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Standardized Ileal Amino Acid Digestibility of Red and White Pigeon Peas (*Cajanus cajan* (L.) Millsp) in Broiler Chicks

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Authors' contributions

This work was carried out in collaboration with all authors. Author AFA designed the study, wrote the protocol while author IOO wrote the first draft of the manuscript. Authors BROO and ROA reviewed the experimental design and all drafts of the manuscript. Authors AFA, IOO and BROO managed the analyses of the study. Authors AFA, IOO and ROA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the standardized ileal amino acid digestibility of two varieties of pigeon pea in broiler chicks.

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Nigeria between May and July, 2019. Two hundred and forty (240) one-day old Abor Acre plus-strain broiler chicks were fed a commercial broiler starter diet for 15 days. On day 16, the birds were weighed, tagged and randomly allotted to 4 dietary treatments (Diet 1: Nitrogen Free Diet (NFD); Diet 2: Highly Digestible Protein (HDP); Diet 3: Red Pigeon Pea (RPP), Diet 4: White Pigeon Pea (WPP) with 6 replicates and 10 birds each in a randomized complete block design.

On day 21, birds were sacrificed and digesta collected from 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 descriptive statistics and ANOVA at $P = .05$.

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Higher EAA flow (*P* < .001) was recorded in lysine and aspartic acid for NFD while endogenous alanine flow was higher in HDP. Arginine had the lowest apparent digestibility values for both WPP and RPP. Apparent digestibility values of histidine, phenylalanine, valine, aspartic acid, glycine, proline and serine in WPP were significantly (*P* = .05) higher than in RPP with their corresponding higher (*P* = .05) SID values. Arginine had the lowest SIAAD value when AIAAD was corrected with HDP or NFD. In conclusion, WPP had higher CP level as well as higher digestibility values for some amino acids than RPP. The method used for standardization did not affect SIAAD values corrected with NFD or HDP for WPP and RPP respectively. Therefore, either of the two methods can be used to correct for endogenous amino acid losses.

Keywords: Broilers; legume; amino acids; precaecal digestibility.

1. INTRODUCTION

Feed accounts for 70% of costs of intensively reared poultry hence a major factor limiting the growth of the poultry industry [1]. The ever-increasing human population has led to a huge competition between animals and humans for feed; leading to scarcity and high cost of conventional protein sources such as soyabean meal, groundnut cake etc. [2]. Therefore, it becomes necessary for animal nutritionists to search for unconventional, less competitive and available feedstuff for sustainable poultry production. However, it is important to have accurate estimates of nutrient availability from these feed ingredients when formulating commercial diets for chickens [3] in order to meet their nutritional requirements. Therefore, assessment of amino acid (AA) digestibility of raw materials is essential if birds are to be fed with balanced diets assuring adequate provision of amino acid available for maintenance and production purposes [4]. Several methods have been used for amino acid digestibility assay such as the analysis of AA in excreta and ileal content. However, apparent ileal amino acid digestibility (AIAAD) values from ileal digesta or excreta do not differentiate between AAs of basal/specific endogenous or dietary origins [5]. Endogenous losses originate from sources such as digestive secretions saliva, bile, gastric, and pancreatic secretions as well as intestinal secretions, mucoproteins, sloughed intestinal epithelial cells, serum albumin, and amides [6] which make it difficult to determine the actual nutrient digestibility and availability. Meanwhile, standardized Ileal Amino Acid Digestibility (SIAAD) coefficient represents AIAAD corrected for Endogenous AA Losses (EAAL). This is considered as an accurate measure of AA digestibility in estimating available AA present in feedstuff.

Pigeon pea (*Cajanus cajan)* are highly nutritious, contains crude protein (CP) of 22%–27 and also crude fiber (CF) of 7.3%–10%, nitrogen-free extract (NFE) of 61.2%, ether extract (EE) of 1.7%–2.1%, ash of 3.1%–4.2%, and lysine of about 7.59% [7,8] and essential amino acids such as methionine, lysine and tryptophan [9,10]. It is a fast growing, hardy, widely adaptable and drought resistant crop and it is an important grain legume commonly grown and consumed in both tropical and subtropical regions and could be a better and cheaper protein alternative as compared to other legume grains [11,8]. However, like other legumes, there are some antinutritional factors such as trypsin and chymotrypsin inhibitors regarding the use of pigeon pea as a feed ingredient in poultry feeding [12] but different processing methods, such as boiling, crushing, soaking, roasting etc., have been employed to inactivate the antinutrients in pigeon pea [13]. The need to improve the utilization of pigeon pea as an alternative feed resource in poultry nutrition and to document standardized ileal amino acid digestibility for red and white pigeon peas becomes imperative. It was therefore the objective of this study to determine the standardized ileal amino acid digestibility of roasted red and white pigeon peas in broiler chicks.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was carried out at the Poultry unit of the Teaching and Research Farm, University of Ibadan, Oyo state in the South West geopolitical zone of Nigeria and within the tropical rain forest region.

2.2 Processing of Test Ingredient

The Pigeon Peas (*Cajanus cajan)* used were purchased from a reputable popular market (Bodija market) in Ibadan, Nigeria. About 200g of

the raw pigeon peas (red and white varieties) were washed, dried and roasted for 6-8 minutes, milled and included in the diets.

2.3 Experimental Diets and Management of Birds

Two hundred and forty (240) one-day old Abor Acre plus-strain of birds were obtained from a reputable commercial hatchery in Ibadan, Nigeria. The birds were fed a commercial starter broiler starter diet formulated to meet the nutrient requirement for broiler chicks according to [14] for 15 days. On day 16, the birds were tagged, weighed and randomly allotted to 4 dietary treatments with 6 replicates and 10 birds per replicate in a randomized complete block design using experimental animal allotment programme (EAAP) of [15]. Diet 1: a Nitrogen-free diet, Diet 2: Highly digestible protein (Casein). Diet 3: Red pigeon pea as the sole source of amino acid and Diet 4: White pigeon pea as the sole source of amino acid. The birds were fed experimental diets from day 16 till day 21 (Table 1). They had free access to feed and water *ad-libitum.*

2.4 Ileal Digesta Collection

On day 21, the birds were sacrificed and the carcasses were opened using surgical blades. The digesta were collected from the ileum into plastic sealed containers, and then transferred into a freeze drier at 68^0C. The dried samples were then taken to the laboratory for amino acid analysis, titanium dioxide analysis, dry matter and crude protein content analysis.

2.5 Chemical Compositions

The proximate composition and amino acid analysis of the diets and digesta samples were determined according to the methods of [16]. Titanium dioxide concentrations in the diets and digesta were analyzed using methods modified by [17].

2.6 Digestibility Calculations

Apparent and standardized ileal AA digestibility values were calculated using the index method according to the following equation:

Apparent ileal AA digestibility (%)

AIAAD = 1- [(TiO_2diet/TiO_2 digesta) X (AA digesta/AA diet) X 100

Standardized ileal AA digestibility (%)

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

Endogenous amino acid flow

$$\text{EAA flow (g/kg/DMI)} = \text{(AA digesta g/kg)} \times \frac{TiO_2\text{diet}}{TiO_2\text{ digesta}}$$

Where,

TiO_2 diet – titanium concentration of dietary intake
TiO_2 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 TiO_2 diet, TiO_2 digesta, AA digesta and AA diet are expressed as mg\kg of DM.

2.7 Statistical Analysis

Data were subjected to descriptive statistics and analysis of variance (ANOVA) using [18] software package. Treatment means were separated using Duncan's multiple range test at $P = .05$ [19].

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

Ingredient	NFD diet	HDP diet	Red pigeon pea	White pigeon pea
Casein	0.00	100.00	0.00	0.00
Red pigeon pea	0.00	0.00	270.00	0.00
White pigeon pea	0.00	0.00	0.00	270.00
Corn starch	272.00	217.00	270.00	270.00
Dextrose	574.00	529.00	392.00	392.00
Soy oil	50.00	50.00	30.00	30.00
Cellulose	60.00	60.00	0.00	0.00
Limestone	16.00	16.00	16.00	16.00

Ingredient	NFD diet	HDP diet	Red pigeon pea	White pigeon pea
Vitamin premix	1.50	1.50	1.50	1.50
Mineral premix	1.00	1.00	1.00	1.00
Table Salt	2.50	2.50	2.50	2.50
Dicalcium phosphate	18.00	18.00	12.00	12.00
Titanium dioxide	5.00	5.00	5.00	5.00
Total	1000	1000	1000	1000
Calculated Nutrients				
Crude Protein	0.00	82.70	60.21	56.70
Metabolizable Energy (Kcal\kg)	3131.33	3058.22	3114.82	3114.82
Ether extract	0.00	2.00	6.21	9.99
Crude Fibre	0.00	3.00	0.17	0.16
Calcium	9.86	9.91	12.11	12.11
Total phosphorus	3.24	3.56	12.15	9.72

**NFD= nitrogen free diet; HDP= highly digestible protein*

3. RESULTS AND DISCUSSION

3.1 Crude Protein and Amino Acid Profile of White and Red Pigeon Peas

The crude protein and amino acid concentrations of white and red pigeon pea is presented in Table 2. The crude protein values obtained were 28.06% and 27.94% in white pigeon pea (WPP) and red pigeon pea (RPP) respectively. These results are in disagreement with the findings of [7], {21.07% CP}; [20], {22.4% CP} and [21], {17.9-24.3% CP} as the CP levels obtained in this study were higher than the reported CP levels by the afore mentioned authors, although the authors did not specify the variety used. The variation observed in CP level of WPP and RPP maybe as a result of the cultivar, geographical location and growth conditions of plants. [12] stated that the chemical composition of pigeon pea could be influenced by the aforestated factors, which is also in agreement with the findings of [22] and [23]. These authors postulated that a wide variability exists in the chemical composition of pigeon pea seeds due to genotype, growth conditions and duration/condition of storage. For indispensable amino acids (AAs), RPP had higher absolute concentration values for arginine, histidine, isoleucine, leucine, lysine, methionine, tryptophan and valine while WPP had higher concentration for phenylalanine and threonine. For dispensable AAs, similar concentration was observed for aspartic acid, cysteine and glutamic acid of WPP and RPP. It was observed that WPP had higher amino acid concentrations in alanine and proline while RPP had a higher concentration in glycine and serine. The amino acid (AA) profile result obtained in this study for roasted WPP (0.36-2.73mg/kg) and RPP (0.36-2.15mg/kg) were lower than the reports of [7] for roasted pigeon pea (0.69-14.21g/16gN). The disparity AA profile values maybe due to varietal differences, processing methods, crops grown in different geographical locations etc.

3.2 Endogenous Ileal Amino Acid Flow in Birds Fed Nitrogen-free or Highly Digestible Protein Diets

Endogenous ileal amino acid (EIAA) flow in birds fed nitrogen-free diet (NFD) or highly digestible protein (HDP) is shown in Table 3. Higher (P <.001) EIAA flow was recorded in lysine and aspartic acid for NFD while endogenous alanine flow was higher in HDP but similar endogenous flow was observed in other amino acids. Several approaches have been used to estimate basal EAA (endogenous amino acid) flow [24,25]. The classical methods that have been used include the nitrogen-free diet [26,27] and feeding of highly digestible protein [27,28]. According to [29], the concentration of dietary protein or amino acids in a feedstuff is a factor that is likely to influence EAA losses in the gut of monogastric animals through increased digestive enzyme secretion and mucin dynamics. 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. [29] averred that variability observed in some digestibility studies as well as in estimating EAA losses could be attributed to the type of index marker included in the diet. The authors stated that although the marker itself might not likely affect the EAA flow, but, passage of the marker, segregation, and different analysis of various markers may contribute to differences between published

values. Similarly, [30], documented that the magnitude of the variations in amino acid digestibility may be associated with the type of index marker used as reported in the meta-analysis. Also, according to [29], the GIT health status of the birds used for the study; when pathogens are present, intestinal microflora turnover can increase quite dramatically and thus influence the endogenous amino acid flow.

Table 2. Crude protein (%) and amino acid profile (mg/kg) of white and red pigeon peas

Parameters	WPP	RPP
DM (%)	95.67	92.00
CP	28.06	27.94
Indispensable amino acids		
Arginine	0.40	1.31
Histidine	1.94	1.95
Isoleucine	0.85	0.86
Leucine	1.52	1.56
Lysine	1.96	2.03
Methionine	1.53	1.56
Phenylalanine	2.73	2.15
Threonine	0.57	0.55
Tryptophan	1.53	1.59
Valine	0.97	0.98
Dispensable amino acids		
Alanine	0.73	0.68
Aspartic acid	0.95	0.95
Cysteine	0.36	0.36
Glutamic acid	0.49	0.49
Glycine	1.70	1.75
Proline	0.64	0.63
Serine	0.99	1.01

**WPP = white pigeon pea; RPP = red pigeon pea; DM = dry matter; CP = crude protein*

Table 3. Endogenous ileal amino acid flow in birds fed nitrogen-free diet or highly digestible protein

Amino acid	NFD	HDP	t value	P value
Indispensable amino acid (mg/Kg)				
Arginine	0.20	0.09	-2.22	0.06
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
Dispensable amino acids (mg/Kg)				
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*

3.3 Apparent Ileal Amino Acid Digestibility of White Pigeon Pea and Red Pigeon Pea in Broiler Chicks

The apparent ileal amino acid digestibility of white pigeon pea (WPP) and red pigeon pea (RPP) diets is shown in Table 4. Significant variations (*P* = .05) were observed in the digestibility values of amino acid among dietary treatments. Generally, apparent digestibility values of histidine, phenylalanine, valine, aspartic acid, glycine, proline and serine in WPP were significantly (*P* = .05) higher than in RPP. Arginine was observed to have the lowest apparent digestibility values for both WPP and RPP.

Apparent digestibility values are usually not corrected for endogenous amino acid flow and may be prejudiced by feed intake levels and protein content of diets. In this study, the apparent ileal amino acid digestibilities (AIAAD) of white and red pigeon peas were estimated and it was observed that the amino acid digestibility values for WPP and RPP were similar except for histidine, phenylalanine, valine, aspartic acid, glycine, proline and serine; the values obtained for WPP were higher than RPP and this could likely due to higher CP level of WPP. According to [29] higher CP has been reported to increase the apparent amino acid digestibility and endogenous amino acid flow in rats and broilers. The result obtained might also have been influenced by varietal differences, plant breeding programs, agronomic conditions as [12] stated that these factors could affect the chemical composition of pigeon pea. There is paucity of published data comparing the AIAAD of different varieties of pigeon pea. However, according to [31], the apparent ileal amino acid digestibility values documented for canola meal (69-86%), chickpeas (58-84%), faba beans (58-81%), field peas (66-83%) and lupins (75-88%) were slightly lower than the AIAAD values of white pigeon pea (66.59-87.59%) and red pigeon pea (62.24-88.44%) values obtained in this study with the exception of arginine.

3.4 Standardized Ileal Amino Acid Digestibility of White Pigeon Pea and Red Pigeon Pea Corrected with Highly Digestible Protein or Nitrogen Free Diet

Results of standardized ileal amino acid digestibility of pigeon peas corrected with highly digestible protein or nitrogen free diet are shown in Tables 5 and 6. In white pigeon pea, there was higher (*P* = .05) standardized ileal digestibility values of histidine, phenylalanine and valine for indispensable amino acids, and aspartic acid, glycine, proline and serine for dispensable amino acids (Table 5). However, higher (*P* = .05) standardized amino acid digestibility values were recorded in histidine, phenylalanine, valine, glycine, proline and serine of white pigeon pea than in red pigeon pea (Table 6). Arginine was observed to have the lowest standardized ileal amino acid digestibility value when apparent ileal amino acid digestibility was corrected with highly digestible protein or nitrogen free diet for birds fed white and red pigeon peas.

According to [32], standardized ileal amino acid digestibility (SIAAD) values of a feedstuff are useful for formulating balanced diets for broiler chickens because they give more accurate estimates of amino acids that are available in feed ingredients. Apparent ileal amino acid digestibility values of white pigeon pea and red pigeon pea were corrected for basal endogenous losses with highly digestible protein and nitrogen free diet to obtain SIAAD values of this feedstuff. Standardization with mean values determined by highly digestible protein and nitrogen free diet method resulted in increased value of amino acid digestibility in the feedstuff. The result obtained for WPP and RPP corrected with HDP or NFD respectively showed that there were significant variations in histidine, phenylalanine, valine, aspartic acid, glycine, proline and serine. This may be due to the higher level of CP in WPP than that of RPP or this might have been influenced by varietal differences. [33] documented lower SIAAD values for chickpeas as compared with values obtained for SIAAD for WPP and RPP, although, the method used to correct apparent ileal amino acid was not stated. However, the SIAAD value obtained for arginine (85%) in chick peas was higher than SIAAD for both WPP (66.64%; 66.66%) and RPP (63.24%; 63.76%) corrected with HDP or NFD respectively [34]. Also, [35] reported the SIAAD values of some legumes fed to broiler chicken and the values obtained for cottonseed meal (65-88%) were slightly similar to the SIAAD values in WPP (66.66-87.65%) and RPP (63.76-88.69%) but with a higher arginine value (93%) in sunflower meal than those recorded for WPP and RPP respectively. However, lupins (82-91%), soybean meal (82-93%) and sunflower meal (80-93%) had a higher SIAAD values when compared to the SIAAD values obtained in the present study.

Table 4. Apparent ileal amino acid digestibility (%) of white pigeon pea and red pigeon pea in broiler chicks

Amino acid	WPP	RPP	T value	P value
Indispensable amino acids				
Arginine	66.59	62.24	-0.98	0.36
Histidine	85.14[a]	74.54[b]	-2.82	0.03
Isoleucine	84.94	87.77	0.79	0.45
Leucine	85.42	70.08	-0.94	0.38
Lysine	85.11	78.82	-1.47	0.18
Methionine	85.32	76.99	-1.59	0.15
Phenylalanine	84.18[a]	64.93[b]	-4.55	0.00
Threonine	85.59	81.65	-0.72	0.49
Tryptophan	82.64	88.44	1.49	0.17
Valine	85.28[a]	65.33[b]	-4.96	0.00
Dispensable amino acids				
Alanine	85.35	87.50	0.61	0.56
Aspartic acid	85.17[a]	74.82[b]	-2.78	0.03
Cysteine	85.59	85.07	-0.14	0.89
Glutamic acid	87.59	84.01	-0.42	0.68
Glycine	84.44[a]	67.10[b]	-2.59	0.03
Proline	85.52[a]	76.49[b]	-2.42	0.04
Serine	85.48[a]	71.12[b]	-3.90	0.01

*[ab]*Means on the same row with different superscripts are significantly (P<0.05) different WPP = white pigeon pea; RPP = red pigeon pea*

Table 5. Standardized ileal amino acid digestibility (%) of white pigeon pea and red pigeon pea corrected with highly digestible protein

Amino acid	WPP	RPP	T value	P value
Indispensable amino acids				
Arginine	66.64	63.24	-0.75	0.48
Histidine	85.32[a]	75.18[b]	-2.69	0.03
Isoleucine	85.07	88.09	0.85	0.42
Leucine	85.56	79.44	-0.92	0.39
Lysine	85.29	79.46	-1.34	0.22
Methionine	85.37	77.44	-1.51	0.17
Phenylalanine	84.40[a]	65.83[b]	-4.35	0.00
Threonine	85.73	81.99	-0.70	0.51
Tryptophan	82.70	88.56	1.53	0.17
Valine	85.51[a]	66.27[b]	-4.75	0.00
Dispensable amino acids				
Alanine	85.49	87.86	0.68	0.52
Aspartic acid	83.35[a]	75.46[b]	-2.65	0.03
Cysteine	85.73	85.45	-0.08	0.94
Glutamic acid	87.68	84.25	-0.41	0.69
Glycine	84.50[a]	67.52[b]	-2.53	0.04
Proline	85.70[a]	77.12[b]	-2.27	0.05
Serine	85.70[a]	71.96[b]	-3.72	0.01

*[ab]*Means on the same row with different superscripts are significantly (P<0.05) different.WPP = white pigeon pea; RPP = red pigeon pea; HDP = highly digestible protein*

Table 6. Standardized ileal amino acid digestibility (%) of white pigeon pea and red pigeon pea corrected with nitrogen free diet

Amino acid	WPP	RPP	T value	P value
Indispensable amino acids				
Arginine	66.66	63.76	-0.63	0.55
Histidine	85.30[a]	75.13[b]	-2.69	0.03
Isoleucine	85.07	88.11	0.85	0.42
Leucine	85.55	79.44	-0.91	0.39
Lysine	85.27	79.41	-1.34	0.22
Methionine	85.38	77.67	-1.47	0.18
Phenylalanine	84.34[a]	65.64[b]	-4.39	0.00
Threonine	85.72	81.99	-0.69	0.51
Tryptophan	82.73	88.69	1.57	0.15
Valine	85.44[a]	66.00[b]	-4.81	0.00
Dispensable amino acids				
Alanine	85.48	87.86	0.68	0.52
Aspartic acid	85.33	74.40	-2.65	0.29
Cysteine	85.72	85.45	-0.07	0.94
Glutamic acid	87.65	84.27	-0.40	0.70
Glycine	84.52[a]	67.72[b]	-2.50	0.04
Proline	85.68[a]	77.07[b]	-2.28	0.05
Serine	85.63[a]	71.73[b]	-3.77	0.01

*[ab]*Means on the same row with different superscripts are significantly (P<0.05) different.WPP = white pigeon pea; RPP = red pigeon pea; NFD = nitrogen free diet*

4. CONCLUSION

The results of this study show that white or red pigeon pea could serve as an alternative feed resource in broiler chicken diet. The standardized ileal amino acid digestibility values of white pigeon pea and red pigeon pea were documented. The methods (nitrogen free diet or highly digestible protein) used to standardize digestibility values did not have much significant effect on the standardized ileal amino acid digestibility of white pigeon pea and red pigeon pea. Therefore, either of the two methods can be used to correct for endogenous amino acid losses.

CONTENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (University of Ibadan ethics policy, revised 2013) were followed. This study complied with the University of Ibadan ethics requirements for animal care and handling.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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