

A comparative assessment of amino acid digestibility of protein feedstuffs in broiler chicks standardized with a nitrogen-free or highly digestible protein diet

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Abstract

The amino acid profile of a protein feedstuff and the extent to which these amino acids are utilized within the animal are primary determinants of its true protein quality. A variety of protocols exist for the assessment of protein quality, and the extent of their impact on amino acid digestibility in broiler chickens was assessed in this study. This study evaluated the apparent ileal amino acid digestibility (AIAAD) and standardized ileal amino acid digestibility (SIAAD) of full-fat black soldier fly larvae (BSFL), soyabean meal, and fish meal in 21d Arbor Acres broiler chickens. Using two standardization approaches- nitrogen-free diet (NFD) and highly digestible protein (HDP) diet—this study also compared basal endogenous amino acid (BEAA) flow profiles. Broiler chickens were assigned to one of five dietary treatments: nitrogen-free and highly digestible protein (casein, 24.59%) reference diets alongside full-fat black soldier fly larvae, soyabean meal, and fish meal diets formulated to contain the protein feedstuffs as the sole protein supply, and titanium dioxide was included as an indigestible marker. Chickens per treatment were allotted to 5 replicates (2 chickens/replicate), fed from 17-21d and sacrificed at 21d to harvest ileal digesta. Amino acids and titanium analyses of feed and digesta showed that BSFL had significantly higher AIAAD and SIAAD values for most amino acids than soyabean meal and were comparable to fish meal, except for isoleucine, threonine, and proline ($p > 0.05$). BEAA flow was significantly higher in birds fed the NFD than those fed the HDP diet for all amino acids ($p < 0.001$). Consequently, the choice of standardization diet significantly influenced SIAAD estimates for several amino acids, with HDP-based values generally lower due to reduced endogenous losses. These findings affirm the potential of BSFL as a viable alternative to conventional protein feedstuffs in broiler nutrition. Also, the HDP diet provides a more physiologically relevant estimate of BEAA flow, making it a preferred method for SIAAD determination in poultry studies.

Keywords: Alternative protein feedstuffs, Endogenous amino acid flow, Meat-type chickens, Precision nutrition, Poultry nutrition

Running title: Standardized amino acid digestibility of protein feedstuffs

Évaluation comparative de la digestibilité des acides aminés des matières premières protéiques chez les poussins de chair standardisée avec un régime sans azote ou à base de protéines hautement digestibles



Résumé

Le profil en acides aminés d'une matière première protéique et la mesure dans laquelle ces acides aminés sont utilisés par l'animal sont des déterminants primaires de sa véritable qualité protéique. Il existe une variété de protocoles pour l'évaluation de la qualité des protéines, et l'étendue de leur impact sur la digestibilité des acides aminés chez les poulets de chair a été évaluée dans cette étude. Cette étude a évalué la digestibilité iléale apparente des acides aminés (DIAAA) et la digestibilité iléale standardisée des acides aminés (DISAA) des larves entières de mouche soldat noire (BSFL), du tourteau de soja et de la farine de poisson chez des poulets de chair Arbor Acres de 21 jours. En utilisant deux approches de standardisation – un régime sans azote (NFD) et un régime à base de protéines hautement digestibles (HDP) – cette étude a également comparé les profils de flux d'acides aminés endogènes basaux (BEAA). Les poulets de chair ont été répartis dans l'un des cinq traitements alimentaires : des régimes de référence sans azote et à base de protéines hautement digestibles (caséine, 24,59 %) ainsi que des régimes de larves entières de mouche soldat noire, de tourteau de soja et de farine de poisson

formulés pour contenir les matières premières protéiques comme seule source de protéines, et du dioxyde de titane a été inclus comme marqueur indigestible. Les poulets par traitement ont été répartis en 5 répétitions (2 poulets/répétition), nourris de 17 à 21 jours et sacrifiés à 21 jours pour prélever le digesta iléal. Les analyses des acides aminés et du titane dans l'aliment et le digesta ont montré que les BSFL avaient des valeurs de DIAAA et DISAA significativement plus élevées pour la plupart des acides aminés que le tourteau de soja et étaient comparables à la farine de poisson, sauf pour l'isoleucine, la thréonine et la proline ($p > 0,05$). Le flux BEAA était significativement plus élevé chez les oiseaux nourris avec le NFD que chez ceux nourris avec le régime HDP pour tous les acides aminés ($p < 0,001$). Par conséquent, le choix du régime de standardisation a influencé de manière significative les estimations de DISAA pour plusieurs acides aminés, les valeurs basées sur HDP étant généralement plus faibles en raison de pertes endogènes réduites. Ces résultats confirment le potentiel des BSFL en tant qu'alternative viable aux matières premières protéiques conventionnelles dans l'alimentation des poulets de chair. De plus, le régime HDP fournit une estimation physiologiquement plus pertinente du flux BEAA, ce qui en fait une méthode préférée pour la détermination de la DISAA dans les études avicoles.

Mots-clés : Matières premières protéiques alternatives, Flux d'acides aminés endogènes, Poulets de type chair, Nutrition de précision, Nutrition avicole

Introduction

Amino acids are the fundamental building blocks of proteins, and they play crucial roles in bird growth, immune function, and overall productivity (Adhikari *et al.*, 2025). Unlike ruminants, poultry lack significant ability for microbial protein synthesis in the gut hence, the choice of dietary protein supplies is a crucial responsibility of the poultry nutritionist (Beski *et al.*, 2015, Moorby and Fraser, 2021). Protein quality is a critical selection criterion when seeking alternative protein feedstuffs, and the extent to which its nutrients are broken down and absorbed within the gastrointestinal environment is measured in digestibility studies (Tahergorabi and Hosseini, 2017). Assessing protein digestibility in ileal digesta rather than excreta in poultry species generates an estimate of apparent ileal digestibility, AID, an index corrected for amino acid digestibility alterations attributable to hindgut proteolysis (Adedokun *et al.*, 2011). The Standardized ileal digestibility (SID) protocol provides a more accurate estimate of protein and amino acids available to chickens as it accounts for endogenous amino acids arising from non-dietary proteins such as digestive secretions (bile, gastric, pancreatic and intestinal secretions), sloughed-off epithelial cells of the gastrointestinal tract, and mucoproteins, expelled in digesta (Barua *et al.*, 2021) and provides a basis for comparing the nutritional value of different feed ingredients on

a more equitable basis. Black soldier fly (*Hermentia illucens*) larva is proposed as an alternative protein for poultry nutrition hence, this study involved the estimation and comparison of the SIAAD of full-fat black soldier fly (*Hermentia illucens*) larvae and other conventional protein feedstuffs in broiler chickens using a nitrogen-free diet and a highly digestible protein diet as standards.

Materials and methods

The experimental protocols adopted in this study were reviewed and approved by the Department of Animal Science, University of Ibadan, Nigeria, according to the guidelines for ethical conduct and reporting of animal research (Jarvis *et al.*, 2005; Kilkenny *et al.*, 2010).

Ingredients and diet formulation

Three protein feedstuffs were used in this study: full-fat black soldier fly (*Hermetia illucens*) larvae obtained from a demonstration insect farm, and soyabean meal and fish meal from a reputable feedstore. Two reference diets were formulated: the semi-purified nitrogen-free diet (NFD) and highly digestible protein (HDP) diets, alongside the full-fat black soldier fly larvae, soyabean meal, and fish meal diets. All diets, except the NFD, were formulated to contain 21.5% crude protein and an energy-protein ratio of 144-169 kcal ME/g crude protein, with the test protein feedstuffs and casein supplying all the amino acids. Titanium dioxide was included as the indigestible marker

at 5g/kg of mash diets. The diet composition and analyzed composition of black soldier fly larvae are shown in Table 1, and the analyzed amino acid composition of the diets is shown in Table 2.

Birds and housing

Unsexed 1d-broiler chicks (Arbor Acres; 40.0 ± 2.0g) were procured from a commercial hatchery and brooded on deep litter with wood shavings as the preferred bedding material. The temperature was maintained at 32°C for the first 7 days, then gradually reduced to 24-29°C. Natural illumination was maintained during the study period, with *ad libitum* water and mash feed provided. From 1-16d, the chicks were fed a typical corn-soyabean meal-based diet containing 3007 kcal/kg metabolizable energy, 23% crude protein, 6.82% crude fat, 4.41% crude fibre, 1.13% calcium, and 0.48%

available phosphorus. On 14d, 40 chickens were selected and randomly assigned to 20 metabolic cages (2 per cage) with dimensions of 148cm×96cm×116cm. On 17d, chickens per cage were randomly offered one of the experimental diets until 21d.

Determination of Ileal Nutrient Digestibility

21d-old chickens were fasted for two hours post-feeding, then immobilized and euthanized by a jugular bleed. Ileal digesta was harvested by excising the section between Merkel's diverticulum and approximately 5mm proximal to the ileo-cecal junction and gently massaging its content into a plastic container. Digesta was pooled per cage and stored at -20°C, then subsequently dehydrated in a forced-air oven at 55°C until constant weight. Diets and digesta were ground in an electric mill and stored in air-tight bags until analyses.

Table 1. Composition and calculated analysis of experimental diets (g/kg, as-is basis), and analyzed composition of Black soldier fly larvae

Ingredients	Diets				
	Nitrogen-free diet, NFD	Highly digestible protein, HDP	Black soldier fly larvae	Soyabean meal	Fish meal
Soyabean meal	-	-	-	483.00	-
Black soldier fly larvae	-	-	410.00	-	-
Fish meal	-	-	-	-	323.00
Casein	-	245.90	-	-	-
Corn starch	766.50	579.30	415.00	359.30	555.50
Dextrose	80.00	40.00	85.50	60.00	28.00
Arbocel ¹	75.00	60.00	55.00	-	55.00
Soya oil	25.00	40.00	5.00	57.00	10.00
Dicalcium phosphate	30.50	15.50	16.50	18.20	14.00
Limestone	7.00	6.80	0.50	10.00	2.00
Sodium bicarbonate	3.50	2.00	2.00	2.00	2.00
Vitamin-mineral premix ²	5.00	3.00	3.00	3.00	3.00
Sodium chloride	2.50	2.50	2.50	2.50	2.50
Titanium oxide	5.00	5.00	5.00	5.00	5.00
Total	1000	1000	1000	1000	1000
Calculated nutrient analysis					
Crude protein (%)	0.54	21.48	21.50	21.50	21.50
ME (kcal/kg)	3207	3629	3143	3097	3155
Energy : Protein		168.95	146.19	144.05	146.74
Digestible methionine (%)	0.08	0.59	0.43	0.34	0.59
Digestible lysine (%)	0.15	1.80	1.26	1.38	1.62
Digestible threonine (%)	0.23	0.95	0.94	0.93	1.00
Analyzed composition of Black soldier fly larvae					
Moisture Content (%)	8.18 ± 0.79				
Crude Protein (%)	51.92 ± 0.09				
Crude Fibre (%)	5.80 ± 0.15				

Ether Extract (%)	15.10 ± 0.20
Ash (%)	18.50 ± 0.35

¹ A fibrillated lignocellulosic fibre concentrate, J. Rettenmaier & Sohne GmbH & Co KG, Rosenberg, Germany

² *Optimix poultry chick nourisher, Animal Care, Nigeria. Provided the following per kg/diet: vitamin A, 20,000 IU; vitamin D3, 4,000 IU; vitamin E, 20 mg; vitamin K3, 4 mg, vitamin B1, 3 mg; vitamin B2, 10 mg; niacin 16 mg; calpan 16 mg; vitamin B6, 6 mg; vitamin B12, 0.03 mg; choline chloride, 200 mg; folic acid, 2 mg; biotin, 0.1 mg; manganese, 120 mg; iron, 40 mg; zinc, 100 mg, copper, 10 mg; iodine, 2 mg; cobalt, 0.4 mg; selenium, 0.4 mg; antioxidant, 250 mg.*

Table 2. Analyzed amino acid composition of the experimental diets (g/100g of feed on as-is-basis)

Item	Diets				
	Nitrogen-free NFD	Highly digestible diet, protein, HDP	Black soldier fly larvae	Soyabean meal	Fish meal
Essential amino acids					
Leucine	0.39	8.86	8.08	6.77	8.76
Lysine	0.30	4.70	4.18	3.53	4.49
Isoleucine	0.33	4.28	2.14	3.16	5.05
Histidine	0.19	2.35	5.36	2.44	2.33
Arginine	0.37	5.36	1.19	5.22	6.10
Methionine	0.30	6.29	3.57	1.24	1.24
Phenylalanine	0.36	4.33	3.18	4.77	3.95
Threonine	0.25	3.44	0.81	2.10	3.33
Tryptophan	0.07	1.09	4.91	0.75	0.94
Valine	0.11	3.91	4.19	4.33	4.41
Nonessential amino acids					
Glycine	0.28	4.54	3.76	2.76	4.08
Alanine	0.35	4.50	4.28	4.54	4.53
Aspartic acid	0.40	9.65	8.34	6.81	9.26
Glutamic acid	0.15	16.18	13.2	13.25	14.77
Cysteine	0.11	1.28	1.12	1.35	1.22
Serine	0.31	4.05	4.16	4.16	3.76
Tyrosine	0.28	3.56	3.45	3.72	3.61
Proline	0.31	4.18	3.91	5.13	3.56
Total amino acids	4.85	88.30	78.60	76.00	85.35

Chemical Analyses

Feed and digesta for amino acid analysis were pretreated by acid (6N HCl) hydrolysis, and the amino acid concentration of hydrolysates determined by high-performance liquid chromatography (AOAC, 2005). Feed and ileal digesta samples for titanium determination were pretreated by acid (7.4M H₂SO₄) hydrolysis, and titanium in the hydrolysate was estimated by a spectrophotometric method using a known

amount of titanium dioxide to prepare common-matrix standards (Short *et al.*, 1996).

Calculations and Statistical Analysis

Basal endogenous amino acid flow estimates were derived from birds fed the nitrogen-free and highly digestible protein diets by the formula of Moughan *et al.* (1992),

$$\text{BEAA flow (mg/kg DMI)} = \text{AA concentration in digesta} \times \left[\frac{\text{titanium in diet}}{\text{titanium in digesta}} \right]$$

while AIAAD and SIAAD were calculated by formula of Lemme *et al.*, (2004). BEAA flow data derived from the NFD and HDP diets was analyzed by studentized t-test, while all other data were subjected to ANOVA and significant means separated by Tukey's Honestly Significant Difference (HSD) in JASP software (JASP 0.18.3). The cage served as the experimental unit, and statistical differences were declared at $p < 0.05$.

Results

The AIAAD of amino acids in 21d broiler chickens fed the black soldier fly larvae, soyabean meal, and fish meal diets is presented in Table 3. The AIAAD of all amino acids in black soldier fly larvae, except leucine, threonine, and proline, were *at par* with fish meal and superior to soyabean meal. No differences in AIAAD of threonine and proline were observed to be due to the test protein feedstuffs assessed ($p > 0.05$). Also, the AIAAD

of leucine in black soldier fly larvae and soyabean meal, 81.42% vs 76.76%, did not differ significantly ($p > 0.05$), however, AIAAD of leucine proved to be superior in fish meal, 83.24%, compared to soyabean meal ($p < 0.01$). The BEAA flow of amino acids derived from the NFD and HDP diets is presented in Table 4. The BEAA flow profile derived from broiler chickens on the HDP diet was markedly lower than the profile derived from chickens on the NDF diet ($p < 0.001$).

A comparison of the SIAAD for the protein feedstuffs—black soldier fly, soyabean meal, and fish meal—corrected for BEAA flow obtained from the NFD and HDP diets is presented in Table 5. The SIAAD profiles for black soldier fly, soyabean meal, and fish meal, derived from either correction method, followed the same trend, with no significant differences observed between the tested protein feedstuffs for threonine and proline ($p > 0.05$).

Table 3. Apparent ileal amino acid digestibility, %, in broiler chicks, fed diets containing different protein feedstuffs

Item	Diets		
	Black soldier fly larvae	Soyabean meal	Fish meal
Essential amino acids			
Leucine	81.41 ± 2.05 ^{ab}	76.76 ± 3.59 ^b	83.24 ± 0.05 ^a
Lysine	82.56 ± 3.27 ^a	73.42 ± 2.28 ^b	83.56 ± 0.03 ^a
Isoleucine	82.76 ± 0.42 ^b	74.11 ± 1.37 ^c	86.61 ± 0.41 ^a
Histidine	86.88 ± 0.97 ^a	80.14 ± 1.96 ^b	86.47 ± 1.99 ^a
Arginine	85.84 ± 1.76 ^a	75.27 ± 3.05 ^b	86.97 ± 0.22 ^a
Methionine	81.95 ± 2.06 ^a	70.89 ± 3.05 ^b	82.53 ± 1.09 ^a
Phenylalanine	85.37 ± 2.11 ^a	74.79 ± 4.05 ^b	84.56 ± 2.33 ^a
Threonine	82.23 ± 1.84	80.11 ± 1.26	82.81 ± 1.84
Tryptophan	86.88 ± 0.97 ^a	80.14 ± 1.96 ^b	86.47 ± 1.99 ^a
Valine	82.62 ± 0.88 ^a	76.39 ± 3.51 ^b	84.67 ± 0.44 ^a
Nonessential amino acids			
Glycine	82.02 ± 2.08 ^a	72.37 ± 3.20 ^b	84.29 ± 0.08 ^a
Alanine	81.73 ± 1.57 ^a	73.81 ± 4.96 ^b	82.67 ± 0.21 ^a
Aspartic acid	81.89 ± 1.66 ^a	76.09 ± 1.27 ^b	83.06 ± 0.60 ^a
Glutamic acid	81.26 ± 1.55 ^a	72.40 ± 3.10 ^b	83.34 ± 0.17 ^a
Cysteine	83.43 ± 0.13 ^a	73.64 ± 4.11 ^b	83.54 ± 1.60 ^a
Serine	83.08 ± 1.41 ^a	75.91 ± 3.69 ^b	82.45 ± 0.58 ^a
Tyrosine	81.29 ± 1.50 ^a	73.96 ± 3.83 ^b	83.23 ± 0.80 ^a
Proline	82.55 ± 1.02	80.77 ± 2.54	82.25 ± 2.00

Table 4. Basal ileal endogenous amino acid flows (mg/kg DM intake) derived from either a nitrogen-free diet, NFD or highly digestible protein, HDP, in 21d broiler chicken

Item	Nitrogen-free diet, NFD	Highly digestible protein, HDP	Probability value
Essential amino acids			
Leucine	499.00 ± 15.95 ^a	122.51 ± 21.40 ^b	< 0.001
Lysine	219.81 ± 4.08 ^a	66.20 ± 12.01 ^b	< 0.001
Isoleucine	241.67 ± 6.54 ^a	54.22 ± 9.45 ^b	< 0.001
Histidine	135.35 ± 1.61 ^a	33.50 ± 5.83 ^b	< 0.001
Arginine	270.71 ± 1.15 ^a	50.79 ± 8.97 ^b	< 0.001
Methionine	79.32 ± 0.18 ^a	18.73 ± 3.42 ^b	< 0.001
Phenylalanine	259.91 ± 12.03 ^a	61.85 ± 10.77 ^b	< 0.001
Threonine	184.12 ± 0.33 ^a	46.21 ± 8.04 ^b	< 0.001
Tryptophan	52.43 ± 3.03 ^a	15.46 ± 2.65 ^b	< 0.001
Valine	221.60 ± 0.52 ^a	55.86 ± 9.79 ^b	< 0.001
Nonessential amino acids			
Glycine	200.57 ± 8.35 ^a	60.58 ± 11.44 ^b	< 0.001
Alanine	251.43 ± 0.04 ^a	64.30 ± 11.28 ^b	< 0.001
Aspartic acid	547.75 ± 14.80 ^a	137.86 ± 24.00 ^b	< 0.001
Glutamic acid	797.61 ± 80.67 ^a	73.95 ± 13.17 ^b	< 0.001
Cysteine	76.41 ± 0.64 ^a	18.29 ± 1.18 ^b	< 0.001
Serine	224.85 ± 2.00 ^a	57.86 ± 10.07 ^b	< 0.001
Tyrosine	200.52 ± 3.20 ^a	50.79 ± 8.97 ^b	< 0.001
Proline	226.76 ± 7.81 ^a	58.81 ± 10.18 ^b	< 0.001

Table 5. Standardized ileal amino acid digestibility derived by either a nitrogen-free diet, NFD, or highly digestible protein, HDP, in broiler chicks fed diets containing different protein feedstuffs

	Nitrogen-free diet, NFD			Highly digestible protein, HDP			Probability value: NFD vs HDP		
	BSFL	Fishmeal	Soyabean meal	BSFL	Fishmeal	Soyabean meal	BSFL	Fishmeal	soyabean meal
Essential amino acids									
Leucine	87.37 ± 1.83 ^b	88.86 ± 0.10 ^{ab}	81.74 ± 3.52 ^c	82.88 ± 1.99 ^y	84.63 ± 0.02 ^{xy}	77.98 ± 3.58 ^z	0.02	<0.00	NS
Lysine	87.97 ± 2.66 ^b	88.46 ± 0.04 ^{ab}	79.30 ± 1.50 ^c	84.19 ± 3.09 ^y	85.04 ± 0.03 ^{xy}	75.19 ± 1.34 ^z	NS	<0.00	0.01
Isoleucine	88.35 ± 0.25 ^b	91.40 ± 0.56 ^a	80.94 ± 1.26 ^c	84.01 ± 0.38 ^y	87.69 ± 0.42 ^x	75.64 ± 1.34 ^z	<0.00	<0.00	0.00
Histidine	92.21 ± 0.77 ^b	91.75 ± 1.82 ^{ab}	85.70 ± 2.01 ^c	88.20 ± 0.92 ^y	87.78 ± 1.87 ^{xy}	81.52 ± 1.97 ^z	<0.00	NS	0.03
Arginine	89.54 ± 1.59 ^b	90.20 ± 0.45 ^{ab}	79.72 ± 2.37 ^c	86.53 ± 1.73 ^y	87.58 ± 0.25 ^{xy}	76.10 ± 2.92 ^z	0.04	<0.00	NS
Methionine	88.20 ± 1.70 ^b	88.76 ± 1.08 ^{ab}	77.37 ± 3.03 ^c	83.42 ± 1.98 ^y	84.00 ± 1.04 ^{xy}	72.42 ± 3.04 ^z	0.01	0.01	NS
Phenylalanine	90.88 ± 1.58 ^b	90.21 ± 2.06 ^{ab}	80.24 ± 4.04 ^c	86.68 ± 1.98 ^y	85.91 ± 2.18 ^x	76.09 ± 4.05 ^z	0.02	NS	NS
Threonine	87.57 ± 1.57	88.10 ± 1.41	86.14 ± 0.79	83.57 ± 1.77	84.14 ± 1.32	81.62 ± 1.11	0.02	0.03	<0.00
Tryptophan	91.29 ± 0.77 ^b	90.67 ± 2.15 ^{ab}	84.93 ± 1.61 ^c	88.18 ± 0.91 ^y	87.71 ± 1.92 ^{xy}	81.56 ± 1.86 ^z	0.00	NS	0.03
Valine	87.70 ± 0.60 ^b	89.69 ± 0.59 ^{ab}	81.52 ± 3.55 ^c	83.90 ± 0.82 ^y	85.93 ± 0.45 ^{xy}	77.69 ± 3.52 ^z	<0.00	<0.00	NS
Nonessential amino acids									
Glycine	87.36 ± 2.01 ^b	89.22 ± 0.19 ^{ab}	78.21 ± 3.15 ^c	83.64 ± 2.06	85.78 ± 0.10 ^{xy}	74.14 ± 3.19 ^z	0.04	<0.00	NS
Alanine	87.42 ± 1.44 ^b	88.22 ± 0.20 ^{ab}	79.35 ± 4.89 ^c	83.19 ± 1.54	84.09 ± 0.20 ^{xy}	75.23 ± 4.94 ^z	0.01	<0.00	NS
Aspartic acid	88.05 ± 1.42 ^b	88.98 ± 0.52 ^{ab}	82.71 ± 1.35 ^c	83.44 ± 1.59 ^y	84.55 ± 0.55 ^{xy}	77.76 ± 1.28 ^z	0.01	<0.00	0.00
Glutamic acid	81.85 ± 1.54 ^b	83.88 ± 0.20 ^{ab}	73.00 ± 3.10 ^c	81.81 ± 1.53 ^y	83.84 ± 0.16 ^{xy}	72.96 ± 3.10 ^z	NS	NS	NS
Cysteine	89.30 ± 0.27 ^b	89.28 ± 1.69 ^{ab}	79.33 ± 4.89 ^c	84.83 ± 0.15 ^y	84.92 ± 1.55 ^{xy}	75.00 ± 4.06 ^z	<0.00	0.03	NS
Serine	88.46 ± 1.43 ^b	88.40 ± 0.57 ^{ab}	81.32 ± 3.55 ^c	85.13 ± 1.42 ^y	84.72 ± 0.54 ^{xy}	77.97 ± 3.64 ^z	0.02	0.00	NS
Tyrosine	86.99 ± 1.39 ^b	88.61 ± 0.72 ^{ab}	79.36 ± 3.73 ^c	82.74 ± 1.47 ^y	84.60 ± 0.75 ^{xy}	75.33 ± 3.81 ^z	0.01	0.00	NS
Proline	88.35 ± 0.93	88.54 ± 2.00	85.19 ± 2.50	84.05 ± 1.00	83.89 ± 1.90	81.92 ± 2.53	<0.00	NS	NS

For all other amino acids except isoleucine, the SIAAD profiles for black soldier fly larvae and fish meal compared favorably but differed significantly from soyabean meal. However, the SIAAD for isoleucine varied significantly among the test feedstuffs, in the order: fish meal > black soldier fly larvae > soyabean meal. The SIAAD profiles for black soldier fly larvae, adjusted using BEAA flow estimates from the NFD and HDP diets in 21-day broiler chickens, differed significantly except for two amino acids: lysine ($p=0.11$) and glutamic acid ($p=0.97$). Similarly, the SIAAD profiles for fish meal, corrected using BEAA flow estimates from the NFD and HDP diets, differed significantly except for five amino acids: histidine ($p=0.07$), phenylalanine ($p=0.08$), tryptophan ($p=0.16$), glutamic acid ($p=0.80$), and proline ($p=0.05$). In contrast, the estimates for the SIAAD of soyabean meal in 21-day broiler chickens differed significantly only for lysine ($p=0.01$), isoleucine ($p=0.00$), histidine ($p=0.03$), threonine ($p<0.001$), tryptophan ($p=0.03$), and aspartic acid ($p=0.00$).

Discussion

Insect protein meals present a promising alternative to conventional protein feedstuffs such as soyabean meal and fishmeal for broiler feeding. Variable processing methods, incomplete characterization of essential amino acid bioavailability and digestibility in insect meals, and its inherent non-digestible components challenge its widespread adoption in broiler ration formulations. The apparent ileal amino acid digestibility, AIAAD, and standard ileal amino acid digestibility, SIAAD of variedly defatted black soldier fly larvae with fat content ranging from 4.6 to 20.7% and chitin content of 5.0 to 6.9% have been well documented (Schiavone *et al.*, 2017; Mwaniki and Kiarie, 2019; Matin *et al.*, 2021), however, there exists limited data recorded for full-fat defatted black soldier fly larvae (De Marco *et al.*, 2015; Abd El-Hack *et al.*, 2020; Mahmoud *et al.*, 2023).

The objectives of this study were thus to:

a. Compare the apparent ileal amino acid digestibility of full-fat black soldier fly larvae

with conventional protein feedstuffs, soyabean meal, and fish meal, in broiler chickens.

In line with this objective, the AIAAD values in the current study were similar to reports by Mwaniki and Kiarie (2019) for all amino acids except histidine (54.6%) and cysteine (57.4%), and were slightly lower than AIAAD values reported for full-fat black soldier fly larvae (Mahmoud *et al.*, 2023). Earlier results of SIAAD of soyabean meal also compare favourably with our results, except for methionine and arginine, which were higher than observed in the current study (Hussain *et al.*, 2024). Yet, AIAAD values considerably underestimate amino acid digestibility; hence, SIAAD is often explored for more precise estimates of dietary amino acid digestibility for diet formulations.

b. Compare the basal endogenous amino acid flow profile derived from a nitrogen-free diet vs. a highly digestible protein diet in broiler chickens.

Basal endogenous amino acid flow represents an estimate of the contribution of endogenous amino acids to apparent amino acid digestibility values and is considered independent of dietary ingredients and composition (Lemme *et al.*, 2004). However, the severe nitrogen imbalance of the nitrogen-free diet suppresses protease production and digestive stimulation while supporting body protein degradation, which is a clear departure from normal digestive physiology (Adeola *et al.*, 2016). These conditions result in markedly increased BEAA flow estimates, particularly from threonine, serine, proline, glutamic and aspartic acid, often arising from mucin glycoproteins (Barua *et al.*, 2021). Basal ileal amino acid flow derived from NFD in the current study was similar to estimates reported by Barua *et al.* (2021), especially for leucine, histidine, arginine, and glutamine, however, markedly lower BEAA flow estimates have been reported in broiler chicks of similar ages (Adedokun *et al.*, 2007b; Mahmoud *et al.*, 2023; Mwaniki and Kiarie, 2019), and a higher BEAA flow profile reported in broilers fed an NFD diet by Kong and Adeola (2013). In the current study, lower BEAA flow

estimates derived from HDP diets were obtained despite the inclusion of casein at 245.9 g/kg of the HDP diet. In another study, the inclusion of casein as the sole supply of amino acids, ranging from 50-150 g/kg HDP diets, steadily increased ileal endogenous flow in 21d chicks (Adedokun *et al.*, 2007b). Although direct studies on calorie-protein ratio and BEAAL are scarce, current knowledge of the relationship between energy-protein ratio and amino acid utilization in poultry clarifies that the “near-standard” calorie-protein ratio of the HDP diet used in the current study (168.9) supports more efficient amino acid utilization and reduced excretion of amino acids of endogenous origin (Barekatin *et al.*, 2021; Musigwa *et al.*, 2024), hence its lower BEAAL flow profile.

c. Derive standardized amino acid digestibility values for black soldier fly larvae, soyabean meal, and fish meal, and the effect of the standardization diet used.

Similar studies in 21d broiler chicks have shown no effect of standardization method, i.e., NFD vs HDP diets, on SIAAD estimates for meat and bone meal (Adedokun *et al.*, 2007a); however, higher estimates were observed for SIAAD of plant feedstuffs, corn, canola meal and DDGS, when standardized with an HDP diet (Adedokun *et al.*, 2008). The SIAAD values obtained for black soldier fly larvae in the current study agree with SIAAD values reported for defatted black soldier fly larvae (Mwaniki and Kiarie, 2019) and full-fat black soldier fly larvae (Mahmoud *et al.*, 2023) except for histidine, glycine, and cysteine. Conversely, SIAAD values reported for soyabean meal in this study were generally higher than published SIAAD values (Iyayi and Adeola, 2014; Ullah *et al.*, 2016; Barua *et al.*, 2020; Kim *et al.*, 2022; Liu *et al.*, 2024) and could be attributed to feedstuff quality/grade and processing conditions. A comparative assessment of SIAAD values for full-fat black soldier fly larvae, fish meal, and soyabean meal pooled from articles earlier cited affirms the results of our study, with fish meal consistently alluding to superior amino acid digestibility, and full-fat

black soldier fly larvae showing higher amino acid digestibility compared to soyabean meal.

Conclusion

In summary, our findings indicate a comparative SIAAD profile for fishmeal and full-fat black soldier fly larvae, superior to the SIAAD profile of soyabean meal for most amino acids, emphasizing the feasibility of black soldier fly larvae as a viable protein feedstuff in broiler diets.

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