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Effects of Fermentation and Enzyme Treatment on Nutritional Composition, *In-vitro* Digestibility, and Cyanide Reduction in Cassava (*Manihot Esculenta*) Root-Leaf Meal

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Abstract

Cassava root-leaf is a readily available energy and vitamin source for use in animal feed, but their high cyanide, crude fiber, low crude protein content, and low digestibility limit their use as pig diets. Therefore, there is a need to reduce the cyanide content and improve the nutrient content of the root-leaf meal to have it as an alternative to maize meal in pig diets. This study evaluated the effect of Aspergillus niger, spontaneous fermentation, Natuzyme® Enzyme, and Lactobacillus brevis treatment on the nutritional composition, In-vitro dry matter digestibility (IVDMD), and cyanide reduction in cassava root leaf meal for use in pig diets. A 4×4 Factorial Completely Randomized experimental design was used to evaluate the nutritive contents of KME 01 cassava variety. Samples of Cassava root-leaf meal (CRLM) were allocated to five treatments (T1–T5) in three replicates. The treatments included untreated cassava root-leaf meal (T1); Cassava root-leaf meal fermented by Aspergillus niger (T2). Cassava root-leaf meal fermented naturally (T3); Cassava root-leaf meal treated with enzyme (Natuzyme ®) (T4); Cassava root-leaf meal fermented with Lactobacillus brevis (T5). The samples were fermented at 37°C for a total of 96 hours. After every 24 hours, samples were collected and analyzed for proximate composition, hydrocyanic acid (HCN) content, and IVDMD. The results showed that Spontaneous fermentation significantly improved cassava root-leaf meal's digestibility and nutritional value from 15.02% to 55.56%. Spontaneous fermentation decreased HCN from 45.00 ppm to 8.00 ppm and crude fibre (CF) from 5.16% to 3.87 %, and increased dry matter (DM) from 93.67% to 98.62%, ether extract (EE) from 0.91% to 1.18 %, and crude protein (CP) from 7.47 % to 11.09 %. These improvements suggested that fermented cassava root-leaf meal is a viable substitute for maize in pig diets. The results indicated that spontaneous fermentation is an effective method to improve digestibility and reduce hydrogen cyanide content. Therefore, the study provided evidence for the inclusion of FCRLM in pig feed as a locally available alternative to maize, promoting sustainable feeding strategies for livestock production. The study recommended that future studies should be conducted to evaluate the effects of fermented cassava root-leaf meal-based diet on pig performance and carcass quality characteristics.

Keywords: Cassava Root Leaf Meal, Grower Pig, Hydrogen Cyanide, In-vitro Dry Matter Digestibility (IVDMD), Spontaneous Fermentation.

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1. Introduction

Cassava (*Manihot esculenta Crantz*) is the second most important root vegetable grown throughout Kenya after the Irish potato. It is a drought-tolerant crop and is the mainstay crop for most rural households to address food insecurity and alleviate poverty (Gatto *et al*., 2021). Cassava production in Kenya is mainly concentrated in a few agroecospheres, including Western Kenya, coastal and eastern regions of the country. In these regions, cassava accounts for 60% of the country's total cassava production (Githunguri *et al*., 2017).

Cassava roots and leaves are used as sources of carbohydrates, protein, vitamins, and minerals. It can potentially completely replace maize as an energy source for pigs. Cassava also contains some anti-nutrients that affect animal health when ingested in high amounts (Bayata, 2019). Cassava is tolerant to poor soils, diseases, and drought; it is the most productive crop under tropical conditions. Despite its low crude protein content, high crude fiber, presence of anti-nutrient factors, and dusty dried meals (Benson *et al*., 2023; Kemboi *et al*., 2023), it can be produced more than 70 tons per hectare and is easily grown by farmers with limited resources (Adebayo, 2023).

Cassava contains hydrogen cyanides (HCN), an anti-nutrient that inhibits body cells' ability to use oxygen. At dangerous doses over 10 mg/kg, HCN can become a respiratory toxic chemical (Kanaabi *et al*., 2024; Akapo *et al*., 2014).

In addition, toxic compounds like hydrogen cyanide and antinutrient factors like phytates, flavonoids, oxalates, and tannins minimize the maximum utilization of feed nutrients like proteins, vitamins, and minerals if fed to animals. These bind the nutrients needed and reduce digestibility (Kemboi *et al*., 2023).

Cassava roots and leaves can be well utilized in pig diets if properly processed to reduce the cyanide content and the anti-nutritional factors. Various studies have recommended solid-state fermentation of cassava pulp, cassava roots, and cassava stems for utilization in animal diets (Barman *et al*., 2023; Knez *et al*., 2023; Padmaja *et al*., 1993; Torres-Pitarch *et al*., 2017). Fermentation improves protein content, lowers crude fiber and HCN content, and enhances the digestibility of cassava root meal for non-ruminants (Morales *et al*., 2020). In this study, solid-state fermentation technology was used to improve the nutritional value, lower anti-nutrient factors through assessing the utilization of different fermentation culture regimes of pure enzyme- Natuzyme® *Enzyme, cultures of Aspergillus Niger and Lactobacillus brevis and natural (spontaneous) fermentation on the Nutritional composition, In-vitro dry matter Digestibility (IVDMD), and cyanide reduction in cassava root leaf meal for use in pig diets.*

2. Materials and Methods

2.1 Statement of Animal Rights

The materials and procedures of this study were approved by the Egerton University Research and Ethics Committee, with approval number EUISERC/APP/376/2024, and the National Commission of Science and Technology of Kenya, under license number NACOSTI/P/24/41460.

2.2 Study Site

The study was conducted at Egerton University, Njoro, Nakuru County, Kenya. Egerton University is 1,800 m above sea level, has a mean annual rainfall of 1000 mm, and a temperature range from 17 to 22^{0}C. The university's coordinates are 0^{o}22’11.0” S and 35^{o}55’58.0” E (Longitude: -0.369734; longitude: 35.932779) (Egerton University Department of Agriculture Engineering Metrological Station, 2019).

2.3 Preparation of the cassava root-leaf meal for screening

Fresh cassava roots and leaves (12 months old, variety of KME-01) said better were obtained from Kenya Agricultural Livestock and Research Organization (KARLO), Njoro, Nakuru, Kenya. They were peeled and cleaned with water. After washing, cassava roots were chopped into pieces of 1 cm using a slicer (Lips $^@$ model 3601150, Zurich, Switzerland) while leaves were chopped using a kitchen knife and then treated for the pre-screening study. Grated and chopped Cassava roots and leaves were allowed to dry to a constant weight in an oven at 60°C, then ground to a meal using a Willy mill with a 5mm sieve. The flour of ground cassava roots and leaves was kept in an airtight container to prevent attracting moisture, and this was analyzed for proximate composition.

2.4 Chemical analysis of cassava samples

Proximate analysis of the samples included dry matter determination by drying in a hot air oven at 105°C for 24 hours (method 934.01; AOAC, 1990), ash by burning samples in a muffle furnace at 550°C for 8 hours (method 942.05; AOAC, 1990), ether extract soxhlet method (using ether) (method 920.39; AOAC, 1990), and total nitrogen for crude protein (N x 6.25) determination was obtained using the micro-Kjeldahl method (method 954.01; AOAC, 1990).

2.5 Determination of Cyanide

HCN content was analyzed at the Kenya Agricultural Livestock and Research Organization (KALRO), Njoro, Nakuru, Kenya, 2185 meters above sea level. The average annual rainfall is 935 mm, and minimum and maximum temperatures are 9.7 °C and 23.5^{o}C, respectively. Cyanide levels were determined using a modified picrate paper testing method described by Ojiambo *et al*. (2017) and Ndung’u *et al*. (2012). Cassava root and leaves samples (100 mg) were weighed and put into a glass vial (250mm outer diameter and 84mm height) with forceps. To extract hydrogen cyanide from each sample, three to five drops of toluene were pipetted onto it. The filter paper (0.7cm by 1.0cm) was dipped in a Sodium picrate solution, which was previously made by combining 25g of anhydrous sodium carbonate and 5g of wet picrate acid in 1 liter of distilled water. The filter paper was then suspended from the cap of the vial without contacting the sample.

The vials were then sealed with a rubber stopper and let to remain at room temperature (20^{0}C–32°C) for 24 hours. A blank was also used as a control. The filter sheets gradually shifted from bright yellow to deep red, depending on the amount of hydrogen cyanide emitted by the samples. After 24 hours, the vials were opened, and the color of the picrate paper was compared to a standard picrate chart on a range of 0- 800 ppm based on the intensity of the red hue. Where 1 ppm corresponds to 1 milligram of hydrogen cyanide per kilogram of cassava. Data was collected in triplicate for each sample and reported as means ± standard deviations.

2.6 *In vitro* dry matter digestibility (IVDMD)

IVDMD determination was conducted for each of the 34 test samples in triplicate. These samples included the untreated cassava root-leaf for two varieties and four treatments (*Aspergillus niger*, Spontaneous fermentation, Enzyme treatment, and *Lactobacillus brevis*). Within four days, with intervals of 24 hours. A two-phase

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in vitro dry matter digestibility method, as described by Syeunda *et al*.(2021), was used.

$$\text{DM digestibility} = \left(\frac{DM\ in - DM\ RS}{DM\ in}\right) X\ 100$$

Where DM-in is the initial DM and DM-RS is the residual DM.

2.7 Preparation of experimental treatments

T1:Untreated cassava root-leaf meal

T2: Fresh cassava root-leaf meal fermented with *Aspergillus niger*

T3: Fresh cassava root-leaf meal fermented using spontaneous fermentation

T4: Fresh cassava root-leaf meal treated with Enzyme (Natuzyme ®)

T5: Fresh cassava root-leaf meal fermented with *Lactobacillus brevis*

The treatment for lower HCN, lower crude fiber, and a better CP and IVDMD was selected to prepare the experimental pig diets.

2.8 Preparation of *Aspergillus niger.* Treated cassava root-leaf meal

2.8.1Preparation of Inoculum

Aspergillus niger inoculum was prepared from fresh mature (three to five days old) cultures grown on Potato Dextrose Agar slants. Spore suspension for inoculation was prepared by adding 10 ml of sterile distilled water on the aerial plate of *Aspergillus niger*, which was produced on a 5-day-old PDA plate. The spores were removed by scraping off with a spatula, and the suspension of the spores was used for inoculation.

2.8.2Solid state fermentation

A sample of fresh cassava root-leaf meal and distilled water was mixed in a 250 mL conical flask to adjust the moisture content to 60%. The mixture in a conical flask was sterilized at 121°C for 15 minutes and cooled at room temperature. Inoculation was done aseptically with 3 ml of spores suspension mixed well and incubated at 37°C, pH of 5.2, within 96 hours. Under these optimized conditions, the inoculum concentration for *Aspergillus niger* was 5% of 10^6 cfu/ml (Muralikandhan & Dhanasekaran, 2020). After incubation, the fermented substrates were dried in an oven at 60°C for two days.

2.9 Spontaneous fermentation of cassava root-leaf meal

Freshly grated cassava root and leaves were mixed at a ratio of 1 kg cassava root meal with 300 g cassava leaves, that mixture with distilled water at a ratio of 1.3:1.3(wt/vol) was incubated in a 3 kg airtight sealed plastic bottle three times at 37^0C for four days under anaerobic conditions (Muremera *et al*., 2022). After four days, a portable pH meter was used to measure the sample's pH. Additionally, the sample was analyzed for proximate composition, *in-vitro* dry matter digestibility, and HCN content.

2.10 Preparation of enzyme-treated cassava root-leaf meal

Three individual samples of 1kg of root and 300 g of leaves were mixed with a powdered enzyme (Natuzyme ®), a multi-enzyme complex containing 12,000 units/g of xylanase, 6000 units/g of cellulase, 1500 units/g of phytase,700 units/g of protease, and 400 units/g of alpha-amylase. The enzyme's inclusion rate was 350mg/kg of the sample in dry form per the manufacturer's instructions and recommendations. After mixing, the individual samples were incubated at a temperature of 37°C under anaerobic conditions and allowed to ferment for four days. The samples were analyzed for proximate composition, *in-vitro* dry matter digestibility, and HCN content.

2.11 Preparation of cassava root-leaf meal fermented with *Lactobacillus brevis*

Three samples of 1kg of cassava root meal and 300g of leaves were mixed with distilled water at 1.3:1.3 (wt/vol). The inoculant containing a single strain of *Lactobacillus brevis* was used as the starter culture and added to the mixture. The experimental samples were inoculated with 5% of 10^6 cfu/ml of the *Lactobacillus brevis*. The inoculated cassava root-leaf meal was then incubated at 37°C in airtightly sealed 3 kg plastic bottles for four days in the laboratory. After four days, the pH of individual samples was measured using a pH meter and recorded to determine if a constant pH was attained. The sample was analyzed for proximate composition, *in-vitro* dry matter digestibility, and HCN content.

2.12 Experimental design and data analysis

The experimental design was a 4 × 4 completely randomized factorial design using the statistical model:

$$Xijk = \mu + \alpha i + \beta j + (\alpha\beta)ij + \varepsilon ijkl$$

Where, Xijk = any observed data, μ = the population mean, αi = the effect of i th type of the microbial inoculation, βj = the effect of j th level of fermentation times, $(\alpha\beta)$ij = the interaction effect of microbial inoculation and time, and εijk= the random error term for replication k.

All measurements were done in triplicate. Data analysis was carried out using SAS 2023, version 9.4 M8. The data obtained were analyzed for mean differences with analysis of variance (ANOVA) using Tukey's Honestly Significant Difference test at a 5% significance level.

3. RESULTS

Chemical analysis of treated and untreated cassava root-leaf meal (CRLM)

Table 1 illustrated the effects of untreated cassava root leaf, *Aspergillus niger*, Spontaneous fermentation, enzyme treatment, and *Lactobacillus brevis* on nutrient composition, HCN and IVDMD.

Table 3.1: Chemical composition of fermented and enzyme-treated CRLM

Nutrient (%)	Untreated	*Aspergillus niger*	Spontaneous fermentation	Enzyme Treated	*Lactobacillus brevis*	P -Value
DM	93.67^b±0.63	97.84^a±0.63	98.61^a±0.63	98.61^a±0.63	97.67^a±0.63	0.0013
CP	3.26^b± 0.68	13.20^a± 0.68	11.10^a± 0.68	11.25^a ± 0.68	12.68^a± 0.68	<.0001
EE	1.02^b±0.68	0.90^b±0.68	1.73^a±0.68	1.69^a±0.68	1.21^b±0.68	<.0001

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CF	4.17^{a}±0.78	3.79^{b}±0.78	3.87^{ab}±0.78	3.55^{b}±0.78	3.79^{b}±0.78	0.0032
HCN	45.00^{a}±0.30	5.67^{c}±0.30	8.00^{b}±0.30	7.33^{b}±0.30	5.33^{c}±0.30	<.0001
%IVDMD	38.73^{c}±0.31	51.04^{b}±0.31	55.56^{a}±0.31	52.28^{b}±0.31	54.84^{a}±0.31	<.0001

DM =Dry matter, CP =Crude protein, EE = Ether extract, CF = Crude Fiber, HCN = Hydrogen Cyanide, IVDMD= *In Vitro* Dry Matter Digestibility.

3.1 Effect of fermentation on Dry matter composition of Untreated and treated cassava root-leaf meal

The dry matter content of cassava root-leaf meal KME 01 variety subjected to different treatment methods and time intervals is presented in Table 3.1. The dry matter content of the treatments varied significantly across all treatments (P<0.05) with the highest dry matter content at 98.62 % in T3 and T4 (KME 01 fermented by spontaneous fermentation and treated with enzyme *(Natuzyme ®)* after 96 hours), and the lowest at 93.67 % in T1 (Untreated KME 01).

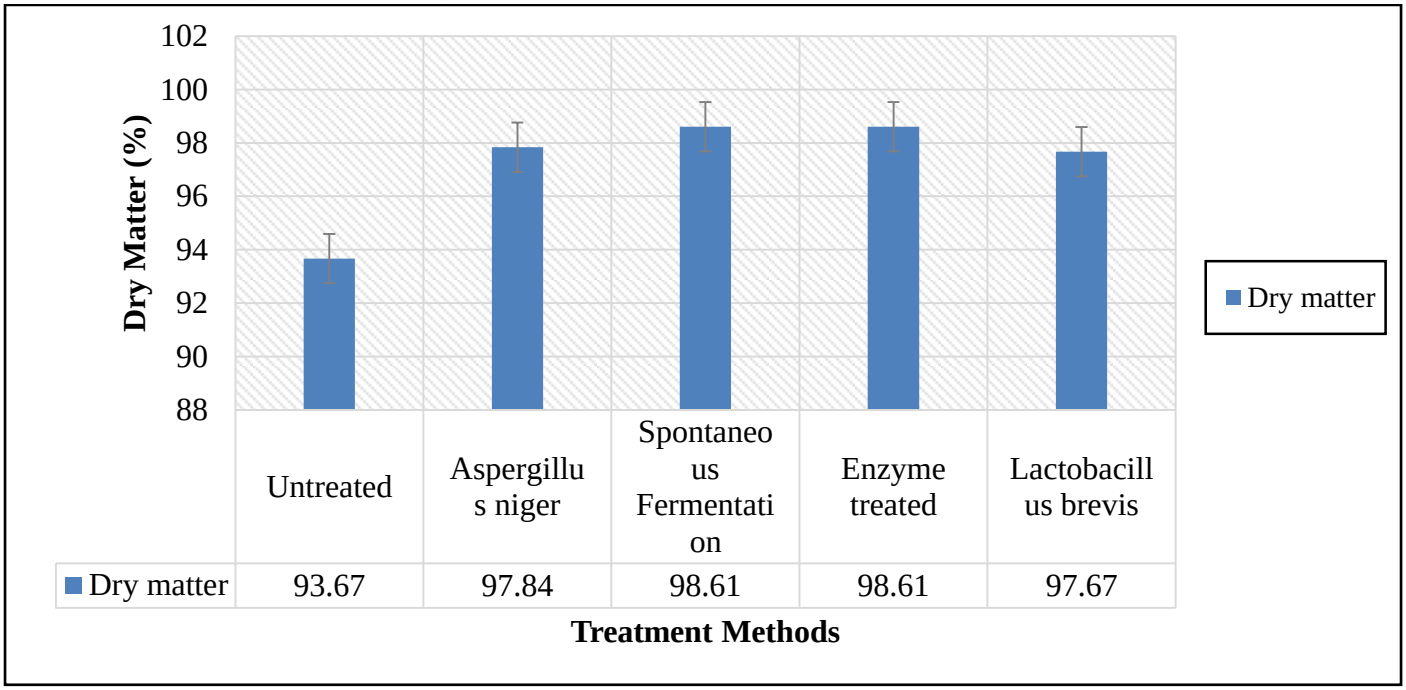

Figure 3.1: Effect of fermentation on dry matter composition of Untreated and treated cassava root-leaf meal

3.2 Effect of fermentation on cyanide content

Table 3.1 showed the cyanide content of treated and fermented cassava root-leaf meal. The cyanide content of the untreated cassava root-leaf of the KME 01 variety was 45 ppm. Through fermentation using inoculum, natural, and enzyme treatment, the cyanide levels showed a significant (p<0.05) steady decrease to below the allowable safe limit of 10 ppm. The levels of cyanide decreased from the beginning of the experiment until 96 hours, which was the end of the experiment.

Cyanide levels decreased from 45.00 ppm to 8.00 ppm with spontaneous fermentation, from 45.00 ppm to 7.33 ppm when treated with enzymes, from 45.00 ppm to 5.67 ppm and ppm and 5.33 ppm with *Aspergillus niger* and *Lactobacillus brevis* inoculum fermentations, respectively. Cyanide levels were higher in the KME 01 fermented spontaneously than in the other processing treatments, but not beyond the safe limit.

The fermentation method, duration, and the cassava variety all interacted with the cyanide levels in the root-leaf meal.

3.3 Effect of fermentation on *in-vitro* dry matter digestibility

The *in vitro* dry matter digestibility (IVDMD) data of the samples are presented in Table 3.1. Before fermentation, the IVDMD for KME 01 was 15.02%, whereas after fermentation using *Aspergillus niger*, Spontaneous, Enzyme treatment, and *Lactobacillus brevis* fermentation, the IVDMD increased and reached 51.04%, 55.56%, 52.28%, and 54.84% respectively at 96 h. Overall, the IVDMD of the cassava root-leaf meal was interactively affected by the fermentation type and fermentation time.

4. Discussion

Proximate analysis of treated cassava root-leaf meal-based diets

The dry matter content of cassava root-leaf meal (KME 01 variety) was significantly influenced by the type of treatment and duration of fermentation (P<0.05), as shown in Table 3.1. The highest dry matter content (98.62%) was recorded in T3 and T4, which were subjected to spontaneous fermentation and enzyme treatment (*Natuzyme* ®) respectively for 96 hours.T1 exhibited the lowest dry matter content (93.67%), indicating a higher moisture level that predisposes the material to rapid spoilage and microbial contamination. This is consistent with earlier findings that high moisture content in feed ingredients can promote fungal growth and mycotoxin production, thereby compromising animal health and feed shelf life (Ezekiel *et al*., 2020). Conversely, all applied treatment methods significantly improved the DM content, indicating effective moisture reduction. Spontaneous fermentation and enzyme treatment resulted in the highest DM content (98.61%). This indicated that natural microbial fermentation and exogenous enzyme application over a 96 hours period effectively reduced moisture content, probably through enhanced breakdown of cellular structures and enhanced water release. Followed closely

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by *Aspergillus niger* (97.84%) and *Lactobacillus brevis* (97.67%) treatments. These improvements can be attributed to the metabolic activities of fermenting microbes and added enzymes, which enhance substrate breakdown and facilitate water loss during processing. This finding aligns with (Oboh *et al*., 2010), who reported that microbial fermentation improves the physicochemical properties of cassava-based feeds, including moisture reduction.

Aspergillus niger, known for its strong enzymatic capabilities, likely contributed to reduced moisture through the hydrolysis of fibrous and carbohydrate matrices, enhancing water release. It also grows vigorously, contributing to the total DM through its mycelial network; additionally, its metabolic activity produces heat, which causes moisture loss, further increasing the DM percentage. Similarly, *Lactobacillus brevis*, a homofermentative lactic acid bacterium, reduces pH and water activity, thereby contributing to feed preservation and dry matter retention (Aguirre *et al*., 2024).

The microbial breakdown of structural polysaccharides into simpler carbohydrates, which the animal can digest more easily, caused this decrease in crude fiber (CF) in fermented cassava. According to Ortega *et al*. (2018) and Ona *et al*. (2019), any type of cassava treatment, including enzyme treatment, can break down complex carbohydrates and even plant fibers. Cassava contains no more than 2% lipids. This is because the majority of fermentative microorganisms, including *Rhizopus oryzae* and lactic acid bacteria, metabolize proteins and carbohydrates more than lipids. These results are consistent with prior research, indicating that the fermentation procedure only slightly alters the lipid content of cassava. Therefore, rather than raising fat levels, fermentation mainly improved the nutritional value of cassava by increasing its DM. It has decreased the HCN content, hence reduced toxicity (Okoth *et al*., 2022).

The CP content varied significantly among the different treatment methods applied to cassava root-leaf meal ($P < 0.0001$). The untreated sample had the lowest CP content (3.26%), which reflects the inherent limitation of raw cassava roots and leaves as protein sources due to their high carbohydrate and fiber content and the presence of anti-nutritional factors (Oboh *et al*., 2010). All the fermentation and enzyme treatment methods significantly increased CP content, with *Aspergillus niger*-treated samples showing the highest value (13.20%), followed by *Lactobacillus brevis* (12.68%), enzyme-treated (11.25%), and spontaneously fermented (11.10%) samples. The high CP in T2 and T5 was attributed to the use of controlled fermentation and inoculum rather than natural fermentation. This agrees with the results of a study by Okoth *et al*. (2022), who reported that controlled fermentation gave a higher final protein content level in the cassava leaves than spontaneous fermentation. Controlled fermentation utilizes specific microbial strains, such as *Levilactobacillus brevis*, which is known for its efficient metabolic activities. These selected microbes proliferate during fermentation, increasing the microbial biomass, which contributes to the overall protein content due to the high protein nature of microbial cells. Additionally, these microbes produce extracellular enzymes that hydrolyze complex proteins and anti-nutritional factors, enhancing protein digestibility and bio-availability. In contrast, spontaneous fermentation relies on naturally occurring microbes, like Lactic acid bacteria (LAB), Yeasts, Bacillus species, and Enterobacteriaceae, leading to inconsistent fermentation outcomes and less efficient protein enhancement. Therefore, the deliberate selection and application of specific microbial cultures in controlled fermentation processes result in a more significant improvement in the protein quality of cassava leaves compared to spontaneous fermentation. In a similar trend, the low CP of 3.26% in T1 agreed with the findings by Zekarias *et al*. (2019), who reported a low protein-to-carbohydrate ratio of cassava.

The increase in CP can be attributed to microbial biomass proliferation during fermentation, which enriches the substrate with microbial proteins (Rajoka *et al*., 2012). In particular, *Aspergillus niger* is known for its rapid growth and high protein-yielding capability due to its ability to synthesize extracellular enzymes and metabolize complex substrates (Cheriaparambil & Grossmann, 2025). This contributes to both enhanced nutrient availability and microbial protein accumulation. The improvement in CP with *Lactobacillus brevis* and spontaneous fermentation may also result from microbial action leading to the breakdown of complex fibers and release of bound nitrogenous compounds, enhancing the apparent protein content. Additionally, fermentation may reduce anti-nutritional factors such as cyanogenic glycosides, which otherwise inhibit protein digestibility (Ram *et al*., 2020). The enzyme-treated samples also demonstrated a significant increase in CP content, which could be due to the enzymatic hydrolysis of cell wall components and the release of protein fractions otherwise bound within fibrous matrices. Enzyme supplementation likely enhanced protein liberation and bioavailability in the fermented cassava meal (Mukhtar *et al*., 2023).

HCN content of the Untreated and Fermented cassava root-leaf meal

Linamarin and lotaustralin are cyanogenic glycosides, which were found in untreated cassava primarily in the leaves, according to reports from Banwo *et al*. (2023), Jakobsen *et al*. (2015), and Xi Zhu *et al*. (2023). The untreated cassava root-leaf meal of KME 01 (T1) contained the highest HCN content of 45 ppm, while all the treated samples contained HCN concentrations ranging from 5-8 ppm, lower than the toxic level of 10 ppm. This decrease in HCN showed that the various treatments denaturized the cyanogenic glycosides. These treatments decreased the anti-nutrient factors in cassava and increased nutrient bio-availability.

***In vitro* dry matter digestibility of untreated, treated, and fermented cassava root-leaf meal**

Treatment of cassava root-leaf by natural, enzyme, and probiotic fermentation under a conducive environment with distilled water at a ratio of 1.3:1.3 (wt/vol), incubated under anaerobic conditions at 37°C for four days, increased concentration of population density of degrading microorganisms, hence a higher IVDMD. These microorganisms enriched the cassava root-leaf meal with microbial protein and enzymes like cellulase, xylanase, and amylase that aided the digestion of cassava fibers, thus a higher IVDMD (Clarke *et al*., 2018; Moran *et al*., 2016; Zeng *et al*., 2018).

Lactobacillus brevis and spontaneous fermentation treatments showed comparable *in-vitro* dry matter digestibility (IVDMD) values (54.84% and 55.56%, respectively). This similarity could be attributed to the dominance of lactic acid bacteria (LAB) in both treatments. During spontaneous fermentation, naturally occurring LAB, such as *Lactobacillus plantarum*, *L. brevis*, and *Leuconostoc* spp., proliferate rapidly under anaerobic, carbohydrate-rich conditions (Holzapfel, 2002). Thus, the spontaneous fermentation likely selected for similar microbial populations and metabolic activities as in the *L. brevis*-inoculated treatment.

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In both treatments, LAB likely produced organic acids like lactic acid, acetic acid, and extracellular enzymes that reduced pH, inhibited undesirable microbes, and contributed to fiber degradation. These microbial processes promote partial hydrolysis of cellulose, hemicellulose, and starches in cassava root-leaf meal, leading to improved digestibility (Muck et al., 2018). Additionally, microbial growth enriches the biomass with microbial protein and enzyme residues such as cellulase and amylase, which further enhance IVDMD.

On the other hand, the relatively lower IVDMD observed in the *Aspergillus niger* (51.04%) and enzyme-treated Natuzyme (52.28%) samples, compared to *Lactobacillus brevis* and spontaneous treatments, could be explained by specific microbial and biochemical dynamics. *Aspergillus niger*, being a filamentous fungus, may require a longer fermentation period to fully colonize and degrade fibrous components of cassava. A 4-day anaerobic incubation may not have been sufficient for optimal enzyme production and fiber breakdown (Bakare *et al*., 2022). While Natuzyme contains a cocktail of enzymes like cellulase, xylanase, and phytase, their activity depends on pH, temperature, and substrate compatibility. The anaerobic condition and pH generated during cassava fermentation may have limited their optimal performance (Adeyemi & Familade, 2003).

5. Conclusions

The study demonstrated that natural fermentation for four days (96 hrs) significantly improved the nutritional quality of cassava root-leaf meal, in terms of *in-vitro* dry matter digestibility, increased CP, DM, and lowered HCN, making it a potential alternative ingredient to formulate the diets for grower pigs. From the results of the treatments, spontaneous fermentation T3 was more suitable more cost-effective, and readily available option compared to other treatment methods because it resulted in a product with moderate Crude protein (CP) content, DM, reduced Crude fiber (CF), high IVDM, and reduction of the level of hydrogen cyanide below 10mg/kg. The application of microbial fermentation technology showed a significant increase in fiber-digesting and CP-enhancing microorganisms, further enhancing action of microorganisms. This enhanced the IVDMD and bio-utilization of the cassava root-leaf meal, thus minimizing the toxicity associated with utilizing cassava-based products in swine nutrition. This study provided information for the knowledge gap in enhancing the nutritive value of cassava root-leaf meal through fermentation. It demonstrated that treatment by spontaneous fermentation resulted in a product with enhanced nutritional profile than treatment by *Aspergillus niger*, *Lactobacillus brevis*, and exogenous enzyme. For smallholder pig farmers in regions where high-cyanide cassava root and leaf varieties are abundant, the adoption of natural fermentation technology of cassava root-leaf products for the production of cassava root-leaf meal-based diets can reduce feed costs, enhance feed quality, and promote sustainable pig production. The study also contributed information to guide on development of agricultural policies aimed at enhancing food security and environmental protection through promoting the utilization of high-cyanide cassava varieties. This will reduce reliance on expensive and highly competitive conventional energy ingredients like maize in pig diets. Future research should be conducted to explore the long-term effects of feeding fermented cassava root-leaf meal (FCRLM) on performance, hematological indices, and meat quality characteristics of grower pigs.

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Author contribution

This study was carried out in collaboration between all the authors. The conception and design of the study were done by Valentine Mutuyimana, Paul A. Onjoro, Anthony M. King'ori, and Joseph W. Matofari. Data collection was performed by Valentine Mutuyimana and Gerald Kizito. Data analysis, writing, proofreading, and publication procedures of the manuscript were handled by Valentine Mutuyimana, Nicholas K. Kibitok, Paul A. Onjoro, Joseph W. Matofari, and Anthony M. King'ori.

Conflict of interest

The authors state that they have no personal, professional, or financial conflicts of interest.

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