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Impact of processing methods and amino acid supplementation of cassava root–leaf meal on performance and caeca microflora of broiler chicks

Impacto dos métodos de processamento e suplementação de aminoácidos da farinha de raiz e folhas de mandioca no desempenho e na microflora dos cecos de frangos de corte

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Abstract: This study investigated the effects of processed cassava root-leaf meal (CRLM) on broiler performance and caecal microflora. A total of 240 Ross broiler chicks were randomly assigned to 10 dietary treatments in a 5 × 2 factorial design, comprising five energy sources: maize, sun-dried CRLM (SCRLM), fermented CRLM (FCRLM), FCRLM with yeast (YFCRLM), and FCRLM with rumen filtrate (RFCRLM) and two amino acid levels (Ideal and National Research Council (NRC) 1994). The NRC diets included methionine and lysine, while the Ideal diets were supplemented with threonine and arginine. Each treatment was replicated three times with eight birds per replicate, and the trial lasted 28 days. Performance parameters were recorded weekly, and on day 28, selected birds were slaughtered for analysis of their gut microflora. The results indicated that diets formulated with Ideal amino acid levels significantly ($P<0.05$) improved weight gain (WG) and feed conversion ratio (FCR). The birds fed RFCRLM-based diets had significantly lower ($P<0.05$) feed intake (FI) and better FCR, comparable to those fed maize-based diets. Notably, the combination of RFCRLM and Ideal amino acids resulted in the lowest ($P<0.05$) FI and the best FCR overall. Microbial analysis revealed that maize-based diets resulted in higher ($P<0.05$) counts of *Bacillus subtilis* and *Proteus mirabilis*, whereas the CRLM-based diet, regardless of amino acid level, resulted in significantly lower ($P<0.05$) total bacterial counts (TBCs). In conclusion, supplementing broiler diets with Ideal amino acids enhanced growth performance, and RFCRLM effectively reduced feed intake without compromising FCR. Moreover, processed CRLM proved beneficial in lowering microbial load when used as an alternative to maize in broiler diets.

Key-words: Alternative feedstuff; fermentation; amino acid; gut microbes.

Resumo: Este estudo investigou os efeitos da farinha de raiz e das folhas de mandioca processada (FRFM) no desempenho de frangos de corte e na microflora cecal. Um total de 240 frangos de corte Ross foram distribuídos aleatoriamente por 10 tratamentos alimentares em um desenho fatorial 5×2, composto por cinco fontes de energia: milho, FRFM seco ao sol (FRFMSS), FRFM fermentado (FRFMf), FRFM com levedura (FRFMl) e FRFM com filtrado ruminal (FRFMfr) e dois níveis de aminoácidos (Ideal e National Research Council

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(NRC) 1994). As dietas NRC incluíam metionina e lisina, enquanto as dietas Ideais foram suplementadas com treonina e arginina. Cada tratamento foi replicado três vezes com oito aves por repetição, e o ensaio teve a duração de 28 dias. Os parâmetros de desempenho foram registados semanalmente e, no dia 28, as aves selecionadas foram abatidas para análise da microflora intestinal. Os resultados indicaram que as dietas formuladas com níveis ótimos de aminoácidos melhoraram significativamente (P<0,05) o ganho de peso (GP) e a taxa de conversão alimentar (TCA). As aves alimentadas com dietas à base de FRFMfr apresentaram uma ingestão de ração (IR) significativamente mais baixa (P<0,05) e uma melhor conversão alimentar, comparável às alimentadas com dietas à base de milho. Notavelmente, a combinação de aminoácidos FRFMfr e Ideal resultou no menor (P<0,05) IR e no melhor TCA global. A análise microbiana revelou que as dietas à base de milho resultaram em contagens mais elevadas (P<0,05) de *Bacillus subtilis* e *Proteus mirabilis*, enquanto a dieta à base de FRFM, independentemente do nível de aminoácidos, resultou em contagens bacterianas totais (CBTs) significativamente mais baixas (P<0,05). Concluindo, a suplementação de dietas para frangos de corte com aminoácidos ideais melhorou o desempenho do crescimento, e o FRFMfr reduziu de maneira eficaz a ingestão de ração sem comprometer a conversão alimentar. Além disso, o FRFM processado mostrou ser benéfico na redução da carga microbiana quando utilizado como alternativa ao milho em dietas de frangos de corte.

Palavras-chave: Alimentos alternativos; fermentação; aminoácidos; micróbios intestinais.

1. Introduction

The considerable increase in the cost of poultry feeds has resulted in a compelling force to search for cheaper sources of dietary energy, which constitute major components of poultry feed to avoid the collapse of the industry. The current feed situation is cause for concern as more than 50 % of the country’s poultry farms particularly, in Nigeria, have closed, and another 30 % have been forced to reduce their production capacity [1]. The increasing cost of feed has been identified as a serious impediment in meeting the demand for animal protein, particularly in developing countries [2] and this situation stimulated the drive to search for alternative energy sources such as cassava. Cassava (*Manihot esculenta* Crantz) root is an inexpensive and sustainable energy feedstuff with the potential to replace the most conventional energy cereal grains in the tropics [3]. The major limitations to its utilisation in poultry rations are its low quantity and poor-quality content, high fibre content, and presence of antinutritional components (cyanogenic glucosides, linamarin and lotaustralin) [3]. The inclusion of leaf meal serves as a source of proteins, vitamins, minerals and carotenoids at a relatively reduced cost [4]. The addition of leaf meal to cassava root meal increased the protein content of the final products to improve protein availability.

An increase in the growth and proliferation of fungal or bacterial complexes in the form of single-cell proteins during fermentation can be employed to enhance fibre degradation, enrich and reduce the toxicity of antinutritional factors in cassava [5]. Hence, fermentation of cassava-based products will help disintegrate the high fibre content, and increase the protein content in the mixture through bacterial cell protein, resulting in increased nutrient availability. Fermentation with additives such as yeast or rumen filtrate has been investigated for its potential to further increase nutrient availability and reduce antinutritional factors [6]. However, despite the benefits of processing, the broiler performance when fed diets containing processed cassava-based ingredients may still depend on appropriate amino acid supplementation, as cassava is typically deficient in key essential amino acids [7]. Amino acid supplementation plays a crucial role in poultry nutrition, as it ensures that protein is utilised efficiently for growth and development [8]. The National Research Council (NRC) is widely used to guide amino acid requirements for poultry, but the "Ideal Protein" concept has gained popularity in recent years for optimizing amino acid profiles which suggests that dietary amino acid levels should be balanced based on the ideal ratios of essential amino acids relative to lysine to achieve improved feed conversion efficiency [9, 8].

In addition to improving performance, the impact of dietary components on gut health is highly important, as it is recognised as critical for bird health and productivity [10]. The composition of the gut microflora can influence nutrient absorption and the immune response, and dietary ingredients such as cassava root-leaf meal (CRLM), may affect the balance of beneficial and harmful bacterial species in the caeca [11]. Fermented feed ingredients, have been shown to modulate the gut microflora, promoting the growth of beneficial bacteria such as *Bacillus subtilis* while reducing the abundance of pathogenic species such as *Proteus mirabilis* [12]. The fermentation strategy makes the use of non-conventional feed stuff and agro-industrial waste products possible, which will limit total dependence on conventional feed materials. Furthermore, it increases digestibility due to the ability to disintegrate lipids, polysaccharides and proteins into their respective simple biochemical components, thereby enhancing nutrient absorption [13]. Fermented sweet potato flour has been used in broiler diet which, resulted in higher weight gain and better feed conversion ratio (FCR) [14]. Feng *et al.* [15] also reported that fermented soyabean meal in diet of broilers increased trypsin and lipase activity in the intestine. Earlier works by Oboh [16] and Aro *et al.* [6] demonstrated the benefits of fermentation for reducing antinutritional factors in cassava, but did not test the interactive effects of different fermentation methods with enhanced amino acid supplementation on both performance and the gut microflora. Previous studies by Apajalahti *et al.* [10] and Torok *et al.* [11] investigated the role of diet on the gut microbiota in poultry, but the focus was primarily on conventional feed ingredients. Therefore, this study aimed to investigate the impact of different processing methods for CRLM and the effects of amino acid supplementation on the performance and caeca microflora of broiler chicks.

2. Material and methods

2.1 Location of the experiment

The study was carried out at the Teaching and Research Farm of the School of Agricultural Technology, Yaba College of Technology, Epe. The site is at a latitude of 6.58 °N and a longitude of 3.98 °E. It lies in the lowland rainforest within the savannah agroecological zones with an annual rainfall of 1694 mm and an average temperature of 27.1 °C [17]. The protocol for the experiment was reviewed and approved (AUCC/23/OL/CPLM/021) by the Animal Use and Care Committee at Yaba College of Technology, Lagos, Nigeria.

2.2 Preparation of test ingredients

2.2.1 Sundried cassava root-leaf meal (SCRLM)

Fresh unpeeled cassava root tubers (TMS 30572) were harvested, washed and ground, while the cassava leaves were removed from the cassava stems and chopped into small pieces via a kitchen knife.

The grated unpeeled cassava and chopped cassava leaves were mixed at a ratio of 1:0.3, i.e., 1 kg of cassava root to 300 g of leaves, according to the procedure described by Olayemi *et al.* [18]. The mixed cassava root-leaf mixture was dried to a constant weight and stored at room temperature in polythene bags.

2.2.2 Fermented cassava root-leaf meal (FCRLM)

The grated unpeeled cassava root and chopped leaves were mixed at a ratio of 1 kg of cassava root to 300 g of cassava leaves (1:0.3), and the mixture was placed in airtight containers and allowed to ferment for 5 days. After 5 days, the fermented blend was dried to a constant weight and stored at room temperature in polythene bags.

2.2.3 Fermentation of cassava root-leaf meal with yeast (*Saccharomyces cerevisiae*) (YFCRLM)

The grated unpeeled cassava root and leaves mixed at a ratio of 1 kg of cassava root to 300 g of cassava leaves (1:0.3) were placed in airtight containers supplemented with yeast (*Saccharomyces cerevisiae*) at 2 g/kg to ferment for 5 days. After 5 days, the fermented blend was dried to a constant weight and stored at room temperature in polythene bags.

2.2.4 Fermented cassava root-leaf meal with rumen filtrate (RFCRLM)

The grated unpeeled cassava root and leaves mixed at a ratio of 1 kg of cassava root to 300 g of cassava leaves (1:0.3) were placed in airtight containers with the inclusion of rumen filtrate at 200 ml/kg for fermentation for 5 days.

2.3 Experimental birds, management and design

A total of 240-day-old broiler Ross® chicks were purchased from a reputable commercial hatchery. The day-old chicks were allotted to 10 dietary treatments with 24 chicks per treatment. Each treatment consisted of 3 replicates with 8 chicks each in a 5 × 2 factorial arrangement of five types of dietary energy sources (maize, SCRLM, FCRLM, YFCRLM and RFCRLM) and two levels of amino acid supplementation (ideal and NRC). Brooding of the chicks was performed in rearing cages, and water and feed were offered ad libitum throughout the experimental period. Appropriate vaccinations and medications were administered as needed by the standard schedule. The feed ingredients were purchased from a reputable feed mill, and experimental diets were formulated to meet the nutrient requirements of broilers [19].

2.4 Experimental diets

Feed was formulated to meet the nutrient requirements of the birds in two phases: pre-starter (0-14d) (Table 1) and starter (15-28d) (Table 2) phases, with 50% processed CRLM as a replacement for maize and amino acid supplementation at ideal and NRC levels. The amino acids included were the limiting amino acids (lysine, methionine, arginine and threonine). Ten experimental diets were formulated such that diets 1-5 represent the IDEAL formulation, whereas diets 6-10 represent the NRC formulation with the inclusion of differently processed cassava at 50 % replacement for maize.

Table 1. Gross composition of the experimental diet (prestarter 0-14days).

	Ideal					NRC				
Diets	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Ingredients										
Maize	56.00	28.00	28.00	28.00	28.00	56.00	28.00	28.00	28.00	28.00
SCRLM	0.00	28.00	0.00	0.00	0.00	0.00	28.00	0.00	0.00	0.00
FCRLM	0.00	0.00	28.00	0.00	0.00	0.00	0.00	28.00	0.00	0.00
YFCRLM	0.00	0.00	0.00	28.00	0.00	0.00	0.00	0.00	28.00	0.00
RFCRLM	0.00	0.00	0.00	0.00	28.00	0.00	0.00	0.00	0.00	28.00
Soybean meal	35.20	34.70	34.70	34.70	34.70	35.40	34.90	34.90	34.90	34.90
Palm oil	2.00	2.50	2.50	2.50	2.50	2.00	2.50	2.50	2.50	2.50
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.00	1.00	1.00	1.20	1.00	1.00	1.00	1.00
DCP-Dicalcium phosphate	1.75	1.75	1.75	1.75	1.75	1.85	1.85	1.85	1.85	1.85
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine	0.40	0.40	0.40	0.40	0.40	0.50	0.40	0.40	0.40	0.40
DL-Methionine	0.35	0.35	0.35	0.35	0.35	0.45	0.35	0.35	0.35	0.35
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-Threonine	0.40	0.40	0.40	0.40	0.40	0.00	0.40	0.40	0.40	0.40
L-Arginine	0.30	0.30	0.30	0.30	0.30	0.00	0.00	0.00	0.00	0.00
Total	100	100	100	100	100	100	100	100	100	100
Calculated nutrients (%)										
Metabolisable Energy (Kcal/kg)	2991.36	3000.40	2998.40	3010.20	2993.26	2991.36	2998.42	2990.40	3080.40	3080.40
Crude protein	23.53	22.64	23.07	21.93	22.01	23.42	22.10	21.30	21.93	22.51
Crude fiber	3.42	3.56	3.40	3.00	3.01	3.42	3.56	3.40	3.00	3.01
Ether extract	3.60	3.21	3.12	3.10	3.15	3.60	3.21	3.12	3.10	3.15
Calcium	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

SCRLM- Sundried cassava root–leaf meal, FCRLM- Fermented cassava root–leaf meal, YFCRLM-Yeast-Fermented cassava root–leaf meal, RFCRLM- Rumen-Fermented cassava root–leaf meal. T1- Maize-based diet with ideal amino acid, T2- SCRLM-based diet with ideal amino acid, T3- FCRLM-based diet with ideal amino acid, T4- YFCRLM-based diet with ideal amino acid, T5- RFCRLM-based diet with ideal amino acid, T6- Maize-based diet with NRC amino acid, T7- SCRLM-based diet with NRC amino acid, T8- FCRLM-based diet with ideal amino acid, T9- YFCRLM-based diet with NRC amino acid, T10- RFCRLM-based diet with NRC amino acid. *Included per Kg total diets: Fe ($FeSO_4$. H_2O), 80mg; Zn ($ZnSO_4$. H2O), 80mg; Mn ($MnSO_4$. H_2O) 80mg; Co ($CoSO_4$. H_2O) 0.5mg; Cu ($CuSO_4H_2O$)10mg; Se (Na_2SeO_3) 0.2 mg; I, (Ca (IO_3). $2H_2O$) 0.9mg; vitamin A, 24,000 IU; vitamin D_3, 6,000 IU; vitamin E, 30 IU; vitamin K, 4 mg; thiamin, 4 mg; riboflavin, 12 mg; pyridoxine, 4 mg; folacine, 2 mg; biotin, 0.03 mg; vitamin B8, 0.06 mg; niacin, 90 mg; pantothenic acid, 30 mg.

Table 2. Gross composition of the experimental diet (starter 15-28 days).

	Ideal					NRC				
Diets	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Ingredients										
Maize	58.00	29.00	29.00	29.00	29.00	58.00	29.00	29.00	29.00	29.00
SCRLM	0.00	29.00	0.00	0.00	0.00	0.00	29.00	0.00	0.00	0.00
FCRLM	0.00	0.00	29.00	0.00	0.00	0.00	0.00	29.00	0.00	0.00
YFCRLM	0.00	0.00	0.00	29.00	0.00	0.00	0.00	0.00	29.00	0.00
RFCRLM	0.00	0.00	0.00	0.00	29.00	0.00	0.00	0.00	0.00	29.00
Soybean meal	35.00	34.30	34.30	34.30	34.30	35.15	34.45	34.45	34.45	34.45
Palm oil	2.00	2.70	2.70	2.70	2.70	2.00	2.70	2.70	2.70	2.70
Fish meal	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	1.00	1.00	1.00	1.00	1.00	1.10	1.10	1.10	1.10	1.10
DCP-Dicalcium phosphate	1.75	1.75	1.75	1.75	1.75	1.85	1.85	1.85	1.85	1.85
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
DL-Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-Threonine	0.10	0.10	0.10	0.10	0.10	0.00	0.00	0.00	0.00	0.00
L-Arginine	0.25	0.25	0.25	0.25	0.25	0.00	0.00	0.00	0.00	0.00
Total	100	100	100	100	100	100	100	100	100	100
Calculated nutrients (%)										
Metabolisable Energy (Kcal/kg)	3160.10	3152.20	3152.12	3152.22	3152.22	3160.20	3152.22	3152.22	3152.22	3152.22
Crude protein	22.01	22.37	22.29	22.10	22.19	22.01	21.57	22.49	22.10	22.19
Crude fiber	3.46	3.69	3.52	3.01	3.12	3.46	3.69	3.52	3.01	3.12
Ether extract	3.68	3.27	3.18	3.16	3.21	3.68	3.27	3.18	3.16	3.21
Calcium	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

SCRLM- Sundried cassava root–leaf meal, FCRLM- Fermented cassava root–leaf meal, YFCRLM-Yeast-Fermented cassava root–leaf meal, RFCRLM- Rumen-Fermented cassava root–leaf meal. T1- Maize-based diet with ideal amino acid, T2- SCRLM-based diet with ideal amino acid, T3- FCRLM-based diet with ideal amino acid, T4- YFCRLM-based diet with ideal amino acid, T5- RFCRLM-based diet with ideal amino acid, T6- Maize-based diet with NRC amino acid, T7- SCRLM-based diet with NRC amino acid, T8- FCRLM-based diet with ideal amino acid, T9- YFCRLM-based diet with NRC amino acid, T10- RFCRLM-based diet with NRC amino acid**.** *Included per Kg total diets: Fe ($FeSO_4$. H_2O), 80mg; Zn ($ZnSO_4$. H2O), 80mg; Mn ($MnSO_4$. H_2O) 80mg; Co ($CoSO_4$. H_2O) 0.5mg; Cu ($CuSO_4$. H_2O)10mg; Se (Na_2SeO_3) 0.2 mg; I, (Ca (IO_3). $2H_2O$) 0.9 mg; vitamin A, 24,000 IU; vitamin D_3, 6,000 IU; vitamin E, 30 IU; vitamin K, 4 mg; thiamin, 4mg; riboflavin, 12 mg; pyridoxine, 4 mg; folacine, 2 mg; biotin, 0.03 mg; vitamin B8, 0.06 mg; niacin, 90 mg; pantothenic acid, 30 mg.

2.5 Chemical analysis of samples

The chemical composition of the processed CRLM was analysed (n=4) by determining the moisture content, crude protein, crude fibre, ether extract, ash, nitrogen-free extract and cyanide content via the standard methods of the Association of Analytical Chemists (AOAC) [20]. The amino acid composition of the cassava products was analysed using High Performance Liquid Chromatography (HPLC) (SSNIFF Spezialdiäten GmbH, Soest, Germany).

2.6 Parameters measured

2.6.1 Growth performance

The initial weight of the birds was measured before the beginning of the feeding trial, the subsequent weights were measured weekly, and the weight gain (WG) was calculated as the difference between the initial and final weights. The feed intake was measured by determining the difference between the feed offered and the remaining feed. The daily feed intake (DFI) and total feed intake (TFI) were also calculated. Records of mortality were monitored daily at both phases of the experiment. The feed conversion ratio (FCR) was calculated as the ratio of feed intake (FI) to weight gain.

2.6.2 Gut microbiota

At the end of the feeding trial (on the 28th day), three birds per treatment were selected and slaughtered. The gastrointestinal tract was excised and dissected into small (duodenum, jejunum and ileum) and large intestinal segments, and fresh digesta samples were collected at the ileocaecal junction into labelled sterile vials. Fresh cecal contents collected from the birds were used for estimation of the gut microbiota according to the methods previously described [21]. One gram of collected sample was dispersed in 9 mL of phosphate-buffered saline solution with 0.5 g/L of cysteine hydrochloride, which was further diluted to a factor of 10-8. The enumeration of the different microbes was carried out via standard selective media in Petri dishes. Counts of microbes were expressed in colony-forming units (CFU) of microorganism per gram of sample.

2.7 Statistical analysis

The data obtained were subjected to two-way analysis of variance in a completely randomised design using the Statistical Analysis Software package [22]. The main effects of five dietary energetic ingredients (maize, processed CRLM (SCRLM, FCRLM, YFCRLM and RFCRLM)) and two levels of amino acid inclusion (ideal and NRC). The significant means considered at levels lower than 5% ($P < 0.05$) were separated via Tukey's test using the same package.

3. Results

The results of the differently processed CRLM samples revealed varying chemical compositions (Table 3). It was observed that SCRLM had the highest crude protein content (15.66 %), while the lowest crude protein content (7.48 %) was recorded in YFCRLM. The carbohydrate content was high in YFCRLM (69.11 %) and low in SCRLM (54.95 %). The crude fibre values varied with processing method (8.36 % - 10.74 %). The fibre level in the SCRLM was high, followed by that in the FCRLM; however, YFCRLM and RFCRLM had decreased fibre values of 8.36 % and 8.75 % respectively. The lowest cyanide content was observed for RFCRLM (3.82 mg/kg), and the highest was observed for SCRLM (9.35 mg/kg).

From the essential amino acids, Methionine levels are low (0.47 – 1.02 g/100g) across the cassava products. Leucine showed the highest concentration across the cassava products, ranging from 5.66

– 7.31 g/100g, and the FCRLM sample had the highest leucine content (7.31 g/100g). Arginine was the second most abundant essential amino acid, ranging from 4.25 – 5.28 g/100g, followed by phenylalanine (2.95 – 4.03 g/100g) and lysine 3.12 – 3.66 g/100g. Histidine ranged from 1.03 to 1.93 g/100g, isoleucine ranged from 2.18 to 3.25 g/100g, and valine ranged from 2.31 to 3.25 g/100g, while YFCRLM had the highest histidine, isoleucine and valine.

The non-essential amino acids show that the concentration of cysteine and glutamine was low across the cassava products, with a range of 0.36 – 0.56 g/100g and 0.76 – 0.98 g/100g, respectively. Glycine was highest in YFCRLM (2.75 g/100g) but lowest in SCRLM (2.07 g/100g). Aspartate and alanine were the most abundant across cassava products, aspartate ranged from 4.52 – 6.12 g/100g, with RFCRLM having the lowest while SCRLM had the highest. Alanine composition ranged from 2.78 – 4.27 g/100g, in which YFCRLM had the highest, while SCRLM had the lowest. The amino acid serine ranged from 2.82 to 3.48 g/100g, the lowest and highest were observed in FCRLM and YFCRLM, respectively. Tyrosine ranged from 2.14 to 2.65 g/100g, and YFCRLM had the highest. Proline ranged from 2.69 to 3.93 g/100g and increased quantity was observed in FCRLM and YFCRLM. The FCRLM and YFCRLM generally yielded higher levels of both essential and non-essential amino acids compared to the SCRLM and RFCRLM treatments.

Table 3. Chemical composition of CRLM after different processing methods.

Parameters (%)	**SCRLM**	**FCRLM**	**YFCRLM**	**RFCRLM**
Moisture	8.73	6.38	6.26	6.06
Crude protein	15.66	11.92	7.84	8.13
Crude fat	2.59	2.29	2.20	2.38
Crude fiber	10.74	10.15	8.36	8.75
Ash	7.33	7.02	6.23	6.81
Carbohydrate	54.95	62.24	69.11	67.87
Cyanide (mg/kg)	9.351	5.809	4.47	3.82
Amino acid (g/100g)				
Essential				
Lysine	3.12	3.19	3.66	3.39
Methionine	1.02	0.47	0.61	0.55
Arginine	4.25	4.72	5.28	4.55
Isoleucine	2.18	2.14	2.85	2.58
Leucine	5.66	7.31	6.65	6.22
Threonine	1.97	1.64	1.69	1.46
Valine	2.31	2.86	3.25	3.03
Histidine	1.03	1.71	1.93	1.55
Phenylalanine	2.95	3.47	4.03	3.66
Non-essential				
Cysteine	0.56	0.36	0.48	0.41
Glutamine	0.98	0.89	1.16	0.76
Glycine	2.07	2.41	2.75	2.66
Alanine	2.78	3.36	4.27	3.98
Serine	2.93	2.82	3.48	3.10
Aspartate	6.12	5.08	4.93	4.52
Tyrosine	2.14	2.48	2.65	2.48
Proline	2.69	3.51	3.93	3.12

SCRLM - Sundried, FCRLM- Fermented cassava root–leaf meal, YFCRLM- Fermented cassava root–leaf meal + yeast, and RFCRLM - Fermented cassava root–leaf meal + rumen filtrate.

From Table 4, it was observed that the weight gain and FCR from 1-4 weeks were significantly affected ($P< 0.05$) with varying levels of limiting amino acids. Chicks at the ideal level of amino acids presented greater WG and better FCR ($P<0.05$). The processing methods had a significant ($P< 0.05$) effect on FI and FCR, with chicks fed the maize and RFCRLM-based diet having lower FI and better FCR.

Table 5 presents the interactive effects of amino acid levels and processing methods on growth performance indices of broiler chicks. The results presented in Table 5 revealed that DFI, TFI and FCR were influenced (P<0.05) by amino acid level and processing method. Broiler chicks fed the RFCRLM-based diet with ideal amino acids presented the lowest DFI and TFI with the best FCR, whereas those fed the SCRLM-based diet with ideal amino acid levels presented increased DFI and TFI (P<0.05). Broiler chicks fed maize-based diets with NRC amino acid levels also presented reduced (P<0.05) DFI and TFI, followed by those fed SCRLM-based diets with NRC amino acid levels. Broilers fed the YFCRLM-based diet with NRC amino acid levels had the worst FCR.

In the analysis of the main effects of amino acid level and processing method on the cecal microflora of broilers (Table 6). It was observed that *Escherichia coli*, Staphylococcus, *Klebsiella oxytoca* and the total bacteria count (TBC) were not (P>0.05) affected by the amino acid level. The abundances of *Pseudomonas fluorescens, Citrobacter* spp, *Bacillus subtilis* and *Enterobacter cloacae* were greater (P<0.05) in broilers fed a diet with an ideal amino acid level, whereas the abundances of *Pseudomonas aeruginosa* and *Proteus mirabilis* were greater in broilers fed a diet with NRC amino acid level. *Escherichia coli* and *Citrobacter* spp. were not (P>0.05) affected by the processing methods, whereas the other bacteria were significantly (P<0.05) affected. Broilers fed the FCRLM-based diet presented the highest (P<0.05) Staphylococcus count, and those fed the maize-based diet presented the lowest Staphylococcus count. Compared with those in the other treatments, the abundances of *Pseudomonas fluorescens*, *Enterobacter cloacae* and TBC were also greater (P<0.05) in broilers fed FCRLM-based diets. Compared with those in the other treatment groups, the *Bacillus subtilis* and *Proteus mirabilis* counts in the maize-based diet group were greater (P<0.05). *Pseudomonas fluorescens* and TBC were lowest (P<0.05) in broilers fed the SCRLM-based diet.

Table 7 shows the interactive effects of amino acid level and processing methods on the microbial count of broilers. All the microbial counts were influenced (P<0.05). The highest (P<0.05) value (0.80×10^6 CFU/mL) of *E. coli* was observed for broilers fed a maize-based diet with an ideal amino acid level and those fed FCRLM and RFCRLM with an NRC amino acid level. The Staphylococcus count (1.13×10^6 CFU/mL) was highest (P<0.05) for broilers fed the FCRLM-based diet with the ideal amino acid level. Under NRC, the FCRLM also presented a relatively high Staphylococcus count (0.80×10^6 CFU/mL), but this count was lower than the ideal amino acid level. Broilers given the SCRLM-based diet with an ideal amino acid concentration presented the highest (0.50×10^6 CFU/mL) concentration of *Klebsiella oxytoca* (P<0.05). Compared with those in the other treatment groups, the broilers in the FCRLM-based diet with NRC amino acid levels presented the highest (P<0.05) *Pseudomonas fluorescens, Pseudomonas aeruginosa, and Enterobacter cloacae* counts. The highest (P<0.05) *Baccilus subtilus* (0.80×10^6 CFU/mL) and *Proteus mirabilis* count (0.60×10^6 CFU/mL) were detected in broilers fed a maize-based diet with an ideal amino acid level. The total bacterial count was greater (P<0.05) for broilers fed the maize-based diet at both amino acid levels than for those fed the diets, but those fed the SCRLM-based diet at both amino acid levels presented the lowest total bacterial count.

Table 4. Main effects of amino acid level and processing methods on performance from 1 to 4 weeks of age.

Parameters	Amino acid		P value	SEM	Processing					P value	SEM
	IDEAL	*NRC*			*Maize*	*SCRLM*	*FCRLM*	*YFCRLM*	*RFCRLM*		
IW (g/bird)	42.67	42.62	0.740	0.10	42.45	42.63	42.81	42.53	42.79	0.417	0.16
FLW (g/bird)	441.86^{a}	403.9^{b}	0.006	8.63	410.61	441.65	426.56	417.67	417.92	0.562	13.65
WG (g/bird)	399.14^{a}	361.28^{b}	0.006	8.64	368.16	398.88	383.75	375.13	375.13	0.571	13.65
DFI (g/bird)	23.98	23.49	0.308	0.33	21.41^{b}	25.04^{a}	24.71^{a}	25.04^{a}	22.48^{b}	0.000	0.52
TFI (g/bird)	671.39	657.71	0.309	9.27	599.58^{b}	700.98^{a}	691.88^{a}	700.98^{a}	629.32^{b}	0.000	14.65
FCR	1.69^{b}	1.83^{a}	0.002	0.03	1.64^{c}	1.76^{abc}	1.81^{ab}	1.88^{a}	1.70^{bc}	0.014	0.05

^{abc}Means in the same row with different superscripts are significantly different (P<0.05), SEM: Pooled standard error of the mean, IW: Initial weight, WG: Weight gain, FLW: Final live weight, DFI: Daily feed intake, TFI: Total feed intake, FCR: Feed conversion ratio, SCRLM- Sundried cassava root-leaf meal, FCRLM- Fermented cassava root–leaf meal, YFCRLM--Yeast fermented cassava root-leaf meal, RFCRLM-- Rumen fermented cassava root-leaf meal, FCR= Feed conversion ratio.

Table 5. Interaction effects of amino acid level and processing methods on performance of broilers from 1 to 4 weeks of age.

Parameters	IDEAL					NRC					SEM	P-value
	Maize	*SCRLM*	*FCRLM*	*YFCRLM*	*RFCRLM*	*Maize*	*SCRLM*	*FCRLM*	*YFCRLM*	*RFCRLM*		
IW (g/bird)	42.22	42.68	43.14	42.53	42.76	42.68	42.58	42.47	42.54	42.83	0.22	0.181
FLW (g/bird)	431.78	445.15	447.08	443.66	441.64	389.43	438.16	406.03	391.67	394.2	19.3	0.787
WG (g/bird)	389.57	402.17	403.94	401.13	398.88	346.75	395.59	363.56	349.13	351.37	19.31	0.781
DFI (g/bird)	21.88^{cd}	26.72^{a}	25.68^{ab}	24.72^{b}	20.88^{d}	20.94^{d}	23.35^{c}	23.74^{bc}	25.35^{ab}	24.07^{b}	0.74	0.003
TFI (g/bird)	612.84^{cd}	748.20^{a}	719.04^{ab}	692.24^{b}	584.62^{d}	586.31^{d}	653.77^{c}	664.73^{bc}	709.72^{ab}	674.02^{b}	20.72	0.003
FCR	1.58^{d}	1.87^{b}	1.78^{bc}	1.73^{bc}	1.47^{e}	1.71^{c}	1.65^{cd}	1.83^{b}	2.03^{a}	1.93^{ab}	0.07	0.001

^{ab}Means in the same row with different superscripts are significantly different (P<0.05). SEM: Pooled standard error of the mean, IW: Initial weight, WG: Weight gain, FLW: Final live weight, DFI: Daily feed intake, TFI: Total feed intake, FCR: Feed conversion ratio, SCRLM- Sundried cassava root–leaf meal, FCRLM- Fermented cassava root–leaf meal, YFCRLM-Yeast fermented cassava root–leaf meal, RFCRLM- Rumen fermented cassava root–leaf meal; FCR= Feed conversion ratio.

Table 6. Main effects of amino acid level and processing on the microbial count of broilers from 1 to 4 weeks of age.

Parameters (x 10^6 cfu/ml)	Amino acid IDEAL	Amino acid NRC	P-value	SEM	processing *Maize*	processing *SCRLM*	processing *FCRLM*	processing *YFCRLM*	processing *RFCRLM*	P-value	SEM
Escherichia coli	0.55	0.63	0.105	0.03	0.60	0.47	0.67	0.57	0.65	0.059	0.05
Staphylococcus	0.31	0.36	0.081	0.02	0.00^{c}	0.20^{b}	0.97^{a}	0.30^{b}	0.20^{b}	0.000	0.03
Klebsiella oxytoca	0.21	0.17	0.105	0.02	0.25^{b}	0.35^{a}	0.00^{c}	0.18^{b}	0.17^{b}	0.000	0.03
PF	0.25^{a}	0.17^{b}	0.012	0.02	0.18^{c}	0.00^{d}	0.42^{a}	0.30^{b}	0.15^{c}	0.000	0.04
PA	0.21^{b}	0.27^{a}	0.029	0.02	0.38^{a}	0.25^{b}	0.25^{b}	0.17^{b}	0.15^{b}	0.000	0.03
Citrobacter spp.	0.17^{a}	0.11^{b}	0.021	0.02	0.15	0.12	0.17	0.13	0.12	0.631	0.03
Bacillus subtilus	0.25^{a}	0.15^{b}	0.002	0.02	0.40^{a}	0.00^{d}	0.17^{c}	0.27^{b}	0.15^{c}	0.000	0.03
Proteus mirabilis	0.12^{b}	0.21^{a}	0.002	0.02	0.43^{a}	0.13^{bc}	0.00^{d}	0.07^{cd}	0.18^{b}	0.000	0.03
Enterobacter cloaca	0.19^{a}	0.11^{b}	0.012	0.02	0.08^{b}	0.13^{b}	0.27^{a}	0.13^{b}	0.15^{b}	0.010	0.03
Total bacteria count	2.26	2.17	0.158	0.05	2.48^{b}	1.65^{d}	2.90^{a}	2.12^{c}	1.92^{c}	0.000	0.07

^{abcd}Means in the same row with different superscripts are significantly different (P<0.05). SEM: Pooled standard error of the mean, SCRLM- Sundried cassava. root–leaf meal, FCRLM-Fermented cassava root–leaf meal, YFCRLM-Yeast fermented cassava root–leaf meal, RFCRLM- Rumen fermented cassava root–leaf meal, *PF= Pseudomonas fluorescens, PA= Pseudomonas aeruginosa.*

Table 7. Interaction effect of amino acid level and processing on the cecal microbial count of broilers.

Parameters (x 10^6 CFU/mL)	*IDEAL* *Maize*	*IDEAL* *SCRLM*	*IDEAL* *FCRLM*	*IDEAL* *YFCRLM*	*IDEAL* *RFCRLM*	NRC *Maize*	NRC *SCRLM*	NRC *FCRLM*	NRC *YFCRLM*	NRC *RFCRLM*	SEM	P value
Escherichia coli	0.80^{a}	0.40^{b}	0.53^{b}	0.53^{b}	0.50^{b}	0.78^{a}	0.53^{b}	0.56^{b}	0.60^{ab}	0.45^{b}	0.07	0.000
Staphylococcus	0.00^{e}	0.00^{e}	1.13^{a}	0.00^{e}	0.40^{d}	0.00^{e}	0.40^{d}	0.80^{b}	0.60^{c}	0.00^{e}	0.05	0.000
Klebsiella oxytoca	0.20^{c}	0.50^{a}	0.00^{d}	0.37^{b}	0.00^{d}	0.30^{bc}	0.20^{c}	0.00^{d}	0.00^{d}	0.33^{bc}	0.04	0.000
PF	0.37^{ab}	0.00^{c}	0.33^{b}	0.27^{b}	0.30^{b}	0.00^{c}	0.00^{c}	0.50^{a}	0.33^{b}	0.00^{c}	0.05	0.000
PA	0.53^{a}	0.50^{a}	0.00^{c}	0.00^{c}	0.00^{c}	0.23^{b}	0.00^{c}	0.50^{a}	0.33^{b}	0.30^{b}	0.05	0.000
Citrobacter spp.	0.00^{b}	0.00^{b}	0.33^{a}	0.27^{a}	0.23^{a}	0.30^{a}	0.23^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.04	0.000
Bacillus subtilus	0.80^{a}	0.00^{c}	0.00^{c}	0.13^{c}	0.30^{b}	0.00^{c}	0.00^{c}	0.33^{b}	0.40^{b}	0.00^{c}	0.04	0.000
Proteus mirabilis	0.60^{a}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.27^{b}	0.27^{b}	0.00^{d}	0.13^{c}	0.37^{b}	0.04	0.000
Enterobacter cloaca	0.00^{e}	0.27^{abc}	0.13^{cd}	0.27^{abc}	0.30^{ab}	0.17^{bc}	0.00^{e}	0.40^{a}	0.00^{e}	0.00^{e}	0.05	0.000
Total bacteria count	3.30^{a}	1.67^{d}	2.47^{b}	1.83^{cd}	2.03^{c}	3.05^{a}	1.63^{d}	2.33^{b}	2.40^{b}	1.80^{cd}	0.10	0.000

^{abcdef}Means in the same row with different superscripts are significantly different (P<0.05). SEM: Pooled standard error of the mean SCRLM- Sundried cassava. root–leaf meal, FCRLM-Fermented cassava root–leaf meal, YFCRLM-Yeast Fermented cassava root–leaf meal, RFCRLM- Rumen-Fermented cassava root–leaf meal; FCR= Feed conversion ratio *PF= Pseudomonas fluorescens, PA= Pseudomonas aeruginosa.*

4. Discussion

The different processing techniques used in this study, i.e., sun-drying, fermentation, yeast-aided fermentation and fermentation with rumen filtrate, resulted in variations in nutrient composition, crude protein, crude fibre, carbohydrate and cyanide contents. The fermentation technology employed reduced the fibre level in CRLM, which agreed with the findings of Adeyemi *et al.* (23) and Dairo *et al.* (24), who reported a reduction in the crude fibre level after fermentation of cassava root meal. Compared with other processing techniques, fermentation generally reduces the residual cyanide content, but the introduction of a rumen filtrate results in the lowest cyanide content, which may be due to the cyanidophilic activity of various protozoa and bacteria in the rumen filtrate (25). However, a reduced crude protein content was observed in YFCRLM and RFCRLM, which could be due to amino acid utilisation by inherent microorganisms for the synthesis of bacterial protein (26). It could also be a result of the proliferation of rumen microbes (16,27). Although a relatively high crude protein value was recorded for the sun-dried method (SCRLM), which may be due to the high crude protein content of cassava leaves and the absence of bacteria, a relatively high cyanide content and poor microbiological properties are associated with the products that could be a source of toxicity to the chicks.

The main effect of amino acid level revealed that the final live weight and WG increased for broilers given the ideal amino acid. This same group of broilers achieved a better FCR. This improvement is due to the ideal quantity of amino acids, which is crucial for accelerating tissue accretion, resulting in improved WG and FCR. It has been reported that the availability of amino acids in adequate quantities in the diet of broilers improves FCR, increases weight gain and positively influences the breast meat yield of broiler chickens (28). This finding is in agreement with the reports of Aburto *et al.* (29) and Mukhtar *et al.* (30), who reported that increasing the level of amino acids in the diet of broilers resulted in better growth performance. The impact of processing methods shows that broilers fed the maize-based diet and those fed a RFCRLM-based diet had reduced feed intake and better FCR, which suggests that the RFCRLM can successfully replace maize in the diet of broilers without negatively affecting performance. The improvement in performance of broiler chicks fed a diet with RFCRLM, which is comparable to that of maize, could be associated with the availability of nutrients such as oligosaccharides, vitamins, and small peptides produced during fermentation, which increased the utilisation of ingested feed as is evident in chicks (31). This improvement could also be due to increases in the activity of gastrointestinal microbes as a result of fermentative microbes in cassava roots fermented with the rumen filtrate.

The growth and proliferation of rumen microbes are responsible for the improvement in chick performance, as they can convert their feed into muscular tissues similar to those fed the control diet. This finding was in agreement with the findings of Adeyemi *et al.* (32), who reported improvements in the performance of broilers fed cassava root meal fermented with rumen filtrate. Akapo *et al.* (33) reported better FCRs in broilers fed fermented cassava root meal. The observed difference in feed intake could be due to varying fibre content due to the effects of processing methods, which may affect nutrient availability and hence influence feed intake (34). The interactive effect of amino acid level and processing methods on the performance of broiler chicks indicates that the DFI and TFI are reduced for broiler chicks fed an RFCRLM-based diet with ideal amino acid. Compared with the other treatment groups, the same group of broilers presented the best FCR. The results can be attributed to the synergistic effect of rumen content fermentation and the ideal amino acid level, which resulted in an improvement in the

FCR. The results of the current study, showing improved FCR with rumen fermentation, suggest that such processing methods can mitigate the negative impacts of antinutritional factors often found in raw cassava leaves, thereby increasing feed efficiency. This finding is in agreement with the study of Palupi *et al.* [35], who reported better feed efficiency in broilers fed a diet fermented with Lactobacillus for five days. The authors stated that fermentation is capable of suppressing the fibre effect, which promotes nutrient availability and utilisation. However, in contrast to the results of the present study, Drażbo *et al.* [36] reported no significant effect of feeding fermented rapeseed cake on the FCR of broilers. These discrepancies could be associated with differences in the properties of the fermented ingredients, processing methods and durations of fermentation. The DFI and TFI were highest for broilers fed the SCRLM-based diet with an ideal amino acid level. The high DFI for SCRLM may also reflect its favourable amino acid profile, which is critical for broiler growth, emphasising the importance of amino acid levels in influencing feed consumption. The FLW and WG were not significantly influenced by dietary treatments however, broilers fed a diet with FCRLM achieved the highest FLW and weight gain under the ideal amino acid levels. The improvement in WG observed could be due to fermentation, which enhances nutrient digestibility. Similarly, Aro *et al.* [37] reported that fermentation positively impacts the nutritional profile of feed ingredients, which aligns with the current findings showing increased FLW and weight gain.

The intestinal microbial population in broilers has significant implications for nutrient utilisation, animal health, immune function and overall poultry productivity. In this study, dietary amino acid levels and feed processing methods significantly influenced the microbial populations in the caeca, with implications for broiler health and performance. The main effects of amino acids were that the abundance of *Escherichia coli*, Staphylococcus, and *Klebsiella oxytoca* and the total bacterial count were not significantly affected. Compared with those in the NRC, Pseudomonas fluorescens counts were greater, with ideal amino acid levels resulting in greater microbial growth, suggesting that ideal amino acid levels may create favourable conditions for certain microbial populations. This agrees with Sugiharto *et al.* [13], who noted that fermentation can selectively increase beneficial or neutral bacteria while suppressing pathogens, depending on the bacterial strain. While *Pseudomonas fluorescens* is generally considered non-pathogenic, its overgrowth could pose risks if pathogenic strains are present. This highlights the importance of microbial monitoring when formulating diets enriched with ideal amino acids. The counts of *Citrobacter* spp, *Bacillus subtilis*, and *Enterobacter cloacum* significantly increased in broilers at the ideal amino acid level. The higher count for these microorganisms could be due to the ideal amino acid level, which encouraged the growth of both beneficial and nonbeneficial microorganisms. However, the concentration of *B. subtilis* was greater than that of *Citrobacter* spp. and *E. cloacae*, the elevated presence of *B. subtilis*, a known probiotic, is particularly beneficial, as it contributes to the competitive exclusion of pathogenic microbes and supports gut health. Its higher abundance relative to *Citrobacter* spp. and *E. cloacae* suggests a dominant and potentially suppressive role in maintaining microbial balance in the gastrointestinal tract (GIT). *B. subtilis* has been reported to enhance gut integrity, modulate immune responses, and reduce pathogen load, ultimately leading to improved nutrient utilisation and growth performance in broilers [38].

The main effect of the processing method was that broilers fed the FCRLM-based diet presented increased Staphylococcus and *Enterobacter cloacal* count, which suggests that processing methods also played a pivotal role in shaping gut microbiota and indicate a potential risk of dysbiosis if not properly managed. However, these organisms were significantly reduced in broilers fed YFCRLM and RFCRLM diets, supporting the idea that co-fermentation with yeast or rumen filtrate may enhance microbial safety and gut health. This finding agrees with the findings of Sugiharto and Ranjitkar [39] who reported that fermentation can be utilised as a strategy to reduce the quantity of harmful microorganisms in the intestine of broilers. Importantly, fermentation suppressed the growth of Pseudomonas aeruginosa, which is a pathogen associated with antibiotic resistance and opportunistic infections in poultry as reported by Pang *et al.* [40]. The reduced abundance could be due to the multiple mechanisms involved in antibiotic resistance such as intrinsic antibiotic resistance, the secretion of antibiotic-inactivating enzymes and production of antimicrobial compounds during fermentation, as well as competitive exclusion by beneficial microbes. Therefore, the lower counts observed in this study suggest that fermentation may help control the growth of potentially harmful microbes.

The gut microbiota plays a significant role in broilers' health, influencing gastrointestinal development, nutrient absorption, immune function and resistance to infection. In this study, both the amino acid levels and the cassava root–leaf meal (CRLM) processing methods significantly influenced the gut microbial profile, with important implications for broiler performance and welfare. The interactive effects of amino acid level and processing methods on the microbial count of broilers revealed that at the ideal amino acid level, the abundance of *E. coli* significantly increased in broilers fed a maize-based diet. A similar trend was observed in broilers fed YFCRLM under NRC levels, suggesting that maize and certain fermented CRLM diets may support *E. coli* growth under specific nutritional conditions. These findings suggest that the processing of cassava root-leaf meals by fermentation generally influences *E. coli*. Although *E. coli* is a normal gut inhabitant, an overgrowth, especially of pathogenic strains, can compromise intestinal integrity and performance, emphasising the need for diet-mediated microbial control. In contrast, fermentation of CRLM, especially when combined with ideal amino acids, generally suppressed *E. coli*, supporting findings by Aro *et al.* [41] and reinforcing the idea that fermentation modulates microbial communities by creating an unfavourable environment for certain pathogens.

Fermentation did not universally suppress all bacteria; the Staphylococcus count was highest for the group of broilers fed the FCRLM-based diet with ideal amino acids and a similar increase was observed for broilers fed the same diet under the NRC. An increase in the count of *E. cloacae* was also observed in broilers fed the RFCRLM or YFCRLM-based diet with ideal amino acids and those fed the FCRLM diet with NRC amino acids. This implies that fermentation may selectively promote the growth of certain commensal or opportunistic microbes bacteria including Staphylococcus depending on the substrate and fermentation conditions. This observation is in agreement with the report of Promthong *et al.* [42], who reported that the solubility of carbohydrate sources affects nutrient availability. The increased solubility of carbohydrates in fermented feeds may provide a more favourable environment for microbial proliferation [43].

Broilers fed the SCRLM-based diet with ideal amino acids presented the highest count of *Klebsiella oxytoca*, but no growth was observed for those fed the FCRLM or RFCRLM-based diet with ideal amino acids or those fed the FCRLM or YFCRLM-based diet with NRC amino acid levels. This illustrates the inhibitory effect of fermentation on potentially harmful bacteria, which is consistent with the report of Russell *et al.* [44], and highlights the potential of fermentation as a natural strategy to enhance gut health.

Interestingly, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*, both associated with spoilage and opportunistic infections, were significantly reduced in birds fed fermented diets with ideal amino acids, reflecting the antimicrobial benefits of fermentation. This finding corroborates the findings of Saleh *et al.* [45], who reported a decrease in the prevalence of pathogenic bacteria with feed fermentation. However, this trend was inconsistent in the NRC groups, indicating that the microbial outcomes of fermentation are also influenced by amino acid balance. The beneficial outcome of ideal amino acid supplementation compared with those in the other treatment groups was the increased abundance of *Bacillus subtilis*, especially in birds fed non-fermented diets. This may be associated with the positive influence of ideal amino acid supplementation on nonfermented feed. As a probiotic, *B. subtilis* contributes to pathogen exclusion, immune modulation, and gut barrier integrity. Its dominance over other bacteria such as *Citrobacter* spp. and *E. cloacae* suggests a stabilising influence on gut microbiota that can improve broiler resilience and productivity [46].

Total bacterial count (TBC) was notably reduced in birds fed fermented diets across both amino acid levels, underscoring the suppressive effect of fermentation on the proliferation of gut pathogenic microbes and implying that fermentation has a stabilising effect on gut microbiology [47]. Interestingly, the lowest TBC was observed in broilers fed SCRLM with ideal amino acids, indicating that sun-drying, like fermentation, can help reduce microbial load, though perhaps less selectively. This is consistent with the observations of Shakouri *et al.* [48], who reported that dietary composition affects the diversity and population of bacterial species in the gut.

5. Conclusion

Fermentation of cassava root-leaf meal (CRLM) effectively reduced cyanide and fibre content. Supplementing broiler diets with ideal amino acids improved weight gain and feed conversion ratio (FCR). While processing methods had little effect on final live weight, fermented CRLM diets with amino acid supplementation reduced feed intake and enhanced FCR, matching maize-based diets. Ideal amino acids increased beneficial bacteria like *Bacillus subtilis*, while fermented CRLM suppressed pathogens such as *Klebsiella oxytoca* and *Pseudomonas aeruginosa*. Overall, fermented CRLM with ideal amino acids supports broiler growth and helps stabilise gut microbiota.

Conflict of interest statement

The authors declare no conflict of interest.

Data availability statement

The data will be provided upon request.

Author contributions

Conceptualization: W.A. Olayemi and A.O. Oso. Data curation: G.A. Williams and W.A. Olayemi. Formal analysis: G.A. Williams. Methodology: A.O. Oso and W.A. Olayemi. Project administration: E.O. Omofunmilola. Software: G.A. Williams. Supervision: W.A. Olayemi. Validation: A.O. Oso. Writing (original draft): W.A. Olayemi. Writing (proofreading, review & editing): G.A. Williams.

References

1. Sowunmi FA, Bello AA, Ogunniyi AI, Omotayo AO. Delving deeper into market concentration of poultry feed and the driving factors for brand switching: evidence from commercial egg producers in Nigeria. Sustainability. 2021; 14(13):8030. https://doi.org/10.3390/su14138030

2. Oloruntola OD, Agbede JO, Onibi GE, Igbasan FA. Replacement value of rumen liquor fermented cassava peels for maize in growing rabbit diets. Arch Zootec. 2016;65(249):89-97. https://doi.org/10.21071/az.v65i249.446

3. Oso AO, Akapo O, Sanwo KA, Bamgbose AM. Utilization of unpeeled cassava (*Manihot esculenta* Crantz) root meal supplemented with or without charcoal by broiler chickens. J Anim Physiol Anim Nutr. 2014;98:431-438. https://doi.org/10.1111/jpn.12088

4. Khieu B, Chhay T, Ogle RB, Preston TR. Research on the use of cassava leaves for livestock feeding in Cambodia. In: Proceeding of the Regional Workshop on the Use of Cassava Roots and Leaves for On-Farm Animal Feeding; 2005; Hue, Vietnam. pp. 17-19.

5. Oboh G, Akindahunsi AA. Biochemical changes in cassava products (flour & gari) subjected to *Saccharomyces cerevisiae* solid media fermentation. Food Chem. 2003;82(4):599-602. https://doi.org/10.1016/S0308-8146(03)00016-5

6. Aro SO, Aletor VA, Tewe OO, Agbede JO. Nutritional potentials of cassava tuber wastes: a case study of a cassava starch processing factory in southwestern Nigeria. Livest Res Rural Dev. 2010;22:1-11. http://www.lrrd.org/lrrd22/11/aro22213.htm

7. Tewe, O.O. 1992. Detoxification of cassava products and effects of residual toxins on consuming animals. In: Machin, D.; Nyvold, S., 1992. Roots, tubers, plantains and bananas in animal feeding. Proceedings of the FAO Expert Consultation held in CIAT, Cali, Colombia 21–25 January 1991; FAO Animal Production and Health Paper - 95

8. Kidd MT. Nutritional modulation of immune function in broilers. Poult Sci. 2004;83(4):650-657. https://doi.org/10.1093/ps/83.4.650

9. Baker DH. Ideal amino acid profiles for swine and poultry and their applications in feed formulations. Biokyowa Tech Rev. 1997;9(1-24). https://books.google.com.br/books/about/Ideal_Amino_Acid_Profiles_for_Swine_and.html?id=GtIxkgAACAAJ&redir_esc=y

10. Apajalahti J, Kettunen A, Graham H. Characterization of gastrointestinal microbial communities with special reference to the chicken. World's Poult Sci J. 2004;60:223-232. https://doi.org/10.1079/WPS20040017

11. Torok VA, Hughes RJ, Mikkelsen LL, Perez-Madonado R, Balding K, MacAlpine R, Percy NJ, Ophel-Keller K. Identification and characterization of potential performance-related gut microbiotas in broiler chickens across various feeding trials. Appl Environ Microbiol. 2011;77(17):5868-5878. https://doi.org/10.1128/AEM.00165-11

12. Aderemi FA, Nworgu FC. Nutritional status of cassava peels and root sieviate biodegraded with Aspergillus niger. Am-Eurasian J Agric Environ Sci. 2007;2(3):308-311. https://idosi.org/aejaes/jaes2(3)/16.pdf

13. Sugiharto S, Ranjitkar S. Recent advances in fermented feeds toward improved broiler chicken performance, gastrointestinal tract microecology, and immune responses: a review. Anim Nutr. 2019;5(1):1-10. https://doi.org/10.1016/j.aninu.2018.11.001

14. Febrianti D, Wahyuni HI, Yudiarti T, Widiastuti E, Agusetyaningsih I, Sartono, TA, Sugiharto S. The use of fermented purple sweet potato flour in broiler chicken feed: Effect on production performance. Livest. Res Rural Dev 2024; Volume 36, Article #24. http://www.lrrd.org/lrrd36/3/3624sgh.html

15. Feng J, Liu X, Xu ZR, Wang YZ, Liu JX. 2007. Effects of fermented Soybean meal on digestive enzyme activities and intestinal morphology in broilers. Poult Sci 2007; 86:1149-1154. https://doi.org/10.1093/ps/86.6.1149

16. Oboh G. Nutrient enrichment of cassava peels using a mixed culture of *Saccharomyces cerevisiae* and Lactobacillus spp. solid media fermentation techniques. Electron J Biotechnol. 2006; 9(1):46-49. https://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0717-34582006000100007

17. Google Earth. Earth maps. 2023. https://google.com/lagos/763331220023114

18. Olayemi WA, Rabiu LA, Oso AO, Akapo OA, Bamgbose AM. Assessment of the nutritive value of fermented cassava root-leaf meal as a reflect on blood profile of ducks. Niger J Anim Prod. 2000; 47(3):194-201. https://doi.org/10.51791/njap.v47i3.155

19. National Research Council (NRC). Nutrient Requirements of Poultry. 9th ed. National Academic Press; 1994

20. AOAC. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists; 2002.

21. Xia MA, Hu CH, Xu ZR. Effects of copper-bearing montmorillonite on growth performance, digestive enzyme activities, and intestinal microflora and morphology of male broilers. Poult Sci. 2004; 83:1868-1875. https://doi.org/10.1093/ps/83.11.1868

22. SAS. Statistical Analysis System Institute User's Guide. 9th ed. SAS Institute; 2002.

23. Adeyemi OA, Eruvbetine D, Oguntona T, Dipeolu M, Agunbiade JA. Enhancing the nutritional value of whole cassava root meal by rumen filtrate fermentation. Arch Zootec. 2007; 56:261-264. https://www.researchgate.net/publication/28181420_Enhancing_the_nutritional_value_of_whole_cassava_root_meal_by_rumen_filtrate_fermentation

24. Dairo FAS, Aina A, Omoyeni L, Adegun MK. Ensiled cassava peel and caged layers manure mixture as energy source in broiler starter diet. J Agric Sci Technol. 2011;1:519-524. https://www.academia.edu/download/60974473/MY_FIRST_DEGREE_JOURNAL20191021-39407-30e508.pdf22

25.Tewe OO. Enhancing the nutritive value of cassava for livestock feeding through microbial degradation. Paper presented at the 3rd International Scientific Meeting of the Cassava Biotechnology Network; 1996; Kampala, Uganda.

26. Laursen BS, Sørensen HP, Mortensen KK, Sperling-Petersen HU. Initiation of protein synthesis in bacteria. Microbiol Mol Biol Rev. 2005;69(1):101-123. https://doi.org/10.1128/MMBR.69.1.101-123.2005

27. Antai SP, Mbongo PN. Utilization of cassava peels as substrate for crude protein formation. Plant Foods Hum Nutr. 1994;46:345-351. https://doi.org/10.1007/BF01088435

28. Kidd, MT, McDaniel CD, Branton SL, Miller ER, Boren BB, Fancher B.I. Increasing amino acid density improves live performance and carcass yields of commercial broilers. J Appl Poult Res. 2004;13(4):593-604. https://doi.org/10.1093/japr/13.4.593

29. Aburto A, Vazquez M, Dale NM. Strategies for utilizing overprocessed soybean meal: II. Lysine supplementation. J Appl Poult Res. 1998;7(2):196-201. http://dx.doi.org/10.1093/japr/7.2.196

30. Mukhtar AM, Mekkawi A, Eltigani M. The effect of feeding increasing levels of synthetic lysine and methionine in broiler chicks. Res J Anim Vet Sci. 2007;2:18-20. https://www.aensiweb.net/AENSIWEB/rjavs/rjavs/2007/18-20.pdf

31. Chen CC, Shih YC, Chiou PWS, Yu B. Evaluating nutritional quality of single-stage and two-stage fermented soybean meal. Asian-Aust J Anim Sci. 2010;25:598-606. https://doi.org/10.5713/ajas.2010.90341

32. Adeyemi OA, Eruvbetine D, Oguntona T, Dipeolu M, Agunbiade JA. Feeding broiler chickens with diets containing whole cassava root meal fermented with rumen filtrate. Arch Zootec. 2008;57:218-255. https://web.archive.org/web/20170706210233/http://www.redalyc.org/pdf/495/49515018017.pdf

33. Akapo AO, Oso AO, Bamgbose AM, Sanwo KA, Jegede AV, Sobayo RA, Idowu OM, Fan J, Li L, Olorunsola RA. Effect of feeding cassava (Manihot esculenta Crantz) root meal on growth performance, hydrocyanide intake, and hematological parameters of broiler chicks. Trop Anim Health Prod. 2014;46(7):1167-1172. https://doi.org/10.1007/s11250-014-0622-5

34. Olowoyeye JC, Agbede JO, Igbasan FA, Oloruntola OD, Ayeni AO. Effect of replacing maize with cassava peel-leaf mixture on growth performance of broiler chickens. Livest Res Rural Dev. 2019;31(10):155. http://www.lrrd.org/lrrd31/10/olugb31155.html

35. Palupi R, Lubis FNL, Pratama ANT, Muhakka. Effects of Lactobacillus-fermented feed on production performance and carcass quality of broiler chickens. J World Poult Res. 2023;13(1):127-135. https://dx.doi.org/10.36380/jwpr.2023.14

36. Drazbo AA, Juśkiewicz J, Józefiak A, Konieczka P. The fermentation process improves the nutritional value of rapeseed cake for turkeys—effects on performance, gut bacterial population, and its fermentative activity. Animals. 2020;10(9):1711. https://doi.org/10.3390/ani10091711

37. Aro SO. Improvement in the nutritive quality of cassava and its by-products through microbial fermentation. Afr J Biotechnol. 2008;7(25). https://www.ajol.info/index.php/ajb/article/view/59672

38. Sugiharto S, Isroli I, Yudiarti T, Widiastuti E, Wahyuni IH, Sartono TA. Effect of two-step fermentation by *Chrysonilia crassa* and *Bacillus subtilis* on nutritional values and antioxidative properties of agro-industrial by-products as poultry feed ingredients. J Adv Vet Anim Res. 2018;5(4):472-480. https://doi.org/10.5455/javar.2018.e301

39. Manafi M, Khalaji S, Hedayati M, Pirany N. Efficacy of **Bacillus subtilis** and bacitracin methylene disalicylate on growth performance, digestibility, blood metabolites, immunity, and intestinal microbiota after intramuscular inoculation with Escherichia coli in broilers. Poult Sci. 2017;96(5):1174-1183. https://doi.org/10.3382/ps/pew347

40. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnol Adv. 2019;37(1):177-192. https://doi.org/10.1016/j.biotechadv.2018.11.013

41. Aro SO, Aletor VA, Oladunmoye MK. Feeding microbe-fermented cassava tuber wastes modulates gut microbiota and fecal characteristics of growing pigs. Ferment Technol. 2017;6:145. https://doi.org/10.4172/2167-7972.1000145.

42. Promthong S, Kanto U, Tiwarattanawanich C, Tongyai S, Isariyodom S, Markvichitr K, Engkagul A. Comparison of nutrient composition and carbohydrate fractions of corn, cassava chip, and cassava pellet ingredients. In: Proceedings of 43rd Kasetsart University Annual Conference; 2005; Thailand.

43. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr. 1995;125:1401-1412. https://doi.org/10.1079/nrr200479

44. Russell SM, Fletcher DL, Pancorbo OC, Merka WC. Effect of lactic acid fermentation on bacterial pathogens and indicator organisms in broiler processing waste. Poult Sci. 1993;72(8):1573-1576. https://doi.org/10.3382/ps.0721573.

45. Saleh AA, Shukry M, Farrag F, Soliman MM, Abdel-Moneim AE. Effect of feeding wet feed or wet feed fermented by *Bacillus licheniformis* on growth performance, histopathology, and growth and lipid metabolism marker genes in broiler chickens. Animals. 2021;11(1):83. https://doi.org/10.3390/ani11010083.

46. Taylor-Bowden T, Bhogoju S, Khwatenge CN, Nahashon SN. The impact of essential amino acids on the gut microbiota of broiler chickens. Microorganisms. 2024;12(4):693. https://doi.org/10.3390/microorganisms12040693.

47. Brenes A, Roura E. Essential oils in poultry nutrition: main effects and modes of action. Anim Feed Sci Technol. 2010;158:1-14. https://doi.org/10.1016/j.anifeedsci.2010.03.007.

48. Shakouri M, Iji P, Mikkelsen L, Cowieson A. Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. J Anim Physiol Anim Nutr. 2009;93:647-658. https://doi.org/10.1111/j.1439-0396.2008.00852.x.