

Utilization of *Calopogonium mucunoides* and Cassava Peels in Feed Waffle Production: Implications for Phytochemical Characteristics and Safe Ruminant Nutrition

Ezimoha C. O¹, Nsidinanya N. O², Nathaniel, J², and Obuji, D. C²

¹Department of Animal production and Livestock Management. College of Animal Science and Animal Production, Michael Okpara university of Agriculture Umudike, Umuahia Abia State Nigeria.

²Department of Animal Breeding and Physiology. College of Animal Science and Animal Production. Michael Okpara university of Agriculture Umudike, Umuahia Abia State Nigeria.

*Corresponding Author: ezimoha.christiana@mouau.edu.ng

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Abstract

The high cost and limited availability of conventional livestock feed ingredients have intensified the search for alternative feed resources that are affordable, sustainable, and nutritionally suitable for ruminant production. This study evaluated the phytochemical composition and anti-nutritional factor profile of ruminant feed waffles formulated with varying inclusion levels of *Calopogonium mucunoides* and cassava peels. The experiment was conducted at the National Root Crops Research Institute (NRCRI), Umudike, Abia State, Nigeria. Four experimental diets were formulated with graded levels of crushed *Calopogonium mucunoides* (0, 7, 14, and 21%) and mashed cassava peels (7, 10, 13, and 16%) designated as S1, S2, S3, and S4, respectively. The feed mixtures were processed into waffles through drying, milling, and baking, after which phytochemical constituents were determined using standard analytical procedures. Results showed significant ($P < 0.05$) differences among treatments for tannin, saponin, oxalate, flavonoid, phenol, phytate, and cyanide concentrations. Tannin content ranged from 0.24 to 0.32 mg/100 g, while saponin values ranged from 1.35 to 1.42 mg/100 g. Oxalate concentration decreased from 3.85 mg/100 g in S1 to 3.65 mg/100 g in S2, whereas phenol content increased from 1.11 to 1.18 mg/100 g across treatments. Alkaloid concentration remained unchanged (3.80 mg/100 g) in all treatments. Cyanide concentration was markedly reduced in the processed diets, decreasing from 12.65 mg/100 g in S1 to 2.25–2.26 mg/100 g in the supplemented diets, indicating the effectiveness of processing in detoxification. The phytochemical values obtained were within tolerable ranges for ruminant animals and suggest minimal risk of adverse effects on animal health. It was concluded that the incorporation of *Calopogonium mucunoides* and cassava peels, coupled with drying, milling, and baking, effectively reduced selected anti-



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nutritional factors and produced feed waffles with acceptable phytochemical characteristics. The S2 formulation exhibited the most favorable balance between phytochemical safety and feed quality, indicating its potential as a sustainable and cost-effective alternative feed resource for ruminant production.

Introduction

The livestock sector plays a critical role in ensuring food security and supplying high-quality animal protein to the growing global population. However, feed costs remain the largest single expense in ruminant production systems, accounting for approximately 60–70% of total production costs in many developing countries. The increasing competition between humans and livestock for conventional feed ingredients such as maize and soybean meal has intensified the search for alternative, affordable, and sustainable feed resources capable of supporting animal productivity without compromising feed quality (Belete et al., 2024).

Forage legumes have received considerable attention as alternative feed resources because of their relatively high protein content, adaptability to tropical environments, and potential to improve ruminant nutrition. *Calopogonium mucunoides* is a perennial tropical forage legume widely distributed in humid and sub-humid regions. The plant is characterized by good biomass production, high crude protein content, and appreciable mineral composition, making it a valuable supplementary feed resource for ruminants (Adebayo et al., 2023). Previous studies have demonstrated that *Calopogonium mucunoides* possesses satisfactory nutritional value and acceptable levels of secondary metabolites that can support livestock production when properly utilized (Ogunlade et al., 2022). Nevertheless, like many forage legumes, it contains phytochemicals that may influence nutrient utilization depending on their concentration and processing methods.

Cassava peels are among the most abundant agro-industrial by-products generated from cassava processing in tropical countries. Their utilization in livestock feeding offers an opportunity to reduce feed costs while

minimizing environmental pollution associated with cassava waste disposal. Cassava peels are rich in carbohydrates and can serve as an important energy source in ruminant diets (Mukhtar et al., 2023). However, their utilization is often constrained by the presence of anti-nutritional compounds such as cyanogenic glycosides, phytates, and oxalates, which may negatively affect animal health and nutrient utilization when present at elevated concentrations (Mijena & Ijara, 2024). Appropriate processing methods including drying, milling, fermentation, and thermal treatment have been reported to significantly reduce these anti-nutritional factors and improve the feeding value of cassava by-products (Mukhtar et al., 2023).

Phytochemicals and anti-nutritional factors are naturally occurring compounds present in many plant-derived feed ingredients. Compounds such as tannins, saponins, flavonoids, alkaloids, oxalates, and phytates may exert both beneficial and detrimental effects on ruminant nutrition depending on their concentration and biological activity. Moderate levels of some phytochemicals have been associated with improved antioxidant status, enhanced rumen fermentation efficiency, and reduced methane emissions, whereas excessive concentrations may impair nutrient digestibility and mineral utilization (Besharati et al., 2022; Olarinre et al., 2022). Consequently, the evaluation of phytochemical composition remains an important aspect of feed quality assessment and feed safety evaluation.

Feed processing technologies have increasingly been explored as practical approaches for improving feed quality, enhancing storage stability, and reducing anti-nutritional factors. The production of feed waffles represents an innovative method of converting unconventional feed resources into compact, easily handled, and potentially more stable feed products. Despite

growing interest in alternative feed ingredients, information regarding the phytochemical characteristics of feed waffles produced from combinations of *Calopogonium mucunoides* and cassava peels remains scarce. Therefore, this study evaluated the phytochemical composition and anti-nutritional factor profile of ruminant feed waffles formulated with varying inclusion levels of *Calopogonium mucunoides* and cassava peels.

Materials and Method

Location of the Experimental Site

The experiment was conducted at the National Root Crop Research Institute, Umudike. Umudike is in Abia State of Nigeria, located at latitude 05° 28' N and longitude 07°31' E and lies at an altitude of 122 meters above sea level. It lies within the tropical rainforest zone characterized by average annual rainfall of 2,177mm in 148-155 rain days. Average ambient temperature was 25.5°C with minimum and maximum temperatures of 22°C and 29°C respectively. Relative humidity ranged from 76-87% (NRCRI, 2004).

Sources and Processing of Experimental Material

Fresh *Calopogonium mucunoides* with their succulent vines were harvested from the western farm of the Michael Okpara University of Agriculture. The plant was air dried for 7 days and crushed. Cassava peels were sourced within Umudike, sorted, washed to remove impurities and mashed. Other feed materials were sourced from Jocan Livestock feed Store in Umuahia, Abia State, thoroughly homogenized according to the treatment groups and placed in a rectangular baking pan measuring 3 by 6 cm for each replicate.

Crushed Calopo was included in the treatments at: 0%, 7%, 14% and 21% for S₁, S₂, S₃ and S₄ respectively, while mashed cassava peels was included in the diets at: 7%, 10%, 13% and 16% for S₁, S₂, S₃ and S₄ respectively. Each sample was replicated two (2) times. 1.4kg of each sample was weighed into the baking pan before baking.

Baking of waffle

Baking of the feed waffles were done in an industrial oven for an extended period of 2 hours, a lower temperature was used to prevent overcooking, nutrient degradation, and waffle darkening. The industrial oven was preheated to 150-160 °C (302-320°F). Then the feed waffles were baked at 140-150°C (284-302°F) for 2 hours, until they reach a light golden brown colour and a crispy texture. The waffles' temperature, texture, and colour was checked every 30 minutes to ensure they don't overcook.

Table 1: Experimental diet containing varying levels of mashed cassava peels and *Calopogonium mucunoides*

Ingredients (%)	S ₁	S ₂	S ₃	S ₄
Maize	55.00	45.00	35.00	25.00
Wheat offal	4.30	4.30	4.30	4.30
Crushed Calopo	0.00	7.00	14.00	21.00
Mashed cassava peels	7.00	10.00	13.00	16.00
Soybean meal	20.10	20.10	20.10	20.10
PKC	10.00	10.00	10.00	10.00
Lysine	0.10	0.10	0.10	0.10
Bone meal	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10
Premix	0.25	0.25	0.25	0.25
Total	100	100	100	100

PKC= Palm kernel cake

Data Collection

Phytochemical Compositions

Quantitative Determination of Alkaloid Content

This was done using the gravimetric method of Harbone, (1993). 5g of the extract sample was weighed into a 250ml beaker and add 200ml of 20% acetic acid in ethanol (1:10) and allow to

stand for 4hr. The mixture was filtered and the concentrate extracted using water bath. Evaporate the extract mixture to one-quarter of its original volume and treat with drop-wise addition of conc. aqueous ammonium hydroxide until alkaloids are precipitated. The whole solution was allowed to settle and the precipitate collected by filtration. Then the precipitate was dried and weigh. The alkaloid content was calculated and expressed as percentage of the weight of sample analyzed. Percentage alkaloids are computed as follows:

$$\text{Alkaloids (\%)} = \frac{(W_2 - W_1)}{\text{Weight of sample}} \times 100$$

Where

(W₂ - W₁) = weight of residue

W₂ = Weight of filter paper + precipitate

W₁ = Weight of filter paper

Quantitative Determination of Tannins:

The method of Van-Burden and Robinson, (1981) is employed. 5g of the sample was weighed into a 100ml plastic bottle. Add 50ml of distilled water and shake for 1 hour in a mechanical shaker. Filter this mixture into a 50ml volumetric flask and make up to the mark with distilled water. Then pipette 5ml of the filtrate into a glass tube and mix with 3ml of 0.1 M FeCl₃ In 0.1N HCL and 0.008M potassium ferrocyanide. Measure the absorbance within 10 minutes at 760nm wavelength using a spectrophotometer. Prepare a blank sample using extraction solution (distilled water). Then prepare a standard solution of tannic acid (5mg/ml). With the blank reagent at zero, the standard absorbance is measured at 760nm wavelength. Tannis are computed:

$$\text{Tannins (mg/100ml)} = \frac{X - \text{Blank}}{\text{Standard} - \text{Blank}}$$

Where X= absorbance

Quantitative Determinations of Flavonoids:

Flavonoids content can be determined using the hydrolysis gravimetric method of Harbone JB (1998). 5g of the sample was weighed into a 100ml plastic bottle and extract repeatedly with

100ml of 80% aqueous ethanol at room temperature. Filter with Whatman no, 42 filter paper into 100ml flask. Then transfer the filtrate into a crucible dish and evaporate to dryness over a water bath. Dry the filtrate further in an oven at 60°C for 30 minutes and cool in desiccators. Weigh the crucible and the content and record.

Percentage flavonoid was calculated thus:

$$\text{Flavonoids (\%)} = \frac{(W_2 - W_1)}{\text{Weight of sample}} \times 100$$

Where: W₀= Weight of the original sample (g)

W₁ = Weight of the empty filter paper or crucible (g)

W₂ = Weight of the filter paper or crucible plus flavonoid residue after drying (g)

Quantitative Determinations of Saponins

This was done according to Obadoni and Ochuko, (2001), where 20g of the finely ground sample was weighed into a conical flask. Add 100mL of 20% aqueous ethanol. Heat the mixture over a water bath at 55°C for 4hours with continuous stirring. Filter the mixture and re-extract the residue with another 200mL of 20% ethanol. Combine the extracts and concentrate to about 40mL on a water bath at 90°C. Transfer the concentrate into a separator funnel and add 20mL diethyl ether .shake vigorously. Discard the ether layer and retain the aqueous layer. Repeat the purification process with diethyl ether twice. Add 60mL n-butanol to the aqueous layer and collect the n-butanol extract. Wash the combined n-butanol extracts twice with 10mL of 5% sodium chloride solution. Evaporate the remaining solution to dryness in a water bath. Dry the residue in an oven to a constant weight.

Saponin concentration is computed thus:

$$\text{Saponin (\%)} = 100 \times \frac{\text{weight of saponin residue}}{\text{Weight of sample}}$$

$$\text{Saponin (\%)} = 100 \times \frac{(W_2 - W_1)}{W_0}$$

Where

W₀= Weight of sample (g)

W₁= Weight of empty dish/crucible (g)

W₂= Weight of dish/crucible + dried saponin residue (g)

Quantitative Determinations of Total Phenolic Contents

This was done using the Folin-Ciocalteu colorimetric method of Harbone JB, (1998) where 0.5g of the sample was weighed and extracted with 10ml of 80% methanol. Centrifuge and collect the supernatant. Pipette 0.5mL of the extract into a test tube. Add 2.5mL of 10%Folin-Ciocalteu reagent. Allow the mixture to stand for 5minutes. Add 2.0mL of 7.5% sodium carbonate solution. Incubate at room temperature for 30 - 40minutes for color development. Measure the absorbance at 765nm using a spectrophotometer. Determine the phenol concentration from a gallic acid standard curve and express the result as mg gallic acid equivalent (GAE/g) sample.

$$\text{Phenols (mg GAE/g)} = \frac{(C \times V)}{M}$$

Weight of sample analyzed

Where:

TPC = Total Phenolic Content (typically expressed as mg GAE/g)

C = Concentration obtained from the standard calibration curve (mg/mL)

V = Volume of extract used (mL)

m = Mass of the sample (g)

GAE = Gallic Acid Equivalents mg GAE/g dry weight (milligrams of gallic acid equivalents per gram of dry sample).

Quantitative Determination of Oxalate

Oxalate content was determined according to the method described by Day and Underwood (1986). The sample was digested with dilute hydrochloric acid, filtered and titrated against standardized potassium permanganate solution (KMnO₄) until a faint pink endpoint persisted for 30 seconds. Oxalate concentration was calculated from the titre value obtained.

Quantitative Determination of Phytate

Phytate content was determined using the method of Wheeler and Ferrel (1971) Colorimetric method. The sample was extracted with hydrochloric acid and phytate was precipitated as ferric phytate. The iron content of

the precipitate was measured spectrophotometrically and converted to phytate concentration using an appropriate factor.

Quantitative Determination of Cyanide (Aoac 2005)

Cyanide content was determined by the alkaline picrate method. The sample extract was reacted with alkaline picrate reagent and incubated for colour development. Absorbance was measured at 490nm using a spectrophotometer and cyanide concentration was calculated from a standard curve.

Experimental Design

The experimental design was a completely randomized design with three (3) replicates per treatment. Twelve (12) samples with three treatments of feed waffles containing varying composition of cassava peels mash and crushed Calopo was replicated 3 times making it three replicates per treatment.

Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS Version 27). Significant differences among treatment means were separated using Duncan's Multiple Range Test (DMRT) at $P < 0.05$.

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

Where:

Y_{ij} =observation the jth observation in the ith group

μ = the overall (grand) mean

T_i = the effect of the ith treatment or group

ϵ_{ij} = the random error associated with the observation

Sample size n=3

Results and Discussions

Results

Table 2 presents the phytochemicals composition of ruminant feed waffles formulated with varying levels of *Calopogonium mucunoides* (Calopo) and cassava peels.

Table 2: Phytochemical composition of ruminant feed waffles containing varying levels of *Calopogonium mucunoides* and cassava peels.

Parameters (mg 100 g ⁻¹ per kg)	S ₁	S ₂	S ₃	S ₄	SEM
Tannin	0.32 ^a	0.24 ^c	0.26 ^b	0.26 ^b	0.012
Saponin	1.42 ^a	1.35 ^c	1.37 ^b	1.36 ^{bc}	0.011
Alkaloid	3.80 ^a	3.80 ^a	3.80 ^a	3.80 ^a	0.000
Oxalate	3.85 ^a	3.65 ^c	3.70 ^b	3.68 ^{bc}	0.030
Flavonoids	4.20 ^a	4.16 ^b	4.17 ^b	4.15 ^{bc}	0.006
Phenol	1.11 ^c	1.18 ^a	1.15 ^b	1.17 ^{ab}	0.010
Phytate	17.82 ^a	14.56 ^a	12.63 ^e	2.29 ^d	0.763
Cyanide	12.65 ^a	2.26 ^b	2.26 ^b	2.25 ^b	1.722

^{a,b,c} Means within the rows with different superscript differ (P<0.05) significantly.

SEM = Standard error of mean.

Discussion

The present study demonstrated that incorporation of *Calopogonium mucunoides* and cassava peels into processed feed waffles significantly modified the phytochemical composition of the diets. Drying, milling, and baking effectively reduced several anti-nutritional compounds, particularly cyanide and oxalate, thereby improving feed safety. Among the formulations evaluated, S₂ exhibited the most favorable balance between phytochemical safety and nutritional suitability. These findings highlight the potential of *Calopogonium mucunoides* and cassava peels as sustainable alternative feed resources for ruminant production in tropical environments.

The phytochemical composition of feed ingredients is an important determinant of their nutritional value, safety, and suitability for livestock feeding. The present study demonstrated that the inclusion of *Calopogonium mucunoides* and cassava peels significantly influenced the concentrations of several phytochemical constituents in the formulated feed waffles. The observed variations among treatments indicate that both ingredient

composition and processing methods contributed to modifications in the anti-nutritional factor profile of the diets.

The reduction in tannin concentrations observed in the supplemented diets compared with the control treatment suggests that the processing procedures employed, including drying, milling, and baking, may have contributed to the degradation or inactivation of tannin compounds. Tannins are known to form complexes with proteins and digestive enzymes, thereby reducing nutrient digestibility when present at excessive concentrations. However, moderate concentrations may improve nitrogen utilization and enhance rumen microbial efficiency (Besharati et al., 2022). The relatively low tannin values recorded in this study indicate minimal risk of adverse effects on nutrient utilization by ruminants and are comparable to values reported for processed forage-based feeds (Olarinre et al., 2022).

Saponin concentrations also declined slightly across the supplemented treatments. Saponins are bioactive secondary metabolites capable of modifying rumen microbial populations and reducing methane production. While excessive concentrations may impair feed intake and microbial activity, moderate levels have been associated with improved rumen fermentation and feed utilization efficiency (Mijena & Ijara, 2024). The values obtained in the present study suggest that the feed waffles contained saponin concentrations unlikely to adversely affect animal performance.

The absence of variation in alkaloid concentration among treatments indicates that the inclusion levels of *Calopogonium mucunoides* and cassava peels had little effect on alkaloid content. The values recorded were relatively low and fall within ranges generally considered safe for ruminant animals. Alkaloids are naturally occurring phytochemicals that may become toxic at elevated concentrations; therefore, the stability of alkaloid levels across treatments suggests minimal toxicological concern (Olarinre et al., 2022).

Oxalate concentrations decreased slightly in the supplemented diets. Oxalates are known to interfere with calcium absorption through the formation of insoluble calcium oxalate complexes, thereby reducing mineral bioavailability. The reduction observed in the present study may be attributed to the effects of drying and thermal treatment during feed waffle production. Similar reductions in oxalate levels following feed processing have been reported for several plant-derived feed materials intended for livestock feeding (Mukhtar et al., 2023).

Flavonoids were detected in all treatments at relatively similar concentrations. Flavonoids are recognized for their antioxidant properties and their role in protecting biological systems against oxidative stress. The presence of these compounds in the feed waffles may provide additional health benefits beyond their nutritional contribution. Likewise, the increase observed in phenolic compounds in some supplemented diets may contribute to improved antioxidant potential without adversely affecting feed utilization. Phenolic compounds have been reported to possess antimicrobial and antioxidant activities capable of enhancing animal health and productivity when present at moderate concentrations (Besharati et al., 2022).

One of the most important findings of the present study was the substantial reduction in cyanide concentration observed in the processed diets. Cassava peels contain cyanogenic glycosides that release hydrogen cyanide upon hydrolysis, posing potential health risks to livestock when inadequately processed. The marked decline in cyanide levels following drying, milling, and baking confirms the effectiveness of these processing techniques in detoxifying cassava-based feed ingredients. Similar reductions in cyanide concentration have been reported following thermal processing of cassava by-products intended for animal feeding (Mukhtar et al., 2023). The cyanide values obtained in this study were within acceptable limits for ruminant nutrition and suggest that the processed feed waffles are safe for inclusion in ruminant diets.

The reduction in phytate concentration, particularly in the higher inclusion diets, is also nutritionally significant because phytates reduce the bioavailability of essential minerals such as calcium, iron, and zinc. Lower phytate concentrations may therefore improve mineral utilization and overall feed efficiency. Collectively, the results indicate that the combination of *Calopogonium mucunoides* and cassava peels, together with appropriate processing methods, can produce feed waffles with acceptable phytochemical characteristics and reduced anti-nutritional factor content suitable for ruminant feeding.

Overall, the findings demonstrate that feed waffle technology can serve as an effective approach for enhancing the utilization of unconventional feed resources while minimizing the potential negative effects of phytochemicals and anti-nutritional compounds. Among the treatments evaluated, S₂ exhibited the most favorable balance between phytochemical safety and feed quality, making it a promising candidate for subsequent feeding trials involving ruminant animals.

Conclusion

The study demonstrated that the inclusion of *Calopogonium mucunoides* and cassava peels significantly influenced the phytochemical composition of ruminant feed waffles. The supplemented diets generally exhibited lower concentrations of tannins, oxalates, saponins, and cyanide compared with the control treatment. Processing methods such as drying, milling, and baking were effective in reducing potentially harmful anti-nutritional factors to acceptable levels.

The phytochemical values obtained in this study were within ranges considered tolerable for ruminant animals, suggesting that the formulated feed waffles can serve as a viable alternative feed resource. The incorporation of Calopo and cassava peels therefore represents a sustainable approach to utilizing locally available feed resources while reducing feed costs.

Recommendation

Based on the phytochemical profile, the S₂ diet containing 7% *Calopogonium mucunoides* and 10% cassava peels is recommended for subsequent feeding trials in ruminants.

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Conflict of Interest Statement

The authors declare that there are no financial, personal, institutional, or commercial conflicts of interest that could have influenced the design, execution, interpretation, or reporting of the findings presented in this study.

Author Contributions

Ezimoha, C.O.: Conceptualization, experimental design, feed formulation, laboratory supervision, data interpretation, manuscript drafting, and correspondence. Nsidinanya, N.O.: Data collection, laboratory analyses, statistical analysis, and manuscript review. Nathaniel, J.: Experimental management, sample preparation, laboratory support, and data validation. Obuji, D.C.: Technical supervision, methodology development, critical review of the manuscript, and final approval of the manuscript for publication. All authors read, reviewed, and approved the final version of the manuscript.

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