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Running Title (within 10 words)	Effects of supplementing amino acids in low protein diet
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Abstract

This study was conducted to determine the effects of amino acid (AA) supplementation in low-protein (LP) diets on growth performance and nitrogen (N) excretion. A total of 175 7-day-old Ross 308 male broilers, with a mean body weight (BW) of 165 g (standard deviation = 11.2 g), were grouped into five blocks by BW and allocated to seven treatments according to a randomized complete block design with five replicate cages at five birds per cage. Dietary treatments comprised a control diet containing 20.0% crude protein (CP) and six LP diets containing either 18.5% or 17.0% CP. These LP diets were supplemented with either no AA supplementation, indispensable AA, or both indispensable and dispensable AA (glutamic acid and glycine). Birds were fed experimental grower diets from day 7 to 21 and then commercial finisher diets until day 28. During the grower period (day 7 to 21), birds fed LP diets supplemented with indispensable AA exhibited greater ($p < 0.05$) BW, body weight gain (BWG), feed intake (FI), and gain-to-feed ratio (G:F) than birds fed LP diets without crystalline AA and were comparable to birds fed the control diet. During the finisher period (day 21 to 28), birds fed LP diets supplemented with indispensable AA showed greater ($p < 0.05$) BW than birds fed LP diets without crystalline AA, and their growth performance was comparable to birds fed the control diet. Throughout the overall period, supplementing indispensable AA in LP diets resulted in elevated ($p < 0.05$) BWG, FI, and G:F more than those of LP diets without crystalline AA and were comparable to those of the control diet. Supplementing indispensable AA in LP diets decreased amount and coefficient of N excretion as much as the control diet. Dispensable AA supplementation in LP diets did not influence growth performance and N excretion. In conclusion, supplementing indispensable AA in LP diets maintains growth performance and N excretion until the dietary CP lowers from 20.0% to 17.0% during the grower period. As long as dietary CP is above 17.0%, dispensable AA may not be deficient in LP diets during the grower period.

Keywords: Amino acid; Broiler; Growth performance; Low protein diet; Nitrogen excretion

Introduction

Soybean meal (SBM) is a prominent protein source in poultry diets and has been included in feed to prevent amino acid (AA) deficiency. However, the price of SBM is expected to soar as climate change disrupts production [1], even as the SBM demand increases alongside broiler meat and egg production [2]. In addition, excessive SBM in diets increases the excretion of nitrogen (N) that is not utilized by animals. The N from excreta is a primary source of environmental pollution in animal production related to land, water, and air contamination [3]. Therefore, low protein (LP) diets supplemented with crystalline AA, reducing SBM use in broiler diets, may contribute to both feed cost reduction and the environmental sustainability of poultry production.

In most cases, crystalline AA supplemented in an LP diet is indispensable AA to meet requirements, although dietary levels of dispensable AA also decrease as SBM dietary concentration decreases. Therefore, specific dispensable AA may be deficient in the LP diet and need to be supplemented to match the growth performance of a standard diet [4]. In previous studies, Hilliar et al. [5] reported that glycine (Gly) supplementation in an 18.5% crude protein (CP) diet improved body weight gain (BWG) and feed conversion ratio compared to an 18.5% CP diet without Gly supplementation. Hofmann et al. [6] reported that a 16% CP diet supplemented with Gly and glutamic acid (Glu) improved growth performance compared to a 16% CP diet without dispensable AA supplementation but had a lower growth performance than the 18% CP diet. However, Hussein et al. [7] illustrated that supplementing Glu in an LP diet did not affect growth performance. These inconsistent results indicate that effects of supplementing dispensable AA in the LP diets require further research for clarification. Therefore, the objective of the present study was to investigate the effects of supplementing indispensable AA as well as dispensable AA on growth performance and N excretion in LP diets.

Materials and Methods

The protocols for this study were reviewed and approved by the Institutional Animal Care and Use Committee at Kyungpook National University (KNU 2021-0212).

Animals and management

A total of 175 day-old Ross 308 male broilers were housed in battery cages (60 cm length × 50 cm width × 60 cm height) and were fed a standard commercial pre-starter broiler diet (21% CP and 12.55 MJ/kg AMEn) for a 7-day pre-experimental period. On day 7, all birds were individually weighed and allocated to seven dietary treatments with five replicate cages in a randomized complete block design based on body weight (BW). From day 21 to 28, all birds were fed a standard broiler finisher diet (18% CP and 13.18 MJ/kg AMEn). All birds had *ad libitum* access to water and feed in an environmentally controlled room, with room temperature maintained at 33℃ for the first three days, then gradually decreased by 3℃ weekly to reach 24℃ by day 28.

Dietary treatments

Experimental diets consisted of seven diets, including one control diet and six LP diets (Table 1). The control diet was formulated to contain 20% CP, and two LP diets were prepared to contain 18.5% and 17.0% CP without crystalline AA supplementation. The other two LP diets were supplemented with indispensable AA, including arginine, histidine, leucine, lysine, methionine, cysteine, phenylalanine, threonine, tryptophan, and valine (11 AA), to meet indispensable AA requirements for broilers. The remaining two LP diets were supplemented with 11 AA, Glu, and Gly (13 AA) to investigate the effect of dispensable AA supplementation. The inclusion levels of Glu and Gly in the two LP diets with 13 AA were maintained at a constant ratio of 4:1 to achieve similar contents as the control diet. Analyzed CP and AA compositions in the experimental diets are presented in Table 2. The control diet and the LP diets, except for LP diets without crystalline AA, were formulated to meet nutrient and AA requirements based on standardized ileal digestible AA according to Hoehler et al. [8] and Aviagen [9] during the grower phase. All experimental diets included 0.5% chromium oxide as an indigestible index for calculating N excretion.

Sample collection

On day 21 and 28, individual BW and feed leftovers per cage were measured and the gain to feed ratio (G:F) was calculated. Excreta from birds fed experimental diets were collected four times every 12 hours from day 19 to 21 of age. Excreta within a cage were pooled and stored at −20℃, then dried in a forced-air oven (JS research, Gongju, Korea) at 50℃ for further analysis.

Chemical analysis and calculation

The experimental diets were ground and analyzed for AA composition following the AOAC International method 982.30E (a, b) [10] and CP concentrations using the Kjeldahl method (Foss Innovation Centre, Hillerød, Denmark). Chromium concentrations in diets were determined using the method described by Fenton and Fenton [11]. Excreta samples were also analyzed for CP and chromium concentration.

The index method was used to determine the N excretion coefficient with the following equations [12]:

$$\text{N retention} = [1 - (Cr_i \div Cr_o) \times (N_o \div N_i)]$$

N excretion coefficient = 1 – N retention

N excretion (g) = Feed intake (g) × Dietary N concentration (%) × N excretion coefficient

where Cr_i and Cr_o represent the chromium concentrations (%) in the diet and excreta, respectively; N_i and N_o represent the nitrogen concentrations (%) in the diet and excreta, respectively.

Statistical analysis

The data were analyzed using the MIXED procedure of SAS software (SAS Inst, Cary, NC, USA). The dietary treatment was considered a fixed variable, and replication was considered a random variable. The experimental unit was a cage. Treatment means were separated using Tukey's HSD, and a statistical significance was set at $p < 0.05$.

Results

The analyzed concentrations of CP and AA in the experimental diets were equal to the calculated values (Table 2). The results of the growth performance and N excretion are presented in Table 3. All growth performances, excluding FI in the finisher period, and N excretion showed significant differences ($p < 0.05$) between dietary treatments.

Growth performance

Excluding FI, the two LP diets without crystalline AA exhibited lower ($p < 0.05$) growth performance during the grower period than the control diet. The LP diet containing 17.0% CP without crystalline AA supplementation yielded lower ($p < 0.05$) FI than the control diet. However, the supplementation of 11 AA in the two LP diets increased ($p < 0.05$) BW, BWG, FI, and G:F compared to the two LP diets without crystalline AA, and their growth performance did not differ from that of the control diet. The two LP diets with 13 AA were not different in terms of the growth performance from the 2 LP diets with 11 AA.

During the finisher period, the two LP diets without crystalline AA exhibited less ($p < 0.05$) BW than the control diet. The BWG of birds fed only the LP diet containing 17.0% CP without crystalline AA was lower ($p < 0.05$) than that of the control diet. For the G:F, birds fed the two LP diets without crystalline AA were not different from birds fed the control diet. However, supplementing 11 AA in the two LP diets increased ($p < 0.05$) BW compared to the two LP diets without crystalline AA, and it was not different from BW of birds fed the control diet. The two LP diets supplemented with 11 AA were not different from the control diet regarding growth performance. Supplementing 13 AA in the two LP diets did not differ from the two LP diets supplemented with 11 AA in terms of growth performance.

Overall, the two LP diets without crystalline AA yielded lower ($p < 0.05$) BWG than the control diet, and the LP diet containing 17.0% CP showed lower FI and G:F than the control diet. However, supplementing 11 AA in the two LP diets increased ($p < 0.05$) BW, BWG, and G:F, reaching a level that was not significantly different from the control diet. Growth performance from supplementing 13 AA in the two LP diets did not differ from the two LP diets with 11 AA.

Nitrogen excretion

The N excretion coefficients differed ($p < 0.05$) between groups. The two LP diets without crystalline AA resulted in higher ($p < 0.05$) N excretion coefficients than the control diet. Supplementing the 11 AA in LP diets reduced ($p < 0.05$) N excretion coefficients compared to the two LP diets without crystalline AA, and these reductions were not different from those observed in the control diet. The amount of N excretion of birds fed the 17.0% LP diet with 11 AA was lower ($p < 0.05$) than that of birds fed the 18.5% LP diet without crystalline AA. Supplementing 13 AA in the LP diets was not different from LP diets supplemented with 11 AA the N excretion coefficient and amount of N excretion.

Discussion

LP diets can lower feed costs and environmental pollution. Previous studies have evaluated the effects of the LP diets on growth performance by supplementing indispensable AA or both indispensable and dispensable AA. Indispensable AA was supplemented in the LP diet to meet AA requirements [13-19], while dispensable AA, such as Asp, Gly, and Glu, were supplemented as N sources for dispensable AA synthesis [4-6,20-24]. In the current study, Glu and Gly were supplemented in LP diets because Glu was considered an N source for dispensable AA synthesis in the LP diet [25-27], and Gly in LP diets was considered a potentially deficient AA [28-30]. The current study evaluated the effects of supplementing indispensable AA and dispensable AA in LP diets.

Supplementing 11 AA in the two LP diets improved BW, BWG, and G:F compared to the two LP diets without crystalline AA during the grower and overall periods. These results might be attributed to satisfying indispensable AA requirements to optimize growth performance. Regarding FI, the two LP diets supplemented with 11 AA were not different from the two LP diets without crystalline AA since the birds might be able to compensate for undernutrition through voluntary feed consumption [31]. During the finisher period, the two LP diets supplemented with 11 AA were not different from the two LP diets without crystalline AA in terms of growth performance, excluding BW. For BW, the two LP diets with 11 AA had a greater BW than the two LP diets without crystalline AA, even though the BWG of the two LP diets with 11 AA during the finisher period was not different from the two LP diets without crystalline AA. This finding is because the BW of the two LP diets supplemented with 11 AA was greater than that of the two LP diets without crystalline AA during the grower period. Regarding BWG, the two LP diets with 11 AA were not different from the two LP diets without crystalline AA since all birds were fed the same standard finisher diet. Additionally, FI was not different between dietary treatments for the same reason. Thus, supplementing of 11 AA in the two LP diets did not increase G:F because it did not alter BWG and FI.

When indispensable AA was supplemented in the LP diet to meet requirements, reducing dietary CP from 20.0% to 17.0% did not lower BW, BWG, FI, and G:F. Previous studies have demonstrated that dietary CP can be practically reduced by up to 3% without adversely affecting growth performance [4,32-35], corroborating this current finding. However, reducing dietary CP may be able to impair growth performance, even with indispensable AA supplementation in the LP diet. Dean et al. [30] found that ADG, final BW, and G:F linearly decrease with the reduction of dietary CP from 22% to 16%. Notably, the true digestible indispensable AA in the experimental diets, excluding Lysine, Threonine, Methionine, and Cysteine, were reduced as the dietary CP was curtailed. Furthermore, Bregendahl et al. [21] reported that despite formulating all diets to meet or exceed indispensable AA requirements based on true digestibility, 18.6% LP diets supplemented with indispensable AA led to less growth performance compared to the control diet (23% CP). These results may be attributed to an increase in ratio of unbound AA to protein-bound AA from a decrease in dietary SBM concentration and utilization differences between unbound AA and AA absorbed from protein in broiler chickens [36]. However, these effects are inconsistent, highlighting the need for further research.

On the other hand, supplementing 13 AA in the two LP diets did not improve growth performance compared to the 2 LP diets supplemented with 11 AA in the current study. This result proposes that dispensable AA was not deficient in the LP diets as long as the CP level was above 17.0% during the grower period. Hussein et al. [7] reported that dispensable AA was not deficient in 17.7% LP diets from day 7 to 21, as adding 5.5% Glu did not impact growth performance. Similarly, Bregendahl et al. [21] showed that adding 1% glutamine and asparagine as N sources to 18.6% LP diets did not affect growth performance. However, Namroud et al. [22] reported that supplementing 1.12% Glu and 0.26% Gly in a 19% LP diet improved growth performance as much as the control diet (23% CP). Corzo et al. [4] reported that all dispensable AA should be added to the 18% LP broiler diets to match the growth performance of the control diet (22% CP). In addition, Dean et al. [30] discovered that from day 0 to 17, 16.2% LP diets supplemented with Gly linearly increased G:F, with increasing concentrations of Gly + serine from 1.80% to 3.00%. The effect of dispensable AA supplementation on growth performance in LP diets may not be consistent with previous experiments due to differences in dispensable AA concentrations, AA precursors, and feed ingredients.

Supplementing 11 AA in the two LP diets resulted in a decrease in the N excretion coefficient compared to the two LP diets without crystalline AA in this study. This result agrees with Deschepper et al. [37] who demonstrated that a 16% LP diet with indispensable AA supplementation exhibited a greater protein accretion than a 21% LP diet with indispensable AA deficiency. These results may be attributed to an AA imbalance due to AA deficiency inhibiting protein synthesis [38]. The AA imbalance can lead to deamination, generating ammonia, which is then excreted via the Krebs cycle as uric acid [36]. Similarly, Macelline et al. [39] reported that supplementing indispensable AA in an LP diet decreased urea N in the plasma. Supplementing indispensable AA may mitigate the effect of the AA imbalance that increases deamination. However, further research is warranted to clarify the impact of reduced N metabolites in the plasma due to abated dietary CP concentration on the N excretion coefficient. Moreover, supplementing indispensable AA and reducing dietary CP might induce to decrease amount of N excretion by lowering N excretion coefficient and intake, primary factors that influence amount of N excretion. Previous research has demonstrated that reducing dietary CP decreased nitrogenous waste in litter [18,22,40-41] supporting current findings.

When indispensable AA was supplemented in LP diets to meet AA requirements for broilers, the 3% reduction in dietary CP did not affect the amount of N excretion and N excretion coefficient. Consistent with the current study, Chrystal et al. [42] demonstrated that there was no linear effect when dietary CP was reduced from 21% to 16.5% on N retention in broilers from day 33 to 35. Hernández et al. [14] also reported that reducing dietary CP from 23% to 20% and 21.5% to 18.5% did not influence N retention for male broilers from day 8 to 21 and day 22 to 35, respectively. These findings suggest that dietary CP level may be able to be reduced from 20.0% to 17.0% without affecting the N excretion coefficient during the grower phase, similar to the current growth performance results.

However, the supplementation of 13 AA in the two LP diets showed no difference in terms of the N excretion coefficient and the amount of N excretion when compared to the two LP diets supplemented with 11 AA. These results are consistent with Deschepper and De Groote [37], who showed that LP diets supplemented with indispensable AA following NRC recommendations [43] were not different from LP diets supplemented with indispensable AA, Glu, and Gly concerning protein accretion. Additionally, Hilliar et al. [23] established that adding Gly to a 19% LP diet did not affect N retention, similar to the current study.

In conclusion, this study demonstrated that supplementing indispensable AA in the LP diet preserved growth performance and N excretion in broilers as long as the CP level was above 17.0% during the grower period. Providing LP diets without supplementation of crystalline AA during the grower period can potentially have a negative impact on growth performance during the finisher period when feeding a standard diet. On the other hand, dispensable AA did not seem to be deficient in the LP diet during the grower period unless the dietary CP level was lower than 17.0%.

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Tables and Figures

Table 1. Ingredient and chemical composition of the experimental diets

Item	CP 20.0%	CP 18.5%[1),2)]			CP 17.0%[1),2)]		
		LP	+11 AA	+13 AA	LP	+11 AA	+13 AA
Ingredient, %							
Corn	59.35	61.86	64.28	64.28	66.06	69.38	69.38
Soybean meal	31.75	30.60	25.46	25.46	26.35	18.76	18.76
Soybean oil	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Corn-starch	-	-	0.50	-	-	1.00	-
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Arginine	0.12	-	0.27	0.27	-	0.44	0.44
L-Histidine	0.00	-	0.05	0.05	-	0.11	0.11
L-Isoleucine	0.14	-	0.23	0.23	-	0.33	0.33
L-Leucine	-	-	0.04	0.04	-	0.18	0.18
L-Lys-HCl	0.41	-	0.57	0.57	-	0.74	0.74
L-Methionine (90 % purity)	0.24	-	0.27	0.27	-	0.30	0.30
L-Cysteine	0.15	-	0.17	0.17	-	0.20	0.20
L-Phenylalanine	-	-	0.05	0.05	-	0.16	0.16
L-Threonine	0.14	-	0.22	0.22	-	0.30	0.30
L-Tryptophan	-	-	0.01	0.01	-	0.05	0.05
L-Valine	0.17	-	0.27	0.27	-	0.37	0.37
L-Glutamic acid	-	-	-	0.10	-	-	0.20
L-Glycine	-	-	-	0.40	-	-	0.80
Limestone	1.41	1.41	1.43	1.43	1.43	1.45	1.45
Dicalcium-phosphate	1.47	1.48	1.53	1.53	1.51	1.58	1.58
Salt	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Vitamin premix[3)]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix[4)]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Calculated value, %							
AME, MJ/kg	12.4	12.4	12.7	12.6	12.6	12.9	12.9
CP	20.00	18.50	18.50	18.85	17.00	17.00	17.71
Ca	0.87	0.87	0.87	0.87	0.87	0.87	0.87
Non-phytate P	0.44	0.44	0.44	0.44	0.44	0.44	0.44
SID AA, %							
Indispensable AA							
Arginine	1.18	1.04	1.18	1.18	0.94	1.18	1.18
Histidine	0.43	0.42	0.43	0.43	0.38	0.43	0.43
Isoleucine	0.78	0.63	0.78	0.78	0.58	0.78	0.78
Leucine	1.33	1.31	1.23	1.23	1.23	1.23	1.23
Lysine	1.15	0.81	1.15	1.15	0.73	1.15	1.15
Methionine	0.47	0.26	0.47	0.47	0.24	0.47	0.47
Phenylalanine	0.77	0.76	0.73	0.73	0.70	0.73	0.73
Threonine	0.73	0.58	0.73	0.73	0.53	0.73	0.73
Tryptophan	0.21	0.20	0.18	0.18	0.18	0.18	0.18
Valine	0.91	0.72	0.91	0.91	0.66	0.91	0.91
Dispensable AA							
Alanine	0.77	0.76	0.69	0.69	0.72	0.62	0.62
Aspartic acid	1.52	1.48	1.29	1.29	1.34	1.05	1.05
Cysteine	0.40	0.25	0.40	0.40	0.24	0.40	0.40
Glutamic acid	2.83	2.79	2.48	2.88	2.57	2.11	2.90
Glycine	0.60	0.59	0.52	0.62	0.54	0.44	0.64
Proline	1.00	0.99	0.89	0.89	0.92	0.79	0.79
Serine	0.76	0.75	0.66	0.66	0.68	0.55	0.55
Tyrosine	0.56	0.56	0.49	0.49	0.51	0.41	0.41

[1)] LP diet was not supplemented with AA, and overall AA were 14.5 % and 21.7% less compared to requirements in 18.5% CP and 17.0% CP diet, respectively.

[2)]LP diets supplemented with 11 AA (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and cysteine) were formulated to meet requirement. LP diets with 13 AA were obtained by supplementing glutamic acid and glycine to LP diets supplemented with 11 AA at the expense of corn-starch.

[3)]Supplies the following quantities per kilogram of diet: vitamin A, 31,500 IU; vitamin D_3, 12,500 IU; vitamin E, 175 mg; vitamin K_3, 8.75 mg; vitamin B_1, 10 mg; vitamin B_2, 30 mg; B_5, 75 mg; vitamin B_6 15 mg; vitamin B_{12}, 0.065 mg; folic acid, 7.5 mg; niacin, 12 mg; biotin, 0.5 mg; proviox50, 10 mg.

[4)]Supplies the following quantities per kilogram of diet: Fe, 250 mg; Cu, 85 mg; Zn, 460 mg; Mn, 5,000 mg; Co, 0.75 mg; Se, 1.5 mg; I, 7.5 mg.

AA, amino acid; AME, apparent metabolizable energy; CP, crude protein; LP, low protein; SID, standardized ileal digestible; P, phosphorus.

Table 2. Analyzed crude protein and amino acid levels of the experimental diets

Item, %	CP 20.0%	CP 18.5%[1),2)]			CP 17.0%[1),2)]		
		LP	+11 AA	+13 AA	LP	+11 AA	+13 AA
CP	20.02	18.96	18.69	18.90	17.35	16.81	17.75
Indispensable AA							
Arginine	1.30	1.17	1.21	1.23	1.01	1.15	1.17
Histidine	0.50	0.48	0.47	0.47	0.44	0.44	0.46
Isoleucine	0.86	0.73	0.83	0.81	0.66	0.78	0.78
Leucine	1.55	1.52	1.44	1.41	1.41	1.36	1.35
Lysine	1.30	0.94	1.21	1.23	0.85	1.22	1.28
Methionine	0.44	0.27	0.43	0.44	0.26	0.48	0.47
Phenylalanine	0.90	0.87	0.83	0.82	0.80	0.79	0.80
Threonine	0.83	0.70	0.79	0.81	0.65	0.76	0.79
Valine	1.02	0.85	0.96	1.00	0.78	0.99	0.95
Dispensable AA							
Alanine	0.89	0.87	0.81	0.80	0.82	0.70	0.71
Aspartic acid	1.88	1.79	1.60	1.58	1.61	1.29	1.30
Cysteine	0.45	0.30	0.41	0.43	0.30	0.43	0.47
Glutamic acid	3.29	3.18	2.89	3.17	2.90	2.43	3.19
Glycine	0.76	0.72	0.66	0.76	0.67	0.55	0.76
Proline	1.13	1.12	1.05	1.04	1.06	0.94	0.92
Serine	0.91	0.88	0.80	0.80	0.80	0.67	0.67
Tyrosine	0.61	0.63	0.52	0.57	0.48	0.43	0.44

[1)]LP diet was not supplemented with AA, and overall AA were 14.5 % and 21.7% less compared to requirements in 18.5% CP and 17.0% CP diet, respectively.

[2)]LP diets supplemented with 11 AA (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and cysteine) were formulated to meet requirement. LP diets with 13 AA were obtained by supplementing glutamic acid and glycine to LP diets supplemented with 11 AA at the expense of corn-starch.

AA, amino acid; CP, crude protein; LP, low protein.

Table 3. Growth performance and nitrogen excretion of experiment diets

Item	CP 20.0%	CP 18.5%[1),2)]			CP 17.0%[1),2)]				
		LP	+11 AA	+13 AA	LP	+11 AA	+13 AA	SEM	*p*-value
Growth performance[3)]									
Grower period									
BW, g	656^{a}	531^{bc}	650^{a}	638^{a}	475^{c}	616^{ab}	655^{a}	21.4	< 0.001
BWG, g	490^{a}	365^{bc}	484^{a}	472^{a}	309^{c}	451^{ab}	490^{a}	19.9	< 0.001
FI, g	710^{ab}	610^{bc}	699^{ab}	678^{abc}	583^{c}	687^{abc}	721^{a}	24.9	0.003
G:F, g/kg	691^{a}	598^{b}	693^{a}	696^{a}	529^{c}	655^{ab}	678^{a}	13.7	<.0001
Finisher period									
BW, g	$1,188^{a}$	$1,019^{bc}$	$1,174^{ab}$	$1,169^{ab}$	903^{c}	$1,105^{ab}$	$1,133^{ab}$	36.4	< 0.001
BWG, g	532^{a}	488^{ab}	525^{a}	532^{a}	428^{b}	488^{ab}	478^{ab}	20.9	0.015
FI, g	803	752	780	810	708	751	763	26.0	0.137
G:F, g/kg	663^{ab}	651^{ab}	672^{a}	657^{ab}	604^{b}	650^{ab}	625^{ab}	14.6	0.041
Overall period									
BWG, g	$1,022^{a}$	854^{bc}	$1,008^{ab}$	$1,004^{ab}$	738^{c}	939^{ab}	968^{ab}	35.1	< 0.001
FI, g	$1,513^{a}$	$1,362^{ab}$	$1,479^{ab}$	$1,488^{ab}$	$1,291^{b}$	$1,438^{ab}$	$1,485^{ab}$	45.3	< 0.001
G:F, g/kg	676^{a}	627^{ab}	682^{ab}	674^{ab}	570^{b}	652^{ab}	651^{ab}	10.1	< 0.001
N excretion[3)]									
Coefficient	0.199^{b}	0.275^{a}	0.205^{b}	0.215^{b}	0.303^{a}	0.211^{b}	0.191^{b}	0.0098	< 0.001
Amount, g	4.6^{ab}	5.1^{a}	4.3^{ab}	4.4^{ab}	4.9^{ab}	3.9^{b}	3.9^{b}	0.25	0.010

[1)]LP diet was not supplemented with AA, and the overall AA were 14.5 % and 21.7% less compared to requirements in 18.5% CP and 17.0% CP diet, respectively.

[2)]LP diets supplemented with 11 AA (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and cysteine) were formulated to meet requirement. LP diets with 13 AA were obtained by supplementing glutamic acid and glycine to LP diets supplemented with 11 AA at the expense of corn-starch.

[3)]Each mean represents five observations.

[a-c]Means within a row without a common superscript differ ($p < 0.05$).

AA; amino acid, BW, body weight; BWG, body weight gain; CP, crude protein; FI, feed intake; G:F, gain to feed ratio; LP, low protein; N, nitrogen; SEM, standard error of the mean.