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Effect of fermentation and enzyme treatment on nutritional quality, digestibility, and hydrogen cyanide reduction in cassava (*Manihot esculenta*) root meal

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Abstract

Cassava (*Manihot esculenta*) root meal is a widely used feed in tropical regions due to its high carbohydrate content, primarily starch, and its dietary fiber. However, its low protein content and hydrogen cyanide (HCN) restrict its use in animal feed. This study investigated the effects of fermentation and enzyme treatment (Natuzyne®) on the chemical composition, digestibility, and HCN levels of cassava root meal. Four treatments were used. T₁: untreated cassava root meal, T₂: enzyme-treated, T₃: *Saccharomyces cerevisiae* fermented and T₄: spontaneously fermented cassava root meal. The study found that both enzyme treatment and fermentation with *Saccharomyces cerevisiae* significantly improved the crude protein content, in-vitro dry matter digestibility and significantly reduced the fiber and HCN content of cassava root meal. These results suggest that both enzyme treatment and fermentation with *Saccharomyces cerevisiae* improves the nutritional quality of cassava root meal, offering a sustainable and cost-effective alternative to maize for poultry feed, helping to alleviate competition for maize as food for human consumption and animal feed.

Keywords: Chicken, cassava root meal, enzyme, fermentation

1. Introduction

Cassava (*Manihot esculenta*) is a common root crop in many tropical and subtropical areas of the world, particularly in sub-Saharan Africa, Southeast Asia, and Latin America, it serves as a major staple diet. Although it is an important source of carbohydrates, its low protein content and lack of certain crucial vitamins and minerals make it frequently nutritionally deficient (Jaramillo *et al.*, 2022) [13]. Despite these limitations, cassava has a major advantage in that it can adapt to difficult soil conditions and drought, which makes it a crucial crop for many low-income nations' food security (Da Costa *et al.*, 2024) [5]. However, one significant health hazard associated with cassava is its concentration of cyanogenic glucosides, especially linamarin, which can yield lethal hydrogen cyanide (HCN) upon consumption (Zidenga *et al.*, 2017) [33]. Increased concentrations of HCN in cassava can result in significant poisoning, neurotoxicity, and potentially fatal outcomes if inadequately processed (Rivadeneira-Domínguez & Rodríguez-Landa, 2019) [29]. To enhance the nutritional quality and safety of cassava, various processing methods, including fermentation and enzyme treatment, have been explored. One of the oldest and most efficient ways to increase the nutritional value and safety of cassava is through fermentation, which uses microorganisms to break down complex substances into simpler, more bioavailable forms that can increase digestibility, promote protein content, and lower toxic substances like cyanogenic glucosides (Halake & Chinthapalli, 2020) [11]. A widely studied microbe for cassava fermentation is *Saccharomyces cerevisiae*, also known as baker's yeast, which breaks down starches and produces metabolites like organic acids and alcohols, enhancing the cassava's texture and nutritional profile (Pimpisai *et al.*, 2024) [28]. The ideal conditions for *Saccharomyces cerevisiae* growth are pH 4, a 5% dissolved oxygen content, temperatures between 30°C and 45°C, and a pH range of 2.5 to 8 (Minden *et al.*, 2022) [20].

Saccharomyces cerevisiae fermentation of cassava root meal lowers the amount of hydrogen cyanide and crude fiber through enzymatic breakdown and detoxifying properties (Khurshida *et al.*, 2025c) ^[15].

In addition to fermentation with *Saccharomyces cerevisiae*, spontaneous fermentation, which depends on the spontaneous growth of naturally occurring microorganisms, has the potential to improve the quality of cassava. This method increases the crude protein content by converting nitrogen sources into protein, improving the nutritional value of the fermented meal, and benefits from microbial diversity, which may help with improved cyanogenic glucoside detoxification and nutrient availability (Egbune *et al.*, 2023) ^[19].

Another approach that shows promise for enhancing cassava's nutritional makeup is enzyme treatment. The breakdown of complex starches, cellulose, and proteins in cassava has been investigated using Natuzyme, a multi-enzyme blend comprising xylanase, phytase, beta glucanase, amylase, cellulase, and protease (LN *et al.*, 2018) ^[17]. These enzymes improve cassava's digestibility and increase the bioavailability of its nutrients by breaking down its indigestible components (Oloruntola, 2020) ^[26]. Additionally, it has been demonstrated that enzyme treatments can help detoxify cyanogenic glucosides, lower HCN levels, and make cassava safer for consumption (Moses *et al.*, 2024) ^[21].

The nutritional value of cassava is largely determined by its proximate composition, which includes its moisture, protein, fat, fiber, and carbohydrate content. These components can be changed by enzyme treatments and fermentation, which use enzymatic and microbiological mechanisms to change the chemical composition and structure of cassava, potentially increasing protein levels, decreasing fiber, and improving the plant's overall digestibility (Ona *et al.*, 2019) ^[27]. By improving the proximate composition, these techniques can make cassava a more balanced food source that offers vital nutrients. *In vitro* dry matter digestibility (DMD), a key indicator of cassava's potential nutritional value, shows how well the nutrients can be absorbed and used by humans or animals. By increasing DMD, fermentation and enzyme treatments can make cassava a more effective and advantageous food source (Dagaew *et al.*, 2021) ^[6]. One of the primary issues with cassava consumption is still cyanogenic toxicity, which can result from eating raw or incorrectly cooked cassava, which can produce significant levels of HCN and cause toxicity and health hazards, especially for people that depend heavily on cassava as a staple food (Nuwamanya *et al.*, 2022) ^[24]. Natuzyme enzyme treatment and *Saccharomyces cerevisiae* fermentation has been demonstrated to lower HCN levels by encouraging the breakdown of cyanogenic glucosides (McMahon *et al.*, 2021) ^[19]. In particular, Cassava is detoxified by *Saccharomyces cerevisiae* fermentation, which breaks down cyanogenic glucosides and makes it safe for consumption (Wang *et al.*, 2024) ^[31]. Similarly, Natuzyme helps to break down cyanogenic chemicals, which increases cassava's nutritional value and safety (Bakare *et al.*, 2021) ^[2]. This study sought to determine how the proximate composition, *in-vitro* dry matter digestibility, and HCN content of cassava root meal were affected by two fermentation processes; *Saccharomyces cerevisiae* and natural fermentation as well as enzymatic treatment with Natuzyme. The purpose of this study was to assess the effects of various techniques on the safety, digestibility, and nutritional value of cassava. This study will help create better cassava processing procedures that increase the crop's potential as a safe and wholesome feed item for chicken diets by analysing the effects of these processing methods.

Materials and Methodology

Study Site

The experiment was conducted at Egerton University, Animal Nutrition Laboratory, Department of Animal Sciences, located in the Njoro Sub-County of Nakuru County at 0° 23' S and 35° 55' N. The region is 1800 meters above sea level with an average annual rainfall of 900-1,200 mm and temperatures ranging from 17°C to 22°C (Egerton University Department of Agriculture Engineering Metrological Station, 2019).

Collection and Preparation of Cassava Root Meal

High HCN, fresh cassava roots (12 months old, variety MH95/0183) were obtained from Kenya Agricultural Livestock and Research Organization (KALRO), Njoro, Nakuru-Kenya. The cassava roots were washed, peeled, and sliced using a slicer to make cassava chips, of various sizes and shapes. They will then naturally sun-dried on a tray dryer, making the cassava chips dry more evenly than drying on a concrete floor. The cassava chips were then ground using a hammer mill with a 5mm sieve size to produce cassava root meal (Lukuyu *et al.*, 2014) ^[18].

Preparation of experimental treatments

Four treatments were used, and each treatment was replicated three times. The treatments included:

- **T₁**: Untreated Cassava root meal (Control).
- **T₂**: Enzyme (Natuzyme®) treated cassava root meal.
- **T₃**: Yeast (*Saccharomyces cerevisiae*) fermented cassava root meal.
- **T₄**: Spontaneously/naturally fermented cassava root meal.

Preparation of enzyme-treated cassava root meal

A Multi-enzyme (Natuzyme®) was procured from Coopers® Kenya Brand Limited. It contained 12,000 parts per gram of xylanase, 6,000 parts of cellulase, 1,500 parts of phytase, 700 parts of beta-glucanase, 700 parts of protease, and 400 parts of alpha-amylases. According to the manufacturer's directions and instructions, the dried cassava root meal was then appropriately mixed with the enzyme in triplicate at a rate of 350 mg/kg for 4 days (Muremera *et al.*, 2022) ^[22]. The sample was analyzed for proximate composition, *in vitro* dry matter digestibility, and HCN content.

Preparation of yeast (*Saccharomyces cerevisiae*) fermented cassava root meal

Dry cassava root meal was mixed with equal amounts of water in a 1:1 ratio, 5% of the yeast powder added, incubated in triplicate at 30°C under anaerobic conditions, and ferment for 4 days. The yeast powder was purchased from the Agro-chemical and Food Company Ltd, Kenya (Muremera *et al.*, 2022) ^[22]. The sample was analyzed for proximate composition, *in vitro* dry matter digestibility, and HCN content.

Spontaneous fermentation of cassava root meal

A 1:1 (wt/vol) mixture of cassava root meal and distilled water was incubated in triplicate at 30°C for four days in a 250 ml airtight sealed plastic bottle (Muremera *et al.*, 2022) ^[22]. 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.

Determination of Nutrient Composition

Proximate analysis was conducted using standard methods of AOAC, (2005). The dry matter (DM), (AOAC, 2005: 934.01), organic matter (OM) (AOAC, 2005: 942.05), and crude protein (CP) (AOAC, 2005: 984.13). To determine the dry matter, samples were dried in a hot air oven at 105°C for 24 hr, method 930.15. The ash content was determined by burning samples in a muffle furnace at 550°C for 8hrs, methods. 942.05, ether extract by Soxhlet method (using ether) method 920.39. Total nitrogen for crude protein (N x 6.25) by Kjeldahl procedure method 984.13, Neutral detergent fibre (NDF), acid detergent lignin (ADL) and acid detergent fibre (ADF) were determined using the Van Soest method.

Determination of *in vitro* digestibility

The experiment was conducted to mimic the digestion in the chicken digestive system as described by (Zhao *et al.*, 2014)^[32]. The treatments were T₁: Untreated Cassava root meal, T₂: Enzyme-treated Cassava root meal, T₃: Yeast-fermented Cassava root meal, and T₄: natural-treated Cassava root meal. A ground Cassava sample (0.4g) was weighed and put in a 100 ml digestibility test tube before simulated gastric fluid was added. To mimic the *in vivo* activity of pepsin in chicken stomach fluid, a fluid containing 1,550 U/mL of pepsin (Sigma 10070; Sigma-Aldrich Co., St. Louis, MO) was used. The gastric buffer solution contains 16.9 mmol/L of NaCl, 9.6 mmol/L of KCl, and 10 mmol/L of HCl to match the *in vivo* ionic concentration of gastric fluid from roosters. The pH was raised to 2.0 at 41°C by adding 200 mmol/L of HCl. Each digestibility test tube was put in 2ml Chloramphenicol C-0378; Sigma-Aldrich Corp., St. Louis, MO, USA (0.5 g/100 ml 19 ethanol) to prevent bacterial growth. The test tubes were then sealed and incubated in a water bath at 39°C with continuous stirring for 2 hours. The first step's mixture was combined with 20 ml of 0.6MNaOH and 80 ml of phosphate buffer (0.2M, pH 6.8). The pH was brought down to 6.8 using 1M HCl or 1M NaOH to establish a stable environment for intestinal enzymes to perform well. To the mixture, 10.6 ml of artificial pancreatin P-1750 Sigma-Aldrich Corp, St. Louis, MO, USA (porcine grade enzyme with 3 x USP activities) containing 100 mg/1litre buffer was added and incubated at 39°C with constant stirring for 4 hours. The remains were put

in 1.5ml centrifuge tubes and centrifuged (12700×g) for 2 minutes. The mixture was carefully withdrawn, washed twice with 20 ml of 95% ethanol and 99.5% acetone, and then rinsed with distilled water. Those that remained were then be dried for 12 hours at 70°C in the oven before weighing.

Calculation of *in vitro* dry matter digestibility

The formula below was used to determine the *in-vitro* dry matter digestibility Boisen & Fernández (1997)^[3].

$$IVDMD = \frac{DM \text{ in feed} - DM \text{ in undigested feed}}{DM \text{ in feed}} \times 100$$

Statistical analysis

Data collected on proximate analysis, HCN content, and *in vitro* DM digestibility of the diets were subjected to the analysis of variance (ANOVA) in a completely randomized design (CRD) using the General linear model procedure of statistical analysis system (SAS 2023) version 9.4 at 5% significance level. Before data analysis, the data were tested for normality using Kolmogorov-Smirnov and Shapiro tests. Turkey's test at ($p < 0.05$) was used to separate means where means are significantly different. The model used for statistical analysis was:

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

Where,

Y_{ij} = Observation of the response variable

μ = Overall mean

t_i = Effect of the i^{th} treatment (fermentation and enzyme treatment)

ϵ_{ij} = Random error

Results

Chemical analysis of treated and untreated cassava root meal (CRM)

Table 1 illustrates the effects of enzyme treatment, *Saccharomyces cerevisiae* fermentation, natural fermentation, and untreated cassava root meal on nutrient composition and HCN levels.

Table 1: Chemical composition of fermented and enzyme-treated CRM

Nutrient (%)	Enzyme treated	Naturally fermented	Untreated	<i>Saccharomyces cerevisiae</i> fermented	P-Value
DM	95.46 ^a ±0.12	94.74 ^b ±0.12	94.11 ^c ±0.12	94.59 ^{bc} ±0.12	<.0001
CP	7.80 ^a ±0.16	4.09 ^b ±0.16	2.30 ^c ±0.16	8.20 ^a ±0.16	<.0001
EE	1.84 ^a ±0.06	1.53 ^{bc} ±0.06	1.36 ^c ±0.06	1.82 ^{ab} ±0.06	<.0001
NDF	13.66 ^c ±0.19	15.26 ^b ±0.19	19.403 ^a ±0.19	13.52 ^c ±0.19	<.0001
ADF	6.45 ^b ±0.21	9.24 ^a ±0.21	8.41 ^a ±0.21	7.09 ^b ±0.21	<.0001
ADL	1.56 ^c ±0.07	2.52 ^b ±0.07	3.33 ^a ±0.07	1.82 ^c ±0.07	<.0001
HCN	5 ^c	10 ^b	100 ^a	5 ^c	<.0001
%DMD	82.30 ^a	64.67 ^b	44.67 ^c	75.20 ^a	<.0001

DM= Dry matter, CP= Crude protein, EE= Ether extract, NDF=Neutral Detergent Fiber, ADF=Acid Detergent Fiber, ADL=Acid Detergent Lignin, HCN=Hydrogen Cyanide, DMD= Dry Matter Digestibility

Enzyme treatment had the highest dry matter and ether extract, lowest ADF, NDF and ADL, and noticeably lower levels of HCN. *Saccharomyces cerevisiae* fermentation significantly increased crude protein while also decreasing HCN levels. Untreated samples had the highest levels of indigestible fiber and toxic HCN, indicating lower safety and nutrient quality. Naturally fermented samples exhibited intermediate results in all parameters.

Dry Matter Digestibility

Enzyme-treated CRM had the highest dry matter digestibility (DMD) of 82.30%, followed by *Saccharomyces cerevisiae* fermented CRM with 75.20%, which are significantly different from the untreated and natural fermentation with 44.67% and 64.67%, respectively (Figure 1).

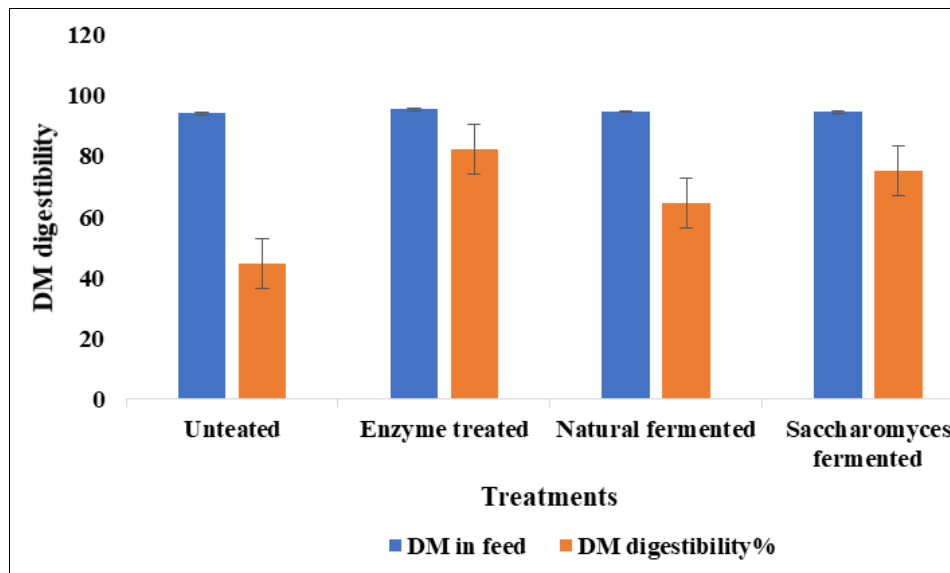


Fig 1: Dry Matter Digestibility of CRM

Discussion

Chemical Composition of Treated and Untreated Cassava Root Meal

There was no significant difference in the crude protein between enzyme treated and *Saccharomyces cerevisiae* fermented CRM however, the increase in *Saccharomyces cerevisiae* fermented CRM could be as a result of *Saccharomyces cerevisiae*'s metabolic processes, which produce extracellular enzymes and assimilate nitrogenous substances during fermentation, both of which aid in protein enrichment (Khurshida *et al.*, 2025) [16]. This is in agreement with Khejornsar & Khejornsart (2021b) [14] who argued that *Saccharomyces cerevisiae* utilises the carbohydrates in cassava pulp as energy sources leading to production of microbial biomass which increases the protein content in the cassava root. In the study by Khurshida *et al.* (2025) [16], the protein content of cassava flour raised from 2.42% to 5.54% after 96 hours of fermentation with *Saccharomyces cerevisiae* at 35°C. This was caused by *Saccharomyces cerevisiae*'s metabolic processes, which produce extracellular enzymes and assimilate nitrogenous substances during fermentation, both of which aid in protein enrichment.

The NDF and ADF content was significantly lower in the enzyme-treated cassava root meal compared to other treatments. This could have been because the complex carbohydrates and fibers were broken down by cellulases, hemicellulases, and pectinases from Natuzyme® enzyme, which break down the cellulose, hemicellulose, and lignin that make up the cell wall's structural elements. As a result of this process, the cassava root meal's fiber content decreased (De Souza & Kawaguti, 2021) [7]. Huang *et al.* (2018) [12] in their study found that enzymatic treatment of cassava residue improved its water-holding capacity and overall quality by reducing its crystallinity and increased that the fraction of soluble dietary fiber. This is also in agreement with Bunterngsook *et al.* (2017) [4] who reported that enzymatic treatment of cassava root meal and other starchy plants decreased the amount of ADF, and NDF significantly through the breakdown of structural carbohydrates by cellulase and hemicellulase. Enzymatic breakdown of the cell wall polysaccharides was identified as the cause of the fiber decrease. Aruwajoye *et al.* (2019) [1] in their work on enzyme-assisted processing of cassava also argued that enzyme treatments decreased the fiber content by breaking down the

structural polysaccharides in the root. The main process that reduced NDF and ADF was the breakdown of cellulose and hemicellulose content.

There was no significant difference in the HCN content between *Saccharomyces cerevisiae* fermented and enzyme-treated cassava root meal. The significant decrease in both *Saccharomyces cerevisiae* fermented and enzyme-treated cassava root meal was because, during fermentation, microorganisms such as *Saccharomyces cerevisiae* species produce enzymes like linamarase and beta-glucosidase which are reported to aid in the breakdown of cyanogenic glycosides, lowering HCN levels (Zidenga *et al.*, (2017b) [34]. This is also in agreement with the findings from Obi *et al.* (2019) [25] who reported that the fermentation process lowers the pH which aids in the breakdown of cyanogenic substances and further lowers the formation of cyanides. An acidic environment encourages the hydrolysis of linamarin and other cyanogenic glycosides. The decrease in HCN content in enzyme treatment of CRM is because the use of exogenous enzymes is believed to degrade cyanogenic glycosides, enhancing the detoxification of cassava root meal, hence lowering the HCN content (Easson *et al.*, 2021) [8].

Dry matter digestibility of cassava root meal

There was no significant difference in the dry matter digestibility (DMD) between enzyme treatment and *Saccharomyces cerevisiae* fermentation of cassava root meal. The significant increase in both enzyme treatment and *Saccharomyces cerevisiae* fermentation and be explained by Emmanuel *et al.* (2024) [10] who in their study of fermenting and enzymatically processing cassava peels to improve their digestibility for non-ruminant animals found out that fermentation process and enzymatic treatments reduced the amount of detrimental anti-nutritional components therefore improving the digestibility and nutritional value of cassava peels as an animal feed ingredient. This method enhanced the growth performance and nutrient consumption of broiler chickens, demonstrating the potential of enzyme treatments to improve diets based on cassava.

The significant increase of DMD in enzyme treatment of CRM could be explained by the breakdown of complex carbohydrates and fibers by cellulase, hemicellulase, and xylanase in Natuzyme® lowering its fiber content and improving the nutrients' accessibility for digestion (Takaeh *et*

al., 2024)^[30]. Huang *et al.* (2018)^[12] in their study found that enzymatic treatment of cassava residue reduced their crystallinity and increased the proportion of soluble dietary fiber, improving their water-holding capacity and overall quality.

These results showed that both Natuzyme[®] enzyme treatment and *Saccharomyces cerevisiae* fermentation, boosted the nutritional value of cassava root meal and decreased its fiber content, making it a more valuable feed item for poultry production.

Conclusion and Recommendations

The findings showed that treating cassava root meal with enzymes increased its protein content (7.80%), improved digestibility (82.30%), and lowered hydrogen cyanide levels (5) compared to *Saccharomyces cerevisiae* fermentation, with digestibility (75.2%), crude protein 8.2%, crude fiber (2.49), hydrogen cyanide (5) and spontaneous fermentation with crude protein (4.09), digestibility (64.67), crude fiber (3.27), and hydrogen cyanide (10). From the results, enzyme treatment and fermentation of CRM with *Saccharomyces cerevisiae* were the best processing method however, there was no significant difference between the two-processing method. Natuzyme[®] enzyme was more cost-effective and readily available option compared to *Saccharomyces cerevisiae*. They are inexpensive and easy to source, making it a practical choice for use in processing cassava root meal.

This research fills the knowledge gap about the nutritional enhancements that enzyme treatment and *Saccharomyces cerevisiae* have on cassava meal, mitigating the limitations of its use in poultry feed. These findings are important because they have the potential applications to enhance the poultry feeding in sustainability of feed supply, reduce competition of use of maize for food and feed. This will contribute to food and nutrition security through an increase in maize supply and poultry products. The study recommended that further studies be conducted to evaluate the long-term effects on poultry performance and health, investigating affordable enzyme sources, and refining enzyme treatment techniques for large-scale manufacturing.

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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 Nasta Chelangat, Anthony M. Kingori and Fred Kemboi. Data collection on proximate composition, digestibility, and hydrogen cyanide reduction analysis was done by Nasta Chelangat. After writing, reviewing, proofreading, and publication procedures of the manuscript were handled by Nasta Chelangat, Anthony M. King'ori, and Fred Kemboi.s

Conflict of interest

There are no personal, professional, or financial conflicts of interest in this study.

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