



Original Article

**Standardized Ileal Amino Acid Digestibility of Bambara Nut
(*Vigna Subterranea* (L.) Verdc) In Broiler Chicks**

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ABSTRACT

This study evaluated the standardized ileal amino acid digestibility of processed Bambara Bambara nut in broiler chicks. Two hundred and forty (240) one-day-old Abor Acre plus-strain birds were fed a commercial broiler starter diet for 15 days. On day 16, the birds were randomly distributed into 4 treatments with 6 replicates and 10 birds each in a randomized complete block design. Diet 1 was a Nitrogen Free Diet (NFD); Diet 2 was a Highly Digestible Protein (HDP); while Diets 3 and 4 comprised roasted Bambara Bambara nut (RBN) and soaked + roasted Bambara Bambara nut (SRBN) as the sole sources of protein. On day 21, birds were sacrificed and digesta was collected from the terminal ileum. The Endogenous Amino Acid Losses (EAAL), Apparent Ileal Amino Acid Digestibility (AIAAD), Standardized Ileal Amino Acid Digestibility (SIAAD) were estimated. Data were analyzed using ANOVA and means separated using Duncan's Multiple Range Test at $\alpha_{0.05}$.

Amino acid with the highest flow was lysine and aspartic acid for nitrogen-free diet, while ileal endogenous alanine was higher in HDP. Overall, the apparent digestibility of soaked + roasted Bambara Bambara nut was significantly lower than birds fed roasted Bambara nut, except for arginine, methionine, tryptophan, glutamic acid, and glycine which did not differ statistically. Birds fed with roasted Bambara nut diet had the highest amino acid digestibility values after standardization except for tryptophan. In conclusion, birds on roasted Bambara nut diet maintained the highest digestibility values after standardization irrespective of the method (NFD or HDP) used for estimating endogenous ileal amino acid losses.

Keywords: Amino acid digestibility, Broiler chicken, Bambara nut, Endogenous amino acid flow, Processing methods.

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INTRODUCTION

Poultry is one of the most efficient converters of feed to animal protein. It has the quickest turnover compared with other livestock species (Longe, 2006). However, the escalating cost of conventional feedstuff which has resulted in the increasing cost of poultry production and consequent increase in the cost of poultry products (Iyayi, 2002) calls for the use of alternative, readily available, and cheaper feedstuff for poultry. In order to meet the nutritional requirements of chickens, it becomes imperative to have precise estimates of nutrient availability from these feedstuffs when formulating commercial diets (Adedokun *et al.*, 2007). However, Apparent Ileal Amino Acid Digestibility (AIAAD) which is used to estimate amino acid digestibility does not differentiate between dietary and endogenous amino acid (AA), thus overestimating AA digestibility. AIAAD corrected by basal endogenous AA losses represents standardized ileal amino acid digestibility (SIAAD) which gives a more accurate estimate of AA in feedstuffs. These estimated SIAAD values are useful for formulating a balanced diet for broiler chickens (Iyayi and Adeola, 2014).

Bambara nut (*Vigna subterranea*) is one of the underutilized indigenous legumes to Africa and is cultivated across the semi-arid sub-Saharan Africa region (Hillocks *et al.*, 2012). It is very hardy and can thrive under harsh environmental conditions. Bambara nut contains approximately 64.4% carbohydrate, 23.6% protein, 6.5% fat, and 5.5% fiber and is rich in minerals and essential amino acids (Azman *et al.*, 2019). However, it contains anti-nutritional factors such as phytic acid, tannins, trypsin inhibitor, etc. that limits its use as feed-in poultry nutrition, but different processing methods, such as boiling, crushing, soaking, roasting, etc., have been employed to deactivate the antinutrients in Bambara nut (Arijeniwa and Omoikhoje, 2004). The need to maximize the available non-conventional feed resource in poultry nutrition and to document standardized ileal amino acid digestibility coefficients for Bambara but becomes necessary. It was therefore the objective of this study to determine the standardized ileal amino acid digestibility of roasted Bambara nut and soaked + roasted Bambara nut in broiler chicks.

MATERIALS AND METHODS

Experimental site

The study was carried out at the poultry unit of the Teaching and Research Farm, University of Ibadan, Oyo State, Nigeria.

Processing of test ingredient

The Bambara nuts used for this study were purchased from a reputable popular market (Bodija market) in Ibadan, Nigeria. A known quantity (200g) of Bambara nut was roasted for 10-12 minutes and ground. Another portion of 200g of Bambara nut was soaked for 24 hours, sun-dried, and roasted for 10-12 minutes.

Management of birds and experimental diets

Two hundred and forty (240) one-day-old Abor Acre plus-strain of broiler chicks were obtained from a reputable commercial hatchery. The birds were fed a commercial broiler starter diet that met the nutrient requirements for broiler chicks (NRC, 1994) for 15 days. On day 16, the birds were tagged, weighed, and randomly distributed into

4 treatments with 6 replicates and 10 birds each in a randomized complete block design using an experimental animal allotment program (EAAP) of Kim and Lindermann (2007). As stated in Table 1, Diet 1 was a Nitrogen-free diet, Diet 2 comprised of Highly digestible protein (Casein). Diet 3 was Roasted Bambara nut as the sole source of amino acid while Diet 4 consisted Soaked + Roasted Bambara nut as the sole source of amino acid. From day 16 to day 21, the birds were fed experimental diets and they had free access to feed and water without restriction

Table 1. Gross composition (g/kg) of experimental broiler starter diets

Ingredients	NFD diet	HDP diet	Roasted Bambara nut	Soaked + roasted Bambara nut
Casein	0.00	100.00	0.00	0.00
Roasted Bambara nut	0.00	0.00	280.00	0.00
Soaked + roasted Bambara nut	0.00	0.00	0.00	300.00
Corn starch	272.00	289.00	260.00	260.00
Dextrose	554.00	579.00	356.00	348.00
Soy oil	50.00	50.00	30.00	20.00
Cellulose	60.00	60.00	60.00	60.00
Limestone	16.00	16.00	16.00	0.00
Vitamin premix	1.50	1.50	1.50	1.50
Mineral premix	1.00	1.00	1.00	1.00
Salt	2.50	2.50	2.50	2.50
DCP	18.00	18.00	16.00	16.00
Titanium dioxide	50.00	5.00	5.00	5.00
Total	1000.00	1000.00	1000.00	1000.00
Calculated nutrients				
Crude protein	0.00	82.70	50.12	63.30
Metabolizable energy (Kcal/kg)	3131.33	3090.04	2701.44	2826.06
Fat	0.00	2.00	14.84	19.50
Crude fibre	0.00	0.00	38.08	17.40
Calcium	9.86	9.91	11.62	11.90
Total Phosphorus	3.24	3.58	3.97	3.18

Collection of ileal digesta

On day 21, the birds were slaughtered and dissected to obtain digesta from the distal two-thirds of the ileum. Digesta samples were obtained by flushing the ileal content with distilled water into tight plastic containers and freeze-drying at 68°C. The dry samples were kept for further chemical analysis.

Chemical composition

The proximate composition and amino acid analysis of the diets and digesta samples were determined according to the methods of AOAC (2000). Titanium dioxide concentrations in the diets and digesta were analyzed using methods modified by Short *et al.*, (1996).

Digestibility calculations**Apparent ileal AA digestibility (%)**

$$\text{AIAAD} = 1 - \left[\left(\frac{\text{TiO}_2 \text{ diet}}{\text{TiO}_2 \text{ digesta}} \right) \times \left(\frac{\text{AA digesta}}{\text{AA diet}} \right) \right] \times 100$$

Standardized ileal AA digestibility (%)

$$\text{SIAAD} = \text{AIAAD}\% + \frac{\text{EAA flow (g/kg/DMI)}}{\text{AA content in feedstuff (g/kg/DM)}}$$

Endogenous amino acid flow

$$\text{EAA flow (g/kg/DMI)} = \left(\frac{\text{AA digesta (g/kg)}}{\text{TiO}_2 \text{ digesta}} \right) \times \text{TiO}_2 \text{ diet}$$

Where,

TiO₂ diet – titanium concentration of dietary intake

TiO₂ digesta – titanium concentration of ileal output

AA digesta - amino acid concentration of ileal output

AA diet – amino acid concentration of dietary intake

All values for TiO₂ diet, TiO₂ digesta, AA digesta, and AA diet are expressed as mg/kg of DM.

Statistical analysis

Data were subjected to descriptive statistics and analysis of variance (ANOVA) using SAS (2012). Treatment means were separated using Duncan's multiple range test at $p = 0.05$.

RESULTS AND DISCUSSION**Crude protein and amino acid profile of processed Bambara nut**

The results of crude protein and amino acid profile of roasted Bambara nut and soaked + roasted Bambara nut were as presented in Table 2. The crude protein level of roasted Bambara nut (RBN) and soaked + roasted Bambara nut (SRBN) observed in this study were 20.74% and 25.23% respectively. These results are in agreement with the findings of Belewu *et al.*, (2008), Akande *et al.*, (2009), and Tan *et al.*, (2020) who reported 24% CP, 20.05-22.54% (raw and roasted Bambara nut), and 23.6% respectively. Overall, leucine had the highest concentration in RBN (0.78mg/kg) and SRBN (0.71mg/kg), while the least concentration was glutamic acid with 0.05mg/kg in RBN and 0.03mg/kg in SRBN respectively. According to Kong and Adeola (2010), the amino acid (AA) concentrations documented for canola meal (0.52-6.7%) and soybean meal (6.6-8.65%) were higher than what was obtained in this study for RBN (0.09-0.78mg/kg) and SRBN (0.03-0.71mg/kg). Variations observed in the reported values may be due to varietal differences, geographical locations, processing methods, etc.

Table 2. Crude protein and amino acid profile of processed Bambara nut

Parameter	RBN	SRBN
DM(%)	96.57	95.12
CP(%)	20.74	25.23
<i>Indispensable amino acid (mg/kg)</i>		
Arginine	0.15	0.21
Histidine	0.67	0.69
Isoleucine	0.33	0.30
Leucine	0.78	0.71
Lysine	0.65	0.68
Methionine	0.06	0.11
Phenylalanine	0.38	0.58
Threonine	0.35	0.33
Tryptophan	0.13	0.14
Valine	0.22	0.23
<i>Dispensable amino acid (mg/kg)</i>		
Alanine	0.42	0.36
Aspartic acid	0.32	0.34
Cysteine	0.23	0.20
Glutamic acid	0.05	0.03
Glycine	0.09	0.12
Proline	0.20	0.22
Serine	0.30	0.31

DM= Dry matter, CP= Crude protein, RBN=Roasted Bambara nut; SRBN= Soaked + roasted Bambara nut

Endogenous Ileal amino acid flow of broiler chicks fed nitrogen-free diet or highly digestible protein

Table 3 represents the endogenous ileal amino acid flow of broiler chicks fed nitrogen-free diet (NFD) or highly digestible protein (HDP). There were no significant differences in the endogenous flow of amino acids in the birds on NFD or HDP diet except for lysine, alanine, and aspartic acid. Amino acid with the highest flow was lysine and aspartic acid for nitrogen-free diet, while ileal endogenous alanine was higher in the HDP diet. Several methods like the fasting of birds for 24 to 48 hours (used only to measure flows in the excreta), feeding of protein-free diet, linear regression (feeding of diets containing graded levels of protein), enzyme hydrolyzed casein and ultrafiltration, feeding of highly digestible protein, e.g. wheat gluten has been used to estimate basal EAA (endogenous amino acid) flow (Moughan *et al.*, 1990; Angkanaporn *et al.*, 1994; Ravindran and Bryden, 1999; Golian *et al.*, (2008); Adedokun *et al.*, 2011). However, there are variations with the published data. It has been reported that the concentration of dietary protein or amino acids in a feedstuff could likely influence EAA losses in the gut of monogastric animals (Adedokun *et al.*, 2011). This was contrary to the findings of the present study as the ileal endogenous amino acid flow of birds fed nitrogen-free diet (NFD) and highly digestible protein (HDP) diets were similar except for lysine, alanine, and aspartic acid.

Table 3. Endogenous Ileal amino acid flow of broiler chicks fed nitrogen-free diet or highly digestible protein

Amino acid	NFD	HDP	t _{value}	p _{value}
<i>Indispensable amino acid (mg/kg)</i>				
Arginine	0.20	0.09	2.22	0.05
Histidine	0.81	0.68	-1.95	0.09
Isoleucine	0.35	0.39	1.81	0.11
Leucine	0.83	0.92	2.01	0.08
Lysine	0.84 ^a	0.54 ^b	-3.39	0.01
Methionine	0.06	0.16	-2.11	0.07
Phenylalanine	0.49	0.39	-2.00	0.08
Threonine	0.35	0.38	2.38	0.44
Tryptophan	0.14	0.16	0.45	0.66
Valine	0.28	0.20	-2.06	0.07
<i>Dispensable amino acid (mg/kg)</i>				
Alanine	0.43 ^b	0.47 ^a	2.35	0.05
Aspartic acid	0.39 ^a	0.29 ^b	3.78	0.01
Cysteine	0.23	0.23	0.02	0.99
Glutamic acid	0.03	0.05	2.10	0.07
Glycine	0.17	0.07	1.92	0.09
Proline	0.25	0.21	1.97	0.08
Serine	0.24	0.26	0.60	0.56

^{ab}Means on the same row with different superscripts are significantly ($P < 0.05$) different. NFD= Nitrogen free diet, HDP= Highly digestible Protein (casein)

Apparent ileal amino acid digestibility of processed Bambara nut in broiler chicks

The apparent ileal digestibility of roasted Bambara nut (RBN) and soaked + roasted Bambara nut (SRBN) fed to broiler chicks is shown in Table 4. There were significant variations ($P < 0.05$) observed in the apparent digestibility values of amino acids (AAs) among dietary treatments. Overall, the apparent digestibility of soaked + roasted Bambara nut was significantly lower than those of the birds fed roasted Bambara nut, except for arginine, methionine, tryptophan, glutamic acid, and glycine which did not differ statistically. According to Fan *et al.*, (1994) apparent amino acid digestibility values are influenced by the level of feed intake and dietary protein concentrations, and are not corrected for endogenous amino acid flow.

Apparent ileal digestibility of phenylalanine (97.15%) and valine (89.11%) were highest in RBN and SRBN respectively while the least was proline (95.33%) in RBN and leucine (55.97%) in SRBN. There is a paucity of published data comparing the AIAAD of treated Bambara nut. However, according to Ravindran *et al.*, (2005), the apparent ileal amino acid digestibility values documented for faba beans (58-81%) and field peas (66-83%) in broiler chickens were slightly lower than the AIAAD values of roasted Bambara nut (92.25-97.15%) and soaked + roasted Bambara nut (64.41-97.13%) obtained in this study except for histidine (64.41%). Similarly, Kong and Adeola (2010) reported lower AIAAD values for canola meal (69.57-87.11%) in

White Pekin ducks compared to RBN and SRBN obtained for broiler chicks except for histidine.

Table 4. Apparent ileal amino acid digestibility of processed Bambara nut in broiler chicks

Amino acid	RBN	SRBN	t _{value}	p _{value}
<i>Indispensable amino acid (%)</i>				
Arginine	92.25	92.40	-0.05	0.96
Histidine	96.33 ^a	63.41 ^b	9.23	0.00
Isoleucine	96.69 ^a	81.18 ^b	5.41	0.01
Leucine	96.53 ^a	55.97 ^b	8.00	0.00
Lysine	96.26 ^a	62.70 ^b	9.26	0.00
Methionine	95.89	92.71	1.53	0.17
Phenylalanine	97.15 ^a	76.57 ^b	6.43	0.00
Threonine	96.68 ^a	84.23 ^a	9.06	0.00
Tryptophan	95.21	95.50	-0.24	0.82
Valine	95.66 ^a	89.11 ^b	4.81	0.00
<i>Dispensable amino acid (%)</i>				
Alanine	96.38 ^a	79.32 ^b	5.28	0.00
Aspartic acid	96.34 ^a	82.33 ^b	7.58	0.00
Cysteine	96.53 ^a	88.39 ^b	5.53	0.00
Glutamic acid	96.61	97.13	-0.48	0.65
Glycine	93.27	91.89	0.50	0.63
Proline	95.33 ^a	88.83 ^b	5.50	0.00
Serine	95.78 ^a	86.29 ^b	5.73	0.00

^{ab}Means on the same row with different superscripts are significantly ($P < 0.05$) different NFD= Nitrogen free diet, HDP= Highly digestible Protein (casein). RBN=Roasted Bambara nut; SRBN= Soaked + roasted Bambara

Standardized ileal amino acid digestibility of processed Bambara nut corrected with highly digestible protein

The standardized ileal amino acid digestibility of roasted Bambara nut and soaked + roasted Bambara nut corrected with highly digestible protein is represented in Table 5. Birds fed with roasted Bambara nut diet had the highest digestibility values after standardization for both indispensable and dispensable amino acids except tryptophan. However, arginine, methionine, and glycine were not significantly different. The need to correct for amino acids of endogenous origin in the estimation of standardized ileal amino acid digestibility (SIAAD) values has also been documented by different authors (Rodehutsord *et al.*, (2004); Lemme *et al.*, (2004); Adedokun *et al.*, (2008) and Iyayi and Adeola, (2014). Standardized ileal digestibility of phenylalanine (97.2%) and tryptophan (99.25%) were highest in RBN and SRBN respectively while the least was tryptophan (95.32%) in RBN and leucine (56.4%) in SRBN. Lemme *et al.*, (2004) documented lower SIAAD values for lupins (83-91%), peas (66-87%), and cottonseed meal (65-88%) compared with values obtained for SIAAD for roasted Bambara nut (92.31-97.2%) corrected with HDP diet.

Table 5. Standardized ileal amino acid digestibility of fed processed Bambara nut corrected with highly digestible protein

Amino acid	RBN	SRBN	t _{value}	p _{value}
<i>Indispensable amino acid (%)</i>				
Arginine	92.31	93.49	-0.49	0.64
Histidine	96.38 ^a	64.22 ^b	9.22	0.00
Isoleucine	96.72 ^a	81.57 ^b	5.40	0.00
Leucine	96.56 ^a	56.40 ^b	6.62	0.00
Lysine	96.31 ^a	63.51 ^b	9.25	0.00
Methionine	95.89	92.71	1.32	0.22
Phenylalanine	97.20 ^a	77.74 ^b	6.40	0.00
Threonine	96.72 ^a	84.63 ^b	9.02	0.00
Tryptophan	95.32 ^b	99.25 ^a	-5.91	0.82
Valine	95.73 ^a	90.34 ^b	4.43	0.00
<i>Dispensable amino acid (%)</i>				
Alanine	96.41 ^a	79.73 ^b	5.27	0.00
Aspartic acid	96.38 ^a	83.14 ^b	7.51	0.00
Cysteine	96.57 ^a	88.85 ^b	5.45	0.00
Glutamic acid	96.64	88.85 ^b	-0.78	0.46
Glycine	93.28	97.40	0.29	0.78
Proline	95.37 ^a	89.63 ^b	5.21	0.00
Serine	95.84 ^a	87.39 ^b	5.53	0.00

^{ab}Means on the same row with different superscripts are significantly ($P < 0.05$) different). RBN=Roasted Bambara nut; SRBN= Soaked + roasted Bambara nut

Standardized ileal amino acid digestibility of processed Bambara nut corrected with nitrogen free diet

The standardized ileal amino acid digestibility of roasted Bambara nut and soaked and roasted Bambara nut corrected with nitrogen-free diet is presented in Table 6. Similar trend was observed in the standardized ileal amino acid digestibility values of RBN and SRBN in broiler chicks corrected with a nitrogen-free diet as with those corrected with highly digestible protein. However, arginine, methionine, glutamic acid, and glycine did not differ significantly. Standardized ileal digestibility of phenylalanine (97.19%) and tryptophan (97.53%) was highest in RBN and SRBN respectively while the least was proline (95.37%) in RBN and leucine (56.4%) in SRBN. There were no significant differences in the values obtained between the two methods of estimating endogenous amino acid losses in this study. However, birds fed with roasted Bambara nut diet had the highest digestibility values after standardization for both indispensable and dispensable amino acids except tryptophan. Lemme *et al.*, (2004) documented lower SIAAD values for sunflower meal (80-93%), soybean meal (82-93%) and canola meal (73-87%) compared with values obtained for SIAAD for roasted Bambara nut (92.33-97.1%) corrected with NFD.

Table 6. Standardized ileal amino acid digestibility of processed Bambara nut corrected with nitrogen free diet

Amino acid	RBN	SRBN	t _{value}	p _{value}
<i>Indispensable amino acid (%)</i>				
Arginine	92.33	94.06	-0.74	0.48
Histidine	96.37 ^a	64.15 ^b	9.22	0.00
Isoleucine	96.72 ^a	81.60 ^b	5.40	0.00
Leucine	96.57 ^a	56.40 ^b	6.62	0.00
Lysine	96.31 ^a	63.44 ^b	9.25	0.00
Methionine	95.90	93.65	1.20	0.26
Phenylalanine	97.19 ^a	77.50 ^b	6.40	0.00
Threonine	96.72 ^a	84.63 ^b	9.02	0.00
Tryptophan	95.90 ^b	97.53 ^a	-5.03	0.00
Valine	95.71 ^a	89.98 ^b	4.55	0.00
<i>Dispensable amino acid (%)</i>				
Alanine	96.41 ^a	79.73 ^b	5.27	0.00
Aspartic acid	96.38 ^a	83.07 ^b	7.51	0.00
Cysteine	96.57 ^a	88.85 ^b	5.45	0.00
Glutamic acid	96.64	97.42	-0.81	0.44
Glycine	93.28	92.80	0.19	0.86
Proline	95.37 ^a	89.57 ^b	5.23	0.00
Serine	95.82 ^a	87.10 ^b	5.58	0.00

^{ab}Means on the same row with different superscripts are significantly ($P < 0.05$) different.

RBN=Roasted Bambara nut; SRBN= Soaked + roasted Bambara nut

CONCLUSION

The results of this study show that roasted or soaked Bambara nut could serve as alternative feed ingredient for broiler chicken. The standardized ileal amino acid digestibility values of processed Bambara nut was documented. Birds on roasted Bambara nut diet maintained the highest digestibility values after standardization irrespectively of the methods (nitrogen-free diet or highly digestible protein) used for estimating endogenous ileal amino acid losses.

CONFLICT OF INTEREST

There is no conflict of interest.

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