Different amino acid supplementation patterns in low-protein diets on growth performance and nitrogen metabolism of goslings from 1 to 28 days of age

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ABSTRACT The investigation aimed to explore the suitable amino acid (AA) supplementation pattern for goslings under low-protein diets. A total of 364 1-day-old male goslings were randomly divided into 4 experimental groups, with 7 pens containing 13 goslings each. The 4 groups were control (CP, 18.55%), LPM (CP, 15.55% + major AA), LPA (CP, 15.55% + all AA), and LPR (CP, 15.55% + AA content reduced proportionally to the control's CP). The corn-soybean meal diets are formulated according to the ideal AA model of goose and its nutritional requirements. The results indicated that the ADG and BW were the lowest, and the F: G was the highest in LPR ($P < 0.05$); the other three groups were not significantly different ($P > 0.05$). The ADFI and mortality were not different among all the groups ($P > 0.05$). Among the AA content in serum and breast muscle, lysine in serum significantly decreased compared with the control ($P < 0.05$). The UREA content was approximately 2-fold higher in the LPR group than in the LPM and LPA groups ($P < 0.05$). No difference in IgA, IgG, IgM, and IgE levels was observed among the groups ($P > 0.05$). The nitrogen excretion was decreased in LPM and LPA compared to the control and LPR ($P < 0.05$). Nitrogen deposition did not differ among groups ($P > 0.05$). Nitrogen utilization was highest in the LPA and LPM groups, followed by the control group and LPR ($P < 0.05$). In conclusion, the patterns of supplementation of major AA and all AA in low-protein diets (CP, 15.55%) had no adverse effect on the growth performance compared with the control (CP, 18.55%) of the goslings. Besides, the two patterns could decrease nitrogen excretion and increase nitrogen utilization. Furthermore, from the perspective of dietary cost and environmental protection, the pattern of supplementing major AA in a corn-soybean meal low-protein diet is suggested.

Key words: low-protein diets, growth performance, nitrogen metabolism, amino acid supplementation pattern, geese

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INTRODUCTION

Approximately 660 million meat geese were produced worldwide in 2021, of which China was the largest consuming and exporting country (FAO, 2021). Feed accounts for 70% of poultry production costs (Donohue and Cunningham, 2009). Modern intensive poultry raising requires a large number of protein ingredients in feeds. However, the shortage of raw protein materials has challenged the feed industry. Higher-protein diets cause waste of protein ingredients and lead to more nitrogen excretion (Hofmann et al., 2019; Macelline et al., 2020) in poultry manure which is a vital factor for environmental pollution. Hence, studying the proper use of low-protein diets is essential for the sustainable development of poultry production.

Many studies on the use of low-protein diets have been conducted on poultry. Reducing CP levels in diets helps to improve the nitrogen utilization ratio and reduced nitrogen excretion in poultry (Applegate et al., 2008). However, low-protein diets without adding amino acids (**AA**) reduced body weight (Shahzad et al., 2011; Cesare et al., 2019) and increased F: G (Hofmann et al., 2020) in broiler chicken. Protein is made of different AA. Studies on adding one or more kinds of essential AA in the feeds of chicken (Bezerra et al., 2016; Hilliar et al., 2019; Zamani et al., 2021), and ducks (Xie et al., 2017) get positive results. Supplementation of AA in low-protein diets could complement the adverse

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effects in growth performance caused by low-protein diets in broilers (Siegert et al., 2016; Attia et al., 2020), ducks (Baeza and Leclercq, 1998), geese (Ashour et al., 2020), turkeys (Boling and Firman, 1997). Studies of different AA supplementation patterns in low-protein diets have not been investigated in geese. So, we hypothesize that the low-protein diet does not negatively affect goslings' growth performance by adding suitable AA in the low-protein feeds.

The nitrogen in poultry feces is one vital factor to cause earth and water pollution. The nitrogen in feces mainly comes from the undigested nitrogenous substances in diets (Chalova et al., 2016; Belloir et al., 2017). It costs lots of money and effort to curb environmental pollution once the nitrogen is discharged into the environment. Thus, reducing nitrogen excretion via low-protein diets is an effective way to reduce environmental pollution. Low-protein diets were proven to reduce nitrogen discharge from poultry (Namroud et al., 2008; Hofmann et al., 2019). Kriseldi et al. (2018) indicated that nitrogen excretion could be significantly reduced by reducing CP levels in diets by supplementing AA. In particular, insufficient and excessive dietary CP and AA reduced performance and increased nitrogen excretion, respectively (Harper et al., 1970; Donato et al., 2016). Thus, we hypothesize that different AA supplementation patterns could influence nitrogen excretion in low-protein feeds.

Therefore, this study aimed to explore the influence of supplementation patterns of AA on growth performance, serum and breast muscle content, serum and immunological variables, and nitrogen metabolism of 1 to 28 d goslings to advocate the least costly and most environmentally beneficial with a low-protein diet.

MATERIALS AND METHODS

Ethics Statement

All animal care and experimental procedures in the study were performed according to the Regulations for the Administration of Affairs Concerning Experimental Animals of the People's Republic of China and approved by the animal care and use committee of Yangzhou University(Yangzhou, China). SYXK (Su) IACUC 2021-0036.

Experimental Diets and Design

Three hundred and sixty-four 1-day-old male healthy Jiangnan White goslings, supplied by the Jiangsu Lihua Animal Husbandry Co., LTD (Changzhou, China), were randomly divided into 4 groups with 7 pens containing 13 goslings each. The 4 groups were the control (CP, 18.55%), LPM (CP, 15.55% + major AA), LPA (CP, 15.55% + all AA), LP (CP, 15.55%, AA content reduced proportionally to control's CP) (major AA: arginine, cysteine, lysine, methionine, threonine, and tryptophan; all AA: arginine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine). The corn-soybean meal diets are formulated according to the ideal AA model of goose and its nutritional requirements (Wang et al., 2010). The goslings were caged on wire nets for the whole experimental period. The stocking density was 18/m^2 from 1 to 14 d and 6/m^2 from 15 to 28 d. The temperature for the growth of goslings varies according to their age (1−5 d, 29°C; 6−10 d, 27°C; 11−15 d, 23°C; 16−20 d, 21°C; 21−28 d, 18°C). The light was provided with yellow lights with 565 to 570 nm wavelength. The goslings were illuminated daily from 1 to 7 d and 14 h a day from 8 to 28 d. The basic dietary nutritional levels were adopted from the NRC (1994), and our laboratory's (Shi et al., 2007; Wang et al., 2010) reports. The basic dietary composition and nutritional levels are shown in Table 1. Water and feeds were given ad libitum.

Table 1. Composition and nutrient levels of the corn-soybean meal diets (air-dry basis)%.

	AA supplementation patterns[1]			
Items	Control	LPM	LPA	LPR
Ingredients, %				
Corn	58.50	63.78	64.17	63.62
Soybean meal	29.77	21.44	22.03	21.04
Wheat bran	4.34	6.04	3.95	7.37
Rice hull	3.50	4.08	4.30	3.92
Limestone	0.80	0.78	0.75	0.80
$CaHPO_4$	1.39	1.50	1.54	1.46
Salt	0.30	0.30	0.30	0.30
Premix[2]	1.00	1.00	1.00	1.00
Methionine	0.19	0.22	0.22	0.14
Lysine	0.02	0.22	0.21	0.06
Arginine	0.00	0.25	0.24	0.04
Histidine	0.00	0.00	0.10	0.02
Isoleucine	0.00	0.00	0.15	0.03
Leucine	0.00	0.00	0.23	0.00
Cysteine	0.19	0.23	0.24	0.15
Phenylalanine	0.00	0.00	0.15	0.01
Tryptophan	0.00	0.00	0.11	0.01
Threonine	0.00	0.12	0.12	0.01
Tryptophan	0.00	0.04	0.04	0.01
Valine	0.00	0.00	0.15	0.01
Total	100.00	100.00	100.00	100.00
Nutrient levels,[3] %				
Metabolizable Energy (MJ/kg)	11.20	11.20	11.20	11.20
Crude protein	18.55	15.55	15.55	15.55
Crude fiber	4.94	4.94	4.94	4.94
Calcium	0.83	0.83	0.83	0.83
Total phosphorus	0.71	0.71	0.71	0.71
Methionine	0.47	0.47	0.47	0.39
Lysine	0.98	0.98	0.98	0.82
Arginine	1.26	1.26	1.26	1.05
Histidine	0.50	0.42	0.52	0.44
Isoleucine	0.77	0.63	0.78	0.65
Leucine	1.60	1.38	1.62	1.38
Cysteine	0.50	0.50	0.50	0.42
Phenylalanine	0.90	0.74	0.90	0.75
Tryptophan	0.63	0.53	0.65	0.54
Threonine	0.70	0.70	0.70	0.59
Tryptophan	0.21	0.21	0.21	0.18
Valine	0.86	0.71	0.86	0.72

[1]Control (CP, 18.55%), LPM (CP, 15.55% + major AA), LPA (CP, 15.55% + all AA), and LPR (CP, 15.55% + AA content reduced proportionally to the control's CP).

[2]Provided per kilogram of premix diet: VA, 900, 000 IU; VD, 300, 000IU; VE, 1800 IU; VK, 150 mg; VB_1, 90 mg; VB_2, 800 mg; VB_6, 320 mg; VB_{12}, 1 mg; nicotinic acid, 4.5 g; pantothenic acid, 1100 mg; folic acid, 65 mg; choline, 45mg; biotin, 5 mg; Fe 6 g; Cu, 1 g; Mn, 9.5 g; Zn, 9 g; I, 50 mg; Se, 30 mg.

[3]Calculated values.

Growth Performance

All goslings were weighed at 1 d and 28 d, and the feed intake of each replicate was recorded weekly during 1 to 28 d, and average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed-to-gain ratio (**F:G**) were calculated at the end of the experiment.

AA Content of Serum and Breast Muscle

The sample was precisely aspirated about 50 mg into a 2 mL centrifuge tube, and 400 μL of 10 % methanol formate solution-H_2O (1:1, V/V) solution was added accurately and vortexed for 30 s. The sample was centrifuged at 12,000 rpm for 5 min at 4°C, 10 μL of the original supernatant was taken, 990 μL of 10 % methanol formate-H_2O (1:1, V/V) solution was added, and vortexed for 30 s. 100 μL of the diluted sample was taken, and 100 μL of the internal isotopic standard with a concentration of 10 ppb was added and vortexed for 30 s. The supernatant was filtered through 0.22 μm membranes, and the filtrate was added to the assay bottle as the assay sample. UPLC-MS/MS analysis was performed using an Agilent 1290-6470 ULPC-MS/MS (Agilent, Santa Clara, CA). Chromatographic conditions, column: Agilent ZORBAX Eclipse Plus C18 (2.1 mm × 100 mm, 1.8 × m), column temperature: 50°C; mobile phase: A: ultrapure water, B: chromatographically pure methanol (containing 0.1 % formic acid); mass spectrometric conditions, sampling mode: positive ion mode (ESI+), multiple reaction monitoring (**MRM**); standard external method for quantification, continuous injection.

Serum and Immunological Variables

Approximately 6 mL of blood was collected from the wing vein before slaughtering and centrifuged at 3,500 r/min for 10 min to produce serum samples. The serum samples were taken and stored at −20°C to determine serum and immunological variables. Serum samples were analyzed for the urea nitrogen (**UREA**), urea acid (**UA**), total protein (**TP**), albumin (**ALB**), globulin (**GLOB**), albumin-globulin ratio (**A/G**), alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), and creatinine (**CRE**) using a machine (Hitachi 7180 Automatic Biochemical Analyzer, Hitachi High-Tech Corporation, Tokyo, Japan). Serum samples were determined using ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) for IgA, IgG, IgM, and IgE with kit numbers ml582431, ml685358, ml388972, and ml965721, respectively.

Nitrogen Metabolism

The total excreta collection method was used to determine nitrogen metabolism CP (N × 6.25) (Matterson et al., 1965). One gosling per treatment was selected and placed in a separate wire floor metabolic cage at 21 d. The housing temperature was maintained at 18 ± 2°C, and goslings were allowed access to water and the diets ad libitum. The experiment involved a 5-d adaptation period of goslings and a 3-d collection period of excreta (feces). During the feces collection process, feathers and dander that fall into the tray are carefully removed to avoid contamination of feces from feathers and scurf's pollution. The excreta nitrogen was fixed by adding l0 mL of 10 % hydrochloric acid per 100 g of feces. The excreta were then dried to a constant weight in an oven at 65°C, regained the moisture in atmospheric moisture for 24 h, weighed, and ground to pass through a 40-mesh sieve. Then nitrogen metabolism-related indicators (nitrogen intake, nitrogen excretion, nitrogen deposition, and nitrogen utilization ratio) were determined by the Kjeldahl method with the Kjeltec System 8400 (FOSS NIRSystems Inc., Hillerød, Denmark). The calculation formula is as follows:

nitrogen intake (g) = feed intake × nitrogen content in feed

nitrogen excretion (g) = fecal output × nitrogen content in feces

nitrogen deposition (g) = nitrogen intake - nitrogen intake

nitrogen utilization ratio (%) = (nitrogen deposition/ nitrogen intake) × 100 %

Statistical Analysis

The result was statistically analyzed by one-way ANONA using SPSS 26.0 (SPSS, Inc., Chicago, IL) software, and Duncan's method was used for multiple comparisons. The data were presented as the mean values and the standard error of the means (SEM). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Growth Performance

The BW, ADG, ADFI, and F/G of goslings fed feeds with different AA supplementation patterns in low-protein diets are shown in Table 2. The ADG and BW were the lowest, the F: G was the highest in LPR ($P < 0.05$), and the F: G did not differ among the control, LPA, and LPM ($P < 0.05$). However, low-protein diets did not affect ADFI or mortality among all the groups during the experimental period ($P > 0.05$).

AA Content of Serum and Breast Muscle

AA content in the serum and breast muscle are shown in Tables 3 and 4, respectively. The serum proline concentration was lower in LPR than in LPM and LPA, and the serum lysine concentration was lower in LPR compared with LPM and the control ($P < 0.05$). The AA supplementation pattern did not affect other serum AA ($P > 0.05$).

Table 2. Effects of different AA supplement patterns in low protein on BW, ADFI, ADG, F/G, and mortality (%) of goslings from 1 to 28 d of age.

	AA supplementation patterns[1] (n = 7)					
Items	Control	LPM	LPA	LPR	SEM	*P*-value
BW (g) 1d	85.60	85.60	85.82	85.71	0.07	0.686
BW (g) 28d	2028.29[b]	2016.11[b]	1973.10[a,b]	1890.60[a]	17.48	0.014
ADG (g/d)	69.38[b]	68.95[b]	67.40[a,b]	64.46[a]	0.62	0.014
ADFI (g/d)	138.04	139.76	130.15	134.36	1.39	0.060
F: G (g/g)	1.99[a,b]	2.03[a,b]	1.93[a]	2.08[b]	1.39	0.032
Mortality (%)	0.00	0.14	0.14	0.57	0.09	0.157

[a-b]Means with different superscripts within the same row differ significantly ($P < 0.05$).
[1]Control (CP, 18.55%), LPM (CP, 15.55% + major AA), LPA (CP, 15.55% + all AA), and LPR (CP, 15.55% + AA content reduced proportionally to the control's CP)

Table 3. Effects of different AA supplement patterns in low protein on serum AA content of goslings at 28 d of age.

	AA supplementation patterns[1] (n = 7)					
Items	Control	LPM	LPA	LPR	SEM	*P*-value
Glycine (nmol/L)	23.95	25.17	24.01	24.75	0.89	0.964
Alanine (nmol/L)	50.71	48.87	43.76	39.73	1.67	0.063
Serine (nmol/L)	75.47	85.68	93.07	65.03	4.65	0.153
Proline (nmol/L)	14.86[a,b]	17.58[b,c]	19.25[c]	13.21[a]	0.81	0.017
Valine (nmol/L)	18.31	15.38	16.90	12.86	0.80	0.078
Threonine (nmol/L)	16.24	18.84	13.93	13.12	1.32	0.457
Isoleucine (nmol/L)	14.02	12.15	12.42	10.54	0.55	0.166
Leucine (nmol/L)	19.28	17.51	19.22	14.78	0.72	0.071
Asparagine (nmol/L)	5.35	6.93	6.20	5.55	0.42	0.585
Aspartic acid (nmol/L)	4.58	4.80	5.12	4.66	0.16	0.698
Glutamine (nmol/L)	48.95	68.49	49.15	55.12	3.07	0.061
Lysine (nmol/L)	20.88[b]	18.43[b]	16.12[a,b]	12.91[a]	1.04	0.022
Glutamic acid (nmol/L)	21.15	19.15	18.16	18.84	0.61	0.368
Methionine (nmol/L)	13.43	17.26	16.15	14.79	0.76	0.328
Histidine (nmol/L)	7.28	8.89	8.56	7.95	0.36	0.433
Phenylalanine (nmol/L)	13.96	14.14	14.27	14.15	0.51	0.998
Arginine (nmol/L)	31.93	36.89	36.69	34.79	1.07	0.347
Tyrosine (nmol/L)	44.23	45.19	38.21	48.74	2.37	0.506
Tryptophan (nmol/L)	9.21	13.25	14.63	12.65	0.88	0.160

[a-c]Means with different superscripts within the same row differ significantly ($P < 0.05$).
[1]Control (CP, 18.55%), LPM (CP, 15.55% + major AA), LPA (CP, 15.55% + all AA), and LPR (CP, 15.55% + AA content reduced proportionally to the control's CP).

Table 4. Effects of different AA supplement patterns in low protein on breast muscle AA content of goslings at 28 d of age.

	AA supplementation patterns[1] (n = 7)					
Items	Control	LPM	LPA	LPR	SEM	*P*-value
Glycine (nmol/L)	198.58	223.43	223.71	213.89	13.77	0.926
Alanine (nmol/L)	377.72	408.35	405.94	351.66	21.32	0.796
Serine (nmol/L)	256.68	249.51	344.24	234.09	20.32	0.220
Proline (nmol/L)	69.78	69.99	80.49	69.77	4.43	0.817
Valine (nmol/L)	69.36	65.85	68.47	61.18	3.91	0.905
Threonine (nmol/L)	54.95	55.86	76.17	53.15	5.47	0.438
Isoleucine (nmol/L)	53.92	53.55	51.97	46.03	3.21	0.840
Leucine (nmol/L)	91.14	89.97	88.91	79.37	5.19	0.875
Asparagine (nmol/L)	51.78	59.81	73.79	59.36	5.39	0.583
Aspartic acid (nmol/L)	67.87	59.74	68.99	71.00	5.59	0.919
Glutamine (nmol/L)	216.82	246.59	299.09	267.44	20.47	0.587
Lysine (nmol/L)	82.16	74.31	91.82	57.76	5.62	0.175
Glutamic acid (nmol/L)	165.44	181.35	190.96	167.64	11.97	0.886
Methionine (nmol/L)	35.95	37.34	38.86	33.26	1.80	0.769
Histidine (nmol/L)	65.84	65.70	79.44	60.12	5.57	0.696
Phenylalanine (nmol/L)	54.34	52.96	53.64	46.70	2.77	0.790
Arginine (nmol/L)	80.93	88.34	96.69	76.75	5.13	0.578
Tyrosine (nmol/L)	78.28	77.20	74.76	72.46	4.37	0.974
Tryptophan (nmol/L)	16.39	16.98	17.16	14.79	0.95	0.840

[1]Control (CP, 18.55%), LPM (CP, 15.55% + major AA), LPA (CP, 15.55% + all AA), and LPR (CP, 15.55% + AA content reduced proportionally to the control's CP).

Table 5. Effects of different AA supplement patterns in low protein serum variables of goslings at 28 d of age.

	AA supplementation patterns[1] (n = 7)					
Items[2]	Control	LPM	LPA	LPR	SEM	*P*-value
UREA (mmol/L)	5.50[a,b]	3.50[a]	4.73[a]	8.40[b]	0.65	0.042
UA (umol/L)	154.00	153.00	179.29	171.71	12.55	0.861
TP(g/L)	38.39[b]	32.71[a]	37.03[b]	37.03[b]	0.61	0.002
ALB(g/L)	17.31	16.40	16.66	16.71	0.14	0.124
GLOB(g/L)	21.07[b]	16.31[a]	20.37[b]	20.31[b]	0.55	0.003
A/G(g/L/g/L)	0.83[a]	1.05[b]	0.82[a]	0.82[a]	0.03	0.009
ALT(U/L)	20.00	23.86	19.43	20.71	1.08	0.497
AST(U/L)	106.43	116.57	99.71	120.86	6.24	0.645
CRE (umol/L)	21.14	17.43	18.71	20.14	1.56	0.861

[a-b]Means with different superscripts within the same row differ significantly ($P < 0.05$).
[1]Control (CP, 18.55%), LPM (CP, 15.55% + major AA), LPA (CP, 15.55% + all AA), and LPR (CP, 15.55% + AA content reduced proportionally to the control's CP)
[2]A/G, albumin-globulin ratio; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRE, creatinine; GLOB, globulin; TP, total protein; UREA, nitrogen; UA, urea acid.

Table 6. Effects of different AA supplement patterns in low protein on the immunological variables of goslings at 28 d of age.

	AA supplementation patterns[1] (n = 7)					
Items	Control	LPM	LPA	LPR	SEM	*P*-value
IgA (g/L)	11.72	28.22	20.31	14.90	2.67	0.134
IgG (g/L)	176.73	245.84	174.40	134.64	20.26	0.282
IgM (g/L)	366.05	377.61	325.37	323.33	29.61	0.894
IgE (g/L)	46.21	41.19	42.60	43.82	2.02	0.858

[1]Control (CP, 18.55%), LPM (CP, 15.55% + major AA), LPA (CP, 15.55% + all AA), and LPR (CP, 15.55% + AA content reduced proportionally to the control's CP).

And the AA supplementation pattern affected none of the 19 AA in the breast muscle ($P > 0.05$).

Serum and Immunological Variables

Table 5 demonstrates the effect of different AA supplement patterns on the serum variables. The UREA was about twice more in LPR than in LPM and LPA ($P < 0.05$). The concentration of TP and GLOB was lower, whereas A/G was higher in LPM than in the other groups ($P < 0.05$). No difference was observed in IgA, IgG, IgM, and IgE among the groups ($P > 0.05$, Table 6).

Nitrogen Metabolism

As shown in Table 7, the nitrogen excretion was decreased ($P < 0.05$) in LPM and LPA as compared with the control and LPR ($P < 0.05$). No effect was observed on nitrogen deposition among groups ($P > 0.05$). Furthermore, the nitrogen utilization rate of LPM and LPA was higher than that of the control group, and the nitrogen utilization rate of the control group was higher than that of the LPR group ($P < 0.05$).

DISCUSSION

Low-protein diets without additional AA lead to decreased performance in poultry (Kamran et al., 2004; Kriseldi et al., 2018; Cesare et al., 2019). In this study, the growth performance reached the control level (CP, 18.55%) when supplementing the major AA pattern and all AA patterns in the low-protein diets (CP, 15.55%) in the goslings. Similar results were observed in low-protein diets in chickens (Bezerra et al., 2016; van Harn et al., 2019; Teng et al., 2021), laying hens (Alagawany et al., 2020; Uyanga et al., 2022), ducks (Xie et al., 2017; Jiang et al., 2018), quail chicks (Elsayed et al., 2021), and goose (Wang et al., 2020). It is concluded that the growth performance would not be adversely affected by low-protein diets when the appropriate amount of AA is supplemented. However, Bregendahl et al. (2002)'s research was not consistent with this study. He found that low-protein diets added with AA failed to support the growth performance equal to high-protein control diets in broilers. The reason might be that the supplements of AA were insufficient in their low-protein diets to maintain a common growth objective in the broilers of their study. Similarly, growth performance was lower

Table 7. Effects of different AA supplement patterns in low protein on nitrogen metabolism of goslings at 27−29 d of age.

	AA supplementation patterns[1] (n = 7)					
Items	Control	LPM	LPA	LPR	SEM	*P*-value
Nitrogen intake (g/d)	3.55[b]	3.08[a]	3.08[a]	3.09[a]	0.06	0.002
Nitrogen excretion (g/d)	1.37[b]	1.05[a]	1.10[a]	1.24[b]	0.03	<0.001
Nitrogen deposition (g/d)	2.18	2.03	1.99	1.85	0.05	0.085
Nitrogen utilization ratio (%)	61.36[a,b]	65.82[c]	64.31[b,c]	59.88[a]	0.82	0.030

[a-c]Means with different superscripts within the same row differ significantly ($P < 0.05$).
[1]Control (CP, 18.55%), LPM (CP, 15.55% + major AA), LPA (CP, 15.55% + all AA), and LPR (CP, 15.55% + AA content reduced proportionally to the control's CP)Q.

in LPR than in control, even if we added a small amount of AA to the LPR. Adopting the pattern of supplementing the major AA and all AA in the low-protein diets (CP, 15.55%) could make the growth performance of the goslings reach the level of the control group (CP, 18.55%).

Serum AA content is an essential parameter in evaluating the nutritional status of whole-body protein (Gustafson et al., 116AD; Liao et al., 2018). Free AA in the blood is mainly influenced by the passage of exogenous proteins and AA through the digestive tract (Windels et al., 1971). The relationship between blood AA concentration and dietary AA intake, as well as the relationship between AA deposition, is usually explored by measuring blood AA concentration in previous studies (Dean and Scott, 1966; Johnson and Anderson, 1982). Insufficient dietary AA can lead to an imbalance in blood AA content (Fisher et al., 1960), which is consistent with the findings of the LPR group in this experiment. In the present study, serum proline and lysine were lowest in the LPR group, consistently lower than in the LPM group. The lower serum proline and lysine may explain why the growth performance was the weakest in the LPR group since lysine (Jespersen et al., 2021) is vital in promoting poultry growth (Lee et al., 2020). Our data demonstrated that the proline might be essential in promoting growth in goslings fed with low-protein diets. However, further studies are needed to reveal how proline improves growth performance in goslings fed with low-protein diets. Our study's AA composition in the breast muscle was not affected by low-protein diets or AA supplementation patterns in feeds. This result indicated that the body has a function to maintain the balance of AA ratio and muscle composition in the muscle. However, the AA percentages of breast muscle are no different. Nevertheless, the body weight was low in LPR and high in LPM and LPA. The data indicated that low-protein diets and AA supplementation patterns might impact growth performance through absolute muscle growth rather than muscle composition.

Blood UA and UREA are often closely related to AA consumption in poultry (Donsbough et al., 2010). In our study, the LPM and LPA groups reduced the UREA level, which was consistent with Ospina-Rojas et al. (2014)'s study main including 3% reduced CP in a low-protein diet supplemented with AA (lysine, methionine, threonine) reduced the serum UA levels. However, Macelline et al. (2020) found that broilers fed higher AA levels did not show elevated UA levels in either environment. Thus, insufficient levels of essential AA in the diet may lead to relative UREA excess. In terms of liver metabolism, the liver produces UA from serine and glycine via the purine pathway, leading to elevated serum UREA levels, which may be further associated with nitrogen deposition. So, reducing the dietary protein level to supplement AA is feasible, but the content should meet gosling's growth and development requirements. In our study, reducing protein levels and supplementing AA did not significantly affect the immunity of goslings from 1 to 28 d of age. In addition, Abou-Elkhair et al. (2020) showed that adding AA to the low-protein diet improved the immunity of broilers. These results showed that the low-protein supplementation of AA had no adverse effect on immunity.

Nitrogen excretion is one of the primary sources of pollution in poultry production. Excessive fecal nitrogen excretion affects poultry's growth performance and health and contributes to air and water pollution (Cappelaere et al., 2021). Many studies on low-protein diets of chickens (Bezerra et al., 2016; Donato et al., 2016) and turkeys (Applegate et al., 2008) have proved that low-protein diets supplemented with AA can effectively reduce nitrogen excretion. Fewer excess of AA in the diet leads to less nitrogen excretion, better utilization of nitrogen, and a higher nitrogen utilization ratio (Surisdiarto, 1991; Hilliar et al., 2019). In this study, the patterns of supplementing major AA and all AA significantly reduced nitrogen excretion and improved nitrogen use efficiency in low-protein diets. In contrast, as observed in LPR, nitrogen excretion or nitrogen utilization ratio was not compensated by supplementing appropriate AA types with an insufficient amount in a low-protein diet. Parr and Summers (1991), Hernández et al. (2012), and Chalova et al. (2016)'s study show the same results as ours. To reduce nitrogen excretion and improve nitrogen utilization rate, the low protein diet supplemented with appropriate AA can meet the growth and development requirements of geese. However, no supplemental or insufficient AA content in the low-protein diet will reduce nitrogen utilization.

In conclusion, the patterns of supplementation of major AA and all AA in low-protein diets (CP, 15.55%) had no adverse effect on the growth performance in goslings from 1 to 28 d of age compared with the higher-protein (CP, 18.55%) diet. Besides, the two patterns could reduce nitrogen excretion and improve nitrogen utilization. Conversely, when the supplemented AA content is insufficient, the nitrogen excretion will be more, and the nitrogen use efficiency will be lower. Furthermore, from the perspective of dietary cost and environmental protection, the pattern of supplementing major AA in corn-soybean meal low-protein feed is suggested.

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DISCLOSURES

No conflict of interest exists in the submission of this manuscript. The work described has not been submitted elsewhere for publication, in whole or in part, and all authors have contributed to, read, and approved the enclosed manuscript.

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