2.24^{a}	1.65^{ab}	1.42^{b}	0.09	0.003	0.002
GSH (mg/g prot)	27.03^{ab}	25.02^{b}	30.27^{b}	39.61^{a}	31.43^{b}	1.06	<0.001	<0.001
CAT (U/mg prot)	84.09	89.86	88.38	87.59	88.07	2.90	0.983	0.798
Day 30								
T-AOC (U/mg prot)	1.31^{cd}	1.25^{d}	1.73^{a}	1.48^{bc}	1.56^{ab}	0.04	<0.001	<0.001
SOD (U/mg prot)	414.51^{c}	443.20^{bc}	440.64^{bc}	460.60^{ab}	480.07^{a}	5.63	<0.001	<0.001
MDA (nmol/mg prot)	2.19^{ab}	2.41^{a}	1.80^{b}	1.23^{c}	2.07^{ab}	0.09	<0.001	0.002
GSH (mg/g prot)	29.64^{bc}	28.56^{bc}	26.07^{c}	33.93^{a}	30.91^{ab}	0.63	<0.001	0.018
CAT (U/mg prot)	82.80	83.98	84.25	88.24	84.92	1.47	0.838	0.442

Data represent the means of five individuals per treatment. $^{a–d}$Means with different superscripts in a row differ significantly ($P < 0.05$). T-AOC = total antioxidant capacity; SOD = superoxide dismutase; MDA = malonaldehyde; GSH = glutathione; CAT = catalase; SEM = standard error of the mean; ANOVA = analysis of variance.

globulin is a critical component of the avian immune response system, which is mainly responsible for preventing diseases and resisting viruses[32]. Different types of immunoglobulins fulfill distinct functions within this system[33]. IgA acts as the defense against pathogen invasion, effectively resisting certain viruses and bacteria. IgM is the earliest antibody generated in the initial immune response. IgG serves as the principal antibody in serum against bacteria, viruses, and antitoxins[34]. In the present study, the puerarin supplementation could increase serum IgM levels in pigeons at 15 days and both serum IgA and IgM levels at 30 days. Similar results were observed in beef cattle, in which 800 mg/kg puerarin significantly elevated serum immunoglobulin (IgA, IgG, and IgM) levels[35]. These findings suggest that puerarin markedly improves immune parameters in pigeons.

In pigeons, alterations in dietary patterns prompt an excessive production of free radicals, leading to impaired antioxidant systems in various tissues[9]. Animals employ antioxidant defense systems to safeguard themselves from both internal and external damage, encompassing non-enzymatic and enzymatic mechanisms[36]. T-AOC, SOD, MDA, GSH, and CAT serve as crucial indicators of antioxidant activity, playing essential roles in mitigating oxidative damage. SOD functions as the primary defense against reactive oxygen species, whereas GSH and CAT can alleviate cellular oxidative damage[37]. Moreover, T-AOC

Table 7. **Effect of dietary puerarin on intestinal morphology in pigeons**

Item	Puerarin level (mg/kg)					SEM	*P*–value	
	0	40	80	120	160		ANOVA	Linear
Day 15								
Duodenum								
VH (μm)	941.98^{b}	921.85^{b}	1075.13^{a}	1101.51^{a}	1066.00^{a}	7.75	<0.001	<0.001
CD (μm)	184.82^{bc}	153.65^{d}	238.66^{a}	200.43^{b}	173.45^{cd}	3.08	<0.001	<0.001
VH/CD	5.55^{b}	6.08^{ab}	4.69^{c}	5.62^{b}	6.47^{a}	0.91	<0.001	0.020
Jejunum								
VH (μm)	509.12^{d}	499.62^{d}	669.91^{c}	775.03^{a}	732.45^{b}	8.32	<0.001	<0.001
CD (μm)	138.89^{b}	146.58^{b}	177.68^{a}	168.27^{a}	174.25^{a}	1.94	<0.001	<0.001
VH/CD	3.76^{b}	3.64^{b}	3.84^{b}	4.68^{a}	4.33^{a}	0.59	<0.001	<0.001
Ileum								
VH (μm)	348.35^{d}	386.84^{c}	444.57^{b}	426.68^{b}	476.24^{a}	3.75	<0.001	<0.001
CD (μm)	134.39	128.67	128.17	135.23	134.90	2.34	0.774	0.640
VH/CD	2.73^{c}	3.08^{bc}	3.62^{a}	3.38^{ab}	3.75^{a}	0.05	<0.001	<0.001
Day 30								
Duodenum								
VH (μm)	967.44^{b}	1023.94^{ab}	1011.69^{ab}	1081.96^{a}	1027.54^{ab}	8.55	0.001	0.003
CD (μm)	183.87^{b}	189.49^{b}	153.98^{c}	179.42^{b}	214.61^{a}	2.39	<0.001	0.001
VH/CD	5.51^{bc}	5.50^{bc}	6.68^{a}	6.22^{ab}	5.04^{c}	0.91	<0.001	0.695
Jejunum								
VH (μm)	871.39^{c}	833.67^{c}	926.85^{b}	953.24^{ab}	994.06^{a}	7.20	<0.001	<0.001
CD (μm)	152.69^{b}	160.85^{ab}	163.06^{ab}	156.81^{ab}	172.93^{a}	1.97	0.016	0.008
VH/CD	5.84^{ab}	5.35^{b}	6.05^{a}	6.24^{a}	5.92^{ab}	0.83	0.010	0.071
Ileum								
VH (μm)	296.75^{c}	371.44^{b}	360.17^{b}	373.06^{a}	437.06^{ab}	3.60	<0.001	<0.001
CD (μm)	139.58^{a}	118.57^{b}	117.99^{b}	135.15^{a}	128.77^{ab}	1.46	<0.001	<0.001
VH/CD	2.17^{c}	3.18^{ab}	3.12^{b}	2.93^{b}	3.51^{a}	0.05	<0.001	<0.001

Data represent the means of five individuals per treatment. $^{a–d}$Means with different superscripts in a row differ significantly ($P < 0.05$). VH/CD = villus height to crypt depth ratio; VH = villus height; CD = crypt depth; SEM = standard error of the mean; ANOVA = analysis of variance.

serves as a measure to assess the oxidative status of animals[38]. MDA functions as a marker for assessing the extent of lipid peroxidation, as it constitutes the final product of this process[39]. In the present investigation, we observed that dietary supplementation with puerarin increased T-AOC, SOD, and GSH activities in the serum and liver and enhanced CAT activities in serum, whereas hepatic MDA content was reduced. Previous research has demonstrated that puerarin alleviates oxidative stress in bovine mammary epithelial cells[40]. Puerarin has also been shown to mitigate the damage to intestinal antioxidant function in pigs and enhance the antioxidant capacity of beef cattle subjected to heat stress[26,35,41]. Collectively, these data indicate that dietary supplementation with puerarin significantly enhances the antioxidant capacity of pigeons, suggesting that puerarin can be a valuable natural antioxidant addition to pigeon diets.

The intestine is an essential organ in pigeons that is required to accomplish digestion, maintain metabolism, and establish immunity; thus, its integrity is important for pigeon health and development. Typically, increased VH and a higher VH/CD ratio are indicative of healthy intestinal morphology[42,43]. In the present study, puerarin treatment exerted a positive effect on intestinal morphology, leading to a significant enhancement in small intestinal villi growth and an associated increase in the VH/CD ratio. In particular, a higher VH/CD ratio can enhance intestinal mucosal absorption and improve the overall intestinal mucosal structure[7,44]. Previous research has demonstrated that puerarin supplementation promotes the proliferation of intestinal mucosal cells and enhances intestinal health in a diquat-challenged piglet model[45]. Moreover, puerarin effectively rescued the reduction in VH and the VH/CD ratio in piglets induced by enterotoxigenic *Escherichia coli*, thus alleviating intestinal damage[41]. Together, these findings indicate that puerarin effectively promotes the development of the pigeon intestine and enhances pigeon adaptability to dietary variations, although the specific regulatory mechanisms remain to be fully elucidated.

In conclusion, this study identified that dietary supplementation of puerarin enhances immune response and antioxidant capacity, and promotes intestinal development in pigeons. The

results of linear regression analysis further indicate that dietary supplementation with puerarin could increase the thymus index, serum IgA, IgM, T-AOC, SOD, GSH, CAT and liver T-AOC, SOD, GSH levels, and reduce liver MDA levels, along with increasing the VH and VH/CD ratio of the duodenum, jejunum and ileum. The data presented herein indicate that puerarin could be a promising supplement for newly-weaned pigeons to enhance health.

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Author Contributions

Runzhi Wang, Tingting Li, and Zaixu Pan designed this study; Zaixu Pan, Bang Bian, and Xixue Lu performed the animal experiments; Runzhi Wang, Tingting Li, Hui Chen, Xixue Lu, and Kai Shi acquired and analyzed the data; Runzhi Wang wrote the original draft; Tingting Li and Hui Chen reviewed and edited the draft; and Shanjin Xu and Guansuo Wu provided in supervision and project administration.

Conflicts of Interest

The authors declare no conflict of interest.

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