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Original Research Article

Effect of age on the standardized ileal amino acid digestibility of soybean meal and canola meal in broilers

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Standardized ileal digestibility coefficients (SIDC) of nitrogen (N) and amino acids (AA) in two protein sources (soybean meal [SBM] and canola meal [CM]) were investigated at six broiler ages (d 7, 14, 21, 28, 35, and 42). Two assay diets were formulated to contain either SBM (413 g/kg) or CM (553 g/kg) as the sole dietary AA source. Titanium dioxide (5 g/kg) was added as an indigestible marker. A total of 696 male broilers at 1 d old were allotted to 12 replicate cages per age group. Each assay diet was offered to birds for 4 d prior to the ileal digesta collection on d 7 (14 birds/cage), 14 (12 birds/cage), 21 (10 birds/cage), 28 (8 birds/cage), 35 (8 birds/cage) and 42 (6 birds/cage), respectively. The apparent digestibility coefficients were standardized using age-specific basal endogenous AA flows. In the SBM group, though the SIDC of N tended to be influenced (quadratic; $P = 0.075$) by age, no linear or quadratic response of age effect was observed on the average SIDC of indispensable (IAA) and total AA (TAA). An age effect (quadratic; $P < 0.05$) was observed on the average SIDC of dispensable AA (DAA) in SBM with the highest value recorded at d 7, followed by a decrease from d 14 to 28, which increased beyond d 35. The SIDC of some individual AA (Arg, Thr, Trp, Cys, Pro) were affected ($P < 0.05$ or $P < 0.001$) in a quadratic manner by age. In the CM, the SIDC of N, average SIDC of IAA, DAA and TAA were influenced (quadratic; $P < 0.05$ or $P < 0.001$) by age. The SIDC of N and average SIDC of DAA and TAA were higher from d 7 to 14, declined at d 21, and then increased beyond d 28. The average SIDC of IAA was low between d 7 and 28 and increased thereafter. The SIDC of individual AA were affected (linear or quadratic; $P < 0.05$ or $P < 0.001$) by different magnitudes by age. The age influence on the SIDC AA was variable, depending on the protein source and AA. The results demonstrate that age-specific SIDC AA data might need consideration in broiler feed formulations.
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1. Introduction

Soybean meal (SBM) is the dominant protein source (PS) used in poultry diets worldwide. Its popularity, compared to other oilseed meals, is due to favourable attributes such as high crude protein (CP) content, an excellent amino acid (AA) profile that complements cereals and high AA digestibility (Ravindran et al., 2014). In a typical corn-SBM broiler diet, SBM contributes up to 70% of dietary CP.

The ever-increasing demand for SBM, fluctuating supply, market volatility, and rising prices have encouraged the poultry industry to explore the use of alternative PS. Canola meal (CM) is relatively high in protein and offers an AA profile close to that of SBM. This makes it a potential PS for poultry. However, the use of CM in poultry diets is restricted because of its higher fiber content than SBM (Newkirk et al., 2000; Kim et al., 2012). The anti-nutritional factors, glucosinolates and erucic acid, have also limited the use of CM in the past,

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but their concentrations in current canola cultivars are reduced to almost zero by plant breeding. Studies have shown that CM could be included at levels up to 167 g/kg in broiler diets balanced for digestible AA without any negative effects on performance (Gopinger et al., 2014).

Formulation of poultry diets to meet the digestible AA requirements is pivotal for reductions in both the feed cost and excess nitrogen (N) release into the surrounding environment (Cowieson et al., 2019). It is now recognized that the use of digestible AA in diet formulations is superior to total AA as it closely represents the amount utilized by the birds for maintenance and production purposes (Rostagno et al., 1995; Lemme et al., 2004). The superiority of measuring AA digestibility at the ileal level is now accepted unquestionably by the poultry industry (Ravindran et al., 1999; Kadim et al., 2002). The ileal AA digestibility can be demonstrated either as apparent ileal digestibility (AID) or standardized ileal digestibility (SID). In the calculation of SID, the AID values are adjusted for the basal endogenous AA (EAA) flows originating from different digestive secretions (bile, pancreatic, enzymatic, and intestinal secretions), serum albumin, mucoproteins, and sloughed intestinal epithelial cells (Ravindran, 2021). The use of SID AA has now become commonplace because it exhibits greater additivity compared to AID in practical feed formulations (Kong and Adeola, 2013; Cowieson et al., 2019; An et al., 2020).

A number of reports (Lemme et al., 2004; Bryden et al., 2009; Blok and Dekker, 2017; Barua et al., 2020; 2021a) are available on the AID and SID coefficients (SIDC) of AA in feed ingredients. However, studies examining the broiler age effects on SIDC AA are limited (Garcia et al., 2007; Adedokun et al., 2007a; 2008; Szczurek et al., 2020) and confined to two or three specific ages. It was hypothesized that the correction of AIDC for age-specific EAA flows might impact the SIDC AA at different ages of broiler. Two previous studies (Barua et al., 2021b; 2021c) reported the broiler age effect on the SIDC AA in different grain sources (wheat, sorghum, corn and barley) from hatch to the end of the growth cycle. The present study aimed to determine SIDC AA in two commonly used PS (SBM and CM) at six broiler ages (d 7, 14, 21, 28, 35, and 42).

2. Materials and methods

2.1. Animal ethics statement

The experimental procedure for this study was conducted in accordance with the Massey University Animal Ethics Committee guidelines and adhered to the New Zealand Revised Code of Practice for the Care and Use of Live Animals for Scientific Purposes.

2.2. Diets and experimental design

Commercially sourced SBM and solvent-extracted CM were processed using a hammer mill to pass through a screen size of 3.0 mm. The SBM was of Argentinean origin and the solvent-extracted CM was of Australian origin. Two experimental diets, based on SBM and CM as the only source of AA, were developed to contain about 180 g/kg dietary protein (Table 1). An indigestible marker, titanium dioxide (5.0 g/kg; Merck KGaA, Darmstadt, Germany) was included in all experimental diets.

The assay diets were subjected to steam conditioning at a temperature of 70 °C for a duration of 30 s, and then these diets were formed into pellets using a pellet mill (Model Orbit 15; Richard Size Limited Engineers, Kingston-upon-Hull, UK). This pellet mill had the capacity to produce 180 kg of feed/h and was equipped with a die ring with 3-mm holes and 35-mm thickness. The pelleted diets were crumbled for feeding young birds during the initial two weeks of the study.

Table 1
Composition and analyzed values of the experimental diets (g/kg, as-fed basis).

Ingredients	Soybean meal	Solvent-extracted canola meal
Soybean meal	413	–
Canola meal	–	553
Corn starch	517	377
Soybean oil	30	30
Dicalcium phosphate	19	19
Limestone	10	10
Titanium dioxide[1]	5.0	5.0
Sodium chloride	2.0	2.0
Sodium bicarbonate	2.0	2.0
Trace mineral premix[2]	1.0	1.0
Vitamin premix[2]	1.0	1.0
Analyzed values		
Dry matter	913	904
Nitrogen (N)	31.7	35.5
Crude protein (N × 6.25)	198	222
Indispensable amino acids (IAA)		
Arg	14.3	12.9
His	5.23	5.51
Ile	9.35	8.79
Leu	15.3	15.1
Lys	12.3	11.5
Met	2.72	4.36
Thr	7.72	9.04
Trp	2.62	2.90
Val	9.82	11.3
Total IAA	79.3	81.3
Dispensable amino acids (DAA)		
Ala	8.80	9.59
Asp	22.6	15.4
Cys[3]	2.51	4.47
Glu	35.9	38.2
Gly[3]	8.46	11.2
Pro	9.88	13.1
Ser	9.65	8.81
Total DAA	97.7	101
Total AA[4]	177	182

The analyzed values for titanium dioxide in soybean meal and canola meal diets were 5.47 and 5.53 g/kg (dry matter basis), respectively.
[1] Merck KGaA, Darmstadt, Germany.
[2] Supplied per kilogram of diet: antioxidant (ethoxyquin), 100 mg; biotin, 0.2 mg; pantothenic acid (calcium pantothenate), 12.8 mg; vitamin D_3 (cholecalciferol), 0.06 mg; vitamin B_{12} (cyanocobalamin), 0.017 mg; folic acid, 5.2 mg; vitamin K_3 (menadione), 4 mg; niacin (nicotinic acid), 35 mg; pyridoxine (pyridoxine HCl), 10 mg; trans-retinol, 3.33 mg; vitamin B_2 (riboflavin), 12 mg; vitamin B_1 (thiamine), 3.0 mg; vitamin E (DL-α-tocopheryl acetate), 60 mg; choline (choline chloride), 638 mg; Co (cobalt sulfate), 0.3 mg; Cu (copper sulfate), 3.0 mg; Fe (iron sulfate), 25 mg; I (calcium iodate), 1 mg; Mn (manganese oxide), 125 mg; Mo (sodium molybdate), 0.5 mg; Se (sodium selenite), 0.2 mg; Zn (zinc sulfate), 60 mg.
[3] Semi-indispensable amino acids for poultry.
[4] Total AA = IAA + DAA.

The SBM and CM samples were analyzed for dry matter (DM), N, fat, CP, neutral detergent fiber (NDF), gross energy (GE), AA, calcium (Ca), phosphorus (P), and ash. The SIDC of N and AA in SBM and CM were determined at six different ages (d 7, 14, 21, 28, 35 and 42) of broilers using the direct method.

2.3. Birds, housing and performance data

A total of 696 male broilers (Ross 308) at 1 d old were procured from a local hatchery. The chicks were reared in floor pens and provided with a commercial broiler starter diet (12.14 MJ/kg apparent metabolizable energy; 225 g/kg CP) from d 1 to 21. Subsequently, they were fed a commercial broiler finisher diet (12.69 MJ/kg apparent metabolizable energy; 190 g/kg CP) from d 22 till d 38 (Table 2) in pelleted forms.

On d 1, a total of 168 chicks were weighed and distributed to 12 separate battery brooders (14 chicks per replicate) so that the average body weight per replicate group was approximately the

Table 2
Composition and calculated analysis (g/kg, as-fed basis) of broiler starter and finisher diets (based on the feed evaluation data from our research laboratory).

Item	Starter diet (0–21 d)	Finisher diet (22–38 d)
Ingredients		
Corn	574.2	660
Soybean meal, 460 g/kg	381.4	295.6
Soybean oil	8.8	13.6
Limestone	11.3	9.9
Dicalcium phosphate	10.7	8.2
DL-Methionine	3.3	3.0
L-Lysine HCl	2.0	1.9
L-Threonine	1.0	0.7
Sodium bicarbonate	2.7	2.5
Sodium chloride	2.5	2.5
Trace mineral premix[1]	1.0	1.0
Vitamin premix[1]	1.0	1.0
Phytase[2]	0.1	0.1
Calculated analysis		
Dry matter	895	889
Apparent metabolizable energy, MJ/kg	12.14	12.69
Crude protein	225	190
Digestible lysine	11.0	9.2
Digestible methionine	6.2	5.6
Digestible methionine + cysteine	9.2	8.3
Digestible threonine	7.2	6.0
Crude fat	32	39
Crude fiber	29.3	27.5
Calcium	9.8	8.5
Available phosphorus	4.9	4.2
Sodium	2.2	2.1
Chloride	2.3	2.3
Potassium	11.5	9.7

[1] Supplied per kilogram of diet: antioxidant (ethoxyquin), 100 mg; biotin, 0.2 mg; pantothenic acid (calcium pantothenate), 12.8 mg; vitamin D_3 (cholecalciferol), 0.06 mg; vitamin B_{12} (cyanocobalamin), 0.017 mg; folic acid, 5.2 mg; vitamin K_3 (menadione), 4 mg; niacin (nicotinic acid), 35 mg; pyridoxine (pyridoxine HCl), 10 mg; trans-retinol, 3.33 mg; vitamin B_2 (riboflavin), 12 mg; vitamin B_1 (thiamine), 3.0 mg; vitamin E (DL-α-tocopheryl acetate), 60 mg; choline (choline chloride), 638 mg; Co (cobalt sulfate), 0.3 mg; Cu (copper sulfate), 3.0 mg; Fe (iron sulfate), 25 mg; I (calcium iodate), 1 mg; Mn (manganese oxide), 125 mg; Mo (sodium molybdate), 0.5 mg; Se (sodium selenite), 0.2 mg; Zn (zinc sulfate), 60 mg.

[2] Ronozyme HiPhos (DSM Nutritional Products, East Wagga, Australia) provided 1,000 phytase units [FYT]/kg diet. Ronozyme HiPhos is a granular 6-phytase preparation expressed by submerged fermentation of *Aspergillus oryzae* and contains more than 10,000 phytase units per gram. Nutrient matrix values (1.5 g/kg non-phytate P and 1.8 g/kg Ca) were used in the diet formulation.

same. The rest of the chicks were raised in floor pens and fed a starter diet, finisher diet or both until the introduction of the experimental diet. The birds were then allotted to total 12 cages (6 cages for each PS) at 5 ages, namely, d 7 (12 birds per cage), d 14 (10 birds per cage), d 21 (8 birds per cage), d 28 (8 birds per cage), and d 35 (6 birds per cage), respectively. After 3 d of the adaptation period, the experimental diets were offered for 4 d; namely d 3 to 7 and 10 to 14 (crumbled); d 17 to 21, 24 to 28, 31 to 35, 38 to 42 (pelleted). Ileal digesta were collected on d 7, 14, 21, 28, 35, and 42 post-hatch.

The birds were provided with ad libitum access to feed and water throughout the whole experimental period. The room temperature was maintained as 32 ± 1 °C on d 1 and gradually reduced to 23 °C and 16 °C by d 21 and 42, respectively. The birds received the following lighting program per day: 24L:0D (d 0); 23L:1D (d 1); 22L:2D (d 2); 21L:3D (d 3); 20L:4D (d 4 to 21); 18L:6D (d 21 to 28); 16L:8D (d 28 to 42). Throughout the 4-d duration of each week's experiment, body weight (BW) of birds and feed consumption were recorded for each individual cage. Additionally, any deaths among the birds were noted on a daily basis.

2.4. Determination of the coefficients of ileal digestibility

At the termination of respective experimental periods (d 7, 14, 21, 28, 35, and 42), all birds were euthanized by intravenous administration (1 mL per 2 kg BW) of sodium pentobarbitone solution (Provet NZ Pty. Ltd., Auckland, New Zealand). Then, the digesta contents were assembled from the lower half of the ileum by softly flushing with distilled water into plastic containers and processed as discussed by Ravindran et al. (2005). The ileum was referred to as the segment of small intestine extending from the Meckel's diverticulum to a point approximately 40 mm proximal to the ileocecal junction. Briefly, the ileum was divided into halves (proximal and distal ileum) and the digesta samples were collected from the lower half towards the ileocecal junction. Digesta from birds within a cage were pooled after collection, frozen immediately, and subsequently lyophilized (Model 0610, Cuddon Engineering, Blenheim, New Zealand). Diet and lyophilized digesta samples were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at 4 °C until laboratory analysis. The digesta samples were analyzed for DM, Titanium (Ti), N and AA.

2.5. Gizzard pH and jejunal digesta viscosity

Two birds from each replicate cage, euthanized for ileal digesta collection, were utilized for the gizzard pH measurement by a digital pH meter (pH spear, Oakton Instruments, Vernon Hill, IL). In brief, the glass probe was inserted through an opening created in the gizzard and positioned directly in the digesta. Three readings were taken from the proximal, middle, and distal areas and the average of these readings were regarded as the final pH measurement. The same birds were used to measure the jejunal digesta viscosity. The digesta collected from the distal jejunum were centrifuged at 3,000 × *g* at 20 °C for 15 min. A 0.5-mL aliquot of the supernatant was used in a viscometer (Brookfield digital viscometer, Model DV2TLV; Brookfield Engineering Laboratories Inc., Stoughton, MA).

2.6. Chemical analyses

All chemical analyses were conducted in duplicate, except for AA, which were analyzed once per sample. The standard procedure (Method 930.15; AOAC International, 2016) was followed to determine the DM content. Titanium was assessed using a UV spectrophotometer (Berthold Technologies GmbH and Co. KG, Bad Wildbad, Germany) following the method stated by Short et al. (1996). An adiabatic bomb calorimeter (Gallenkamp Autobomb, Weiss Gallenkamp Ltd, Loughborough, UK) standardized with benzoic acid was employed for GE analysis. Nitrogen content was ascertained by combustion (Method 968.06; AOAC International, 2016) using a carbon nanosphere-200 carbon, N and sulfur auto analyzer (LECO Corporation, St. Joseph, MI). The CP content was calculated as N × 6.25. Fat content was determined using the Soxhlet extraction method (Method, 2003.06; AOAC International, 2016). Neutral detergent fiber was determined (Method, 2002.04; AOAC International, 2016) using Tecator Fibertec (FOSS Analytical AB, Höganäs, Sweden). Samples were measured for ash by subjected to ashing in a muffle furnace at 550 °C for 16 h (Method 942.05; AOAC International, 2016). Calcium and P concentrations were measured using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument (Thermo Jarrell Ash Corporation, Franklin, MA).

Amino acids analysis was carried out following standard procedures (Method 994.12; AOAC International, 2011). The samples underwent hydrolysis in glass bottles in an oven, utilizing 6 mol/L HCl containing phenol for 24 h at a temperature of 110 ± 2 °C. The AA were measured using an AA analyzer (ion exchange) with ninhydrin post-column derivatization. The chromatograms captured at 570 and 440 nm were integrated using dedicated software (Agilent Open Lab software, Waldbronn, Baden-Württemberg, Germany). Cys and Met, the sulfur-containing AA, were analyzed as cysteic acid and methionine sulfone, respectively, by oxidation with performic acid-phenol for 16 h at 0 °C before the hydrolysis process.

For Trp analysis, the samples underwent saponification in an alkaline environment using barium hydroxide solution in the absence of air at 110 °C for 20 h in an autoclave. Following hydrolysis, the α-methyl Trp as internal standard, was introduced into the mixture. After adjusting the hydrolysate to pH 3.0 and diluting with 30% methanol, Trp and the internal standard were separated by reverse phase chromatography (RP-18) on a HPLC column (CORTECS C18 Column; 2.7 μm, Waters Corporation, Dublin, Ireland). Detection was selectively performed using a fluorescence detector to avoid any potential interference by other AA and components.

2.7. Calculations

The AID coefficients (AIDC) of AA were calculated from the dietary ratio of AA to Ti relative to the corresponding ratio in the ileal digesta using the formula provided.

$$\text{AIDC of AA} = [(\text{AA/Ti})_d - (\text{AA/Ti})_i]/(\text{AA/Ti})_d,$$

where $(\text{AA/Ti})_d$ = ratio of AA to Ti in the diet, and $(\text{AA/Ti})_i$ = ratio of AA to Ti in the ileal digesta.

Apparent digestibility data for N and AA were then transformed into SIDC, using the age-specific basal endogenous N and AA estimates (EAA; grams per kilogram of DM intake [DMI]) measured at different ages (d 7, 14, 21, 28, 35 and 42) in a prior experiment (Barua et al., 2021d).

$$\text{SIDC} = \text{AIDC} + [\text{Basal EAA (g/kg DMI)/Ing. AA (g/kg DM)}],$$

where SIDC = standardized ileal digestibility coefficient of the AA, AIDC = apparent ileal digestibility coefficient of the AA, Basal EAA = basal endogenous AA loss, and Ing. AA = concentration of the AA in the ingredient.

2.8. Data analysis

The data pertaining to each PS were analyzed by one-way ANOVA using the General Linear Model Procedure of SAS (version 9.4; 2015; SAS Institute Inc., Cary, NC). The cages were treated as the experimental unit. Significance of differences was determined at $P < 0.05$. Additionally, orthogonal polynomial contrasts were conducted to analyze the linear and quadratic effects of broiler age. The relationships between SIDC AA and other parameters were evaluated using Pearson correlation.

3. Results

3.1. Proximate and nutrient composition

Table 3 presents the proximate and AA composition of SBM and CM, with SBM exhibiting a greater amount of CP (446 g/kg) and total AA (TAA; 391 g/kg) compared to CM (383 g/kg CP and 315 g/kg TAA).

Table 3
Proximate and amino acid composition of soybean meal and solvent-extracted canola meal (g/kg, as is basis).

Item	Soybean meal	Canola meal
DM	885	896
Nitrogen (N)	71.4	61.2
CP (N × 6.25)	446	383
Fat	12.3	48.5
NDF	84.6	250
GE, MJ/kg	17.7	17.8
Ash	65.4	72.5
Calcium	3.26	5.78
Phosphorus	6.61	10.5
Indispensable amino acids (IAA)		
Arg	32.3	22.4
His	11.7	9.61
Ile	20.7	15.3
Leu	33.6	26.0
Lys	27.3	19.8
Met	5.95	7.64
Thr	17.0	15.8
Trp	5.99	5.14
Val	21.6	19.5
Total IAA	176	141
Dispensable amino acids (DAA)		
Ala	19.3	16.4
Asp	49.9	26.5
Cys[1]	5.58	7.95
Glu	79.3	66.7
Gly[1]	18.7	19.0
Pro	21.2	22.1
Ser	21.4	15.3
Total DAA	215	174
Total AA[2]	391	315

CP = crude protein; GE = gross energy; NDF = neutral detergent fiber.
[1] Semi-indispensable amino acids for poultry.
[2] Total AA = IAA + DAA.

3.2. Growth performance, gizzard pH and jejunal digesta viscosity

Table 4 summarizes the data on the performance, gizzard pH, and jejunal digesta viscosity of birds that were fed SBM- or CM-based diets.

Mortality was minimal, as only 3 out of 696 birds died, and these deaths were not associated with any specific treatment. As the birds grew older, an effect ($P < 0.001$) was observed in the daily feed intake (DFI) and the daily weight gain (DWG) in both SBM and CM. The DFI exhibited a quadratic pattern for SBM and a linear pattern for CM, with both showing significant increases ($P < 0.001$). The daily weight gain (DWG) increased (quadratic; $P < 0.001$) with advancing broiler age in both PS.

In both SBM and CM, gizzard pH and jejunal viscosity were affected ($P < 0.001$) by age. Gizzard pH showed a quadratic age effect ($P < 0.01$) in both PS, with higher values observed at d 7, a decline at d 14, and an increase afterwards. Jejunal viscosity tended to be influenced quadratically by age in SBM ($P = 0.060$), while in CM, a linear increase ($P < 0.001$) was observed with advancing age.

3.3. Ileal digestibility coefficients of N and AA in soybean meal

Tables 5–7 present the impact of broiler age on AIDC, SIDC, and SID content of N and AA in SBM over the 42-d experimental period.

In SBM, significant effects of broiler age were observed in the AIDC of N, average digestibility of IAA, DAA, and TAA and all individual AA except Leu, Asp and Glu ($P < 0.05$; Table 5). As bird age increased, a linear increase was observed in the AIDC of N ($P < 0.01$), average digestibility of IAA ($P < 0.01$), DAA ($P < 0.01$), and TAA ($P < 0.01$). The AIDC of N and average AIDC of IAA were lower at d 7, increased to d 14, and leveled off until d 28, after which a further

Table 4
Daily feed intake (DFI), daily weight gain (DWG), gizzard pH and viscosity in jejunal digesta of broilers fed soybean meal- and canola meal-based diets at different ages.[1]

Item	Age, d						ANOVA		Orthogonal polynomial contrasts	
	7	14	21	28	35	42	Pooled SEM	*P*-value	Linear	Quadratic
Soybean meal										
DFI[2], g/bird per day	18.2[f]	48.9[e]	89.8[d]	115.0[c]	172.0[b]	202.0[a]	1.19	0.001	0.001	0.001
DWG[2], g/bird per day	16.6[d]	38.2[c]	69.9[b]	70.8[b]	85.0[a]	88.2[a]	1.60	0.001	0.001	0.001
Gizzard pH[3]	3.17[cd]	2.92[d]	3.30[c]	3.43[c]	3.87[b]	4.31[a]	0.108	0.001	0.001	0.001
Viscosity[3], cP	2.85[cd]	3.37[ab]	3.58[a]	2.80[d]	2.90[cd]	3.12[bc]	0.109	0.001	0.368	0.060
Canola meal										
DFI[2], g/bird per day	20.3[f]	53.0[e]	92.4[d]	121.0[c]	176.0[b]	198.0[a]	1.49	0.001	0.001	0.580
DWG[2], g/bird per day	17.6[e]	41.9[d]	72.8[c]	73.5[c]	90.1[b]	97.5[a]	0.99	0.001	0.001	0.001
Gizzard pH[3]	3.16[b]	2.70[c]	3.16[b]	3.21[b]	3.76[a]	3.78[a]	0.095	0.001	0.001	0.002
Viscosity[3], cP	2.02[d]	2.25[bc]	2.47[a]	2.29[b]	2.13[cd]	2.58[a]	0.056	0.001	0.001	0.424

[a-f] Means in a row not sharing a common letter are significantly different ($P < 0.05$).
[1] Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7-, 14- and 21-d-old birds, respectively; 8 birds per replicate for 28- and 35-d-old birds; and 6 birds per replicate for 42-d-old birds).
[2] Measured during 4-d feeding of experimental diets.
[3] Calculated as the mean of six replicates (2 birds per replicate).

Table 5
Apparent ileal digestibility coefficients of nitrogen (N) and amino acids of soybean meal at different ages of broilers.[1]

Item	Age, d						ANOVA		Orthogonal polynomial contrasts	
	7	14	21	28	35	42	Pooled SEM	*P*-value	Linear	Quadratic
N	0.838[c]	0.848[abc]	0.851[ab]	0.841[bc]	0.859[a]	0.857[a]	0.0045	0.012	0.004	0.966
Indispensable amino acids										
Arg	0.916[b]	0.908[b]	0.907[b]	0.913[b]	0.931[a]	0.916[b]	0.0035	0.001	0.016	0.244
His	0.876[b]	0.878[b]	0.876[b]	0.878[b]	0.895[a]	0.884[ab]	0.0040	0.015	0.011	0.755
Ile	0.859[b]	0.866[b]	0.865[b]	0.862[b]	0.883[a]	0.870[ab]	0.0046	0.021	0.021	0.823
Leu	0.858	0.868	0.866	0.863	0.879	0.865	0.0044	0.058	0.088	0.309
Lys	0.879[c]	0.891[b]	0.894[ab]	0.892[b]	0.903[a]	0.892[b]	0.0034	0.001	0.001	0.012
Met	0.869[b]	0.895[b]	0.887[a]	0.886[a]	0.895[a]	0.890[a]	0.0046	0.004	0.010	0.053
Thr	0.793[c]	0.796[bc]	0.801[bc]	0.794[bc]	0.821[a]	0.810[ab]	0.0055	0.007	0.002	0.746
Trp	0.843[b]	0.843[b]	0.846[b]	0.845[b]	0.872[a]	0.866[a]	0.0049	0.001	0.001	0.176
Val	0.844[b]	0.856[b]	0.856[b]	0.849[b]	0.872[a]	0.857[b]	0.0048	0.009	0.019	0.388
IAA	0.859[c]	0.867[bc]	0.866[bc]	0.865[bc]	0.884[a]	0.872[ab]	0.0043	0.011	0.002	0.426
Dispensable amino acids										
Ala	0.838[b]	0.857[a]	0.856[a]	0.853[a]	0.865[a]	0.855[a]	0.0048	0.013	0.017	0.033
Asp	0.855	0.850	0.849	0.846	0.864	0.852	0.0047	0.169	0.636	0.378
Cys[2]	0.732[b]	0.726[b]	0.723[b]	0.725[b]	0.776[a]	0.769[a]	0.0077	0.001	0.001	0.005
Glu	0.899	0.896	0.893	0.898	0.909	0.898	0.0039	0.099	0.211	0.661
Gly[2]	0.819[c]	0.817[c]	0.822[bc]	0.823[bc]	0.847[a]	0.836[ab]	0.0050	0.001	0.001	0.432
Pro	0.847[b]	0.844[b]	0.843[b]	0.845[b]	0.869[a]	0.856[ab]	0.0050	0.007	0.009	0.275
Ser	0.839[b]	0.839[b]	0.850[ab]	0.847[ab]	0.860[a]	0.853[ab]	0.0052	0.045	0.005	0.541
DAA	0.833[b]	0.833[b]	0.834[b]	0.834[b]	0.856[a]	0.845[ab]	0.0049	0.010	0.004	0.475
TAA	0.848[b]	0.852[b]	0.852[b]	0.851[b]	0.871[a]	0.861[ab]	0.0045	0.011	0.004	0.881

DAA = average digestibility of dispensable amino acids; IAA = average digestibility of indispensable amino acids; TAA = average digestibility of all amino acids.
[a-c] Means in a row not sharing a common letter are significantly different ($P < 0.05$).
[1] Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7-, 14- and 21-d-old birds, respectively; 8 birds per replicate for 28- and 35-d-old birds; and 6 birds per replicate for 42-d-old birds).
[2] Semi-indispensable amino acids for poultry.

increase was observed beyond d 35. The average AIDC of DAA and TAA plateaued between d 7 and 28 and increased from d 35 onwards.

For IAA, with the exception of Leu, the AIDC of all individual IAA in SBM increased (linear or quadratic; $P < 0.05$ or $P < 0.001$) with broiler age. A tendency of age effect was observed on the AIDC of Leu (linear; $P = 0.088$). The AIDC of Lys was influenced in a quadratic manner ($P < 0.05$), while a linear effect ($P < 0.05$ or $P < 0.001$) was observed for the AIDC of all other IAA. The AIDC of His, Ile, Thr, and Trp increased linearly ($P < 0.05$ or $P < 0.001$) at d 35, with no further increase until d 42.

Except for Asp and Glu, the AIDC of all individual DAA was affected by age, showing either linear or quadratic effects with P values ranging from less than 0.05 to 0.001. The AIDC of Ala and Cys demonstrated a quadratic effect ($P < 0.05$), increasing beyond d 14 and d 35, respectively. The AIDC of Gly, Pro, and Ser showed a linear increase ($P < 0.05$ or $P < 0.001$) as age advanced.

Table 6 shows that the bird age tended to influence the SIDC of N in SBM ($P = 0.059$), while a tendency for quadratic response ($P = 0.075$) was also observed. The average SIDC of IAA, DAA and TAA were influenced ($P < 0.05$) by age. With the exception of Ile, Leu, Lys, Met and Ala, an impact of broiler age was noticed in the SIDC of all individual AA ($P < 0.05$ or $P < 0.001$). The average SIDC of IAA and TAA did not show any linear or quadratic response to age. For individual IAA, the bird age influenced the SIDC of Arg ($P < 0.05$), Thr ($P < 0.01$), and Trp ($P < 0.05$) in a quadratic manner. The SIDC of Arg and Thr was higher at d 7, decreased at d 14, and stayed consistent until d 28; it increased at d 35 and then declined at d 42. The SIDC of Trp increased at d 35 and did not show any further changes until d 42.

The age of the broilers had a quadratic influence ($P < 0.05$) on the average SIDC of DAA, as well as the SIDC of Cys and Pro in SBM. Specifically, the values were higher at d 7, decreased at d 14, with no change until d 28, and then increased at d 35. The SIDC of Cys

Table 6
Standardized ileal digestibility coefficients[1] of nitrogen (N) and amino acids of soybean meal at different ages of broilers.[2]

Item	Age, d						ANOVA		Orthogonal polynomial contrasts	
	7	14	21	28	35	42	Pooled SEM	P-value	Linear	Quadratic
N	0.882	0.871	0.873	0.864	0.882	0.873	0.0045	0.059	0.529	0.075
Indispensable amino acids										
Arg	0.934[a]	0.917[b]	0.916[b]	0.923[b]	0.940[a]	0.922[b]	0.0035	0.001	0.666	0.042
His	0.898[ab]	0.890[b]	0.887[b]	0.889[b]	0.906[a]	0.891[b]	0.0040	0.021	0.633	0.237
Ile	0.887	0.880	0.879	0.876	0.895	0.878	0.0046	0.058	0.992	0.530
Leu	0.884	0.880	0.879	0.877	0.892	0.874	0.0044	0.098	0.617	0.851
Lys	0.899	0.900	0.903	0.902	0.912	0.898	0.0034	0.067	0.381	0.137
Met	0.908	0.913	0.905	0.906	0.913	0.900	0.0046	0.342	0.282	0.510
Thr	0.863[a]	0.835[b]	0.838[b]	0.829[b]	0.858[a]	0.838[b]	0.0055	0.001	0.159	0.008
Trp	0.874[bc]	0.860[c]	0.864[c]	0.865[c]	0.890[a]	0.879[ab]	0.0049	0.001	0.008	0.037
Val	0.878[ab]	0.874[b]	0.873[b]	0.867[b]	0.889[a]	0.869[b]	0.0048	0.047	0.897	0.841
IAA	0.892[ab]	0.883[b]	0.882[b]	0.882[b]	0.899[a]	0.883[b]	0.0043	0.036	0.908	0.396
Dispensable amino acids										
Ala	0.872	0.874	0.873	0.871	0.882	0.866	0.0047	0.339	0.788	0.310
Asp	0.880[a]	0.863[bc]	0.862[c]	0.859[c]	0.876[ab]	0.860[c]	0.0047	0.011	0.124	0.081
Cys[3]	0.807[a]	0.768[b]	0.763[b]	0.764[b]	0.818[a]	0.802[a]	0.0077	0.001	0.072	0.001
Glu	0.918[a]	0.905[b]	0.902[b]	0.908[ab]	0.919[a]	0.904[b]	0.0039	0.015	0.496	0.198
Gly[3]	0.857[ab]	0.836[c]	0.841[c]	0.843[bc]	0.867[a]	0.850[bc]	0.0050	0.002	0.172	0.051
Pro	0.885[a]	0.864[b]	0.864[b]	0.864[b]	0.888[a]	0.870[b]	0.0050	0.002	0.945	0.019
Ser	0.883[a]	0.862[b]	0.873[ab]	0.869[ab]	0.884[a]	0.870[ab]	0.0052	0.039	0.909	0.343
DAA	0.872[ab]	0.853[c]	0.854[c]	0.854[c]	0.876[a]	0.860[bc]	0.0049	0.006	0.798	0.039
TAA	0.883[ab]	0.870[bc]	0.870[bc]	0.869[c]	0.889[a]	0.873[bc]	0.0045	0.016	0.854	0.147

DAA = average digestibility of dispensable amino acids; IAA = average digestibility of indispensable amino acids; TAA = average digestibility of all amino acids.
[a-c] Means in a row not sharing a common letter are significantly different ($P < 0.05$).
[1] Apparent digestibility values were standardized using the following basal ileal endogenous flow values (g/kg dry matter intake), determined by the feeding nitrogen-free diet at different ages (Barua et al., 2021d): d 7: N, 3.59; Arg, 0.68; His, 0.29; Ile, 0.63; Leu, 0.97; Lys, 0.64; Met, 0.27; Thr, 1.35; Trp, 0.21; Val, 0.81; Ala, 0.75; Asp, 1.41; Cys, 0.47; Glu, 1.71; Gly, 0.78; Pro, 0.91; and Ser, 1.06; d 14: N, 1.87; Arg, 0.30; His, 0.16; Ile, 0.33; Leu, 0.49; Lys, 0.30; Met, 0.12; Thr, 0.73; Trp, 0.12; Val, 0.43; Ala, 0.37; Asp, 0.73; Cys, 0.27; Glu, 0.80; Gly, 0.39; Pro, 0.48; and Ser, 0.55; d 21: N, 1.79; Arg, 0.31; His, 0.15; Ile, 0.32; Leu, 0.49; Lys, 0.28; Met, 0.12; Thr, 0.71; Trp, 0.12; Val, 0.43; Ala, 0.36; Asp, 0.72; Cys, 0.26; Glu, 0.80; Gly, 0.40; Pro, 0.48; and Ser, 0.56; d 28: N, 1.82; Arg, 0.36; His, 0.16; Ile, 0.34; Leu, 0.55; Lys, 0.32; Met, 0.14; Thr, 0.67; Trp, 0.13; Val, 0.45; Ala, 0.41; Asp, 0.74; Cys, 0.25; Glu, 0.89; Gly, 0.43; Pro, 0.48; and Ser, 0.54; d 35: N, 1.81; Arg, 0.32; His, 0.15; Ile, 0.30; Leu, 0.49; Lys, 0.28; Met, 0.12; Thr, 0.71; Trp, 0.12; Val, 0.42; Ala, 0.37; Asp, 0.72; Cys, 0.26; Glu, 0.81; Gly, 0.40; Pro, 0.47; and Ser, 0.56; d 42: N, 1.29; Arg, 0.20; His, 0.10; Ile, 0.20; Leu, 0.31; Lys, 0.18; Met, 0.07; Thr, 0.52; Trp, 0.09; Val, 0.29; Ala, 0.25; Asp, 0.49; Cys, 0.21; Glu, 0.53; Gly, 0.28; Pro, 0.34; and Ser, 0.40.
[2] Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7-, 14- and 21-d old birds, respectively; 8 birds per replicate for 28- and 35-d-old birds; and 6 birds per replicate for 42-d old birds).
[3] Semi-indispensable amino acids for poultry.

Table 7
Influence of age on standardized digestible protein (CP) and amino acid contents[1] (g/kg) of soybean meal, as is basis.

Item	Age, d						ANOVA		Orthogonal polynomial contrasts	
	7	14	21	28	35	42	Pooled SEM	P-value	Linear	Quadratic
CP	394	389	390	385	394	390	2.0	0.059	0.529	0.075
Indispensable amino acids										
Arg	30.1[a]	29.6[b]	29.5[b]	29.8[b]	30.3[a]	29.7[b]	0.11	0.001	0.666	0.042
His	10.5[ab]	10.4[b]	10.3[b]	10.4[b]	10.6[a]	10.4[b]	0.05	0.021	0.633	0.237
Ile	18.4	18.2	18.2	18.1	18.5	18.2	0.09	0.058	0.992	0.530
Leu	29.7	29.6	29.5	29.5	30.0	29.4	0.15	0.098	0.617	0.851
Lys	24.5	24.5	24.7	24.6	24.9	24.5	0.09	0.067	0.380	0.137
Met	5.41	5.43	5.39	5.39	5.43	5.36	0.027	0.341	0.282	0.509
Thr	14.7[a]	14.2[b]	14.3[b]	14.1[b]	14.6[a]	14.2[b]	0.09	0.001	0.159	0.008
Trp	5.23[bc]	5.15[c]	5.18[c]	5.18[c]	5.33[a]	5.27[ab]	0.029	0.001	0.008	0.037
Val	18.9[ab]	18.8[b]	18.8[b]	18.7[b]	19.2[a]	18.8[b]	0.10	0.047	0.897	0.841
IAA	158[ab]	156[b]	156[b]	156[b]	159[a]	156[b]	0.7	0.026	0.983	0.482
Dispensable amino acids										
Ala	16.8	16.9	16.8	16.8	17.0	16.7	0.09	0.339	0.788	0.310
Asp	43.9[a]	43.1[bc]	43.0[c]	42.9[c]	43.7[ab]	43.0[c]	0.23	0.011	0.124	0.081
Cys[2]	4.51[a]	4.29[b]	4.26[b]	4.26[b]	4.56[a]	4.47[a]	0.043	0.001	0.072	0.001
Glu	72.7[a]	71.8[b]	71.5[b]	72.1[ab]	72.8[a]	71.7[b]	0.31	0.015	0.496	0.199
Gly[2]	16.0[ab]	15.6[c]	15.7[c]	15.8[bc]	16.2[a]	15.9[bc]	0.09	0.002	0.172	0.051
Pro	18.8[a]	18.3[b]	18.3[b]	18.3[b]	18.9[a]	18.5[b]	0.11	0.002	0.945	0.020
Ser	18.9[a]	18.4[b]	18.7[ab]	18.6[ab]	18.9[a]	18.6[ab]	0.11	0.039	0.909	0.343
DAA	192[a]	190[b]	188[b]	189[b]	192[a]	189[b]	0.9	0.025	0.330	0.161
Total AA	349[ab]	345[bc]	344[c]	345[bc]	351[a]	345[bc]	1.7	0.021	0.797	0.235

DAA = total digestible dispensable amino acid contents; IAA = total digestible indispensable amino acid contents; Total AA = total digestible content of all amino acids.
[a-c] Means in a row not sharing a common letter are significantly different ($P < 0.05$).
Standardized digestible amino acid content (g/kg) = [ingredient amino acid content (g/kg) × standardized ileal digestibility (%)]/100.
[1] Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7-, 14- and 21-d-old birds, respectively; 8 birds per replicate for 28- and 35-d old birds; and 6 birds per replicate for 42-d old birds).
[2] Semi-indispensable amino acids for poultry.

remained unaffected until d 42, while the SIDC of Pro decreased further at d 42. There was a tendency for a quadratic age effect on the SIDC of Asp ($P = 0.081$) and Gly ($P = 0.051$).

In Table 7, it was noted that the SID protein content of SBM remain unaffected by age. However, an effect ($P < 0.05$ - 0.001) was observed in the SID contents of total IAA, DAA, total AA and all individual AA except Ile, Leu, Lys, Met and Ala. The broiler age tended to quadratically influence ($P = 0.075$) the SID protein content of SBM, while no quadratic or linear response of age effect was observed for the SID contents of total IAA, DAA, and total AA. Among the individual AA, the SID contents of Arg, Thr, Trp, Cys, and Pro were all influenced in a quadratic manner ($P < 0.05$) by age. Moreover, there was a tendency for a quadratic age effect on the SID contents of Asp ($P = 0.081$) and Gly ($P = 0.051$).

3.4. Ileal digestibility coefficients of N and AA in canola meal

Tables 8–10 present the influence of broiler age on the AIDC, SIDC, and SID content of N and AA in CM.

An age effect was observed in the AIDC of N, IAA, DAA, TAA and all individual AA ($P < 0.001$, Table 8). The AIDC of N, the average AIDC of DAA and TAA, were influenced in a quadratic manner ($P < 0.05$), while the average AIDC of IAA showed a linear pattern ($P < 0.001$). The AIDC of N, average of IAA, DAA and TAA increased only at d 35 and then plateaued until d 42. With increasing broiler age, the AIDC of all individual AA (except Leu, Met, and Ala) remained constant from d 7 to 28, followed by an increase at d 35 with no further changes to d 42, as indicated by linear or quadratic ($P < 0.05$ or $P < 0.001$) patterns. The lowest AIDC of Leu (linear; $P < 0.001$), Met (quadratic; $P < 0.05$), and Ala (linear; $P < 0.001$) was recorded at d 7, which increased between d 14 and 28, followed by a further increase at d 35 with no change afterwards.

A significant effect of age was observed in the SIDC of N and average SIDC of IAA, DAA and TAA and all individual AA in CM ($P < 0.05$ or $P < 0.001$; Table 9). The SIDC of N and average SIDC of IAA, DAA and TAA in CM were found to increase in a quadratic manner with age ($P < 0.001$). The SIDC of N and average SIDC of DAA and TAA remained constant from d 7 to 14, decreased on d 21, and then increased again after d 28. The average SIDC of IAA was consistently lower from day 7 to 28, but increased and remained steady between d 35 and 42.

Among the IAA, the SIDC of Arg, His, Lys, Thr, Trp and Val showed a quadratic response to broiler age ($P < 0.05$ or $P < 0.001$). However, the SIDC of Ile, Leu and Met increased linearly ($P < 0.05$ or $P < 0.001$) with age. With the exception of Ala (linear; $P < 0.001$) and Ser (linear; $P < 0.05$), the SIDC of individual DAA were influenced in a quadratic manner ($P < 0.001$) with age.

The SID content of protein, IAA, DAA, total AA and all individual AA in CM were affected by broiler age ($P < 0.01$; Table 10). The SID protein content was influenced in a linear manner ($P < 0.001$), ranging from 290 to 295 g/kg between d 7 and 28 and increasing thereafter. The SID content of IAA increased linearly ($P < 0.001$) by age. As for DAA and total AA, the SID contents plateaued from d 7 to 28 and increased thereafter (linear; $P < 0.001$). With the exception of Cys, the SID contents of all individual AA in CM increased (linear or quadratic; $P < 0.05$ or $P < 0.001$) with advancing broiler age. The SID content of Cys was influenced in a quadratic manner ($P < 0.001$), with a higher value at d 7, decreasing at d 28, and increasing beyond d 35.

3.5. Uplift in digestibility coefficients due to correction for age-specific endogenous amino acid losses

Table 11 presents the changes in the digestibility coefficients of N and AA after adjusting AIDC for basal endogenous N and AA losses at different broiler ages.

Regardless of age, the correction for age-specific endogenous losses resulted increased the SIDC estimates over AIDC AA, and this extent of increase decreased with increasing broiler age. Correcting AIDC values led to a rise in the average TAA digestibility coefficients in SBM by 4.13% (d 7), 2.24% (d 14), 2.11% (d 21), 2.12% (d 28), 2.07% (d 35), and 1.39% (d 42). Similarly, the corresponding increases in

Table 8
Apparent ileal digestibility coefficients of nitrogen (N) and amino acids of canola meal at different ages of broilers.[1]

Item	Age, d						ANOVA		Orthogonal polynomial contrasts	
	7	14	21	28	35	42	Pooled SEM	*P*-value	Linear	Quadratic
N	0.725^{b}	0.733^{b}	0.722^{b}	0.733^{b}	0.767^{a}	0.768^{a}	0.0067	0.001	0.001	0.023
Indispensable amino acids										
Arg	0.845^{b}	0.839^{b}	0.839^{b}	0.848^{b}	0.878^{a}	0.880^{a}	0.0033	0.001	0.001	0.001
His	0.803^{b}	0.804^{b}	0.794^{b}	0.800^{b}	0.832^{a}	0.830^{a}	0.0043	0.001	0.001	0.001
Ile	0.754^{b}	0.769^{b}	0.768^{b}	0.770^{b}	0.804^{a}	0.797^{a}	0.0057	0.001	0.001	0.571
Leu	0.784^{c}	0.803^{b}	0.800^{b}	0.800^{b}	0.830^{a}	0.822^{a}	0.0050	0.001	0.001	0.946
Lys	0.741^{b}	0.749^{b}	0.742^{b}	0.752^{b}	0.779^{a}	0.778^{a}	0.0057	0.001	0.001	0.089
Met	0.839^{d}	0.863^{bc}	0.862^{c}	0.861^{c}	0.875^{a}	0.873^{ab}	0.0037	0.001	0.001	0.046
Thr	0.678^{b}	0.688^{b}	0.680^{b}	0.688^{b}	0.728^{a}	0.722^{a}	0.0073	0.001	0.001	0.096
Trp	0.762^{b}	0.761^{b}	0.753^{b}	0.762^{b}	0.809^{a}	0.804^{a}	0.0059	0.001	0.001	0.001
Val	0.750^{b}	0.762^{b}	0.758^{b}	0.761^{b}	0.794^{a}	0.789^{a}	0.0057	0.001	0.001	0.252
IAA	0.773^{b}	0.782^{b}	0.777^{b}	0.782^{b}	0.814^{a}	0.811^{a}	0.0048	0.001	0.001	0.073
Dispensable amino acids										
Ala	0.768^{c}	0.791^{b}	0.786^{b}	0.789^{b}	0.818^{a}	0.812^{a}	0.0054	0.001	0.001	0.884
Asp	0.721^{b}	0.716^{b}	0.715^{b}	0.714^{b}	0.755^{a}	0.752^{a}	0.0066	0.001	0.001	0.006
Cys[2]	0.691^{b}	0.690^{b}	0.701^{b}	0.699^{b}	0.725^{a}	0.736^{a}	0.0062	0.001	0.001	0.038
Glu	0.843^{b}	0.847^{b}	0.839^{b}	0.847^{b}	0.871^{a}	0.870^{a}	0.0038	0.001	0.001	0.008
Gly[2]	0.735^{b}	0.730^{b}	0.718^{b}	0.731^{b}	0.772^{a}	0.777^{a}	0.0061	0.001	0.001	0.001
Pro	0.706^{b}	0.704^{b}	0.692^{b}	0.704^{b}	0.746^{a}	0.748^{a}	0.0066	0.001	0.001	0.001
Ser	0.699^{b}	0.713^{b}	0.718^{b}	0.717^{b}	0.754^{a}	0.744^{a}	0.0070	0.001	0.001	0.898
DAA	0.738^{b}	0.742^{b}	0.739^{b}	0.743^{b}	0.778^{a}	0.777^{a}	0.0054	0.001	0.001	0.016
TAA	0.758^{b}	0.764^{b}	0.760^{b}	0.765^{b}	0.798^{a}	0.796^{a}	0.0050	0.001	0.001	0.035

DAA = average digestibility of dispensable amino acids; IAA = average digestibility of indispensable amino acids; TAA = average digestibility of all amino acids.
[a-d] Means in a row not sharing a common letter are significantly different ($P < 0.05$).
[1] Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7-, 14- and 21-d-old birds, respectively; 8 birds per replicate for 28- and 35-d-old birds; and 6 birds per replicate for 42-d old birds).
[2] Semi-indispensable amino acids for poultry.

Table 9
Standardized ileal digestibility coefficients[1] of nitrogen (N) and amino acids of canola meal at different ages of broilers.[2]

Item	Age, d						ANOVA		Orthogonal polynomial contrasts	
	7	14	21	28	35	42	Pooled SEM	*P*-value	Linear	Quadratic
N	0.778[ab]	0.760[bc]	0.748[c]	0.760[bc]	0.794[a]	0.787[a]	0.0067	0.001	0.008	0.001
Indispensable amino acids										
Arg	0.873[b]	0.851[d]	0.852[d]	0.862[c]	0.891[a]	0.889[a]	0.0033	0.001	0.001	0.001
His	0.830[bc]	0.819[cd]	0.807[d]	0.815[d]	0.846[a]	0.840[ab]	0.0043	0.001	0.001	0.001
Ile	0.791[b]	0.788[b]	0.787[b]	0.790[b]	0.822[a]	0.809[a]	0.0057	0.001	0.001	0.136
Leu	0.818[c]	0.820[bc]	0.817[c]	0.819[bc]	0.847[a]	0.833[ab]	0.0051	0.001	0.001	0.368
Lys	0.771[bc]	0.762[c]	0.755[c]	0.766[c]	0.792[a]	0.786[ab]	0.0057	0.001	0.001	0.011
Met	0.870[c]	0.878[bc]	0.870[bc]	0.878[bc]	0.889[a]	0.881[ab]	0.0037	0.034	0.007	0.446
Thr	0.755[a]	0.730[b]	0.720[b]	0.726[b]	0.768[a]	0.751[a]	0.0073	0.001	0.105	0.001
Trp	0.798[b]	0.781[c]	0.775[c]	0.784[bc]	0.830[a]	0.819[a]	0.0059	0.001	0.001	0.001
Val	0.788[bc]	0.783[c]	0.778[c]	0.782[c]	0.813[a]	0.802[ab]	0.0057	0.001	0.001	0.036
IAA	0.810[bc]	0.801[c]	0.796[c]	0.802[c]	0.833[a]	0.823[ab]	0.0050	0.001	0.001	0.001
Dispensable amino acids										
Ala	0.809[c]	0.811[bc]	0.806[c]	0.811[bc]	0.839[a]	0.826[ab]	0.0054	0.001	0.001	0.272
Asp	0.769[a]	0.741[b]	0.740[b]	0.739[b]	0.780[a]	0.769[a]	0.0065	0.001	0.046	0.001
Cys[3]	0.744[ab]	0.743[ab]	0.731[bc]	0.726[c]	0.754[a]	0.759[a]	0.0056	0.002	0.035	0.001
Glu	0.866[bc]	0.858[cd]	0.850[d]	0.859[cd]	0.882[a]	0.877[ab]	0.0038	0.001	0.001	0.001
Gly[3]	0.771[b]	0.748[c]	0.737[c]	0.751[c]	0.791[a]	0.790[a]	0.0061	0.001	0.001	0.001
Pro	0.743[bc]	0.723[d]	0.712[d]	0.724[cd]	0.766[a]	0.762[ab]	0.0066	0.001	0.001	0.001
Ser	0.762[bc]	0.746[c]	0.751[bc]	0.749[bc]	0.787[a]	0.767[ab]	0.0070	0.002	0.015	0.086
DAA	0.778[b]	0.769[bc]	0.762[c]	0.766[bc]	0.800[a]	0.793[a]	0.0058	0.001	0.001	0.004
TAA	0.796[bc]	0.787[cd]	0.781[d]	0.786[cd]	0.819[a]	0.810[ab]	0.0052	0.001	0.001	0.001

DAA = average digestibility of dispensable amino acids; IAA = average digestibility of indispensable amino acids; TAA = average digestibility of all amino acids.
[a-d] Means in a row not sharing a common letter are significantly different ($P < 0.05$).
[1] Apparent digestibility values were standardized using the following basal ileal endogenous flow values (g/kg dry matter intake), determined by feeding an N-free diet at different ages (Barua et al., 2021d); see Table 6.
[2] Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7-, 14- and 21-d-old birds, respectively; 8 birds per replicate for 28- and 35-d-old birds; and 6 birds per replicate for 42-d old birds).
[3] Semi-indispensable amino acids for poultry.

Table 10
Influence of age on standardized digestible protein (CP) and amino acid contents[1] (g/kg) of canola meal, as is basis.

Item	Age, d						ANOVA		Orthogonal polynomial contrasts	
	7	14	21	28	35	42	Pooled SEM	*P*-value	Linear	Quadratic
CP	292[b]	291[b]	290[b]	295[b]	304[a]	302[a]	1.9	0.001	0.001	0.084
Indispensable amino acids										
Arg	19.3[bc]	19.1[cd]	19.1[cd]	19.4[b]	20.0[a]	19.9[a]	0.08	0.001	0.001	0.001
His	7.87[b]	7.87[b]	7.82[b]	7.91[b]	8.13[a]	8.07[a]	0.035	0.001	0.001	0.024
Ile	11.9[d]	12.0[cd]	12.2[bc]	12.2[bc]	12.6[a]	12.4[ab]	0.08	0.001	0.001	0.222
Leu	20.9[c]	21.3[b]	21.4[b]	21.5[b]	22.0[a]	21.7[b]	0.12	0.001	0.001	0.079
Lys	14.9[d]	15.0[d]	15.1[cd]	15.3[bc]	15.6[a]	15.5[ab]	0.09	0.001	0.001	0.865
Met	6.58[c]	6.70[b]	6.72[ab]	6.71[ab]	6.79[a]	6.73[ab]	0.029	0.001	0.001	0.024
Thr	11.6[bc]	11.5[c]	11.6[c]	11.7[bc]	12.1[a]	11.9[ab]	0.09	0.001	0.001	0.239
Trp	4.03[b]	4.01[b]	4.02[b]	4.08[b]	4.27[a]	4.21[a]	0.026	0.001	0.001	0.037
Val	15.1[d]	15.3[cd]	15.3[cd]	15.4[bc]	15.8[a]	15.6[ab]	0.10	0.001	0.001	0.554
IAA	112[d]	113[cd]	113[cd]	114[bc]	117[a]	116[ab]	0.6	0.001	0.001	0.869
Dispensable amino acids										
Ala	13.0[d]	13.2[c]	13.3[bc]	13.4[bc]	13.7[a]	13.5[ab]	0.08	0.001	0.001	0.103
Asp	19.9[bc]	19.7[c]	19.8[c]	19.9[c]	20.7[a]	20.4[ab]	0.16	0.001	0.001	0.111
Cys[2]	5.92[ab]	5.91[ab]	5.81[bc]	5.78[c]	6.00[a]	6.04[a]	0.044	0.002	0.035	0.001
Glu	57.1[bc]	57.2[bc]	57.0[c]	57.7[b]	58.9[a]	58.5[a]	0.23	0.001	0.001	0.152
Gly[2]	14.4[bc]	14.3[bc]	14.2[c]	14.5[b]	15.1[a]	15.0[a]	0.09	0.001	0.001	0.001
Pro	15.9[c]	16.3[b]	16.0[c]	16.3[b]	16.9[a]	16.8[a]	0.09	0.001	0.001	0.035
Ser	11.3[c]	11.4[c]	11.6[bc]	11.6[bc]	12.1[a]	11.8[b]	0.09	0.001	0.001	0.318
DAA	138[b]	138[b]	138[b]	139[b]	143[a]	142[a]	0.7	0.001	0.001	0.144
Total AA	250[b]	251[b]	254[b]	253[b]	261[a]	258[a]	1.3	0.001	0.001	0.379

DAA = total digestible dispensable amino acid contents; IAA = total digestible indispensable amino acid contents; Total AA = total digestible content of all amino acids.
Standardized digestible amino acid content (g/kg) = [ingredient amino acid content (g/kg) × standardized ileal digestibility (%)]/100.
[a-d] Means in a row not sharing a common letter are significantly different ($P < 0.05$).
[1] Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7-, 14- and 21-d-old birds, respectively; 8 birds per replicate for 28- and 35-d-old birds; and 6 birds per replicate for 42-d old birds).
[2] Semi-indispensable amino acids for poultry.

Table 11
Percentage increase (%) in the standardized ileal digestibility coefficients of nitrogen (N) and amino acids in soybean meal and canola meal after correction of apparent ileal digestibility coefficients for age-specific endogenous amino acid losses of broilers.

Item	Soybean meal						Canola meal					
	Age, d						Age, d					
	7	14	21	28	35	42	7	14	21	28	35	42
N	5.25	2.71	2.59	2.73	2.68	1.87	7.31	3.68	3.60	3.68	3.52	2.47
Indispensable amino acids												
Arg	1.97	0.99	0.99	1.10	0.97	0.66	3.31	1.43	1.55	1.65	1.48	1.02
His	2.51	1.37	1.26	1.25	1.23	0.79	3.36	1.87	1.64	1.87	1.68	1.20
Ile	3.26	1.62	1.62	1.62	1.36	0.92	4.91	2.47	2.47	2.60	2.24	1.51
Leu	3.03	1.38	1.50	1.62	1.48	1.04	4.34	2.12	2.12	2.37	2.05	1.34
Lys	2.28	1.01	1.01	1.12	1.00	0.67	4.05	1.74	1.75	1.86	1.67	1.03
Met	4.49	2.01	2.03	2.26	2.01	1.12	3.69	1.74	0.93	1.97	1.60	0.92
Thr	8.83	4.90	4.62	4.41	4.51	3.46	11.4	6.10	5.88	5.52	5.49	4.02
Trp	3.68	2.02	2.13	2.37	2.06	1.50	4.72	2.63	2.92	2.89	2.60	1.87
Val	4.03	2.10	1.99	2.12	1.95	1.40	5.07	2.76	2.64	2.76	2.39	1.65
IAA	3.84	1.85	1.85	1.97	1.70	1.26	4.79	2.43	2.45	2.56	2.33	1.48
Dispensable amino acids												
Ala	4.06	1.98	1.99	2.11	1.97	1.29	5.34	2.53	2.54	2.79	2.57	1.72
Asp	2.92	1.55	1.53	1.54	1.39	0.94	6.66	3.49	3.50	3.50	3.31	2.26
Cys[1]	10.30	6.13	5.53	5.38	5.41	4.29	7.67	7.68	4.28	3.86	4.00	3.13
Glu	2.11	1.00	1.01	1.11	1.10	0.67	2.73	1.30	1.31	1.42	1.26	0.80
Gly[1]	4.64	2.33	2.31	2.43	2.36	1.67	4.90	2.47	2.65	2.74	2.46	1.67
Pro	4.49	2.37	2.49	2.25	2.19	1.64	5.24	2.70	2.89	2.84	2.68	1.87
Ser	5.24	2.74	2.71	2.60	2.79	1.99	9.01	4.63	4.60	4.46	4.38	3.09
DAA	4.68	2.42	2.40	2.40	2.34	1.78	5.42	3.64	3.11	3.10	2.83	2.06
TAA	4.13	2.24	2.11	2.12	2.07	1.39	5.01	3.01	2.76	2.75	2.63	1.76

DAA = average digestibility of dispensable amino acids; IAA = average digestibility of indispensable amino acids; TAA = average digestibility of all amino acids.
[1] Semi-indispensable amino acids for poultry.

the average TAA digestibility coefficients in the CM were 5.01% (d 7), 3.01% (d 14), 2.76% (d 21), 2.75% (d 28), 2.63% (d 35), and 1.76% (d 42), respectively.

4. Discussion

4.1. Performance, gizzard pH and jejunal digesta viscosity

Irrespective of the PS, the DFI and DWG increased as the birds advanced in age. The decrease in gizzard pH at d 14, compared to d 7, in both the SBM (3.17 vs. 2.92) and CM (3.16 vs. 2.70) was in agreement with the findings of David et al. (2020). The cause of reduction in gastric pH is likely to be due to increased gastric secretions in the proventriculus from d 2 to 15 (Rynsburger, 2009). Though concurrent secretions of pepsin and hydrochloric acid (HCl) from the proventriculus enhance with age (Nitsan et al., 1991), the pH increases in gizzard digesta after d 14 in both PS could be explained by greater feed intake as the birds grow (Ravindran, 2013). With advancing age, the increased load of feed with pH close to neutral in the gastrointestinal tract (GIT) dilutes the HCl elevating the gizzard pH. The dietary proteins are exposed to HCl in the proventriculus and the acidic conditions initiate protein digestion by denaturing the protein and exposing the peptide bonds to enzyme hydrolysis. The first step in protein digestion is the conversion of pepsinogen to pepsin that requires an acidic environment (Rynsburger, 2009). Therefore, the expectation was that the elevation of gizzard pH with advancing broiler age would negatively influence protein digestion. However, no correlation (r = 0.158; P > 0.05) was observed between the gizzard pH and the average SIDC of TAA in SBM in the current study. On the other hand, in the case of CM, a positive correlation (r = 0.553; P < 0.001) was exhibited, a finding that is difficult to explain.

A tendency (quadratic; P = 0.060) for age effect was observed for the jejunal digesta viscosity in birds fed the SBM diet. In the CM, the viscosity increased from d 7 to 21 and then decreased from d 28 to 35, followed by an increase at d 42. The CM contains 14.3 g/kg soluble non-starch polysaccharides (NSP; Meng and Slominski, 2005). According to Choct and Annison (1992a), the soluble NSP is positively related to digesta viscosity. Increased feed intake by age and, consequently, increased intake of dietary soluble NSP may have contributed to the gradual rise of intestinal viscosity until d 21. The decreased viscosity after d 21 may be due to the increased resilience of older birds to soluble NSP because of the increased activity and stability of gut microbiota (Choct and Annison, 1992b). The cause of higher viscosity at d 42 in CM is hard to explain. It is noteworthy that the jejunal viscosity in both SBM (r = −0.202; P > 0.05) and CM (r = −0.038; P > 0.05) was not correlated with the average SIDC of TAA. Besides the gizzard pH and viscosity, numerous other factors may potentially affect the AA digestibility at different broiler ages.

4.2. Ileal digestibility coefficients of nitrogen and amino acids

According to a limited number of reports (Huang et al., 2005; Adedokun et al., 2007a; 2008), the AIDC AA varies based on the age of broilers. In the present study, the AIDC of N, and the average AIDC of IAA, DAA and TAA in SBM increased as the birds aged. With the exceptions of Leu, Asp and Glu, an increase was also recorded in the AIDC of all individual AA. The average AIDC of TAA from d 7 to 28 was 2.3% lower than that of d 35 and 1.2% lower than d 42. The reason for lower AIDC AA in SBM at an earlier age may be partly due to the presence of α-galactosides (raffinose, verbascose, stachyose), which cannot be digested by young broilers (Carre et al., 1995).

The trend of increased AIDC of N and AA with age in SBM is in agreement with previous findings (Noy and Sklan, 1995; Huang et al., 2005; Adedokun et al., 2008). Noy and Sklan (1995) reported an increase in the ileal N digestion from d 4 (78%) to 21 (92%) following the feeding of corn-SBM-based diets to broilers. Uni et al. (1995), in a study with corn-SBM-based diets, reported an increase in N digestion in both heavy and light strain chicks from 70% on d 4 to 90% on d 14. Adedokun et al. (2008) reported the AIDC AA in SBM and CM at two ages of broilers (d 5 vs. 21). The AIDC of TAA in SBM were reported to be 0.810 (d 5) and 0.860 (d 21), respectively. The AIDC AA in CM also increased by age. Huang et al. (2005), investigating the age effect (d 14, 28 and 42) on the AIDC AA in a wide range of ingredients (wheat, sorghum, corn, SBM, CM, meat and bone meal, cotton seed meal and mill run), concluded that the AIDC AA increased in older birds though the age effect varied depending on the ingredient and AA. In line with present findings, and compared to d 14, the AIDC AA was found to be higher at d 28 and 42 in SBM and CM. The average AIDC of TAA in SBM at d 28 and 42 was similar and 2.35% higher than those at d 14.

In the current study, the AIDC of N, average AIDC of IAA, DAA and TAA in CM increased with advancing broiler age. The digestibility estimates were lower from d 7 to 28 and increased thereafter. Compared to the average AIDC of TAA from d 7 to 28, the AIDC of TAA at d 35 and d 42 were 4.72 and 4.46% higher, respectively. The AIDC of individual AA increased at different magnitudes. Toghyani et al. (2015) reported a decrease in the AIDC of CP and most AA at d 10 compared to d 24 of broiler in expeller extracted CM processed at different temperatures (90, 95 and 100 °C). A study by Huang et al. (2005) found that the average AIDC of TAA in CM at d 14 was 1.23% lower compared to either d 28 or 42 with no differences in the digestibility estimates between these two ages. Adedokun et al. (2008) observed 3.59% lower AIDC of TAA in CM at d 5 compared to d 21. Canola meal contains relatively high contents of fiber and phytate; both can lower AA utilization (Cowieson et al., 2009). The protein-phytate complex in CM is poorly hydrolyzed decreasing the availability of some AA (Bell and Keith, 1991). The dietary phytate also increases the EAA flows at the ileal level of birds by increasing secretion and/or the reduction of digestion and reabsorption of AA (Cowieson and Ravindran, 2007; Cowieson et al., 2009). Edwards et al. (1989) reported that older birds can utilize phytate-P more than young chicks. The greater endogenous phytase activity in the GIT of older birds might enhance the utilization of phytate (Ravindran et al., 1995), thus removing the adverse impacts on AA.

Three explanations may be provided for the increased AIDC AA in SBM and CM with age. First, the GIT and digestive physiology are immature at hatch, and development in these systems with age is the key factor contributing to increased AA digestibility (Ravindran and Abdollahi, 2021). Nitsan et al. (1991) found that the pancreatic weight of newly hatched chicks increased at d 8 and 23 by 10- and 30-fold, respectively. The corresponding increases in small intestinal weight were recorded to be 10-fold (d 8) and 20-fold (d 23), respectively. The insufficient secretion and reduced activity of proteases (trypsin, chymotrypsin) in the pancreas and small intestine during the early stage of development is a notable factor contributing to the compromised AA digestibility (Nitsan et al., 1991). Second, digesta passage rate is slower through the small intestine (Hurwitz and Bar, 1966; Sklan et al., 1975) and GIT (Hillerman et al., 1953; Shires et al., 1987) in older birds than young birds. The digesta passage rate directly influences nutrient digestion by regulating the contact time among the nutrients, digestive enzymes and intestinal surface (Mateos et al., 1982; Shires et al., 1987). Third, trends in the AIDC AA in SBM and CM are clearly affected by the contribution of AA of endogenous origin in the ileal digesta. As observed in our previous study (Barua et al., 2021d), the EAA flow varied depending on broiler age. The EAA flow was higher at d 7, declined on d 14 with no further change until d 35, and decreased again on d 42. The EAA flow is measured as a balance between the secretion and reabsorption of AA. The reduction in EAA flow with advancing age has been attributed to decreased mucin production, longer digesta retention, and increased digestion and re-absorption of EAA (Adedokun et al., 2007b; Barua et al., 2021d).

The AIDC of N and AA increased with broiler age in SBM, but the SIDC of N, average SIDC of IAA and TAA, remained stable throughout the growth cycle as demonstrated by the lack of quadratic or linear effects of age. The average SIDC of DAA and the SIDC of some individual AA (Arg, Thr, Trp, Cys and Pro) showed a quadratic pattern of change with age. The findings indicate that the influence of age on the SIDC AA in SBM was masked by the EAA flows, particularly in younger broilers. Similarly, the effect of age on SIDC estimates in CM was also inconsistent. The SIDC of N, average SIDC of DAA and TAA were higher at d 7 to 14, declined at d 21, and then increased from d 28 to 42. The average SIDC of IAA in CM from d 7 to 28 was lower than that at d 35 (3.72%) and d 42 (2.55%). Toghyani et al. (2015) reported a similar finding to the present study, indicating higher SIDC for most AA at d 24 compared to d 10 in the CM.

Striking differences observed in age effect trends between the AIDC and SIDC AA estimates for both PS aligned with findings from prior research (Adedokun et al., 2008; Szczurek et al., 2020; Barua et al., 2021b; 2021c). Adedokun et al. (2008) found that AIDC AA in SBM and CM increased with broiler age (d 5 vs. 21), while the SIDC AA in SBM and CM was unaffected.

Some studies indicated opposing effects, where there was a reduction of AA digestibility in older birds (Fonolla et al., 1981; Zuprizal et al., 1992). The above contradictions may be attributed to the site of measurement (ileal vs. excreta; Ravindran and Bryden, 1999) and reflect modifications in AA by hindgut microbial fermentation and urinary contamination. Adedokun et al. (2007a), using ileal measurements, found that the apparent digestibility of TAA in beef-originated meat and bone meal at 5 d (0.705) was higher than that of 21 d old broilers (0.595). Correction for age-specific endogenous AA failed to make any difference and the SIDC of TAA at d 5 was 20.3% higher than d 21. Conversely, the AIDC of TAA in pork-originated meat and bone meal was 32.1% higher at d 21. The SIDC of TAA also increased with age and the estimated values were 0.588 and 0.752 at d 5 and d 21, respectively.

Although the AIDC AA estimates have limitations, they are still presented in this study for two reasons. Firstly, most of the existing AA digestibility data are based on AIDC. Secondly, it can help in comprehending the extent of age impact on the SIDC AA after standardizing the AIDC data with age-specific EAA flows. The differences between the average AIDC and SIDC of TAA in SBM and CM, respectively, with the use of age-specific EAA flows, are clearly depicted in Figs. 1 and 2.

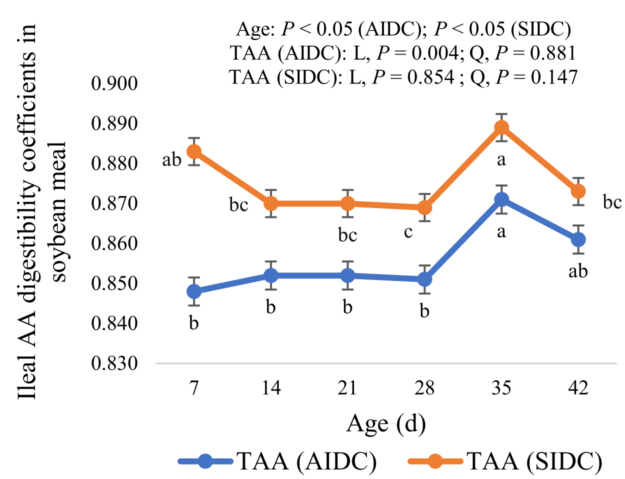

Fig. 1. Apparent and standardized ileal digestibility coefficients of total amino acids (TAA) in soybean meal (bars represent means ± SE) as influenced by broiler age. [a, b, c] Values not sharing a common superscript differ significantly ($P < 0.05$). AIDC = apparent ileal digestibility coefficients; SIDC = standardized ileal digestibility coefficients; L = linear; Q = quadratic.

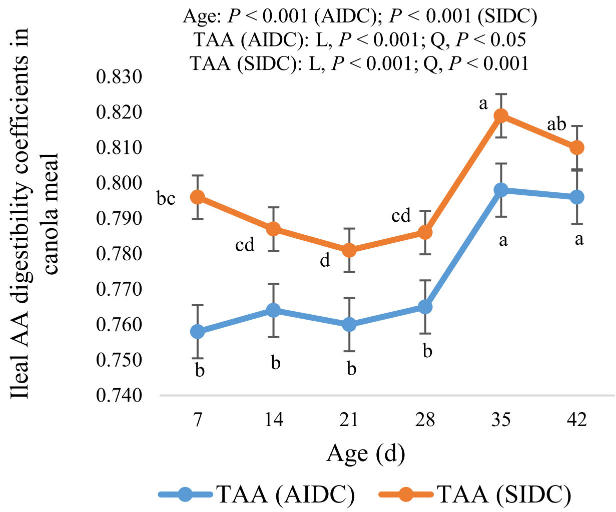

Fig. 2. Apparent and standardized ileal digestibility coefficients of total amino acids (TAA) in solvent-extracted canola meal (bars represent means ± SE) as influenced by broiler age. [a, b, c, d] Values not sharing a common letter differ significantly ($P < 0.05$). AIDC = apparent ileal digestibility coefficients; SIDC = standardized ileal digestibility coefficients; L = linear; Q = quadratic.

The results revealed significant variations in the percentage increase of SIDC AA estimates compared to AIDC data at different ages. In SBM, the increase in average SIDC of TAA was the highest at d 7 (4.13%), followed by a decrease at d 14 to 35 (2.07% to 2.24%), and further decline at d 42 (1.39%). Similarly, in the CM, the percentage increase in the SIDC of average TAA at d 7 was 5.01%, which decreased at d 14 to 35 (2.63% to 3.01%) and d 42 (1.76%). These findings indicate that the magnitude of increase in SIDC AA over AIDC AA reduces as age progresses. Therefore, using a single EAA flow to standardize AIDC data at different ages may underestimate SIDC AA at early ages and overestimate it at later ages.

5. Conclusions

For the first time, this study provides information on the age-related changes in the AIDC AA and SIDC AA in SBM and CM throughout the entire growth cycle of broiler chickens. The AIDC AA in both PS increased as the broiler age increased, but the trends in SIDC AA differed between the PS. In SBM, no patterns of age influence were observed in case of the average SIDC of IAA and TAA. However, inconsistent patterns of age effects were observed for the average SIDC of DAA and the SIDC of some individual AA (Arg, Thr, Trp, Cys and Pro). In CM, an increase in the average SIDC of DAA and TAA was seen from d 7 to 14, which then decreased at d 21, followed by an increase after d 28. The average SIDC of IAA, however, remained lower until d 28 and increased beyond d 35. These results suggest that the effect of age on the SIDC AA varies based on the PS and the specific AA, and that the age effect on AA digestibility needs to be considered in ingredient matrices for precise feed formulation.

Author contributions

M. Barua: conceptualization, designing, animal trial, investigation, writing - original draft preparation, data curation; **M. R. Abdollahi**: conceptualization, study design, feed formulation, supervision, reviewing and editing; **F. Zaefarian**: supervision, data curation, editing; **C. K. Girish**: methodology, supervision; **T. J. Wester**: supervision, manuscript review, **P. V. Chrystal**: manuscript review; **V. Ravindran**: data evaluation, revising critically and providing comments on manuscript, editing.

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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