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Research note: Comparison of apparent and standardized ileal digestibility of amino acids in corn-soybean meal-based diets for laying hens at different ages

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ABSTRACT

This study aimed to assess and compare the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of amino acids (AA) in laying hens at different ages. A total of 180 28-wk-old Hy-Line Brown laying hens were randomly assigned to 3 age groups of 30, 50, and 70 wks. At each designated age, 60 hens were randomly allotted to 2 treatment diets: nitrogen-free diet (NFD) for estimating basal endogenous losses (BEL) of AA and corn-soybean meal-based diet (CSD). It was observed that the BEL of most AA increased with age, with significant increases ($P < 0.05$) for Arg, Lys, Thr, Asp, and Ser by an average of 118.3 % for these 5 AA in 70-wk-old hens than in 30-wk-old hens. The AID and SID of most AA in CSD decreased as hens were aged, with 70-wk-old hens exhibiting significant reductions ($P < 0.05$) in the AID of Arg and Lys by an average of 10.4 %, and in the SID of these 2 AA by 7.6 % compared with 30-wk-old hens. The AID and SID of Met were also lower ($P < 0.05$) in 70-wk-old hens by an average of 12.5 % and 10.7 %, respectively, than in both 30-wk-old and 50-wk-old hens. In conclusion, the BEL of AA increased, whereas AA digestibility decreased with advancing age in laying hens. Specifically, 70-wk-old hens showed decreased digestibility of limiting AA (Arg, Lys, and Met) compared with younger hens. Therefore, age-dependent AA digestibility in laying hens should be considered to improve the precision of feed formulation.

Introduction

Accurate estimation of amino acid (**AA**) availability in poultry diets is essential to improve the precision of feed formulation, ensuring that AA requirements are satisfied while minimizing nitrogen excretion. The AA availability in poultry diets is commonly assessed using apparent ileal digestibility (**AID**) or standardized ileal digestibility (**SID;** Kong and Adeola, 2014). It is well-documented that AA digestibility in poultry is influenced by various factors, including methodology used for digestibility determination, feed compositions, and animal characteristics such as sex, genotype, and health status (Bryden and Li, 2010; Blok et al., 2017; Parsons, 2020). One potential animal factor affecting AA digestibility is the age because the ability of poultry to digest and absorb dietary AA varies with age (Bryden and Li, 2010). It has been recognized that AA digestibility generally increases with advancing age in broiler chickens, likely due to progressive improvements in digestive physiology (Bryden and Li, 2010). However, despite the potential effect of age on AA digestibility in laying hens, relevant information is largely limited. A previous study using cecectomized hens reported that increasing age of hens improved apparent total tract digestibility of AA (Rezvani et al., 2007). In contrast, Gu et al. (2021) observed decreased apparent total tract retention of DM and CP, along with increased AA excretion as laying hens were aged. This contradictory finding may result from variations in methodology, feed compositions, and animals between these studies (Parsons, 2020). However, these studies examined the effect of age on apparent AA digestibility without accounting for endogenous AA losses, potentially complicating AA digestibility in laying hens at different ages. The SID of AA is considered a more accurate estimate of AA digestibility than AID of AA in poultry (Kong and Adeola, 2014; Parsons, 2020). However, to our knowledge, no studies have investigated the effect of age on the SID of AA in laying hens. Therefore, this study aimed to assess and compare the AID and SID of AA in laying hens across 3 different ages.

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Materials and methods

Experimental design

All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Chung-Ang University (Approval No. 202401030073). A total of 180 28-wk-old Hy-Line Brown laying hens (average BW = 1.93 ± 0.07 kg) originated from the same flock were randomly assigned to 3 age groups at 30, 50, and 70 wks of age. All hens were individually housed in a cage (24 × 36 × 39 cm) in an environmentally-controlled room and fed a commercial layer diet until the designated age for determination of AA digestibility. At each age, 60 hens of each age group were randomly allotted to 2 treatment diets: nitrogen-free diet (**NFD**) and corn–soybean meal-based diet (**CSD**). The NFD treatment had 3 replicates with 8 hens per replicate, whereas the CSD treatment had 6 replicates with 6 hens per replicate. The NFD was prepared to estimate the basal endogenous losses (**BEL**) of AA (An and Kong, 2022; Table 1). The CSD was formulated as a typical layer diet primarily with corn and soybean meal (**SBM**) according to the nutrient requirement estimates for laying hens (Hy-Line International, 2024). The identical formulation for CSD was maintained across all age groups to eliminate possible factors other than age. However, the prolonged duration of this study (i.e., 40 wks) precluded the use of a single batch of corn and SBM for all age groups. Accordingly, to minimize nutritional variability, corn and SBM from the same country of origin (i.e., imported corn from USA and domestic SBM from South Korea) with comparable particle size and nutritional compositions were used in all age groups. The analyzed concentrations of CP and AA in corn, SBM, NFD, and CSD for each age are presented in Table 1. Chromium oxide was used as an indigestible marker at 0.50 % in all diets. Environmental conditions, including ambient temperature and photoperiod, were controlled according to the management guideline of Hy-Line Brown International (2024).

Ileal digesta collection and chemical analysis

At each age, hens in CSD treatments were fed CSD for 10 d, whereas those in NFD treatment were fed NFD for 5 d before ileal digesta collection (Kong and Adeola, 2014; An and Kong, 2022). Hens were given free access to diets and water. Sample collection and digesta processing were performed as described previously (An and Kong, 2022).

The treatment diets and ileal digesta were analyzed for DM (method 930.15; AOAC, 2005) and CP (method 984.13; AOAC, 2005). The concentrations of AA in diets and digesta were quantified using an L-8900 AA analyzer (Hitachi, Tokyo, Japan; method 982.30; AOAC, 2005).

Table 1
Ingredients and chemical composition of experimental diets for laying hens at different ages[1].

Ingredients, %		30 wk of age			50 wk of age			70 wk of age		
	NFD	Corn	SBM	CSD	Corn	SBM	CSD	Corn	SBM	CSD
Corn grains				62.00			62.00			62.00
Soybean meal (45.0 % CP)				25.00			25.00			25.00
Cornstarch	35.00									
Sucrose	50.00									
Soybean oil	2.00			2.00			2.00			2.00
Limestone	2.00			8.30			8.30			8.30
Monocalcium phosphate	2.20			1.30			1.30			1.30
Cellulose	5.00									
$NaHCO_3$	0.70			0.30			0.30			0.30
NaCl	0.50			0.20			0.20			0.20
Chromic oxide	0.50			0.50			0.50			0.50
Choline (50 %)	0.25			0.10			0.10			0.10
MgO	0.25									
K_2CO_3	0.50									
KCl	0.50									
Vitamin premix[2]	0.30			0.15			0.15			0.15
Mineral premix[3]	0.30			0.15			0.15			0.15
Total	100.00			100.00			100.00			100.00
Calculated AME_n, kcal/kg	3,394			2,807			2,807			2,807
Analyzed composition, %										
CP	0.73	8.87	46.06	16.81	8.25	45.06	16.50	8.51	45.37	16.10
Arg		0.47	3.22	0.99	0.35	3.25	1.01	0.41	3.20	0.98
His		0.25	1.19	0.41	0.22	1.16	0.44	0.24	1.20	0.42
Ile		0.25	1.88	0.58	0.20	1.89	0.58	0.23	1.93	0.61
Leu		0.94	3.53	1.40	0.84	3.47	1.42	0.88	3.51	1.41
Lys		0.39	2.94	0.83	0.24	2.83	0.85	0.29	2.82	0.84
Met		0.17	0.54	0.23	0.15	0.57	0.24	0.14	0.53	0.22
Phe		0.41	2.38	0.79	0.33	2.34	0.81	0.36	2.41	0.81
Thr		0.42	1.87	0.66	0.28	1.84	0.64	0.31	1.84	0.63
Trp		0.08	0.90	0.27	0.07	0.91	0.25	0.07	0.94	0.25
Val		0.39	2.12	0.66	0.33	1.98	0.67	0.34	2.06	0.68
Ala		0.65	2.05	0.85	0.53	2.05	0.85	0.56	2.04	0.82
Asp		0.64	5.29	1.59	0.50	5.20	1.64	0.57	5.22	1.60
Cys		0.21	0.66	0.26	0.21	0.67	0.27	0.17	0.64	0.26
Glu		1.56	8.47	2.96	1.37	8.40	2.96	1.46	8.47	2.91
Gly		0.45	1.97	0.69	0.30	1.95	0.68	0.33	1.96	0.66
Pro		0.76	2.29	0.98	0.68	2.29	1.01	0.64	2.27	0.95
Ser		0.45	2.48	0.83	0.36	2.46	0.85	0.40	2.43	0.82
Tyr		0.26	1.39	0.50	0.20	1.34	0.49	0.23	1.31	0.47

[1] NFD, nitrogen-free diet; CSD, corn-soybean meal-based diet.
[2] Provided per kg of the complete diet: vitamin A, 12,000 IU; vitamin D_3, 4,000 IU; vitamin E, 80.0 mg; vitamin K_3, 4.0 mg; vitamin B_1, 4.0 mg; vitamin B_2, 10.0 mg; vitamin B_6, 6.0 mg; vitamin B_{12}, 20.0 μg; folic acid, 2.0 mg; biotin, 200 μg; niacin, 60 mg.
[3] Provided per kg of the complete diet: iron, 60 mg ($FeSO_4$); zinc, 100 mg ($ZnSO_4$); manganese, 120 mg (MnO); copper, 16 mg ($CuSO_4$); cobalt, 1,000 μg ($CoSO_4$); selenium, 300 μg (Na_2SeO_3); iodine, 1.25 mg [$Ca(IO_3)_2$].

Briefly, samples were hydrolyzed with 6 N HCl at 110 °C for 24 h, concentrated, buffered, and filtered before ion-exchange chromatography with post-column ninhydrin detection. Sulfur-containing AA were measured after performic-acid oxidation, whereas Trp was analyzed from separate alkaline hydrolysates. The concentrations of Cr were measured after wet-acid digestion by inductively coupled plasma–optical emission spectrometry (ICP-OES; Optima 5300 DV, PerkinElmer, Shelton, CT; method 985.01; AOAC, 2005). All analyses were conducted in triplicate.

Calculations and statistical analysis

The values for BEL, AID, and SID of AA were determined on a DM basis, following calculations of ileal AA digestibility based on the index method (Kong and Adeola, 2014; An and Kong, 2022).

All data were analyzed by ANOVA using the MIXED procedure of SAS (SAS Institute, Cary, NC). Each replicate was considered the experimental unit. The LSMEANS procedure was used to calculate treatment means and Tukey's honestly significant difference test was used to separate treatment means. Significance for statistical tests was set at $P < 0.05$.

Results and discussion

The BEL of most AA in laying hens generally increased with age from 30 to 70 wks, although significance was observed only for Arg, Lys, Thr, Asp, and Ser (Table 2). The BEL of Arg, Thr, and Ser was greater ($P < 0.05$) in 70-wk-old hens than in 30-wk-old hens by 74.1 %, 72.1 %, and 113.2 %, respectively, with 50-wk-old hens showing intermedicate values. However, the BEL of Lys and Asp in hens at both 50 and 70 wks of age was greater ($P < 0.05$) than in hens at 30 wk of age. Compared with 30-wk-old hens, 50-wk-old hens exhibited increases in the BEL of Lys and Asp by 149.1 % and 110.3 %, respectively, whereas 70-wk-old hens showed even greater increases of 177.5 % and 154.5 %.

The predominant AA in the BEL of laying hens were Glu, Asp, Leu, Thr, and Ser, despite the slight age-related variation in this study, which is consistent with the previous findings (Blok et al., 2017). The observation of increased BEL of most AA with age may result from age-related

Table 2
Basal endogenous losses (BEL) of amino acids (AA) and digestibility of AA in corn-soybean meal-based diets for laying hens at 30, 50, and 70 wks of age[1].

Items	BEL of AA, mg/kg DM intake				
	30 wk	50 wk	70 wk	SEM	*P*-value
Essential AA					
Arg	220[b]	358[ab]	383[a]	35.1	0.034
His	148	174	206	13.9	0.069
Ile	258	312	378	30.6	0.084
Leu	429	506	597	46.6	0.110
Lys	169[b]	421[a]	469[a]	47.9	0.009
Met	76	83	109	7.7	0.052
Phe	247	290	354	33.2	0.151
Thr	355[b]	526[ab]	611[a]	40.8	0.012
Trp	88	73	93	11.4	0.479
Val	343	367	451	39.7	0.209
Non-essential AA					
Ala	351	373	428	42.1	0.467
Asp	312[b]	656[a]	794[a]	70.5	0.007
Cys	251	250	273	21.4	0.706
Glu	607	872	1,024	94.4	0.053
Gly	312	367	435	41.2	0.190
Pro	294	364	423	44.9	0.205
Ser	287[b]	481[ab]	612[a]	52.5	0.013
Tyr	169	243	198	30.6	0.299

Digestibility[2], %

Items	AID					SID				
	30 wk	50 wk	70 wk	SEM	*P*-value	30 wk	50 wk	70 wk	SEM	*P*-value
Nitrogen	85.1[a]	84.7[a]	78.0[b]	1.22	0.001	89.1[a]	88.2[ab]	84.0[b]	1.22	0.023
Essential AA										
Arg	90.1[a]	87.2[ab]	82.8[b]	1.40	0.008	92.2[a]	90.5[ab]	86.3[b]	1.40	0.027
His	85.4	83.5	80.7	1.78	0.201	88.7	87.3	85.2	1.77	0.380
Ile	82.7	79.5	76.8	2.26	0.205	86.8	84.5	82.3	2.27	0.399
Leu	86.1	84.1	82.2	1.77	0.314	89.0	87.3	86.0	1.77	0.496
Lys	86.2[a]	82.6[ab]	75.3[b]	2.03	0.006	88.1[a]	87.2[ab]	80.3[b]	2.02	0.030
Met	87.3[a]	87.6[a]	76.5[b]	1.49	<0.001	90.4[a]	90.8[a]	80.9[b]	1.48	<0.001
Phe	86.6	84.0	81.6	1.85	0.195	89.5	87.3	85.5	1.84	0.333
Thr	79.1	75.3	72.1	2.52	0.193	84.2	82.9	80.9	2.52	0.662
Trp	84.2[a]	83.4[ab]	79.9[b]	1.09	0.031	87.3	86.1	83.3	1.09	0.054
Val	81.6	78.6	75.1	2.36	0.184	86.5	83.6	81.1	2.36	0.296
Non-essential AA										
Ala	85.0	83.0	79.5	1.87	0.138	88.9	87.0	84.2	1.86	0.228
Asp	84.7	82.0	79.7	1.93	0.220	86.6	85.7	84.2	1.93	0.688
Cys	67.9	71.2	73.1	1.92	0.236	76.9	79.8	82.5	2.10	0.197
Glu	89.3	86.8	85.2	1.45	0.162	91.3	89.5	88.4	1.45	0.390
Gly	80.5	76.7	74.1	2.57	0.239	84.7	81.7	80.0	2.56	0.439
Pro	84.8	82.8	83.3	1.78	0.718	87.6	86.1	87.3	1.77	0.827
Ser	83.8	80.4	79.0	2.17	0.317	87.0	85.7	85.8	2.17	0.886
Tyr	84.5	82.2	82.4	1.89	0.637	87.7	86.8	86.2	1.88	0.856

[a,b]Means with different superscripts in a row differ significantly ($P < 0.05$).
[1] Basal endogenous losses are the means of 3 replications, whereas digestibility values are the means of 6 replications.
[2] AID, apparent ileal digestibility; SID, standardized ileal digestibility.

alterations in intestinal structure and function of laying hens. Aged hens have been reported to show decreased efficiency of nutrient digestion and absorption, leading to a compensatory increase in intestinal morphology and enzyme secretion (Gu et al., 2021). These compensatory changes may enhance intestinal cell losses and digestive enzyme excretion, thereby elevating the BEL of AA. In particular, age-dependent increases in the BEL of Arg, Lys, Thr, Asp, and Ser were significant. This effect on Thr, Asp, and Ser may be caused by their high abundance in mucins and related secretions, with advancing age leading to enhanced mucosal turnover (Gu et al., 2021). However, the reason for significant increases in the BEL of Arg and Lys is not clear; however, it may be related to decreased expression of intestinal cationic AA transporters with aging (Gilbert et al., 2007), which may reduce reabsorption of endogenous Arg and Lys losses.

The AID and SID of most AA in CSD decreased with age, with 4 AAs (Arg, Lys, Met, and Trp) being significantly affected. In 70-wk-old hens compared with 30-wk-old hens, the AID of Arg and Lys decreased ($P < 0.05$) by 8.1 % and 12.6 %, respectively, while the SID of Arg and Lys also declined ($P < 0.05$) by 6.4 % and 8.9 %. However, the AID and SID of Arg and Lys in 50-wk-old hens did not differ from other age groups. The AID and SID of Met were lower ($P < 0.05$) in 70-wk-old hens than in hens at both 30 and 50 wks of age, with average reductions of 12.5 % and 10.7 %, respectively. The AID of Trp in CSD in 70-wk-old hens was lower ($P < 0.05$) by 5.1 % than in 30-wk-old hens despite no differences compared with 50-wk-old hens; however, the SID of Trp did not differ among hens at different ages.

A previous study reported increased AA digestibility with advancing age in cecectomized hens from 27 to 57 wks, possibly due to increasing development of intestinal structure and function (Rezvani et al., 2007). In contrast, Gu et al. (2021) reported that increasing age of hens from 27 to 75 wks decreased apparent DM and CP retention with elevated AA losses in excreta as a consequence of decreased efficiency of nutrient digestion and absorption. This result is consistent with the current findings of decreased AID of N and most AA with age. In this study, the BEL of most AA increased with age, which may partly explain decreased AID of AA with age because an increase in the BEL of AA typically contributes to decreased AID of AA (Kong and Adeola, 2014). However, after correcting AID for the BEL of AA measured at each age, the SID of most AA also decreased with age, indicating that AA digestion and absorption actually decreased with advancing age of hens. This observation is likely attributable to the reduction in digestive enzyme secretion and its activity with increasing age of hens (Gu et al., 2021). Moreover, decreased expressions of intestinal AA transporters with age (Gilbert et al., 2007) may also be possible reason for these findings. Nevertheless, the information regarding age-dependent changes in AA absorptive capacity is lacking, especially in laying hens, which limits further interpretation. In this study, 70-wk-old hens showed a significant decline in the SID of Arg, Lys, and Met, typically considered limiting AAs for chickens, compared with 30-wk-old hens. The reason for significant reductions in only these 3 AAs remains unclear; however, it may indicate that application of a single SID value for these AAs in layer diet formulations, regardless of age, may lead to an under- or over-estimation of most essential AA concentrations in diets because essential AAs are formulated based on ideal protein concepts.

In conclusion, the BEL of most AA in laying hens increased with age, whereas the AID and SID of most AA in CSD decreased. In particular, compared with 30-wk-old hens, 70-wk-old hens exhibited a greater BEL of AA, averaging a 57.8 % increase, while decreased AID and SID of AA in CSD by an average of 6.0 % and 4.0 %, respectively, suggesting that applications of age-dependent AA digestibility in laying hens are necessary to improve the precision of feed formulations.

CRediT authorship contribution statement

Min Sung Kang: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ryun Ha Kim:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Ju Hye Kim:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Ye Jin Jeong:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Dong Yong Kil:** Writing – review & editing, Writing – original draft, Validation, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Disclosures

The authors declare no conflict of interest for the data presented in this study.

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References

AOAC International, 2005. Official Methods of Analysis of AOAC International, 18th ed. AOAC Int, Gaithersburg, MD.

An, S.H., Kong, C., 2022. Influence of age and type of feed ingredients on apparent and standardized ileal amino acid digestibility in broiler chickens. J. Anim. Sci. Technol. 64, 740–751. https://doi.org/10.5187/jast.2022.e43.

Blok, M.C., Jansman, A.J.M., Makkink, C.A., 2017. Amount and Amino Acid Composition of Basal Endogenous Protein Losses at the Terminal Ileum of Broilers. Wageningen Livestock Research, Wageningen, the Netherlands. https://doi.org/10.18174/426332. CVB Documentation Report 60.

Bryden, W.L., Li, X., 2010. Amino acid digestibility and poultry feed formulation: expression, limitations and application. Rev. Bras. Zootec. 39, 279–287. https://doi.org/10.1590/S1516-35982010001300031.

Gilbert, E.R., Li, H., Emmerson, D.A., Webb, K.E., Wong, E.A., 2007. Developmental regulation of nutrient transporter and enzyme mRNA abundance in the small intestine of broilers. Poult. Sci. 86, 1739–1753. https://doi.org/10.1093/ps/86.8.1739.

Gu, Y.F., Chen, Y.P., Jin, R., Wang, C., Wen, C., Zhou, Y.M., 2021. A comparison of intestinal integrity, digestive function, and egg quality in laying hens with different ages. Poult. Sci. 100, 100949. https://doi.org/10.1016/j.psj.2020.12.046.

Hy-Line International. 2024. Hy-Line Brown Commercial Layers (International Standards): Performance Guide. Accessed Feb. 16, 2024. https://www.hyline.com/Hy-Line-Products/.

Kong, C., Adeola, O., 2014. Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australas. J. Anim. Sci. 27, 917–925. https://doi.org/10.5713/ajas.2014.r.02.

Parsons, C.M., 2020. Unresolved issues for amino acid digestibility in poultry nutrition. J. Appl. Poult. Res. 29, 1–10. https://doi.org/10.1016/j.japr.2019.12.007.

Rezvani, M., Kluth, H., Woitow, G., Rodehutscord, M., 2007. Studies on the effect of age and caecectomy on amino acid excretion and digestibility in laying hens. Eur. Poultry Sci. 71, 241–246. ISSN 0003-9098.