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Original Research Article

Comparison of methods for estimating basal endogenous losses of amino acids and additivity of digestibility of amino acids in corn and soybean meal for broilers

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ABSTRACT

This study was to compare the estimates of basal endogenous losses (BEL) of amino acids (AA) determined by 3 methods including feeding a nitrogen-free diet (NFD) or a low-casein diet (LCD, containing casein at 30 g/kg diet) or using the regression method. Another objective was to investigate whether the ileal AA digestibility of corn calculated from a casein-supplemented corn diet is additive for a corn-soybean meal (SBM) mixed diet in broilers. On d 31 of age, 168 Ross 308 male broilers were assigned to 8 dietary treatments with 6 replicates in a randomized complete block design. An NFD and 3 diets containing 30, 60, or 90 g/kg of casein were formulated to determine the BEL of AA and ileal AA digestibility of casein. The other 4 diets consisted of a corn diet, SBM diet, casein-supplemented corn diet, and corn-SBM mixed diet. On d 35 of age, digesta from the distal section of the ileum were collected. The BEL of AA in birds fed the LCD were greater ($P < 0.05$) than those of the NFD and the regression method. There were no differences in the BEL of AA determined between the NFD and the regression method. Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of AA for corn calculated from the casein-supplemented corn diet were greater ($P < 0.05$) than those of the corn diet. The predicted AID of Thr in the corn-SBM mixed diet based on the AID of AA for corn in the corn diet was lower ($P < 0.05$) than the measured AID. However, the predicted AID of AA in the mixed diet based on the AID of AA for corn in the casein-supplemented corn diet did not differ from the measured AID. The predicted SID of AA in the mixed diet did not differ from the measured SID irrespective of casein supplementation. In conclusion, feeding an NFD or using the regression method yields similar BEL of AA, but not feeding an LCD. Casein supplementation in the corn diet increases the ileal AA digestibility for corn, which is additive for the corn-SBM mixed diet.

1. Introduction

The ileal digestibility of amino acids (AA) has been used to express the availability of AA in feed ingredients for poultry. The standardized ileal digestibility (SID) of AA in feed ingredients is calculated by correcting the apparent ileal digestibility (AID) of AA for basal endogenous losses (BEL) of AA. To adequately evaluate feed ingredients, accurate estimation of the BEL of AA is required to calculate the SID of AA.

Several methods, such as feeding a nitrogen-free diet (NFD; Kong and Adeola, 2013b) or a highly digestible protein (Adedokun et al., 2007b), or using the regression method (Golian et al.,

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2008), have been used to estimate the BEL of AA. Feeding an NFD to pigs and birds is the preferred method for estimating the BEL of AA, because of its simplicity (Kong and Adeola, 2014). However, a limitation of this method is that AA deficiency results in an abnormal physiological condition in birds, which may alter the estimates of the BEL of AA (Adeola et al., 2016). As an alternative method, low inclusion levels of highly digestible proteins such as casein have been added to NFD to alleviate AA deficiency in pigs and birds (Golian et al., 2008; Park and Adeola, 2020). This method assumes that the AA content in casein is 100% digestible (Adeola et al., 2016) and the regression method involving multiple levels of casein has been applied to estimate the BEL of AA by extrapolating to zero AA intake (Adedokun et al., 2007b; Golian et al., 2008). However, comparative studies on the BEL of AA in broilers determined using these 3 methods are limited.

Additivity of AA digestibility is the fundamental assumption for feed formulation that the amount of digestible AA in a mixed diet is equal to the sum of digestible AA derived from each feed ingredient (Ravindran, 2007). The SID, which is independent of dietary AA intake, is additive in the mixed diet and is important for feed formulation on a digestible AA basis (Stein et al., 2007). However, a previous study showed that the AID may not provide a predictable value for the mixed diet (Cowieson et al., 2019).

The ileal AA digestibility of feed ingredients has been estimated using the direct method and an experimental diet containing the test ingredient as the sole source of protein (Zhang and Adeola, 2017). Low dietary AA intake can occur in experimental diets containing low-crude protein (CP) ingredients. The AID of low-CP ingredients can be underestimated because the low dietary AA intake increases the relative contribution of the BEL of AA to total AA outflow (Lemme et al., 2004). Underestimation of the AID for low-CP ingredients leads to the lack of additivity for AID. Moreover, the low AA content in diets containing low-CP ingredients can lead to AA deficiency and imbalance in birds. Therefore, there is a concern that the impaired physiological condition of birds may affect the ileal AA digestibility of low-CP ingredients.

Casein is a high-quality protein that contains high concentrations of CP and AA and has the greatest AA digestibility among protein sources (Cervantes-Pahm and Stein, 2010). Casein supplementation in the diet containing feed ingredients can improve dietary AA intake and composition, increasing the ileal AA digestibility for feed ingredients. In particular, diets containing low-intake or low-quality protein ingredients may benefit from casein supplementation. Park et al. (2018) reported that casein supplementation in a low-quality protein diet improved dietary AA composition, thus increasing the SID of AA for low-quality protein ingredients.

The hypotheses were that the BEL of AA determined by 3 different methods are similar and casein supplementation in a corn diet increases the ileal AA digestibility of corn. Therefore, the objectives of the present study were to compare the BEL of AA between the 3 methods and to investigate whether the ileal AA digestibility for corn calculated from a casein-supplemented corn diet provides predictable values for a corn-soybean meal (SBM) mixed diet.

2. Materials and methods

2.1. Animal ethics statement

Experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Kyungpook National University, Republic of Korea (approval number: KNU 2021-0194) and animal experiments complied with the ARRIVE guidelines.

2.2. Ingredients and experimental diets

The analyzed concentrations of CP and AA in corn, SBM, and casein are shown in Table 1. The ingredients and chemical compositions of experimental diets are shown in Table 2. An NFD and 3 semi-purified diets containing 30, 60, or 90 g/kg of casein at the expense of cornstarch were formulated. The BEL of AA were estimated from 3 different methods including an NFD, a low-casein diet (LCD) containing 30 g/kg of highly digestible casein, and the regression method involving diets containing 3 levels of casein. The ileal AA digestibility of casein was determined using the regression method using diets containing 3 levels of casein. The other 4 diets consisted of a corn diet, a casein-supplemented corn diet, an SBM diet, and a corn-SBM mixed diet. Corn, SBM, and casein were the source of AA in the experimental diets. To investigate the effect of casein supplementation on the ileal AA digestibility of corn, digestibility values for corn in the casein-supplemented corn diet were calculated using the difference procedure using the ileal AA digestibility of casein derived from the regression method (Kong and Adeola, 2014). The SBM diet and either the corn or casein-supplemented corn diet were used to predict the ileal digestibility of AA in the corn-SBM mixed diet. The nutrient content in diets except for CP and AA met or exceeded the recommended nutritional specifications for broilers (Aviagen, 2019). Chromium oxide was included in all experimental diets at 5 g/kg as an indigestible index.

2.3. Animals, feeding, and experimental design

Day-old Ross 308 male broilers were obtained from a local hatchery (Samhwa Breeding, Hongseong, Korea) and individually tagged with identification numbers. Birds were fed with a pre-starter diet from d 1 to 7 (22% CP), a starter diet from d 7 to 21 (20% CP), and a grower diet from d 21 to 31 (18% CP), respectively. On d 31, 168 birds were assigned to 8 dietary treatments with 6 replicate cages based on body weight in a randomized complete block design, using the Experimental Animal Allotment Program of Kim and Lindemann (2007). To collect sufficient ileal digesta samples, the NFD and 3 diets containing 30, 60, and 90 g/kg of casein were given to 4 birds per cage, whereas the other 4 diets

Table 1
Analyzed concentrations of crude protein (CP) and amino acids (AA) in corn, soybean meal (SBM), and casein (as-fed basis, g/kg).

Item	Ingredient		
	Corn	SBM	Casein
CP	77	464	835
Indispensable AA			
Arg	3.4	31.6	32.9
His	2.2	12.4	25.5
Ile	2.3	19.9	46.4
Leu	8.0	33.4	84.9
Lys	2.4	28.2	70.0
Met	1.4	6.3	23.8
Phe	3.3	22.4	45.0
Thr	2.7	18.6	38.4
Val	3.4	21.5	57.8
Dispensable AA			
Ala	4.8	18.8	28.2
Asp	4.8	51.7	64.1
Cys	1.8	6.8	4.3
Glu	12.4	81.8	190.6
Gly	2.8	18.6	17.2
Pro	6.0	23.7	89.5
Ser	3.4	22.9	50.0
Tyr	2.3	13.8	50.3

Table 2
Ingredients and chemical compositions of experimental diets (as-fed basis, g/kg).

Item	Dietary treatments							
	NFD	Casein, g/kg			Corn diet	CCD	SBM diet	Corn-SBM mixed diet
		30	60	90				
Ingredients								
Corn	–	–	–	–	909.0	766.1	–	627.8
SBM	–	–	–	–	–	–	388.3	284.1
Sucrose	640.0	640.0	640.0	640.0	–	–	–	–
Casein	–	30.0	60.0	90.0	–	145.0	–	–
Cornstarch	200.7	171.2	141.7	112.3	–	–	523.4	–
Cellulose	50.0	50.0	50.0	50.0	–	–	–	–
Soybean oil	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Limestone	13.6	14.0	14.4	14.8	14.6	16.4	12.5	13.5
Dicalcium phosphate	18.2	17.3	16.4	15.4	14.9	11.0	14.3	13.1
Salt	–	–	–	–	4.5	4.5	4.5	4.5
Potassium carbonate	3.0	3.0	3.0	3.0	–	–	–	–
Magnesium oxide	2.0	2.0	2.0	2.0	–	–	–	–
Sodium bicarbonate	12.0	12.0	12.0	12.0	–	–	–	–
Potassium chloride	3.0	3.0	3.0	3.0	–	–	–	–
Vitamin premix[1]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Mineral premix[2]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Choline chloride	2.5	2.5	2.5	2.5	2.0	2.0	2.0	2.0
Chromium oxide	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Calculated nutrient and energy								
AME, kcal/kg	3,580	3,577	3,574	3,571	3,393	3,477	3,395	3,127
CP	0.0	25.0	50.1	75.1	70.0	180.0	180.0	180.0
Calcium	8.10	8.10	8.10	8.10	8.10	8.10	8.10	8.10
Non-phytate phosphorus	4.05	4.05	4.05	4.05	4.05	4.05	4.05	4.05

NFD = nitrogen-free diet; CCD = casein-supplemented corn diet; SBM = soybean meal; AME = apparent metabolizable energy; CP = crude protein.
[1] Vitamin premix supplied the following per kilogram of diet: vitamin A, 24,000 IU; vitamin D_3, 8000 IU; vitamin E, 160 mg; vitamin K_3, 8 mg; vitamin B_1, 8 mg; vitamin B_2, 20 mg; vitamin B_6, 12 mg; pantothenic acid, 40 mg; folic acid, 4 mg; niacin, 12 mg.
[2] Mineral premix supplied the following per kilogram of diet: iron, 120 mg; copper, 320 mg; zinc, 200 mg; manganese, 240 mg; cobalt, 2 mg; selenium, 0.6 mg; iodine, 2.5 mg.

were fed to 3 birds per cage. Water and feed were provided ad libitum throughout the experiment. Birds were housed in battery cages (60 cm × 50 cm × 60 cm) in an environmentally controlled room under 24 h of light. Room temperature was maintained at 33 °C on d 0 and gradually decreased by 2 °C weekly up to 5 weeks.

2.4. Sample collection and chemical analysis

At the end of the experiment (d 35), all birds were euthanized by carbon dioxide asphyxiation. The distal two-thirds of the ileum were collected from Meckel's diverticulum to 1 cm proximal to the ileo-cecal junction. Ileal digesta samples were gently rinsed with distilled water, pooled within a cage, and stored at −20 °C prior to further analyses.

The ingredients and experimental diets were ground using a mill grinder (CT 293 Cyclotec, Foss Ltd., Denmark) and dried at 135 °C for 2 h to measure the dry matter (DM) content (method 930.15; AOAC, 2016). Freeze-dried ileal digesta samples were ground using a mortar and pestle. The nitrogen content of the ingredients was analyzed using the Kjeldahl method (Kjeltec 8400, Foss Ltd., Denmark; method 984.13; AOAC, 2016) and the CP concentration was calculated by multiplying the concentration of nitrogen by 6.25. The dried samples of the ingredients, experimental diets, and ileal digesta samples were analyzed for AA contents according to method 982.30 E (a and b) (AOAC, 2016). The experimental diets and ileal digesta samples were analyzed for chromium content using the method described by Fenton and Fenton (1979).

2.5. Calculations

The analyzed concentrations of AA in experimental diets are shown in Table 3. The analyzed AA concentrations were used to calculate the ileal AA digestibility of experimental diets and the BEL of AA in the present study. The AID and SID of AA in experimental diets and the BEL of AA in broilers were calculated using following equations (Kong and Adeola, 2014):

$$\text{AID (\%)} = 100 - [(Cr_{diet}/Cr_{digesta}) \times (AA_{digesta}/AA_{diet})] \times 100,$$

$$\text{BEL (g/kg of DM intake)} = (Cr_{diet}/Cr_{digesta}) \times AA_{digesta},$$

$$\text{SID (\%)} = \text{AID} + (\text{BEL}/AA_{diet}) \times 100,$$

where Cr_{diet} and $Cr_{digesta}$ are the concentrations (g/kg) of chromium in the diet and ileal digesta, respectively; AA_{diet} and $AA_{digesta}$ are the concentrations (g/kg) of AA in the diet and ileal digesta, respectively. In the SID calculation, the AID was corrected using the BEL of AA determined by feeding an NFD.

The apparent ileal digestible AA (g/kg of DM) was calculated as the product of AID and dietary AA intake (g/kg of DM). The apparent ileal digestible AA was regressed against the dietary AA intake (g/kg of DM) to determine the ileal AA digestibility (%) in casein and the BEL of AA (g/kg of DM intake) in broilers. The ileal AA digestibility was calculated by multiplying slopes by 100. The BEL of AA determined using the regression method were estimated as the y-intercept of the equations.

The AID and SID of AA for corn in the casein-supplemented corn diet were calculated according to the difference procedure described by Kong and Adeola (2014), using the ileal AA digestibility of casein determined by the regression method as follows:

$$AID_{corn}\ (\%) = ID_{casein} + [(AID_{corn\text{-}casein} - ID_{casein})/P_{corn}],$$

where AID_{corn} is the AID of AA for corn in the casein-supplemented corn diet; ID_{casein} is the ileal AA digestibility (%) of casein determined using the regression method; $AID_{corn\text{-}casein}$ is the AID (%) of

Table 3
Analyzed concentrations of amino acids (AA) in experimental diets (as-fed basis, g/kg).

Item	Dietary treatments							
	NFD	Casein, g/kg			Corn diet	CCD	SBM diet	Corn-SBM mixed diet
		30	60	90				
Indispensable AA								
Arg	0.01	1.00	1.80	3.00	3.20	7.40	15.30	12.00
His	0.00	0.84	1.60	2.60	2.00	5.70	5.70	5.10
Ile	0.01	1.60	2.80	4.70	2.30	9.30	10.40	8.20
Leu	0.04	2.80	5.00	8.50	8.10	20.10	17.60	16.80
Lys	0.01	2.40	4.30	7.10	2.10	12.80	13.40	10.00
Met	0.02	0.88	1.50	2.40	1.40	4.80	2.90	2.70
Phe	0.02	1.50	2.70	4.40	3.20	9.50	11.10	9.20
Thr	0.01	1.30	2.20	3.60	2.30	7.30	8.20	6.80
Val	0.02	2.10	3.60	5.80	3.30	11.50	10.80	9.10
Dispensable AA								
Ala	0.02	0.97	1.70	2.80	4.80	8.20	9.70	9.40
Asp	0.03	2.10	3.90	6.20	4.30	13.00	23.90	18.30
Cys	0.04	0.25	0.34	0.46	1.40	1.70	2.80	2.60
Glu	0.04	6.50	11.50	19.10	11.90	38.60	38.70	33.00
Gly	0.02	0.60	1.00	1.70	2.70	4.80	9.20	7.70
Pro	0.01	3.10	5.70	9.40	5.70	19.70	11.30	11.10
Ser	0.02	1.50	2.60	4.30	2.80	8.70	9.90	8.20
Tyr	0.00	1.10	2.10	3.50	2.30	8.00	6.60	5.80

NFD = nitrogen-free diet; CCD = casein-supplemented corn diet; SBM = soybean meal.

AA in the casein-supplemented corn diet; P_{corn} is the proportional AA contribution of corn to the casein-supplemented corn diet. The SID_{corn} was calculated using the same equation by substituting AID with SID.

The predicted AID and SID of AA were calculated to test the additivity of ileal AA digestibility for the corn-SBM mixed diet. The predicted AID and SID were calculated as described by An et al. (2020):

$$\text{Predicted AID (\%)} = (AA_{corn} \times AID_{corn} + AA_{SBM} \times AID_{SBM}) / (AA_{corn} + AA_{SBM}),$$

where AA_{corn} and AA_{SBM} are the AA concentrations (g/kg of DM) contributed from corn and SBM in the mixed diet, respectively. AID_{corn} and AID_{SBM} are the AID (%) of AA from corn and SBM diet, respectively. The predicted SID was calculated using the same equation by substituting AID with SID.

2.6. Statistical analysis

The data for the BEL, AID, and SID of AA in the experimental diets were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model included dietary treatment and block as fixed variables. Tukey's honestly significant difference test was used for pairwise comparisons between the treatment means. Orthogonal polynomial contrasts were conducted to investigate the linear and quadratic effects of casein and to compare the treatment means. The REG procedure of SAS was used for the linear regression analysis to estimate the ileal AA digestibility (%) of casein and the BEL of AA (g/kg of DM intake). The data between the predicted and measured values were analyzed by 2-sample 2-tailed *t*-test using the TTEST procedure of SAS. The experimental unit was a cage, and the statistical difference was set at $P < 0.05$.

3. Results

3.1. Comparison of 3 methods for estimating the BEL of AA

The BEL of 9 out of 17 AA (Ile, Thr, Val, Ala, Asp, Cys, Glu, Pro, and Ser) were different among the 3 methods ($P < 0.05$; Table 4). The BEL of Ile, Glu, and Ser in birds fed NFD were lower ($P < 0.05$) than in birds fed the LCD but were not different from the BEL determined using the regression method. The BEL of Thr, Val, Ala, Asp, Cys, Glu, Pro, and Ser determined using the regression method were lower ($P < 0.05$) than those of birds fed the LCD but were not different from birds fed the NFD. The BEL of AA except for Cys did not differ between birds fed the NFD and the regression method.

Table 4
BEL (mg/kg of DM intake) of AA of 35-d-old broilers determined by feeding a NFD or LCD or by REG.[1,2]

Item	Methods			SEM	*P*-value
	NFD	LCD	REG[3]		
Indispensable AA					
Arg	108.3	122.3	80.0	12.64	0.101
His	55.7	65.8	46.4	5.59	0.094
Ile	125.2[b]	198.2[a]	136.7[ab]	16.10	0.020
Leu	170.2	197.9	135.4	17.21	0.079
Lys	121.2	155.2	97.0	15.15	0.062
Met	44.0	67.1	49.2	6.53	0.072
Phe	107.2	113.6	75.4	10.75	0.066
Thr	243.7[ab]	284.4[a]	202.6[b]	16.30	0.017
Val	238.1[ab]	297.6[a]	209.6[b]	17.36	0.014
Dispensable AA					
Ala	133.1[ab]	170.1[a]	119.1[b]	12.15	0.036
Asp	256.6[ab]	323.9[a]	224.7[b]	22.43	0.030
Cys	104.5[a]	108.5[a]	65.7[b]	9.55	0.018
Glu	339.6[b]	591.3[a]	413.1[b]	45.68	0.008
Gly	146.7	163.7	119.0	12.03	0.070
Pro	177.4[ab]	217.8[a]	158.0[b]	12.51	0.020
Ser	204.5[b]	316.4[a]	231.9[b]	21.15	0.010
Tyr	96.9	107.6	81.8	8.89	0.170

BEL = basal endogenous losses; DM = dry matter; AA = amino acids; NFD = nitrogen-free diet; LCD = low-casein diet; REG = the regression method; SEM = standard error of the mean.
[a, b]Least squares means within a row without a common superscript differ significantly ($P < 0.05$).
[1] Each least squares mean represents 6 replicates.
[2] Diet containing 30 g/kg of casein was considered the LCD.
[3] REG was conducted by regressing the dietary AA intake (g/kg of DM intake) against the apparent ileal digestible AA (g/kg of DM intake) in diets containing 30, 60, and 90 g/kg of casein (18 observations).

3.2. Ileal AA digestibility of casein

The AID of AA in diets containing 30, 60, and 90 g/kg of casein is shown in Table 5. The AID of AA in casein increased linearly ($P < 0.01$) as the dietary levels of casein increased. The SID of AA in casein was not affected by dietary levels of casein (Table 6). The SID of indispensable AA in casein ranged from 94.2% for Ile in birds fed the diet containing 60 g/kg of casein to 99.6% for Phe in birds fed the diet containing 30 g/kg of casein. The ileal AA digestibility in casein determined using the regression method ranged from 84.6% (for Cys) to 97.9% (for Met and Pro). The average SID of Cys in diets containing 30, 60, and 90 g/kg of casein was greater ($P < 0.01$) than the value obtained using the regression method (96.1% vs. 84.6%), but the SID of other AA were not different.

3.3. Ileal AA digestibility in corn, SBM, and casein-supplemented corn diets

The AID and SID of AA in the corn, casein-supplemented corn diet, and SBM are shown in Table 7. The AID of indispensable AA in corn and SBM were lowest for Thr (75.6% and 89.4%) and the highest for Leu (93.2%) and Arg (96.1%), respectively. The SID of indispensable AA in corn and SBM were lowest for Thr (84.9% and 92.1%) and the highest for Leu (95.0%) and Arg (96.8%), respectively. The AID of AA except for Ala and Cys in the casein-supplemented corn diet was greater ($P < 0.05$) than the AID in the corn diet. Birds fed a casein-supplemented corn diet had greater ($P < 0.05$) SID of AA, except for Ala, Cys, Gly, and Ser, than birds fed the corn diet.

3.4. Effect of casein supplementation in the corn diet on ileal AA digestibility for corn

The AID of Arg, Lys, Thr, Val, and Asp for corn in the casein-supplemented corn diet was greater ($P < 0.05$) than that in the corn diet (Table 8). The SID of Arg, Lys, Phe, Thr, Val, Asp, and Tyr for corn in the casein-supplemented corn diet was greater ($P < 0.05$) than that in the corn diet. The AID of AA, except for Cys, Glu, Pro, and Ser, for corn in the casein-supplemented corn diet was not different from the SID of AA for corn in the corn diet.

Table 5
Apparent ileal digestibility (%) of amino acids (AA) in diets containing 30, 60, and 90 g/kg of casein fed to broilers.[1]

Item	Casein, g/kg			SEM	P-values[2]	
	30	60	90		Linear	Quadratic
Indispensable AA						
Arg	88.0	91.1	93.1	0.91	0.003	0.625
His	92.3	94.6	95.8	0.53	0.001	0.468
Ile	87.8	89.9	92.7	1.04	0.007	0.753
Leu	93.0	94.8	96.1	0.49	0.001	0.757
Lys	93.6	94.9	96.1	0.47	0.004	0.936
Met	92.5	94.6	95.9	0.59	0.002	0.571
Phe	92.5	94.6	95.7	0.51	0.001	0.509
Thr	78.5	83.5	87.8	1.16	<0.001	0.801
Val	86.0	88.6	91.8	0.95	0.002	0.826
Dispensable AA						
Ala	82.7	86.6	90.1	1.08	0.001	0.880
Asp	84.8	88.8	91.5	0.99	0.001	0.608
Cys	57.2	67.7	70.1	2.33	0.003	0.189
Glu	91.0	92.6	94.7	0.69	0.004	0.726
Gly	73.1	79.2	85.1	1.68	0.001	0.970
Pro	93.1	95.1	96.3	0.34	<0.001	0.392
Ser	79.2	83.1	88.2	1.57	0.002	0.783
Tyr	90.4	93.5	95.2	0.70	0.001	0.446

SEM = standard error of the mean.
[1] Each least squares mean represents 6 replicates.
[2] Linear = linear effect of casein; Quadratic = quadratic effect of casein.

Table 6
Standardized ileal digestibility (SID, %) of amino acids (AA) in diets containing 30, 60, and 90 g/kg of casein and ileal AA digestibility (%) in casein determined using the regression method (REG) for broilers.[1]

Item	Casein, g/kg			REG[2]	SEM	P-values[3]		
	30	60	90			Linear	Quadratic	SID vs. REG
Indispensable AA								
Arg	98.6	96.9	96.6	95.6	1.00	0.169	0.602	0.145
His	98.8	98.0	97.9	97.5	0.57	0.298	0.558	0.315
Ile	95.5	94.2	95.3	95.4	0.89	0.888	0.292	0.726
Leu	99.0	98.1	98.0	97.6	0.50	0.184	0.474	0.195
Lys	98.6	97.7	97.7	97.4	0.49	0.237	0.405	0.273
Met	97.4	97.5	97.7	97.9	0.59	0.712	0.928	0.593
Phe	99.6	98.4	98.1	97.4	0.58	0.095	0.579	0.066
Thr	96.9	94.3	94.4	93.1	1.25	0.177	0.395	0.173
Val	97.2	95.1	95.8	95.1	0.86	0.253	0.208	0.362
Dispensable AA								
Ala	96.2	94.3	94.8	94.1	1.12	0.370	0.386	0.465
Asp	96.8	95.2	95.5	94.9	0.97	0.341	0.430	0.395
Cys	98.4	97.7	92.2	84.6	3.18	0.191	0.550	0.007
Glu	96.2	95.4	96.4	96.7	0.67	0.800	0.310	0.419
Gly	97.2	93.5	93.5	91.7	1.75	0.155	0.406	0.156
Pro	98.7	98.1	98.2	97.9	0.36	0.308	0.471	0.354
Ser	92.6	90.8	92.8	93.1	1.43	0.943	0.297	0.544
Tyr	99.0	98.0	97.9	97.5	0.69	0.278	0.563	0.301

SEM = standard error of the mean; DM = dry matter.
[1] Each least squares mean represents 6 replicates.
[2] REG was conducted by regressing the dietary AA intake (g/kg of DM intake) against the apparent ileal digestible AA (g/kg of DM intake) in diets containing 30, 60, and 90 g/kg of casein (18 observations).
[3] Linear = linear effect of casein; Quadratic = quadratic effect of casein; SID vs. REG = contrast between the SID of AA in diets containing 30, 60, and 90 g/kg of casein and the ileal AA digestibility of casein determined using the REG.

Table 7
Apparent and standardized ileal digestibility (%) of amino acids (AA) in corn, casein-supplemented corn diet (CCD), and soybean meal (SBM).[1]

Item	AID			SEM	P-value[2]	SID			SEM	P-value[2]
	Corn	CCD	SBM			Corn	CCD	SBM		
Indispensable AA										
Arg	91.7	95.4	96.1	0.53	0.001	94.7	96.7	96.8	0.60	0.045
His	90.6	95.7	94.4	0.74	0.001	93.1	96.6	95.3	0.76	0.008
Ile	85.9	93.5	93.4	0.89	<0.001	90.7	94.7	94.5	0.94	0.013
Leu	93.2	96.5	93.5	0.67	0.006	95.0	97.3	94.4	0.68	0.044
Lys	80.5	95.9	94.1	1.87	<0.001	85.6	96.8	94.9	1.82	0.001
Met	90.5	96.6	94.8	0.88	0.001	93.3	97.5	96.2	0.82	0.005
Phe	90.7	96.4	94.0	0.83	0.001	93.6	97.4	94.8	0.86	0.011
Thr	75.6	89.7	89.4	1.36	<0.001	84.9	92.6	92.1	1.53	0.005
Val	83.1	93.1	91.2	0.88	<0.001	89.5	95.0	93.2	0.96	0.002
Dispensable AA										
Ala	91.4	93.0	92.9	0.69	0.123	93.8	94.4	94.1	0.70	0.538
Asp	84.2	92.9	92.0	1.10	<0.001	89.4	94.7	92.9	1.13	0.008
Cys	84.8	86.8	89.2	1.46	0.371	91.4	92.2	92.6	1.45	0.699
Glu	91.7	95.2	95.2	0.57	0.001	94.2	96.0	96.0	0.55	0.048
Gly	82.7	88.8	91.6	1.39	0.011	87.5	91.5	93.0	1.41	0.071
Pro	90.4	96.1	93.2	0.71	<0.001	93.2	96.9	94.6	0.74	0.006
Ser	82.7	89.6	92.4	0.96	0.001	89.2	91.7	94.3	1.00	0.110
Tyr	88.9	96.3	93.5	0.90	<0.001	92.6	97.4	94.8	0.90	0.004

AID = apparent ileal digestibility; SID = standardized ileal digestibility; SEM = standard error of the mean.
[1] Each least squares mean represents 6 replicates.
[2] P-value for contrast between corn and CCD.

3.5. Additivity of ileal AA digestibility for the corn-SBM mixed diet

When the AID of AA in the corn-SBM mixed diet was calculated from the AID of AA for corn determined by the corn diet, the

Table 8
Apparent and standardized ileal digestibility (AID and SID, %) of amino acids (AA) for corn in broilers fed a corn diet (− Casein) or casein-supplemented corn diet (+ Casein).[1]

Item	AID		SID		SEM	P-values		
	− Casein	+ Casein[2]	− Casein	+ Casein[2]		AID	SID	AID (+ Casein) vs. SID (− Casein)[3]
Indispensable AA								
Arg	91.7	94.9	94.7	98.3	0.98	0.034	0.020	0.870
His	90.6	92.0	93.1	94.6	0.86	0.279	0.212	0.386
Ile	85.9	86.3	90.7	92.1	2.30	0.901	0.680	0.197
Leu	93.2	94.3	95.0	96.6	0.90	0.383	0.245	0.586
Lys	80.5	88.4	85.6	93.8	2.55	0.045	0.039	0.449
Met	90.5	92.7	93.3	96.0	1.05	0.160	0.084	0.706
Phe	90.7	94.0	93.6	97.4	1.15	0.058	0.033	0.818
Thr	75.6	82.0	84.9	91.5	1.04	0.001	<0.001	0.064
Val	83.1	87.2	89.5	94.6	1.12	0.020	0.005	0.175
Dispensable AA								
Ala	91.4	91.8	93.8	94.8	0.70	0.648	0.354	0.064
Asp	84.2	88.7	89.4	94.3	1.08	0.010	0.006	0.634
Cys	84.8	87.0	91.4	93.2	1.25	0.233	0.338	0.025
Glu	91.7	91.3	94.2	94.2	0.97	0.771	0.984	0.049
Gly	82.7	85.7	87.5	91.3	1.44	0.155	0.082	0.407
Pro	90.4	90.6	93.2	93.8	0.66	0.844	0.553	0.015
Ser	82.7	82.3	89.2	88.7	1.99	0.885	0.862	0.028
Tyr	88.9	92.6	92.6	97.1	1.42	0.084	0.042	0.999

SEM = standard error of the mean.
[1] Each least squares mean represents 6 replicates.
[2] AID and SID of AA for corn in + Casein were calculated by the difference procedure using the ileal AA digestibility in casein determined by the regression method.
[3] *P*-value for contrast between AID for corn in + Casein and the SID for corn in − Casein.

predicted AID of Thr was lower ($P = 0.025$) than the measured AID (Table 9). However, when the AID of AA in the mixed diet was calculated from the AID of AA for corn estimated by the casein-supplemented corn diet, the predicted AID of AA did not differ from the measured AID. The predicted SID of AA except for Cys in the mixed diet was not different from the measured SID when the SID of AA for corn was determined by corn diets irrespective of casein supplementation (Table 10).

4. Discussion

The analyzed concentrations of CP and AA in corn, SBM, and casein were within the range of reported values (An and Kong, 2020; An et al., 2020; Cervantes-Pahm and Stein, 2010). The analyzed AA concentrations in the experimental diets were comparable to those calculated from the analyzed AA concentrations in corn, SBM, and casein. Adedokun et al. (2011) reported that excessive watery excreta in broilers fed NFD with 219 mEq of electrolyte balance may be the result of excessive water intake. The electrolyte balance value of the NFD in the present study was 168 mEq, which was not greatly different from the suggested value (108 to 130 mEq; Adedokun et al., 2011). Consequently, no watery excreta were observed in the present study.

Previous studies have estimated the BEL of AA in birds and pigs by feeding an LCD (50 g/kg of casein in diet) or using the regression method involving diets containing graded levels of casein (Adedokun et al., 2007a, 2007b; Golian et al., 2008; Zhang et al., 2002). The ileal endogenous AA outflows in birds increase as

Table 9
Predicted and measured values for apparent ileal digestibility (AID, %) of amino acids (AA) in a corn-soybean meal mixed diet using the AID for corn calculated from the corn diet (− Casein) or casein-supplemented corn diet (+ Casein).[1]

Item	− Casein		SE	P-value	+ Casein		SE	P-value
	Predicted	Measured			Predicted	Measured		
Indispensable AA								
Arg	95.3	91.3	3.11	0.387	95.9	91.3	3.12	0.321
His	93.3	93.4	0.44	0.799	93.7	93.4	0.52	0.746
Ile	91.9	92.5	0.55	0.471	92.0	92.5	0.85	0.685
Leu	93.4	93.0	0.47	0.591	93.8	93.0	0.58	0.388
Lys	91.8	92.6	0.54	0.313	93.1	92.6	0.59	0.565
Met	93.3	93.4	0.53	0.926	94.1	93.4	0.73	0.504
Phe	93.1	93.4	0.33	0.615	94.0	93.4	0.46	0.395
Thr	85.6	87.7	0.55	0.025	87.4	87.7	0.65	0.775
Val	89.1	89.9	0.52	0.304	90.2	89.9	0.67	0.794
Dispensable AA								
Ala	92.4	92.4	0.38	0.945	92.5	92.4	0.48	0.858
Asp	90.6	90.9	0.44	0.568	91.4	90.9	0.56	0.587
Cys	87.1	86.4	0.64	0.449	88.2	86.4	0.88	0.190
Glu	94.3	94.3	0.36	0.960	94.2	94.3	0.47	0.847
Gly	89.3	89.6	0.58	0.781	90.1	89.6	0.68	0.589
Pro	92.2	92.1	0.34	0.922	92.2	92.1	0.46	0.859
Ser	89.7	90.9	0.52	0.131	89.5	90.9	0.77	0.254
Tyr	92.2	92.6	0.36	0.433	93.2	92.6	0.53	0.446

SE = standard error.
[1] Each least squares mean represents 6 replicates.

Table 10
Predicted and measured values for standardized ileal digestibility (SID, %) of amino acids (AA) in a corn-soybean meal mixed diet using the SID for corn calculated from the corn diet (− Casein) or casein-supplemented corn diet (+ Casein).[1]

Item	− Casein		SE	*P*-value	+ Casein		SE	*P*-value
	Predicted	Measured			Predicted	Measured		
Indispensable AA								
Arg	96.4	92.1	3.10	0.353	97.1	92.1	3.11	0.285
His	94.6	94.4	0.45	0.738	95.1	94.4	0.52	0.375
Ile	93.7	93.9	0.55	0.865	94.0	93.9	0.79	0.911
Leu	94.6	93.9	0.48	0.354	95.1	93.9	0.60	0.202
Lys	93.4	93.7	0.54	0.645	94.7	93.7	0.63	0.296
Met	95.2	94.8	0.45	0.598	96.1	94.8	0.67	0.190
Phe	94.5	94.4	0.36	0.840	95.5	94.4	0.48	0.151
Thr	90.2	90.9	0.59	0.416	92.0	90.9	0.63	0.244
Val	92.3	92.3	0.51	0.984	93.6	92.3	0.65	0.184
Dispensable AA								
Ala	94.0	93.7	0.40	0.529	94.4	93.7	0.50	0.350
Asp	92.3	92.2	0.44	0.843	93.2	92.2	0.57	0.233
Cys	92.0	90.0	0.62	0.043	92.9	90.0	0.90	0.048
Glu	95.5	95.2	0.38	0.592	95.5	95.2	0.49	0.684
Gly	91.6	91.3	0.58	0.675	92.6	91.3	0.68	0.208
Pro	94.1	93.5	0.37	0.323	94.3	93.5	0.46	0.271
Ser	92.8	93.1	0.55	0.754	92.7	93.1	0.76	0.724
Tyr	94.2	94.1	0.39	0.892	95.4	94.1	0.58	0.143

SE = standard error.
[1] Each least squares mean represents 6 replicates.

dietary inclusion levels of casein increase, resulting in higher BEL of AA in birds fed LCD compared to birds fed NFD (Adedokun et al., 2007b; Golian et al., 2008). Previous studies have shown that the increased BEL of AA in birds fed LCD is likely due to increased secretion of endogenous protein in the intestine induced by dietary protein. On the other hand, no difference was observed in the BEL of AA determined between feeding an NFD and the regression method (Golian et al., 2008). However, Adedokun et al. (2007b) reported that the BEL of Lys, Met, and Glu determined using the regression method were lower than those obtained from feeding an NFD. This discrepancy between feeding an NFD and the regression method may be attributed to increased estimation error when extrapolating dietary AA intake to zero level in linear regression analysis. Estimates outside the regression line may not fit the linear regression model (Park et al., 2018). Adedokun et al. (2007b) concluded that the discrepancy among the 3 methods may be related to the inclusion levels of casein. Therefore, the experimental diets in the present study were formulated to contain casein at lower inclusion levels (30, 60, and 90 g/kg) to minimize extrapolation errors and overestimation of the BEL of AA estimated by feeding the LCD. The present study hypothesized that there is no difference in the BEL of AA in birds estimated using the three different methods.

In the present study, the estimated BEL of AA in birds fed an LCD were greater than those determined by feeding an NFD or using the regression method. Adedokun et al. (2011) suggested that the BEL of AA in birds fed an LCD could be elevated by increasing endogenous secretion or increasing enterocyte turnover in response to the stimulatory effect of dietary proteins. Moreover, the increased BEL of AA in birds fed an LCD might be attributed to the undigested AA content from dietary casein. Even if the ileal AA digestibility of casein was close to 100%, it does not guarantee complete digestion of AA in dietary casein. The undigested AA content, which was calculated as the product of the ileal AA indigestibility (100% − digestibility) of casein estimated using the regression method and dietary AA intake, was the greatest in Glu, followed by Asp, Ser, Val, Thr, and Ile. These AA had greater BEL of AA in birds fed LCD compared to the BEL of AA determined by feeding NFD or using the regression method. This finding may support the overestimation of the BEL of AA in birds fed LCD. Park and Adeola (2020) noted that the increased BEL of Glu in pigs fed LCD may be likely due to the relatively higher Glu concentrations in casein, which is consistent with the finding of the present study.

In the present study, the BEL of AA in birds fed the NFD were lower than those reported in previous studies (Adedokun et al., 2007b; An and Kong, 2020; Barua et al., 2021; Ravindran et al., 2004; Soleimani et al., 2010). Differences in the BEL of AA in birds fed the NFD might be attributed to the dietary composition and electrolyte balance of the NFD as well as the age of the birds (An and Kong, 2022). A quadratic decrease in the BEL of AA with increasing bird age has been reported, where the BEL of AA decreased up to d 14, then plateaued until d 35, and further decreased at d 42 (Barua et al., 2021). In addition, several factors, including different cornstarch-to-dextrose ratios and electrolyte balance values of the NFD, may influence the estimate of the BEL of AA (Adedokun et al., 2011; Kong and Adeola, 2013b). Glutamic acid was the most abundant AA in the BEL, followed by Asp and Thr, whereas Met and His showed the lowest quantities in the present study, which is consistent with the results reported by Golian et al. (2008). High concentrations of Glu, Asp, and Thr are found in intestinal and pancreatic secretions and mucoproteins, which are major components of endogenous proteins (Ravindran, 2021).

The ileal AA digestibility values of casein in the present study were consistent with those reported previously. The AID of AA in casein increased linearly, but not quadratically, as dietary levels of casein increased. A similar tendency was observed by Park et al. (2018), there was a linear increase in the AID of AA in casein in pigs fed 60, 100, and 140 g/kg of casein in the diet. Adedokun et al. (2011) revealed that the SID of AA in casein for birds did not increase linearly with an increase in dietary casein from 50 to 150 g/kg. In the present study, the average SID of AA in casein was approximately 97% for broilers, which is greater than the average SID value (94%) reported by Adedokun et al. (2011). This result might be attributed to the differences in the age of the birds, inclusion levels of casein in diets, and the source of casein used. The ileal AA digestibility of casein derived from the regression method was not different from the SID of AA in casein except for Cys. The levels of Cys in diets containing 30, 60, and 90 g/kg of casein were markedly lower (0.25, 0.34, and 0.46 g/kg in the diet, respectively) than the other AA because of the low Cys concentration in casein. Therefore, the ileal digestibility of Cys in casein determined using the regression method might be underestimated due to the decreased AID of Cys in diets containing low Cys concentrations.

The AID and SID of AA for corn and SBM in birds were slightly greater than the reported range in previous studies (An et al., 2020; Cowieson et al., 2019; Kong and Adeola, 2013a). The lowest AID of Thr in corn and SBM was consistent with the reported values in previous studies, which could be attributed to the high Thr concentration in BEL. The AID and SID of most AA were greater in the casein-supplemented corn diet compared to the corn diet. Because casein had greater ileal AA digestibility than corn, the digestible AA fraction derived from casein increased the AID and SID values in the casein-supplemented corn diet.

Casein supplementation in the corn diet increased the AID and SID of AA for corn. The AID of AA for corn was underestimated by the low dietary AA intake, which made the BEL of AA more important (Kong and Adeola, 2013a). Casein supplementation in the corn diet increased dietary AA intake, alleviating the underestimation of AID for corn. The SID of AA for corn tended to increase similarly to the AID of AA because the SID was calculated from the AID. The increased SID could also be explained by the fact that the casein supplementation in the corn diet could improve the dietary protein quality and high-quality protein sources could induce the activity and expression of peptide or AA transporters in the small intestine. Gilbert et al. (2008) reported that the abundance of peptide transporter (PepT1) and Na^{+}-independent cationic and zwitterionic AA transporter ($b^{0,+}$AT) mRNA expression in broilers was associated with a high-quality SBM diet compared with a low-quality corn gluten meal diet. Moreover, Osmanyan et al. (2018) reported that jejunal PepT1 and $b^{0,+}$AT mRNA expression increased with an increase in digestible AA levels from 100% to 114% in broilers, which was also supported by a previous study on broilers (Chen et al., 2005). Lysine, His, and Cys or di- and tripeptide are considered substrates for $b^{0,+}$AT or PepT1, respectively (Bröer, 2008). Meanwhile, the results of the present study showed that the AID of indispensable AA for corn in the casein-supplemented corn diet was not different from the SID for corn in the corn diet. Casein supplementation in the corn diet might be an alternative approach to estimate the SID of indispensable AA for corn instead of using NFD to standardize the AID. However, applying this approach requires further studies to obtain consistent ileal AA digestibility for casein.

Additivity of AA digestibility could be verified by identifying significant differences between predicted and measured AA digestibility values in the mixed diet. A significant difference would indicate that the AA digestibility of single ingredient is not additive to the mixed diet and therefore could not be used to predict the AA digestibility for the mixed diet. In the present study, the AID of Thr was not additive in the corn-SBM mixed diet when the AID for corn was calculated from the corn diet. The lack of additivity for AID of Thr was attributed to the underestimated AID of Thr in the corn diet due to low dietary Thr intake. This result was in agreement with previous studies in which significant differences were observed between the predicted and measured AID of AA in 24-, 26-, and 28-d-old broilers fed a corn-SBM mixed diet (An et al., 2020; Cowieson et al., 2019; Kong and Adeola, 2013a). In turn, when the AID of AA for corn was calculated from the casein-supplemented corn diet, the AID of AA for corn was additive for the mixed diet. It was likely because casein supplementation in the corn diet mitigates the underestimation of AID for corn by increasing dietary AA intake. As expected, the SID of AA for corn except for Cys was additive in the corn-SBM mixed diet, regardless of casein supplementation in the corn diet used to calculate the SID of AA for corn. An et al. (2020) calculated the relative proportion of BEL of AA to total ileal AA outflow (RBEL) to investigate the effect of BEL on the SID of AA in feed ingredients. Cysteine in corn and SBM diets had the third and first highest RBEL values, respectively (An et al., 2020; Kong and Adeola, 2013a). Similar to previous studies, the RBEL of Cys in the present study was second highest (7.2%) in corn and the highest (3.6%) in SBM. Thus, the higher predicted SID of Cys in the mixed diet might be likely due to the high RBEL of Cys in corn and SBM, which is consistent with the result of Kong and Adeola (2013a).

5. Conclusions

The BEL of AA determined by feeding an LCD were greater than those determined by feeding an NFD or using the regression method. However, feeding an NFD or using the regression method yields similar BEL values for AA. Casein supplementation in the corn diet improved dietary AA intake and composition, and these digestibility values for corn provided predictable values for the corn-SBM mixed diet in broilers.

Author contributions

June Hyeok Yoon: Conceptualization, Data curation, Formal analysis, Methodology, Investigation, Writing-Original draft preparation; **Changsu Kong:** Conceptualization, Methodology, Project administration, Writing-Reviewing and Editing, Supervision, Funding acquisition.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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