Dietary L-arginine supplementation enhances intestinal antioxidative capacity in yellow-feathered chickens

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Abstract

This study investigated the effects of dietary Arginine (Arg) on intestinal antioxidative capacity in Chinese yellow-feathered chickens. One thousand and two hundred 1-day-old female Qingyuan partridge chickens were randomly assigned to 5 groups with 6 replicates of 40 birds each. Chickens were fed diets with 5 levels of total Arg (8.5, 9.7, 10.9, 12.1, and 13.3 g/kg) without antibiotics for 30 days. Dietary Arg level had a linear ($P < 0.05$) or quadratic ($P < 0.05$) effect on the gene expression of glutathione peroxidase 1, heme oxygenase 1, nuclear factor erythroid 2-related factor 2, and the activities of glutathione peroxidase and total antioxidative capacity in the jejunum and ileum. A diet containing 12.1 g Arg/kg promoted intestinal antioxidation in yellow-feathered chickens.

Key words: arginine, intestinal antioxidation, yellow-feathered chickens

Introduction

In poultry, arginine (Arg) is an essential amino acid and is also a functional amino acid due to the lack of carbamoyl-phosphate synthetase and ornithine carbamoyl transferase in the urea cycle (Wu et al., 2009). Arginine and/or its derivatives enhance growth performance, reproduction, digestive enzymes secretion, nutrient transporters expression, antioxidative status, intestinal barrier function, and immunity (Duan et al., 2015; Hu et al., 2016; Gao et al., 2017; Xu et al., 2018; Castro et al., 2019; Zhang et al., 2020).

Intestinal antioxidation, immunity, and structure of the enteric microbial community in young broiler chickens are all associated with health of the birds (Tang et al., 2019; Diaz et al., 2019). Previous studies have shown that Arg supplementation alleviated oxidative stress and improved the antioxidative capacity (Cao et al., 2016; Liang et al., 2018). Chickens fed diets deficient in Arg have decreased protein accretion resulting in problems in growth, antioxidation, and immunity (Kwak et al., 1999; Jahanian, 2009; Xu et al., 2018).

The Qingyuan partridge chicken is an important indigenous slow-growing breed in China that is very popular for its superior meat quality. To the authors' knowledge, nothing is known about the impact of Arg on intestinal antioxidation in Qingyuan partridge chickens. Therefore, the current study aimed to determine the effect of dietary Arg levels on antioxidative capacity in Qingyuan partridge chickens reared without in-feed antibiotics and the optimal dietary Arg requirements in a dose-dependent manner.

Materials and methods

Experimental design, diets, and bird husbandry

All experimental procedures were approved by the Animal Care and Use Committee of the Institute of Animal Science, Guangdong Academy of Agriculture Sciences and performed in accordance with animal welfare and ethics (GAASI-SA-2019-019). The trial used a completely randomized block design with 5 dietary levels of total Arg. The control diet used corn gluten meal to achieve a low level of Arg (calculated 8.5 g/kg) but otherwise satisfied the nutritional requirements for Qingyuan partridge chickens (Table 1). The 4 additional treatments were the basal diet supplemented with 1.2, 2.4, 3.6, and 4.8 g/kg L-Arg (98.5% purity, CJ CheilJedang Co., Ltd, Shanghai, China) to make the dietary Arg level of 9.7, 10.9, 12.1, and 13.3 g/kg of diet.

Table 1. **Composition and nutrient content of the basal diet for yellow-feathered chickens (as-fed basis)**

Component	Content, g/kg	Nutrient composition[2]	Level, g/kg
Corn	606.0	Metabolizable energy, MJ/kg	11.9
Wheat bran	135.0	CP	192.6
Soybean meal	90.0	Ca	9.5
Corn gluten meal	120.0	Total P	6.7
L-Lysine·HCl (98.5%)	4.7	Available P	4.4
DL-Methionine (99%)	0.9	Total Lys	9.9
L-Threonine hydrochloride (98.5%)	1.3	Total Met	4.1
L-Tryptophan (98%)	0.2	Total Met+Cys	8.0
Limestone	13.2	Total Thr	7.2
Dicalcium phosphate	16.8	Total Trp	1.6
Sodium chloride	3.0	Total Ile	7.0
Premix[1]	10.0	Total Arg	8.7
Total	1000.0		

[1]The premix provided per kilogram of diet: vitamin A, 3,000 IU; vitamin D3, 600 IU; vitamin E, 20 mg; vitamin K3, 0.5 mg; vitamin B1, 3.8 mg; vitamin B2, 4.0 mg; vitamin B6, 3.5 mg; vitamin B12, 0.01 mg; choline, 1,300 mg; nicotinic acid, 25 mg; pantothenic acid, 10 mg; folic acid, 0.55 mg; biotin, 0.15 mg; Fe, 80 mg; Cu, 7.0 mg; Mn, 60 mg; Zn, 70 mg; I, 0.35 mg; Se, 0.23 mg

[2]Total Arg, Lys, Met, Met+Cys, Thr, Ile, and CP were measured values in the mixed feed. Each value is based on triplicate determinations. Other nutrient compositions are calculated values.

A total of 1,200 one-day-old female Qingyuan partridge chicks were randomly assigned to the 5 dietary treatments, each with 6 replicates of 40 birds. Each replicate was housed in 1 of 30 identical galvanized steel floor pens with 8 water nipples and 2 feeders. All chicks were handled in accordance with the Qingyuan partridge chicken management guidelines for lighting, ad libitum feeding and allowed access to non-antibiotic tap water from 1 to 30 d. The temperature of the room was maintained at 32 to 35 ℃ for the first week and then reduced by 2 to 3 ℃ per week to a final temperature of 26 ℃.

Sample collection and preparation

At the end of the 30-d experiment, 2 chickens per replicate were sampled at random. The birds were stunned and exsanguinated. Samples of the mid-portions of jejunum and ileum were collected, rinsed rapidly with ice-cold PBS (pH 7.4), snap-frozen in liquid N_2 and stored at -80 °C. for further analysis. Ileal digesta samples were collected, and snap-frozen as well.

Antioxidative indices in jejunum and ileum

The jejunal and ileal tissues were homogenized in ice-cold PBS and then centrifuged at 10,000 × g for 10 min at 4 °C, and the supernatants were stored at -80 °C. The activities of total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), total antioxidative capacity (T-AOC), and the content of malondialdehyde (MDA) were measured using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturers' instructions.

RNA Isolation, Reverse Transcription, and Real-Time Quantitative PCR

Total RNA was isolated from the frozen jejunum and ileum samples by TRIzol reagent (Invitrogen, Carlsbad, CA) followed by quality measurement on 1.0% denaturing agarose gel and yield determination on a Nano-Drop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The cDNA was synthesized by reverse transcription using a PrimeScript RT Regent Kit with gDNA Eraser (TaKaRa, Dalian, China). The primers (Table 2) were based on chicken sequences and were purchased from Sangong Biological Engineering Co., Ltd. (Shanghai, China).
Real-time quantitative PCR were performed on a CFX 96 real-time PCR Detection System (Bio-Rad,Hercules, CA). The qPCR procedure was as follows: denaturation at 95°C for 30 s, followed by 40 cycles of 95°C for 15 s, and annealing and amplification for 30 s. Amplification was performed in a total volume of 20 μL containing 10 μL of SYBER Green PCR Master Mix (TakaRa), 2 μL of 10 × cDNA mix, 1 μL of each primer, and 7 μL nuclease-free water. All measurements were carried out in triplicate, and the average values were obtained. The $2^{-\triangle\triangle Ct}$ method was used to analyze the relative mRNA expression of each target gene (Livak and Schmittgen, 2001). β-actin was used as an endogenous control to normalize the expression of the targeted genes. Data are shown with further normalization to values obtained from the basal diet.

Results

Antioxidative indices and related gene expression in the jejunum and Ileum of yellow-feathered chickens

Jejunal and ileal activities of GSH-Px and T-AOC increased linearly and quadratically ($P < 0.05$) as dietary Arg concentration increased (Table 3). However, the jejunal content of MDA decreased linearly with Arg supplementation ($P < 0.001$). Dietary Arg levels increased the jejunal and ileal abundance of glutathione peroxidase1 (GPX1), heme oxygenase 1 (HMOX1), and nuclear factor erythroid 2-related factor 2 (NRF2) transcripts in a linear ($P < 0.05$) and quadratic ($P < 0.05$) manner. There were no significant effects ($P > 0.05$) on the jejunal and ileal expression of superoxide dismutase 1 (SOD1).

Table 2. Primers of target genes used for quantitative real-time PCR.

Transcript[1]	Accession number	Primer sequence (5′-3′)	Annealing temperature (°C)
GPX1	NM_001277853.2	F: AAGTGCCAGGTGAACGGGAAGG	60
		R: AGGGCTGTAGCGGCGGAAAG	
SOD1	NM_205064.1	F: GGTGCTCACTTTAATCCTG	60
		R: CTACTTCTGCCACTCCTCC	
HMOX1	NM_205344.1	F: CTCAAGGGCATTCATTCG	56
		R: ACCCTGTCTATGCTCCTGTT	
NRF2	NM_205117.1	F: ATCACCTCTTCTGCACCGAA	60
		R: GCTTTCTCCCGCTCTTTCTG	
IL1B	NM_204524.1	F: GAAGTGCTTCGTGCTGGAGT	60
		R: ACTGGCATCTGCCCAGTTC	
TNF-α	NM_204267.1	F: AATTTGCAGGCTGTTTCTGC	60
		R: TATGAAGGTGGTGCAGATGG	
COX-2	NC_001323.1	F: TGCAACGATATGGCTGAGAG	57
		R: CTGCGATTCGGTTCTGGTAT	
MYD88	NM_001030962.4	F: AGCATTACCAGGGCTGAGTT	59
		R: TGGTACCATGCCAGCAGTTA	
TLR4	NM_001030693.1	F: AGTCTGAAATTGCTGAGCTCAAAT	60
		R: GCGACGTTAAGCCATGGAAG	
TICAM1	NM_001081506.1	F: CAACTGGCCCTCCTCCTTTA	56
		R: CAAGTCAGCTGGTTGTGTCC	
β-actin	NM_205518	F: GAGAAATTGTGCGTGACATCA	55~60
		R: CCTGAACCTCTCATTGCCA	

[1]GPX1 = glutathione peroxidase 1; SOD1 = superoxide dismutase 1; HMOX1 = heme oxygenase 1; NRF2 = nuclear factor erythroid 2-related factor 2; IL1B = interleukin 1, beta; TNF-α = tumor necrosis factor-alpha; COX2 = cyclo-oxygenase 2; MYD88 = myeloid differentiation primary response 88; TLR4 = toll like receptor 4; TICAM1 = toll like receptor adaptor molecule 1.

Table 3. Effects of dietary L-arginine levels on the antioxidant indices in jejunum and ileum of yellow-feathered chickens at 30 days of age[1]

Indices[2]	Dietary Arg level, g/kg					SEM	*P*-value		
	8.5	9.7	10.9	12.1	13.3		Arg	Linear	Quadratic
Jejunum									
GSH-PX (U/mg prot)	4.81^{b}	6.32^{b}	11.36^{a}	11.88^{a}	7.55^{a,b}	1.33	<0.001	0.02	0.00
T-SOD (U/mg prot)	743.2	764.9	735.1	749.9	765.9	71.5	NS	NS	NS
T-AOC (U/mg prot)	1.73^{b}	1.87^{b}	1.95^{a,b}	2.11^{a}	2.08^{a}	0.10	0.07	0.01	0.06
MDA (nmol/mg prot)	0.595^{a}	0.353^{a,b}	0.349^{a,b}	0.297^{b}	0.281^{b}	0.07	0.03	0.07	NS
Ileum									
GSH-Px (U/mg prot)	7.18^{c}	13.85^{b,c}	22.32^{a,b}	26.85^{a}	16.76^{b}	2.48	<0.001	<0.001	0.00
T-SOD (U/mg prot)	754.50	684.60	793.10	705.00	731.30	79.08	NS	NS	NS
T-AOC (U/mg prot)	1.99^{b}	2.20^{a,b}	2.19^{a,b}	2.66^{a}	2.24^{a,b}	0.14	0.02	0.02	0.04
MDA (nmol/mg prot)	0.223	0.197	0.181	0.172	0.225	0.035	NS	NS	NS

[1]Means are based on 2 birds per pen and 6 replicate pens per diet.
[2]GSH-PX = glutathione peroxidase; T-SOD = total superoxide dismutase; T-AOC = total antioxidative capacity; MDA = malondialdehyde.
NS = not significant.
[a-c]Means with different superscripts within a main effect differ significantly ($P < 0.05$).

Dietary Arg requirements of Qingyuan partridge chickens estimated by quadratic regression analysis are shown in Table 4. The dietary Arg requirements for chickens aged 1 to 30 d for optimizing ileal activity of GSH-Px was 11.0 g/kg.

Table 4. **Effects of dietary L-arginine level on the relative jejunal and ileal expression of genes related to antioxidation in yellow-feathered chickens at 30 days of age[1]**

Indices[2]	Dietary Arg level, g/kg					SEM	*P*-valuet		
	8.5	9.7	10.9	12.1	13.3		Arg	Linear	Quadratic
Jejunum									
GPX1	1.02^{b}	1.12^{b}	1.20^{b}	1.60^{a}	1.42^{a,b}	0.110	0.021	0.083	0.005
SOD1	1.02	1.08	1.20	1.39	1.14	0.212	NS	NS	NS
HMOX1	1.04^{b}	1.3^{b}	2.04^{a}	2.15^{a}	1.80^{a,b}	0.227	0.013	0.005	0.031
NRF2	1.02^{b}	1.35^{b}	1.97^{a,b}	2.39^{a}	1.82^{a,b}	0.266	0.035	0.004	0.022
Ileum									
GPX1	1.01^{b}	1.62^{a,b}	2.17^{a}	2.43^{a}	2.24^{a}	0.247	0.02	<0.001	0.015
SOD1	1.04	1.08	1.14	1.3	1.31	0.182	NS	NS	NS
HMOX1	1.03^{b}	0.97^{b}	1.08^{b}	2.57^{a}	2.17^{a}	0.185	0.008	<0.001	0.022
NRF2	1.05^{b}	1.38^{b}	1.66^{a,b}	2.75^{a}	1.86^{a,b}	0.320	0.003	0.004	0.017

[1]Means are based on 2 birds per pen and 6 replicate pens per diet.
[2]GPX1 = glutathione peroxidase 1; SOD1 = superoxide dismutase 1; HMOX1 = heme oxygenase 1; NRF2 = nuclear factor erythroid 2-related factor 2.
NS = not significant.
[a,b]Means with different superscripts within a main effect differ significantly (P < 0.05)

Discussion

Arg is considered to contribute significantly to antioxidative potential. It increases antioxidative ability, reduces superoxide release, and ameliorates lipid peroxidation (Liang et al., 2018). Atakisi et al. (2009) revealed that diet supplemented with 5 mg Arg/kg for a month improved antioxidative capacity and decreased plasma MDA in quail, which was consistent with the present findings in Chinese yellow-feathered chickens. Also, in aged broiler breeder hens, adding 4.0 g Arg/kg diet increased activity of GSH-Px and T-AOC in egg yolk of breeders and in the serum, liver, and breast muscles of their hatchling offspring, while concentrations of MDA decreased in all of these tissues (Duan et al., 2015). In the current study, dietary Arg increased expression in jejunum and ileum of GPX1, HMOX1, and NRF2 genes. Heme oxidase-1 (HMOX-1) participates in the antioxidative defense system by its a pivotal role in generating biliverdin and bilirubin, whereas the transcription factor, NRF2, is a key transcription factor for antioxidative mechanisms against oxidative stress. It can upregulate antioxidative responsive element depezndent gene expressions, such as SOD, GPX, catalase, glutathione S-transferase, NAD(P)H: quinone oxidoreductase 1 (Loboda et al., 2016). The present findings indicate that Arg enhanced the intestinal antioxidative defense system and reduced lipid peroxidation via regulating the NRF2 signaling pathway. In Carp fish, dietary Arg supplementation enhanced GPX mRNA expression by activating NRF2 signaling pathway (Wang et al., 2015).

Conclusions

Optimal Arg levels for maximizing ileal activity of GSH-Px were 11.0 g/kg in Qingyuan partridge chickens. Dietary Arg enhanced intestinal antioxidative capacity.

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