Dietary L-arginine supplementation enhances growth performance, intestinal antioxidative capacity, immunity and modulates gut microbiota in yellow-feathered chickens

D. Ruan,[*] A. M. Fouad,[*,†] Q. L. Fan,[*] X. H. Huo,[*] Z. X. Kuang,[‡] H. Wang,[‡] C. Y. Guo,[§] Y. F. Deng,[§] C. Zhang,[*] J. H. Zhang,[*] and S. Q. Jiang[*,1]

*Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Key Laboratory of Animal Nutrition and Feed Science in South China, Ministry of Agriculture and Rural Affairs, State Key Laboratory of Livestock and Poultry Breeding, Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangzhou 510640, China; †Department of Animal Production, Faculty of Agriculture, Cairo University, Giza 12613, Egypt; ‡Guangdong Aijiankang Biotechnology Co., Ltd., Qingyuan 511500, China; and §CJ International Trading Co., Ltd., Shanghai 201107, China

ABSTRACT This study investigated the effects of dietary Arginine (**Arg**) on performance, intestinal antioxidative capacity, immunity, and gut microbiota in Chinese yellow-feathered chickens. One thousand 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 d. The ADFI, ADG, and feed conversion ratio were improved with dietary Arg levels ($P < 0.05$). The proportions of $CD3^+$ and $CD4^+/CD8^+$ lymphocytes responded in a linear ($P < 0.05$) manner and those of $CD4^+$ in a linear or quadratic ($P < 0.05$) manner as dietary Arg levels increased. 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. The relative expression of IL-1β, myeloid differentiation primary response 88, and Toll-like receptor 4 decreased linearly ($P < 0.05$) in the ileum with increasing dietary Arg levels; secretory IgA contents were increased. In addition, sequencing data of 16S rRNA indicated that dietary Arg increased the relative abundance of Firmicutes phylum, *Romboutsia* and *Candidatus Arthromitus* genera, while decreased that of *Clostridium sensu stricto 1*. A diet containing 12.1 g Arg/kg promoted growth performance, intestinal antioxidation, and innate immunity and modulated gut microbiota in yellow-feathered chickens.

Key words: arginine, intestinal antioxidation, immunity, gut microbiota, yellow-feathered chicken

INTRODUCTION

In poultry, arginine (**Arg**) is an essential amino acid and is also a functional amino acid owing to the lack of carbamoyl-phosphate synthetase and ornithine carbamoyl transferase in the urea cycle (Wu et al., 2009). It is an important substrate for protein synthesis and a precursor of many molecules such as nitric oxide, creatine, ornithine, glutamate, glutamine, polyamines, proline, and agmatine (Wu and Morris, 1998). 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., 2017a; Xu et al., 2018; Castro et al., 2019; Zhang et al., 2020). The Arg requirements for modern strains of broiler chickens showed that their requirements of Arg depend on age, sex and other variables (Cuca and Jensen, 1990; Kidd et al., 2001; Labadan et al., 2001; Chamruspollert et al., 2002). These studies mentioned previously have shown that Arg requirements vary greatly among species. To date, very little is known about the Arg requirements of Chinese yellow-feathered broiler chickens.

Intestinal antioxidation, immunity, and structure of the enteric microbial community in young broiler chickens are all associated with health of the birds

(Diaz Carrasco et al., 2019; Tang et al., 2019). Previous studies have reported that Arg modulates T cell metabolism and enhances survival (Geiger et al., 2016). Others have shown that Arg supplementation alleviated oxidative stress and improved the antioxidative capacity (Cao et al., 2016; Liang et al., 2018). Furthermore, dietary Arg supplementation enhanced innate immune responses, alleviated gut injury, and normalized the ileal microbiota of Arbor Acres broiler chickens challenged with *Clostridium perfringens* or *Salmonella enterica* (Zhang et al., 2017, 2018, 2020). Dietary supplementation of Arg in turkeys also increased turkey's cecal *Lactobacillus* counts and reduced small intestinal *Salmonella* counts (Oso et al., 2017). 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 of the impact of Arg on intestinal antioxidation, immunity, and the related ileal microflora community in Qingyuan partridge chickens. Therefore, the present study aimed to determine the effect of dietary Arg levels on growth performance, antioxidative capacity, innate immunity, and gut microbiota 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 (GAASISA-2019-019). The trial used a completely randomized block design with 5 grade contents 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 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. These are identified by their calculated Arg content throughout this article. The Arg concentrations in hydrolyzates of the prepared diets were analyzed using a Hitachi L-8900 Amino Acid Analyzer (Hitachi High Technologies Corporation, Tokyo, Japan). The analyzed levels of dietary Arg were 8.7, 9.9, 11.0, 11.9, and 13.4 g/kg of diet.

A total of 1,200 1-day-old female Qingyuan partridge chicks (Guangdong Aijiankang Biotechnology Co., Ltd., Qingyuan, China) 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 (length 160 cm × width 140 cm × height 40 cm; Guangzhou Muxing Poultry Equipment Co., Ltd., Guangzhou, China) 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 nonantibiotic tap water from 1 to 30 d. The temperature of the room was maintained at 32°C to 35°C for the first week and then reduced by 2°C to 3°C per week to a final temperature of 26°C.

Growth Performance Determination

The birds were weighed, by pen, at day 1 and 30 d of the experiment, and consumed feed was recorded daily to determine the ADFI, ADG, and feed conversion ratio (**FCR**; feed: gain, g: g). Mortality was recorded daily and was used to adjust the total number of birds per replicate to exclude them from calculations of ADFI and FCR.

Sample Collection and Preparation

At the end of the 30-d experiment, 2 chickens per replicate were sampled at random (excluding obvious outliers in BW). Fresh heparinized blood was collected from the wing vein for immediate T lymphocyte phenotype analysis. The birds were stunned and exsanguinated. Samples of the midportions of the 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.

Peripheral Blood Lymphocytes Isolation and Flow Cytometric Analysis of T-Lymphocyte Phenotypes

Peripheral blood lymphocytes were isolated from heparinized peripheral blood samples using lymphocyte separation medium (Solarbio, Beijing, China) as per the manufacturer's protocol. The isolated peripheral blood lymphocytes were incubated with mouse anti-chicken CD3-FITC (Southern Biotech, Birmingham, AL) and mouse anti-chicken CD4-APC (Southern Biotech) or mouse anti-chicken CD8a-PE (Southern Biotech) at 37°C for 30 min, then washed twice with PBS to remove excess antibodies. The percentage of $CD3^+$, $CD4^+$, and $CD8^+$ cells were analyzed with a FACSCalibur flow cytometer (Becton Dickinson and Company, Franklin Lakes, NJ). Cell phenotypic data were expressed as percentages of gated lymphocytes.

Determination of IgG, IgM, and IgA Contents in Jejunum and Ileum

The contents of IgG, IgM, and secretory IgA (**sIgA**) were assayed using chicken elisa kits (Beijing Equation Biotechnology Co., Ltd., Beijing, China) with a spectrophotometer (Thermo Electron, Rochester, NY). Protein

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	ME, MJ/kg	11.93
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	1,000.0		

[1]The premix provided per kilogram of diet: vitamin A, 3,000 IU; vitamin D_3, 600 IU; vitamin E, 20 mg; vitamin K_3, 0.5 mg; vitamin B_1, 3.8 mg; vitamin B_2, 4.0 mg; vitamin B_6, 3.5 mg; vitamin B_{12}, 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.

concentrations were measured with the use of bicinchoninic acid protein assay kit from Beyotime Institute of Biotechnology (Haimen, China).

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, glutathione peroxidase (**GSH-Px**), total antioxidative capacity, and the content of malondialdehyde (**MDA**) were measured using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) as per 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,

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

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

Abbreviations: *GPX1*, glutathione peroxidase 1; *HMOX1*, heme oxygenase 1; *IL1B*, IL 1, beta; *MYD88*, myeloid differentiation primary response 88; *NRF2*, nuclear factor erythroid 2–related factor 2; *SOD1*, superoxide dismutase 1; *TICAM1*, Toll-like receptor adaptor molecule 1; *TLR4*, Toll-like receptor 4; *TNF-α*, tumor necrosis factor-alpha.

Table 3. Effects of the dietary *L*-arginine level on the growth performance of yellow-feathered chickens in the starter phase.[1]

Indices	Dietary Arg level, g/kg					SEM	P-value		
	8.5	9.7	10.9	12.1	13.3		ANOVA	Linear	Quadratic
1 d BW (g)	31.30	31.34	31.44	31.38	31.41	0.067	NS	NS	NS
30 d BW (g)	262.2[c]	281.6[b]	289.8[a,b]	290.4[a,b]	296.9[a]	3.018	<0.001	<0.001	0.008
ADFI (g)	17.89[b]	18.23[a,b]	18.70[a,b]	18.97[a,b]	19.40[a]	0.326	0.017	0.002	NS
ADG (g)	7.97[c]	8.63[b]	8.91[a,b]	8.93[a,b]	9.16[a]	0.104	<0.001	<0.001	0.009
FCR (g feed/g gain)	2.25[a]	2.12[b]	2.10[b]	2.12[b]	2.12[b]	0.029	0.047	0.014	0.014
Mortality (%)	1.67	1.25	0.00	0.82	0.42	0.203	NS	NS	NS

[a-c]Means with different superscripts within a main effect do not differ significantly ($P < 0.05$).

Abbreviations: FCR, feed conversion ratio; NS, not significant.

[1]Means are based on 40 birds per pen and 6 replicate pens per diet.

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 $2^{-\Delta\Delta Ct}$ 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.

16S rRNA Sequencing of Gut Microbiota

Microbial community genomic DNA was extracted from pooled samples of ileal digesta of birds (n = 4) fed the control diet and pools of samples from birds fed the Arg-supplemented diets (ArgM, 10.9 g/kg and ArgH, 13.3 g/kg) using the Mag-Bind Soil DNA Kit (Omega Biotec, Norcross, GA) as per the manufacturer's instructions. The V3-V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F (5′-ACTCC-TACGGGAGGCAGCAG-3′) and 806R (5′-GGAC-TACHVGGGTWTCTAAT-3′) using an ABI GeneAmp 9700 PCR thermocycler (Thermo Fisher Scientific). The 16S rRNA genes were sequenced on the Illumina MiSeq platform as per the standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai,

China). The raw reads were demultiplexed, quality-filtered by Trimmomatic, and merged by FLASH to obtain the effective reads. Operational taxonomic units with 97% similarity cutoff were clustered using UPARSE (version 7.1; http://drive5.com/uparse/). The taxonomy of each operational taxonomic units representative sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/). Alpha diversity (Observed species, Chao1, ACE, Shannon, Simpson, and Coverage) and beta diversity (principal coordinates analysis [**PCoA**] and unweighted UniFrac full tree method) were calculated by analysis of similarity (**ANOSIM**). Differentially abundant taxa among the treatments were identified using laser diffraction analysis (**LDA**) effect size analysis ($\alpha = 0.05$, LDA score > 4).

Statistical Analysis

Replicate (n = 6) served as the experimental unit. Except where noted otherwise, averages of the 2 sampled birds per replicate were used. Effects of dietary Arg were analyzed by 1-way ANOVA using the GLM procedure in SAS (SAS Institute Inc., Cary, NC). Differences among means were assessed using Duncan's multiple range tests at $P < 0.05$ probability levels. The orthogonal comparisons were applied for linear and quadratic effects of Arg. Quadratic regressions ($Y = c + bX + aX^2$) were fitted to the responses of the dependent variables to dietary Arg content. The dietary concentration of Arg at which the response first reached 95% of the maximum was used to estimate the requirement (Dozier et al., 2009).

Table 4. Effects of the dietary *L*-arginine level on the percentages of blood lymphocytes expressing cell-surface antigens for CD3$^+$, CD4$^+$, and CD8$^+$ of yellow-feathered chickens at 30 d of age.[1]

Indices	Dietary Arg level, g/kg					SEM	P-value		
	8.5	9.7	10.9	12.1	13.3		Arg	Linear	Quadratic
CD3$^+$ (%)	29.48[b]	31.22[a,b]	36.74[a,b]	39.32[a]	38.07[a,b]	3.724	0.037	0.035	NS
CD4$^+$ (%)	20.72[b]	24.67[a,b]	26.33[a,b]	29.30[a]	28.79[a]	1.397	0.019	0.005	0.013
CD8$^+$ (%)	9.05	10.08	11.74	9.82	10.50	1.239	NS	NS	NS
CD4$^+$/CD8$^+$	2.03[b]	2.39[a,b]	2.43[a,b]	3.04[a]	2.79[a,b]	0.382	0.062	0.018	NS

[a-c]Means with different superscripts within a main effect do not differ significantly ($P < 0.05$).

Abbreviations: CD3, cluster of differentiation 3; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; NS, not significant.

[1]Means are based on 2 birds per pen and 6 replicate pens per diet.

Table 5. Effects of the dietary *L*-arginine level on the antioxidant indices in the jejunum and ileum of yellow-feathered chickens at 30 d of age.[1]

	Dietary Arg level, g/kg						P-value		
Indices	8.5	9.7	10.9	12.1	13.3	SEM	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]	1.329	<0.001	0.016	0.004
T-SOD (U/mg prot)	743.2	764.9	735.1	749.9	765.9	71.46	NS	NS	NS
T-AOC (U/mg prot)	1.73[b]	1.87[b]	1.95[a,b]	2.11[a]	2.08[a]	0.099	0.074	0.008	0.061
MDA (nmol/mg prot)	0.595[a]	0.353[a,b]	0.349[a,b]	0.297[b]	0.281[b]	0.071	0.0264	0.067	NS
Ileum									
GSH-Px (U/mg prot)	7.18[c]	13.85[b,c]	22.32[a,b]	26.85[a]	16.76[b]	2.480	<0.001	<0.001	0.004
T-SOD (U/mg prot)	754.5	684.6	793.10	705.00	731.3	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]	0.138	0.022	0.019	0.035
MDA (nmol/mg prot)	0.223	0.197	0.181	0.172	0.225	0.035	NS	NS	NS

[a-c]Means with different superscripts within a main effect do not differ significantly ($P < 0.05$).

Abbrevaitions: GSH-PX, glutathione peroxidase; MDA, malondialdehyde; NS, not significant; T-SOD, total superoxide dismutase; T-AOC, total antioxidative capacity.

[1]Means are based on 2 birds per pen and 6 replicate pens per diet.

RESULTS

Growth Performance

As shown in Table 3, ADFI increased in a linear manner ($P < 0.001$) with increasing dietary Arg inclusion. Means for 30 d BW, ADG, and FCR increased in a linear and quadratic manner ($P < 0.05$) as dietary Arg concentrations increased.
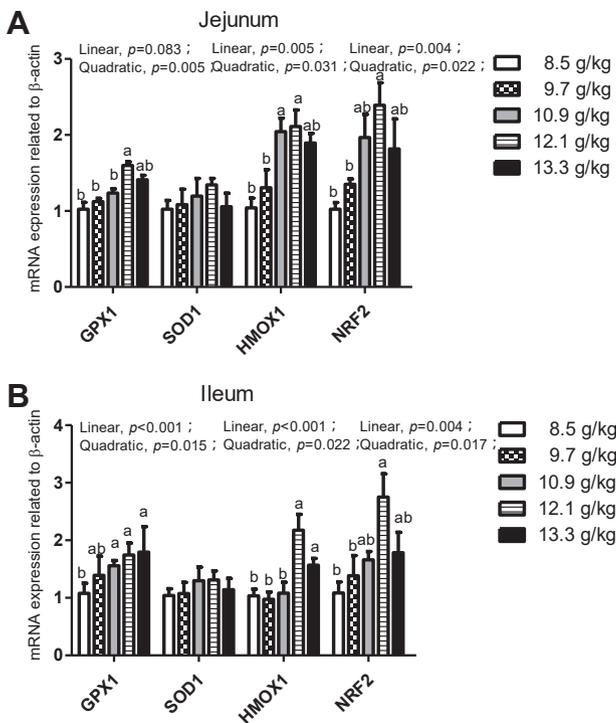

Figure 1. Effect of dietary *L*-arginine levels on the expression genes related to antioxidation in the jejunum and ileum. n = 6. (A) Relative mRNA abundances of glutathione peroxidase 1 (*GPX1*), superoxide dismutase 1 (*SOD1*), heme oxygenase 1 (*HMOX1*), and nuclear factor erythroid 2–related factor 2 (*NRF2*) in the jejunum. (B) Relative mRNA abundances of *GPX1*, *SOD1*, *HMOX1*, and *NRF2* in the ileum. Changes in mRNA abundances of *GPX1*, *SOD1*, *HMOX1*, and *NRF2* are normalized to β-actin and expressed relative to the 8.5 g/kg *L*-arginine group. Data are means ± SE, n = 6. Means with different letters (superscripts a, b) differ ($P < 0.05$).

T Lymphocyte Phenotypic Analysis

As shown in Table 4, the proportions of CD3^{+} and CD4^{+}/CD8^{+} lymphocytes responded in a linear ($P < 0.05$) manner and those of CD4^{+} in a linear and quadratic ($P < 0.05$) manner as dietary Arg concentrations increased. There were no significant effects ($P > 0.05$) of increasing Arg on the proportions of CD8^{+} cells.

Antioxidative Indices and Related Gene Expression in the Jejunum and Ileum of Yellow-Feathered Chickens

Table 5 shows that the jejunal and ileal activities of GSH-Px and total antioxidative capacity increased linearly and quadratically ($P < 0.05$) as dietary Arg concentration increased; jejunal content of MDA showed a linear decrease ($P < 0.001$). As shown in Figure 1, dietary Arg levels increased the jejunal and ileal abundance of glutathione peroxidase1, heme oxygenase 1, 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.

Contents of Ig and Expression of Inflammatory Genes in the Jejunum and Ileum of Yellow-Feathered Chickens

Table 6 shows that dietary Arg levels increased the jejunal content of IgG in a linear ($P < 0.05$) manner and increased the ileal content of sIgA in a linear ($P < 0.05$) and quadratic ($P < 0.05$) manner. As shown in Figure 2B, with increased dietary Arg, there were decreasing trends in ileal expression of IL 1 beta (*IL1B*) ($P = 0.004$), Toll-like receptor 4 (*TLR4*) ($P = 0.082$) and myeloid differentiation primary response 88 ($P = 0.062$). There were no influence ($P > 0.05$) of Arg on the ileal expression of *tumor* necrosis factor-alpha, or Toll-like receptor adaptor molecule 1. Furthermore, there were no significant effects ($P > 0.05$)
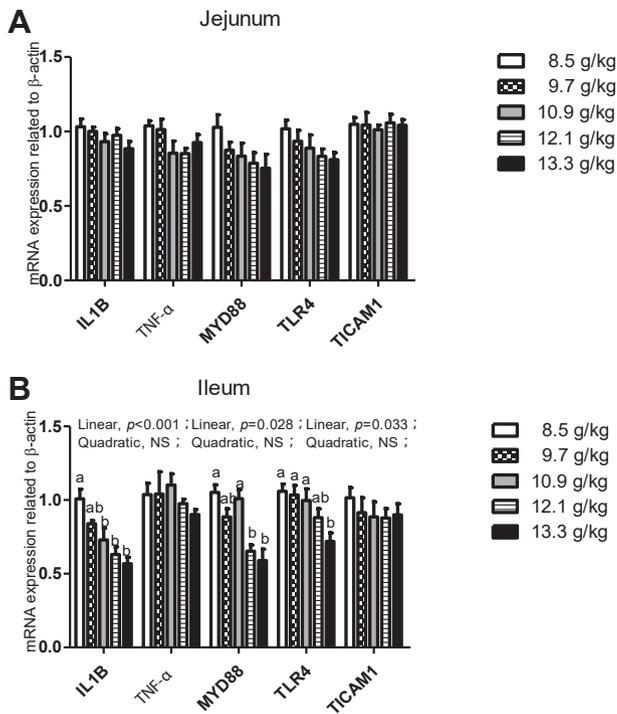
Figure 2. Effect of dietary *L*-arginine levels on the expression genes related to immunity in the jejunum and ileum. n = 6. (A) Relative mRNA abundances of IL 1, beta (*IL1B*), *tumor* necrosis factor-alpha (*TNF-α*), myeloid differentiation primary response 88 (MYD88), Toll-like receptor 4 (TLR4), and Toll-like receptor adaptor molecule 1 (*TICAM1*) in the jejunum. (B) Relative mRNA abundances of *IL1B*, *TNF-α*, *MYD88*, *TLR4*, and *TICAM1* in the ileum. Changes in mRNA abundances of *IL1B*, *TNF-α*, *MYD88*, *TLR4*, and *TICAM1* are normalized to *β*-actin and expressed relative to the 8.5 g/kg *L*-arginine group. Data are means ± SE, n = 6. Means with different letters (superscripts a, b) differ ($P < 0.05$).

on the jejunal expression of myeloid differentiation primary response 88, *IL1B*, *tumor* necrosis factor-alpha, *TLR4*, and Toll-like receptor adaptor molecule 1 (Figure 2A).

Gut Microbiome

Beta diversity analysis was illustrated by PCoA and ANOSIM in Figure 3A. Principal coordinates analysis

of operational taxonomic units, based on the unweighted UniFrac full tree method indicated there was distinct separation of ileal microbiota between the controls and Arg-supplemented birds ($P = 0.058$).

As shown in Supplementary Figure 1, Arg addition significantly increased the relative abundance of ileal Firmicutes ($P < 0.05$) and tended to decrease the relative abundance of ileal Proteobacteria ($P < 0.05$). Birds fed Arg-supplemented diets showed notable increase in the relative abundance of ileal *Romboutsia* and *Candidatus Arthromitus* genera ($P < 0.05$, Figures 1B and 1C) compared with birds fed the control diet. The relative abundance of ileal *Clostridium sensu stricto 1* genus was significantly influenced by Arg supplementation ($P < 0.05$, Figures 1B and 1C).

Estimates of the Dietary Arginine Requirements for Yellow-Feathered Chickens

Dietary Arg requirements of Qingyuan partridge chickens estimated by quadratic regression analysis are shown in Table 7. The dietary Arg requirements for chickens aged 1 to 30 d for optimizing ADG, FCR, ileal activity of GSH-Px, and its content of sIgA were rather consistent, 12.2, 11.0, 11.0, and 11.9 g/kg, respectively.

DISCUSSION

In the present study, dietary Arg content had favorable effects on the ADFI, ADG, FCR, and 30-d BW in Qingyuan partridge chickens. Dietary Arg deficiency causes lack of appetite, thus suppressing growth and development in broiler chickens and ducks (Kidd et al., 2001; Wang et al., 2014). Castro et al. (2019) reported that dietary Arg supplementation increased ADFI and improved ADG and feed efficiency in Ross 308 broiler chicks. Similarly, Xu et al. (2018) found improvements in ADG after increasing dietary Arg level in Arbor Acre broiler chicks. The results obtained here with a Chinese Yellow strain were consistent with the studies cited previously. In contrast, other studies (Corzo et al., 2003;

Table 6. Effects of the dietary *L*-arginine level on the immunologic indices in the jejunum and ileum of yellow-feathered chickens at 30 d of age.[1]

Indices	Dietary Arg level, g/kg					SEM	*P*-value		
	8.5	9.7	10.9	12.1	13.3		Arg	Linear	Quadratic
Jejunum									
IgG (μg/mg prot)	18.97[b]	20.43[a,b]	21.62[a,b]	26.24[a]	28.20[a]	2.338	0.057	0.013	NS
IgM (μg/mg prot)	3.59	5.06	5.07	5.36	5.95	0.982	NS	NS	NS
SIgA (μg/mg prot)	2.05	2.47	2.35	2.63	1.92	0.518	NS	NS	NS
Ileum									
IgG (μg/mg prot)	19.58	17.54	21.33	21.74	22.79	1.891	NS	NS	NS
IgM (μg/mg prot)	3.87	3.12	3.68	4.04	4.57	0.425	NS	NS	NS
SIgA (μg/mg prot)	1.06[b]	1.29[a,b]	1.76[a]	1.99[a]	1.78[a]	0.235	0.034	0.011	0.047

[a,b]Means with different superscripts within a main effect do not differ significantly ($P < 0.05$).

Abbreviaitons: NS, not significant; sIgA, secretory immunoglobulin A.

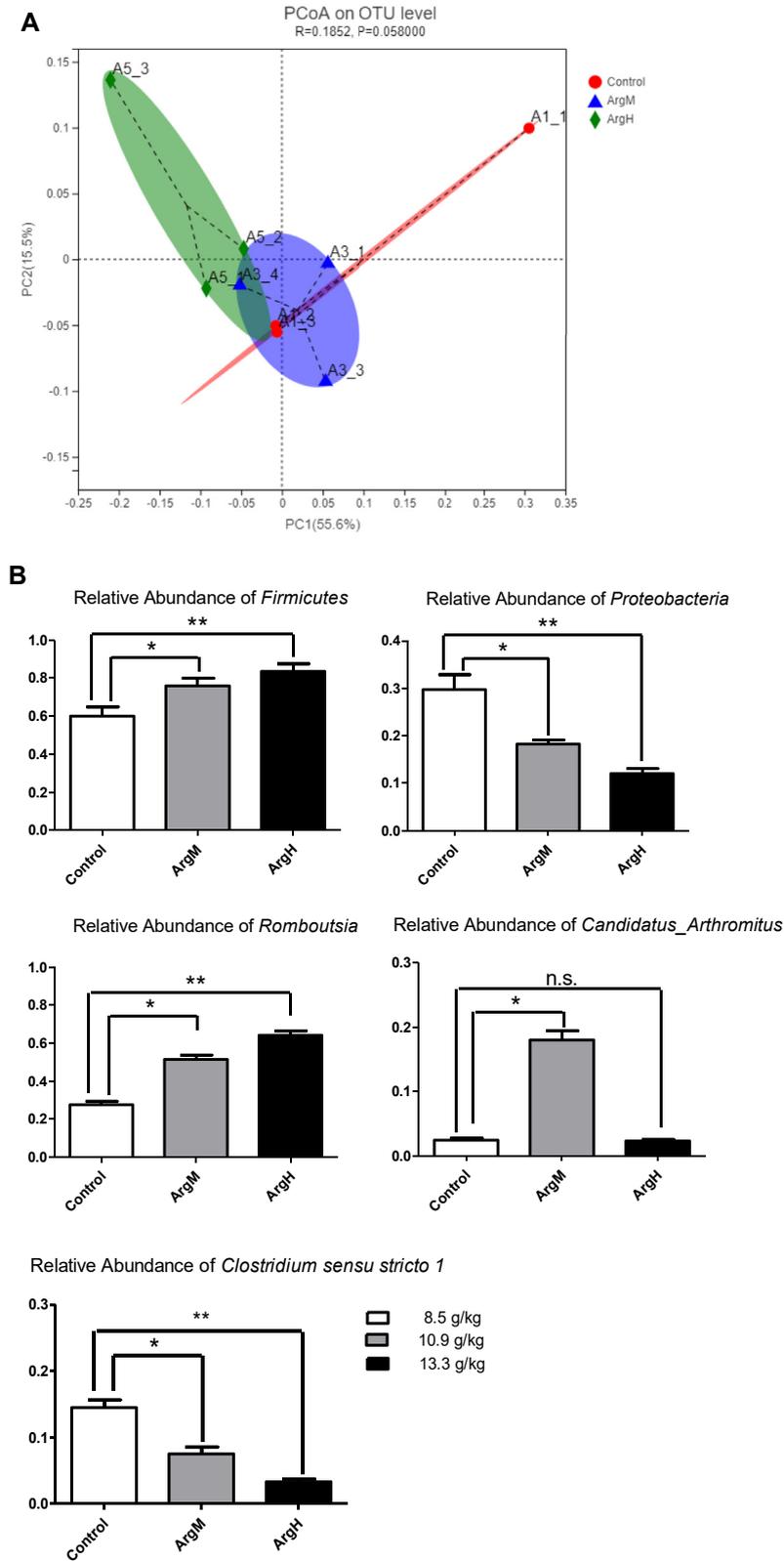[1]Means are based on 2 birds per pen and 6 replicate pens per diet.

Figure 3. Effect of dietary *L*-arginine on microbiome composition in the ileum of yellow-feathered chickens (n = 4). (A) Principal coordinates analysis (PCoA); (B) relative abundance of Firmicute (at the phylum level), Proteobacteria (at the phylum level), *Romboutsia* (at the genus level) and *Candidatus_ Arthromitus* (at the genus level), *Clostridum_ sensu_ stricto 1* (at the genus level); (C) relative abundance of top 15 genus in each group. Data in (B) were analyzed with unpaired *t* test, * means $P < 0.05$, ** means $P < 0.01$, ns means not significant. Abbreviations: ArgH, chickens received an experimental diet with 13.3 g/kg arginine; ArgM, chickens received an experimental diet with 10.9 g/kg arginine; Control, chickens received a basal diet with 8.5 g/kg arginine; OTU, operational taxonomic units.

C

Relative Abundance in Genus Level
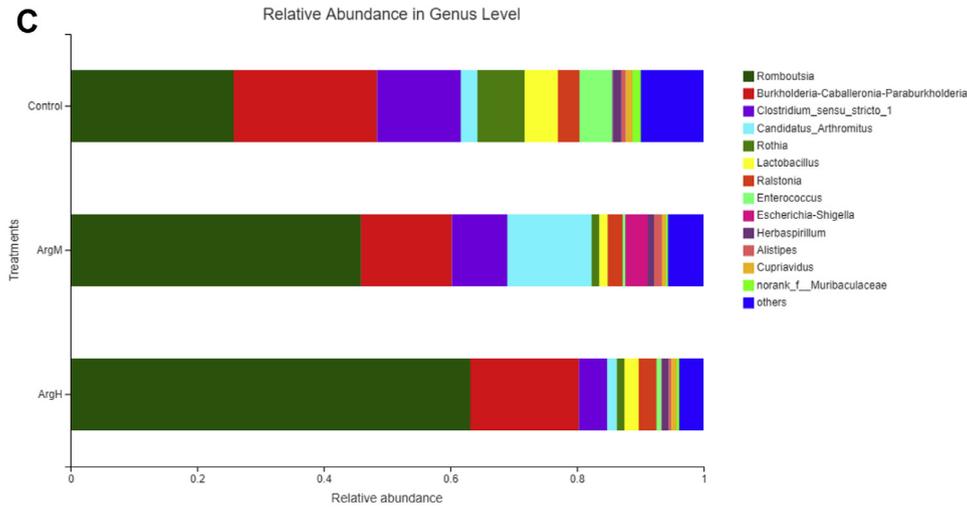
Figure 3. Continued

Ebrahimi et al., 2014) failed to demonstrate the various level of Arg supplementation had any effect on ADFI in broilers. Jahanian and Khalifeh-Gholi (2018) showed that high concentrations of Arg (13.75 g/kg) had no effect on ADG and FCR in Ross 308 broiler chicks aged 1 to 21 d, compared with recommended levels (NRC, 1994). In previous studies, Cuca and Jensen (1990) and the NRC (1994) have suggested that Arg levels for Arbor Acre broiler chicks of 12.5 g/kg (1–21 d) and 11.0 g/kg (22–42 d) are appropriate. Castro et al. (2019) recommended that Arg levels for maximum BW gain of Ross 308 broiler chicks were 14.3 g/kg (1–10 d) and 13.4 g/kg (11–24 d). Ebrahimi et al. (2014) showed that the concentration of digestible Arg inducing the highest growth and muscle gain in Ross chicks were 22.0 g/kg (1–10 d) and 20.0 g/kg (11–24 d). Previous studies using various birds, strains, and/or basal diets with different Arg concentrations have provided inconsistent requirements for dietary Arg level. The optimal dietary Arg concentration found here for maximum BW gain in Qingyuan partridge chickens (1–30 d) was 12.2 g/kg. In avian species, optimal concentration of Arg maximized protein biosynthesis and minimized protein degradation, which could explain the improvement in BW gain by dietary Arg supplementation (Yuan et al., 2016; Miao et al., 2017). Therefore, previous studies using different birds, strains, and/or Arg concentrations in the basal diet varied in responses to increasing dietary Arg level. As the prevalent meat chickens in southern China, optimizing nutrient provision for *Qingyuan* partridge chickens is of economic importance, so the present findings are relevant.

Arginine 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 a diet supplemented with 5 mg Arg/kg for a mo improved antioxidative capacity and decreased plasma MDA in quail, which was consistent with the present findings in Chinese yellow-feathered chickens. In addition, in aged broiler breeder hens, adding 4.0 g

Arg/kg Arg diet increased activity of GSH-Px and total antioxidative capacity 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 present study, dietary Arg increased expression in the jejunum and ileum of *GPX1*, *HMOX1*, and *NRF2* genes. Heme oxidase-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 dependent gene expressions, such as *SOD*, *GPX*, catalase, glutathione S-transferase, and 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).

Arginine is known to play an important role in the development of T cells. Increasing Arg levels from 11.0 to 15.0 g/kg during the first 21 d of broilers age did not affect proportions of $CD4^+$, $CD8^+$ cells, and the ratio between them (D'Amato and Humphrey, 2010). In addition, diets contain 10.5 to 19.0 g/kg Arg during the first 21 d of broiler chickens age did not show significant enhancements in $CD3^+$, CD^{11+}, and CD^{14+} cells compared with basal diet with 10.5 g/kg Arg (Tan et al., 2014b). By contrast, diets containing a high level of Arg (21.3 g/kg) tended to increase $CD3^+$ and significantly increased $CD4^+$ and $CD8^+$ cells compared with basal diets with 9.9 g/kg Arg (Tan et al., 2015). The present findings showed that the basal diet (8.5 g/kg Arg) resulted in the lowest proportion of $CD3^+$ and $CD4^+$ cells, and the diet containing 12.1 g/kg Arg maximized the proportion of these cells. Our findings and previous studies showed that effects of Arg on cell-surface antigens for T cells depended on the concentration of Arg in the diet. An Arg-deficient diet retarded the

Table 7. Estimations of the dietary L-arginine requirements based on non-linear regressions of ADG, feed conversion ratio, glutathione peroxidase (GSH-PX) activity, and secreted IgA (sIgA) content on dietary arginine concentrations.

Dependent variables	Regression equation[1]	R^2	P	Dietary Arg Requirement, g/kg[2]
ADG	$Y = -0.155 + 1.434X - 0.056X^2$	0.957	<0.001	12.2
Feed conversion ratio	$Y = 4.103 - 0.346X + 0.149X^2$	0.886	0.047	11.0
Ileal activity of GSH-PX	$Y = -227.2 + 43.18X - 1.858X^2$	0.882	<0.001	11.0
Ileal content of sIgA	$Y = -0.055 + 1.382X - 0.055X^2$	0.921	0.034	11.9

[1]Y is the dependent variable and X the dietary Arg concentration, g/kg.
[2]Dietary Arg requirement = the optimal dietary Arg concentration, g/kg.

development of lymphoid organs in broiler chickens (Kwak et al., 1999), whereas in ovo injection of 17.5 mg Arg at the incubation day enhanced growth of lymphoid organs at 21 d of age (Gao et al., 2017b), which could explain why dietary Arg supplementation improved CD3$^+$, CD4$^+$, and CD8$^+$ T cell populations in healthy chicks. Cytokine and chemokine responses play crucial roles in the immune defense against infection (Withanage et al., 2005). The pattern recognition receptor TLR4 is a transmembrane protein that activates intracellular nuclear factor-κB and mitogen-activated protein kinases, resulting in the production of proinflammatory cytokines such as IL-1β and IL-6 (Kawai and Akira, 2007). Young chickens are susceptible to intestinal inflammation and infection. The present findings indicated that Arg supplementation attenuated the ileal expression of *IL1B* and *TLR4*. Hu et al. (2016) reported that dietary Arg supplementation increased the expression of proinflammatory cytokine *IL1B*, *IFN-γ*, and *tumor* necrosis factor-alpha in the spleen. Tan et al. (2014a) revealed that dietary Arg tended to decrease the jejunal *IL1B* and myeloid differentiation primary response 88 expression in birds infected with coccidiosis. Secretory IgA is the primary immunologic barrier preventing intraluminal pathogens from colonizing to the intestinal mucosa, and this aids in maintaining homeostasis with the commensal microbiota (Johansen and Kaetzel, 2011). The present study showed that ileal sIgA levels measured on day 30 were increased by Arg supplementation; this would be expected to increase protection of the intestinal mucosal surface. High concentrations of Arg (18.7 g/kg) enhanced jejunal but not ileal sIgA in Arbor Acres broilers at 28 d of age (Zhang et al., 2017). In ovo delivery of Arg increased concentrations of sIgA in all sections of broiler intestines at 21 d of age (Gao et al., 2017a). In addition, increasing Arg levels from 11.1 to 20.2 g/kg had significant impact on jejunal sIgA but not sIgG (Tan et al., 2014b). Wu et al. (2020) reported that 4 g/kg Arg supplementation promoted sIgA production in the mouse ileum, possibly mediated by cytokines associated with T cell–dependent pathways. These results indicate that Arg increased sIgA production and reduced that of proinflammatory cytokines production. The differences among present findings and previous studies could be due to the time of supplementation and/or strain of chickens being used.

The complex communities of gut microbiota harbored by individuals play an important role in intestinal health and disease. They influence the host in the regulation of homeostasis, organ development, nutrient metabolism, and immune response (Tremaroli and Bäckhed, 2012). In the present study, PCoA and ANOSIM analysis revealed that moderate or high Arg supplementation (ArgM and ArgH) of Qingyuan partridge chickens had similar composition of the ileal microbiota, distinct from that in the control treatment. Compared with the controls, there were more Firmicutes and less Proteobacteria in chickens with supplemental Arg. A relative increase in Firmicutes would be expected to benefit gut health. Firmicutes are predominant bacteria in the intestinal tract and are capable of oxidizing sugars by lactic acid fermentation (Eckburg et al., 2005); most bacteria that produce short-chain fatty acids also belong to Firmicutes. With the increase of Firmicutes, the intestinal barrier functions are strengthened, and inflammatory responses are diminished (Huang et al., 2018). Proteobacteria, a major phylum of gram-negative bacteria, include a wide variety of pathogenic genera, such as *Escherichia* and *Salmonella* (Madigan et al., 1997). At the genus level, dietary Arg markedly increased relative abundance of *Romboutsia* and *Candidatus Arthromitus*, while decreased that of *C. sensu stricto 1*. *Romboutsia* contains a versatile array of metabolic capabilities related to carbohydrate utilization, fermentation of single amino acids, anaerobic respiration, and metabolic end products (Gerritsen, 2015). *Candidatus Arthromitus* is the collective name for segmented, filamentous, nonculturable gram-positive bacteria. They are able to specifically modulate host immunity by T cell responses including the T helper (Th17) cell differentiation, induction of IgA plasma cells, and intestinal sIgA secretion (Robino et al., 2019). Yang et al. (2019) reported that overgrowth of *C. sensu stricto 1* was the major microbiotic contributor to necrotic enteritis and infection by Eimeria such as *Coccidia*. Zhang et al. (2017) showed that Arg supplementation could inhibit *C. perfringens* overgrowth in the ileum by means of production of nitric oxide, and the present results are consistent with such an

explanation. In summary, in Qingyuan partridge chickens, dietary Arg was shown to stimulate intestinal epithelial cells, enhance intestinal antioxidative enzyme activities and enteric barrier function, and modulate the composition of gut microbiota.

CONCLUSIONS

In conclusion, the present study with Qingyuan partridge chickens demonstrated that growth performance was improved by increasing dietary Arg from 8.5 to approximately 12.0 g/kg. The optimal Arg levels for maximizing ADG, FCR, ileal activity of GSH-Px, and content of sIgA were 12.2, 11.0, 11.0, and 11.9 g/kg, respectively. Dietary Arg enhanced intestinal antioxidative capacity and immune function and improved the population structure of gut microbiota, favoring intestinal health.

ACKNOWLEDGMENTS

DISCLOSURES

The authors declare no conflicts of interest

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.09.042.

REFERENCES

Atakisi, O., E. Atakisi, and A. Kart. 2009. Effects of dietary zinc and L-arginine supplementation on total antioxidants capacity, lipid peroxidation, nitric oxide, egg weight, and blood biochemical values in Japanses quails. Biol. Trace. Elem. Res. 132:136–143.

Cao, W., L. Xiao, G. Liu, T. Fang, X. Wu, G. Jia, H. Zhao, X. Chen, C. Wu, J. Cai, and J. Wang. 2016. Dietary arginine and N-carbamyl glutamate supplementation enhances the antioxidant statuses of the liver and plasma against oxidative stress in rats. Food Funct. 7:2303–2311.

Castro, F. L. S., S. Su, H. Chio, E. Koo, and W. K. Kim. 2019. L-Arginine supplementation enhances growth performance, lean muscle, and bone density but not fat in broiler chickens. Poult. Sci. 98:1716–1722.

Chamruspollert, M., G. M. Pesti, and R. I. Bakalli. 2002. The influence of labile dietary methyl donors on arginine requirement of young broiler chicks. Poult. Sci. 81:1142–1148.

Corzo, A., E. T. Moran, and D. Hoehler. 2003. Arginine need of heavy broiler males: Applying ideal protein concept. Poult. Sci. 82:402–407.

Cuca, M., and L. S. Jensen. 1990. Arginine requirement of starting broiler chicks. Poult. Sci. 69:1377–1382.

D'Amato, J. L., and B. D. Humphrey. 2010. Dietary arginine levels alter markers of arginine utilization in peripheral blood mononuclear cells and thymocytes in young broiler chicks. Poult. Sci. 89:938–947.

Diaz Carrasco, J. M., N. A. Casanova, and M. E. Fernández Miyakawa. 2019. Microbiota, gut health and chicken productivity: what is the connection? Microorganisms 7:374.

Dozier, W. A., A. Corzo, M. T. Kidd, P. B. Tillman, and S. L. Branton. 2009. Digestible lysine requirements of male and female broilers from fourteen to twenty-eight days of age. Poult. Sci. 88:1676–1682.

Duan, X., F. Li, S. Mou, J. Feng, P. Liu, and L. Xu. 2015. Effects of dietary L-arginine on laying performance and antioxidant capacity of broiler breeder hens, eggs, and offspring during the late laying period. Poult. Sci. 94:2938–2943.

Ebrahimi, M., A. Z. Shahneh, M. Shivazad, Z. A. Pirsaraei, M. Tebianian, C. A. Ruizferia, M. Adibmoradi, K. Nourijelyani, and F. Mohamadnejad. 2014. The effect of feeding excess arginine on lipogenic gene expression and growth performance in broilers. Br. Poult. Sci. 55:81–88.

Eckburg, P. B., E. M. Bik, C. N. Bernstein, E. Purdom, L. Dethlefsen, M. Sargent, S. R. Gill, K. E. Nelson, and D. A. Relman. 2005. Diversity of the human intestinal microbial flora. Science 308:1635–1638.

Gao, T., M. M. Zhao, L. Zhang, J. L. Li, L. L. Yu, P. A. Lv, F. Gao, and G. H. Zhou. 2017a. Effects of in ovo feeding of l-arginine on the development of lymphoid organs and small intestinal immune barrier function in posthatch broilers. Anim. Feed. Sci. Tech. 225:8–19.

Gao, T., M. Zhao, L. Zhang, J. Li, L. Yu, P. Lv, F. Gao, and G. Zhou. 2017b. Effect of in ovo feeding of L-arginine on the hatchability, growth performance, gastrointestinal hormones, and jejunal digestive and absorptive capacity of posthatch broilers. J. Anim. Sci. 95:3079–3092.

Geiger, R., J. C. Rieckmann, T. Wolf, C. Basso, Y. Feng, T. Fuhrer, M. Kogadeeva, P. Picotti, F. Meissner, M. Mann, N. Zamboni, F. Sallusto, and A. Lanzavecchia. 2016. L-arginine modulates T cell metabolism and enhances survival and anti-tumor activity. Cell 167:829–842.

Gerritsen, J. 2015. The genus Romboutsia: genomic and functional characterization of novel bacteria dedicated to life in the intestinal tract. PhD thesis. Wageningen University, Wageningen.

Hu, Y., J. Tan, J. Qi, and H. Zhang. 2016. Regulatory effects of dietary L-Arg supplementation on the innate immunity and antioxidant ability in broiler chickens. J. Inter. Agr. 15:2578–2587.

Huang, Y., X. Shi, Z. Li, Y. Shen, X. Shi, L. Wang, G. Li, Y. Yuan, J. Wang, Y. Zhang, L. Zhao, M. Zhang, Y. Kang, and Y. Liang. 2018. Possible association of Firmicutes in the gut microbiota of patients with major depressive disorder. Neuropsych Dis. Treat. 14:3329–3337.

Jahanian, R. 2009. Immunological responses as affected by dietary protein and arginine concentrations in starting broiler chicks. Poult. Sci. 88:1818–1824.

Jahanian, R., and M. Khalifeh-Gholi. 2018. Marginal deficiency of dietary arginine and methionine could suppress growth performance and immunological responses in broiler chickens. J. Anim. Physiol. Anim. Nutri. 102:11–20.

Johansen, F. E., and C. Kaetzel. 2011. Regulation of polymeric immunoglobulin receptor and IgA transport: new advances in environmental factors that stimulate pIgR expression and its role in muscosal immunity. Mucosal. Immunol. 4:598.

Kawai, T., and S. Akira. 2007. Signaling to NF-κB by toll-like receptors. Trends. Mol. Med. 13:460–469.

Kidd, M. T., E. D. Peebles, S. K. Whitmarsh, J. B. Yeatman, and R. F. Wideman. 2001. Gowth and immunity of broiler chicks as affected by dietary arginine. Poult. Sci. 80:1535–1542.

Kwak, H., R. E. Austic, and R. R. Dietert. 1999. Influence of dietary arginine concentration on lymphoid organ growth in chickens. Poult. Sci. 78:1536–1541.

Labadan, M. C., K. N. Hsu, and R. E. Austic. 2001. Lysine and arginine requirements of broiler chickens at two-to three-week intervals to eight weeks of age. Poult. Sci. 80:599–606.

Liang, M., Z. Wang, H. Li, L. Cai, J. Pan, H. He, Q. Wu, Y. Tang, J. Ma, and L. Yang. 2018. L-arginine induces antioxidant response to prevent oxidative stress via stimulation of glutathione synthesis and activation of Nrf2 pathway. Food Chem. Toxicol. 115:315–328.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408.

Loboda, A., M. Damulewicz, E. Pyza, A. Jozkowicz, and J. Dulak. 2016. Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. Cell. Mol. Life Sci. 73:3221–3247.

Madigan, M. T., J. M. Martinko, and J. Parker. 1997. Brock Biology of Microorganisms. Prentice Hall, Upper Saddle River, NJ.

Miao, L. P., C. Yuan, X. Y. Dong, X. Y. Zhang, M. Y. Zhou, and X. T. Zou. 2017. Effects of dietary L-arginine levels on small intestine protein turnover and the expression of genes related to protein synthesis and proteolysis of layers. Poult. Sci. 96:1800–1808.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Oso, A. O., G. A. Williams, O. O. Oluwatosin, A. M. Bamgbose, A. O. Adebayo, O. Olowofeso, V. Pirgozliev, A. A. Adegbenjo, S. O. Osho, J. O. Alabi, F. Li, H. Liu, K. Yao, and W. Xin. 2017. Effect of dietary supplementation with arginine on haematological indices, serum chemistry, carcass yield, gut microflora, and lymphoid organs of growing turkeys. Livest. Sci. 198:58–64.

Robino, P., I. Ferrocino, G. Rossi, A. Dogliero, V. Alessandria, L. Grosso, L. Galosi, C. Tramuta, L. Cocolin, and P. Nebbia. 2019. Changes in gut bacterial communities in canaries infected by macrohabdus ornithogaster. Avian Pathol. 48:111–120.

Tan, J. Z., Y. M. Guo, T. J. Applegate, E. C. Du, and X. Zhao. 2015. Dietary L-arginine modulates immunosuppression in broilers inoculated with an intermediate strain of infectious bursa disease virus. J. Sci. Food Agri. 95:126–135.

Tan, J., S. Liu, Y. Guo, T. J. Applegate, and S. D. Eicher. 2014a. Dietary L-arginine supplementation attenuates lipopolysaccharide-induced inflammatory response in broiler chickens. Br. J. Nutr. 111:1394–1404.

Tan, J., T. J. Applegate, S. Liu, Y. Guo, and S. D. Eicher. 2014b. Supplemental dietary L-arginine attenuates intestinal mucosal disruption during a coccidial vaccine challenge in broiler chickens. Br. J. Nutr. 112:1098–1109.

Tang, D., J. Wu, H. Jiao, X. Wang, J. Zhao, and H. Lin. 2019. The development of antioxidant system in the intestinal tract of broiler chickens. Poult. Sci. 98:664–678.

Tremaroli, V., and F. Bäckhed. 2012. Functional interactions between the gut microbiota and host metabolism. Nature 489:242–249.

Wang, B., Y. Liu, L. Feng, W. D. Jiang, S. Y. Kuang, J. Jiang, S. H. Li, L. Tang, and X. Q. Zhou. 2015. Effects of dietary arginine supplementation on growth performance, flesh quality, muscle antioxidant capacity and antioxidant-related signaling molecule expression in young grass carp (Ctenopharyngodon idella). Food Chem. 167:91–99.

Wang, C., S. S. Hou, W. Huang, T. S. Xu, G. H. Rong, and M. Xie. 2014. Arginine affects appetite via nitric oxide in ducks. Poult. Sci. 93:2048–2053.

Yang, W., Y. Lee, H. Lu, C. Chou, and C. Wang. 2019. Analysis of gut microbiota and effect of lauric acid against necrotic enteritis in Clostridium perfringens and Eimeria side-by-side challenge model. PLoS One 14:e0205784.

Withanage, G. S. K., P. Wigley, P. Kaiser, P. Mastroeni, H. Brooks, C. Powers, R. Beal, P. Barrow, D. Maskell, and I. McConnell. 2005. Cytokine and chemokine responses associated with clearance of a primary Salmonella enterica Serovar Typhimurium infection in the chicken and in protective immunity to rechallenge. Infect. Immun. 73:5173–5182.

Wu, G., F. W. Bazer, T. A. Davis, S. W. Kim, P. Li, J. M. Rhoads, M. C. Satterfield, S. B. Smith, T. E. Spencer, and Y. Yin. 2009. Arginine metabolism and nutrition in growth, health and disease. Amino Acids 37:153–168.

Wu, G., and S. M. Morris. 1998. Arginine metabolism: nitric oxide and beyond. Biochem. J. 336:1–17.

Wu, M., H. Xiao, F. Shao, B. Tan, and S. Hu. 2020. Arginine accelerates intestinal health through cytokines and intestinal microbiota. Int. Immunopharmacol. 81:106029.

Xu, Y. Q., Y. W. Guo, B. L. Shi, S. M. Yan, and X. Y. Guo. 2018. Dietary arginine supplementation enhances the growth performance and immune status of broiler chickens. Livest. Sci. 209:8–13.

Yuan, C., X. C. Bu, H. X. Yan, J. J. Lu, and X. T. Zou. 2016. Dietary L-arginine levels affect the liver protein turnover and alter the expression of genes related to protein synthesis and proteolysis of laying hens. Poult. Sci. 95:61–267.

Zhang, B., G. Li, M. S. Suhaib, L. Gan, H. Fan, Z. Lv, and Y. Guo. 2020. Dietary L-arginine supplementation ameliorates inflammatory response and alters gut microbiota in broiler chickens infected with Salmonella enterica serovar Typhimurium. Poult. Sci. 99:1862–1874.

Zhang, B., Z. Lv, H. Li, S. Guo, D. Liu, and Y. Guo. 2017. Dietary L-arginine inhibits intestinal Clostridium perfringens colonization and attenuates intestinal muscoal injury in broiler chickens. Br. J. Nutr. 118:321–332.

Zhang, B., Z. Lv, Z. Li, W. Wang, G. Li, and Y. Guo. 2018. Dietary L-arginine supplementation alleviates the intestinal injury and modulates the gut microbiota in broiler chickens challenged by Clostridium perfringens. Front. Microbiol. 9:1716.