Influence of dietary crude protein on growth performance and apparent and standardized ileal digestibility of amino acids in corn-soybean meal-based diets fed to broilers

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ABSTRACT This study aimed to examine the influence of dietary CP on the apparent ileal digestibility (**AID**) and standardized ileal digestibility (**SID**) of amino acids (**AA**) and test the additivity of AA digestibility in corn-soybean meal-based diets fed to broilers. Six experimental diets comprising a nitrogen-free diet and five corn-soybean meal-based diets containing 6.0%, 9.5%, 13.0%, 16.5%, and 20.0% CP were prepared. Increments in CP and AA concentrations were achieved by increasing the inclusion rate of corn and soybean meal at the expense of cornstarch. All diets contained 0.5% chromic oxide, which was included as an indigestible index. A total of 960 Ross 308 male broilers 19-day-old male broilers (Ross 308), with a mean BW of 628 g (SD = 58.0), were allocated to six dietary treatment groups in a randomized complete block design, with each treatment group have eight replicate cages and 20 birds per cage. All birds were fed the experimental diets for 4 days. On d 23, individual BW and feed intake were recorded, followed by collection of ileal digesta samples from the distal ileum. Regarding growth, the final BW, weight gain, feed intake, and gain to feed ratio increased linearly ($P < 0.001$) as dietary CP concentrations increased. With the increase in dietary CP concentrations from 6.0% to 20.0%, the AID of all AA, except Arg, increased linearly ($P < 0.05$). However, the SID of all AA, except Arg, Cys, and Pro, remained unaffected by CP concentrations in the diets. This study indicated that dietary CP concentrations from 6.0% to 20.0% have an effect on the growth performance of birds and the AID of most AA; however, the SID of most AA was not affected by dietary CP concentrations in the corn-soybean meal-based diets. In conclusion, the SID of AA is more additive than the AID of AA in poultry diets containing CP in the range of 6.0% to 20.0%.

Key words: amino acid, crude protein, growth performance, ileal digestibility, broiler

2023 Poultry Science 102:102505
https://doi.org/10.1016/j.psj.2023.102505

INTRODUCTION

Proteins are the building blocks of body tissues and aid in the development and maintenance of body muscles of birds. Dietary CP and amino acid (**AA**) supplementation are essential for diverse physiological functions, including muscle metabolism, and for the growth and maintenance of broilers. Providing low-CP diets to broilers adversely affects their normal body growth. Therefore, changes in dietary CP concentrations have a profound impact on the growth performance of broilers (Chrystal et al., 2020a). In addition, dietary protein cannot be completely digested by birds because of antinutritional factors and indigestible fractions in feed ingredients (Beski et al., 2015) or limited secretion of digestive enzymes in birds (Al-Qahtani et al., 2021). Therefore, for an accurate feed formulation to provide appropriate amounts of dietary AA, it is necessary to define an appropriate approach for estimating the accurate availability of AA in feed ingredients or mixed diets for broilers.

The accuracy of estimating ileal digestibility has an impact on the accurate formulation of feed. Apparent ileal digestibility (**AID**) has been widely used to estimate digestible AA concentrations in mixed diets or single feed ingredients for poultry (Kong and Adeola, 2010). However, some researchers have demonstrated that the AID of AA in pigs (Donkoh et al., 1994; Fan et al., 1994; Liu et al., 2018) and broilers (Widyaratne and Drew, 2011; Chrystal et al., 2020b) can be influenced by dietary CP concentrations. For example, Liu et al. (2018) reported that AID of CP and

Received September 4, 2022.
Accepted January 10, 2023.

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AA influenced by the dietary CP concentrations when birds were fed the different types of feed ingredients-based diets containing CP in the range of 8% to 16%. Widyaratne and Drew (2011) also reported the increased AID of AA of broilers during the grower (d 7−21) period with increasing dietary CP concentrations from 17% to 20%. In their study, the highest AA digestibility was observed in birds fed a diet containing 18.5% of CP concentration. These results may be attributed to the high contributions of basal endogenous losses (**BEL**) of CP and AA to the total AA flow when dietary CP and AA concentrations are low (Kong and Adeola, 2013). To overcome this issue, Kong and Adeola (2014) suggested that diets should be formulated based on the standardized ileal digestible AA, which is corrected for the BEL of AA. Theoretically, the standardized ileal digestibility (**SID**) of AA is not affected by dietary CP concentrations; however, there is limited information regarding the effect of dietary CP concentrations on the SID of AA in broilers. Several researchers have demonstrated that the SID of AA is additive in feed ingredients or mixed diets in Pekin ducks (Kong and Adeola, 2013) and broilers (Cowieson et al., 2019; Osho et al., 2019; An et al., 2020). Previous studies confirmed the additivity of the SID of AA using single ingredients and mixed diets containing multiple feed ingredients. Furthermore, assuming that the SID of AA is not affected by dietary protein concentrations compared with the AID of AA, Liu et al. (2018) evaluated the additivity of AA digestibility in pigs using diets with various dietary protein concentrations. However, there is a dearth of research on this topic in poultry.

Therefore, the present study aimed to examine the effects of various dietary CP concentrations on the growth performance of broilers and determine the AID and SID of AA in a corn-soybean meal (**SBM**)-based diet.

MATERIALS AND METHODS

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Konkuk University, Republic of Korea (approval number: KU16025).

Animals and Experimental Design

In total, 960 male broilers (Ross 308) were used in a 4-d digestibility study. Birds received commercial prestarter (d 1−10) and starter (d 11−18) diets, respectively. On d 19, birds (average BW: 628 ± 58.0 g) were weighed individually, grouped into eight blocks (replicates) by BW, and randomly allocated to 6 dietary treatment groups with 20 birds per cage in each block using a randomized complete block design. Feed and water were supplied ad libitum from d 19 to 23. The experimental period was determined according to the recommended feeding length of nitrogen-free diet (**NFD**) reported in previous studies (Kong and Adeola, 2014; Adedokun et al., 2017) to avoid harmful impact on bird's health caused by long-term feeding NFD.

Dietary Treatments

Six dietary treatments comprising a NFD and 5 corn-SBM-based diets with graded concentrations of CP ranging from 6.0% to 20.0% (Table 1). The increments in corn and SBM concentrations were equally spaced, and the concentrations were increased at the expense of cornstarch to achieve the desired graded CP concentrations among the 5 experimental diets. An NFD and 5 corn-SBM-based diets were formulated to meet or exceed the estimated nutrient requirements (NRC, 1994), except for dietary AA concentrations. Chromic oxide (Cr_2O_3; 0.5%) was added to all diets as an indigestible index used for calculating ileal digestibility.

Management

Growth responses, including weight gain, feed intake, and gain to feed ratio (**G:F**), of birds from the age of 19 to 23 d were calculated using individual BW and the amount of feed supply and feed leftover per cage. Mortalities were recorded during the entire experimental period. Further, growth performance data were corrected using BW and the estimated individual feed intake of dead birds, as modified by Sung and Adeola (2022). The following equation suggested by Noblet et al. (2015) was used to calculate the metabolizable energy required for maintenance (kcal/d): 131 kcal × $BW^{0.70}$.

Sample Collection

On d 23, all birds were euthanized via CO_2 asphyxiation, and ileal digesta samples were collected from the distal two-third of the ileum by flushing with distilled water. The ileal digesta samples collected from all birds within a cage were pooled and stored at −20°C until further analysis.

Chemical Analyses and Calculations

The collected ileal digesta samples were freeze-dried (Vacuum freeze dryer; SFDTS10K, Samwon Freezing Engineering Co., Busan, Korea). All experimental diets and freeze-dried ileal digesta samples were ground using the Foss Cyclotec mill (CT 293 Cyclotec, Foss, Eden Prairie, MN) and a coffee grinder (KWG-150, He Shan Co., Ltd., Guangdong, China), respectively. The diets and ileal digesta samples were analyzed for DM (method 930.01; AOAC, 2016) using a forced air-drying oven, CP (method 984.13; AOAC, 2016) using the Kjeldahl method (Kjeltec 8400 analyzer, Foss Tecator AB, Höganäs, Sweden), and AA (method 982.30 E [a, b]; AOAC, 2016). Chromium concentrations in the experimental diets and ileal digesta samples were determined using the method reported by Fenton and Fenton (1979). The AID, BEL, and SID of CP and AA were calculated using the following equations (Kong and Adeola, 2014):

Table 1. Ingredient and chemical compositions of experimental diets, as-fed basis.

	Dietary crude protein, %					
Ingredients, %	0	6.0	9.5	13.0	16.5	20.0
Corn	—	24.80	33.75	42.70	51.65	60.60
Soybean meal	—	8.03	13.68	19.33	24.98	30.63
Cornstarch	29.67	59.92	45.42	30.92	16.41	1.92
Soybean oil	3.50	2.00	2.00	2.00	2.00	2.00
Monocalcium phosphate	2.15	1.97	1.87	1.78	1.69	1.59
Limestone	1.88	1.88	1.88	1.87	1.87	1.86
Vitamin−mineral premix[1]	1.00	0.50	0.50	0.50	0.50	0.50
Chromium oxide	0.50	0.50	0.50	0.50	0.50	0.50
Salt	—	0.40	0.40	0.40	0.40	0.40
Sucrose	54.61	—	—	—	—	—
Cellulose	5.00	—	—	—	—	—
Choline chloride	0.25	—	—	—	—	—
Sodium bicarbonate	0.75	—	—	—	—	—
Potassium chloride	0.30	—	—	—	—	—
Magnesium oxide	0.09	—	—	—	—	—
Potassium carbonate	0.30	—	—	—	—	—
Total	100.0	100.0	100.0	100.0	100.0	100.0
Calculated composition						
AME[2], kcal/kg	3,537	3,633	3,480	3,328	3,175	3,023
Crude protein	—	6.0	9.5	13.0	16.5	20.0
Calcium	1.0	1.0	1.0	1.0	1.0	1.0
Non-phytate phosphorus	0.45	0.45	0.45	0.45	0.45	0.45
Total amino acids						
Arg	—	0.37	0.60	0.84	1.07	1.30
His	—	0.16	0.25	0.35	0.44	0.53
Ile	—	0.24	0.39	0.53	0.68	0.83
Leu	—	0.55	0.85	1.15	1.45	1.75
Lys	—	0.30	0.49	0.68	0.87	1.06
Met	—	0.10	0.15	0.21	0.26	0.31
Met + Cys	—	0.20	0.31	0.42	0.53	0.64
Phe	—	0.28	0.45	0.62	0.78	0.95
Phe + Tyr	—	0.51	0.82	1.12	1.42	1.73
Thr	—	0.22	0.35	0.49	0.62	0.75
Trp	—	0.07	0.12	0.17	0.22	0.26
Val	—	0.28	0.44	0.60	0.76	0.92

[1]Vitamin−mineral premix supplied the following per kilogram of diet: vitamin A, 5,484 IU; vitamin D_3, 2,643 IU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; thiamine mononitrate, 2.2 mg; riboflavin, 5.49 mg; D-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B_{12}, 13.2 μg; biotin, 55.2 μg; folic acid, 990 μg; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg as potassium iodide; Mn, 66.1 mg as manganese oxide; Cu, 4.44 mg as copper sulfate; Fe, 44.1 mg as iron sulfate; Zn, 44.1 mg as zinc oxide; Se, 0.3 mg as sodium selenite.

[2]AME = Apparent metabolizable energy.

$$\text{AID (\%)} = \left[1 - \left(\frac{Cr_i}{Cr_o}\right) \times \left(\frac{CPo\ or\ AA_o}{CP_i\ or\ AA_i}\right)\right] \times 100$$

$$\text{BEL (mg/kg DM intake)} = \left(\frac{Cr_i}{Cr_o}\right) \times AA_o$$

$$\text{SID (\%)} = \text{AID} + \left(\frac{\text{BEL}}{CP_i\ or\ AA_i}\right) \times 100$$

where CP_i or AA_i and CP_o or AA_o represent the CP or AA concentration (% DM) in the diet and ileal digesta samples, respectively; Cr_i and Cr_o represent the chromium concentration (% DM) in the diet and ileal digesta samples, respectively. The AID, BEL, and SID of AA were calculated according to 8 observations from each treatment group.

Statistical Analyses

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Dietary treatments were considered as fixed variables, and replicates were considered as random variables. The experimental unit was a cage, and the least squares means were presented. Tukey's significant difference test was used to compare the least squares means of all treatments. The linear and quadratic effects of dietary CP and AA concentrations on both AID and SID were determined using orthogonal polynomial contrasts. Significance was set at an α-level of 0.05.

RESULTS

All birds used in this study were healthy throughout the experimental period and readily consumed the experimental diets. Four birds died during the experimental period, indicating a mortality rate of 0.41%. The calculated and analyzed values of CP and AA concentrations in the experimental diets are presented in Tables 1 and 2, respectively. The calculated CP concentrations in the five experimental diets were 6.0%, 9.5%, 13.0%, 16.5%, and 20.0%, which were fairly equal to the analyzed values (7.1%, 10.3%, 12.8%, 17.4%, and 21.7%,

Table 2. Analyzed chemical composition (%) of experimental diets, as-fed basis.

	Dietary crude protein concentrations, %					
Item, %	0	6.0	9.5	13.0	16.5	20.0
Dry matter	97.3	91.6	91.2	90.6	90.3	89.4
Crude protein	0.3	7.1	10.3	12.8	17.4	21.7
Indispensable amino acids						
Arg	0.02	0.33	0.52	0.72	1.00	1.29
His	0.01	0.14	0.21	0.28	0.39	0.49
Ile	0.02	0.18	0.27	0.37	0.55	0.68
Leu	0.06	0.46	0.72	0.99	1.43	1.69
Lys	0.02	0.28	0.43	0.58	0.81	1.06
Met	0.02	0.08	0.12	0.16	0.23	0.24
Phe	0.04	0.26	0.40	0.54	0.76	0.95
Thr	0.02	0.23	0.35	0.46	0.64	0.82
Val	0.03	0.22	0.33	0.43	0.59	0.75
Dispensable amino acids						
Ala	0.04	0.29	0.42	0.57	0.79	0.95
Asp	0.06	0.53	0.82	1.12	1.58	2.02
Cys	0.04	0.10	0.14	0.19	0.27	0.28
Glu	0.08	0.96	1.42	1.90	2.67	3.31
Gly	0.03	0.23	0.34	0.46	0.63	0.81
Pro	0.04	0.38	0.54	0.72	0.97	1.20
Ser	0.03	0.30	0.44	0.58	0.80	1.01
Tyr	0.01	0.15	0.24	0.35	0.52	0.64

respectively). The effects of dietary CP concentrations on growth performance are shown in Table 3. The final BW, weight gain, feed intake, and G:F of birds improved ($P < 0.001$) linearly with increasing dietary CP concentration. In addition, the G:F quadratically increased ($P < 0.001$) as dietary CP concentration increased.

The BEL of CP and AA are shown in Table 4. The BEL of AA were major in Glu, Val, Thr, Ser, Asp, and Pro, with the values of 311, 286, 280, 263, 245, and 211 mg/kg DM intake, respectively. The BEL of these AA represented 58.6% of the total BEL of AA. As shown in Table 5, the increase in dietary CP concentrations from 6.0% to 20.0% influenced the AID of CP and AA and resulted in a quadratic increase in the AID of CP and 8 of 17 AA including Lue ($P = 0.026$), Met ($P = 0.001$), Phe ($P = 0.017$), Thr ($P = 0.017$), Cys ($P = 0.0004$), Pro ($P = 0.035$), Ser ($P = 0.031$), and Tyr ($P = 0.002$). The AID of CP and AA, except Arg ($P = 0.132$), increased linearly ($P < 0.05$) with increasing dietary CP concentrations. However, the SID of most AA, except Met ($P = 0.042$), did not exhibit quadratic responses when dietary CP concentrations increased from 6.0% to 20.0% (Table 6). Moreover, the SID of 3 of 17 AA including Arg ($P = 0.023$), Cys ($P = 0.006$), and Pro ($P = 0.042$) increased linearly with increasing dietary CP concentrations.

DISCUSSION

This study demonstrated that all growth responses, including BW, weight gain, and G:F, improved linearly with the increase in dietary CP concentrations from 6.0% to 20.0%. In contrast, Kamran et al. (2008) reported that the weight of birds increased from 692 g/bird when fed on 19% CP-containing diets to 761 g/bird when fed on 22% CP-containing diets. However, the feed intake decreased as dietary CP concentrations increased but the growth rate and feed intake remained unchanged with diets containing less than 21% CP. This result is similar to that reported by Chrystal et al. (2020b). In the present study, there was an increase in the feed intake of birds who were fed the high-CP diet (20.0% CP) compared with those who were fed the low-CP diet (6.0% CP), which may be attributed to the decrease in dietary energy concentration from 3,633 to 3,023 kcal/kg. Despite the high dietary energy density in the low-CP diet, the feed intake of birds was reduced, which might be attributed to the physical limitation of birds (Kamran et al., 2008) because differences in dietary energy concentration (Taylor et al., 2021) and relative body capacity may influence the amounts of voluntary feed intake in different birds (Tallentire et al., 2016).

In this study, the estimated BEL of AA in broilers fed a NFD at 23 days of age were lower than those reported in previous studies (Adedokun et al., 2007; Golian et al., 2008; Woyengo et al., 2010; Kong and Adeola, 2013; Adeola et al., 2016; Ravindran, 2021). However, the ratios of AA in the BEL of broilers fed NFD was comparable with values reported by Ravindran (2021) who reviewed 18 published studies. The BEL of AA may be influenced by the age of birds (Barua et al., 2021), techniques used to determine the BEL of AA at the recovery site (site for collecting ileal digesta samples from the ileum; Blok et al., 2017), ingredient composition of experimental diets (Zhou et al., 2022), and DM intake (Blok et al., 2017). The BEL of AA varies depending on these factors; hence, it is difficult to explain these losses in the present study compared with those reported in previous studies.

This study showed an overall increase of 2.9% unit in the average AID of 17 AA (87.9% vs. 90.8%) with the increase in dietary CP concentrations from 6.0% to 20.0%. This result is consistent with that reported in a study by Hilliar et al. (2019) that showed a 3.2% unit increase in the average AID of AA with an increase in

Table 3. Effecht of dietary crude protein concentration on growth performance of broilers from 19 to 23 d posthatching.

	Dietary CP concentration, %						*P* values	
Item	6.0	9.5	13.0	16.5	20.0	SEM[2]	Linear	Quadratic
Initial BW, g (at 19 d)	628	628	628	628	629	0.2	0.427	0.913
Final BW, g (at 23 d)	655	707	780	843	894	2.9	<0.001	0.249
Weight gain, g/bird	27	78	152	214	265	2.9	<0.001	0.251
Feed intake, g/bird	298	319	367	399	414	4.1	<0.001	0.084
G:F, g/kg	88	243	413	538	640	5.9	<0.001	<0.001

[1]Data are presented as least squares means of eight observations per treatment.
[2]SEM = standard error of the mean.

Table 4. Basal ileal endogenous crude protein and amino acids in 23-day-old male broilers fed a nitrogen-free diet (mg/kg dry matter intake).[1]

	Basal endogenous losses of amino acids	
Item	Mean	SD
Crude protein	382	57.1
Indispensable amino acids		
Arg	85	18.3
His	37	8.0
Ile	99	21.4
Leu	158	30.0
Lys	80	18.1
Met	34	5.8
Phe	143	25.2
Thr	280	39.5
Val	286	40.1
Dispensable amino acids		
Ala	122	23.0
Asp	245	43.2
Cys	100	15.7
Glu	311	53.9
Gly	133	24.0
Pro	211	27.2
Ser	263	37.5
Tyr	137	25.2

[1]Data are presented as least squares mean of eight observations.

CP concentrations from 19.1% to 22.7% in a wheat-SBM-based diet fed to 21-day-old broilers. The reduction in the AID of CP and AA in the low-CP diet (6% CP) compared with that in the high-CP diet (20% CP) observed in the present study might be attributed to the relatively high contribution of the BEL of CP and AA in the low-CP diet, which is due to the low contribution of high-CP-containing feed ingredients. The AID of CP and 8 of 17 AA in the present study increased in a quadratic manner with the increase in dietary CP concentrations from 6.0% to 20.0%. Similar observations were reported in other experimental studies using rats (Donkoh et al., 1994), and pigs (Fan et al., 1994; Eklund et al., 2010). The quadratic responses of the AID of CP and AA to dietary CP concentrations may be attributed to the decreased relative contribution of BEL to the total ileal outflow with increasing dietary CP concentrations (Fan et al., 1994; Eklund et al., 2010).

In contrast to the AID results, the SID of CP and most AA remained constant in response to dietary CP concentrations. There is limited information regarding the SID of CP and AA in response to dietary CP concentrations in broilers; therefore, direct comparison between previous studies is not possible. However, the results of the present study are consistent with those of several studies conducted using adult Leghorns (Kamisoyama et al., 2010), rats (Donkoh et al., 1994), growing pigs (Furuya and Kaji, 1989; Zhai and Adeola, 2011; Liu et al., 2018), and piglets (Eklund et al., 2008).

An accurate determination of digestible AA in feed ingredients and mixed diets is essential to provide a precise dietary AA composition for the bird's requirements with accurate feed formulation. The underlying assumption for feed formulation is that the total amount of digestible protein in the mixed diet is equal to the sum of the protein compositions in individual feed ingredients. The total AA and digestible AA contents vary depending on the type of feed ingredients. The effect of dietary CP concentrations on AA digestibility can be observed in the AID and SID of AA, but it is more evident when diets are formulated based on the apparent ileal digestible AA content (Kong and Adeola, 2013). For AID, the assumption of additivity is not always exhibited in individual feed ingredients and mixed diets for broilers (Angkanaporn et al., 1996) and pigs (Stein et al., 2005,2007). When feed ingredients in low-digestible protein or low-protein diets were used, the relative contribution of the BEL of AA to the total ileal outflow increased, which in turn caused the lack of additivity of

Table 5. Apparent ileal digestibility (%) of crude protein and amino acids in corn-soybean meal-based diets fed to broilers.[1]

	Dietary crude protein concentration, %						*P* values	
Item	6.0	9.5	13.0	16.5	20.0	SEM[2]	Linear	Quadratic
Crude protein	88.5	89.6	90.5	91.3	90.5	0.49	0.001	0.049
Indispensable amino acids								
Arg	93.9	94.6	94.7	95.1	94.5	0.33	0.132	0.080
His	91.4	92.1	92.3	93.2	92.4	0.44	0.039	0.200
Ile	88.4	89.8	90.7	92.3	91.5	0.52	<0.001	0.073
Leu	91.3	92.4	92.8	93.9	92.7	0.43	0.005	0.026
Lys	91.0	91.2	91.8	93.0	92.6	0.49	0.003	0.745
Met	90.8	92.9	93.9	95.3	93.8	0.49	<0.001	0.001
Phe	89.4	91.1	91.6	92.8	92.1	0.45	<0.001	0.017
Thr	80.4	83.6	85.3	88.0	87.5	0.68	<0.001	0.017
Val	81.2	83.3	85.0	87.9	87.4	0.67	<0.001	0.126
Dispensable amino acids								
Ala	90.2	91.0	91.5	92.7	91.4	0.50	0.013	0.076
Asp	87.6	88.6	89.0	90.5	89.8	0.46	<0.001	0.180
Cys	81.6	84.3	85.6	87.8	84.0	0.80	0.003	<0.001
Glu	92.0	92.7	92.9	93.9	93.0	0.36	0.009	0.104
Gly	86.0	87.1	87.8	89.3	88.5	0.59	0.001	0.177
Pro	89.1	90.2	90.9	92.0	91.1	0.45	0.001	0.035
Ser	85.2	87.4	88.4	90.3	89.8	0.53	<0.001	0.031
Tyr	85.5	88.8	90.7	93.0	92.0	0.61	<0.001	0.002

[1]Data are presented as least squares means of eight observations per treatment.
[2]SEM = standard error of the mean.

Table 6. Standardized ileal digestibility (%) of crude protein and amino acids in corn-soybean meal-based diets fed to broilers.[1]

Item	Dietary crude protein concentration, %					SEM[2]	P values	
	6.0	9.5	13.0	16.5	20.0		Linear	Quadratic
Crude protein	93.7	93.2	93.1	93.3	92.1	0.49	0.056	0.528
Indispensable amino acids								
Arg	96.2	96.1	95.8	95.9	95.1	0.33	0.023	0.514
His	93.9	93.7	93.5	94.0	93.1	0.44	0.394	0.638
Ile	93.6	93.2	93.1	93.9	92.8	0.52	0.553	0.808
Leu	94.5	94.4	94.2	94.9	93.5	0.43	0.305	0.289
Lys	93.6	92.9	93.0	93.9	93.3	0.49	0.851	0.624
Met	94.8	95.4	95.8	96.6	95.1	0.49	0.280	0.042
Phe	94.5	94.4	94.0	94.5	93.4	0.45	0.187	0.474
Thr	91.7	91.0	90.8	91.9	90.6	0.68	0.571	0.975
Val	92.8	91.3	91.0	92.2	90.8	0.67	0.152	0.506
Dispensable amino acids								
Ala	94.0	93.7	93.4	94.1	92.6	0.50	0.130	0.483
Asp	91.8	91.3	91.0	91.9	90.9	0.46	0.402	0.921
Cys	91.2	90.8	90.4	91.1	87.2	0.80	0.006	0.061
Glu	95.0	94.7	94.4	94.9	93.9	0.36	0.077	0.653
Gly	91.4	90.7	90.4	91.2	89.9	0.59	0.214	0.990
Pro	94.2	93.8	93.6	93.9	92.7	0.45	0.042	0.508
Ser	93.3	92.9	92.5	93.3	92.2	0.53	0.278	0.901
Tyr	93.8	94.1	94.2	95.4	93.9	0.61	0.396	0.303

[1]Data are presented as least squares means of eight observations per treatment.
[2]SEM = standard error of the mean.

the AID of AA in the mixed diet for pigs (Stein et al., 2005,2007) and broilers (Kong and Adeola, 2013; An et al., 2020).

Furthermore, several studies (Lemme et al., 2004; Stein et al., 2007; Kong and Adeola, 2013) have reported that the SID of AA is more additive in individual feed ingredients or mixed diets than the AID of AA because the SID of AA is calculated by correcting the BEL of AA from the total ileal output. According to the diets used in previous studies (D'Mello, 2003; Lemme et al., 2004; Stein et al., 2007), the SID of AA is not affected by dietary CP and AA concentrations in mixed diets. Consistent with this result, the present study confirmed that the SID of AA was not affected by the increase in dietary CP concentrations from 6.0% to 20.0%. Therefore, consistent estimation of the SID of CP and AA in response to dietary CP concentrations is a more appropriate method for formulating a diet for broilers than the estimation of the AID of AA.

DISCLOSURES

The authors have no conflicts of interest to report.

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