

Growth curves, body composition, and carcass energy partitions of broiler chickens fed low crude protein cassava diets supplemented with limiting amino acids

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Abstract

The strategic integration of low crude protein (CP) diets in broiler production systems is considered a means to reduce feed costs. This study evaluated the effects of a low CP cassava–soybean diet with or without supplementation of methionine, lysine, and threonine on growth curves, carcass composition, and energy partitioning in broiler chickens. A total of 180 10-day-old chicks were assigned to three treatments: a standard corn–soybean control diet (CP 21.5–23.0%), a low-CP cassava-based diet (CP 16.0–17.8%), and the same low-CP diet supplemented with limiting amino acids; lysine, methionine, and threonine. Growth data fitted to a logistic model showed no significant differences in asymptotic weight (W_a), growth rate constant (k), or age at inflection (t_i) among treatments ($p > 0.05$). At 42 d, carcass composition was markedly altered. Birds on the low-CP cassava diet had significantly lower carcass water (61.31 ± 5.55 g/100 g) and protein (8.34 – 10.30 g/100 g) but higher fat, and carcass energy compared with controls (protein 13.86 ± 2.74 g/100 g; water 68.15 ± 4.05 g/100 g). Amino acid supplementation improved water (64.69 ± 2.92 g/100 g) and reduced fat accumulation but failed to restore protein deposition. Regression analysis showed carcass energy (kcal) = $-227.45 + 2.38$ fat (g) + 2.31 protein (g) ($R^2 = 0.376$, $p < 0.001$), with fat contributing disproportionately in low-CP diets. These findings highlight that supplementation with only the first three limiting amino acids is insufficient to sustain lean accretion and energy efficiency in cassava-based low-CP feeding systems.

Keywords: Carcass energy density, Lean tissue accretion, Fat deposition, Alternative energy feedstuffs

Courbes de croissance, composition corporelle et répartition de l'énergie de la carcasse chez des poulets de chair nourris avec des régimes pauvres en protéines brutes à base de manioc supplémentés en acides aminés limitants



Résumé

L'intégration stratégique de régimes pauvres en protéines brutes (PB) dans les systèmes de production de poulets de chair est considérée comme un moyen de réduire les coûts alimentaires. Cette étude a évalué les effets d'un régime à base de manioc-soja pauvre en PB, avec ou sans supplémentation en méthionine, lysine et thréonine, sur les courbes de croissance, la composition de la carcasse et le partitionnement de l'énergie chez des poulets de chair. Au total, 180 poussins âgés de 10 jours ont été répartis en trois traitements : un régime témoin standard maïs-soja (PB 21,5–23,0 %), un régime pauvre en PB à base de manioc (PB 16,0–17,8 %), et le même régime pauvre en PB supplémenté avec les acides aminés limitants ; lysine, méthionine et thréonine. L'ajustement des données de croissance à un modèle logistique n'a montré aucune différence significative entre les traitements pour le poids asymptotique (W_a), la constante de taux de croissance (k) ou l'âge au point d'inflexion (t_i) ($p > 0,05$). À 42 jours, la composition de la carcasse était nettement modifiée. Les oiseaux du régime pauvre en PB à base de manioc présentaient une teneur en eau ($61,31 \pm 5,55$ g/100 g) et en protéines ($8,34$ – $10,30$ g/100 g) de la carcasse significativement plus faible, mais une teneur en lipides et en énergie de la carcasse plus élevée par rapport aux témoins (protéines $13,86 \pm 2,74$ g/100 g ; eau $68,15 \pm 4,05$ g/100 g). La supplémentation en acides aminés a amélioré la teneur en eau ($64,69 \pm 2,92$ g/100 g) et réduit l'accumulation de lipides, mais n'a pas permis de rétablir la déposition protéique. L'analyse de régression a montré que l'énergie de la carcasse (kcal) = $-227,45 + 2,38$ lipides (g) + $2,31$ protéines (g) ($R^2 = 0,376$, $p < 0,001$), les lipides contribuant de manière disproportionnée dans les régimes pauvres en PB. Ces résultats soulignent qu'une supplémentation avec seulement les trois premiers

acides aminés limitants est insuffisante pour maintenir l'accrétion de tissu maigre et l'efficacité énergétique dans les systèmes d'alimentation pauvres en PB à base de manioc.

Mots-clés : Densité énergétique de la carcasse, Accrétion de tissu maigre, Dépôt de lipides, Sources d'énergie alimentaires alternatives

Introduction

The synergy between successful broiler breeding efforts and high-quality feeds drives the production efficiency of broiler enterprises. Feed expenditure typically represents the largest share of total production costs (Jie *et al.*, 2024); hence, reducing feed expenditure is desirable. Nutritionists recognize that low crude protein diets supplemented with essential amino acids are an appropriate way to meet the rising demand for poultry meat flocks in an environmentally sustainable manner, while reducing feed costs (Chrystal *et al.*, 2020; Chrystal *et al.*, 2020a; Chrystal *et al.*, 2021; Attia *et al.*, 2022; Aderibigbe *et al.*, 2024). Reducing crude protein in broiler diets without adequate amino acid supplementation can impair growth performance, increase fat deposition, and alter nutrient metabolism (Chrystal *et al.*, 2020; Cordero *et al.*, 2025). The supply of limiting amino acids, particularly lysine, methionine, and threonine, is critical to support lean tissue growth, intestinal health, and optimal feed efficiency (Zampiga *et al.*, 2021; Gong *et al.*, 2023). Price volatility of key feed ingredients such as grains and oilseeds has continued to fuel the surge in feed prices, riding on the competition between feed, food, and biofuel, supply chain disruptions, climate change, and the drastic weather changes it brings (Donohue and Cunningham, 2009; Rojas-Reyes *et al.*, 2024; Tchoukouang *et al.*, 2024). The strategic integration of non-conventional feedstuffs in broiler feeds can reduce feed costs significantly and promote sustainability in broiler enterprises (Vlaicu *et al.*, 2024).

Cassava (*Manihot esculenta* Crantz) is widely cultivated across tropical regions, including sub-Saharan Africa (Chaunaronng *et al.*, 2009), and represents an abundant, inexpensive source of dietary energy. Its high starch content and adaptability to marginal soils make it a promising substitute for cereal grains in broiler diets (Morgan and Choct, 2016), and cassava

root products have been successfully used at about 50% of broiler diets to reduce the cost of production (Bhuiyan and Iji, 2015). However, cassava is characterized by extremely low protein content and deficiencies in essential amino acids such as lysine, methionine, and threonine (Nassar and Sousa, 2007; Olugbemi *et al.*, 2010). These limitations raise concerns about the adequacy of protein deposition and overall nutrient utilization when cassava replaces conventional energy sources in poultry feeding programs (Morgan and Choct, 2016). The focus of studies assessing low crude protein diets and essential amino acid supplementation in broiler chickens has centered on bird performance and carcass slaughter yield, with limited literature focused on body or carcass composition of broiler chickens in response to these conditions (Bregendahl *et al.*, 2002). While some studies indicate low CP diets can impact breast meat characteristics and abdominal fat (Chrystal *et al.*, 2020b; Benahmed *et al.*, 2023), a lack of comprehensive analysis on detailed carcass composition components leaves a gap in understanding the full impact. It is well documented that the growth curve of livestock species can be manipulated through management variables such as environmental temperature, rearing systems, stocking density, and feeding management, in addition to genotype and nutritional challenges (Gbangboche *et al.*, 2008; Şekeroğlu *et al.*, 2013; Van Der Klein *et al.*, 2017; Faraji-Arough *et al.*, 2023; Menchetti *et al.*, 2024). Hence, the objective of this study was to quantitatively assess the response of broiler chickens' growth curves, body composition, and body energy partitions to a low crude-protein cassava-soybean-based diet and the effect of supplemental administration of the first three-limiting amino acids, methionine, lysine, and threonine in this low crude-protein diet.

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).

Diets, animals, experimental design, and management

Two diets were formulated to be isocaloric but differed in crude protein content. The control was a conventional corn-soybean meal-based

diet formulated to meet breeders' recommendations (Aviagen, 2019), and the second diet (negative control, NC) was formulated with cassava chips as the primary energy source, totally replacing maize in the diet. The negative control diet was then balanced for the first three limiting amino acids: methionine, lysine, and threonine, creating a third diet. The diets were fed as a mash for 32 days in two phases: starter (11 - 21d) and grower (22 - 42d). The gross composition of the experimental diets and calculated and analyzed nutrients are shown in Table 1.

Table 1. Gross composition (g/1000g) and calculated nutrients of experimental diets used in this study

Ingredients	Starter diets			Grower diets		
	Control diet	Low crude protein Cassava diet	Low crude protein Cassava diet + LAA ^a	Control diet	Low crude protein Cassava diet	Low crude protein Cassava diet + LAA ^a
Maize	439.0	-	-	504.0	-	-
Cassava (dehydrated chips)	-	550.0	550.0	-	580.0	580.0
Soybean meal	240.0	181.0	181.0	214.0	170.0	170.0
Groundnut cake	145.0	165.0	160.0	132.3	137.2	131.7
Corn bran	100.6	-	-	78.0	-	-
Soya oil	30.0	50.0	48.0	33.5	64.0	62.5
Dicalcium phosphate	12.0	31.1	31.1	6.0	29.0	29.0
Limestone	17.0	6.5	6.5	17.6	5.2	5.2
Vitamin-Min. mix ^b	2.5	2.5	2.5	2.5	2.5	2.5
Lysine-HCl	6.1	6.1	9.45	5.1	5.1	8.4
DL-Methionine	2.7	2.7	3.85	2.35	2.35	3.55
L-Threonine	2.6	2.6	5.1	2.15	2.15	4.65
NaCl	2.5	2.5	2.5	2.5	2.5	2.5
Carbohydrase	+	+	+	+	+	+
Protease	+	+	+	+	+	+
Phytase	+	+	+	+	+	+
Total	1000	1000	1000	1000	1000	1000
Calculated Analysis						
Metabolizable energy, kcal/kg	3002.18	3004.30	3000.62	3100.35	3102.19	3101.41
Crude protein, %	23.03	17.79	18.13	21.51	15.96	16.28
Crude fat, %	7.22	7.00	6.76	7.53	8.12	7.92
Crude fibre, %	4.62	2.68	2.65	4.35	2.42	2.38
Calcium, %	0.97	0.96	0.96	0.87	0.87	0.87
Av. Phosphorus, %	0.49	0.48	0.48	0.44	0.45	0.44
Methionine, %	0.56	0.45	0.56	0.51	0.40	0.51
Lysine, %	1.45	1.18	1.44	1.29	1.03	1.29
Threonine, %	0.97	0.73	0.97	0.88	0.64	0.88

^a Limiting amino acid: methionine, lysine, and threonine

^b Composition of vitamin mineral premix per kg/diet: vitamin A, 10,000 IU; vitamin D3, 2,000 IU; vitamin E, 32 mg; vitamin K3, 1.6 mg; vitamin B1, 2.5 mg; vitamin B2, 4.4 mg; niacin, 44 mg; calcium pantothenate, 9.2 mg; vitamin B6, 4 mg; vitamin B12, 0.02mg; choline chloride, 400 mg; folic acid, 0.8 mg; biotin, 0.064 mg; manganese, 96 mg; iron, 80 mg; zinc, 64 mg; copper, 6.8 mg; iodine, 1.2 mg; cobalt, 0.24 mg; selenium, 0.096 mg; antioxidant, 96 mg.

A total of 180 pre-tagged 10-day-old chicks were selected from a production flock and allotted to three treatments, each consisting of six replicates of ten chicks each. Chicks in each treatment were offered one of the experimental diets throughout the study period. Natural lighting (12L: 12D) and ventilation were used in an open-sided poultry house, wood shavings were the bedding material of choice, stocking density was 10 birds/m², and water and feed were offered *ad libitum*.

Data collection

Growth data collection

Chicks were weighed individually and body weight data collected at eight timepoints during the study period; at 1, 10, 17, 21, 24, 31, 38 and 42d of age for growth curve analyses.

Body composition analysis

At the end of the study period, two chickens per replicate were randomly selected, fasted for 6 hours to empty the gastrointestinal tract of undigested feed, weighed, killed by cervical dislocation, and stored at -20 °C (with feathers, shanks, blood, and all organs intact) until whole carcass analysis.

Sample processing and chemical analysis

The whole chicken carcasses (feathers, shanks, blood, and all organs) were individually processed by chopping and coarse grinding in an industrial blender. Aliquot portions were then dried in a forced-air oven at 105 °C. Water (moisture) was determined as the difference between the whole carcass weight before and after drying. Carcass samples were further milled and analyzed for crude protein and ether-extractable fat using the Kjeldahl and Soxhlet extraction methods (AOAC, 2005), and gross energy by bomb calorimetry, with benzoic acid as a calibration standard (AOAC, 1995).

Calculations and statistical analyses

Body weight data were fitted to a three-parameter logistic non-linear regression model (Aggrey, 2002; Robertson, 1923):

$$W_t = W_a / [1 + \exp(-K(t - t_i))]$$

where W_t = body weight of individual birds at time t (in days), W_a = asymptotic (mature) body weight, K = exponential growth rate, and t_i = age at the inflection point, i.e., age when growth rate is maximum. Individual growth curves were fitted for birds subjected to the different dietary treatments using the PROC NONLIN procedure of SAS software version 9.4 (SAS Institute Inc, 2023).

Water, crude protein, fat, and REST components of the whole carcass were considered for body composition. REST in this study was assumed to consist mainly of minerals, glycogen, and the variability of the analysis (Caldas *et al.*, 2019), and was estimated as

$$REST = 100 - (\text{water} + \text{crude protein} + \text{fat})$$

Carcass energy was reported as gross energy corrected for dry matter, and the protein-to-fat ratio (protein:fat) of the whole carcass was also estimated.

A multiple linear regression model was fitted to derive the calorific coefficients of protein and fat, with the slopes of the equation considered the calorific contributions of protein and fat to whole carcass energy. The model used to describe this relationship is:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \epsilon$$

where: Y = body energy content, kcal; β_0 = intercept; β_1 = partial regression coefficient for body fat; X_1 = body fat content, g; β_2 = partial regression coefficient for body protein; X_2 = body protein content, g, and ϵ = error term.

Data from body composition analysis, calorific contributions of protein and fat, and growth curve function parameters were subjected to descriptive statistics, means were compared using the analysis of variance, ANOVA, procedure, and significance based on a 5%

probability level. All analyses were performed using JASP version 0.18.3 (JASP, 2024).

Results

Growth curves

The effect of low crude protein cassava diets and supplemental limiting amino acids on

growth curves of broiler chickens is presented in Figure 1. Growth estimates derived from the Logistic growth model, Asymptotic weight, *A*, kg, growth rate constant, *k*, and time at inflection, *ti*, days, were not significantly modified by the dietary treatments ($p>0.05$).

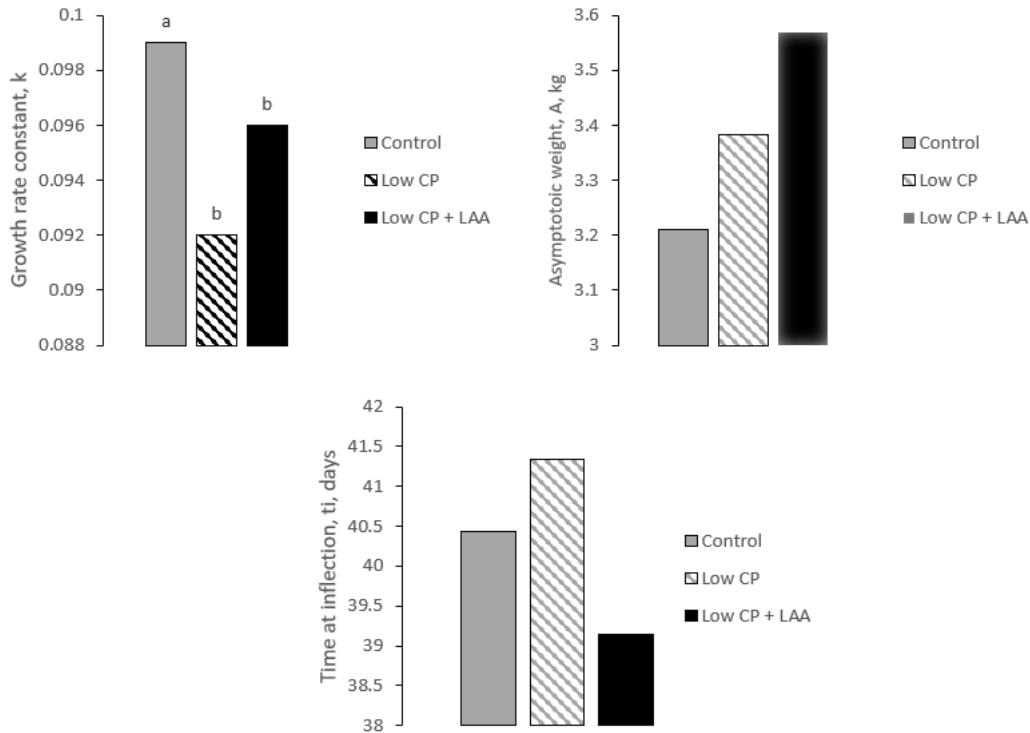


Figure 1. Effect of low crude protein cassava diets and supplemental limiting amino acids on growth estimates: Asymptotic weight, *A*, kg, growth rate constant, *k*, and time at inflection, *ti*, days, derived from the Logistic growth model for 42d-old broiler chickens.

Body composition

The effect of low crude protein cassava diets and supplemental limiting amino acids on body composition of broiler chickens fed a low crude

protein cassava-soybean meal-based diet are presented in Figure 2.

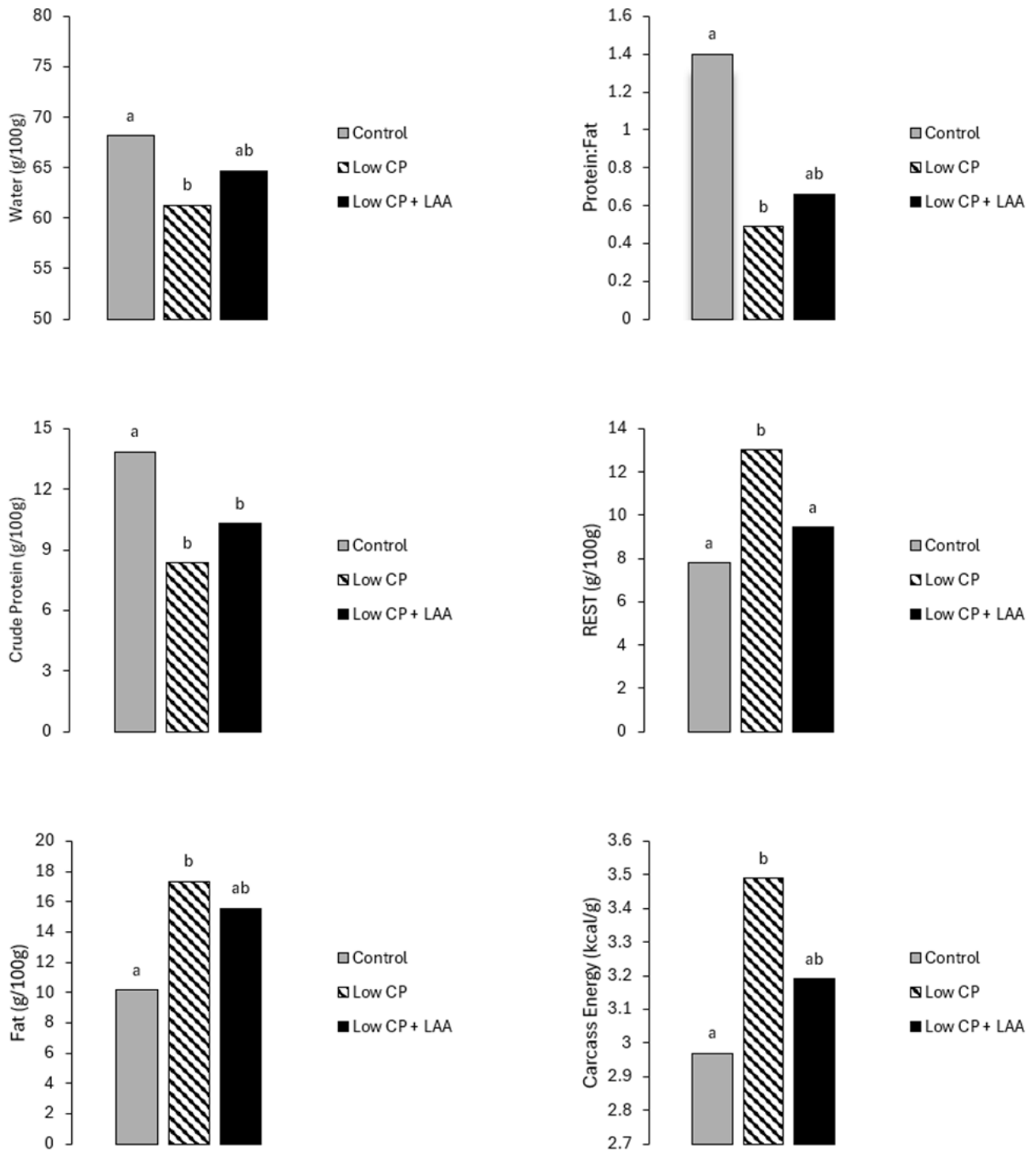


Figure 2. Effect of low crude protein cassava diets and supplemental limiting amino acids on body composition; water, crude protein, fat, Protein:fat, REST, and carcass energy, of 42d-old broiler chickens.

The water fraction of 42-d whole carcass was significantly lower in the low crude protein diet group (61.31 ± 5.55 g/100g). Limiting amino acid supplementation of the diet increased its water fraction (64.69 ± 2.92 g/100g), similar to the control (68.15 ± 4.05 g/100g). A similar trend was observed for carcass fat, carcass

energy, and carcass protein-to-fat ratio, as the low-CP cassava diet supplemented with limiting amino acids compared favourably with the control group. However, the low crude protein cassava diet compromised carcass crude protein, and this was not improved by limiting amino acid supplementation, as carcass protein

in the control group was significantly higher (13.86 ± 2.74 g/100g, $p < 0.001$) than the low crude protein diets, which ranged from 8.34-10.30 g/100g. The REST fraction was significantly highest in the low crude protein group (13.01 ± 2.66 g/100g), while the control and low-CP cassava diet supplemented with limiting amino acids compared favourably and ranged from 7-81-9.45 g/100g.

Calorific contributions of protein and fat

The linear relationship of carcass energy, with whole carcass protein, and fat composition was described by the regression equation:

$$\text{Carcass energy, kcal} = -227.45 + 2.38 \text{ fat, g} + 2.31 \text{ protein, g}$$

The overall model predicted about 38% variance in carcass energy, $R^2 = 0.376$, $F = 9.95$, $p < 0.001$ RMSE = 147.57. Positive significant associations were observed between carcass energy and fat ($B = 2.38$, $SE = 0.07$, $p < 0.001$, 95%CI = 2.25 to 2.52) and protein ($B = 2.37$, $SE = 0.06$, $p < 0.001$, 95%CI = 2.26 to 2.48). The effect of dietary treatments on calorific contributions of fat and protein to carcass energy, derived from the slopes of the regression equation, is presented in Figure 3.

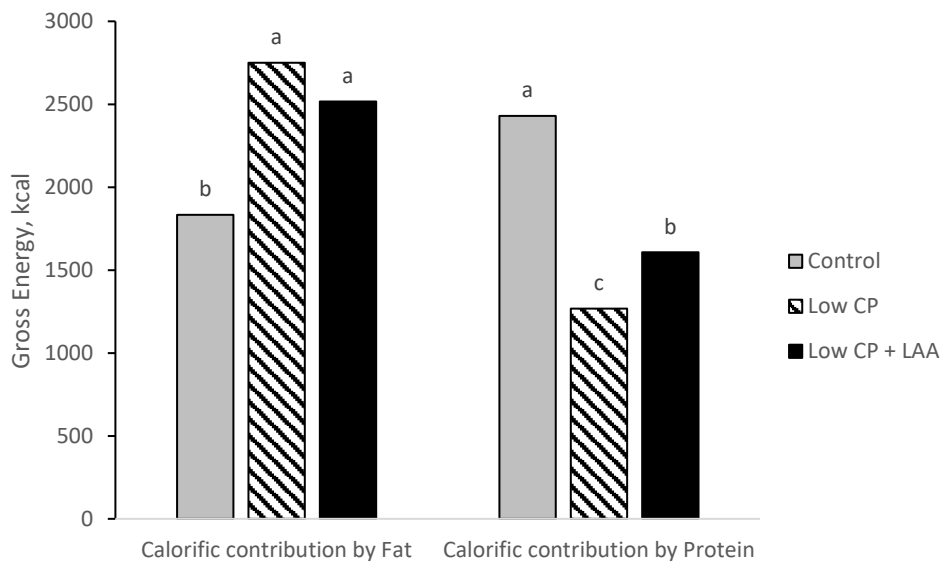


Figure 3. Effect of low crude protein cassava diets and supplemental limiting amino acids on estimates of calorific contribution of fat and crude protein to carcass energy derived for 42d-old broiler chickens. The linear regression used was: Carcass energy, kcal = $\beta_0 + \beta_1 X_1 + \beta_2 X_2$, where; β_0 = intercept, β_1 and β_2 = partial regression coefficient for carcass fat and protein, respectively, X_1 and X_2 = carcass fat and protein content, g. Carcass energy was analysed as gross energy in whole carcass, and the proportion of the input descriptors; fat and protein composition of whole carcass, were corrected for liveweight, assuming fat and protein as the only energy contributors.

The low CP cassava significantly influenced the calorific contribution of fat to carcass energy of low CP dietary treatments, with a 49.23% increase compared to the control treatment. However, limiting amino acid supplementation of the low CP diet did not impact the calorific contribution of fat to carcass energy ($p > 0.05$), 2750.45 kcal vs 2516.68 kcal, respectively. Conversely, the low-CP cassava diet and the low-CP cassava diet supplemented with limiting amino acids elicited a progressive decrease in the calorific contribution of protein

to carcass energy of 47.8% and 33.84%, respectively.

Discussion

The current study demonstrated that supplementing a low-CP cassava-based diet with limiting amino acids; methionine, lysine and threonine, did not significantly affect growth curve estimates of broiler chickens. Despite a dearth of studies focused on the effect of low-CP diets and supplemental limiting amino acids on growth curves, a 2023 meta-analysis of 106 trials asserted that reducing CP

of broiler chicken diets without consideration for essential amino acid balance decreased average daily gain, ADG (de Rauglaudre *et al.*, 2023). However, in datasets obtained from low-CP diet trials in which amino acid-to-lysine ratios of the diets complied with the ideal protein profile, average daily gain compared favourably with control treatments. Similar multi-flock studies also corroborate that low-CP diets preserve the growth of broiler chickens within adequate free amino acid supply (Saleh *et al.*, 2021; Cho *et al.*, 2024; El-Far *et al.*, 2024) but are sensitive to electrolyte balance and glycine-equivalent supply (van Harn *et al.*, 2019; Chrystal *et al.*, 2020a; Aguihe *et al.*, 2024).

Water is the most abundant constituent of the body, and its fraction of body weight is variable and dependent on relative proportions of lean muscle to fat. Muscle tissue relies on its protein matrix to bind water; hence, a lower level of protein accretion is naturally associated with reduced structural water-holding capacity, ultimately impacting the economics of meat production (Van Laack and Solomon, 1994). In broiler chickens fed low-CP diets, excess dietary energy that would normally support protein synthesis is instead diverted into *de novo* lipogenesis, shifting carcass composition toward fat at the expense of protein and moisture (Bregendahl *et al.*, 2002). Low crude protein diets in broiler chickens are also associated with reduced water intake and water-to-feed ratio (Brink *et al.*, 2022; Lambert *et al.*, 2023). The marked reduction in carcass crude protein of chickens offered the low-CP diets, despite amino acid supplementation, indicates that amino acid balance was insufficient to fully support protein synthesis and lean tissue accretion. Preferential *de novo* lipogenesis is reflected in a higher fat-to-protein ratio, increased calorific contribution of fat, and decreased contribution of protein to carcass energy in chickens fed the low-CP diet and amino acid-supplemented low-CP diet in the current study. Current literature suggests that non-supplemented amino acids such as isoleucine, valine, and glycine + serine equivalents may have become limiting as dietary CP decreased (Chrystal *et al.*, 2020; Chrystal *et al.*, 2020a) indicating that meeting

methionine, lysine and threonine needs alone may not fully restore the amino acid profile needed for maximal protein accretion when crude protein is markedly reduced. The reduced protein deposition and increased fat accretion observed in this study may also lower processing yield quality and economic returns in markets that prioritize lean meat. The shift from lean tissue to fat deposition in chickens on the low-CP diet may also be deemed responsible for the higher carcass energy concentration in this group compared to the control. Increase in REST% of broiler chickens fed the low-CP diet compared to chickens fed the control and essential amino acid supplemented low-CP diets may reflect increased proportions of bone relative to muscle (bone-to-meat ratio) (Havenstein *et al.*, 2003). The fitted multiple regression model relating carcass energy to protein and fat content accounted for 38% of the total variation in carcass energy. While this coefficient of determination is modest, both predictors were statistically significant, indicating that protein and fat content remain meaningful explanatory variables for carcass energy content in broiler chickens. The relatively low coefficient of determination may reflect the influence of other carcass constituents—such as water, minerals, and residual glycogen—that contribute to total carcass mass but are not explicitly included in the model and contribute no energy, resulting in a dilution effect. The observed nutrient partitioning shifts align with energy–protein interaction theory, where inadequate amino acid supply constrains protein synthesis, leading to greater fat accretion (Namroud *et al.*, 2008; Belloir *et al.*, 2017; Benahmed *et al.*, 2023). These findings highlight the critical role of complete amino acid balancing to avoid inefficiencies in energy utilization and undesirable carcass fat deposition in low-CP feeding systems.

Conclusion

An unfulfilled expectation in this study was that supplementation of a low crude protein cassava-based diet with the first three limiting amino acids, lysine, methionine, and threonine, would be sufficient to not only preserve growth but also maintain carcass quality of broiler

chickens. However, fat accretion increased at the expense of carcass protein, and calorific contribution of fat and protein to carcass energy was influenced by the low crude protein treatments, suggesting that a broader approach incorporating other amino acids of relevance to lean tissue accretion beyond the first three limiting amino acids is required for optimal protein accretion and energy efficiency in cassava-based low-CP diets.

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