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Effects of dietary arginine supplementation on production performance, serum biochemicals, antioxidant capacity, and immunity of laying Wulong geese

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ABSTRACT This study investigated the effects of dietary arginine supplementation on the production performance, serum biochemicals, antioxidant capacity, and immunity of laying Wulong geese. A total of 150 Wulong geese (34-wk old) with similar body weights were randomly divided into 6 groups with 5 replicates and 5 geese each (1 male and 4 female). The geese in the control group were fed a corn-rapeseed meal basal diet, and the geese in the treatment groups were fed the basal diet supplemented with 0.1, 0.2, 0.3, 0.4, and 0.5% arginine. The experiment lasted for 17 wk. Our results showed that dietary arginine increased the egg production rate (**LR**) and average egg weight (**AEW**) of geese in a quadratic manner ($P < 0.05$). Dietary arginine had a quadratic effect on the contents of total protein (**TP**) and triglyceride (**TG**) ($P < 0.05$) in the serum. Dietary arginine quadratically decreased the content of malondialdehyde (**MDA**) and increased the activity of total superoxide dismutase (**T-SOD**) ($P < 0.05$). Dietary arginine supplementation linearly and quadratically increased the contents of immunoglobulin A (**IgA**) and immunoglobulin G (**IgG**), and linearly increased the content of nitric oxide (**NO**) ($P < 0.05$). In conclusion, dietary arginine supplementation can significantly improve the production performance, serum biochemicals, antioxidant capacity, and immunity of laying Wulong geese. Therefore, 0.3% arginine (actual content: 1.02%) is recommended in the diet.

Key words: arginine, laying goose, production performance, antioxidant capacity, immunity

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INTRODUCTION

Arginine is a basic amino acid in humans and animals. There are 2 types of arginine, that is, D-arginine and L-arginine, and L-arginine plays a variety of physiological roles in livestock and poultry (Mateo et al., 2007; Yuan et al., 2016). L-arginine is a functional amino acid and plays a critical role in the synthesis and metabolism of nutrients. Almost all tissues in the body use L-arginine to synthesize cytoplasmic proteins and nucleoproteins. Arginine can promote muscle protein synthesis, cell division, wound recovery, hormone secretion, and other physiological processes.

Arginine can improve the production performance, antioxidant function, and immune function in livestock and poultry (Evoy et al., 1998; Jabłecka et al., 2012; Silva et al., 2012; Cao et al., 2016). Arginine plays an important role in the synthesis of urea, creatine, glutamic acid, proline, polyamines, NO, and hormones. Arginine can be decomposed into urea and guanylate by arginase, while guanylate is the precursor of polyamines, which can regulate cell growth and differentiation (Alimohammadi et al., 2015). Moreover, arginine is considered an essential amino acid for poultry due to the lack of a complete urea cycle and the low activity of key enzymes required for arginine synthesis (Geng et al., 2011; Khajali et al., 2013). Arginine can promote muscle protein synthesis, polyamine, and hormone production, thereby improving poultry production and nutrient absorption. Recent studies have shown that dietary arginine supplementation can improve laying performance and feed conversion rate in chickens (Najib and Basiouni, 2004; Silva et al., 2012). Huang et al. (2009) found that dietary arginine supplementation can improve the activity of antioxidant enzymes, alleviate oxidative stress injury, and increase antioxidant capacity in rats. Arginine is not only involved in the synthesis and catabolism of various nutrients but also acts as an immunoregulatory mediator to regulate immune function. Perez-Carbajal et al. (2010) found that dietary supplementation with 0.30 and 0.60% arginine can increase serum IgM and IgG levels, stimulate lymphocyte proliferation, and improve immunity in broilers challenged with *Eimeria*. Moreover, arginine is a potent NO precursor for the synthesis of NO, which is actively involved

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in various biological processes, such as growth and reproduction. Uyanga et al. (2022) reported that the serum NO content had a linear response with arginine supplementation in laying hens.

Up to now, the studies of dietary arginine supplementation have mainly focused on pigs, mice, and chickens. However, little is known about the potential effects of dietary arginine supplementation in breeding geese. In this study, geese were fed diets supplemented with different levels of arginine during the laying period, and we measured the production performance, serum biochemical, antioxidant capacity, and immune indexes of laying geese in order to determine the optimal dose of arginine. The findings provide evidence for the application of dietary arginine supplementation in laying geese during the laying period.

MATERIALS AND METHODS

Animals and Experimental Treatments

This study was approved by the Animal Care and Use Committee of Qingdao Agricultural University. A total of 150 laying Wulong geese (34-wk old) with similar body weights were randomly divided into 6 groups with 5 replicates, 5 geese per replicate (1 male and 4 female). The geese in the control group were fed a corn-rapeseed meal basal diet, and the geese in the treatment groups (Arg0.1, Arg0.2, Arg0.3, Arg0.4, and Arg0.5) were fed basal diets supplemented with 0.1, 0.2, 0.3, 0.4, and 0.5% arginine, respectively. The level of arginine in the basal diet was 0.72%. Arginine (purity: 99%) was purchased from Qingdao Puxing Biotechnology (Qingdao, China). The basal diet was formulated based on the National Research Council (1994)) Requirements of Poultry, and the composition and nutritional levels are shown in Table 1. The geese were raised in 30 separate floor pens, with free access to food and water. The experiment lasted for 17 wk.

Table 1. Composition and nutrient levels of the basal diet (air-dry basis, %).

Ingredients	Content (%)	Nutrients[2]	Level (%)
Corn	64.12	ME (MJ/Kg)	12.01
Rapeseed meal	5.10	CP	18.03
Bran	3.99	Ca	2.27
Soybean meal	1.95	P	0.66
Chrysanthemum stalks powder	2.89	CF	2.61
Corn gluten meal	11.10	L-Arg	0.72
Soybean oil	2.20	Lys	0.73
Limestone	5.65	Met	0.47
$CaHPO_4$	1.28		
NaCl	0.34		
Met	0.11		
Premix[1]	0.77		
L-alanine	0.5		
Total	100		

[1]The premix provided the following per kg of diets: VA 9,000 IU, VD_3 2,000 IU, VE 40 mg, VK_3 0.8 mg, VB_1 2.0 mg, VB_2 4.0 mg, nicotinic acid 30 mg, pantothenate 11 mg, VB_{11} 0.5 mg, VB_6 4.0 mg, biotin 0.2 mg, VB_{12} (1%) 12 ug, Lys 2.5g, Fe 80 mg, Se 0.5 mg, I 0.3 mg, Mn 30 mg, Cu 4.0 mg, Zn 65 mg.

[2]CP, Ca, P, and L-Arg were analyzed values; others were calculated values.

Sample Collection

At the end of treatment, 30 female geese (1 goose per replicate) were randomly selected and feed-deprived for 6 h. Blood samples were collected from the wing veins and the geese were then slaughtered by jugular vein exsanguination. The blood samples were centrifuged at 3,000 r/min for 10 min at 4°C, and the serum was collected and stored at −80°C for further analysis.

Production Performance Determination

During the experiment, feed consumption was recorded daily to determine the average daily feed intake (**ADFI**), average egg weight (**AEW**), egg production rate (**LR**), and feed-to-egg ratio (**F/E**). The number of deaths was recorded daily and used to adjust the total number of birds per replicate to exclude them from the calculation of ADFI and F/E.

Measurement of Serum Biochemicals

The concentrations of total protein (**TP**), albumin (**ALB**), and blood urea nitrogen (**BUN**), and the contents of triglycerides (**TG**), total cholesterol (**T-CHO**), uric acid (**UA**), high-density lipoprotein cholesterol (**HDL-C**), and low-density lipoprotein cholesterol (**LDL-C**) in the serum were determined using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China).

Measurement of Antioxidant Indices

The total superoxide dismutase (**T-SOD**), glutathione peroxidase (**GSH-Px**), total antioxidant capacity (**T-AOC**) activities, and malondialdehyde (**MDA**) level in the serum were measured using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China).

Immunoglobulin Measurement

The levels of immunoglobulin A (**IgA**), immunoglobulin G (**IgG**), and immunoglobulin M (**IgM**) in the serum were determined using enzyme-linked immunosorbent assay (**ELISA**) kits, and the level of nitric oxide (**NO**) in the serum was determined using a commercial kit (Jiancheng Bioengineering Institute, Nanjing, China).

Statistical Analysis

The results are presented as the mean and pooled SEM. Data are analyzed by 1-way ANOVA procedure using SPSS statistical software (version 25.0, SPSS, Chicago, IL). Duncan's multiple range test was used to determine significant differences among the treatment means. Polynomial contrasts were used to test the linear

Table 2. Effects of dietary arginine supplementation on production performance indexes in Wulong geese.

								P value		
Treatment	Control	Arg0.1	Arg0.2	Arg0.3	Arg0.4	Arg0.5	SEM	ANOVA	Linear	Quadratic
ADFI (g/d)	170.85	174.68	178.95	174.35	175.55	161.23	1.86	0.087	0.161	0.012
AEW (g)	109.33^{c}	112.59^{bc}	116.33^{ab}	120.70^{a}	117.07^{ab}	112.22^{bc}	0.35	0.015	0.143	0.005
F:E ratio	4.04	3.68	4.11	3.54	3.84	3.56	0.07	0.081	0.075	0.935
LR (%)	38.97^{c}	40.92^{abc}	40.97^{abc}	42.70^{a}	41.51^{ab}	40.18^{bc}	1.06	0.041	0.089	0.002

Abbreviations: ADFI, average daily feed intake; AEW, average egg weight; F/E, feed-to-egg ratio; LR, egg production rate.
$^{a-c}$Means within the same row with different superscripts differ significantly ($P < 0.05$).

and quadratic effects of arginine supplementation levels. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Production Performance

As shown in Table 2, the AEW and LR in Group Arg0.3 were significantly higher than those in the control group ($P > 0.05$). The ADFI and F/E were not significantly affected by arginine ($P > 0.05$).

Serum Biochemicals

As shown in Table 3, dietary arginine supplementation quadratically increased the serum TP content ($P < 0.05$), and the highest serum TP content was found in Group Arg0.3. Dietary arginine supplementation decreased the serum TG content in a quadratic manner ($P < 0.05$), and the lowest TG content was found in Groups Arg0.2 and Arg0.3. There were no differences in the concentrations of ALB, BUN, TG, T-CHO, UA, HDL-C, and LDL-C between the control group and the treatment groups ($P > 0.05$).

Antioxidant Function

As shown in Table 4, dietary arginine supplementation quadratically decreased the serum MDA concentration ($P < 0.05$), whereas the activity of T-SOD was significantly increased in Group Arg0.3 compared with the control group ($P < 0.05$). The levels of T-AOC in the treatment groups were higher than that in the control group, but the difference was not statistically significant ($P > 0.05$). The activity of GSH-Px showed no significant change between the control group and the treatment groups ($P > 0.05$).

Immunity

As shown in Table 5, dietary arginine supplementation significantly increased the serum IgA level in linear and quadratic manners ($P < 0.05$) and increased the

Table 3. Effects of dietary arginine supplementation on serum biochemical parameters in Wulong geese.

								P value		
Treatment	Control	Arg0.1	Arg0.2	Arg0.3	Arg0.4	Arg0.5	SEM	ANOVA	Linear	Quadratic
ALB (g/L)	12.92	16.35	14.42	14.95	14.01	12.83	0.56	0.358	0.555	0.146
TP (g/L)	19.54^{c}	21.35^{abc}	23.24^{ab}	24.63^{a}	23.89^{ab}	20.79^{bc}	0.52	0.012	0.098	0.001
BUN (mmol/L)	1.22	1.71	1.28	1.48	1.75	1.27	0.18	0.931	0.886	0.637
UA (mmol/L)	61.16	60.69	52.32	45.92	62.11	56.39	2.43	0.356	0.602	0.193
T-CHO (mmol/L)	6.04	7.26	4.60	4.43	5.33	3.58	0.48	0.295	0.068	0.954
TG (mmol/L)	13.46^{a}	11.75^{ab}	10.07^{b}	9.50^{b}	12.09^{ab}	13.67^{a}	0.46	0.025	0.854	0.001
LDL-C (mmol/L)	0.94	0.87	0.73	0.64	0.74	0.73	0.07	0.883	0.573	0.784
HDL-C (mmol/L)	0.05	0.04	0.04	0.05	0.06	0.05	0.06	0.970	0.342	0.466

Abbreviations: ALB, albumin; BUN, blood urea nitrogen; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T-CHO, total cholesterol; TG, triglycerides; TP, total protein; UA, uric acid.
$^{a-c}$Means within the same row with different superscripts differ significantly ($P < 0.05$).

Table 4. Effects of dietary arginine supplementation on antioxidant indexes in Wulong geese.

								P value		
Treatment	Control	Arg0.1	Arg0.2	Arg0.3	Arg0.4	Arg0.5	SEM	ANOVA	Linear	Quadratic
MDA (nmol/mL)	9.86^{a}	8.28^{ab}	7.24^{ab}	5.79^{b}	9.08^{a}	9.59^{a}	0.44	0.042	0.957	0.004
T-SOD (U/mL)	91.91^{b}	90.93^{b}	99.63^{ab}	111.26^{a}	110.56^{a}	88.05^{b}	2.80	0.033	0.313	0.013
T-AOC (mM)	0.23	0.34	0.34	0.42	0.40	0.35	0.02	0.252	0.070	0.112
GSH-Px (U/mL)	251.11	268.15	283.03	278.73	265.31	250.19	11.01	0.950	0.943	0.312

Abbreviations: GSH-Px, glutathione peroxidase; MDA, malondialdehyde; T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase.
^{a,b}Means within the same row with different superscripts differ significantly ($P < 0.05$).

Table 5. Effects of arginine on the immunity in Wulong geese.

								P value		
Treatment	Control	Arg0.1	Arg0.2	Arg0.3	Arg0.4	Arg0.5	SEM	ANOVA	Linear	Quadratic
IgA (μg/mL)	382.91^{c}	493.08^{bc}	522.90^{abc}	646.60^{a}	585.10^{ab}	522.35^{abc}	23.13	0.015	0.001	0.009
IgM (μg/mL)	2014.79	2088.20	2225.76	2228.71	2198.92	2282.25	75.12	0.112	0.320	0.734
IgG (μg/mL)	1000.30^{c}	1060.50^{abc}	1155.34^{abc}	1239.22^{a}	1200.18^{ab}	1027.16^{bc}	27.67	0.044	0.213	0.005
NO (mmol/L)	35.27^{b}	43.86^{ab}	46.77^{ab}	57.92^{a}	54.83^{a}	51.03^{a}	2.27	0.037	0.006	0.065

Abbreviations: IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M.
$^{a-c}$The values within the same row with different superscripts differ significantly ($P < 0.05$).

serum IgG level in a linear manner ($P < 0.05$), but it did not affect the serum IgM level compared to the control group ($P > 0.05$). The serum NO levels in Groups Arg0.3, Arg0.4, and Arg0.5 were significantly higher than that in the control group ($P < 0.05$).

DISCUSSION

Arginine can be hydrolyzed by arginase to produce ornithine, and it can also be used to synthesize NO through nitric oxide synthases. Furthermore, ornithine and NO can promote the release of growth hormones to regulate the metabolism of proteins and amino acids. Ornithine is converted by ornithine decarboxylase into polyamines, which can improve nucleic acid stability and regulate cell proliferation (Alimohammadi et al., 2015). Duan et al. (2015) reported that dietary arginine supplementation had significant linear and quadratic effects on the LR in broiler breeders because arginine could stimulate the secretion of luteinizing hormone, which directly acted on ovaries and follicles. Silva et al. (2012) found that dietary arginine supplementation linearly increased egg weight in broiler breeders, and the egg production rate had a significant quadratic correlation with the level of dietary arginine, which was in accordance with our result. In the present study, dietary arginine supplementation significantly increased the LR and AEW in geese, which peaked at 0.3% of arginine supplementation (actual level: 1.02%). We speculated that arginine could directly act on the hypothalamus and promote the release of endocrine hormones (such as growth hormone) to increase protein synthesis but reduce protein catabolism, thereby improving egg production performance (Frank et al., 2007; Wu et al., 2007).

Serum TP is an important indicator of protein metabolism in poultry. High-quality animal feed can increase the synthesis of protein. It is reported that dietary arginine supplementation increases serum total protein levels, promotes protein precipitation in cells, and accelerates production performance in rabbits (Ahmed, 2021). Similar phenomena were observed in our study, in which dietary arginine supplementation significantly increased the level of serum TP in geese. As a major source of nitrogen in the body, arginine is actively engaged in protein synthesis and metabolism. In addition, arginine may participate in protein regulation by stimulating amino acid-sensitive targets (Alimohammadi et al., 2015), thereby affecting serum protein levels.

TG levels reflect the body's lipid metabolism, and TG can be hydrolyzed into fatty acids for energy production. Li et al. (2021) demonstrated that arginine supplementation, especially 1 and 2% arginine, decreased lipid accumulation in the liver of Nile tilapia and reduced serum TG levels, indicating that arginine can improve lipid metabolism, regulate plasma lipid levels, and reduce the risk of metabolic diseases. Fouad et al. (2013) found that the level of TG was significantly lower in broiler chickens fed diets supplemented with 0.25 or 1.00% L-arginine than those fed the basal diet, suggesting that L-arginine promotes the conversion of TG to glycerol. In this study, TG levels quadratically decreased as dietary arginine levels increased, which was similar to the studies mentioned above. There were 2 possible reasons for the changes in TG levels. One reason is that arginine can regulate lipid metabolism by controlling fatty acid synthesis and metabolism-related enzymes (Li et al., 2021). Another reason is that NO produced by arginine metabolism can regulate the activity of acetyl-CoA carboxylase and inhibit the synthesis of fatty acids (García-Villafranca et al., 2003).

The levels of MDA, T-SOD, T-AOC, and GSH-Px are important indicators of the antioxidative system in the body. In general, MDA levels are inversely proportional to the antioxidant capacity, which reflects the degree of lipid peroxidation and oxidative damage (Luo et al., 2011). T-SOD can protect the biofilm from oxidative damage caused by hydrogen peroxide through the disproportionation reaction and enhance disease resistance (Maksimenko, 2005). In addition, arginine can increase the activity of antioxidant enzymes and reduce the levels of MDA in rats (Huang et al., 2009; Liang et al., 2018). Our data showed that diets supplemented with 1.02% arginine could significantly increase the activity of T-SOD and reduce the level of MDA in the serum, but had no significant effects on the activities of T-AOC and GSH-Px. Similarly, arginine can alleviate oxidative stress in rats by promoting NO production, increasing SOD activity, and reducing MDA levels (Dasgupta et al., 2006). Nevertheless, high doses of arginine can suppress the antioxidant capacity of Wulong geese. Excessive free radical accumulation can easily induce lipid peroxidation and oxidative stress, thereby causing tissue damage Arginine can play an antioxidative

role through multiple pathways, such as the arginine-NO pathway, the glutathione synthesis pathway, and so on and produce an appropriate amount of NO to eliminate oxygen free radicals, increase the activity of antioxidant enzymes, improve glutathione synthesis efficiency, reduce the content of MDA, relieve oxidative stress, and improve the antioxidant capacity. However, excessive NO produced by high doses of arginine will cause oxidative damage to the body to a certain extent (Dasgupta et al., 2006; Huang et al., 2009; Devine et al., 2012).

Immunoglobulins (IgA, IgM, and IgG) are the main antibodies that mediate humoral immunity by specifically binding to antigens. The levels of these immunoglobulins can reflect the body's disease resistance (Fathi et al., 2017; Wu et al., 2021). IgA protects the mucosal surface from damage by directly neutralizing viruses or preventing the binding of viruses to the mucosal surface (Schroeder and Cavacini, 2010). IgG exerts antibacterial and antiviral activities and regulates immune function by agglutinating and precipitating antigens (Wu et al., 2021), and IgG levels can reflect the health and immune status of the body. In addition, dietary supplementation with 0.4 to 0.8% arginine increases serum IgM and IgG levels in piglets, possibly resulting from a complex interaction between arginine and other dietary nutrients in regulating the synthesis of NO and polyamines (Tan et al., 2009). Similarly, our results demonstrated that dietary supplementation with 0.3% arginine (actual level: 1.02%) could significantly increase serum IgA and IgG levels in geese. The results indicated that arginine could act as an immunomodulatory agent and undergo hydrolysis by arginase to promote the production of immunoglobulins from metabolites (Fathi et al., 2017). In addition, arginine is essential for lymphocyte development, and the immune function of the body is reduced when the diet is deficient in arginine. Arginine can promote the differentiation of pre-B lymphocytes and the release of B lymphocytes in bone marrow, thereby promoting the secretion of immunoglobulins by B lymphocytes (Liu et al., 2019).

Arginine can be catalyzed by nitric oxide synthase to generate a cellular signaling molecule NO, which is an important immunomodulatory factor in immune responses (Birmani et al., 2019). Zeng et al. (2008) found that arginine supplementation significantly increased the plasma concentration of NO in rats, indicating that dietary arginine supplementation could promote the synthesis of NO by nitric oxide synthase using arginine as a substrate. In the current study, we found that dietary supplementation with arginine linearly increased serum NO levels in geese. Similarly, Uyanga et al. (2022) found that the serum NO level had a linear response with arginine supplementation in laying hens. The results indicate that arginine is a limiting substrate in NO generation and participates in immune regulation through the nitric oxide pathway (Jobgen et al., 2006). Arginine can be decarboxylated by arginine decarboxylase to generate agmatine, which interacts with NO to regulate the serum level of arginine. However, low or excessive levels of arginine will exceed the body's regulation range, unable to generate an appropriate amount of agmatine to regulate NO, resulting in a decrease in serum NO concentration and affecting the immune function (Piletz et al., 2013).

In conclusion, this study demonstrated that dietary arginine supplementation could promote protein metabolism, lipid metabolism, production performance, antioxidant capacity, and immune functions in geese. The optimal dosage of 1.02% arginine in diets was recommended for laying Wulong geese.

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DISCLOSURES

The authors declare that they have no conflicts of interest.

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